B76 Highly Multiplexed Analysis of STRs and SNPs Using Massively Parallel Sequencing: Concordance With Current Methodologies

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After attending this presentation, attendees will be informed about next generation sequencing applied to forensic DNA analysis and whether data generated from sequencing is concordant with capillary electrophoresis data.

This presentation will impact the forensic science community by exploring the advantages of Next Generation Sequencing (NGS) to forensic DNA analysis as well as its concordant results with current methodologies.

Capillary Electrophoresis (CE) is the predominant method for DNA analysis currently employed by forensic laboratories and has been regarded as the gold standard for many years. Data from this technique has been admitted into court since 1996. Although this is an effective technique, it is limited in the amount and type of information that can be generated efficiently. Although Short Tandem Repeats (STRs) can give sufficient information for human identification, it is often useful in forensic cases to be able to analyze other variants including Single Nucleotide Polymorphisms (SNPs) and Y-chromosomal Short Tandem Repeats (Y-STRs), which can provide valuable information to law enforcement. Analyzing SNPs using CE is difficult due to multiplexing constraints and is not often performed in forensic laboratories.

Whole Genome Sequencing (WGS) is now more widely available with the advent of NGS techniques. WGS allows analysts to analyze STRs as well as other informative variants in the DNA sequence simultaneously. With NGS systems, scientists are able to obtain an accurate sequence of a genome in a relatively short amount of time due to the massively parallel sequencing that is utilized by NGS platforms. Each base is sequenced 30 or more times to ensure accurate calls are being made. NGS can also be used for targeted sequencing to study specific genetic regions of interest according to application.

As technology continues to expand, it would be useful to consider if these advanced technologies could help forensic laboratories gain more information from their often difficult and limited samples. Illumina's® Prototype ForenSeq™ DNA Signature Prep Kit is an NGS kit targeted for forensics. With this system, one workflow is required to analyze a multitude of variants including autosomal STRs, SNPs (identity, phenotype, and ancestry informative), Y-STRs, and X-chromosomal Short Tandem Repeat (X-STRs). The kit targets all STRs required for the Combined DNA Index System (CODIS) and European Standard Set (ESS) of STR loci, as well as many more. Because of the system's ability to distinguish indexed samples, 32 to 96 samples also can be run concurrently with NGS on a single run. This approach could tremendously impact existing backlogs in forensic laboratories. Sequencing by NGS has the advantage of being able to analyze more loci at a time, thereby generating more information for the analyst.

This study was performed to check concordance of sequencing data performed on Illumina's® MiSeq™ FGx using the Prototype ForenSeq™ DNA Signature Prep Kit against data obtained using traditional CE methods. Concordance was also checked between SNP data from the Prototype ForenSeq™ kit against Illumina's® WGS data from the Platinum Genome Project. This study focused on concordance between the 95 identity informative SNPs, 29 autosomal STRs, 9 X-STRs, 24 Y-STRs, 22 phenotypic informative SNPs, and 56 ancestry informative SNPS that are included in the Illumina's® Prototype ForenSeq™ DNA Signature Prep Kit. Full profiles were generated for all 23 samples run simultaneously with the Prototype ForenSeq™ DNA Signature Prep Kit. A total discordance of 0.001% was observed for this study. Showing concordance between this new technology and the existing technologies would confirm the possibility of implementation of the ForenSeq™ DNA Signature Prep Kit into forensic laboratories.

Next Generation Sequencing, Concordance, Forensic DNA Analysis