B139 Use of the Ion PGM™ System for Typing Human Identity Marker Systems

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After attending this presentation, attendees will understand the methodology and sequencing chemistry used in massively parallel sequencing on the Ion Personal Genome Machine® (PGM™) platform and the capability of this system to sequence and interpret large genetic marker panels.

This presentation will impact the forensic science community by providing current progress on the use of massively parallel sequencing as it pertains to forensic DNA analysis. In addition, attendees will learn about the quantity and quality of the data generated by Ion PGM™ system.

Massively Parallel Sequencing (MPS), also known as next generation sequencing, technologies provide the scientific community with novel and enhanced approaches to DNA typing. While capillary electrophoresis-based technologies have been considered the gold standard for human identity typing applications, the current technology has notable limitations in intra-repeat variation resolution, sample scalability, and throughput of markers that can be typed. MPS has the potential to overcome these limitations with its ability to multiplex different types of forensically relevant genetic markers, analyze a large number of markers simultaneously, and sequence multiple samples per run. Additionally, MPS data may offer a new avenue for interpretation of mixtures. The Ion Torrent™ PGM™ is a benchtop sequencer that uses semiconductor sequencing chemistries and laboratory workflows that enable high-throughput and quick run times at a reasonable cost.

Twelve genomic samples, containing total DNA ranging from ~42ng to 280ng, were provided by a third party (the Green Mountain Conference) for a blinded genetic study. The mitochondrial genome and three Ion PGM™ panels containing human identity Single Nucleotide Polymorphisms (SNPs), ancestry informative SNPs, and Short Tandem Repeats (STRs) were sequenced on the Ion PGM™ system and analyzed for these 12 samples. Sequencing and genetic analysis for all four genetic systems were completed in a reasonably quick time frame by one individual. Completeness of genetic profiles, depth of coverage, strand balance, and allele balance were evaluated as informative metrics for the quality and reliability of the data produced. The autosomal SNPs from the human identity SNP panel reached an average read depth of 2,233. For this study, 99% of these SNPs had an average strand balance of 60% to 100%, and 100% of these SNPs had an average allele coverage ratio of 30% to 50%. The Y-chromosome Single Nucleotide Polymorphisms (Y-SNPs) from the human identity panel reached an average read depth of 975 while 97% of these SNPs had an average strand balance of 60 to 100 percent. The SNPs from the ancestry SNP panel reached an average read depth of 1,511 and 98% of these SNPs had an average strand balance ratio of 60% to 100% and 94% of these SNPs had an average allele coverage ratio of 30% to 50%. The large number of SNPs in these panels provides information on individual identification, familial relationships, and population background for the samples analyzed. The average allele coverage ratios for the STR markers ranged from 70% to 100% and the STR genotypes generated by MPS were in complete concordance with genotypes generated by standard capillary electrophoresis-based technologies. Additionally, intra-STR SNPs identified by MPS offer the potential for increased discriminatory power and improvement in mixture analysis. MPS coverage across the entire mitochondrial genome ranged from 489 to 7,029 in read depth. The average percent positive strand coverage for the entire mitochondrial genome ranged from 30% to 86% and indicated that sequence data from both strands of the mitochondrial DNA were captured. Mitochondrial variants identified among this sequence data were used to generate haplogroups for each sample and ultimately provide information on population background and maternal relationships. Sample information provided by Green Mountain subsequent to the blinded study supported that reliable results had been produced for all 12 genomic samples. The strength and depth of these results warrant expanded validation studies of current MPS technologies (which are ongoing) and continued development of tools for data analysis.

Massively Parallel Sequencing, Forensic DNA Typing, Human Identity