B101 Massively Parallel Sequencing (MPS) of Short Tandem Repeats (STRs) and Microhaplotypes for Mixtures

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After attending this presentation, attendees will understand how to utilize microhaplotypes and STR data generated by MPS.

This presentation will impact the forensic science community by presenting a novel method using microhaplotypes to determine the number of contributors in a mixture sample as well as infer biogeographical ancestral information.

Primers, library and template preparation chemistries, and algorithms were designed to analyze these markers. The AmpliSeq™ library preparation chemistry was developed to handle up to 100ng of input DNA, along with using a high-multiplex panel, which is important to increase the probability of recovering the minor contributor.

Microhaplotypes are multi-allelic genomic markers that contain more than one SNP residing in genetic proximity, whereby each haplotype is represented as statistically phased SNP genotypes.[1] These markers provide an ideal tool for mixture analysis, with a lower baseline and better intralocus balance over STRs in an MPS context. Furthermore, microhaplotypes are surrounded by conserved sequences, which can be sequenced with high accuracy, so therefore lack the complexity and interpretation issues of stutter. On the other hand, MPS exposes the additional diversity in compound and complex STR loci when repeat and flanking sequence is compared.[2-4] To investigate the sensitivity of the two marker types for mixture detection, a range of major to minor donor ratios and number of individuals was sequenced.

Seventy-eight microhaplotypes that have been previously typed on 83 populations showing high numbers of alleles and ancestry informativeness were targeted for design in multiplex with 31 autosomal STRs and 4 Y-markers.[5] DNA was extracted from samples taken from individuals of different biogeographic ancestries. Mixtures were created at ratios of 1:1, 1:3, 1:10, 1:20, and 1:50, along with mixtures containing up to four donors. Libraries for MPS were barcoded with both manual and automated Ion Chef™ library preparation methods. The libraries were then quantified and subsequently run through template preparation on the Ion Chef™, then sequenced on the Ion S5™. Using GlobalFiler™, fragment analyses were also performed on the same mock mixture samples to gather STR concordance and performance data. Reads were aligned to target regions of the reference human genome and haplotypes, biogeographic ancestry, number of contributors, mixture ratios, and minor and major contributors were determined. Converge™ 2.0 software was used to visualize sequence, STR profiles, strand bias, and number of contributors.

With the capacity to sequence many markers in parallel, MPS underscores the power of microhaplotypes and STR sequence as powerful forensic markers for handling mixture samples when Capillary Electrophoresis (CE) systems are not capable of generating conclusive results.

Reference(s):

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2. Guo, Fei et al. Evaluation of the Early Access STR Kit v1 on the Ion Torrent PGM™ platform. *Forensic Science International: Genetics.* Volume 23, 111-120.
3. Børsting, C. and Morling, N. (2015). Next generation sequencing and its applications in forensic genetics. *Forensic Science International: Genetics.* 18 IS, 78-89.
4. Bottino, C.G., Chang, C.W., Wootton, S., Rajagopalan, N., Langit, R., Lagacé, R.E., et al. (n.d.). STR genotyping using ion torrent PGM and STR 24-plex system: Performance and data interpretation. *Elsevier Ireland Ltd.* 5 IS -, e325-e326.
5. Kidd, Kenneth K. et al. Evaluating 130 microhaplotypes across a global set of 83 populations. *Forensic Science International: Genetics.* Volume 29 , 29-37.

Microhaplotypes, Massively Parallel Sequencing, Mixtures

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