DEVELOPMENT OF AN IMPROVED METHOD FOR THE RECOVERY AND TYPING OF NUCLEAR DNA FROM HIGHLY DEGRADED BONES

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One of the primary missions of the Armed Forces DNA Identification Laboratory is to support the Central Identification Laboratory Hawaii (CILHI) in the identification of skeletal remains from previous military conflicts through mitochondrial DNA testing. One of CILHI's greatest challenges lies in the identification of more than 8100 servicemen still unaccounted for from the Korean War.

Between 1991 and 1995, two hundred and eight sets of skeletal remains were unilaterally turned over to the United States government by the Democratic People's Republic of North Korea. At the time of transfer, it was believed that these 208 sets of remains, termed the 'K208', represented 208 individuals. However, anthropological and preliminary mtDNA investigations revealed that multiple individuals were represented among any one set of remains, and that the remains of any single individual were likely spread among many sets. Based on further analysis of the skeletal elements represented, it is now believed that over 300 individuals are represented among the 'K208', and that the remains are widely commingled.

In terms of DNA testing, skeletal remains from the Korean War era are typically treated as mitochondrial DNA cases, due to sample degradation and the resulting difficulty with nuclear DNA typing. Unfortunately, the discriminatory power of mitochondrial DNA is limited for large batches of commingled remains. In this situation, there is strong potential for random mtDNA matches between bones of different individuals because common mtDNA types are often present. The problem is confounded when an unknown number of individuals are represented. Thus, in order to assist CILHI with the re-association of remains, AFDIL hopes to conduct both mitochondrial and nuclear DNA testing. For the unique mtDNA types encountered, skeletal re-association will likely be possible with mtDNA data alone. However, for the many cases in which shared common mitochondrial DNA types will be encountered, nuclear DNA typing would permit positive association among the skeletal elements. In these instances, nuclear DNA testing will be used to re-associate skeletons, and mtDNA testing will be utilized primarily to associate the remains with matching family references.

Thus far, standard mitochondrial DNA testing on the majority of 'K208' specimens submitted has been highly successful. Complete HVI/HVII sequence data has been generated in the majority of cases from relatively large (~250 bp) amplicons, suggesting that the recovery and typing of nuclear DNA may indeed be possible. As a result, the AFDIL research section has sought to establish a robust protocol for the recovery and typing of nuclear DNA from highly degraded bones.

Protocol improvement efforts have focused on three areas: extraction modifications, amplification modifications, and post-amplification modifications. Extraction modifications, including an extended de-calcification/dialysis step, have resulted in DNA recoveries up to 100 times greater than with a standard phenol chloroform protocol. The dialysis step results in complete demineralization of the bone, without the loss of DNA that occurs in many demineralization protocols. At the amplification stage, increases in Taq concentration and cycle number have resulted in improved STR profiles, and post-amplification modifications of both the PCR product and the Applied Biosystems 3100 injection time have improved data quality. Experiments to demonstrate reliability and reproducibility of the modified protocols are ongoing, and the latest results will be presented.