

**Inoculating *Aiptasia* with algae (FIZ or cultured)  
Prepared by Santiago Perez; 3/22/2006**

First you need healthy aposymbiotic (apos) anemones and the best way to get apos is to cold shock them:

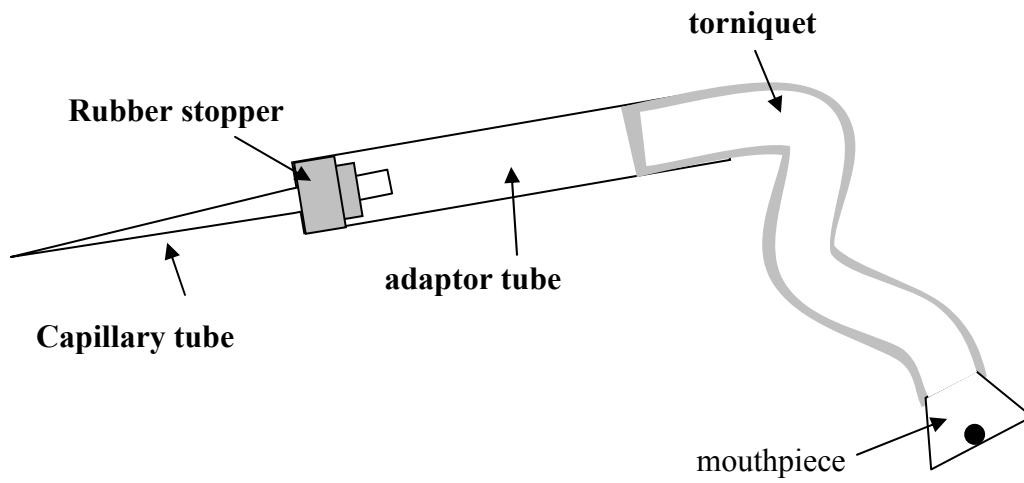
- Cool seawater without the anemones to 4°C.
- Change their room temp. seawater (I keep them in glass bowls) with the cold water.
- Place them in the fridge (at 4°C) in the dark for 4 hours.
- Replace the water back again with room temp water and keep them in the DARK (this is critical!) at room temp.
- Its better if the anemones have not fed for at least a week before shocking them. Change their water every day as they spit out algae.
- As they start to open again and look 'happier' you may want to repeat the procedure to get more of the remaining algae out.
- Once 'stabilized', keep them in the dark and feed them 1-3 times a week with freshly hatched brine shrimp.
- Sometimes it takes a while to get 100% of the algae out (i.e check under the dissecting scope with good magnification).
- To get the last few algae out I have experimented by incubating them for 30 min in 50µM of 3-(3,4-Dichlorophenyl)-1,1 dimethylurea DCMU (Sigma D2425; see below for prep instructions) in the dark, then blasting them with the strongest lights you have for another 30min (watch out overheating them)

**Inoculating anemones.**

- Simply draw some FIZ or cultured algae with a capillary tube previously drawn to a fine point with a flame.
- Insert into mouth of anesthetized anemones (see below) and gently blow out algal suspension. It is best if the anemone is securely attached so that manipulating the tube doesn't push the anemone around. Look for expansion of anemone body wall as you inject the algal suspension. Repeat as necessary.
- Remove anemones from anesthetic and maintain in the light. Feed as necessary. Feeding greatly increases the rate of algal up-take.

**Inmobilizing anemones:**

A magnesium chloride solution works great! Make a 1:1 solution of regular seawater (filtered/sterile optional) and 0.37M MgCl<sub>2</sub>. Place anemones in the solution and within 10 minutes or less they start expanding and become unresponsive to touch. If you are having trouble getting the mouth to open, or inserting the pipette tip, try adding a few drops near the anemone of 10mM succinylcholine chloride (Sigma S8251). This may relax the mouth more.



**DCMU recipe:**

To make stock first make 0.0313 M solution in 95% ethanol, or 0.0073g DCMU into 50ml 95%EtOH. To get 50 $\mu$ M add 32 $\mu$ l of stock to 200ml of seawater.