B146 Optimized Extraction of Nuclear DNA From Hair Shafts: Part 2

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After attending this presentation, attendees will learn a method to extract nuclear DNA from hair shafts (without roots) on both head and pubic hairs

Traditionally, it is known that nuclear DNA from hair shafts is minimal to non-existent in the absence of a root or adhering epithelial cells. This method proves that nuclear DNA can be obtained from hair shafts. This method can be implemented in forensic DNA labs equipped mainly for STR DNA testing. It can also be used in labs also online for forensic mtDNA testing to use in conjunction with the STR profile to increase the statistics on the profile obtained whether it be for use in criminal cases or for missing persons. The profile or profiles can then be uploaded to CODIS.

This research initially began as work to test hair samples recovered from the World Trade Center Disaster and the hair samples received as references provided by the families as well as direct reference hair samples from the victims. The experiments during Part 1 explored decontamination methods, application of various enzymes for digestion, enzyme concentrations, different volumes of incubation buffer, amplification strategies, and varied run parameters on the ABI Prism 3100 Genetic Analyzer. Part 2 focuses on comparing two modified extraction protocols using Promega's DNA IQ versus a direct lysis extraction.

Head and pubic hairs were collected. The hairs were cut to ensure that no roots were present. If the hairs were not cut, they were examined macroscopically and microscopically to ensure that no roots were present. The hairs were washed using 5% Terg-a-zyme, 0.9% NaCl solution, and 100% ethyl alcohol and air dried overnight. The dried head hair was milled into a powder using the Spex Certi-Prep Freezer Mill and measured out in 20mg amounts. The pubic hairs were washed in the same way and each cut to 2.5 cm in length. The pubic hairs were pooled together to 12.5 cm and 25 cm lengths. The hairs were then incubated in 1.8 mg/ml Proteinase K, 0.25M DTT, 0.5% SDS, and DNA IQ incubation buffer containing 10mM Tris (pH 8.0), 100mM NaCl, and 50mM EDTA overnight at 56°C. The volume of incubation buffer varied depending on the type of hair sample-milled hair or pubic hair strands. For the milled head hair, the supernatant was taken and divided evenly between the optimized DNA IQ and direct lysis extraction. For DNA IQ, the recommended amount of Resin was used followed by a Microcon 100 cleanup. For the direct lysis extraction, the extract was purified through either a Bio-Rad Micro Bio-Spin Chromatography or a Centri-Sep column followed by a Microcon 100 clean-up.

The amount of DNA was quantified using a real-time PCR *Alu*-based assay (Nicklas and Buel). Attempts at quantifying the amount of nuclear DNA were initially done using the slot-blot method (Quantiblot). As expected, no DNA was detected since the sensitivity of Quantiblot is insufficient to less than 150 picograms. The use of the real-time technology is advantageous for quantifying the low level amount of nuclear DNA in the hair shaft extraction, to detect if melanin is inhibiting amplification, to check the "success" of the positive and negative controls, and also to predict the success of STR amplification.

Results from head hair and pubic hair are compared as well as the differences in the amount of DNA recovered from blond, brown, and black colored hairs. Initial results for 20 mg of milled head hair show that naturally blond hair yields a stronger signal in real-time PCR than brown or black colored hairs for both extraction methods. This further proves that melanin can act as a PCR inhibitor. Comparing the two extraction methods, the optimized DNA IQ protocol is generally more successful at "capturing" about three times more the amount of DNA than by using the direct lysis method. Initial STR data based on Promega's PowerPlex® 16 show the expected amount of DNA degradation with drop out of the larger loci.

Future work will discuss the results of the testing on pubic hair strands, additional STR results using PowerPlex®16, and the viability of the extract and success for mtDNA sequencing.

Hair Shafts, Extraction, Nuclear DNA

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