Y9 Testing of a Probe Capture Next-Generation Sequencing Assay for the Analysis of Nuclear Short Tandem Repeat (STR) and Single Nucleotide Polymorphism (SNP) Markers

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Learning Overview: The goal of this presentation is for attendees to recognize the value of probe capture enrichment Next Generation Sequencing (NGS) assays in the analysis of genetic markers from single DNA shotgun libraries.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing attendees an alternative method to Polymerase Chain Reaction (PCR) amplification for analyzing challenging samples, including highly degraded samples and mixtures.

DNA from biological samples in forensic casework may be mixed or in degraded condition. In samples with highly degraded DNA, both PCR primer binding sites may not be present on the template DNA fragments, and conventional PCR amplification and Capillary Electrophoresis (CE) analysis of STR may fail. Probe capture enrichment utilizes overlapping biotinylated probes to capture fragmented DNA. Since STR regions cannot directly be targeted for capture, DNA regions flanking the STRs were targeted using probe capture enrichment.

Previously, it was demonstrated that the SNP probe capture/NGS system can capture and sequence DNA fragments as short as 35 base pairs with input as low as 0.5ng while yielding 99%–100% reportable SNP genotypes.[1] Reportability is determined by meeting the minimum threshold requirement for read depth of >500 reads per base. This study tested the performance of SNP (v3.0) and STR (v1.0) probe capture panels with the same shotgun libraries. Three commercial control DNA samples, Promega's® 2800 M, the National Institute of Standards and Technology's SRM2391-c, and Coriell Cell Repositories' NA24149, and 12 blood-derived DNA population samples were prepared using a "shotgun" approach and given unique dual-indexed barcode sequences. DNA shotgun libraries were pooled for probe capture enrichment. The enriched products were sequenced on Illumina® MiSeq®. SNP and STR sequence data were analyzed using NextGENe® and GeneMarker® HTS Software.

Ninety-six percent of SNPs (*n*=496) were reportable using this custom SNP (v3.0) probe capture panel in samples with input amounts of DNA at 25ng. Some of the STR loci could be analyzed by inspection of the sequence read "pile-ups" with reads that spanned the STR corresponding to the known length variants. This "proof of concept" study with this STR (v1.0) panel identified sequence polymorphisms that would be undetectable by conventional CE analysis. The successful development of probe capture NGS for both STR and SNP assays would provide practitioners an alternative method to PCR amplification for analyzing challenging samples, including highly degraded samples and mixtures.

Reference(s):

1. Bose, Nikhil, Katie Carlberg, George Sensabaugh, Henry Erlich, and Cassandra Calloway. Target Capture Enrichment of Nuclear SNP Markers for Massively Parallel Sequencing of Degraded and Mixed Samples. *Forensic Science International*: Genetics 34 (2018): 186–96.

Next Generation Sequencing (NGS), Single Nucleotide Polymorphism, Short Tandem Repeat (STR)

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