DATA SHEET

Illumina RNA Prep with Enrichment

A fast, integrated workflow for accurate, unbiased transcript detection

- Achieve high sensitivity from only 10–20 ng total RNA from fresh, frozen, or FFPE samples
- Prepare libraries in nine hours with less than two hours of hands-on time
- Multiplex up to 384 samples in a single run with unique dual indexes

illumina®

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 methods include:

- Targeted RNA-Seq analyzes expression in a focused set of genes. Enrichment enables cost-effective RNA exome analysis using sequence-specific capture of the coding regions of the transcriptome. It is ideal for low-quality, formalin-fixed paraffin-embedded (FFPE) samples
- 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

Illumina RNA Prep with Enrichment, (L) Tagmentation offers a streamlined solution for targeted RNA-Seq. The kit offers high flexibility regarding input type and amount to support a range of RNA-Seq applications, enabling detection and discovery studies such as allele-specific expression, fusion detection, biomarker screening, and more. Combining Illumina RNA Prep with Enrichment with the Illumina Exome Panel provides a comprehensive view of the coding transcriptome for maximum discovery power at a fraction of the sequencing depth.

Fast and simple RNA enrichment workflow

Illumina RNA Prep with Enrichment uses on-bead tagmentation followed by a single, simplified 90-minute hybridization step to provide a rapid workflow (Figure 1). On-bead tagmentation features enrichment bead-linked transposomes (eBLT) optimized for RNA (eBLTL) that mediate a uniform tagmentation reaction, eliminating the need for separate fragmentation steps to save time.

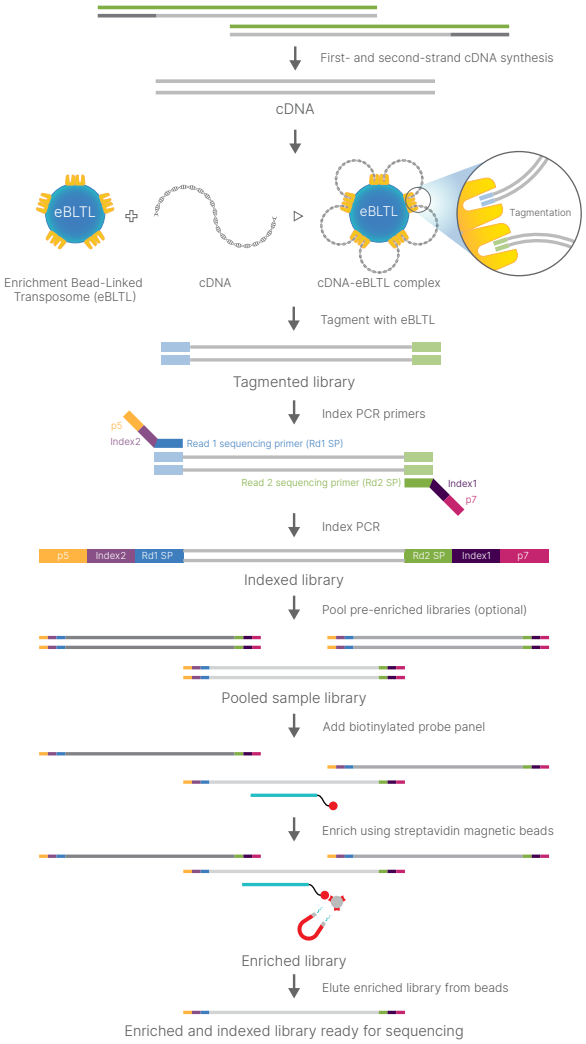

Figure 1: Illumina RNA Prep with Enrichment chemistry—After cDNA synthesis, a uniform tagmentation reaction mediated by eBLTLs followed by a single, 90-minute hybridization reaction enables a fast and flexible workflow.

Combined with innovations to the hybridization reaction, the workflow features fewer steps, shorter incubation times, and numerous safe stopping points and a total assay time that is > 50% faster than TruSeq™ RNA Exome (Figure 2). In addition to manual preparation, Illumina RNA Prep with Enrichment is designed to be 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 2: Illumina RNA Prep with Enrichment delivers a fast workflow—On-Bead Tagmentation and a single, 90-minute hybridization step combine to deliver a faster workflow with fewer steps compared to TruSeq RNA Exome.

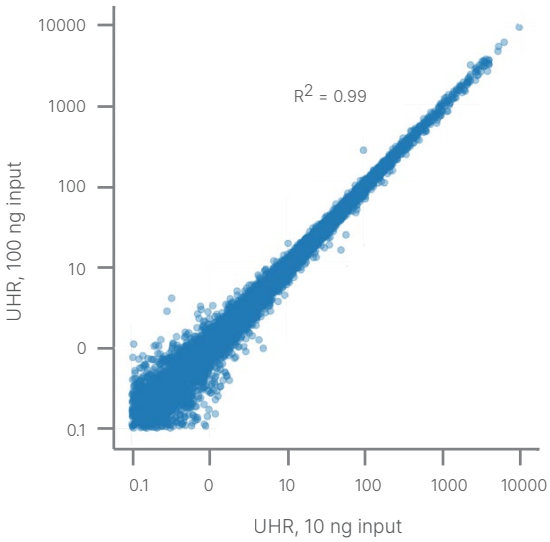

Figure 3: High-quality data from low input samples—Illumina RNA Prep with Enrichment achieves high data concordance between input amounts of 10 ng and 100 ng total RNA from universal human reference (UHR). UHR RNA libraries were sequenced on the NovaSeq 6000 System, subsampled to 25M clusters per library. Data were analyzed with the BaseSpace RNA-Seq Alignment app v 1.1.1.

High-quality data

Highly accurate data from low-input and FFPE samples

High capture efficiency and coverage uniformity minimize the required sequencing depth to determine expression levels accurately and without bias. Starting with as little as 10 ng total RNA, Illumina RNA Prep with Enrichment produces quality data with high concordance between varying amounts of input RNA from fresh or frozen samples (Figure 3). Tumor–normal biopsy specimens or FFPE archival tissue samples provide a rich source of biological information for gene expression profiling; however, they can be difficult to study due to nucleic acid degradation from the fixation and storage process.[1] Illumina RNA Prep with Enrichment produces quality data with input as low as 20 ng of input RNA from FFPE samples. Together, these results demonstrate that Illumina RNA Prep with Enrichment is an ideal solution for degraded samples with limited starting material.

Gene fusion detection in low-input and FFPE samples

To demonstrate the ability of Illumina RNA Prep with Enrichment to recognize structural variants within RNA transcripts, fresh-frozen and FFPE samples were enriched using the Illumina Exome Panel and sequenced on the NovaSeq™ 6000 System. Results showed a 100% call rate for *BCR-ABL1* (Figure 4) and *TPM3-NTRK1* gene fusions across six replicates of the K-562 cell line (RNA integrity number, RIN = 7.4, DV200 = 90%) and a colorectal cancer cell line (RIN = 2.5, DV200 = 85%), respectively (Table 1).

Table 1: Gene fusion detection

Fusion (source)	RIN	RNA input	Detection
BCR-ABL1 (K-562)	7.4	10 ng	6/6 replicates (100%)
TPM3-NTRK1 (colorectal cancer)	2.5	20 ng	6/6 replicates (100%)

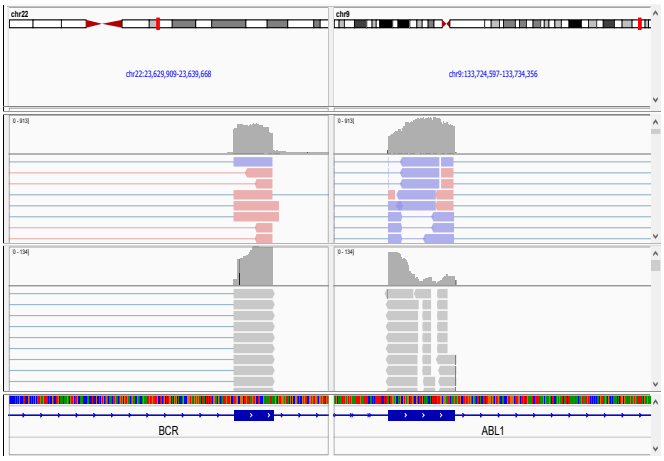

Figure 4: Detection of *BCR-ABL1* gene fusion—Libraries prepared from 10 ng K-562 cell line RNA using Illumina RNA Prep with Enrichment and the Illumina Exome Panel resulted in successful detection of the *BCR-ABL1* gene fusion, using the Broad Integrative Genomics Viewer (IGV). Top alignment track shows all reads; bottom track shows only reads supporting the *BCR-ABL1* fusion.

Focused, affordable RNA-Seq

Exceptional exonic coverage

Illumina RNA Prep with Enrichment can be used with the Illumina Exome Panel, which features a highly optimized probe set that delivers comprehensive coverage of coding RNA sequences (Table 2).

Table 2: Illumina Exome panel specifications

Coverage specifications	Illumina Exome Panel
No. of target genes	21,415
No. of target exonic regions	214,126
No. of probes	425,437
RefSeq exome percent covered	98.3%

To evaluate the performance of Illumina RNA Prep with Enrichment for exome sequencing, libraries were prepared from universal human reference (UHR) RNA and FFPE RNA using Illumina RNA Prep with Enrichment. Resulting libraries were sequenced on a NovaSeq 6000 System at 2 × 100 bp (25 M reads). Data analysis with the Enrichment App in BaseSpace™ Sequence Hub revealed that Illumina RNA Prep with Enrichment resulted exceptional exonic coverage with > 85% of the bases covered aligning to coding sequence and untranslated regions (UTR) of RNA, comparable to TruSeq RNA Exome (Figure 5, Figure 6).

These results demonstrate that Illumina RNA Prep with Enrichment provides high capture efficiencies that focus sequencing efforts on the high value content of RNA coding regions. By working with more focused content, Illumina RNA Prep with Enrichment requires lower sequencing depths and produces smaller data sets that result in time and cost savings.

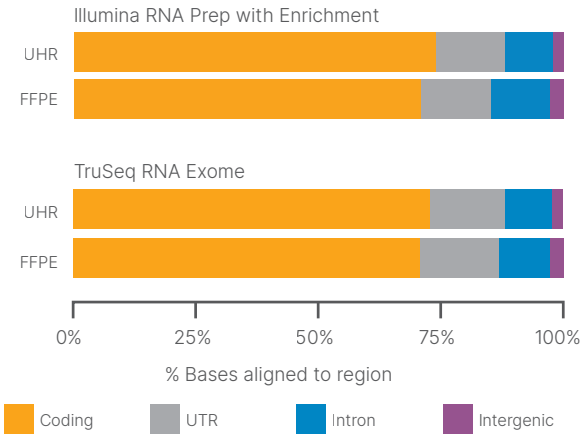

Figure 5: Coverage of coding regions with Illumina RNA Prep with Enrichment—Libraries prepared from 10 ng UHR RNA and 20 ng FFPE RNA using Illumina RNA Prep with Enrichment and the Illumina Exome Panel show more than 85% of the data aligned to coding and UTRs. Data from TruSeq RNA Exome libraries are shown for comparison. Libraries were sequenced on a NovaSeq 6000 System at 2 × 100 bp, subsampled to 25 M reads.

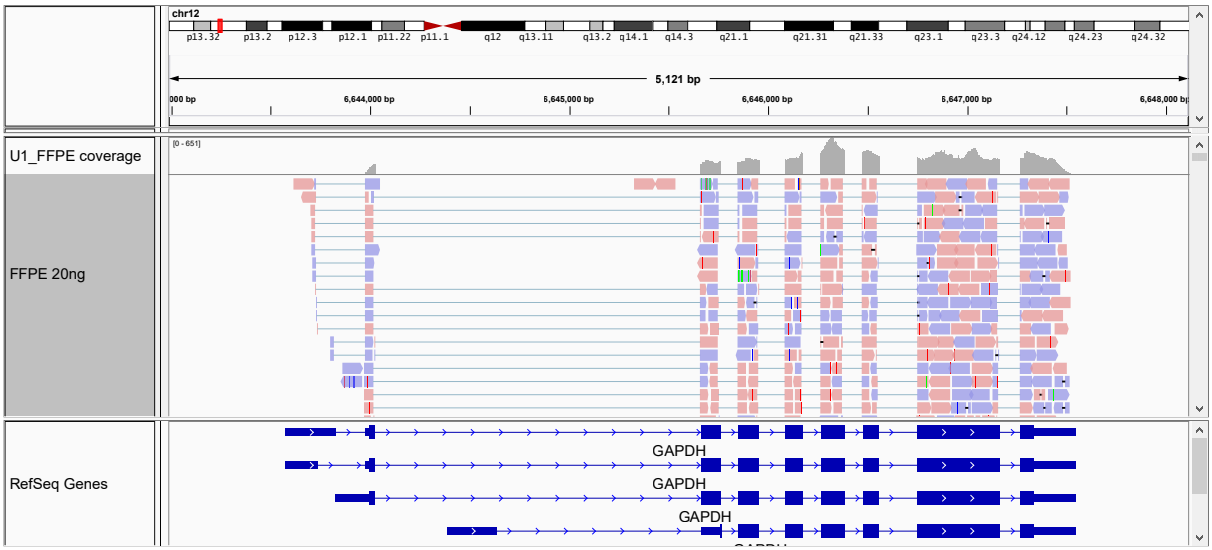

Figure 6: Coverage of coding regions Illumina RNA Prep with Enrichment—A library prepared from 20 ng of low-quality FFPE sample and enriched with the Illumina Exome Panel was sequenced at 25M reads. Coverage of the *GAPDH* control gene using Broad IGV is displayed, showing reads aligning across coding exons and demonstrating good target capture.

Concordance with TruSeq RNA Exome

Comparison of data generated from Illumina RNA Prep with Enrichment libraries to data from TruSeq RNA Exome libraries, a standard solution for RNA enrichment, showed high concordance (Figure 7). Of note, the sigmoid shape of the data plot is the result of index hopping from adapters used with TruSeq RNA Exome. Importantly, Illumina RNA Prep with Enrichment uses unique dual indexes (UDIs), which are designed to eliminate index recombination.

Flexible and scalable throughput

By combining Illumina RNA Prep with Enrichment and Illumina mid- or high-throughput sequencing systems, including 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 RNA Prep with Enrichment supports multiplexing with 384 unique dual indexes (UDIs). In addition to eliminating index misassignment, UDIs help to decrease sequencing costs by allowing up to 384 samples to be loaded on a single NovaSeq 6000 S4 flow cell for significantly increased throughput.

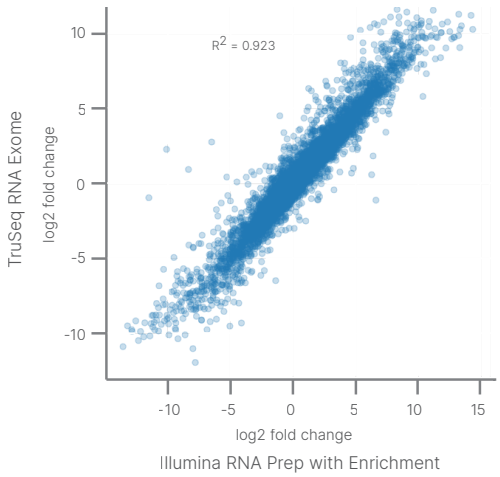

Figure 7: Concordance with TruSeq RNA Exome—Illumina RNA Prep with Enrichment Plot shows high concordance with TruSeq RNA Exome, as measured by log2 fold change for UHR RNA (Agilent, Catalog no. 740000) vs. Human Brain Total RNA (ThermoFisher Scientific, Catalog no. AM7962). All libraries were prepared from 10 ng input. TruSeq RNA Exome libraries were enriched as 4-plex, Illumina RNA Prep with Enrichment libraries were enriched as 3-plex. All data are downsampled to 25M clusters per library. Data were analyzed with the BaseSpace Cufflinks Assembly & DE app v 2.1.0.

Modular design for a broad range of RNA applications

By combining RNA library preparation and enrichment performance with the proven accuracy of Illumina sequencing by synthesis (SBS) chemistry,[2] Illumina RNA Prep with Enrichment supports both fixed and custom panels of varying sizes for advanced study designs in a variety of areas. Examples include the Illumina Exome Panel for analysis of coding regions of the transcriptome and the more focused Respiratory Virus Oligos Panel, which features ~7800 probes designed to detect respiratory viruses, recent flu strains, and SARS-CoV-2. Validation and protocol adjustments may be required when combining Illumina RNA Prep with Enrichment and a custom panel.

Summary

Illumina 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 low input amounts. The modular design supports a wide range of RNA-Seq applications across regions of interest, eg, with the Illumina Exome Panel and Respiratory Virus Oligos Panel, enabling detection and discovery studies such as allele-specific expression, fusion detection, biomarker screening, and more.

Learn more

Illumina RNA Prep with Enrichment

Ordering information

Product	Catalog no.
Illumina RNA Prep with Enrichment, (L) Tagmentation (16 samples)[a]	20040536
Illumina RNA Prep with Enrichment, (L) Tagmentation (96 samples)[b]	20040537
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 Exome Panel	20020183
Illumina Respiratory Virus Oligo Panel v2	20044311
Viral Surveillance Panel, RUO (96 reactions)	20088154
Pan-Coronavirus Panel, RUO (96 reactions)	20088155

a. Kit includes reagents for 1-plex, 16 enrichment reactions.
b. Kit includes reagents for 3-plex, 32 enrichment reactions.

References

1. von Ahlfen S, Missel A, Bendrat K, and Schlimpberger M. Determinants of RNA quality from FFPE samples. *PLoS ONE*. 2007;2(12): e1261. doi: 10.1371/journal.pone.0001261
2. 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.

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