## B176 Massively Parallel Sequencing (MPS) of Microhaplotypes for Forensics

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After attending this presentation, attendees will better understand the fundamentals of MPS and its application to forensics, particularly the use of microhaplotypes for determining biogeographic ancestry and for resolving mixture and kinship scenarios.

This presentation will impact the forensic science community by demonstrating microhaplotype multi-allelic genomic markers as a forensic solution to be used in conjunction with other Single Nucleotide Polymorphism (SNP) and Short Tandem Repeat (STR) markers in an MPS context. This presentation will show analysis from experiments designed to test the sensitivity of mixture detection, to define the ancestry prediction resolution, and to measure the accuracy of kinship analysis.

Recent studies demonstrating the use of MPS in forensics have shown a new methodology for interrogating a wide range of genomic markers beyond STRs, including mitochondrial DNA(mtDNA), messenger RNA (mRNA), and SNPs for identity, biogeographic ancestry, and phenotype.[1,2] The flexibility of a small amplicon, highly multiplexed, genomic assay enables the analysis of degraded or low template DNA samples. The additional resolution of sequence differences plus fragment length for an STR locus supplements the analysis of familial relationships and the deconvolution of mixtures.

When multiple SNPs reside within a single sequencing read (~<300bp), there may be multiple haplotypes represented as statistically phased SNP genotypes.[3] These multiallelic markers, like STRs, provide an excellent tool for kinship and mixture analysis. Additionally, some microhaplotypes may be used to derive biogeographic ancestry when there is large enough allele frequency variation between global populations.[3]

Forty-five microhaplotypes with high heterozygosity were selected using a set of criteria defined by Dr. Kenneth Kidd.[3] All of these have been run on a set of 54 populations by the Kidd laboratory. Primers were designed to amplify all markers in multiplex. DNA was extracted from a number of sample sources taken from individuals of different biogeographic ancestries. Mixtures were created at ratios of 1:1, 1:3, 1:7, 1:15, and 1:30. Samples were also selected to represent a number of kinship scenarios. Libraries for MPS were created by ligating barcoded sequencing adaptors. The barcoded libraries were sequenced on the Ion Torrent™ PGM™. Sequencing reads were aligned to target regions of the reference human genome; haplotypes were determined; and tertiary analysis was performed to type biogeographic ancestry, mixture ratios, minor and major contributors, and familial relationships. The multiplex was able to amplify and sequence all intended targets with <10% off-target amplicons. There was no ambiguity-calling haplotypes, and further analysis showed promise for resolving mixture components, ancestry, and kinship.

With the capacity to sequence many markers in parallel, MPS underscores the power of microhaplotypes as a forensic marker. Its use in multiplex with STRs and lineage, phenotype, and ancestry-informative SNPs could be investigated for a comprehensive forensic solution.

**Reference(s):**

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2. Zubakov D. et al. Towards simultaneous individual and tissue identification: A proof-of-principle study on parallel sequencing of STRs, amelogenin, and mRNAs with the Ion Torrent PGM. *Forensic Science International: Genetics*. 2015. Volume 17: 122 – 128
3. Kidd K.K. et al. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic Science International: Genetics*. 2014. Volume 12: 215 - 224

**Massively Parallel Sequencing, Microhaplotypes, Ancestry**





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