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Protocols Learned and Developed

Crepidula fornicata: **Total RNA extraction**

Developed by Jon Henry (University of Illinois), Antje Fischer (Marine Biological Laboratory), and Alejandro Sanchez Alvarado (Stowers Institute for Medical Research)

To extract RNA for sequencing or cDNA synthesis

1) Pipet embryos for extraction into a 1.5 mL tube with 100% filtered seawater (FSW), remove FSW, add 100µl Trizol, and store at -80°C until use for total RNA extraction.

2) Homogenize in 1ml Trizol and incubate 5 min at RT, vortex every 2 min

- Homogenize the sample, which contains 100µl Trizol and the embryos, with a pestle.
- When the embryos have been adequately homogenized, add the remaining amount of Trizol (900µl) and pipet up and down with an aerosol-free pipet tip, which further aids in homogenization. Additionally, vortex this mixture to make sure everything is mixed well. Incubate 5 min at RT.

3) Add 200ul chloroform, vortex vigorously for 15 seconds, incubate 10 min at RT, vortex every 2-5 min

4) Centrifuge 30 min at 4°C at max speed, then transfer aqueous phase to fresh tube - be very careful here not to disturb the interphase

5) Add 500ul Isopropanol and 2µl linear acryl amid, invert to mix and incubate for 30 min at RT, vortex every 5 min

6) Centrifuge 20 min at 4°C and remove supernatant.

- Note that at this step it is often very difficult to see the pellet. For some samples we just have to assume it's there and be careful not to disturb anything at the bottom of the tube.

7) Add 70% ethanol and very carefully and centrifuge for 5 min at 4°C (DO NOT VORTEX)

8) Remove ethanol wash carefully and dry pellet 5-6 minutes (don't over-dry)

9) Dilute RNA pellet in 21µl of dH_2O and take a spec reading

- The volume of water in which the RNA is resuspended can vary, depending on what the RNA will later be used for (BioAnalyzer, NanoDrop, cDNA for probes, sequencing, etc.)