OPTIMIZATION OF A METHOD FOR THE EXTRACTION OF DNA FROM HUMAN SKELETAL REMAINS

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Bone tissue is often the only physical evidence available for forensic DNA analysis. Bone is comprised of a matrix consisting principally of the structural protein collagen and the mineral hydroxyapatite, both of which are known inhibitors of PCR. As with many forensic samples, it is often challenging to obtain STR profiles from nuclear DNA extracted from bone due to potential low copy number as well as DNA degradation as a result of prolonged environmental exposure. Although STR profiling is preferred due to its discriminatory power, mitochondrial DNA (mtDNA) analysis is often utilized in these cases. MtDNA is a small, maternally inherited chromosome residing in high copy number in the mitochondria. The matrilineal inheritance and lack of genetic recombination allows mtDNA to be used to trace maternal lineages, which is particularly relevant in forensic casework in the absence of reference material.

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 (Coble *et al.* 2004). Our laboratory has developed a multiplex PCR approach that enables amplification of the entire mitochondrial genome in 9 PCR reactions. This, combined with current-generation sequencing technologies, will allow for rapid generation of whole genome sequence data.

An efficient extraction protocol is required to obtain sufficient quantities of amplifiable DNA from bone samples while minimizing the coextraction of PCR inhibitors. In this work, we describe initial efforts for the optimization of a DNA extraction method particularly suited for calcified tissues. This method is modeled after a protocol developed in our laboratory for the extraction of DNA from human hair shafts. Using this method, we have shown that a particular commercially available lysis buffer is most effective at maximizing DNA recovery from 2 cm hair shafts when combined with Life Technologies™ PrepFiler® Forensic DNA Extraction Kit (Thermo Fisher Scientific, Waltham, MA). DNA extraction from bone can be thought of as 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 (2.5 cm x 2.5 cm) of bone tissue were excised from a human femur (Skulls Unlimited International Inc. Oklahoma City, OK). The tissue was pulverized using a Spex 6770 Freezer Mill (Spex Sample Prep Metuchen, NJ). Bone powder (0.1 g) was demineralized using a chelating EDTA solution to disrupt the structural matrix of bone (Loreille et al. 2007). Resulting sequestered divalent metal cations (Ca^{2+}) 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, whereas lysis buffer C is commonly used in forensic casework

and is prepared in our laboratory. Lysates were purified with the commercially available solid phase purification kit, QIAamp® DNA Mini Kit (Qiagen Hilden, DE). Purified extracts were quantified using a human mitochondrial DNA quantitative PCR (qPCR) assay (Kavlick *et al*. 2011).

Preliminary data suggests that buffer A performs the most consistently and often yields higher DNA recovery from bone samples than the other buffers studied, although additional work is needed to further refine these results. Future method development will include evaluation of different purification methods including 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.

Coble MD, Just RS, O'Callaghan JE, Letmanyi IH, Peterson CT, Irwin JA, Parsons TJ. Single nucleotide polymorphisms over the entire mtDNA genome that increases the power of forensic testing in Caucasians. *Int J Legal Med* 2004;118:137–46.

Kavlick MF, Lawrence HS, Merritt RT, Fisher C, Isenberg A, Robertson JM, Budowle B. Quantification of human mitochondrial DNA using synthesized DNA standards. *J Forensic Sci* 2011;6:1457-63.

Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. *Forensic Sci Int: Gen* 2007;1:191–5.