NextSeq® Series RNA-Seq Solution

An accessible, cost-effective solution that fully characterizes RNA transcript changes for a deeper understanding of biology.

Highlights

- **Clearest, Most Complete Transcriptome View for Any Species**
  Accurate transcriptome-wide abundance measurement and discovery of gene fusions, cSNPs, and novel transcript isomers
- **Accessible End-to-End Solution**
  Comprehensive suite of library preparation solutions and simple-to-use data analysis pipeline
- **Speed, Power, and Flexibility**
  Complete runs in less than 18 hours with ability to switch between 2 output modes to support full range of project needs
- **End-to-End Illumina Scientific Support**
  Illumina scientists and engineers are there every step of the way, providing installation, training, applications, and data analysis support

Introduction

The NextSeq Series RNA Sequencing (RNA-Seq) Solution delivers the clearest, most complete view of the transcriptome, making it more accessible than ever before. It leverages industry-leading Illumina next-generation sequencing (NGS) technology, the broadest range of library preparation solutions, and a new data analysis suite that delivers expert-preferred tools in a simple, intuitive user interface. The flexible and scalable NextSeq Series of sequencing systems can be tailored to meet researchers' changing needs as projects evolve. Ensuring the optimal balance of run time, read budget, and sample throughput, the NextSeq Series RNA-Seq Solution supports a range of studies, from basic gene expression profiling to complex whole-transcriptome analysis.

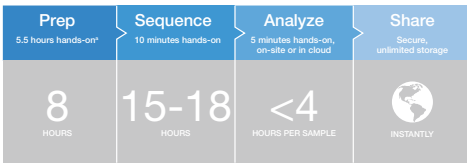

Figure 1: NextSeq Series Sample-to-Answer RNA-Seq Sequencing Workflow*—The NextSeq Series features a simple workflow that delivers highly accurate sequencing data.

* Times vary by experiment and assay type. Details shown are for an mRNA expression profiling experiment assuming 2 × 75 bp on instrument. Analysis results include differential expression and identification of alternative transcripts.

A Flexible RNA-Seq Solution

The NextSeq Series RNA-Seq solution enables rapid profiling, and deep investigation of the whole transcriptome (Figure 1). Researchers can choose from a complete suite of library preparation kits, enabling RNA-Seq studies to be tailored for whole-transcriptome or focused RNA analysis of any species, across a wide range of sample types.

Figure 2: NextSeq System—The NextSeq Series of sequencing systems leverages the latest advances in SBS chemistry and the industry's simplest workflow.

The NextSeq Series provides the power and versatility needed to address the full range of transcriptome analysis needs (Figure 2). Dual sequencing output modes enable researchers to select the optimal balance between sample number and reads per sample. For example, gene expression profiling (the measurement of gene-level abundance across known features) can be performed efficiently at high-throughput capacity with up to 40 samples† in a single run. Offering the power to discover novel features, whole-transcriptome analysis can query coding and noncoding RNA at up to 8 samples per run; researchers can also analyze coding RNA at up to 16 samples per run (Table 1).

Data analysis can be performed in BaseSpace®—the Illumina cloud and onsite genomics computing environment—using intuitive apps developed for analyzing Illumina RNA-Seq data. A part of the Illumina industry-leading NGS ecosystem, the NextSeq Series affords researchers with access to the world's largest collection of commercial and open source data analysis software tools for performing additional downstream analysis.

The NextSeq Series offers cross-application flexibility, enabling researchers to transition easily between sequencing projects (Figure 3). The system is compatible with the industry's widest range of library preparation kits from Illumina and third parties, enabling an easy transition between Illumina RNA-Seq, whole-genome sequencing (WGS), exome sequencing, and other applications. For example, researchers can pair RNA-Seq with exome sequencing on the NextSeq Series to assess whether coding variants impact transcript

† Expression profiling assumes 10 M reads per sample

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expression or perform ChIP-Seq to analyze DNA-protein interactions and better characterize functional regulation. With the NextSeq 550 System, researchers can perform NGS and array scanning on the same platform for further exploration or confirmation of copy number variants detected through sequencing.

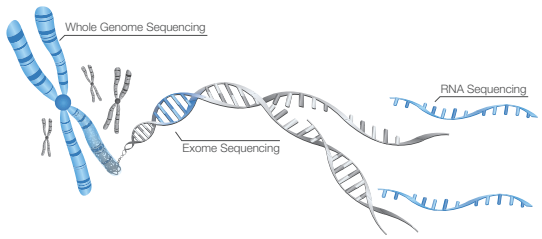

Figure 3: NextSeq Series Sequencing Applications—The flexible NextSeq Series enables researchers to transition seamlessly between applications to advance their research.

Broad Suite of RNA Library Preparation Options

The NextSeq Series RNA-Seq Solution offers an extensive portfolio of library preparation solutions addressing the full range of transcriptome study needs. Researchers can choose the kit that best fits their experimental design goals, while addressing common challenges, including poor RNA quality or limited available sample input amount.

The TruSeq® Stranded RNA suite of library preparation kits deliver precise measurement of strand orientation and the highest uniformity of coverage across transcripts, ensuring the most efficient use of read output. TruSeq Stranded mRNA provides a cost-efficient option for coding RNA-focused analysis, while TruSeq Stranded Total RNA enables whole-transcriptome analysis, capturing coding and multiple forms of noncoding RNA to obtain a complete picture of biology. TruSeq Stranded Total RNA configurations also offer highly robust performance in low quality, formalin-fixed, paraffin-embedded (FFPE) samples.

Additional solutions from Epicentre® (an Illumina company) provide complementary fits for specialized application needs. ScriptSeq™ kits provide fast total assay time and whole-transcriptome solutions for yeast and bacterial studies.

Advantages of RNA-Seq Over Gene Expression Arrays

RNA-Seq is increasingly the platform of choice for thought leaders, providing a detailed snapshot of the transcriptome at a given point in time. It offers numerous advantages over gene expression arrays, including:

- Requires no *a priori* knowledge of the transcriptome
- Provides qualitative and quantitative transcriptome analysis:
- Sequence and variant information
- Greater accuracy of fold-change measurement
- Better specificity
- May be applied to any species, even if a reference sequence is not available
- A better value, delivering advantages at lower price / sample than many gene expression arrays

Industry-Leading Read Quality

With the highest read output of any desktop sequencer, the NextSeq Series generates higher read counts per sample, enabling researchers to empower their studies with increased dynamic range, more accurate fold-change estimates, and more sensitive detection of genes, transcripts, and differential expression. New NextSeq v2 reagent kits are optimized to improve base calling and data quality even further.

The NextSeq Series dual output modes enable researchers to optimize study designs based on sample number and output requirements. If higher sample throughput is needed, studies can be scaled up to hundreds of samples per run using the Illumina HiSeq® 2500 System (Table 1).

Value of Paired-End Sequencing

With the versatile NextSeq Series, researchers can perform single-read or paired-end sequencing. Single-read sequencing is a highly economical option for gene expression profiling. However, paired-end RNA-Seq offers critical advantages. Read depth information generated from both ends of an insert allows transcript isomers to be differentiated effectively, providing more accurate

Table 1: Illumina RNA-Seq Solutions

Study	Measurement	Typical Reads Per Sample	Samples Per Run			
			NextSeq Series		HiSeq 2500 System	
			Mid Output	High Output	Rapid Run	High Output
Gene Expression Profiling	Gene-level abundance across known features	10 M (1 × 75 bp)	13	40	60	400
mRNA-Seq	Coding RNA abundance and discovery	25 M (2 × 75 bp)	4	16	24	160
Total RNA-Seq	Coding and noncoding RNA abundance and discovery	50 M (2 × 75 bp)	2	8	12	80

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detection and quantification of transcript-level abundance. Paired-end sequencing also enables potential duplicate reads to be retained, increasing the percentage of aligned reads. Paired-end information substantially enhances the sensitivity to detect gene fusions and insertion/deletion variants.

Simplified Bioinformatics in BaseSpace

Transcriptome data generated on the NextSeq Series is instantly and securely transferred, stored, and analyzed in BaseSpace (Cloud or Onsite), delivering transcript profiles in a single day (Figure 4). New Illumina RNA-Seq Software Apps in BaseSpace provide expert-preferred data analysis tools (TopHat/Cufflinks) packaged in an intuitive, click-and-go user interface designed for informatics novices. These apps deliver preconfigured workflows that support a range of common transcriptome data analysis needs. TopHat 2 provides high-confidence alignment for abundance measurement and the detection of splice junctions, gene fusions, and cSNPs. CuffDiff enables sensitive transcript discovery and differential expression analysis. TopHat Fusion delivers robust, high-confidence detection of gene fusions, while the Illumina Isaac[1] pipeline delivers reliable variant calling. Simple-to-follow prompts guide users through the entire process, starting from selecting the files generated by the sequencer, to filtering and visualizing analyzed data and results. RNA-Seq Apps Software generates output files that can be directly input into a broad range of available downstream analysis tools.

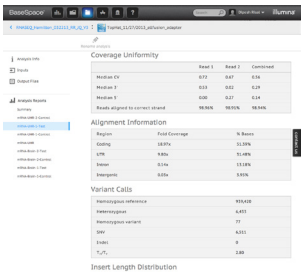

Figure 4: Storage and Analysis of NextSeq Series Data in the BaseSpace Cloud—NextSeq Series data can be securely and seamlessly uploaded to the BaseSpace cloud for fast, cost-effective analysis and storage.

The BaseSpace environment includes a growing community of BaseSpace app software tools for visualization, analysis, and sharing. Because Illumina NGS technology is the most established and broadly adopted sequencing solution, users can leverage the industry-leading Illumina NGS ecosystem, the world's largest collection of commercial and open-source data analysis software tools.

To learn more about RNA-Seq Software Apps, go to www.illumina.com/transcriptome.

Summary

The NextSeq Series RNA-Seq Solution offers a true solution, providing unprecedented access to the clearest, most complete view of the transcriptome. Dual output modes ensure cost-efficiency across transcriptome project types, from gene-level expression profiling to whole-transcriptome discovery. Combining the power, speed, and flexibility of the NextSeq Series with the industry's most comprehensive library preparation portfolio and user-friendly RNA-Seq Software Apps, the NextSeq Series RNA-Seq Solution enables researchers to gain a deeper understanding of the transcriptome.

Learn More

Go to www.illumina.com/rna to learn more about the next revolution in RNA sequencing.

Join the Illumina Community

With a NextSeq System in their laboratory, researchers join a worldwide community of over 60,000 scientists using Illumina technology for their research studies. Illumina schedules community events throughout the year, bringing researchers together to share ideas. User group meetings, scientific symposiums, and blog forums provide venues to discuss new research methods and breakthrough studies.

An integral part of the Illumina community is our dedicated service and support team, consisting of more than 300 people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the NextSeq System is delivered, with Illumina scientists and engineers assisting with system installation and setup, and the training of laboratory personnel. They are there 24/7 globally to answer questions every step of the way, giving researchers the peace of mind to focus on their next research study.

As researchers' needs change, new systems are brought into the laboratory, or new methods are undertaken, the Illumina support and training teams are there to provide assistance. In addition to on-site support, training courses (via webinar or at an Illumina facility) are available to bring laboratory personnel quickly up to speed.

Table 2: NextSeq Series Performance Parameters

Flow Cell Configuration	Read Length (bp)	Output (Gb)	Run Time	Data Quality	Required Input
High-Output Flow Cell Up to 400 M single reads Up to 800 M paired-end reads	2 × 150	100–120	29 hours	> 75% higher than Q30 at 2 × 150 bp	100 ng–1 µg with TruSeq RNA Library Prep Kits
	2 × 75	50–60	18 hours		
	1 × 75	25–30	11 hours		
Mid-Output Flow Cell Up to 130 M single reads Up to 260 M paired-end reads	2 × 150	32–39	26 hours		
	2 × 75	16–19	15 hours		

Total times include cluster generation, sequencing, and base calling on a NextSeq System. Install specifications are based on Illumina PhiX control library at supported cluster densities (between 129 and 165 K/mm² clusters passing filter). Actual performance parameters may vary based on sample type, sample quality, and clusters passing filter. The percentage of bases > Q30 is averaged over the entire run.

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Table 4: NextSeq Series Specifications

Instrument Configuration
RFID tracking for consumables
Instrument Control Computer (Internal)[a]
Base Unit: Dual Intel Xeon ES-2448L 1.8 GHz CPU
Memory: 96 GB RAM
Hard Drive: 750 GB
Operating System: Windows 7 embedded standard
Operating Environment
Temperature: 19°C to 25°C (22°C ± 3°C)
Humidity: Noncondensing 20%–80% relative humidity
Altitude: Less than 2,000 m (6,500 ft)
Air Quality: Pollution degree rating of II
Ventilation: Up to 2,048 BTU/hr @ 600 W
For Indoor Use Only
Light Emitting Diode (LED)
520 nm, 650 nm; Laser diode: 780 nm, Class IIIb
Dimensions
W×D×H: 58.5 cm × 53.4 cm × 63.5 cm (23.0 in × 21.0 in × 25 in)
Weight: 83 kg (183 lbs)
Crated Weight: 151.5 kg (334 lbs)
Power Requirements
100–120 VAC 15 A
220–240 VAC 10 A
Radio Frequency Identifier (RFID)
Frequency: 13.56 MHz
Power: Supply current 120 mA, RF output power 200 mW
Product Safety and Compliance
NRTL certified IEC 61010-1
CE marked
FCC/IC approved

a. Computer specifications are subject to change.

Reference

1. Raczy C, Petrovski R, Saunders CT, et al. Isaac: Ultra-fast whole genome secondary analysis on Illumina sequencing platforms. *Bioinformatics.* 2013;29:2041-2043.

Ordering Information

System Name	Catalog No.
NextSeq 500 System	SY-415-1001
NextSeq 550 System	SY-415-1002
Output Kit Name	**Catalog No.**
NextSeq 500 Mid-Output Kit (150 cycles)	FC-102-1001
NextSeq 500 Mid-Output Kit (300 cycles)	FC-404-1003
NextSeq 500 High-Output Kit (75 cycles)	FC-404-1005
NextSeq 500 High-Output Kit (150 cycles)	FC-404-1002
NextSeq 500 High-Output Kit (300 cycles)	FC-404-1004
NextSeq 500 Mid-Output v2 Kit (150 cycles)	FC-404-2001
NextSeq 500 High-Output v2 Kit (150 cycles)	FC-404-2002
NextSeq 500 Mid-Output v2 Kit (300 cycles)	FC-404-2003
NextSeq 500 High-Output v2 Kit (300 cycles)	FC-404-2004
NextSeq 500 High-Output v2 Kit (75 cycles)	FC-404-2005
Library Preparation Kit Name	**Catalog No.**
TruSeq Stranded mRNA LT Set A	RS-122-2101
TruSeq Stranded mRNA LT Set B	RS-122-2102
TruSeq Stranded mRNA HT	RS-122-2103
TruSeq Stranded Total RNA LT (with Ribo-Zero Human/Mouse/Rat) Set A	RS-122-2201
TruSeq Stranded Total RNA LT (with Ribo-Zero Human/Mouse/Rat) Set B	RS-122-2202
TruSeq Stranded Total RNA HT (with Ribo-Zero Human/Mouse/Rat)	RS-122-2203
TruSeq Stranded Total RNA LT (with Ribo-Zero Gold) Set A	RS-122-2301
TruSeq Stranded Total RNA LT (with Ribo-Zero Gold) Set B	RS-122-2302
TruSeq Stranded Total RNA HT (with Ribo-Zero Gold)	RS-122-2303
TruSeq Stranded Total RNA LT (with Ribo-Zero Plant) Set A	RS-122-2401
TruSeq Stranded Total RNA LT (with Ribo-Zero Plant) Set B	RS-122-2402
TruSeq Stranded Total RNA HT (with Ribo-Zero Plant)	RS-122-2403
TruSeq Stranded Total RNA LT (with Ribo-Zero Globin) Set A	RS-122-2501
TruSeq Stranded Total RNA LT (with Ribo-Zero Globin) Set B	RS-122-2502
TruSeq Stranded Total RNA HT (with Ribo-Zero Globin)	RS-122-2503
TruSeq RNA Sample Prep Kit v2 Set A (48 reactions)	RS-122-2001
TruSeq RNA Sample Prep Kit v2 Set B (48 reactions)	RS-122-2002
TruSeq Small RNA Library Prep Kit Set A	RS-200-0012
TruSeq Small RNA Library Prep Kit Set B	RS-200-0024
TruSeq Small RNA Library Prep Kit Set C	RS-200-0036
TruSeq Small RNA Library Prep Kit Set D	RS-200-0048

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