Total RNA extraction from *Caenorhabditis elegans*

December 17, 2019

This protocol describes a method to extract total RNA from nematode worms (*Caenorhabditis elegans* strain SX3254, descendent from N2 wildtype). The worms were grown in NGM (nematode growth media) with *E. coli* as a food source. The total RNA extractions were performed using TRIzol®. The sequencing performance was assessed using the PCR-cDNA Sequencing Kit.

Materials

- *C. elegans* (strain SX3254) grown on an *E. coli* plate
- TRIzol®
- Chloroform
- M9 buffer (0.85 M Na_2HPO_4, 0.4 M KH_2PO_4, 0.3 M NaCl, 1 mM $MgSO_4$)
- Isopropanol
- 15 ml Falcon tubes
- Ethanol
- TE buffer (1 mM EDTA, pH 8.0)
- Refrigerated centrifuge (with capacity for 15 ml Falcon tubes)
- TissueRuptor II and probes

Method

Step 1

Harvest the C. elegans in M9 buffer as described in this protocol (steps 1–2). Keep the pellet frozen at –80°C until ready to use.

Step 2

Add 1 ml of TRIzol® to a frozen worm pellet (~800 µl) in a 15 ml Falcon tube and allow the pellet to thaw at room temperature.

Step 3

Once the pellet is thawed, homogenise using the TissueRuptor II for 6 cycles of 5 seconds each, speed set to 2, and allowing an interval of 5 seconds between each homogenisation cycle.

Step 4

Add 9 ml of TRIzol® and invert the tube 15 times to mix. Incubate at room temperature for 15 minutes.

Step 5

Add 2 ml of chloroform and invert the tubes 15 times to mix and incubate at room temperature for 5 minutes.

Step 6

Centrifuge at 4000 x g at 4°C for 15 minutes.

Step 7

Transfer and retain the supernatant to a new 15 ml Falcon tube and discard the pellet.

Step 8

Add an equal volume of ice-cold isopropanol to the retained supernatant.

Step 9

Invert 15 times to mix and incubate at room temperature for 15 minutes.

Step 10

Centrifuge at 4000 x g at 4°C for 10 minutes.

Step 11

Discard the supernatant and retain the pellet.

Step 12

Add 10 ml of ice-cold 70% ethanol to the retained pellet and invert 5 times.

Step 13

Centrifuge at 4000 x g at 4°C for 5 minutes.

Step 14

Discard the supernatant, retain the pellet and use a sterile wipe to remove the ethanol leftovers from the tube walls, being careful not to disturb the pellet.

Step 15

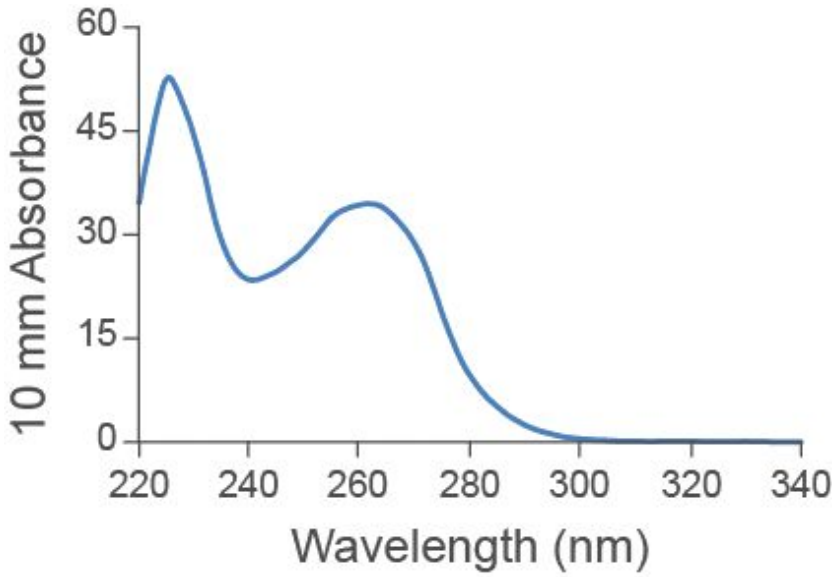
Add 500 µl of TE to the pellet and mix by pipetting up and down.

Step 16

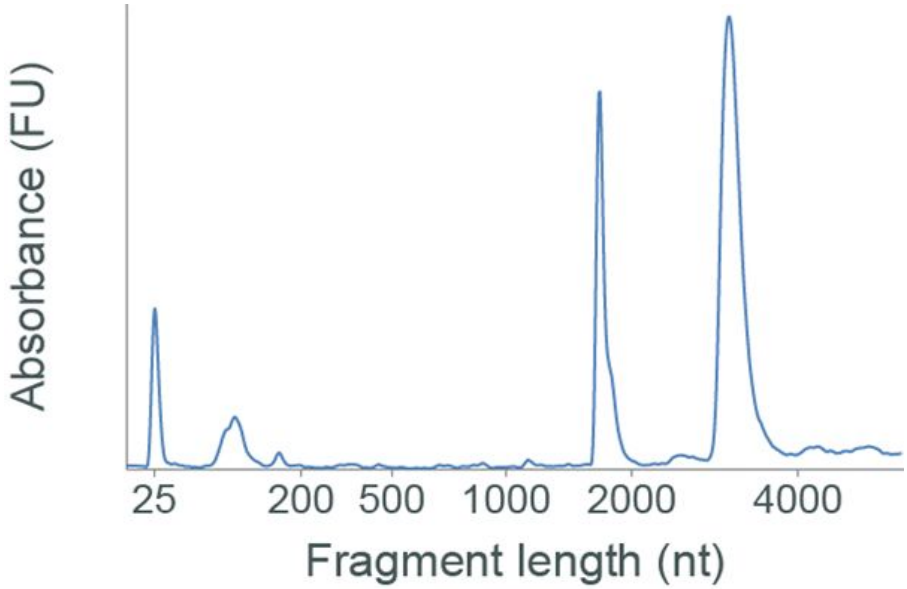
Sub-aliquot as necessary and store the aliquots at −80°C.

Results

- **Yield:** 400–500 µg
- **A260/280:** 2.02
- **A260/230:** 0.67



- Agilent Bioanalyzer RNA 6000 Nano Kit, RIN: 10



Sequencing performance

Libraries for nanopore sequencing were prepared from 100 ng of total RNA ± 200 pg of Spike-In RNA Variant Control (SIRV) Set 3, using the PCR-cDNA Sequencing Kit (SQK-PCS109).

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

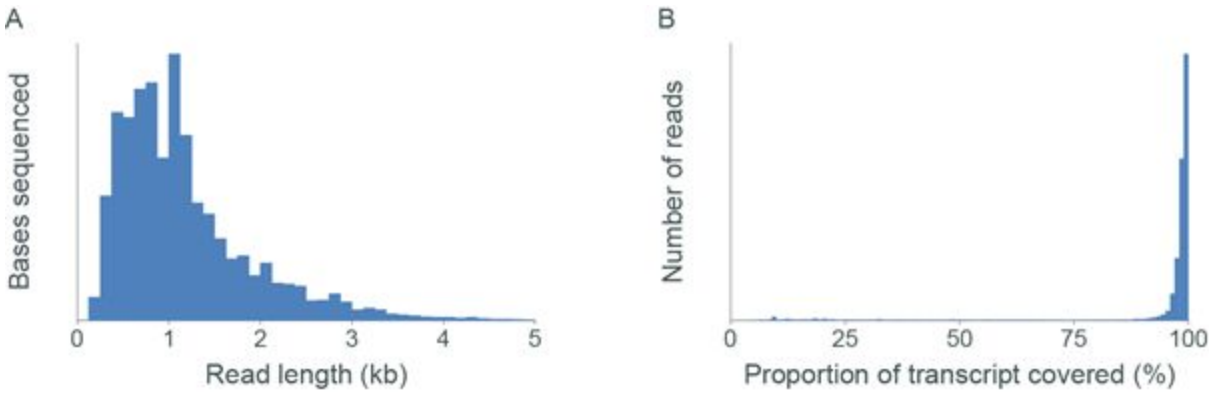


Figure 1. The length distribution of reads that align to *C. elegans* and the ERCC transcripts. Panel A: the length distribution of reads that align to the *C. elegans* reference genome. Panel B: the proportion of the ERCC transcript covered by a read aligning to that transcript. The observed lengths 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 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 >0.92, further suggesting that there is very little bias in the library preparation and sequencing.