

IMPROVEMENTS IN BONE EXTRACTION FOR mtDNA ANALYSIS

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Mitochondrial DNA (mtDNA) analysis of forensic samples can be performed when the quantity and quality of DNA are insufficient for nuclear DNA analysis or when DNA analysis through a maternal lineage is required. Bone can be one of the more challenging forensic samples because of the low levels of DNA present, the likelihood of DNA degradation, and the potential presence of PCR inhibitors. Several extraction and purification strategies for efficiently obtaining highly purified DNA from bone have been analyzed. These include decalcification, the use of chaotropic agents, phenol-chloroform extraction, and the use of silica beads for DNA purification. In addition, the incorporation of pre-extraction wash steps to clean the bone samples have been evaluated.

Since the cellular contribution to bone mass is small, the successful extraction method should allow for extrication of DNA from the other components of bone. Hydroxyapatite is a major constituent of bone that can interfere with DNA extraction because calcium present in hydroxyapatite has a high affinity for the phosphate groups on the DNA backbone. Thus, decalcification should increase the amount of DNA that can be extracted from the bone. In fact, the process of decalcification is routinely used with DNA extraction in the field of molecular anthropology. This study has demonstrated that decalcification of bone samples prior to DNA extraction can enhance the amount of DNA that can be extracted and amplified from the sample.

The standard operating protocol (SOP) at the FBI for bone extraction utilizes a detergent/proteinase K incubation step followed by phenol-chloroform extraction and Microcon®-100 filtration. Alternatives to the detergent/proteinase K extraction have been with silica for DNA purification. In this study, DNA extraction from bone using protocols from Qiagen and Organon Teknika and a method described in Boom *et al.*

(J. Clinical Microbiology 28:495-503) have been evaluated. Each of these procedures was used to extract DNA from a bone sample. The quantity of DNA extracted (measured by slot blot), the quantity of DNA amplified (measured by capillary electrophoresis), and the quality of sequence produced from the extracted DNA was examined. It has been shown that each method provides DNA that can be successfully amplified and sequenced. Further, a comparison of the DNA extraction by the chaotropic methods and SOP has been performed. Preliminary results suggest that decalcification followed by a combination of organic extraction and the use of silica for DNA purification may provide for the most efficient recovery of high quality DNA.