SEP 29, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.14egn36nyl5d/v1

Protocol Citation: Pacific Biosciences, Adam AB Bates, Caroline Howard 2023. Sanger Tree of Life HMW DNA Extraction: Manual Mollusc Nanobind®. **protocols.io** https://dx.doi.org/10.17504/protocols.io.14egn36nyl5d/v1

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

Created: Sep 04, 2023

Sanger Tree of Life HMW DNA Extraction: Manual Mollusc Nanobind®

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ABSTRACT

This protocol describes the manual extraction of HMW DNA from mollusc samples intended for long-read sequencing using the Nanobind® tissue kit and following the 'Extracting HMW DNA from black mystery snail tissue using Nanobind® kits' procedure from PacBio. This process is effective for mollusc species covered by the Tree of Life Programme. The output of this protocol is high quality and quantity HMW DNA, which can be directed towards the HMW DNA Fragmentation: Diagenode Megaruptor®3 for LI PacBio protocol.

Acronyms

HMW: high molecular weight
LI: low input

Last Modified: Sep 29, 2023

PROTOCOL integer ID: 87303

Keywords: HMW DNA extraction, mollusc extraction, Nanobind, manual DNA extraction, reference genome, long read sequencing

GUIDELINES

- This protocol uses the 'Extracting HMW DNA from black mystery snail tissue using Nanobind® kits' procedure, with the inclusion of the sample preparations, standard sample inputs and standard elution volumes used by Sanger Tree of Life.
- This protocol works best with cryogenically disrupted material of around 30–50 mg input weight. If the tissue has not been cryogenically disrupted, place in a chilled petri dish and on ice, cut the tissue into smaller chunks, then powermash the sample in the CT buffer.
- Before starting the protocol, keep samples on dry ice to maintain temperature and prevent nucleic acid degradation.
- An experienced operator can expect to comfortably process 8 samples with a start to finish period of 4 hours. This estimation excludes overnight incubation at room temperature and subsequent QC checks.

Additional Notes:

- FluidX tubes are used throughout the Tree of Life programme in order to track samples, therefore rather than the microcentrifuge tubes which have been mentioned in this protocol for DNA storage, all routine DNA extracts are stored in FluidX tubes.

MATERIALS

- Nanobind® tissue kit (Cat. no. 102-302-100)
- 2 mL DNA LoBind microcentrifuge tubes (Eppendorf Cat. no. 0030108078)
- 100% absolute ethanol
- 100% absolute isopropanol

Equipment:

- Pipettes for 0.5 to 1000 µL and filtered tips
- Wide-bore pipette tips (200 µL, filtered if available)
- Scalpel (Thermo Fisher Scientific Cat. no. 22-079-712)
- DynaMag™-2 magnetic rack (Cat. no. 12321D) or similar
- Eppendorf ThermoMixer C (Cat. no. 5382000031)
- Eppendorf SmartBlock 2.0 ml (Cat. no. 5362000035)
- Mini centrifuge (Cat. no. SS-6050)
- Eppendorf Centrifuge 5425/5425 R (Cat. no. 5405000263)
- HulaMixer Sample Mixer (Cat. no. 15920D)
- Vortexer (Vortex Genie™ 2 SI-0266)
- Timer

Protocol PDF:

Sanger Tree of Life HMW DNA Extraction_ Manual Mollusc Nanobind.pdf

SAFETY WARNINGS

- The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol. Cotton glove liners are strongly recommended when handling the samples on dry ice.
- Waste needs to be collected in a suitable container (e.g. plastic screw-top jar or Biobin) and disposed of in accordance with local regulations.
- Liquid waste needs to be collected in a suitable container (e.g. glass screw-top jar) and disposed of in accordance with local regulations.

BEFORE START INSTRUCTIONS

- Add 100% ethanol to the Buffers CW1 and CW2 as per manufacturer's instructions.
- Set a heat block to 55 °C.
- Pre-chill the centrifuge to 4 °C.

Laboratory protocol

1 Transfer tissue to a 2 mL microcentrifuge tube or a powermash tube if not cryoprepped and add 750 µL ice cold buffer CT; powermashing if required.

2 Centrifuge 6,000 × *g* at 4 °C for 5 minutes.

3 Discard supernatant and add fresh 1 mL cold buffer CT.

4 Pulse vortex 10 times for 1s (or mix with wide-bore tip) to dislodge pellet.

5 Pellet homogenate by centrifuging at 6,000 × *g* at 4 °C for 5 minutes.

6 Discard supernatant.

7 Add 20 µL of Proteinase K to the pellet and pulse vortex 1s twice to dislodge the pellet.

8 Add 150 µL of buffer CLE3 and pipette mix 10 times using a wide-bore tip.

9 Incubate at 55 °C and 900 rpm for 30 minutes on the heat block.

10 Spin on a mini-centrifuge to collect liquid from the lid.

11 Add 20 µL of RNAse A.

12 Incubate at 55 °C and 900 rpm on the heat block for another 30 minutes. This incubation can be longer, up to 2 hours, if there are still visible large tissue pieces remaining.

13 Add 60µL buffer SB and pulse vortex 5 times to mix (wide-bore mixing is also acceptable).

14 Centrifuge 10,000 × *g* at room temperature for 5 minutes.

15 Transfer up to 300 µL of the supernatant to a new freshly labelled 2 mL microcentrifuge tube.

16 Add 50 µL of buffer BL3 to the collected supernatant and inversion mix 10 times (this can also be done with a wide-bore tip).

17 Spin on a mini-centrifuge to collect liquid from the lid.

18 Add the Nanobind disk to the lysate.

19 Add 350 µL isopropanol and inversion mix 10 times.

20 Allow 15 minutes at room temperature for the DNA to bind to the disk.
This step should be performed on a platform rocker, alternatively, you could manually invert the tube every 3 minutes or place the sample on a HulaMixer at 25 rpm for 15 minutes with the reciprocal angle set at 50°.

21 Place the tubes on the magnetic rack.

22 Discard the supernatant, either using a pipette and taking care to avoid touching the disk or by tipping the supernatant out into a reservoir, ensuring that the disk and DNA are bound to the magnet.

23 Add 500 µL of Buffer CW1, remove the tubes from the magnet and inversion mix 4 times, place back on the rack and discard the supernatant.

24 Repeat step 23.

25 Add 500 µL of Buffer CW2, remove the tubes from the magnet and inversion mix 4X, place back on the rack and discard the supernatant.

26 Repeat step 25.

27 Spin on a mini-centrifuge to collect liquid from the lid.

28 Place on the magnetic rack and remove any residual supernatant.

29 Add 75–100 µL Buffer EB directly to the nanobind disk and incubate for 10 min at room temperature.

30 Collect supernatant using a wide-bore pipette tip.

31 Allow 24 hours at room temperature for the DNA to relax before QC.