OPTIMIZATION AND VALIDATION OF A DNA EXTRACTION METHOD FROM SKELETAL REMAINS

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Skeletal remains (bones and teeth) have been one of the most challenging biological samples from which to extract amplifiable DNA for forensic purposes. Some factors that can contribute to the inability to obtain a mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) profile include contamination, DNA damage, amount of available skeletal material, and presence of polymerase chain reaction (PCR) inhibitors. In the mtDNA Unit at the FBI Laboratory, approximately 30% of questioned origin evidentiary items are skeletal remains. Therefore, improving DNA extraction efficiency by minimizing sample consumption, maximizing DNA yield, and providing one DNA extract that can be used to generate both mtDNA and nDNA profiles is an important goal.

To accomplish this goal, several different combinations of the amount of calcified tissue, DNA extraction and demineralization buffers, concentration devices, and DNA clean-up methods were tested. The resulting DNA extracts were evaluated using both mtDNA and nDNA-specific qPCR assays (each with an internal positive control to assess inhibition). The quality of the mtDNA and nDNA profiles obtained from the extracts was also assessed by sequencing and STR analysis, respectively. Preliminary examinations were conducted using multiple extractions from three different bones of varying quality. A method similar to that published by Loreille, et al (FSI Genetics, 1:191-195, 2007) provided the best results in terms of greatest yield, least inhibition, and consumed the least amount of sample (0.2g bone/tooth powder).

A validation study with 50 calcified tissue samples was then conducted comparing the selected method to the current FBI Laboratory mtDNA Unit process for skeletal remains. Each bone or tooth sample was extracted with both methods concurrently. Samples extracted using the modified Loreille, et al. method consistently produced higher mtDNA and nDNA yields. Details of the preliminary and validation studies are presented.