B179 Comparison of DNA Repair Methods for Improved Success With Next Generation Sequencing (NGS) of Compromised Skeletal Remains

Erin M. Gorden, MFS, Armed Forces DNA Identification Lab, 115 Purple Heart Drive, Dover AFB, DE 19902; Charla Marshall, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; and Kimberly S. Andreaggi, MFS*, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902*

After attending this presentation, attendees will be aware of the existence of DNA damage in common forensic samples in both Sanger sequencing and NGS data. This presentation will address ways to overcome this damage using DNA repair kits.

This presentation will impact the forensic science community by demonstrating how DNA damage from common forensic samples (such as bones and teeth) can impact analysis of both Sanger and, to a larger degree, NGS results. This presentation will also exhibit the ability of various kits to repair the damage.

The Armed Forces DNA Identification Laboratory (AFDIL) was established with the primary purpose of employing DNA techniques to assist in the identification of remains of United States service members. The identification of compromised skeletal remains by the AFDIL's past accounting section is achieved by using a combination of analysis types: mitochondrial DNA (mtDNA), autosomal Short Tandem Repeat (STR), and Y-chromosomal Short Tandem Repeat (Y-STR) testing; however, the condition of skeletal remains, including exposure to environmental insults and chemical treatment, can present a challenge in obtaining results due to DNA degradation and Polymerase Chain Reaction (PCR) inhibition. NGS can aid in the identification process by yielding results for such damaged and degraded samples in a more cost-effective and high-throughput manner than current methodologies. For these reasons, NGS methods are currently being optimized and validated for degraded DNA casework at the AFDIL.

Several skeletal elements that spanned a quality range typically encountered in past accounting cases as well as appropriate controls were enriched for the mtDNA control region using mini-primer sets (125bp-180bp). Following PCR enrichment, library preparation was performed for sequencing on the Illumina® MiSeq®. The NGS data for the positive controls were of good quality and generated the expected profiles; however, erroneous variants were detected at nearly every position across the targeted regions for the low-quality casework samples. These errors were observed at variant frequencies as high as 30% and consisted mostly of GàA and CàT mutations, which is indicative of DNA damage due to cytosine deamination. Sanger sequence data confirmed the presence of DNA damage, but at a level that did not impact variant calling due to the qualitative nature of this type of analysis. Furthermore, a decrease in Guanine-Cytosine (GC) content was observed in the sequences from the skeletal samples, providing additional evidence of DNA damage.

To address the DNA damage in casework samples, two methods of DNA repair were evaluated: the NEBNext® Formalin-Fixed Paraffin-Embedded (FFPE) DNA Repair Mix and Uracil-Specific Excision Reagent (USER™) Enzyme. In an initial study, improved sequence results were obtained from six casework samples that underwent repair treatment in comparison to the untreated extracts. Approximately 20% of sequences in repaired samples showed a shift in GC, indicating damage, compared to 70% in untreated samples. The number of mapped reads generated with NEBNext® FFPE repair-treated samples was closest to that of the untreated samples (two to three times higher than those treated with the USER™ enzyme), resulting in greater coverage across the targeted regions. The low number of reads obtained with the USER™ protocol is most likely the result of a required clean-up step in which DNA loss is expected. This step is not necessary in the FFPE protocol, making it a more attractive option for use with low-quantity samples. In most cases, unauthentic low-level variants observed in the untreated samples were eliminated in the repaired extracts. The results of this work suggest that DNA repair of compromised samples will not only be necessary for NGS processing but will also be beneficial for current Sanger sequencing of skeletal remains performed at the AFDIL. As the forensic community continues to look toward NGS for its increased sensitivity and low-level variant detection capability, DNA damage in low-quality specimens must first be addressed in order to generate reliable data.

The opinions or assertions presented herein are the private views of the authors and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the United States Army Medical Research and Materiel Command or the Armed Forces Medical Examiner System.

Next Generation Sequencing, DNA Damage, DNA Repair

*Presenting Author