LEXOGEN

Enabling complete transcriptome sequencing

SPLIT RNA Extraction Kit

The SPLIT RNA Extraction Kit enables a fast and highly efficient extraction of RNA that is free of genomic DNA contamination. The RNA can be recovered as total RNA or split into a large and a small RNA fraction, facilitating the analysis of e.g. mRNA and miRNA from the same sample. Thus the RNA obtained is ideal for seamlessly preparing libraries for Next Generation Sequencing of total RNA or its large and small fractions.

Small and Large RNA Fractions

The SPLIT kit can be used for the extraction of either total RNA or the isolation of the large RNA fraction, whereby the small RNA fraction is optionally purified as a separate fraction. This allows for the separate analysis of miRNA, siRNA, shRNA, and snRNA (Figure 1).

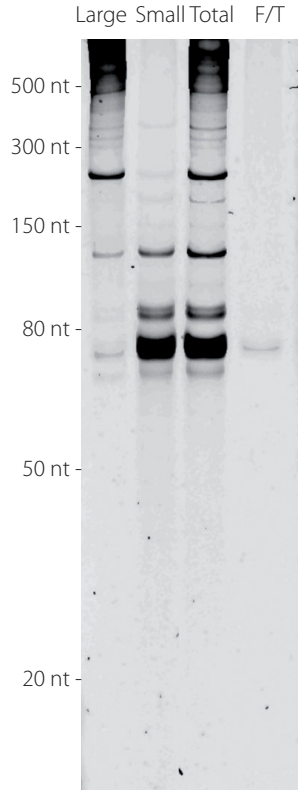

Figure 1 . Separation of SPLIT RNA samples on a polyacrylamide gel, demonstrating the splitting of large and small RNA at a threshold of ~150 nt. The total RNA sample comprising small and large RNA is shown as comparison.

High Quality RNA

RNA extracted with the SPLIT RNA Extraction Kit has a high RIN quality score for all types of samples. A RIN of 10 and a 28S / 18S rRNA ratio of 2.7 can be obtained from cell culture. Extractions from tissue samples usually result in RNA with a RIN of 8.0-9.5.

No Genomic DNA Contamination

Genomic DNA (gDNA) content in the extracted RNA sample is negligible comparing to a conventional method (Figure 2).

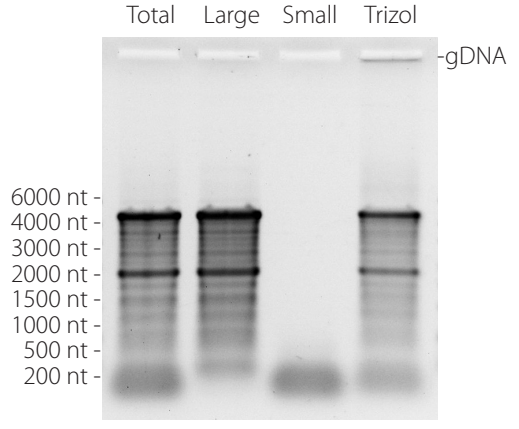

Figure 2. Agarose gel anaylsis of RNA samples extracted with the SPLIT kit or by a Trizol / isopropanol precipitation method. In the Trizol extracted sample genomic DNA is visible as a slot-retained band, whereas RNA obtained with the SPLIT kit is free from detectable genomic DNA contamination.

No Degradation of RNA

The SPLIT protocol does not require DNase treatment which is often used for the removal of genomic DNA in the sample and can be a reason for degradation of RNA.

Workflow

The SPLIT RNA Extraction Kit has enough reagents for the isolation of total RNA or the large RNA fraction from 48 samples, or small and large RNA fractions from 24 samples.

Cell/tissue homogenization is performed in an isolation buffer that is highly chaotropic to facilitate effortless and complete solubilization.

The RNA or this homogenate is then purified in a phenol-chloroform extraction. An acidic phenol is added to create a monophasic solution, a step that is essential for the efficient separation of genomic DNA into the organic phase. An acidic buffer and chloroform are added, and phases are cleanly separated using phase-lock gel tubes. The use of these tubes mitigates the risk of contaminating the upper aqueous phase that contains the RNA with the lower phenol phase that contains the DNA.

Purification of RNA is done on a silica column to eliminate trace amounts of phenol and to optionally fractionate the RNA. By adding an equal volume of isopropanol to the aqueous phase the entire total RNA will precipitate onto the silica carrier. When using only 1/3 volume isopropanol large RNA with a lower limit of about 150 nt will bind whereas the small RNA will be in the flow-through. By adding another volume of isopropanol to this flow-through also the small RNA can be recovered on another silica column.

SPLIT RNA Extraction Kit is appropriate for RNA extraction from (human) cell culture and animal tissue, and the extraction protocol can be easily adapted to a variety of RNA sources.

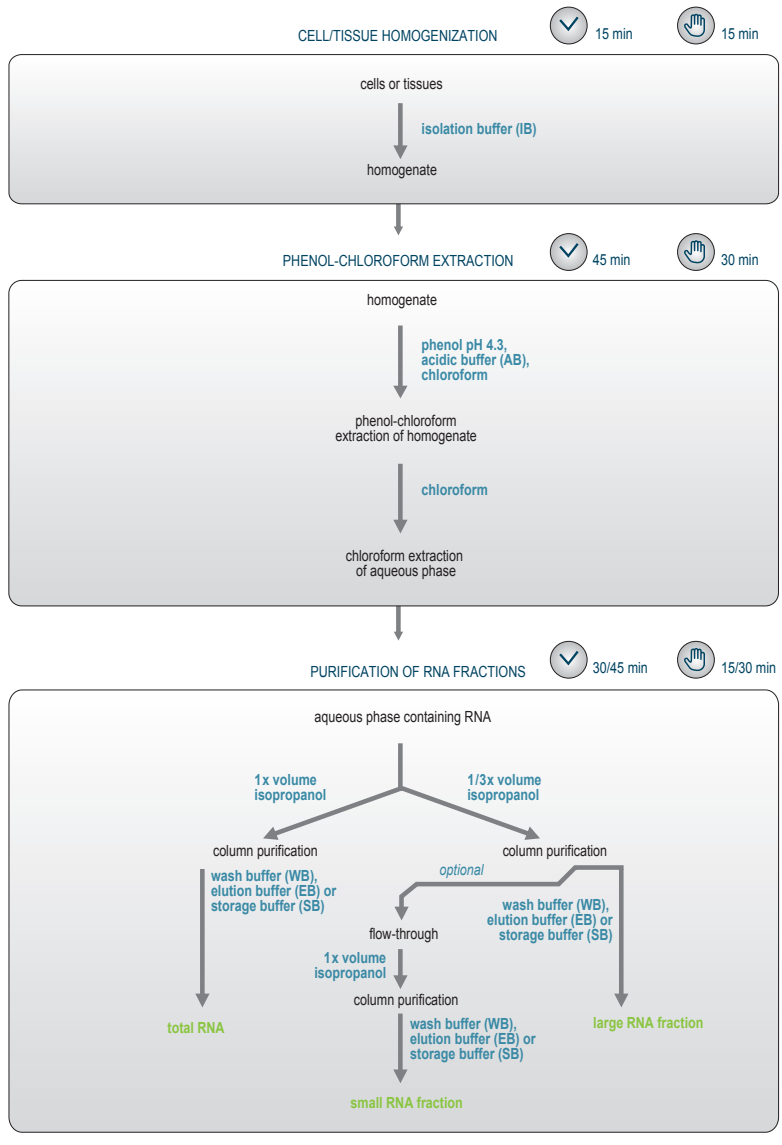

Figure 3. Schematic overview of the SPLIT workflow. Times given are for the parallel RNA extraction of up to 8 samples.

Find more about SPLIT at www.lexogen.com.
Contact us at info@lexogen.com or +43 1 345 1212-41.

SPLIT™
Fractions for pure RNA sequencing