B50 Performance of Microhaplotype and Short Tandem Repeat (STR) Biomarkers in Mixture Detection and Deconvolution

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Learning Overview: After attending this presentation, attendees will understand the use of different biomarkers for mixture detection and deconvolution.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by proposing microhaplotype markers as a supplemental tool to conventional STR typing analysis.

Microhaplotypes (microhaps) are emerging biomarkers defined by two or more closely linked Single Nucleotide Polymorphisms (SNPs) in <300bp displaying multiple allelic combinations.[1] The presence of multiple SNPs within the same locus augments the information over a stand-alone SNP locus. The multi-allelic nature of microhaps, although less polymorphic than STRs, makes these markers a promising versatile toolset as it offers certain advantages over STRs. These are absence of stutter, same-size alleles within a locus, low mutation rate, and ancestry informative alleles. Microhap multiplex assays can reach the same power of discrimination of STRs and also be used for different forensic applications, including human identification, mixture deconvolution, relationship testing, and ancestry prediction.[2] Unlike the standard Sanger sequencing methodology, which does not determine the *cis/trans* relationship among individual SNPs, Massively Parallel Sequencing (MPS) allows determining the parental SNP haplotypes by clonal sequencing of individual amplicons originating from each individual strand, thus distinguishing each parental allele at a locus. This study evaluated the mixture performance of a 74 microhap locus-assay on the Thermo Fisher Scientific™ Ion Torrent S5™ system and compared the results to standard sized-based STR and sequenced-based STR analysis.[3,4]

The detection limit of the bioassay was tested in triplicate using a 2ng to 25pg range of input DNA. The performance of the microhap panel was evaluated in parallel with the GlobalFiler™ kit on Capillary Electrophoresis (CE) on the ABI® 3500 Genetic Analyzer and the Precision ID GlobalFiler™ NGS STR Panel v2 on the S5™ platform. To simulate casework-like DNA samples, a large series of two- to five-person mixtures was simulated at 1-10ng input DNA and at different contribution ratios. Examples of the tested ratios included 10:1 to 40:1 for two-person mixtures, 5:5:1 and 10:5:1 for three-person, 20:5:1:1 for four-person mixtures, and 5:5:1:1:1 for five-person mixtures. For genotyping of microhap loci, Microhaplotyper Plugin v8.1 was used while STR sequence data and CE STR data were analyzed with Converge and GeneMapper® ID-X software, respectively.

The 74-locus panel was found to be sensitive to approximately 50pg input DNA (equivalent of eight diploid cells). Overall the deconvolution and interpretation of CE-based STR mixtures was challenging for imbalanced and high order mixtures. However, sequencing data demonstrates that the deconvolution of these latter can be enhanced by MPS. This approach allows detecting additional minor alleles masked by stutter with conventional CE but distinguishable by sequence and displaying repeat pattern variation or due to the presence of microvariants. It is worth noting that both sequencing of microhap and STR mixtures displayed imbalanced coverage between different loci in all mixtures tested using an input amount of 1–10ng DNA. For microhaps and, in particular, for two-person mixtures, a full minor profile was reported at 1:10 ratio with minimal allele and locus dropout at 20:1, and more significant locus dropout at 40:1 ratio at both 1ng and 10ng DNA inputs. At the same input DNA amount for three- to five-person mixtures, full microhap profiles could be reported for the minor DNA contributors at all tested mixture ratios with the exception of some microhap loci that underperformed.

These findings suggest that microhap biomarkers can enhance the deconvolution of mixed DNA samples and complement both conventional size-based and sequence-based analysis of STRs.

Reference(s):

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2. Bennett L., Oldoni F., Long K., Cisana S., Madella K., Wootton S., Chang J., Hasegawa R., Lagace R., Kidd K.K., Podini D. Mixture deconvolution by massively parallel sequencing of microhaplotypes *International Journal of Legal Medicine* (2019) 133:719-729.
3. Oldoni F., Kidd K.K., Podini D. Microhaplotypes in forensic genetics. *Forensic Science International: Genetics* (2019) 38:54-69.
4. Kidd K.K., Speed W.C., Pakstis A.J., Podini D.S., Lagacé R., Chang J., Wootton S., Haigh E., Soundararajan U, Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic Science International: Genetics* (2017) 29:29-37.

Microhaplotypes and STRs, Mixture Deconvolution, Massively Parallel Sequencing

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