Ectocarpus *RNA extraction method*

This protocol is based on the method described by Apt et al. (1995). Expect a yield of approximately 100 µg of RNA per gram of tissue (although yields can be quite variable).

1. Freeze tissue in liquid nitrogen (can be stored at -80°C).

2. Grind tissue in a mortar and pestle with a small amount of sand (sable de Fontainebleau) under liquid nitrogen (This step has a critical influence on the final yield.).

3. Add 10 ml of extraction buffer per g of tissue (wet weight), mix vigorously at RT for at least 30 min.

4. Extract with one volume of chloroform/isoamyl alcohol (24/1) for several minutes (a large quantity of precipitate may form).

5. Spin at 10,000g for 20 min at 20°C and recover the (upper) aqueous phase

6. Slowly add 0.2 - 0.3 volumes of ethanol with swirling (This step is to eliminate polysaccharides. It is important to swirl so that the local concentration of ethanol does not become high enough to precipate the nucleic acids.).

7. Extract with 1 volume of chloroform, spin 10,000 g 20°C 20 min (With some algae a large quantity of polysaccharide may precipitate but this is not usually the case for *Ectocarpus*. This also removes the ethanol).

8. Recover the (upper) aqueous phase and add 0.25 vol of 12 M LiCl and mercaptoethanol to 1% final concentration, mix vigorously and place at -20° (freezer) overnight.

9. Spin at 10,000 g and 4°C for 1 hour, carefully remove the supernatant and invert the tube on some absorbent paper (The DNA can be precipitated from the supernatant at this stage with isopropanol and then purified on a caesium chloride gradient).

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Reagents
Extraction buffer
12M LiCl
3 M NaAc pH 5.5
TE
phenol/chloroform (1:1)
chloroform/isoamyl alcohol (24:1)
β mercaptoethanol
100% ethanol
80% ethanol
water

Additional information:

Apt KE, Clendennen SK, Powers DA, Grossman AR *(1995) The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga Macrocystis pyrifera. Mol Gen Genet* ***246****: 455-464.*

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10. Dissolve the pellet in 1 ml of TE on ice and transfer to an eppendorf tube.

11. Extract with phenol/chloroform (50/50 using "RNA" phenol equilibrated at pH 4.3).

12. Precipitate the RNA from the aqueous phase by adding 0.1 volumes of 3M sodium acetate and 2.5 volumes of ethanol and placing at -20°C for at least 6 hours before centrifuging for 20 minutes à 10,000g (The precipitated RNA can be stored at -20°C).

13. Wash the pellet 80% ethanol and re-spin, resuspend the RNA in TE or ultrapure water.

SOLUTIONS

EXTRACTION BUFFER

Reagent	Quantity (for 10 ml)	
100 mM Tris pH 7.5	1 ml of 1 M	1M: 24.22g/200ml (121.1 base)
1.5 M NaCl	3 ml of 5 M	5M: 58.44g/200ml (58.44)
2% CTAB	2 ml of 10%	10%: 24.22g/200ml (364.5)
50 mM EDTA pH 8	1 ml of 0.5 M	0.5M: 37.22g/200ml (372.2)
H_2O	2.5 ml	
50 mM DTT (add before use)	0.5 ml of 1 M	24.22g/200ml (154.2)

CTAB is hexadecyltrimethyl ammonium bromide

12 M LiCl

Reagent	Quantity (for 200 ml)	Final concentration
LiCl (MW= 42.39 g/mol)	101.736 g	12 M

Take care during the dissolving step as this is very exothermic.

Filter the solution at 0.45 µm and autoclave.

3 M NaAc

Reagent	Quantity (for 200 ml)	Final concentration
NaAc (MW= 82.03 g/ml)	49.218 g	3 M

Dissolve the sodium acetate in 120 ml of water and adjust pH to 5.5 with glacial acetic acid. Autoclave.

TRIS-EDTA

Reagent	Quantity (for 1 l)	Final concentration
Tris-HCl (pH 7.5) MW=121.14g/mole	1.21g	10 mM
EDTA-4Na-2H2O MW=416.2g/mole	0.42g	1 mM

Adjust to pH 7.5 and autoclave.