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Microbial DNA extraction from the brown seaweed *Ascophyllum nodosum*

Molecular Ecology Resources

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We use this protocol and it's working

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Abstract

This protocol is the result of optimizing microbial DNA extraction from the brown alga *Ascophyllum nodosum*. This protocol includes three different DNA purification steps to remove polysaccharide contaminants and other potential PCR inhibitors. This protocol takes 1.5 days for 22 samples

✅ Short- and long-read sequencing
✅ High molecular weight is obtained (>40 kb)
✅ Sucessfully tested for metagenome sequencing (skip step 1, carry out step 2 in liquid nitrogen, and use 10 mg wet weight of sample in triplicate, and skip the bead beater)

This protocol has been successfully applied to other brown seaweeds:
✅ *Sargassum*

Materials

Consumables & Chemicals

Reagents / Buffers:

- Acetone (100%)
- CTAB 10% (Cetyltrimethylammonium bromide)
- PVPP (Polyvinylpolypyrrolidone)
- NaCl 5M (Sodium Chloride)
- Tris HCL pH 8
- EDTA pH 8
- DIECA 0.1M (Sodium Diethyldithiocarbamate, ref. 228680, SigmaAldrich)
- β-mercaptoethanol (ref. M31148, SigmaAldrich)
- Chloroform/isoamyl alcohol (24:1)
- Ethanol 100%
- Ethanol 70% (with Biological grade water)
- Sodium acetate solution 3M
- Biological grade water (ref. 46-000-CV, Corning, USA)
- NucleoSpin Plant II kit (MachereyNagel, Germany)
- Agencourt AMPure XP beads (Beckman Coulter, Brea, CA, USA)

Disposable materials:

- 1.5 mL standart Eppendorf tubes (standard)
- 2 mL standart Eppendorf tubes (standard)
- 2 mL Safe-Lock Eppendorf tubes (ref. 0030120094)
- Parafilm
- Steel beads for TissueLyser

Lab Equipments:

- Freeze-dryer (lyophilizer) (Qiagen, Hilden, Germany)
- Mortar (for manual grinding)
- Qiagen TissuLyser II (bead beater)
- Vortex mixer
- Centrifuges
- Incubator
- DynaMag™-2 Magnet (ref. 12321D, ThermoFisher)
- Manual pipettes
- Dry ice
- -80°C freezer
- -20°C freezer

Sample prepration

1m

1 Dry matter in freeze-dryer at least 48:00:00

2
- In sterile condition, grind sample by hand with sterile mortar and pestle until a very fine powder is obtained

> Note
>
> Clean with **ethanol 70%** mortar and pestle to avoid contaminations between samples

- Take 10 mg of ground algal tissue, put into 2 mL Eppendorf tube with 1 steel bead
- Put in Qiagen TissueLyser II bead beater (00:01:00 , 30 Hz)

Sodium diethyldithiocarbamate (CTAB) Buffer preparation (for 22 samples)

10m

3 Prepare in a 50 mL Falcon tube:

	Reagent	Initial concentration	Final concentration	Volume to put
	CTAB 10%	10	2	4.6 mL
	PVPP (g)	100	2	0.46 g
	Tris-HCl pH 8	1000	100	2.3 mL
	NaCL 5M	5	1.4	6.44 mL
	EDTA pH 8	500	20	0.92 mL
	Sterile H20			8.74 mL
				Total: 23 mL

Mix the preparation

> Note
>
> Keep CTAB buffer at 21°C or higher to prevent CTAB from precipitating
>
> Use fresh buffer each extraction

Polyphenol Cleaning

16m

4

- Add 1 mL **acetone (100%)**
- Vortex, 10 min
- 12800 x g, 21°C, 00:01:00
- Remove acetone by pipetting
- Repeat the previous steps

1m

5

Dry between 00:05:00 to 00:10:00 to remove any remaining acetone at Room temperature

15m

First DNA purification - Remove polysaccharides

3h 30m

6

Remove bead from each tube by reversing the tube to make it roll away (bead added step 2)

7

In 2mL Eppendorf Safe-Lock Tubes:

- add 1 mL of **CTAB buffer** to each sample
- Add 35 µL of **0.1M DIECA** (Sodium Diethyldithiocarbamate) + 10 µL of **β-mercaptoethanol**
- Incubation during 01:00:00 at 65 °C
- Under Sorbonne, add 1 mL of **chloroform/isoamyl (24:1, v/v)**

1h 30m

> Note
>
> Add parafilm around each Eppendorf Safe-Lock Tube to avoid chloroform vapours

- Vortex, 10 min
- 13200 rpm, 21°C, 00:30:00
- Collect the **upper phase** (750 µL) in a new 2mL Eppendorf Safe-Lock Tube. Be careful to not collect the interphase

8

To the upper phase:

- Add 225 µL of **ethanol 100%** drop by drop and mix by pipette agitation inside the liquid
- Add 975 µL of **chloroform (100%)**

20m

Note

Add parafilm around each Eppendorf Safe-Lock Tube to avoid chloroform vapours

- Vortex, 1 min
- 13200 rpm, 21°C, 00:20:00
- Collect the **upper phase** (550 µL) in new 2mL Eppendorf Safe-Lock Tubes. Be careful to not collect the interphase

9 To the upper phase: 1h 30m

- Add 110 µL of **acetate sodium 3M** solution
- Add 1.1 mL of **ethanol 100%** to precipitate DNA (mixing by tube inversion)
- Put samples in -80 °C during 01:00:00

Note

Prepare 50 mL of **ethanol 70%** and put the tube on ice

- 13200 rpm, 4°C, 00:30:00
- Carefully remove liquid phase without disturbing DNA pellet

10 10m

- Add 500 µL of **cold ethanol 70%**
- Vortex, 20s
- 13200 rpm, 4°C, 00:10:00
- Carefully remove liquid phase without disturbing DNA pellet
- Repeat step 10 once

Note

Pellet could be translucide, white or yellow

11

- Spin down to collect remaining ethanol
- Remove the upper phase by pipetting
- Dry pellet during 00:05:00 at Room temperature to remove remaining ethanol

12 Add 50 µL of **biological grade water** and mix by pipetting 10 times

Note

STOPPING POINT -20 °C

Second DNA purification (with Nucleospin Plant Kit II) 13m

13

Note

All of the following steps are processed in sterile conditions and performed according to the manufacturer's instructions (except for step 17)

Place **Buffer PE** at 65 °C

14
- Add 400 µL of **biological grade wate**r to the previously extracted DNA and mix by pipetting 10 times 2m
- Add 400 µL in purple Nucleospin column
- 11000 x g, 21°C, 00:02:00
- Keep the flow-through which contains the DNA
- Add 450 µL of **Buffer PC**
- Mix by pipetting 5 times

15
- Add 450 µL in green NucleoSpin Column 2m
- 11000 x g, 21°C, 00:01:00
- Discard the flow-through.
- Add the remainder in green NucleoSpin Column
- 11000 x g, 21°C, 00:01:00
- Discard the flow-through.

16
- Add 400 µL of **buffer PW1** to the green column 1m
- 11000 x g, 21°C, 00:01:00
- Discard the flow-through.

17 2m

- Add 700 µL of **buffer PW2** to the green column
- 11000 x g, 21°C, 00:01:00
- Discard the flow-through
- Repeat the previous step
- Add 200 µL of **buffer PW2** to the green column
- 11000 x g, 21°C, 00:01:00
- Discard the flow-through

18 6m

- Put the green column on a clean 1.5 mL Eppendorf
- Elution process: Add 25 µL of **Buffer PE** on the green column membrane
- Incubation at 65 °C during 00:05:00
- 11000 x g, 21°C, 00:01:00
- Keep the flow-thought which contains the DNA
- Repeat the elution process

Note

STOPPING POINT -20 °C

Thrid DNA purification (with Protocol Agencourt AMPure XP) 40m 30s

19 30m

Note

All the following steps are processed in sterile conditions and performed according to the manufacturer's instructions

1. Place the bottle of **Agencourt AMPure XP** at Room temperature 00:30:00 before use and shake it to resuspend any magnetic particles.

2. Prepare fresh 70% ethanol with biological grade water

20 5m
- Add 90 µL of **Agencourt AMPure XP** (according to sample reaction volumes: Volume AMPure = 1.8 x sample volume)
- Mix by pipetting 10 times. Color must be homogenous
- Incubation at Room temperature during 00:05:00

21 2m 30s
On DynaMag™-2 Magnet:
- Put Eppendorf on magnetic plate during 00:02:00. Wait for the solution to clear
- Removing slowly and discard the upper phase. Leave a few µL of supernatant behind, otherwise beads are drawn out with the supernatant.
- Add 200 µL of **ethanol 70%**
- Incubation 00:00:30 at Room temperature
- Slowly remove the supernatant by pipetting and discard
- Repeat the previous steps

22 Remove the reaction plate from the magnet plate, dry the pellet to remove any trace of ethanol
Wait until the pellet is a little cracked

23 2m
- Add 50 µL of elution **buffer PE** (from kit NucleoSpin)
- Mix by pipetting 10 times
- Incubation during 00:02:00 at Room temperature

24 1m
On DynaMag™-2 Magnet:
- Put Eppendorf on magnetic plate during 00:01:00
- Transfert the DNA elution (upper phase) into a new 1.5 mL Eppendorf

25
Note

The end.
Short-reads: Conservation at -20 °C
Long-reads: Conservation at -80 °C

Quality control

26 DNA must be measured by Qubit Fluorometer (Thermo Fisher Scientific) or equivalent

Protocol references

Panova, M. et al. 2016. DNA Extraction Protocols for Whole-Genome Sequencing in Marine Organisms, pp. 13–44. *In* Bourlat, S.J., ed, Marine Genomics: Methods and Protocols. Springer, New York, NY.

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