

## Modernizing DNA-Assisted Identification of Missing U.S. Service Members with NGS Technologies

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The Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL) performs DNA testing to assist with the identification of missing U.S. service members from current and past conflicts. The majority of AFMES-AFDIL casework supports the past accounting mission, which is largely focused on World War II and Korean War casualties. This type of historical missing persons casework is ill-suited for traditional forensic DNA analysis with autosomal STRs for two reasons. First, the samples are, predominantly, bone and tooth samples with endogenous DNA that is degraded, damaged, and plagued with environmental contaminants. Secondly, many of the missing service members from conflicts in the mid-twentieth century did not leave direct descendants; hence DNA reference sample donors are often one to two generations removed from the missing individual, and thus second- or third-degree relatives.

Consequently, lineage markers (mtDNA and Y-DNA) shared between distant kin have been the crux of DNA casework at the AFMES-AFDIL. MtDNA analysis can target small amplicons within the mitochondrial control region using Sanger sequencing methods. And low copy number amplification techniques for Y-STR testing has been implemented at the AFMES-AFDIL for enhanced detection. Although autosomal STR analysis is utilized when possible, it is limited to cases with requisite DNA preservation and appropriate family reference sample donors. Hence, to date, the vast majority of past accounting casework has relied on lineage markers as DNA evidence for their exclusionary power. DNA is used to support the anthropological analysis, historical records, and circumstantial evidence that together can lead to an identification.

The recent adoption of Next-Generation Sequencing (NGS) at the AFMES-AFDIL can modernize the use of DNA evidence in the past accounting mission. In 2016, NGS of the mitochondrial genome was implemented, thereby maximizing the discrimination power of the mtDNA locus. And now that the foundation has been laid for NGS workflows targeting degraded DNA, the laboratory can implement other assays that will enable distant kinship inference, such as genome-wide SNP analysis. By providing a kinship statistic from nuclear DNA, the DNA evidence alone can provide the statistical weight to support an identification. This unlocks the potential to shift the past accounting mission from a DNA-assisted effort to more a *DNA-centered effort* to provide the fullest possible accounting of the nation's missing service members.

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