A135 Best Practice Procedures for Sampling Differentially Burned Bone for Successful DNA Recovery

Katelyn L. Bolhofner, PhD, Texas Tech University, Lubbock, TX 79410; Matthew V. Emery, PhD, Arizona State University, Tempe, AZ 85281; Jane E. Buikstra, PhD, Arizona State University, Tempe, AZ 85287-2402; Laura C. Fulginiti, PhD, Forensic Science Center, Phoenix, AZ 85007; Anne Stone, PhD, School of Human Evolution & Social Change, Tempe, AZ 85287-2402*

Learning Overview: The goal of this presentation is to offer best-practice procedures for the recovery of skeletal samples from burned human remains 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 for determining which samples from incinerated skeletal remains are likely to produce the best results for Short Tandem Repeat (STR) testing, mitochondrial genome sequencing, and genome-wide Single Nucleotide Polymorphism (SNP) analysis.

Forensic anthropologists are often asked to evaluate burned human remains resulting from a variety of circumstances. When seeking to determine the identity of individuals found in these scenarios, DNA recovery can provide essential information. However, fragments of burned remains often are not amenable to standard DNA recovery and identification, and it is difficult to predict which samples would be most successful in producing useful results. To maximize resources and minimize the destruction of remains, establishing the conditions under which burned bones are most likely to produce DNA is crucial. By examining variables such as heat-induced color change and type/location of element sampled, the research presented here addresses these issues by offering new standards for the recovery of skeletal samples from burned human remains.

Previous experimental studies have reported that bone changes color in a predictable sequence in response to the duration of exposure to heat. This progression is associated with increased fragmentation and loss of collagen, making the bone increasingly not amenable to DNA preservation and recovery. However, some researchers have argued that DNA can be recovered from even calcined bone. Further complicating the sampling strategy for burned skeletal elements is that many burned remains show variable levels of heat alteration across the body. To assess how different degrees of charring/burning affect DNA recovery, this study obtained and documented 80 samples from 27 fire death and cremation cases. Samples were chosen with the further goal of obtaining statistically significant results from different skeletal elements. For remains that were burned only on the extremities, samples were taken from affected long bones with at least two different levels of burning as well as unburnt tissue (n=32 samples). In cases in which the cranium was burned, where possible the petrous portion of the temporal bone, the cranial vault, and the dentition were sampled (n=14 cases). In severely burned cases in which the remains were calcined, a range of skeletal elements were sampled (n=11 cases).

As this research also explores the degree to which visible markers upon thermally altered human remains can be used to predict DNA preservation, the skeletal samples collected were classified into five levels of burning recorded by Munsell Color Charts (I: well-preserved, unburnt; II: semi-burnt, black/brown; III: black burnt; IV: blue-gray burnt; and V: blue-gray-white burnt). For the dental sample, the published classification system (I: well-preserved, unburnt; II: carbonized, black; III: brown/olive; IV: gray; and V: calcined white) was employed.[1] From these elements, DNA was successfully extracted from 68 samples (including blanks) using two different DNA extraction protocols: one currently used to obtain DNA from ancient remains, and another commonly used in forensic DNA analysis.[2,3]

Overall, skeletal elements belonging to burn levels I through III produced the highest yields. Using the total demineralization forensic protocol, those samples in level V yielded no useful DNA. However, the ancient DNA-based extraction technique resulted in recovery of DNA across all five burn levels, demonstrating that DNA may be recovered from calcined bone in some cases with this method. The parietal bone, tibia, metatarsal, metacarpal, and phalanges produced sufficiently high DNA yields (>/=1ng/uL) for STR genotyping and next-generation sequencing analysis. Of these elements, the highest yields were obtained from the parietal bone. Contrary to previously published research, the petrous portion of the temporal produced very low yields, as did the dentition sampled.[4]

The results of this study will further the ability of forensic scientists to target those samples from burned human remains most likely to produce high DNA yields, thus increasing the likelihood of positive identification of individuals in these cases.

Reference(s):

1. Symes S.A., L'Abbé E.N., Pokines J.T., Yuzwa T., Messer D., Stromquist A., and Keough N. 2014. Thermal Alteration to Bone. In: Pokines J.T., and Symes S.A., editors. *Manual of Forensic Taphonomy.* Boca Raton: CRC Press. p 367-402.
2. Dabney J., Knapp M., Glocke I., Gansauge M.T., Weihmann A., Nickel B., Valdiosera C., Garcia N., Paabo S., Arsuaga J.L., et al. 2013. Complete Mitochondrial Genome Sequence of a Middle Pleistocene Cave Bear Reconstructed From Ultrashort DNA Fragments. *Proc Natl Acad Sci USA.* 110(39):15758-15763.
3. Loreille O.M., Parr R.L., McGregor K.A., Fitzpatrick C.M., Lyon C., Yang D.Y., Speller C.F., Grimm M.R., Grimm M.J., Irwin J.A., et al. 2010. Integrated DNA and Fingerprint Analyses in the Identification of 60-Year-Old Mummified Human Remains Discovered in an Alaskan Glacier. *Journal of Forensic Sciences.* 55(3):813-818.
4. Gamba C., Jones E.R., Teasdale M.D., McLaughlin R.L., Gonzalez-Fortes G., Mattiangeli V., Domboroczki L., Kovari I., Pap I., Anders A., et al. 2014. Genome Flux and Stasis in a Five Millennium Transect of European Prehistory. *Nature Communications.* 5:5257.

Burned Bone, Cremated Bone, Identification