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TruSeq® Stranded Total RNA Library Preparation Kit with Ribo-Zero™ Plant

Capture a comprehensive view of the transcriptome for gene expression studies and transcriptome discovery applications.

Highlights

- **Precise Measurement of Strand Orientation**
  Enables detection of antisense transcription, enhances transcript annotation, and increases alignment efficiency

- **Total RNA Analysis**
  Ribo-Zero chemistry removes cytoplasmic, mitochondrial, and chloroplast rRNA for analysis of a range of plant species

- **Unparalleled Coverage Quality**
  High coverage uniformity optimizes sequencing efficiency and enhances discovery of novel features

- **Integrated and Scalable Workflow**
  RNA-Seq provides the fastest time to answer for a range of sample types and applications

Figure 1: TruSeq Stranded Total RNA Library Preparation Kit with Ribo-Zero Plant—TruSeq Stranded Total RNA kits with Ribo-Zero Plant enable the capture and quantification of both coding and noncoding transcripts without prior knowledge, providing a comprehensive view of the plant transcriptome.

Introduction

RNA sequencing (RNA-Seq) is a powerful method for discovering, annotating, and quantifying RNA transcripts that is revolutionizing the field of agrigenomics.[1-9] RNA-Seq does not require species- or transcript-specific probes, enabling precise quantification of both known and novel transcripts without prior knowledge. 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 rare and novel transcripts.

As the complexities of gene regulation become better understood, a need for capturing additional data has emerged. Strand information identifies from which of the 2 DNA strands a given RNA transcript was derived. These data provide increased confidence in transcript annotation—particularly for nonhuman samples—and may serve to increase the percentage of uniquely alignable reads, reducing sequencing costs per sample. Maintaining strand orientation also enables identification of antisense expression, an important mechanism of gene regulation. Most important agronomic crops contain complex polyploid genomes, and stranded RNA-Seq expression analysis has shown that antisense expression-mediated gene regulation is utilized extensively in polyploid species.[3]

TruSeq Stranded Total RNA Library Preparation Kits with Ribo-Zero Plant (Figure 1) provide quick and efficient capture of both coding and noncoding RNA through Ribo-Zero ribosomal reduction chemistry, offering a comprehensive view of the transcriptome. 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. A rapidly growing body of literature supports important roles for various noncoding RNA forms in regulating an expanding range of biological systems and processes. TruSeq Stranded Total RNA kits with Ribo-Zero Plant enable efficient interrogation of coding and multiple forms of noncoding RNA across a broad range of plant species, requiring as little as 100 ng of input RNA. Ribo-Zero chemistry is also compatible with low-quality and partially degraded RNA, enabling accurate interrogation of samples that are commonly difficult to isolate or of agronomically important stored tissues, such as seed stocks.

Total RNA Analysis with Enhanced Ribo-Zero Chemistry

TruSeq Stranded Total RNA kits with Ribo-Zero Plant couple proven ribosomal RNA reduction and library 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 noncoding RNA species, including long intergenic noncoding RNA (lincRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA).

The rapid, highly specific removal of cytoplasmic, mitochondrial, and chloroplast rRNA from leaf, seed, and root tissues ensures efficient analysis across a broad range of both monocot and dicot species, including Arabidopsis and rice (Table 1).

Table 1: Stranded and rRNA Removal Parameters

Species	% Aligned to Cytoplasmic rRNA				% Aligned to Chloroplast rRNA				% Aligned to Mitochondrial rRNA	
	25S	18S	5.8S	5S	23S	16S	5S	4.5S	18S	5S
Arabidopsis	0.322%	0.133%	0.003%	N/A	0.148%	0.037%	0.002%	N/A	0.179%	N/A
Rice	0.108%	0.028%	0.002%	N/A	0.041%	0.011%	0.001%	N/A	0.002%	N/A

These samples were prepared using TruSeq Total RNA Library Preparation with Ribo-Zero Plant and data were generated from an indexed, 2 × 50 bp cycle run on 1/3 of a lane on the HiSeq® 2000 system, delivering highly precise stranded alignment.

Greater Coverage Uniformity

In addition to providing coverage of both coding and noncoding RNA species, Ribo-Zero ribosomal reduction chemistry delivers greater coverage uniformity through its ability to capture fragments of partially degraded transcripts without dependence on polyA tail association. Given the unstable nature of RNA, this capability provides an advantage in a wide range of samples, particularly those for which input RNA quality is low. Figure 2 compares the levels of coverage uniformity provided by TruSeq Stranded Total RNA with Ribo-Zero Plant and polyA-based methods on high-quality Arabidopsis RNA samples.

High-Quality Stranded Information and Capture of Noncoding RNA

TruSeq Stranded RNA kits deliver unmatched quality (Table 1). Figure 3 illustrates examples of the detected expression of coding and noncoding RNA transcribed from opposite strands at the same locus in both Arabidopsis and rice. In both cases, noncoding RNA species were not captured by polyA-based methods. Highly accurate strand information also increases the percentage of uniquely alignable reads, particularly in the assembly of poorly annotated transcriptomes, and enables accurate mapping and quantification of overlapping transcripts (Figure 4).

Flexible and Integrated Workflow

With an automation-friendly workflow, these kits provide fast and easy rRNA reduction and RNA library preparation. Each kit includes 2 workflows: the high-throughput protocol is ideal for projects with ≥ 48 samples, and the low-throughput protocol is best suited for projects with ≤ 48 samples. The plate-based assay and barcoding solution enable simultaneous processing of up to 96 samples, providing a scalable approach to support a range of study designs.
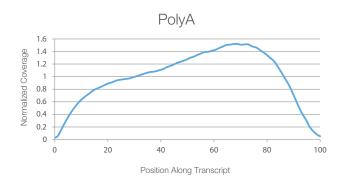

PolyA

Ribo-Zero

Figure 2: Even Coverage Across Transcripts— TruSeq Stranded Total RNA kits deliver uniform coverage across transcripts. Arabidopsis samples prepared using Ribo-Zero chemistry show increased coverage uniformity when compared to samples prepared using polyA-based methods.
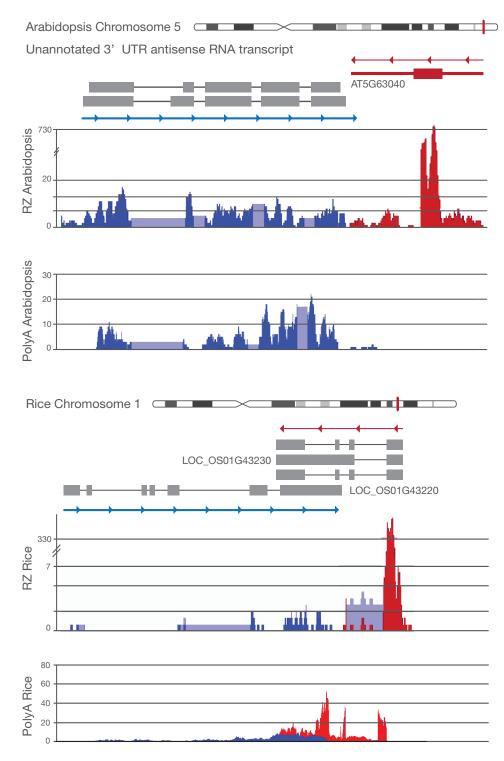
Figure 3: Identification of Antisense Noncoding RNA Transcripts—When prepared using either Ribo-Zero (RZ) or polyA-based mRNA methods (upper and lower panels, respectively, for each species), the expression of protein-coding mRNA transcripts is detected in both Arabidopsis and rice tissues (shown in blue). However, in each case, only Total RNA with Ribo-Zero prep also detects the expression of noncoding RNA from opposite strands (shown in red, AT5G63040 in Arabidopsis and LOC_Os01g43230 in rice).
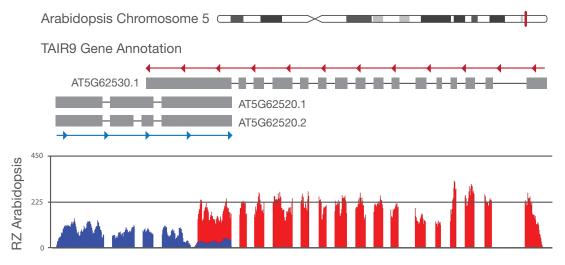
Figure 4: Strand Specificity Enables Accurate Alignment and Mapping— The ability to differentiate between sense and antisense expression enables accurate quantification of gene expression. This figure illustrates how strand information enables accurate quantification of overlapping genes. In the example above, 2 Arabidopsis mRNA transcripts that are encoded on opposite strands and overlap at the 3' end are accurately differentiated. Expression levels for AT5G62520 are shown in blue, and expression of AT5G62530 is shown in red.

Summary

TruSeq Stranded Total RNA Library Preparation Kits with Ribo-Zero Plant deliver a comprehensive, clear view of the plant transcriptome. RNA-Seq technology provides precise measurement of strand orientation, uniform coverage, and high-confidence discovery of features such as alternative transcripts and allele-specific expression. These kits couple the data quality of TruSeq library preparation with the efficient capture of both coding and noncoding RNA enabled by Ribo-Zero ribosomal reduction chemistry, providing a robust and scalable end-to-end solution for whole-transcriptome analysis compatible with a wide range of plant species.

Ordering Information

Product	Catalog No.
TruSeq Stranded Total RNA LT Library Preparation Kit with Ribo-Zero Plant, Set A (48 samples)	RS-122-2401
TruSeq Stranded Total RNA LT Library Preparation Kit with Ribo-Zero Plant, Set B (24 samples)	RS-122-2402
TruSeq Stranded Total RNA HT Library Preparation Kit with Ribo-Zero Plant (96 samples)	RS-122-2403

References

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