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O DNA-extraction of Daphnia and symbionts

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

working

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Abstract

This protocol was designed for DNA extraction of about 50 adult female Daphnia magna, it should also work for 1-150 animals but with adjusted reagent volumes. For achieving HMW DNA or maximizing yield, some modifications are indicated as substeps. Before DNA extraction, animals can be freed from microbes using antibiotics (https://www.evolution.unibas.ch/ebert/lab/daphnia_dna.htm) and should be dehydrated and snap-frozen with liquid nitrogen for archiving HMW DNA.

Attachments



General DNA Extracti...

19KB

Protocol materials

Cell Lysis Solution Qiagen Catalog #1045696 In 2 steps Protein Precipitation Solution Qiagen Catalog #1045697 Step 9 Step 18 SRE Kit PacBio Catalog #102-208-300 Step 16.1



Tissue lysis and digest 19h 31m 5s 1 Add 🔊 animals and 🚨 200 µL 🔯 Cell Lysis Solution Qiagen Catalog #1045696 1.5 ml tube. (See abstract for how to prepare the animals.) 1.1 For HMW DNA, use snap-frozen animals and pre-cool lysis solution and tube with ice. 2 Grind animals with a clean, DNA(ase)-free plastic pestle, matching the shape of the 1.5 ml tube 10s to maximize tissue maceration. 2.1 For HMW DNA, use cold a pestle and only move it up and down 10 times (no twisting). 3 Add ∡ 300 µL Cell Lysis Solution Qiagen Catalog #1045696 and vortex shortly. 4 Add 🚨 20 µL ProtK [M] 20 mg/mL and carefully invert 25 times. 30s 5 Incubate at \$\mathbb{g} 55 \cdot C \quad \text{while shaking at } \mathbb{G} 400 \text{ rpm} \quad \text{overnight} \text{ (\frac{1}{2}) Overnight} \quad \text{incubation} 17h increases yield dramatically). 6 Put sample & On ice, add 🚨 20 µL RNAse A [M] 20 mg/mL to the cooled sample, and 30s carefully invert 25 times. 7 Incubate at 37 °C while shaking at 5 400 rpm for 6 01:00:00. 1h 8 Put sample on \ On ice for \ 00:01:00 \. 1m 9 Protein Precipitation Solution Qiagen Catalog #1045697 and vortex for Add <u></u> 300 µL 15s 00:00:15 X

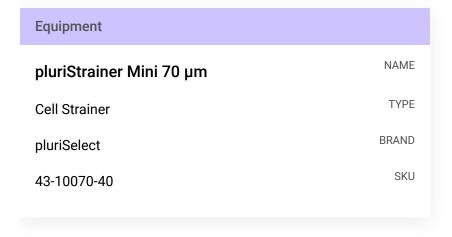


10 Centrifuge for (5) 00:04:00 at (8) 16000 x g.

- 4m
- 10.1 If the pellet is not tight, put tube On ice for 00:05:00 and 50 go to step #10 or precool centrifuge at 4 °C.
- 5m

11 Pipette supernatant (Δ 800 μ L | – Δ 1.000 μ L) to a 2 ml tube. Discard tissue.

11.1 For HMW DNA, use a 70 µm mesh.



12 Add the same amount of isopropanol (Δ 800 μ L | - Δ 1.000 μ L) to the supernatant and carefully invert 25 times.



12.1 For HMW DNA, carefully invert 50 times.



12.2 For maximum yield (but not HMW), use cold isopropanol and add 🚨 2 µL glycogen. Then, put the sample in the freezer for 01:00:00.



13 Centrifuge for 600003:00 at 60000 x g .







14 Discard supernatant, add 4 500 µL 70 % ethanol, and carefully invert until the pellet dislodges.



14.1 For maximum yield (but not HMW), use cold ethanol.



15 Centrifuge for (5) 00:01:00 at (8) 16000 x g .



16 Discard supernatant.

- 16.1 Apply SRE Kit PacBio Catalog #102-208-300 for HMW DNA and repeat step 14.-16. twice for purification.
- 15m

17 Put the open tube in the vacuum centrifuge for 00:15:00.



18 Add 🚨 80 µL 🔯 DNA Hydration Solution Qiagen Catalog #1045698 and incubate in the dark overnight. If fewer animals are being used or less DNA yield is expected, add less (∆ 20 µL - ∆ 50 µL)

