Jul 11, 2024

DNA extraction - Zooplankton - 50 tubes

DOI
dx.doi.org/10.17504/protocols.io.36wgqnm9ygk5/v1

coline.royaux[1,2,3], Nicolas Rabet[1,2,3], Céline Bonillo[1,2,3], Myriam Georges[2]

[1]Sorbonne Université; [2]Muséum National d'Histoire Naturelle; [3]UMR BOREA

Coline Royaux
Université Pierre et Marie Curie (Paris VI), Muséum National...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.36wgqnm9ygk5/v1

Protocol Citation: coline.royaux, Nicolas Rabet, Céline Bonillo, Myriam Georges 2024. DNA extraction - Zooplankton - 50 tubes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.36wgqnm9ygk5/v1

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

Created: July 10, 2024

Last Modified: July 11, 2024

Protocol Integer ID: 103142

Keywords: DNA, Extraction, crustacean, freshwater, PNDB, BOREA

Abstract

This protocol is derived from the QIAamp DNA micro kit (QIAGEN) protocol and was used to extract DNA from whole or parts of zooplanktonic freshwater crustaceans (Copepoda, Branchiopoda, ...) from New Caledonia.

Materials

QIAamp (R) DNA Micro kit (50), QIAGEN

Before start

Make sure to properly prepare your buffers (e.g. heating, adding ethanol)

1 Prepare your 50 tubes with one individual per well. Alternate genus in the wells to detect eventual contamination between wells. 1d

1.1 Collect one individual from a sample 1m

1.2 Note its genus and determine its sex with a binocular microscope 1m

1.3 For big individuals (more than 5 mm), dissect a few legs and put it in the well. Be careful not to damage the rest of the body and put it in a tagged Eppendorf tube.
For little individuals (less than 5 mm), put the whole body. 3m

Note

If necessary, use alcool to get the biological material to fall at the bottom of the well

1.4 When all tubes are filled, the biological material has to dry before going to lysis 12h

Note

If necessary, use a micropipette to empty an excess of alcool in the well

Safety information

Make sure the plate is closed when you want to transport it elsewhere

2 Prepare the lysis 15m

2.1 Add 180 µL ATL buffer in each well and equilibrate to room temperature 10m

2.2 Add 20 µL K proteinase in each well, mix by pulse-vortexing for 00:00:15 and briefly centrifuge 15s

3 Put your tubes in a proofer at 56 °C Overnight (6h or more) to lyse the tissues 6h

4 Perform the DNA extraction manually

> **Note**
>
> When manually extracting DNA alone, do not do all 50 tubes at once as timing is short between each step. A maximum of twenty tubes can be done simultaneously.

4.1 **Optional steps, if RNA carrier is needed** : Quantity for twenty tubes (using 20 volumes + 2 margin to avoid errors = 22 volumes)

- Mix 22 µg solid RNA carrier with 22 µL AE buffer and mix until dissolved.
- Mix 22 µL of the AE + RNAse mix with 4400 µL AL buffer (200 µL per volume) and mix.

4.2 Add 200 µL AL buffer (+RNAse if suited) in each tube and mix by pulse-vortexing for 00:00:15 15s

4.3 Add 200 µL 96-100% ethanol in each tube, mix by pulse-vortexing for 00:00:15 , incubate at room temperature for 00:05:00 . 5m 15s

4.4 Briefly centrifuge to remove drops from the lid.

4.5 Prepare all the eluting columns by puting each in a 2mL collection tube.

4.6 Transfer the prepared DNA lysate into the eluting column with a pipette, centrifuge the column 8000 rpm, 00:01:00 , discard the collection tube containing the flow through and place the column in a clean 2mL collection tube. 1m

Safety information

Make sure the column is empty of liquid. If not, centrifuge at higher speed until it is.

4.7 Add 500 µL AW1 buffer in the column, centrifuge 8000 rpm, 00:01:00 , discard the collection tube containing the flow through and place the column in a clean 2mL collection tube. 1m

4.8 Add 500 µL AW2 buffer in the column, centrifuge 8000 rpm, 00:01:00 , discard the collection tube containing the flow through and place the column in a clean 2mL collection tube. 1m

4.9 Centrifuge , 00:03:00 , Full speed to dry the membrane completely. 3m

4.10 Place the column in a clean, closable, eppendorf tube.

4.11 Add 50 µL AE buffer in the column, incubate at room temperature for 00:01:00 and centrifuge , 00:01:00 , Full speed . The DNA extract in the clean eppendorf tube, discard the column. 2m