Microalgal culture V.2

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

A brief guide to subculturing marine eukaryotic microalgae such as prasinophytes, diatoms and dinoflagellates.

PROTOCOL CITATION

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GUIDELINES

Carry out all subculturing under a laminar flow hood to minimise the risk of contamination.

MATERIALS TEXT

MATERIALS

L1

medium **NCMA Catalog #MKL150L**

K

medium **NCMA Catalog #MKK50L**

f/2

medium **NCMA Catalog #MKF250L**

Transfer Pipette **Globe Scientific Catalog #139118**

T-25 CytoOne Non-Treated Flasks with Vented Filter Caps **StarLab**

BEFORE STARTING

Ensure suffucient media appropriate for the culture(s) being transferred are prepared.

Media preparation

1 Microalgal culture media are comprised of **macronutrients** (nitrates, phosphates, sulphates), **micronutrients** (trace metals) and **vitamins**.

For media preparation, it is best to prepare stock solutions of the macro- and micronutrients and vitamins at concentrations higher than those needed in the final medium. This gives a consistent source for the medium and reduces the need to weigh chemicals etc.

Once you have prepared your stock solutions (in deionised water), they can be stored refrigerated (between **2 °C** and **9 °C**) for long periods. You can also seal the stock solutions with parafilm to prevent evaporation which may alter concentrations. Vitamin stocks can also be frozen and re-frozen without degradation.

2

To make medium for marine microalgae, either use artificial seawater (ASW) or aged, filtered and autoclaved seawater (FSW).

Filter aged seawater using a **0.45 µm membrane filter** or **0.7 µm GF/F filter** and autoclave.

Don't forget to loosen the lid of the bottle(s) prior to autoclaving.

Once you have FSW, it should be kept refrigerated and in the dark.

3

Media recipes:
NCMA
Roscoff Culture Collection
CCAP

Add the macro- and micronutrients in the required quantities for the media recipe being used to the cooled ASW or FSW (following autoclaving). Mix by inverting the bottle several times and autoclave.

Don't forget to loosen the lid of the media bottle(s) prior to autoclaving.

Once autoclaved, precipitation can be reduced by rapidly cooling the medium.

4 Using a laminar flow hood, pour a small amount of vitamin stock solution into a beaker.

Using a syringe and **0.2 µm filter**, filter sterilise the vitamins into a clean beaker.

Pipette the required quantity of sterilised vitamins into the cooled medium and mix well.

5 You can check the pH of the medium using a pH meter if culturing strains that are sensitive to pH changes.

Keep the medium refrigerated and in the dark when not subculturing.

Preparation

6 Work under a laminar flow hood.

Label a new T25 culture flask (vented cap, non-treated) with strain ID, species name, medium used, temperature of incubation and date of subculturing.

Subculturing

7 Using a pipettor, add **20 mL** of medium to the new culture flask.

7.1 If required, add **20 µl** of antibiotic solution to the medium for 1/1000 dilution.

Antibiotics for algaculture
by Victoria Jackson,
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PREVIEW RUN

7.1.1

To **10 mL** of MilliQ water **Contributed by users** , add any 3 of:

- **0.5 g Ampicillin**
- **0.1 g Gentamicin**
- **0.2 g Kanamycin**
- **1 g Neomycin**
- **0.2 g Streptomycin**

It is best not to use all ABX in every solution, but to use a selection of 2-3 so that you can change the combinations in future solutions in case of bacterial resistance developing.

7.1.2 Mix the solution well and filter using a 0.2 µM filter into a sterile bottle.
Store in the fridge at **4 °C** up to **1 month**.

8 Using a transfer pipette, transfer **2 mL** of the old culture to the new flask and gently shake to mix.

Culture Growth 2w

9

Screw the lid on the flask and incubate the culture at the appropriate temperature and light regime.

10 Culture growth can be monitored by microscopy, fluorescence using a plate reader, flow cytometry, or by simply

observing culture colour.

11 Repeat approximately every ~2 weeks, depending on culture growth.