Prepare RNA-seq libraries from FFPE samples

- rRNA removal and SMARTer cDNA synthesis:
  Remove rRNA sequences and produce sequencing libraries from small amounts (10–100 ng) of degraded RNA (RIN 2–3)
- RNA-seq data from FFPE samples:
  Obtain complete transcriptome coverage from FFPE samples, using RiboGone - Mammalian and SMARTer random priming methods

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.

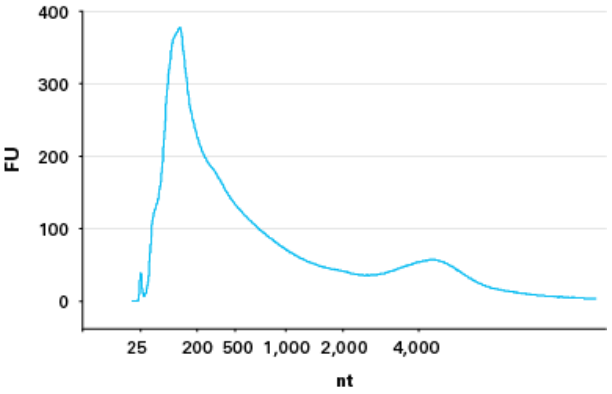
Random-primed cDNA synthesis is an ideal solution for transcriptome analysis from FFPE tissue and other samples containing fragmented RNA; however, ribosomal RNA (which makes up ≥90% of total RNA) must be removed from these samples prior to cDNA synthesis. The RiboGone - Mammalian kit uses hybridization 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, which excels at cDNA amplification from low-input, fragmented RNA such as that found in FFPE tissue. The cDNA can then be prepared for sequencing using either the Illumina-specific ThruPLEX DNA-seq library preparation kit or Ion Torrent library preparation kits.

Results

RNA extraction from FFPE tissue

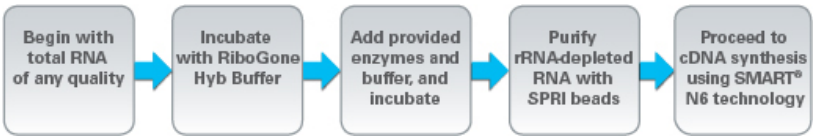
Total RNA extracted from curls of breast carcinoma FFPE tissue (Cureline), using the NucleoSpin totalRNA FFPE kit according to its protocol, is degraded. The profile of the RNA is illustrated on an electropherogram trace with a broad peak at <200 bp.



Example electropherogram showing the degraded nature of FFPE RNA. Extracted RNA was validated on an Agilent 2100 Bioanalyzer with a RNA 6000 Pico Chip.

rRNA removal & random-primed SMARTer cDNA synthesis from low-input FFPE RNA

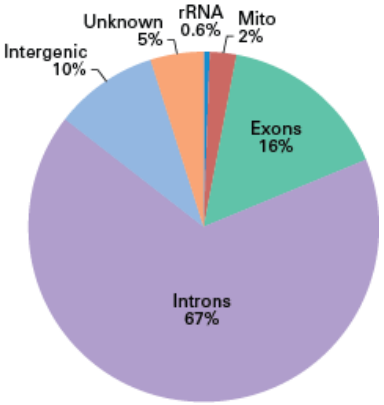
The RiboGone - Mammalian kit was used to clear rRNA from total RNA extracted from FFPE tissue. rRNA-depleted FFPE RNA was converted to cDNA with the SMARTer Universal Low Input RNA Kit for Sequencing according to the kit protocol. Illumina adapters and indices were added using the Low Input Library Prep Kit according to its protocol.



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.

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. RiboGone treatment and random-primed SMARTer cDNA synthesis preserve transcriptome data while eliminating rRNA.



Sequencing data generated from FFPE samples. rRNA reads were reduced to 0.6% of total reads. 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.

Conclusions

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. 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.

Methods

Total RNA was extracted from curls of breast carcinoma FFPE tissue (Cureline) using the NucleoSpin totalRNA FFPE kit according to its protocol, using lysis method B with a 75-minute incubation at 56°C and the optional on-column DNase treatment. 30 ng of the extracted total RNA was cleared of rRNA using the RiboGone - Mammalian kit according to the RiboGone kit protocol. 8 µl of rRNA-depleted FFPE RNA was converted to cDNA with the SMARTer Universal Low Input RNA Kit for Sequencing according to the kit protocol, 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 (now discontinued; replaced by the ThruPLEX DNA-Seq Kit) according to its protocol.

The RNA-seq library was sequenced on an Illumina MiSeq Platform with 1 x 50 bp reads. 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.

Related products

Cat. #	Product	Size	License	Quantity	Details
634938	SMARTer® Universal Low Input RNA Kit for Sequencing	10 Rxns		*	

The SMARTer Universal Low Input RNA Kit for Sequencing contains the components needed to synthesize high-quality cDNA from as little as 200 pg of RNA, and includes the Advantage 2 PCR Kit for PCR amplification and validation. The kit utilizes both SMART technology and random priming, and has been designed and validated to prepare cDNA samples for sequencing and quantification with next-generation sequencing platforms. While SMART technology offers unparalleled sensitivity and unbiased amplification of cDNA transcripts, random (universal) priming allows for amplification of damaged RNA and maintains the true representation of the original mRNA transcripts. Both of these factors are critical for transcriptome sequencing and gene expression analysis.

Documents	Components	You May Also Like	Image Data

634940	SMARTer® Universal Low Input RNA Kit for Sequencing	25 Rxns		*	
634846	RiboGone™ - Mammalian	6 Rxns		*	
634847	RiboGone™ - Mammalian	24 Rxns		*	

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