Gaotian Zhang 2022
Formatted August 2023

RNA Isolation Protocol

Wear gloves and use RNAse-free reagents and plastic:

Prep: Label tubes, prepare sand aliquots

1. Remove worm (100 µL) tube from the freezer (-80°C).
2. Add 1 mL TRIzol (cn 15596018, Thermo Fisher) to each frozen sample.
    a. If > 100 µL worm pellet present, for example, 300µL worms in a microcentrifuge tube, add 1 mL TRIzol first and let the frozen pellet thaw fully. Mix well and aliquot into three microcentrifuge tubes, adding ~600 µL TRIzol to each tube.
    b. If the 300 µL worm pellet was in a 15 mL conical, add 3 mL TRIzol, thaw and aliquot into three microcentrifuge tubes. Only do RNA isolation on one tube of worms. Put 2 microcentrifuge tubes of worms-TRIzol mix back into the -80°C freezer as a backup.
    c. Each 100µl worms could give 50-150 µg total RNA.
3. Add 100 µL of prepped sand.*
4. Vortex vigorously for 10 minutes at room temperature.
5. Add 0.2 mL chloroform.
6. Vortex for 3 minutes.
7. Spin for 3 minutes at full speed.
8. Transfer the aqueous layer (500-600 µL) to a new labeled tube.
9. Add 0.5 mL isopropanol.
10. Mix by short vortex and incubate for 8 minutes at room temperature.
11. Transfer to ice for 2 minutes.
12. Centrifuge at full speed for 10 minutes.
13. Remove supernatant and add 1 mL 75% ethanol (made with RNAse-free water).
14. Vortex vigorously and spin at full speed for 3 minutes.
15. Remove supernatant.
16. Centrifuge at full speed for 30s to spin down the residual wash. Pipette to remove the residual wash. Be careful not to disturb the RNA pellet.
17. Air dry for 3 minutes or until the pellet appears almost completely dry.
18. Resuspend in 50 µL of RNAse-free water. Make sure RNA is fully suspended. More water can be added.
19. Aliquot 15 µL to a separate tube for QC and store on ice. Transfer the master RNA tube to -80ºC freezer.
20. Assay RNA concentration using 10 µL on the Qubit with Qubit™ RNA XR Assay Kit (cat. Q33224, Invitrogen via Life Technologies).
21. Dilute the remaining 5µl to a concentration of 50ng/µL to 500ng/µL, for quality and integrity of the assay by Bioanalyzer and Nano chip. Each chip can measure 11 user samples.

*Sand is from Sigma (#274739). To prep sand, wash 2x in 1 M HCl , wash ~8x in RNAse-free water (until pH is ~7.0), bake to dry in an 80°C oven for 2 hours or more. Predispense ~100 µL aliquots before starting RNA prep so they are handy for quick addition to each sample.