B128 The Evaluation of the ForenSeq™ MainstAY Kit for Mixed Forensic DNA Samples

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Learning Objective: After attending this presentation, attendees will have a better understanding of a plexity in next generation sequencing, how commonly encountered mixtures and challenging sample types may affect flow cell capacity, and recommendations for maximizing run efficiency.

Impact Statement: This presentation will impact the forensic science community by addressing a potential obstacle for labs interested in implementing NGS for casework and by providing suggestions aimed at making that transition easier and more accessible.

Next Generation Sequencing (NGS) has been widely considered to be the future of forensic casework, with increasing numbers of labs beginning to implement it into their workflows alongside, or even in place of, traditional Capillary Electrophoresis (CE). There are numerous advantages to NGS such as shorter amplicons, increased sensitivity, the potential detection of isoalleles, and the massive number of markers and marker types that can be run simultaneously. One main limiting factor, though, is plexity, the number of samples that can be run at once. Plexity is affected by the physical capacity of the flow cell, which facilitates the formation of clusters that each represent a distinct PCR target (DPT). Since only so many DPTs can fit on the flow cell, the read coverage at each locus in an NGS run is highly dependent on the flow cell's capacity, the number of markers in the kit, and the number of DPTs generated during the PCR process. The total number of DPTs includes things like forward and back stutter as well as the alleles. More loci and higher numbers of contributors result in higher DPTs.

Verogen's™ recently released ForenSeq™ MainstAY kit is specifically aimed at a forensically relevant set of 27 autosomal STR and 25 Y-STR markers. It uses the MiSeq® FGx Reagent Micro Kit, with a flow cell capable of five million reads. This combination of markers and flow cell capacity should allow full coverage of a run of 96 single-source samples. However, forensic casework samples are rarely this simple. Labs regularly process samples that contain a mixture of two or more contributors, often with low-level trace donors. Mixed samples can multiply the number of DPTs present, causing a 96-sample run that should fall within the capacity of the flow cell to exceed it, reducing the effective coverage of each locus. In addition, the increased sensitivity promised by NGS has not yet been fully explored for mixed samples, especially for batched samples such as sexual assault swabs, which commonly have very high female-to-male mixture ratios. Such samples, if included in a full run of 96 libraries, must compete for space on the flow cell for detection.

The study presented here aimed to test the limits of MainstAY's plexity by simultaneously running a variety of sample types and mixtures that would be typically encountered in forensic casework. The presentation will explore the trade-off of more rare genotypes due to isoalleles against detection challenges due to extreme plexity and competition for a spot on the flow cell. Additionally, properties unique to NGS such as sequence-specific stutter models will be explored. Last, the presentation will provide recommendations for optimal batching of samples for an efficient NGS case processing workflow.

Next Generation Sequencing; Plexity; Isoalleles

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