RNA-Seq with SMARTer Universal cDNA

Ion Torrent Sequencing of cDNA from the SMARTer® Universal Low Input RNA Kit

that's GOOD science!™

- Generate cDNA from low initial amounts of RNA or degraded RNA samples
- Use cDNA generated with the SMARTer Universal kit to obtain transcriptome data using your choice of Illumina® or Ion Torrent sequencing platforms

Introduction

Next Generation Sequencing (NGS) has allowed transcriptome analysis to achieve high sensitivity and a wide dynamic range. The SMARTer Universal Low Input RNA Kit for Sequencing is a random-primed cDNA synthesis kit ideal for analysis of both coding and non-coding RNA. The cDNA generated with the SMARTer Universal kit can be further processed using NGS platform-specific library preparation methods. The choice of sequencing platforms may be limited by access; therefore, having cDNA that is compatible with multiple sequencing platforms is an advantage. We have previously shown that the cDNA generated by the SMARTer Universal kit is compatible with Illumina sequencing platforms, and in this application note we show that it is also compatible with Ion Torrent platforms.

Generate cDNA from low input samples that is compatible with Ion Torrent platforms

SMART™ (**S**witching **M**echanism **a**t 5' End of **R**NA **T**emplate) technology, which forms the basis of the SMARTer Universal Low Input RNA Kit for Sequencing, generates robust and reproducible cDNA. This SMARTer kit is specifically designed to work well with degraded RNA, non-polyadenylated RNA, and/or low input RNA samples. The SMARTer Universal Low Input RNA Kit for Sequencing has the sensitivity to work optimally with 200 pg–100 ng of input material. Like all random primed cDNA synthesis kits, the SMARTer Universal kit requires ribosomal RNA depletion prior to use. When combined with the new RiboGone™ - Mammalian rRNA depletion kit, the SMARTer Universal kit generates reliable cDNA from mammalian total RNA, including low-quality RNA.

In this application, we treated 10 ng of total, sheared RNA (Human Universal Reference RNA, Agilent) with the RiboGone - Mammalian kit following the kit protocol. The resulting rRNA-depleted material was used as input for the SMARTer Universal Low Input RNA Kit for Sequencing. cDNA was generated following the SMARTer Universal kit protocol.

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2 ng of the cDNA was then used as input to create barcoded Ion Torrent-compatible libraries using the Ion Plus Fragment Library Kit (Life Technologies, Cat. No. 4471252) and the Ion Xpress Barcode Adapter 1–16 Kit (Life Technologies, Cat. No. 4471250). The Ion Xpress Plus Fragment Library Preparation User Guide was slightly modified by increasing the number of PCR cycles from 8 to 14 in order to accommodate the use of a lower amount of input cDNA. Fragments were size selected to 270 bp with an E-Gel Agarose Gel Electrophoresis System. Templated beads were made using an Ion OneTouch 2 system and loaded on an Ion 318 Chip. Sequencing was conducted using the Ion PGM system. The data resulting from Ion Torrent sequencing show that the SMARTer Universal Low Input RNA Kit for Sequencing generates cDNA from low concentrations of starting material that is compatible with Ion Torrent library prep kits and sequencing platforms (Table I).

Summary

Next generation sequencing is creating new opportunities to study whole transcriptome changes. Driving this development are the rapid advancements in sequencing technologies which lower the cost and time involved in acquiring transcriptome data. Along with the advancements in sequencing technology, the ability to generate cDNA for sequencing has also expanded to capture information from less than perfect, and low concentration, RNA samples such as the sheared, low-input sample used in this application. The SMARTer Universal Low Input RNA Kit for Sequencing, along with the new RiboGone - Mammalian kit, provides quality cDNA libraries and consistent data from low starting concentrations. Here we report that cDNA generated with SMART technology has the flexibility to be compatible with other library prep kits, such as the Ion Plus Fragment Library Kit, and Ion Torrent sequencing (Table I). Combined with earlier studies highlighting compatibility with Illumina platforms, these results show that SMART technology is truly universal (Table I).

Table I: Sequence Alignment Metrics		
Sample: Human Universal Reference RNA		
No. of reads	2,511,295	
Mapped to RefSeq	1,428,109	(74%)
Mapped uniquely to RefSeq	1,309,906	(68%)
Exons	601,101	(42%)
Introns	827,008	(58%)
No. of genes with at least 0.1 RPKM	12,551	

Table I. Sequencing alignment metrics for Ion PGM sequencing data generated from Human Universal Reference RNA. A 10 ng sample of sheared Human Universal Reference RNA was rRNA-depleted using the RiboGone - Mammalian kit. A cDNA library, created with the SMARTer Universal Low Input RNA Kit for Sequencing, was used in the Ion Plus Fragment Library Kit with the Ion Xpress Barcode Adapter 1–16 Kit and was sequenced on the Ion PGM sequencer. Reads were trimmed by CLC Genomics Workbench and mapped to rRNA (9.4% of total reads) and the mitochondrial genome (12% of total reads) with CLC. 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 reads that map to introns or exons is a percentage of the reads successfully mapped to RefSeq.

PRODUCTS

Cat. #	Product	Package Size
634846 634847	RiboGone - Mammalian	6 Rxns 24 Rxns
634938 634940	SMARTer Universal Low Input RNA Kit for Sequencing	10 Rxns 25 Rxns