B133 Utility of a Novel and Sensitive DNA Multiplex for Highly Degraded Missing Persons Samples

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After attending this presentation, attendees will understand the application of a novel DNA marker system for use with missing persons samples.

This presentation will impact the forensic science community by serving as a key aspect for the analysis of degraded forensic DNA samples, as it can augment or replace traditional DNA testing systems in cases where those systems do not produce results due to the presence of highly degraded DNA.

The Missing Persons unit of the UNT Center for Human ID (UNTCHI) annually processes more than 2,600 specimens including human remains, family reference samples, and direct reference samples. The ability to successfully type these human remains samples using DNA analysis is of utmost importance for their identification. In these cases, identification is achieved primarily through Short Tandem Repeat (STR), Y-chromosomal Short Tandem Repeat (Y-STR), and mitochondrial DNA analyses; however, this approach is often restricted by common haplotypes and the excessively degraded condition of samples, thereby limiting the discrimination power of DNA using these technologies. To address these limitations, the UNTCHI strives to optimize and validate new techniques to assist with the identifications in missing persons cases. This presentation focuses on the utilization of a novel DNA marker system, for use with the types of samples commonly encountered in the Missing Persons unit at UNTCHI.

InnoTyper™ is a marker system that utilizes Retrotransposon Insertion Polymorphisms (RIPs). Retrotransposable Elements (REs) consist of Long Interspersed Nuclear Elements (LINEs) and Short Interspersed Nuclear Elements (SINEs). This group of bi-allelic markers can be useful for human identity testing. Among the advantages of using RIPs are that they do not yield stutter artifacts due to slippage during the PCR, there are no known genetic mutations since they are identical by descent only, they are present in very high copy numbers, and they have a well-defined genetic lineage, which makes RIPs useful for relationship determinations; however, until recently due to the inherent size difference (>300bp) associated with insertion and null alleles, the use of RIPs has not been practical for forensic applications. To circumvent the allele size disparity, a novel primer design methodology was used to remove the intra-specific locus competition that occurs in heterozygotes. This innovative primer design allows for the amplicon size to be reduced to a size smaller than currently used STR markers, such that substantially degraded DNA samples can be analyzed. Utilizing this primer design, a more simplified, rapid, and automated typing technology can be applied to RIP typing.

The multiplex utilized in this study consisted of 13 RE markers plus the gender identifying marker, amelogenin. All amplicons in this PCR multiplex range in size between 50bp to 125bp. To test the utility of this system in a forensic casework missing persons laboratory, several studies were performed, including sensitivity and testing on non-probative human remains. Results show the system to be highly robust and sensitive. Complete 13 RE marker profiles were obtained with as little as 16pg. These results were compared to the low copy procedure used by UNTCHI. The small amplicon sizes result in an extremely sensitive, rapid, and useful multiplex for typing highly degraded forensic samples as well as for high-quality DNA samples.

Data to be presented supports the usefulness of this system for analyzing missing persons samples which did not produce usable STR results but did provide InnoTyper™ results with high discrimination power. This system will prove very useful for analyzing single-source degraded DNA samples such as those found in mass disasters and other human identification efforts.

DNA, Degradation, Retrotransposon