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

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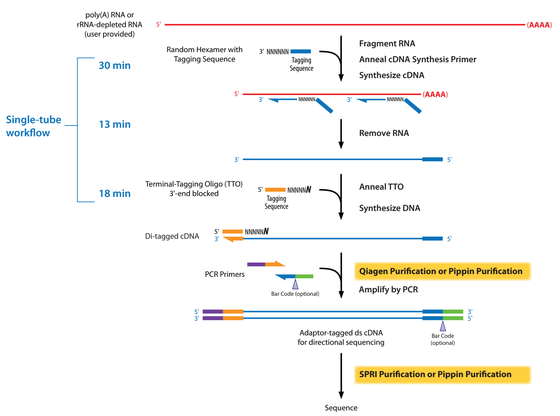
Introduction

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.

Methods Overview

Figure 1. Schematic overview of the ScriptSeq™ v2 method.



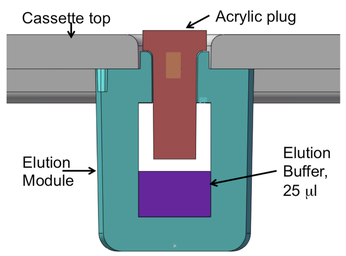
A patented terminal-tagging process generates directional RNA-seq libraries in approximately 4 hours from 500 pg to 50 ng of RNA. ScriptSeq Index PCR Primers (1-12) are available to add an Illumina® Index (barcode) to an RNA-Seq library prepared using the ScriptSeq v2 RNA-Seq Library Preparation Kit.

Automation of cDNA purification (before PCR) and library purification (after PCR) by the Pippin Prep results in libraries that are reproducible and tightly size-selected. Automation reduces hands-on time and is easy to set up.

Figure 2. The Pippin Prep™ system.

The Pippin Prep system is an automated, preparative electrophoresis system that includes a disposable five-channel precast agarose gel cassette and a computerized instrument that combines a power supply for electrophoresis with a fluorescence-based DNA detection unit. The cassette lanes are physically isolated from each other to prevent sample cross-contamination. DNA for library formation is collected in a membrane-delimited elution module. Fractionated DNA products are recovered in liquid buffer, and no gel extraction is required. Timing of DNA collection is determined by the onboard computer, which uses optical data from a DNA marker lane to determine the mobility of DNA through the cassette. Cassettes are manufactured in agarose concentrations of 3% (range 50-300 bp), 2% (75-600 bp), 1.5% (300-1,500 bp), and 0.75% (>1,500 bp).

Figure 3. Plug method to reduce Pippin Prep elution volumes.

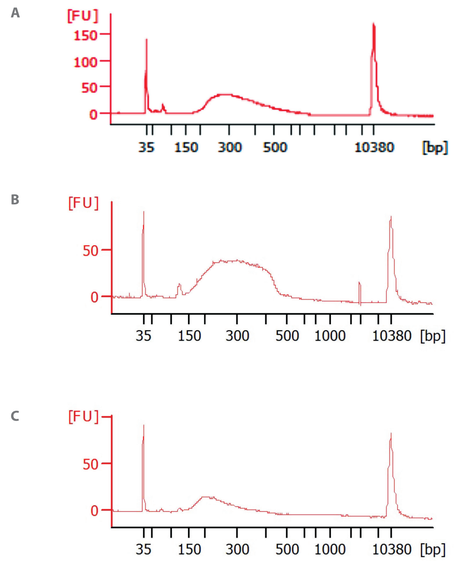


An acrylic plug inserted into the module reduces the air space above the elution buffer, concentrating the DNA.

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™ cDNA 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.

Figure 4. Tight size selection of libraries.



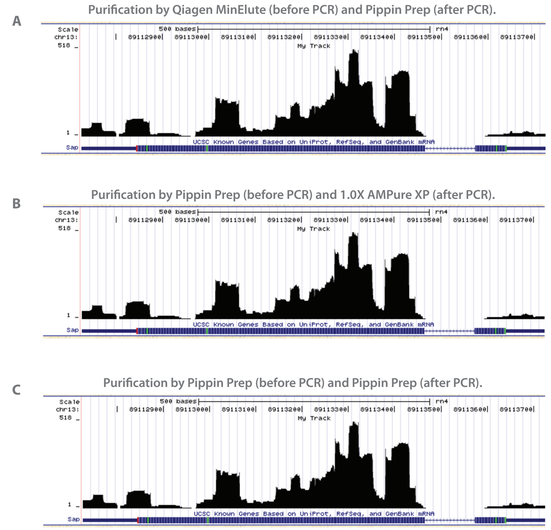
DNA was purified on the Pippin Prep using a selection range of (A) 76-500 nt for Qiagen replacement, (B) 135-500 nt for AMPure XP replacement, or (C) for replacement of both. Nucleotides and adaptor/primer artifacts are removed to ensure high-quality sequencing data.

Table 1. Summary of sequencing metrics.

Sample (cDNA purification, library purification	Reads (M)	Reads Passing Filter (%)	Reads >Q30 (%)	Total Mapped Reads (%)
Pippin, AMPure	46.5	93.7	96.1	73.6
Qiagen, Pippin	59	90.6	94.3	66.3
Pippin, Pippin	59.6	91.8	95	66.8

Libraries were sequenced 1 x 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.

Figure 5. Transcript coverage.



Transcript coverage from libraries purified on the Pippin Prep matches transcript coverage from libraries purified by Qiagen MinElute or 1.0X AMPure XP.

Conclusions

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