B70 The Development of the Precision ID GlobalFiler™ Next Generation Sequencing (NGS) Short Tandem Repeat (STR) Panel

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After attending this presentation, attendees will be aware of the development of sequencing STRs on an NGS platform and better understand visualizing and analyzing data with a new software platform.

This presentation will impact the forensic science community by presenting an NGS system developed and optimized for STR sequencing and data analysis. The resulting STR NGS profiles can be used in adjunct with partial Capillary Electrophoresis (CE) STR profiles from difficult casework samples.

Not unlike CE STR kit development, extensive studies were performed for new primer and master mix designs. Since these designs are made for NGS, they were developed to be compatible with library and template preparation techniques. Additionally, novel bioinformatics algorithms and software were invented to visualize and analyze data and allow analysts to transition from CE and NGS data and vice versa.

The Precision™ ID GlobalFiler™ NGS STR Panel targets 31 autosomal STRs and 4 sex-determining markers. The Precision™ ID DL8 Kit for Library Preparation on Ion Chef™, the Ion S5™ Precision™ ID Chef & Sequencing Kit for Template Preparation, and Ion S5™ Sequencing Systems for sequencing were developed to sequence STRs. Additionally, there are 53 Single Nucleotide Polymorphisms (SNPs) In Flanking (SIFs) regions surrounding the repeat. These SIFs allow for further discrimination of mixtures between major and minor contributors, as well as what may look like a stutter with CE versus a minor contributor allele.

In concert, the new Converge 2.0 Software provides an optimized pipeline for STR panel sequence analysis and reporting. Developed with a GMID-X-like interface, with browser-based navigation, Converge can be directly or indirectly connected to the Ion Torrent™ server. This allows secondary analysis data to be automatically transferred to Converge after sequencing runs are complete.

A study utilizing 34 known samples were sequenced with the Precision™ ID system workflow and concordance with previously generated CE genotyping assessed. In addition, 32 of the samples were used to create 1:10 and 1:20 mock mixture sample sets of both genders (male:female, male:male, and female:female). The mixture samples were a stress test for both the STR bioinformatic algorithms and detection of a low-level male contributor(s). High levels of concordance between the CE genotype and Massively Parallel Sequencing (MPS) genotype were observed and resulted in accurate results displayed in simplified STR and International Society of Forensic Genetics (ISFG) long-sequence nomenclature. Stutter ratios in the MPS system are elevated in comparison to those in a CE fragment length-based system due to the multiple rounds of PCR performed; however, even with higher stutter, the more complex and compound STRs allow for determining between stutter and minor contributor when the repeat motif of the minor contributor differs significantly from the stutter allele of the major contributor.

Next Generation Sequencing, Massively Parallel Sequencing, Mixture Analysis

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