

EVALUATION OF THE FORENSEQ MAINSTAY KIT WITH CHALLENGING SAMPLES

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The majority of DNA profiles are generated using a standard workflow: extraction, quantitation, amplification, and separation and detection using capillary electrophoresis (CE). These techniques still provide results for the majority of DNA case working laboratories. However, there continue to be examples where recovered DNA is insufficient to produce complete STR profiles due to low quality, degradation, or mixtures. In these cases, the need to recover more information has grown, and one technique that is more sensitive and yields more genetic data is Next Generation Sequencing (NGS). Unlike CE platforms, NGS platforms are not limited by dye channel real-estate, and as a result, more amplicons can be simultaneously sequenced and analyzed. In addition, this increased number of amplicons provides analysts more power of discrimination to analyze samples more effectively.

This study focuses on a new NGS assay from Verogen, the ForenSeq MainstAY kit. This kit contains approximately one-fourth the number of markers available in the ForenSeq DNA Signature Prep kit. The MainstAY kit focuses on identification and only includes established autosomal STRs and Y-STRs accepted by most global databases. The decrease in marker complexity increases sensitivity compared to the ForenSeq DNA Signature Prep Kit and may provide more robust data. In this study, we evaluated the MainstAY kit compared to data previously collected using both GlobalFiler™ (Thermo Fisher) and Investigator 24plex QS (QIAGEN) kits. A sensitivity study consisted of varying concentrations of control DNA between 1ng – 8pg. Additionally, various challenging samples were tested, including bone, blood, saliva, and hair samples.

Results showed the genotypes of the shared loci for the challenging samples were concordant between the two methods (NGS and CE). However, the MainstAY kit had similar or improved percent recovery compared to the capillary electrophoresis methods for these samples. In instances with reduced percent recovery, the MainstAY kit still recovered more loci and thus more information than the CE method. Additionally, the MainstAY chemistry was determined to be sensitive and reliable, with few alleles dropping out at an input DNA amount of 31pg and approximately 50% of loci still recovered at only 8pg, making it an effective tool for casework samples.