# Total RNA extraction from *Arabidopsis* leaves (*Arabidopsis thaliana* L*Er*)

**12th March, 2020**

This protocol describes a method to extract total RNA from *Arabidopsis* (*Arabidopsis thaliana* L*Er*) leaves, as an example of plant leaves. The plants were grown in a lab with LED lights and were cut into ~5 $mm^2$ chunks after collection. They were then stored at -80°C in bags containing 100 mg of leaf material. The total RNA plant extraction was performed using the Spectrum™ Plant Total RNA Kit. The PCR-cDNA Sequencing Kit (SQK-PCS109) was used to generate the libraries and the sequencing performance was determined using a MinION Mk 1B.

## Materials

- 100 mg of *Arabidopsis* leaves (cut into ~5 $mm^2$ chunks and stored at -80°C)
- Spectrum™ Plant Total RNA Kit
- TE buffer (1 mM EDTA, pH 8.0)
- Mortar and pestle
- Microcentrifuge
- Ethanol
- 2 ml Eppendorf tubes
- Incubator or water bath with capacity for 56ºC
- Vortex

# Method

**Note:** Steps 1-9 must be performed in the fume hood due to the handling of β-mercaptoethanol.

### Step 1

Pre-cool the mortar and pestle at -80°C for at least 30 minutes before starting the extraction. Store in an ice bucket with crushed ice to keep cool.

### Step 2

Add 100 mg of frozen leaves to the mortar and grind to a fine powder.

### Step 3

Transfer the leaf powder to a 2 ml Eppendorf tube with the lysis solution supplemented with β-mercaptoethanol, as outlined in the recommended protocol (step 3, page 5).

### Step 5

Vortex the tube for 30 seconds.

### Step 6

Incubate at 56°C for 5 minutes.

### Step 7

Centrifuge at 16,000 x g for 3 minutes.

### Step 8

Follow the recommended protocol from step 3-8 (pages 6-8).

### Step 9

Warm TE buffer at 56°C and pipette 50 µl directly into the matrix inside the column.

## Step 10

Incubate at room temperature for 5 minutes.

## Step 11

Centrifuge at 16,000 x g for 1 minute.

## Optional step 12

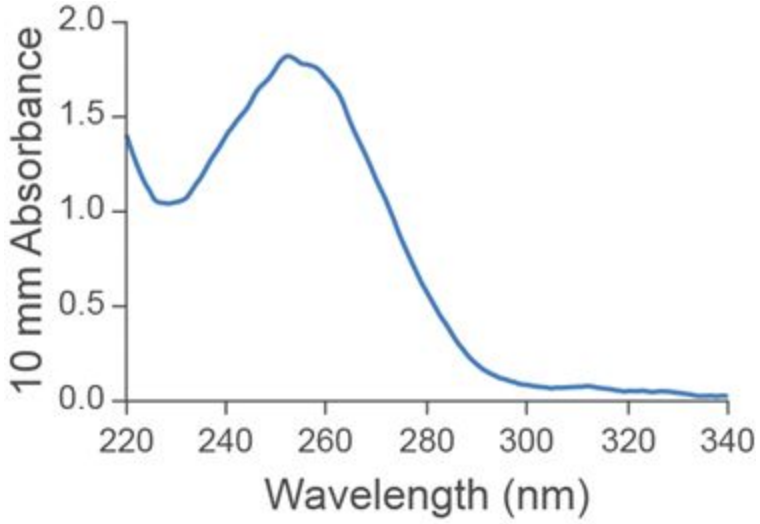
A second elution may be performed by repeating steps 9-11. The first elution should retrieve 20-25 µg of total RNA and the second elution should yield 3-6 µg.

## Step 13

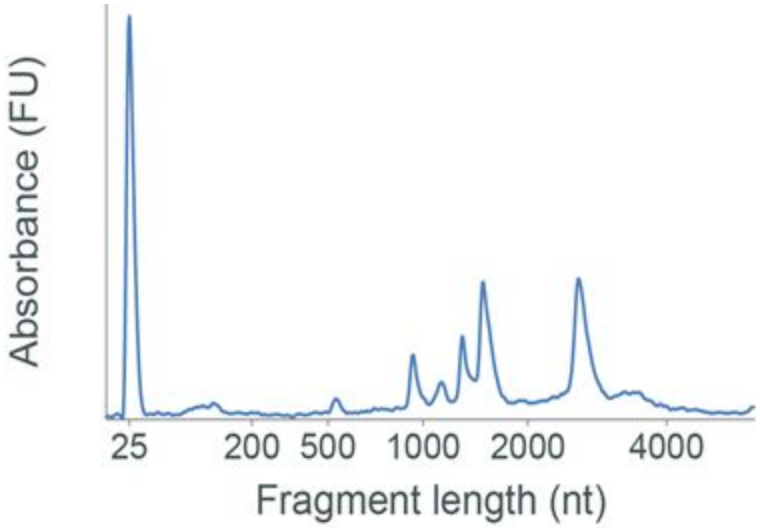
Sub-aliquot as necessary and store aliquots at -80°C.

## Results

- **Yield:** 25–30 µg
- **OD 260/280:** 2.07
- **OD 260/230:** 2.18




Agilent BioAnalyser RNA 6000 Nano Kit, RIN: 7.8



## Sequencing performance

Libraries for nanopore sequencing were prepared from 50 ng of total RNA ± 200 pg of ERCC RNA panel, using the PCR-cDNA Sequencing Kit (SQK-PCS109).

- Typical throughput: ★★★ (8+ Gb or 7-12+ reads in 48 hours on FLO-MIN106D flow cells).
- Read length profile:

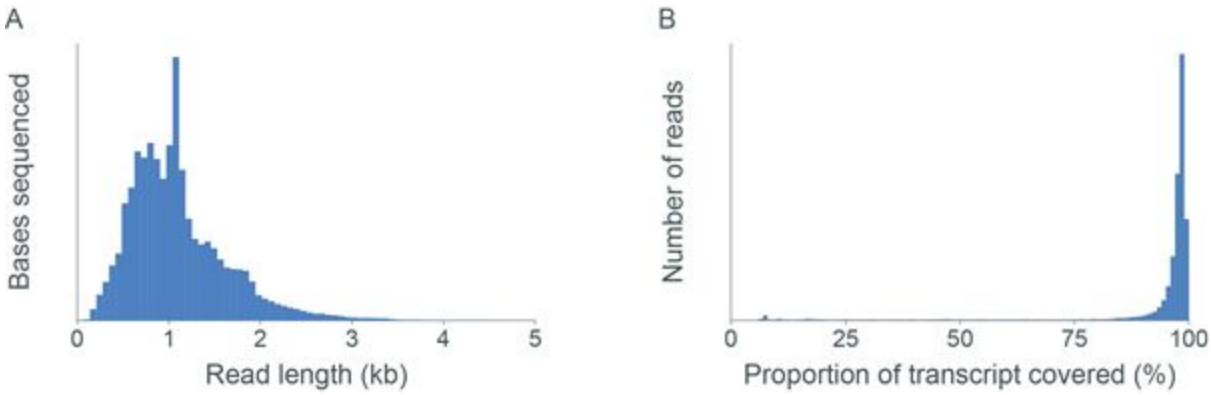



**Figure 1. The length distribution of reads that align to *Arabidopsis thaliana* and the ERCC panel.** Panel A) length distribution of reads that align to the *Arabidopsis* reference genome. Panel B) the proportion of the ERCC transcript covered by a read aligning to that transcript. The observed lengths of the reads that aligned to the ERCC panel show the majority of reads cover almost the entire transcript (as is expected, as the SQK-PCS109 kit enriches for full-length RNA template molecules). This suggests that the length distribution of reads in Panel A is representative of the length of the RNA molecules present in the sample and is not being biased during the library preparation process. The Spearman's rank correlation coefficient (rho) and the coefficient of determination ($r^2$) for the ERCC alignments were >0.94, further suggesting that there is very little bias in the library preparation and sequencing.