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High molecular weight DNA extraction from algae : SoCK (Sorbitol + CTAB + KAc)

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

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Abstract

High Molecular Weight DNA extraction protocol from algae for Long Read sequencing.
Extraction is performed from flash frozen algal fragments stored at -80° C.
The protocol is adapted for an extraction of 1 g of algal fragment.
The protocol is based on washing the algae with Sorbitol, followed by a conventional extraction with CTAB and then a purification with Potassium acetate coupled with chloroform.
This protocol is specifically adapted to all types of algae (green, red or brown).

Guidelines

Use only wide bore tips.
Work in a chemical hood when using 2-mercaptoethanol.
Allow the DNA to resuspend for at least of 24 hours before proceeding with QC.

Materials

Reagents :

- Tris HCl Buffer 1M Solution, Sterile pH 8.0 **Bio Basic Inc.** **Catalog #**SD8127.SIZE.450ml
- EDTA (0.5 M), pH 8.0 **Life Technologies** **Catalog #**AM9260G
- CTAB (Hexadecyltrimethylamm onium bromide) **BBI Biotech** **Catalog #**CB0108-100g
- Sodium chloride **P212121**
- PEG-8000
- Sorbitol **P212121**
- Polyvinylpyrrolidone (PVP-40) **Bio Basic Inc.** **Catalog #**PB0436.SIZE.250g
- 1X TE buffer (10 mM Tris-HCl pH 8.0 1 mM EDTA)
- 70% Ethanol
- Sodium acetate **P212121**
- 2-Propanol (IsoPropanol) **Bio Basic Inc.** **Catalog #**PC8601.SIZE.4L
- Chloroform Isoamyl alcohol 24:1 **Merck**
- Potassium Acetate
- Ethanol absolute
- RNaseA **Thermo Fisher** **Catalog #**12091021
- 2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M6250
- Liquid Nitrogen

Equipment :

- Eppendorf ThermoMixer C **pipette.com** **Catalog #**2231000667
- Mortar and Pestels
- Vortex Mixer

Chemical fume hood

Protocol materials

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- Ethanol absolute
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- CTAB (Hexadecyltrimethylamm onium bromide) **BBI Biotech Catalog #**CB0108-100g
- Mortar and Pestels
- Sodium chloride **P212121**
- PEG-8000
- Sorbitol **P212121**

Preparation of reagents

1 **Sorbitol pre-wash buffer** : the buffer can be stored for 6 months at 4°C

	Reagents	Final Concentration
	Tris HCl ph8	100 mM
	EDTA	5 mM
	Sorbitol	700 mM
	PVP 40	1%
	H2O	Qsp 100 ml

2 **Lysis Buffer**

	Reagents	Final Concentration
	Tris HCl ph8	100 mM
	EDTA	20 mM
	CTAB	2 %
	NaCl	1.4 M
	PEG 8000	1 %
	H2O	Qsp 20 ml

Sorbitol Wash

39m

3 Add **20 ml of Sorbitol pre-wash Buffer** in a 50 ml tube.

20 mL Sorbitol buffer

4 Put a **mortar in ice.**

10m

Cool the mortar and pestle with **liquid nitrogen** until the bubbling stops.

Liquid Nitrogen

5 **Grind 1g of frozen sample** to a fine powder (approx. 2 min), without adding liquid nitrogen. 2m
1 g 00:02:00

6 **Transfer powder** to sorbitol pre-wash buffer

7 Add **20 µl of 2-Mercaptoethanol** and vortex 5 s
2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250

8 **Centrifuge** 5 minutes with low deceleration 5m
5000 x g, Room temperature, 00:05:00 , Acc 9 / Dec 7

9 Carrefully **remove the supernatan**t without resuspending the pellet. 2m

10 **Perform a second sorbitol wash** as above (step 3 + 7 + 8 +9) 20m

DNA extraction and purification 5h 30m

11 Resuspend the pellet with **20 ml of Lysis buffer**.

12 Add **50 µl of 2-Mercaptoethanol** and **40 µl of RNaseA** (100mg/ml) and vortex 5 s
2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250
RNaseA **Thermo Fisher Catalog #**12091021

13 **Incubate 1h at 65°C** with low agitation (300 rpm) 1h
01:00:00 65 °C

14 **Centrifuge** 10 minutes with low deceleration 10m
8500 x g, 4°C, 00:10:00 , Acc 9 / Dec 7

15 Gently **transfer the supernatant** to a new 50 ml tube.

16 Add :
- 0.1 vol of **absolute ethanol** Ethanol absolute
- 0.25 vol of **KAc 3M** Potassium Acetate
- 1 vol of **chloroform isoamyl alcohol** Chloroform Isoamyl alcohol 24:1 **Merck**

17 Vortex 5 sec and **incubate 20 min at -20°C** 20m
00:20:00 -20 °C

18 **Centrifuge** 20 minutes with low deceleration 20m
8500 x g, 4°C, 00:20:00 , Acc 9 / Dec 7

19 Gently **transfer the aqueous phase** to a new 50 ml tube.

20 Add :
- 0.8 vol of **isopropanol**
2-Propanol (IsoPropanol) **Bio Basic Inc. Catalog #**PC8601.SIZE.4L
- 0.1 vol of **NaAc 3M** Sodium acetate **P212121**
- 0.2% of **2-Mercaptoethanol**
2-mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M6250

21 mix gently by inversion 10 times and **incubate 1h at -20°C** 1h
01:00:00 -20 °C

22 **Centrifuge** 20 minutes with low deceleration 20m
8500 x g, 4°C, 00:20:00 , Acc 9 / Dec 7

23 Carefully **discard the supernatant, gently resuspend the pellet** with 1 ml of cold 70% ethanol.
70% Ethanol

24 **Transfer the resuspended DNA** to a 1.5 ml DNA LoBind tube.

25 **Centrifuge** 10 minutes with low deceleration
5000 x g, 4°C, 00:10:00 , Acc 9 / Dec 7
10m

26 Carefully **discard the supernatant.**
Air dry the pellet at RT for about 10 minutes. 00:10:00
10m

27 **Resuspend the pellet** with 50-100 µl of 1X TE buffer.
1X TE buffer (10 mM Tris-HCl pH 8.0 1 mM EDTA)

28 **Allow the DNA to resuspend** for 2 hours at 55°C or ON at RT.
02:00:00 55 °C
2h

29 **Store the DNA at 4°C.**

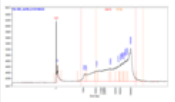
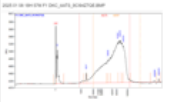
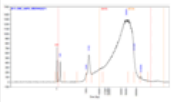
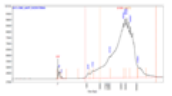
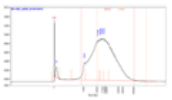
Sample quality control

30 Quantify your sample with a Qubit DNA HS assay.
Check the purity of the sample with a Nanodrop (measurements of 260/280 and 260/230 absorbance ratios).
Estimate the molecular weight of the sample with a Tapestation and/or a Femto pulse

31 Depending on the DNA concentrations and DNA length profiles, deplete short DNA molecules using SRE size selection kits (SRE XS, SRE or SRE XL kits).

Results

32 **QC results** obtained on different species. (3 green algae and 3 red algae)

Phylum	Species	DNA quantity (µg)	Extraction Yield (µg DNA/g organism	Femto pulse kb	Profile Femto pulse
Chlorophyta	Codium saccatum	4,5	2,3	60	
	Codium sp. 1	9,5	9,5	56	
	Chaetomorpha linum	13	11,3	50	
Rhodophyta	Gracilaria dura	2,6	2,6	61	
	Phymatolithon calcareum	4,8	4,6	20	
	Griffithsia corallinoides	1,8	1,6	74