B11 An Evaluation of the Illumina® ForenSeq™ DNA Signature Prep Kit and Promega® PowerPlex® Fusion System in the Evaluation of Degraded Identical Twin Samples

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After attending this presentation, attendees will better understand the Illumina® ForenSeq™ DNA Signature Prep kit and Promega® PowerPlex® Fusion systems' abilities to analyze naturally degraded samples.

This presentation will impact the forensic science community by providing an understanding of both kits' limitations in evaluating degraded samples when degradation levels are considered.

A comparison of Short Tandem Repeat (STR) profile quality generated by both Massively Parallel Sequencing (MPS) and traditional Polymerase Chain Reaction-Capillary Electrophoresis (PCR-CE) will be presented. The analysis of phenotypic, ancestry-informative, and identity-informative Single Nucleotide Polymorphisms (SNPs) from degraded samples will also be discussed.

MPS enables an increased amount of forensically beneficial genetic information to be obtained over traditional CE. The ForenSeq™ DNA kit simultaneously amplifies up to 231 genetic markers, namely 27 autosomal STRs (aSTRs), 24 Y-chromosomal Short Tandem Repeats (Y-STRs), 7 X-chromosomal Short Tandem Repeats (X-STRs), 95 identity-informative SNPs (iiSNPs), 22 phenotypic-informative SNPs (pSNPs), and 56 ancestry-informative SNPs (aSNPs). MPS amplicon sequencing allows for small amplicon sizes, hypothetically making MPS technologies more beneficial when evaluating degraded samples; however, minimal research has been conducted on the efficiency of the ForenSeq™ DNA kit in conjunction with environmentally degraded samples to test this hypothesis. This research sought to explore this hypothesis by comparing the ForenSeq™ kit with that of a traditional forensic chemistry, Promega® PowerPlex® Fusion, in the evaluation of naturally degraded samples.

Seventy-six degraded buccal samples were quantified using Applied Biosystem's® Quantifiler® Trio DNA Quantification Kit to obtain information on the samples' degradation levels. The samples' Degradation Index (DI) values ranged between 2.93 and 110.12, with values greater than 10 indicating severe degradation. The samples were then processed using the PowerPlex® Fusion and ForenSeq™ kits with DNA inputs ranging between 0.250ng and 1ng. Comparative analysis of the PowerPlex® Fusion and ForenSeq™ DNA kits were performed for the overlapping loci, while the additional ForenSeq™ loci were assessed to evaluate the kit's ability to provide additional individualizing information.

As expected, the quality of the profiles decreased with increased DI values. With some exceptions, MPS generated higher average percent profiles, exhibited less allelic drop-out, and produced better heterozygous balance, while PCR-CE produced better inter-locus balance. There was no significant difference observed for profile uniqueness between the systems when average Probabilities Of Identity (POI) values were calculated. All samples generated biogeographical ancestry estimations with three observed discordances from donor self-reported ancestry. Hair color estimations were only possible for the least degraded sample. Several previously unreported STR sequence variants were observed in aSTRs, Y-STRs, and X-STRs when samples were processed with MPS; however, no genetic differentiation between identical twin siblings was observed in either system.

Overall, the ForenSeq™ kit produced more forensically beneficial information for the degraded samples in this study, demonstrating that it is highly valuable for the analysis of degraded samples; however, the kit is limited in its ability to produce investigative leads from degraded samples due to the number of pi-SNPs required to generate phenotypic estimations and the ForenSeq™ Universal Analysis Software's incorrect estimations of donor biogeographic ancestry groups.

Massively Parallel Sequencing, Degraded DNA, STR

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