THE USE OF MICROHAPLOTYPES IN THE ANALYSIS AND DECONVOLUTION OF MIXED DNA SAMPLES

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Short tandem repeat (STR) analysis of mixed samples can be challenging, particularly in imbalanced mixtures when stutters of major alleles overlap with alleles of the minor contributor. Targeted sequencing of STRs with Massively Parallel Sequencing (MPS) technologies has shown that differences in complex repeats are detectable and can contribute to identification. However experiments have shown that interpretation of STR *via* MPS is not as straightforward as originally anticipated, especially when dealing with imbalance mixtures. Single alleles from complex repeats (like D21S11 or D12S391) can generate multiple stutter sequences. These artifacts, when in the same range of one or more minor contributors, will complicate interpretation. Microhaplotypes (MHs) are loci of two or more SNPs within a short distance from each other (<300 nucleotides i.e. 'micro') with three or more allelic combinations ('haplotypes'). Conventional Sanger sequencing does not allow determining the *cis/trans* relationship between individual SNP alleles (i.e. the haplotype). MPS methods instead, when SNPs are in the same amplicon, allow sequencing of individual strands and haplotype detection 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 low mutation rate make these markers potentially effective on degraded samples and in familial testing respectively. Dr. Ken Kidd has identified numerous MHs that are promising forensic markers, and inferred allele frequencies through PHASE.
In this study, forensic type samples were genotyped using both MPS (Precision ID GlobalFiler™ Mixture ID Panel – Thermo Fisher) as well as CE fragment size analysis (GlobalFiler ™) and the results were compared. Genotype concordance was observed for all STRs in single source samples with starting DNA concentrations down to 125pg. Interestingly, several samples first analyzed by CE were potentially composed of multiple genetic contributors, though the interpretation using CE alone was questionable. MPS of STRs in these samples was equally inconclusive as potential minor contributors fell below analytical thresholds and/or into stutter ranges. MH genotyping, however, conclusively indicated multiple contributors, as more than 2 alleles were observed in at least 50% of the MH loci. These results support the potential of MHs as a tool for enhancing mixture deconvolution capabilities in complex forensic samples.