B179 Examination of Methods for Amplification of Nuclear DNA From Human Telogen Hairs

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After attending this presentation, attendees will gain an understanding of the processes of obtaining a genetic profile from single telogen hairs using reduced-size STR primer sets.

This presentation will impact the forensic community and/or humanity by providing information on the methodology required for obtaining genetic profiles from single human telogen hairs collected as evidence as well as the likelihood of producing a profile from these types of samples.

Shed telogen hairs can be an important form of forensic evidence. Such samples are usually reserved for mtDNA analysis due to the assumption that little nuclear DNA is present. Unfortunately due to its small size and haploid nature, mtDNA lacks the probative value of nuclear DNA. Since telogen hairs are not expected to contain much nuclear DNA, such samples are reserved for mtDNA testing or are not amplified and typed. Few studies have probed the absolute amounts of nuclear DNA in hair. This was due to a lack of a suitable quantitative technique to analyze such samples. In spite of this problem, several studies 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.

In a previous study, after sampling over 500 individual hairs, it was determined that the majority of subjects (70%) had on average less than 500 pg of total recoverable DNA per hair. In addition, through the use of different size *Alu* primers and real time quantitative PCR, it was demonstrated that the DNA extracted from telogen hairs is highly degraded. Both of these conditions present a problem for obtain a genetic profile from shed telogen hair evidence. Additionally, PCR inhibitors such as melanin which may be present in hair can interfere with the amplification and reduce the possibility of obtaining a DNA profile. In the case of low amounts of DNA, dilution or purification methods such as sodium hydroxide or silica based spin columns, which typically result in a loss of DNA, are not feasible for these types of samples.

In this study, the focus was on the methods required to produce a profile from nuclear DNA extracted from human telogen hairs using the Miniplex reduced-size STR primer sets. Concentration of samples and collection of samples in lower volumes were both evaluated as possible solutions to low concentrations produce in the original method. For new extractions, single hairs from different individuals were extracted using a protocol that included a differential extraction to remove exogenous DNA. Previously extracted samples were concentrated and collected in 10 µL (instead of 60). The extracted DNA was quantified by real time PCR using an 82 bp *Alu* amplicon.

Because of the limited amounts of recoverable DNA present in the hair and due to its high degree of degradation, changes in several PCR reaction mix components were investigated. These included the concentration of primers and other PCR reagents as well as addition of various concentrations of bovine serum albumin (BSA) to relieve inhibition. Three ranges of extracted and quantified DNA were tested for amplification success: high (>500 pg), medium (100-500 pg) and low (< 100 pg). The high range samples were tested with all three Miniplexes sets while the lower level concentrations were amplified with a single set of reduced size amplicons. The results are discussed in terms of the potential compromises necessary when utilizing samples containing low levels of degraded DNA.

Reduced Size STRs, Degraded DNA, Human Telogen Hairs

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