B105 Advances in Microhaplotypes (Microhaps) as a Comprehensive Forensic Marker

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Learning Overview: After attending this presentation, attendees will understand the currents status of the research conducted on microhaps for forensics applications.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by explaining how microhaps are a comprehensive forensic DNA tool and how their implementation can complement conventional Short Tandem Repeat (STR) -based analysis.

Microhaps are biomarkers less than 300 nucleotides long that display multiple allelic combinations.[1] The main advantages of microhaps over conventional STRs include the absence of stutter, same-size alleles within each locus, lower mutation rate, and ancestry informative alleles. These forensically relevant loci can yield a power of discrimination similar to STRs while enhancing Human Identification (HID), mixture deconvolution, and biogeographic ancestry prediction. Sanger sequencing does not allow determining the cis/trans relationship among closely related SNPs while Massively Parallel Sequencing (MPS) allows determining the parental haplotypes at each locus by clonally sequencing of each DNA molecule if they are included in the same amplicon. Currently, STR panels are used for mixture deconvolution and SNP assays are used for ancestry inference. Microhaps can be used for both functions allowing, for example, to infer the ancestry of a minor contributor to a mixture (DNA intelligence). In this study, the current status of the research conducted in the George Washington-Forensic Molecular Lab (GW-FMB) lab and elsewhere on this new multi-function DNA marker and its potential impact to the field of forensic genetics will be discussed.[2]

A newly developed MPS assay of 74 microhap loci was evaluated on the Thermo Fisher Scientific™ Ion Torrent S5™ system to address different forensic research questions including human identification, mixture deconvolution, and ancestry inference.[3-5] For mixture deconvolution, two- to five-person mixtures at different DNA input were simulated, with each donor with a distinct ancestry and contribution ratio to simulate casework-like DNA samples. Mixture results were compared to conventional capillary electrophoresis-based STR and sequence-based STR analysis. To assist in the interpretation of microhap mixed profiles, two Probabilistic Genotyping (PG) software, LRMixStudio v2.1.4 (semicontinuous) and DNAView MixtureSolutions v18-6-20 (continuous), were adapted to microhap mixture data intake to evaluated the output compared to STRs.

The MPS assay improved the deconvolution of all tested mixtures and complemented results of size-based and sequence-based STR analysis. Overall, PG software facilitated and reduced the analysis time of microhap mixed profiles, proving the amenability of microhap data to casework implementation. These results also point toward the rapid development of new mixture deconvolution tools for the implementation of microhap profiling in casework in the near future as new and extremely polymorphic markers are being discovered. Rare variants have been identified that increase the power of discrimination of the assay and the ability to correctly infer the number of contributors to a mixture. Furthermore, biogeographic ancestry of the minor DNA contributor detected in a 10:1 and 20:1 two-person mixtures was accurately inferred by considering the unique minor alleles reported.

These results indicate that the emerging microhaps are an effective and comprehensive biomarker tool, which can be used to enhance and broaden forensic DNA investigations.

Reference(s):

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Massively Parallel Sequencing, Microhaplotypes, Forensic Applications

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