INTERNAL VALIDATION OF THE FORENSEQ™ DNA SIGNATURE PREP KIT FOR REFERENCE SAMPLES: SUCCESSES AND LESSONS LEARNED

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Massively parallel sequencing (MPS) provides DNA laboratories an option to overcome the limitations of capillary electrophoresis. In an effort to align with today's demands in solving cold or difficult cases which are likely a combination of multiple sources and often lack sufficient biological material to enable the generation of full DNA profiles; the incorporation of commercially available massively parallel typing kits will soon become an essential tool in any laboratory workflow. Illumina's MPS Forenseq™ DNA Signature kit is comprised of over 200 markers in one multiplex spanning autosomal, Y- and X-STRs in addition to identity, phenotypic and ancestry informative SNPs. This kit offers increased efficiency, the ability to interrogate multiple types of markers in one system, higher resolution and sensitivity, visibility of sequence variations that could aid in mixture interpretation and markers designed to provide potential investigative leads. An internal validation of reference samples was conducted to investigate whether the Forenseq™ DNA Signature Prep kit, specifically primer panel B, has the capability to provide consistent and accurate typing/sequencing data. The studies conducted included repeatability, reproducibility, concordance to orthogonal CE data, and other next generation sequencing chemistries, contamination and sensitivity. As part of the validation, the Forenseq™ Universal Analysis Software was evaluated - software issued flags were studied, necessary read coverage for successful sample typing was determined, interpretation thresholds were established and prediction tools were evaluated for accuracy and compared to other available software packages for concordance. This validation provides deeper understanding of the chemistry and software behavior and will ultimately serve as the foundation to additional validation efforts with case-type samples.