B73 An Analysis of Challenging Forensic Samples Using Probe Capture Next Generation Sequencing (NGS)

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After attending this presentation, attendees will understand the advantages and application of a customized probe capture NGS system designed for forensically challenging samples.

This presentation will impact the forensic science community by illustrating how the custom probe capture NGS method is useful for the recovery and sequencing of the whole mitochondrial genome of forensically challenging samples (e.g., telogen hairs and touch DNA).

Forensic biological samples that are highly degraded, limited, and mixed (such as telogen hair and touch DNA) can be challenging for conventional Short Tandem Repeat (STR) genotyping. An alternative strategy for analyzing forensically challenging samples is analysis of mitochondrial DNA (mtDNA); however, conventional mtDNA sequencing methods such as Sanger Sequencing are limiting in discrimination power because they often fail to detect low level heteroplasmy or mixtures that are common in forensic samples. NGS methods have the potential to overcome many of the limitations of conventional methods used for analyzing mtDNA markers due to the high-throughput massively parallel clonal sequencing nature. In conjunction with using NGS, this study developed a custom probe capture enrichment system targeting the entire mitochondrial genome and 451 nuclear Single Nucleotide Polymorphism (SNP) markers for highly degraded and mixed samples. This approach uses DNA probes to enrich targeted regions from randomly fragmented DNA libraries for clonal, massively parallel sequencing, thereby maximizing recovery of short DNA fragments characteristic of forensic samples.

This custom probe capture NGS assay was successful in recovery and sequencing of the entire mitochondrial genome from forensically challenging samples, including 33 telogen hair roots, 22 telogen hair shafts, and 19 touch DNA samples recovered from spent cartridges using the double-swab method with cotton and flocked swabs. Thirty of 33 telogen hair roots yielded 100% coverage of the mitochondrial genome while 3 telogen hair roots had >80% coverage of the mitochondrial genome at >100x read depth. Seventeen of the 22 telogen hair shafts yielded 100% coverage of the mitochondrial genome while 5 showed >80% coverage of the mitochondrial genome at >100x read depth. Of these 5 telogen hair shafts, 4 failed conventional PCR-based amplification while 1 showed weak results. Furthermore, a subset of the DNA libraries of these telogen hair roots were captured and sequenced using a customized SNP probe capture assay, yielding 65.1% to near 100% coverage of the 451 nuclear SNPs. In addition to telogen hairs, touch DNA collected using cotton swabs exhibited an average of 93.7% coverage of the mitochondrial genome while touch DNA collected using flocked swabs exhibited 100% coverage of the mitochondrial genome at >100x read depth. A subset of these touch DNA samples collected using flocked swabs exhibited 100% coverage of the mitochondrial genome at >500x read depth. The major mtDNA variant sequences were consistent for all touch DNA samples and with the subject's profile. The mtDNA haplogroup was determined to be H7 for all four of the touch DNA samples and the reference.

In conclusion, this probe capture NGS system is shown to be useful for recovery and sequencing of the entire mitochondrial genome for forensically challenging samples, including telogen hair roots, telogen hair shafts, and touch DNA recovered from spent cartridge casings. Furthermore, both nuclear SNP and mtDNA markers can be analyzed from a single DNA library prepared from challenging samples that are limited in quantities and compromised in qualities. Therefore, when a sample with degraded or a low amount of DNA is encountered, this probe capture NGS method will allow for both SNP and mtDNA analyses without consuming more DNA extract.

Probe Capture, Next Generation Sequencing, Touch DNA

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