

SARS-CoV-2 research applications using the Ion GeneStudio S5 system

Ion Torrent™ next-generation sequencing (NGS) solutions are part of complete sample-to-answer workflows that include intuitive and robust tools for easy analysis to help researchers focus on the biological meaning of their data, regardless of bioinformatics experience. In this white paper, we present the use of the Ion GeneStudio™ S5 Series System for multiple infectious disease research applications, including:

- **Complete sequencing of the SARS-CoV-2 genome** for studying virus evolution, contact tracing, infection severity, zoonotic transmission, wastewater epidemiology, and more
- **Molecular profiling of the immune repertoire** for deeper insight into the immune system, which could influence the immune response to infection and vaccination
- **Analysis of additional factors that may influence host responses to infection**, such as the human microbiota, the transcriptome, and gene expression

Introduction

The SARS-CoV-2 crisis has highlighted the need for urgent research in many scientific disciplines to better understand the origin of the virus, its mechanisms and transmission, and its impacts on society. New discoveries in virology, immunology, and vaccine development research will be needed to combat the spread of the virus.

Targeted NGS provides rapid, comprehensive, and scalable solutions for many of these research areas and has been a trusted tool to study the impact of other infectious disease agents such as Ebola, Zika, H1N1, and other coronaviruses (e.g., MERS).

The Ion GeneStudio S5 system, assays, and software make up a complete targeted NGS solution that enables the speed and simplicity required for infectious disease research studies that go beyond single-gene analyses.

Comprehensive SARS-CoV-2 research solutions for the Ion GeneStudio S5 Series System

Research area	Viral discovery, typing, and epidemiology research	Host genetics, immune response, mechanisms, and progression	Vaccine development research
Research applications	<ul style="list-style-type: none"> • Viral identification and life cycle • Viral genome sequencing • Viral surveillance and epidemiology, including wastewater epidemiology • Infection severity • Zoonotic transmission 	<ul style="list-style-type: none"> • General host response • Biomarkers for infection severity • Targeted immune profiling • T cell and B cell characterization • Microbiome characterization 	<ul style="list-style-type: none"> • Vaccine response characterization • Prediction of adverse effects • Biomarkers for vaccine efficacy
Ion Torrent solutions for Ion GeneStudio S5 Series System	<ul style="list-style-type: none"> • Targeted sequencing—Ion AmpliSeq™ SARS-CoV-2 Research Panel • RNA sequencing—Ion Torrent Total RNA-Seq Kit 	<ul style="list-style-type: none"> • Immune repertoire analysis—Ion Torrent™ OncoPrint™ BCR IGH and TCR beta assays* • Microbiome analysis—Ion AmpliSeq™ Microbiome Health Research Kit • Gene expression and transcriptome analysis—Ion AmpliSeq™ Transcriptome Gene Expression Kit* 	

* Available for human and mouse species.

Flexible and fast targeted NGS workflow for infectious disease research

Ion Torrent™ semiconductor sequencing is based on ultrahigh-multiplex PCR—the same technology that enables public health agencies to closely monitor viral infections.

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

- **Scalable**—throughput capability and wide application breadth to perform assays for SARS-CoV-2 viral research, the immune repertoire, microbiome and transcriptome analysis, and more, all on a single platform
- **Rapid turnaround time**—get sequencing data in as little as 2.5 hours

- **Automated workflows**—minimizes errors by reducing user intervention, enables easier adoption of NGS, and improves lab efficiency
- **Accuracy of variants**—lower rate of substitution errors for SNVs
- **Superior sensitivity and higher success rates**—analyze samples with viral loads as low as 20 copies
- **End-to-end workflow**—from nucleic acid to analysis, including an intuitive and robust analytics solution for simple and accessible analysis regardless of bioinformatics expertise

With Ion Torrent targeted NGS, easily go from nucleic acid to data in less than 24 hours with ~45 minutes of hands-on time (Figure 1).



Figure 1. Targeted NGS workflow using the Ion GeneStudio S5 Series System. Ion AmpliSeq™ libraries are prepared manually or with the Ion Chef™ System. Libraries are then placed in the Ion Chef System for emulsion PCR, enrichment, and loading onto Ion S5™ Chips.

Key challenges in SARS-CoV-2 research

Challenge	Benefits of Ion Torrent NGS
SARS-CoV-2 samples vary in viral load and sample quality	<ul style="list-style-type: none"> • Highly sensitive and accurate—Ion AmpliSeq technology works well with low input amounts (as low as 1 ng of total RNA input) and can detect viral loads as low as 20 copies • Start with biological samples—instead of the isolated virus that may be required by other methods
Research labs that have pivoted to study the virus may not have the components or personnel required for traditional RNA-Seq platforms	<ul style="list-style-type: none"> • Complete solution—each Ion Torrent assay is part of a nucleic-acid-to-answer workflow • Automation capabilities—Ion Torrent workflows have automation capabilities that reduce user intervention, minimizing user errors*
Research labs that have pivoted to study the virus may not have bioinformatics expertise	<ul style="list-style-type: none"> • Powerful and intuitive analysis tools—make NGS data analysis accessible for any lab regardless of bioinformatics experience
Time is of the essence during this crisis	<ul style="list-style-type: none"> • Fast—only ~45 minutes of hands-on time; go from nucleic acid to data in less than 24 hours • No need to outsource

* The Ion AmpliSeq SARS-CoV-2 Research Panel is also available for the Ion Torrent™ Genexus™ System, which delivers results in a single day with just two user touchpoints. To learn more about the Genexus System, contact a sales representative or visit [thermofisher.com/coronavirus-genexus](https://www.thermofisher.com/coronavirus-genexus)

Ion AmpliSeq SARS-CoV-2 Research Panel for strain identification and tracking viral spread

The Ion AmpliSeq SARS-CoV-2 Research Panel consists of 2 pools with amplicons ranging from 125 to 275 bp for complete coverage of >99% of the viral genome and variants. This assay is part of an end-to-end solution that includes a plug-in suite developed in collaboration with scientists at the forefront of the SARS-CoV-2 crisis. The complete analysis solution enables variant annotation and reference-assisted or *de novo* sequence assembly of SARS-CoV-2-related samples.

Performance data from Ion AmpliSeq SARS-CoV-2 Research Panel early-access sites demonstrate:

- Superior performance in two key metrics for targeted sequencing efficiency—on-target reads and coverage uniformity.** High-efficiency sequencing optimizes the total amount of sequencing needed, which reduces the overall project costs and timeline. This performance was demonstrated by collaborators with biological samples that were positive for SARS-CoV-2 infection. The data in Figure 1A show over 95% reads on-target, and coverage uniformity for RNA isolated from nasopharyngeal swabs and for different concentrations of viral isolates.
- The ability to generate high-quality sequencing results from very low DNA or RNA inputs** is one of the advantages of amplicon-based targeted resequencing. The Ion AmpliSeq SARS-CoV-2 Research Panel provides over 90% coverage uniformity across a range of input viral copy numbers from 20 to 20,000 (Figure 1B). This level of sensitivity gives confidence in results for samples with even very low amounts of viral material.

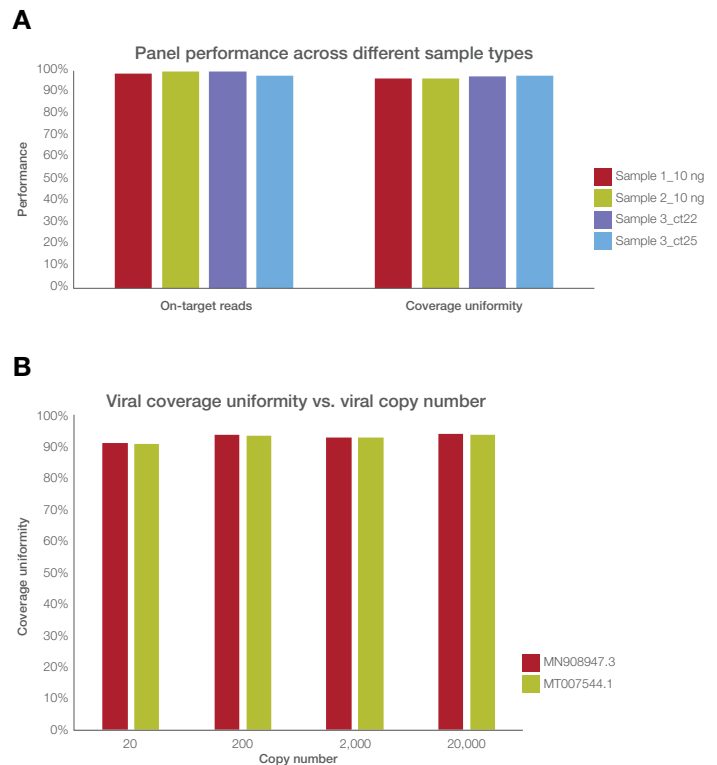


Figure 1. The Ion AmpliSeq SARS-CoV-2 Research Panel demonstrates superior performance and sensitivity. (A) High percentages of on-target reads and coverage uniformity were obtained with 10 ng of input RNA from nasopharyngeal swabs (red and green bars) and from serial dilutions of viral isolates (purple and blue bars). N = 3. **(B)** More than 90% coverage uniformity was demonstrated with the Ion AmpliSeq SARS-CoV-2 Research Panel on samples with known viral copy numbers. N = 2.

Complete workflow for Ion AmpliSeq SARS-CoV-2 Research Panel on Ion GeneStudio S5 system

Add sample	Prepare	Run sequence	Analyze data
<p>Start with as little as 1 ng of total RNA input</p> <p>1–10 ng cDNA input per pool</p> <p>Handles variety of sample types, including FFPE for retrospective studies</p>	<p>Ion Chef instrument</p> <ul style="list-style-type: none"> cDNA synthesis Automated library preparation Automated template preparation <p>Ion AmpliSeq™ Library PLUS Kit or Ion AmpliSeq™ Kit for Chef DL8</p>	<p>Ion GeneStudio S5 system</p> <p>200 bp sequencing</p> <p>3 samples (Ion 510™ Chip) to 130 samples (Ion 550™ Chip)</p>	<p>Torrent Suite software</p> <p>Plug-ins:</p> <ul style="list-style-type: none"> COVID19AnnotateSnpEff IRMAreport AssemblerTrinity Variant Caller Coverage analysis

Learn more about the Ion AmpliSeq SARS-CoV-2 Research Panel and request a software demo at thermofisher.com/coronavirus-genestudio



Genomic epidemiology of SARS-CoV-2 from nextstrain.org. Data are from December 2019 through May 2020. Researchers contributing data to nextstrain.org have used the Ion AmpliSeq SARS-CoV-2 Research Panel to provide rapid and accurate results. Data shown with permission under a Creative Commons Attribution 4.0 International Public License.

Global tracking of SARS-CoV-2

A major challenge for researchers when exploring a cure is tracking and monitoring the differences across viral strains as they rapidly evolve during transmission. Tracking the virus allows us to identify its source, develop future treatments based on how it evolves, and put appropriate preventive measures in place to limit its distribution.

Read the blog post at thermofisher.com/ngs-sarscov2globaltracking

Case study: Implications of viral sequencing at Children’s Hospital Los Angeles

While tracking of the SARS-CoV-2 viral crisis on a global scale is important, it’s also imperative to track sources of virus transmission within local institutions, such as research hospitals. As a case study of this application, the Center for Personalized Medicine at Children’s Hospital Los Angeles (CHLA) performed NGS sequencing with the Ion AmpliSeq SARS-CoV-2 Research Panel and the Ion GeneStudio S5 system to analyze the phylogenetic relationship between 6 SARS-CoV-2 positive samples.

Individual SARS-CoV-2 particles mutate as the virus spreads in the community, but because the Ion AmpliSeq SARS-CoV-2 Research Panel enables identification of unique mutations, the researchers were able to determine that there were 2 unique lineages present in the samples. The first set of samples was from 4 health care workers who tested positive for SARS-CoV-2 by polymerase chain reaction (PCR), and the second set of samples was from a mother and son.

The Ion GeneStudio S5 system enabled NGS sequencing data to be generated quickly, enabling CHLA to draw conclusions about potential hospital viral spread within 48 hours of sample procurement. Library prep was performed by both manual and automated methods on the Ion Chef system, template prep was automated on the Ion Chef System, and sequencing was done using the Ion 510™ Chip. The automated workflow reduced imbalances between pools and improved panel performance.

Comparative analysis showed that, not unexpectedly, the mother and son had nearly identical strains of the virus that originated in Utah (with links to Europe), while sequences from the 4 health care workers were only distantly related to each other and to the sequences from the family (Figures 2 and 3). This confirmed that there was no transmission between the family and the health care workers.

This study demonstrates the utility of the Ion AmpliSeq SARS-CoV-2 Research Panel on the Ion GeneStudio S5 workflow for epidemiological research in a clinical research setting.

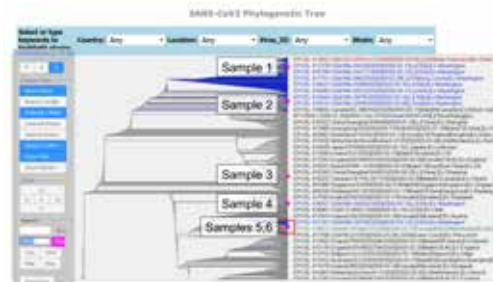


Figure 2. Phylogenetic tree showing the relationships among the 6 SARS-CoV-2 samples from CHLA.



Figure 3. The mother and son have closely related viral sequences.

Get the details for this study and watch the webinar at thermofisher.com/coronavirus-genestudio

Emerging research area: Wastewater-based epidemiology of SARS-CoV-2

Researchers studying the spread of SARS-CoV-2 may need to look beyond direct ways of tracking infections, such as testing and contact tracing in a geographic area. Wastewater-based epidemiology (WBE) may be another way to monitor SARS-CoV-2, as it provides information on viral prevalence in a population from asymptomatic people.

WBE is possible because SARS-CoV-2 RNA has been detected in fecal matter from individuals, which in turn is detectable in untreated wastewater samples. The high throughput and sensitivity of NGS is an ideal tool for WBE, as this method enables direct sequencing of samples to rapidly understand viral spread. A unique advantage of NGS for these types of studies is that variant analysis and strain identification would allow researchers to detect various SARS-CoV-2 strains

and their emergence and decline within a population over time.

Researchers in Milano, a hotspot for SARS-CoV-2 within northern Italy, utilized Ion Torrent sequencing technology to study the prevalence and spread of SARS-CoV-2 [1]. Data showed an increasing presence of the virus in human biological samples in late February, with a peak in mid-March, followed by a decline. Analysis of wastewater samples for the virus correlated with human sample data during that same time period.

The study indicates that wastewater-based epidemiology could provide practical markers to predict transmission of the virus in the current crisis and potential future crises.

Read the blog post at thermofisher.com/ngs-wbe

Rapid analysis of zoonotic transmission

As the human population grows, greater contact with wildlife may occur and result in the increasing probability of introducing novel diseases to humans that our immune system cannot defend us against. Identifying possible zoonotic sources of the disease is important in order to understand how to fight the current SARS-CoV-2 crisis and reduce the chances of a resurgence.

It has been speculated that SARS-CoV-2 may have originated in bats, and that another intermediate species may have been involved in the transfer. The Ion GeneStudio S5 sequencer and Ion AmpliSeq SARS-CoV-2 Research Panel enable rapid pathogen identification and can be used to determine whether or not the intermediate host was the pangolin or another species. Other opportunities for studying zoonotic transmission include research into how the virus may spread to household pets or animal farms.



Targeted NGS for immune response and vaccine development research

In addition to the transmission of infectious agents like SARS-CoV-2, the versatile Ion GeneStudio S5 system can be used to study the immune system, human gut microbiome, and transcriptome for a more complete understanding of how the body responds to infection or vaccination, and to discover biomarkers that may provide clues to why certain populations have severe, mild, or no adverse responses.

For deeper insight into the immune system

Ion Torrent™ TCR and BCR assays can be used for research on general host response and to identify disease severity biomarkers, perform targeted immune profiling, and characterize T cells and B cells to predict immune-related adverse events, as well as help to understand and improve vaccine efficacy by characterizing response to vaccines.

* Available for human and mouse species.

Learn more at thermofisher.com/ngs-immuneresponse

With the easy-to-use software suite developed specifically for immune repertoire analysis, researchers can easily analyze their own data and generate publication-quality graphs without a background in computational biology or bioinformatics.

BCR IGH assays*

- Identify and quantify somatic hypermutations
- Assess clonal expansion, clonal evolution, and isotype abundance
- Evaluate vaccine efficacy

TCR beta assays*

- Discover disease severity biomarkers
- Obtain deeper insights into TCR clonality and convergence
- Identify T cells that are responding to antigenic challenge

Scientist spotlight: TCR and BCR sequencing applications for SARS-CoV-2 research at National Jewish Health

The Genomics Facility of the Center for Genes, Environment and Health at National Jewish Health has been using Ion Torrent targeted NGS to study TCRs in T cell populations since 2013. They aim to examine interactions between TCRs and target antigens in various conditions in order to understand why severity of the immune response varies across different populations.

With the ongoing SARS-CoV-2 crisis, there is an opportunity to explore how the presence of the virus may affect T cells and B cells at a clone level in respiratory diseases such as sarcoidosis and cystic fibrosis.

To learn more about the research at National Jewish Health, watch the webinar titled “TCR and BCR sequencing: applications to clinical research” at thermofisher.com/ngs-immunology



“A huge advantage of the Ion GeneStudio S5 system is ... the software on the back end. It has been a game changer for our research at National Jewish Health.”

Brian P. O'Connor, PhD, Associate Professor and Scientific Director of Genomics, Center for Genes, Environment and Health, National Jewish Health

Expression analysis: Cytokines may be an indication of susceptibility to SARS-CoV-2

In a recent study, Broggi et al. [2] performed a retrospective analysis of SARS-CoV-2 positive samples and noted elevated levels of type III interferons. Type III interferons are a type of cytokine, a signaling protein released by cells as a response to viral infection, serving to increase the defense of neighboring cells.

To address this, researchers used mouse models and targeted sequencing with the Ion GeneStudio S5 system and the Ion AmpliSeq™ Transcriptome Mouse Gene Expression Kit to determine the molecular mechanisms elicited by interferons and their impact on immune pathology. Using this simple assay, expression analysis showed that interferon lambda (INF-λ) can interfere with lung tissue repair by inhibiting epithelial cell proliferation. In their studies, wild-type mice became more susceptible to bacterial superinfections after INF-λ induction. While more research is needed, these results illustrate how the presence of cytokines may be an indication of susceptibility to SARS-CoV-2 and may potentially impact the severity of infection.

Ion AmpliSeq Transcriptome Mouse Gene Expression Kit

- **Low sample input**—as little as 10 ng of total RNA
- **Fast gene expression profiling with simple workflow**—go from RNA to data in <2 days and only ~45 minutes of total hands-on time with the automated workflow of the Ion GeneStudio S5 Series and Ion Chef systems
- **Streamlined data analysis**—export data with Torrent Suite™ Software into existing analysis software

Learn more about gene expression and transcriptome analysis for human and mouse at [thermofisher.com/iontranscriptome](https://www.thermofisher.com/iontranscriptome)

Potential role of the gut microbiome as a biomarker for immune response and infection severity

The gut microbiome plays an integral role in viral infection and the immune response, triggering unique immune signaling pathways to combat viruses like SARS-CoV-2, but the mechanisms are poorly understood. It is thought that alterations to the relative abundance of particular taxa within the gut, namely those that offer antiviral immunity, may play a critical role in the future success of vaccines and susceptibility to infection.

With the Ion AmpliSeq™ Microbiome Health Research Kit, species-level sensitivity and specificity is enabled with an easy sample-to-answer workflow.

When used with the Ion GeneStudio S5 workflow, this assay enables cost-effective yet comprehensive profiling of the human gut microbiome and includes fully integrated data analysis tools for robust analytics to enable biomarker research on gut flora from stool samples.

Learn more about the Ion AmpliSeq Microbiome Health Research Kit at [thermofisher.com/ngsmicrobiome](https://www.thermofisher.com/ngsmicrobiome)

The Ion AmpliSeq SARS-CoV-2 Research Panel is also available for use on our latest innovation, the Ion Torrent™ Genexus™ Integrated Sequencer, enabling a nucleic-acid-to-variant report in under a single 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, comprehensive portfolio of assays, and analysis tools support multiple infectious disease research applications all on a single system, making it easy for any lab to adopt targeted NGS for SARS-CoV-2 viral research, host immune response research, and vaccine development research.

Ion Torrent targeted NGS provides high-resolution information to distinguish pathogen strains that differ by as little as a single nucleotide, opening up applications such as viral evolution and surveillance research. In addition to the Ion AmpliSeq SARS-CoV-2 Research Panel, other assays are available for related research areas such as host genetics, immune response, vaccine research, and microbiome and whole-transcriptome analysis.

References

1. Rimoldi S, Stefani F, Gigantiello A et al. (2020) Presence and vitality of SARS-CoV-2 virus in wastewaters and rivers. medRxiv preprint. <https://doi.org/10.1101/2020.05.01.20086009>
2. Broggi A, Ghosh S, Sposito B et al. (2020) Type III interferons disrupt the lung epithelial barrier upon viral recognition. bioRxiv preprint. <https://doi.org/10.1101/2020.05.05.077867>

Explore targeted NGS solutions for SARS-CoV-2 research at thermofisher.com/ngscoronavirus

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