B63 Sodium Phosphate Enhanced DNA Extraction From Bone

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After attending this presentation, attendees will have learned a rapid alternative method to extract DNA from bone.

This presentation will impact the forensic community and/or humanity by demonstrating improved methods of dealing with the calcified matrix from compact bone during DNA extraction will be useful for forensic practitioners in human identification.

The forensic community frequently uses bone tissue for DNA typing the remains of missing persons, crime victims, and victims of mass disasters (Hochmeister et al. 1991, Hagelberg et al. 1991, Gill et al.1994, Holland et al. 2003). Extraction of DNA from bone can be difficult due to a compact calcified matrix combined with a relatively low cellular content. Consequently, improved methods of dealing with bones' calcified matrix during DNA extraction should prove beneficial to assist forensic practitioners in human identification.

The predominant inorganic component of bone is hydroxyapatite (HA), a stable calcium phosphate compound ([Ca5(PO4)3OH]2) that is the major contributor to bones' structure and compact matrix. Nucleic acids have a strong affinity for HA that has been utilized for chromatographic separation and purification of nucleic acids (Bernardi 1965). Since DNA likely binds endogenous HA of bone following cell lysis, several current forensic protocols include extensive incubations in a high concentration of EDTA to alter this interaction (Hagelberg et al. 1991). While this process improves the DNA recovery in many cases, decalcification extends the extraction protocol and requires the removal of EDTA, a potent PCR inhibitor, prior to PCR amplification. With this in mind, alternative methods to disrupt the interaction between DNA and HA during the extraction step were explored.

The affinity between DNA and HA can be regulated using various phosphate buffers (Sambrook and Russell 2001). Historically, the manipulation of phosphate concentrations has exploited HA as a method of purifying and separating DNA from various samples. Studies from the anthropological community first applied this technique to DNA extraction by using phosphate buffers on prehistoric bone (Persson 1992). This was later supported by a comparative study of several extraction buffers, in which phosphate buffer was the most successful in extracting DNA from both synthetic HA and bovine bone samples (Götherstrom & Lidén 1996).

In this study, a faster, alternative method of disrupting the interaction between DNA and HA was pursued. By incorporating high concentrations of sodium phosphate (NaP) in the extraction buffer, the binding of DNA to the endogenous HA of bone may be blocked or reversed. Preliminary work showed that this extraction method is suitable for downstream mitochondrial DNA (mtDNA) analyses and can be completed in as little as two hours. More in depth studies included optimization of NaP concentration and DNA purification conditions. Depending on the bone, this extraction method can yield a 2,000-fold increase in mtDNA copy number concentration when compared to protocols that do not disrupt the HA-DNA interaction. In addition, initial work indicates that STR profiles can be obtained from these extracts by optimizing PCR conditions. In summary, application of this protocol to forensic science has the potential to dramatically reduce the amount of time required to isolate DNA from bone samples, increase mtDNA yields, and possibly improve chances of obtaining STRs from skeletal remains.

mtDNA, Extraction, Skeletal Remains

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