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Ribo-Zero Gold Kit: improved RNA-seq results after removal of cytoplasmic and mitochondrial ribosomal RNA

Ribosomal RNA (rRNA) constitutes the majority (>98%) of total RNA preparations. To avoid wasting sequencing reads, it is necessary to remove this abundant RNA before preparing RNA libraries for deep sequencing. We previously developed Ribo-Zero™ technology for cytoplasmic rRNA removal from intact and degraded RNA. Here, we describe the Ribo-Zero Gold Kit: an improvement to the original Ribo-Zero method that allows removal of mitochondrial as well as cytoplasmic rRNA from total RNA preparations.

Introduction

Deep sequencing of cDNA prepared from total RNA (RNA-seq) or mRNA (mRNA-seq) has become the method of choice for transcript profiling, discovery of new transcripts and identification of alternative splicing events. In particular, the quantitative nature, depth and dynamic range of mRNA-seq has made it a useful method for so-called digital gene expression compared with microarray-based methods[1].

However, a standard whole-transcriptome approach to RNA-seq faces a significant challenge, as the vast majority of reads map to rRNA. One solution is to enrich the sample RNA in polyadenylated transcripts using oligo(dT)-based affinity matrices, but such poly(A) enrichment eliminates other biologically relevant RNA species, such as microRNA and other noncoding RNAs. To overcome these challenges, we previously developed the Ribo-Zero family of kits for rRNA removal. Unlike a competitive hybridization-based rRNA removal technology, the Ribo-Zero kits provide excellent removal of cytoplasmic rRNA even from degraded or archived RNA samples.

In addition to cytoplasmic rRNA, total RNA preparations contain mitochondrial ribosomal RNA (mtrRNA). The abundance of mtrRNA is highly variable, because the expression of mitochondrial 12S and 16S rRNA genes, as well as the number of mitochondria, vary widely within a given cell type and stage of differentiation[2]. As with rRNA reads, mtrRNA reads are often filtered when performing analysis of RNA-seq data. Therefore, we improved on the original Ribo-Zero technology to develop a new kit (the Ribo-Zero Gold Kit) that removes mtrRNA as well as cytoplasmic rRNA from total RNA extracted from mammalian cells.

Methods overview

The Ribo-Zero Gold Kit uses a proprietary method that is optimized for removal of all sizes of rRNA. Intact or degraded total RNA samples (100 ng to 5 µg) are mixed with the rRNA Removal Reagents in solution (25 minutes). The mixture is then added to Ribo-Zero Gold Microspheres and incubated for 20 minutes, followed by removal of the Microspheres with a spin-filter column (2 minutes). The rRNA-depleted RNA is recovered either by ethanol precipitation or a column-purification method of choice.

For this analysis, total RNA was isolated from MCF-7 cells using Trizol® reagent and 5 µg was used as input for either the standard Ribo-Zero or the Ribo-Zero Gold Kit. Following rRNA removal, the RNA was used to prepare cDNA libraries using the ScriptSeq™ mRNA-Seq Library Preparation Kit (Epicentre). Libraries were sequenced on Illumina® GAIIx and HiSeq 2000 platforms.

Removal of rRNA and mtrRNA

Overall, we observed good removal of rRNA after using the Ribo-Zero Gold Kit as shown by Bioanalyzer profiles in **Figure 1**. Libraries prepared from RNA treated with a standard Ribo-Zero Kit and the Ribo-Zero Gold Kit showed similar results on analysis of RNA-seq data. As shown in **Table 1**, the number of reads passing filter and alignable reads for both sets of libraries are within normal limits for Illumina sequencing.

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APPLICATION NOTES

Table 1 | Summary of RNA-seq results from libraries prepared after treatment with the Ribo-Zero and Ribo-Zero Gold Kits.

Run	Total number of reads	Reads passing filter (PF)	Reads PF alignable	% PF	% PF alignable
1: Ribo-Zero	39195935	31945984	20916020	81.50	65.47
2: Ribo-Zero Gold	75183911	67698522	38938707	90.04	57.52
3: Ribo-Zero Gold	20482965	16393068	9562481	80.03	58.33

Run 1, 76-base reads on GAIIx. Run 2, 105-base reads on HiSeq 2000. Run 3, 150-base reads on GAIIx.

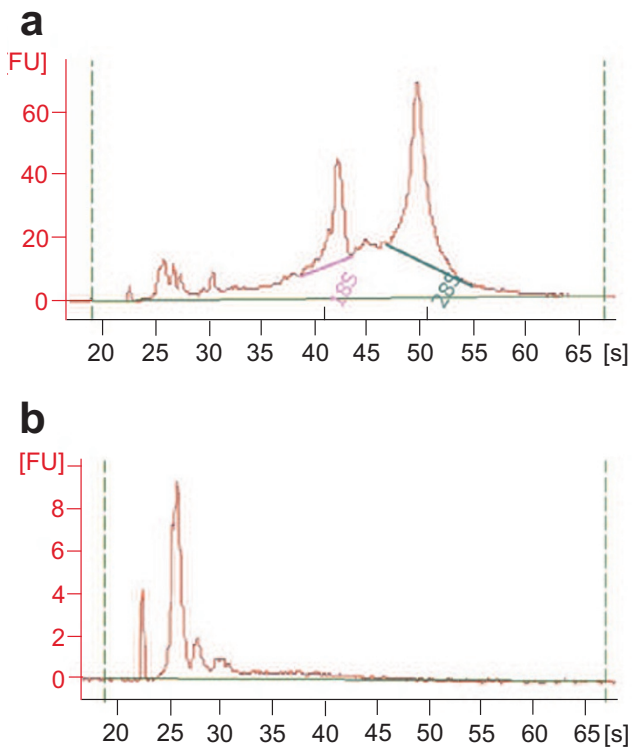

Figure 1 | Removal of ribosomal RNAs from total RNA. (**a,b**) Total RNA from MCF-7 cells was examined using a nanochip on a Bioanalyzer 2100 before (**a**) and after (**b**) treatment with the Ribo-Zero Gold Kit.

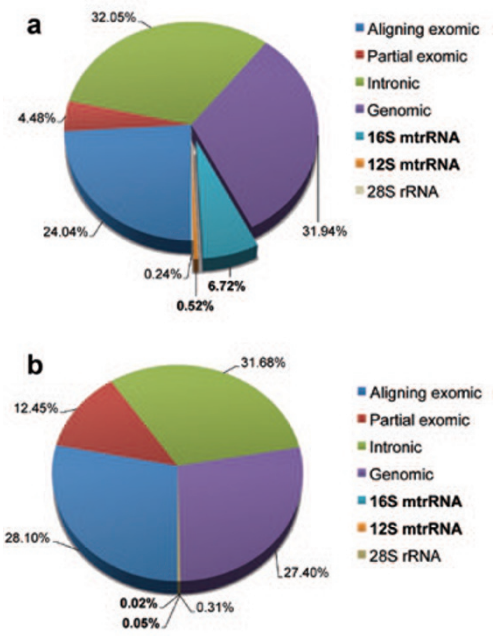

Figure 2 | (**a,b**) Profiles of RNA-seq libraries prepared after treatment with the Ribo-Zero (**a**) and Ribo-Zero Gold (**b**) Kits.

Improved mapping of exomic reads

We performed further analysis of the RNA-seq data using Genomatix RegionMiner to classify reads into partial exomic, complete exomic, intronic and mtrRNA sequences (**Fig. 2**). We observed that the library prepared after treatment of RNA with the Ribo-Zero Gold Kit was enriched in reads aligning to exomic sequences. In comparing mtrRNA reads, we observed reduction from 7.2% to less than 0.5% with the Ribo-Zero Gold Kit.

It is worth noting that conventional RNA-seq library preparation with poly(A)-enriched RNA appears to give a higher percentage of exomic reads than the libraries prepared in our analysis using Ribo-Zero technology. This is because the Ribo-Zero method permits the inclusion of many other nonexomic but biologically relevant RNA species that are lost when using poly(A) enrichment. Thus, the overall percentage of exomic reads in libraries prepared after Ribo-Zero treatment will appear lower compared with those prepared from poly(A)-enriched RNA.

Conclusions

The Ribo-Zero Gold Kit improves RNA-seq results by removing mtrRNA as well as cytoplasmic rRNA, allowing improved mapping of exomic reads. The kit delivers rRNA removal performance comparable to that of the original Ribo-Zero Kits and can be used with intact and degraded RNA. The Ribo-Zero Gold Kit offers a benefit to those researchers who wish to maximize the number of useful RNA-seq reads from total RNA derived from mammalian cells. Ribo-Zero Gold Kits for other organisms are under development.

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