Growth of Dunaliella salina on artificial seawater.

Larry Klobutcher

Abstract

Citation: Larry Klobutcher Growth of Dunaliella salina on artificial seawater.. **protocols.io**
dx.doi.org/10.17504/protocols.io.gwpbxdn
Published: 06 Jan 2017

Protocol

Step 1.

For artificial seawater, add 36 g Reef Crystals (Aquarium Systems, Mentor, Ohio; note that some other brands of artificial seawater salts contain algae inhibitors!) to 1 liter of deionized or distilled water in a 1 liter Erlenmeyer and dissolve by stirring.

Step 2.

Add 1 ml Walne's solution, 1 ml of 10 ug/ml $FeSO_4$, and 0.2 ml of 2 mg/ml thiamine.

Recipe for Walne's Solution (all solutions stored at 4°C)**:**

Solution A (dissolve in order given):

100 g $NaNO_3$

45 g EDTA (Na)

33.6 g H_3BO_3

20 g $NaH_2PO_4 \cdot H_20$

0.36 g $MnCl_2$

1.8 g EDTA (Fe)

H_2O to 1000 ml

Solution B:

2.1 g $ZnCl_2$

2.0 g $CoCl_2 \cdot 6H_20$

0.3 g $(NH_4)_6Mo_7O_{24} \cdot 4H_20$

2.0 g $CuSO_4 \cdot 5H_2O$

H_2O to 100 ml

The complete Walne's solution is then made by mixing 1000 ml of solution A with 1 ml of solution B.

Step 3.

Aerate the artificial seawater for at least 15 min by inserting a piece of Tygon tubing connected either to an aquarium pump or the laboratory air-line (include a trap).

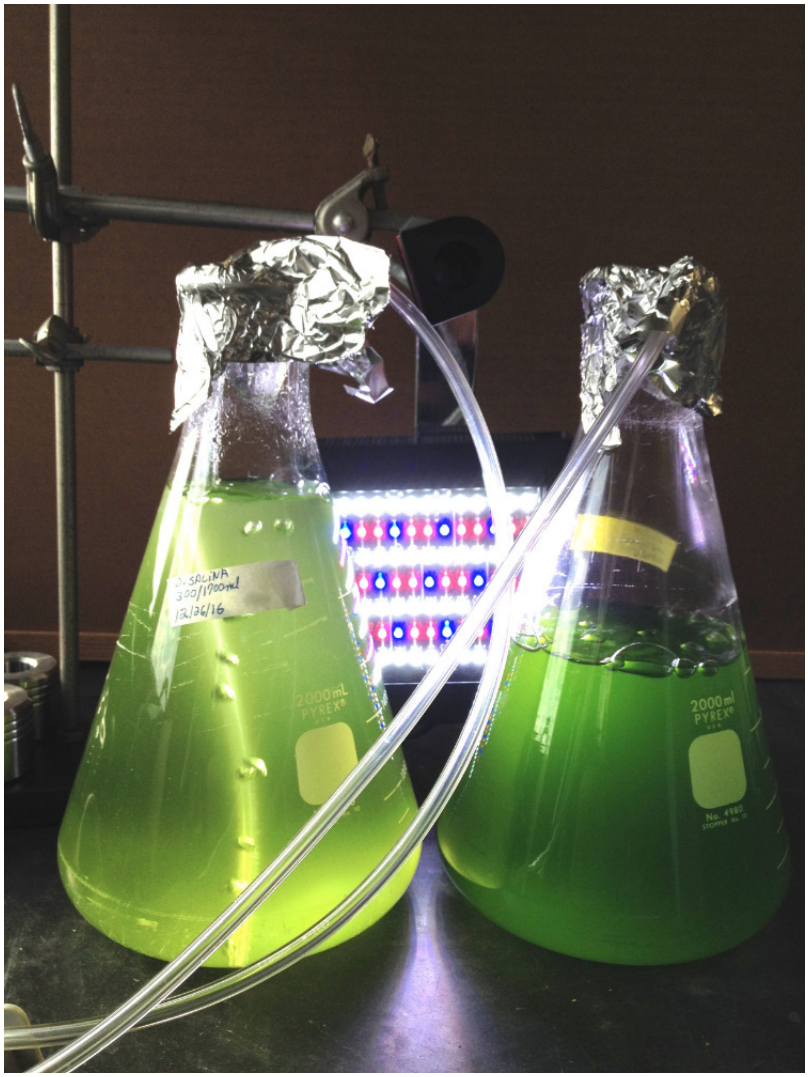
Step 4.

Remove and discard 150 ml of the artificial seawater and replace with 150 ml of a dense algae culture.

Step 5.

Incubate flask at room temperature with continued aeration under a plant grow light [Fluval Nano Aqua Life And Plant Performance Led Lamp, or Agro-Lite Fluorescent Lamps (Phillips)] using a 12 hour light:12 hour dark cycle with continued aeration.

Photo shows two 2-liter algae cultures with aeration tubes in front of a Fluval LED plant grow lamp.



Step 6.

After one week, culture should be opaque and dark green in color. Subculture following the steps above.

NOTES:

We typically maintain at least two 1-liter cultures at different stages of growth. That is, when we set up a new algae culture, we retain the culture from which it was inoculated. This provides an algal culture at suitable density for *Euplotes* growth throughout the week.

While cultures are typically grown in 1 or 2 liter Erlenmeyer flasks, this can easily be scaled up. In cases where we want to grow large amounts of algae (20 liters), we use polycarbonate carboys.

For culturing *Euplotes*, one wants algae cultures that are deep green and opaque; dark green cultures should be avoided, as dead and clumped algae will soon arise. Finally, algae that is used as the inoculum can filtered through 15 um Nitex Filtration Cloth (Tetko, Inc. Kansas City, Missouri 1-800-283-8182) to remove dead algae clumps and undissolved material if build up of such material should occur. This filtration can also done prior to using the algae to culture *Euplotes crassus* to help

produce cleaner cultures.

Procedure is adapted from: Roth, M., Lin, M., and Prescott, D.M. (1985) Large scale synchronous mating and the study of macronuclear development in *Euplotes crassus*. J. Cell Biol. 101:79-84.