B5 Alkaline Digestion of Head and Pubic Hairs for Nuclear and Mitochondrial DNA Analysis

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After attending this presentation, the attendees will learn the use- fulness of utilizing an alkaline extraction technique to obtain genetic infor- mation from shed head and pubic hairs.

This presentation will impact the forensic community and/or humanity by demonstrating current forensic examination of shed head and pubic hairs is limited to microscope comparisons with known hairs or to mtDNA sequence analysis. Alkaline extraction of shed hairs is a much simpler means of isolating DNA, as it requires less "hands on" technician time, reducing potential contamination. During the incubation in concen- trated sodium hydroxide, DNA remains intact while the hair itself is com- pletely dissolved. Once the DNA is isolated, several routes of genetic analysis can be performed for both mitochondrial and nuclear markers.

Hair is a common form of evidence found at crime scenes, and may be the sole trace evidence to tie a suspect or victim to a location or crime. Hair comparisons via light microscopy are effective for excluding an indi- vidual as a source of a hair, but not for identification purposes.

Obtaining DNA data from biological material associated with a crime is invaluable as evidence. Isolating DNA from head or pubic hairs is an attractive means of placing a suspect and/or victim at a crime scene. If the root of a hair is present, STR analysis may be successful, but it is more common that shed (telogen) head or pubic hairs are found at a scene. Nuclear DNA is thought to be degraded or not present in these samples, making STR analysis using commercially available kits difficult or impos- sible. MtDNA analysis of hair shafts is often successful, but many labs have not validated the method, sending samples out for DNA sequencing can be expensive, and in the end, it is not an absolute identifier.

Likewise, DNA isolation from hair shafts involves laborious extraction techniques, which can increase the likelihood of contamination. An alternative to standard DNA isolation from hair shafts is alkaline extraction, in which keratin from hair is hydrolyzed but DNA is kept intact. In the current study, this method has been used to extract DNA from head and pubic hair shafts. Hairs are washed in an enzymatic detergent, and then rinsed with ethanol and water. The hairs are then incubated in concentrated sodium hydroxide until completely dissolved. Following the incubation, the solution is neutralized and the DNA eluted in TE on a spin column. The suitability of alkaline extracted hair DNA was tested on mtDNA, as well as nuclear markers. MiniSTRs were examined, which have shown a greater chance of successfully amplifying highly degraded DNA. High copy number nuclear loci were tested to compensate for the small amount of nuclear DNA found in hair shafts. Quantitative PCR and nested PCR tech- niques were also examined.

Alkaline Extraction, Hair Shafts, Degraded DNA

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