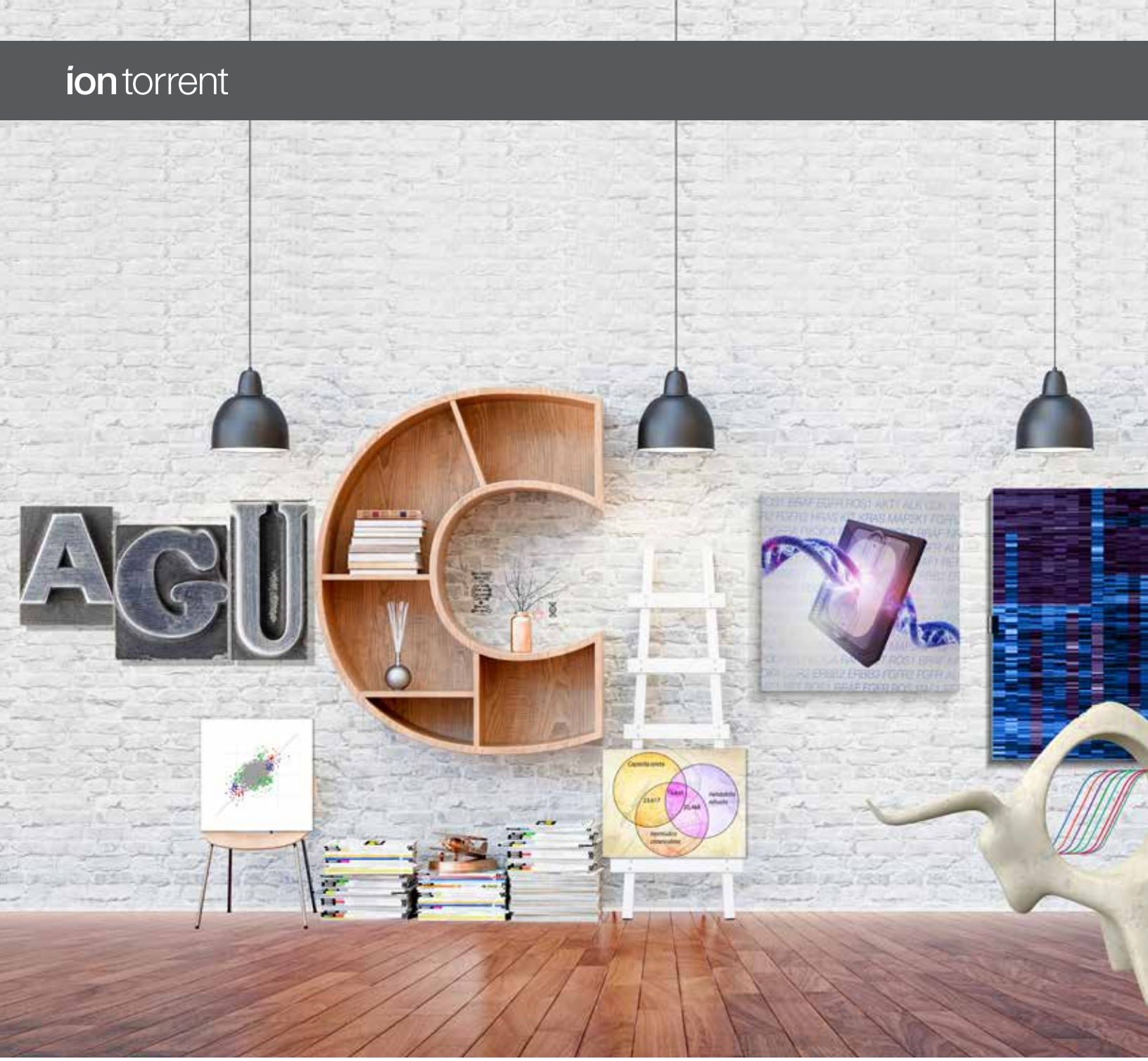
ion torrent



Simple, rapid, and reliable RNA sequencing

ThermoFisher SCIENTIFIC

RNA 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 (Figure 1). RNA sequencing enables:

- Understanding of disease at the transcript level
- Transcriptome sequencing, which is more complete and cost-efficient for various sample types
- The flexibility to interrogate the whole transcriptome, targeted genes, or regulatory elements

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

Benefits of Ion Torrent™ technology

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

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

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

Sample compatibility
Detect high- and low-abundance transcripts in formalin-fixed, paraffin-embedded (FFPE) samples from as little as 5 ng input RNA with Ion AmpliSeq™ targeted panels.

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

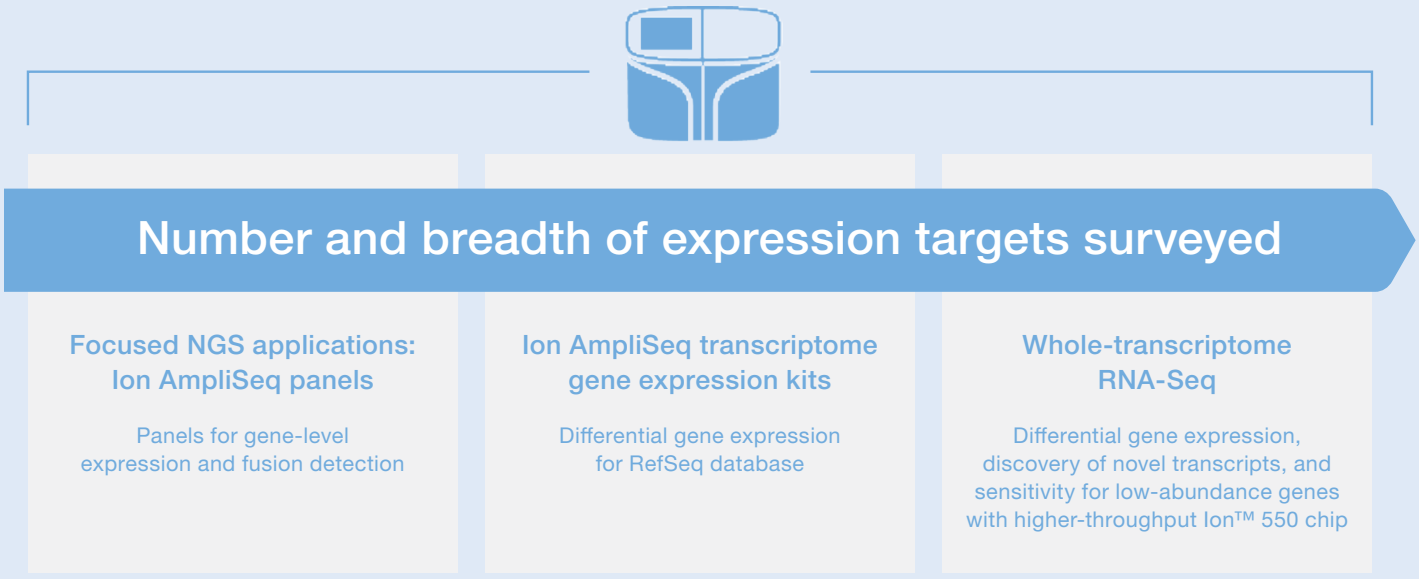


Figure 1. Ion Torrent technologies provide a spectrum of solutions for RNA sequencing, from focusing on specific regions of the genome or transcriptome to a global survey of the human transcriptome.

Focused NGS applications

Use Ion AmpliSeq™ RNA panels to analyze a specific expressed sequence of interest to look for expression of a particular mutation or fusion transcript.

Ion AmpliSeq panels are a fast and simple gene expression method comprised of targets selected from over 20,000 well-annotated RefSeq genes. They help empower high-throughput gene expression analysis with high sensitivity and specificity, and they are straightforward to implement because they take advantage of the simple and fast sequencing workflow of the Ion GeneStudio™ S5 System and the Ion Chef™ Instrument. This makes Ion AmpliSeq panels a simple and cost-effective alternative to whole-transcriptome sequencing.

Predesigned community panels

Our made-to-order panels predesigned by our community include the following menus:

- Ion AmpliSeq RNA MAPK Pathway Research Panel
- Ion AmpliSeq RNA WNT Pathway Research Panel
- Ion AmpliSeq RNA Human Oncology Pathway Research Panel
- Ion AmpliSeq RNA Breast Cancer Research Panel
- Ion AmpliSeq RNA Pancreatic Adenocarcinoma Research Panel
- Ion AmpliSeq RNA Stem Cell Research Panel
- Ion AmpliSeq RNA Inflammation Response Research Panel
- Ion AmpliSeq Long Non-coding RNA Research Panel

See the full list of panels at
thermofisher.com/ionampliseqpanels

Ready-to-use panels

We offer the following Ion AmpliSeq ready-to-use panels:

- Ion AmpliSeq Transcriptome Human Gene Expression Kits
- Ion AmpliSeq Transcriptome Mouse Gene Expression Kits
- Ion AmpliSeq RNA Fusion Lung Cancer Research Panel

Design your own Ion AmpliSeq RNA panel at
thermofisher.com/customampliseqrna

"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
Director of Genomics, Laboratory of Milan Radovich, PhD
Department of Surgery
Indiana University School of Medicine, USA

"RNA-Seq on the Ion [GeneStudio] 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

Ion AmpliSeq transcriptome panels

Explore particular pathways or sets of expressed genes by looking at multiple RNA and transcripts at the same time in a single, targeted NGS panel for RNA. Choose from a range of predesigned, ready-made panels or design your own.

Key benefits include:

Highly correlated results

Differentially expressed genes (DEGs) identified using the Ion AmpliSeq Transcriptome Human Gene Expression Kit correlate well with MicroArray Quality Control (MAQC), RNA-Seq, and qPCR data.

Robust performance with limited or degraded samples

The power of Ion AmpliSeq technology enables reproducible detection of high- and low-abundance transcripts from difficult samples such as FFPE tissue sections or from samples with limited input—as little as 10 ng total of RNA.

Cost-effective

Helps reduce the cost and complexity of gene expression analysis through the targeted sequencing of 20,802 genes and up to 8 multiplexed samples per Ion 540™ Chip.

Simple, automated workflow

The simplicity and speed of PCR enables a rapid method for gene expression profiling with a ≤2-day workflow from sample to analysis. Intuitive bioinformatics through point-and-click run setup and automated secondary data analysis in Torrent Suite Software with an associated plug-in, the AmpliSeq RNA, enables unambiguous gene expression analysis and visualization from sequence-read data.

Ion AmpliSeq Trancriptome Human or Mouse Gene Expression Panels in Chef-Ready Kits

Fully automated production of Ion AmpliSeq transcriptome libraries with less than one hour total of hands-on time from sample to data	Construct libraries during the day; prepare templates and load chips overnight
Same chemistry and equivalent performance as a manual workflow	32 samples per kit; optimal configuration is 8 samples per Ion 540™ Chip

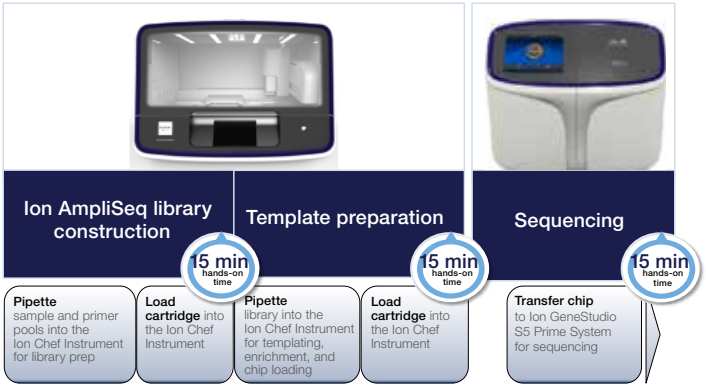
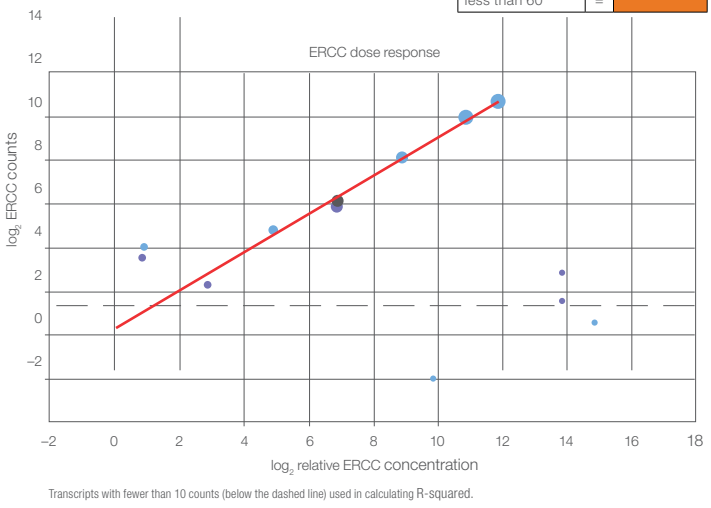


Figure 2. Streamlined workflow for gene expression analysis. Fully automated workflow from cDNA to gene expression data requires only 45 minutes of hands-on time using the Ion Chef System and Ion GeneStudio S5 Prime System.

% of total reads			Regression analysis			Map quality legend		
ERCC (pct)	=	0.19	R-squared	=	0.99	Mean Mapq	=	Dot color
Other (pct)	=	99.81	Slope	=	0.87	more than 90	=	
Raw ERCC counts	=	12,211	Y-intercept	=	2.28	less than 90	=	
			N	=	8	less than 80	=	
						less than 70	=	
						less than 60	=	



Transcripts with fewer than 10 counts (below the dashed line) used in calculating R-squared.

Figure 3. Ion AmpliSeq RNA ERCC Companion Panel will be availableto run ERCC spike-in controls for any Ion AmpliSeq Transcriptome Panel.

Whole-transcriptome RNA-Seq

Transcriptome analysis using the Ion Total RNA-Seq Kit v2 allows you to sequence noncoding RNA, splice variants, and alternative transcripts. Your starting material (e.g., ribosomal RNA (rRNA) or messenger RNA (mRNA)) will determine your output.

Key benefits include:

Increased specificity and sensitivity
Detect more RNA species, including fusion transcripts and closely related isoforms.

Wider dynamic range
With sufficient read depth, the full breadth of biologically relevant expression changes can be detected using NGS approaches without the signal compression typical of microarray platforms.

Digital output
NGS read count provides a representation of absolute expression, enabling you to identify and characterize low-abundance transcripts.

Detect both known and unknown transcripts and variants
The unbiased nature of NGS coverage enables true transcriptome-wide biomarker discovery that does not rely on prior knowledge of the genome.

The Ion 550 Chip generates 100–130 million sequencing reads on the Ion GeneStudio™ S5 Plus and Prime Systems using the automated workflow of the Ion Chef System. This chip is the fifth addition to our sequencing chip series, allowing scalability with a single instrument for clinical research applications. The Ion 550 Chip is not compatible with Ion GeneStudio S5 System.

The Ion 550 Chip offers:

- **Greater throughput of the Ion GeneStudio S5 Plus and Prime Systems**—a good choice for exome and transcriptome sequencing, liquid biopsy, or large oncology panels
- **Flexible Ion 550 Kit-Chef workflow**—1 or 2 loaded chips per Ion Chef run, with no loss of reagents

Whole-transcriptome sequencing with a simple and complete workflow

Target	Enrichment strategy	Number of reads needed	Research applications
Whole transcriptome	rRNA depletion	30–40 M	• Expression analysis at both gene and transcript level • Detection of gene fusions, noncoding RNA, alternative splicing, and novel genes
mRNA sequencing	poly(A) selection	15–25 M	• Gene detection and expression quantitation
Smal RNA or miRNA sequencing	Small RNA enrichment	2–5 M	• Small RNA detection and expression quantitation

Figure 4. Schematic representing the whole-transcriptome sequencing workflow steps and corresponding application specifications.

FFPE-compatible

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

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

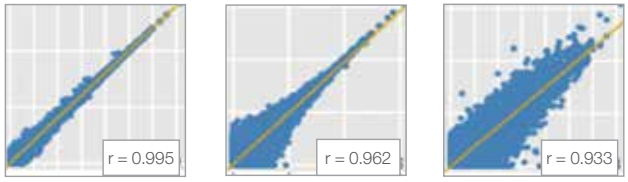


Figure 5. Using the Ion AmpliSeq Transcriptome Human Gene Expression Kit with 10 ng of total RNA input, sequencing data were generated from matched FFPE and fresh frozen samples. Shown are **(A)** sequencing metrics from Ion Proton sequencing reads and **(B)** correlations between fresh frozen replicates, FFPE replicates, and fresh frozen tissue vs. FFPE samples, respectively.

Fast

Go from RNA to quantitated genes in less than 2 days with minimal hands-on time (Figure 6).

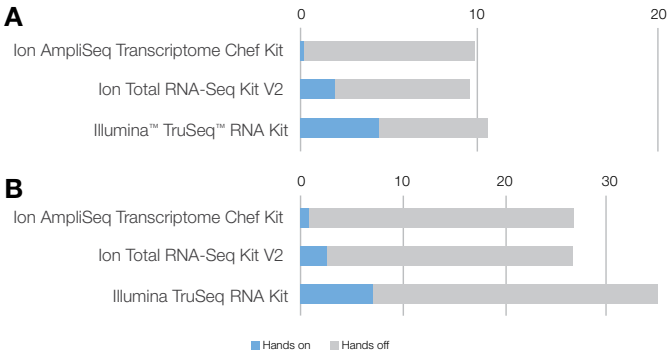


Figure 6. Comparison of hands-on and total turnaround time (in hours) for leading gene expression analysis platforms. **(A)** NGS library prep. **(B)** Entire workflow, from sample to data files.

Easy analysis

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

Figure 7. Use Torrent Suite Software and hardware for customizable, automated data analysis and storage. Ion Reporter™ Software characterizes sequencing variants efficiently and securely, enabling you to focus on finding the biological meaning of your data.

Better performance

Exceed microarray sensitivity for detection of differentially expressed genes (DEGs), with high correlation to microarray quality control (MAQC) array data (Figure 8).

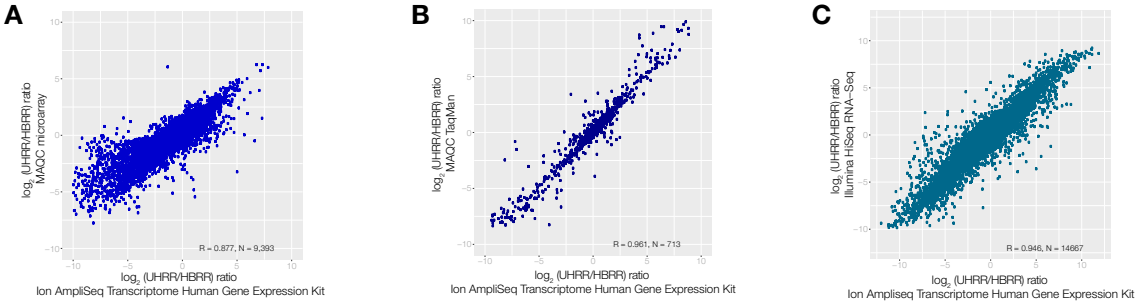


Figure 8. Representation of strong correlation for DEGs among Ion AmpliSeq Transcriptome Human Gene Expression Kit data with whole-transcriptome data, relative to MAQC array and qPCR data. Data from 8 UHRR and HBRR samples multiplexed on a single Ion 540 Chip were used to calculate differential gene expression for Ion AmpliSeq Transcriptome Human Gene Expression Kit data. **(A)** Scatter plot comparison of $\log_2$ (UHRR/HBRR) ratios from the Ion AmpliSeq Transcriptome Human Gene Expression Kit and MAQC microarray expression data. A total of 9,393 RefSeq genes were highly correlated, with Pearson correlation coefficients (R) of 0.88 and 0.91 for Ion AmpliSeq gene expression kit and MAQC data, respectively. **(B)** Scatter plot comparison of the Ion AmpliSeq Transcriptome Human Gene Expression Kit and MAQC qPCR data. Differential expression with a Pearson correlation coefficient (R) of 0.96, demonstrating that the qPCR and Ion AmpliSeq Transcriptome Human Gene Expression Kit datasets are highly correlated. **(C)** Scatter plot comparison of Illumina™ HiSeq™ whole-transcriptome RNA-Seq and Ion AmpliSeq Transcriptome Human Gene Expression Kit data. Differential expression for the two methods demonstrated a Pearson correlation coefficient (R) of 0.95, demonstrating that whole-transcriptome RNA-Seq and Ion AmpliSeq Transcriptome Human Gene Expression Kit datasets are highly correlated.

Scalable

Five 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.

Ion GeneStudio S5 System

Single sequencer. Multiple RNA sequencing applications.

+ Ion 510 Chip	+ Ion 520 Chip	+ Ion 530 Chip	+ Ion 540 Chip	+ Ion 550 Chip
2–3M reads*	4–6M reads*	15–20M reads*	60–80M reads*	100–130M reads*

Targeted and small RNA sequencing

Transcriptome sequencing and RNA-Seq

* Read counts shown are per chip. Dependening on the chip size and Ion GeneStudio S5 system used, up to two chips can be run per day.

Find out more about targeted transcriptome sequencing at **thermofisher.com/ampliseqtranscriptome**

RNA applications—important considerations

Dynamic range	• Up to 130M reads per run: 4x greater number of reads than Illumina™ MiniSeq™ and Illumina™ MiSeq™ sequencers • Greater number of reads provide high dynamic range for discovery and profiling applications
Application flexibility	• Ion Torrent 500 series chip format allows cost-effective discovery and profiling applications • Allows applications from mRNA gene expression profiling to whole-transcriptome sequencing
Sample throughput flexibility	• Flexible chip format enables you to cost-effectively process samples In increments without waiting to batch—sequence when ready, no need to wait!
Price per sample	• Greater number of reads allows lower price per sample • Chip formats allow for an affordable price per sample without having to batch >100 samples per run
Workflow and turnaround time	• Move from total RNA to data in less than 24 hours • Simple and automated data analysis • Leverage familiar microarray-based analysis tools that are optimized for Ion Torrent™ data

Figure 9. RNA sequencing considerations. Customers have choices that will result in an Ion Torrent RNA sequencing solution that suits their individual lab requirements.

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Ordering information

Product	Quantity	Cat. No.
Ion GeneStudio S5 System	1 system	A38194
Ion GeneStudio S5 Plus System	1 system	A38195
Ion GeneStudio S5 Prime System	1 system	A38196
Ion S5 XL System	1 system	A27214
Ion Chef Instrument	1 system	4484177
Ion 540 Chip Kit	8 chips	A27766
Ion 540 Kit-Chef	8 reactions	A30011
Ion 550 Chip Kit	8 chips	A34538
Ion 550 Kit-Chef	8 reactions	A34541
Ion AmpliSeq Transcriptome Human Gene Expression Kit	24 reactions	A26325
	96 reactions	A26326
	384 reactions	A26327
Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit	32 reactions	A31446
Ion AmpliSeq Transcriptome Mouse Gene Expression Kit	24 reactions	A36553
	96 reactions	A36554
	384 reactions	A36555
Ion AmpliSeq Transcriptome Mouse Gene Expression Panel, Chef-Ready Kit	32 reactions	A36412
Ion AmpliSeq ERCC Companion Panel	96 reactions	A36552
Ion Total RNA-Seq Kit v2	12 reactions	4475936
	48 reactions	4479789
Ion Total RNA-Seq Kit for AB Library Builder System	13 reactions	4482416
Ion Xpress RNA-Seq Barcode 1-16 Kit	160 reactions	4475485
Applied Biosystems MagMAX FFPE DNA/RNA Ultra Kit	1 kit	A31881
Invitrogen SuperScript VILO cDNA Synthesis Kit	50 reactions	11754050

Reference

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

Request a quote at **thermofisher.com/genestudio**

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

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