MOSES SCHANFIELD MEMORIAL SYMPOSIUM – POSTER PRESENTATIONS

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AN EXPLORATORY AI APPROACH COMBINING INTERPRETABLE MACHINE LEARNING (ML) AND SELF-SUPERVISED DEEP LEARNING FOR DIFFERENTIATING NAIL SCRATCH PATTERNS ON PORCINE SKIN

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The study explored the identifiability of nail-shape-related signals using interpretable ML and AI-based image analysis. The study included 8 participants: 4 with rounded and 4 with square gel nails. Each participant performed 5 controlled scratch strokes on a different porcine skin piece. The length and maximum width of each of the three individual traces per stroke were measured using a digital caliper, and the mean width/ length ratio was calculated. For each trace, edge morphology and line continuity were assessed and photographed. A regularized logistic regression (LR) model with class balancing was trained and evaluated using leave-one-participant-out cross-validation (LOPO-CV) to account for intra-participant dependency. To independently assess whether shape-related information was present in the photographs, a self-supervised vision transformer (DINO ViT-S/8) was used with CLAHE contrast enhancement and attention-weighted patch feature extraction and evaluated under identical LOPO-CV conditions. The morphological LR achieved an accuracy of 0.76 (AUC 0.83), with a sensitivity of 0.84 for rounded nails and a specificity of 0.68 for square nails. Scratch width was the most influential predictor: wider traces were associated with square nails, whereas smooth edges and continuous lines were associated with rounded nails. The DINO classifier achieved an accuracy of 0.74 (AUC 0.83, sensitivity 0.68, specifically 0.79). The results suggest that scratch morphology encodes nail shape information detectable by both feature-engineered and pixel-level methods. The differences likely reflect contact mechanics: square nails (flatter edges, sharper lateral transitions) distribute force more broadly, producing wider and irregular traces, while rounded nails concentrate force, resulting in narrower, smoother, and more continuous marks. The comparable AUCs across methods support signal consistency, though the findings are exploratory and require further validation.

Keywords: scratch marks, nail morphology, porcine skin model, interpretable machine learning, self-supervised deep learning

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WHEN ALGORITHMS TESTIFY: AIDRIVEN DNA ANALYSIS, EVIDENTIARY STANDARDS, AND CRIMINAL JUSTICE REFORM

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Forensic DNA analysis has already influenced criminal justice, and serves as a powerful tool and double-edged sword for both conviction and exoneration. Despite its scientific foundations and wide application, DNA evidence is vulnerable to factors as interpretive error, methodological limitations, and cognitive bias, as demonstrated by numerous wrongful convictions identified through the Innocence Project. Recent advances in artificial intelligence (AI) promise to improve the interpretation of complex DNA samples, including mixed, low template, and degraded profiles. At the same time, the growing use of AI driven forensic analysis raises significant legal, ethical concerns, as well as procedural challenges in terms of whether it can be used as a direct evidence in the procedure. This article examines the implications of AI based DNA interpretation for criminal justice, with particular attention to evidentiary reliability, due process, institutional accountability, and emerging policy responses in the U.S. and Europe. Further it draws on parallels with clinical genomics and documented forensic applications of AI, where the authors argue that AI can enhance forensic accuracy and fairness only if integrated within transparent, validated, and ethically governed frameworks that respect fundamental legal protections.

Keywords: probabilistic genotyping, artificial intelligence, innocence, fair trial rights

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HELIXCROSS – AN AI-POWERED CROSS-EXAMINATION SIMULATION FOR FORENSIC EXPERT TESTIMONY TRAINING

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Forensic expert testimony faces intensified scrutiny as methods have grown more complex, probabilistic reasoning has become central to evidence interpretation, and admissibility standards have strengthened. Testimony preparation, however, remains largely informal – conducted in-house, lacking standardization, and rarely providing the adversarial practice needed to develop reliable courtroom competence. HelixCross is a prompt-engineered large language model (LLM) agent designed to address this gap. It simulates defense counsel cross-examination and provides structured, on-demand coaching for forensic practitioners in a controlled, repeatable environment at no cost. The flagship module targets forensic DNA interpretation, focusing on areas that routinely generate courtroom challenges: probabilistic genotyping, likelihood ratio communication, mixture interpretation assumptions, number-of-contributors sensitivity, and admissibility framing under legally permissible standards. The curriculum is discipline-agnostic and intended for extension to other forensic domains. HelixCross uses a Retrieval-Augmented Generation (RAG) architecture, anchoring questioning and coaching to a curated knowledge base of forensic science standards and legal frameworks rather than relying on general training data alone. The curriculum spans nine progressive phases, from voir dire and foundations through to reliability and disclosure challenges at peak difficulty. The simulation alternates between two modes: In-court mode simulates adversarial questioning, while Coach mode delivers phase-by-phase debriefs with exploitable-statement analysis, court-safe rewrites, and a grading rubric across ten performance dimensions. The tool is freely deployed on OpenAI ChatGPT and Google Gemini. Future work will evaluate educational effectiveness and scoring reliability through a controlled empirical study.

Keywords: expert testimony, simulation-based education, large language model, probabilistic genotyping, artificial intelligence

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DEEP LEARNING-BASED AUTOMATED SKELETAL SEGMENTATION FOR FORENSIC RECONSTRUCTION: A CRITICAL EVALUATION OF AI RELIABILITY AND HUMAN-IN-THE-LOOP NECESSITY

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Automated AI segmentation offers a promising approach for building virtual skeletal reference datasets where osteological collections are absent, yet systematic assessment of its forensic limitations remains sparse. This study evaluated the reliability and failure modes of Total-Segmentator, a MONAI-based deep learning plugin for 3D Slicer, applied to lower-extremity segmentation of clinical MSCT data. Ninety-one MSCT angiography scans (UH Merkur Zagreb; UHC Split) were processed on a Ryzen 9 8945HX (8 GB GDDR6 VRAM), with mean runtimes of 108.63 s (femur, cropped protocol) and 75.62 s (tibia/patella). The femur was the primary target given its preservation frequency, pronounced sexual dimorphism, and utility for stature estimation. Automated segmentation generated STL surface meshes reviewed for anatomical validity. Of 91 cases, 79 (86.8%) produced valid models; of 16 failures, 8 were due to incomplete acquisition, 1 to non-skeletal protocol scope, 3 to unexplained cortical artifacts, and 4 to voxel-level metal- bone misclassification in endoprosthetic cases (25% of such cases; 4.4% of total), producing STL models fusing bone and implant geometry. The key finding is not proportion but the invisibility: endoprosthetic hardware was not consistently detectable at pre-selection, so corrupted models entered the pipeline undetected, producing systematically erroneous bone anatomy. No DICOM metadata differences (acquisition parameters, reconstruction kernel, or protocol descriptors) were identified between cases with and without endoprosthetic hardware, confirming implant-related errors cannot be anticipated or filtered by metadata alone. These findings confirm that, despite its efficiency for virtual collection building, deep learning-based segmentation requires structured human-in-the-loop validation. Implant-related misclassification represents a non-obvious but forensically consequential risk to data integrity that pre- screening alone cannot eliminate.

Keywords: MSCT, automatic segmentation, deep learning, skeletal collections, femur

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ANALYSIS OF POST-MORTEM BLOOD MICROBIOME: HIGH-RESOLUTION IDENTIFICATION OF PSEUDOMONAS TOLAASII USING PACBIO 16S FULL-LENGTH SEQUENCING

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Accurate identification of the thanatomicrobiome is crucial for estimating the post-mortem interval (PMI) and understanding decomposition processes. Traditional culture-based methods, such as MicroSeq ID, often fail to detect non-culturable or fastidious microorganisms, limiting the comprehensive profiling of the post-mortem microbial community. In this study, we analyzed DNA extracted from post-mortem blood samples (n=20) using third-generation sequencing (TGS). We employed the PacBio Sequel system for 16S rRNA full-length sequencing to achieve species -level resolution. The results were compared with previous data obtained via culture-based MicroSeq ID analysis to evaluate the diagnostic improvement. Peripheral blood samples were collected from 20 cadavers during autopsies conducted within a post-mortem interval (PMI) of 1 to 3 days. To ensure analytical consistency, the extracted DNA underwent PacBio 16S full-length sequencing across two independent runs. Among the 20 samples analyzed, *Pseudomonas tolaasii* was identified in 5 cases, while it remained undetected. The consistent detection of this species in a significant majority of samples collected within a narrow, early PMI window suggests that *P. tolaasii* may be a characteristic component of the early-stage thanatomicrobiome in peripheral blood, a finding that was previously unattainable through conventional culture-based MicroSeq ID systems. *Pseudomonas tolaasii* was identified in 5 out of 13 samples (38.5%) through PacBio full-length sequencing. Notably, this species had never been detected in the same samples using the MicroSeq ID system. The high-fidelity long-read sequencing provided sufficient phylogenetic markers to distinguish *P. tolaasii* from other closely related Pseudomonas species, which is often challenging with short-read NGS or phenotypic assays. The detection of *P. tolaasii* highlights the superior sensitivity and resolution of third-generation sequencing in forensic investigations.

Keywords: thanatomicrobiome, *pseudomonas tolaasii*, forensic microbiology, post-mortem interval (PMI)

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INTERPRETATION OF TWO INDEPENDENT AUTOSOMAL STR ANOMALIES IN A COURT-ORDERED PATERNITY TESTING CASE

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This case study describes a court-ordered paternity investigation involving two children, in which an exceptionally rare discordance was observed in one confirmed father-child pair. The anomaly consisted of two independent autosomal STR inconsistencies, highlighting challenges in interpreting atypical inheritance patterns in paternity testing. DNA from buccal swabs was analyzed using a 24 STR loci system. To exclude technical artifacts (e.g., allele dropout, amplification failure, or sample mix-up), additional testing with alternative STR kits and sequence-based analysis was performed, confirming the initial findings. For the first child, full concordance was observed among the child, mother, and alleged father across all loci. The second child matched the mother but showed discordance with the alleged father at two loci, sharing 21 of 23 obligate alleles. Despite this, the probability of paternity was 99.85%. Comparison between siblings showed concordance at 19 of 23 loci, supporting a full sibling relationship (99.84%). At the vWA locus (chromosome 12), the second child was homozygous for the maternal allele, lacking the paternal allele. A similar pattern was observed at D7S820 (chromosome 7), without broader chromosomal abnormalities. These findings suggest rare biological mechanisms beyond simple STR mutations, including multistep germline mutation, gene conversion, or localized loss of heterozygosity. Sequence analysis of vWA showed the child's allele matched the maternal microvariant, supporting a locus-specific anomaly. Analysis of D7S820 confirmed previously identified alleles, consistent with mutation or gene conversion. This rare case of dual STR anomalies in a confirmed father-child pair highlights the complexity of STR interpretation and the importance of confirmatory and sequence-based analyses in resolving atypical results.

Keywords: paternity testing, autosomal STR, sequence-based analysis, inheritance anomalies

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PRELIMINARY VALIDATION OF THE A27PLEX STR DETECTION KIT AND CONCORDANCE STUDY OF THE NEW GENERATION MULTIPLEX STR KITS

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Short tandem repeats (STRs), also referred to as microsatellites, are polymorphic genetic markers consisting of short repetitive sequences, typically 2-7 base pairs in length, that are consecutively repeated a variable number of times at a given locus. The number of repeats varies greatly among individuals, making STRs ideal markers for DNA profiling and a powerful tool for forensic identification, as well as for paternity and relatedness testing. With the expansion of STR marker use in forensic identification, commercial multiplex STR kits have become widely available. These kits are designed to analyze multiple autosomal STR loci in a single PCR reaction. The purpose of this study was to validate a novel A27Plex Fluorescence Detection Kit, produced by Nanjing Superyears Gene Technology Co., Ltd., and to conduct a concordance study comparing this commercial kit with those currently used in forensic analyses in Croatia. The A27Plex Fluorescence Detection Kit is a 6-dye STR multiplex assay that amplifies 25 autosomal STR loci, one Y-chromosome STR locus, and an insertion-deletion (indel) sex-determining marker. In this preliminary study, standard samples with previously determined DNA profiles were analyzed. Detected loci, intra-locus balance, interlocus balance, stutter ratios, and concordance with other kits used in Croatia were evaluated. Based on the obtained results, it can be concluded that this kit is suitable for forensic identification in the Croatian population and produces results comparable to those of other kits routinely used in this region. This study represents the first validation of the A27Plex Fluorescence Detection Kit in Croatia.

Keywords: short tandem repeats (STRs), DNA profiling, forensic identification, multiplex PCR, concordance

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PRELIMINARY VALIDATION OF THE Y41SE STR DETECTION KIT AND CONCORDANCE STUDY OF THE NEW GENERATION MULTIPLEX STR KITS

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Y-chromosome short tandem repeats (Y-STRs) are genetic markers consisting of short repetitive sequences, typically 1 to 6 base pairs in length, distributed throughout the Y chromosome and inherited as a haplotype along the paternal line. They are primarily used to trace male lineage in forensics, anthropology, and genealogy. Commercial multiplex Y-STR kits are designed to analyze multiple Y- chromosome STR loci in a single PCR reaction. The aim of this study was to validate the novel Y41se STR Fluorescence Detection Kit, produced by Nanjing Superyears Gene Technology Co., Ltd., and to perform a concordance study comparing this kit with those currently used in forensic analyses in Croatia. The Y41se STR Fluorescence Detection Kit is a 6-dye multiplex assay that amplifies 40 Y-chromosome STR loci (33 low-mutation-rate and 7 rapidly mutating loci) and one Y-chromosome insertion–deletion (INDEL) marker. In this preliminary study, selected samples with previously determined Y-STR profiles were analyzed. Detected loci, stutter ratios, and concordance with other kits used in Croatia were evaluated. Based on the obtained results, it can be concluded that this kit is suitable for use in the Croatian population and produces results comparable to those of other kits routinely used in this region. This study represents the first validation of the Y41se STR Fluorescence Detection Kit in Croatia.

Keywords: Y-chromosome STRs (Y-STRs), forensic genetics, male lineage, multiplex PCR, concordance

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IN HOUSE OPTIMISED METHOD OF NON-DESTRUCTIVE AUTOMATED DNA EXTRACTION FROM FORENSIC TOOTH SAMPLES

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At the Forensic Science Centre "Ivan Vucetic," routine methods for DNA extraction from dental/skeletal remains often involve time-consuming procedures and the destructive grinding of samples into powder. However, recent forensic guidelines for the identification of human remains prioritize less invasive methods. To address this shift, previously published protocols for DNA extraction from ancient/archaeological intact tooth samples were reviewed and optimized, to establish a rapid, effective, and non-destructive method for obtaining short tandem repeat (STR) profiles. Tooth samples from five cases of unidentified human remains received in 2025 were processed using the optimized protocol: chemical cleaning, washing, drying, UV irradiation, followed by demineralization and lysis. The last two steps of the described protocol were achieved by immersing the intact teeth in 0.5 M EDTA at pH 8, supplemented with proteinase K, under continuous mixing for 24 hours. The resulting supernatants were centrifuged at 5000 rpm using Amicon Ultra 15 filter units until a lysate volume of approximately 300 µl was reached. Automated purification was performed on a BioRobot EZ1 using a large-volume protocol, yielding 50 µl of eluate. DNA quality and quantity were evaluated via Real-Time PCR, followed by STR typing using the GlobalFiler™ PCR Amplification Kit resulting in complete DNA profiles for all samples. Subsequently, to validate the efficacy of this non-destructive approach, the same samples were grounded into powder and processed according to the laboratory's validated protocol, which produced consistent results. Comparison with reference DNA profiles obtained from biological relatives and personal belongings further confirmed the accuracy of the findings. Overall, this study demonstrates the applicability of the established automated, non-destructive extraction method for the forensic identification of human remains, ensuring the preservation of evidence, resulting in a usable STR profile.

Keywords: forensic identification, intact tooth samples, automated DNA extraction, STRs analysis

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ANALYSIS OF DEGRADED FORENSIC SAMPLES USING MASSIVELY PARALLEL SEQUENCING: ADVANTAGES AND CHALLENGES

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Forensic casework samples are often challenging. PCR- and capillary electrophoresis (CE)-based analysis methods may result in partial DNA profile or no profile at all when analyzing inhibited and/or degraded samples. Massively parallel sequencing (MPS) offers analysis of many different types of loci, short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs), in a single reaction, thus conserving the sample while extracting the maximum amount of information. In this study, degraded samples from commercial test kit (containing 500, 300 and 150 bp fragments; Qiagen) were amplified in duplicate using Primer Mix B from ForenSeq™ DNA Signature Prep (Illumina®/Verogen). One replicate of samples underwent library preparation according to manufacturer's instructions, while preparation of the other replicate was modified thusly: 30 µL of purified libraries (instead of 20 µL) were added into normalization, with proportionately increased magnetic beads volume. The total of 18 casework samples (tissue, bone, teeth, blood) were also prepared for sequencing using unmodified protocol. Libraries were sequenced on the MiSeq FGx® instrument (Illumina/Verogen). ForenSeq® Universal Analysis Software (Verogen) and Microsoft Excel were used for data analysis. Detection of STR loci (particularly long allele variants) was considerably reduced in 150bp-samples, which was expected. Impact of degradation on detection of SNPs was not significant, due to their short loci lengths. Most prominent differences between 20µL- and 30µL-libraries were observed in numbers of imbalanced heterozygous loci and detected alleles above analytical threshold, with small gain in favor of 30µL-libraries. For casework samples, only 4/18 resulted in poor MPS profiles, while others benefited from larger number of detected markers and additional information of ancestry and phenotype, clearly underlining the advantages of MPS in comparison to CE-based STR genotyping.

Keywords: degraded forensic samples, MPS preparation modification

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EVALUATION OF SIMULATED FORENSIC MIXED SAMPLES: COMPARISON AND ADVANTAGES OF MASSIVELY PARALLEL SEQUENCING TECHNOLOGY OVER CAPILLARY ELECTROPHORESIS

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Forensic samples from multiple donors present significant challenges in evaluation and interpretation. Massively parallel sequencing (MPS) enables simultaneous analysis of many genetic markers, including short tandem repeats (STRs) and single nucleotide polymorphisms (SNPs), while also providing detailed sequence information for each allele. The ability to distinguish alleles with identical STR repeat numbers but different sequences enhances the resolution and interpretation of mixed DNA profiles. In this study, we aimed to evaluate the analytical performance of an MPS kit in comparison to capillary electrophoresis (CE) results for simulated forensic mixtures. Samples were prepared by mixing the male positive control 2800M and the female positive control 9947A at ratios of 1:1, 1:2, 1:5, 1:10, and 1:20. Each mixture was amplified in triplicate using Primer Mix B from the ForenSeq® DNA Signature Prep kit (Verogen), targeting 231 loci. Libraries were subsequently prepared, pooled, and sequenced on the MiSeq FGx® platform (Illumina/Verogen) according to the manufacturer's instructions. Data analysis was performed using the ForenSeq® Universal Analysis Software (Verogen) and Microsoft Excel. Mixture ratios calculated for STR and identity SNP loci without shared alleles generally showed lower female-to-male average values than expected theoretical values and CE results. Identity SNPs were consistently more reliable than other loci. Significant dropout of male alleles was observed across all loci at ratios of 1:10 and 1:20. For certain loci (e.g., D1S1656, vWA, DXS10103, and four Y-STRs), allele dropout occurred even at a 1:1 ratio, with D22S1045 being particularly problematic, showing markedly deviating average ratios. In conclusion, MPS offers advantages over CE in mixture analysis; however, results should still be interpreted cautiously, as sequencing artefacts may overlap with minor contributor ratios.

Keywords: mixed samples, evaluation, massively parallel sequencing, ForenSeq, MiSeq FGx

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DNA-BASED PREDICTION OF EYE, HAIR, AND SKIN COLOR IN MEDIEVAL INDIVIDUALS FROM BOSNIA AND HERZEGOVINA

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Human skeletal remains provide valuable biological information, but many aspects of an individual's physical traits remain unknown without genetic analysis. Recent developments in DNA phenotyping now allow researchers to reconstruct key pigmentation features from ancient remains. In that regard, we analyzed DNA from ten skeletal remains recovered from three medieval necropolises in Bosnia and Herzegovina, alongside five contemporary individuals used as controls. A set of 40 SNP markers linked to pigmentation was examined using the HIrisPlex-S system, and the results were expressed as probabilities of specific traits. The predictions for the modern samples matched their known physical characteristics, supporting the reliability of the method when applied to ancient remains. Among the medieval individuals, blue eyes were predicted in four, and brown eyes in six. Hair color was mostly brown, with some individuals showing darker or lighter shades. Most were also predicted to have intermediate skin pigmentation, while a smaller number likely had lighter skin. Overall, this study offers the first genetic-based insight into the pigmentation traits of medieval individuals from Bosnia and Herzegovina, improving our understanding of their biological characteristics.

Keywords: DNA phenotyping, medieval Bosnia, ancient DNA, pigmentation traits

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MOLECULAR APPROACH TO AGE ESTIMATION: EXPLORING THE POTENTIAL OF TELOMERE LENGTH IN FORENSICS

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With the growing application of age estimation in modern forensics, the potential of telomere length (TL) as a biomarker of molecular age should not be overlooked, given the continuous shortening of telomeres throughout life. This study aimed to establish and refine methodological approaches for TL assessment in human samples, evaluate its applicability in a forensic context, and investigate its correlation with chronological age, as well as the potential influence of sex on this relationship. A total of fifty unrelated, healthy adult non-smokers (aged 22-73 years) provided buccal epithelial cells and peripheral venous blood samples, along with relevant personal, lifestyle, and health information. Genomic DNA was subjected to absolute TL estimation using quantitative Real-Time PCR. Absolute telomere length per diploid genome was determined using standard curves, with the 36B4 gene serving as the reference marker. In addition, Fluorescence In Situ Hybridization (FISH) was employed to visualize telomere structure, length, and potential chromosomal aberrations in metaphase spreads from selected samples representing the highest and lowest TL values. Statistical analysis revealed significant differences in telomere length between lymphocytes and buccal epithelial cells, while no significant associations were observed with age or sex, regardless of the tissue type used for DNA extraction. FISH analysis demonstrated stronger fluorescent signals in samples with previously determined longer telomeres, supporting the reliability of qPCR-based estimation. Although telomere length alone may have limited reliability as a predictor of age, it may still serve as a complementary biomarker alongside epigenetic and other molecular markers. Future studies with larger and more diverse cohorts, as well as integrated multi-marker approaches, are needed to improve the accuracy and practical applicability of molecular age prediction in forensic settings.

Keywords: telomeres, absolute telomere length, molecular age, Real-Time qPCR, FISH

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AN ANALYSIS OF THE EFFICIENCY AND RELIABILITY OF PHOTOGRAPH-BASED LINEUP RECOGNITION WITH AI-GENERATED IMAGES

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This study examined the reliability of photograph-based lineups composed of AI-generated fillers, as well as the relationship between recognition accuracy and characteristics of the event, the witness, and the identification procedure. The study included 161 participants who were shown four video recordings of simulated criminal acts, two violent and two non-violent. After viewing each video, participants were asked to identify the perpetrator from a five-person photographic lineup consisting of one real perpetrator and four AI-generated fillers, while control questions were used to introduce a short delay between observation and identification. Overall identification accuracy across all scenarios was 25%, with participants correctly identifying the perpetrator in an average of one video. Accuracy was significantly higher in scenarios involving violent events (39.13%) compared to non-violent events (12.42%). The highest accuracy was observed in simulated robbery (44.10%) and aggravated theft (34.16%), whereas identification in bicycle theft (16.77%) and drug delivery (8.07%) remained at or near chance level. Multivariate analysis showed that identification accuracy was influenced by the presence of violent elements, the order of video presentation, the position of the perpetrator in the lineup, participant age, and prior police-related experience. The results indicate that AI-generated images can be effectively used to construct fair lineup fillers, as participants did not treat them differently from real faces, supporting their application in forensic practice. However, overall identification accuracy remains limited and is influenced by both procedural and psychological factors, which should be considered in future applications.

Keywords: eyewitness identification, lineup recognition, photo lineup, ai-generated images, recognition accuracy

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KIT-DEPENDENT ALLELIC DISCORDANCE AT THE D1S1656 STR LOCUS: A FORENSIC CASE REPORT

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In forensic science, human identification relies on DNA profiles. Short Tandem Repeats (STRs) are repetitive DNA sequences that vary in length among individuals, and are widely used for identification purposes e.g. in criminal investigations and paternity testing. Misinterpretation of alleles at any locus may lead to incorrect conclusions and have significant downstream consequences. Here we report a case of discordant results at D1S1656 STR locus obtained with different PCR kits. Genomic DNA was extracted from a buccal swab sample using two methods: EZ1 Investigator Kit (Qiagen) and Chelex® 100. Real-time PCR with Quantifiler® Human DNA Quantification Trio Kit (Applied Biosystems) and Investigator Quantiplex Pro RGQ Kit (Qiagen) were used for quantification. STR amplification was performed using GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific), PowerPlex® Fusion 6C System (Promega), and Investigator 24plex Kit (Qiagen), with subsequent fragment separation by capillary electrophoresis on 3500 Genetic Analyzer (Applied Biosystems). Data analysis was performed in GeneMapper® ID-X v1.7. The resulting DNA profile from Investigator 24plex kit exhibited a tri-allelic pattern at D1S1656 and a single peak at D12S391. In contrast, bi-allelic peaks at D1S1656 and D12S391 were obtained with GlobalFiler™ and PowerPlex® Fusion 6C kits. The remaining profile was concordant across kits, no contamination was detected in negative controls, and positive controls were as expected. Re-extraction from the same sample yielded consistent results. These findings highlight the advantage of using multiple STR amplification kits: in cases of extremely short or extremely long allele variants appearing at loci that are closely located in one kit, which may cause misinterpretation, it is greatly beneficial to use another kit with different loci arrangement. Any atypical patterns need to be carefully evaluated to prevent erroneous interpretation of forensic DNA evidence.

Keywords: short tandem repeats, human identification, misinterpretation, allelic discordance, tri-allelic peak

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TRUSTWORTHY AI IN FORENSIC GENETICS STARTS WITH CONTAMINATION GOVERNANCE: A EUROPEAN EDB PERSPECTIVE

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Artificial intelligence (AI) applications in forensic and anthropological genetics increasingly depend on the quality, traceability, and governance of upstream laboratory and casework data. Yet discussions on AI readiness often underemphasize a foundational issue: contamination control. This presentation argues that elimination DNA databases (EDBs) should be understood not only as quality assurance tools, but as core components of trustworthy AI-enabling infrastructure in forensic genetics. Building on a narrative review with scoping elements and a policy-focused synthesis of empirical contamination studies, accreditation practice, and European legal frameworks, we identify harmonized minimum requirements for EDB operation. The analysis integrates forensic contamination evidence, ISO/IEC 17025-aligned quality management principles, and European data protection constraints (including strict purpose limitation and safeguards against function creep). Particular attention is given to governance separation from criminal/intelligence databases, auditable workflows, role-based inclusion and retention, and monitoring metrics that improve comparability across jurisdictions. The central claim is that robust EDB governance improves not only contamination detection and incident handling, but also the reliability of downstream data ecosystems on which AI-supported forensic decision systems may rely. In the context of expanding cross-border forensic data exchange in Europe, harmonized EDB standards can therefore be framed as both a quality assurance priority and a precondition for ethically and legally trustworthy AI deployment in forensic genetics.

Keywords: forensic genetics, elimination DNA database (EDB), DNA contamination control, trustworthy AI

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FORENSIC INVESTIGATION OF INTENTIONAL ANIMAL POISONING: AN INTEGRATED APPROACH IN CRIME PREDICTION AND RESOLUTION

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Animal poisoning, particularly in urban environments, frequently presents a significant challenge for investigative authorities due to the difficulty of linking the toxic agent with the perpetrator's intent. This paper presents a forensic investigation of a case involving the serial poisoning of cats, where a combination of forensic necropsy, toxicology, and molecular identification played a pivotal role. Following several suspicious deaths of cats, a forensic examination of one carcass was performed. The necropsy raised reasonable suspicion of carbofuran poisoning (a carbamate pesticide) based on external findings of vomit around the mouth, the presence of bluish granules with a distinctive odour in the digestive tract, and systemic findings such as generalised extensive congestion and haemorrhage within parenchymal organs. Toxicological analysis using gas chromatography-mass spectrometry confirmed carbofuran in the gastric contents and its derivatives in the liver. To establish the biological origin of the partially digested bait, DNA barcoding was performed targeting four mitochondrial markers (cyt b, COI, 12S, and 16S rRNA). This molecular evidence created a critical link between the preparation of the poison and the criminal act. Proving the method of poisoning and identifying the biological origin of the bait are essential steps in establishing the elements of the criminal offense "Killing or Torturing Animals." The synergy of these techniques, further enhanced by artificial intelligence for predictive mapping and pattern recognition, not only elucidates the cause of death but provides irrefutable evidence of a deliberate act, thereby facilitating the identification of perpetrators and prevention of future incidents.

Keywords: animal poisoning, forensic necropsy, forensic toxicology, DNA barcoding, crime prediction

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AI ASSISTED CLASSIFICATION OF HUMAN, DOG AND CAT HAIR USING MICROSCOPY IMAGES: A PILOT STUDY

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The aim of this study was to evaluate the performance of a simple, freely available AI-based image classification tool for differentiating human, dog, and cat hair. Hair samples were mounted in glycerol and analysed under 400× magnification using a DigiCyte NE930 microscope. Four images were captured from different regions of each hair shaft using focus stacking. Medulla type was recorded, and the medullary index (MI) was calculated as the ratio of medulla width to total hair width. A total of 120 images (10 hair samples per category, 4 images per sample) were used to train the model using the Teachable Machine platform. Model performance was evaluated on an independent test set of 60 images (15 hair samples; 5 per category). The model achieved an overall accuracy of 85%. Human hair was correctly classified in all cases (20/20), while cat hair was correctly classified in 19 out of 20 cases. Dog hair showed lower classification accuracy (12/20), with all misclassified dog samples assigned to the cat category. Correct classifications were associated with higher confidence values (mean 0.97), whereas misclassified samples showed lower confidence (mean 0.78). Analysis of misclassified samples indicated that darker hair colour and borderline medullary index values may contribute to classification errors. Human hair predominantly exhibited absent or fragmented medulla and lower MI values (mean 0.24), whereas animal hair showed continuous and wider medulla, with higher values in dog (0.41) and cat hair (0.69). Dog hair exhibited intermediate medullary index values, which may explain lower classification accuracy and increased variability in model confidence. These results demonstrate that simple AI models can effectively differentiate human from animal hair while classification between animal species remains challenging, likely due to overlapping morphological features. Future studies should include larger datasets and additional species to improve model robustness.

Keywords: forensic hair analysis, microscopy, artificial intelligence, machine learning, image classification

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INTERNAL VALIDATION AND COMPARATIVE ANALYSIS OF OSIRIS AND GENOPROOF SOFTWARE FOR STR PROFILE INTERPRETATION

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Accurate interpretation of STR profiles is essential for reliable forensic DNA analysis. The evaluation of a broad spectrum of forensic samples can be complicated by the presence of artifacts such as stutter peaks, pull-up peaks, dye blobs, off-ladder alleles, and others. This study presents an independent validation and comparative assessment of the open-source software OSIRIS v2.4 and the commercial platform GenoProof Suite, using GeneMapper® v5 as the reference standard. A total of 113 human DNA profiles (comprising approximately 3400 alleles), including both single-source and mixed samples, were analyzed using raw .fsa data generated on a SeqStudio™ Genetic Analyzer (Applied Biosystems) with the NGM Select™ kit. The dataset consisted of 68 concordant profiles and 45 problematic cases ("flags") involving artifacts, mixed samples, or degraded DNA. All profiles were independently interpreted in the three software platforms. Results showed over 99% concordance in allele calling, with no significant differences in allele designation between the tools. However, OSIRIS consistently reported higher RFU values across all loci compared to GeneMapper and GenoProof. This behavior is attributed to OSIRIS's use of adaptive baseline modeling and mathematical curve fitting, which normalize raw data to a zero baseline and apply iterative fitting of idealized parametric curves to the signal. In contrast, GeneMapper and GenoProof utilize fixed-window baseline estimation, which may result in lower peak intensities or uncalled alleles. These findings suggest that OSIRIS is a highly effective and cost-efficient alternative for allele designation and artifact identification, potentially reducing interpretation errors in forensic casework. However, as all profiles were manually reviewed, this study does not validate any automated expert system functionality.

Keywords: GeneMapper, GenoProof Suite, OSIRIS, STR profiling, artifact interpretation

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COMPARISON OF CONVENTIONAL AUTOSOMAL STR ANALYSIS AND NEXT-GENERATION SEQUENCING (NGS) IN DEGRADED BONE SAMPLES FROM MASS GRAVE VICTIMS AND BLOOD SAMPLES FROM PHENOTYPICALLY KNOWN INDIVIDUALS

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Identification of degraded human remains represents one of the greatest challenges in forensic genetics, particularly in samples obtained from mass graves and other aging environments, where degradation and inhibitors compromise conventional STR genotyping methods. Previous studies have shown that numerous PCR inhibitors, including humic acids, heavy metals (iron, copper, cadmium, and lead), and microbial contamination, may interfere with DNA analysis and reduce amplification efficiency. The aim of this study was to compare the results obtained by capillary electrophoresis and next-generation sequencing (NGS) in degraded bone samples from mass grave victims and blood samples of persons with known phenotypes. The previous study included 30 bone samples from war crime mass graves analyzed in 2002, 2003, and 2007. In the initial analysis, DNA was extracted using a phenol-chloroform-based protocol, followed by autosomal STR genotyping using the PowerPlex® 16 System on an ABI Prism 310 capillary electrophoresis platform. In the present study, the same samples were reprocessed, and DNA was re-isolated using the PrepFiler BTA Forensic DNA Extraction Kit (Thermo Fisher Scientific) following the manufacturer's protocol. Subsequent genotyping was conducted using next-generation sequencing (NGS) after library preparation with the MiSeq FGx System Identification Library Prep Kit, targeting forensic identification markers. Blood samples of persons with known phenotypes were also analyzed using the same methodology (NGS).

Keywords: forensic genetics, next-generation sequencing (NGS), degraded bone samples, mass grave identification, STR genotyping