RiboGone™ kit for Mammalian Samples

Highly Effective rRNA Removal for Transcriptome Analysis

that's GOOD science!®

- Remove rRNA and mtRNA sequences from mammalian total RNA prior to cDNA synthesis
- Suitable for full-length or degraded RNA
- Obtain highly accurate transcriptome analysis data (based on MAQC data comparison)

Introduction

Next Generation Sequencing (NGS) is a key tool for expression analysis of the entire transcriptome, with high sensitivity and a wide dynamic range. Random-primed cDNA synthesis kits, such as the SMARTer® Universal Low Input RNA Kit for Sequencing and the SMARTer Stranded RNA-Seq Kit, are ideal for transcriptome analysis from all types of input RNA, including compromised RNA samples and bacterial RNA.

Prior to cDNA synthesis with any random-primed cDNA synthesis kit, it is important to remove ribosomal RNA, which makes up ≥90% of total RNA. The RiboGone - Mammalian kit uses hybridization technology and RNase H digestion to bind and specifically deplete *5S*, *5.8S*, *18S*, and *28S* nuclear rRNA sequences and *12S* mitochondrial rRNA sequences from full length or sheared total RNA derived from human, mouse, or rat samples. (This kit does not deplete *16S* mitochondrial RNA sequences, which share significant homology with some nuclear genes.) Following RiboGone - Mammalian treatment, the RNA sample is ready for cDNA synthesis and RNA-Seq.

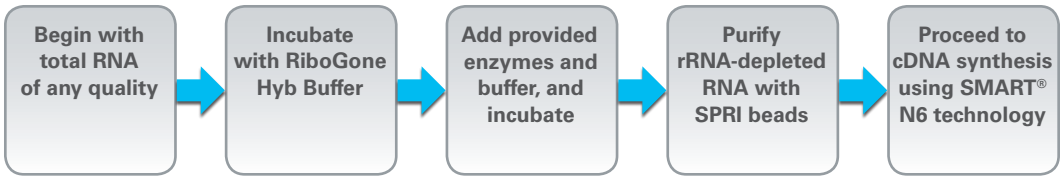

Figure 1. Workflow for RiboGone rRNA depletion. This kit is designed for use with low input samples containing 10–100 ng of total RNA, and works with full-length or degraded RNA. Samples processed using the RiboGone kit are ready for cDNA synthesis with any random-primed SMARTer RNA-Seq kit, including the SMARTer Stranded RNA-Seq Kit, SMARTer Universal Low Input RNA Kit for Sequencing, and SMARTer Universal Low Input RNA Library Prep Kit.

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rRNA removal, cDNA library production, and sequencing

The RiboGone - Mammalian kit was used to clear rRNA from 10 ng samples of Human Universal Reference RNA (HURR; Agilent) and Human Brain Reference RNA (HBRR; Ambion), the same RNAs used in the MicroArray Quality Control (MAQC) project (1), according to the RiboGone kit protocol (Figure 1). The rRNA-cleared samples were then converted to cDNA with the SMARTer Stranded RNA-Seq Kit according to the SMARTer Stranded RNA-Seq protocol, using 18 cycles of PCR. The HURR and HBRR libraries were sequenced on an Illumina® MiSeq® instrument, with ~7M 1x50 bp single end reads. The HURR and HBRR libraries both yielded a high number of reads, with 75–76% mapped, 66–70% uniquely mapped, and over 13,800 genes identified. As expected after treatment with the RiboGone - Mammalian kit, less than 1% of reads mapped to rRNA (Table I).

Table I: Sequence Alignment Metrics

	Human Universal Reference RNA (HURR)		Human Brain Reference RNA (HBRR)	
No. of reads	6,829,540		7,728,850	
Mapped to rRNA	62,792	(0.9%)	49,844	(0.7%)
Mapped to mitochondrial RNA	318,006	(4.7%)	224,939	(2.9%)
Mapped to RefSeq	4,871,900	(76%)	5,515,264	(75%)
Mapped uniquely to RefSeq	4,435,123	(70%)	4,888,340	(66%)
Exons	2,311,575	(47%)	2,712,444	(49%)
Introns	2,560,325	(53%)	2,802,820	(51%)
Genes identified	14,563		13,839	

Table I. Sequencing alignment metrics for HURR and HBRR libraries. 10 ng samples of intact HURR and HBRR were rRNA-depleted using the RiboGone - Mammalian kit, and cDNA libraries were created with the SMARTer Stranded RNA-Seq Kit. Libraries were sequenced on an Illumina MiSeq Platform with ~7M 1x50 bp single end reads per library. Reads were trimmed by CLC Genomics Workbench and mapped to rRNA and the mitochondrial genome with CLC (% reads indicated). The unmapped reads were subsequently mapped with CLC to the human genome with RefSeq masking, producing mapped reads and uniquely mapped reads. The number of genes identified in each library was determined by the number of genes with an RPKM (read per kilobase of exon per million of reads) of at least 0.1. The number of reads that map to introns or exons is a percentage of the reads successfully mapped to RefSeq.

RNA-Seq data is highly correlated with MAQC qPCR data

In order to measure the accuracy of RNA-Seq data obtained after RiboGone rRNA depletion and cDNA synthesis with SMART technology, we compared differential expression data for HURR and HBRR (obtained as described above) with qPCR data for the same RNAs obtained through the MAQC study. The high level of correlation with MAQC (R = 0.860) suggests that the RNA-Seq data was not affected by rRNA depletion with the RiboGone kit (Figure 2).

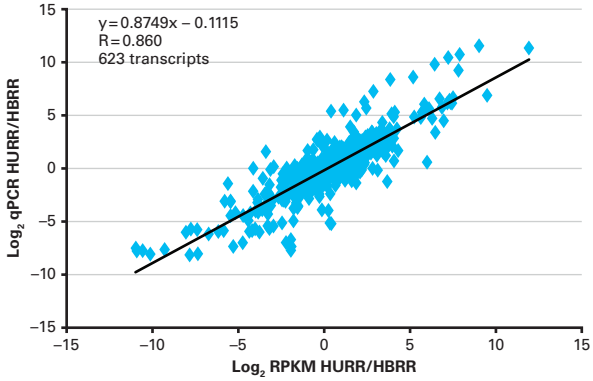

Figure 2. High correlation between SMARTer RNA-Seq data and MAQC qPCR data. A scatter plot was used to compare differential expression data obtained from SMARTer transcriptome analysis of HURR and HBRR cDNA libraries (Table I) and qPCR data for HURR and HBRR from the MAQC project. The slope (0.846) and correlation (0.860) for the comparison line of expression ratio (in RPKM) and qPCR ratio (in C_t) are plotted for HURR and HBRR, in a $\log_2$ scale. The transcripts used in this analysis were the 623 of ~900 transcripts present in the MAQC data set that were also detected in both the HURR and HBRR RNA-Seq data sets.

Summary

Random priming extends the applicability of transcriptome analysis to include samples which contain non-polyadenylated and/or compromised input RNA. However, in order to maximize RNA-Seq data quality and quantity, random priming RNA-Seq kits must be paired with rRNA removal methods. The RiboGone - Mammalian kit specifically removes *5S*, *5.8S*, *18S*, and *28S* rRNA sequences (as well as *12S* mitochondrial rRNA sequences) from human, mouse, or rat total RNA. In this study, rRNA reads were reduced to less than 1% of the RNA-Seq reads and mtRNA reads were reduced to less than 5% of the RNA-Seq library reads (Table I). A comparison of differential expression levels measured using RNA-Seq data and MAQC data confirmed that RiboGone rRNA depletion followed by SMARTer random-primed cDNA synthesis provide highly accurate and reliable data (Figure 2).

For more information about this and other kits for NGS, please visit: www.clontech.com/NGS

References

1. MAQC Consortium (2006) *Nat. Biotechnol.* **24**(98):1151–1161.

PRODUCTS

Cat. #	Product	Package Size
634846	RiboGone - Mammalian	6 Rxns
634847		24 Rxns
634836	SMARTer Stranded RNA-Seq Kit*	12 Rxns
634837		24 Rxns
634838		48 Rxns
634839		96 Rxns
634947	Low Input Library Prep Kit**	12 Rxns
634938	SMARTer Universal Low Input RNA Kit for Sequencing	10 Rxns
634940		25 Rxns
634945	SMARTer Universal Low Input RNA Library Prep Kit*	10 Rxns
634946		25 Rxns

* Illumina platform only. No separate Library Prep Kit required.
** Library prep kit for Illumina platforms, for use with Cat. Nos. 634938 and 634940. Included as a part of Cat. Nos. 634945 and 634946.

Notice to Purchaser

RUBICON GENOMICS breaking the sample barrier

The Low Input Library Prep Kit and SMARTer Universal Low Input RNA Library Prep Kit contain ThruPLEX-FD technology developed and manufactured by Rubicon Genomics Inc., Ann Arbor, Michigan, USA and protected by US Patent 7,803,550; EP1924704; and US and international patents pending.