B189 The Use of Microhaplotypes in the Analysis and Deconvolution of Mixed DNA Samples

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After attending this presentation, attendees will understand some of the advantages of using Massive Parallel Sequencing (MPS) of regions containing multiple Single Nucleotide Polymorphisms (SNPs), known as "Microhaplotypes (MHs)," to analyze and deconvolute complex forensic samples that are composed of DNA from multiple contributors.

This presentation will impact the forensic science community by providing further understanding of additional methods that can be used in the analysis of forensic DNA samples.

Short Tandem Repeats (STRs) have been the standard DNA markers used in human identification due to the ease of amplification and their highly polymorphic nature, which leads to a high power of discrimination. Additionally, several software packages have been developed that allow the analyst to easily identify and interpret the results. While STRs have been vital in forensic DNA analysis, several characteristics have led to limitations in their use. STR fragments are amplified and labeled using fluorescent probes; the number of loci that can be analyzed in one assay is determined by the size of the fragments and number of dyes. Current Capillary Electrophoresis (CE) methods allow determining the size of the fragment but not the sequence; however, STR loci have complex repeats that vary in sequence, making alleles indistinguishable by size discernable only by sequence. Lastly, during amplification of the repeats, stutter effects can occur for some loci over 20% of the time, leading to the production of n-4 (also n-8/+4) fragments indistinguishable from true alleles of the same size.

MHs are loci of two or more SNPs within a short distance of each other (<250 nucleotides (i.e., micro)) with three or more allelic combinations (haplotypes). Conventional Sanger sequencing does not allow determination of the *cis/trans* relationship between individual SNP alleles (i.e., the haplotype). In contrast, MPS methods, when SNPs are in the same amplicon, allow sequencing of individual strands and, therefore, the detection of haplotypes at a locus. As MHs are sequence variations, stutter effects are of no concern, making MHs a potential resource for the analysis and deconvolution of imbalanced mixtures. Other characteristics, such as small amplicon size and lower mutation rate, make these markers potentially effective on degraded samples and in familial cases, respectively. Numerous MHs have been identified that are promising forensic markers; these MHs are currently being further characterized for their use in mixture deconvolution.

In this study, synthetic mixtures and forensic type samples were genotyped using both MPS (Thermo Fisher HIDS Early Access Ion AmpliSeq™ GlobalFiler™ Mixture ID Panel) as well as CE size analysis (GlobalFiler™) and the results were compared. Results from samples first analyzed by CE suggested they were potentially composed of multiple genetic contributors, though the interpretation using CE alone was questionable as potential minor

contributors fell below analytical or stochastic thresholds and into stutter ranges. MPS of STRs of these samples showed sequence variations that provided more support for the presence of a mixture; however, MH genotyping conclusively indicated the presence of multiple contributors in mixtures of two, three, and four persons at 40:1, 10:1:1, and 10:1:1:1 ratios, respectively. Additionally, several forensic-type samples were found to contain DNA from more than one contributor, with the minor contributor(s) more easily identified using MHs. Using preliminary MH allele frequencies, work is being conducted to determine random match probabilities for minor contributors found in mixed forensic samples.

These results illustrate the capabilities of MHs in the detection of mixtures and suggest the ability to genotype multiple contributors in complex forensic samples.

DNA, Sequencing, Microhaplotypes