B175 High Efficiency DNA Extraction From Bone by Total Demineralization

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After attending this presentation, attendees will learn about bone extraction and how to get better STR profiles from bones.

This presentation will impact the forensic community and/or humanity by demonstrating how this new demineralization protocol can significantly enhance the quantity and quality of DNA extracted from degraded skeletal remains. This technique has great potential to recover authentic DNA sequences from extremely challenging samples that repeatedly failed when using the standard forensic protocol.

In historical cases, missing persons' identification, mass disasters, and ancient DNA investigations, bone and teeth samples are often the only, and almost always the best, biological material available for DNA typing. This is because of the physical and chemical barrier that the protein:mineral matrix of bone poses to environmental deterioration and biological attack. DNA is generally best preserved in dense cortical bone, and a recent study indicates that very high quality DNA may be locked away in small, extremely dense crystalline aggregates that are highly resistant to chemical infusion (Salamon et al). Evidence and reason both suggest, then, that the most abundant and best preserved DNA in bone is also the most difficult to access and extract.

Most bone extraction protocols utilized in the forensic community involve an incubation period of bone powder in extraction buffer for digestion, followed by the collection of the supernatant, and the disposal of large quantities of undigested bone powder (and unextracted DNA). Alternatively, some bone extraction methods utilize high volume EDTA washes to partly or completely demineralize the bone, resulting in more complete digestion of the bone powder. That DNA is also liberated, and discarded, during the washing stepshas been demonstrated.

Presented here is an extremely efficient means for recovery of DNA by full demineralization, resulting in full physical digestion of the bone sample. This is performed in a manner that retains and concentrates all the reagent volume, so that released DNA is recovered.

Fifteen bone fragments were extracted side-by-side with this new demineralization protocol and the standard extraction protocol in use at AFDIL. A real-time quantification assay based on the amplification of a 143bp mtDNA fragment showed that this new demineralization protocol significantly enhances the quantity of DNA that can be extracted and amplified from degraded skeletal remains. This technique has been used to successfully recover authentic DNA sequences from extremely challenging samples that failed repeatedly using the standard protocol. The better preserved samples were tested for STR analysis and the number of loci characterized almost doubled between this demineralization extract and the standard extract.

Reference:

Salamon M, T. N., Arensburg B, Weiner S, (2005). "Relatively well preserved DNA is present in the crystal aggregates of fossil bones." Proc Natl Acad Sci U S A. 102(39): 13783-8.

Bone, STR, Demineralization

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