H131 Maximum DNA Recovery From Cold Case Victims Using Ancient and Forensic Extraction Methods

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Learning Overview: The goal of this presentation is to compare quantitative Polymerase Chain Reaction (PCR), Short Tandem Repeat (STR), and preliminary whole-mitochondrial genome results from the skeletal remains of cold case victims from Maricopa County, AZ, using comparative DNA extraction techniques.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees about the results of comparisons of different methods for DNA recovery for Combined DNA Index System (CODIS) marker and mitochondrial genome analyses in the context of degraded DNA.

Human identification using DNA analysis is a central focus of forensic science. However, environmental, microbiological, and climatic factors often limit the amount of recoverable DNA (i.e., short DNA fragments and nucleotide damage) following death, especially in circumstances where only hard tissues, such as bones and teeth, are available for study. Despite these limitations, the field of ancient DNA (aDNA) analysis has advanced DNA methodology remarkably, allowing for the rapid quantification and genome-wide reconstruction of ancient samples (e.g., human, megafauna, and pathogen) dating as far back as the Middle Pleistocene (781,000 to 126,000 years ago). As a result, the applications of aDNA techniques to forensic cold case victims using skeletal material recovered from harsh, desert environments from Maricopa County, AZ, have the potential to recover the complete genetic profiles of these previously unidentified victims. These individuals remained unidentified because traditional attempts failed to produce adequate DNA yields. This study examines the results of quantitative PCR, STR, and whole-mitochondrial genome analyses generated from two different DNA extraction techniques used to recover DNA from the skeletal remains of cold case victims from Maricopa County, AZ.

In collaboration with Arizona's Maricopa County Office of the Medical Examiner, this study obtained 75 bone and tooth samples representing 75 open and/or missing persons cases. This study obtained DNA from these victims using two different DNA extraction methods: one devised by Dabney and colleagues (ancient DNA) and another by Loreille and colleagues (forensic DNA). Absolute DNA concentrations were measured using the Quantifiler® Trio DNA Quantification Kit, and STR profiles were generated using Promega's® PowerPlex® Fusion 6C System, a kit designed to amplify the expanded CODIS core loci (i.e., 23 autosomal STRs, 3 Y-chromosomal Short Tandem Repeats (Y-STRs), and amelogenin). In addition, this study built double-stranded DNA libraries and performed a long hybridization (48 hours) targeted enrichment using mitochondrial RNA baits. Libraries were pooled and sequenced on an Illumina® MiSeq® platform, and haplogroups were called using an in-house custom mitochondrial DNA computational pipeline. The analysis suggests that aDNA extraction methods yield higher quantities of DNA for cold case identification than traditional forensic DNA extraction methods. The integration and adoption of optimized aDNA protocols, such as the Dabney DNA extraction protocol, in forensic case analyses may improve the success rate of acquiring adequate STR profiles and whole-mitochondrial genome assembly to identify cold cases in circumstances where traditional extraction techniques have failed.

Genetics, CODIS, MtDNA

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