B147 Evaluation and Quantification of Nuclear DNA From Human Telogen Hair

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After attending this presentation, attendees will be familiar with the procedures utilized in the extraction, quantification and amplification of nuclear DNA from single telogen hairs.

This presentation will impact the forensic community and/or humanity by providing information on the practicality of extracting and amplifying nuclear DNA from human telogen hair.

This paper presents data on the extraction and quantification of nuclear DNA from naturally shed hairs such as those that would be present at crime scenes. Additional goals of this project are: to determine the significance of exogenous DNA on hair; to evaluate method for the removal of exogenous DNA from the hair; and to evaluate the state of degradation of the DNA using real time PCR. The quantified DNA was amplified using small amplicon STR kits (Miniplexes) and a commercial STR typing kit.

Many experiments have been published on the extraction and amplification of DNA from hair. While some success has been reported for amplification of DNA from hair, accurate quantification of the extracted DNA has been a problem. Before the use of real time PCR for quantification, DNA analysts have relied on methods which lack the sensitivity required to detect the minute amounts of DNA found in hair. Therefore, information on the actual amount of nuclear DNA that can be recovered from hair is scarce. The use of real time PCR for quantification allows for detection of DNA in the picogram range, and this method is suitable for a study of the amount of DNA that can be recovered from telogen hairs. In order to ensure that the DNA quantified comes from within the hair, methods that can remove exogenous DNA must be utilized. The use of differential extraction is a method that allows removal of epithelial cells from the surface of hair, and also allows for the evaluation of the impact of exogenous DNA through quantification of the recovered DNA and evaluation of contamination by amplification of the exogenous DNA.

Direct evaluation of the state of degradation of DNA can normally be accomplished through agarose gel separation and ethidium bromide staining. However, this method required microgram amounts of DNA. When only picogram or nanogram amounts are recovered, real time PCR can be used to determine the number of small and large fragments through amplification with different primers. Comparison of the amounts of large and small fragments will indicate if degradation is present in the sample.

When DNA recovered from hair is degraded, success of amplification of the DNA using multiplex kits with large amplicons can be limited. Instead, Miniplex kits specifically designed for use with degraded DNA can be used as an alternative for such samples.

In this project, DNA was extracted from telogen hairs (3 cm in length) from numerous volunteers. Eight to nine hairs from each individual were extracted using a published protocol consisting of a calcium based extraction buffer system with DTT, phenol chloroform separation, and Microcon® YM-30 filtration. The extracted DNA was quantified by real time PCR using the Corbett Rotor-Gene 3000, a 124 base pair *Alu* amplicon, and SYBR Green I dye. In order to evaluate the amount of exogenous DNA contamination, a differential extraction buffer without DTT was used to remove epithelial cell DNA, and the DNA removed was extracted, quantified, and amplified with the Miniplex kits. Samples with the highest concentrations were concentrated further using Microcon® filters. The concentrated extracts were quantified with two different sized *Alu* primers (124 and 280 base pairs) and the quantification results were compared to determine the state of degradation of the extracted DNA. The concentrated extracts were then amplified with the Miniplex kits, and the results of the quantification were compared to the profiles to determine the effect of the degradation on genotyping.

The amount of DNA recovered from hair using this method varies greatly, with a range of 1-90 pg/1L. The amount of exogenous DNA present in hair also varies, and the exogenous DNA appears to have little contamination from outside sources. DNA recovered from hair does show degradation upon evaluation by real time PCR, but the DNA can be successfully amplified using reduced size STRs.

Nuclear DNA, Telogen Hair, Real Time PCR Quantification

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