B8 Evaluation and Optimization of mtDNA Hair Extraction Methods

Mark F. Kavlick, BS, and Helen S. Lawrence, MS, Federal Bureau of Investigation, CFSRU, Building 12, FBI Academy, Quantico, VA 22135; Constance Fisher, PhD, Federal Bureau of Investigation, Investigation Parkway, Quantico, VA 22135; and Kerri A. Dugan, PhD, Federal Bureau of Investigation, CFSRU, Building 12, FBI Academy, Quantico, VA 22135*

After attending this presentation, attendees will learn the results of a study designed to compare four methods to recover mtDNA from hair.

This presentation will impact the forensic community and/or humanity by identifying a means to increase hair evidence through mtDNA analysis and enhance efficiency.

Forensic mitochondrial DNA (mtDNA) analysis has proven useful for biological evidence that contains small or degraded quantities of DNA. Extraction of DNA from fresh hair that includes a living root or a tissue tag should yield ample quantities of DNA; however, extraction from hair shaft alone, which contains non-living, keratinized tissue, can be challenging. An optimized protocol should make analysis more efficient without sacri- ficing data quality and consuming limited evidence samples.

The FBI Laboratory's current standard operating protocol (SOP) for DNA extraction from hair begins by mechanically grinding the hair in extraction buffer using a glass micro tissue grinder. DNA is then released from the disrupted hair by the action of DTT and proteinase K at 56° C during a two hour to overnight incubation period. Following the DNA extraction is a two-step purification process that includes an organic extraction step to remove proteinaceous material and a micro filtration step to further purify the DNA and remove potential PCR inhibitors. Extractions from hair fragments approximately 2 cm in length by this method yield dependable mtDNA analyses. However, the SOP is a time- consuming method with lengthy incubations, exposure of laboratory staff to harmful, volatile organic solvents and requires expensive, singly used glass tissue grinders.

Several commercial kits for extraction of DNA from hair have become available and these were investigated to determine whether any might provide advantages over the SOP. The Hair mtDNA Extraction Kit (Marligen Biosciences) and a user-adapted protocol for hair extraction using the QIAamp DNA Mini Kit (Qiagen) both involve chemical digestion of hair followed by column purification of DNA. The Tissue and Hair Extraction Kit (Promega Corporation) also chemically digests the hair but purifies the DNA using a paramagnetic resin. Each of the commercial methods instruct shorter incubation periods, which could result in a time savings and therefore allow higher throughput of evidence. In addition, the elimination of mechanical hair grinding should provide a cost savings. Finally, the nonuse of organic solvents would create a safer laboratory envi- ronment.

The SOP and the three commercial methods were evaluated for their ability to extract mtDNA from Caucasian head hair specimens. The amount of DNA recovered from hair after processing by each method was assessed by both pre-amplification DNA quantification and post-amplifi- cation DNA quantification. Amplicons subjected to DNA sequencing and the resultant electropherograms were evaluated to determine the quality of the derived sequence data. Additional preliminary experiments included incorporation of a hair-grinding step prior to beginning each commercial method and determination of the percent recovery for each method.

These data revealed that the SOP and Qiagen methods provided the best results among all four methods. Therefore these methods were further evaluated by extracting DNA from additional hair types including pubic hair, African American head hair, and dyed and bleached Caucasian head hairs. The resultant pre- and post-amplification quantification data showed that the SOP was the best method. Further evaluation of the SOP through a series of time course experiments showed that extraction for as little as two hours may be sufficient to provide high quality mtDNA.

The commercial methods of hair mtDNA extraction examined showed varied results; however, the SOP provided the best results, or among the best results, for all evaluations conducted. Therefore, despite the potential cost and time savings afforded by the available commercial hair extraction kits, the SOP consistently produces the highest yield of extracted DNA from hair shafts. Furthermore, if the time course experi- ments confirm initial observations, a reduction to a two-hour initial incu- bation with the SOP will still result in a substantial time savings and increased evidence throughput without sacrificing DNA yield.

mtDNA, Extraction, Hair

 ** Presenting Author*