LEXOGEN

Enabling complete transcriptome sequencing

SPLIT RNA Extraction Kit

Technical Note: Recovery of small RNA

Introduction

Lexogen's SPLIT RNA extraction kit is designed to extract RNAs of all lengths from various sources, either as total RNA or split by size into a large RNA fraction and a small RNA fraction. Size-selection is achieved by adjusting the isopropanol volume used to precipitate the RNA onto the silica column.

Experiment

To test for efficient recovery of RNAs in the size range of siRNAs and miRNAs, mouse liver was homogenized and spiked with either single-stranded or double-stranded small RNA (miRNA or siRNA markers, New England Biolabs). Four homogenate aliquots were extracted according to the SPLIT User Guide, three of them included a silica column purification step with 1x, 1.5x or 1.75x volumes isopropanol to obtain total RNA. The respective recovery profiles were assessed using denaturing gels and microfluidics (Agilent 2100 Bioanalyzer).

Small RNA recovery in total RNA

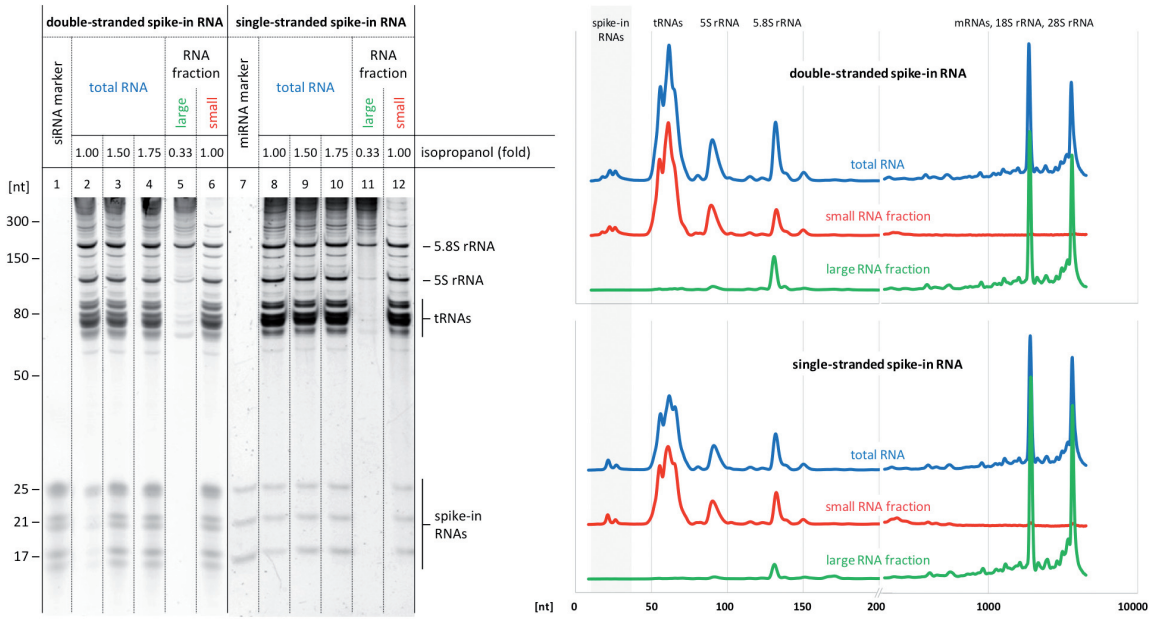
Binding of total RNA with 1.75x volumes isopropanol to the silica column results in quantitative recovery of both, single-stranded and double-stranded markers down to 17 nt (see figure). While adding 1x volume is already sufficient to obtain most of the small single-stranded RNA but fails to bind double-stranded small RNA efficiently. Increasing isopropanol to 1.5x volumes significantly improves recovery but not to the maximum.

Small RNA recovery in RNA fractions

From a fourth homogenate aliquot, the large RNA fraction was purified using 0.33x volume isopropanol, and the flow-through of this binding step was silica column purified by adding another 1x volume to yield the small RNA fraction (cumulative isopropanol volume: 1.66x).
In this "split" set-up, small RNAs down to 17 nt were efficiently recovered. Conversely, only RNA > 150 nt is quantitatively eluted in the large RNA fraction.

Conclusion

The SPLIT kit is ideally suited to recover miRNA-sized small RNAs either in a small RNA fraction or as part of the total RNA sample.



Denaturing polyacrylamide gel (LEFT). Total RNA extracts with 1x, 1.5x or 1.75x volumes isopropanol in the silica binding step are shown alongside large RNA fractions and small RNA fractions. The theoretical maximum spike-in RNA recovery amount of 81 ng (dsRNA, siRNA) or 108 ng (ssRNA, miRNA) was loaded in lane 1 and lane 7, respectively, to enable a semi-quantitative comparison. **Bioanalyzer traces (RIGHT).** Total RNA samples (1.75x volumes isopropanol), small RNA fractions and large RNA fractions were analyzed on an Agilent Bioanalyzer on a small RNA chip (10-200 nt, linear scale) and on an RNA 6000 pico chip (200-5000 nt, log scale). The combination of the traces is shown for illustrative purposes only but do not imply a quantitative correspondence.

Lexogen GmbH · Campus Vienna Biocenter 5 · 1030 Vienna, Austria
For more information visit www.lexogen.com.
Correspondence should be addressed to info@lexogen.com.

SPLIT™ Fractions for pure RNA sequencing