

## **PERFORMANCE OF THE HID-ION AmpliSeq™ ANCESTRY PANEL WITH FORENSIC-TYPE SAMPLES ON THE ION TORRENT PGM™ AND ION S5™ SYSTEMS**

Ashley M. Cooley<sup>1</sup>, Kelly A. Meiklejohn<sup>1</sup>, [James M. Robertson](#)<sup>2</sup>, Tracey Dawson Cruz<sup>3</sup>

<sup>1</sup>Visiting Scientist Program, Research Support Unit, Federal Bureau of Investigation Laboratory Division

<sup>2</sup>Research Support Unit, Federal Bureau of Investigation Laboratory Division

<sup>3</sup>Department of Forensic Science, Virginia Commonwealth University

Single nucleotide polymorphisms (SNPs) can provide information on evidence such as a suspect's biogeographical ancestry, which cannot be gleaned from traditional DNA analysis using short tandem repeats (STRs). Thermo Fisher Scientific released the HID-Ion AmpliSeq™ Ancestry Panel, a 165 SNP panel for ancestry prediction that was initially compatible with the manufacturer's massively parallel sequencer (MPS), the Ion Torrent Personal Genome Machine® (PGM™). The workflow using the panel with the PGM™ involved several, time-consuming manual steps, including making the templating solutions and loading the chips. Several reports (n, 5) have been published that evaluated the ancestry panel with forensic-type and high-quality samples on the PGM™. In 2014, the manufacturer released the Ion Chef™ robot, followed by the Ion S5™ MPS in 2016. The robot performs the templating using reagent cartridges and loads the chips. The chemistry was altered to allow higher-throughput of the S5™, although the overall molecular biology remained unchanged. The objective of the work reported here was to compare the performance of the two MPS systems, the Ion PGM™ and Ion S5™ and to ascertain if the changes in the workflow and chemistry produced different results.

For performance comparison of the two systems, mock forensic-type samples (e.g., toothbrush, razor and freshly shed hair, nail clippings, cigarette butts, personal water bottles, soda cans, chewing gum and bone; n,16) were used to manually make the libraries using 1.0, 0.5, 0.2 and 0.05 ng DNA. For controls, libraries were made from high-quality samples (e.g., blood, saliva and sexual fluid of the donors in the mock series and run on the PGM™; n,6) . Libraries were templated either with the Ion One Touch™ 2 System (for the PGM™) or on the Ion Chef™ (for the S5™). Sequencing was performed with 8 chips on the PGM™ and with 4 chips on the S5™. Resulting sequencing data were analyzed with the manufacturer's plug-in (HID SNP Genotyper).

The chip metrics (e.g., percent ISP loading, usable reads, final library, low quality, adapter dimer, clonal and enrichment) were similar for the two systems. The total coverage per SNP was higher for the S5™ as expected due to the higher number of wells per chip and SNP quality was higher. Predictions were 99.3% concordant for the mock forensic-type samples sequenced on both MPS systems and between chips. The results show that the sequencing process has not changed with updates in chemistry and workflow; further, our work supports the conclusions from earlier reported work using the PGM™.