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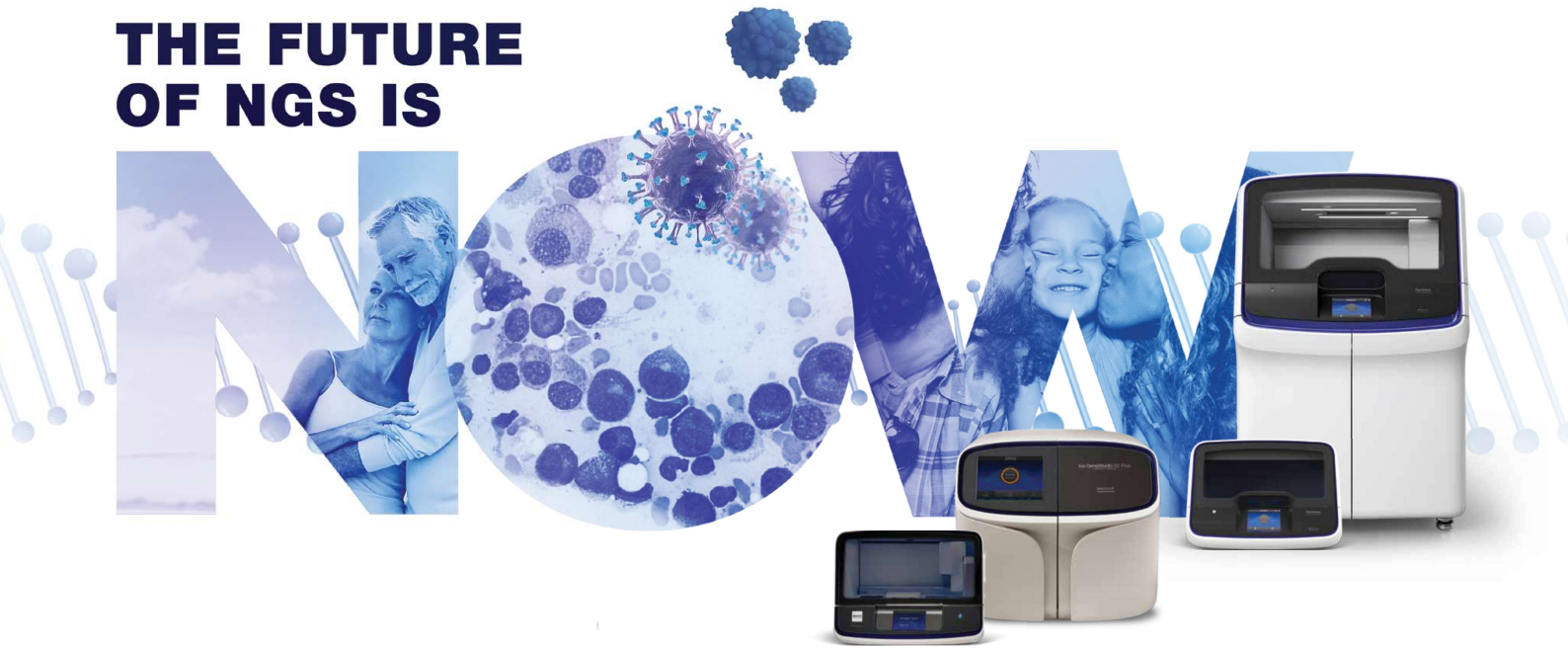

Introduction to next-generation sequencing

Ion Torrent technology and instrumentation

ion torrent

Introduction

DNA sequencing involves determining the order of nucleic acid residues in biological samples, and it is key to understanding the way that genomes function. Sequencing information forms the basis of much of modern biological inquiry, and it is integral to a great variety of research and clinical applications [1]. The techniques and technology of DNA and RNA sequencing have been developed over more than 50 years, and many of the technical improvements available today were instigated by the Human Genome Project [2]. Since completion of that project, the speed of sequencing has increased even as the costs have decreased, making the most current technological advances available to a wider range of laboratories than ever before.

Sequencing is an important component of biological research because it informs so many areas of biology, medicine, epidemiology, and forensics. Just a few of the applications of sequencing include the determination of the roles of genes and gene regulation in infectious and inherited diseases and in cancers, the analysis of phylogenetic relationships of various organisms, and forensic examination of highly complex biological samples. One of the most exciting developments has been the invention of next-generation sequencing, or NGS. NGS was first introduced for commercial use in 2005, and it would not be an overstatement to say that it has revolutionized the various fields that utilize genetic analysis [3]. NGS allows for the interrogation of up to thousands of genes at a single time from multiple samples and the discovery and analysis of a broad variety of features of the genome. It has the advantages of requiring low amounts of sample input, high accuracy, and the ability to detect low-frequency variants [3].

Thermo Fisher Scientific has developed a wide array of Ion Torrent™ products for NGS, including instrumentation, assay kits, reagents, and consumables. This ebook describes the different types and methods of NGS that are currently available, and the applications to which they are relevant. In particular, we highlight the use of Ion Torrent products to address a broad range of questions that may be answerable with NGS data.

References

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Chapter 1: Why NGS matters

Overview

The speed, throughput, and accuracy of next-generation sequencing (NGS) have revolutionized genetic analysis and enabled new applications in genomic and clinical research, precision medicine, reproductive health, and beyond.

NGS is a technology for determining the sequence of DNA or RNA to study genetic variation associated with diseases or other biological phenomena. NGS was initially introduced for commercial use in 2005. It was known then as "massively parallel sequencing" because it enabled the sequencing of many DNA strands simultaneously, instead of one at a time as with traditional Sanger sequencing by capillary electrophoresis (CE). Both NGS and Sanger sequencing have utility in today's genetic analysis environment. Sanger sequencing is best for analyzing small numbers of gene targets and samples and can be accomplished in a single day. NGS results are often verified using Sanger sequencing because the latter is considered the gold-standard sequencing technology.

NGS enables the interrogation of hundreds to thousands of genes at one time in multiple samples. It also allows for the discovery and analysis of different types of genomic features in a single sequencing run, from single-nucleotide variants (SNVs) to copy number and structural variants, and even RNA fusions. NGS allows ideal throughput per run, and studies can be performed quickly and cost-effectively. Additional advantages of NGS include lower sample input requirements and the ability to detect variants at lower allele frequencies than with Sanger sequencing.

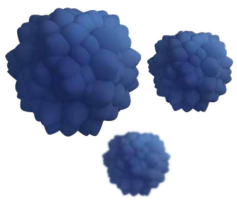
Genomic aberrations detected by NGS

NGS can be used for many biomarkers and biomarker types (e.g., mutations, fusions, and copy number variations) simultaneously with a single test.

- Translocations are migrations of DNA from one chromosome to another. They lead to the origination of new fusion genes and proteins and play a significant role in disease development. Only those translocations that are transcribed to RNA can potentially become functional, and therefore RNA sequencing is the preferred method for fusion testing.
- Constitutional copy number variants (CNVs) are DNA segments present at a variable copy number in comparison to the number in a normal genome. CNVs lead to gene amplification and deletion, which can disrupt protein production, biological pathways, and cell function; they can potentially lead to disease development.
- Insertions and deletions (indels) are additions or deletions of one or more nucleotides in a DNA sequence. They are the second most common aberrations in the human genome (after SNVs) and are well known to contribute to disease.
- Single-nucleotide variants (SNVs) are single-base changes in DNA sequences responsible for genetic diversity that also may contribute to the development of complex diseases, such as cancer.

Advantages of NGS

- **Time savings:** A comprehensive molecular profile can be available in as little as 24 hours, accelerating research that has the power to advance science.
- **Tissue savings:** NGS can provide comprehensive genomic profile results from one small sample with as little as 10 ng of DNA or RNA.
- **Cost and resource savings:** If three or more biomarkers are required, it can be cheaper to run one NGS test than multiple single-gene assays. NGS also allows savings by streamlining training and technical experience requirements, as well as instrumentation maintenance, on one platform. Additionally, the use of a single platform saves on space requirements.
- **High specificity and sensitivity:** NGS enables detection of genomic variants even if they are present in extremely low fractions of cells in the sample (such as in liquid biopsies) and allows researchers to distinguish between different cells.
- **Reduced sample handling errors:** The more tests that are performed to achieve the required result, the more potential there is for errors to occur. Consolidation into one NGS test can reduce error rates.



Next-generation sequencing methods

Genomics researchers have multiple NGS methods to choose from when designing and implementing their studies. General NGS methods include whole-genome sequencing (WGS) and targeted sequencing, which is further subdivided into exome sequencing and gene- or region-specific panels (Table 1.1). Although all these methods can yield gene sequences of interest to a researcher, they are far from interchangeable.

While whole-genome and whole-exome sequencing are suited to discovery-based questions, targeted NGS is preferred for the study of known variants or rare alleles. Additionally, within each of these approaches, there are specialized techniques tailored to specific sample types, organisms, diseases, or regions of the genome. Table 1.1 summarizes the pros and cons of each method and includes examples and applications.

Table 1.1. Comparison of DNA sequencing methods.

	Whole-genome sequencing (WGS)	Targeted sequencing: exome sequencing	Targeted sequencing: gene- or region-specific panels
Description	Sequencing of the entire genome	Sequencing of only exons (protein-coding regions)	Sequencing of regions of interest such as disease-associated genes or genomic hotspots
Pros	• Most comprehensive genome coverage • Detects the widest range of features: SNVs, indels, structural and copy number variants, and regulatory elements • No bias from PCR amplification or probe hybridization • Best for discovery research	• Exomes constitute 1% of the human genome—much less data to analyze than for WGS • Faster workflow than that of WGS • Used to multiplex a small number of samples • Medium sample input (50 ng–1 µg depending on library prep method)	• Highly flexible, customizable designs • Data are focused specifically on regions or genes of interest • Lowest sample input (10 ng) • Compatible with FFPE tissue samples • Used to multiplex large numbers of samples • Better for detecting rare alleles
Cons	• Great amount of potentially unnecessary data from noncoding and nonfunctional regions • Data are very complicated • Multiplexing is usually not possible	• Only sequences data in exons (may miss functionally relevant variants) • May generate too much extra data if one only needs to study a small number of genes	• Only obtains data on targeted regions (may miss relevant variants if not in the design)
Speed of results	Slow	Medium	Fast
Cost	$$$	$$	$
Data volume	Large	Medium	Small
When to use	• Complete coverage of genome needed • *De novo* assembly • Discovery of unknown genomic variants associated with a disease • Aneuploidy detection (preimplantation genetic testing)	• Disease-specific research projects • Clinical research sample sequencing	• Clinical research sample sequencing • Disease-specific research projects • *In vitro* diagnostic (IVD) testing • Creation of laboratory-developed tests (LDT) • Inherited disease detection and characterization • Oncology applications • Immune repertoire analysis • Liquid biopsy samples

Whole-genome sequencing

WGS is the most comprehensive sequencing method that enables an in-depth analysis of entire genomes, including exons, noncoding regions, and structural variants. WGS libraries are typically prepared using fragmentation or enzymatic digestion of genomic DNA. WGS does not require prior knowledge of the genome sequence being analyzed, so it is the best method for discovery of new genetic variations associated with disease, *de novo* genome assembly, microbial sequencing, and low-pass genome sequencing for copy number and aneuploidy determination.

While WGS has advanced discovery and human health, certain complex regions of the genome are difficult to analyze with this approach. It should be pointed out here that WGS in humans has resulted in a population sequencing bias, with existing databases noted to be neither complete nor accurate. For many research applications, the cost of computational processing, informatics, and data storage needs for whole-genome analysis is a burden. The extra cost is of little benefit when studying a specific region of interest associated with a disease or in translational research applications. To address this issue, many researchers use targeted sequencing approaches, such as exome sequencing or smaller gene- or region-specific panels to improve sequence coverage and reduce their total sequencing workflow costs.

Targeted sequencing

A targeted NGS approach leverages current knowledge of the genome and uses molecular biology methods to enrich it for specific sequences. The targeted approach enables researchers to focus their studies on the individual genes or genomic regions that are most relevant to their research. Using this targeted method, obtaining sequence coverage of challenging genomic regions is now possible, including for regions from difficult-to-sequence or limited samples, such as degraded DNA or RNA from clinical samples, or circulating DNA from blood samples.

By sequencing only what you need, cost benefits are realized from less intensive computational processing, informatics, and data storage; shorter workflows and sequencing time; and higher depth of coverage for rare variants that occur at low allelic frequency. More samples can be processed simultaneously in a single sequencing run for a faster time-to-result, whether for an individual sample or a cohort. Whole-exome sequencing (WES) and target-specific panels are the two most common targeted sequencing approaches.

- WES enables analysis of all the exons (the protein-coding regions of the genome). About 20,000 genes constitute the human exome, which accounts for approximately 1% of the entire genome. WES allows researchers to focus on content that may be more relevant to disease compared to the whole genome sequence.
- Target-specific NGS panels are the most flexible option because they can be designed to sequence any gene or region of interest in a genome and can include structural and copy number variation, as well as RNA transcript analysis. Unlike broader approaches such as WES or WGS, targeted panels generate smaller and more manageable data sets, which reduces the data analysis burden for researchers. Using target-specific panels is the fastest and most cost-effective NGS method, making them more suitable for clinical research applications.
- Targeted NGS libraries enriched for specific genomic regions can be made using two techniques: hybridization capture or amplicon-based enrichment.

Hybridization capture uses molecules complementary to target regions to act as probes that select the target molecules from the sample. These capture probes are either immobilized on a solid substrate in an array-based format or used directly in solution. In the array-based format, the DNA sample is applied to the solid surface and the targeted DNA fragments hybridize to the immobilized capture probes. Any unbound molecules are washed away, leaving the desired targets on the surface. The desired isolated genetic material is now eluted away and amplified for sequencing. With solution-based hybridization, the probes are biotinylated and hybridized targets are isolated and purified for subsequent amplification using streptavidin magnetic beads.

Amplicon-based enrichment uses highly multiplex PCR that is carefully designed to amplify regions of interest from the DNA or complementary DNA (cDNA) sample (Figure 1.1). This workflow is much shorter than the hybridization capture workflow. After multiplex PCR, the resulting amplicon library is purified from the sample material, ligated to sequencing adaptors containing barcodes, and used for sequencing. Amplicon-based enrichment techniques have several advantages compared to hybridization capture methods:

Three advantages of amplicon-based approaches

1. PCR specificity allows researchers to enrich for target gene regions from low sample input amounts.
Limited sample sources with trace amounts of DNA and/or RNA, such as FFPE tissue, fine needle aspirates, or circulating tumor DNA (ctDNA), can now be sequenced for biomarker discovery and retrospective clinical trials.

2. Amplicon-based enrichment can be used to discriminate between two highly homologous regions in the genome because PCR primers can be designed to specifically target the desired region.
In contrast, hybridization capture would have difficulty distinguishing between the two homologous regions in the genome, resulting in nonspecific enrichment. For example, the *PTEN* gene is a known tumor suppressor gene that controls cell growth and division. *PTEN* is one of the most commonly mutated suppressor genes in cancer. *PTENP1* is a processed pseudogene very similar in sequence, with a missense mutation in the initiation methionine codon that prevents translation of the normal PTEN protein. The ability to distinguish and target the correct gene clearly plays an important role in cancer research. The same concept applies when trying to target low-complexity regions that are prevalent in whole genomes, such as dinucleotide and trinucleotide repeats.

3. Amplicon-based enrichment can be used to better detect known insertions and fusion events than capture hybridization.
Since capture hybridization requires developing complementary capture probes against a known reference genome, unknown genetic mutations could disrupt the hybridization process and result in a failure to enrich for a target region of interest. This issue is particularly relevant for genetic regions that have many variants near one another. Hypervariable regions, such as the T cell immune repertoire, can be sequenced more effectively using amplicon-based enrichment, providing translational researchers a tool to discover predictive biomarkers for immunotherapy.

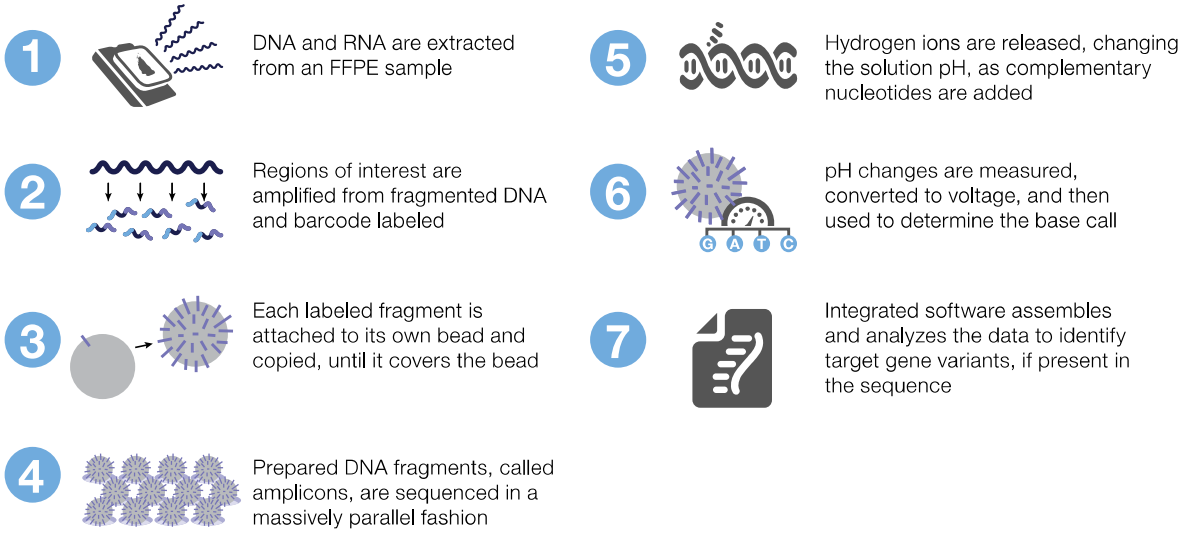


Figure 1.1. Workflow for amplicon-based NGS.

The NGS workflows

Prepare the sample and library

Begin the NGS workflow by preparing a sequencing library from the tissue sample. First, extract and purify the DNA and/or RNA, depending on the assay. Using PCR, amplify regions of interest to generate an amplicon sequencing library. You can either prepare a library from a single specimen or combine multiple specimens into a mixed library. In a process called sample multiplexing, each specimen is tagged with a specific barcode so it can be analyzed independently downstream. Multiplexing enables you to optimize efficiency by maximizing the number of samples processed in each sequencing run.

Create the template

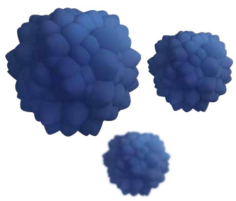
After the sequencing library is prepared, generate a template in preparation for sequencing. This entire process can be easily automated. During template generation, the library is settled onto a solid substrate and further amplified. For Ion Torrent™ technology, the substrate is a semi-conductor microchip that enables the sequence of each amplicon in the library to be read independently.

Sequence

Once the template is ready, simply load the chip into the sequencer and initiate a sequencing run. The sequence of each amplicon in the library will be read during this process, and the data will be digitally transmitted to a computer for downstream analysis.

Analyze data and interpret results

Integrated analysis software assembles the amplicon sequencing data and calls any identified variants. Using a decision support tool, gene variants detected in the sequences can be matched against databases of known relevant biomarkers, associated therapies, clinical trials, and guidelines.



How to select the optimal NGS method

While NGS is the ideal platform for testing multiple biomarkers and preserving precious sample tissue, not all NGS is the same. These are the six key factors to consider when choosing the NGS technology for your laboratory:

1. Adequate portfolio of applications and panel coverage—In precision medicine research, one size does not fit all. You need a panel that includes genes relevant to the disease of interest and that can profile your sample format. For example, if you are testing non-small cell lung cancer (NSCLC) samples, you need a panel that includes *ALK*, *ROS1*, *EGFR*, *BRAF*, *NTRK*, and *RET,* and likely also offers the possibility to profile liquid biopsy samples, as in many cases there is not enough tissue available. For immuno-oncology clinical research, both tumor mutational burden (TMB) sequencing and T cell receptor (TCR) sequencing are becoming important.

2. Panel design—Depending on the variant to be detected, either DNA- or RNA-based sequencing is preferable. Often, an assay that can do both at once is required. RNA-based methodology is optimal for testing fusions, as it directly detects translocation events between the target gene and partner gene. It should also be able to detect not only all the known, but also the novel, driver and partner combinations.

3. Sample requirements—Often the amount of tissue that is available is very limited. Different NGS methods vary significantly in the amount of sample required, ranging from 10 to 500 ng of nucleic acid (RNA or DNA), which can have a direct impact on your ability to successfully test all samples.

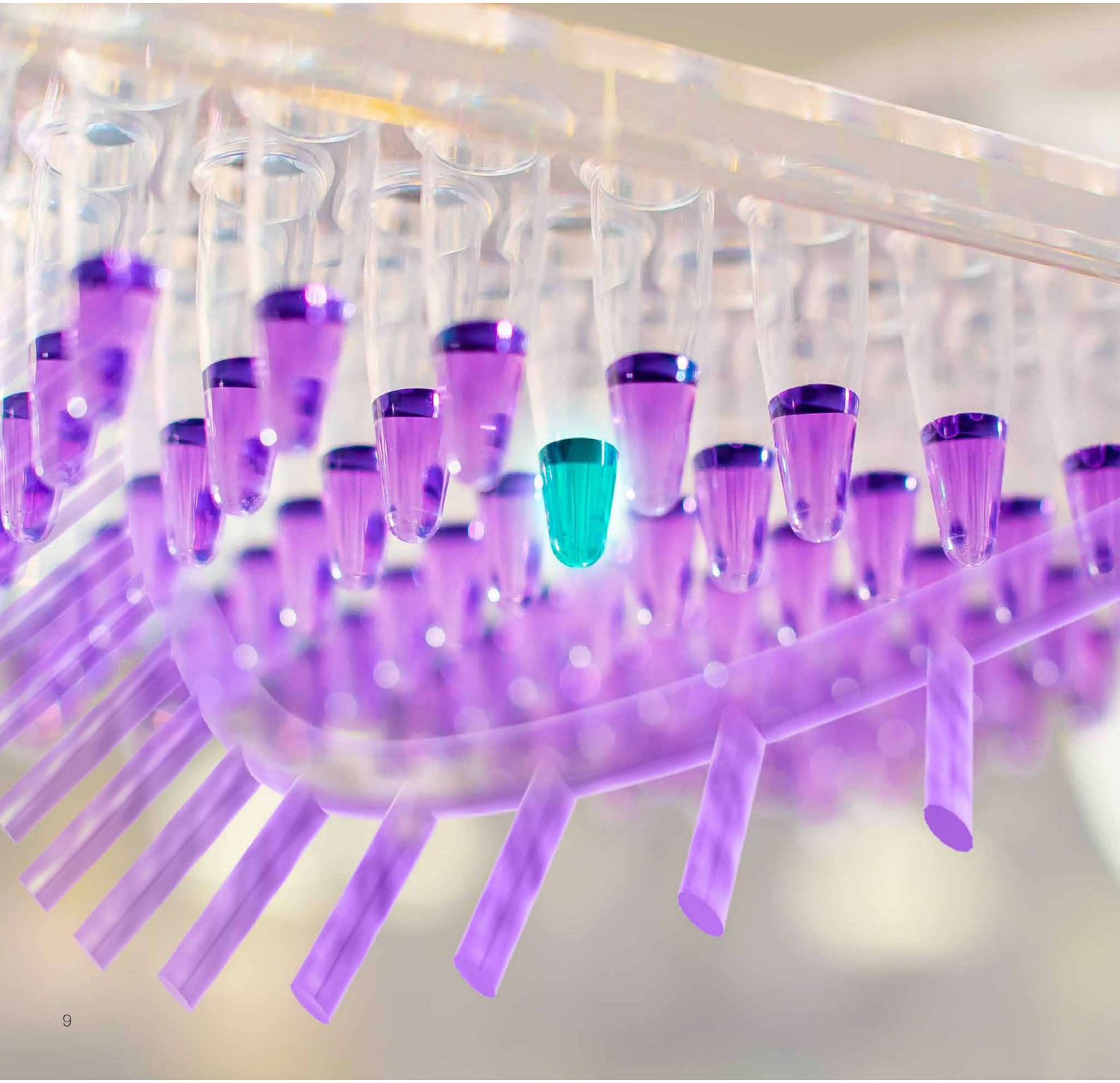
4. Completeness and automation level of the workflow—NGS workflows can be complex. An easy-to-use and highly automated workflow from sample to report simplifies laboratory operation and test implementation.

5. Analytical validation support—High-touch consultation service and support from the vendor help accelerate a laboratory's validation process to implement the test in a time-efficient manner and save costs.

6. Robustness and key performance characteristics—Not all NGS technologies can handle all tissue types equally well. For example, FFPE material can be challenging, especially when there is insufficient tumor sample. Look for evidence of the sequencing success rate, or the opposite—failure rate and "quantity not sufficient" frequency. Specificity and sensitivity are very important, as cell populations may be heterogeneous, and a particular aberration may be present only in a small proportion of cells, although the patient might still benefit from the corresponding therapy. High reproducibility is also required, so you can generate consistent results.

Conclusion

There are many possible research applications for NGS. These include WGS to identify associations of genomic variation with different diseases as well as targeted NGS, in which the whole power of NGS is used to interrogate a defined number of targets with possible significance in disease pathogenesis.



Chapter 2: Introduction to Ion Torrent sequencing

Overview

Ion Torrent™ technology from Thermo Fisher Scientific takes a unique approach to next-generation sequencing (NGS). This approach marries simple chemistry to proprietary semiconductor technology, providing a fast, simple, affordable, and scalable sequencing solution.

How Ion Torrent sequencing works

Ion Torrent technology directly translates chemically encoded information (A, C, G, or T) into digital information (0 or 1) on a semiconductor chip.

The sequencing process starts when a sample of DNA is cut into millions of fragments. Each fragment is then attached to its own bead and is copied until it covers the bead. This automated process covers millions of beads with millions of different DNA fragments. These beads then flow across the chip, each depositing into a well (Figure 2.1). The Ion Torrent™ next-generation sequencer then sequentially floods the chip with nucleotides to initiate base calling.

How the technology is used to call a base

In nature, when a nucleotide is incorporated into a strand of DNA by a polymerase, a hydrogen ion is released as a byproduct. This is how an Ion Torrent system sequences DNA.

If a nucleotide is added to a DNA template and is then incorporated into a strand of DNA, a hydrogen ion will be released. The charge from that ion will change the pH of the solution in the well. Our proprietary ion sensors beneath the well measure the change in pH and convert it to voltage. In essence, each well works as the world's smallest pH meter.

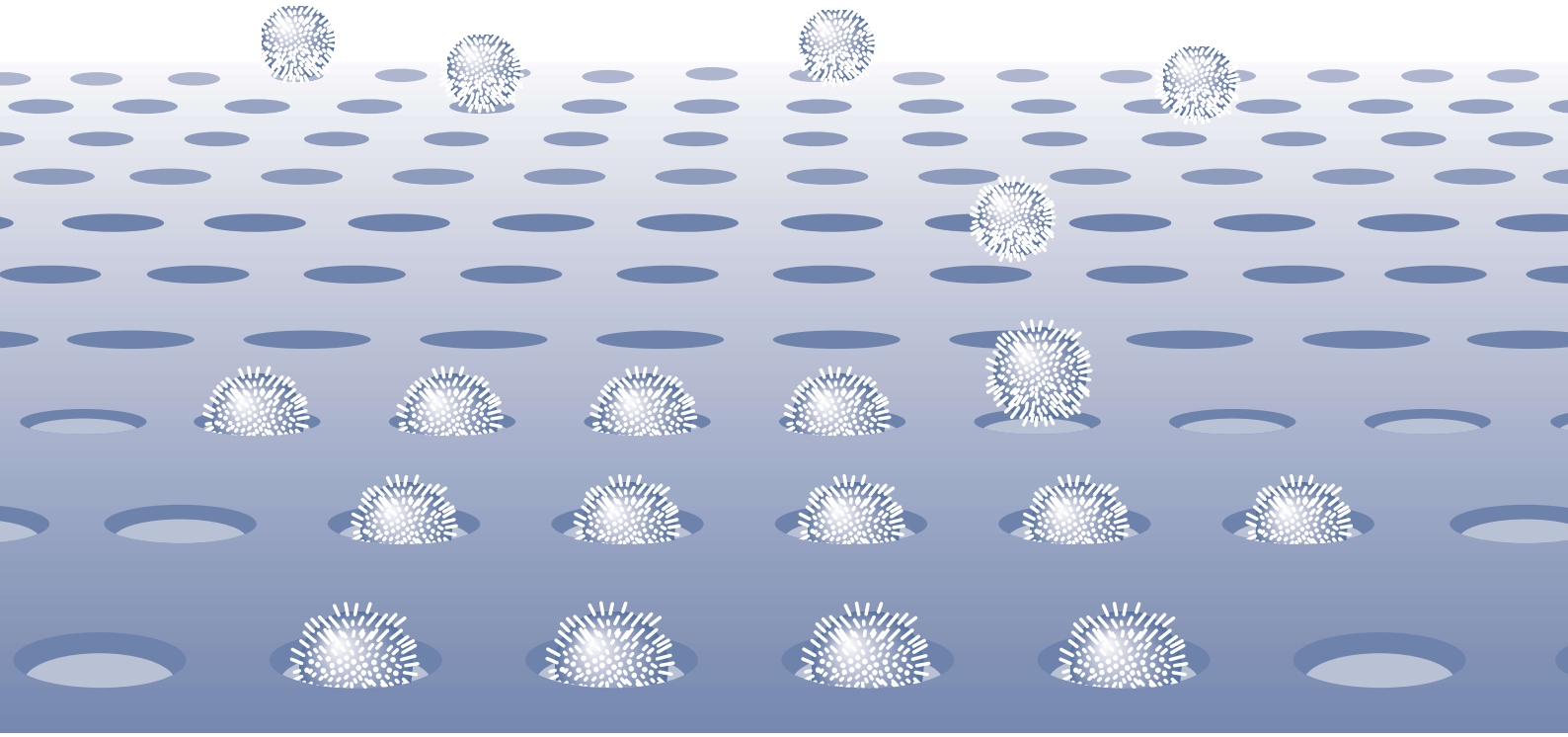


Figure 2.1. Ion Torrent technology. Beads covered with DNA fragments flowing across the chip and depositing into a well.

The sequencer records the voltage, indicating the nucleotide was incorporated and the base was called, going directly from chemical information to digital information. For example, if a polymerase incorporates a C nucleotide in a DNA strand when a complementary G molecule is present, a hydrogen ion is released and there is a voltage change (Figure 2.2A). If the next nucleotide that floods the chip is not a match, no voltage change will be recorded, and no base will be called (Figure 2.2B). If there are two identical bases next to each other on the DNA strand, two nucleotides are incorporated, the voltage will be double, and the chip will record two identical bases (Figure 2.2C).

Because this is direct detection—no scanning, no cameras, no light—each nucleotide incorporation is recorded in seconds. The process is repeated every 15 seconds with a different nucleotide washing over the chip, and it occurs simultaneously in millions of wells, which is why it is often described as massively parallel sequencing.

The Ion Torrent™ chips help you scale the workflow to your research needs so that you can run both small- and large-scale projects without the need to change platforms. The semiconductor approach helps you implement a significantly faster NGS workflow compared with other sequencing technologies. Ion Torrent technology harnesses the power of targeted NGS to make sequencing faster, more scalable, and more accessible than ever before.

The semiconductor has transformed every industry it has touched. Just as the microprocessor enabled desktop computing to displace the mainframe, semiconductor technology has the ability to democratize research. In the future, Ion Torrent technology may be able to provide diagnostics that are less expensive and more reliable, helping to improve human health around the world.

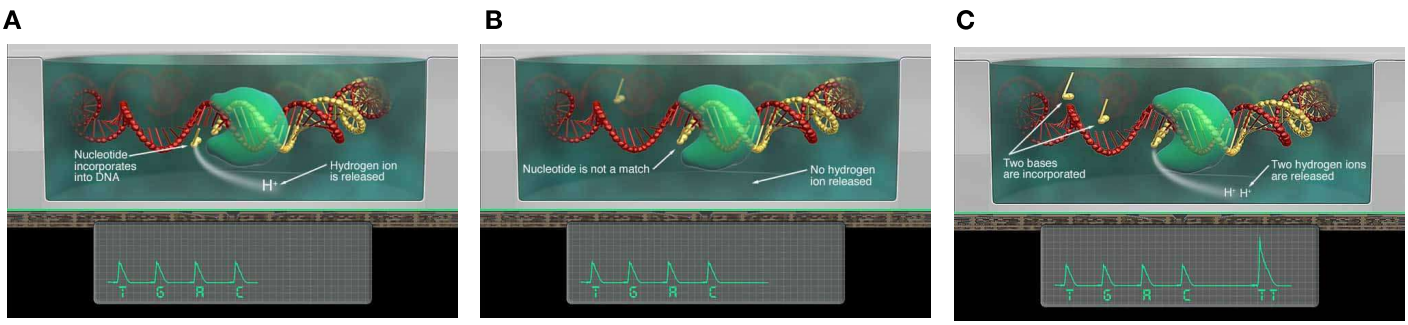


Figure 2.2. Base calling using Ion Torrent technology. (A) As a nucleotide is added to a DNA template and incorporated into a strand of DNA, a hydrogen ion is released, leading to a voltage change. **(B)** If the next nucleotide is not a match, no voltage change will be recorded. **(C)** Two identical bases that are incorporated next to each other will lead to the release of two hydrogens, doubling the voltage change.

Chapter 3: NGS instruments—the Ion GeneStudio S5 series

Overview

The Ion GeneStudio™ S5 systems, combined with the Ion Chef™ System for automated library and template preparation, enable a streamlined next-generation sequencing (NGS) workflow for targeted sequencing with flexibility and scalability.

There are three models to choose from, giving customers options of different sequencing and analysis speeds at different price points: the Ion GeneStudio™ S5 System, Ion GeneStudio™ S5 Plus System, and Ion GeneStudio™ S5 Prime System.

Key features of Ion GeneStudio S5 systems

Chips

The Ion GeneStudio S5 systems use sequencing chips to help streamline workflows, while offering flexibility for a wide range of research applications. What differentiates the chips is the amount of throughput they can deliver, ranging from 2 to 130 million reads per run. This versatility enables you to run both small- and large-scale projects without the need to change platforms (Table 3.1).

Figure 3.1. Throughput of the chips for Ion GeneStudio S5 systems.

Table 3.1. Turnaround times of the Ion GeneStudio S5 systems.

Chip type	Number of reads	Read length (output)*	Ion GeneStudio S5 System	Ion GeneStudio S5 Plus System	Ion GeneStudio S5 Prime System
			Turnaround time (sequencing run** and analysis time)		
Ion 510 Chip	2–3M	200 bp (0.3–0.5 Gb)	4.5 hr	3 hr	3 hr
		400 bp (0.6–1 Gb)	10.5 hr	5 hr	5 hr
Ion 520 Chip	4–6M	200 bp (0.6–1 Gb)	7.5 hr	3.5 hr	3 hr
		400 bp (1.2–2 Gb)	12 hr	5.5 hr	5.5 hr
	3–4M	600 bp (0.5–1.5 Gb)	12 hr	5.5 hr	5.5 hr
Ion 530 Chip	15–20M	200 bp (3–4 Gb)	10.5 hr	5 hr	4 hr
		400 bp (6–8 Gb)	21.5 hr	8 hr	6.5 hr
	9–12M	600 bp (1.5–4.5 Gb)	21 hr	8 hr	7 hr
Ion 540 Chip	60–80M	200 bp (10–15 Gb)	19 hr	10 hr	6.5 hr
		200 bp (20–30 Gb); 2 runs in 1 day	NA	20 hr	10 hr†
Ion 550 Chip	100–130M	200 bp (20–25 Gb)	NA	11.5 hr	8.5 hr
		200 bp (40–50 Gb); 2 runs in 1 day	NA	NA	12 hr†

* Expected output with >99% aligned or measured accuracy. Output depends on read length and application.
** Sequencing run times are between 2.5 and 4 hr.
† Analysis of the first run occurs concurrently with the second sequencing run.

Application highlights of the Ion GeneStudio S5 systems

With an extensive catalog of NGS assays covering all major application areas, the Ion GeneStudio S5 systems are ready to meet the needs of laboratories no matter what sample comes through the door. The application areas include:

- **Cancer research**—gene panels for SNPs, indels, gene expression, and gene fusion analysis
- **Hemato-oncology research**—profile for multiple relevant driver genes in myeloid malignancies in a single test
- **Reproductive health research**—aneuploidy detection
- **Inherited disease research**—panels for targeted gene or whole-exome analysis
- **Gene expression analysis**—whole-transcriptome RNA sequencing, targeted RNA sequencing, and small RNA sequencing
- **Microbiology and infectious disease research**—SARS-CoV-2, microbial whole genomes, microbial genotyping, and metagenomics
- **Microbiome research**—profiling of microbial diversity in the human gut microbiome

Small sample input

Low input DNA from challenging sample types such as formalin-fixed, paraffin-embedded (FFPE) tissue, retrospective samples from fine needle aspirates, and cell-free DNA (cfDNA) extracted from blood can be difficult to sequence on next-generation sequencers from other suppliers. With Ion AmpliSeq™ technology and the Ion GeneStudio S5 series instruments, you can use as little as 1 ng of input DNA or RNA.

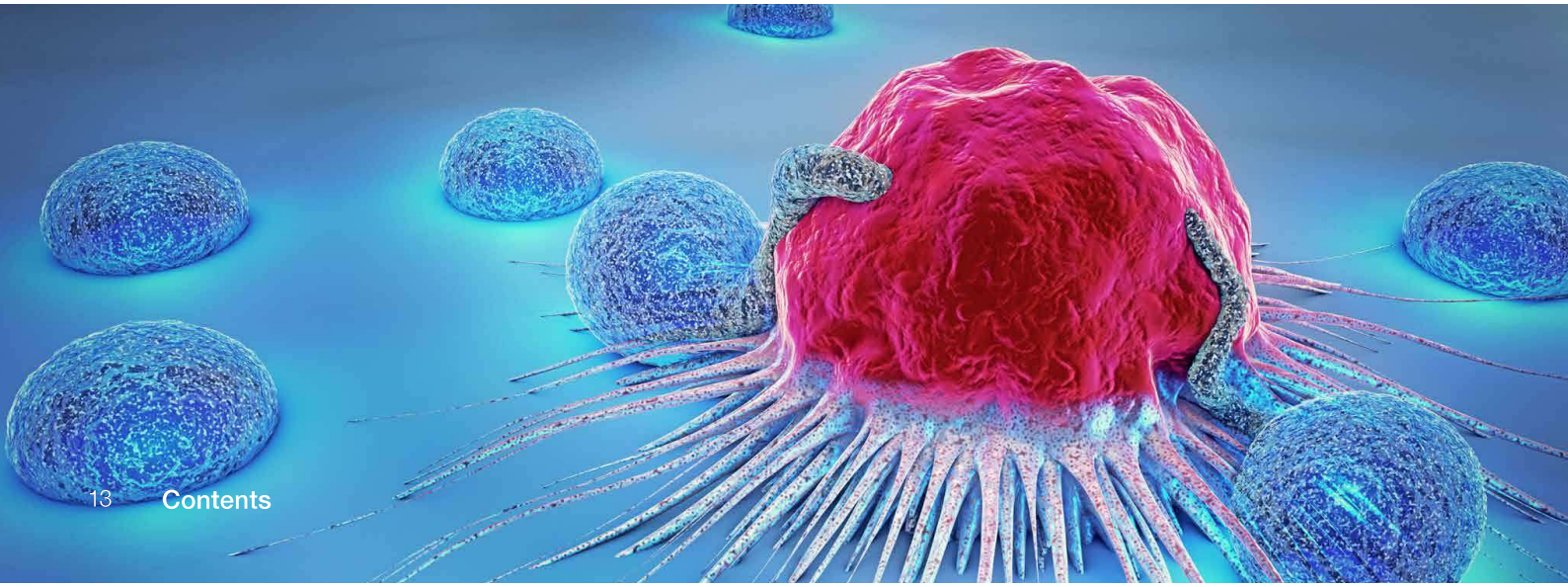
Simple analysis and storage solutions

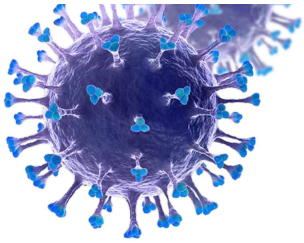
Torrent Suite™ Software and Ion Reporter™ Software make NGS easy for beginners and experts alike. Plan, monitor, track, and analyze your runs using Torrent Suite Software (preinstalled on the Ion PGM™ Torrent Server). Integrate, annotate, and interpret variants using Ion Reporter Software (Thermo Fisher™ Connect Platform or local options available based on your needs).

Cancer research

The Ion GeneStudio S5 systems can aid your oncology research when combined with an Ion Torrent™ Oncomine™ assay design that focuses on clinical research. Oncomine assays are part of an end-to-end workflow that includes simple, scalable sequencing with optimized bioinformatics and reporting—designed for cancer research.

- Easily detect mutations in tumor-associated genes from as little as 1 ng of tumor DNA with Ion AmpliSeq™ cancer research gene panels, or create custom assays with the online Ion AmpliSeq™ Designer tool
- Identify single-nucleotide variants (SNVs), indels, copy number variants (CNVs), and gene fusions starting from precious clinical research samples using our growing menu of Oncomine assays for precision oncology research, such as the Ion Torrent™ Oncomine™ Comprehensive Assay Plus
- Perform analysis on small, archived FFPE solid tumor and fine needle aspirate samples





Complex and inherited disease research

The Ion GeneStudio S5 systems deliver an end-to-end NGS solution for researchers studying complex and inherited diseases. Using Ion AmpliSeq targeted assay technology, thousands of amplicons, including the complete exome, can be rapidly amplified by PCR, followed by sequencing using the Ion GeneStudio S5 System. Ready-to-use and customizable Ion AmpliSeq assays are available to target genetic loci of interest for inherited disease research, including hereditary cancers, neurodegenerative disorders, and cardiovascular diseases. Primer pools can also be created to target any customer-defined region of interest within a reference sequence.

- Predesigned assays support cost-effective detection of single-nucleotide variants (SNVs), indels, and copy number variants (CNVs) for inherited disease research.
- Customize existing inherited disease research panel designs at the gene, region, or amplicon level, and deliver just the content needed by leveraging the simple-to-use Ion AmpliSeq Designer tool.

Reproductive health research

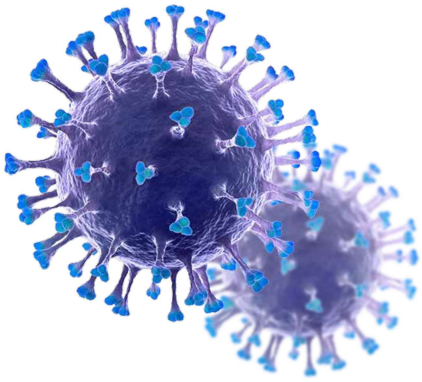
Understanding risk factors for inherited genetic disorders is an important step in evaluating embryos. Expanded carrier screening (ECS) research and preimplantation genetic testing (PGT) offer scientific insights that may increase probability of success for future pregnancies and healthier future generations. Ion GeneStudio S5 systems facilitate scalable, precise sequencing solutions for ECS and PGT research.

- **Comprehensive ECS for research of a broad range of inherited disorders**—The Ion Torrent™ CarrierSeq™ ECS Kit contains a 420-gene panel that targets full coding regions, enabling the analysis of >36,000 non-benign ClinVar variants for SNVs, indels, and CNVs by NGS.
- **Combined PGT from a single biopsy**—The Ion Torrent™ ReproSeq™ PGS kit for PGT-A (aneuploidy) and Ion Torrent™ PGD-SEQ™ kit for PGT-M (monogenic disease) can be used to simultaneously study chromosomal abnormalities and variants linked to single-gene disorders, in one convenient NGS workflow.

Microbiology and infectious disease research

Uncover microbial diversity, study pathogen outbreaks, and identify mutations that may be associated with antibiotic resistance. Take advantage of high throughput and accuracy with long reads to produce rapid and accurate sequencing of microbes with streamlined sample preparation and a simple, scalable, and optimized workflow for data analysis.

- Conduct rapid and affordable bacterial and viral typing research during disease surveillance, using archived samples from investigations and disease etiology studies, such as studies of SARS-CoV-2, MERS, H1N1, and Ebola.
- Study microbial communities and conduct metagenomic research leveraging 16S rRNA sequencing or other targeted panels with Ion AmpliSeq technology. Based on ultrahigh-multiplex PCR, Ion AmpliSeq technology requires as little as 1 ng of input DNA and uses a simple, streamlined workflow.



Conclusion

The ability of the Ion GeneStudio S5 systems to work with multiple read lengths helps you achieve fast and consistent results irrespective of the size of your project. You can save your precious samples by using quantities as low as picograms and nanograms. The Ion GeneStudio S5 systems are great for customers who are seeking flexibility and a wide array of application options. In the next chapter, you will learn about the Ion Torrent™ Genexus™ System, which uses the same sophisticated sequencing technology as the Ion GeneStudio S5 systems but delivers an advanced level of automation and ease of use.

Chapter 4: NGS instruments—the Genexus System

Overview

The Ion Torrent™ Genexus™ System automates sample and library/template preparation, sequencing, analysis, and reporting. The Ion Torrent™ Genexus™ Purification System and Genexus™ Integrated Sequencer with Genexus™ Software work together seamlessly, tracking sample information and results automatically throughout the process.

This integrated system can deliver an end-to-end workflow from the biological specimen all the way to the report in a single day with just two user touchpoints and 20 minutes of total hands-on time.

- The Genexus Purification System extracts and quantifies nucleic acids in approximately 2–4 hours.
- The Genexus Integrated Sequencer automates library preparation, templating, and sequencing.
- The Genexus Software fully integrates the purification system with the sequencer, reducing the possibility of human error and making reporting simple. Data files can be exported for third-party analytics, or analytic tools from Thermo Fisher Scientific can be used to generate customizable report formats based on guidelines, clinical trials, curated markers, and novel variants.

The Genexus Purification System

The Genexus Purification System automates nucleic acid extraction, purification, and quantitation on a single platform to enable a consistent and efficient workflow solution for next-generation sequencing (NGS) sample preparation.

Extract, purify, and quantify nucleic acid samples—all on a single automated platform

The system helps minimize user processing errors and increases the reproducibility of your results by requiring just one touchpoint with 10 minutes of hands-on time. In as little a 2 hours, the system will provide you with a plate of purified and quantified nucleic acid sample that is ready to be loaded onto the Genexus Integrated Sequencer for downstream applications. The Genexus Purification System supports multiple specimen types to enable NGS applications (Table 4.1).

Innovative features of the Genexus Purification System include:

- **Easy operation and automated setup for error detection**—Work simply with prefilled reagents that require only one touchpoint and confirm correct reagent placement with onscreen guidance for loading consumables onto the instrument. The automated barcode scanning helps identify consumable expiration dates and detect errors.
- **Verified protocols**—Produce reliable data for downstream NGS on the Genexus Integrated Sequencer.
- **Rapid turnaround**—Go from specimen to purified and quantified nucleic acid that is ready for NGS analysis in as little as 2 hours.
- **Manufactured at an FDA-registered and ISO 13485–certified facility**—Have total confidence in the quality of your instrument.

Table 4.1. Ion Torrent™ Genexus™ kits for sample preparation.

Kit	Specimen type	Output nucleic acid	Reactions per kit
Genexus FFPE DNA and RNA Purification Kit (sequential extraction of DNA and RNA)	Lysate from FFPE tissue (surgical resection, core needle biopsy, and fine needle aspiration)	DNA and RNA, DNA only, or RNA only	48
Genexus Cell-Free Total Nucleic Acid Purification Kit	Plasma	cfTNA	24
Genexus Multisample DNA Purification Kit	Whole blood, PBLs, lysate from fresh-frozen tissue, and bone marrow	DNA only	48
Genexus Total RNA Purification Kit	Whole blood, PBLs, lysate from fresh-frozen tissue, and bone marrow	RNA only	48

The Genexus Integrated Sequencer

The Genexus System enables a workflow from a biological specimen all the way to the final report, with results delivered in as little as a single day. The Genexus Integrated Sequencer can also be used as a stand-alone instrument to automate NGS library preparation, sequencing, and analysis on a single platform.

Using the multilane Ion Torrent™ GX5™ Chip, which is designed to support variability in sample intake, samples can be cost-effectively processed as they come in.

Innovative features of the Genexus Integrated Sequencer:

- **Hands-off automation**—Library prep, sequencing, and analysis all happen on one instrument with a simple set-up-and-go workflow.
- **Streamlined on-instrument analysis**—Integrated reporting capabilities are supported, no server required.
- **Easy operation**—The instrument uses prefilled reagents and preset protocols, a single touchpoint, and 10 minutes of hands-on time.*
- **Rapid turnaround**—Go from nucleic acid to report in as little as a single day.**
- **Flexibility to accommodate small sample batches**—On-instrument reagent and chip stability supports sample intake variability.
- **Onboard vision system**—Understand reagent placement and detect errors through automated barcode scanning.
- **Manufactured at an FDA-registered and ISO 13485–certified facility**—Have total confidence in your instrument's quality.
- **Innovative multilane chip**—Simultaneously process up to four compatible assays in a single run.

Application highlights of the Genexus System

Learn how scientists, clinical researchers, and other laboratory personnel use automated workflows and the Genexus System to complete NGS.

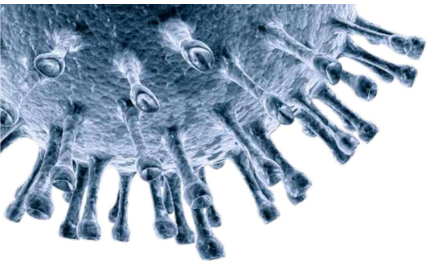
Oncology clinical research

Ion Torrent™ Oncomine™ assays for the Genexus System feature a complete NGS testing workflow. Now every laboratory can go from specimen to report in a single day, providing a comprehensive genomic profile. This allows you to deliver NGS results at the same time as other single-gene methods, such as immunohistochemistry (IHC).

The limited hands-on, set-up-and-go workflow makes NGS accessible even if your laboratory is new to the technology. With the lowest sample input requirement of any NGS solution, you can successfully sequence more of the samples that come through your laboratory.

* 10 minutes of hands-on time when running in integrated mode with the Genexus Purification System. A few more minutes to accommodate pipetting step time are needed when using as a stand-alone instrument.

** Processing times for individual samples vary.



Microbial and infectious disease research

Ion AmpliSeq™ targeted NGS panels run on the Genexus Integrated Sequencer provide an ultrasensitive, rapid, and accessible solution for infectious disease researchers. The panels provide a fast and accurate platform for sequencing of microbial genomes, suitable for bacterial and viral typing, epidemiological studies, and disease surveillance.

Benefits of using targeted NGS and the Genexus Integrated Sequencer for studying infectious diseases include:

- **Rapid turnaround time**—our fastest NGS workflow for infectious disease research for time-sensitive applications
- **Automation**—the set-up-and-go workflow enables minimal user touchpoints, which is designed to reduce user variability and increase the reproducibility of results
- **Accuracy of variants**—lower substitution errors for single-nucleotide variants (SNVs)
- **Low input requirements**—need as little as 1 ng of input nucleic acid to target regions of interest
- **Custom panel design options**—customize your own infectious disease research panel using the Ion AmpliSeq Designer tool at ampliseq.com

Coronavirus (SARS-CoV-2) research

A major challenge for microbiologists and virologists is the prediction of patterns of evolution and emergence of disease agents. RNA viruses like coronaviruses share the feature of high genetic variability, which causes them to phylogenetically cluster as clouds of mutants. Coronavirus variants also emerge through antigenic shifts within animal reservoirs, such as bats, snakes, and pangolins.

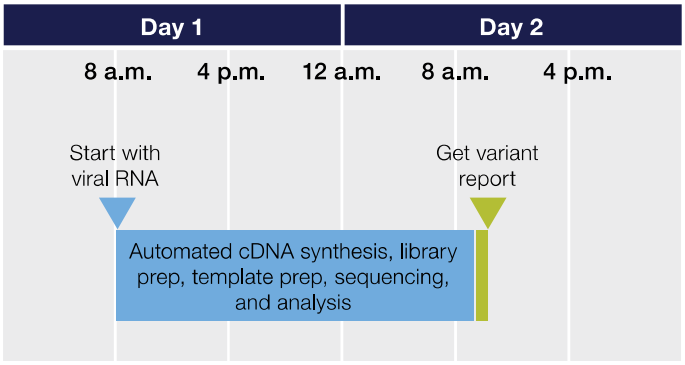
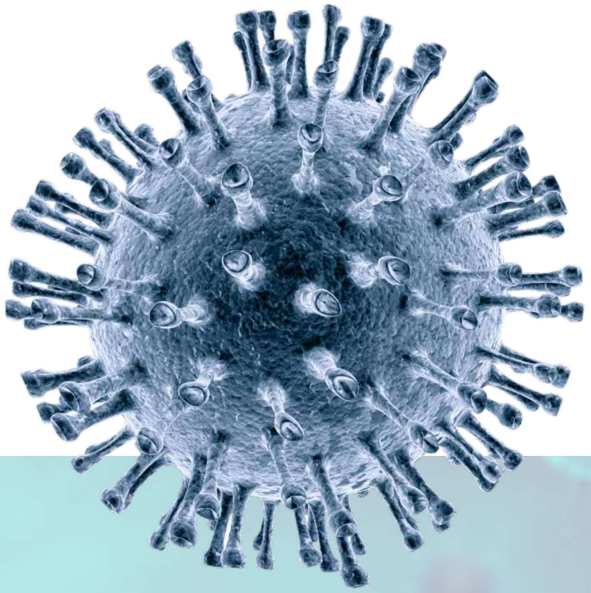
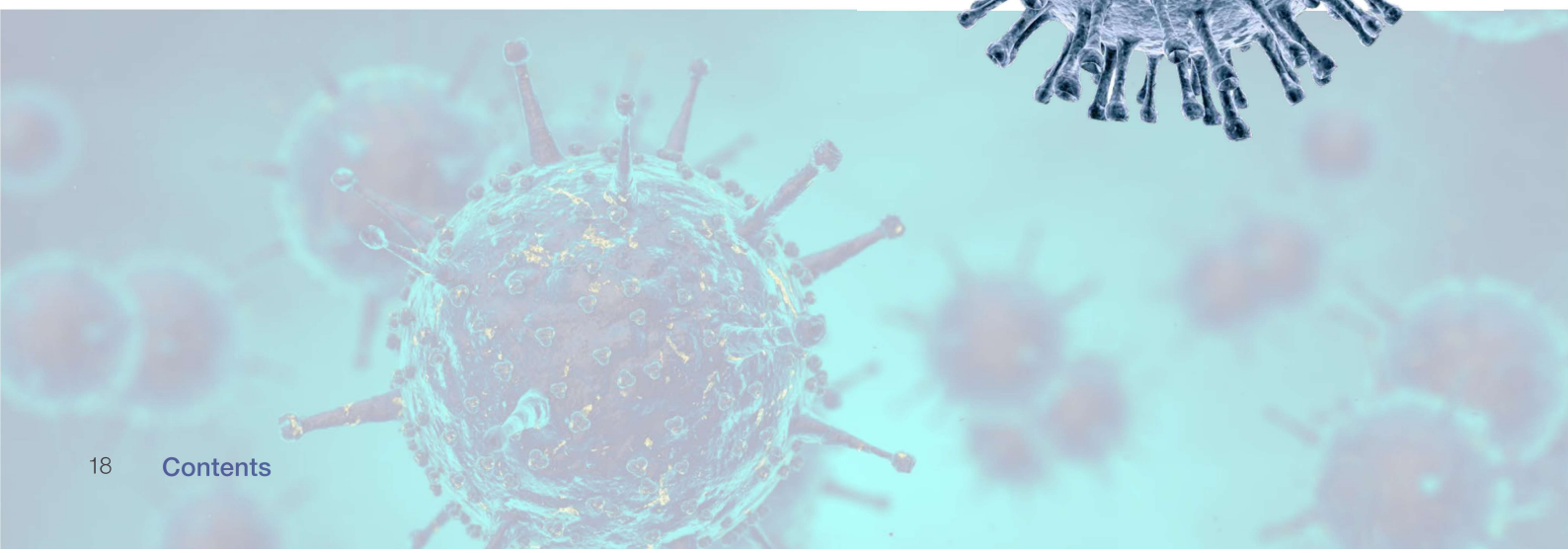


Figure 4.1. A typical workflow for analyzing 16 samples using the Ion AmpliSeq SARS-CoV-2 panel on the Genexus Integrated Sequencer, featuring rapid NGS automation.

Through the use of a set of highly specific, universal coronavirus primers in combination with a high-fidelity master mix, all genomic segments are amplified, sequenced, and analyzed in an automated workflow on the Genexus Integrated Sequencer. This NGS solution enables you to rapidly go from nucleic acid to variant report in under a single day with minimal hands-on time (Figure 4.1).

The Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay consists of two pools with amplicons 125–275 bp in length for complete coverage of over 99% of the SARS-CoV-2 genome, including all variants [1].





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

- **Rapid turnaround time**—our fastest NGS workflow for infectious disease research for critically time-sensitive applications
- **Automation**—the set-up-and-go workflow enables minimal user touchpoints, reducing user variability and increasing reproducibility of results
- **Accuracy of variants**—fewer substitution errors for SNVs
- **Higher success rates**—directly analyze samples that have low viral loads
- **Higher-resolution and longer-read NGS**—accurate and rapid virus typing for surveillance and epidemiology investigations

Inherited disease clinical research

Since 2011, Ion Torrent products for NGS have supported clinical research of human diseases with leading Ion AmpliSeq technology.

Now, Ion AmpliSeq™ On-Demand Panels are available for the Genexus System. Together, they provide a powerful and targeted NGS workflow that enables any laboratory to go from specimen to variant report in a single day, providing accessibility, efficiency, and convenience at a speed never possible before (Figure 4.2).

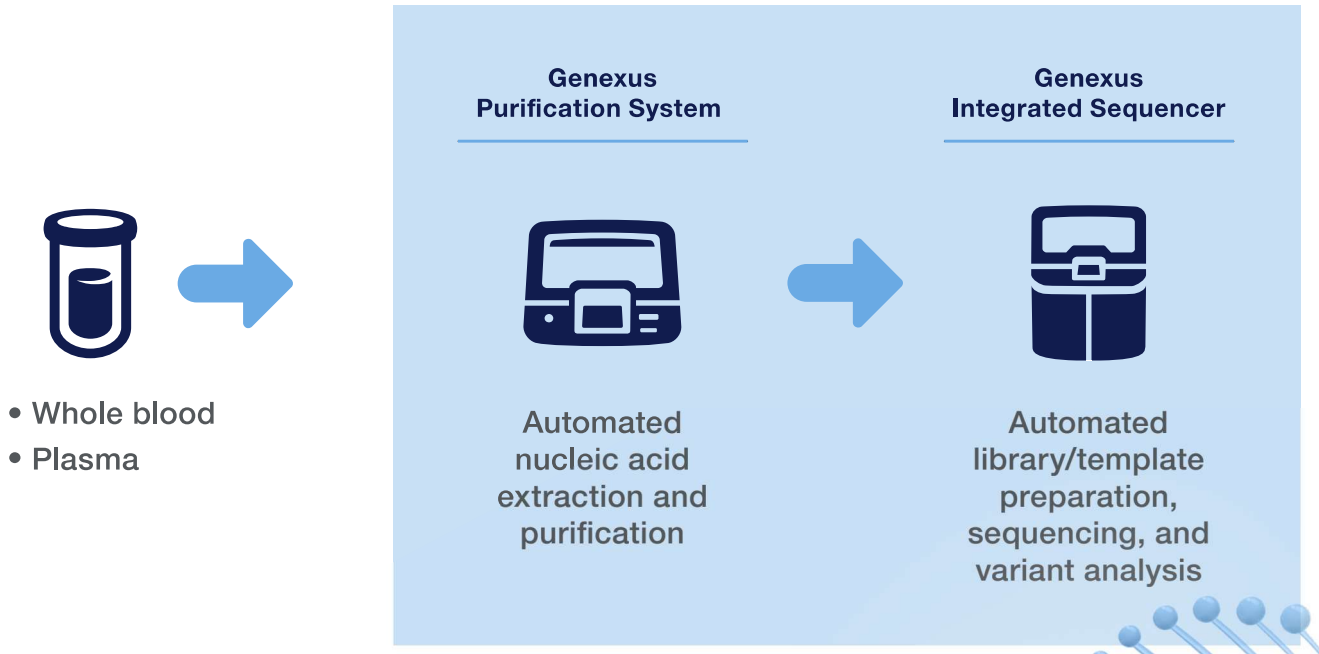


Figure 4.2. Workflow for specimen to variant report using the Genexus System.

The Genexus System integrates and automates nucleic acid extraction and purification, library/template preparation, sequencing, and analysis in a single software ecosystem. This highly flexible system lets you process samples—even just one—cost-effectively as they come in. With just 20 minutes of hands-on time and two touchpoints, users can get up and running quickly with significantly less training. The set-up-and-go workflow makes NGS accessible, even if your laboratory is new to the technology.

Conclusion

Next-generation sequencing is a powerful tool that has a wide variety of research applications. Targeted sequencing can be used to identify variations associated with individual organisms, genes, or gene-fragments; these variations can inform our understanding of disease mechanisms and immunity to infectious diseases, as well as the role of genes and gene variants in inherited diseases. Ion Torrent technology is accessible to laboratories of nearly any size and budget, and can be purchased alongside a full set of reagents, assays, and consumables from Thermo Fisher Scientific.

Chapter 4 reference

1. Thermo Fisher Scientific. Ion AmpliSeq SARS-CoV-2 Insight Research Assay. **https://www.thermofisher.com/us/en/home/life-science/sequencing/dna-sequencing/microbial-sequencing/microbial-identification-ion-torrent-next-generation-sequencing/viral-typing/coronavirus-research.html.**

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