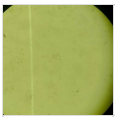

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Algal DNA extraction for HMW Nanopore sequencing

Robert Auber[1], Jennifer Wisecaver[1]

[1]Purdue University

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MinION user group for high molecular weight DNA extraction from all kingdoms Wisecaver Lab

Robert Auber

ABSTRACT

This is a protocol adapted for a haptophte algae. It is a single cellular organism lacking any type of silica or calcium carbonate armor. If your organism doesn't lyse by a simple snap freeze, some type of grinding step is recommended. Confirmation of properly extracting the nuclei can be done with propidium iodide staining and an epiflourescent microscope. The actual DNA extraction gives ultra long reads (greater than 200kb) by using a circulomics NanoBind kit.

MATERIALS TEXT

Supplies

50 ml conical tubes
LoBind 1.5 ml microcentrifuge tubes
1000 µl and 100 µl wide bore pipette tips
Nylon mesh filters: 100 um,70 µm and 40 µm
Liquid nitrogen

Prepared Buffers & Reagents

Nucleus Isolation Buffer (Must be cooled to 0°C for > 1 hour before use):
10 mM Tris pH 9.5
10 mM EDTA 100 mM KCL
500 mM Sucrose
4 mM Spermidine
1 mM Spermine
0.1% BME (add day of extraction)

10% Triton X-100
2-Mercaptoethanol (BME)
Isopropanol
100% Ethanol
Proteinase K (> 600 mAU/mL solution)
RNase A (100mg/mL)

Prepare equipment and reagents

1 Set water bath to 74°C

2 Chill centrifuge to 4°C

3 Add BME to premade NIB (Per Sample 35 µl BME into 35 ml premade NIB + Aliquot 1.8 ml NIB and add 200 µl Triton X-100)

4 Cool NIB to 0°C

5 Cool NIB + Triton X to 0°C

6 Get liquid nitrogen

7 Chill four 50 ml conical tubes (per sample) on ice

Cell Lysis

8

9 Transfer 20-50 ml of culture to a pre-chilled 50 ml conical tube and centrifuge at 4°C at 2000g for 10 min to pellet cells.

10 Discard the supernatant and snap freeze the conical tube in liquid nitrogen.

11 Place cells on ice and add 10 ml of ice cold NIB.

12 Mix by slowly pipetting up and down with a 5 ml pipettor until the mixture is smooth and not clumpy

Filter to remove particulate material

13 Place a 100 µm filter on top of a pre-chilled 50 ml conical tube

14 Slowly run the sample through the filter into the clean tube

15 Wash the filter with 10 ml of ice cold NIB

16 Place a 70 µm filter on top of a second pre-chilled 50 ml conical tube

17 Slowly run the sample through the filter into the clean tube

18 Wash the filter with 10 ml of ice cold NIB

19 Place a 40 µm filter on top of a third pre-chilled 50 ml conical tube

20 Slowly run the sample through the filter into the clean tube (do not wash with fresh NIB)

21 Add 1.5 ml of ice cold NIB+TritonX

22 Centrifuge at 4°C at 500g for 10 min to pellet nuclei.

23 Discard the supernatant

Extract DNA from Nuclei

24 D. Extract DNA by referring to Circulomics Plant Nuclei Big DNA Kit protocol
https://www.circulomics.com/store/Nanobind-Plant-Nuclei-Big-DNA-Kit-Alpha-Version-p99924200