Extraction of high quality genomic DNA from Ectocarpus

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This protocol was taken from Coehlo et al. (2012). For some applications, such as genome sequencing (Cock et al. 2010) and high-throughput genotyping with multiple markers (Heesch et al. 2010), it is necessary to use high quality genomic DNA. This protocol describes how to obtain several micrograms of high quality, caesium chloride purified DNA from one gram of Ectocarpus filaments. We also recommend using DNA of this quality for quantitative RT-PCR control reactions. Simpler, more rapid, kit-based methods are however preferable for experiments that involve the treatment of large numbers of individuals, such as genotyping large populations with a small number of markers or PCR screening of large populations.

1. Grind about 1 g of frozen filaments in a mortar and pestle under liquid nitrogen for about 15 min.
2. Transfer the powder to a 40 ml teflon tube and add 2 ml of 2% CTAB buffer. Mix well, but gently.
3. Further grind 1 ml aliquots in a Wheaton grinder (5 min per aliquot) and transfer the aliquots to a new teflon tube using a 1 ml filter tip. After all the aliquots have been ground, rinse the Wheaton several times with 1 ml aliquots of CTAB buffer, recover these rinses in the teflon tube and make the total volume up to 15 ml with CTAB buffer. Mix vigorously for 10 min.
4. Add 25 units of Proteinase K. Mix gently and incubate at 55˚C for 2 hours.
5. Add 1 volume of chloroform / isoamylalcohol, (24:1) mix gently and centrifuge at 10,000 rpm (12,000 g) in a JA20 rotor at 12˚C for 20 min. Transfer the upper phase into a new teflon tube and measure the volume.
6. Add 0.3 volumes of 100% ethanol drop by drop whilst stirring gently. Mix well and add 1 volume of chloroform / isoamylalcohol.

This step removes polysaccharides. The ethanol must be added gradually to prevent precipitation of the DNA.

7. Centrifuge at 12,000 rpm (10,000 g) in a JA20 rotor at 12˚C for 20 min, transfer the upper phase to a new teflon tube and measure the volume.
8. Add 0.25 vol. of 12 M lithium chloride and mix it vigorously. Add 1% β-mercaptoethanol and mix well. Incubate at -20˚C overnight.

This step precipitates RNA.

<u>Additional information</u>:

Cock J. M., Sterck L., Rouzé P., Scornet D., Allen A. E., Amoutzias G., Anthouard V., Artiguenave F., Aury J. M., Badger J. H. *et al. 2010. The Ectocarpus genome and the independent evolution of multicellularity in brown algae. Nature* ***465****: 617-621.*

Heesch S., Cho G. Y., Peters A. F., Le Corguillé G., Falentin C., Boutet G., Coëdel S., Jubin C., Samson G., Corre E. *et al. 2010. A sequence-tagged genetic map for the brown alga Ectocarpus siliculosus provides large-scale assembly of the genome sequence. New Phytol* ***188****: 42-51.*

Coelho SM, Scornet D, Rousvoal S, Peters N, Dartevelle L, Peters AF, Cock JM. *2012. Extraction of High-Quality Genomic DNA from Ectocarpus. Cold Spring Harbor Protoc doi: 10.1101/pdb.prot067967.*

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9. Centrifuge at 10,000 rpm (12,000 g), in a JA20 rotor at 4˚C for 30 min, transfer the supernatant to a new teflon tube and measure the volume.

A pellet of RNA should be visible on the side of the tube. The RNA can be recovered by adding 500 μl of water and stored at -20°C.

10. Add 0.8 vol. of 100% isopropanol and incubate at -20˚C for at least 2 hours (ideally overnight). Centrifuge at 10,000 rpm (12,000 g) in a JA20 rotor at 4˚C for 30 min, discard the supernatant and retain the pellet.

11. Add 500 μl of TE buffer pH 8 or water and dissolve the pellet gently. Transfer the DNA solution to a 2 ml tube and measure the volume. Add 0.5 volumes of phenol pH 8.0 and 0.5 volumes of chloroform / isoamylalcohol and mix vigorously. Centrifuge at 13,000 rpm (17,950 g), 12˚C, for 10 min, transfer the supernatant into a new 2 ml tube and measure the volume.

12. Repeat the phenol / chloroform / isoamylalcohol extraction.

13. Add 1 volume of chloroform / isoamylalcohol and mix gently. Centrifuge at 13,000 rpm (17,950 g), 12˚C, for 10 min, transfer the supernatant into a new 2 ml tube and measure the volume.

14. Add 2.5 volumes of 100% ethanol and a tenth volume 3M sodium acetate pH5.2. The DNA should precipitate as a white fluffy material. Incubate at -20˚C overnight, centrifuge at 13,000 rpm (17,950 g), 4˚C, for 30 min and discard the supernatant. Wash the pellet with 200 µl of 80% ethanol. Centrifuge at 13,000 rpm (17,950 g), 4˚C, for 15 min and discard the supernatant. Dry the pellet and dissolve in 200 to 500 µl of Tris-EDTA buffer (pH 7.5).

15. Weigh out 1.818 g of caesium chloride into an Eppendorf tube and add 1 mL of TE pH 8. Add at least 20 µg of DNA in TE pH 8 buffer solution and adjust the volume to 1.5 ml. Add 7.4 µl Hoechst N° 33258 bis-benzimide, mix well and check that the caesium chloride is fully dissolved (if not add more TE pH 8 buffer).

16. Transfer the solution to a 2 ml Polyallomer bell-top tube and weigh the tube. Adjust the weight with TE pH 8 buffer so that pairs of tube differ in weight by less than 5 mg. Place the small metal cap on the tube and heat seal.

17. Spin the sample in an ultracentrifuge at 90,000 rpm (650,000 g), 20 ˚C, for at least 24 hours.

Note that larger preparations in 13.5 ml tubes should be centrifuged for at least 40 hours.

18. Remove the tubes from the rotor and visualise the DNA on a UV table. There should be two bands, the upper band corresponding to organelle DNA and the lower to nuclear DNA.

19. Make a small hole in the top of the tube with a 26 G needle, then puncture the tube with a 18 G needle attached to a 2 ml syringe just underneath the desired DNA band. Carefully aspirate the DNA and transfer it to a 2 ml Eppendorf tube.

20. Add 1 volume of TE-saturated butanol. Mix well, and centrifuge 2 min at 13,000 rpm (17,950 g) to allow the two phases to separate and discard the upper phase (which is the butanol with the extracted Hoechst dye). Repeat this extraction 2-3 times and then check the DNA solution on a UV transilluminator. The solution will fluoresce blue if Hoechst is still present, if this is the case carry out 2 or 3 more butanol extractions.

21. Measure the volume of the DNA solution, add 2.5 volumes of 100% ethanol and precipitate the DNA overnight at -20 °C. Centrifuge at 13,000 rpm (17,950 g), 4 °C, for 30 min and discard the supernatant. Wash the pellet with 50 µl of 80% ethanol and centrifuge at 13, 000 rpm (17,950 g), 4 °C, for 5 min. Discard the supernatant and dry the pellet to remove any ethanol. Dissolve the pellet in 50 ul of Tris-EDTA buffer (pH 8.0). Check the quantity and quality of the DNA using a Nanodrop machine and by running an aliquot on a 0.8% agarose gel.
22. To check the stability and the digestibility of the DNA dilute about 100 ng of DNA to 30 µl with an appropriate restriction digestion buffer and incubate samples either with or without added restriction enzyme (we usually use *Xho*I or *Sal*I) at 37°C overnight. Check the two samples on a 0.8% agarose gel. The undigested sample should produce a band of about 25 kbp whereas the digested sample should have a smeared appearance.

<u>Reagents</u>

Liquid nitrogen

2% CTAB extraction buffer

Proteinase K

Chloroform:isoamylalcohol (24:1, CIA)

Ethanol (100%, 80%)

12 M LiCl

β-mercaptoethanol

Isopropanol

Tris-EDTA (TE, pH8)

Phenol (for DNA)

3 M sodium acetate (NaAc, pH 5.2)

CsCl

Hoechst N° 33258 bis-benzimide

TE saturated butanol

Isopropanol

Distilled water

<u>Equipment</u>

Mortar and pestle

5 teflon tubes

Wheaton grinder (1 ml)

2 ml Eppendorf tubes

55˚C water bath

Pipettes

Filter tips

Centrifuge

Ultracentrifuge

Weighing balance

Polyallomer bell-top tube

Vacuum centrifuge

Minicentrifuge

Beckman heater (for ultracentifuge tube sealing)

Parafilm

26G needle

2 ml syringe with 18G needle

Aluminium foil

UV illuminator

Filter tips

Pipettes

SOLUTIONS

2% CTAB EXTRACTION BUFFER

Reagent	Quantity (for 30 ml)
100 mM TrisHCl (pH 6)	3 ml of 1M stock
1.5 M NaCl	9 ml of 5M stock
2% CTAB	3.75 ml of 16% stock
50 mM EDTA (pH 7)	6 ml of 250 mM stock
50 mM DTT	0.231g

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 5.2 with glacial acetic acid. Autoclave.

TE SATURATED BUTANOL

Reagent	Quantity (for 200 ml)	Final concentration
Butanol	100 ml	50%
Tris-EDTA buffer (pH 8)	100 ml	50%

The saturated butanol is the upper phase.

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 8 and autoclave.