B102 Investigating Rates of Mitochondrial DNA (mtDNA) Heteroplasmy in Different Haplogroups Using Massively Parallel Sequencing (MPS)

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After attending this presentation, attendees will understand MPS analysis of mtDNA haplotype and heteroplasmy and whether rates of heteroplasmy are linked to populations of different haplogroups.

This presentation will impact the forensic science community by introducing a robust MPS approach to sequencing mtDNA and enhancing the discrimination potential of mtDNA typing by evaluating rates of heteroplasmy across haplogroups.

MPS, a high-throughput form of next generation sequencing, allows increased resolution of mtDNA heteroplasmy and is at the forefront of efforts to expand the utility of forensic mtDNA typing. Maternally inherited mtDNA is present in hundreds to thousands of copies within one cell, has a high mutation rate, and passes through multiple bottlenecks, which allows for a range of variant percentages in the mtDNA sequence. Heteroplasmy is a heterogeneous collection of sequence variants in the cytoplasm of the cell. Heteroplamic rates from a population of European haplogroups (for example, H, J, K, T, and U) have provided evidence that heteroplasmy is common in the Control Region (CR) of the mitochondrial genome (mtgenome). A maternal lineage of mtDNA will share the same collection of major variant Single Nucleotide Polymorphisms (SNPs) and insertions/deletions (indels) called the haplotype. Certain haplotypes are common in population groups and may be shared among unrelated individuals.[1] In line with this, a haplogroup consists of similar haplotypes that have risen from related ancestral lineages. It is hypothesized that there is potential for differences in rates of heteroplasmy linked to population haplogroups, based on assumption and empirical observation that the position and rate of heteroplasmy may be linked to the haplotype sequence.

This current project has used a robust MPS approach to measure, analyze, and report rates of heteroplasmy on a per sample and per nucleotide basis for 750 samples in population groups reporting to be non-European (NIJ-2016-DN-BX-0171). Buccal cells were collected from 750 unrelated non-European individuals and MPS analysis conducted on the CR using Nextera® XT library preparation and 300X300 paired-end reads on an Illumina® MiSeq®. Secondary analysis was performed using GeneMarker® High-Throughput Sequencing (HTS) software to evaluate haplotype and heteroplasmy, and HaploGrep to determine haplogroups.

The forensic science community requires further validation of MPS analysis of mtDNA to encourage adoption of this technology into working laboratories and investigations. This project utilized a reliable MPS workflow developed in a laboratory, along with proper software for evaluation of MPS mtDNA data. Implementation of this optimized procedure combined with establishment of rates of heteroplasmy across the CR in different haplogroups will significantly enhance the accessibility to, and discrimination potential of, mtDNA typing. This highly resolved reporting will encourage the forensic science community to increase implementation of mtDNA analysis as a whole.

Reference(s):

1. Carracedo A, Bär W, Lincoln P, Mayr W, Morling N, Olaisen B, Schneider P, Budowle B, Brinkmann B, Gill P, Holland MM, Tully G, Wilson M. DNA commission of the International Society of Forensic Genetics: Guidelines for mitochondrial DNA typing. *Forensic Sci. Int.* 2000;110:79-85.

Mitochondrial DNA, Massively Parallel Sequencing, Heteroplasmy

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