H39 DNA Quantification of Burned Skeletal Tissue

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After attending this presentation, attendees will understand the effects of temperature on gross morphology and DNA degradation in bone. The study will in turn provide an aid in producing a standardized protocol for extracting and processing DNA from burnt bone.

This presentation will impact the forensic community by demonstrating high temperatures promote DNA degradation in skeletal tissue and that DNA is unlikely to be extracted from bone exposed to temperatures above 200°C. The use of PTB in DNA extraction has shown to increase DNA yields. PTB is a potential agent in increasing DNA yields for DNA profiling from small bone samples.

Forensic anthropologists are often enlisted to utilize investigative tech- niques for the identification of human remains. Identifying human remains using conventional anthropological methods does not necessarily produce a successful positive identification. In such cases, other techniques, including DNA typing, may be pursued. Recovered human remains may have become too fragmented or may have lost their integrity for successful physical iden- tification or may be accompanied by soft tissues that are too decomposed for DNA typing. As the only viable sample that may be available for iden- tification, skeletal tissue is an extremely important source of DNA. Identi- fying individuals using DNA profiles derived from bone samples is becoming increasingly important and has been used to successfully identify victims from a wide range of scenarios including natural disasters, terror attacks and armed conflicts. Like all forensic samples, skeletal tissue can be recovered from a wide variety of environments. After death, nucleic acids can undergo spontaneous degradation, which can be accelerated by envi- ronmental factors including pH, temperature and other chemical exposure. These can all hinder DNA profiling. It is well documented that high temper- atures promote DNA degradation in bone. However, the literature concerning DNA typing from skeletal tissue exposed to high temperature has thus far been inconclusive and lacks suitable controls. This study will compare two different DNA extraction techniques and determine the correlation between DNA degradation and temperature. This study will in turn provide an aid in producing a standardized protocol for extracting and processing DNA from burnt bone and establish potential links with gross morphology as a possible predictor of quality and quantity of DNA that can be extracted.

Using *Bos taurus* as an animal model, eight radii were burnt using a muffle furnace at 100 °C, 200°C, 350 °C and 500 °C for one and three hours. An untreated sample of fresh radii was used as a control. Using two different extraction techniques, one using phenol: chloroform: isoamyl alcohol and the other using phenol: chloroform: isoamyl alcohol plus N-phenacylthia- zolium bromide (PTB), DNA was extracted from each sample and compared. Samples were then semi-quantified on an agarose gel with appropriate ladders.

From the results it is clear that an increase in temperature and time decreases the volume of DNA that can be extracted. High molecular weight DNA was extracted from untreated samples and samples that were exposed at 100°C for both one and three hours. Though lower molecular weight DNA was extracted from samples exposed to 200°C for one and three hours there was a distinct decrease as the exposure period increased. DNA from samples that have been exposed to temperatures of 350°C and 500°C for both one and three hours could not be extracted suggesting that the majority of DNA has been degraded. There was also a clear difference in gross morphology. Samples where DNA was extracted had little color change and intact integrity, while those where DNA extraction was unsuccessful had charcoal appearance and lateral cracking.

There was also a difference in the volume of DNA extracted when using the two different protocols. PTB is an agent that cleaves advanced-glycation end-products (AGEs) and results from nonenzymatic glycosylation. Glucose and other reducing sugars have been shown to react nonenzymatically with DNA and have been shown to affect both the physical and biological prop- erties of DNA. AGE formation increases with age and temperature and may explain the fact that, of all the samples where DNA was extracted, the yield of DNA was increased when using PTB.

This study has shown that temperature has a detrimental effect on DNA degradation. It has been seen to increase DNA yields from those where DNA was extant. PTB therefore may be an ideal reagent to increase DNA yields from small sample sets.

DNA, Bone, Temperature

 ** Presenting Author*