APPLICATION NOTE

NextSeq™ 1000 & NextSeq 2000 RNA sequencing solution

Cost-effective, integrated workflow for gene expression and transcriptome analysis

- Innovative library prep portfolio enables transcriptome-wide analyses with sample and input flexibility
- Scalable sequencing throughput supports a broad range of RNA-Seq applications
- Streamlined pipeline algorithms overcome bottlenecks in data analysis

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Introduction

The NextSeq 1000 and 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, the latest advances in sequencing by synthesis (SBS) chemistry, a broad portfolio of library preparation solutions, and data analysis tools to deliver streamlined workflows (Figure 1). The flexibility and scalability of the NextSeq 1000 and NextSeq 2000 Sequencing Systems (Figure 2) enable users to process a range of sample volumes efficiently, ensuring the optimal balance of read budget and sample throughput. The NextSeq 1000 and 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 1000 and NextSeq 2000 Sequencing Systems—The NextSeq 1000 and NextSeq 2000 systems harness the latest advances in SBS chemistry and streamline sequencing workflows.

Advantages of RNA-Seq

RNA-Seq has quickly emerged as the paramount approach to high-throughput transcriptome profiling.[1,2] RNA-Seq provides a detailed snapshot of the transcriptome at a given point in time and offers numerous advantages over quantitative PCR, including:

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

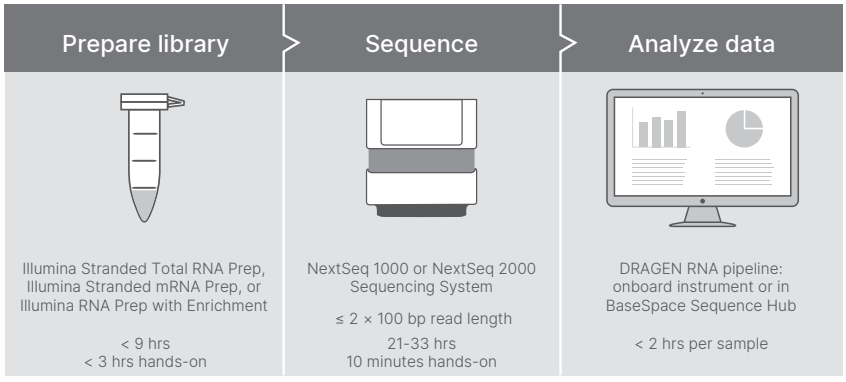

Figure 1: NextSeq 1000 and NextSeq 2000 RNA-Seq workflow—The NextSeq 1000 and NextSeq 2000 Sequencing Systems are part of a simple, integrated NGS workflow that delivers highly accurate RNA sequencing data. Times vary by experiment and assay type.

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Integrated NGS workflow

The NextSeq 1000 and NextSeq 2000 Sequencing Systems support 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 availability.

Advanced Illumina RNA library preparation

With well-established experience in RNA-Seq, Illumina offers trusted and proven solutions for RNA library preparation. 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 (Table 1):

- **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 when working with low-quality, formalin-fixed paraffin-embedded (FFPE) samples.
- **Illumina Stranded mRNA Prep** provides a cost-efficient option for coding RNA–focused analysis.
- **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 the NextSeq 1000 and NextSeq 2000 Sequencing Systems, the resulting nonstranded RNA data provide valuable insights across a large number of genomic positions.

For users of BaseSpace™ Clarity LIMS, 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 1000 and NextSeq 2000 Sequencing Systems.

Table 1: Illumina RNA library preparation kits

	Illumina Stranded Total RNA Prep	Illumina Stranded mRNA Prep	Illumina RNA Prep with Enrichment
Method	Ligation with Ribo-Zero Plus	Ligation	(L) Tagmentation
Detection	Coding and noncoding transcriptome	Coding transcriptome with polyA tail	Targeted coding regions[c]
FFPE compatibility	Yes	No	Yes
Input	1-1000 ng[b]	25-1000 ng	10 ng non-FFPE 20 ng FFPE
Total assay time[a]	7 hrs	< 7 hrs	< 9 hrs
Hands-on time[a]	< 3 hrs	< 3 hrs	< 2 hrs
Automation friendly	Yes	Yes	Yes

a. Hands-on and total time based on manual processing of up to 24 samples for Illumina Stranded Total RNA and mRNA workflows and 1 sample on enrichment workflow
b. Minimum input for high-quality RNA shown; 10 ng minimum recommended for optimal quality and FFPE for Illumina Stranded Total RNA Prep
c. Tested with Illumina Exome Panel and Respiratory Oligos Panel v2; Illumina RNA Prep with Enrichment does not provide strand information

The NextSeq 1000 and NextSeq 2000 Sequencing Systems

The NextSeq 1000 and NextSeq 2000 Sequencing Systems provide power and versatility to address a full range of transcriptome analysis needs. Three available flow cell configurations enable researchers to select the optimal balance between sample number and reads per sample (Table 2). 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 120 samples* in a single run. Whole-transcriptome analysis enables discovery of novel features by interrogating coding and noncoding RNA at up to 24 samples per run; researchers can also analyze coding RNA at up to 48 samples per run (Table 2, Table 3).

The NextSeq 1000 and NextSeq 2000 Sequencing Systems offer cross-application flexibility, enabling researchers to transition easily between sequencing projects. The systems are 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 1000 or NextSeq 2000 system to assess whether coding variants impact transcript expression or perform ATAC-Seq† to analyze chromatin accessibility and better characterize functional regulation.

Discover more with industry-leading SBS read quality

With the highest read output and lowest price per read of any Illumina benchtop sequencer, the NextSeq 1000 and NextSeq 2000 Sequencing Systems enable 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 the NextSeq 1000 and NextSeq 2000 Sequencing Systems, the reduction in cost per million reads coupled with additional sequencing capacity provides:

- More reads per sample to capture information about lower abundance transcripts
- More samples to 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

† ATAC-Seq, assay for transposase-accessible chromatin with sequencing

Table 2: Illumina RNA-Seq solutions and throughput per system

Method	Measurement	Typical read pairs per sample[a]	Library prep	No. of samples per run						
				NextSeq 1000 and NextSeq 2000			NovaSeq 6000			
				P1	P2	P3[c]	SP	S1	S2	S4
Gene expression quantification	Gene-level abundance across known features	10M	Illumina Stranded mRNA Prep[b]	10	40	120	80	160	410[d]	1K[d]
mRNA-Seq	Coding RNA abundance and discovery	25M	Illumina RNA Prep with Enrichment	4	16	48	32	64	164	400[d]
Total RNA-Seq	Coding and noncoding RNA abundance and discovery	50M	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	2	8	24	16	32	82	200

a. Recommended read lengths are 2 × 75 bp for Illumina Stranded Total RNA Prep and Illumina Stranded mRNA Prep and 2 × 100 bp for Illumina RNA Prep with Enrichment
b. Illumina Stranded mRNA Prep is not compatible with FFPE samples; for low-quality or FFPE samples, Illumina RNA Prep with Enrichment is recommended
c. P3 Reagents are available for the NextSeq 2000 System only
d. A maximum of 384 unique dual indexes is available

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The multiple flow cell configurations of the NextSeq 1000 and NextSeq 2000 systems enable researchers to optimize study designs based on sample number and output requirements. For example, 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. 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 1000 and NextSeq 2000 Sequencing Systems, 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

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. The Illumina DRAGEN RNA pipeline takes output from the NextSeq 1000 and NextSeq 2000 Sequencing Systems 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 1000 and NextSeq 2000 onboard DRAGEN hardware.

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

Table 3: NextSeq 1000 and NextSeq 2000 performance parameters

Flow cell configuration	Read length	Output[a]	Run time[b]	Data quality[c]	Required input
NextSeq 1000/2000 P1 Reagents	2 × 150 bp	30 Gb	~19 hrs	≥ 85% bases above Q30 at 2 × 100 bp and 2 × 150 bp	10 ng–1 µg with Illumina RNA Prep
NextSeq 1000/2000 P2 Reagents	2 × 150 bp	120 Gb	~29 hrs		
	2 × 100 bp	80 Gb	~21 hrs		
	2 × 50 bp	40 Gb	~13 hrs		
NextSeq 2000 P3 Reagents[d]	2 × 150 bp	360 Gb	~48 hrs	≥ 90% bases above Q30 at 1 × 50 bp and 2 × 50 bp	
	2 × 100 bp	240 Gb	~33 hrs		
	2 × 50 bp	120 Gb	~19 hrs		
	1 × 50 bp	60 Gb	~11 hrs		

a. Output specifications based on a single flow cell using Illumina PhiX control library at supported cluster densities
b. Run time includes cluster generation, sequencing, and base calling on the NextSeq 1000 and NextSeq 2000 systems
c. Quality scores are based on an Illumina PhiX control library; performance may vary based on library type and quality, insert size, loading concentration, and other experimental factors; the percentage of bases > Q30 is averaged over the entire run
d. P3 Reagents are available for the NextSeq 2000 System only

range of available downstream analysis tools. Beyond the DRAGEN platform, BaseSpace Sequence Hub includes a growing community of software tools for visualization, analysis, and sharing.

Comprehensive Illumina technical support

Illumina provides a world-class support team comprised of experienced scientists who are experts in library preparation, sequencing, and analysis. This dedicated team includes highly qualified field service engineers (FSE), technical applications scientists (TAS), field applications scientists (FAS), system support engineers, bioinformaticians, and IT network experts, all deeply familiar with NGS and the applications that Illumina customers perform around the globe. Technical support is available via phone five days a week or via online support 24/7, worldwide and in multiple languages.

With this unmatched service and support, Illumina helps users maximize the efficacy of their NextSeq 1000 or NextSeq 2000 Sequencing Systems, train new employees, and learn the latest techniques and best practices.

Summary

The NextSeq 1000 and NextSeq 2000 RNA-Seq solution offers a streamlined RNA-to-results workflow for high-throughput transcriptome analysis. The workflow combines the power, speed, and flexibility of the NextSeq 1000 and NextSeq 2000 Sequencing Systems with an advanced RNA library preparation portfolio and user-friendly RNA-Seq software applications. Three flow cell configurations ensure cost-efficiency across RNA-Seq project types, from gene expression profiling to whole-transcriptome discovery. RNA sequencing on the NextSeq 1000 and NextSeq 2000 Sequencing Systems enables researchers to drive insight through a deeper understanding of the transcriptome.

Learn more

RNA sequencing, illumina.com/techniques/sequencing/rna-sequencing.html

Illumina RNA library preparation, illumina.com/techniques/sequencing/ngs-library-prep/rna.html

NextSeq 1000 and NextSeq 2000 Sequencing Systems, illumina.com/systems/sequencing-platforms/nextseq-1000-2000.html

DRAGEN Bio-IT Platform, illumina.com/products/by-type/informatics-products/dragen-bio-it-platform.html

References

1. Geraci F, Saha I, Bianchini M. Editorial: RNA-Seq Analysis: Methods, Applications and Challenges. *Front Genet*. 2020;11:220. doi:10.3389/fgene.2020.00220
2. Corchete LA, Rojas EA, Alonso-López D, De Las Rivas J, Gutiérrez NC, Burguillo FJ. Systematic comparison and assessment of RNA-seq procedures for gene expression quantitative analysis. *Sci Rep*. 2020;10(1):19737. doi:10.1038/s41598-020-76881-x

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Ordering information

Product	Catalog no.
NextSeq 2000 Sequencing System	20038897
NextSeq 1000 Sequencing System	20038898
NextSeq 1000 to NextSeq 2000 upgrade	20047256
NextSeq 1000/2000 P1 Reagents (300 cycles)	20050264
NextSeq 1000/2000 P2 Reagents (100 cycles)	20046811
NextSeq 1000/2000 P2 Reagents (200 cycles)	20046812
NextSeq 1000/2000 P2 Reagents (300 cycles)	20046813
NextSeq 2000 P3 Reagents (50 cycles)	20046810
NextSeq 2000 P3 Reagents (100 cycles)	20040559
NextSeq 2000 P3 Reagents (200 cycles)	20040560
NextSeq 1000/2000 Read and Index Primers	20046115
NextSeq 1000/2000 Index Primer Kit	20046116
NextSeq 1000/2000 Read Primer Kit	20046117
NextSeq 1000/2000 Recycling Tool	20050631

Ordering information

Product	Catalog no.
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (16 samples)	20040525
Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero Plus (96 samples)	20040529
Illumina Stranded mRNA Prep, Ligation (16 samples)	20040532
Illumina Stranded mRNA Prep, Ligation (96 samples)	20040534
Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples)	20040536
Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples)	20040537
Illumina Exome Panel	20020183
IDT for Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)[a]	20040553
IDT for Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)	20040554
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20027213
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20027214

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

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