CULTURING ALGAE
Prepared by Santiago Perez; 3/22/06

f/2 agar plates
(for culturing/purifying zooxanthellae)

Need:
35ppt Millipore-filtered seawater (MFSW)
Agar (DIFCO BactoAgar; Fisher DF0140-15-4)
Concentrated silica-free f/2 media (Sigma No. G0154) see appendix for original recipe (or see here: http://ccmp.bigelow.org)

Petri dishes

- Calculate the volume of MFSW to use based on the number of plates to be filled. Each plate is typically filled ½ to ¾ of the way.
- You may wish to make different agar concentrations (e.g. 0.5, 1.0, 1.5 or 2.0%). Add the necessary amount of agar to the MFSW
- Heat agar solution until it dissolves using a hotplate stirrer/stirbar)
- Autoclave at 121°C, 15psi for at least 15 minutes. If you are autoclaving several large bottles it is recommended to autoclave for longer times (e.g. 30min).
- Have sterile Petri dishes ready.
- Let cool a bit but without causing the agar to solidify. You can use a water bath set at 50°C.
- If using Sigma solution, add 50X f/2 media to a final concentration on 1X (1:50 dilution).

Note: This media comes sterile. Be careful to maintain its sterility. If you suspect that this step is contaminating the preparation, filter-sterilize the media first. – However it may mean that the media is ruined. Do not autoclave f/2 media

- Quickly dispense enriched agar onto the plates on a sterile surface next to a flame.
- Cover dishes and stack to let cool.
- Once the agar solidifies, store plates in the refrigerator. Some precipitate in the plates is normal and doesn't affect the growth of algae.

To culture algae from anemones:

- Homogenize anemones and isolate algae by repeated centrifugation followed by resuspension in sterile f/2 media until little host tissue remains. This takes about 2-4 times with *Aiptasia*. Other 'bulkier' species may take more.
- I then add the algal suspension to your culture vessels with the F/2 media. I usually inoculate 1ml (approx 1 million cells per ml. -I count algae with a hemocytometer) into 50ml sterile clear plastic conical tubes. 250ml sterile flasks work well too.

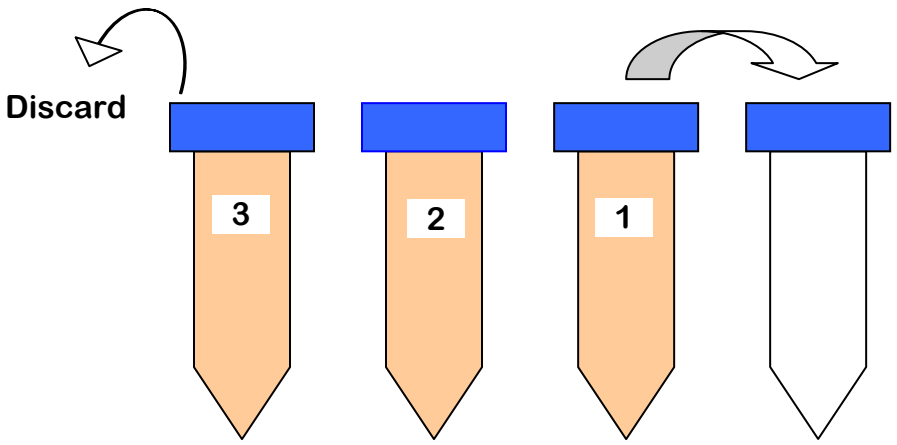
- I keep them at 25C chamber with a 12/12 hour light/dark cycle. Cool white fluorecent bulbs work well - I try to get 50-100 uE of light intensity.
- It can take 1-3 months for them to get going -be patient.
- I turn cultures over once a month in the 50ml tubes.
  To get clones you can either try to plate algae from the freshly isolated algal suspension or from algae that have been growing already.
- I inoculate
  these with varying amounts of algae and wait patiently to see if they form colonies.
- Keep plates in the light (same environmetal conditions as above).
  Sometimes they don't like to grow on plates. You'll get all kinds of 'unwanted' growth of cyanobacteria and other. I have found that the zoox usually grow best on plates when some of these 'contaminants' are present. Perhaps they are getting some nutrient from them -who knows (?!)

For f/2 liquid media

- Filter natural seawater through 0.45uM Millipore filter
- Sterilize filtered seawater in autoclave.
- Let sterile seawater cool to room temperature (or colder) overnight
- Add concentrated f/2 media using asceptic technique and mix well
- Note: use silica free f/2 for culturing algae other than diatoms.
- Dispense enriched seawater to sterile containers to be used for growing algae
- Inoculate as necessary.

For maintenance cultures of zoox:

- You can use 50ml sterile falcon tubes and inoculate with up to 1ml of 1 month old culture.
- Maintain a 2 month-old culture, a 1 month-old culture together with the newly started culture. On the next round discard the oldest culture (the now 3 month-old culture)
- Grow at 25 C and adequate light on a 12h/12h Light/Dark cycle.



APPENDIX

f/2 Medium and Derivatives
(Guillard & Ryther 1962, Guillard 1975)

Below are recipes for f/2 medium, its derivatives (e.g, f/2 agar, f/2-Si, f/2 + Se, f/4, f/50) and related media (e.g., Black Sea). F/2 is listed first, followed by derivatives of f/2.

---

f/2 Medium

(Guillard & Ryther 1962, Guillard 1975)

To 950 mL filtered seawater add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
1 mL	$NaNO_3$	75 g/L dH_2O	8.83×10^{-4} M
1 mL	$NaH_2PO_4 \cdot H_2O$	5 g/L dH_2O	3.63×10^{-5} M
1 mL *	$Na_2SiO_3 \cdot 9H_2O$*	30 g/L dH_2O*	1.07×10^{-4} M*
1 mL	f/2 trace metal solution	(see recipe below)	-
0.5 mL	f/2 vitamin solution	(see recipe below)	-

Make final volume up to 1 L with filtered seawater and autoclave.
***Note:** Autoclaved f/2 medium produces extensive silica precipitate. We delete silicate when it is not required by the alga (see f/2-Si medium below).

f/2 Trace Metal Solution

(Guillard & Ryther 1962, Guillard 1975)

To 950 mL dH_2O add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
3.15 g	$FeCl_3 \cdot 6H_2O$	-	1×10^{-5} M
4.36 g	$Na_2EDTA \cdot 2H_2O$	-	1×10^{-5} M
1 mL	$CuSO_4 \cdot 5H_2O$	9.8 g/L dH_2O	4×10^{-8} M
1 mL	$Na_2MoO_4 \cdot 2H_2O$	6.3 g/L dH_2O	3×10^{-8} M
1 mL	$ZnSO_4 \cdot 7H_2O$	22.0 g/L dH_2O	8×10^{-8} M
1 mL	$CoCl_2 \cdot 6H_2O$	10.0 g/L dH_2O	5×10^{-8} M
1 mL	$MnCl_2 \cdot 4H_2O$	180.0 g/L dH_2O	9×10^{-7} M

Make final volume up to 1 L with dH_2O. Autoclave.

---

f/2 Vitamin Solution

(Guillard & Ryther 1962, Guillard 1975)

To 950 mL dH_2O add:

Quantity	Compound	Stock Solution	Molar Concentration in Final Medium
1 mL	Vitamin B_{12} (cyanocobalamin)	1.0 g/L dH_2O	1×10^{-10} M
10 mL	Biotin	0.1 g/L dH_2O	2×10^{-9} M
200 mg	Thiamine · HCl	-	3×10^{-7} M

Make final volume up to 1 L with dH_2O. Autoclave and store in refrigerator. **Note**: Vitamin B_{12} and biotin are obtained in a crystalline form. When preparing the vitamin B_{12} stock solution, allow for approximately 11% water of crystallization (for each 1 mg of

Vitamin $B_{12,}$ add 0.89 mL dH_2O). When preparing the biotin stock solution, allow for approximately 4% water of crystallization (for each 1 mg of biotin, add 9.6 mL dH_2O).

---

f/2 Derivatives

Black Sea Medium: For brackish water organisms (16 psu, half-strength nutrients). Combine 500 mL f/2 medium and 500 mL dH_2O. Autoclave.

f/2 agar: Prepare 1 liter of f/2 medium and dissolve 9g Bacto-agar (heat and mix). For test tubes, dispense dissolved agar medium into tubes, autoclave, and then cool with tubes slanted at an angle. For Petri plates, autoclave in a flask, cool almost to the gelling point, and then aseptically dispense into sterile Petri plates. **Note:** Agar can be added to other media (e.g., f/50 agar), and agar concentration can be varied to produce softer or firmer substrates.

f/2-Si: Prepare as for f/2 medium but omit $Na_2SiO_3 \cdot 9H_2O$. This is preferred over f/2 medium for organisms with no silica requirement because less precipitation forms.

f/2 + Se: Extra silicon and selenium are beneficial to several diatom strains. Prepare 1 L of f/2 medium but use 2 mL of silicate stock, then add 1.0 mL of selenium stock solution (1.29 mg H_2SeO_3 /L distilled H_2O). Autoclave.

f/2 (11 psu): For brackish water organisms. Mix 650 mL distilled H_2O and 350 mL filtered seawater. Add f/2 medium nutrients and autoclave.

f/2-Si (24 psu): Mix 750 mL distilled H_2O and 250 mL filtered seawater. Prepare as for f/2 medium but omit $Na_2SiO_3 \cdot 9H_2O$.

f/4: Add 500 mL f/2 medium to 500 mL filtered seawater, then autoclave.

f/4-Si: Autoclave 1 L of filtered seawater. When cool, aseptically add f/2-Si nutrients at half concentration (i.e., 0.5 mL).

f/20-Si: Autoclave 1 L of filtered seawater. When cool, aseptically add f/2-Si nutrients at one tenth concentration (i.e., 100 µL).

f/50-Si: This is more than a 1/25 dilution of f/2-Si medium. We autoclave 1 L of seawater in a Teflon-lined bottle. Wait for the autoclaved seawater to cool to room temperature (important). Aseptically add 40 µL of sterile f/2 nutrients (20 µL of vitamins).

f/50-Si + CCMP1320 as food: Prepare f/50 and aseptically add 50 µL of healthy, moderately dense culture of CCMP1320.

f/2m: To 1L f/2 medium add 1 g methylamine · HCl, mix until dissolved and autoclave. This medium is used to test for contamination by methylaminotrophic bacteria.

f/2p: To 1 L f/2 medium, add 1 g Bacto-peptone, mix until dissolves and autoclave. This medium is used to test for contamination by non- methylaminotrophic bacteria and fungi.

f/2pm: To 1L f/2 medium add 1 g Bacto-peptone and 1 g methylamine · HCl, mix until dissolved and autoclave. This general medium is used to test for contamination by bacteria and fungi.

f/2 + NPM: Add f/2 nutrients to 900 mL of seawater and autoclave. After cooling, aseptically add 100 mL of the following organic stock solution. Dispense aseptically into test tubes.