illumina®

TruSeq™ Stranded mRNA and Total RNA

Gain a clear and comprehensive view of the transcriptome with a streamlined, cost-efficient, and scalable solution for mRNA or whole-transcriptome analyses.

Highlights

- **Precise Measurement of Strand Orientation**
  Enables detection of antisense transcription, enhances transcript annotation, and increases alignment efficiency
- **Excellent Coverage Quality**
  Provides accurate and comprehensive mapping of alternative transcripts and gene fusions
- **Compatible with Multiple Sample Types**
  Analyze various samples including low-quality, formalin-fixed, paraffin-embedded (FFPE), and blood samples
- **Exceptional Flexibility**
  Provides exceptional flexibility in experimental design with up to 96 unique dual indexes (UDIs) for confident sample multiplexing

Introduction

RNA sequencing (RNA-Seq) is a powerful method for discovering, profiling, and quantifying RNA transcripts. Using Illumina next-generation sequencing (NGS) technology, RNA-Seq does not require species- or transcript-specific probes, meaning previous assumptions about the transcriptome do not bias the data. Therefore, RNA-Seq enables hypothesis-free experimental designs of any species, including species with poor or missing genomic annotation. Beyond the measurement of gene expression changes, RNA-Seq can be used for discovery applications such as identifying alternative splicing events, gene fusions, allele-specific expression, and examining rare and novel transcripts.

As the complexities of gene regulation become better understood, a need for capturing additional data has emerged. Stranded information identifies from which of the two DNA strands a given RNA transcript was derived. This information provides increased confidence in transcript annotation, particularly for nonhuman samples. Identifying strand origin increases the percentage of reads that align, reducing sequencing costs per sample. Maintaining strand orientation also allows identification of antisense expression, an important mediator of gene regulation.[1] The ability to capture the relative abundance of sense and antisense expression provides visibility to regulatory interactions that might otherwise be missed.

As the important biological roles of noncoding RNA (ncRNA) continue to be recognized, whole-transcriptome analysis, or total RNA-Seq, provides a broader picture of expression dynamics. Total RNA-Seq enabled by ribosomal RNA (rRNA) reduction is compatible with FFPE samples, which contain potentially critical biological information. TruSeq Stranded RNA provides a unique combination of exceptional data quality for both mRNA and whole-transcriptome analyses. Also, the workflow enables robust interrogation of both standard and low-quality samples and is compatible with a wide range of study designs (Figure 1).

Figure 1: TruSeq Stranded RNA—TruSeq Stranded mRNA and Total RNA allow robust interrogation of both standard and low-quality samples, and is compatible with a wide range of study designs.

Effective Ribosomal Reduction

TruSeq Stranded RNA (Table 1) combines proven ribosomal reduction and library preparation chemistries into a single, streamlined protocol. Unlike polyA-based capture methods, Ribo-Zero™ kits remove rRNA using biotinylated probes that selectively bind rRNA species. Magnetic beads capture the probe-rRNA hybrid and are removed by pulldown, leaving the desired rRNA-depleted RNA in solution. This process minimizes ribosomal contamination and maximizes the percentage of uniquely mapped reads covering both mRNA and a broad range of ncRNA species of interest, including long intergenic noncoding RNA (lincRNA), small nuclear (snRNA), small nucleolar (snoRNA), and other RNA species.[2]

Table 1: RNA Species Targeted for Reduction

Targeted RNA Species	Library Preparation
• Cytoplasmic rRNA	TruSeq Stranded Total RNA with Ribo-Zero Human/Mouse/Rat
• Cytoplasmic rRNA • Mitochondrial rRNA	TruSeq Stranded Total RNA with Ribo-Zero Gold
• Cytoplasmic rRNA • Mitochondrial rRNA • Globin mRNA	TruSeq Stranded Total RNA with Ribo-Zero Globin

High-Quality Stranded Information

TruSeq Stranded RNA chemistry delivers exceptional data quality. The stranded measurement, or the percentage of uniquely mapped reads that return accurate strand origin information based on well-characterized universal human reference (UHR) RNA, is ≥ 99% using TruSeq Stranded mRNA and ≥ 98% using TruSeq Stranded Total RNA. This highly accurate information serves to increase the percentage of unique reads that align in the assembly of poorly annotated transcriptomes and provides sensitivity to detect antisense expression. Consistent, precise measurement of RNA abundance is reflected by high reproducibility between technical replicates (Figure 2, $R^2 = 0.9783$).

TruSeq Total RNA for Low-Quality Samples

TruSeq Stranded RNA enables robust and efficient interrogation of FFPE and other low-quality RNA samples. Coverage across transcripts is high and balanced in both fresh-frozen (FF) and FFPE samples prepared with TruSeq Stranded Total RNA (Figure 3). The optimized Ribo-Zero rRNA removal workflow provides a viable, highly scalable solution for efficient whole-transcriptome analysis across samples that have been historically difficult to analyze.

RNA Analysis of Blood Samples

TruSeq Stranded Total RNA with Ribo-Zero Globin enables efficient, robust interrogation of coding and ncRNA isolated from blood samples. A streamlined, automation-friendly workflow applies Ribo-Zero chemistry to remove globin mRNA along with both cytoplasmic and mitochondrial rRNA simultaneously in a single, rapid step (Table 1). The workflow combines globin mRNA removal, rRNA removal, and library preparation to optimize sequencing output. It also reduces total assay time, eliminates the need for additional removal chemistry, and reduces cost per sample.

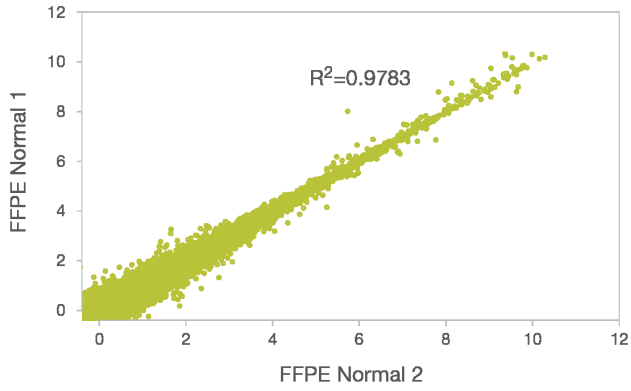

Figure 2: High Concordance Between Technical Replicates—Technical replicates of FFPE tissue show high concordance, indicating robust library prep performance. Axes are log2 fragments per kilobase million (FPKM) gene expression plots. R^2 value is shown.

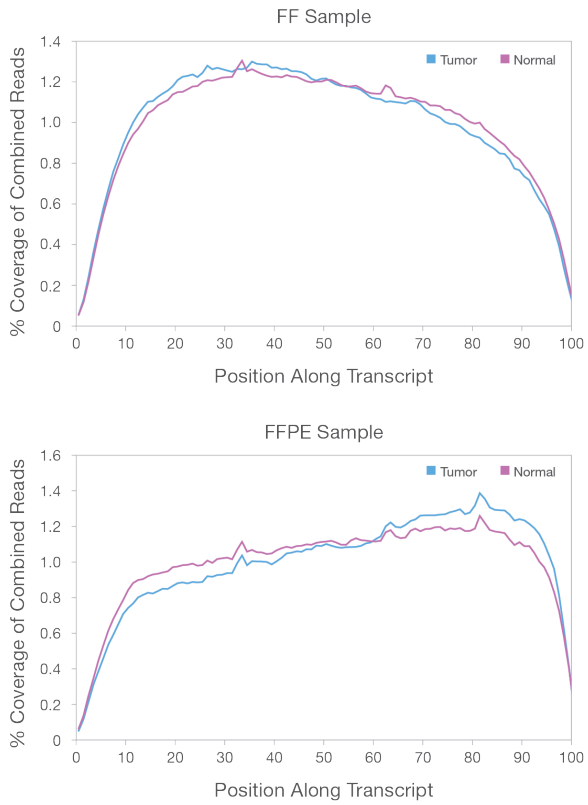

Figure 3: Even Coverage Across Transcripts—TruSeq Stranded Total RNA provides excellent coverage across the top 1000 expressed transcripts in both FF (top) and FFPE (bottom) tumor and matched normal breast tissue, with > 98% aligned stranded reads.

Differential Expression of Noncoding RNA

Maintaining strand information of RNA transcripts is important for many reasons, including detection of differentially expressed transcripts. RNA-Seq analyses of breast tumor and normal tissue was compared between TruSeq Stranded Total RNA with Ribo-Zero and a standard polyA-based method for library prep. Both TruSeq Stranded Total RNA and polyA-prepared libraries detected the differential expression of *ATP5H* between tumor and normal samples. However, using TruSeq Stranded Total RNA, differential expression in reverse orientation at the position of pseudogene transcript AC087651.1 is also detected in the expected, opposite strand orientation (Figure 4). TruSeq Stranded Total RNA also enables reliable detection of differential expression across multiple forms of ncRNA, including lincRNA, snRNA, snoRNA, and other RNA species (miscellaneous RNA) between tumor and normal tissues (Figure 5).

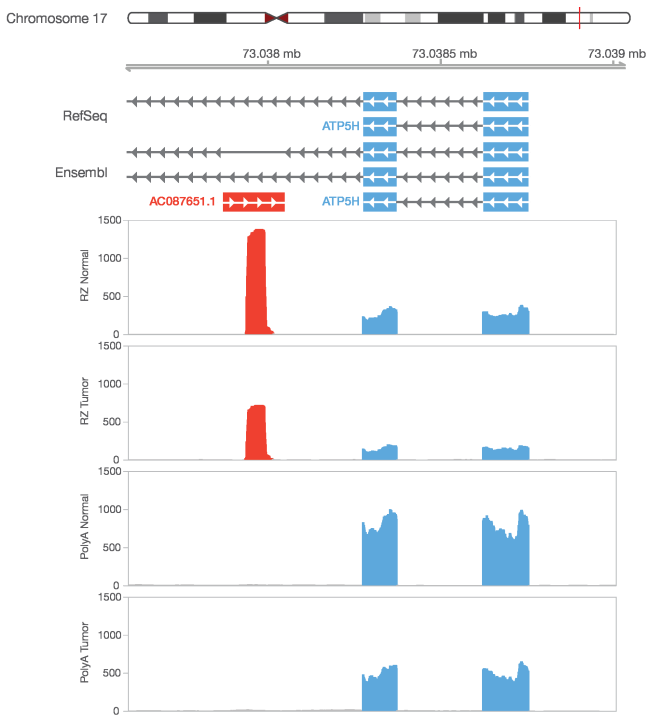

Figure 4: Differential Expression of ncRNA Transcripts—*ATP5H* expression from chromosome 17 is differentially expressed in breast tumor vs. normal tissue. Using two different library preparation methods (RZ; Ribo-Zero for total RNA or PolyA; polyA-based mRNA) shows differential expression in tumor vs. normal tissues in both preps (Blue). However, only TruSeq Stranded Total RNA with Ribo-Zero reveals differential expression at the locus of a pseudogene (Red, AC087651.1), for which reads are detected in the opposite orientation, as expected. This stranded information would have been lost in a standard mRNA prep.

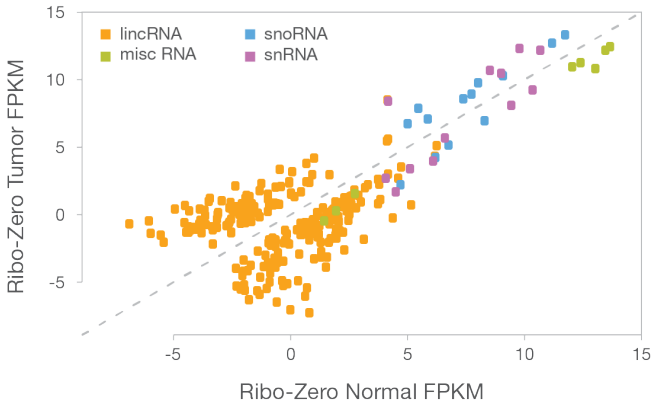

Figure 5: Detection of ncRNA Expression—With TruSeq Stranded Total RNA, differential expression across a range of ncRNA species, including long intergenic noncoding RNA (lincRNA), small nuclear (snRNA) and small nucleolar (snoRNA) and miscellaneous RNA (misc RNA) can be detected between tumor and normal tissues (four replicates per sample, false discovery rate (FDR) = 0.05).

Flexible Workflow Configurations

TruSeq Stranded mRNA and Total RNA offer solutions optimized for individual experimental needs. Each workflow includes low- and high-throughput protocols that are ideally suited for projects with ≤ 48 samples and ≥ 48 samples, respectively. Stranded Total RNA configurations are available for targeting the removal of cytoplasmic rRNA only, or both cytoplasmic and mitochondrial rRNA. In a comparison using UHR RNA, TruSeq Stranded Total RNA with Ribo-Zero Human/Mouse/Rat and Gold both reduced cytoplasmic rRNA to < 2% of aligned reads. TruSeq Stranded with Ribo-Zero Gold also reduced mitochondrial rRNA from 7% to only 0.02% of aligned reads.

Efficient Sample Multiplexing

Using a simple procedure, indexes are added to sample cDNA fragments to provide an innovative solution for sample multiplexing. For the greatest operational efficiency, up to 96 preplated, uniquely indexed samples can be pooled and sequenced together in a single flow cell lane on any Illumina sequencing platform. After sequencing, the indexes are used to demultiplex the data and accurately assign reads to the proper samples in the pool.

TruSeq Stranded RNA can use a single indexing strategy or a dual-indexing strategy that uses a unique combination of two indexes to demultiplex. The unique dual index (UDI) adapters were developed in a collaboration between Integrated DNA Technologies, Inc. (IDT) and Illumina (available separately) and employ unique pairs of indexes to demultiplex. The newly introduced UDIs (24 and 96) offer increased plexity that enables accurate assignment of reads and efficient use of flow cells. Using UDI combinations is a best practice to make sure that reads with incorrect indexes do not impact variant calling.

Ordering Information

Product	Ribosomal Removal	Configuration	Catalog No.
TruSeq Stranded mRNA Library Prep	N/A	48 samples	20020594
		96 samples	20020595
TruSeq Stranded Total RNA Library Prep Human/Mouse/Rat	Cytoplasmic Ribosomal RNA	48 samples	20020596
		96 samples	20020597
TruSeq Stranded Total RNA Library Prep Gold	Cytoplasmic and Mitochondrial Ribosomal RNA	48 samples	20020598
		96 samples	20020599
TruSeq Stranded Total RNA Library Prep Plant	Cytoplasmic and Chloroplast Ribosomal RNA	48 samples	20020610
		96 samples	20020611
TruSeq Stranded Total RNA Library Prep Globin	Cytoplasmic and Mitochondrial Ribosomal RNA and Globin mRNA	48 samples	20020612
		96 samples	20020613

Indexes	Configuration	Catalog No.
TruSeq RNA Single Indexes Set A	12 indexes, 48 samples	20020492
TruSeq RNA Single Indexes Set B	12 indexes, 48 samples	20020493
TruSeq RNA CD Indexes	96 indexes, 96 samples	20019792
IDT for Illumina–TruSeq RNA UD Indexes	24 indexes, 96 samples	20020591
IDT for Illumina–TruSeq RNA UD Indexes	96 indexes, 96 samples	20022371

Summary

TruSeq Stranded mRNA and Total RNA provide a clear, comprehensive view of the transcriptome, enabling precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as alternative transcripts, gene fusions, and allele-specific expression. TruSeq Stranded Total RNA combines all the benefits of TruSeq RNA library prep with Ribo-Zero ribosomal reduction chemistry, providing a robust and highly scalable solution preparing sequencing-ready libraries for whole-transcriptome analysis compatible with a wide range of samples, including nonhuman and FFPE.

References

1. Nagai K, Kohno K, Chiba M, et al. Differential expression profiles of sense and antisense transcripts between HCV-associated hepatocellular carcinoma and corresponding noncancerous liver tissue. *Int J Oncol*. 2012(40):1813-20.
2. Benes V, Blake J, Doyle K. Ribo-Zero Gold Kit: Improved RNA-Seq results after removal of cytoplasmic and mitochondrial ribosomal RNA. *Nat Methods*. 2011(8):10.1038/nmeth.f.352.

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