**A Comprehensive Illumina Library Preparation Method Suitable for Any Forensic Amplification Approach**

Joseph D. Ring[1,2], Kimberly Sturk-Andreaggi[1,2], Erin M. Gorden[1,2], Jennifer D. Higginbotham[1,2], Cassandra R. Taylor[1,2], Charla Marshall[1,2]
[1]*Armed Forces Medical Examiner System's Armed Forces DNA Identification Laboratory (AFMES-AFDIL), 115 Purple Heart Drive, Dover AFB, DE, 19902, United States*
[2]*SNA International, 525 Wythe Street, Alexandria, VA, 22314, United States*

Next generation sequencing (NGS) is becoming more readily used as a tool in forensic human identification. Although novel assays for the sequencing of mitochondrial DNA (mtDNA), short tandem repeat (STR) markers, and autosomal single nucleotide polymorphisms (SNPs) have been developmentally validated, each uses a separate workflow that involves individual library preparation procedures and sequencing platforms. This can lead to high reagent cost, increased storage space needs, the quality control (QC) of a variety of reagents, and an implementation burden to validate the myriad methodologies along with training of scientific staff.

Presented here is a streamlined workflow involving the Roche KAPA HyperPlus and Hyper Prep library preparation kits that enable Illumina library preparation from any starting forensic amplification target. This workflow can accept not only enriched targets from NGS-focused kits (e.g. Precision ID SNP, STR, and mtDNA panels), but also kits that are intended for capillary electrophoresis-based STR typing (e.g. PowerPlex Fusion, Yfiler, and MiniFiler). Additionally, long-range mitochondrial genome (mitogenome) and whole genome shotgun sequencing can be accommodated through the utilization of an enzymatic fragmentation step included in HyperPlus. By funneling all forensically relevant marker sets into one workflow, sample processing is simplified and reagent requirements are minimized. The presentation will also show the efficacy of combining separate amplification targets into a single sample library for even further reduction of the necessary reagent costs. Furthermore, this workflow was automated on a Hamilton STARplus liquid handling instrument to improve upon efficiency and throughput, which has enabled over 2,100 mitogenomes to be processed by two analysts in the span of approximately 250 days. To date, over 2,500 samples have been processed using this library preparation method (approximately 130 STR, 100 SNP, and 2,300 mtDNA).

Through a streamlined, automated workflow for library preparation, forensic laboratories will gain efficiency not only in processing time, but also in training, reagent costs, and QC. It will also be easier for validation of new enrichment strategies, as the same library preparation workflow can be employed.

*Disclaimer: The opinions and assertions presented hereafter are the private views of the authors and should not be construed as official or as reflecting the views of the United States government.*