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DNA extraction - Zooplankton - 96 wells V.1

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

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Abstract

This protocol was used to extract DNA from whole or parts of zooplanktonic freshwater crustaceans (Copepoda, Branchiopoda, ...) from New Caledonia.

1 Prepare your 96-well extraction plate 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 96 wells are filled, the biological material has to dry to go 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 Mix 18 mL T1 buffer and 2.5 mL K proteinase in a Multi-Channel Reservoir and distribute 200 µL of the mix with a multimicropipette in each well 10m

Note

180 µL T1 buffer and 25 µL K proteinase in each well

2.2 Close your extraction plate with a heated aluminium foil and an adhesive plastic film 3m

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

4 Perform the DNA extraction with a DNA extraction robot

4.1 Remove the adhesive film and aluminium foil from the plate and put it in the robot

4.2 It will deposit 200 µL BQ1 buffer and 200 µL ethanol in each wells and mix it

4.3 Then, 600 µL of the wells content (lysate, BQ1, ethanol) are transfered on the tissue binding plate. Reagents excess are emptied in a waste container.

4.4 The tissue binding plate is then dried by a 00:05:00 aspiration to bind DNA to the silica membrane of the binding plate 5m

4.5 The silica membrane is then washed with 600 µL BW buffer and twice with 900 µL B5 buffer per well. Each wash is intercalated by a 00:05:00 aspiration dry. 5m

4.6 The waste container is then removed from under the binding plate which is dried again by a 00:10:00 aspriration 10m

4.7 An empty extraction plate is placed under the binding plate to retrieve the genomic DNA from it

4.8 DNA is eluted from the tissue binding plate with 100 µL BE buffer in each well and is collected in the new extraction plate underneath

4.9 After a 00:03:00 rest, the binding plate is dried for 00:02:00 and the elution is repeated with 100 µL BE buffer 5m

4.10 Retrieve the new extraction plate containing the genomic DNA and discard the rest