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RNA Extraction Protocol for Coral Larvae
Last updated: 17 March 2009 by Kevin Portune

Protocol

-Larvae should be stored in RNA later (or some preservative)

1. Label 2 ml screw cap tubes.

2. Fill 2 ml tubes with 0.3 ml each of 0.1 mm glass and 0.5 mm zirconia/silica beads.

3. Pipet off RNA later from larval samples and transfer larvae to 2 ml tubes using a spatula.

4. Add 1 ml Qiazol.

5. Bead-beat for 2 min using Biospec Mini-Beadbeater.

6. Spin at 12,000 x g for 10 minutes at 4ºC to pellet larval waste.

7. Transfer supernatant to new tube.

8. Let sit for 5 min at RM temp.

9. Add 1/5 vol (200 µl) chloroform, shake for 15 s and let sit for 3 min at RTºC.

10. Spin for 15 min at 13,000 x g at 4ºC.

11. Transfer aqueous phase (~500 µl) to 2 ml RNase-free centrifuge tubes.

12. Add 1 vol. (~500 µl) chloroform, shake for 15 s and let sit for 3 min at RTºC.

13. Spin for 15 min. at 13,000 x g at 4ºC.

14. Transfer upper aqueous phase (approx 400 µl) to 2 ml RNase-free centrifuge tubes, add 1 vol. isopropanol (~400-500 µl), and vortex.

15. Incubate for 10 min at RTºC, then spin for 20 min at 13,000 x g at 4ºC.

16. Discard supernatant, add 1 ml of EtOH (75%), flick tubes to wash the pellet.

17. Spin for 5 min at 13,000 x g at 4ºC.

18. Repeat wash step.

19. Air-dry pellet for a couple of minutes and resuspend in 100 µl RNase-free water.

20. Measure concentration on Nano-drop.

21. Purify total RNA with RNeasy kit (Qiagen).