B131 Effect of Skeletal Sampling Technique on DNA and Elemental Analysis Results

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After attending this presentation, attendees will understand the relationship between skeletal sampling technique and recovery of nucleic acids and elemental materials and the reduction in the quality of Short Tandem Repeat (STR) results due to DNA damage from laboratory processing and Polymerase Chain Reaction (PCR) inhibition from co-isolated elements.

This presentation will impact the forensic science community by demonstrating the effect of skeletal sampling technique on the DNA recovered and mineral constituents co-isolated from DNA extraction of human skeletal remains. Presented data include measurement of DNA quality and quantity, genetic analyses, and determination of elemental contents.

The goal supported by this work is to identify more people from skeletal remains by developing improved procedures for extracting DNA from bone samples. Bones are considered one of the most challenging sample types due to laborious laboratory processing that may result in incomplete genetic results or amplification failure. Causes of poor results may include DNA damage, inhibition of PCR, and/or inefficient extraction. These experiments will determine whether skeletal sampling technique contributes to poor quality DNA analysis results and resulting data will provide a foundation for the development of improved procedures to extract DNA from skeletal remains.

Cadaver long bones were obtained through the Willed Body Program at the University of North Texas (UNT) Health Science Center and samples were cut from the diaphysis of a left tibia. Three extraction sets, each comprised of five samples and a reagent blank, were tested for DNA and elemental contents. The three sets included the following: whole bone pieces weighing between 0.837 and 0.935 gram, numerous bone cuttings with a total sample weight between 0.887 and 0.915 gram, and pulverized samples weighing between 0.905 and 0.912 gram. DNA extraction was performed using the UNT Center for Human Identification "Demineralization Extraction of Skeletal Remains" protocol, a modified version of the Loreille et al. method.[1] Samples were weighed and placed into tubes. Demineralization buffer and proteinase K were added to each sample and incubated overnight. An equal volume of Phenol-Chloroform-Isoamyl Alcohol (PCIA) was added and samples were vortexed and centrifuged. A 1mL aliquot was removed from the aqueous phase for ethanol purification and subsequent testing and the remaining aqueous phase was transferred to a centrifugal filtration device. Samples were centrifuged through the device and the retentate was purified using a chaotropic salt solution and a centrifugation column.

Two fractions were examined: the aqueous phase following PCIA purification, subsequently purified with ethanol; and the fully purified samples, consisting of the retentate from the centrifugal filtration and purified using a second column. Contents of the aqueous phase formed crystals as a result of ethanol purification. These samples were dissolved in TE^{-4} buffer and the DNA content evaluated using a microfluidic platform. The crystals were again formed with ethanol and examined by X-ray diffraction. Fully-purified samples were tested for DNA content using the following methods: sizing on a microfluidic platform in duplicate; two qPCR DNA quantification kits in triplicate; STR amplification in triplicate; and, genetic analysis on two models of capillary electrophoresis instruments. Elemental analysis of both fractions was performed using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS). Baseline and subsequent analyses were performed in solution mode ICP/MS. Acid digestion was used for baseline sample preparation; approximately 100mg of sample was dissolved in concentrated nitric acid (HNO_3), and then diluted with 1% HNO_3. Dilution factors varied between 20 and 100 for aluminum, calcium, copper, iron, nickel, and lead. All samples were measured in triplicate.

Results were successfully obtained for each assay attempted. DNA quantification yields for pulverized bone were 16.7 to 93.3 times higher than whole or cut bone pieces; however, STR profiles obtained for the cut bone pieces were superior to whole or pulverized bone samples. STR profile quality was assessed by determining the percent of fluorescence exhibited by the highest molecular weight allele versus the lowest molecular weight allele for a dye channel. The range of percent recovery of the highest molecular weight allele for whole bone was 1.72% to 35.66%, cut bone was 4.82% to 49.79%, and pulverized bone was 0.96% to 18.05%.

Reference:

1. Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. *Forensic Sci Int Genet*. 2007 Jun;1(2):191-5.

Skeletal Remains, DNA Extraction, Elemental Analysis