Total RNA Extraction from *C. elegans*

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[Abstract] This protocol describes total RNA extraction from worms with or without using commercial RNA extraction kits.

Materials and Reagents

1. *C. elegans*
2. Trizol (Life Technologies, Gibco®, catalog number: 15596-026)
3. DEPC treated H_2O (Life Technologies, Ambion®)
4. Turbo DNase (Life Technologies, Ambion®, catalog number: AM2238)
5. RNeasy Mini kit (Life Technologies, Gibco®, catalog number: 15596-026)
6. Ethanol
7. Chloroform
8. Isopropanol
9. Liquid nitrogen
10. RNase-free EDTA
11. KH_2PO_4
12. Na_2HPO_4
13. NaCl
14. $MgSO_4$
15. M9 buffer (see Recipes)

Equipment

1. 15-ml Corning tubes (Corning)
2. RNase-free eppendorf tubes (Eppendorf)
3. Filter tips (Eppendorf)
4. Dissecting microscope
5. Water bath

Procedure

1. Wash worms off plates with M9 buffer and collect them in 15-ml Corning tubes.
2. Wash 2 – 3 x with M9 buffer to get rid of bacteria.

3. Add 10 ml Trizol for every ml of packed worms (typically - add just 1 ml Trizol for ≤ 100 µl worms).
   Note: At this step, you can freeze tubes in liquid nitrogen immediately and store them at -80 °C until you are ready to proceed the experiment.
4. Vortex tubes for 30 sec, then place in liquid nitrogen, let thaw at 37 °C, and repeat several times (3 – 6 x).
5. Thaw frozen worms/Trizol mixture and vortex 30 sec then put on ice for 30 sec. Repeat this 6 - 7 x.
6. Most worms (not 100%) should appear disrupted under a dissecting microscope.
7. Move disrupted worm sample to RNase-free Eppendorf tubes (alternatively, move worm samples to RNase-free Eppendorf tubes before freezing at step 3).
8. Let tubes stand at room temperature (RT) for 5 min.
9. Chloroform extraction (working in hood).
   a. Add 2 ml chloroform per 1 ml of packed worms (typically 200 µl).
   b. Invert tubes 15 sec, let tubes sit 3 min RT for phase separation.
   c. Spin 15 min at 12,000 *x g* at 4 °C. RNA is in the aqueous supernatant.
10. Isopropanol precipitation (working in hood).
    a. Transfer top aqueous phase to new RNase-free eppendorf tube.
    b. Add 0.7 volumes (of what is already in tube) isopropanol (typically 400 - 500 µl).
    c. Gently invert tubes several times to mix.
    d. Leave tubes at RT for 10 min.
    e. Spin at 12,000 *x g* for 10 min at 4 °C.
    f. A small white RNA pellet at the bottom of tubes should be visible. Carefully pipet out supernatant.
    g. Wash pellet with ice cold 75% EtOH (use DEPC-treated H_2O to make EtOH solution).
    h. Spin 12,000 *x g* at 4 °C for 5 min.
11. Pipet out EtOH. When almost all the ethanol has evaporated (faint halo around pellet), resuspend the pellet in 25-100 µl DEPC-H_2O by pipetting and incubate the sample at 60 °C (water bath) for 10 min (if you have been using larger tubes, now transfer to RNAse-free eppendorf tube).
12. Set up Turbo DNase reaction.
    a. Dilute the 10x Turbo buffer in the RNA sample to 1x.
    b. Add 10 units of Turbo DNase per ml of sample (1 µl per 100 µl is sufficient).
    c. Incubate the sample at 37 °C for 30 min.
    d. Add RNase-free EDTA (pH 8.0, use DEPC water) to a final concentration of 5 mM and incubate at RT 10 min.

13. Take absorption readings at 260 and 280 nm absorption readings. If the A260/A280 ratio is 2.0-1.8, the sample is good (little contamination of protein). Expect to get 1-4 mg RNA /per gram of worms.
14. Store RNA sample at -20 °C (or -80 °C for long-term storage).

Notes

1. Steps 10-12 can be replaced by using Qiagen RNeasy mini kit as described below:
   a. Transfer top aqueous phase to new 1.5 ml RNase-free eppendorf tube.
   b. Slowly add an equal volume of 70% EtOH and mix by inverting tubes.
   c. Transfer the mixture to a Qiagen RNeasy spin column and follow manufacture's instructions (see Qiagen RNeasy Mini Handbook).

Recipes

1. 1 liter M9 buffer
   3 g KH_2PO_4
   6 g Na_2HPO_4
   5 g NaCl
   Add H_2O to 1 liter. Sterilize by autoclaving.
   After solution cools down, and 1 ml autoclaved/sterile 1 M $MgSO_4$.