Procedures for isolating *Prochlorococcus* from the wild

LR Moore, AF Post, G Rocap, and SW Chisholm (2002).
Utilization of different nitrogen sources by the marine cyanobacteria, *Prochlorococcus* and *Synechococcus*.
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Culturing the marine cyanobacterium *Prochlorococcus.*
Limnol. Oceanography: Methods 5: 353-362.

Protocol

1. Collect seawater in Go-Flo bottles using "acid-clean" tubing and amber Teflon bottles.
2. Protect seawater from high sunlight exposure.
3. Gravity filter 250 mL of seawater through two 0.6-µm or 0.8-µm polycarbonate filters (stacked) using an acid-clean Nuclepore filter funnel and acid-clean 1-L flask.
4. Add PRO2 nutrients aseptically and mix gently.
5. Transfer enriched seawater (~10 mL) to sterile glass or polystyrene culture tubes using sterile serological pipettes.
6. Place tubes in a light and temperature environment that closely matches the conditions of the native environment of the isolates.
7. Most of the cultures should be free of *Synechococcus* and other larger phytoplankton, but they will not be free of heterotrophic bacteria.
8. Monitor growth via fluorescence and flow cytometry
9. Remove heterotrophic bacteria by plating (Saito 2001), serial dilution (Rippka et al, 2000), and flow cytometric sorting (Moore et al. 2005).

PRO2 recipe

Nutrient	Concentration
NH_4Cl	50 µM
$(NH_2)_2CO$ (urea)	100 µM
NaH_2PO_4	10 µM
Trace metal mix	
EDTA	1.17 µM
$ZnCl_2$	8 nM
$CoCl_2$	5 nM
$MnCl_2$	90 nM
Na_2MoO_4	3 nM
Na_2SeO_3	10 nM
$NiCl_2$	10 nM
$FeCl_3$	1.17 µM