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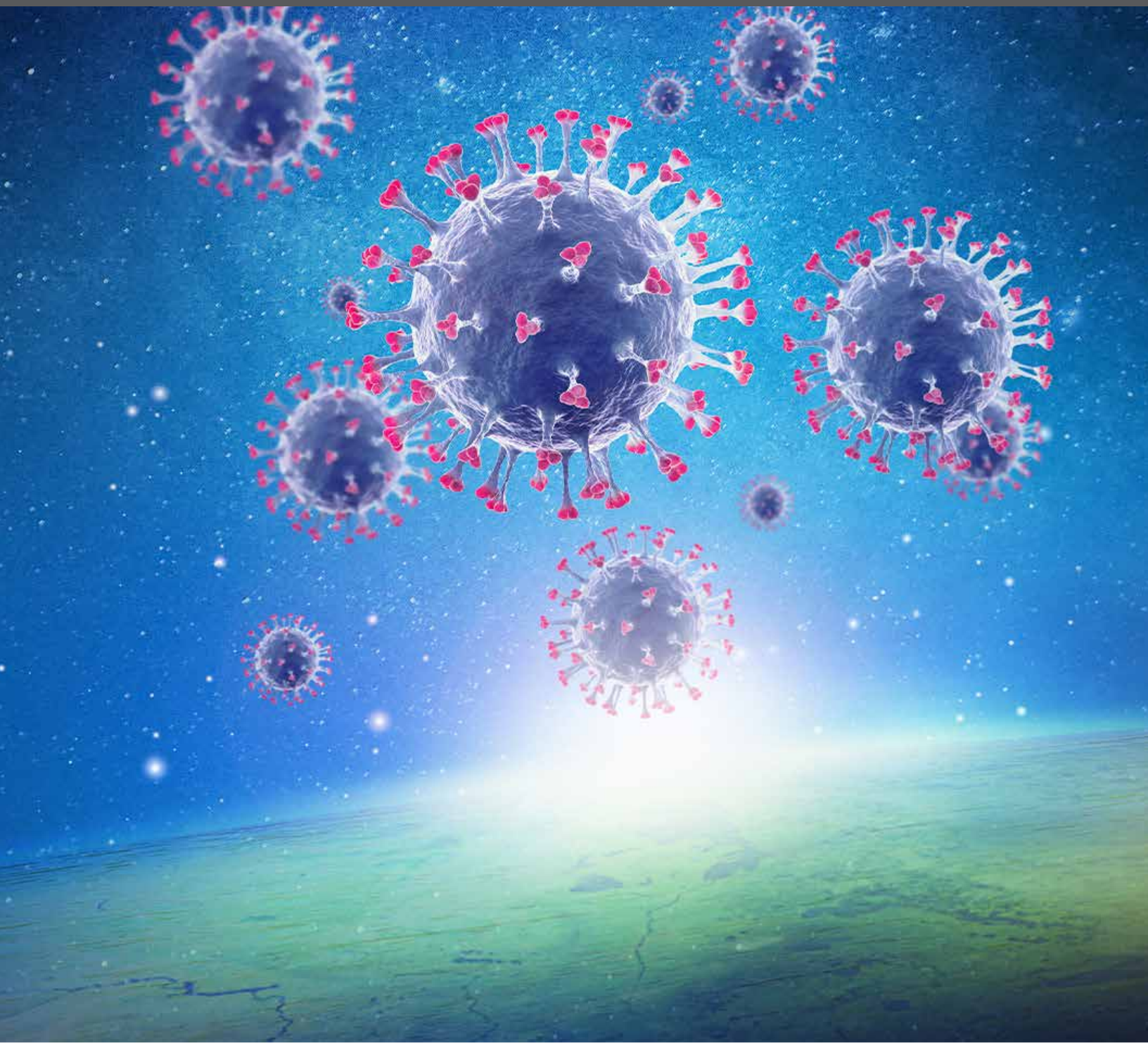


Advances in epidemiological research using next-generation sequencing

ThermoFisher SCIENTIFIC

Contents

Chapter 1—Epidemiology and the value of next-generation sequencing

The United States (US) Centers for Disease Control and Prevention (CDC) defines epidemiology as "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems." Epidemiology has been an important part of public health practice to aid in determining potential disease prevention strategies.

Some of the key questions epidemiologists look to address are: Where did an infectious disease originate and how is it being transmitted? What is the mutation rate of the pathogen? Are new strains emerging, and if so, what is the change in virulence across these strains? How do we use the existing data to predict where and when the next health crisis will occur?

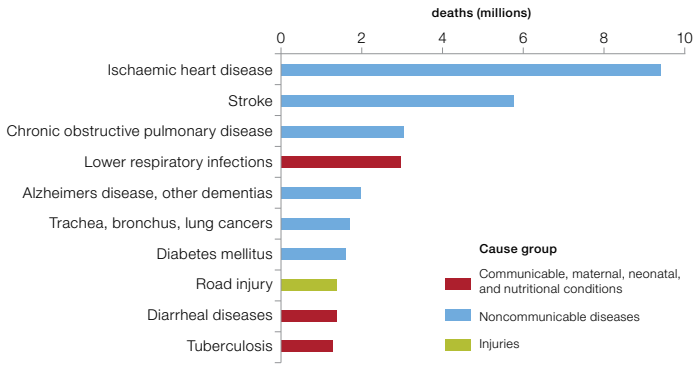


Figure 1. Top 10 causes of deaths in 2016.

Early on, several studies were carried out using two different strategies—case-control and cohort—to investigate a disease outbreak. Case-control studies use a retrospective design to look for disease-associated factors by comparing a disease group (the cases) with a control group. A cohort study design identifies the effects of a risk factor on a group of individuals exposed to a disease versus an unexposed group.

While epidemiology can be applied to many types of diseases (e.g., Alzheimer's disease and leukemia), epidemiological research has gotten significant attention in recent years due to acute outbreaks of emerging infectious disease agents such as swine flu (H1N1), Ebola, zika, and most recently SARS-CoV-2.

In fact, three of the top 10 global causes of deaths (acute lower respiratory infections including pneumonia and influenza, diarrheal diseases, and tuberculosis) are due to pathogenic (bacterial or viral) infection (Figure 1).

The global spread of infectious diseases as well as new technological advancements in the life sciences such as qPCR and next-generation sequencing (NGS) have brought about the burgeoning field of epidemiological research—molecular epidemiology. This area of research focuses on the contribution of genetics and environmental risk factors that can be identified using molecular biology methods.

The value of NGS

For molecular epidemiological studies that simply call for the detection of a disease, qPCR has been the common method of choice. However, with innovations in sequencing technology and advancements in bioinformatic analysis capabilities, NGS now enables a growing number of applications in epidemiology research. With improved, streamlined, and easy-to-use automated workflows, even researchers new to sequencing can conduct experiments in applications like the ones below.

Disease and health factors

Pathogen biology and retrospective analysis

To understand a disease, it is critical for epidemiologists to get insight into the biology of a pathogen, which paves way to understanding the process of disease transmission and also helps determine strain virulence.

For retrospective analyses, researchers use biobanked formalin-fixed, paraffin-embedded (FFPE) samples. Retrospective studies allow for a better understanding of virulence and host factors.

Advances in NGS technology will help enable parallel processing of many samples with a rapid and automated workflow and facilitate complete genome sequencing of the pathogen strain, including all variants and potential serotypes.

Contact tracing and tracking

Contact tracing has become a critical process to help local and state health departments implement and assess the effectiveness of infection control strategies. Once a confirmed case of infection has been identified, NGS and bioinformatics approaches can be used to sequence samples from individuals who may have come in direct "contact" with the infected person and thus map or "trace" the spread of infection. It is imperative that this process be fast and scalable with reliable accuracy to ensure proper mitigation of infection.

"The ability to very quickly run mulitple samples and accurately decipher key changes in the virus' genetic code will be crucial for the global scientific community to stay ahead of SARS-CoV-2 and to develop strategies against it."

Dr. Maria Rosaria Capobianchi,
Head of the Virology Department,
Lazzaro Spallanzani National Institute
for Infectious Diseases, Italy

Dr. Maria Rosaria Capobianchi saw the value of NGS for the discovery and understanding of novel pathogens such as SARS-CoV-2. The challenge was to find an NGS workflow that enables speed and accuracy and is simple and easy to implement.

Find out more at
thermofisher.com/sarsandngs

Monitoring

NGS also enables monitoring for the presence of diseases in an environment, as well as tracking transmission across species.

Wastewater-based epidemiology

Wastewater-based epidemiology (WBE) monitors community wastewater to check the status of public health. The high throughput and sensitivity of NGS is ideal for WBE; 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 strains and track their emergence and decline within a population over time.

Zoonotic transmissions

Zoonotic transmission involves the spreading of a disease from the source animal to humans via an intermediate species that carries the pathogen without getting sick. NGS can help identify the strain that can live in the intermediate host and may mutate and possibly transfer to humans.

Phylogenetic analysis/viral typing

Phylogenetic analysis is important to support identification of the initial source of infection, track geographic spread, and to enable development of disease control strategies. A wide variety of research methods can be used to differentiate viral variants, though molecular techniques are used most frequently. By sequencing a specific variable region of the viral genome, one can investigate whether selected isolates of a certain species are phylogenetically related.

Chapter 2—Considerations for utilizing NGS during a pandemic

NGS has proven to be a revolutionary tool in the quest to assess human health and understand disease. Until recently, a rapid and reliable method for identification of pathogens has been challenging. However, with NGS, millions of gigabases of sequence can be produced per day at a low cost and comprehensive full sequences of the possible pathogen, including multiple variants or serotypes, can be determined. The concern for those considering NGS as a new methodology is that the workflow will require skilled technicians and can take several days to complete, delaying the turnaround time. In order to address the needs of the lab, there are several criteria to consider when evaluating an NGS workflow.

Simplicity

When evaluating the adoption of an NGS workflow, it's important to consider how easy and intuitive the process is to learn and integrate into your research. You need to have confidence that the technology will work in your hands.

Productivity

It's also important that you reliably and consistently get robust and accurate results. This means that you will have minimal failures not only of the runs but also of individual sample processing.

When it comes to productivity, an automated workflow could optimize and reduce the number of steps, thus decreasing the risk of human error.

Innovation

For a technology innovation to endure, it is critical to consider its ability to evolve. To gain a competitive edge in the fields of science and business, continuous improvement of the NGS technology with regards to turnaround time, higher output and reads, and data processing is necessary. This will also help prevent the innovation being outpaced by newer solutions.

Overall workflow

When evaluating a new workflow for implemention in a lab, the main consideration is the value it adds. It is important to speculate whether the automated workflow is able to free up a significant amount of labor cost and enable time for possibly more relevant research activities.

The Ion AmpliSeq SARS-CoV-2 panel and Genexus Integrated Sequencer solution

Fortunately, many of the considerations that have been presented can be addressed by the innovations in the Ion Torrent™ Genexus™ Integrated Sequencer. The Genexus Integrated Sequencer enables a streamlined, automated workflow for complete genome sequencing and epidemiological study of SARS-CoV-2 that is simple to use even for researchers new to sequencing and only requires two user touch points from nucleic acid to report, without compromising the sequence coverage or accuracy.

Ion AmpliSeq SARS-CoV-2 Research Panel

The Ion AmpliSeq™ SARS-CoV-2 Research Panel consists of two pools with amplicons 125–275 bp in length for complete coverage of over 99% of the viral genome and variants.

Benefits of the Ion AmpliSeq SARS-CoV-2 Research Panel for the Genexus Integrated Sequencer include:

- **Rapid turnaround time**—our fastest NGS workflow for critically time-sensitive infectious disease research applications
- **Automation**—initial setup is followed by a hands-off workflow, enabling minimal user touch points, lowering user variability and increasing reproducibility of results
- **Accuracy of variants**—lower substitution errors for SNVs
- **Higher success rates**—analyze samples directly with low viral loads, as little as 1 ng of RNA input
- **Higher-resolution and longer-read NGS**—accurate and rapid viral typing for viral surveillance and epidemiology investigation

Genexus Integrated Sequencer

Rapid and efficient prediction of patterns of evolution and emergence of SARS-CoV-2 is essential in slowing the spread of the virus. Researchers need an easy-to-adopt solution to quickly and accurately sequence the SARS-CoV-2 genome to understand how the virus is evolving, assist with contact tracing efforts, and inform vaccine development research.

The Genexus Integrated Sequencer combined with the Ion AmpliSeq SARS-CoV-2 Research Panel provides a highly automated nucleic acid–to–variant report NGS workflow in under a single day with minimal hands-on time, enabling labs to survey the complete SARS-CoV-2 genome at a speed never possible before (Figure 2). With unmatched ease of use and less operational hands-on time than other technologies, this new solution makes the power of NGS accessible to labs that want to easily and quickly adopt the technology for epidemiological studies.

As targeted NGS evolves, the ability to create reproducible, high-quality libraries becomes increasingly important. Ion AmpliSeq™ technology has proven useful to reliably generate quality libraries from as little as 1 ng of nucleic acid (Figure 3).

Starting with the nucleic acid sample, there is less than 5 minutes of hands-on time required to set up the fully automated sequencing process.

Fully automated sequencing

The Genexus Integrated Sequencer automates all steps from cDNA synthesis through post-run analysis.

Assay definition used	Total turnaround time
SARS-CoV-2 low-titer research assay	24 hr 23 min
SARS-CoV-2 research assay	28 hr 12 min

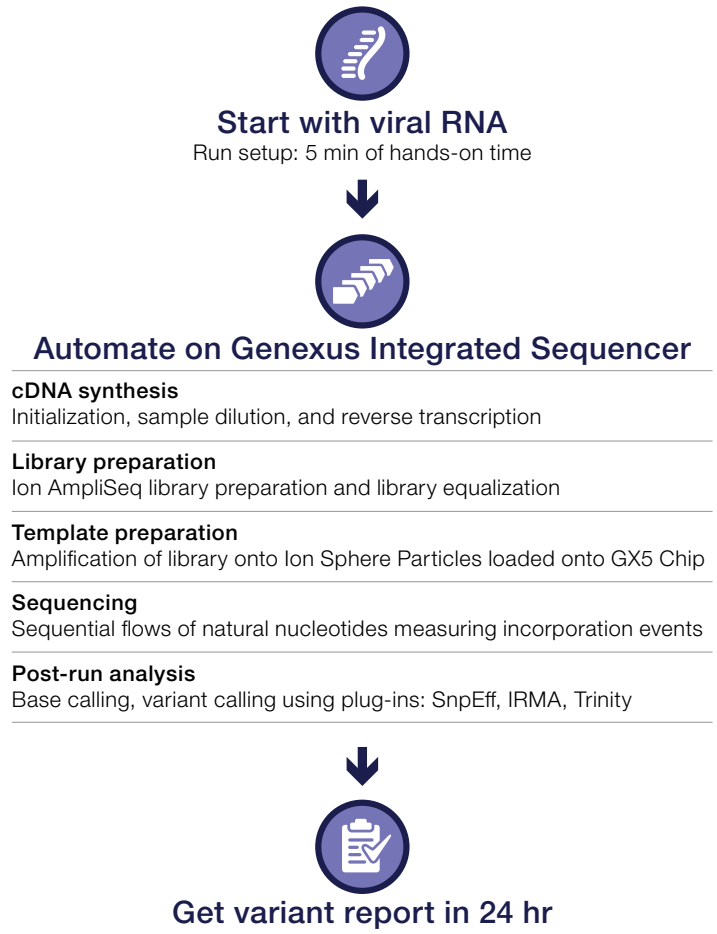


Figure 2. Automated workflow on the Genexus Integrated Sequencer. The Genexus Integrated Sequencer automates all steps from cDNA synthesis through post-run analysis. Approximate turnaround times shown are for 16 SARS-CoV-2 research samples run in 2 lanes on the Ion Torrent™ GX5™ Chip. Post-run analysis time scales are based on total reads returned. The next run can be started on the Genexus Integrated Sequencer while analysis from the previous run completes.

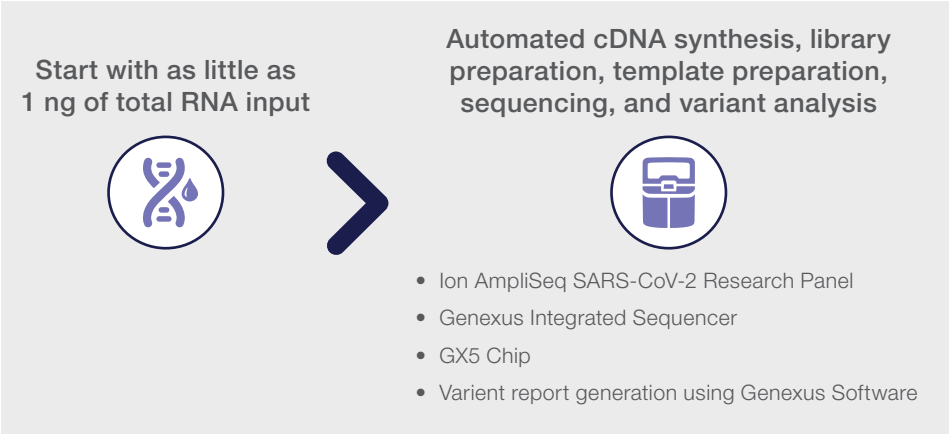


Figure 3. Genexus System workflow with fully automated Ion AmpliSeq SARS-CoV-2 Research Panel.

Data analysis

The Genexus Integrated Sequencer also comes with the Ion Torrent™ Genexus™ Software 6.2, which has multiple plug-ins to provide a complete informatics suite for analysis of consensus sequence, assembly construction, and variant annotation, as well a report of key metrics (Figure 4). The integrated and automated plug-ins have been updated to allow for faster analysis time and improved assembly and variant calling accuracy. The software also has enhanced download functionality and is compatible with third-party analysis software, such as the CosmosID™ bioinformatics platform and Qiagen™ CLC Genomics Workbench.

NGS workflows

Other NGS technologies can take weeks to get results, similar to outsourcing samples, which may delay answers. With the Genexus System, you can go from a biological specimen to report in just one day.* This allows you to provide NGS results at the same time as other, single-gene methods such as immunohistochemistry (IHC). Workflows from other suppliers can include extended hours of hands-on time, several user touch points, and longer turnaround time (Figure 5).

* Specimen-to-report workflow will be available after the Ion Torrent™ Genexus™ Purification System and integrated reporting capabilities are added in 2021.

Single-day turnaround time—comparison with other

Figure 4. Complete informatics suite for the Ion AmpliSeq SARS-CoV-2 Research Panel on the Genexus Integrated Sequencer.

Find out more at **thermofisher.com/sarspanel**

Genexus Integrated Sequencer

1 touch point; 5 min of hands-on time
Total turnaround time: 1 day

Company I's NGS systems

>10 touch points; ~7 hr of hands-on time
Total turnaround time: 3–4 days

Figure 5. Comparison of the targeted NGS workflows for SARS-CoV-2 on the Genexus Integrated Sequencer and company I's NGS system. The Genexus Integrated Sequencer enables labs to go from nucleic acid to report in a single day with minimal user intervention.

Unmatched ease of use

The hands-off, build-and-go workflow of the Genexus System makes the technology accessible even if your lab is new to NGS. With just 5 minutes of hands-on time and one touch point, all users can get up and running quickly with significantly less training. With less operational hands-on time than other technologies, the Genexus System can help improve your lab's productivity.

Targeted sequencing for NGS

Having coverage is clearly important to ensure that the genomic region of interest can be studied with high confidence. For regions with little-to-no coverage, researchers frequently increase the sequencing throughput for their studies (to obtain more sequencing reads and data to increase coverage for a genetic region). However, this method is inefficient, increases costs, and does not address the underlying reasons for the poor coverage. By increasing throughput, genomic regions with sufficient coverage will now be overrepresented and the reads are in effect, wasted. Areas with zero coverage before may not have coverage after sequencing more sample. A more efficient way to address coverage is by using a targeted sequencing approach.

While whole human genome sequencing has advanced discovery with regards to human health, the cost of whole genome sequencing can be a burden for many epidemiological researchers, particularly when you take into account the computational processing and informatics requirements. This added cost and complexity would afford little benefit when studying a specific region of interest for disease and translational research applications. Through targeted sequencing, researchers can focus on their regions of interest instead of sequencing the entire genome. This confers the additional benefit of ensuring sufficient coverage, including in parts of the genome that may not have been accessible previously, with lower sequencing costs.

Researchers have turned toward targeted enrichment approaches to help sequence specific regions of interest at much higher depths of coverage and at lower cost. Compared to hybridization capture, amplicon-based enrichment methods can target difficult genomic regions with lower input amounts to enable discovery. Ion AmpliSeq technology is the industry leader in target enrichment, providing higher multiplexing and coverage in a single assay than other methods (Figure 6).

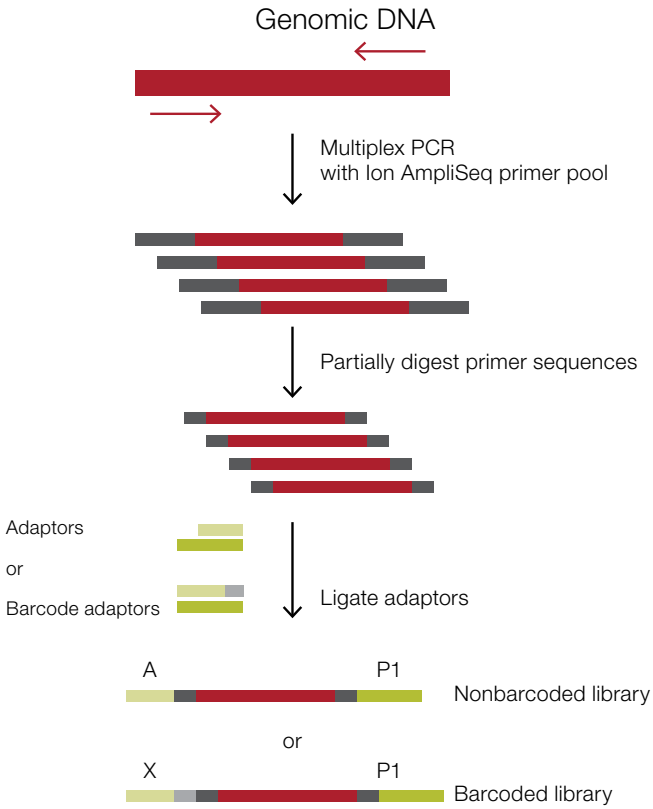


Figure 6. Amplicon-based target enrichment using Ion AmpliSeq technology.

Chapter 3–SARS-CoV-2 and customer spotlights

According to the World Health Organization (WHO), the SARS-CoV-2 viral infection that was originally identified in Wuhan, China back in December 2019 has spread to over 200 countries with >25 million cases and >850,000 deaths as of September 2020.

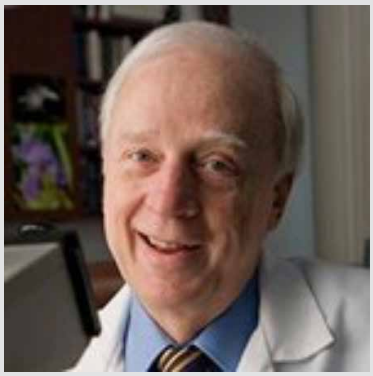
This crisis has highlighted the diverse applications that NGS can play a role in. From disease research and contact tracing and tracking to zoonotic transmission and wastewater-based epidemiology, there have been many NGS applications that researchers use to gain key insights to further their SARS-CoV-2 research.

Global tracking of SARS-CoV-2 using targeted NGS

NGS is a tool that enables researchers to track thousands of mutations across samples in parallel with a rapid time to actionable results in less than a day. Viral surveillance and epidemiological research allows us to better understand the virus and its spread. A major challenge for researchers when exploring a cure is tracking and monitoring the differences across strains as the virus rapidly evolves during transmission. A targeted approach with an automated sample-to-answer workflow is ideal for researchers looking to get information on strain to aid in determining the effectiveness of transmission control measures.

Read the blog:
thermofisher.com/ngs-sarscov2globaltracking

Rapid NGS contact tracking and tracing in the hospital



Timothy J. Triche, MD, PhD
Co-Director, Center for Personalized Medicine, Children's Hospital Los Angeles

Tim Triche, MD, PhD, co-director, Center for Personalized Medicine, Children's Hospital Los Angeles, utilized the Ion AmpliSeq SARS-CoV-2 Research Panel to help trace the virus when patients and some of the frontline workers exhibited symptoms of SARS-CoV-2 infection. The rapid results showed the strains from the health care workers were distantly related and that no transmission occurred between the patient and the health care workers.

Dr. Triche commented: "The NGS sequence data permitted Children's Hospital Los Angeles to draw conclusions within 48 hours of sample procurement from the six individuals. This information gave leaders at our institution greater confidence in our ability to provide a safe environment for our patients and our team members."

Read the press release:
thermofisher.com/ngscontacttrackingblog

Watch the webinar:
thermofisher.com/ngssarswebinar

NGS-based wastewater sequencing may provide clues to SARS-CoV-2 prevalence and spread

Researchers in the province of Milano in Northern Italy are using NGS to monitor the wastewater for SARS-CoV-2. Due to the sensitivity of NGS, scientists are able to detect SARS-CoV-2 RNA from fecal matter in wastewater. Not only does the robustness of the Ion AmpliSeq SARS-CoV-2 Research Panel enable the scientists to conduct wastewater surveillance to help them monitor strain prevalence early on, but they can also study the sequence for variant analysis.

Read the blog:
thermofisher.com/ngs-wbe

Understanding zoonotic transmission of SARS-CoV-2 using RNA sequencing

The origin of the SARS-CoV-2 pandemic is reported to be via zoonotic transmission of the virus from wild animals to humans. Although bats are thought to be a possible source, there have been reports of an intermediate species in the transmission process. Determining the possible zoonotic source of this virus could potentially prevent future spread of similar viruses. There has been recent research to study related coronaviruses in other species such as the Malayan pangolin. NGS can simplify the study of zoonotic transmission.

Read the blog:
thermofisher.com/ngs-zoonotictransmission

Summary

NGS technology has revolutionized epidemiological research, especially in the area of emerging infectious disease where there is a need for a rapid automated workflow with highly accurate results.

Ion Torrent™ targeted NGS allows for a simple sequencing solution for researchers new to NGS. Our NGS systems are designed to enable a broad range of targeted NGS applications with a fast and simple workflow that scales to your research needs with speed, high-throughput capabilities, less hands-on time, and accuracy. The holistic and flexible methodology is easy to use, rapid, and rigorous, and analyses are user friendly with an integrated analysis software solution.

The most recent targeted NGS system launched by Thermo Fisher Scientific, the Genexus System, is the first automated NGS platform developed to provide researchers—regardless of expertise level—access to the power of NGS technology. When combined with the Ion AmpliSeq SARS-CoV-2 Research Panel, the platform provides laboratories with a powerful specimen-to-report workflow to carry out infectious disease studies using minimal amounts of sample input.

Service and support

An innovative technology requires advanced and comprehensive services and support to succeed. We understand that when research is critical, there is simply no time for instrument downtime. That's why our instrument service plans include digital service innovations to help keep your instruments and your lab running smoothly. These include pioneering on-demand tools and capabilities such as remote support using augmented reality technology, instrument-driven support, and on-demand instrument training. We're constantly looking ahead so your lab never falls behind.

In addition to on-demand tools, we also provide access to:

- **Remote monitoring and diagnostics service**—problem detection and fast service resolution improves instrument uptime by resolving issues proactively
- **Interactive, self-paced education**—whether you're still going into the lab or you're working from home, explore our eLearning and 3D experiences
- **Instrument Connect**—use Connect, our cloud-based platform, to get connected to your data anytime, from anywhere

Note: The content provided herein may relate to products that have not been fully validated by Thermo Fisher Scientific.

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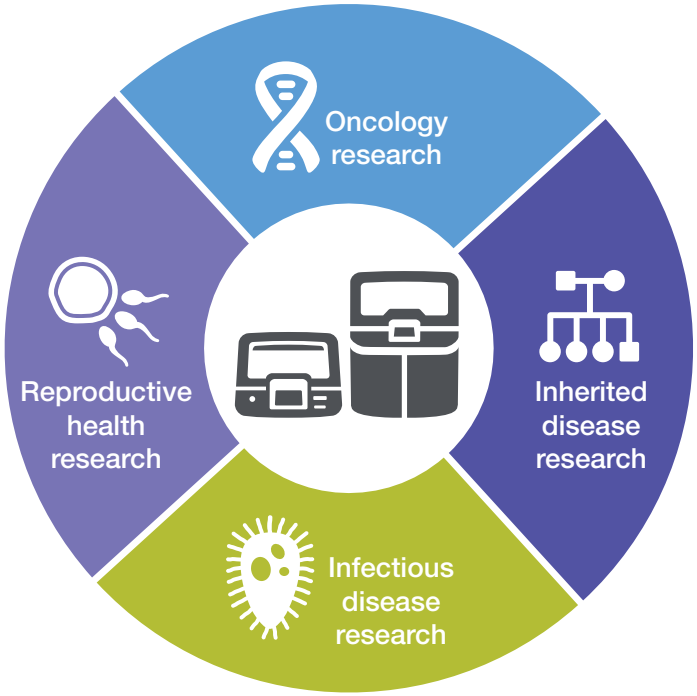

Learn more about the Genexus Integrated Sequencer

Learn more about the Ion AmpliSeq SARS-CoV-2 Research Panel

Resources

Use our comprehensive resources on the Genexus System across clinical research applications, from infectious disease and oncology to inherited disease, and more.

Application note

- **Rapid, automated NGS solution to survey the complete SARS-CoV-2 genome for epidemiological investigation**
  Learn about the workflow and performance of the Ion AmpliSeq SARS-CoV-2 Research Panel on the Genexus Integrated Sequencer
  https://assets.thermofisher.com/TFS-Assets/CSD/Application-Notes/rapid-automated-sars-cov-2-workflow-genexus-app-note.pdf

Webinar

- **SARS-CoV-2 Research: Implications of Viral Sequencing—The Children's Hospital Los Angeles Experience**
  Dr. Timothy J. Triche MD, PhD, Co-Director of Center for Personalized Medicine at Children's Hospital Los Angeles (CHLA), shares how he and his team used sequencing data generated from the Ion AmpliSeq SARS-CoV-2 Research Panel to analyze phylogenetic relationships among a cluster of six SARS-CoV-2-positive samples.

Publications

- **Evaluation of the Ion AmpliSeq SARS-CoV-2 Research Panel by Massive Parallel Sequencing**
- **High-density amplicon sequencing identifies community spread and ongoing evolution of 2 SARS-CoV-2 in the Southern United States**
- **Genomic variations in SARS-CoV-2 genomes from Gujarat: Underlying role of variants in disease epidemiology**
- **Geographic reconstruction of the SARS-CoV-2 outbreak in Lombardy (Italy) during the early phase**

Additional assays on Ion Torrent platforms

- Learn about **Ion AmpliSeq™ TCR and BCR assays** for studying immune response
- Learn about the **Ion AmpliSeq™ Microbiome Health Research Panel**

Find out more at **thermofisher.com/coronavirus-genexus**

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