Genetic crosses in Ectocarpus

This protocol (Coelho et al. 2012) describes how to carry out crosses between different strains of Ectocarpus. Crossing gametophytes to obtain the sporophyte generation is technically challenging because diploid sporophytes have to be distinguished from the haploid partheno-sporophytes that result from the parthenogenetic germination of unfused gametes. This requires careful monitoring of the progeny of the genetic cross until they have developed sufficiently to be transferred to a separate Petri dish. Genetic crosses allow several classical genetic methodologies to be applied in Ectocarpus, including allelic complementation tests, backcrosses, combination of different genetic mutations and outcrosses to create mapping populations.

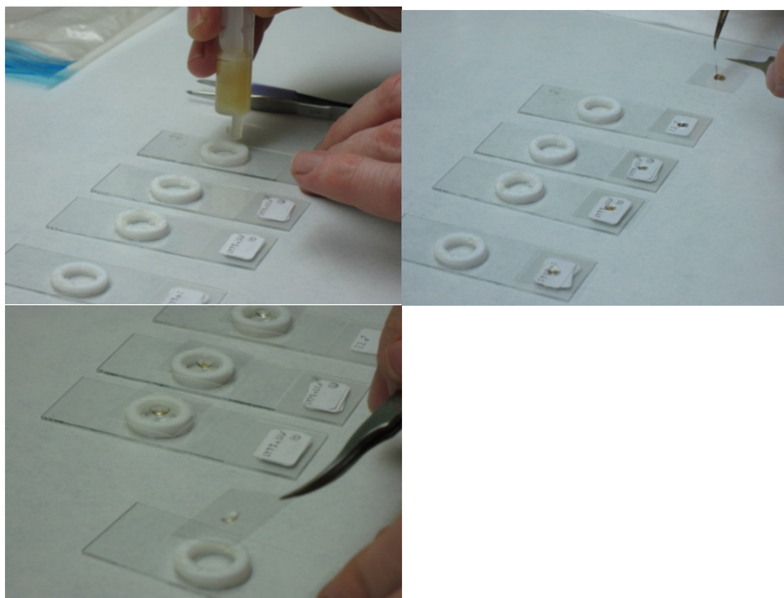
Peters AF, Dartevelle L, Scornet D, Coelho SM, Cock JM
Bezhin Rosko, France
Station Biologique de Roscoff, France

Reagents
Provosoli enriched seawater (PES)
Microscope immersion oil (Zeiss)

Equipment
22 mm coverslips
Fine forceps
Pasteur pipettes
Vaseline
White plastic plumber's washers (1 mm thick, outer diameter 18 mm, inner diameter 12 mm)
60 mm Petri dishes
Steel needles

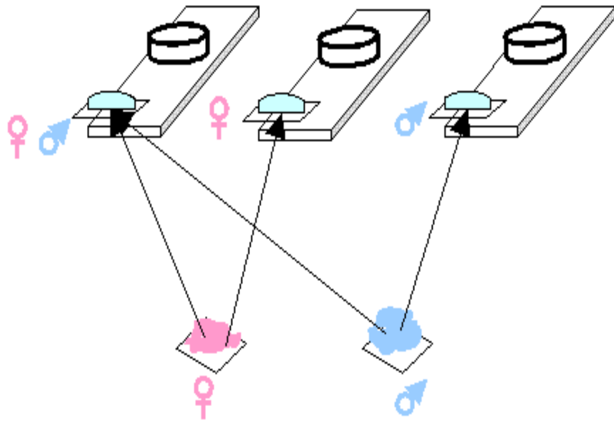
Additional information
Coelho SM, Scornet D, Rousvoal S, Peters N, Dartevelle L, Peters AF, Cock JM. *2012. Genetic crosses between Ectocarpus strains. Cold Spring Harbor Protoc doi: 10.1101/pdb.prot067942*

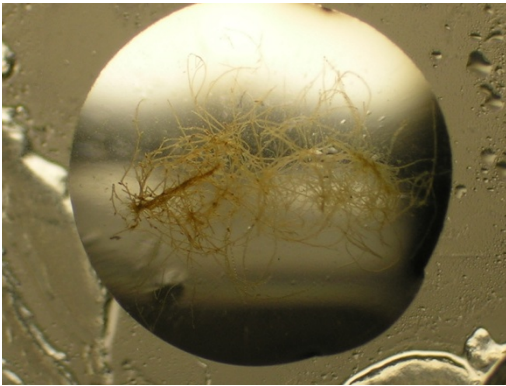
1. Use Vaseline to stick a plastic washer to a microscope slide and put some more Vaseline along the top of the washer so that a coverslip can be stuck to the top of the ring. Keep the cultures at 10°C whilst preparing this material so that they do not release their gametes before the cross is set up.



2. Place three coverslips (22 mm) on a surface that will allow them to be picked up easily and spot a drop (about 10 µl) of PES onto each coverslip.

3. Place small pieces of filament into each drop, two drops should just receive one of each of the parents, whilst the third will receive both parents. The two uniparental units are controls, to verify that both of the parent gametophytes release their gametes. Be careful not to cross contaminate between the three drops. Adjust the size of the drop to about 20 µl by adding medium.

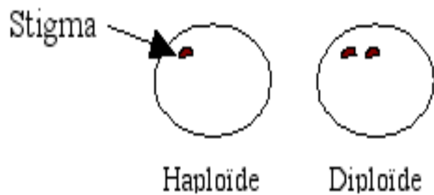



Fragments of male and female gametophytes

4. Gently but rapidly invert the coverslip and place it on the plastic ring. The bottom of the drop must not touch the microscope slide .

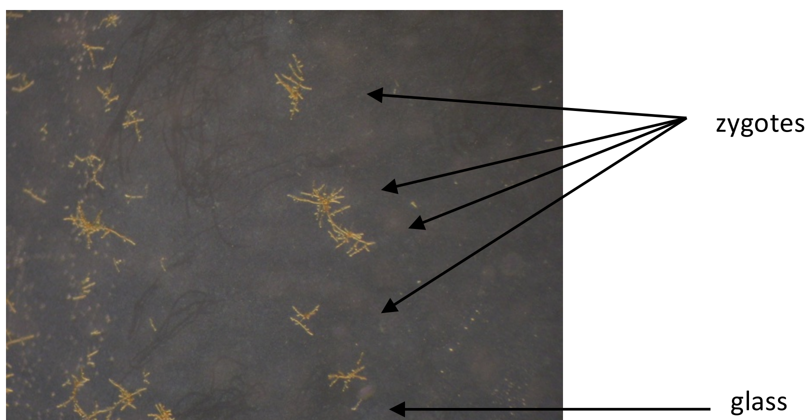
5. Incubate overnight at 13°C and then analyse under a microscope to determine whether zygotes have formed. Zygotes are identified by their larger size and confirmed (100x magnification may be required) by the presence of two stigmata. The stigmata will not be visible later (after 24-48h) so it is necessary to draw a sketch of the distribution pattern of the germlings. This sketch will allow the diploid sporophytes to be located and isolated later so that they can be transferred for culturing.

   Zygotes with 3 or more stigmata can be formed as a result of polyspermy; this is rare but it does occur.



6. After about a week, invert the coverslip and place it in a Petri dish with PES and incubate at 13°C. Note that the microscope oil can be toxic (use Zeiss oil) so remove it from the slide with alcohol before placing the coverslip in culture. Remove the parthenogenically-derived germlings that surround the zygote-derived germlings with a fine-tipped steel needle (sharpened using abrasive paper and rinsed with ethanol) so that the latter will be easier to isolate later. Under an inverted microscope, centre a zygote in the field using 10x or 40x, then work at 4x to remove parthenosporophytes with the needle. Monitor the configuration of the zygotes with respect to the drawing (individuals grow and some die).

7. When the zygote-derived germlings have grown sufficiently, they can be isolated and placed in culture. Transfer the growing sporophytes to 60 mm Petri dishes in PES by detaching with a steel needle then transferring using a Pasteur pipette.



SOLUTIONS

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.