A131 Y-STR Typing Strategy for Challenging Samples: Validation and Application to Historical Cases

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After attending this presentation, attendees will be familiar with an aggressive amplification protocol to obtain Y-chromosomal data from degraded skeletal remains. Attendees will learn the results of various experiments conducted to validate this protocol, in addition to learning how this method has already been applied to cases at AFDIL.

This presentation will impact the forensic community by describing a genetic assay that can complement or even replace DNA-based methods currently used in the identification of missing persons.

The identification of degraded skeletal remains at the Armed Forces DNA Identification Laboratory (AFDIL) is primarily achieved by mitochondrial DNA (mtDNA) typing. However, the forensic utility of mtDNA data is limited by the molecule's uniparental inheritance and lack of recombination, which can sometimes result in a low power of discrimination. Mitochondrial DNA testing also requires either direct or maternal references for evidentiary comparison, and in some cases these types of references are unavailable. When specific limitations of mtDNA testing such as these are encountered, data from alternative DNA markers in the nuclear genome would benefit the overall identification effort. Unfortunately, the poor quality and limited quantity of nuclear DNA present in degraded skeletal remains has historically restricted the use of autosomal and Y-chromosomal short tandem repeat (STR) data in such cases. Recently, modified or so-called "low copy number" (LCN) STR typing protocols[1] have shown great promise on the degraded skeletal elements typically encountered at AFDIL[2], particularly when the modified amplification is coupled with an improved DNA extraction.[3] As a result, AFDIL has begun to validate a LCN STR typing strategy for Y-chromosomal loci.

The application of Y-chromosome STR (Y-STR) typing to degraded skeletal samples can provide additional genetic information that may assist in missing persons investigations. For many cases submitted to AFDIL, Y-STR typing will be valuable in confirming gender, reassociating commingled skeletal elements and, of course, supporting identifications. In fact, the option of testing Y-STRs in these cases should facilitate the overall identification effort by expanding the pool of potential family references that can be used for DNA comparisons. Although Y-STRs do not provide the discriminatory power of autosomal STRs, the fact that distant paternal relatives can provide reference material is of great importance in these decades-old cases for which the necessary family references are unavailable for standard autosomal and mitochondrial comparisons. Additionally, data interpretation issues typically encountered with autosomal STRs from poor quality specimens, such as peak imbalance, allele drop-out and allele drop-in at potentially heterozygous loci, tend to be reduced for Y-STRs because of the haploid nature of the Y-marker.

The commercially-available Y-STR amplification kit used at AFDIL includes 17 loci located on the non-recombining portion of the Y- chromosome and targets amplicons ranging in size from 90 to 330 base pairs. For most STR kits, the optimal template input is approximately 1.0ng. However, the degraded skeletal elements typically processed at AFDIL yield too little DNA to produce usable data with standard amplification conditions. As a result, modifications have been made to the manufacturer's suggested amplification protocol. The recommended Taq concentration has been doubled to overcome potential inhibition from the large volumes of extract added to the PCR (extract volumes were maximized in order to maximize allele sampling and recovery). In addition, the standard polymerase chain reaction (PCR) cycles has been increased by six (for a total of 36 cycles) to facilitate maximum allele detection from limited amounts of amplification template. In order to confirm data authenticity, all amplifications are conducted in triplicate and only alleles observed in the majority of amplifications are included in any finalized, consensus profile [1].

As part of the validation process, the modified Y-STR amplification protocol was evaluated for sensitivity, mixture detection and effectiveness on non-probative case samples. Data generated with both the standard and the modified protocols were utilized to characterize the overall authentic data recovery. The results of these experiments and the forensic implications of these results will be presented. Finally, in order to demonstrate the practical utility of the modified Y-STR typing strategy in cases regularly encountered at AFDIL, a number of interesting historical cases that have benefited from Y-STR data will also be presented.

The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the United States Department of Defense or the United States Department of the Army.

References:

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Short Tandem Repeat, Y-Chromosome, Low Copy Number

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