ion torrent

RNA sequencing solutions

Simple, fast, and affordable

ThermoFisher SCIENTIFIC

Transcriptome sequencing applications

RNA sequencing provides fundamental insights into how genomes are organized and regulated—giving us valuable information about the internal state of cells and transcriptional networks.

Understanding disease at the transcript level

Next-generation sequencing (NGS) is a powerful tool that can be used to understand the molecular mechanisms of gene transcription and explore the human genome in an unprecedented way.

RNA research interests

The key goals of RNA research are to:

- Catalog complete sets of transcripts in the genome [1]
- Quantify changing expression levels of genes and transcripts during development and under different conditions [1]
- Discover novel fusion transcripts, alternatively spliced isoforms, and biomarkers
- Monitor gene pathway flux

Gene transcription is an intricate and dynamic process that generates a variety of RNA species, including short RNAs, polyadenylated RNA, and nonadenylated transcripts. Transcriptome sequencing is the most complete and cost-efficient method for identifying and quantifying RNA from multiple types of starting material. An important consideration for the application of NGS in human disease research is whether to interrogate the whole transcriptome, targeted genes, or regulatory elements.

Whole transcriptome analysis

Whole transcriptome sequencing and analysis is of growing importance in understanding how altered expression contributes to complex diseases such as cancer, diabetes, and heart disease. Analysis of differential RNA expression provides researchers with greater insight into mechanisms that regulate cell fate, development, and disease progression.

The unprecedented depth of coverage and hypothesis-free approach afforded by NGS facilitates the identification of novel transcribed regions, undiscovered alternatively spliced variants, and low-abundance fusion transcripts from coding and noncoding RNA.

Gene expression analysis

Quantitative and differential 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) and amplification-based (qRT-PCR) technologies. However, both approaches have limitations: neither method enables absolute quantification of transcripts, hybridization-based approaches suffer from compressed fold-change accuracy and low sensitivity, and the workflow associated with amplification-based methods does not enable parallel analysis of large numbers of genes (for example, as defined by a pathway or disease). Additionally, analysis of degraded samples, such as RNA from formalin-fixed, paraffin-

embedded (FFPE) tissues, poses additional challenges when considering these methods. In contrast, RNA sequencing using NGS technology provides a representation of absolute expression, and can identify and characterize low-abundance transcripts even from FFPE RNA.

Biomarker discovery

The depth of NGS sequencing coverage also enables transcriptome-wide biomarker discovery. Identifying regions of the human genome that may be linked to specific diseases through detection of transcriptomic alterations may enable researchers to identify markers associated with a particular disease. This is especially helpful for research studies where genomic resources are scarce.

Small RNA analysis

Small noncoding RNAs can be grouped into different classes and are involved in biological processes such as translation, RNA splicing, and gene regulation. Studies focusing on how dysregulation and variation of small RNAs influence mRNA expression can provide critical insight into disease causation and progression.

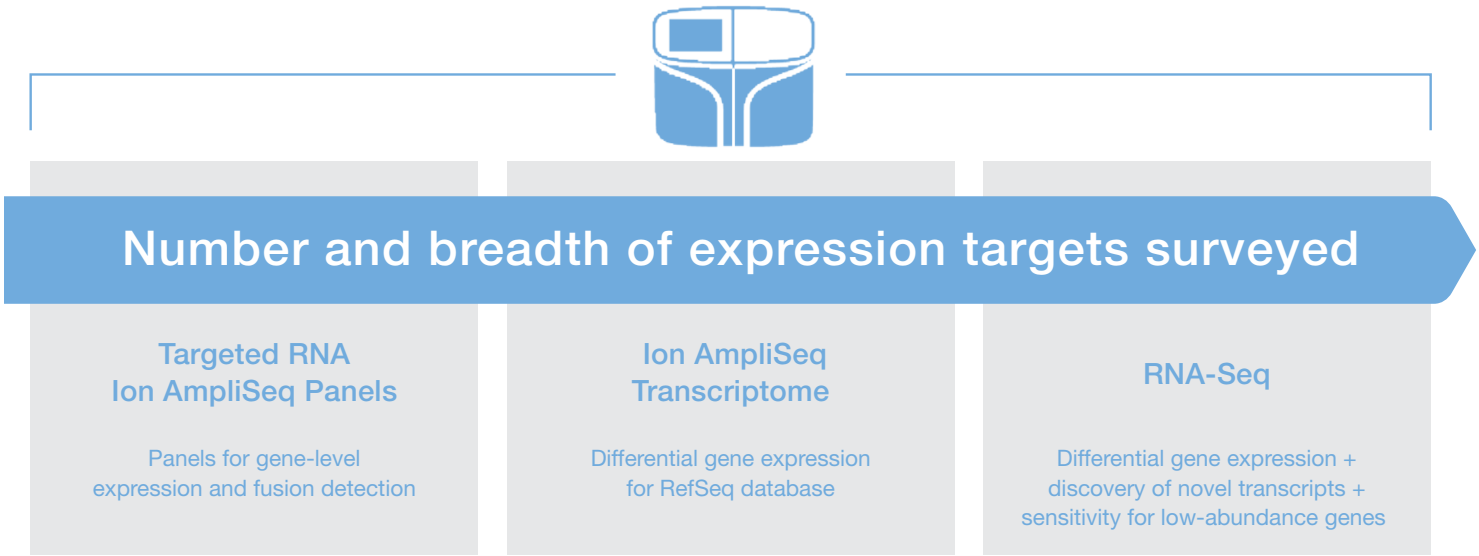


Figure 1. Ion Torrent™ technologies provide a spectrum of solutions for RNA sequencing, from focusing on specific regions of the genome or transcriptome to a global survey of the human transcriptome.

Ion Torrent
sequencing for all

The Ion S5™ and Ion Chef™ sequencing systems, combined with Ion AmpliSeq™ panels and Invitrogen™ RNA purification and library construction kits, offer a reliable sequencing workflow that integrates simple sample and library preparation, intuitive data analysis, and flexible chip output for ultimate project flexibility—giving you one of the best benchtop solutions for RNA analysis. No other benchtop solution offers this combination of rapid turnaround time, simple workflow, and scalable throughput.

In contrast to microarray analyses that have limited sensitivity and complex workflows, transcriptome sequencing provides workflows with a greater quantitative linear dynamic range and improved detection at the extremes of the quantitation range. With flexible solutions, including a simple FFPE-compatible workflow for targeted transcriptome sequencing or whole transcriptome sequencing for a hypothesis-neutral approach to interrogate all the transcriptional content of a genome, these fast and affordable sequencing approaches provide valuable insights into how genomes are organized and regulated.

Scalable

Three chip types are available to support your varying application and sample throughput needs. This flexibility also means you don't have to batch samples to achieve optimum cost efficiency.

FFPE-compatible

Detect high- and low-abundance transcripts in FFPE samples from as little as 5 ng input RNA (Figure 3).

Better performance

Exceed microarray sensitivity for detection of differentially expressed genes (DEGs), with high correlation to microarray quality control (MAQC) array data (Figure 2).

Go from sequence data to biological interpretation quickly and easily with powerful statistical and visualization tools, an intuitive user interface, and pipelines built for the way biologists think.
To learn more, go to **thermofisher.com/ioninformatics**

Easy analysis

Leverage existing NGS, qRT-PCR or microarray pipelines for simple automated differential expression data analysis.

Fast

Go from RNA to quantitated genes in less than 2 days with minimal hands-on time (Figure 4).

Unlock your precious samples with the Ion AmpliSeq transcriptome solution

Ion Torrent sequencing

discover more genes

Torrent Suite™ Software, in conjunction with analysis plug-ins, generates simple, easy-to-use data files for gene expression analysis. Perform simple statistical analysis or import files into third-party software, such as Bioconductor™ or Partek™ software, for more comprehensive data analysis.

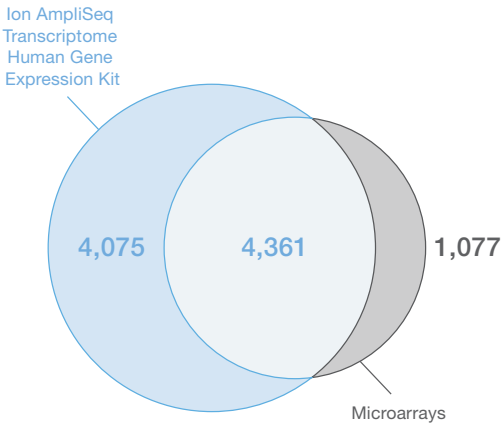
Flow Genomics Suite

"RNA-Seq on the Ion S5 System enabled us to perform rapid detection of fusion transcripts in our cancer research, enabling us to find results we would not be able to find with microarray technology."

Adam Ameur
Department of Immunology, Genetics and Pathology
Uppsala University, Sweden

Far greater sensitivity than microarrays

A



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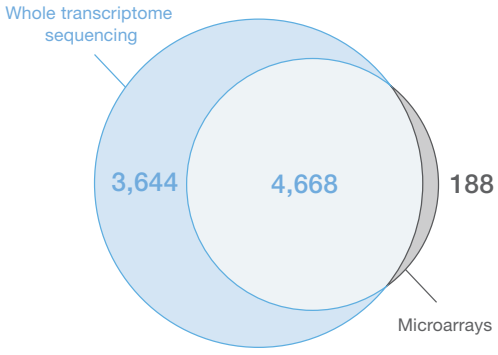


Figure 2. Detection of significantly differentially expressed genes (DEGs). Venn diagram showing the concordance for DEGs (≥2-fold change between universal human reference RNA (UHRR) and human brain reference RNA (HBRR)) between MAQC microarray data and **(A)** data from the Ion AmpliSeq Transcriptome Human Gene Expression Kit with ~10 million mapped reads, or **(B)** data from whole transcriptome sequencing with 35 million reads. All data were generated on the Ion Proton™ platform.

Targeted, quantitative gene expression analysis at your fingertips

With simultaneous amplification of more than 20,000 RefSeq genes in a single tube starting from as little as 10 ng of total RNA, the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit transforms cancer and translational research for tumor profiling and biomarker discovery by unlocking access to precious FFPE or limited-quantity samples.

FFPE-compatible

A

Sample name	Mapped reads	Valid reads	Targets detected
Fresh frozen #1	11.6 M	88.0%	73.9%
Fresh frozen #2	11.6 M	88.0%	73.9%
FFPE #1	9.2 M	77.8%	67.3%
FFPE #2	9.8 M	77.7%	67.9%

B

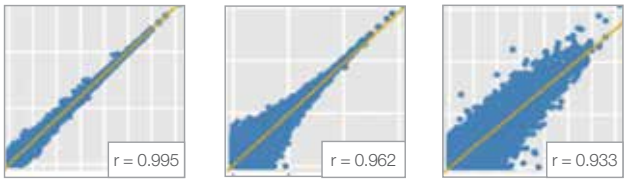
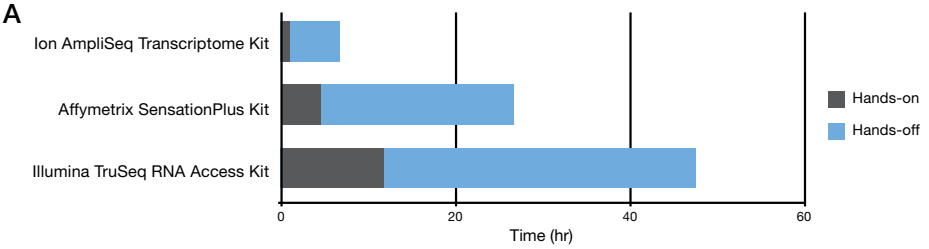


Figure 3. Using the Ion AmpliSeq Transcriptome Human Gene Expression Kit with 10 ng of total RNA input, sequencing data were generated from matched FFPE and fresh frozen samples. Shown are **(A)** sequencing metrics from Ion Proton sequencing reads and **(B)** correlations between fresh frozen replicates, FFPE replicates, and fresh frozen tissue vs. FFPE samples, respectively.

Fast and simple workflow

A



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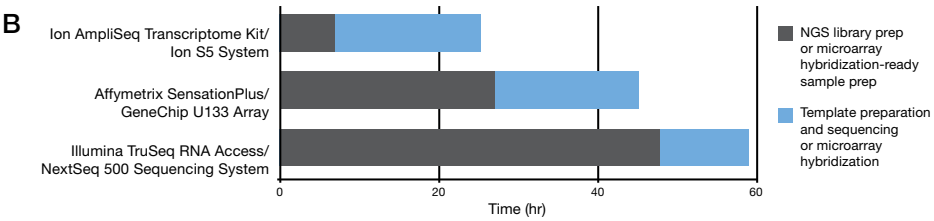

Figure 4. Comparison of hands-on and total turnaround time for leading gene expression analysis platforms. (A) NGS library prep or microarray hybridization-ready sample step only. **(B)** Entire workflow, from sample to data files.

> "The Ion AmpliSeq™ transcriptome technology is a very useful research tool for any group analyzing low-input or FFPE samples using RNA-Seq. Our highly degraded, low-yield, or microdissected samples that could not be successfully processed in the past now have a dependable and efficient conduit for library preparation."

Brad Hancock
Laboratory of Milan Radovich, PhD
Department of Surgery
Indiana University School of Medicine, USA

Create custom RNA analysis panels in three easy steps

RNA research considerations

RNA enrichment

The key to successful RNA sequencing is enrichment, so that you get the quality and quantity needed to build a library. The type of RNA enrichment depends on your application and the amount of RNA available (Table 1).

rRNA depletion

Ribosomal RNA accounts for over 80% of total RNA. The efficient removal of rRNA is paramount for high-quality whole transcriptome sequencing, as it increases the coverage of both coding and non-coding RNAs and improves the sensitivity to detect low-abundance transcripts. The Invitrogen™ RiboMinus™ Eukaryote System v2 provides a robust and efficient method for removal of cytoplasmic rRNA (5S, 5.8S, 18S, and 28S) and mitochondrial rRNA (12S and 16S) from 1–5 µg of total RNA in a single round of depletion. The system utilizes probe designs from highly conserved regions of rRNA, which enables use for several eukaryotic species such as human, mouse, and rat. The Invitrogen™ Low Input RiboMinus™ Eukaryote System v2 improves upon the previous system by removing significantly more rRNA, starting with as little as 100 ng total RNA.

mRNA enrichment

To separate coding RNAs from noncoding RNA, the 3´ poly(A) tail is targeted for enrichment. The Invitrogen™ Dynabeads™ mRNA DIRECT™ Micro Purification Kit uses this approach to purify mRNA from total RNA with oligo(dT)-coupled magnetic beads in less than 1 hour. Using total RNA in the Dynabeads mRNA DIRECT Micro Purification Kit procedure offers a robust, rapid method to enrich for poly(A) RNA with less ribosomal RNA (rRNA) carryover.

PCR-based enrichment

Using Ion AmpliSeq™ technology, the fast and simple Ion AmpliSeq Transcriptome Human Gene Expression Kit and Ion AmpliSeq™ RNA panels use 10 ng or less of total RNA for reverse transcription, followed by an ultrahigh-multiplex PCR reaction to amplify transcripts of interest. The Ion AmpliSeq Transcriptome Human Gene Expression panel targets >20,000 RefSeq transcripts with as little as 1 hour of hands-on time, while custom Ion AmpliSeq RNA panels targeting up to 1,200 transcripts in a single tube can be quickly and easily designed using a free online tool, Ion AmpliSeq™ Designer.

Strandedness

Transcribed RNA has “strandedness” in the sense that a given RNA element is synthesized from a particular strand of the genomic DNA. Retaining this strand information aids in the understanding of transcriptional regulation, the potential role of sense and antisense transcription, and elucidation of transcribed loci with complex patterns of overlapping transcription. RNA-mediated silencing or activation is often sequence-specific, and knowledge of strand specificity provides more accurate target prediction.

Proprietary Invitrogen™ RNA library construction technology attaches adapters in a directional manner that preserves strand information in the libraries. As a result, mapped reads are aligned based on the direction of transcription relative to the chromosomal strand, which aids in the understanding of the structure and expression levels of the transcript and in the discovery of novel transcription regions.

1. Log into **ampliseq.com** with your **thermofisher.com** login.
2. Start a new design, selecting your application of choice (RNA Gene Expression or RNA Gene-Fusion).
3. Add genes of interest and submit for design.

Coverage required

The appropriate depth needed for a particular transcriptome experiment depends on the purpose of the project. For example, if the project's purpose is to look at the expression profile of poly(A) RNA samples, that may require only a modest depth of sequencing. If the purpose is discovery of novel transcripts or low-abundance transcripts, a higher number of reads will facilitate this.

Smart informatics

The simplicity of using Ion Torrent™ sequencing instruments is enhanced by the easy-to-use, pre-installed Torrent Suite Software that runs on the Torrent Server. This software suite provides automated data analysis workflows that take you from raw data to high-quality sequencing reads and alignments. Also included in Torrent Suite Software is Torrent™ browser: a web-based interface for planning, monitoring, and viewing results from a sequencing run. Plug-ins provided in Torrent Suite Software and on the Ion Community offer a powerful means to manage a full range of additional applications and analyses.

Multi-sample differential gene expression analysis

- Whole transcriptome RNA-Seq: RNASeqAnalysis plug-in enables automated QC, genome mapping, gene/transcript quantification, differential gene/transcript expression analysis, and report generation with easy-to-interpret visualization
- Targeted RNA sequencing: AmpliSeqRNA plug-in enables automated QC, genome mapping, gene quantification, differential gene expression analysis, and report generation with easy-to-interpret visualization
- Small RNA/miRNA sequencing: smallRNA analysis plug-in enables automated analysis including run QC, reading mapping, small RNA quantification and visualization

All workflows offer intermediate BAM or gene expression result files that can be exported to open-source tools such as Bioconductor or third-party applications such as Partek™ software.

Table 1. Recommended products for enrichment.

Application	Input amount	RNA enrichment	Products
Whole transcriptome RNA-Seq	100 ng–5 µg	Selective ribosomal RNA reduction from total RNA	RiboMinus Eukaryote System
Small RNA/miRNA sequencing	500ng–20 µg	Selective small RNA enrichment	Ion Total RNA-Seq Kit v2
mRNA-Seq	100 ng–1 µg	mRNA enrichment from total RNA	Dynabeads mRNA DIRECT Micro Purification Kit
Targeted RNA-Seq	10 ng	Ultrahigh-multiplex PCR	Ion AmpliSeq Transcriptome Human Gene Expression Kit
	500 pg–5 ng	High-multiplex PCR	Ion AmpliSeq RNA Cancer Panel
	500 pg–5 ng	High-multiplex PCR	Ion AmpliSeq RNA Apoptosis Panel
	500 pg–5 ng	High-multiplex PCR	Ion AmpliSeq RNA Custom Panel

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

RNA sequencing workflow

The Ion Torrent™ RNA research solution offers streamlined sample preparation, application-specific data analysis, and the lowest cost per sample on a benchtop sequencer. The combination of affordable instrument pricing with scalable chips enables the easy implementation of transcriptome sequencing for studies with sample size variability or requiring experimental fine-tuning for the detection of low-abundance transcripts and rare RNA types.

We offer a full solution for RNA sequencing that is easy to implement—with a rapid workflow from RNA to quantitated genes in less than 2 days. Torrent Suite Software automatically provides the sequence reads in a format that can be uploaded to third-party software for easy analysis and data interpretation.

Select targets

Application	Products
Whole transcriptome RNA-Seq (ribosomal depletion)	Low Input RiboMinus Eukaryote System v2 or RiboMinus Eukaryote System v2
mRNA-Seq (poly(A) selection)	Dynabeads mRNA DIRECT Micro Purification Kit Ion AmpliSeq Transcriptome Human Gene Expression Kit
Targeted RNA-Seq	Ion AmpliSeq RNA Cancer Panel Ion AmpliSeq RNA Apoptosis Panel Ion AmpliSeq RNA Custom Panel

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.

 Construct library	 Prepare template	 Run sequence	 Analyze data
Ion Total RNA-Seq Kit v2 or, for less hands-on time, Ion Total RNA Seq-Kit for AB Library Builder™ System **Optional** Ion Xpress™ RNA-Seq Barcode 1–16 Kit	Ion OneTouch™ 2 System and Ion PI™ Template OT2 200 Kit or Ion Chef™ System and Ion PI™ IC 200 Kit	Ion S5™ Sequencer and Ion 540™ Chip Kit	Torrent Suite Software and RNASeqAnalysis plug-in **Optional** Partek Flow software
Ion AmpliSeq™ Library Kit Plus **Optional** Multiplexed sequencing Ion Xpress™ Barcode Adapters 1–16 Kit			Torrent Suite Software and AmpliSeqRNA plug-in **Optional** Partek or Bioconductor software
Ion Chef Instrument Ion AmpliSeq Kit for Chef DL8 or Ion AmpliSeq RNA Library Kit **Optional** Ion Xpress Barcode Adapters 1–16 Kit	Ion Chef Instrument and Ion PGM IC 200 Kit or Ion OneTouch 2 System and Ion PGM Template OT2 200 Kit	Ion S5 System and Ion 520 Chip Kit or Ion 530 Chip Kit or Ion 540 Chip Kit	Torrent Suite Software and AmpliSeqRNA plug-in

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RNA sequencing with Ion Torrent sequencing systems

FFPE compatibility

Detect high- and low-abundance transcripts in FFPE samples from as little as 5 ng input RNA with the Ion AmpliSeq™ targeted panels.

Rapid workflow

Go from RNA to gene quantitation in less than 2 days.

Easy analysis

Use Torrent Suite Software and associated plug-ins, or utilize existing NGS, qRT-PCR, or microarray pipelines for simple, automated differential expression data analysis.

Better performance

Exceed microarray sensitivity for detection of differentially expressed genes, with high correlation to MAQC microarray and qPCR data.

Flexibility

Survey differential gene expression profiles and discover novel biomarkers with the power of a single sequencing platform.

Reference

1. Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet* 10:57–63.

Learn more about transcriptome sequencing at
thermofisher.com/iontranscriptome

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