

Heat shock/bleaching experiments

Prepared by Santiago Perez; 3/22/2006

1. Place anemones in individual wells of plates or Petri dishes.
2. Treat anemones as necessary; for heatshock alone, incubate anemones in 30-34°C for 24 hours or more to get measurable bleaching.
3. After incubation, collect medium into adequate centrifuge tubes using a pipette vigorously to get all the released algae. Rinse chamber as necessary and add to collected sample.
4. Add several drops of Lugol's solution (Sigma L15675-7) and mix well.
5. Centrifuge well to get a good pellet and remove supernatant thoroughly but without disrupting the pellet.
6. Re-suspend pellet thoroughly in a **known volume** of filtered sterile seawater. 1 ml works well. Use .5 ml to rinse
7. Transfer re-suspension into a 2ml microfuge tube. (Store at 4°C or freeze)
8. Rinse anemones and freeze for later processing if desired.
9. Homogenize anemones in filtered sterile seawater using the smallest possible unit. Fisher sells very useful microtube pellet pestles that work great with tinies (Fisher; Kontes pellet pestle, K749520)
10. Collect homogenate into a centrifuge tube, rinse homogenizer well and add rinsate to homogenate. Add several drops of Lugol's
11. Centrifuge well to pellet the algae and remove supernatant.
12. Resuspend pellet with seawater and centrifuge again.
13. Repeat until the pellet is free of most animal tissues (may require 2-3 iterations)
14. Treat the final pellet as you did for the expelled algae (step #6)
15. Count algae using a haemocytometer (see protocol)
16. Add the total number of algae expelled to the total number of algae remaining in the host and calculate %expulsion as number of expelled algae divided by the sum of expelled and in host algae and multiply by 100%