RNA EXTRACTIONS to obtain algae-free host RNA

Protocol author: Wendy Phillips
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OR

Weis, V. M. and W. S. Reynolds. 1999. Carbonic anhydrase expression and synthesis in the sea anemone *Anthopleura elegantissima* are enhanced by the presence of dinoflagellate symbionts. *Physiol. Zool.* 72(3): 307-316.

SOLUTIONS

PBS	Solution D	3M Na acetate
100mM Na phosphate, pH 7.4	Guanidine thiocyanate 50 g	pH 5.2
0.45 M NaCl	N-lauryl sarcosine 0.5g	DEPC treated
DEPC treated	1 M Na citrate pH7.0 2.5 ml	
	Final volume should be 100ml	
	Heat and stir to dissolve. Store in dark.	

1. Estimate approximately how much grinding solution and Solution D you will need for the extraction.
2. Add 2-mercaptoethanol to the Solution D that you will need at a rate of 7ul 2-mercaptoethanol/1 ml of solution D. Mix up enough Solution D for the grinding solution and what you will use to dilute the grinding solution. Put Solution D on ice.
3. Make grinding solution: Mix 2 parts PBS with 1 part Solution D. Then add 1/20 of that volume of Ribonucleoside Vanadyl complexes (RVC) 200mM. Keep this solution on ice.
4. Remove animal from –80° freezer and weigh.
5. As soon as the anemone begins to thaw, quickly mince with new razor blade on the parafilm so it is in very small chunks but not so long that it is a mush. (It helps to use a pipet tip to keep the tissue where you want it.) Transfer tissue to grinder.
6. Add 4 x (mg of animal) ul of grinding solution to the grinder and thoroughly grind anemone. (Example: if anemone weighs 200 mg, add 800 ul grinding solution.)
7. Pour solution into 15 ml centrifuge tube. Keep on ice.
8. Add approximately ¾ original grinding volume of grinding solution to the tissue grinder. (As in example above, add 600 ul more grinding solution.) Reinsert pestle and grind briefly. Pour solution into tube with first set of sample.
9. Centrifuge sample 8000g, 6 min, 4° C.
10. Pipet supernatant to new tube, being careful not to transfer any of pellet. Centrifuge again 5 min.

11. Pipet supernatant to new 15 ml polypropylene tube being careful not to disturb algal pellet.
12. Determine approximate volume of sample by comparing to a tube with volumes lined out.
13. Add 2 volumes of Solution D. Mix.
14. Add 1/10 the new volume of 3M Sodium acetate pH 5.2. Mix. If the solution volume is greater than 4 ml, divide into two tubes.
15. Add an equal volume of phenol pH 4.3. Cap very tightly and mix vigorously ~ 2 min.
16. Add 1/3 of the volume after step 14 of chloroform. Cap and mix vigorously.
17. Let sit on ice 15 min.
18. Centrifuge 9500g, 15 min, 4° C.
19. Remove supernatant to new tube being extremely careful not to contaminate sample with any of phenol phase or with they layer between phases. Estimate volume against marked tube.
20. Add an equal volume of chloroform. Cap tightly and mix vigorously.
21. Centrifuge 9500g, 20 min, 4° C.
22. Remove supernatant to new tube. Estimate volume against marked tube.
23. Add an equal volume of isopropanol. Store at -20° C overnight.
24. Centrifuge 9500 g, 30 min, 4° C. Pour super off of pellet.
25. Add ~6 ml 70% ethanol. Mix briefly. Centrifuge 10 min.
26. Remove all of 70% ethanol and air dry. (Takes upwards of an hour.)
27. Resuspend in 50-200 ul of DEPC treated water or whatever you want to resuspend it in.