The Power of mtDNA Sequencing and auSTR and YSTR's to Identify Missing Service Members from Highly Comingled Remains

Timothy P. McMahon, PhD[1]; Suzanne Barritt-Ross, MS[1]; Suni Edson, MS[1]; and Christopher Los, MS[1]

Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, DAFB, DE 19902

Since the creation of the Armed Forces DNA Identification Laboratory (AFDIL) in 1992, AFDIL has endeavored to provide world-class DNA analysis to aid the Joint POW/MIA Command – Central Identification Laboratory (JPAC-CIL) in the identification of missing U.S. service members. Over the past 22 years, DNA processing methodologies have been constantly adapted and improved, to stay at the cutting edge of Forensic DNA testing. This presentation will show how mitochondrial DNA (mtDNA) sequencing and autosomal and Y-STR testing have been used to segregate and identify missing US service members from extensively comingled remains.

Between 1990 and 1994 the North Korean government unilaterally returned 208 sets of skeletal remains thought to be from US service members listed as Missing in Action (MIA) or Prisoners of War (POW). Mitochondrial DNA sequencing of the 208 sets of skeletal remains has: 1) identified 589 unique mtDNA sequences; 2) allowed for the segregation of remains by sequence and region; and 3) has demonstrated that the remains are comingled. However, many of the sequences matched the most common Caucasian HV1/HV2 mitotypes. This has hindered the identification of the minimal number of individuals represented in the 208 sets of skeletal remains as well as the identification of individual service members. The development and validation of a demineralization protocol in 2006 allowed AFDIL to: 1) reduce sample size by 20 fold; 2) completely dissolve all bone material; 3) recover both nuclear and mtDNA; and 4) develop, validate and implement more discriminating auSTR, YSTR and LCN-YSTR testing methodologies to aid in the identification of these extremely degraded skeletal fragments. The ability to utilize all three testing methodologies has been the key to segregation and identification of missing service members from comingled remains. However, the ability to use a specific testing method is dependent on what family references are available for comparison. The extraction and testing methods developed and optimized over the past 14 years for the segregation and identification of individuals from comingled remains are directly applicable for use in testing remains recovered from WWII where the specific loss may not be attributed to a single individual (multi-person air loss or mass grave) or a unilateral turn over with no context associated to the specimen.

In addition to utilizing conventional testing methods, AFDIL's Emerging Technology Section has developed and is currently validating a next generation (NGS) mtDNA hypervariable sequencing protocol that uses hybridization capture to generate sequencing results from Korean War unknowns whose remains where chemically modified by immersion in formaldehyde followed by aggressive mortuary practices prior to burial. This NGS method will be the first time that mtDNA sequencing results will be consistently produced from these highly degraded samples and is the culmination of a decade of work.