SARS-CoV-2

Highly sensitive detection of SARS-CoV-2 variants with the Ion GeneStudio S5 System

Ion Torrent™ next-generation sequencing (NGS) solutions are part of a complete specimen-to-report workflow that includes intuitive and robust tools that can facilitate analysis and help researchers with limited bioinformatics experience interpret their data (Table 1). Here we describe how the Ion GeneStudio™ S5 System can be used for SARS-CoV-2 surveillance and highlight additional solutions for infectious disease research.

Introduction

Recurring SARS-CoV-2 outbreaks have underscored the need for genomic surveillance to monitor viral transmission and quickly identify emerging mutations and variants. The SARS-CoV-2 genome continues to evolve while selective pressure increases. Some variants have acquired mutations that can increase transmissibility. These variants have the potential to reduce the effectiveness of public health measures. It is thus important to understand more about how the virus is evolving to combat its spread.

The Ion GeneStudio S5 system, Ion Torrent™ software, and Ion AmpliSeq™ assays are a complete solution for targeted NGS of the SARS-CoV-2 genome. The Ion GeneStudio S5 System provides the high sensitivity needed for effective SARS-CoV-2 surveillance, and it is compatible with automation to monitor mutations in real time.

Comprehensive infectious disease research solutions for the Ion GeneStudio S5 System

	Research area: Pathogen surveillance	Research area: Drug/antimicrobial resistance and pathogen identification
Ion Torrent solutions for Ion GeneStudio S5 System	• Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay* • Ion AmpliSeq™ Ebola Research Panel • Ion Torrent™ Total RNA-Seq Kit	• Ion AmpliSeq™ Antimicrobial Resistance (AMR) Research Panel • Ion AmpliSeq™ Pan-Bacterial Research Panel • Ion AmpliSeq™ TB Research Panel

Table 1. Key challenges in SARS-CoV-2 surveillance.

Challenge	Benefits of Ion Torrent NGS solutions
Variable viral loads and SARS-CoV-2 sample quality	The highly sensitive and accurate IonTorrent system is designed specifically for samples with low viral loads. The Ion AmpliSeq SARS-CoV-2 Insight Research Assay™ can be used to sequence samples with C_t of 28 or higher, allowing you to expand sample testing eligibility. You can also add biological samples directly to your workflow rather than isolating the virus.
Lack of resources for traditional RNA sequencing platforms	Each Ion Torrent assay is part of a specimen-to-report workflow that requires no additional resources, and Ion Torrent workflows are compatible with automation. Fewer manual steps are required in an automated workflow, reducing the risk of error and making cross-training easier.
Lack of bioinformatics expertise	Powerful and intuitive tools make NGS data analysis accessible to any laboratory, even laboratories without bioinformatics expertise. Streamlined uploading to sequence databases enables you to easily share your data with the research community.
Rapid turnaround times needed for outbreak management	Requires only ~45 minutes of hands-on time, and you can go from nucleic acid to data in less than 24 hours. There is no need to outsource with the complete in-house workflow—go all the way from a biological sample to a sequencing report.

* The Ion AmpliSeq SARS-CoV-2 Insight Research Assay is also compatible with the Ion Torrent™ Genexus™ System, which can deliver results in a single day with just two user touch points. To learn more about the Ion AmpliSeq SARS-CoV-2 Insight Research Assay, contact a sales representative or visit thermofisher.com/sarscov2insight.

Rapid and flexible workflow for targeted NGS for SARS-CoV-2 research

Semiconductor sequencing on the GeneStudio S5 System is based on Ion AmpliSeq™ HD technology for ultrasensitive NGS, which is enabling public health agencies to closely monitor transmission of SARS-CoV-2.

Benefits of the Ion GeneStudio S5 System for SARS-CoV-2 research:

- Superior sensitivity enables analysis of samples with low viral titers (C_t > 28).
- Interchangeable chips provide scalability and allow 3–200 samples to be run on a single platform for your specific application.
- Sequencing data can be obtained in as little as 2.5 hours.
- Automated workflows require minimal user intervention, which helps reduce training time and the potential for error. Automation also makes it easier to adopt NGS and improve laboratory efficiency.
- Using the Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay to detect single-nucleotide variations helps minimize the chance of substitution errors.
- The specimen-to-report workflow is intuitive, and robust analytics software makes SARS-CoV-2 surveillance accessible to laboratories without bioinformatics expertise.

Ion Torrent targeted NGS allows you to easily go from nucleic acid isolation to data analysis in less than 24 hours and requires only ~45 minutes of hands-on time (Figure 1). The complete workflow for the Ion AmpliSeq SARS-CoV-2 Insight Research Assay on the Ion GeneStudio S5 System is summarized in Table 2.

Figure 1. Workflow for targeted NGS on the Ion GeneStudio S5 System. Ion AmpliSeq™ libraries can be prepared manually or with the Ion Chef™ Instrument for emulsion PCR, enrichment, and loading onto Applied Biosystems™ Ion S5™ chips.

Table 2. Complete workflow for the Ion AmpliSeq SARS-CoV-2 Insight Research Assay on the Ion GeneStudio S5 System.

Sample	Library preparation with Ion Chef Instrument	Run sequence on GeneStudio S5 System	Data analysis
• Start with as little as 1 ng total RNA • 1–10 ng cDNA input per pool Handles a variety of sample types, including FFPE samples for retrospective studies.	• cDNA synthesis • Automated library preparation • Automated template preparation Use the Ion AmpliSeq™ Library Kit Plus or the Ion AmpliSeq™ Kit for Chef DL8.	• 200-base reads Run 3 samples with the Ion 510™ Chip or up to 200 samples with the Ion 550™ Chip.	Ion Torrent Suite Software plug-ins: • SARS-CoV-2_ coverageAnalysis for metrics reporting and visualization • SARS_CoV_2_variantCaller for variant calling • SARS-CoV-2_lineageID for variant annotation • generateConsensus for consensus sequence generation in FASTA format • SARS-CoV-2_lineageID for lineage assignment using Pangolin™ plug-in

Ion AmpliSeq SARS-CoV-2 Insight Research Assay for variant identification and transmission tracking

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay consists of 2 pools with amplicons ranging from 125 to 275 bp. With a control strategy for >99% coverage of the viral genome, even samples with low viral titers (C_t > 28) can be analyzed. This assay is part of a complete analysis solution that includes a plug-in package for variant identification, annotation, and coverage visualization. SARS-CoV-2 genome sequences can also be assigned lineages with an integrated Pangolin™ plug-in.

Data obtained with the Ion AmpliSeq SARS-CoV-2 Insight Research Assay

Nasopharyngeal swabs were analyzed after testing positive for SARS-CoV-2 with an orthogonal Sanger sequencing assay. The performance of the Ion AmpliSeq SARS-CoV-2 assay was excellent based on two key metrics: the percentage of on-target reads and coverage uniformity. Over 98% of reads were on target for samples that had high C_t values (Figure 2A), and coverage was uniform for samples with varying concentrations of the virus (Figure 2B).

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay provided over 90% coverage uniformity in an analysis of nasal swabs with viral copy numbers ranging from 50 to 10^6. Coverage even reached 100x in one run. This level of sensitivity can allow you to be confident in your results even when samples contain very little viral material. Using a targeted amplicon sequencing strategy enables high-quality sequences to be obtained with very little DNA or RNA. Highly efficient sequencing reduces the total amount of sequencing required, which in turn reduces overall cost and time requirements.

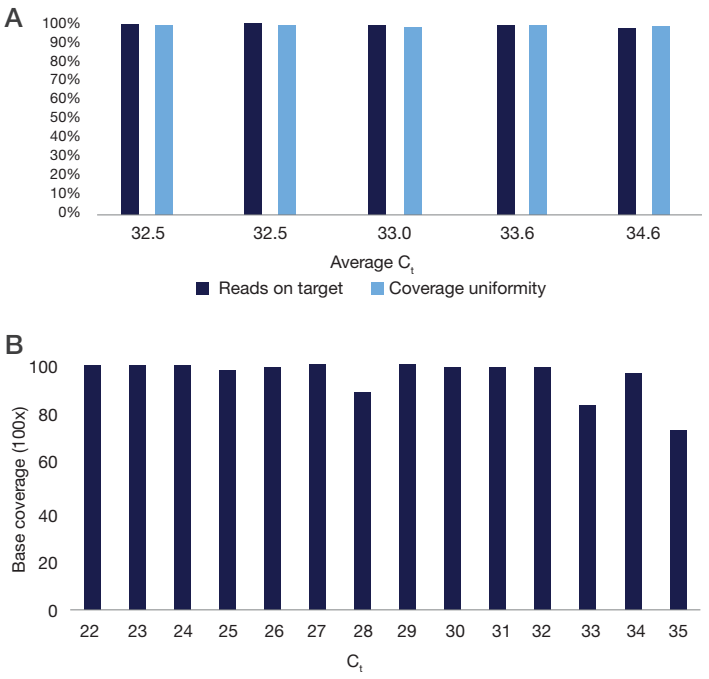

Figure 2. Performance and sensitivity of the Ion AmpliSeq SARS-CoV-2 Insight Research Assay. (A) Nasopharyngeal swabs with C_t ranging from 32.5 to 34.6 were analyzed in duplicate. Over 98% on-target reads and coverage uniformity were observed. **(B)** Up to 100x base coverage was observed for 21 shallow nasal swabs with high C_t values.

Ion AmpliSeq panels for infectious disease research

Other accurate and cost-effective Ion AmpliSeq panels are available for monitoring and surveillance of various pathogens. Our targeted solutions for infectious disease research include the Ion AmpliSeq Ebola Research Panel, the Ion AmpliSeq Pan-Bacterial Research Panel, the Ion AmpliSeq Antimicrobial Resistance (AMR) Research Panel, and the Ion AmpliSeq™ TB Research Panel (Table 3). Custom Ion AmpliSeq™ NGS panels can also be tailored to your specific research. Ion AmpliSeq technology is flexible enough to suit your needs whether you are interested in a few targets or hundreds, and it can be used to analyze any genome for infectious disease research.

Table 3. Features of Ion AmpliSeq panels for infectious disease research.

Ion AmpliSeq panel	Key features
Ion AmpliSeq Antimicrobial Resistance (AMR) Research Panel	• Detects 478 antimicrobial resistance genes against 28 classes of antibiotics. The panel can be used to analyze environmental and biological samples. • DNA panel with 2 pools for a total of 815 amplicons.
Ion AmpliSeq Pan-Bacterial Research Panel	• Detects 21 bacterial species and 364 resistance genes against 31 classes of antibiotics. • The DNA panel contains 2 pools. One pool contains 269 amplicons to detect 21 species, and 716 amplicons to assess the presence of 364 antibiotic resistance genes. The other contains 24 amplicons for 16S profiling of up to ~400,000 16S sequences in the Greengenes database.
Ion AmpliSeq TB Research Panel	• Identifies variants in *Mycobacterium tuberculosis* genes associated with antimicrobial resistance in tuberculosis (TB). • The DNA panel of 2 pools with 109 amplicons can be used to assess 8 genes related to antimicrobial resistance (*embB*, *eis*, *gyrA*, *inhA*, *katG*, *pncA*, *rpoB*, *rpsL*).
Ion AmpliSeq Ebola Research Panel	• Detects Ebola virus (EBOV). • The RNA panel has 150 amplicons in 2 pools that cover 99.49% of the EBOV genome.
Ion AmpliSeq custom NGS panels	• DNA and RNA panels can be customized for your specific research needs.

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay is also available for use on our latest innovation, the Ion Torrent™ Genexus™ Integrated Sequencer. A nucleic acid–to-variant report can be generated in about a day with minimal hands-on time. Find out how to bring simple, practical NGS to your lab.

Request a virtual demo at thermofisher.com/genexus

Conclusion

The Ion GeneStudio S5 System and our comprehensive portfolio of assays and analysis tools support multiple research applications. Streamlined workflows and a single system make it easy to adopt targeted NGS in any laboratory.

The Ion AmpliSeq SARS-CoV-2 Insight Research Assay can identify single nucleotide variations between sequences, which enables the detection of SARS-CoV-2 variants to study the evolution of the virus. The Ion AmpliSeq family of products also includes sequencing solutions for antimicrobial resistance research, pathogen identification, and more. The Ion GeneStudio S5 System and Ion AmpliSeq assays and panels provide fast, robust, and easy-to-use NGS workflows to support your infectious disease research.

Learn more about Ion AmpliSeq solutions at
thermofisher.com/ampliseq