A Rapid Method for Ribosome Profiling

The TruSeq® Ribo Profile Kit streamlines library prep.

Introduction

Traditional RNA-Seq experiments are effective for surveying the transcribed regions of the genome but are not designed specifically to measure mRNA involved in active translation. Recent interest in this area of research has led to the development of ribosome-profiling techniques to examine the transcripts associated with the translation machinery directly. One technique is to isolate and sequence the nuclease-resistant ribosome-protected fragments or footprints (RPFs), which comprise approximately 30 nucleotides of mRNA bound to the ribosome during translation.

Existing ribosome-profiling protocols in the literature[1] require multiple polyacrylamide gel electrophoresis (PAGE) purifications and can take 5–7 days from cell lysis to a sequencer-ready library. In contrast, Illumina has streamlined these protocols to develop the TruSeq Ribo profile Kits. The protocol requires only two PAGE purifications and significantly reduces the time and labor to move from cell lysis to a sequencer-ready library in 3–4 days. Besides eliminating half of the PAGE purifications, the method introduces the use of size-exclusion chromatography (SEC) spin columns, instead of sucrose gradients or cushions, to purify RPFs. These columns do not require the use of specialized equipment, such as an ultracentrifuge, gradient stations or fractionation collectors. As a result, the workflow reduces bench time for purifying the RPFs from approximately 1 day to 1.5 hours. This application note provides an overview of the workflow and shows typical results obtained with the TruSeq Ribo Profile Kits.

Methods Overview

The TruSeq Ribo Profile Kits contain 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 an 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 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.

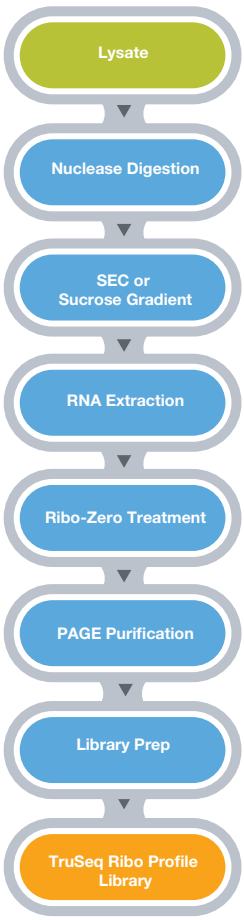

Figure 1: Workflow for the TruSeq Ribo Profile Kit.

Results and Data Analysis

Comparison of Sucrose and SEC Methods

TruSeq Ribo Profile libraries of HEK293 cells were constructed using sucrose gradient and SEC columns for isolating the monosomes. The sequencing data were aligned and annotated using open-source RNA-Seq tools such as TopHat and Cufflinks. Data quality was measured using several common RNA-Seq metrics, using a nonfootprinted Ribo-Zero–treated total RNA library as a reference. Sucrose gradient and SEC column techniques produced comparable results (Table 1). The relative percentage of reads aligning to coding transcripts, as well as the overall levels of expected contaminants such as rRNA and tRNA, are similar for both RPF purification methods (Table 1 and Figure 2A). In contrast, the nonfootprinted total RNA sample resulted in less sequencing contaminants than the RPF samples, demonstrating that ribosome-enriched samples pose a particular challenge when they undergo depletion of short rRNA fragments generated by nuclease treatments. After filtering the data of these expected contaminants, the resulting reads that align to mRNA are of high quality and can be further categorized to highlight the differences between footprinted samples versus more traditional total RNA–type samples.

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.

Characteristics and Distribution of Aligned Reads

Because RPF samples are derived from the translationally active fraction of the cell, most of the reads are expected to map to coding regions of the transcriptome, rather than to untranslated regions. Indeed, this expectation is demonstrated in Figure 2A. Additionally, regardless of whether the sucrose gradient or SEC method was used to isolate the RPFs, the distribution and enrichment of coding sequence over other regions of the transcripts are very similar. Illumina also examined if the type of monosome purification method used affected coverage distribution across transcripts (Figure 2B). Nonfootprinted, total RNA samples showed fairly even coverage across transcripts with very little 5′ or 3′ bias. Conversely, both the sucrose gradient and SEC column samples demonstrated a distinct bias toward the 5′ end of transcripts. This characteristic of footprinted samples is due to a combination of the nuclease digestion and treatment with cycloheximide, the agent used to inhibit translation elongation and lock ribosome positions.

Conclusions

Ribosome profiling is a powerful technique capable of defining the proteome of complex organisms by systematic monitoring of cellular translation processes. The TruSeq Ribo Profile Kits provide a simple alternative to isolating RPFs by using SEC columns. This method is rapid, scalable, and does not require specialized equipment, unlike sucrose gradient or cushion techniques. With these improvements, the TruSeq Ribo Profile Kits are now the preferred method for investigating translational control.

Reference

1. Ingolia NT, Ghaemmaghami S, Newman JRS, Weissman JS. Genome-wide analysis *in vivo* of translation with nucleotide resolution using ribosome profiling. ***Science*** 2009;324:218–223.

Figure 2: Distribution of Aligned Reads. A) Percent of reads that align to coding regions, untranslated regions (UTRs), introns, or intergenic regions. B) Coverage as a function of distance from the 5′ end of transcripts. The total RNA sample demonstrates a fairly equal distribution of reads from 5′ to 3′. In contrast, samples prepared from footprinted RNA show 5′ bias and very little coverage near transcript 3′ ends, characteristic of ribosome footprints. Libraries from both SEC and sucrose gradient samples demonstrate very similar profiles.

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