

POWER OF THE ILLUMINA® FORENSEQ™ DNA SIGNATURE PREPARATION KIT IN HUMAN IDENTITY DNA TYPING

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Introduction:

The introduction of massively parallel sequencing (MPS) technologies almost a decade ago has changed the scientific community's approach to DNA sequencing as they offer improvements to forensic identification's current capillary electrophoresis (CE)-based methodologies. MPS technologies enable multiplexing of different types of forensically-relevant genetic markers and can sequence a larger number of markers simultaneously compared with that of CE technologies. This increase in markers translates into additional information for development of investigative leads and more robust associations may be generated from database searches. These additional markers may facilitate analysis of challenged samples and address novel investigative questions. MPS allows for identification of sequence-based variation among STR alleles in addition to the traditional nominal repeat length-based alleles. Sequence variants provide greater discrimination power which in turn may facilitate mixture interpretation and kinship analyses. Finally, MPS provides a sensitivity of detection comparable to current CE technologies.

The Illumina ForenSeq DNA Signature Prep Kit is a PCR-based library preparation method used for simultaneous targeted amplification and sequencing of 59 STRs, 95 identity SNPs, 56 ancestry SNPs, and 22 phenotypic SNPs on the MiSeq desktop sequencer (Illumina). This MPS kit was evaluated for high throughput genotyping of reference, population, and challenged samples.

Methods:

The methodology used in this study is described in detail in Churchill et al. 2016.

Results:

- Informative metrics such as genotype accuracy, depth of coverage, allele coverage ratios, and sequence coverage ratios indicated reproducible and reliable data had been produced.
- The ForenSeq kit produced STR data concordant with current CE methods.
- Sensitivity studies illustrated accurate and full profiles were obtained with DNA input amounts of 1 ng, and 94% of alleles were observed with DNA input amounts as low as 100 pg.
- Alleles from the minor contributor of a mixture were observed in a 1:19 ratio. The mixture study illustrated the added utility of intra-allelic sequence variants for interpretation.
- Results from analysis of challenged samples indicated that more markers and a higher success rate could be obtained compared with CE results.

- Population data were generated on approximately 800 Caucasian, African, Hispanic, and Asian samples.

Conclusion:

Studies such as the one herein describe the strengths and limitations of MPS systems designed for forensic analyses. The results support the utility of MPS. Since the technology is evolving rapidly, there is every indication that the MPS technology will continue to advance and improve. The promising nature of the results from the beta tests and population studies supports continuing onward with full validation studies of Illumina's MiSeq FGx Forensic Genomics System. These studies support that the MiSeq FGx System is a viable MPS system for forensic genetic analysis and has the potential for incorporation in forensic genetic laboratories.

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