LEXOGEN

Enabling complete transcriptome sequencing

099PF199V0100

NEW

SPLIT RNA Extraction Kit for Blood

- Total RNA from blood samples in less than 1 hour
- Efficient depletion of globin mRNA
- Option to split into large RNA and small RNA fractions
- High RNA integrity and purity, free of genomic DNA
- Excellent extraction efficiency

Introduction

The SPLIT RNA Extraction Kits enable easy, fast, and reliable extraction of RNA that is free of genomic DNA (gDNA) contamination (without DNase treatment which may damage RNA). 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. The obtained RNA is ideal for demanding applications such as Next Generation Sequencing (NGS) library preparation, full-length cDNA generation, RT-PCR, or microarray analysis. The SPLIT RNA Extraction Kit for Blood was specifically developed for RNA extraction from fresh human blood and enables concomitant depletion of globin mRNAs from blood samples in low volumes (50 - 250 µl).

Workflow

The SPLIT workflow (Fig. 1) generally enables RNA extraction from a variety of materials (plasma, tissue, cell lines) from different organisms (mammals, plants, insects, fungi, and bacteria). The samples are homogenized in a highly chaotropic Isolation Buffer (IB) for RNase deactivation and easy solubilization. This is followed by acidic phenol-chlorform extraction aided by phase lock gel tubes for a clean separation of the aqueous phase (containing the RNA) from the organic and inter-phase (comprising DNA and proteins). RNA fractions are purified via silica column purifications which enable the recovery of either total RNA or large and/or small RNA fractions.

The SPLIT for Blood workflow additionally includes a red blood cell lysis step (using the Blood Lysis Buffer, BLB) prior to sample homogenization in IB. This step adds 25 minutes to the conventional SPLIT RNA Extraction protocol, with only 5 minutes of hands-on time.

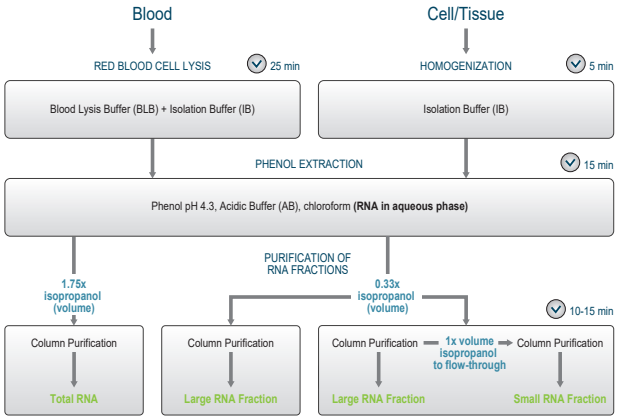

Figure 1 I Schematic overview of the SPLIT workflow. The cutoff between the large and small RNA fraction is at ~150 nucleotides. A supplementary protocol for FFPE samples is available on demand.

miRNA- and mRNA-specific NGS Library Preparations from the Same Sample

RNA Sequencing is a demanding application with its own special input RNA requirements. Samples extracted with the SPLIT kits deliver the whole range of RNA sizes for RNA-Seq, from miRNAs (down to 17 nucleotides) to mRNAs of over 10,000 nucleotides length.

gDNA Removal and RNA Integrity

The SPLIT RNA Extraction protocols yield gDNA-free RNA of high 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 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.

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

High-Integrity RNA from Blood Samples

The SPLIT for Blood protocol yields high-integrity RNA from fresh human blood (Fig. 2). After red blood cell lysis, samples can be re-suspended in Isolation Buffer and stored for up to three months at -80 °C for later RNA extraction.

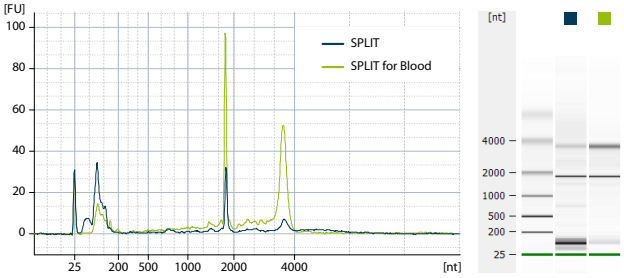

Figure 2 | The SPLIT for Blood protocol improves the RNA quality from whole blood extractions. Bioanalyzer traces of RNA extracted from fresh human blood using the SPLIT RNA Extraction Kit for Blood (green, RIN 8.7) or the conventional SPLIT protocol without prior red blood cell lysis (blue, RIN 7.0).

Efficient Depletion of Globin mRNA from Human Blood

The red blood cell lysis causes efficient depletion of globin mRNAs from the blood samples (Fig. 3).

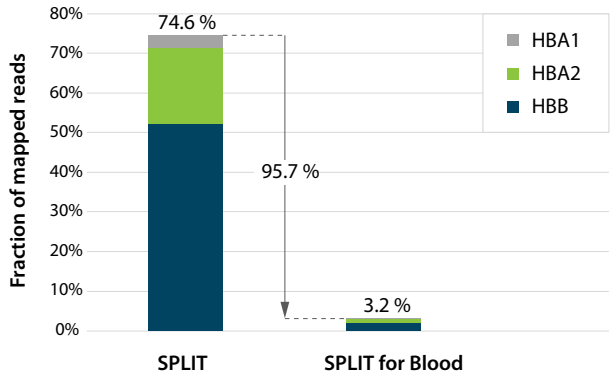

Figure 3 | The SPLIT for Blood protocol depletes >95 % of globin mRNA species from the extracted RNA samples. RNA was extracted from fresh human blood using the SPLIT and SPLIT for Blood protocols, respectively. RNA-Seq libraries were prepared with Lexogen's QuantSeq 3′ mRNA-Seq (FWD) kit and the percentage of reads mapping to globin mRNAs was calculated.

Increased Gene Detection after Globin mRNA Depletion

The efficient depletion of the predominant globin mRNA species from the blood RNA samples frees up sequencing space and results in increased gene discovery rates, saving sequencing costs.

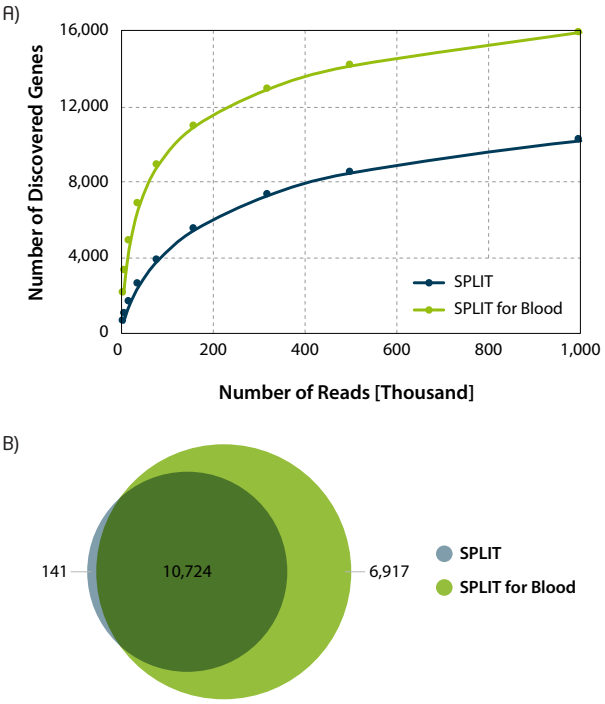

Figure 4 | Increased gene detection in human blood QuantSeq libraries using SPLIT RNA Extraction Kit for Blood. Libraries were prepared as described in Fig. 3. **A) Gene Discovery Plot.** The number of discovered genes was calculated from CPM (Counts Per Million) normalized read counts (threshold >0.5 CPM). **B) Venn Diagram** depicting the number of genes detected in libraries from RNA extracted using the SPLIT and SPLIT for Blood protocols and the overlap between the two.

Ordering Information

Catalog Numbers:
008.48 (SPLIT RNA Extraction Kit, 48 extractions)
099.48 (SPLIT RNA Extraction Kit for Blood, 48 extractions)

SPLIT™
Fractions for pure RNA sequencing