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HMW DNA extraction for amphipods V.1

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Protocol adapted from Qiagen's genomic DNA handbook protocol for the HMW extraction of live or flash-freezed amphipods.

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HMW, Nanopore, Amphipods, DNA Extraction

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To preserve large DNA sizes, never use Vortex and only use Wide-Bore tips.

Reagent :

Genomic-tip

20/G **Qiagen Catalog #ID: 10223** Step 9

Buffer

G2 **Qiagen Catalog #1014636** Step 1

Buffer QBT **Contributed by users** Step 10

Buffer QF **Contributed by users** Step 13

Buffer QC **Contributed by users** Step 12

RNase A **Qiagen Catalog #19101** Step 1

Proteinase K (2 ml) **Qiagen Catalog #19131** Step 7

2-propanol **Sigma Aldrich** Step 14

Ethanol 70% [Note: freshly prepared] **Contributed by users** Step 15

Tris-EDTA (TE) buffer pH 8.0 1X **Contributed by users** Step 18

Consumables :

2 mL Eppendorf **Contributed by users** In 2 steps

1.5 mL Eppendorf tubes **Contributed by users** Step 15

15 mL Falcon tubes **Contributed by users** Step 9

ART Wide-Bore tips 1000 µl **Contributed by users Catalog #2079GPK**

ART Wide-Bore tips 200µl **Contributed by users Catalog #2069GPK**

Equipment:

Dounce Homogenizer, 2mL
Tissue Grinder

Kimble **885300-0002**

2 mL with Pestles A and B

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ThermoMixer
Benchtop Incubator

Eppendorf **5382000023**

Any heat block will suffice

Centrifuge 4K15
Sigma

Centrifuge **BLE1700081**

Tissue homogenization 10m

1 Prepare the **lysis buffer** by adding 3 µL of **RNase A** (100 mg/mL) to 1.5 ml of **Buffer G2** per sample.

RNase

A **Qiagen Catalog #19101**

Buffer

G2 **Qiagen Catalog #1014636**

2 place live or flash-freezed amphipod in 2ml douncer and add 1 ml of **lysis buffer**

3 gently up and down **10 times** with the piston

4 **transfer the lysate** to a 2 ml tube

2 mL Eppendorf **Contributed by users**

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5 rinse the douncer with 0.5 ml lysis buffer and transfer to the 2 ml tube

Lysis 2h 30m

6 Incubate for 30 minutes at 37°C. 30m

37 °C **00:30:00**

7 Add 75 µL of **Proteinase K** (20 mg/mL) and incubate with inversion at 50°C for 2 hours. 2h

Proteinase K (2 ml) **Qiagen Catalog #19131**

02:00:00 **50 °C**

8 Gently transfer in a new 2 ml tube the supernatant with a 1000 µl **wide-bore tip**, avoiding touching the pelleted debris by gravity.

2 mL Eppendorf **Contributed by users**

DNA binding and washing 1h

9 Place a **genomic-tip 20G** column on a 15 ml tube

15 mL Falcon tubes **Contributed by users**

Genomic-tip 20/G **Qiagen Catalog #ID: 10223**

10 **Equilibrate the column** with 1 mL Buffer QBT. Wait for the Genomic-tip to drain by gravity.

Buffer QBT **Contributed by users**

11 Carefully **apply the lysate to the Genomic-tip** with a 1000 µl wide-bore tip. Wait for the Genomic-tip to drain by gravity.

12 **Wash** the QIAGEN Genomic-tip with 3 x 1 mL Buffer QC.

Buffer QC **Contributed by users**

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13 **Elute the DNA** into a new 15 mL tube with 2 x 1 mL of Buffer QF prewarm to 50°C

Buffer QF **Contributed by users**

DNA recovery 1h 50m

14 Add 1.4 mL of room-temperature isopropanol to the éluate, mix by inversion about 10 times, and centrifuge for 30 min at 5,500g at 4°C to pellet DNA. set centrifuge deceleration speed to level3 30m

2-propanol **Sigma Aldrich**

5500 x g, 4°C, 00:30:00 , Acc 9 / Dec 3

15 Gently remove the supernatant using a P1000
Gently resuspend the pellet with 1 ml of cold 70% ethanol using a 1000µl wide-bore tip.
Transfer to a new 1.5 ml tube.

Ethanol 70% [Note: freshly prepared] **Contributed by users**

1.5 mL Eppendorf tubes **Contributed by users**

16 Centrifuge for 10 min at 5,000g at 4°C to pellet DNA. set centrifuge deceleration speed to level3 10m

5000 x g, 4°C, 00:10:00 , Acc 9 / Dec 3

17 Remove as much supernatant as possible, avoiding touching the pellet. 10m
Air dry the pellet 10 min at RT.

00:10:00 **Room temperature**

18 Resuspend the pellet with 50 µL of TE 1x prewarm to 50°C

Tris-EDTA (TE) buffer pH 8.0 1X **Contributed by users**

19 Incubate for 1hour at 50°C, then overnight at RT. 1h

01:00:00 **50 °C**

Overnight **Room temperature**

Sample QC

20 Quantify your sample with a Qubit HS.

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Analyse 1 µL in a UV spectrophotometer (e.g. Nanodrop).

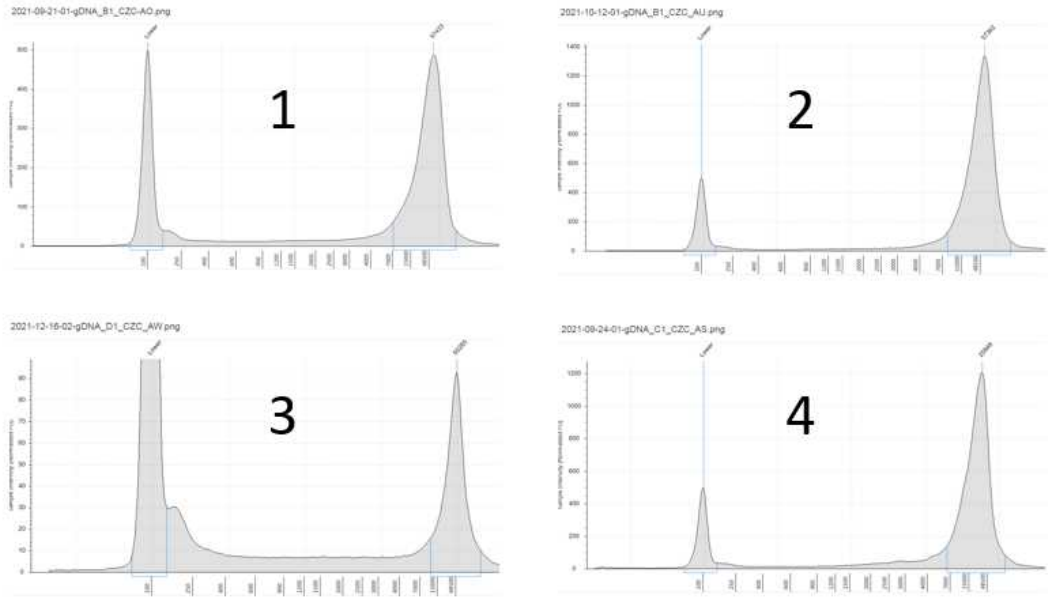
Visualise 1 µL of sample to estimate the molecular weight. (Tapestation)

Results

21 QC results of extraction of 4 individuals

Weight mg	[c] ng/µl	Nanodrop A260/A280	Nanodrop A260/A230	Tapestation kb
63	39.4	1.82	1.86	57
40	30.9	1.88	2.20	57
65	82.1	1.78	1.82	50
43	197	1.86	2.33	26

22 Tapestation profiles



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