

Maintaining *Symbiodinium* Cultures: Fall 2008

Each month new cultures of the *Symbiodinium* need to be made using the previous months' cultures. Make sure to leave time for all the prep work before you intend to make the new cultures!

Preparing Autoclaved Glassware

1. Clean the oldest flasks in the incubator and allow to dry overnight.
2. Stuff top of the flask with cotton and then cover with a double layer piece of aluminum foil.
3. Autoclave flasks for 20-25 minutes with drying for 10-15 minutes.

Preparing Artificial Sea Water

1. Fill buoy in cabinet beneath incubator with DI water (black tap)
2. Add the appropriate amount of salt: want 35-36g/L, which is equivalent to about 9 scoops.
3. Allow to sit overnight while aerating with tube from anemone tank.
4. The next morning check the density using the refractometer and adjust as necessary.
5. Store the carboy in the dark to prevent growth of diatoms until further use.

Preparing the F2 Media

1. Filter the artificial sea water using a 0.45 micron filter.
2. Autoclave the filtered sea water for 20 minutes with 5 minutes of drying
3. Salts for making the F2 media are in the fridge in the wet lab. Add 1ml/L of each of the nitrates, phosphates, vitamins, and trace metals, but NO silicates (only used for diatoms).
4. Store in the fridge until future use.
5. One day before use, place media in the incubator overnight.

Preparing the new *Symbiodinium* cultures

1. Place 50ml of F2 media and 1ml from the appropriate previous culture and place back in incubator.

Note: The algae tend to stick to the glass so pipette up and down gently to free before transferring 1ml to the new culture.