A56 Validation of an Alternative for DNA Extract Purification Using the QIAamp® DNA Mini Kit in mtDNA Analysis

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After attending this presentation, attendees will learn that the QIAamp DNA mini kit is a viable alternative for extract purification from hairs, blood, and buccal samples for mtDNA analysis.

This presentation will impact the forensic scientific community by providing a study that compares extraction methods through mtDNA amplification yields.

The purpose of this study was to determine if an alternative method for DNA extract purification using the QIAamp® DNA Mini Kit is comparable to the current method of Phenol Chloroform Isoamyl Alcohol (PCIA) purification for hair, blood, and buccal samples. Side-by-side comparison of the QIAamp® purification with the PCIA purification method was assessed by yield of amplified mitochondrial DNA, overall sequence quality, and contamination susceptibility.

To test DNA extraction from hairs, 12 hairs from 10 different individuals of known sequence were extracted and purified with both methods (exception: 2 hairs from extensions made from human hair were not previously sequenced). Hair cuttings effectively represented samples of 2 cm, 1.5 cm, 1 cm, and 0.5 cm in length. Twice these lengths were initially cut (no root material was used), ground in a microtissue grinder in Stain Extraction Buffer (SEB), and incubated at 56°C for two hours to overnight. The extract volume was split into two—one purified with PCIA and the other purified using the manufacturer's instructions for the QIAamp® DNA Mini Kit. The hair samples were amplified for two subregions of HV1 and HV2: "HV1b" spanning positions 16160-16390 and "HV2b" spanning positions 178-408 using 36 cycles. Post-amplification yields were measured using the DNA 1000 assay on the Agilent Technologies 2100 Bioanalyzer instrument. Cycle sequencing was performed using the Big Dye Terminator v.1.1 Cycle Sequencing kit. Post-cycle sequencing clean up was performed using the CentriSep™ columns and then run on a 3130xl Genetic Analyzer capillary electrophoresis instrument for visualization of the sequence products.

In addition, 10 total blood and buccal samples from different individuals of known sequence were extracted and purified with both methods using a similar experimental design to the one described above. For blood samples, approximately 1.5mm^2 cuttings were taken, and ¼ of a swab was cut for buccal samples. The DNA extracts from the blood and buccal samples were amplified for the Whole Control Region (WCR, spanning nucleotide positions 15998-616) using 32 cycles. Post-amplification yields were measured using the DNA 7500 assay on the Agilent Technologies 2100 Bioanalyzer instrument.

There was no effective difference in the yield of amplified mitochondrial DNA based on length of starting material for the hairs between the extracts purified with the different methods. In fact, no overall pattern emerged in the data to indicate one method consistently provided a higher yield over the other for hairs, blood, or buccal samples. A vast majority of the samples exhibited little to no difference in the amount of amplified mitochondrial DNA when comparing the two procedures. One exception was a single observation of a threefold higher difference in amplified mitochondrial DNA yield for one bloodstain sample purified with PCIA. However, the extract from the corresponding QIAamp® purification for that bloodstain appears to have been inhibited, since dilution of the extract was required for successful amplification. It is also important to note that two other bloodstains had a 1.7-1.9 fold higher yield with QIAamp®. Both these observations, taken with the fact that all yields for the blood samples were relatively low, indicate caution is necessary when making the decision as to which treatment to use when processing bloodstains. There was virtually no difference in the sequence quality generated or the number of length variants detected between the two purification methods. The results also indicate little difference between the two methods with respect to the incidence of contamination, despite the higher number of manual manipulations with the QIAamp® columns. Therefore, using the QIAamp® DNA Mini Kit for purification for hair, blood, and buccal samples is a viable alternative to PCIA purification.

Extraction, mtDNA, Comparison

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