APPLICATION NOTE

NextSeq™ 1000 & NextSeq 2000 RNA sequencing solution

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

- Innovative library prep portfolio for transcriptome-wide analyses with sample and input flexibility
- Scalable sequencing throughput to support a broad range of RNA-Seq applications
- Integrated DRAGEN secondary analysis for optimal workflow efficiency

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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, optimized sequencing by synthesis (SBS) XLEAP-SBS™ chemistry, a broad portfolio of library preparation solutions, and data analysis tools to deliver streamlined and efficient 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 RNA applications, from basic gene expression profiling to complex whole-transcriptome analyses.

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:

Figure 2: NextSeq 1000 and NextSeq 2000 Sequencing Systems—The NextSeq 1000 and NextSeq 2000 Systems harness XLEAP-SBS chemistry and onboard secondary analysis to streamline sequencing workflows.

- 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

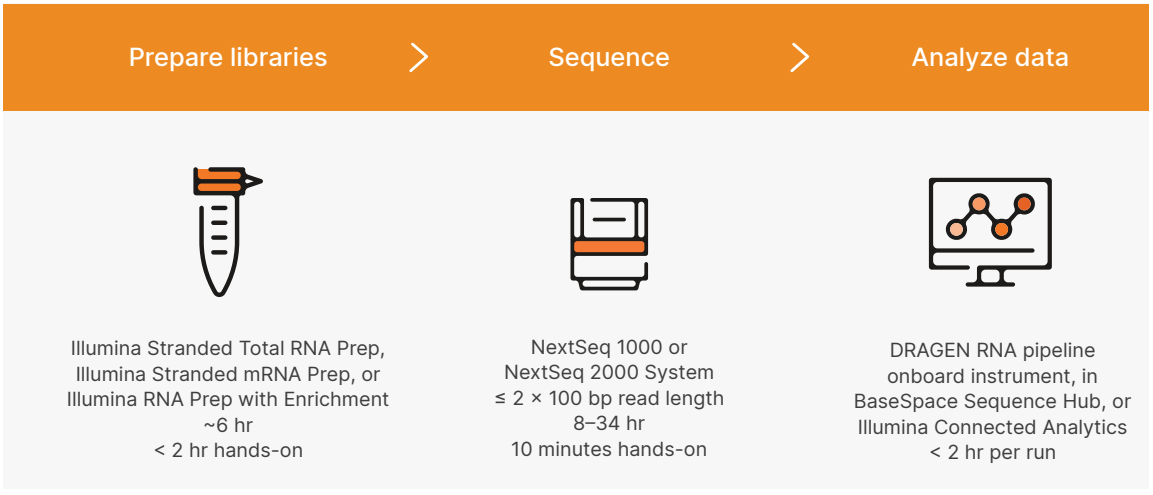

Figure 1: NextSeq 1000 and NextSeq 2000 RNA-Seq workflow—The NextSeq 1000 and NextSeq 2000 Systems are part of a simple, integrated NGS workflow that delivers highly accurate RNA sequencing data. Times vary by experiment and assay type.

Integrated NGS workflow

The NextSeq 1000 and NextSeq 2000 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 Stranded mRNA Prep works with any eukaryotic RNA from high-quality 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 the NextSeq 1000 and NextSeq 2000 Systems, the resulting nonstranded RNA data provide valuable insights across a large number of genomic positions.

* RNA integrity number (RIN) > 8. For lower quality or FFPE samples, use Illumina RNA Prep with Enrichment or Illumina Stranded Total RNA Prep.

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[a]	Ligation	(L) Tagmentation
Detection	Coding and noncoding transcriptome	Coding transcriptome with polyA tail	Targeted coding regions[b]
FFPE compatibility	Yes	No	Yes
Input	1–1000 ng[c]	25–1000 ng	10 ng non-FFPE 20 ng FFPE
Total assay time[d]	7 hr	< 7 hr	< 9 hr
Hands-on time[d]	< 3 hr	< 3 hr	< 2 hr
Automation friendly	Yes	Yes	Yes

a. The included Ribo-Zero Plus removes abundant RNA from multiple species, including human, mouse, rat, bacteria, and epidemiology samples.
b. For human samples only. Tested with Illumina Exome Panel and Respiratory Oligos Panel v2. Illumina RNA Prep with Enrichment does not provide strand information.
c. Minimum input for high-quality RNA shown. 10 ng minimum recommended for optimal quality and FFPE for Illumina Stranded Total RNA Prep.
d. 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.

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 Systems.

NextSeq 1000 and NextSeq 2000 Systems

The NextSeq 1000 and NextSeq 2000 Systems provide flexibility and scalability to address a full range of transcriptome analysis needs. Four available flow cell types 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 180 samples† in a single run. Whole-transcriptome analysis enables discovery of novel features by interrogating coding and noncoding RNA at up to 36 samples per run; researchers can also analyze coding RNA at up to 72 samples per run (Table 2, Table 3). Illumina recommends consulting the primary literature for your field and organism for the most up-to-date guidance on experiment design and sequencing depth.

† Expression profiling assumes 10M reads per sample.

The NextSeq 1000 and NextSeq 2000 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, whole-transcriptome sequencing, 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 XLEAP-SBS chemistry

With the NextSeq 1000 and NextSeq 2000 Systems, researchers can enrich their studies with greater read depth, resulting in more accurate fold-change estimates and high sensitivity in detection of genes, transcripts, and differential expression. The NextSeq 1000 and NextSeq 2000 Systems are powered by XLEAP-SBS chemistry, the fastest, highest quality, and most robust Illumina SBS chemistry to date. XLEAP-SBS reagents enable the highest read output and lowest price per million reads of any

‡ ATAC-Seq, assay for transposase-accessible chromatin with sequencing.

Table 2: Illumina RNA-Seq solutions and sample throughput per flow cell

Method	Measurement	Typical read pairs per sample[a]	Library prep	No. of samples per flow cell per run						
				NextSeq 1000 and NextSeq 2000				NovaSeq X Series[d]		
				P1	P2	P3[c]	P4[c]	1.5B	10B	25B
Gene expression quantification	Gene-level abundance across known features	10M	Illumina Stranded mRNA Prep[b]	10	40	120	180	150	~1K[e]	~2.5K[e]
mRNA-Seq	Coding RNA abundance and discovery	25M	Illumina RNA Prep with Enrichment	4	16	48	72	~60	~400[e]	~1K[e]
Total RNA-Seq	Coding and noncoding RNA abundance and discovery	50M	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	2	8	24	36	~30	~200	~520[e]

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 or Illumina Stranded Total RNA Prep is recommended.
c. P3 and P4 reagents are available for the NextSeq 2000 System only.
d. The NovaSeq X Plus System is capable of single flow cell runs or dual flow cell runs. The NovaSeq X System is capable of single flow cell runs.
e. A maximum of 384 unique dual indexes is available. For NovaSeq X Series, independent lane loading allows for multiplexing of more samples.

Table 3: NextSeq 1000 and NextSeq 2000 performance parameters for RNA-Seq

Flow cell	Single-end reads[a]	Read length	Output[a]	Run time[b]	Data quality[c]	Required input
NextSeq 1000/2000 P1 XLEAP-SBS Reagents	100M	2 × 150 bp	30 Gb	17 hr	≥ 90% bases above Q30	10 ng–1 µg with Illumina RNA Prep
NextSeq 1000/2000 P2 XLEAP-SBS Reagents	400M	2 × 100 bp	80 Gb	19 hr		
NextSeq 2000 P3 XLEAP-SBS Reagents[d]	1.2B	2 × 100 bp	240 Gb	31 hr		
NextSeq 2000 P4 XLEAP-SBS Reagents[d]	1.8B	2 × 100 bp	360 Gb	34 hr		

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 and P4 reagents are available for the NextSeq 2000 System only.

Illumina benchtop sequencing system. 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

The multiple flow cell configurations of the NextSeq 1000 and NextSeq 2000 Systems (Table 3) enable researchers to optimize study designs based on sample number and output requirements. For example, the additional sequencing capacity afforded by the NextSeq 2000 P4 flow cell makes it easier and more economical to design appropriately powered studies and 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™ X Series (Table 2).

Value of paired-end sequencing

With the NextSeq 1000 and NextSeq 2000 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 is necessary for stranded information and 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™ secondary analysis

RNA-Seq data analysis can be performed using tools from Illumina DRAGEN secondary analysis, a suite of accurate, comprehensive, and efficient data analysis pipelines.§ The Illumina DRAGEN RNA pipeline performs accurate RNA alignment to a reference genome, variant calling and quantification of genes, and characterization of splice junctions and candidate gene fusions (Figure 3). The DRAGEN RNA pipeline can be configured as part of run setup to launch on cloud with Illumina Connected Analytics or BaseSpace Sequence Hub, or as an on-instrument workflow using the NextSeq 1000 and NextSeq 2000 onboard DRAGEN hardware.

§ DRAGEN hardware is included onboard the NextSeq 1000 and NextSeq 2000 Systems. A DRAGEN license is included with the instrument and does not need to be purchased separately.

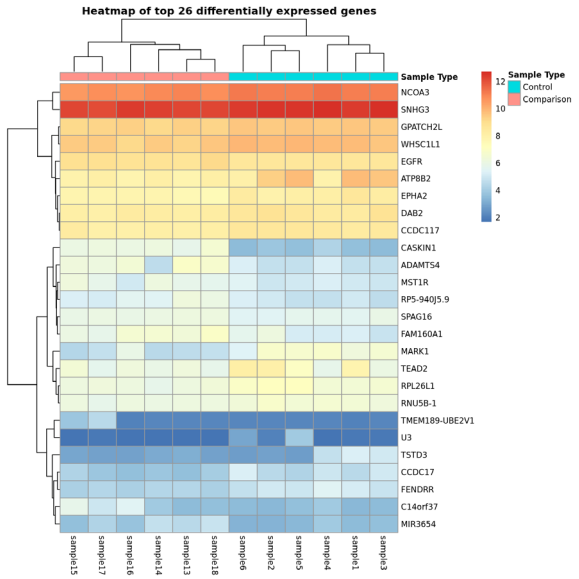

Figure 3: DRAGEN RNA pipeline—Screenshot example of differential expression heatmap with DRAGEN RNA pipeline, available onboard the NextSeq 1000 and NextSeq 2000 Systems or on cloud with BaseSpace Sequence Hub or Illumina Connected Analytics.

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. Beyond the DRAGEN platform, Illumina Connected Analytics and BaseSpace Sequence Hub provide 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 efficacy of their NextSeq 1000 and NextSeq 2000 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 that 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. Four flow cell types ensure cost-efficiency across RNA-Seq project types, from gene expression profiling to whole-transcriptome discovery.

Learn more

RNA sequencing

Illumina RNA library preparation

NextSeq 1000 and NextSeq 2000 Sequencing Systems

DRAGEN secondary analysis

Demo data on BaseSpace Sequence Hub

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

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 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100983
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100982
NextSeq 1000/2000 P1 XLEAP-SBS Reagent Kit (600 cycles)[a]	20100981
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100987
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100986
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100985
NextSeq 1000/2000 P2 XLEAP-SBS Reagent Kit (600 cycles)[a]	20100984
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100990
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100989
NextSeq 2000 P3 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100988
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (50 cycles)[a]	20100995
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (100 cycles)[a]	20100994
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (200 cycles)[a]	20100993
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (300 cycles)[a]	20100992
NextSeq 1000/2000 XLEAP-SBS Read and Index Primers[a]	20112856
NextSeq 1000/2000 XLEAP-SBS Index Primer Kit[a]	20112858

Ordering information

Product	Catalog no.
NextSeq 1000/2000 XLEAP-SBS Read Primer Kit[a]	20112859
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
Illumina RNA UD Indexes Set A, Ligation (96 indexes, 96 samples)[b]	20091655
Illumina RNA UD Indexes Set B, Ligation (96 indexes, 96 samples)[b]	20091657
Illumina RNA UD Indexes Set C, Ligation (96 indexes, 96 samples)[b]	20091659
Illumina RNA UD Indexes Set D, Ligation (96 indexes, 96 samples)[b]	20091661
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)[b]	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)[b]	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 indexes, 96 samples)[b]	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 indexes, 96 samples)[b]	20091660

a. XLEAP-SBS reagent kits for the NextSeq 1000 and NextSeq 2000 instruments are shipped and stored at the same temperature as standard SBS reagent kits.
b. 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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