B74 An Innovative Massively Parallel Sequencing (MPS) 74-Microhaplotypeplex Forensic Assay for Improved Deconvolution of Mixed DNA Samples

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After attending this presentation, attendees will appreciate the potential of using MPS for short DNA regions containing multiple Single Nucleotide Polymorphisms (SNPs), called Microhaplotypes (MHs), to deconvolute DNA mixtures.

This presentation will impact the forensic science community by proposing a novel analytical approach on an MPS platform to improve the forensic analysis of mixed DNA samples.

Microhaplotypes are novel genetic markers with two or more SNPs within a short distance of each other (<300 nucleotides), which jointly define a multi-allelic locus. MHs are useful for identification purposes and prediction of biogeographic ancestry, but also demonstrate great potential for mixture deconvolution as they are characterized by having important differences from conventional Short Tandem Repeat (STR) markers, such as same size alleles, lower mutation rate, and the absence of stutter peaks.[1] As the mainstay Sanger sequencing does not allow determining the cis/trans relationship between individual SNP alleles (i.e., the haplotype), the MPS technology overcomes such limitations by clonal sequencing of individual strands and the detection of the haplotype of interest within a specific locus.[2]

In this study, an innovative MPS multiplex panel of 74 MH loci, with optimum characteristics for improved deconvolution of DNA mixtures, was developed and tested on the Ion Chef/Ion S5™ MPS platform. To simulate a range of scenarios typically encountered in casework samples, a series of unbalanced two- to four-person DNA mixtures characterized by different ratios between contributors was prepared. The performance of the assay in targeting one or more minor contributors in the presence of up to 160-fold excess of major contributor(s) was tested using different amounts (1ng-10 ng) of input of genomic DNA (gDNA).

For data analysis of the Ion AmpliSeq™ sequencing results, the latest released beta version of Microhaplotyper Plugin was used for genotyping the DNA contributors. The MPS 74-MHplex forensic assay was shown to detect the minor contributor at higher mixture ratio than conventional autosomal STR markers, regardless of the sex of the individual. For two-person mixtures, a full MH profile of the minor contributor was reported at 1:10 and 1:20 ratios, whereas an increased number of allele/locus drop-out events was observed proportionally at 1:40, 1:80, 1:100, and 1:160 ratios; fewer were also reported at lower mixture ratios for three- and four-person DNA mixtures. Albeit allele drop-outs reduced the total number of exploitable MH loci, the Random Match Probability (RMP) calculated for the minor contributor(s) was higher or within the range of values typically obtained from full/partial autosomal STR profiles at low and high mixture ratios, respectively. In addition, MH mixture profiles of two to six persons were statistically simulated to determine the global distribution of alleles detected for 74 MH loci. Interestingly, promising results suggest the possibility of estimating the potential number of contributors based on the total allele count observed for each specific two-, three-, four-, five-, and six-person mixture-group.

These findings indicate that the MPS 74-MHplex assay is an insightful forensic DNA tool for improving the deconvolution of mixed samples and simultaneously extrapolating useful biogeographic ancestry information of the detected contributors.

Reference(s):

1. Kidd KK, Pakstis AJ, Speed WC, Lagacé R, Chang J, Wootton S, Haigh E, Kidd JR. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic Science International: Genetics.* (2014) 12:215-224.

2. Kidd KK, Speed WC. Criteria for selecting microhaplotypes: mixture detection and deconvolution. *Investigative Genetics.* (2015) 6(1):1.

Microhaplotypes, Mixture Deconvolution, Massively Parallel Sequencing

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