B130 An Alternate Workflow for Preparing Precision ID Identity and Ancestry Panel Libraries for Illumina® Sequencing

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Learning Overview: After attending this presentation, attendees will better understand about a verified workflow that can be used to generate Single Nucleotide Polymorphism (SNP) genotypes using Precision ID primer panels, third-party library construction reagents, and Illumina® sequencing instrumentation and reagents. This presentation will also highlight a published data analysis workflow that uses quality control measures and flagging to aid in genotype confirmation.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by informing attendees how to incorporate this method as a cost-effective alternative to the complete commercial workflow, based on their laboratory's requirements and existing instrumentation. Given that the workflow presented is flexible, attendees could make adjustments in order to optimize scale and utilize accessible core sequencing lab instrumentation.

SNPs have been under development for human identification purposes for over a decade. Although they are not compatible with criminal casework databases such as the Combined DNA Index System (CODIS), they offer some advantages over the more traditionally typed Short Tandem Repeat (STR) markers: (1) they can be designed as very small Polymerase Chain Reaction (PCR) amplicons, offering greater success with degraded samples; (2) they do not generate stutter, an artifact of STR amplification that complicates data interpretation; and (3) they can offer predictions of biographic ancestry and phenotype, which could be valuable for generating investigative leads in criminal cases. The Precision ID NGS System, a commercially available workflow offered by Thermo Fisher Scientific™, offers a streamlined solution for genotyping forensically relevant identity and ancestry SNPs using next-generation sequencing. The Precision ID Identity and Ancestry Panels combined target 289 SNPs, and the sensitivity, reproducibility, and accuracy of the panels have been already evaluated by the forensics community. One potential limitation to the broad use of these panels is that genotyping using Thermo Fisher Scientific™ reagents and instrumentation requires a significant financial commitment. Thus, the aim of this study was to find an alternative workflow compatible with Illumina® sequencing chemistry for genotyping SNPs using the Precision ID Identity and Ancestry Panels, given the laboratory and nearly all core sequencing facilities have Illumina® instruments. Commercially available genomic DNA samples (n = 3) were amplified with both panels in separate reactions using three commercially available uracil-tolerant polymerase master mixes. All resulting amplicons were prepared into libraries using the KAPA™ Hyper Prep Kit and sequenced via Illumina's® MiniSeq Sequencing System. Sequencing reads were analyzed using a previously published QIAGEN's® CLC Genomics Workbench workflow, and a Python® script was used to compile final genotypes and coverage information. Phusion™ U Multiplex PCR Master Mix statistically outperformed the other polymerase master mixes tested, with respect to the number of SNPs genotyped and associated coverage. To ensure that a workflow using the Phusion™ U Multiplex PCR Master Mix would be compatible across diverse sample types, this study optimized the number of PCR cycles using commercially available genomic DNAs, along with DNA from reference buccal swabs and environmental samples (total n = 12). Optimized conditions yielded 98.2 ± 0.45% autosomal SNP loci recovery in positive controls. The developed workflow should be straightforward to implement by forensic laboratories and suitable for processing reference and casework samples.

Next Generation Sequencing, Single Nucleotide Polymorphisms, Alternate Workflow