Apr 23, 2024

DNA-extraction of Daphnia and symbionts

DOI
dx.doi.org/10.17504/protocols.io.5jyl82n96l2w/v1

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl82n96l2w/v1

Protocol Citation: Pascal Angst, Peter D. Fields 2024. DNA-extraction of Daphnia and symbionts. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl82n96l2w/v1

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

Created: March 27, 2024

Last Modified: April 23, 2024

Protocol Integer ID: 97452

Keywords: DNA Extraction, Daphnia, Crustacean

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

DOCX

General_DNA_Extracti...

19KB

Protocol materials

Cell Lysis Solution **Qiagen Catalog #**1045696	In 2 steps
Protein Precipitation Solution **Qiagen Catalog #**1045697	Step 9
DNA Hydration Solution **Qiagen Catalog #**1045698	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 to a 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 to maximize tissue maceration. 10s

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 55 °C while shaking at 400 rpm overnight (Overnight incubation increases yield dramatically). 17h

6 Put sample On ice , add 20 µL RNAse A [M] 20 mg/mL to the cooled sample, and carefully invert 25 times. 30s

7 Incubate at 37 °C while shaking at 400 rpm for 01:00:00 . 1h

8 Put sample on On ice for 00:01:00 . 1m

9 Add 300 µL Protein Precipitation Solution **Qiagen Catalog #**1045697 and vortex for 00:00:15 . 15s

10 Centrifuge for 00:04:00 at 16000 x g . 4m

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

11 Pipette supernatant (800 µL – 1.000 µL) to a 2 ml tube. Discard tissue.

11.1 For HMW DNA, use a 70 µm mesh.

Equipment

pluriStrainer Mini 70 µm	NAME
Cell Strainer	TYPE
pluriSelect	BRAND
43-10070-40	SKU

12 Add the same amount of isopropanol (800 µL – 1.000 µL) to the supernatant and carefully invert 25 times. 30s

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 . 1h

13 Centrifuge for 00:03:00 at 16000 x g . 3m

14 Discard supernatant, add 500 µL 70 % ethanol, and carefully invert until the pellet dislodges. 10s

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

15 Centrifuge for 00:01:00 at 16000 x g . 1m

16 Discard supernatant.

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

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

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)