A56 Evaluating the Applicability of Direct Polymerase Chain Reaction (PCR) Techniques to Samples from Human Skeletal Remains

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After attending this presentation, attendees will understand the possibility of using direct polymerase chain reaction (PCR) methods to perform genetic analysis on human skeletal remains. The ability to conduct direct PCR would potentially reduce the amount of biological sample destroyed for DNA isolation, as well as decrease the time and cost constraints of creating a genetic profile from skeletal DNA for positive identification purposes.

This presentation will impact the forensic science community by introducing a new tool that can potentially be employed in the positive identification of human skeletal remains via DNA analysis. It is important that the forensic community be aware of such advances that can decrease the amount of valuable biological evidence destroyed in attempts at producing a genetic profile.

Positive identification of skeletonized human remains using DNA analysis is becoming more and more frequent within the forensic community. While the forensic anthropologist plays an important role in estimating the descendant's biological characteristics such as age, sex and ancestry, as well as other essential tasks like ascertaining perimortem trauma, estimating the postmortem interval and identifying factors that have altered the remains since the time of death, the actual positive identification of human skeletonized remains is often the job of the forensic odontologist or DNA analyst. The process of obtaining analyzable DNA from bone has traditionally been a destructive, timely and costly process. While the starting amount of osseous material required for DNA analysis has decreased significantly over the past decade, many forensic DNA labs still require one or two whole bones from the decedent for analysis. As an expert in the human skeleton, the forensic anthropologist is often consulted regarding the appropriate bones or amount of bone to be submitted for DNA analysis.

The isolation of DNA from human cells entails the disruption of the structural components of the cells enclosing the nuclear and mitochondrial DNA. This process is simpler in a soft tissue cells in which the components are mostly fluid and membranous. The process becomes increasingly difficult in bone cells as the structural components also consist of a calcified extracellular matrix. Isolation of skeletal DNA has traditionally required the disruption of the inorganic hydroxyapatite structure of the osseous tissue, followed by a disruption of the organic collagen component of the osseous tissue, and finally the purification of the genetic material. Dependent upon the particular lab protocols, kits or chemical reagents used the process can require several grams of powderized bone, several weeks of preparation and hundreds of dollars

per sample. Recent advances in DNA analysis from soft tissue have led to the development of direct PCR techniques in which a microscopic piece of tissue, with no prior DNA purification, could be added directly to a tube prepared for PCR amplification. Such a scientific advancement saves copious amounts of time, expense and sample destruction.

This pilot experiment was designed to test the Finnzymes Phire® Animal Tissue Direct PCR Kit (New England BioLabs) using human bone as a source of template DNA. The study employed bone samples excised from two temporal periods: a modern bone from a recent death and an historic bone dating to a 19th century Euro-American cemetery. A small piece of bone (less than 0.2 grams) was powderized, and several grains of bone powder were directly added to the PCR tube. PCR amplifications targeted both a nuclear DNA locus and a mitochondrial DNA locus, as both are commonly employed in forensic DNA analyses. The direct PCR technique was found to amplify both loci from both temporal samples, although the results were not consistently repeatable. The preliminary results indicate that while it is possible to employ direct PCR techniques to human skeletal material it still requires significant optimization. **Skeletal DNA, Direct PCR, DNA Amplification**

 ** Presenting Author*