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Total DNA extraction from microalgae strain samples using NucleoSpin Plant modified kit (Macherey Nagel)

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This DNA extraction protocol allows to get both eukaryotic and prokaryotic DNA from microalgae strains, so the microbiome diversity can be studied in cultures by using this protocol.

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protocol

Camara dos Reis M., Romac S. et al. Microbiome assembly in axenic Emiliania huxleyi cultures is influenced by the source community composition and is resilient to disturbance. In prep.

DNA, Microalgae

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You can perform this protocol in safety conditions on the bench.

-NucleoSpin PlantII (Macherey-Nagel - ref 740770.50 (50 preps);
-Proteinase K 100 mg (Macherey-Nagel - ref 740506) ;
-Optionnal : Lysozyme (Sigma - ref L6876-1G).
-TE 1x buffer sterile (Dominique Dutscher – ref 348619).

-Centrifuge 5427R (Eppendorf, ref 5409000210).
-Thermomix - magnetic stirrer (Eppendorf – ref 5382000015) equipped with thermoblock 1.5-2mL (Eppendorf - ref 3880000151)

- Wear a labcoat and nitrile gloves during all the process.

-Clean all the area with DNA away.
-Heat the Incubator to 55°C for lysis.
-Heat buffer PL1 at 50°C for at least 30min before to start the extraction to dissolve SDS precipitant.
-Equilibrate buffer PE to 65°C.
-**Proteinase K** : add 2500 µl Proteinase K buffer PB to adjust concentration to 20 mg/mL, incubate 1min at RT. Don't vortex! Do aliquots of 250µL. Store at -20°C. Don't freeze/defreeze aliquots more than 3 times.
-Thaw/prepare **Lysozyme** (**20mg/ml**) in TE-Buffer.
-**Wash Buffer PW2**: add 100ml EtOH 96-100%

Cell Lysis

1 Transfer 2mL from the strain in a sterile 2mL microtube.
Centrifuge 5 min at 5000g to pellet the strain.
Discard the supernatant.
Pellet are ready for extraction or can stored at -20°C until extraction.

2 Add 400µL **PL1** + 25µL **proteinase K 20 mg/mL**.
Vortex.

If you don't need to extract prokaryotic DNA, skip step 3 et directly go to step 4.

3 **Optionnal :**

If you also want to extract the prokaryotic DNA to study microbiome associated to the eukaryote strain, add 100µl **Lysozyme 20mg/mL.**
Vortex and incubate for 5 min at room-temperature.

Incubate in the thermomixer 60 min @ 55°C, 900 rpm.

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Clarification of crude lysate

5 After lysis, short spin the lysate and transfer the lysate on a NucleoSpin Filter column (purple column).
Centrifuge 5min @ 11 000g.

6 Transfer the eluate on a new sterile 2mL microtube. If a pellet is visible, only transfer the supernatant without touching the pellet.

Precipitation/Purification

7 Add 550 µL **PC buffer**, mix by pipetting up and down at least 5 times.
Transfer all liquid onto a green column (max 650µL).
Centrifuge 1min @ 11 000g. Discard the flow-through.
Repeat this step until all liquid has been loaded on the green column.

8 Add 400µL **PW1** to the membrane of the column.
Centrifuge 1min @ 11 000g. Discard the flow-through.

9 Add 700µL **PW2** to the membrane of the column.
Centrifuge 1min @ 11 000g. Discard the flow-through.

10 Add 200µL **PW2** to the membrane of the column.
Centrifuge 2min @ 11 000g. Discard the flow-through.

11 Put the column on a new sterile 1.5mL microtube correctly labeled (name, date, operator).

DNA Elution

12 Add 50µL **PE** (preheated 65°C) onto the membrane. Incubate 5 min @ 65°C.
Centrifuge 1min @ 11 000g.

13 Add again 50µL **PE** (preheated 65°C) onto the membrane.
Centrifuge 1min @ 11 000g.

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14 After centrifugation, store the DNA at -20°C.

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