NEXT GENERATION SEQUENCING OF THE HUMAN MITOCHONDRIAL GENOME USING A MULTIPLEXED PCR STRATEGY AND ILLUMINA® NEXTERA® XT

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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 (Andreasson *et al.* 2007).

Thus, the objective of this research is to expand the amount of information gleaned from a limited sample type, such as a short hair shaft fragment using by combining a multiplexed PCR approach with Next Generation Sequencing (NGS) methods. In this study, mtDNA was extracted from two centimeters of hair shaft using an optimized extraction method designed in our laboratory. Extracts were quantified with a human mtDNA-specific qPCR assay (Kavlick *et al.* 2011) 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. 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. Following amplification, amplicons were diluted, pooled, and processed using Illumina® Nextera XT®. This sample preparation method requires only 1 ng of total dsDNA, making it extremely useful in forensic applications. This method enzymatically fragments amplicons and tags them with Illumina®-specific adaptors and indexes. These processed samples were then sequenced on the Illumina® MiSeq™ platform. Sequencing data was evaluated using the 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.