

Scaling a High Throughput Next Generation Sequencing Laboratory for Community Surveillance of Sars-Cov-2 Viral Variants During the Public Health Crisis

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Introduction: As threats to global health evolve, it is imperative that clinical laboratories are prepared to quickly respond through rapid method validation and laboratory scaling for diagnostics and surveillance of emerging infectious diseases. Here we report an optimized workflow using the Illumina® COVIDSeq RUO Kits and the NovaSeq 6000 sequencer for high throughput sequencing of SARS-CoV-2 positive patient specimens. Sample preparation for high quality next-generation sequencing (NGS) includes multiple, time-consuming steps to reverse transcribe cDNA, to amplify and index the genome targets, and to pool, quantify, and normalize sequencing libraries. These manual workflows can take up to 48 hours before the samples are loaded to the sequencer. In March 2021, Aegis Sciences BioPharma Laboratory validated a modified workflow to prepare 3072 samples for sequencing in just 10 hours.

Methods: The Illumina® COVIDSeq RUO reference guide was used with modifications. Sequencing libraries were generated at 4X efficiency by prepping samples in 384-well PCR plates with modified reaction volumes instead of the recommended 96-well format. Furthermore, custom scripts for Tecan® EVO liquid handlers automated the manual pipetting steps resulting in faster and concurrent upstream workflows and lower error rates, and automated sample pooling allowed team members to simultaneously quantify and dilute libraries for loading to the sequencer the same day. Lastly, custom applications were developed to generate plate maps for quality control and sequencing sample sheets for immediate demultiplexing and FASTQ generation following sequencing.

Results: This customized procedure for use with the Illumina® COVIDSeq RUO Kits and the NovaSeq 6000 sequencer allows four team members to prepare (10 hours), sequence (36 hours), analyze (10 hours), and report (8 hours) 3072 high-quality SARS-CoV-2 sequences with an average turnaround time of 4 days. With additional NovaSeq 6000 sequencers (3) and concurrent workflows (8 team members), Aegis can process and report ~27,000 sequences in one week.

Conclusions: As of November 2021, Aegis has generated >250,000 SARS-CoV-2 genome sequences, accounting for 14% of the United States' submissions to the GISAID database. These data include over 400 different SARS-CoV-2 lineages identified in 50 states and Puerto Rico. Through the ability of Aegis Sciences BioPharma Laboratory to rapidly implement and scale this SARS-CoV-2 surveillance sequencing assay, we have made significant contributions to the emergence, identification, and community spread of SARS-CoV-2 variants.