B48 A Preliminary Sensitivity Assessment Comparing Two Next Generation Sequencing (NGS) Laboratory Workflows for Forensic Analysis

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The goal of this presentation is to educate the forensic science community concerning the progress of NGS and the performance of two specific workflows in regard to use in forensic DNA analysis.

This presentation will impact the forensic science community by sharing information about the potential of these two specific workflows in forensic crime laboratories.

DNA evidence recovered from a crime scene is rarely ample in quality or quantity. Therefore, it is vital to know the limits of any assay used for forensic DNA analysis. NGS DNA sequencing technology is now being implemented and investigated in forensic laboratories. Integration of NGS into forensic DNA analysis workflows provides value by its ability to: (1) successfully process degraded samples; (2) reduce the cost of materials, labor, and time by multiplexing a large number of DNA markers into one sequencing run; (3) better distinguish individual profiles from mixtures; and, (4) use phenotypic markers to identify persons by physical characteristics. It is important to perform a sensitivity assessment to ultimately validate this new technology.

The Scientific Working Group on DNA Analysis Methods (SWGDAM) recommends various studies be conducted in order to evaluate the limits of an assay. With these evaluations having been performed for current technology (i.e. capillary electrophoresis) in the forensic setting, SWGDAM guidelines are serving as a guide for the assessment and validation of the NGS technology.

For this experiment, the Promega® PowerSeq™ Auto/Y System Prototype and Illumina® ForenSeq™ DNA Signature Prep kits were evaluated as both use the MiSeq® instrument platform. Initially, two high-quality DNA samples were assessed at different titrations, ranging in amplification input from 500pg to 15.6pg. The same samples were processed with both laboratory workflows being compared according to the manufacturer's instructions. Following this study, eight mock casework samples were tested with both workflows on separate sequencing runs. Data were analyzed using default analytical threshold settings using software platforms recommended in the manufacturer's instructions. Preliminary results indicated full DNA profiles were obtained for autosomal Short Tandem Repeats (STRs) at lower amplification input levels in samples processed with the PowerSeq™ workflow than samples processed with the ForenSeq™ DNA Signature Prep kit. Similar trends were observed with the mock casework samples. Genotypes for each marker were deemed concordant when the correct allele or alleles were the most abundant for homo- and heterozygous loci, respectively. Correct alleles were obtained from capillary electrophoresis data previously generated.

It is recommended that more sensitivity and forensic casework-like studies be performed within the community to further supplant these initial findings. This assessment will be valuable for the forensic casework and academic research communities in order to demonstrate the capability of NGS to assist in their selection of an appropriate methodology. This preliminary study can assist laboratories that are seeking to integrate this new technology to gain an initial idea of which kit and workflow are best suited for their needs.

Next Generation Sequencing, Sensitivity Assessment, Validation