Extraction of High-Quality Genomic DNA from *Ectocarpus*

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Protocol

Extraction of High-Quality Genomic DNA from *Ectocarpus*

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For some applications, such as genome sequencing and high-throughput genotyping with multiple markers, it is necessary to use high-quality genomic DNA. This article describes how to obtain several micrograms of high-quality, cesium chloride-purified DNA from 1 g of *Ectocarpus* filaments. We also recommend using DNA of this quality for quantitative RT–PCR control reactions. However, simpler, more rapid, kit-based methods are 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.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

RECIPES: Please see the end of this article for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Agarose (0.8%)
Cesium chloride
Chloroform:isoamylalcohol (24:1)
CTAB (2%) extraction buffer <R>
Ectocarpus (frozen filaments)
Ethanol (100% and 80%)
Hoechst 33258 (bisbenzimide)
Isopropanol
Liquid nitrogen
Lithium chloride (12 M)

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Take care when dissolving the LiCl because this step is exothermic. Filter the solution through a 0.45-µm membrane and autoclave.

β-Mercaptoethanol

Phenol (pH 8.0)

Proteinase K

Restriction enzyme and buffer (e.g., XhoI or SalI; see Step 22)

Sodium acetate (3 M, pH 5.2)

TE-saturated butanol

Prepare 200 mL of a 1:1 butanol:TE (pH 8) solution. The saturated butanol is the upper phase.

TE buffer (pH 7.5 and 8.0)

Equipment

Centrifuge with JA20 rotor and adjustable temperature (4°C and 12°C)

Equipment for agarose gel electrophoresis

Filter tips (1 mL)

Heat sealer (for sealing ultracentifuge tubes) (Beckman)

Microcentrifuge tubes (2 mL)

Mortar and pestle

NanoDrop spectrophotometer

Needles (18G and 26G)

Polyallomer bell-top tubes

Syringe (2 mL)

Teflon tubes (40 mL)

Ultracentrifuge (see Step 17)

UV illuminator

Water bath (set at 55°C)

Weighing balance

Wheaton grinder (1 mL)

METHOD

1. Grind about 1 g of frozen *Ectocarpus* filaments in a mortar and pestle under liquid nitrogen for ~15 min.
2. Transfer the powder to a 40-mL Teflon tube and add 2 mL of CTAB (2%) extraction buffer. Mix well, but gently.
3. Further grind the extract in 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 vessel several times with 1-mL aliquots of CTAB (2%) extraction buffer, recover these rinses in the Teflon tube, and make the total volume up to 15 mL with CTAB (2%) extraction buffer. Mix vigorously for 10 min.
4. Add 25 U of proteinase K. Mix gently and incubate for 2 h at 55°C.
5. Add 1 volume of chloroform/isoamylalcohol (24:1), mix gently, and centrifuge at 10,000 rpm (12,000*g*) in a JA20 rotor for 20 min at 12°C. Transfer the upper phase into a new Teflon tube and measure the volume.

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6. Add 0.3 volumes of 100% ethanol drop by drop while 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 for 20 min at 12°C, transfer the upper phase to a new Teflon tube, and measure the volume.

8. Add 0.25 volumes of 12 M lithium chloride and mix vigorously. Add β-mercaptoethanol to a concentration of 1% and mix well. Incubate overnight at −20°C.

   This step precipitates RNA.

9. Centrifuge at 10,000 rpm (12,000*g*) in a JA20 rotor for 30 min at 4°C. 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 volumes of 100% isopropanol and incubate for at least 2 h (ideally overnight) at −20°C. Centrifuge at 10,000 rpm (12,000*g*) in a JA20 rotor for 30 min at 4°C. Discard the supernatant and retain the pellet.

11. Add 500 µL of TE buffer (pH 8.0) 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*) for 10 min at 12°C. 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*) for 10 min at 12°C. Transfer the supernatant into a new 2-mL tube and measure the volume.

14. Add 2.5 volumes of 100% ethanol and 0.1 volumes of 3 M sodium acetate (pH 5.2). (The DNA should precipitate as a white fluffy material.) Incubate overnight at −20°C. Centrifuge at 13,000 rpm (17,950*g*) for 30 min at 4°C and discard the supernatant. Wash the pellet with 200 µL of 80% ethanol. Centrifuge at 13,000 rpm (17,950*g*) for 15 min at 4°C and discard the supernatant. Dry the pellet and dissolve in 200–500 µL of TE buffer (pH 7.5). Determine the concentration of DNA in the tube.

15. Weigh out 1.818 g of cesium chloride into a microcentrifuge tube and add 1 mL of TE (pH 8.0). Add at least **20 µg of DNA in TE buffer (pH 8)** and adjust the volume to 1.5 mL. Add 7.4 µL of Hoechst 33258 (bisbenzimide), mix well, and check that the cesium 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 8.0) buffer so that pairs of tubes differ in weight by <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*) for at least 24 h at 20°C.

    Note that larger preparations in 13.5-mL tubes should be centrifuged for at least 40 h.

18. Remove the tubes from the rotor and visualize 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 microcentrifuge tube.

20. Add 1 volume of TE-saturated butanol. Mix well, and **centrifuge at 13,000 rpm (17,950*g*) for 2 min** at room temperature 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 two or three 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*) for 30 min at 4°C and discard the supernatant. Wash the pellet with 50 µL of 80% ethanol and centrifuge at 13,000 rpm (17,950*g*) for 5 min at 4°C. Discard the supernatant and dry the pellet to remove any ethanol. Dissolve the pellet in 50 µL of TE buffer (pH 8.0). Check the quantity and quality of the DNA using a NanoDrop spectrophotometer 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 XhoI or SalI) overnight at 37°C. Check the two samples on a 0.8% agarose gel.

    The undigested sample should produce a band of about 25 kb whereas the digested sample should have a smeared appearance.

RELATED INFORMATION

For further details regarding the biology of *Ectocarpus* and the development of genomic and genetic tools for this organism, see ***Ectocarpus*: A Model Organism for the Brown Algae** (Coelho et al. 2012).

RECIPE

CTAB (2%) Extraction Buffer

Reagent	Quantity (for 30 mL)	Final concentration
Tris–HCl (1 M, pH 6)	3 mL	100 mM
NaCl (5 M)	9 mL	1.5 M
Hexadecyltrimethylammonium bromide (CTAB) (16%, w/v)	3.75 mL	2% (w/v)
EDTA (250 mM, pH 7)	6 mL	50 mM
DTT	0.231 g	50 mM

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