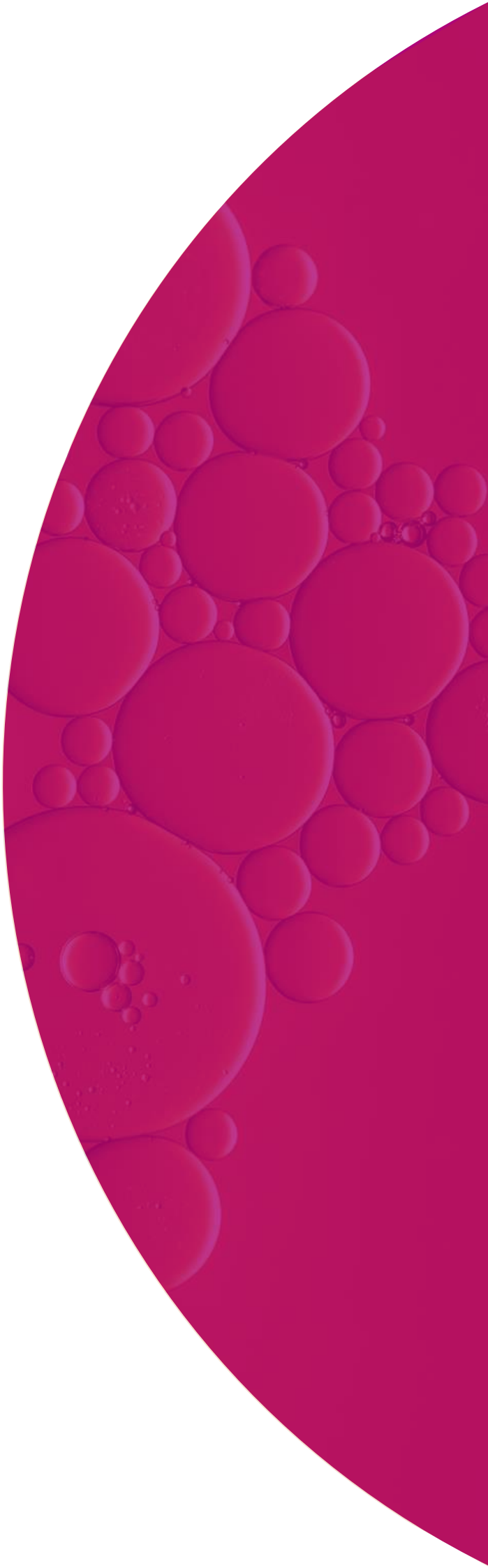
PacBio

Extracting HMW from mammalian liver using Nanobind® kits

Procedure & checklist



User supplied equipment and reagent list

Equipment	Model
Nanobind® tissue kit	PacBio® (102-302-100)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
TissueRuptor II	Qiagen (9002755)
Surgical scalpel	Fisher Scientific (22-079-712)
ThermoMixer	Eppendorf (5382000023)
Platform rocker	Thermo Scientific (M48725Q)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431081)
2.0 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431102)
14 mL round bottom tubes	Fisher Scientific (14-956-3B)
Wide bore 200 µL pipette tips	USA Scientific (1011-8410)
Ethanol (96–100%)	
Isopropanol (100%)	
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantitation	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits

*Eppendorf Protein LoBind tubes (Eppendorf #022431081 and #022431102) are highly recommended for all extractions to reduce protein contamination from tube carryover. Protein LoBind tubes are more effective in reducing carryover contamination than DNA LoBind tubes or other tubes and will result in improved UV purity.

Kit storage

RNase A and buffer CT should be stored at 4°C upon arrival.

Nanobind Disks and all other buffers should be stored at room temperature (15–30°C).

Safety precautions

Buffer BL3 and Buffer CW1 contain guanidine hydrochloride. Warning! Guanidine hydrochloride is harmful if swallowed or inhaled and causes skin and eye irritation. DO NOT mix with bleach or acidic solutions.

Product use

Nanobind tissue kits are intended for research use only.

Mammalian liver

This Procedure & checklist describes an example extraction of HMW DNA from mammalian liver tissue. This example provides sample specific details along with any modifications to the standard protocol.

For the standard Nanobind HMW tissue kit protocols, see either the Procedure & checklist – Extracting HMW DNA from standard Dounce homogenizer tissue using Nanobind kits or Procedure & checklist – Extracting HMW DNA from animal tissue using TissueRuptor. Please use the Guide & overview – Nanobind tissue kit to determine which protocol is suitable for a given sample type and to find general information regarding the kit.

Sample notes

- Liver tissue can generate high quality HMW DNA but must be fresh or have been frozen quickly after resection.
- DNA in liver tissue seems to degrade quickly, compared to other tissue types.
- Liver tissue typically results in high amounts of DNA per mg input because this tissue has a moderately high density of nuclei.
- This sample was validated on the PacBio Sequel® system.

Protocol notes

- This Procedure & checklist uses the **Nanobind tissue kit** (102-302-100).
- This Procedure & checklist describes DNA extraction from 23 mg of mouse liver using TissueRuptor homogenization for disruption.
- Dounce homogenization is also fine for liver disruption.
- This liver tissue Procedure & checklist uses centrifugation speeds in steps 7 and 9 that are faster (6,000 x *g*) than in the standard tissue protocol.
- Due to the moderately high nuclei density of liver tissue, the supernatant in step 20 may have very concentrated DNA that forms a gel-like matrix – **this gel-like matrix contains all the DNA.** Transfer the entire gel-like matrix in step 20.
- Formation of this gel-like matrix may be mitigated by using a lower starting input.

Protocol

1. Place a 14 mL round bottom tube on ice and chill the centrifuge to 4°C.
2. Place 23 mg mouse liver tissue on a clean, chilled surface, and finely mince to ≤1 mm^3 pieces using a scalpel.
   - A plastic weigh boat cleaned with 70% EtOH can be placed on an upside-down, aluminum dry bath incubator heat block sitting in ice.
3. Transfer minced tissue to the chilled 14 mL round bottom tube. Keep the tube on ice during the entire disruption process.
4. Add 750 µL of cold Buffer CT.
   - Buffer CT should be kept on ice when removed from the refrigerator.
5. Submerge the TissueRuptor probe tip in the buffer and blend at max speed for 10 s.
   - Some foam may form. Be sure to transfer any foam in the next step.
6. Transfer homogenate and any foam to a 2 mL Protein LoBind microcentrifuge tube.
7. Pellet homogenate by centrifuging at 6,000 x *g* and 4°C for 5 min. Discard supernatant.
   - Sometimes for mammalian liver samples, the pellet is not visible after this spin. In these cases, remove the supernatant carefully and avoid pipetting from the very bottom of the tube.
8. Add 1 mL of cold Buffer CT and pipette mix 10X with a wide bore P200 pipette to resuspend tissue.
9. Pellet homogenate by centrifuging at 6,000 x *g* and 4°C for 5 min. Discard supernatant.
   - Sometimes for mammalian liver samples, the pellet is not visible after this spin. In these cases, remove the supernatant carefully and avoid pipetting from the very bottom of the tube.
10. Pulse vortex for 1s x 2 times (max setting) to dislodge pellet.
11. Add 20 µL of Proteinase K to the previous pellet.
12. Add 150 µL of Buffer CLE3 and pipette mix 10X with a wide bore P200 pipette.
13. Incubate on a ThermoMixer at 55°C and 900 rpm for 30 min.
14. Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.
15. Add 20 µL of RNaseA.
16. Incubate on a ThermoMixer at 55°C and 900 rpm for 30 min.
17. Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.
18. Add 60 µL of Buffer SB and pulse vortex for 1s x 5 times (max setting) to mix.
19. Centrifuge at 10,000 x *g* and RT (15–30°C) for 5 min.
20. Transfer up to 250 µL supernatant to a new 1.5 mL Protein LoBind microcentrifuge tube using a wide bore P200 pipette. (Discard the 2 mL Protein LoBind microcentrifuge tube containing the precipitated pellet.)
    - Typical supernatant volumes will be 200–270 µL.

Quick tip

Thorough tissue disruption is key to efficient lysis. It is also important to keep the tissue cold during the entire disruption process.

Quick tip

The 2 mL tube is essential for efficient lysis because of its shape; the narrow taper of a 1.5 mL tube prevents proper mixing of the lysate during thermomixing.

Quick tip

If there are still visible, undigested tissue pieces after step 13, the incubation may be extended up to 2 h. However, if tissue is appropriately disrupted in steps 1-5, then 30 min should be sufficient.

Quick tip

The narrow taper of the 1.5 mL tube is critical for proper removal of wash buffer in steps 32 & 33 and for thorough recovery of eluate in step 36.

- Liver usually results in a small, firm pellet after this spin.
- Due to the high DNA content of this liver sample, the supernatant contained a gel-like matrix that comprised the DNA. All of the matrix was transferred.

21. Add 50 µL of Buffer BL3 to the previous supernatant and inversion mix 10X.
    - Solution became cloudy but cleared up in step 24.
22. Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.
23. Add Nanobind disk to lysate and add 300 µL of isopropanol. Inversion mix 10X.
    - The Nanobind disk must be added before isopropanol.
    - A large, cloudy mass appeared upon addition of isopropanol and inversion mixing; this adhered to the Nanobind disk and became clear during the next step.
24. Mix on a platform rocker at 20 rpm for 15 min at RT.
25. Place tube rack on the magnetic base using the method described in the Guide & overview – Nanobind tissue kit Magnetic Rack Handling Procedure section.
26. Discard the supernatant with a pipette using the method described in the Guide & overview – Nanobind tissue kit Pipetting section, taking care to avoid pipetting the DNA or contacting the Nanobind disk.
27. Add 500 µL of Buffer CW1, remove tube rack from magnetic base, inversion mix 4X, replace the tube rack on the magnetic base, and discard the supernatant.
28. Repeat step 27.
29. Add 500 µL of Buffer CW2, remove tube rack from magnetic base, inversion mix 4X, replace the tube rack on the magnetic base and discard the supernatant.
30. Repeat step 29.
31. Pipette out any residual liquid from the tube cap.
32. Spin the tube on a mini-centrifuge for 2 s. With the tube rack already on the magnetic base and right-side-up, place tube on tube rack and remove residual liquid.
    - If the Nanobind disk is blocking the bottom of the tube, gently push it aside with the tip of the pipette towards the magnet.
33. Repeat step 32.
34. Add 75 µL of Buffer EB directly onto the Nanobind disk and incubate at RT for 10 min.
    - The Nanobind disk does not need to be fully immersed in Buffer EB – it need only be wetted and sitting atop the liquid.
35. Collect DNA by transferring eluate to a new 1.5 mL microcentrifuge tube using a wide bore P200 pipette.
    - Either Protein LoBind or DNA LoBind tubes can be used in this step.
    - Avoid Axygen tubes as these have been shown to interfere with PacBio sequencing.
36. Spin the tube containing the Nanobind disk on a mini-centrifuge for 5 s. Use a standard P200 pipette to combine any additional liquid that comes off the disk with the previous eluate. Repeat if necessary.
    - For 23 mg of mouse liver, step 36 had to be performed 3 times to get all the DNA off of the disk.

Quick tip

The Nanobind disk only needs to be wetted in the elution step: **THE DISK DOES NOT NEED TO BE FULLY SUBMERGED IN BUFFER EB**.

Quick tip

This **5 s spin is CRITICAL** for recovering the DNA. We do not recommend a 2nd elution.

- Tissue types with moderate DNA content such as liver may result in a dense, clear gel that adheres strongly to the Nanobind disk. **This clear gel is DNA.** For these tissue types, this spin step is critical for recovering all the DNA. Repeat until all the clear gel has spun off of the Nanobind disk.

37. Pipette mix 5X with a standard P200 pipette to homogenize the eluate and disrupt any unsolubilized "jellies" that may be present.
    - Limited pipette mixing will not noticeably reduce DNA size or sequencing read lengths but is important for accurate quantitation and consistent sequencing performance.
    - Take care to disrupt any regions that feel more viscous than other regions.
38. Let eluate rest overnight at RT to allow DNA to solubilize.
    - Visible "jellies" should disperse after resting.
    - The extracted HMW DNA can be heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The bigger the DNA, the more this will be apparent.
39. Following overnight rest, pipette mix 5X with a standard P200 pipette and perform triplicate NanoDrop measurements by sampling the top, middle, and bottom of the eluate.
    - If the concentration %CV exceeds 30%, 5X pipette mix with a standard P200 pipette and allow DNA to rest at RT for 1 hour to overnight. Take care to disrupt any regions that feel more viscous than other regions. Remeasure with NanoDrop.
    - Limited pipette mixing will not noticeably reduce DNA size or sequencing read lengths but is important for accurate quantitation and consistent sequencing performance.
    - We routinely see A260/A280 in the range of 1.83–1.88 and A260/A230 in the range of 1.76–2.22 for liver samples.
40. Use Qubit dsDNA BR Assay to determine DNA concentration.
    - We recommend making multiple measurements from the top, middle, and bottom of the eluate for an accurate DNA concentration reading.
41. Run pulsed field gel electrophoresis (PFGE) or Agilent Femto Pulse to size the HMW DNA.

Quick tip

The DNA will solubilize after resting at RT or by coaxing it into solution using standard P200 pipette mixing. For samples that need to be used immediately, we recommend needle shearing.

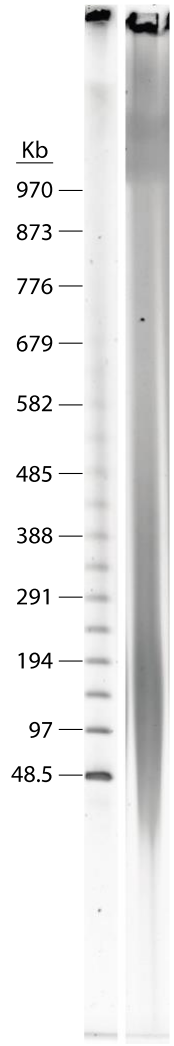
DNA extraction yield and purity

- Mouse liver samples result in moderate to high extraction yields.
- Purities obtained from mouse liver were very good.

Sample	Sample input	dsDNA yield	% RNA	A260/A280	A260/A230
Mouse liver - TissueRuptor	23 mg	18 µg	9.4	1.87	2.10
Mouse liver – Dounce	23 mg	31.9 µg	7.8	1.88	2.09

DNA size

- Size of DNA extracted from mouse liver is typically 50–300+ kb.



PFGE of DNA extracted from mouse liver homogenized with TissueRuptor.

Revision history (description)	Version	Date
Initial release	01	July 2022
Minor updates throughout	02	December 2022