LEXOGEN

Enabling complete transcriptome sequencing

SPLIT RNA Extraction Kit: Pure Fractions for Demanding Applications

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 from tissue, cells, fluids, and other sources 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. The obtained RNA is ideal for demanding applications such as Next Generation Sequencing library preparation, full-length cDNA generation, RT-PCR, or microarray analysis.

The SPLIT workflow

The protocol is designed to be completed in 30-35 min. and starts with cell / tissue homogenization in a highly chaotropic buffer that also readily dissolves solid tissues (Fig. 1). Cell debris, proteins and DNA are then separated from the RNA in an acidic phenol extraction. This step is aided by the use of phase-lock gel tubes, significantly facilitating the handling and increasing safety, separation and recovery. The organic extraction together with the ensuing silica-column based purification ensures an isolation of RNA without significant genomic DNA background (Fig. 2).

Importantly, the RNA can be eluted either as Total RNA covering the complete size range or split into two fractions. A double purification allows for the separate elution of the large RNA and the small RNA fractions with a cutoff at ~ 150 nt (Fig. 2). These fractions are ideally suited for the evaluation of RNA populations that require specific analysis pathways such as mRNAs, lncRNAs and miRNAs.

gDNA removal and RNA integrity

No further gDNA removal processes are necessary, preserving the extracted RNA. Other methods designed to control gDNA contamination mostly rely on enzymatic removal, whereby the application itself (especially on-column DNase digestion) or the enzyme inactivation (e.g., by heat denaturation) can severely compromise RNA integrity. Similarly, size-filtration based methods such as gDNA removal columns result in either ineffective gDNA removal or exclusion of longer RNA molecules (Fig. 3).

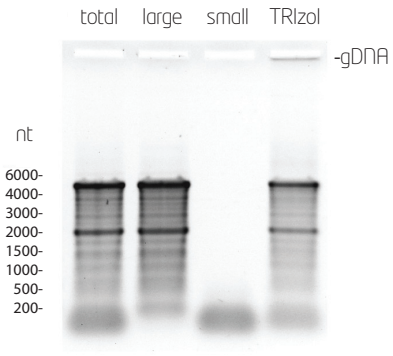

Figure 2 I Total RNA and RNA fractions obtained with the SPLIT kit. SPLIT samples are free from genomic DNA, as assessed on a denaturing agarose gel. A TRIzol-extracted control sample shows a significant amount of genomic DNA in this analysis.

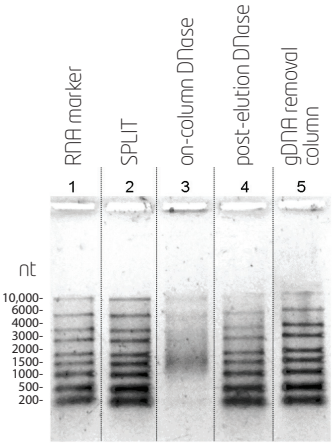

Figure 3 I The SPLIT protocol recovers the complete RNA size range without gDNA-removal associated adverse effects. A transcript RNA marker (0.2 – 10kB) was mock-extracted using RNA kits employing different gDNA removal methods. Effects on RNA integrity and size bias were assessed on a denaturing agarose gel.

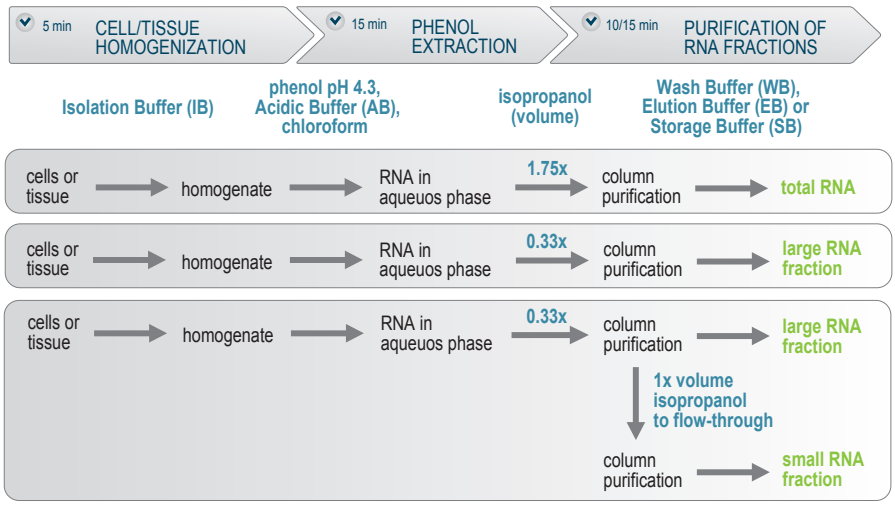

Figure 1 I SPLIT workflow.

The SPLIT RNA Extraction Kit features

- **Extraction of total RNA:** complete RNA size range from < 17 nt to > 10,000 nt
- **Extraction of RNA fractions:** Split into large RNA and small RNA fractions with a cutoff at ~150 nt
- **Highest RNA integrity and purity:** RIN up to 10, full-length RNAs suitable for virtually all applications
- **Genomic DNA-free:** no DNase treatment necessary
- **High yield:** up to 100 µg RNA per extraction
- **Convenience:** phase-lock gel tubes for efficient, comfortable and safe phase separation
- **Universal and fast protocol:** RNA extraction in 30-35 min. from tissues, cells, whole organisms, fluids, etc

Table 1 I Yield and integrity of extracted RNA. RNA samples were assessed by photometry and microfluidics. A_{260}/A_{280} and A_{260}/A_{230} ratios were > 2 for all samples. Yield and RIN values (RNA Integrity Number calculated by Agilent's Bioanalyzer software) are the average of duplicates (CoV < 0.2). * Tissue / cells were stored in RNAlater (Life Technologies), homogenization was in a tissue grinder and yield is given in µg RNA / mg tissue except: [1]yield in µg RNA / 1 million cells, [2]yield in µg RNA / mg pelleted cells, [3]homogenization in liquid N_2; yield in µg / seedling, [4]extracted from pelleted jellyfish.

Organism	Source	Total RNA		Large RNA		Small RNA
		Yield *	RIN	Yield	RIN	Yield *
Human	Cell culture			7.75 µg[1]	10.0	
	Cell culture			2.2 µg[2]	10.0	
	Brain	n.d.	8.1			
Mouse	Liver	5.0	8.2	3.7	8.3	1.2
	Brain	1.5	8.1	0.8	8.5	0.3
	Lung	0.9	7.7	0.5	7.9	0.3
	Heart	1.4	8.8	0.9	8.5	0.4
	Thymus	3.4	8.9	1.7	9.0	0.8
A. thaliana	Seedling			2.5-3.6 µg[3]	8.2	1.5-2.2 µg[3]
Nemostella[4]	Adult			n.d. 4	10.0	
Aurelia	Adult			n.d. 4	10.0	

miRNA- and mRNA-specific NGS library preparations from the same sample

Next Generation Sequencing of transcripts (RNA-Seq) has become the showcase of transcriptome research with its own special input RNA requirements. Samples extracted with the SPLIT kit deliver the whole range of RNA sizes for RNA-Seq, from miRNAs to mRNAs of over 10,000 nt length. Efficient recovery of siRNA and miRNA down to 17 nt in the total RNA or in the small RNA fraction has been shown in spike-in experiments with small RNA markers (Fig. 4).

Extraction efficiency and RNA quality

The homogenization step of the SPLIT workflow is highly efficient and designed to obtain RNA of highest integrity (Table 1). The RNA quality almost exclusively depends on the quality of the input. A RIN of 10 can be easily obtained, from, e.g., freshly harvested tissue culture cells, and RNA from tissue preserved with an RNA stabilizing reagent routinely yields a RIN of 8.0 – 9.5.

The workflow can be adapted to suit a wide range of input materials, and Lexogen strives to continuously expand this range with protocols given in the User Guide and online at www.lexogen.com.

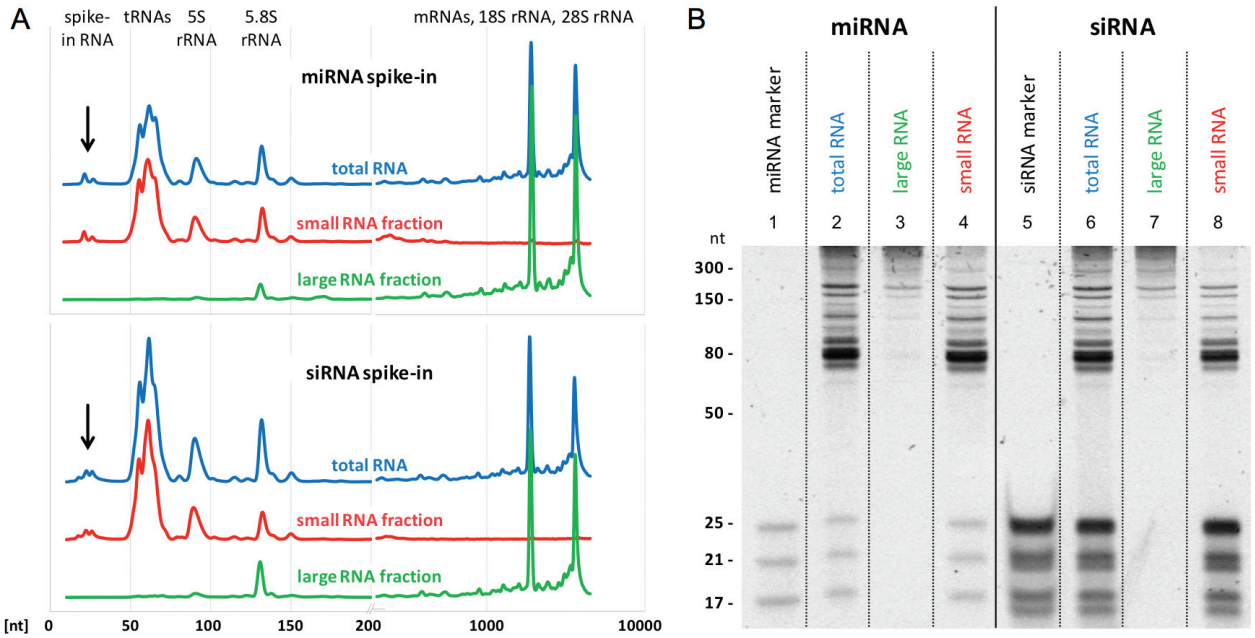

Figure 4 I miRNA-sized RNAs are efficiently recovered in the total RNA elution and in the small RNA fraction. Mouse liver homogenate was spiked with a single-stranded miRNA marker or a double-stranded siRNA marker. Total RNA or small RNA and large RNA fractions were extracted with the SPLIT RNA Extraction Kit. **(A)** The RNA samples 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, the Y-axes do not correspond quantitatively. **(B)** Polyacrylamide gel analysis of RNA samples. The theoretical maximum spike-in RNA recovery amount was loaded in lane 1 and lane 5, respectively, to enable a semi-quantitative comparison.

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SPLIT™
Fractions for pure RNA sequencing