SEQUENCING CHEMISTYR CHANGES GREATLY IMPROVE HID-STR PERFORMANCE ON Ion PGM™ and Ion S5™

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As next-generation DNA sequencing (NGS) technology ushers in the era of precision medicine, Ion Torrent™ is emerging as a key player in the industry. Using semiconductor technology, we have developed a fast, low-cost, and accurate massively parallel sequencing (MPS) method which utilizes the by-product of a well-defined biochemical process – the release of a hydrogen ion upon the incorporation of a nucleotide by a polymerase. One exciting application for the Ion Torrent™ platform is in the field of human identification (HID), which is a major component of forensics. HID is in part based on the detection of short tandem repeat markers (STRs) which vary in length from person to person. It requires sequencing of templates containing significant regions of secondary structure that are refractory to most methods of amplification and in general are difficult to amplify.

Secondary structures and complex sequences can lead to stutter or polymerase slippage which keeps the NGS workflow from achieving CE-like quality. To ameliorate polymerase stutter and increase the ability to sequence full allele range for various markers we have examined different polymerase mutants, cycling and sequencing conditions. Additionally alternate library amplification methods and nucleotide flows were investigated for improvements in stutter and overall performance.

By supplementing CE (the gold standard in HID) with NGS capability, we allow for analysis of samples intractable to the traditional workflow. Unlike CE, NGS is not limited in the number of loci that can be simultaneously detected. The increased loci detection and the ability to detect sequence variation with NGS allows improved capability to identify mixtures and degraded DNA. Moreover, future addition of phenotypic and ancestry markers with the NGS platform will, over time, greatly expand the utility of HID.