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DNA Extraction - Qiagen BioSprint® 96 Workstation using the Biosprint 96 tissue protocol UIBK eWHALE

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Lauren Rodriguez[1], Bettina Thalinger[1]

[1]University of Innsbruck

Lauren Rodriguez
University of Innsbruck

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

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Abstract

This DNA extraction protocol is used in Rodriguez et al., 2024 (in prep) for the extraction of environmental DNA from samples collected in the North-East Atlantic and Mediterranean Sea as a part of the eWHALE project. The protocol outlines lysis from both Sylphium (**https://sylphium.com/eng/**) and Smith-Root (**https://www.smith-root.com/**) eDNA filters and DNA extraction of lysates using the Qiagen BioSprint® 96 Workstation automated extraction robot + BioSprint 96 DNA blood Kit (ID: 940057). For further information on this protocol, please refer to Qiagen (2012) BioSprint® 96 DNA Handbook. Available via Qiagen: https://www.qiagen.com/us/resources/resourcedetail?id=64902c5d-9c3c-4fe3-a3f7-668c4704d9eb&lang=en

Materials

- Pipettes : monochannel p10, p100, p1000, p5000 and corresponding filter tips
- 1.5 mL and 2 mL microcentrifuge tubes
- 3 mL, 5 mL, or 10 mL syringes
- Ethanol (96-100%)
- BioSprint 96 DNA blood Kit (ID: 940057. Qiagen)
- Spin columns with silica membrane removed (from this kit ID: 69504, Qiagen)
- S-Blocks (ID: 19585, Qiagen)
- Microplates (ID: 1031656, Qiagen)

Safety warnings

Lysis should be done in a laminar flow hood.
Always change pipette tips while working with different lysates.

Ethics statement

Not applicable to this study.

Before start

Sample collection and preparation:
Seawater was collected in the waters surrounding the Azores Islands as well as in the Ligurian Sea filtered using Sylphium filter capsules with 0.45µm filters. All filters were stored at -20 °C until being shipped or transported to the laboratory at the University of Innsbruck (Austria). Once arriving at the laboratory, filters were again stored at -20 °C.

Lab work:
Clean all workbenches with bleach and ethanol prior to use.
Thaw all samples.

Lyse sample

6h 10m

1 Smith-Root Filters

1.1 Label 2 mL tube for each Sample (+ control)

1.2 Remove filter housing from the plastic bag. The yellow half of the filter should be facing the bottom of the bag. Use an Erlenmeyer flask to hold the filter upright while the filter is being opened.

1.3 Discard the beige filter top.

1.4 Using two pairs of forceps and a pair of scissors, all which have been sterilized by flame 3x each, fold the "top" filter paper from each Sample into a triangle and put it into the corresponding 2 mL reaction tube (tip is at the bottom end).

1.5 Pipette 380 µL TES buffer (an alternative to Qiagen DNeasy lysis buffer) and 20 µL Proteinase K (total lysis buffer mixture = 400 µL) to all 2 mL reaction tubes, taking care to saturate the filter paper.

Note

It's easiest to prepare a mixture of these two liquids for all samples in a falcon tube (always calculate with extra volumes: 1 for negative control, one-two for pipetting errors).

Note

300 µL of lysis buffer mixture would suffice.

1.6 Vortex filters/turn them upside down a few times.

1.7 Incubate all 2 mL tubes at 56 °C for 03:00:00 3h

1.8 Remove from incubator, briefly to spin down lysate.

1.9 Transfer filter into a plastic inlet* and place the inlet + filter back into the original 2 mL tube in which the filter came from.

> Note
>
> Make sure to remove the silica membrane from the plastic inlet!!!

1.10 14000 rpm, 00:10:00 Centrifuge tubes with the 2 mL lid open. 10m

1.11 Throw away the filters from each tube - lysate for extraction remains at the bottom of the 2 mL tube.

2 Sylphium Filters

2.1 Make sure that all filter capsules are labelled and closed properly on the top and bottom.

2.2 Incubate filters at 56 °C for 03:00:00 3h

2.3 Label 2 mL for each Sample (+ control)

2.4 Use 3 mL to 10 mL syringes (which fit to Luer Locks) to evacuate the lysis buffer from each capsule. Hold the filter vertically, attach syringe to the bottom cap, unscrew the top cap then suck the lysate out.

> Note
>
> Use a new syringe for each filter!

DNA Extraction using Qiagen BioSprint® Workstation

3 Prepare extraction reagents according.

3.1 Pipette 650 µL of AW1 Buffer into each well of Wash Plate 1 (S-Block).

3.2 Pipette 500 µL of AW1 Buffer into each well of Wash Plate 2 (S-Block).

3.3 Pipette 500 µL AW2 Buffer into each well of Wash Plate 3 (S-Block).

3.4 Pipette 500 µL AW2 Buffer into each well of Wash Plate 4 (S-Block).

3.5 Pipette 500 µL RNase free water (MilliQ water) + Tween 20 (Final concentration of 0.02%) to each well of Wash Plate 5 (Microplate).

4 Pipette 100 µL Buffer TE or AE into each well of the Elution Plate (Microplate).

5 Prepare lysate bind plates (for DNA uptake).

Note

Each bind plate can accommodate 300 µL of lysate. Depending on the amount of lysate in your sample, prepare several bind plates.

5.1 Pipette 300 µL AL Buffer and 300 µL Isopropanol to each well in each bind plate.

5.2 Add 30 µL MagAttract magnetic particles to each well in the FIRST bind plate.

Note

Vortex the MagAttract bottle for a few seconds prior to pipetting.

5.3 Add 200 µL lysate to each of the bind plates. Fill up any missing lysate volume (i.e., not up to 200 µL) with TES buffer (or DNeasy Lysis Buffer).

6 Place a new Rod Cover into the first bind plate. Start the appropriate program on the BioSprint ("DNA uptake") according to the number of bind plates (2-7 are available).

Note

At the end of the DNA uptake program, the DNA and Rod Cover will be in the last bind plate.

7 Using the last bind plate with DNA + Rod Cover as well as the other plates with all reagents, start the "eDNA extract" program on the BioSprint.

8 Pipette processed extracts (in the elution plate!) into Eppendorf tubes®

Cleanup

9 Carefully dispose of toxic BIoSprint waste into appropriate receptacle.

10 Wash all blocks/plates several times with water and subsequently incubate in a bleach-water bath for 1 day.

11 After 1 day, wash the blocks/plates in the dishwasher for reuse.