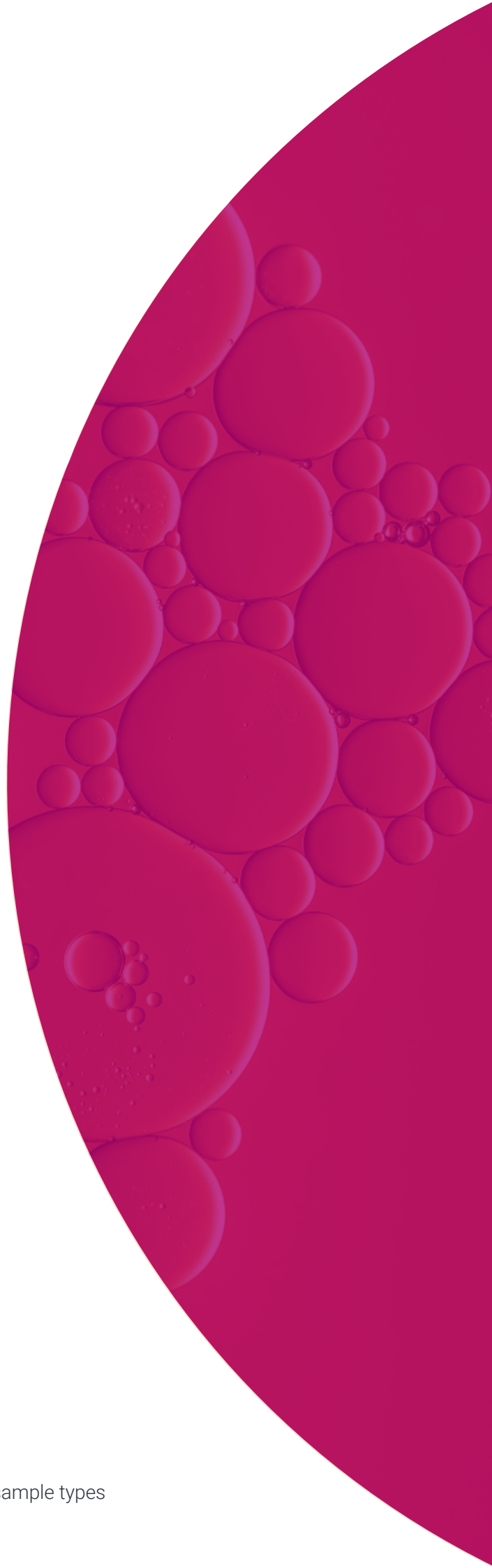
PacBio

Nanobind® PanDNA kit

Guide & overview

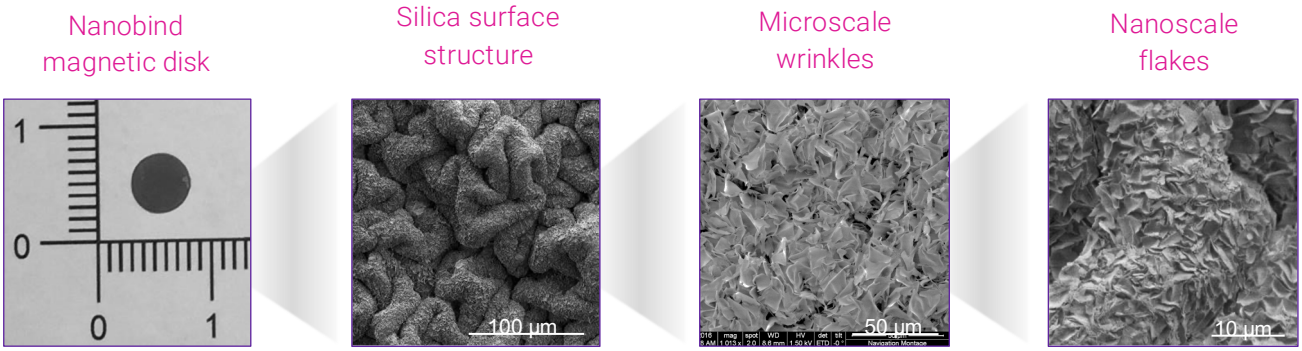


For extraction of HMW (50–300+ kb) genomic DNA from diverse sample types

Introduction

This guide and overview describes the PacBio® Nanobind PanDNA kit which can be used for diverse sample types including cultured cells and bacteria, human blood 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.

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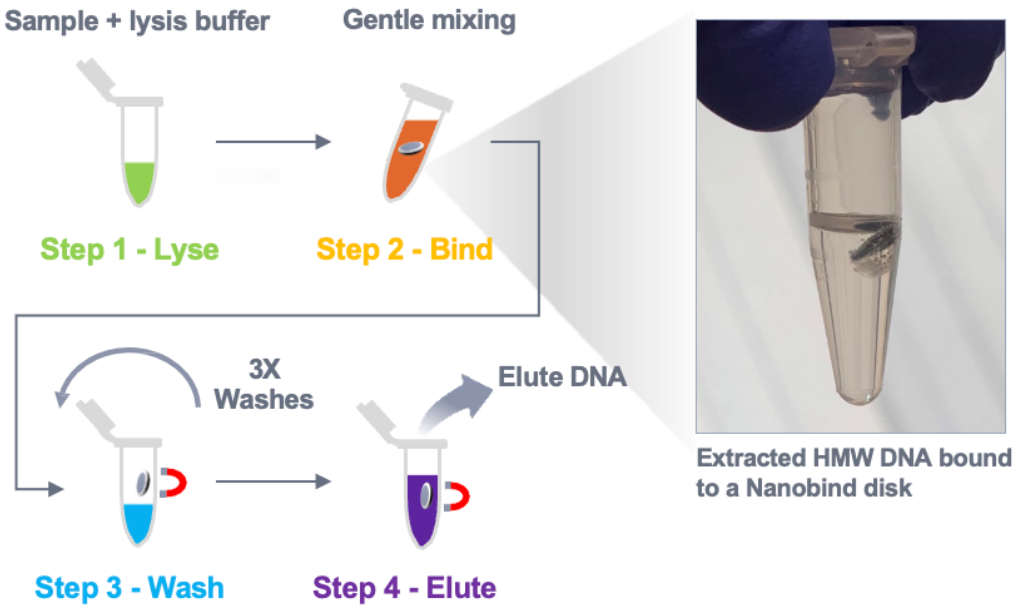
Nanobind PanDNA kit principle

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, 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. The CBB kit only contains 2 wash buffers (CW1 and CW2). 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.

Protocol / Buffer	Cultured Cells & Bacteria	Human Whole Blood	Human & Animal Tissue	Plant Nuclei	Insect	RBC Lysed Human Whole Blood
PK	✓	✓	✓	✓	✓	✓
RNase A	✓	✓	✓	✓	✓	✓
CLE3	✓		✓			
BL3	✓	✓	✓		✓	✓
CW1	✓	✓	✓		✓	✓
CW2	✓	✓	✓		✓	✓
LTE	✓	✓	✓	✓	✓	✓
CT			✓		✓	
SB			✓			
NPL				✓	✓	
PW1				✓		
RBC 10X						✓

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.

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 Documentation page. Below is an overview of all sample types for input quantity and expected results.

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

Sample type	Starting material	Recommended input	Example input	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	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)	2.5 – 20 µL	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	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
Animal tissue	Diverse tissue types	2 – 100 mg	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	>20 mg	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	0.25 – 5 g	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	1 x 10^6 – 5 x 10^6 diploid human cells	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	1 x 10^6 – 5 x 10^6 diploid human cells	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	5 x 10^8 – 5 x10^9 bacterial cells	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	5 x 10^8 – 5 x10^9 bacterial cells	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)).

[1] The expected "normal" range for 260/280 and 260/230 ratios will vary depending on sample type. UV purities outside of these expected ranges may indicate abnormalities in the extraction process. **Note:** Some sample types that consistently yield lower UV purities can still sequence well.

Nanobind PanDNA kit example performance

Example Nanobind PanDNA DNA extraction QC and HiFi sequencing performance results[1]

Sample	Input material	DNA yield	DNA mode size	HiFi mean read length	HiFi yield	Median QV
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[2])	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

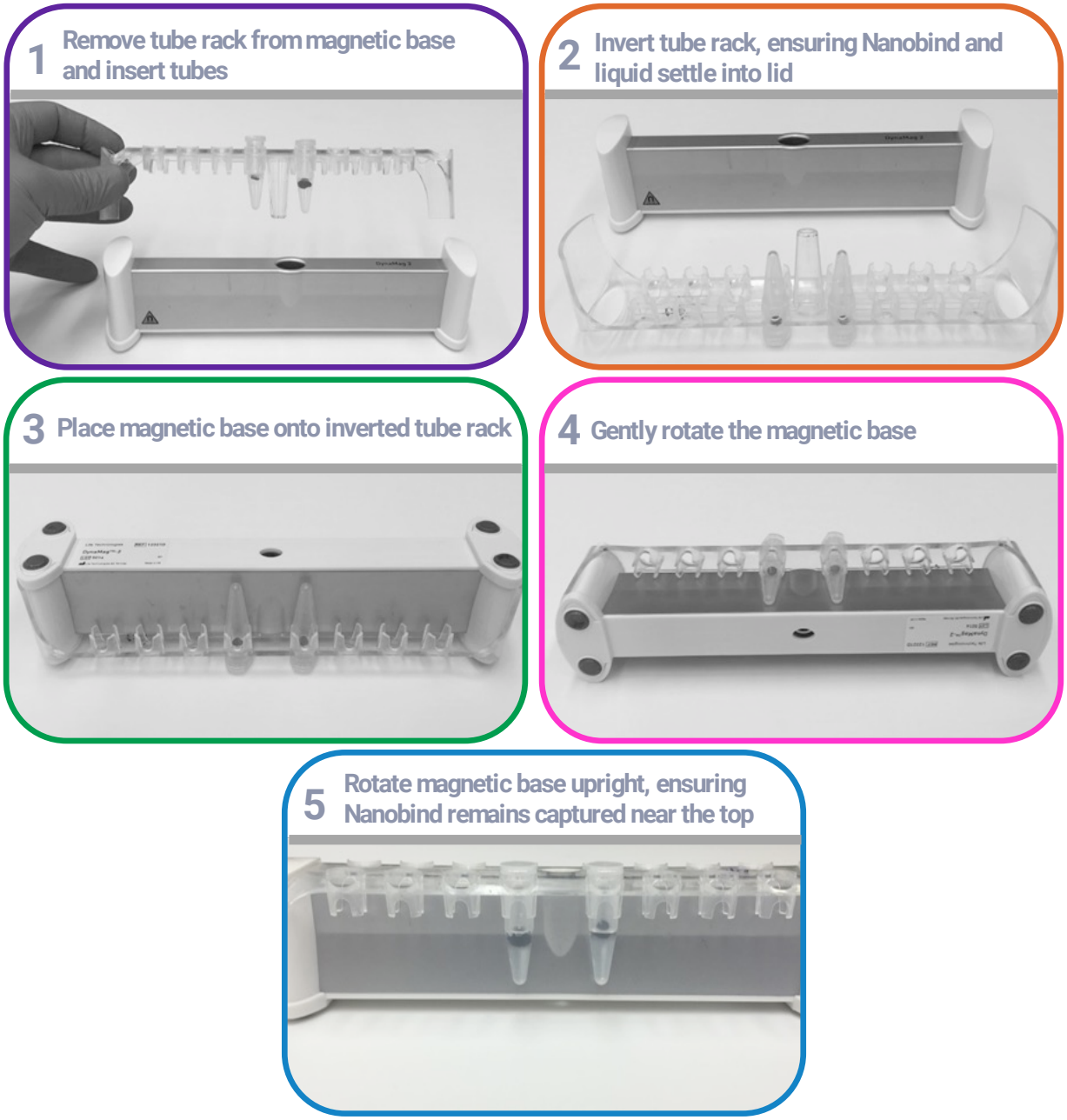
[1] See ***Brochure – Nanobind PanDNA kit*** (102-326-604).

[2] Note: For DNA isolation from insects, we generally recommend using the thorax – however, for insects such as crickets or grasshoppers where the hindlegs contain a substantial amount of muscle, the hindlegs are recommended for DNA isolation. See ***Extracting HMW DNA extraction from insects using the Nanobind PanDNA kit*** (102-377-400).

Processing tips

Magnetic rack handling with tubes containing Nanobind disks

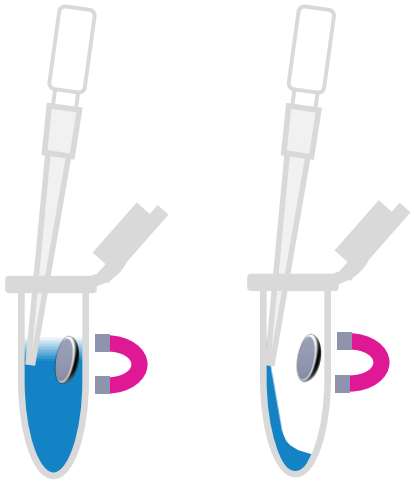
To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that is used with a magnetic base. Although DNA is bound quite robustly, proper pipetting and handling will ensure thorough washing and minimize disturbance of the bound DNA. For best results, 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

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