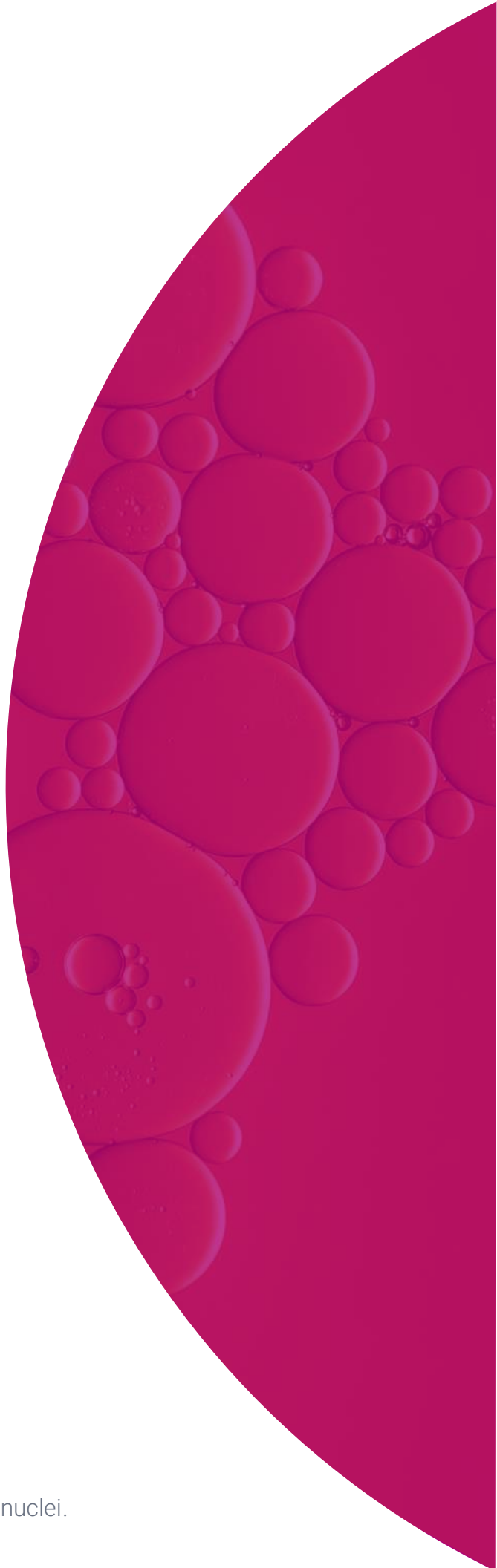
PacBio

Nanobind® plant nuclei kit

Guide & overview



For extraction of HMW (50–300+ kb) genomic DNA from plant nuclei.

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Prior to starting

Buffer PW1 is supplied as a concentrate. This kit uses PW1 with a 70% final ethanol concentration. Before using, add the appropriate amount of ethanol (96–100%) as indicated on the bottle.

Storage

The Nanobind plant nuclei kit 4C (102-207-900) should be stored at 4°C upon arrival.

The Nanobind plant nuclei kit RT (102-207-900) should be stored at room temperature (15–30°C).

Product use

Nanobind plant nuclei kits are intended for research use only.

For all protocols

Eppendorf Protein LoBind tubes (Eppendorf #022431081) are highly recommended for all extractions to reduce protein contamination from tube carryover. Protein LoBind tubes are more effective in reducing carryover contamination than DNA LoBind tubes or other tubes and will result in improved UV purity.

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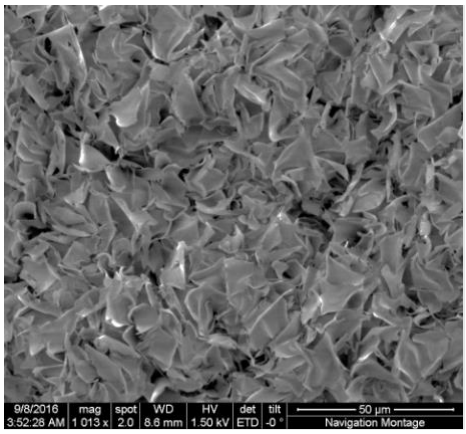
Equipment and reagent list

Equipment/reagent	Manufacturer (part number)
Nanobind plant nuclei kit	PacBio® (102-302-000)
Microcentrifuge	Eppendorf (5415R)
HulaMixer	Thermo Fisher (15920D)
Magnetic tube rack	Thermo Fisher DynaMag-2 (12321D)
Mini-centrifuge	Ohaus Mini-Centrifuge (FC5306)
ThermoMixer	Eppendorf (5382000023)
1.5 mL Protein LoBind microcentrifuge tubes	Eppendorf (022431081)
Isopropanol (100%)	
Ethanol (96–100%)	
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA quantification	Thermo Qubit 3.0, dsDNA BR and RNA BR assay kits
26G blunt end needle	SAI Infusion (B26150)
1 mL syringe	Fisher Scientific (14-823-30)

Introduction

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



SEM image of Nanobind's silica surface structure.

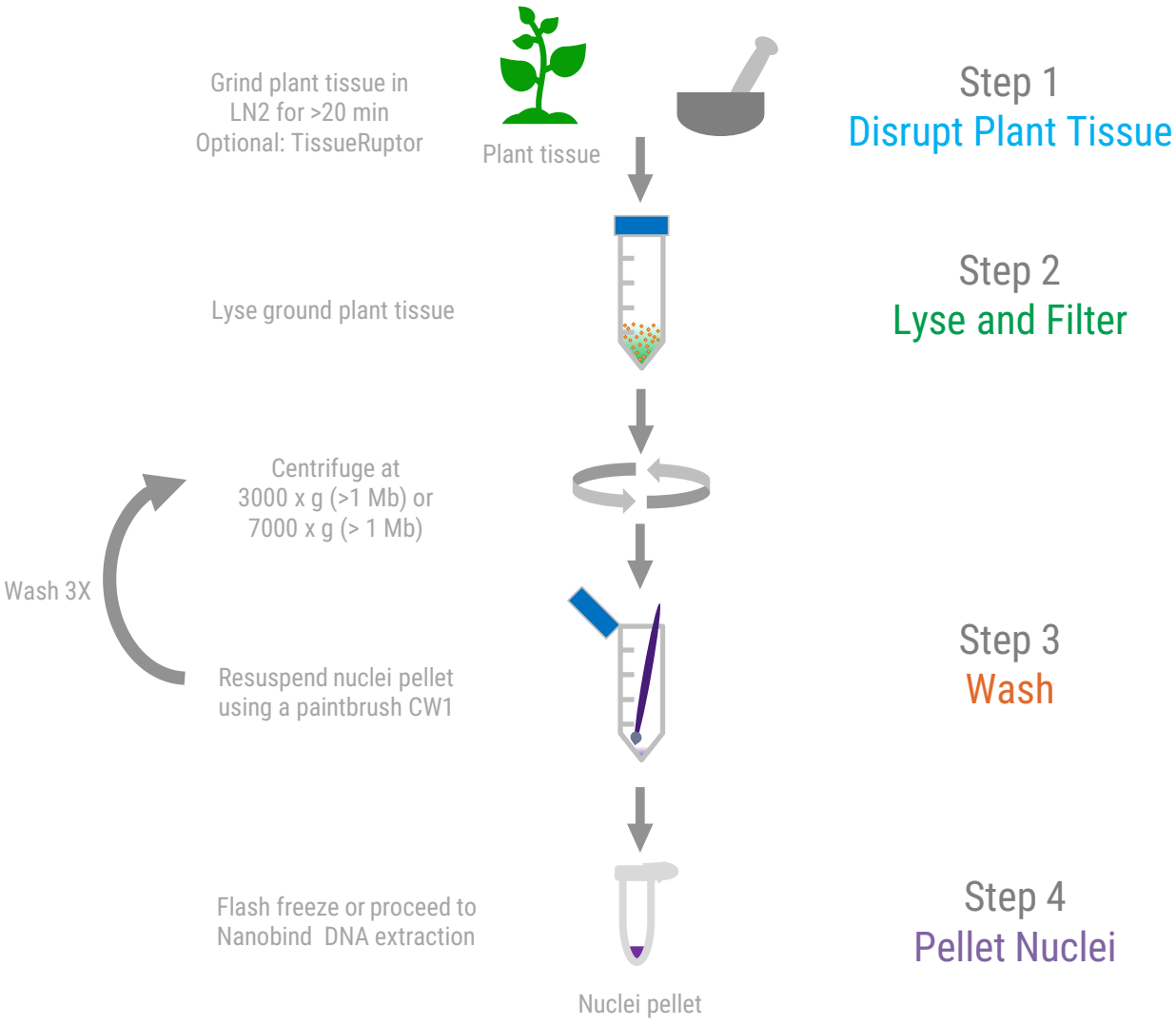
Kit overview

The Nanobind plant nuclei kit is used for extraction of HMW (50–300+ kb) DNA from plant nuclei. First, nuclei are isolated from 1–5 g of plant tissue using one of the recommended nuclei isolation protocols. Then, HMW DNA is extracted from the nuclei using Nanobind disks. Each of the two purification steps (i.e., nuclei isolation + Nanobind extraction) removes different contaminants from the sample, resulting in clean, HMW DNA from even the most challenging plant species.

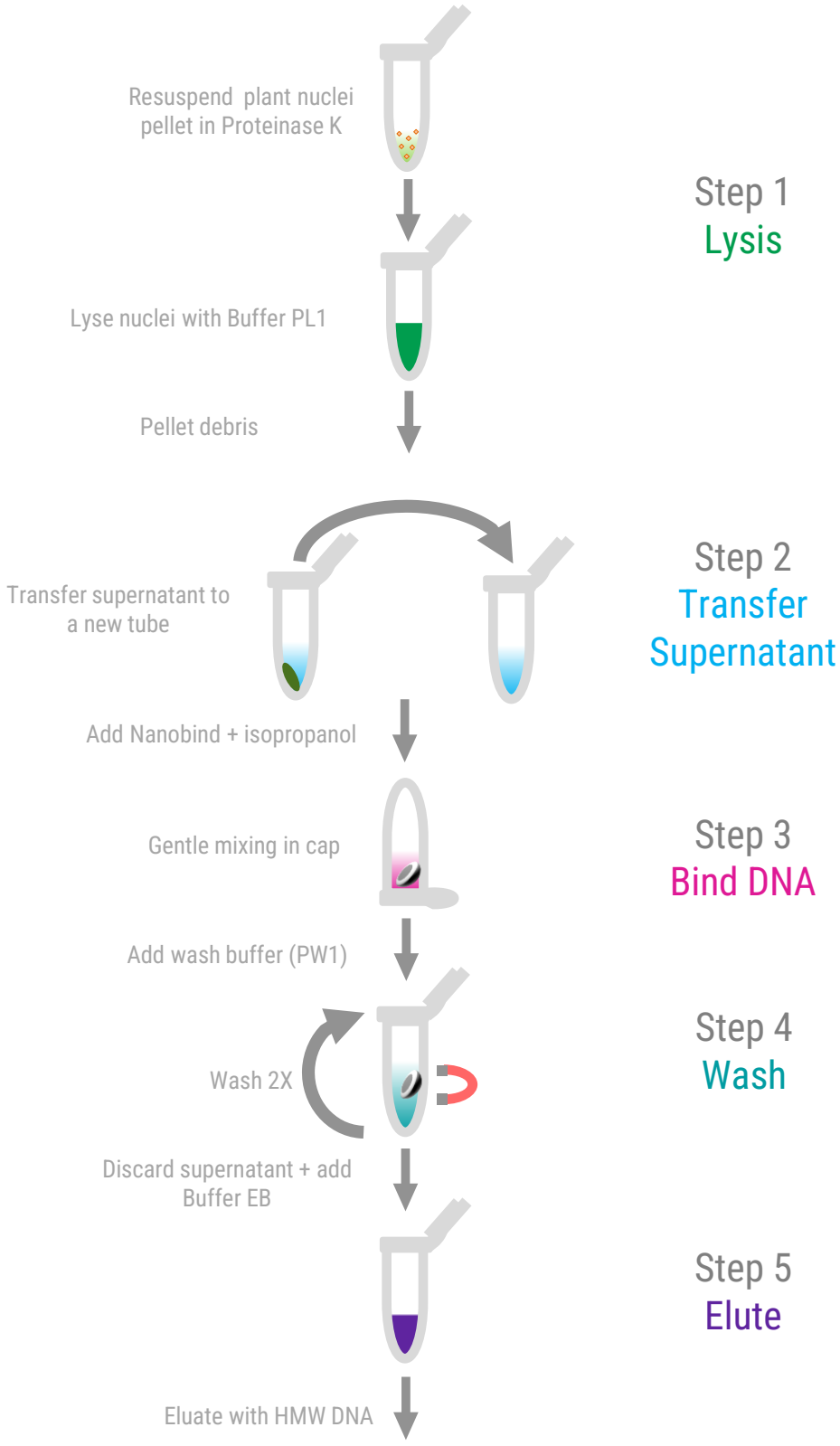
The extracted DNA is suitable for HiFi sequencing on PacBio systems. Process time is approximately 2–3 hours for the nuclei isolation and 60 minutes for the Nanobind DNA extraction.

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

Workflow – nuclei isolation



Workflow – Nanobind DNA extraction



Sample information

The following tables illustrate extraction data from a variety of plant species. All samples have been validated for PacBio sequencing.

Each sample was extracted using either the Liquid Nitrogen (LN2) and/or TissueRuptor II (TR) nuclei isolation Protocol. These protocols differ primarily in the upfront homogenization approach. For most plant species, either protocol may be used with similar results. If only a single protocol is listed, this does not indicate that the other protocol is incompatible.

Tissue disruption with TissueRuptor is faster and typically results in higher extraction yields than LN2 grinding. For some plant species, LN2 grinding may result in improved DNA size. It is recommended that users start with the TissueRuptor protocol.

Either fresh or frozen plant material can be used. 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.

Nanobind plant nuclei kit – validated plant tissues					
Sample	**Nuclei isolation protocol tested**	**tissue input1**	**DNA yield (µg)**	**260/280**	**260/230**
Arabidopsis thaliana[2]	LN2, TR*	3 g	7.4	1.8	1.6
Baby's Breath[3]	LN2	1 g	7.2	1.7	1.7
Banana leaf	LN2, TR*	1 g	13.2	1.8	1.7
Brazilian Hyacinth[4]	LN2	3 g	26.0	1.8	2.0
Coastal Redwood[5]	LN2	1 g	23.0	1.8	1.4
English Holly	LN2	0.5 g	5.6	1.9	2.0
Giant Sequoia[5]	LN2	1 g	13.3	1.8	1.4
Lavender[6]	LN2*, TR	1 g	6.2	1.8	1.8
Melon[7]	LN2, TR*	1 g	23.8	1.9	2.1
Pepper leaf[7]	LN2*, TR	1 g	7.3	1.8	1.9
Rapeseed[8]	LN2, TR*	1 g	12.5	1.8	1.9
Rice[4]	LN2	3 g	12.0	1.9	1.7
Setaria spp.[2]	LN2*, TR	1 g	3.6	1.8	1.9
Spinach	LN2	1 g	1.9	1.9	1.5
Sugar beet[7]	LN2, TR*	1 g	13.9	1.9	1.8

LN2 Liquid nitrogen nuclei isolation protocol

TR TissueRuptor nuclei isolation protocol

* Denotes the protocol used to generate the data presented in the table.

1 Input levels can be scaled up or down as necessary. Optimization may be required.

2 Work performed in collaboration with Peter Thielen at Johns Hopkins University Applied Physics Laboratory.

3 Work performed in collaboration with Dr. Charlyn Partridge at Grand Valley State University.

4 Work performed in collaboration Arizona Genomics Institute at University of Arizona.

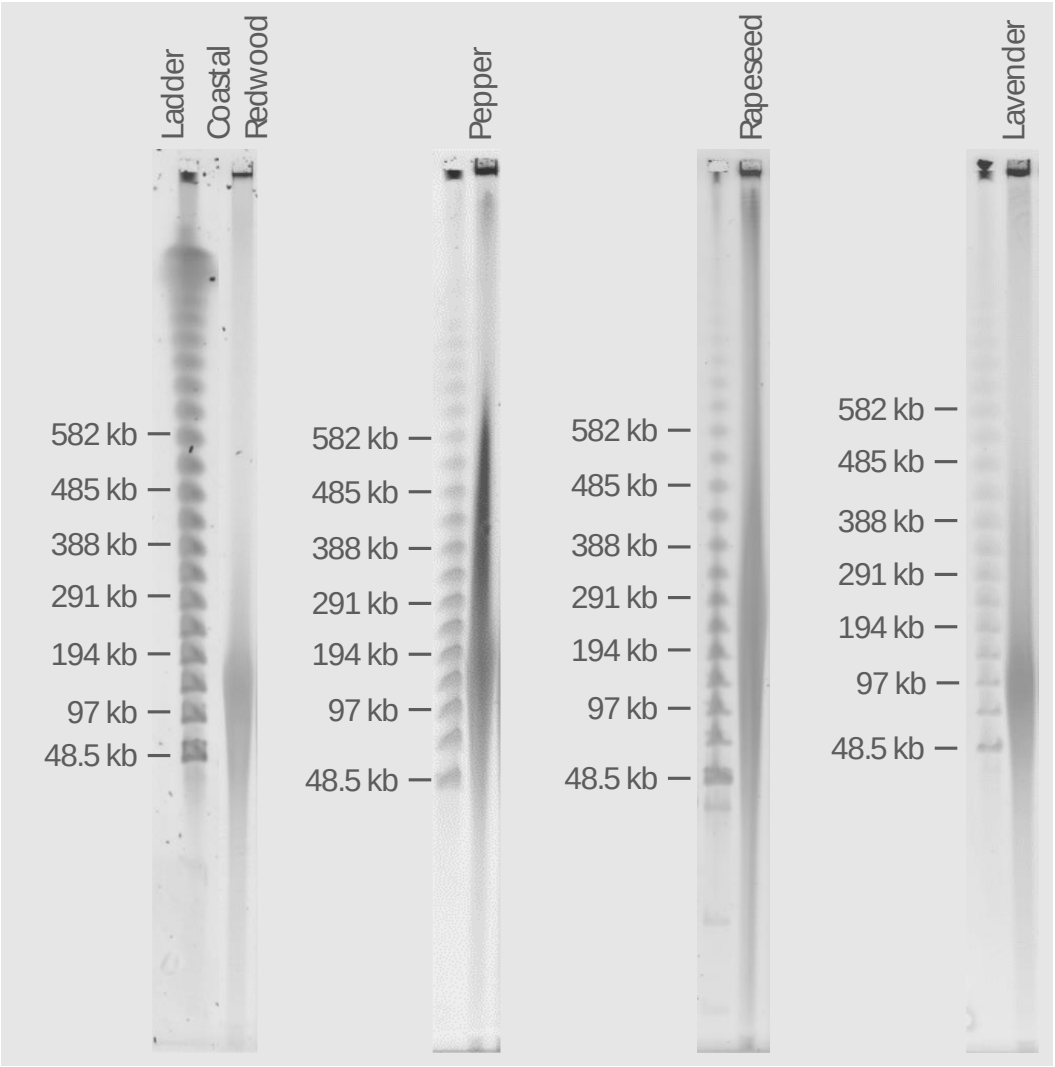
5 Work performed in collaboration with Timp Lab at Johns Hopkins University.

6 Work performed in collaboration with Buell Lab at Michigan State University.

7 Work performed in collaboration with KeyGene N.V.

DNA size

The size of the genomic DNA will vary depending on plant species, the quality of the starting material, the nuclei isolation protocol used, and processing parameters during Nanobind purification. The HMW DNA extraction protocol typically yields DNA in the 50–300+ kb size range, with some samples sizing larger and some samples sizing smaller.

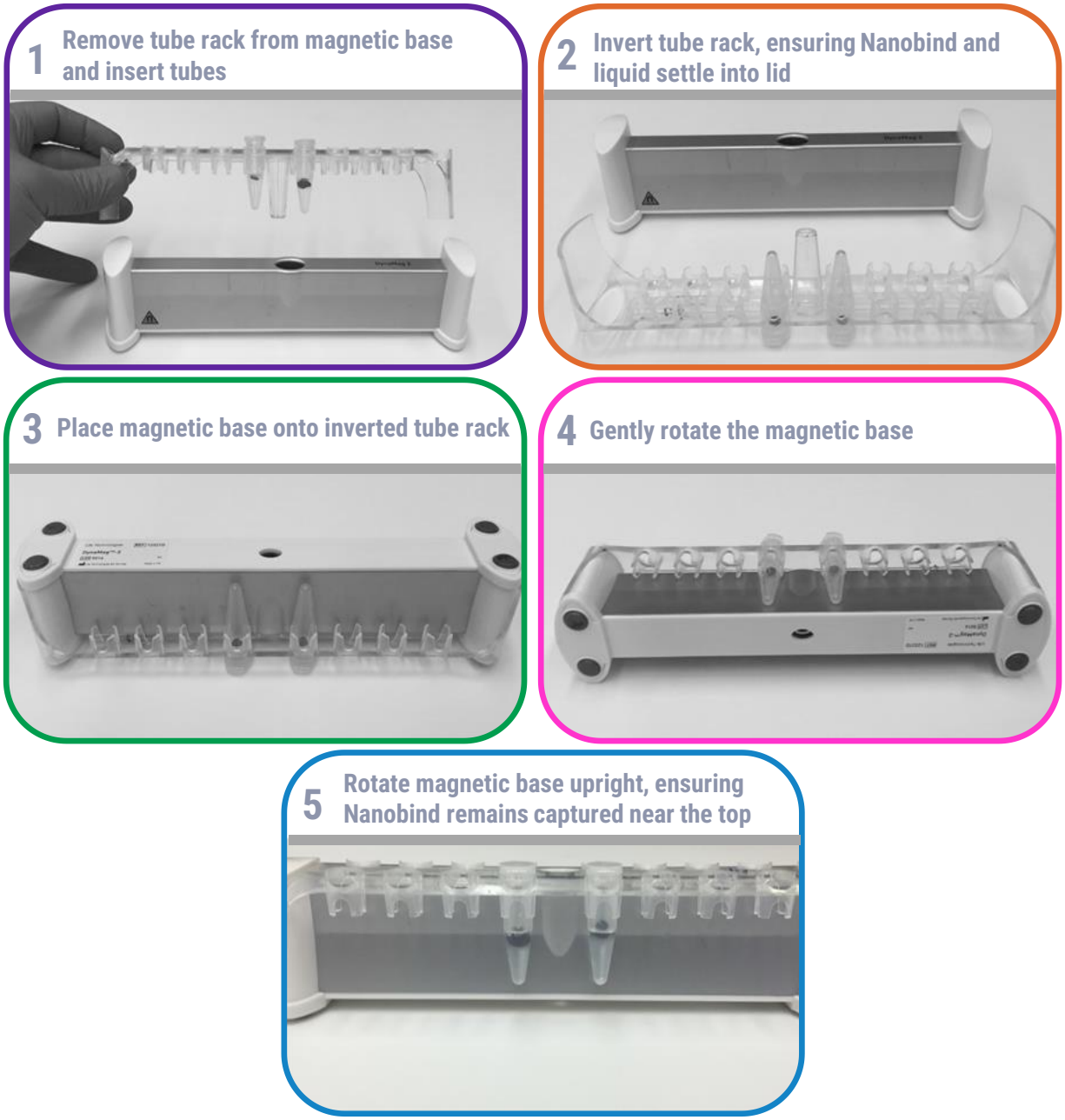


Pulsed Field Gel Electrophoresis (PFGE) illustrating DNA extracted using the Nanobind Plant Nuclei Extraction Kit.

Processing tips

Magnetic rack handling procedure

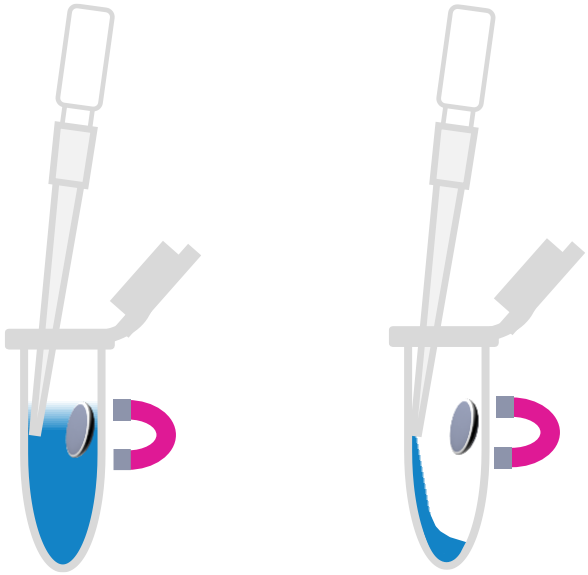
To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that is used with a magnetic base. Although DNA is bound quite robustly, proper pipetting and handling will ensure thorough washing and minimize disturbance of the bound DNA. For best results,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



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

Pipetting

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



Pipetting procedure for removal (left) and addition (right) of liquid during wash steps.

Heterogeneity and viscosity

The extracted HMW DNA can be highly viscous and heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The heterogeneity and viscosity of the DNA eluate will vary depending on sample type, DNA size, sample input, and processing parameters. More gentle processing will yield larger DNA size but will also result in higher heterogeneity and larger amounts of highly viscous, unsolubilized "jellies." Processing that is too gentle can dramatically reduce DNA purity and yield. To minimize the challenges of heterogeneity and viscosity, we recommend that new users err on the side of being overly aggressive. Listed below are tips for working with HMW DNA.

Following elution of the HMW DNA:

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

In some cases, the extracted DNA will be very heterogeneous and contain large amounts of unsolubilized “jellies”:

The most common reason for high sample heterogeneity and low purity is insufficient mixing during lysis. More aggressive mixing will result in samples with improved purity due to more efficient lysis and digestion. Improved sample purity will lead to improved DNA homogeneity and reduced “jellies.” Aggressive mixing during lysis will not significantly impact DNA length.

To accurately quantify the HMW DNA:

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

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

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

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

Shearing HMW DNA

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

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

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

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

Nuclei isolation protocols

As of the document release date, the following protocols are available for nuclei isolation from plant samples. They have been optimized for use in downstream HMW DNA extraction with the Nanobind plant nuclei kit (102-302-000).

Tissue disruption with TissueRuptor is faster and typically results in higher extraction yields than LN2 grinding. For some plant species, LN2 grinding may result in improved DNA size. It is recommended that users start with the TissueRuptor protocol.

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

Isolating nuclei from plant tissue using TissueRuptor disruption (102-574-900)

This protocol describes the isolation of nuclei from plant tissues using QIAGEN TissueRuptor II for tissue disruption. This protocol has been validated on plants including banana, lavender, melon, pepper, rapeseed, and sugar beet.

Isolating nuclei from plant tissue using LN2 disruption (102-574-800)

This protocol describes the isolation of nuclei from plant tissues using liquid nitrogen grinding for tissue disruption. This protocol has been validated on plants including baby's breath, coastal redwood, English holly, lavender, melon, pepper, rapeseed, and sugar beet.

HMW (50–300+ kb) DNA extraction protocols

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

Extracting HMW DNA from plant nuclei using Nanobind kits (102-574-200)

This protocol describes the extraction of HMW DNA from plant nuclei. It is recommended for all standard long-read sequencing applications on PacBio instruments. This protocol has been validated on baby's breath, banana, coastal redwood, English holly, fescue, giant sequoia, lavender, melon, pepper, rapeseed, rice, and sugar beet. This protocol requires the Nanobind plant nuclei kit (102-302-000).

QC procedures

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

See individual HMW 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.

Revision history (description)	Version	Date
Initial release	01	July 2022
Minor updates throughout	02	July 2022
Minor updates, deleted old PacBio sequencing information	03	December 2022
Minor updates, deleted UHMW DNA information	04	May 2023