B149 DNA Profiling of Rootless Hair Shafts Utilizing Massively Parallel Sequencing and Bi-Allelic Assays

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Learning Overview: After attending this presentation, attendees will understand the potential for rootless hair shafts to be used as genetic evidence for forensic purposes. Additionally, the utility of assays that include loci amplicons smaller than those found in traditional Short Tandem Repeat (STR) -based amplification assays will be discussed.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by illustrating the potential use of rootless hair shafts as discriminatory genetic evidence. With additional samples identified as viable sources of probative information, additional samples can be successfully processed during routine laboratory operations.

Shed hairs are often underutilized as genetic evidence. These hairs are often passively lost and do not contain a hair root, where intact DNA is usually found. However, with advances in genotyping techniques and instrument technologies, the remaining hair shaft may be more viable as evidence than previously thought. Using naturally shed hair samples, STR analysis often fails to provide profiles. However, there remains the possibility of DNA that is both low template and highly degraded by the keratinization process of hair formation remaining in or on a hair shaft. This remaining DNA, though unusable for STR analysis, may still be impactful in the forensic setting. Utilizing alternative methods for processing these sample types may provide information that can still be highly discriminatory.

Currently, complementing unsuccessful samples with mitochondrial genome sequencing is the best method for processing challenging rootless hair samples. Mitochondrial DNA is inherited maternally, with the lack of recombination in mitochondrial inheritance results in low discriminatory power for resulting genetic information. This makes exclusion or inclusion of possible donors difficult in cases where potential sources are related or share mitochondrial haplotypes by chance. The current limitations using the mitochondrial genome suggest a need for additional sample processing strategies. With current advances in available amplification chemistries and instrumentation, additional processing options are readily available. These novel options can be used in tandem with already established methods to increase the discriminatory power of DNA extracts obtained from rootless hairs.

This presentation will cover a preliminary investigation into alternative methods identified as promising for the genetic analysis of rootless hair shafts. In this research, samples of hairs were collected with informed consent. The rooted portion of hair samples was then removed before extraction with the InnoGenomics® Hair Extraction Kit. Sample extracts were amplified using three methods. As a control, STR analysis was attempted with the GlobalFiler® PCR Amplification Kit. Alternative sample processing strategies include amplification with the retrotransposon insertion polymorphism marker-based InnoTyper™ 21 chemistry, which was then analyzed using capillary electrophoresis. An additional test method applied massively parallel sequencing using the MiSeq® FGx. Samples were processed using the ForenSeq™ Signature DNA Prep kit with primer set B, which includes a number of bi-allelic single nucleotide polymorphisms that can be used for identification, ancestry estimation, or phenotyping.

Rootless Hair Shafts, Massively Parallel Sequencing, Bi-Allelic Markers

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