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DNA extraction NucleoSpin Tissue INRAE eWHALE

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

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Abstract

This DNA extraction protocol aims at extracting total genomic DNA from filtered seawater samples suspended in Tris-EDTA-SDS Buffer.

Our protocol mostly follows the NucleoSpin Tissue protocol (qr.mn-net.com/qr/(IFU)740952), section 5 (Standard protocol for human or animal tissue and cultured cells). It starts at step 3, with 200µL of sample, and adds 25µL Proteinase K to the original step. To maximize DNA concentration, Buffer BE is heated at 70°C, and elution is repeated twice with the same 100µL of Buffer BE with 3 min incubation.

Materials

- Pipettes : monochannel p100, p200, p1000 and corresponding filter tips
- 1.5 mL microcentrifuge tubes
- Ethanol (96-100%)

Before start

Sample collection and preparation:
Seawater was filtrated using Sylphium pods with 0.45µm filters. Using a syringe, water was removed and filters dried. To lyse cells and preserve DNA, 3mL of Tris-EDTA-SDS Buffer was added to each Sylphium pod. Sylphium pods were freezed quickly after.

Lab work:
Prepare Buffer B5 and Proteinase K.
Put all equipment (micropipettes, disposable tips, microtubes, columns, ethanol, B3, BW and B5) in UV cabinet and light UV for 20 mins.
Set incubator to 70°C and heat Buffer BE to 70 °C.
Vortex and spin samples.

Lyse sample

1 Add 200 µL of Sample to a microcentrifuge tube. 10m
Add 200 µL Buffer B3 and 25 µL Proteinase K solution.
Vortex vigorously.
Incubate at 70 °C for 00:10:00 .
Vortex briefly.

Adjust DNA binding conditions

2 Add 210 µL ethanol (96 – 100 %) to the sample and vortex vigorously.

Bind DNA

3 For each Sample , place one NucleoSpin Tissue Column into a Collection Tube. 1m
Apply the Sample to the column.
Centrifuge for 00:01:00 at 11,000 x g.
Discard Collection Tube with flowthrough and place the column in a new Collection Tube.

Wash silica membrane

4 Add 500 µL Buffer BW. 2m
Centrifuge for 00:01:00 at 11,000 x g.
Discard flowthrough and place the column back into the Collection Tube.
Add 600 µL Buffer B5 to the column and centrifuge for 00:01:00 at 11,000 x g.
Discard flowthrough and place the column back into the Collection Tube.

Elute highly pure DNA in two steps with Buffer BE heated at 70°C 8m

5 Place the NucleoSpin Tissue Column into a 1.5 mL microcentrifuge tube and add 100 µL Buffer BE. 8m
Incubate at room temperature for 00:03:00 .
Centrifuge 00:01:00 at 11,000 x g.
Add ***the same*** 100 µL Buffer BE back in the NucleoSpin Tissue Column.
Incubate at room temperature for 00:03:00 .

Centrifuge 00:01:00 at 11,000 x g.

Discard NucleoSpin Tissue Column.