Targeted sequencing solutions

Accurate, scalable, fast

TARGETED SEQUENCING

Ion Torrent™

life technologies

Sequencing for every lab, every budget, every application

Ion Torrent™ semiconductor sequencing

Ion Torrent™ technology has pioneered an entirely new approach to sequencing by combining simple chemistry and semiconductor technology—translating chemical signals into digital information. The ability to sequence faster, more simply, and affordably enables every researcher to take advantage of the power of next-generation sequencing.

The Ion PGM™ System makes affordable, high-quality next-generation sequencing accessible to scientists around the world. The Ion PGM™ System is a reliable sequencing platform that combines simple sample preparation and data analysis solutions with scalable chip output, for ultimate project flexibility. With this system, human disease researchers can perform a variety of targeted gene sequencing applications, such as variant detection in cancer and genetic disorders.

IMPROVE ACCURACY

Accurately call variants of interest, including those within difficult sequence contexts

MINIMAL SAMPLE INPUT

Interrogate precious research samples using only 10 ng of DNA or 5 ng of RNA

GENOME SEQUENCING | EXOME SEQUENCING | TRANSCRIPTOME SEQUENCING | **TARGETED SEQUENCING**

SCALABLE

Analyze hundreds of genes simultaneously with ultrahigh-multiplex PCR using up to 6,144 primer pairs in a single primer pool

FAST

Address time-sensitive samples with single-day assays, from samples to annotated variants

Join the worldwide Ion Torrent™ development community at **lifetechnologies.com/ioncommunity**

Targeted sequencing applications

Understanding disease at the genomic level

Next-generation sequencing (NGS) is a powerful tool used to extract and analyze molecular information and explore the human genome in an unprecedented manner. Routinely used in basic human disease research, NGS provides a genetic survey of samples by systematically identifying genetic alterations, including single-nucleotide variants, insertions and deletions (indels), copy number variations, and large genomic rearrangements. In particular, the heterogeneity and complexity of genetic alterations linked to cancer poses unique challenges for researchers, especially in determining variants among closely related samples.

Researchers now utilize NGS to identify specific changes in DNA by rapidly and simultaneously sequencing multiple gene targets within multiple samples. In contrast to whole-genome or whole-transcriptome sequencing, targeted DNA or targeted RNA sequencing studies focus the analysis on specific areas of interest (Figure 1).

The key to targeted sequencing is to amplify genomic regions of interest using PCR and specific sets of pooled primers. Before now, the tasks of designing such primers and optimizing PCR conditions were labor-intensive and time-consuming, taking weeks

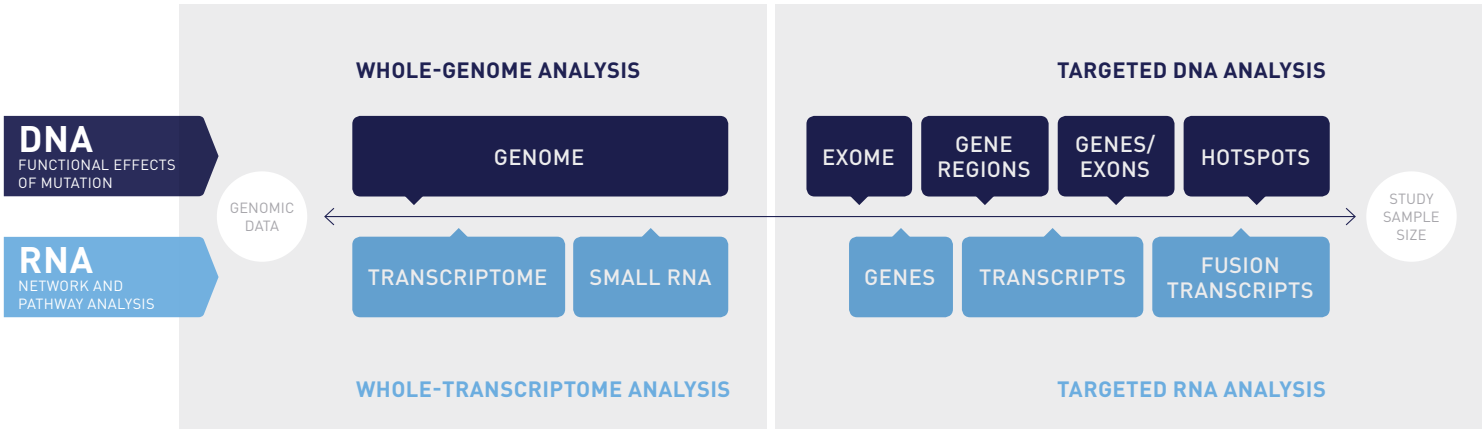


Figure 1. Next-generation sequencing (NGS) can provide a global genetic survey of the human genome and transcriptome, or it can be restricted to specific regions of the genome or transcriptome.

The Ion Community allows researchers to openly share methods and data, to both evaluate the technology and build on it. We have opened our protocols, datasets, and source code to the world to enable the community to drive application development.

> "We've created four custom Ion AmpliSeq™ panels, and all of them are performing extremely well on FFPE-derived DNA. We routinely screen dozens of genes from very small biopsy samples that would otherwise be insufficient for analysis."
>
> **CHRISTOPHER CORLESS, MD, PhD**
> KNIGHT DIAGNOSTIC LABORATORIES
> KNIGHT CANCER INSTITUTE
> OREGON, USA

and allowing you to sequence only a few genes at a time. Now Ion AmpliSeq™ targeted technology allows you to produce assay panels of preselected genes by designing the primers for each of your selected genes of interest—enabling you to construct your own customized targeted library. Using the designed primers and as little as 10 ng of DNA or 5 ng of RNA, you can amplify your samples using simple PCR and then sequence the library using the Ion PGM™ Sequencer. The ability to focus on a region to sequence, and then analyze the selected and potentially clinically relevant genes, makes targeted sequencing a practical application in clinical disease research. This sequencing approach can help uncover rare variants occurring at low allelic frequency, verify germline and somatic variants across a large number of samples, and allow you to discover similar shared mutations in genes driving the disease.

Cancer genomics

Genomics plays an essential role in cancer research since inherited genetic variations and acquired somatic mutations are integral to cancer pathogenesis. Researchers utilizing NGS have already contributed to an explosion of genetic information that is critical to determining the effects of mutations at a functional level. The use of NGS is also anticipated to contribute significantly to cancer research that may in the future provide information to more accurately diagnose, predict, and monitor disease progression and treatment outcomes. While capillary electrophoresis (CE)–based Sanger sequencing has traditionally been the gold standard in cancer research, it has limitations in throughput, speed, and resolution, and also does not easily scale to projects with large numbers of genes or samples (Figure 2). Targeted sequencing of tens or even hundreds of genes provides a more comprehensive picture of the cancer being studied.

Transform your research with Ion AmpliSeq™ target selection technology

Cancer mutation analysis

Cancer can be characterized by numerous somatic mutations, although only a subset may contribute to tumor progression. Distinguishing these "driver" mutations from neutral "passenger" mutations can help explain the phenotypic diversity in cancer and provide increased understanding of tumor initiation, maintenance, progression, and metastasis. NGS is a powerful tool for detecting and characterizing mutations as drivers or passengers.

Routine sequencing of FFPE tissue samples

One of the major challenges in cancer research is working with DNA and RNA isolated from formalin-fixed, paraffin-embedded (FFPE) tissue samples. Most archived cancer samples are FFPE-based, and can yield purified DNA that is degraded and contains base modifications. The availability of archived cancer samples is also limited, making low sample input requirements critical. Ion AmpliSeq™ technology transforms targeted sequencing by allowing you to interrogate precious samples for hundreds of genes simultaneously using as little as 10 ng of DNA or 5 ng of RNA from FFPE samples. The ability to process DNA and RNA from FFPE samples using targeted sequencing enables you to focus on selected sets of genomic regions using limited sample input, resulting in reduced cost per sample. With a wide variety of preselected, community-designed fixed panels and custom options, Ion AmpliSeq™ technology coupled with the Ion PGM™ Sequencer and Ion Reporter™ Software allows single-day processing of FFPE samples to annotated variants.

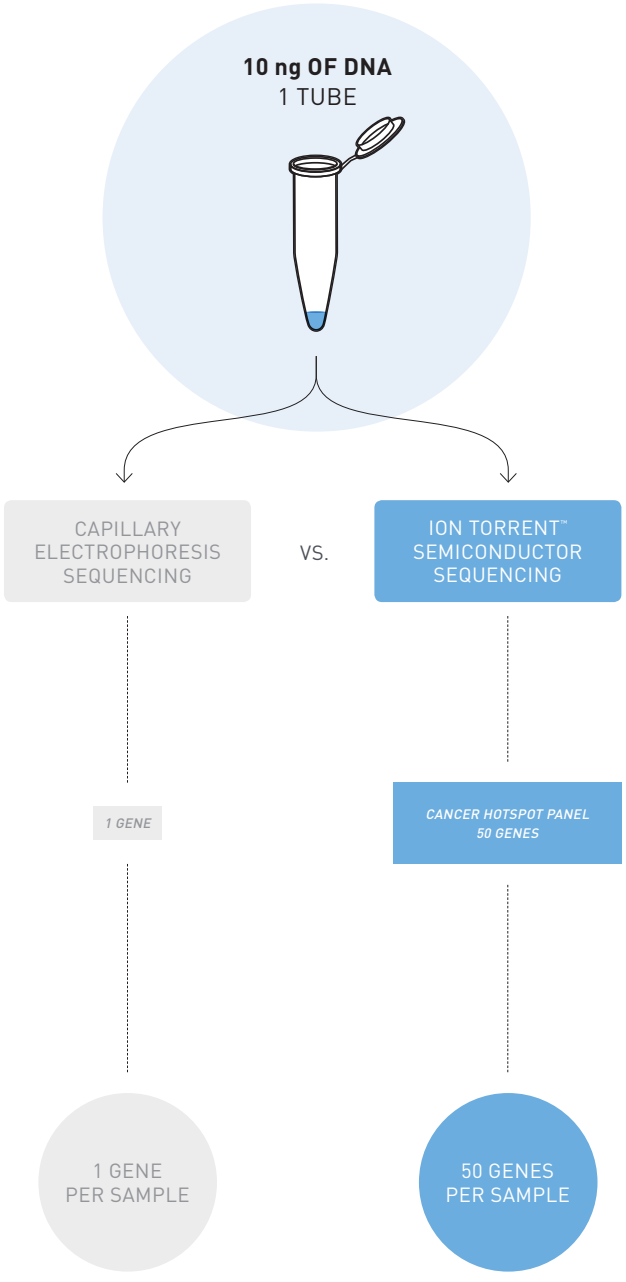


Figure 2. Methods such as CE-based Sanger sequencing interrogate a single gene per sample, requiring large-scale availability of sample input, time, and cost. In contrast, our most highly cited panel, the Ion AmpliSeq™ Cancer Hotspot Panel v2, interrogates variants of 50 genes in a single tube, requiring just 10 ng of DNA.

Gene panel sequencing finally enabled. Using just 10 ng of FFPE DNA and one pool of 6,144 primer pairs, variants can be identified in a single day using the Ion AmpliSeq™ custom workflow. Alternative NGS workflows use 25 times as much DNA, yet have 1/15 the multiplexing capacity, thus requiring 375 times as much DNA in all for the same project.

Genetic disorders research

Only one quarter of genetically inherited diseases are linked to regions of the human genome. In addition, while Mendelian disorders (monogenic disorders) are caused by mutations in a single gene, other complex disorders (polygenic disorders) can have a significant number of underlying causative genes.

Identifying regions of the human genome linked to specific diseases includes characterizing variants such as single-nucleotide polymorphisms (SNPs) and copy number variations. Evolutionary forces add complexity by introducing new variants in each generation. There is a need to interrogate genes in many different samples to not only find potential mutations but also determine which of these mutations may play a role in a specific disease. Since traditional methods such as Sanger sequencing are not ideal for analyzing large sets of genes, NGS provides the perfect tool to survey hundreds of genes across hundreds of research samples to find rare mutations in a single run.

The Ion AmpliSeq™ Inherited Disease Panel was developed to match genes targeted by clinical molecular geneticists studying inherited diseases with genes listed in the NIH Genetic Testing Registry. The panel employs over 10,000 primer pairs in just 3 tubes to amplify the exons of 328 genes. These genes are associated with over 700 inherited diseases according to the NCBI ClinVar database, allowing you to quickly begin comprehensive research studies for disease-causing mutations.

"The advantage of the Ion PGM™ System with Ion AmpliSeq™ technology is that you can get results on tens of genes by using 10 ng of DNA from FFPE tissue."

DR. NICOLA NORMANNO
CENTRO DI RICERCHE ONCOLOGICHE DI MERCOGLIANO
MERCOGLIANO (AV) AND INT-FONDAZIONE PASCALE
NAPLES, ITALY

Torrent Suite™ Software: most accurate for multiple gene panels

Copy number in the human genome

Copy number variations (CNVs) represent a class of genomic variation in which large regions (>1 kb) of the genome are duplicated or deleted. Inherited and *de novo* CNVs of chromosomal regions have been associated with many diseases, including cancer and neurodevelopmental diseases such as autism and schizophrenia. There are a variety of impacts of CNVs, including deletions that unmask recessive mutations, duplications and deletions that alter the copy number of genes, and duplication of regions containing driver mutations affecting the expression and regulation of genes involved in cancer.

Until recently, researchers mainly employed methods such as array comparative genomic hybridization (aCGH) or fluorescence *in situ* hybridization (FISH) to detect CNVs and other types of genomic alterations. However, these methods are limited by the requirement of array design, or they detect only known CNVs and SNPs, with no ability to discover novel variations.

Furthermore, since relative changes in copy number are measured with limited dynamic range, only gross-level changes can be detected and smaller variations are missed. Finally, many of these methods are costly and rely on subjective analysis.

Targeted NGS not only allows discovery of novel copy number variants but also provides important genetic information to discover variants in biological pathways and disease in the future. Ion AmpliSeq™ technology and the Ion PGM™ Sequencer, coupled with specific CNV workflows within Ion Reporter™ Software, enable simultaneous identification of substitutions, insertions and deletions, and now copy number variants across hundreds of genes within a sample in a single day (Figure 3). The detection and discovery of CNVs in concert with other variants in a single experiment helps reduce total cost, time, and complexity, and enables you to gain broader insight into disease from a limited amount of sample.

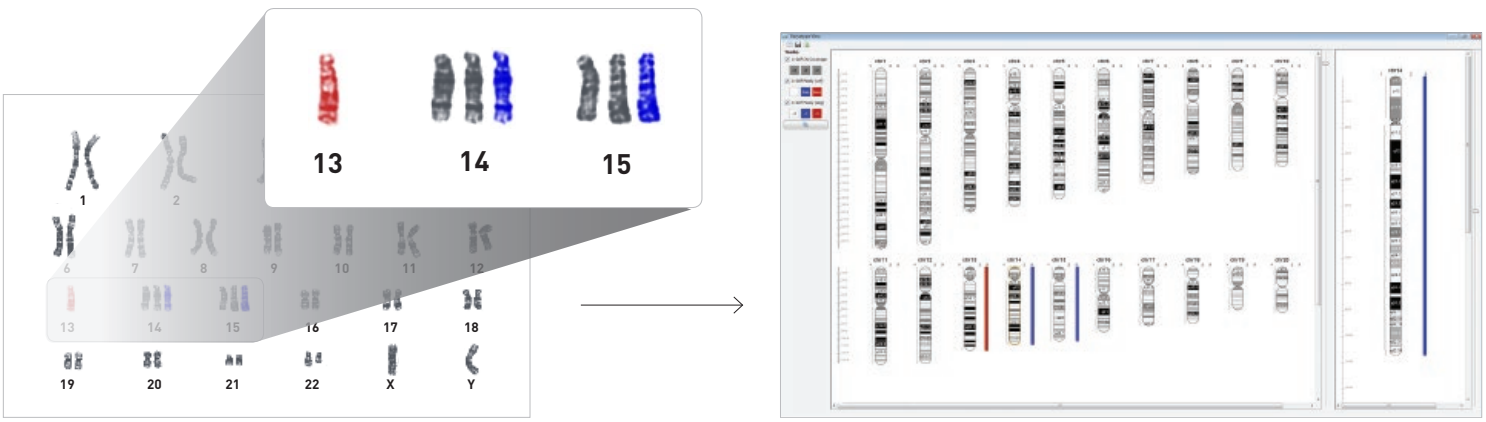


Figure 3. A monosomy of chromosome 13 and a trisomy of chromosome 14 and 15 as detected and shown in Ion Reporter™ Software.

Confidence in calling variants in gene panel sequencing is paramount for disease researchers. Torrent Suite™ Software is an essential component—with a variant caller optimized for Ion AmpliSeq™ ready-to-use and community panels, easily tunable parameters and preset analysis modes for high or low stringency, and specific features for germline or somatic studies.

Quantitative gene expression analysis

Common genetic variants that influence gene expression are implicated in complex diseases. Quantitative gene expression analysis is fundamental to many biological research applications, including gene regulation analysis, correlating gene expression with phenotypic information, and cellular pathway analysis. Conventional gene expression profiling methods include hybridization-based (microarray) or amplification-based (qRT-PCR) technologies. However, these methods present challenges in accuracy and absolute quantification of transcripts, in addition to only being able to target known array designs. RNA sequencing using NGS technology not only provides a digital representation of absolute expression but also can identify and characterize low-abundance transcripts.

Using Ion AmpliSeq™ technology, you can profile up to 1,200 customer-defined mRNA or noncoding RNA targets in a single amplification reaction. Importantly, the Ion AmpliSeq™ targeted RNA workflow utilizes very low amounts of input RNA—500 pg of unfixed RNA or 5 ng of RNA isolated from FFPE samples. Targeted RNA sequencing also allows you to detect fusion transcripts from chromosomal rearrangements, critical in the initiation of tumorigenesis in some cancers.

"The idea is to use Ion AmpliSeq™ RNA panels to detect changes in RNA settings, like gene fusion, and use DNA panels to detect genomic variations, SNPs, indels, and copy number variants."

DR. IAN CREE
PROFESSOR OF PATHOLOGY
THE UNIVERSITY OF WARWICK
COVENTRY, THE UNITED KINGDOM

Customized gene panels

If the ready-to-use Ion AmpliSeq™ panels do not match gene targets of interest, researchers can easily create customized panels for their disease area using Ion AmpliSeq™ Designer. Ion AmpliSeq™ Designer is a free online tool that allows researchers to create and order Ion AmpliSeq™ DNA custom panels comprising human or mouse genes of interest. Incorporating ultrahigh-multiplex PCR with up to 6,144 primer pairs in a single pool, Ion AmpliSeq™ custom panels enable scalable panel design from a few genes to hundreds of genes per panel. Leveraging more than a decade of expertise powering the TaqMan® custom assay design pipeline, Ion AmpliSeq™ Designer produces optimized primer designs in just hours. The Ion AmpliSeq™ workflow is based on a transformative technology that simplifies ultrahigh-multiplex PCR amplification and library construction and requires as little as 10 ng of DNA or 5 ng of RNA per pool. You now have full flexibility to analyze hundreds of genes of your choice, such as those implicated in particular disease states or representing specific biochemical pathways.

With three sequencing chips to choose from, you can select the amount of sequencing throughput required for your specific application—saving you time and money.

Targeted sequencing
single-day workflows

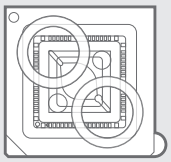
Streamlined sample preparation and application-specific data analysis solutions have further simplified targeted sequencing, enabling significant breakthroughs across all areas of human disease research.

We offer a full solution for targeted sequencing for cancer and genetic disorders research. These workflows help speed your time-to-results with automated template preparation, fast sequencing runs, and data analysis packages optimized for your research. The Ion PGM™ Sequencer coupled with Ion AmpliSeq™ gene panels provides a fast and cost-effective gene panel sequencing solution. The integrated software solutions provide tunable accuracy and results in a single day—all on your benchtop.

SELECT TARGETS

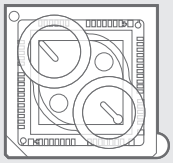
<2 HR

APPLICATION	PRODUCTS
Cancer driver mutations	Ion AmpliSeq™ DNA panels • Ready-to-use panels • Community panels • Custom panels
Inherited disease mutations	
Quantitative gene expression	Ion AmpliSeq™ RNA panels • Ready-to-use panels • Custom panels



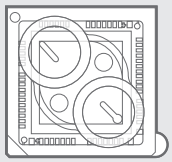
314

Ion 314™ Chip
1 million wells
400–550 thousand reads
for 200-base sequencing



316

Ion 316™ Chip
6 million wells
2–3 million reads
for 200-base sequencing



318

Ion 318™ Chip
11 million wells
4–5.5 million reads
for 200-base sequencing

CONSTRUCT LIBRARY	PREPARE TEMPLATE	RUN SEQUENCE	ANALYZE DATA
5.5 HR	4 HR	4.5 HR	0.5 HR
Ion AmpliSeq™ DNA Library Kit Ion Library Equalizer™ Kit Ion AmpliSeq™ Sample ID Panel Ion Xpress™ Barcode Adapters Kit	Ion One Touch™ 2 System and Ion Library Equalizer™ Kit OR Ion Chef™ System and Ion PGM™ IC 200 Kit	Ion PGM™ Sequencer and Ion PGM™ Sequencing 200 Kit v2 AND ANY OF THE FOLLOWING Ion 314™ Chip Kit v2 Ion 316™ Chip Kit v2 Ion 318™ Chip Kit v2 For use with Ion Chef™ System (sequencing reagents are provided as part of the Ion PGM™ IC 200 Kit) Ion 314™ Chip Kit v2 BC Ion 316™ Chip Kit v2 BC Ion 318™ Chip Kit v2 BC	Torrent Suite™ Software Ion Reporter™ Software
Ion AmpliSeq™ RNA Library Kit Ion Xpress™ Barcode Adapters Kits			Torrent Suite™ Software Ion Reporter™ Software • Copy Number Variation workflow
			Torrent Suite™ Software • Coverage Analysis Plugin Partek® Flow® Software

Targeted sequencing solutions with the Ion PGM™ System

Accurate:
Call variants of interest, including those within difficult sequence contexts

Minimal sample input:
Interrogate precious research samples using as little as 10 ng of DNA or 5 ng of RNA

Fast:
Address time-sensitive samples with single-day assays

Scalable:
Target hundreds of genes simultaneously with ultrahigh-multiplex PCR

"The Ion AmpliSeq™ Cancer Hotspot Panel and custom gene panels on the Ion PGM™ System offer a great advance in cancer research by allowing the simultaneous detection of multiple gene mutations and, potentially, chromosomal rearrangements with high throughput and in a short period of time."

MARINA NIKIFOROVA, MD
ASSOCIATE PROFESSOR OF PATHOLOGY
PATHOLOGY DEPARTMENT, UNIVERSITY OF PITTSBURGH
PENNSYLVANIA, USA

Learn more about targeted sequencing using the Ion PGM™ System at **lifetechnologies.com/iontargeted**

life technologies
A Thermo Fisher Scientific Brand