Nanobind Plant Nuclei Big DNA Kit Handbook

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

circulomics

Table of Contents

Kit Specifications

Contents

Nanobind Plant Nuclei Big DNA Kit Part Number Number of Samples	v1 NB-900-801-01 20
Nanobind Disks	20
Proteinase K	0.66 mL
RNase A	0.25 mL
Buffer PL1	2.0 mL
Buffer PW1 Concentrate (Dilute to 70% final ethanol concentration as indicated on the bottle)	7.5 mL (25 mL after EtOH)
Buffer EB	5 mL

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

RNase A should be stored at 4 °C upon arrival.

Nanobind disks and all other buffers should be stored at room temperature (18–25 °C).

Product Use

Nanobind Plant Nuclei Big DNA Kits are intended for research use only.

Version History

See Nanobind Big DNA Kit Version History Document (www.circulomics.com/support-nanobind.com) for a list of kit and protocol changes.

Equipment and Reagent List

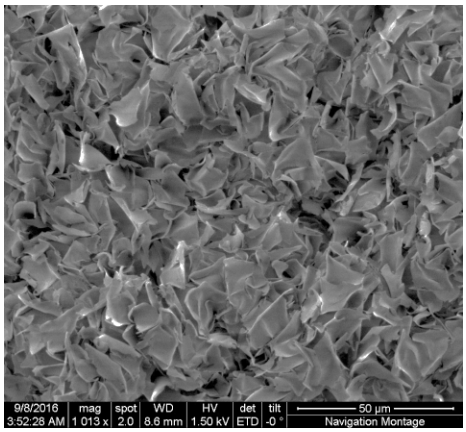
Equipment/Reagent	Manufacturer (Part Number)
Microcentrifuge	Eppendorf (5415R)
HulaMixer	Thermo Fisher (15920D)
Magnetic Tube Rack	Thermo Fisher DynaMag-2 (12321D)
Mini-Centrifuge	Ohaus Mini-Centrifuge (FC5306)
ThermoMixer	Eppendorf (5382000023)
1.5 mL Protein LoBind Microcentrifuge Tubes	Eppendorf (022431081)
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)

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.

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

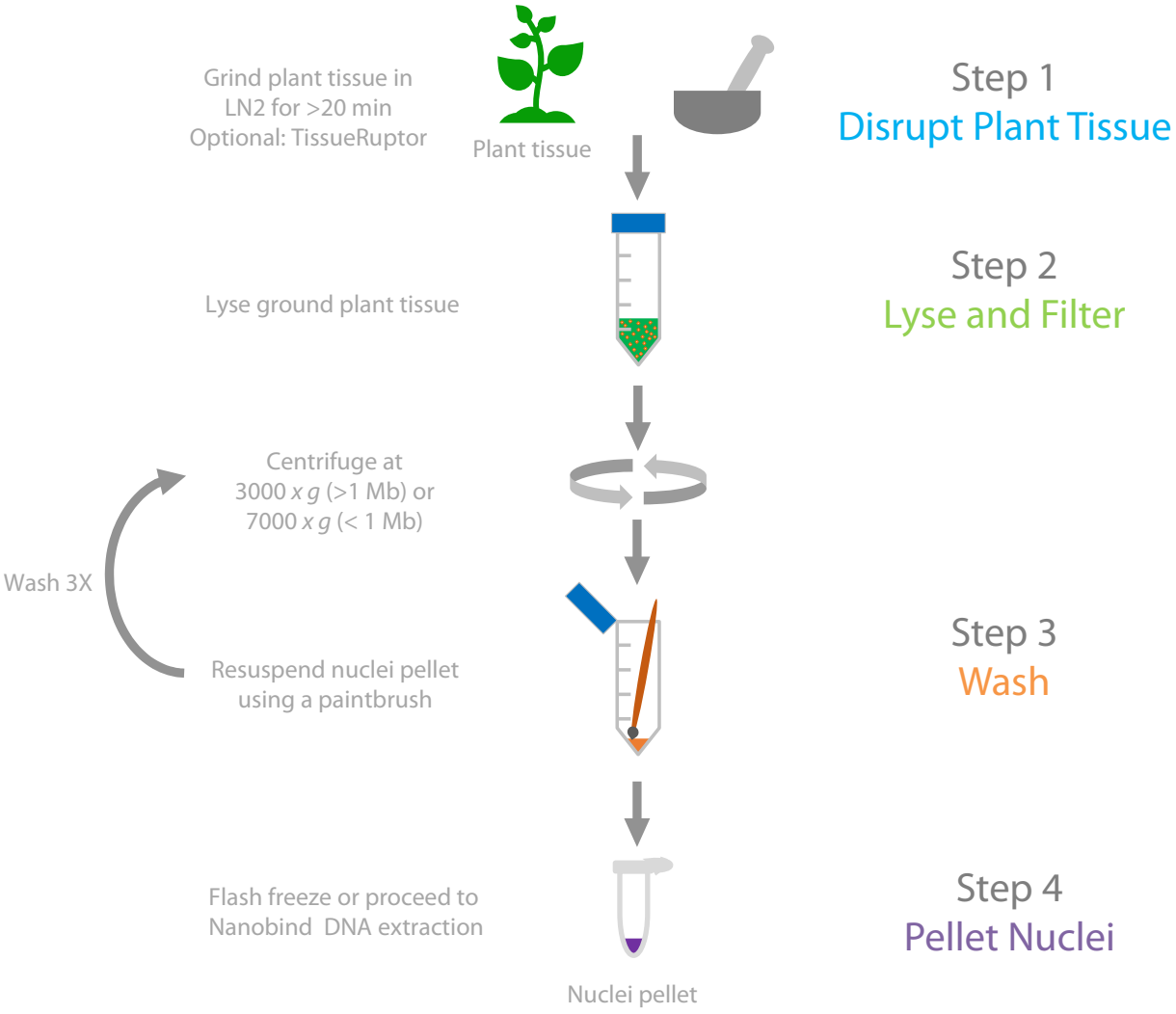
The Nanobind Plant Nuclei Big DNA Kit is used for extraction of HMW (50 kb – 300+ kb) DNA from plant nuclei. First, nuclei are isolated from 1–5 g of plant tissue using one of the recommended nuclei isolation protocols. Then, HMW DNA is extracted from the nuclei using Nanobind disks. Each of the two 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 analysis on long-range genomics platforms including PacBio RSII/Sequel/Sequel II, Oxford Nanopore MinION/GridION/PromethION, and Bionano Genomics Irys/Saphyr. Process time is approximately 2–3 hours for the nuclei isolation and 60 minutes for the Nanobind DNA extraction.

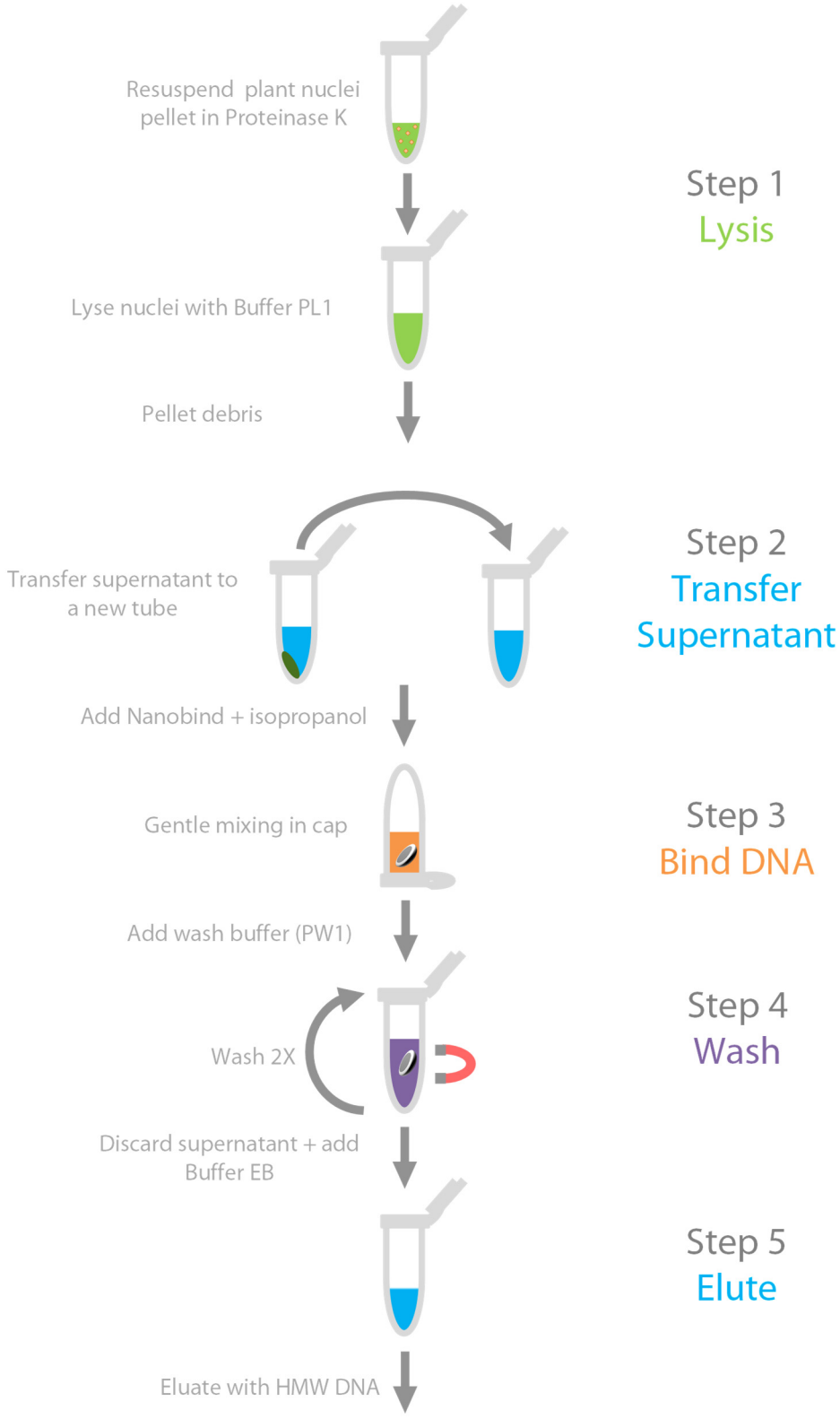
The **Sample Information** section provides example extraction and sequencing results from a wide variety of plant species.

Protocols listed in the **Nuclei Isolation Protocols** and **HMW (50 kb – 300+ kb) DNA Extraction Protocols** sections are updated frequently so please check the Nanobind Support page for the most up-to-date list and for the current version of the protocol (http://www.circulomics.com/support-nanobind).

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 by PacBio and/or Oxford Nanopore 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 protocols 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. Please contact us for additional details.

Nanobind Plant Nuclei Big DNA Kit – Validated Plant Tissues

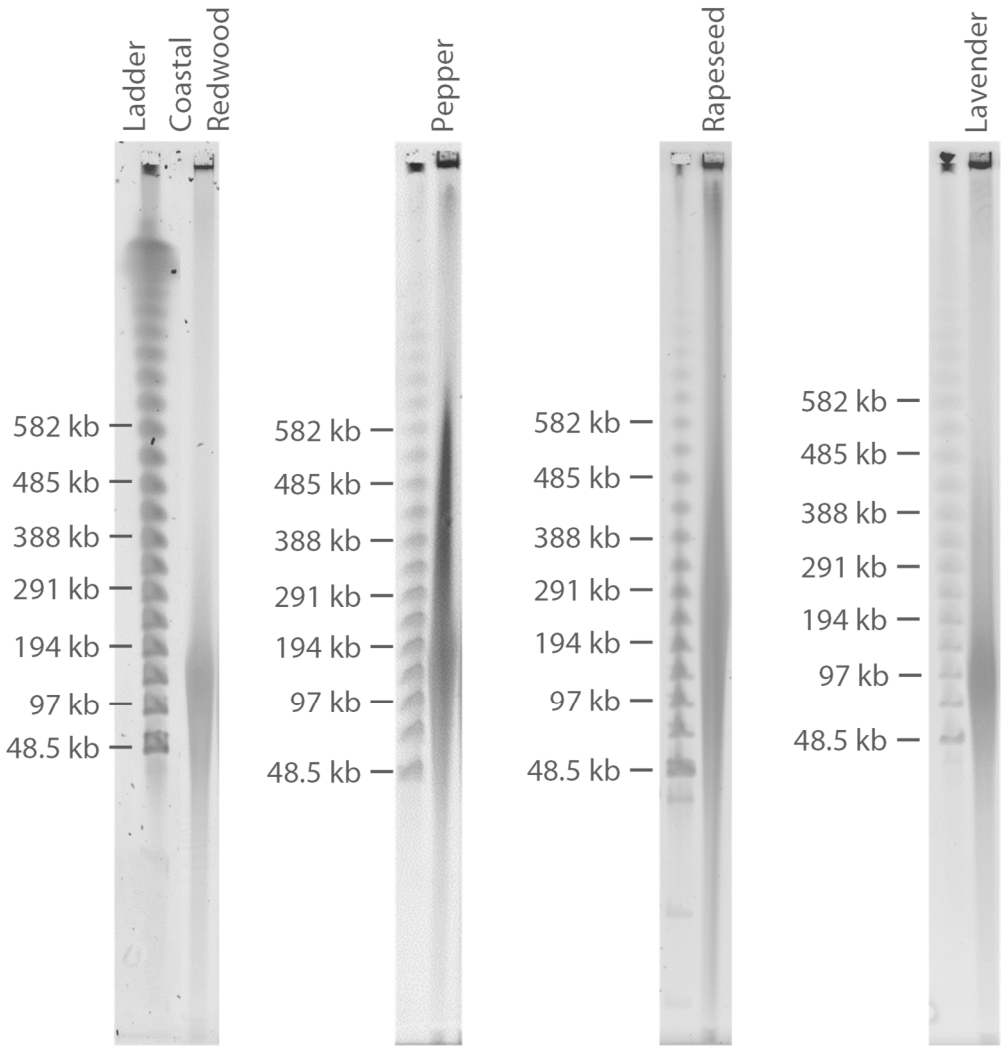
Sample	Nuclei Isolation Protocol Tested	Tissue Input[1]	DNA Yield (µg)	260/280	260/230	PacBio Validated	ONT Validated
Arabidopsis thaliana[2]	LN2, TR*	3 g	7.4	1.8	1.6		Y
Baby's Breath[3]	LN2	1 g	7.2	1.7	1.7	C	Y
Banana leaf	LN2, TR*	1 g	13.2	1.8	1.7	C	
Brazilian Hyacinth[4]	LN2	3 g	26.0	1.8	2.0	C	
Coastal Redwood[5]	LN2	1 g	23.0	1.8	1.4	C	Y
English Holly	LN2	0.5 g	5.6	1.9	2.0		Y
Giant Sequoia[5]	LN2	1 g	13.3	1.8	1.4		Y
Lavender[6]	LN2*, TR	1 g	6.2	1.8	1.8		Y
Melon[7]	LN2, TR*	1 g	23.8	1.9	2.1		Y
Pepper leaf[7]	LN2*, TR	1 g	7.3	1.8	1.9		Y
Rapeseed[8]	LN2, TR*	1 g	12.5	1.8	1.9		Y
Rice[4]	LN2	3 g	12.0	1.9	1.7	C	
Setaria spp.[2]	LN2*, TR	1 g	3.6	1.8	1.9	C	
Spinach	LN2	1 g	1.9	1.9	1.5		Y
Sugar beet[7]	LN2, TR*	1 g	13.9	1.9	1.8		Y

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.
Y Yes
C Customer extracted and sequenced

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 kb – 300+ kb size range, with some samples sizing larger and some samples sizing smaller.



Pulsed Field Gel Electrophoresis (PFGE) images illustrating DNA extracted from nuclei isolated from coastal redwood, pepper, rapeseed, and lavender using the Nanobind Plant Nuclei Big DNA Extraction Kit.

PacBio Sequencing

HMW DNA was extracted from various plant samples. The samples were then sequenced on PacBio RS II, Sequel I, or Sequel II using CLR or HiFi workflows. HMW DNA is recommended for standard CLR and HiFi sequencing.

PacBio Sequel I CLR Sequencing					
Sample	**Protocol**	**Library Prep**	**Polymerase Length N50 (bp)**	**Subread Length N50 (bp)**	**Total Data (Gb)**
Coastal Redwood[1]	LN2	5X NS, 30 kb BP Express Template 1.0	35,558	35,558	5.6
Rice[2]	LN2	No shear, 30 kb BP Express Template 1.0	31,750	29,750	8.5
Brazilian Hyacinth[2]	LN2	No shear, 30 kb BP Express Template 1.0	31,750	29,750	11.0
Baby's Breath[3]	LN2	No shear, 10-50 kb BP Express Template 1.0	N/A	24,000	9.0

PacBio Sequel II HiFi Sequencing						
Sample	**Protocol**	**Library Prep**	**Total Bases (Gb)**	**≥Q20 Mean Read Length (bp)**	**≥Q20 Read Yield (Gb)**	**≥Q20 Median Read Quality**
Setaria spp[4]	TR	SageELF Express Template 2.0	380	14,894	26.0	30
Coastal Redwood[5]	LN2	SageELF Express Template 2.0	-	24,397	23.1	27

LN2 Liquid nitrogen nuclei isolation protocol
TR TissueRuptor nuclei isolation protocol
NS Needle shear
BP BluePippin size selection
1 Work performed in collaboration with Timp Lab at Johns Hopkins University
2 Data generated in collaboration Arizona Genomics Institute at University of Arizona and PacBio
3 Work performed in collaboration with Dr. Charlyn Partridge at Grand Valley State University.
4 Work performed in collaboration with Peter Thielen at Johns Hopkins University Applied Physics Laboratory.
5 Data generated by PacBio

Oxford Nanopore Sequencing

HMW DNA was extracted from various plant samples. The samples were then sequenced on Oxford Nanopore MinION and GridION using the Ligation Sequencing Kits. HMW DNA is recommended for standard long-read sequencing using the Ligation Sequencing Kit (SQK-LSK108/109/110) and Rapid Sequencing Kit (SQK-RAD004).

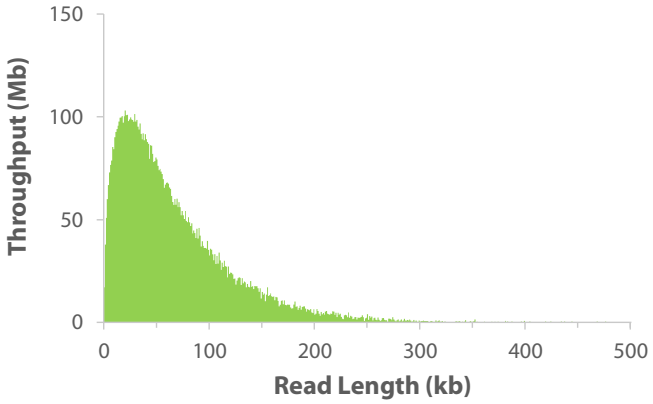
Oxford Nanopore MinION/GridION				
Sample	**Library Prep**	**Read Length N50 (bp)**	**Longest Read (bp)**	**Total Data (Gb)**
Giant Sequoia[1]	Megaruptor (8 kb), No SS LSK109, FLO-MIN106D, 48 h	7,039	121,596	7.0
Coastal Redwood[1]	Covaris G-tube (8 kb), No SS LSK109, FLO-MIN106D, 48 h	6,567	78,241	10.1
Coastal Redwood[1]	5X NS, No SS LSK109, FLO-MIN106D, 48 h	29,196	227,249	3.3
Raspberry[1]	5X NS, No SS LSK109, FLO-PRO002, 48 h	14,616	821,689	47.9

NS Needle shear
BP BluePippin size selection
1 Work performed in collaboration with Timp Lab at Johns Hopkins University

Nanobind Ultra Long Sequencing

Nanobind Ultra Long Sequencing was performed on a pepper leaf sample and sequenced on GridION. These data are preliminary and shown for reference purposes.

- UHMW DNA was extracted from 5 g of pepper leaf using the Nuclei Isolation – LN2 Plant Tissue (NUC-LNP-001) and HMW DNA Extraction – Plant Nuclei (EXT-PLH-002) Protocols.
- A 3X library was prepared and sequenced on a R9.4 MinION Flow Cell (FLO-MIN106D) using the Nanobind Library Prep – Ultra Long Sequencing Protocol (LBP-ULN-001).
- Development is underway to enhance read length and throughput.
- Data generated in collaboration with Keygene NV.

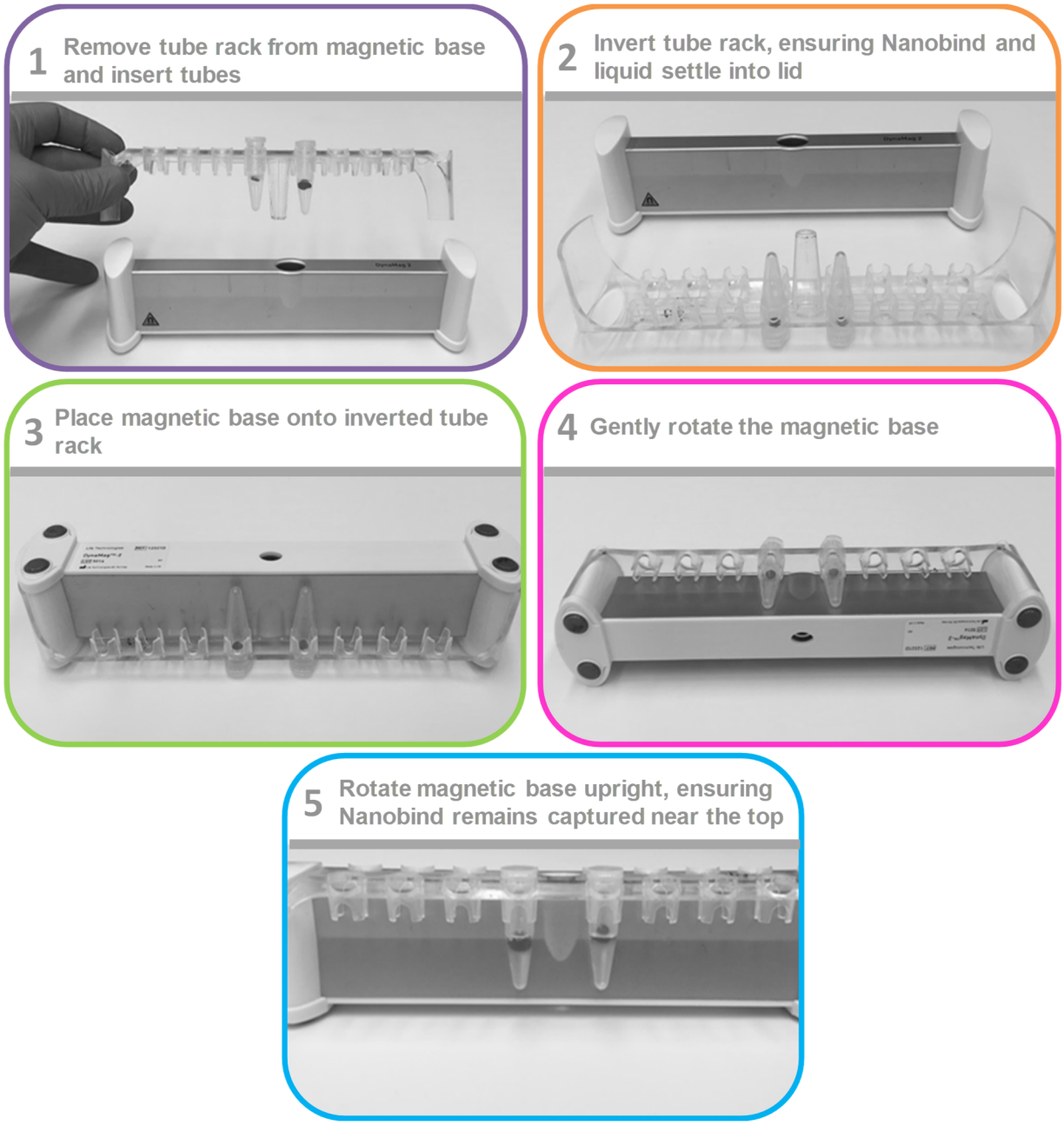


Nanobind Ultra Long Sequencing – Pepper						
Instrument	**Total Bases (Gb)**	**N50 (kb)**	**Gb >100 kb**	**Gb >200 kb**	**Number Reads > 1 Mb**	**Longest Read (Mb)**
GridION	9.0	51.5	1.9	0.3	0	0.52

Processing Tips

Magnetic Rack Handling Procedure

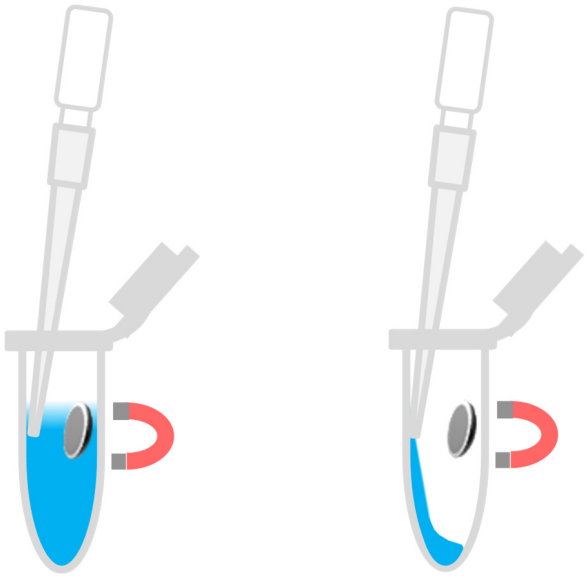
To capture the Nanobind disk and enable simple processing, the microcentrifuge tubes are placed in a tube rack that is used with a magnetic base. Although DNA is bound quite robustly, proper pipetting and handling will ensure thorough washing and minimize disturbance of the bound DNA. For best results,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

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

Following elution of the HMW DNA:

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

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

The most common reason for high sample heterogeneity and low purity is insufficient mixing during lysis. More aggressive mixing will result in samples with improved purity due to more efficient lysis and digestion. Improved sample purity will lead to improved DNA homogeneity and reduced "jellies." Aggressive mixing during lysis will not significantly impact DNA length. Mixing may be carefully scaled back by skilled users to achieve bigger DNA. For UHMW DNA, users should follow the appropriate UHMW DNA extraction protocol.

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.

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 and UHMW DNA extraction with the Nanobind Plant Nuclei Big DNA Kit (NB-900-801-01).

Protocols are updated and added frequently. ***Please refer to the Circulomics Support Page (https://www.circulomics.com/support-nanobind) for the latest versions and the appropriate Nanobind Kit Handbook for additional data and guidance.***

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.

Contact us if you have any questions about which protocol should be used (support@circulomics.com).

Nuclei Isolation – TissueRuptor Plant Tissue (NUC-TRP-001)

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.

Nuclei Isolation – LN2 Plant Tissue (NUC-LNP-001)

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 kb – 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 CLR and HiFi sequencing and nanopore sequencing using Oxford Nanopore Ligation and Rapid Sequencing Kits. A preliminary protocol is also included for Nanobind Ultra Long Sequencing.

Protocols are updated and added frequently. ***Please refer to the Circulomics Support Page (https://www.circulomics.com/support-nanobind) for the latest versions and the appropriate Nanobind Kit Handbook for additional data and guidance.***

Contact us if you have any questions about which protocol should be used (support@circulomics.com).

HMW DNA Extraction – Plant Nuclei (EXT-PLH-001)

This protocol describes the extraction of HMW DNA from plant nuclei. It is recommended for all standard long-read sequencing applications on PacBio and Oxford Nanopore 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 Big DNA Kit (NB-900-801-01).

HMW DNA Extraction – Plant Nuclei (EXT-PLH-002)

This protocol describes the extraction of HMW DNA from plant nuclei. It is recommended only for ultra long sequencing on Oxford Nanopore instruments. This protocol has been validated on pepper. This protocol requires the 1) Nanobind Plant Nuclei Big DNA Kit (NB-900-801-01), 2) Nanobind UL Library Prep Kit (NB-900-601-01), and 3) UHMW DNA Aux Kit (NB-900-101-01).

QC Procedures

Accurate quantification of HMW and UHMW 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 and UHMW 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 2.0.

1. **Isolate HMW DNA using Nanobind Big DNA Kit.**
2. **Perform a 5X needle shear using a 26g blunt end needle.**
    - Light needle shearing will improve sequencing throughput, DNA handling, and AMPure recovery during library preparation without noticeably affecting read length. The DNA concentration during needle shearing should be >100 ng/µL. Lower DNA concentration will result in increased rates of shearing.
    - More aggressive shearing to 10–30 kb using Covaris G-tube or Diagenode Megaruptor can be used to obtain a fixed insert size.
3. **Prepare DNA for sequencing using Procedure & Checklist - Preparing gDNA Libraries Using the SMRTbell Express Template Preparation Kit 2.0**
    - For BluePippin size selected CLR libraries, begin with 5 µg of HMW DNA input and use a >30 kb cutoff.
    - For SageELF or BluePippin size selected HiFi libraries, begin with 20 µg of sheared HMW DNA.
    - For Short Read Eliminator size selected CLR or HiFi libraries, begin with 5 µg of HMW DNA input.

Oxford Nanopore Sequencing Recommendations

Below are our standard conditions and tips for sequencing on Oxford Nanopore MinION/GridION using the 1D Ligation Sequencing Kits (SQK-LSK109).

1. **Isolate HMW DNA using Nanobind Big DNA Kit.**
2. **Perform a 5X needle shear using a 26g blunt end needle.**
    - Light needle shearing will improve sequencing throughput, DNA handling, and AMPure recovery during library preparation without noticeably affecting read length. The DNA concentration during needle shearing should be >100 ng/µL. Lower DNA concentration will result in increased rates of shearing.
    - More aggressive shearing to 10–30 kb using Covaris G-tube or Diagenode Megaruptor can greatly increase throughput at the cost of read length.
3. **Optional – Size select sample using Circulomics Short Read Eliminator.**
    - Size selection can be used to improve read length distribution by depleting short DNA.
4. **Prepare DNA for sequencing using the 1D Genomic DNA by Ligation (SQK-LSK109) Protocol.**
    - For size selected samples, we generally begin library preparation with 2–4 µg of size selected HMW DNA (42–83 ng/µL). After library preparation, we generally end up with 700–1500 ng of DNA ready for sequencing.
    - For non-size selected samples, we generally begin library preparations with 1–1.5 µg of HMW DNA (21–31 ng/µL). After library preparation, we generally end up with 200–500 ng of DNA ready for sequencing.
5. **Library Preparation Notes**
    - DNA precipitation may be visible during the adapter ligation step, especially when large amounts of HMW DNA are input into the reaction. This does not appear to have any significant impact on sequencing throughput.
    - We typically elute the AMPure cleanups for 10 minutes and 20 minutes after the end-prep and ligation steps, respectively.
6. **Load a constant molar concentration of library onto the flow cell.**
    - For a size selected DNA sample, we recommend loading 800–1000 ng of DNA onto the flow cell.
    - For a non-size selected DNA sample, we recommend loading 300–500 ng onto the flow cell.
    - For a moderately sheared DNA sample (i.e. 30 kb shear), we recommend loading 300 ng or less onto the flow cell.
    - For consistent throughputs, more should be loaded for libraries with higher average size and less should be loaded for libraries with shorter average size. Both overloading and underloading of the flow cell will decrease sequencing throughput as a constant molar concentration of DNA library is ideal.