B97 Methods for Accurate STR Sex Typing of Ancient Bone and Tissue Samples

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The goal of this presentation is to discuss current methods including two new ones for generating sex markers for ancient bone and muscle.

This presentation will impact the forensic science community by stimulating thought and discussion as to problems and issues observed with different methods.

The Smithsonian Institution provided two ancient bone samples and two ancient muscle samples. These samples were taken from skeletons found within a cave in the southern Mongolian Gobi desert. Radiometric dating revealed the bones originated between AD 1270 and 1400. The first sample, designated 1-G, consists of muscle tissue from the mid-shaft of the right humerus. Sample 1-G represents the lower portion of a skeleton and, based on position, matches with the skeleton of the second muscle tissue sample, designated 1-A/D. Sample 1-A/D is from the distal end of the right femur from a skeleton consisting of an upper body with male characteristics, including a penis. Sample 1-G, on the other hand, was determined to have derived from a female skeleton based on examination of the pelvic region. The two bone samples, labeled 1-D-1 and 1-D-2, are rib fragments from a skeleton determined to be female based on morphological characteristics. The purpose of DNA testing in this presentation was to clarify the gender of the sample and to use 1-D-1 as a control female sample.

Samples 1-G and 1-D-1 were pulverized using the SPEX Freezer/Mill 6770. The Freezer/Mill 6770 freezes the samples and then crushes the material to a fine powder. A forensic DNA extraction kit by Invitek (Invitek, Germany) was used to obtain DNA for amplification following the manufacturer's protocol. Additionally, longer digestion times and double the volume of the bone lysis enhancer were used to obtain more DNA fragments. DNA profiles were obtained using either the Identifiler or Minifiler STR amplification kits (Applied Biosystems, Inc., Foster City, CA) and detected with an ABI 310 DNA Sequencer and GeneMapper software (Applied Biosystems, Inc., Foster City, CA).

Identifiler did not provide any useful information about the DNA extracted from sample 1-G regarding sex typing. Minifiler, on the other hand, revealed a large X allele peak with a very small Y allele peak for the amelogenin locus. Based on the relative amplitude (RFU) of the peaks, it is probable that the presence of a Y allele is due to surface contamination. The next step for this problem is to type the Y-chromosome of male laboratory personnel and the sample with Y-Filer (Applied Biosystems, Inc.). The Y allele peak was so small compared to the X allele peak that it is likely the sample is derived from a female skeleton.

When additional PCR cycles were added to the process, the 1-G sample typed as exclusively female. This result confirms the results of anthropological analysis determination of sex. However, this raises some more anthropological questions since the upper portion of this skeleton contained a penis and male characteristics.

Minifiler results posed a similar problem for the bone sample 1-D- 1. The amelogenin locus revealed a large peak representing the X allele and a very small peak representing a Y allele. Further testing will be done to determine whether the Y allele is simply due to contamination or if the allele is actually an accurate representation of the STR profile. However, like sample 1-G, the sample is probably female since the X allele peak is much larger than the Y allele peak. Other possibilities include a mutation in the primer binding site which can be determined by DNA sequencing or an artifact of the Minifiler kit and the age of our samples.

Unlike DNA from bone samples, the DNA from the muscle tissue is more likely to contain contaminant DNA. The DNA of the bone samples is protected, and often sealed, from surface contamination due to collection and handling. Additionally, since the samples are hundreds of years old and the location of the cave is well known among nearby residents, the amount of potential surface contamination is infinite. With ancient samples it can be difficult to determine whether the results are true representations of the genetic profile. The authors are in the process of establishing a standard procedure for DNA typing of ancient muscle and bone samples using two new kits in combination with standard procedures such as grinding methods and extended PCR cycles. In addition, to obtain cleaner STR profiles, post-PCR purification steps will be implemented because it has been shown to significantly reduce background noise in low copy number DNA samples and may eliminate the small Y peak that we observed with Minifiler.

A combination of these new technologies and methods can provide enough information to more conclusively determine the sex of each sample and whether the two samples, 1-G and 1-A/D, were derived from the same skeleton and, subsequently, the same person. These technologies have a tremendous impact on the forensic community by providing useful information about difficult and highly degraded samples that have been nearly impossible to analyze in the past.

DNA Typing, Degraded DNA, PCR and Bone or Muscle

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