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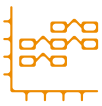
DATA SHEET

Illumina Rapid mRNA Prep

Fast and easy workflow for coding transcriptome analysis

Three-hour library prep, including poly(A) capture, with under 1.5 hours hands-on time

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



High-quality nonstranded data for mRNA-Seq applications and a wide range of input amounts

Fast and accurate mRNA-Seq

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for precise profiling of RNA transcripts—the dynamic indicators of cell types and states. Messenger RNA (mRNA)-Seq provides a comprehensive and quantitative readout of the coding transcriptome, with sensitive detection of isoforms, gene fusions, and allele-specific expression. However, mRNA-Seq library preparation can be long and labor-intensive, limiting throughput.

Illumina Rapid mRNA Prep offers a fast and simple tagmentation-based workflow that supports low sample inputs. This streamlined method delivers high-accuracy nonstranded data for well-annotated genomes,* ideal for mRNA-Seq applications such as:

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

Save time with a streamlined workflow

Illumina Rapid mRNA Prep offers the fastest and easiest mRNA-Seq library prep solution in the Illumina portfolio, reducing turnaround time and hands-on time by half compared with Illumina Stranded mRNA Prep (Table 1). Perform capture of polyadenylated (poly(A)) mRNA with the Illumina mRNA Capture Kit. Then, the Illumina Rapid RNA Prep kit uses a streamlined, 30-minute reaction that combines cDNA synthesis and on-bead tagmentation in a single step (Figure 1). 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 three hours to generate a sequencing-ready mRNA library, helping researchers get answers more quickly. Fewer pipetting steps with less than 1.5 hours hands-on time reduces opportunities for error, resulting in reproducible data.

* 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 mRNA Prep. Illumina Stranded mRNA Prep is a ligation-based library prep kit for accurate, unbiased mRNA-Seq with precise measurement of strand information.

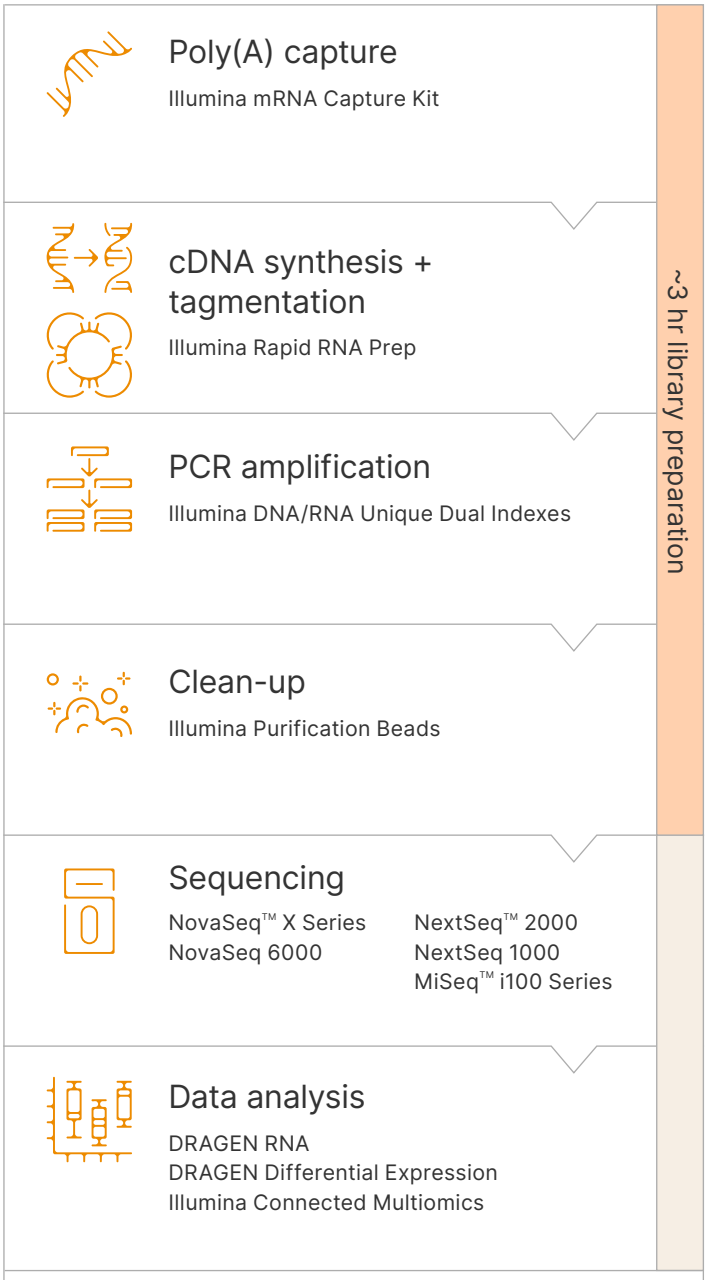


Figure 1: Illumina Rapid mRNA Prep delivers a fast library preparation workflow with reduced hands-on time
The Illumina Rapid mRNA Prep workflow starts with poly(A) capture using poly(dT) magnetic beads with the Illumina mRNA Capture Kit. Next, Illumina Rapid RNA Prep uses a single-tube reaction of cDNA 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.

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

	Illumina Stranded mRNA Prep	Illumina Rapid mRNA Prep[a]
Total turnaround time	6.5 hr	~3 hr
Hands-on time	< 3 hr	< 1.5 hr
Clean-up steps	3	1
RNA input amount	25–1000 ng	1–1000 ng
Library prep method	Ligation	Tagmentation
Strand specificity	Stranded	Nonstranded

a. For Illumina Rapid mRNA Prep, the Illumina Rapid RNA Prep and Illumina mRNA Capture Kit are sold separately.

High-quality gene expression data across input amounts

Illumina Rapid mRNA Prep is optimized for performance across a wide input range from 1 ng to 1000 ng of high-quality RNA† (Figure 2, Figure 3, Figure 4). For traditional RNA-Seq workflows, clean-up steps following cDNA synthesis or ligation may lose a significant amount of the sample library. Illumina Rapid mRNA Prep eliminates those clean-up steps, resulting in more diverse and complex mRNA libraries for detection of low-abundance transcripts.

Illumina Rapid mRNA Prep demonstrates excellent library complexity with high detection of unique transcripts and low duplicate rates across coverage depths and inputs (Figure 2). The Illumina mRNA Capture Kit achieves efficient purification of poly(A) RNA with over 90% of reads mapped to known transcripts and less than 5% mapped to abundant ribosomal RNA or mitochondrial RNA (data not shown). Data from Illumina Rapid mRNA Prep shows high mapping efficiency and coverage uniformity (Figure 3). High-accuracy, uniform coverage across complete transcripts is critical for sensitive applications such as alternative splicing and isoform analysis. Gene expression profiles also show high consistency between high and low RNA input amounts and concordance among technical replicates (Figure 4).

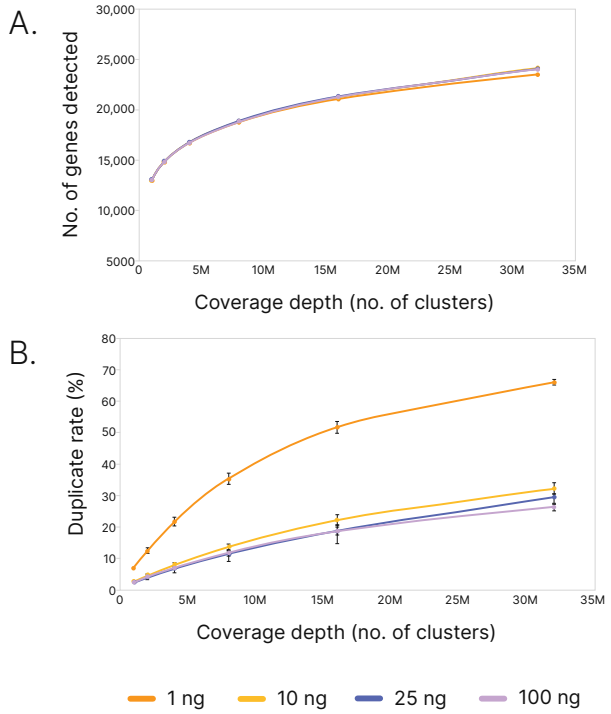


Figure 2: Illumina Rapid mRNA Prep library complexity across input amounts

mRNA-Seq data from 1 ng, 10 ng, 25 ng, and 100 ng of universal human reference (UHR) RNA shows (A) the number of unique genes detected, defined as having ≥ 5 mapped reads, and (B) the percentage of duplicates, across sequence depths downsampled to 1M–32M clusters or library fragments (2M–64M paired-end reads). Despite the 1 ng input showing higher duplicates, the number of genes detected is consistent with the higher inputs. Because UHR material is from a mix of 10 diverse cell types, it is harder to achieve saturation of unique gene expression. For most cells and tissue types, 10M to 20M clusters should be sufficient. Traditionally, the recommended sequence depth for mRNA-Seq applications is 25M clusters.

† Illumina Rapid RNA Prep for mRNA-Seq is not compatible with formalin-fixed, paraffin-embedded (FFPE) samples.

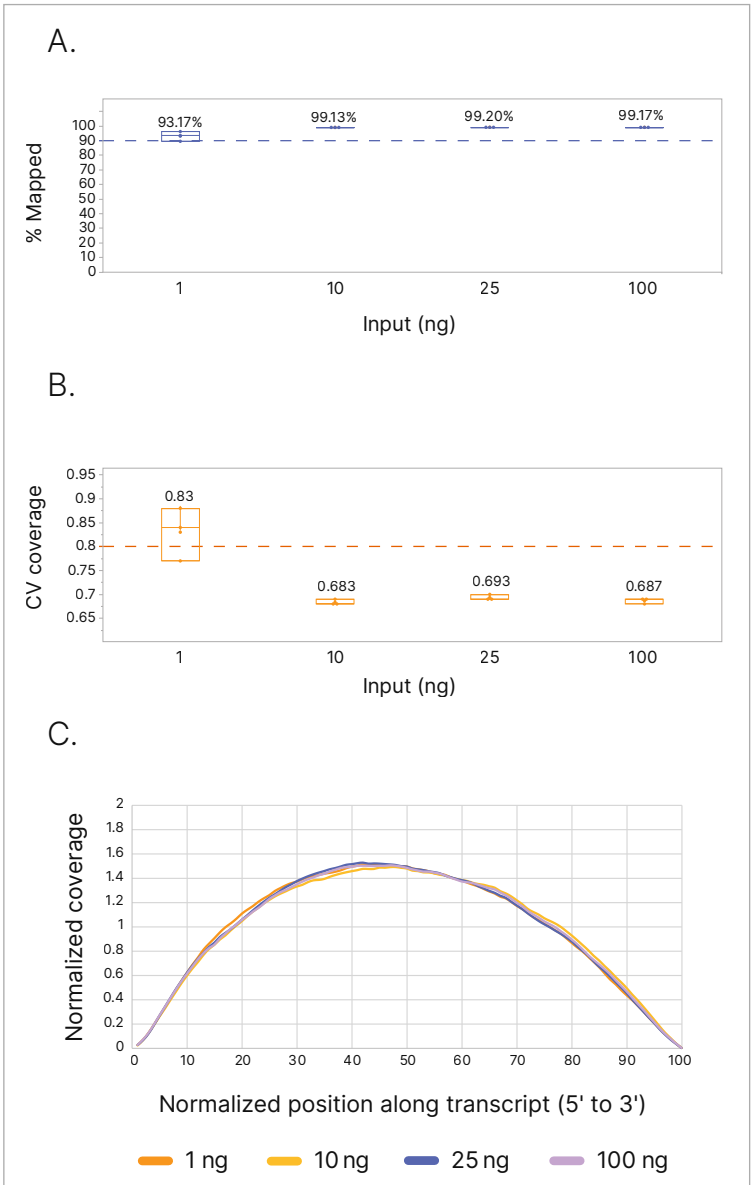


Figure 3: Illumina Rapid mRNA Prep coverage uniformity across input amounts

mRNA-Seq data from 1 ng, 10 ng, 25 ng, and 100 ng of universal human reference (UHR) RNA, downsampled to 20M clusters (40M paired-end reads) shows (A) high percentage of mapped reads (≥ 99% for inputs ≥ 10 ng) and (B) low median coefficient of variation (CV) for coverage across transcripts. CV is the standard deviation divided by mean coverage of the 1000 most highly expressed transcripts. Only the 1 ng data is exceeding the CV recommended upper limit of 0.8 (orange dotted line). (C) Consistent normalized coverage distributed along the length of transcripts.

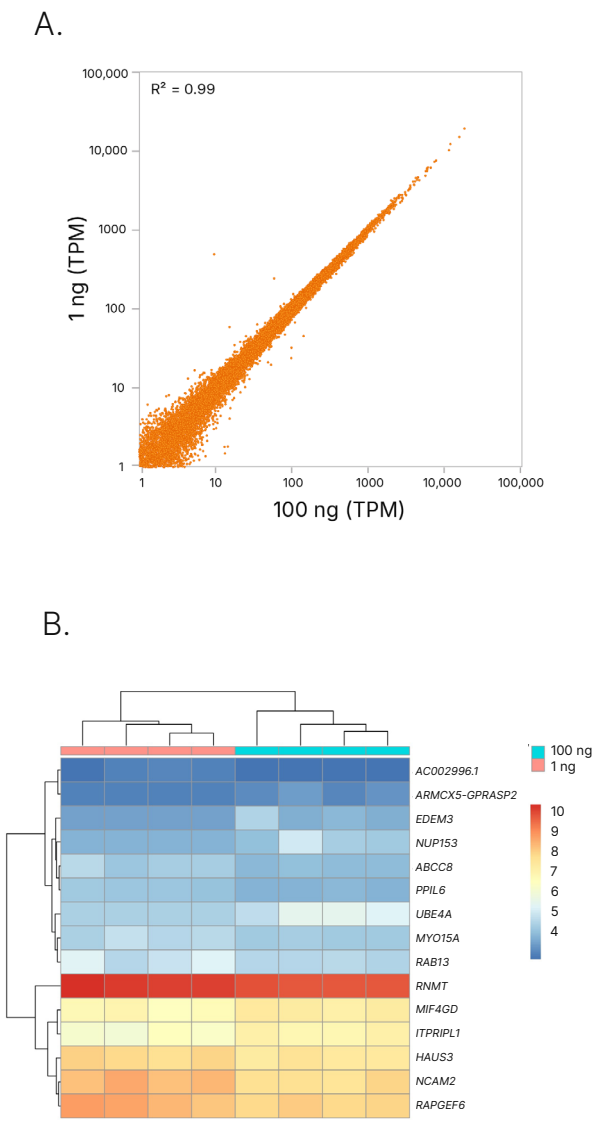


Figure 4: Illumina Rapid mRNA Prep achieves high reproducibility across high and low inputs

mRNA-Seq data from 1 ng and 100 ng of input universal human reference (UHR) RNA, downsampled to 20M clusters (40M paired-end reads) shows (A) correlation of normalized gene counts between technical replicates and input amounts (R^2 = 0.99). Transcripts per million (TPM) generated by DRAGEN RNA v4.4. (B) Gene expression heat map output from the DRAGEN Differential Expression pipeline shows the top 15 differentially expressed genes between 1 ng and 100 ng inputs, with highly similar profiles.

Comprehensive mRNA-Seq solution

Illumina Rapid mRNA Prep is part of a comprehensive solution for coding 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 mRNA-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.

Summary

Obtain fast and accurate gene expression results for well-annotated genomes with Illumina Rapid mRNA Prep. Poly(A) capture followed by cDNA synthesis and on-bead tagmentation in a single step reduces turnaround time by half, enabling high-quality mRNA-Seq with an efficient RNA-to-answer workflow.

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

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
Illumina mRNA Capture Kit (16 samples)[a]	20040893
Illumina mRNA Capture Kit (96 samples)[a]	20040894
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 mRNA Prep, the Illumina Rapid RNA Prep and Illumina mRNA Capture Kit are sold separately.

Learn more →

Illumina Rapid mRNA Prep

DRAGEN RNA

DRAGEN Differential Expression

Illumina Connected Multiomics

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