A160 Next Generation Sequencing of Human Mitochondrial DNA Extracted From Hair Shafts Using a Multiplexed PCR Strategy and Illumina® Nextera® XT

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After attending this presentation, attendees will gain an understanding of how a multiplex Polymerase Chain Reaction (PCR) approach and next generation sequencing methods can be used to sequence whole mitochondrial genomes from challenging sample types such as short hair shaft fragments.

This presentation will impact the forensic science community by providing insight into how next generation sequencing methods are being used to increase the discriminatory power of human mitochondrial DNA analysis by providing high-quality sequence data that extends to regions of the mtGenome that are not routinely analyzed in forensics laboratories.

Forensic scientists are often faced with the challenge of limited or degraded samples, where a nuclear DNA (nDNA) profile may be difficult to obtain. In these instances, mitochondrial DNA (mtDNA) analysis can be particularly useful, as mtDNA is more easily recoverable from challenging sample types such as hair shafts and bone. Traditional methods focus on sequencing of two hypervariable regions (HV1 and HV2) located in the non-coding control region of the mtGenome; however, HV1/HV2 comprise only about 4% of the mtGenome and mtDNA is less discriminatory than nDNA, which limits the current utility of mtDNA analysis. Studies have shown that expanding analyses to the coding region of the mtGenome can lead to a dramatic reduction in previously unresolved individuals.[1]

Thus, the objective of this research was to expand the amount of information gleaned from a limited sample type, such as a short hair shaft fragment, by combining a multiplexed PCR approach with Next Generation Sequencing (NGS) methods. In this study, mtDNA was extracted from two centimeters of hair shaft collected from two donors using an optimized extraction method designed in WCU's forensics laboratory. Extracts were quantified with a human mtDNA-specific qPCR assay and then amplified using a multiplexed PCR strategy consisting of forty-six total primer sets in ten reactions (four- or five-plexes) and covering the entire mtGenome.[2] The multiplex reactions were designed using previously described primer sets (Applied Biosystems® MitoSEQr™ Kit) with slight modifications for NGS. Primer set combinations were chosen based on a variety of factors including primer melting temperatures, amplicon size, position in the mtGenome, and evaluation of secondary structure formation. Various amplification conditions and enzymes were attempted during method development using positive control DNA and the Roche® FastStart™ High Fidelity PCR System was found to be most effective. Multiplexed reactions were evaluated with the Agilent® 2100 Bioanalyzer for concentration and sizing information. Amplicons were diluted, pooled in various ways, and processed using Illumina® Nextera XT®, which enzymatically fragments amplicons and tags them with Illumina®-specific adaptors and indexes. This library preparation method requires only 1ng of total dsDNA, making it extremely useful in forensic applications. These processed samples were then sequenced on the Illumina® MiSeq™ platform using a 300-cycle, paired-end v2 reagent kit. Sequencing data was evaluated using Illumina® MiSeq™ Reporter and CLC Bio Genomics Workbench software packages and results were compared to reference sequences generated using blood or buccal samples and traditional Sanger sequencing methods. A total of nine mtGenomes were sequenced in one NGS run with an average coverage of 7,000x across all reported positions, and 89.1% of the five gigabases sequenced exhibited a Phred Q-score ≥ 30.

References:

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mtDNA, Next Generation Sequencing, Multiplex Amplification

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