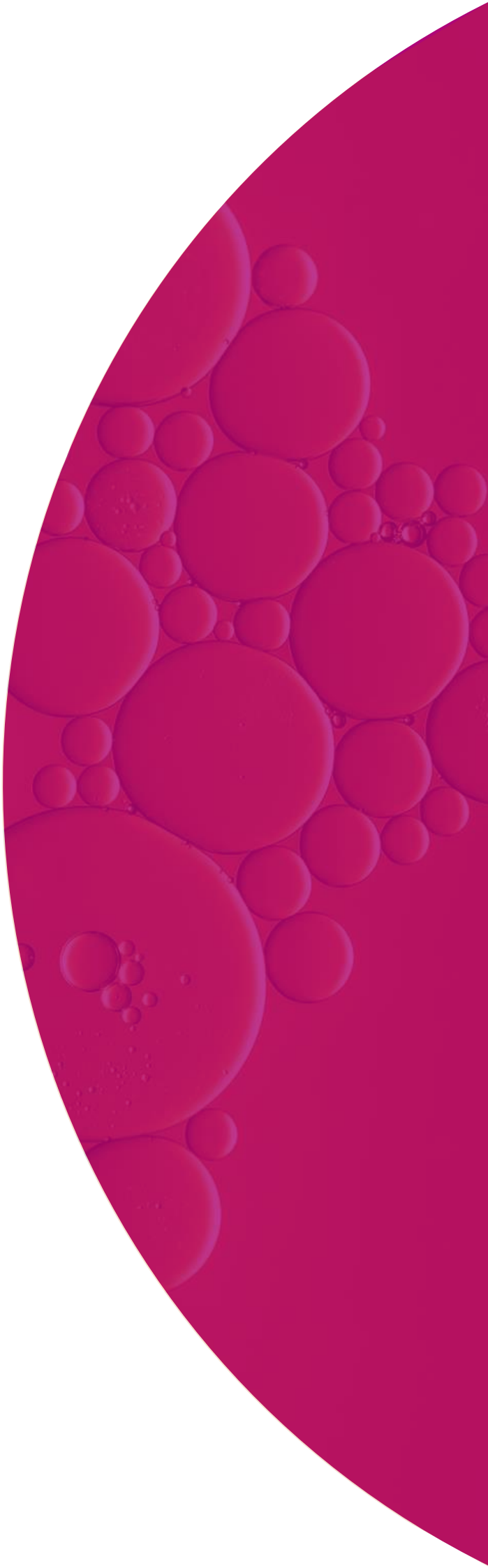
PacBio

Nanobind® tissue kit

Guide & overview



For extraction of HMW (50–300+ kb) genomic DNA from tissue

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Prior to starting

Buffer CW1 and CW2 are supplied as concentrates. This kit uses CW1 with a 60% final ethanol concentration. This kit uses CW2 with a 60% final ethanol concentration. Before using, add the appropriate amount of ethanol (96–100%) to Buffer CW1 and Buffer CW2 as indicated on the bottles.

Kit storage

The Nanobind tissue kit 4C (102-208-800) should be stored at 4°C upon arrival.

The Nanobind tissue kit RT (102-208-000) 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.

Equipment and reagent list

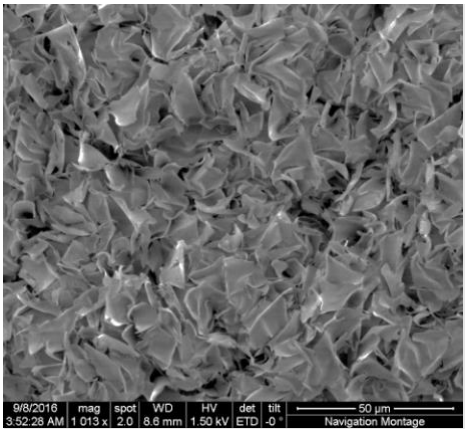
Equipment/reagent	Manufacturer (part number)
Nanobind tissue kit	PacBio® (102-302-100)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
Wheaton 1 mL Dounce tissue grinder with tight and loose pestles	Fisher Scientific (06-434)
TissueRuptor II	Qiagen (9002755)
Surgical scalpel	Fisher Scientific (22-079-712)
ThermoMixer	Eppendorf (5382000023)
Heat block (or water bath)	Fisher Scientific (11-715-125DQ)
Mini-centrifuge	Ohaus (FC5306)
Micro-centrifuge	Eppendorf (5404000413)
Platform rocker	Thermo Scientific (M48725Q)
Mini-tube rotator	Fisher Scientific (05-450-127)
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)
Wide bore 1000 µL pipette tips	Thermo Scientific (2079G)
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
26G blunt end needle	SAI Infusion (B26150)
1 mL syringe	Fisher Scientific (14-823-30)

For all protocols

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.

Introduction

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 and RNA. 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, to yield DNA up to megabases in length.



SEM image of Nanobind's silica surface structure.

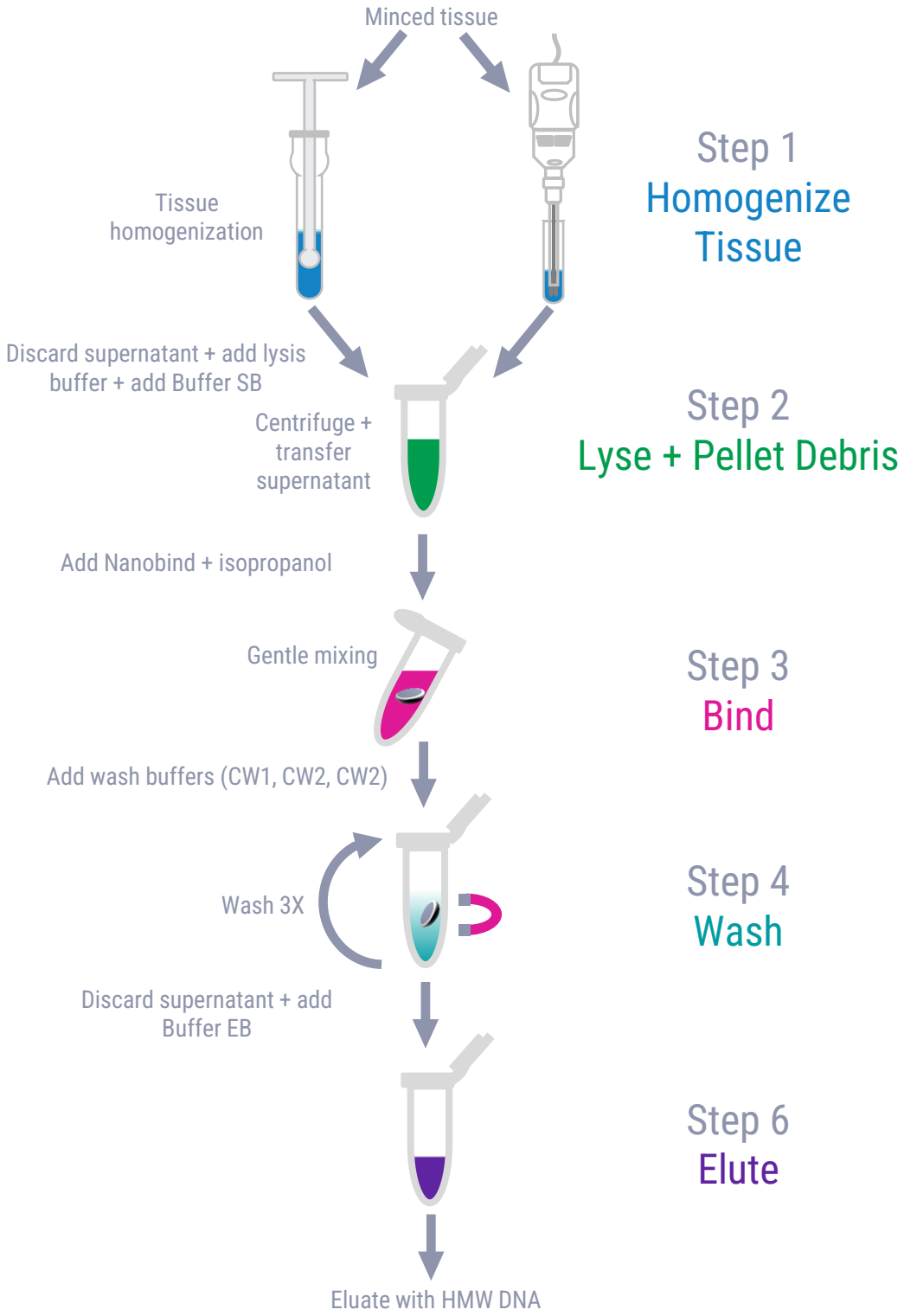
Kit overview

The Nanobind tissue kit is used for the extraction of HMW (50–300+ kb) DNA from animal tissue samples. It has been used to sequence a variety of animals including mammals, fish, birds, sharks, crustaceans, and mollusks. The extracted DNA is suitable for HiFi sequencing on PacBio systems. The Nanobind tissue kit can also be used to process insect samples using the appropriate aux kit. Finally, it can be used to process samples covered by the Nanobind CBB kit including cultured cells, cultured bacteria, whole blood, and nucleated blood. Depending on sample type, process time is approximately 2 h for HMW DNA.

The Sample information section provides extraction and sequencing results from a variety of sample types.

Protocols listed in the HMW (50—300+ kb) DNA extraction protocols section are updated frequently so please check the PacBio Documentation page for the most up-to-date list and for the current versions of the Procedures & checklists (P&C). These protocols are recommended for PacBio HiFi sequencing.

Workflow



Sample information

The following tables illustrate extraction data from a variety of animal species and tissue types. All samples have been validated by PacBio sequencing.

Each sample was extracted using either the TissueRuptor II (A), Dounce homogenizer (B), and/or cryoPREP protocols (C). These protocols differ only in the upfront homogenization approach.

For most tissues, any of the protocols may be used with similar results. If only a single protocol is listed, this does not indicate that the other protocols are incompatible. It only indicates that the listed P&C were tested.

Tissues that require modifications to the standard P&C are indicated in the tables below with asterisks (*).

See the relevant Sample specific Procedures & checklists for additional insight into experimental design and expected results.

Fresh frozen samples generate the best results, but tissues preserved in RNAlater and ethanol may also be used as described in the preservation methods section.

Nanobind tissue kit - Validated mammalian tissues

Sample type	Tested P&C	Input (mg)	DNA yield (µg)	260/280	260/230	Sample specific P&C
Human skeletal (FZ)	A, B, C	25	8.3	1.85	2.04	
Human colon (FZ)	A, B, C	24	18	1.86	2.15	
Human uterine fibroid (FZ)	A, B, C	25	10.9	1.87	2.19	
Human kidney (FZ)	A, B, C	30	16	1.86	2.20	
Human breast (FZ)	A*, B*, C	20	60.6	1.88	2.33	1
Mouse brain (FZ)	A*	22	5.3	1.85	2.03	2
Mouse liver (FZ)	A*, B*	32	23	1.88	2.22	3
Mouse spleen (FZ)	A*	19	143	1.86	2.30	4
Pig brain (FZ)	B	30	30	1.84	2.32	2
Pig liver (FZ)	B	25	17.1	1.90	2.05	3
Skunk ear punch (FZ)	A	33	9.9	1.81	1.81	
Vole heart (FZ)	A	47	21.3	1.87	2.16	
Vole kidney (RL)	A	38	45	1.87	2.17	

Nanobind tissue kit - Validated avian sample

Sample type	Tested P&C	Input (mg)	DNA yield (µg)	260/280	260/230	Sample specific P&C
Zebra finch muscle (FZ)	A	35	6.6	1.85	1.66	

Nanobind tissue kit - validated fish samples						
Sample type	**Tested P&C**	**Input (mg)**	**DNA yield (µg)**	**260/280**	**260/230**	**Sample specific P&C**
Teleost fin clip (FZ)	C	95 (P)	9.1	1.87	2.03	
Teleost liver (FZ)	C	25	9.0	1.74	1.3	
Stickleback brain/muscle (FZ)	C	50	126	1.84	2.14	
Stickleback liver (RL)	C	15	8.5	1.82	1.84	
Antarctic icefish muscle (FZ)	A*	206 (P)	10.2	1.70	1.65	8
Skate testis (FZ)	A*, B*	80	112.5	1.86	2.32	5
Tilapia heart (FZ)	A	25	13.1	1.86	2.18	10
Tilapia fin (FZ)	A	27	11.6	1.82	2.13	10
Tilapia testis (FZ)	A*	25	83.7	1.86	2.31	5, 10
Tilapia spleen (FZ)	A	25	97.2	1.87	2.33	10
Tilapia kidney (FZ)	A	22	16.1	1.86	2.22	10

Nanobind tissue kit - validated mollusk samples						
Sample type	**Standard P&C**	**Input (mg)**	**DNA yield (µg)**	**260/280**	**260/230**	**Sample specific P&C**
Black mystery snail (FZ)	B*	107	16.1	1.79	1.41	6
Aplysia muscle (FR)	A	43	9.1	1.87	2.07	11

Nanobind tissue kit - validated crustacean samples						
Sample type	**Standard P&C**	**Input (mg)**	**DNA yield (µg)**	**260/280**	**260/230**	**Sample specific P&C**
Dungeness crab Leg	B*	100	6.6	1.85	1.79	7
Red king crab leg (FZ)	A	116 (P)	112.4	1.87	2.28	
Snow crab leg (FZ)	A	550 (P)	15.6	1.44	0.92	
Tanner crab leg (FZ)	A	750 (P)	3.8	1.14	0.63	
Prawn	A	30	2.7	1.81	1.66	8

Nanobind tissue kit - validated blood samples						
Sample type	**Standard P&C**	**Input (µL)**	**DNA yield (µg)**	**260/280**	**260/230**	**Sample specific P&C**
Human blood	D	200	5.6	1.90	1.90	
Tuna blood	E	10	N/A	N/A	N/A	9

Procedures & checklists (P&C)

A	Nanobind HMW tissue TissueRuptor Procedures & checklists
B	Nanobind HMW tissue Dounce Procedures & checklists
C	cryo PREP tissue homogenization Precedures & checklists
D	Nanobind HMW mammalian whole blood Precedures & checklists
E	Nanobind HMW nucleated blood Precedures & checklists

Sample specific Procedures & checklists

1	See Human breast example Procedure & checklist
2	See Mammalian brain example Procedure & checklist
3	See Mammalian liver example Procedure & checklist
4	See Mammalian spleen example Procedure & checklist
5	See Fish testis example Procedure & checklist
6	See Black mystery snail example Procedure & checklist
7	See Crab muscle example Procedure & checklist
8	See Fish skeletal muscle example Procedure & checklist
9	See Nanobind HMW Nucleated blood Procedure & checklist
10	See Fish tissue selection guide Procedure & checklist
11	See Aplysia example Procedure & checklist

Abbreviations

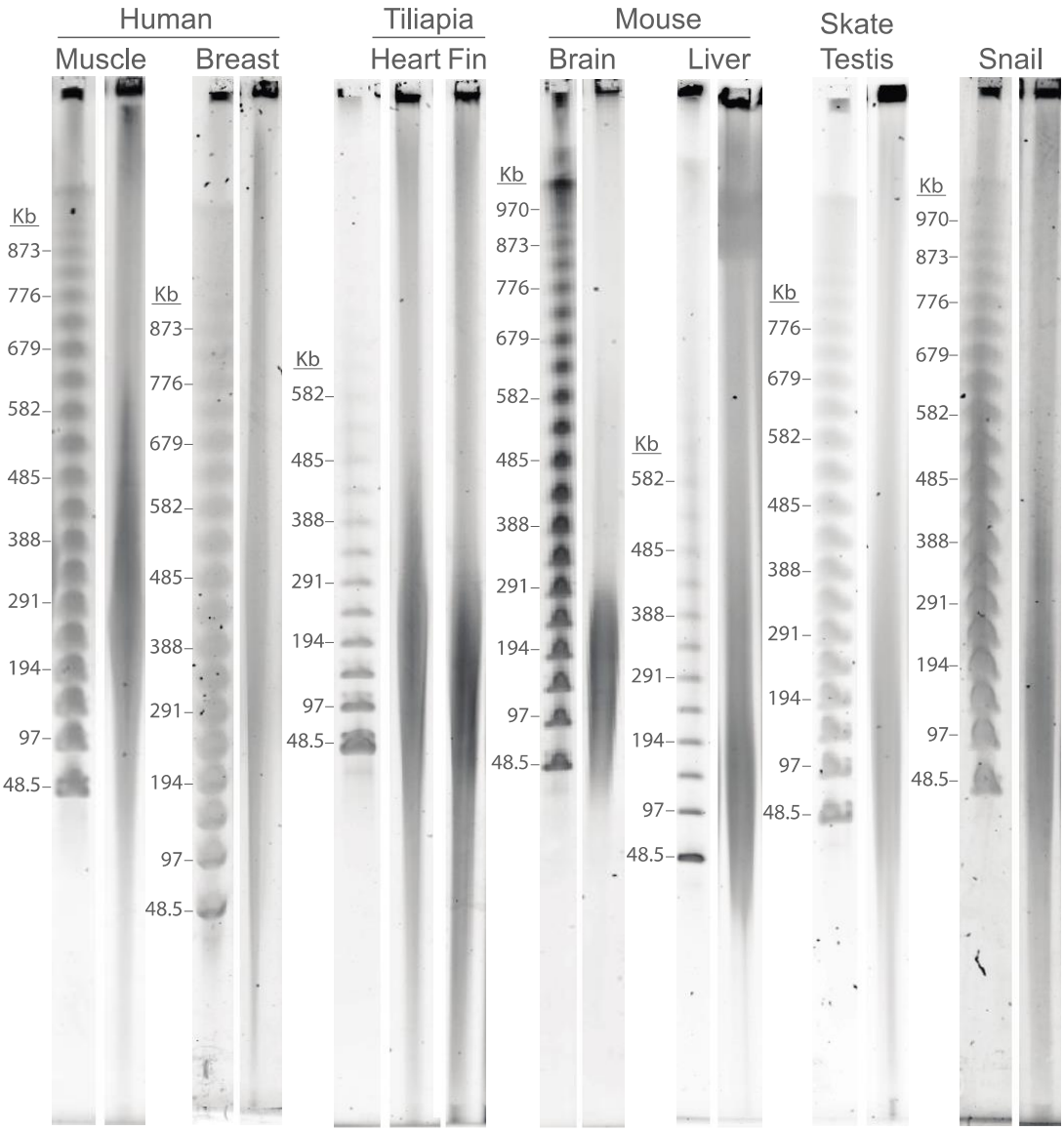
*	Modifications are needed to the standard protocols and are described in the Sample-specific Procedures & checklists.
FR	Fresh tissue
FZ	Frozen tissue
RL	RNAlater preserved
P	Pooled extractions

Insect samples

Insect samples are not officially supported at this time with the tissue kit. Select insects can be processed with insect aux kit. Contact us for more details.

DNA size

The HMW DNA extraction protocols typically yield DNA in the 50–300+ kb size range. The exact size will vary depending on sample type, the quality of the starting material, and processing parameters. For most long-read sequencing applications, superior sequencing performance will be obtained using the HMW DNA extraction protocols. This includes PacBio HiFi sequencing.



Pulsed Field Gel Electrophoresis (PFGE) images of HMW DNA extracted from various tissues.

Preservation methods

High quality tissue samples are the key to obtaining high quality DNA. Fresh frozen tissues generate the highest quality DNA and are the preferred sample type. However, other preservation methods such as storage in ethanol or RNAlater can be also used. With all samples, ischemic times before preservation or freezing should be avoided to minimize tissue DNA degradation.

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 appropriate extraction protocol.

Ethanol-preserved nucleated blood

Nucleated blood preserved in ethanol (e.g.,1:10 blood to ethanol) can be used by first centrifuging the blood at 10,000 x *g* for 2 minutes to pellet the cells and then following the nucleated blood Procedure & checklist.

Tissue disruption strategies

Choosing the appropriate disruption strategy

Most tissues may be processed with the standard TissueRuptor or Dounce homogenizer protocols. Tissues that require modifications to the standard protocols are indicated in the **Sample information** tables and accompanied by Sample-specific Procedures & checklists.

In addition, we provide a Procedure & checklist describing tissue disruption using a Covaris cryoPREP instrument.

We have tested alternative disruption methods including liquid nitrogen grinding and homogenization with pellet pestle. We do not recommend these homogenization methods as they do not consistently and sufficiently disrupt the tissue, leading to decreased yields, reduced purity, and diminished sequencing performance.

Homogenization tips and tricks

Dounce

It is important to use the Dounce homogenizer with the tight pestle. We do not recommend the loose pestle as this may not result in sufficient homogenization of the tissue.

When using the Dounce homogenizer, push the tissue firmly into the bottom of the Dounce chamber with each stroke. Try to keep the tissue between the tip of the pestle and the bottom of the Dounce chamber for thorough homogenization. If the tissue becomes trapped above the bulb of the pestle, pull the pestle completely out of the chamber and let the tissue settle back into the chamber by gravity.

It is not necessary to twist the pestle at the bottom of the Dounce chamber to disrupt the tissue. The compression and suction forces on the tissue that are created when moving the pestle up and down are sufficient to disrupt the tissue.

The homogenate may become foamy with some tissue types. This is normal and will not cause issues with downstream processing. If foam forms, be sure to transfer all of it in step 6.

TissueRuptor II

Use the TissueRuptor on its maximum setting. Ten seconds should be sufficient for all tissues as long as the sample was adequately minced beforehand.

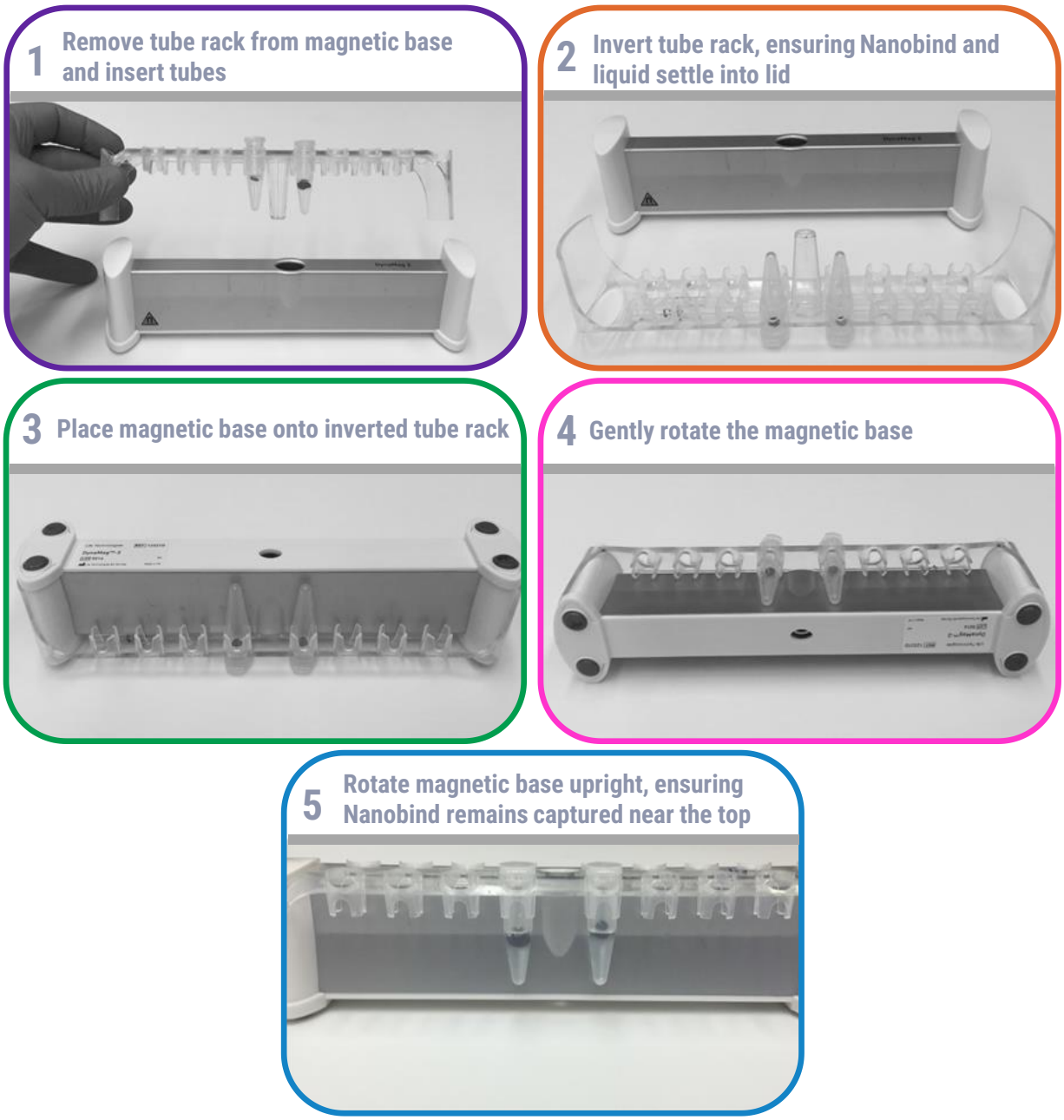
cryoPREP

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 application note for details.

Processing tips

Magnetic rack handling procedure

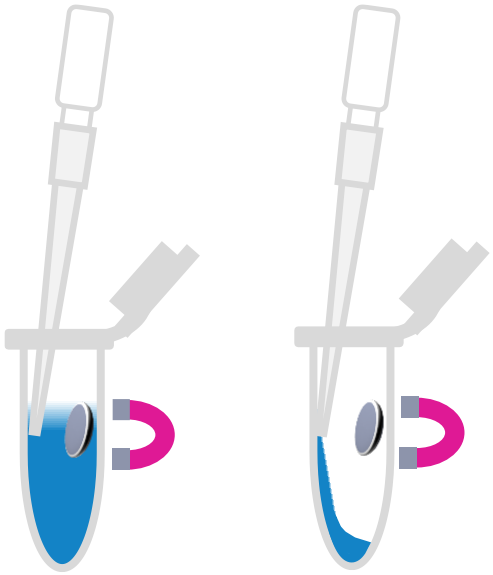
To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that is used with a magnetic base. Although DNA is bound quite robustly, proper pipetting and handling will ensure thorough washing and minimize disturbance of the bound DNA. For best results,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

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. More gentle processing will yield larger DNA size but will also result in higher heterogeneity and larger amounts of highly viscous, unsolubilized “jellies.” Processing that is too gentle can dramatically reduce DNA purity and yield. To minimize the challenges of heterogeneity and viscosity, we recommend that new users err on the side of being overly aggressive. Listed below are tips for working with HMW DNA.

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.

To accurately quantify the HMW DNA:

Pipette mix the DNA 5X with a standard P200 pipette again. Perform triplicate Nanodrop readings by sampling the top, middle, and bottom of the eluate. If the concentration %CV > 30, perform an additional 5X pipette mixing using a standard P200 pipette. Let the DNA rest for at least 1 hr and repeat the Nanodrop measurements.

To accurately determine the concentration of dsDNA, we recommend making triplicate measurements using the Qubit dsDNA BR assay.

If the extracted DNA needs to be used immediately after extraction:

The extracted DNA can be sheared 5X using a 26G blunt stainless-steel needle and 1 mL syringe. The needle-sheared DNA can be used immediately for library preparation. Moderate amounts of needle shearing will not significantly impact DNA length. Nearly all samples we sequence have been 5X needle sheared.

Shearing HMW DNA

Concentrated, HMW DNA can be difficult to shear with either the Corvaris g-TUBE or Diagenode Megauptor 3 Hydropore - long. In some cases, the viscous DNA could clog the shearing consumables. For these samples, we recommend trying one or more of the following:

1. Pre-shear the HMW DNA 5X using a 26G blunt end stainless-steel needle and 1 mL syringe.
2. Pre-shear using the Megaruptor 3 DNAFluid+ Kit. We recommend 100 µL of sample, concentrations <500 ng/µL, and a speed setting of 59.

Both of these options will help to decrease the sample viscosity without negatively affecting sequencing performance.

Follow the shearing guidelines outlined in the appropriate library prep Procedure & checklist.

HMW (50–300+ kb) DNA extraction protocols

The following protocols are available for HMW (50–300+ kb) DNA extraction. They are recommended for most long-read sequencing applications. This includes PacBio HiFi sequencing.

Two standard tissue protocols are provided. These protocols differ only in which upfront homogenization method was used, TissueRuptor II or Dounce homogenizer. Most tissues can be processed using either protocol.

Sample-specific protocols are provided on our website.

The **Sample information** section provides additional guidance regarding protocol selection.

Extracting HMW DNA from animal tissue using TissueRuptor (102-574-600)

This protocol describes the extraction of HMW DNA from animal tissue using Qiagen TissueRuptor II for sample homogenization. This protocol has been validated for many mammalian, avian, and fish tissue types including heart, liver, spleen, kidney, and colon. This protocol requires the Nanobind tissue kit (102-302-100).

Extracting HMW DNA from standard Dounce homogenizer tissue using Nanobind kits (102-573-700)

This protocol describes the extraction of HMW DNA from animal tissue using a Dounce homogenizer for sample homogenization. This protocol has been validated for many mammalian, avian, and fish tissue types including gonad, kidney, liver, brain, and colon. This protocol requires the Nanobind tissue kit (102-302-100).

Extracting HMW DNA from cultured cells using Nanobind kits (102-573-600)

This protocol describes the extraction of HMW DNA from cultured cells. This protocol has been validated on several cell types including GM12878 and MCF-7. This protocol requires the Nanobind CBB kit (102-301-900) or Nanobind tissue kit (102-302-100).

Extracting HMW DNA from Gram-negative bacteria using Nanobind kits (102-573-800)

This protocol describes the extraction of HMW DNA from Gram-negative bacteria. This protocol has been validated on several Gram-negative bacterial species including *E. coli, S. sonnei,* and *S. enterica*. This protocol requires the Nanobind CBB kit (102-301-900) or Nanobind tissue kit (102-302-100).

Extracting HMW DNA from Gram-positive bacteria using Nanobind kits (102-573-900)

This protocol describes the extraction of HMW DNA from Gram-positive bacteria. This protocol has been validated on several Gram-positive bacterial species including *L. monocytogenes*, *S. aureus*, and *E. faecalis*. This protocol requires the Nanobind CBB kit (102-301-900) or Nanobind tissue kit (102-302-100).

Extracting HMW DNA from mammalian whole blood using Nanobind kits (102-573-500)

This protocol describes the extraction of HMW DNA from 200 µL of mammalian whole blood. It has been validated using fresh and frozen whole blood. Other volumes can also be used with modification to the protocol. This protocol requires the Nanobind CBB kit (102-301-900) or Nanobind tissue kit (102-302-100).

Extracting HMW DNA from nucleated blood using Nanobind kits (102-574-000)

This protocol describes the extraction of HMW DNA from 5–30 µL of nucleated blood. It has been validated on fish, bird, and lizard blood including fresh, frozen, and ethanol preserved blood samples. This protocol requires the Nanobind CBB kit (102-301-900) or Nanobind tissue kit (102-302-100).

QC procedures

Accurate quantification of HMW DNA can be challenging due to sample inhomogeneity, often leading to concentration measurements with high concentration CVs. We recommend performing replicate Nanodrop UV/Vis, replicate Qubit BR DNA assay measurements, and a single, optional Qubit BR RNA assay measurement.

See individual HMW extraction protocols for detailed guidance.

Storage of DNA

DNA can be stored in Buffer EB at 4°C for several months. Long term storage at -20°C or -80°C can be used if necessary. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Troubleshooting FAQ

See individual DNA extraction protocols for details.

Revision history (description)	Version	Date
Initial release	01	July 2022
Minor updates throughout	02	August 2022
Minor updates throughout	03	December 2022
Minor updates throughout, deleted UHMW DNA information	04	May 2023
Updated table of contents	05	June 2023