B104   On the Migration Route: Challenges in the Forensic Application of Massively Parallel Sequencing (MPS) in Human Body Identification

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Learning Overview: After attending this presentation, attendees will have an overview of the data obtained using MPS in the personal identification of migrants who perished on their way to Europe.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing insights into the challenges of forensic identification as consequences of the migrant crisis in Europe.

The recovery of DNA profiles from unidentified individuals is an essential part of the identification process. The limits of traditional DNA typing, which is based on matching the DNA profile of the deceased to the family references or antemortem samples, is evident in cases without any available sample for comparison. Emerging technologies such as MPS generate more data from the DNA sample, without the need for a reference sample for comparative analysis. Moreover, the assessment of externally visible characteristics and biogeographical ancestry may be of assistance in the police investigation. The latter is additionally relevant in cases of missing persons and unidentified migrants who die when crossing borders. The Western Balkan Route has been one of the predominate migration pathways from Africa and the Middle East into Europe. Croatia is the most southeastern European Union member state on the abovementioned migration route. Recently, the Forensic Biology Laboratory (FBL) of Forensic Science Center "Ivan Vučetić" has received an increased number of human identification cases in which remains were recovered from the river borders between Croatia and neighboring countries. The aim of this study is to assess the applicability of the MPS method in the forensic identification of human remains without any family references and antemortem data.

Blood samples were obtained from well-preserved bodies and teeth and tissue samples from decomposed bodies, respectively. DNA typing was performed using the Applied Biosystems® GlobalFiler® and Y Filer® Plus PCR Amplification kits on AB3500 Capillary Electrophoresis (CE) instrument. Laboratory procedures and data interpretation using Gene Mapper® ID-X v.1.4 were completed according to the accredited workflow at the FBL.

In addition, a library was prepared using the Verogen ForenSeq™ DNA Signature Prep Kit B. A positive 2800M control, negative amplification control, and reagent blank were included. The library pool was sequenced on the Illumina® MiSeq® FGx Instrument. All steps were performed following the manufacturers' instructions. Subsequent data analysis was performed using ForenSeq™ Universal Analysis Software (UAS) v.1.3 with default threshold settings. In order to estimate biogeographic ancestry and phenotype, the ForenSeq™ UAS and Snipper app v.2.5 were compared. In addition, paternal inheritance was evaluated using Whit Athey's algorithm using a Bayesian approach.

The run quality metrics (cluster density, clusters passing filters, phasing, and prephasing) were within the recommended range. In total, 231 Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) loci were typed for each sample, without contamination observed during the DNA typing process. Autosomal and Y-chromosomal Short Tandem Repeat (Y-STR) data obtained using MPS showed concordance with CE data. ForenSeq™ UAS classified four out of five samples in admixed American populations and one sample had European ancestry. However, Snipper assigned one sample in the European cluster, two samples between Europe and South Asia, one sample in the South Asia, and one sample slightly displaced toward the African cluster. This was consistent with the prevalence of determined Y-chromosome lineages. The UAS and Snipper prediction models agreed on the prediction of hair and eyes color. Moreover, the hair color of each individual was concordant with the data obtained from the medicolegal reports while eye colors could not be verified due to body decomposition.

The application of MPS can provide valuable data for personal identification in challenging forensic samples, such as human remains of migrants who die when crossing borders. The prediction of hair color, autosomal STR, and Y-STR data was consistent. However, the discordance was evident in ancestry prediction due to the lack of certain Eurasian population databases such as the Middle East. In the meanwhile, an individual of European ancestry has been identified and, for four remaining individuals, the analysis conducted here may contribute to the ongoing investigation.

Massively Parallel Sequencing, Human Identification, Migration

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