Technical overview – HMW DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and SRE kits

Sequel II and IIe systems ICS v11.0

Revio system ICS v13.1

SMRT Link v13.1

PN 103-401-100 Rev 01 | April 2024

High-molecular weight (HMW) DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and Short read eliminator (SRE) kits

Technical Overview

1. Nanobind PanDNA kit configuration and workflow overview
2. Nanobind reagent and sample handling best practices
3. Short read eliminator (SRE) kit configuration and workflow overview
4. Technical documentation & applications support resources

Nanobind HMW DNA extraction for PacBio long-read sequencing: Getting started

Nanobind literature → **Nanobind protocols** → **Nanobind technical overview** → **DNA sample extraction, library prep & sequencing**

PacBio NANOBIND® PANDNA KIT

Sample the tree of life with a single kit

The all-new PacBio® Nanobind® PanDNA kit consolidates the capabilities of our existing sample-specific offerings into a single solution for DNA extraction. This kit provides users with an all-in-one extraction experience that can be used with a wide range of sample types. The PanDNA kit supports high-quality extraction from cultured cells, bacteria, whole blood, tissue, plant nuclei, and insect samples.

Higher DNA quality means better HiFi data

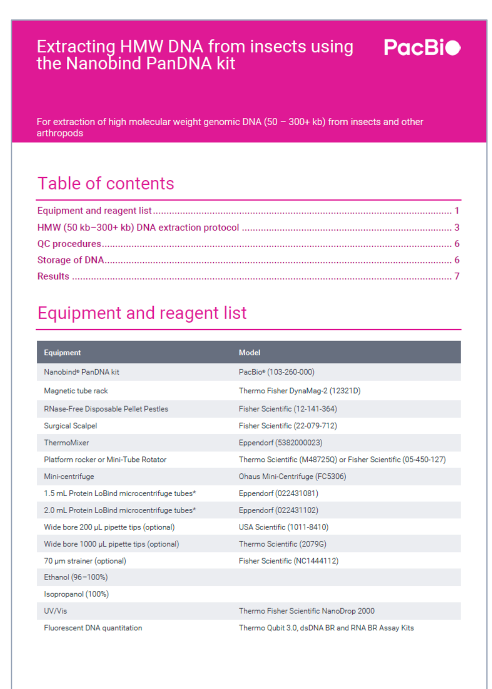
Using the Nanobind PanDNA kit on a diverse set of plant and animal samples demonstrates extraction yields between 4–26 µg and mode fragment size greater than 100 kb for the majority of samples (and greater than 65 kb for insect). Sequencing these samples on the Revio™ system yields an average of 103 Gb HiFi data per SMRT® Cell and optimal read lengths with a mean of 15–17 kb.

Sample type	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	27 mg	5.3 µg	67 Kb	16,034 bp	86 Gb	Q34
Cricket (legs)	44 mg	10.5 µg	118 Kb	15,731 bp	113 Gb	Q35
Tobacco leaf (nuclei prep)	1 g	16.5 µg	140 Kb	14,649 bp	90 Gb	Q34
Pepper leaf (nuclei prep)	1 g	26.1 µg	118 Kb	15,440 bp	94 Gb	Q34
Apple leaf (nuclei prep)	1 g	11.3 µg	112 Kb	15,598 bp	106 Gb	Q35

Learn more: pacb.com/nanobind

Brochure– Nanobind PanDNA kit (102-326-604)

Summary overview of Nanobind PanDNA kit for high-quality HMW DNA extraction from cultured cells, bacteria, whole blood, tissue, plant nuclei, and insect samples.



Extracting HMW DNA from insects using the Nanobind PanDNA kit

For extraction of high molecular weight genomic DNA (50 – 300+ kb) from insects and other arthropods

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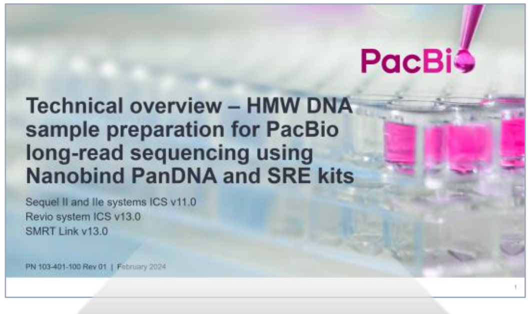
Equipment and reagent list

Equipment	Model
Nanobind® PanDNA kit	PacBio® (103-260-000)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
RNase-Free Disposable Pellet Pestles	Fisher Scientific (12-141-364)
Surgical Scalpel	Fisher Scientific (22-079-712)
ThermoMixer	Eppendorf (5382000023)
Platform rocker or Mini-Tube Rotator	Thermo Scientific (M48725Q) or Fisher Scientific (05-450-127)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431081)
2.0 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431102)
Wide bore 200 µL pipette tips (optional)	USA Scientific (1011-8410)
Wide bore 1000 µL pipette tips (optional)	Thermo Scientific (2079G)
70 µm strainer (optional)	Fisher Scientific (NC1444112)
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

Nanobind Procedures & checklists and Guide & overview – Nanobind PanDNA kit (103-394-800)

Refer to PacBio's Documentation website for a full list of Nanobind HT HMW DNA sample preparation protocols for use with different automated sample purification systems.



PacBio

Technical overview – HMW DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and SRE kits

Sequel II and IIe systems ICS v11.0
Revio system ICS v13.0
SMRT Link v13.0

PN 103-401-100 Rev 01 | February 2024

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

Technical overview – HMW DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and SRE kits (103-401-100)

Technical overview presentation describes protocol details for using Nanobind PanDNA kit for HMW DNA extraction SRE kit for size selection, and also shows example sequencing performance data.

HMW DNA sample extraction (Nanobind PanDNA kit)

Use Nanobind PanDNA kit for high-quality HMW DNA extraction from cultured cells, bacteria, whole blood, tissue, plant nuclei, and insect samples.

SMRTbell library preparation (SMRTbell prep kit 3.0)

Follow Procedure & checklist to construct a SMRTbell library using purified HMW DNA

SMRT sequencing (Sequel II/IIe & Revio systems)

Prepare samples for long-read sequencing by following SMRT Link sample setup instructions

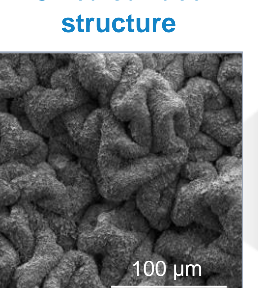
Nanobind DNA extraction technology overview

Nanobind technology enables extraction of high-molecular weight DNA from common samples as well as more challenging samples such as animal tissue, insects and plants

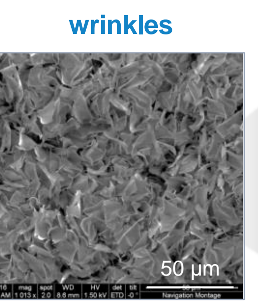
Nanobind is a novel magnetic disk (3 – 5 mm) covered with a high density of micro- and nanostructured silica

Nanobind magnetic disk

Silica surface structure

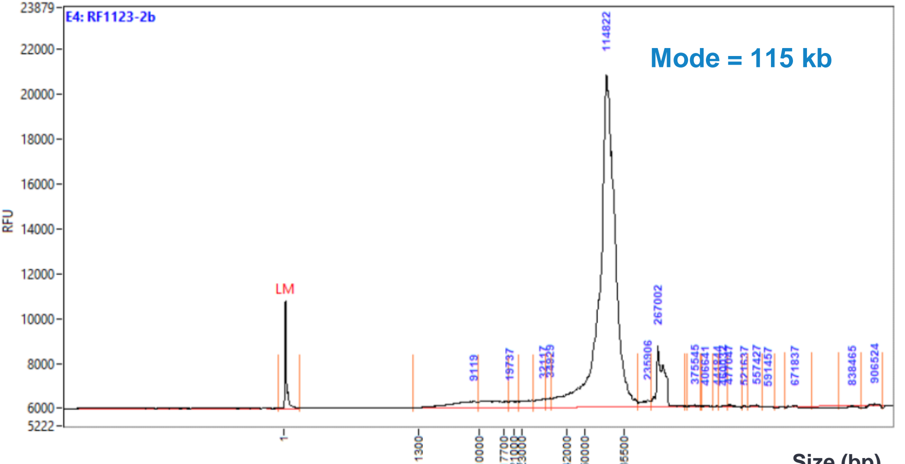


Microscale wrinkles



Nanoscale flakes

10 µm

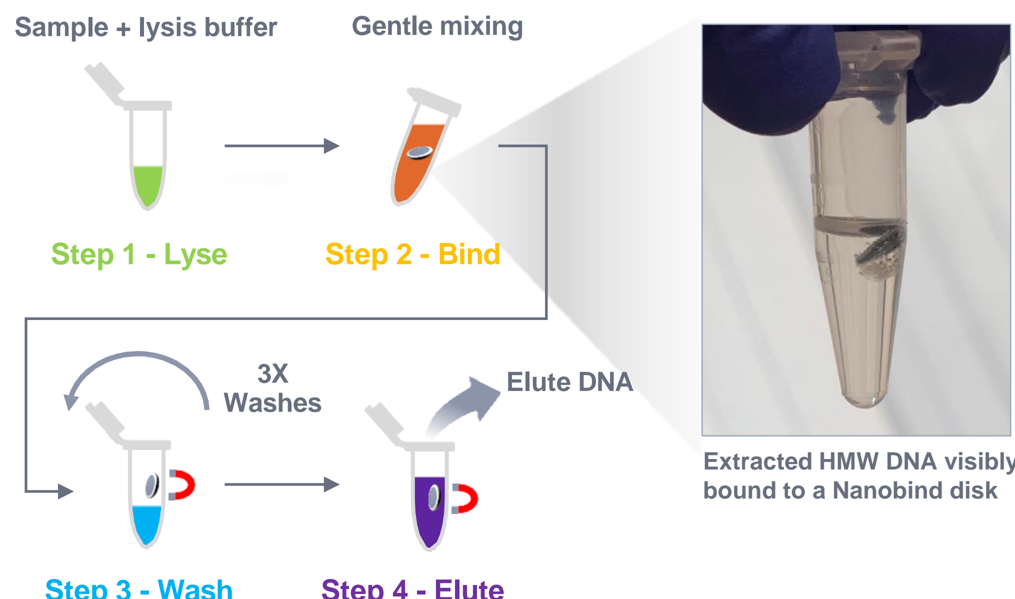


Nanobind disks bind and release DNA without fragmentation, yielding HMW DNA 50–300+ kb

Femto Pulse DNA sizing QC analysis of unsheared gDNA isolated from $5x10^6$ GM12878 cells using Nanobind technology.

Rapid magnetic purification procedure

- Rapid and simple bind, wash, and elute protocol
- Can perform manual processing using a magnetic separation rack
- Nanobind disks are automation compatible[1] for high-throughput applications



Extracted HMW DNA visibly bound to a Nanobind disk

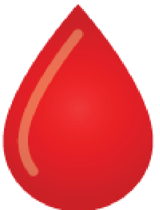
[1] See *Technical overview – Automated high-throughput HMW DNA extraction for PacBio long-read sequencing using Nanobind HT kits* (103-401-700).

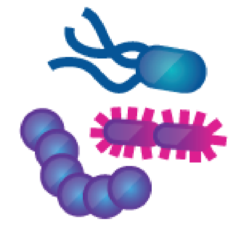
PacBio

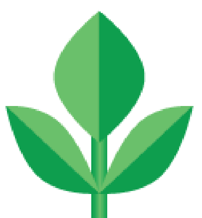
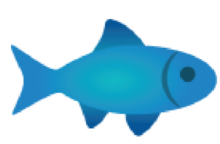
Nanobind PanDNA kit configuration and workflow overview

Nanobind PanDNA kit provides an all-in-one DNA extraction kit that can be used with a wide range of sample types for PacBio HiFi sequencing

Nanobind PanDNA kit enables high-quality HMW DNA extraction from cells, blood, bacteria, tissues, insects, and plant nuclei





Easy-to-use solutions built on Nanobind technology

The all-new PacBio Nanobind PanDNA kit consolidates the capabilities of our existing sample-specific Nanobind kit product offerings into a single solution for DNA extraction.[1,2]

- Cultured mammalian cells
- Human whole blood
- Animal blood (mammalian & non-mammalian)
- Cultured bacteria
- Animal tissues
- Plant nuclei
- Insects
- RBC lysed human whole blood

Nanobind PanDNA kit (103-260-000)

Supports 24 reactions per kit and includes:

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

Using the Nanobind PanDNA kit on a diverse set of plant and animal samples demonstrates extraction yields between ~3–26 µg and mode fragment sizes >100 kb for the majority of samples (and >65 kb for insect samples)[3]

[1] Nanobind CBB kit (102-301-900) is also available for HMW DNA extraction from cultured mammalian cells, blood, and bacterial samples.

[2] **Note:** Fungal, lichen, algae and microalgae sample types are unsupported with the Nanobind PanDNA kit.

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

Available Nanobind PanDNA HMW DNA extraction protocols (cont.)

Select the appropriate Nanobind Procedure & checklist to use based on sample type and starting material[1]

Sample type	Starting material	Sample input	Workflow time	Procedure & checklist
Blood	Human whole blood	200 µL	~1 hr	*Extracting HMW DNA from human whole blood using Nanobind kits* (102-573-500)[2]
	Nucleated red blood cells (nRBCs)	2.5 – 20 µL	~1 hr	*Extracting HMW DNA from nucleated red blood cells using Nanobind kits* (102-574-000)
	Human whole blood with RBC lysis	400 µL	<1.5 hrs	*Extracting HMW DNA from human whole blood with RBC lysis using Nanobind kits* (103-377-500)
Animal tissue	Diverse tissue types	2 – 100 mg	~2.5 hrs	*Extracting HMW DNA from animal tissue* (102-574-600)[3]
Insect tissue	Insect whole body or segment	>20 mg	~2.5 hrs	*Extracting HMW DNA extraction from insects using the Nanobind PanDNA kit* (102-377-400)
Plant tissue	Isolated plant nuclei	0.25 – 5 g	~1.5 hrs[4]	*Extracting HMW DNA from plant nuclei using Nanobind kits* (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 using Nanobind kits* (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 using Nanobind kits* (102-573-600)
Cultured bacteria	Gram-negative bacteria	5 x 10^8 – 5 x10^9 bacterial cells	~1 hr	*Extracting HMW DNA from Gram-negative bacteria using Nanobind kits* (102-573-800)
	Gram-positive bacteria	5 x 10^8 – 5 x10^9 bacterial cells	~1 hr	*Extracting HMW DNA from Gram-positive bacteria using Nanobind kits* (102-573-900)

[3] ***Procedure & checklist – Extracting HMW DNA from animal tissue*** (102-574-600) describes the extraction of HMW DNA from animal tissues using a TissueRuptor tool for tissue disruption. If a TissueRuptor tool is unavailable, then ***Procedure & checklist – Extracting HMW DNA from standard Dounce homogenizer tissue using Nanobind kits*** (102-573-700) may alternatively be used.

[1] For a complete list of supported Nanobind HMW DNA extraction procedures, refer to the PacBio Documentation website.

[2] Note that this manual Nanobind procedure (102-573-500) does not support processing of other types of (non-human) mammalian blood samples that are non-nucleated.

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

PacBio

Nanobind HMW DNA extraction procedure for insect samples

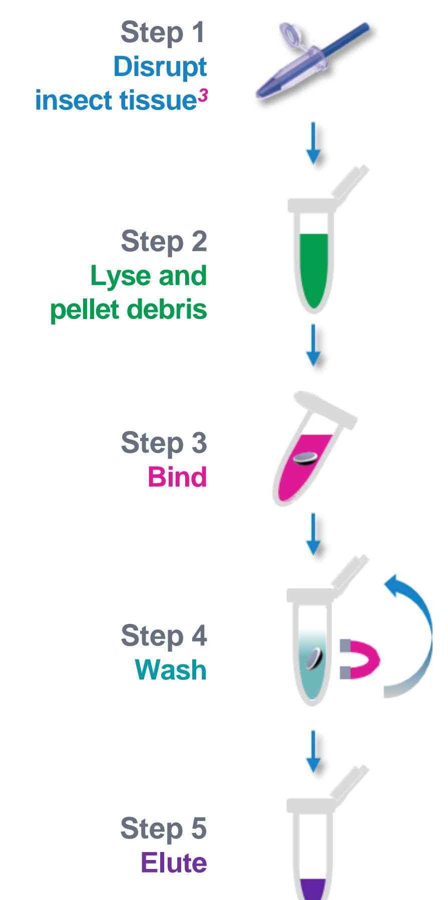
Procedure & checklist – Extracting HMW DNA from insects using the Nanobind PanDNA kit (102-377-400)

Procedure & checklist 102-377-400 describes the extraction of HMW DNA from insects and other arthropods[1] using the **Nanobind PanDNA kit** for PacBio HiFi sequencing workflows.

Sample input requirements

- Use a sample input amount (~20 – 100 mg[2]) that yields 3–30 µg of DNA (optimal input mass will vary by tissue type and by insect species)
    - Using too high of an input amount will overload lysis chemistries and negatively impact DNA yield & quality
- For large insects: Ideally use thorax body part → Remove any wings or legs
- Use pupa(e) or larva(e) stage insects → Have less chitin and typically less pigment than adults
- Use fresh or flash frozen insects → Avoid storage in ethanol for insects

If the insect is big enough to dissect into body parts, we first recommend using the thorax and then the head (but be aware that eye pigments may interfere with DNA binding during processing). Use abdomen only if there is no other option available since this body part has a higher concentration of gut microbes compared to the thorax or head. See ***Technical note – Insect DNA extraction*** (102-326-612).



Extracting HMW DNA from insects using the Nanobind PanDNA kit

PacBio

For extraction of high molecular weight genomic DNA (50 – 300+ kb) from insects and other arthropods

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Equipment and reagent list

Equipment	Model
Nanobind® PanDNA kit	PacBio® (103-260-000)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
RNase-Free Disposable Pellet Pestles	Fisher Scientific (12-141-364)
Surgical Scalpel	Fisher Scientific (22-079-712)
ThermoMixer	Eppendorf (5382000023)
Platform rocker or Mini-Tube Rotator	Thermo Scientific (M48725Q) or Fisher Scientific (05-450-127)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431081)
2.0 mL Protein LoBind microcentrifuge tubes*	Eppendorf (022431102)
Wide bore 200 µL pipette tips (optional)	USA Scientific (1011-8410)
Wide bore 1000 µL pipette tips (optional)	Thermo Scientific (2079G)
70 µm strainer (optional)	Fisher Scientific (NC1444112)
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

PacBio Documentation (102-377-400)

[1] This procedure is also suitable for DNA extraction from other types of arthropod species such as crustaceans (e.g., shrimps, crabs, lobsters, etc.)

[2] Note that the exoskeleton (largely composed of chitin, wax, and protein) may contribute significantly to sample mass but does not contain any DNA.

[3] Use a pellet pestle (do not use TissueRuptor or Dounce homogenizer) to break open exoskeleton so that inner tissue (containing DNA) will be exposed to lysis and digestion reagents.

Nanobind HMW DNA extraction procedure for RBC lysis samples

Procedure & checklist – Extracting HMW DNA from human whole blood with RBC lysis using Nanobind kits (103-377-500)

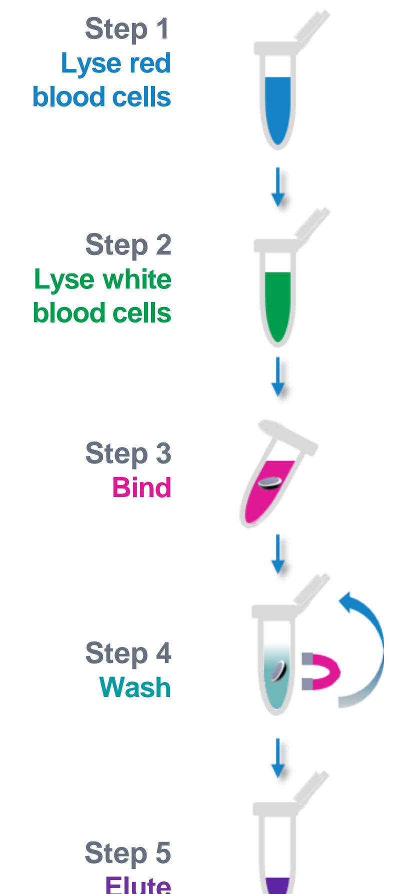
Procedure & checklist 103-377-500 describes the extraction of HMW DNA from RBC lysed human whole blood using the **Nanobind PanDNA kit** for PacBio HiFi sequencing workflows.

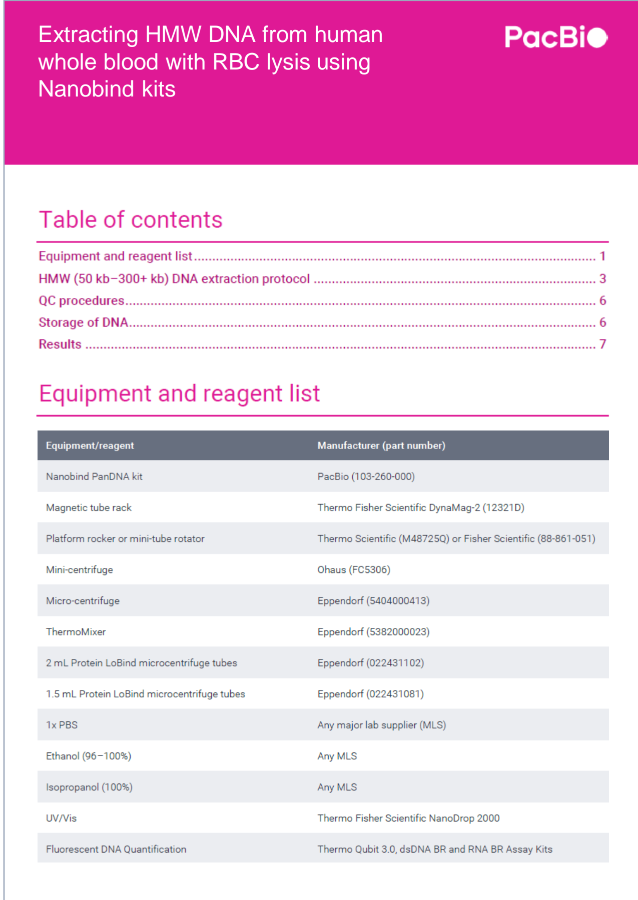
Sample input requirements

- Sample input volume: 400 µL of human whole blood → Typical DNA yield is ~3–25 µg based on donor WBC concentration
- Stored blood should be frozen as quickly as possible after being drawn
    - Storage at 4°C should be limited to 2 days or fewer to prevent sample degradation
    - Blood samples should be aliquoted to avoid repeated freeze-thaws
- No systematic difference has been observed in DNA QC or sequencing results between fresh and frozen blood samples
- For frozen blood samples, we recommend thawing at 37°C for 15 minutes
- K2 EDTA is the recommended anticoagulant[2]

Red blood cell lysis step

- Mammalian red blood cells (RBCs) typically do not contain nuclei and thus cannot be used for DNA extraction
- In RBC lysis method, RBCs are first lysed and removed from the blood sample and then DNA is extracted from the white blood cells (WBCs)
- DNA extracted using RBC lysis method allowing for extraction from higher volumes and amounts of blood without having to use large quantities of DNA extraction reagents





Extracting HMW DNA from human whole blood with RBC lysis using Nanobind kits

PacBio

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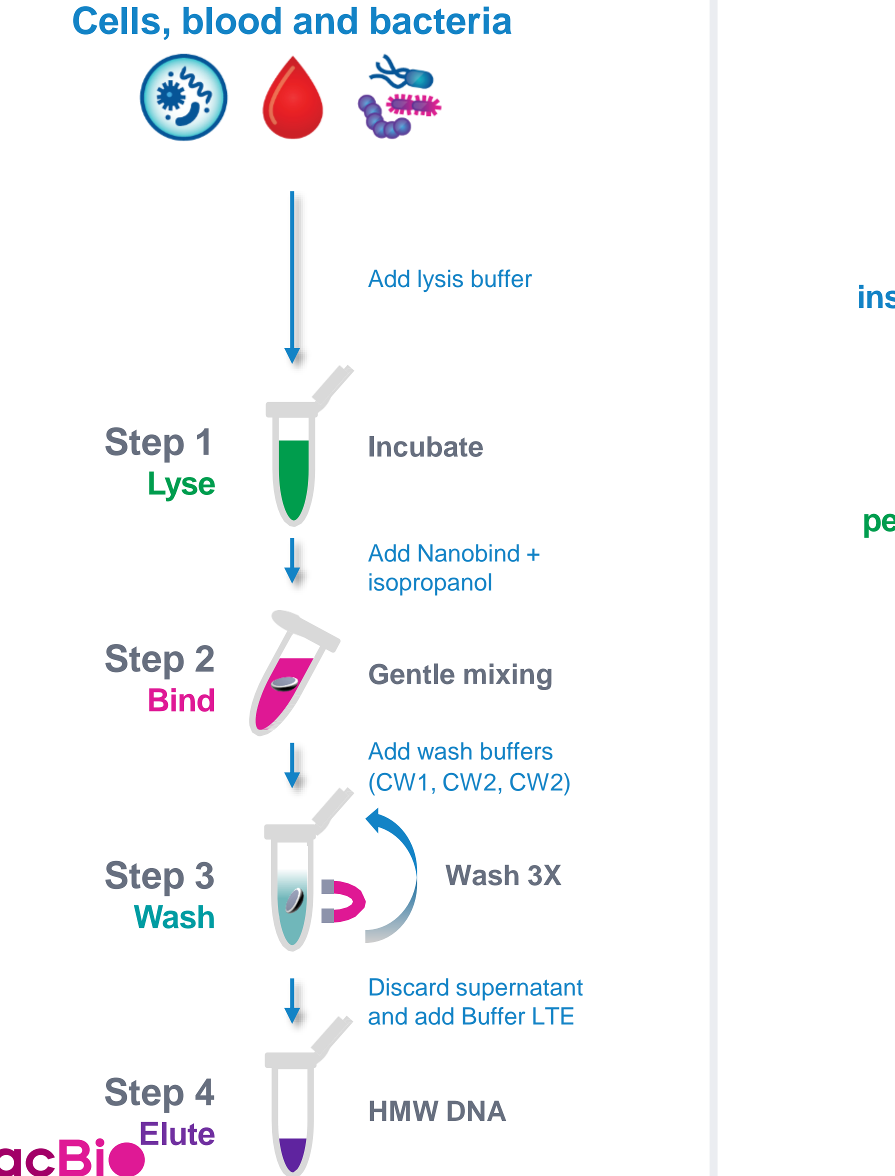
Equipment and reagent list

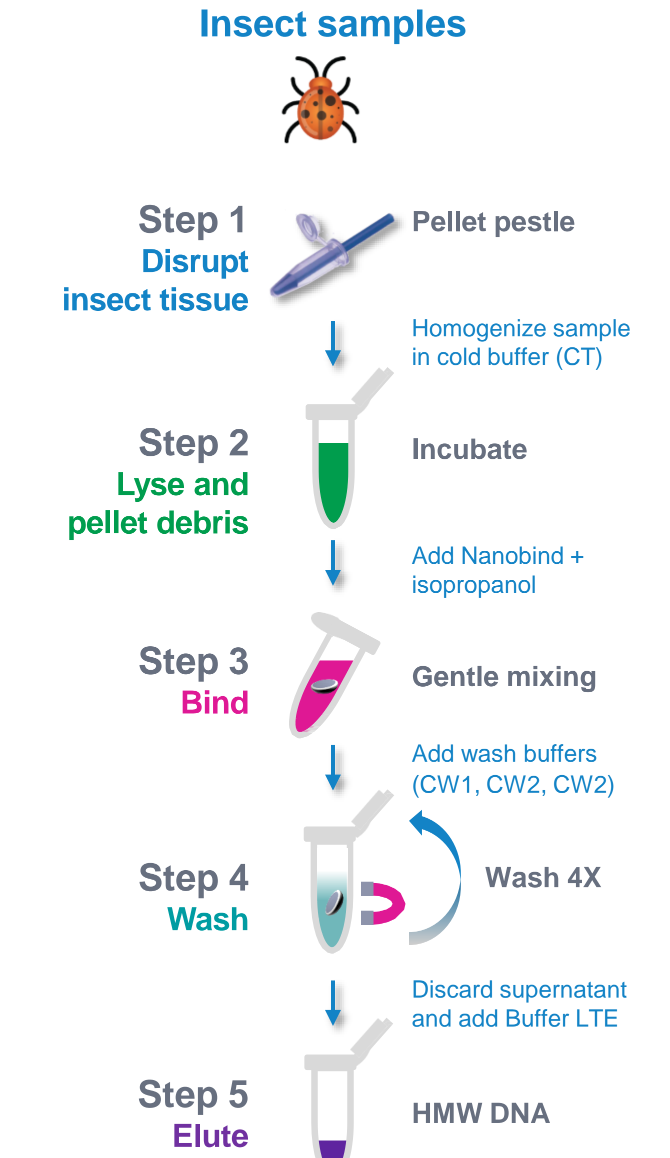
Equipment/reagent	Manufacturer (part number)
Nanobind PanDNA kit	PacBio (103-260-000)
Magnetic tube rack	Thermo Fisher Scientific DynaMag-2 (12321D)
Platform rocker or mini-tube rotator	Thermo Scientific (M48725Q) or Fisher Scientific (88-861-051)
Mini-centrifuge	Ohaus (FC5306)
Micro-centrifuge	Eppendorf (5404000413)
ThermoMixer	Eppendorf (5382000023)
2 mL Protein LoBind microcentrifuge tubes	Eppendorf (022431102)
1.5 mL Protein LoBind microcentrifuge tubes	Eppendorf (022431081)
1x PBS	Any major lab supplier (MLS)
Ethanol (96–100%)	Any MLS
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA Quantification	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits

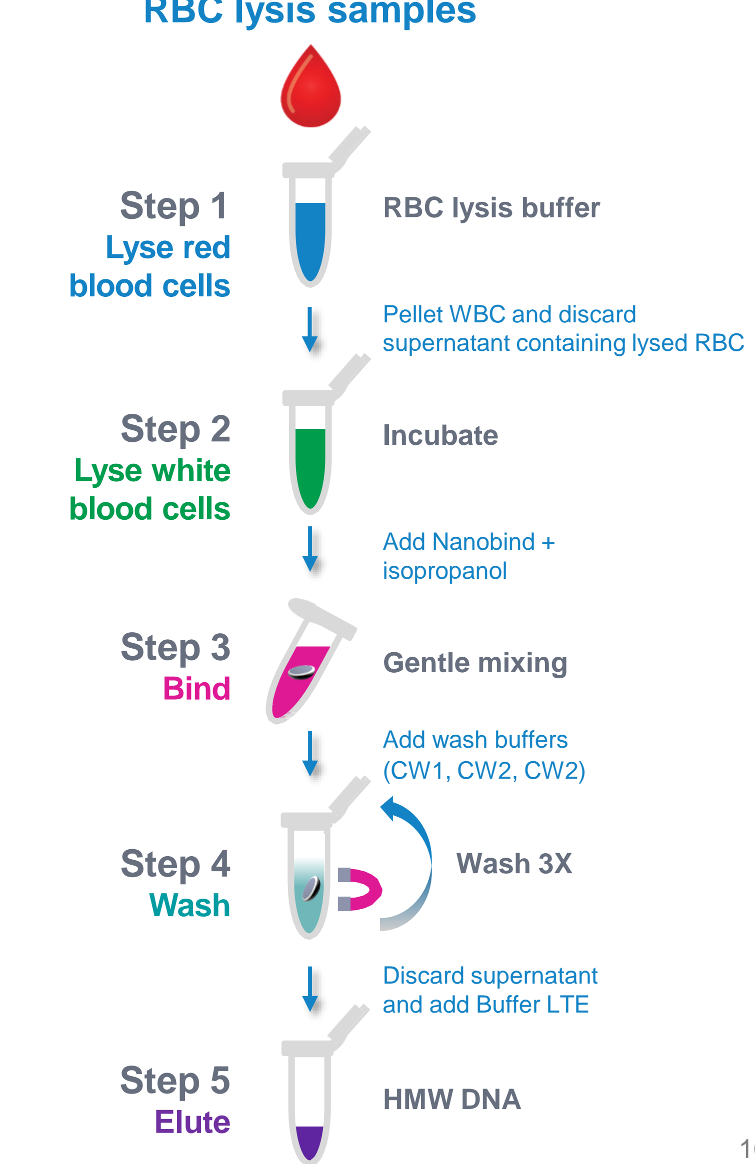
PacBio Documentation (103-377-500)

Nanobind PanDNA HMW DNA extraction workflow overview

Key DNA extraction workflow processing steps for standard Nanobind PanDNA procedures





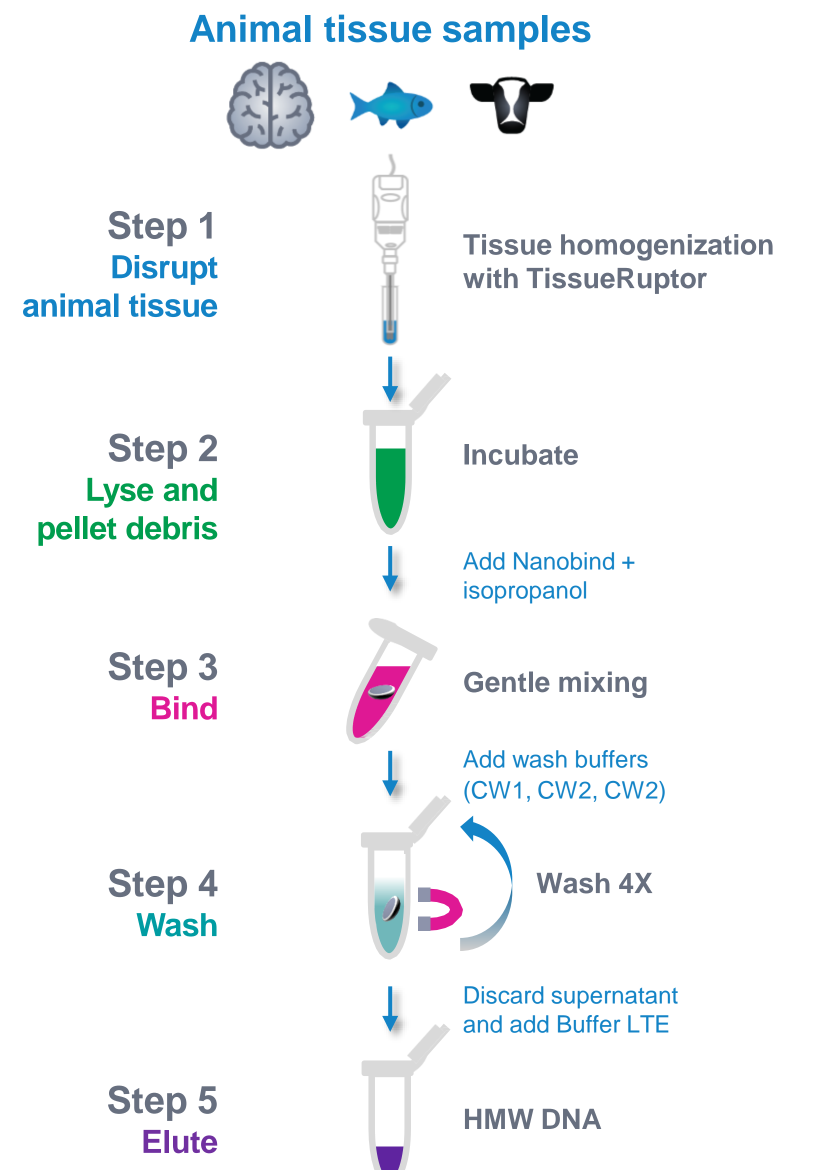


PacBio

Nanobind PanDNA HMW DNA extraction workflow overview

Key DNA extraction workflow processing steps for standard Nanobind PanDNA procedures

Animal tissue samples



Plant tissue samples

1 Plant nuclei isolation

Step 1 Disrupt plant tissue — Tissue homogenization with TissueRuptor

Step 2 Lyse and filter — Lyse ground plant tissue

Centrifuge to pellet nuclei

Step 3 Wash — Resuspend nuclei pellet and wash 3X

Centrifuge to pellet nuclei

Step 4 Pellet nuclei — Proceed to DNA extraction

2 Nanobind DNA extraction

Step 1 Lyse

Step 2 Transfer supernatant

Add Nanobind + isopropanol

Step 3 Bind — Gentle mixing in cap

Add wash buffer (PW1)

Step 4 Wash — Wash 2X

Discard supernatant and add Buffer LTE

Step 5 Elute — HMW DNA

PacBio

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).

PacBio

Nanobind reagent and sample handling best practices

Nanobind kit general best practices

Use Protein LoBind tubes

- Eppendorf Protein LoBind tubes are highly recommended for Nanobind extraction procedures to reduce protein contamination
- Protein LoBind tubes are more effective in reducing carryover contamination than DNA LoBind tubes and can result in improved UV purity

Protein LoBind tubes (Eppendorf PN 022431102 (2 mL[1]) and 022431081 (1.5 mL)) are highly recommended to reduce protein contamination from tube carryover.

Prepare wash buffer working solutions prior to starting

- Nanobind PanDNA kit wash buffers CW1, CW2 and PW1 are supplied as concentrated stocks
- CW1 and CW2 working solution contains 60% final ethanol concentration, while PW1 working solution contains a 70% final ethanol concentration
- Prepare working solutions prior to starting DNA extractions by adding the appropriate amount of ethanol (96–100%) to each buffer stock as indicated on bottles
- **Note:** Not all buffers are used in all DNA extractions protocols
  - E.g., only the plant nuclei DNA extraction protocol uses Buffer PW1

Perform cell lysis steps using a ThermoMixer

- We recommend using a ThermoMixer device for cell lysis incubation steps
- If a ThermoMixer is not available, a heat block or water bath can instead be used with periodic agitation to ensure lysis

A ThermoMixer (Eppendorf PN 5382000023) is recommended for cell lysis incubation steps in Nanobind DNA extraction procedures.

Follow kit storage recommendations

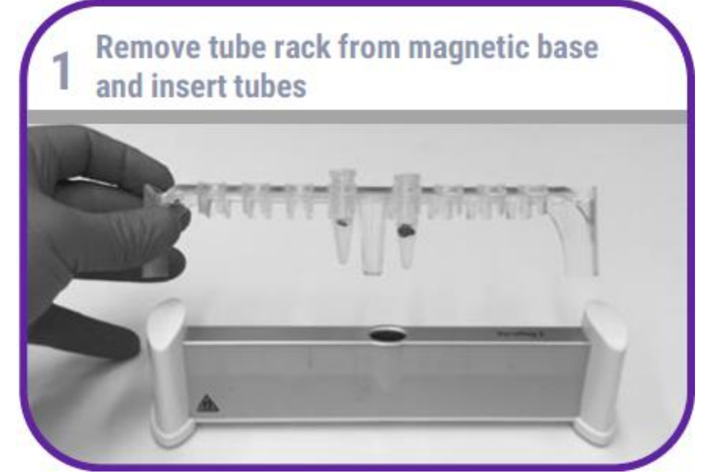
- Store Buffer CT, Buffer RBC 10X, & RNase A at 4°C
- Store Nanobind disks and all other buffers at room temperature (RT, 18–25°C)
- Buffer NPL may form precipitates if stored at <RT
  - If this happens, precipitates will return to solution when stored at RT
  - Alternatively, buffer can be warmed in a water bath to re-dissolve precipitates

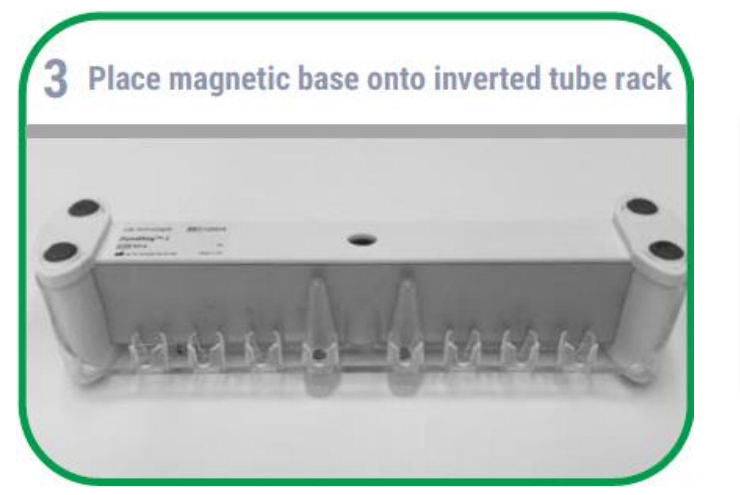
[1] **Note:** For selected protocols (e.g., ***Procedure & checklist – Extracting HMW DNA extraction from insects the Nanobind PanDNA kit*** (102-377-400)), using the 2 mL tube size is essential for efficient lysis of samples.

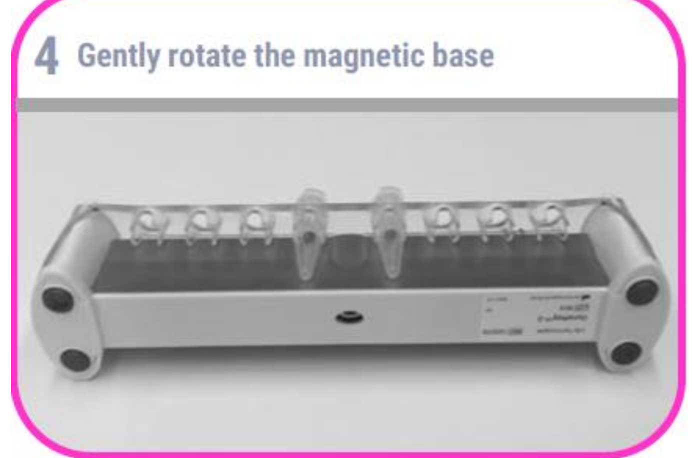
Nanobind kit general best practices (cont.)

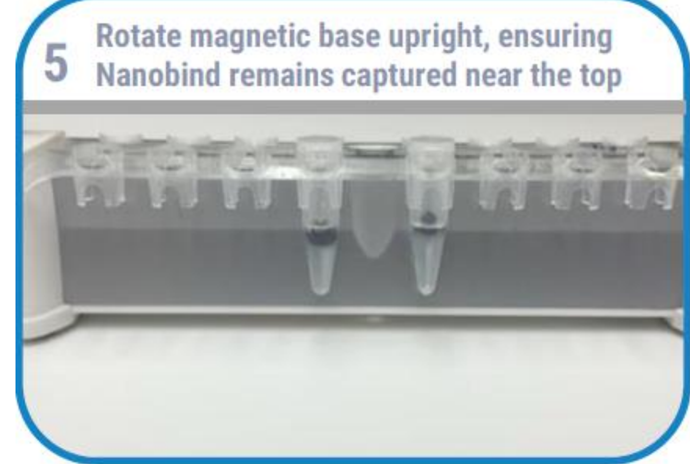
Recommended magnetic rack handling procedure for Nanobind kits

Thermo Fisher Scientific DynaMag-2 (12321D) is recommended for Nanobind DNA extraction procedures





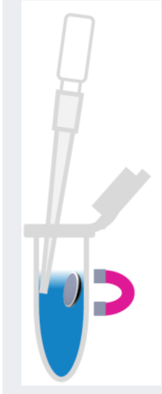




Recommended procedure for capturing Nanobind disk on a tube rack and magnetic base. This procedure ensures that the Nanobind disk is captured near the top of the liquid interface, minimizing disturbance of the bound DNA and facilitating processing.

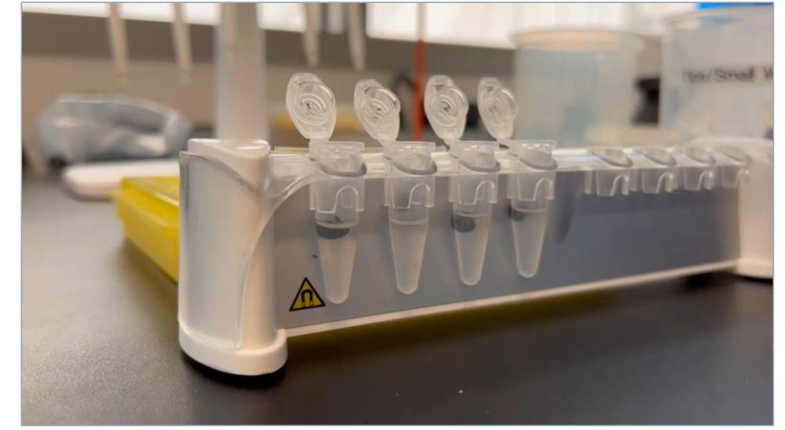
Nanobind kit general best practices (cont.)

Recommended pipetting procedure for Nanobind kits



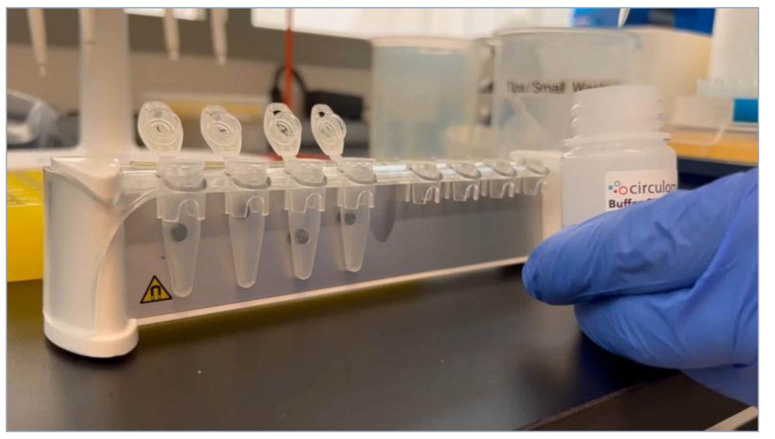
Removing liquid from microcentrifuge tubes containing a Nanobind disk

- Nanobind disk should not be disturbed
- Carefully insert pipette tip against the wall opposite to the Nanobind disk and remove liquid by pipetting from the liquid surface
  - → This will minimize the chances of accidentally pipetting bound DNA



Adding liquid to microcentrifuge tubes containing a Nanobind disk

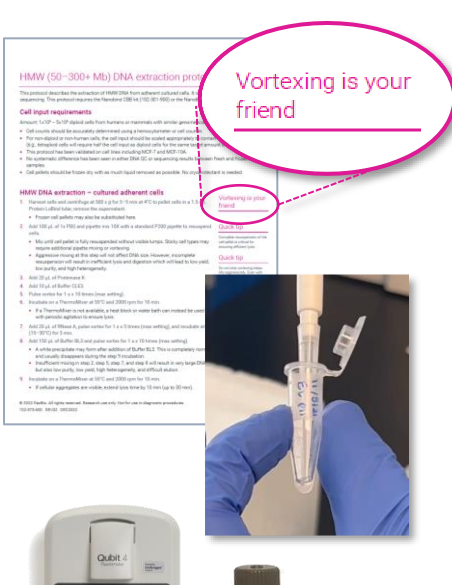
- Nanobind disk should not be disturbed
- Dispense liquid against the wall opposite the Nanobind disk.



Nanobind kit general best practices (cont.)

High-molecular weight DNA heterogeneity and viscosity considerations

- Extracted HMW DNA can be highly viscous and heterogeneous – *This is normal and is one of the challenges of working with HMW DNA*
- 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
- Use aggressive mixing during DNA extraction steps to minimize the challenges of heterogeneity and viscosity



Use aggressive mixing during cell resuspension and lysis steps to improve extracted DNA sample purity

- 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 and homogeneity due to more efficient lysis and digestion[1]

Pipette mix to help bring viscous extracted DNA samples into solution

- Pipette mix the extracted DNA 5–10X with a standard P200 pipette to help loosen and coax 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

Measure extracted DNA sample concentration using a Qubit BR dsDNA assay kit

- Pipette mix the DNA 5X with a standard P200 pipette and perform triplicate DNA concentration readings by sampling the top, middle, and bottom of the eluate
- To accurately determine the concentration of dsDNA, we recommend using a Qubit dsDNA assay kit[2]

[1] Note: Aggressive mixing during lysis will not significantly impact DNA fragment length.

[2] We recommend using 2 µL of DNA sample for quantification using the Qubit Broad Range dsDNA assay kit and performing triplicate Qubit dsDNA BR Assay measurements from top, middle, and bottom of tube to determine DNA concentration.

PacBio

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows

Lysis procedure

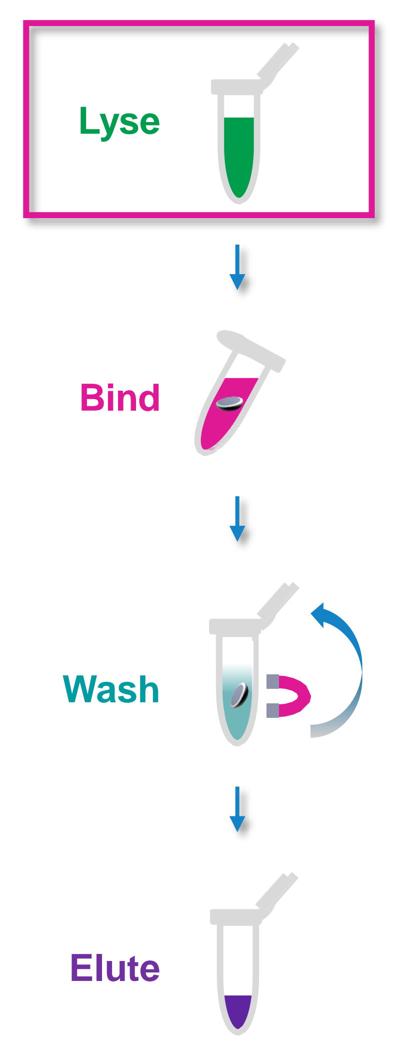
Lyse → Bind → Wash → Elute

Example lysis workflow for processing cultured cells, blood & bacterial samples

Lysis workflow	Step	Processing tips	Demonstration
1. Resuspend cells	1. Add PBS buffer 2. Pipette mix 10X with standard P200 pipette to resuspend cells	• Be aggressive in resuspending cells! Complete resuspension of the cell pellet is critical for ensuring efficient lysis • DNA will not become sheared at this step • Pipette more than 10X if necessary to properly resuspend • Proper resuspension is critical to ensure efficient lysis – especially important for sticky cells (some bacteria, fibroblasts, white blood cells)	
2. Proteinase K digestion	1. Add Proteinase K 2. Add other required reagents if needed (e.g., Buffer CLE3) 3. Pulse vortex (10 x 1 s at max setting) 4. Incubate on ThermoMixer (55°C, 900 rpm, 10 min)	• Be aggressive with vortexing – vortexing is critical to ensure efficient lysis! • Do not skip vortexing steps → Mix aggressively; even with aggressive vortexing, the DNA will be hundreds of kilobases in length • We encourage customers to err on the side of being overly aggressive • We strongly recommend using a ThermoMixer for incubations	
3. RNase A digestion	1. Add RNase A 2. Pulse vortex (5 x 1 s at max setting) 3. Incubate at RT for 3 min	• Ribonuclease A specifically digests RNA (but not DNA) • We recommend performing RNase A digestion for all sample types • Be aggressive with vortexing – even with aggressive vortexing, the DNA will be hundreds of kilobases in length	
4. Lysis	1. Add Buffer BL3. 2. Pulse vortex (10 x 1 s at max setting) 3. Incubate on ThermoMixer (55°C, 900 rpm, 10 min)	• Be aggressive with vortexing – vortexing is critical to ensure efficient lysis! • Do not skip vortexing steps → Foaming is normal at this step • We encourage customers to err on the side of being overly aggressive • We strongly recommend using a ThermoMixer for incubations • Check lysate for unlysed material following incubation → Extend lysis time if necessary	

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

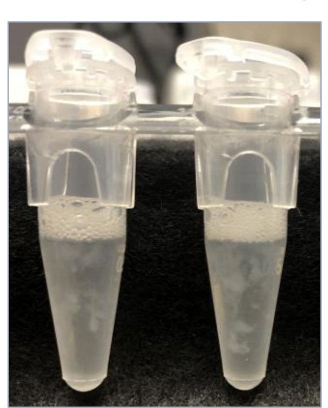
Lysis procedure



Clear lysate→ Successful lysis

- Clear, homogeneous lysate
- No visible "cloud" or flakes

Uneven, cloudy lysate → Incomplete lysis



- Inhomogeneous, cloudy patches

Example Gram-positive bacterial lysates

Listeria monocytogenes

- Lysis readily in lysozyme
- Clear lysate
- No visible "cloud" or flakes

Efficient lysis

***Staphylococcus aureus*[1]**

- Does not lyse in lysozyme
- Very cloudy
- Will not yield usable DNA

Poor lysis

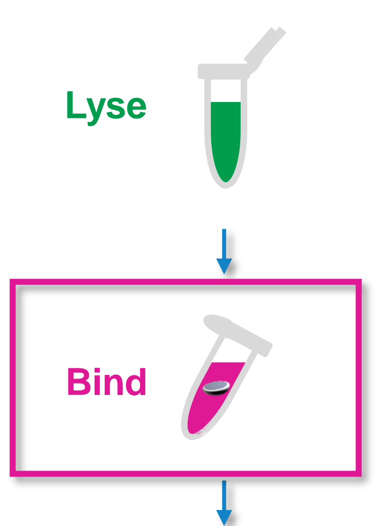
- Lysis readily in lysostaphin
- Clear lysate
- No visible "cloud" or flakes

Efficient lysis

[1] For some Gram-positive bacteria, such as *S. aureus*, lysostaphin (Sigma-Aldrich #L7386) should be substituted for lysozyme at 0.15 mg/mL final concentration in ***Procedure & checklist – Extracting HMW DNA from Gram-positive bacteria using Nanobind kits*** (102-573-900).

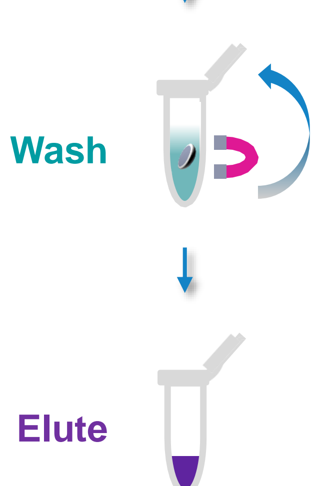
Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

Binding procedure



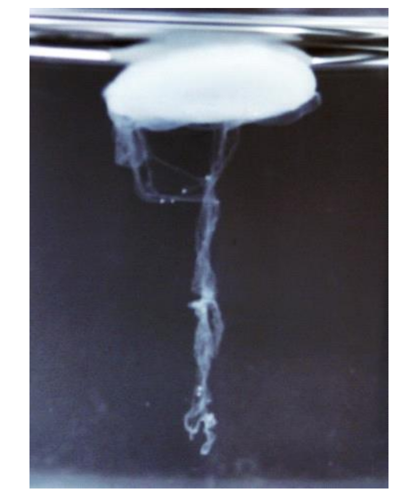
Example binding workflow for processing cultured cells, blood & bacterial samples

Binding workflow	Step	Processing tips	Demonstration
1. Bind DNA to Nanobind disk	1. Add Nanobind disk to lysate and then add isopropanol (IPA). 2. Inversion mix by hand 5X 3. Mix on tube rotator (9 rpm, RT, 15 min)	• Nanobind disk must be added **before** adding isopropanol • Invert mix by hand after addition of IPA • End-over-end mixing is recommended over rocking or shaking mixing • In some cases, can immediately start to see DNA binding to the Nanobind disk.	



Example images of DNA visibly bound to Nanobind disk

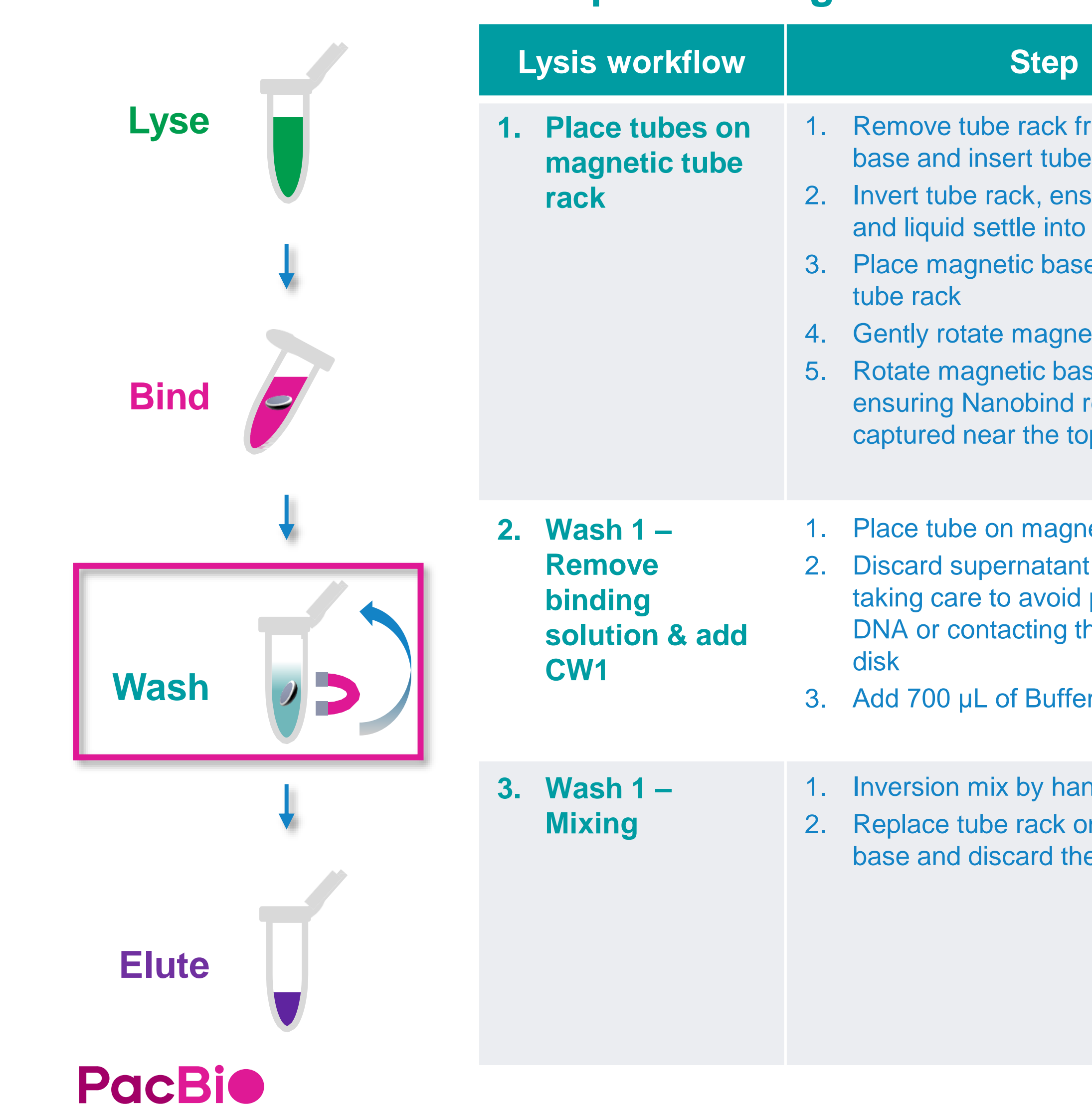
DNA yield



- **Note:** Actual amount of visible DNA bound to Nanobind disk will depend on sample input starting amount and DNA recovery efficiency
- For many sample types, **when starting with lower sample input amounts the bound DNA may not be clearly visible as a cloud on the disk** (but Qubit dsDNA assay shows that DNA is indeed present)

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

Washing procedure



Example washing workflow for processing cultured cells, blood & bacterial samples

Lysis workflow	Step	Processing tips	Demonstration
1. Place tubes on magnetic tube rack	1. Remove tube rack from magnetic base and insert tubes 2. Invert tube rack, ensuring Nanobind and liquid settle into lid 3. Place magnetic base onto inverted tube rack 4. Gently rotate magnetic base 5. Rotate magnetic base upright, ensuring Nanobind remains captured near the top	• To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that can be separated from the 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	
2. Wash 1 – Remove binding solution & add CW1	1. Place tube on magnetic tube rack. 2. Discard supernatant with a pipette, taking care to avoid pipetting the DNA or contacting the Nanobind disk 3. Add 700 µL of Buffer CW1	• Avoid accidental pipetting of DNA bound to Nanobind disk • Pipette against the front wall of tube to remove liquid • Dispense wash buffer against the front wall of tube	
3. Wash 1 – Mixing	1. Inversion mix by hand 4X 2. Replace tube rack on the magnetic base and discard the supernatant	• Remove tubes from magnetic rack • Completely invert tubes to fully wash disk and tube • Do not need to be overly careful, but do not vigorously shake • Loss of DNA during this mixing step is possible but is very rare – usually occurs when sample is very dirty or lysis was inefficient.	

PacBio

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

Washing procedure

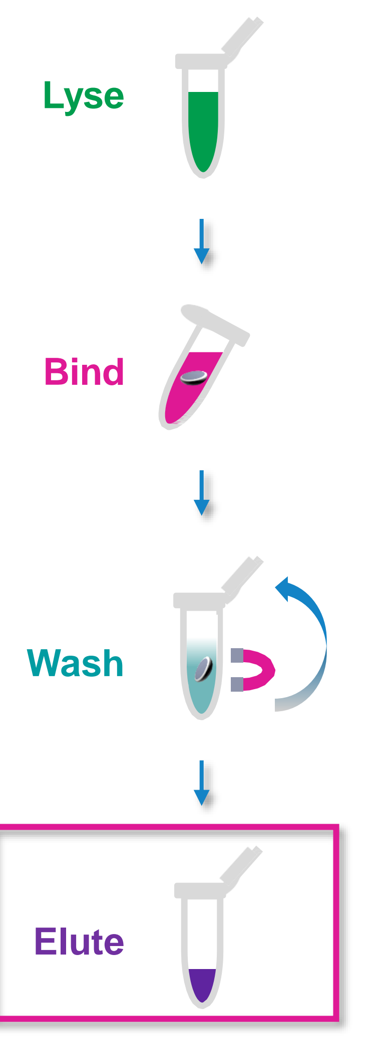
Lyse → Bind → **Wash** → Elute

Example washing workflow for processing cultured cells, blood & bacterial samples (cont.)

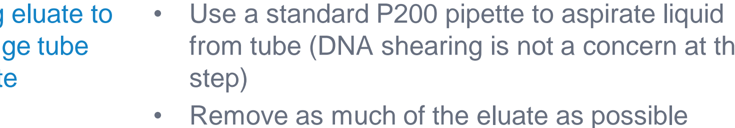
Lysis workflow	Step	Processing tips	Demonstration
4. Wash 2 – Mixing	1. Add Buffer CW2 2. Remove tube rack from magnetic base, inversion mix 4X, replace tube rack on the magnetic base, and discard the supernatant	• Same key points as previous step (Wash 1) • DNA will tighten onto disk after addition of CW1 → less likely to accidentally pipette DNA	
5. Wash 3 – Mixing	1. Add Buffer CW2 2. Remove tube rack from magnetic base, inversion mix 4X, replace tube rack on the magnetic base, and discard the supernatant	• Same key points as previous step (Wash 2)	
6. Remove residual Liquid	1. Quick-spin the tube on a mini-centrifuge for 2 s 2. With the tube rack already on the magnetic base and right-side-up, place tube on tube rack and remove residual liquid	• Remove as much liquid as possible • Do not air dry • Do not allow disk to over-dry • Repeat if necessary to remove excess liquid	

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

Elution procedure

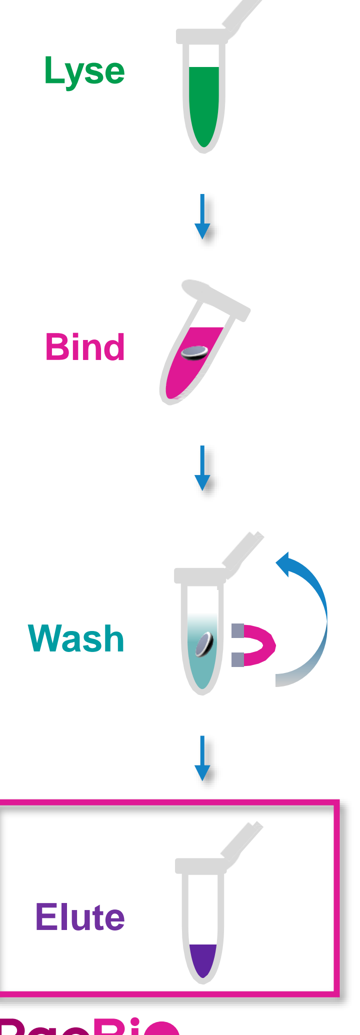


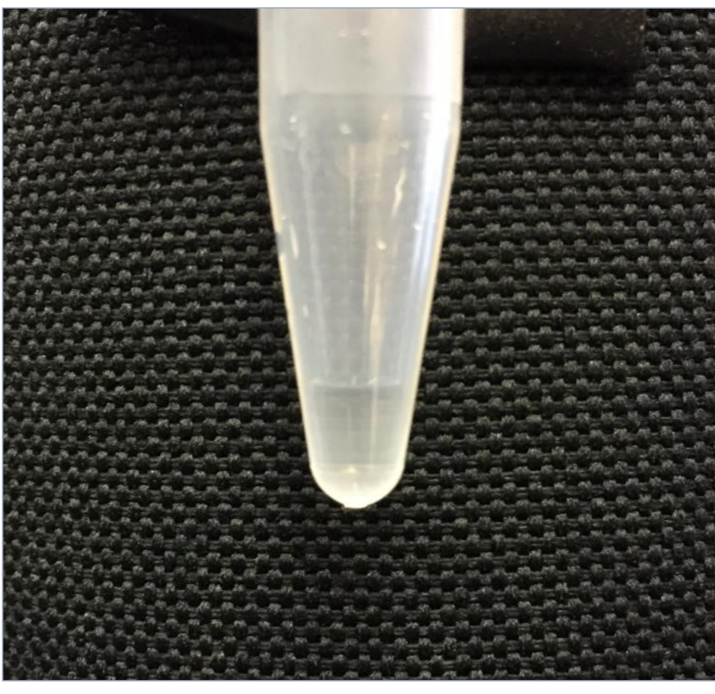
Example elution workflow for processing cultured cells, blood & bacterial samples (cont.)

Lysis workflow	Step	Processing tips	Demonstration
1. Elution	1. Add Buffer EB directly onto the Nanobind disk 2. Incubate at RT for 10 min on benchtop	• Add EB directly on the Nanobind disk • Quick-spin in a mini-centrifuge to remove any air bubbles • 75 µL is the recommended minimum elution volume • It is not necessary to incubate for longer than 10 min	
2. Remove eluate	1. Collect DNA by transferring eluate to a new 1.5 mL microcentrifuge tube with a standard P200 pipette	• Use a standard P200 pipette to aspirate liquid from tube (DNA shearing is not a concern at this step) • Remove as much of the eluate as possible 	
3. Spin to recover remaining DNA	1. Spin the tube containing the Nanobind disk on a micro-centrifuge at 10,000 x *g* for 15 s	• After spinning, use a standard P200 pipette to aspirate liquid from tube • Remove as much liquid as possible • Eluate may be viscous and difficult to remove from the Nanobind disk → if needed, can use tip of pipette to scrape off and collect any DNA adhering to tube walls • Repeat spin if necessary	

Nanobind processing tips for core lysis, bind, wash & elute procedures in standard HMW DNA extraction workflows (cont.)

Elution procedure





Example image of HMW DNA eluate. The appearance of the eluate solution should be clear.

- 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 at this stage will not significantly impact DNA fragment length
    - Pipette mixing is a standard part of our Nanobind DNA elution process
- For greater accuracy, the pipette mixed DNA should be left overnight at RT before quantifying the concentration using a Qubit dsDNA assay kit

Nanobind processing tips for HMW DNA extraction from animal tissues

Animal tissue disruption methods

- We recommended using a TissueRuptor (QIAGEN) tool for animal tissue homogenization
- If a TissueRuptor tool is unavailable, then a Dounce homogenizer may be used
  - We do not recommend alternative disruption methods such as liquid nitrogen (LN2) grinding for animal tissues since these methods do not consistently and sufficiently disrupt tissue samples, leading to decreased yields, reduced purity, and diminished sequencing performance
- TissureRuptor disruption or Dounce homogenization methods can be used on fresh frozen, ethanol-preserved, and RNAlater-preserved tissue samples → **Note:** Tissues preserved in EtOH prior to freezing or storage require pre-treatment before extraction to remove EtOH
- Animal tissue samples should be finely minced with a scalpel prior to disruption and aliquoted to avoid repeated freeze-thaws

Recommended animal tissue disruption method

TissueRuptor[1]
(QIAGEN)

- Recommend using a tissue sample input mass (~2 – 100 mg) that will yield 3−30 µg of extracted DNA
  → This will vary by tissue type and by animal species)
- Use the TissueRuptor on its maximum power setting
- 10 seconds should be sufficient for all tissues as long as the sample was adequately minced beforehand

Note: For **insect** samples, we recommend using a pellet pestle[3]

Alternative animal tissue disruption method <u>if</u> TissueRuptor tool is unavailable

Dounce homogenizer[2]
(with tight pestle)

- 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, try to keep the tissue between the tip of the pestle and the bottom of the Dounce chamber for thorough homogenization.

[1] See *Procedure & checklist – Extracting HMW DNA from animal tissue* (102-574-600).

[2] See *Procedure & checklist – Extracting HMW DNA from animal tissue using Dounce homogenization with Nanobind kits* (102-573-700)
[3] See *Procedure & checklist – Extracting HMW DNA from insects using the Nanobind PanDNA kit* (102-377-400)

Nanobind processing tips for HMW DNA extraction from plant tissues

Plant tissue disruption methods

- We recommended using a TissueRuptor (QIAGEN) tool for plant tissue homogenization during upstream nuclei isolation step
- Liquid nitrogen (LN2) disruption may optionally be used if a TissueRuptor tool is unavailable
  - ***Important!*** If using LN2 disruption method, then manual grinding MUST be performed for **25 – 30 minutes**[2] to achieve expected DNA yields and purity
- TissueRuptor disruption method is faster and typically results in higher extraction yields than LN2 grinding
  - We recommend that users start with the TissueRuptor protocol
- TissueRuptor disruption or LN2 grinding methods can be performed using fresh or frozen plant tissues
- Young leaves, preferably grown in a greenhouse or growth chamber, will produce the highest quantity and quality of DNA
  - Mature plants grown in more natural environments tend to require larger tissue inputs and more wash steps to obtain equivalent DNA yields and purity

Recommended plant tissue disruption method

TissueRuptor[1]
(QIAGEN)

- Maximum plant tissue mass that can be processed in one 50 mL conical using TissueRuptor is 4 g

Alternative plant tissue disruption method <u>**if**</u> TissueRuptor tool is unavailable

Liquid nitrogen (LN2) disruption[2]
(in LN2-cooled mortar)

- Maximum plant tissue mass we recommend processing at once is 5 g

[1] See ***Procedure & checklist – Isolating nuclei from plant tissue using TissueRuptor disruption*** (102-574-900).
[2] See ***Procedure & checklist –Isolating nuclei from plant tissue using LN2 disruption*** (102-574-800).

Nanobind QC procedures

We recommended that QC be performed on extracted DNA samples after the DNA has been allowed to rest at RT overnight and appears homogeneous under visual examination and when pipetting

QC measurement	QC tool	QC procedure	QC notes
DNA purity and total nucleic acid concentration		Perform triplicate NanoDrop UV/VIS measurements from top, middle, and bottom of tube to determine total nucleic acid concentration as well as purity (A260/A280, A260/230)	• HMW DNA is inherently difficult to work with as viscosity and inhomogeneity are often issues • We recommend taking at least three measurements, sampling from the top, middle, and bottom of the tube, to get an accurate concentration reading • We typically see concentration %CV values of <20%; however, if the DNA is very large, the %CV can exceed 30–40%[1]
DNA concentration		Perform triplicate Qubit dsDNA BR Assay measurements from top, middle, and bottom of tube to determine DNA concentration	• We recommend taking the average of multiple measurements to ensure an accurate DNA concentration reading • We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the dsDNA BR assay kit
RNA concentration		Perform a single Qubit RNA BR Assay measurement to determine RNA concentration (optional)	• We recommend taking a single measurement to get an approximate RNA concentration reading • We recommend the Qubit 3.0 (Thermo Fisher Scientific) with the RNA BR assay kit
DNA size distribution		Use Agilent Femto Pulse for HMW DNA size QC	• We recommend diluting the sample to 250 pg/μL • Follow Agilent instructions for diluting the sample • Use the Genomic DNA 165 kb Kit (Agilent Technologies) for unsheared gDNA

[1] If the DNA is very heterogeneous or contains large amounts of unsolubilized "jellies", refer to the Nanobind Guide & overview "Heterogeneity and viscosity" and individual Nanobind DNA extraction protocol '**Troubleshooting FAQ**' sections.

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] 260/280	Example absorbance ratio[1] 260/230	Expected DNA size range	Notes
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.

PacBio

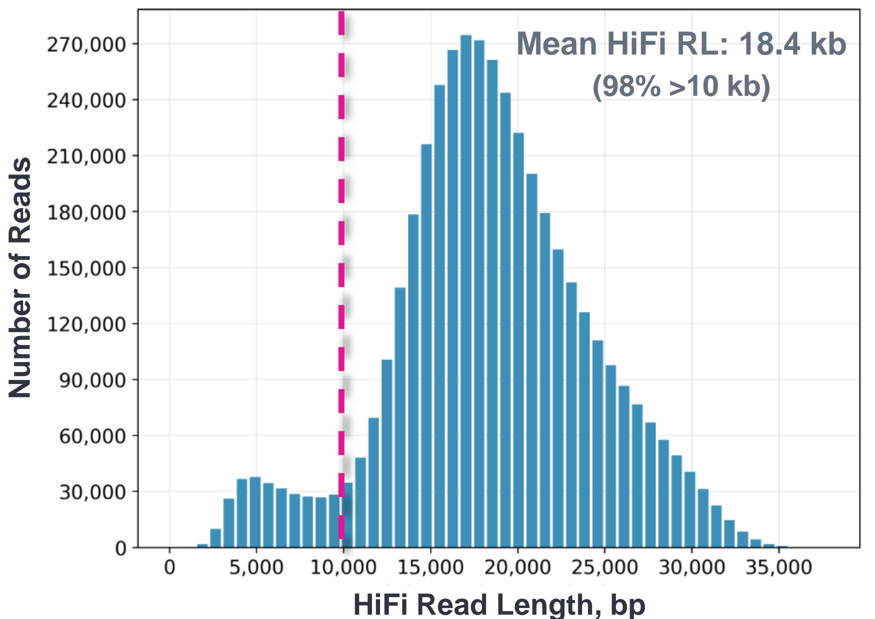
Short read eliminator (SRE) kit configuration and workflow overview

Short read eliminator (SRE) kits for genomic DNA sample cleanup for PacBio HiFi sequencing

PacBio Short read eliminator kits[1] can be used can be used for rapid high-pass size selection of gDNA samples

- **SRE kit (102-208-300)** can be used for easy and rapid size selection of (unsheared) HMW gDNA samples[2] prior to HiFi library preparation
- Uses a simple centrifugation procedure similar to standard ethanol precipitation techniques

Short read eliminator (SRE) kit (102-208-300) contains reagents for removal of DNA fragments **<10 kb** using size-selective precipitation.



Example HiFi read length distribution profile for a HG002 WGS library prepared by performing selection using the SRE kit (102-208-300) on the unsheared starting input HMW DNA.

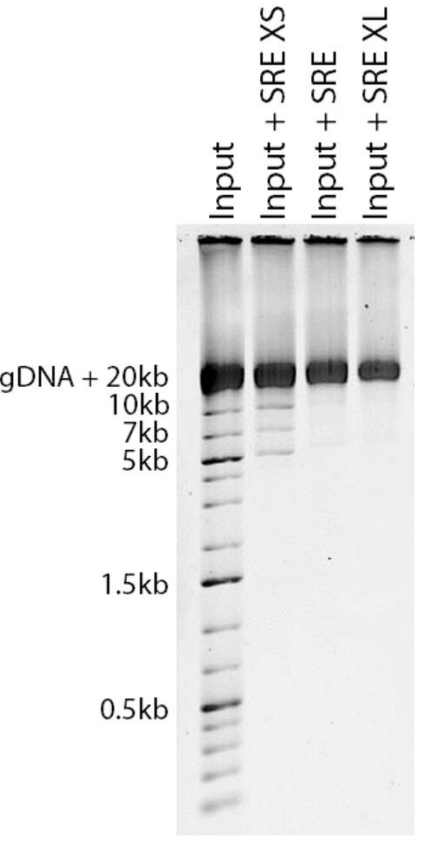
SRE kit (102-208-300) can significantly enhance mean HiFi read lengths by depleting short DNA fragments <10 kb

[1] See **Guide & overview – Nanobind short read eliminator kit family** (102-582-400) for recommended use cases and input DNA quality & DNA concentration requirements.
[2] SRE kit is recommended for HiFi library prep workflows – see ***Procedure & checklist – Removing short DNA fragments with the Short Read Eliminator (SRE) kit*** (102-982-300)

Short read eliminator kit options

SRE kit (102-208-300) is the recommended default kit for PacBio HiFi whole genome sequencing workflows

SRE kit	DNA depletion range	DNA input[1]	Recovery efficiency	Notes
SRE XS (24 rxn) (102-208-200)	Progressive depletion: <10 kb Near complete depletion: <5 kb	60 µL 25–150 ng/µL	50–90%	• Suitable for use on sheared or fragmented input DNA and long amplicons • Can also be used for treating degraded input DNA samples • Process up to 24 samples in 2 hrs (10 min hands-on time)
SRE (24 rxn) (102-208-300)	Progressive depletion: <25 kb Near complete depletion: <10 kb	60 µL 10–150 ng/µL	50–70%	• Requires high-quality HMW (unsheared) DNA (50% ≥30 kb) • **Recommended for PacBio HiFi WGS workflows** • Process up to 24 samples in 2 hrs (10 min hands-on time)
SRE XL (24 rxn) (102-208-400)	Progressive depletion: <40 kb Near complete depletion: <10 kb	60 µL 50–150 ng/µL	40–50%	• Requires very high quality HMW (unsheared) DNA (>>48 kb) • Only for ultra-long DNA use cases (not recommended for PacBio HiFi WGS workflows) • Process up to 24 samples in 2 hrs (10 min hands-on time)



1% Agarose gel separation of size-selected DNA with size cutoffs demonstrated using a spiked-in ladder.[2] Input is 50 ng/µL gDNA extracted from GM12878 cells using Nanobind CBB kit + 20 ng/µL ladder.

[1] **Note:** SRE kits should be used on starting genomic DNA material – NOT on final SMRTbell libraries.

[2] Ladder spike-in = Thermo Scientific GeneRuler 1 kb Plus Ladder (#SM1334).

SRE DNA size selection workflow overview

SRE kits use a fast and easy centrifugation-based procedure similar to standard ethanol precipitation techniques

Step 1 – Add Buffer SRE

HMW gDNA

Step 2 – Centrifuge

Discard supernatant and add Buffer EB

Step 3 – Resuspend

Size-selected HMW DNA

HMW DNA + 1 kb Plus Ladder

HMW DNA + 1 kb Plus Ladder + SRE XS kit

500bp

1.5kbp

5kbp

10kbp

20kbp

Example performance of the Short read eliminator XS kit on HMW DNA spiked with 1 kb Plus Ladder.[1] DNA <5 kb in length is removed to trace levels as detected by gel or a Femto Pulse system.

[1] Ladder spike-in = Thermo Scientific GeneRuler 1 kb Plus Ladder (#SM1334).

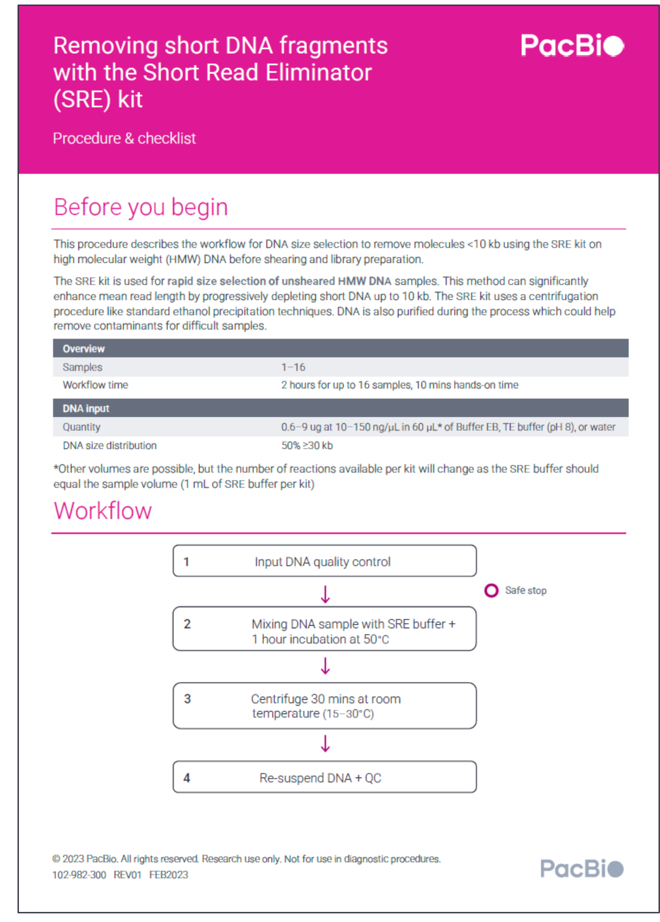
Short Read Eliminator (SRE) kit procedure description

Procedure & checklist – Removing short DNA fragments with the Short Read Eliminator (SRE) kit (102-982-300) describes the workflow for DNA size selection to remove molecules <10 kb using the SRE kit on high molecular weight (HMW) DNA before shearing and library preparation.

Overview	
Samples	1 – 24
Workflow time	2 hours for up to 24 samples, 10 mins hands-on time
DNA input	
Quantity	0.6–9 ug at 10–150 ng/µL in 60 µL* of Buffer EB, TE buffer (pH 8), or water
DNA size distribution	50% ≥30 kb

* Other volumes are possible, but the number of reactions available per kit will change as the SRE buffer should equal the sample volume (1 mL of SRE buffer per kit)

- SRE kit is used for rapid size selection of unsheared HMW gDNA samples
- SRE method can significantly enhance mean read length by depleting short DNA up to 10 kb[1]
- SRE kit uses a centrifugation procedure similar to standard ethanol precipitation techniques
- DNA is also purified during the SRE procedure, which can help remove contaminants for "difficult" samples



Removing short DNA fragments with the Short Read Eliminator (SRE) kit

PacBio

Procedure & checklist

Before you begin

This procedure describes the workflow for DNA size selection to remove molecules <10 kb using the SRE kit on high molecular weight (HMW) DNA before shearing and library preparation.

The SRE kit is used for **rapid size selection of unsheared HMW DNA** samples. This method can significantly enhance mean read length by progressively depleting short DNA up to 10 kb. The SRE kit uses a centrifugation procedure like standard ethanol precipitation techniques. DNA is also purified during the process which could help remove contaminants for difficult samples.

Overview	
Samples	1–16
Workflow time	2 hours for up to 16 samples, 10 mins hands-on time
DNA input	
Quantity	0.6–9 ug at 10–150 ng/µL in 60 µL* of Buffer EB, TE buffer (pH 8), or water
DNA size distribution	50% ≥30 kb

*Other volumes are possible, but the number of reactions available per kit will change as the SRE buffer should equal the sample volume (1 mL of SRE buffer per kit)

Workflow

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102-982-300 REV01 FEB2023

PacBio

PacBio Documentation (102-982-300)

[1] Short read eliminator (SRE) kit (102-208-300) contains reagents for achieving near complete depletion of DNA <10 kb and progressive depletion of DNA <25 kb using size-selective precipitation.

SRE kit DNA size selection workflow timing overview

Procedure & checklist – Removing short DNA fragments with the Short Read Eliminator (SRE) kit (102-982-300)

Step	SRE step	DNA QC	Walk-away time
1 Input DNA quality control	N/A	Qubit dsDNA HS assay Femto pulse gDNA165 kb kit	N/A
Safe stop			
2 Mix SRE buffer with DNA sample	Add SRE buffer to DNA sample	N/A	1.0 hr
3 Centrifugation	Centrifuge at 10,000 x *g* (RT)	N/A	0.5 hrs
4 Re-suspend DNA + QC	Resuspend DNA in Buffer EB	Qubit dsDNA HS assay Femto Pulse gDNA 165 kb kit	20 min

~2 hrs[1] (steps 2–4)

[1] **Excludes** hands-on time for setting up reaction steps and additional time required to perform DNA sizing QC and DNA concentration QC.

SRE kit general best practices

Input DNA quantity and quality recommendations

- Start with an input DNA concentration between 10–150 ng/µL (as determined by a Qubit assay[1]) in 60 µL
  - This represents a total input DNA mass amount from 0.6–9.0 µg
  - Starting DNA sample must be in Buffer EB (or Buffer LTE), TE buffer (pH 8) or water[2]
- At least 50% of DNA should be ≥30 kb, as measured on the Femto Pulse system
  - This corresponds to a genome quality number (GQN) of 5.0 or higher with a 30 kb cutoff
- Post-SRE recovery yield should be ~50% or higher

Input DNA heterogeneity and viscosity considerations

- SRE kit recovery efficiency and size selection performance depends on input DNA being homogeneous and fully in solution
- Sample homogeneity can be evaluated by performing triplicate DNA concentration measurements using a Qubit dsDNA assay and verifying that the concentration CV is <20%
- If HMW DNA sample is inhomogeneous, we recommend needle shearing 5-10X with a 26G needle and then allowing DNA to rest at room temperature overnight before beginning SRE size selection procedure

Reagent and sample handling

- Eppendorf DNA LoBind tubes (Eppendorf PN 022431021) are recommended for SRE kit applications
- All buffers should be stored at room temperature

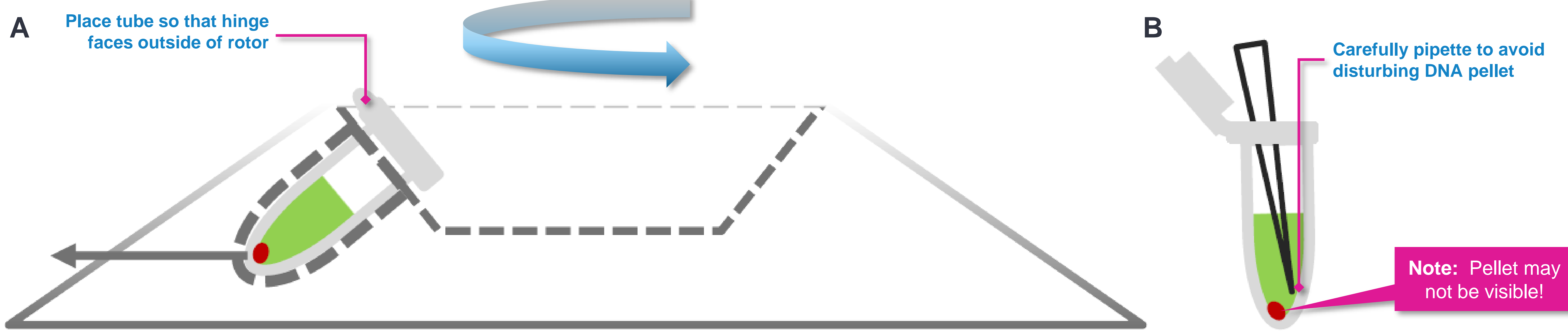
[1] It is essential that DNA concentration is determined by a Qubit system or PicoGreen assay. Using concentrations derived solely from UV-Vis measurements will often result in low recovery, as the DNA concentration will be over-estimated due to RNA that may also be present in the sample.

[2] The DNA sample should be in TE buffer (pH 8), the supplied Buffer EB, or water. If the sample buffer differs significantly or contains high levels of salt, the SRE size selection properties and recoveries may be affected.

SRE kit general best practices (cont.)

SRE DNA size selection pipetting procedure

- Load tube into centrifuge[1] with hinge of tube facing toward the outside of rotor
  - This will help to avoid disturbing the pellet if it cannot be seen
- After centrifugation, the DNA pellet will have formed on the bottom side of the microcentrifuge tube under the hinge region
- Carefully pipette on opposite side towards the thumb lip of tube to avoid disturbing the pellet

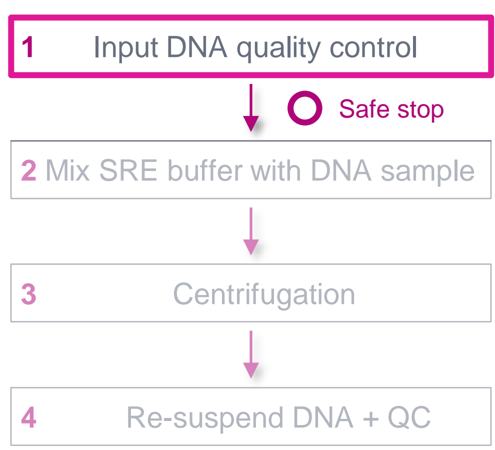


A. Note orientation of tube in centrifuge. Pellet will form on side of the tube facing outwards, in this case underneath the hinge region. B. Carefully pipette from opposite side of tube on the thumb lip side to avoid disturbing pellet. Note: Pellet may not be visible.

[1] Centrifuge at 10,000 x *g* at room temperature (RT). Turn off any built-in temp. control function by specifying a target temp. set point higher than ambient RT.

SRE kit procedural notes – Input DNA quality control

Before you begin, evaluate the quantity and size distribution of input DNA to determine whether it is suitable for the SRE size selection protocol



Input DNA quality control

✔	Step	Instructions
	1.1	Bring the Qubit 1X dsDNA BR working solution and standards to room temperature.
	1.2	Pulse vortex or pipette mix each sample to homogenize the DNA in solution.
	1.3	Quick-spin each sample to collect liquid.
	1.4	Measure DNA concentration with a Qubit fluorometer using the 1X dsDNA BR kit.
	1.5	Dilute each aliquot to 250 pg/µL in Femto Pulse dilution buffer based on the Qubit reading.
	1.6	Measure DNA size distribution with a Femto Pulse system using the gDNA 165kb analysis kit.
	1.7	Proceed to the next step of the protocol if sample size distribution is acceptable (GQN(30k) > 5.0).

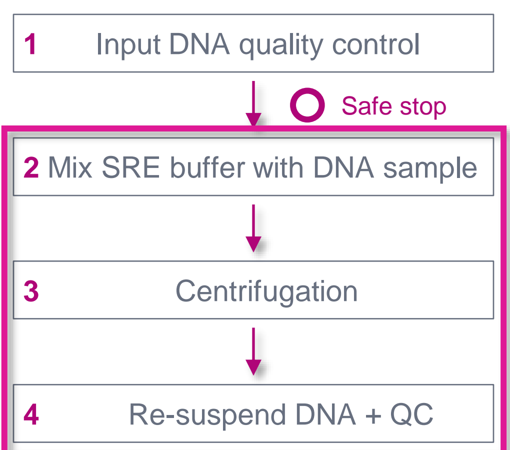
SAFE STOPPING POINT – Store at 4°C

- Bring Qubit 1X dsDNA BR assay reagents to **room temperature**

- This protocol requires high-quality, high molecular weight DNA with 50% of fragments ≥30 kb (GQN at 30 kb ≥5.0)

SRE kit procedural notes – DNA size selection

Carefully perform SRE size selection pipetting steps to avoid disturbing the pelleted DNA and maximize recovery yields



Troubleshooting tip[3]

Possible reasons for low SRE recovery frequently include:

- Input DNA already contains a lot of fragments below the SRE size cutoff
- Starting input DNA concentration was too low
- DNA was accidentally pipetted and discarded

SRE size selection

✔	Step	Instructions
	2.1	Adjust the DNA sample to a total volume of 60 µL and a Qubit DNA concentration between 10–150 ng/µL. Pipette sample into a 1.5 mL Eppendorf DNA LoBind tube. • This concentration MUST be measured using Qubit dsDNA Broad Range Assay or equivalent. • Dilute sample using TE buffer (pH 8), Buffer EB, or water.
	2.2	Add 60 µL of Buffer SRE to the sample. Vortex to mix for 5 seconds at max speed.
	2.3	Incubate the tube for 1 hour at 50°C in heating block.
	2.4	Load tube into centrifuge with the hinge facing toward the outside of the rotor.
	2.5	Centrifuge at 10,000 x *g* for 30 mins at room temperature. • If using a centrifuge with temperature control (i.e., cooling function), turn this function off by specifying a target temperature set point higher than ambient room temperature (e.g., 29°C or 30°C).
	2.6	Carefully remove supernatant from tube without disturbing the pellet. Place the pipette tip on the thumb tip side of the tube (see Figure 1). • The DNA pellet will have formed on the bottom of the tube under the hinge region but may not be visible. • Leaving up to 10 µL is acceptable to be sure the pellet is not disturbed.
	2.7	Add 50–100 µL of Buffer EB to the tube and incubate at room temperature for 20 minutes.
	2.8	After incubation, pipette-mix 20 times and vortex the tube for 5s to ensure that the DNA is properly re-suspended and mixed.
	2.9	Analyze the recovery and purity of the DNA by NanoDrop and Qubit. • If the recovery is lower than 50% repeat pipette-mixing 20 times and vortex for 5s.
	2.10	Buffer volume may be adjusted to achieve desired concentration.
	2.11	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.
		PROTOCOL COMPLETE

- DNA sample concentration should be **10–150 ng/µL**[1]

- **Note orientation of tube in centrifuge**
  → Tube should be placed with hinge facing toward outside of rotor

- Centrifuge at 10,000 x *g* at **room temperature** (RT)
  → Turn off any built-in temp. control function by specifying a target temp. set point higher than ambient RT

- Carefully remove supernatant **without disturbing the DNA pellet**
  → DNA pellet will have formed on bottom of the tube under the hinge region but **may not be visible**[2]

- Pipette-mix 20 times and vortex the tube for 5s to ensure that the DNA is **properly re-suspended**
- **Note:** Longer DNA can take more time to re-suspend
  → Heating to 50°C or eluting for more time can help increase recoveries.

[1] It is essential that DNA concentration is determined by a Qubit system or PicoGreen assay. Using concentrations derived from UV-Vis measurements without accounting for RNA concentrations will adversely affect yields..

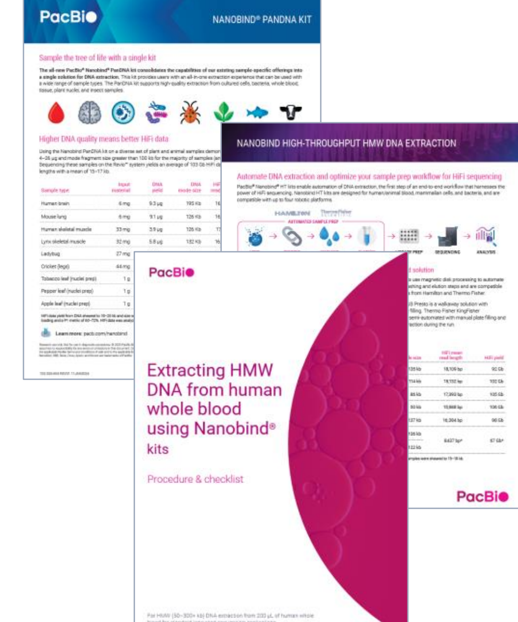
[2] The DNA pellet may not be visible. Placing the tube and pipetting in the directed orientations will prevent accidentally aspirating the DNA pellet.

[3] Refer to ***Guide & overview – Short Read Eliminator (SRE) XS and XL kits*** (102-582-400) and other available SRE documentation for further troubleshooting guidance.

PacBio

Technical documentation & applications support resources

DNA sample extraction documentation & other literature



Brochures

- Brochure – Nanobind high-throughput HMW DNA extraction (102-326-565)
- Brochure – Nanobind PanDNA kit (102-326-604)

Technical notes

- Technical note – High-throughput DNA extraction (102-326-611)
- Technical note – Insect DNA extraction (102-326-612)
- Technical note – Preparing DNA for PacBio HiFi sequencing – Extraction and quality control (102-193-651)
- Technical note – Sample preparation for PacBio HiFi sequencing from human whole blood (102-326-500)

Nanobind kit protocols and Guides & overviews

- Guide & overview – Nanobind CBB kit (102-572-200)
- Guide & overview – Nanobind PanDNA kit (103-394-800)
- Nanobind Procedures & checklists – see PacBio Documentation
- Technical overview – HMW DNA sample preparation for PacBio long-read sequencing using Nanobind PanDNA and SRE kits (103-401-100)

Nanobind high-throughput (HT) automation kit[1] protocols and Guides & overviews

- Guide & overview – Nanobind HT kits (103-028-100)
- Nanobind HT Procedures & checklists – see PacBio Documentation
- Technical overview – Automated high-throughput HMW DNA extraction for PacBio long-read sequencing using Nanobind HT kits (103-401-700)

Short Read Eliminator (SRE) kit protocols and Guides & overviews

- Guide & overview – Short Read Eliminator (SRE) XS and XL kits (102-582-400)
- Procedure & checklist – Removing short DNA fragments with the Short Read Eliminator (SRE) kit (102-982-300)

[1] Contact PacBio Technical Support for information about the latest available Nanobind HT robotic automation scripts.

DNA sample extraction documentation & other literature (cont.)

Nanobind PanDNA kit HMW DNA extraction procedures for PacBio HiFi sequencing[1]

Sample type	Starting material	Sample input	Procedure & checklist
Blood	Human whole blood (non-nucleated RBCs)	200 µL	*Extracting HMW DNA from human whole blood using Nanobind kits* (102-573-500)
	Nucleated red blood cells (nRBCs)	2.5 – 20 µL	*Extracting HMW DNA from nucleated red blood cells using Nanobind kits* (102-574-000)
	Human whole blood with RBC lysis	400 µL	*Extracting HMW DNA from human whole blood with RBC lysis using Nanobind kits* (103-377-500)
Animal tissue	Diverse tissue types	2 – 100 mg	*Extracting HMW DNA from animal tissue* (102-574-600)[2]
Insect tissue	Insect whole body or segment	>20 mg	*Extracting HMW DNA extraction from insects using the Nanobind PanDNA kit* (102-377-400)
Plant tissue	Isolated plant nuclei	0.25 – 5 g	*Extracting HMW DNA from plant nuclei using Nanobind kits* (103-378-200)
Mammalian cultured cells	Suspension cell culture	$1 \times 10^6 – 5 \times 10^6$ diploid human cells	*Extracting HMW DNA from cultured suspension cells using Nanobind kits* (103-394-500)
	Adherent cell culture	$1 \times 10^6 – 5 \times 10^6$ diploid human cells	*Extracting HMW DNA from cultured adherent cells using Nanobind kits* (102-573-600)
Cultured bacteria	Gram-negative bacteria	$5 \times 10^8 – 5 \times 10^9$ bacterial cells	*Extracting HMW DNA from Gram-negative bacteria using Nanobind kits* (102-573-800)
	Gram-positive bacteria	$5 \times 10^8 – 5 \times 10^9$ bacterial cells	*Extracting HMW DNA from Gram-positive bacteria using Nanobind kits* (102-573-900)

[2] ***Procedure & checklist – Extracting HMW DNA from animal tissue*** (102-574-600) describes the extraction of HMW DNA from animal tissues using a TissueRuptor tool for tissue disruption. If a TissueRuptor tool is unavailable, then ***Procedure & checklist – Extracting HMW DNA from standard Dounce homogenizer tissue using Nanobind kits*** (102-573-700) may alternatively be used.

[1] *See **Brochure – Nanobind PanDNA kit*** (102-326-604). For a complete list of supported Nanobind HMW DNA extraction procedures, refer to the PacBio Documentation website.

DNA sample extraction documentation & other literature (cont.)

Nanobind HT kit HMW DNA extraction procedures for PacBio high-throughput HiFi sequencing workflows using robotic automation systems

Automation system	Procedure & checklist	Sample type
KingFisher Duo	Extracting HMW DNA using the Nanobind HT CBB kit for mammalian cultured cells on KingFisher Duo Prime system [102-996-200]	Mammalian cells
	Extracting HMW DNA using the Nanobind HT CBB kit for 200 µL human whole blood on KingFisher Duo Prime system [102-995-800]	Human whole blood
	Extracting HMW DNA using Nanobind HT 1 mL blood kit for human whole blood on KingFisher Duo Prime system [102-995-400]	Human whole blood
KingFisher Flex	Extracting HMW DNA using the Nanobind HT CBB kit for mammalian cultured cells on KingFisher Flex system [102-996-300]	Mammalian cells
	Extracting HMW DNA using the Nanobind HT CBB kit for 200 µL human whole blood on KingFisher Flex system [102-995-900]	Human whole blood
	Extracting HMW DNA using Nanobind HT 1 mL blood kit for human whole blood on KingFisher Flex system [102-995-500]	Human whole blood
KingFisher Apex	Extracting HMW DNA using the Nanobind HT CBB kit for mammalian cultured cells on KingFisher Apex system [102-996-100]	Mammalian cells
	Extracting HMW DNA using the Nanobind HT CBB kit for 200 µL human whole blood on KingFisher Apex system [102-995-700]	Human whole blood
	Extracting HMW DNA using the Nanobind HT CBB kit for bacteria on the KingFisher Apex system [103-377-600]	Cultured bacteria
	Extracting HMW DNA using the Nanobind HT CBB kit for non-human mammalian blood (NHMB) on the KingFisher Apex system [103-397-300]	Non-human mammalian blood
	Extracting HMW DNA using the Nanobind HT CBB kit for nucleated red blood cells (nRBCs) on the KingFisher Apex system [103-377-800]	Non-mammalian blood (nucleated RBCs)
	Extracting HMW DNA using Nanobind HT 1 mL blood kit for human whole blood on KingFisher Apex system [102-995-300]	Human whole blood

DNA sample extraction documentation & other literature (cont.)

Nanobind HT kit HMW DNA extraction procedures for PacBio high-throughput HiFi sequencing workflows using robotic automation systems

Automation system	Procedure & checklist	Sample type
Hamilton NIMBUS Presto	Extracting HMW DNA using the Nanobind HT CBB kit for mammalian cultured cells on Hamilton NIMBUS Presto system [102-996-400]	Mammalian cells
	Extracting HMW DNA using the Nanobind HT CBB kit for 200 µL human whole blood on Hamilton NIMBUS Presto system [102-996-000]	Human whole blood
	Extracting HMW DNA using the Nanobind HT CBB kit for bacteria on Hamilton NIMBUS Presto system [103-397-400]	Cultured bacteria
	Extracting HMW DNA using the Nanobind HT CBB kit for non-human mammalian blood (NHMB) on the Hamilton NIMBUS Presto system [103-377-700]	Non-human mammalian blood
	Extracting HMW DNA using the Nanobind HT CBB kit for nucleated red blood cells (nRBCs) on the Hamilton NIMBUS Presto system [103-397-500]	Non-mammalian blood (nucleated RBCs)
	Extracting HMW DNA using the Nanobind HT 1 mL blood kit for human whole blood on Hamilton NIMBUS Presto system [102-995-600]	Human whole blood

Nanobind HMW DNA extraction common technical challenges

Common technical challenges encountered with all sample types[1]

A common root cause for low DNA yields is an issue with the starting material itself

1. Low DNA recoveries

- Sample input material was improperly collected or stored → Follow recommended tissue collection procedure to ensure correct tissue type is selected and correct storage conditions are used
- Incorrect Nanobind protocol used → Understand your sample type to ensure that correct Nanobind protocol is used
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too low → Increase sample input amount
- Sample input amount is too high → decrease sample input amount

2. Viscous, heterogeneous DNA

- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too high → Decrease sample input amount

3. Eluate is not clear

- Sample input amount is too high → Decrease sample input amount
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube

[1] See individual Nanobind DNA extraction protocol documentation for troubleshooting guidance & FAQs for specific sample types.

[2] Even with aggressive vortexing, HMW DNA extracted with Nanobind protocols will be hundreds of kilobases in length.

Nanobind HMW DNA extraction common technical challenges (cont.)

Common technical challenges encountered with animal tissue samples[1]

A common root cause for low DNA yields is an issue with the starting material itself

1. Low DNA recoveries

- Sample input material was improperly collected or stored → Follow recommended tissue collection procedure to ensure correct tissue type is selected and correct storage conditions are used
- Incorrect Nanobind protocol used → Understand your sample type to ensure that correct Nanobind protocol is used
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too low → Increase sample input amount
- Sample input amount is too high → decrease sample input amount

2. Viscous, heterogeneous DNA

- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too high → Decrease sample input amount.

3. Eluate is not clear

- Sample input amount is too high → Decrease sample input amount.
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube

4. Animal tissue sample is not disrupting well

- Ensure tissue is finely minced with a scalpel
- Use recommended tissue disruption method (TissueRuptor)

[1] See individual Nanobind DNA extraction protocol documentation for troubleshooting guidance & FAQs for specific sample types.
[2] Even with aggressive vortexing, HMW DNA extracted with Nanobind protocols will be hundreds of kilobases in length.

Nanobind HMW DNA extraction common technical challenges (cont.)

Common technical challenges encountered with plant tissue samples[1]

A common root cause for low DNA yields is an issue with the starting material itself

1. Low DNA recoveries

- Sample input material was improperly collected or stored → Follow recommended tissue collection procedure to ensure correct tissue type is selected and correct storage conditions are used
- Incorrect Nanobind protocol used → Understand your sample type to ensure that correct Nanobind protocol is used
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too low → Increase sample input amount
- Sample input amount is too high → decrease sample input amount
- Plant nuclei isolation prep issue → Ensure all nuclei isolation procedure steps are followed correctly

2. Viscous, heterogeneous DNA

- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube
- Sample input amount is too high → Decrease sample input amount

3. Eluate is not clear

- Sample input amount is too high → Decrease sample input amount
- Insufficient mixing during lysis step → Do not skip vortexing steps (do mix aggressively[2]) and use the correct size tube

4. Plant tissue sample is not disrupting well (or other problems with the nuclei isolation prep)

- Use recommended tissue disruption method (TissueRuptor)

5. Plant nuclei pellet is very large

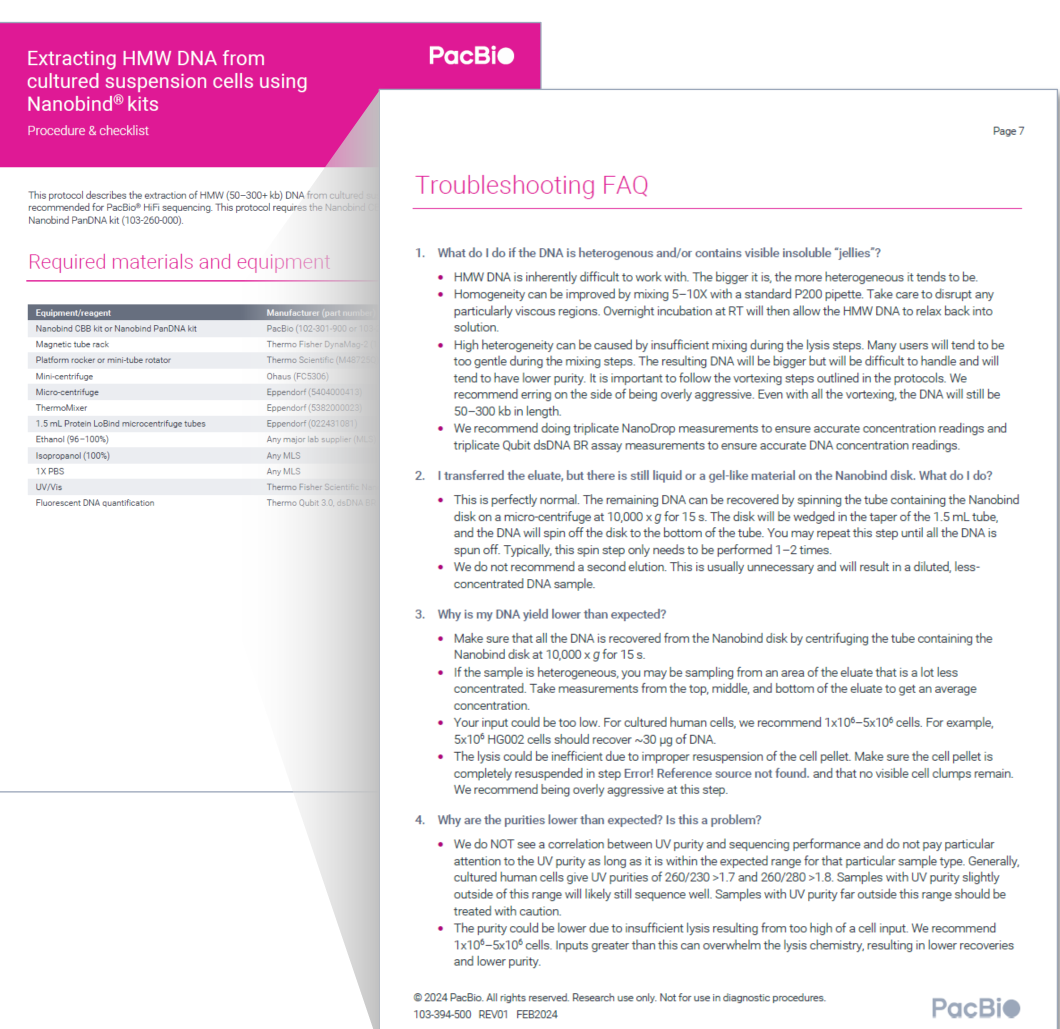
- Plant tissue sample input amount is too high → Decrease sample input amount
  - Up to 5 g of plant material can be input into the LN2 protocol and up to 4 g of plant material can be put into the TissueRuptor protocol
  - A nuclei pellet containing up to ~20 µg of DNA can be input into each Nanobind DNA extraction process
  - For higher yields, parallel extractions can be performed or the Nanobind DNA extraction processes can be scaled up

[1] See individual Nanobind DNA extraction protocol documentation for troubleshooting guidance & FAQs for specific sample types.

[2] Even with aggressive vortexing, HMW DNA extracted with Nanobind protocols will be hundreds of kilobases in length.

Nanobind HMW DNA extraction troubleshooting tips

Refer to individual Nanobind DNA extraction protocol documentation for detailed troubleshooting guidance & FAQs for specific sample types[1]



Extracting HMW DNA from cultured suspension cells using Nanobind® kits

Procedure & checklist

PacBio

This protocol describes the extraction of HMW (50–300+ kb) DNA from cultured ... recommended for PacBio® HiFi sequencing. This protocol requires the Nanobind ... Nanobind PanDNA kit (103-260-000).

Required materials and equipment

Equipment/reagent	Manufacturer (part number)
Nanobind CBB kit or Nanobind PanDNA kit	PacBio (102-301-900 or 103-...
Magnetic tube rack	Thermo Fisher DynaMag-2 ...
Platform rocker or mini-tube rotator	Thermo Scientific (M48721...
Mini-centrifuge	Ohaus (FC5306)
Micro-centrifuge	Eppendorf (5404000413)
ThermoMixer	Eppendorf (5382000023)
1.5 mL Protein LoBind microcentrifuge tubes	Eppendorf (022431081)
Ethanol (96–100%)	Any major lab supplier (MLS)
Isopropanol (100%)	Any MLS
1X PBS	Any MLS
UV/Vis	Thermo Fisher Scientific Na...
Fluorescent DNA quantification	Thermo Qubit 3.0, dsDNA B...

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Troubleshooting FAQ

1. What do I do if the DNA is heterogenous and/or contains visible insoluble "jellies"?
   - HMW DNA is inherently difficult to work with. The bigger it is, the more heterogeneous it tends to be.
   - Homogeneity can be improved by mixing 5–10X with a standard P200 pipette. Take care to disrupt any particularly viscous regions. Overnight incubation at RT will then allow the HMW DNA to relax back into solution.
   - High heterogeneity can be caused by insufficient mixing during the lysis steps. Many users will tend to be too gentle during the mixing steps. The resulting DNA will be bigger but will be difficult to handle and will tend to have lower purity. It is important to follow the vortexing steps outlined in the protocols. We recommend erring on the side of being overly aggressive. Even with all the vortexing, the DNA will still be 50–300 kb in length.
   - We recommend doing triplicate NanoDrop measurements to ensure accurate concentration readings and triplicate Qubit dsDNA BR assay measurements to ensure accurate DNA concentration readings.
2. I transferred the eluate, but there is still liquid or a gel-like material on the Nanobind disk. What do I do?
   - This is perfectly normal. The remaining DNA can be recovered by spinning the tube containing the Nanobind disk on a micro-centrifuge at 10,000 x *g* for 15 s. The disk will be wedged in the taper of the 1.5 mL tube, and the DNA will spin off the disk to the bottom of the tube. You may repeat this step until all the DNA is spun off. Typically, this spin step only needs to be performed 1–2 times.
   - We do not recommend a second elution. This is usually unnecessary and will result in a diluted, less-concentrated DNA sample.
3. Why is my DNA yield lower than expected?
   - Make sure that all the DNA is recovered from the Nanobind disk by centrifuging the tube containing the Nanobind disk at 10,000 x *g* for 15 s.
   - If the sample is heterogeneous, you may be sampling from an area of the eluate that is a lot less concentrated. Take measurements from the top, middle, and bottom of the eluate to get an average concentration.
   - Your input could be too low. For cultured human cells, we recommend $1 x 10^6$–$5 x 10^6$ cells. For example, $5 x 10^6$ HG002 cells should recover ~30 µg of DNA.
   - The lysis could be inefficient due to improper resuspension of the cell pellet. Make sure the cell pellet is completely resuspended in step Error! Reference source not found. and that no visible cell clumps remain. We recommend being overly aggressive at this step.
4. Why are the purities lower than expected? Is this a problem?
   - We do NOT see a correlation between UV purity and sequencing performance and do not pay particular attention to the UV purity as long as it is within the expected range for that particular sample type. Generally, cultured human cells give UV purities of 260/230 >1.7 and 260/280 >1.8. Samples with UV purity slightly outside of this range will likely still sequence well. Samples with UV purity far outside this range should be treated with caution.
   - The purity could be lower due to insufficient lysis resulting from too high of a cell input. We recommend $1 x 10^6$–$5 x 10^6$ cells. Inputs greater than this can overwhelm the lysis chemistry, resulting in lower recoveries and lower purity.

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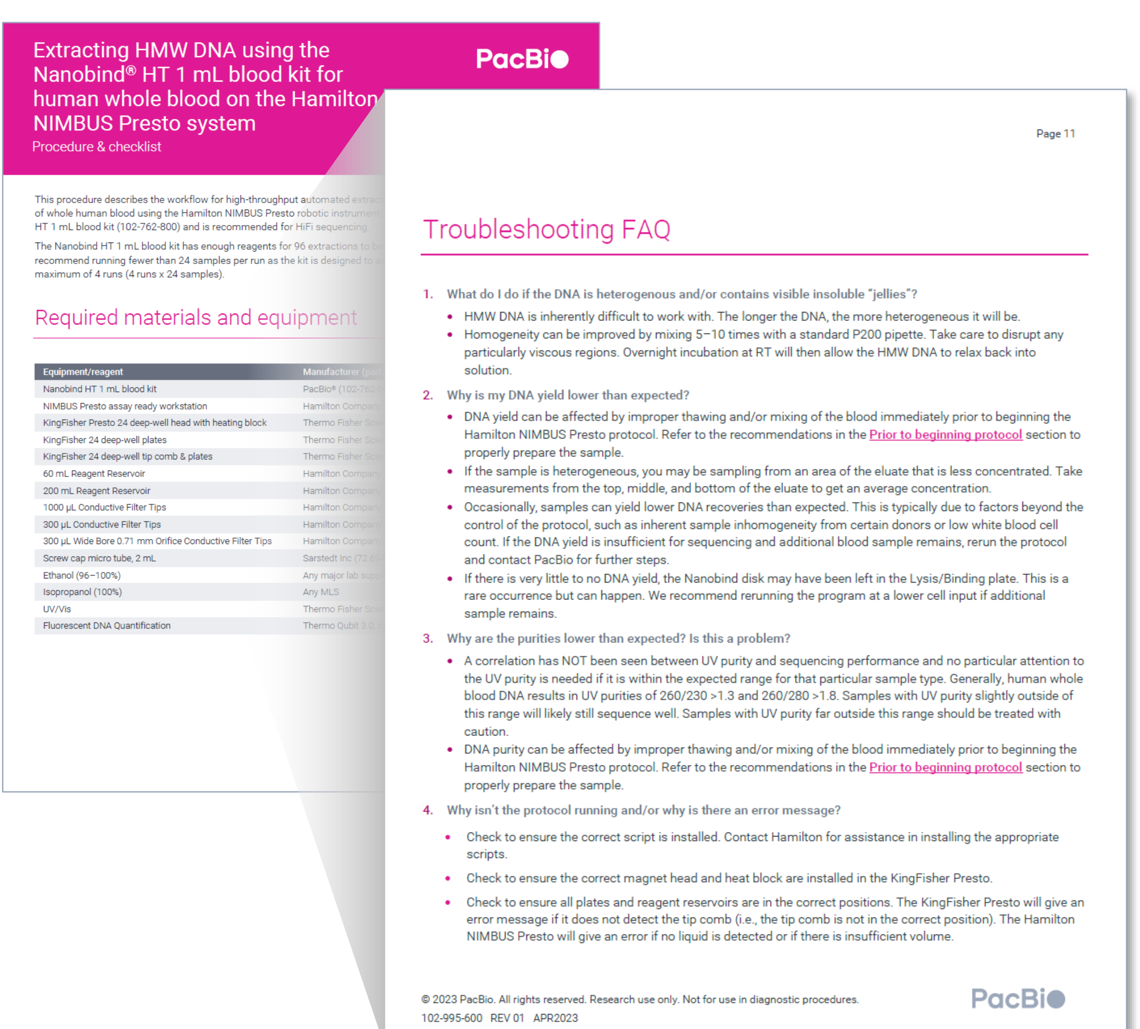

Extracting HMW DNA using the Nanobind® HT 1 mL blood kit for human whole blood on the Hamilton NIMBUS Presto system

Procedure & checklist

PacBio

This procedure describes the workflow for high-throughput automated extra... of whole human blood using the Hamilton NIMBUS Presto robotic instrument... HT 1 mL blood kit (102-762-800) and is recommended for HiFi sequencing.

The Nanobind HT 1 mL blood kit has enough reagents for 96 extractions to b... recommend running fewer than 24 samples per run as the kit is designed to ... maximum of 4 runs (4 runs x 24 samples).

Required materials and equipment

Equipment/reagent	Manufacturer (part number)
Nanobind HT 1 mL blood kit	PacBio® (102-762-...
NIMBUS Presto assay ready workstation	Hamilton Compan...
KingFisher Presto 24 deep-well head with heating block	Thermo Fisher Sci...
KingFisher 24 deep-well plates	Thermo Fisher Sci...
KingFisher 24 deep-well tip comb & plates	Thermo Fisher Sci...
60 mL Reagent Reservoir	Hamilton Compan...
200 mL Reagent Reservoir	Hamilton Compan...
1000 µL Conductive Filter Tips	Hamilton Compan...
300 µL Conductive Filter Tips	Hamilton Compan...
300 µL Wide Bore 0.71 mm Orifice Conductive Filter Tips	Hamilton Compan...
Screw cap micro tube, 2 mL	Sarstedt Inc (72.6...
Ethanol (96–100%)	Any major lab sup...
Isopropanol (100%)	Any MLS
UV/Vis	Thermo Fisher Sci...
Fluorescent DNA Quantification	Thermo Qubit 3.0...

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Troubleshooting FAQ

1. What do I do if the DNA is heterogenous and/or contains visible insoluble "jellies"?
   - HMW DNA is inherently difficult to work with. The longer the DNA, the more heterogeneous it will be.
   - Homogeneity can be improved by mixing 5–10 times with a standard P200 pipette. Take care to disrupt any particularly viscous regions. Overnight incubation at RT will then allow the HMW DNA to relax back into solution.
2. Why is my DNA yield lower than expected?
   - DNA yield can be affected by improper thawing and/or mixing of the blood immediately prior to beginning the Hamilton NIMBUS Presto protocol. Refer to the recommendations in the Prior to beginning protocol section to properly prepare the sample.
   - If the sample is heterogeneous, you may be sampling from an area of the eluate that is less concentrated. Take measurements from the top, middle, and bottom of the eluate to get an average concentration.
   - Occasionally, samples can yield lower DNA recoveries than expected. This is typically due to factors beyond the control of the protocol, such as inherent sample inhomogeneity from certain donors or low white blood cell count. If the DNA yield is insufficient for sequencing and additional blood sample remains, rerun the protocol and contact PacBio for further steps.
   - If there is very little to no DNA yield, the Nanobind disk may have been left in the Lysis/Binding plate. This is a rare occurrence but can happen. We recommend rerunning the program at a lower cell input if additional sample remains.
3. Why are the purities lower than expected? Is this a problem?
   - A correlation has NOT been seen between UV purity and sequencing performance and no particular attention to the UV purity is needed if it is within the expected range for that particular sample type. Generally, human whole blood DNA results in UV purities of 260/230 >1.3 and 260/280 >1.8. Samples with UV purity slightly outside of this range will likely still sequence well. Samples with UV purity far outside this range should be treated with caution.
   - DNA purity can be affected by improper thawing and/or mixing of the blood immediately prior to beginning the Hamilton NIMBUS Presto protocol. Refer to the recommendations in the Prior to beginning protocol section to properly prepare the sample.
4. Why isn't the protocol running and/or why is there an error message?
   - Check to ensure the correct script is installed. Contact Hamilton for assistance in installing the appropriate scripts.
   - Check to ensure the correct magnet head and heat block are installed in the KingFisher Presto.
   - Check to ensure all plates and reagent reservoirs are in the correct positions. The KingFisher Presto will give an error message if it does not detect the tip comb (i.e., the tip comb is not in the correct position). The Hamilton NIMBUS Presto will give an error if no liquid is detected or if there is insufficient volume.

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102-995-600 REV 01 APR2023

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[1] See individual PacBio protocol documentation for guidance on troubleshooting low DNA yield or other unexpected DNA isolation issues encountered with manual or automated high-throughput Nanobind HMW DNA extraction workflows.

SRE kit troubleshooting tips

Possible reasons for low SRE recovery or unexpected SRE size selection performance

If SRE recovery is lower than expected

- **Highly fragmented gDNA.** When using the SRE kit, recovery will be low if DNA is not HMW. Verify that the starting input DNA shows a size distribution with at least 50% of fragments ≥30 kb (GQN at 30 kb = 5.0) by using a Femto Pulse system.
- **Low input DNA concentration.** Recovery will be low if dsDNA concentration is <50 ng/µL. Verify the input gDNA concentration using Qubit dsDNA broad range assay or equivalent. Using concentrations derived solely from UV-Vis measurements will often result in low recovery as the estimated DNA concentration will not account for RNA that is also present in the solution. Try increasing concentration of input DNA up to the maximum of 150 ng/µL.
- **Incorrect centrifugation speed.**
- **Incorrect centrifugation temperature.** Recovery will be impacted if centrifugation is performed at low temperature (e.g., 4°C). Verify that the centrifuge is not cooling by turning off cooling or setting the temperature above ambient (e.g., 29°C).
- **Heterogeneous input sample.** If input sample is heterogeneous and contains fractions of DNA that are not fully solubilized, recovery will be affected. Verify homogeneity by pipetting to ensure that no viscous jellies exist in the sample. Homogeneity can also be determined by performing triplicate concentration measurements and verifying that the CV <20%. If the sample fails these tests, needle shear the input DNA 10X using a 26G needle or pipette mix 10X using a standard P200 pipette and allow to rest overnight at RT before proceeding.
- **Non-standard DNA buffer.** This method has only been tested using DNA in solubilized in TE buffer (pH 8), Buffer EB, or water. If the DNA sample contains high levels of contaminants or salts, recovery may be affected.
- **Handling error.** The DNA pellet is often invisible. If the pellet is disturbed during the wash steps, it is possible to accidentally aspirate it into the pipette tip. Ensure that proper care is taken with tube orientation during centrifuge and pipetting steps such that pipetting is always performed on the opposite side of the tube from the pellet.

[1] Refer to ***Guide & overview – Short Read Eliminator (SRE) XS and XL kits*** (102-582-400) and other available SRE documentation for further troubleshooting guidance.

SRE kit troubleshooting tips (cont.)

Possible reasons for low SRE recovery or unexpected SRE size selection performance

If unexpected SRE size selection performance is obtained

- **Non-standard DNA buffer.** This method has only been tested using DNA in water, TE buffer, or Buffer EB. If the DNA sample contains high levels of contaminants or salts or compounds that affect DNA solubility/precipitation, size selection performance may be affected.

[1] Refer to ***Guide & overview – Short Read Eliminator (SRE) XS and XL kits*** (102-582-400) and other available SRE documentation for further troubleshooting guidance.

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