B121 The Detection of Cryptic Single Nucleotide Polymorphism (SNP) Variants for Enhancing Human Identification Capabilities

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Learning Overview: After attending this presentation, attendees will be able to appreciate the advantages of using Massively Parallel Sequencing (MPS) technology to improve human identification capabilities.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating that the detection of additional rare SNP variants within a microhaplotype region can increase the power of discrimination of DNA evidence.

Microhaplotypes (microhaps) are loci with two or more SNPs within an expanse of 300 nucleotides associated in multiple allelic combinations.[1] These biomarkers have small amplicon size, ancestry informative alleles, no stutter, and lower mutation rates than Short Tandem Repeats (STRs), which make them useful for human identification, relationship testing, mixture deconvolution, and ancestry inference.[2] Sanger sequencing does not provide the *cis/trans* relationship between individual SNP alleles while MPS allows distinguishing the parental haplotypes by clonal sequencing of each individual DNA strand. The detection of rare (cryptic) SNP variants can potentially increase the power of discrimination of these markers and the ability to correctly infer the number of contributors to a mixture. This study explored the potential of a newly developed pipeline to identify rare SNP variants within microhaplotype alleles and their impact on human identification.

A MPS panel of 74 microhap loci was implemented and evaluated on the Thermo Fisher Scientific™ Ion Torrent™ S5™ system.[3] To expand the potential of microhaps to human identification, a custom bioinformatic pipeline was developed (Microhaplotyper_CR_v1.0), which uses an alignment-specific tool to enable the detection of the SNP defining the microhap loci while identifying additional SNP variants potentially present in between the SNPs that define the locus. These extra variants can be highly informative if detected as they are generally rare and consequentially increase the power of discrimination of the overall profile when present. To evaluate the performance of the pipeline, 18 single-source samples of African origin were selected and run on the Ion S5™ platform. All DNA samples were genotyped using the Microhaplotyper_CR_v1.0 pipeline, which was installed as a plug-in on the S5™ server. The output file identifies the alleles based on the SNPs that define the locus and also detects all other variants present within the amplicon. The frequency of the additional SNPs of interest was queried on the public database SNP (dbSNP), which includes allele frequencies of global populations from multiple data sets.

On average, this study identified eight additional SNP variants per each individual tested. Preliminary analysis showed that six rare SNP variants were observed only once within the sample set tested at a frequency of 0.1%–4.6% in African populations reported on dbSNPs. Eighteen different additional SNP variants were detected at high allele frequencies in African populations (10%–40%) and frequencies ranging from 1%–9% in other global populations, including Europeans, East and South Asians, and Americans. For instance, one of the identified SNP variants (rs79763993) was found at 19.4% in the African population, 0.01% in the European, 1% in the American, and undetected in East and South Asian populations. Comprehensive analyses on a set of more than 800 individuals from 14 global populations are ongoing to identify additional rare SNP variants.

Preliminary findings suggest that rare SNP variants are present within the targeted microhap regions and can be identified with the newly developed pipeline. Detecting these variants will increase the discrimination power of a profile and will increase the mixture deconvolution capabilities of this microhap assay.

Reference(s):

1. Kidd K.K., Pakstis A.J., Speed W.C., Lagacé R., Chang J., Wootton S., Haigh E., Kidd J.R. Current sequencing technology makes microhaplotypes a powerful new type of genetic marker for forensics. *Forensic Science International: Genetics* (2014) 12: 215–224.
2. 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 *Forensic Science International Genetics Supplement Series* (2017) 6: e513-e515.
3. Kidd K.K., Speed W.C., Pakstis A.J., Podini D.S., 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.

Microhaplotypes, Massively Parallel Sequencing, Rare SNPs

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