TruSeq® Ribo Profile Kit

A rapid, powerful technique to study mRNA translation.

Highlights

- **Sequence ribosome-protected mRNA**
  Predict protein abundance, investigate translational control, and measure gene expression
- **Rapid, scalable spin-column method**
  No ultracentrifuge required, eliminating time-consuming sucrose gradients/cushions
- **Study lower and higher eukaryotes**
  TruSeq Ribo Profile Kits are available for yeast and mammalian samples

Sequence Actively Translated mRNA

The TruSeq Ribo Profile Kits provide a powerful method for studying mRNA translation by next-generation sequencing. The kits enable sequencing of mRNA fragments actively undergoing translation by ribosomes. These mRNA fragments are called footprinted or ribosome-protected fragments (RPFs) and provide a snapshot of the active ribosomes in a cell. Biological samples collected at different times or stages of the cell cycle often show changes in translation. Samples treated with different chemicals also show different translation patterns.

Workflow

The TruSeq Ribo Profile Kits contain needed reagents and protocols to lyse cells, isolate RPFs, and convert them into an Illumina-compatible sequencing library. To generate the RPFs, cells are lysed with the included polysome buffer. The lysate is treated with a nuclease and passed through a size-exclusion chromatography (SEC) column. This step is followed by RNA extraction from the monosome fraction. The RNA samples are then treated with the Illumina Ribo-Zero™ Kit to deplete the samples of as much rRNA contamination as possible before polyacrylamide gel electrophoresis (PAGE) purification of the ~30 nt RPFs. Following reverse transcription, the cDNA is circularized to create a template for PCR. Indexed PCR primers are used during amplification to permit multiplexing. Figure 1 summarizes the kit workflow.

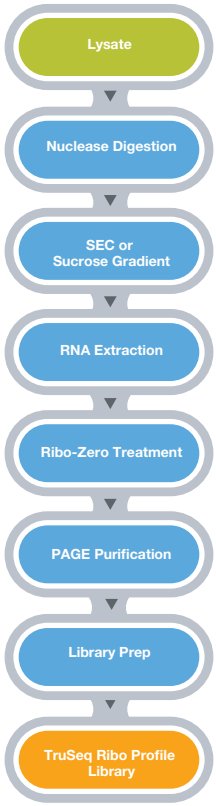

Figure 1: Workflow for the TruSeq Ribo Profile Kit.

Size-Exclusion Method

The TruSeq Ribo Profile method uses a size-exclusion column (SEC) to enrich for genomic and splice junction regions. Sucrose gradient and SEC column techniques produced comparable results (Table 1).

Table 1: Sequencing Metrics.

Method	rRNA*	tRNA*	Genome and Splice Junctions*
SEC columns	30.0%	7.6%	44.2%
Sucrose gradient	44.2%	2.7%	36.6%
Total RNA	2.5%	3.4%	45.0

*Percent of reads aligning to specified areas of the genome.

Sequence Only Protein-Coding Regions

Samples prepared with the TruSeq Ribo Profile Kits are enriched for open-reading frames (ORFs) and devoid of untranslated region (UTR) sequences (Figure 2). The start and stop codons are easily seen. Sequences are focused on protein-coding regions.

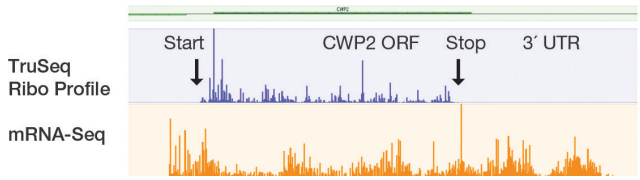

Figure 2: TruSeq Ribo Profile Kits Identify Actively Translated mRNAs. Compared to mRNA-Seq, ribosomal profiling samples are enriched for ORFs and lack untranslated regions.

The TruSeq Ribo Profile method captures the translationally active fraction of the cell. The majority of the sequencing reads map to protein-coding regions of the transcriptome. Very few sequencing reads map to untranslated regions.

Coverage patterns are different between TruSeq Ribo Profile and total RNA samples (Figure 3). TruSeq Ribo Profile samples contain a high amount of coding reads.

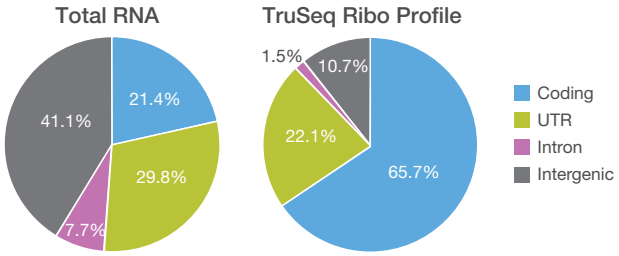

Figure 3: Distribution of Reads from Total RNA-Seq and TruSeq Ribo Profile Kits. Percent of reads that align to coding regions, untranslated regions (UTRs), introns, or intergenic regions.

Ordering Information

Product	Size	Catalog No.
TruSeq Ribo Profile Kit—Yeast	12 rxn	RPYSC12116
TruSeq Ribo Profile Kit—Mammalian	12 rxn	RPHMR12126
Ribo-Zero Magnetic Gold Kit (Yeast)	6 rxn	MRZY1306
	24 rxn	MRZY1324
Ribo-Zero Magnetic Gold Kit (Human/Mouse/Rat)	6 rxn	MRZG126
	24 rxn	MRZG12324

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