DEVELOPMENTAL VALIDATION OF FORENSEQ MAINSTAY LIBRARY PREP, SEQUENCING AND SOFTWARE FOR ANALYSIS OF AUTOSOMAL AND Y STRS

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Next generation sequencing (NGS, sometimes called [KS1][BL2]) massively parallel sequencing (MPS) has been well established as a viable solution for forensic human identification (HID) and an alternative to methods utilizing capillary electrophoresis (CE). It has also been well described that NGS detected sequence variation in short tandem repeat alleles of the same length offer discriminatory enhancements compared to traditional CE based genotyping as well as the ability to use the sequence to identify the amplicons instead of size to allow the use of small amplicons to assist with typing severely degraded DNA. However, widespread adoption of NGS workflows for HID purposes have been slowed by a number of factors such as perceptions of high cost per sample, reluctance of forensic practitioners to adopt novel markers such as single nucleotide polymorphisms into downstream analysis pipelines, and analysis methods that have a steep learning curve compared to CE based methods.

To address these concerns, we have developed the ForenSeq MainstAY assay and Universal Analysis Software for MiSeq FGx™ Sequencing System.

In this presentation we describe the SWGDAM prescribed developmental validation of the ForenSeq MainstAY workflow. The ForenSeq MainstAY workflow is a “sample to profile” system for reliably and robustly generating 52 of the most common globally used Autosomal and Y STR genotypes along with amelogenin for sex determination. The polymerase chain reaction (PCR)-based library prep generates full profiles with as little as 62.5pg of input gDNA, crude cell lysates, or FTA punches. Sequencing data is analyzed, and reports are automatically generated in about one hour following the completion of sequencing. Genotypes are viewed and can be edited in the user-friendly, GUI based, Universal Analysis Software. Studies have been performed to assess the robustness of the system with environmental and situational variables (hair roots, manually degraded DNA, and dental remains), assessments of sensitivity, DNA mixture detection, and concordance with genotypes determined using orthogonal methods.

The ForenSeq MainstAY assay and Universal Analysis Software for MiSeq FGx™ Sequencing System is an effective method that has been validated for use with routine casework samples.