H7 The Utility of Baking Bone to Increase Skeletal DNA Yield

Megan E. Madonna, BS, 1216 E Castle Avenue, Indianapolis, IN 46227; Krista E. Latham, PhD, University of Indianapolis, Biology Dept, 1400 E Hanna Avenue, Indianapolis, IN 46227; and Stephen P. Nawrocki, PhD, University of Indianapolis, Archeology & Forensics Lab, 1400 E Hanna Avenue, Indianapolis, IN 46227-3697*

After viewing this presentation, attendees will understand whether artificially baking bones will increase skeletal DNA yields obtained during the skeletal DNA extraction process and whether baking bones impacts the quality of the DNA required for subsequent genetic analysis.

This presentation will impact the forensic science community by contributing information on a new technique aimed at increasing DNA yields obtained during the skeletal DNA extraction process. The ability to increase skeletal DNA yield by artificially heating the bone may provide the analyst with a greater DNA yield for subsequent genetic analyses and identification efforts.

It is well established that DNA can be extracted from both soft and hard tissues. Once the body dies the cells and cellular components, including the DNA, begin to degrade. The body's soft tissues will decompose at a faster rate than the osseous tissues resulting, in many cases, in the skeleton being the only biological material remaining at the crime scene. For this reason, the skeleton is an alluring target for genetic analyses aimed at individual identification. However, isolating DNA from osseous tissue can be challenging. On the one hand, bone affords some protection to the skeletal DNA by providing a physical barrier to environmental agents that could potentially act to degrade the molecular material. On the other hand, the skeletal DNA becomes chemically bound to the inorganic portion of the bone and this chemical modification makes skeletal DNA more difficult to purify than soft tissue DNA. There is a growing body of research aimed at improving DNA yields obtained during the skeletal extraction process. While heat tends to negatively impact biological preservation at both the gross and molecular levels, recent information suggests heating bone may be beneficial in increasing skeletal DNA yields by making the osseous material more brittle and therefore better able to release the DNA bound to the mineral phase. However, this hypothesis had never been systematically investigated until now.

This research utilized a sample of seven dry (unmacerated) pig femora of adult or near adult age collected from the ground surface of an Indiana farm. Ten small 1x1cm squares of cortical bone were cut from each shaft, resulting in a final sample size of 70 skeletal DNA extractions. Nine samples from each individual were baked in a mechanical airflow oven at 90°C for different increments of time (3, 6, 9, 12, 18, 24, 36, 48, and 72 hours). The last segment of each bone served as an unbaked control to standardize for possible variation in the starting levels of DNA. After extraction, the DNA yields were quantified and the extracted DNA was Polymerase Chain Reaction amplified to determine DNA quality. PCR amplification employed pig-specific DNA primers that target nuclear Short Tandem Repeats (STRs). Pig STRs were targeted primarily because they span a region that consists of a variable number of repeating units similar to the human STRs that are routinely used in forensic DNA analysis. Several null hypotheses were tested: (1) DNA yield from baked bones will not differ from those that are unbaked; (2) DNA yield is not correlated with accumulated heat as measured by Accumulated Degree Hours (ADH); and, (3) PCR success is not dependent on accumulated heat.

Results indicate that artificially heating bones by baking, on average, increases skeletal DNA yield compared to the unheated controls. A *t*-test was used to determine whether yield from baked bones differed from the unbaked sample and was found to be significant (t= -3.81, p=0.027). After plotting the mean yields versus time, a pattern of increasing yields was evident as heating time increased. A Pearson's correlation between quantity of extracted DNA and ADH was significant as well (r= 0.298, p=0.016). However, a drop-off point at which heating became detrimental was not evident. Lastly, a Spearman's correlation used to test whether there was an association between heating and PCR success was not significant. This research has found that there may be some benefit in baking bones before skeletal DNA extraction.

Skeletal DNA, Bone Baking, DNA Yield

 ** Presenting Author*