A37 DNA Isolation and Analysis From Skeletal Remains: Novel Methods for Removing PCR Inhibitors

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After attending this presentation, attendees will appreciate common difficulties encountered during DNA analysis of skeletal remains and understand the extent to which soil microbial DNA isolation kits can act as an advantageous extraction method for buried skeletal remains. Attendees will also be informed about which DNA extraction methods are most useful for recovering amplifiable DNA from buried human skeletal remains and removing Polymerase Chain Reaction (PCR) inhibitors.

This presentation will impact the forensic science community by disseminating whether microbial DNA isolation kits are more effective at purifying DNA away from PCR inhibitors than are commonly used DNA isolation methods, as these kits have never been assessed for their effectiveness in extracting clean DNA from skeletal remains.

DNA analysis of skeletal remains is crucial in order to identify missing persons, victims of war, and individuals in cases of mass disaster. Unlike reliable sources of DNA such as buccal swabs, challenges arise with skeletal DNA analysis for numerous reasons. The harsh conditions skeletal remains are often recovered from are not conducive to DNA preservation, such as wet environments or the heat of a fire. The presence of PCR inhibitors is also a common hindrance with skeletal remains, particularly if they have been buried or are otherwise in prolonged contact with soil. Likewise, components of the bone itself, such as calcium or collagen, can inhibit PCR, and thus the removal of PCR inhibitors during DNA extraction is critical for successful forensic DNA analysis.

Previous researchers have compared DNA extraction methods from skeletal material, including standard phenol-chloroform organic extraction and commercially available kits. In these studies, the kits had low DNA recovery, instances of PCR inhibition, and resulted in poor quality STR profiles.[1, 2] Organic extraction recovered higher quantities of DNA; however, this method also resulted in PCR inhibition.[2] Although commercial DNA isolation kits are easy to use and claim to remove PCR inhibitors, none have been optimized for the highly compromised skeletal remains that are often encountered by forensic scientists, which is presumably why they have been found lacking for skeletal analyses. This led to the question of whether commercially available kits that are specifically designed to isolate and purify DNA from soil samples that are high in PCR inhibitors such as humic and fulvic acids might be advantageous when testing buried skeletal remains.

In the research presented here, the ability of microbial DNA isolation kits to recover amplifiable bone DNA and remove PCR inhibitors was compared with other common extraction methods. DNA extraction systems included a PowerSoil® DNA Isolation Kit (MoBio), a SoilMaster™ DNA Extraction Kit (EpiCentre), a standard organic extraction, and a QIAamp® DNA Investigator kit (QIAGEN), which has a specific protocol for DNA isolation from bone. Since the soil kits are not designed for extraction of human materials, a preliminary study was conducted to determine if reagents contained in the kits were contaminated with human DNA. Blank extracts from the soil DNA kits failed to amplify with human mtDNA primers. Next, DNA extractions were performed on bone powder obtained from drilling cow femur segments to determine whether the standard protocols supplied with each are adequate for DNA isolation from bone. The PowerSoil® kit standard protocol did not consistently result in amplifiable DNA. The protocol was then optimized by altering the mechanical/chemical digestion step, including substituting a hot lysis for the mechanical digestion.

In addition to DNA recovery, each extraction method was tested for its ability to remove the PCR inhibitors calcium, collagen, and humic acid, which are associated with buried skeletal remains. Inhibitor removal was assessed by amplification or no amplification of control mitochondrial DNA, by adding the purified extract to the reaction. Each extraction method was then used on bone powder produced by drilling cow femur sections buried in soil for a range of time: one day, seveb days, thirty days, and three years. DNA was quantified by a real-time PCR TaqMan assay targeting the Melanocortin-1 Receptor gene, developed by Lindquist *et al.*[3] Inhibition was assessed by comparing cycle threshold values of the internal positive control. Since failure to amplify DNA is a common challenge encountered with skeletal remains, successful amplification of both mitochondrial and nuclear DNA was compared for each extraction method to see which recovered amplifiable DNA more often. The four extraction methods were then tested on various human skeletal remains, including bones from the medieval period, which had previously shown PCR inhibition during DNA analysis. After comparing the microbial DNA isolation kits to organic extraction and a standard DNA extraction kit for inhibitor removal, quantity of bone DNA recovered, and success of mitochondrial and nuclear DNA amplification, the effectiveness of microbial DNA extraction kits for use on skeletal remains was determined, as well as the most optimal extraction method.

References:

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DNA, Skeletal Remains, PCR Inhibition

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