Prep degraded samples for RNA-Seq with RiboGone™ rRNA removal & SMARTer® cDNA synthesis

Prepare RNA from FFPE Samples for Transcriptome Analysis Studies

- Remove rRNA sequences and produce libraries from small amounts (10–100 ng) of degraded RNA (RIN 2–3)
- Obtain complete transcriptome coverage from FFPE samples, using RiboGone - Mammalian and SMARTer N6 priming methods

that's GOOD science!®

Introduction

Next Generation Sequencing (NGS) is a key tool for transcriptome analysis, with high sensitivity and a wide dynamic range. One challenge in NGS transcriptome analysis studies centers around FFPE (formaldehyde fixed paraffin embedded) tissue, in which the RNA is typically degraded.

Random-primed cDNA synthesis is an ideal solution for transcriptome analysis from FFPE tissue and other samples containing fragmented RNA; however, ribosomal RNA (which makes up ≥90% of total RNA) must be removed from these samples prior to cDNA synthesis. The RiboGone - Mammalian kit uses hybridization technology and RNase H digestion to identify and specifically degrade/eliminate *5S, 5.8S, 18S,* and *28S* nuclear rRNA sequences and *12S* mitochondrial RNA sequences from RNA derived from human, mouse, or rat tissues.

Following RiboGone - Mammalian treatment, the RNA sample is ready for random-primed cDNA synthesis with the SMARTer Universal Low Input RNA Kit for Sequencing or the SMARTer Stranded RNA-Seq Kit, both of which excel at cDNA amplification from low-input, fragmented RNA such as that found in FFPE tissue.

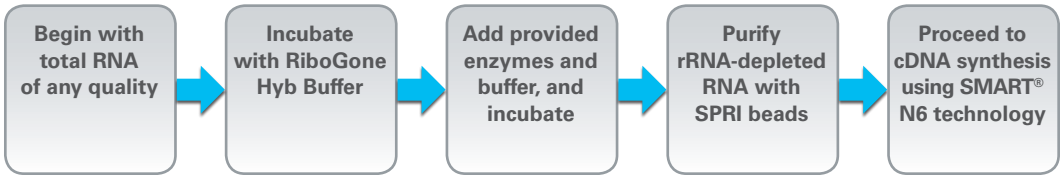

Figure 1. Workflow for RiboGone rRNA depletion. This kit is designed for use with low input samples containing 10–100 ng of total RNA, and works with full-length or degraded RNA. Samples processed using the RiboGone kit are ready for cDNA synthesis with any random-primed SMARTer RNA-Seq kit, including 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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RNA extraction & rRNA removal from FFPE tissue

Total RNA was extracted from curls of breast carcinoma FFPE tissue (Cureline) using the NucleoSpin totalRNA FFPE kit (Cat. Nos. 740982.10, 740982.50, and 740982.100) according to its protocol, using lysis method B with a 75-minute incubation at 56°C and the optional on-column DNase treatment. The profile of the RNA is illustrated on an electropherogram trace, with a broad peak at <200 bp (Figure 2). The RiboGone - Mammalian kit was then used to clear rRNA from 30 ng of the extracted total RNA according to the RiboGone kit protocol (Figure 1).

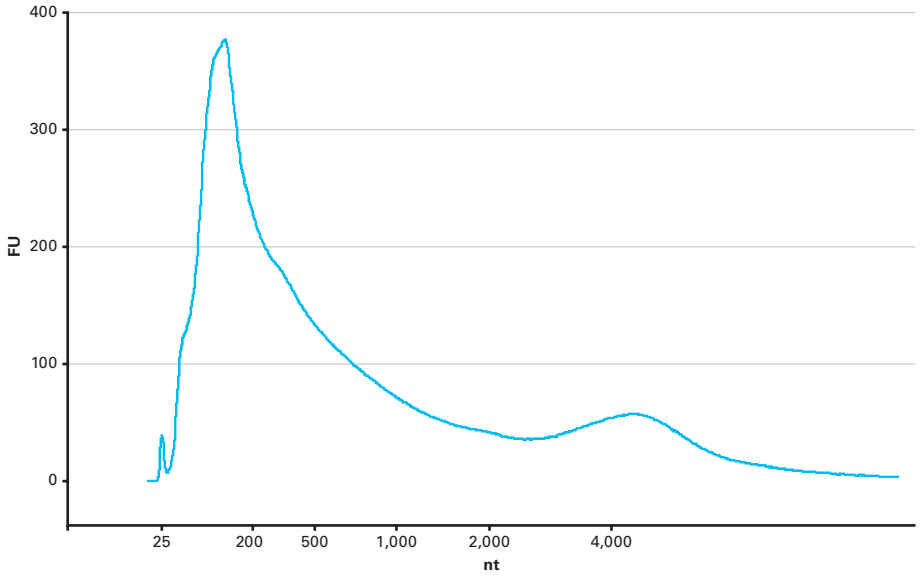


Figure 2. Degraded nature of FFPE RNA. Total RNA was extracted from FFPE tissue using NucleoSpin totalRNA FFPE and 1 µl of the extracted RNA was validated on an Agilent 2100 Bioanalyzer with a RNA 6000 Pico Chip. As expected for FFPE tissue, the electropherogram trace peaked at less than 200 bp in length, indicating degraded RNA.

Random-primed SMARTer cDNA synthesis from low-input FFPE RNA

In this application, 8 µl of rRNA-depleted FFPE RNA was converted to cDNA with a SMARTer Universal Low Input RNA Kit for Sequencing (Cat. Nos. 634938 and 634940) according to the kit protocol (Figure 3), using 18 PCR cycles for ds cDNA amplification due to the small amount and degraded nature of the RNA extracted from the FFPE curls. Illumina® adapters and indices were added using the Low Input Library Prep Kit (Cat. No. 634947) according to its protocol.

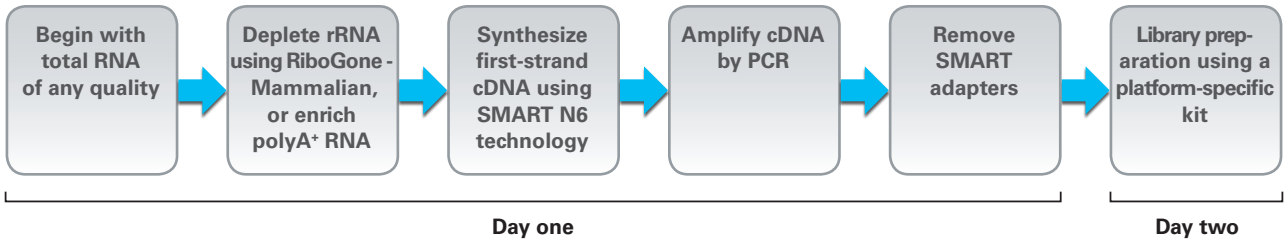
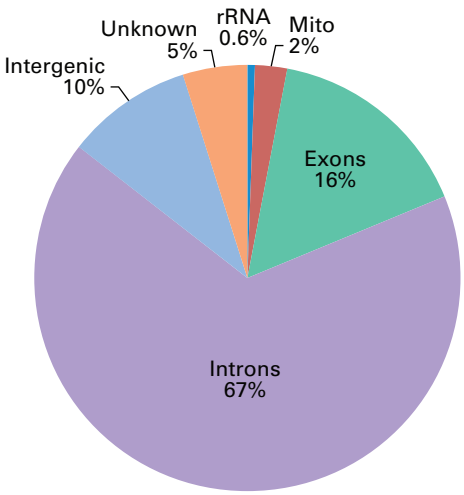


Figure 3. Workflow for random-primed cDNA synthesis with SMARTer Universal Low Input RNA kits. SMARTer Universal cDNA synthesis is random-primed, which makes it ideal for use with compromised mammalian RNA samples, e.g. RNA from FFPE tissue. Ribosomal RNA must be removed with RiboGone - Mammalian prior to SMARTer Universal cDNA synthesis.

Random primed cDNA synthesis kits (such as the SMARTer Universal Low Input kit used in this application) are essential when preparing compromised or non-poly-A RNA for transcriptome analysis. In order to generate stranded RNA-Seq data from this type of sample, we recommend using the SMARTer Stranded RNA-Seq Kit, which includes Illumina indexes and primers and identifies each transcript's strand of origin with >99% accuracy. All of our SMARTer kits are listed at http://www.clontech.com/smarter-ngs to help you select the best cDNA synthesis kit for your experiment.



High quality RNA-seq data from FFPE samples

The library was sequenced on an Illumina MiSeq® instrument with ~6M 1 x 50 bp paired end reads. rRNA reads were reduced to 0.6% of total reads, and 16,463 genes were identified. The number of reads that mapped to introns, exons, intergenic regions, rRNA, mitochondrial RNA, and unknown sources are shown as percentages of the total reads (Figure 4).

Figure 4. RiboGone treatment and random-primed SMARTer cDNA synthesis preserve transcriptome data while eliminating rRNA. Total RNA was extracted, cleared of rRNA, converted to cDNA and prepared for Illlumina sequencing as described above. The cDNA was sequenced on an Illumina MiSeq Platform with 1 x 50 bp reads, and the reads were trimmed by CLC Genomics Workbench and mapped to rRNA, the mitochondrial genome, and the human genome with RefSeq masking using CLC (% reads indicated). 16,463 genes were identified with an RPKM (reads 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 total reads.

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 primed 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, 16,463 genes were identified with an RPKM ≥ 0.1, while rRNA and mtRNA reads were reduced to <1% and ~2% of the RNA-Seq library reads, respectively (Figure 4). These data indicate that SMARTer random-primed cDNA synthesis paired with RiboGone rRNA depletion yields high-value RNA-Seq data, even from challenging samples such as small quantities of FFPE tissue.

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

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

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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.

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