A Complete Solution for Generating Stranded RNA-Seq Libraries from High-Input Total RNA

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Abstract
Expression analysis of the entire transcriptome by RNA sequencing (RNA-seq) can benefit greatly from high sensitivity, a wide range of sample input amounts, and easy-to-use protocols. A streamlined workflow, combining time-saving techniques with high-performance reagents, provides the opportunity to push each experiment to its fullest potential, improving efficiency and accuracy. Traditionally, the high amounts of ribosomal RNA (rRNA) in the starting material, and lengthy protocols required to incorporate platform-specific adaptors via ligation are major challenges to the generation of RNA-seq libraries from total RNA. The SMART<sup>®</sup> Stranded Total RNA Sample Prep Kit - Hi Mammalian is a unique and simplified solution for generating indexed cDNA libraries from mammalian total RNA suitable for next-generation sequencing (NGS) on any Illumina<sup>®</sup> platform. In this poster, we illustrate the method for efficient RNA removal and ligation-free library preparation used in this kit. We present data showing a high correlation between RNA-seq expression levels and qPCR data from the MAQC (Microarray Quality Control) analysis. Additionally, we present ERCC (External RNA Control) spike-in experiments demonstrating the dynamic range and accuracy of libraries prepared using this method. Finally, we probe the range of input RNA by comparing sequencing metrics from libraries generated using 100 ng–1 µg of total RNA with RNA integrity numbers (RINs) between 3 and 7. In total, these results demonstrate the high reproducibility, minimal bias, and high accuracy obtained using the SMART<sup>®</sup> Stranded Total RNA Sample Prep Kit - Hi Mammalian.

Introduction
The SMART<sup>®</sup> Stranded Total RNA Samples Prep Kit - Hi Mammalian uses inherently stranded SMART<sup>®</sup> (Switching Mechanism at 5’ End of RNA Template) technology (1) to both preserve strand information and add Illumina adapters during cDNA synthesis. This total RNA-seq kit also seamlessly integrates RiboGone™ technology—which uses RNase H digestion to specifically remove rRNA—to the workflow prior to cDNA synthesis. The combination of these two technologies into a single kit decreases the total time needed for RNA removal, cDNA synthesis, and Illumina library preparation down to approximately 5 hours, which is significantly shorter than other methods for generating total RNA-seq libraries that preserve strand of origin information.

Sequence Analysis
Libraries generated using the SMART<sup>®</sup> Stranded Total RNA Sample Prep Kit - Hi Mammalian from HRRR (Human Universal Reference RNA; Agilent) and HBRR (Human Brain Reference RNA; Ambion) were aligned with STAR against mm10 with Ensembl annotation. Libraries generated from Mouse Liver Total RNA (Clontech) were aligned with STAR against mm10 with Ensembl annotation. The percentage of reads that mapped to rRNA, exonic regions, intronic regions, intergenic regions, and the correct strand were defined by Picard analysis.

References

Table 1: Sequence Alignment Metrics

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<thead>
<tr>
<th>RNA Source</th>
<th>Human Universal</th>
<th>Human Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total reads</td>
<td>52,100,000</td>
<td>51,900,000</td>
</tr>
<tr>
<td>Mapped to genome (%)</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>Mapped uniquely to genome (%)</td>
<td>91</td>
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<tr>
<td>Exonic (%)</td>
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<td>43</td>
</tr>
<tr>
<td>Intronic (%)</td>
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<tr>
<td>Intergenic (%)</td>
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<td>24</td>
</tr>
<tr>
<td>Number of genes identified</td>
<td>12,172</td>
<td>12,172</td>
</tr>
<tr>
<td>Number of ERCC transcripts</td>
<td>4,549</td>
<td>4,549</td>
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</tbody>
</table>

Table 2: Sequence Alignment Metrics from RNA of Varying Quality

<table>
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<tr>
<th>RNA Source</th>
<th>Human Universal</th>
<th>Human Brain</th>
</tr>
</thead>
<tbody>
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<td>RIN</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Total reads</td>
<td>52,100,000</td>
<td>51,900,000</td>
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<tr>
<td>Mapped to genome (%)</td>
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<td>96</td>
</tr>
<tr>
<td>Mapped uniquely to genome (%)</td>
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<td>12,172</td>
</tr>
<tr>
<td>Number of ERCC transcripts</td>
<td>4,549</td>
<td>4,549</td>
</tr>
</tbody>
</table>

Sequence alignment metrics from different RNA sources: RNA-seq libraries were generated from two samples of 100 ng–1 µg of Universal Human Reference RNA (HRRR) and three samples of Universal Human Reference RNA (HBRR) with ERCC RNA Spike-In Mix 1 (Life Technologies) added to HRRR and HBRR. Libraries were sequenced on an Illumina MiSeq instrument generating 8.5 million paired-end reads (2 x 150 bp). Alignment data are displayed for both libraries, with the percentage of reads that mapped to rRNA, exonic regions, intronic regions, intergenic regions, and the correct strand.

Conclusions
The SMART<sup>®</sup> Stranded Total RNA Sample Prep Kit - Hi Mammalian is a complete solution for preparing indexed illumina sequencing libraries from 100 ng–1 µg of mammalian total RNA. This kit incorporates key RiboGone and SMART<sup>®</sup> technologies that significantly reduce hands-on time and increase efficiency while maintaining the high-quality sequencing data of low-input SMART<sup>®</sup> Stranded RNA-Seq KIts.

- Simplified protocol—a single kit for RNA removal, cDNA synthesis, and indexed Illumina library preparation
- High reproducibility—Consistent data across replicates as well as between RNA of different quality
- High accuracy and wide dynamic range—High correlation with MAQC data and accurate detection of ERCC controls

To download this poster visit: www.clontech.com/ABRF2015-Total-RNA-seq

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