PacBio

# Nanobind® CBB kit

## Guide & overview

For extraction of HMW (50 kb – 300+ kb) genomic DNA from cultured cells, cultured bacteria, and blood.

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## Prior to starting

Buffer CW1 and CW2 are supplied as concentrates. This kit uses CW1 and CW2 with 60% final ethanol concentrations. 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 CBB kit 4C (102-207-700) should be stored at 4 °C upon arrival.

The Nanobind CBB kit RT (102-207-600) 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 CBB kits are intended for research use only.

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# Equipment and reagent list

| Equipment/reagent | Manufacturer (part number) |
|---|---|
| Nanobind CBB kit | PacBio® (102-301-900) |
| Magnetic tube rack | Thermo Fisher Scientific DynaMag-2 (12321D) |
| Mini-tube rotator | Fisher Scientific Mini Tube Rotator (05-450-127) |
| Heat block (or water bath) | Fisher Scientific Isotemp dry bath incubator (11-715-125DQ) |
| Mini-centrifuge | Ohaus (FC5306) |
| Micro-centrifuge | Eppendorf (5404000413) |
| ThermoMixer | Eppendorf (5382000023) |
| 1.5 mL Protein LoBind microcentrifuge tubes | Eppendorf (022431081) |
| Ethanol (96–100%) | |
| Isopropanol (100%) | |
| 1X PBS | |
| UV/Vis | Thermo Fisher Scientific NanoDrop 2000 |
| Fluorescent DNA quantification | Invitrogen Qubit 3.0, dsDNA BR and RNA BR assay kits |
| Tris-HCl, 1 M, pH 8.0 | Invitrogen (15568025) |
| Ethylenediaminetetraacetic acid (EDTA), 0.5 M, pH 8.0 | Thermo Fisher Scientific (15575020) |
| Sucrose | Fisher Scientific (BP220) |
| Triton X-100 | Sigma-Aldrich (X100) |
| Lysozyme | MP Biomedicals (100831) |
| Lysostaphin | Sigma-Aldrich (L7386) |
| 26g blunt end needle | SAI Infusion (B26150) |
| 1 mL syringe | Fisher Scientific (14-823-30) |

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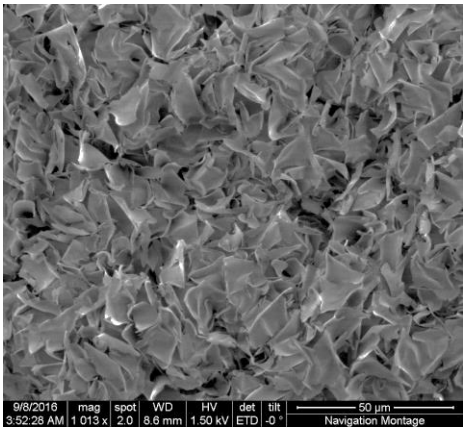
# Introduction

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) and ultra high molecular weight (UHMW) 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 CBB Kit is used for the extraction of HMW (50 kb – 300+ kb) DNA from cultured cells, cultured bacteria, and blood. It has been used for both mammalian and non-mammalian cell culture samples, a diverse array of Gram-negative and Gram-positive bacteria, mammalian blood from a variety of species, and nucleated blood from birds, fish, and reptiles. The extracted DNA is suitable for HiFi sequencing. Process time is approximately 45–90 minutes.

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

Protocols listed in the **HMW (50 kb – 300+ kb)** DNA extraction protocols sections are updated frequently so please check the PacBio Documentation page for the most up-to-date list and for the current versions of the protocols.

The **HMW (50 kb – 300+ kb) DNA extraction protocols** are recommended for PacBio HiFi sequencing.

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| | HMW DNA extraction protocols |
|---|---|
| **Extraction processing** | Thermomixing<br>vortexing |
| **Pipetting** | Standard tips |
| **Typical DNA size** | 50 kb – 300+ kb |
| **Sample heterogeneity** | Low to Moderate<br>(Conc CV = 5–25%) |
| **Recommended applications** | PacBio HiFi sequencing |

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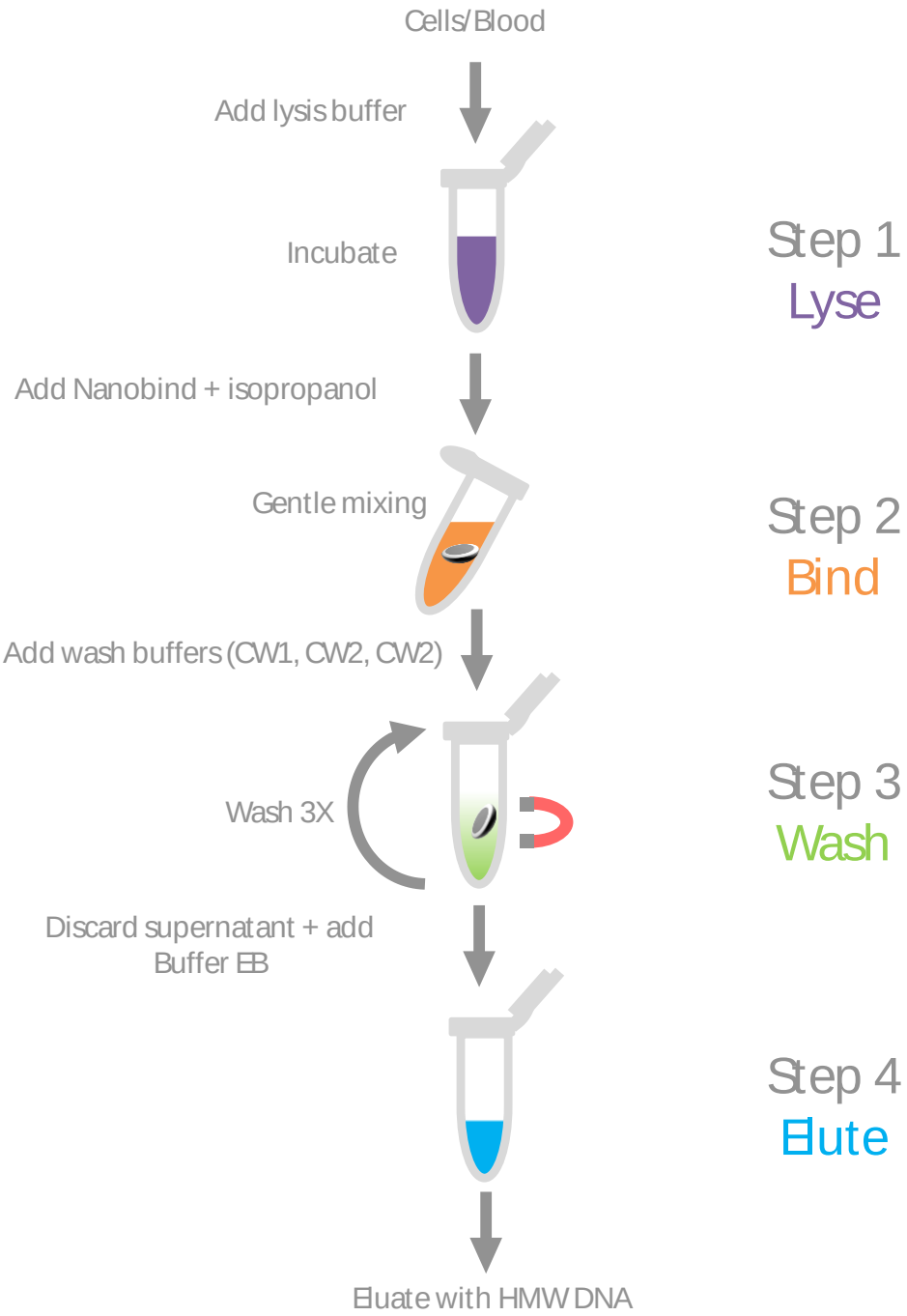
# Workflow

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# Sample information

Yields of HMW genomic DNA will vary depending on the sample being processed. The following table provides suggested input ranges and expected yields for the validated sample types. Each sample has been validated by long read sequencing.

| Expected yields | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Suggested input[1]** | **Example input** | **Example 260/280** | **Example 260/230** | **ExampleYield (µg)** |
| MCF-7 cells (tetraploid) | $0.5x10^6$ – $10x10^6$ cells | $1x10^6$ cells | 1.9 | 2.0 | 13.5 |
| MDA-MB-231 (tetraploid) | $0.5x10^6$ – $10x10^6$ cells | $1x10^6$ cells | 1.9 | 2.0 | 14.9 |
| MCF-10A cells (diploid) | $0.5x10^6$ – $10x10^6$ cells | $2x10^6$ cells | 1.9 | 2.0 | 12.3 |
| GM12878 cells (diploid) | $0.5x10^6$ – $10x10^6$ cells | $5x10^6$ cells | 1.9 | 2.2 | 37.2 |
| *Escherichia coli*[2] | $5x10^8$ – $5x10^9$ cells | 1 mL | 1.8 | 1.4 | 18.3 |
| *Shigella sonnei*[2] | $5x10^8$ – $5x10^9$ cells | 0.25 mL | 1.8 | 1.4 | 27.7 |
| *Salmonella enterica*[2] | $5x10^8$ – $5x10^9$ cells | 0.25 mL | 1.8 | 1.5 | 23.4 |
| *Listeria monocytogenes*[2] | $5x10^8$ – $5x10^9$ cells | 1 mL | 1.8 | 1.9 | 21.7 |
| Human whole blood | 200 µL – 1 mL | 200 µL | 1.9 | 1.9 | 5.6 |

[1]Higher input levels can be used with appropriate optimization of buffer volumes and enzyme levels.

[2]Input based on cell pellet from stated volume of overnight culture.

| Blood anticoagulant validation | | | |
|---|---|---|---|
| **Sample** | **260/280** | **260/230** | **DNA yield (µg)** |
| Whole blood – K2 EDTA | 1.9 | 2.0 | 5.5 |
| Whole blood – Sodium citrate | 1.9 | 2.0 | 5.0 |
| Whole blood – Heparin | 1.9 | 1.9 | 5.1 |
| Whole blood – ACD | 1.9 | 1.8 | 4.4 |

Nanobind was used to extract HMW DNA (up to 300 kb) from 200 µL of fresh human whole blood (n=3). All four samples showed similar yields and UV purity. HiFi sequencing performance was best for samples stored in potassium EDTA (K2 EDTA). Samples stored in Heparin and sodium citrate also performed well in limited testing.

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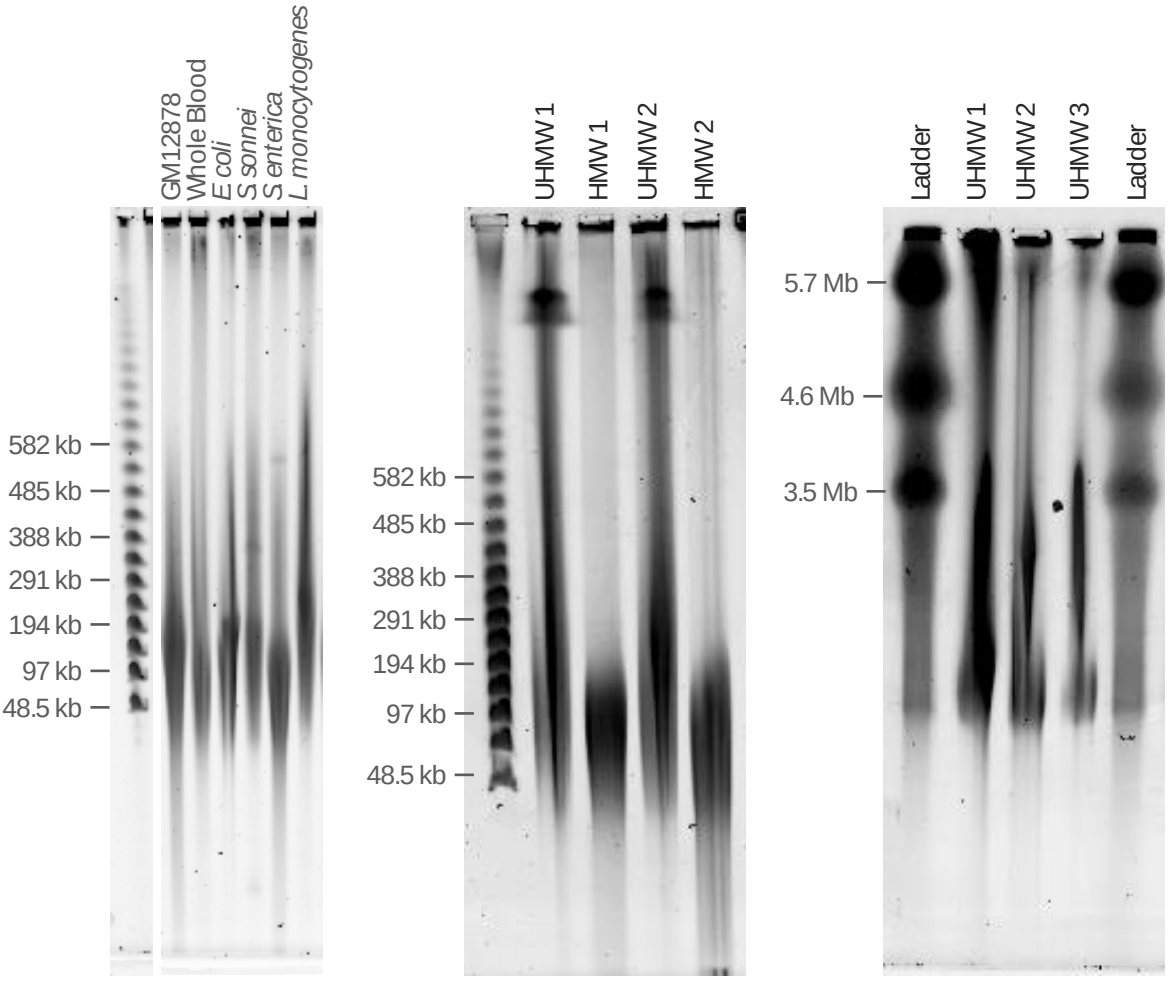
## DNA size

The HMW DNA Extraction Protocols typically yield DNA in the 50 kb – 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.




Pulsed Field Gel Electrophoresis (PFGE) comparison of DNA extracted using the HMW DNA Extraction Protocol from various sample types (left). Pulsed Field Gel Electrophoresis (PFGE) comparison of HMW (center) and UHMW (center, right) DNA extracted from MCF-7 cells. The HMW protocols typically result in DNA from 50 – 300+ kb. The UHMW protocols are capable of obtaining megabase DNA surpassing 5.7 Mb in length.

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## Preservation methods

High quality samples are the key to obtaining high quality DNA. Either fresh or frozen samples can be used equivalently. However, care should be taken to minimize freeze-thaws and to minimize the time samples spend at ambient or at 4 °C.

## Fresh vs. frozen cells

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen cell pellets. Cell pellets should be frozen in minimal liquid after harvesting. No cryoprotectant is necessary. When using frozen cell pellets, it is important to fully resuspend the cell pellets before processing.

## Fresh vs. frozen blood

No systematic difference has been seen in either DNA QC or sequencing results between fresh and frozen blood samples. Blood samples should be frozen as quickly as possible after being drawn. Storage at 4 °C should be limited to 2 days from collection to extraction to prevent sample degradation. Blood samples should be aliquoted to avoid repeated freeze-thaws.

## Blood Anti-coagulant

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

## 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 protocols.

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# PacBio® sequencing

HMW DNA was extracted from cultured cells, bacteria, and blood samples. The samples were then sequenced on PacBio RS II, Sequel®, or Sequel II systems using CLR or HiFi workflows. HMW DNA is recommended for HiFi sequencing.

**PacBio Sequel/Sequel II system CLR sequencing**

| Sample | Library prep | Subread length N50 (bp) | Max subread length (bp) | Total data (Gb) |
|---|---|---|---|---|
| **GM12878 cells** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 32,813 | 95,946 | 10.4 |
| **GM12878 cells** | 5X NS, SRE<br>Express template 2.0, Sequel II | 26,789 | N/A | 142 |
| **E. coli** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 29,611 | 80,713 | 6.8 |
| **S. sonnei** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 35,251 | 83,705 | 9.0 |
| **S. enterica** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 33,420 | 93,614 | 10.5 |
| **L. monocytogenes** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 32,010 | 95,382 | 10.2 |
| **Human Whole Blood (200 µL)** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 28,443 | 95,679 | 6.7 |
| **Human Whole Blood (1 mL)** | 5X NS, 30 kb BP<br>Express template 1.0, Sequel | 26,325 | 89,360 | 6.4 |

**PacBio Sequel II system HiFi sequencing**

| Sample | Library prep | Total bases (Gb) | ≥Q20 Mean read Length (bp) | ≥Q20 read yield (Gb) | ≥Q20 median read quality |
|---|---|---|---|---|---|
| GM12878 cells | 17 kb shear, SRE<br>Express template 2.0 | 276 | 15,320 | 18.8 | Q32 |
| GM12878 cells | 17 kb shear, SRE<br>Express template 2.0 | 299 | 14,589 | 19.2 | Q32 |
| GM12878 cells | 21 kb shear, SRE<br>Express template 2.0 | 325 | 15,697 | 20.7 | Q31 |

Data generated in collaboration with PacBio®

NS Needle shear

BP BluePippin size selection

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## PacBio multiplex microbial sequencing

HMW DNA was extracted from a panel of Gram-negative and Gram-positive bacteria. The samples were then sequenced in multiplex on PacBio Sequel II using **Procedure & Checklist – Preparing Multiplexed Microbial Libraries Using SMRTbell® Express Template Prep Kit 2.0 (101-696-100 Version 07).** All bacteria assembled to a single chromosomal contig as expected. Detailed data is provided in the **Microbial Multiplexing Workflow on the Sequel System using the SMRTbell Express Template Prep Kit 2.0 Application Note.**

**PacBio Sequel II system microbial multiplexing**

| Barcode | Sample | Gram status | Genome size (bp) | Filtered subread coverage | Max contig length (bp) | Chromosomal contigs (#) | Concordance w/ NCBI (QV) |
|---|---|---|---|---|---|---|---|
| BC1001 | *E. coli K12* | – | 4,653,240 | 64x | 4,642,499 | 1 | 56 |
| BC1002 | *E. coli K12* | – | 4,653,240 | 55x | 4,642,500 | 1 | 57 |
| BC1009 | *K. pneumonaie* | – | 5,781,501 | 50x | 5,435,746 | 1 | 48 |
| BC1010 | *B. cereus* | + | 5,427,083 | 59x | 5,408,315 | 1 | 35 |
| BC1012 | *L. monocytogenes* | + | 3,032,269 | 66x | 3,043,149 | 1 | 46 |
| BC1015 | *S. sonnei* | – | 5,062,953 | 53x | 4,813,454 | 1 | 62 |
| BC1016 | *N. meningitidis* | – | 2,194,961 | 74x | 2,213,947 | 1 | 50 |
| BC1018 | *S. aureus* | + | 2,806,345 | 92x | 2,778,860 | 1 | 56 |
| BC1019 | *E. coli Strain W* | – | 5,005,347 | 68x | 4,898,327 | 1 | 44 |
| BC1022 | *S. aureus HPV107* | + | 2,901,406 | 82x | 2,962,786 | 1 | 33 |

Data generated in collaboration with PacBio®. All samples other than BC1001 were extracted using the Nanobind CBB kit.

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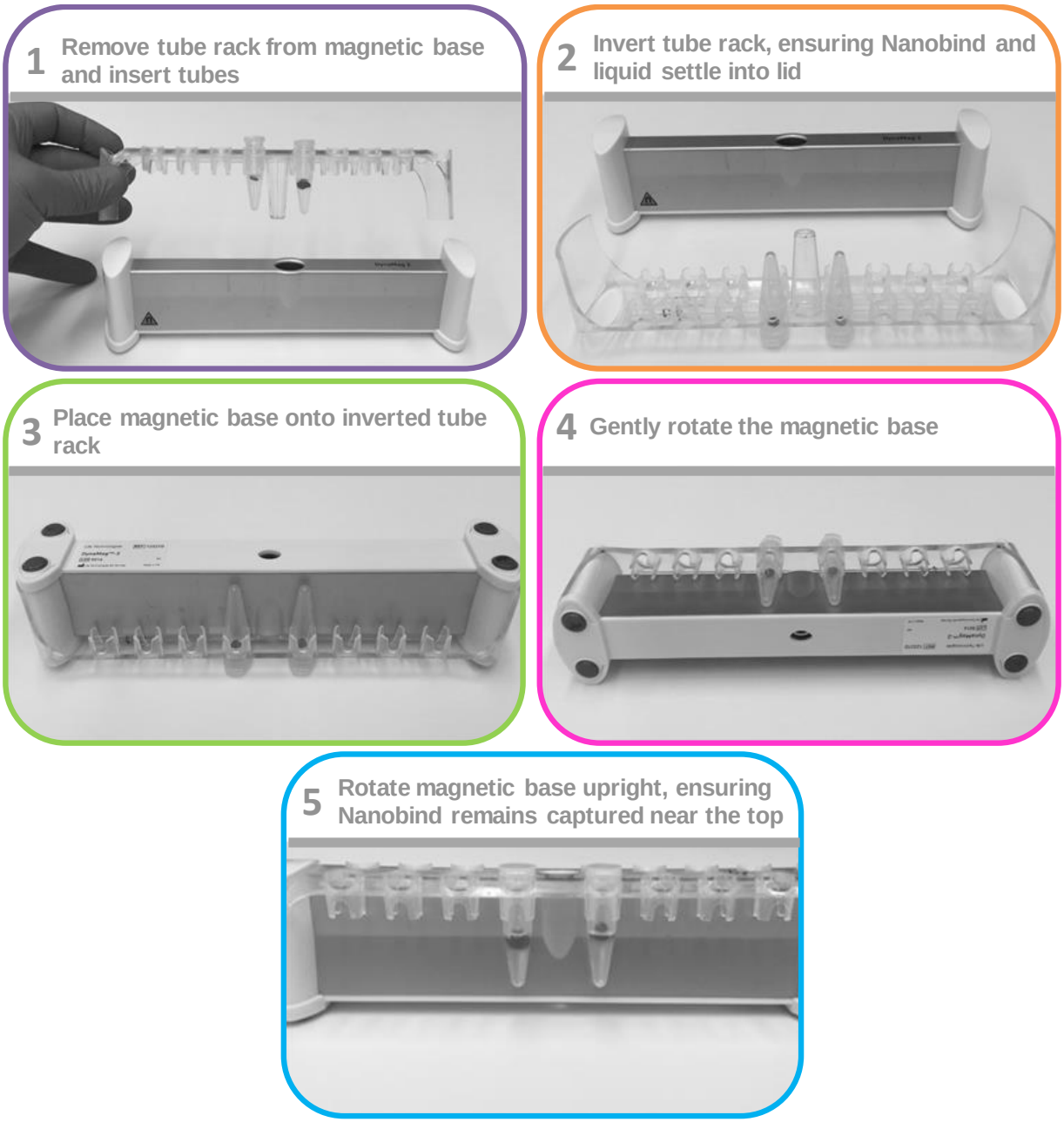
# Processing tips

## 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.

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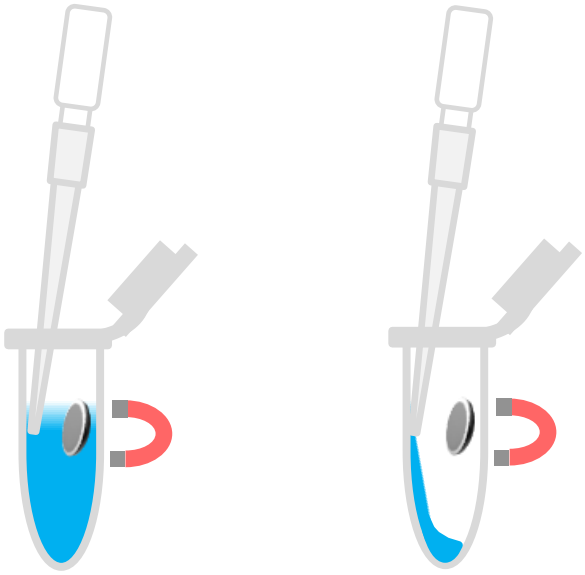
## Pipetting

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.

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## 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. 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.

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# HMW (50 kb – 300+ kb) DNA extraction protocols

As of the document release date, the following Procedures & checklists are available for HMW (50–300+ kb) DNA extraction. They are recommended for most long-read sequencing applications. This includes PacBio CLR and HiFi sequencing.

## HMW DNA extraction – cultured cells (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).

## HMW DNA extraction – gram-negative bacteria (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).

## HMW DNA extraction – gram-positive bacteria (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)).

## HMW DNA extraction – 200 µL mammalian whole blood (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).

## HMW DNA extraction – nucleated blood (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).

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# QC procedures

Accurate quantification of HMW 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 DNA 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.

# PacBio Sequel sequencing recommendations

Below are our standard conditions and tips for sequencing on PacBio Sequel using the SMRTbell express template prep kit SPK3.

1. Isolate HMW DNA using Nanobind CBB kit.
2. Prepare DNA for sequencing using procedure & checklist - Preparing gDNA libraries using the SMRTbell express template preparation kit SPK3.

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