DATA SHEET

Illumina Stranded Total RNA Prep, Ligation with Ribo-Zero™ Plus

Sensitive and accurate analysis of the transcriptome with a fast and flexible solution

- Achieve high sensitivity from as little as 1 ng high-quality RNA or 10 ng RNA from degraded FFPE samples
- Remove rRNAs from human, mouse, rat, and bacterial species and globin RNAs in a single-tube reaction
- Prepare libraries in 7 hours, including only 3 hours of hands-on time

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Introduction

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for discovering, profiling, and quantifying RNA transcripts. Advantages of key RNA-Seq approaches include:

- Total RNA-Seq provides an unbiased, hypothesis-free approach for comprehensive analysis of the transcriptome. It accurately measures gene and transcript abundance and detects both known and novel features in coding and multiple forms of noncoding RNA
- Messenger RNA (mRNA)-Seq sensitively and accurately quantifies gene expression, identifies known and novel isoforms in the coding transcriptome, and measures allele-specific expression
- Targeted RNA-Seq analyzes gene expression in a focused set of genes of interest. Targeted RNA-Seq with enrichment enables cost-effective RNA exome analysis using sequence-specific capture of the coding regions of the transcriptome

TruSeq™ Stranded Total RNA provides a robust solution for whole-transcriptome analyses for standard and low-quality samples. However, a relatively high input requirement, long total assay and hands-on times, and narrow application flexibility have limited its use in total RNA-Seq applications. To overcome these challenges, Illumina developed Illumina Stranded Total RNA Prep (Table 1). This advanced solution offers streamlined, rapid ligation-based library preparation that supports low sample inputs and a wide range of RNA-Seq applications.

To focus studies on high-value sequences, Illumina Stranded Total RNA Prep includes the Illumina Ribo-Zero Plus rRNA Depletion Kit*, which provides efficient removal of ribosomal RNA (rRNA) from multiple species, including human, mouse, rat, and bacteria, in a single reaction (Figure 1).

* For metatranscriptomics studies, Illumina Stranded Total RNA Prep can be combined with the Illumina Ribo-Zero Plus Microbiome Depletion kit, which provides robust depletion of abundant rRNA in complex microbial samples.

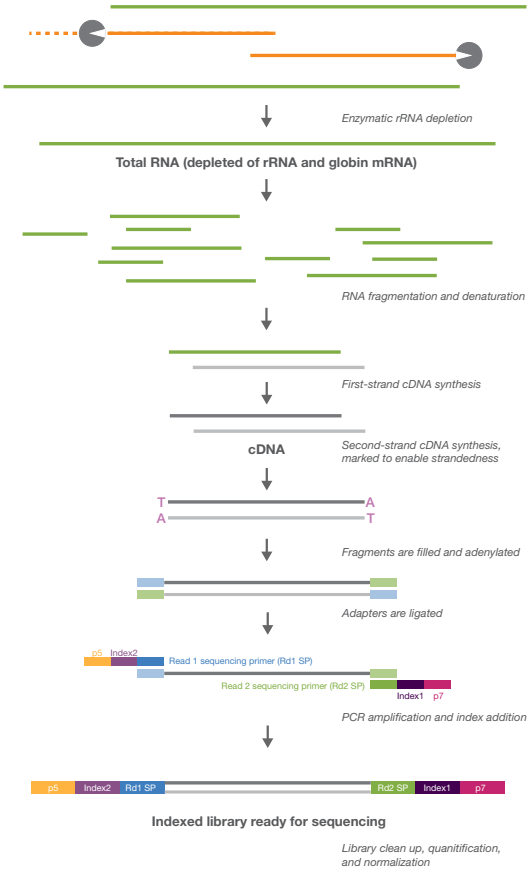

Figure 1: Illumina Stranded Total RNA Prep with Ribo-Zero Plus— After rRNAs and abundant globin mRNAs (orange lines) are depleted and cDNA synthesis is complete, adapters are ligated and unique dual indexes are added by PCR amplification to produce high-quality libraries that are quantified and normalized prior to sequencing.

Effective, multispecies ribodepletion with Ribo-Zero Plus

Removal of abundant RNAs, including rRNAs and globin RNAs, prior to RNA-Seq enables researchers to focus on analyzing high-value, informative portions of the transcriptome while lowering sequencing costs. Illumina Stranded Total RNA includes the Ribo-Zero Plus rRNA Depletion Kit, which facilitates rich transcriptome analyses by removing rRNAs and globin RNAs. The single-tube enzymatic ribodepletion method is compatible with low inputs (1 ng) and reduces rRNA from prokaryotic and eukaryotic species (Table 2).

Table 1: Illumina Stranded Total RNA specifications

Feature	TruSeq Stranded Total RNA	Illumina Stranded Total RNA Prep
Abundant RNA depletion	Human, mouse, rat rRNAs or globin mRNAs	Human, mouse, rat, bacteria rRNAs and globin mRNAs
Max UDI	96	384
RNA input amount	100–1000 ng	1–1000 ng RNA[a]
Total assay time	11.5 hours	7 hours
Hands-on time	5.5 hours	< 3 hours
FFPE compatible	Yes	Yes
Kit configuration	48 or 96 samples	16 or 96 samples

a. 1–1000 ng high-quality RNA (RIN > 7), 10–1000 ng degraded RNA (RIN 2–7) or FFPE RNA (DV200 > 55). For best performance, 10 ng input RNA is recommended.
b. Abbreviations: UDI, unique dual indexes; RIN, RNA integrity number.

Table 2: RNA species targeted for reduction

Sample	rRNAs targeted
Human cytoplasmic rRNAs	28S, 18S, 5.8S, 5S
Human mitochondrial rRNAs	12S, 16S
Human Beta Globin transcripts	HBA1, HBA2, HBB, HBG1, HBG2
Mouse and rat rRNA	16S, 28S
Gram (-) bacterial rRNAs	*E. coli* 5S, 16S, 23S
Gram (+) bacterial rRNAs	*B. subtilis* 5S, 16S, 23S

Abundant rRNA and globin RNA is removed from total RNA by targeted hybridization to DNA probes and subsequent RNase H-mediated cleavage (Figure 2, Table 3). Ribodepleted samples then undergo library preparation. To evaluate rRNA depletion and library preparation performance with Illumina Stranded Total RNA with Ribo-Zero Plus, a range of total RNA inputs were tested against TruSeq Stranded Total RNA with Ribo-Zero. Illumina Stranded Total RNA with Ribo-Zero Plus showed superior performance, especially at low inputs (Figure 3, Table 4).

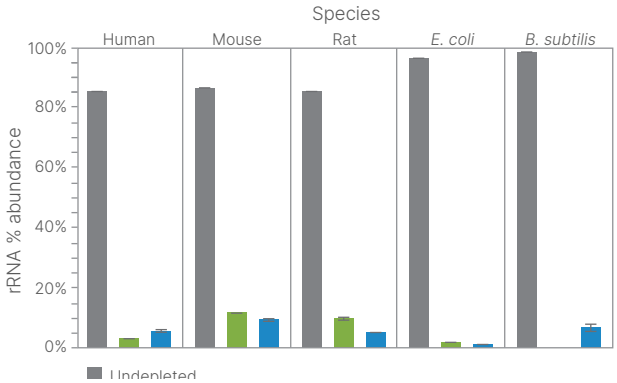

Figure 2: Multispecies ribodepletion with Illumina Stranded Total RNA Prep with Ribo-Zero Plus—Illumina Stranded Total RNA Prep with Ribo-Zero Plus effectively depletes rRNA levels for human, mouse, rat, and bacterial species in a single-tube reaction. Results are compared to TruSeq Stranded Total RNA paired with Ribo-Zero Gold for mammalian species and Ribo-Zero Bacteria for *E. coli* (*B. subtilis* data not shown).

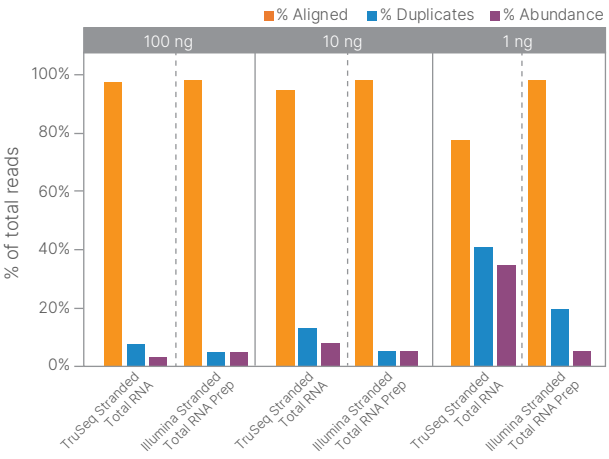

Figure 3: Comparison of library preparation performance—Illumina Stranded Total RNA Prep with Ribo-Zero Plus was compared against TruSeq Stranded Total RNA with Ribo-Zero. Illumina Stranded Total RNA Prep with Ribo-Zero Plus was more effective, with increased aligned reads and fewer duplicates and rRNA abundance, particularly at low inputs of 10 ng and 1 ng total UHR RNA. Libraries were sequenced on a NextSeq 550 System, subsampled to 30M reads. Percent duplicates were calculated by subsampling to 4M reads and analyzed using the BaseSpace™ RNA-Seq Alignment App v2.0.

Table 3: Globin mRNA depletion from human peripheral blood leukocytes with Illumina Stranded Total RNA Prep with Ribo-Zero Plus

	100 ng total RNA input			10 ng total RNA input		
Gene	Nondepleted	Depleted	% Depleted	Nondepleted	Depleted	% Depleted
HBA1	7489	2	99.97%	13,685	4	99.97%
HBA2	66,045	18	99.97%	110,406	16	99.99%
HBB	154,614	78	99.95%	173,704	86	99.95%
HBG1	22	0	96.29%	37	1	99.69%
HBG2	203	0	100%	143	0	100%

Table 4: Performance metrics for Illumina Stranded Total RNA Prep with Ribo-Zero Plus[a]

	100 ng total RNA input		10 ng total RNA input		1 ng total RNA input	
	TruSeq Stranded Total RNA with Ribo-Zero	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	TruSeq Stranded Total RNA with Ribo-Zero	Illumina Stranded Total RNA Prep with Ribo-Zero Plus	TruSeq Stranded Total RNA with Ribo-Zero	Illumina Stranded Total RNA Prep with Ribo-Zero Plus
% rRNA (28S/18S)	2.0	3.8	7.2	4.4	32.8	4.5
% Strandedness	99	99	99	99	99	99
Median CV of coverage	0.44	0.46	0.48	0.47	0.52	0.51
% Duplicates	7.5	4.5	12.8	5.3	40.9	19.2
% Aligned	96.9	96.9	94.2	97.5	76.6	97.5
% Abundance	3.0	4.9	8	5.2	35.8	5.0

a. Data analysis was performed using BaseSpace RNA-Seq Alignment App v2.0.1.
b. Percent duplicates are reported after subsampling to 4M paired-end reads passing filter (PF).

High-quality data

Coverage uniformity

Illumina Stranded Total RNA Prep produces sequencing libraries that result in highly uniform transcript coverage using high-quality and degraded universal human reference (UHR) input RNA (Figure 4A) and with low input amounts of FFPE RNA (Figure 4B).

Gene discovery efficiency

To compare the performance of Illumina Stranded Total RNA Prep to TruSeq Stranded Total RNA for gene discovery applications, varying amounts of input UHR RNA were sequenced at 30M paired-end reads and the number of genes with 1× and 10× coverage was assessed. Results show that Illumina Stranded Total RNA Prep enables greater gene detection at low input amounts of only 1 ng total RNA (Figure 5).

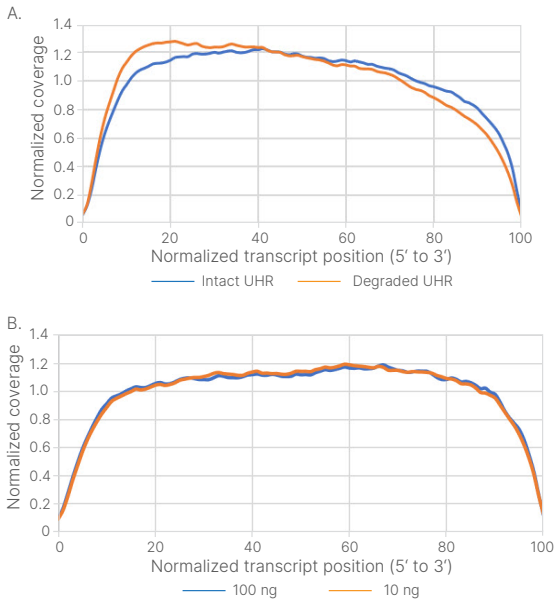

Figure 4: High coverage uniformity—Illumina Stranded Total RNA Prep provides high coverage uniformity for (A) high-quality and synthetically degraded UHR RNA (RIN=2) and (B) FFPE RNA at input levels of 100 ng and 10 ng. The FFPE sample had a DV_{200} quality score of 55%. All libraries were sequenced on a NovaSeq 6000 System at 50M reads. Data analysis was performed using the BaseSpace RNA-Seq Alignment App v2.0.1.

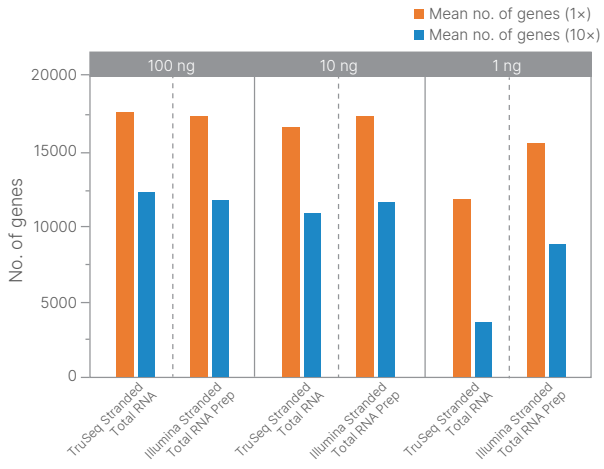

Figure 5: Greater gene discovery at low input—Illumina Stranded Total RNA Prep enables greater gene detection with low RNA inputs, as compared to TruSeq Stranded Total RNA, as measured by the number of genes detected at 30M subsampled paired-end reads PF. More genes detected at 1× with Illumina Stranded Total RNA Prep is an indicator of greater sensitivity.

Exceptional data concordance

Illumina Stranded Total RNA Prep produces quality data with high concordance between varying amounts of input UHR RNA (Figure 6A) and between technical replicates of low input amounts of RNA from FFPE samples (Figure 6B). These results demonstrate that Illumina Stranded Total RNA Prep is an ideal solution for degraded samples with limited starting material. Also, Illumina Stranded Total RNA Prep shows high data concordance with TruSeq Stranded Total RNA, both with equivalent inputs (Figure 7A) and reduced input (Figure 7B).

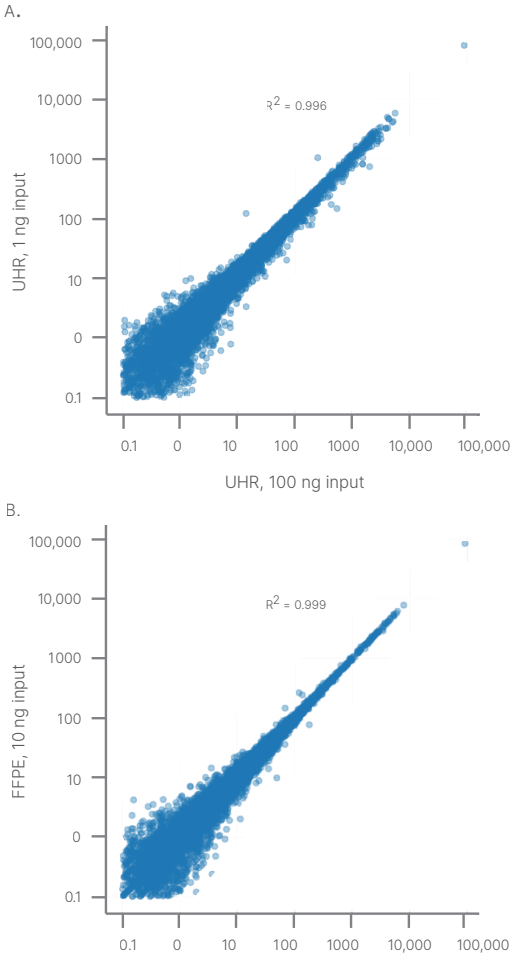

Figure 6: High data concordance—Illumina Stranded Total RNA Prep achieves high data concordance between (A) input amounts of 1 ng and 100 ng UHR RNA and (B) technical replicates of 10 ng FFPE RNA. Libraries were sequenced on a NovaSeq 6000 System at 2 × 74 bp. Data analysis was performed using the BaseSpace RNA-Seq Alignment App v2.0.1.

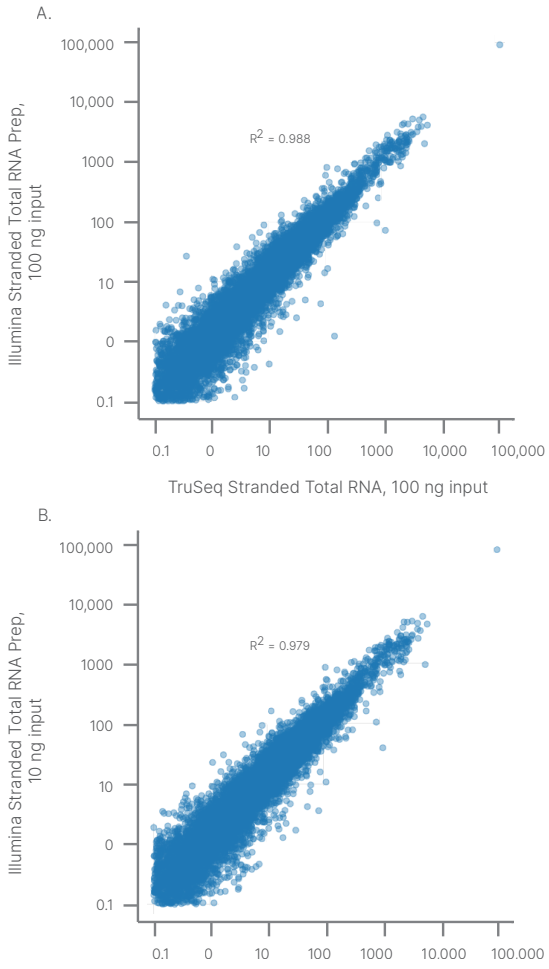

Figure 7: High concordance with legacy kit—Illumina Stranded Total RNA Prep produces highly concordant data with TruSeq Stranded Total RNA at (A) equivalent and (B) lower inputs.

Streamlined library preparation workflow

Illumina Stranded Total RNA Prep uses a fast and flexible workflow for ligation-based preparation of RNA libraries (Figure 1). Innovations to the workflow, including shorter incubation times and reduced sample cleanup steps, result in a total assay time that is > 40% faster than TruSeq Stranded Total RNA (Figure 8).

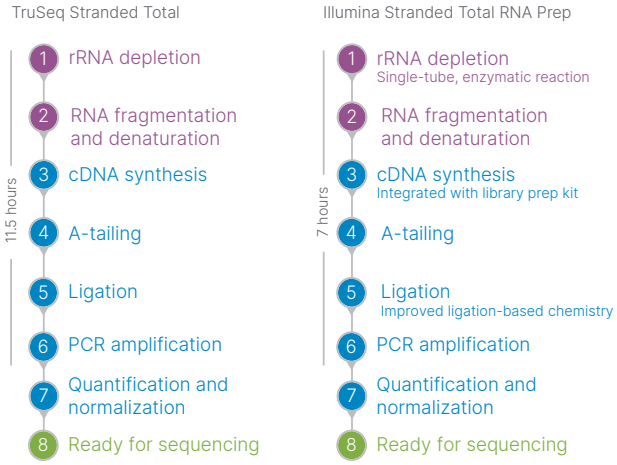

Figure 8: Illumina Stranded Total RNA Prep with Ribo-Zero Plus workflow—Illumina Stranded Total RNA Prep delivers a fast workflow with reduced hands-on time. Times may vary depending on equipment used, number of samples processed, automation procedures, or user experience.

Increased throughput with unique dual indexes

By combining Illumina Stranded Total RNA Prep with Ribo-Zero Plus and high-throughput instruments such as the NextSeq™ 550 and NovaSeq™ 6000 Systems, laboratories can sequence significantly more samples per run without compromising data quality. For additional increases in sample throughput, Illumina Stranded Total RNA Prep supports multiplexing with 384 unique dual indexes (UDIs). In addition to eliminating the impact of index misassignment, or index hopping, UDIs help decrease sequencing costs by allowing up to 384 samples to be loaded on a single NovaSeq 6000 S4 flow cell for significantly increased throughput.

Summary

Illumina Stranded Total RNA Prep offers a streamlined RNA-Seq solution for clear and comprehensive analysis across the transcriptome. This solution offers extraordinary flexibility for input type and supports low input amounts, down to 1 ng of high-quality RNA. Compatibility with the Ribo-Zero Plus rRNA Depletion Kit enables highly effective removal of interfering rRNA from multiple species, including human, mouse, rat, and bacteria. Illumina Stranded Total RNA Prep enables precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as alternative transcripts, gene fusions, and allele-specific expression.

Learn more

Illumina Stranded Total RNA Prep with Ribo-Zero Plus or Ribo-Zero Plus Microbiome

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 Total RNA Prep, Ligation with Ribo-Zero Plus Microbiome (96 samples)	20072063
Illumina RNA UD Indexes Set A, Ligation (96 Indexes, 96 Samples)	20091655
Illumina RNA UD Indexes Set B, Ligation (96 Indexes, 96 Samples)	20091657
Illumina RNA UD Indexes Set C, Ligation (96 Indexes, 96 Samples)	20091659
Illumina RNA UD Indexes Set D, Ligation (96 Indexes, 96 Samples)	20091661

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1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

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