SARS-CoV-2

Highly sensitive detection of SARS-CoV-2 variants with the Genexus Integrated Sequencer

Ion Torrent™ next-generation sequencing (NGS) solutions are part of a complete specimen-to-report workflow that features intuitive, robust analysis tools to help researchers with limited bioinformatics experience interpret their data. Herein we describe how the Ion Torrent™ Genexus™ Integrated Sequencer (Cat. No. A45727) can be used to obtain full SARS-CoV-2 genome sequences for variant surveillance in less than a day with automated analysis and minimal hands-on time.

Introduction

As SARS-CoV-2 continues to evolve, there is an ongoing need for genomic surveillance to quickly identify emerging variants and monitor viral transmission. SARS-CoV-2 variants can potentially reduce the effectiveness of public health measures, so it is important to understand more about how the virus is evolving to combat its spread. Studying variants like Omicron is also necessary for the development of new treatments and vaccines as well as for understanding how disease severity changes.

Variant surveillance requires full genome sequencing to detect new mutations across the SARS-CoV-2 genome. Unfortunately, most NGS workflows require specialized training and equipment and can take several days to deliver results. This can potentially delay a public health response. The Genexus Integrated Sequencer provides a rapid and highly automated NGS workflow for SARS-CoV-2 surveillance that can enable laboratories to go from sample to variant report in approximately one day with minimal hands-on time. This set-up-and-go workflow increases reproducibility, improves laboratory efficiency, and makes it easy to adopt NGS for genomic surveillance. Here we explain how to run the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay GX (Cat. No. A51307) on the Genexus Integrated Sequencer for robust and highly sensitive SARS-CoV-2 variant detection.

Workflow features

- The Genexus Integrated Sequencer enables complete workflow automation—from nucleic acid to report—for SARS-CoV-2 sequencing in as little as 24 hours (Figure 1).
- The Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX provides 99.9% coverage of the SARS-CoV-2 genome. It is designed to be highly sensitive, so the assay provides excellent genomic coverage even when viral titers are low (C_t >28).
- Ion Torrent™ Genexus™ Software automates post-sequencing run analysis for variant annotation (SARS_CoV_2_annotateSnpEff), FASTA consensus sequence generation for streamlined epidemiological database uploads (generateConsensus), and lineage assignment (SARS_CoV_2_lineageID).

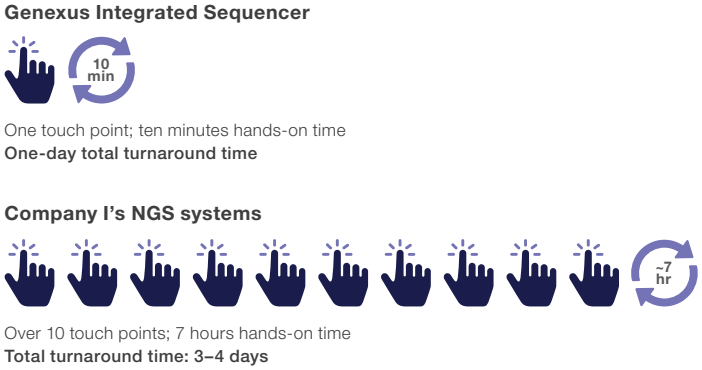

Figure 1. Comparison of the Genexus Integrated Sequencer targeted NGS workflow for SARS-CoV-2 sequencing and a different supplier's NGS workflow. The Genexus Integrated Sequencer enables laboratories to go from nucleic acid to variant report in a single day with minimal user intervention.

ion torrent

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX is a fast, accurate, and comprehensive whole-genome sequencing assay for surveillance of SARS-CoV-2, including variants of concern. Ion AmpliSeq™ technology is based on multiplex PCR and provides flexibility for total RNA inputs of as low as 1 ng. The intelligent assay design includes variant-tolerant primers that enable detection of emerging variants, and it offers high sensitivity for detection of the virus in low-titer samples. The assay consists of two primer pools that target 237 amplicons 125 to 275 bp in length, which are tiled across the SARS-CoV-2 genome. The assay also includes five primer pairs that target human controls. The 237 viral amplicons provide >99% coverage of the SARS-CoV-2 genome (~30 kb), while the other primer pairs serve as gender identification controls and internal controls for library generation.

Materials and methods

Two purified Thermo Scientific™ AcroMetrix™ Coronavirus 2019 (COVID-19) RNA Controls (RUO) (Cat. No. 954519) and an ATCC™ heat-inactivated SARS-CoV-2 control (VR-1986HK) were used to demonstrate the simple workflow and excellent performance of the Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX on the Genexus Integrated Sequencer. Serial dilutions of the RNA controls were prepared and spiked into 5 ng of human RNA to obtain final viral copy numbers ranging from 50 to 500.

Sample plates were placed on separate Genexus sequencers along with the primers for the Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX, the Ion Torrent™ GX5™ Chip and Genexus™ Coupler (Cat. No. A40269), and consumables from the Ion Torrent™ Genexus™ GX5™ Starter Pack-AS (Cat. No. A40279). The setup could support 16 samples, or 32 library reactions, across two sequencing lanes per instrument. After entering the sample information in the Genexus Software interface, runs were created and executed using one of two preinstalled assay definition files. We used the SARS-CoV-2 Low Titer Research Assay file for Plate 1 (0–200 copies) and the SARS-CoV-2 Research Assay file for Plate 2 (0–200,000 copies). Quality control (QC) metrics and the base and variant calling results were then reviewed. The results were automatically generated by the built-in SARS_CoV_2_annotateSnpEff, SARS_CoV_2_ControlStat, coverageAnalysis, generateConsensus, and SARS_CoV_2_lineageID plug-ins.

The total turnaround time from RNA to variant report for each run is in Table 1. All of the automated steps of the Genexus Integrated Sequencer are shown in Figure 2. Figure 3 summarizes analysis of the SARS-CoV-2 genome with Genexus Software.

Table 1. Total turnaround times for the Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX run on the Genexus Integrated Sequencer using low-titer and standard run templates.*

Run template	Total turnaround time
SARS-CoV-2 Low Titer Research Assay file	25 hr 11 min
SARS-CoV-2 Research Assay file	24 hr 57 min

* Total turnaround time includes automated cDNA synthesis, library preparation, template preparation, sequencing, and post-run analysis for 16 samples run across two lanes on the GX5 Chip. Post-run analysis time is based on the total number of reads returned. Faster turnaround times can be achieved with smaller sample batches.

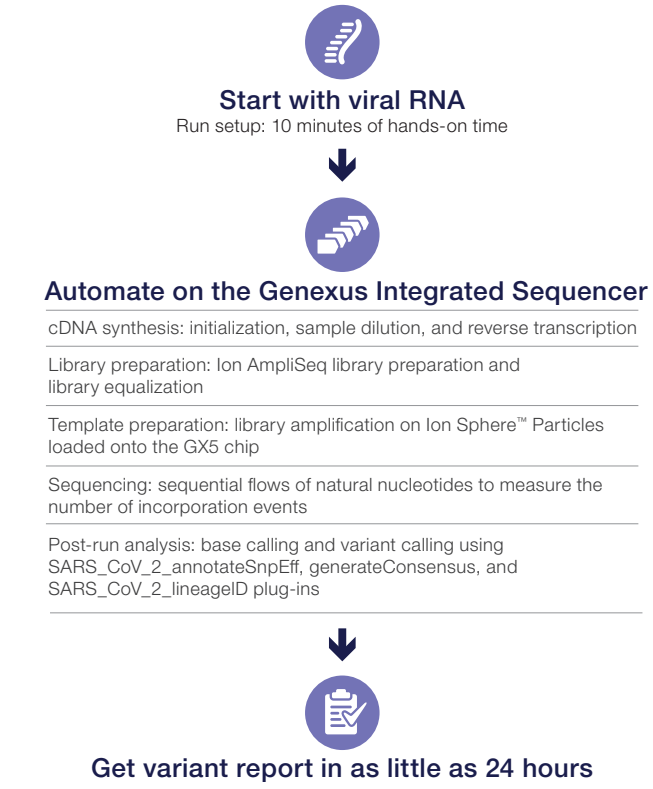

Figure 2. Automated workflow for the Genexus Integrated Sequencer. The Genexus Integrated Sequencer automates all steps from cDNA synthesis through post-run analysis. The approximate turnaround time shown is for 16 SARS-CoV-2 research samples run in two lanes on the GX5 chip.The next run can be started on the Genexus Integrated Sequencer while analysis from the previous run is completed.

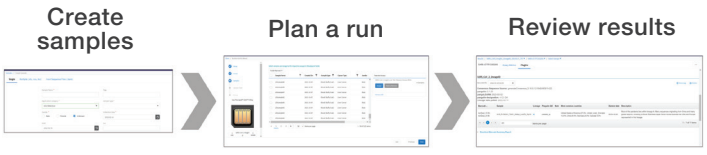

Figure 3. Analyze the SARS-CoV-2 genome in three simple steps with Genexus Software. Set up samples with Genexus Software using the Create Samples menu. Then, plan a sequencing run by selecting the appropriate template for standard or low-titer samples. Once sequencing is complete, Genexus Software will automatically run plug-ins to analyze your results. Simply review the QC metrics and data to complete your analysis.

Results

All samples across both runs returned reads that mapped to the five human expression controls for the Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX, which indicated that automated library generation on the Genexus Integrated Sequencer was successful. Reads that did not map to the human expression controls potentially represented non-human sequences and were mapped against the SARS-CoV-2 reference to determine the percentage of on-target base reads (Figure 4). The percentage of on-target reads ranged from 98% to 99.9% for all viral copy numbers tested across both controls.

The impact of base coverage on resolution depth for variant analysis was also evaluated. Average target base coverage versus copy depth was analyzed for samples with low titers (Table 2). The assay yielded robust results with >98% coverage at 20x and 500x coverage depths, even for samples that contained 50 viral copies. The high degree of resolution and coverage provided superior sensitivity for sequencing genomic variants.

Conclusion

Rapid and efficient detection of new SARS-CoV-2 variants is essential to slow the spread of the virus. Researchers need an easy-to-adopt solution to quickly and accurately sequence the SARS-CoV-2 genome for variant surveillance. Pairing the Genexus Integrated Sequencer with the Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX provides a highly automated NGS workflow from nucleic acid to report in a single day. The workflow will enable laboratories to survey the entire SARS-CoV-2 genome faster than ever before. With unmatched ease of use and lower hands-on time requirements than other technologies, this new solution makes the power of NGS accessible to laboratories that want to implement SARS-CoV-2 surveillance quickly and easily.

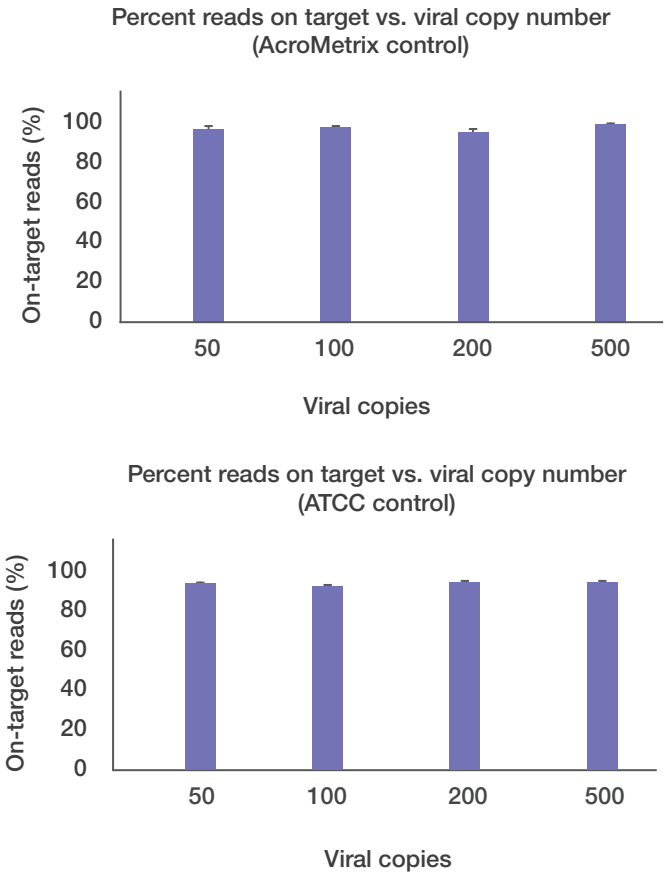

Figure 4. Percentage of on-target base reads versus viral copy number for the AcroMetrix (A) and ATCC (B) controls. The AcroMetrix RNA control and the ATCC contrived control were spiked into 5 ng of human RNA, and samples were prepared in duplicate. The data were generated using the low-titer Ion AmpliSeq SARS-CoV-2 Insight Research Assay GX template with 16 samples in two lanes on the GX5 chip.

Table 2. Average target base coverage for samples with low viral copy numbers analyzed on the Genexus Integrated Sequencer.

Coverage depth	50 copies	100 copies	200 copies	500 copies
20x	100.0%	100.0%	100.0%	100.0%
500x	99.1%	98.9%	99.5%	99.4%

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