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NextSeq™ 2000 RNA-Seq Solution

Enabling cost-effective characterization of RNA transcript changes for a deeper understanding of biology.

Highlights

- **Integrated, streamlined NGS solution**
  Workflow delivers fast library preparation, push-button sequencing, and simple-to-use data analysis options
- **Innovative library preparation**
  Library prep portfolio enables transcriptome-wide analyses with flexibility for species, sample type, and input amount
- **Flexible, scalable sequencing system**
  Ability to switch between two output modes and multiple read lengths supports a full range of project needs
- **Complete Illumina technical support**
  Illumina scientists and engineers provide installation, training, and support for customers through the entire workflow, from library preparation through data analysis

Introduction

The NextSeq 2000 RNA sequencing (RNA-Seq) solution delivers a clear, complete view of the transcriptome, making it more accessible than ever before. The solution uses industry-leading Illumina next-generation sequencing (NGS) technology,[1] the latest advances in sequencing by synthesis (SBS) chemistry, a broad range of library preparation solutions, and data analysis tools that deliver streamlined workflows (Figure 1). The flexibility and scalability of the NextSeq 2000 Sequencing System (Figure 2) enable it to be tailored to process a range of sample volumes efficiently. Ensuring the optimal balance of read budget and sample throughput, the NextSeq 2000 RNA-Seq solution supports a range of bulk RNA applications, from basic gene expression profiling to complex whole-transcriptome analyses.

Figure 2: NextSeq 2000 Sequencing System—The NextSeq 2000 Sequencing System harnesses the latest advances in SBS chemistry and streamlines sequencing workflows.

Advantages of RNA-Seq

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

- Hypothesis-free experimental design, requiring no previous knowledge of the transcriptome
- Higher discovery power to detect known and novel transcripts
- Higher throughput capability to quantify hundreds to thousands of regions in each assay
- Broader dynamic range, providing more accurate measurement of gene expression
- More data per assay, providing full sequence and variant information

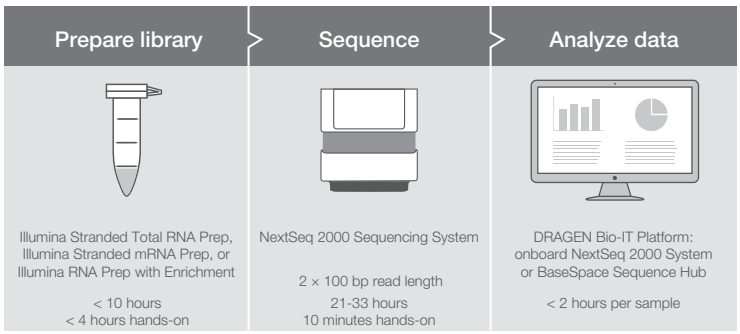

Figure 1: NextSeq 2000 RNA-Seq workflow—The NextSeq 2000 Sequencing System is part of a simple, integrated NGS workflow that delivers highly accurate RNA sequencing data. Times vary by experiment and assay type.

Integrated NGS workflow

The NextSeq 2000 Sequencing System supports an extensive portfolio of library preparation solutions, addressing a wide range of transcriptome study needs. Researchers can choose from various library prep kits, enabling them to find one that best fits their experimental needs and helps overcome common challenges such as poor quality starting RNA or limited sample availabity.

Illumina RNA library preparation advances

Advances in the Illumina portfolio of RNA library preparation kits deliver the high-quality data researchers require, with a streamlined workflow that can be completed within one standard working shift. Illumina offers three RNA library prep kits:

- **Illumina Stranded mRNA Prep** provides a cost-efficient option for coding RNA-focused analysis.
- **Illumina Stranded Total RNA Prep** enables whole-transcriptome analysis using Ribo-Zero™ Plus, capturing coding and multiple forms of noncoding RNA to obtain a comprehensive picture of biology. Illumina Stranded Total RNA Prep also offers robust performance in low-quality, formalin-fixed paraffin-embedded (FFPE) samples.
- **Illumina RNA Prep with Enrichment** brings bead-linked transposome (BLT) technology to RNA-Seq and provides a fast single-day RNA enrichment workflow with minimal hands-on time (< 2 hours). When sequenced on an Illumina sequencing platform, such as the NextSeq 2000 Sequencing System, the resulting nonstranded RNA data provide valuable insights across a large number of genomic positions.

For users of BaseSpace™ Clarity LIMS, new preset protocols for Illumina Stranded mRNA Prep, Illumina Stranded Total RNA Prep, and Illumina RNA Prep with Enrichment are available to use with the NextSeq 2000 Sequencing System.

The NextSeq 2000 Sequencing System

The NextSeq 2000 Sequencing System provides the power and versatility to address a full range of transcriptome analysis needs. Two available sequencing output modes enable researchers to select the optimal balance between sample number and reads per sample (Table 1). 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. Whole-transcriptome analysis enables discovery of novel features by interrogating 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 2).

The NextSeq 2000 Sequencing System offers cross-application flexibility, enabling researchers to transition easily between sequencing projects (Figure 3). The system is compatible with a range of library preparation kits from Illumina and third parties, enabling an easy transition between bulk RNA-Seq, single-cell RNA-Seq, exome sequencing, and other applications. For example, researchers can pair RNA-Seq with exome sequencing on the NextSeq 2000 Sequencing System to assess whether coding variants impact transcript expression or perform ATAC-Seq to analyze chromatin accessibility and better characterize functional regulation.

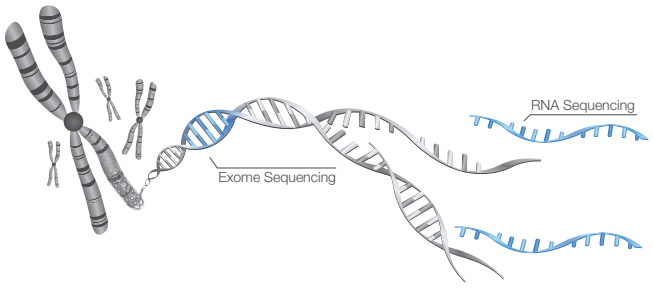

Figure 3: NextSeq 2000 sequencing applications—The NextSeq 2000 Sequencing System enables researchers to transition seamlessly between applications to advance research.

Industry-leading SBS read quality

With the highest read output and lowest price per read of any desktop sequencer,[2] the NextSeq 2000 Sequencing System enables researchers to enrich their studies with greater read depth resulting in more accurate fold-change estimates and superior sensitivity in detection of genes, transcripts, and differential expression. With NextSeq 2000 Sequencing System, the reduction in cost per gigabase of output coupled with additional sequencing capacity together provides:

- More reads per sample to capture information about lower-abundance transcripts
- More samples power more robust experimental design within a given research budget
- More comprehensive methods to capture more complex facets of the RNA landscape to drive more discovery

* Expression profiling assumes 10M reads per sample.

Table 1: NextSeq 2000 performance parameters

Flow cell configuration	Read length (bp)	Output (Gb)	Run time	Data quality	Required input
P2 flow cell Up to 400M reads	2 × 150	120	29 hours	≥ 75% bases above Q30 at 2 × 150 bp	10 ng–1 µg with Illumina RNA Prep
	2 × 100	80	21 hours		
	2 × 50	40	13 hours		
P3 flow cell Up to 1000M reads	2 × 150	300	48 hours		
	2 × 100	200	33 hours		
	2 × 50	100	19 hours		

Total times include cluster generation, sequencing, and base calling on a NextSeq 2000 System. 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.

The additional sequencing capacity afforded by the NextSeq 2000 P3 flow cell makes it easier than ever to design appropriately powered studies to make RNA-Seq a routine part of any lab's repertoire of molecular tools. The NextSeq 2000 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 NovaSeq™ 6000 System (Table 2).

Value of paired-end sequencing

With the NextSeq 2000 Sequencing System, researchers can perform single-read or paired-end sequencing. Single-read sequencing is an economical option for gene expression profiling. However, paired-end RNA-Seq offers key advantages. Read depth information generated from both ends of an insert allows transcript isomers to be differentiated effectively, providing more accurate detection and quantification of transcript-level abundance. Paired-end information substantially enhances the sensitivity to detect gene fusions and insertion/deletion (indel) variants.

Simplified analysis solutions from Illumina

The DRAGEN™ Bio-IT Platform

RNA-Seq data analysis can be performed using tools from the Illumina DRAGEN Bio-IT Platform, a suite of fast and accurate data analysis pipelines, and a broad ecosystem of commercial and open-source data analysis software tools. The Illumina DRAGEN RNA Pipeline takes output from the NextSeq 2000 Sequencing System and performs accurate RNA alignment to a reference genome, variant calling and quantification of genes, and characterization of splice junctions and candidate gene fusions. The DRAGEN RNA Pipeline can be launched in BaseSpace Sequence Hub, the Illumina cloud-based genomics computing environment, or as an on-instrument workflow using the NextSeq 2000 onboard DRAGEN hardware.

Whether in the cloud or on-instrument, the DRAGEN RNA pipeline provides high-quality data packaged in an intuitive user interface. Simple-to-follow prompts guide users through the entire process, starting from selecting the files generated by the sequencer, to viewing analyzed data and results. Output from the DRAGEN RNA pipeline can be directly input into a broad range of available downstream analysis tools, including the RNA Differential Expression App in BaseSpace Sequence Hub. Beyond the DRAGEN platform, BaseSpace Sequence Hub includes a growing community of software tools for visualization, analysis, and sharing.

Complete Illumina technical support

With a NextSeq 2000 Sequencing System in their laboratory, researchers join a worldwide community of thousands of 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 hundreds of people worldwide, 75% of whom have advanced degrees. Illumina technical support begins when the NextSeq 2000 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.

Summary

The NextSeq 2000 RNA-Seq solution provides a clear, comprehensive 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 2000 Sequencing System with an extensive library preparation portfolio and user-friendly RNA-Seq software applications, RNA sequencing on the NextSeq 2000 Sequencing System enables researchers to gain a deeper understanding of the transcriptome.

Learn more

To learn more about RNA sequencing, visit www.illumina.com/rna

To learn more about RNA-Seq analysis, visit www.illumina.com/rna-analysis.

Table 2: Illumina RNA-Seq solutions

Method	Measurement	Typical read pairs per sample	Library prep	Samples per run				Data analysis
				NextSeq 2000 System		NovaSeq 6000 System		
				P2	P3	SP	S1	
Gene expression quantification	Gene-level abundance across known features	10M (2 × 100 bp)	Illumina Stranded mRNA Prep[a]	40	100	80	160	DRAGEN RNA (onboard, server, or cloud) + RNA-Seq Differential Expression App (cloud only)
mRNA-Seq	Coding RNA abundance and discovery	25M (2 × 100 bp)	Illumina RNA Prep with Enrichment	16	40	32	64	
Total RNA-Seq	Coding and noncoding RNA abundance and discovery	50M (2 × 100 bp)	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	8	20	16	32	

a. Illumina Stranded mRNA Prep is not compatible with FFPE samples. For low-quality or FFPE samples, Illumina RNA Prep with Enrichment is recommended.

NextSeq 2000 Sequencing System specifications
Instrument configuration
Self-contained, dry instrument with integrated DRAGEN Bio-IT field-programmable gate array technology (FPGA) secondary analysis
Instrument control computer
Base Unit: 2U Microserver located inside the instrument Memory: 288 GB Hard Drive: 3.8 TB SSD Operating System: Linux CentOS 7.6
Operating environment
Temperature: 15°C-30°C Humidity: 20%-80% relative humidity, non-condensing Altitude: 0-2000 meters For Indoor Use Only
Laser
Wavelengths: 449 nm, 523 nm, 820 nm Safety: Class 1 Laser Product
Dimensions
W×D×H: 60 cm × 65 cm × 60 cm Weight: 141 kg **Crated Dimensions** Crated W×D×H: 92 cm × 120 cm × 118 cm Crated Weight: 232 kg
Power requirements
Instrument Input Voltage: 100 VAC to 240 VAC Instrument Input Frequency: 50/60 Hz
Bandwidth for network connection
200 Mb/s/instrument for internal network uploads 200 Mb/s/instrument for BaseSpace Sequence Hub uploads 5 Mb/s/instrument for instrument operational data uploads
Product safety and compliance
NRTL certified IEC 61010-1 CE marked FCC/IC approved

References

1. Based on a comparison of the top three industry-leading NGS platforms. Data calculations on file. Illumina, Inc., 2016.
2. Ross MG, Russ C, Costello M, et al. Characterizing and measuring bias in sequence data. *Genome Biol*. 2013;14(5):R51.

Ordering information

System	Catalog no.
NextSeq 2000 Sequencing System	20038897
Sequencing reagents	**Catalog no.**
NextSeq 1000/2000 P2 Reagents (100 cycles)	20038899
NextSeq 1000/2000 P2 Reagents (200 cycles)	20040557
NextSeq 1000/2000 P2 Reagents (300 cycles)	20040558
Library preparation kits	**Catalog no.**
Illumina Stranded Total RNA Prep with Ribo-Zero Plus (16 samples)	20040525
Illumina Stranded Total RNA Prep with Ribo-Zero Plus (96 samples)	20040529
Illumina Stranded mRNA Prep (16 samples)	20040532
Illumina Stranded mRNA Prep (96 samples)	20040534
Illumina RNA Prep with Enrichment (16 samples)	20040536
Illumina RNA Prep with Enrichment (96 samples)	20040537
Library preparation accessory components	**Catalog no.**
Illumina Ribo-Zero Plus rRNA Depletion Kit (16 samples)	20040526
Illumina Ribo-Zero Plus rRNA Depletion Kit (96 samples)	20037135
Indexes	**Catalog no.**
IDT for Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)	20040553
IDT for Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20040554
IDT for Illumina RNA UD Indexes Set C, Ligation (96 indexes, 96 samples)	20040555
IDT for Illumina RNA UD Indexes Set D, Ligation (96 indexes, 96 samples)	20040556
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20026121
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20026930
IDT for Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)	20042666
IDT for Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)	20042667
Informatics solutions	**Catalog no.**
Illumina Analytics - 1 iCredit	20042038
Illumina Analytics Starter Package - 1000 iCredits	20042039
Illumina Analytics - 5000 iCredits	20042040
Illumina Analytics - 50,000 iCredits	20042041
Illumina Analytics - 100,000 iCredits	20042042
BaseSpace Sequence Hub Professional Annual Subscription	20041109
BaseSpace Sequence Hub Enterprise Annual Subscription	15066411
BaseSpace Sequence Hub Consumption Billing	20012931

Ligation indexes are compatible with total and mRNA prep kits; tagmentation indexes are compatioble with DNA and RNA enrichment prep kits.

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