A093 Sequencing the Novel Coronavirus Using the Ion AmpliSeq™ SARS-CoV-2 Research Panel

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Introduction: Tracking the spread and evolution of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is important to help address the global crisis. SARS-CoV-2 sequencing allows real-time epidemiology, phylogenetic studies, virus variant detection and antigen evolution tracking. Here we present our experience with the Ion AmpliSeq™ SARS-CoV-2 Research Panel which targets 237 amplicons specific to the SARS-CoV-2, and 5 human gene expression controls. **Methods:** Nucleic acid was isolated from nasopharyngeal or oropharyngeal swab specimens. We tested two paired methods of extractions/quantification: (1) EZ1 Virus Mini Kit v2.0 for extraction, followed by the CDC 2019-Novel Coronavirus Real-Time reverse transcriptase polymerase chain reaction Diagnostic Panel and (2) Abbott RealTime SARS-CoV-2 assay for both the extraction and quantification. Samples were then normalized based on their calculated viral copy number. After reverse transcription, libraries were prepared on the Ion Chef™ Instrument using the Ion AmpliSeq™ Kit for Chef DL8. Positive genomic RNA controls were obtained from the BEI Resources Repository (NR-52285). Following templating and chip loading, samples were sequenced using the Ion GeneStudio S5 System. Variant annotation was performed with SARS-CoV-2 plugins in Torrent Suite™ Software using NC_045512.2 as a reference sequence. The panel, with an amplicon length range of 125–275 bp, provides >99% coverage of the SARS-CoV-2 genome. **Results**: Sequencing was successful with both paired extraction-quantification methods. A major advantage of the Abbott RealTime SARS-CoV-2 assay is its ability to test a high volume of patients due to automation. Various viral copy numbers were used for the starting point of library preparation. Quality passing data were obtained with a viral copy number of 50, but we routinely used 500 copies. For the run performance metrics, we aimed for one million reads per sample with a uniformity > 90% and reads on target >99%. We also confirmed that the ratio of viral versus human reads is >1. The BEI positive control had consistent metrics through several runs, with three expected variants detected each time. **Conclusions:** The Ion AmpliSeq™ SARS-CoV-2 Research Panel proved to be a flexible method for viral sequencing from a variety of sample types and viral loads, leading to a better understanding of the epidemiological impact of the virus as well as to help therapeutic development.