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HMW DNA extraction from red algae for Long Read Sequencing

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

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Abstract

This protocol describes High Molecular Weight DNA extraction for Long Read Sequencing.
This protocol was adapted from Ramakrishnan et al. (2017; doi: 10.1007/s13205-017-0992-2), with some modifications.
This protocol is designed for the extraction of DNA from 200 mg of material that has been flash-frozen in liquid nitrogen and stored at –80 °C.
The procedure involves cell lysis in a Sarkosyl-based buffer supplemented with PVP and β-mercaptoethanol, followed by purification using potassium acetate and chloroform:isoamyl alcohol.

Guidelines

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

Protocol materials

- Liquid Nitrogen
- RNAse A (DNAse free) 100mg/ml **Qiagen** **Catalog #**19101
- 70% ethanol
- DNA LoBind Tubes 1.5 ml **Eppendorf** **Catalog #**022431021
- Beta-mercaptoethanol **Catalog #**97622-10X1ML
- Isopropanol
- Sodium acetate, 3M **Fisher Scientific** **Catalog #**J61928
- Chloroform:Isoamyl Alcohol [24:1] **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**25666
- Ethanol absolute
- Potassium Acetate
- Buffer TE 1x

Preparation of reagents

30m

1 **Extraction Buffer** : Prepare 10 mL of extraction buffer (per 200g of material) in a 50 mL tube

30m

	Reagents	Final concentration
	Tris-HCl ph8	100 mM
	EDTA	20 mM
	NaCl	1.5 M
	Sarkosyl	1%
	PVP40	2%
	H2O	QSP 10 ml

DNA Extraction

1h 26m

2 Add **10 ml of extraction buffer** to a 50 ml tube.

10 mL Extraction buffer

1m

3 Put a mortar in ice.
Cool the mortar and pestle with liquid nitrogen until the bubbling stops.

Liquid Nitrogen

5m

4 **Grind 200 mg of frozen sample** to a fine powder (approx. 2 min).

200 mg Sample

2m

5 **Transfer the powder** to the extraction buffer

1m

6 Add **20 µl of Beta-mercaptoethanol** to the extraction buffer and vortex 5 sec.

Beta-mercaptoethanol **Catalog #**97622-10X1ML

1m

7 **Incubate at room temperature for 1 h** with agitation (300 rpm).

1h

8 **Centrifuge** with low deceleration
8500 x g, 4°C, 00:10:00 , Acc 9 / Dec 6
15m

9 **Transfer the supernatant** to a new 50 ml tube.
1m

DNA Purification
2h 4m

10 Add to the supernatant :
0.1 volume (1 ml) of **absolute ethanol** Ethanol absolute
0.25 volume (2.5 ml) of **3M potassium acetate** Potassium Acetate
1 volume (10 ml) of **chloroform isoamyl alcohol**
Chloroform:Isoamyl Alcohol [24:1] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**25666
Vortex 2× 5 sec
1m

11 **Incubate for 20 min at -20°C**
20m

12 **Centrifuge** with low deceleration
8500 x g, 4°C, 00:20:00 , Acc 9 / Dec 6
25m

13 Gently **transfer the aqueous phase** to a new 50 ml tube.
1m

14 Add **2µl of RNase A** (100mg/ml)
RNAse A (DNAse free) 100mg/ml **Qiagen Catalog #**19101
1m

15 **Incubate for 30 min at 37°C**
30m

16 Add 1 volume (10 ml) of **chloroform isoamyl alcohol**
Chloroform:Isoamyl Alcohol [24:1] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**25666
Vortex 2× 5 sec
1m

17 **Incubate for 20 min at -20°C**
20m

18 **Centrifuge** with low deceleration
8500 x g, 4°C, 00:20:00 , Acc 9 / Dec 6
25m

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

DNA précipitation

4h 2m

20 Beta-mercaptoethanol **Catalog** #97622-10X1ML Add to the supernatant :
1m
0.8 volume (≈ 8 ml) of **isopropanol** Isopropanol
0.1 volume (≈ 1 ml) of **3M sodium acetate**
Sodium acetate, 3M **Fisher Scientific Catalog** #J61928
0.2% (≈ 2 µl) of **β-mercaptoethanol**
Beta-mercaptoethanol **Catalog** #97622-10X1ML

21 **Homogenise by inversion and incubate for 1 hour at -20°C.**
1h

22 **Centrifuge** with low deceleration
8500 x g, 4°C, 00:20:00 , Acc 9 / Dec 6
25m

23 Carefully **discard the supernatant**, gently resuspend the pellet with **1 ml of cold 70% ethanol.**
70% ethanol
5m

24 **Transfer the resuspended DNA** to a 1.5 ml DNA LoBind tube.
DNA LoBind Tubes 1.5 ml **Eppendorf Catalog** #022431021
5m

25 **Centrifuge** with low deceleration
5000 x g, 4°C, 00:10:00 , Acc 9 / Dec 6
15m

26 Carefully **discard the supernatant.**
Air dry the pellet at RT for about 5-10 minutes.
10m

27 **Resuspend the pellet** in 50-100 µl of 1X TE buffer.
Buffer TE 1x
1m

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

29 **Store the DNA at 4°C.**

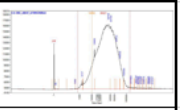
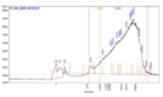
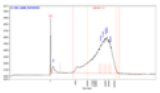
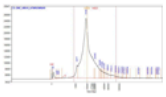
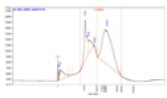
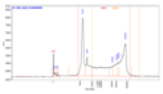
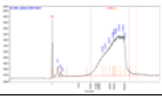
Sample quality control

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.

Phylum	Taxon	DNA quantity µg	Extraction yield µg/g	Femto pulse
Rhodophyta	Gulsonia nodulosa	15	37,5	38 kb 
Rhodophyta	Dasya sp. 1	16	26,7	82 kb 
Rhodophyta	Lythophyllum incrustans	5,1	8,5	40 kb 
Ochrophyta	Sporochnus pedunculatus	4,3	15,0	14 kb 
Ochrophyta	Striaria attenuata	1,5	3,75	18 kb 
Chlorophyta	Cladophora laetevirens	21	52,5	41 kb 
Porifera	Cymbastela cantharella	62	310	73 kb 
Streptophyta	Prunus ramburei	5,6	14	128 kb