W23 Considerations for Implementing Next Generation Sequencing (NGS) Technologies Into a Forensic Laboratory

Kimberly S. Andreaggi, MFS, ARP/AFDIL, 115 Purple Heart Drive, Dover AFB, DE 19902; Alice Briones, DO*, 599 Phillips Drive, Magnolia, DE 19962; Katherine B. Gettings, PhD*, NIST, 100 Bureau Drive, MS 8314, Gaithersburg, MD 20899; Erin M. Gorden, MFS*, Armed Forces DNA Identification Lab, 115 Purple Heart Drive, Dover AFB, DE 19902; Richard A. Guerrieri, MS*, 1 Corduroy Court, Stafford, VA 22554; Jennifer L. Higginbotham, MFS*, 115 Purple Heart Drive, Dover AFB, DE 19902; Christina M. Neal, MS*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover, DE 19902; Walther Parson, PhD*, Muellerstraße 44, Innsbruck A-6020, AUSTRIA; Michelle A. Peck, MFS*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; Joseph D. Ring, MS*, 115 Purple Heart Drive, Dover AFB, DE 19902; Peter M. Vallone, PhD*, 100 Bureau Drive, Gaithersburg, MD 20899-8311; Charla Marshall, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover AFB, DE 19902; and Timothy P. McMahon, PhD*, Armed Forces DNA Identification Laboratory, 115 Purple Heart Drive, Dover Air Force Base, Dover, DE 19902*

After attending this presentation, attendees will understand NGS methodologies that can be applied to typical forensic specimens, as well as appreciate the considerations specific to the validation of NGS technologies.

This presentation will impact the forensic science community by discussing the benefits and challenges of implementing NGS into a forensic laboratory.

Over the past two decades, the gold standard for nucleic acid sequencing has been the chain-termination technique developed by Edward Sanger and colleagues in the late 1970s, now known as Sanger sequencing. In forensic laboratories, sequencing has historically been performed for mitochondrial DNA (mtDNA) typing, which is most applicable in cases with minimal quantities of nuclear DNA or in those lacking appropriate references for direct identification; however, there is strong forensic interest in the adoption of NGS technologies for wider use within the laboratory. NGS platforms enable massively parallel sequencing to generate millions of DNA sequence reads simultaneously. As such, NGS facilitates high-throughput DNA sequencing of multiplexed samples and DNA targets, which can be automated to streamline the laboratory workflow. Moreover, NGS data are quantitative and amenable to hands-off analysis within an expert system bioinformatic software package. This benefit thereby eliminates the need for visual assessment of electropherogram images that can slow the process of data review. The recent development of low-cost, high-throughput NGS platforms and commercial kits for forensic applications has made sequencing more accessible to forensic laboratories. Consequently, a demand has driven the forensic community to implement NGS technologies for routine use.

Forensic DNA laboratories around the world have begun the task of validating NGS technologies for missing persons and criminal casework as well as databasing efforts; however, these advances pose significant hurdles as traditional typing methods are traded for NGS assays, quantitation instruments, and sequencing platforms. First, NGS will require a transition from traditional length-based DNA typing methods to the sequencing of core forensic DNA markers. Consequently, sequence variation present in Short Tandem Repeats (STRs) will require an establishment of nomenclature for STR sequence analysis. NGS enhances the feasibility of entire mtDNA sequencing and enables Single Nucleotide Polymorphism (SNP) characterization as a feasible tool for genetic discrimination. In turn, the adoption of NGS sets the stage for ethical and legal discussions surrounding the use of phenotypic markers in forensics. Although other fields have adopted this technology successfully, forensic laboratories are beholden to strict guidelines and legal challenges that affect NGS implementation.

This presentation will provide a snapshot of the current progress of forensic DNA laboratories in the implementation of NGS technology. First, a historical perspective on DNA typing technologies will be presented to situate NGS within the context of methodological advancement. The presentation will follow with an overview of NGS methods available to the forensic community, and a discussion of laboratory infrastructure as it transitions to meet NGS requirements. Several presentations will focus on data generated from NGS workflows, including an evaluation of quantification systems as well as STR sequencing kits. The presentation will then turn to mitochondrial DNA sequencing, data analysis, and interpretation. Considerations surrounding the selection of NGS workflows and the challenges to the validation of NGS technology will be discussed. The final portion of this presentation will take the pulse of the broader forensic DNA community as it works to adopt NGS technologies in the laboratory.

The opinions or assertions presented hereafter are the private views of the author(s) 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.

DNA Analysis, Next Generation Sequencing, Validation

*Presenting Author