FORENSIC GENETICS

FORENSIC AND COMPARATIVE GENETICS

Presentation number: FG 01 Abstract number: ABS-5-ISABS-2024

REVISITING THE MYSTERY OF THE CHIDLOW MAN IN WESTERN AUSTRALIA – STRATEGIES FOR DNA ANALYSIS OF 45-YEAR-OLD HUMAN SKELETAL REMAINS

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In August 1979, the remains of a man were found in a semi-rural area northeast of Perth. The male had a gunshot wound to the chest and had been deceased for approximately 30-50 days before being discovered. As DNA techniques were not available in 1979 to assist in identifying the remains, no samples were taken from the body specifically for DNA analysis at the time. Subsequent attempts at extracting DNA from the deceased clothes and bones from the body after exhumation were unsuccessful. Specialized techniques are needed to improve DNA profiles, especially when no other biological material is available due to the age of the remains or exposure to adverse environmental conditions. Despite bones and teeth being more resilient, they still undergo DNA degradation, aggravated by high mineral content hindering extraction and resulting in suboptimal outcomes. The DNA extraction method was optimized to withstand harsh conditions and soil compositions prevalent in the Western Australian region, guided by recent scientific advancements. Briefly, bone samples were ground, then either extracted using the PrepFiler Express BTATM method, or subjected to a full demineralization protocol followed by PrepFiler Express BTATM extraction. The standard PrepFiler Express BTATM method yielded lower-quality DNA extracts, with all PowerPlex® 21 loci falling below reportable thresholds. On the other hand, bone fragments processed using the PrepFiler Express BTATM full demineralisation method resulted in higher quality DNA and reportable PowerPlex® 21 profiles. The optimised full demineralisation method proved effective in this cold case, where despite significant degradation, a clavicle bone produced a good quality DNA sample that was suitable for Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) analysis which enabled further investigative options including Familial searching and Forensic Genetic Genealogy for the unresolved murder inquiry.

Keywords: demineralisation, DNA bone extraction, human remains, DNA profiling, PrepFiler Express BTA

Presentation number: FG 02 Abstract number: ABS-29-ISABS-2024

NONINVASIVE PRENATAL PATERNITY TESTING: A NEW CONTRIBUTION FROM DIP-STR MARKERS

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Noninvasive prenatal paternity testing (NIPPT) plays an important role in forensic analysis, especially in cases of suspected post-rape pregnancy. It allows for the determination of fetal paternity within weeks of gestation. This project aims to contribute to the field of NIPPT for forensic applications by developing a novel and robust method. The goal is to leverage Next-Generation Sequencing (NGS) technology to analyze 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 even under extreme DNA imbalances (up to 1000-fold). They have already shown promises 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. We will present promising preliminary results regarding the application of the multiplex to single-source profiles. Efforts are currently being dedicated to ensuring the results' reproducibility. 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. 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.

Keywords: NIPPT, NGS, DIP-STR markers, multiplex, forensic genetics

Presentation number: FG 03 Abstract number: ABS-73-ISABS-2024

IMPLEMENTING LIMITS OF DETECTION IN FORENSIC DNA ANALYSIS BY MASSIVELY PARALLEL SEQUENCING

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Goal was to define and characterize a sequence-aware approach to establishing lower limit of detection (LOD) thresholds based on positive control data. Compare and contrast the novel positive control-based method to the traditional negative control-based method. LODs were modeled from both negative and positive controls using a total of 53 negative controls and 100 positive controls analyzed using the OmniSTR (NimaGen) and ForenSeq DPMA (QIAGEN) reagent kits, and the MiSeq (QIAGEN) and NextSeq (Illumina) sequencing platforms. All analysis was performed using R (R Core Team) and MixtureAce forensic software (NicheVision Forensics). Statistical models were built on positive or negative control data analyzed without a threshold to assure observation of the complete distributions of noise reads. Noise models were built on contamination in negative controls according to published methods, and on artifacts in positive controls according to a novel approach. Briefly, artifacts other than back-one LUS (longest uninterrupted stretch) stutter were used to calculate thresholds in positive controls. Back-one LUS stutter can be considered informative signal, along with true alleles, in cases where back-one LUS stutter is included in probabilistic genotyping models. Models were also tested where back-one LUS stutter was included in the artifacts, and where back-one SLUS stutter was excluded. LODs determined from negative and positive controls ranged from 10-200 reads and 50-150 reads respectively. LODs based on negative controls were more variable due to the stochastic nature of contamination. Selected STR loci can generate significant back-one SLUS stutter which can exceed calculated thresholds. LODs based on negative controls can be set per sequencing run or transformed to dynamic thresholds when being used with samples having different levels of coverage.

Keywords: sequencing, LOD, STR, stutter, LUS

Presentation number: FG 04 Abstract number: ABS-175-ISABS-2024

EFFECT OF SURFACE ROUGHNESS ON THE DEVELOPMENT OF LATENT FINGERMARKS USING DIFFERENT FINGERPRINT POWDERS

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Aim was to investigate the effect of surface roughness on the effectiveness of latent fingerprint visualization using the two most commonly used fingerprint powders. For the experiment, we used a white polystyrene board, which we cut into four sections. Three sections were treated with sandpaper (grit sizes 60, 240, and 600), while one remained in its original state. We determined the mean roughness depth (Rz) for all surfaces using the Mitutoyo device (Mitutoyo, Japan). They were classified as very smooth (Rz = 0.0092 µm), smooth (Rz = 0.1493 µm), medium to smooth (Rz =2.1251), and of a medium roughness (Rz = 4.2839 µm). Each section was then separated into two halves, and eight participants left one thumb fingermark on each surface. To develop fingermarks, the first halves were treated with Jet-Black Special Powder (BVDA, The Netherlands) and the second with Magnetic Jet Black (BVDA, The Netherlands). The quality of developed fingermarks was scored on a scale of 0 – 4, where scores 3 and 4 were considered suitable for the identification. Fingerprints developed with black special powder were suitable for identification in 71.9% (23/32) cases, and those developed with magnetic powder were suitable in 96.9% (31/32). Fingermarks on very smooth and smooth surfaces were almost all suitable for identification, despite the powder used. On the medium to smooth surfaces, magnetic powder provided identifiable marks for all cases, while the efficiency of special powder dropped to 62.5%. On the surface of medium roughness, magnetic powder was efficient in 87.5 cases, while special powder could provide 37.5% of prints suitable for identification. The study findings imply that selecting fingering powder could be less important when dealing with very smooth and smooth surfaces. On the other hand, Magnetic powder could be more effective on rough surfaces than Special powder.

Keywords: surface roughness, fingermark development, identification, fingerprint powder, visualization

Presentation number: FG 05 Abstract number: ABS-76-ISABS-2024

EVALUATION OF AUTOSOMAL, X- AND Y-CHROMOSOME STR LOCI SEQUENCING PERFORMANCE WITH FORENSEQ® SIGNATURE PREP KIT ON MISEQ FGX® INSTRUMENT

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Massively parallel sequencing is steadily finding its place within routine forensic workflows, thus enabling extraction of more forensically relevant information from challenging samples. ForenSeq® DNA Signature Prep kit (Verogen), with its Primer Mix B, targets over 200 markers of different types, including 27 autosomal STR (aSTR), 7 X-STR and 24 Y-STR loci. In this study, we aimed to evaluate genotyping performance of 58 STR markers by testing sensitivity, repeatability, reproducibility and concordance, as part of internal validation. Genomic DNA was extracted according to our validated laboratory procedures, and samples of positive control 2800M were used as provided by the manufacturer (Verogen). Libraries were prepared, pooled and sequenced using ForenSeq® DNA Signature Prep kit (Verogen) on MiSeq FGx® (Illumina/ Verogen) sequencing platform, according to the manufacturer's instructions. Subsequent data analysis was performed in ForenSeq® Universal Analysis Software (Verogen) and Microsoft Excel. Sequencing quality parameters were within optimal range for all sequencing runs. Complete aSTR and Y- STR genotypes were obtained for DNA inputs down to 125 pg, while dropouts were observed at X-STRs already with 250 pg input. Results were repeatable and reproducible for all replicates and were 100% concordant with capillary electrophoresis results. When testing reference samples, concordance rate was 99% for aSTRs, 100% for X-STRs, and 98% for Y-STRs, due to allele dropouts observed in D22S1045 and ambiguous allele calls in DYS392 and DYS612. Stutters n-1 comprised most non-allelic signals (71%) and exceeded default stutter thresholds in 11/58 loci, while other stutters (n+1, n+2, n-2, n- 3) were also detected but with lower intensities (<10%). Overall, this study presents analysis thresholds and interpretation guidelines for sequencing 58 STR loci as part of in-house validation of ForenSeq® DNA Signature Prep kit.

Keywords: massively parallel sequencing, evaluation, ForenSeq, autosomal STRs, X- and Y-STRs

Presentation number: FG 06 Abstract number: ABS-87-ISABS-2024

ABOUT ETHYLENDIAMINETETRAACETIC ACID (EDTA) POSSIBILITY TO INCREASE THE SELECTIVITY OF COMBUR TEST E FOR FORENSIC BLOOD ANALYSIS

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Recently published method validation reported increased selectivity of tetramethyl-benzidine (TMB)-based presumptive tests, by adding 0.5M ethylendiaminetetraacetic acid (EDTA) to a reaction pad. Chelator EDTA binds positively charged metal ions and other substances preventing them to react with TMB in pad, i. e. eliminating possible false positives. Hence, the main goal of this study was to verify these data for subsequent upgrade of ours validated protocols. In addition, possible interference of the 0.5M EDTA on DNA analysis was evaluated. Twenty-one substances/surfaces were tested. Whole blood (known donor) was used as a positive control, and test strips moistened with ultrapure water as negative control. All testing was done in triplicates, with methods of EDTA application previously described. Presence/absence of colored reaction was recorded at 30s and 60s and interpreted as positive or negative after 60s. Rust, brass, brick, soil-1, tomato pureè/paste and saliva samples of known donor exhibited negative results for test strips moistened with water and 0.5M EDTA. Contrary to previously published, majority of tested samples including plant leaf, copper surface and bleach, showed false positives with 0.5M EDTA. Negative results with EDTA for wood branch, soil-2 and banana pointed out possible influence of EDTA on increased selectivity since positive results were obtained with water. Finally, all EDTA tested blood samples resulted in complete DNA profile using the GlobalFiler™ PCR Amplification Kit. Overall findings showed considerable discrepancy when compared with previously published, indicating that further study about influence of EDTA on selectivity of Combur3 Test® E are required. Sodium hypochlorite as a major compound of comercially available bleaches, remains important substance causing false positives. Nevertheless, Combur3 Test® E is distinguished as highly sensitive, cheap, easy to use and STRs analysis compatible presumptive test for blood.

Keywords: blood, Combur3 Test® E, selectivity, EDTA, STRs analysis

Presentation number: FG 07 Abstract number: ABS-84-ISABS-2024

IMPACT OF TREATED VS. UNTREATED HAIR SHAFTS AND CHEMICAL CLEANING APPROACHES ON THE YIELD OF MITOCHONDRIAL DNA

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In forensic science, traces such as hair are common pieces of evidence. Although nuclear DNA is known to be present in hair shafts, it's been found to be very fragmented. Mitochondria have been observed to be a common component of keratinizing hair shafts, therefore, providing a rich source for mitochondrial DNA (mtDNA). It has been shown that heating and chemical hair treatments can affect the physical structure of the hair shaft. For heating treatment, a critical temperature of 140C caused modifications that were irreversible. These modifications included a change in appearance of the cuticle and gradual disappearance of the scales of the cuticle. Chemical treatments can include lightening, dying, relaxers, and perms. These changes, particularly of the scales along the shaft, can lead to external biological material being trapped within the cuticle scales. The DNA of the trapped material will be extracted along with the DNA of the hair if the biological material cannot be adequately removed. A study demonstrated that 3% NaClO, bleach, worked very well to remove biological contaminants. However, this study didn't examine whether the NaClO damages the endogenous DNA. In addition, no studies have assessed whether this type of NaClO cleaning would be effective when conducting analysis on treated hairs. Since heat and chemical treatments affect the hair's physical characteristics, it is plausible that the entirety of the shaft of treated hairs might allow the NaClO to penetrate further into the hair, ultimately causing unwanted DNA damage and degradation. This research will be conducted in two phases. The first phase will assess whether there is an impact on mtDNA yield on treated vs. untreated hairs and the second phase will assess whether NaClO impacts mtDNA yield when compared to Terg-a-zyme, a cleaning detergent containing protease enzymes. A custom mtqPCR assay will be used for quantification. Significant differences between groups in both phases will be assessed.

Keywords: hair, mtDNA, treatment, cleaning, yield

Presentation number: FG 08 Abstract number: ABS-134-ISABS-2024

Y-HAPLOGROUP ANALYSIS OF MEDIEVAL BOSNIAN POPULATION: DISCOVERIES FROM ARCHAEOLOGICAL REMAINS

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Archaeological sites in modern Bosnia and Herzegovina provide evidence of continuous life during the Middle Ages. This study aimed to ascertain Y-haplotypes, predict Y-haplogroups, and assess their frequencies in a sample of the medieval Bosnian population. DNA samples were collected from 42 male remains across 12 archaeological sites dating to medieval Bosnia. DNA extraction was performed using phenol-chloroform extraction from bones and teeth, followed by Y-STR analysis using the PowerPlex® Y23 System. Y-haplogroups were predicted using online software. Statistical analysis was conducted using the 2 test with a significance level of $p<0.05$. The most frequently detected haplogroups were I2a, R1a, R1b, and J2a. The predominant haplogroup in both the medieval Bosnian and contemporary Bosnian and Herzegovinian populations was I2a. However, the European haplogroup E1B1b, present in 10% of the recent population, was absent in the medieval samples. This discrepancy may be attributed to factors such as limited successfully amplified Y-STR profiles, small sample size, and stochastic effects. The 2 test revealed no significant differences in haplogroup frequencies between the medieval and contemporary populations. Based on the obtained Y-haplotypes, Y-haplogroups were detected for the first time, and their frequency in a sample of the medieval Bosnian population was determined.

Keywords: ancient DNA, Y-STR markers, medieval Bosnia, archaeology

FORENSIC DNA DATABASES

Presentation number: FG 09 Abstract number: ABS-31-ISABS-2024

MITOGENOME SEQUENCING OF 5,000 POPULATION SAMPLES USING THE PACBIO SEQUEL II SYSTEM AND DEEP DIVE INTO THE DATA ANALYSIS

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In forensic investigations where nuclear DNA cannot be obtained for damaged or degraded samples, sequence analysis of mitochondrial (mt) DNA plays an important role. Large population databases of mitogenome sequences for forensic purposes are not yet available, reducing the value of a mtDNA profile match since statistical analysis is based on the size of the database. Laboratories around the world utilize the European DNA profiling group (EDNAP) forensic mtDNA population (EMPOP) database in their casework. The primary goal of our research is to produce 10,000 mitogenome sequences from blood samples to be included in the EMPOP database and used for forensic and population studies. Extraction of the mtDNA from blood samples is being performed using the Zymo Quick-DNA Miniprep Plus kit. The mitogenomes are amplified using primer sets targeting two, overlapping 8.5kb amplicons. SMRTbell adapters are added to the DNA amplicons prior to running them on the PacBio Sequel IIe instrument. The sequence files are analyzed using GeneMarker (GM) HTS software to produce mitotypes. The sequences are analyzed before being sent to EMPOP. GM HTS data analysis is performed with the help of Haplogrep to obtain a detailed analysis of the population data, addressing confirmation of haplotype information and haplogroup assignments, and addressing anomalies in sequence data related to misalignment and noise patterns or amplification errors. Analysis also includes confirmation of single nucleotide polymorphisms (SNPs), whether they are part of the sequence or noise generated within the sequenced mitogenomes. Finally, before uploading the samples to EMPOP they are interpreted for mitochondrial DNA sequence variation and checked for quality and error using the SAM2 algorithm. By obtaining the haplotypes of the samples and ensuring the sequenced mitogenomes meet the standards of the EMPOP database, the sample data is then incorporated into EMPOP to increase statistical power of mtDNA matches.

Keywords: mitogenome, PacBio Sequel IIe, mitochondrial DNA, haplotypes, EMPOP, next generation sequencing

Presentation number: FG 10 Abstract number: ABS-146-ISABS-2024

DEVELOPING FORENSIC DATABASES BY Y-CHROMOSOME HAPLOGROUPS FOR MEGALOPOLISES CONSIDERING MIGRATION

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The goal was to analyze migration influence on dynamics of Y-chromosome haplogroups in megalopolis populations and consider migration role for developing DNA forensic databases for megalopolises. In the samples from population of three megalopolises (Moscow, Novosibirsk and Sankt-Petersburg), Y-chromosome haplogroups were detected by means of Whit Athey's Haplogroup Predictor basing on 18 STR haplotypes. Simultaneously, questionnaire data for residents of megalopolises was collected, including data and place of birth, ethnic affiliation, and their ancestors in two previous generations. In three megalopolises, distribution of Y-chromosome haplogroups (R1a, N, I1, I2, E1b1b. R1b, J1 and J2) characteristic for the Russian population was revealed, heterogeneity of the frequency profiles was observed. In the previous years, intensive migration to megalopolises took place from Northern Caucasus and Middle Asia, that resulted in accumulation of the Southern origin haplogroups (C3, G2a, G2c, J1, J2, L, O2, O3, Q, R2 and T). During three generations of Moscow population, frequency of the Southern origin haplogroups increased from 11 to 21% with parallel decrease of frequencies for the most widely spread haplogroups (R1a and N). Molecular data obtained was in good agreement with the questionnaire data. Our results demonstrate necessity of developing DNA forensic databases by Y-chromosome for each megalopolis taking in consideration geographical position and genetic demographic processes, especially, migration. Individual affiliation to concrete generation should be considered, as different generations are under action of different migration flows. The DNA forensic databases for megalopolis should be updated considering migration processes.

Keywords: megalopolis, migration, forensic databases, Y-chromosome haplogroups

Presentation number: FG 11 Abstract number: ABS-152-ISABS-2024

FORENSIC DNA ANALYSIS AND GENETIC PRIVACY IN CRIMINAL PROCEEDINGS: PERSPECTIVES FROM THE INNOCENCE PROJECT CROATIA ON WRONGFUL CONVICTIONS IN CROATIA

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The emergence of the Innocence Project in Croatia in 2015 has significantly highlighted the challenges and potential of post-conviction DNA examinations in addressing wrongful convictions. Established experimentally at the Faculty of Law in Zagreb in 2020 with support from the Croatian Science Foundation, this initiative plays a pivotal role in raising public awareness about miscarriages of justice and advocating for essential legal reforms to aid defendants in reopening their cases. Within the framework of the Innocence Project Croatia, several regional and national conferences and workshops have been organized, partnerships have been formed, and the prison population has been notified about this project and actively participates in it. Currently, there are 27 cases on the docket of the Innocence Project Croatia from prisoners who consider themselves innocent. These cases have raised significant issues regarding the treatment of DNA evidence and genetic privacy in criminal trials, underscoring the need for improved legal standards and practices in the handling of such sensitive information. This paper explores the handling of DNA evidence, focusing on its retention, use, and the challenges of ensuring genetic privacy in criminal investigations. Through a detailed analysis of the European Court of Human Rights jurisprudence, this paper evaluates how DNA data is managed in Croatian national criminal legislation and its implications for individual privacy and the presumption of innocence. Additionally, the paper discusses forensic errors and the significant risks they pose to justice, as well as received cases from inmates as part of the Innocence Project Croatia initiative. By incorporating theoretical, comparative, and case study methodologies, this article suggests potential legislative reforms to optimize the use, storage, and ramifications of DNA data in Croatia.

Keywords: DNA evidence, innocence project, wrongful convictions, genetic privacy, croatian legal reforms, European Court of Human Rights

FORENSIC DNA PHENOTYPING

Presentation number: FG 12 Abstract number: ABS-75-ISABS-2024

ASSESSMENT OF THE GENOTYPING AND PREDICTION PERFORMANCE OF PISNPS AND AISNPS USING FORENSEQ® DNA SIGNATURE PREP KIT

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The application of massivelly parallel sequencing (MPS) in forensic genetics has enabled the analysis of larger number and types of markers in a single multiplex assay. Development of single nucleotide polymorphisms (SNPs) genotyping technologies provided the prediction of externally visible characteristics and the inference of biogeographical ancestry based on genetic data that may be used as investigative lead. The ForenSeq® DNA Signature Prep Kit includes 24 phenotypic (piSNPs) and 54 ancestries informative (aiSNPs) with two markers common to both categories. The aim of this study was to assess the genotyping and prediction performance of piSNPs and aiSNPs using ForenSeq® DNA Signature Prep Kit (Verogen) with Primer Mix B on MiSeq® FGx Forensic Genomics System (Illumina). Genomic DNA was extracted from 83 buccal swabs according to the validated laboratory procedures. Libraries were prepared, pooled, and sequenced following the manufacturer's recommendations. Pooled libraries were quantified before sequencing using Qubit™ dsDNA HS Assay Kit and Qubit 3.0 Fluorometer (Thermo Fisher Scientific). Results were analyzed with ForenSeq Universal Analysis Software v.1.3 (UAS). Sequencing performance was assessed using UAS. Run metrics passed across all runs. The kit was able to produce complete piSNP and aiSNP genotypes using DNA inputs of positive control (PC2800M) from 1.0 ng down to 0.25 ng. As expected, SNP coverage imbalances became more significant with the reduction of the DNA input. Inter-replicate discordance was observed in terms of allele and locus drop-out. Reference- type samples showed a high call rate with the generally balanced heterozygous reads in piSNPs (0.85). However, sample-to-sample variation was observed for 9 piSNPs (<0.6) that may impact phenotype prediction. As a part of in-house validation, this study presents the application of piSNPs and aiSNPs genotyping and potential challenges in phenotype estimation.

Keywords: massively parallel sequencing, phenotype informative SNPs, ancestry informative SNPs, ForenSeq DNA Signature Prep kit, MiSeq FGx

Presentation number: FG 13 Abstract number: ABS-166-ISABS-2024

NEXT-GENERATION FORENSIC DNA TOOLS: A LEAP FORWARD IN CRIMINAL JUSTICE

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The field of forensic science is rapidly evolving, particularly in DNA phenotyping, which holds the promise of revolutionizing crime scene investigations. This technique allows for the prediction of a suspect's physical appearance based on associated genetic markers, thus providing a valuable tool when a DNA database for comparison is lacking. By integrating DNA evidence with crime methodologies, biogeographical ancestry, medical information, and familial genetic traits, DNA phenotyping can significantly aid criminal investigations. In Taiwan, there is a growing need for law enforcement agencies to become familiar with this technology. Through a comprehensive literature review, this paper discusses the intricacies of DNA phenotyping, its application alongside genetic technologies and databases, and the legal challenges that may arise in the future. The literature reveals that DNA phenotyping can construct predictive models of appearance, such as eye color, hair color, and skin tone, and refine these predictions with minor physiological features like eye spacing, nose width, earlobe formation, eyelid type, and thumb curvature. The accuracy of DNA phenotyping could be further enhanced by integrating it with national health care databases, which could narrow down investigation scopes, clarify case details, and greatly assist the criminal justice system in apprehending the true culprits or exonerating the wrongfully convicted. As this technology advances, it will be imperative to address the ethical and privacy concerns associated with accessing and utilizing personal genetic information.

Keywords: forensic science, DNA phenotyping, physical characteristics, genetic phenotypes, minor physiological features

GENETIC ANALYSIS OF FORENSIC NON-HUMAN MATERIAL

Presentation number: FG 14 Abstract number: ABS-26-ISABS-2024

WILDLIFE POISONING INVESTIGATION - THE IMPORTANCE OF USING MOLECULAR IDENTIFICATION METHODS IN THE INVESTIGATION PROCEDURES.

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The poisoning of wild animals continues to be one of the main methods of illegal killing. Recently, reports of poisoning by biological baits and poisoned carcasses of domestic animals have been increasing. This type of poisoning is mainly directed against predators such as foxes, jackals, and wolves, which cause damage to game in hunting areas, or against livestock kept outdoors, indirectly harming birds of prey that feed on poisoned carcasses. Investigating the facts and circumstances of such illegal killing is a complex investigation that necessarily requires the involvement of experts in the field of veterinary medicine and experts in the field of forensic investigation, research, and expertise, in addition to the action of the competent authorities. In addition to establishing the cause of death through the forensic veterinary examination and the presence of poison in the organs and tissues of the poisoned animal, the investigating authorities sometimes have an interest in determining the origin of the bait that served as the source of an excellent or individual identification. In the case that took place in January 2020, which the media called the case of Mazin poisoning, poisoning with carbofuran was confirmed in a fox (Vulpes vulpes) and a strictly protected species the wolf (Canis lupus) and the bald eagle (Aquila chrysaetos) in two separate incidents in different locations and in a time interval of 14 days. In both cases, cattle carcasses were used as bait, which was confirmed by molecular identification of stomach content samples from fox, wolf, and bald eagle by determining species-specific polymorphisms on the cytochrome b (cyt b) and cytochrome c oxidase subunit I (COI) genes. In addition, each individual was genotyped to determine a unique DNA profile assessment of the Thermo Scientific Bovine Genotypes Panel 3.1 Kit panel of 12 STRs to determine the calves possible connection to the cow and connection to the potential herd.

Keywords: wildlife poisoning, baits, carbofuran, DNA barcoding, ST

Presentation number: FG 15 Abstract number: ABS-88-ISABS-2024

A META-ANALYSIS OF DNA EXTRACTION METHODS IN FORENSIC SCIENCES

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This study presents the findings of a meta-analysis conducted in February 2024, focusing on DNA extraction methods from human and animal samples within the field of forensic sciences. Utilizing the Web of Science database, we systematically searched for papers using the keywords "DNA extraction" OR "DNA isolation" AND "forensic*" covering the period from 2013 through 2023. The analysis included 491 papers from 14 high-ranking journals in the field of Forensic Sciencesof, of which 313 articles met the requirements of our meta-analysis, the rest of the articles were excluded mainly because of missing or insufficiently described methodology, or because the article referred to the source of another article that was no longer available. We carefully examined and summarized the most frequently utilized extraction kits and methods. This comprehensive review offers valuable insights into the current landscape of DNA extraction practices, shedding light on the predominant approaches employed in forensic investigations. The results of this meta-analysis serve as a useful resource for forensic scientists, aiding in the selection of optimal DNA extraction techniques for a wide range of sample types.

Keywords: extraction kits, forensic samples, tissue, swabs

GENOME-BASED APPLICATIONS IN FORENSIC SCIENCE

Presentation number: FG 16 Abstract number: ABS-141-ISABS-2024

COMPARISON OF FST AND RST COEFFICIENT OF GENETIC DIFFERENTIATION IN THE ANALYSIS OF POPULATION SUB-STRUCTURE

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Coefficient of genetic differentiation, F_{ST}, is a measure of population differentiation due to genetic structure. The analog coefficient, R_{ST}, adapted to microsatellite data, is also used. The aim of this study was to perform a meta-analysis of synthesized data and investigate the influence of specific intrapopulation genetic structures on interpopulation relationships. Special focus was the influence of island population isolation on the substructuring of the Croatian population, and the influence of regional population groups on the substructuring of Southeast Europe. Autosomal STR loci of microsatelite DNA were analyzed in the sample of 590 non-related adult individuals of insular (Cres, Ugljan, Pašman and Dugi Otok) and continental populations of Croatia (Baranja) and Slovenia were integrated with the data on 952 individuals available at the Institute for Anthropological Research, and the data on 1335 individuals available from the database for South-Eastern Europe. The calculation of F_{ST} and R_{ST} coefficient of genetic differentiation was performed using the statistical package Arlequin version 3.5.1.2. software. Finally, we performed comparison of F_{ST} and R_{ST} coefficient of genetic differentiation at aforementioned levels. Based on two mentioned coefficients of genetic differentiation lower genetic differentiation was detected at the higher level of grouping of SE European populations (F_{ST}/R_{ST} = 0,002) than at the level of sub-populations of Croatia (F_{ST}/R_{ST} = 0,005), due to the decreased influence of endogamy. The comparison of F_{ST} and R_{ST} coefficient between different (sub)populations of Croatia and Southeast Europe indicates that the specific features of (sub)populations and certain rare alleles in their gene pool affects the values of this statistical parameters.

Keywords: STRs, genetic sub-structuring, coefficient of F_{ST}, R_{ST}, Croatia, Southeast Europe