How to cultivate *Ectocarpus*

This protocol (Coelho et al., 2012) describes the standard procedure for growing Ectocarpus *in the laboratory. Standard growth conditions are 13°C with a 12h:12h day:night cycle and 20 µmol photons.m^{-2}.s^{-1} irradiance using daylight type fluorescent tubes (Philipps TL-D). The algae can be cultivated either in plastic Petri dishes or in 10 l bottles with bubbling if large amounts of biomass are required. The standard medium is Provasoli-enriched natural seawater (PES) but* Ectocarpus *can also be grown in artificial seawater allowing a more precise control over the culture conditions. The protocol starts with partheno-sporophyte (or sporophyte) material because this is the stage that is usually maintained as stock. All manipulations of cultures should be carried out in a clean environment, if possible under a laminar flow hood. Forceps should be dipped in ethanol and allowed to dry under the hood. Using a combination of this protocol and protocol ASSEMBLE-JRA1-Protocol-07.00 (Genetic crosses between* Ectocarpus *strains), the sexual life cycle can be completed in 3 to 4 months, depending on the strain.*

Susana M. Coelho, Delphine Scornet, Laurence Dartevelle, Akira F. Peters, J. Mark Cock
Station Biologique de Roscoff, France
Bezhin Rosko, France

Reagents
Natural or artificial seawater
Provasoli solution

Equipment
140 mm diameter Petri dishes
60 mm diameter Petri dishes
Fine forceps
70% ethanol

Additional information:
Coelho SM, Scornet D, Rousvoal S, Peters N, Dartevelle L, Peters AF, Cock JM. *2012. How to cultivate Ectocarpus. Cold Spring Harbor Protoc doi: 10.1101/pdb.prot067934.*

Müller D. G. 1963. *Die Temperaturabhängigkeit der Sporangienbildung bei Ectocarpus siliculosus von verschiedenen Standorten. Pubbl. staz. zool. Napoli* ***33****: 310-314.*

Starr R. C., and Zeikus J. A. *1993. UTEX-The culture collection of algae at the University of Texas at Austin. J Phycol* ***29 (Suppl.)****: 1-106.*

1. Inoculate autoclaved seawater supplemented with Provasoli solution with small pieces (1 mm) of (partheno-)sporophyte filaments. Culture should be at low density (just a few pieces of filament per Petri dish) to ensure that there are sufficient nutrients (cultures grown at high densities will not produce unilocular sporangia). The sporophyte filaments will stick to the bottom of the dish and in a few weeks they start to produce upright filaments. Change the medium regularly (once a fortnight for a 140 mm Petri dish culture).

> *Temperature induction of unilocular sporangia (10-13°C) has been reported for some strains (*E. siliculosus sensu strictu *(Müller 1963). However, this temperature shift may not induce unilocular sporangia in other* Ectocarpus *strains.*

2. About 2 days after the uprights appear, plurilocular sporangia are produced. These contain mito-spores that allow asexual (clonal) reproduction of the sporophyte. About 1 week later, the unilocular sporangia that contain the meio-spores will appear. The meio-spores are the initial cells of the gametophyte generation.

3. Working under a sterile hood with a stereomicroscope, use a sterile Pasteur pipette to carefully dissect off one or two unilocular sporangia. Put a drop (30 µl) of PES containing the unilocular sporangium on a coverslip that has been

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placed on a drop of medium in a Petri dish. Add 4-8 drops of PES around the sides of the Petri dish. This creates a moist chamber (Fig. 1) and prevents the drop containing the unilocular sporangium from drying up. A few hours after isolation, the unilocular sporangium should release its meio-spores into the medium. When this happens, discard the piece of filament bearing the empty unilocular sporangium using forceps or a Pasteur pipette under the stereomicroscope and fill the Petri dish with PES. Gametophytes should develop in about two weeks (100 to 200 per unilocular sporangium).

> *Meiospore germlings can be kept under very low light (2 µmol photons.m^{-2}.s^{-1}) for up to 2 months. Under these conditions, they will not develop, and can be returned to standard growth conditions when necessary.*

4. Isolate the gametophytes under the stereomicroscope. Be careful that they are not contaminated by sporophytes (a proportion of the meio-spores will develop as sporophytes, a phenomenon known as heteroblasty). Approximately ten gametophytes can be cultivated in PES in a 140 mm Petri dish. The gametophytes should become fertile in about 2 weeks, producing gametes in plurilocular gametangia.

5. Monitor the gametophytes under the stereomicroscope until they become mature (i.e. produce plurilocular gametangia). Remove most of the PES so that they are in a small drop and incubate in the dark at 13°C overnight. In the morning, add 1-2 ml of PES to the drop and place under strong light for ten minutes. This treatment induces synchronous gamete release. The release of the swimming zoids can be followed under the stereomicroscope.

SOLUTIONS

ARTIFICIAL SEA WATER

Reagent	Quantity (for 1 litre)	Final concentration
NaCl	26.29 g	450 mM
KCl	0.74 g	10 mM
$CaCl_2$	0.99 g	9 mM
$MgCl_2$ (6H2O)	6.09 g	30 mM
$MgSO_4$ (7H2O)	3.94 g	16 mM

Adjust to pH 7.8, autoclave and store at 4°C.

PROVASOLI SOLUTION

Solution 1 (10x)	Quantity (for 1 litre)	Final concentration
H_3BO_3 (MW=61.83)	1.9 g	30.7 mM
$FeCl_3$ (MW=162.21)	0.05 g	0.3 mM
$MnSO_4(H_2O)$ (MW=169.02)	0.273 g	1.6 mM
$ZnSO_4(7\ H_2O)$ (MW=287.54)	0.0367 g	0.127 mM
$CoSO_4(7\ H_2O)$ (MW=281.1)	0.008 g	28 µM
0.5 M EDTA pH8 (MW=292.24)	11.4 ml	5.7 mM

Solution 2 (10x)	Quantity (for 500 ml)
Vitamin B12 (cyanocobalamine)	3.35 mg
Thiamine hydrochloride (vitamin B1) (MW=337.27)	165 mg
Biotin $C_{10}H_{16}N_2O_3S$ (MW=244.31)	1.65 mg
TRIS = Trisma base $C_4H_{11}NO_3$ (MW=121.14)	166.5 g

Solution 3 (10x)	Quantity (for 1 litre)	Final concentration
$(NH_4)_2Fe(SO_4)_2(6H_2O)$ (MW=392.14)	1.17 g	3 mM
0.5 M EDTA pH8	6.8 ml	3.4 mM

Solution 4 (10x)	Quantity (for 1 litre)	Final concentration
NaNO3 (MW=84.99)	23 g	270 mM

Solution 5 (10x)	Quantity (for 1 litre)	Final concentration
$C_3H_7Na_2O_6P(5H_2O)$ "glycerophosphate" (MW=216.04)	3.33 g	15.4 mM

Prepare each stock solution separately, autoclave and store at 4°C in a glass bottle. Use a dark bottle for solution 2.
For 1 litre of Provasoli solution add 100 ml each of solution 1, 3, 4 and 5 plus 10 ml of solution 2 to milliQ water (starting pH should be between 9.6 and 9.8).
Adjust to pH 7.8 with concentrated HCl (37%) and adjust the volume to 1 litre with milliQ water.
Aliquot into small glass bottles (20, 50, 100 or 200 ml), autoclave and store at 4°C.

PROVASOLI ENRICHED SEAWATER (PES) (Starr and Zeikus 1993)

Reagent	Quantity (for 1 litre)
Natural seawater	1 l
Provasoli solution	20 ml

If possible seawater should be collected by boat at some distance from the coast. Filter the seawater at 5 µm. Aliquot into Nalgene™ bottles (in glass bottles a precipitate can form), autoclave and store at 13°C. We use half-strength PES, i.e. 10 ml of Provasoli solution is added to 1l of autoclaved seawater.

The filtered seawater and the Provasoli solution are autoclaved separately in order to avoid precipitation.