RECOVERY OF HIGHLY FRAGMENTED nDNA FROM SKELETAL MATERIAL FOR SNP-BASED MPS ANALYSIS

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Identification of skeletal remains that have been discovered after an extended period of time is becoming an increasingly important part of the forensic science field. Examples of this are the missions of the International Commission on Missing Persons (ICMP) and the Armed Forces DNA Identification Laboratory (AFDIL). These types of remains have usually been exposed to the elements and tend to be older than what may be seen in a typical forensic identification case; although a significant percentage of the unidentified remains in medical examiner's offices across the country fall into this category. Extended exposure to environmental insults results in increased DNA degradation, leading to fragmentation of the DNA, in some cases to less than 150 base pairs in length. Current human identification methods use a combination of mitochondrial (mt) DNA and short tandem repeat (STR) analysis. These techniques target segments of DNA ranging from 100 to 500 bps in length. Therefore, due to the highly fragmented nature of the DNA recovered from more degraded skeletal remains, identification is not always possible with these techniques. Single nucleotide polymorphism (SNP) analysis is ideal for these types of samples as the targets are short regions of DNA (40 to 70bps). SNPs can be used for identity, and can also provide phenotypic and ancestry information that would help with human identification. Unfortunately, current methods of DNA extraction from skeletal material have not been optimized to recover nuclear (n)DNA fragments that would serve as templates for SNP analysis. In order to evaluate which parameters to address for optimization, a modeling experiment was performed that utilized sheared, pristine DNA. Through this modeling the efficiency of different binding buffers and columns was evaluated in order to identify an optimized protocol. The results indicated little variance between the MinElute column and QIAquick column, but an increase in recovery with a binding buffer consisting of 5M GuHCl and 50% Isopropanol. A change from a 100K amicon filter to a 30K filter during the concentration step was also incorporated into the optimized protocol to retain smaller fragments of DNA. The resulting protocol was compared to the current protocol used by the ICMP with skeletal material from the 7th-9th century, medieval era and 17th to 18th century. Massively parallel sequencing (MPS) SNP analysis was conducted with the Illumina ForenSeq kit on the MiSeq instrument.