End Report: Advancing DNA Sequencing Methods for Degraded Forensic Samples

The research collaboration between the Institute of Legal Medicine (GMI) of the Medical University of Innsbruck and the Armed Forces DNA Identification Laboratory (AFDIL) successfully took place with the objective of exchanging knowledge and enhancing the performance of massively parallel sequencing (MPS) methods for highly degraded DNA in decomposed and ancient samples.

Considering the extensive experience in developing and applying forensic DNA analysis tools of AFDIL, the opportunity to assess and compare the respective MPS methods at both institutions provided valuable insights for future developments. The visit took place from the 15th to the 29th of March 2025 and allowed for a detailed comparison of the techniques used in each laboratory, offering the chance to learn from their approaches and identify potential improvements for these forensic applications.

At GMI, the focus is on the use of the Primer Extension Capture (PEC) method, which has been successfully implemented for forensic applications, particularly in cases involving degraded DNA. This method has proven effective in complex cases, including the identification of victims in unsolved criminal investigations. However, there was a need to explore additional sequencing methods to extract more information when facing extremely challenging samples.

To address such cases, AFDIL utilizes hybridization capture technology, particularly for sequencing mitochondrial DNA (mtDNA). This approach has been validated for forensic use and has shown success in retrieving valuable genetic information from even the most compromised DNA. Both the PEC and hybridization capture methods work well for their respective applications, but when dealing with highly degraded samples, the goal of this collaboration was to investigate whether shotgun sequencing could provide further insight, particularly for samples where conventional methods may be insufficient.

For this evaluation, we used a shotgun sequencing approach that utilizes a single-stranded method for library preparation prior to sequencing. This technique offers several advantages, particularly for hair samples, which are expected to be devoid of bacterial DNA, thereby eliminating the need for capture. Because of multiple washing steps involved in the capture approach, there is a potential risk of DNA loss during the capture process of hair samples.

Given the extreme degradation often encountered in forensic samples, this method was considered a promising strategy to extract additional genomic information from these challenging specimens. The primary goal was to assess whether this single-stranded shotgun sequencing method could improve the recovery of DNA, potentially surpassing the limitations of other MPS approaches such as PEC and hybridization capture. In addition, another library preparation strategy with a double-stranded library was also used to apply an alternative method.