Procedural Improvements in Mitochondrial DNA Analysis: Validation and Implementation of a New Hair Extraction Procedure and a Modified Amplification/Sequencing Primer

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An increasingly high number of recent forensic DNA cases involve the submission of single hairs as evidence samples. Extracting mtDNA from single hair shafts can be an extremely tedious, labor intensive procedure. Many of the hair extraction protocols that are currently in use by the forensic community involve numerous tube transfers, the use of tissue grinders, and the introduction of many different reagents. A hair extraction protocol that can eliminate a number of tube transfers and diminish the amount of handling and addition of different reagents would be ideal for reducing the potential of contamination in this procedure. However, the quality and quantity of DNA yield should also not be compromised. While our current hair extraction protocol was a more simplified procedure, we were encountering difficulty in consistently obtaining substantial DNA yield. To investigate this issue, variations of our current hair extraction protocol were compared. The ability of these different methodologies to successfully undergo PCR amplification and give reliable, accurate results were used as selection criteria.

The best results were achieved by using a modified hair extraction procedure that involves an initial wash step with sterile water and then ethanol, a 2-3 hour incubation with TE buffer, Proteinase K, and DTT, and additional 2-3 hour incubation with more Proteinase K and DTT, heating the samples to 95°C to inactivate the Proteinase K, followed by Microcon® purification. The resulting extract can then immediately undergo PCR amplification. This hair extraction procedure was successfully validated by producing accurate results from six single hairs of known mtDNA sequence. This hair extraction protocol has also produced excellent results from the first two hair cases (involving single hair shafts) that have been attempted since the validation of this new procedure.

An improved primer for amplifying and sequencing mtDNA has also been recently validated. It was discovered that the primer known by the forensic DNA community as R306 (if naming by the 5' end) or R285 (if naming by the 3' end) has one additional thymine, that may possibly interfere with primer binding. The R306 primer has seven (7) thymines, where the corresponding primer binding site on the Anderson standard sequence has six (6) adenines. The majority of observed mtDNA sequences also have six (6) adenines at this location. A side by side comparison of the standard R306 primer and a new, modified R306 primer with six (6) thymines was conducted. It was shown that the new R306 primer gave enhanced PCR amplification results, most likely due to increased specificity of primer binding.