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TruSeq® Stranded mRNA and Total RNA Sample Preparation Kits

The clearest and most complete 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
- **Unparalleled Coverage Quality**
  High coverage uniformity enables most accurate and complete mapping of alternative transcripts and gene fusions
- **Configurations Compatible with Many Sample Types Including Low-Quality, FFPE, and Blood Samples**
  Leverage the power of RNA-Seq for previously inaccessible samples

Introduction

RNA sequencing (RNA-Seq) is a powerful method for discovering, profiling, and quantifying RNA transcripts. Using Illumina next generation sequencing technology, RNA-Seq does not require species- or transcript-specific probes, meaning the data are not biased by previous assumptions about the transcriptome. RNA-Seq enables hypothesis-free experimental designs of any species, including those 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 non-human samples. Identifying strand origin increases the percentage of alignable reads, 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 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 formalin-fixed paraffin embedded (FFPE) samples, which contain potentially critical biological information. The family of TruSeq Stranded Total RNA sample preparation kits provides a unique combination of unmatched data quality for both mRNA and whole-transcriptome analyses, robust interrogation of both standard and low-quality samples and workflows compatible with a wide range of study designs (Figure 1).

Effective Ribosomal Reduction

TruSeq Stranded Total RNA kits couple proven ribosomal reduction and sample preparation chemistries into a single, streamlined workflow. Unlike polyA-based capture methods, Ribo-Zero kits remove ribosomal RNA (rRNA) using biotinylated probes that selectively bind rRNA species. The probe:rRNA hybrid is then captured by magnetic beads and removed, 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 non-coding RNA species of interest, including long intergenic noncoding RNA (lincRNA), small nuclear (snRNA), small nucleolar (snoRNA), and other RNA species[2].

High Quality Stranded Information

TruSeq Stranded RNA kits deliver unmatched data quality. As shown in Table 1, 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 Stranded mRNA and ≥ 98% using Stranded Total RNA. This highly accurate information serves to increase the percentage of uniquely alignable reads 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. 9873).

Figure 1: TruSeq Stranded RNA Sample Preparation Kits

The TruSeq Stranded mRNA and Total RNA Kits allow robust interrogation of both standard and low-quality samples, and include workflows compatible with a wide range of study designs.

Table 1: Stranded Parameters

RNA	TruSeq Sample Prep Method	Percent Aligned	Percent Stranded
Universal Human Reference	mRNA*	84.9%	99.6%
Human Reference Brain	mRNA	79.6%	99.0%
Universal Human Reference	Total RNA with Ribo-Zero**	79.0%	98.6%
Human Reference Brain	Total RNA with Ribo-Zero	73.0%	98.6%

Indexed, 2 x 75 cycle run on HiSeq® 2000; *Four mRNA samples per lane; ** Two Ribo-Zero samples per lane.

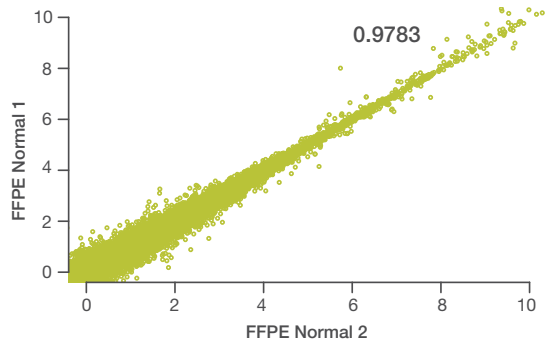
TruSeq Total RNA for Low-Quality Samples

TruSeq Total RNA enables robust and efficient interrogation of FFPE and other low-quality RNA samples. As shown in Figure 3, coverage across transcripts is high and even in both fresh-frozen (FF) and FFPE samples prepared with the TruSeq Stranded Total RNA kit. 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

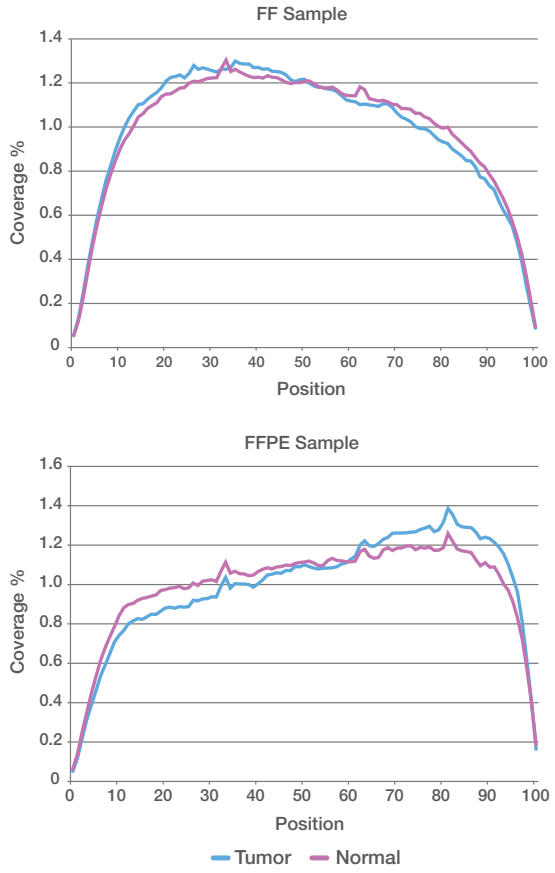
TruSeq Stranded Total RNA kits with Ribo-Zero Globin enable the efficient, robust interrogation of coding and noncoding RNA isolated from blood samples. A streamlined, automation-friendly workflow applies Ribo-Zero chemistry to simultaneously remove globin mRNA along with both cytoplasmic and mitochondrial rRNA in a single, rapid step (Table 2). In comparison to library preparation after ribosomal RNA reduction only, TruSeq Stranded Total RNA kits with Ribo-Zero Globin reduced globin mRNA levels generated from commercially obtained, blood-derived RNA from 28% to only 0.3% of aligned reads. These kits combine globin mRNA removal, rRNA removal, and library preparation to optimize sequencing output while reducing total assay time, eliminating the need for additional removal chemistry and reducing costs per sample.

Figure 2: Technical Replicates



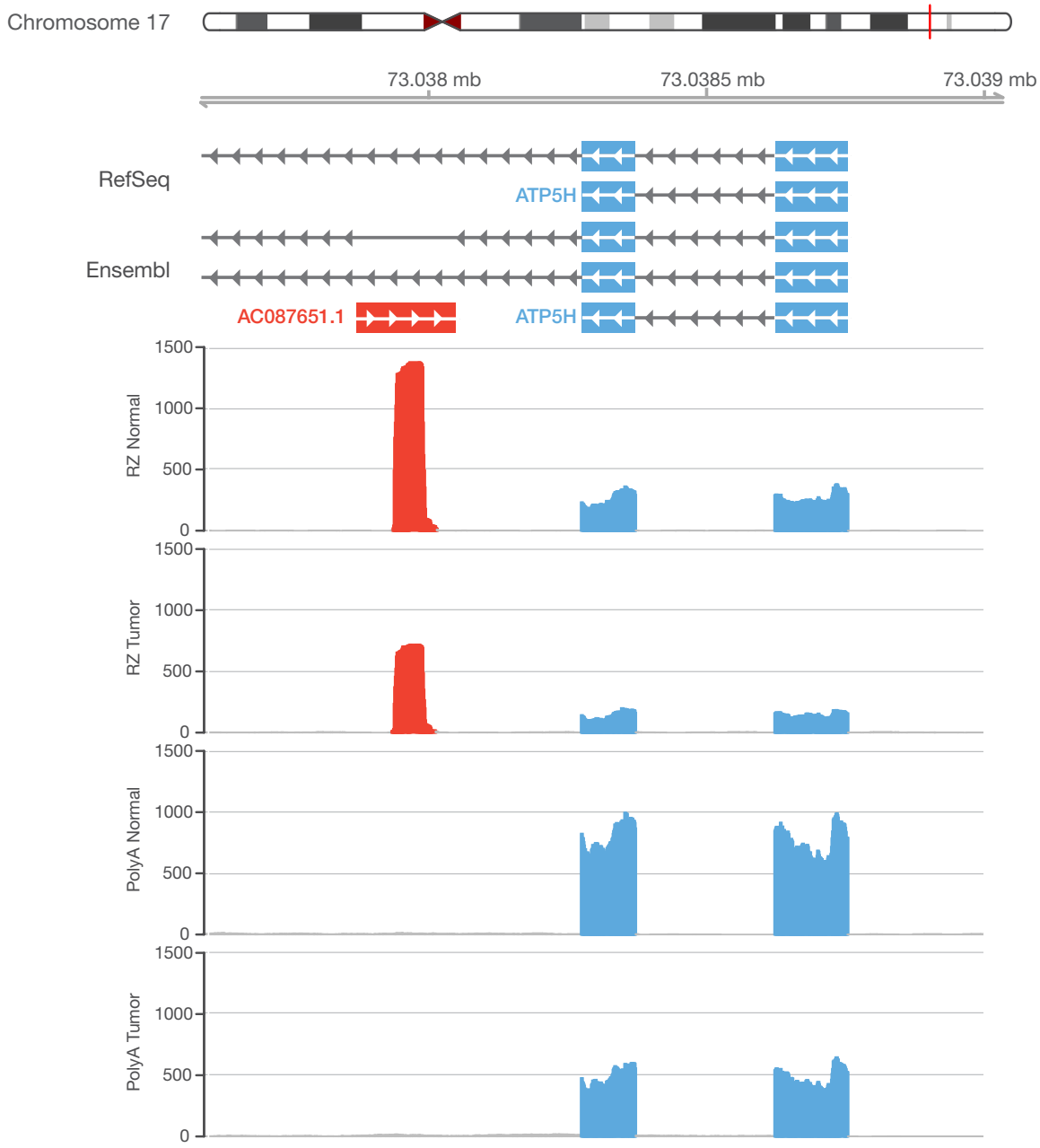
Technical replicates of FFPE tissue show high concordance, indicating robust sample prep performance. Axes are log2(FPKM). R^2 value is shown.

Figure 3: Even Coverage Across Transcripts



TruSeq Stranded Total RNA gives excellent coverage across the top 1,000 expressed transcripts in both fresh-frozen (FF, top) and FFPE (bottom) tumor and matched normal breast tissue, with > 98% aligned stranded reads. X-axis: position along transcript, Y-axis = percent coverage of combined reads.

Figure 4: Differential Expression of ncRNA Transcripts



ATP5H expression from chromosome 17 is differentially expressed in breast tumor vs. normal tissue. Using two different sample preparation methods (RZ; Ribo-Zero for total RNA or PolyA-based mRNA) shows differential expression in tumor vs. normal tissues in both preps (Blue). However, only 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.

Table 2: Targeted RNA Species

Kit Name	Cytoplasmic rRNA	Mitochondrial rRNA	Globin mRNA
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Human/Mouse/Rat	Targeted	Not targeted	Not targeted
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Gold	Targeted	Targeted	Not targeted
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Globin	Targeted	Targeted	Targeted

Several TruSeq Stranded Total RNA with Ribo-Zero kit configurations are available to suit a range of study designs, providing highly efficient removal of cytoplasmic rRNA, cytoplasmic and mitochondrial rRNA, or both forms of rRNA in addition to globin mRNA.

Differential Expression of Noncoding RNA

Maintaining strand information of RNA transcripts is important for many reasons. The example in Figure 4 shows a differentially-expressed transcript of the *ATP5H* gene in breast tumor and normal tissue prepared using the TruSeq RNA with Ribo-Zero compared to a standard polyA-based method. Both TruSeq Stranded Total RNA and polyA-prepared samples detect the differential expression of ATP5H between tumor and normal samples. However, using the Stranded Total RNA sample preparation kit, differential expression in reverse orientation at the position of pseudogene transcript AC087651.1 is also detected in the expected, opposite strand orientation.

The example in Figure 5 shows that TruSeq Stranded Total RNA enables reliable detection of differential expression across multiple forms of ncRNA, including lincRNA, snRNA, snoRNA, and other RNA species.

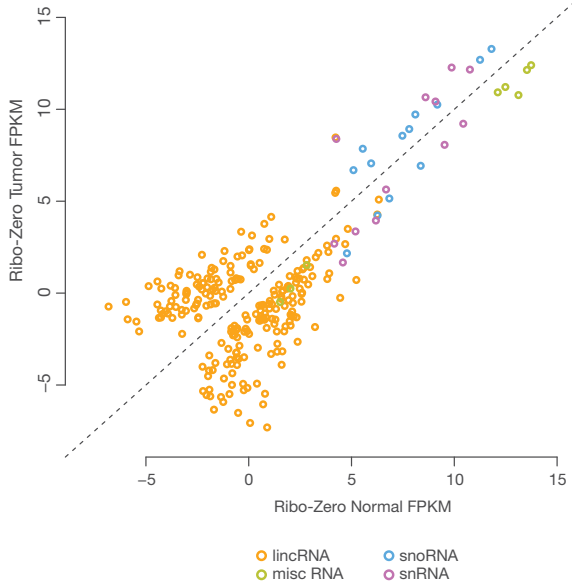
Flexible Workflow Configurations

The TruSeq Stranded mRNA and Total RNA kits offer solutions optimized for your individual experimental needs. Each kit includes two workflows: the high throughput protocol is ideally suited for projects with ≥ 48 samples, and the low throughput protocol is best suited for projects with ≤ 48 samples. Stranded Total RNA configurations are available for targeting the removal of either cytoplasmic rRNA only, or both cytoplasmic plus mitochondrial rRNA (Table 3). In a comparison using Universal Human Reference RNA, TruSeq Stranded Total RNA kits with Ribo-Zero Human/Mouse/Rat and Gold both reduced cytoplasmic rRNA to < 2% of aligned reads, whereas those with Ribo-Zero Gold additionally reduced mitochondrial rRNA from 7% to only 0.02% of aligned reads.

Conclusion

TruSeq Stranded mRNA sample prep kits provide the clearest, most complete view of the transcriptome, providing 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 kits couple all of the benefits of TruSeq RNA preparation kits with Ribo-Zero ribosomal reduction chemistry, providing a robust and highly scalable end-to-end solution for whole-transcriptome analysis compatible with a wide range of samples, including non-human and FFPE.

Figure 5: Detection of ncRNA Expression



With TruSeq Stranded Total RNA sample preparation, differential expression across a range of non-coding RNA species, including long intergenic noncoding RNA (lincRNA), small nuclear (snRNA) and small nucleolar (snoRNA) and other species (misc RNA) can be detected between tumor and normal tissues (four replicates per sample, false discovery rate (FDR) = 0.05).

Table 3: Ordering Information

Kit Name	Ribosomal Removal	Configuration	Catalog No.
TruSeq Stranded mRNA Sample Preparation Kit	N/A	Set A (48 samples, 12 indexes)	RS-122-2101
		Set B (48 samples, 12 indexes)	RS-122-2102
		High throughput (96 samples, 96 indexes)	RS-122-2103
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Human/Mouse/Rat	Cytoplasmic ribosomal RNA	Set A (48 samples, 12 indexes)	RS-122-2201
		Set B (48 samples, 12 indexes)	RS-122-2202
		High throughput (96 samples, 96 indexes)	RS-122-2203
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Gold	Cytoplasmic and mitochondrial ribosomal RNA	Set A (48 samples, 12 indexes)	RS-122-2301
		Set B (48 samples, 12 indexes)	RS-122-2302
		High throughput (96 samples, 96 indexes)	RS-122-2303
TruSeq Stranded Total RNA Sample Preparation Kit with Ribo-Zero Globin	Cytoplasmic and mitochondrial ribosomal RNA	Set A (48 samples, 12 indexes)	RS-122-2501
		Set B (48 samples, 12 indexes)	RS-122-2502
		High throughput (96 samples, 96 indexes)	RS-122-2503

References

1. Nagai K, Kohno K, Chiba M, Pak S, Murata S, et al. (2012) Differential expression profiles of sense and antisense transcripts between HCV-associated hepatocellular carcinoma and corresponding non-cancerous liver tissue. Int J Oncol 40(6):1813–20.
2. Ribo-Zero Gold Kit: Improved RNA-Seq results after removal of cytoplasmic and mitochondrial ribosomal RNA. Nature Methods Application Note, 2011.

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