PacBio

Nanobind® PanDNA kit

Guide & overview

103-394-800 REV04 OCT2024

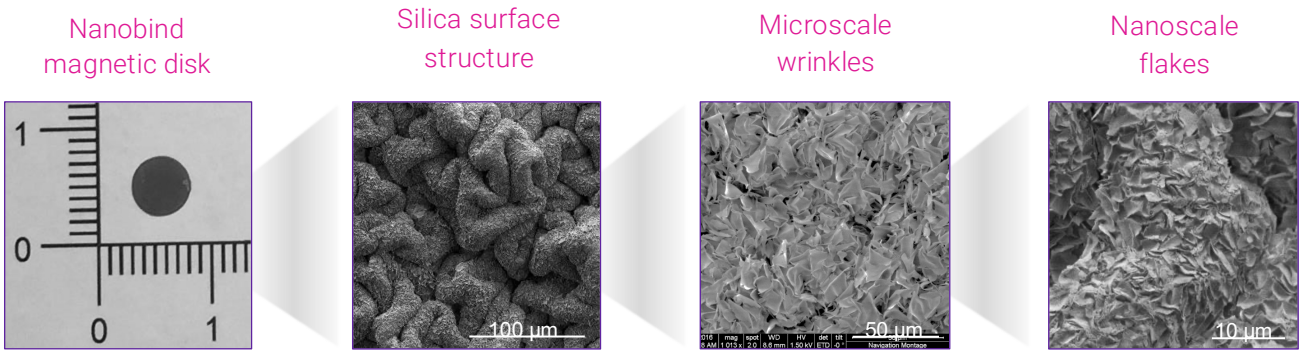
Table of contents

103-394-800 REV04 OCT2024

Introduction

This Guide & Overview describes the PacBio® Nanobind PanDNA kit which can be used for diverse sample types including cultured cells and bacteria, human blood, saliva and tissue, animal blood and tissue, insects, and plant nuclei. For each sample type, a detailed Procedure & Checklist can be found at the PacBio Documentation page.

Nanobind is a novel magnetic disk covered with a high density of micro- and nanostructured silica that can be used for rapid extraction and purification of high-quality DNA. The high surface area and unique binding mechanism give it an extraordinary binding capacity, allowing isolation of high purity, high molecular weight (HMW) DNA in a microcentrifuge tube format. It uses a standard lyse, bind, wash, and elute procedure that is common for silica DNA extraction technologies. A single disk is used in each tube. However, unlike magnetic beads and silica spin columns which shear large DNA, Nanobind disks bind and release DNA without fragmentation, yielding DNA up to megabases in length.

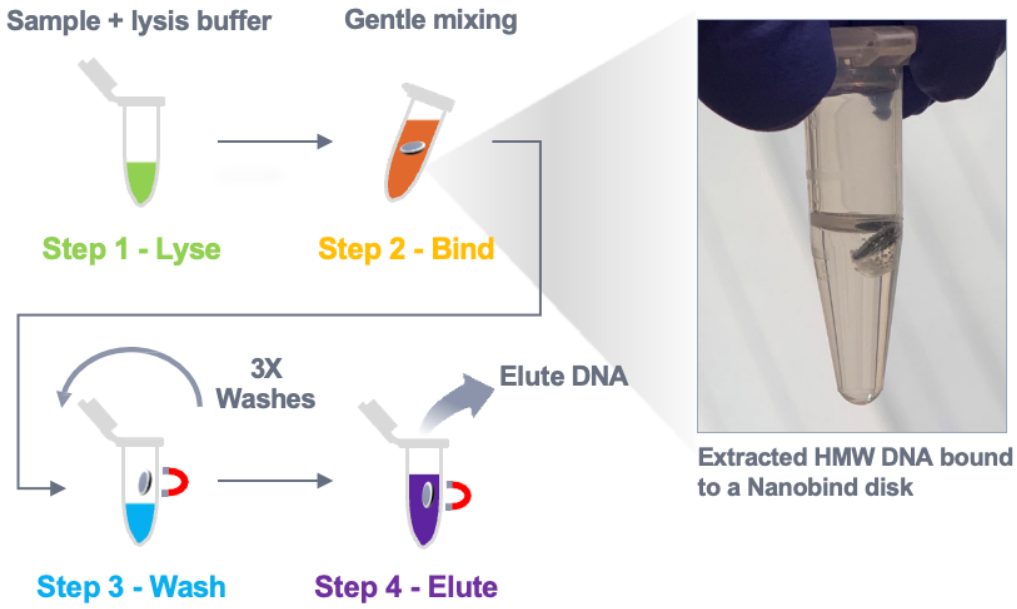


SEM images of Nanobind's silica surface structure.

Nanobind PanDNA kit principle

The Nanobind PanDNA kit provides an all-in-one HMW DNA extraction solution that can be used with a wide range of sample types for PacBio HiFi sequencing. This kit enables high-quality HMW DNA extraction from cells, blood, saliva, bacteria, tissues, insects, and plant nuclei. Depending on the sample type, the processing time is approximately 2 h and yields 3–25 µg HMW DNA.

Nanobind disks are automation compatible. High-throughput workflows using Nanobind HT kits are available for multiple sample types and can be found at the Documentation page.



Nanobind HMW DNA extraction workflow and picture of DNA bound to Nanobind disk in 1.5 mL Eppendorf tube.

Kit composition & workflow

Kit contents

Nanobind PanDNA kit (PN 103-260-000) supports 24 reactions and is comprised of two parts:

1. Nanobind PanDNA kit RT (103-260-300)
2. Nanobind PanDNA kit 4C (103-260-400)

Both parts are shipped at ambient temperature and the Nanobind PanDNA kit 4C pouch should be stored at 4°C upon delivery.

Prior to starting

The PanDNA kit contains 3 wash buffers (CW1, CW2 and, PW1) to extract various sample types. Buffers CW1, CW2, and PW1 are supplied as concentrates. CW1 and CW2 are used with a 60% final ethanol concentration. PW1 is used with a 70% final ethanol concentration. Before using, add the appropriate amount of ethanol (96–100%) to Buffers CW1, CW2, and PW1, as indicated on the bottles.

Note: Not all buffers are used in every procedure. The table below details which buffers are used for different protocols.

Protocols						
Buffer	**Cultured cell & bacteria**	**Human whole blood & Nucleated red blood cells & Saliva**	**Human whole blood with RBC lysis**	**Animal tissue**	**Insect**	**Plant nuclei**
PK	x	x	x	x	x	x
RNase A	x	x	x	x	x	x
CLE3	x			x		
BL3	x	x	x	x	x	
CW1	x	x	x	x	x	
CW2	x	x	x	x	x	
LTE	x	x	x	x	x	x
CT				x	x	
SB				x		
NPL					x	x
PW1						x
RBC 10X			x			

Kit storage

Buffer CT, Buffer RBC 10X, and RNase A should be stored at 4°C upon arrival.

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

Buffer NPL may form precipitates if stored cooler than room temperature. If this happens, precipitates will return to solution when stored at room temperature. Alternatively, the buffer can be warmed in a water bath to re-dissolve precipitates.

Safety precautions

Refer to the Safety Data Sheet (SDS) for information on reagent hazards and protocols for safe handling, use, storage, and disposal.

Product use

Nanobind PanDNA kits are intended for research use only.

Extraction protocols

Select the appropriate Nanobind PanDNA Procedure & checklist to use based on sample type and starting material:

Sample type	Starting material	Samples input	Workflow time	Procedure & checklist
Blood	Human whole blood	200 µL	~1 hr.	*Extracting HMW DNA from human whole blood* (102-573-500)
	Nucleated red blood cells (nRBCs)	2.5–20 µL	~1 hr.	*Extracting HMW DNA from nucleated red blood cells* (102-574-000)
	Human whole blood with RBC lysis	400 µL	<1.5 hrs.	*Extracting HMW DNA from human whole blood with RBC lysis* (103-377-500)
Saliva	Human saliva collected in DNA Genotek™ Oragene™ devices	500 µL	~2 hrs.	*Extracting HMW DNA from saliva collected in DNA Genotek™ Oragene™ devices (103-544-000)*
Animal tissue	Diverse tissue types	2–100 mg	~2.5 hrs.	*Extracting HMW DNA from animal tissue* (102-574-600)
Insect tissue	Insect whole body or segment	>20 mg	~2.5 hrs.	*Extracting HMW DNA extraction from insects* (102-377-400)
Plant tissue	Isolated plant nuclei	0.25–5g	~1.5 hrs.[1]	*Extracting HMW DNA from plant nuclei* (103-378-200)
Mammalian cultured cells	Suspension cell culture	1 x 10^6–5 x 10^6 diploid human cells	~1 hr.	*Extracting HMW DNA from cultured suspension cells* (103-394-500)
	Adherent cell culture	1 x 10^6–5 x 10^6 diploid human cells	~1 hr.	*Extracting HMW DNA from cultured adherent cells* (102-573-600)
Cultured bacteria	Gram-negative bacteria	5 x 10^8–5 x 10^9 bacterial cells	~1 hr.	*Extracting HMW DNA from Gram-negative bacteria* (102-573-800)
	Gram-positive bacteria	5 x 10^8–5 x 10^9 bacterial cells	~1 hr.	*Extracting HMW DNA from Gram-positive bacteria* (102-573-900)

[1]Upstream plant nuclei prep isolation procedure (e.g., 102-574-900 or 102-574-800) typically take ~3 hours to complete.

Examples of extraction results and sequencing data

The Nanobind PanDNA extraction protocols typically yield ~5–25 µg of HMW DNA in the 50–300+ kb size range. These ranges will vary depending on the sample type, quality of the starting material, and processing parameters. For the highest quality DNA, it is critical to start with optimal input material. Detailed guidance for each sample type and additional insight into experimental design and expected results can be found in the Nanobind PanDNA kit protocol Overview. Below is an overview of all sample types for input quantity and expected results.

Additional information for HMW DNA extraction from insects can be found in this Technical note.

Example of DNA QC metrics for Nanobind HMW DNA extracted from different sample types

Sample type	Starting material	Input material	Example DNA yield	Example absorbance ratio[1]		Expected DNA size range	Notes
				260/280	260/230		
Blood	Mammalian whole blood (non-nucleated RBC)	200 µL fresh or frozen human blood	3 – 10 µg	≥1.7	≥1.5	50 – 300+ kb	Blood samples need to be ≥4 x 10^6 WBC cells/L to give ≥3 µg HMW DNA yield
	Nucleated red blood cells (nRBCs)	5 µL tuna or chicken blood	15 – 20 µg	~1.8	1.7 – 2.1	50 – 300+ kb	Nucleated red blood cells are found in most vertebrate animals, with the exception of mammals
	Human whole blood with RBC lysis	400 µL human blood + RBC lysis	3 – 25 µg	1.8 – 2.0	1.9 – 2.3	50 – 300+ kb	Yield for human whole blood will vary from 3–25 µg based on donor WBC concentration
Saliva	Human saliva collected in DNA Genotek™ Oragene™ devices	500 µL saliva	1 – 50 µg	1.4 – 2.0	1.0 – 1.7	50 – 300+ kb	Saliva samples need to be collected using Genotek™ Oragene™ devices and contain > 2 µg of DNA in 500 µL for efficient Nanobind extraction. DNA content can be checked prior to extraction by Qubit BR measurement
Animal tissue	Diverse tissue types	25 mg heart tissue	8 – 13 µg	1.7 – 2.0	1.6 – 2.3	50 – 300+ kb	DNA yield is organ type-dependent due to differences in tissue cellularity (cell size & cell density)
Insect tissue	Insect whole body or segment	50 mg bulk fruit flies	9 – 10 µg	1.7 – 2.0	1.0 – 2.2	50 – 300+ kb	A260/230 can be low due to high amounts of pigments but mat not necessarily impact HiFi data yields
Plant tissue	Isolated plant nuclei	1 g leaf tissue	5 – 10 µg	1.7 – 2.0	1.1 – 2.3	50 – 300+ kb	Ensure nuclei isolation prep is carried out correctly prior to starting Nanobind DNA extraction
Cultured cells	Suspension cell culture	2 x 10^6 HG002 cells	~10 µg	1.8 – 2.0	1.7 – 2.2	50 – 300+ kb	Input cell counts should be accurately determined using a hemocytometer or cell counter
	Adherent cell culture	2 x 10^6 MCF-10A cells	~10 µg	1.8 – 2.0	1.7 – 2.2	50 – 300+ kb	Input cell counts should be accurately determined using a hemocytometer or cell counter
Cultured bacteria	Gram-negative bacteria	0.5 – 1 mL (1.0 OD600)	18 – 27 µg	~1.8	1.2 – 1.8	50 – 300+ kb	Different bacterial species will have different cell counts for a given OD600 value
	Gram-positive bacteria	0.5 – 1 mL (1.0 OD600)	~20 µg	~1.8	1.2 – 1.8	50 – 300+ kb	Different bacterial species will have different cell counts for a given OD600 value

Note: High UV absorbance values are ***not*** always a guarantee of optimal sequencing performance (Not all inhibitors absorb at 230 and 280 nm). Conversely, low UV absorbance values are ***not*** always a guarantee that non-optimal sequencing performance will be obtained → gDNA samples with A260/280 ≥1.7 and A260/230 ratios ≥1.0 can still generate excellent HiFi sequencing performance (see ***PacBio Technical Note: Preparing DNA for PacBio HiFi sequencing – Extraction and quality control*** (102-193-651)).

Example Nanobind PanDNA DNA extraction QC and HiFi sequencing results

Sample	Input material	DNA yield	DNA mode size	HiFi mean read length	HiFi yield	Median QV
RBC lysed human whole blood	400 µL	9.5 µg	97 Kb	17,518 bp	108 Gb	Q34
Saliva	500 µL	3.8 µg	146 Kb	14,756 bp	120 Gb	Q35
Human brain	6 mg	9.3 µg	195 kb	16,164 bp	119 Gb	Q36
Mouse lung	6 mg	9.1 µg	126 kb	16,975 bp	121 Gb	Q35
Human skeletal muscle	33 mg	3.9 µg	126 kb	17,170 bp	101 Gb	Q33
Lynx skeletal muscle	32 mg	5.8 µg	132 kb	16,945 bp	98 Gb	Q31
Ladybug (whole insect)	27 mg	5.3 µg	67 kb	16,034 bp	86 Gb	Q34
Cricket (hindlegs)	44 mg	10.5 µg	118 kb	15,731 bp	113 Gb	Q35
Tobacco leaf nuclei	1 g	16.5 µg	140 kb	14,649 bp	90 Gb	Q34
Pepper leaf nuclei	1 g	26.1 µg	118 kb	15,440 bp	94 Gb	Q34
Apple leaf nuclei	1 g	11.3 µg	112 kb	15,598 bp	106 Gb	Q35

HiFi data yield from gDNA samples size selected with the PacBio SRE kit and sheared to 18–20 kb. HiFi sequencing was performed on the Revio system (225 pM loading concentration and a *P1* metric of 60–72%).

SMRTbell prep 3.0 (SPK 3.0) WGS SMRTbell libraries constructed with Nanobind PanDNA-extracted DNA show excellent HiFi sequencing performance

Preservation methods

Animal tissue

High quality tissue samples are the key to obtaining high quality DNA. Fresh and immediately-frozen tissues generate the highest quality DNA. With all samples, ischemic times before preservation or freezing should be avoided to minimize tissue DNA degradation. Other preservation methods such as storage in ethanol or RNAlater can be also used, but these samples require pre-treatment prior to beginning DNA extraction.

Frozen tissues

Tissues that are frozen without a preservation medium do not require pre-treatment before extraction.

RNAlater-preserved tissues

Tissues that are preserved in RNAlater prior to freezing or storage should have excess RNAlater solution removed. After placing the tissue on a clean, chilled surface, wick away excess RNAlater liquid using a Kimwipe.

Ethanol-preserved tissues

Tissues that are preserved in ethanol prior to freezing or storage require pre-treatment before extraction to remove the ethanol.

1. Prepare EtOH removal buffer
   - 400 mM NaCl
   - 20 mM Tris, pH 7.5
   - 30 mM EDTA
2. Place tissue into a 50 mL conical tube containing 45 mL of EtOH removal buffer and incubate on a rocker or tube rotator for 30 min at room temperature.
3. Remove and discard EtOH removal buffer.
4. Add 45 mL of fresh EtOH removal buffer and incubate on a rocker or tube rotator for 30 min at room temperature.
5. Remove and discard EtOH removal buffer, and wick excess liquid with a Kimwipe to remove as much liquid as possible.
6. Proceed into step 1 of the tissue extraction protocol.

Cultured mammalian and bacterial cells

Frozen cells

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen cell samples. If freezing, store cells in small, single-extraction aliquots (e.g., $1x10^6$–$5x10^6$ diploid human cells, $0.5x10^9$–$5x10^9$ bacterial cells) to avoid repeated freeze-thawing. To make aliquots, spin cultured mammalian cells at 500 x *g* for 3–5 minutes at 4°C or cultured bacterial cells at 16,000 x *g* for 1 minute at 4°C. Remove as much of the supernatant as possible and store the pellets at −80°C.

Nucleated red blood cells (nRBCs)

Ethanol-preserved nucleated red blood cells

nRBCs preserved in ≥70% ethanol performed well in limited testing. Use the equivalent of 5 µL of blood. For example, the volume equivalent of 5 µL of unpreserved nRBCs is ~17 µL if the nRBCs are preserved with 70% ethanol or 50 µL if the nRBCs are preserved with 90% ethanol.

Human whole blood

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen blood samples.

Fresh blood

Storage at 4°C should be limited to 2 days or fewer to prevent sample degradation.

Frozen blood

When freezing, blood should be frozen as quickly as possible after being drawn and in small aliquots to avoid repeated freeze-thaws. Prior to beginning DNA extraction, frozen blood must be thawed at 37°C for 15 minutes and inversion mixed thoroughly.

Blood anti-coagulant

K2 EDTA is the preferred anti-coagulant. Samples stored in sodium heparin (NaHep) and citrate (NaCit) also performed well in very limited testing.

Saliva

Saliva collected in Oragene devices is stable at RT up to 5 years as described in this white paper from DNA Genotek.

Insects

Frozen insects

We recommend freezing insects at −80°C after capture/harvesting. We do not recommend storage in ethanol, as this may lead to difficulty isolating HMW DNA.

Tissue disruption strategies

For the protocol Extracting HMW DNA from animal tissue (102-574-600)

Effective tissue disruption is key to producing the highest yield and highest quality HMW DNA.

The first critical step in tissue disruption is fine mincing with a surgical scalpel. This is particularly important for rubbery samples such as cartilage and leiomyomas and for tough, stringy samples such as tendons and ligaments. The more finely minced these samples are, the more efficient lysis will be.

For the next step in tissue disruption, we recommend the Qiagen TissueRuptor. We have also tested alternative disruption methods including Dounce homogenization, liquid nitrogen grinding, and pulverization with the Covaris CryoPREP. However, disruption with the TissueRuptor will provide the highest consistency across replicates and samples with the most convenience.

TissueRuptor II

Use the TissueRuptor on its maximum setting. Ten seconds should be sufficient for all tissues as long as the sample is adequately minced beforehand. Tissues that are rubbery or tough may require another ten-second round with the TissueRuptor.

Dounce homogenization

We have extracted and sequenced various human and animal tissues using a 2 mL Dounce homogenizer with a tight pestle for upfront disruption. Please refer to the Dounce homogenization protocol for details.

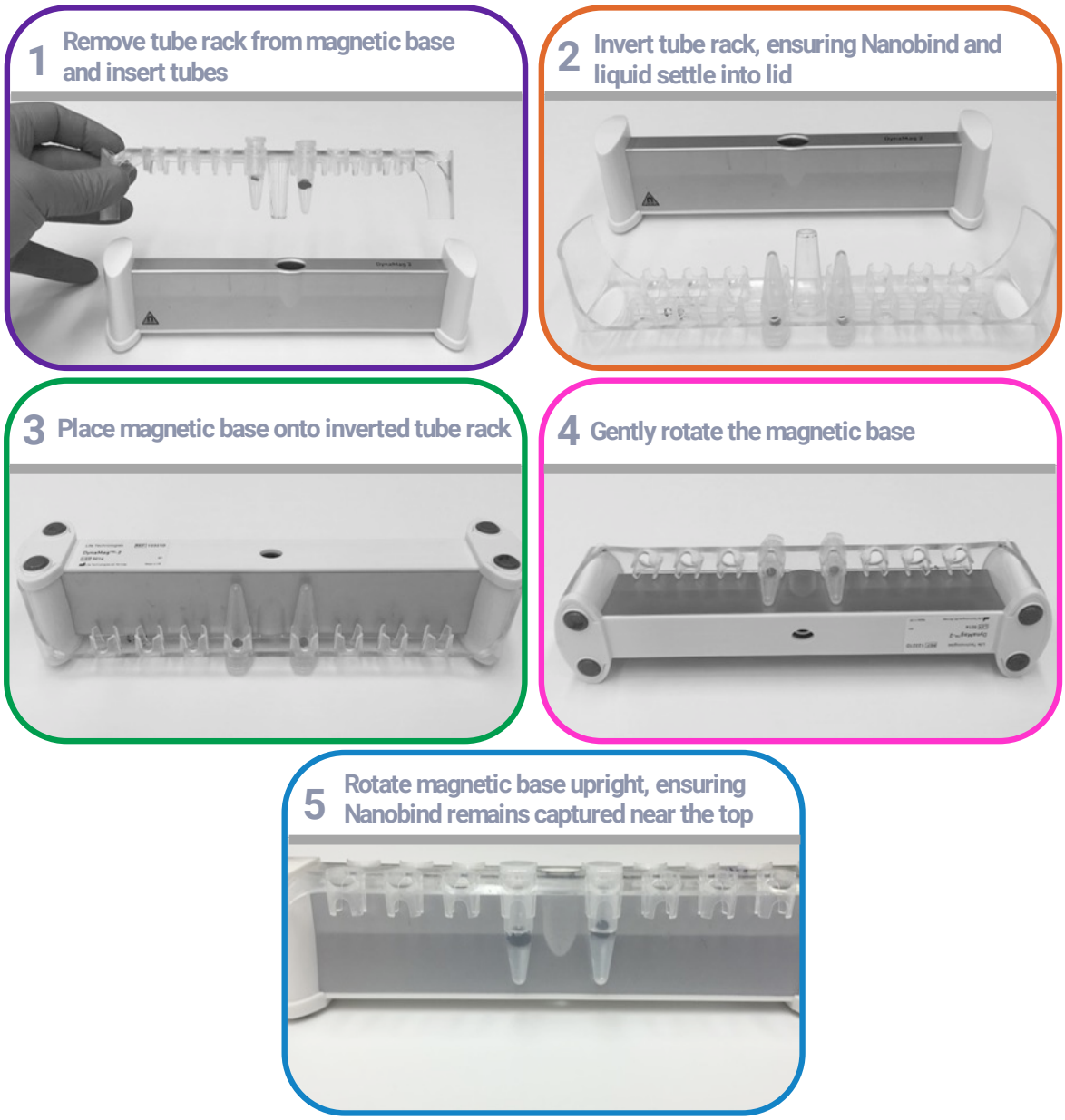
cryoPREP

We have extracted and sequenced various human tissues using the Covaris cryoPREP (CP02) automated dry pulverizer for upfront disruption. Please refer to the cryoPREP tissue homogenization protocol for details.

Processing tips

Magnetic rack handling with tubes containing Nanobind disks

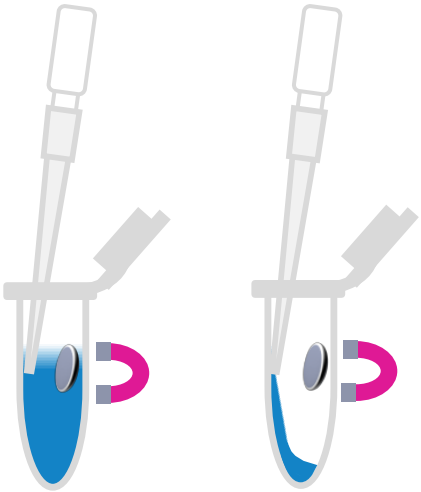
To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that is used with a magnetic base. Although DNA is bound quite robustly, proper pipetting and handling will ensure thorough washing and minimize disturbance of the bound DNA. For best results, the Nanobind disk should be captured near the top of the tube so that fluid can be easily removed from the bottom of the tube. The following procedure is recommended.



Recommended procedure for capturing Nanobind disk on a tube rack and magnetic base. This procedure ensures that the Nanobind disk is captured near the top of the liquid interface, minimizing disturbance of the bound DNA and facilitating processing.

Pipetting

When removing liquid from the microcentrifuge tube, the Nanobind disk should not be disturbed. Carefully insert the pipette tip against the wall opposite the Nanobind disk and remove liquid by pipetting from the liquid surface. This will minimize the chances of accidentally pipetting bound DNA. Likewise, when adding liquid, dispense against the wall opposite the Nanobind disk.



Pipetting procedure for removal (left) and addition (right) of liquid during wash steps. Avoid disrupting the Nanobind disk and bound nucleic acids.

Heterogeneity and viscosity

The extracted HMW DNA can be highly viscous and heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The heterogeneity and viscosity of the DNA eluate will vary depending on sample type, DNA size, sample input, and processing parameters.

Following elution of the HMW DNA: Pipette mix the extracted DNA 5–10X with a standard P200 pipette. Pipette mixing will help to loosen and coax the viscous DNA into solution. Moderate amounts of pipette mixing will not significantly impact DNA length. Pipette mixing is a standard part of our DNA elution process; we routinely use it for all long-read sequencing and optical mapping applications. For greater accuracy, the pipette mixed DNA should be left overnight at RT before quantifying the concentration.

In some cases, the extracted DNA will be very heterogeneous and contain large amounts of unsolubilized “jellies”: The most common reason for high sample heterogeneity and low purity is insufficient mixing during lysis. More aggressive mixing will result in samples with improved purity due to more efficient lysis and digestion. Improved sample purity will lead to improved DNA homogeneity and reduced “jellies.” Aggressive mixing during lysis will not significantly impact DNA length.

Shearing

Follow the shearing guidelines outlined in the appropriate library prep Procedure & checklist or consult Technical notes for experimental conditions for shearing whole blood, microbial, and human, plant, and animal samples.

Troubleshooting FAQ

See individual DNA extraction protocols for details.

Revision history (description)	Version	Date
Initial release	01	February 2024
Updated to include guidance on preservation methods and tissue disruption	02	April 2024
Updated to include links to all extraction protocols	03	August 2024
Updated to include saliva protocol	04	October 2024