B108 A Newly Developed Massively Parallel Sequencing (MPS) Microhaplotype Forensic Assay for Mixture Detection and Deconvolution and Ancestry Prediction

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Learning Overview: After attending this presentation, attendees will understand the use of massively parallel sequencing (MPS) for the analysis of microhaplotypes (MHs).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by suggesting a new forensically relevant MPS-MH typing approach to enhance both mixture deconvolution and ancestry prediction.

Microhaplotypes are markers defined by two or more SNPs located within a short distance from each other (<300 bp) associated in multiple allelic combinations within a locus. These markers have the potential to improve human identification, enhance mixture deconvolution capabilities while enabling for ancestry inference.[1-3] In addition, MHs have some advantages over short tandem repeats (STRs) including the absence of stutter peaks, alleles within a locus all having the same size, and a lower mutation rate than conventional forensic markers. Altogether they can achieve discrimination power like that of STRs while providing a greater amount of information. Sanger sequencing is unable to determine the *cis/trans* relationship of SNP alleles within a MH locus while MPS enables determining the parental haplotypes at each locus by clonal sequencing of each DNA strand.[3] In this study, we defined the detection limit of a novel panel of 74 MH loci analyzed on the Ion Chef™/Ion S5™ (Thermo Fisher Scientific) MPS platform, explored the assay efficiency in mixture analysis and compared the results to conventional capillary electrophoresis (CE)-STR typing, and evaluated ancestry inference capabilities of the detected minor contributor.[4]

The sensitivity limit of the MPS-MH assay was tested by typing three samples in triplicate from 2 ng to 25 pg input DNA range. The sensitivity and mixture studies were conducted in parallel comparing MPS of MHs and CE of STRs (GlobalFiler™ kit, Thermo Fisher Scientific) on the same mixed samples. The analysis of MH and STR mixtures was performed using a wide range of artificial mixed-source samples to mimic forensic scenarios commonly encountered in caseworks. These included approximately 100 simulated two-to-five-person mixtures at 1-10 ng input DNA, with each donor having a distinct ancestry origin and contribution ratio. Representative examples of tested ratios included 10:1 to 80:1 for two-person mixtures, 10:1:1 and 5:5:1 for three-person, 10:1:1:1 and 5:5:1:1 for four-person mixtures, and 10:1:1:1:1 and 5:5:1:1:1 for five-person mixtures. For genotyping of MH loci, the latest released Microhaplotyper Plugin v8.1 (Thermo Fisher Scientific) was used. In addition, a set of approximately 400 individual representatives of four major American population groups (US African, US European, US Asian, and South West Hispanics) was genotyped and allele frequency tables for estimating the biogeographic ancestry of the minor donor were generated.

Overall the MPS-MH assay was sensitive down to 50 pg input DNA with minimal allele dropout at 25 pg input DNA. For two-person mixtures, full MH profile of the minor donor was reported at a 1:10 ratio while few allele dropouts were observed at a 1:20 ratio. Moreover, the random match probability (RMP) calculated for the minor donor was higher than that obtained for the same mixtures analyzed with STRs. For the three-to-five-person mixtures, full MH profile was reported for all minor donors within the full range of mixture ratios tested. For these mixtures, STR profiles of the minor donors were fully or partially detectable. However, due to the level of complexity of the mixtures the minor contributor(s) would have been considered not suitable for comparison. In addition, ancestry of the minor donor of two-person mixtures was correctly predicted by dividing the highest RMP value obtained using different populations by the second and third highest. The value obtained is indicative of how much more likely it is to observe the profile of interest if it originated from an individual from the population at the numerator than if it originated from an individual from the population at the denominator.

These results suggest that the 74plex MPS-MH assay is an effective and versatile forensic tool, which allows for mixture deconvolution and ancestry inference. Current work is also focused on the comparison of MPS-MH to MPS-STR typing on the same mixed samples.

Reference(s):

1. Kidd KK, Speed WC. Criteria for selecting microhaplotypes: mixture detection and deconvolution. *Investigative genetics* (2015) 6(1):1.
2. 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.
3. Kidd KK, Speed WC, Pakstis AJ, Podini DS, 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.
4. Oldoni F, Hart R, Long K, Maddela K, Cisana S, Schanfield M, Wootton S, Chang J, Lagace R, Hasegawa R, Kidd K, Podini D, Microhaplotypes for ancestry prediction (2017) *Forensic Science International Genetics Supplement Series* 6: e513-e515

MPS of Microhaplotypes, Mixture Deconvolution, Ancestry Inference