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O-01

Advancing Forensic Genetics with Ancient DNA Techniques: Comparative Assessment of Three SNP Genotyping Approaches

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Forensic genetic analysis often copes with degraded samples and standard STR typing may prove insufficient. Massively parallel sequencing (MPS) of forensically relevant SNPs has emerged as a powerful tool to overcome these limitations. MPS assays enables high-density SNP analysis, crucial for various forensic applications including forensic investigative genetic genealogy, assessment of distant kinships and prediction of phenotype and ancestry. Drawing parallels with ancient DNA (aDNA) research, we integrated aDNA practices into forensic genetics, aiming to enhance DNA analysis of challenging samples.

Our study included 20 skeletal bone and tooth samples from authentic missing person cases, reflecting varying sample qualities, including complete, partial, or absent STR profiles. Employing a modified silica-based DNA extraction method common in aDNA research, we conducted three distinct downstream analyses using different SNP genotyping technologies.

Firstly, we utilized the FORCE panel, including ~5500 forensically relevant SNP markers. DNA libraries targeting these SNPs were built with the QIAseq Targeted DNA Panel and sequenced on a MiSeq FGx. Secondly, we employed the Twist ancient DNA panel, targeting ~1.35 million SNPs via hybridization capture-based target enrichment, sequenced on a NovaSeq X Plus. Finally, we performed whole-genome sequencing on a NovaSeq X Plus. Bioinformatic analysis for all three methods was performed with CLC Genomics Workbench.

Through genotype comparison across these three methods, we evaluated their potential in generating usable SNP profiles for further analysis. We observed improved DNA information in 13 samples, demonstrating significant potential in integrating aDNA techniques into forensic genetics, offering improved outcomes in human identification from challenging samples. This research presents valuable data supporting alternative genotyping approaches to overcome limitations encountered in traditional forensic analysis methods.

O-02

Challenging the performance of the FORCE hybridization capture assay with difficult samples

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Traditional forensic molecular assays have focused on the analysis of STRs using capillary electrophoresis. Since a large portion of the samples found in routine forensic casework harbour only low quantity and quality DNA, alternative, more sensitive markers and assays have been applied successfully such as sequencing the mitochondrial DNA (mtDNA). Despite its high copy number that confers it more sensitivity, mtDNA as an haploid marker conveys less discrimination power than nuclear DNA does, which limits its usefulness for human identification and kinship cases (excluding a maternal transmission).

With the democratization of massively parallel sequencing, many assays were developed in the past years to improve the genetic information content present in highly degraded and low-copy DNA samples. The FORCE panel is one of this kind employing hybridization capture to investigate some 5422 DNA markers for identification, biogeographical ancestry, phenotypic traits, X- and Y-chromosomal SNPs sequencing. The FORCE panel was originally developed for Illumina-based technology.

Here, we aim to 1) adapt the FORCE panel to the Ion Torrent technology and optimize it for improved results; and 2) to test the assay with very challenging samples either by their technical complexity and using a complicated kinship constellation. For our second objective we decided to use hair shafts as a degraded and low-copy DNA source to really challenge the assay with minimum nuclear DNA amounts. Furthermore, to assess the assay's capacity to handle complex kinship tests, we opted to test it with famous historical cases that so far produced frail results.

O-03

Leveraging mid-density SNP panels to aid in the large-scale identification of the missing in Vietnam

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The 1955-1975 war in Vietnam resulted in millions of deaths. While many remains have been returned to their families, hundreds of thousands are still unidentified. DNA-led identification is challenged by the now 50-70 years old skeletal remains that have been buried in a tropical environment, resulting in extreme degradation of any remaining DNA. Most close family members are now deceased, so more distant relatives must be relied on to provide reference samples for kinship comparisons. These compounding challenges mean that a standard STR profiling workflow is not viable – instead next generation sequencing (NGS) of single nucleotide polymorphism (SNP) panels are being evaluated. A collaboration between the International Commission on Missing Persons (ICMP) and Vietnam's Academy of Science and Technology (VAST) has been established to jointly evaluate NGS workflows for highly degraded Vietnamese samples with the aim of permitting identifications on a large scale.

100 unidentified bone samples of varying visual quality were selected to represent the range of samples encountered. DNA was extracted using a full demineralization method, optimized to retain the shorter fragments characteristic of highly degraded samples. Subsets of extracts were subsequently processed using one or both of two mid-density NGS-SNP workflows: the MPSplex panel targeting 1439 SNPs using QIAseq single-base extension chemistry and unique molecular indexes (UMIs); the FORCE panel targeting 5422 bi-allelic SNPs using hybridization capture. Resulting data were evaluated for the number of SNPs recovered, percentage of sequencing reads aligned to the human genome, and average length of the mapped reads. Kinship simulations were performed to determine the theoretical matching power of these initial results in the Vietnam context.

Full and partial MPSplex and FORCE SNP profiles, capable of allowing extended kinship comparisons with up to third degree relatives, were generated. However, large percentages of off-target, exogenous DNA and short on-target DNA fragments limited profiling success of lower quality samples. Regardless, these initial results show that NGS workflows incorporating mid-density SNP panels and highly sensitive library preparation strategies show promise for the identification of highly degraded Vietnamese skeletal remains.

O-04

Impact of DNA Degradation on the Accuracy of Probabilistic Genotyping and Imputation of SNPs

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The integration of next generation sequencing into forensic casework has expanded the quality and types of substrates that can be used to generate DNA profiles. Recent analyses have revealed that DNA recovered from skeletal remains less than 70 years old can share the same degradation characteristics (decreased fragment sizes and deamination) as ancient hominin remains >30,000 years old. This not only emphasizes the challenges in working with degraded remains in forensic casework, but also raises questions around best practices for the downstream analysis of degraded DNA. How much coverage is required? Is it necessary to alter computational pipelines to minimize the impacts of degradation on genotype accuracy? Does genetic ancestry impact accuracy when using probabilistic genotyping and imputation? In this study we answer these questions using a simulation framework to directly interrogate how degradation impacts accuracy for different genotyping and imputation methods across genetic ancestries.

We simulated sequencing data of three different qualities in relation to DNA fragment size distribution (averages of 40 to 150 base pairs) and deamination (20% to 0%) across five different SNP panels (FORCE, MPS-plex, 25K, 95K, and Human Origins) for coverages between 0.1 to 10X. Genetic variation was then introduced from forty individuals representative of the genetic diversity in the 1000 genomes and TOPMed Study phs001644 (n=11,996) databases. We show that using mapping and filtering settings used in ancient DNA studies increases genotype concordance and retention of informative sequences. Testing of the ATLAS, GATK, and Samtools genotypers found that at least 10X coverage was needed to reach 90% concordance across DNA qualities. We also show that ATLAS consistently results in more accurate genotypes, in particular for low-coverage and degraded sequencing data. ATLAS continues to outperform the other genotypers even after genotype refinement, requiring only 5X coverage to reach 90% concordance. BEAGLE 5.4 and GLIMPSE were then compared for imputation, where we demonstrate that accuracy increases with a more diverse reference panel compared to one with closer genetic affinity to the individual in question (for example using a European reference panel for imputing the DNA profile of a European individual). Finally, we present these results using statistics that describe the genetics of an individual without using traditional population binning. The outputs of this study provide benchmarks for the analysis and interpretation of low-quality sequencing data.

O-05

Enhancing Forensic Investigations: Development of a Multiplex PCR Method with 12 Markers for Estimating the Time Since Deposition of Blood Stains

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A DNA profile can only provide information on the potential identity of the donor; it does not provide any context on how or when it was deposited. Thus, in many cases, the court is dependent on additional information to link a DNA profile to a crime. In these cases, knowing how or when the stain was deposited is the essential question. To date, there is no reliable method to accurately determine the time since deposition (TSD) of a blood stain for use in criminal cases. However, some studies have demonstrated a correlation between time since deposition and the ratio between pairs of fast and slow degrading RNA targets.

In a previous study, we have tested the preferential degradation of the 5’ end over the 3’ end of a selection of mRNA markers using quantitative PCR analysis. In this study, we present a newly-developed PCR-multiplex consisting of 12 markers for TSD estimation on the capillary electrophoresis platform. The multiplex further exploits the differential degradation of the 5’ and 3’ ends and is based on 8 previously tested mRNA markers with new primers designed to facilitate multiplexing. In addition, 4 new markers are included. The performance of the multiplex was tested in a time series of aged blood stains from 8 participants aged from 0 to 8 months after deposition, and on mock stains collected at other time points from different environmental conditions. A correlation between the ratio of marker degradation and TSD was observed, but certain markers showed a higher correlation than others. Additionally, inter-participant variation was noticed. The time series dataset was further used in a statistical framework to provide a model for estimating the TSD of a stain.

O-06

Denoising of microhaplotype MPS by computational correction and its application to mixed DNA analysis

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With the advent of phase-known sequencing enabled by massively parallel sequencing (MPS), research on microhaplotypes (microhaps), multi-SNPs within short fragments, has been widely reported in the forensic genetics. However, MPS data contains PCR and MPS errors, still posing challenges in distinguishing between noise and minor contributors in mixed DNA analysis. To mitigate the background noise of MPS, DADA2, a computational denoising package that tracks and corrects Illumina sequencing errors, was introduced and has become the most popular tool for microbial community analysis. Nevertheless, computational corrections for denoising MPS in forensic identity testing remain unexplored. In this study, we constructed an in-house MPS panel to amplify 24 multipurpose microhaps suggested by Kidd et al. (2022) for providing additional information on various forensic questions. It adopted high fidelity PCR system and unique dual indices to mitigate noise generated during library preparation. Subsequently, we employed DADA2 to analyze microhap amplicon sequencing data from Illumina MiSeq platform and assessed noise levels for 50 single-source DNA samples. DADA2 dramatically reduced noise levels compared to those reported by STRait Razor, decreasing from 20.8 % to just 0.1 %. Furthermore, we investigated the allele recovery rate of the minor contributors across 40 two-person DNA mixtures at various ratios (1:10, 1:20, 1:50 and 1:100), with 1 ng of input DNA. At 1:10, 1:20 and 1:50 mixtures, an average of 92.6 %, 80.3 % and 64.4 % of alleles from minor contributors were recovered, respectively. Even at 1:100 mixtures, 36.0 % of recovery rate was observed, with a low average false positive rate of 7.5 %. We also evaluated performance of the developed system by calculating precision, sensitivity and specificity. Finally, we will discuss the significance of denoising in mixture deconvolution using microhaplotype MPS system.

O-07

Progress of the Microhaplotype Working Group towards the selection of a consensus core set of loci for forensic use.

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The Microhaplotype Working Group (MWG) is composed of 28 individuals, who responded to the call initiated at the 2022 ISFG meeting in Washington, D.C. The group convened over the following year and a half to define selection criteria for a core set of microhaplotype (MH) loci to streamline their integration into forensic casework. A comprehensive report detailing these discussions is currently under development. This report outlines the initial groundwork for a broader, more comprehensive initiative. It envisions the future formation of an ISFG Commission, to finalize the MH core pool selection, engage investigators in preliminary testing, and develop recommendations for the implementation of the panel/s into routine casework.

An MH locus is defined by a set of polymorphic haplotype-defining SNPs in close proximity, with the first and last SNP of the set defining the limits and size in base pairs of the reported region. Flanking sequence real estate should be considered for efficient and specific primer binding. Although haplotypes and their frequencies are determined by a specific set of SNPs, the entire sequence should be recorded as rare variations may be valuable for forensic exclusions or inclusions. Utilizing short descriptors such as canonical SNP strings and sequence identifiers (SIDs) could be a practical solution for secondary allele nomenclature. The main locus selection metric should be the highest effective number of alleles (Ae) within and across populations. Loci of varying sizes serve different purposes: smaller loci (up to ≈100 bp) are best for analyzing degraded DNA samples, while mid-range loci (≈100 to ≈200 bp) are suitable for general forensic applications. Larger loci (≈200 to ≈250 bp) enhance mixture analyses and fine-scale biogeographic ancestry estimation. Loci exceeding ≈250 bp are discouraged. Selection criteria also involve avoiding insertion/deletion polymorphisms, homopolymers, and tandem repeats. Loci overlapping SINES and LINES, despite their high Ae, together with regions containing duplications, are not recommended. The selection of loci from the core set can then be customized based on the intended application and characteristics of the sequencing technology selected.

O-08

Recommendations of the ISFG DNA Commission on STR Sequence Nomenclature and Challenges with Implementation

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The ISFG DNA Commissions and their resulting reports/recommendations are one mechanism to accomplish the aim of the Society: to promote scientific knowledge in the field of genetic markers as applied to forensic science. These Commission reports serve as a benchmark of current knowledge, opinion and procedure, as agreed by the contributing panel of scientific experts, including the ISFG Executive Board. The ISFG DNA Commission on STR Sequence Nomenclature Recommendations convened in 2021, and the resulting report was published in early 2024 [1], containing recommendations on: STR sequence reporting range; STR sequence proxies; resources for nomenclature information; guidance on formatting new STR loci; and considerations for future databasing of STR sequences.

Any such recommendations will generate discussion in the affected scientific field. The objective of this presentation is to discuss the challenges in implementing some of these recommendations. The challenges were largely known during the course of the Commission; they were discussed at length and weighed against their benefits. The guiding principle of the Commission was to recommend what the members believed would be best scientifically, based on current knowledge. In the context of STR sequence nomenclature, this included recommendations aimed at reducing two potential sources of error. The first recommendation aims to reduce ambiguity in sequence string reporting which could confound inter-kit / inter-laboratory comparisons and database searching. Decisions resulting from this aim, namely the recommended "ISFG minimum reporting range", now result in implementation challenges due to the current inability of existing assays to meet this range for varying loci, and the effort required to reformulate and revalidate these assays. The second recommendation in the report aims to reduce the opportunity for human error when sequence proxies are needed. Recommendations related to this aim, specifically changes in the "bracketed motif" (e.g., ATAG[12] to GATA[12]) can result in a disconnect between previously published data and data generated going forward. Other, lesser challenges also exist.

Subsequent to the publication of this DNA Commission report, the STRAND working group and other Commission members have regularly been discussing implementation of these recommendations with relevant casework laboratories and commercial kit vendors. While an easy answer may not be possible for each challenge, both interim solutions and long-term ideas will be presented.

References

[1] Gettings, K.B. et al, Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on short tandem repeat sequence nomenclature, Forensic Sci Int Genet, Vol 68 (2024) https://doi.org/10.1016/j.fsigen.2023.102946.

O-09

NIST Research Grade Test Materials: A New Collaborative Effort to Address Measurement Challenges

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As DNA typing technologies have advanced and sensitivity improved, measurement and interpretation issues related to low template, degraded, and complex DNA mixtures encountered in forensic laboratories have evolved. In turn, the forensic DNA typing community has requested more relevant reference materials to support the validation of their methods. The stochastic nature of these measurements, in tandem with the goal of reproducibly providing “casework-like" sample types, poses a challenge in meeting the robust characterization and the long-term stability required for a Standard Reference Material classification. NIST has introduced a new category of exploratory material called a Research Grade Test Material (RGTM) to collaboratively evaluate fit-for-purpose needs within a community. The material is sent to requesting laboratories at no cost, with an agreement that data collected from the samples will be shared with NIST. A data portal hosted through STRBase (https://strbase.nist.gov/Information/RGTM_10235) was created to receive and publicly share results.

The RGTM is composed of a set of eight DNA extracts quantified by digital PCR methods. Components include three single source, two UV-degraded single source, and three DNA mixture samples. As part of the RGTM production process, automated methods for bottling, alternative sample tube types, and the addition of carrier RNA to stabilize low template samples were investigated. Additionally, the samples were collected with specific informed consent to guide their use and subsequent data sharing by the forensic genetics community.

This presentation details the material production and the initial quantification results submitted by multiple users employing various qPCR methods. Results of replicate amplifications of the three mixtures followed by interpretation using probabilistic genotyping and subsequent assignment of likelihood ratio values will be discussed for the three mixture samples. The goal is that this sample resource will support validation efforts, facilitate data sharing, and guide further development of forensically relevant reference materials.

Oral Communications 2.1

General Session 2.1: New Technologies and Human Identification

General Session 2.1 New Technologies and Human Identification

Abstracts Oral Communication

O-10

Micromanipulated single cell subsampling, genetic analysis and probabilistic genotyping for complex mixture deconvolution

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Biological evidence is often found at crime scenes and can comprise of DNA from the victim(s) and perpetrator(s) to a crime as well as from individuals with no direct relationship to the incident. This can complicate analysis as DNA mixtures are one of the more difficult sources of biological evidence to interpret. Probabilistic genotyping (PG) has greatly aided in mixture analysis. However, even with PG, standard bulk mixture approaches do not always result in probative results as allele overlap, artifacts, or low-level minor contributors inevitably cause genotype information loss. Therefore, deconvolution of forensic DNA mixtures into their individual component DNA (geno)types is of great investigative value.

In the present work, enhanced single cell DNA typing conditions consisting of reduced reaction volumes and increased PCR cycle number were optimized and paired with a simplified micro-manipulation technique resulting in a subsampling scheme referred to as direct single cell subsampling (DSCS). DSCS allows for various clustering methods to be employed to group resulting genotypes according to donor (e.g., mixture-to-mixture matching or by donor LR). Furthermore, the PG systems STRmixTM and EuroForMix were validated for use with both standard bulk DNA mixtures as well as with 1-5 cells. The DSCS approach was applied to various complex mixture scenarios including equimolar 2-6 person mixtures, mixtures comprised of 1st degree relatives, and mixtures in which a minor donor is virtually undetectable (~1:50) resulting in a probative gain of information compared to the standard mixture methods. Specifically, with the 5- and 6- person complex mixtures analyzed, DSCS recovered highly probative LRs (> 10^20) from donors that had returned non-probative LRs (< 10^3) by standard methods. With familial mixtures, DSCS prevented the false inclusion of non-donor relatives seen with standard methods. This approach was further applied to Y-STR mixture analysis.

O-11

Air DNA Forensics: A potential new tool in an investigator's kit

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Modern techniques can generate highly discriminatory DNA profiles from minuscule biological samples, providing valuable information in criminal investigations and court proceedings. However, trace and touch DNA samples, due to their nature, often have lower success rates than other biological materials such as blood. Further, forensically aware criminals can utilise gloves and meticulously clean the crime scene to remove DNA traces of themselves from contacted surfaces.

Air sampling offers a novel approach to the collection of human DNA that has a potential to bypass some of these issues. This study reports on the results of research into the prevalence and persistence of human DNA in the air and describes the progress towards optimising the collection method. The ability to collect human DNA from the air was investigated utilising an AirPrep Cub Sampler ACD220 in different spaces, with and without the presence of individuals, for various durations and with multiple collection filter types.

Results of this study demonstrate that the type of filter, filter sample size, sampling duration, level of occupation and time since last occupation each have an influence on quantity and quality of DNA recovered. The data produced from this study will provide insights into the utility and optimal conditions of air sampling for human DNA for forensic applications such as providing samples from the most recent occupants of crime scenes, increases awareness of contamination risks, and suggests further avenues for improvement.

O-12

Advancing forensic investigations in aquatic environments through environmental DNA (eDNA) analysis

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This study investigated the forensic application of environmental DNA (eDNA) techniques in aquatic environments, with a specific focus on detecting human DNA. The aim was to develop a model to assist forensic investigators in locating human DNA, or narrowing down search areas in water where human biological evidence may be present. To achieve this, detection patterns surrounding point sources of human biological material were identified. A mesocosm laboratory experiment within an 800 L tank 240cm (L) x 35cm (W) x 80cm (H) was conducted using 5 mL of food dye as a proxy for human white blood cells, deposited at different locations, each location tested in two replicate trials. GoPro cameras were strategically positioned in x, y, and z dimensions, to determine approximate dispersion patterns of eDNA in aquatic environments. Subsequently, another mesocosm laboratory experiment used 3 mL of human blood spiked into an 800 L freshwater tank, with the concentration of human mitochondria DNA (mtDNA) measured in each quadrant for up to 2 hours. Data from the trials, including the area of the food dye plume and the concentration of human DNA from the blood trial, were used to develop the model. Following this, a field experiment was conducted at a dam on a rural property to simulate a crime scene and validate the eDNA dispersion rate model in a real-world setting. Human blood (30 mL) was poured in at the top of the water surface at the dam's shoreline with 4 lateral transects aligned to the source (two bankside and two midstream), with 6 sampling points along each transect: 1, 5, 10, 15, 20, 30 and 40 m away from the source. A 1 L water sample was taken from a 0.5 m depth of the water at each point along the transect at 0 – 1, 6, 24, 48, and 72 hours following the deposition of human blood. The distribution patterns and concentrations of human mtDNA generated under field conditions were compared with those predicted using the model. Further refinement of the model will ultimately provide a practical forensic tool which can be used to enhance search efforts for human biological evidence, including bodies and items containing human biological materials, in aquatic environments.

O-13

Exploring the potential of FORmics: “Forensic microbiome and metabolomics-based” approach for body fluid identification

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1. Objectives:

Determining the bodily origin of a biological trace from crime scenes is important in forensic investigations to decipher activities that led to the crime and towards formulating activity-level propositions in casework. We systematically evaluate microbiome and metabolomic signatures for body fluid identification (BFI) leveraging machine learning tools. Ultimately, our goal is to establish a novel BFI method that integrates microbiome and metabolomic signatures. An integrated method could therefore provide additional lines of evidence in the investigation of complex cases including sexual assault cases.

2. Material and methods:

We examined a total of 700 control and mock samples from 9 forensically relevant body fluids, namely: saliva, semen, urine, feces, vaginal fluid, menstrual blood, fingerprick blood, skin from hands (palmar region), and penile skin. Mock samples included aged samples, mixed samples, samples deposited on substrates in the lab, and underwear samples from women. Microbiome data was generated through sequencing of the V4-V5 region of the 16S rRNA gene. Read data was processed and a random forest classifier with 5-fold cross-validation was trained with QIIME2 (v2023.5). Untargeted metabolomics data was obtained using a high-resolution liquid chromatography-quadrupole time of flight mass spectrometer (LC-QTOF-MS, Sciex 6600) instrument. The metabolomics data was analysed using MSDial (v4.9.22) and the mixomics package in R was used to train an sPLS-DA model. R Studio (v4.2.1) was used for generating plots and statistical analyses for both signatures.

3. Results and conclusions:

Overall, high prediction probabilities were observed for the random forest classifier trained on microbiome data with an overall accuracy of 0.88. Metabolomics analyses using sPLS-DA model also revealed body site distinction with an overall accuracy of 0.7. Interestingly, the two signatures were complementary to each other, especially for body fluids frequently encountered in sexual assault cases. For instance, the microbiome classifier could distinguish among saliva, skin from hands and urogenital samples such as semen or vaginal fluid. Meanwhile, the metabolomics model could resolve the urogenital samples especially semen from all other body fluids. In addition, the metabolites could distinguish between blood samples from different origins i.e. menstrual versus fingerprick or venous in control samples. These results provide compelling evidence to explore the microbe-metabolite associations with mmvec and correlation-based network analyses. Furthermore, combining the predictive capabilities could open new avenues to determine crime-related activities and potentially formulate activity-level propositions.

O-14

Forensic Genetics in Historical Context: Identifying 1742 Murder Suspects

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The investigation into two bodies unearthed in 1946, suspected to be those of an infamous pair of siblings executed in 1742 for murder, has undergone comprehensive genetic investigations. We prepared whole genome libraries from DNA extracted from bones in quadruplicates and sequenced each library to 30X theoretical depth. The data suggest high bacterial content and a high amount of unspecific sequence, with only 5-10% of reads mapping to the human genome, as well as high duplication levels (10-50%).

With the aid of a dedicated group of local historians, ten distant relatives descended from the accused duo were identified and sampled. We analyzed the 10 reference samples and 35 unrelated individuals from the Norwegian population using a GSA microarray covering an excess of 600,000 SNP markers.

To augment the data and provide a higher overlap between the whole genome sequence data from the bones and the reference individuals, we performed imputations using the 1000Genomes individuals.

Using a combination of so-called segment matching and traditional likelihood ratio approaches, we determine the identity of the unidentified remains.

O-15

Applying hyperplex PCR for mRNA-based cell type identification

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Body fluid and tissue identification of biological crime scene stains may provide increased evidential values, e.g., in the evaluation of activity level propositions. Methods for mRNA-based cell type identification are generally based on RT-PCR and either capillary electrophoresis or massively parallel sequencing. The recently invented hyperplex PCR offers an alternative methodology for forensic mRNA analysis, potentially enabling quicker analyses with lower limits of detection. We have developed and evaluated a hyperplex PCR assay for cell type identification, using mRNA markers and primers from a published 19-plex developed at the Netherlands Forensic Institute (van de Berge et al, FSI Genetics, 2016). The method consists of 1) a one-step multiplex RT-PCR, 2) target specific padlock probes that, upon a perfect match to the amplicons, form circular DNA molecules using a ligase, 3) rolling circle amplification (RCA) to generate products that contain repetitions of the padlock probe sequence, 4) labelling of the RCA products using fluorescently labelled probes, called nanopixels, with a unique fluorescent fingerprint for each marker, and 5) detection and counting of individual RCA products using fluorescence microscopy and a custom software. The assay targets mRNA markers for blood, saliva, nasal mucosa, semen, vaginal mucosa and menstrual secretion. Samples with known body fluids, including mixtures, were analysed at different RNA amounts to evaluate the specificity and limit of detection of the hyperplex PCR assay. Overall, the target specific padlock probes and end-point detection of RCA products using nanopixels enabled confident detection of the cell types at acceptable limits of detection. Hyperplex PCR is thus a promising technique for cell type identification, and may also find other applications in forensics.

Oral Communications 2.2

General Session 2.2: Human Identification

General Session 2.2 Human Identification

Abstracts Oral Communication

O-16

Distinguishing between monozygotic twins in a victim-perpetrator DNA mixture

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In this study we describe a method to distinguish between the DNA of two monozygotic twin brothers in order to identify which brother contributed DNA to a mixed trace. In the case that we report upon, mixed DNA profiles had been obtained from a victim's clothing containing the victim's DNA and DNA of an unknown male, presumably her offender. A subsequent database search with the autosomal DNA profile pointed towards a man in the database. However, this person had a monozygotic twin brother who, based on other case circumstances, became the suspect in this case. With both men denying all involvement, the court requested to try and differentiate between the twins DNA in order to establish which brother contributed to the trace sample.

Obviously almost all of the DNA of twin brothers is identical. To find differences between the DNA of both brothers, we searched for particular mutations on the twins' DNA. Ideally, these should be identifiable as pre-twinning or early post-twinning , such that both men have become mosaic for such a mutation (carrying both the mutant and regular DNA sequence), but with the degree of mosaicism being sufficiently different between them to be useful for identification purposes. We identified five useful mutations. Subsequently the trace profiles were investigated for these mutations, and we obtained PCR products in which we could measure, for each locus, the proportions of mutant alleles. We then set up a new statistical model for the interpretation of such results, accounting for various sources of uncertainty (e.g. in the number and relative fraction of mutant alleles pre and post-PCR). We verified that the model was able to predict post-PCR fractions of mutant alleles in agreement with observations. This model yielded very strong evidence in favor of the suspect having been the origin of the male DNA in the trace samples. In addition, the model allows to speculate on how much trace DNA, and how many mosaic mutations, are needed to differentiate between the DNA of monozygotic twins in general.

The NFI report describing these results was accepted as evidence in court. During this talk we will discuss the DNA evidence, the statistical model and the court acceptance.

O-17

OneShot: Developing a novel methodology for simultaneous genetic and epigenetic sequencing for forensic applications

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In the past decade, significant strides have been made in utilizing epigenetics for forensic purposes, particularly in age prediction and body fluid identification (BFID). Various markers have been identified to estimate age and differentiate between body fluids. The current practice entails a dedicated workflow to convert and quantify methylation levels: notably bisulfite conversion, followed by sequencing. Enzymatic conversion was also explored as a solution to differentiate methylated from unmethylated cytosines, mostly for clinical purposes. However, current methods often involve separate workflows for genetic and epigenetic analyses, presenting a challenge for comprehensive forensic profiling.

In this proof-of-concept study, we developed a novel approach integrating genetic and epigenetic sequencing into a single workflow. Our methodology combines enzymatic-based library preparation with hybridization capture, targeting core STR loci and CpG markers relevant for BFID and age prediction. The library prep involves hairpin addition, splitting, copy strand synthesis, adapter ligation, protection, deamination, and amplification. For target enrichment, 584 probes were custom designed to capture our markers of interest, followed by sequencing on a MiSeq FGx. A custom bioinformatic pipeline was developed for base resolution via a two-base coding mechanism where R1 and R2 reads are compared to resolve for A, T, G, C, and methylated C followed by data interpretation via IGV and MixtureAce software programs.

To validate our methodology, we collected blood, semen, buccal, and vaginal fluid samples with informed consent and a control sample. DNA extracts were sonicated down to 250bp on average (M220 focused-ultrasonicator, Covaris), spiked with methylation controls, library prepared (duet multiomics solution +modC, biomodal), pooled, and captured (Fast Hybridization Target Enrichment, Twist Bioscience), then sequenced on a Micro kit followed by analysis.

Our results demonstrate a highly efficient conversion exceeding 96% in sensitivity and 99.8% in specificity in modified Cytosine calls, with average coverage of 30x for CpGs and up to 10x for most STR loci. Target enrichment was successful with differential methylation observed across the four fluids on all tested markers. Additionally, accurate partial STR profiles were achieved.

This study represents a pioneering effort for simultaneous sequencing of STRs and CpGs. It will serve as the foundation for an exciting area of development to provide the forensic community with a novel solution that potentially will save on time, resources, and sample consumption. The integration of genetic and epigenetic profiling in one workflow can be customizable as needed and holds potential to enhance efficiency in forensic analyses.

O-18

DNA methylation in the AHRR gene can accurately identify tobacco smokers and may also be useful in predicting other lifestyle behaviours of a perpetrator

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DNA methylation analysis makes it possible to find traces of environmental influences in the human genome. This allows the selection of epigenetic markers of lifestyle behaviours and the development of predictive models to better describe the profile of an unknown individual. It is an important step forward in the use of DNA analysis for forensic DNA intelligence purposes.

In this study, the DNA methylation profiles of the blood of almost 800 people for whom lifestyle information was collected were examined using Illumina's MethylationEPIC 850 MicroArray.
We found 459 significant CpGs involved in smoking. The top smoking marker, cg05575921 in AHRR (p=4.5e-32), and three additional CpGs all showing hypomethylation in smokers, were selected for prediction modelling. The developed new compact 4-CpG prediction model allowed smoking to be inferred with an accuracy of AUC = 0.8 in a binary model. AHRR played a key role in a predictive analysis, explaining 21.5% of the variation in smoking and, when used alone, predicted smoking outcome with an AUC of 0.76. When three smoking categories were considered during model training, AUC values were 0.8 for never smokers, 0.69 for light smokers, and 0.88 for heavy smokers. Importantly, former smokers displayed the intermediate DNA methylation profiles compared to current and never smokers, and our results indicate the potential reversibility of DNA methylation after smoking cessation. In addition, the AHRR methylation was analyzed for association with several other modifiable lifestyle factors and showed significance for sleep (p=0.004), physical activity (p=6.2e-5), education (p=2.2e-5), and vegetable consumption (p=0.001), confirming its power as a lifestyle biomarker for forensic purposes.

O-19

DIVIANA: A free, user-friendly app for complex DVI cases

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1. Objectives
We present DIVIANA, a user-friendly and comprehensive program for disaster victim identification (DVI). DVI constitutes a branch of kinship testing, where a main task is to match DNA from a set of victims against relatives of the missing individuals. The need for accurate and robust DVI methods is increasing, but current software solutions do not adequately cover some of the complexities often seen by case workers. In particular, cases with related victims are challenging with existing methods, typically requiring sub-optimal stepwise approaches. Our program aims to alleviate these problems, by exploiting recent advances in the statistical treatment of DVI.

2. Material and methods
DIVIANA combines multiple techniques for DVI, including pairwise likelihood ratios, and joint (global) analysis in families with multiple missing individuals (Vigeland & Egeland, 2021). We introduce a generalised likelihood ratio (GLR) as a novel statistical measure to help interpretating and reporting the joint results.

Supported input formats include files created with GeneMapper and Familias. A core feature is an interactive pedigree builder, which significantly simplifies the task of assigning relationships in the ante mortem data. Results may be exported to Excel. Other novel features include relatedness estimation to check for pedigree errors (not only genetic inconsistencies) in the reference data, visualisation of solutions and post-analysis pedigree reconstruction to assess the identifications. DIVIANA is made in R/shiny and builds on the pedsuite packages for pedigree analysis in R (Vigeland, 2021).

3. Results and conclusions
DIVIANA offers a comprehensive pipeline for DVI analysis, wrapped in a user-friendly graphical interface. It expands on existing methods and addresses several challenges encountered in real-life DVI cases, especially those involving related victims. The app is freely available, open-source, and runs on all platforms.

O-20

Comparison of forensically relevant large scale SNP panels for complex kinship analysis

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Targeting Single Nucleotide Polymorphism (SNP) loci for human identification in forensics offers several well documented advantages over traditional DNA typing methods. SNP amplicons are shorter than those of Short Tandem Repeat (STR) markers, making them useful for the analysis of degraded DNA, where larger fragments may not be recoverable. They also have a lower mutation rate over the course of generations than STRs, which is useful for kinship analysis, especially in the context of complex pedigrees. Through the application of Next Generation Sequencing (NGS), many SNPs can be multiplexed at once, easily providing a power of discrimination equal to or far exceeding that of a traditional STR test.

The demand for larger scale SNP panels for complex kinship analysis and unidentified human remains (UHR) applications has grown in recent years, and today, the question arises – "how many SNPs do we need?". The more distant the relationship being queried, the more SNPs would in theory be required. Unlike the long-range kinship analysis used for Forensic Investigative Genetic Genealogy (FIGG), UHR cases are often closed scenarios, with available family references. Currently, there is a lack of empirical data to show how the kit configuration affects the likelihood ratio (LR) of a given pedigree. This is especially true for low input, low quality samples, often encountered in UHR cases and disaster victim identification scenarios.

In this work, data was compared for a repurposed commercially available panel developed for FIGG and a community developed panel. Samples were analysed using the 10,230 SNPs in the ForenSeq Kintelligence kit and the 5,500 SNPs in the QIAseq FORCE panel. Pairs of samples obtained for the purpose of complex kinship analysis were run, with the aim to test these different workflows at the outer limits of the relationship scenarios that might realistically be encountered in UHR cases. Direct comparison of real-world performance between the methods on the same samples was performed, factoring in kit specific locus and allele dropouts when calculating LR values on samples containing compromised DNA quality and/or quantity. This direct comparison can be used to guide laboratories regarding which SNP panel configuration to choose in differing scenarios.

Oral Communications 3

General Session 3: Population Genetics and Forensic DNA Phenotyping

General Session 3 Population Genetics and Forensic DNA Phenotyping

Abstracts Oral Communication

O-21

Optimizing Ancestry Informative Markers selection for inferring biogeographical origin of migrant victims along the Central Mediterranean route

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Introduction
Ancestry Informative Markers (AIMs) can play a pivotal role in various stages of the identification of migrants who have perished in maritime incidents during passage across the Mediterranean Sea. AIMs can aid in accessing and searching for ante mortem data, determining allele frequencies for likelihood ratio calculations and assessing prior odds.

Material and methods
In this study, we focused on the main regions of origin of migrants following the Central Mediterranean route, including North and East Africa, Middle East and South Asia regions. We developed a machine learning pipeline for selecting optimal markers for the smallest possible panel for ancestry classification. First, we compared Rosenberg's Index (In) and random forest feature importance for marker prioritization. Then, we combined the most informative markers into expanding AIM sets until reaching a required accuracy with Naïve Bayes classifiers.

Results and conclusions
Using SNP array data of 1088 individuals, we identified two AIM sets: one composed of 30 SNPs compatible with traditional capillary electrophoresis approaches and a larger set of 100 AIMs for massive parallel sequencing analysis. Despite the high genetic similarity of some populations (e.g., Middle East and North Africa), we achieved cross-validated balanced accuracy scores of 0.805±0.036 with 30 AIMs, and 0.884±0.027 with 100. Notably, East Africa showed the highest cross-validated AUCs (0.983 with 30 AIMs and 0.998 with 100), followed by South Asia (0.956 and 0.983), Middle East (0.918 and 0.967) and North Africa (0.873 and 0.928).

By evaluating whole sets of AIMs, our method intrinsically balances marker informativeness for discriminating each pair of populations and avoids redundancies, thus highlighting the importance of tailored marker selection for accurate ancestry inference in populations with overlapping ancestries.

O-22

From familial haplotype to evolutionary haplogroup: Are haplogroup predictions based on Y-chromosomal short tandem repeats accurate enough to identify biogeographical roots for forensics?

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The male Y-chromosome serves as an interesting tool in population and forensic genetics. Its rather conserved paternal inheritance provides valuable insights into human ancestry, biogeographical origins and DNA kinship investigations. Through slowly mutating single nucleotide polymorphisms (Y-SNPs; 10^-9-10^-8 mutations/generation), twenty evolutionary Y-haplogroups (A-T) can be assigned. Nevertheless, with Y-SNP typing being time-consuming and costly, there has been a growing interest in seeking alternative approaches for haplogroup assignment. Interestingly, haplogroups often share similar sets of more rapid mutating familial Y-chromosomal short tandem repeats (Y-STRs; 10^-4-10^-2 mutations/generation), which are usually determined in forensic casework. This implies that a men's Y-STR haplotype might successfully estimate his haplogroup, offering efficiency regarding labour, time, and cost. In the last decades, various Y-STR haplogroup predictors have been developed. Unfortunately, their effectiveness for use in forensics remains uncertain. Therefore, we investigated the most promising predictors for Y-haplogroup assignment from Y-STR profiles, including the Whit Athey's, Stephen P. Morse, NevGen, and PredYMaLe Predictors. This validation was based on Y-DNA data from 2,476 men in our CSY-database. Both their Y-(sub)haplogroup (81 Y-SNPs) and Y-STR haplotype (38-46 Y-STRs) were typed using capillary electrophoresis. This database is representative for the West-European population, showing a Y-haplogroup distribution of R (62%), I (22%), E (6%), J (5%), G (4%), and H, L, N, Q, T (1%). Y-STR haplotypes were entered into the Y-STR haplogroup predictors and the predictions were then compared to the Y-SNP-derived haplogroups, offering a means to assess the prediction's accuracy. Furthermore, the impact of the number of input Y-STR loci was evaluated. Our results showed that the Whit Athey's Predictor with Main 111-Marker Program is the preferred Bayesian allel-frequency approach as it resulted in more accurate haplogroup predictions in only several seconds per sample. Moreover, comparing the commercially available Yfiler® Plus Kit (27 Y-STRs) with the in house developed YForGen Kit (46 Y-STRs) revealed that adding more Y-STRs results in a higher prediction's accuracy. However, to predict hundreds of Y-haplogroups, the PredYMaLe Predictor should be considered as it is a machine learning-based approach which is beneficial regarding time efficiency. Its analyses will be finalised in the upcoming weeks. By our in-depth investigation of the prediction's accuracy in terms of Y-(sub)haplogroups and number of input Y-STR loci, we provide valuable insights into the effectiveness of the Y-STR haplogroup prediction tools. This sheds light on Y-chromosomal variation, aiding in refining and facilitating cost-efficient methodologies for genetic classifications and forensic DNA investigations.

O-23

Classification in Biogeographical Analysis: When Does it Make Sense and What are the Alternatives?

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In forensic analysis, a common task is the analysis of Biogeographical Ancestry (BGA), which involves classifying individuals into populations. Available tools include software such as SNIPPER, which comes with a graphical PCA-analysis and a classifier, both using a curated publicly available dataset of SNPs in a set of individuals with known geographic origin. However, individuals might be admixed. Specifically, their genome comprises contributions from multiple populations, e.g. one half of the genome comes from Africa and the other half from Oceania. For such samples the question arises if it makes sense to use a classifier, or if the analysis using STRUCTURE (or ADMIXTURE) should be preferred.

We implemented a statistical test, where the null hypothesis asserts the suitability of classification into non-admixed populations, while the alternative hypothesis states that the individual is admixed. Furthermore, our investigation revealed that employing too many markers in SNIPPER lead to worse estimations, contrasting with the performance of STRUCTURE. As a compromise between classification and admixture analysis, we propose a novel approach that classifies the parents of the DNA trace into populations. This approach is similar to SNIPPER with the difference that the (unknown) parents can be classified into different populations.

We showed that our statistical test and our novel model work very well using both, real data from the 1000 Genomes Project and simulated data. As a result, we advocate the use of the statistical test to decide whether the classification of the trace into one population makes sense. The recent classifier provides more information than the state-of-the-art approach and it also takes admixed individuals into account.

O-24

Face-ing the Unknown: 3D Facial Prediction for Forensic Identification

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Facial approximation plays a crucial role in helping to identify victims in forensic cases involving skeletal remains. However, manual 3D facial reconstruction approaches such as the Combination Manchester method are time-consuming and subjective, relying on limited numbers of anthropological facial soft tissue thickness (FSTT) and facial muscle measures. While available computational methods offer potential objectivity, they are held back by insufficient databases and a lack of standardized, easily applicable methods. To address these challenges, we describe a novel computational approach built from a dataset of both skull and tissue facial masks obtained in the same space using Cone-Beam-Computed-Tomography (CBCT) facial scans of 100 European individuals. The approach uses the open-source program Meshmonk to mask both hard and soft tissue structures with identical landmarks so that FSTT can be measured by calculating the distance between these corresponding points. This generates a clear FSTT metric at approximately 7000 points across the craniofacial region which we use to ascertain the average depth for prediction. Our approach can generate facial approximations within 10 minutes, incorporating and adjusting these measures with information on age, sex, height, and weight. We also provide an error adjustment gauge to highlight areas across the face that are more prone to variation, generating several possible visuals. Additionally, we integrate DNA prediction knowledge from Single Nucleotide Polymorphisms (SNPs) associated with nose shape, as nose morphology is not accurately determined by underlying hard tissue, to complete the visual renditions. At present this method is limited to a European scale, however the goal of this work is to highlight its proof of concept and utility. We believe that providing standardized masks to measure population FSTT variation will enhance data comparability and sharing amongst research groups, with the idea of creating a larger standardized database of measures that captures both global and local variation. Finally, we show the application of our method to the skulls of several unknown skeletal remains to highlight its use in the forensic field with the goal of developing a streamlined pipeline for implementation in research and investigative facilities.

O-25

Towards DNA-based prediction of facial appearance – identification of novel loci shaping facial morphology

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The ability to develop a forensic sketch through DNA analysis is currently one of the most intensively researched topics in forensic science, as it has the practical value of enabling the perpetrator to be apprehended or the victim to be identified more quickly. DNA-based methods for predicting pigmentation, ancestry and age are available to forensic DNA experts, but the most useful would be to predict facial appearance. The main difference and difficulty arising from the analysis of the facial morphology seems to be its three-dimensionality, the multiplicity of the factors influencing the phenotype (sex, age, ancestry, BMI, environment) and the fact that it is controlled by many genes. For nasal phenotypes alone, the GWAS catalog lists 266 reported traits (both 2D and 3D) with 332 genetic associations.

In our research we explore a new dataset that includes 3D facial scans, genetic (Infinium® Global Screening Array, Illumina) and epigenetic (MethylationEPIC 850 MicroArray, Illumina) data for 767 research participants from the Polish population. To capture the facial morphology, we used 43 landmarks selected based on a face recognition software that were augmented with additional anthropometric points. These coordinates were then used in the association analysis using PLINK. The anthropological analysis confirmed that some of the facial features are gender and/or age-related and thus the analysis was adjusted for these variables.

Linear regression analysis identified 53 SNPs significantly associated with 25 facial measurements and indices. The identified polymorphisms are located within 22 genes and 24 intergenic regions. The results included 7 previously known genes involved in facial shape and 46 novel associations. Notably, SNPs within the CCDC65 and SCAPER genes were found to be significantly associated with more than one trait. The highest number and the strongest associations were found for nose width, total facial index and lower facial contour.

Further studies will focus on predictive modelling that will include SNPs for various face characteristics and DNA methylation data which is a source of age and BMI markers. We aim to investigate the genetic basis of craniofacial shape and the potential use of hard tissue structure in predicting facial shape.

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Oral Communications 4

General Session 4: Forensic DNA Phenotyping

General Session 4 Forensic DNA Phenotyping

Abstracts Oral Communication

O-26

Evaluation of DNA methylation sites neighboring STRs and iSNPs for potential body fluid determination and contributor assignment

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When analyzing mixtures, a highly relevant question is the assignment between sample contributors and corresponding body fluid. Earlier studies started to analyze combined person-identifying single nucleotide polymorphisms (iSNP) and tissue-specific differentially methylated DNA regions (tDMR) to link mixture contributors to “their” body fluid. However, short tandem repeats (STRs) are the gold standard for individualization and identification of mixture contributors as they exhibit a higher variation and thereby discriminative power. Therefore, we additionally investigated DNA methylation sites in close proximity of STRs for potential body fluid-specific methylation.

We selected 18 autosomal STR systems, including nine CODIS systems, to investigate their neighboring DNA methylation sites. In addition, our approach was extended with an evaluation of eight known tDMRs for blood, semen, saliva and vaginal fluid and iSNPs in close proximity.
Blood, semen, saliva, vaginal fluid, buccal mucosa, nasal secretion and menstrual blood was collected from volunteers. DNA was extracted from these samples and quantified using qPCR. After bisulfite conversion, an additional qPCR was performed to verify the successful conversion and ssDNA quantity. The converted DNA was analyzed using amplicon-based massive parallel sequencing (MPS) including regions surrounding the selected STR and iSNP sites.

We present the successful MPS analysis of STR systems and iSNPs of bisulfite-converted DNA. We identified potential STR-flanking sites with varying DNA methylation levels between body fluids. Besides, we confirmed the tissue-specificity of known tDMRs and the potential to use flanking iSNP for identification of known contributors. Thus, we show a set of combined individual and tissue-specific positions that form the basis for linking sample contributor and biological source type in mixtures.

0-27

Analysis of genetic and epigenetic data to infer an individual's perceived age

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Objectives: Epigenetic age estimation is an important application of DNA methylation analysis. Due to observed differences in individual rates of biological aging, estimation of perceived age, i.e., assessed based on an individual's appearance, may be of added value in forensic science. Phenotypic aging, as manifested by facial wrinkling and other symptoms of a gradual deterioration of skin functions, is determined by genetic and epigenetic variability, and obtaining information on facial aging can complement the profile of the perpetrator of a crime. Material and methods: In the present study, we collected a set of phenotypes from the analysis of 3D facial scans and dermatological evaluation, including perceived age, which was estimated by the examiner through a visual assessment of the participant's facial appearance without prior knowledge of chronological age. Genome-wide SNP and DNA methylation data were generated using Illumina GSA and EPIC arrays for 741 blood samples. Results and conclusions: We showed that accelerated epigenetic age correlated well with higher facial wrinkle count and perceived older age ($p<0.001$). Using the genetic and epigenetic markers identified through a series of GWAS and EWAS analyses, predictive models were developed for estimating perceived age with a mean absolute error of approximately 4 years and for predicting deep facial wrinkles with high accuracy as determined by AUC-ROC at the 0.95 level. These models hold promise as valuable tools in forensic investigations and can serve as eyewitness testimony in non-suspect cases when age and facial information are unavailable.

0-28

Genetic prediction model of male-pattern hair loss based on DNA variants

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Male pattern hair loss (MPHL), also known as androgenetic alopecia (AGA), is the most common type of alopecia worldwide. Recently, genome-wide association studies (GWAS) in large European cohorts like UK Biobank identified numerous of single nucleotide polymorphisms (SNPs) significantly associated with MPHL, and genetic MPHL predictive models were constructed based on those SNPs. However, few MPB-associated genetic variants have been validated or used for MPB prediction in East-Asian. In this study, we examined the replicability of 594 previously reported SNPs in 825 Han Chinese males (Nmphl=400, Nnormal=425) and validated 66 SNPs significantly associated with MPHL using lasso-regression. Subsequently, we performed a genome-wide association study (GWAS) and identified 14 independent SNPs associated with MPHL (P<1E-6). To identify an effective group of MPHL predictive variants, a bidirectional stepwise regression analysis was carried out and 40 SNPs was selected for MPHL risk prediction (R2=0.48). We then trained a logistic regression model considering the genotypes of 40 SNPs in 825 Chinses males, without age as additional predictor, and preformed 10-fold cross-validation to estimate the prediction accuracy. The model demonstrated that MPB risk is predictable at an accuracy level of 0.860, measured using the area under the receiver-operating characteristic curves (AUC). External validation in an independent dataset of 2300 Chinese males (Nmphl=910, Nnormal=1390) showed a robust accuracy as AUC of 0.813. Our model was highly informative for MPHL genetic risk evaluate in East-Asian and has great potential for forensic DNA phenotyping (FDP) technology development.

O-29

DNA methylation-based organ tissue identification: marker identification, SNaPshot multiplex assay development, and interlaboratory comparison

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Identifying body fluids and organ tissues is highly significant as they can offer crucial evidence in criminal investigations and aid the court in making informed decisions, primarily through evaluating the biological source and possibly at the activity level up to death or fatal damage. In this study, organ tissue-specific CpG markers were identified from Illumina's methylation EPIC array data of nine organ tissues, including epidermis, dermis, heart, skeletal muscle, blood, kidney, brain, lung, and liver, from autopsies of 10 Koreans. Through the validation test using 43 samples, 18 hypomethylation markers, with two markers for each organ tissue type, were selected to construct a SNaPshot assay. The developed multiplex demonstrated high accuracy, achieving 100.0% correct detection of the presence of nine organ tissue types in 88 samples from autopsies of 10 Asians. An interlaboratory comparison using 80 autopsy samples (heart, skeletal muscle, blood, kidney cortex, kidney medulla, brain, lung, and liver) from 10 individuals in Germany revealed overall comparable results with correct detection of the presence of eight organ tissue types in 92.5% samples (74 of 80 samples). In the case of six samples, it was impossible to determine the correct tissue successfully due to drop-outs of unmethylation signals at target tissue marker loci. The observed differences might be due to differences in sample collection during routine autopsy, technical differences due to the PCR cycler, and the threshold used for signal calling. To sum up, the developed SNaPshot multiplex assays will be valuable for forensic investigators dealing with organ tissue identification, as well as for prosecutors and defense aiming to establish the circumstances that occurred at the crime scene.

O-30

“Barcoding” the phenotype with PhenoTrivium+ Panel. A novel MPS assay for enhancing forensic DNA phenotyping of single cells

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The use of single cells in forensics is a growing trend in the field. Currently, their application has mainly been focused on improving the deconvolution of DNA mixtures with STR typing. However, new directions are also being explored, including SNP and transcriptome sequencing. Our group used the former to test a pipeline for deconvoluting DNA mixtures submitted to forensic DNA phenotyping. Conducted experiments were successful, but they also revealed limitations. To obtain the most reliable ancestry and phenotype predictions, it is necessary to perform them with the most complete and correct genotypes possible. This can be achieved by creating consensus profiles, which can be challenging when working with biallelic SNP markers. To enhance the process of building reliable consensus genotypes, we have developed a new MPS-based assay that combines our previously introduced Ion AmpliSeq™ PhenoTrivium Panel with STR markers. The newly presented PhenoTrivium+ comprises over 200 autosomal SNPs associated with ancestry and phenotype, 18 autosomal STRs used to “barcode” the single cells, and 3 Y-chromosomal SNPs to confirm the sex. The aim of the study was to assess whether STR sequencing could be used to reliably assign multiple individual cells collected from a trace to one contributor and thus help to generate consensus SNP profiles for ancestry and phenotype prediction. The DEPArray™ Platform was used to collect groups of 20, 10, and 5 cells and 10 single cells (leukocytes) from one individual, which were then sequenced with the PhenoTrivium+ to test the new assay's sensitivity. Using the same workflow, single cells were then collected from 1:1 DNA mixture and used to predict ancestry and phenotype of both contributors. Our results demonstrate that “barcoding” the single cells with STR markers improved the predictions by helping to reliably generate consensus SNP genotypes. The PhenoTrivium+ is a tool which can be successfully used for the forensic DNA phenotyping of mixed traces.

0-31

Exploring legal age estimation using DNA methylation

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Europe faces a challenge regarding unaccompanied minors who arrive at its borders. Many of them have no means to prove their legal age and are submitted to medical and non-medical procedures to establish if they are minors and therefore, whether they are protected by the International Convention on the Rights of the Child, or not. Currently, medical procedures consist of X-rays to analyse bones and teeth to estimate their age. However, since there is no medical rationale, use of X-rays should be avoided. For this reason, in recent years, the application of DNA methylation to predict legal age has been proposed instead of X-rays.

This study aims to explore the possibility of epigenetic clocks substituting X-ray analysis and examine whether DNA methylation is an acceptable tool to predict if someone is a minor or not. To do this, an age prediction model based on blood samples ranging from 14 to 94 years of age was created, with a median absolute error (MAE) of ±3.58 years. A reduced prediciton model was also created using only samples from 14 to 25 years of age with a MAE of ±1.22 years. These models were tested using a testing-set with 1464 samples from both dizygotic and monozygotic 18-year-old twins (732 pairs). The MAE obtained for this testing-set using the reduced model was ±1.08 years. Looking at the age of twins predicted by the model, we saw that 51.37% of the twins were predicted as minors and 48.63% predicted as over-age. Amongst the total twin pairs analysed, 30.19% produced predictions where one was classified as a minor and the other was not.

The model is still being improved in order to reduce the MAE, allowing us to see if epigenetic clocks are suitable for legal age prediction or not. If they predict age as well as or better than X-ray analysis, this would mean that radiating young immigrants would no longer be necessary to estimate their age, protecting their safety and health.

O-32

Towards quantitative eye color prediction from DNA using biometrically secure visual iris images

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Current DNA-based eye color prediction methods make categorical inferences about iris pigmentation, limiting information held in the full, continuous spectrum of iris coloration. In addition, the iris as a biometric identifier has prompted privacy and security concerns regarding iris image use in research. We aim to overcome these limitations by moving DNA-based eye color prediction into the quantitative realm, and using synthetically generated iris images to provide accurate visualizations of its predicted phenotype. In pursuit of this goal, we describe how we created a large database of realistic, biometrically unique colored iris images using an open-source diffusion framework. We began with an internal set of 6989 DSLR-captured RGB images collected with institutional ethical approval (IU IRB 1409306349). To quantify the color of training set irises, a Support Vector Machine (SVM)-based Quantification Tool was used to determine the percentage of four pigment classes: blue-grey, green, light brown, dark brown. To ensure high quality diffusion model outputs, 1757 iris images survived pre-processing and were rotated 11 times by 30 degrees resulting in a training set of 21084. This helped train our model to better generalize the iris colors and structures, and prevent overfitting using OpenAI's codebase from the article 'Diffusion Models Beat GANS on Image Synthesis'. To ensure that our generated iris images were statistically independent from the training set images, we first employed a 'traditional', Gabor filter based iris identification technique, showing that 10,236 of 10,240 sampled iris images with confirmed unique identity. To confirm that model generates realistic iris color distributions, we then employed color quantification comparisons involving Euclidian distance, ILR transform, and PCA. With iris image generation complete, we are now in the process of assessing the effects of more than 1500 DNA variants in over 5000 individuals for their contribution towards a quantitative iris prediction model using several modeling approaches: from regression to multivariate analyses, and deep learning. We aim to provide an update to the community on the development of IrisPlex-QT, a next generation eye color DNA predictive tool.

Oral Communications 5

General Session 5: Statistics and Interpretation

General Session 5 Statistics and Interpretation

Abstracts Oral Communication

O-33

Likelihood ratio calculations based on sequencing error analysis of shotgun sequencing data and dynamic SNP marker sets for human identification

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Degraded biological trace samples with short DNA fragments and low amounts of DNA are challenging for standard short tandem repeat (STR) genotyping. Single nucleotide polymorphism (SNP) markers can be amplified on short PCR fragments and used to generate SNP profiles from low-quality DNA samples. However, the stochastic results from low-quality DNA samples may result in frequent locus drop-outs and insufficient numbers of SNP genotypes for human identification. Shotgun DNA sequencing is a method that analyses all DNA fragments in a sample in contrast to the targeted PCR sequencing methods. Besides giving genome-wide information, shotgun sequencing also works on DNA samples of very low quality, e.g., ancient DNA samples. Thus, shotgun DNA sequencing, which does not rely on an initial PCR and a predefined set of SNPs, may be used in the forensic setting for low-quality DNA samples. Since low-quality samples have an increased probability of genotyping errors, we statistically modelled a call error probability based on sequencing, alignment, and SNP variant calling analysis. Results from replicated DNA sequencing of various sample types were arranged in a genotype-call confusion matrix to estimate the call error probability by the maximum likelihood method. We developed formulas for the evidential weight as a likelihood ratio (LR) that uses a dynamic set of SNPs from shotgun DNA sequencing data, e.g., all SNP results considered relevant for a given comparison. An error probability of zero resulted in the commonly used LR formulas. When considering a single marker's contribution to the LR, error probabilities larger than zero reduced the LR contribution of a match and increased the LR contribution of a mismatch from zero (occurring with an error probability of zero) to positive values above zero, as the mismatch may be due to genotyping errors.

O-34

Predicting the LR value of the mixed DNA profile using random forest regression for database searching

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Objectives: Comparison of multiple reference individuals of a DNA database with a mixed DNA profile from an evidentiary trace is an important approach for forensic investigation. Several search methods exist for accurately, but time-consumingly, identifying potential suspects through qualitative or quantitative LR calculations. In this study, the Random Forest Regression algorithm was used to analyze the mixed DNA profiles for rapid LR prediction.

Material and methods: The mixed DNA profiles of the PROVEDIt dataset, generated by four kits, with two to three contributors, varying injection times, and different assay platforms, were used to calculate the log10(LR) for each contributor separately by Euroformix. Kit-specific and injection time-specific AT values, as well as drop-in parameters, were determined using single-source profiles. Degradation and backward stutters were considered during computation. A total of 6,305 pieces of data were input into the algorithm. After feature selection, algorithm training, and testing, the model's performance was evaluated.

Results and conclusions: The ten most relevant features were filtered out, all of which were generated from the profile information. The top three features included the count of contributor's alleles detected in the mixture profile, the dropout rate of contributor's alleles, and the ratio of the total peak heights of contributor's alleles to the sum of peak heights in the profile. 80% of the data was divided into a training set while 20% into a testing set for model evaluation. The coefficient of determination R2 was 0.98 for the training set and 0.87 for the testing set, indicating a strong model fit as the values approach one. In the testing set, 99.8% of the predictions for log10(LR) were above zero in favor of the prosecutor’s hypothesis. Only three with predicted values below zero supported the defendant's hypothesis. These log10(LR) prediction values were -0.0354, -0.0263, and -0.0032, respectively, corresponding to actual values of 0.0390, 0.0037, and 0.2809. The difference between the predicted and actual values in a testing set was normally distributed with a mean of 0.0124. Fifty percent of the difference was concentrated in the range of [-1.2611, 1.6102], while ninety percent of the data fell within the interval of [-4.7736, 4.0190]. The Random Forest Regression offers a rapid approach for predicting the LRs of multiple suspects within mixed DNA profiles, aiding in the evaluation of a potential suspect's contribution to an evidence profile. This provides a concise and fast method to target suspects in large databases for forensic investigation.

O-35

Searching national DNA databases with complex DNA profiles: an empirical study using probabilistic genotyping

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1. Objectives
In most National DNA databases (NDNADB) only single source DNA profiles, sometimes 2 person DNA mixtures, can be searched provided a minimum number of loci is available. DNA profiles that do not meet these criteria (~10% of the traces analyzed in Western Switzerland) can only be compared locally with candidates upon request from police services or remain unused. With the advent of probabilistic genotyping (PG), such complex DNA profiles can be compared to those stored in NDNADB based on LR. In this study, mock traces and casework DNA profiles were used to evaluate the performance of the DBLR™ “Search database” tool using data from the Swiss NDNADB.

2. Material and Methods
In a first part, 40 mock DNA mixtures (2 to 5 contributors) from 15 volunteers were compared to a database containing the DNA profiles of the volunteers and 175,389 person DNA profiles of the Swiss NDNADB. In a second part, 160 DNA mixture profiles from casework (2 to 5 contributors) that had previously been locally compared were searched using the same conditions.

3. Results and Conclusions
In the first part, using LR thresholds of 10^3/10^6, sensitivity and specificity were respectively 90.0/57.1% and 99.9/100%. For the lower LR threshold, this represented 52 adventitious matches on more than 24 million pairwise comparisons. In the second part, with the 10^3 LR threshold, 380 associations were retrieved: 194 were expected associations as they were previously made through local comparisons with known persons and 186 were new. With the 10^6 LR threshold, 199 associations were recovered with 180 that were expected and 19 new. This demonstrates that even with complex DNA profiles (up to 5 contributors) all expected associations were retrieved with a limited number of candidates per trace. Database searches for complex mixtures allow to produce leads early in the investigation for DNA profiles that would remain otherwise poorly used. Next steps for the integration of DBLR™ or similar software within an operational context requires discussions on legal, financial and technical aspects between the stakeholders.

O-36

Mixtures and Baskerville data – what isn't there can exonerate

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Objective –
This presentation exposes a bias in the evaluation of DNA evidence to which nearly all probabilistic mixture software is susceptible. Situations in which the bias would manufacture a case against a suspect are probably not rare. A current criminal case is an example.

Reasoning –
Probabilistic mixture analysis generally defines an “analytic threshold” below which epg data is ignored as unreliable. That rule is subtly but significantly illogical: A visible sub-threshold signal at an allelic position may be from DNA, or merely garbage. We don’t know which so it is not reliably DNA data. Fine. However, lack of signal – a void – is also data but similar logic does not apply: Where nothing is seen there is no garbage and no DNA. In particular there is no DNA.

A better rule would be that a sub-threshold signal is unreliable, but a sub-threshold void is reliable data. (A caveat is omitted). And the void may well be exculpatory evidence. For example, if the suspect has a particular allele p, looking under the threshold and seeing no p is relatively strong exculpating evidence compared to not looking.

Result and conclusions –
Analysis of a shell casing DNA mixture was presented in a current criminal case. The analysis, using a dropout-capable mixture program as recommended, calculated a hefty likelihood ratio of 825 connecting the defendant to the casing. But consideration of the sub-threshold pattern of the data led to the new insights and a likelihood ratio favoring the defendant.

The issue of Baskerville data arises with all mixture software that purports to deal with dropout and that filters the data with a threshold. That includes virtually all of the available continuous and semi-continuous model programs.

An occasionally stated dictum is the wisdom that while sub-threshold information should not be used as inculpatory evidence it can be used to exclude. How that might be done was not explained until now.

0-37

The Best of Everything: Modularizing and Combining Statistical Methods Into the Ultimate Forensic-Genetic Statistics Toolbox

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In the past two decades, probabilistic genotyping systems (PGS) have evolved from simple binary matching methods via qualitative models (employing drop-in and drop-out probabilities) to quantitative models that also use peak height information in the DNA profile. In each category of PGS, multiple alternative models with subtly different approaches exist, such as the qualitative models LRmix and MixCal and the quantitative models EuroForMix and STRmix. Moreover, software implementing these models have different properties and features, such as the ability to include simple genetic relations or pedigrees in the calculation, the way uncertainty in model parameters is handled, the possibility to use the software as a database searching tool, availability of a graphical or programmatic interface, and the ability of the software to leverage specialized computer hardware.

Here, we show how PGS can be decomposed into their main building blocks and how these building blocks are subsequently combined into a powerful forensic-genetic statistics toolbox that enables combined usage of components of different PGS. For example, one may combine a pedigree-based algorithm to generate possible contributor genotypes with a quantitative model to calculate the likelihood of obtaining the observed peak heights in a (possibly mixed) DNA profile. Similarly, a quantitative model can be used together with a method to assess a mixture of Y-STR haplotypes, such as the Discrete Laplace method. Finally, we illustrate an example of the unprecedented joint statistical analyses that become possible: an LR-based familial search in a DNA database with a mixed trace typed with autosomal and Y-STR kits using a quantitative model. With all these building blocks implemented as modules in a single software, future modelling advancements achieved in one area benefit the others.

O-38

SMapper: Visualising spatial prevalence data while accounting for data sparseness and incompleteness

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Available spatial prevalence data e.g., Y-SNP based haplogroup data used in forensic genetics for paternal biogeographic ancestry inference, are often limited by sparse geographic coverage and insufficient sample size. Not accounting for sparseness and incompleteness in the data visualization, as done by most previously developed bioinformatic tools, carries the risk of severe misinterpretation e.g., concluding an apparently restricted paternal ancestry while the true ancestry region maybe much larger. To overcome these limitations, we developed the SMapper bioinformatic tool to visualize spatial prevalence data including those that are sparse and incomplete. In its spatial visualization at the worldwide level, SMapper clearly separates geographic regions where the feature of interest, such as a Y-haplogroup, was observed at some frequency, from those regions not covered by the data, and those where, although covered, the feature was not observed (zero frequency). Moreover, the uncertainty of a zero frequency observation is visualized by taking the respective sample size into account. This way, SMapper overcomes interpretational issues known for existing tools that are caused by data limitations. Here, we introduce SMapper and focus the demonstration of its forensic usefulness on forensically-relevant data and applications, while applications in other fields such as in epidemiology and anthropology will be mentioned. For widespread applications, we made SMapper publicly available as a web service as well as a native Linux installation and a singularity container at https://rhodos.ccg.uni-koeln.de/smapper/.

O-39

TrACE: An Expert-Driven Proficiency Test for Forensic Genetics and Genomics

Prof. Marielle Vennemann[1], TrACE-Team[1], Prof. Dr. Walther Parson[2], Prof. Dr. Katja Anslinger[3], Dr. Martin Eckert[4], German Stain Commisison
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For many years, the German Stain Commission was the scientific backbone of the well-recognised GEDNAP proficiency tests (PT). Since this PT turned into a solely economic enterprise without consultation by a scientific commission, the German Stain Commission developed updated practical and scientific requirements for PTs. Core elements are an expert-driven processing of the PT from sample selection to results evaluation. The PT comprises the analysis of realistic forensic sample types that represent casework challenges such as low DNA quantities and high degradation states. Transparent, anonymous overviews of all results are provided for participants to encourage discussion and enhance learning and standardisation in the field.

Based on a survey among laboratories across Europe, the concept of TrACE (=Trace Analysis Collaborative Exercise) was developed. A first round of proficiency tests was conducted in 2023/2024 with 45 participating laboratories across Europe offering six different modules, each of which were directed by an expert module coordinator:

Module 1: Advanced Trace Analysis (Coordinator: Marielle Vennemann)
Module 2: Advanced mtDNA Analysis (Coordinator: Walther Parson)
Module 3: Paperchallenge (Coordinator: Marielle Vennemann)
Module 4: Advanced Statistical Analysis (Coordinator: Martin Eckert and international advisors)
Module 5: Forensic DNA Phenotyping (Coordinator: Katja Anslinger)
Module 6: Epigenetic Age Estimation (Coordinator: Katja Anslinger)

This presentation will outline the concept of TrACE and detail the results of the first round of TrACE.

O-40

PHYLOIMPUTE – Impute missing data on non-recombining DNA using SNP phylogeny

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Many biallelic SNPs on haploid DNA, like the human Y chromosome, are transmitted over generations in a traceable order due to the lack of recombination. This order can be represented in a phylogenetic tree, utilized for population and evolutionary genetic studies. However, incomplete information of the allelic states of SNPs is commonly encountered in low quality data, such as from ancient samples, or due to sample and data processing, such as targeted sequencing and compiling data from multiple sources. Missing or incomplete data impedes representation of allele frequencies or distributions and is detrimental for the analysis of populations and evolutionary processes. Since SNPs represented in a phylogenetic tree harbor information on the allelic states of the remaining SNPs in the same tree, missing data can be imputed when the phylogenetic order is known.

Thus, we introduce the software Phyloimpute, which leverages the phylogenetic nature of SNPs reported in a phylogenetic tree to impute incomplete data within seconds. Phyloimpute assumes that all SNPs in a phylogenetic clade leading up to a SNP with a derived allele are derived as well, while SNPs on parallel tree branches are ancestral. For each sequence, Phyloimpute requires the allelic state of SNPs located in a phylogenetic tree, which can be provided by the user or selected from Phyloimpute's preprocessed phylogenetic trees.

The outcome are the observed and imputed allelic states for the initially reported SNPs complemented with the SNPs in the phylogenetic tree, while distinguishing between recurrent SNPs. Further, Phyloimpute cross-references the allelic states of all observed SNPs with the phylogenetic tree to verify the accuracy of the phylogenetic tree and the sequencing data, allowing to identify sequencing errors and backmutations.

In short, Phyloimpute complements SNP data on haploid DNA by leveraging the SNPs' phylogeny, thus providing more and accurate genetic information for downstream analysis.

O-41

The Challenge of X Chromosome Aneuploidies in Kinship Analyses

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X chromosome aneuploidies, such as Trisomy X, Klinefelter or Turner syndromes, have a high incidence in the population. For example, 1 in 500 to 1,000 males are expected to have the 47, XXY karyotype, which characterizes the Klinefelter syndrome. Moreover, it is noteworthy that a significant proportion of individuals with X-chromosomal aneuploidies remain undiagnosed, either because they are asymptomatic or present mild symptoms, which indicates that incidence rates may be underestimated. Indeed, this work was triggered by a real kinship casework problem involving a female with an undiagnosed Trisomy X syndrome.

In any case, individuals with an aneuploidy introduce a new challenge to kinship analyses, when it is convenient to analyze X chromosomal markers. These markers may be crucial to solve complex kinship problems, and also to complement the information provided by the autosomes due to their unique characteristics, such as its mode of transmission and recombination patterns. Currently, the formal and theoretical framework that evaluates pairwise kinship analyses considers only euploid individuals, failing to address those with an X chromosome aneuploidy. To overcome this obstacle, we developed a novel framework capable of weighting the DNA evidence of both linked and unlinked X chromosomal markers for kinship analyses involving two non-inbred individuals, one euploid (46, XX or 46, XY), and the other presenting a pure (non-mosaic) X chromosome aneuploidy. By integrating the linked markers in the analysis, our study will allow obtaining results with a higher statistical power, outstanding the use of independent markers alone. Moreover, our approach extends to several kinship hypotheses involving two individuals of both sexes (male and female), and it covers every possible genotypic configurations. We explored the challenges posed by considering the type of error that occurred and its parental origin. Our findings unveil that algebraic formulae depend not only on the kinship hypotheses considered but also on the genotypic configurations of the individuals, contrarily to what occurs when assuming euploidy. Besides, the prospect that a non-inbred individual with an extra X chromosome can have a pair of identical-by-descent alleles in their genotype, adds a new level of complexity to the calculations, as this is only possible in euploid individuals if they are inbred. Our research will then enhance the evaluation of kinship analyses when one of the individuals has an X chromosome aneuploidy in forensic and medical genetics fields, offering new insights into the interpretation of DNA profiles and its evaluation.

O-42

Evaluation of DNA results considering activities: practical challenges

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For many years, DNA scientists have been asked questions about DNA transfer in court. Giving a meaningful answer when the issue regards how or when the DNA was transferred is difficult. As such it requires regulations and adoption of new procedures.

In this presentation, we discuss the challenges of evaluation of biological results given alleged activities from a caseworkers' perspective. We present how we integrated the recommendations of professional organisations (e.g., ISFG, ENFSI) and how DNA scientists have acquired specific knowledge to account for the possibility of transfer in their evaluation. We further review and discuss the practical challenges encountered for implementation of the approach in casework. Indeed, in practice, caseworkers still face many difficulties: from assessing the results with the available data to communicating results, moreover within accreditation requirements.

We share solutions, if any, and raise questions for the future: both from an administrative perspective (peer review process, quality assurance, use of ressources) and from a scientific point of view (e.g., access to relevant data through collaboration between casework and research).

O-43

Whole transcriptome sequencing and investigative genetic genealogy – Is it possible?

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In recent years, RNA has emerged as a promising molecule in forensic genetics with applications such as body fluid identification, time since deposition determination, and more recently human identification. RNA has some advantages over DNA. Whereas each cell only contains two copies of autosomal DNA, RNA species may be present and expressed in high copy numbers. Although RNA is more easily degradable than DNA, the higher copy number of RNA is expected to, in part, compensate for the RNA degradation that occurs after a biological stain has been deposited ex vivo. Furthermore, a direct link between body fluid and donor of a biological stain is obtained with RNA, which is not possible using DNA.
In this study, we expand the current knowledge of RNA in forensic genetics, by showing how RNA also can be used for investigative genetic genealogy (IGG) purposes and inference of distant relationship. We exemplify this by investigating whole transcriptome sequencing of low template blood samples (corresponding to 50 pg gDNA). Transcriptome-based genotypes were determined using a genotype likelihoods approach and augmented using imputations. To determine the accuracy, the transcriptome-based genotypes were compared with DNA genotyping using the Omni4.5 array. Genetic data was simulated for relationships ranging from full siblings down to third cousins and subsequently intersected with the available transcriptome-based genotypes. Since transcriptome-based genotypes are primarily found in coding regions of the genome, a large proportion of the inferred loci had low variant allele frequencies across individuals and populations. We developed an approach to take this into consideration as this would otherwise lead to an inflation in shared segments and hence an artificially closer relationship.
In conclusion, we present a proof-of-concept study on how transcriptome-based genotypes in combination with imputed genotypes may be used to infer reliable relationships for IGG purposes.

O-44

Driving policy and engagement through an International Roundtable for Forensic Investigative Genetic Genealogy

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Objectives
The development of a consistent operational and procedural methodology for Forensic Investigative Genetic Genealogy (FIGG) requires an international approach to help address what commentators regard as a lack of consistency and absence of regulatory frameworks in most jurisdictions.[i,ii,iii,iv]
In 2023, supported by the Australian Federal Police and Australia's FIGG Project Working Group, representatives from Oceania, North America and Europe attended an International Roundtable on FIGG. The objective of the Roundtable was to share experience, scientific, operational and policy-related, and to work towards establishing an international network of law enforcement agencies to promote ongoing information exchange.

Material and methods
The inaugural International Roundtable was held in November 2023 in Sydney, Australia. Participants represented agencies from newly established FIGG units to those with a significant resource investment. The Roundtable covered topics from training and scientific advances, 'assessment of SNP genotyping methodology for FIGG, opportunities to standardise case and sample data collection, to how FIGG can operate within mutual assistance and international police-to-police cooperative frameworks.
In addition to the scientific and policy discussion, participants in the Roundtable had the opportunity to work together on a FIGG case. Ten agencies participated, bringing their FIGG experience to an Australian unidentified human remains investigation. This piloting of an international approach could prove beneficial for complex cases, given that genealogy knows no borders.
To ensure that international considerations continue to progress in a timely manner, several smaller working groups were established with international representation, and will report to the second International Roundtable scheduled for September 2024.

Results and conclusions
This presentation will provide insights into the high-level outcomes of the first and second International Roundtables, including collaboration opportunities and other lessons learnt. This will cover opportunities identified, in both technical developments and policy and legal considerations at an international level.

i. Glynn, C. L. (2022). "Bridging Disciplines to Form a New One: The Emergence of Forensic Genetic Genealogy" Genes 13(8): 1381.
ii. Gurney, D., et al. (2022). "The Need for Standards and Certification for Investigative Genetic Genealogy, and a Notice of Action" Forensic science international: 111495.
iii. Granja, R. (2023). "Citizen science at the roots and as the future of forensic genetic genealogy" International Journal of Police Science & Management: 14613557231164901.
iv. Scudder, N., et al. (2020). "Operationalising forensic genetic genealogy in an Australian context" Forensic science international 316: 110543.

Oral Communications 6

General Session 6: Genetic Genealogy

General Session 6 Genetic Genealogy

Abstracts Oral Communication

O-45

Getting intelligence with Kintelligence: analysis of known pedigrees and progress report on ongoing cases

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Investigative Forensic Genetic Genealogy (iFGG) has recently emerged as a method for generating investigative leads for cold cases and cases with unidentified human remains. The ForenSeq Kintelligence Kit has been designed for (up to 6th degree) kinship analysis of forensic samples [1] that, by nature, are often low in DNA quantity and quality (e.g degraded, contaminated with microbial DNA).

Over ten unidentified human remains cases, that didn't yield any investigative leads using conventional DNA analysis and one-to-one as well as familial search in the Estonian DNA database, were chosen. Forensic samples, incl. bones, 20-year-old blood stains on filter paper and fresh blood (degradation index of the extracted DNA from forensic samples was in the range of 0.7 – 16.5), were analysed with the Kintelligence Kit and uploaded to Gedmatch Pro and FamilyTree DNA databases. Possible genetic relatives (PGRs) that qualified for the genealogical research (individuals that were found in the Estonian or Finnish population registries) were identified for two samples. As the amount of shared DNA indicated a 6th or beyond degree relationship (77-139 cM), effort was made to verify the reliability of the matches. Both PGRs were contacted for collecting samples and/or additional data that could aid the investigations. The construction of family trees is underway.

Additionally, in order to understand better the effect of 6th and 7th degree relationship on the Kintelligence results, pedigrees of three unrelated Estonian families with up to 3rd cousin (7th degree) members were constructed, volunteers genotyped with microarrays and uploaded to Gedmatch. Up to two family members from each pedigree were genotyped with the Kintelligence Kit and the matches between known family members reported.

The progress report on these cases and the analysis of known pedigrees will be presented at the ISFG 2024.

[1] Verogen, Inc., „ForenSeq Kintelligence Kit. Reference Guide. Document # VD2020053 Rev. B". märts 2021.

O-46

Analysing the Impact of Information Loss on Kinship Analysis with the ForenSeq® Kintelligence Kit

Ms. Jessica Watson[1,2], Prof. Dennis McNevin[1,2], Dr. Kelly Grisedale[2], Mrs. Louise Coakley[2,3], Dr. Jodie Ward[1,2]
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Medium and long-range kinship analysis using single nucleotide polymorphism (SNP) genotyping allows law enforcement to generate investigative leads to identify an unknown individual through their close and distant genetic relatives. However, if SNPs are not detected or incorrectly assigned, the ability to detect and accurately classify genetic relationships may be compromised.

The ForenSeq® Kintelligence Kit provides forensic laboratories with the ability to apply a suite of genetic markers to forensic samples by targeting 10,230 SNPs. Resulting Kintelligence profiles can be uploaded to GEDmatch PRO™ for one-to-one comparisons or forensic investigative genetic genealogy using the one-to-many database searching. Two volunteer family groups were sampled to compare the ability to detect and classify genetic relationships between Kintelligence profiles (Group 1: Kintelligence vs Kintelligence) and between a Kintelligence profile and a microarray profile (Group 2: Kintelligence vs microarray). The Kintelligence profiles uploaded for comparison were edited to simulate information loss through locus and allele dropout. Group 1 was also analysed using the DBLR™ kinship software to assess the impact on likelihood ratios.

The loss of up to 30% of SNP loci from the profile did not significantly impact the ability to detect and accurately classify first to fifth order relatives. However, fifth order relatives were not detected with allele dropout greater than 10%. The relationship classification accuracy also decreased for all orders beyond parent/offspring with allele dropout at 25%. This study highlights the robustness of the GEDmatch PRO™ kinship algorithms and the suitability of the Kintelligence Kit for medium-range kinship analysis, with the algorithm maintaining the ability to detect and accurately classify relationships despite increasing information loss.

Oral Communications 7

General Session 7: Ethics

General Session 7 Ethics

Abstracts Oral Communication

O-47

Disclosure of Biological Sex May Impact Gender and Other Privacy Concerns

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Human identification by forensic DNA profiling primarily relies on the analysis of short tandem repeat markers (STRs) and Amelogenin or other sex determining markers. The resultant DNA profiles can be compared directly between evidence and reference samples or indirectly (i.e., kinship) between human remains and family reference samples. Amelogenin, although being a phenotypic marker for biological sex, appears to be considered innocuous and the biological sex derived from this marker is often reported and/or uploaded to national DNA databases. However, biological sex does not necessarily align with gender identity and chromosomal anomalies may affect presentation of biological sex. Biological sex is genetically determined and assigned at birth based on anatomical features, whereas gender identity is an expression of an individual, which may change over time, and may not correspond with biological sex. This presentation will highlight how the differences between biological sex and gender identity may potentially impinge on individual privacy. Gender differences aside, genetic anomalies related to presentation of biological sex also can occur, albeit relatively rarely, and the consequences of revealing those anomalies may be far reaching for the individual in question. Additionally, the concept of gender privacy will be formally introduced, along with a proposed definition. Through the presentation of various case scenarios, it will be demonstrated that while knowledge of biological sex may be important for operational forensic DNA laboratories and even critical in certain cases, in many instances, it is not germane to criminal investigations, courtroom deliberations and/or public disclosure. The presentation will challenge all of us to understand that the dissemination of biological sex data in the public domain, particularly in contexts where such disclosure is unnecessary, can impact gender privacy.

In conclusion, in light of the current understanding and impact of gender privacy, it will be recommended that (1) ‘biological sex’ and ‘gender’ are recognised as separate concepts not to be used synonymously and interchangeably, (2) definitions of a DNA profile be reviewed and more clarity added, and (3) policies and protocols be developed to seclude such information, when not relevant, from reports and the courtroom (i.e., public arena) to reduce unwarranted intrusion into gender privacy

O-48

Dealing with incidental findings in Forensic Genetics

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Following the impressive development of sequencing techniques in the last decade, the analytical scope of forensic genetics has significantly broadened with, among others, the validation and legal approval of Forensic DNA Phenotyping, mitochondrial whole genome analysis and Investigative Genetic Genealogy based on large-scale SNP array data. It can be assumed that this development has not yet reached its end. It is evident that privacy intrusion becomes more serious and that data protection issues become more important when more genetic data is analyzed. Particularly with the paradigm shift from the analysis of non-coding to coding regions of DNA, we can foresee that incidental findings with potential health implications for the concerned individuals will become more frequent in the future, even though efforts are made in forensic genetics to avoid such information to arise. The treatment of incidental findings seems to have been largely neglected in forensic genetics, whereas human geneticists in the medical field are well aware of the problem and have developed guidelines for the correct handling of incidental findings. This talk gives a brief overview over the treatment of incidental findings in the medical field and tries to present some solutions for forensic genetics, to improve data fairness for convicted offenders, suspects, victims and other individuals from whom genetic data may be analyzed for forensic purposes.

O-49

Ethical Standards in Forensic Population Genetics - A Systematic Review of Forensic Science International: Genetics Publications

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In the statistical evaluation of forensic DNA profiles, accurate information on genetic variability in relevant human populations is indispensable, to avert the risk of overestimation of the "weight of evidence". However, this may raise ethical concerns, especially when vulnerable groups and minorities are involved. Recently, the methods of constructing and managing forensic population databases have been subject to criticism, which has sometimes led to removal of data and withdrawal of related scientific publications.

To assess the effectiveness of countermeasures taken by the ISFG over the years, we conducted a systematic review of population genetics studies published between 2007-2022 in Forensic Science International: Genetics, categorizing them according to risk parameters identified by the Forensic Databases Advisory Board (FDAB).

Out of 624 retrieved studies, 27% listed law enforcement / state laboratories among the co-authors, 4% included judicial case samples, and over one third focused on populations for which the FDAB broad definitions of minority or vulnerable group may apply. The overall percentage of studies conducted exclusively by foreign institutions without involvement of local researchers was 6%, raising to 33% in specific geographic areas such as Africa. A positive correlation was observed between publication year and statements about informed consent and ethics committee approval ($p < 0.001$). "High/medium risk" studies, which were >90% at the beginning of the journal publication and ~70% after the first editorial guidelines on forensic population data (2010), dropped to 35% following the 2020 recommendations on ethical publishing.

Despite an evident improvement in the ethical quality of forensic population genetics studies, achieving a 100% low-risk classification remains a goal. It is hoped that the collected data can contribute to raise full ethical awareness in scientists contributing to the peer review process of human genetic variability researches, especially when they concern at-risk communities facing historical or cultural discrimination.

O-50

Informed consent for forensic population studies: a call for harmonization

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The adherence of population studies for forensic genetic frequency databases to ethical principles has received public and scientific scrutiny in recent years. A group of experts from diverse fields affiliated with forensics has joined forces and created the Forensic Databases Advisory Board (FDAB). In its first report (2023) the FDAB offers a framework to ethically assess implications of presenting data from diverse population studies and groups in the ISFG-endorsed frequency databases EMPOP, STRidER, and YHRD. The report extends to data acquisition and among other prerequisites discusses donor-signed informed consent (IC) based on the existence of respective guidance at the time of sampling. Consistently, IC is fundamental for quality assessment of population datasets and their inclusion into the frequency databases. In addition to the report, considerations on IC content in the era of forensic genomics and example forms from numerous medical fields have been published, but little information is available with regard to the actual nature of IC forms currently in use for forensic frequency databasing purposes.

We analyzed 60 IC forms submitted to STRidER and EMPOP from a broad range of contributors across worldwide legislations in the past two years, irrespective of the quality of the associated genetic data. We report the common ground of the IC forms, their specific content and differences, and outline to what extent they contain suggested components.

The ‘reality check’ of authentic IC diversity adds to the discussion on formal aspects to be covered at the time of sampling. This evaluation may expedite future harmonization in forensic population studies through elaboration of standardized IC text elements, assuring ethical principles and the applicability of precious sample sets for a broad range of investigations across genetic disciplines.

Posters Topic 1

Human Identification

1. Human Identification

Abstracts Poster

P-002

Comparison of DNA preservation between petrous bone and tooth cementum analysing five skeletons from the modern era archaeological site

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1. Objectives
Proper sampling strategy is important to obtain sufficient DNA for successful identification of aged skeletal remains. The petrous bone is the highest DNA yielding bone in human body. Because its DNA extraction is highly destructive and petrous bone is not always part of the uncovered remains, the demand for alternative sources of DNA is of significant importance. When investigating aged skeletal remains, teeth are usually preserved and DNA can be found in dental pulp, dentin and cementum. Similar to petrous bone, highly destructive grinding of whole tooth is employed in standard extraction procedure. Recent studies have shown that DNA may be best preserved in the dental cementum that surrounds the surface of the tooth root apex. To extract the DNA from cementum, non-destructive method without grinding was optimized and DNA yield, degradation rate and STR typing success were compared to petrous bones sampled from the same skeletons. Petrous bones and teeth from five skeletons from the 13th to 19th century Christian cemetery Črnomelj were analysed.
2. Material and methods
Bones and teeth were chemically cleaned and UV irradiated. The dense part of the petrous bone was ground into a fine powder, and 0.5 g of powder was used for DNA extraction. To obtain the DNA from tooth cementum, non-destructive method without grinding was optimised. Full demineralization was employed to obtain the DNA and the qPCR PowerQuant System (Promega) was used to determine the quantity and quality of DNA. To evaluate the suitability of tooth cementum in comparison to petrous bones for forensic human identification purposes and archaeological kinship analyses, also the STR typing was performed.
3. Results and conclusions
Results showed that even if DNA yield was higher in all petrous bones, higher STR typing success was reached in tooth cementum because of lower DNA degradation. Some contamination issues were observed in two petrous bones but not in cementum extracts, indicating higher exposure of endogenous DNA to contamination with modern DNA during grinding process. Profiles obtained from petrous bones matched the profiles from tooth cementum sampled from the same skeleton, confirming endogenous DNA, together with no elimination database match and clean negative controls. This study contributes valuable insights into the potential use of the tooth cementum as a reliable source of DNA for investigation of aged skeletal remains, highlights the importance of non-destructive extraction method for DNA analysis, and offer practical implications for forensic and archaeological investigations.

P-003

Evaluation of DNA from teeth subjected to various extreme ante-mortem degradation factors: preliminary study

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Objective: In cases of tragic events that require the analysis of highly degraded tissues, DNA analysis is sometimes the only option for identification. Mineralized tissues such as teeth are used for this purpose because they are the most resistant tissue in humans. Various factors may influence the success of this analysis, and they can be classified according to whether they are ante-mortem (related to the tooth and individual) or post-mortem. Among the ante-mortem factors are age, pathological changes, and type of tooth. Literature suggests that molars and premolars, without dental treatments or pathologies, are ideal for DNA analysis. However, these characteristics are sometimes absent. Therefore, the question arises whether DNA recovery is possible when an ideal tooth is unavailable. Therefore, the aim of this research is to determine the performance of DNA recovery in teeth with "non-ideal" ante-mortem factors.
Material and method: Ten samples of permanent teeth will be taken from the Emergency and Maxillofacial Surgery Clinic of the Faculty of Dentistry (University of Concepción-Chile) where therapeutic extractions are performed. Samples will be collected according to the ethical procedures of the Scientific Ethical Committee of the Concepción Health Service. The age and sex of the donor, type of tooth, and pathologies associated with the tooth will be recorded. DNA extraction from pulverized teeth will be performed using QIAamp DNA Investigator Kit. Then, DNA will be quantified by Real-Time PCR system using Quantifiler Trio Kit (Life Technologies) with AriaMx Real-Time PCR System (Agilent Technologies). Subsequently, statistical analysis will be performed using the IBM Statistical Package for the Social Sciences SPSS V2 software.
Results and conclusion: The literature indicates that certain conditions must be met for DNA analysis in teeth. In our case, teeth that do not meet these conditions will be analyzed. It has been observed that the highest amount of DNA is extracted from the pulp-dentin complex and cementum. However, according to other studies, DNA can still be extracted when there are pathologies or changes in these tissues that affect their composition. Therefore, variable quantities/quality of DNA are expected to be extracted from these samples, allowing us to evaluate which pathology, dental treatment, or type of tooth most affect genetic analysis. Data obtained from the study will help in carrying out targeted and correct sampling, depending on the situation we face.

P-004

Evaluation of the usefulness of insertion-null markers in critical skeletal remains

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Forensic DNA analysis in compromised skeletal remains may pose challenges due to DNA degradation, often resulting in partial or negative autosomal STRs profiles. To address this issue, alternative approaches such as mitochondrial DNA or SNPs typing may be employed; however, they are labour-intensive and costly. Insertion-null alleles (INNULs), short interspersed nuclear elements, have been suggested as a valuable tool for human identification in challenging samples due to their small amplicon size. A commercial kit including 20 INNULs markers along with amelogenin (InnoTyper® 21) has been developed. This study assesses its utility using degraded skeletal remains (n=70), comparing the results obtained (the number of detected alleles, RFU values, PHR, and the number of reportable markers) to those obtained using GlobalFiler™. Subsequently, the random match probability of the two profiles for each sample was determined using Familias version 3 to evaluate the power of discrimination of the results obtained from each kit. In every sample, InnoTyper® 21 yielded more alleles, higher RFU values, and a greater number of reportable loci. However, in most cases, both profiles were similarly informative. In conclusion, InnoTyper® 21 serves as a valuable complement to the analysis of challenging samples in cases where a poor or negative profile was obtained.

P-005

Identification of decades-old bone samples by microhaplotype markers

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STR-CE typing of autosomal markers is the gold standard for personal identification of skeletal remains, with the chance of successful identification depending on the number of STR markers typed in the bone samples and the availability of a suitable reference database. In addition, special procedures need to be adopted in statistically evaluating bone remains belonging to the DVI scenarios. Therefore, inconclusive LR values can be achieved even when the ante mortem reference samples are represented by 1st or 2nd-degree relatives.
In this study, we describe the use of microhaplotype (MH) markers as an additional tool for assessing the LRs in five kinship analyses where the use of the autosomal markers included in the PowerPlex Fusion kit (Promega) provided inconclusive or low ($<3.1x10^3$) LRs. The questioned full profiles were yielded from 5 bone samples found in a Second World War mass grave; the reference database included 6 subjects (4 males and 2 females) from 5 independent Italian families missing one first-degree, four second-degree and one third-degree relatives, who were likely buried in that mass grave.
A panel of 76 MHs, designed for degraded DNA (amplicons length <145bp), was analysed by MPS using Ion GeneStudio S5 Systems (TFS). About 0.12-1 ng of DNA was amplified through 22 and 25 cycles of PCR. Good results in terms of quality and average coverage of sequenced libraries were obtained despite sample degradation. For data analysis, the HID Microhaplotype plugin (v1.5, TFS) and Integrative Genomic Viewer were used. Overall, most samples showed a complete profile, and samples with partial profiles showed a “haplotype calling” in more than 90% of the MH loci tested, even with input DNA amounts as low as 0.12 ng.
The MH data were then processed by the DVI option of the Familias software (v.3.2.9). In agreement with the high informativity of the MH panel used, LR values from 6.3×10^3 to 2.3×10^{21} were computed in three kinship analyses. Inconclusive LR values were computed in the remaining two cases: locus and allelic dropout phenomena and the availability of second-third-degree relatives as reference samples are the likely explanations for these preliminary results. Finally, we are evaluating the correctness of combining the informativity of STR and MHs markers.

P-006

Head over Heels? Your DNA says otherwise: insight into ICMP's unique bone database.

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The International Commission on Missing Persons (ICMP) has supported governments, civil society and partner institutions in the large-scale recovery, analysis and DNA-led identification of skeletal remains since the 1990s. Varied and extensive operations around the globe over nearly three decades have generated an unparalleled dataset containing over 78,000 skeletal samples that today informs decision making on sampling different bone types based on DNA recovery and profiling success.

This study focuses on evaluating DNA recovery of 4214 skeletal samples from different types of bone processed from 2018 onwards. It encompasses a wide diversity of samples from several geographic locations, various post-mortem intervals and conditions. The samples included were processed using different STR kits, with a range of 16 to 24 loci. DNA quantity and quality will also be evaluated for bones at different degradation stages.

The workflow used for the processing of skeletal samples at ICMP’s DNA Laboratories is well established. It involves cleaning, cutting and grinding of the bone into fine powder, which undergoes full demineralisation. The lysate is extracted and purified using a silica-column based method. The resulting purified DNA extracts are then quantified, amplified with the relevant STR kit and analysed using capillary electrophoresis (CE).

Out of the 4214 skeletal samples considered in this study, successful DNA STR profiles were obtained from 3404 samples; demonstrating a success rate of 81%. Samples were categorised into teeth and 48 different types of bones. 95% of femurs resulted in successful DNA STR profiles, followed by scapula (93%) and tarsal bones (90%). Ribs and clavicles gave the lowest STR profiling success rates, with 50% and 37% respectively.

DNA STR profiling results led to 2432 matches, 882 unique identifications of missing persons and 1550 reassociations to existing profiles.

P-007

12 real forensic caseworks solved by the DNA STR-typing of skeletal remains exposed to extremely environment conditions, without the conventional bone pulverization step

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DNA identification of human skeletal remains plays a valuable role in forensic field, especially in missing persons and mass disasters investigation. Hard tissues, such as bones and teeth, represent a very common kind of samples analyzed in forensic laboratories because of they are often the only biological materials remaining. However, the major limitation in using these compact samples rely on the extremely time – consuming and labor – intensive treatment of grinding them into powder, before proceeding with the conventional DNA purification and extraction step. In this context, a novel DNA extraction assay, called the TBone Ex kit (DNA Chip Research Inc.), was developed to digests bone chips without powdering [Kitayama et al., 2010]. Here, we simultaneously analyzed bone and tooth samples arrived to our police laboratory and belonged to 15 different forensic caseworks, occurred in Sardinia (Italy). The total of 26 samples were recovered from different scenarios and were exposed to extreme environmental factors, including sunlight, seawater, soil, fauna, vegetation and high temperature and humidity. The TBone Ex kit was used prior to the EZ2 DNA extraction kit on the EZ2 Connect Fx instrument (Qiagen), and high quality autosomal and Y-chromosome STRs profiles were obtained for the 87% of the caseworks, in an extremely short time frame.
This study provides additional support for the use of the TBone Ex kit for digesting bone fragments/whole teeth as an effective alternative to pulverization protocols. We empirically demonstrated the effectiveness of the kit in processing multiple bone samples simultaneously, largely simplifying the DNA extraction procedure, and the good yield of recovered DNA for downstream genetic typing in highly compromised forensic real specimens. In conclusion, this study turns out to be extremely useful for police force laboratories, to which the various actors of the criminal justice system – such as potential jury members, judges, defense attorneys and prosecutors – required an immediate feedback.

P-008

The Improved Ancient DNA Extraction Method was Applied to Human Remains Research

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The successful extraction of endogenous DNA from sclerous tissues is pivotal for the identification of human remains, but the retrieval of DNA from bone and tooth presents greater challenges compared to that from fresh tissues or bodily fluids. Existing methods or commercial kits for bone and tooth DNA extraction in forensic often struggle with aged, poorly preserved, chemically treated, or heat-exposed samples. Ancient DNA offer advanced extraction methods capable of isolating DNA from bone remains or sediments tens of thousands of years old, primarily utilizing silica adsorption. The samples in the two fields have different characteristics and the downstream analysis methods are not consistent, so the direct application of ancient DNA extraction methods to forensic may not produce the best results. The objective of this study is to develop the most suitable extraction protocol for forensic degraded samples by improving the ancient DNA extraction method. In this study, 90 case samples and 15 simulated samples were used to explore the optimal experimental conditions in pyrolysis and purification processes, and 8 aged bones and 20 heat-treated teeth were used to test the application potential of optimized ancient DNA extraction methods. The results show that increasing pyrolysis temperature, reducing pyrolysis time and avoiding additional decalcification steps are more suitable for highly degraded samples in forensic research. Centrifuge column was found to be more suitable for manual extraction during purification and the higher proportion of binding buffer helps to retain short fragments of DNA. The advantage of ancient DNA extraction method was more significant for tooth samples burned for a long time. All in all, the research proves that the improved extraction methods of ancient DNA is more suitable for extracting highly degraded human remains in forensic, which helps to augment the success rate of human remains analyses.

P-009

AN ASSESSMENT OF DNA RECOVERY FROM TEETH SUBMERGED IN A SEMI-NATURAL MARINE ENVIRONMENT

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1. Objectives
DNA recovery from human remains submerged in marine environments present challenges for forensic human identification, with 12.8% of bodies from the ocean surrounding Cape Town, South Africa remaining unidentified after autopsy. There is limited prospective research with longitudinal study designs on DNA quantity and quality recovered from hard tissue, particularly directly after submersion. This study aimed to assess the DNA concentration, integrity, and statistical informativeness obtained from human teeth submerged in a simulated marine environment for forensic DNA profiling purposes.
2. Materials and methods.
Sixteen pairs of third molars from consenting donors were used, each providing one control and one experimental tooth. Experimental teeth were submerged in continuously replenished seawater tanks. Daily sampling occurred for 10 days, with additional sampling on days 12, 14, 16, 18, 20, and 30. Control teeth were maintained at room temperature for the same duration. Real-time PCR and forensic DNA profiling via capillary electrophoresis were performed.
3. Results and conclusion
DNA concentration decreased consistently over time, from 0.37 - 0.001 ng/mg in experimental teeth and 0.52 - 0.01 ng/mg in controls. None of the samples exhibited DNA degradation or PCR inhibition. Extended submersion of experimental samples diminished the completeness of forensic DNA profiles, with the likelihood ratio decreasing at a greater pace than the control samples. Experimental profiles became statistically uninformative by day 30.
These findings indicated that despite initial similarities to control samples, the decrease in forensic DNA profiling quality accelerated in experimental samples with prolonged submersion. This decline was attributed to low-copy number DNA rather than DNA degradation. A baseline for forensic DNA profiling in medico-legal cases was thus established for samples from marine environments, enhancing our understanding of DNA recovery dynamics in these taphonomic recovery contexts.

P-010

DNA profiling of burnt teeth: Effects of burning temperature on DNA quantification and profiling success

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Fires are a frequent cause of death, with individuals often burnt beyond the point of visual recognition. Teeth maintain their structure and can withstand high temperatures; making them a possible source of DNA from burnt human remains. Despite this, limited literature pertaining to DNA profiling of burnt teeth exists. The aim of this study was thus to evaluate the success of forensic DNA profiling of teeth burnt at different temperatures.

Tooth samples from 25 donors (4 teeth per donor) were subjected to four temperature conditions, one tooth was left unburnt to act as a control and three teeth were each burnt in a muffle furnace at 100 ˚C, 200 ˚C, and 300 ˚C for 10 min. The colour of each tooth was recorded before and after burning using a Munsell colour chart. DNA was extracted using an optimised procedure, which included a demineralisation step. Extracted DNA was quantified through real-time PCR and profiled using capillary electrophoresis with the Promega PowerPlex® ESX 16 system.

Teeth burnt at 100 ˚C resulted in the highest profiling success (96%; n = 24/25), followed by teeth burnt at 200 ˚C (84%; n = 21/25). Teeth burnt at 300 ˚C resulted in significantly lower profiling success ($p < 0.01$), with 88% failed profiles (n = 22/25). Overall, DNA concentration was significantly lower ($p < 0.01$) and significantly more degraded ($p < 0.01$), compared to control samples and those burnt at lower temperatures. There were significant associations ($p < 0.05$) between tooth colour and DNA profiling success at 300 ˚C, suggesting that tooth colour along with the quantification results may be useful predictors of downstream DNA profiling success of burnt teeth. These results suggest that conventional DNA profiling methods and the DNA extraction method used herein are suitable for obtaining full DNA profiles from teeth exposed to temperatures as high as 200 ˚C, however, more sensitive methods such as targeted next generation sequencing (NGS) would be recommended to obtain more insight into highly degraded and fragmented samples, such as those burnt at 300 ˚C.

P-011

Y-chromosomal Analysis of Highly Degraded DNA from Skeletal Remains over 70 Years Old

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Objetives
The goal of this study is to clarify whether the skeletal remains over 70 years old from missing persons and their alleged relatives shared identical Y-STR loci.
Material and methods
Six samples of human teeth over 70 years old (postmortem) were bathed in 5 % Na-hypochlorite for 5 minutes and rinsed once with distilled water. Then the tooth samples were soaked in absolute ethanol and left to air-dry overnight. Before drilling the teeth into powder, all samples were exposed to UV-light for 30 minutes on each side. Approximately 50 mg of powder was drilled and incubated for 24 hours at 37℃ in 1 mL of extraction buffer containing 0.45 M EDTA (PH 8.0) and proteinase K (0.25 mg/ml) with 0.05 % Tween 20. Subsequently, the supernatant was transferred into 13 mL of binding buffer containing quinidine hydrochloride and purified with silica-based MinElute spin columns (Qiagen, Hilden, Germany), then the DNA was eluted in 50 µL of elution buffer. DNA quantification of the extracts using Qubit™ 1X dsDNA High Sensitivity (HS) assay kits was done for the purpose of evaluating the final concentration of DNA. Three different kits for the amplification of Y-STRs. Amplified DNA products were separated by size and detected on an ABI 3500 Genetic Analyzer.
Results and conclusions
Y-STR genotypes obtained from these alleged relatives were identical to each other and to the alleles of missing persons' consensus profiles at more than 22 loci examined, while not being found in Y-STR population database from Y Chromosome STR Haplotype Reference Database (YHRD). Therefore, missing person No.7 and missing person No.18 have a patrilineal relationship with reference samples from Family1 and Family2, respectively. In addition, the fact that Y-STR haplotypes obtained from skeletal remains of missing persons and reference samples are not found in the Han Chinese people from East Asian demonstrates its rarity and further supports a paternal lineage relationship among them.

P-012

Genetic identification of human remains in Mexico – is the 8-dye PowerPlex® 35GY System suitable for degraded tissues?

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In Mexico, almost 100,000 people are currently considered missing and about 52,000 bodies are unidentified. Officials speak of a humanitarian and forensic crisis that places a heavy burden on those affected, society and the rule of law. The state of preservation of the mortal remains makes it difficult to identify the bodies. The investigation of human remains is important in order to solve committed crimes and counteract the social depression. This also includes the subsequent identification of unknown deceased, which nowadays is mainly based on genetic profiling.
PCR and CE based STR analysis is the gold standard for genetic identification. However, STR analysis of postmortem altered tissue is a challenge due to several parameters. One limiting factor is DNA degradation. Environmental influences such as humidity, temperature or microbial infestation can enhance the degradation degree, often given parameters in a Mexican environment. In classical STR analysis, DNA fragments of different sizes, usually up to 400 bp, are analysed. STR analysis of loci located in the target region for long fragments often fails due to the fragmentation of the DNA of postmortem altered tissue. Therefore, the total number of STR loci is often not sufficient for identification. To increase the amount of amplified STR loci of highly degraded tissues, the novel PowerPlex® 35GY System (Promega, Madison, USA) was tested and compared to other commercially available STR systems. The STR Multiplex is an 8-Dye system consisting of 23 autosomal and 11 Y-STR loci as well as amelogenin. The detection of 8 colours allows 15 of the autosomal STR loci have a size of less than 250bp, which favours the analysis of degraded DNA.
During a research stay Mexico, as part of the CoCiMex project (DAAD), DNA samples from body parts exhumed from a mass grave were preserved, including bones, teeth, tendons, hairs, nails and miscellaneous tissues. In order to test the performance of the 8-Dye STR kit, several of these degraded tissue samples were analysed on the Spectrum Compact CE System (Promega, Madison, USA) using the PowerPlex® 35GY System. The results were compared with other common commercial 5-Dye available STR Kits (e.g. PowerPlex® Fusion/ PowerPlex® ESI (Promega, Madison, USA)).
Overall, the number of STR loci called in the 8-Dye STR system exceeded that of the 5-Dye STR systems resulting in a gain of autosomal and Y-STR loci for each sample. Implications for the identification of the unknown deceased in Mexico are discussed.

P-013

Molecular analysis of ancient human remains belonging to multiple unknown burials discovered in an Italian middle age archaeological site

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In forensics, the study of the archaeological site where ancient remains were discovered is of considerable importance to understand the dynamics of burials, kinship scenarios and unravel historical questions. A molecular study was conducted on bone and dental remains of some individuals belonging to an unknown burial site found outside the cloister at the former convent of S. Francesco in the city of Mirandola, Modena, Italy. During the excavation works, the portion of a late-medieval burial ground was discovered, with infant and adult graves dating back to a period between the 14th and 16th centuries. At approximately 1.5 m depth from the walking surface, nearly 30 burials were identified, placed in pits dug into the ground, organized on several overlapping levels and separated only by a couple of centimeters of soil, oriented along an east-west axis in the direction of the rising sun and without funeral equipment, according to the Christian tradition. The historical explanation for this discovery is currently poorly known, no archive records are available about the origin and identity of the buried. This genetic study is intended to integrate the archaeological and anthropological studies already underway on the site, trying to establish some kind of biological relationship among the individuals, confirm the exact number of subjects and explaining their peculiar arrangement and location. In order to verify the feasibility, a preliminary analysis was conducted on a molar tooth belonging to a single subject (grave n°15). After the promising results, the analyses were extended to the entire group of remains. After a decalcification step, genomic DNA was isolated using a powder-free extraction method, subsequently to a decalcification step. Different STR multiplex kits for both autosomal and Y markers were used in order to confirm the results. With the aim of extending the analysis also to Externally Visible Characteristics (EVCs), the HIrisPlex system was used for genetic phenotyping with a single multiplex SNaPshot assay, targeting 24 eye and hair colour predictive SNPs. The HIrisPlex webtool was used for phenotypic traits inference. These findings could be helpful to deep the knowledge of the city's history during the medieval period and to give possible information about the population of origin.

P-014

The contribution of seawater in DNA degradation: comparison of genetic results from skeletal remains affected by different taphonomic conditions in forensic contexts

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In the field of forensic genetics, the examination of compromised biological samples is a common investigative challenge. It is crucial to thoroughly assess the thanatological and taphonomic events affecting human samples for genetic analysis, including the environment surrounding the corpses or the skeletal remains, which, in the cases studied, is represented by seawater.
This research aims to compare different conditions to determine the extent to which cadaveric submersion in seawater affects endogenous DNA, by evaluating the degree of degradation and quantity of DNA through the analysis of reaction artifacts.
Considering the advanced state of cadaveric decomposition, genetic profiling was conducted on the skeletal remains using Short Tandem Repeat (STR) polymorphisms. The subjects of this study were derived from two distinct situations, each characterized by variations in post-mortem damage, time intervals, causes of death, durations of seawater immersion for the exposed samples, and the time between recovery from the marine environment and the genetic analysis. These factors potentially influence the DNA's qualitative and quantitative properties differently.
In the first scenario, victims remained submerged in seawater for periods ranging from 3 to 15 months post-mortem, while the second situation involved the collapse of a cemetery in to the sea, over half of the cadavers were submerged in the adjacent seawater. The remains not exposed to the water were also selected for control comparisons. In the second scenario, cadaveric submersion was exclusively post-mortem and significantly shorter, with a maximum duration of a few weeks.

P-015

Evaluation of Genomic Methods for Sex Prediction of Historical Remains

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Sex determination of skeletal remains may be critical to human identification associated with archeological investigations and missing persons cases. Traditional methods of sexing such as osteological analysis have limitations, particularly when dealing with highly fragmented or degraded remains. As a result, genomic methods for more accurate and reliable sex determination have been developed with early DNA methods focused on targeting of sex-specific markers. Advancements in next-generation sequencing (NGS) have enabled the development of more sensitive sexing techniques suitable for degraded DNA and/or samples with limited template DNA molecules. These sexing methods evaluate the number of NGS reads mapping to the human genome, specifically the proportion of reads that map to the X- and/or Y-chromosomes. The sex of the individual is then inferred based on established references values.

In this study, the sex of 15 individuals from the Vasa warship, which sank in 1628, was predicted based on low coverage whole genome sequencing (WGS) data using three published methods (Skoglund 2013, Mittnik 2015 and Anastasiadou 2024). Initial testing produced inconclusive or unexpected results with the Skoglund and Anastasiadou methods for most individuals. It was found that this was due to the specific bioinformatic pipeline applied, which required modifications to the published reference values. Reference values were updated for the specific WGS analysis workflow. Also, appropriate reference values were established for three targeted approaches that utilized hybridization capture panels.

The sex predicted for each Vasa individual was consistent across genomic methods and targeted approaches using the updated reference values. However, the sex for two individuals was inconsistent with previous osteological analyses. Further anthropological investigations supported the genomic determination of sex in these cases and provided important information about the people on the Vasa. These genomic methods have also been applied to NGS data from additional human remains dating from 750 to that late 1800s, further demonstrating the usefulness of sex prediction in historical contexts. Yet, this study also showed the need to evaluate the published reference values associated with each genomic sexing method to ensure the suitability for specific NGS workflows.

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P-016

Testing Methods for the Maximum Recovery of DNA from Degraded Remains

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Objectives
Degradation of human remains presents technical challenges for their identification by researchers and government agencies alike. To reduce the number of unidentified individuals, we attempt to answer whether advances in Ancient DNA extraction methods from archaeological contexts can be applied to difficult forensic cases, and to examine how they compare to protocols developed for forensic samples. We also investigate how the two methods perform in downstream analysis such as genome-wide SNP and mtDNA genome recovery, and STR profile generation quality. Finally, since it is known that different skeletal elements yield more data than others, we examine how DNA concentration, depth of coverage, damage, and profile generation differ across samples.

Materials and Methods
For this study, the Maricopa County Office of the Medical Examiner provided 75 skeletal samples representing 42 individuals who have remained unidentified by standard forensic procedures. DNA from bone and teeth samples was extracted using the Dabney et al. (2013) protocol optimized for ancient DNA extraction as well as a forensic protocol developed by Loreille et al. (2007) for degraded samples. The DNA extracted by both techniques was typed using Promega's Powerplex Fusion 6C amplification kit, which contains 27 loci including the core CODIS loci. The extracted DNA was also used to create double-stranded libraries for mitochondrial DNA capture using Arbor Bioscience's myBaits target capture kit. SNP capture was completed using a custom SNP panel targeting ~4200 SNPs (Daicel Arbor Biosciences, Ann Arbor, MI). The enriched libraries were then sequenced using the Illumina Miseq and Novaseq Plus. The resulting reads were processed using EAGER pipeline (Peltzer et al., 2016), haplogroups were assigned using Haplogrep 3 (Schonherr et al., 2023) and EMPOP (parson et al., 2014)

Results and Conclusions
We found that the Dabney extraction method resulted in an average 4.4-fold improvement in DNA yield when compared to the Loreille extraction method. STR Profiles for 13 individuals were consistent across 13 or more loci for two independent DNA extracts. From the double-stranded DNA libraries, we generated mitochondrial genomes ranging from 0.3-246.8x depth of coverage, Sequencing reads were not recovered from 13 samples, likely due to lack of sufficient DNA. STR loci recovery does not seem to be correlated with depth of mtDNA genome coverage ($p = > 0.05$), when samples do not yield an STR profile NGS of the mtDNA genome could still be viable. Analysis of Genome Wide SNP sequencing data i

P-017

Optimisation and evaluation of forensic DNA recovery from post-mortem nail samples

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The identification of deceased human remains is often challenging and poses significant challenges for many forensic services worldwide. Bones or teeth are typically used as the DNA source for genetic identification in decomposed cases, however, these sample types do not always yield informative DNA profiles. Previous studies have shown that nails could be a valid alternative DNA source. However, these studies used varying protocols and obtained diverse results. The current study aimed to optimise and evaluate the recovery of DNA from nails from unidentified human remains for use by the state's Forensic Pathology Services.

First, the input weight of nail (2 mg, 5 mg and 10 mg) and the DNA extraction incubation time (2 hours and 16 hours) were assessed using the QIAamp® DNA Investigator kit (Qiagen, Hilden) on nails from living donors (n = 5). Further optimisation using nails from cadavers (n=6) indicated that 2 mg nail with 16 hours incubation consistently yielded informative DNA profiles. The optimised protocol was then assessed on nail clippings from ten unidentified human remains from Salt River Mortuary, and full DNA profiles (concordant with control buccal swabs) were obtained for all cases. Lastly, the protocol was assessed on stored nail samples from an additional 45 unidentified human remains from 2018-2021, where bodies were no longer available for further sampling.

Out of the 55 forensic cases, full DNA profiles were obtained for 100% of nail samples. The time between death and DNA extraction, time between death and sample collection and recovery environment of the body did not have a significant impact on the DNA concentration or degradation index. These results, combined with the ease of sampling, advocate for the use of nail samples as an identification tool to help reduce the burden of unidentified human remains.

P-026

Inference of the number of contributors and involved kinship in complex DNA mixtures using machine learning

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In complex DNA mixtures involving kinship, the degree of allele sharing increases due to more contributors and kinship, making it challenging for the number of contributors (NoC) inference. The possibility of simultaneously inferring the NoC and the kinship to extract more information from the DNA mixture profiles and obtain more reliable results is worth exploring. In this study, we simulated a large number of complex DNA mixture profiles based on a 60-plex MH panel to train the machine learning model for co-inference of the NoC and the kinship. The NoC ranged from 2 to 5, with the involving kinship comprising unrelated individuals (RM type), parent-offspring pairs (PO type), full-siblings (FS type), and second-degree kinship pairs (SE type). Our results showed that when the NoC was 2, the accuracy of NoC inference for RM, PO, FS, and SE was 98.3%, 100%, 100%, and 100% respectively, while the accuracy of co-inference was 90.1%, 99.8%, 88.2%, and 88.5% respectively. However, when NoC was 5, the accuracy of NoC inference for RM, PO, FS, and SE was 99.8%, 91.2%, 87.4%, and 97.5% respectively, whereas the accuracy of co-inference significantly decreased to 65.9%, 27.4%, 37.5%, and 29.4%. In summary, our results demonstrated the potential of MHs combined with machine learning in co-inference of the NoC and kinship in complex DNA mixtures.

P-039

Recurring PCR artefacts observed in forensic STR typing of human skeletal remains: possible explanations and interpretation strategies

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Personal identification of skeletonized human remains heavily relies on genotyping of STR loci by means of capillary electrophoresis (CE). There is growing evidence that environmental genetic material can be co-extracted, and in some cases, co-amplified with human DNA isolated from bones, due to non-specific binding of primers included in multiplex PCR kits used for STR genotyping. This can cause non-specific CE peak artefacts in genetic profiles, leading to interpretation problems, especially in degraded DNA samples. While such observations are assumed to be sporadic, here we describe a series of PCR artefacts recurring in skeletal remains recovered at different times and in distinct locations in Piedmont, Northern Italy.

Off ladder peaks in the amplicon range of locus D21S11 (~213 bp) and D13S317 (~190 bp) were observed in STR profiles from two exhumed bodies (2019 and 2021) amplified with the AmpFℓSTR Identifiler Plus kit. A ~438 bp peak in the amplicon range of SE33, labelled by genotyping software as allele 33.2, was found in skeletal remains of two individuals recovered in woodlands (2020 and 2022) and analyzed with the Powerplex ESI 17 Fast kit. Notably, the artefacts were always present in DNA isolated from exposed bones, but not in samples protected from contact with soil, as petrous bone and tooth.
Molecular characterization of the observed artefacts is hampered by proprietary nature of primer sequences. In order to identify the source of unexpected peaks, we explored different strategies, including NGS analysis of microbial and fungal DNA from bone and associated soil and sequencing of purified PCR artefacts. A bio-informatics tool to test in silico any set of multiplex primers of interest against a wide host of organisms was also developed to assist the preliminary validation of multiplex PCR forensic assays.

Our results highlight the importance to always consider the possibility of artefactual effects of exogenous DNA in environmental forensic samples.
The identification of artefacts and responsible microorganisms will be useful for the forensic scientific community in order to avoid interpretation problems, especially in low template DNA samples.

P-093

Evaluation of mutation rates in Nigerian father-son pairs using Y-STR markers

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The male-specific Y-STR markers are a useful complement to autosomal STR testing, especially in cases of sexual assault. However, mutation rates can alter interpretations and thus require careful evaluation. One of the Y-STR kits, the Yfiler™ Plus PCR Amplification Kit, contains 25 Y-STR loci, including 6 RM-Y markers with mutation rates exceeding $1.0x10^{-2}$.

The mutation rate of Y-STR markers from the Yfiler Plus Kit was determined using Nigerian samples. Buccal swabs were collected from 148 Nigerian father-son pairs, and DNA was extracted using the QIAamp DNA Mini Kit. Paternity was confirmed by calculating the CPI (with a 10,000 threshold) using the VeriFiler™ Express PCR Amplification Kit. The samples were then amplified using the Yfiler Plus kit, and the mutation rate and binomial 95% confidence interval (CI) were calculated based on the observed mutations and the number of meioses. These results were compared with mutation rates reported in the YHRD.

Paternity was confirmed for all 148 pairs, with no mismatches observed. The analysis of the Yfiler Plus Y-STRs revealed a total of 29 one-step mutations across 15 markers, with the highest mutation rates observed at loci DYS518 (0.0338, 95% CI: 0.0111-0.0771), DYS458, and DYS449 (0.0270, 95% CI: 0.0074-0.0678). Approximately 48% of the mutations were repeat gains, while 52% were losses. All mutations appeared once in different father-son pairs, except for 3 pairs where 2 mutations were found. The YHRD mutation rates for DYS458 and DYS518 were found to be below the 95% CI obtained in the Nigerian dataset. Additionally, the rapidly mutating markers DYS627, DYS570, and DYF387S1 showed mutation rates lower than the 0.01 rate indicated by the manufacturer. However, this study was limited by the small number of father-son pairs analysed. This study suggests that evaluations of Y-STR mutation rates should be extended and population-specific rates established to support the use of the kit for forensic and relationship testing.

P-098

Forensic and population genetic analysis of the X-STR loci in the ForenSeq™ Signature Prep Kit in the Ghanaian population

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Forensic Genetic studies of the X-chromosome are limited for the populations of Sub-Saharan Africa. Presently, little forensic genetic data is available for the X-chromosome in Ghana to characterise the population. This study uses the ForenSeq™ Signature Prep kit to generate length and sequence-based data for 100 unrelated male samples (25 per sub-population) from the four major ethnic groups in Ghana; the Akan, Ewe, Mole-Dagomba and Ga-Adangbe.
The total average DoC for the X-STRs for all the samples in this study was calculated as 4882.5 with the highest per locus average DoC of 1608.62 seen in DXS10074. The number of alleles generated were 179, 211, and 217, for length, repeat, and flanking region sequence datasets, respectively, with allele ranges between 7 to 40.2.
The DXS10074 and DXS10135 loci were the most informative markers while DXS7423 was the least. The observed number of alleles per locus for the length-based X-STRs ranged from 4 for the DXS8378 and DXS7423 loci, to 19 for DXS10135. The repeat region sequenced data generated alleles in the range of 4 for the markers DXS8378 and DXS7423 to 44 for DXS10135. The increase in the number of alleles and heterozygosity impacted and enhanced the power of discrimination.
The pairwise FST showed the Mole-Dagomba, and the Akan presented low FST values of 0.02 for both the length and sequenced-based data. The Mole-Dagomba, Ewe, and Ga-Adangbe sub-populations when compared, all had low FST values ranging from 0.01 to 0.02. Due to the low FST values, all the four sub-populations clustered in a PCA.
Lastly, the study proved that the X-STRs are effective and suitable for complex Kinship analysis and complementing the autosomal STR profiling for human identification in the Ghanaian population.

P-102

Unraveling genetic variants: isoalleles at the DXS10146 locus may cause genotyping errors

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The X chromosome has distinctive characteristics owing to its haplodiploid mode of inheritance, providing a unique role in population-genetic studies. Predominantly, X-STR markers are used in particular cases of kinship analyses exclusively or by complementing autosomal STR information.
In a study using a Portuguese population sample for estimating the mutation rates of the X-STRs included in the Investigator Argus X-12 kit, multiple off-ladder microvariants (genotyped as putative 39.1 alleles) were observed for the DXS10146 locus. To obtain further insights on this observation, qualitative information on the sequence composition of the allele and locus structure of DXS10146 was studied for both 39.1 as well as for the adjacent 39.2 allele that is represented in the allelic ladder. In addition, the structure stability of this X-STR was also analyzed through primate-human comparisons.
Fourteen DNA samples from Portuguese individuals genotyped as 39.1 and 39.2 were amplified by PCR, followed by Sanger sequencing. Results revealed that all sequenced alleles were 39.2 and therefore may be classified as isoalleles. The group of “putative 39.1” alleles that were reclassified as 39.2 showed the same structure among each other, but were different when compared to the other sequenced 39.2 alleles. The structural differences observed may have altered the conformation of the DNA molecule, consequently changing the rate of migration during electrophoresis.
Six samples from different primates were submitted to sequencing following the same methodology and comparisons with human reference sequences support the stability of the DXS10146.
This study highlights the importance of sequencing STRs: i) to uncover genetic variation that might pose problems during genotyping; ii) to clarify doubtful genotyping questions and maintain accuracy in genotyping, as this locus is one of the most used X-STR markers and; iii) to infer on a more accurate nomenclature for DXS10146.

P-111

Optimizing Template Input: Complex Family pedigrees M-FISys with LIMS

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Currently, various databases and software tools assist analysts in using reference DNA to identify unidentified human remains (UHRs) through Direct or Kinship matching. M-FISys (Mass-Fatality Identification System) employs direct and indirect profile matching, originally designed for identifying remains from the 9/11 attack on NYC's World Trade Center. Our lab specializes in identifying victims of the Spanish Civil War (1936–1939) and postwar Andalusian victims. The identification of skeletal remains from the Spanish Civil War and postwar is conducted through kinship matching using M-FISys. Given a pedigree of family members for a missing person, the database can be searched for samples that are consistent with all family members, to calculate the joint probability likelihood ratio (JPLR) indicating the likelihood of the match being related to all family members as presented. M-FISys constructs pedigrees using specified relationships in input files. Two templates in M-FISys are used: Basic Family and Complex Family. The Basic Family template, requiring no effort, automatically draws pedigrees through pre-established relationships in the template, such as father, mother, sibling, etc. The Complex Family template involves defining parents for each member, with columns including Family Code, Father/Mother Fname, Lname, and Donor Code.

Manually filling the Complex Family template is time-consuming and prone to errors. We implemented a script designed in the Laboratory Information Management System (LIMS) and used the free online software QuickPed to semi-automate, obtaining personalized Lab-codes for pedigrees.

Optimization allowed exporting ready-to-import files from LIMS to M-FISys, containing all necessary data, including genetic profiles. Moreover, this optimization through LIMS allowed traceability of the samples from DNA extraction until obtaining the genetic profiles necessary for laboratories undergoing accreditation based on the ISO/IEC 17025 standard.

P-112

Use of 3915 kinship SNPs-Microarrays in missing person identification in the absence of two generations

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In missing person identification, the statistical power that arises from the genetic STR profiles of a family group is crucial for establishing clear differences between the person of interest and non-related people. In particular, those cases of identifying missing persons where only second and/or third-degree relatives are available, the statistical power is very low, and STR markers do not provide enough information.
Our main objectives are: (i) create a frequency table of the Argentine population based on 3915 SNPs; (ii) analyze the statistical power of identification of missing grandchildren and great grandchildren using these markers to solve complex cases such as those involving incomplete family groups.
For the SNP marker typing, microarray technology was utilized with the Illumina Infinium Global Screening Array-24 Kit (Illumina, San Diego, CA, USA), which includes a panel of approximately 650,000 SNPs. As the panel is primarily designed for medical diagnosis, based on the FORCE panel designed by Tillmar et al. (2021), we selected 3915 SNPs linked to kinship analysis. To create a frequency table of the Argentine population, 30 samples of unrelated individuals were analyzed. Three previously solved cases with STRs were used for conditioned simulations on SNPs. For this, we used Merlin software to calculate likelihood ratios (LR) for the tested hypotheses, selecting 1, 2, and up to 3 pedigree members of second and third-degree relatives. Both simulated and empirical LRs were compared to validate the strategy. Here, we present the first population frequency table of Argentina for kinship SNPs markers obtained by Microarrays technology. Additionally, we demonstrated that the panel of 3915 kinship SNPs allows for good identification power, even when only one second or third-degree relative of the sought individual is available.

P-114

Kinship analysis for human identification of the korean war victims

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Higher density targeted SNP sets are the important to analyze long range relationships in unidentified and missing person especially the old skeletal remains. The purpose of this study is analysis more SNPs using more challenging samples with minimize errors in kinship analysis and make sure accurate human identification. In this study, we selected bones from various parts of excavated Korean War victims and saliva samples from their family members. For obtaining purified DNA more effectively, a modified organic extraction method was used to get gDNA above 0.04 ng/µL. To protect the individual genetic information, we constructed the local DB software for direct matching solution which only accessible to compare with local references in private database instead of using global and public cloud database. Prior to application this system for the old skeletal remains, we sequenced on a MiSeq FGx Sequencing system using Kintelligence kit which 10,230 SNPs to compatible human identification and genetic genealogy. The derived data is accumulated using analysis of family reference samples into the ForenSeq Universal Analysis Software(UAS) and upload the reference SNP profiles in local DB. Next, targeted samples are analyzed and likelihood ratios are calculated with reference samples. Finally, the relationship level was obtained by comparing 9,867 kinship SNPs included in the case sample and reference data, and the family relationship capable of maximum analysis was confirmed to 4th degree(1st cousin once removed). This method could serve as improved result for identification of old skeletal remains. In cases where family relationships unidentified through STR analysis alone and maternal or paternal lineage cannot be confirmed, the accuracy of kinship probability is expected to improve through this analysis.

P-120

A systematic approach for interpreting siblingship: A pilot study on genetically secluded Lebanese population

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Siblingship testing is a type of complex kinship analysis where individuals are compared to determine whether they are full siblings (share both parents) or half siblings (have one parent in common). This type of testing becomes required in human identification, forensic casework or familial DNA search when neither parents nor any of the individual's belongings are available. The degree of confidence of siblingship testing has always been under question. In this study, we probe a methodology for interpreting siblingship cases in the inbred Lebanese communities by calculating the likelihood ratio (LR), following the standard methods, of 99 full sibling pairs, 254 half sibling pairs and 22,473 non-sibling pairs. The accuracy, sensitivity and specificity were tested using 15, 23 and 28 STR markers. The LR cutoffs were set in order to reduce the margin of uncertainty in siblingship testing in the Lebanese population. Yet, the exceptional high LR values in non-sibling pairs, supporting relatedness, and on the other hand the very low LR in some true sibling pairs, supporting non-relatedness, suggested the application of a grey zone and the evaluation of shared alleles to infer siblingship between individuals in an inbred population with more confidence.
In conclusion, LR values below 0.04 and 0.16 are considered negative for full and half siblingships, respectively, and LR values above 64 and 15.13 are considered positive for full and half siblingships, respectively. Values in between fall into the grey zone and the number of shared alleles is further assessed. The combination of LR and, the number of two allele shared loci (which is 7 loci for cases of full siblingship), and all shared alleles (which is 23 alleles for cases of half siblingship), increased the degree of confidence of siblingship testing in a population where genetic relatedness is abundant due to increased consanguineous and endogamous marriages.

P-123

Noninvasive prenatal paternity testing: a new contribution from DIP-STRs markers

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1. Objectives
Noninvasive prenatal paternity testing (NIPPT) is an important forensic genetics analysis, especially in cases of suspected post-rape pregnancy. It allows the determination of fetal paternity within weeks of
gestation. Current NIPPT methods are based on the analysis of cell-free fetal DNA (cffDNA) circulating in the blood of pregnant mothers. However, these methods face challenges due to the interference from maternal DNA in the analysis.
This project aims to contribute to the field of NIPPT for forensic applications by developing a novel and robust method.

2. Materials and methods
The goal is to apply Next-Generation Sequencing (NGS) technology to the analysis of DIP-STR (Insertion/Deletion Polymorphism coupled with Short Tandem Repeat) markers. These genetic markers have demonstrated an enhanced ability to deconvolute mixtures of DNA from two contributors, such as the blood of pregnant mothers, by specifically targeting the alleles of the minor contributors. They have already shown potential for NIPPT up to 7 weeks of gestation using capillary electrophoresis analysis. To maximize their potential, a panel of 27 DIP-STRs has been optimized for NGS analysis on an Illumina sequencing platform.

3. Results and conclusion
We will present here the sequencing results for the DIP-STRs markers panel tested on both single source DNA profile as well as simulated DNA mixtures.
Upon completion of optimization, the panel of markers will be validated through the analysis of plasma samples collected from a cohort of 100 pregnant women at three different gestational intervals.
The novel sequencing approach applied to DIP-STRs markers is expected to enhance sensitivity, specificity, and multiplexing capability, thus improving the performance of these markers in NIPPT. Moreover, it will address the challenge of maternal DNA interference in cffDNA analysis.

P-124

Impact of additional STR loci and maternal inclusion on the accuracy of duo and trio paternity tests: A comparative study

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A DNA paternity test is a genetic analysis comparing a child's DNA with that of a presumed father to ascertain if a biological relationship exists between them. This typically involves analysing the alleles present in the STR regions. The method relies on the fact that half of an individual's STR alleles are inherited from their mother and the other half from their father. A duo paternity test only involves testing the DNA of the child and the alleged father, whereas a trio paternity test also includes the mother's DNA. With the increasing demand for duo paternity tests, the issue of reduced specificity arises, as the child's maternal alleles cannot be determined without the mother's DNA. This is further complicated by null alleles or mutations, leading to potential misinterpretation in the test results, such as false exclusions or inclusions. Additionally, using a limited number of STR loci may exacerbate these issues.

This study utilised DNA samples from 156 inclusion and 57 exclusion cases previously identified through trio paternity testing. Buccal swabs from these cases were processed using the Prep-n-Go™ buffer, and the DNA samples were analysed using both the Identifiler™ kit (15 STR loci) and the Verifiler™ Express kit (23 STR loci). The Combined Paternity Index (CPI) was calculated for both duo and trio paternity scenarios, with a CPI threshold exceeding 10,000 indicating paternity inclusion.

The comparison of results, conducted via a t-test (with a p-value ≤ 0.05 indicating statistical significance), showed no significant difference (p-value 0.067) between the trio paternity tests carried out with both kits. However, a notable difference (p-value 0.031) was observed in the duo paternity tests when additional loci were analysed. This highlights the crucial role of the mother's participation for more conclusive results. Furthermore, the use of additional loci in duo exclusion paternity cases demonstrated an average increase of 4.4 incompatible loci. This finding emphasises the importance of including additional loci in duo paternity tests when the mother's DNA is not available. Nonetheless, to reinforce these hypotheses, future research should focus on a larger sample size and an expanded investigation into cases involving mutations.

P-125

Exploring the Long-Term DNA Stability on Buccal 4N6FLOQSwabs® in Simulated Paternity Cases using a Traditional and an Alternative Forensic Workflow

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Introduction

Accurate paternity determination is pivotal in legal proceedings and required complete DNA profiles, which serve as the foundation for establishing biological relationships between individuals. Copan buccal 4N6FLOQSwabs® (4N6FS) are commonly utilized for DNA collection due to their non-invasive nature and efficiency of use.
This study aimed to explore the long-term DNA stability on buccal 4N6FS stored at room temperature (RT) for over 5 years, in six simulated paternity cases. Additionally, an alternative workflow involving microFLOQ® direct (MD) was evaluated to expedite the analytical process, eliminating the need for DNA extraction and quantification steps.

Materials & Methods

Starting from 2018, six simulated paternity cases have been set-up, each with a different Time to Analysis (TTA) interval for the collected buccal 4N6FS:

- Case 1 (07/2018–TTA:5 years and 8 months): Daughter, Presumptive Mother
- Case 2 (11/2019–TTA:4 years and 4 months): Son, Mother, Presumptive Father
- Case 3 (01/2020–TTA:4 years and 2 months): Son, Mother, Presumptive Father
- Case 4 (09/2020–TTA:3 years and 6 months): Son, Mother, Presumptive Father
- Case 5 (07/2021–TTA:2 years and 8 months): Daughter, Presumptive Father
- Case 6 (10/2021–TTA:2 years and 4 months): Son, Presumptive Father

One 4N6FS per individual was processed with the standard workflow including: DNA extraction using QIAamp DNA Investigator Kit on QIAcube; DNA quantification by real-time PCR with Quantifiler® Trio Kit on AB 7500; profiling with GlobalFiler™ kit on Veriti™ Dx 96-well thermal cycler and AB 3500 Dx Genetic Analyzer.
Before proceeding with DNA extraction, each 4N6FS was subsampled using a MD previously pre-wetted with 1ul of PCR grade water. Each MD was directly amplified using GlobalFiler™ Express kit on Veriti™ Dx 96-well thermal cycler and AB 3500 Dx Genetic Analyzer.

Results

Full and concordant DNA profiles from the same individuals were obtained using both the traditional method with 4N6FS and the MF alternative workflow. The average peaks height and DNA profile quality fall within the parameters recommended by the profiling kit manufacturer for the standard method, with higher variability observed for the direct workflow.

Conclusion

Results demonstrated that after prolonged RT storage for over 5 years, buccal 4N6FS allowed for successful generation of full DNA profiles using both the traditional method and MF alternative workflow.
The implementation of the MF workflow offers potential advantages in expediting the paternity testing process, also dealing with samples stored at RT for prolonged periods and preserving the original buccal 4N6FS for further analyses.

P-129

Kinship testing using X-STR linkage groups on a Tamil pedigree

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X-STR markers can be useful supplements to autosomal STR testing in kinship and complex cases such as deficiency paternity and incest. Among the commercially available kits for X-chromosome markers, the Investigator® Argus X-12 QS Kit offers multiplex typing of 12 X-STRs clustered into 4 linkage groups, producing 3-allele mini-haplotypes from male samples. Further research into the utilisation, behaviour, and mutation of X-STRs is required to fully support their use in forensic studies.
For this reason, we decided to evaluate the utility of the Argus X-12 QS Kit for kinship testing on a Tamil pedigree. This pedigree comprised 8 males from 3 sets of full siblings, sharing the same maternal grandparents, resulting in pairs of 8 full siblings and 20 first cousins. Samples from the mothers were also used to confirm maternity and to conduct full sibling tests among them. Buccal swab samples from all individuals were extracted using Prep-n-Go™ Buffer and amplified using the Argus X-12 QS Kit. Confirmatory relationship tests were performed with the VeriFiler™ Express Kit, using maternity and full sibling probability thresholds of 99.99% and 95%, respectively.
All maternity and full sibling relationships were confirmed. The X-STRs results showed that the full male siblings shared either 1 or 2 linkage groups. When pairs of first cousins were examined, 5 pairs showed no shared linkage groups, while 8 pairs shared 1, 4 pairs shared 2, and 3 pairs shared 3 linkage groups. This demonstrates the possibility of first cousins with the same maternal grandparents sharing more linkage groups than full siblings. In addition, 2 rare events were observed: a recombination event within a linkage group and a mutation. Thus, this study underlines the importance of careful evaluation when using the Argus X-12 QS Kit to supplement the resolution of pedigrees including first cousins, while highlighting the potential co-occurrence of rare events.

P-133

Simplified DE Method for the RapidHIT ID to Obtain Investigative Leads from Sexual Assault Evidence

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Rapid DNA integrates sample analysis workflow from extraction to capillary electrophoresis, generating autosomal STR profiles in as little as 90 minutes without the need for a DNA laboratory. The FBI allows profiles developed from reference samples on rapid DNA instruments to be uploaded to CODIS, but not from crime scene samples. Reference samples collected from qualifying arrestees during booking are enrolled to and automatically searched against unsolved crimes. This is expanding nationwide due to its ability to link arrestees to unrelated crimes while still in custody.

While the FBI is working with vendors to enable analysis of crime scene samples by early 2025, many law enforcement agencies have been using rapid DNA independent of CODIS. Rapid DNA is rarely used for SA cases because the instruments do not perform differential extractions (DE). We sought to develop an off-instrument DE method for the RapidHIT™ ID system (RapidINTEL™ Plus cartridges) to enable law enforcement to leverage the speed of rapid DNA in SA cases, with the goal of compatibility with non-technical users and potential point-of-collection environments.

The outcome is a simple workflow that utilizes a 1-hour differential lysis to preferentially lyse epithelial cells leaving sperm cells intact. After a few brief washes, the epithelial cell fraction is separated, and the remaining sperm pellet is briefly incubated with 1-thioglycerol. Five microliters of the 30 µl sperm fraction is added to a HydraFlock® swab and run on the RapidHIT™ ID system (low lysis volume protocol). Single source male DNA profiles can be obtained from as little as 1 µl of semen, with the full sensitivity of the method still being evaluated. Mock mixtures using both buccal and vaginal epithelial cells were evaluated representing possible casework scenarios of vaginal and oral assaults. Successful results were also obtained from volunteer donated 12-24 hr post coital samples.

P-134

Developmental Validation of the Maxwell® DE System for Efficient Sperm Cell Separation in Sexual Assault Sample Analysis

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The testing of sexual assault samples often involves the task of separating sperm cells from non-sperm cells, a process known as differential extraction. Traditional methods for differential extraction are demanding in terms of labor, time, and skill. The Maxwell® DE System introduces a streamlined solution, employing a new paramagnetic particle that binds sperm cells following an initial lysis of non-sperm cells. Subsequently, using the hands-off automation of the Maxwell® instrument, sperm cells attached to the paramagnetic particles are washed to remove non-sperm DNA, enabling delivery of an enriched, lysed sperm fraction. This lysed sperm DNA fraction is optimized for use with Promega's PowerQuant® and PowerPlex® Systems, allowing for immediate DNA quantification and STR analysis without the need for further purification steps, facilitating faster results with Promega systems.

To ensure the efficacy and reliability of the Maxwell® DE System, a developmental validation was performed. These developmental validation studies included sensitivity studies with diluted semen on vaginal swabs to evaluate the system's ability to separate sperm cells at varying concentrations; precision and accuracy studies to assess reproducibility of results; and mock case-type sample analysis representative of real-world forensic samples. Additionally, mixture studies were conducted to determine the system's efficiency in separating cell types in mixed biological samples, along with a contamination assessment to evaluate the risk and impact of potential cross-contamination in the workflow.

These studies validate the Maxwell® DE System's capability to consistently deliver high-quality, enriched sperm DNA fractions, ready for immediate analysis. The Maxwell® DE System provides forensic laboratories with a reliable, streamlined approach to the analysis of sexual assault samples.

P-135

Exploring Immunomagnetic Isolation of Spermatozoa

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High incidence of rape and sexual assault crimes have created a need for efficient, high-throughput processing methods of sexual assault kits. The evidence recovered from these kits, typically collected from the bodies of female victims, contain a mixture of mostly female epithelial cells with some male cells such as spermatozoa. Separation of these cell types is typically performed using differential extraction methods such as preferential lysis extraction. However, because preferential lysis is time-consuming and labor-intensive, there is a demand for exploring alternative methodologies, including immunomagnetic-based cell isolation.

Sperm-specific antibodies conjugated to magnetic beads have been shown to successfully isolate spermatozoa. In this study, a cocktail of antibodies for sperm-specific proteins were conjugated to magnetic beads through either carboxylic acid or epoxy residues to capture spermatozoa. Expression of these spermatozoa proteins and antibody-binding capability were confirmed by immunocytochemistry. However, even in the absence of antibodies, both the magnetic beads with carboxylic acid residues and those with epoxy residues exhibited a binding affinity towards both spermatozoa and vaginal epithelial cells despite the integration of blocking agents such as bovine serum albumin. These non-specific interactions increased with additional incubation between cells and beads. While previous studies have shown varying levels of success in immunomagnetic isolation of sperm cells from single source and mixed source samples, it is critical to address these issues of non-specific binding and cross-reactivity with vaginal epithelial to fully exploit the cell-isolation capabilities of magnetic beads coupled with specific antibodies. While immunological methods are currently employed in forensic laboratories for screening purposes, caution is advised for future implementation of immunological methods for differential cell selection.

P-136

Single cell sorting of spermatozoa: a validation study for integration in forensic casework

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A crucial aspect of identifying sexual assault offenders often involves the detection and separation of spermatozoa in samples collected from victims. While differential lysis is the conventional method for the isolation of spermatozoa, it is time-consuming and often ineffective in generating male DNA profiles from samples with an unfavourable epithelial/sperm cell ratio. This study aims to develop a methodology specifically designed for analysing samples with a low number of spermatozoa, such as those collected long after the alleged sexual assault. Fluorescence-activated cell sorting (FACS) is a well-established technology utilised in clinical settings to enrich rare target cells from a mixed cell population based on phenotypical characteristics detected by flow cytometry. Although its application in forensics has previously been reported on a limited basis, reliable validation is essential for its integration into forensic casework. This research established a methodology for sorting sperm from epithelial cells using FACS, followed by direct lysis and PCR for subsequent DNA profiling. Following this procedure, as few as 3 and 10 sorted sperm cells are sufficient to produce highly informative and full DNA profiles, respectively. The performance of this method was compared with standard differential lysis through parallel testing of samples with different epithelial/sperm cell ratios and post-coital swabs. Using the sorting method, male DNA profiles were acquired at a higher epithelial/sperm cell ratio and at a longer post-coital interval, making it a viable alternative to differential lysis. This comparative study is therefore followed by a systematic validation considering the sensitivity, specificity, reproducibility, and repeatability, along with DNA quality parameters such as profile completeness and purity, stutter ratio, drop-in rate, and heterozygote balance. Upon completion of validation, this methodology will be implemented for analysing casework samples where differential lysis is expected to be inadequate, such as those with a long interval between the alleged assault and collection. By employing more sensitive methods, the success rate of identifying perpetrators could significantly improve, and therefore potentially have a major impact on the conviction rate of sexual offenders.

P-147

Hair root staining and STR typing.

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DAPI-stained visible nuclei counting has been shown to be an invaluable technique in the selection of hair samples for short tandem repeat (STR) typing. However, it is critically important to establish visible nuclei count threshold, defined as the minimum number of hair root nuclei required to yield an STR profile suitable for National DNA Index System (NDIS) upload. To achieve this goal, 183 hair samples were collected and assigned a growth stage after microscopic examination by a qualified Forensic Trace Examiner. The samples were DAPI-stained and classified into five bins based on the number of visible nuclei detected in hair roots: bin 1 (0 nuclei), bin 2 (1 – 24 nuclei), bin 3 (25 – 49 nuclei), bin 4 (50 – 99 nuclei) and bin 5 (> 100 nuclei). Nuclear DNA (nuDNA) were extracted followed by quantification of DNA extracts. The quantification results showed a strong linear trend and a positive correlation between visible nuclei bins and nuDNA yield (R2 = 0.92, p-value = 0.009). As such, counting of DAPI-stained nuclei is a predictor of nuDNA yield and can be regarded as a quantitative technique to assess nuDNA yield based on nuclei amounts in hair roots prior to nuDNA extraction. STR profiles developed from DNA extracts showed hair roots containing at least 25 visible nuclei could produce STR profiles that are suitable for NDIS upload.

P-150

Inference of forensic body fluids/tissues based on mitochondrial DNA copy number: a preliminary study

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Objetives: The inference of body fluids and tissues is critical in reconstructing crime scenes and inferring criminal behaviours. Nevertheless, present methods are incompatible with conventional DNA genotyping, and additional testing might result in excessive consumption of forensic scene materials.

Material and methods: This study aims to investigate the feasibility of distinguishing common body fluids/tissues through the difference in mitochondrial DNA copy number (mtDNAcn). Five types of body fluids/tissues were analyzed in this study - blood, hair, saliva, semen, and skeletal muscle. mtDNAcn was estimated by dividing the read counts of mitochondrial DNA to that of nuclear DNA (RRmt/nu).

Results and conclusions: There were significant differences in RRmt/nu between the five types of body fluids/tissues. Specifically, hair samples exhibited the highest RRmt/nu (log10RRmt/nu: 4.3 ± 0.28), while semen samples showed the lowest RRmt/nu (log10RRmt/nu: -0.1 ± 0.28). RRmt/nu values of blood samples without extraction were notably higher (approximately 2.9 times) than those obtained after extraction. However, no significant difference in RRmt/nu was observed between various age and gender groups. Hierarchical clustering and Kmeans clustering analyses showed that body fluids/tissues of the same type clustered closely to each other and could be inferred with high accuracy. In conclusion, this study demonstrated that the simultaneous detection of nuclear and mitochondrial DNA made it possible to perform conventional DNA analyses and body fluid/tissue inference at the same time, thus catching two flies in one catch. Furthermore, mtDNAcn has the potential to serve as a novel and promising biomarker for the identification of body fluids/tissues.

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P-155

Usefulness of one-off DNA database searches in Switzerland

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1. Objectives

Forensic DNA databases (DNADB) are efficient to provide investigation leads. However, DNA profiles that are uploaded in such DNADB must meet specified criteria to lower the risk of adventitious matches. For example, in Switzerland, only single-source DNA profiles with a minimum of 6 (out of 16) loci and two contributors mixtures with a minimum of 8 loci can be transmitted to the national DNADB. DNA profiles that do not meet these criteria represent about 10% of the 15'000 DNA profiles analyzed each year in Western Switzerland. These profiles can be manually compared with candidates upon request from police services. Alternatively, one-off searches can be undertaken in the national DNADB. Complex DNA profiles can be compared once (without being stored) within the database using information from the DNA profile selected by the DNA expert. One-off searches may provide lists of candidates, that can be reduced according to the information contained within the whole DNA profile.

2. Material and Methods

In this study, we reviewed all the one-off search cases performed in western Switzerland between 2014 and 2023.

3. Results and Conclusions

This showed that the number of requests for one-off searches has more than tripled and is still increasing, particularly since the introduction of probabilistic genotyping in our laboratory in 2019. Since then, 79 compatibilities with LR > 1 were highlighted out of 327 requests, representing investigation leads for more than 20% of the cases. Here, we used this analysis to determine the usefulness as well as the limitations of one-off searches.

P-156

Expanding the Base: Adding STR sequencing into STRBase

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In the last year, STRBase (strbase.nist.gov) has undergone major changes. The website has been reorganized and made more user-friendly. Content continues to be added including information related to Short Tandem Repeat (STR) sequencing; specifically, STR allele sequences and sequencing kit information. As STR sequencing methods are being implemented in the forensic community, public resources are needed for continuity between nomenclature reported by laboratories. A curated collection of known sequences that is easy for the community to access and search will facilitate this continuity. In an ongoing effort since 2017, STR sequencing data is being collected, processed, and submitted to the NCBI's GenBank resource creating individual GenBank records for sequenced alleles. The STRSeq BioProject [1] at NCBI houses these records in an organized nested set of BioProjects. The structured records include additional forensic information as compared to typical GenBank records. In addition, these records have also undergone their own format and content updates in the last year, incorporating the 2024 ISFG nomenclature recommendations [2].

These GenBank records can be downloaded as flat text files that are easily manipulated computationally but are not easily accessible for an overall review of multiple sequences at once. STRBase is the perfect platform to include STR sequences in locus-specific sets. A new locus tab for sequences is being developed to include nomenclature and sequence information in an easy-to-search and view structure. It will also connect to the STRSeq BioProject records. Additional updates to the general information tab for each locus will bring the information into line with the 2024 ISFG nomenclature recommendations. This includes minimum ranges and updated bracketing structures. A search function to allow the community to check individual sequences and determine if they are present in the STRSeq catalog is in development. The goal of STRBase has been to bring together useful information for the forensic community, and incorporating this wealth of STR sequence information into the site is the next step.

1. The STR Sequencing Project (human), BioProject Accession: PRJNA380127, https://www.ncbi.nlm.nih.gov/bioproject/380127, Accessed Mar. 27, 2024
2. Gettings, K.B. et al., "Recommendations of the DNA Commission of the International Society for Forensic Genetics (ISFG) on short tandem repeat sequence nomenclature." 2024, 68:102946.

P-157

The importance of staff genetic profiles in DNA databases

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The Portuguese Law no. 5/2008, published on the 12th of February of 2008, approved the creation of the DNA profile database for civil and criminal investigation. The first DNA profile was inserted in 2010 and in December of 2023 the DNA Database had a total of 23775 genetic profiles, 67% of which from convicted offenders, 30% from crime scene evidence for criminal investigation and 2% from staff. The 1% left are profiles for civil identification proposes as well as volunteers for civil and criminal investigations.
The primary objective of forensic DNA databases is to help criminal investigations, by producing matches to enable the identification of profiles from crime scene evidences, and to help civil identification through matches between profiles of unidentified corpses and profiles obtained from missing person objects or relatives of missing persons.
Until December 2023, Portuguese DNA database produced 989 matches at national level, 39% were between profiles obtained from evidence samples of criminal investigation, around 60% resulted between profiles from crime scene evidence with convicted offenders and around 1% were between profiles from collected evidence and staff.
The Portuguese DNA database counts with 564 staff profiles, which leaded to nearly 1% of the national matches. From these eight matches obtained from crime scene investigation, three were with single profiles and the other five were with mixture profiles. One of the mixture profiles matched with two different staff profiles, resulting this combination in a perfect match.
According to the ENFSI's recommended guidelines, every person who could introduce cross-contamination to the investigated DNA samples, should have their profile inserted in a laboratory elimination DNA database and in the national DNA database.
The aim of this work is to emphasize the importance of the inclusion of the staff profiles in the national DNA database, leading to identify genetic profiles that were assumed as evidence and therefore could help to solve an investigation. However, as a result of contamination, should not be considered.

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Diagenetic research of factors influencing the performance of biomolecular investigations of human skeletal remains from Spanish Civil War graves.

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During the Spanish Civil War (1936-1939) and the posterior dictatorship (until 1970s), a huge number of people disappeared during the war or in episodes of repression. More than 80 years later, a large number of victims remain unidentified, despite the efforts made and scientific advances in identification techniques. One of the main limitations of these analyses is the difficulty of obtaining informative STRs profiles from the remains, limiting the identification of missing persons. This may be because the quantity and quality of DNA recovered from skeletal remains are affected by the extrinsic and intrinsic conditions to which remains have been exposed, which in turn influenced the degradation and modification of genetic material. With this aim, this work focuses on multidisciplinary research to investigate the relationship between the factors (physical-chemical and biological) that influence the state of preservation (anthropological and genetic) in order to understand the influence of these factors and increase the number of identifications of victims in the context of the Spanish Civil War.
For this purpose, we have collected and analyzed the physicochemical composition of 27 mass graves from Spanish Civil War and performed the anthropological analysis of the skeletal remains buried in the graves. Subsequently, the autosomal and Y-chromosome STRs of the skeletal remains were analysed.
Finally, we developed a predictive statistical model to determine the most influential variable in obtaining an informative profile of autosomal STRs (> 12STRs).

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bs-LIMS®: a unique tool for mass human identification applied to LR threshold calibration for kinship analyses

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During the night of the 2nd to 3rd October 2020, the storm named Alex generated an exceptional Mediterranean episode causing considerable damage in the valleys of Nice's hinterland (France). Two cemeteries from the valleys of the Vésubie and the Roya were ravaged by the flood, and the corpses contained in the cemeteries were washed away. Following this tragedy, our lab was selected by the prefecture of Alpes-Maritimes to perform DNA analysis on the remains of the deceased through the comparison with their next of kin. The identification of the corpses was performed in collaboration with the BioSilicium company, by the use of the bs-LIMS® software, based on the DNA recognition between human remains from the cemeteries and their next of kin. The genetic profiles of both the deceased (56 remains plus 9 profiles from police services) and the next of kin (84 reference profiles) were run through the "national base" module of the bs-LIMS® software. DNA traces were compared with reference DNA following two criteria: the concordance by homology and the comparison by the calculation of the likelihood ratio (LR) in various kinship testing.The interpretation of the results raised the question of LR calibration according to familial relation types. The study of LR distribution for the different kinships led us to create a specific auto-calibration module. This module limits LR ranges depending on allelic frequencies used in the lab to facilitate interpretation of the data. The identification of 19 people was thus achieved and the human remains were returned to their families.

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A comparison of likelihood ratios between STR sequencing and capillary electrophoresis for a set of complex mixtures

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The wide application of massively parallel sequencing (MPS) in forensic DNA profiling has in part been hampered by the limited knowledge on the possible information gain compared to the established capillary electrophoresis (CE) methods. MPS analysis of short tandem repeats (STRs) offers increased discriminatory power due to the inclusion of sequence variants which potentially generate increased weights of evidence for true contributors, in addition to the often higher number of STR markers. In this study, the Mainstay sequencing kit (27 autosomal STRs, Qiagen) was compared to the CE assay PowerPlex Fusion 6C (23 autosomal STRs, Promega), with respect to likelihood ratios (LR) following analysis of mixtures. Specifically, a set of 20 complex mixtures with two to four contributors in various proportions (1:9, 1:1:8, 1:1:18, 1:1:2:8 and 1:1:2:18) were analyzed with both methods. LR calculations were performed using the continuous model EuroForMix for both sequencing and CE results applying various filters. LRs were calculated both for all markers included in the kits and for the 23 autosomal STR markers that the kits share, to study the effects of increased allele discrimination and higher number of markers separately. This study contributes with knowledge regarding when sequencing is preferable over CE analysis, thus aiding forensic laboratories in the decision to implement STR sequencing.

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Developmental Validation Testing of the Integrated Quantifiler™ Trio-HRM Mixture Screening Assay

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The forensic DNA analysis process is currently incapable of providing information about the number of contributors in an evidentiary DNA sample at an early stage. To meet this need we integrated a high-resolution melt (HRM) mixture screening assay into the qPCR step of the forensic workflow producing the integrated Quantifiler™ Trio-HRM assay. This assay, when coupled with a sequential statistical prediction tool, allows for an overall prediction accuracy of ~80% in the identification of the contributor status (single-source vs mixture) of samples.

Many factors determine the complexity of a mixture, including the number of contributors, quantity of DNA from each contributor, and the quality of the DNA. Further, all new methods used for forensic DNA analysis must undergo developmental validation prior to being implemented in labs. Thus, a select group of essential developmental validation studies were conducted to assess a variety of sample conditions and determine the performance limits of the newly developed integrated Quantifiler™ Trio-HRM assay. First assessing reproducibility, the assay was found to be 59% reproducible across runs and 65% within run. Sensitivity of the integrated Quantifiler™ Trio-HRM assay was assessed by testing single source and mixture samples that ranged in DNA concentration from 0.01-60ng/µl. While mixture detection remained robust at very low DNA concentrations, the data indicated that the integrated Quantifiler™ Trio-HRM assay performs optimally for unknown samples with DNA concentrations between 0.025ng/µl-0.5ng/µl. Ten of the previously evaluated 2-person mixtures were remade across a range of mixture ratios spanning 1:2-1:100. Additionally, five of the existing 2-person mixtures were remade with equal contributions from a 3rd, 4th, and 5th contributor. These studies indicated that the integrated Quantifiler™ Trio-HRM assay is very robust in the identification of DNA mixture samples, regardless of the number of contributors or the ratio of the major and minor contributors. Further, mixture prediction accuracies were improved well over random chance (6.25%) even when the major:minor ratios were 100:1, which is well beyond the sensitivity of traditional CE analysis. Finally, when mock evidence samples were evaluated, prediction accuracies were consistent with previously tested buccal swabs, and all limitations noted in the reported validation studies held true.

With a user-friendly web interface (in development), the application will be ready to deploy to accredited practitioner laboratories for external testing. Upon successful validation and implementation, this tool will provide forensic examiners with a powerful way to screen and triage evidence items prior to the end point of analysis.

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Internal Validation of MaSTR™ and NOCIt® Software: A Casework Lab Perspective

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Mixture analysis is a complicated process that has the potential to be influenced by bias if strict rules or objective software aren't used. NOCIt® software to determine the number of contributors and MaSTR™ probabilistic mixture analysis software are two programs that can be used to assist analysts with this process, as the programs utilize objective information including allele calls, peak heights, drop-in/out, and stutter. NOCIt® advantages include specification of assumed contributor resulting in a hypothesis consistent with probabilistic genotyping, unimodal result distribution, calibration using laboratory sensitivity data, and incorporation of more information than decision tree methods for NOC determination. Software used in forensic labs require developmental validation following SWGDAM guidelines. Peer reviewed publications validating NOCIt® software up to six contributors and MaSTR™ software up to five contributors along with degraded samples were published independently by forensic researchers (2019 – 2022). Additionally, laboratories undergo internal validation using data prepared by the same methods as casework samples, also per SWGDAM Guidelines.

This presentation summarizes the internal validation approach and conclusions of a forensic laboratory for NOCIt® and MaSTR™ software. DNA was extracted using the Promega DNA IQ™ System, and amplified using the Promega PowerPlex® Fusion 5C kit. Capillary electrophoresis and short tandem repeat (STR) genotyping were performed on an ABI 3500 Genetic Analyzer and GeneMapper™ID-X software. Mixture design for the validations include combinations of two, three, four, and five contributors of known single source samples. The two and three contributor mixtures were created to reflect minimal or maximal allele sharing amongst the selected contributors.
In addition to summarizing the internal validation approach and conclusions of the validation using CE STR data, the status of preliminary studies analyzing massively parallel sequencing (MPS) of STRs with NOCIt® and MaSTR™ software using publicly available data will also be presented.

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Ge.F.I. UPDATED RECOMMENDATIONS ON THE COLLECTION OF BIOLOGICAL SAMPLES FOR FORENSIC GENETIC TESTING IN THE MEDICAL LEGAL CARE OF VICTIMS OF SEXUAL VIOLENCE AND/OR ABUSE

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International guidelines and scientific literature suggest that individuals who have experienced sexual violence often seek medical assistance, even if they fail to disclose the event itself. The purpose of the Ge.F.I. updated recommendations is to guide the intervention of healthcare professionals involved in the collection of biological samples from victims of sexual violence and/or maltreatment for forensic genetic testing purposes considering the new DNA technologies and the increase in the analytical sensitivity. The sample collection procedure is crucial to avoid contamination or degradation of DNA as well as the assessment of the chain of custody to maintain evidence integrity. The recommendations serve as the "minimum standards" that can be included in the practices of healthcare providers and response units to sexual violence and abuse.

According to the Italian Law 219/2017, informed consent from victims of sexual violence and/or abuse must be properly recorded before receiving medical care or participating in any body sampling activity. Healthcare professionals—even those appointed by the Judicial Authority—must ensure that individuals seeking assistance in cases of sexual violence and/or abuse receive comprehensive and unambiguous information about the protocols for the collection of biological specimens used for forensic genetic testing. Guiding criteria for handling, sampling, and storing forensic evidence, including body samples and clothing/other material of interest from the victims, are outlined in a table readily accessible for consultation in the emergency room. In addition, illustrative photographs showing the proper techniques for collection of skin samples and subungual material from abused victims are annexed. Finally, standard forms for informed consent, sample collection, chain of custody, and a list of materials/supplies useful in the collection of forensic evidence in hospital emergency departments are provided to assist further the documentation process.

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The ICRC's Forensic Genetic Centre: Operational Setup for DNA Application in Missing Person Identification in South Caucasus

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As a leading humanitarian agency, the ICRC works in situations of conflict and violence globally. In highly complex operational environments and contexts, the ICRC's Forensic Unit helps strengthen the ICRC's humanitarian response for affected people and closely collaborates with local forensic experts and institutions to enhance their skills and capacity. ICRC's forensic programs are focused on ensuring the protection of the deceased and the rights of the families to know the fate and whereabouts of their relatives.

There was a growing need to provide additional support to the Georgian authorities and the de facto authorities in Abkhazia and South Ossetia in locating, recovering, identifying, and returning the deceased, who went missing during the 1990s and 2008 armed conflicts, to their families. To this end, in 2019, the ICRC established the Forensic Genetic Centre (FGC), based in Tbilisi, to provide dedicated in-house genetic expertise. Since 2020, the FGC has also acted as a global resource for the ICRC Forensic Unit, supporting various contexts engaged in Forensic Human Identification and focusing on strengthening and building sustainable local forensic genetic capacities. The team includes four genetic specialists with expertise in forensic DNA analysis, DNA matching, statistics, and data management.

We present DNA data management, established procedures, operational activities, international collaborations, and encountered challenges. The FGC has contributed to more than 160 DNA reports, the quality control, analysis and management of over 2500 postmortem samples and 4400 family reference samples related to Georgia proper/Abkhazia/South Ossetia identification efforts, thus increasing the number of families for whom the fate and whereabouts of their loved ones were clarified and who have had remains returned for a dignified burial.

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Burial practices in Southern Italy and DNA analysis: state of the art and future perspectives

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Burial practices represent an integral aspect of cultural heritage, offering invaluable insights into historical traditions, societal beliefs, and the evolution of human civilization. The preservation of human remains, inherent in various burial methods, profoundly impacts the quality of extracted DNA. This work aims to elucidate how burial practices in Italy can affect DNA preservation and future perspectives in forensic casework.

In Italy, burial practices are regulated by the Italian Presidential Decree (DPR) No. 285, 10th September 1990. The conventional burial involves interring the deceased in a wooden coffin, which is then sealed. Subsequently, after a period ranging from 5 to 10 years, the corpse is either enveloped in a linen blanket or, if decomposition has progressed to skeletal remains, placed within a miniature zinc ossuary. This arrangement facilitates the final placement of the remains within a stone niche. According to DPR 285/90 regulations, the corpse could be directly enclosed in a zinc container and placed within a wooden coffin. Subsequently, the prepared remains are allocated to a stone niche for permanent placement.

In addition to the burial practices, it is noteworthy to highlight the significant increase in cremation rates in Italy. Over the past decades, the prevalence of cremation has soared, rising from a mere 0.2% in 1970 to a substantial 33.2% by 2020. Furthermore, preceding the cremation process in Italy, individuals must undergo a buccal swab collected and stored – on an FTA card - by the Local Health Authority (LHA) for a minimum of 10 years. However, beyond this timeframe, concerns emerge regarding the availability of genetic material for genetic investigations in the future.

Will these samples be subject to disposal due to administrative protocols? The potential loss of such valuable genetic data challenges future research and forensic investigations.

One solution may involve extending the storage period. Another futuristic option could involve establishing a BioData Bank that collects the genetic profiles of all cremated people.

According to our experience (since 1990), DNA extraction was not affected by the two different burial techniques proposed by DPR 280/90, while it was negatively impacted by cremation, where we could not obtain a valuable genetic profile for forensic purposes. As we navigate this evolving landscape, it becomes imperative to devise strategies that ensure the preservation and accessibility of genetic data for generations to come.

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WHEN SCIENCE FACES THE LEGISLATIVE LABIRITNH: AN ITALIAN CASE REPORT

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This abstract explores how to communicate and use unexpected genetic outcomes within the court context. It digs into the challenges forensic geneticists face when presenting findings that diverge from preconceived notions. A man (F), father of seven daughters (D1-D7), had an affair with a young woman (W). Subsequently, (F) and his wife died. Three years after (F) burial, (W) took legal action to demonstrate that (F) was the biological father of her son (S). The Judge ordered the man's exhumation and the DNA comparison to establish the kinship. The analysis of 15 autosomal STRs (LR=1,27e-27) and Y-STRs (3,24e-6) excluded (F) as (S) 's father. The case would be closed, but a new scenario took place because (W) 's lawyer sustained the hypothesis of the possible body replacement. This theory was supported by several signs of alteration on the coffin he noticed, albeit he did not mention this objection during the collection of (F) 's femur. The Judge accepted the (W) 's lawyer's request. Thus, to assess that the body in the coffin belonged to (F), he ordered that all the seven daughters' DNA profiles had to be compared to that obtained from the exhumated man. Five out of seven daughters' autosomal profiles showed a paternity probability (PP) of over 0,9999, while the last two of them showed a (PP) lower than 0,00000000001. A supplementary X haplotype analysis confirmed the precedent outcomes. Since the request of the Judge was to assess whether the body in that coffin belonged to (F) or not, we were not authorized to use the genetic data to report a paternity exclusion between each single daughter and (F). Then, we referred to the genetic profiles of two daughters (D6-D7) as genetic discrepancies, and we did not use them to resolve the case. Instead, we used the genetic profiles of only five daughters (D1-D5) to confirm that no cadaver replacement was performed. The body belonged to (F). Although the case was closed, many questions were still seeking answers (e.g. What could happen if no one of the daughters was a biological daughter?) With our contribution, we would like to stimulate more debate among judges, lawyers, and expert witnesses about scenarios like the one we presented. Finally, this paper may be helpful as a source of insights into the strategies and best practices forensic geneticists can employ to navigate these challenging situations.

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Ongoing research to assess the recovery of nuclear DNA from seminal fluid applied to mock human sexual assault victims exposed to high-intensity, low duration fires

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From 2022 to 2023, four test fires were conducted at the Western Carolina University (WCU) Forensic Osteology Research Station (FOREST) in conjunction with the Bureau of Alcohol, Tobacco, Firearms, and Explosives (ATF). In preparation for three fires, full-scale wood-frame flashover cells were constructed that displayed a three-wall configuration with ceilings. Each was furnished as a carpeted bedroom with typical household furnishings. Fire four occurred in a two-story, abandoned farmhouse. In all cases, unembalmed, human cadavers were placed in a supine position on a bed and human seminal fluid was pipetted into the anal, vaginal (if present), and oral cavities to mimic coital deposition. Cadavers were from WCU's willed body donation program and consented to destructive and DNA testing. All fires were started using an open flame to ignite a cellulose fuel load placed in a wastebasket. Fires were allowed to intensify, transition through flashover, then burn in post-flashover conditions for 8-12 minutes before being extinguished.

Post-fire, cadavers were noted to have extensive exterior charring with some bone exposure in areas with minor soft tissue shielding, but organs appeared to remain intact. Swabs were obtained from the perineum, and vaginal, anal, and oral cavities using dry Copan 4N6 FLOQSwabs®. Differential DNA extractions were performed using the Qiagen EZ1 Investigator kit on all swabs obtained. Purified DNA was quantified using the QuantiFiler™ Trio DNA Quantification kit. The average quantity of male DNA recovered from sperm fractions was 9.19 ng/µL (SD = 13.31). QuantiFiler™ data also showed that DNA was only slightly degraded with average degradation indices (DI) of 1.21 (SD = 0.43). DNA was amplified using the GlobalFiler™ PCR Amplification kit and fragment analysis was performed on a SeqStudio™ or 3500 xL Genetic Analyzer. High-quality profiles concordant with the reference sample from the seminal fluid donor were obtained for 56% of swabs tested with some showing a mixture of two individuals. Secondary profiles were concordant with cadaver reference profiles. Samples that failed to yield data were those that were exposed to high temperatures including perineum and oral swabs. A total of 86% of sperm fractions from anal and vaginal swabs yielded full STR profiles matching the seminal fluid donor. These results indicate that DNA from a perpetrator can be recovered from internal cavities of burned human remains. Therefore, swabbing of burn victim orifices can reveal cases where a perpetrator of a sexual assault has attempted to destroy evidence by staging a fire.

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Crassify: A Tool for Identifying CrAss-like Phages, the Predominant Human Gut Bacteriophages, as Indicators of Fecal Environmental Contamination

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CrAss-like phages are a recently discovered human gut bacteriophage. Current analyses estimate crAss-like phages to be present in 73% of global human gut metagenomes, where they can comprise up to 99% of the viral metagenomic reads. Ubiquitous in the human population, crAss-like phages have been detected even in remote populations such as rural Malawi and from the Amazonas of Venezuela. Consequently, there has been growing interest in using crAss-like phages as a marker for human fecal contamination in environmental samples. Here we introduce a tool to identify crAss-like phages from fragmented metagenomic data.

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Forensic genetic and genomic testing: A review of the law, current and emerging methods their perception and their social and ethical aspects

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Background: The development of new technologies whose impact is direct on the behavior of criminality has led to the advent of new means of criminal commission. At the same time, the techniques of research and identification of crimes authors have evolved. In this context, DNA fingerprinting technology has become the new form of scientific evidence used in criminal investigation and generally accepted in all legal worldwide system. DNA is used not only to aid with identification of perpetrators of violent crimes such as murders and rape, but in paternity cases and in identification of the remains of missing persons. More and more courts admit extensively the DNA based evidence to resolve these forensics cases. This article provide a comprehensive landscape on the status of the forensic DNA testing in the Moroccan and worldwide context, the current and emerging technologies used for forensic DNA profiling and elucidate the consequence of admissibility of DNA as evidence in the judicial procedure with special focus on their privacy, social and ethical aspects.

Main body: Since its first discovery, DN fingerprinting is more used in judicial procedures. It has been widely used as an investigation tool in various forensic situations for more than three decades now. Over the years, many proposed methods have been invented and described which helped in the detection and profiling of DNA as evidence. Although, there is no legislation on the use of DNA in the judicial realm in many countries, largely, the promoters of forensic DNA testing have anticipated that DNA tests are nearly infallible and DNA technology could be the greatest single advance steps in search of truth, conviction of the perpetrator and acquittal of the innocent.

Conclusion :This article provide a comprehensive landscape on the status of the forensic DNA testing in the Moroccan and worldwide context, the current and emerging technologies used for DNA profiling evidence and elucidate the consequence of admissibility of DNA as evidence in the judicial procedure with special focus on their privacy, social and ethical aspect.

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Analysis of Allelic Alteration in STR and InDel Markers for Human Identification: the challenge of Tumoral Tissue.

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In the field of forensic genetics, personal identification has been traditionally performed through the analysis of short tandem repeats (STR). However, in the presence of degraded DNA, additional small-sized markers such as insertion/deletion polymorphisms (InDels) have been introduced. In some cases of deficient kinship test, there may be a need to use tumor samples. In these circumstances, it is essential to consider the genomic instability that characterizes such samples and how this may impact forensic genetic analyses. To this end, the present study analyzed and compared samples of healthy and tumor tissue of different origins (hepatic, gastric, breast and colorectal) from 66 patients. Genomic instability was assessed at three levels: partial loss of heterozygosity (pLOH), complete loss of heterozygosity (cLOH), and miscosatellite instability (MSI). Two kits were used specifically to amplify STR and InDel, respectively, AmpFℓSTR® NGM SelectTM and InDelPlex Kit. In the case of STRs, colorectal cancer has emerged as the most genetically unstable tumor type and some polymorphisms (D18S51, FGA and Se33) have been found to be particularly susceptible to these events. As regards the InDels, a high level of genomic instability was observed such that 92.4% of the samples were characterized by at least one mutation, without any significant difference based on the tumor origin. These mutations are equally distributed on the different markers with the exception of only one polymorphism which does not present any difference in tumor tissue compared to healthy tissue. This study therefore highlights the importance of using STRs when working with tissues of tumor origin, even if these appear degraded, as STRs were found to be less affected by mutational events capable of compromising the genotyping attribution. Indeed, in conclusion for STR only the 6% of the samples were affected by this type of mutations compared to the 71.2% of samples for Indels.

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Shaping the French scientific police genetic units to deal with massive analyses

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The FNAEG is the French police DNA database which stores different kind of genetic profiles. Among them are those obtained from individuals involved in criminal acts and those obtained from trace evidences collected in crime scenes. This DNA database was originally built to identify individuals involved in sexual-assault cases. However since 2003, the spectrum of infractions that requiring a genetic registration to the FNAEG has been dramatically extended by French laws. Nowadays, even low impact criminal acts such as burglaries (more than 290 000 cases in 2021 in France) are considered for genetic investigations. Therefore, since 2003, the genetic units of the French scientific police laboratories have faced a massive increase of samples to process, whether from individuals and from evidences collected on crime scenes.

To deal with this massive amount of samples, two highly automated genetic units have been created in the laboratory of Lyon.
The SI unit is dedicated to the genotyping of samples collected from individuals and deposited on FTA® cards. FTA punches are submitted to direct multiplex PCR followed by the separation of DNA fragments using 24-capillary genetic analyzers.
The SDM unit is dedicated to the treatment of low-impact criminal cases. Samples collected from trace evidences are submitted to magnetic beads DNA extraction and purification. Recovered DNA is quantified and normalized using Real time quantitative PCR. 21 STRs of interest are then amplified using multiplex PCR. Finally, DNA fragments are separated using a 24-capillary genetic analyzer.
In both units, data are processed using GeneMapperTM software. To ensure optimized data delivery, the generation of analysis reports is also mainly automatized and validated genetic profiles are sent to the FNAEG every 2 hours through electronic transmission.

These two genetic units harbor strong performances. In 2023, the genetic profiles of more than 221 000 individuals have been established and registered to the FNAEG by the SI unit. In the same time, more than 42 000 low-impact criminal cases were investigated by the SDM unit, which involves the processing of more than 77 000 samples.

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Single nucleotide polymorphism–short tandem repeats analysis system for Japanese people

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Objectives: In forensic practice, human identification is generally performed by capillary electrophoresis targeting short tandem repeats (STRs). As an alternative, a single nucleotide polymorphism (SNP)-STR system has been reported that uses an amplification refractory mutation system to identify STRs and adjacent SNPs simultaneously [1,2]. This system has better discriminatory power than the traditional system and advantageously does not require expensive massively parallel sequencing equipment [2]. We aimed to develop a specific SNP-STR system for common SNPs in Japanese people.

Materials and methods: From a dataset of Japanese people [3], nine SNP-STR loci were selected as targets. Two fluorophore labeled allele-specific SNP primers (wild-type and mutant) and one reverse (or forward) primer were designed according to a previous report [1]. DNA extracts from the blood of volunteers with known SNP-STR types were used as test samples. Multiplex PCR was performed and electrophoresis was carried out using a genetic analyzer (SeqStudio, Thermo Fisher Scientific). Analysis was performed using Gene Mapper ID-X (version 1.6, Thermo Fisher Scientific).
Results and conclusions: Seven out of nine loci were accurately genotyped. Wild-type alleles were detected from both primer sets at rs25768-D5S818, and mutant alleles were not detected at rs149466976-D3S4529. Adjustment of the primer concentration or PCR conditions was needed to establish this SNP-STR system. We plan to continue development to improve the accuracy of DNA typing.

References: [1] Li Wang et al. Forensic Sci. Int. Genet. Suppl. Ser. 2013; 4 e206. [2] Jian H et al. Front Genet. 2021; 12:636821. [3] Ohuchi et al. Leg Med (Tokyo). 2022; 59:102151.

P-228

Testing forensic efficacy of most recent available asSTRs and Y-STR markers in Indian population

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Multiplex kits are made available after published forensic validation as per SWGDAM guidelines for the purpose of forensic DNA examination. Available various multiplex kits are used for the purpose of identification in criminal cases and also in population based genetic studies. Prior to their use these kits are not tested specifically on the populations on which the kits are being used. An attempt is made to test the most recent asSTRs (new CODIS) and Y-STR (23 or more loci) multiplex systems on Indian population to test their forensic efficacy and forensic evaluation and to decipher best set of asSTR and Y-STR markers in Indian population.

Keywords: Forensic DNA; population; efficacy; CODIS marker; asSTRs; Y-STR ; marker set; India

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A multiplex amplification system for methylation age prediction based on droplet digital PCR

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Objetives：Age estimation has always been a crucial aspect of forensic research, with DNA methylation level being acknowledged as a more precise method for age prediction. Given the variations in methylation levels across different body fluid samples, most existing age estimation models are based on a single sample. However, considering the diversity of body fluid samples at forensic sites, it is crucial to develop a universal age estimation model for forensic field body fluid samples. In addition, the droplet digital PCR (ddPCR) can measure DNA methylation levels more quickly and accurately, and has great application prospects in forensic trace and degraded samples.
Material and methods：In this study, three CpGs located on the ELOLVL2, KLF14, and FHL2 genes were screened for high correlation with age in various body fluids. Two multiplex amplification systems based on ddPCR were established for age estimation. The double compound and quadruplex amplification systems are age estimation models constructed based on the methylation levels of 127 and 101 blood samples, respectively. The two systems constructed three age estimation models (MLR, SVR, and RF). After the best model was selected, the performance of the model was evaluated and the sensitivity was verified using real saliva samples and real blood samples.

Results and conclusions：Based on the double compound amplification system, the RF model is identified as the best performer (MAD=2.88 years, R2=0.98, and RMSE=1.82 years). The MAD for saliva samples was determined to be 2.5 years and for bloodstain samples, it was found to be 2.2 years. The optimal age estimation model using the quadruple duplication amplification system was also RF (MAD=3.04 years, R2=0.98, and RMSE=1.78 years). MAD was 2.65 and 1.93 years in saliva and bloodstain samples, respectively. Both composite amplification systems demonstrated their capability in achieving accurate age inference even when subjected to bisulfite conversion at DNA concentrations as low as 0.5ng DNA, MAD was 2.61.

In conclusion, the multiplex multiplex amplification system constructed in this study can quantify the methylation level of three CpG sites simultaneously, to realize the age prediction with a single reaction, which is an innovative method. The age estimation model has high accuracy and stability and applies to various sample types, such as blood, saliva, and blood stains. This model is more suitable for situations where the number of forensic samples is limited. Compared with single target amplification, it saves time and biological materials while improving experimental efficiency and data reliability.

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Small RNA Sequencing Reveals Age-Associated miRNAs in Human Blood Samples

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Age estimation is a critical aspect of forensic genetics, essential for individual identification and case investigation. Traditional anthropological methods, such as teeth and bones analysis, may present margins of error. Among contemporary molecular markers, DNA methylation stands out as the most accurate for age estimation, however, the practical application is constrained by stringent requirements for sample quality. Up to date, there remains a lack of standardized method for age determination. Recent studies have explored miRNA variations during the developmental stages of various species, indicating the potential for studies related to human aging. Nevertheless, the related studies remain under-researched and merits further exploration. In this study, we aimed to acquire the miRNA expression profile using small RNA sequencing of blood samples, screen for age-related miRNAs, and subsequently establish age prediction models using the machine learning methods.
A total of 1422 miRNAs were detected through high-throughput sequencing, and 31 miRNAs showed differential expression in the group-to-group comparisons every five years. To find more age-associated miRNAs, we conducted additional pearson correlation analysis and linear regression analysis, resulting in 85 highly correlated miRNAs. Five predictive algorithms were applied to establish the model, and the random forest regression (RFR) performed the best, with mean absolute error (MAE) of 5.79 years and a root mean square error (RMSE) of 7.12 years. In conclusion, our research introduced a new model for age estimation based on blood miRNAs, which may holds promise for routine forensic applications.

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Investigation into the bacteria from the skin and touched substrate for personal identification

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Objective: Microbiome has been an emerging field in forensic science whose studies include personal identification. The human skin has many microbiotas that have been proven to be influenced by many individual factors, which can easily transfer to surfaces, and this microbial exchange between humans and their surroundings could aid in identifying individuals in criminal investigations by recovering specific bacteria from various settings. With the pilot study, we want to explore whether the skin bacteria from humans and the transferred bacteria from touched objects can successfully match or not.

Material and methods: We recover the skin bacteria from the participants' whole palm and the knife that is held by participants and explore the skin bacterial community composition via sequencing of the 16S rRNA gene of V3-V4 and V1-V9 region. We performed a comparative analysis of the bacterial profiles of swabs from palms and knife handles, and analyzed differences in the effectiveness of the 16S rRNA gene of V3-V4 and V1-V9 regions.

Results and conclusions: According to the results of taxa composition, sequencing on the 16S rRNA gene V1-V9 region gets more taxa at the species level than that on the V3-V4 region. The principal coordinate analysis based on the whole bacterial community composition shows it mainly clusters by the sampling site (palm and surface of knife handles). We will analyze the concrete taxa at the genus and species level to find some individual special taxa that can aid in matching the palm and the knife handle. This study can observe the different effectiveness of 16S rRNA gene V3-V4 and V1-V9 regions for personal identification and the dissimilarity and similarity of bacteria between the skin and touched substrate that can be used to link a suspect to a crime scene.

P-269

Exploration of the Application Value of Oral and Intestinal Metagenomics in Occupational Prediction: Taking Teachers and Construction Workers as Examples

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Objective: Accurately inferring the occupational category of individuals based on forensic evidence can help narrow down the scope of investigation and improve efficiency. Whether occupation, as a complex individual characteristic, affects the composition of the human microbiome has not been reported. Material and methods: This study collected fecal and saliva samples from 100 teachers in Hebei province universities and 100 construction workers, and analyzed the differences in microbial community diversity and functional activity between the two occupational groups using metagenomic sequencing. Recursive feature elimination combined with machine learning algorithms was used to construct a predictive model for occupational category. A detailed questionnaire survey was conducted to record personal information, living habits, and dietary patterns of the participants, and permutation multivariate analysis of variance was used to quantify the impact of individual characteristics on the oral and intestinal microbiomes. Results: Brushing frequency, breakfast habits, dietary patterns, and sleep time had a greater impact on the oral microbiome than the gut microbiome, while milk consumption habits, educational level, drinking frequency, BMI, and occupation had a greater impact on the gut microbiome than the oral microbiome. Smoking had an equal impact on the composition of both the oral and gut microbiomes. The study successfully developed 12 models that accurately distinguished between the two occupational groups, with the best accuracy rates of 100% and 88.33% for the training and test sets, respectively. Conclusion: Microbial species and functional genes have the potential to serve as valuable biomarkers in the field of population information forensics. Recursive feature elimination can help filter out less important features, thereby establishing a distinction model with the fewest features and the highest accuracy. This study not only deepens our understanding of the relationship between occupational activities and microbial community structure but also provides a scientific basis for the development of occupation classification tools based on the microbiome.

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Forensic study of environmental and human skin microbiome differences in college dormitory based on 16S rRNA

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Objective: This study aims to screen microbial biomarkers in university dormitory through 16S rRNA sequencing technology, mainly exploring the forensic ability of individual microbiome matching with dormitory microbiome and expanding the potential of microbial characteristics in forensic science research and application. Material and methods: A total of 128 samples were collected from skin swab samples of 26 healthy unrelated student individuals, as well as from dormitory items and environment swab samples. Use TGuide S96 Magnetic Soil/Stool DNA Kit to extract microbial DNA. The 16S rRNA V3-V4 regions of dormitory microbiota were sequenced by Illumina sequencing platform. Results: The α diversity analysis showed that there were significant differences in microbiome among the six dormitory groups, and the female dormitory group was significantly higher than the male dormitory group, N6(Room for 6 people) ＞ N4(Room for 4 people)＞ N3(Room for 3 people). The β Diversity analysis also showed significant differences in the overall microbiome between different groups. According to SourceTracker, hand-associated environmental surface microbiota, such as door handle and light switch buttons, exhibited a higher degree of similarity with those found on the palms of dormitory residents. Conversely, floor microbiota played a more significant role in shaping the microbial composition of shoe soles. A random forest prediction model was constructed to predict the gender, number of residents and dormitory by using the dormitory environmental microbiome. The prediction accuracy of gender was more than 92%, the prediction accuracy of number was more than 78%, and the prediction accuracy of dormitory was more than 83%. Conclusions: This study analyzed the microbial composition of six dormitory environment and the skin microbiota of the residents, and found significant differences in the microbiomes based on gender, number of occupants, and dormitory. Based on these findings, we constructed a random forest model for predicting sex, number of occupants and dormitory. The findings demonstrate a clear correlation between the human microbiota and the residential environment, suggesting that the microbiota holds potential for geographical location tracing, thereby offering novel insights of the research and application for forensic microbiology analysis.
Keywords: Environmental microbiota, Skin microbiota, University dormitories, 16S rRNA, Forensic science

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Performance Comparison of Two RapidHIT™ ID Cartridge Types for Forensic Leads

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Prompt identification of crime suspects via DNA profiling allows swift suspect apprehension while they are still within the jurisdiction. The ability to generate quick forensic leads by DNA analysis is augmented by the development of rapid DNA platforms, such as the RapidHIT™ ID system. This system requires about 100 minutes for DNA analysis from sample input to data generation. The system has two types of sample processing cartridges. The RapidHIT™ ID ACE GlobalFiler™ Express cartridge is designed for DNA-rich, single-source samples. The newly-launched RapidINTEL™ Plus cartridge is designed to process crime samples with two additional PCR cycles and reduced amount of lysis solution. Studies on the performance of the new cartridge type is limited. This study therefore aims to compare the performance of the two RapidHIT™ cartridge types with various sample input amounts. Mock casework samples with blood and saliva were prepared. To allow better control of sample input amount, a sub-sampling procedure was evaluated in this study. This procedure involved brushing a new swab against the casework swab for varied number of times to collect different amounts of blood or saliva. The new swabs were then subjected to RapidHIT™ processing. Sub-sampling of biological materials from the casework swab can help prevent sample overloading for RapidHIT™ processing and conserves the remaining material for the standard analysis workflow. STR profiles from the RapidHIT™ ID system were assessed for result repeatability, peak height, peak height ratio, artefact occurrence and concordance with reference profiles. Results from this study can: i) demonstrate the performance of the RapidINTEL™ Plus cartridge on blood and saliva-based samples; ii) provide a direct comparison of the performance of the two RapidHIT™ cartridge types with the same samples; and iii) help RapidHIT™ users optimize the sub-sampling procedure given certain sample types and avoid sample overloading.

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Revolutionizing Forensic DNA Analysis: The PowerPlex® 18E System

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Forensic laboratories across Europe, faced with the challenge of analyzing complex and degraded samples, require robust solutions that can deliver precise and reliable results. The PowerPlex® 18E System addresses these needs by incorporating all European Network of Forensic Science Institutes (ENFSI)-recommended loci, along with Amelogenin and DYS391 for sex determination in a single 8-color STR multiplex kit.

One of the standout features of the PowerPlex® 18E System is the inclusion of all the ENFSI loci as mini-STRs (less than 325bp) in a 8-color format. This mini-STR design enhances the system's ability to process degraded and inhibited samples, outperforming existing 5- and 6-color STR multiplexes. Furthermore, the system includes a redesigned SE33 locus, achieving the smallest amplicon size available on the market, which significantly improves amplification efficiency for this highly discriminatory locus.

The PowerPlex® 18E System is rapid and reliable producing results in approximately one hour and is designed for use on the Spectrum and Spectrum Compact CE Systems,. These features, coupled with the inclusion of two quality indicators (QIS and QIL), facilitate the identification of poor-quality profiles due to degradation, inhibition, or absence of human DNA, ensuring the reliability of results.

This presentation will explore the results of the developmental validation study of the PowerPlex® 18E System, highlighting its innovative features, performance advantages, and potential to transform forensic DNA analysis.

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Can human DNA aid in the investigation of animal abuse?

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Human DNA profiling is frequently used due to its ability to inculpate and exculpate individuals in criminal investigations and legal proceedings. However, it is rarely considered or used in animal abuse cases. Recently, it was shown that companion dogs and cats harbour human DNA, picked up from within their environment, and that this DNA is readily transferred to and from animals during contact.
Here we investigate the prevalence and origin of human DNA collected from areas on companion dogs that are known to be contacted during abuse situations (tail, legs and neck), but rarely during normal interactions.
Samples were collected from the tails, legs and necks of 15 dogs following three different situations: a) no contact (by sampling an area mirroring or adjacent to those sampled in situations b and c); b) after owner contact with the tail, neck and back, mimicking an abuse by a household member and c) after an unknown person contact with the tail, neck and back, mimicking an abuse by a non-household member.
The results of the study show that animals can serve as reservoirs of human DNA and that particular areas can be targeted for sampling in criminal investigations to provide probative information when animal abuse is suspected by either an owner or a person unknown to an animal. The outcome of this study demonstrates the potential utility of sampling for human DNA from dogs, and other animals, to assist criminal investigations of animal abuse.

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Bidirectional Transfer of DNA in Controlled Experimental Settings: Providing New Insights into Transfer Events

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Advancements in DNA technologies, including increased sensitivity of detection and complex mixture interpretation, has increasingly shifted the questions relating to DNA evidence from identity to mode of deposition. DNA transfer, persistence, prevalence and recovery research aims to answer questions about how DNA came to be present (or absent) on a surface. Extensive research has been reported on transfer of DNA in one direction, however there is a gap in knowledge surrounding the effect of background DNA and factors affecting bidirectional DNA transfer in controlled conditions.
To address this knowledge gap, experiments were carried out which investigated the effects of biological material, surface type and type of contact to a surface, by placing DNA on both starting surfaces and measuring the levels of bi-directional transfer. To achieve this, touch or blood samples were deposited on two substrates, either cotton or plastic, and these substrates, both with biological material, were placed together (touch to touch, or touch to blood) for 60 seconds, under passive or friction contact.
This study investigates the effect of DNA being on both starting surfaces and compares that to matched studies where only one surface was dosed with DNA. It also investigates the dependence between the two simultaneous DNA transfers, which is essential information (currently lacking in literature) for proper modelling of DNA transfer events in some activity level evaluation scenarios.

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Illicit Drug Distribution: Evaluation of DNA Transfer between Ziplock Bags and Capsules

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The damage caused by the illicit drug trade on individuals is profound and widespread. Clandestine laboratories are notoriously filthy and unhygienic, leaving ample opportunity for DNA contamination of the drugs, capsules, and their packaging. The ability to obtain biological information from drugs and related items can aid in forensic investigations and intelligence efforts.

To evaluate the transfer of DNA between individuals involved in making, packing, and transporting capsules within ziplock bags two pathways were analysed with ten ziplock bags each. A two-person chain was created where person A made and packaged the capsules with person C then carrying the bags. To be more realistic, a three-person chain was also devised where person A made the capsules and placed them in a container, person B placed the capsules in bags, and person C carried the bags. The ziplock bags were opened and sampled on the inside, the inner portion of the opening, and the outside surface for DNA. The exterior of capsules yielded informative profiles and there was evidence of secondary transfer of DNA from the capsules onto the ziplock bags. DNA profiles were also obtained from swabbing the bottom of a container used to store capsules for three days after making, but before packaging, again resulting from secondary DNA transfer.

Analysis of different locations within a ziplock bag yielded significantly different results not only relating to the concentration of DNA present but also who's DNA is recovered. The outside of ziplock bags yielded complex mixtures, however, the inside of the bag and the exterior of capsules had greater protection, yielding profiles with only one to two contributors. This highlights that the inside of the bags and exterior of capsules could be targeted to identify individuals involved in the early packaging stages of the illicit drug pathway.

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Relevant sampling areas on firearms for the reconstruction of shooting scenario involving two individuals

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DNA traces recovered from firearms can provide valuable information for identifying individuals involved in firearm-related events. However, there is a scarcity of empirical data on transfer, prevalence, persistence, and recovery of touch DNA traces from firearms under realistic case-work conditions. This study consisted of the experimental replication of a real case. During a fight between two individuals, one person was holding his loaded gun. The second person (Occasional Shooter) took the gun and allegedly fired two shots. The gun was dropped to the ground, where it was recovered. The study aimed to determine which sections of a handgun's surface could yield informative results for identifying the Occasional Shooter, provided they were the last handler. Seven semi-automatic handguns were provided by their owners. The surface of each handgun was divided into ten sampling areas and traces were collected in two phases. Phase A investigated the amount and composition of traces recovered from guns handled solely by their owners. Phase B took place at a nationally accredited shooting range and involved simulating the shooting scenario. Contact traces were recovered by double swabbing and extracted using the phenol-chloroform method. Eluates were quantified with the PowerQuant® System (Promega) and amplified with the PowerPlex® ESX17 Fast (Promega). Statistical calculations were performed using EuroForMix and R. The traces' quantitative and qualitative characteristics, including DNA yield, relative mixture proportion, likelihood ratio, profile completeness, were evaluated per each sampling area. The study identified the most informative areas for successfully retrieving the individual who loaded the gun and the Occasional Shooter in a shooting scenario. It also described the prevalence of the owner's self and non-self DNA on the guns, as well as the background DNA composition. Secondary DNA transfer events from the gun owner's close associates were observed.

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When secondary DNA transfer is crucial to solving the case

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Our laboratory was asked to analyse the swimsuit bottoms of a six-year-old child who reported being sexually assaulted by an entertainer working at the campsite where she was on holiday with her parents. The man had requested that the little girl follow him into his room, where he licked her genitals and attempted to kiss her, before she managed to run away and seek refuge with her father in their bungalow. Upon hearing his daughter's report, the father immediately contacted the police, who accompanied her to the emergency room (ER). The emergency room staff conducted routine medical examinations, but refrained from taking biological samples from the child's body because, according to the child, there was no evidence of penetration. Fortunately, the child's swimsuit bottom was secured as evidence and sent to our laboratory.

We sampled seven different areas of the swimsuit bottom by cutting. Saliva was detected with the RSID™ Saliva Kit (Indipendent Forensics) on three of the sections, which were located on the central, inner tassel. The cuttings were extracted with the organic phenol/chloroform method, quantified with the PowerQuant® System (Promega), and amplified with the PowerPlex® Fusion 5C (Promega). DNA analysis of the samples revealed a mixed trace, consisting of the child's and the perpetrator's genetic profiles.

In this case, the secondary transfer of saliva traces from the child's skin to the swimsuit bottom compensated for the failure of the emergency staff to collect biological samples from the child's external genitalia and led to the identification of the camping entertainer as the perpetrator.

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Investigating Touch DNA Success Rates in Vehicle Sites for Hit-and-Run Casework

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This study investigated Touch DNA success rates across four vehicle sites—Steering Wheel (SW), Gear Shift (GS), Interior Door Handle (IDH), and Exterior Door Handle (EDH)—for hit-and-run casework. A total of 1,769 samples from 359 vehicles involved in incidents spanning 2020-2023 were collected and analyzed. Significant variations in Touch DNA quantity and quality were observed across sampling sites ($p < 0.05$). The Steering Wheel yielded the highest success rates, followed by the Gear Shift. Conversely, the Exterior Door Handle exhibited the lowest efficacy, with the Interior Door Handle closely behind. The study underscores the importance of strategic sampling in forensic investigations and highlights the potential of Touch DNA analysis in hit-and-run cases. Findings suggest the Steering Wheel and Gear Shift as primary sites for DNA recovery. Future research should explore alternative collection methods and investigate potential contamination sources to enhance forensic practices in hit-and-run investigations.

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Transfer of DNA during a slap and a punch of a face

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Touch DNA is often collected to assist investigations of alleged criminal activities, often from surfaces contacted by hands. While most hand contacts of interest relate to the palmar side of a hand, there are some where the dorsal side is of relevance, such as during investigation of a coward's punch, also known as a king hit or one-punch attack.

There is awareness of the quantities of DNA, the proportional presence of self versus non-self DNA, and the origin of non-self DNA, present on the palmar side of hands, and what is transferred from and to the palmar side of hands when contacting a surface. However, there is a lack of this information in respect to the dorsal side. There is also a general lack of information regarding skin-to-skin DNA transfer.

A preliminary investigation was undertaken of the quantity of DNA, and composition of the profiles generated, from samples taken from four distinct areas of the palmer side of hands and four distinct mirror areas of the dorsal side of hands. Mock assaults were also performed during which a person grabbed the biceps of a second and third person sequentially. The first person then firmly contacted both sides of a fourth person's face with a clenched fist on one side and an open palm on the other Samples were then taken from each of the 8 areas of the hands of the 'offender', both sides of the 'victim's' face, and the grabbed areas of arms, to assess the detectability of direct, indirect, and bidirectional transfer during the activity performed.

We found that there is variation in the composition of DNA present in different areas of the palmar and dorsal sides of a hand, and that detectable levels of DNA were transferred during a grab of an arm, a slap to a face and a punch to a face. The information gained may assist investigators of such alleged skin-to-skin assaults, in respect to sample targeting and activity level evaluations.

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Elucidating the dynamics of trace DNA evidence in pickpocket scenarios

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Trace DNA is latent DNA found on an object or individual deposited through direct or indirect contact. This type of evidence often contains low amounts of DNA and multiple donors. This study was designed to investigate the dynamics of trace DNA in pickpocket scenarios and the relative contribution of DNA recovered from objects with different substrate-types handled/worn by a primary and secondary user for varying durations.

Three simulations were designed, and nine pairs of male and female participants were recruited to act as the primary or secondary handler/wearer of four objects, which included a pair of sunglasses, credit card, money clip, and wallet. The primary user handled/worn the object for 40 minutes each day for 4 consecutive days and the second user handled/worn the object within 24 hours after the primary user for 1, 3, and 20 minute(s) in three separate simulations. All samples were processed using the standard PCR-to-CE workflow and STR profiles were analyzed using DNAStatistX probabilistic genotyping software for calculation of mixture proportions and likelihood ratio values. Participants were considered a contributor to a sample with an LR of 10^4 or higher supporting inclusion.

Overall, 72 trace samples were collected and analyzed. In 50 out of 72 samples DNA from both first and second user was recovered. Additional unknown contributor DNA was detected in all trace samples and ranged from 2% to 8% across all objects. The relative percent contribution of the secondary user increases as the handling time increases, as reported in previous studies. In addition, the persistence of DNA on an object was also affected by the substrate type of the object as the major contributor tends to be the primary handler for porous substrates and the secondary handler for non-porous substrates.

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DNA TRANSFER IN THE WASHING MACHINE. ANALYSIS WITH DIFFERENT DETERGENTS.

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Introduction: Different studies have shown the existence of DNA transfers in washing machine. Transfer can be direct or through a vector, in which case it will be indirect.
Material and methods: This study aims to analyse the effect of detergents with different pHs on direct and indirect transfer in washing machine washes. For this purpose, blood samples taken intravenously in a tube without anticoagulants from two individuals, a man, and a woman of similar ages, were used. In a 2x2 cm cotton cloth was added to 50l of this blood, which was placed in the washing machine together with a clean cloth with the same characteristics. All the fabrics had previously been irradiated for 48h with UV. All the fabrics were impregnated with blood on the same day. They were left to dry for 48 hours. All the fabrics were impregnated with blood on the same day. They were left to dry for 48 hours. The washing time has been considered as a variable, so T0 corresponded to the first wash and T6 corresponded to the sixth day of washing. A total of 80 washes were performed, 40 with Ariel detergent (pH 7.4) and 40 with Kiriko (pH 8-9), using for both a fast cycle (1 hour) at 60ºC and with a total of 160 tissues, two for each wash (with blood and clean), for a total of 40 tissues per individual. Subsequently, DNA was extracted from the tissues by the FTA BioScience protocol (2022); (Whatman BioScience) and quantified by qPCR (Applied Biosystems™ Quantifiler™ Human DNA Quantification Kit). Finally, the data obtained were analysed with the SPSS v.29 program.

Results and conclusions: Significant differences in DNA concentrations were obtained between the detergents used (Ariel-Kiriko) and between stained and clean fabrics. The absence of quantification results with Ariel allows us to assume that among its components there is some PCR inhibitory molecule. It cannot be determined whether it also has a DNA-degrading effect. With Kiriko detergent, we obtained high DNA concentrations in both fabrics, although much higher in the stained fabrics.

P-290

Investigation into the Effects of Gunshot Residue on STR PCR Inhibition in DNA Testing from Firearms

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In 2021, firearms were implicated in 85.7% of homicide cases, highlighting the critical need for law enforcement to enhance forensic investigative capabilities regarding touch DNA evidence recovered from firearms. Forensic DNA laboratories routinely gather touch DNA from various parts of firearms, such as grips, slide serrations, magazines, triggers, and other components, to facilitate the identification of individuals involved in criminal activities. However, the impact of inhibitory compounds from gunshot residue (GSR) on DNA analysis using modern technologies remains inadequately understood. Firearms-related samples often yield complex DNA profiles, posing challenges for forensic analysts. These profiles frequently manifest as mixtures involving three or more individuals, yet little is known about the potential effects of co-eluting metallic compounds from DNA extraction on polymerase chain reaction (PCR) efficiency and/or inhibition levels. This lack of understanding extends to the physical location on the firearm (grips vs. slide serrations, etc.) and the presence of GSR during DNA collection, which may vary in dosage and inhibit PCR reactions to varying degrees.

Our current study addresses these gaps by examining the varying levels of PCR enhancement and/or inhibition associated with touch DNA collections from firearms. We aim to illustrate how the quantity of GSR-related inhibition varies based on the site of sample collection and the analytical methodology employed for DNA profiling. While advancements in DNA typing technologies have improved sensitivity, these systems are still susceptible to limitations imposed by inhibitory substances co-eluting with DNA during extraction and purification. By identifying and quantifying metallic species from the primer, cartridge cases, bullet construction, and other potential GSR combustion products present in samples collected from firearms, forensic practitioners can refine DNA sampling techniques and biochemical testing methods. This approach aims to enhance the quality and reliability of touch DNA profiles recovered from firearms, thereby aiding law enforcement agencies in criminal investigations.

P-292

DNA Typing from chewing tobacco Naswar

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At a crime scene, blood stains, semen, and other biological traces are found very often, and any of these could be used as evidence. Touched DNA samples play an essential role in forensic casework analysis. They enable analysts to collect and analyse trace amounts of DNA left at the scene of a crime by individuals who might have touched or handled an item involved in the crime. Several factors are associated when analysing touched samples, including low DNA amounts, DNA degradation, and the contribution of DNA from multiple individuals. Generating a good profile from these samples is a great challenge, especially when receiving a challenge rare evidence to collect DNA like chewing tobacco small black balls “Naswar”. The present case shows our approach and the results obtained from the cells collected from Naswar. Cells were collected using Bode SecureSwab 2 (Bode Technology) and extracted via the PrepFiler Express BTA™ Forensic DNA Extraction Kit (ThermoFisher Scientific). The DNA was quantified using Quantifiler™ Trio kit (ThermoFisher Scientific), and the amplification was conducted with GlobalFiler™ IQC PCR Amplification Kit (ThermoFisher Scientific). All samples were successfully typed, and as expected, complete and mixed DNA profiles possibly with two individuals were generated, showing the great possibility of collecting touch DNA from these types of evidence and performing DNA typing.

P-300

Evaluating the effects of environmental insults on presumptive and confirmatory tests and DNA recovery from blood samples

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Presumptive tests are routinely used in forensic casework to establish the likely presence of a particular body fluid such as blood. Confirmatory tests are also used in some situations where the presence of a particular body fluid is important in the context of the case. Whilst there has been an increasing number of research on the sensitivity of different tests, limited data is available to the effectiveness of different presumptive and confirmatory tests following environmental insult.
The impact of the environment was evaluated on presumptive and confirmatory tests in the Emirate of Ras Al Khaimah in the UAE, using blood in a pilot study. Liquid blood samples were deposited on cotton, glass, and metal allowed to dry and exposed to the environment in direct sunlight. They were then collected over a period of 48 days at intervals of 72 hours. Each collected sample was subjected to two presumptive and two confirmatory tests for blood, namely the Kastle-Mayer and Hemastix® (Bayer Diagnostics) presumptive tests and the Hexagon® OBTI (Gesellschaft fur Biochemica und Diagnostic) and the RSID™-Blood (Independent Forensics) confirmatory tests. DNA was extracted using Chelex-100 method and DNA concentrations were measured using the Quantifiler Human kit; samples from selected time points were also profiled using the Identifiler plus PCR Amplification Kit. Both presumptive tests continued to give positive results for the duration of the experiment (1425.5 accumulated degree days (ADD)). On glass and metal the Hexagon OBTI failed to give positive results from day 45 (1357 ADD), while the RSID™-Blood kit gave negative results from day 29 (809 ADD). DNA profiling of blood samples deposited on glass and cloth materials gave full profiles using the Identifiler Plus kit for the duration of the experiment (1425.5 ADD) even when confirmatory tests provide a negative result. Blood samples deposited on metal showed typical signs of degradation such as peak imbalance and allele drop out from day 33 (943 ADD).

False negative confirmatory tests due to environmental insult could lead to misleading decision making and sample analysis prioritization. Similarly, presumptive tests may yield positive results long after any meaningful DNA can be recovered.

P-302

Comparison of single-cell whole genome amplification and direct low volume PCR for detecting STR and mtDNA HV Ⅰ and II region

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Objetives In this study, we investigate the detection rate of STR loci and mtDNA after whole genome amplification (WGA) and direct low volume PCR (LVPCR) without WGA.

Material and methods Oral epithelial cells of 5 volunteers and skin shed cells of 1 volunteer were collected, and the mixed samples of epithelial cells and skin shed cells of 2 volunteers were prepared. 1, 2, 3 and 5 oral epithelial cells were isolated by laser capture microdissection (LCM) method. The whole genome of single oral epithelial cells was amplified using MALBAC (MALBAC whole genome amplification kit, China Ekang Gene), and the STR loci was amplified using GSTARTM 25 kit (Beijing Jinma Shenghe Technology Co., LTD.) with routine 10μl PCR. The amplified products were separated by ABI 3500 genetic analyzer for STR typing. After cell isolation, STR loci were directly amplified by low volume (3μl) (LVPCR) for capillary electrophoresis typing. After WGA of single oral epithelial cells, mitochondrial DNA (mtDNA) hypervariable region I (HV Ⅰ nt16047-16464) and II and (HV II nt29-408) fragments were amplified by PCR, and Sanger sequencing.

Results and conclusions The results showed that the STR loci allele detection rate of 1, 2, 3 and 5 oral epithelial cells after WGA treatment was lower than that of LVPCR method, the STR allele detection rate of single-cell LVPCR method was 99.13%, and the STR allele detection rate of 2, 3 and 5 cells was 100%. The detection rates of STR alleles after WGA were 83.48%, 80.87%, 78.26% and 93.91%, respectively. After WGA of single oral epithelial cells, mitochondrial DNA (mtDNA) hypervariable region I (HV Ⅰ nt16047-16464) and II and (HV II nt29-408) fragments were amplified by PCR, and Sanger sequencing showed that the mtDNA haplotypes detected were consistent with the reference samples. The detection rate of single cell STR detected by LVPCR was higher than that by WGA treated. However, the results of mtDNA detection using single-cell WGA DNA were stable.

P-306

Mitochondrial DNA sequencing for forensic human identification on a sample of the Saudi Arabian Population

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Mitochondrial DNA (mtDNA) has some unique features that make it a particularly useful forensic marker such as having a higher copy number per cell compared to nuclear DNA, these include absence of recombination, maternal inheritance, accumulation of mutations over time and multiple copies per cell.

The creation of regional reference databases is recommended for all forensic loci, but even more important for lineage markers. While there is already a large amount of mtDNA sequence data produced from different global and regional populations, limited mtDNA data exists for the Kingdom of Saudi Arabia, with most previous studies focusing on medical genetics aspects of the population, and so they do not necessarily reflect forensic genetics population studies.

This study investigated the Saudi population from a mitochondrial perspective and aimed to establish a mtDNA forensic database. A total of 305 samples were analysed and they were collected from Saudi nationals. After the samples were collected, the DNA was extracted and quantified. After primer optimization using the chosen sample and primer concentration, samples generated a high quality mtDNA control region dataset. The entire control region (CR) was amplified, sequenced, and analysed by STS using six forward and reverse sequencing primers generated full coverage with overlapping sequences across the entire mtDNA control region, representing 66.2% unique haplotypes and 7.5% shared. Whereas, with MPS of the whole mtDNA of a total of 60 sample, 47% of these mitochondrial types were unique and 19% haplotypes were shared. The polymorphic sites of the whole mtDNA genome MPS were found in the CR with a total of 119 polymorphic sites and 85 in CR sanger analysis, indicating a large number of unique mitochondrial haplotypes, these finding suggest that the high mtDNA, especially in mtDNA control region for forensic identification context. Haplogroups were assigned and showed that J, R0 and H are the major haplogroup with almost half of the population samples then L2, L3 and M. Those major haplogroups were similar to sanger control region sequencing and, the percentage of each of the haplogroup differed. In conclusion, the frequency distribution of mitochondrial haplogroups shows that Saudi Arabia population is genetically related to those population from the Middle East and Africa which were expected due to its geographical location.

P-309

Mitochondrial DNA heteroplasmy patterns in hair shaft samples: Insights from Whole Mitogenome Sequencing using the Precision ID mtDNA Whole Genome Panel

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Mitochondrial DNA (mtDNA) is a robust marker in forensic casework, particularly crucial for forensic casework involving samples containing small amounts of nuclear DNA (nuDNA), such as hair shafts. However, the interpretation of mtDNA faces challenges, notably in the reporting of heteroplasmy. In this study, we aim to identify heteroplasmy patterns in hair shaft samples using whole mitogenomes sequenced with massively parallel sequencing and compare these heteroplasmy patterns with those obtained in reference samples.

Blood and buccal reference samples from 36 unrelated individuals, alongside 317 hair segments (each measuring 2 cm) were included in this study. DNA extractions were performed using in-house pre-treatment protocols followed by DNA extraction with the EZ-1 extraction robot (Qiagen). Quantification of nuDNA in the reference samples was performed using the Quantifiler Trio DNA quantification Kit (ThermoFisher Scientific, TFS), while mtDNA (and nuDNA if present) in hair samples was quantified using the qPCR SD quants assay (Xavier et al., 2019). All quantification experiments were performed on the 7500 Real-Time PCR system (Applied Biosystems). Hair samples with less than 100 mtDNA copies were up-concentrated and re-quantified. The Precision ID mtDNA Whole Genome Panel and the Ion Torrent Ion Gene Studio S5 sequencing platform with Ion 530 chip (both TFS) were used for library preparation and sequencing.

Successful amplification of mtDNA was obtained for all samples, even those with low mtDNA copy numbers. Our study provides insight into the heteroplasmy patterns observed in hair samples, revealing a higher frequency of heteroplasmy events in hair compared to those obtained in reference samples. Furthermore, variations in heteroplasmy patterns exist both within and between hairs from the same individual. These findings advance our understanding of mtDNA dynamics and hold promise to enhance the interpretation and reporting of mtDNA data in forensic contexts.

P-328

Constructing a microbial model for differentiating monozygotic twins using the combination of feature selection and likelihood ratio when there are two suspects

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Objectives: To refine forensic genetic identification for monozygotic twins (MZT), by harnessing the discriminative power of microbial markers, known for their personal specificity and long-term stability. Through the application of a genetic algorithm to pinpoint the most informative microbiota units and the use of likelihood ratios to address the “fall-off-the-cliff effect”, we aim to develop an accurate model to assist in cases involving two MZT suspects for a query sample.

Material and methods: Saliva samples from 10 MZT pairs were collected at four time points (TP1-TP4), with TP2-TP4 collected 12, 13, and 14 months after TP1. After sequencing the 16S rRNA V3–V4 region, microbiota information was obtained. The MZT pairs were randomly divided into training and test sets at an 8:2 ratio. The 8 samples of each MZT pair can form 28 sample pairs, for each of which specific distance parameters were calculated based on different data basis. Twenty-four types of distance distributions were estimated using the Kernel Density Estimation method, considering the relationship between samples (Self or MZT), time intervals (≥1 year or ≤2 months), data basis (Amplicon sequence variants, ASVs or Operational taxonomic unit, OTUs), and distance parameter selection (Jaccard distance, JD, Bray-Curtis distance, BC, or Hellinger distance, HD). Based on these distributions, 12 identification models were constructed and evaluated. Each model utilized 96 groups from the training set, with a pair of MZT samples as suspects (A or B) and another sample from the corresponding MZT pair with a specific time interval with them as the query. Likelihood ratios were calculated for each group, based on the distance distribution estimated for MZT or Self pairs. An AUC-based parameter was calculated to evaluate the identification value of the model. A genetic algorithm process was employed to select high-value ASVs or OTUs and to improve the models’ accuracy.

Results and conclusions: From the 80 saliva samples, 369 OTUs and 1130 ASVs were detected. After the feature selection process, an ASV-JD model and an ASV-HD model can provide best identification value for short-interval and long-interval identifications, respectively, where all query samples’ originating suspects were correctly pointed. Our findings suggested the potential of the microbiota solution to the two-suspect MZT identification problem and highlight the importance of feature selection in improving model performance.

P-330

A strategy for screening high-quality variants from low-depth whole genome sequencing data by bloodstains to discriminate monozygotic twins

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Objetives: Monozygotic (MZ) twins have a high degree of genetic similarity but are not entirely identical. The DNA mutations accumulated after the zygote splitting, which are potential biomarkers for MZ twin discrimination, could be detected by massively parallel sequencing (MPS). However, it's still a formidable challenge to reliably identify authentic discrimination through millions of variants called by pairwise whole genome sequence (WGS) data.

Material and methods: In our study, we selected six pairs of MZ twins in actual cases and sequenced their whole genome at approximately 10× coverage using bloodstains on the MGISEQ-2000 sequencer (PE100 mode). Calling of MZ twins' single-nucleotide variants (SNVs) was performed according to the best practice workflow proposed by GATK. Our strategy to screen for authentic discordant SNVs between MZ twins is based on a simple hypothesis: if we set stricter thresholds in terms of variant quality along a certain gradient, the ratio of the number of discordant SNVs to the total number of SNVs, which we defined as the discordance ratio, will gradually decrease, thereby reserving the authentic SNVs with greater potential for validation.

Results and conclusions: Overall, we found that all six pairs of MZ twins had the minimum discordance ratio when the threshold was set as the depth of SNVs (DP) ≥ 20, frequency of major allele ≥ 0.95 in homozygous, and frequency of minor allele ≥ 0.4 in heterozygous. The average number of discordant SNVs under this threshold is 46. This strategy provides a solution to dynamically screen high-quality variants sets from WGS data for subsequent Sanger sequencing validation. With its use of bloodstains, low-depth WGS, and rapid screening of variants sets, our strategy is suitable for practical application in forensic genetics.

P-332

Improved identification of victims from the Spanish Civil War with NGS technology

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OBJECTIVES
Next-generation sequencing (NGS) technology has offered new opportunities in forensic genetics, providing several advantages over traditional methodologies in terms of: (1) an increased number of loci can be multiplexed and multiple samples can be sequenced simultaneously, (2) higher discrimination power as a consequence of the access to full amplicon sequences (3) shorter amplicons for a more effective analysis of degraded samples, and (4) lesser background noise due to instrumentation. In this study, we assess the applicability of NGS technology for the identification of victims from 2 mass graves of the Spanish Civil War (1936–1939) and Francoism located in Valencia. These mass graves were previously analyzed by capillary electrophoresis (CE), allowing the identification of 30 victims.

MATERIAL AND METHODS
Here, we present the second genetic identification of 102 post-mortem remains and 76 alleged relatives sequenced using the ForenSeqTM DNA Signature Prep Kit. To carry out the kinship analyses, we used the Disaster Victim Identification (DVI) module of the Familias software.

RESULTS AND CONCLUSIONS
Overall, a success of DNA profiling was reached, because informative genetic markers were obtained in 63.28% (combining 27 autosomal STRs and 94 SNPs) of the skeletal remains. Only around 31.37% of the samples did not provide an informative profile (<50% of aSTRs and SNPs), probably due to the presence of degradation and low DNA concentration. Up to now, we have identified 15 victims who were not reported by CE. These identifications were obtained after the comparison with reference samples from relatives: first-degree (4 identifications), second-degree (5), third-degree (5), and second- and third-degree (1). As expected, parent-child relationship was robustly identified compared to other relationships. However, the highest LR value was obtained by the match performed with second- and third-degree relatives (LR= 1.84E+17). The main limitations for the identification of victims from the mass graves are the low number of first-degree living relatives and the high levels of DNA degradation. Despite this, NGS-based genotyping provides numerous advantages that improve the interpretation of challenging or degraded DNA and has the potential to increase the power of discrimination for identification and kinship analyses. We conclude that NGS allows increasing the number of identifications reported previously, even with second- and third-degree relatives, in situations with low sequencing performance and important levels of allelic drop-out.

P-333

Identification of monozygotic twins by microbial polymorphism

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Objectives: Monozygotic twins (MZ) are widely believed to be genetically identical, and traditional DNA typing methods are insufficient to identify MZ twins. Therefore, the identification of MZ twins has become a difficult problem in forensic medicine. Microbial communities vary due to genetic, environmental, lifestyle and other influences, which can be used for forensic personal identification and paternity testing.This study aims to establish a method for analyzing microbial communities to distinguish MZ twins.

Material and methods: Saliva, buccal swab and wrist epidermal swab samples of three pairs of twins are collected, in which 16S rRNA genes of microorganisms are sequenced and analyzed. The V4 region of 16S rRNA is amplified using a universal primer. After sample extraction, the purity and concentration of the collected samples are then tested using Nanodrop. The isolated DNA is frozen at -20℃ and used for microbial sequencing and data analysis.

Results and conclusion: The results show that there are differences in microbial communities and quantity in MZ twin pairs, indicating the role of microorganism in the recognition of MZ twins.

P-334

Exploration of mitochondrial genome sequencing: insights from ATOPlex Kit on pedigrees and twins

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Objetives: Previous studies have shown that compared to focusing solely on control regions, sequencing the mitochondrial genome (mtGenome) increases the individual identification efficiency and improves the accuracy of haplogroup prediction.

Material and methods: The ATOPlex Mitochondrial Full-Length Library Preparation Kit (ATOPlex), based on the sequencing platform of the MGI, is a novel massive parallel sequencing (MPS) panel targeting the mtGenome. 154 pairs of short amplicons spanning 133 ~ 198bp (average 178bp) were designed to cover the mtGenome, with overlap lengths between amplicons ranging 38bp ~ 112bp (average 69bp). Prior to sequencing, the amplicons are randomly enzymatically fragmented into ~ 100bp fragments to increase sequence randomness and improve sequencing accuracy. In this study, targeted sequencing was performed on 2 positive controls DNA (NA12878) and blood samples from a 6-person three-generation matrilineal pedigree and 4 pairs of monozygotic (MZ) twins.

Results and conclusions: The findings indicated an average sequencing depth across all samples of 33303 × ± 21915 ×, with read lengths spanning from 29 to 100bp and on average 79bp ± 13bp. For depths exceeding 300 × and an analysis threshold of 0.05, the homozygous and heteroplasmic mutation sites of the two positive controls exhibited complete consistency. The predominant genotypes were consistent for the six individuals from the same maternal lineage, and three identical point heterozygosities (PHPs) were observed in all six samples. Similarly, the primary genotypes observed in the 4 pairs of MZ twins remained uniform, yielding consistent haplotype predictions, although a discrepancy of 2 PHPs was noted within one pair. In conclusion, the ATOPlex kit can produce reliable whole mitochondrial DNA sequencing results based on blood stain samples, which can be used for maternal kinship testing and discrimination of monozygotic twins in forensic medicine.

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P-343

Performance comparison between a novel pentameric STR panel and well-established STR kits for kinship and mixture resolution

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A novel STR panel, consisting of six highly polymorphic pentameric STRs, was developed to complement routine STRs kits when the discrimination power achieved fails to resolve complex and distant kinship cases. This panel has been used to genotype 302 Iberian samples in order to estimate allele ranges, allele frequencies and forensic informativeness indices for each marker. Kinship cases of different degrees of relationship have been analyzed with this novel STR panel in combination with well-established STR kits, and here we present discrimination power comparisons in terms of the likelihood ratio values obtained.

Another presumed advantage of pentameric STRs is their lower stutter ratio, a feature that could be of great interest when these loci are applied to DNA mixtures analysis. We have compared the stutter ratio obtained with tetrameric and these novel pentameric STR markers, confirming an improvement in the stutter proportions for pentameric repeat loci. Taking together the high level of polymorphism and the reduced stutter ratio, the panel could be highly applicable to mixture analysis, improving the detail of interpretation possible by enhancing the discrimination between the stutter peaks and alleles from a minor component and estimating the number of contributors. Both theoretical and analytical approaches have being carried out to determine and evaluate the performance of the pentameric panel for mixture evaluation.

Finally, the pentameric STR panel has been applied to compromised DNA samples, and we recorded a wide variety of results, depending on the DNA concentration and levels of degradation seen in the test DNA extracts. An optimized protocol is proposed for recovering profiles in these types of degraded or low-quality samples.

P-344

Metabolomics, but not proteomics, efficiently discriminate monozygotic twins in peripheral blood

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Monozygotic (MZ) twins cannot be distinguished using conventional forensic STR typing because they present identical STR genotyping. However, MZ twins do not always live in the same environment and often have different dietary and other lifestyle habits. Metabolic profiles are deyermined by individual characteristics and are also influenced by the environment in which they live. Therefore, they are potential markers that are capable of identifying MZ twins. Moreover, the production of proteins varies from organism to organism and is influenced by both the physiological state of the body and the external environment. Hence, we used metabolomics and proteomics to identify metabolites and proteins in peripheral blood to discriminate MZ twins. We identified 1749 metabolites as known metabolites and 622 proteins in proteomic analysis. The metabolic profiles of four pairs of MZ twins revealed minor differences in intra-MZ twins and major differences in inter-MZ twins. Each pair of MZ twins exhibited distinct characteristics, and four metabolites—methyl picolinate, acesulfame, paraxanthine, and phenylbenzimidazole sulfonic acid—were observed in all four MZ twin pairs. The four differential exogenous metabolites conincidently show that the different external environments and life styles can be well distinguished by metabolites, considering that twins have not all the same eating habits, living environments, etc. Moreover, MZ twins showed different protein profiles in serum but not in whole blood. Thus, our results show that differential metabolites provide the potential biomarkers for the personal identification of MZ twins in forensic medicine.

P-370

Evaluation of three sampling methods for the recovery of touch DNA from metal items

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Objectives: It is well documented that forensic DNA analysis of samples taken from metal surfaces is problematic due to inhibition at various stages along the DNA profiling process. It is therefore of the utmost importance that as much DNA as possible is recovered from the metal surface at the time of sampling. This is especially important when dealing with touch DNA, as only low-levels of DNA are expected to be present in the sampling area. The aim of this study was to evaluate the effectiveness of three different sampling methods on DNA deposited via touch (touch DNA) on six different types of metals.

Material and methods: Three sampling methods a) swabbing with a buffer wetting solution, b) swabbing with a water wetting solution and c) minitaping, were tested for their effectiveness in recovering touch level DNA (diluted control DNA and true touch DNA) from six different metals (lead, copper, brass, bronze, stainless steel, and aluminium). The percentage of the expected alleles detected and the average peak heights (relative fluorescence units) of the alleles detected were used in order to assess the efficiency of each sampling method per metal and over all metal data.
Results and conclusions: The results from the combined data indicated there was no statistical significance in the effectiveness of the sampling methods in relation to the percentage of expected alleles detected. However, when evaluating the average peak heights of the alleles detected for the combined data, buffer and minitapes were determined to be the most effective methods, with water being the lest effective method.

Whilst all three sampling methods were equally effective for the percentage of expected alleles detected, the effectiveness of the average peak height parameter is equally important, as the higher the alleles detected, the more likely it is that a useable DNA profile may be obtained from the sample. Therefore, overall when considering the data from the second round of results, the findings indicate the buffer and minitape methods are more effective when sampling touch DNA from metal surfaces than the water method.

The results from all of the metals combined, specifically relating to true touch DNA, indicate sampling via minitapes or swabbing with a buffer solution are more effective than sampling with water as a wetting solution.

P-371

Comparison of DNA Extraction Methods for Telogen Hair Samples

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Telogen hair samples are valuable sources of DNA for forensic genetics and commonly found during crime scene investigations. The most common marker to genotype in extracts from telogen hair is mitochondrial DNA (mtDNA). With the introduction of shotgun sequencing, small, highly degraded fragments of nuclear DNA (nuDNA) from hair may be used for human identification. Here, we tested the efficacy of three DNA extraction methods designed specifically for telogen hair, and we examined and compared yields of both mtDNA and nuDNA. The methods were commonly used for isolating mtDNA in forensic genetics and ancient DNA research.

Two fragments were analysed from each telogen hair: A 2 cm fragment including the root and a 2 cm fragment immediate distal to the root fragment. The hair fragments were cleaned using an ultrasonic bath and a proteinase solution. Lysis was performed according to the published protocols. The lysis buffer contained varying amounts of Proteinase K and DTT, and the lysis incubation time ranged from 30 minutes to overnight. The extraction methods also varied in the purification step, where the techniques for isolation of DNA were based on either glass fibres or silica attached to magnetic particles or immobilised in a column.

The results confirmed that both mtDNA and nuDNA is present in hair samples. The preliminary results indicated that glass fibres used for DNA isolation increased the yield of DNA, particularly nuDNA. Overall, our study provides valuable insights into DNA extraction methods for telogen hair samples, highlighting the importance of method selection based on the specific research objectives and resource availability.

P-372

DNA Extraction from One Step Urine Drug Screen Test for STR Typing

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Introduction

In scenarios where lateral flow immunoassays (LFIs) serve as the initial method for drug usage screening, there may emerge concerns regarding potential misuse. The ability to identify the sample donor becomes critical in these instances. Short Tandem Repeat (STR)-based DNA profiling, would provide an objective means to either confirm or refute the suspicion of misuse. To date, according to the literature reviewed, there appears to be an absence of research specifically focused on the feasibility of generating STR profiles from LFIs.

Aim

The objective of this study is to ascertain the feasibility of extracting DNA from utilized urine lateral flow immunoassays (LFIs) and subsequently generating Short Tandem Repeat (STR) profiles from these extracts. Furthermore, this research seeks to determine whether the ability to establish an STR profile is influenced by the timing of sample collection.

Methods

We collected anonymous LFIs used for the preliminary screening of drug use, which were used under real conditions and stored at room temperature for various durations. The time interval between the date of sample collection and the date of DNA extraction was recorded. Half of the samples were extracted using the Chelex method, while the other half were extracted using the DNeasy Blood & Tissue Kit from QIAGEN. DNA was amplified by AmpFLSTR NGM Select (Thermo Fisher Scientific). The data collected from the electrophoresis were analyzed using GeneMapper ID (Thermo Fisher) software. The software used for statistical evaluation was SPSS Statistics (IBM).

Results and Conclusions

There is a noticeable gap in both domestic and international literature regarding the establishment of Short Tandem Repeat (STR) profiles from lateral flow immunoassays (LFIs) utilized in the preliminary screening for drug usage. We were able to detect partial profiles from the tests, the Blood and Tissue kit offered a higher recovery rate over the Chelex-100 resin.
Consequently, this research aims to furnish valuable insights into the potential of these assays to serve as an objective tool for confirming or refuting suspicions of misuse that may arise during their application.

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Degradation of DNA Extracts Stored Under Different Conditions: What We Know and What Is New

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Preserving the integrity of forensic evidence through the stabilisation of the biological signature contained in DNA extracts is particularly important, specifically when re-analysis samples after a certain period of time. This translates into the needs for appropriate storage of DNA extracts to ensure the successful outcome of forensic genetic analyses, including human identification through STR typing and the application of phenotype and ancestry panels to infer the morphological characteristics and geographical origin of the contributor. Although guidelines shared by the international scientific community recommend freezing of the DNA extracts for long-term storage, it is possible that samples are preserved in sub-optimal conditions for a variety of reasons (e.g., faulty freezers, moving samples to court office at the case closure). In these circumstances, little is known about the survival of DNA and its suitability for future STR and SNP analyses.

In this study, we evaluated the preservation of DNA extracts obtained from buccal swabs collected from two subjects of different sexes using the QIAamp DNA Investigator Kit (Qiagen). The extracts were quantified (Quantifiler™ Trio DNA Quantification Kit – Thermo Fisher Scientific) and aliquots containing 1 ng/µl of DNA were created. In addition, mixtures were set up from the female and male DNA extracts, in a ratio of 1:15 (male/female. All the aliquots were stored at three temperatures (+20°C, +4°C and -20°C) for a period of 90 days. As the results showed a surprising DNA survival rate also at room temperature, revealing similar completeness of the STR profile (GlobalFiler™ PCR Amplification Kit – Thermo Fisher Scientific) at both -20°C and +20°C conditions, the experiment was further extended to 400 days. In addition, after the initial 90 days experiment, a subset of samples was moved to uncontrolled temperature conditions. Surprisingly, DNA extracts did not degrade up to 400 days under any condition, and the ratio in the mixed samples remains unchanged. It was also noted that storing the extracts at +4°C and +20°C causes their evaporation and consequent concentration up to 20-fold. Overall, these results could open new avenues for the analysis of samples from cold cases not always preserved frozen.

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From dactyloscopy to the Minimum Surface Requirement (MSR). A preliminary study to assess DNA genotyping from fingermarks.

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Maximizing the quantity and quality of genetic information that can be obtained from fingermarks has become one of the primary objectives in criminal cases. On certain occasions, friction ridge impressions may hold little discriminatory power due to their low quality or the insufficient area extension of such patterns. In these instances, an appropriate human DNA collection and a high-quality DNA extraction could become key steps to yield a genetic identity from an unsuitable fingermark pattern. To date, no research has yet explored the (potential) quantity of DNA that could be isolated per area extension of a ridge pattern. In the current study, the authors have standardized the fingermark deposition process and DNA collection methods to establish the Minimum ridge Surface extension Requirement (MSR) necessary to obtain a meaningful human short tandem repeats (STR) profile. The study involved the comparison of two fingermark “recharging-deposition-collecting” methods: the original experiment (OE) and an improved version (IE) in which two swabs for DNA collection with distinct structural and physical characteristics were evaluated: cotton swabs from Delta Lab (Spain) and 4N6FLOQSwabs® from Copan (Italy). After standardizing the fingermark deposition process, participants were instructed to wash their hands and then allowed to naturally "recharge" while applying gentle finger, neck, and forehead tapping to homogenize skin and sweat compounds. The index, middle, and ring fingers from each hand were imprinted onto a glass surface through a predesigned cutout mold. This was followed by DNA extraction (DNeasy Blood & Tissue kit, Qiagen) and quantification (Quantifiler™ Human, ThermoFisher Scientific). The DNA concentration per cm2 of deposited fingermark was calculated before the amplification step with AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit on a VeritiPro™ Thermal Cycler (ThermoFisher Scientific). Samples were then genotyped using capillary electrophoresis, with STR analysis performed using GeneMapper software (AppliedBiosystems). The OE revealed that DNA extraction from flocked swabs was more effective than cotton swabs. Indeed, most STR profiles obtained from cotton swabs were not suitable for forensic purposes and displayed several deficiencies that were less commonly detected when using 4N6FLOQSwabs®. Subsequently, the collection method was improved using only the 4N6FLOQSwabs®. IE’s results indicated an increase in human DNA quantities through the combination of some improvements in the collection method (i.e., washing hands, recharging time, and collection device), resulting in the isolation of meaningful STR profiles.

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Effects of Fingerprint Enhancement Techniques on Forensic DNA Recovery

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Latent fingerprints are common traces found at crime scenes and typically form an essential part of the evidential materials gathered. Analysis of fingerprints is an important tool in criminal investigations, which often requires the application of enhancement techniques. Meanwhile, advancements in the sensitivity of DNA recovery have enabled the possibility of obtaining complete DNA profiles from the few cells left behind with fingerprint deposits. Therefore, to maximise evidence recovery, DNA recovery techniques can also be performed after visualisation and recording of fingerprint details. However, there are concerns about potential interference of fingerprint enhancement processes on subsequent DNA analysis, including extraction, amplification, and profiling. In this study, experiments were conducted to assess the risk of interference from various fingerprint enhancement techniques on DNA recovery. The effects of these techniques on trace DNA recovery were examined on commonly encountered articles in casework, such as paper, acrylic, plastic bag, duct tape, cellophane tape, and glass. We have compared the quantity of extracted DNA and the quality of obtained DNA profiles from treated fingerprint samples with the control. Our results showed that the application of DFO and ninhydrin on the paper strongly interfered with DNA recovery, and we have observed an average 76% decrease in extracted DNA quantity and a 59% decrease in percentage profiles after fingerprint enhancement. In contrast, other examined fingerprint enhancement techniques did not significantly affect DNA recovery from the corresponding articles, including the application of gentian violet on the adhesive side of the duct tape, magnetic powder on the acrylic, and cyanoacrylate fuming and fluorescent dye on the plastic bag. In conclusion, this study demonstrates that fingerprints developed by various techniques can be successfully utilized for DNA analysis. It also provides guidance for crime investigators to select fingerprint enhancement processes that are compatible with DNA recovery whenever possible.

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Unveiling MSR: The Genesis of a Device for the Assessment of Fingermarks and their Genetic Identification

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The identification of individuals through the extraction of DNA from fingermarks (DFMs) has represented a significant breakthrough in forensic science, enabling the resolution of crime cases that would have been otherwise unsolved. Often, the genetic discriminatory power of friction ridge impressions is limited due to insufficient extension of ridge areas. Therefore, comprehensive knowledge of the minimum surface necessary for a successful DFMs identification and the proper selection of DNA collection methods at crime scenes are essential for obtaining genetic identities from latent fingermark patterns. The main objective of this preliminary study is to determine the Minimum ridge Surface extension Requirement (MSR) necessary to achieve meaningful genotypes from human Short Tandem Repeat (STR). To accomplish this, a standardized fingermark deposition template or mold was developed. Ten participants, comprising both biological sexes, provided ink prints of their index, middle, and ring fingers from both hands to establish an average finger size for analysis. Three pressure ranges were applied: low (180-220 g), middle (480-520 g) and high (780-1000 g). These impressions were measured manually and digitally using PhotoshopTM. A statistical analysis revealed consistency between the two methods. An average of each finger area was calculated, facilitating the design of the template. Then, the template was superimposed to a non-porous surface (glass) to test if each of the six fingers' templates (three fingers from each hand) was suitable for obtaining STR profiles. For this, twelve volunteers were asked to follow a washing-recharging-deposition protocol for DFMs collection using the 4N6FLOQSwabs® from Copan (Italy). DNA extraction was conducted using the QIAamp® DNA Blood Mini kit (Qiagen) following manufacturer's instructions. After, DNA quantification was run on a 7500 Real-Time PCR System (AppliedBiosystems) with the Quantifiler™Human DNA Quantification kit (ThermoFisher Scientific). DNA amplification was completed with Identifiler™ Plus (ThermoFisher Scientific). The amplified samples were processed with capillary electrophoresis, and STRs profiles analyzed by GeneMapper ID 3.2 software (AppliedBiosystems). This preliminary study successfully yielded reliable human STR genotypes which was the primary mission, marking the initial step in a new investigative path aimed at determining the minimum surface area required for obtaining meaningful STR genotypes.

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Microhaplotypes: from exploration to application in forensic genetics

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The usefulness and varied applications of microhaplotyes (microhaps or MHs) in forensic genetics are no longer under question. Indeed, in the last ten years several studies reported that MHs are highly informative for identification of individuals, testing biokinship, typing degraded DNA, inference of ancestry, and interpretation of mixtures. Therefore, after the exploratory period, the time has come for assessing the effective informative power of microhaps to be used in forensic laboratories. For this purpose, we present a study on selecting, developing and validating a panel of 77 MHs designed for studies of degraded DNA.

Markers have been taken in part from published literature, while others are new markers. The main selection criteria for the MHs were first to keep the distance between the two outermost SNPs under 86 bp to reduce PCR amplicon sizes. The other main requirement was for Ae values for each MH to average above 3 among the populations studied to enhance the panel's ability to distinguish individuals. Indeed, the random match probability (RMP) values of the 77 MHs panel, based on data from 26 populations of the 1000 Genomes (1KG) Consortium, range from 1.81E-59 to 3.87E-74. The Ae values for those 26 populations range from 2.47 to 5.39 with an average of 3.52. Moreover, STRUCTURE results on the same 1KG dataset show that the panel also does a good job of characterizing ancestry across major continental regions.

The Ion AmpliSeq Designer tool (TFS) was used for panel design, estimating amplicons length below 145bp. Libraries were prepared with Precision ID Library chemistry and sequencing was performed on the Ion GeneStudio S5 Systems. For data analysis, the HID Microhaplotype plugin (v1.5, TFS) and IGV were used. Testing was on serial dilutions of standard DNA, artificially degraded DNA and Italian population samples.

Sensitivity studies find a very good performance of the MHs panel, with only two allelic drop out events at 25 pg of input DNA.

Good results in term of quality and average coverage were obtained despite low input DNA and sample degradation. Finally, the statistical power of the panel in relationship tests and individual identification were assessed based on the frequencies of the MH dataset based on 1KG and preliminary Italian population data.

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A 25 Loci Mini-STR System to Facilitate Analysis of Degraded DNA

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Objectives--- DNA extracted from crime scene evidence is often degraded. Amplification of degraded DNA may yield incomplete profile, i.e., drop out of loci or allele, which compromises DNA evidence reliability. A lot of factors including biological ones, apoptosis and microbial growth, and environmental ones, sunlight, heat, humidity, contribute to the degradation. Large fragments in a forensic DNA analysis kit are more prone to drop out. In order to get profiles of those lost loci / allele, small fragment analysis kit has been developed to complement a primary analysis kit with larger amplicons.

Methods--- Here we describe a different approach to analyze degraded DNA. The new approach is a kit with two sets of primers to analyze 25 loci. Panel A contains amelogenin and 13 original CODIS loci, and Panel B contains 7 expanded CODIS loci, Penta-D, Penta-E, D6S1043, 1 Y-indel (rs199815934), and three loci from Panel A (Amel, CSF1PO, and D21S11). The three loci are used to verify consistency of the two panels. Panel A and Panel B PCR share same thermal cycling parameters and can be performed at the same time.

Results--- Amplification takes less than 55 minutes to complete. The largest amplicon of the 25 loci is less than 280 bp. The amplification system is optimized to analyze difficult samples, low copy number, degraded and highly inhibitory. Full profile can be generated from as little as 50 pg human genomic DNA. It has also been used to analyze DNA from cold case samples.

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ANALYSIS OF FORENSIC SAMPLES USING THE INVESTIGATOR QUANTIPLEX PRO RGQ KIT (QIAGEN) AT HALF VOLUMES

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Objectives: The Investigator Quantiplex ProRGQ kit (Qiagen) uses a fast-cycling technology for the quantification, in around 1 hour of total human DNA and male DNA, using the Real Time instrument Rotor-Gene Q (Qiagen). The assay also provides information about inhibitors persistence, DNA degradation status and mixture condition. With the aim of improving the number of samples that may be analyzed with a single kit, in the present study we checked the performance of the Investigator Quantiplex Pro RGQ Kit, using half of the reaction volumes recommended by the Manufacturer.

Material and methods: 10 samples (oral swab, cigarette butt, bloodstain onto white cotton, bloodstain on blue denim, semen on toilet paper, vaginal swab, knife handle, hair root, 5 year femur, 5 year molar) were selected as representative of forensic samples. All samples, taken in accordance with internal procedures and informed consent, were extracted, by the EZ1 Advanced XL DNA Investigator kit (Qiagen) using the biorobot EZ1 Advanced XL and the Trace protocol. Quantification was performed using either standard than half of the reagents (Quantiplex Pro RGQ Reaction Mix and Primer mix) reaction volumes, without altering the components ratio. Data obtained were analysed by the software Q-Rex (Qiagen) using the Quant Assay Data Handling Tool: quantification results, Inhibition Index (IC), Degradation Index (DI), Mixture Index (MI) were evaluated.

Results and conclusions: differences in DNA quantity and quality were found among samples, due to the nature of substrates and the consequent different ability to preserve and to release biological material. Anyway, in all cases, we observed concordance per each sample between quantification data obtained using half and regular reaction volumes. The present study confirmed the Investigator Quantiplex Pro RGQ Kit provides accurate and precise results even using half of the recommended reaction volumes. That allows the quantification of a larger number of samples than with the standard protocol, reducing laboratory costs without compromising the quality of the analysis.

P-385

Preparations for shotgun sequencing analyses in forensic genetics

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Shotgun sequencing (SGS) of forensic samples has at least three important purposes: 1) Increase the evidential weight in cases involving close relatives, 2) human identification (HID) of DNA from trace samples that are too degraded for standard short tandem repeat (STR) analyses, and 3) provide single nucleotide polymorphism (SNP) profiles for forensic investigative genetic genealogy (FIGG). SGS potentially provides genotypes of millions of SNP loci covering the entire human genome. However, the genotypes obtained from SGS will not include the standard STRs for HID and thus, searches in the traditional forensic STR databases will not be possible. Instead, the SNP profile of the trace sample can be used to compare it to that of a known reference sample.

SGS of forensically relevant reference samples (whole blood and buccal swap) was investigated using DNA extracts from the four commonly used extraction methods, Chelex®, PrepFiler™ Automated Forensic DNA Extraction Kit (Thermo Fisher Scientific), and EZ1&2 DNA Investigator Kit (Qiagen) with either punch from Whatman FTA® cards or whole blood. For library conversion, three protocols (two for double-stranded DNA (dsDNA) and one for single-stranded DNA (ssDNA)) were tested. In total, ten combinations of extraction methods and library conversions were investigated on a NovaSeq 6000 (Illumina). SGS data were analysed using an in-house pipeline with BWA-MEM and GATK.

The results showed that DNA extracts from Chelex® and PrepFiler™ gave most reads of the highest quality with the ssDNA protocol. The extracts from the EZ1&2 methods gave the best results with dsDNA protocols. Here, we provide recommendations for SGS analysis of reference samples using common DNA extraction methods. Also, we set criteria for evaluating genotypes based on read depth (DP) and GATK genotype quality (GQ). Stringent criteria for DP and GQ are needed for HID, whereas more relaxed criteria might be sufficient for FIGG searches.

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Enhancing Trace DNA Recovery in Forensic Casework Samples, Evaluation of Amplicon RxTM Post-PCR Clean-up Kit on GlobalFiler™ PCR Products

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This study aimed to evaluate the effectiveness of the Amplicon RX™ Post-PCR Clean-up Kit for enhancing trace DNA profile recovery in forensic casework samples amplified using the GlobalFiler™ PCR Amplification Kit. The impact of post-PCR clean-up on allele recovery and signal intensity was assessed. The results indicated that the Amplicon RX™ method and the 30 cycles protocol showed comparable performance in terms of allele recovery ($p = 0.79$). However, the Amplicon RX™ method exhibited higher signal intensity, indicating improved sensitivity in detecting trace DNA profiles ($p = 0.0119$). The study highlights the potential of the Amplicon RX™ Post-PCR Clean-up Kit for enhancing trace DNA analysis in forensic casework samples. Further research is warranted to validate these findings and explore its broader applicability in forensic DNA analysis.

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STR isoalleles detection in Capillary Electrophoresis

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For an inter-laboratory project, two independent laboratories analysed the same sample in replicate using the Investigator Argus X-12 QS kit (Qiagen) and CE was performed on the SeqStudio Genetic Analyzer (Thermo Fisher Scientific). Both laboratories detected a slight mobility shift, of around 0.43 – 0.47 bp, for allele 23 at locus DXS10135. Considering that two independent entities, whose sequencers have been internally validated and calibrated, encountered the same issue, that the shift was localised and was consistently observed among replicates, instrument-dependent problems were excluded. For confirmation, the stain was then sequenced with the ForenSeq DNA Signature Prep Kit (Verogen) on the MiSeq FGx Sequencing System (Verogen). The observed allele 23 at the locus DXS10135 showed an A→G transition in four repeats compared to the reference, leading to part of the sequence being [AAGA]14 [AAGG]4 instead of [AAGA]18. We suggest that the mobility shift for the observed allele 23 is due to the greater molecular weight (MW) of G compared to that of A, producing a fragment slightly heavier than the allele 23 of the allelic ladder. The results led to the analysis of other samples presenting the allele in question and of cases where a localised mobility shift was observed. Our study demonstrates how, albeit not being as informative as NGS techniques, more modern and sensitive CE instruments, coupled with more stringent polymers, can assist in the detection of STR isoalleles.

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Influence of fragment lengths in DNA mixtures for mitochondrial sequence analysis

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Mitochondrial DNA (mtDNA) analysis is a fundamental part of today's forensic sciences. mtDNA analyses are especially valuable for samples with low quality and quantity of DNA, as it is the case in highly degraded samples, e.g. cold cases and ancient DNA (aDNA) studies.

Massively Parallel Sequencing (MPS) further enhances the possibilities of mtDNA analyses as it allows detecting small fragments that are not readable with conventional Sanger sequencing. As a consequence, relevant for forensic applications, MPS might produce successful mtDNA sequencing results that would not be achieved with the Sanger method.

We have observed that mixtures can result in discrepant mtDNA sequences depending on the chosen method and the quantity and degradation state of the contributing mtDNA copies. This was the case for Sanger sequencing data from hypervariable segments obtained from hair shafts that yielded mitotypes which could not be reproduced when sequencing the same DNA extract with Primer Extension Capture or hybridization capture MPS. Apparently, the portion captured with MPS was too small in fragment size to generate successful Sanger sequences.

This phenomenon, that could occur with old DNA and modern contamination, was simulated in our study with mixtures of degraded and non-degraded DNA. For this purpose, we compared three different sequencing methods. The mixtures were subjected to two amplicon-based approaches, Sanger sequencing with amplicon lengths exceeding 400 bp and the Precision ID mtDNA Whole Genome Panel (Thermo Fisher Scientific) with average fragment lengths of approximately 175 bp, and a Primer Extension Capture method targeting significantly smaller fragments.

The results and implications are discussed with forensic interpretation guidelines in mind.

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An Optimised MPSplex QIAseq Workflow for Highly Degraded Post-Mortem Bone Samples.

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To date, the International Commission on Missing Persons (ICMP) has received >120,000 independent reports of missing persons, for which c.65% have resulted in post-mortem (PM) bone sample recovery for DNA profiling. Largescale, DNA-led missing persons investigations like this can be presented with a range of challenges, including low sample quality and complex pedigrees for comparison. To facilitate DNA profiling for all cases, a range of DNA testing methods is needed. For DNA profiling of low-quality samples and kinship matching to more distant relatives, ICMP uses a custom, mid-density SNP sequencing panel – the "MPSplex". The panel includes 1,439 tri-allelic SNP sites and uses QIAGEN's QIAseq chemistry, which is designed for genetic variant detection in cells, tissues and biofluids. The chemistry defines an ideal input between 10 to 80 ng DNA, which is rarely encountered at our organisation. Most samples processed at ICMP are PM bone samples, and samples selected for Next Generation Sequencing testing are highly degraded, averaging an input of 400 pg. This poster presents the successful optimisation of the QIAseq Targeted DNA Chemistry for low-quality samples and determination of a sample quality threshold for triaging purposes.

Optimisation experiments were carried out on a selection of 50 to 70-year-old Vietnamese bone samples from the Vietnam war, which represent some of the lowest quality samples received at ICMP: highly degraded, often with no detectable human DNA (average human DNA quantity = 13 pg/µL, average read length mapped = 50 bp). DNA was extracted using a full demineralisation method, optimised to retain the shorter fragments characteristic of highly degraded samples, and quantified using QIAGEN's Investigator QuantiPlex Pro Kit. QIAseq chemistry optimisations focused on changes to fragmentation settings and bead-to-sample purification ratios.

These modifications showed significant improvements in the retention of shorter DNA fragments, genotyping success and mapping efficiency. Full, or close-to-full profiles (≥1,200 SNPs), were obtained from a DNA input of 300 pg. Partial, but meaningful profiles (≥400 SNPs) were obtained with a DNA input upwards of 35 pg. Matching simulations were performed to confirm the strength of the DNA profiles obtained when using the MPSplex panel. Besides the improved ability to profile challenging samples, optimisation of the workflow has resulted in a better understanding of the assay and has indicated where a potential quality threshold for triaging purposes could be introduced.

P-402

MPS Library preparation optimization and quality control with QIAxcel Connect System for low template DNA samples

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Massively parallel sequencing (MPS) is a powerful tool in Forensic Genetics that can complement information gained by traditional capillary electrophoresis systems (CE). One of the advantages of MPS technology is its ability to deal with low template and degraded DNA samples.

However, challenges may arise during library preparation of these types of samples. When the starting material is of limited quantity or highly degraded, there can be an increase in the formation of adapter dimers, which compete with target DNA fragments during sequencing. To mitigate this risk, it is important to perform library quality controls (QC) to identify the presence of adapter dimers and assess the quality of each library before sequencing. This study provides preliminary insights about the efficiency of the QIAxcel Connect system (Qiagen) for library QC of low template DNA samples.

Thirty DNA samples, characterized by low quantity and quality, were analyzed using both CE and MPS systems. QIAxcel Connect System (Qiagen) was used to assess the quality of purified ForenSeq DNA Signature Prep libraries before sequencing on the MiSeq FGx system.

DNA profiles were obtained from all sequenced samples, concordant to those resulting from standard CE analysis. However, further optimization of library preparation and quality control strategies are recommended to improve sequencing results from challenging DNA samples and prevent costly run failures.

P-403

Investigation into the enhancement of unique molecular identifiers applied in the MH-MPS panel for genotyping unbalanced mixed DNA

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Detecting unique alleles of minor contributors in unbalanced DNA mixtures can be challenging. When the mixture ratio is too high, the sequencing depth of these unique alleles may be similar to sequencing errors. To address this issue, unique molecular identifier (UMI) has been incorporated into the library construction process for next-generation sequencing (NGS) to help reduce sequencing errors. However, the joint application of UMIs and microhaplotypes (MHs) for genotyping unbalanced DNA mixtures has not been previously reported. In this study, we developed a highly polymorphic MHs multiplex system with an average Ae value of 6.9 using the UMI-based NGS library construction method. Unbalanced DNA mixtures with mixing ratios ranging from 1:1 to 160:1 were prepared, and the number of unique alleles detected was determined. The mixture proportion (Mx) was calculated separately using allele reads and UMI reads. The results demonstrated that UMI aided in identifying sequencing errors and improving the accuracy of genotyping alleles in unbalanced DNA mixtures. Furthermore, there was a strong correlation ($r^2 = 0.96$) between UMI amounts and template amount, and the distribution of Mx values obtained using UMI reads was more consistent across different MHs compared to allele reads. Additionally, UMI effectively reflected the initial DNA template mixing ratio, with a correlation of $r^2 = 0.85$. Overall, our study highlights the potential of UMIs in reducing PCR and sequencing bias, thereby enhancing the capability of MH for typing unbalanced mixed DNA. UMIs represent a valuable tool for analyzing DNA mixture data based on NGS reads.

P-406

Evaluation of the effectiveness of microhaplotypes in analyzing degraded DNA specimens

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The analysis of degraded DNA samples in some circumstances is still challenging, with partial STR profiles resulting in inconclusive LR values. The microhaplotyes (microhaps or MHs) markers were found to be highly useful in degraded DNA typing, due to the reduced PCR amplicon sizes. Moreover, microhaps results highly informative also for identification purposes, DNA mixture interpretation, kinship analysis and ancestry inference.

In this study, we describe the usefulness of a panel of 77 MHs in the analysis of DNA degraded specimens which come from real forensic caseworks. Samples were chosen on the basis of their DNA content and degradation level, assessed by a real time PCR assay. Moreover, samples had been already typed with conventional autosomal STRs, in order to compare the effectiveness of the two different marker types in degraded DNA typing. Different biological specimens, including blood trace, bone, teeth, fingernails and buccal swab, were tested.

MPS libraries were prepared with Precision ID Library chemistry and sequencing was performed on the Ion GeneStudio S5 Systems (TFS). For data analysis, the HID Microhaplotype plugin (v1.5, TFS) and IGV were used.

Primary analysis showed good results in term of quality, average base coverage depth and uniformity of base coverage, despite low level DNA and sample degradation. Indeed, the Depth of Coverage (rDoC) analysis showed no correlation between amplicons size and depth coverage.

The majority of the samples analyzed showed complete microhaps profiles, even in degraded samples, while partial profiles were observed in samples with low amounts of DNA. The results obtained point to the effectiveness of this MHs panel as a tool for enhancing the typing outcomes of challenging forensic samples encountered at crime scene.

Finally, probabilistic genotyping on microhaplotypes genotypes data has been used to calculate LRs and assess the informative power of the used MHs panel in forensic identification casework.

P-408

Inter-platform evaluation of a large-scale tri-allelic SNP panel for forensic identification: the MPSplex panel

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OBJECTIVES
The MPSplex panel incorporates a total 1,270 tri-allelic SNPs (1,241 autosomal and 29 X-SNPs), plus 46 microhaplotypes and 55 ancestry informative SNPs, designed for the identification of missing persons. MPSplex has been implemented on a QIAseq Targeted DNA Panel (Qiagen), incorporating the use of UMIs (Unique Molecular Indices). To evaluate the performance of the multiplex, we have analysed a set of control samples for its forensic validation in three different massively parallel sequencing (MPS) platforms and as common samples across different laboratories.

MATERIALS AND METHODS
The performance of the panel was tested on the most widely used MPS platforms in forensics – the Ion S5, the MiSeq and the GeneReader. A standard interlaboratory validation plan included a set of reference samples used to evaluate the panel's performance in terms of the obtained sequences and assess the concordance of our results with publicly available SNP genotype data; a dilution series to gauge sensitivity to low level DNA; and a mixture study. In addition, a distant kinship case was analysed as a proof of concept of the applicability of the panel for this application.

RESULTS AND CONCLUSIONS
The results obtained show a reliable and sensitive genotyping performance of the panel in each platform for all evaluations, indicating high levels of sensitivity, accuracy and capacity to identify DNA mixtures. In addition, a high order of magnitude LR value was obtained in the kinship study, confirming the utility of MPSplex applied to cases of distant kinship where the use of routine forensic markers such as STRs is not always able to provide sufficient power of discrimination.

P-413

Development and Validation of A MPS-based 512-plex SNP Panel for Degradative Forensic Samples

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1.Objetives: In forensic practice, degraded samples are one of the most challenging types for Short Tandem Repeat (STR) typing. Due to the limitations in the length of STR amplicons, it is difficult to obtain STR profiles of degraded samples for further forensic identification. Single Nucleotide Polymorphism (SNP), as a biallelic genetic marker, has shorter amplicons, making it more suitable for degraded forensic samples. In this study, we developed a novel panel using massively parallel sequencing (MPS), consisting of 507 common autosomal SNPs, 5 Y-InDel gender identification loci, and Amelogenin.

2.Material and methods: Our team selected SNP loci with a Minor Allele Frequency (MAF) >0.4 in the East Asian population from the 1000 Genomes Project database. Additionally, we included SNP loci from the Verogen ForenSeq DNA Signature Prep Kit, Precision ID Identity Panel, and BGI's forensic SNP detection system for autosomal loci. To ensure optimal performance, closely located loci were excluded to maintain a minimum distance of 1 million base pairs between SNP loci. During the primer design phase, special attention was given to shortening the amplification fragment length to fall within the 81-116bp range. We also collected 74 population samples for population genetic analysis. Furthermore, we treated a batch of DNA samples with heating at 95°C to induce degradation, using the degradation index (DI) as the assessment criterion to evaluate the extent of degradation. Subsequently, the composite system underwent rigorous analysis for degraded samples, sensitivity, population samples, and species-specificity validation utilizing the Illumina NovaSeq 6000 sequencing platform.

3.Results and conclusions: The results indicate that the system exhibits species specificity, with an individual identification probability of 1-(5.513E-143). Even under challenging conditions with a total input of 0.5ng degraded DNA, all 507 SNP loci within the system were detectable in mildly, moderately, and severely degraded samples. The gender identification loci yielded precise results, with consistent genotyping outcomes observed across two repeated experiments. Notably, even in severely degraded samples, the locus with the fewest reads still had 113 reads. Additionally, degraded samples often present in trace amounts. In the sensitivity test using 9948 as the standard sample and a total DNA input of 0.015625ng, repeated three times, the average number of reads for the locus with the lowest reads was 20.66.

In conclusion, the system meets the requirements of SWGDAM and can be utilized in forensic practice, providing new insights for handling degraded samples in forensic work.

P-438

Can useable male DNA profiles be obtained from seminal fractions where no spermatozoa have been visualised after differential lysis and microscope slide preparation?

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The processing of intimate swabs from sexual assault cases frequently involves a preparation of a microscope slide to facilitate a microscopic examination for spermatozoa. Although extraction methods vary, all protocols essentially involve releasing cellular material from the swab matrix and spotting a portion of the recovered material onto a glass microscope slide. This may be done before or after differential lysis and can involve a variety of different staining methods. However, the objective is the same – to visualise spermatozoa. In instances where, we fail to visualise spermatozoa after two independent searches on a post-lysis microscope slide, our current laboratory policy is to NOT to send the seminal fraction (pellet) for autosomal DNA analysis. In this study we examined whether useable autosomal DNA profiles attributable to a male contributor could be obtained from such material notwithstanding the apparent lack of spermatozoa.

P-439

Automation and LIMS development for the PowerSeq CRM mtDNA Assay

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Though automated processes and laboratory information systems (LIMS) are routinely employed for CE STR typing, few reports describe automation of mtDNA processes and/or NGS processes. For mtDNA, very few labs have the type of case volume requiring automation and, for those labs that do, automation has been primarily restricted to sequencing of high quality and quantity reference samples that can typically be handled in a more standardized fashion than low quantity/quality evidence samples (Taylor et al. 2020). For NGS STRs, automated processes for both high- and low-quality samples have been described (Montano et al. 2018, Hollard et al. 2019); but because STR testing is less prone to contamination, STR workflows tend to be more amenable to automation than mtDNA workflows. Here, we report on the development of automated protocols and laboratory information management system (LIMS) modules for next generation sequencing of mitochondrial DNA typing of evidence specimens.

Automated protocols were developed on Tecan workstations to handle evidence processing, with the pre-PCR steps of mtDNA qPCR set-up and PowerSeq CRM amplification performed on a Tecan Evo, and the post-PCR steps of bead purification, PowerSeq Quant MS set-up and DNA library normalization and pooling performed on a Tecan Fluent. All steps of the process are recorded in a custom LIMS, which provides sample/batch tracking via unique barcodes, reagent tracking, file import/export functionality with instrumentation, and data import/storage of mtDNA profiles. In addition, a specific reagent tracking utility was developed to monitor individual index combinations and index volumes to ensure that each index combination is unique to its respective reaction.

The details of the system development and validation, as well as the changes required to adapt the manually validated chemistry to an automated workflow will be discussed.

P-450

Massively parallel sequencing of trace DNA – better than CE?

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Massively parallel sequencing (MPS) applications offer several advantages over the current gold standard, capillary electrophoreses (CE), in the field of forensic genetics. These advantages include the provision of additional sequence information, shorter amplicon lengths, and the simultaneous analysis of a large number of markers, which result in a reduced number of reactions necessary while improving the amount of data obtained, thereby conserving valuable sample extracts. This innovative approach may prove particularly advantageous for the analysis of trace DNA. The present study aimed to assess the sensitivity and suitability of MPS for STR typing in low template samples, namely skin and hair. MPS data was generated using Promega's PowerSeq® 46GY System, which concurrently amplifies amelogenin, 22 autosomal STRs, and 23 Y-STR loci, followed by sequencing on an Illumina MiSeq® System. The obtained results were compared with those obtained through amplification with the PowerPlex® Fusion 6C and PowerPlex® Y23 (Promega) followed by capillary electrophoresis. The MPS approach resulted in an increase in the number of alleles concordant to reference genotypes, with donor alleles being more frequently identified as the major contributor. Consequently, a higher proportion of samples met the requirements for a database entry, potentially providing critical information in investigations where the use of CE alone might have resulted in data loss. Furthermore, mixtures and allele drop-outs were overall less frequently observed in MPS data. However, the benefits of this MPS workflow are accompanied by some drawbacks, including a longer duration for library preparation and sequencing runs, as well as higher associated costs.

P-453

The Growing Impact of Rapid DNA Testing on Disaster Victim Identification

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Objectives: 1) To describe how Rapid DNA is being utilized as the primary DVI identification modality in a wide range of mass disasters. 2) To present the validation of Rapid DNA technology for DVI based on data from the Maui wildfire and several other mass casualty events.

Methods: DNA has historically been the approach of last resort in disaster victim identification(DVI), with fingerprints, odontology, and radiology the identification modalities of choice. Until recently, avoiding DNA in DVI made good sense—Short Tandem Repeat (STR) based identifications were slow (often taking years to result), costly, and labor-intensive. In the 2018 Camp Fire, the deadliest wildfire in California history, Rapid DNA was applied in a DVI setting for the first time. Rapid DNA, the fully-automated generation of an STR profile in <2 hours, was performed on human remains in a van located outside the Sacramento County Coroner's Office and on Family Reference samples in a family assistance center. A series of SOPs were developed to apply Rapid DNA more broadly to DVI, and Rapid DNA results were validated by comparison to results from other identification modalities.

Results and Conclusions: In August 2023, a wildfire ripped through Maui, including the historic capital of Lahaina. Ultimately, over 100 people perished in the flames, making the Maui wildfire the deadliest US fire of the past century. Unlike other mass disasters that employed Rapid DNA analysis for DVI, the Maui response incorporated Rapid DNA essentially immediately into the identification efforts. Remains were highly degraded, and bones were frequently the only sample type that generate useful STR data. Of 101 victims, 77 were identified by Rapid DNA (most on the day FRS became available) and 22 by other identification modalities (note that of these 22, 13 cases generated Rapid DNA STR profiles but did not have corresponding FRS). Detailed results from the Maui wildfire and the Rapid DNA validation testing program will be presented. In conclusion, Rapid DNA is now being utilized, typically as the primary identification modality, in many mass casualty events including the 2019 Conception dive boat fire, the 2020 Calabasas helicopter crash, the 2020 California wildfires, the 2021 Tennessee floods, the 2022 (ongoing) Ukraine War, and the 2024 Chile wildfires. As more experience with Rapid DNA is gained, its application to DVI will become increasingly widespread.

P-454

Recommendations for identifying disaster victims from the “Mass Grave Project”: forensic genetics issues

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Disaster victim identification poses serious challenges to forensic scientists, including bodies or body parts requiring identification in remote places with limited laboratory access. In these settings, samples for genetic analysis may be collected at a later, after freezing of the corpse. Therefore, given the ease of collection and the possibility of preservation under harsh conditions, swabs can be a viable alternative to tissue and bone sampling. As DNA yield varies by anatomical region, maximum DNA recovery and sampling as many tissues as possible are important to maximize the success of the analysis.

As part of the Mass Grave Project, an interdisciplinary taphonomic study simulating primary and secondary clandestine mass and single graves with whole body donors at the outdoor research facility of the Forensic Anthropology Center, Texas State University (FACTS), nine body donors were examined with the aim of proposing sampling strategies and recommendations for the human identification through DNA analysis. Swabs were collected upon arrival of donors at FACTS and after freezing (between 12-455 days). The donors were then buried for 18 months, six in a mass grave and three in single graves. After burial, biological material in the form of swabs (skin, oral, rectal, periocular) and tissues (muscle, nails, internal organs, cartilage) was sampled.

The inter/intra-individual variation in DNA quantity and quality was explored through STR typing. The results show a progressive loss of information within defrosted and decomposed bodies, unaffected by the observed degree of decomposition of the tissues nor by the position of the body within the mass grave.

Our analyses show that freezing and burial can affect personal identification, so it is good practice to sample as many biological tissues/fluids as possible even considering 'uncommon' sampling such as rectal swabs, and proceeding as quickly as possible to successfully identify the remains of unknown individuals.

P-455

From Death to Discovery: Forensic Biology at Human Decomposition Facilities

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Objectives
Human decomposition facilities provide unique and valuable resources for forensic research, teaching, and training. Currently there are eight human taphonomic facilities in the USA and three internationally (Australia, Canada, The Netherlands) that work closely with the forensic community to conduct novel research, enrich the learning experience of students, and provide “real-world” training opportunities for law enforcement, forensic, and the medico-legal communities.

Although the primary focus and responsibility of these facilities has traditionally been to build as large and representative skeletal collection as possible for forensic anthropology-based research, the scope of research being conducted at these facilities has greatly expanded to encompass almost all fields of forensic science; from forensic chemistry, forensic biology, crime scene investigation, pattern and trace analysis, to entomology, microbiology, and geology.

This presentation will showcase the diverse range of forensic biology-related research being conducted using human cadavers at the Southeast Texas Applied Forensic Science (STAFS) facility at Sam Houston State University in Huntsville, Texas, USA. However, data and results from select projects using the most innovative forensic biology methodologies for human identification (HID) and investigative applications will be the focus of this presentation.

Materials & Methods
Data from various projects demonstrating alternate workflows for tissues sampled from decomposing human cadavers will be presented. These include various DNA extraction methods for bone and challenging samples, recovery of human DNA from feeding maggots, quantitative colour analysis for screening burned bones, rapid DNA (via the RapidHIT ID System), and next generation sequencing panels for HID (phenotype and ancestry predictions). We will also present how human decomposition facilities can assist with research using emerging technologies such as proteomics/metabolomics/lipidomics and microbiome approaches (for determination of sex, post-mortem interval, or age at death), and Forensic Genetic Genealogy (FGG) using the ForenSeq Kintelligence kit and FORCE panels.

Results & Conclusions
These projects highlight how the fields of forensic biology, forensic anthropology, and pathology meet in the setting of a human decomposition facility to investigate current forensic issues using the most realistic samples possible when mimicking routine forensic casework and other scenarios such as mass disasters and missing persons cases.

P-456

DNA Preservation in highly degraded skeletal remains from the Vietnam assessed by various extraction methods, quantitative PCR, and MPS testing with nuclear SNP panels

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Since the 1990's, DNA testing of combatants missing in action from the Vietnam War has played an outsized role in the development of DNA identification methods from highly degraded skeletal remains. Hundreds of identifications of US missing were made using then-pioneering mtDNA methods, in combination with significant non-DNA evidence. However, today there are still some 300,000 Vietnamese soldiers missing from the conflict. The limited identification power of mtDNA renders it unsuitable for such large numbers and limited non-DNA information. Moreover, the DNA in skeletal remains from the hot, humid environment of Vietnam is too highly degraded for large scale success with nuclear STR typing, due to the large size of the required amplicons. A collaborative program has been established between the Vietnam Academy of Science and Technology (VAST) and the International Commission on Missing Persons (ICMP) to assess the applicability of modern DNA methods spanning forensic genetics and ancient DNA analysis to resolve cases in Vietnam.

While generally hot and humid, Vietnam spans over 1500 kms of latitude with a wide range of micro-climates from mountainous to coastal. Bone samples subject to investigation therefore exhibit a broad range of morphological condition. A system has been devised to categorize bone samples according to numerous parameters pertaining to external visual characteristics and internal characteristics assessed during processing of the bone sample. 100 representative bone samples have been categorized and analyzed via alternative DNA extraction methods from the forensic and ancient DNA fields. We will present and discuss DNA preservation/recovery as assessed by total DNA yield, qPCR of human-specific DNA, and the ratio of human to exogenous microbial DNA, in light of various optimization approaches to DNA extraction. Detailed analysis of the sample and DNA extract characteristics will be compared to the outcome of testing with MPS-based medium-density SNP panels performed at the ICMP laboratory.

DNA yields indicate that a sizeable proportion of Vietnam cases will be amenable to identification testing via MPS SNP panels. However, some samples are essentially devoid of DNA, and the efficiency of library preparation is sometimes strongly affected by massive quantities of co-purifying microbial DNA. We will present an analysis of correlation between the various measures of DNA preservation to the morphological categorization of the bone samples to assess the potential for a triage approach to sample selection.

P-457

You Can Only Test One – A Study of over 11,000 NGS Reactions for Skeletal Materials

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Next Generation Sequencing (NGS, aka MPS) has gained focus as a form of DNA testing for many types of forensic samples. NGS of the mitochondrial DNA genome (mtG) has gained predominance in many forensic laboratories for degraded skeletal samples. While mtG is a lineage marker, it is remarkably useful for the sorting of commingled remains in immediate and slow mass fatality events. Optimization of the NGS process begins with the selection of the ideal skeletal element coupled with the most efficient DNA extraction protocol.

The Armed Forces DNA Identification Laboratory (AFDIL, aka AFMES-AFDIL) implemented the use of an NGS capture protocol for the mtG in 2016. The process was originally designed in-house to test formalin treated remains from the Korean War. Since then, the process has been modified and improved over time to produce results in over 75% of those samples tested. Many of these samples have been subjected to extensive environmental insult. In addition to the NGS protocol itself, the DNA extraction protocols have been tweaked and pushed to find the best to be fit for use.

In an effort to further optimize the testing strategy, over 11,000 individual extraction events were assessed for a wide range of variables: location of recovery, time since death, skeletal element, DNA extraction protocol, concentration, and overall success. The choice to seek out the best element for DNA testing is a long-standing goal in forensics and is oft debated. The goal of this study is to present research that will aid in the streamlining of NGS processes for skeletal remains. NGS/MPS is an expensive testing strategy and is often out of reach of a regular crime lab, making the testing of retained skeletal remains unfeasible or extremely limited. This study will provide guidance to the forensic community as a whole for selection of an optimal skeletal element for NGS/MPS testing as well as recommendations for a DNA extraction protocol.

P-459

Preliminary results of classic Sanger and Next Generation Sequencing mitotypes comparison of Second World War victims' skeletal remains

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1. Objectives

Several properties of mitochondrial DNA (mtDNA) make mitotypes relevant for use in forensic genetics, such as abundant copy number per cell, no recombination, and maternal inheritance. As new technologies come into play it is crucial to evaluate them by comparing them to the old ones in order to establish credibility of the results and their informativeness. To accomplish that, certain considerations based on specific laboratory needs must be addressed, such as sample and analysis type. Here we present typing results for mtDNA hypervariable regions (HV) I and II which were compared between two different technologies – classic Sanger, and Next Generation Sequencing (NGS). The aim was to check for concordance between the results of the two technologies that were used, and identify possible discrepancies or additional beneficial information provided that could be used in victim identification process.

2. Materials and methods

Poorly preserved bones of Second Word War victims, found in Konfin I mass grave in Slovenia were used as a model of forensically relevant samples. Parts of right femurs of 5 victims were cleaned mechanically as well as chemically, then grinded into fine dust, which was followed by demineralization of 0.5 g of bone dust, extraction and purification. After quantification, ABI PRISM™ 3130 Genetic Analyser (Applied Biosystems) and Ion Torrent™ HID Ion GeneStudio™ S5 System (Thermo Fisher Scientific) were used for typing. Data was anayzed using AB DNA Sequencing Analysis Software v 5.2 (AB) and Converge Software (TFS). For HVI sequences were compared in the range 16030-16400, and for HVII sequences were compared in the range 55-407.

3. Results and conclusions

Majority of the analyzed variants were in concordance between the two technologies, and the most often found differences originated from various types of heteroplasmies. Minor variants were reported more often using NGS, as the threshold for detection was lower due to the higher sensitivity of the technology. Moreover, a few single-nucleotide inconsistencies were found in length heteroplasmies. The latter could be potentially attributed to either C-stretch resolution limitations of the NGS technology used (making the determination of the number of consecutive cytosines in such cases unreliable), or on the other side the aforementioned higher sensitivity. We therefore advise caution and conclude that when comparing mtDNA sequences typed with these two technologies, heteroplasmies should be excluded from the comparison, as they could steer towards misguided findings.

P-460

DNA analysis in human remains identification – Croatian (Hi)Story

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Identification of human remains is always a challenge. DNA analysis was a game changer in the process of identification since DNA fingerprinting was established. In Croatia, in 1990s, appeared a huge need for implementation of DNA analysis in forensic cases dealing with identification of human remains. Therefore, we have started using using genomic and Y STR markers analysis and techniques that were available at that time.

Few years after, switching from tedious and time-consuming PAA gel electrophoresis followed by silver staining method to fluorescent technology and automated sequencer was like a revolution. The implementation of mtDNA analysis, at the end of 90s, for special identification cases is an achievement we are proud of. Nowadays, using MPS technology as a method of choice is astonishing. A match or positive identification depends on many elements such as condition of human remains, quantity and quality of extracted DNA, availability of missing person's relatives and their willingness to provide sample for DNA analysis, possible mutations and/or unrelatedness, statistical analysis and final interpretation of the results.

During this period of more than 30 years, we were dealing with different types of samples, usually degraded and with minute amount of DNA. Many remains of of Croatian Homeland war victims, sometimes burned and commingled, found in mass graves (primary, secondary even tertiary), DVI after plane crashes, samples from WWII and older, as well as from other armed conflicts were successfully identified.

Here we present several cases of human remains identification showing different DNA analysis methods used due to the technology development at the time of process. The need for improvement and the challenge of new technologies were always present. The lesson learned is that the crucial thing is never giving up. There are cases that still could not be solved and remains identified but hopefully it would be possible in the future.

P-465

Effect of adhesive removal solvents on touch DNA

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1) There is increasing focus on holistic consideration of different investigation modalities within forensic science. This is especially notable when considering DNA, due to the potential for DNA recovery methods to impact recovery of other forensic modalities (and vice versa). This research therefore examined solvents for removing tape from forensic exhibits when recovering DNA/fingerprints.

2) This research aimed to assess the impact of four solvents (un-du® VOC compliant, HFE 7100, heptane and ethanol) to determine whether any proposed solvents had altered effects on downstream DNA recovery, ideally with minimal chemical hazard.

3) HFE 7100 exhibited significantly improved downstream DNA recovery compared to un-du®, whilst also being less chemically hazardous: however, it was not able to successfully remove tape. In contrast, heptane did not exhibit a significant difference in DNA recovery, whilst being more chemically hazardous: it did however successfully remove tape. Heptane and un-du® solvents therefore allow combined preservation of both fingerprints and DNA for a mutually beneficial combined workflow. un-du® is therefore the least detrimental tape removal solvent from a combined DNA and fingerprint perspective, with heptane as a possible alternative should un-du® formulation change in future. This work showcases the value in combined research activity between different forensic modalities for improved outcomes for all.

P-470

AN ALTERNATIVE METHOD: A SIMPLE POSTMORTEM DNA SAMPLING AND TYPING FOR EFFICIENT DVI AND MISSING PERSONS IDENTIFICATION

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The identification of human remains belonging to missing persons is one of the main challenges for the forensic genetics DNA analysis from unidentified human remains. It consists of many steps, but the extraction and optimized recovery of DNA is of paramount importance in this process. Since the beginning in 2003, our laboratory has focused exclusively on STR DNA from a bone. FTA card is unfortunately rarely used to collect samples from decomposed bodies. The number of samples submitted for body identifications to our laboratory by the law enforcement has increased in the last few years. Hence the ability to efficiently and rapidly extract DNA from a variety of post-mortem sample types and perform matching with family or direct reference samples in a short period of time is one of the main challenges that our laboratory has been facing. The goal of this paper is to provide sampling and DNA typing guidelines that would make it both easier and more cost effective than methods currently used in such cases (e.g. DNA typing from bones/teeth). Rib cartilage samples were used for a DNA identification of 10 bodies that were autopsied at the Institute of Forensic Medicine. Extraction of DNA was performed using QIAamp DNA Micro Kit (QIAGEN), 5% Chelex 100 (Bio-Rad) and InstaGene™ Matrix (Bio-Rad). DNA profiles were generated from DNA extracts using GlobalFiler™ PCR Amplification kit (Thermo Fisher Scientific). It was of particular importance to obtain complete DNA profiles irrespective of environmental conditions from which the bodies were recovered. Our work has shown that with post-mortem DNA sampling and simple extraction protocol, using more sensitive and robust new amplification kits we were able to overcome the challenges associated with processing compromised skeletal remains and to perform faster, easier and more cost effective analysis of these types of samples.

P-483

Concurrent detection of mitochondrial DNA and nuclear DNA in hair shaft samples using an using an all-in-one multiplex system

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Objectives: Rootless hair shaft is a type of important biological evidence in crime scenes, where both mitochondrial DNA (mtDNA) and nuclear DNA (nuDNA) were demonstrated to exist. However, the highly degraded nature of DNA fragments in hair shafts poses challenges for the detection of nuDNA through capillary electrophoresis-based STR genotyping.

Material and methods: An all-in-one multiplex system based on MPS technology, encompassing Amelogenin, STRs, SNPs and mtDNA hypervariable regions (HVRs) in one reaction, was employed to simultaneously detect both mtDNA and nuDNA of hair shafts. A total of 370 hair shafts, together with 180 blood samples as the references, were typed using two DNA extraction protocols (Investigator method and the MinElute method) aimed at recovering different sizes of DNA fragments. Population polymorphisms of these loci in the Guangdong Han Chinese, mtDNA heteroplasmy including both point heteroplasmy (PHP) and length heteroplasmy (LHP), as well as depth of coverage (DoC) for nuDNA and mtDNA amplicons along the length of the hair shaft were also studied.

Results and conclusions: The mtDNA analysis of 110 unrelated blood samples unveiled a total of 150 homoplasmic variants and 105 distinct haplotypes, revealing population polymorphisms in the Guangdong Han Chinese. The mtDNA heteroplasmy analysis revealed that 8.18% and 16.36% of individuals have PHPs in blood and hair shafts samples, respectively. Additionally, LHPs were observed in 61.82% of individuals. Sequencing of 370 hair shafts exhibits remarkable differences in coverage between nuDNA and mtDNA amplicons. In addition, a noteworthy difference in the average DoC was observed between samples extracted using the Investigator method and those using the MinElute method. In the longitudinal research, a gradual decrease in the total DoC of mtDNA fragments along the length of the hair shaft was observed, ranging from the proximal to the root to the distal end. In contrast, the DoC of nuDNA exhibited a relatively stable pattern along the hair shaft, from the proximal to the root to the distal end. The study contributes valuable insights into the simultaneous detection of nuDNA and mtDNA in hair shafts, emphasizing the need for optimized DNA extraction and detection methods for these highly degraded samples.

P-490

Investigation of a Y-chromosome deletion affecting amelogenin-based sex typing in forensics using CE and MPS methods

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Sex determination in forensic science is particularly important in human identification cases and sexual offence crimes. The amelogenin marker (AMEL), which enables sex typing, was described in the early 1990s and immediately found widespread use in human identification studies. Its analysis is based on standard capillary electrophoresis (CE) and can be combined with analysis of STR markers. The presence of the Y chromosome (ChrY) amelogenin variant in the genetic profile clearly indicates male sex. However, cases of lack of detection of the ChrY allele, known as AMELY-null, have been reported. This can lead to misidentification of the female sex, resulting in misleading information being reported. The aim of the project was a thorough investigation of the AMELY-null case observed in a forensic sample, using CE and massively parallel sequencing (MPS) methods. DNA profiling using NGM and GlobalFiler kits showed lack of the Y allele within the amelogenin marker. Analysis of ChrY STR markers (Y-filer Plus and PowerPlex Y23 kits) revealed deletion of 6 markers closely located on the short arm of ChrY. MPS using ForenSeq kit confirmed the occurrence of mutation in the tested sample. Further interpretation of the obtained results revealed a large deletion encompassing 7 loci (DYS570, DYS576, DYS522, DYS458, DYS449, DYS481, DYS627) in the analyzed sample, which is consistent with the common observation of susceptibility of certain regions of the Y chromosome to structural mutation events. The use of various Y-STR multiplex kits containing different marker primers and different DNA analysis techniques proves that the presence of the AMELY-null allele is not due to primer binding site mutations. Our study confirmed that AMELY sex typing can be misleading and the female sex should be interpreted in the light of other markers useful in sex typing including ChrY-STRs, ChrY-indels, and those analysed at the stage of evaluation of DNA concentration with e.g., Quantifiler Trio DNA Quantification method. The occurrence of ChrY deletions involving amelogenin locus is rare but particular caution is recommended in sex typing for populations more susceptible to AMELY deletions (e.g. South Asian region).

P-494

Is it really necessary to include the Y-STRs in autosomal STR multiplex kits to help determine the number of mixture contributors?

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The ability to indicate whether a sample comes from a male or a female source and to distinguish the contributors of a mixture is particularly relevant for identifying the evidence of the victim and the perpetrator(s), especially in cases of sexual assault.

Since its introduction in 1994, the Amelogenin (AMEL) system has been the gold standard for gender determination also because it can be amplified with autosomal and gonosomal short tandem repeats (aSTRs, gSTRs) markers.

Over the years, cases have been reported where deletion at position p11.2 of the Y-chromosome (Y-Chr) has resulted in failed amplification of AMEL Y, leading to gender misinterpretation. To prevent this occurrence, since 2014, the DYS391 locus, located at q11.21 on the long arm of the Y-Chr, has been introduced in autosomal STR kits as support for AMEL Y.

During a validation and population data acquisition study on 400 individuals in Northern Italy using the PowerPlex® Fusion 6C System, in a sample for which the AMEL Y deletion was already known, the non-amplification for the two Rapidly Mutating Y-STRs (RM Y-STRs) DYS570 and DYS576 was also revealed.

On first examination of the electropherogram of this sample, it appeared to belong to a female subject, given the absence of alleles for AMEL Y and the two RM Y-STRs, and the single peak in DYS391 could be considered an artefact (e.g. spike). The analysis was repeated twice to confirm the aSTRs profile. Then, the DNA sample was amplified with PowerPlex® Y23 System and Investigator® Argus Y-28 QS Kit, proving the deletion at DYS570 and DYS576 loci and revealing a further deletion at DYS458.

These results raise the question of whether using Y-STRs in close proximity to the amelogenin gene really contributes to gender determination in mixtures or whether it would be more appropriate to use Y-STRs in positions less affected by deletion events.

Although deletions on the short arm of Y-Chr at the p11.2 position are a rare occurrence, they have been documented several times in the literature, and therefore, they have to be taken into consideration.

Hence, it would be more advisable to include Y-STRs in the multiplex kits, away from Amelogenin and preferably in a Y-chromosome location characterized by better stability and less susceptibility to deletions. This approach could better meet forensic requirements, ensuring greater consistency of results.

P-495

Developmental validation of multiplexes featured by multicopy and rapidly-mutating Y-STRs

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Objetives
As an essential tool of paternity identification, Y-STR have been applied overwhelmingly in forensic areas, especially in solving sexual assault cases. However, discrimination capacity may be restricted since the widely used Y-STR kits contain mostly single-copy markers with moderate mutating rates. Here, we developed two sets of Y-STRs featured by multicopy and rapidly-mutating loci to complement the currently available Y-STR kits, in order to improve discrimination power especially on differentiating male relatives.

Material and methods
Multicopy and rapidly-mutating loci were selected that are genetically diverse in populations. Multiplex I includes 12 multicopy Y-STR loci (DYF371, DYF383S1, DYS385, DYF387S1, DYS389I/II, DYF399S1, DYF404S1, DYF409S1, DYF411S1, DYS464, DYS526, DYS527), 1 single-copy Y-STR (DYS391), and Amelogenin. Multiplex II includes 13 rapidly-mutating Y-STRs (DYS449, DYS518, DYS547, DYS570, DYS576, DYS612, DYS626, DYS627, DYF387S1, DYF399S1, DYF403S1, DYF404S1, DYS526). The multiplexes were constructed based on five-dye fluorescence labeling technique. Validation studies were performed to test their sensitivity, species specificity, stability, male specificity and size precision.

Results and conclusions
Both the two multiplexes were of species-specific, sensitive and robust to tolerate high concentrations of PCR inhibitors (i.e., humic acid, hematin and EDTA). The Y-STR systems also displayed strong male specificity even if the male/female ratio was down to 1:800 (multiplex I) or 1:4000 (multiplex II). In all, the two Y-STR sets we developed are of great value to assist the differentiation of males for forensic use.

P-496

Forensic Genetic Identification of Human Remains – A quantitative workflow approach adapted for DNA profiling and haplotyping.

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Hard tissues are often the only biological material available for human remains identification, well preserved through time yet variably efficient for genetic analysis. In many cases, old human bones found in harsh environments stay unidentified, due to the difficulty of obtaining good quality DNA. In this work, we present a quantification-based workflow approach to improve genetic identification results of human skeletal remains, in terms of generating the most genetic data accessible. First, we show the importance of sample selection according to discovery site and climatic conditions. Then we address sample preparation and present comparative results of the most suitable DNA extraction methods. The critical metric streamlining this workflow, is the triple RT-PCR quantification results, which simultaneously evaluate the yield of total human and human male DNA, as well as the degradation level. Based on these values, empirical assessments are shown for STR profiling and Y-STR and mitochondrial haplotyping. For the profiling pathway, we compared three relevant autosomal STR amplification strategies for the purpose of achieving the most complete set of data from degraded samples. Concordantly, we established three alternative mtDNA amplification designs that can be selected in line with quantification results, to generate a full Control Region mitochondrial DNA haplotype. Finally, prior to sequencing, amplified mtDNA fragments are checked by electrophoresis in a microfluidic chip, which enables to simultaneously obtain quantitative and qualitative assessment. Through this combined approach, we attempted to conveniently accommodate quantifiable metrics, in order to lead the forensic workflow to the best methodologies, and to integrate a larger set of genetic data for challenging samples in view of a positive identification.

P-497

The Quick TargSeq 1.0 integrated system for rapid forensic DNA analysis: sample collection to DNA profile

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Autosomal short tandem repeat typing is a global standard for human identification, which is used routinely for associating or excluding individuals with biological evidence left at a crime scene. Haplotypes composed of Y-chromosomal short tandem repeat polymorphisms (Y-STRs) are used to characterize paternal lineages identification of unknown male trace donors, especially suitable when males and females have contributed to the same trace, such as in sexual assault cases. Traditionally, STR typing requires tedious process and specialized laboratory instrument, which are somewhat labor intensive and time consuming. To enhance process steps from sample to DNA profiles, several groups including us have reported the successful development of integrated microfluidic devices. Our group developed a rapid DNA microsystem (Quick TargSeq 1.0 integrated system) that is fully integrated the processes of DNA extraction, PCR amplification, electrophoresis separation, and data analysis in a single self-contained system. One important property of this microsystem is the flexibility modular design for handling different DNA samples, for buccal swab, a direct sample preparation chip cartridge was employed, for blood stain, an extraction sample preparation chip cartridge was used. Previously, we have demonstrated the efficiency of Quick TargSeq 1.0 integrated system in utilizing the RTyper21 chip cartridge for individual identification. Here, we explored its application in the field of identifying males and male lineages in forensic practice via Y-STR analysis. This further research showed that simultaneous detection by installation of RTyper21 and RTyper Y27 reagents in two channels of the same chip cartridge yields results of two distinct genetic markers within approximately two hours. This parallel detection method can obtain individual identification and paternity testing simultaneously, thereby significantly enhancing the efficiency of case detection. A rigorous environmental examination conducted in plateau conditions has validated the resilience of the Quick TargSeq 1.0 integrated system. The device has demonstrated its robustness in withstanding altitudes ranging from 2500m to 3800m, and in weather conditions characterized by rain, snow, and humidity levels exceeding 85%. It has also proven its durability in temperatures ranging from -1℃ to 5℃, and under the vibrational and random impact forces generated by regular vehicular movement in plateau regions, which has the capacity to work optimally while in transit. This test is the first attempt in the field of forensic DNA rapid testing that has not been reported by other three commercial rapid DNA devices.

P-518

Assessment of simultaneous DNA/RNA/Protein extraction from human remains

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DNA isolation from human remains is of utmost importance to be able to identify a victim. However, there are situations in which, apart from the STR profile, other parameters need to be estimated, like the time-since-death. Recent advances in this field point to “-omics” technologies to improve these estimates, particularly in skeletonized remains. Among them, transcriptomics and proteomics are emerging as promising techniques for this purpose. However, this requires the extraction of RNA and proteins from the remains along with the DNA, which is particularly challenging in this kind of sample exposed to different environmental conditions. This study aimed to assess the efficiency of simultaneous DNA/RNA/protein extraction from teeth, the hardest tissues in the human body and most resistant to decomposition. Towards this goal, six teeth were processed, separating dentin and pulp and creating aliquots of 200 ng of dentin. The Zymo Quick-DNA/RNA™ Microprep Plus Kit was used for the simultaneous DNA/RNA/protein extraction. Different incubation times on the digestion buffer and proteinase K were evaluated. DNA/RNA/protein concentrations were assessed through Qubit fluorescent quantification, and human-specific quantification was carried out by applying the Promega PowerQuant® kit. According to our results, both Qubit and PowerQuant quantification showed an increase in DNA yield after 24 hours of incubation, although not significant, obtaining average concentrations around 30 ng/ul. However, the degradation ratios of the DNA also increased with time. Respective to RNA, time did not impact the RNA yield, obtaining average concentrations around 50 ng/ul. In contrast, for proteins, 2 hours of incubation increased the protein yield, obtaining average concentrations around 1 ug/ul. To the best of our knowledge, this is the first study assessing the efficiency of simultaneous DNA/RNA/proteins from human remains, demonstrating that it is possible to isolate these three molecules, obtaining appropriate yields, not only for human identification but also for time-since-death estimation.

P-520

Shotgun RNA sequencing of low template blood samples provides information for multiple forensic genetic purposes

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Biological trace samples of low quality or quantity constitute a challenge to standard forensic DNA profiling. The transcriptome retains much of the genomic variation replicated in numerous copies, why RNA may be an alternative to DNA if the sample consists of very few cells (low template samples). Shotgun RNA sequencing generates sequence information of every RNA molecule present in a sample, including highly degraded fragments and pre-mRNAs. Thus, both exonic and intronic single nucleotide polymorphisms (SNPs) may be genotyped.

This study aimed to examine if human identification (HID) from low template blood samples (50 pg gDNA) was possible using RNA sequencing. By focusing on the 100 protein-coding genes with the most sequencing reads, we looked for bi-allelic SNPs able to discriminate individuals at a global level. The performance of these markers was assessed in both whole blood samples and blood stain samples with relatively low RNA quality. Whole blood samples from 41 Europeans were analysed in duplicates by shotgun RNA sequencing on a NovaSeq 6000, as were blood stains (seven individuals at six time points) subjected to degradation at room temperature for up to two weeks to simulate forensic trace samples. Concordance was assessed by DNA genotyping using the Infinium Omni5-4 SNP BeadChip.

We developed a prototype RNA-HID-SNP selection set consisting of 24 bi-allelic SNP markers with gnomAD minor allele frequencies (MAF) > 0.1 in the African/African American, East Asian, and (non-Finnish) European populations. The RNA-HID-SNPs demonstrated concordant typing in both whole blood samples and degraded blood stains, yielding a mean match probability of 4.5 · 10^-9. Moreover, we identified genes and additional SNP markers that may be useful for body fluid identification and ancestry inference, respectively.

This proof-of-concept study demonstrated how shotgun sequencing of RNA from low template samples holds promise for various forensic genetics purposes.

P-523

Massively parallel sequencing of mRNA-cSNPs: a typing assay for human identification for hair shaft

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Objetives: At the crime scene, hair is one of the common samples found for individual identification, which has trace nuclear DNA and abundant mitochondrial DNA. However, the fallen hair often shows no follicle, resulting in a failed STR detection. Recent years, RNA has showed great potential for forensic genetics. Our previous study has indicated that hair shaft possess the detectable RNA.
Material and methods: In this study, a massively parallel sequencing assay was established, including 385 coding region SNPs (cSNPs) from 75 genes based on BGI seq T7 platform. Hair shaft samples were collected from 50 Chinese individuals. 10 pieces of 6cm hair shaft was used to extract total RNA using RNAiso Plus with some modifications. The multiplex assay was evaluated for sensitivity and species specificity.

Results and conclusions: The minimum threshold for amplicon read counts was set at 100. Genes failing to meet this threshold were excluded from the panel. Ultimately, 63 genes containing 338 cSNPs were retained. The polymorphism was observed in 295 SNPs. The combined discrimination power of the assay was 0.999999999999996 in the Shanxi population. When one piece of 6cm hair shaft was used, 53%-70% SNPs could be detected. Results of hair from four common animals (cat, dog, rabbit and rat) showed the excellent species specificity. This study provide a new strategy for the human identification of hair shaft .

P-534

FAR FROM HOME: GENETIC IDENTIFICATION OF SPANISH CIVIL WAR VICTIMS

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The genetic study of 70 victims of the Spanish Civil war (1936-1939) and posterior dictatorship (until 1970s) for identification purposes is reported. The victims buried in graves in the cemetery of Orduña in North of Spain (Basque Country), came from the central prison established in that moment in the locality. According to the official reports more than two hundred males died in this prison as a result of the terrible conditions of insalubrity and hunger. Most of the prisoners were from southern regions of Spain and were sent to the north as part of the dispersion of prisoners far from home. Informative autosomal STR and/or Y-chromosome STR profiles were obtained from the skeletal remains of the victims, and were compared to those from relatives of more than 50 families. A total of 10 identifications have been reached at the moment. However, the search for new relatives of reference is continuing in order to identify the largest possible number of victims.

P-535

A multi disciplinary approach to the identification of sandy point man

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Objectives
In Australia, the identification of human remains is paramount to medico-legal death investigations, where DNA plays an integral role. When human remains are located, case-related contextual information is used to generate an identification hypothesis. However, in some cases there is no contextual information available. These are known as Unidentified Human Remains (UHR) cases, which often require a complex process of identification reliant on a multidisciplinary and collaborative approach.

Here we present the case of a near complete human skeleton located at Sandy Point in Victoria, Australia. As there was no case related information that could be used to form an identification hypothesis for this case, forensic experts at the Victorian Institute of Forensic Medicine (VIFM) applied all possible means of identification.

Material and Methods
This included what are considered standard identification methodologies such as DNA profiling (nuclear and mitochondrial DNA profiling) as well as anthropology and odontology. Ultimately, the identification of the Sandy Point Man (as he become known) relied on the integration of anthropology, odontology, molecular biology, radiocarbon dating, historical research, and Forensic Investigative Genetic Genealogy (FIGG).

Results and Conclusions
While this case relied on the application of DNA analyses that generated forensic DNA intelligence, it highlights that the identification of UHRs is a process that necessarily requires a multidisciplinary and collaborative approach.

P-536

Genetic typing of Italian soldiers found in a Second World War mass grave: a preliminary report

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In this work, we present the preliminary results of the genetic typing of skeletal remains found in a Second World War (WWII) mass grave on the Isle of Cres (Croatia), where 27 Italian soldiers supposed to be buried according to historical records. Anthropological and medico-legal examinations identified a minimal number of 31 individuals. In total, 131 bone samples and 16 molar teeth were used for DNA extraction, allowing the quantification of DNA in 75.2 % of the samples (from 100 % in petrous bones to 6.3 % in dental samples).

PowerPlex Fusion kit was used for 129 PCRs producing full profiles in 46.5 % of the tests, whereas partial profiles (≥ 12 markers) were yielded in 36.4 % of the tests. The PowerPlex Y-23 kit provided full profiles in 17 out of 48 tests, whereas partial profiles (≥ 12 markers) were recovered in 12 tests. In total, 23 different autosomal consensus male profiles and four unexpected female profiles were currently identified.

Post-mortem data of the 23 male individuals were then compared with the antemortem database, which included 21 subjects (8 males and 13 females) from 14 unrelated Italian families missing 4 first degree, 7 second degree, and 3 third-four degree relatives, who were killed and likely buried in that mass grave. The DVI (Disaster Victim Identification) module of the Familias software was used for computing the LRs and the posterior probabilities (PP). Conclusive results (PP > 99.9 %) were obtained in four cases, while two cases showed PP > 90%, and the remaining cases showed inconclusive results (PP < 77%).

Although the combination of autosomal and lineage markers in forensic identification is controversial, it is commonly performed in similar cases of historical interest. By combining autosomal and Y-chromosome results for the six pedigrees with a male descendant, conclusive results were achieved in 10 cases (only in a single case, where the reference sample was a full-sister, the PP remained 99.6 %).

The results of this study confirm that petrous bone outperforms other bone elements in yielding genetic data and that PCR-CE analysis of STR markers is an excellent tool for identifying WWII victims. Still, the availability of close family members is the major issue in such cases. Thus, this work further addresses cooperation among anthropologists, geneticists and historians in solving WWII cold cases.

P-537

LR values in the identificacion of victims of the Spanish Civil War (1936-1939). The experience of a big consortium.

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Objectives
The aim is to present the identification data of the study promoted by the General Directorate of Democratic Memory of the Generalitat de Catalunya, between July 2017 and the present, to identify those who died as a result of the Spanish Civil War.

Material and methods
A database of 3936 potential living descendants is available in the Clinical and Molecular Genetics Area of the Vall d'Hebron Hospital, Barcelona, Spain. Three centres, including Universitat Pompeu Fabra, Universitat Autónoma de Barcelona and Universidad Complutense de Madrid, have provided a total of 579 (plus 62 with no results) genetic profiles of deceased people based on human remains excavated by the company Iltirta S.L. The identifications were made using the DVI module from Familias 3.2 software, first, and then LR value calculation was based on individual comparisons between suspected samples.

Results
As of March 2024, there have been a total of 24 cases with identificative results, all of them belonging to males. In 6 cases, the identification was made through autosomal STRs with GlobalFiler PCR Amplification Kit (Thermo Fisher Scientific)(LR>>100000) and subsequent confirmation with Yfiler Plus PCR Amplification Kit (Thermo Fisher Scientific), and in 2 cases with subsequent confirmation with mitochondrial haplotype. In addition, in 5 cases there could only be a patrilineal match probability by Y chromosome and in 1 case a matrilineal match by mitochondrial haplotype. Regarding kinship, in 11 cases daughters or sons were used, in 2 cases siblings, in 4 cases grandchildren, in 1 case great-grandchildren and in 6 cases nephews or nieces.

Conclusions
Elevated LR values mostly correspond to parent-child comparisons that will decrease in frequency over time. Therefore, it is necessary to incorporate more polymorphism into the genetic profiles in order to be able to make comparisons between more distant relatives.

P-543

FAMILY REUNIFICATION THROUGH DNA ANALYSIS OF SURVIVORS OF THE GREATEST NATURAL DISASTER IN COLOMBIA IN ARMERO-TOLIMA (1985).

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Objective:
To describe the efforts for family reunification after the greatest natural disaster in Armero Colombia (1985).

On November 13, 1985 at 9:20 p.m., the Nevado del Ruiz Volcano erupted (A Glacier Snowy Volcano 17457 fts above sea level). The lahar, melting ice and landslide devastated the town of Armero located 45 kilometers away in a matter of minutes, in the Tolima department of Colombia,. More than 25,000 people died or were reported missing. This tragedy is the largest natural disaster to date in Colombia.
The Armando Armero Foundation is a Non-Governmental Organization (NGO) that brings together survivors of this tragedy.

Different strategies are used to find matches in our database. A personal interview is carried out with each individual in order to establish family relationships and to gather information as possible. In some instances, the Armando Armero foundation has carried out additional research to find documents, and possible links among individuals. Thus, in some cases, oriented searches had been carried out and in other cases, each individual is matched against the entire database.

Materials and Methods: A total of 266 samples have been collected with prior informed consent. Of these samples, 41 samples correspond to individuals that were given up in adoption following the disaster. The remaining correspond to individuals and family members looking to find their lost children or relatives. They have been typed for Powerplex 23 (Promega Corporation) or by GlobalFiler (Thermo Fischer Scientific) and analyzed in ABI 3500 or ABI 3130 XLS genetic analyzers. Genetic profiles are then uploaded into our database for search and match.
79 Y Chromosome STR samples have been analyzed using Powerplex Y23 or Yfiler Plus kits following manufacturer's recommendations.
mtDNA have been carried out in selected cases for HV1 and HV2 regions, or f for HV1, HV2 and HV3 control regions using standardized protocols and DNA sanger sequencing using BigDye Terminator 3.1 cycle sequencing (Thermo Fischer Scientific) and analyzed in a 3500 genetic analyzer using SeqScape v2.7 software. The revised CRS is used as reference following ISFG recommendations and guidelines for haplotype and nomenclature.
All DNA testing has been carried out in our 17025:2015 accredited laboratory for Autosomal, Y Chromosome and mtDNA analysis.

Results
Thus far, we have reunited 4 Armero's adopted children with their relatives through DNA testing, using autosomal and uniparental markers. We continue collecting samples to reunite as many families as we can.

P-544

DNA-assisted Identification of human remains in the Iraqi context

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Iraq has experienced multiple conflicts over the last few decades with hundreds of thousands of deaths, and many of the victims have not been identified. The Medico Legal Directorate, which falls under the Ministry of Health, is authorized and mandated in law along with the Martyrs' Foundation, to carry out the exhumation and identification of human remains. While DNA analysis plays a critical part in most identifications the MLD has also developed and incorporates anthropological analysis, which is especially important in cases involving commingling.

Notable episodes of conflict and violence that have resulted in large numbers of deceased include: the Iraq-Iran war (1980-1988) with over 50,000 Iraqis still missing; the Al-Anfal campaign (1978-1988) where between 50,000 and 100,000 Kurds were killed; the Gulf War (1990-1991) with over 50,000 Iraqi Service men killed; the Shia Uprising against Saddam Hussein's regime (1991) where between 400,000 and 1,000,000 were killed with most placed into mass graves, and the civil conflict (2005 - 2008) that is still politically very sensitive. In its recent history between 2014 and 2017 ISIS occupied approximately one third of the country and during this time committed many atrocities, including Camp Spicher (2014) where 1,700 air cadets were killed, Badoush Prison (2014) where over 600 Shia prisoners were executed and the persecution of the Yazidi population, where an estimated 5,000 males have been killed.

Each context presents it own challenges. Recovery of DNA from much of the skeletal material is difficult and degradation is advanced in remains that have been recovered from mass graves dating to the 1991 uprising, and also in remains from the ISIS period, especially when the decease individuals have been left on the surface. Biological reference sample collection has been difficult in some contexts, with large numbers of relatives among the deceased and the challenges of tracing relatives following internal displacement and emigration, and the security and location of some mass grave sites has hampered the recovery of human remains.

Despite the challenges the MLD have been successful in identifying large numbers of individuals, for example, with the victims of Camp Spicher 1,139 bodies from the 1,700 victims have been identified and returned to their families. Here we will discuss the incorporation of DNA in the identification process and provide more detail on the identification of the victims of the Yazidi persecution and Camp Spicher.

P-545

Genetic Identification of victims of the Spanish Civil War and Dictatorship in the Cuelgamuros Valley (Madrid – Spain).

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1. Objetives
The recovery and identification of skeletal human remains related to the Spanish Civil War (1936-1939) and the Dictatorship (1939-1975) is a complex process that has mainly been overtaken since the late 20th century by Scientific Associations with the participation of family members. In some cases, the “Instituto Nacional de Toxicología y Ciencias Forenses” (INTCF), a public research center under the Ministry of Justice of Spain, has collaborated in the identification of human remains from the Civil War and the Dictatorship context.
In this poster, we present the first case of genetic identification of human remains exhumed from the “Valle de Cuelgamuros”, formerly “Valle de los Caídos” or Valley of the Fallen, the major monument of Franco's regime, commissioned by the dictator Francisco Franco to celebrate his military victory.

2. Material and methods
The skeletal remains contained in Columbarium 198, found on level 0 of the “Capilla del Santo Sepulcro” (Holy Sepulcher Chapel) at the Cuelgamuros Valley, were analyzed. According to historical research, this collective box contained 12 bodies with known identities and, therefore, with living descendants who claimed the bodies.
The skeletal remains of 12 individuals (12 femurs and 12 tibias) arrived at the INTCF, from which DNA was extracted and they were genetically analyzed (A-STRs, Y-STRs, mtDNA), according the internal protocols of the INTCF.
Reference samples were also obtained from 13 relatives, with whom the genetic comparison was carried out. The relatives were sons/daughters (5), as well as grandchildren (6), a grandnephew and a great niece, both on the maternal and paternal lines.

3. Results and conclusions
To date, 11 bodies have been genetically identified from this Columbarium (with LRs ranging from 2,10E+01 to 5,54E+08). The remains were returned to the relatives in an institutional ceremony so that they could be worthily buried and honored.
Thanks to the Democratic Memory Law of Spain (Law 20/2022, of October 19), the victims of the Civil War and the Dictatorship are being given new meaning, giving “the recognition of those who suffered persecution or violence, for political, ideological, thought, opinion, conscience, religious belief, sexual orientation or identity reasons".

P-546

Monitoring success rates of touch DNA samples in cases of volume crime in a high-throughput German police lab

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Volume property crime cases (e.g. burglary and vehicle break-ins) are characterized by a high number of contact traces considered as low-template touch DNA. These contact traces form a substantial part of the workload for the high-throughput forensic DNA laboratory of the Lower Saxony State Office of Criminal Investigation. Decades of research and development improved sensitivity of DNA typing methods tremendously, but still, analysis of touch DNA presents with numerous challenges. Success rates of contact traces remain low due to limited DNA quantity / quality, complex mixture interpretation, and the absence of DNA reference samples. However, facing social and political pressure to increase success rates in cases of volume property crime, law enforcement agencies frequently request the analysis of contact traces aiming to provide the criminal justice system with objective and reliable evidence. Aiming to depict a realistic valuation of touch DNA evidence in volume crime investigation, we started to examine our contact trace success rates.

In 2017, we have implemented a continuous process of monitoring results of analysing touch DNA samples in real cases of volume property crime. The entire workflow of contact trace processing follows accredited routine protocols and the concluding success rate data is collected manually. For burglary, we are individually focusing on pry marks, (glove) swipe marks, and drill holes. Looking at cases of vehicle break-in, we concentrate on infotainment systems (e.g. contact traces from various infotainment connectors and plugs) as well as screws.

From 2017 to 2024, 2671 contact traces belonging to 1802 cases have been analysed and evaluated. Altogether, 65 DNA profiles were sent to the German DNA database resulting in 19 hits. Seeing this low likelihood of success, requests for pry marks, (glove) swipe marks, and car-infotainment plugs are typically rejected (except for well-justified reasons). In comparison, success rates for drill holes (often accompanied by saliva stains) are superior justifying their analysis.

The police intranet of Lower Saxony is provided annually with the success rates of each contact trace type. This data collation offers objective statistics to better evaluate the potential outcome of contact DNA evidence in volume crime investigation for the investigative phase in the criminal justice system of Lower Saxony. In accordance with that, recent trends show a drop in this type of investigation requests.

To further address the challenges of manual and time-consuming data collection, we are planning to automate success rate estimations using our Laboratory Information Management System (LIMS).

P-548

A case of tetragametic chimerism identified during a routine paternity testing case. A Case Report.

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Objective: Report and create awareness about tetragametic chimerism cases.
Human chimerism is the presence of two or more genetically distinct cell populations in an individual containing genetic material derived from two or more zygotes.

Material and methods: We identified a case of tetragametic chimerism during a routine paternity testing case. Due to an unexpected maternal exclusions in two STR´s, and the presence of an XY amelogenin profile in the mother's sample, a phenotypically normal female, prompted us to further investigate the case.

Tetragametic chimerism was confirmed based on cytogenetics analysis, as well as STR analysis of different tissues, along with segregation analysis using the mother's samples and her brothers to reconstruct the parental genetic profiles. Our analysis included blood, hair, buccal swabs, and saliva samples, mtDNA analysis, Y chromosome STR analysis and autosomal STR analysis's using Powerplex 16, Powerplex 18 and Powerplex 21 STRS. In addition, analysis of two additional children also showed maternal exclusion when compared to their mother´s blood sample. Chimeric tetragametic individuals' range in the spectrum from phenotypically normal males (chimeric XY/XY or XY/XX), disorders of sex development (only XY/XX) to phenotypically normal females (Chimeric XX/XX or XX/XY).

Conclusions: Although tetragametic chimerism cases are considered low occurrence events, our result highlight the importance of reporting cases to the forensic community, to create awareness due to the profound legal and social implications of such cases.

P-555

Prüm implementation in the UK: An example of success through collaboration

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After conducting a successful pilot and receiving subsequent parliamentary approval the Metropolitan Police Service (MPS) went live with its step 1 Prüm DNA service in July 2019 initially connecting with Austria followed quickly by Germany, the Netherlands and then Spain. Since then connections to further European countries have been established such that currently the UK MPS Prüm DNA team is now routinely sharing DNA biometric data with 25 countries and performing scientific verification of all matches prior to reporting to UK forces and engaging with the UK National Crime Agency (NCA) as the UK Step 2 partner for exchange of demographics. This review will consider the journey from pilot to go-live as well as highlighting the positive impact the implementation has had on the MPS, the UK and also partners across Europe since implementation as a crime fighting intelligence tool.

Posters Topic 2

New Technologies and Applications

Abstracts Poster

2. New Technologies and Applications

P-018

Exploring the Effects of Temperature-Dependent Mitochondrial DNA Sequencing from Incinerated Tooth Samples: A Preliminary Study

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1. Objectives
The objective of this study is to perform mitochondrial DNA sequencing on incinerated tooth samples, especially in cases where results from nuclear DNA profiling are either partial or unreliable.

2. Material and Methods
A total of 30 molar tooth samples were collected, where three samples were obtained from each subject. These samples were then incinerated at different temperatures ranging from 100-300ºC using a muffle furnace for 15 mins. Subsequently, the samples were pulverized into thin powder using a tissue lyser. All the samples were decalcified by using 0.5M EDTA for 72hrs, followed by isolation and extraction of DNA using an organic extraction method. The quantity and quality of the extracted DNA were estimated by using a Nanodrop 1000 spectrophotometer and 1% agarose gel, respectively. These samples were analyzed for STR profiling to detect genetic markers. After STR profiling, multiple overlapping primer pairs were used on extracted DNA for amplification of control region of mitochondrial DNA. These amplicons were visualized on a 2% agarose gel. The samples were then processed for Sanger sequencing using the Genetic Analyzer 3500xL, and sequences analysis were conducted using SeqA6 software, and haplotypes were determined using Seqscape3 software.

3. Results and Conclusions
It was observed on the agarose gel that extracted DNA was degraded. Although, complete STR profiles of the samples incinerated at 100ºC and 200ºC were obtained, indicating sufficient nuclear DNA quantity. However, samples subjected to 300ºC exhibited only partial profiles, suggesting an insufficient quantity of nuclear DNA. Nonetheless, mitochondrial DNA amplification was successfully achieved across all temperature conditions.

This study concludes that mitochondrial DNA can be recovered and sequenced from incinerated samples exposed to high temperatures, especially in cases where results obtained from STR profiling are either partial or unreliable for identification purposes.

P-019

The recovery and analysis of DNA from burned bone and tissue.

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Objectives:
DNA identification of burned human remains is often difficult due to inadequate quantities of DNA for downstream analysis and inhibition due to charring and other fire residues. In this project, we tested different extraction and library construction protocols to see what increases the likelihood of successful downstream DNA identification using STRs and mitochondrial and genome-wide SNP capture and sequencing.

Materials and Methods:
Because ancient DNA (aDNA) research is also heavily invested in optimization of DNA recovery from challenging samples, we evaluated both aDNA (Velsko et al. 2020) and forensic (Loreille et al. 2007) extraction methods for bone sampled from donors subjected to controlled burning at the University of Tennessee's Forensic Anthropology Center (FAC) over a three-year period. Samples (n=109) were selected from different skeletal tissues and the level of burning of each element was characterized based on the observed discoloration of the bone. For a subset of the bone samples (n = 45), we also extracted DNA from adhering overlying charred tissue using a modified protocol for the Qiagen DNeasy® Blood and Tissue kit. For 109 bone samples, we constructed both double-stranded and single-stranded DNA libraries and then used these for the targeted enrichment of the mitochondrial DNA genome (H. sapiens Representative Global Diversity Panel) and genome-wide single nucleotide polymorphisms (FORCE-v2, Daicel Arbor BioSciences).

Results and Conclusions:
We found that charred tissue samples, when available, generally returned higher concentrations of both total DNA (Qubit HS DNA assay and Agilent TapeStation D5000 HS) and endogenous DNA (Quantifilier Trio) than bone extracted with either method. Also, the absolute quantities returned by these quantification assays were not a reliable predictor of successful data recovery in downstream methods, likely due to high levels of co-extracted inhibitors. Our preliminary analyses suggest inhibitors also affected NGS results, reducing recovery in burn category 2.5 but with lessening effects in categories 3 and 3.5. DNA recovery and mapped reads dropped significantly at temperatures greater than 350oC (burn categories 4 and 5). Innovative molecular methods can improve DNA recovery from burned samples for human identification, but further efforts are needed to identify and remove inhibitors generated during the burning process as well as to examine the rare conditions under which DNA recovery is possible in the highest burn categories.

P-022

Species-level thanatomicrobiome analysis in human intestinal tissue for forensic PMI estimation

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Estimating the post-mortem interval (PMI) accurately remains a challenging aspect of forensic investigations. Recent progress in microbial genomics and the development of DNA sequencing technologies have highlighted the thanatomicrobiome as a valuable resource for PMI estimation. In this study, we employed 2bRAD-M, an efficient metagenomic sequencing technique, to examine the human intestinal microbiome. Our study developed an artificial neural network model to explore the correlation between microbial succession and PMI in human intestinal decomposition, showing that species-level thanatomicrobiome data significantly improve PMI prediction accuracy. While the model demonstrated high predictive accuracy, especially at the species level with the goodness-of-fit (R2) value of 73.21%, future research will aim to increase sample sizes to reduce overfitting and further refine the model's performance in PMI estimation.

P-024

AI-based, automated detection of spermatozoa in standard forensic specimens.

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1. Objectives

In the field of image analysis, artificial intelligence, particularly using deep neural networks (DNNs), has shown significant effectiveness. These networks, trained on large collections of categorized image data, achieve high accuracy, for example, in detecting and identifying objects within digital images of microscopic samples.

This advancement holds particular importance in forensic investigations related to suspected sexual offenses, where analyzing Rape Kits and other evidence for the presence of sperm is a standard procedure. Traditionally, forensic labs rely on manual microscopy, a process that is both time-consuming and labor-intensive, requiring the expertise of specially trained personnel. The manual approach contributes significantly to backlogs in case processing due to its demands on time and resources.

2. Material and Methods

To address these issues, we have developed an automated workflow leveraging two DNNs trained for sperm detection and classification, aimed at streamlining the analysis of trace evidence. These networks were trained on forensic specimens prepared using common staining techniques, including "Christmas Tree" stain, Baecchi stain, and H&E, through supervised learning.

The detection network was trained on approximately 20,000 objects, while the classification network was trained on nearly 80,000 objects. The two deep neural networks are seamlessly incorporated into the imaging procedure, and the evaluation occurs simultaneously as the software examines the slides.

3. Results and Conclusions

Internal examinations using Baecchi-stained samples from a standard forensic laboratory demonstrated a detection sensitivity of 98.7% and a classification accuracy of 98.4%. A direct comparison of manual evaluation methods and DNN-based automatic microscopy on 80 specimens in a European forensic laboratory showed that 10 cases, previously identified as negative through manual microscopy, were actually positive.

These results indicate that our AI-enhanced method for identifying sperm on microscopic slides presents a viable option for decreasing the time and resources required by conventional microscopy. This, in turn, could help alleviate the backlog problem faced by forensic laboratories.
Our workflow integrates two DNNs with automated microscope imaging software to significantly improve the microscopy process in forensic labs. It generates a gallery of detected and classified objects, sorted by their likelihood of being sperm, providing forensic experts with a comprehensive, machine-generated document for rapid on-screen verification. Additionally, the workflow saves the captured image fields, enhancing documentation.

We will present the workflow's details and its application results in routine lab operations on standard preparations.

P-031

Geolocation of Chinese soil samples using eDNA and machine learning for forensic purposes

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When human DNA was not found at crime scenes or human DNA evidences could not provide effective information of the perpetrator, environmental DNA (eDNA) evidences may lead directions for solving crimes. The microbial DNA in the soil or dust transferred by the suspect to crime scenes may reveal unique microbial community structural characteristics related to geographical sources. The purpose of this study is to develop a Chinese soil DNA source inference model for forensic application scenarios based on machine learning methods. 16S rRNA sequencing data from 3,186 soil samples within China were collected, covering different environmental types such as forests, farmland, and grasslands. The data were categorized based on the Köppen-Geiger climate zones, the seven major geographical regions of China, and the administrative provinces. Five machine learning algorithms, namely Random Forest, Support Vector Machine, LightGBM, CatBoost, and XGBoost, were employed in the study. After hyper-parameter optimization, the models were evaluated based on metrics such as accuracy, recall, and F1 score. A total of 696,397 amplicon sequence vatiants were obtained through amplicon analysis of all samples. In predicting the classification of five Köppen-Geiger climate zones, the support vector machine model employing a radial basis function kernel achieved an overall inference accuracy of 94.24%. In the prediction of the seven major geographical regions in China, the LightGBM model achieved an inference accuracy of 93.83%. The study also used provinces as classification criteria. Among the 28 provinces covered by the samples, the linear kernel function support vector machine model achieved an accuracy of 88.91%. Based on the above results, we will further evaluate the inference performance of the model for other common environmental types, such as soil in urban residential areas.

P-038

A bioinformatic mixture study approach as an alternative to lab-based mixtures for massively parallel sequencing data

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1. Mixture studies are a crucial part of assessing new methods and required by accreditation standards for validation. Completing the lab processing of such an assessment can be costly and time consuming, particularly with massively parallel sequencing (MPS) methods or when many scenarios should be evaluated. An artificial, or bioinformatic (BI), mixture approach using existing single source MPS data to mimic a variety of mixtures would represent a substantial efficiency gain.

2. This approach was tested using a 95,000 SNP capture panel for extended kinship comparisons between family reference samples (FRS) and degraded case samples. To test the viability of a BI mixture approach, two anonymized FRS were used to create mixed DNA extracts which were processed in-lab and sequenced along with the single source extracts. To generate the BI mixtures, single source sample MPS data were down-sampled to target specific mixture ratios such that the total number of reads per BI mixture were consistent with the single source sample data. Analysis of both BI and lab-generated mixture data was performed in the Parabon Fx Forensic Analysis Platform (Fx).

3. Heterozygous proportion (HT%) of all SNPs was identified as a suitable metric for mixture detection, but HT% of X and Y SNPs were useful for determining sex and/or relatedness. The HT% was consistent between the lab-based and BI approaches, as was the mixture detection sensitivity. Mixtures were identifiable to a 1:19 ratio based on total SNP HT% greater than 33%. Results from additional two person BI mixture scenarios were used to determine that female:male and related male mixtures have imbalanced X SNP versus Y SNP HT% values, and female mixtures had X SNP HT% greater than 37.5% regardless of relationship. A similar approach could be used with a wide variety of data types and analysis programs, giving results that are needed to establish panel-specific interpretation thresholds.

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P-067

Loop Mediated Isothermal Amplification (LAMP) and Nanopore Sequencing as a Presumptive and Confirmatory Tool for the Identification of Endangered Species Common to the Illegal Wildlife Trade

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Wildlife crime can be described as any unlawful activity associated with the exploitation and trade of wildlife and constitutes the third most lucrative global illegal business behind narcotics and weapons. Unlawful activities include harvesting, transporting, exchange (money or goods), and end use of wildlife or wildlife-derived products. Rare or endangered species are often sought after by collectors as these items are typically the most prestigious and valuable. Globally, the illegal wildlife trade is regulated by the Convention on the International Trade in Endangered Species of Flora and Fauna (CITES, 1973), and domestically in New Zealand by the Trade in Endangered Species Act (1989). Currently, species identification of suspicious items is conducted using morphology or DNA sequencing-based methods, however there is a demand for a rapid, portable, and cost-effective screening tool that could be used at the border by Customs staff. Loop mediated isothermal amplification (LAMP) is an amplification technique that occurs at a constant temperature, mitigating the need for expensive laboratory equipment. This study evaluates the use of a colorimetric LAMP assay as a presumptive screening tool for the detection of species prevalent in the illegal ivory trade. LAMP products were then sequenced using nanopore sequencing to determine if this could provide a confirmation species identification result. Novel LAMP assays were developed to detect 8 CITES-listed species and 7 common adulterants in 30 minutes or less. The developed assays were tested for their specificity, sensitivity, and high amounts of non-target DNA. The results of this study provides new insights into the utility of this amplification and sequencing method as a rapid and simple presumptive or confirmation tool for the identification of species common in the illegal wildlife trade.

P-068

Highly portable, specific and sensitive platform for fast-track wildlife species identification.

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Wildlife preservation is paramount for maintaining ecological balance, regulating climate, and supporting sustainable livelihoods crucial for human well-being. However, the illegal trafficking of endangered animals and their products remains a formidable global challenge, recognized under the UN Sustainable Development Goals. Wildlife trafficking, with its intricate networks spanning international borders, poses significant hurdles for law enforcement due to differing legal systems and capacities among nations. Despite these challenges, the lucrative nature of the trade, driven by demand for rare species and their products, continues to incentivize traffickers. Insufficient resources for enforcement and conservation efforts further hinder effective protection of habitats and patrols.

A holistic approach involving collaboration among governments, law enforcement agencies, conservation groups, communities, and international stakeholders is imperative. This approach should prioritize enhancing enforcement capacity, reducing demand through education and sustainable alternatives, strengthening legal frameworks, and addressing underlying issues such as poverty and corruption.

At SERATEC, we are developing novel and highly sensitive diagnostic kits for animal species identification in close collaboration with government authorities specialized in combating wildlife crimes. Our project aims to significantly reduce the cost of identifying endangered species by offering a solution that demands reduced skilled labour and provides a cost-effective equipment-consumable kit, up to 5-6 times cheaper than current market alternatives.

Our efforts focus on pioneering a groundbreaking platform that integrates features of digital-PCR and isothermal amplification of DNA for wildlife species identification. This innovative approach promises swift and accurate detection crucial for combatting illegal wildlife trafficking and preserving biodiversity. By leveraging patented technology, we aim to revolutionize wildlife monitoring, enabling rapid and reliable species identification even in resource-constrained environments. This advancement holds immense potential for conservationists, researchers, and wildlife authorities, facilitating informed decision-making and effective management strategies against wildlife crimes. Our primary objective is to significantly contribute to the protection and sustainable management of wildlife populations globally while fostering a novel market in this field. A presentation at ISFG will provide invaluable exposure to our project, showcasing its innovative approach to wildlife species identification and highlighting its potential impact on combating illegal wildlife trafficking. This platform will facilitate networking opportunities, collaboration, and potential partnerships with experts and stakeholders in the field, accelerating project progress and adoption.

P-069

The Canine DNA Recovery Project: Current Findings and Next Steps

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The Canine DNA Recovery Project (CDnaRP) is a collaborative forensic project that aims to develop best practice methods for the collection and analysis of dog DNA recovered from attacked livestock and wildlife. The project works closely with police and forensic practitioners to optimise and standardise methods and also works with special interest groups including vets, rural insurers, farmers, and charities to ensure stakeholder awareness of the research and how it relates to livestock and wildlife offences involving canines.

Our research to date has developed and validated a robust qPCR assay for canine DNA quantification. The method acts as an important quality control step in the forensic DNA analysis pipeline and has allowed us to assess the effectiveness of different DNA recovery techniques such as swabbing, taping, and cutting. Other areas of our research have revealed the extent to which PCR inhibitors and livestock DNA prevent PCR amplification and canine DNA profile interpretation and has identified mitigation steps leading to improved data quality.

To build on these findings, our project has developed an Early Evidence Kit (EEK) for police and rural stakeholder groups to use in the event of a livestock attack. The distribution of these kits will allow a greater number of samples to be collected for the research and will begin to understand whether non-enforcement groups such as farmers themselves can collect admissible evidence in the future. Our presentation looks at the validation of this kit and considers their application in the UK Criminal Justice System.

P-070

NanoSSID: a bioinformatics pipeline for species identification in forensic genetics

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Objetives: Species identification has special forensic value in many cases, such as combating wildlife smuggling and tracking food adulteration. The mitochondrial 12S rRNA gene has sequence information characteristics of low intra-species variation and high inter-species variation, making it suitable for species identification.

Material and methods: In the present study, the 12S rRNA gene sequences of over 100 different species obtained from public database were used to develop an automated nanopore sequencing-based species identification pipeline, called NanoSSID. We sequenced 64 of these species and compared their sequencing results with each other to evaluate sequence similarity, and the NanoSSID was used to determine whether the detected species can be correctly assigned to the corresponding species. In addition, we assessed the ability of this pipeline to distinguish samples mixed with different species.

Results and conclusions: Most species exhibited over 80% similarity, and some species showed 95% to 99.5% similarity with 1 to 20 base differences. Species with significant differences can be correctly assigned to their respective species by the NanoSSID, while species with high similarity can only be identified by examining the different bases among similar species. Furthermore, this pipeline can successfully identify different components in mixed samples. Overall, the NanoSSID exhibited strong species identification capability and can play an effective role in the future.

P-078

Absolute Quantification of Bacterial DNA in Degraded Forensic Samples: A Comparative Evaluation of Real-Time PCR and Digital PCR

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Objectives: Quantifying bacterial load in degraded crime scene samples is essential for forensic investigations. Real-Time PCR is a widely used method, but its accuracy can be compromised in challenging samples. This study compared the performance of Real-Time PCR and digital PCR for quantifying bacterial DNA in highly degraded samples.

Material and methods: Sensitivity, precision, and accuracy of both methods were assessed using samples spiked with known concentrations of bacterial DNA. Additionally, inhibitor tolerance was evaluated by adding known PCR inhibitors to the samples.

Results and conclusions: Real-Time PCR showed a broad detection range but overestimated bacterial load, particularly in high concentration samples. Digital PCR demonstrated higher sensitivity and precision, especially for low bacterial loads. The limit of detection (LOD) and limit of quantification (LOQ) were lower for digital PCR, indicating better performance in degraded samples. Digital PCR also exhibited greater tolerance to PCR inhibitors compared to Real-Time PCR. Real-Time PCR limitations likely stem from factors like primer-dimer formation and non-specific amplification. Digital PCR's absolute quantification approach minimizes these issues, leading to more accurate results, particularly in degraded samples with low bacterial loads.

Digital PCR offers superior sensitivity, precision, and inhibitor tolerance compared to Real-Time PCR for quantifying bacterial loads in highly degraded crime scene samples. This makes it a more reliable and accurate tool for forensic applications.

P-080

Bacterial and Fungal Succession in the Oral Cavity and Soil Surrounding Decomposing Pig Carcasses

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Post-mortem interval (PMI) estimation is a key challenge frequently encountered in forensic science. Microorganisms are strongly associated with the decomposition of bodies and have been shown to harbour great potential acting as a "microbial clock". To contribute to a better understanding of the underlying dynamics of microbial succession and its applicability in practice, we investigated the temporal changes in microbial communities during the decomposition of six pig carcasses over a five-month period, quantified using the Total Body Score (TBS) system, in a temperate Swiss forest. 96 buccal swab samples and 94 soil samples were characterized in their bacterial and fungal community compositions by full-length 16s rRNA and ITS PacBio Sequel IIe sequencing. Our long fragment amplicon dataset allowed for a high taxonomic resolution with a classification at species level between 50.2 – 63.5% over all ASVs. We found trends linking certain microbial taxonomic groups with specific TBSs, which is also reflected in PCoA analyses based on Bray-Curtis dissimilarities between samples, highlighting a continuous and consistent shift in community compositions in both sample types with increasing TBS. PERMANOVA testing showed a strong association of buccal microbiomes with PMI ($R2 = 0.437 – 0.456$; p-value < 0.001) and soil communities with TBS ($R2 = 0.167 – 0.118$; p-value < 0.001). Our results, coinciding with previous studies on exposed bodies, also show the incomplete recovery of native soil microbial communities after a period of five months. Based on the consistency of our results and predictability of microbial propagation, we believe that long fragment metabarcoding approaches have considerable potential in criminal investigations. We believe this to be especially valuable when examining outdoor crime scene where the body has been relocated and for resolving cases with extensively decomposed remains.

P-083

Using the microbiome in soil to locate the origin of soil stains

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Methods that give information that complements or contextualizes human DNA evidence or provide information in cases where no human trace material is found are especially important in forensics. Genomic analysis of microbial composition has the potential to be such a method. One use of microbiomics in forensic science is to investigate the origin site of a soil stain from suspects' clothes or shoes, which can help to link the trace to the crime scene.

To integrate such a method in the forensic toolkit, the techniques used needs to be standardized and accredited, and the limits of the method needs to be thoroughly understood, to establish under which circumstances it may provide useful information.
This research focuses on understanding under which conditions microbiomics can be used to tie a soil sample to an area of origin. The specific aim is to explore variation in the microbiome at a site over time and investigate whether soil stains deposited on clothes can be accurately tied back to their original site.

Soil samples have been collected from 13 sites in the Oslo area with two weekly repetitions. In addition, mock soil stains on clothing were created and collected from each area at the first time point of soil collection. To investigate seasonal variations a selection of sites were sampled in the summer in addition to the main sampling in the fall. The microbial composition in each of these samples was determined with amplicon sequencing of the variable region 4 of the ribosomal 16S gene. Differences in the microbial composition between locations were assessed, and samples collected from the same site at different dates tended to cluster together. Supervised learning was used to classify mock cloth stain samples back to their original location.

Keywords: Microbiomics, environmental traces, method development

P-087

Development of Species-Specific cf-DNA Markers for Improved Accuracy in Wildlife Forensics

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The need for precise and dependable methods of species identification arises from wildlife conservation's growing reliance on non-invasive monitoring approaches. Without direct capture, environmental DNA (eDNA), and more especially cell-free DNA (cfDNA), presents a viable method for identifying cryptic or elusive species. However, universal primers used in traditional eDNA analysis frequently amplify DNA fragments from a wide variety of taxa, which may result in misidentification or decreased sensitivity for target species. The creation of species-specific cfDNA markers for increased precision in animal forensics is presented in this work.

To find informative genetic areas with high sequence divergence between target and non-target species, we used a focused method. Analyzing whole-genome sequencing data from a variety of animal species that are important to conservation efforts was part of this. Short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs) with substantial intraspecific variability and low interspecific variance within the target species were identified using bioinformatics workflows. Afterward, the primer design and possible amplification efficiency of these candidate markers were assessed in silico.

The most promising species-specific markers were chosen for wet lab testing after in-silico validation. Real-time PCR experiments with cfDNA isolated from a variety of environmental materials, including soil, water, and air, were used to evaluate the performance of these markers. Tests of specificity were carried out with cfDNA derived from non-target species to guarantee low levels of amplification. Furthermore, sensitivity tests were carried out to establish the target species' cfDNA detection limit.

It is anticipated that the outcomes will show how useful species-specific cfDNA markers are for improving wildlife forensics' accuracy. Through the reduction of non-target DNA amplification, these markers will provide more accurate species identification from environmental sample data. Numerous applications related to wildlife protection will be significantly impacted by this increased accuracy, including:

1. Population Monitoring: Species-specific cfDNA markers improve accuracy in estimating population size and mapping species dispersion.
2. Tracking Species Movements: Analysis of cfDNA reveals insights into animal dispersal patterns and habitat connectivity.
3. Fighting Wildlife Crime: Precise identification of species from poacher materials enhances law enforcement efforts against illegal wildlife trade.

The development of species-specific cfDNA markers marks a significant advancement in wildlife forensics, promising enhanced conservation strategies and revolutionizing non-invasive animal monitoring. This innovation opens avenues for future research exploring the application of these markers in diverse ecological contexts, contributing to the growing body of knowledge on eDNA utilization for wildlife conservation.

P-122

NEWPAT: Development of a non-invasive paternity test with high specificity

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The non-invasive prenatal paternity test (NIPAT) leverages small amounts of cell-free fetal DNA (cffDNA) present in maternal blood. Conventional non-invasive tests rely on single nucleotide polymorphisms (SNPs), but face challenges such as low fetal DNA fractions and high error rates stemming from targeted next-generation sequencing (NGS). To enhance accuracy, we introduced a pioneering NIPAT approach (NEWPAT) employing double nucleotide polymorphisms (DNPs). We selected 978 genome-wide DNPs from the Genome Aggregation Database, amplified these markers using the Ion AmpliSeq™ Kit Plus from Thermo Fisher Scientific, and sequenced them with the Ion Torrent™ S5 platform. System validation encompassed: 1) 5000x sequencing across 4 families, to assess Mendelism and DNP amplification bias; 2) re-sequencing at 500-1000x, to evaluate sensitivity; and 3) ultra-high depth (10000x) sequencing of 12 pregnant women and the putative fathers, to evaluate system reliability and estimate combined paternity index (CPI) in real-word scenarios. Since DNPs are not routinely used as markers in most genomic analyses, a standardized bioinformatic workflow for their analysis from NGS data has yet to be established. We developed an ad hoc pipeline and applied rigorous quality control measures to ensure precise allele calling at each DNP position while safeguarding against contamination. Our methodology significantly enhanced the discrimination between cffDNA alleles and sequencing artefacts, crucial for paternal allele detection. Sequencing error rates for most DNPs were negligible and significantly lower compared to SNPs, highlighting the precision of our approach. For all the pregnancies tested, we obtained CPIs > 10^{25}, far exceeding the thresholds for paternity attribution. This innovative methodology represents a pivotal advancement in prenatal diagnostics, offering a new level of accuracy and reliability in genetic testing.

P-126

Expansion of familial searching capabilities through simultaneous searches and Y STR based searches

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Since 2012 the NFI has conducted familial searches in the Dutch criminal DNA database in 150 serious criminal offence cases and unidentified human remains cases. Approximately 10% of the searches result in new investigative leads for law enforcement.

In 2023 two projects were started with the objective to increase (1) the number of familial searches conducted each year and (2) the reach of the familial searches through Y STR searching.

Until now, cases eligible for familial searching were handled one by one and the legal procedures were labor-intensive and time-consuming. A project team consisting of representatives from the Dutch Police, Dutch Prosecution and NFI has proposed to conduct a pilot for simultaneous familial searching in 100 selected cases from one of the police forces. Handling cases as a batch without the full legal assessment will increase efficiency and reduce turnaround time. A legal analysis was presented to an investigative judge. If the proposal is legally approved, simultaneous familial searches will be conducted using the ProbRank search method, integrated in the DNAxs expert system. If this simultaneous approach is successful, the pilot will be expanded to all police forces in The Netherlands. Details on the legal aspects and software solutions will be presented.

Familial searches based on autosomal STRs are limited to finding potential first degree relatives (parents, children, siblings) in the criminal DNA database. Inclusion of Y STRs will expand the reach to the whole male pedigree, including uncles, cousins, nephews etc. To accomplish this, the NFI received funding to upgrade DNA profiles from suspects and convicted offenders in the Dutch criminal DNA database with Y STR profiles. The goal is to have an additional 60.000 Powerplex Y23 profiles available for familial searching by the end of 2024. Results of optimizing high throughput Y STR profiling and impact on familial searching results will be presented.

P-127

Unravelling Familial Bonds: Differential Profiling of Closely Related Males from a single family (Satuniya-Patel Family) of India using 26 Rapidly Mutating Y-STR Markers

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Objective
Rapidly Mutating Y-STRs (RM Y-STRs) offer a promising avenue for differentiating paternally related individuals, overcoming the limitations of standard Y-STR markers. This study investigates the forensic applicability and mutation dynamics of RM Y-STRs within a single family hailing from Gujarat state in India.

Methodology
The Microreader™ 26 RM-Yplex ID System, comprising 26 recently developed RM Y-STRs, was employed to assess its forensic utility to differentiate the closely related males. Blood samples (5.0 ml) were collected from a cohort of 65 males from Satuniya – Patel family, and DNA extraction was performed using DNEasy Blood and Tissue Kit. Quantification was carried out using the Bio Tek Cytation-5 Imaging plate reader, followed by DNA amplification with the RM Y-STR markers. Fragment separation was achieved using the 3500 Genetic Analyzer, and data analysis was conducted using Gene Mapper ID-X v1.5 software.

Results and discussion
Our findings revealed significant differentiation between paternally related men, with the Microreader system exhibiting increased resolution with successive meiotic generations. The differentiation percentages ranged from 50% (1st meiosis) to 72.7% (4th meiosis), with a mean differentiation rate of 65.6%.

Furthermore, the study evaluated the mutation rates of RM Y-STRs. Analysis targeting inter-generational differences indicated slightly higher mutation rates in third-generation differentiations, followed by second and first meiosis. Specifically, the mean mutation rate among father-son pairs on RM Y-Chromosomes was found to be 0.02 (SD = 0.037), significantly higher than the expected rate of 0.01 ($t(33) = 2.82$, $p = 0.008$).

Conclusion
These findings underscore the forensic significance of RM Y-STRs, particularly in addressing cases involving complex DNA mixtures, such as sexual assaults. Additionally, the observed mutation rates highlight the dynamic nature of RM Y-STRs within the same family. Future research should explore the broader applicability of RM Y-STRs across diverse populations and investigate the factors influencing mutation dynamics.

P-137

Test of the Hy-liter system for the identification of spermatozoa in sexual assault cases

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The detection of spermatozoa in forensic samples, for instance vaginal swabs, gives information on the presence of sperm. Immunochromatographic tests, such as PSA, are subject to false positive and false negative results. Contrary to that, the observation of several spermatozoa is a strong indication of the presence of semen. In the present study we compared the fluorescent detection of spermatozoa, using the Hy-Liter kit from Independant Forensics, with the Christmas tree staining routinely used in our laboratory. Sets of mock and real samples were compared in term of ease of staining and spermatozoa identification, time needed to screen whole slides, specificity of the staining in presence of mixed biological material which can be found on sexual assault samples, stability of a positive event detection over time, reproducibility of staining, as well as reagents handling, storage and price. The different results obtained during the comparisons presented here have led to the Christmas Tree staining being retained in our laboratory as a method of choice for the time being.

P-138

Development of a new automated sexual assault sample processing kit

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The analysis of sexual assault samples is often challenging as insufficient separation of cells from the involved persons leads to mixed profiles. Since identification of the perpetrators in all conventional methods is based on the DNA analysis of sperm cells, successful testing is largely determined by the relative amount of sperm DNA recovered. Consequently, new methods need to improve sperm cell recovery, precision of separation between sperm cells and DNA coming from non-sperm cells, and efficiency of sperm cell lysis to add value. A new sexual assault sample processing kit has been developed to address these requirements. The kit is based on the principle of differential extraction, which uses the different properties of sperm cells and all other cells that occur in sexual assault samples. This enables the generation of individual profiles of the perpetrator from which the sperm cells originated.

The new sexual assault sample processing kit is automated and uses a newly developed lysis buffer for the efficient and specific lysis of non-sperm cells. The kit provides improved sperm DNA recovery and efficient separation between sperm cells and DNA coming from non-sperm cells. The automated workflow provides reduced hands-on time, high ease-of-use and highly reproducible results.

We will present data from our current development.

P-139

Alkaline lysis of sperm cells from forensic sexual assault samples

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Differential extraction is typically used for forensic sexual assault samples in order to separate sperm cells (from the perpetrator) from other cells (predominantly from the victim). Sperm cells are more robust compared to e.g. white blood cells and epithelial cells and therefore require tougher lysis conditions. Traditionally, the reducing agent DTT in combination with Proteinase K is used in the digestion of sperm cells in forensic sexual assault samples. Since DTT may inhibit the downstream PCR process, the extracts must be purified. Purification may be performed using e.g. phenol/chloroform, magnetic bead-based chemistries or filter devises. Application of any of these methods increases time and cost and leads to DNA loss. In this study, we evaluated alkaline lysis as an alternative to the traditional lysis using DTT and Proteinase K. In the alkaline lysis protocol, NaOH is used to disrupt the cell and nucleus membranes and denature nucleases. This is followed by the addition of Tris-HCl to neutralize the lysate. The effect on DNA yield of different molar concentrations of NaOH and Tris-HCl was evaluated to determine the optimal lysis conditions. Overall, the chosen alkaline protocol showed a higher DNA recovery compared to lysis using DTT/Proteinase K (mean values of 0.075 vs. 0.035 ng/µL, p=0.025). The quality of the STR profiling results was equivalent for the two lysis principles. Quantification and STR typing showed no indication of degradation after storage in refrigerator or freezer for up to two weeks. In addition, there was no need for a purification step when using alkaline sperm cell lysis, reducing the hands-on time and total processing time. By introducing alkaline lysis, the laborious differential lysis DNA extraction protocol can be substantially simplified and streamlined, leading to shorter turnaround times and lower cost.

P-140

Cellular Selfies – Optimisation of Flow Cytometry-Based Methodologies for Identification and Isolation of Epithelial Cells in the Context of Forensic Science

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Linking a DNA profile to a source of biological material has significant implications in forensic investigations and judicial considerations, as it can provide important information about the alleged crime. For example, in an alleged sexual assault, the identification of a specific cell type on the penis (e.g. cells from the vagina, saliva or other skin areas) may provide support for either the prosecution or the defence. 'Touch DNA' samples are largely comprised of epithelial cells and represent a large proportion of the samples processed from crime scenes. However, there remains an unmet need for confirmatory tests to accurately distinguish and isolate epithelial cells from different anatomical regions. Flow cytometry represents a potentially reliable and accessible method to discriminate between epithelial cell types. Given that samples collected as part of casework differ from those routinely analysed by flow cytometry (e.g. cancer cells), we sought to determine the optimal conditions that yield intact epithelial cells for subsequent analysis/isolation by flow cytometry. This study describes the progress towards the development and optimisation of methods for the collection and preparation of skin, vaginal, penile and buccal samples that enable epithelial cells to be distinguished and isolated using fluorescence-activated cell sorting (FACS) and imaging flow cytometry (IFC).

P-141

Mass spectrometry-based proteomics for source-level attribution of body fluids after DNA extraction

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When biological traces are recovered from crime scenes, the ability to report the nature of the body fluid (source level attribution) is valuable evidence, as this can assist investigations and legal deliberations. While some forensic serological techniques currently accepted by the forensic community have been successful, they pose certain limitations, including their nature as individual tests performed separately, targeting one biological fluid at a time. This may be particularly challenging when dealing with limited sample availability.

The method described in this research addresses these limitations and allows for the multiplex analysis of body fluids, where multiple analytes in one sample can be targeted simultaneously from a forensic biology laboratory waste stream. The method involves protein isolation from the non-DNA component of the DNA extraction process for biological fluids collected from consenting volunteers. This means there is no need to sample individually for DNA and then for proteins. The overall outcome of this study will lead to a single assay confirmatory test to detect multiple biological fluids simultaneously from a DNA profiling waste stream, which enhances the efficiency of forensic analysis in cases involving biological evidence. The method includes acetone precipitation, protein isolation using the single-pot solid-phase-enhanced sample preparation (SP3) technique, peptide digestion, and separation and analysis by nanoflow liquid chromatography coupled to a hybrid quadrupole-orbitrap mass spectrometer.

Samples included human blood, saliva, semen, urine, and menstrual blood, typical of forensically relevant stains, each from multiple individuals. The resulting data files were searched against the Human Proteome Database, and the analysis of these samples led to the characterisation of proteins specific to body fluids and candidate peptide biomarkers to be targeted for distinguishing between body fluids for identification purposes.

P-144

Efficiency of differential extraction techniques: automated versus manual separation of sperm from non-sperm cells

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Differential extraction (DE) is a well-known approach to separate non-sperm cells and sperm cells. The DE method is based on an initial mild lysis step to digest non-sperm cells. After separating the non-sperm fraction (NF) from the sperm cells, the sperm cell fraction (SF) is subjected to a more stringent buffer to lyse the sperm cells, resulting in separate DNA extracts of both the NF and the SF fraction of the sample.

At the Netherlands Forensic Institute (NFI), the majority of the sexual assault samples are processed using a manual DE method resulting in approximately 150 samples subjected to DE each month. Although this manual approach is effective, the lab workflow would benefit from a (semi-)automated DE protocol, lowering the hands-on-time and reducing the risk of human-errors.
In this study, results are presented comparing our manual DE with (semi-)automated DE. The performance of the manual and automated extraction is evaluated using a set of identically prepared mock samples along with more realistic casework samples (individually taken vaginal swabs), where more sample variation is to be expected. The NF and SF separation efficiency is compared based on DNA quantification and autosomal STR-typing results.

Preliminary results are promising and show an improved separation efficiency especially for the SF of the automated procedures, that now mainly contain alleles of the sperm donor, and almost no alleles matching the non-sperm donor.

P-145

mRNA profiling and donor association of mock case samples: Results of two EDNAP collaborative exercises

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The European DNA Profiling Group (EDNAP) has previously evaluated the performance, robustness, and reproducibility of various mRNA markers for identifying body fluids using capillary electrophoresis (CE) and massively parallel sequencing (MPS) methods. MPS of mRNA targets is used for body fluid identification and provides information on who contributed which body fluid to a binary mixture, thereby adding important contextual aspects to a crime scene investigation. The analysis of coding region SNPs (cSNPs) in body fluid-specific transcripts allows the association of an individual's DNA cSNP profile with the body fluid-specific RNA cSNP genotype.
The Zurich Institute of Forensic Medicine in Switzerland organized two consecutive collaborative exercises within the EDNAP group to evaluate the performance of two targeted mRNA sequencing assays: the BSS cSNP assay (for blood, saliva and semen) and the 6F cSNP assay (for six fluids/tissues, including BSS and additionally vaginal secretion, menstrual blood and skin). Each cSNP RNA assay was accompanied by a genomic DNA assay to genotype the cSNPs in the person(s) of interest.
Eleven laboratories participated in one or both of the EDNAP collaborative exercises. In each exercise, 16 mock case samples were provided by the organizers, and the laboratories could analyze additional, self-prepared stains. Participants could use either the Ion S5 or the MiSeq sequencing platform. Here, we present the compiled results of the two collaborative exercises. We investigated DNA and RNA yields, STR profiles, and RNA profiles for body fluid identification and body fluid - donor association. While the mock case samples provided information on the performance of the assays, the self-prepared stains were a blind test for the organizers to identify the body fluids and the contributors.

P-146

Connecting the dots: matching a genetic profile with its cell type in mixed evidence, at a single cell level

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Objectives
Complex DNA profiles occur when genotyping a mixture of DNA of different individuals. Each individual can contribute in the mixture with different body fluids and the mixture might be composed of the same type of fluid (e.g. blood of more individuals) or of different type (blood and semen). To generate the genetic profile, cells in the biological mixture must be lysed to release their DNA, losing the possibility to assign the profile to cell phenotype, thus to the corresponding biological fluid. Isolation of intact cells, prior to genotyping, can be the tool to match phenotype and genotype, down to single cell level.

Material and methods
Biological mixtures were created by adsorbing 2 µl of semen, 20 µl of blood, and 80 µl of saliva, from different donors, on flocked swabs, obtaining a mixture semen:blood:saliva of 1:10:40.
Swabs were processed using the DEPArray™ Forensic SamplePrep Kit, staining simultaneously sperm (SC), epithelial (EC) and white blood cells (WBC) with fluorescent labelled antibodies, for their image-based identification and isolation at the DEPArray™. Genotyping was performed with PowerPlex® Fusion 6C (Promega), followed by analysis at GeneMapper® ID-X (threshold at 50 RFU).

Results and conclusions
Out of 242 recovered single cells (52 EC, 66 SC, 124 WBC), 237 (98%) passed the acceptance criteria. For each profile, concordance, completeness, specificity and stutter presence were calculated with respect to their reference gDNA profile.
Average single cells profile completeness was 73%, while keeping profile's accuracy (mean accordance = 96%), and negligible non-specific peaks and stutters. Each single cell profile could be assigned to the corresponding cell type and the single cell fluorescent image coupled unequivocally to its genetic profile.

Single cells constitute the perfect specimen in which genetic information of an individual is present entirely. Genotype and phenotype can be matched at a single cell level, offering a new powerful tool to unravel the biological origin of the evidence.

P-148

Identification of body fluids using RT-LAMP isothermal amplification coupled with CRISPR-Cas12a

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While often necessary in sexual assault cases, confirmatory identification of body fluids can be a lengthy and/or costly process. In particular, the detection of vaginal fluid and menstrual blood in forensic casework is limited to endpoint reverse-transcription PCR to detect fluid-specific messenger RNA (mRNA) markers. It is not currently possible to detect rectal mucosa, the presence of which would provide probative value in cases of alleged anal penetration. Reverse-transcription loop-mediated isothermal amplification (RT-LAMP) is an alternative technique that enables detection of mRNA at a single temperature (usually 60-65℃) for 10-30 minutes and has comparable sensitivity to PCR. We will describe the coupling of RT-LAMP amplification with CRISPR-mediated fluorescent detection of the body fluid specific mRNA markers MMP3 (menstrual fluid), CYP2B7P (vaginal material), TNP1 (spermatozoa), KLK2 (semen), and MUC12 (rectal mucosa).

Following temperature optimisation and final selection of CRISPR-RT-LAMP assays, specificity across circulatory blood, buccal, menstrual fluid, vaginal material, semen, and rectal mucosa was established. Assays were specific for their target body fluid, although MMP3 and CYP2B7P were detected in some rectal mucosa samples. A range of assay approaches were investigated to develop a protocol suitable for use in a forensic screening laboratory. This included determination of fluorescent assay results by eye, use of lyophilised reagents, and RT-LAMP and CRISPR reactions undertaken in one-tube in a lower resource setting.

P-149

Rapid On-site Screening of Male, Saliva, and ABO blood types using Direct Loop-mediated Isothermal Amplification (LAMP) and Real-time Polymerase Chain Reaction (PCR)

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Distinguishing bloodstains from male suspects at crime scenes where most bloodstains are from female victims and distinguishing between impact spatters and expiratory bloodstains containing saliva, which are morphologically similar, are very important for efficient DNA analysis and crime scene reconstruction. Additionally, ABO blood typing is a useful method for screening suspects. However, it takes a lot of time, money, and labor to analyze all biological evidence. Therefore, it is necessary to select valid evidence that provides investigative information from a lot of evidence. Typical PCR-based DNA analysis take a long time and require expensive equipment, making it difficult to use at the crime scene and extracting DNA is also very difficult. In this study, we established optimal conditions for loop-mediated isothermal amplification (LAMP) and multiplex real-time polymerase chain reaction (PCR) without a DNA purification. The SRY gene, located on the Y chromosome, only found in males, and the MetAP gene, specific to Streptococcus salivarius, only found in saliva, were selected as male and saliva-specific markers. Three SNP sites (261, 803, and 526) in the ABO gene were selected as markers to identify ABO genotypes. Four commercial lysis buffers (Rapi:Direct™ Lysis Buffer (Genesystem), One-Step DNA/RNA Extraction Buffer (CHAI), MightyPrep for DNA (Takara), and REDExtract N-Amp™ Blood PCR Kit (Sigma Aldrich)) were selected for direct amplification, and blood and saliva were serially diluted in five steps for direct DNA extraction with each buffer, D.W., and NaOH. LAMP reactions were performed by two methods: colorimetric and real-time fluorescent, and real-time PCR was performed for ABO genotyping. All reactions were optimized for 30 min at 65°C. The colorimetric LAMP kit for male or saliva identification of blood and/or saliva samples amplified only in D.W. among the 6 solutions, as the pH of the lysis solution affected the phenol red. The fluorescent LAMP kit showed the highest sensitivity for NaOH-treated samples. Positive results were obtained within 30 minutes, even at dilutions as low as 1/125 for blood and 1/25 for saliva. For screening with ABO genotyping, the real-time PCR and LAMP kits were also able to fully analyze the six genotypes only in NaOH-treated samples. The detection sensitivity allowed analysis of saliva diluted down to 1/125. Commercial buffers were not suitable for use in terms of sensitivity and cost. Therefore, direct LAMP and direct real-time PCR with D.W. and NaOH are expected to enable quick and easy screening of valid evidence at crime scenes.

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Developmental validation of an mRNA-cSNP profiling panel for body fluids identification and individualization

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Objetives: Forensic analysis of body fluid stains is extremely important, which helps reconstruct crime scenes and determine the guilt of suspects. It involves two main challenges: body fluid identification and individualization. The former aims to ascertain the type/origin of the body fluid stain, while the latter tries to assign the stain to a specific donor. Generally, they are addressed through RNA and DNA analyses, respectively, but this can be time-consuming, laborious, and sometimes yield suboptimal results, especially mixed body fluid stains.

Material and methods: We developed an innovative mRNA-based sequencing panel with a dual function. This system comprises 34 body fluid-specific mRNA genes and 39 cSNP markers (amplicon length: 50-90 bp), enabling a direct link between specific body fluids and their donors. We thoroughly validated the integrated system according to the guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM) on the MGI massively parallel sequencing (MPS) platform.

Results and conclusions: As a result, this panel showed robust identification efficiency for body fluids at a 20 ng RNA input, and the range of cumulative discrimination power (CDP) value was 0.802445047~0.999310789 in five types of body fluid. Furthermore, it had been employed in actual cases and provided the guidance. In conclusion, this system can efficiently, rapidly, and accurately perform body fluid identification and individualization for single-source, mixed, and degraded samples, meeting the daily investigative demands in forensic genetics.

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Evaluating novel and conventional cell-separation techniques for sexual assault investigations and first applications

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Biological evidence from sexual assaults frequently contains few male and numerous female cells. In practice, their genetic analysis usually begins with separating the victim's from the perpetrator's cells using either conventional differential extraction (DE) or more advanced cell capturing techniques. Thus, this study aimed to evaluate the advantages and drawbacks of standard and novel methods in sexual assault cases, providing a comprehensive understanding of their merits and postulating potential applications of modern, image-based approaches.

A descriptive study on simulated sexual assault samples was carried out by the recruitment of ten heterosexual, monogamous couples. Post-coital swabs were collected before and after consensual sexual intercourse, with a sampling period of up to 96 h, and subjected to analysis to detect, quantify, and genotype adhering sperms by three distinct cell-separation techniques: DE, laser capture microdissection (LCM), and DEPArray™.

The methods differed in their sperm detection and genotyping efficacy, while foreign DNA was generally identifiable up to 96 h, with time since intercourse and individuals being statistically significant influences. While DE is an efficient, well-established enrichment tool, more attention should be paid to presumptive tests on seminal fluids, which yielded false positive results in half of the references. Furthermore, we will present a pivotal sperm enrichment step for LCM analysis, and demonstrate an additional demand for the more complex DEPArray™ analysis. Lastly, to bridge the DEPArray™ application to casework, we will show relevant optimizations (e.g., substrate, sample count), and address storage-induced degradation effects.

Overall, these image-based applications support conventional ones in scenarios where DE is inappropriate, such as cases involving cell-like mixtures or crimes with multiple offenders or when traditional approaches yield inconclusive or insufficient results.

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A new strategy for associating donors with forensic body fluids based on STRs and biofluid-specific CpG markers

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The identification of body fluids in biological stains at crime scenes and their association with donors increasingly impacts the validity of evidence for case investigations and prosecutions. While autosomal STR and DNA methylation markers can effectively analyze personal identification and body fluid origin respectively, there currently lacks a system that integrates STR typing (sub-source level) and biofluid identification (source level) for simultaneous detection and analysis. This study has developed an analytical strategy for simultaneous personal identification and biofluid origin determination using capillary electrophoresis, based on DNA methylation markers (CpG) and an STR amplification system. The biofluid-specific CpG markers, which were selected from the previous reports, were verified using a methylation sensitive restriction enzyme-PCR (MSRE-PCR) technology. The 5 candidate biofluid-specific CpG markers, along with 2 control markers were integrated into a 7-plex amplification system. Finally, amplification products of the 7-plex CpG amplification system and a commercial 21-plex STR amplification system were mixed and co-detected through capillary electrophoresis. The results showed that a mixed detection system based on 5 candidate biofluid-specific CpG markers and 21 STR loci can effectively identify five types of body fluids from single sources—peripheral blood, semen, saliva, menstrual blood, and vaginal secretion—with the cumulative discrimination power (CDP) for individual identification reaching 1-3.51198×10-25. Semen and vaginal secretion stains with 0.1 ng DNA input or stored at room temperature for 5 months could still be identified efficiently. Moreover, it could produce successful DNA methylation profiles in mixed samples consisting of two to four body fluids in varying proportions. This study provides a good strategy for simultaneous identification of body fluids and their donors in forensic science.

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Automation in DNAxs makes large scale DNA forensic intelligence on criminal cooperation and organization a reality

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Additional developments in automation via DNAxs makes it possible to routinely perform large scale DNA forensic intelligence investigations at The Netherlands Forensic Institute (NFI). In 2019 the NFI started the first large scale DNA forensic intelligence case with the aid of DNAxs, consisting of approximately 300 thousand comparisons between 550 complex mixed DNA profiles (from 50 preexisting cases) and 540 persons. However, this still required a lot of manual steps and comparisons, resulting in a time consuming investigation. Despite of these difficulties this first case was a success and prompted the Police and Prosecution Office in The Netherlands to request more large scale DNA forensic intelligence cases to undermine criminal cooperation and organization. To be able to handle more and even larger cases additional development in automation (DNAxs) was needed. To automatically upload reference DNA-profiles of persons of interest and traces, automatically search the DNA database with mixed DNA-profiles up to four persons (with ProbRank) and the facilitation of large DNA comparison datasets, is now possible in DNAxs. Thus increasing efficiency, reducing error and aiding the decision making of the DNA scientist.

These developments make DNA forensic intelligence less time consuming, increase the possibility of finding additional donors or connections and allow for even larger scale DNA comparisons. Thus providing the Police and Prosecution Office with a powerful DNA intelligence tool for insights into criminal cooperation and organization.

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Fast DNA IDentification Line 2.0, an automated software expert system for (mixed) DNA-profile analysis, interpretation, comparison and reporting in criminal casework

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Today there is a need for more and faster availability of DNA results in criminal cases. Laboratories have invested in faster procedures and automation and to handle more samples. This can result in bottlenecks in DNA analysis/interpretation and reporting. The Netherlands Forensic Institute (NFI) has therefor developed a fully automated software system for DNA profile analysis, interpretation, comparison and reporting 'Fast DNA IDentification Line (FIDL)'. The FIDL automatically generates DNA profiles after capillary electrophoreses, removes artifacts, does a cross-contamination check and compares DNA profiles of a trace with DNA profiles in the case or DNA database and report the results. This automated workflow functions with complete oversight by experts. The processing time, from receiving the trace at the NFI to reporting the automated results, is reduced to three working days. Allowing the police and prosecuting authorities to act faster on the bases of DNA results.
The FIDL 1.0, only reported results of derived major or single DNA profiles and was tested and used in practice for 2,5 years with one regional police unit. In the 2.0 version mixed DNA-profiles up to four persons can now be searched and rank based on peak heights with ProbRank (based on DNAStatistX). This new version is able to report more potential donors of mixed DNA-profiles than the 1.0.
The FIDL 1.0 has already proven its ability to increase the efficiency and effectiveness of the whole criminal justice system, by decreasing the time needed from crime scene to court. We can now include mixed DNA profiles in FIDL 2.0, allowing an even greater impact in fighting crime not only for Rotterdam but the whole of the Netherlands. Results will be presented for the development, validation and performance in real casework for FIDL 2.0.

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Increasing Open-Source Applied Genetics Software Adoption through a Community-Based Development Project: Forensic Genetics Open-Source Software (F-GOSS®).

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Objectives:
Science for Social Good CIC, headquartered in Manchester, UK, is a non-profit organisation promoting vocational training programs and advancing open-source software development. The organisation supports existing tools via its website, SSG-CIC.org, through collaboration with open-source authors, volunteers, hobbyists, academics, and service laboratories engaged in applied genetic testing.

Open-source software development is widely recognised for enhancing collaboration, innovation, and accessibility across various knowledge-based sectors, particularly in applied genetics. However, the rapid influx of new open-source software in this field raises concerns about reusability and long-term development. Specifically, within forensic genetics, ensuring the reusability of open-source software is paramount to prevent redundancy, facilitate building upon existing work, and expedite advancements in forensic science practice. Although frameworks such as FAIR4RS[1] (Findable, Accessible, Interoperable, and Reusable for Research Software) and FAIR-BioRS (FAIR-Biomedical Research Software)[2] provide guidelines and actionable instructions, the unique medico-legal implications of forensic genetic software necessitate ongoing discussion and refinement to establish agreeable standards of practice.

Approach:
Through our poster at the ISFG 2024 congress, we aim to

(1) Demonstrate the proposed F-GOSS® Platform, featuring a Forensic Genetics Workbench (FGW™) that integrates freely available, open-source Forensic Genetics Validation and Verification resources developed by Science for Social Good with existing tools such as Oskar Hansson's STRvalidator software[4].

(2) Raise awareness within the forensic genetics community about our approach to organising various open-source tools, including software, plug-ins, pipelines, and knowledge bases, within the F-GOSS platform.

Call to Action:
We invite contributors, developers, software experts, and users to join us in building a community and ensuring the widespread accessibility of these resources - increasing their adoption and further refinements. We are committed to providing frontline support through our helpdesk, managing back-end maintenance tasks, and incorporating strategic insights from the community (i.e. in the form of feature requests, integration bottlenecks, reporting bugs and proposing solutions or more efficient alternatives, to name a few) to shape the future roadmap for open-source software development. In this process, we will also engage with the community to discuss policy matters relating to software use, fair recognition of authors and developers, reducing unnecessary commercial software spending through training and awareness campaigns, incentivising contributors through boot camps, hackathons/competitions and other opportunities to make it rewarding.
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(1) Patel, B., Soundarajan, S., Ménager, H. et al. Making Biomedical Research Software FAIR: Actionable Step-by-step Guidelines with a User-support Tool. Sci Data 10, 557 (2023). https://doi.org/10.1038/s41597-023-02463-x
(2) https://fair-biors.org/docs/getting-started/intro
(3) https://www.software.ac.uk/blog/fair-research-software-principles-after-two-years-adoption-update
(4) https://sites.google.com/site/forensicapps/strvalidator

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A comprehensive, reusable, scalable, and secure bioinformatics platform for validated forensic science

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Advances in massively parallel sequencing (MPS) including whole genome sequencing (WGS) have had a transformative impact on forensic genetics, opening new possibilities in analyzing complex biological evidence, thereby enhancing our understanding and interpretation of forensic genomic data. Here we present Foundry, a comprehensive bioinformatics platform developed in collaboration with UMass Chan Medical School, and its application as an enabling technology in forensic genetics. Foundry provides a comprehensive and versatile bioinformatics platform designed to facilitate MPS data analysis pipeline design, development, and execution, and provides an innovative suite of tools tailored for the advanced analysis of forensic samples through NGS data. Foundry provides a user-centric interface democratizing bioinformatics, allowing forensic scientists to run hundreds of pre-existing genomic data analysis workflows and to easily design new pipelines without requiring bioinformatics expertise. Central to forensic genetics research and casework, including forensic investigative genetic genealogy (FIGG), Foundry provides no-code automated validated pipelines for WGS data preprocessing (variant calling, imputation), and a plethora of downstream forensic genetics analysis pipelines, including forensic DNA phenotyping, biogeographic ancestry analysis, mixture analysis and deconvolution, deep kinship analysis, body fluid identification (e.g., from RNA-seq data), age estimation from DNA methylation data, and others. Foundry also provides no-code workflows for microbiome and environmental DNA (eDNA) analysis, democratizing cutting edge analytical techniques for microbial community analysis, aiding in crime scene reconstruction and post-mortem interval estimation. We present Foundry as a new technology and application for accelerating forensic science research as well as a platform for which contemporary forensic genetics analysis tools can be deployed in a forensic casework laboratory. Foundry separates forensic genetics analytical workflow implementation from actual execution environment: Foundry can be deployed across many different computing environments including all the major cloud computing providers (Amazon, Google, Microsoft), local high performance computing (HPC), air gapped servers, and personal workstations, enabling flexible and secure deployment adhering to applicable state or national regulatory guidelines. In conclusion, we present Foundry as a novel enabling technology at the forefront of integrating new technologies and applications in forensic genetics, offering a robust, user-friendly, scalable, and secure platform for the analysis of large MPS/WGS data across a wide range of forensic genetics applications. This integration of cutting-edge validated computational tools with forensic science deployed in a scalable, flexible, and secure environment will accelerate the pace of forensic science research and will enable the integration of these new technologies into forensic casework investigations.

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Navigating the DNA Database Landscape in India: Development, Implications for Privacy, Justice, and Ethical Considerations

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DNA databases have become indispensable tools for global law enforcement, aiding forensic investigations and combating crime. In India, with a population nearing 1.4 billion in 2024 and a vast, intricate landscape, implementing such databases is pivotal. However, unlike countries like the UK, USA, Australia, and Germany, India's efforts are in nascent stages, hindered by a lack of robust legislation and ethical concerns.

The Umid Project at All India Institute of Medical Sciences (AIIMS), New Delhi, in collaboration with the Indian Council of Medical Research (ICMR) and Crime Branch India (CBI) CFSL has hit the ground running with its efforts to create a civil database for unidentified bodies (a person that cannot be identified) and missing persons in India. This effort started out as a pilot project in 2018 within AIIMS, New Delhi and since then, expanded to include other laboratories in various states. The Umid portal that shows the phenotypic information of the deceased, is available for access by the public and law enforcement agencies and aims to utilise DNA technology to generate DNA profiles of the family member(s) (known as claimants) to match with the profiles of the deceased previously generated and uploaded on to the database.

This presentation delves into the intricate balance between the potential for justice and the protection of individual rights. The socio-cultural fabric of India presents unique challenges in the implementation of a DNA database. It will explore the diverse perspectives from legal experts, policymakers, and the public. Understanding these viewpoints is crucial in formulating a database that aligns with India's democratic values and respects its citizens' rights to privacy and dignity. The literature was obtained from published papers regarding other DNA databases and similar techniques were employed to collect, store and analyse biological samples.

Four cases have been matched till date and the database is being upgraded to accommodate more profiles. Drawing from international experiences, it offers insights into potential models for the establishment and governance of a national DNA database in India. It emphasises the need for interdisciplinary collaboration between geneticists, legal scholars and policymakers to navigate the complexities of genetic data within the Indian context. With progressions in Artificial Intelligence/Machine Learning, databases will play a pivotal role in discussions surrounding trust, reliability, repeatability, and ethical considerations. Advancements in molecular DNA technology will lead a thoughtful re-examination of validation and verification process, alongside deliberations on the ethical implications of public-access DNA databases.

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Multiplex Loop-mediated Isothermal Amplification combined with Lateral Flow Device for Simultaneous Species and Sex Identification

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Bloodstains are the most common biological specimens in forensic scenes. Rapid identification of species and sex from bloodstains can significantly expedite the process of crime investigation. Taking advantages of speediness and conveniences, loop-mediated isothermal amplification (LAMP) has been used for bloodstains analysis by targeting characteristics gene. However, these methods could only detect single target per reaction, leading to specimen consuming and limited throughput. In this study, an integrated biosensor is first developed for simultaneous species and sex identification from bloodstain without DNA purification. Based on the combination of multiplex LAMP and lateral flow device, the straightforward “sample to result” work-flow avoids any sophisticated apparatus, making it more suitable for on-site performing. By functionalizing gold magnetic nanoparticle (GMNP) as nanoprobe for amplicons hybridization, the results can be interpreted by naked eye. Meanwhile, the interference on colorimetric signal from pigments in bloodstains is effectively prevented by the filtration of nitrocellulose membrane. Although the amplicon detection requires solution transfer with uncapping, the aerosol contamination is prevented by uracil-DNA glycosylase, while holding the consistent temperature. Additionally, by labeling inner primer with nontarget hapten of GMNP, the false positive result caused by primer dimers is effectively prevents. As a demonstration, species and sex of bloodstains can be accurately identified within 1 hour by targeting cytochrome b and Y-chromosomal amelogenin. The identification results of the dated bloodstains stored for more than 6 years exhibits a 100% concordance with the truth, indicating the great potential of this biosensor for forensic nucleic acid analysis in point-of-care test scenarios.

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Opening the archives of aviation mishaps investigation: Analysis of the detection levels of microRNAs for molecular pathology diagnostics using long-term storage tissue specimens.

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Background: Comprehensive medico-legal investigation of death victims from aircraft accidents relies on a panel of different methods including autopsy, histology, and with increasing frequency molecular pathology diagnostics. Studying nuclear-acid based markers of health vs. chronic and acute disease provides insight into a pilot's aeromedical status prior to the deadly event. Unlike morphology-based techniques, genetic analysis is not impaired from secondary changes of the body resulting for instance from blunt force trauma along with the crash of the aircraft. To investigate highly special cases of aviation mishaps, in particular from military aviation, prospective studies should be combined with retrospective surveys to ensure sufficient numbers of cases for verified conclusions.

Material and methods: To validate the use of tissue samples after long-term storage for molecular pathology assessment, formalin-fixed paraffin-embedded (FFPE) specimens from 21 victims of military aircraft accidents between the years 1967 and 2019 were retrieved from the archives of Branch I 4 (Legal Medicine and Mishaps Investigation) of the German Air Force Centre of Aerospace Medicine. The tissue blocks were freshly cut into a predefined number of 10 µM thick sections. Five different protocols for de-paraffinization and RNA extraction using in-house solutions as well as commercial chemistry (Promega, Mannheim, and Qiagen, Hilden, Germany) were applied. The respective RNA yields were measured employing the Quanti-Fluor® system (Promega). The sensitivity for individual RNAs was studied using RT-PCR detecting spiked-in miRCURY® controls (Qiagen) on a Quant-Studio 5™ thermal cycler (Thermo Fisher, Darmstadt, Germany).

Results: RNA extraction from identical numbers of 10 µM thick sections consecutively retrieved from the same tissue block demonstrated higher yields in silica-column based procedures vs. magnetic-beats based techniques. Very low recovery rates of RNA insufficient for subsequent RT-PCR analysis almost exclusively resulted from magnetic-beat based extractions. No relevant differences were observed between de-paraffinization protocols using commercially available chemistry or xylol-ethanol-washing. The RT-PCR results for control microRNAs documented detection levels of nuclear acids spiked-in with concentrations down to 0.00002 femtomol per µL.

Discussion: Silica-column based RNA extraction procedures using FFPE tissue sections previously de-paraffinized with either commercial chemistry or xylol-ethanol-washing allow successful molecular pathology diagnostics employing more than 50 years old sample material. The inclusion of retrospective data in studies on rare and very special scenarios of military aviation mishaps is reliably possible. Hence, lessons to improve flight safety can be learned from a more significant cohort including retrospective and prospective casework.

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Medical laboratory sciences and lectures a veritable tool in forensic science: A comparative review study of rape in Nigeria.

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Rape is an age-long crime; its rapid identification is of utmost importance in criminal justice. Considering the high rape caseload in Nigeria and the required high-level expert investigations, the researcher explores the evidence in rape crime with a ranking based on easy availability at the crime scene using medical laboratory scientists (MLS). Search with the phrase: rape and evidence, evidence reliance, and common rape evidence conducted on Web of Science, ..., and ScienceDirect. The search outcome was discussed professionally by two groups comprising ten members each as Group-1 (MLS lecturers) and Group-2 (MLS laboratory). From the discussions, the matrix of evidence and sources were: Blood (Teeth bite and weapon injury), Saliva (mouth as drop of salivas and area leaked by mouth), Hair (Pubic and head hairs), Semen (Ejaculation), Fingerprint and Footprint (Contacts with the finger and foot), Sweat (Skin especially from the armpits), Fibre (Cloth) and skin cell (skin). Ranking from ease of availability posited Blood (2.2:10%), Saliva (6.2: 27.5%), Hair (5.8: 31.25%), Semen (2: 11.25%), Fingerprint and footprint (2.4: 13.75%), Sweat (1: 2.5%), Fibre (0.6: 1.875%) and Skin cell (0.175: 2.1875%) as percentage availability at the scene. The most available evidence is Hair and Saliva. It may be that their release is uncontrollable and not easily noticed. The Fibre is the least of all the evidence available for crime. Fibre may not drop depending on the material. Semen may be good evidence, but physical barriers used as protection make it less available evidence. The MLS's proficient analytical skills are supportive of the Jury.

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A novel droplet digital PCR system with five different fluorescent dyes for DNA methylation detection of four CpGs

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Objectives: Age prediction is one of the most necessary investigation directions in forensic science. To date, DNA methylation stands out as a leading genetic indicator for age prediction. The newly raised technology, droplet digital Polymerase Chain Reaction (ddPCR) has been found its utility in DNA methylation and age prediction with merits of absolute quantification and no PCR bias. However, its weakness is also evident, as it is constrained by its dual fluorescence channel and dual-site methylation detection, rendering it incapable of simultaneously detecting multiple methylation loci. To address the challenge, leveraging the unique characteristics of amplifications at terminal dilution, where typically only a singular target is detectable per droplet, along with the advancements in the novel quintuple-fluorescence ddPCR instrumentation, we have devised a ddPCR system capable of concurrently interrogating four methylation sites.

Material and methods: Four age-associated CG dinucleotides (CpGs) of blood were screened out, which were CCDC102B, ASPA, MEIS1-AS3, and IGSF11, and the special probes were designed to either detect the methylated or the non-methylated sequence with four different fluorescent dyes. ROX for the methylated sequence of CCDC102B, VIC for the non-methylated sequence of MEIS1-AS3, FAM for the methylated sequence of ASPA, and Cy5 for the non-methylated sequence of IGSF11. Fluorescence HEX was employed for the reference gene C-LESS-C1.

Results and conclusions: The result showed that all of the fluorescent dyes could be detected and the absolute values could be determined by Poisson distribution, which indicated that the novel droplet digital PCR system was effective for DNA methylation studies.

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DNA and proteins united: Molecular age prediction clocks in bones

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The identification of bodies is an important task in the field of forensics. The methodological possibilities of chronological age prediction of a deceased depend on the availability of tissues, whereby bones are preserved for a long time due to their mineralization under normal environmental conditions, and are often-used materials for generation of the individualizing DNA profile. With age, changes in DNA methylation (DNAm) as well as the accumulation of pentosidine (Pen) and D-aspartate (D-Asp) occur, and can be useful molecular markers for age prediction.
In this study, the potential of these three molecular clocks for age prediction was investigated using bone material. Furthermore, the parallel analysis allowed us to investigate a possible improvement of age prediction by combination of molecular clocks.

Parietal calotte from deceased individuals (18- 96 years) without signs of decay were split into a training (86) and test dataset (44). The procedure for cleaning of bone material and pre-processing was harmonized to allow a parallel subsequent DNA and protein analysis from the same bone powder material. DNAm of 6 CpG sites in ELOVL2, KLF14, PDE4C, RPA2, TRIM59, and ZYG11A was analyzed using MPS. The D-Asp and Pen amount were determined with HPLC. The same molecular markers were analyzed in 48 bone samples of putrefied bodies.

Age prediction models based on ridge regression were developed, resulting in preliminary mean absolute errors (MAEs) for univariate models of around 5 years (DNAm), 8 years (Pen) and 12 years (D-Asp) in the test set. Unsurprisingly, a general lower accuracy was observed in samples from putrefied bodies. This reduced accuracy could be caused by multiple factors with different impact on each molecular clocks. However, an improvement by the combination of all three clocks was possible, reducing especially the deviation in case of outliers. The results demonstrate the general potential in a combined analysis of different molecular clocks.

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The utility of the diatom test through metagenomic analysis

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[Objectives] The diatom test is used in forensic autopsies to determine drowning. This test involves the microscopic examination of organs after they are broken down with strong acids or digested enzymatically using proteinase K, a process that is harmful to health and/or demands expert techniques. Conversely, metagenomic analysis targeting aquatic bacteria has been recognized for its usefulness. Therefore, our study focused on diatoms to assess the value of data derived from diatom metagenome analysis.
[Material and methods] We collected 137 water samples from 51 locations (rivers: 42 locations; seas: 9 locations). Sediments from 2 ml of water samples were pulverized with beads, followed by DNA extraction using an EZ1&2 DNA Investigator Kit. The 18S rRNA region was amplified through PCR and sequenced using a MiSeq v2 500 cycles. Metagenomic analysis was conducted using Qiime2 software.
[Results and Discussion] The heatmap indicated that clusters were generally closer together for geographically proximate locations. However, there were instances where clusters were distant despite the proximity of locations, indicating the challenge in determining geographic origins through metagenomic analysis. Almost all diatoms that inhabit freshwater were detected only in freshwater and brackish water samples, and almost all diatoms that inhabit seawater were detected only in seawater and brackish water samples, implying that the primary utility of metagenomic analysis may lie in differentiating between freshwater and seawater. Especially, Skeletonema was detected in all seawater and brackish water samples, indicating that this genus is useful for identifying seawater. The ability to perform diatom metagenomic analysis with as little as 2 ml of water suggests the potential for detecting and analyzing diatoms in minimal samples, such as those remaining at crime scenes, including on clothing.

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Emerging technologies for hair examination and analysis: Assessing the profiling capabilities of genetic analysers

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Biological traces play a critical role in DNA casework; however, despite the significance of DNA profiling, its efficacy is often hampered by the limited availability of DNA. Standard DNA analysis has predominantly focused on nuclear DNA and has overlooked other potential biological traces, such as microbiome signatures. Hair samples pose a challenge as they may offer limited nuclear DNA information. This study investigated a novel approach for the analysis of hair samples, integrating traditional nuclear DNA identification with microbial forensic analysis to obtain a comprehensive profile for identification.

This research employed the emerging technologies RapidHIT™ ID for nuclear DNA detection and a SeqStudio® Genetic Analyzer for processing biological traces. Initially, DNA-containing material on hair samples was detected using Diamond™ Nucleic Acid Dye (DD) and real-time Extended Depth of Field (EDF) imaging to visualize and count nuclei. Detection of DNA containing material was an integral step to determine hair suitability for DNA analysis. Hair samples, both shed and plucked, were imaged using the Optico N300F LED Fluorescent Microscope and a MIchrome 5 Pro camera. Subsequently, the samples underwent processing through the RapidHIT™ID platform, recollected and processed through standard DNA analysis.

This study explored microbial signatures in follicle tissue to gauge their discriminatory potential for hairs with limited nuclear DNA and STR profiling results. Bacterial isolates were PCR-amplified and sequenced using the 16S rRNA gene, analysed via MicrobeBridge™ software for forensic relevance. The SeqStudio® platform enabled combined sequencing and STR profiling on a single plate. These findings could significantly impact casework by enhancing hair examination for forensic purposes.

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Genetic diagnosis of plant and fungal evidence using nrDNA ITS2

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Plant and fungal evidence can be found at the scene of the crime, of an undetermined sudden death (USD), as a cause of intoxication with judicial procedure, illegal species trading, being of relevance as evidence of prosecution. The ITS region is the most widely used nuclear marker in phylogenetic reconstructions at the species level, being recommended as a barcode. The ITS2 region of nuclear ribosomal DNA (nrDNA ITS2) as a minibarcode is here suggested for current diagnostic use of taxa (species) belonging to the kingdoms Plantae (superior plants) and Fungi (larger fungi or macromycete). The identification of control specimens was carried out with the aim of demonstrating the identifying power of the sequences. Likewise, recent casuistry is presented in the resolution of USDs and poisonings of forensic interest. A total of 50 taxa were diagnosed out of 23 cases and 30 control samples, where mostly 100% identity values could be reached. Within the ITS2 region, a review is carried out on the different primers that can optimize the identification of difficult samples, such as: plants with fungal endophytes, macromycetes with minor fungi presence, basidiomycetes versus ascomycetes, gastric contents and degraded specimens.

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Optimisation of A Metagenomic Analysis Method - Evaluating Microbiome Variation in Forensically Relevant Samples

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Advances in forensic DNA typing have allowed individuals to be linked to a crime from just a few cells containing damaged human DNA. However, instances where no suspect can be identified using the human genetic material, samples taken from a crime scene hold much more information in the form of environmental DNA, which has the potential to provide valuable “intelligence” to further the forensic investigation. Metagenomics is a field of study that focuses on understanding the diversity and implications of environmental DNA. Within a sample, non-human DNA will be present and this may include DNA from bacteria, archaea, fungi, animals and more.

This work presented here aims to optimise a metagenomic method for use in a forensic setting. Currently, much of the literature surrounding metagenomics is focused on its application in a clinical or environmental setting, and as such, use of this type of analysis in a forensic context has not been widely explored. During the initial stages of this study, the performance of two commercially available DNA extraction kits (ZymoBIOMICS and Qiagen) and two different processing methods (shotgun sequencing and random priming paired with massively parallel sequencing) were compared. The bioinformatic pipeline, Kraken2, was used to analyse the data produced. Results were analysed to assess efficiency of extraction and correlation of taxa abundance using the different workflows with those values that were expected.

Once an optimised method has been established it will be used to assess how metagenomic profiles vary with several parameters.

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Real-time and STR data in one reaction: a means to optimise PCR

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Trace DNA can be crucial evidence in the investigation of criminal and terrorist acts. Yet the rate of success when generating DNA data from such compromised evidence types is low. A possible process to generate STR data from limited and compromised DNA samples, such as touched items, is to alter the PCR cycling conditions in real time to optimise amplification. On a per-sample basis this has the potential to make maximal use of enzyme activity, improve DNA profile quality, counter effects of inhibition or degradation, or make the PCR program quicker and more cost effective. To realise this goal requires a means to monitor amplification in real-time, assess its performance, and learn to react when required.

We have successfully combined qPCR with STR amplification without compromising overall profile quality. GlobalFiler™ STR amplifications were performed in the same reaction with an Investigator Quantiplex Pro® qPCR to generate both sets of data. All amplifications were performed on an open-source qPCR machine to generate both real-time data and STR alleles in the same reaction, providing real-time STR PCR monitoring ability. The usually invariant cycling conditions were modified to alter the time at denaturation and the time at annealing/amplification. Analyses of the resulting fluorescence data allowed feedback metrics to be created. These are the first steps towards the endpoint of generating a ‘smart PCR’ that can modify the cycling conditions based on amplification data at each cycle to optimise STR profiles.

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RapidHit ID: RESULTS OF STUDIES USING DIFFERENT KINDS OF SAMPLES

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1. Objectives:

The aim of this work is to present the results of a study in which different kinds of samples have been processed in the RapidHit ID System for validation in the forensic routine analysis in caseworks. The RapidHit ID System equipment is a completely automated system which integrates sample preparation lysis, DNA amplification and capillary electrophoresis in approximately 90 minutes workflow with minimal human intervention.

2. Material and methods:

For this study we processed 40 samples. We used reference samples (buccal swabs and blood in Nucleic-CardsTM), samples from identified and unidentified bodies (blood, cartilage from decomposed and carbonized bodies, muscle, nails, vitreous humor etc) and samples such as blood on fabric clothes, nails, semen on swab and on fabric, ear swab, etc from caseworks.
For reference samples it was used ACE GlobalFiler Express Sample Cartridge and for the other samples it was used INTEL GlobalFiler Express Sample Cartridge.
Samples were processed in the RapidHit ID System. It was used the RapidLink Software. The profiles obtained were analyzed with the GeneMarker HID Software.
The samples were also processed in a traditional workflow of the laboratory (DNA extraction, quantification, amplification and electrophoresis on a 3500xL)

3. Results and conclusions

The results from this study have been excellent, considering all samples processed, highlighting the quality of the genetic profiles and data obtained comparing with the profiles obtained using the traditional workflow and methods. With this study we have validated the use of RapidHit ID System for the samples above mentioned.

Some caseworks of this study were related to missing persons cases in which it was possible to identify dead bodies decomposed or carbonized, using cartilage samples, in less than two hours, without processing hard tissues such as theet and bones using traditional methods that would take much more time.

The equipment RapidHit ID System has been extremely useful and important, especially in response time in urgent cases, approximately 90 minutes, without interrupting the traditional workflow and routine of the DNA laboratory, which would take much more time and human resources.
Whether for identifying a perpetrator or exonatering an innocent person or for identifying a missing person (living or deceased) in various contexts such as disaster victim identification, DVI, kidnapping, abduction, human trafficking, exchange of new born in maternities, the equipment RapidHit ID System has been very helpful with a rapid response, supporting justice and law enforcent.

P-275

STR genotyping by real-time PCR using QueSTR probes

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Forensic genotyping using short tandem repeat (STR) analysis is predominantly conducted through capillary electrophoresis (CE) within specialized laboratories. However, in scenarios like crime scenes or mass disasters, having a rapid and portable method would speed up the process of identifying individuals involved. To address this need and enable miniaturized lab-on-a-chip STR profiling as an alternative to CE, we developed QueSTR probes. QueSTR probes are implemented in a hybridization-based genotyping assay that relies on the recognition and cleavage of an RNA:DNA duplex facilitated by the RNase H2 enzyme. The cleavage of RNA:DNA duplex releases the quencher allowing the emission of fluorescence. For each allele of an STR locus targeted for genotyping, a corresponding DNA probe containing an RNA nucleotide is designed. We modified the existing two-step QueSTR probe hybridization curve-based assay for use as a hydrolysis probe assay during real-time PCR. An asymmetric real-time PCR is performed with QueSTR probes and RNase H2 in the master mix, during which the fluorescence is recorded. Reactions containing probes with matching alleles resulted in lower threshold cycle values and steeper incline of fluorescence curves compared to non-matching probes, indicating accurate genotype. The QueSTR qPCR assay was used to successfully genotype four CODIS core loci (D16S539, D7S820, TPOX, and TH01) in 12 samples, with one exception. The integration of amplification and detection in a single reaction supports the use of QueSTR probes for miniaturizing STR genotyping, thereby complementing CE-based analyses in centralized labs.

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The Quick TargSeq 1.0 integrated system for rapid DNA analysis with sample-in–answer-out capability

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Standard forensic DNA analysis based on short tandem repeats (STRs), or other types of polymorphic DNA markers, such as single nucleotide polymorphisms (SNPs), to identify the donor of a human biological trace found at a crime-scene by comparing DNA typing data. Current DNA typing involves a trained forensic worker operating several specialized instruments in a controlled laboratory environment, which takes 6-8 hours. We have developed a Quick TargSeq 1.0 integrated system (hereinafter abbreviated to Quick TargSeq 1.0) for automated generation of STR and SNP profiles from common biological samples, such as buccal swab, blood stains, and seminal stain. The system fully integrated the processes of DNA extraction, PCR amplification, electrophoresis separation by microfluidic biochip technology, which significantly decreased processing time of routine laboratory. The internal validation studies were performed using RTyper 21, RTyper Y27, or Y-SNP chip cartridge with single source reference sample. These results indicated that the Quick TargSeq 1.0 could process reference samples and generates STR profiles or SNP profiles in approximately 2 h, and the profiles were concordance with traditional STR or SNP analysis methods. Reproducible and concordant DNA profiles were obtained from reference samples, with the success rate and calling accuracy of 98.6% and 100%, respectively. The Quick TargSeq 1.0 produces full profiles from buccal swabs with 1 swipes, or dried blood spot cards with two 2 mm disks, or 5 ng of purified DNA. All the results demonstrated that the Quick TargSeq 1.0 can rapid generate reliable DNA profiles in an automated manner and has the potential for use in the field and forensic laboratory.

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A “sample-in-answer-out” system for integrated and automated short tandem repeat genotyping

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Short tandem repeat (STR) typing serves as a “golden standard” method for human identification in forensic DNA analysis. A routine process for STR typing includes tedious manual operations of sample lysis, DNA extraction, multiple PCR and electrophoresis.

In this article, microfluidic technology was introduced into forensic DNA analysis for “sample-in-answer-out” test of various types of samples. The microfluidic was modular designed, with a plastic microfluidic module to conduct PCR directly from swab samples, and a general capillary microfluidic chip made from glass wafer to perform electrophoresis. The two function modules were then combined and integrated through a uniquely designed microfluidic interface. An integrated instrument system was developed to conduct the whole automatic on-chip analysis. The core detection assembly of such instrumentation is comprised of a laser confocal system based on linear scanning and a compact color splitting detection component.

In this system, the modular construction and the introduction of microfluidic technology enhanced the performance of system’s environmental tolerance. The fragile tradition polymer-coated glass capillary was replaced by an on-chip one. And the complicate and easily disturbed fluidic system used for matrix injection was also removed due to the on-chip and in-capillary storage of all the electrophoresis reagents such as matrix, buffer, formamide and sieving standards. The whole system was applied to several kinds of extreme environments to verify its performance. The test results indicated that this rapid DNA analyzer is capable of operation in an outdoor environment even under some bad weather conditions. The concept of “anytime & anywhere” forensic DNA analysis was launched by the development of this new test method and system.

Key words:
Forensic genetics, Microfluidic, Capillary electrophoresis, “Sample-in-answer- out”, Short tandem repeat

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Shedding more Light on Shedders

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What is your shedder status? There is currently no agreed way to answer this. We report on the use of a simple cell-staining and cell-counting method to assess shedder status. Our previous work involved the same method and a small number of donors (11but it indicated that there may be a continuum of shedder types rather than the two usually referred to in the literature (heavy and light). We have now expanded the time points post-handwashing to 0, 15, 30, 60, and 180 minutes. Triplicate samples were collected from both the right and left thumbs of the volunteers to create 6 samples per person. Samples were collected by donors placing a thumb on a clean glass slide and then adding a DNA binding dye. The number of cells were recorded within three separate square millimetre areas (cells/mm2) at 220x magnification. Triplicate samples collected on three different days gave a total of 1,392 thumbprints from which 4,176 observed frames were recorded across the entire dataset. Forty (15 males and 25 females) of the donors were tested at all 5 time points post washing and a further 60 (42 females and 18 males) at the 30-minute time point. Of the 100 donors, 98 gave consistent and reproducible cell number deposition. There was no difference between the number of cells deposited by the left and right thumbs (13 of 15 tested); males tended to deposit more cells than females. The results obtained from all donors (n = 100) showed: 8 heavy shedders (5 male and 3 female); 40 intermediate (24 males and 16 females); and 52 light (41 females and 11 males). If applying arbitrary boundaries based on a cell count to definitively determine shedder status, then many of the donors fell within two categories. This study based on 100 individuals underlined that shedder status is a continuum phenomenon, shedder type was highly reproducible, and that there are no clear boundaries between shedder types.

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Single-cell analysis in forensic science - the SCAnDi project

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The analysis of evidential samples arising from multiple contributors remains a significant hurdle in forensic science, often requiring computational deconvolution of the mixed DNA profile after it is generated.

In the SCAnDi (Single-Cell Analysis for DNA identification) project, we are bringing together expertise in single-cell genomics, microfluidics and AI with forensic researchers and stakeholders from across the UK criminal justice system to explore how single-cell approaches may be applied to casework.

Our project uses single-cell approaches to isolate and amplify individual cells to study DNA transfer and persistence and also link cell-of-origin information with the DNA profile of the same cell. This information could be critical in deconvoluting mixed samples - especially higher order mixed samples, ascribing a narrative to where a DNA molecule came from, when it was transferred and by whom and potentially resolving a major challenge for complex mixed samples.

This work aims to bring together remarkable technical advances in single-cell analysis with a fundamental challenge in forensic science, uniquely within a framework where stakeholder engagement has been made a central objective.
We will use both commercial approaches (imaging cell sorting) and custom microfluidics to isolate individual cells, followed by the application of single-cell genomics approaches to measure STR loci using conventional and NGS-based approaches.

Additionally, we are developing AI-based approaches that will allow the classification of cell types based on imaging data acquired during cell sorting, e.g. sperm cells and seminal/vaginal epithelial cells, enabling linkage between cell-of-origin and the resultant DNA profile.

This multidisciplinary project was developed as a result of the UKRI Sandpit in Forensic Science for the Justice System, and began in April 2024. In this presentation we will give an overview of the project, our initial findings and anticipated outcomes.

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Retrospective inference of human behavior using environmental DNA analysis techniques

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Introduction: Retrospective inference of human behavior is crucial in forensic science. We used environmental DNA (eDNA) analysis techniques to determine whether human behavior could be inferred from human DNA collected from indoor environments.

Materials and methods: Water and dust present in indoors were used as targets for eDNA collection. Six beakers filled with 1 L water were prepared. Four beakers placed on a shared desk in an office were collected 1, 2, 3, and 4 weeks later. Two beakers placed on two participants' desks were collected at the end of 4 weeks. Dust samples were collected from 15 areas of the office floor using a roll cleaner at baseline and 2 and 12 months later. Dust samples were also collected from the floors of several participants' rooms using wet clothes or a vacuum cleaner. Additional dust samples were collected from 21 elevated locations in the office, ~2 m above the floor, using a roll cleaner or wet cotton swabs. DNA was extracted from all the collected samples and 24 loci (21 autosomal short tandem repeats, amelogenin, DYS391, and Y indel) were amplified for DNA typing.

Results: In all the samples, the detected alleles were mixed. Many alleles were detected in areas used by numerous persons. However, those that matched the DNA type of individuals who frequently used the area were more often detected and the peak heights of such alleles in the electropherograms were higher.

Discussion: Our analyses reveal the relation between human behavior and human eDNA analysis, such as the frequency at which a person uses a particular place and whether a particular place is used by many people or occupied by a specific person. These results suggest that human eDNA analysis can be used to retrospectively infer human behavior even in indoor environments without security cameras.

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Comparison of Fetal DNA Extraction Techniques for Paternity and Fetal Sex Determination: Implications for Forensic Genetics.

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Background: Forensic genetics significantly depends on precise and reliable techniques to determine paternity, which is often crucial in legal proceedings1. The prediction of fetal sex also plays a vital role in various forensic investigations, especially in cases involving crimes against women or infants. Cell-free fetal DNA, circulating in maternal blood, provides a non-invasive method for paternity analysis and fetal sex determination 2,3. Objective: the objective of this study was to evaluate various techniques for fetal DNA extraction, assessing the advantages and disadvantages of each method and focusing on DNA concentration and quality to ensure the certainty and precision of forensic genetic analyses, particularly in determining paternity and predicting fetal sex.

Material and methods: the methods evaluated for fetal DNA extraction included the use of Phenol-Chloroform techniques, a Quick-cfDNA™ Serum & Plasma Kit (Zymo Research, USA), and a QIAamp MinElute ccfDNA Mini Kit (Qiagen, Hilden, Germany).

Results and Conclusions: The evaluation revealed that the QIAamp MinElute ccfDNA Mini Kit was the most effective method for fetal DNA extraction, demonstrating high DNA concentration and quality compared to other techniques. The concentration of cell-free fetal DNA in maternal plasma samples was a key consideration, with optimal results obtained using 4 ml of plasma. Plasma processing before DNA extraction was also addressed, emphasizing the importance of immediate processing or appropriate storage conditions, such as -80°C. The concentration of cell-free fetal DNA in maternal plasma samples, plasma volume, and extraction method emerged as critical factors influencing the quality and viability of extracted DNA. In conclusion, careful attention to these factors is essential for obtaining fetal DNA of sufficient quality and concentration, thereby ensuring the reliability of forensic genetic analyses, particularly in cases involving paternity determination and fetal sex prediction.

Keys words: Fetal DNA, paternity, fetal sex, forensic genetics.

Bibliography

1. Arenas M, Pereira F, Oliveira M, et al. Forensic genetics and genomics: Much more than just a human affair. Published online 2017. doi:10.1371/journal.pgen.1006960
2. Devaney SA, Palomaki GE, Scott JA, Bianchi DW. Noninvasive Fetal Sex Determination Using Cell-Free Fetal DNA: A Systematic Review and Meta-analysis. Published online 1997. doi:10.1001/jama.2011.1114
3. Ordoñez E, Rueda L, Cañadas MP, Fuster C, Cirigliano V. Clinical Study Evaluation of Sample Stability and Automated DNA Extraction for Fetal Sex Determination Using Cell-Free Fetal DNA in Maternal Plasma. Biomed Res Int. 2013;2013. doi:10.1155/2013/195363

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Exploring the efficacy of distant-degree Kinship testing using nanopore sequencing with portable devices

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Objetives
Single nucleotide polymorphism (SNP) which is the most preferred genetic marker in investigating the distant degree of familial relationships, enables higher-degree relationship identification when combined use of a large number. In some special application scenarios where complex kinship determination of samples needs to be identified in the field, rapid DNA analysis device provides a solution for this kind of situation. The MinION MK1B nanopore sequencing device (Oxford Nanopore Technologies, Oxford, UK) is the smallest commercially available sequencer and can be used outside of conventional laboratories. In our previous study, the SNP genotyping accuracy based on nanopore sequencing data was evaluated over 99%. However, there were no studies have been conducted on the detection of SNPs of the 10,000 order of magnitude by nanopore sequencing technology to identify distant degrees of complex kinship identification.

Material and methods
In this study, combining xGen™ Human Copy Number Variant (CNV) Backbone Hybridization Panel with Liquid-phase hybridization capture technology for enrichment of over 9000 SNP sites. Then the enriched library was sequenced and genotyped on the MK1B nanopore sequencer. The detected SNPs are screened, including the removal of sites of poor sequencing quality and polymorphism, no accordance with Hardy Weinberg equilibrium, with genetic linkage. The testing efficacies of these SNPs were studied in different degrees of relationship based on the likelihood ratio model.

Results and conclusions
This research explored the feasibility of nanopore sequencing for the detection of SNP sites of the 10,000 order of magnitude and provided valuable methods, data, and results for the application of nanopore sequencing in the field of complex kinship determination.

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Validation of the Ion AmpliSeq™ MH-74 Plex Microhaplotype Research Panel as a supplementary assay for kinship testing

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Microhaplotypes are a new set of markers defined as short DNA stretches of approximately 300 bp that contain 2 or more SNPs and indels. The short DNA sequence length allows identification of the phase of the SNPs included in the microhaplotype using PCR-MPS techniques. After testing the performance of the Ion AmpliSeq™ MH-74 Plex Microhaplotype Research Panel, both with in vitro and in silico experiments, our laboratory decided to validate the MH-74 panel for kinship testing. Four of the most relevant populations for our casework (i.e. population samples from Denmark, Greenland, Somalia, and Afghanistan) were typed using the Ion GeneStudio S5 System, the Torrent Suite™ software, and the 'HID-Microhaplotype-Research-PluginV1.5'. In addition to the 222 SNPs defined in the original MH hotspot file, 73 relatively polymorphic SNPs were observed in the typed populations and included in the MHs. The validation process included duplicated genotyping of 454 samples, comparison of the results with an in house developed software for microhaplotype calling (MHinNGS), and investigation of possible inconsistent results. Also, population genetic analyses, including allele frequencies, Hardy-Weinberg and linkage equilibrium, and population structure, were performed. Population specific sequence variants, typically close to hotspots or in flanking regions, can reduce testing performance and need to be considered for correct genotyping. Population structure and linkage disequilibrium were observed in the population sample from Greenland. These observations are considered in kinship calculations.

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Analysis of paternity cases with a single exclusion in a genetic marker using precision ID Globalfiler™ NGS STR Panel v2

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Paternity test results can sometimes evidence incompatibilities in the allelic transmission from parents to children, such as the presence of a single exclusion in one specific genetic marker, revealing a mismatch between the genetic profiles of the biological parent and the offspring. In these cases, it is important to determine whether the exclusion could be the result of a mutation or other factors as null or silent alleles.

Capillary electrophoresis (CE) is the traditional method used in forensic genetics to analyze STRs (Short Tandem Repeats), however it is not possible to know the exact allele number variation due to the lack of sequence data. The application of Next-Generation Sequencing (NGS) technology may provide additional information, since it allows to detect and sequence simultaneously SNPs (Single Nucleotide Polymorphisms) present in the flanking regions and also distinguish isometric alleles with the same length but different sequences, that were misinterpreted as homozygous.

In this study, a set of reference samples (buccal swabs and blood stains), previously amplified with the GlobalFiler™ PCR Amplification Kit and sequenced by CE on the 3500 Genetic Analyzer, were selected from paternity cases with a single exclusion, reported after GeneMapper ID-X Software analysis. All samples were automatically prepared with the Precision ID GlobalFiler™ NGS STR Panel v2 on the Ion Chef™ System, followed by sequencing on the Ion S5™ System and finally Converge™ Software analysis, according to the manufacturer's instructions. The aim was to verify if the NGS methodology provides valuable information in these paternity cases and to identify the parental origin of a mutant allele.

The NGS results were in concordance with those obtained by CE. In addition, this methodology demonstrated to be useful to clarify the paternity cases, because it enables a higher power of discrimination through 9 additional multi-allelic STRs, in a total of 35 markers instead of 24 markers of the GlobalFiler™ PCR Amplification Kit used in the traditional method. Therefore, the Precision ID GlobalFiler™ NGS STR Panel v2 shows to be a powerful method for kinship analyses and typing reference samples.

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Innovating Forensic Application: Digital Microfluidic Integration for Non-Invasive Prenatal Paternity Testing

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Objectives: In forensic studies employing high-throughput sequencing, the library preparation often necessitates stringent laboratory conditions and cumbersome workflows. For forensic applications requiring higher cleanliness levels, such as non-invasive prenatal paternity testing (NIPPT), the library preparation demands become even more complex. Besides that, the commonly used probe-based methods usually take several days. Therefore, we developed a library preparation method for NIPPT based on digital microfluidic technology, which consolidates the entire library preparation process onto one chip, significantly enhancing the efficiency and reliability of NIPPT.

Material and Methods: We designed a two-step PCR process to prepare libraries for 2010 SNPs, each SNP with a minor allele frequency above 0.35 and no inter-SNP linkage, coupled with a digital microfluidic system tailored for this process. The process from DNA sample input to library output is completed within a fully enclosed digital microfluidic environment, operators only needed to load reagents and samples. We employed 12 real family samples with gestational weeks ranged 6~30 weeks, including 8 paternity Inclusions and 4 exclusions, with duplicate experiments. Libraries were prepared with 1ng of parental DNA (gDNA) and 2ng of maternal cell-free DNA (cfDNA), and we also explored the system's minimum detection limit for fetal DNA concentration across a 0.5%~5% gradient. All libraries were sequenced on the DNBSEQ-G99 platform, and the data were subjected to analysis after being capped at a maximum of 5 million reads for gDNA and 25 million for cfDNA.
Results and Conclusions: The entire process from DNA to results was completed within 13 hours, with all 27 family test groups successfully sequenced. The gDNA data used for analysis ranged 1.98~5 million reads, with an average of 4.61 million, and cfDNA data ranged 22.07 ~25 million reads, averaging 24.83 million. Uniformity(0.1×) and coverage(100×) of gDNA were 95.16%~98.39%, averaging 96.96% and 96.60%~98.94%, averaging 98.20%, respectively. Uniformity(0.1×) and coverage(1000×) of cfDNA were 95.73%~98.35%, averaging 97.34% and 96.32%~98.55%, averaging 97.55% respectively. The paternity determinations were consistent with known results, even in samples simulating 0.5% fetal DNA concentration and as early as 6 weeks of gestation. These results demonstrate that our digital microfluidic NIPPT system not only ensures performance but also accelerates the testing process and simplifies experimental operations, making it more suitable for large-scale use.

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STR allele dropout and flanking SNP analysis with The Precision ID GlobalFilerTM NGS STR Panel v2 kit in monozygotic twins

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NGS (Next Generation Sequencing) strategies are currently under validation in the field of human identification for forensic purposes, aiming to overcome some limitations of Capillary Electrophoresis (CE), allowing a comprehensive evaluation of STRs' sequences, their isoalleles and flanking regions. Nevertheless, there are still no reports of using STR genotyping by this technique for differentiation of monozygotic twins (MZ), which are indistinguishable by standard CE procedures. Objectives: Herein, we evaluated the possibility to distinguish MZ using The Precision ID GlobalFilerTM NGS STR Panel v2 by analyzing STRs and their flanking regions. Material and Methods: First, CE and NGS profiles of 32 MZ pairs were compared. After that, the NGS results were analyzed using different software. Results and conclusions: Allele length results were concordant between CE and NGS, except for one volunteer from one pair, which showed an allele dropout on Penta D on NGS results (Converge software), confirmed by Integrative Genomic Viewer (IGV software). However, STRait Razor software was able to detect the given allele despite its low coverage (19 reads). In addition, isoalleles were observed in eight different markers; although they did not allow the differentiation within each pair, they do increase the discrimination power in populational studies. Next, by analyzing the flanking regions, Converge software indicated two flanking unbalanced heterozygous SNP genotypes that allowed the discernment of the individuals from two pairs: rs560609904 in TPOX (individual G016A) and rs569521603 in D6S1043 (individual G027B). However, after a comprehensive sequencing quality and error rate analysis with STRaitRazor and IGV, and by performing Sanger Sequencing, we concluded that these unbalanced genotypes pointed out by Converge were in fact sequencing errors rather than somatic mutations. In conclusion, despite being unsuccessful in differentiating monozygotic twins in this current sample, we have observed that the Precision ID GlobalFilerTM NGS STR Panel v2 kit is indeed effective at identifying isoalleles, which increases the combined discrimination power. Notwithstanding, we strongly recommend routine analysis of NGS data with more than one genotype calling software in order to avoid allele dropout events and to confirm the results, taking extreme care to tell apart sequencing errors from actual genetic variability. Acknowledgements: FAPESP(20/08092-5), CAPES, HCFMUSP/LIM40, ThermoFisher.

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Distinguishing monozygotic twins from trace DNA by using whole genome amplification strategies

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Although monozygotic twins could accumulate sequence variations after the zygote splits, current DNA testing utilizing STR typing technology cannot effectively differentiate between them. Previous case studies have shown the potential of detecting sequence variants to distinguish identical twins. However, distinguishing identical twins based on trace DNA left behind at crime scenes poses a significant challenge in forensic science. To address this issue, experiments involving simulated trace DNA, whole genome amplification, and high-throughput sequencing have been conducted to establish a systematic technical approach for identifying identical twins from trace DNA. This will establish a robust scientific foundation and provide crucial technical support for the identification of monozygotic twins from trace DNA.

P-340

Combining Targeted SNP Sequencing and Local Kinship Analysis for the Identification of Human Remains

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The identification of human remains can provide closure to those searching for missing family members and justice for families when death is not accidental. In the case of mass fatality events or historical remains identification, this process can be challenging given the large number of victims and the potential loss of integrity of the available biological material. Short Tandem Repeat (STR) markers have been used successfully in the past for human identification, but their utility is somewhat limited for this application. Namely, STRs cannot identify relatives further out than second degree, and samples which are degraded and subjected to environmental insults do not always provide a full profile.

To address these limitations and enable laboratories working on the identification of human remains, the ForenSeq Kintelligence HT Library Prep Kit was developed. Single Nucleotide Polymorphism (SNP) amplicons are shorter than STR amplicons, making them well suited for the analysis of degraded DNA. This presentation will demonstrate the power of combining the 10,230 SNPs of the original Kintelligence kit with increased multiplexity to support higher throughput applications, and a local software solution to establish the degree of relatedness between unknown samples and family reference samples. Performance data will be presented for post-mortem (PM) and ante-mortem (AM) samples sequenced at plexities of 12 and 36, respectively, with determination of relationships out to the 3rd order. At these plexities, an average of 5600 loci were detected at 100 pg input for AM samples and 3000 loci detected at 12.5 pg input for PM samples. DNA extracted from bones subjected to different insults, dental remains, and artificially degraded and low input DNA were used to simulate PM samples. DNA from diverse populations were typed to simulate AM samples. Local database management and pedigree tools developed and integrated into the Universal Analysis Software will also be presented.

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A decade of mRNA body fluid and organ typing in casework: outcomes of Dutch court decisions

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Inferring what cell types reside in evidentiary samplings can assist courts in their decision making. mRNA typing provides a sensitive, human-specific method, applicable to both single source or mixed samples to deduce which body fluids or what organs are present in a trace. mRNA molecules can be remarkably stable, allowing the analysis of minute and degraded forensic stains.

At the Netherlands Forensic Institute, mRNA profiling has been applied in casework since 2010 and hundreds of samples have been analysed. Body fluid typing is mostly applied to sexual assault cases in which the presence of vaginal mucosa cells is disputed, while organ tissue identification is mainly requested for objects involved in violent crimes.

The ultimate value of RNA typing can be assessed by regarding verdicts after trial procedures. In the Netherlands, part of the court rulings are published (anonymously) online in the Dutch registry of verdicts. This registry was searched for court decision in which the mRNA typing results were actively considered by the judge, and around 30 court decisions were found. We studied examined amongst others the alternative scenarios that were put forward by the defence. These debates can be grouped into four main categories of alternative scenarios: 1) Time since deposition; 2) secondary transfer; 3) an innocent explanation and 4) another person performed the crime. Details on how mRNA typing results were used and other lessons learned will be discussed.

P-350

A comparative study of mRNA profiling in normal and inflammatory vaginal secretions

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After more than two decades of research and application, the utilization of mRNA for identifying the tissue origin of common forensic body fluid stains has reached a level of maturity. Various mRNA markers have been validated for different types of body fluid stains, including peripheral blood, menstrual blood, vaginal secretions, saliva, and semen. Nonetheless, not all evidence samples in practical cases conform to the ideal fluid sample criteria, and the physiological condition of the contributor of the body fluid stain may impact mRNA profiling.

Objetives: Investigate the impact of pathological (inflammatory) status on the efficacy of vaginal secretion mRNA testing.

Material and methods : After obtaining approval from the ethics committee, this study collected 100 vaginal swabs from the hospital's obstetrics and gynecology department, including 50 samples of normal vaginal secretions and 50 samples of inflammatory (pathological) vaginal secretions. Total RNA were extracted by RNeasy Micro Kit(Qiagen), performed reverse transcription and endpoint PCR amplification of specific markers, to investigate the differences in mRNA testing under inflammatory conditions. The study analyzed a total of four commonly used vaginal secretion-specific mRNA markers - CYP2B7P1, MUC4, HBD1, and L.cri - and then conducted statistical analysis of the test results.

Results and conclusions: The findings of the research suggest that there is no statistically significant variance between the two types of vaginal secretion samples in terms of marker detection rates, amplification product peak heights, and other parameters. This implies that the pathological condition of an individual is unlikely to have a substantial impact on the testing of vaginal secretions in real-world forensic examinations. However, it is important to note that this study only examined two distinct states of vaginal secretions. Factors such as tumors, medication, and others may also influence the analysis of mRNA testing, necessitating further investigation in future research endeavors.

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Differentiation of five forensically relevant body fluids using a small set of miRNA markers

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The identification of body fluids in forensic investigations can aid in the activity-level interpretation of biological evidence. Over the past two decades, significant research efforts focused on the development of molecular methods for this purpose. MicroRNAs (miRNAs) hold great promise due to their tissue-specific expression, lack of splice variants and stability, making them advantageous for identifying body fluids even in degraded samples.

Despite promising findings, challenges remain in achieving consistent results across studies. To address this, we selected 18 miRNA candidates and tested them on six body fluids commonly encountered in forensic cases: peripheral blood, menstrual blood, saliva, semen, vaginal secretions, and skin. Using reverse transcription-qPCR analysis, we confirmed eight miRNAs candidates (miR 144-3p, miR 451a, miR205-5p, miR 214-3p, miR 888-5p, miR 891a-5p, miR 193b-3p, miR 1260b) with high tissue specificity and four (miR 203a-3p, miR 141-3p, miR 200b-3p, miR 4286) with lesser discrimination ability but still contributing to body fluid differentiation.

Through principal component analysis and hierarchical clustering, the set of 12 miRNAs successfully distinguished all body fluids including the challenging discrimination of blood from menstrual blood and saliva form vaginal secretions. This research offers a reliable method for identifying common body fluids in forensic contexts, paving the way for improved handling of stains and reconstruction of criminal activities in laboratories.

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Validation of novel mRNA semen-specific markers for forensic fluid identification

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In crime scene investigations, the identification of body fluids using (m)RNA is crucial to provide details about the crime. While DNA profiling gives information on the identity of the donor of a biological stain (by confirming or excluding the donor), RNA analysis, on the contrary, allows for the detection of expressed genes by different cell types. This way the identification of the tissue from which the sample originated, without compromising further DNA analysis (due to co-extraction of RNA and DNA from the same sample), is possible. In the specific context of sexual violence, semen is one of the most relevant fluids. In a scenario where an ejaculation has occurred, the investigation depends mainly on the cytological detection/ visualisation of sperm. However, the absence of sperm does not always indicate the absence of semen.

The main goal of the present study was to validate four recently and newly described mRNA markers for potential identification of semen in forensic settings. For this, a multiplex system containing the mRNAs (ODF1, SMCP, TcP11 and TNP1) as well as two commonly used markers (PRM1 and SEMG1) was developed. The potential application of this multiplex in samples from forensic backgrounds was evaluated through testing of: i) sensitivity using RNA concentration inputs ranging from 5ng/µl to 0.02 ng/µl; ii) specificity by cross reactivity assessment on vaginal secretions, saliva and menstrual blood samples; and iii) degradation assessment on semen samples up to 18 months old since collection. The preliminary data obtained support the use of the new four mRNA markers in cases of sexual assault. The proposed markers, ODF1, SMCP, TcP11 and TNP1, can serve as a possible molecular alternative for semen identification to the traditional techniques since high sensitivity, high specificity as well as detection in aged samples was confirmed.

P-353

The use of rectal mucosa mRNA markers in forensic casework

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At the Netherlands Forensic Institute, mRNA-based body fluid identification is used to infer the presence of for example vaginal mucosa in sexual assault cases. Recent studies have shown cross-reactivity of genes that are currently used for the identification of vaginal mucosa and menstrual secretion, when analysing rectal mucosa-containing samples. This may present for instance a risk of incorrect inference of vaginal contact as previously seen with nasal mucosa-containing samples. Here, we show the results of a study aiming to gain insight in this cross-reactivity by analysing rectal mucosa, faeces, and anal samples from both male and female donors. Results highlight the importance of introducing rectal mucosa-specific markers to currently used assays, to avoid the risk of falsely concluding the presence of vaginal mucosa cells in samples containing rectal mucosa or faeces.

After evaluating three potential rectal mucosa-specific genes, two genes were incorporated in an updated version of our body fluid identification assay. The addition of these markers allows for the evaluation of faecal matter or rectal mucosa, allowing to answer casework questions that could previously not be answered by use of mRNA-profiling. This updated assay, has since then been used in casework at our laboratory where we use an RNA LR tool to report results with an accompanying weight of evidence presented in a verbal scale. The added value of the rectal mucosa markers is evaluated by performing a retrospective study on cases that have since then been subjected to mRNA-based body fluid identification.

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Comprehensive Body Fluid Identification and Contributor Assignment by integrating Targeted Sequencing of mRNA and coding region SNP

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Forensic genetic analyses aim to retrieve as much information as possible from biological trace material recovered from crime scenes. While standard short tandem repeat (STR) profiling is critical to individualize biological traces, its significance is diminished in crime scenarios where the presence of a suspect's DNA is acknowledged by all parties. In such cases, forensic (m)RNA analysis can provide crucial contextualizing information on the source level about a trace's composition, i.e., body fluids/tissues, and has therefore emerged as a powerful tool for modern forensic investigations. However, the question which of several suspects donated a specific component (body fluid) to a mixed trace cannot be answered by RNA analysis using conventional capillary electrophoresis-based methods. Massively parallel sequencing (MPS) poses a promising alternative, not only offering higher multiplex capacity, but also typing individual coding region SNPs (cSNPs) to enable the assignment of contributors hence reducing the risk of association fallacies.

Herein, we describe the development and validation of an extensive mRNA/cSNP panel for targeted sequencing on the IonTorrent S5 platform. Comprising 30 markers for the detection of six body fluids/tissues (blood, saliva, semen, skin, vaginal and menstrual secretion), along with 70 linkage-controlled cSNP for contributor assignment, our panel exhibited high detection sensitivity with RNA inputs of down to 75 pg and resulted in a conservatively calculated probability of identity of 0.03 – 6 % for individual body fluid-specific cSNP profiles. Limitations and areas for future work include RNA-related allele imbalances, including markers to detect rectal mucosa and the optimization of specific markers.

In summary, our new panel is intended to be a major step forward to interpret biological evidence at source level (based on cSNP attribution of a body fluid component to a suspect or victim), and in a suitable criminal case scenario, even at activity level.

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Evaluation of DNA Extraction Efficiency Method for Forensic Application on Trace Levels and Degraded Body Fluid Substrate Stains

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Body fluid substrate stains recovered at crime scene are often at trace levels and non-pristine conditions. Stains from such conditions mostly impacted DNA profiling failure. The advent of two newly designed DNA extraction kits (QIAamp DNA investigator kit – QIAGEN and PrepFiler™ Express BTA kit chemistries kit coupled in AutoMate Express system – Applied Biosystems) had made genetic profiling success for vast varieties of previously compromised body fluid substrate stain samples. Despite this improvement still genetic profiling success for vast varieties of body fluid substrate stains are compromised. This research study utilises optimization procedure for the protocol of the two DNA extraction kits to improve their DNA extraction efficiency. The two improved and optimised DNA extraction kits protocols were tested using simulated non-degraded and UV-degraded trace body fluid substrate stains. The data results from the two extraction test studies were compared using the DNA recovery yield and STR loci/alleles profiles peak heights intensities (rfu) parameters. The evaluation study based on the comparison analysis studies had indicated that DNA extraction method using PrepFiler™ Express BTA Kit chemistries protocol coupled in AutoMate Express system had the best DNA recovery and purification yield and efficient STR loci/alleles profiles peak heights intensities as compared with using QIAamp DNA investigator kit protocol method. Based on this research outcomes and observations, DNA extraction method using PrepFiler™ Express BTA Kit chemistries protocol coupled in AutoMate Express system method was therefore recommended as the best and efficient DNA extraction method for trace and forensically challenged crime scene stains samples.

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Inference of the Saliva Donor in Body Fluid Mixtures by cSNP Analysis

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In addition to standard DNA analysis for individualization, determining the different kinds of tissues or body fluids present in a forensic sample by mRNA-based trace contextualization has emerged to a valid and routinely used method in forensic laboratories.

One disadvantage of this approach is the inevitable separation of the DNA and RNA fraction, hence, the contributors to a trace mixture can be determined by DNA, and the different (e.g.) body fluids by RNA analysis; yet it is not possible to assign a person to the respective body fluid if no sex specific secretion such as ejaculate is present. However, this information could provide important insights into the course of a crime or validate/negate claims made by the persons involved.

Lately, the analysis of insertions or deletions (InDels) and single nucleotide polymorphisms (SNPs) in the coding region of mRNA transcripts has been introduced as a promising approach to match a body fluid to its respective donor by comparing these polymophisms with a reference sample. Still, the vast majority of this experiments have been conducted using MPS, which is not present or affordable for many laboratories working on routine crime cases. A more feasibly method is performing a SNaPshot assay using capillary electrophoresis.

In this study, we aim to establish and validate a set of polymorphic SNPs in the coding region of mRNAs that are specific for the forensically relevant and biologically sex-independent body fluid saliva. A literature and database search was conducted to identify potential SNP candidates in different transcripts with a variant frequency of at least 0.25 for the minor variant. After confirming the specificity of the transcripts without cross-reactions, the most promising candidates were combined to a multiplex SNaPshot assay and validated for their forensic usability, performing several mixture and sensitivity experiments. Here, our preliminary results and outlook are presented.

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DNA methylation direct sequencing in body fluid identification

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DNA methylation is widely applied in forensic genetics for body fluid identification, age estimation, facial feature prediction, and more. Typically, DNA methylation detection requires conversion and amplification, with the bisulfite conversion method, considered the gold standard, also presenting disadvantages like high losses, stringent reaction conditions, and sample degradation. The emergence of single-molecule sequencing enables direct detection of the methylation of genomic DNA (gDNA) without additional sample processing, avoiding sample loss, and offering higher throughput. In this study, based on QNome nanopore sequencing technology, methylation sequencing was performed on gDNA and amplified DNA products. The preliminary target region for detection consists of 16 body fluid-specific CpGs in our previous research. The methylation results of gDNA and amplified DNA were compared with the results obtained from Capillary Electrophoresis (CE) platform to evaluate the accuracy of direct sequencing. The raw reads were filtered by NanoFilt with Q-value > 7 and Q-value > 12, which precision inferred is approximately around 80% and 90% respectively. Methylation values were calculated by measuring methylation signals on reads. The results showed that the methylation levels of the two types of samples exhibited consistent trends. Direct methylation sequencing of gDNA yielded methylation levels that were generally consistent with reference CE results, but the low sequencing depth due to low throughput resulted in significant differences in CpG methylation levels. PCR samples were analyzed using Q7 and Q12 data, with similar results under both filtering conditions. The analysis results with Q12 data were closer to the reference CE results. The results indicate the potential use of DNA methylation combining single-molecule sequencing technology for body fluid identification. In the future, it is hoped that detection of larger DNA methylation regions will enable differentiation of a greater variety of body fluids.

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Microbial profiling in postmortem organ tissues: insights from 2bRAD-M analysis in cases of sudden death and hanging events

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The thanatomicrobiome, which analyzes microbial shifts after death, is crucial in forensic science for accurately identifying causes of death, complementing traditional methods. In our research, we applied 2bRAD-M to study the thanatomicrobiome in multiple organs from two sudden death cases and one hanging case. The results revealed varied microbial species compositions across different organ tissues, with specific bacteria like Escherichia coli, Prevotella copri, and Streptococcus pseudopneumoniae dominating in certain organs of cadavers with differing ages and causes of death, reflecting potential age-related microbial patterns and their association with death mechanisms. These findings, alongside observed microbial abundances in cardiac tissues, underscore the utility of advanced microbiome sequencing techniques like 2bRAD-M in forensic science to discern distinct microbial markers that could shed light on the causes of death.

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Exploring the Forensic Potential of Oral and Nasal Microbiota for Estimating Post-Mortem Intervals

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Microbiota have become an innovative tool for estimating the post-mortem interval (PMI) in forensic science. The roles of oral and nasal microbiota in cadaver decomposition are crucial, yet their distributions within human cadavers remain underexplored. In our research, we collected 98 swab samples from the oral and nasal cavities of 10 healthy volunteers and 38 cadavers. By employing high-throughput sequencing of the 16S rRNA gene, we conducted comprehensive analyses of alpha diversity, beta diversity, and the distribution of relative abundance to delineate the microbial communities in both healthy individuals and cadavers across varied PMIs and freezing durations. Our results indicate that the microbial communities in the oral and nasal cavities of living individuals display significantly higher richness and diversity compared to those in cadavers, with oral microbiota showing greater levels than their nasal counterparts. At the phylum level, Firmicutes predominated within the oral microbial communities, while Proteobacteria was the leading phylum in post-mortem nasal communities, contrasted by Actinobacteriota in healthy nasal communities. At the genus level, Streptococcus and Haemophilus were notably present in the oral microbiota of healthy individuals; conversely, Corynebacterium and Staphylococcus were prominent in healthy nasal microbiota. In cadavers, Streptococcus and Klebsiella were predominant in the oral and nasal microbial communities, respectively. Importantly, our analysis revealed that the dominance of these genera varies in distinct patterns as PMIs lengthen. Additionally, the process of freezing was found to have a minimal effect on the distribution of post-mortem microbiota. These findings highlight the significant potential of leveraging oral and nasal microbiota for forensic applications, particularly in the estimation of PMI. This opens new pathways for advancing forensic methodologies and enhancing the accuracy of PMI determinations.

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Development of a multiplex system for blood and menstrual blood identification based on the microbial markers

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Objectives: Identification and detection of the source of body fluids is critical in criminal investigations as it provides information about the crime's nature and the DNA's origin. One effective method to accomplish this is using microbial markers, which can trace the origin of body fluids based on their site specificity and time stability. Previous studies have indicated that the microbial community composition of blood and menstrual blood is significantly different. Therefore, we aimed to use microbial markers to distinguish between blood and menstrual blood.

Material and methods: We initially collected 30 blood samples and 30 menstrual blood samples to construct a fluorescence multiplex amplification detection system based on the capillary electrophoresis platform. This system was composed of several microbial markers with the potential to distinguish between the two body fluids, which could exhibit different characteristic peaks in blood samples and menstrual blood samples. We then constructed a machine learning model for prediction based on the analyzed data.

Results and conclusions: The Prediction model established in this study can initially distinguish peripheral blood and menstrual blood samples, which are commonly confused at the scene of forensic cases, to guide the direction of case investigation quickly.

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What do forensic geneticists ignore? Two alternative, non-toxic methods for FFPE tissue deparaffinization

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In forensic genetics focusing on kinship analysis and individual identification, it is sometimes necessary to employ biological tissue fixed in formalin and subsequently embedded in paraffin (FFPE) as a source of DNA.

Although FFPE tissues allow samples to be stored for long periods at room temperature, reducing maintenance and storage costs, a significant problem arises in forensic genetics due to formalin and paraffin's chemical degradation and retention effects on DNA.

In the FFPE tissue pre-treatment step, deparaffinization plays a crucial role as it can significantly influence the outcome of the subsequent DNA extraction step.

Currently, the standard dewaxing procedure involves using xylene to remove paraffin from FFPE tissues. As it is an organic solvent highly toxic to the operator, different deparaffinization protocols using non-toxic solvents, such as white mineral oil and D-limonene, were compared in this study. For this purpose, one FFPE lung tissue taken after the autopsy of a male body was used. The sample was cut into twenty slices by a microtome to allow the dewaxing protocols to be performed (five protocols, each repeated four times), following which DNA extraction from the tissue samples was performed using the QIAmp DNA Mini kit. The extracted DNA was quantified and normalized to 1 ng/µl, after which the amplification was performed using the PowerPlex® Fusion 6C System. Amplicons were genotyped with the SeqStudio Genetic Analyzer for HID, and fragment analysis was performed using GeneMapper ID-x v1.6.

Based on the results of this preliminary study, it is apparent that the degree of informativeness of the genetic profiles varied depending on the deparaffinization technique employed. Pre-treatment of FFPE tissue with white mineral oil showed greater efficacy than using xylene, generating an average of 18 out of 27 accurately identified markers; in contrast, d-limonene produced results superimposed on those obtained with xylene.

Although further validation on a more extensive and more diverse set of FFPE tissues is undoubtedly required, as well as an increase in the time between fixation in formalin and inclusion in paraffin and the time of sample analysis, it seems legitimate to suggest that alternative solvents, with equivalent or higher efficacy but reduced or absent toxicity levels, may serve as viable substitutes for xylene in deparaffinization processes.

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Development and Validation of the 9-dye Fluorenscent Y chromosome and autosomal Multiplex STR Amplification System

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Abstract Objective To deveplope a 9-dye fluorenscent autosomal + Y STR multiplex amplification system and varify its performance.Methods Using the 9-dye fluorescent labeling technology, construct a multiplex amplification system that using high genetic polymorphism autosomal and Y chromosome loci, then evaluate its accuracy, sensitivity, species specificity and other system performance index. Results A 9-dye fluorescence amplification system containing 70 loci including Amel、D5S818、D21S11、D7S820、CS1FPO、D2S1338、D19S433、D8S1132、D2S441、VWA、D8S1179、D16S539、D10S1435、PentaD、D3S3045、TPOX、TH01、D22S1045、D18S51、FGA、D19S253、D10S1248、D6S477、D6S1043、D13S317、D12S391、D15S659、PentaE、D1S1656、D3S1358、DYS460、DYS389I、DYS390、DYS533、DYS392、DYS518、DYS557、DYS570、DYS458、DYS437、DYS385、GATA-
H4、DYS576、DYS643、DYS508、DYS627、DYS456、DYS391、DYF404S1、DYS438、DYS448、DYF387S1、DYS596、DYS549、DYS399S1、DYS393、DYS635、DYS439、DYS19、DYS444、DYS449、DYS481、DYS527a / b and DYS447 was successfully constructed. The various loci in this system are well balanced, with a sensitivity of 0.0625ng and a certain ability to detect mixed samples. Conclusion This system can obtain rich locus information through a single test, and meet the needs of the practical inspection of forensic cases and database building. It has a good practical significance in forensic science.

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Development and validation of a new Investigator Quantiplex Pro variant for high sensitivity forensic workflow applications

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Purified human DNA from various trace types must be assessed for quantity, quality and integrity before STR analysis using CE or NGS, because STR assays are complex systems that require a narrowly defined range of input DNA and template quality to perform precisely and accurately. Since DNA quantification is the only step preceding the STR-PCR, it is essential to extract as much information as possible from this reaction to aid correct setup of STR reactions. Ideally, quantification decides whether the sample is suitable for further processing, which leads to sample triage and can therefore save significant costs.

The new Investigator Quantiplex Pro FLX quantification kit enables the use of a flexible sample template volume of 1-18 µL per reaction and thus achieves up to ~10 times higher sensitivity compared to standard assays. The PCR chemistry is already available as a lyophilized master mix in optical 96-well plates, the handling of which has been optimized for the corresponding throughput in the laboratory. Also, it free-up freezer capacities since it can be stored sustainably at room temperature. Screening of sexual assault samples, where maximum sensitivity of male DNA is crucial even in the presence of high amounts of female DNA, or high-throughput applications in routine laboratories are just two possible applications where the new Investigator Quantiplex Pro FLX has advantages over traditional quantification assays with liquid chemistry and a fixed input volume of 2 µL.

We will present data from the development and validation.

P-382

A Comprehensive Evaluation of Commercial Small-Amplicon Mitogenome Enrichment Kits for Massively Parallel Sequencing

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Massively parallel sequencing (MPS) of mitochondrial DNA (mtDNA) is critical in both historical and forensic cases involving degraded remains and/or distant, maternal relatives. The commercial development of small-amplicon (~100-250 bp) based approaches for mitochondrial genome (mitogenome) enrichment allows MPS for mtDNA analysis to be implemented by laboratories on a wider scale.

To assess these assays, a comprehensive study was conducted to compare the five small-amplicon mitogenome MPS kits following their respective manufacturers' protocols: Precision ID mtDNA Whole Genome Panel, ForenSeq mtDNA Whole Genome Kit, PowerSeq WGM System, QIASeq Targeted DNA Human Mitochondrial Panel, and NimaGen IDseek Mitochondrial DNA Full Genome Sequencing. A total of 96 samples were processed with each kit, including a variety of substrates and extraction methods, non-human samples, mixtures, serial dilutions, and population samples from a range of haplogroups. Sequencing was performed on the Ion S5 for the Precision ID panel, while all other assays were sequenced on a MiSeq FGx. Data were analyzed using the manufacturers' recommended software where applicable; otherwise, CLC Genomics Workbench was the default. Analysis workflows were optimized to minimize the impact of reference bias and artifacts on variant calling to ensure the generation of accurate and high-quality mitogenome haplotypes. Additionally, overall performance was evaluated based on metrics such as haplotype concordance, heteroplasmy detection, and breadth and depth of coverage.

This performance assessment combined with a cost-benefit analysis may provide guidance to forensic practitioners looking to implement this technology in their own laboratory. Furthermore, this comprehensive study addresses a majority of the requirements for an internal validation of each commercial mitogenome MPS kit.

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P-388

A highly integrated next-generation sequencing detection method for forensic DNA genetic markers and RNA molecular markers

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Forensic DNA and RNA markers are significant in individual identification and body fluid identification. However, DNA and RNA markers are separately detected in most forensic experiments, which entail large sample consumption, complex procedures, and weak bio-information correlation. While some integrated methods based on capillary electrophoresis and next-generation sequencing technologies are presented, these methods can neither detect large numbers of markers nor simplify experimental procedures. In this study, we developed a highly integrated next-generation sequencing method for DNA and RNA simultaneously testing, including DNA and RNA co-extraction, reverse transcription, library co-construction, and co-sequencing. Next, we used this method to construct a multiplex panel including 55 DNA markers (10 autosomal short tandem repeats and 45 autosomal single nucleotide polymorphisms) and eight message RNA markers (two blood-specific, four saliva-specific, and two housekeeping markers), which were available for human identification and body fluid identification from blood and saliva derived samples. The high accuracy of the panel was confirmed through a series of experiments. To sum up, the novel integrated method can be used to sequence forensic DNA and RNA markers in one simplified procedure, which was a promising method to comprehensively reveal DNA and RNA bioinformation in samples.

Key words: forensic genetics; DNA; RNA; integrated detection method; next generation sequencing

P-391

MVCall: An automatic nanopore sequencing analysis pipeline for variation and heterogeneity identification of mitogenome

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Objetives: Mitochondrial DNA (mtDNA) analysis has gradually evolved from control region sequencing to whole genome sequencing with the development of massively parallel sequencing (MPS) technology, greatly promoting the application of mtDNA analysis in the fields of biomedicine, anthropology, and forensic science. However, the accuracy of MPS-based mtDNA analysis can be reduced due to uneven amplification and splicing of short fragments. Moreover, the heterogeneity resolution of MPS platforms is limited, approximately 10%. Nanopore sequencing technology has outstanding ability to sequence long continuous DNA fragments, making the detection of mitogenome without splicing possible.

Material and methods: In this study, we developed an automatic analysis pipeline, named MVCall, based on nanopore sequencing data for identifying variation and heterogeneity in mitogenome. Compared with CmVCall, the mtDNA control region analysis pipeline built in our previous study, MVCall added an insertion/deletion (InDel) identification module, a consistency sequence generation module and a vcf file generation module, and optimized the analysis parameters of the polymer region.

Results and conclusions: The sequence information and heterogeneity level of whole mitogenome can be obtained simultaneously by this pipeline. Overall, the MVCall provides a useful tool for mtDNA profiling in large-scale samples.

P-394

Forensic potential of low-frequency, single-base substitutions in human mitochondrial genome

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Mitochondrial (mt) genome is circular, maternally-inherited extranuclear DNA fragment, which replicates independently from nuclear DNA, thus generating multiple haploid copies per cell. Consequentially, point mutations at each mtDNA position can expand to anywhere between 0% and 100% within a tissue. This phenomenon, termed point heteroplasmy (PHP), exhibits forensic relevance by increasing variability of mitochondrial genomes within maternal lineage. Due to technical limitations of massively parallel sequencing, little is known about the pattern of PHPs with minor allele frequency (MAF) below 1% in different human tissues. Therefore, with the aim to explore potential forensic pertinence of low-frequency alleles at heteroplasmic positions, we sequenced whole mitochondrial genomes at approximately 7,700x coverage, with multiple technical and biological replicates of longitudinal blood and buccal swab samples from 11 human donors (159 libraries in total). Reproducible results were obtained by using two independent Illumina® systems: MiSeq FGxTM and NextSeq®500; and two independent data analysis solutions: Illumina® BaseSpace® Sequence Hub and an in-house bioinformatics pipeline. We found that PHP signature between 0.1% and 1% MAF in buccal swabs and blood samples exhibit high inter-individual prevalence at polymorphic positions of mtDNA control region (CR), tissue preference, different potential of CR PHP expansion and short-term PHP instability in mitotically active tissues. Our findings represent a step further towards the application of low-level PHPs as forensic markers in questions of identity, tissue-specificity, age and maternal kinship.

P-395

Advancing Semen Detection Techniques: Utilizing STK®Sperm Tracker to Emphasize Traces and Potentially Define Time Since Deposition (TSD)

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Introduction:
Recently, a new innovative technology has emerged for semen detection: the STK®Sperm Tracker, developed by AXO Science (Villeurbanne, France). This reagent is a non-toxic presumptive test, specifically designed to highlight male acid phosphatase (PA) present in human seminal fluid. Upon interaction with this enzyme, the reagent triggers a reaction that, after subsequent exposure to UV-365 nm lamp irradiation, enables the observation of persistent blue fluorescence.

Objectives:
This study aims to evaluate the efficiency of STK®, particularly the STK®Skin, in emphasizing and detecting semen traces across various scenarios and environments. Specifically, the study seeks to assess potential effects on DNA after with STK® treatment, with the goal of improving the evidence collection phase without compromising the subsequent analyses.

Material and Methods:
Mock samples were created using pig ears, widely recognized for their faithful resemblance to human skin, along with natural and bleached human hairs. Different deposition times and environmental conditions were simulated. The emphasized traces were then photographed in a standardized manner using VILBER VL-6.L UV lamp (365nm) for exposure. Subsequently, exploratory analyses, including pixel-level and image-level approaches on RGB raw data, along with Principal Component Analysis (PCA), were conducted.

Results and Conclusion:
The results demonstrate the consistent efficacy of STK® in semen detection across various environmental conditions and time intervals. Additionally, interesting time-dependent color changes were observed, suggesting a potential technique for time since deposition (TSD) estimation. Ideas for next improvement include integrating advanced imaging systems, conducting precise segmentation, and studying the specificity of STK® Sperm Tracker reagent over longer periods.

P-396

Comparison of MPS methodologies for Individual identification based in STRs, mitochondrial DNA and SNPs in ancient DNA samples

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Background and Objectives
The forensic scientific community has been struggling to obtain quality DNA from ancient samples since the first sequencing technologies appeared. Moreover, it is not only the difficulty in obtaining the DNA from the remains what's been addressed but also the challenges of the subsequent amplifications of regions of interest for human individual identification.

Our aim here is to improve DNA extraction techniques from bone ancient samples, more than 80-year-old, and afterwards compare different commercially available kits to analyze STRs, SNPs and/or mtDNA for human individual identification by massively parallel sequencing (MPS). Our final goal is to correlate the efficiency of these kits to the quantity and quality of the obtained DNA to stablish an efficient protocol of analysis for every sample.

Material and Methods
Samples have been obtained from the remains of several mass graves in Catalonia from the Spanish Civil War under the project "Pla de Fosses" driven by the Department of Justice, Rights and Memory from the Government of Catalonia. DNA was extracted from tooth powder produced with different mechanisms and subsequently quantified with QuantifilerTrio. Available MPS kits such as ForenSeq TM DNA Signature Prep Kit (Verogen), Power Seq Auto/Mito/YSTR (Promega) and xGen™ Human Mitochondrial DNA (mtDNA) Hybridization Panel (IDT), have been used to create genetic profiles from these samples and compared to those obtained by capillary electrophoresis.

Results and Conclusions
Though there isn't much room for improvement in the obtention of quality DNA from degraded ancient samples, there are many changes in the library preparation and sequencing protocols that can be applied to generate valuable information from this sort of samples.
One of the limitations of current available kits is the amplicon size and the polymerases used, that are intended for modern DNA. For damaged and fragmented DNA, the chemistry should be adapted to the singularities of those samples avoiding long amplicons or designing short captured regions and adding polymerases that preserved damage.

P-397

An NGS-based Typing Panel of Human Multi-source Genetic Markers

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Presently, such an increasing demand emerges for the identification of complex genetic relationships in forensic practice that requires the combination of a variety of genetic markers, e.g., STRs, X/Y specific indicators, SNPs and mitochondrial DNA. Next generation sequencing (NGS) approach is able to integrate multiple genetic markers into one detection system, excelling the traditional capillary electrophoresis (CE) technology in overcoming the cumbersome operational procedures and experimental handling. Here, a typing panel, designated as DNATyper NGSPanel v1.0, was developed with enclosure of human 29 autosomal STRs, 36 Y-STRs, 32 X-STRs, 71 Y-SNPs and the whole genome of mtDNA. Its validation was implemented under the guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM), having evaluated the items of repeatability, accuracy, consistency, sensitivity, mixture and species specificity. For the developed DNATyper NGSPanel v1.0, a consistency of 99.72% was shown to the detection with CE, plus the complete identical results coming true about its componential loci shared with the Forenseq™ DNA Signature Prep Kit. No allelic loss occurred to the DNA template of 0.5~10 ng, yet the respective 2 and 9 loci being lost with the 0.25ng and 0.125ng of template. Regarding to the mixture of male and female substances, the mixed ratio of 2:1 had the the female's begun to appear allelic loss, contrasting that the mixed ratios of 9:1 ~ 1:1 brought the positive detections to 54.72%, 81.13%, 98.11% and 100%, and the mixed ratio of 1:4 made the male's happen to allelic loss, opposite the mixed ratios of 1:1 ~ 1:9 rendering the positive detections to 100%, 100%, 90.24% and 82.93%. The test of species specificity proved that the developed DNATyper NGSPanel v1.0 hardly amplified the DNA from pig, cattle, mouse, either the cynomolgus monkey or the rhesus one. In conclusion, the developed DNATyper NGSPanel v1.0 is of high detection throughout, high sensitivity and stability, accurate and repeatable typing and good ability for mixed sample to detect. Also, it can deliver the genetic information related to paternity and maternity at the same time in just a single run of test with any common biological samples, hence eligible for boosting the capability of individual identification and efficiency of consanguinity determination.

P-398

A survey of human microhaplotype variation and panel design with MicroHapDB

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Interest in microhaplotypes (MHs) as genetic markers for numerous forensic, anthropological, and medical applications continues to grow, but stakeholders within the forensic genetics community have yet to converge on a definitive MH panel (or set of panels) for development and standardization. Improved accounting of human genetic variation has driven both the discovery of new polymorphic MH loci as well as the recharacterization of previously described MHs to incorporate additional SNP targets, increasing the scale and complexity of published MH data considerably. The availability of highly polymorphic candidate markers is no longer a bottleneck to MH implementation: as of March 2024, more than 3000 MH markers have been proposed in the forensic genetics literature. Rather, effective informatics and data management have become the primary limiting factor. Automated procedures are needed to aggregate MH data from disparate sources, apply and enforce consistent nomenclature, and ultimately support the selection of optimal loci for further investment. MicroHapDB has maintained a comprehensive catalog of MH marker and frequency data since 2018 as an open-access community resource. This presentation will provide a global survey of published MH markers, present changes to MH nomenclature recently adopted by an international working group to address data provenance challenges, and describe how MicroHapDB is being utilized in the design of candidate panels for development and eventual standardization.

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P-400

Detection of mitochondrial genome in degraded and inhibitor samples with ForenSeq mtDNA Whole Genome Kit

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1. Objectives
Mitochondrial DNA typing is commonly used in forensic practice to obtain genetic information from seriously degraded and ancient samples. This study assesses the efficiency of the ForenSeq mtDNA Whole Genome Kit in degraded and inhibitor samples.

2. Material and methods
Degraded samples were prepared using muscle tissue soaked in tap water at room temperature for 3, 6, and 9 days and extracted using AutoMate Express Forensic DNA Extraction System. Inhibitor samples were mocked using swab samples with 100 µM hematin and extracted using Automatic 96 Channel Micro DNA Extraction Workstation. Genomic DNA was quantified on QuantStudio 5 Real-Time PCR System. Each sample is divided into two 50 pg reactions. Library preparation was performed using ForenSeq mtDNA Whole Genome Kit, which included 12 samples, 1 HL60, and 1 negative control. Template clustering and MPS were performed on MiSeq FGx System. Raw data was processed by ForenSeq UAS v2.5.1 with AT > 6% and IT > 10%.

3. Results and conclusions
Quality metrics in ForenSeq UAS were within the recommended range, except for phasing (> 0.25%). This suggests that the efficiency of sequencing synthetase is reduced, which is common in the case of outdated reagents and pooled libraries containing too many inhibitors. Upon conducting mass spectrometry detection, hematin residues were found in each purified library of inhibitor samples, as well as pooled libraries. Sample intensity averaged 0.067M reads, far lower than the recommended minimum number of reads (0.4M). It indicates that Automatic 96 Channel Micro DNA Extraction Workstation did not remove the inhibitors well, thereby affecting the downstream workflow.
Degraded samples were obtained with 29 identical variants and assigned to haplogroup F2d, with 0.71 (positive control), 4.52 (Day 3), 63.84 (Day 6), and 146.01 (Day 9) degradation indexes (DIs). Interestingly, the number of reads was progressively generated with DIs increased, and more artificial heterozygous variants were incrementally observed. This may be due to the method used to quantify gDNA to infer mitogenome content (50 pg gDNA ~ 6000 mitogenome copies). Mitogenomes were put more than expected because they may be less degraded than nuclear genomes.
Overall, this study shows that ForenSeq mtDNA Whole Genome Kit is well-suited for the detection of mitogenomes of severely degraded samples (DI > 10). However, it is important to ensure that inhibitor samples must be well purified since hematin residues not only inhibit the first PCR but also subsequent sequencing reactions.

P-404

Development of an automated workflow for library preparation of ForenSeq assays

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Compared to historic methods like capillary electrophoresis (CE), massively parallel sequencing (MPS) enables the simultaneous analysis of more genetic markers, and provides a higher detailed genetic profile. However manual library preparation for sequencing is an obstacle and broader adoption for routine use, needs affordable and convenient automation for low to mid throughput. Here we describe the development of such an automated workflow, based on the EZ2 Connect Fx and the QIAgility. In this workflow, the QIAgility can be used to perform the PCR setup and library pooling steps. The EZ2 Connect Fx is used for library purification and normalization in a single protocol. The prefilled EZ2 cartridges increase reproducibility and significantly reduces hands-on time.
We will present data from the development of automated workflows for ForenSeq MainstAY, ForenSeq MainstAY SE, and ForenSeq DNA Signature Prep Kits.

P-405

Assessing the suitability of a commercial microhaplotypes assay for casework use

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Since microhaplotypes (MHs) first entered the forensic lexicon a decade ago, research and development in the area has advanced from the initial identification of candidate markers and generation of allele frequency data to wide-ranging marker discovery and characterization, publication of multiplex panels, and the establishment of community resources such as an international MH working group and the comprehensive, multi-purpose MicroHapDB. Interest in MHs continues to grow, in part due to one specific advantage of MHs over STRs: the simplified analysis and interpretation of mixtures because of the absence of stutter. Yet, for caseworking laboratories that may be interested in utilizing MHs, there have been few available options in the form of commercial kits. To help facilitate the implementation of MHs, we investigated the performance of a new, commercially available MH assay. The OmniHap™ Global Microhaplotype Profiling Kit (NimaGen), which targets 29 MH loci, employs reverse-complement PCR to perform amplification and library preparation in the same step, and is compatible with Illumina MiSeq sequencing. Using the open-source program MicroHapulator for data analysis, we evaluated the accuracy, precision, and sensitivity of the OmniHap kit. Additionally, we assessed aspects of assay performance that could affect allele detection and downstream interpretation, including inter-locus balance, heterozygote balance, primer carryover, and the impact of insertion-deletion polymorphisms within the marker definition for a MH locus. This presentation will describe the results of these examinations and consider the suitability of the assay for validation and casework implementation from an operational perspective.

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P-407

Advantages of using MPS in routine cases in forensics: more than just increased discriminatory power.

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1. Objectives
Regarding human identification purposes, forensic genetics has primarily relied upon a core set of autosomal and Y chromosome short tandem repeat (STR) markers that are amplificated and subsequently separated and detected using capillary electrophoresis (CE). While STR typing performed on this way is well-advanced and robust, advances in molecular biology, specifically massive parallel sequencing (MPS), provides brand new blessings in comparison to CE. One of the main advantages of MPS upon CE is that it allows us to amplify autosomal STR and Chromosome Y STR in the same reaction, while CE commercial kits just report either about autosomal STR or Y-STRs and Amelogenin. This feature allows us to discriminate correctly forensic cases where the chromosome Y may have mutations. Another advantage is the number of STR loci that it allows us to amplify compared to commercial CE kits, enabling a higher degree of discrimination in kinship cases. In this study, cases were compared where MPS provided us with information beyond what CE did.

2. Materials and Methods
DNA from the samples was extracted using the automated extraction protocol with EZ1 ADVANCED XL and quantified using real-time PCR. Subsequently, 22 different markers were amplified from each sample using NGM-SElect and AmpFLSTR Identifiler Plus. On the other hand, library preparation and amplification of marker loci were performed using the ForenSeq™ MAinstAY Kit (Verogen), which allows the analysis of 52 Short Tandem Repeat (STR) markers.

3. Results and conclusions
We present two cases where, the profile of the samples (reference, identified as males) was firstly analyzed using CE. In both cases, only the X marker for Amelogenin was detected. However, when analyzed using MPS, Y-STRs were amplified, chromosomally identifying individuals as males, whereas traditional sequencing methods would have identified these individuals as females. This is a key feature in forensic and identification cases where a misidentification error can alter the course of an investigation.

Additionally, we also found several cases where MPS has allowed us to be more discriminatory in kinship cases, where the sequencing of 27 autosomal STRs compared to the 15 or 16 loci amplified has allowed us to establish kinship relationships more accurately.

In conclusion, MPS emerges as a key tool in forensic genetics, providing large benefits over capillary electrophoresis in terms of efficiency, sensitivity, and discrimination for forensic analysis.

P-409

Forensic validation of the MPS Forenseq MainstAY assay

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Massively Parallel Sequencing (MPS) has emerged as a powerful tool for overcoming some of the current challenges of forensic genetics due to its improved sensitivity, higher discrimination power from the capacity to evaluate component marker's sequence variation (e.g., for STRs and microhaplotypes) and enhanced mixture interpretation. However, despite the increasing accessibility of MPS assays and platforms, results from forensic internal validations are not easily available, so it is difficult to evaluate the performance of STR analysis with MPS in real forensic routine casework.

The Forenseq® MainstAY SE assay is composed of 28 autosomal STRs (aSTRS), including the highly polymorphic SE33 marker, and 25 Y-chromosome STRs (Y-STR), offering the benefits of combined analysis of autosomal and Y STRs in one assay, and expanding the capabilities of the current 6-dye capillary electrophoresis kits. Here we present a forensic validation pipeline for the MainstAY SE assay, and we evaluate its performance on forensic routine cases. Protocol optimization using reduced volumes has been explored. Sensitivity analyses using dilution series, common control sample capillary electrophoresis-MPS genotype concordance evaluations, and analysis of prepared mixtures with different proportions of DNA from several donors. These evaluations have allowed us to define the limits of the MainstAY assay and to identify underperforming markers. Sequence coverage, stutter ratios, heterozygous balance, length/sequence variation and stacking artifacts were among the parameters evaluated for determining individual STR performance. Finally, comparative analysis in terms of performance and forensic informativeness were carried out using a range of casework samples, including DNA extracts from sexual assault samples, ancient skeletal remains and complex kinship cases.

P-411

Cas9-guided target nanopore sequencing and profiling of forensic short tandem repeats

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Objectives:
The novel gene editing technique known as CRISPR-Cas9 leverages the specificity of guide RNA to induce the cas9 protein to attach to particular locations in DNA, allowing for the precise cutting of specific DNA sequences. Prior research has demonstrated that CRISPR-Cas9 combined with high-throughput sequencing technologies can more effectively enrich microsatellite areas while lowering amplification-related noise. Furthermore, CRISPR-Cas9 in conjunction with nanopore sequencing technology has the potential to enrich the entire mitochondrial genome and address issues related to joint point mutations and long-range phasing. However, the enrichment of short tandem repeats panel by CRISPR-Cas9 with nanopore sequencing has not been reported.

Material and methods:
In this pilot project, we developed a STR enrichment method based on Cas9 technology in conjunction with a nanopore sequencing platform(Cas9-seq). We then analyzed seven STRs (D18S51, FGA, TPOX, D16S539, vWA, CSF1PO, and TH01) of NA12878 and 293T, assessed typing accuracy and stutter, and compared the results with an amplicon sequencing method (amplicon-seq).

Results and conclusions:
In both samples, the average sequencing depth of STR was 167X and 241X, respectively, and the enrichment efficiency was 556-fold and 803-fold, respectively. The average positive/negative chain ratio under the cas9-seq approach was 89.9%±7.6%, significantly greater than that under the amplicon-seq method (38.4%±6.7%). The average allele noise ratio of the remaining five STR sites, based on the Cas9-seq approach, was lower than that of amplicon-seq (9.8% VS 11.3%), except for FGA and D18S51. We also identified three point mutations that emerged in amplicon-seq by analyzing flanking sequences, which may be due to the amplification. In conclusion, the Cas9-seq approach can successfully enrich target fragments, opening up a new avenue for the identification of forensic genetic markers.

P-414

A massively parallel sequencing based forensic DNA analysis system: Four-in-One solution

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Fluorescence-labeled capillary electrophoresis (CE) is widely used to detect the length polymorphism of short tandem repeats (STRs) in forensic DNA identification. Recent over ten years, massively parallel sequencing (MPS) presents prominent advantages for STR genotyping, including much more genetic loci, higher polymorphisms (length and sequence) and larger sample throughput.

Here, we present a MPS based forensic DNA analysis system, which contains a DNA nanoball based sequencing platform, a four-in-one multiplex panel, an automatic forensic data analysis system, and a semi-automated DNA library preparation system. From DNA samples to genotypes, all steps are carried out within 2 days, including DNA library construction, sequencing and data analysis.

The four-in-one multiplex DNA typing panel contains four kinds of forensic genetic markers, including 48 STRs in autosomes, 62 STRs in Y chromosome, 32 STRs in X chromosome and three hypervariable regions of Mitochondrial genome. We can detect them simultaneously in one amplification. Using the panel, over 140 genetic markers are genotyped, which can generate much more polymorphisms and higher statistical power. It supports more complicated kinship inferences in forensic DNA analysis. In this system, we employ an efficient and flexible workstation with 96-channel pipettes to perform DNA library preparation. It reduces hands-on work from operators with standardized operation for as many as 192 samples per run. The quantitated DNA libraries were then pooled equally and sequenced on the DNBSEQTM sequencing platform at a read-length of 400 bp single-ends within 24 hours. The platform allowed dual flow cells to be operated with different read lengths independently or concurrently. For data analysis, we built an automatic forensic data analysis system, FGID. This system is linked with sequencer, realizes synchronous data transmission and carries out data analyses automatically. It provides not only the analyses from raw data (FASTQ) to genotype, but also the multiple forensic statistical inferences, including individual identification, paternity test and complicated kinship calculations.

The run time of the whole process from template amplification to forensic DNA report is less than 30 hours. Only one lab technician can detect hundreds of samples by using this system. It will make forensic DNA analyses more flexible, informative, accurate, and efficient.

P-415

Massive sequencing in molecular autopsy. Gene panel vs whole exome sequencing

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1. Objetives
Molecular autopsy is presented as a fundamental tool to elucidate sudden unexplained death (SUD) cases. In the last decade, the detection of genetic variants has gone from Sanger sequencing to the most modern massive sequencing approaches allowing large gene panels to be analysed. The evolution of technology, together with the reduction in costs, is currently favoring the choice of whole exome sequencing (WES) as a molecular autopsy strategy. In this study we will try to evaluate the efficacy of re-evaluating by WES cases of SUD that were previously analysed for a panel of genes with inconclusive results.

2.Material and Methods
In our study we started from 86 cases of sudden cardiac death (62 men and 24 women) with ages ranging from 0 to 62 years. All cases underwent a gene panel by massive sequencing that included the main genes associated with cardiomyopathies and channelopathies. In 14 cases in which no variant was found that could be associated with cardiac pathology, whole exome sequencing was performed, and then prioritized to search for pathogenic variants using a virtual panel of 565 genes associated with cardiac pathology.

3.Results and conclusions
Analysis of 86 cases with the gene panel showed pathogenic or likely pathogenic variants in relation to the risk of sudden cardiac death in 16% of them. Among the negative cases, the sequencing study was extended to WES in 14 cases with structurally normal heart at autopsy or with mild findings that do not justify the diagnosis of structural heart disease. Data analysis performed using a virtual panel of 565 genes related with cardiac diseases, showed no genetic variants related to sudden cardiac death according to current evidence.

Our study supports the efficacy of molecular autopsy in the search for the genetic cause of unexplained sudden cardiac death cases, showing a probable genetic cause in 16% of cases. However, it also suggests that the increase of genes beyond those definitively associated with cardiopathies and channelopathies, does not improve the efficacy of molecular autopsy. As long as there are no new genes with a definitive association to sudden cardiac death, new approaches should be directed towards the use of technologies that allow the detection of other genetic variants not detectable with WES, such as whole genome sequencing studies.

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Identification and characterization of Multi-Indels from whole genome data

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The analysis of mixtures has consistently been a significant challenge in the field of forensic science, especially when dealing with varying proportions of components and extremely imbalanced states. The amplification advantage of higher proportion components during the PCR process further magnifies this imbalance, potentially leading to the loss of alleles in subsequent genotype profiles. However, utilizing traditional genetic markers to address such issues proves challenging. Recently, some studies have reported the use of compound genetic marker to separate and detect the mixture based on the allele-specific amplification methods. Multi-Indel, a generalized microhaplotype formed by two or more closely linked indels within 200-300bp, has emerged as a promising candidate. Compared to other compound genetic markers, its advantage lies in its widespread distribution across the genome. Moreover, it exhibits length polymorphism, making it compatible with CE platforms without the need for introducing mismatches or other techniques. Therefore, we screened Multi-Indels from the whole genome and conducted allele-specific amplification. By designing two forward primers targeting different states of indel （insertions/deletions） and sharing the same reverse primer, we aim to selectively amplify the minor components in two-person imbalanced mixtures. Simultaneously, by restricting the amplicon lengths of different allele genes, individual haplotypes could be directly determined on CE platforms. And we simulated various proportions of mixture (1:50, 1:100, 1:200, 1:500, 1:1000, 1:1500) and applied it to practical cases of non-invasive prenatal testing. In summary, this study screened Multi-Indels from the whole genome using the data of 1000 Genomes Project and successfully established an allele-specific multiplex amplification system containing 16 loci. This system can amplify the minor components in imbalanced mixtures with high sensitivity and enhance the effectiveness of DNA mixture interpretation.

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Progress in Standardization of Forensic Next Generation Sequencing in China

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Next generation sequencing (NGS), also known as massively parallel sequencing (MPS), allows the simultaneous detection of various forensic genetic markers and expands the information content of a DNA sample. Chinese authorities and experts have already explored to applicate this emerging technology on practical case work. At least 80 Chinese forensic organizations have NGS platform, covering public security organizations at the provincial, municipal and county levels. Meanwhile more than 30 kinds of commercial NGS kits for various forensic purposes have emerged on the market. Indeed, it is urgent to establish national standards and public security industry standards to guide and standardize forensic NGS applications.

This report discusses three forensic NGS standards released by DNA Working Group of National Technical Committee for Standardization of Criminal Technology of China. The first standard focused on the use of NGS technology for human DNA genotyping by genetic markers, including STR, SNP, InDel and mitochondrial DNA. It outlines recommendation and requirements of the laboratory environment, equipment and kit, especially workflow, quality control, and analysis pipeline for NGS. The standard recommends assessing and filtering the quality of raw sequencing data and adopting a validated percentage of sequencing depth, rather than fixed depth value, as an analytical threshold for forensic practice. The second standard addresses the issue of nomenclature for sequence-based STR alleles, specifically the inconsistency in naming format. The proposal suggests using numerical values and a limited number of symbols to simplify naming while preserving complete sequence information for each allele's repeat and flanking regions. This approach ensures unique naming results and compatibility with length-based genotyping results. The standard includes reference repeat structures for the 167 forensic STR loci, along with examples to illustrate and aid in understanding the nomenclature rules. The third standard applies to all types of DNA genetic markers, including STR, SNP, microhaplotypes, and mitochondrial DNA, as well as kits based on various NGS platforms, such as Illumina, Ion Torrent, and MGI. It focuses on 12 performance indicators and qualification requirements for reagent quality testing, providing guidance for R&D, quality control and quality evaluation.

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Developmental Validation of the IDseek® OmniSTR™ Global autosomal STR profiling kit

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Forensic science takes advantage of population variability in autosomal Short Tandem Repeat (STR) lengths to establish human identification. The most common method for DNA profiling by STR is based on PCR, where the highly polymorphic STR regions are amplified and analysed using Capillary Electrophoresis (CE). STR analysis can also be performed using Massively Parallel Sequencing (MPS), in which besides the repeat length, also the repeat structure and variations in the flanking regions are determined. Additionally, MPS allows for greater multiplexing and smaller amplicon designs, making it more suitable for degraded samples. While MPS is superior in discriminatory power compared to CE, the relatively laborious multi-step sample/library preparation presents a certain barrier for routine application of MPS.

Reverse Complement PCR (RC-PCR) is a novel, more sophisticated PCR based MPS library preparation method, combining indexing and PCR amplification in a single closed-tube reaction. As a direct consequence of the single-step library prep the risk of sample swapping, PCR contamination and pipetting errors is drastically minimized. Additionally, the reaction kinetics of RC-PCR results in high sensitivity and specificity, because target-specific primers are synthesized during the reaction the concentrations of primers and available template are more aligned.

This poster discusses the complete developmental validation of the IDseek® OmniSTR™ Kit: an RC-PCR based MPS library preparation kit for autosomal STR profiling which includes all the US and European expanded core loci, plus SE33, D4S2408, D6S1043, D9S1122, D17S1301, D20S482, PentaD, PentaE and DYS391. All targets have been designed to generate short amplicons, while maintaining the most informative sites in the flanking regions. Performance of the kit was assessed on aspects such as concordance, sensitivity, inhibitor tolerance and mixtures with a ratio up to 1:20.

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Advancing Forensic Age Estimation: Insights from Fluorescence Intensity and Time-Resolved Fluorescence Spectroscopy on Human Semen Stains

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Biological evidence, including blood, semen, and sweat stains, is crucial in forensic investigations by providing DNA for the identification of suspects or victims. Equally vital is the estimation of the age of these body fluid satins, offering critical insights into the timeline of a crime.

This study employed two fluorescence spectroscopy techniques, namely fluorescence intensity and time-resolved fluorescence, to estimate the age of human semen stains. The semen stains deposited on glass surfaces for various durations (0, 1, 2, 3, 5, 7, and 14 days). Preliminary results indicated that fluorescence intensity exhibited significant changes in the long term, whereas time-resolved changes occurred in the short term. A trend line for these techniques was calculated using a mathematical model. The fluorescence intensity technique produced low R-squared values at 0.45, indicating a weak predictive equation and suggesting the need for additional data to enhance accuracy in the future study. Conversely, the time-resolved fluorescence technique produced high R-squared values at 0.89, indicating a more robust predictive capability.

The integration of fluorescence lifetime with fluorescence intensity in the prediction model showed substantial potential for estimating the age of semen stains. Moreover, this methodology shows promise in estimating the age of other biological evidence and could potentially be incorporated into forensic light sources (FLS) as equipment for estimating body fluid age in the future.

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Assessing the Utility of STR Profiling for Combating COVID-19 Insurance Fraud Involving Lateral Flow Test Kits in Thailand

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During the COVID-19 pandemic in Thailand, insurance companies offered benefits upon positive virus detection. However, accessing real-time PCR (RT-PCR) tests and results proved challenging, delaying treatment and insurance claims. Consequently, the government allowed positive lateral flow test (commonly known in Thailand as antigen test kit or ATK) results for medical certificates. This created a vulnerability for insurance fraud, as individuals could potentially use another person's positive ATK result to obtain a certificate and claim benefits. Estimates suggest this resulted in over 500 million Baht in fraudulent claims.

1. Objective
This study aims to investigate the feasibility of using Short Tandem Repeat (STR) profiling to verify the authenticity of COVID-19 ATKs.

2. Material and Methods
ATKs with negative results from a volunteer will be used to assess the quantity and quality of DNA recoverable from various ATK segments and its suitability for STR analysis. Comparisons will be drawn between profiles generated from DNA extracted using conventional procedure and direct PCR protocol. Additionally, used ATKs stored for 7, 14, 30 days, and more than one year, will be examined.

3. Results and conclusions
It is expected that the results could provide a valuable tool for law enforcement and insurance companies to combat fraudulent claims associated with ATKs used for COVID-19 testing.

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Expanding the capacity of conventional fragment analysis with virtual channels

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The most widely used technic for forensic genotyping is fragment analysis based on capillary electrophoresis. This method is cheap, fast and robust, but its capacity is limited by the number of detection channels. Most of the currently used capillary analyzers are able to detect simultaneously up to six fluorescent dyes. This limits the number of markers in the multiplex analyzed and also puts restrictions on the size of PCR products. Ideally, a forensic laboratory would need a multiplex assay that makes it possible to analyze all the markers of interest in one reaction with the shortest possible target. This is achieved by use of mass parallel sequencing (MPS) approach, but MPS is more expensive, more time and labor consuming, and is less available for most forensic laboratories.

We have developed a new approach to extend the capabilities of conventional, PCR based fragment analysis by introducing additional virtual detection channels. New virtual detection channels are created by double labeling fragments with a combination of dyes. Double labeled fragments are detected by the instrument simultaneously in different dye channels. Our specially developed software recognizes these double detected fragments and analyzes the data in a new virtual channel. Each combination of two dyes creates a new virtual channel. We can get up to fifteen new virtual channels in addition to the existing six. Thus, we can use 21 detection channels instead of six. This increases the power of the method by 3.5 times.

We have successfully tested the virtual channel concept with our prototype of forensic mini-STR multiplex assay. The virtual channel concept allows the detection of all CODIS/ESS markers in the shortest possible amplicons in a single reaction. Further validation of the method is required to evaluate the advantages of the proposed approach in various forensic scenarios, e.g. on degraded samples.

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EFFECT OF CHELATING AGENTS ON THE QUALITY OF DNA PROFILING: A FORENSIC APPROACH

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Deoxyribonucleic acid (DNA) is presently considered as one of the most significant forms of biological evidence, and it has a long history of forensic successes. In DNA Extraction, they are shortcomings of preservation of DNA specially, the degraded DNA which cannot be used to generate full genomic profile due to breakage of nucleotide sequences. To overcome the shortcomings, in order to extract High molecular weight Deoxyribonucleic acid, the activity of the enzyme DNAse which catalyses DNA hydrolysis by cleaving DNA's phosphodiester links should be regulated. Human DNAse I (hDNAse I) need bivalent ions of magnesium (Mg2+) and calcium (Ca2+) to operate effectively in its catalytic function and the synthesis of oligonucleotides with phosphate at the 5’ end. The anticoagulant ethylenediamine tetra acetic acid (EDTA) is an indirect deterrent of DNAse I that functions through chelation of divalent ions (Ca2+ and Mg2+) that are crucial to the structure of the enzyme and its enzymatic activity in general. The use of the above-mentioned synthetic chelating agent is a well-known Environmental contaminant which persist in the environment because it is poorly biodegradable, to address the problem we introduced a novel chelating agent. We assessed the DNA concentration extracted using novel chelating agent, Sodium Gluconate and a comparative study with the age old ethylenediamine tetra acetic acid (EDTA) in 50 samples ranging from Fresh Blood to Blood preserved for 15 days before extraction. We developed a new extraction process using novel chelating agent which provides better concentration of DNA in Fresh blood and also the use of Proteinase K is been omitted, and irrespective of that procedural omission, the extraction process gives high yield of DNA which is relatively purer than the traditional method as with alliance to Protein Purity, where Sodium Gluconate shows a significant better purity value around 1.8, showing less fluctuation for entire sample collection treated with Sodium Gluconate rather than EDTA ($p = 0.052$). As the scientific community continues to refine methodologies and practices, the findings of this dissertation work contribute to the ongoing dialogue on DNA extraction protocols for enhanced accuracy and cost-effectiveness in genetic research.

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Strategies of Solving Unbalanced DNA Mixtures by DIP-STR Technology

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Objectives: With the development of forensic DNA technology, forensic genetics has played an increasingly important role in solving cases as well as brought huge technical challenges. Accompanied by the increasing sensitivity of STR testing, it becomes more and more common to obtain DNA mixture profiles in daily testing. In DNA mixtures, when the DNA are significantly different between major and minor contributors, the phenomenon of DNA annihilation from minor contributor is prone to occur, which results in unusable or weakened evidence of DNA mixtures. In 2013, Castella et al. firstly reported to use a kind of new linked genetic marker combined by DIP and STR in DNA mixture tests, and then screened new DIP-STR marker sites and constructed a composite amplification system which is suitable for the Swiss population. Inspired by this, our group also performed to screen and verify DIP-STR markers suitable for the Chinese population. Material and methods: Firstly, referenced to the screening conditions of DIP-STR markers proposed by Oldoni et al , the method of computer programming was used to output the DIP-STR sequence and related site information from the UCSC database which met the 5-point screening conditions, and 100 sites were totally obtained. Secondly, design primers and make further screening of the 100 primers and get over 10 maker sites by discarding the maker sites which the target fragment is too long, the primer annealing temperature has big difference, or there are too many other SNP, DIP or STR sites in the DIP-STR site. Then, verified these sites by PCR and agarose gel electrophoresis. After ensuring the corresponding length of amplified fragments appears, a large number of samples were verified and tested for sensitivity by CE electrophoresis. Results: We have obtained over 5 DIP-STR markers suitable for the Chinese population. Finally, we tested the obtained sites in unbalanced DNA mixture samples and found that these sites all performed well in distinguishing individuals from unbalanced DNA mixtures.

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Implementation of an AI system for automated sperm identification

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Microscopy for identification of spermatozoa is a labour-intensive task in forensic laboratories. Automation of imaging and image interpretation has the potential to greatly improve throughput. Automation may also increase quality by helping to ensure consistency between operators or laboratories.

We describe the evaluation, validation and ongoing implementation of Metasystems Metafer, an automated microscope system which includes robotic feeding of microscopic slides and automated image analysis by artificial intelligence. The system can be loaded with up to 80 microscopic slides, read the slides unsupervised, assign a score to potential sperm in each slide and present those to an operator in order ranked by likeness to a spermatozoon. The operator can then make a judgement on whether to accept the presented objects as sperm or not.

We have investigated how to optimize specificity and sensitivity with regards to the operator effort needed. We find that imaging in multiple focus planes considerably increases specificity for spermatozoa, reducing the number of objects that must be reviewed. We have also mapped the distribution of scores for sperm and non-sperm objects, allowing the laboratory to decide on a well-informed policy on how many potential sperm objects that must be reviewed to find positive samples with sufficient sensitivity. Whether certain sample types are unsuitable for automated analysis is also investigated. Finally, we have performed an in-house validation, finding that sensitivity using the automated system surpasses that of manual microscopy.

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Validation of sperm detection scanning system by Metasystems

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Manually searching sperm cells from microscopy slides of trace samples of sexual assault cases is a routine process in forensic laboratories. Often the case samples contain very few or no sperm cells, which makes it crucial to methodically cover the whole search area. This makes it time consuming and susceptible to human error.

Deep neural network algorithm based automated sperm detection has been established by Metasystems, for searching Christmas Tree-stained sperm cells on microscope slides prepared from forensic casework samples. The current study was performed to validate the new system as a method to be used routinely in the Estonian Forensic Science Institute.

Validated parameters were concordance, repeatability, reproducibility, and sample traceability. Concordance was tested by comparing the previously gained casework analysis results of manual search to the new method, where samples were searched and scored by Metasystems Sperm Finder and confirmed by an expert. In addition, mock samples were prepared and compared using manual and automated search.

Repeatability of the system was evaluated by analysing dilution series of mock samples in 5 replicas. For reproducibility, the same slides were scanned again on a different day. The system classifies the findings into three categories (sperm candidates, unsure, background). For repeatability and reproducibility, the number of sperm candidates were compared.

Test slides were prepared using Christmas Tree staining and scanned using single plane focus and 20x objective. The findings were verified by the expert from the scanned images in the gallery or using the microscope with 20x or 50x objective if focus required manual adjustment.

We describe the results obtained and draw conclusions on the suitability of the method for routine forensic casework.

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Establishment and validation of automated microscopic sperm detection using Deep Neural Network-based analysis software in a forensic routine laboratory

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Objectives
The Institute for Forensic Genetics in Berlin receives about 20,000 applications for DNA analysis every year, including at least 400 sexual offenses. Despite the possibility of performing immunological tests for proteins of semen fluid (PSA, Semenogelin) as well as differential lysis of sperm and epithelial cells, microscopic sperm detection in our laboratory is still routine. Among other things, because courts often only recognize microscopic representation as unambiguous sperm detection.
However, the traditional microscopic assessment of up to 20 specimens per case is labor- and personnel-intensive as well as time-consuming. For example, in the case of conventional analysis, up to 2 hours per preparation may be needed to rule out the presence of sperm, depending on the type of sample. In addition, success depends on subjective influences brought in by the person performing the process (amount of material used, preparation, staining, time required, concentration, experience, assessment matrix).

Material and methods
In order to narrow down some of these individual factors and at the same time to be able to work efficiently and time-saving, we have established and validated the use of an automatic microscope (Zeiss) and a Deep Neural Network (DNN) based analysis software from MetaSystems (Metafer) and integrated it into the workflow.

Results and conclusions
Here we report on the implementation process of automated sperm detection in which DNNs learn from example images that are labeled by experts. We show the adaptation of the DNNs with multiple rounds of re-training to detect and classify objects specified by the user (e.g., sperm, sperm head, doubtful sperm head). We demonstrate, how the definition of more refined object classes influences correctness and predictive value of the resulting assignment based on relative assignment scores (0 to 100). Introduction of reference scans that have been examined independently by several persons using conventional microscopy allowed for validation of the automated sperm detection by direct comparison of classification success rate (true positives vs. false positives). In addition, the improvement of the specific network applied can be assessed by changes in the relative scores achieved for respective object classes.
Our validation data show, that sensitivity and objectivity of the microscopic detection method could demonstrably be increased by routine implementation of automated sperm detection and in addition, that time and manpower required for examinations were significantly reduced.

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Interpreting Microhaplotype Data

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BACKGROUND: Microhaplotype markers consist of multiple SNP markers covered by a single sequencing read. This class of marker is rapidly gaining interest in the forensic community because of the simplicity of analysis relative to sequence based STRs. Microhaplotype loci exhibit high heterozygosity like sequence based STRs, and microhaplotype multiplex genotypes exhibit similar allele counts. Microhaplotype alleles can be defined as SNP-SNP microhaplotypes, whereas sequence-based STR alleles can be described as SNP-STR microhaplotypes. Therefore, much of the same analysis philosophy can be applied to both marker types. While fewer artifacts are observed in SNP-SNP microhaplotype analysis, genotype profiles of these markers are not artifact-free.

OBJECTIVES: To compare microhaplotype and sequence based STR analysis, to describe options for microhaplotype allele and genotype displays, and to demonstrate microhaplotype data interpretation.

METHODS: DNA samples were obtained from standard sources including NIST and Coriell. Profiles were generated using the OmniHAP™ and OmniSTR™ kits (NimaGen) and MiSeq™ sequencer (Illumina). All analysis was performed using MixtureAce™ software (NicheVision).

RESULTS: Microhaplotype alleles can be presented in several optional formats including as raw sequence strings (for evidence preservation), SID labels (for ease of communicating allele profiles), strings of the canonical SNP states, and canonical SNPs supplemented by additional non-reference SNP states. An approach is demonstrated for managing microhaplotype allele frequencies for kits that cover less than the full set of canonical SNPs. This same approach can be used for comparing profiles from different kits covering different genomic ranges for the same microhaplotype alleles. Techniques are demonstrated for managing artifacts arising from homopolymer stutter, sequencing error, and PCR misincorporation error. An approach is demonstrated for recovering full length phased microhaplotype alleles when using sequencing cycles shorter than the length of the defined microhaplotype. Comparisons are made between microhaplotype allele and sequence based STR profile displays.

CONCLUSIONS: Issues surrounding microhaplotype analysis are presented and discussed and procedures for implementing microhaplotypes in forensic casework are demonstrated.

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MPS vs CE STR data, similarities and differences and the impact on LR calculations using probabilistic genotyping systems

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The number of commercial kits for Massively Parallel Sequencing (MPS) of STRs for human identification is rapidly increasing, and lab protocols are becoming easier and more suitable for high throughput use. While multiple studies have shown advantages of MPS STR sequencing compared to Capillary Electrophoresis (CE) analysis, only few studies have addressed the statistical likelihood ratio (LR) calculations on MPS STR data. The transition from CE peak heights to MPS read counts maintains many similar factors to be taken into account for probabilistic LR calculations, but some subtle differences between MPS and CE data and their corresponding laboratory workflows, can impact the manner in which current CE-based statistical models handle MPS data.

In this study, we address the impact of differences between MPS and CE data and lab workflows on probabilistic LR calculations. Specifically, we focus on differences between replicates of the same mixture when, for instance, generated in different MPS runs. LRs were calculated using DNAStatistX for 54 complex 3-, 4- and 5-person DNA mixtures that were analysed in triplicate using the IDseek® OmniSTR™ Global kit. Differences between the triplicate analyses often impacted the LR calculations.

In this presentation, we discuss the different approaches that have been explored and provide recommendation for addressing MPS specific factors that impact the LR calculations.

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Development of a Nicking Endonuclease Dependent Amplification Method and Its Use in STR Analysis of Trace DNA

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Objectives
Detection of trace DNA from crime scene samples is a great challenge in forensic DNA analysis. Some samples, especially touch samples, contain only trace amount of DNA. Amplification of those samples often result in loss of loci / alleles or even total failure. To improve detection of trace DNA, various preamplification technologies, including WGA, MDA, RCA, nested PCR etc have been developed and applied to trace DNA analysis. Unfortunately, none of those technologies is faithful enough to be used as a valid method to assist trace DNA analysis. The unfaithful amplification is mostly caused by primers used in preamplification, either random hexamers / octamers or locus specific primers.

Methods
Here we describe development of NDA (Nicking endonuclease Dependent Amplification), an isothermal whole genome amplification method and its application in trace DNA analysis. Two essential enzymes used in NDA are: one nicking endonuclease which recognizes a specific sequence of a double stranded DNA and generates a nick only on one of the strands, and one DNA polymerase with strand displacement activity which can extend the nicked DNA. Upon extension of the nicked strand, the existing strand is displaced, a new strand is synthesized, and the nick is sealed. Then nicking and extension processes are repeated. Consequently DNA in the NDA system is linearly amplified.

Results and conclusions
The NDA technology has good accuracy without changing trace DNA's genotype.We have verified that the average amplification efficiency of AMEL, 12 autosomal STRs and 19 Y-STRs loci can be increased by more than 5 times based on NDA. These autosomal STRs loci are D19S433, D2S441, D8S1179, TH01, D22S1045, FGA, D5S818, D16S539, vWA, D18S51 and D10S1248, respectively. These Y-STRs loci are DYS576, DYF387S1ab, DYS635, DYS449, DYS627, DYS393, DYS481, DYS389I, DYS460, DYS456, DYS385ab, DYS437, DYS458, DYS439, DYS518, DYS557, DYS389II, DYS444 and DYS392, respectively. The detection rate of trace samples under the LOD can be significantly improved.

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Determining the Best Strategy in Processing Low Level DNA Samples from Fired Cartridge Cases (FCCs) and Firearms Given Quantifiable Metrics of Recoverable DNA

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Between 2017 and 2021, the National Integrated Ballistic Information Network (NIBIN) observed a staggering 103% surge in cases, totaling 843,719 casings, emphasizing the alarming rise in firearm-related crimes in the United States. This underscores the critical necessity for forensic laboratories to efficiently process fired cartridge casings (FCCs) and firearm (FA) swabs.

DNA profiles obtained from these low-level sample types can provide important information to criminal investigations and possibly identify suspect(s) where there are little or no other investigative leads. With novel DNA testing methods like Massively Parallel Sequencing (MPS) now being used in the forensic community, this allows laboratories to utilize another tool to obtain DNA profiles from low levels of DNA.

This study aims to address this pressing need by developing a decision tree to guide laboratories in processing low-level DNA samples from FCCs and FA swabs effectively. It evaluates whether traditional DNA methods with capillary electrophoresis (CE) or MPS methods should be employed based on DNA quantity and quality metrics.

FCCs and FA swabs collected at four locations (slide, magazine clip, trigger, and handle) were processed using a customized extra-large volume method on QIAGEN's EZ2 Connect instrument. This instrument facilitates substantial lysate volumes from two FCCs (4000µL), and allows for low elution volumes (20µL), enhancing DNA fragment recovery with ethanol. Quantification was conducted using the Quantiplex Pro RPQ Kit with average quantification values and degradation indices (DI) calculated.

Samples underwent processing using the Investigator® 24plex QS Kit on the 3500 Genetic Analyzer and the ForenSeq™ MainstAY kit on the MiSeq FGX. Data analysis was carried out on GeneMapper ID-X (CE) and Universal Analysis Software (MPS).

The quantification values for FCCs ranged from 1.7 pg/µL to 10.9 pg/µL, alongside degradation indices varying between 1.2 and 12.3. Notably, the Investigator® 24plex results revealed that FCCs successfully retrieved 89% of alleles from the contributor. For FAs, quantification values ranged from 8.9 pg/µL to 95.4 pg/µL, with degradation indices ranging from 3.8 to 10. The Investigator® 24plex yielded a complete recovery of alleles (100%) from the contributor in FA samples. Expected outcomes are anticipated with the MainstAY results, which, along with data from additional donors tested, contribute to the development of a comprehensive decision tree tailored for low-level DNA samples. This study demonstrates an optimized extraction method for FCCs and a guidance on choosing the most suitable analytical method for low-level DNA, crucial for addressing the escalating firearm-related crimes.

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Evaluation of a new collection technique for touch DNA: the DNA-Buster

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Biological material is often secured as potential evidence for crime investigation by rubbing with swabs or by taping. Since current research indicates an interaction between substrate properties and collection methods, research efforts are focusing on the development of new collection techniques, such as suctioning. At the Institute of Forensic Medicine Basel, Switzerland, the DNA-Buster has been developed, a new dry suction device for forensic use. It is mobile and can be used both stationary and on the crime scene, allowing access to hard-to-reach areas like crevices or vehicle seats.
For evaluating performance regarding DNA yield and profile quality, the DNA-Buster was compared to standard methods (swabbing, taping) and the wet vacuum method (M-Vac®) on five different substrates (carpet, cotton sweater, stone, tiles, and wood) prepared with touch DNA. Despite conventional methods yielding better results on carrier materials such as wood and stone, the results demonstrate, firstly, the suitability of the DNA-Buster as a superior DNA collection tool for textiles, and secondly, the need for optimization for its use on other carrier materials. Thirdly, the data emphasize the dependence of the collection method and the substrate. Therefore, the results provide essential insights for determining the optimal method for the precise and targeted collection of genetic material from forensic trace carriers.

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Evaluation of a novel tapelift-applicator to elevate DNA recovery by tapelifting

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Whilst swabs are used globally for DNA recovery, their performance is impacted by various factors, such as application pressure and angle, wetting agent volume and type, swab type, etc. Use of adhesive tapelifts to collect DNA can eliminate some of these factors and be as effective as swabbing, even for non-porous surfaces that are not routinely sampled by tapelifts. However, tapelifts can be prone to user contamination due to excessive handling when applying a tapelift to a surface and placing it into an extraction tube for DNA processing. In response to a call for the development of a field-ready DNA collection method that minimises user contamination, is easy to use and is equal to or more effective than current methods, we developed an applicator for use with tapelifts to meet these aims. Evolution of the applicator's design was informed by multiple rounds of testing its performance against manual tapelifting for DNA recovery from substrates seeded with human saliva dilutions. Testing of the final design demonstrated that the applicator can perform as well or better than the manual tapelift method for cotton, denim, tile and synthetic leather, but varied results were obtained with brass and polyester. Here, we present testing of the applicator to examine whether user experience impacts its performance and investigate how it could perform in casework. Four experienced crime scene examiners and four novices used the applicator with tapelifts to recover DNA from cotton and tile substrates seeded with saliva dilutions. Each test was performed with four replicates, and examiners answered questions on their experience using the applicator. Performance of the tapelift-applicator was then compared to the routine DNA recovery method for various mock exhibits and surfaces within vehicles and residences. Results from these experiments provide insights to the feasibility of introducing the applicator into casework to facilitate DNA recovery by tapelifting.

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Evaluation of recovery of DNA from blood, saliva and touch samples deposited of different states of acetaminophen

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The manufacture, distribution and use of illicit substances requires the handling of various chemicals, and the final product. It is possible the DNA of people handling such samples may be present on the substrate, and can therefore be useful within forensic investigations for identifying persons of interest. However, to date, there has been no research that explores the possibility of collecting DNA from different biological sources after they have been deposited on such substrates. This study aimed to determine if DNA could be recovered from blood (20 µL), saliva (20 µL) and epithelial (touch) samples (10 seconds from single finger) deposited on acetaminophen prepared in different states; large crystalline, powder and residue form. Acetaminophen was chosen as it can readily form large crystals and is a known excipient in drug manufacture. The quantity and quality of recovered DNA was investigated. For the blood and saliva samples, all returned full profiles that corresponded to the reference profile, thus highlighting the potential for use for identification purposes in casework. For touch samples, 33% returned no detectable alleles, yet the remaining 66% of samples did have alleles that corresponded to the reference profile, thus it can still be informative within forensic investigations, including in an exclusionary capacity. It was discovered that irrespective of biological material, the highest amount and quality of DNA was recovered from residue substrates. Touch samples were found to have the highest percentage recovery when compared to the other biological samples yet were significantly more degraded. No inhibition was detected in any samples. This research can be used as a stepping stone to help understand the impacts of drugs on DNA profiling and in the consideration of targeted sampling of biological materials. This can further assist bringing justice to those engaging in such illegal acts, and aid in safeguarding the public from drug related crime.

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Optimizing the Recovery of DNA from Exhaled Breath Devices for Human Identification

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Recovering DNA from exhaled breath may have some interesting forensic applications, but it presents unique challenges. The objective of this study was to investigate whether DNA could be captured from exhaled breath using two different collection devices: Breath Explor® and SensAbuse®. These devices are typically employed for in-field drug testing and then posted by mail for further analysis in a laboratory; emphasizing the importance of maintaining sample integrity to safeguard the chain of custody. Processing these breath devices for both drugs of concern and DNA to confirm sample identity could hold considerable value.

Various DNA collection methods were evaluated to determine if quality STR profiles could be retrieved from the mouthpieces and/or internal filters of these devices. Cotton and microFLOQ® swabs were used to sample the mouthpieces of both devices. The filters underwent swabbing with a microFLOQ™ swab or were subjected to two different soaking methods; one using EZ1 Investigator® chemistry, and the other a method established by the San Diego Police Department. All samples were quantified using the Quantiplex Pro (QIAGEN) kit and genotyped with the Investigator™ 24 plex kit on a 3500 Genetic Analyzer. However, these approaches yielded suboptimal DNA recovery and partial STR profiles.

In an effort to improve the capture of exhaled breath, we tried inserting wet or dry FTA® card punches into the mouthpiece of the Breath Explor® device. This strategy failed to yield any significant improvements, with less than 10% of samples exhibiting detectable DNA quantities. Finally, the use of Diamond Dye® was explored in an attempt to visualize cell or cell-free DNA transfer from exhaled breath onto the devices, allowing for targeted sample collection.

In summary, this study confirms that collecting DNA from exhaled breath is difficult; however, in cases where the person being drug tested with these devices is in question, laboratories should prioritize swabbing the inside of the mouthpiece for the most successful STR typing results.

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Comparing Extraction and Direct Amplification Methods for Enhanced Touch DNA Profiling

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The study investigates the comparative effectiveness of two distinct methods for DNA collection and profiling, namely traditional extraction and direct amplification, in the context of Touch DNA profiling. Various surface sizes and materials were utilized to assess the impact of method selection on DNA yield and profile quality. Results demonstrate that direct amplification, especially when employing MicroFLOQ™ Direct swabs and SceneSafe Fast™ minitape, exhibits superior performance in scenarios involving smaller surface areas. Conversely, extraction proves advantageous for larger surface areas. The research highlights the critical importance of method choice based on surface size, providing insights to enhance the efficiency of forensic DNA analysis. These findings offer valuable guidance in the optimization of Touch DNA profiling techniques, contributing to the advancement of forensic science.

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Internal Validation of the Investigator 26Plex QS Amplification Kit: a high-throughput multiplex assay for reference and low copy number DNA samples

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The Investigator® 26plex QS Amplification Kit, from QIAGEN, provides reliable and rapid DNA profiles while enabling the multiplex amplification of 24 STRs, 2 Quality Sensors and a gender-determining marker, Amelogenin. The Quality Sensors incorporated in this kit provide insight into the sample quality and the PCR's success while alerting to the presence of inhibitors.

Through an extensive internal validation study, this research sought to implement the Investigator® 26plex QS in the laboratory routine of the Forensic Genetic and Biology Service, Central branch (SGBF-C) of the Portuguese National Institute of Legal Medicine and Forensic Sciences. Here we detail the procedures and parameters applied which followed the SWGDAM guidelines, as well as report the results obtained throughout.

Firstly, it was crucial to establish unique analytical criteria that would be the baseline for the interpretation of the results obtained. To accomplish such, analytical, stochastic, heterozygous balance and stutter thresholds were defined. Thereupon, this study intended to gauge the kit's performance, by evaluating its concordance with normalized methodologies while assessing its sensitivity, specificity, robustness, and precision, besides its efficiency in the presence of degraded and/or inhibited samples. Lastly, in favour of optimizing the laboratory workflow, an assay was carried out to test half volume reactions and direct amplification on reference samples.

In this study a wide range of sample types were analysed, which made it possible to acquire robust data pertaining to the performance of the assay. The laboratory methodology applied comprehended: DNA extraction using Prep-n-Go™ Buffer and PrepFiler Express™ Forensic DNA Extraction Kit, quantification with the Quantifiler™ Trio Quantification Kit, followed by the amplification with the Investigator® 26plex QS. Capillary electrophoresis was performed in the Applied Biosystems™ 3500 Genetic Analyzer, and the electropherograms' were analysed using the GeneMapper™ ID-X Software.

Through this work, it was possible to characterize the main advantages of this amplification kit, as well as its limitations. The validation data demonstrated that this system produces reliable profiles in the presence of minute DNA quantities and identifies the minor contributor in a mixture up to a 1:50 ratio. The results inferred a high human specificity, robustness, and sensitivity, while producing concordant and reproducible results with optimized protocols for both reference and low copy number DNA samples. Through concordance studies it was further shown that there is a high consistency between this novel amplification kit and those currently implemented in the SGBF-C, thus proving its suitability for the analysis of forensic samples.

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Straight Outta the Gel: direct PCR from agarose and polyacrylamide gels

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The separation of DNA fragments of varying lengths constitutes a fundamental step in genetics, often accomplished through agarose or polyacrylamide gel electrophoresis. If the further analysis of a given DNA fragment is necessary, it is usually excised from the gel and the DNA is extracted from the gel slab. DNA isolation methods, such as the crush and soak, or commercial gel extraction kits are available, but it might be possible to forgo this step.

In this study, we propose a novel approach employing direct polymerase chain reaction without DNA extraction for downstream applications (e.g. sequencing, cloning). Comparative analysis was conducted to evaluate the efficiency of amplification post-gel extraction using established DNA elution techniques and a commercially available DNA elution kit, juxtaposed with the efficacy of direct PCR from gel-incorporated DNA.

Our findings indicate no discernible difference in amplification efficiency among the tested methodologies, including the commercial kit, the traditional crush and soak method, and the direct PCR approach, irrespective of whether agarose or polyacrylamide gel electrophoresis was employed. Cooling (4 °C) or freezing (-20 °C) the excised piece of gel did not change the results.

Notably, direct PCR emerges as a time-saving and cost-effective alternative, offering comparable results to conventional techniques. This study underscores the potential of direct PCR to streamline gel electrophoresis workflows while maintaining analytical fidelity, thus presenting a promising avenue for molecular biology research.

P-475

Enhancing Forensic DNA Analysis Through the Utilization of HiMedia's Insta NX® Mag32 Automated Nucleic Acid Extractor with HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32

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Objectives
Forensic DNA analysis plays a pivotal role in criminal investigations, providing critical evidence for identifying perpetrators and exonerating the innocent. Traditional DNA extraction methods often involve complex procedures and are prone to contamination, leading to potential inaccuracies and delays in case resolution. HiMedia's Insta NX® Mag32 Automated Nucleic Acid Extractor offers a cutting-edge solution for streamlining the DNA extraction process in forensic applications. This system employs magnetic particle technology to efficiently isolate high-quality DNA from various forensic samples, including blood, blood-stained fabric, saliva, hair, tissue, bone, teeth, etc. using HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32.

Material and methods
Validation of HiMedia's Insta NX® Mag32 Automated Nucleic Acid Extractor with HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 were performed in accordance with the guidelines outlined by the Scientific Working Group on DNA Analysis Methods (SWGDAM) and European Network of Forensic Science Institutes DNA Working Group (ENFSI). The validation described includes different parameter studies viz., sensitivity, mixture analysis, reproducibility, etc.

Results and conclusions
The results from the sensitivity study indicate that DNA can be extracted up to 0.0003 ng/µL. Furthermore, this system enables the extraction of DNA from challenging sample types, such as degraded or low-yield samples, which are frequently encountered in forensic casework. In addition, the system shows the ability to extract DNA by removing the potential inhibitors. By optimizing DNA recovery and purity, this system enhances the sensitivity and accuracy of downstream DNA profiling techniques, such as short tandem repeat (STR) analysis. The STR profiles from different samples were much better when compared with Applied BiosystemsTM AutoMate Express Forensic DNA Extraction System, Qiagen's EZ1 Advanced XL and Promega's Maxwell® RSC Instrument. Its automated workflow minimizes human error, reduces hands-on time, and enhances reproducibility, thereby improving the reliability and efficiency of forensic DNA analysis. Overall, the results demonstrate that DNA extracted on the HiMedia's Insta NX® Mag32 Automated Nucleic Acid Extractor with HiPurA® Multi Sample Pre- filled Plates for Insta NX® Mag32 kit generates profile results that are accurate, reproducible, precise in sizing and free from human DNA contamination.

P-476

A High Performance PCR Assay System Capable Of Cleaning Carry-Over Contamination

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Objectives
Longo conceptualized dUTP / UDG method to control carry-over contamination in PCR in 1990. Since then dUTP / UDG has been widely used in PCR practice. It also safeguards automatic PCR systems. Conventional PCR DNA polymerase fails to incorporate dUTP efficiently in a high multiplex STR amplification especially those loci with high A/T content. Control of carry-over contamination in STR analysis has been relying on carefully designed and constructed laboratory and good laboratory practice. Lack of a dUTP / UDP PCR system also hinders effort to develop an automatic STR analysis system except cartridge based systems such as RapitHit ID and ANDE 6C which work well with high DNA content samples such as buccal swabs and blood stains but suit not well with touch DNA.

Methods
We developed a high performance dUTP / UDG PCR system based on a novel engineered Taq DNA polymerase. The engineered polymerase utilizes dUTP more efficiently than wild type Taq DNA polymerase. A high performance 25 loci (20 CODIS loci, Penta D, Penta E, D6S1043, Amel, and a Y-indel) assay has been developed. A 2-step 29-cycle amplification is completed in about 75 minutes.
Results

Full DNA profile is generated from as low as 62.5 pg human genomic DNA. As much as 104 copies of carry-over contamination could be eliminated by UDG. The system also works well with inhibited samples. In conclusion, the performance of this cleaning carry-over contamination PCR system is as same as original STR multiplex PCR kit with dTTP.

P-477

The use of non-thermal plasma for DNA decontamination in forensic settings

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The validity of DNA profiles in forensic cases are always subject to scrutiny. This is why it is crucial to prevent the contamination in forensic casework. . This study reports on the method used to evaluate the effectiveness of non-thermal plasma in the destruction of human cellular DNA for the purpose of decontamination in a forensic laboratory setting.

Previous research in agricultural and medical sciences proves that non thermal plasma can be used as a sterilisation tool to destroy unwanted pathogens and microorganisms. Despite the low cost, lack of solvents involved and potential to reach areas out of the line of sight the use of non-thermal plasma for DNA decontamination in forensic settings has not been thoroughly explored.

In this preliminary study we use a human upper respiratory track cell line to spike artificial saliva with known concentrations of DNA. A range of DNA concentrations (0.5ng – 25ng) underwent exposure to the plasma under different settings such as time, power and pressure. The samples were then collected using nylon flocked swabs and extracted from using Qiagen Investigator Extraction Kit. The additional step of the QiaShredder Column was added to try to insure the maximum recovery of DNA possible. Results were quantified using the Qubit 3 and RT – PCR. Preliminary results show at least a 10-fold decrease in DNA concentration under tested conditions. Overall, this study showed promise in the use of non – thermal plasma to prevent DNA contamination in forensic laboratories.

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Detection of DNA-containing material in vehicles using fluorescent in situ detection

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Identifying, collecting, and analysing DNA evidence has become an essential and expanding part of forensic investigations. Motor vehicles can be involved in criminal activities ranging from robberies to hit and runs. Due to the size, motor vehicles can pose challenges to investigators locating areas that have DNA-containing material present. Many DNA samples, such as swabs and tape lifts, need to be collected to determine where DNA may be located. Utilising alternative light sources in crime scene investigations has proven useful in locating and enhancing forensic evidence by utilising different wavelengths of light. However, using light sources can be highly impacted by environmental conditions, including external lighting. While DiamondTM Nucleic Acid Dye has been shown to help visualize DNA-containing material on surfaces in a laboratory setting, the limitations of this technique outside of the laboratory are currently unknown. This study proposes the use of DiamondTM Dye to aid with visualisation of DNA-containing materials on varying vehicle surfaces. Alternative light sources, including the Crime-lite®, Polilight® Flares, and Crime-lite Auto will be investigated to determine which light source yields the best result. The effectiveness of visualising the fluorescence emitted by DiamondTM Dye will also be compared under different lighting conditions, including natural light, synthetic lighting, and the absence of light. It is hoped that DiamondTM Dye will be robust outside of laboratory conditions and successfully visualise DNA-containing material on and within vehicle surfaces.

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Comparative study for the effect of different vectors and pretreatment methods on DNA typing efficiency

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Objectives: To obtain carriers and pretreatment methods suitable for the preservation of trace blood stain, and provide a new idea and method for the long-term preservation, DNA extraction and reliable genotype of trace blood stain. Material and methods: the most common peripheral blood samples in practical cases were taken as our research object, and the storage time was set to one month using the currently commonly used FTA card and medical gauze as carriers. Samples were preprocessed by Tris EDTA saline buffer solution (STE) and phosphate buffered saline (PBS). And DNA was extracted by QIAamp DNA blood mini kit and genotyped by Human STRtyper-21G amplified fluorescence detection kit on 3130 genetic analyzer. Results and conclusions: Meaningful results showed that STE was better than PBS when FTA card was used as carrier and there was no significant difference between STE and PBS when using medical gauze. When STE was used, medical gauze could obtain higher peak height and bigger peak area than FTA card, but the intra locus balance was poor. When PBS was used, there was no significant difference between the two vectors for any parameters. When simultaneously considering the pretreatment method and carrier, it was found that there was no significant difference between the FTA card pretreatment with STE and the medical gauze pretreatment with PBS especially focused on the intra locus balance. In conclusion, the carrier and pretreatment method screened in this research can effectively preserve and dispose the trace peripheral blood samples. And PBS is recommended for medical gauze, STE is recommended for FTA card. More carriers, different samples, longer storage time as well as more pretreat methods are suggested in the future researches.

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Assessment of Diamond™ Nucleic Acid Dye as predictive method for targeting hair follicles suitable for DNA profiling in casework

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In forensic casework is common to find human hair samples, however successful rate of nuclear DNA profiling is low in most of them. Microscopic examination of hair roots reveals the growth stage of the hair and if there are skin cells attached, indicative of the genotyping success. This is a necessary step, since most of the hairs found in crime scenes are telogen hairs, which were naturally shed, and rarely produce informative STR profiles. Staining of hair roots with fluorescent dyes has shown to be a proper method to observe the nuclei present in the sample. The number of observed nuclei predicts the possibility to achieve the complete STR genotype of the hair.

Here we present the tests performed by our laboratory to assess the use and possible implementation in routine casework of hair staining with Diamond™ Nucleic Acid Dye and fluorescent microscopy observation as screening method to prioritise samples with high probability of genotyping success. After nuclear hair staining and microscopy examination, DNA was extracted, quantified by real-time PCR and genotyped for autosomal STRs. The method turned out to be quick and easy, and results showed that hairs, in which numerous nuclei were visible, yielded enough amount of DNA to obtain informative STR profiles. Thus, it has proved to be a suitable method for implementation in our routine casework processes.

P-484

NOVEL METHOD TO RELIABLY RECOVER NUCLEAR DNA FROM HUMAN HAIR SHAFT

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Hair strands are frequently encountered as potential evidence at crime scenes, but they are currently of limited utility for forensic analyses due to the challenges associated with DNA analysis. The DNA contained in hair shaft is of low quality and quantity, and pigment from dark hairs can co-purify with the DNA. We have adapted a method originally developed by Biochain Institute Inc. for extraction of cell free DNA to recover DNA from hair shaft. This approach has previously been used in forensic applications in the extraction of touch DNA (Burrill et al, FSIG 51:10242), and we modified that protocol to extract DNA from approximately 5 cm of hair from 5 hair donors. The yields of DNA ranged from 16-160 pg/cm hair, levels of recovery sufficient for subsequent procedures used in human identification, including PCR, STR analysis, library preparation and capture hybridization for sequencing. The DNA recovered from hair shaft using this method has successfully served as a template for all these applications, for both nuclear and mitochondrial DNA targets. When dark hairs are used, there is no residual pigment visually apparent in the DNA solution, and no inhibition of PCR reactions is noted. The extraction is completed in one day and requires no specialty equipment other than that found in a typical forensic DNA laboratory. The ability of researchers and analysts to reliably extract sufficient nuclear DNA from hair shaft to pursue forensic DNA analyses removes a major barrier to the use of hairs recovered in crime scenes or sites containing human remains.

P-485

Reproducible Artificial DNA Degradation for Forensic Purposes by UV-light

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Degraded DNA is frequently encountered in forensic casework samples due to mechanisms that favor the decomposition process and thus the fragmentation of DNA due to extreme environmental conditions. The reduced DNA fragment size can be a decisive factor for the feasibility of genetic tests. For this reason, it is important to include degradation experiments during the evaluation and validation of new markers and new technologies.

This study presents a method to reproducibly generate artificially degraded DNA of forensically relevant quality in only 5 minutes. For degradation, different concentrations and volumes of DNA extracted from blood were exposed to UV-C light. This led to a gradual decrease in DNA fragment size. Increasing the volume had little effect on the degradation efficiency, whereas DNA quantity had an effect on the observed degradation patterns. The process was assessed using degradation-sensitive quantitative real-time PCR and Short Tandem Repeat analysis. Repeated experiments revealed highly comparable degradation patterns. We found this technique suitable for mimicking case samples to investigate, evaluate and validate forensic genotyping applications.

P-486

A simple DNA extraction method to selectively purify high molecular weight and low molecular weight DNA

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Any method for extracting DNA from evidence samples balances the goal of removing non-DNA substances which may affect downstream analyses and maximizing the quantity of DNA recovered. In the Qiagen QIAamp® DNA Micro and DNA Investigator extraction methods, the amount of ethanol added to the lysate before loading the mixture onto the binding column affects the binding efficiency which in turn affects the size of the DNA fragments recovered. Increasing the amount of ethanol added may also result in the co-elution of inhibitory or other undesired substances. However, modern amplification kits are more robust with respect to the ability to overcome most common PCR inhibitors. Our studies demonstrate that modifying the relative volume of ethanol added to the lysate can increase the overall yield of samples containing severely degraded/fragmented DNA by increasing the binding efficiency to the silica membrane. This modified method was used to obtain partial autosomal STR profiles from hair shafts and significantly increase the yield of severely degraded samples obtained from fired cartridge cases. In addition, with a simple modification to the protocol, the low molecular weight DNA and high molecular weight DNA can be purified separately from the same initial lysate. This size selective purification (SSP) is a simple two-step process. In mixtures containing contributions of high quality and severely degraded DNA, the studies demonstrate that an almost complete separation of the degraded DNA from high quality DNA is possible. The ability to separate high quality DNA from degraded DNA has multiple applications such as separating old/ancient DNA from recent contamination which would overwhelm the authentic DNA in the sample or confound profile interpretation. Another potential application is with criminal evidence samples that contain mixtures of high quality/quantity (victim blood) and degraded (handler) DNA, for example, firearms or fired cartridge cases, and fragments of improvised explosive devices. Separating, or at a minimum, enriching the high molecular weight and low molecular weight components, may allow for interpretable profiles where this was not previously possible.

P-487

DNA degradation potential on Body Fluid stains under various conditions: A preliminary approach

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Numerous body fluids, such as blood, semen, saliva, and vaginal fluid, are frequently encountered at crime scenes. This makes it crucial to use forensics to examine these fluids in order to learn more about the crime, including who committed it, what it was for, who the victim was, how long it had been since it was deposited, and more. Since every person's DNA profile is thought to be unique including identical twins, DNA typing of biological samples can establish a suspect's guilt or innocence in criminal cases and even help clear innocent people who have been unfairly convicted. The degradation of DNA poses a severe challenge to forensic science. DNA degradation is the process through which DNA breaks down into smaller pieces. DNA breaks down when hydrogen bonds or the connections between nucleotides are broken. Numerous methods, such as hydrolysis, oxidation, radiation, and heat, can cause this. Crime scenes and other sites exposed to the environment over time are familiar sources of DNA evidence. Environmental factors can seriously degrade DNA, which makes extraction and analysis difficult. Extracting and analysing DNA evidence may be challenging due to DNA degradation. Numerous elements, such as temperature, pH, moisture, sunlight, and the abundance of enzymes, can influence DNA degradation. The present study aims to evaluate the degraded DNA profiles of different body fluids like blood, semen, and saliva according to typical crime scene environments like muddy, moist, grassland, heat and room temperature and to determine how they impact DNA profiling in forensic investigations.

P-491

Improving forensic Y-chromosome DNA analysis on multiple levels

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The Y-chromosome serves a vital role in forensic DNA analysis, particularly when standard autosomal STR profiling proves impractical, such as in many male-female DNA mixtures in cases of sexual assault. While the non-recombining nature of the Y chromosome is highly suitable for identifying male lineages, it presents challenges for male individualization.

Over the years, our research groups have dedicated considerable efforts in overcoming these challenges with the discovery and utilization of rapidly mutating (RM) Y-STR markers, the development and validation of RM Y-STR analysis tools, and the forensic interpretation of the outcomes. Due to their increased mutation rate, RM Y-STRs provide largely improved differentiation of (un)related males, as has already been shown in multiple populations from several continents. RM Y-STRs have the potential to move forensic Y-chromosome analysis beyond male lineage identification towards male individual identification.

Here, we present our recent efforts to further improve forensic Y-chromosome analysis with new developments on multiple levels. These include the search for additional
RM Y-STRs, the development of a framework and computer tool for weight of evidence calculations focusing on individuals rather than lineages, and the exploration of RM Y-STRs for familial searching. Moreover, we will discuss moving beyond (RM) Y-STRs by reflecting on the benefits of other types of Y-chromosomal polymorphisms for male relative differentiation and individual male identification.

We believe that current forensic Y-chromosome analysis is far from exploiting the full benefits the Y-chromosome has to offer for forensic applications, which we will highlight by sharing our recent achievements in moving the field from current male lineage identification to future male individual identification.

P-492

Seeking Efficiency: Developmental Validation of the One-Tube Reaction IDseek® mYSTR Y-Chromosomal STR Profiling Kit

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Advances in massively parallel sequencing (MPS) technologies have provided the forensic community with an enhanced analytical tool for DNA profiling beyond traditional methods. The current MPS-based chemistry entails a multi-step nested PCR to enrich the targets of interest (e.g. STRs). Reverse Complement PCR (RC-PCR) is an alternative target enrichment method that streamlines the laborious processes of MPS. By enabling simultaneous amplification and tagging of targeted sequences in a single tube, RC-PCR minimizes the risk of sample switches and contamination. Moreover, its synthesis of specific primers during the reaction ensures heightened sensitivity and specificity, enhancing congruence between primer and amplicon concentrations. The IDseek® mYSTR™ Y-Chromosomal STR Profiling kit leverages the use of RC-PCR technology to amplify 30 Y-chromosomal STR markers (including three rapidly mutating Y-STRs) alongside a sex marker in a single multiplex, closed tube reaction.

This study involved a full developmental validation following FBI QAS and SWGDAM guidelines utilizing the MiSeq FGx and MixtureAce™ analysis software. A population study to evaluate the distribution of genetic markers across African, Caucasian, Hispanic, and Asian populations (N=100) was performed. Specificity for humans was confirmed through analysis of seven non-human eukaryotic species and one bacterial strain, while sensitivity was assessed from 4 ng down to 8 pg total DNA input. Direct amplification feasibility was tested with 1, 2, and 3 punches of blood and saliva. Stability evaluations included exposure to three inhibitors (hematin, humic acid, tannic acid) at varying concentrations and heat-degraded blood samples. Case-type samples (including hair, fingernails, semen, and mock sexual assault kits) were examined, along with two-person mixtures (male:male and male:female). Precision and accuracy were examined via concordance against AB Yfiler™ Plus and PowerPlex® Y23 profiles, along with repeatability and reproducibility assessments.

The utilization of RC-PCR-based sequencing technology in MPS workflows presents a compelling array of advantages, including enhanced reliability of results, decreased manual intervention, and increased recovery of genetic information. These merits are especially pertinent in complex forensic scenarios such as sexual assault investigations or kinship analyses. The ongoing developmental validations of emerging sequencing methodologies further expand the repertoire of options available to crime laboratories, empowering them to select and implement sequencing-based approaches tailored to their specific casework requirements.

P-498

Visual identification for species and sex derived from bloodstain based on phosphate-mediated isothermal amplification colorimetric system

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Species and sex confirmation of the biological specimen play a crucial role in crime investigation. However, the specimen found in the scene is always trace quantity, which is hard to be analyzed by current methods. Moreover, the time-consuming DNA extraction, sophisticated apparatus, and complex data processing make it difficult to satisfy the demand of speediness and convenience for point-of-care tests. In this study, we first exhibit a phosphate-based visual system for field-based species and sex identification derived from trace bloodstain. By introducing phosphate ion-based colorimetry into loop-mediated isothermal amplification (LAMP) for result interpretation, not only the bloodstain can be directly submitted to mitochondrial variant amplification owing to the enhanced amplification efficiency by pyrophosphate ion hydrolyzation, but also the colorimetric signal can be recognized by the naked eye for result output within 30 min through molybdophosphate generation. Aerosol contamination, the major conflict of LAMP, has been solved once and for all by integrating uracil-DNA glycosylase into this system that still holds on a constant temperature. As a demonstration, cytochrome b and Y-chromosomal amelogenin are employed to identify species and sex respectively, which has achieved a highly sensitive and specific distinguishability under a strong interferential background. Accurate results can be obtained from both the simulative degraded and dated specimen, which indicates that this novel system may serve as a promising tool in forensic practice.

P-499

Bridging towards ISO 17025 accreditation: Validating automated NGS-based technology with Qiagen ForenSeq MainstAY (MainstAY) and ForenSeq mtDNA Control Region (mtDNA-CR) kits at Bordeaux's Forensic Hematology Laboratory (LHML), a first in France.

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1.Objectives:
For the first time in France, our laboratory initiated a large-scale study to validate NGS-based analyses of forensic casework samples collected from evidences and the identification of human genomic and mitochondrial DNA.
The aim of this study is to obtain ISO 17025 accreditation for this method and to use it routinely for all types of forensic casework samples.

2.Material and Methods:
First, the validation of the MainstAY kit, mtDNA-CR kit, related ForenSeq Universal Analysis Software (UAS) and an automated Hamilton-based platform protocol for the preparation of the samples has been undertaken. Using mock forensic DNA samples (n=91 and n=82 for MainstAY and mtDNA-CR kits validation respectively) extracted from all types of supports (swab, FTA card, bone, sperm, hair, blood, tissue), we have determined the kit's accuracy, reproducibility, repeatability and sensitivity. Then, challenging forensic casework samples collected from evidences including DNA mixtures, degraded or low-concentrated DNA samples (n=339 and n=110 for MainstAY and mtDNA-CR kits validation respectively) were analysed in parallel with the NGS- and the capillary electrophoresis-based approaches in order to assess the robustness and performance levels of the NGS-based kits and the reliability of the UAS for the analysis of the generated sequences.

3.Results and Conclusion:
First, the results from our accuracy, reproducibility, repeatability and sensitivity studies allowed us to validate the kits and the protocol for the preparation of the samples.
Furthermore, for the first time, challenging forensic casework samples were used to characterize the robustness and performance levels of the kits. Regarding the MainstAY kit, our results demonstrate that more information is obtained within a single reaction compared to the use of two capillary electrophoresis-based kits combined (autosomal- and Y-STR analyses). This is explained by a higher performance and a higher sensitivity on challenging DNA samples. Concerning the mtDNA-CR kit, concordant results with the reference method were obtained, even starting from samples with low genomic DNA concentrations (< 1.5 pg/µl). Finally, the UAS appeared to be a straightforward and robust data interpretation system and this study allowed us to configure the software in accordance with our DNA samples.
In conclusion, both kits are robust and allow forensic laboratories to collect more information from very challenging DNA samples (mixtures, highly degraded or low-concentrated) compared to capillary electrophoresis-based methods. Therefore, these NGS-based methods will be applied routinely in our laboratory and ISO 17025 accreditation for these new methods will improve our investigative capacity for criminal justice casework.

P-500

Effects of DNA polymerases with different properties on error types and rates in PCR and sequencing of STR markers

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Polymorphic Short Tandem Repeat (STR) markers are used globally in forensic DNA profiling. The repetitive nature of STR markers makes them highly susceptible for slipped strand mispairing in Polymerase Chain Reaction (PCR), resulting in stutter artefacts. Stutters are problematic as they may conceal the detection of alleles from minor contributors in mixed samples or lead to faulty allele calling. Base substitution is another type of error occurring in PCR and sequencing. Little is known about the mechanisms controlling error formation and the impact of applying DNA polymerases with different properties. The objective of this study was to investigate error types and rates of six commercial DNA polymerases in STR analysis: AccuPrime Pfx, AccuPrime Taq, ExTaq HS, Immolase, Phusion, and SuperFi II. Applying Unique Molecular Identifiers (UMIs) and a seven STR multiplex SiMSen-Seq assay, errors were analyzed in relation to the different properties of the DNA polymerases used. SiMSen-Seq consists of library preparation in two PCR steps. The first PCR employs a low number of PCR cycles, extended annealing times, and low polymerase and primer concentrations to label each template molecule with a UMI. The second PCR then amplifies the UMI labelled STR fragments, creating UMI families representing the original template molecules. This design enabled the study of errors emanating from individual template molecules, as well as the overall types and rates of generated errors. AccuPrime Taq and Immolase showed substantially lower amplification efficiencies in the first PCR compared to Phusion and SuperFi II, whereas AccuPrime Pfx showed the lowest amplification efficiency when applied in the second PCR. AccuPrime Pfx and Immolase gave the largest proportions of incorrect sequences, especially base substitution errors when applied in the second PCR, whereas AccuPrime Taq, ExTaq HS and Phusion revealed similar performances with respect to both proportions of errors and types of errors. Understanding and minimizing errors during PCR and sequencing is essential to achieve optimal limits of detection and to enable the identification of minor contributors in complex mixtures.

P-508

Inferencing Fingerprints TsD Based on Copy Number Differences of Multiple mtDNA markers

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Fingerprints are commonly encountered contact materials at crime scenes, and the determination of their time since deposition (TsD) is pivotal in establishing the timeline of the crimes, which is a challenging issue urgently awaiting resolution in forensic science. Mitochondrial DNA (mtDNA), with its structurally stable and multicopy nature within cells, can offer application advantages in fingerprints TsD.

1. Objectives: The primary aim is to develop a reliable method for inferring the fingerprints TsD by exploiting the advantages of mtDNA. Longer DNA fragments are more susceptible to degradation than shorter ones. Thus, by comparing the copy numbers of mtDNA markers with different lengths, we seek to establish quantitative indexes for fingerprint degradation, providing a more precise method for ascertaining the TsD of fingerprints encountered at crime scenes.

2. Material and Methods: We initially screen for TsD mtDNA markers, followed by the meticulous design of specialized primers and the TaqMan MGB probe tailored to these markers. Markers should exhibit length polymorphism, possess specificity to the human species, and be devoid of NUMTs; the primers should have high amplification specificity and efficiency, and demonstrate high genetic conservation within Chinese populations. Then, after preparing fingerprints at different time points, DNA was extracted using the magnetic bead method. The qRT-PCR standards were prepared using total DNA from peripheral blood quantified on a digital PCR platform. Finally, the candidate mtDNA markers of fingerprints were quantified on the ABI QuantStudio3 platform. The copy number ratios of selected markers were used as the degradation indexes to evaluate the degradation degree of mtDNA, and further assess the fingerprint TsD.

3. Results and Conclusions: Three mtDNA markers within the mitochondrial CO1 gene were initially selected with varying lengths (~60bp, ~140bp, and ~350bp). The quantitative analysis revealed that the concentration of three CO1 markers in aged fingerprints (21 days) were lower than that in fresh samples, indicating potential degradation of the mtDNA in fingerprints over time. Analysis based on the degradation indexes ln(350bp/60bp) and ln(140bp/60bp) indicated a discernible degradation pattern in some fingerprint samples after 21 days, with the degradation pace for the ln(350bp/60bp) index notably surpassing that of ln(140bp/60bp). However, certain fingerprints did not exhibit expected degradation patterns, possibly due to interference from fingerprint lipids and other factors. This study underscores the potential of mtDNA markers as reliable biomarkers for fingerprints TsD inference, offering significant promise for forensic applications.

*Jianbo Li is the corresponding author

P-511

TrACES of Time: Transcriptomic Analyses for the Contextualization of Evidential Stains – towards estimating the time of deposition using targeted RNA sequencing

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In forensic casework, identifying the donor of a biological trace regularly needs to be complemented with additional intelligence regarding the context of trace deposition to reconstruct the true course of events.

The human transcriptome in biological materials encodes a complex body of information on the trace's nature and condition. Transcriptomic analyses such as body fluid identification via the detection of cell-type specific mRNA markers have become highly relevant and are now commonly used in forensic laboratories in several countries. In parallel, research groups are exploring the potential of exploiting the transcriptome to answer further questions of forensic relevance. In a recent study, we assessed whether information regarding the time of day of blood stain deposition may be gathered from the transcriptome. Using whole-transcriptome sequencing we identified a set of 81 RNA biomarkers potentially predictive of the time of day.

Here, we present results of a follow-up study in which we quantified the expression of previously identified RNA biomarkers in an independent sample set of fresh and aged dried blood stains collected at different times of day using a newly developed targeted sequencing assay on an Ion S5 benchtop sequencing device. We furthermore tested and compared different statistical approaches to devise a model to estimate time of day of sample deposition based on gene expression data.

P-512

Using the temporal variations of both microbial AsVs and metabolic functional pathways improved the accuracy of TSD estimation for saliva stains

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In recent years, microbiological profiling has emerged as a promising biomarker for specific challenges in forensic DNA investigations, such as determining the origin of body fluids and inferring the post-mortem interval (PMI). Predicting the time since deposition (TSD) of body fluids is crucial for verifying suspect confessions, reconstructing crime scenes, and establishing a comprehensive chain of evidence. In this study, we monitored alterations in the microbial community of saliva stains exposed to a laboratory environment for 60 days by sequencing full-length 16S rRNA gene of bacteria. We observed changes in the microbial community structure of saliva stains with increasing TSD. Additionally, we constructed the potential relationships between the temporal variations of the microbial communities with the TSD of saliva stains using the abundance of microbial genera, amplicon sequence variant (AsV) and the predicted metabolic functional pathways, respectively. We first showed that the metabolism function alterations of the microbial communities could potentially contribute to the TSD estimation of saliva stains. A 14-day deposition of the saliva stain could be the possible key factor that led to the alterations in the microbial communities of the saliva stain. We also demonstrated that by combining 47 feature-selected microbial AsVs with microbial metabolic functional pathway abundance and applying an ensemble learning algorithm to construct the model, the prediction accuracy of saliva stain TSD could be improved. Specifically, the least mean absolute error (MAE) of the ensemble learning model based on the blend strategy was 9.51 days in the 60-day deposition interval. To sum up, this study underscored that the microbes could serve as valuable biomarkers for estimating the TSD of saliva stains. It also confirmed that an ensemble learning strategy, which analyzed multiple data patterns (like AsVs and metabolic functional pathways) could improve the accuracy of TSD prediction.

P-519

Assessment of DNA and mRNA stability under different conditions

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The stability DNA and mRNA are important to know under different conditions. Saliva was examined under different conditions. Saliva was stored dried on glass, fabric, and cotton swabs alongside liquid saliva at ambient temperature. DNA and mRNA yields were tested at time points between 0 hours and up to 6 months.

Saliva was also dried on a few additional non-absorbent surfaces; glass beads, plastic counters and metal discs. Samples were tested at a number of different time periods from 0 hours and 28 days. The impact of storage temperature on the stability of DNA and mRNA was assessed with vaginal swabs and saliva swabs. Storage at ambient temperature, 4°C and -20°C were tested over a period of up to 6 months.

Samples were extracted and DNA was quantified with the PowerQuant® (Promega) system. mRNA recovery was assessed using an RT-qPCR assay targeting the FDCSP marker for saliva and the CYP2B7P1 marker for vaginal material.

The data showed that mRNA transcripts are generally stable, but storage on certain sample matrices (specifically hard, non-absorbent surfaces in the case of the FDCSP transcript) can lead to rapid degradation of mRNA, whilst DNA remains stable. Furthermore, DNA in liquid saliva can degrade with little to no impact on mRNA.

P-522

A nanopore sequencing-based mRNA set for body fluids and personal source analysis

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Objetives: Personal identification can determine the donor of a biological trace left at the crime scene, and body fluid identification helps to link the donor to criminal behavior, both of which are crucial to the investigation and prosecution of a case. Recent studies have shown that the differential expression patterns of mRNA can identify body fluids, and its sequence variation can be used to distinguish donors. In this study, we designed a mRNA set with an average length exceeding 700bp to include more potential sequence variations.

Material and methods: The mRNA set contains 14 biomarkers, including CD3G and CD93 for peripheral blood, MMP7, MMP10 and MMP11 for menstrual blood, PRB4 and STATH for saliva, SEMG1 and SEMG2 for semen, FAM83D and DKK4 for vaginal secretion, LCE1C for skin and two reference genes (ACTB and GAPDH). By optimizing the nanopore sequencing process and developing automated analysis plugins, we can simultaneously obtain the sequences and expression levels of each marker.

Results and conclusions: The results demonstrated that all mRNAs were present in corresponding target body fluids at a moderate to high expression level. Peripheral blood and semen showed little cross-reactivity with the selected markers. However, vaginal secretion markers FAM83D and DKK4 were expressed in saliva and menstrual blood respectively, peripheral blood marker CD93 and skin marker LCE1C were expressed in vaginal secretion. Despite the presence of cross-reactivity, each of the body fluid has a unique mRNA profile, allowing for effective identification of body fluids. In addition, we found a set of coding region SNPs (cSNPs) in the 12 specific mRNA transcripts, which helps to establish a link between the body fluids and the donors.

P-524

Optimizing RNA Extraction from Formalin-Fixed Tissues for Forensic Gene Expression Analysis: A Pilot Study

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In forensics, RNA extracted from formalin-fixed tissues poses a challenge for gene expression analysis due to the effect of formalin on nucleic acids. Formalin causes protein-to-protein and protein-to-nucleic acid crosslinking, which prevents effective extraction of significant amounts of intact RNA. The recovered RNA appears to be fragmented and chemically altered due to the formalin fixation. Therefore, our pilot study aimed to optimize the RNA extraction process by adjusting the proteinase K volume and incubation period to counteract the chemical changes caused by formalin fixation. We used three postmortem cardiac tissue samples collected from sudden unexplained nocturnal death syndrome victims during autopsies at Chulalongkorn University's Department of Forensic Medicine, Faculty of Medicine in Thailand. The Maxwell RSC RNA FFPE kit (Promega) was used to extract RNA from the tissue sections. Our revised protocol modified the proteinase K volume and incubation period. Specifically, we increased the proteinase K solution from 25 µl to 50 µl and extended the incubation period to 24 hours. Our research showed a significant increase in the concentration of total RNA, with an average value of 24.3 ng/ul and a mean A260/280 ratio of 2.04. In summary, our results imply that increasing the yield of total RNA extraction and achieving suitable purity is possible by adjusting the volume of proteinase K and the incubation time.

P-525

A sequencing assay incorporating coding region SNPs for body fluid identification

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Objetives: Body fluid identification (BFID) and tracing the donors of specific components are crucial aspects of forensic practice. Recently, coding region SNPs (cSNPs) in body fluid-specific mRNAs have emerged as important molecular markers for BFID, allowing for direct association of certain body fluids with their respective donors. In this study, cSNP analysis using a highly sensitive targeted RNA sequencing assay was conducted on a condom sample collected from the crime scene to identify the case nature and criminal processes. Material and methods: In our recent work, we screened body fluid-specific mRNAs containing cSNPs with a Minor Allele Frequency (MAF) >0.05 in the East Asian population. Candidate mRNAs were subsequently validated for specificity in their target body fluids. Finally, a Massive Parallel Sequencing (MPS) panel comprising 31 cSNPs within 16 mRNAs was developed and applied. Results and conclusions: We successfully identified the body fluid types from both the inner and outer surfaces of the condom. Furthermore, by comparing the genotypes of cSNPs from the condom with the reference gDNA of the suspect/victim involved in the case, each body fluid was assigned to the correct donor directly. While the assay shows promise, additional validation tests are warranted. In conclusion, we developed a MPS cSNP panel containing novel BFID mRNA markers and cSNPs. Its application in samples from a criminal case demonstrated that cSNPs analysis can provide solid evidence to support the act of vaginal penetration and distinguish rape from molestation.

P-526

Persistence of Proteomic Body Fluid Signals in DNA-workflows and in an In Vitro Model of the Post-Coital Interval.

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Objectives: Identification of the source of a biological stain adds important context to evidence and the corroboration of testimony. Current methods of body fluid identification however present significant challenges: they are presumptive, qualitative, and have sensitivity and specificity issues. When evidence is limited, investigators have to choose between body fluid identification or maximizing DNA-isolation and human identification. Interpretation of these choices as exclusive is based on assumptions about the persistence of protein signals in body fluid samples. In the case of sexual assault evidence, it is assumed that endogenous proteases, mainly prostate specific antigen (PSA), fully degrade semen proteins in vivo. It is also assumed that DNA isolation workflows, many of which rely on the Proteinase K (PK), fully eliminate protein as a source of forensic information. Both assumptions were tested.

Materials and Methods: Assumptions about body fluid identification were tested by: 1) An in vitro model of the post-coital interval using equal volumes of neat semen and vaginal fluid at 37C over a 5-day period (n = 3). Aliquots were assayed using Quantifiler-Trio, Coomassie-staining on SDS-PAGE, and proteomic mass spectrometry up to day 3. 2) Protein recovery in DNA isolation workflows, in the presence and absence of DTT and PK, were measured with Coomassie staining and mass spectrometry.

Results and Conclusions: 1) From day 2 to 5 of the post-coital interval Coomassie staining was stable (33±15% yield). From days 1 to 3, Semelogenin-1 (SEMG1) and -2 (SEMG2) yields were stable at 29±4% and 61±10% respectively, and the amount of the protein sequenced decreased from 91% to 82% and 96 to 86%. 2) DNA isolation workflows were tested in the presence and absence of DTT and PK. In the absence of PK there was no change in protein quality or yields (n = 4, 101±21%). Discarded lysis buffers without PK therefore are a rich source of proteomic information. As expected, PK digestion fully eliminated intact protein structures and Coomassie staining. However, bioinformatic processing of the PK-peptides identified SEMG1 and SEMG2 at coverage of 72% and 70% respectively. These data demonstrate that the above assumptions are false. Protein body fluid signals persist in an in vitro model of the post-coital interval. Protein persists in DNA-isolation workflows, regardless of whether PK is used or not. Proteomic body fluid identification is therefore relevant to post-coital samples. Discarded material from DNA-isolation workflows are a rich source of easily accessible proteomic information.

P-538

Large scale SNP analysis for the identification of victims from the Spanish Civil War

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1.Objectives
Across Spain, there is a drive to identify victims from the Spanish Civil War (1936-1939). Forensic Investigative Genetic Genealogy (FIGG) relies on searching an unknown genetic profile against a global consumer database to identify distant relatives. Although FIGG has been used successfully to solve cold cases, it may not always be appropriate in situations of unidentified human remains (UHR) for legislative or ethical reasons. Additionally, in scenarios like the identification of Spanish Civil War remains, family references are often available for direct comparison. However, as time passes, these family references are more likely to be more and more distantly related, requiring a large number of genetic markers for kinship analysis.

As an ISO/IEC 17025 accredited laboratory for Massively Parallel Sequencing (MPS) in forensics and working in missing persons projects, we have already studied more than 200 ~90 years old bone samples, obtaining full profiles (27 aSTRs and 25 Y STRs) from degraded and low concentrated samples (quant values ranging from 10-2 to 10-3 ng/µL) with different degradation indexes, using the ForenSeq MainstAY kit. These studies have enabled us to identify victims, with relatively high likelihood ratio (LR) values (up to 49500 in grandfather-grandson cases) based on autosomal STRs, and in many cases, also supported by Y-STRs results.
Despite obtaining full STR profiles using MPS, the absence of direct relatives and even second-degree relatives from many of the victims led to a number of inconclusive results.

2. Material and Methods
The 10,230 SNPs targeted by the ForenSeq Kintelligence kit, and the algorithms for kinship analysis used in GEDmatch PRO, have been repurposed into a solution for UHR processing – the Kintelligence HT kit. In this work, antemortem and postmortem samples were processed using the ForenSeq Kintelligence or ForenSeq Kintelligence HT kits.

3.Results and conclusions
This presentation will show the first results obtained from applying this large-scale SNP set and local kinship database on the identification of victims of the Spanish Civil War. We demonstrate that even for highly degraded bones, over 7000 SNPs were typed in the majority of cases. This meant that the SNP overlap between the bone samples and relative samples remained high enough to perform robust kinship calculations, with data evaluated using kinship coefficients and LRs for stated pedigrees.

Results show that using 10,230 SNPs, combined with strong kinship algorithms can be used successfully to identify missing persons using degraded remains when no direct relatives are available.

Posters Topic 3

Epigenetics

3. Epigenetics

Abstracts Poster

16S rRNA and metagenomics sequencing to decode human thanatomicrobiome

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Accurately predicting the postmortem interval (PMI) is of great significance in forensic research. Microorganisms play a crucial role in carcass decomposition. The successional change of microbial communities during the progress of cadaver decay and decomposition could aid in determining the PMI. However, previous studies have primarily focused on animal remains. Insufficient research has been conducted on the microbiota of human cadavers and mainly on the gut and skin. In the present study, we examined the microbiota in nine distinct body regions (including oral and nasal cavities, hearts, livers, spleens, lungs, kidneys, muscles and guts) of eight cadavers at different stages of decomposition using 16S rRNA and metagenomic sequencing. We compared microbial composition and diversity differences between organs and evaluated the efficacy of these two sequencing methods. The results showed that the oral cavity group had the highest microbial diversity in the community, followed by the nasal cavity group. The microbial compositions of samples from the oral and nasal cavity groups were relatively similar and quite different from other groups of samples. The results also showed that the host contamination ratio was relatively substantial in the metagenomic sequencing, resulting in a subsequent loss of data for analysis. The 16S rRNA sequencing was the most cost-effective method for the study of cadavers in the early stages of decomposition, whereas this technique was not highly accurate at the species level and could be subsequently improved by full-length sequencing (V1-V9).

P-021

DNA methylation as a marker for post-mortem interval (PMI) estimation in Sus scrofa in southern Ontario (Dfb)

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Accurate post-mortem interval (PMI) estimation is key in forensic investigations, but it still constitutes a challenge. DNA is a widely used source of biological material for identification in forensic science; however, testing of DNA as a method to determine PMI has only recently begun.

1. Objectives
Recently, DNA methylation has been successfully used as a marker to estimate individuals' biological age. Since the study of changes in methylation levels has been applied to estimating the passing of time, the current study aims to use DNA methylation as a potential marker for PMI in the humid continental climate of southern Ontario (Köppen classification Dfb) in Sus scrofa as a model organism.

2. Material and methods
For this purpose, biopsy punches were collected at regular intervals and 27.032 pig-specific CpG sites were selected from an array of 37.492 to quantify total levels of DNA methylation during a period of 0 to 182 days post-mortem during winter, spring and summer. The biological age and the sex of the pigs were estimated at different times of the PMI. The age was calculated using epigenetic clocks and the sex was based on known sex-specific CpG sites, with the goal of exploring how long after death it’s possible to obtain an accurate age and sex estimate. Total Body Scores (TBS) and Accumulated Degree Days (ADDs) were also calculated at regular intervals and correlated with the known PMI. The TBS systems used were: Megyesi et al, the pioneer scoring system, Ribéreau-Gayon et al, a system developed in the Dfb climate zone, and Keough et al, a system developed specifically for pigs. The process of decay was monitored with pictures.

3. Results and conclusions
Our preliminary results provide insight into the potential of using DNA methylation as a marker for post-mortem interval in Sus scrofa. A consistent decrease in overall DNA methylation levels was observed during the PMI. One of the study subjects presented substantial differences between the samples collected in the right and left side, which can be explained by asymmetric sun exposure. Trends were observed between the correlations of TBS and ADDs, as well as ADDs with the PMI. This research is intended as proof-of-concept that can identify patterns in the post-mortem modifications of DNA methylation in pigs that will contribute to a more accurate and quantifiable PMI estimation in humans.

P-236

Application and evaluation of the existing DNA methylation-based age prediction models in postmortem autopsy blood samples

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Age prediction of biological evidence e.g., autopsy blood from an unidentified body can be useful in the progress of investigation by limiting the search range or by providing additional information. To date, a DNA methylation-based biological age predictor has been established and found to be highly associated with chronological age. The age prediction model using the methylation value at 5 CpG sites in the ELOVL2, FHL2, KLF14, C1orf132, and TRIM59 genes showed high prediction accuracy in peripheral blood. The model was constructed by the SNaPshot assay-based method using on genetic analyzer-the Applied Biosystems 3130 but the prediction accuracies were decreased when methylation data obtained from 3500 genetic analyzer. To address this issue, adjusting models of the 3130 genetic analyzer, the 3500 genetic analyzer specific model and genetic analyzer type independent model have been suggested recently. In this study, the performance of these age prediction models was tested in 300 postmortem autopsy blood samples using the 3500 genetic analyzer. The 3500 genetic analyzer specific model demonstrated the strongest correlation between DNA methylation and chronological age. However, the overestimated age prediction values from the chronological age were showed in some autopsy blood samples where drugs such diabetes or blood pressure were detected. It is implied that age acceleration is linked to several aging-related factors, including infectious diseases, medical history, lifestyle, or environmental hazards. These data would have important implications for age prediction modeling in forensic casework analysis.

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Age estimation based on mitochondrial DNA methylation

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Mitochondrial DNA (mtDNA) mirrors the cellular capability for generating and utilizing energy and it may also be associated with aging. Due to the high cost and technical limitations, age estimation using the mtDNA methylation markers has not been understood. To evaluate whether mtDNA methylation pattern could be considered reliable markers of age estimation, the mtDNA CpG methylation levels from 147 peripheral blood samples (aged 19-81 years) were determined. To avoid the interference of nuclear mitochondrial DNA segments (Numts), only the reads uniquely mapped to mitochondria were selected for further methylation analysis. We found low levels of mtDNA CpG methylation using the high coverage sequencing, despite general low levels, higher beta values were found among the younger people. The 147 samples were split into a training set (n = 111) and a validation set (n = 36), and a least absolute shrinkage and selection operator (LASSO) regression model including 545 mtDNA CpG sites were conducted. The final model resulted in a root mean squared error (RSME) of 12 years and an adjusted R2 of 0.49 between the predicted age and chronological age. In conclusion, the outcomes from the analysis of mtDNA CpG sites suggested the potential for estimating the age of an unknown individual. However, further investigation is needed to assess its applicability in forensic casework, particularly in the detection of degraded samples.

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Forensic age estimation from human blood by combining DNA methylation and microRNA markers using droplet digital PCR

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1. Objectives:
Age prediction is significant in forensic practices. Recently, several studies have successfully used DNA methylation or transcriptome markers for age prediction. However, there are few reports on combining these two kinds of markers for age prediction. In this study, based on DNA and microRNA co-isolation, a rapid and specific 4-plex droplet digital PCR (ddPCR) assay for simultaneous quantification of 4 genes for age prediction, including an age-related CpG site cg14361627 (KLF14), an age-related miRNA miR-106b-5p, and two reference genes (C-CLESS-C1 and RNU6B) from blood samples was developed and its application value in forensic age prediction was evaluated.

2. Material and methods:
Bisulfite-converted DNA and complementary DNA were in a single ddPCR reaction system and varying the concentration of TaqMan-MGB probes with different fluorescence-modified reporter groups enabled the 4-plex ddPCR assays for the simultaneous detection of 4 genes. To evaluate the accuracy of this assay, 4-plex ddPCR and dual-plex ddPCR were simultaneously detected10 blood samples, and the percent agreement rate of the quantitative results was analyzed. Blood samples from 132 healthy individuals (60 females, and 72 males; aged 18 – 58 years old) were examined to calculate methylation levels of the KLF14 and the relative expression levels of miR-106b-5p and evaluate the association with age. The age prediction models were established using four machine learning algorithms based on DNAm and microRNA.

3. Results and conclusions:
No significant difference was observed between the dual-plex and the 4-plex ddPCR assays. The KLF14 showed a relatively high association with age (R2 = 0.8428) compared to miR-106b-5p (R2 = 0.2348). The mean absolute deviation for the training set and testing set all ranged from 3 to 4 years for each age prediction model. Models using both DNA methylation and miRNA expression level can increase the accuracy of age estimation compared to models using one type of predictor. In conclusion, a stability method for the detection of KLF14, miR-106b-5p, C-CLESS-C1, and RNU6B by 4-plex ddPCR was successfully established. This demonstrates the possibility of combining transcriptome with DNA analysis for forensic age prediction based on a 4-plex ddPCR assay and may provide useful information in case investigations.

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A Five-Locus Multiple regression Model for Human Chronological age Estimation from Buccal swabs. A pilot study on DNA Methylation level.

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In recent years, various techniques have been explored at the forensic level for personal identification and for the extrapolation of different characteristics from unidentified traces. Some of these new techniques analyse epigenetic variations, in particular the predictive potential of DNA methylation. For this study, buccal swabs samples were collected from 60 Italian volunteers aged between 23 and 70 years. On this pool of biological samples, methylation levels for five specific genes (ELOVL2, FHL2, KLF14, C1orf132, and TRIM59) were analysed to predict age. Initially, a test was performed using a regression model already developed on biological blood samples, for both the five-loci model and the four-loci model, whose results lead to a significant overestimations of predicted age given a MAD (Mean Absolute Deviation) of, respectively, 10.28 years and 13.07 years. Consequently, it was necessary to develop an analysis tool for the study of buccal swabs samples. First, the sample pool was divided into test set and training set, for the construction of a multivariate linear regression model. On the training set, good correlation values between predicted age and chronological age was demonstrated. At this point, a validation was carried out by entering the test samples into the created model through the training set, obtaining a final MAD of 3.49 years. This multiple regression model, including all five genes, revealed a strong correlation between age and methylation levels with R2 coefficients of 0.938 for the test set and R2 of 0.822 for the training set. Overall, this study provides an overview of the complexity of DNA methylation models for the application in the forensic field, contributing to the development of more accurate tools that can be used in investigations.

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Age prediction of burned human remains: application of a blood age estimation model through DNA methylation pattern analysis

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The identification of burned human remains represents a challenging task in forensic medicine. In such cases, the creation of a biological profile is crucial to link the body to possible identities. In this scenario, the recent advance on DNA methylation pattern analysis could be a promising support in the definition of the age-at-death, although no data is available on the effect of postmortem alterations.

To this purpose, the present study aims to investigate the DNA methylation pattern in blood samples collected from burned human remains and to evaluate the applicability of an age estimation model in such cases.

A blood age estimation model was developed analysing the 5 most informative CpGs (ELOVL-2, FHL-2, KLF-14, C1orf-132, TRIM-59) by a SNaPshot assay in a training set of 72 living individuals of known age (mean age±SD: 49.3±18.9, min-max: 18-85), using multiple linear regression (adjusted R^2: 96.2, Mean Absolute Error, MAE: 2.84). Repeated k-fold cross validation was performed to evaluate the performance of the model. Subsequently, the same CpG sites were investigated in blood samples from 27 burned human remains of known age (mean age±SD: 48.1±14.4, min-max: 24-75) and different stages of thermal alterations according to anthropological classification systems. Age prediction was computed for all the samples, resulting in a MAE of 7.03 and a correct prediction in 78% of the cases (95% prediction interval). Statistical analyses detected a significant association between DNA degradation (DI) and wrong prediction, identifying a strong relation with DI greater than 2 (wrong classification in 57% of the cases). No evidence suggested the burning stages and fire environments as variables influencing the model performance.

In conclusion, the present study suggests the possibility of obtaining molecular data suitable for age estimation from burned human remains and the reliability of the blood age estimation model, especially for cases with DI equal/lower than 2.

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Estimation of Legal Age by Quantification of DNA Methylation

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Mass migration in the European Union has highlighted the need to estimate the age of unaccompanied migrant children in order to guarantee their asylum rights. Current legal age estimation techniques, based on skeletal and dental radiographic analysis, are invasive and have limitations in terms of precision and objectivity. Epigenetics, in particular DNA methylation, offers a promising alternative.

In the last decade, the relationship between DNA methylation and the chronological age of people has driven the development of the so-called epigenetic clocks, which rely on DNA methylation patterns to estimate age. However, most of these estimation models are designed for adults and blood samples, and have limitations in their application to pediatric populations (children and adolescents) due to differences in DNA methylation patterns and sample availability. Therefore, it is crucial to develop specific models for these populations.

Our project focuses on developing a legal age estimation method based on DNA methylation, investigating the potential of buccal epithelial cells as an accessible and homogeneous DNA source, and analyzing DNA methylation data from representative cohorts spanning relevant ages (between 14 and 23 years). Machine learning algorithms offer the possibility to identify methylation markers that are robustly correlated with chronological age and to develop mathematical models that enable the most precise estimation of legal age, taking into account ethical aspects such as the minimization of false categorizations of minors as adults. While our work is still in its early stages, our preliminary methodologies and results show promising potential.

Through comprehensive analysis of DNA methylation data and advanced machine learning algorithms, we aim to establish robust models that can be useful in facilitating the forensic tasks involved in asylum petitions. This goal not only seeks the enhancement of the reliability in age assessment but also contributes to the broader field of forensic sciences, paving the way for more efficient methods that ensure justice and protection for vulnerable migrant populations.

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Strategic Approaches to Address Discrepancies in DNA Methylation Values Across Different Analysis Platforms

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Targeted bisulfite sequencing utilizing single-base extension (SBE) and Massively Parallel Sequencing (MPS) have been typically used to measure DNA methylation for age prediction in forensic laboratories. However, discrepancies arise when employing different analysis platforms, even when using different genetic analyzers with different software settings for SBE analysis, resulting in significant prediction errors. To address this issue, the study proposes and compares two approaches: (1) developing new age prediction models specific to each analysis platform, and (2) developing universal age prediction models applicable across different analysis platforms through bias calibration. Platform-specific models were constructed using actual body fluid DNAs with linear regression analysis. Bias calibration was performed by using bisulfite converted control DNAs with varying ratios (0%-100%) to adjust observed methylation values to the ideal values. Bias-adjusting equations were derived by plotting the observed values on the x-axis against the ideal values on the y-axis and establishing a regression line (trendline). Then, the universal model was constructed using calibrated methylation data of body fluid samples. A total of eight age-predictive CpGs for blood, saliva, and semen DNA (ELOVL2, FHL2, KLF14, MIR29B2CHG/C1orf132, TRIM59, TTC7B, LOC401324/cg12837463, and LOC729960/NOX4) were used to deal with genetic analyzer-specific DNA methylation measurement, and six age predictive CpGs for blood DNA (ELOVL2, FHL2, KLF14, MIR29B2CHG, TRIM59, and PDE4C) [Woźniak et al., 2021] were used to deal with the difference between the SNaPshot and MPS. The comparison results indicated that platform-specific models showed the highest accuracy. However, universal age prediction models through bias adjustment also yielded accurate results, suggesting its potential as an alternative in situations with multiple constraints.

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Development of DNA-Methylation based age prediction model in blood samples using snapshot methodology

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Objectives: In this study, we aim to define a mathematical model for chronological age estimation, which would be routinely used in forensic investigations.

Materials and methods: 163 blood samples from both male and female volunteers aged from 18 to 70 years were collected. DNA extracted from the blood was quantified and subjected to bisulfite conversion, then amplified by PCR and purified. Purified PCR fragments were subjected to SnaPshot and capillary electrophoresis.

Results and conclusion: Methylation fractions of 40 CpG sites located in promotor regions of 7 genes (ELOVL2, FHL2, TRIM59, KLF14, C1orf132, PDE4, and EDDARAD) have been analyzed using 8 newly designed SNaPshot multiplex primers sets, and one multiplex primer set from previously published data. In the first phase of the study, 48 blood samples from both male and female volunteers aged from 22 to 70 years were analyzed, and 10 CpG sites that showed the strongest correlation with aging (rs>0,85) were selected for further analysis. In the second phase, an additional 86 blood samples from both male and female volunteers aged from 18 to 70 years were analyzed, and 5 CpG sites with the strongest correlation with aging were selected for building an age prediction model using multivariate linear regression. The model showed high prediction accuracy with MAD=2.66 years and RMSE=3.9 years, and a very strong correlation between actual and predicted age (rs=0.96). There was no significant difference in predicted age between male (MAD=2.84 years) and female (MAD=2.53 years) volunteers. Model validation was performed through the 5 different age groups and an additional validation sample set of 29 volunteers (MAD=1.82 years, RMSE=3.59 years). Given the aforementioned results, we concluded that the newly designed age prediction model could be used as a reliable forensic tool for age prediction in criminal cases.

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Epigenetic Age Prediction for Forensic Investigations

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DNA methylation is the most commonly occurring DNA modification and is mostly seen at cytosines in a CpG dinucleotide context. Studies have shown that about one-third of methylated sites in the genome are affected by age. These discoveries have led to the attempted usage of age-related differentially methylated regions as age markers for the prediction of chronological age using selected sets of CpG sites, which are used to train “epigenetic age clocks”. An epigenetic age estimator is best described as a mathematical model constructed using a machine learning method that regresses chronological age on a selected set of the most informative CpG sites for age prediction. This model estimates epigenetic age by converting the methylation status of the selected CpGs into units of years. A handful of studies have developed their own mathematical models for epigenetic age prediction; however, most methods require measurement assays that require hundreds of nanograms of DNA. Additionally, these models do not take into consideration missing data which introduces bias to the epigenetic age estimates. Recently, epigenetic age clocks have also been considered for use as investigative leads in forensic cases to predict the age of an unknown individual from biological material left behind at a crime scene. Here, we present an epigenetic clock using a binomial estimator approach from whole genome bisulphite sequencing data of cfDNA from plasma to predict gestational age at the trimester level. As ethically obtained forensic samples are difficult to possess, this data is used as a proxy for samples that can be found in forensic casework due to its varying degrees of coverage and sparseness. Preliminary results show that of the eight samples that were randomly selected, six of the samples were accurately assessed for an accuracy rate of 75%. The model presented here serves to provide a proof of principle so that a similar model may be considered for low DNA input forensic samples as degraded DNA is becoming more frequently analyzed among law enforcement. This work has led us to further introduce the use of droplet digital PCR as a method for low template samples in age estimation across varying biogeographical ancestries.

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Impact of lifestyles and presence of disease on age estimation

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The application of DNA methylation as a biomarker for age prediction has become increasingly widespread in the forensic field. To date, multiple age prediction models based on different tissues of forensic interest have been developed reaching errors of around ± 3 years. At the same time, the study of this biomarker has been consolidated, identifying various factors that can produce variations in human methylation patterns. Among these factors, the influence of lifestyle and diseases on DNA methylation has been highlighted, generating new fields of research. These studies have shown, in some cases, an association with accelerated epigenetic ageing patterns, which could affect the predictions obtained with both new and already developed age prediction models.

In the current study, the influence of various conditions on the age prediction models were evaluated. These conditions were alcohol intake, cocaine intake, depression, HIV and schizophrenia. Predicted age was calculated using the following epigenetic clocks: Horvath's clock; Hannum's clock; Horvath's SkinBlood clock; and Levine's clock. Additionally, an adaptation of the USC clock (1) for Illumina HumanMethylation data was also analysed. Comparisons of predicted age using the previous clocks will be presented in terms of prediction errors and correct classification rates.

1. A Freire-Aradas, L Girón-Santamaría, A Mosquera-Miguel, A Ambroa-Conde, C Phillips, M Casares de Cal, A Gómez-Tato, J Álvarez-Dios, E Pospiech, A Aliferi, D Syndercombe Court, W Branicki, M V Lareu. 2022. A common epigenetic clock from childhood to old age. Forensic Sci Int Genet: 60: 102743. doi: 10.1016/j.fsigen.2022.102743.

P-250

Impact of cancer on methylation-based forensic age estimation in blood

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Forensic age estimation through DNA methylation analysis has been a widely researched topic within the field of forensic DNA phenotyping in recent years. As its implementation is currently underway in many laboratories across Europe, it is crucial to investigate the performance of existing age estimation tools and models in different scenarios.

Methylation patterns are not only tissue- and age-dependent at specific CpG sites but are also influenced by environmental factors and disease. One of the medical conditions with the highest incidence in the human population is cancer. Both the cancer itself and treatments targeting epigenetic regulation have long been reported to alter DNA methylation patterns, yet their impact on forensic age estimation tools has only scarcely been studied.

In our study, we hypothesize that the presence of cancer might affect the accuracy of forensic age estimation in human blood samples. Therefore, we test an existing massively parallel sequencing (MPS) based age estimation tool on blood samples from cancer patients. The tool and statistical model use six target CpG sites from different markers (ELOVL2, PDE4C, KLF14, MIR29B2C, TRIM59, FHL2) to estimate the chronological age. For our study cohort, we collect blood samples from both healthy individuals as well as people suffering from a variety of cancer entities, including mamma carcinoma, pancreas carcinoma, prostate carcinoma, lymphoma, and others.

We will compare the accuracy of age estimation between the blood samples from different cancer entities and the healthy control group. Based on these results, we will report whether an adjustment to the estimation model is necessary to improve the estimation accuracy. Additionally, this study aims to provide some initial guidance as to whether forensic age estimation models in general need to account for the potential effect of cancer.

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Development of a methylation-based age prediction model using Next Generation Sequencing

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Extended DNA analysis can offer information on a person's visible characteristics, biogeographic origin, and age, and thereby extending the spectrum of forensic casework and significantly aiding forensic investigation. This proves especially vital in cases where traditional methods such as short tandem repeats (STR) analysis have reached their limits. Age estimation holds particular importance within this field, as aging involves molecular modifications, influenced by genetic and environmental stimuli. Previous research has identified several biomarkers suitable for predicting chronological age, with DNA methylation emerging as the most promising, but with known limitations such as high DNA input concentrations, a mean absolute deviation (MAD) error of 3 to 4 years, and a lack of standardization. As the current law in Switzerland has been changed recently, allowing the extended DNA analysis, the primary objective of this study is to develop and implement a robust DNA methylation-based age prediction model using Next Generation Sequencing techniques on site.
A comprehensive study of DNA methylation markers, including ELOVL2, FHL2, PDE4C, and ASPA, among others, will be conducted to identify age-related changes in tissue-specific methylation status. Whole blood and saliva samples will be collected from donors aged 20 to 80 years, which will be extracted and then quantified using the Maxwell® FSC DNA IQ™ Casework Kit (Promega) and the PowerQuant System (Promega), respectively. After this comes bisulfite conversion using the MethylEdge Bisulfide Conversion System (Promega) followed by library preparation involving target amplification, adapter ligation, library purification, normalization, and library pooling steps. Next Generation Sequencing will be conducted using the Illumina MiSeqFGx system (Illumina), after which an age-prediction regression model will be established. Depending on the progress and success, a novel approach will be presented.

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Array-based age prediction from bloodstains deposited for up to two weeks

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DNA methylation (DNAm) at specific CpG sites is correlated with the chronological age. Various DNAm-based age prediction models, commonly referred to as epigenetic clocks, have been proposed and applied to various tissues. These models have obtained interest in forensic casework, where estimation of the age of an unknown sample donor may provide important leads in police investigations and used to narrow down the suspect pool. Despite these advances, the impact of time since deposition (TsD) on the accuracy of DNAm age estimation remains unexplored.

In this study, we investigated bloodstains with TsDs of 0, 1, 6, and 14 days from seven healthy Danish individuals (age range: 28-55 years). DNAm was analyzed using the Illumina Infinium MethylationEPIC v2.0 BeadChip Array (input DNA: 250 ng), and chronological age was subsequently estimated using five different array-based epigenetic clocks: Horvath (353 CpGs), Hannum (71 CpGs), skinHorvath (391 CpGs), BLUP (319,607 CpGs), and EN (514 CpGs). Overall, none of the five epigenetic clocks showed a decrease in age prediction accuracy with increasing TsD. The BLUP clock produced the lowest mean absolute error (MAE) in age prediction for all samples irrespective of the TsD (MAE at day 0 = 1.22 years, MAE at day 1 = 1.08 years, MAE at day 6 = 1.70 years, and MAE at day 14 = 1.15 years). To further investigate the BLUP clock in a larger sample set, we analyzed an independent dataset generated with the EPIC v1.0. We found a similar low MAE of 2.50 years in blood samples of 63 Danish individuals (age range: 32-63 years). In conclusion, our study shows that array-based epigenetic clocks provide accurate chronological age predictions and are robust for bloodstains with a TsD of up to 14 days. Future work will focus on assessing DNAm age prediction in highly degraded blood samples.

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Transferring MPS-based epigenetic age prediction to SNaPshot – introducing technical protocols and predictive models for bone and cartilage tissues

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Predicting age from DNA, in addition to appearance and ancestry, is an essential element in developing reliable clues for investigating authorities. The use of DNA methylation analysis allows accurate age estimation, which has led to the development of a number of forensically useful methods. A simple and convenient method for measuring DNA methylation is the SNaPshot analysis available in any forensic DNA laboratory. However, with regard to the quantitative measurement of DNA methylation, the application of existing predictive models requires further research to minimize the prediction error related to method-to-method bias.

The aim of our work was to develop new tools for SNaPshot epigenetic age estimation, based on methods previously developed from MPS-based methylation data collected for 8 VISAGE markers, comprising EDARADD, TRIM59, ELOVL2, MIR29B2CHG, PDE4C, ASPA, FHL2 and KLF14.

In a first step, existing MPS-based DNA methylation analysis protocols for bone and cartilage tissues were transferred to the SNaPshot mini-sequencing methods. The sensitivity varied depending on the marker, but the study showed that 10 ng of PCR input is safe for reliable age estimation. Next, we collected new data for bone and cartilage samples and used them for prediction models' reconstruction. The model already completed for predicting age in bone includes markers ELOVL2 (chr6:11044655), KLF14 (chr7:130734372), PDE4C (chr19:18233105), ASPA (chr17:3476273). We expect that the cartilage model we are working on will allow age estimation with similar accuracy. The new methods may find application in any forensic DNA laboratory for reliable age prediction in bone and cartilage tissues.

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The oral microbial community and its potential for age prediction

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Objectives: Age prediction is a critical field of forensic science that can provide valuable information applicable to criminal and anthropological investigations. To date, there are a number of age prediction methods, such as examining bones morphologically, analyzing the amino acid racemization of teeth or using the age-related alterations in the DNA methylation of certain CpG sites. However, these genetic biomarkers have not achieved a high level of precision (the mean absolute error is about 4 years) and are difficult to further improve. Moreover, these markers are greatly affected by the degradation of DNA extracted from evidentiary materials found at crime scenes. Therefore, the use of new genetic markers is highly necessary. It is widely known that the oral microbial DNA is better protected than human DNA and more resistant to degradation. Researches from publicly available databases initially found some correlation between the composition of the oral microbial community and the age of the individual.

Material and methods: Oral microbial DNA was extracted from 42 Chinese participants, and the salivary microbiome was characterized using high-throughput sequencing of the V3–V4 region of the 16S rRNA gene. The participants were divided into 5 age groups (1~19years: 6; 20~29years: 10; 30~39years: 7; 40~49years: 9; 50~59years: 10). The composition of oral microorganisms was analyzed, and the α and β diversity between different age groups were calculated using the Chao1 index and the Jaccard distance, respectively. Principal co-ordinates analysis and permutational multivariate analysis of variance were performed to assess the distinguishability of different age groups based on oral microbial composition.

Results and conclusions: The composition of oral microorganisms was unique in different age groups, and the α and β diversity both found significant differences ($p < 0.05$). By means of principal co-ordinates analysis, it was possible to distinguish clearly between different age groups, and permutational multivariate analysis of variance demonstrated that age could explain the overall difference, which was statistically significant ($p = 0.001$). These findings suggest that individuals of different ages possess unique oral microbial communities, highlighting the potential application of oral microbiota in forensic age prediction.

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Genome-wide DNA methylome profiling to identify between identical twins for forensic use

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In the current forensic scenario, Short Tandem Repeat (STR) profiling originated from genomic difference is highly accurate and widely used to distinguish suspects. However, if the suspect is an identical twin, STR profiling cannot be used. Because DNA methylation patterns known to vary even among twins offer promise, currently LINE-1 and Alu sequences are utilized as DNA methylation markers. Moreover, distinct DNA methylation patterns have been observed among the three types of monozygotic twins (such as dichorionic-diamniotic (DCDA), monochorionic-diamniotic (MCDA), and monochorionic-monoamniotic (MCMA)), and these epigenetic patterns are known to change with environmental factors and age. Hence, there is an unmet need for markers applicable regardless of twin types, environment, age, or gender.

We collected placental blood samples from 54 pairs of monozygotic twins for methylation profiling using the Human MethylationEPIC v2.0 platform.

Differences observed among the three types of monozygotic twins predominantly involved immune-related regions, consistent with previous findings. Both MCDA and MCMA types showed enrichment in 'cytokine signaling' and 'interleukin signaling'-related regions. Conversely, DCDA types exhibited enrichment in immune-related regions along with differences in regions related to 'PPI at synapse' and the 'neuronal system'. Lastly, we suggested useful combinations of concurrently and differentially methylated CpG sites in all 54 pairs of monozygotic twins, which were validated with an average accuracy of 98.8% across two independent datasets.

Traditionally, markers applicable to distinguish monozygotic twins were scarcely identified or required lengthy sequences. However, the markers proposed here require the measurement of methylation levels at a minimum of four CpG sites, enabling validation with small volumes of crime scene samples. These markers maintain their differences from fetuses to elderly ages, implying stability against environmental changes.

P-355

New miRNA markers to distinguish saliva from vaginal secretion in body fluid source identification

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We previously established a forensic body fluid source identification system comprising 12 miRNAs, and modeled it using Naive Bayes (NB) and Partial Least Squares Discriminant Analysis (PLS-DA), achieving high prediction accuracy. The model included three saliva-specific miRNAs (miRNA-223-3p, miR-205-5p, miR-1246) and three vaginal secretion-specific miRNAs (miR-203a-3p, miR-378a-3p, miR-1260b). However, among these six miRNAs, only miR-223-3p and miR-1246 showed differential expression between saliva and vaginal secretion samples, both being higher in saliva, indicating a lack of vaginal secretion-specific miRNAs. We screened three novel vaginal secretion-specific miRNAs through microarrays: miR-26a-3p, let-7a, and miR-762. The expression of these sites in ten vaginal secretion samples and ten saliva samples was measured with the LNA RT-qPCR system, showing significantly higher expression in vaginal secretions than in saliva samples. The expression of these three miRNAs was also measured in ten samples each of peripheral blood, menstrual blood, and semen, forming a dataset including 15 miRNAs in 50 samples. Prediction models were built using multiple logistic regression, neural networks, and random forests, with leave-one-out validation accuracies of 96%, 94%, and 96%, respectively. Various methods were used to assess the contribution of each miRNA to the model, and the top six contributing miRNAs were used to rebuild the models. The leave-one-out validation accuracies for multiple logistic regression, neural networks, and random forests were 92%, 96%, and 94%, respectively. The six miRNAs in the highest-performing neural network model were: miR-126-3p, miR-144-3p, miR-205-5p, miR-497-5p, miR-223-3p, and miR-762. Our results suggest a neural network model constructed with six miRNAs achieves high prediction accuracy, indicating miRNA is a promising complement in forensic body fluid source identification.

P-359

The potential application of Enzyme conversion of DNA methylation in body fluid identification

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The common conversion method for DNA methylation is bisulfite conversion, which resulting high losses, stringent reaction conditions, and sample degradation. Recently, a new enzyme conversion method was proposed, which has been reported with high conversion efficiency, simple and mild reaction conditions, in which methylated cytosine undergoes oxidation to yield 5-carboxycytosine (5caC) catalyzed by ten-eleven translocation dioxygenase 2 (TET2). Subsequently, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) deaminates cytosine, without affecting 5caC, yielding a sequence akin to those generated after bisulfite treatment. The gentle reaction conditions facilitated by enzyme conversion hold promise for the identification of forensic challenging samples. Here, we applied the enzyme conversion method in body fluid identification, aiming to explore the application of the enzyme conversion method in forensic practice. Furthermore, we have conducted a comparative assessment of two methods with detected methylation values, the analysis of mixtures, trace and degraded samples. The results show that the methylation values obtained after enzymatic conversion exhibit more significant differences. After analyzing the methylation values obtained from two methods through model analysis, there is slight difference in the accuracy of determining body fluids. In the analysis of mixtures, 27.5% of the samples treated with enzyme conversion were successfully identified one component, while 72.5% of the samples were identified both two components. The results of degraded samples showed that samples after enzyme conversion can detect more sites than bisulfite conversion. When DNA input was low (≤10 ng), methylation values were more likely to overestimate methylation after bisulfite conversion. The above results indicate that enzymatic conversion method holds promise for the identification of forensic challenging samples.

P-360

Saliva and Semen Identification by DNA Methylation Analysis Using Droplet Digital PCR

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Determination of the type and origin of body fluid can be of great value in crime scene reconstruction by establishing a crucial link between the donor, the cell type, and the activities that occurred. For example, if the DNA source collected from a crime scene is blood, it can imply that there was a strong physical contact. There are various methods for body fluid identification, such as chemical tests, immunologic assays, mRNA assays, and DNA methylation analysis. The chemical tests and immunologic assays sometimes result in false-positive or false-negative outcomes. RNA is more unstable than DNA and single-base extension or pyrosequencing for DNA methylation analysis is time-consuming and costly. To overcome these limitations, droplet digital PCR (ddPCR) was applied to methylation-based saliva and semen identification in this study. Saliva (n=11), semen (n=13), and blood (n=11) were collected in a conical tube from the healthy individuals. DNA was extracted using the QIAamp DNA mini kit (QIAGEN) and then bisulfite-converted through the EZ DNA methylation gold kit (Zymo Research). Two body fluid-specific CpG markers (cg09652652-2d, and cg17610929) were selected and utilized in QX600 droplet digital PCR system (Bio-Rad) to analyze the methylation status of saliva and semen. EpiTect PCR Control DNA Set (QIAGEN) was used as a positive control and a negative control to assess the validity of the experiment. In sensitivity test, except for 1 saliva sample (SA3) which is less than 5 copies/µl, all saliva and semen samples were successfully quantified. Thus, limit of quantification (LoQ) was 5 copies/µl. When the concentration was not higher than 5 copies/µl, the data interpretation was difficult because of the rains. In specificity test, 11 blood samples were tested for cross-reactivity with saliva and semen samples. The result was that both markers weren't amplified in blood and were amplified specifically for each saliva and semen. We also found the fact that DTT affects the number of droplets generated through a comparative experiment. When qMSP (quantitative methylation specific PCR) was performed, the Ct values were over 30. It can be confusing when interpreting the data. However, in ddPCR using the same samples, the result was clear. Moreover, ddPCR enables the absolute quantification because there is no need to make a standard curve. There are also other advantages such as saving time and money. As a result, it can be widely used as a new method for DNA methylation analysis in the field of forensic science.

P-366

tsRNAs — a novel small RNA molecule with potential in forensic body fluid source identification

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Transfer RNA-derived small RNA (tsRNA) is a new class of small RNA molecules with a length of 14-40nt and its application in forensic medicine hasn't been reported. We used microarrays to detect the expression of small RNAs (miRNA, tsRNA, tRNA, snoRNA) in saliva and vaginal secretion samples. The results showed significant differences in the tsRNA expression profiles between the two types of body fluid samples, with a higher number of differential abundance tsRNAs compared to miRNAs. Using a standard of FC>=1.5 and P<0.05, 796 tsRNAs were highly expressed in vaginal secretions, and 720 tsRNAs were highly expressed in saliva. Subsequently, we used stem-loop reverse transcription-SYBR Green method to detect 20 tsRNAs, and after sequencing the PCR products using TA cloning, sequences of 18 tsRNAs were correct. The expression of 18 tsRNAs in ten saliva samples and ten vaginal secretion samples were detected (with U6 snRNA as the reference), and results showed the expression of eight tsRNAs were significantly different. Then ten samples each of semen, peripheral blood, and menstrual blood samples were detected, and using multiple logistic regression and neural network methods to train predictive models (expression data of 18 tsRNAs in 50 samples), leave-one-out validation showed predictive accuracies of the two models were 94% and 100%, respectively. Reducing the number of tsRNA to six, the leave-one-out validation accuracy of both predictive models was 86%. The sensitivity of tsRNA could be as low as 3.9pg. Our study results confirm that tsRNA is a potential small RNA molecule that can be used in forensic body fluid source identification.

P-504

Linking BGA prediction to epigenetic age estimation. A pilot study on the potential of BGA-specific epigenetic age estimation (P)

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Forensic DNA Phenotyping (FDP) has become an integral part of forensic molecular biology in recent years. Extensive research work has not only improved the prediction models for individual phenotypic traits, biogeographic ancestry (BGA) and chronological age, but also optimized the experimental workflows for efficient application of the tools in forensic casework. Since many phenotypic traits show regional patterns, the interpretation of phenotyping results taking BGA into account is much more reliable than without BGA. In a recent study, we showed that a person's ancestry can also have a significant influence on age-dependent DNA methylation, which is investigated in epigenetic age estimation. We compared the age-dependent methylation change in the CpGs of ELOVL2, MIR29B2CHG, FHL2, KLF14 and TRIM59 between a population from Central Europe (EU) and the Middle East (ME) and found an effective estimate difference of more than four years between the two populations. It is worth mentioning that subjects from the respective populations were selected based on their place of birth and residence and have not yet been tested for their actual biogeographic ancestry. In the context of trace investigations, the person being examined is unknown, that information about the person's ancestry information can only be obtained through genetic analysis. Therefore, in this follow-up study, we performed a BGA analysis on the ME and EU test samples from the aforementioned study to determine whether the samples could be reliably assigned to the respective population-specific estimation models using BGA analysis. To our knowledge, this study represents the first attempt to combine BGA analysis with population-specific epigenetic age estimation. The results of this work provide a first impression of the potential, the challenges and consequently the criteria such as specific thresholds that need to be considered for reliable BGA-specific age estimation.

P-510

Fingertip blood deposition time of day estimation utilizing circadian microRNAs and mRNAs

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Objectives: Determining the time since deposition of a biological stain can clarify the time of the case and correlate the biological material associated with the crime event. Bloodstains are one of the most common pieces of evidence at a crime scene. The currently available methods for estimating the time of stain deposition have limitations with different types and magnitudes.

Material and methods: This study evaluated several circadian mRNA and miRNA molecules for estimating the deposition time. Blood samples (300 µl) were collected by finger pricks from nine healthy male volunteers aged 24-27 years (mean age ± SD = 28 ± 2.5 years) at 3-hour intervals within 1 day under controlled sleep-laboratory conditions. The relative expression values of 11 microRNAs (miRNAs) and five mRNAs that have been reported to exhibit circadian rhythms were detected by RT-qPCR. The day/night distinction is indicated using the binary Logistic regression model. For each prediction model, the agreement between the predicted and actual categories is used to create the area under the receiver operating characteristic (ROC) curve (AUC), indicating accuracy. The Leave-one-out classification approach was used to cross-validate the Fisher discriminant analysis model.

Results and conclusions: A total of four RNA biomarkers (two miRNAs: hsa-miR-150-5p, hsa-miR-140-5p, two mRNAs: TIMELESS, CLOCK) showed significant day/night differential expressions. Time estimation models were established based on these rhythmic RNA markers, using miRNAs only, mRNAs only, or both, for predicting the categories of deposition time. The binary logistic regression model was built to infer the day/night categories. The AUC value of the training set is 0.905 for the combined model of miRNAs (hsa-miR-150-5p, hsa-miR-192a-5p, and hsa-miR-140-5p) and mRNAs (TIMELESS, PER3, and CLOCK), and all samples are classified correctly in the testing set. Fisher discriminant analysis was performed to predict three-time categories, namely early morning-morning (6:00-11:59), noon-evening (12:00-20:59), and before midnight-early morning (21:00-5:59) with an accuracy of 70.4% for self-validation and 64.2% for cross-validation. The evaluation of degradability indicated that miRNAs show good stability in 28 days and mRNAs are slightly less stable. Our study, for the first time, demonstrated the potential role of the circadian rhythm miRNA combined with mRNA biomarkers in estimating blood deposition time for forensic applications. In light of the less stability of mRNA, we suggest using circadian mRNA profiling to supplement miRNA-based deposition time categories estimation. Future studies using additional samples and more markers of temporal specificity and other forensically relevant tissues shall be further validated.

P-517

Evaluation of mRNA profiling from DNA/RNA co-extraction method and effect of RNA stabilizer treatment.

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1. Evaluation of DNA/RNA co isolation method for applying forensic field samples and effect of RNA stabilizer treatment.

Over the past 20 years, DNA analysis results have become decisive evidence in proving crimes. Therefore, development of body fluid identification methods has been crucial to enhance the credibility of testimonies. This study aims to test mRNA profiling for body fluid identification and RNA stabilizer treatment.

2. Material and methods

- 2.1 Sample collection: A set of control samples (Semen, Saliva, Blood) from 10 volunteers(approved by IRB committee), 2 set of field samples(vaginal mucosa, menstrual secretion)
- 2.2 DNA/RNA extraction followed by Allprep DNA/RNA extraction kit(Qiagen) according to the manufacturer's manual.
- 2.3 RNA treatment process
  DNase treatment with Turbo DNA-Free solution (ThermoFisher), cDNA synthesis according to the Superscript IV First Strand Synthesis system(ThermoFisher).
- 2.4 cDNA multiplex PCR primers
  PCR primers (Blood- HBB, CD93, ALAS2, Saliva-HTN3, STATH, BP1FA1, Sperm-KLK2, SEMG1, PRM1, Vaginal secretion-CYP2BP1, MUC4, MYOZ1, Menstrual secretion-MMP7, MMP10, MMO11, Skin-CDSN, LCE1C) were used.
- 2.5 RNA stabilizer treatment

Remain samples were treated by RNAlater(ThermoFisher) solution to stabilize and stored in refrigeration. RNA Later treated samples were reanalyzed at intervals 1 month, 3 months and 6 months.

3. Results and conclusions

- 3.1 mRNA profiling result
  Two markers of semen(SEMG1, PRM1) , three markers of blood(ASLAS2, CD93, HBB), two markers of saliva(HTH3, STATH) were successfully detected from the control samples.
  Three markers of menstrual secretion(MMP7, MMP10, MMP11), three markers of vaginal secretion(CYP287P1, MUC4, MYOZ1) were detected from forensic field samples.

- 3.2 Results of RNAlater treated samples
  mRNA profiling results of RNA treated samples were almost identical to the first analysis. Simultaneous DNA/RNA extraction method applying to the forensic field samples is very useful to obtain not only STR results but also mRNA profiling results. As a result of testing of the RNA later reagent, the mRNA profiling results were stable even after 6 months, it would be useful for storing and reanalyzing remaining mRNA.

Posters Topic 4

Forensic DNA Phenotyping

4. Forensic DNA Phenotyping

Abstracts Posters

P-045

Further assessment of biogeographical ancestry in Eurasian populations

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Inference of biogeographic ancestry forms an important part of the DNA intelligence tools available to help police investigators, particularly where there are no suspects or no matches with the DNA databases. A lot of research has been done to improve the informativeness of forensic ancestry tests, and while intercontinental ancestry differences have been well described, constraints appear when dealing with individuals with admixed backgrounds and/or regions of the globe that are not so well defined by geographic barriers. This is the case for the extensive Eurasian regions. As reported in a previous study (1), the Eurasiaplex panel combined with an established 34plex ancestry SNP set can successfully differentiate the geographic extremes of Eurasia (Europe versus South Asia), however, regions in between, including North Africa, Mediterranean regions as well as Middle East regions, need further development of SNP analysis regimes.

In the present study we recompiled the Eurasiaplex + 34plex training sets by replacing the previous Middle East reference group (based on three CEPH Israeli Arab populations) with the recently reported Middle East populations from the study of Almarri et al. (2) (comprising Emirati, Iraqi, Jordanian, Omani, Saudi, Syrian and Yemeni samples). Using the recompiled training set, samples from a range of European and Middle East populations have been tested. Results from STRUCTURE and the Snipper forensic classifier show improved classification success rates and increased likelihoods with the novel Middle East reference group compared to the previous CEPH-based Middle East reference datasets.

(1) C. Phillips, A. Freire Aradas, AK. Kriegel, M. Fondevila, O. Bulbul, C. Santos, F. Serrulla Rech, MD. Perez Carceles, Á. Carracedo, PM. Schneider, MV. Lareu. Eurasiaplex: a forensic SNP assay for differentiating European and South Asian ancestries. Forensic Sci Int Genet. 2013; 7: 359-66.

(2) MA. Almarri, M. Haber, RA. Lootah, ..., HC. Martin, Y. Xue, C. Tyler-Smith. The genomic history of the Middle East. Cell. 2021; 184, 4612–4625.

P-046

Quo vadis, BGA? A reflective assessment of the status of forensic predictions of biogeographical ancestry

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Forensic tests for predicting biogeographical ancestry (BGA) of an unknown sample work well technically, but it has been shown that interpretation of marker genotypes and their pattern of population distribution can be challenging. There is no doubt that BGA analyses are proven to give informative data for population genetics and historical identification cases. The impact of BGA is potentially even more powerful when applied to the investigation of crime cases without immediate suspect leads. The legal situation concerning BGA varies between countries, from those where it is explicitly forbidden, through to those where there are no laws governing the prediction of biogeographical ancestry, to countries that have already introduced legislation permitting the use of this type of analysis. In addition to data privacy issues, the reluctance to use BGA analysis is also due to the limitations of the genotyping techniques used. Forensic BGA prediction panels comprise up to ca. 300 SNPs to ensure sensitivity levels typical of forensic samples. The relatively small number of genetic markers tested, together with limited reference population data, makes interpretation of the results challenging. This presentation discusses data from donor samples with self-declared grandparental ancestry selected to highlight the challenges faced by forensic scientists working with BGA. Nine samples were sequenced using forensic panels with varied numbers and types of ancestry informative markers: VISAGE Basic Tool, VISAGE Enhanced Tool and Ion AmpliSeq™ PhenoTrivium Panel. The latter two assays include selected ancestry informative Y-SNPs in addition to autosomal SNPs, and the Enhanced Tool also includes X-SNPs. All panels contain the HIrisPlex-S SNP sets for predicting eye, hair and skin colour. In addition, the mitogenomes of all samples were sequenced using the Precision ID mtDNA Whole Genome Panel. The data obtained was used to create an interpretational framework for forensic scientists to experience and learn from the data analysis and interpretation of samples with undisclosed ancestry, as if these were real cases. This study is the first exercise aimed at evaluating an expanded BGA analysis. We show how intradisciplinary forensic analysis combining a full range of markers informative for BGA, can influence and aid data interpretation, making forensic DNA phenotyping a valid and informative proxy for traditional eyewitness descriptions of possible suspects in an investigation.

P-047

Biogeographical ancestry prediction in forensic investigations: Creation of reference population data sets to support operational decision making

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Biogeographical ancestry (BGA) prediction is a valuable Forensic DNA Intelligence (FDI) tool used to provide investigators with additional information about the donor of biological evidence. FDI is particularly useful in cases where routine DNA analysis methods, using short tandem repeats, has not led to an identification.

Over the last two decades, substantial research effort has been directed towards the development of new panels of ancestry informative markers (AIMs) and analysis tools for BGA prediction. These efforts have supported the successful application of BGA prediction in casework across a number of international jurisdictions, including Australia.

One of the remaining challenges is the reliable detection and resolution of admixture (ancestral contributions from more than one population) which complicates interpretation. Limitations also exist in the availability of suitable reference population datasets for use with BGA prediction tools, resulting in deficiencies for some populations of interest.

Given these known deficiencies, an informed approach to the collation of suitable reference population samples, which recognises the diversity of global populations, would be beneficial to enhance operational decision making. This presentation describes a novel approach to the creation of reference data sets for BGA prediction in which population and sub-population groupings are informed by outputs from existing BGA prediction tools, including i) Q matrices from STRUCTURE and ii) results of the likelihood ratio test function incorporated in the GenoGeographer tool. Performance characteristics of reference data sets created using this approach will be discussed in the context of the operational implementation of BGA prediction methods and strategies will be proposed for improved practice and application.

P-048

A Compact 12-Microhaplotype Panel for Enhanced Ancestry Inference

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Microhaplotypes (MHs) have emerged as a powerful tool in forensic genetics, offering significant advantages over traditional STRs, particularly when analysing degraded DNA samples. In this study, we report a new panel consisting of 12 MHs which were selected meticulously through a stepwise conditional genome-wide association study (SCGWAS) using deep sequencing data of 2,504 individuals from the phase 3 of the 1000 Genomes Project (1kGP) for ancestry inference between five super populations. SCGWAS successfully identified 46 genome-wide significant ancestry-informative SNPs which leads to the development of a 46-MH panel. This panel demonstrated outstanding accuracy, achieving an overall accuracy rate of 99.68% and an average area under the curve (AUC) of 99.96% in leave-one-out cross-validation (LOOCV). To address the practical challenges posted by larger MH panels in forensic contexts, especially when dealing with limited and degraded DNA samples, we further reduced the size to a 12 MH panel via a backward selection. Despite its small size, this panel retained remarkable performance, as indicated by an accuracy of 95.85% and an average AUC of 99.78% in LOOCV. In comparison with existing similar sized MH panels, such as Chen's 10-MH and Zhu's 13-MH panels, our 12-MH panel showed superior performance, particularly in admixed populations. The accuracy of our models was further validated at similarly high accuracies in 698 individuals from the May 2020 expansion of the 1kGP who were not included in our panel development. Our 12-MH panel thus represents an ideal equilibrium between panel size and predictive accuracy which opens new avenues for more precise and efficient analysis of complex DNA samples in routine forensic investigations. Our computer tool for ancestry inference using 12 to 46 MHs is freely available at https://github.com/Fun-Gene/Ancestry_46MH.

P-049

Unlocking the Potential of the ForenSeq Kintelligence Kit SNPs for Biogeographical Ancestry Inference

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The resolution of the Golden State Killer case underscored the transformative potential of forensic investigative genetic genealogy (FIGG). In response, VEROGEN developed the ForenSeq Kintelligence Kit with 10k carefully chosen SNPs, tailored for integration with the GEDmatch PRO database to enable familial searches. Despite these advancements, not every search on GEDmatch PRO database produces actionable leads, potentially wasting law enforcement resources. Our research explores a novel application of the Kit's genotypic data for biogeographical ancestry (BGA) inference, addressing the unexplored potential of these markers in accurate BGA identification.

To assess the performance of the ForenSeq Kintelligence Kit for BGA inference, we used the 600k Human Origins (HO) array SNPs and a reference database collated from nine previous publications. The reference dataset contains ~6,500 individuals categorized into 22 geographic regions. Due to the use of the HO SNPs, only an overlapping subset of ~2,600 SNPs was employed for ancestry inference assessment. We split the dataset 80/20 for training/testing and simulated first-order admixed individuals using pyAdmix, to conduct an exhaustive evaluation with GENOGEOGRAPHER.

Our initial results demonstrate the ForenSeq Kintelligence Kit's exceptional accuracy in identifying admixed ancestries and achieving a level of ancestry resolution beyond what traditional BGA panels can offer. These results suggest that, even in cases where GEDmatch PRO fails to provide investigative leads, the genotypic data collected can be repurposed for BGA inference, thereby extending the potential of the ForenSeq Kintelligence Kit beyond its initial scope. Lastly, we provide a custom GENOGEOGRAPHER database created from our reference dataset, which allows fast ancestry inference without the need for bioinformatics expertise.

P-056

Alternatives to multinomial logistic regression data input in the outcome of pigmentation traits forensic prediction.

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INTRODUCTION: The usage of informative molecular markers to predict human visible characteristics is widely found in the current forensic literature. Regarding the prediction of eye and skin colour, the current gold standard is the IrisPlex system, which has been tested in several scenarios, yielding different results according to the tested population. Notoriously, Irisplex and other pigmentation prediction tools have low performance when evaluating intermediate classes. According to the authors, IrisPlex is based on a multinomial logistic regression, where the independent variable is the number of minor alleles of the k-th SNP. Such definition indicates that the independent variable is treated as an integer variable with domain within [0,2]. However, such approach has a major drawback, once the prediction probability based on the number of alleles assumes a linear effect, meaning that the model predicts each distinct homozygous genotypes as presenting the same distance and biological effect in relation to the heterozygous genotype. Such assumption ignores the dominance and epistatic effects usually presented by pigmentation associated traits.

OBJETIVE: We present in this work the mathematical and the practical consequences of this approach, employing a genetic/phenotypical database based on the Irisplex system and comprised of on admixed Brazilian subjects.

RESULTS AND CONCLUSIONS: Our results indicate that alternative statistical approaches to the imputing the genetic data as categorical variables in the logistic regression may seems to better model the biological pigmentation process, since minor changes on the mathematical approach within the same data provide a significantly better classification performance, especially considering intermediate phenotypical classes. Even though such disparities can be explained by differences between the original population used in Irisplex development and the one used in our study, alternatives to the currently used prediction variables of multinomial logistic regression should be considered. Improvement in the sensibility of intermediate classes prediction is a benefit, in addition to the adoption of models which better represent the underlying biological mechanism and present additional interpretability.

P-057

Use of MicroRNA Expression in DNA extracts to Predict Body Weight Class in Forensic Samples

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Identification of phenotypic traits such as hair, skin and eye color along with biogeographic ancestry are rapidly becoming mainstream forensic analysis methods to provide investigative leads for unsolved criminal cases. Externally visible characteristics (EVCs) that include both genetic and environmental factors including facial structure, age, height and weight have proven to be more difficult to accomplish, but remain important physical traits for identification of persons of interest to a case. While admittedly an imperfect measurement, estimation of body weight classification (underweight, normal, overweight, and obese) would provide important information for visual characterization of an unknown individual, whether that be an assailant for a crime or an unidentified victim. This area of characterization of body weight prediction of an individual from forensic evidence is completely unexplored.

RNA extracts from 24 blood and 24 saliva samples from donors of both genders, varying ethnicities, and body weight classes were subjected to small RNA sequencing on the Illumina platform. From these data, candidate miRNAs predictive of body weight class were identified using DNA extracts and tested for prediction accuracy using RT-qPCR analysis. Future work will test those miRNA markers in a larger sampling of the population, and to explore additional markers (if necessary) for improved accuracy. Continued testing of prediction models will utilize machine learning methods for classification modeling and utilize standard forensic developmental validation tests for precision, reliability, robustness, sensitivity and error rate, once accurate markers are identified in both body fluids.

The impact that this project will have on the forensic science community could be significant. Alone, body mass or weight classification is a minor advance for forensic biology; however, as part of a broader phenotypic panel (which is already available for use in casework), we begin to see a more comprehensive picture of the unknown person. By using DNA extracts for determining miRNA expression for body weight class prediction, the stage is set for inclusion of the markers into a commercial high-throughput sequencing primer set that already includes other EVC markers. Ultimately, providing tools to DNA laboratories that can provide additional information about an unknown assailant or victim benefits society, the crime laboratory, and the criminal justice system by providing information to investigators for a more speedy and conclusive investigation.

P-058

Integrating physical anthropological techniques and emerging methodologies using a quasi-landmark mesh to decode the upper facial region from DNA

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Genetic identification currently requires comparing genetic data from remains against reference profiles of indubitable individuals or stored in forensic databases. However, lack of a matching genetic profile or an individual with whom to match the DNA profile found on a trace means that some cases remain unsolved. In such circumstances, advances in Forensic DNA Phenotyping, that seeks to determine externally visible characteristics (EVCs) from the DNA, could assist in guiding the individual identification. Among all EVCs, the facial shape stands out as particularly useful for this issue. Although recent GWAS have shed light on possible SNPs associated with facial traits, offering insights into determining individual appearance, further research is required because of its high level of complexity. Addressing this issue, the present study has selected a set of candidate SNPs and assessed their correlation with facial phenotype in the Spanish population, focusing on the upper facial region as it contains the ocular area. This is one of the most striking and informative regions where we tend to look initially and more frequently when considering a specific face. From a collection of 412 individuals, we performed two different strategies: one considering different measurements and indexes used in physical anthropology and using logistic regression analyses, and the other considering a spatially dense mesh of quasi-landmarks and using canonical correlation analyses. Results revealed significant associations between several SNPs and different upper facial metrics. Interestingly, SNPs and their associated facial areas identified in both strategies differed. These findings underline the importance of methodology in facial description when conducting genetic studies, and most notably, when considering forensic applications.

P-059

Forensic DNA Profiling and Phenotyping from Tobacco Sticks and Nails with Semi-Permanent Polish Samples

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The significant increase in the use of Heat not Burn devices for smoking has enhanced the chance of finding the so-called tobacco sticks as evidence at the crime scene. Similarly, the worldwide popularity of nail beautification may lead to analyze nails with semi-permanent polish for the identification of badly preserved corpses.

The aim of this study was to explore the possibility of forensic DNA profiling and phenotyping of eye, hair, and skin colour features using an MPS assay consisting of the 41 SNPs included in the HIrisPlex system on 22 samples of which 16 tobacco sticks and 6 clipped fingernails with semi-permanent nail polish. The MPS assay was designed by the Ion AmpliSeq Designer web tool with amplicons size <180 bp, to allow analysis of degraded DNA. The MPS libraries were performed using Precision ID Library kit, and sequenced using Ion GeneStudio S5TM System. The average coverage of sequenced libraries was 2511.5 and the uniformity of coverage of 97% (mean). Genotype data obtained was then uploaded on DNA Phenotyping web tool (https://hirisplex.erasmusmc.nl/) to predict eye, hair, and skin colour. Full STR profiles and phenotype prediction were obtained for all the samples subjected to analysis and eye, hair and skin traits of samples donors were predicted with high accuracy.
The new technologies and DNA-based approaches for personal identification are suitable to follow the evolution of costumes and personal behaviors allowing to obtain useful information for criminal investigation even from new items.

P-060

Phenotype prediction in Croatian population using ForenSeq® DNA Signature Prep kit

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Prediction of externally visible characteristics (EVC) based on single nucleotide polymorphism (SNP) genotyping data may generate investigative lead in forensic casework. The ForenSeq® DNA Signature Prep kit targets 24 phenotype-informative SNPs (piSNPs) included in HIrisPlex system predicting hair and eye colour. As part of in-house validation, the aim of this study was to assess the genotyping and prediction performance of ForenSeq® DNA Signature Prep kit (Verogen) on MiSeq® FGx™ system (Illumina) in Croatian population. Genomic DNA was extracted from buccal swabs obtained from unrelated volunteers (n=83) according to the validated laboratory procedure. Sequencing was performed following the manufacturer's recommendations. Run metrics and sequence data were processed by ForenSeq Universal Analysis Software v.1.3 (UAS). Sequencing performance was assessed using UAS default quality metrics. The prediction accuracy for eye and hair colour was determined based on self-declared data. Run metrics passed across all runs. The kit was able to produce high quality SNP profiles. Eye colour was predicted correctly in 72% of tested individuals and all inconsistent predictions corresponded to individuals who were predicted or self-declared intermediate eye colour. The majority of individuals with self-declared intermediate eye colour were predicted to have blue eye colour. We observed four individuals with predicted intermediate eye colour and two of them were correctly assigned. The Croatian study population displayed high frequency of brown (58%) and blue (37%) eyes, as well as blond (65%) and brown hair (30%). Regarding the hair colour, 69% of predictions were accurate, and the majority of inconsistencies resulted from predicting blond hair in individuals with brown hair. Due to known limitations of ForenSeq system, it is important to test phenotype prediction accuracy of the specific population prior to the implementation in forensic casework.

P-061

Prediction of hair colour from genotype in the Argentine population: a preliminary study

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Hair colour is an external visible phenotypic trait, which is determined by a set of genes that are variable among individuals and among populations. It is possible to predict the physical appearance of an individual with a certain probability, by genotyping a biological sample, being a valuable tool for the identification of persons in the field of forensic genetics.

Based on previous studies of phenotyping, systems for predicting phenotype using genetic data have been developed, such as HIrisPlex-S and Snipper, among others. However, these studies have been carried out mainly on populations of European origin, and may not be directly applicable to admixed populations, as is the case of Argentineans, where genotype-phenotype association could differ, given their multiple ancestry components (Eurasian, Native, and Sub-Saharan).

The aim of this work is to describe the genetic variation underlying hair colour in Argentina population. We obtained DNA from 158 samples of mouthwash from donors from the province of Buenos Aires. Five SNPs were genotyped for MC1R gene, rs11547464, rs1805006, rs1805007, rs1805008, rs1805009, and one for SLC45A2 gene rs28777. Samples were visually classified into two colour categories, and on the other hand, using Snipper as a bayesian classifier. The coincidence rate was 56.96%, indicating a correlation between the Snipper results and observations in that percentage of instances, with a higher incidence among individuals with dark hair (58.9% for dark-haired individuals and 41.1% for blondes).

Our results suggest the need for improving the values of prediction of hair colour from genotype information for this admixed population. Given the substantial amount of genetic and phenotypic variability of this population, influenced by various ancestry components, it is crucial to increase the accuracy and applicability in forensic investigations by continuing these studies with more markers and a bigger sample of individuals.

P-062

DNA methylation-based models for predicting multiple phenotypes

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DNA methylation, which provides a comprehensive reflection of an individual's acquired traits, has been considered a promising biomarker for forensic DNA phenotyping to aid in the identification of persons of interest (POI), categorizing individuals biologically. Therefore, we aimed to develop DNA methylation-based models capable of analyzing various phenotypes ranging from age estimation to lifestyles like smoking habits and alcohol consumption.

An epigenome-wide association study (EWAS) of CpG sites associated with age, smoking habits, and alcohol consumption was performed using Infinium MethylationEPIC array data of 2,350 Koreans from the Korean genome and epidemiology study (KoGES) or 1,008 Korean data from the Gene-EnviroNment IntEraction and phenotype (GENIE) cohort, taking into account the distribution of samples with each phenotype. The significance threshold of $P < 9.42 \times 10\text{-}8$ and several criteria were applied to select highly associated CpGs with phenotypes. Subsequently, the models for age, smoking habits (never smokers vs current smokers), and alcohol consumption (non-to-moderate vs heavy drinkers), were trained respectively using LASSO regression or stepwise regression in the same discovery set. The developed models were then validated using the other independent cohort dataset.

The EWAS for age, smoking habits, and alcohol consumption identified 99,802 13,070, and 629 CpGs respectively according to the threshold, and each prediction model for the phenotypes was composed of 687, 7, and 41 CpGs. The age prediction model yielded an error of 3.8 years with an R squared value of 0.69. The prediction models for smoking habits and alcohol consumption showed an accuracy of 0.89 and 0.64 with an AUC value of 0.864-0.907 and 0.62-0.69, respectively. The identified CpGs and developed prediction models will contribute to the advancement of forensic DNA phenotyping.

P-065

Genetic distribution on 5 SNPs associated with ear morphology in the Han Chinese population

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Objectives: The external human ear is considered to be a polymorphic and polygenic structure with individual uniqueness that serves as an essential target in Forensic DNA Phenotyping studies. Multiple loci and SNPs for variation in human ear characteristics have recently been reported; however, these genetic sites have seldom been validated in the Chinese population. To fill this gap, the present study assessed the genotypes and allele frequencies of 5 SNPs associated with ear morphology in Chinese cohorts.

Material and methods: We collected oral swabs from 50 unrelated Han Chinese (25 males and 25 females) living in Sichuan Province, followed by DNA extraction using the Chelex-100 method. For genotyping, the SNaPshot multiplex assays containing 5 SNPs (rs3827760, rs17023457, rs263156, rs1619249, rs1960918) that have been previously validated in Pakistani ear morphology were developed.

Results and conclusions: The allele frequencies of G and A in rs3827760 were 0.87 and 0.13, respectively, while no genetic polymorphism was found in the Pakistani population. In the Chinese population, the rs17023457 exhibits allele frequencies of 0.36 for C and 0.64 for T, respectively, which has shown predictive potential for earlobe size, tragus size, and the antihelix superior crus among Pakistanis. The allele frequencies of T were lower than those of both allele C in rs263156 (0.65) and allele G in rs1960918 (0.71), while the rs1619249 demonstrated nearly equal allele frequencies of A (0.48) and G (0.52). Our study indicated that the genetic distribution of 5 SNPs existed difference between the Chinese and the Pakistani, further providing a reference for the application of Chinese ear phenotypic markers in forensic investigations.

P-079

Identification of genome-wide polymorphic microsatellites in cattle (Bos taurus)

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Objectives: A trustworthy approach to determine cattle paternity testing is essential given that domestic cattle in underdeveloped agricultural zones is primarily grassland grazing without properly regular monitoring, resulting in an increasing number of theft cases and property dispute cases. In such scenarios, short tandem repeats (STRs) are a commonly utilized genetic marker because of their robust reproducibility and considerable polymorphism. However, artifacts known as stutters typically cause confusions regarding the genotype of STRs, particularly dinucleotide microsatellites (di-STRs), whereas tetranucleotide microsatellites (tetra-STRs) tended to be comparatively insusceptible to stutters.

Material and methods: Whole genome sequencing (WGS) data of cattle (Bos taurus) available on NCBI were obtained. Tandem Repeat Finder (TRF) software was employed to search for genome-wide STRs in cattle reference genome and of which identified STRs were then profiled with HipSTR software. Based on population results, the profiling rate and polymorphic information content (PIC) of these STRs were further assessed. We ultimately validated the efficiency of identified tetra-STRs by randomly sampling and massive parallel sequencing on indigenous cattle.

Results and conclusions: 775,444 STRs in cattle (Bos taurus) reference genome were identified by TRF software. These STRs were profiled and filtered by HipSTR software on open-source WGS data of 111 samples, revealing an average profiling rate of 78.8%. The PIC of high-resolution STRs that had available genotypes on more than 80% samples was subsequently calculated, indicating a rich variability on tetra-STRs. A significantly positive correlation was additionally found between mean allele length and PIC of STRs. Furthermore, a set of 20 identified tetra-STRs were randomly selected and validated on 100 indigenous cattle, confirming the reliability of current STR identification method. In the end, we offered a qualified cattle STR database comprising tetra-STRs that had a profiling rate >0.8 and allele numbers >3, prospectively facilitating paternity testing and other genetic research involving cattle.

P-081

An NGS-based Panel of Cattle (Bos taurus) Multi-source Genetic Markers: Development and Validation

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Objetives
As one of the most common animals in the livestock industry, cattle are also the main target in many civil disputes and offences. Currently, the kinship identification of cattle is mainly done by polymorphism analysis of dinucleotide STR (di-STR) motifs by capillary electrophoresis. However, the di-STR locus will produce many stutters that affect the interpretation of genotyping results, and different laboratories and even different identifiers may get different conclusions. Whereas, the tetranucleotide STR (tetra-STR) generally have less stutter and the genotyping results of these markers were more easer to be interpreted than di-STR. Therefore, we would like to construct a multi-source genetic markers typing system for individual identification and paternity testing of cattle and evaluate its performance for forensic applications.

Material and methods
We selected cattle tetra-STR loci that meet the requirements of single copy, high polymorphism, and clear chromosome location with inhouse python script. The commonly used di-STR loci recommended by the FAO (FAO) and the International Society (ISAG) were also included. Mitochondrial DNA fragments locating on d-loop for maternal lineage analysis and DNA barcode for species identification were also included in this panel. Thus a multi-source, multi-purpose genetic marker detection system (named Cattle DNAtyper NGSPanel) was construct based on Next Generation Sequencing (NGS) platform BGI. The sensitivity, reproducibility, consistency, and species specificity of the Cattle DNAtyper NGSPanel were validated according to the validation guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM). In this study, 120 unrelated individuals and 15 pairs of mother-child cattle blood samples (Neimenggu, China) were collected for population experiments and paternity identification experiments, and the blood samples were subjected to DNA extraction using the QIAamp DNA Investigator Kit.

Results and conclusions
Based on NGS platform, we developed the Cattle DNAtyper NGSPanel, which has 34 di-STRs, 53 tetra-STRs,3 X-STRs,1 COI,1 CYTB,2 RAG1, and 7 D-LOOP genetic markers. Cattle DNAtyper NGSPanel contains a variety of genetic markers commonly used in forensic medicine, with high detection throughput, high sensitivity and stability, accurate typing, good repeatability, strong detection ability of mixed samples, and can be used for cattle species identification, individual identification and paternity testing.

P-201

In vitro splicing assay for exon trapping analysis of rare RyR2 variants

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Being a major mediator of cardiac calcium-induced calcium release, as a calcium release channel in sarcoplasmic reticulum, the cardiac Ryanodine receptor 2 (RyR2) plays a key role in regulation of intracellular calcium influx and calcium homeostasis in cardiomyocytes. For this reason, RyR2 is a main regulator of excitation contraction coupling (ECC) and therefore, associated with this, it is essential for a controlled heart-beat and –rhythm. Alterations in ryanodine receptor 2 gene get associated with arrhythmogenic diseases and cases of sudden cardiac death (SCD).

Initially, patients DNA from so far unexplained deaths got analysed by next generation sequencing (NGS), which revealed various rare RyR2 splice variants. Since the variants could affect the recognition of the splicing motif, in silico prediction tools were used to assess the potential impact and relevance on RNA splicing. According to the prediction programs used, an effect of the nucleotide exchange of the variants can be considered as probable.

Within this experimental approach, the splicing motif of these different splice-variants of Ryanodine receptor 2 gene (RyR2) of so far unexplained deaths got investigated using exon-trapping experiments. Therefore, in order to assess the functional influence of the base change at exon intron boundaries, we performed an in vitro minigene splicing assay, using the pSPL3 exon trapping vector system. Through multiple cloning sites in the intron sequence of the vector, an exon with flanking intronic regions can be inserted using in vitro splicing assay. The assay serves the investigation whether a change in splice site sequence leads to altered RNA splicing by expressing wildtype and variants in human embryonic kidney cells (HEK293) and subsequently the resulting RNA products get analysed.

The performed exon-trapping analysis gives valuable insights into the structure and function of the analysed RyR2 variants, investigating their respective splice motive. This enabled the identification of exons included in the mature mRNA, thus characterizing the impacts of these variants on protein structure and function.

The in vitro splicing assay thus provided further indications on the potential cause of death of the deceased persons.

P-249

Age Prediction from Semen Samples via DNA Methylation Analysis and Bisulfite DNA Quality Method Implementation

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Age prediction remains one of the foremost pursuits in forensic DNA phenotyping tools. The current standard method for age prediction involves detecting cytosine methylation using pyrosequencing or massively parallel sequencing, both relying on bisulfite conversion. However, the harsh and destructive nature of the bisulfite reaction poses limitations, particularly in analyses involving DNA of low quality and/or quantity found in forensic traces. The absence of a universally accepted method for evaluating the quality of bisulfite conversion presents a challenge, as incomplete conversion can significantly distort downstream results.

In this project, we opted to employ the BisQuE method, a multiplex quantitative real-time PCR technique for bisulfite conversion quality control (doi: 10.3389/fgene.2021.618955), and to develop an age prediction model for semen samples in the Czech population based on massively parallel sequencing.

Here, we introduce several modifications to the BisQuE workflow, including incorporating salmon sperm DNA into both the DNA standard dilution series and the C- and T-indicator dilution series and allowing both dilution series to incubate overnight to facilitate proper equilibration. We also present our initial results, including performance characteristics (n > 20).

Our age prediction model for semen samples, suitable for sexual assault cases, utilizes six CpGs in six genes (SH2B2, SYT7, NOX4, TTC7B, TBX4, and GALR2). This model was constructed using a dataset comprising 46 samples (35 volunteers from a fertility clinic and 11 healthy volunteers). We present the performance characteristics of this model, along with the results of testing the effect of cryoprotective medium (n = 10) and differences between the methylation profiles from sperm and non-sperm fractions of ejaculate.

P-259

Forensic Age Estimation:transcriptome analysis and modeling in Peripheral Blood

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As research into the human aging transcriptome advances rapidly, differentially expressed mRNAs have emerged as potential biomarkers for age estimation. To enhance its accuracy and applicability as a forensic age predictor, additional biomarkers from diverse datasets are necessary. In this study, the peripheral blood samples were collected from 127 unrelated Chinese individuals and sequenced using the RNA-seq approach. To adjust batch effects, the output data underwent basic normalisation to ensure consistency and reliability across all samples. In total, over 50,000 mRNAs were identified as the potentially age-related. Among them, 1427 up-regulated genes and 1197 down-regulated genes were found to be age-associated based on Spearman's correlation. A total of 260 genes showed a significant p-value ($p<0.05$) and an absolute Spearman correlation coefficient above 0.4, which were selected as candidate biomarkers for further research. Several popular machine learning (ML) algorithms, such as —support vector regression, random forest regression, XGBoost, lasso regression, and stepwise regression, —were used to establish and evaluate the age prediction models. By comparing the root-mean-square-error (RMSE) and mean-absolute-error (MAE), random forest regression was determined the best, with a RMSE of 9.29 years and MAE of 7.23 years. In summary, the results derived from this model fitting showed an improvement over the previous reports, which demonstrated a new mRNA-derived model for the prediction of biological age.

P-260

Forensic Age estimation and the potential use of 5-hydroxymethylcytosine

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DNA methylation has been studied intensively over the last decades in many health-related fields such as cancer research, mortality risk analysis and for a better understanding of aging. Some studies analysed age-dependent changes in DNA methylation to create epigenetic clocks to estimate an individual's biological age and potential health risks. Forensic science, on the other hand, strives to predict an individual's age as closely to chronological age as possible. Most of these studies investigate DNA methylation using sodium bisulfite conversion, the current gold standard for DNA methylation detection. It cannot discriminate between 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC), resulting in methylation levels consisting of the sum of both modified cytosines.

Paired oxidative bisulfite sequencing (oxBS) can be applied to differentiate between 5mC and 5hmC by comparing the results of each analysis. This study investigated the methylation levels of buccal swabs of 108 volunteers. 5mC and 5hmC levels at 42 CpG sites of eight genetic markers (EDARADD, PDE4C, SST, KLF14, SLC12A5, TOX2, LRRN2, and STK12A) were analysed via pyrosequencing.
Our results show that some of the markers established in epigenetic age estimation comprise a considerable proportion of 5hmC, which can also be age-dependent. In this presentation, we show how well 5hmC levels correlate with age and discuss the general influence of 5hmC on age estimation models.

P-261

Forensic age estimation by epigenetic-based age prediction models from blood and semen samples in the French population.

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Objectives.
Identifying the origin of human biological stains detected at crime scenes by comparing DNA profiles to national or international forensic databases is instrumental to provide new orientations to police investigations. Among the biological stains frequently collected by forensic investigators, blood and semen traces are of primary interest in sexual offence and crime cases, and often lead to the extraction of usable genetic material. When unknown profiles are established, DNA phenotyping offers additional investigation leads by inferring key aspects of individual's visible appearance and geographical origins. Interestingly, recent studies have defined a correlation between the levels of epigenetic modifications of specific cytosine residues in the genome and the chronological age of individuals. As a forensic laboratory of the French National Police, we therefore developed distinct DNA intelligence methodologies to predict the biological age from blood or semen samples based on the analysis of DNA methylation of human genomic regions.

Materials and methods.
We established a first cohort of blood samples obtained from 170 French male and female donors aged from 0 to 101 years old, and a second cohort of semen samples from 284 French males aged from 18 to 74 years old. Using a primer-extension based assay, we analyzed the methylation status of 5 and 8 age-associated CpG sites for blood and semen samples, respectively. DNA methylation-based age prediction models were developed for each biological matrix, based on multiple regression analyses and data transformation. Their respective predictive performances were finally validated on independent sample sets.

Results and conclusions.
We therefore developed an approach compatible with routine genetic analyses in the police forensic laboratories in France to predict the biological age of individuals strictly based on the methylation levels of 2 panels of age-related CpG sites. We first established an optimized statistical model for blood samples that can explain 97% of age variation with a mean absolute error (MAE) of 3.45 years between the estimated biological and chronological age of individuals. Interestingly, this model demonstrated similar predictive performances for both males and females. Finally, we also defined a separate semen-optimized age prediction model that explains 70% of age variation with a MAE of 3.76 years.

P-410

The development and validation of a novel forensic STR panel (155 STRs) on massively parallel sequencing platforms

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Short tandem repeats (STRs) are utilized broadly in various forensic communities, as the mainstream biomarkers. However, conventional assays of measuring genotypes fail to provide sufficient discriminative power in several scenarios. Massively parallel sequencing is able to detect a large number of forensic STRs simultaneously, focusing on not only length variations but also base polymorphisms. In our study, we developed a novel panel including 156 loci (67 autosomal-STRs, 57 Y-STRs, 31 X-STRs and Amelogenin) and performed the validation following the guidelines of SWGDAM on MiSeq FGxTM Forensic Genomics System. The sequencing outputs were called using GRCh38 by STRait Razor v3.0 and STRinNGS v2.1 respectively and counted by homemade statistical scripts. Three types of standard DNA samples were measured to monitor the repeatability and reproducibility, sensitivity, species specificity, the influence of PCR inhibitors, mixture analysis and tissues specificity, and one hundred unrelated healthy samples were sequenced for studies of population genetics and forensic parameters. The 156-multiplex system showed fair concordance in our study, more than 90% of minor contributor alleles were called definitely in a 1:19 male-female mixture and when the inputs of humic acid and tannin reached 100ng/μL and 40ng/μL respectively, allele dropouts were observed in several loci. What's more, the panel showed a favorable inter-loci balance and heterozygosity balance, and credible results could be obtained with a minor data volume (around 200 M per sample). Based on population studies, we found the numbers of alleles increased significantly compared to PCR-CE platforms, attributed to the alleles with identical length but varying bases, with several loci showing an increase of more than 300%. Our results demonstrated the new panel would be an ideal tool for STR genotyping, meeting several forensic demands such as pedigree investigation and mixture analysis, with its reliable performance and excellent discriminative power. And in the future, we will apply the panel on the other sequencing platforms.

P-421

Limitations of low-depth WGS data in forensic applications

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1. Objetives: forensic scientists have successively undergone capillary electrophoresis and targeted amplification methods based on the next generation sequencing technology platform for DNA typing. For forensic scientists, only accurate DNA typing of biological samples can ensure the accuracy of personal identification and paternity testing conclusions. Whole genome sequencing technology can simultaneously obtain STR and SNP information for an individual, and even obtain many SNP loci with genetic polymorphism that we have not paid attention to. With the more mature and gradually reduced cost and price of whole genome sequencing technology, the application of whole genome sequencing in forensic medicine has gradually attracted the interest of researchers. However, it is unclear how effective the application of whole genome sequencing in forensic medicine will be. There are many methods for high-throughput sequencing, such as whole genome sequencing and amplicon sequencing. At present, the application of high-throughput sequencing in forensic science still uses amplicon sequencing. However, there is a lot of whole genome sequencing data both in public database or some scientific research. Can these whole genome sequencing data be used for personal identification or parent-child identification? 2. Material and methods: this study simultaneously used traditional capillary electrophoresis (CE), whole genome sequencing, and amplicon sequencing (ForenSeq™ DNA Signature Prep Kit with Illumina MiSeq FGxTM instrument) to detect 21 samples and compare their detection effects. 3. Results and conclusions: results showed that both traditional capillary electrophoresis and amplicon sequencing performed well and could achieve forensic application effects. However, low-depth whole genome sequencing data cannot be applied in STR typing, and the accuracy in SNP detection can reach approximately 70-90%. This study indicates that low-depth whole genome sequencing data is difficult to use for forensic personal identification, and also suggests that SNP loci detected by low-depth whole genome sequencing need to be further validated using other methods.

P-472

Comparation of Lyophilized and Normal DNATyperTM 30 6-dye PCR Amplification Kit

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1.Objective To test the technical performance indicators of DNATyperTM 30 lyophilized reagent and evaluate its application ability in practical forensic cases.

2.Material and methods A test plan was developed to test the detection rate, sensitivity,stability and other aspects of the lyophilized reagent by setting different storage conditions and time ranges. Standard DNA 9947A and case samples were used as template. A parallel comparison was carried out between liquid reagent and lyophilized reagent.

3.Results DNATyperTM 30 lyophilized reagent had accurate typing results,repeatability and high sensitivity(0.125ng). It had good adaptability to trace amounts of obsolete test materials and could be stored at room temperature over 1 month.

Conclusions DNATyperTM 30 lyophilized reagent with easy operation and high sensitivity can significantly increase the loading quantity of sample,extend the storage time at room temperature, which can meet the needs of DNA tracing,long-distance transportation and carrying at room temperature.

P-501

Internal evaluation of the uses and limitations of externally visible characteristics (EVCs) and biogeographical prediction tools for forensic applications

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As the next generation sequencing tools and resources continue to grow, more laboratories in the United States are exploring the use of chemistries that go beyond just STRs. Single nucleotide polymorphisms can be used to predict externally visible characteristics (EVCs) and biogeographical information which could provide investigators with information, intelligence and/or investigative leads that could be of particular benefit when no other information about a sample is available. In this study, we aimed to evaluate the precision, accuracy and ultimate utility of the ForenSeq UAS, HIrisPlex, and STRUCTURE prediction tools for i3 forensic purposes. A total of one hundred samples obtained from distinct individuals were typed using the Forenseq Signature Prep kit following manufacturer recommendations. This kit targets 22 phenotypic and 56 biogeographical SNPs which are used by the on-board analysis software to predict eye/hair color and ancestry. Sample donors were asked to self-report their natural eye and hair color as well as their ancestry and that of their predecessors. This data served as the ground-truth to establish the accuracy of the EVC and biogeographical ancestry extrapolations. The genetic data was initially analyzed in the Forenseq UAS using the default manufacturer parameters and subsequently used to develop independent predictions from both the HIrisPlex and STRUCTURE software tools. A comparison between the results from each of the tools to each other and from the results of the independent tools to the 'truth' data was performed. The effects of incomplete genotype data on the predictive tools was also assessed by either utilizing samples for which the complete set of phenotypic or ancestry SNPs failed to be developed or by manually excluding information from the generated genotypes. Iris photographs were obtained from individuals whose self-reported and predicted phenotypes were discrepant in order to help resolve the inconsistency or assist with the development of guidelines for the interpretation of the data.

The examined tools were shown to be precise, though not always accurate when predicting eye and hair color. The data also suggested that though the broad biogeographical extrapolations were usually accurate, the same was not true for more refined biogeographical predictions. Given this, it became important to establish a percent error for the results in order avoid providing misleading information to investigators. Finally, it was determined that the predictive algorithms were capable of yielding consistent results whether a full or partial genetic profile was obtained. However, which genetic information was missing was relevant.

P-502

Enhancing Signal Precision in SNaPshot Multiplex Kit Genotyping of IRISPLEX Targets

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Background:
Accomplishing specificity in multiplex polymerase chain reactions (PCR) and single base extension (SBE) reactions poses a significant challenge. Despite meticulous primer design efforts, residual non-specific nucleotide interactions often persist, impacting the accuracy of genotyping results. Using established protocols for genotyping IRISPLEX targets, we encountered multiple non-specific peaks. A maximum of 12 signal peaks were expected, but a total of 23 peaks were observed, suggesting the presence of at least 11 non-specific peaks.

Objectives:
This study aims to address the specificity issues associated with multiplex PCR and SBE reactions, specifically targeting the genotyping process of the IRISPLEX tool. The primary objective is to optimize the genotyping workflow to minimize non-specific peaks while maximizing the strength of signal peaks, thereby improving the reliability and precision of genotyping outcomes.

Materials and Methods:
The study employed established protocols for genotyping IRISPLEX targets using the SNaPshot multiplex assay, which involves the SBE of oligonucleotides targeting specific SNPs within multiplex PCR products. Signal peaks were identified based on comparison with single plex PCR and single plex SBE reactions, while no DNA template multiplex PCR followed by SBE revealed primer-dimers. Optimization strategies included implementing touchdown multiplex PCR (67 °C to 60 °C), varying PCR primer concentrations (0.05 µM to 0.4 µM), and introducing ammonium sulfate (50 mM to 100 mM) to the SBE reaction. The effectiveness of each optimization technique was evaluated by quantifying the reduction in non-specific peaks and assessing the strength of signal peaks relative to standard protocols.

Results and Discussion:
Optimization efforts yielded a substantial decrease (>90%) in non-specific peaks, consequently enhancing specificity within the genotyping process. Techniques such as touchdown multiplex PCR and primer concentration adjustments effectively mitigated non-specific peaks, likely by destabilizing less stable primer-dimer interactions. Moreover, the addition of ammonium sulfate in the SBE reaction further minimized non-specific peaks, possibly by disrupting these interactions. Adjustments in primer concentrations within the SBE reaction compensated for signal loss attributed to ammonium sulfate, ensuring the retention of all signal peaks. These findings underscore the successful optimization of the genotyping workflow, resulting in improved specificity and reliability of IRISPLEX genotyping procedures.

P-503

Determination of Human Facial Morphology: Eye and Forehead Structure

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Objectives

Forensic DNA phenotyping (FDP) is a relatively new branch of forensic genetics that focuses on predicting an individual's physical appearance or biogeographical ancestry using genetic information extracted from DNA samples. FDP holds significant potential in criminal investigations where traditional methods, such as eyewitness accounts or conventional DNA profiling, may be insufficient. The main objective of this study is to identify SNP loci that play a crucial role in determining the shape of the eyes and forehead, highlighting individual variations, which can aid in identifying suspects.

Material-Methods

Blood and/or swab samples were collected from 100 unrelated individuals aged 25-45 who consented to DNA analysis. The individuals had no facial trauma or aesthetic procedures. Standardized photographic documentation was taken under optimal conditions, including frontal and lateral views. Facial measurements were conducted using ImageJ2 software. A panel of 20 highly correlated SNP loci associated with eye and forehead morphology was selected following a literature review. The SNaPshot® methodology was used to analyse the panel. Using SSPS software, a multinomial logistic regression analysis will be conducted to examine the relationships between morphological traits and genotypes, revealing variations that can be attributed to different genotypes.

Result and conclusion

This study successfully assessed individuals' genotypes and phenotypic characteristics using SNP markers specific to eye and forehead regions derived from blood/buccal swab samples. Optimization and validation of the 20 SNP panel were successfully made. The population study, which will be conducted using the developed panel, aims to create a model for generating a molecular facial composite in cases where suspects are absent and missing persons need to be identified. This innovative approach holds potential for aiding investigations in cases where suspects are absent and in the detection of missing persons, offering a promising avenue for enhancing forensic identification methods.

P-547

The role of NGS mitogenomes and DNA phenotyping in the forensic investigation: casework of newborn abandoned in a dumpster

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Inside a large bag collected in a dumpster, a newborn was found, noticed due to the cries heard by passersby. When discovered in May 2013, the female infant, still alive, was wrapped in several towels soiled with widespread bloodstains. Additionally, the placenta and umbilical cord were also recovered near the baby. At first, the placenta samples from the mother's side were analyzed to genotype autosomal DNA profile and mtDNA haplotype using the Sanger method for the HVS-I and HVS-II regions to determine the potential ancestral background of the unknown mother. The predicted haplogroup based on mutations in the control region was H27, predominantly found in northern Europe. In addition, biogeographic ancestry and DNA phenotyping analysis were performed using the Precision ID Ancestry Panel which targets 165 ancestry-informative autosomal SNPs and the 41 HIrisPlex-S markers respectively, on the Ion GeneStudio S5 Prime System.

The results showed the mother's sample to have a predicted predominant European ancestry with Southwest Asian admixture. To increase informativity, a massive parallel sequencing (MPS) experiment was conducted using the Ion PGM System to analyze the entire mitogenome. The results revealed new mutations in the coding regions, leading to the assignment of a different haplogroup (H2a5), exclusively found in the Franco-Cantabrian area.

The HIrisPlex-S webtool predicted dark brown hair with a light shade, green eye color, and an intermediate skin phenotype.

This casework is an example of the impact of the development of new forensic markers and technologies considering that DNA-based inference of biogeographic ancestry should include ancestry-informative autosomal, Y-chromosomal and mitochondrial DNA markers for inference a person’s subcontinental ancestry.

A combined DNA analysis of externally visible characteristics and biogeographic ancestry can increase the informational yield of a criminal investigation.

Posters Topic 5

Population Genetics

5. Population Genetics

Abstracts Poster

P-050

Exploring STRs as an approach to continental biogeographical ancestry inference

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Bio-geographical ancestry (BGA) inference provides valuable information to guide investigators when there is no database hit, or no suspects are available for comparison. This approach is usually carried out through the analysis of panels that collect different ancestry informative markers (AIMs), centering on SNPs, but also including microhaplotypes or InDels, as markers showing different allele frequencies between populations.

The use of STRs for BGA analysis is limited, due to their higher mutation rate and degree of polymorphism, despite being the standard markers in the forensic genetics field. Nowadays, the arrival of massively parallel sequencing (MPS) and increasingly sophisticated capillary electrophoresis (CE) systems enable larger STR multiplexing capabilities, providing greater discriminatory power while opening new possibilities.

This study aims to explore BGA informativeness for the different STR kits available for human identification, including CE and MPS panels. We applied different population data analysis tools to explore the degree of differentiation at continental level achieved by the panels, using the HGDP-CEPH panel as sample set, and performed a comparative evaluation of the results.

We consider the use of the above kits for dual purposes: individual human identification and initial approximation to a continental bio-geographical ancestry, which can help avoid sample depletion from multiple analyses, or when all DNA has been used and only STR profiles are available. Moreover, STRs - as multiallelic markers - are easier to deconvolute in comparison to AIM-SNPs, prompting the possibility of BGA inference of the major and minor components in simple DNA mixtures.

P-051

Highlighting the Potential of Microhaplotypes for Eastern Asian Ancestry Inference via Machine Learning Approach

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In forensic investigations where there is no match, inferring the ancestral source of DNA evidence recovered from crime scenes can provide critical supplementary information. To aid in such ancestry inference, numerous panels of ancestry informative markers (AIMs) have been developed to cluster populations at the continental level. However, distinguishing biogeographic origins at a finer scale still remains challenging. Microhaplotypes, exhibiting multiple variations within small genomic regions, are highly polymorphic, and thus can be effectively utilized as informative genetic markers for ancestry inference. Here we evaluated the ability of microhaplotypes to infer biogeographic origins at a finer level within East Asia using machine learning methods.

We assessed the capacity of 56 microhaplotypes to discriminate Asian populations into three subgroups (Northeast, Southeast, and Southwest Asian) based on their geographical residence. Genotyping of 376 Asian samples were perfomed on the MiSeq platform with an in-house multiplexing microhaplotype panel developed by Yonsei University, and microhaplotype profiles were generated using open-source tools, STRait Razor and Visual Microhap. The genetic structure of populations was investigated using the STRUCTURE and Harvester software. Additionally, two machine learning classification models, XGBoost and Random Forest, were constructed using Python. The genetic structure revealed three distinct clusters (k=3) based on the microhaplotype profiles. The XGBoost model achieved a mean accuracy of 0.81 ± 0.00, outperforming the Random Forest model model with 0.78 ± 0.02. In both classifiers, the Southwest Asian group was clearly distinguishable from the East Asian populations, with approximately 80% classification accuracy within the East Asian ancestries. Notably, the mh02KK-003 and mh15KK-104 markers were particularly informative for both classifiers. These findings suggest that microhaplotypes, in conjunction with machine learning classification, are promising for distinguish biogeographical origins within Eastern Asia.

P-089

Phylogeography of Y-chromosome haplogroup I-P37.2 in Serbian population groups originating from the Balkan Peninsula

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Objectives: Genetic structure of the contemporary Serbian population was shaped by a long history of turbulent historical and demographical events. The most important migrations of Serbs towards present day Serbia, in the recent history, occurred between the 15th to the 18th century from the regions of Old Herzegovina and Kosovo and Metohija. In the present study, we analyzed 464 Serbian male samples, previously classified as haplogroup I-P37.2, from three geographical regions in the Balkan Peninsula inhabited by Serbs: present-day Serbia, regions of Old Herzegovina and Kosovo and Metohija in order to determine phylogenetic and phylogeographic analysis of haplogroup I-P37.2. Material and methods: These samples were surveyed for a total of 22 binary markers, analyzed in a hierarchical way using single base extension (SBE) reactions, within three multiplexes.

Results and conclusion: Based on SNP typing all samples in the Old Herzegovina and present-day Serbia dataset and 122 out of 128 samples from Kosovo and Metohija were assigned to haplogroup I-L621. Further SNP typing revealed very similar haplogroup distribution in all datasets, with the predominant haplogroup being I-PH908, followed by haplogroup I-Z17855. Further analysis within haplogroup I-PH908 distinguished haplogroup I-FT14506 as the most frequent in the Kosovo and Metohija dataset, while haplogroup I-FT16449 was the most frequent in the Old Herzegovina dataset. In the present-day Serbia dataset, more equal occurrence of haplogroups I-FT14506 and I-FT16449 was detected, compromising 40.2% and 34.4%, respectively. Low level of differentiation, within haplogroup I-PH908, was detected between all datasets, with the lowest one detected between present-day Serbia and Old Herzegovina datasets and highest one between Kosovo and Metohija and Old Herzegovina datasets. Furthermore, median-joining network analysis and shared haplotypes statistics revealed closer genetic relationship between Old Herzegovina and present-day Serbia haplotypes. Results obtained within this study support the thesis that migrations from historical region of Old Herzegovina and geographical region of Kosovo and Metohija, had great contribution on the present-day Serbian population genetic structure. Furthermore, here presented results, gave insight into geographic distribution of detected haplogroups I-Z17855, I-Y4460, I-PH908, I-Y5596, I-Y4882, I-FT14506, I-FT16449 and I-A5913 and analyzed SNPs, enabling further improvement of the geographic resolution of paternal ancestry inference.

P-090

The genetic male component of Afro-descendant from Pará (Brazil)

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Y-chromosomal markers are widely used in population genetics, to infer paternal ancestry and male-mediated migratory movements between populations, due to their non-recombining nature. For instance, studies of the paternal lineages of South American populations have been useful for building genetic databases that capture their high genetic diversity. Although the majority of the current Brazilian population is admixed, there are native and African-Brazilian communities called indigenous communities and Quilombos, respectively. Quilombos are communities formed by enslaved Africans and their descendants who resisted exploitation during slavery. Currently, Brazil has more than 2.807 communities legally certified as Quilombos, each with a particular history of formation. The present work aimed to increase our knowledge of the origin and diversity paternal lineages of Quilombos from Pará (a State in the Amazonian region of Brazil). In this way, 23 Y-STR markers were characterized in 222 individuals using the PowerPlex® Y23 system. A high haplotype diversity was observed (0.9954 +/- 0.0010). The haplotype data obtained and those available from previous studies of South American populations were compared through genetic distance analysis. This analysis revealed greater proximity between the Pará, Manaus and other admixed populations from Brazil, Colombia, and Afro-descendants from Ecuador, all very close to the Iberian populations analyzed. To infer continental contributions in the population sample, haplogroups were predicted based on haplotypes, using the NEVGEN predictor. The high frequency of European paternal lineages (>65%) detected in the present study was also observed in other studies of admixed populations from South America. However, contrary to expectations, given the history of the formation of Quilombos and their supposed isolation, paternal lineages of Native American origin were more frequent than those from Africa (~21% and >13%, respectively).

P-091

The Qatari population’s genetic structure and gene flow as revealed by the Y chromosome

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The Y-chromosome is widely used in forensic genetics and human population studies due to its uniparental origins. We conducted a study on the Qatari population, comparing it to databases from the Arabian Peninsula, the Middle East, and Africa. Using the PowerPlex Y23 kit, we genotyped 379 unrelated males from Qatar, a country at a migration crossroads. The most polymorphic locus in the kit was DYS458, with a genetic diversity value of 0.85 and a haplotype diversity of 0.998924. We used Athey's Haplogroup Predictor tool to predict haplogroups in the Qatari population. The dominant haplogroup J1 (49%) in Qatar formed a star-like expansion cluster in a median-joining network. The Q-matrix, developed using Y-STR data from 38 Middle Eastern and 97 African populations, revealed stronger sub-groupings within each ethnic group and the impact of Arabs on the indigenous Berbers of North Africa. Bayesian coalescence theory in the Migrate-n program estimated the migration rate between Qatar and other Arabian populations, with the main route being from Yemen to Kuwait through Qatar. This research, utilizing the PowerPlex Y23 database, highlights the significance of gene diversity, regional and social structures, and their impact on demographic and forensic databases.

P-092

Mutation Rates of 23 Y-Chromosome Short Tandem Repeats (Y-STRs) in an Ecuadorian Male Population

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The Y chromosome Short Tandem Repeats (Y-STRs) represent a crucial tool in the fields of paternity testing, forensic science, ancestral characterization, and individual identification. These genetic markers specifically target male DNA, allowing for precise discrimination and detection (1,2). The present study was focused on an Ecuadorian population sample to elucidate the mutation rate of commonly used Y-STRs. Blood samples were collected from four hundred father-son pairs who agreed to participate in genetic population research. The samples were processed using 23 Y-STRs according to the manufacturer protocol, and the resulting data was analyzed to report the mutation rates within this specific population. The analysis revealed notable mutation events across several Y-STRs markers, including DYS385, DYS389, DYS391, DYS438, DYS439, DYS448, DYS456, DYS458, DYS570, DYS576, and DYS635. Interestingly, a higher mutation rates were observed compared with a previous study (3). Moreover, one father-son pair exhibited two mutations in DYS448 and DYS389 II. In conclusion, Y-STRs play a pivotal role in genetic investigations, particularly in paternity testing. Our Ecuadorian population study sheds light on mutation rates to interpret Y-STR mutation events accurately.

1. Gaviria A, Sánchez ME, Morejón G, Vela M, Aguirre V, Burgos G, et al. Characterization and Haplotype analysis of 11 Y-STR loci in Ecuadorian population. Forensic Sci Int Genet Suppl Ser. 2013;4(1):e310–1.
2. Toscanini U, Gaviria A, Pardo-Seco J, Gómez-Carballa A, Moscoso F, Vela M, et al. The geographic mosaic of Ecuadorian Y-chromosome ancestry. Forensic Sci Int Genet. 2018 Mar 1;33:59–65.
3. Sánchez ME, Burgos G, Gaviria A, Aguirre V, Vela M, Leone PE, et al. Y STRs mutation events in father-son pairs in Ecuadorian individuals. Forensic Sci Int Genet Suppl Ser. 2015 Dec 1;5:e310–1.

P-094

Genetic characterization of Y-chromosomal STRs in Santander department of Colombia.

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1. Objetives
The use of uniparental markers such as those located in the non-recombinant region of the Y chromosome can be very useful to solve some forensic cases. Namely, Y-STRs are relevant in investigations of sexual assaults since autosomal markers can be difficult to analyze in evidence with mixed contributions from the victim and the aggressor. Despite its importance, the use of Y-chromosomal markers in Colombia is limited. Colombia is an admixed population with a high ancestry variation, where the Native, European, and African contributions vary widely among population from different regions. Nevertheless, there is a lack of Y-STR haplotypic databases representative of most country regions. Moreover, the few haplotypic profiles in the YHRD only include 17 Y-STRs, with no data available for the most recent kits. These population databases are important for understanding the genetic diversity of the country and its substructure, as well as to estimate the Y-haplotype frequencies in each region. The aim of this study was to contribute with data from 97 unrelated individuals born in the department of Santander (northeast Colombia).

2. Materal and methods
Samples were typed for 23 Y-STR markers using the PowerPlex® Y23 system, following the manufacturer's instructions. Haplotype frequencies and parameters of forensic relevance were determined.

3. Results and conclusions
A total of 96 haplotypes were found in our sample, of which 95 were unique. A high haplotype diversity was observed 0.9998 ± 0.0015. Haplogroup predictions based on Y-STR haplotypes (using the NevGen tool) point to the prevalence of European haplogroups (85.6%), followed by Native American haplogroups (13.4%). Only one haplotype was assigned to a sub-Saharan African haplogroup.

P-095

Exploring Y-DNA haplogroup diversity in the British Isles

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The Yfiler™ Plus PCR Amplification Kit is a Y-chromosome STR kit that facilitates specific amplification of male samples. This feature renders it particularly useful in sexual assault cases and paternal lineage studies. Furthermore, its high inter-population variability makes it ideal for investigating biogeographical differences and migration patterns. Consequently, this kit was utilised to examine the haplogroups of English, Scottish, Welsh, and Irish populations to support the development of population-specific Y-STR databases and advance our understanding of the genetics of the British Isles population.

Buccal swabs were collected from 888 unrelated male individuals; 461 English, 112 Scottish, 124 Welsh, and 191 Irish. The DNA was extracted using Prep-n-Go™ Buffer and amplified using the Yfiler Plus kit. The obtained data passed the YHRD quality checks and were uploaded to the database. The haplogroups were determined using the Y-DNA Haplogroup Predictor NevGen online tool. Haplogroup diversity was calculated using Excel as $n(1-\sum pi^2)/(n-1)$.

A total of 46 haplogroups were observed, with R1b M269 being the most common haplogroup in all populations (64% in English, 73% in Scottish, 93% in Welsh, and 85% in Irish). I1 was the second most common haplogroup in both the English (13%) and Scottish (6%), while I2a2a M223 was the second most common in both the Welsh (2%) and Irish (6%). R1a M198 and E1b1b > V13 were the other two most observed haplogroups overall, while the remaining 41 were found less than 5 times across all the tested population samples. Although the Scottish population showed an increased number of haplogroups in proportion to the samples, greater diversity was observed in the English (0.57) and Scottish (0.46) compared to the Irish (0.28) and Welsh (0.14). This study highlights the differences between the 4 populations, although these observations should be confirmed with larger sample sizes.

P-096

Haplogroup-specific differences in mutation rates and detected polymorphisms of 30 RM Y-STR loci in father-son pairs from Serbia

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Objectives: Rapidly mutating Y-chromosomal short tandem repeats (RM Y-STRs) are a powerful tool in forensic genetics, anthropological genetics and genetic genealogy. Due to their high mutation rates (≥ 10e-2 per locus per generation), they are expected to improve male individualization in unbalanced male-female mixtures in criminal cases, even when they include close male relatives. However, little is known about the extent to which the frequency of mutation events, at these markers, correlates with particular haplogroup background. Some studies have identified allele size distribution as the most important cause of variation between haplogroup-specific mutation rates. The aim of this study was to investigate the haplogroup-specific background of RM Y-STR mutability and to determine differences in average mutation rates between the three most prominent Y-tree branches in father-son pairs from Serbia.

Material and Methods: We performed an analysis of 30 RM Y-STRs included in RMplex in 415 genetically confirmed father-son pairs from Serbia. To determine Y-chromosomal haplogroup membership, samples were genotyped with Y-STRs from the commercial kit Investigator Argus Y28. Results and Conclusion: The average RM Y-STR mutation rates for the three most frequently detected branches of the Y-tree in the Serbian sample (I&J - 13.3x10e-3, E - 11.1x10e-3, R - 11.6x10e-3) showed no statistically significant difference, neither in comparison to each other nor to the overall mutation rate (13.1x10e-3). However, a separate analysis of locus-specific mutation rates and mean allele sizes revealed a certain discrepancy depending on haplogroup affiliation. In addition, the occurrence of non-consensus polymorphisms, such as microvariant alleles (DYS1003, DYS1005, DYS1010, DYS1007 and DYS547) and multiallelic patterns (DYS93S1, DYR88 and DYS724), showed a clear haplogroup-specific distribution. This study provides the first report of comprehensive analyses of haplogroup-specific RM Y-STR mutation rates performed on Serbian samples. According to our results, the average haplogroup-specific mutation rates in the Serbian samples did not differ significantly, suggesting that differences in mean allele sizes have moderate effects on Y-STR mutability. However, newly discovered microvariant alleles and multiallelic polymorphisms detected with high frequency in certain haplogroups might provide more information about the mutational capacity of RM Y-STRs and their haplogroup context

P-097

A Study of the X-chromosome's Role in Population Genetics on a Worldwide Scale

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X-chromosome short tandem repeats (X-STRs) are a valuable tool in population genetics, anthropology, and complex kinship analyses. The genetic makeup and population differentiation of 46 different ethnic groups (36 populations) were studied. To show the corrected genetic differences within and between groups, average pairwise distances were computed. The FST/FSTmax ratio was used to ascertain the ancestral makeup of the five principal geographic regions, which are: Africa, the Middle East, East Asia, Europe, and South America. The findings of this analysis revealed that the Middle Eastern populations exhibited the lowest the FST/FSTmax ratio, measuring at 0.23243, indicating a relatively lower ancestral diversity. Conversely, the European populations showcased the highest the FST/FSTmax ratio, measuring at 0.27122, indicative of a greater ancestral diversity within this region. Additionally, the allelic richness indicators, namely distinctive and private alleles, indicated that Africa and the Middle East displayed the highest levels, while Far East Asia exhibited the lowest. This analysis supports the hypothesis of repeated founder effects during outward migrations, as evidenced by both the ancestry variability and the allelic richness. Consequently, the findings of this study have important implications for forensic genetics and population genetics research, particularly in relation to the consideration of genetic predispositions within specific ethnic groups.

P-099

Exploring the Genetic Landscape: Population Diversity of Rapidly Mutating Y-STRs for the Five Major Ethnic Groups in Ghana Using the Novel 26 RM Yplex Kit

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1. OBJECTIVES

This research aims to explore the genetic landscape of the five major ethnic groups in Ghana, focusing on the population diversity of Rapidly Mutating Y-STRs using the Novel 26 RM Yplex Kit.

a. Evaluate Population Diversity and Haplotype Distribution:
   i. Assess the population diversity of Rapidly Mutating Y-STRs across the aforementioned ethnic groups, generating haplotype data through the 26 RM Yplex Kit.
   ii. Examine the distribution of Y-STR haplotypes within each ethnic group, identifying unique patterns that contribute to the overall genetic diversity

b. Compare Ethnic Groups for Genetic Relationships:
   i. Conduct a comparative analysis of Rapidly Mutating Y-STR profiles among the five major ethnic groups, emphasizing distinctive genetic signatures.
   ii. Explore genetic relationships and differences between ethnic groups through comprehensive Y-STR variation analysis.

c. Validate 26 RM Yplex Performance and Genotyping:
   i. Validate the performance of the 26 RM Yplex Kit in accurately capturing the variation in Rapidly Mutating Y-STRs within diverse Ghanaian populations.
   ii. Assess the sensitivity, specificity, and reproducibility of the kit in detecting and genotyping Y-STR markers.

2. MATERIALS AND METHOD

435 samples from unrelated males representing the Akan, Mole-Dagbon, Ewe, Ga-Dangme, and Guang ethnic groups using the 26 RM Yplex Kit will be genetically characterized using the PrepFiler Express kit and the AutoMate Express Forensic DNA Extraction System (Thermo Fisher Scientific) and quantified using the Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific) using the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific). Haplotype data will be generated to assess population diversity and distribution within each ethnic group. Comparative analysis will be conducted to explore genetic relationships and differences among the ethnic groups.

RESULTS & CONCLUSION

Results: Anticipated outcomes include the elucidation of population-specific Y-STR haplotype distributions, identification of unique genetic signatures, and comparison of genetic relationships among the five major ethnic groups in Ghana. This comprehensive analysis will provide insights into the genetic diversity and population structure of Ghana's diverse ethnic groups.

Conclusion: Integration of the 26 RM Yplex Kit into our research promises to provide valuable insights into the genetic diversity of Y chromosome markers, contributing to the reliability of forensic investigations and enhancing our understanding of population genetics in Ghana. This study not only aims to advance forensic genetics methodologies but also to deepen our knowledge of the genetic landscape of Ghana's diverse population, potentially informing future forensic investigations and population studies in Ghana.

P-101

Characterization of 94 Identity-Informative SNPs in a North-Eastern Italian population using ForenSeq™ DNA Signature Prep Kit

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Genotyping of identity-informative single nucleotide polymorphisms (iiSNPs) by massively parallel sequencing (MPS) provides information on the sequence variations characterizing individual populations, which supports the capillary electrophoresis (CE) analysis of short tandem repeats (STRs) in some challenging forensic cases. Nonetheless, population data on iiSNPs are relatively scarce, especially concerning the Italian population.

Using the MPS technique, 93 unrelated native subjects from North-East Italy were genotyped in this study. The ForenSeq™ DNA Signature Prep kit that amplifies 94 iiSNPs (Verogen - Qiagen) was used for library preparation. Sequencing was performed using the MiSeq FGx™ System (Illumina), and data analysis was carried out with Universal Analysis Software (UAS) v1.2. Subsequently, the output FASTQ files were reanalyzed using STRait Razor v3.0 (SR3) to confirm UAS's target iiSNP genotypes and reveal sequence variations. Furthermore, allele frequencies and relevant forensic statistic parameters were calculated.

Compared to 8742 expected call genotypes for the 93 subjects typed with the set of 94 iiSNPs, 8571 genotypes were obtained (corresponding to a call rate of 98%) with a failure of 171 genotypes. Examining the read depth, it has been revealed low sequencing coverages for rs1736442 and rs1031825, that were genotyped in only 60 and 66 subjects, respectively.

No discrepancies in target iiSNP genotypes called by UAS and SR3 were found when the number of reads for each iiSNP met the fixed detection thresholds for both software.

SR3 identified in 32 out of the 94 iiSNPs (equal to 34%) microhaplotypes (MHs), characterized by further SNPs in the flanking region of the target SNP. Ten of these iiSNPs presented two or three unique MH alleles, while the remaining 22 loci each had one unique MH allele. In addition, eight loci had singleton MHs for which SR3 failed to identify flanking SNPs, probably because the nucleotide mutations observed in this study were not present in its sequence string-matching database.

A total of 44 unique MH alleles were detected, of which six have never been reported previously in the literature. The highest number of MHs were revealed at the rs4606077 and rs727811 loci, with frequency values of 97.80% and 100%, respectively.

Overall, the occurrence of the 966 genotypes with MH alleles converts the SNP polymorphism from a biallelic to a multiallelic marker, which, in this study, generated a 23% increment in the typologies of unique sequences found, leading to an increase in the discrimination power of the panel of 94 iiSNPs.

P-103

Investigation to the mutation of 26 RM Y-STRs based on Han population in Sichuan, China

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Objectives: With mutation rates ≥10e-2, rapidly mutating Y-STRs (RM Y-STRs) revealed better polymorphism compared to Y-STRs with lower mutation rates (<10e-2), which could be used to improve the power of discrimination when male individuals in the same paternal lineage been involved. In this study, mutation rates and father-son discrimination rates of 26 RM Y-STRs (DYS627, DYS576, DYF387S1, DYS518, DYS570, DYS449, DYF404S1, DYF399S1, DYS547, DYS526a/b, DYS626, DYF403S1a/b, DYS612, DYS1003, DYS1007, DYS712, DYS711, DYS724, DYF1002, DYR88, DYS713, DYF1001, DYF1000, DYS1012, DYS1010) have been investigated based on 100 confirmed father-son pairs from Sichuan Han population. Materials and methods: 100 father-son's fingertip blood have been collected for the DNA information after full ethic review by the committee of the school and informed consent to each individual. The amplification of the 26 RM Y-STRs was performed using a kit and PCR primer sequences as reported, followed by electrophoresis using the 3500 Genetic Analyzer.

Results and conclusions: A total of 49 mutations have been observed, with 47 one-step mutations (95.9%) and 2 two-step mutations (4.1%). The mutation rates ranged from <10.0×10e-3 to 50.0×10e-3, with an average mutation rate of 18.8×10e-3. RM Y-STRs DYS712 and DYF1001 indicated the highest mutation rates among the 26 ones, while no mutation was observed in DYS1007, DYS626, and DYF404S1. The father-son discrimination rate of the 26 RM Y-STRs in Sichuan population (42%) was lower than that reported in Europeans (44%). Our data of the 26 RM Y-STRs suggest a promise in identifying Chinese male individuals in the same paternal lineage.

P-104

Rapidly mutating Y-STRs implemented in the RMplex tool significantly improve discrimination capacity in clan-structured populations

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Y chromosome STRs are mainly used in cases of sexual assaults, where the presence of male DNA from the perpetrator might be significantly overshadowed by the female DNA of the victim. The low discrimination capacity (DC) of Y-STRs, mainly due to the lack of Y chromosome recombination, has been partially overcome by introducing a class of STRs characterized by a higher mutation rate (RM Y-STRs). However, the discrimination capacity of RM Y-STRs in populations structured into patrilineal clans, where low paternal diversity is expected, has not yet been thoroughly investigated.
As a case study, we analyzed six clans of Pakistani Pashtun (N = 583), characterized by strong stratification into patrilinear groups.

Using the commercial Y-STR multiplex Yfiler Plus, encompassing 25 loci including 6 RM Y-STRs, we observed a surprisingly low discrimination capacity (DC = 0.782), with one-third of males sharing haplotypes (186 males, 31.9%). At the clan level, the DC ranged from 0.527 in the Bettani to 0.908 among the Yousafzai. Haplotype sharing between males belonging to different clans occurred only once, with a strong inter-clan diversity (RST = 0.350).

Pashtun males sharing Yfiler Plus haplotypes were further analyzed using 30 RM Y-STRs included in the recently developed RMplex tool (Ralf et al. 2021). Using this multiplex, we were able to discriminate about 90% of the residual males. Specifically, the number of shared haplotypes decreased from 60 to 8, and the number of males still sharing haplotypes dropped from 186 to 19 (3.3% of the global sample of 583 subjects), resulting in an increase in DC from 0.782 obtained with the Yfiler Plus to 0.981.

Our study encourages the forensic Y-chromosome community to apply the RMplex tool for male differentiation, especially in challenging scenarios where the Y chromosome diversity is expected to be reduced due to factors such as isolation, patrilinearity, and/or genetic drift.

P-105

Rapidly mutating Y chromosomal STRs in deep-rooted endogamous pedigrees from punjab pakistan

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Y-chromosome short tandem repeat polymorphisms (Y-STRs) are important in many areas of human genetics. Y chromosomal STRs being normally utilized in the field of forensic exhibit low haplotype diversity in endogamous populations and fail to discriminate among male relatives from same pedigree. Rapidly mutating Y-STRs (RM Y-STRs) have been paid much attention in last decade. These 13 RM Y STRs have high mutation rates (>10-2) and have considerably higher haplotype diversity and discrimination capacity than conventionally used Y-STRs showing remakeable power, when it comes to differentiation in paternal lineages in endogamous populations.Previously, we have analyzed 2–4 generation, 99 pedigrees covering 1568 pairs of men covering 1–6 meioses from all over Pakistan and 216 male relatives from 18 deep rooted endogamous Sindhi pedigrees covering 1-7 meioses. Here we are presenting 861 pairs of men from 63 endogamous pedigrees covering 1-6 meioses from Punjabi population of Punjab, Pakistan. Mutations were frequently observed at DYF399 and DYF403 while no mutation was observed at DYS526a/b. The rate of differentiation ranged from 29.70% (first meiosis) to 80.95% (fifth meiosis) while overall (1 to 6 meiosis) differentiation was 59.46%. Combining previously published data with newly generated data, an overall differentiation rate was 38.79% based on 5176 pairs of men related by 1–20 meioses, while Y-filer differentiation was 9.24% based on 3864 pairs.Using father-son pair data from the present and previous studies, we also provide updated RM Y-STR mutation rates.

P-106

FORENSIC CHARACTERISTICS Y-STRs IN THE SERBIAN POPULATION USING THE NOVEL 26 RT Y-STRs MULTIPLEX ASSAY

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Recent research with Rapidly Mutating Y-STRs (RM Y-STRs) have shown that these loci provide substantially higher haplotype diversity and haplotype discrimination capacity in worldwide populations when compared with the Y-STRs commonly used in genetic forensics. The discovery of these markers happened in two stages, first stage was in 2010 when 13 markers were discovered, whereas the second stage was the reveal of 12 additional RM Y-STRs in 2021. In our earlier studies, we have estimated a mutation rate for 13 RM-YSTR in 85 pairs of male relatives in the population of Serbia (74 father-son pairs, and 11 twin pairs) to evaluate the capacity of distinguishing between male subjects within a single lineage. In this study, a total of 210 male individuals' samples from population of Serbia were analyzed using 26 RM-Yplex. The assay was developed and validated to amplify 26 markers on Y-Chromosome consisting of 17 single-allelic markers and 10 multi-allelic markers, brining the total number of amplified alleles to 40. The same set of samples was analysed using previously designed 13 RM Y-STR multiplex assay. Therefore in this study we will demonstrate the impact of the additionally released 12 RM Y-STRs compared to the previously revealed 13 TM-Y-STRs and also we will be able to assess the efficiency of the newly designed 26 RM-Yplex assay in differentiating close male relatives based on the analysis of the 26 RM Y-STRs simultaneously. Our findings are encouraging and concur with previous studies showing that by RM Y-STR typing the discrimination power of close male relatives could be considerably increased in comparison to every Y-STR markers commonly used in forensic genetics.

P-108

Molecular diversity of 23 Y-STR Genetic Markers in Endogamous Ramgharia Population of Punjab, India

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Background: In forensic investigations and population studies, Y-STR analysis has been shown to be quite useful. The present study aimed to evaluate genetic polymorphism in Ramgharia population of Punjab. Two hundred unrelated healthy male individuals were typed using the Powerplex® Y-23 PCR amplification kit.

Results: A total of 170 haplotypes were obtained among which 146 were found to be unique. Multidimensional scaling (MDS) Plot was used to study the Intra-population relationship between the studied population data and other reported populations of India, which revealed that Ramgharia population of Punjab lies in a cluster with the Bhil Population of Madhya Pradesh. A total of 140 different alleles were reported with allelic frequency ranging from 0.006 to 0.765. The genetic diversity for 23 Y-STRs ranged from 0.375 (DYS391) to 0.836 (DYS635).

Conclusion: This 23 Y-STR marker data can be used as reference for determining genetic relatedness among various Indian populations and with the other populations worldwide. Overall, the data obtained will enrich the forensic database of endogamous populations of India.

P-109

GENETIC DIVERSITY AND HAPLOTYPE ANALYSIS OF THE YOUSAFZAI TRIBE IN SWAT DISTRICT PAKISTAN

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This study delves into the genetic characteristics of the Yousafzai tribe residing in the Swat district, with a specific focus on the mtDNA. A cohort of 400 unrelated individuals was subjected to molecular and bioinformatics analysis. The predominant haplogroup identified was H2, accounting for 35% of the individuals, followed by haplogroup H1 at 15%, and a distinct haplotype associated with haplogroup H observed in 12.5% of the samples. The observed distribution of haplogroups paints a genetic mosaic of Yousafzai ancestry, showcasing influences from West Eurasia, South Asia, and a limited representation from East Asian haplogroups. Notably, the study unveils 11 new haplotypes not previously reported in Pathan populations. The findings contribute valuable insights into the genetic diversity and relationships within the Yousafzai tribe. This research goes beyond the specific population under investigation, offering significant implications for understanding broader tribal affinities within and across global populations. The insights garnered from this study hold relevance for disciplines such as anthropology, population genetics, and the exploration of human migration and evolution, making it a noteworthy contribution for presentation at this conference.

P-110

Reconstructing the genetic admixture history of Roma people: Insights from genome-wide SNP data from Punjab, Pakistan.

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This study presents a comprehensive genetic analysis of the Roma population in Punjab, Pakistan, aiming to elucidate their complex admixture history and migratory patterns. Utilizing genome-wide SNP data, we conducted an array of genetic analyses including PCA, STRUCTURE, f3, f4, qpAdm, qpWave, and uni-parental markers mtDNA and Y Chromosome analysis to investigate the genetic diversity and ancestral origins of the Roma. Our findings highlight significant admixture events, showcasing a rich mosaic of South Asian, West Eurasian, and additional ancestries that correlate with historical migrations from the Indian subcontinent through West Asia into Europe. Notably, the analyses reveal substantial contributions from Anatolian and Caucasus Hunter-Gatherer ancestries, alongside evidence of interactions with European groups, as indicated by the presence of Iberian Hunter-Gatherer genes. The mtDNA and Y Chromosome analyses provide further insights into the maternal and paternal lineages, supporting the hypothesis of an Indian subcontinent origin while also revealing diverse interactions along their migratory routes. Our results contribute to a deeper understanding of the Roma's genetic legacy, reflecting their unique position as a bridge between disparate populations and highlighting the complex interplay of genetics, history, and migration. This study not only adds to the growing body of knowledge on the genetic diversity of the Roma but also underscores the importance of comprehensive genetic analyses in uncovering the intricate history of human populations.

P-130

Pairwise kinship testing of first-degree to seventh-degree relatives using 5940 SNPs

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Objetives:
Single nucleotide polymorphism (SNP) has been used by many scientists to infer distant degree relatives because of their wide distribution in the genome and low mutation rate. Previous studies have shown that an increase in the number of SNPs can have a positive effect on the accuracy of the inference of relatives and the level of inference of distant relatives. Hybridization capture technology can enrich a large number of SNP sites and is suitable for degradation sample, which can be used in forensic pairwise kinship inference.

Material and methods:
In this study, SNPs were detected using the xGen™ Human Copy Number Variant (CNV) Backbone Hybridization Panel combined with next generation sequencing technology. The LR and IBS methods were used to analysis the effectiveness of the kit in the inference of distant degree relatives.

Results and conclusions:
This enabled over 7,000 SNP sites to be sequenced at high quality across the genome. A total of 80 samples from two families were tested in this study, including 1st to 7th degree relatives. In this study, we investigated the efficacy of 5,940 SNPs to infer distant degree relatives. The panel performs well in predicting 1st to 4th degree relatives, while the prediction accuracy was underperformed for relatives of 5th to 7th degree. We also provided methods and results for the application of hybridization capture combined with next generation sequencing technology to infer distant degree relatives.

P-162

oYSTR: A new Y-chromosomal ancestor estimator to improve speed, accuracy and versatility for forensic Y-DNA kinship investigations

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The Y-chromosome serves as a valuable indicator of paternal kinships in forensic DNA investigations. To reconstruct genealogies, the time to the most recent common ancestor (tMRCA) among paternal relatives can be estimated through in-depth analysis of short tandem repeats (Y-STRs). The previously developed 'YMrCA' calculator has taken a remarkable advancement by including individual and haplogroup-specific Y-STR mutation rates, while accounting for multi-step changes and hidden, back, or parallel mutations. After validation, the YMrCA demonstrated a significantly improved success rate (96%, +20%) and reduced generation error (7 generations, -3) compared to state-of-the-art kinship models. However, computational demands leading to lengthy runtimes (30 minutes per genealogical pair) and the absence of sequencing data integration, motivated us to enhance the existing calculator. A groundbreaking collaboration between CSY-Belgium (KU Leuven) and the Netherlands Forensic Institute, has resulted in the development of a novel kinship estimating tool, named ‘oYSTR’ (Objective Y-Simulator Towards Relatives). This advanced prediction model seamlessly integrates the ability to include hundreds of Y-STRs, allele-specific mutation rates as well as Y-STR motif-specific mutation rates, and even Y-STR sequence data. Despite the integration of these additional Y-characteristics, oYSTR is capable of estimating kinships for hundreds of generations without requiring a supercomputer. To do so, the calculator uses discrete convolutions, automatically accounting for parallel mutations. The oYSTR prediction tool was validated using our extended CSY-database containing 1,120 biologically related males confirmed by 46 Y-STRs (capillary electrophoresis) covering 18,109 generations in total. Promising results showed that oYSTR estimates 160,000 times faster than YMrCA with a runtime of 0.016 seconds per genealogical pair. oYSTR successfully provided tMRCA estimations for all genealogical pairs, demonstrating its ability to effectively estimate kinships between males with a high number of Y-mutations and/or larger multi-step mutations. Currently, in terms of tMRCA estimation accuracy, we observed no significant difference with the state-of-the-art YMrCA calculator. However, further in-depth analysis of Y-STR mutation rate characteristics and inclusion of genealogical pair Y-STR sequence data remains necessary to investigate the impact on tMRCA estimation accuracy. Given its dynamic, rapid, and user-friendly nature, oYSTR stands as a future-proof calculator, ready to integrate additional Y-STR mutation rate data as they become available. With more accurate knowledge, for example on allele-specific mutation rates, complex Y-STR motif mutation rates and even haplogroup-specific mutation rates, oYSTR will be able to estimate kinships closer to reality. Therefore, oYSTR offers the next step towards more objective tMRCA estimation for forensic DNA kinship investigations.

P-205

Genome-wide association study of substance use disorders

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Substance use disorders (SUDs) are neuropsychiatric disorders where there is compulsive reiteration in taking certain substances, regardless of any disruptive outcome. SUDs imply both abuse and dependence and have become of acute, worldwide public health concern, with huge socioeconomic burden.

Genetic determinants have a part in SUD etiology. Twin and family studies have already demonstrated strong familial inheritance patterns of certain SUDs. More recently genome-wide association studies (GWAS) with massive parallel sequencing (MPS) have become the current standard used to address the case of genetic variants associated with SUDs.

Identifying additional markers may be of great help in preventing and possibly treating SUD.
We here report on a multidisciplinary project enrolling several genetics and forensic toxicology laboratories in whose context a pilot association study was carried out in order to find SUD-linked phenotypes in the Galician population. All samples were genotyped with the “Axiom Spanish Biobank” array, which contains 757,836 markers.

As a result of our study we found a variant showing statistically significant association ($p < 5x10^{-8}$) with SUDs in the SMOC2 gene. We can also report the case of several other implied variants ($p<5x10^{-6}$) within genes that are already known to correlate for the disorder. The whole of our study strongly supports the assumption that SUD has a strong background of genetic predisposition. We plan to carry out further studies, enlarge the database and possibly investigate the underlying genetic pathways and neurobiological mechanisms.

P-206

Unusual phenomena of 12058 individuals DNA profiling using commercial “A” reagent kit

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The Biology and DNA Subdivision, which is a laboratory of the Central Police Forensic Science Division under the Royal Thai Police, is responsible for testing DNA evidence from crime scenes and people using autosomal Short Tandem Repeat (STR) in DNA comparison testing. STR is used to link evidence to evidence or evidence to a person. The current testing loci, according to CODIS 2017, are 20 loci. For human DNA testing, the laboratory performs the commercial “A” reagent kit that has 24 testing loci: Amelogenin X, Amelogenin Y, DYS391, D1S1656, D2S441, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1248, D12S391, D13S317, D16S539, D18S51, D19S433, D21S11, D22S1045, CSF1PO, FGA, SE33, TH01, TPOX, and vWA. Those testing loci are stored in the DNA database system. Unusual phenomena, such as the occurrence of additional alleles and the disappearance of alleles, can occur in each DNA test. Therefore, the researcher is interested in the unusual phenomena in the commercial “A” test kit. The purpose of the research is to determine the number of unusual phenomena occurring with the commercial “A” reagent kit. The research was conducted by testing the DNA of 12,058 people using the commercial “A” reagent kit. The test results found that there were two unusual phenomena. The first one was that the allele of the locus D1S1656 had disappeared, but it was shown in the locus DYS139. Another one was that the alleles of locus DYS139 were missing while they were displayed at locus TPOX. The first unusual phenomenon was by 114 people (0.95% of 12,058 persons), which were 80 Thai and 34 Myanmar. The second phenomenon was conducted with 12 people (0.10% of 12,058 persons), of whom 11 were Thai, and one was from Myanmar. Both unusual phenomena may be caused by the absence of an allele, which is a locus D1S1656 and locus DYS139, and the presence of an allele, which is a locus DYS139 and locus TPOX. These phenomena may occur due to the lack of a supporting bin. The research findings show that it is necessary to be careful when interpreting DNA using commercial kits.

P-209

A landmark African achievement: First-time insight into forensically relevant STR data for the South African population using massively parallel sequencing

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South Africa is home to both an exceedingly high crime rate and a public health crisis regarding the number of bodies left unidentified. Human identification in South Africa has primarily relied on traditional DNA profiling methods, known for its limitations when processing challenging samples, and the emergence of massively parallel sequencing (MPS) has overcome these limitations. With forensic laboratories in developing regions showing proclivity towards a seemingly impossible adoption of MPS, sequence-based population databases are sorely needed to leverage emerging advancements.

This study characterised sequence data for the Black African (n=216) and Admixed (n=247) populations in South Africa using the ForenSeq™ DNA Signature Prep Kit for 27 autosomal short tandem repeat (STR) markers on the MiSeq FGx™ system. Alleles were characterised using a short designator naming system in collaboration with Kings College London. In the Black African population, a 70% increase was observed in the number of length- to sequence-based alleles (295 to 500), with 52 alleles observed through flanking region sequences. In the Admixed population, an increase of 81% was observed from length- to sequence-based alleles (327 to 591). Flanking region assessment resulted in an added 59 alleles. Length-based random match probability decreased from 3.88E-28 and 3.04E-29 to 4.17E-33 and 1.93E-34 when including sequence data for Black African and Admixed populations respectively.

In both populations, 81 previously undescribed alleles were observed. Novel findings included the presence of previously undescribed flanking region variation in the FGA marker. In conservative markers such as CSF1PO, the first and only microvariant sequence for CSF1PO was characterised. The novel findings and richness of variation observed in South African populations exceeds what has been previously characterised and warrants further research into characterising additional forensically relevant markers.

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AFRICAN GENETIC AND LINGUISTIC ORIGINS OF PALENQUE, COLOMBIA

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The Colombian town of San Basilio de Palenque, established as a refuge for enslaved Africans more than 400 years ago, has maintained African culture and traditions that include the Afro-Spanish Creole language Palenquero. It was previously discovered that Palenquero has a single African substrate: the Yombe language of the Pointe-Noire region in the Republic of the Congo (Ansari-Pour, N. et al., 2016). We characterized the African subcontinental origins of Palenque individuals through fine-scale analysis of WGS data from Palenque donors along with African reference. While individuals from Palenque show the highest levels of Bantu (Loango Coast) ancestry, it is also clear that they have substantial contributions from all three of the African regions that participated in the transatlantic slave trade to Colombia: 51% Bantu, Loango Coast, ancestry, 35% of Bight of Benin ancestry, and 14% of Senegambia ancestry. Palenque also shows substantial genetic diversity within the Bantu genetic ancestry component. The Yombe language corresponds to the Kongo-like population group that constitutes 25% of the Palenque ancestry, while the remaining Bantu population groups represent 26% of the Palenque ancestry. With our results we could conclude that the African origins of the Palenque population is actually more complex than previously imagined, with contributions from multiple African regions. Our results could also be used to motivate further studies and potentially new discoveries about the African substrate of the Palenquero language.

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Genetic Relationship between two Sub-population within the Akan ethnic groups in Ghana.

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Short tandem repeats (STRs) continue to be the golden standard used in forensic genetics. STRs have proven to be highly discriminatory for human identification and are widely accepted in legal proceedings. While many population studies have been published data on sub-Saharan African populations is limited. In Ghana, there are 75 recognised ethnic groups, but limited studies on the genetic relationship between these populations. The largest ethnic group is the Akan, who make up nearly 50% of the population.

This study involved sampling two populations of the Akan population, one from the Ashanti region and one from the Eastern region, with a total of 196 samples from unrelated males and females (98 samples each). These were extracted and analysed using the GlobalFiler™ Amplification kit. Data were analysed to test for Hardy-Weinberg equilibrium (HWE) and population differentiation using STRUCTURE, STRAF and Arlequin.

The computation of pairwise FST was performed using the 21 loci autosomal STRs for the two sub-populations of the Ghanaian Akan ethnic group. A FST value of 0.0013 between the Ashanti Akan and Eastern Akan populations within Ghana, indicated a relatively low genetic differentiation. STRUCTURE analysis did not detect any significant differences between the two populations.

Comparative analysis was also carried out using datasets from other African groups. These included Guinea Bissau in West Africa, Angola in Central Africa, Cape Coloured, AmaXhosa, Afrikaner, and Asian Indians in South Africa.

The data support the use of a single allele frequency database to represent the Akan population. Future work will investigate the genetic relationships between different Ghanaian ethnic groups.

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ANALYSIS OF THE ANCESTRY AND ADMIXTURE PROPORTIONS IN PERUVIAN MESTIZO POPULATIONS USING AN INSERTION DELETION MULTIPLEX

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Objectives: The objective of the present investigation was to estimate African, European, Native American and Asian ancestry proportions in Peruvian mestizo populations, using 46 Insertion/Deletion polymorphisms (INDELs).

Material and methods: A total of 172 unrelated mestizos from the 3 main geographic regions of Peru (36 from the Coastal area, 8 from the Amazon rainforest and 128 from the Andes highlands) were analyzed and compared with data from 273 African, 346 European, 64 Native American and 291 Asian individuals. All samples were genotyped for 46 INDELs in a single PCR multiplex. The amplicons were separated by capillary electrophoresis and detected using an Applied Biosystems® 3500XL Series Genetic Analyzer. Automated allele calls were obtained with the GeneMapper v1.6 software.

Results and conclusions: In the mestizo Peruvian population, the following ancestry proportions were identified: 0.051 African, 0.236 European, 0.663 Native American and 0.050 Asian. The Native American ancestry predominate in the three studied regions, namely in the Coast, Andes highlands and Amazon rainforest areas of Peru (0.484, 0.713 and 0.663, respectively). Meanwhile, Asian proportion presented the lowest values in the Coastal area and Andes highlands (0.069 and0.041, respectively), and African proportion was the lowest in the Amazon rainforest (0.042). The European ancestry showed the second-highest proportion values in individuals from the Coastal area and Andes highlands (0.369 and 0.201 respectively). The Asian proportion was higher in the Amazon rainforest (0.1063) than in the other two regions. Based on the results, the proportion of Native Americans predominates in the Peruvian mestizo population, indicating that despite the migrations Peru has experienced (from Europe, Africa and Asia), it still maintains a high proportion of the Native American component.

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Tracing the African maternal origins in Brazilian Quilombos

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Brazil carries a complex history of admixture among Native Americans, European colonizers and enslaved Africans. Urban populations in the country show high European admixture, while small scattered native communities still retain Native American genetic background. There are also several communities known as Quilombos, formed by remnant groups of Afrodescendants who escaped from slavery during colonial times. This study aimed to investigate the possible African ethnolinguistic or geographic origins of the maternal lineages of five Quilombos from the State of Alagoas in the Northeast region of Brazil: Bom Despacho, Pau D'Arco, Quilombo, Tabuleiro dos Negros and Vila Santo Antônio. All these communities maintain high levels of maternal African ancestry (68%). The main non-African contribution is of Native American origin (28%), and only eight sequences were attributed to European input. Statistically significant genetic distances were found among all quilombos. However, when only African lineages were analysed, the Quilombo and Bom Despacho communities showed genetic similarities. Such proximity may be attributed to their geographic closeness. All other comparisons between quilombos increased when non-African lineages were excluded, which can be due to possible genetic drift effects. Signs of genetic flow were found, with the presence of shared haplotypes among communities: (1) Pau D'Arco and Tabuleiro dos Negros and (2) Bom Despacho and Quilombo. Preliminary results on shared haplotypes between quilombola communities and African populations point to a diverse origin of the observed lineages, with matches with haplotypes from Ghana, Angola, Ivory Coast, Togo, Mozambique, Chad, Niger, Nigeria, and Rwanda. In summary, the heterogeneity of the African maternal lineages in quilombos can be due to different origins as well as genetic events or drifts involved in the foundation of these communities.

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ALLELIC INCONSISTENCIES BETWEEN COMMERCIAL KITS OF AUTOSOME STR MARKERS: TECHNICAL-LEGAL IMPLICATIONS AND RECOMMENDATIONS

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Autosomal STRs (A-STRs) are the currently recommended markers for investigating biological paternity testing. Commercially there are several A-STRs kits that share many of their markers. In this work we studied the concordance of the profiles obtained with A-STRs kits from different commercial companies in 22,114 cases of biological paternity research from Genes SAS and 9,195 cases from the Laboratory of Molecular Genetics of the Ecuadorian Red Cross.

Genetic profiles of 11,560 cases from 31,309 paternity tests attended in both laboratories from July 2019 to February 2024, initially studied with the VeriFiler Express kit (ThermoFisher) and subsequently verified with their counter-samples using the PowerPlex Fusion kit (Promega), were comparatively analyzed to confirm paternity exclusion or verify any new paternal and/or maternal mutation.

We found 114 discordances distributed in 13 markers among the genetic profiles obtained with the different commercial kits in the 11,560 cases verified, 94 cases presented absence of an allele in a marker for one of the kits, D19S433 (23), D8S1179 (20) and D16s539 (19) presented more events. Two markers presented different genotypes and 18 markers presented alleles outside the range of their allelic ladder.

It is concluded that both laboratories that investigate paternity tests, complex biological relationships and forensic cases using this type of STR kits, as well as commercial companies, are obliged to know and communicate the behaviour of the genetic markers used in these kits, thus avoiding erroneous interpretations of this type of events. Likewise, commercial companies are recommended to make adjustments in the formulation of the kits in order to achieve perfect concordance of genetic profiles in individuals. Besides it is important to include the polymorphisms found in different populations to make the kits more inclusive.

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Analysis of autosomal forensic markers in Southern African Bantu speaking populations

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Genetic markers have been extensively used in forensic investigations, kinship testing, and human identification. Although human populations in Africa are known to possess a high level of genetic variation, data are scarce in this region.

This study evaluated the diversity and the population structure in South Africa and Lesotho ethnic groups using the GlobalFiler™ Express Kit.

A total of 179 new samples from the Swati and Tsonga ethnic groups in South Africa and baPhuthi from Lesotho were genotyped with the GlobalFiler STR panel. These samples were analyzed along with approximately 932 previously genotyped samples, which includes 541 samples from South Africa (Ristow et al., 2016). Additionally, the comparative analysis was limited to the marker in the Identifiler Plus Kit with the addition of 992 samples from Botswana (Tau et al., 2017).

We report novel variations: five off-ladder alleles and one D19S433 tri-allele in the Phuthi people of Lesotho; one off-ladder allele and two tri-alleles (in TH01 and TPOX) in South Africa. We report allelic frequencies, forensic summary statistics, and genetic diversity parameters.

The population structure was analyzed using unsupervised cluster analysis performed by STRUCTURE and DAPC, and population genetic distances by MDS, and NJ. There was a distinct lack of substructure between the Bantu populations of South Africa and Lesotho. The Bantu differentiated from the out-of-Africa populations such as the Coloured, Afrikaner, and Indian. The distance-based methods evidenced geographical and linguistic affinities between populations.

Novel variants and off-ladders would require the update of bins or allelic ladder panels for genotyping African profiles. Recommendations for a single Southern African Bantu allele frequency reference database for kinship analysis are presented.

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Sequence-based North-East Italy population data of five autosomal STRs exclusively included in the ForenSeq™ DNA Signature Prep Kit

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To date, there are several massively parallel sequencing (MPS) platforms available that allow the simultaneous typing of short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs). One of the forensic kits used for MPS is the ForenSeq™ DNA Signature Prep Kit, for which several validation studies have been performed demonstrating its advantages in forensic practice, but population data on the five autosomal STRs exclusive of the kit are still scarce, precluding the use of these loci in complex kinship analysis.

This study aims to estimate allele frequencies and relevant forensic parameters for D4S2408, D6S1043, D9S1122, D17S1301, and D20S482.

DNA extracted from buccal swabs of 255 unrelated autochthonous individuals from North-East Italy was used for library preparation with the ForenSeq™ DNA Signature Prep Kit/Primer Mix A (Verogen – Qiagen). Sequencing was performed on the MiSeq FGx™ Forensic Genomics System, and data analysis was carried out by Universal Analysis Software (UAS) v1.2 (Verogen). The FASTQ files automatically generated by UAS were then re-analyzed using STRait Razor v3.0 (SR3), which can detect isoalleles in homozygous, such as UAS, but also in heterozygous genotype.

Therefore, particular focus was given to the variation in the power of discrimination (PD) of each of the five STRs when genotyping was performed based on sequence variations detected by UAS and SR3.

At the D6S1043 locus, SR3 only found isoalleles in heterozygous genotypes in four subjects, and this did not significantly affect the PD value, which was already high (0.9354) due to the many length-based alleles detected at this locus.

For the D20S482, UAS identified three isoalleles in twelve individuals with homozygous genotypes. In comparison, SR3 found a further sequence variation, revealing the four isoalleles in homo- and heterozygous genotypes in 37 subjects, and this increased the PD from 0.885 to 0.903.

It was observed that D9S1122 is characterized by the presence of three isoalleles in homozygosity and of five more isoalleles in heterozygosity detected exclusively by SR3, leading to an increase in the PD from 0.870 to 0.946.

No significant differences in PD value were disclosed for both D17S1301 and D4S2408 markers due to the detection of isoalleles by both UAS and SR3 in a minimal number of subjects.

These findings suggest how UAS tends to underestimate the presence of allele sequence variations when present in heterozygous genotypes, to the detriment of PD.

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Genetic variability of Roma population in Serbia: The Perspective From Autosomal STR Markers

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Objectives: Genetic variability of Roma population was shaped by the strong influence of genetic drift and gene flow during the migrations from their ancestral homeland in Indian subcontinent towards Europe. In addition, social stigmatization in many European countries, which was a consequence of different cultural heritage and social practices, induced further genetic differentiation and substructuring within the population. Although many population genetic studies on European Roma were carried out, the genetic structure of the Serbian Roma has not been described yet, since only the modest number of individuals from this territory was analyzed. The main aim of this study was the characterization of genetic variability of the Roma and the assessment of intrapopulation genetic differentiation based on the analysis of 21 autosomal STR loci.

Material and Methods: In this study 259 self-identified unrelated Roma individuals from different regions of the Republic of Serbia were genotyped using the Investigator 24plex QS kit (Qiagen, Germany). For intrapopulation analysis samples were classified into different groups based on the geographical region of origin and religion of the previous generation, while the interpopulation analysis was performed using the population data for the countries across their migration route.

Results and conclusion: Intrapopulation analysis revealed divergence of Roma groups illustrating the effect of the historical events after their arrival on Balkan Peninsula and emphasizing significance of the religious affiliation on admixture with autochthonous population. Genetic distance analysis showed the greatest similarity of the studied population with the Middle Eastern populations, while South Asian and European population were more distant. Our results demonstrate that Roma groups in this region of Balkan Peninsula do not represent completely isolated, but rather admixed populations with different proportion of gene flow with other Roma and non-Roma groups.

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ANALYSIS OF 23 AUTOSOMAL STRs IN COASTAL, SIERRA AND JUNGLE POPULATIONS FROM PERU FOR HUMAN IDENTIFICATION PURPOSES

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Objective: To analyze 23 autosomal STR markers and obtain a database of allele/locus frequencies of populations residing in three regions of Peruvian, assess the significance of forensic genetic results, and to improve forensic practices. This research has been approved by the Ethics Committee N°042-2023-CBE-FCB-UNMSM of the Faculty of Biological Sciences of the Universidad Nacional Mayor de San Marcos (Peru).

Materials and Methods: The STR profiles of 524 unrelated Peruvian mestizo individuals (coastal (n=309), sierra (n=181) and jungle (n=34) were generated and analyzed. With the data obtained, an analysis of forensic statistical parameters was conducted, including discrimination power (PD), exclusion power (PE), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), and departures from Hardy-Weinberg equilibrium.

Results and Conclusion: In the populations studied all loci were highly polymorphic. All the autosomal STRs markers met Hardy-Weinberg equilibrium expectations, except for D3S1358, TPOX, D19S433, D6S1043 and Penta D in the Peruvian mestizo population, TPOX, Penta E, D22S1045, D6S1043, D12S391, D2S1338 and D2S1338 in the coastal population, D13S317 in the sierra population and D3S1358, D21S11, D5S818, D6S1043 and D1S1656 in the jungle population. After applying the Bonferroni correction, only the D6S1043locus in the Peruvian and Coastal population significantly departed from expectations. No detectable population genetic subdivision was observed among the population sample of Peruvian mestizo, coastal, sierra and jungle compared with Ecuador, Hispanos, and the Aymara people. However, there are differences with the population sample of Peruvian mestizo, coastal, sierra and jungle compared and population samples of Mexico, Bolivia, the Ashaninka people, and the departments of Loreto, Amazonas, and Madre de Dios in Peru. A database of allele frequencies for 23 autosomal STR markers typical of the Peruvian population is presented.

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Insight into sequence variants of autosomal STR loci in Croatian population with ForenSeq® DNA Signature Prep kit

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Alleles of autosomal short tandem repeat (aSTR) loci display both length-based (LB) and sequence-based (SB) variations. The latter have become easier to explore with massively parallel sequencing technology and commercially available kits, such as ForenSeq® DNA Signature Prep kit (Verogen). The aim of the study was to sequence Croatian population samples (n=83) in order to observe sequence variants at 27 aSTR loci. Buccal swabs underwent DNA extraction and purification on EZ1® Advanced XL instrument (Qiagen). Quantity of 1 ng genomic DNA was input into library preparation using ForenSeq® DNA Signature Prep kit, which was conducted according to the manufacturer's instructions. Libraries were sequenced on MiSeq FGx® (Illumina/Verogen) platform. Data analysis was performed in ForenSeq® Universal Analysis Software (UAS) and Microsoft Excel. All sequence variants were checked against National Center for Biotechnology Information (NCBI) STRSeq BioProject database. Sequencing quality parameters were within optimal range, and all samples exhibited coverage >85000 reads. Alleles detected by sequencing were 99.8% concordant to capillary electrophoresis results (due to allele dropouts, most prominent in D22S1045). Loci D2S1338, D3S1358, D12S391 and D21S11 showed the greatest increase in the number of detected SB versus LB variants (110% to 150%), while at 11 loci no sequence variations were detected. Two novel SB variants were identified that are not present in the NCBI database: one allele 26 at D12S391 and one allele 29 at D21S11. In as much as 42.2% of samples isometric heterozygotes (isoalleles) were detected, occurring at 1 to 4 loci. Observations herein clearly show that sequence information can make valuable contribution to the power of discrimination in forensics. Through this study we have gained insight into common and rare aSTR sequence variants occurring in Croatian population, which is an important step towards implementation into forensic casework.

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Genetic Analysis based on 23 autosomal STRs of the Moroccan population

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Background
Morocco has long been a subject of interest in genetic research because of its high heterogeneity manifested through various ethnic groups (Arabs, Berbers, and Sahraouis). This study describes, for the first time, the genetic profile of the mixed population located in center of Morocco.
Methods and results

23 STR was used on a random sample of 750 unrelated healthy individuals from different regions in Morocco. Based on Nei genetic distance, a Neighbor-Joining tree and a Principal coordinate analysis (PCoA) were implemented. For a more detailed approach, a locus by locus pairwise genetic distance (Fst) was calculated. After Bonferroni's correction, only two loci (TH01 and D18S51) deviated from Hardy–Weinberg equilibrium. The Moroccan population was clustered with Northwest African populations. It was the closest to the Berber population of Azrou. It shared close genetic affinities with all North African populations, with a west to east gradient emphasizing varying degrees of Berber or Arab ancestry contributions, as well as with Sub-Saharan populations and Iran. The genetic affinity with Arabian populations was weak and no clear evidence of significant genetic contribution from European populations.
Conclusions

The Moroccan population seems to embody major North African characteristics, strongly related to a Berber ancestry, with a relatively minor influence from an Arabian origin, as well as a close genetic affinity with Sub-Saharan populations and, surprisingly, with Iran.
Keywords: STR, Population data, Morocco.

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Genetic variation of Chanca population using 23 autosomal STR markers

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Objective: To analyze the genetic variation of 23 autosomal STR markers in the Chanca population residing in the province of Abancay-Peru. This research has been approved by the Ethics Committee N°042-2023-CBE-FCB-UNMSM of the Faculty of Biological Sciences of the Universidad Nacional Mayor de San Marcos (Peru).

Materials and Methods: The STR profiles of 150 unrelated Chanca individuals (Abancay-Peru) were generated and analyzed. Various forensic parameters were analyzed including discrimination power (PD), exclusion power (PE), polymorphic information content (PIC), observed heterozygosity (Ho), expected heterozygosity (He), and departures from Hardy-Weinberg equilibrium. Genetic distances between regionally close populations were calculated

Results and Conclusion: The PENTA E marker presented the highest PD (0.9770), PIC (0.8918) and PM (0.2956) values. The combined PD was >0.99999999, and the combined PE was 0.99999994. The largest distance, based on Fst values, was between the Chanca population and the Amazonas population (0.08517), and the smallest distance was with the populations of Central Peru (0.00023) and Southern Peru (0.00030). This study provides STR population data from the Chanca population, one of ethnic groups of Peru. The results provide additional population data for statistical calculations in human identity testing cases in Peru.

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Genetic polymorphism and forensic evaluation of high-resolution HLA typing in Han population from Guangdong Province, Southern China

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Owing to the high degree of polymorphism, the human leukocyte antigen (HLA) genes may have great power in forensic and medicine applications. However, the potential of the HLA system for forensic DNA typing has not yet been fully evaluated. Recently, Wu et al. studied the suitability of HLA-based typing for kinship testing, which have provided an important starting point for reintroducing HLA genes back into forensic analysis.

As an essential part in evaluating the power of genetic markers, detection of forensic genetics and diversity characteristics of population data is necessary. In this research, we selected 728 unrelated individuals in kinship testing from Han population in Guangdong Province, Southern China. Then we studied the genetic polymorphisms of HLA-A, B, C, DQB1 and DRB1 with the sequence-based typing (SBT) method and calculated the forensic parameters. Totally, 150 alleles were detected, and the corresponding allelic frequencies ranging from 0.0014~0.2081 for the 5 loci. The statistic analysis indicates that the 5 HLA loci were in accordance with the Hardy-Weinberg's equilibrium ($p > 0.05$). The locus with highest polymorphism was HLA-DRB1 (PIC value was 0.9057) and the one with lowest was HLA-A (PIC value was 0.8061). The combined power of discrimination (CPD) was 1–1.73688E-08 and the combined power of exclusion (CPE) was 0.990 508 74 and 0.999 994 01 for trio and duo cases, respectively. However, the significant linkage disequilibrium was detected among the 5 loci and when we regard the loci as one haplotype, 678 unique phenotypes were detected in the population. Meanwhile, one phenotype was shared by five individuals and another one was shared by eight, with a discrimination power (DP) value of 0.999 775 08.

The results indicate that HLA-A, B, C, DQB1 and DRB1 are polymorphic among the Han population in Guangdong Province, China. Using some methods with higher resolution and accuracy, such as next generate sequencing (NGS), more alleles might be detected and would provide a powerful tool for relationship testing and individual identification.

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The genetic structure of the Maltese analysed with genome-wide SNP array data

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The Maltese archipelago spans just 320 sq km and is home to a population of 536,343. The islands boast a rich demographic history, with the genetic landscape of the Maltese shaped by migrations of populations in the Mediterranean and subsequent invasions at different time periods. While historical records trace population origins back to the Temple people (5000 – 4100 BCE), contemporary Maltese are descendants from those who re-populated the islands at the turn of the first millennium AD, with a later influx of people from Arab-ruled Sicily.

Following an initial study with a very small sample set, we conducted a much larger randomised sample of the Maltese population to investigate the genetic structure and assess potential signals of the origins of contemporary Maltese. The Affymetrix Genome-Wide Human Axiom Spanish Biobank Array was used to genotype 791K genome-wide markers in 286 Maltese samples. Analysis of the SNP array data utilised a variety of population genetics algorithms including ADMIXTURE software, to identify patterns of population structure and genetic clusters present in Malta. Comparisons were made to reference continental populations in and around the Mediterranean, with compiled genotypes for the same SNPs. Preliminary results suggest a predominant proportion of European ancestry in Maltese, with a minor contribution from North and sub-Saharan African ancestry. However, Maltese present a singular profile within the Mediterranean context when compared to other Mediterranean datasets.

The SNP array-based analyses presented here have proven informative in assessing the genetic impacts of historical events and population movements that have shaped the current distribution of genomic variation in the Maltese.

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Analysis of NGS-based forensic markers in a Spanish population

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OBJECTIVES
Next-generation sequencing (NGS) techniques have revolutionized biological and biomedical disciplines, including forensic genetics. But their application depends on the availability of newly generated data and their comparison with previous sources of information. We have used NGS in our ongoing analyses to identify remains from the Spanish Civil War (1936-1939) in mass graves of the Comunitat Valenciana (CV) region.

MATERIAL AND METHODS
We have used the ForenSeq DNA Signature Prep Kit to obtain genetic profiles at 94 SNPs and 58 STR loci (27 autosomal, 24 Y-linked, and 7 X-linked) in 689 living relatives of 384 victims. Here, we report the allele frequencies and initial population genetic analyses of the 121 autosomal and X-linked loci from those relatives. Data were analyzed with STRAF and library poppr in R.

RESULTS AND CONCLUSIONS
Autosomal STRs had from 5 to 17 alleles, with expected heterozygosity (He) ranging from 0.65 to 0.90. All the SNPs were biallelic, with He in the range 0.28 - 0.50. All the loci were in Hardy-Weinberg equilibrium after correction for multiple testing and their power of discrimination ranged from 0.82 to 0.98. When compared to the reference population for STRs (Barrio et al. 2019; 496 individuals), Fst values were very low (avg(Fst) = 0.0004, range = 0.0 - 0.0019), thus indicating no differentiation between the two populations. Reference values for SNP loci were extracted for the Iberian and European populations from Ensembl. Again, no significant differentiation was observed between the CV and the Iberian populations (avg(Fst) = 0.0139, range = 0.0 - 0.0015), and the structuring was slightly higher with the European one (avg(Fst) = 0.0205, range = 0.0 - 0.0016)). The analysis of X-linked markers showed no significant differences in allele frequencies between males and females (avg(Fst) = 0.0014, range = 0.0006 - 0.0033), thus conforming to equilibrium expectations. We have provided allele and genotype frequencies for SNPs and STR markers in the CV useful for forensic analyses and verified that they correspond to expectations under panmictic reproduction.

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Evaluation of STR Markers allele frequency distributions in Czech population utilizing NGS Technology with Verogen MainstAY SE Kit

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Understanding the allele frequency distributions of STR markers in the Czech population is crucial for forensic DNA analysis, aiding in criminal investigations and justice. In this ongoing study, we have already analyzed 100 samples and are midway through assessing the frequency distribution and sequence polymorphism of Short Tandem Repeat (STR) markers within the Czech Republic population. Leveraging Next-Generation Sequencing (NGS) technology and the Verogen MainstAY SE kit, we have conducted comprehensive sequencing to explore the diversity of STR markers and map the distribution and frequency of iso-alleles for each marker.

In this abstract, we showcase preliminary results from our analysis of the first 100 samples. Through targeted sequencing, we have captured detailed variations in STR markers, including allele and iso-allele lengths, sequence motifs, and frequencies in the Czech population. The terminology used is based on the latest recommendations of the ISFG. Additionally, ongoing efforts include examining mutations in the flanking regions of the STR markers to understand their impact on the accuracy and discriminatory power of forensic DNA analysis.

Preliminary findings indicate a significant increase in discriminatory power compared to traditional length-based capillary electrophoresis (CE) data approaches. This enhancement underscores the potential of NGS technology in revolutionizing forensic genetics and advancing forensic DNA analysis methodologies. As our study progresses and we expand our sample size, we anticipate further insights into the genetic diversity of the Czech population and its implications for forensic applications.

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Genetic relationships of semi-feral sub-populations of Ecuadorian highland creole horse “Paramero” (Caballo Paramero Ecuatoriano)

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In the Ecuadorian Andes, Paramero Creole horses are adapted to conditions from high altitude areas known as "paramos" (heights of 3300 meters above sea level). They are deeply rooted in the lives and traditions of Andean peoples. These semi-wild herds roam the paramo and are regularly managed by local peoples. The present study aimed to genotype and compare feral populations of Paramero Creole horses from four Andean provinces of Ecuador through microsatellite markers. DNA was extracted from the blood of 182 feral horses from herds in the paramos of Carchi (40), Pichincha (61), Cotopaxi (50), and Chimborazo (33) provinces. Genetic information was accessed through 29 STR molecular markers. Allelic frequencies, diversity, and F statistics were estimated. The results were analyzed with reference population data from the BIOHORSE consortium.

All four populations exhibited high genetic diversity. F statistics indicated little variability between Parameros and other horses (FIT=0.066-0.102), horse subpopulations were homogeneous (FIS=0.009-0.036) and therefore did not show significant genetic differentiation (FST=0.055-0.071) which is somewhat expected due to the history of the spread of Creole horses.

The dendrogram revealed a close relationship between the Paramero horse and other Creole breeds, such as Colombian and Peruvian “Paso Fino”, the Salvadoran Creole, Pantaneiro from Brazil, and the Panamanian Creole. The short branch length of Paramero suggests no evidence of directed selection, unlike other breeds such as Thoroughbred, Retuertas, and Argentinian Creole.

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STRAF 2: An improved version of the STR population data analysis software.

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Population data in forensic genetics must be checked for a variety of statistical parameters before it can be employed for case work. Several tools exist to perform such tasks. However, it can become challenging to obtain results due to the number of software to use and the broad range of input formats. Furthermore, a substantial amount of experience is required to use some of these programs. To overcome these difficulties, we have developed STRAF (STR Analysis for Forensics), a convenient online tool to analyse STR data in forensic genetics. Since its first release in 2017, it has been used in many studies to report allele frequencies, forensic and population genetics parameters, and to interactively explore STR datasets through a user-friendly interface. We introduce the latest version of the STRAF software and the improvements we have implemented over the last years. It includes several new features, such as new statistical methods (multidimensional scaling, comparison to a reference population, haplotype diversities and frequencies) and file conversion utilities. Performance and user experience have also been improved and documentation has been extended.

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Study of rare allelic versatility in the population of Rajasthan (India) to propose population specific best set of polymorphic STR markers for the forensic purposes

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STR markers are well established gold standard genetic markers for the forensic DNA applications. Despite of population diversity across worldwide, still many forensic laboratories are using non population specific set of STR markers, which reflect into allelic challenges in the form of off ladder (OL) peaks, peak in the outside marker region (OMR), non amplification or difference in repeat number at a particular locus in various multiplex STR kits due to use of manufacturer specific different primer and allelic ladder designing. Therefore, to find out rare allelic versatility in the population of Rajasthan based on new CODIS and SE33 STR loci to propose population specific best set of polymorphic STR markers, a pilot study, using 694 randomly selected unrelated individual was undertaken. Rajasthan population was considered for this study, as it contribute 5.84 % population of “India”, the most populous the country across the globe; and it cover 10.4% of total geographical area of the country. Among the studied population, a total of 284 allele ranged from 0.001 to 0.459 was observed. The allele 11 of extended CODIS marker D2S441 was found most common allele as the frequency of o.459. Various microvariant alleles, AMEL Y deletion, novel alleles and off ladder (OL) peaks were observed in the studied population. Six samples showed OL allele at German core SE33 locus, three samples exhibited OL allele at extended CODIS marker D12S391 and one sample showed OL allele at CODIS marker TH01. Few occurrence of false tri-allelic pattern were also seen during analysis of data. Heterozygosity was found to be more than 0.7 except CODIS marker D2S441 (o.644) and TH01 (o.698). Overall, forensically important parameters viz., Power of discrimination (PD), Power of exclusion (PE) and Polymorphic information components (PIC), were found highest for the SE33 markers among the studied loci. The most significant statistical combined matching probability of the studied 21 loci was observed 1.20X10-25 and combined power of exclusion was 0.999999997239.

Thus, this pilot study, showed huge allelic versatility in the population of Rajasthan. Therefore, it is suppose to be population specific genetic markers with suitable allele range, marker should be universal primer sequence, Multiplex STR marker kit should contain autosomal STR marker (20- 25), Sex determination marker, Y chromosomal STR (2-3), X chromosomal Markers (2-3) and SNP marker (1-2) along with quality sensors.

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A phylogenetic study of Hungarian-speaking Drávaszög (Baranja, Croatia) and Zobor region (Slovakia) populations

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This study included 168 contemporary Hungarian speaking individuals, the samples were collected from villages of Drávaszög (Baranja, Croatia) and the Zobor region (Slovakia) and genotyped for Y-chromosomal haplotypes and haplogroups. The most frequent haplogroups of the Zobor, Slovakia population were R1a-Z280, R1a-M458, R1b-P312, G2a-L156.

In the case of the Drávaszög (Baranja, Croatia) males, the most frequent haplogroups were I2a-P37, R1a-Z280, I1-M253, R1b-P25 and E1b1-M78. Genetic make-up of the Drávaszög from Baranja, Croatia and Zobor population from Slovakia tell us different histories. However, more than 17% of the present-day Drávaszög population may be related to the ancient Hungarians and the Caucasus populations, whereas the present-day Hungarians in the Zobor may not be only but also related to the Caucasus populations (10%).

The haplotype and haplogroup diversities of the Drávaszög group were 0.99938 and 0.90586, respectively, whereas these values for the Zobor were 0.98974 and 0.81154, respectively.
In this study, we present new Y-STR and Y-SNP data of two Hungarian-speaking populations from Croatia and Slovakia and compare them to contemporary Eurasian and available aDNA data to gain further knowledge about the genetic legacy of these populations.

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Uncovering Novel Plastome Variants in Trafficked Cannabis sativa in Brazil: A Potential Tool for Drug Origin Traceability

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1. Introduction:
Cannabis sativa, commonly known as marijuana, is the most consumed illicit drug in Brazil. According to the Brazilian Federal Police (BFP), the illicit market for C. sativa in Brazil is primarily supplied by drugs originating from Paraguay and the Northeast region of Brazil, where the Marijuana Polygon is located. These two known routes, along with the increasing trend of indoor cultivation supported by online markets and drugs sourced from Uruguay and Colombia, are currently under scrutiny by BFP. Samples from different seizures are necessary to investigate trafficking routes into and within Brazil, with determining the crop origin potentially providing crucial leads.

2. Objectives:
- Contribute to the genomic characterization of chloroplast DNA (cpDNA) and mitochondrial DNA (mtDNA) of Cannabis sativa samples trafficked in Brazil;
- Explore polymorphic regions within the cpDNA and mtDNA of C. sativa seized in Brazil;
- Evaluate common barcoding markers documented in the literature in seized samples in Brazil;
- Discover novel hotspot regions conducive to biogeographic determination, specifically tailored to the Brazilian context.

3. Material and Methods:
45 samples representing the four main origins of the drug underwent whole-genome sequencing on a NovaSeq platform. Specifically, the sample distribution comprised 15 from Paraguay, four from Colombia, 12 from the Marijuana Polygon region, and 14 commercial samples. The project will incorporate published cpDNA and mtDNA genomes of C. sativa, integrating haplotypic assessment, phylogenetics, interpopulational comparison, pairwise evaluations, and principal component analysis.

4. Perspectives:
This comprehensive approach aims to provide insights into genetic diversity and origins of trafficked C. sativa, thereby facilitating the implementation of effective regulatory measures and aiding in the development of strategies to address illicit drug trafficking in Brazil.

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Alternative Mitogenome PCR Enrichment Methods for Reference-Type Samples

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The whole mitochondrial genome (mitogenome) of reference and population samples are often generated utilizing PCR enrichment with two long range (LR) amplicons (~8500 bp) and massively parallel sequencing (MPS). Unfortunately, collection method and long-term storage of reference-type samples may cause DNA degradation that limit LR amplification success. As a result, an alternative mitogenome enrichment method with smaller amplicons may be required. The mitogenome may be targeted using an approach with shorter amplicons to improve amplification success.

Thirteen reference-type samples that previously failed to generate LR amplification results were tested with two alternative methods with reduced amplicon sizes: a four amplicon (~4000 bp each; 4-amp) and an eight amplicon (1200-2500 bp each; 8-amp) approach. One representative amplicon from three mitogenome enrichment strategies (LR, 4-amp and 8-amp) was tested to assess the DNA degradation in the failed samples. The amplicons were quantified on a Fragment Analyzer or Bioanalyzer to determine amplification success.

Five out of 13 samples generated LR amplicons with replicate processing, while 4-amp PCR products were successfully generated for 11 samples. Due to the smaller amplicon size, all samples except for one generated 8-amp PCR products. By evaluating these alternative methods, a workflow may be developed to generate data from a range of sample types and quality. Commercial MPS kits that utilize small amplicons (~200 bp) to target the mitogenome could be employed to overcome degradation. However, smaller amplicons may complicate analyses due to co-enrichment of nuclear psuedogenes. Therefore, a comprehensive assessment including a cost-benefit analysis should be conducted in order to determine the optimal mitogenome enrichment method within a laboratory's MPS workflow.

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Disclosing the maternal genetic background of Andean Mestizos from Ecuador

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Ecuador's genetic makeup reflects complex admixture events shaped by European and African influences since the colonial era. This study analyses the maternal genetic contributions to the Andean Mestizos from Ecuador, exploring post-colonial migrations at a regional level. The maternal ancestry was assessed through complete mtDNA control region sequencing of 210 samples from Andean Mestizos, categorized into North, Center, and South subgroups. High haplotype diversity values were found in the three Andean regions (>0.9960). Native American haplogroups (A2, B2, B4, C1, D1, D4) prevailed in the whole sample with differences in the proportion of some lineages among regions. African (L1, L2, L3) and Eurasian (U4c1, HV, R) haplogroups were detected at low frequencies in North and South Andes, but were absent in Central Andes, where most Native American communities are established. Despite some variation in haplogroup distribution among Andean sub-regions, there were no significant differences in haplotype composition. However, a statistically significant difference observed between the Andes and a Pacific Coast population highlighted a possible interregional heterogeneity. In a South American context, our samples showed genetic similarity to West Andean populations, driven by elevated haplogroup B frequency, suggesting a distinct evolutionary path to the populations from the Eastern South American regions.

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mitoTree: Advancing mtDNA research through updated Phylogeny and Haplogroup Guidelines

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1. Introduction & Objectives

The field of mitochondrial DNA (mtDNA) haplogrouping faces challenges arising from the uncontrolled growth of mitogenome sequences and associated haplogroups in the absence of standardized rules for their definition. Given the lack of Phylotree updates since 2017, there is an urgent requirement for an up-to-date framework capable of accommodating the accumulating body of data. To address these issues, we introduce mitoTree, a novel initiative designed to update the human mtDNA phylogeny by updating the quality-controlled mitogenome database, standardizing criteria for haplogroup definition, evaluating contemporary tree-building methods and providing a curated repository.

2. Material & Methods

mitoTree employs a multi-faceted approach that includes more than 60,000 mitogenomes retrieved from the NCBI database and underwent rigorous quality control. The cleaned dataset was used to update the current phylogeny of human mtDNA and to serve as a basis for the definition of criteria for haplogroup assignment. We assessed the applicability of contemporary methods including maximum likelihood and Bayesian inference, in comparison to the traditional maximum parsimony approach.

3. Results & Conclusions

The initiative has yielded an increased and thoroughly vetted mtDNA mitogenome database, guidelines for haplogroup definition, a refined phylogenetic tree incorporating new mitogenomes, and led to launching an accessible repository for efficient retrieval and exploration of mtDNA haplogroup data.

mitoTree meets a crucial demand across multiple disciplines by providing a comprehensive framework for mtDNA haplogrouping. It closes the gap caused by outdated repositories and ambiguous haplogroup definition. Its impact on mtDNA research exceeds the current results, providing researchers with reliable data and advances future developments in mitochondrial haplogroup analysis.

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Direct Evidence about Founding Populations: Mitogenomes from the first residents of Porto Alegre, Southern Brazil

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INTRODUCTION: Porto Alegre is the main city and capital of the Brazilian southern state of Rio Grande do Sul. Officially founded in 1772, original settlers comprised Portuguese Azorean immigrants, southeastern Brazilian farmers and enslaved Africans and Native Americans. In 2012, an excavation revealed the town's first graveyard location.

OBJETIVES: Human remains were recovered and submitted to genetic analyses in order to contribute to the retrieval of information regarding the matrilineal origins of Porto Alegre's founding populations. Until then, only historical documentary and archaeological analyses had been used to detail the history of the first residents.

MATERIAL AND METHODS: All laboratory work was performed in dedicated clean facilities following ancient DNA protocols and strict standards to avoid external contaminations.

RESULTS AND CONCLUSIONS: We generated complete mitogenome sequences for 22 individuals buried between 1772 and 1850, here named the 'Ancient Southern Brazil' sample. The complete sample set was evaluated regarding haplotype composition and distribution. No identical sequences were found in the present dataset. Interpopulational analysis comparing haplotypic diversity among Ancient Southern Brazilians and a set of 26 worldwide populations, was also performed. In addition, a collection of Brazilian full mitogenome sequences belonging to distinct biogeographical regions was included in the comparison to assess possible differences between Ancient Southern Brazil and the rest of the country. Also, a modern Southern Brazilian population was included to compare the current population with the ancient one. The total sample revealed a high percentage of African mtDNA lineages, followed by Native American and European lineages. Haplotype distribution in this Ancient Southern Brazilian sample seems to be similar to the remaining of the country, with a larger contribution of maternal lines with African origin, especially from west-central and southeastern Africa. FST values demonstrate that the Ancient Southern Brazilian sample, is most closely related to the modern Brazilian population than to the modern Southern Brazilian population. These results corroborate with the historical records regarding the territorial occupation dynamics of Southern Brazil and configure the first mitogenomes of the most ancient bones from residents of Porto Alegre, Brazil.

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Analysis of the Mitochondrial and Y Chromosomal DNA Variation in Hispaniola

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Hispaniola is an island full of diverse history, originating with the native Taíno people who migrated over from what is now Venezuela six to seven thousand years ago. In 1492, Christopher Columbus landed on the island and claimed it for Spain. Spanish, and eventually French, colonists established settlements on Hispaniola, and later forcibly brought more than 800,000 slaves from Africa to the island. By the mid-19th century, the island of Hispaniola was split into two independent countries - the Dominican Republic and Haiti. Because of differences in regional history, each country has their own unique demography. Most Dominicans self-identify as mixed-race (Native American, African and/or European), whereas the Haiti's population is predominantly of African origin.

Buccal swabs were collected with informed consent from a total of 240 maternally unrelated individuals from the two countries of Hispaniola, the Dominican Republic (n=101) and Haiti (n=139). The extracts were amplified using PCR enrichment to target the entire mitochondrial genome (mitogenome) and 884 Y-chromosomal SNPs (Y-SNPs). After PCR enrichment an automated half reaction library preparation was performed followed by sequencing on a MiSeq FGx. Mitogenome data were analyzed in CLC Genomics Workbench and mitochondrial haplogroups were predicted based on PhyloTree Build 17. Y-SNP data were analyzed with YLeaf v3.2.1, establishing Y haplogroups based on Yfull tree.

Over half of the 43 male Dominican samples had mitochondrial and Y haplogroups of different origins, indicating a heavily admixed population of African, European and Native American ancestries. Over 75% of Haitian samples were assigned to African haplogroups, with only minor representation of European and Native American lineages within the population. These haplogroup distributions were expected given the histories and current demographics of each country.

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Expanding population data for mtDNA haplogroups in the Republic of North Macedonia by using mtDNA set of SNPs in SNP microarray analysis

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The analysis of mtDNA SNPs has become a widely used method in disease analysis, forensic genetics, molecular anthropology, but also in population genetic studies. While routine procedures for mtDNA analysis is by sequencing the control region, in recent years there have also been used several other techniques to analyse single nucleotide polymorphisms (SNPs) on the mtDNA genome, for example pyrosequencing and the most common used method SNaPshot techniques. Mitochondrial DNA haplogroups are mainly discriminated by coding region SNPs, but covering the whole mtDNA genome provides more comprehensive understanding of the genetic variation including subclades within major haplogroups.

Here we present the results of the analysis of 1765 mtDNA SNPs throughout the whole mitochondrial genome which are part of SNP microarray - Global Screening Array (GSA) v3 microarray, (Illumina, USA), that also covers nuclear DNA SNPs which primarily are used for detection of variants in different health conditions.

The size of the sample group for determination of the mtDNA haplogroup frequencies was 100 individuals from the Macedonian ethnic group from the Republic of North Macedonia. Raw data from the SNParray were analyzed by Plink v1.90b7.2. The mitochondrial sequences were imported in the mthap software Data Version 17.9 – (2016-02-18), and annotated variants were imported in EMPOP database (v4/R13) for haplogroup identification.

The obtained results were in concordance with previously published data for mtDNA haplogroups in the Republic of Macedonia. In our study the most common haplogroup was H with 37%, followed by haplogroup U with 24%, and intermediate frequencies of haplogroups J (9%), K (7%), N (5%) and T (4%). Within haplogroups, subclades were determined among which 3 subclades from the L macrohaplogroup. Microarray SNPs covering the whole mtDNA genome allow determination of the subclades of haplogroups and also give a possibility to enrich the population data of the country with additional information.

P-311

Mitochondrial DNA Genome polymorphism analysis in the Mexican-Mestizo Population from Mexico City Using Next-Generation Sequencing.

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Mitochondrial DNA (mtDNA) is used to aid in human identification due to its characteristics that make it suitable for forensic purposes. mtDNA is an extranuclear genome that has several copies in each cell and can be extracted from teeth, bone, and hair without shafts. Commonly, for human identification, the mtDNA hypervariable regions 1 (HVR1) and 2 (HVR2) are analyzed through Sanger sequencing. Nevertheless, the power of discrimination (PD) when analyzing these two regions is low compared to autosomal STRs markers. An approach to increase the PD of mtDNA would be through whole mtDNA sequencing with next-generation sequencing (NGS). Moreover, obtaining whole genome mtDNA population data is mandatory for forensic statistical calculations of this genetic marker. To establish an mtDNA genome database of the Mexican Mestizo population useful for forensic purposes, we sequenced mtDNA genomes of 79 unrelated individuals from Mexico City using Illumina NGS technology. First, from each individual, the whole mtDNA genome was amplified in two amplicons by long PCR, and both amplicons were pooled in a single tube to prepare the libraries for NGS. These libraries were sequenced in a Verogen MiSeq sequencer, and the sequences were aligned with the revised Cambridge Reference Sequence (rCRS) to identify the polymorphisms using the software. mtDNA Haplogroup assignment was accomplished using the EMPOP mtDNA database. A total of 79 different haplotypes were identified in the analyzed samples. This contrasts with the 73 haplotypes found when considering only the HVR1 and HVR2 regions. Moreover, the haplotype diversity was higher analyzing the whole mtDNA genome compared to only these two hypervariable regions (1 vs. 0.9980). The Amerindian haplogroups represented 88.6% of the haplogroups found, represented by the haplogroup A (35.4%), B (24.1%), C (20.3%), and D (8.9%). In fact, the most common mtDNA haplogroups found in our sample were the haplogroups B2x (n=5), C1b1 (n=5), and A2u1 (n=4). An analysis of a larger sample size would be necessary to accurately evaluate the forensic parameters of whole mtDNA genome polymorphism in Mexican Mestizo population. It is noteworthy that haplogroup A could be one of the most diverse haplogroups in this population.

P-312

A comprehensive review of HVS-I mitochondrial DNA variation of 19 Iranian populations

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Iran is located along the Central Asian corridor, a natural artery that has served as a cross-continental route since the first anatomically modern human populations migrated out of Africa. We compiled and reanalyzed the HVS-I (hypervariable segment-I) of 3840 mitochondrial DNA (mtDNA) sequences from 19 Iranian populations and from 26 groups from adjacent countries to give a comprehensive review of the maternal genetic variation and investigate the impact of historical events and cultural factors on the maternal genetic structure of modern Iranians. We conclude that Iranians have a high level of genetic diversity. Thirty-six haplogroups were observed in Iran's populations, and most of them belong to widespread West-Eurasian haplogroups, such as H, HV, J, N, T, and U. In contrast, the predominant haplogroups observed in most of the adjacent countries studied here are H, M, D, R, U, and C haplogroups. Using principal component analysis, clustering, and genetic distance-based calculations, we estimated moderate genetic relationships between Iranian and other Eurasian groups. Further, analyses of molecular variance and comparing geographic and genetic structures indicate that mtDNA HVS-I sequence diversity does not exhibit any sharp geographic structure in the country. Barring a few from some culturally distinct and naturally separated minorities, most Iranian populations have a homogenous maternal genetic structure.

P-315

Signature AIMs - a new kind of forensic ancestry marker

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We have developed a novel panel of forensic ancestry-informative marker (AIM) SNPs, which we name Signature AIMs. These markers exhibit variation with absolute population specificity - defined as SNP sites having zero, or close-to-zero frequencies (set at a maximum value of 0.001), for the alternative allele - i.e., the ALT non-reference allele - in all worldwide populations, except the target population (which show ALT-allele frequencies in the range 0.05-0.35). Such patterns of variation provide a characteristic 'signature' of a particular population-of-origin in an individual when a relatively large number of genotypes with population specific alleles are detected. We identified several thousand candidate SNPs and balanced the final panel of ~340 selected markers to provide an average of between 19 to 22 specific alleles in any one individual across eight population groups (the five main continental population groups plus South Asia, Middle East, and North Africa). Prior to embarking on multiplex designs to construct a massively parallel sequencing assay, we have collected Signature AIMs profiles from 4234 globally distributed population samples to confirm patterns of ALT-allele specificity in each marker and in each targeted population. The efficiency of Signature AIMs to detect and characterise co-ancestry proportions in individuals with admixed backgrounds was also evaluated by comparing our observed genotype patterns to 'ground-truth' information obtained from the 1000 Genomes Project's own analyses of co-ancestry in their six admixed population samples (ASW, ACB, PUR, CLM, PEL, MXL) using whole-genome-sequence SNP datasets.

P-316

Estimating biogeographic ancestry – being certain of uncertainty

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While estimating the phenotype of a potential suspect is legalised in the field of so-called ‘extended forensic DNA analysis’ in Germany (§§81e.g STPO) since 2019, the estimation of the biogeographic ancestry is still not permitted. To address prospective questions about the uncertainty in the estimation of the biogeographic ancestry several panels (VISAGE Basic Tool, Verogen ForenSeq DNA Signature Kit, The Force Panel AIMs subset, and TFS Precision ID Ancestry Panel) were compared to evaluate their performance in distinguishing continental and sub-continental populations. Migrants from the Middle East show the second highest proportion of migrants in Germany next to migrants from European countries. Therefore, particular attention was centred on the differentiation between Europe and the Middle East. With the SNIPPER Naïve-Bayes classifier it was possible to estimate the Divergence of markers from these panels. With this information, we could calculate the I_n Informativeness Metric of each marker and the delta Δ providing information on the performance of any one marker to differentiate various pairs of populations. By comparing these panels, we showed that the performance in distinguishing continental and sub-continental regions differs and that there is no panel able to cover all continents equally well. With the obtained information from SNIPPER we wanted to evaluate an in-house designed panel in silico with the goal to address this gap to have the possibility for better distinction of admixed individuals. Therefore, we developed an algorithm, which solves the optimization problem for the combination of markers for a panel which can distinguish continental and sub-continental population groups equally well, and can enhance the investigation of admixture individuals. We investigated this panel and established ancestry panels (as listed above) using STRUCTURE with admixture individuals (African Carribbeans in Barbados, Americans of African ancestry, Puerto Ricans, Colombians, Mexican ancestry from Los Angeles and Peruvians from Lima) and compared the similarity between each of them. We present our results and outline the marker assessment algorithm with which we compiled the new panel.

P-317

Genetic ancestry of a Montubios rural population from Ecuador unveils East Asian contribution.

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The Montubios constitute about 7.4% of the Ecuadorian population and were officially recognized based on their ethnography in the 2010 census but are one of the least studied ethnic groups. They are genetically similar to the Mestizos group as they also have admixed origin from Native American, European, and African contributors, but the Montubios are settled around the central area of the Pacific coast. In this work, we examine the autosomal genetic ancestry of Montubios rural communities in the Santa Elena province of Ecuador.

A total of 53 individuals were analyzed using 46 autosomal ancestry-informative markers (AIM-indels) through multiplex PCR followed by capillary electrophoresis. We then compared Montubio's genetic profiles with those of four reference populations that represent possible main sources of ancestry: African, European, East Asian, and Native American. We performed Bayesian clustering analyses using the STRUCTURE software with the Admixture model to estimate the individual and global ancestry proportions for each population group.

Our results show that the Montubios have a predominantly Native American ancestry (87.1%), followed by European (5.7%), East Asian (5.4%), and African (1.9%) contributions. These findings are consistent with the essentially tri-hybrid Native American, European, and African admixture model known for Ecuadorian populations; while also showing a noticeable East Asian input in these rural communities from Santa Elena province. Our study is the first to describe such genetic makeup of the Montubios population and contributes to the understanding of the complex genetic diversity of Ecuador and Latin America.

P-319

Evaluating the effect of marker panel sizes on estimation of bio-geographical co-ancestry proportions in admixed individuals

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Individuals with admixed backgrounds will have differing degrees of co-ancestry contributions from two or more ancestral populations. Such patterns represent a challenge when performing biogeographical ancestry (BGA) analysis as these two scenarios cannot be easily distinguished: (i) that the individual belongs to an admixed population, or (ii) that the individual has first or second order antecedents from different ancestral populations. The correct estimation of the co-ancestry proportions can help distinguish these situations by discounting the second option if estimates do not match a ~50%-50% or ~75%-25% pattern. However, several factors affect the accuracy of the co-ancestry proportion estimates, including the balance of the population-specific divergence amongst the selected SNPs and the size of the ancestry panel used.

In this work, we investigate the effect of the number of SNPs in the panel on estimates of co-ancestry proportions. For this purpose, we selected a range of panels with different sizes, and including both BGA-dedicated and kinship marker panels. We compared the co-ancestry proportion estimations obtained with these panels for a set of >500 admixed individuals from the 1000 Genomes Project with those obtained with the Affymetrix Human Origins panel, an array-based set of >600,000 SNPs which have been specifically selected to reduce ascertainment bias.

P-489

Deletion events at DYS448 locus: Exploring rearrangement patterns in the AZFc region of the Y chromosome

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The ampliconic sequence of the human Y chromosome contains abundant palindromes and inverted repeats. This repetitive structure disposes to deletions or duplications, which are located mainly on the long arm of the Y chromosome, in the three domains of the azoospermia factor regions called AZFa, AZFb and AZFc. Since the AZFc region consists almost entirely of ampliconic segments, it is more prone to structural rearrangements leading to deletions and/or duplications of large chromosomal portions. Analysis with different sets of Y-STRs revealed duplication/deletion patterns, whose presence may lead to misinterpretation of Y-STR profiles. Here, we report Y-chromosomal variations in five unrelated men, in whom deletions at the DYS448 locus were observed with three different commercial kits: PowerPlex Y-23; Investigator Argus Y-27; and Y-filer Plus. Previous studies identified partial deletions in AZFc involving DYS448, due to recombination between b1/b3 and b2/b3 palindromic segments. To further explore rearrangement patterns within the AZFc region, the five Y chromosomes with a deletion at the DYS448 were analyzed using 6 sequence-tagged site (STS) markers. In this work, we will discuss the causes of unexpected rearrangements within the AZFc, associated with the deletion of the DYS448 locus; and (b) provide an estimative of its frequency in three South American populations (one admixed, one Native and one Afro-descent).

Posters Topic 6

Statistics and Interpretation

6. Statistics and Interpretation

Abstracts Posters

P-023

Abnormal Inheritance Patterns Detection and Identification in Paternity Testing using Bayesian Networks

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Bayesian networks have emerged as a valuable tool in forensic genetics for many contexts, including paternity and kinship determination. They provide a probabilistic framework to analyze complex family relationships based on genetic data. Within this context, Bayesian networks integrate prior knowledge, such as allele frequencies and mutation rates, with DNA evidence. Recent studies using high-density genotypic data or whole-genomic sequencing suggest that abnormal genetic inheritance patterns may occur more frequently than previously recognized. Among such biological phenomena, uniparental disomy (UPD), a situation where an individual carries both homologous of a specific chromosome pair from a single parent, was recently shown to be almost as common as STR mutational events and therefore should be considered when genetic divergences are observed in a case. The correct identification of uniparental disomy occurrence may have a critical impact on accurate paternity determination since observed genetic data in such cases may not be compatible with the widely employed stepwise mutation model used in paternity determination. In this study, we introduce Object-Oriented Bayesian Networks (OOBE) tailored for paternity testing, capable of accommodating additional abnormalities in STR inheritance patterns. While simultaneously evaluating genetic data and providing identical Combined Paternity Index (CPI) results to traditional statistical analysis, the proposed networks were projected to distinguish and identify the underlying abnormal genetic mechanism (including both mutation and uniparental disomy). The weight of evidence toward the hypotheses (including the putative paternity and the abnormal mechanism alternatives) is presented as likelihood ratios. The proposed networks can evaluate cases of both uniparental iso and heterodisomies. Current work is underway to allow the detection of partial UPD cases, in addition to complete UPD.

P-034

A comparison of likelihood ratios obtained from EuroForMix and STRmix™ for two and three contributor mixture profiles

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The use of probabilistic genotyping software (PGS) for the calculation of the likelihood ratio (LR) has become very widespread for the study of mixed genetic profiles of variable and increasing complexity.
At this point it is an undisputed that continuous software is the best-performing method for the study of the mixtures, especially those in which the ratio of the contributors is unbalanced.
Even though there is no single or best mathematical model, nor consequently an absolutly "correct" value of the LR related to a given mixture, in any case it is important that the value of the calculated LR be solid and reliable, especially if it is reported in court.

1. The aim of the present work was to compare the LR values obtained with two different types of software (SW) EuroForMix (v. 3.3.4–4.0.8) and STRmix™ (v. 2.9.1 demo-2.11).

   The two types of SW have a continuous approach, but use different mathematical models. EFM assumes maximum likelihood estimation (MLE) or Bayesian inference approaches and uses the gamma distribution for peak height modeling. STRmix™ is based on the Bayesian approach, uses Markov Chain Monte Carlo (MCMC) and assumes a log-normal distribution for peak height.

2. We selected 50 mixtures with 2 (2PM) and 3 (3PM) contributors from the PROVEDIt dataset, with various Mixture Ratio (1:1, 1:2, 1:4, 1:9, 1:1:1, 1:2:2, 1:9:1 and 1:9:9), degradation and DNA input. We calculated the LR for each contributor for 4 pairs of hypotheses [H1/H2], the LRbase with the basic hypotheses [POI+U/U+U or POI+U+U/U+U+U] and three LRsFam, replacing an unknown person with a relative of the POI (father/son or sibling or cousin) in the H2.

   The LR values obtained with both types of SW were compared with each other, and also considering some parameters, such as the number of drop-outs, the DNA input, the degradation and the degree of overlapping, in order to study and point out the probable impact on the different calculation methods.

3. From the comparison of the obtained results, it was possible to highlight a good agreement in most of the mixtures analyzed, especially in the case of 3PM (log10LRbase average variation =1.09) compared to 2PM (log10LRbase average variation=1.94).
   The set of mixtures with the greatest divergence of orders of magnitude in the log10LRbase value was the 2PM 1:1 (log10LRbase average variation=2.52).

The most divergent results are currently under further investigation in order to better identify the possible reasons for these differences.

P-035

An open source tool for simulating single source and mixed DNA profiles involving relatives and linked loci

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Recent years have seen an increased uptake of sequencing technologies, including extended SNP panels for kinship testing. Meanwhile, probabilistic genotyping of STR profiles is continuing to be introduced into routine casework in forensic laboratories. Validation studies and research regarding both techniques are challenged by a lack of comprehensive ground truth known datasets. Simulated profiles may in some circumstances be used to validate systems or to research certain properties of systems applied in casework. We introduce an open source tool that can be used to generate synthetic data involving single source or mixed DNA profiles where the sample contributors may be related according to a pedigree.

The tool is made available as an open source R-package. The tool supports simulation of STRs, SNPs and microhaplotypes for single source or mixed profiles with possibly related contributors. There is support for linked markers in the simulations and a peak height model of choice can be incorporated. Genotypes are sampled by randomly drawing alleles and dropping these down the pedigree taking linkage into account. The number of contributors per profile, template amounts per contributor and levels of degradation can be controlled. An important application of the tool is estimation of the power of discrimination for an identification or relationship testing workflow. Moreover, various hypotheses around the reliability of systems when pushed to their limits can be investigated using in silico generated data.

P-040

KongohPlus: An R-shiny package for probabilistic genotyping software based on an MCMC approach

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The interpretation of DNA mixtures and small amounts or degraded DNA samples is challenging due to difficulties in estimating mixture proportions and genotype combinations. The previously developed probabilistic genotyping software “Kongoh” cannot estimate mixture proportions continuously and degradation parameters per contributor. To address this issue, we developed a new probabilistic genotyping software “KongohPlus” based on an MCMC approach.

KongohPlus is an R-shiny package with interactive graphical user interface application. The MCMC models are written in the Stan codes and CmdStanR interface is used to run the codes. The parameters of the MCMC are mixture proportions per contributor, degradation parameters per contributor, total peak height that reflects the total DNA amount, and locus-specific amplification efficiencies. The expected allele-specific stutter ratios for back stutters, forward stutters, double-back stutters, and minus 2-nt stutters are determined based on the user-prepared experimental data. All possible genotype combinations are considered in each MCMC step; therefore, the number of warm-up steps and sampling steps are relatively small. The Rhat values (also known as the Gelman-Rubin diagnostics) of the likelihood and each parameter are used for convergence diagnostics.

The software KongohPlus was run as a pre-trial using some 2–4 person mixed DNA profiles typed by the GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific). In the conditions that the number of warm-up steps is 1,200, the number of sampling steps is 300, and the number of chains is 4, the convergence diagnostics have been achieved (i.e., all Rhat values were smaller than 1.1). The computational time was approximate 20 sec in 2-person mixtures, approximate 10 min in 3-person mixtures, and approximate 10 hours in 4-person mixtures. The computational time was expected to be significantly reduced if rare stutter types (e.g., double-back stutters) were not considered. We are going to perform the developmental validation of KongohPlus preparing the experimental profiles typed by the GlobalFiler, which is generally used for actual caseworks in Japan.

P-041

Bridging expertise and data-driven approaches in forensic analysis: Validation of the DNA Decision Support Tool

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The interpretation of complex DNA profiles can vary both within and among laboratories. Decisions such as whether to perform rework, the type of rework to perform, whether to submit or search within the criminal database, or to conduct a weight of evidence calculation are influenced by a combination of guidelines, expert knowledge, and experience. To our knowledge, no laboratory systematically utilises historical casework data to assist in these decisions. To address this gap, we have developed a DNA Decision Support Tool[1].

In its current version, the tool allows users to upload a DNA profile, search for profiles with similar characteristics, and review the actions taken and their outcomes in previous cases. Additionally, it estimates the number of additional peaks that could be obtained if the profile was analysed using lower analytical thresholds (i.e., a type of rework utilised in our laboratory). The tool, along with the underlying database, also facilitates research inquiries aimed at optimising or automating laboratory processes.

The database undergoes nightly updates, incorporating new data from a variety of sources. These sources include the DNA eXpert System (DNAxs), our Laboratory Information Management System (LIMS), GeneMarker HIDauto, and electropherograms. Currently, it contains more than 60,000 initial profiles and over 15,000 rework profiles. This study validates the DNA Decision Support Tool and highlights its potential to facilitate more data-driven methodologies in the examination and quality control of DNA casework.

[1]Benschop et al. Using previous DNA casework data to aid decision making in the process of DNA profile interpretation. Forensic Sci. Int. Genet. Suppl. Ser. 8 (2022) 251-253.

P-160

FamLink2: A Comprehensive Tool for Kinship Analysis in Forensic Genetics

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In forensic genetics, there is a never-ceasing demand for robust software capable of performing relationship calculations using genetic markers. The applications are versatile ranging from a simple paternity case to more general inferences of relationship. As sequencing technologies continue to evolve, they yield denser sets of SNP markers which in turn present challenges relating to marker dependencies. Moreover, working with sequence data of varying quality and limited quantity introduces uncertainty regarding the true genotype. To address these challenges, we present an efficient implementation of a three-layered model for likelihood computations in kinship analysis.

Population Model (Top Layer):
In this layer, we account for allele frequencies and population substructure. It serves as the foundation for subsequent analyses.

Inheritance Model (Second Layer):
Here, we efficiently handle linked markers using an IBD (Identity by Descent) model. This layer ensures accurate representation of genetic inheritance patterns.

Observational Level (Bottom Layer):
At the observational level, we model the likelihood of genotypes based on underlying reads and error parameters. This versatile model extends beyond kinship questions and can be applied to ancestry and phenotype inference, accounting for uncertainties in true genotypes.

To demonstrate the potential of our approach, we utilize a real case involving low-coverage sequencing data. Additionally, we provide validation examples, exploring the impact of different parameters within the model.

FamLink2, freely available at https://famlink.se, enables end-users to jointly address genetic linkage, genotype uncertainty, and population substructure within arbitrary pedigrees containing typed individuals.

P-161

Investigating the utility of large-scale SNP panels for distinguishing between degrees of relatedness within kinship investigations

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Single nucleotide polymorphisms (SNPs) offer several advantages over short tandem repeats (STRs), such as exhibiting a lower mutation rate, a greater abundance of potential loci within the genome and the ability to design smaller amplicons. These advantageous properties have led to the development of a number of forensically relevant large scale SNP panels that have shown great promise for kinship analysis applications, particularly for evaluating long-range relationships. This study aims to investigate the efficacy of the ForenSeq Kintelligence kit and the FORCE community panel (using the QIAseq chemistry) when trying to resolve between different degrees of relatedness, for example whether two individuals are related as either half-siblings or first cousins. Eight buccal swabs from four previously processed cases were selected, and libraries were prepared using these two panels. SNP profiles were analyzed using different data analysis pipelines to examine optimal workflows. Statistical calculations were conducted to evaluate the ability of the panels to resolve the tested relationship scenarios.

P-168

Evaluation of uncertainties in investigations of different type of kinship scenarios

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Most currently used CE autosomal STR kits are well-suited for simple kinship investigations (i.e.: parent/child duos or trios), where high LR values (above 100.000 to 1) are usually achieved. The picture is however quite different when it comes to kinship calculations in complex cases, where LR values obtained with autosomal STR kits alone are typically in a much lower range for the true related (H0) scenario. Depending on the scenario it is in some cases possible to achieve a higher certainty for the true hypothesis by including other type of markers, such as non-autosomal STRs, microhaplotypes or SNPs. In this study, we aimed to investigate which LR value ranges ought to be considered as inconclusive for the kinship scenarios most often requested to our laboratory. Simulated pedigrees for 31 kinship scenarios were generated using the Familias software v. 3.3.1, using a Danish allele frequency database for the 21 autosomal STRs included in the GlobalFiler™ Express (Thermo Fisher) kit. For each kinship scenario 50.000 simulations were performed and the LR values for true related (H0) versus true unrelated (H1) were plotted in two density curves. The overlapping area of the two curves represents the LR values for which it is not possible to discern between the two tested hypothesis for the given scenario. Values within this LR range should therefore be regarded as being uncertain. The proportion of LR values of each hypothesis within this region, as well as the percentages and median LR values for H0 and H1 were calculated. For most of the tested kinship scenarios, the overlapping region was observed within a LR range of 0.01 and 100.

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A general approach for combining non-genetic and genetic data to solve complex kinship cases

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Objectives: In forensic investigations, particularly for missing person cases, the combination of genetic and non-genetic data is becoming increasingly important [1]. This study focuses on the development and application of methods to integrate these two types of data effectively. The primary goal is to enhance the accuracy and efficiency of identifying missing persons, primarily in large scale searches.

Material and methods. Genetic data, mainly derived from DNA analysis, forms the cornerstone of modern forensic identification. However, its utility can be limited in cases with insufficient or degraded samples, or in large-scale cases with many unidentified individuals [2]. Moreover, in cases where for example two brothers are missing, distinguishing which of the unidentified person corresponds to the missing requires additional information. Here, non-genetic data, such as eyewitness reports, personal items, features (like e.g. a tatoo) and environmental clues, become valuable. We considered simulated scenarios to show how this information could be used. Our approach involves applying statistical models to merge genetic and non-genetic data. We calculate likelihood ratios to assess the strength of the combined evidence. These ratios compare the probability of the data alignment under various investigative hypotheses. This method helps to quantify the evidential value of the combined data, supporting more informed decision-making in identification cases. We also address potential challenges in integrating diverse data types, like varying data quality and errors due to unreliable testimonies or data entry. Our models consider these factors to ensure the reliability of the combined data analysis.

Results and conclusions. We show how the integration of these data types enhances the overall effectiveness of the investigative process. Importantly, our results point out that considering an integrative statistical framework in complex kinship cases is beneficial in situations with both low and high statistical power considering DNA-based identifications. We present this methodology and the related computational tools as an open-source R statistical package, aiming to make this integrated approach widely accessible and practical for forensic applications.

[1] Marsico, F. L., & Caridi, I. (2023). Incorporating non-genetic evidence in large scale missing person searches: A general approach beyond filtering. Forensic Science International: Genetics, 66, 102891.

[2] Vigeland, M. D., & Egeland, T. (2021). Joint DNA-based disaster victim identification. Scientific Reports, 11(1), 13661.

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Informativity offered by 12 X-STRs (Argus X-12 kit) and 7-XSTRs (Forenseq DNA Signature prep) from analysis of real kinship cases

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Informativity from real kinship cases confirms theoretical expectations besides offering a worthy landscape for paternity testing casework. This is important for auxiliary and more recently implemented genetic systems, such as the Investigator ArgusX-12 kit (Qiagen) or the 7-XSTRs included in ForenSeq™ DNA Signature Prep Kit (Verogen), the last one analyzed by MPS (Massive parallel sequencing). Objective. To describe the informativity from complex kinship cases solved with 12-XSTRs and 7-XSTRs. Material and methods. 24 complex kinship cases including 11 grandmother-granddaughter, 8 full sisters, and 5 paternal half-sisters were analyzed with the Argus X-12 QS kit. LR (ikelihood ratio) values were estimated with an update of the Mexican database (n=1115) for the FamlinkX software for 12 X-STRs and the subset of 7 X-STRs included in the Forenseq™ kit. Results. For each kinship case, we estimated the LR average and range among the corresponding X-STRs, and by linkage group (LG). Some extremely large LR values were observed in different cases for some loci, particularly between full sisters (e.g. LR-DXS10101= 289159), which increased the LR average for these eight cases (LR= 5680). Based on 12 X-STRs, the combined exact LR was larger for full-sister cases (9.5E+17), followed by grandmother-granddaughter (1.1E+08), and paternal half-sisters (6.4E+04). Conversely, considering LR >1000 (W ≥ 99.9%) as a conclusive result, full sisters showed the lowest success rate 75% (6/8), whereas 100% of the paternal half-sister and grandmother-granddaughter cases reached conclusive results. On the other hand, a considerable LR decrease was observed using only 7-XSTRs to analyze these kinship cases ($p<0.05$). This is evident with the low success rate based on conclusive results: 37.5% (3/8) for full sisters, 40% (2/5) for paternal half-sisters, and 36.4% (4/11) for grandmother-granddaughter cases. Conclusions. Full-sister cases showed huge LR values in different X-STRs, but with 12 X-STRs they displayed the lowest conclusive result rate (75%) regarding paternal half-sister and grandmother-granddaughter cases (100%). Evaluation of these cases with 7 X-STRs, diminished significantly the LR values and the conclusive result rate in these kinship cases (average LR= 38%).

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CONSIDERATIONS FOR THE IMPLEMENTATION OF EXPANDED MARKER SETS IN COMPLEX KINSHIP ANALYSIS

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When carrying out kinship investigations involving more complex or distant relationships, genotype data from a large number of forensically-relevant genetic loci (e.g. >30 STRs) is often required to resolve the case with sufficient statistical support. With electrophoretic methods, currently it is possible to target up to ~50 autosomal STR loci when combining data from multiple commercially available kits. If using a massively parallel sequencing (MPS)-based approach however, it is possible to analyse many thousands of SNP loci with assays such as the ForenSeq Kintelligence kit or the FORCE community panel as well as smaller scale marker sets. Whilst increasing the number of genetic data points can increase the likelihood of resolving a case, an important consideration when analysing more markers is the chance of loci being genetically linked. Genetic linkage refers to the propensity of two loci that are close together (i.e. molecularly close on the same chromosome) to be inherited together without recombination occurring between them.
Traditionally, during kinship calculations, genetic loci are assumed to be independent. However, if a recombination event does not occur between two loci, then this assumption is no longer true. STR loci vWA and D12S391 are well-known examples of “linked” loci and when both markers came into use within kinship testing laboratories, a common approach was to disregard data from one of the loci. An analysis strategy that relies on ignoring data from one of each pair of linked loci however, is not reasonable, as it counteracts the overarching aim of testing more markers. The initial apprehension of laboratories to include data from both loci was largely based on the perceived complexity of accounting for genetic linkage within statistical calculations. However, with the development of user-friendly software solutions such as FamLink, and availability of data resources such as genetic maps of forensic loci, including genetic linkage adjustments within kinship calculations is now much more approachable.
This work aimed to explore the impact of genetic linkage on kinship statistics obtained, both in relation to the number of loci targeted as well as the specific kinship scenarios investigated (i.e. the degree of relatedness). This included a comprehensive evaluation of whether genetic linkage corrections significantly influenced the reporting outcome of individual cases, demonstrating that such corrections are critical when analysing linked loci within these forensically-relevant marker panels. Technical details of how such linkage adjustments can be incorporated into a routine data analysis workflow will be discussed.

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Exploring different software options for likelihood ratio calculations in paternity cases with low template DNA.

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Introduction & Objectives
In low template DNA (LT-DNA) samples, the interpretation of STR profiles by calculation of likelihood ratio (LR) must consider the possibility of stochastic artefacts (allelic drop-out and drop-in). Several probabilistic genotyping software were developed for this purpose with main focus on direct identification of contributors to mixed stains. Less attention has been given to kinship cases requiring the analysis of LT-DNA specimens (paternity testing on deceased individuals, identification of human remains through relatives).

With this in mind, we evaluated the performances of three software -Familias, EuroForMix and EFMrep- in a set of paternity cases that required the analysis of LT-DNA samples.

Materials & Methods.
Samples from 25 paternity cases including formalin fixed, paraffin embedded archival tissues, bone specimens, and personal items were studied. Tested pedigrees consisted of both trios and duos (missing maternal information). LT-DNA samples were classified as "highly" (HD) or "mildly" (MD) degraded, based on the quality of STR profiles (longest amplicon with peak height associated to a <5% risk of drop-out, estimated through a logistic model, below or above 200 bp, respectively). Calculations were performed using two PCR results from each LT-DNA sample (two PCR replicates of the same STR kit for Familias and EuroForMix, two different STR kits for EFMrep).

Results & Conclusions
It was observed that in duo-HD, duo-MD and trio-HD cases, average LR values obtained with EFMrep were significantly higher compared to Familias and EuroForMix. In 50% duo-HD, 91% duo-MD, and 60% trio-HD cases, LRs obtained with EFMrep exceed the threshold beyond which paternity is considered proven according to current Italian guidelines (LR>10,000). In trio-MD cases, Familias generated average LR values significantly higher than EuroForMix and EFMrep, although the proportion of LRs>10,000 was equal for all the three software (89%).
These preliminary results can represent the basis for the development of a standardized analytical and interpretative flow-chart in kinship cases involving LT-DNA samples.

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Extracting the value of evidence from a two person Y chromosome mixture by a quantitative computational approach

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Mixtures are of ever-growing concern to the forensic geneticist. Whereas autosomal mixtures have been extensively studied by at least two distinctive viewpoints (the semi-continuous and the quantitative Bayesian principle), a robust theoretical framework to be applied to Y chromosome mixtures interpretation is still to be achieved. A series of hurdles complicate the interpretation of this latter class of forensic evidence:

a) Mixed Y electropherograms let the analyst lose contact with the original constitutive chains of haplotypes;
b) reconstructing haplotype under the perspective of a growing number of contributors makes interpretation increasingly difficult;
c) most haplotypes to be reconstructed by mere combinatorial work starting from the free combination of individual alleles may have never existed;
d) extracting haplotype frequencies from the current databases is considerably time-consuming.

We here introduce a computational procedure enabling to estimate the value of evidence to draw from a two contributors' scheme of interpretation based on a 10-loci protocol of Y typing. All possible haplotypes generated by a free combination of individual alleles are generated by a genotype permutation matrix, containing two columns (two contributors) and 1024 records (210 combinatorial states) allocate into an Excel MS spreadsheet. The spreadsheet is of universal use.
A quantitative calculation based on peak height values and haplotype frequencies is illustrated. The effectiveness of our method of calculation is illustrated by presentation of a few practical examples.

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Instant LR Calculations of Y-STR Matches with the Discrete Laplace Method

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The discrete Laplace (DL) method for calculating the evidential weight of a Y-STR DNA-profile match was developed to avoid overestimating haplotype frequencies, particularly for singleton profiles. The DL method is implemented in the YHRD and has been used in court cases in Germany, Norway, and Switzerland. Recently, we found that, in a reference database with approximately 1,000 Y-STR profiles, adding the Y-STR profile of the person of interest had a negligible impact on the estimated population frequency using the DL method. This means that once the DL model of the Y-STR reference database has been established, the LR of a Y-STR profile can be calculated in a second. We typed 1,022 male individuals in Danish paternity cases with the AmpFISTR Yfiler™ Plus kit (Thermo Fisher Scientific) and estimated DL models for 8, 12, and 17 Y-STRs, respectively. The distributions of Y-STR haplotype clusters were visualised in STRUCTURE-like plots. We wrote a software app in C# with an intuitive GUI. The relevant DL models are uploaded to and saved by the app. The Y-STR profile is uploaded to the app as a text file. The app calculates the haplotype frequency and likelihood ratio within a second and exports the results in a PDF format. Likelihood ratios were calculated for 86 Y-STR profiles from biological stains from Danish criminal cases and compared to those obtained with the counting and Kappa methods. For the 17-marker model, the DL LRs were above 1,000,000 for 73%, above 100,000 for 84%, and above 10,000 for 95% of the Y-STR profiles. For the 12-marker model, the LRs were above 1,000,000 for 53%, above 100,000 for 74%, and above 10,000 for 90% of the profiles. For the 8-marker, model, the LRs were above 1,000,000 for 34%, above 100,000 for 56%, and above 10,000 for 87% of the profiles. Using the Kappa method, the maximum LRs were 4,388, 5,248, and 7,355 for the 8, 12, and 17-marker models, respectively.

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Probabilities of finding trace profile donors and their paternal relatives in Y-STR reference databases

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Forensic investigative genetic genealogy using Y-chromosome short tandem repeat (Y-STR) DNA profiles can give investigative leads in criminal cases by searching for the Y-STR trace profile or very similar Y-STR profiles in relevant Y-STR databases. We conducted a simulation study with Yfiler™ Plus Y-STR profiles to estimate the probabilities of finding matches and near-matches in Y-STR databases and quantified the success rate of finding the trace profile donors or their close relatives. We used the malan R software package to simulate the populations based on the Wright-Fisher model with a variance in reproductive success of 0.2 and a constant size per generation followed by a 2% growth per generation. We generated Y-STR databases by randomly drawing Y-STR profiles from a Yfiler™ Plus Y-STR population data set. In a population of 500,066 individuals, a database size of 0.2% of the population resulted in a Y-STR database match probability of ca. 2%. Increasing the database size to 2% of the population resulted in a Y-STR database match probability of ca. 20%. When a Y-STR match was found in the database, the probability of the matching individual being related within five meioses to the actual donor of the DNA was 50% (including the cases where the Y-STR profile originated from the donor). The search efficiency regarding matches and near-matches, the number of genetic inconsistencies between Y-STR profiles, the database-to-population ratio, etc., were quantified and will be discussed.

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The Unexpected Contradictory Effects of Co-ancestry Coefficient on the Weight of Genetic Evidence

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The shared evolutionary history of a population can be measured through the empirical coancestry coefficient parameter, which indicates the probability of a pair of alleles, each one randomly chosen from a pair of individuals, having the same ancestral allele. It is thus expected that for smaller, more isolated, and/or substructured populations, the coancestry will be higher and the genetic variation between individuals smaller than otherwise. Thus, the proper consideration and estimation of this parameter are crucial for both the accurate inference of profile frequencies and the weight of the evidence in forensic genetics problems.

In this work, we measured the impact of disregarding the population coancestry on the quantification of the weight of the evidence in identification problems involving a reference sample that cannot be excluded as a contributor of a mixture of two or three contributors.

We compared the likelihood ratios (LRs) obtained for 21 STRs when disregarding population coancestry or considering it equal to 0.01 (a reasonable value for modern populations), using two different approaches and sets of data: i. 2x106 pairs of sample data simulated in R Studio were analyzed with algebraic expressions under simplified assumptions, and ii. 156 pairs of complex real casework data were analyzed through specific software considering the associated complicating factors. The first approach allowed the identification of correlations between both coancestry and allele frequencies and genotypic configurations and their impact on the quantification of the evidence. The second aimed at more complex scenarios, including analytical factors. For both approaches, the results obtained were then analyzed considering each marker separately as well as the complete genotypic information of the individuals and mixture samples.

When a reference sample cannot be excluded as a contributor to the problem sample, an inverse correlation between the coancestry coefficient and the LR would be expected. Increasing the coancestry coefficient implies that pairs of unrelated individuals share identical alleles with greater likelihood, decreasing the LR value, also as previous studies have shown. For most analyzed cases, the results were congruent with these expectations but for some cases, an opposite trend was observed.

This study emphasizes the importance of proper estimation of coancestry coefficient and its consideration in identification problems even in modern populations, as the existence of great and/or counterintuitive differences cannot be disregarded. Undoubtedly, a greater impact of this parameter in statistical analyses would be expected if populations with higher coancestry coefficient were considered.

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MitoMetrics: Characterisation of mitochondrial DNA heteroplasmy in hair shafts and its incorporation on likelihood ratio calculations

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1. Objectives
Interpretation of mitochondrial DNA (mtDNA) evidence in a forensic context faces challenges, particularly when reporting heteroplasmy. Segregation of mtDNA in hair, for example, shows tight bottlenecks that result in different heteroplasmic ratios between and along hair shafts, and between hair and other reference tissues of the same donor.

Current forensic interpretation guidelines for mtDNA are based on conventions that derive from practical observations. Most legislations consider two discrepancies between samples as exclusion and one difference as inclusion or as inconclusive. Phylogenetic knowledge suggests that such concepts are outdated and should be replaced by scientific procedures based on ground truth data.

2. Material and methods
The international, collaborative initiative MitoMetrics attempts to collect this body of data for various phylogenies and tissues. In the current phase, we focus on the variation between mtDNA profiles from hair shaft samples from diverse countries compared to those from blood and/or buccal samples. The levels of heteroplasmy detected in the different tissues were annotated, together with the frequency data for these observations.

3. Results and conclusions
We suggest a preliminary model for calculating the evidential value of mtDNA-based evidence by likelihood ratio, moving the field from conventional decision-making to data-based modelling.
The work represents the first attempt to model these events, when reporting mtDNA in a forensic context.

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It’s my car but the drugs are not mine!

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News media often report a person of interest (POI) being arrested with drug packages in his car. Typically, the POI will claim that the drugs do not belong to him, he has no knowledge of it, and/or he was not involved in packing the drugs. The claim that he did not pack the drugs forms the context of phase 1 of this study which involves evaluating the probability of finding the POI’s DNA on the drug packages. The DNA profile recovered may arise from either (i) direct transfer from the POI during contacts unrelated to the packing of the drug package, (ii) indirect transfer from the POI’s car, and/or (iii) direct transfer from another person (non-POI) packing the drug package. Ten cars and their respective drivers were involved in this study. A set of four DNA-free plastic bags, each containing a DNA-free 200 g salt-filled ‘drug packet’ was prepared. The plastic bags were placed in one of four locations in each car – (a) boot, (b) rear passenger seat, (c) floor of front passenger seat, and (d) front glove compartment – for either a short (~2 hours) or a long (2 days) duration. Subsequently, the exterior, handles and interior of each plastic bag were swabbed for DNA. Additionally, an area in the car beside which each of the plastic bag had been placed was also swabbed as a background control. The alternative proposition that the POI had packed and transported the drug packages forms the context of phase 2 of this study. Ten participants were each asked to prepare a ‘drug packet’ with their bare hands, place the packet into a plastic bag, and carry the bag for a walking journey of 5 minutes. The results of this study serve to inform on the probabilities of finding POI’s DNA on drug packages found in cars if he had been involved in its preparation, was merely a courier, or an innocent driver with no idea who left the packages in his car and would facilitate development of likelihood ratio for the above-mentioned claims.

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Interpretation and education: (sub) source and activity issues, similar challenges?

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The purpose of this presentation is to present the mechanisms for success in acquiring skills and competencies in evaluation and reporting. We show that the curriculum needs to be tailored to the role of the participant. We discuss the different means of online and accredited/certifying education: at one extreme, Massive Open Online Courses (MOOC) or short single-topic video clips to raise awareness among scientists and the public. At the other end of the spectrum are certifying, in-depth, longer-term learning courses with individual feedback and tutoring to prepare DNA scientists for the challenges of forensic interpretation in casework and research.

As practical examples of problems best addressed by the latter type of education, we present theoretical and practical concepts that can help scientists formulate more meaningful propositions (hypotheses), for example, for the joint evaluation of autosomal and non-autosomal results. We also discuss problematic reporting that is often observed in the context of alleged activities, particularly when the nature of the biological material is at issue. More generally, we illustrate the similarities among the challenges DNA scientists face in reporting the value of their findings, regardless of the level of propositions.

While much progress has been made in recent years through the use of specialised software (especially probabilistic genotyping), publications and court transcripts indicate that the concept of likelihood ratio is still misunderstood by many forensic scientists. This illustrates that education in forensic DNA interpretation by academics specialising in this field is both necessary and timely. We argue that the future should include the continuing education of DNA scientists/researchers and the education of key players (investigators, prosecutors, defence attorneys, judges) through the creation of flexible learning pathways.

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ENFSI ReAct project: Results of transfer experiments performed by 23 laboratories

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There are many studies on DNA transfer, but generally the number of experiments is small, restricted to one laboratory and the data difficult to access as there is no standardisation on how to present them.

Objectives: ReAct is a project that includes cooperation between 23 forensic laboratories from 20 countries and is funded by the European Network of Forensic Science Institutes (ENFSI) Monopoly programme funding 2020. The purpose of the project is to execute an experimental design that allows comparison of multiple laboratories and provides data to assign probabilities of recovering DNA when activities implying direct or indirect transfer are performed. These probabilities can be incorporated into Bayesian Networks to compute likelihood ratios given activity level propositions. The BNs are incorporated into “Shiny React”, open-access software to facilitate calculations.

Materials and Methods: The experiments simulated burglaries where multiple individuals held a large screwdriver according to different scenarios. EuroForMix was used to calculate mixture proportions of contributors and background (unknown contributors). The “Shiny React” software computes likelihood ratios given activity level propositions for any given DNA quantities. Sensitivity (bootstrap with replacement) analysis is incorporated to help account for small sample sizes. In total, laboratories carried out >2,700 experiments.

Results and conclusions: Differences in recovery rates occurred between different laboratories confirming tendencies observed in other publications and proficiency tests. This means that some caution is needed if laboratories are to base reports on each-others’ findings.
The Shiny React software is used to both compile data and to compute likelihood ratios. It is open source, and the results of tests are open access to help facilitate collaborative research and knowledge transfer in an open-science format. This will also help to promote standardisation of methods.

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Human Factors Recommendations for the Interpretation and Expression of DNA Results

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The study of human factors in forensic science informs our understanding of the interaction between humans and the systems they use. The National Institute of Standards and Technology (NIST)/National Institute of Justice (NIJ) Expert Working Group (EWG) on Human Factors in Forensic DNA Interpretation used a systems approach to conduct a scientific assessment of the effects of human factors on forensic DNA interpretation with the goal of recommending approaches to improve practice and reduce the likelihood of errors. This effort resulted in 44 recommendations. The EWG designed many of these recommendations to improve the production, interpretation, evaluation, documentation, and communication of DNA comparison results. Additional discussions include:

- The potential for cognitive bias and how to reduce it.
- DNA transfer, persistence, prevalence, and recovery.
- Work environments and how they can impact productivity and morale.
- Building a more equipped workforce through investment in centralized forensic education and training.
- How forensic science service provider management and leadership can foster a culture whereby errors are seen as a learning opportunity and not treated punitively.
- Future research and funding directions in forensic DNA interpretation.

In this presentation, we will discuss recommendations and future directions related to the interpretation and expression of DNA results.

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Assessing full-length and sub-regional 16S rRNA gene sequencing for skin and saliva microbiome profiling in forensic analysis

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The human microbiota, with its distinct signatures akin to fingerprints, has emerged as a promising tool for forensic applications, especially for individual identification through skin and saliva bacterial profiles. Despite the potential, the absence of standardized guidelines for selecting optimal 16S rRNA gene sub-regions has resulted in inconsistent outcomes. To address this, we performed comprehensive full-length 16S rRNA gene sequencing on 31 skin and saliva microbiota samples utilizing the PacBio platform, complemented by both in silico and sequence-based evaluations. Our findings underscore the superior taxonomic resolution afforded by full-length 16S sequencing, with the V1-V3 region nearly matching the resolution of the full V1-V9 sequence, outperforming other variable regions such as the V4. Although the community structure analysis at the genus level and clustering assessments through PCoA and NMDS indicated minimal differences among sample types across various hypervariable regions, pronounced distinctions were observed at the species level. We also discovered that primer binding sites across different hypervariable regions exhibit a range of base variations, often extending beyond the capabilities of degenerate primers. Consequently, we advocate for the strategic targeting of specific 16S sub-regions for skin microbiome research, focusing on taxonomic analyses at the genus level or higher. Moreover, we encourage microbiological studies in diverse settings to adopt third-generation sequencing techniques, such as full-length 16S or metagenomic sequencing, to increase the precision and practicality of forensic microbiology.

P-440

Interpretation strategies for microhaplotype DNA profiles generated by next-generation sequencing

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Objectives: Microhaplotypes (MHs) appear to be useful for forensic DNA analysis, such as individual identification, kinship analysis, ancestry inference, and DNA mixture deconvolution. This study aims to explore and establish a complete pipeline for typing and deconvoluting mixed DNA MHs profiles generated by next generation sequencing.

Material and methods: Using a panel of 66 microhaplotypes to analyze a range of known single-source and mixed DNA samples on the Illumina platform. This included a sensitivity assessment using NA12878 DNA dilutions with DNA target masses varying from 0.015625ng to 5ng.We interpreted the characteristics of these MHs DNA profiles in terms of heterozygote balance, noise, inter-locus balance, and allele dropout. And we presented a statistical model for the quantitative read depth, incorporating allele dropout, noise and efficiency of marker-specific amplification. Through this statistical model, we calculated LR values for MHs profiles with varying mixture ratios and completed the deconvolution of the mixtures.

Results and conclusion：The model could be incorporated into continuous probabilistic interpretation approaches for mixed DNA MHs profiles. Notably, noise emerged as a critical factor affecting genotyping accuracy, particularly in scenarios with large mixture ratios.

P-441

Enhanced interpretation of complex DNA mixtures using multiple highly polymorphic MPS-based microhaplotype panels

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Interpreting DNA mixtures has long presented challenges in forensic practice, particularly with traditional genetic markers and techniques offering limited resolution. This limitation often hinders the discriminatory power required to distinguish contributors and non-contributors within complex DNA mixtures, especially in scenarios with limited DNA input or unbalanced mix ratios. However, advancements in novel genetic markers, sequencing technology, and probabilistic genotype software have significantly accelerated and simplified DNA mixture interpretation. In this study, we employed massively parallel sequencing (MPS) to assess the performance of three highly polymorphic microhaplotype panels in detecting complex DNA mixtures, including those from multiple individuals (up to 4 persons), low-template mixtures (as low as 0.05ng), and unbalanced mixtures (as low as 1:40). These panels comprised a 55-plex panel (average effective allele number (Ae) of 7.43), a 67-plex panel (average Ae of 5.34), and a combined 87-plex panel (average Ae of 7.02) formed by merging the aforementioned two panels. Utilizing probabilistic genotyping software EuroForMix, we analyzed likelihood ratio (LR) outcomes in various scenarios, including different degrees of kinship of contributors as the person of interest (PoI), overestimation or underestimation of the number of contributors, varying numbers of known contributors, and differing allelic dropout rates due to low DNA template amounts. The results underscored the superior performance of the three MPS-based panels in analyzing DNA mixtures, even at low DNA template amounts and in unbalanced mixtures. Additionally, we explored variations in LR across different numbers and Ae of loci, utilizing theoretical microhaplotypes from the 1000 Genomes Project, aiming to investigate the LR status under ideal conditions. In summary, our research promises to enhance the effectiveness of DNA mixture interpretation, thereby advancing forensic practice.

P-444

A Software Package for Designing and Interpreting Forensic DNA Validation Studies

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Internal validation of measuring and interpreting single-source and mixture DNA profiles is essential in every forensic DNA laboratory. Validation is not a one-time process as laboratories continue to revalidate when changes or upgrades are introduced to their workflows. This process often generates large amounts of data; forensic practitioners face the challenges of designing validation experiments, as well as formatting, analyzing, and understanding the validation results. Currently, existing software programs analyze (i.e., call alleles), deconvolute, and assign LRs for DNA profiles. However, the field lacks much-needed open-source software that can assist in designing validation experiments and interpreting the resulting data. Here, we present the development of software accessible to users through an easy-to-use graphical user interface (GUI) that can help practitioners in (1) designing validation studies to adequately cover a user selected factor space and (2) interpreting and visualizing the validated data.

The source codes developed for the software are in Python and R programming language formats that will be publicly available. In-house datasets available at the NIST Applied Genetics Group will be used to test the codes and interface. An online community platform hosted on STRBase will be initiated where users can submit questions, report issues, and request additional functionalities. The software consists of the following modules (1) simulation of candidate mixture genotype combinations (up to six persons) from provided ground truth single-source profiles, (2) summary statistics describing the complexity for each possible mixture profile, (3) instructions on calculating how much of each contributor's DNA is required to make the final mixture volume of desired concentration and mixture ratios, and (4) interactive visualization of the validated data. All codes for the different functionalities are wrapped in a drag-and-drop standalone (i.e., not running over a network) user interface. The developed software (1) will aid forensic practitioners in automating the design and interpretation of validation experiments without having to be an expert in programming languages or statistics, and (2) will free the analysts from the technicalities of manual data formatting and processing. To enhance accessibility, the tool will be accompanied by instructions describing the installation of the software, tutorials, and on-demand videos demonstrating the use of the tool.

P-445

A mixture simulation method based on single source DNA profiles

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Objetives:
Simulation studies are a cost-effective and time-efficient alternative to experimental studies. Simulation studies play a crucial role in forensic DNA research, such as validation and evaluation of the probabilistic genotyping (PG) systems. Most in silico DNA simulation methods involve numerous redundant parameters and generate simulated profiles with lower variability than the noisier experimental data. A strategy for simulating mixtures is proposed based on laboratory-generated profiles that take into account the high variability of peak height in experimental DNA profiles.

Material and methods:
The strategy assumes additivity of peak height in gamma modeling and generates simulated mixture profiles using single-source DNA profiles through regression analysis. To create a simulated mixture profile, single-source experimental profiles are multiplied by a coefficient and superimposed. The gamma model is employed to fit the peak height, while Weighted Least Squares (WLS) linear regressions are used to fit the peak height expectation and template. The relationship between the coefficient and the quotient of peak height expectation to template of DNA mixtures was determined through WLS regression analysis.

Results and conclusions:
The simulated profiles generated by this method exhibited a high level of agreement with experimental profiles. Our proposed simulation method differs from other strategies in that it does not require complex parameter considerations and can generate mixtures with higher variability. The simulated method can also generate mixtures that are not commonly available, such as series of mixtures with common contributors or mixtures with related contributors. This provides the necessary support for typing complex DNA mixtures with kinship participation and for the bunching and merging cases.

P-446

The impact of the drop-out in the evaluation of LR with the continuous approach

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1. Objectives
In the context of our internal validation of mixed genetic profiles and the related bio-statistical calculation, trying to identify the limits in the application of a probabilistic genotyping software (PGS), we asked ourselves: how much and in what ways can the loss of genetic information relating to the POI impact on the calculation of the LR and its reliability? Does a maximum number of drop-outs exist beyond which the LR may not be reliable?

2. Material and methods
We selected two mixed genetic profiles of 2 contributors (2PM) from our routine criminal cases, with the ground truth reasonably certain, and we mimicked a progressive loss of genetic information (sequential drop-out from 1 to 21), up to a loss of approximately 50% of the profile of the minor contributor. Then we calculated the LRbase for the basic hypothesis [H1/H2=POI+U/U+U] and 3 LRsFam, replacing in H2 a relative of the POI (father/son or brother or cousin).

We also repeated the study on ten 2PM from the PROVEDIt dataset (with various Mixture Ratio, degradation and DNA input), studying the profiles with 0, 6, 12, 21 drop-outs, corresponding to 0%, 15%, 30% and 50% loss of genetic information of the POI.

The PGS used for the study was EuroForMix (vers. 3.3.1/4.0.8), with the allele frequencies of the Italian reference population and a theta factor equal to 0.01.

3. Results and conclusions
The different decreases in LR, as the number of drop-outs increases, allowed us to observe that there is no possibility of determining a common threshold value for the drop-outs with which to consider the LR not reliable.

The loss of information does not impact the LR calculation in the same way in all the mixtures studied, and also it changes also in relation to: the NOC, if the POI is the major or the minor contributor, the quality of the profile (degradation, LT-DNA), the frequencies of the remaining alleles, as well as, in the real cases, the available information and circumstances regarding the case.

The analyses of these LR results have suggested the possibility of using the LRsFam in the final report for the court, if they go to neutral value or veer to support at H2. These values could be considered as a warning, above all in those criminal cases (e.g., burglary, robbery) in order to use the most objective, impartial and transparent explanatory approach.

P-447

An overlooked treasure; the relationship between the back stutter rate and the double back stutter rate

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In order to understand the process of DNA stuttering, many researches have been conducted using simulations. In these simulations the Stepwise Mutation Model (SMM) is usually used. MPS This study shows that, using only the back-stutter ratio (r1), the double back-stutter ratio (r2) in SMM can be estimated as 1/2*r1^2. This approximation is in good agreement with the MPS data. In SMM, a multinomial distribution with three parameters is used to calculate the number of back-stutters: the probability of not being amplified (p1), amplified with no stutter (p2), and amplified with a back-stutter (p3). Using these PCR probability parameters, and the initial template number (N0), and the number of PCR cycles (C), the mean amplicon copy number without stutter (a0) can be estimated as N0*(1+p2)^C. Similarly, the mean amplicon copy with back-stutter (a1) is estimated as N0*C*(1+p2)^(C-1)*p3. This estimation can be explained as that the number of chances to produce back-stutter is C and the amplification effeicency is (1+p2)^(C-1)*p3. When we approximate r1 as a1/a0, r1 is estimated as C*p3/(1+p2). In the SMM model, the double back stutter is produced through two back stutter PCR processes. The number of chance to produce double back stutter is calculated as 1/2*C*(C-1) and the amplification effeicency is (1+p2)^(C-2)*p3*pp3, where pp3 represents the second back-stutter producing probability. Therefore, the mean amplicon copy with double-back-stutter (a2) is estimated as N0*1/2*C*(C-1)(1+p2)^(C-2)*p3*pp3. By approximating C-1 as C and pp3 as p3, a2 can be approximated as 1/2*a1^2/a0. To the best of our knowledge, this relation is not published. Thus, when we obtain information about both a0 and a1, the double-back-stutter (a2) can be estimated without any other information. Similarly, r2 can be estimated as 1/2*r1*(C-1)*{pp3/(1+p2)}. Using the previous approximation, it boils down to 1/2*r1^2. Using MPS data, this approximation is shown to be mostly correct. However, the experimental data are usually larger than the estimate. This suggests that small a2 is not observed due to the analytical thresholding and that there are processes which produce double-back stutter in a cycle. It is known that r1 depends linearly on the number of repeats (l). With using parameter a and l0, p3 can be expressed as a*(l-l0), and pp3 can be substituted by a*(l-1-l0), since the template length is one repeat shorter. The r2 can be expressed as 1/2*C*(C-1)*{a*(l-l0)/(1+p2)}*{a*(l-1-l0)}/(1+p2)}. This implies that r2 is a quadratic function of l. This approximation fits well the data from MPS experiment.

P-448

DNA mixture interpretation: A guiding principle to identify the most supported propositions

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Over the past decade, the field of DNA mixture interpretation has seen substantial advancements in statistical and computational methodologies, greatly influencing casework workflow and interpretation practices. These technological developments necessitate a revision of existing guidelines for mixture interpretation in DNA casework. In this poster, we share the procedure for DNA mixture interpretation as employed within our laboratory, which is centered around a guiding principle: identifying the propositions that are best supported by the data within the context of the case. This principle is versatile and can be applied across all steps of interpretation. By logical reasoning one can decide on the necessity to perform weight of evidence calculations, to decide on what relevant possible explanations for the DNA profile must be considered and to decide how to report the evidence. Unlike traditional methods that limit the interpretation to a predefined pair of propositions, this approach advocates for a dynamic evaluation process. Here, propositions can be customized and refined based on emerging insights from the statistical analysis, leading to a more nuanced understanding of the DNA evidence. We argue for this flexible propositions-driven approach and discuss its implications for forensic casework. Additionally, we present practical casework examples where this method has been successfully applied, demonstrating its effectiveness in the DNA mixture interpretation process.

P-449

An empirical comparison of qualitative vs quantitative models in the statistical evaluation of forensic genetic evidence

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Likelihood Ratio (LR) estimation between two opposing hypotheses is a routine part of forensic genetics laboratory tasks when analysing biological contact traces from crime scenes. This calculation can become challenging when considering all the possible stochastic effects that could affect a DNA sample, such as drop-out, drop-in and stutter. These effects require use of complex mathematical models.

Such models can be classified as qualitative or quantitative depending on the information employed: the former only uses allelic presence/absence, while the latter considers the intensity of the signal, which in traditional STR analysis translates to Capillary Electrophoresis (CE) peak height. This intensity is positively correlated to the amount of DNA contributed by each mixture donor and could be useful in the analysis of complex mixtures, as quantitative models can infer to which contributor an allele is most likely to belong based on its peak height relative to the system's whole fluorescent signal. Although promising, quantitative models must be explored and validated for their use in forensic routine.

We conducted an internal validation process for assessing the quantitative model implemented in the software EuroForMix, by comparison with the results obtained using the qualitative model implemented in LRmix Studio. We investigated the effect of each model using different scenarios and complex mixtures comparing their accuracy, sensitivity, and specificity, as well as their effect on the magnitude of the LR values obtained. Moreover, using DNA mixtures of known ratios, we compared the estimated contribution percentage of each donor from EuroForMix with the expected values.

P-479

Comparison of three DNA quantification methods for forensic blood samples

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1. Objectives
Suicide is one of the leading causes of death worldwide and its research towards prevention is critical. Many factors can contribute to suicidal behaviour, including social, psychological, and psychopathological aspects. In this paper, we aim to identify genomic and epigenetic risk factors associated. The first step, in order to reach our goal, is to establish which DNA quantification method is the most reliable for sample handling. The Institut de Medicina Legal i Ciències Forenses de Catalunya (IMLCF) provided blood samples from suicide victims in the Barcelona metropolitan area for quantifying and analysing.

2. Material and methods
We considered three different quantifying methods: Quantifiler Human DNA Quantification Kit, a selective method, and Qubit, and Nanodrop, non-selective methods. The first technique is based on measuring amplifiable human DNA through a software that analyses spectral data. Qubit is a fluorimeter that detects fluorescent fluorochromes while Nanodrop uses a UV-Visible spectrophotometer for micro-volume analysis.

3. Results and conclusions
48 samples were analysed using Quantifiler, Qubit, and Nanodrop. Quantifiler showed significantly higher values of DNA compared to Nanodrop and Qubit, concluding that while it may not be the most suitable method for quantification, it can be useful in predicting STR profiles. On the other hand, Nanodrop and Qubit did not show significant differences between them. Our statistical and analytical tests show that these two techniques are the most viable options. Knowing that UV quantification in Nanodrop can be less precise, we conclude that Qubit is the most suitable method for quantifying DNA blood samples.

P-493

A practical proposal for interpreting X-STR profiles from the Forenseq DNA Signature Prep (MPS kit) based on ArgusX-12 population databases

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The Forenseq DNA Signature Prep kit (Verogen) analyzed by Massive parallel sequencing (MPS) includes 7 X-STRs that work as a complementary tool to disclose complex kinship cases. However, X-STR databases of repeat sequence-based (RSB) alleles from MPS are not currently representative of most worldwide populations. Thus, larger databases of length-based (LB) alleles can work better for forensic casework interpretation; in this case, 7 X-STR databases can be completed from those available in the ArgusX-12 kit, preferably in the FamlinkX format, the specific software for statistical interpretation of X-STR profiles. Objectives. 1) To update the Mexican population database for ArgusX12; 2) to generate a practical LB database for interpretation of 7 X-STR profiles (Forenseq kit); 3) To evaluate the impact of population database updating from the interpretation of different kinship cases; 4) To evaluate the impact of diminishing the number of X-STRs from the interpretation of different kinship cases. Material and Methods. We obtained 222 length-based (LB) haplotypes with the Investigator Argus X-12 kit QS (Qiagen). We estimated different forensic parameters and completed two updated FamlinkX population databases based on 7 X-STRs and 12 X-STRs. Results. For both 7 and 12 X-STR kits, we describe allele and haplotype frequencies, forensic informativity parameters (MEC, Het, PIC, and PD), and FamlinkX databases with the updated sample size (from 933 to 1115). Based on the original (Cortes-Trujillo et al. 2016) and updated databases, we estimated the likelihood ratios (LRs) of four complex kinship cases: Maternal and Paternal half-sisters, Paternal half-sisters (data mother), and Paternal grandmother-granddaughter. The LR values from 7 X-STRs comprised -on average- only 0.8% regarding those based on 12 X-STRs. Conversely, the updated Mexican population database increased significantly the LRs estimated in these kinship cases (average 183.1%). Conclusion. We demonstrated how LB allele population databases constitute a practical option for interpreting complex cases analyzed with the 7 X-STRs of the Forenseq kit in Mexico, but probably also be useful for other Latin American populations. We showed a landscape of the statistical interpretation impact by reducing the number of X-STRs and updating the population sample size.

Posters Topic 7

Investigative Genetic Genealogy

7. Investigative Genetic Genealogy

Abstracts Poster

P-025

Forensic Kinship Classification Based on CE Genotyping Platform from the Machine Learning Perspective: A Pilot Study on A Newly Validated Six-Dye InDel Panel

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Kinship analysis in trace amounts and degraded biological samples has consistently posed a challenge in forensic practice. With shorter amplicons and no stutter peak, Insertion/Deletion polymorphisms (InDels) have been proven to offer unique advantages in the forensic identification of aged and degraded biomaterials, significantly impacting kinship analyses of deceased individuals and their potential living relatives, as well as the pedigree analysis of natural disaster victims. Despite the efficacy of these markers in forensic paternity testing, room for improvement remains in identifying 2nd-degree and more distant kinships, due to the current likelihood ratio (LR) cutoff threshold-based classification strategy. Based on population genetics data of 15 East Asian populations and multiple kinship parameters including identity-by-state (IBS) scores, identity-by-descent (IBD) scores, and LR values, this pilot study replicated the common workflow based on LR cutoff thresholds and applied a recently validated InDel panel to preliminarily develop a machine learning (ML) workflow for forensic kinship multi-classification. Our results reveal distinct data distribution patterns between the same pair of individuals under different kinship parameter feature combinations, providing multidimensional information for kinship analysis. In the binary classification of 2nd-degree relatives and unrelated pairs, when the common workflow and the machine learning workflow achieved a similar accuracy rate of 0.9194, the ML method had a conclusiveness rate (CR) of 1.0 compared to the former's 0.7066. In the multiclass task without preset relationships, the common workflow had a macro f1 score of 0.5212 and a CR of 0.7046. However, the ML-based workflow showed that the optimal model-feature combination (XGBoost-IBDscores+LR) could reach a definitive conclusion for all the samples, with a macro f1 score of 0.9020. Additionally, in both the training and validation sets, optimal models were predominantly ensemble learning models like LightGBM and XGBoost, which exhibited higher macro f1 scores for kinship multiclassification (> 0.9) as well as faster fitting speeds (<1s). In summary, the ML-based kinship analysis workflow enhanced the forensic kinship analysis efficiency based on the InDel genotyping system and provided a reference for future kinship analysis combining multiple parameters.

P-037

A new algorithm for robust detection of IBD segments from challenging DNA samples

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Objetives: Identity-by-descent (IBD) segments are widely used for kinship inference. However, existing methods for IBD segment detection are powerless for challenging materials.

Material and methods: Here, we propose a new method, clusIBD, which is an error-aware algorithm for IBD segment detection using unphased genotype data. We evaluated and compared the performance of clusIBD with IBIS, TRUFFLE and IBDseq using simulated data, artificial challenging materials and ancient DNA samples.

Results and conclusions: The results showed that all the four methods could detect IBD segments and infer a relationship with high accuracy when the genotype error was low (<0.5%). However, the accuracy decreased with increasing genotyping error, dropping to ~0 when the error was 5% for IBIS, TRUFFLE, and IBDseq. In contrast, clusIBD was still able to robustly detect long IBD segments (> 20 cM) at an error of 5%. clusIBD succeeded in classifying many relationships with input DNA of 0.1ng and for samples with fragment sizes of ~150bp, while the values for all the other three methods were 0. Furthermore, clusIBD could identified more relationships from ancient DNA samples, which had been assigned as unrelated in the previous study. In conclusion, clusIBD could robustly detect IBD segments from materials in various quality and quantity and may be a promising tool for ecological studies, investigative genetic genealogy, and ancient DNA analysis.

P-064

A Profile of Unidentified Human Remains Cases Solved Using Forensic Genetic Genealogy

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The National Missing and Unidentified Persons System (NamUs) is the largest federal database in the United States for missing, unidentified, and unclaimed persons cases. NamUs is the nation's only centralized federal program that offers free forensic, investigative, and analytical services to resolve any long-term case, regardless of age, ethnic affiliation, biological sex, gender identity, or circumstances of death to assist with matching long-term missing persons with unidentified decedents to resolve cases and provide families with resolution. With over 14,000 unidentified human remains cases housed within the NamUs database, a retrospective analysis was conducted to evaluate the use of FGG for unidentified decedent casework. This presentation will focus on over 700 cases submitted for FGG and biogeographic ancestry analyses. All samples are initially screened for quality, prior to genome-wide single nucleotide polymorphism (SNP) generation. Data was evaluated to better predict case outcomes based on metrics including degradation index, quality assessment, and number and degree of relatives available in databases. Additionally, the accuracy of anthropological assessment of race/ethnicity as compared to biogeographic ancestry estimations were evaluated. At least 25% of the examined cases had uncertain biological ancestry measures, using anthropological methods alone. An additional 15% of the database had two anthropologically measured designations, the most common dual assessment being "White / Caucasian" and "Hispanic / Latino". A combination on MDLP-based classification and traditional PCA methods, enabled assignment biogeographical ancestry very accurately, even for cases that had ambiguous anthropological assessments.

P-100

A case study of a male inferred to be of Japanese origin based on Y-STRs haplotypes.

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[Introduction]
Even today, about 80 years after World War II, children born to Japanese men and Filipino women before and after the war (Japanese orphans left behind in Philippines) are still alive and reaching old age. In fact, according to a survey conducted by the Ministry of Foreign Affairs (FY 2018), there are approximately 1,000 survivors whose Japanese nationality has not been restored and who remain stateless, regardless of whether their father's identity has been determined or not. One of them (Mr. A), with the cooperation of a certain NPO, filed a lawsuit with the court, however, the case was dismissed because there was no documentary proof such as family register. A company was asked to test the Y-STR haplotype (y-ht) and the Y haplogroup (y-hg), but the y-ht did not match any possible person in the investigation. We appealed to the high court without any new and glaring evidence, but the appeal was dismissed. Accordingly, I was consulted from a NPO to see if there was any procedure to analyze the situation.

[Materials and Methods]
We used 15 alleles from 17 Y-STRs of Mr. A's Yfiler tested by a certain company as y-ht. As controls for Japanese and Filipinos, data of 1280 and 790 y-hts, respectively, were analyzed by excluding y-hts including null and duplicated alleles from the literature and web databases. A total of 2071 y-hts of data from these 15 loci were used for principal component analysis and network analysis.

[Results and Discussion].
Comparing the y-hts of Mr. A with those of Japanese and Filipinos, the y-hts of Mr. A were characteristic of Japanese. As a result, it was presumed the Y-chromosome inherited in Mr. A was derived from Japanese, not Filipinos. Additionally, in the response letter as an expert opinion, it would be possible to estimate the generation to some extent by genome-wide analysis, etc., if their DNA samples were collected before their death because such orphans become elderly. This written response was submitted to the High Court as new evidence and a retrial was requested, which was granted, and Mr. A was granted Japanese citizenship. Unfortunately, however, about a year after the resolution, Mr. A passed away due to illness.

P-113

Assessing a High Throughput SNP Assay for Closed Scenario Kinship Investigation

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Experiments were conducted using the ForenSeq Kintelligence HT Kit, a highly multiplexed single nucleotide polymorphism (SNP) assay targeting more than 10,000 SNP loci using the MiSeq FGx System. The increased throughput assay is intended for use in closed scenarios such as disaster victim identification, facilitated by Universal Analysis Software (UAS, version 2.6) running a local database for sample comparison, pedigree construction, and kinship coefficient calculation. The Kintelligence HT kit is designed for a capacity of 36 high-quality reference samples or 12 challenging samples per sequencing flow cell, including positive and negative controls. Experiments were completed in four sequencing runs. The first two runs consisted of a dilution series of single-source reference quality DNA (100 pg, 500 pg, and 1000 pg DNA input) processed in triplicate. The third run examined ten samples: six artificially degraded mock postmortem samples with degradation indices ranging from 2.4 to 43.5, as assessed using qPCR methods, and four non-degraded samples. Run four included 34 antemortem samples, a subset of which were related to those in run three. Related samples included two parent-child trios with the child samples present in moderately and highly degraded forms on run three and parents as references on run four. Eight samples with previous support for a full sibling relationship were sequenced in runs three and four, with one of the sibling pairs on each run. Two unrelated samples were run as artificially degraded or intact on runs three and four respectively to assess the impact on SNP call rates and kinship metrics for self-comparison. Kinship coefficients calculated by the UAS reflected the known relationships between the samples. Analysis of degraded DNA yielded a slightly lower SNP locus call rate of 90.1 % (± 3.8 %), relative to non-degraded samples (93.7 % ± 0.7 %) on the same sequencing run. Low-input samples resulted in lower SNP locus call rates at 100 pg input (68.0 % ± 5.3 %) than samples run with 500 pg input (85.7 % ± 13.6 %) or 1000 pg input (97.5 % ± 0.4 %). Lower SNP call rates resulted in an apparent decrease in the amount of shared DNA between relatives, which in turn produced more distant relatedness estimates. However, in a closed scenario investigation we expect minimal impact from underestimating the degree of kinship. Challenging degraded or low DNA input samples were able to be searched in the UAS 2.6 database, with successful kinship attribution.

P-115

It’s all relative: A multi-generational family study using Kintelligence

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The successful application of Forensic Investigative Genetic Genealogy (FIGG) to the identification of perpetrators of serious crime and unidentified human remains in the United States (US) has led to a growing interest in its use internationally and particularly in Australia. To date, FIGG has required whole genome single nucleotide polymorphism (SNP) data from whole genome arrays (WGAs) or sequencing (WGS) which is uploaded to law enforcement permitted commercial DNA databases to search for genetic relatives of a donor. WGAs are cost-effective but have limited success with low quantity and quality forensic samples. WGS overcomes these limitations but at a higher cost and requirement for bioinformatics expertise while both methods generate medical/health related data.

In the last decade, massively parallel sequencing (MPS) platforms and assays for human identification and investigative purposes have been developed and used operationally. In 2021, an FIGG assay, ForenSeq® Kintelligence (Qiagen), was designed as targeted amplicon sequencing assay on the MiSeq FGx Sequencing system to overcome the challenges associated with WGA and WGS SNPs. Of the 10,230 Kintelligence SNPs for ancestry, phenotype and identity analysis, and Y and X for biological sex determination, kinship association is performed using 9,867 SNPs of those SNPs. Kintelligence SNPs were selected to overlap with commonly used WGAs and to exclude medically relevant data. The small amplicon size (<150bp) and 1ng DNA input assists in the recovery and analysis of degraded DNA.

A comparative study of a multi-generational family (n=12) with 1st to 6th degree relationships was undertaken using Kintelligence to compare the relationship prediction outcomes with a commonly used WGA, the Illumina Global Screening Array-24 version 3.0 Beadchip. Using the GEDMatch Pro (GM Pro) user interface, Kintelligence consistently predicted 1st-3rd degree relationships correctly and while 4-5th degree relationships were within the correct shared centimorgan (cM) ranges for the known relationship, the GM Pro user interface generally indicated more distant relationships. The 6th degree relationship, not detected using Kintelligence, was detected using GSA as a more distant relationship.

Kintelligence is a viable ‘in-house’ alternative to external service provision for FIGG that can be analysed using the MiSeq FGx validated for forensic use. Additional assessments of compromised, low quantity DNA samples and extended studies of related individuals are required to accurately determine the limits of its application for criminal and coronial investigations.

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How to Talk to Your Genetic Genealogist: The Language of Kinship

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1. Objectives: to clarify terminology and definitions within the field of FGG. While genetic genealogy has the power to help solve previously unsolvable cases as well as uncover new types of crimes, its relative newness means that there may not always be commonly understood terms to describe the work being performed. In addition, many of the terms used in the field—especially in communication between scientists, law enforcement, and genealogists—are inaccurate, misleading, or confusing. Labs are often described as performing genetic genealogy when, in reality, the labs are processing DNA to generate a DNA profile so that it can be uploaded and used by genetic genealogists to perform their research.

2. Materials and methods: Genetic genealogy involves identifying the DNA contributor's genetic relatives, building family trees, and finding connections between the trees. Genetic genealogy is not "new technology;" the DNA profile isn't "uploaded to family tree databases over months;" neither law enforcement nor the genealogists have "access to troves of genetic information" that they "can then link to the DNA of unknown suspects;" and they don't interview hundreds of people in a family tree.

Additionally, terminology is important. While scientists and others sometimes describe the familial relationship between two people in terms of steps, degrees, STRSs, SNPs, or ranges, genetic genealogists use centimorgans (cMs) and familial relationship terms including grandparents, aunts, uncles, and cousins. A "second degree relationship," for example, means one thing to a scientist and another to a genealogist. Accuracy and consistency in terminology is key to having productive discussions about a case.

3. Results and conclusions: Describing what it is that genetic genealogists actually do and understanding the terminology they use will be useful in creating a multicultural, multilingual, inclusive environment where we can communicate effectively and not proliferate false information. Common definitions and terminology are key to making this a success.

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UnKintelligible: Two Genealogists' First Look at Kintelligence

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Objective
Verogen's ForenSeq® Kintelligence kit, released in February 2021, was designed for use with Forensic Genetic Genealogy (FGG). While it claims to address issues of privacy and is "explicitly curated" for FGG, the linear, scientific approach has inadvertently left genealogists out of the loop. In this presentation we assess the results of a Kintelligence kit head-to-head with consumer DNA database match lists and discuss the practical application and possible limitations of kinship determination with a bracketed approach from a genealogist's perspective.

Materials and Methods
Using two genealogists' DNA processed through Kintelligence Kits, we will compare those results to corresponding results across the various consumer DNA platforms and databases. Comparisons will be done on various factors such as the quantity of matches, distance of matches, and similarity of centiMorgans (cMs) across databases for the same matches. Once the depth, range, and consistency of the Kintelligence matches have been established, we will analyze how these factors compare to law enforcement cases we work on, with the goal of answering several questions:

- How many matches would these cases have gotten using Kintelligence?
- Would the highest match in FTDNA / GEDmatch have been included in the Kintelligence results?
- What would the most distant match have been in Kintelligence?

Results and Conclusions
Based on this analysis we will discuss the many variables that exist when doing genetic genealogy research and assess the usefulness of Kintelligence for law enforcement cases:

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Research on distant kinship estimation using Single Nucleotide Polymorphism: utilizing the ForenSeq® Verogen Kintelligence Kit

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There are several methods used to identify family relationships between individuals based on genetic information. These methods use various genetic markers, such as Single Nucleotide Polymorphisms (SNPs) or Short Tandem Repeats (STRs), to infer the degree of relatedness. Each approach has its strengths and limitations, and the choice of method depends on factors such as the genetic markers available, the required computational resources, and the complexity of the relationship.

SNP genotyping technologies is currently used for forensic purpose such as human identification, phenotype, ancestry estimation and kinship analysis. SNPs, small genetic variations occurring within individual chromosomes, offer higher genetic information compared to traditional STR methods. Based on this premise, we aim to validate the effectiveness of SNPs system for confirming distant kinship relationships using the Verogen® Kintelligence Kit (Verogen Inc.).

The Kintelligence Kit, developed by Verogen Inc., utilizes Next Generation Sequencing (NGS) technology and Single Nucleotide Polymorphism (SNP) analysis to estimate kinship relationships. Kinship coefficients are calculated from the data to provide quantitative measures of relatedness between individuals within and across family groups.

In this study, we aim to validate the effectiveness of the Verogen® Kintelligence Kit by analyzing genetic data from 4 family groups, comprising 23 participants. The family relationships were appropriately inferred. In regions where cM (centimorgan) overlaps, such as between children, parents, and siblings, we could appropriately differentiate relationships by utilizing biological information such as age. We anticipate that our research findings will demonstrate the efficacy of the SNP-based system for confirming distant kinship relationships.

P-119

Construction of a new multiplex system and integrated mutation rate analysis of 11 Y-STRs in father-son pairs

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Objectives: In recent years, forensic genetic genealogy (FGG) has grown rapidly as an emerging field of forensic genetics. Particularly since 2010, rapidly mutating Y-STRs (RM Y-STRs) have shown great potential in distinguishing paternal-related males owing to their high mutation rates. Combining Y-STRs with high mutation rates holds the promise of differentiating between male relatives on the same paternal line and thus identifying suspected criminals within the family. Therefore, we were committed to screening more RM Y-STRs for paternity identification.

Material and methods: In this study, we have screened 11 Y-STR markers and constructed a multiplex system to analyze the pattern of mutations in 108 DNA-confirmed father-son pairs. The system used the five-dye chemistry, which includes FAM, HEX, TAM, ROX, and the Marker SIZ-500, and all PCR products were detected by the Applied Biosystems 3500 Genetic Analyzer.

Results and conclusions: The results showed that a total of 7 mutational events were detected in 108 father-son pairs, and all were one-step mutations. The average estimated mutation rate of these 11 Y-STRs was 5.9 × 10-3 (95% CI 2.4 × 10-3 - 12.1 × 10-3). Among them, there are 6 Y-STR markers with no mutation, including DYS447, DYS443, DYS513, DYS484, DYS452 and DYS510. It’s noteworthy that DYS630 and DYS516 have the highest mutation rates, with a mutation rate of 18.5 × 10-3 (95% CI 2.2 × 10-3 - 65.3 × 10-3), which meets the mutation rate screening criteria for RM Y-STRs.

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Mixture Deconvolution of Kintelligence Profiles and Kinship Inference Assessment

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ForenSeq Kintelligence SNP profiles can be used to perform kinship inference with publicly available SNP databases, such as GEDmatch. Currently, this is limited to single-source profiles, which excludes mixed samples commonly encountered in forensic casework. However, probabilistic genotyping software, such as EuroForMix (EFM), can perform deconvolution of mixed samples, generating SNP profiles of individual contributors that can be uploaded to GEDmatch PRO to perform kinship inference. We have developed a method utilizing EFM to perform mixture deconvolution of two-person mixtures. This presentation will focus on the development of the method and the optimization of parameters to determine what mixture conditions and degree of relatedness (DOR) it could be applied to. After this presentation, attendees will understand the capabilities and limitations of this method.

The method consists of initial stratification of Kintelligence SNPs, performing mixture deconvolution in EFM on each SNP set, and applying specific allele probability thresholds to the deduced genotypes to generate a final SNP profile to upload to GEDmatch PRO. Mixtures can have a wide range of conditions that can vastly affect the ability of the software to successfully deconvolute the individual contributor profiles and thus affect the ability to correctly match and predict the DOR to genetic relatives. To identify the ranges of eligible mixture conditions, we performed a comprehensive assessment using mixed samples with a wide range of ratios and DNA input amounts. For comparison purposes, contributors to the mixed samples had known SNP genotypes and genetic relatives in GEDmatch ranging from first to fifth DOR. For each deduced SNP genotype, we ran the one-to-many kinship tool in GEDmatch PRO to evaluate the capability to match both the known genetic relatives and predict the correct DOR, but also to evaluate any matches to individuals of unknown relation.

We were able to identify the correct genetic relatives and DOR for a range of mixture conditions. While some mixtures were able to identify the correct genetic relative, the DOR was off by one degree (i.e., second degree predicted to be third), and other mixtures were not able identify the relatives. Better results were observed with conditioned analysis (providing genotypes of a known contributor) versus unconditioned (two unknown contributor profiles); and utilizing replicate samples versus a single sample for deconvolution. In conclusion, this method has the potential to expand sample eligibility for Investigative Genetic Genealogy to mixed samples for a range of mixture conditions to identify potential genetic relatives.

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Forensic complex kinship inferences with a combination of STRs and MHs accounting for linkage

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In forensic genetics, kinship identification is pivotal for criminal investigations, inheritance disputes, finding missing persons, and identifying remains. It involves analyzing DNA markers in biological samples and determining relationships based on genetic principles. Linkage is a consequence of the biological phenomenon recombination occurring within a pedigree, and the effect of which was minimal on average, but more considerable in individual cases for kinship analysis. In this study, we included 19 short tandem repeats (STRs) from AGCU EX22 kit, 53 microhaplotypes (MHs) in Panel A, and 67 MHs in Panel B, and contracted a theoretic SNP panel with 9,622 loci selected from the whole genome. The system power in kinship analysis for STR + Panel A, STR + Panel B, Panel A & B, and STR + Panel A & B with fully accounting for linkage were assessed based on 198 family samples and simulated data. STR + Panel A & B exhibited a higher system power of all for distinguishing 1st- to 5th-degree related form unrelated, follows as Panel A & B > STR + Panel A ≥ STR + Panel B. When threshold |t| = 4, STR + Panel A & B was sufficient for the discrimination of full-sibling (FS), and the effectiveness for 2nd-degree were over 0.96 with small overlaps (< 0.06%). For 3rd-degree relatives, the effectiveness was over 0.75 when |t| = 2 with the false testing level (FTL) of 5.37%. The 9K panel with 9,266 SNPs was sufficient to separate 3rd-degree, while not for 4th-degree with effectiveness of 0.966 when |t| = 4. Discriminating 2nd- and 3rd-degree or within 2nd-degree relatives of half sibling (HS), uncle/aunt-nephew/niece (UN) and grandparents-grandson/granddaughter (GS) seems very challenging, even with the utilization of the 9K Panel. The inclusion of a carefully selected additional relative (AR) which was GS for the first person and unrelated for the other (GS-un) and AR (PC-UN), might have potential improvements in the discrimination power between GS and UN/HS. Similarly, the inclusion of AR (PC-GS) might show promise in discriminating UN duos from GS/HS. However, there was only a little enhance in efficiency when including an additional relative for HS duos. The outcomes of our study offer a valuable strategy and guidance applicable in forensic practice, particularly in addressing complex kinship analysis by the combination of STRs and MHs.

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Investigating the correlation between mutations and meiosis in Chinese pedigrees based on 30 newly suggested Y-STRs with high mutation rates

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Objectives: In recent years, a new multiplex genotyping panel termed RMplex, which comprises 26 rapidly mutating Y-STRs (RM Y-STRs) and 4 fastly mutating Y-STRs (FM Y-STRs) was introduced and validated in multiple populations. There is no research in forensic genetic genealogy on whether a correlation exists between mutations of Y-STRs and the number of meiosis at higher levels of patrilineal relatedness, which might have the potential to distinguish paternal kinship degree.

Material and methods: To investigate this correlation, 281 males from 29 family lineages were studied. These samples covered 423 pairs separated by 1-5 meiosis, totaling 1264 meiosis. Based on the number of meiosis, the number of mutant loci and the cumulative number of mutant steps of the sample pairs in the RMplex system were respectively counted. Scatter plots were graphed and the linear regression was constructed to explore their relevance with the number of meiosis.

Results and conclusions: The findings indicated that the mutations in 30 Y-STRs showed a linear increase in the number of meiosis. In particular, the slope of the line was almost constant in 1-4 meiosis, and slightly decreased in the 5 meiosis. It is suggested that there is a correlation between the mutation of the RMplex system and the number of meiosis in deep-rooting Chinese pedigrees.

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Measures to Mitigate Genetic Privacy Risk and Support of Defensible Hypothesis Testing by Forensic Genetic Genealogy in Publicly Accessible Judicial Proceedings

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Massively parallel sequencing (MPS) is revolutionizing the forensic field by greatly expanding analytical throughput which in turn is increasing investigative leads even with some of the most intractable of samples. Forensic genetic genealogy (FGG) leverages the power of MPS and volunteer donated profiles housed in genetic genealogy databases to associate near and distant kinship relationships that can be used through genealogical research to identify crime scene sample donors or unknown human remains (UHRs). Additionally, dense SNP profiles can be compared directly between evidence samples and a person of interest or an antemortem sample and UHRs generating likelihood ratios that far exceed that of any STR-based system. Several countries (United States, Sweden, Australia, Norway, New Zealand, Finland, Estonia, etc.) have either applied or are investigating implementation of FGG to develop leads in cases in which standard DNA technology (i.e., short tandem repeat markers) and national DNA database searching have been unsuccessful. While FGG is powerful and is being embraced for investigating some of the most recalcitrant of cases, the genetic data generated in a FGG investigation contain private and sensitive information. As has been raised for molecular autopsy data and reporting biological sex, sensitive, private genetic data in reports and presentation in court proceedings become publicly accessible and thus pose a real risk to personal genetic privacy. Guidelines are needed to mitigate potential risks to individual privacy. KinSNP (kinSNP.com) was used to illustrate methods and systems for protecting genetic data presented in reports and court proceedings. Shared data can be displayed in a one-to-one manner via skyline plots showing full, partial and non-matching regions and various kinship relations determined but without chromosomal locations and in an encrypted and obfuscated manner to minimize reverse engineering of private genetic data. Thus, the interpretation and findings still can be reported, and good documentation is maintained, which allows tracing back to case files and analysts to effectively recall the bases for their conclusions. Furthermore, third-party candidates that may be sequenced to strengthen tree hypotheses or to develop alternate tree hypotheses can be compared offline reducing the risk of improper access to genetic data. These proposed guidelines address efficient targeted testing, minimization of use of on-line tools, especially for third party targets, and data encryption and obfuscation for handling sensitive data in the public arena which in combination with physical security, can be part of an overall good governance program for privacy risk mitigation.

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EXPLORING GENETIC GENEALOGY IN FORENSIC SCIENCE: A PERSPECTIVE FROM AOTEAROA NEW ZEALAND

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Forensic genetic genealogy describes the use of direct-to-consumer databases in conjunction with bespoke DNA profiles derived from forensic case samples to identify possible relatives of unidentified sources of DNA found associated with crimes and other discoveries of human remains. The use of this technology presents technical challenges, challenges of data security and data sovereignty and multiple social and ethical concerns. The objective of our research was to explore these aspects and the potential of genetic genealogy to extend capability equitably for the benefit of all New Zealanders. We present our evaluation of the Kintelligence Forensic Genomics solution, including aspects of sensitivity, reliability and data management solutions as an in-house technical option. We describe our findings from social systems research we have conducted surfacing social, ethical, Indigenous Māori, and equity concerns and considerations. We set all of this in an Aotearoa New Zealand context incorporating whanaungatanga – recognising the importance of kinship and lasting relationships and kaitiakitanga – valuing stewardship and guardianship of things that are precious, such as our relationships with our ancestors, the natural environment, and with each other.

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Massive Parallel Sequencing of Maternal Lineages: mtDNA Variations and Haplogroups in the United Arab Emirates Mother-Child Pairs

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Mitochondrial DNA (mtDNA) is inherited solely from the mother and contains important genetic information required for cellular energy generation and metabolic function.

Variations in mtDNA can alter phenotypic features and susceptibility to numerous illnesses, but the direct transmission patterns and phenotypic consequences in closely related people remain unknown. This study focuses on mitochondrial DNA (mtDNA) variants in 45 individuals as mother-child to discover patterns of maternal inheritance and how they relate to phenotypic features. Utilizing massive parallel sequencing technology, we started by analyzing the mtDNA control region for common variations and then proceeded to the whole mtDNA sequence if no significant variations were found. To identify major genetic impacts, we sequenced mtDNA, heteroplasmy, and haplogroup features and compared them to phenotypic and medical variables. In total, a significant number of variants were observed across numerous nucleotide positions from a substantial sample of individuals. Notably, all maternal pairs exhibited identical homoplasmic single nucleotide polymorphisms (SNPs) and haplotypes. Rigorous criteria were applied to mitigate the risk of false positives arising from sequencing errors and nuclear mitochondrial pseudogene contamination, emphasizing the importance of maternal genetic legacy in a daughter's health and physiological traits. In conclusion, this study improves our understanding of mtDNA inheritance patterns between mothers and daughters and clarifies their possible consequences for forensic investigations. Our results, which reveal unique mtDNA polymorphisms and haplogroups linked with individuals, give crucial insights for forensic investigators looking to establish maternal ancestry and identify human remains. Furthermore, the complete study of mtDNA sequences emphasizes the need to include both the control region and the coding regions in forensic mtDNA analysis, which improves the accuracy and reliability of identifications.

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Problematic practices and potential policy options for investigative genetic genealogy: results of a modified policy Delphi study

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Background and objectives: Investigative genetic genealogy (IGG) is a pioneering technique for human identification that law enforcement has now used to identify hundreds of violent criminal perpetrators and unidentified human remains. As IGG is integrated into investigative workflows around the world, policymakers have begun to regulate its practice. For example, in the United States, the Department of Justice has adopted an interim policy for IGG, two states (Maryland and Utah) have adopted comprehensive IGG legislation, and other states (such as Texas) have adopted broad genetic privacy statutes that impact IGG. Meanwhile, new professional bodies, including the National Technology Validation and Implementation Collaborative and the IGG Accreditation Board, are developing standards and guidelines for agencies, laboratories, and genetic genealogists. We sought to inform these policy efforts and to promote coherence in the system of IGG governance as it expands using expert engagement and elicitation methods. Methods: From 2023 to 2024, we conducted a modified policy Delphi with 34 experts in forensic science, law enforcement, genetic genealogy, law and ethics, database operations, and victim advocacy. The Delphi occurred over four rounds that included real-time engagement sessions and questionnaires. The rounds were structured to identify and prioritize IGG practices for policy attention and to develop and evaluate policy options to address relevant ethical and legal concerns. Results and conclusions: In the first two rounds, participants identified 33 IGG practices that at least some believed warrant policy attention and prioritized 9 of them. These top priority practices relate to: case eligibility and criteria; consent and notification; data management, privacy, and security; and governance and accountability. Although participants did not reach complete consensus with respect to any of the practices that they considered, convergence of opinions was strongest with respect to law enforcement participation in genetic genealogy databases against terms of service, which the majority opposed and almost half evaluated as top priority for policy attention. In the last two rounds, participants identified over 40 policy options to address the 9 prioritized practices and evaluated their advantages and disadvantages. Our study demonstrates the feasibility and value of engaging with diverse experts over an extended period of time to surface potential points of agreement, and persistent points of disagreement, on a pressing matter of public policy and provides a useful empirical foundation for IGG policymaking.

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Overcoming Budget Constraints to Obtain Sustainable Funding for Forensic Genetic Genealogy

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Forensic science plays an integral role in providing long-awaited answers and justice to countless families burdened by years, even decades, of trauma and uncertainty. Forensic genetic genealogy (FGG) is the most recent tool to provide leads that seemed unsolvable with standard DNA typing procedures. FGG combines genealogical research with advanced DNA technologies, providing a powerful tool in helping solve challenging active and cold cases. A dense SNP profile from evidence or human remains is compared against a database(s) of profiles from volunteers who consented to allow law enforcement to use their DNA to identify potential kinship associations. Such associations can guide investigators toward suspects in violent crimes, or aid in naming unidentified human remains, can streamline investigations, and most importantly, offer long awaited resolution. Actually, the application of FGG is not bottlenecked by scientific or technical barriers but rather by real and perceived funding constraints. Budget constraints often mean that the technology's benefits are not uniformly accessible, leaving some cases and thus victims, families, and communities unassisted. First, the cost of sequencing on a per sample basis may appear to be greater than current STR typing by capillary electrophoresis. However, sequencing costs have decreased and continue to drop such that a cost/benefit analysis could demonstrate that FGG is cost effective, based on a greater success in typing challenging DNA samples, less sample consumption, increased number of leads, efficient investigative times and resource usage, and tangible and intangible benefits to victims, families, and government budgets. The criminal justice system should assess FGG in a cost/benefit fashion so this important tool can be democratized to the benefit of all in society. Second, even armed with an effective cost/benefit analysis some jurisdictions may not readily embrace FGG again citing budget constraints. An alternate approach to obtain funding for these serious stalled cases is demonstrated with DNASolves (www.dnasolves.com) - a program focused on generating funding support by embracing citizen science and community support. The success of DNASolves is demonstrated by cases that have remained unsolved for years, or even decades, but have achieved resolution shortly after being crowdfunded. Success through a DNASolves program can be leveraged to solicit dedicated funding locally or nationally from government, private sector partnerships, and further community involvement. Innovative approaches, advocacy and cost/benefit analyses are avenues to obtain sustainable funding to realize the full potential of FGG, ultimately making it a standard, routine, and broadly accessible tool in forensic investigations.

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Unraveling Forensic Genetic Genealogy: Advances and Challenges in the EU Context

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Forensic Genetic Genealogy (FGG) represents a groundbreaking advancement in forensic science, offering new avenues for unravelling the identities of unidentified human remains. In this study, we sought to harness the potential of FGG methodologies to address the longstanding challenge of identifying individuals whose remains have defied traditional forensic methods. Our approach integrated Next-Generation Sequencing (NGS) technology, the MiSeqFGx instrument, and the Verogen Kintelligence Kit to analyze DNA samples extracted from unidentified human remains.

Our objectives were multifold: to assess the feasibility of FGG techniques in resolving cases involving unidentified human remains, to evaluate the efficacy of FGG as a complementary tool to traditional forensic methods, and to navigate the ethical considerations inherent in utilizing genetic genealogy in forensic investigations.

Through our comprehensive analysis using FGG, we successfully progressed with the identification of an individual previously classified as an unknown victim of homicide. By employing FGG techniques, we reconstructed familial pedigrees and traced the genetic ancestry of the unidentified remains.

However, our study also uncovered significant complications stemming from communication barriers among police agencies across the European Union (EU) region. These challenges hindered the timely exchange of information and coordination of investigative efforts, underscoring the need for enhanced collaboration mechanisms on the Europol/Interpol level to implement standardized protocols for data sharing in cross-border forensic genealogic investigations.

Despite these hurdles, our findings underscore the transformative potential of FGG in forensic science. The successful identification achieved in this study highlights the invaluable role of FGG in resolving cold cases and bringing closure to families of missing persons. Furthermore, our study emphasizes the critical importance of overcoming bureaucratic and regulatory barriers to maximize the impact of FGG in forensic investigations.

Moving forward, collaborative efforts among law enforcement agencies, forensic practitioners, and policymakers are essential to address these challenges and promote the responsible and effective utilization of FGG in the EU space. By fostering greater collaboration and implementing standardized protocols, we can unlock the full potential of FGG to advance the field of forensic science and promote public safety.

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On the use of Forensic Investigative Genetic Genealogy in criminal cases in Sweden

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Evidently, the use of commercial genealogy databases to solve crime and identify human remains took the world, as well as the forensic DNA community, by storm. Even though Swedish law enforcement started off early being first outside north America in using forensic investigative genetic genealogy (FIGG), implementation has been delayed awaiting necessary legal amendments. Following a successful pilot study on a cold case double murder the supervisory authority IMY (Swedish Authority for Privacy Protection) stated that legal support was lacking for the police authority to use FIGG. Likely, due to legal, privacy and practical concerns there has been an evident and rather long delay in using FIGG in most countries outside the United States. In Sweden, a governmental committee on biometrics presented their report in June 2023 which included recommendations on the use of FIGG to solve crime. The committee argue that FIGG is a tool of last resort that should be of limited and restricted use, applied only in the most severe cases (murder and aggravated rape), but not until other forensic and investigative measures has proved unsuccessful. The suggestion of the Biometric committee is that new legislation should come active January 1st 2025. Whether this timeline is doable is unclear. Also, the details on the use of FIGG remains to be seen, as the legal bill is still being prepared by the Justice department, after which it needs to pass a parliament vote before a law is in place and the tool can be implemented. Irrespective the situation, while awaiting legal amendments, the police authority is preparing implementation of FIGG by e.g. shaping policy documents. The policy documents will comprise significant criteria on the use of FIGG and aim to complement the coming legislation in order to direct the use of the method towards suitable cases. These criteria include availability of traces/DNA (quantity/quality), SNP data requirements (quantity/quality), genealogy assessment of relevance given estimated ancestry of the stain donor versus coverage of database users, a FIGG advisory board and more. The presentation will discuss the legal landscape, proposed criteria and coming practice in using FIGG in Sweden.

P-341

Microarray is not for forensics. We disagree!

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Investigative Forensic Genetic Genealogy (iFGG) has recently emerged as a method for generating investigative leads for cold cases and cases with unidentified human remains. Databases used for iFGG (Gedmatch Pro and Family Tree DNA) are built on DNA data generated with microarray technology, that, according to the manufacturer, requires 200 ng of high-quality DNA input. As microarray produced data allows detecting associations beyond 6th degree relationships, analyzing forensic samples with the microarray technology is tempting.

Two variations of the Global Screening Arrays (GSA, Illumina) were tested with the dilution series (200 ng – 0.1 ng) of reference buccal swab and blood samples. The percentage of concordant, discordant and no call SNPs was examined. Generated profiles were uploaded to Gedmatch and compared against known relatives genotyped with direct-to-consumer kits. Additionally, a number of forensic case work samples - old bone, old blood of filter paper and fresh blood - were genotyped with the GSA using the maximum available DNA input and DNA input of 1 ng. The effect of DNA degradation on the SNP call rate was examined.

Based on the reference samples, it was reported that the number of falsely typed and no call positions increased with the decrease of DNA amount and that the majority of discordant positions were homozygotes that were genotyped as false heterozygotes. DNA input of 1 ng resulted in >90% call rate, and the same shared DNA amount between known family members compared to 200 ng DNA input. Correct relationships were reported with 0.25 ng and 0.1 ng, however not as the most probable relationships. Based on the analysis of forensic samples, it was seen that even slight degradation has a negative effect on the call rate and one ng of input DNA was not sufficient for getting >90% call rate. In case of one forensic sample with the degradation index of 3.22, microarray analysis resulted in >90% call rate when 115 ng (the maximum available amount) of input DNA was used.

P-342

Comparisons of four common approaches for kinship inference in FIGG

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Objectives: As a novel tool for kinship inference, forensic investigative genetic genealogy (FIGG) has developed rapidly in recent years. However, in the face of complicated crime scene samples, it is still a troubling question for investigators to determine which approach to choose in forensic practice, especially when faced with challenging criminal samples.

Material and methods: We selected four common approaches (one MoM estimator and three IBD segment-based tools), and compared their performance on varying numbers of SNPs (from 5 million to 5,000) and genotyping errors (from 0.1% to 10%) with simulated 1st to 7th degree relatives and unrelated pairs. Then we explored the possibility of making robust kinship inference for samples with ultrahigh genotyping errors by integrating different methods.

Results and conclusions: Results showed that decreasing the number of SNPs had little effect on kinship inference for both MoM and IBD segment-based methods when the number of SNPs was more than 164,000; while its effect became nonnegligible for IBD segment-based methods when the number was fewer. We also found that genotyping errors had a significant effect on kinship inference, especially when it exceeded 1%. In contrast, MoM performed much more robustly to genotyping errors. Furthermore, we found that integrating MoM and IBD segment methods with sufficient number of SNPs increased the overall accuracy for inference, showing it has the potential to improve the tolerance to genotyping errors without additional optimization. These findings were validated using real samples. In conclusion, this study shows that different methods perform differently and different methods should be chosen for different scenarios. More importantly, our models by combining the outputs of different methods can improve the robustness for kinship inference, which is meaningful for forensic use.

P-345

An innovative genetic genealogy approach to the identification of three victims of World War I and World War II: The new Forenseq Kintelligence kit

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The identification of unknown human remains is still dependent on the availability of comparison samples from potential leads and the use of STR profiling. In cold cases, or cases where remains have been recovered long after death, establishing a list of leads is an added difficulty. The absence of external clues to the identity of the individual, or the original failure of identification given the initial data, imply that leads must be established using either the genetic data itself, or contextual clues. Moreover, kinship confirmation further than the first degree is unreliable using autosomal STR alone. The Forenseq Kintelligence kit by Qiagen (formerly Verogen) is presented as a solution to both problems, since it proposes the comparison and kinship testing (the so-called genetic genealogy) up to the fifth degree of SNP profiles recovered from unknown degraded remains with the large SNP databases produced by commercial test providers such as MyHeritage, 23andMe, GEDmatch, Ancestry and others, as gathered into the GEDmatch Pro database, on a voluntary basis.

We applied the Kintelligence kit to two skeletonised bodies recovered from the battlefield of Verdun and a body that was buried alongside members of the French Résistance, executed by the nazis in 1944. In all three cases, we obtained usable genotyping data, with between 6230 and 9591 SNP, out of the 9867 SNP dedicated to kinship inference that are included in the Kintelligence panel. The comparison with the GEDmatch Pro database produced between 3 and 500 matches with our samples, all around 200cM in genetic distance.

Our efforts to use these matches as leads to identify these remains were limited by the lack of information provided by GEDmatch Pro, with aliases and email addresses (many obsolete) constituting the bulk of data. The few genealogies linked to matches being mostly anonymous for individuals born after 1920, we relied on contacting participants in the database using email and parsing their responses, looking for a profile that would fit the soldiers or member of the Résistance. We present the results of the combined genetic and genealogical approach in recovering the identity of remains of long-deceased individuals and propose improvements to both the GEDmatch database and the process of investigation itself.

Posters Topic 8

Ethics

8. Ethics

Abstracts Poster

P-189

Forensic DNA examination : Indian Perspective

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Forensic DNA examination is considered to be the most believed technique in forensics. There has been an extensive demand for forensic DNA examination in India. This has resulted in to very high backlog of all variety of cases in most of the Indian forensic DNA laboratories. Status of forensic DNA examination, adopted scientific procedures, validations, SOPs, statistical evaluation of results, status of population data, maintenance of examination records, proficiency of examiners, cognitive bias and judgements passed by Honorable courts on DNA examination is reviewed in Indian context. The analysis and obtained results of this extensive exercise on present status of DNA examination suggests thorough overhauling of forensic DNA examination strategy in India to maintain sanctity and belief on the golden technology.

P-322

Familial search as a complementary tool for identification of unknown offender - case report

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Familial searching is a strategy that can be of great importance in solving crimes. This tool can be used for establishing first degree family relationships between DNA profiles already present in the database and a DNA sample of unknown donor. Identification of potential relatives can lead to identification of the unknown offender in the crime case. Numerous countries have implemented familial search mechanism in their DNA databases mostly to search for missing persons, where the aim is to use this tool to establish kinship between unidentified human remains and a potential relative that is registered in DNA elimination database. Still the use of familial searching in criminal investigations is relatively limited raising some legal, ethical and scientific issues regarding the effectiveness of its use. The legislation for familial searches in Republic of North Macedonia is still not precisely determined, so under specific circumstances and depending on the seriousness of the crime, the public prosecutor can mandate familial search of an unidentified crime scene DNA profile. Case study: A man was arrested with a large amount of drugs wrapped in plastic bags. The surface of the plastic bags was swabbed and a mixture DNA profile was obtained from one of the bags. The man arrested did not match the mixture DNA profile. Actually, the DNA mixture matched with a trace DNA profile from another case in the DNA database. The law enforcement officers with operative investigations came to information for a potential suspect who refused to give reference sample for comparison. Having no other evidence to connect this trace with the potential suspect but knowing that his brother's DNA profile was already in the database, the prosecutor mandate familial search of the unknown trace. As a result of these search, a list of possible kinship was established between the donor of the sample under investigation and the DNA profiles from the database. The brother of the suspect was first on the list and based on this information the prosecutor was able to mandate collection of reference sample of the suspect. His DNA profile matched with unknown trace from the DNA database and subsequently with the DNA mixture.

Even though the familial search is still controversial technique that raises a lot of legal and ethical questions it can be very useful investigative tool in solving crimes.

Posters Topic 9

Others

9. Others

Abstracts Poster

P-027

Estimation of time of death from RNA integrity

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Abstract: Objectives: Estimation of time of death is one of the most urgent problems in the field of forensic science. In recent years, RNA was used as a good biomarker for predicting postmortem interval (PMI). However, it is currently not possible to estimate time of death accurately. The research tries to develop an effective method for inferring PMI. Material and Method: In this study, mice were euthanized by cervical dislocation, then placed in a constant temperature and humidity chamber (25℃, 50%humidity). 48 mice (C57BL/6J, 10-12 weeks) were evenly divided into 8 groups at 0 hours, 6 hours, 12 hours, 18 hours, 24 hours, 30 hours, 36 hours, and 48 hours postmortem. Eight types of tissues (heart, liver, spleen, lung, kidney, testes, brain, skeletal muscle) were extracted from each mouse for RNA isolation using Trizol, and RNA Integrity Number (RIN) was determined using Agilent 4200 analyzer. We integrated RIN data from 380 samples with nine different machine learning algorithms to develop predictive models for inferring postmortem interval (PMI). Nine machine learning algorithms include Support Vector Machine (SVM) Model with four distinct kernel functions (sigmoid, linear, polynomial, and radial), Random Forest Model, ElasticNet, Generalized Regression Neural Network, K-newerest Algorithm Model and Artificial Neural Network Model. 80% of the samples were used as the training set, while the remaining 20% were used as the prediction set. Results and conclusions: The results showed that on the prediction set, R2 ranged from 0.92 to 0.98, MAE ranged from 1.47 hours to 3.47 hours, RMSE ranged from 2.18 hours to 4.33 hours. The most accurate method is SVM (radial), R2=0.98, MAE=1.47 hours and RMSE=2.18 hours. The findings highlight the efficacy of RIN data from various organs with machine learning algorithms to enhance the accuracy of PMI. This study introduces several machine learning algorithm methods for more accurate PMI inference, especially SVM (radial).

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P-028

A Machine Learning Algorithm for Modelling Y-STRs' Mutations

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The analysis of Y-chromosomal markers provides invaluable insights regarding germinal mutation modeling. Due to its haploid mode of transmission, it allows the inference of which parental allele originated which filial one. In contrast, for diploid and haplodiploid markers not only hidden mutations can occur, as also multistep mutations can be misinterpreted as single step ones, which biases the modelling of the phenomena.

Mutation rates of microsatellites (or short tandem repeats, STRs) are known to be correlated with the parental sex, age, and allele size and sequence. Nonetheless, corresponding estimates are generally computed simply considering the marker-specific ratio between the number of Mendelian incompatibilities and transmissions observed. This naïve approach hides the variation in germinal mutation rates within each marker, dependent on the allele, sex and age of the individual.

Under the framework of a GHEP-ISFG working commission, father-son segregation data for 28 Y-STRs were analyzed, and a machine-learning model was developed, where logistic regression analyses were computed to estimate marker specific mutation rates depending on paternal age and/or allele length. Statistical significance was reached for both predictors for three markers out of the 25 analyzed, with allele length showing greater contribution than age (from 5 to 16 times greater). Greater subsets of data were able to be analyzed when considering only the allele length as predictor, which allowed statistical significance to be reached for 18 Y-STRs out of the 28 analyzed. For each case, algebraic expressions were provided for estimating marker specific mutation rates depending on paternal age and/or allele length.

These results support that machine learning algorithms may be used to improve mutation modelling, statistical significance depending on the available data to be used as training and test sets. As for any other rare event, a huge amount of data is needed for the proper estimation of mutation parameters. Therefore, interlaboratory studies are crucial to produce and gather important amounts of data, in parallel to the establishment of publication guidelines to assure the release of data with the proper level of detail. To circumvent the limitation inherent to the scarce data available and increase its potential, in this work we evaluate the possibility of gathering data from different markers with the same structure of the repetitive motif for modelling mutation rates considering also as predictors the parental allele and/or age.

P-029

Comparative study of three convolutional neural network models for DNA profile prediction based on electropherogram images

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The convolutional neural network (CNN) is a kind of deep learning methods to recognize images, video and behavior with high accuracy and low error rate. In this study, we have conducted prediction of DNA profiles of image obtained from electropherogram using the CNN. 12,600 DNA profiles images from DNAs of 10 reference samples were used for CNN model as a dataset. A dataset was divided into the train, validation and test data set. Test data set is composed of single source DNA, serial diluted DNA and mixed DNA. To compare the model performance, we have tested using three CNN models: VGGNET, AlexNet, LeNet, and an accuracy of model has been estimated by k-fold cross validation. As the result, all three CNN models showed accuracy close to 100%. In addition, accurate predictions were made even in low concentration DNA and mixed DNA. It is considered that DNA profile prediction using CNN can be used in laboratories where it is difficult to build a database.

P-030

Predicting Ancestry with the help of Autosomal STR Profiles

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The aim of this study was to test the validity of a predictive model of ancestry affiliation based on Short Tandem Repeat (STR) profiles. Frequencies of 29 genetic markers from the Promega website for four distinct population groups (African-Americans, Asians, Caucasians, Hispanic Americans) were used to generate 360,000 profiles (90,000 profiles per group), which were later used to train and test a range of machine learning algorithms with the goal of establishing the most optimal model for accurate ancestry prediction. The chosen models (Decision Trees, Support Vector Machines, XGBoost, among others) were deployed in Python, and their performance was compared. The XGBoost model outperformed the others, displaying significant predictive power with an accuracy rating of 94.28% for all four classes, and an accuracy rating of 98.82% in the differentiation between Asian, African American, and Caucasian subsamples. The accuracy rating for different population groups was balanced, demonstrating the model's discriminatory abilities. Evaluating the impact of training set size revealed that model accuracy peaked at 94% with 90,000 profiles per category but decreased to 83% as profile quantity was reduced to 500, particularly affecting the precision in distinguishing between Caucasian and Hispanic subgroups. The study further investigated the impact of the number of markers on the model's accuracy, finding that using 21 markers, commonly available in commercial amplification kits, resulted in an accuracy of 96.3% for African Americans, Asians, and Caucasians, and 88.28% for all four groups combined. These findings underscore the potential of STR-based models in forensic analysis and hint at the broader applicability of machine learning in genetic ancestry determination, with implications for enhancing the precision and reliability of forensic investigations, particularly in heterogeneous environments where ancestral background can be a crucial piece of information.

P-052

Development of a new microhaplotype panel for ancestry inference

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Microhaplotypes (MHs) are a novel class of genetic markers, exhibiting features that position them as a preferential choice over STRs and SNPs in addressing challenges commonly encountered in forensic investigations. Several publications detail the performance of selected and newly discovered MHs, as well as the development of panels for human identification, mixture deconvolution, kinship testing, or biogeographic ancestry inference. However, MH panels with the sole purpose of biogeographic ancestry inference are scarce and show some problems that are hindering a broader acceptance within the community. One of the key issues lies on the incipient laboratory testing of the selected MHs, especially considering that some were solely identified based on data from the 1000 Genomes Project, without having been further subject to amplification and sequencing in human samples. Here, we entail the development of a new ancestry informative MH panel named MHappaMundi. The MHs were chosen from MicroHapDB with the purpose of clustering five population groups: Sub-Saharan Africans, Europeans, South Asians, East Asians, and Native Americans. The selection method involved locus-by-locus AMOVA analyses between each pair of population groups. The most informative MHs for each pair were recruited for the final panel, which was complemented with others that allowed to balance the FST values across all population pairs. A total of 100 MHs encompassing 293 SNPs were included in the ancestry panel, which already has demonstrated good performance when applied to the 1000 Genomes data. Sensitivity, half-volume, marker performance tests were conducted to validate the panel, as well as the sequencing of samples from European, Sub-Saharan African, and East Asian populations.

P-053

Comparison of genome-based and anthropological-based ancestry estimations for unidentified human remains

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Objective: Identification of unidentified human remains (UHRs) is crucial yet challenging, especially with traditional forensic techniques. Forensic anthropological examinations can yield ancestry estimations; however, the utility of these estimates is limited by the data points that can be collected from partial remains, complexities of admixture, and variation of phenotypic expression due to environmental effects. While it is generally known that anthropological estimates can be imprecise, the performance of these methods has not been studied at scale.

Materials and methods: Genome-wide SNP testing is an orthogonal approach for estimating ancestry and offers a unique opportunity to measure the magnitude of anthropological ancestry misattribution. Genetic-based ancestry techniques leverage ancestry proportions inferred from putative reference populations and principal component analysis. In this study, these two approaches were leveraged, along with a large set of SNP markers, to compare genetic ancestry estimations with those determined anthropologically. DNA from more than 600 UHRs was subjected to genome-wide SNP testing, and ancestry and admixture proportions were calculated.

Results and conclusions: The genetic ancestry approach, validated against reference population samples, offers robust ancestry calculations for major population groups. The National Missing and Unidentified Persons System (NamUs) contains anthropologically estimated ancestry information on many of these same samples. A comparison of the ancestry estimations revealed discordance in a number of cases, especially in cases labeled as ‘Uncertain’. This study indicates that genome-based approaches can inform better regarding ancestry attribution. Additionally, based on the magnitude of anthropological estimation errors current policy and practices for reporting ancestry for UHRs should be revisited to reduce potential misinformation.

P-054

Evaluation of genome-wide microhaplotypes derived from the 1000 Genomes Project for ancestry inference

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Microhaplotype (MH), as a novel genetic marker, has been applied in biogeographic ancestry inference. However, screening MHs from the 1000 Genomes Project (1kGP) is still burdensome, and the overall picture of genome-wide MHs in terms of population structure remains unknown. We aim to provide a reference dataset for screening MHs in terms of ancestry inference and evaluated the effectiveness of genome-wide MHs. In our study, we constructed a genome-wide MHs dataset with MH lengths within 350 bp based on 1kGP phase3 (GRCh38) using the strategy applied in our previous study. Within a 350 bp fragment, the longest MH was retained, and other subset MHs (first and last SNPs were included in that MH) were deleted, but loci that partially overlapped with that longest MH were retained to obtain all possible and longest MHs on a genome-wide basis. We took the population with the largest sample size from each of the five super-populations and calculated forensic parameters, fixation index (Fst) values and informativeness (In) for genome-wide MHs in these five populations (CHS, IBS, GIH, PUR, and GWD). Our study generated a dataset of 24,211,569 MHs covering 90.56% of the whole genome. The average effective number of alleles (Ae) for 5 populations ranged from 1.14 to 1.22, with Fst -0.96 – 0.33. Considering that MHs with Ae ≥ 3 had a higher application value, we further screened for loci with Ae ≥ 3 and Fst ≥ 0. Each of the five populations preserved between 15,781 (GWD) and 27,941 (PUR), which had mean Fst values of 0.03 to 0.05, mean Ae values between 3.46 and 3.51, and mean heterozygosity values between 0.71 and 0.72. Among these loci, 3484 to 6757 loci had Fst greater than or equal to 0.05 and could be used to correct population substructure. Our results demonstrate the wide distribution of MHs and the efficacy of these loci in ancestry inference.

P-071

Saving Bambi: Whole genome sequencing of Dama dama (fallow deer) and an investigation into their profound lack of genetic diversity

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On average, 55,000 deer are illegally killed in the UK each year. Lack of genomic tools for identification mean that more often than not, the perpetrators get away with their crimes, free to commit again. In order to protect the species, this study set out to develop an STR multiplex for individual identification. In order to identify polymorphic loci and develop species specific primers, the entire genome of the fallow deer species was sequenced.
The Genome is 3,108,385,535 bp in length with the sequence assembled in to 35 Chromosomes including an assembly of the X and Y allosomes. The annotated genome was found to consist of 34,891 genes, 22,157 of which are protein coding genes. The final BUSCO v4.1.4 completeness score for the annotation was 99%. Predicted genes have an average transcript length of 37,945bp and on average each gene has 10 exons.
A total of 21,580 tetra and penta nucleotide STRs were located within the genome. 92 tetranucleotide loci were tested and only 9 were found to be polymorphic. The lack of polymorphic loci discovered can be attributed to the low genetic diversity of the fallow deer species. It is well documented that Dama dama have low genetic diversity caused by a genetic bottleneck during the Mesolithic (8000 BC – 2700 BC) and Neolithic (10,200 BC - 2000 BC) periods. This lack of genetic diversity, which is still observed today, has implications for the successful development of a forensically useful STR multiplex for fallow deer identification. Hardy Weinberg equilibrium was investigated at 6 loci. Results are concordant with low genetic diversity and high levels of inbreeding with significant deviation from HWE observed at all loci.
This project is the first to fully sequence the genome of fallow deer and provides the groundwork for further research involving this species. The result of this project benefits the welfare of the fallow deer species across the world, contributing to the global effort to combat wildlife crime as well as the conservation of the species.

P-072

Development and validation of a fluorescence PCR assay for the species identification of ten animals

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1. Objetives To develop a rapid and reliable multiplex fluorescence PCR assay to simultaneously identify ten different animal species (goat, cattle, donkey, chicken, pig, horse, sheep, goose, mouse, and duck).

2. Material and methods Based on the complete mitochondria genome sequence of the ten animals in the GenBank database, a comparative genomics analysis strategy was used to screen DNA sequences with both intra-species conservation and inter-species specificity. Species-specific primers were designed to construct a multiplex fluorescence PCR assay that can simultaneously identify ten animal species. Subsequently, the performance of the assay, including species specificity, sensitivity, accuracy, reproducibility, and concordance, was validated for use in forensic analysis according to the guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM). In addition, simulated mixed meat samples were also detected using this assay.

3. Results and conclusions Ten pairs of species-specific primers were designed to amplify the partial region of mitochondria genome (D-loop, 16S rRNA, NADH2, Cytb, CO I, ATP6, NADH4 and NADH6) of the ten animals. The assay was constructed by combining of these primers to identify species through multiplex fluorescence PCR and capillary electrophoresis. The results of developmental validation indicated that this new assay is reproducible, accurate and reliable. Specifically, the species specificity study showed that this assay did not exhibit cross-reactivity with a variety of common animal species or human beings. The sensitivity study demonstrated that detection limit of the present assay were 0.03125 ng for ten animals. Additionally, the system was able to detect as low as 0.5% duck component from the duck and beef mixture. The developed novel multiplex fluorescence PCR assay can successfully achieve the purposes of species identification for ten common animals, which will be a valuable tool for the detection of meat fraud and adulteration.

P-073

Molecular Tools for species-specific qPCR and STR-based Individual Identification of Lynx lynx in Forensic Casework

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The Eurasian lynx (Lynx lynx) is included in CITES Appendix II and protected under the Bern Convention and the EU Habitats Directive. Lynx is a frequently targeted species in the wildlife crime, and this highlights the pressing need for reliable methods of identification. Our study focuses on species determination and DNA quantification targeting the Eurasian lynx using quantitative real-time PCR. The quantification multiplex system Lynx Qplex also effectively distinguishes the Eurasian lynx from other Feliformia based on mitochondrial and nuclear targets, plus it contains an internal positive control. Additionally, the study presents the results of developmental validation of the Lynx STRplex system for individual identification and databasing. The study followed the ISFG recommendations for the non-human DNA testing, as well as the recommendations for developmental validations. With the Eurasian lynx facing threats from illegal activities, the development of accurate and efficient molecular tools is crucial for conservation efforts.

P-074

Species identification using mtDNA Minibarcoding

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Wildlife forensic analysis often encounters highly degraded DNA samples, such as tanned hides, conserved tissues in formalin-fixed paraffin-embedded (FFPE) blocks, and specimens preserved in conservation fluids. These samples pose significant challenges for traditional DNA barcoding approaches due to the difficulty in amplifying sufficiently long DNA fragments. To address this limitation, we developed a set of five PCR primer pairs designed to amplify short mtDNA fragments – around 120 base pairs (bp) long. When aligned collectively, these fragments span approximately 500 bp of the cytochrome c oxidase subunit I (COI) gene, which is one of the most common species barcoding targets. These primers for short COI amplicons are universal across mammalian species. We subjected the primers to a comprehensive validation process, including in silico analysis and experimental verification using various non-degraded samples of animal tissues, as well as degraded wildlife forensic samples. Here, we demonstrate the efficacy and reliability of our DNA minibarcoding method. By overcoming the challenges associated with traditional DNA barcoding approaches, this method offers enhanced resolution and accuracy in species identification and forensic investigation, thereby contributing to the conservation and management of wildlife populations.

P-075

Little Red Riding Hood and the bad wolf (or lynx, or maybe even bear …)" - on the return of large predators in Germany and the influence on forensic casework

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The number of wolves in Germany and many other European countries has increased continuously over the last twenty or so years. It is estimated that well over 1000 grey wolves live in German forests, with additional animals just passing through. Some areas in Germany are densely populated by the predators, the German state of Brandenburg, for example, is home to more animals than all of Sweden. Other large predators such as bears and lynxes are also returning to Central Europe.
With the increasing number of such predators, not only livestock and pets, but also humans are in danger of close encounters with these animals that might lead to serious and sometimes even fatal injuries, and may have implications for forensic casework. The same also applies to post-mortem feeding of animals or displacement of body parts.

Currently, in Germany and some other European countries, molecular genetic examinations of traces that are secured from people who are suspected to have been attacked and injured by a wolf are exclusively analyzed at an institute specializing in wildlife biology without forensic expertise. In addition, the injuries are rarely examined by a forensic pathologist and are often poorly documented. We are of the opinion that a forensic documentation and description of such injuries, as well as a professional securing of DNA traces on victims of suspected wild animal attacks – especially in cases of questionable wolf involvement – are indispensable, and suggest the early involvement of forensic specialists in such cases.
We present typical cases of suspected predator attacks, and discuss how an improved workflow can ensure legal certainty.

P-082

The use of canine autosomal STRs for breed determination

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The reporting of animal DNA evidence is influenced by the availability of species-specific databases and reporting guidelines. Dogs are one of the most common companion animals on the planet, with over 10 million dogs present in the UK alone; despite their prevalence in households, the lack of genetic databases that can characterise canine markers used in individual identification is problematic. Amongst the collected evidence at crime scenes, items of animal derivation are typically blood and saliva from wounds or bites, and hair shafts which dogs shed generously. The aim of this research is to close the identified gap and create canine databases of short tandem repeats (STRs) and mitochondrial DNA. The databases have been created to be representative of the most common UK breeds, aligning to the canine UK breed distribution. Moreover, it could be extremely informative if more intelligence information could be gathered from this evidence left at the scene, such as the breed of origin of the animal in question that contributed to the retrieved specimens. The newly created database has over 1200 participant dogs spanning 80 breeds with 10 individuals per breed on average; further, for the 10 most common breeds in the UK an additional 40 dogs have been tested. DNA profiling was obtained with the typing of 13 autosomal STR markers via capillary electrophoresis and population substructure was observed via the implementation of the software STRUCTURE.

Here we present the results of the population substructure investigation, with the discovery of breed-enriched genetic signatures that may be useful in identifying the breed of the animal. This has the potential to provide forensic intelligence should the breed of the dog be unknown, with the goal of supporting the judicial system and giving confidence to the reporting of animal evidence.

P-084

Standardization of Molecular Analysis Techniques for DNA Identification of Bird Species from Eggshells

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Advancements in genetic analyses have been increasingly contributing in our understanding of ecological, evolutionary, and phylogenetic aspects of animals. Examples of this progress can be seen in the forensic field. In Brazil, wildlife traffic stands out as one of the most prevalent environmental crimes, with significant impacts on avian populations. For these animals, obtaining genetic material through blood is more advantageous due to the presence of nucleated red blood cells. However, this method can induce stress and alter the behavior of these animals, proving more challenging for threatened and elusive species. Consequently, non-invasive techniques for obtaining genetic material have been increasingly studied. The present study aimed to standardize molecular analysis techniques in bird eggshells. For standardization, incubated eggshells of Common Gallinule (Gallinula galeata) were used, from which fragments were collected weekly over 30 days post-hatch and separated into three categories ("shell," "membrane," and "shell + membrane"). DNA was extracted and purified using sodium acetate, and the quantity and quality of the molecule were evaluated by fluorimetry and 0.8% agarose gel electrophoresis. Polymerase chain reaction (PCR) amplifications of the nuclear marker Chromodomain Helicase DNA Binding Protein (CHD) and the mitochondrial marker Cytochrome B (CytB) were conducted for genetic determinations. Forty-five samples were analyzed, from which DNA extraction was successful in 91% (n=41). DNA from shells was not detected after the third week post-hatch. The highest DNA concentration was obtained in membranes (2.074±2.073 µg/ml). Success rates for markers were 51.1% for CytB (n=23) and 44.4% for CHD (n=20). No amplification was observed for CytB after three weeks post-hatch (n=9), regardless of the biological material used. The tested methodological conditions were suitable for molecular analysis from eggshells with and without membranes, with DNA extraction possible even four weeks post-hatch, albeit with a lower success rate for CytB amplifications. We hope to conclude this observation for a larger number of samples and markers.

P-085

The "Bear" Necessities: A multi-agency database for DNA identification of American black bears

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The Smoky Mountains of the United States, one of the most-visited wilderness areas in the country, averages more than 330 reported negative bear-human encounters annually. Wildlife officials rely on DNA identification to conclusively identify bears involved in an attack and to pinpoint repeat nuisance bears. Tribal, federal, and state officials have collaborated with Western Carolina University's Forensic DNA laboratory to build a black bear DNA database for the Smoky Mountain and southern Blue Ridge region. The goal of the database is to expedite the accurate identification of offending bears, particularly repeat offenders. For DNA identification, we use a black bear specific STR panel that has been validated for use in forensic casework. At present, the database houses more than 100 DNA profiles of bears across the southern Blue Ridge region of North Carolina, Tennessee, and the sovereign nation of the Eastern Band of the Cherokee Indians. As part of an internal validation, we performed empirical analyses to establish analytical and interpretation thresholds. We present descriptive population genetic statistics, allele frequencies, probability of identity and likelihood ratios to demonstrate the efficacy of the database for forensic DNA identification of black bears. Implementation of the database will help prevent needless euthanasia of innocent bears and will instill public confidence that offending bears have been accurately identified.

P-086

Forensic genetics in the conservation of Guiana Dolphins

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Cetaceans are fully aquatic animals predominantly occupying the long Brazilian coast, with Guiana dolphin Sotalia guianensis (van Béneden, 1864) being the most frequently stranded and, consequently, found in zoological collections. This overspreading distribution with anthropogenic activities results in pressures such as habitat loss, pollution, and accidental/incidental captures. Carcasses recovery is preponderant for documenting some biological conditions, such as sex; however, the accelerated decomposition in tropical regions and the absence of marked skeletal sexual dimorphism make these records difficult. Different behaviours between females and males, juveniles and adults, and even between populations, suggest that exposure to threats should represent differences in habitat use. In this study, we aim to develop a forensic protocol for DNA extraction from bone tissue, verifying the effectiveness of diverse structures, i.e., humerus, vertebra, sternum, and the periotic-tympanic complex. Subsequently, molecular analysis was done using nuclear and mitochondrial DNA markers in a specimen of Guiana dolphin of known sex. DNA extractions were done using phenol chloroform and then quantified with the fluorimeter Qubit®. The quantification revealed the higher effectiveness for the vertebra and humerus, followed by the periotic-tympanic complex and sternum. Amplification of nuclear and mitochondrial DNA fragments was done through polymerase chain reactions based on the literature for cetaceans and humans. Bone fragments for DNA extraction are relevant, as these parts are the most available in zoological collections. Molecular data on the Guiana dolphin has contributed, mainly, to studies of systematic, taxonomy, and evolution, with emphasis on conservation, which has eight of the twelve Management Units on the Brazilian coast and was recognized as ‘Near Threatened’ by the International Union for Conservation of Nature throughout its distribution in Central and South America.

P-166

Comprehensive data management thought a MySQL database for forensic studies

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For the proper management and storage of genetic data obtained through massive sequencing from relatives and skeletal remains of victims of the Spanish Civil War and Francoism in the Valencian Community, and to facilitate subsequent identification work, a web application type LIMS (Laboratory Information Management System) has been developed specifically designed to track sample traceability and store and manage genetic profiles in a MySQL database. The application, primarily programmed in PHP, provides a user-friendly and secure interface for efficient manipulation of genetic data.

A standout feature of this application is its ability to execute various scripts in R-base directly from the web interface. These scripts are designed to load genetic profiles into the database directly from the output files of the ForenSeq Universal Analysis Software. It is also capable of preparing the necessary files for analysis in the Familias 3 software, calculating allelic frequencies, and automatically drafting kinship reports from the information stored in the database. This integration with R-base significantly extends the analytical capabilities of the application, allowing for detailed analysis and precise and efficient report generation.

The application provides a comprehensive solution for the management of genetic data in laboratory environments, offering functionalities for the loading and querying of genetic profiles. Additionally, the automatic generation of reports streamlines the analysis process and provides consistent and reproducible results.

In summary, this LIMS web application offers a robust and versatile platform for the storage and analysis of genetic profiles, with the additional capability of executing scripts in R-base that facilitate subsequent analysis and allow for automatic report generation.

P-192

Some Like It Hot – Feasibility studies on the collection of evidence and DNA analysis of fire accelerants and burned objects at crime scenes

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According to the latest police crime statistics, the number of police-recorded cases of arson and creating a fire hazard in Germany is approximately 20,871 (about 0.4 % of all recorded crimes). Nearly half of these cases remain unsolved. The motives behind arson can vary: some perpetrators are indeed interested in the destruction of objects, while others aim to cover up previous crimes, such as murder.

Fires complicate the search for and collection of evidence at crime scenes and significantly limit the range of available forensic methods for investigation. Therefore, it is pivotal to gain deeper knowledge about the possibilities and limitations of evidence collection and DNA analysis at such crime scenes.

To this end, two feasibility studies were conducted at the Federal Criminal Police Office. These feasibility studies aimed to demonstrate whether DNA analysis can be conducted at crime scenes affected by fire and to better understand the different factors that can influence DNA recovery. Both are encouraged by genuine case work scenarios:

- The first study scenario represents an arson attack where firelighters were left at the crime scene. Here, the impact of different evidence collection methods on DNA recovery was examined. Preliminary results demonstrate that DNA recovery is low, regardless of the type of firelighter or the sample collection technique (swabbing versus taping) that was used.

- In the second study, arson was conducted in order to cover up a committed murder. Here, various substrates (with smooth and structured surfaces) were prepared with fingerprints and bloodstains and exposed to fire. To remove the soot, the objects were then covered with liquid latex. The objects as well as the latex coating were subjected to various preliminary blood detection tests as well as molecular genetic analysis.

P-195

Identification of cell death biomarkers in early and late postmortem intervals

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The determination of the postmortem interval (PMI) is an important and, at the same time, a challenging issue in forensics sciences. Over the years, innovative approaches have been tried, from physical evaluation to molecular methods, without being validated as successful for accurate PMI estimation. Postmortem interval estimation relies on the assessment of the effect of various processes occurring in the body after death. Traditionally, postmortem interval has been classified into early and late PMI. Early PMI (up to 3 days after death) have been most frequently estimated using physical postmortem changes. On the other hand, late PMI (more than 3 days after death), referring to the time when decomposition and putrefaction begin, is a more challenging task as environmental factors and the presence of insects and scavengers can accelerate or slow down these processes; consequently, methods different to physical evaluation have been developed. Decomposition process is triggered by cellular autolysis, which in turn induces destructive changes inside the cell culminating in cell death. The present work aimed to assess postmortem changes at the cellular level over time, as a potential tool to improve PMI estimates. To address this, RNA was isolated from buccal swab samples from human subjects of postmortem intervals from 1 to 22 days-after-death under natural environmental decomposition conditions. The experiment was carried out at the Forensic Osteology Research Station (FOREST) facilities in Cullowhee, North Carolina. Based on the molecular cell death signaling processes, we studied the expression patterns of 18 genes involved in cell death by quantitative Polymerase Chain Reaction (qPCR). Remarkably, we observed expression of both housekeeping (GAPDH) and key genes involved both in apoptosis and necrosis processes up to 22 days-after-death. Additionally, a constant downregulation of the expression of FasL, FasR, DR4, DR5, and Caspase-9 genes was observed for all timepoints compared to day 1 after death, along with similarity in their expression patterns from 2-10 days after death. Furthermore, Bcl-2, RIPK-1 and NF-κβ genes´ expression was found to be upregulated for all timepoints compared to day 1 after death, together with similar expression patterns from 2 to 10 days after death.

Further research is needed to validate these genes as PMI estimation biomarkers by evaluating them in a larger number of individuals and conditions.

P-196

Differentiation of cannabis types using seven tetrahydrocannabinolic acid synthase (THCAS) gene's single nucleotide polymorphisms (SNPs)

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Cannabis sativa is a vegetal species cultivated for industrial purposes, for its fibers, or as a seed source. Moreover, it is largely used for recreational purposes as well as medical due to the presence of the psychoactive cannabinoid tetrahydrocannabinol (THC). Based on the THC concentration, two different variants of this species can be discerned. Low THC plants (hemp), whose cultivation is promoted in most countries, and high THC plants (marijuana). Which are not allowed by the law in many European countries. Currently, the legality of a cannabis plant is determined based on the levels of tetrahydrocannabinol (THC) by using chemical techniques. However, these methods are not useful when the samples to be analyzed are small fragments of vegetal tissues, seeds, or are degraded. DNA-based methods could assist in the analysis of those kinds of cannabis specimens. Therefore, it is of forensic interest to find a DNA identification method for these cases.

Forty cannabis samples were analyzed. Vegetal DNA was isolated using the DNeasy® Plant mini kit (Qiagen) following the manufacturing procedure. The DNA was then quantified by real-time PCR and amplified on VeritiPro™ Thermal Cycler (Applied Biosystems). Therefore, PCR amplicons were cleaned up by using the ExoSAP-IT™. Afterwards, the SNaPshot™ multiplex kit was used to combine seven tetrahydrocannabinolic acid synthase (THCAS) gene's SNPs in a single assay. Developing a single-nucleotide polymorphism (SNP) assay for the differentiation of hemp and marijuana plants. Four previously reported SNPs for determining the C. sativa crop type were optimized, and three new ones were discovered.

This SNPs-based assay was tested on 40 cannabis plants, which included blind samples, and different haplotypes for hemp and marijuana were generated. According to the results, this SNaPshot™ assay may be a helpful method to differentiate cannabis types and could be run for forensic purposes.

P-200

Identifying the common characteristics and biological correlates of sudden unexplained death susceptibility genes

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Objectives: Sudden unexplained death (SUD) constitutes a considerable portion of unexpected sudden natural death in children and young adults. Though primary arrhythmia syndromes have been proposed as the most important risk factor for SUD, its pathophysiology and genetic background needs to be further elucidated. In the past decade, molecular autopsy has proved to be an efficient diagnostic tool in the multidisciplinary management of SUD. By post-mortem genetic testing, pathogenic/likely pathogenic (P/LP) variants in cardiovascular and metabolic genes were shown to exist in about one third of the SUD cases. Based on currently available data, we aim to identify the common characteristics of SUD susceptibility genes and the most affected canonical pathways and biological processes.

Material and methods: In this study, we have analyzed the published datasets derived from several studies focusing on the genetic predisposition of SUD, in order to systematically characterize the distribution pattern of rare P/LP variants. Subsequently, a comprehensive assessment of the constraint metrics and intolerance scores of genes that harbor P/LP variants was performed to investigate the role of selective pressure in shaping the genetic features of SUD. In addition, the Gene Ontology enrichment analysis and protein-protein interaction network analysis was conducted to identify the genes with strongest correlation to SUD.

Results and conclusions: In brief, 102 different genes with a mean Loss-of-function observed/expected upper bound fraction (LOEUF) value of 0.71 and a mean Residual variation intolerance score (RVIS) of -0.74 was identified from a total of 519 SUD cases. The mean Genomic evolutionary rate profiling (GERP) percentile in the coding region and non-coding region of these genes were 40.28 and 41.42, respectively. A calcium channel encoding gene, RYR2, was found to be the most frequently affected gene with a total of 6 P/LP variants. Among enriched pathways and processes, striated muscle contraction was identified as the most relevant biological process. Our findings could provide insights for a better interpretation of molecular autopsy findings and the investigation of novel genetic risk factors for SUD.

P-202

The significance of molecular autopsy in Sudden and Unexpected Death of individuals with Epilepsy (SUDEP)

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SUDEP is the Sudden, Unexpected Death of someone with Epilepsy, who was otherwise healthy. Each year, more than 1 in 1000 people with epilepsy die from SUDEP. These deaths occur mainly during sleep or at rest and the individual doesn't appear to have had a convulsive seizure. The cause of death in SUDEP is still unknown and it may differ between cases. Among the most involved factors in SUDEP, there are cardiac factors such as channelopathies, cardiomyopathies, aortopathies, cardiac arrhythmias and long QT syndrome (LQTS). Therefore, in forensic medicine framework, the well-known DNA markers involved in cardiac sudden and unexpected death would aid in the understanding the cause of SUDEP and in finding cardiac risk markers in patients with epilepsy. Research on SUDEP are supported by genetic analysis and recent studies focused on molecular autopsy, identified pathogenic/likely pathogenic variants in ion channel or arrhythmia-related.
The purpose of this study was to identify through next generation sequencing (NGS or MPS) technique genetic variants involved in cases of SUDEP investigated at the Legal Medicine section of Ancona (Italy). The clinical picture of the cases taken into account in this study showed the absence of structural abnormalities in the heart or brain after autopsy, histological analysis and toxicology test. Therefore, a genetic analysis (so called molecular autopsy) has been carried out to attempt to understand the cause of the sudden deaths.

We sequenced about one hundred genes correlated to inherited cardiac diseases by a MPS assay. The biological specimens collected for the genetic analysis were peripheral blood samples and FFPE cardiac tissues. Different DNA extraction methods were performed according to different starting materials and the DNA quantity was assessed through a real time-PCR assay.

MPS libraries were prepared with Ion AmpliSeq™ Library Kit 2.0 (TFS) and sequencing was performed on the Ion PGM System and Ion GeneStudio S5 Systems (TFS). For data analysis, the Torrent Suite (v 5.12.3, TFS), the Integrative Genomics Viewer tool, the Alamut® Visual Plus software (v 1.9) and Human Gene Mutation Database (HGMD) were used.

Bioinformatics analysis showed some genetic variants that have been evaluated according to ACMG standards and applying in silico prediction tools to verify if the observed variants can be considered as pathogenic, likely pathogenic, benign, likely benign or VUS.

P-207

Precision Touch DNA Sampling on Plastic Bag Knots for Improved Profiling of Packer and Holder Contributions

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In forensic DNA analysis, evidence sampling stands as a pivotal step setting the ground for the quality of the forensic profiling. The collection of touch DNA from objects, when guidelines are scarce or absent, is usually governed by ad hoc decisions based on the available case circumstances. In our laboratory, in the context of illicit drug-related crimes, similar objects are frequently encountered, offering an opportunity for the standardization of evidence treatment. This study aims to develop an effective method for sampling touch DNA from knots on plastic bags. We examine both the exposed and hidden areas of knots, considering the latter as "protected" zones less likely to accumulate biological material during subsequent handling. The study contrasts a single sample method (whole knot surface sampling, Method 1) with dual-sample methods that separate exterior (exposed) and interior (hidden) surfaces of the knot. Notably, our study consistently reveals higher DNA yields from exterior surfaces of the knots as opposed to interior samples. Importantly, our findings demonstrate that utilizing a single sample may produce DNA profiles that are not interpretable, while employing a dual-sample approach may allow for the differentiation between the genetic contributions of the person who tied the knot, the packer, from the person who held the package, the holder. We have refined the dual-sample method to reduce holder DNA in the interior sample while maintaining it on the exterior, also allowing the packer's DNA to be detected on both surfaces. We explore four dual-sample collection methods. Method 2 involves taking the first sample from the exterior and the second from the interior of an untied knot. Method 3 visually differentiates between the original exposed and hidden surfaces for precise sampling. Method 4 employs tools to open the knot for interior sampling. Method 5 uses Diamond dye to highlight cell-free DNA on both surfaces before sampling. In conclusion, this study not only clarifies the complex dynamics of touch DNA transfer and collection on plastic bag knots but also offers insights into standardizing evidence collection in similar cases.

P-254

Prediction of Gender and Age of Criminals using Saliva Microbiome Analysis

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Microbiome, comprising microorganisms present in various environments, such as on and in the human body, are increasingly recognized for their potential use in forensic investigations due to their ubiquity and ease of collection compared to other biological evidence. In this study, the saliva microbiome in South Korean participants was examined to identify potential biomarkers that could indicate an individual's age and biological gender. Saliva samples were collected from 72 participants, 12 males and 12 females, targeting three age groups: 20s, 35-44s, and 50s. DNA was extracted with ARA MagNA Tissue DNA Isolation Kit (LAS, Gimpo, Republic of Korea), followed by next-generation sequencing (NGS) with MiSeq (Illumina, San Diego, CA, USA). Data analysis was carried out using QIIME2 and MicrobiomeAnalyst platforms. The comparison of microbial composition at the phylum level revealed that the Actinobacteriota was directly proportional to age. Alpha diversity analysis using the Chao1 index was performed to measure microbial richness in the samples, which showed higher alpha diversity in individuals in their 50s. Moreover, to identify statistically significant biomarkers between biological groups, Linear discriminant analysis Effect Size (LEfSe) analysis was conducted. As a result, specific species unique to different age groups were observed: Porphyromonas pasteri in the 20s age group; Leptotrichia hongkongensis and Kingella potus in the 35-44 age group; and Porphyromonas gulae and Filifactor alocis in the 50s age group. Furthermore, variations in saliva microbiome composition were examined across different biological genders within the same age group. Notably, specific species were identified exclusively in certain biological genders in different age groups. Lachnoanaerobaculum orale was found in males in their 20s. For females aged 35 – 44, eight species, including Prevotella pallens and Prevotella nigrescens were prevalent, whereas females in their 50s exhibited seven species, including Leptotrichia goodfellowii and Prevotella nigrescens. Consequently, the presence of Prevotella nigrescens was notably consistent among females aged 35 and above. This study characterized the distinct saliva microbiome of individuals in three different age groups, while also identifying biological gender-specific microbiome within each age group. The saliva specimens utilized in this research were obtained under controlled laboratory conditions only from consenting Korean participants. However, saliva samples collected from crime scenes can be influenced by environmental factors. Thus, further studies using saliva samples exposed to other conditions are needed. Moreover, expanding the sample size to encompass a broader demographic spectrum, including individuals from diverse backgrounds, such as foreigners living in South Korea, is imperative.

P-270

GENETICS ENHANCING FORENSIC ENTOMOLGY

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In the forensic laboratories of Dubai police faith has been restored in forensic entomology through two pivotal projects :

- The first being a three year screening of the different ecologies in Dubai for carrion insects which allows for the construction of an accurate and usable database

- The second which is genomic data basing of insect species found in the first project which are absent from the NCBI Genome Database . Here long read sequencing is done using Oxford Nanopore Technology to create denovo assemblies .

The second project is a great enhancer for forensic entomology as it allows for species conformation even at the larval stage when found at a crime scene hence decreasing waiting time for a result from a potential two weeks to a few days. An example of two species that are being researched at the moment is Dermestes Frischii and Dermestes Maculatus both of which appear in all five of the ecologies studied in Dubai in different months of the year. Accuracy , Time and Comprehensiveness improvements to the discipline of forensic entomology through genetics has certainly increased faith and usability at Dubai Police .

P-291

Development, characterization and qualification of a cellular target to track biological trace transfers for forensic purposes

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Human identification through DNA analysis has known a switch in 1997 with the possibility to recover DNA and obtain a genetic profile following an object manipulation (van Oorshot & Jones, 1997). Those types of traces, known as 'Touch DNA', are defined as invisible biological traces transferred from a donor to an object or another person by physical contact. They have generated growing interest since then, due to their challenging nature, but also because they represent the majority of samples received in forensic laboratories. Although Touch DNA composition has been described as biological material from endogen and exogenous origin (anucleate cells -corneocytes-, nucleated cells, cell free-DNA and free-nuclei), its exact content remains debated (Burrill et al., 2019). Among the challenges associated with Touch DNA, their detection, transfers and associated dynamics still need to be better understood. Recently, our team brought a proof-of-concept by tracking cell shreds instead of DNA only, assuming that targeting larger cell-derived biological compounds can lead to an increased chance of Touch DNA detection (Recipon et al., 2023). They showed that persistent matrix-derived proteins and glycosylations on the skin cells (keratinocytes cells -corneocytes precursors- and corneocytes from fingermarks), can be sustainable targets. Herein, our aim was to expand this existing cell-derived fragments' markers panel with a new target: lipids. In the skin, particularly the stratum corneum, lipids are located in the lipid envelope and the lipid extracellular matrix surrounding corneocytes. Several lipid stains were chosen regarding their previous applications for fingerprint detection and user safety. Our results showed that all of them are usable to detect either calibrated keratinocytes deposition or corneocytes from fingermarks. For field use, they were submitted to CrimeLite Auto® use, two of them worked with calibrated keratinocytes, but only one showed fluorescence emission with fingermarks. Both were tested with our DNA profiling chain and showed no negative impact on the profiles obtained. In the end, one of the lipid staining fulfilled the specifications expected. Thus, we demonstrated that lipids can be added to the panel of targets for Touch DNA detection and used for sampling or transfer monitoring with quantitative imaging during mock scenarios.

P-296

It's all because of a kiss: transfer and persistence of the kisser's DNA in the kissed mouth

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Studying DNA transfer and persistence of exogenous DNA has become increasingly important, due to the increasing sensitivity of DNA detection methods and to ensure that sound expert opinion evidence is provided. During sexual assaults the perpetrator could constrain the victim to kissing, resulting in the transfer of DNA, which could be used both for personal identification and to try to establish the time when the fact happened.

In our study ten voluntary pairs were tested at seven different times after kissing: samples were collected from four different sites (perilabial and labial site, teeth and tongue) to assess the presence of exogenous DNA. Extracted DNA was quantified by real time PCR and autosomal and Y-STRs were analized.

Results obtained from DNA quantification showed an extreme interindividual variability in the transfer of DNA. The larger amount of male DNA was recovered on the perilabial and labial sites of the female partner. Male DNA amount was constant over time on the perilabial site and, to a lesser extent, on the labial site, while it rapidly decreased both on teeth and tongue.

Autosomal DNA analysis showed that transfer and persistence of male DNA on the female partner is higher than that of female DNA on the male partner. In accordance with quantification results, mixed DNA profiles were obtained from the perilabial and labial areas after 120 minutes since the kiss, while mostly single profiles corresponding to the recipient of the kiss were obtained from teeth and tongue samples. Y-STR analysis showed a good correlation with male DNA quantification results: mostly full profiles were obtained from samples recovered on female perilabial and labial sites; teeth and tongue samples gave mostly partial profiles already in samples recovered after only 5 minutes; in one sample a full Y-STR profile was obtained from the teeth at 120 minutes and at 30 minutes from the tongue.

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A retrospective study of mRNA analyses in casework after the first year of implementation.

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In April 2023 the Department of Forensic Biology at Oslo University Hospital implemented mRNA analyses as a part of the presumptive test panel for different body fluids in routine case work. Based on specific requests and case information given by the police, exhibits and/or swabs collected in a total of approx. 50 cases were evaluated as suitable and forwarded to mRNA analyses. The assumption is that these analyses will provide additional information connected to the cell type deposited on a specific body surface or an item. Here we review both the mRNA- and DNA-results of the analysed samples, specifically we study the success rate of samples collected from different evidence types and evaluate the correlation between a positive result for a given body fluid and the DNA results from the person of interest (POI).

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Experiences of the use of combined DNA and mRNA analysis in criminal cases

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In forensic criminal investigations, a DNA result may not be sufficiently informative in itself unless there is additional information about the body tissue or cell type from which the extracted DNA originates from. Especially in criminal cases in which the suspect and victim has had social or physical contact before the assault it can be of vital importance for the case to investigate the nature or possible source of the DNA findings. One method that may be used is DNA analysis followed by cell type specific mRNA analysis. For the past years the Netherlands Forensic Institute (NFI) has performed DNA/mRNA analysis on request by the Swedish National Forensic Centre (NFC). In total, samples from more than 60 rape cases was analyzed between 2015-2023. In the vast majority of cases, swabs from the suspects fingers/nail scrapings were analyzed in order to detect possible presence of DNA from the victim. If relevant DNA was detected a subset of the samples was further analyzed with mRNA typing for the possible presence of predominantly vaginal cells and/or menstrual secretion. In approximately half of the criminal cases, DNA of interest was detected and samples subjected to RNA typing analysis. Approximately in half of these cases, an indication of the presence of RNA for a specific cell type was found and the weight of evidence calculated and reported in verbal terms. An overview will be given on the subsequent use of results in court proceedings and in which some of the findings provided valuable evidence. This study contributes to knowledge on the use of DNA/mRNA approaches in criminal cases and experiences gained.

P-365

Environmental microbiotas from substrate might interfere microbiome-based identification of forensically relevant body fluids: a pilot study

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1. Objetives: Microbiome was considered as a promising tool to identify body fluids. Body fluids can be deposited to various substrates at crime scene. The body fluids collected from crime scenes could not be entirely free from microbes of substrate. However, the effects to microbiome-based identification of body fluids were not been well understood.

2. Material and methods: In the current study, five body fluids (peripheral blood, menstrual blood, nasal secretion, saliva and semen) were deposited to sterile cotton swab, bedspread and floor at indoor exposure condition for 7 days. The microbial communities of samples were charactered by 16S rRNA gene sequencing.

3.Results and conclusions: The results of FEAST showed the microbes of mock saliva samples were mainly from pure body fluids (51.53% for and 63.04%) but not substrates (25.70% and 18.92%). A contrary result was observed in peripheral blood, mock nasal secretion and semen samples. All samples were mainly clustered based on substrate but not type of body fluids in the PCoA visualization. The PERMANOVA result showed that substrate explained more variance ($R2 = 0.211$, $P < 0.001$) compared to type of body fluids ($R2 = 0.152$, $P < 0.001$). MicroDecon was applied to remove contamination of microbes of substrate from mock body fluid samples. Then, the PCoA and PERMANOVA was reperformed using decontaminated data. The results showed that samples were no longer clustered based on the substrate, and the type of body fluids ($R2 = 0.240$, $P < 0.001$) explained more variance of microbial communities of samples compared to the substrate ($R2 = 0.108$, $P < 0.001$). Our results suggested that environmental microbiotas from substrate might interfere microbiome-based identification of forensically relevant body fluids. Decontamination could decrease the effect of substrate on the microbial communities of samples and enhanced the ability to distinguish types of body fluids to some extent. This pilot study is valuable for promoting application of microbiome-based stain analyses in forensics.

P-416

Systemic approach: A review of forensic genetic programs working on Missing Person Identification in humanitarian contexts.

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Background: The Identification of Missing Persons (MPI) in communities affected by armed conflicts and other situation of violence (OSV) is a fundamental pillar to address humanitarian needs. Furthermore, the integration of forensic genetic programs into the MPI has become an added value to this multidisciplinary process. The approach of strengthening medicolegal systems - the systemic approach - has been described as an effective strategy for the resolution of MPI (M.D. Morcillo, 2024). This article reviews under the systemic approach forensic genetic programs working in MPI.

Methods: The six requirements described by M.D. Morcillo for the proper functioning of medicolegal systems were used as indicators to review the functioning of forensic genetic programs working on MPI in three humanitarian contexts: conflict, post conflict and OSV as follows: 1) Policy, 2) Procedures, 3) Human Resources, 4) Infrastructure, 5) Family/Beneficiaries, 5) Information Management, 6) Quality control/assurance.

Results: Common challenges in the functioning of forensic genetic programs working on MPI were identified in the three humanitarian contexts. Implementation of policies and the importance to address Families/beneficiaries needs has been observed as the most common gap/challenge in these contexts impacting the resolution of MPI.

Conclusions: The functioning of forensic genetic programs working in MPI requires to be integrated, understood, and discussed under the umbrella of a systemic approach, which allows to better identify gaps, provides a sustainable impact, an effective prevention and the resolution of challenges in MPI.

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The exploration of the AMI(Acute Myocardial Infarction) related piRNA in plasma exosomes based on high-throughput sequencing

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OBJECTIVE: Acute myocardial infarction (AMI) is an important cause of sudden cardiac death. but the markers for judging myocardial injury are mostly small molecules of proteins and enzymes, with low specificity and high false positive rates. At present, multiple PIWI-interacting RNA (piRNA) in plasma exosomes have been found to be involved in the occurrence of cardiovascular diseases, but the piRNAs showed a highly association with the development of AMI had not been reported yet.
METHODS: An AMI model with different times of infarction was established by ligating the anterior descending branch of the left coronary artery of rats, which was divided into three groups, namely, Sham, AMI 30 min, and AMI 60 min. The plasma exosomes and the RNAs there in were extracted,the piRNAs which were differentially expressed in the plasma exosomes of the rats were investigated using high-throughput sequencing technology.

RESULTS: There were 10909 piRNAs down-regulated and 8088 piRNAs up-regulated in the AMI 30 min group and 9100 piRNAs down-regulated and 15634 piRNAs up-regulated in the AMI 60 min group compared with the sham group. There were 18,299 piRNAs upregulated and 7,745 piRNAs downregulated in the AMI 60 min group compared with the AMI 30 min group. With the prolongation of AMI time, 212 piRNAs showed an upward trend and 88 piRNAs showed a downward trend. Prediction of the first few piRNAs target genes with more significant up- and down-regulation revealed that analysis of piRNAs with higher association with AMI, for example, piR-2490770 and piR-282889 have the same target gene, Mterf3, which is involved in negative transcriptional regulation in both rats and humans.

CONCLUSION: The expression of plasma exosomal piRNAs changes at the onset of AMI.This study contributes to the development of novel molecular markers suitable for early diagnosis of AMI, providing new ideas for the identification and prevention of AMI.

Keywords: acute myocardial infarction, exosomes, high-throughput sequencing, differential diagnosis, piRNA

P-425

Assessing the impact of inhibitors on the viability of biological samples for forensic DNA analysis: A pilot study

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The value of DNA evidence in forensic cases is tremendous. However, the presence of inhibitors in biological samples can sometimes present an obstacle for forensic scientists. This study aims to evaluate the impact of inhibition on forensic DNA analysis, highlighting limitations of current DNA quantification and amplification protocols.

This study utilised control human genomic DNA K562 (Promega) diluted at 8 different concentrations (100, 33, 11, 3.7, 1.23, 0.41, 0.14, and 0.05 ng/µl). Two common inhibitors, haematin and humic acid, were separately combined with the DNA dilutions at various concentrations. Haematin was added in 4 concentrations (2,000, 200, 20, 2 µg/µl), and humic acid in 3 (1.5, 0.15, 0.015 µg/µl). DNA was quantified using the Quantifiler™ Trio DNA Quantification Kit and amplified using the VeriFiler™ Express PCR Amplification Kit. The effects of inhibition on quantification were evaluated as amplification failure or Internal Positive Control (IPC) Ct > 30. The Degradation Index (DI) was also calculated. The effects on DNA profiling were determined based on the quality of the obtained electropherograms.

The results showed that both haematin and humic acid, at their highest concentrations, inhibited DNA quantification by preventing amplification or delaying the IPC Ct to beyond 30. Furthermore, DI > 1 were obtained from samples containing 2,000 µg/µl of haematin. An increased inhibitory effect was also observed when DNA concentrations of 33 and 100 ng/ µl were combined with any quantity of haematin. On the other hand, the quality of the electropherograms appeared unaffected by its presence. Similarly, a limited inhibitory effect was revealed in the presence of humic acid, even though a decrease in peak height was observed at 1.5 µg/µl. In conclusion, this investigation underlines the heightened impact of inhibitors on forensic DNA quantification rather than amplification, while highlighting the potential inhibitory effect of high DNA concentrations.

P-426

Investigating cardiac genetic background in sudden infant death syndrome (SIDS)

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Objectives: Sudden unexpected infant death (SUID) encompasses any unforeseen fatal event occurring during infancy, thereby including both explained and unexplained deaths. When a comprehensive case investigation yields no conclusive findings, it is termed as “sudden infant death syndrome” (SIDS). The term SIDS is applied to unexpected and unexplained deaths of infants <1 year old, typically occurring during sleep. Although the pathophysiological mechanisms of SIDS have not yet been fully elucidated, recent advancements have implicated specific genetic variants in cardiac structural proteins and ion channels among adults, suggesting a potential monogenic cause of death also in certain SIDS cases. The aim of our investigation was to evaluate whether a genetic predisposition to cardiac conditions could provide an unambiguous explanation for the fatal event in SIDS cases.

Material and methods: In the current study, 76 SIDS cases underwent full autopsy and thorough analysis using Next-Generation Sequencing (NGS) with a custom panel of genes associated with Sudden Cardiac Death (SCD). Rare genetic variants were classified according to the guidelines of the American College of Medical Genetics and Genomics (ACMG).

Results and conclusions: Genetic analyses revealed that a substantial portion of cases exhibited variants in SCD-related genes. A significant number of rare genetic variants were identified, primarily associated with genes encoding structural proteins. Only a few cases hosted at least a pathogenic or likely pathogenic variant in genes associated with structural or structural/arrhythmogenic functions. Additionally, most variants were classified as of uncertain significance (VUS). It’s noteworthy that the observed difference in variant distribution among gene groups based on function did not attain statistical significance. Despite this, most of the variants involved structural genes supposed to have a close interaction with ion channels, potentially explaining the arrhythmic event. The findings reported in our study provide evidence supporting the utility of post-mortem genetic testing in SIDS cases. However, the primary challenge associated with the most advanced and comprehensive post-mortem genetic testing lies not in identifying variants, but rather in interpreting their pathogenicity and assigning them a causal role in the infant’s death. The designation of a variant as pathogenic carries significant implications for relatives. Therefore, the clinical translation of laboratory results should be performed carefully and co-segregation analysis in family members is strongly recommended.

P-427

Alleged genetic-driven criminal impulses and free will: an underaddressed forensic research frontier

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Objetives
Genetic predisposition to aggressive-impulsive antisocial behavior is frequently used as line of defense in criminal trials in both Italy and US, especially in murder cases. This evidence is aimed to claim that some neurochemical drives due to abnormal genetic variants may overcome free will and sharply predispose the carrier to antisocial behavior. Even if in non-impulsive crimes (e.g., premeditated murder) this line of defense is easy to rebut, in impulsive crimes (e.g., unprovoked interpersonal violence, indecent exposure) this hypothesis must be verified.

Material and methods
Scientific literature regarding relationship between variants of genes involved into key neurochemical factors (e.g., MAO-A, COMT) and predisposition to aggressive-impulsive behavior
of criminal interest will be reviewed. Since the broad scope, a non-systematic approach will be used.

Results and conclusions
Current evidence of a (sometimes statistically relationship) between some genetic variants and antisocial behavior may be biased by significant confounding variables (e.g., the socio-economical context, the choice of the study sample). In order to overcome this issue, our research group proposed to compare carriers and non-carriers of the mainly involved variants in populations affected by (clinically non-sever) conditions that can strongly reduce control over impulses (hence, specific forms of dementia). Pros and cons of this approach and its endpoints will be critically discussed.

P-428

The effect of luminol on blood presumptive testing and DNA analyses

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Blood is the most frequently found evidence at the crime scene in case of violent crimes like murder, rape, and assaults. To hide evidence, perpetrators can wash bloodstains, making them invisible to the naked eye. Luminol based solution is an effective presumptive reagent for detecting latent blood at the crime scene and its chemiluminescent property has been used in forensic science for over 50 years to detect and photograph bloodstains.

From all the luminol commercial products, that are widely available, Estonian regional police agencies have decided to use Lumiscene (Loci Forensics B.V.). For this reason, Lumiscene was chosen for this study. In addition, Lumicene Ultra (Loci Forensics B.V.), a more concentrated and therefore more sensitive version of the regular Lumiscene, was tested.

Dilution series of blood stains were made on commonly used fabric. Stains were treated with Lumiscene and Lumicene Ultra solutions and left to dry. DNA samples were taken on different time points – on the first day, and after one, two, three and six months. DNA yield, degradation, and the quality of the DNA profile was assessed. Also, blood presumptive test Tetrabase, which is the main test used in our laboratory to detect blood, was carried out at the same time points to see if luminol treatment influences Tetrabase test results.

In addition, effect of luminol treatment on touch samples was evaluated. Fingerprints were left on the same fabric, treated with Lumiscene and Lumicene Ultra solutions and left to dry. DNA samples were taken on the same time points that is described above. DNA yield, degradation, and the quality of the DNA profile was assessed.

The results of the study will be described and a conclusion on the effect of luminol on Tetrabase presumptive test, DNA analysis, and contact traces will be reported.

P-429

Species-specific (human, dog and cat) identification and quantification using droplet digital PCR in forensic cases

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As the number of households raising companion animals increases worldwide, animal-related crimes have increased. Crime scenes where companion animals are present often contain a mixture human and animal DNA. In forensic case samples, it is important to know the species of origin of the sample, so it is necessary to accurately identify and quantify target DNA in mixed-species samples.

In this study, we designed and evaluated a droplet digital PCR (ddPCR) method for animal species identification and quantification of humans, dogs and cats. HLA-DRA was chosen as a human-specific marker, OR6D7 as a dog-specific marker, and FLAI-K as a cat-specific marker. The species specificity of each target was confirmed. Sensitivity testing revealed detection limits of 0.0098 ng/µL for human DNA, 0.0195 ng/µL for dog DNA, and 0.00244 ng/µL for cat DNA. Additionally, some PCR inhibitors had no effect on ddPCR quantitation. Each DNA was independently and accurately detected in samples containing a mixture of human, dog and cat DNA. This ddPCR method was also able to identify animal species from forensic casework samples and is thus useful tool for animal species (human, dog and cat) identification and quantification simultaneously in forensic cases.

P-430

Viral Detection from Formalin-Fixed Paraffin-Embedded Tissue in Autopsy Cases of Viral Pneumonia

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1.Objetives

Viral respiratory infections are a common cause of death, particularly among infants and the elderly. Accurate viral identification is crucial for diagnosis, as well as understanding and preventing the epidemiology. Detecting the virus in the lungs and trachea is essential for identifying the specific virus responsible in forensic autopsies. This study aimed to assess the feasibility of detecting viruses in formalin-fixed paraffin-embedded (FFPE) tissue by comparing the viral load and nucleic acid degradation in lung tissue from autopsy cases of viral pneumonia with that in frozen tissue.

2. Material and methods

Four cases of viral pneumonia were confirmed through histopathological examination and real-time PCR. Frozen lung tissue was stored at -80°C post-autopsy, and FFPE lung tissue was stored at room temperature for 8 months to 2 years after formalin fixation for 1 month. The FFPE lung tissue was sectioned at 10 µm, and only the tissue was extracted. DNA or RNA was extracted from 10 mg of each sample, and the virus was quantified using digital PCR. Nucleic acid degradation was evaluated using the DNA Integrity Number (DIN) or RNA Integrity Number (RIN) value.

3. Results and conclusions

Human Boca virus (HBoV) was detected in 2 cases, Human Respiratory Syncytial Virus A (HRSV A) in 1 case, and Enterovirus (EV) in another case, in both frozen and FFPE tissues. However, viral loads were significantly lower in FFPE tissues than in frozen tissues, and viral nucleic acids were more degraded in FFPE tissues. The detection rate of viral nucleic acids in FFPE tissues depends on fixation time, storage temperature, and duration. Additionally, the viruses targeted in this study were single-stranded DNA or RNA viruses, which are structurally less stable and more susceptible to degradation than double-stranded viruses.

Although frozen tissue storage is recommended for viral identification in viral pneumonia, FFPE tissues can be a viable option when the fixation time is minimized, and the analysis is prompt.

P-471

Degradation of nuclear DNA in different types of bloodstain depositions along with increasing time frames of ultraviolet light (UV) exposition. Initial results from an experimental setting using highly- UV-reflecting military garment with a desert-type camouflage pattern.

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Background: Bloodstain depositions on clothing can be the single source of nuclear DNA for the identification of unknown subjects involved in violent incidents in a significant number of cases. The yield of nuclear DNA for short tandem repeat (STR)- or single nucleotide polymorphism (SNP)-based identification can be critically low in degraded samples, for instance as a consequence of ultraviolet light (UV) exposition. Science-based time frames and radiation intensities of UV exposure leading to partial or complete destruction of DNA evidence need to be established.

Material and Methods: An experimental design involving desert-type military garment with highly efficient UV-reflecting properties and a maximum possible DNA-destructive UV light source from a laboratory work bench (LaboGene ScanLaf Mars 1800™) was established. Blood depositions including large and small drip stains from 400µL and 20 µL of blood as well as spatter patterns were generated using blood samples from healthy volunteers. Mixed stains with predefined ratios of male (M) and female (F) blood were used (10%F/90% M, 30%F/70%M and 50%F/50%M). To recover sample material, two different systems (Sarstedt Forensic Swab, Copan 4N6 FLOQ-Swab), moistened with 20µl of forensic grade DNA-free water immediately prior to sampling were employed. Specimens were obtained prior to and after 1, 2, 5, 10, 15, 20, and 30 minutes of UV exposure. For DNA extraction, the Maxwell® FSC DNA IQ™ Casework Kit was used on a Maxwell CSC 48™ platform (Promega). Quantification of DNA yield employed the Quantifiler Trio™ Kit (Thermo Fisher) and the QuantiFluor dsDNA System™ (Promega). To demonstrate the thresholds between complete, incomplete, or lost STR profiles and/or SNP genotypes, specimens from drip stains and spatter patterns were further analysed using the Verogen ForenSeq DNA Signature Kit™ on a MiSeq FGx system™ (Qiagen).

Results: In over 80% of respective samplings, higher DNA yields using the Copan 4N6 FLOQ-Swab were demonstrated. Impairment of the STR- and SNP-results along with increasing times of UV-exposition differed between the types of bloodstains. Small depositions from 20 µL of blood and spatter patterns showed a complete loss of DNA amplification and STR-/SNP-recovery after 2 minutes to 5 minutes of UV exposure. Large depositions from 400µl of blood enabled complete STR profiles even after 15 minutes of UV impact.

Discussion: Larger sized blood depositions and flocked-surface swabs are recommended for sampling from military garment. UV exposure destroying trace evidence in small drips and spatter stains may be effective already within 2 minutes.

P-480

Long-term storage of blood and saliva on five different forensic swab types over 56 months

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Efficient sampling of DNA traces is essential for the success of the subsequent DNA analysis. The choice of the optimal sampling device can significantly impact the outcome of DNA profiling. Swabs are commonly used for the collection of traces in forensic casework. However, since DNA analysis is not always conducted immediately after trace sampling, storage time can influence DNA quality and quantity. Therefore, we examined different swab types by storing and analyzing them at specific time points spanning more than four and a half years.

For this study, blood and saliva were applied separately on five different forensic swab models (ForensiX Evidence Collection Kit, ForensiX SafeDry, Copan Genetics, Copan Crime Scene and abf diagnostics A-Swab). Swabs were dried and stored at room temperature, representing the average storage conditions for real casework samples. DNA was extracted by Maxwell® RSC and DNA IQ Casework Kit (Promega) at defined time points for up to 56 months after sampling. DNA quantity as well as the degradation index and IPC shift were measured using Real-Time PCR with the PowerQuant® System Kit (Promega). Multiplex PCR was performed with the NGM Detect Kit (Applied Biosystems).

Results show that DNA concentrations of saliva decreased more rapidly compared to blood samples. The Copan Crime Scene swab exhibited lower DNA concentrations for saliva (but not blood) at the initial sampling time points compared to all other swabs although the same sample amounts were applied on all of them. The in-house degradation threshold was exceeded for three out of five swabs for saliva, but only for one swab for blood samples after varying time spans. PCR results will also be discussed.

This study highlights the importance of swab choice, especially in light of the novel extended storage duration beyond 4 years. Additionally, we observed significant differences between blood and saliva samples, alongside the crucial impact of sample substrate on DNA stability.

P-507

Evaluation of an mRNA targeted assay to determine time since deposition

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These days, contextualization of a biological crime scene stain, such as the time since deposition (TsD), is one of the main research areas in forensic genetics. Recently, transcriptome analysis has become a priority in forensic genetic research. Although RNA is thermodynamically unstable compared to DNA, it can be recovered from biological evidence in sufficient quality and quantity for analysis. Several studies have shown that transcriptional decay of specific mRNA gene transcripts can provide information on the age of a stain. We designed a targeted assay using 22 relatively “stable” or “degrading” genes, most of which were identified in one of our previously published studies. The complex design of this marker panel allows us to follow both an inter- and intra-transcript degradation assessment approach for TsD inference. We will share some of our experience in evaluating the performance of this marker panel on a time series of mock case samples, that included four forensically relevant body fluids (blood, semen, menstrual blood, and vaginal secretion).

P-509

The Necrobiome as an Indicator of Postmortem Interval in a Northern Temperate Climate

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When human remains are discovered, it is critical to establish the time since death, or postmortem interval (PMI), to help identify the deceased, witnesses, and suspects in cases of suspicious deaths. By analyzing the collection of microbes associated with decomposing remains (the necrobiome), statistical models can predict PMI; however, these are location-specific and additional research is required from representative environments before necrobiome data can be validated for use in a legal context. To date, there have been no human necrobiome studies conducted in climates like Canada with extreme seasonal temperature fluctuations, including freeze-thaw cycles and prolonged exposure to snow.

This study examined the bacterial taxa associated with human remains decomposing in a novel Canadian climate (Köppen–Geiger climate classification: Dfb). Working at the Research in Experimental and Social Thanatology (REST) facility in Bécancour, Quebec, we collected gravesoil and nasal swabs associated with 19 human donors with PMI spanning from 48 hours to >3 years. Libraries of the V4 region of the 16S rRNA marker were prepared following the Earth Microbiome Project 16S Illumina Amplicon Protocol, followed by paired-end sequencing on an Illumina MiSeq instrument. Our results provide preliminary insight into necrobiome membership and variability in a novel environment and support the use of microbial community data in estimating PMI.

P-513

Constructing the Time since Deposition (TsD) Model in Saliva Stains Based on Full-length Sequencing Technology and Microbial Markers

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Determining the time since deposition (TsD) and gender of saliva stains is helpful to reveal the time of crime and determine the nature of the case, and improve the efficiency of case detection. However, there are no relevant studies on long-term saliva stains based on microbial markers. The purpose of this study was to explore the succession pattern of long-placed human saliva stains microbial communities and identify relevant microbial markers for estimating TsD and identifying host gender, in order to be an effective alternative tool for solving practical forensic cases. In this study, a total of 20 subjects, including 10 males and 10 females, aged 19-29 years old, were enrolled. We set 9 time points (one day, one week, two weeks, four weeks, six weeks, eight weeks, twelve weeks, sixteen weeks and twenty weeks) and set blank controls. Saliva stains exposed to indoor environmental conditions for up to 140 days were collected and 16S rRNA full-length sequencing was performed using single-molecule real-time sequencing technology based on the PacBio sequencing platform. The original disembarkation subreads are corrected to obtain Circular Consensus Sequencing (CCS) (SMRT Link, version8.0), then lima (v1.7.0) software was used to identify CCS sequences of different samples by barcode sequence and remove chimeras to obtain high quality CCS sequences. Dada2 in QIIME2 was applied to denoise sequences, generating amplicon sequence variants (ASVs). The study found that the saliva stains microbial communities was relatively stable, although after long-term placement, the change of core microbiota was low, and there were significant differences between male and female saliva stains microbiota. Then, we constructed a TsD estimating model for microbial community markers based on random forest, and 24 genera were included in the model , and the final test set had an R2 of 0.92 and MAE of 9.59 days.The accuracy of sex classification model based on stepwise logistic regression model and 4 bacterial markers was 84.21%. This indicates that the long-placed saliva stains still have strong forensic practical value, and microbial markers can be used to infer the TsD of dried saliva stains and host gender identification

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Internal Validation of a Modified SALIgAE Saliva Test Protocol

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Objectives: The recovery and presumptive identification of biological material such as saliva, blood and prostate specific antigen/PSA from crime scenes is instrumental in forensic biology as it contributes directly to the crime investigation as a source of genetic material and may provide support towards or refute activity level scenarios proposed by defence and prosecution in the context of a court case examination. An ideal method for the identification of biological material should fulfill specific validation criteria and includes sensitivity and specificity for the particular biological material derived from humans, it should be rapidly and easily implemented in order to accommodate the time constraints of urgent case work and preferably with minimal or no sample destruction. The present report describes the internal validation of a modified, SALIgAE Saliva Identification (Abacus Diagnostics) protocol for “clean” cotton swabs and cuttings which does not consume additional evidentiary material other than that which will undergo subsequent DNA extraction. Materials & Methods: The modified protocol used 20 µl of reagent to test each item for α-amylase, followed by DNA extraction with QIAGEN Investigator Kit, DNA quantitation, STR PCR and capillary electrophoresis to detect for possible inhibition of the downstream molecular analysis by the SALIgAE reagent. Results & Conclusions: The sensitivity of detection in the modified protocol is increased 10-fold compared to that of the manufacturer protocol and as a direct consequence, the number of samples that can be analyzed by the supplied reagent vials is extended rendering the kit more cost-effective. Satisfactory specificity as that documented by the manufacturer is verified and the possibility of inhibitory effects of the SALIgAE reagent on DNA extraction and STR amplification was also excluded. A quality control procedure has also been devised to test each batch prior to use in routine sample testing since the kit is not certified DNA free. The modified protocol has been successful in consecutive annual external proficiency tests since its validation and routine implementation and has been accredited with the ISO 17025 Quality Assurance Standard. This modification is therefore recommended due to multiple benefits demonstrated.

P-521

Comparative evaluation of targeted RNA sequencing protocols for gene expression quantification with and without the utilization of unique molecular indices (UMIs)

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1 Objectives
Interest in forensic RNA analysis has increased over the last years. Analyzing differential gene expression to identify body fluids and/or organ tissues present in biological stains is now routinely performed by several laboratories worldwide and other potential applications of forensic RNA analysis are currently being researched.

Gene expression can accurately be quantified via quantitative PCR (qPCR), however, due to the limited number of markers that can be assayed simultaneously per reaction, qPCR is less suitable for applications requiring gene expression quantification of large marker sets. Few years ago, massively parallel targeted RNA-sequencing (RNAseq) allowing to simultaneously and accurately quantify several hundreds of markers had been added to the forensic genetic tool set. However, typical RNAseq protocols include a multiplex-PCR-step to amplify selected targets which potentially introduces bias and limits accurate gene expression quantification.
Unique Molecular Indices (UMIs) have been invented to overcome this limitation and have been implemented in protocols from some vendors.

2 Material and Methods
In this study, we compared two targeted RNAseq protocols assaying expression of a set of 121 forensically relevant mRNA biomarkers: The Ion Ampliseq targeted RNA sequencing panel (Thermo Fisher Scientific), which employs a multiplex-PCR without the use of UMIs, and the QIAseq targeted RNA panel (QIAGEN), which uses UMIs prior to multiplex amplification.
Both protocols were tested on replicated samples and dilution series and compared with respect to sensitivity and accuracy of gene expression quantification.

3 Results and discussion
The UMI-based protocol exhibited decreased sensitivity in comparison to the non-UMI-based alternative, however, making use of UMI technology greatly improved gene expression quantification accuracy. We thus recommend the use of UMI-based protocols for targeted RNA sequencing for applications requiring accurate gene expression quantification.

P-527

Reverse Body Fluid Identification Workflow: A Direct to DNA Approach

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When forensic DNA laboratories receive evidence from a crime scene their first task is to check for the presence of biological material, namely blood, semen or saliva; the same principle is applied to clothing or swabs related to victims from sexual assault cases. Examination of the exhibits by naked eye or using a forensic light source is done in order to detect the presence of body fluid stains. Many laboratories perform preliminary tests on items where biological material is potentially present before sending a cutting or swab for extraction and subsequent DNA typing.

To identify the presence of body fluids our laboratory has implemented presumptive and/or confirmatory assays to detect semen, blood and saliva and, until the end of 2022, all samples selected for DNA extraction and posterior amplification were also tested to determine the type of biological evidence in question (whenever enough sample was available) in two independent workflows.

For semen identification, all presumptive positive results were then tested in order to visualize sperm cells. However, in sexual assault cases there are many samples with a semen presumptive positive result but with a negative confirmatory test, meaning that this biological fluid cannot be confirmed. On the other hand, it was detected that in several situations the analysed samples did not present probative DNA results and, consequently, it would not have been necessary to test them for the presence of bodily fluids.

The aim of this study was to propose a more efficient workflow to be applied to all forensic samples 647 samples from sexual assault crimes (male/female victims), occurred between 2020 and 2021, were selected and the results obtained in both previously mentioned workflows were evaluated and compared with a Direct to DNA approach, in which DNA analysis is performed prior to body fluids identification (carried out only on samples with eligible DNA profiles for the criminal case under study).

The results revealed that if a Direct to DNA approach was adopted, only 256 samples (39,6%) would have been tested for the presence of semen (confirmed in 80 samples). Therefore, this workflow (currently implemented in our laboratory) is less laborious and time-consuming, allows standardization of the techniques implemented and, above all, no loss of information relevant to the judicial process was detected.

P-528

PSA and Semenogelin effectiveness to detect semen in sexual assault investigations.

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Semen detection at crime scenes is essential for sexual assault investigations. There are several approaches to detecting body fluids at the crime scene. Lateral flow immunochromatographic (LFI) tests, based on antigen-antibody reactions, are widely used for this purpose. Currently, there are two types of LFI for the detection of semen: those based on prostate-specific antibodies (PSA), produced in the prostatic epithelium; and those based on semenogelin (Sg), produced in the seminal vesicles, the vas deferens, the prostate and the epididymis, both of which are secreted in semen. The aim of this study was to evaluate the effectiveness of PSA and Sg LFI tests to detect seminal fluid, evaluating the factors that could affect the identification of these proteins and assessing the possibility of obtaining DNA. To do this, PSA and Sg LFI tests were analyzed in four types of semen samples: single and pool donors, vasectomized and 30-year-old frozen pool donor (to simulate a cold case). Serial dilutions of the samples were carried out from 1:2 to 1:32000. Our results indicated that both tests were positive in all samples, except that Sg began to be negative from 1:2000 in the 30-year-old group sample and from 1:8000 in the rest of the samples. Conversely, PSA began to be negative from 1:16000 in the fresh pool donor and from 1:32000 in the 30-year-old group, indicating better PSA performance than Sg, even in vasectomized samples. These results were independent of the presence of sperm cells, still being positive without them. Regarding DNA analysis from 1:2000 onwards, it was not possible to obtain quantifiable DNA to perform the STR profile. PSA was not detected in other dilutions of body fluids by the LFI PSA test. Future research will aim to correlate the positivity of these tests with DNA concentration and identifying the Y chromosome for the presence/absence of male, to arrive at an unambiguous and powerful identification.

P-529

Identification of Postmortem Blood through detection of D-dimer

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There are different approaches to identifying blood at a crime scene. Depending on the scenario, it may be important to determine whether a person was alive at the time of blood deposition. To identify potential biomarkers for postmortem blood, we focus on the fibrinolysis pathway. Fibrinolysis is the natural process that breaks down blood clots after healing a vascular injury. Among the fragments produced in fibrinolysis are soluble fibrin monomers crosslinked between two adjacent outer D domains detectable in plasma, known as D-dimers. Currently, D-dimer detection is used to distinguish menstrual blood from peripheral blood, as fibrinolysis plays an essential role in menstruation. Based on the rapid onset of fibrinolysis after death, D-dimer could be considered a biomarker for postmortem blood. SERATEC® has developed the PMB immunochromatographic assay to simultaneously detect human hemoglobin and D-dimer. This study aimed to assess the possibility of using the PMB test to detect postmortem blood. In addition, assessments of D-dimer levels in peripheral, menstrual, and postmortem blood were carried out, and the ability to obtain STR profiles from postmortem blood was evaluated. Our results showed that all postmortem samples reacted positively for the presence of hemoglobin using the SERATEC® PMB test, and all but one degraded sample showed a positive result for D-dimer. All peripheral blood samples from living individuals showed positive results for hemoglobin and negative results for D-dimer detection, except for one sample with a weak positive. Menstrual blood samples gave positive results for hemoglobin but variable results for D-dimer. The DIMERTEST® Latex assay was used for semi-quantitative measurement of D-dimer concentrations, with postmortem and menstrual blood yielding higher D-dimer concentrations compared to antemortem peripheral blood. Full STR profiles were developed for all postmortem samples tested except for one degraded sample, pointing to the possibility of not only detecting postmortem blood at the crime scene but also the identification of the victim.

P-530

Identification and characterization of extrachromosomal circular DNA profiles of four body fluids

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Objectives: As one of the emerging biomarkers, extrachromosomal circular DNA (eccDNA) is being revealed in an increasing number of diseases. The double-stranded, circularized, and tissue-heterogeneous features give it a more stable theoretical structure and the possibility of body fluid identification. To find potential markers with body fluid specificity was the aim of this study.
Material and methods: To demonstrate the eccDNA expression profile in different body fluids, we collected peripheral blood (PB), menstrual blood (MB), semen (SE), and saliva (SA) samples (each = 3) from healthy individuals for Circle-seq and RNA-seq. Validation was performed by outward PCR and Sanger sequencing.

Results and conclusions: In total, 263036 eccDNAs were identified, with considerably more eccDNAs in MB and SE samples than the other two. Most eccDNAs were within 1,000 base pairs (bp) in size. Mapping identified eccDNAs to genomic regions, the normalized data indicated that they were significantly enriched in the core promoter region. However, eccDNAs was highly heterogeneous among individuals compared to mRNA data results, which poses a challenge in the search for body fluid-specific markers. Conjoint analysis of the eccDNAs and mRNAs results found that 1375, 2261, and 2235 genes showed synchronous up- and down-regulation of expression in two-by-two comparisons of PB, MB, and SE, respectively ($|log2FC| > 1$, $p < 0.05$). We validated selected eccDNAs by outward PCR, and the subsequent Sanger sequencing exhibited that the junction sequence predicted by Circle-seq could be close to the actual one but with several bp deviation. In short, this study elucidated, for the first time, the eccDNA profiles of four body fluids in healthy individuals. However, more cost-effective and efficient eccDNA enrichment methods need to be established in future studies. Meanwhile, stable eccDNA markers that break the high heterogeneity among individuals require discovery.

P-531

The Impact of Temperature and Humidity on the Persistence, Detection and Recovery of Semen.

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1.
Over the years, there have been an increasing number of studies investigating the persistence of semen deposits. However, there is little information currently available on how climate conditions affect the persistence, detection, and recovery of this biological material. Semen is often a key piece of evidence in sexual assault investigations and may be found on surfaces exposed to unfavourable weather conditions for various periods of time. This work aimed to investigate the impact of constant exposure to temperature and humidity combinations on the persistence, detection and recovery of semen that has been deposited on cotton for different periods of time.

2.
Semen (50 µL) was deposited onto swatches cut from new underwear and left in a climatic chamber for designated time periods, under pre-determined temperature and humidity conditions. Specific temperature and humidity combinations were chosen to reflect climates at various times of the year. Exposed samples were tested utilizing commonly used presumptive and confirmatory tests , i.e. acid phosphatase (AP) reagent, lateral flow immunochromatographic assay to detect the presence of prostate specific antigen, and hematoxylin and eosin (H&E) staining to detect sperm. DNA was extracted, quantified, amplified, and genotyped, with the resulting DNA profile compared to the reference profiles of semen donors.

3.
As crimes of a sexual nature may not get reported until days after the event has occurred, evidence such as clothing may be exposed to environmental insults, including potentially harmful weather conditions, prior to collection and analysis. During this time, some forensically relevant components of semen may become degraded, and are therefore not detected using common forensic tests. The results of this work will be useful for forensic investigators as it will help them to determine which samples may be suitable for analysis, and provide an explanation for why false negative test results for semen may have occurred.

P-532

Mass spectrometry-based proteomics as a tool for vaginal fluid identification in forensic casework

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The identification of forensically relevant body fluids, in particular semen, saliva, blood, and vaginal secretions, serves as meaningful evidence in the investigation of sexual offenses. With semen and blood being body fluids that can be identified highly reliably, investigators face a challenge when aiming to confirm the presence of vaginal fluid. While preliminary and confirmatory tests exist that detect saliva in a sample, there is currently no readily available method available that allows for positive confirmation that vaginal fluid is present. Furthermore, vaginal fluid frequently reacts false-positive to common forensic saliva tests, hence distinguishing vaginal fluid from saliva poses a challenge.

With the goal to identify protein markers that allow for the differentiation of vaginal fluid and saliva, cheek and vaginal swabs were collected from 30 female participants. The proteome of both body fluids was characterized using high-resolution UPLC-MS/MS. Protein identification and quantification was performed using Progenesis QI (WatersTM).

From 481 detected proteins in total, 12 proteins could be identified as potential markers for vaginal fluid identification and 24 proteins could be identified as potential markers for saliva identification, and were investigated in depth for forensic purposes.

P-539

Enhancing Crime Scene Investigation Practices and Training through the Use of Artificial DNA

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Applying artificial DNA to valuables as a deterrent has demonstrated efficacy as a preventative measure. Utilizing these products enables the accurate matching of objects tagged with artificial DNA to their rightful owners and frequently establishes a connection between the perpetrator and the crime scene.

Additionally, artificial DNA provides the opportunity to develop, educate, and enhance the technical work of crime scene investigators on-site.

The issue of DNA contamination and transfer by CSI personnel is widely acknowledged as a significant concern. It is also proved, that with proper training and education, the risks associated with both phenomena can be effectively mitigated.

In our study, were examined the possibilities of using artificial DNA in the activities criminal technicians conducted within a test environment in scenario-based training. We used rooms specifically equipped for the training of forensic technicians to study the rate of DNA transfer and contamination among both experienced and student technicians, using artificial DNA.

The aim of our study with employing artificial DNA is to gain a deeper understanding of the reasons behind errors in crime scene investigation, which could assist in improving both training programs and practical work in the field. Since the use of artificial DNA allows for the demonstration and practice of these protocols without the risk of contaminating real evidence, our study could also contribute to improving contamination prevention protocols.

P-552

MPS Proficiency testing results of the GHEP-ISFG program on STR, Y-STR, and mtDNA markers

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The increased number and types of genetic markers that can be analysed simultaneously, the higher throughput of samples and the improvement of results in cases of degraded DNA, among others, are the main reasons to the expansion of the Massive Parallel Sequencing (MPS) technology in the field of forensic genetics. Once validated, this technique, a forensic DNA laboratory has to implement quality controls and monitor that it is working properly. In 2023, a MPS Proficiency. testing, using the samples of the basic level of the EIADN Exercise coordinated by the National Institute of Toxicology and Forensic Sciences (INTCF) and organized within the Spanish and Portuguese Speaking Group of the ISFG was offered to kinship and forensic laboratories. Results of the analysis of 3 reference items and two forensic items were compared to those obtained by capillary electrophoresis. Twelve laboratories took part analysing STR markers and additionally seven performed analysis of the mitochondrial DNA control region. In general results were quite good. Regarding autosomal STRs, there was one laboratory that reported one repeat less in all genotypes for the D6S474 system with respect to the other laboratories. Moreover, it was observed that not all laboratories reported results using the same strand direction. As for the Y chromosome markers, no consensus was reached for the marker DYS612 due to different nomenclatures used. Finally errors in Mitochondrial DNA analyses were due to items swap, low quality sequence, transcription error or due to wrong interpretation of the AC repeat in HV3 region. It is expected that MPS costs will be more accessible to forensic laboratories, and thus, more labs will be able to incorporate MPS into their forensic routine. The development of MPS Proficiency testing is essential to evaluate the performance of laboratories, identify inter-laboratory differences and initiate actions within the forensic community for improvement.

P-553

What is the most efficient DNA extraction method? A thorough review of Gednap extraction efficiency data.

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In forensic molecular genetics, DNA extraction is a crucial step, because crime scene samples are often limited in the amount of cells collected with evidentiary tools. That is why professional human identification labs aim for high efficiency in their extraction method. The higher the yield of extracted DNA, the more DNA available for downstream PCR and genotyping. The success of downstream genotyping results is strongly dependent on a laboratory's extraction efficiency.

The Gednap proficiency test (www.gednap.org) is a semi-annual program that includes an extraction efficiency module, enabling participating labs to compare their extraction efficiency against others. To achieve this goal, Gednap sends out identical samples to all participants and then the participants return those samples after processing with their extraction technique. Gednap will quantify the returned extracts and anonymously publish the results of the participating labs. It is a good way for each lab to see if their extraction method is efficient and economical or inefficient and wasteful. This can be valuable to inform actions to improve or change a poor extraction method.

Starting in 2012, there have been multiple Gednap extraction efficiency tests. The data obtained are an important resource to evaluate if there is a general trend towards a favorable extraction technique or if there is a dependency of extraction techniques and carrier materials used (e.g., swabs, cellulose card etc.). In this presentation, we will share the Gednap extraction efficiency data over recent years to show comparative results and trends, and discuss potential learnings for forensic genetics laboratories.

P-554

Successful performance of the Genetic Database of Mendoza, Argentina

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Since the law approval in 2016, a new DNA Database has been steadily growing in Mendoza, Argentina. Our laboratory underwent several changes to accommodate the increasing number of samples received and processed, including reference samples and evidence from crime scenes. The database saw a rapid increase in genetic profiles, encompassing convicted offenders, suspects, law enforcement personnel, laboratory staff, and evidence from unknown sources reaching over 83.500 profiles uploaded to CODIS. This represent over the 4% of the local population, making it one of the most important DNA databases of South-America. As a result, we began to see the first benefits for criminal investigation. Serious crimes, such as serial rapes and murders, were successfully solved through hits, totalizing over 950 matches with an outstanding 66% of success rate. This includes Pedigree ranks for single-source evidences, providing new investigative leads. In this poster, we present the outcomes derived from the application of a comprehensive DNA database law. We explore the success rate by analyzing the relationship between the number of evidences and hits. Additionally, we emphasize the importance of comprehensive legislation that covers all types of crimes or offenses as a prerequisite for an individual's inclusion in the database. Finally, we discuss the significant impact this tool has on justice administration, as well as the reduction in time and costs associated with criminal prosecution.

P-556

Towards a Common Body of Knowledge for Forensic Genetics: the Most Valuable Publications List and the INTERPOL DNA Reviews

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Effective training and continuing education are crucial to keep up with evolving forensic genetics technologies and applications. Forensic laboratories invest in the continuing education of their staff. Universities with forensic science programs seek to prepare their students to be future contributing caseworkers and researchers. Stakeholders in the criminal justice system (e.g., law enforcement personnel, lawyers, and judges) using DNA results also benefit from regular training and continuing education to understand capabilities and limitations of methods and practices.

A common body of knowledge in forensic genetics is expected to benefit forensic scientists, students, and stakeholders. However, it is challenging to keep up with the thousands of publications in dozens of peer-reviewed journals that exist on the topic of forensic genetics. This ever-growing body of scientific literature becomes increasingly challenging to monitor, much less incorporate into forensic laboratory training programs. For case-working forensic scientists, understanding which research publications are most informative would be helpful. This is one of the reasons that the INTERPOL reviews are prepared and shared every few years (e.g., [1,2]). DNA technical leaders and analysts can benefit from receiving regular updates on useful articles and creation of lists of valuable articles in specific areas of interest to forensic DNA casework. An effort to identify and describe some of the most valuable publications (MVPs) in the field has also been made with the initial MVP list containing almost 500 informative publications across 26 topic categories [3]. This list builds upon references cited in the July 2020 SWGDAM Training Guidelines [4] and efforts from the OSAC Biology Scientific Area Committee [5]. Approaches taken to develop the two latest INTERPOL reviews and the MVP list will be described.

References Cited

[1] Butler JM, Willis S (2020) INTERPOL review of forensic biology and forensic DNA typing 2016-2019. Forensic Sci. Int.: Synergy 2:352-367. doi: 10.1016/j.fsisyn.2019.12.002.
[2] Butler JM (2023) Recent advances in forensic biology and forensic DNA typing: INTERPOL review 2019-2022. Forensic Sci. Int.: Synergy 6:100311. doi: 10.1016/j.fsisyn.2022.100311.
[3] See https://strbase.nist.gov/NIST_Resources/Presentations/2021/AAFS2021-W19-Handouts.pdf
[4] SWGDAM Training Guidelines (2020). Available at https://www.swgdam.org/publications
[5] See https://www.nist.gov/osac/biology-scientific-area-committee

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