Total RNA extraction from a single *Drosophila melanogaster* fruit fly

December 17, 2019

This protocol describes a method to extract total RNA from a single fruit fly (*Drosophila melanogaster*). The flies were kept at −80°C until extraction. The total RNA extraction was performed using Ambion® RNAwiz reagent. The sequencing performance was assessed using the PCR-cDNA Sequencing Kit.

Materials

- Single fruit fly stored at –80°C
- Ambion® RNAwiz reagent as a component of RiboPure™ kit
- Chloroform
- RNase-free disposable cell pestle
- Nuclease-free water
- Merck™ Novagen™ Pellet Paint™ co-precipitant
- Isopropanol
- Ethanol
- TE buffer (1 mM EDTA, pH 8.0)
- Refrigerated mini centrifuge
- 1.5 ml Eppendorf DNA LoBind tubes

Method

Step 1

Add one frozen fruit fly to a 1.5 ml Eppendorf DNA LoBind tube and immediately add 50 µl of RNAwiz, allowing the fly to thaw in the solution, at room temperature.

Step 2

Homogenize the fly using a disposable cell pestle (around 50 strikes) and incubate at room temperature for 15 minutes.

Step 3

Add 10 µl of chloroform. Invert the tube 10 times to mix, and incubate at room temperature for 10 minutes.

Step 4

Centrifuge at 12,000 x g for 5 minutes at 4°C.

Step 5

Transfer the supernatant to a new 1.5 ml Eppendorf DNA LoBind tube and add 20 µl of water, 2 µl of Pellet Paint™ co-precipitant, and 50 µl of ice-cold isopropanol.

Step 6

Invert the tube 10 times and incubate at room temperature for 15 minutes.

Step 7

Centrifuge at 12,000 x g for 5 minutes at 4°C.

Step 8

Discard the supernatant, retain the pellet and add 100 µl of ice-cold 70% ethanol to the pellet.

Step 9

Invert the tube 10 times and centrifuge at 12,000 x g for 5 minutes at 4ºC.

Step 10

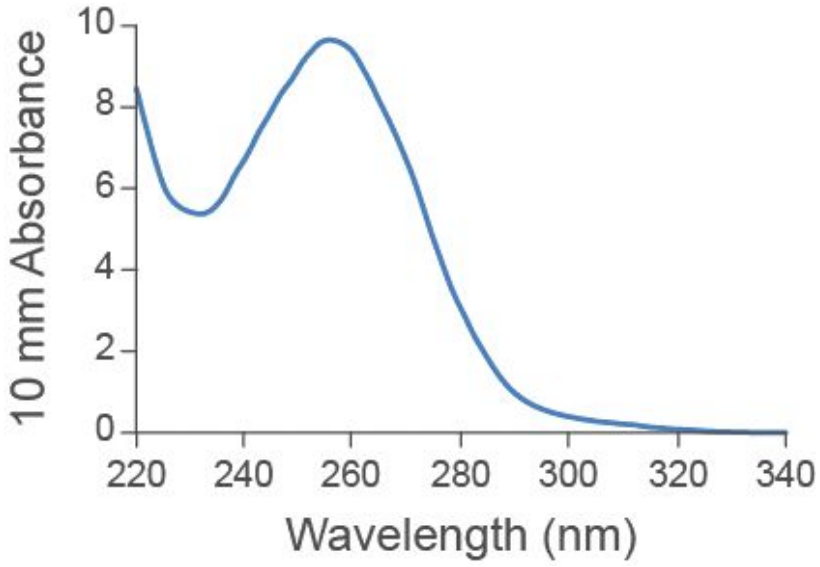
Discard the supernatant, retain the pellet and carefully dry the tube walls with a sterile paper wipe, taking care not to disturb the pellet.

Step 11

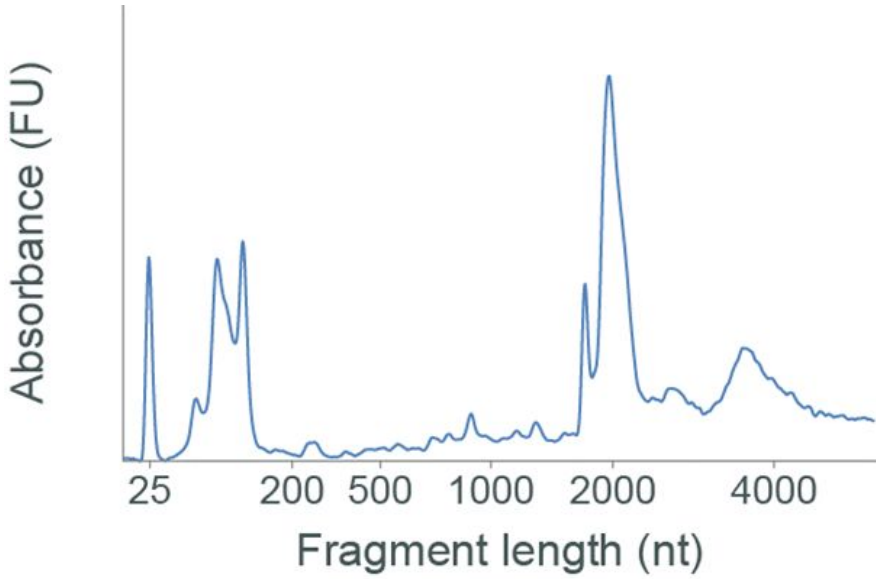
Elute in 10 µl of TE buffer.

Results

- **Yield:** 1–2 µg
- **A260/280:** 2.06
- **A260/230:** 1.77



- Agilent Bioanalyzer RNA 6000 Nano Kit, RIN: 7.5



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 distribution:

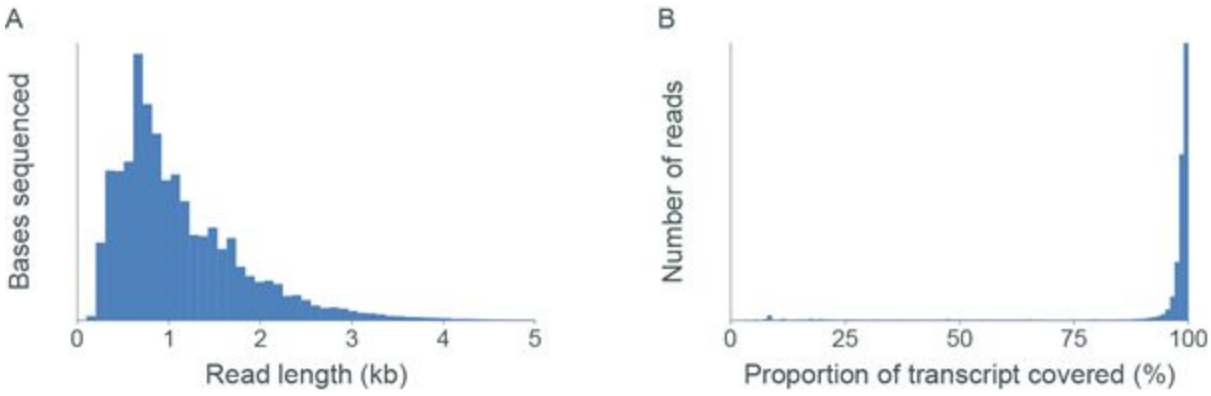


Figure 1. The length distribution of reads that align to *D. melanogaster* and the ERCC transcripts. Panel A: the length distribution of reads that align to the *D. melanogaster* reference genome. Panel B: the proportion of the ERCC transcript covered by a read aligning to that transcript. The observed 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 >0.93, further suggesting that there is very little bias in the library preparation and sequencing.