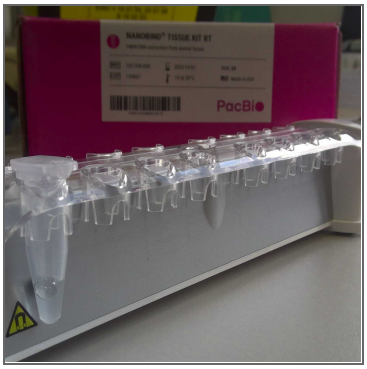

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Protocol status: Working
We use this protocol and it's working

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Sanger Tree of Life HMW DNA Extraction: Manual Nucleated Blood Nanobind®

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Tree of Life at the Wellcome Sanger Institute

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ABSTRACT

This protocol describes the manual extraction of HMW DNA from nucleated blood samples intended for long-read sequencing using the Nanobind® tissue kit and following the 'Extracting HMW DNA from nucleated blood using Nanobind® kits' procedure from PacBio. This process is effective for any species with nucleated blood within the Chordate group, covered by the Tree of Life Programme, however difficulties can arise with samples where preservation has not been optimal. 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

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PROTOCOL integer ID: 87304

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

GUIDELINES

- This protocol uses the 'Extracting HMW DNA from nucleated blood using Nanobind® kits' procedure, with the inclusion of the standard sample inputs and the standard elution volumes used by Sanger Tree of Life.
- This protocol is suitable for nucleated blood from birds, amphibians or fish that has been either flash frozen or stored in ethanol.
- 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 4 samples with a start to finish period of 1 hour. 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

- 1.5 mL Protein LoBind microcentrifuge tubes (Eppendorf Cat. no. 0030 108.116)
- Nanobind® tissue kit (Cat. no. 102-302-100)
- 100% absolute ethanol
- 100% absolute isopropanol
- 1x phosphate-buffered saline (PBS)
- 15 mL or 50 mL centrifuge tubes

Equipment:

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

Protocol PDF:

Sanger Tree of Life HMW DNA Extraction_ Manual Nucleated Blood 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.

Laboratory protocol

1 Add 10–20 µL of nucleated blood to a 1.5 mL Protein LoBind tube.

2 Add 180–190 µL of 1 X PBS for a total volume of 200 µL.

3 Add 20 µL of Proteinase K.

4 Add 20 µL of RNase A.

5 Pulse vortex the blood sample 10 times for 1 second at the maximum setting, and then spin the blood sample tube on a mini-centrifuge for 2 seconds.

6 Incubate the blood sample at room temperature for 3 minutes.

7 Add 200 µL of Buffer BL3 and pulse vortex 10 times for 1 second at the maximum setting, then spin the blood sample on a mini-centrifuge for 2 seconds.

8 Incubate the blood sample on a heat block at 55 °C at 900 rpm for 10 minutes.

9 Pulse vortex the blood sample 3 times for 1 second at the maximum setting, and then spin the blood sample on a mini-centrifuge for 2 seconds.

10 Add a Nanobind disk to the blood sample tube.

11 Add 350 µL of 100% isopropanol to the blood sample and Nanobind disk.

12 Inversion mix the blood sample 5 times, then place the sample on a rotating mixer at 9 rpm for 15 minutes at room temperature.

13 Place the blood sample tube on the magnetic rack to allow for disk (and therefore HMW DNA) capture.

14 Discard the supernatant with a pipette, trying not to disturb the Nanobind disk or pipette the DNA. To minimise carryover contamination, remove any excess liquid in the tube cap.

15 Add 700 µL of Buffer CW1, then remove the blood sample tube from the magnetic rack, inversion mix 4 times, before placing the blood sample tube back on the magnetic rack and discarding the supernatant. To ensure that the Nanobind disk is captured towards the top of the tube, first place the tube in the rack separated from the magnet, then flip the rack so that the lid of the tube is on the benchtop. Add the magnet into the rack, then flip the now-assembled magnetic rack so that the sample is the right side up.

16 Add 500 µL of Buffer CW2, then remove the blood sample tube from the magnetic rack, inversion mix 4 times, before placing the blood sample tube back on the magnetic rack and discarding the supernatant. To ensure that the Nanobind disk is captured towards the top of the tube, first place the tube in the rack separated from the magnet, then flip the rack so that the lid of the tube is on the benchtop. Add the magnet into the rack, then flip the now-assembled magnetic rack so that the sample is the right side up.

17 Repeat step 16 for a total of two Buffer CW2 washes.

18 Spin the blood sample tube on the mini-centrifuge for 2 seconds, then place the blood sample tube back on the magnetic rack.

19 Remove any residual buffer that may be present in the tube; if the Nanobind disk is blocking access to the bottom of the tube to remove this liquid, it can be gently pushed towards the magnet with the tip of the pipette.

20 Repeat steps 17 and 18 to ensure that there is no residual buffer in the tube.

21 Remove the sample from the magnetic rack, then add 200 µL of Buffer EB directly onto the Nanobind disk and incubate the sample for 10 minutes at room temperature.

22 Transfer the eluate containing the HMW DNA to a new 1.5 mL microcentrifuge tube with a standard p200 pipette and 200 µL pipette tip. Repeat until all the eluate is transferred.

23 Spin the sample tube containing the Nanobind disk on a centrifuge at 10,000 × *g* for 15 seconds, then combine any additional liquid that comes off the disk with the previously transferred eluate.

24 Repeat step 23 for a total of 2 spins on the centrifuge if any visible DNA remains on the disk.

25 Pipette mix the eluate 10 times with a standard p200 pipette and 200 µL pipette tip to homogenise the DNA within the sample and disrupt any viscous regions.

26 Allow the eluate to rest overnight at room temperature to allow the DNA to solubilise.

27 Following the overnight rest, pipette mix 10 times with a standard p200 pipette and 200 µL pipette tip, then perform the required QC.

28 Store the DNA at 4 °C.