B178 Methods for the Assessment of the Suitability of Catagen Hairs for Nuclear DNA Profiling Using Nuclear Fast Red Staining and Polarized Light Microscopy

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After attending this presentation, attendees will learn how to use Fast Nuclear Red stain and polarized light microscopy to assess the suitability of hair roots for PCR amplification for nuclear STR analysis.

This presentation will impact the forensic community and/or humanity by making the forensic community aware of simple assessment techniques for determining the suitability of a hair root for the nuclear STR analysis.

Hairs may be found at crime scenes and can provide useful identifying information. While mitochondrial DNA is commonly extracted from hairs, nuclear DNA can also be available for short- tandem repeat (STR) analysis. STR analysis is forensically more informative than mitochondrial DNA sequencing and therefore should be done if possible. Not all evidentiary hairs are suitable for the recovery of nuclear DNA. Sufficient nuclear DNA for STR analysis can be obtained from an actively growing anagen hair root. On the other hand, telogen hairs lack growing cells and sufficient nuclear DNA for STR analysis may not be recovered from them. Catagen hairs are those hairs that are in the process of switching from the actively growing anagen phase to the fully keratinized telogen phase. It would be useful for the forensic molecular biologist to be able to determine if a catagen hair is likely to yield sufficient nuclear DNA for STR analysis. Two approaches for assessing a catagen hair root's suitability for STR analysis were explored: polarized light microscopy (PLM) and staining with Nuclear Fast Red stain. In the catagen phase of hair growth progressive keratinization of the root bulb results in an increase in the retardation of plane polarized light so that the interference colors observed with PLM should be indicative of how close the hair root is to the telogen phase. On the other hand, staining with Nuclear Fast Red stain (the first stain in the 'Christmas Tree' staining procedure frequently used in the microscopical identification of spermatozoa) should disclose the presence of cell nuclei in the root tissue of a catagen hair. Nuclear Fast Red staining does not interfere with subsequent STR analysis.

Plucked hair samples were obtained from a male donor. Those hairs lacking readily observable follicular tissue were subjected to Nuclear Fast Red staining by immersing the roots for several minutes in the staining solution. The hairs were removed from the staining solution and rinsed with distilled, deionized water. The hair roots were then temporarily mounted on microscope slides in distilled, deionized water. The hair roots were photographed under normal transmitted light and between crossed polarizing filters.

DNA was isolated using an organic extraction and quantitated with a Real-Time PCR assay for nuclear autosomal DNA. The results indicated a correlation between the intensity of Nuclear Fast Red staining and the amount of DNA that could be extracted from the hair root. To facilitate the interpretation of the Real-Time PCR assay the stained roots were grouped in four categories defined by the amount and intensity of Nuclear Fast Red staining: (a) no visible staining of the follicle; (b) slight staining (thin area around the hair root with a pink/light red color); (c) clear and well defined staining around the hair root; and (d) a thick dark red stained area around the hair root. These four categories showed consistent results in DNA concentrations, yielding respectively (a) less than 0.01 ngm/µL, (b) between 0.01 ngm/µL and 0.09 ngm/µL, (c) between 0.1 ngm/µl and 0.5 ngm/µl, and (d) more than 0.5 ngm/µL. PLM did not yield any clear-cut categories. Evidently the process of root keratinization does not correlate closely with the quantity of actively growing cells attached to the root.

The data obtained suggest that observing the quantity/intensity of the staining of hair root treated with Nuclear Fast Red stain can allow the forensic molecular biologist to predict the amount of nuclear DNA that can be extracted and amplified from the sample. This technique could be applied as an initial screening tool for hair samples classified as catagen hairs and allow DNA laboratories to optimize their resources by ruling out samples with very low likelihood of yielding sufficient nuclear DNA for an STR profile. Samples that are determined to be unlikely to yield an STR profile can be submitted for mitochondrial DNA sequencing.

Hairs, Real-Time PCR, DNA

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