**RNA isolation protocol**

Protocol edited by Nikita Jhaveri, 8th May 2025

(Original protocols: Erik Andersen 2010, Gaotian Zhang 2023)

Wear gloves and use RNase-free reagents and plastic
Wipe down your bench and pipettes with 70% ethanol before proceeding
Use filter tips for RNA isolation

**Collection of worms**

1. Wash the animals off the plates with M9 in a clean, labeled 15 mL conical tube.
2. Centrifuge at 254 g for one minute (Andersen lab: Eppendorf 5810R, 1100 rpm).
3. Discard the supernatant and add ~10 mL M9. Repeat step two.
4. Discard the supernatant. Add 1 mL of M9, mix well by pipetting up and down 5 times, and transfer the animals to a clean, labeled microcentrifuge tube.
5. Centrifuge in the tabletop centrifuge (Andersen lab: Eppendorf 5417C) at 10,000 rpm for one minute.
6. Discard the supernatant.
7. Add four volumes (of the worm pellet) of Trizol (Invitrogen, catalog # 15596018).
8. Vortex vigorously until completely resuspended.
9. Store at -80℃ until all samples are ready to be processed.

**RNA isolation**

10. Thaw the samples from -80℃ at 37℃.
11. Flash freeze the slurry in liquid nitrogen or dry ice.
12. Thaw at 37℃ and vortex for 30 seconds.
13. Repeat steps 11 and 12.
14. Add two volumes (of the starting worm pellet) of Trizol and vortex vigorously.
15. Add 100 µL of prepped sand*.
16. Vortex vigorously for 10 minutes at room temperature (22℃, 1500 rpm).
17. Add 0.2 mL of chloroform (Fischer, catalog # C298-500) and vortex for 3 minutes at room temperature (22℃, 1500 rpm).
18. Centrifuge (Andersen lab: Eppendorf 5417C) at full speed (14,000 rpm) for 3 minutes.
19. Transfer the top aqueous layer to a new labeled microcentrifuge tube, taking care not to disturb the interphase.
20. Add 0.5 mL of isopropanol and mix the tube well by short vortex.
21. Incubate the tube at room temperature for 8 minutes.
22. Transfer the tube to ice for 2 minutes.
23. Centrifuge (Andersen lab: Eppendorf 5417C) at full speed (14,000 rpm) for 10 minutes.
24. Remove the supernatant and add 1 mL of 75% ethanol (made with RNase-free water).
25. Vortex vigorously and spin at full speed (Andersen lab: Eppendorf 5417C) (14,000 rpm) for 3 minutes.
26. Remove supernatant.
27. Centrifuge again at full speed (Andersen lab: Eppendorf 5417C) for 30 seconds to spin down the residual wash. Pipette to remove the residual wash. Be careful not to disturb the RNA pellet.

28. Air dry for 3 minutes or until the pellet appears almost completely dry.
29. Resuspend in 50 µL RNase-free water. Make sure RNA is fully suspended.
30. Aliquot 15 µL to a separate tube for QC and store on ice. Transfer the master RNA tube to the -80℃ freezer.
31. Assay RNA concentration using 10 µL on the Qubit with Qubit™ RNA XR Assay Kit (cat. Q33224, Invitrogen via Life Technologies).
32. Dilute the remaining 5 µl to a concentration of 50 ng/µL to 500 ng/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. Pre-dispense ~100 µL aliquots before starting RNA prep so that they are handy for quick addition to each sample.