Improved RNA-seq Library Quality and Workflow Enabled by Automated Preparative Gel Electrophoresis

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Methods Overview

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Epicentre’s ScriptSeq™ v2 Kit provides high-quality, directional RNA-Seq libraries with minimal hands-on time, and only two library purification steps. Studies were carried out to evaluate the Sage Science Pippin Prep™ system for the library purifications steps. The Pippin Prep is a preparative gel electrophoresis system that offers several potential advantages for this application, including further reduction in hands-on time, efficient removal of low molecular weight library contaminants (nucleotides, primer/adaptor artifacts), better reproducibility, and accurate size-selection of the library. ScriptSeq v2 workflow and library quality were compared between protocols using the Pippin Prep and protocols using standard column- and bead-based cleanup methods.

Results

Duplicate ScriptSeq v2 libraries were prepared from 5-ng samples of rat liver poly(A) mRNA (Stratagene). During the library generation process, the Qiagen MinElute™ DNA purification step was replaced with the Pippin Prep. Purified cDNA was amplified by 15 cycles of PCR and purified by the Pippin Prep for subsequent sequencing. All Pippin Prep purifications were performed on a 2% agarose cassette, no overflow, detection, and tape sealed over the elution port. Libraries were visualized on an Agilent® BioAnalyzer: Duplicates were highly reproducible.

Table 1. Summary of sequencing metrics.

<table>
<thead>
<tr>
<th>Sample (DNA purification, Library purification)</th>
<th>Reads (B)</th>
<th>Reads Passing Filter (%)</th>
<th>Reads %Q20 (%)</th>
<th>Total Mapped Reads (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pippin, AMPure</td>
<td>46.5</td>
<td>93.7</td>
<td>94.1</td>
<td>63.6</td>
</tr>
<tr>
<td>Qiagen, Pippin</td>
<td>63.4</td>
<td>91.8</td>
<td>98.3</td>
<td>70.8</td>
</tr>
<tr>
<td>Pippin, Pippin</td>
<td>59.6</td>
<td>91.6</td>
<td>95.5</td>
<td>68.8</td>
</tr>
</tbody>
</table>

Libraries were sequenced 1x 51 bp on an Illumina GAIIx. Percent mapped to reference was calculated by Bowtie with no more than two mismatches in the first 28 bases.

Conclusions

- Automation of purification steps in the ScriptSeq v2 workflow produces reproducible libraries of tight size-selection.
- Transcript coverage is the same among Qiagen MinElute, 1.0X AMPure XP, and Pippin Prep purifications.
- RNA-Seq libraries can be made from 500 pg to 50 ng of RNA with automated, reproducible purification.