Mar 01, 2024

HMW gDNA purification proto

PLOS One

Peer-reviewed method

DOI
https://dx.doi.org/10.17504/protocols.io.j8nlkoz2xv5r/v1

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PLOS ONE Lab Protocols | Spotlight series

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkoz2xv5r/v1

External link: https://doi.org/10.1371/journal.pone.0301680

Protocol Citation: Ivette Cornejo Corona, Devon Boland, Timothy Devarenne 2024. HMW gDNA purification proto. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkoz2xv5r/v1

Manuscript citation:
Cornejo-Corona I, Boland DJ, Devarenne TP (2024) Method for isolation of high molecular weight genomic DNA from Botryococcus biomass. PLOS ONE 19(7). doi: 10.1371/journal.pone.0301680

Protocol status: Working
We use this protocol and it's working

Created: March 01, 2024

Last Modified: November 12, 2024

Protocol Integer ID: 95973

Keywords: high molecular weight DNA, Botryococcus braunii, long-read sequencing , hmw gdna purification proto, rich microalga botryococcus, hmw gdna, oxford nanopore technologies platform, nanopore, read sequencing

Disclaimer

Spotlight Video

The video below is a supplement with extra context and tips, as part of the protocols.io Spotlight series, featuring conversations with protocol authors.

https://www.youtube.com/embed/im47jGbfuXo?si=2UuE5lV70aGH2SpI

Abstract

Optimized protocol for efficient extraction of HMW gDNA from the polysaccharide-rich microalga *Botryococcus*, enabling long-read sequencing on the Oxford Nanopore Technologies platform.

Attachments

PDF

HMW gDNA PLOS ONE S1...

116KB

Guidelines

Maintain Frozen Samples: It is crucial to keep samples frozen during maceration using liquid nitrogen. Prepare sterile mortar, pestle, and spatulas for this step.
Handle Homogenization with Care: Exercise caution with the use of liquid nitrogen during maceration and sampling preparation.
Optimize Polysaccharide Removal: For effective polysaccharide removal with minimal DNA damage, keep the sonication step prior to cell lysis brief and at a low power setting.
Monitor Pellet Size: After each sorbitol wash step, check for an increase in pellet size.
Buffer Preparation: Prepare and sterilize buffers in advance to streamline the process.
Warm DNA Extraction Buffer: Warm the DNA extraction buffer to 65°C for at least 5 minutes before use for optimal results.

Materials

A. Buffer preparation

1. Sorbitol wash buffer. Autoclave and store this buffer at 4°C for no more than six months.
• 100 mM Tris-HCl pH 8.0
• 0.35 M Sorbitol
• 5 mM EDTA pH 8.0
• 1 % (W/V) Polyvinylpyrrolidone molecular weight 40,000 (PVP-40)
• 1% (V/V) 2-Mercaptoethanol (β-ME). Note: Add after autoclaving and before use. It is best to aliquot the amount of buffer needed and add β-ME to this aliquot.

2. DNA extraction buffer. Autoclave and store this buffer at room temperature for no more than six months.
• 100 mM Tris-HCl pH 8.0
• 3M NaCl
• 3% CTAB
• 20 mM EDTA
• 1% (W/V) Polyvinylpyrrolidone
• 1% (V/V) 2-Mercaptoethanol (β-ME). Note: Add after autoclaving and before use. It is best to aliquot the amount of buffer needed and add β-ME to this aliquot.

3. 24:1 CHCl3/IAA buffer. Store this buffer at 4°C.
• 96 ml Chloroform
• 4 ml Isoamyl Alcohol.

4. 3M Sodium acetate buffer
• 408.3 g sodium acetate
• 3H2O per L
• pH to 5.2
• autoclave

5. 1x TE (Tris EDTA) Buffer
• 1mM EDTA, pH 8.0
• 10 mM Tris-HCl, pH 8.0

B. Culturing *Botryococcus* culturing
• Culture *Botryococcus* species of choice in 1 L roux flasks with 750 ml modified Chu 13 medium, pH 7.5.
• Maintain at 22°C under continuous aeration with 2.5% CO2.
• Grow cultures for 6 weeks under a 12 h light:12 h dark cycle using 13 W compact fluorescent 65 K lighting at an intensity of 280 µmol photons/m2/s.

Before start

Ensure Bench Cleanliness: Prior to starting the protocol, thoroughly sanitize lab bench and instruments.

Biomass harvesting and HMW gDNA isolation

1 **Biomass preparation**
• Harvest the biomass by filtration using a 10 µm nylon net.

1.1 • Collect small amounts of biomass from the mesh using a rubber spatula and immediately freeze by placing in a 50 ml Falcon tube containing liquid nitrogen.
• Repeat until all biomass is collected into a single Falcom tube.
• Store at -80°C until needed.

1.2 • Place small amount of frozen biomass into mortar and pestle with liquid nitrogen.
• Grind biomass until a fine powder is formed, keeping frozen at all times.
• Weigh out ~100 mg aliquots, pace in 1.5 ml eppendorf tube, and store at -80°C.

Biomass pre-wash

2 • Add 1 ml sorbitol wash buffer to 1.5 ml eppendorf tube containing ~100 mg ground biomass.
• Allow sample to thaw while vortexing for 10 seconds
• Keep samples on ice and sonicate for 25 seconds at 30% of power.
• Centrifuge at 2,500 x *g* for 5 minutes at room temperature. Discard the liquid phase by aspiration or decanting. Save the pelleted and floating biomass.
• Repeat the biomass pre-wash step three times.

Extraction process

3 • Add 700 µl DNA extraction buffer pre-warmed to 65°C, homogenize by vortexing for 10 seconds.
• Incubate at 65°C for 30 minutes mixing by inversion every 10 minutes.
• Incubate samples at room temperature for 5 minutes.
• Add 700 µl CHCl3:IAA buffer, vortex for 10 seconds, and centrifuge at 2,500 x *g* for 10 minutes at room temperature.
• Carefully transfer the upper aqueous phase (approximately 500 µl) to a new 1.5 ml eppendorf tube and keep on ice.

RNA digestion

4 • Add 2 µl RNase A (25 mg/ml), and incubate at 37°C for 15 minutes mixing by inversion every 5 minutes.
• Add 500 µl CHCl3:IAA buffer, vortex 5 seconds, and centrifuge at 13,000 x *g* for 10 minutes at 4°C. • Transfer the upper phase to a new 1.5 ml eppendorf tube and keep on ice.

HMW gDNA precipitation

5 • Precipitate the HMW gDNA by adding 0.1 volumes of 3M sodium acetate pH 5.2 and 0.66 volumes of cold (-20°C) isopropanol.
• Incubate samples overnight at -20°C.
• Centrifuge at 13,000 x *g* for 10 minutes at 4°C, and discard supernatant by aspiration or decanting. • Dry pellet by resting inverted on paper towels at room temperature.

6 • Wash dried pellets with 1 ml of 70% ethanol and invert several times.
• Centrifuge at 13,000 x *g* for 10 min at 4°C. Remove the supernatant by aspiration to avoid pellet disturbance.
• Dry samples in a vacuum centrifuge for 10 min at 36°C.
• Resuspend HMW gDNA by adding 100 µl 1x TE buffer. Incubate at room temperature for 10 min then gently homogenize by inversion.
• Avoid pipetting that will shear the DNA.
• Store at -80°C until needed.