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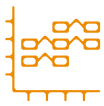
DATA SHEET

Illumina Rapid Total RNA Prep

Fast and easy workflow for whole-transcriptome analysis

3.5-hour RNA-Seq library prep using Ribo-Zero™ Plus for depletion of abundant RNAs

Proprietary chemistry that allows for cDNA synthesis and adapter tagging in a single step



High-quality total RNA-Seq nonstranded data from low inputs or poor-quality samples

Fast and accurate total RNA-Seq

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for discovering, profiling, and quantifying RNA transcripts. 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. However, total RNA-Seq library preparation can be long and labor-intensive, limiting throughput.

Illumina Rapid Total RNA Prep offers a fast and simple tagmentation-based workflow that supports NGS library preparation from low RNA inputs and formalin-fixed, paraffin-embedded (FFPE) samples. The Illumina Rapid RNA library prep reaction combines cDNA synthesis and adapter tagging with bead-linked transposomes into a single step (Figure 1). This streamlined method delivers high-accuracy nonstranded data for whole-transcriptome sequencing of well-annotated genomes.*

Total RNA-Seq applications include:

- Differential gene expression analysis
- Transcript counting
- Gene fusion detection
- Isoform/alternative splicing analysis
- Allele-specific expression analysis
- Single nucleotide variant (SNV) analysis
- Pathway analysis

Save time with a streamlined workflow

Illumina Rapid Total RNA Prep, using Ribo-Zero Plus, is the fastest and easiest total RNA-Seq library prep solution in the Illumina portfolio, reducing turnaround time and hands-on time by half compared with Illumina Stranded Total RNA Prep with Ribo-Zero Plus (Table 1).

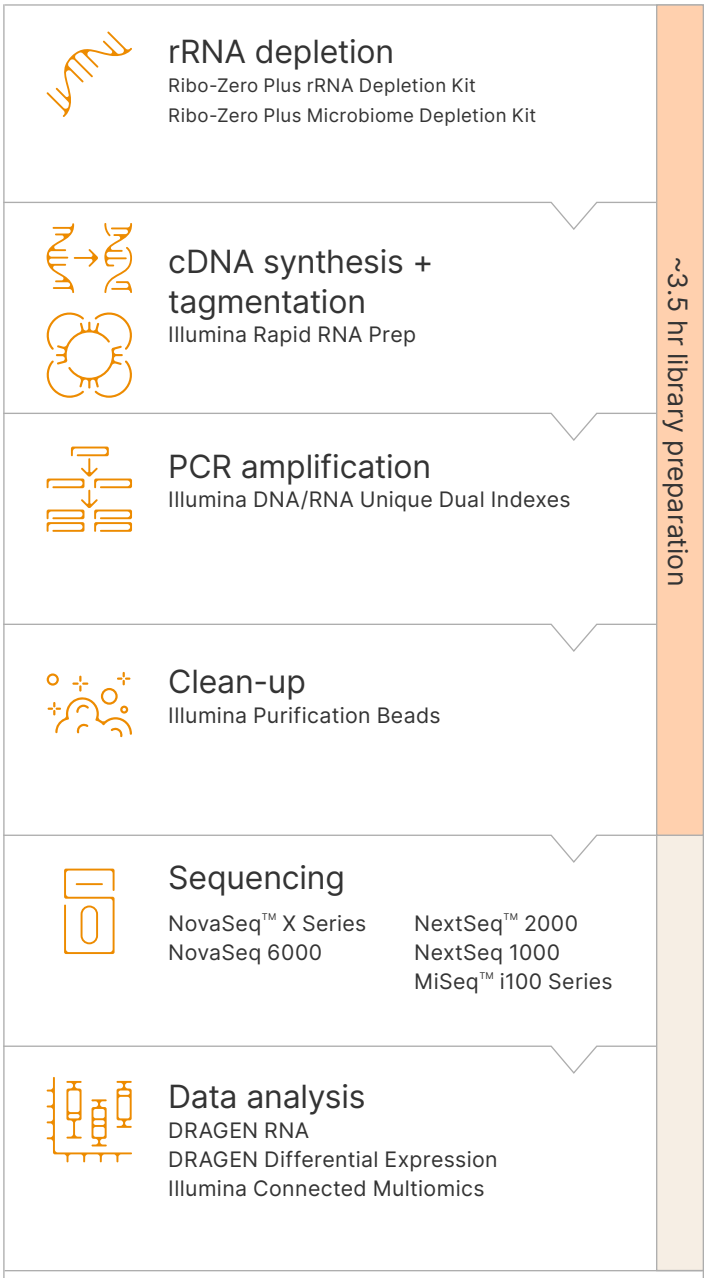


Figure 1: Illumina Rapid Total RNA Prep delivers a fast library preparation workflow

The Illumina Rapid Total RNA Prep workflow starts with rRNA depletion using Ribo-Zero Plus. Next, Illumina Rapid RNA Prep uses a single-tube reaction of cDNA synthesis reagents and bead-linked transposomes to synthesize first- and second-strand cDNA and tag the cDNA with adapters. The combined reaction requires ~30 minutes of incubation time and is followed by a PCR step that adds indexed adapter sequences to the ends of the cDNA fragments. Illumina Rapid RNA Prep kits include Illumina Purification Beads for a final library clean-up step. Libraries can be sequenced on an Illumina sequencing system. Analyze data using the DRAGEN RNA and DRAGEN Differential Expression apps and visualize with Illumina Connected Multiomics.

* For applications where transcript strand information is important, such as novel discovery of new RNAs or for organisms without well-annotated genomes, we recommend Illumina Stranded Total RNA Prep with Ribo-Zero Plus. Illumina Stranded Total RNA Prep is a ligation-based library prep kit for accurate, unbiased total RNA-Seq with precise measurement of strand information.

Table 1: Illumina Rapid Total RNA Prep reduces library preparation time and hands-on time by half

	Illumina Stranded Total RNA Prep with Ribo-Zero Plus[a]	Illumina Rapid Total RNA Prep (using Ribo-Zero Plus)[b]
Total turnaround time	7 hr	~3.5 hr
Hands-on time	< 3 hr	< 1.5 hr
Clean up steps	4	2
RNA input amount	1–1000 ng[c]	1–1000 ng[c]
Library prep method	Ligation	Tagmentation
Strand specificity	Stranded	Nonstranded

a. Illumina Stranded Total RNA Prep with Ribo-Zero Plus includes the Ribo-Zero Plus rRNA Depletion Kit (or Ribo-Zero Plus Microbiome Depletion Kit).
b. For Illumina Rapid Total RNA Prep, the Illumina Rapid RNA Prep and Ribo-Zero Plus rRNA Depletion Kit (or Ribo-Zero Plus Microbiome Depletion Kit) are sold separately.
c. 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.

The Ribo-Zero Plus rRNA Depletion Kit starts with removal of prokaryotic and eukaryotic ribosomal RNA (rRNA) and globin RNA in a single reaction to allow rich transcriptome analysis. Next, Illumina Rapid RNA Prep uses a single-tube, 30-minute reaction for combined cDNA synthesis and on-bead tagmentation. PCR amplification adds index adapters, then a single clean-up step with included Illumina Purification Beads completes the library prep. The entire simplified protocol requires only 3.5 hours to generate a sequencing-ready total RNA library, helping researchers get answers more quickly. Fewer pipetting steps with less than 1.5 hours of hands-on time reduces opportunities for error, resulting in reproducible data.

Effective depletion of abundant RNA species

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. For total RNA-Seq, Illumina Rapid RNA Prep is used with the Ribo-Zero Plus rRNA Depletion Kit,† which efficiently removes rRNA from multiple species, including human, mouse, rat, and bacteria. The single-tube enzymatic ribodepletion method uses targeted hybridization to DNA probes, followed by RNase H-mediated cleavage. Illumina Rapid RNA Prep used with Ribo-Zero Plus shows effective depletion of human rRNA, globin RNA, and microbiome bacterial rRNA (Figure 2, Figure 3).

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

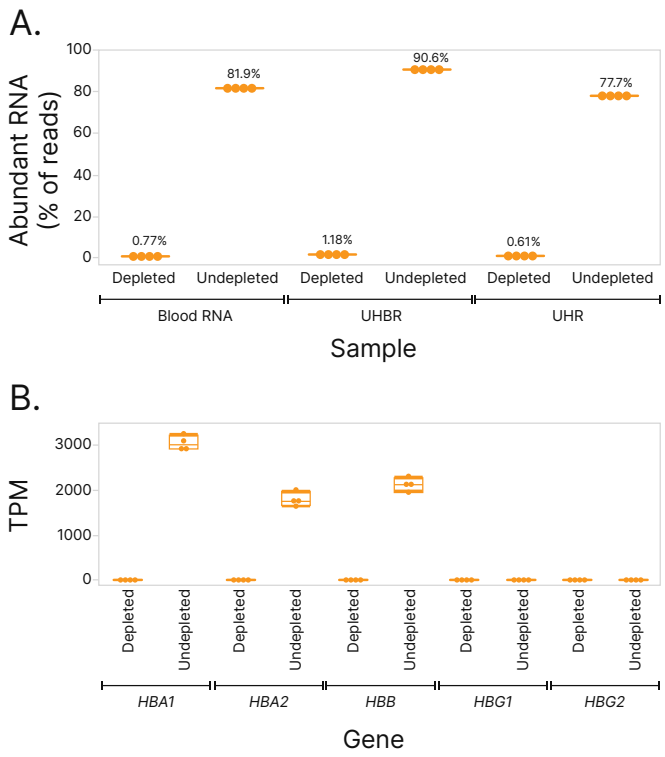


Figure 2: Effective depletion of rRNA and globin RNA using the Ribo-Zero Plus rRNA Depletion Kit
(A) Percent of total reads that align to abundant RNA in depleted and undepleted samples: 10 ng blood RNA, 10 ng universal human brain reference (UHBR) RNA, and 10 ng universal human reference (UHR) RNA. (B) Normalized transcripts per million (TPM) for globin genes: *HBA1/HBA2* (alpha), *HBB* (beta), and *HBG1/HBG2* (gamma) for 10 ng depleted and undepleted blood samples. *HBG1/HBG2* are fetal-specific genes, not expected to be expressed in blood samples.

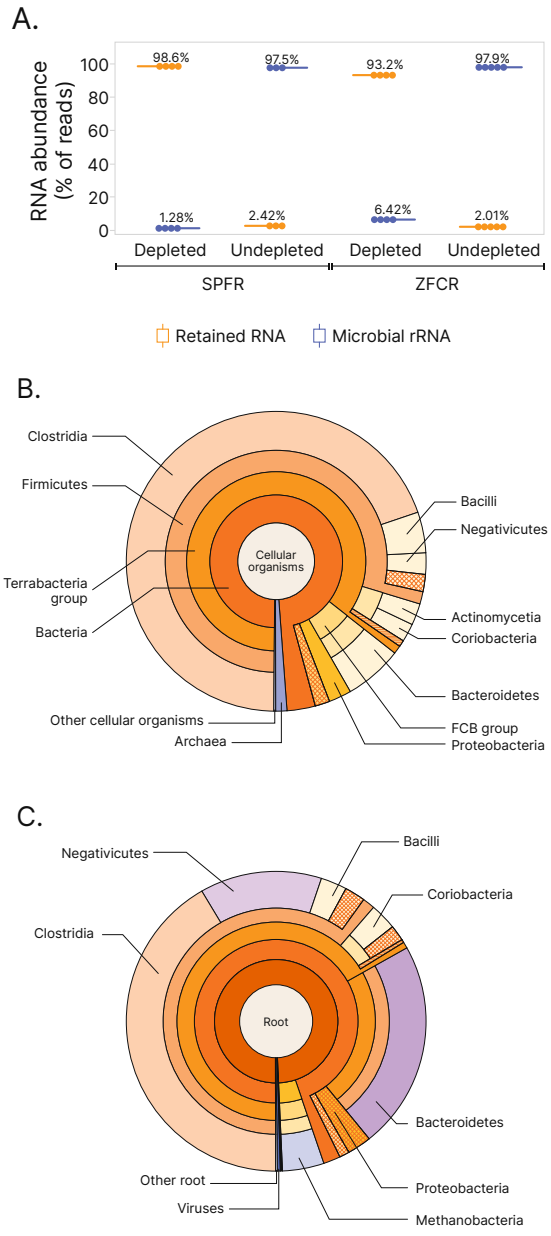


Figure 3: Effective depletion of microbiome RNA using the Ribo-Zero Plus Microbiome Depletion Kit

(A) Percentage of reads that align to microbial rRNA (blue) or retained non-rRNA microbial transcripts (orange) in depleted and undepleted samples: 100 ng stool pool fecal RNA (SPFR) collected from six donors, and 100 ng ZymoBIOMICS Fecal Reference RNA (ZFCR) (Zymo Research, Catalog no. D6323). Kronographs of microbial species detected in depleted samples: (B) SPFR and (C) ZFCR. Data analysis generated using Microbiome Metatranscriptomics app v1.0.2.

High-quality data across input amounts

Illumina Rapid Total RNA Prep is optimized for performance across a wide input range from 1 ng to 1000 ng high-quality RNA for high-confidence gene expression studies (Figure 4, Figure 5, Figure 6). For traditional RNA-Seq workflows, clean-up steps following cDNA synthesis or ligation may lose a significant amount of the sample library. Illumina Rapid Total RNA Prep eliminates those clean-up steps, resulting in more diverse and complex libraries for detection of low-abundance transcripts.

Illumina Rapid Total RNA Prep demonstrates excellent library complexity with high detection of unique transcripts and low duplicate rates‡ across coverage depths and inputs (Figure 4). Data from Illumina Rapid Total RNA Prep shows high mapping efficiency and robust coverage uniformity (Figure 5). The CV for coverage§ was 0.7 for all samples. High-accuracy, uniform coverage across complete transcripts is critical for sensitive applications such as alternative splicing and isoform analysis. Gene expression profiles show high concordance between RNA input amounts and among technical replicates (Figure 6). Illumina Rapid Total RNA Prep produces high-quality data from partially degraded samples (Figure 7) and can detect known oncogene fusions important for cancer research (Table 2).

Comprehensive RNA-Seq solution

Illumina Rapid Total RNA Prep is part of an RNA-to-answer solution for whole-transcriptome analysis that includes Illumina sequencing systems, DRAGEN™ RNA and Differential Expression secondary analysis pipelines, and data visualization and discovery with Illumina Connected Multiomics (Figure 1).

The DRAGEN RNA app enables analysis of both stranded and nonstranded RNA library preps, resulting in high-accuracy alignment and fast analysis time for total RNA-Seq projects. DRAGEN RNA outputs critical RNA-Seq metrics for assessing library prep quality and sample complexity. The DRAGEN RNA app also provides normalized gene expression and transcript quantification¶ for differential analysis pipelines.

‡ Duplicates, a normal byproduct of RNA library prep and sequencing, are included in differential gene expression analysis, but can be excluded for more accurate quantification of gene fusions, splice variants, and SNVs.

§ Coefficient of variation (CV) is the standard deviation divided by mean coverage of the 1000 most highly expressed transcripts.

¶ Gene expression normalization uses transcripts per million (TPM), a measure of relative transcript abundance across samples and replicates.

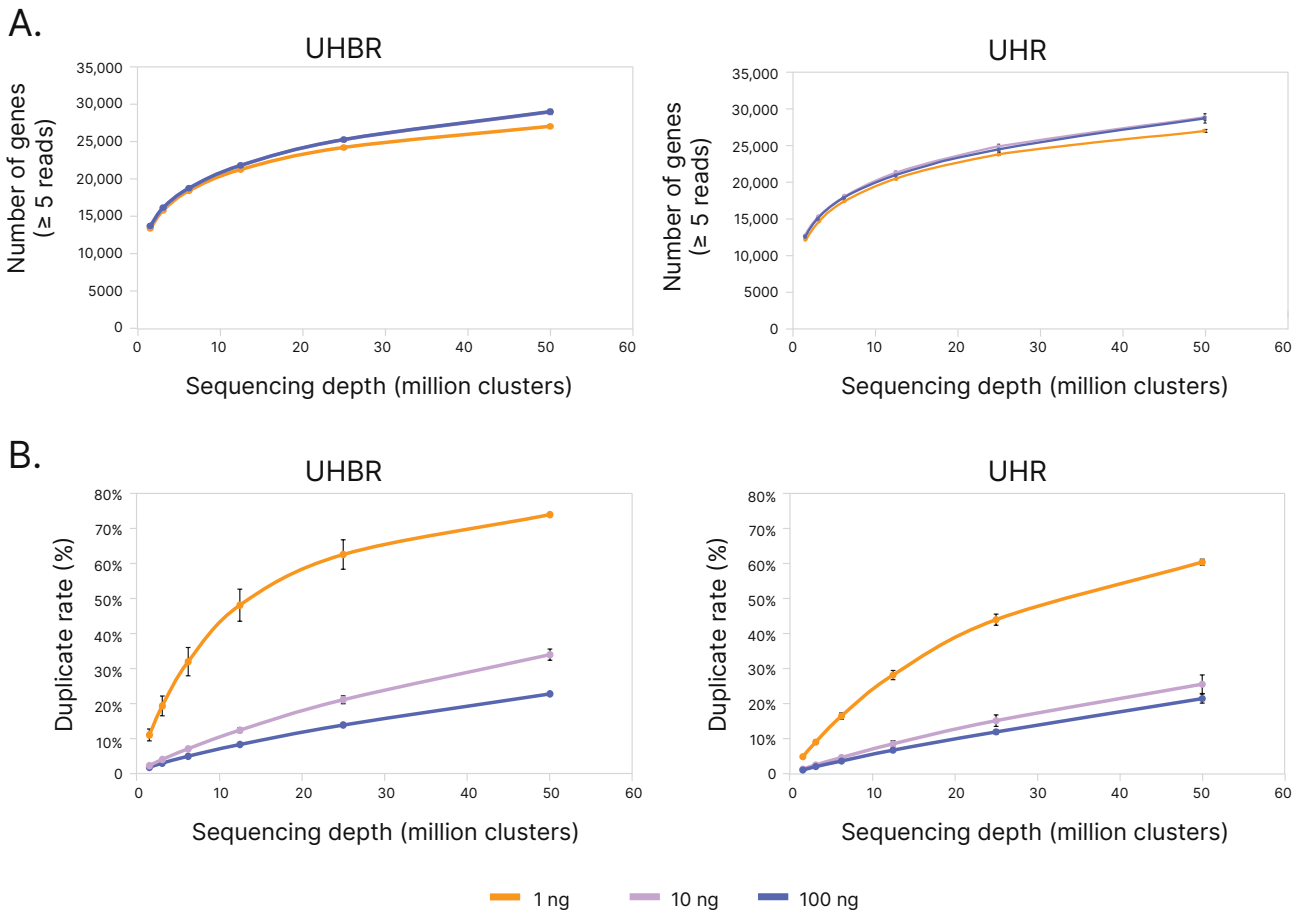


Figure 4: Illumina Rapid Total RNA Prep produces high-complexity libraries across input amounts

Total RNA-Seq data from 1 ng, 10 ng, and 100 ng of universal human brain reference (UHBR) RNA and universal human reference (UHR) RNA shows (A) similar numbers of unique genes detected (defined as having ≥ 5 mapped reads) and (B) low duplicate rates across sequence depths downsampled to 1M–50M clusters (2M–100M paired-end reads). Low-input samples are inherently lower complexity and show higher relative duplicate rates. Despite the 1 ng inputs showing higher duplicates as expected, the number of genes detected is consistent with the higher inputs. Because UHR material is from a mix of 10 diverse cell types,[1] it is harder to achieve saturation of unique gene expression. For most cells and tissue types, 20M to 40M clusters should be sufficient. Traditionally, the recommended sequence depth for total RNA-Seq applications is 50M clusters.

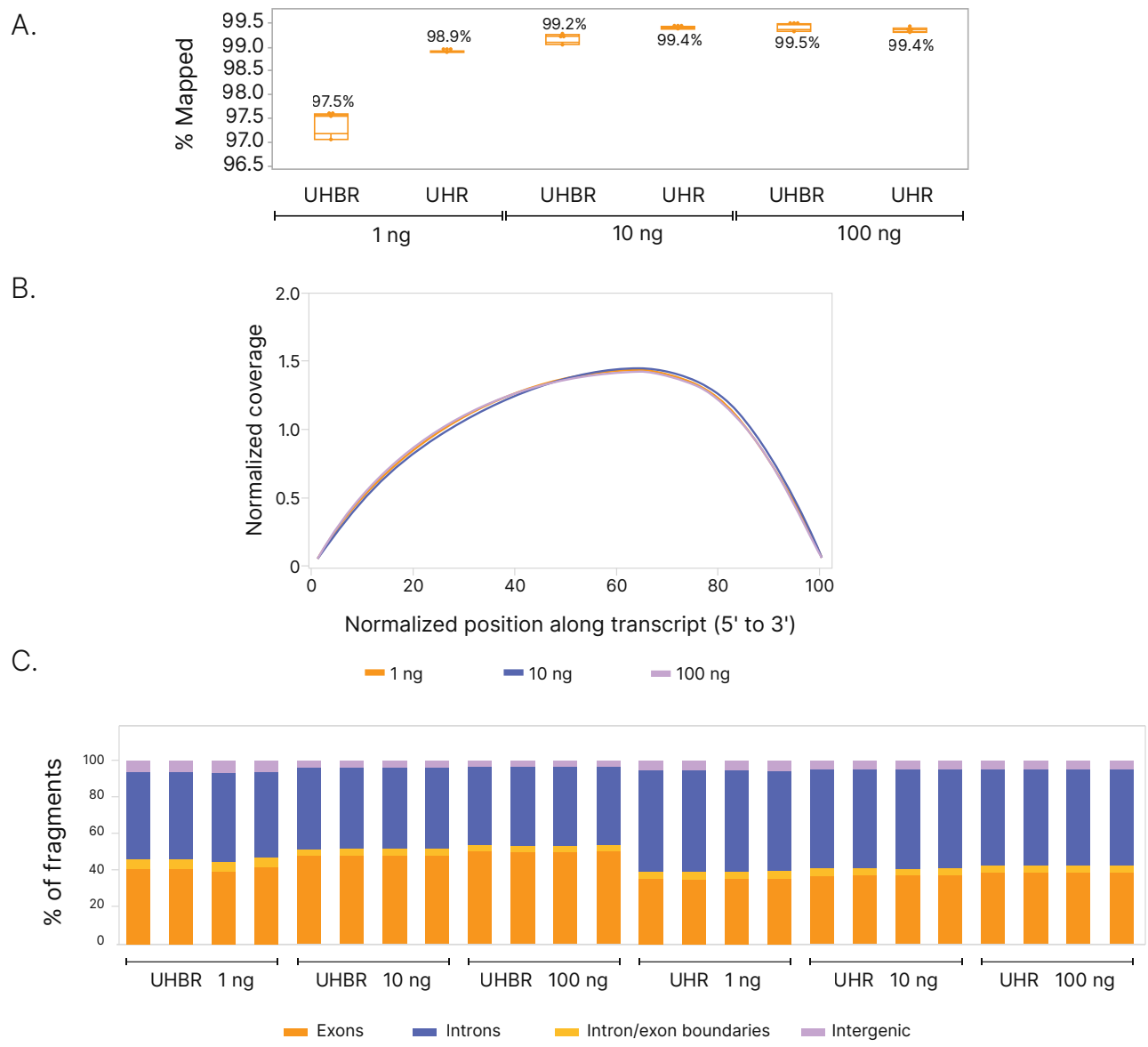


Figure 5: Illumina Rapid Total RNA Prep shows high coverage uniformity across input amounts

Total RNA-Seq data from 1 ng, 10 ng, and 100 ng of universal human brain reference (UHBR) RNA and universal human reference (UHR) RNA shows (A) high percentage of mapped reads and (B) consistent normalized coverage distributed along the length of transcripts. (C) Read alignment to transcript regions: Exons (orange), introns (blue), intron/exon boundaries (yellow), and intergenic regions (purple). Exon reads include reads mapping to forward, reverse, mismatch, or both orientations. Intron reads map to a gene but do not overlap exons. Intron/exon boundary reads overlap an exon but do not match a known transcript, representing reads crossing splice junctions or novel transcript splice sites. Intergenic reads do not overlap with any gene. The percentage of reads mapping to exons increased slightly with greater input for both UBHR and UHR samples. Data generated by DRAGEN RNA v4.4.

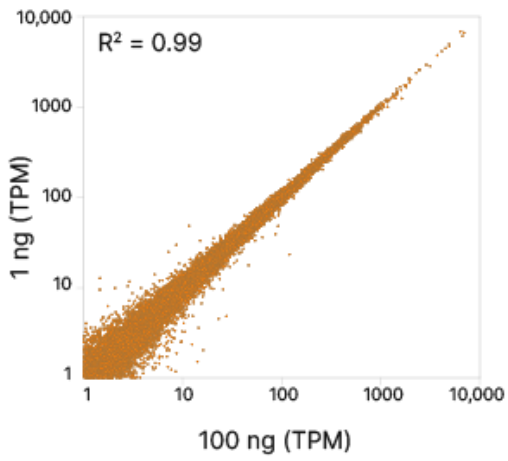


Figure 6: Illumina Rapid Total RNA Prep achieves high reproducibility
Total RNA-Seq data from 10 ng and 100 ng of high-quality universal human reference (UHR) RNA shows high correlation of gene expression (R^2 = 0.99). Transcripts per million (TPM) generated by DRAGEN RNA v4.4.

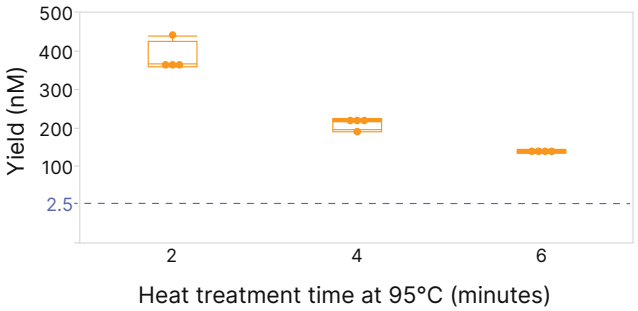


Figure 7: Illumina Rapid Total RNA Prep produces high-quality data even with partially degraded samples
RNA samples were heat treated for 2 min, 4 min, or 6 min resulting in increasing amounts of fragmentation. Following heat treatment, libraries were prepared and the yields were measured using a Qubit instrument. Regardless of fragmentation time, the library yields were far above 2.5 nM (blue dotted line), the minimum amount needed for two rounds of the denature and dilute protocol before loading the NovaSeq X instrument.

Table 2: Illumina Rapid Total RNA Prep detects T-cell receptor and cancer cell line gene fusions in UHR[a]

UHR gene fusion detected	Total passing fusion detection for 12 replicates
BCAS4-BCAS3[b]	12
TRBV10-3-TRBJ2-5[c]	12
TRBV20OR9-2-TRBJ2-1[c]	12
TRBV20-1-TRBJ2-1[c]	9
GAS6-RASA3	6
NSD3-FGFR1[d]	2
ARFGEF2-SULF2[b]	2
MLLT10-MALRD1[e]	2
MLLT10-PICALM[e]	1
SULF2-PRICKLE2[b]	1
DEPDC1B-ELOVL7[b]	1

a. Universal Human Reference RNA (Agilent, Catalog no. 740000) is a mix of 10 cancer cell lines.[1] Passing fusions detected by the DRAGEN RNA app v4.4 from 1–100 ng UHR input sequenced at high depth (≥ 30M clusters).
b. Fusion associated with breast cancer (MCF7 cell line).
c. T-cell receptor (TCR) beta chain gene.
d. Fusion associated with T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML).
e. Fusion associated with lung squamous cell carcinoma (LUSC) and Merkel cell carcinoma (MCC).

Summary

Obtain fast and accurate whole-transcriptome results for well-annotated genomes with Illumina Rapid Total RNA Prep. Removing rRNAs and globin RNAs facilitates rich transcriptome analysis. Combining cDNA synthesis and on-bead tagmentation in a single step reduces turnaround time by half, enabling high-quality total RNA-Seq with an efficient workflow.

Learn more →

Illumina Rapid Total RNA Prep

DRAGEN RNA

DRAGEN Differential Expression

Illumina Connected Multiomics

References

1. Gong B, Li D, Łabaj PP, et al. Targeted DNA-Seq and RNA-Seq of reference samples with short-read and long-read sequencing. *Sci Data*. 2024;11(1):892. doi:10.1038/s41597-024-03741-y

Ordering information

Product	Catalog no.
Illumina Rapid RNA Prep, (S) Tagmentation (16 samples)[a]	20158823
Illumina Rapid RNA Prep, (S) Tagmentation (96 samples)[a]	20158824
Ribo-Zero Plus rRNA Depletion Kit (16 samples)[a]	20040526
Ribo-Zero Plus rRNA Depletion Kit (96 samples)[a]	20037135
Ribo-Zero Plus Microbiome Depletion Kit (96 samples)[a]	20072062
Illumina DNA/RNA UD Index Set A, Tagmentation (96 indexes, 96 samples)	20091654
Illumina DNA/RNA UD Index Set B, Tagmentation (96 indexes, 96 samples)	20091656
Illumina DNA/RNA UD Index Set C, Tagmentation (96 indexes, 96 samples)	20091658
Illumina DNA/RNA UD Index Set D, Tagmentation (96 indexes, 96 samples)	20091660

a. For Illumina Rapid Total RNA Prep, the Illumina Rapid RNA Prep and Ribo-Zero Plus rRNA Depletion Kit (or Ribo-Zero Plus Microbiome Depletion Kit) are sold separately.

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