A69 Improved Extraction Efficiency of Human Mitochondrial DNA From Hair Shafts and Its Implications for Sequencing of the Entire mtGenome From a Single Hair Fragment

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After attending this presentation, attendees will gain an understanding of a novel protocol for extraction of mitochondrial DNA (mtDNA) from hair shafts, learning how this improved extraction method, when coupled with whole-genome amplification strategies, can increase the discriminatory power of mtDNA analysis by providing sequencing data that extends to regions of the mtGenome that are not routinely analyzed in forensic science laboratories.

This presentation will impact the forensic science community by providing insight into how optimized extraction and whole genome pre-amplification methods for difficult sample types can improve the utility of mitochondrial DNA analysis using both traditional Sanger sequencing and next generation sequencing platforms.

Forensic scientists are often faced with the challenge of limited or degraded samples, where a full nuclear DNA 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. Existing extraction protocols generally yield enough mtDNA from two centimeters of hair shaft to reliably sequence two hypervariable regions (HV1 and HV2) located in the non-coding control region of the mtGenome. However, HV1 and HV2 comprise only about 4% of the entire mtGenome. This, coupled with the fact that mtDNA is, by nature, less discriminatory than nuclear DNA, limits the current utility of mtDNA analysis. The main objective of this research is to maximize the mtDNA extracted from two centimeters of hair shaft so that more mtGenome sequence information may be obtained for comparison to a reference sample, leading to a higher discriminatory power of mtDNA analysis.

In this study, a novel method for extracting mtDNA from hair shafts is described, which combines traditional methods with two kit-based extraction methods (Qiagen® QIAamp® DNA Investigator and Applied Biosystems® PrepFiler® Forensic DNA Extraction Kits). Comparison studies were conducted as follows: 13 two-centimeter hair fragments were subjected to the traditional manual grinding/organic extraction method and 14 two-centimeter hair fragments were processed using the newly developed extraction method.[1] All purified hair extracts were quantified using a custom real-time quantitative PCR (qPCR) assay specific for human mtDNA.[2] Hair fragments processed using manual grinding/organic extraction yielded an average of 31,440 copies per extract (60µl elution volume), while those processed using the optimized method yielded an average of 270,100 copies per extract (50µl elution volume). Not only do these results indicate a consistent nine-fold increase in mtDNA concentration, but the optimized method is also less time-consuming and can be completed in approximately four hours.

Following optimized hair extraction, targeted PCR methods were used to prepare the mtDNA for traditional Sanger sequencing. Preliminary results indicate that 84.6% of the entire mtGenome can be amplified from a single hair extract using these methods. The resulting amplicons were sequenced on an Applied Biosystems® 3130*xl* Genetic Analyzer. In addition, mtDNA was enzymatically fragmented and tagged with adaptors using Nextera® XT DNA Sample Preparation Kit, and then sequenced using an Illumina® MiSeq® Personal Sequencer. Hair extracts were also subjected to whole genome amplification (WGA) using the Qiagen® RepliG® Mini Kit in an attempt to further increase the analyzable amount of mtDNA for downstream applications. While previous efforts to pre-amplify mtDNA using various WGA methods were largely unsuccessful using extracts processed with manual grinding and organic extraction, a two-fold to 16-fold increase in mtDNA concentration has routinely been achieved when using extracts processed with the optimized method. WGA pre-amplified material was also fragmented using Nextera® XT DNA Sample Preparation Kit and sequenced using an Illumina® MiSeq® Personal Sequencer. Results of all Sanger and Next Generation sequencing studies were compared to reference sequences generated using blood or buccal samples obtained from donors and the Applied Biosystems® MitoSEQr™ Resequencing System protocol and will be presented.

References:

1. Wilson MR, Polanskey DP, Butler J, DiZinno JA, Replogle J, Budowle B. Extraction, PCR amplification, and sequencing of mitochondrial DNA from human hair shafts. Biotechniques 1995:14:662–669.
2. Kavlick MF, Lawrence HS, Merritt T, Fisher C, Isenberg A, Robertson JM, Budowle B. Quantification of human mitochondrial DNA using synthesized DNA standards. J Forensic Sci 2011:56(6):1457-63

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