APPLICATION NOTE

NextSeq™ 550 RNA sequencing solution

RNA-to-results workflow for gene expression profiling to whole-transcriptome analysis

- Comprehensive library preparation portfolio enables broad range of RNA-Seq applications
- Tunable sequencing platform with mid- and high-output modes supports faster turnaround times
- Best-in-class pipeline algorithms overcome bottlenecks in data analysis

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Introduction

The NextSeq 550 RNA sequencing (RNA-Seq) solution offers a robust, RNA-to-results workflow for obtaining a clear, comprehensive view of the transcriptome. The solution includes a suite of advanced RNA library preparation kits, push-button sequencing on the proven NextSeq 550 System (Figure 1), and streamlined data analysis tools (Figure 2). The NextSeq 550 RNA-Seq solution leverages industry-leading Illumina sequencing by synthesis (SBS) chemistry and next-generation sequencing (NGS) technology[1] to deliver highly accurate data for various RNA-Seq applications—from basic gene expression profiling to complex whole-transcriptome analyses. With minimal hands-on time and tunable output on the NextSeq 550 System, this solution can help more labs add RNA-Seq to their repertoire of molecular methods.

Figure 1: NextSeq 550 System—Proven platform offering the accuracy of SBS chemistry as part of a streamlined RNA-Seq workflow.

Streamlined RNA-Seq solution

The NextSeq 550 RNA-Seq solution begins with RNA library preparation using Illumina Stranded Total RNA Prep, Illumina Stranded mRNA Prep, or Illumina RNA Prep with Enrichment.* Prepared libraries are loaded into a reagent cartridge and then onto the NextSeq 550 System for sequencing. The NextSeq 550 System features dual output modes (mid and high) that enable labs to scale RNA-Seq studies according to their needs. Data analysis, including alignment, fusion detection, and gene quantification, is easily performed on a local server or in the cloud with the DRAGEN™ RNA Pipeline or other BaseSpace™ Sequence Hub apps. Data generated provides full sequence and variant information across a broad dynamic range, and can be used to identify isoforms, novel transcripts, and gene fusions.

* The open-platform NextSeq 550 System can accommodate library preparation kits developed by third-party providers.

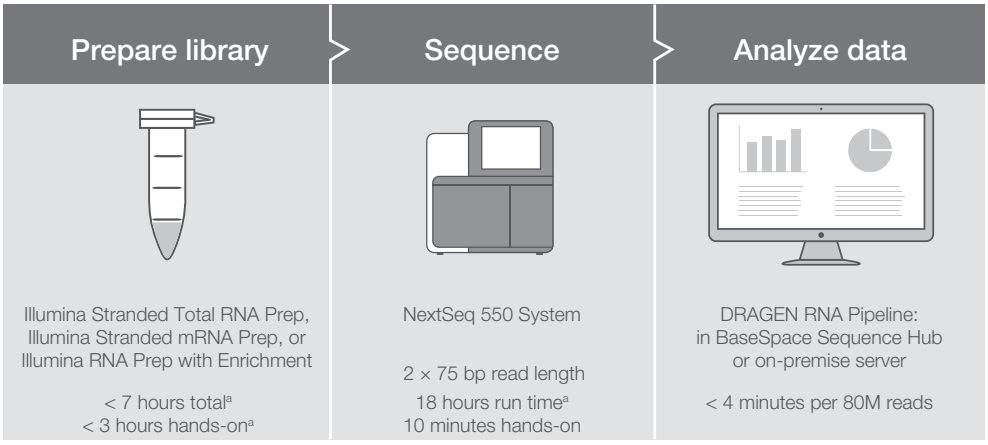

Figure 2: NextSeq 550 RNA-Seq workflow—A simple workflow that delivers highly accurate data with minimal hands-on time. The integrated NGS workflow includes library prep, push-button sequencing, and streamlined data analysis.

a. Assay times vary based on library prep method and number of samples; times indicated are for mRNA-Seq with Illumina Stranded mRNA Prep for 16 samples.

Advanced RNA library preparation portfolio

The NextSeq 550 System supports an extensive portfolio of RNA library preparation solutions, addressing a wide range of transcriptome applications. Users can choose from various library prep kits from Illumina or third-party providers to overcome common challenges such as poor-quality starting RNA or limited sample availability.

Advances in the Illumina portfolio of RNA library preparation kits (Table 1) deliver the high-throughput that many labs require, with a streamlined workflow that can be completed within one standard working shift. Illumina offers three RNA library prep kits:

- **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 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 550 System, the resulting nonstranded RNA data provide valuable insights across 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 for use with the NextSeq 550 System.

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 poly-A 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 hours	< 7 hours	< 9 hours
Hands-on time	< 3 hours	< 3 hours	< 2 hours
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 workflow.
c. Tested with Illumina Exome Panel and Respiratory Oligos Panel v2. Illumina RNA Prep with Enrichment does not provide strand information.

Tunable sequencing performance

The NextSeq 550 System provides robust sequencing power and tunability to address a full range of transcriptome analysis needs. High- and mid-output sequencing modes enable users to select the optimal balance between sample number and output requirements (Table 2). For example, gene expression profiling (the measurement of gene-level abundance across known features) can be performed efficiently at high-output 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 eight samples per run. Users can also analyze coding RNA at up to 16 samples per run.

Fast, accurate RNA-Seq workflow

The NextSeq 550 System streamlines the RNA-Seq workflow. It takes less than 10 minutes to load and initiate the system. Sequencing is completed in as few as 18 hours using the high-output mode and paired-end 75 bp read lengths (Table 3). The NextSeq 550 mid-output and high-output options enable users to increase turnaround time based on sample number. When timing is critical, labs can analyze smaller numbers of samples without waiting for larger batches.

Industry-leading SBS read quality

At the core of the NextSeq 550 System is proven Illumina SBS chemistry, which is used to generate > 90% of the world's sequencing data.[1] The NextSeq 550 System delivers industry-leading sequencing accuracy of > 80% of sequenced bases over Q30‡ at 2 × 75 bp. It enables users to generate high-quality results with increased dynamic range, accurate fold-change estimates, and sensitive detection of genes, transcripts, and differential expression.

Simplified RNA-Seq analysis solutions

The DRAGEN Bio-IT Platform

Labs can perform RNA-Seq data analysis using tools from the Illumina DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform. The DRAGEN Bio-IT Platform

† Expression profiling assumes 10M reads per sample.

‡ Q30 = 1 error in 1000 base calls or an accuracy of 99.9%.

Table 2: Illumiina RNA-Seq Solutions on the NextSeq 550 System

Method	Measurement	Typical read pairs per sample	Library prep	Samples per run		Data analysis
				Mid-output	High-output	
Gene expression profiling	Gene-level abundance across known features	10M (1 × 75 bp)	Illumina Stranded mRNA Prep[a]	13	40	DRAGEN RNA Pipeline (server or cloud) + RNA-Seq Differential Expression App (cloud only)
mRNA-Seq	Coding RNA abundance and discovery	25M (2 × 75 bp)	Illumina RNA Prep with Enrichment	5	16	
Total RNA-Seq	Coding and noncoding RNA abundance and discovery	50M (2 × 75 bp)	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	2	8	

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

Table 3: NextSeq 550 System sequencing performance

Flow cell configuration	Read length	Output	Run time	Data quality	Required input
High-output flow cell Up to 400M single reads Up to 800M paired-end reads	2 × 150 bp	100-120 Gb	29 hours	> 80% bases above Q30 at 2 × 75 bp > 75% bases above Q30 at 2 × 150 bp	10 ng–1 µg with Illumina RNA Prep
	2 × 75 bp	50-60 Gb	18 hours		
	1 × 75 bp	25-30 Gb	11 hours		
Mid-output flow cell Up to 130M single reads Up to 260M paired-end reads	2 × 150 bp	32.5-39 Gb	26 hours		
	2 × 75 bp	16.25-19.5 Gb	15 hours		

Run time includes cluster generation, sequencing, and base calling on the NextSeq 550 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.

uses best-in-class pipeline algorithms to help users overcome bottlenecks in data analysis and reduce reliance on external informatics experts. The DRAGEN RNA Pipeline takes output from the NextSeq 550 System and performs accurate RNA mapping and alignment to a reference genome, fusion detection, and gene quantification. Users can launch the DRAGEN RNA Pipeline in BaseSpace Sequence Hub or on-premise using a DRAGEN Server. Whether in the cloud or onsite, the pipeline provides high-quality data packaged in an intuitive user interface.

An ecosystem of apps in BaseSpace Sequence Hub

Output from the DRAGEN RNA Pipeline can be input directly into a wide selection of downstream analysis tools available in BaseSpace Sequence Hub, including the RNA-Seq Differential Expression App (Figure 3). Beyond the DRAGEN platform, BaseSpace Sequence Hub includes a growing community of Illumina and third-party bioinformatics tools for visualization, analysis, and sharing.

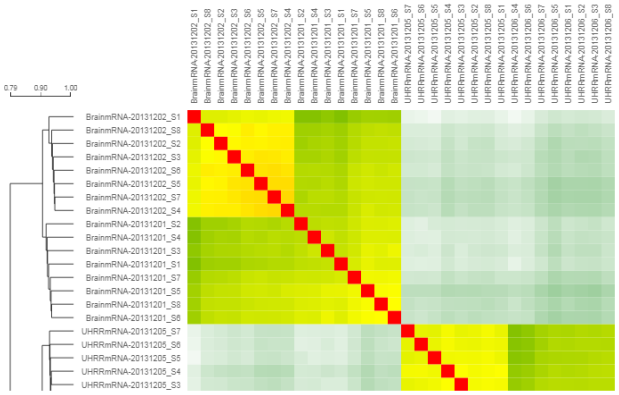

Figure 3: RNA-Seq Differential Expression App on BaseSpace Sequence Hub—Perform differential expression analysis for RNA-Seq data generated on the NextSeq 550 System and processed with the DRAGEN RNA Pipeline.

World-class service and 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 application 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 access online support 24/7, worldwide and in multiple languages.

With this unmatched service and support, Illumina helps users maximize the effectiveness of their NextSeq 550 System, train new employees, and learn the latest techniques and best practices.

Summary

The NextSeq 550 RNA-Seq solution offers a robust, RNA-to-results workflow for transcriptome analysis. With an advanced RNA library prep portfolio and tunable sequencing output, the NextSeq 550 RNA-Seq solution supports dynamic throughput levels across RNA-Seq project types, from gene expression profiling to whole-transcriptome discovery. Adopted by leading genomics centers and referenced in ample transcriptome-related publications, the NextSeq 550 RNA-Seq solution enables users to drive insight through a deeper understanding of the transcriptome.

Learn more

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

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

References

1. Data calculations on file. Illumina, Inc., 2017.

Ordering information

Product	Catalog no.
NextSeq 550 System	SY-415-1002
NextSeq 500/550 Mid-Output v2.5 Kit (150 cycles)	20024904
NextSeq 500/550 Mid-Output v2.5 Kit (300 cycles)	20024905
NextSeq 500/550 High-Output v2.5 Kit (75 cycles)	20024906
NextSeq 500/550 High-Output v2.5 Kit (150 cycles)	20024907
NextSeq 500/550 High-Output v2.5 Kit (300 cycles)	20024908
TG NextSeq 500/550 Mid-Output Kit v2.5 (150 cycles)[a]	20024909
TG NextSeq 500/550 Mid-Output Kit v2.5 (300 cycles)	20024910
TG NextSeq 500/550 High-Output Kit v2.5 (75 cycles)	20024911
TG NextSeq 500/550 High-Output Kit v2.5 (150 cycles)	20024912
TG NextSeq 500/550 High-Output Kit v2.5 (300 cycles)	20024913

a. TG-labeled consumables have features that help customers reduce the frequency of revalidation. These consumables are available only under supply agreement and require customers to provide a binding forecast. Contact your account manager for more.

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)[b]	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

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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