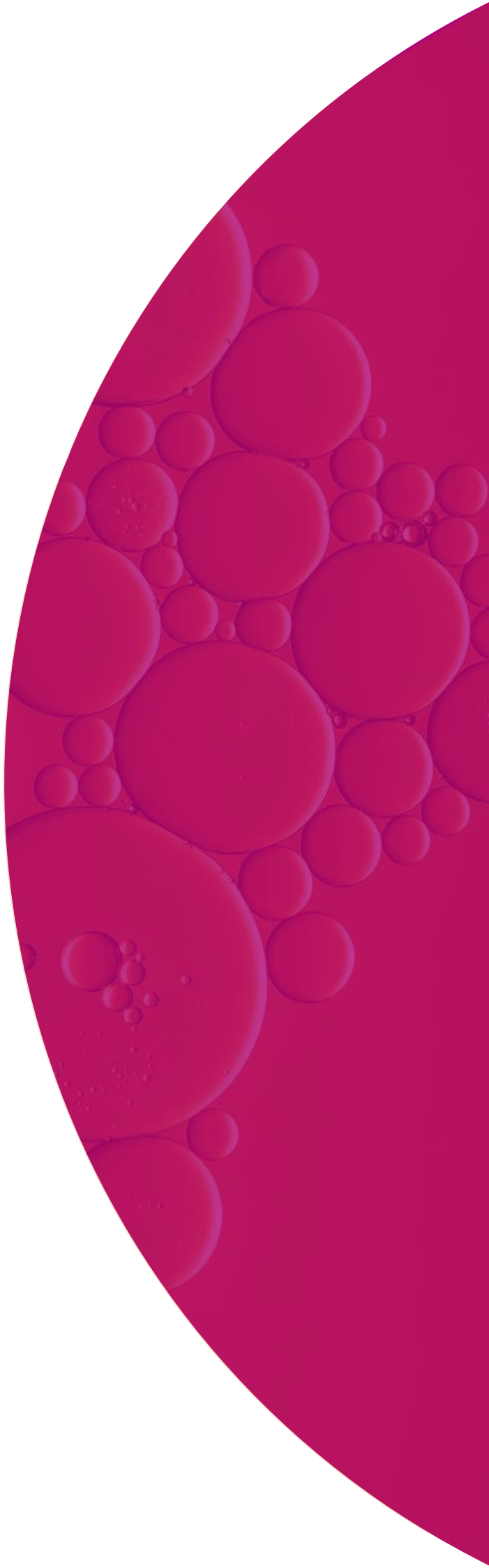


Extracting HMW DNA from tilapia testis using Nanobind[®] kits

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.

Tilapia testis tissue

This application note describes an example extraction of HMW DNA from frozen tilapia testis 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

- Testis tissue results in very high amounts of DNA per mg input because this tissue has a very high density of nuclei.

Protocol notes

- This protocol uses the **Nanobind tissue kit** (102-302-100).
- This protocol describes DNA extraction from 25 mg of tilapia testis using TissueRuptor homogenization for disruption.
- Dounce homogenization would also be fine for testis disruption.
- Testis does not require mincing prior to disruption due to its loose, liquid-like consistency.
- Due to the very high nuclei density of testis tissue, the supernatant in step 19 will 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 19.
- Formation of this gel-like matrix formation may be mitigated by using a lower starting input amount.
- Due to the exceptionally high DNA content of fish testis tissue, we diluted the eluate with Buffer EB to a final volume of 150 μ L after performing QC in step 40.

Protocol

1. Place a 14 mL round bottom tube on ice and chill the centrifuge to 4°C.
2. Transfer 25 mg tilapia testis tissue to the chilled 14 mL round bottom tube. Keep the tube on ice during the entire disruption process.
 - Our tilapia testis sample was frozen, and when it thawed, it had a liquid consistency and did not require mincing.
 - If your sample does not have a liquid consistency, finely mince the tissue to $\leq 1 \text{ mm}^3$ pieces using a scalpel.
 - Testes have very high DNA content, so using less than the standard 25 mg input is fine and preferable.
 - Using more than 25 mg will probably make elution and DNA solubilization quite difficult.
3. Add 750 μL of cold Buffer CT.
 - Buffer CT should be kept on ice when removed from the refrigerator.
4. Submerge the TissueRuptor probe tip in the buffer and blend at max speed for 10 s.
 - Blending the testis tissue generated a lot of foam. All of the foam was transferred in the next step.
5. Transfer homogenate and all foam to a 2 mL Protein LoBind microcentrifuge tube.
6. Pellet homogenate by centrifuging at 1,500 x g and 4°C for 5 min. Discard supernatant.
7. Add 1 mL of cold Buffer CT and pipette mix 10X with a wide bore P200 pipette to resuspend tissue.
8. Pellet homogenate by centrifuging at 1,500 x g and 4°C for 5 min. Discard supernatant.
9. Pulse vortex pellet 1s x 2 times (max setting) to dislodge pellet.
10. Add 20 μL of Proteinase K to the previous pellet.
11. Add 150 μL of Buffer CLE3 and pipette mix 10X with a wide bore P200 pipette.
12. Incubate on a ThermoMixer at 55°C and 900 rpm for 30 min.
13. Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.
14. Add 20 μL of RNaseA.
15. Incubate on a ThermoMixer at 55°C and 900 rpm for 30 min.
16. Spin the tube on a mini-centrifuge for 2 s to remove liquid from the cap.
17. Add 60 μL of Buffer SB and pulse vortex for 1s x 5 times (max setting) to mix.
18. Centrifuge at 10,000 x g and RT (15–30°C) for 5 min.
19. 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 200–250 μL .
 - This extraction of 25 mg of testis did not result in a visible pellet after this spin.

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 12, the incubation may be extended up to 2 h. However, if tissue is appropriately disrupted in steps 1-4, 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.

- Due to the high DNA content of testis tissue, the supernatant contained a gel-like matrix that comprised the DNA. Make sure all of this matrix is transferred.
20. Add 50 μL of Buffer BL3 to the previous supernatant and inversion mix 10X.
 - The solution became very cloudy with noticeable precipitates.
 21. Incubate on a ThermoMixer at 55°C and 900 rpm for 10 min.
 - This extra incubation step was added to help solubilize the precipitates.
 - Following the 10 min incubation, a few white precipitates remained, but cleared 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.
 - Testis tissue can result in very high DNA yields. If the DNA concentration exceeds 1000 ng/ μL after performing QC in step 40, the eluate can be diluted with additional Buffer EB. Alternatively, the elution volume can be increased to 150 μL in subsequent extractions.
 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.

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.**

- 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 25 mg of tilapia testis, step 36 had to be performed 4 times to get all the DNA off of the disk.
 - Tissue types with high DNA content such as testis 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 1.85–1.86 and A260/A230 in the range of 2.26–2.35 for fish testis 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) of Agilent Femto Pulse to size the HMW DNA.

Quick tip

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

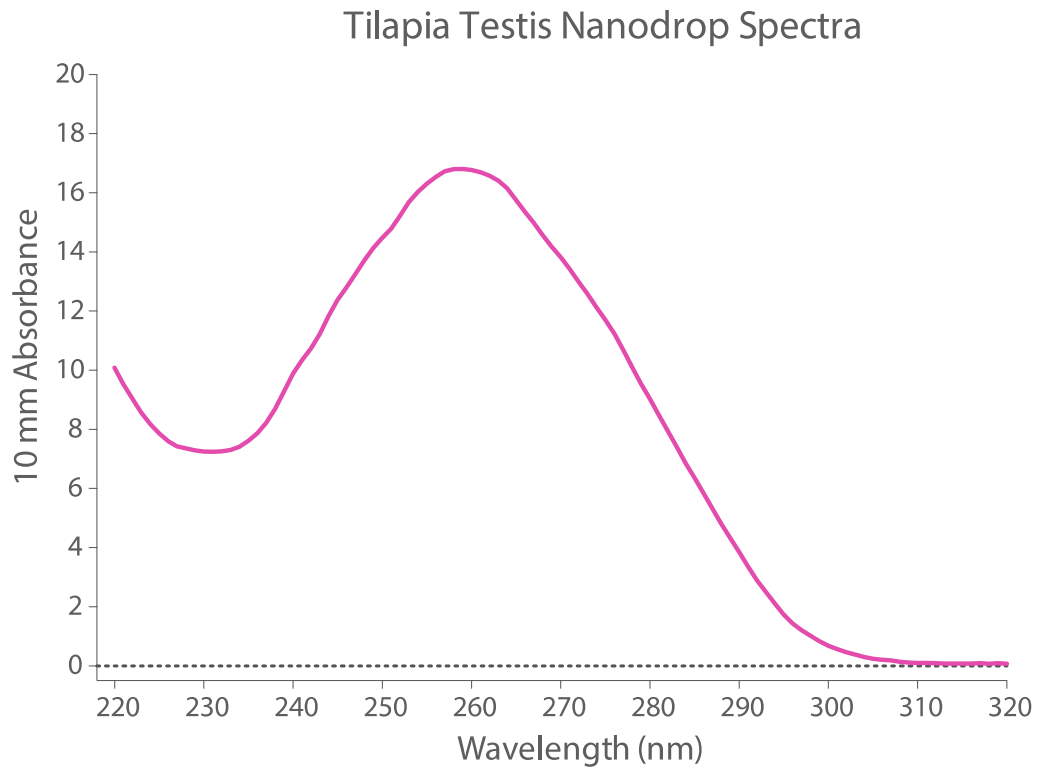
Quick tip

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

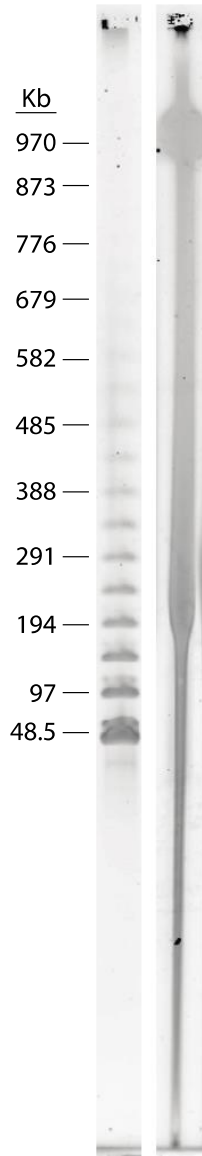
- Testis samples result in very high extraction yields.
- Purities obtained from tilapia testis were good.

Sample	Sample input	dsDNA yield	% RNA	A260/A280	A260/A230
Tilapia Testis	25 mg	83.7 µg	9.3	1.86	2.31



DNA size

- Size of DNA extracted from tilapia testis is 50–400+ kb.



PFGE of DNA extracted from tilapia testis.

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

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