DATA SHEET

Illumina Rapid RNA Prep with Enrichment plus Illumina Custom Enrichment Panel v2

Highly flexible, rapid RNA sequencing applications

Prepare libraries in 6.5 hours with less than 1.5 hours hands-on time

Generate high-quality data for RNA-Seq applications from a wide range of input amounts

Achieve high sensitivity from as low as 1 ng total RNA and using various sample types, including FFPE tissues

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Rapid RNA sequencing

RNA sequencing (RNA-Seq) with next-generation sequencing (NGS) is a powerful method for precise profiling of RNA transcripts that serve as 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 RNA Prep with Enrichment offers a fast and simple workflow that supports low sample inputs and diverse sample types. Illumina Custom Enrichment Panel v2 can be used with Illumina Rapid RNA Prep with Enrichment to provide a simple tagmentation-based workflow that delivers high-accuracy data for RNA-Seq applications such as:

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

Fast and simple RNA enrichment workflow

Illumina Rapid RNA Prep with Enrichment uses a novel and effective method to combine first- and second-strand cDNA synthesis with on-bead tagmentation in a single reaction that is followed by a single, simplified 90-minute hybridization step to create a rapid workflow (Figure 1). This approach eliminates multiple pipetting and purification steps to provide fast and effective conversion of RNA samples into indexed libraries ready for hybrid capture.

Combined with innovations to the hybridization reaction, the Rapid RNA Prep with Enrichment workflow features fewer steps, shorter incubation times, and numerous safe stopping points and a total assay time that is ~30% faster than the Illumina RNA Prep with Enrichment protocol. In addition to manual preparation, Illumina Rapid RNA Prep with Enrichment is compatible with liquid-handling platforms for an automated workflow, providing highly reproducible sample handling, reduced risk of human error, and less hands-on time.

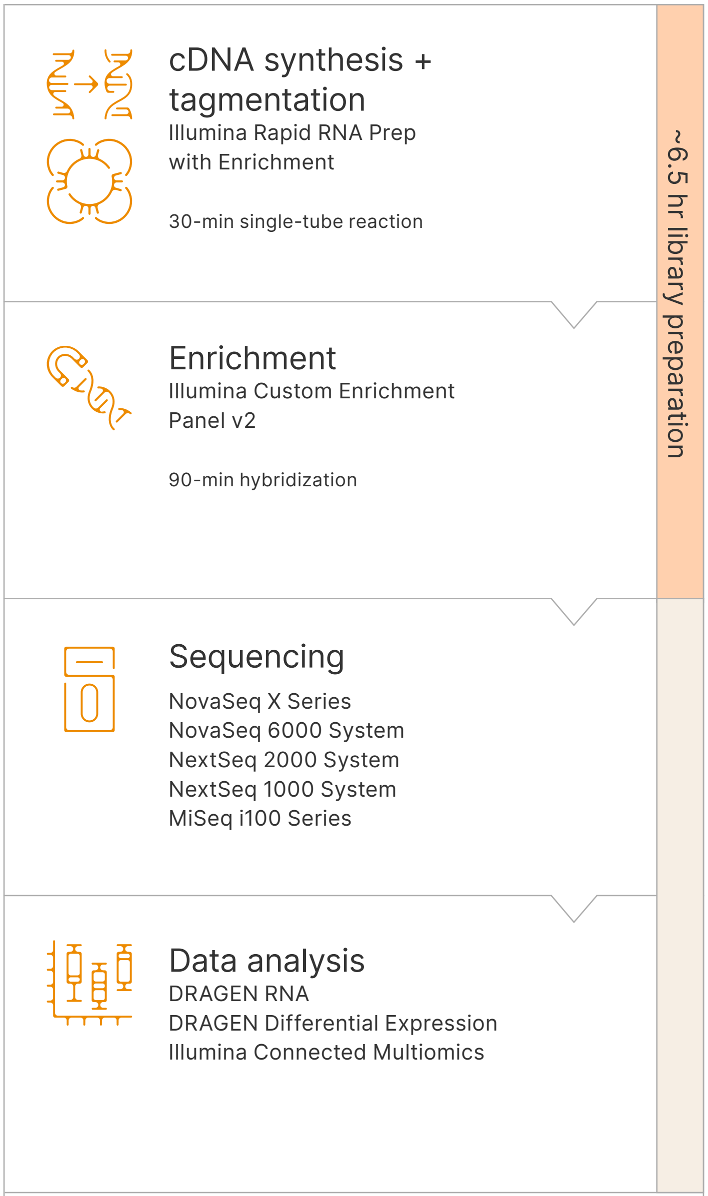

Figure 1: Illumina Rapid RNA Prep with Enrichment delivers a fast library preparation workflow

Illumina Rapid RNA Prep with 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 min 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 Illumina Custom Enrichment Panel v2 focuses sequencing on selected RNA targets. Libraries are sequenced on an Illumina sequencing system. Data is analyzed using the DRAGEN Enrichment and DRAGEN RNA apps and visualized with Illumina Connected Multiomics.

Modular design for a broad range of RNA applications

Illumina Rapid RNA Prep with Enrichment combines exceptional RNA library preparation and enrichment performance with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry[1] to support fixed and custom panels of varying sizes. This flexibility enables advanced study designs across applications such as oncology and infectious disease research, including use with degraded FFPE samples.

Illumina recommends designing enrichment panels with a minimum of 20 genes for optimal performance. Custom enrichment panels for human RNA applications can be designed using the free Illumina DesignStudio™ portal. Probes are placed in an exon-informed manner that avoids spanning exon–exon junctions, enabling the same custom panel to be used for both gene expression analysis and detection of known and novel gene splice isoforms or fusions. Design support for nonhuman content is available through the Illumina Concierge design team.

Custom panel performance and coverage requirements

To evaluate performance, four enrichment panels containing probes against 20 to 109 genes (Table 1) were used to prepare libraries from three samples: universal human reference (UHR) RNA, Seraseq Fusion RNA Mix v2 (SeraCare, Catalog no. 0710-0127), and Seraseq FFPE Tumor Fusion RNA Reference Material v2 (SeraCare, Catalog no. 0710-0129) reference samples. The resulting libraries were sequenced on a NovaSeq™ 6000 System at 1 × 151 bp; data were trimmed to 2 × 101 bp for subsequent analysis. Data analysis was performed with the DRAGEN™ RNA app and DRAGEN Enrichment app v4.3.

Illumina Rapid RNA Prep with Enrichment v2 produces uniform insert sizes (Figure 2) from both high-quality UHR samples and degraded FFPE samples, excellent padded read enrichment (Figure 3), and duplicate read performance, especially with larger panels and more diverse samples (Figure 4). High duplicate rates are expected at this coverage depth across target regions, which enables detection of low-frequency somatic variants and accounts for variable gene expression within the sample.

Illumina Custom Enrichment Panel v2 can be used to enrich for RNA variants, including gene fusions, splice variants, and single nucleotide variants (SNVs). Detection of somatic variants in formalin-fixed, paraffin-embedded (FFPE) samples with variable populations of cancer cells requires high coverage depth to detect variants present at low copy numbers.

Seraseq Fusion RNA Mix v2 and Seraseq FFPE Tumor Fusion RNA Reference Material v2 reference samples were sequenced using the four custom RNA enrichment panels. For comparisons, data were downsampled to an average target coverage of 400× using the DRAGEN FASTQ Toolkit v2.0.3 App in BaseSpace™ Sequence Hub, resulting in an average of 4000 clusters or RNA fragments per gene (Table 1).

Table 1: Custom RNA enrichment panels used for performance evaluation

Panel	No. of probes	Panel target size (bp)	Custom panel description	Downsampling for data analysis[a]	
				Clusters/ sample[b]	Clusters/ gene[b]
20-gene	792	50,850	Transcription factors and splicing factors involved in stress response	68,000	3000
42-gene	1770	128,195	Genes associated with cancer, a subset of the 79-gene and 109-gene panels	171,000	4000
79-gene	3302	247,951	Genes associated with cancer research	331,000	4000
109-gene	3794	292,896	Genes associated with cancer and fusion targets	391,000	4000

a. Achieved with downsampling to 400x-target mean coverage read depth for each panel.
b. Values are rounded to the nearest 1000 place.

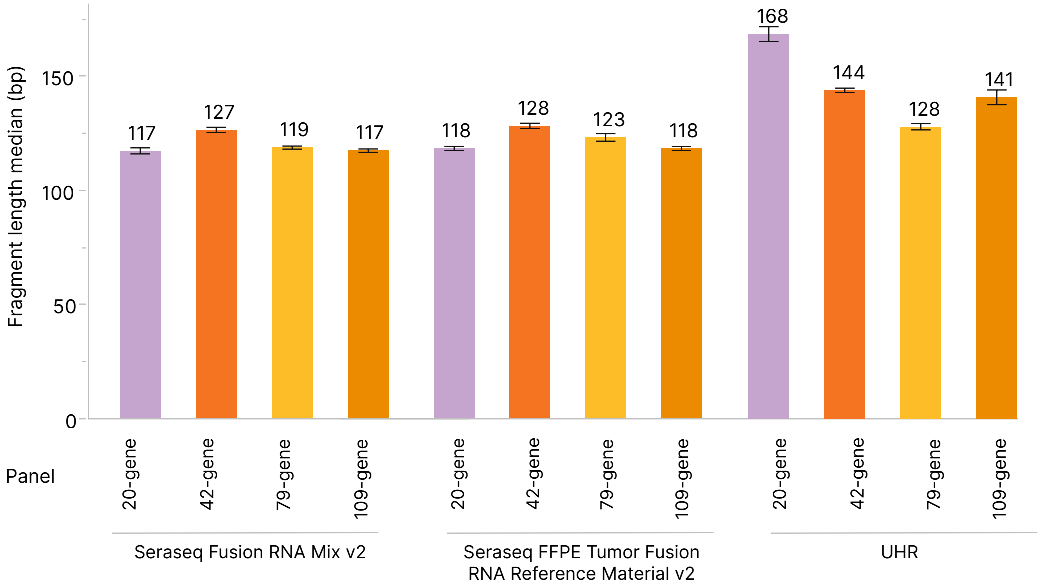

Figure 2: Illumina Rapid RNA Prep with Enrichment observed median fragment length

Four RNA enrichment panels containing 20 to 109 genes were evaluated (Table 1). Seraseq Fusion RNA Mix v2 (20 ng), Seraseq FFPE Tumor Fusion RNA Reference Material v2 (20 ng), and universal human reference (UHR, 10 ng) RNA samples were sequenced on the NovaSeq 6000 System at 2 × 151 bp paired-end reads, with data trimmed to 2 × 101 bp for DRAGEN analysis. For these samples, enrichment hybridization time was 12 hr overnight. Data analysis was performed with DRAGEN Enrichment app v4.3.

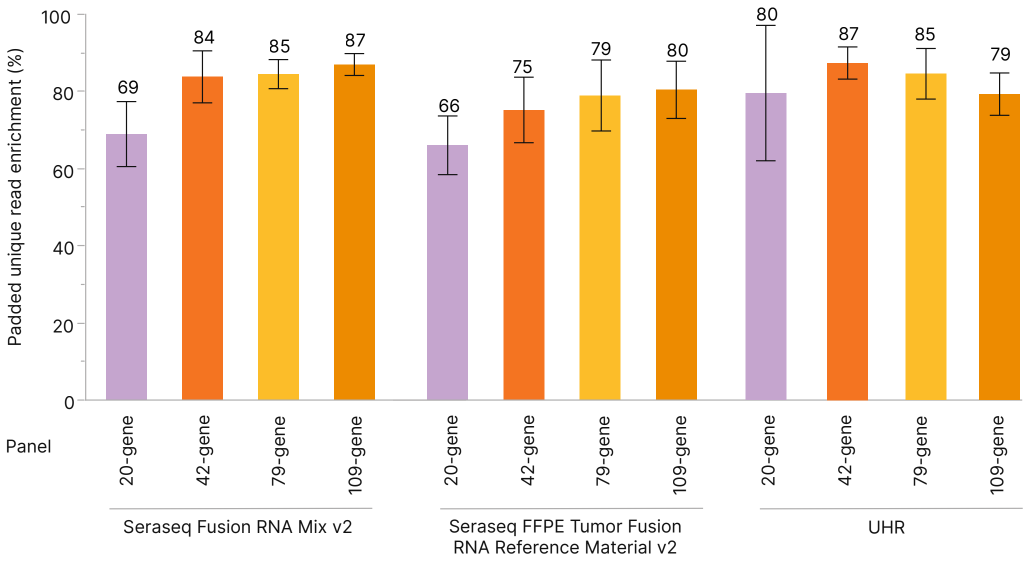

Figure 3: Illumina Rapid RNA Prep with Enrichment observed padded read enrichment performance

Four RNA enrichment panels containing 20 to 109 genes were evaluated (Table 1). Seraseq Fusion RNA Mix v2 (20 ng), Seraseq FFPE Tumor Fusion RNA Reference Material v2 (20 ng), and universal human reference (UHR, 10 ng) RNA samples were sequenced on the NovaSeq 6000 System at 2 × 151 bp paired-end reads, with data trimmed to 2 × 101 bp for DRAGEN analysis. For these samples, enrichment hybridization time was 12 hr overnight. Data analysis was performed with DRAGEN Enrichment app v4.3.

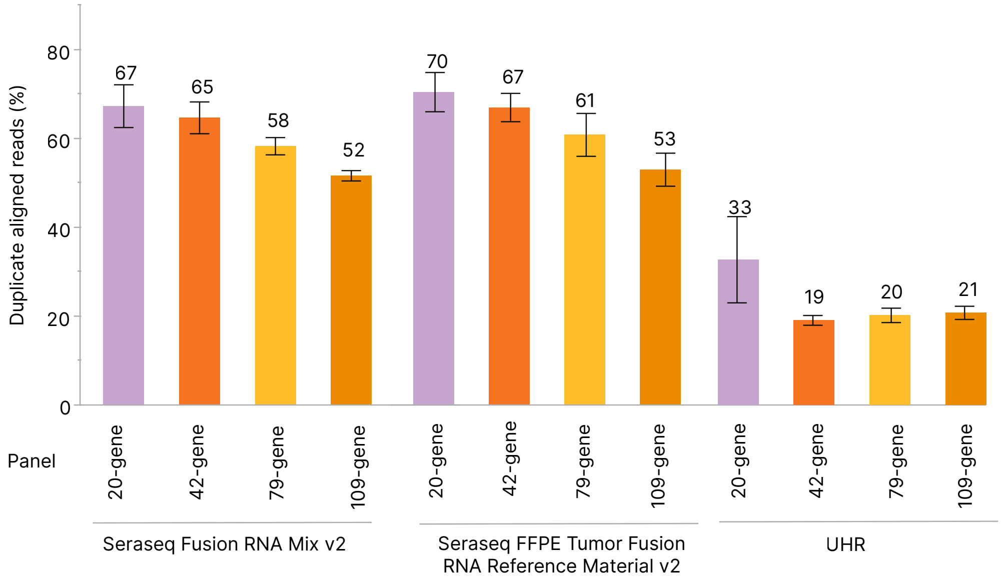

Figure 4: Illumina Rapid RNA Prep with Enrichment observed duplicate read enrichment performance

Four RNA enrichment panels containing 20 to 109 genes were evaluated (Table 1). Seraseq Fusion RNA Mix v2 (20 ng), Seraseq FFPE Tumor Fusion RNA Reference Material v2 (20 ng), and universal human reference (UHR, 10 ng) RNA samples were sequenced on the NovaSeq 6000 System at 2 × 151 bp paired-end reads, with data trimmed to 2 × 101 bp for DRAGEN analysis. For these samples, enrichment hybridization time was 12 hr overnight. Data analysis was performed with DRAGEN Enrichment app v4.3.

Target coverage

Illumina Rapid RNA Prep with Enrichment delivers focused target coverage that can be configured to the exact needs of RNA-Seq studies. When targeting 400× mean coverage for the FFPE samples, over 70% of the panel targets are covered with a mean ≥ 100× coverage and over 80% with mean ≥ 50× coverage, reflecting variability in coverage driven by differences in gene expression across targets.

The UHR control is a mix of total RNA from 10 human cell lines and is commonly used for RNA-Seq applications. In contrast to FFPE samples, UHR samples represent diverse cell types and gene expression profiles, and were able to achieve > 80% of targets with > 100× mean coverage and > 88% of targets with > 50× mean coverage (Figure 5). In the UHR reference sample, while targeting 400× mean coverage of the entire panel, only ~80% of targets achieved ≥ 100× mean coverage. Accordingly, aiming for a higher sequencing depth than the desired mean coverage of most targets should be considered to account for variation in gene expression across panel targets and within samples.

Illumina recommends targeting ≥ 5000 clusters or fragment reads per gene for custom RNA enrichment to detect somatic variants in FFPE samples. Variation in gene expression across samples should be considered when determining target sequencing depth requirements.

Specific targeting of expressed RNA

Illumina Rapid RNA Prep with Enrichment with Custom Enrichment Panel v2 produces high on-target specificity for RNA transcripts (Figure 6). The probe design strategy places capture probes directly adjacent to splice junctions to allow for detection of novel splice variants, which results in capturing reads covering intron/exon boundaries from prespliced transcripts. The custom panels of 20–109 genes showed 85–90% reads mapping to exons and another 8–10% mapping to intron/exon boundaries. Reads mapping to introns only or intergenic regions were < 2–3%. Illumina recommends designing custom panels with a minimum of 20 genes to produce diverse libraries and strong on-target enrichment metrics. Additional targets can be included to increase panel complexity.

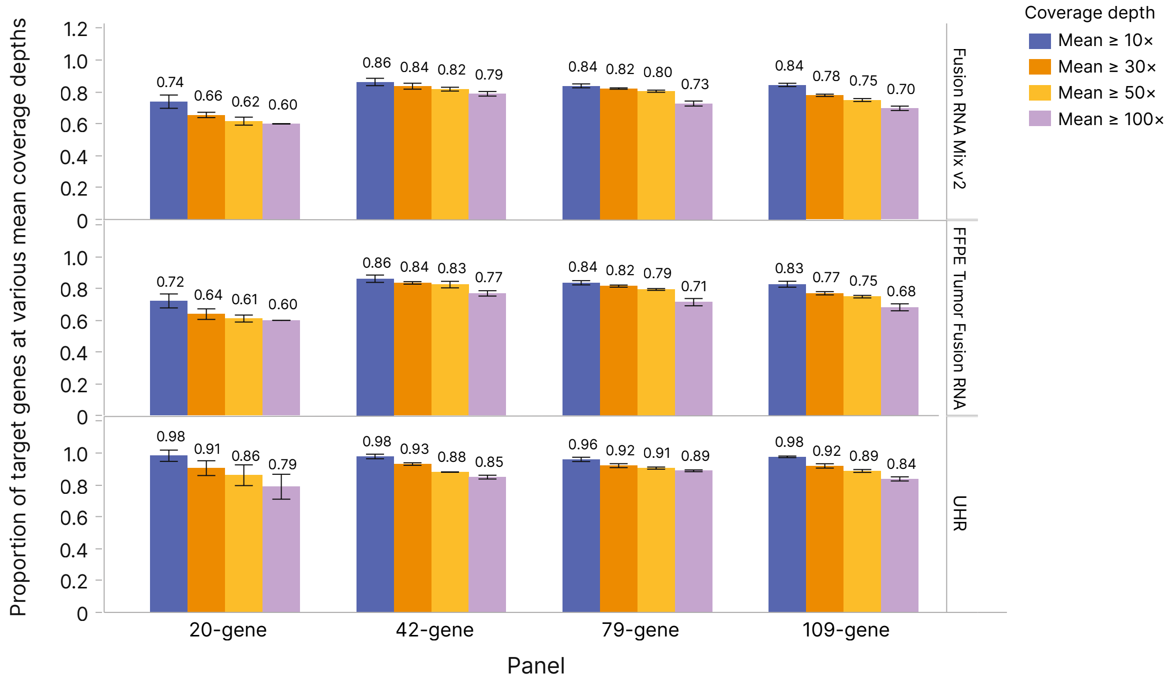

Figure 5: Illumina Rapid RNA Prep with Enrichment custom panel genes at various coverage depths

Four RNA enrichment panels containing 20 to 109 genes were evaluated (Table 1). Seraseq Fusion RNA Mix v2, Seraseq FFPE Tumor Fusion RNA Reference Material v2, and universal human reference (UHR) RNA samples were sequenced on the NovaSeq 6000 System at 2 × 151 bp paired end reads, with data trimmed to 2 × 101 bp for DRAGEN analysis. For these samples, enrichment hybridization time was 12 hr overnight. Data analysis was performed with DRAGEN Enrichment App v4.3.

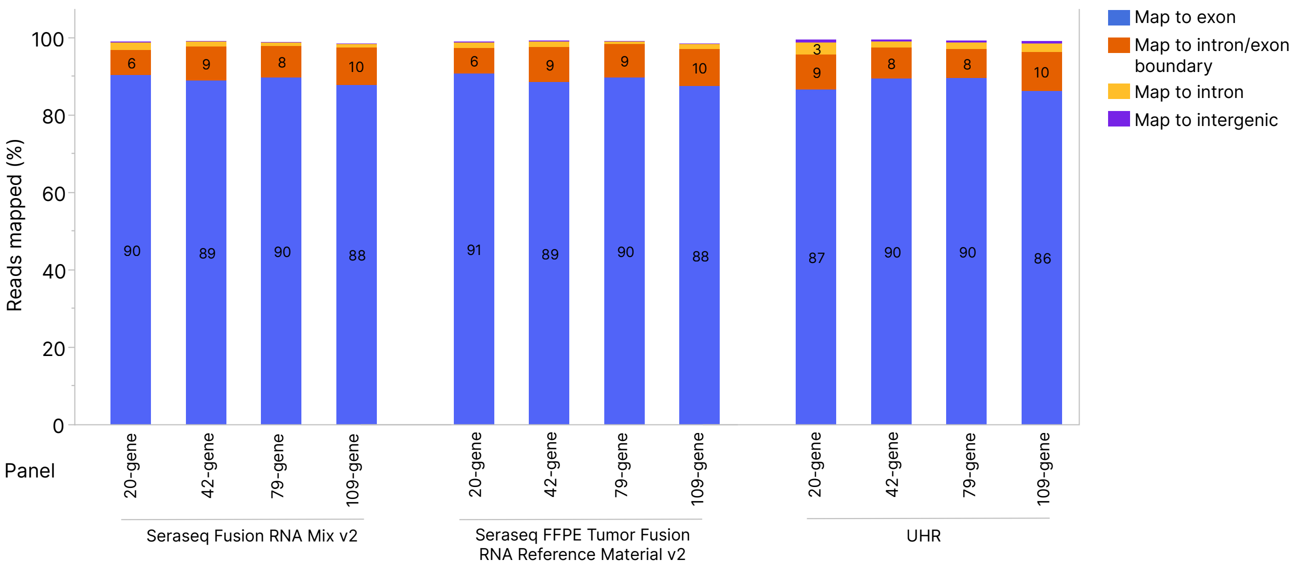

Figure 6: Reads mapping to gene transcript regions

Exonic alignment metrics show that > 90% of reads aligned to exons and > 95% aligned at least partially to exons, including intron/exon boundary reads. Four RNA enrichment panels containing 20 to 109 genes were evaluated (Table 1). Seraseq Fusion RNA Mix v2, Seraseq FFPE Tumor Fusion RNA Reference Material v2, and universal human reference (UHR) RNA samples were sequenced on a NovaSeq 6000 System using 2 × 101 bp paired-end reads. For these samples, enrichment hybridization time was 12 hr overnight. Data analysis was performed with DRAGEN Enrichment App v4.3.

Accurate fusion detection

With appropriately designed panels, Illumina Rapid RNA Prep with Enrichment with Illumina Custom Enrichment Panel v2 can be used for accurate detection of gene fusions. Detection of fusions was performed using 20 ng of SeraSeq Fusion Reference Material v2 or Seraseq FFPE Tumor Fusion RNA Reference Material v2 sequenced on the NovaSeq 6000 System with 2 × 101 bp paired-end reads.

The 109-gene custom panel allowed accurate detection of known gene fusions in the SeraSeq Fusion Reference Material v2 and Seraseq FFPE Tumor Fusion RNA Reference Material v2 samples (Table 2). The reference samples include a *TMPRSS2–ERG* gene fusion, which is not included in the 109-gene panel, enabling a negative control for this panel.

Detection of SNVs and Indels

Illumina Rapid RNA Prep with Enrichment is capable of targeted SNV and indel detection in samples of varying quality. More small variants were detected in the diverse UHR sample compared to the SeraSeq Fusion Reference Material v2 and Seraseq FFPE Tumor Fusion RNA Reference Material v2 sequenced on the NovaSeq 6000 System (Table 3).

Table 2: Illumina Rapid RNA Prep with Enrichment fusion detection in reference samples

Gene fusion	Detection across 9 replicates using 109-gene panel		Included in panel
	Seraseq Fusion RNA Mix v2	Seraseq FFPE Tumor Fusion RNA Reference Material v2	
EML4–ALK	9/9	9/9	Yes
KIF5B–RET	9/9	9/9	Yes
NCOA4–RET	9/9	9/9	Yes
CD74–ROS1	9/9	9/9	Yes
SLC34A2–ROS1	7/9	3/9	Yes
TPM3–NTRK1	9/9	9/9	Yes
FGFR3–BAIAP2L1	9/9	9/9	Yes
PAX8–PPARG	9/9	9/9	Yes
FGFR3–TACC3	9/9	9/9	Yes
ETV6–NTRK3	9/9	9/9	Yes
LMNA–NTRK1	9/9	9/9	Yes
SLC45A3–BRAF	9/9	9/9	Yes
TMPRSS2–ERG[b]	0/9	1/9	No
EGFR–SEPT14	9/9	9/9	Yes

a. Sequencing of libraries prepared using a 109-gene custom panel and SeraSeq Fusion Reference Material v2 samples was performed on the NovaSeq 6000 System using 2 × 101 bp paired end reads.

b. A *TMPRSS2–ERG* fusion is included in the reference sample but not part of the 109-gene custom panel so it serves as a negative control.

Table 3: Average number of variants identified using the DRAGEN Enrichment app[a]

	SeraSeq Fusion Reference Material v2		Seraseq FFPE Tumor Fusion RNA Reference Material v2		UHR	
Panel	**SNV**	**Indel**	**SNV**	**Indel**	**SNV**	**Indel**
20-gene	12	2	14	3	35	2
42-gene	35	18	37	18	69	22
79-gene	64	38	69	34	118	42
109-gene	72	36	74	39	107	34

a. Values are calculated from the averages of nine samples for each sample type.

Flexible design of custom panels for all RNA enrichment applications

Compatibility with Illumina Custom Enrichment Panel v2 provides labs with the flexibility to cover the targets they need at the read depths required to support advanced study designs in a variety of areas, including oncology, immunology, and infectious disease research.

Kits are available in a modular format with Enrichment reagents available in a 16-sample format for single-plex hybridization reactions or 96-sample format for 32 three-plex hybridization reactions. The library prep and enrichment modules can be purchased separately to support various enrichment plexities. Validation and protocol adjustments may be required when using plexities that are not recommended.

Summary

Illumina Rapid RNA Prep with Enrichment offers a streamlined solution and simple, rapid workflow for targeted RNA-Seq. It offers extraordinary flexibility for input type, including degraded samples, and supports input amounts as low as 1 ng. The modular design supports a range of RNA-Seq applications with flexible custom enrichment content or available fixed panels, enabling detection studies such as allele-specific expression, fusion detection, biomarker research, and more.

Illumina Rapid RNA Prep with Enrichment is part of a complete RNA sequencing and analysis workflow that includes Illumina sequencing systems, DRAGEN RNA applications for secondary analysis, and Illumina Connected Multiomics for biological interpretation and visualization.

Learn more →

Illumina Rapid RNA Prep with Enrichment

Illumina Custom Enrichment Panel v2

DesignStudio Assay Design tool information

DesignStudio Assay Design Tool

DRAGEN RNA app

DRAGEN Differential Expression app

Illumina Connected Multiomics app

Ordering information

Product	Catalog no.
Illumina Rapid RNA Prep, (S) Tagmentation (16 samples)	20158823
Illumina Rapid RNA Prep, (S) Tagmentation (96 samples)	20158824
Illumina RNA Fast Hyb Enrichment (16 samples)	20040540
Illumina RNA Fast Hyb Enrichment (96 samples)	20040541
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
Illumina Custom Enrichment Panel v2 (32 µl, 120 bp)[c]	20073953
Illumina Custom Enrichment Panel v2 (384 µl, 120 bp)[c]	20073952
Illumina Custom Enrichment Panel v2 (1536 µl, 120 bp)[c]	20111339
Illumina Purification Beads, 30 ml[d]	20119944
Illumina Purification Beads, 100 ml[d]	20060057
Illumina Purification Beads, 400 ml[d]	20060058

[a] The kit includes reagents for 1-plex, 16 enrichment reactions.

[b] The kit includes reagents for 3-plex, 32 enrichment reactions.

[c] Custom enrichment panels for human designs can be designed through the Illumina DesignStudio portal, a free online design tool. design support for nonhuman content is enabled through the Illumina Concierge Design team. Contact your Illumina sales representative for more information about Concierge design services.

[d] Illumina Purification beads are included in Illumina Rapid RNA Prep kits but can be purchased separately in large volumes for additional library cleanup and purification applications.

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References

1. Bentley DR, Balasubramanian S, Swerdlow HP, et al. Accurate whole human genome sequencing using reversible terminator chemistry. *Nature*. 2008;456:53-59. doi: 10.1038/nature07517.