

Host free - algal DNA extraction

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Paper citation: Reynolds, W. S., Schwarz, J. A., and V. M. Weis. 2000. Symbiosis-enhanced gene expression in cnidarian-algal association: Cloning and characterization of a cDNA, *sym32* encoding a possible cell adhesion protein. *Comp. Biochem. and Physiol.A* 126:33-44.

1. If possible, start with a couple large LIVE anemones (*Anthopleura elegantissima*) full of algae. Cut them into chunks with a razor blade saving algal rich parts and tossing algal poor tissue.
2. Using the largest glass grinder with the pestle attached to a drill, put anemone chunks in grinder with some room temp sterile filtered sea water (SFSW). Grind like hell. Occasionally put the grinder on ice briefly if it seems to be getting quite warm. It may be necessary to pour off some of the super, keeping chunks in the grinder, adding more SFSW to the chunks and grinding until all big chunks are gone. All this ground anemone/SFSW will go into 2 - 50 ml conical tubes filled $\frac{1}{2}$ - $\frac{3}{4}$ with ground slurry. By hand, shake tubes as vigorously as possible 30 sec. Weigh tubes to balance exactly for centrifugation.
3. Centrifuge in the swinging bucket rotor 600x g, 1 m, 14°
4. Pour off supernatant and resuspend pellet in SFSW. Note, sometimes there is a significant amount of floating algae. Pipet this into the grinder before pouring off the unwanted super.
5. Repeat steps 2-4 for a total of 4 times.
6. Finally resuspend pellet in SFSW. Incubate at 14° in 2 - 50 ml conical tubes filled $\frac{1}{2}$ - $\frac{3}{4}$. (They can be floated upright in the tank with the help of some styrofoam. Try to shade them a little.)
7. If possible, do daily changes of SFSW. Centrifuge in the swinging bucket rotor 600x g, 1 m, 14°. Resuspend pellets in fresh SFSW and grind by hand in large grinder. Pour into new 50 ml tubes. Shake vigorously. Centrifuge in the swinging bucket rotor 600x g, 1 m, 14°. Resuspend pellets in fresh SFSW. They should hang out at 14° for 5 days with at least 3 changes of water.
8. After 5 days, do a final rinse with SFSW. Resuspend pellet in DNA extraction buffer and put in a glass tube with glass beads. Vortex to break open algae. This may require 5 to 10 minutes of vortexing, chilling on ice, vortexing, chilling on ice.

DNA extraction can be by a homespun proteinase K/phenol:chloroform type protocol or by using a Qiagen DNeasy kit.