B9 Improvements in DNA Extraction From Bone

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After attending this presentation, attendees will learn of improve- ments in DNA extraction from bone.

This presentation will impact the forensic community and/or humanity by identifying improvements in DNA extraction methods.

The ability to extract DNA from various tissue types has improved over time, although some tissues continue to present challenges. Extraction of DNA from bone can be especially difficult due to a compact calcified matrix combined with a relatively low cellular content. Interestingly, the same compact calcified matrix feature that complicates DNA extraction from bone also presents a stable and durable tissue that remains intact in environments where no other tissue types survive. For example, ancient skeletal remains recovered from archeological sites often provide the only resource for possible DNA analyses. Similarly, the forensic community frequently uses bone for DNA typing the remains of missing persons as well as those of crime victims. Because of bone's permanence, improve- ments in DNA extraction protocols from bone are tremendously useful for forensic practitioners.

The predominant inorganic component of bone is hydroxyapatite (HA), a stable calcium phosphate compound that contributes to bone's structure. Nucleic acids have a strong affinity for HA, and DNA likely binds to the HA of bone following cell lysis. To minimize the loss of DNA, current protocols remove the HA prior to DNA extraction using extensive incubations in a high concentration of EDTA to decalcify the bone. While decalcification with EDTA does improve DNA recovery from bone, the process extends the extraction protocol a minimum of eight hours and requires the removal of this potent PCR inhibitor prior to amplification. Furthermore, there is the possibility of DNA loss during this EDTA incu- bation in some bone samples, particularly with extended periods of incu- bation. As an alternative to this lengthy decalcification process using EDTA, the possibility of interfering with the interaction between DNA and HA during the extraction step was investigated in this study.

The affinity of the DNA/HA complex can be regulated using various phosphate buffers. Molecular techniques have been established in which DNA elutes from an HA matrix when sodium phosphate (NaP) concentra- tions exceed a specific threshold. For example, Sambrook and Russell report that double stranded DNA couples tightly to HA and requires phos- phate concentrations in excess of 0.4 M for elution.[1] Alternatively, buffers containing sodium fluoride (NaF) may be used to alter the interaction between DNA and the HA matrix. Using this information as groundwork, a modification to DNA extraction protocols from bone that reduces the time required for extraction, while simultaneously maintaining or potentially increasing the yield of DNA recovered, is reported. By adding NaP or NaF to the extraction buffer, the binding of DNA to the endogenous HA of bone is effectively blocked. Consequently, 500 mM of NaP or NaF mimics the effects of decalcification without extensive EDTA incubations prior to DNA extraction. Preliminary results show that this method is appropriate for downstream nuclear and mitochondrial DNA analyses. In summary, this protocol, which requires as little as two hour extraction incubations, dramatically reduces the amount of time required to isolate DNA from bone samples with a potential increase in DNA yield.

References:

1. Sambrook, J. and Russell, D. in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press; 3rd edition (2001)

mtDNA, Extraction, Hair

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