Preparation of plugs of Ectocarpus *material for pulse field electrophoresis*

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Algae: Gametophyte filaments give better results than sporophyte.

Harvesting the tissue

1. Harvest 100 to 200 mg of fresh tissue and incubate for one hour at 4°C in 10 ml of PBS-4% formaldéhyde (v/v). Note: use gametophytes if possible
2. Freeze in liquid nitrogen.

Plug preparation

3. Grind the tissue with a mortar and pestle under liquid nitrogen (do not add any sand).
4. Transfer the ground tissue into 10 ml of lysis buffer on ice and grind further using a Wheaton.
5. Centrifuge for 7 min at 400 g and 4°C.
6. Discard the supernatant and resuspend the pellet in 10 ml of lysis buffer.
7. Centrifuge for 7 min at 400 g and 4°C.
8. Discard the supernatant and resuspend the pellet in 300 µl of 1% GTG agarose.
9. Pour le agarose into a PFGE mould on ice to make 3 or 4 plugs.

Proteinase K digestion

10. Incubate the plugs overnight at 50°C in 1mg/ml proteinase K / 0.5% SDS / 0.5 M EDTA.
11. Inhibit the proteinase K by washing twice for 30 min at room temperature with PMSF (isopropanol stock diluted to 40 µg/ml in TE).
12. Wash the plugs well with TE (two washes, each of at least on hour minimum).
13. Store the plugs at 4°C in 0.5 M EDTA

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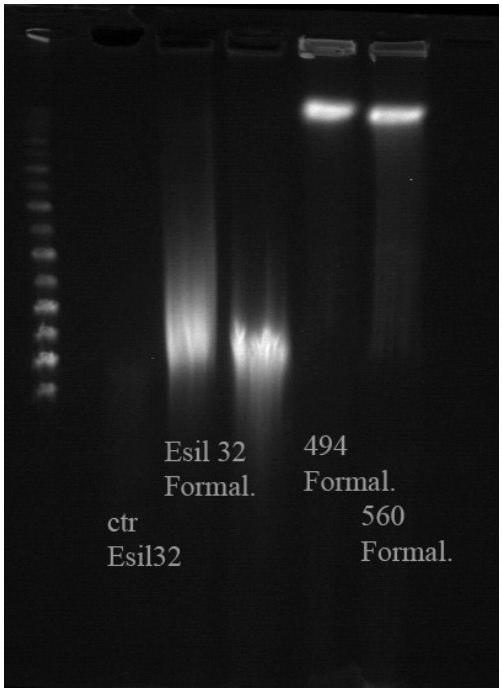
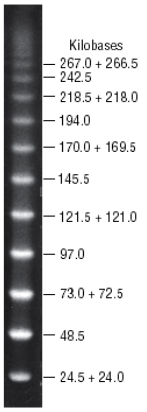
SOLUTIONS

LYSIS BUFFER

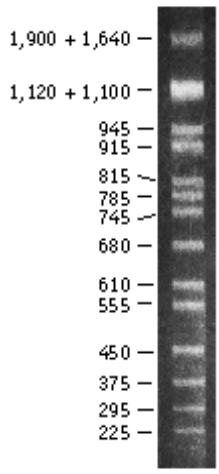
Reagent	Quantity (for 100 ml)
140 mM NaCl	2.8 ml of 5 M stock
2.8 mM KCl	140 µl of 2 M stock
1 mM EDTA	200 µl of 0.5 M stock
10 mM Tris HCl pH 7.5	1 ml of 1 M stock
1% v/v Triton X100	1 ml
12 % w/v Saccharose	12 g
Adjust to pH 8.3	

Example of a PFGE separation:





MidRange II marker



Yeast Chromosome PFG Marker