B74 The Application of Probabilistic Genotyping Software Analysis for Mixture Deconvolution Using a New Massively Parallel Sequencing (MPS) Panel for Microhaplotypes

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Learning Overview: After attending this presentation, attendees will have a better understanding of Probabilistic Genotyping (PG) software for mixture deconvolution utilizing Microhaplotype (MH) markers.

Impact on the Forensic Science Community: The presentation will impact the forensics community by demonstrating the efficacy of semi-continuous and continuous PG models when applied to deconvolution of complex mixtures generated using Massively Parallel Sequencing (MPS) of Microhaplotype (MH) loci.

Microhaplotypes are novel markers defined by two or more single nucleotide polymorphisms (SNPs) located within less than 300 nucleotides from one another. These SNPs can be associated in multi-allelic combinations within a locus, thereby generating a haplotype.[1] MHs are useful markers for human identification and bio-geographic ancestry prediction, while also enabling enhancing mixture deconvolution capabilities when coupled with probabilistic genotyping considering the abundance of information provided by these markers.[1-3]

A novel panel of 74 MH loci was developed and implemented on the Ion Chef/Ion S5™ (Thermo Fisher Scientific) massively parallel sequencing (MPS) platform and its sensitivity assessed using 2ng to 25 pg input DNA.[4] This sensitivity study not only establishes the limitations of the method but also provides an essential means of determining the stochastic behavior of the minor contributor for the continuous model of probabilistic genotyping. Deconvolution was explored using artificial mixtures of two to five contributors of different contribution ratios and ancestries, which were genotyped in parallel for MPS of MHs. The significance of the deconvolution was quantified by the PG software with likelihood ratios (LRs) which evaluate the strength of two competing hypotheses to quantify which hypothesis provides the best explanation for the data present in the mixture.

The two distinct models of probabilistic genotyping available to assist in mixture interpretation utilize different approaches to address deconvolution. The semi-continuous model represents a binary-like approach in that alleles are absent or present with the occurrence of drop-in and drop-out considered during the binary statistical evaluation.[5] However, this model requires manual assessment of contributor number, which in complex mixture it is essentially unknown and requires an assumption be made by the analyst. The continuous model, on the other hand, is non-binary in that it does not examine mainly the presence or absence of peaks but also uses the peak heights and stochastic variability in its deconvolution process.[5] Unlike the semi-continuous model, the continuous model used in this study does not require a number of contributors assumption by the analyst and instead only requires an assessment of stochastic behavior through a sensitivity study. In this study we evaluated the potential application of the semi-continuous LRMixStudio (version 2.1.4) and continuous MixtureSolutions (version 18-6-20) PG software for the analysis of mixture profiles using MH loci.

LRMixStudio produced LR values for the minor contributor(s) that were consistent with the expected LR range of MH profiling. In particular, an LR value of 9.90×10^{14} was obtained for a 10:1 mixture while, 5.26×10^{8} and 1.55×10^{9} for the two minor contributors at a 10:1:1 ratio. Finally, LR values of 2.57×10^{4}, 1.28×10^{5} and 8.53×10^{7} were generated for each contributor, respectively at 10:1:1:1 mixture ratio. MixtureSolutions was able to produce LR value of 2.0×10^{24} for the minor contributor at 1:10 ratio while a 10:1:1 mixture produced a 6.0×10^{40} LR for the first minor contributor and a 4.0×10^{46} LR for the second minor when a stochastic ratio of 6.1 was applied to the analysis. A 5:1:1:1 mixture was able to produce the following LRs for each minor contributor: of 4.0×10^{54}, 4.0×10^{63}, and 2.0×10^{71} applying a stochastic ratio of 6.6 for the statistical analysis.

Preliminary results indicate that MH loci are amenable to PG software analysis, which enhances mixture deconvolution capabilities and assists forensic DNA practitioners in the interpretation of higher-order mixed MH profiles with promising LR values for the inclusion of minor contributors within imbalanced mixtures. This analysis represents the first iteration of mixture deconvolution employing MH specifically with PG and will continue to develop as the software and methods improve but demonstrate the viability of this approach to addressing the issues associated with complex mixture interpretation.

Reference(s):

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Microhaplotypes, Probabilistic Genotyping, Mixture Deconvolution