H33 Optimization of a Method for the Extraction of DNA From Human Skeletal Remains

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After attending this presentation, attendees will better understand the challenges regarding the recovery of amplifiable DNA from bone and the development of a method for maximizing DNA yield from bone while reducing the coextraction of inhibitory substances. Additionally, attendees will learn how the use of multiplex Polymerase Chain Reaction (PCR) and current-generation sequencing technologies to amplify and sequence the entire mitochondrial genome can increase the discriminatory power of mitochondrial DNA (mtDNA).

This presentation will impact the forensic science community by illustrating how maximizing DNA recovery from challenging bone samples with an efficient extraction method will allow for amplification and sequencing of the entire mitochondrial genome with decreased labor and cost than Sanger-type sequencing methods.

It is often challenging to obtain Short Tandem Repeat (STR) profiles from DNA extracted from bone as a result of the low amounts of nuclear DNA present or due to DNA degradation as a result of prolonged environmental exposure. Although STR profiling is preferred due to its discriminatory power, mtDNA analysis is often utilized in these cases. The matrilineal inheritance and lack of genetic recombination enables use of mtDNA to trace maternal lineages, which is particularly relevant in forensic casework in the absence of reference material; however, these attributes also limit the discriminatory power of mtDNA analysis.

The forensic community currently focuses on the analysis of the non-coding control region of mtDNA. The control region contains two hypervariable regions (HV1 and HV2), where the majority of differences between individuals are found. Shared polymorphisms present within the human mitochondrial genome are used to define haplogroups or population lineages. In some instances, it is challenging to discriminate between individuals who share polymorphisms in their hypervariable regions. Expanding analysis of mtDNA beyond the HV region has been shown to increase resolution of common haplogroups that are not resolvable with analysis of the HV regions alone.[1] A multiplex PCR approach has been developed that enables amplification of the entire mitochondrial genome in nine PCR reactions. This, combined with current-generation sequencing technologies, will allow for rapid generation of whole genome sequence data. Sufficient quantities of amplifiable mtDNA must be obtained to utilize this method.

An efficient extraction protocol is required to maximize DNA yield from bone samples while minimizing the coextraction of PCR inhibitors naturally present in bone, such as calcium and collagen. DNA extraction from bone can be thought of in three discrete steps: demineralization, lysis, and purification. Determining which lysis buffer is most effective for bone tissue is a critical first step in optimizing a method for DNA extraction from bone.

Two cross sections (2.5cm x 2.5cm) of bone tissue were excised from a human femur. The tissue was pulverized using a SPEX® 6770 Freezer/Mill®. Bone powder (0.1g) was demineralized using a chelating Ethylenediaminetetraacetic acid (EDTA) solution to disrupt the structural matrix of bone.[2] Resulting sequestered divalent metal cations were then washed away, and the remaining cellular material was incubated overnight in one of three different lysis buffers (buffers A-C). Lysis buffers A and B were purchased from commercial suppliers. Lysis buffer C is commonly used in forensic casework and is prepared in the laboratory. Lysates were purified using the QIAamp® DNA Mini Kit. Purified extracts were quantified using a human mtDNA quantitative PCR assay.[3]

Preliminary data suggests that buffer A exhibits consistent performance and often yields higher DNA recovery from bone samples than the other buffers studied, although additional work is needed to further refine these results. Furthermore, sample preparation methods appear to impact DNA recovery. A significant increase in yield was observed when polycarbonate components were used rather than stainless steel components during pulverization. Future method development will include evaluation of different purification methods, specifically the use of commercially available silica spin columns and magnetic bead-based purification systems. A final protocol will then be used to extract DNA from weathered skeletal remains, which will better represent bone specimens encountered in forensic casework. Maximizing DNA recovery will make whole genome mtDNA analysis possible which will result in greater discriminatory power of mtDNA sequence analysis.

References:

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2. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. (2007): High efficiency DNA extraction from bone by total demineralization. Forensic Sci Int: Gen 1:191–5.
3. Kavlick MF, Lawrence HS, Merritt RT, Fisher C, Isenberg A, Robertson JM, Budowle B. (2011): Quantification of human mitochondrial DNA using synthesized DNA standards. J Forensic Sci 6:1457-63.

Bone, Extraction, mtDNA