RNA and DNA Extraction from *Aiptasia pallida*

by Dr. Anderson Mayfield

modified by Pei-Ciao Tang

Sample collection

Place one anemone directly into 500 µl TRIzol or comparable extraction reagent.

Process immediately or store sample at 4°C for short-term or at -80°C for long-term preservation.

RNA extraction

1. Grind anemone with plastic pestle in 1.5 ml microcentrifuge tube and add Trizol up to 1 ml.
2. Incubate samples at room temperature for 15 min or 4°C for overnight.
3. Add 200 µl chloroform.
4. Incubate samples at room temperature for 15 min.
5. Spin samples at 12,000 rpm for 15 min at 4°C.
6. Remove aqueous phase (~650 µl) and add to 250 µl isopropanol
7. Add 250 µl high salt solution (0.8 M Na citrate, 1.2 M NaCl), vortex, and incubate at room temperature for 10 min.
8. Spin at 12,000 rpm for 10 min at 4°C.
9. Decant supernatant and wash pellet (by pipetting) with 1.ml 75% ethanol.
10. Spin at 12,000 rpm for 10 min at 4°C and decant supernatant.
11. Repeat steps 10 and 11.
12. Dry pellets inverted on benchtop for about 30 min.
13. Add ~50 µl DEPC-treated water to resuspend RNA pellets and mix by either pipetting or tapping the tube.
14. Assess quantity on the NanoDrop (2µl/reading).
15. Electrophorese ~5µl RNA on a 0.8 % agarose gel stained with ethidium bromide. Alternatively, formaldehyde (denaturing) gel may be used to better estimate 18S and 28S bands.

DNA extraction

1. Add 500 µl back extraction buffer (BEB, 1 M Tris Base, 4 M guanidinium thiocyanate, and 50 mM Na citrate) to the tube from the step 7 of RNA extraction.
2. Vortex and place tubes on shaker table for 10-15 min.
3. Spin at 12,000 rpm for 10 min at 4°C.
4. Transfer aqueous phase (~650 µl) to new tube.
5. Add 72 µl ammonium acetate (final conc. 0.5 M) and 2 µl GlycoBlue Co-precipitant (final conc. 50 µg/ml).
6. Add 1 vol isopropanol or 2 vol 100% ethanol.
7. Incubate at -20°C for 15 min.
8. Centrifuge samples at 13,000 rpm for 15 min. A significant amount of GlycoBlue Copreciptant precipitates with the nucleic acid, resulting in blue pellets).
9. Wash with 1 ml 75% ethanol.
10. Spin at 12,000 rpm for 10 min at 4°C, and decant supernatant.
11. Dry DNA pellet inverted on benchtop for 30-60 min.
12. Resuspend DNA in ~50 µl I would use 1/10 TE in water or some other diluted Tris buffer.
13. Assess quantity on the NanoDrop (2µl/reading)