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DNA extraction - Zooplankton - 50 tubes

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We use this protocol and it's working

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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 alcohol 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 alcohol 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 $^{\circ}\text{C}$  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 putting 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 μ L AW1 buffer in the column, centrifuge  8000 rpm, 00:01:00 , 1m
discard the collection tube containing the flow through and place the column in a clean 2mL collection tube.
- 4.8 Add  500 μ L AW2 buffer in the column, centrifuge  8000 rpm, 00:01:00 , 1m
discard the collection tube containing the flow through and place the column in a clean 2mL collection tube.
- 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 μ L AE buffer in the column, incubate at room temperature for  00:01:00 2m
and centrifuge  , 00:01:00 , Full speed . The DNA extract in the clean eppendorf tube, discard the column.