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Daphnia magna: DNA extraction for downstream whole genome NGS

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Albano Pinto[1], Joana Luísa Pereira[1]

[1]CESAM - Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro, Portugal

Joana Luísa Pereira
University of Aveiro

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Abstract

This protocol provides optimised procedures for the extraction of DNA from the freshwater microcrustacean *Daphnia magna*, based on the use of a commercial kit (MasterPure Complete DNA and RNA Kit, Epicentre, Biosearch Technologies), added indications for preliminary quality control. By running this protocol, one should obtain sufficient DNA yield towards downstream next-generation whole-genome sequencing applications, e.g. base-level DNA methylation assessment (Methyl-seq); as well, samples should withstand good quality levels for the purpose, namely low-to-minimal contaminants and high molecular weight (low fragmentation).

Materials

Apart from the MasterPure Complete DNA and RNA Kit (Epicentre, Biosearch Technologies), the following materials will be needed to carry out the protocol:

Isopropanol, 99.5%, molecular biology grade **Thermo Fisher Scientific Catalog #**327272500
Ethanol 70%
SYBR Safe DNA Gel Stain **Invitrogen - Thermo Fisher**
10X TBE Buffer **Thermo Fisher Scientific**
6X DNA Loading Dye **Thermo Fisher Scientific**
GeneRuler 1 kb Plus DNA Ladder **Thermo Fisher Catalog #**SM1331

Sterile 1.5-mL Eppendorf tubes
Sterile 15 mL Falcon tubes
Sterile pipette tips
Sterile pestles matching 1.5-mL Eppendorf tubes
Ice

Thermoblock set to 65°C
Refrigerated centrifuge (4ºC)
Microcentrifuge for short spins
Vortex
Qubit fluorometer (Qubit 3.0, Invitrogen)
Nanodrop spectrophotometer (NanoDrop 1000 spectrophotometer, Nano-Drop Technologies, Wilmington, DE, USA)
Electrophoresis system

Daphnids

Protocol materials

Isopropanol, 99.5%, molecular biology grade **Thermo Fisher Scientific Catalog #**327272500
Ethanol 70%
SYBR Safe DNA Gel Stain **Invitrogen - Thermo Fisher**
6X DNA Loading Dye **Thermo Fisher Scientific**
GeneRuler 1 kb Plus DNA Ladder **Thermo Fisher Catalog #**SM1331
10X TBE Buffer **Thermo Fisher Scientific**

Before start

Note the materials, equipment and conditions required for the protocol, especially those items that are required apart from the kit. Ensure also the proper environment to carry out the protocol, including disinfected surfaces, established flows in handling and transfer of samples to prevent cross-contamination, availability of general labware, including disposal vessels, sterile vessels, tube holders as needed, labelling material, gloves, etc.

Tyssue lysis and digestion

5h 41m 20s

1 Prepare the lysis solution: dilute 1 µl of Proteinase K (50 µg/µl) into 300 µl of Tissue and Cell Lysis Solution (both supplied by the kit) per sample. 5m

2 Add 150 µl of Tissue and Cell Lysis solution with proteinase K to each tube with the Daphnids and grind the daphnids using a pestle. 20s

3 Add more 150 µl of Tissue and Cell Lysis solution with proteinase K to each Daphnids tube and mix by inverting the tubes several times. 30s

4 Incubate the Daphnids tubes at 65°C for 15 min in the thermoblock, and vortex the tubes every 5 minutes to mix. 20m

5 Cool the Daphnids down to 37°C 20m

6 Add 1 µl of 5 µg/µl RNAse A (supplied by the kit) to each sample, then mix by inverting the tubes several times. Spin-down the samples. 2m

7 Incubate the Daphnids tubes at 50°C for 3 hours in the thermoblock. 3h

8 Incubate the Daphnids tubes on ice for 5 minutes. 5m

9 Add 150 µl of MPC protein precipitation solution (supplied by the kit) to each Daphnids tube. Mix by vortexing for 10 seconds. 30s

10 Centrifuge at 4 °C for 10 minutes at 10000xg. 15m

11 Discard the pellet (do not disturb the pellet) and transfer the supernatant to new sterile 1.5-mL eppendorf tubes.

12 Add 500 µl of
Isopropanol, 99.5%, molecular biology grade **Thermo Fisher Scientific Catalog** #327272500
. Mix by gently inverting 40 times.
3m

13 Centrifuge at 4 °C for 10 minutes at 13,000 rpm.
15m

14 Discard the supernatant (isopropanol) **using a micropipette**, carefully to not disturb the pellet. Centrifuge the tubes again for 10 seconds at room temperature and remove the remaining supernatant with a smaller bore pipette tip. Check if the DNA pellet (white) is visible.

15 Add 500 µl of refrigerated Ethanol 70% , **gently**, to avoid lifting the pellet. Spin-down the tubes at 4 °C if the pellet is loose. Discard the supernatant (ethanol) **using a micropipette**. Repeat this step 2 times.
5m

16 Dry the pelleted DNA at RT for 30 minutes in the tubes with the lids open.
30m

17 Resuspend the DNA with 35-37 µl of autoclaved UP water. Leave the DNA at 4 °C for 30 minutes to aid in DNA suspending.
35m

18 Aliquot the samples (≈15 µl) for quality control checks.
5m

19 Store DNA at -80°C or keep it at 4°C if preservation for a short period is intended before further steps.

gDNA quantity and quality check
1h 25m 20s

20 Run DNA quantification using fluorometry (recommended)

20.1 Prepare a 1-20 µL aliquot of sample (commonly 2 µL) and add the proprietary kit reagent for DNA quantification (dsDNA BR/HS) with Qubit to complete a final reading volume of 200 µL. For samples starting from higher biomass dilution might be needed before this step (check the expected DNA concentration vs. reading range).
5m

20.2 Read the solution in the Qubit for DNA quantification. The outcome will be the DNA concentration of the sample in ng/µL. If sample dilution applied, mind it to record the DNA concentration of the sample. For the reference samples as indicated in this protocol (Daphnids), the expected DNA concentration is generally within 5-10 ng/µL. 20s

21 Run DNA quality screening using spectrophotometry

21.1 Set the Nanodrop spectrophotometer software for nucleic acids assessment 2m

21.2 Pipette a drop (2 µL recommended volume) of the sample into the holder in the reader. No dilution is needed if using similar biomass as indicated for the reference sample in this protocol (Daphnids) 30s

21.3 Read the sample. The outcome will be:
(i) Total nucleic acids concentration, typically within the range of 150-300 ng/µL if using samples similar to the reference sample in this protocol (Daphnids). As there is no consistent quantification of gDNA concentration only, this concentration is not particularly informative.
(ii) Absorbance ratio 260/280 (~1.8 should be the target; appreciably lower ratios may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm). With this protocol, similar samples to that indicated as a reference (Daphnids) and using the indicated kit, ratios around 1.9 are commonly reached.
(iii) Absorbance ratio 260/230 (2.0-2.2 should be the target; appreciably lower ratios indicate the presence of contaminants which absorb at 230 nm, such as EDTA, carbohydrates, phenol and some extraction reagents, e.g. TRIzol). With this protocol, similar samples to that indicated as a reference (Daphnids) and using the indicated kit, ratios around 2.0 can be reached, but more commonly the ratios are within 1.7-1.9; this poses little to no issues in most downstream NGS applications. 30s

21.4 Clean and repeat the read at least once for consistency check. Some variability in readings is relatively common. 2m

22 Run DNA quality screening via electrophoresis

22.1 Prepare a 0.8% agarose gel stained with Invitrogen SYBR Safe DNA Gel Stain **Invitrogen - Thermo Fisher** , following the manufacturer 20m

instructions.

22.2 Place the gel in an electrophoresis tank filled with an appropriate electrophoresis buffer (e.g. 10X TBE Buffer **Thermo Fisher Scientific**, diluted to 1x) and load the samples with an appropriate loading dye (e.g., 6X DNA Loading Dye **Thermo Fisher Scientific**, diluted to 1x). In the first slot add the ladder (e.g., GeneRuler 1 kb Plus DNA Ladder **Thermo Fisher Catalog #**SM1331). 10m

22.3 Run an electrophoresis at 100V for 30 min. 40m

22.4 Take your gel to a gel documentation platform and check the bands pattern. Ideally, integer DNA fragments should be on the top of the gel, above the 10,000 bp ladder band. 5m

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