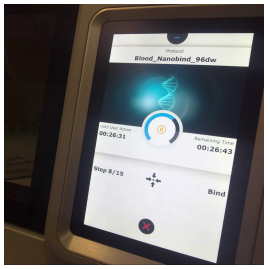
Sep 03, 2024

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



In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.6qpvr8453lmk/v1

Pacific Biosciences[1], Adam Bates[2], Amy Denton[3], Caroline Howard[3]

[1]Pacific Biosciences; [2]Wellcome Sanger Institute;
[3]Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA

Amy Denton
Tree of Life, Wellcome Sanger Institute, Hinxton, Cambridges...

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.6qpvr8453lmk/v1

Protocol Citation: Pacific Biosciences, Adam Bates, Amy Denton, Caroline Howard 2024. Sanger Tree of Life HMW DNA Extraction: Automated Nucleated Blood Nanobind®. **protocols.io https://dx.doi.org/10.17504/protocols.io.6qpvr8453lmk/v1**

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

Created: May 20, 2024

Last Modified: September 03, 2024

Protocol Integer ID: 100134

Keywords: HMW DNA extraction, Automated nucleated blood extraction, Nucleated blood extraction, Nanobind extraction, Automated Nanobind extraction, reference genome, long read sequencing, PacBio sequencing, KingFisher extraction

Funders Acknowledgement:
Wellcome Trust
Grant ID: 206194

Abstract

This protocol describes the automated extraction of HMW DNA from nucleated blood samples intended for long-read sequencing using the Nanobind® tissue kit and the Thermo Fisher KingFisher™ Apex. It follows the "Nanobind® HMW DNA Extraction from 5 µL nucleated red blood cell on KingFisher Apex" R&D protocol, which was developed by Pacific Biosciences and validated by the Tree of Life Core Laboratory using bird, fish and reptile nucleated blood samples. 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. In addition to allowing a higher throughput of nucleated blood samples, this protocol is also particularly beneficial for nucleated blood samples where clumping has occurred, with higher yields obtained compared to processing them using the Manual Nucleated Blood Nanobind® protocol. The output of this protocol is HMW DNA of high quality and quantity, which can be directed towards the HMW DNA Fragmentation: Diagenode Megaruptor®3 for LI PacBio protocol.

Acronyms:
HMW: high-molecular weight
LI: low input

Guidelines

- This protocol follows the Nanobind® HMW DNA Extraction from 5 µL nucleated red cell blood on KingFisher Apex R&D protocol, 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, reptiles 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.
- Ensure that samples are spaced adequately from one another in the 96-well deep-well plate - e.g. two to three empty wells between each sample - this will prevent contamination if the Nanobind disc falls from the tip comb during movement between plates.
- An experienced operator can expect to comfortably process 8 to 16 samples with a start to finish period of 1 to 2 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.

Troubleshooting:

- **Nanobind disc is not present in the Elution Plate after the completion of the protocol -** The Nanobind discs occasionally remain on the tip comb after the elution and are returned to the Tip Plate at the end of the protocol. Check the Tip Plate for the Nanobind disc and if it is present, continue to QC with this sample, as this should not have any effects on the sample.
  If the Nanobind disc is not in the Tip Plate, then this means that the disc became dislodged from the tip comb during the protocol and likely does not contain any DNA. For these samples, if there is material remaining, the protocol may be re-run with a new sample. Alternatively, if the Nanobind disc has been dropped in one of the wash plates, it is possible to recover the disc from this plate and continue with the protocol manually, following Sanger Tree of Life HMW DNA Extraction: Manual Nucleated Blood Nanobind.
- **Two Nanobind discs are present in one sample well of the Elution Plate after the completion of the protocol -** If two Nanobind discs have ended up in one sample well of the Elution Plate, then one of the discs has been dislodged from the tip comb and ended up in another sample well. This therefore means that there is contamination. If there is material remaining for the affected samples, then the protocol should be re-run with new samples. Ensure that there is sufficient distance between the sample wells to prevent this from recurring.

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
- Thermo Fisher KingFisher™ 1 mL 96-well Deep-well plates (Thermo Fisher Cat. no. 95040450)
- Thermo Fisher KingFisher™ 96 Tip Comb (Thermo Fisher Cat. no. 97002570)

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)
- Thermo Fisher KingFisher™ Apex instrument (Cat. no. 5400930)
- Timer

KingFisher™ Apex DNA Extraction Protocol:

KFX file: Blood_Nanobind_96dw.kfx 2KB

1. Pick Up Tip - Tip Plate
2. Lysis I - Lysis Plate
   Heating & Cooling: On 55°C Pre-heat: Off
   Mixing 1# 00:20:00 Fast
   Postmix: Off
3. Post-Lysis Cooling - Lysis Plate
   Heating & Cooling: On 25°C Pre-heat: Off
   Mixing 1# 00:00:00
   Postmix: Off
4. Pick-up discs 1 - Nanobind Disc Storage Plate
   Pre-collect beads: On
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:00:03 Paused Tip Position: Tip edge in liquid
   Postmix: Off
   Collect beads: Off
5. Wet Nanobinds - Lysis Plate
   Pre-collect beads: On

Release beads: Off
Heating & Cooling: Off
Mixing 1# 00:00:10 Medium
Postmix: Off
Collect beads: Off

6. Add IPA - Lysis Plate
   Step Type: Dispense
   Custom naming: Add 300µl of isopropanol to Lysis/Binding Plate
   Dispense to plate: Isopropanol 300µl
7. Mix 1 - Lysis Plate
   Pre-collect beads: On
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:00:05 Fast
   Postmix: Off
   Collect beads: Off
8. Bind - Lysis Plate
   Pre-collect beads: On
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:05:00 Slow Looping: 6
   2# 00:00:10 Paused Tip Position: Tip edge in liquid
   Postmix: Off
   Collect beads: Off
9. Pause 1 - Lysis Plate
   Pre-collect beads: Off
   Release beads: Off
   Heating & Cooling: Off
   Mixing 1# 00:30:00 Paused Tip Position: Above well
   Postmix: Off
   Collect beads: Off
10. CW1 Wash 1 - Wash Plate 1
    Pre-collect beads: On
    Release beads: Off
    Heating & Cooling: Off
    Mixing 1# 00:00:45 Medium Looping:1
    2# 00:00:15 Fast
    3# 00:00:10 Slow
    Postmix: Off
    Collect beads: Off
11. CW2 Wash 1 - Wash Plate 2
    Pre-collect beads: On
    Release beads: Off

Heating & Cooling: Off
Mixing 1# 00:00:20 Medium Looping:1
2# 00:00:10 Fast
3# 00:00:10 Slow
Postmix: Off
Collect beads: Off

12. CW2 Wash 2 - Wash Plate 3
    Pre-collect beads: On
    Release beads: Off
    Heating & Cooling: Off
    Mixing 1# 00:00:20 Medium Looping:1
    2# 00:00:10 Fast
    3# 00:00:10 Slow
    Postmix: Off
    Collect beads: Off
13. Dry - Elution Plate
    Duration: 00:01:00 Dry Type: Outside Well
14. Elute - Elution Plate
    Pre-collect beads: Off
    Release beads: On 00:00:00
    Heating & Cooling: Off
    Mixing 1# 00:02:00 Medium Looping: 1
    2# 00:02:00 Paused Tip Position: Above Well
    3# 00:08:00 Fast
    Postmix: Off
    Collect beads: Off
15. Leave Tip - Tip Plate

Protocol: Sanger Tree of Life HMW DNA Extra… 89KB

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.
- Do not open the door of the KingFisher™ Apex instrument whilst it is in operation.

Before start

- Add 100% ethanol to the Buffers CW1 and CW2 as per manufacturer's instructions.
- Set heat block to 37 °C (if using frozen blood) and incubate for 15 minutes to thaw the sample thoroughly.

Laboratory Protocol

1 In one Thermo Fisher KingFisher™ 1 mL 96-well deep-well plate, labelled Lysis Plate, add the following reagents into each well for the number of samples being processed:

1.1 Add 20 µL of Proteinase K.

1.2 Add 5 to 10 µL of nucleated blood.

1.3 Add 190 to 195 µL of PBS.

1.4 Add 20 µL of RNAse A.

1.5 Add 150 µL of Buffer BL3. Note: add this reagent gently against the side of the well, as adding it directly into the lysis solution may affect the extraction performance.

2 Set up the six remaining Thermo Fisher KingFisher™ 1 mL 96-well deep-well plates required for the protocol as detailed in the table below. Ensure that the reagents are added into the wells corresponding to those in which samples were added into the Lysis Plate:

Plate	Reagent(s) required per well
Tip Plate	96-well tip comb (no reagent)
Nanobind Disc Storage Plate	One 3 mm Nanobind disc
Wash Plate 1	700 µL Buffer CW1
Wash Plate 2	700 µL Buffer CW2
Wash Plate 3	700 µL Buffer CW2
Elution Plate	100 µL Buffer EB

3 Select the required DNA extraction protocol in the protocol list on the KingFisher™ Apex (details below in KingFisher™ Apex DNA Extraction Protocol section/attached file) and select using the play button.

4 Load the filled plates onto the instrument following the instructions provided on screen. Once the final plate is loaded, the protocol will automatically begin; this takes approximately 75 minutes.

5 Approximately 22 minutes into the protocol, the instrument will prompt the user to remove the Lysis Plate from the instrument and add 300 µL of isopropanol to each well containing a sample.
Ensure that the isopropanol is added gently against the side of the well rather than directly into the lysis solution, as this may affect extraction purity.
Once this has been done, re-insert the plate into the instrument and press 'Next' to resume the protocol.

6 Once the protocol has completed, follow the on-screen instructions to remove plates from the instrument.

7 Using a standard P200 pipette and 200 µL pipette tips, transfer eluates from the Elution Plate to microcentrifuge tubes for storage.

8 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.

9 Allow the eluates to incubate at room temperature overnight to allow the DNA to solubilise.

10 Following the overnight rest, pipette mix 10 times with a standard P200 pipette and 200 µL pipette tip, then perform the required QC.

11 Store the DNA at 4 °C.

Protocol references

High-throughput HMW DNA animal blood extraction and sequencing on the PacBio Revio system - PacBio - Moine, D. et al 2024