E102 Examination of Genetic Drift in Mitochondrial DNA (mtDNA) Heteroplasmy in Hair Samples Using Massively Parallel Sequencing (MPS) Approach

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After attending this presentation, attendees will understand the manner in which mtDNA heteroplasmy drifts in human hair shafts when employing an MPS approach.

The use of an MPS approach to forensic mtDNA analysis enhances the discrimination potential of the testing method. This presentation will impact the forensic science community by providing information on how the ratio of heteroplasmic sequence variants drifts between hair samples when interpreting MPS data.

MtDNA is commonly analyzed in forensic hair samples due to its high copy number, as the amount of nuclear nDNA is typically below what is needed for Short Tandem Repeat (STR) analysis. While mtDNA analysis is not as discriminating as nDNA testing, the ability to resolve heteroplasmic sequences (the presence of more than one mtDNA variant in a cell) can increase the weight of the mtDNA analysis. Differences in the ratio and presence of heteroplasmic variants exist between maternally related family members, allowing for more discrimination between relatives. Variations can also exist within a single individual. For example, the cells that make up hair follicles form in a clonal manner, creating a bottleneck effect during fetal development, allowing for genetic drift among hair follicles within an individual. The differences in variant ratios within an individual may pose a challenge when interpreting heteroplasmy in forensic cases. Rates of heteroplasmy in a population and at each nucleotide position are required to properly generate statistical weight estimates in support of a match between evidence and a reference. A better understanding of the patterns of drift in the heteroplasmic ratios within an individual are needed to determine their impact on interpretation criteria.

This study focuses on characterizing the drift in variant ratios among hair samples from different regions of the scalp from single individuals with varying levels of heteroplasmy. Sequencing has historically been performed with the Sanger method (Sanger-Type Sequencing (STS)). This method sequences multiple mtDNA strands at a time, reporting the predominant sequence, or haplotype, for mtDNA analysis. If a heteroplasmic variant is present in at least 10% of the mtDNA strands, it can be detected in STS data; however, the individual variants cannot be resolved. To detect lower levels of heteroplasmy and resolve the variants, MPS has the ability to identify variants as low as 1%.

Hair samples were collected from human subjects exhibiting known levels of heteroplasmy. Two groups of subjects were studied: (1) five donors who exhibited low levels of heteroplasmy, (2%-5% in their blood and buccal swabs); and, (2) five donors who exhibited higher levels of heteroplasmy (>10%). Five hair samples were collected from each of the ten donors from different regions of the scalp, including the forehead, neck, left temple, right temple, and crown. The DNA from each hair shaft was extracted using an optimal lysis and magnetic bead purification method, amplified using the Promega® PowerSeq™ Mito System protocol/kit (a 10-plex amplification method for the entire mtDNA control region), library preparation was conducted using the TruSeq® method from Illumina®, and the library was run through MPS analysis on the MiSeq® from Illumina®. The level and nature of genetic drift was assessed for the hairs collected from each set of donors. In particular, drift was assessed in relation

to donors with low or high amounts of known heteroplasmy, determined from reference data of blood and/or buccal cells. In addition, the observation of unexpected variants was cataloged and evaluated.

Mitochondria, Heteroplasmy, Hair