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DATA SHEET

Illumina Rapid RNA Prep with Exome 2.5 Enrichment

Optimized RNA exome capture sequencing

Rapid workflow that combines cDNA synthesis and adapter tagging in a single-tube reaction

Focused coverage of the coding exome using a comprehensive and up-to-date enrichment panel

High-quality data from low-input or degraded samples, including FFPE tissue

Rapid and focused RNA exome sequencing

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for precise profiling of RNA transcripts, providing a quantitative readout of isoforms, gene fusions, and allele-specific expression. RNA-Seq with exome enrichment targets specific gene sequences, instead of polyadenylated messenger RNA (poly(A) mRNA), to capture the coding transcriptome. Because it does not rely on the presence of a poly(A) tail, RNA exome capture is ideal for RNA-Seq with low-quality samples or limited starting material.

Illumina Rapid RNA Prep with Exome 2.5 Enrichment offers a fast and easy tagmentation-based workflow and an optimized enrichment panel for focused coverage of the RNA exome. This streamlined method achieves high-quality data from degraded samples, including formalin-fixed, paraffin-embedded (FFPE) tissues, and is ideal for RNA-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 RNA Prep with Exome 2.5 Enrichment offers a fast and easy workflow to prepare exome-enriched RNA libraries for sequencing (Figure 1).* First, a streamlined, 30-minute reaction combines cDNA synthesis and on-bead tagmentation in a single step, and indexes are added with PCR. Then enrichment with the included Twist Bioscience for Illumina Exome 2.5 Panel[1] can be done in a 90-minute hybridization step, completing the workflow in as little as 6.5 hours with less than 1.5 hours hands-on time. This approach has fewer pipetting and purification steps compared to traditional protocols, reducing opportunities for error and resulting in reproducible data.

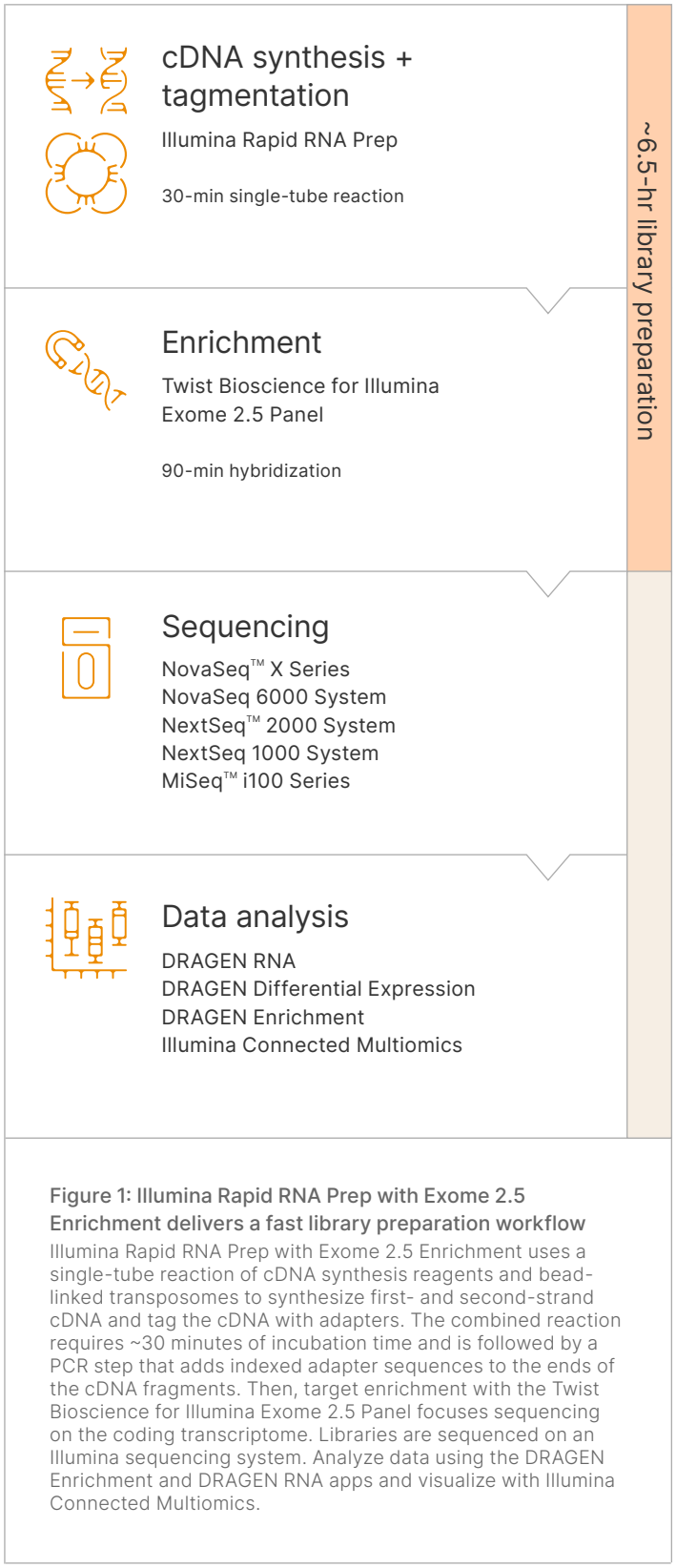

Figure 1: Illumina Rapid RNA Prep with Exome 2.5 Enrichment delivers a fast library preparation workflow
Illumina Rapid RNA Prep with Exome 2.5 Enrichment uses a single-tube reaction of cDNA synthesis reagents and bead-linked transposomes to synthesize first- and second-strand cDNA and tag the cDNA with 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. Then, target enrichment with the Twist Bioscience for Illumina Exome 2.5 Panel focuses sequencing on the coding transcriptome. Libraries are sequenced on an Illumina sequencing system. Analyze data using the DRAGEN Enrichment and DRAGEN RNA apps and visualize with Illumina Connected Multiomics.

* Illumina Rapid RNA Prep with Exome 2.5 Enrichment includes the Illumina Rapid RNA Prep kit, the Twist Bioscience for Illumina Exome 2.5 Panel, hybridization reagents, and Illumina Purification Beads.

Illumina Rapid RNA Prep with Exome 2.5 Enrichment is part of a comprehensive RNA sequencing and analysis workflow that includes Illumina sequencing systems, DRAGEN™ RNA apps for secondary analysis, and Illumina Connected Multiomics for biological interpretation and visualization (Figure 1).

Focused exome coverage

High capture efficiency and coverage uniformity minimize the required sequencing depth to determine gene expression levels accurately and without bias. Illumina Rapid RNA Prep with Exome 2.5 Enrichment includes the Twist Bioscience for Illumina Exome 2.5 Panel, a focused, up-to-date probe set that delivers comprehensive coverage of coding RNA sequences, especially for variants reported in public databases (Table 1). Illumina Rapid RNA Prep with Exome 2.5 Enrichment produces exceptional exonic coverage with over 98% on-target reads (~87% of bases align to coding sequence and ~11% align to reads crossing intron/exon boundaries in prespliced transcripts) (Figure 2).

Highly accurate data from low-input and FFPE samples

Illumina Rapid RNA Prep with Exome 2.5 Enrichment is optimized for performance across a wide range of RNA input amounts and sample types (Figure 2, Figure 3, Figure 4). Targeted enrichment of the coding RNA exome with 1 ng to 100 ng universal human reference (UHR) RNA and 25 ng FFPE tumor fusion RNA reference and fusion RNA mix samples† shows high aligned reads and target coverage (Figure 3).‡ Both short (90 minutes) and long (12 hours) hybridization times yielded equivalent results with high reproducibility ($R^2 > 0.96$) (Figure 2, Figure 3), supporting flexibility to complete library prep with time to start sequencing on the same day. Gene expression profiles show high consistency between high and low RNA input amounts and concordance among technical replicates (Figure 4). Illumina Rapid RNA Prep with Exome 2.5 Enrichment also allows high-confidence fusion gene detection in FFPE reference samples (Table 2) and sensitive variant detection (Table 3).

† Seraseq FFPE Tumor Fusion RNA Reference Material v2 (SeraCare, Catalog no. 0710-0129) and Seraseq Fusion RNA Mix v2 (SeraCare, Catalog no. 0710-0127).
‡ DRAGEN RNA does not output data for mean target coverage. Picard metrics option is only available with DRAGEN Enrichment v4.3.13 (not v4.4.4).

Table 1: Coverage of coding regions represented in key databases with the Twist Bioscience for Illumina Exome 2.5 Panel

Reference databases	Exome panel	Twist Bioscience for Illumina Exome 2.5 Panel	Illumina Exome Panel
	Size	37.5 Mb	45 Mb
RefSeq CDS[2]		99.1%	98.2%
CCDS CDS[3]		99.9%	99.5%
ACMG 73 genes CDS[4]		99.9%	99.3%
COSMIC Cancer Gene Census CDS[5,6]		99.9%	99.3%
OMIM[7]		99.1%	97.7%

RefSeq, Reference Sequence database; CDS, coding sequence; CCDS, Consensus CDS database; ACMG, American College of Medical Genetics and Genomics; COSMIC, Catalog of Somatic Mutations in Cancer; OMIM, Online Mendelian Inheritance in Man.

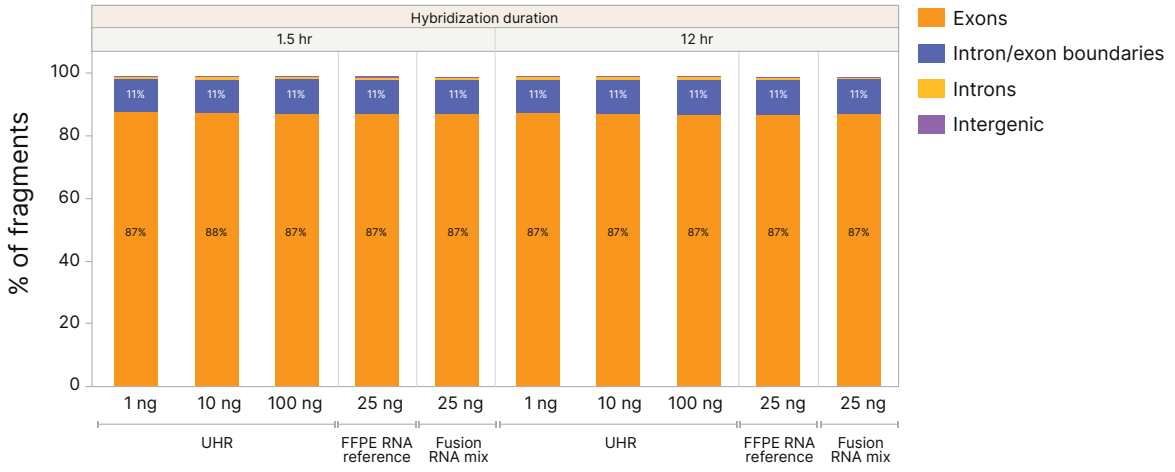

Figure 2: Efficient enrichment of coding transcripts using Illumina Rapid RNA Prep with Exome 2.5 Enrichment

Mapping metrics for exome-enriched RNA-Seq data show high on-target rates across a range of inputs: 1 ng, 10 ng, and 100 ng universal human reference (UHR) RNA and 25 ng each of commercial samples: Seraseq FFPE Tumor Fusion RNA Reference Material v2 (FFPE RNA reference) and Seraseq Fusion RNA Mix v2 (Fusion RNA mix). For both short and long hybridization times, alignment is ~87% to exons (orange) and ~11% to reads overlapping intron/exon boundaries (blue), with less than 2% alignment to introns (yellow) and intergenic regions (purple). Exome-enriched RNA-Seq libraries were sequenced on the NovaSeq 6000 System, S4 flow cell, 2 × 101 bp read length, and data downsampled to 25M clusters (50M paired-end reads). Data generated with DRAGEN RNA v4.4.6.

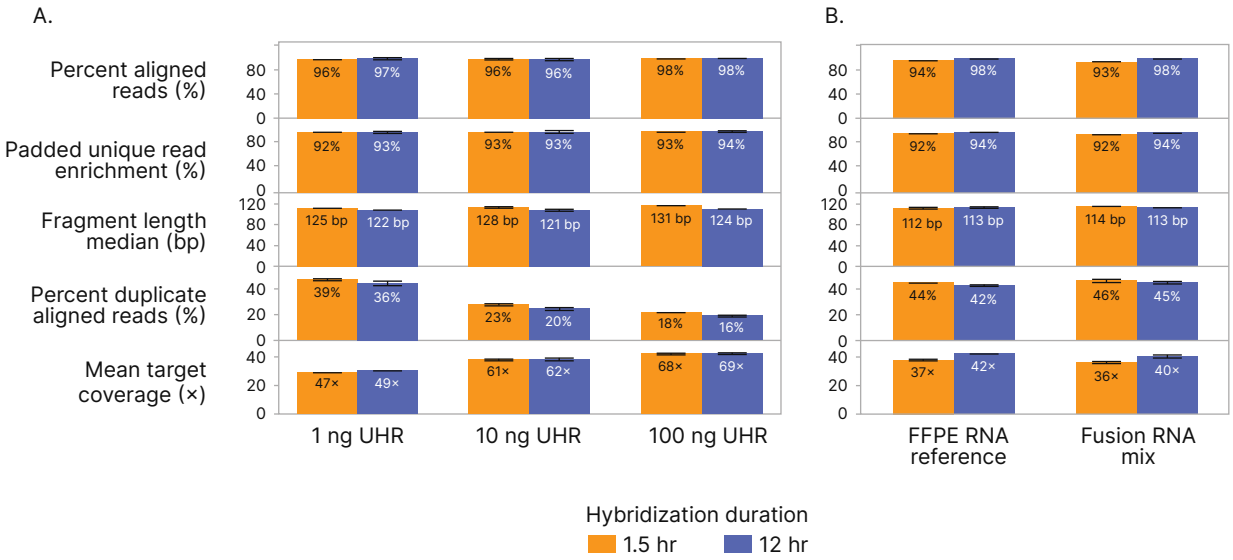

Figure 3: Illumina Rapid RNA with Exome 2.5 Enrichment shows equivalent enrichment metrics for long and short hybridization times

Percent aligned reads, padded unique read enrichment (PURE), median fragment length, duplicate rate, and mean target coverage for hybridization durations of 90 min (orange) and 12 hr (blue) across a range of inputs: (A) 1 ng, 10 ng, and 100 ng universal human reference (UHR) RNA and (B) 25 ng each of commercial samples: Seraseq FFPE Tumor Fusion RNA Reference Material v2 (FFPE RNA reference) and Seraseq Fusion RNA Mix v2 (Fusion RNA mix). Exome-enriched RNA-Seq libraries were sequenced on the NovaSeq 6000 System, S4 flow cell, 2 × 101 bp read length, and data downsampled to 25M clusters (50M paired-end reads). Picard enrichment metrics generated by DRAGEN Enrichment v4.3.13. The DRAGEN Enrichment app removes duplicates when calculating mean target coverage. Low-input samples inherently have lower library complexity with higher duplicate rates. FFPE samples are more degraded and produce less complex libraries than high-quality intact RNA. While PURE is similar across RNA inputs, mean target coverage appears lower in the 1 ng UHR and the FFPE samples because of the higher duplicate rates.

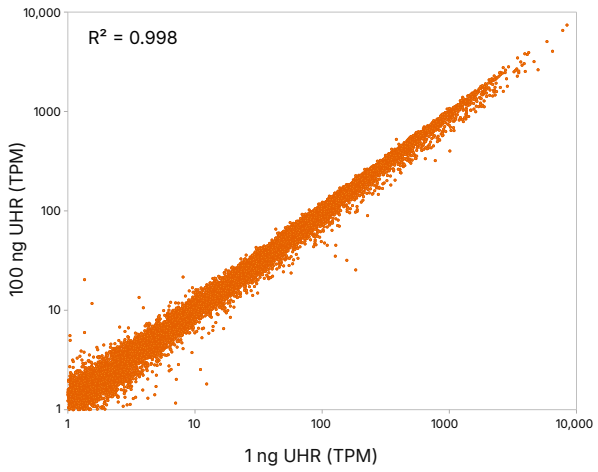

Figure 4: Illumina Rapid RNA Prep with Exome 2.5 Enrichment achieves high reproducibility across high and low inputs

Exome-enriched RNA-Seq data from 1 ng and 100 ng of input universal human reference (UHR) RNA downsampled to 25M clusters (50M paired-end reads) shows strong correlation of normalized gene counts between technical replicates and input amounts (R^2 = 0.998). Transcripts per million (TPM) generated by DRAGEN RNA v4.4.6.

Table 2: High reproducibility of detection for fusion genes covered by Illumina Rapid RNA Prep with Exome 2.5 Enrichment[a]

Gene fusions	No. of times detected across replicates	
	FFPE RNA reference[b]	Fusion RNA mix[b]
EML4-ALK	9/9	9/9
KIF5B-RET	9/9	9/9
NCOA4-RET	7/9	8/9
CD74-ROS1	8/9	7/9
SLC34A2-ROS1	8/9	8/9
TPM3-NTRK1	8/9	8/9
FGFR3-BAIAP2L1	8/9	9/9
PAX8-PPARG	9/9	9/9
FGFR3-TACC3	8/9	8/9
ETV6-NTRK3	9/9	7/9
LMNA-NTRK1	9/9	9/9
SLC45A3-BRAF	7/9	4/9
EGFR-SEPTIN14	6/9	8/9

a. Exome-enriched RNA-Seq libraries were sequenced on the NovaSeq 6000 System, S4 flow cell, 2 × 101 bp read length, and data downsampled to 25M clusters (50M paired-end reads). Gene fusion detection data generated with DRAGEN RNA v4.4.6.

b. FFPE RNA reference, Seraseq FFPE Tumor Fusion RNA Reference Material v2; Fusion RNA mix, Seraseq Fusion RNA Mix v2.

Table 3: Variant detection with Illumina Rapid RNA Prep with Exome 2.5 Enrichment

	UHR		FFPE RNA reference		Fusion RNA mix	
	SNVs	Indels	SNVs	Indels	SNVs	Indels
Total variants[a]	27,730	3818	15,683	3475	15,809	3703
Standard deviation[b]	173	79	485	127	150	55

a. Number of variants identified using DRAGEN Enrichment v4.3.13.

b. Standard deviation of nine replicates of each sample.

UHR, universal human reference RNA; FFPE RNA reference, Seraseq FFPE Tumor Fusion RNA Reference Material v2; Fusion RNA mix, Seraseq Fusion RNA Mix v2; SNVs, single nucleotide variants; indels, insertions-deletions.

Summary

Illumina Rapid RNA Prep with Exome 2.5 Enrichment offers an optimized solution for RNA exome capture sequencing. The streamlined workflow combines cDNA synthesis and on-bead tagmentation in a single step, reducing turnaround time to less than a day. A comprehensive exome panel focuses sequencing on the high value content of RNA coding regions, providing high-quality RNA-Seq data, even from low-input and degraded samples.

Learn more →

Illumina Rapid RNA Prep with Exome 2.5 Enrichment

DRAGEN RNA

DRAGEN Differential Expression

DRAGEN Enrichment

Illumina Connected Multiomics

References

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Ordering information

Product	Catalog no.
Illumina Rapid RNA Prep with Exome 2.5 Enrichment, (S) Tagmentation (96 samples)[a]	20158825
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. Illumina Rapid RNA Prep with Exome 2.5 Enrichment includes the Illumina Rapid RNA Prep kit, the Twist Bioscience for Illumina Exome 2.5 Panel, hybridization reagents, and Illumina Purification Beads.

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