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High molecular weight DNA extraction for marine macroalgal tissue

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We use this protocol and it's working

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Abstract

This protocol details high molecular weight DNA extraction for marine macroalgal tissue. Marine macroalgae contain a variety of unique cell wall components including sulfated polysaccharides and polyphenolics. These components often co-elute with high molecular weight (HMW) DNA and lead to reduced library prep and sequencing outcomes. This protocol incorporates polyvinylpolypyrrolidone (PVPP) and β-mercaptoethanol (BME) to reduce polyphenolic contamination, and an early salting out step with potassium acetate (KOAc) to address polysaccharides. This protocol is largely adapted from an Oxford Nanopore HMW DNA extraction from Arabidopsis leaves, which incorporates the QIAGEN Blood and Cell Culture DNA Midi Kit for column cleanup. The DNA product often requires additional cleanup after elution, and we suggest the BluePippin 15kb size selection for all HMW applications.

Attachments



711-1533.pdf

55KB



Guidelines

Marine macroalgae contain a variety of unique cell wall components including sulfated polysaccharides and polyphenolics. These components often co-elute with high molecular weight (HMW) DNA and lead to reduced library prep and sequencing outcomes. This protocol incorporates polyvinylpolypyrrolidone (PVPP) and βmercaptoethanol (BME) to reduce polyphenolic contamination, and an early salting out step with potassium acetate (KOAc) to address polysaccharides. This protocol is largely adapted from an Oxford Nanopore HMW DNA extraction from Arabidopsis leaves, which incorporates the QIAGEN Blood and Cell Culture DNA Midi Kit for column cleanup. The DNA product often requires additional cleanup after elution, and we suggest the BluePippin 15kb size selection for all HMW applications.

Additional tips:

- In the field or in lab, it is vital to scrape off all surface epiphytes and wash the sample in clean water before flash freezing to reduce contaminants common in the marine environment that confound genome assembly.
- Marine macroalgae are incredibly diverse in biochemical content, so individual seaweeds may require troubleshooting. Suggested alterations include varying input tissue type or quantity, increasing CTAB or BME percent, or adding a second chloroform separation.
- It may be necessary to carry out extractions of the same tissue in parallel to yield sufficient DNA, especially when large losses from BluePippin are expected. It is not suggested to combine multiple extractions onto the same column, as this may lead to overloading and a dirty sample. This protocol as written, paired with BluePippin, has produced sequencing-quality DNA for Nanopore from a red alga Porteria hornemanii and a brown alga Macrocystis pyrifera. For P. hornemanii, a single 🚨 20 mL extraction produced sufficient DNA for sequencing, but for *M. pyrifera*, three parallel extractions of ____ 20 mL were necessary.



Materials

Equipment:

- Lyophilizer
- Stir plate
- Heat block or water bath
- Vortex
- Mortar and pestle
- Refrigerated centrifuge for spins up to 3,500 xg with

 4 50 mL
- Suggested: Sage Science BluePippin

Consumables:

- Stock solution: [M] 1 Molarity (M) Tris-HCl, Opt 9.5
- Stock solution: [M] 5 Molarity (M) sodium chloride (NaCl)
- Stock solution: [M] 500 millimolar (mM) ethylenediaminetetraacetic acid (EDTA)
- Stock solution: [M] 5 Molarity (M) potassium acetate (KOAc)
- Cetyltrimethylammonium bromide (CTAB)
- Polyethylene glycol (PEG) 8000
- β-mercaptoethanol (BME)
- Polyvinylpolypyrrolidone (PVPP)
- RNase A, <u>A</u> 100 mg/mL (eg. QIAGEN Mat. #1007885)
- 100% isopropanol
- 95-100% ethanol
- Nuclease-free water
- 🔀 Blood & Cell Culture DNA Mini Kit (25) Qiagen Catalog #13323
- Tris-EDTA (TE) buffer
- 4 50 mL Falcon Tubes
- **I** DNA LoBind Tube 1.5ml **Eppendorf Catalog** #022431021
- Suggested: Sage Science High Pass Plus Cassette (BPLUS10 or BPLUS03) for BluePippin

Troubleshooting



Lyophilizing algal tissue

- 1 Flash-freeze algal tissue in liquid nitrogen (target $\geq 45 \text{ g}$ wet tissue).
- 2 Quickly transfer sample to lyophilization container and freeze dry for 36-48 hours.
- Macerate the tissue with a clean spatula to increase surface area and put on the lyophilizer for another 24:00:00.
- Remove and refrigerate with desiccant for immediate use, or store at longer periods.

Setting up the DNA extraction

- 6 Pre-heat a heat block or water bath to \$\mathbb{\mod}\mod}\mathbb{\m
- For each extraction, transfer 4 20 mL of Carlson lysis buffer to a 50-ml Falcon tube.
- Scoop 0.5 teaspoons lyophilized plant tissue into a clean mortar and add

 50-100 mg powdered PVPP. Grind with pestle for ~ 00:00:30 , until tissue is powdered and combined, but not long enough to introduce significant moisture. Move immediately into DNA extraction.

Lysis and first precipitation

30m

1d

1d



13

- 10 Pour tissue into the warm buffer. Invert 5 times.
- 11 Add \perp 40 μ L of RNase A and vortex for \bigcirc 00:00:05.

5s

Optional: If using a heat block with mixing, set the block (still at 65 °C) to mixing at



Incubate for (5) 01:00:00 at \$ 65 °C .



13.1 Invert 10 times every 15 minutes.

45 300 rpm, 00:05:00

13.2 At 30 minutes, add another Δ 40 μ L of RNase A, inverting 10 times to combine.



Allow the tubes to cool down to Room temperature for 00:10:00.



Add 4 20 mL chloroform and vortex for two pulses of 00:00:05 each.



16 Centrifuge the tubes at 3500 x g, 4°C, 00:15:00.

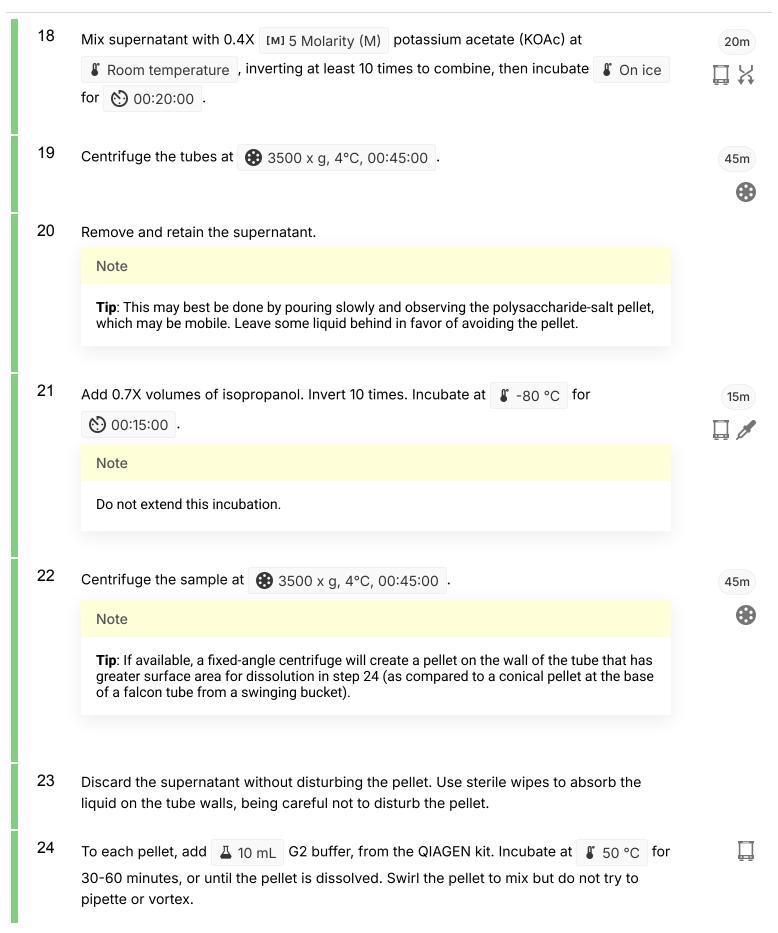


In a fume hood, transfer the top layer of lysate from each tube to a new 50-ml Falcon tube, without disturbing the interphase.

Note

Tip: The lysate layer should be $\[\] 4-18 \]$ of solution, but it is recommended to use widebore tips, transferring $\[\] 1 \]$ at a time. Tips can also be widened by cutting standard P1000 tips.







Column cleanup

- 25 Equilibrate a QIAGEN Genomic-tip 100/G column with 4 mL of Buffer QBT.
- Pour the DNA in G2 buffer through the equilibrated column and allow it to flow through with just gravity.
- Once all the lysate has passed through, wash the column with 4 8 mL of Buffer QC.
- 28 Repeat the wash with another 4 8 mL of Buffer QC.
- Place the column over a clean 50-mL Falcon tube, and elute the genomic DNA with 45 mL of Buffer QF, pre-warmed to 45 s 55 °C.
- Allow the eluate to cool down to Room temperature.
- 31 Add 🚨 3.5 mL of isopropanol to the eluted DNA and mix by inverting the tube 10 times.
- 32 Incubate the tube at 4 -20 °C for at least 3 hours, or 🖒 Overnight .

Final precipitation

- 33 Centrifuge the tube at 3500 x q, 4°C, 00:45:00.
- 34 Discard the supernatant without disturbing the pellet.
- Add 4 mL of ice-cold 70% ethanol to the pelleted DNA and invert the tube 10 times.

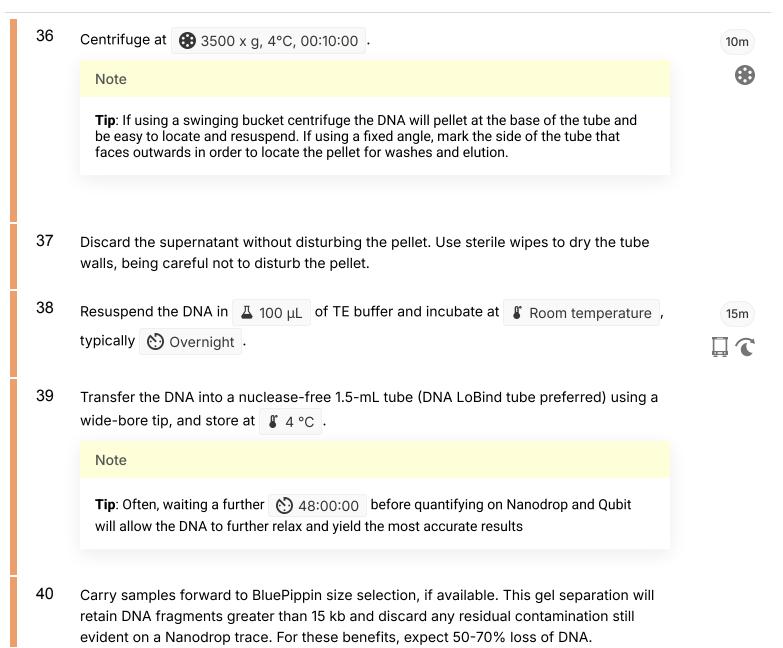
8 %

15m

1h 10m

45m





Protocol references

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