B148 DNA Analysis From Human Skeletal and Tooth Remains: A Comparison of the Recent Isolation Methods for Removing Polymerase Chain Reaction (PCR) Inhibitors

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Learning Overview: The goal of this presentation is to offer best-practice procedures for the isolation of DNA and removal of PCR inhibitors from human burned remains and to recover DNA useful for identification.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing a predictive model to determine which incinerated human remains are likely to produce the best results for the removal of PCR inhibitors during extraction procedures.

DNA analysis of human bone remains is a crucial process for identifying missing persons and individuals in cases of mass disasters and wars. In this study, the ability of microbial DNA isolation kits to recover amplifiable bone DNA and remove PCR inhibitors was compared to other common extraction methods. The generation of a DNA profile from bone samples is an important part of the identification process, both in cases of mass disasters and in cases of unidentified persons.[1] As bones and teeth are often the only biological evidence remaining after exposure to challenging environmental conditions, intense heat, certain traumatic events, and in cases in which a significant amount of time has passed since the death of the individual, the ability to purify reasonable amounts of DNA from such hard tissues is always beneficial.[2,3]

Because sampling procedures from hard tissues for genetic analysis is a destructive process, it is important to understand the environmental and intrinsic factors that will contribute to the preservation of DNA.[4] DNA extraction systems consisted of several methods and kits, including a standard organic extraction and a QIAamp® DNA research kit. A preliminary study was conducted to determine whether the reagents contained in the kits were contaminated with human DNA. The standard protocol did not result in consistently amplifiable DNA. The protocol was optimized by altering the digestion step. In addition to DNA recovery, each extraction method was tested to determine its ability to remove the calcium, collagen, and humic acid PCR inhibitors associated with buried and burned skeletal remains. Since lack of DNA amplification is a common challenge encountered with bone debris, successful amplification of nuclear DNA for each extraction method was compared to see which amplifiable DNA was most frequently recovered. The five extraction methods were tested on several remains of human skeletons, including bones and teeth, that had previously shown inhibition of PCR during DNA analysis. After comparing the human DNA isolation kits with organic extraction and a standard DNA extraction kit for inhibitor removal, the amount of bone DNA recovered and the success of nuclear DNA amplification, the effectiveness of the DNA extraction kits for use in bone remains was determined, as well as the most convenient extraction method. Improving the ability to interpret DNA results from challenging samples will be an important aspect of the future of the field. Finding ways to reduce the cost of genetic analyses will reduce delays and allow more samples to be processed. Finally, adequate training and funding must be provided to recognize that sound research based on the scientific method is the key to advancement in any field of forensic sciences.

Reference(s):

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4. Gamba, Cristina et al. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications* 5 (2014): 5257.

Burned Bone, Burned Teeth, Forensic Identification

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