Nanobind Plant Nuclei Big DNA Kit

Handbook v0.18 (10/2018)

For extraction of high molecular weight genomic DNA (50–300+ kb) from plant nuclei

Table of Contents

Kit Specifications

Contents

Nanobind Plant Nuclei Big DNA Kit Part Number Number of Samples	Alpha Version 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.

Note: Buffer CW2 was renamed to Buffer PW1 to minimize confusion across kits. Buffer CW2 at 70% final ethanol concentration is equivalent to Buffer PW1 at 70% final ethanol concentration. For plant nuclei, these wash buffers should only be used at 70% final ethanol concentration.

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.

User Supplied Equipment and Reagent List

Equipment	Model
Magnetic Tube Rack	Thermo Fisher DynaMag-2 (12321D)
HulaMixer	Thermo Fisher (15920D)
ThermoMixer	Eppendorf (5382000023)
Mini-Centrifuge	Ohaus Mini-Centrifuge (FC5306)
1.5 mL Protein LoBind Microcentrifuge Tubes	Eppendorf (022431081)
200 µL Wide Bore Pipette Tips	USA Scientific (1011-8410)
Ethanol (96–100%)	
Isopropanol (100%)	
UV/Vis	Thermo Fisher Scientific NanoDrop 2000
Fluorescent DNA Quantification	Thermo Qubit 3.0, dsDNA BR and RNA BR Assay Kits

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 (**Figure 1**). The Nanobind disk can be used for rapid extraction of high quality DNA and RNA. It uses a standard lyse, bind, wash, and elute procedure that is common for silica DNA extraction technologies (see **Workflow**). However, unlike magnetic beads and silica spin columns which shear large DNA, Nanobind binds and releases DNA without fragmentation, resulting in high purity, high molecular weight (HMW), and ultra high molecular weight (UHMW) DNA. The high surface area and unique binding mechanism give it an extraordinary binding capacity, allowing isolation of >200 µg of DNA in a 1.5 mL format.

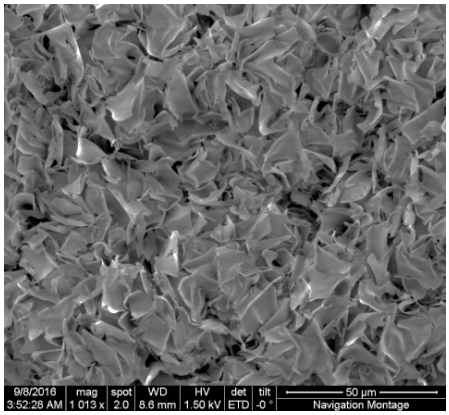


Figure 1. SEM image of Nanobind's silica surface structure.

The Nanobind Plant Nuclei Extraction kit can be used for the extraction of HMW genomic DNA from intact plant nuclei. Process time is approximately 60 minutes. A protocol is provided for extraction of HMW (50 kb – 300+ kb) DNA (**Figure 2**).

The extracted DNA has been used on a variety of genomics platforms including PacBio RSII/Sequel, Oxford Nanopore MinION/GridION/PromethION, and 10X Genomics Chromium.

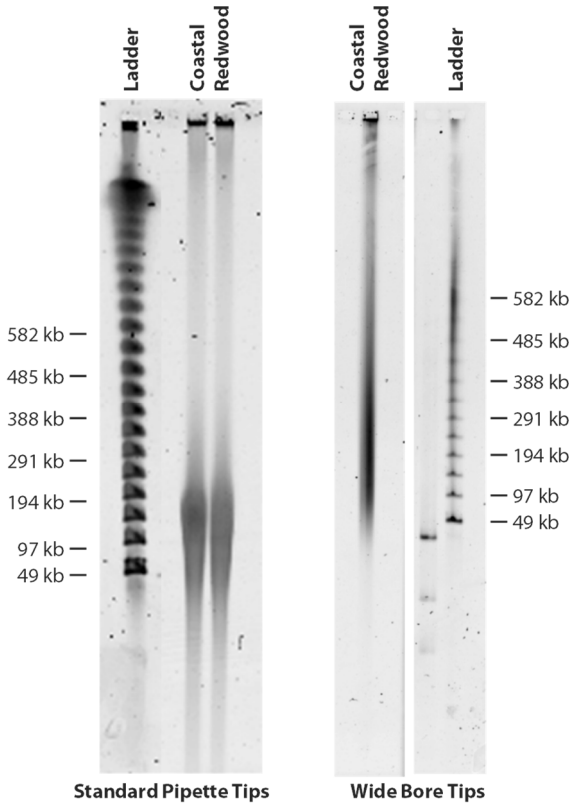


Figure 2. Pulsed Field Gel Electrophoresis (PFGE) illustrating HMW DNA extracted from coastal redwood nuclei using the Nanobind Plant Nuclei Big DNA Extraction Kit. The gel images show HMW DNA processed with standard (left) vs. wide bore-pipette tips (right).

Sample Information and Expected Yields

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.

Nanobind Plant Nuclei Big DNA Kit – Expected Yields				
Sample	**Example Input[1]**	**Example 260/280**	**Example 260/230**	**Example Yield (µg)**
Giant Sequoia[2]	~1 g of plant material	1.8	1.4	13.3
Coastal Redwood[2]	~1 g of plant material	1.8	1.4	23.0
Maize[3]	~1 g of plant material	1.9	1.9	5.8
Rice[4]	~3 g of plant material	1.9	1.7	12.0
Brazilian Hyacinth[4]	~3 g of plant material	1.8	2.0	26.0

[1]Higher input levels can be used, though optimization of buffer volumes and enzyme levels will be necessary. Optimization may be necessary based on plant species.

[2]Nuclei extraction performed using method of Workman *et al*. Protocol Exchange (2018) DOI:10.1038/protex.2018.059. Work performed in collaboration with Timp Lab at Johns Hopkins University.

[3]Nuclei extraction performed using method of Workman *et al*. Protocol Exchange (2018) DOI:10.1038/protex.2018.059. Work performed in collaboration with Buell Lab at Michigan State University and Timp Lab at Johns Hopkins University.

[4]Work performed in collaboration Arizona Genomics Institute at University of Arizona and PacBio®.

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 (**Figure 3)**, with some samples sizing larger and some samples sizing smaller.

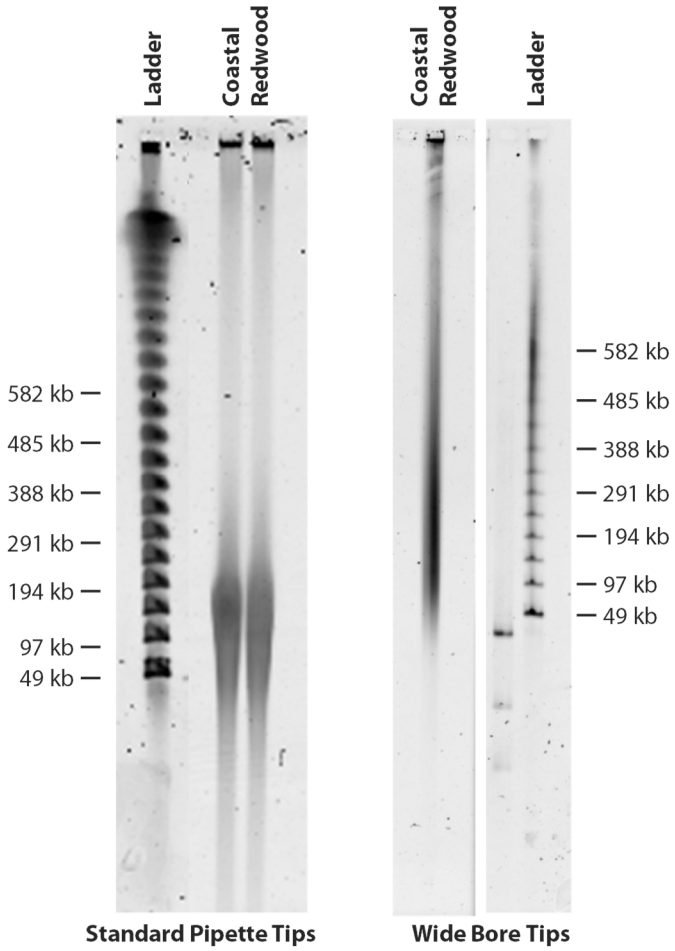


Figure 3. Pulsed Field Gel Electrophoresis (PFGE) illustrating DNA extracted from coastal redwood nuclei using the Nanobind Plant Nuclei Big DNA Extraction Kit. The gel images show HMW DNA processed with standard (left) vs. wide-bore pipette tips (right).

PacBio Sequencing

Sequencing validation of HMW DNA extracted from several sample types using the Nanobind Plant Nuclei Big DNA Kit was performed using the PacBio® Sequel® long read sequencing platform. The following tables show typical sequencing results.

Nanobind Plant Nuclei Big DNA Kit – PacBio Sequel				
Sample	**260/280**	**260/230**	**Subread Length N50 (bp)**	**Total Data (Gb)**
Coastal Redwood[1]	1.8	1.7	35,558	5.6
Rice[2]	1.9	1.7	29,750	8.5
Brazilian Hyacinth[2]	1.8	2.0	29,750	11.0

HMW DNA (up to 300 kb) was extracted using the Nanobind Plant Nuclei Big DNA Kit and used with the PacBio SMRTbell® Express Template Prep Kit to prepare >30 kb libraries. The libraries were then sequenced on the PacBio Sequel® System (10-hour movie, Sequel® Sequencing Kit 2.0, Sequel® Binding Kit 2.1). The coastal redwood samples were needle sheared 5X before entering library preparation.

[1]Nuclei extraction performed using method of Workman *et al*. Protocol Exchange (2018) DOI:10.1038/protex.2018.059. Data generated in collaboration with Timp Lab at Johns Hopkins University and PacBio®.

[2]Data generated in collaboration Arizona Genomics Institute at University of Arizona and PacBio®.

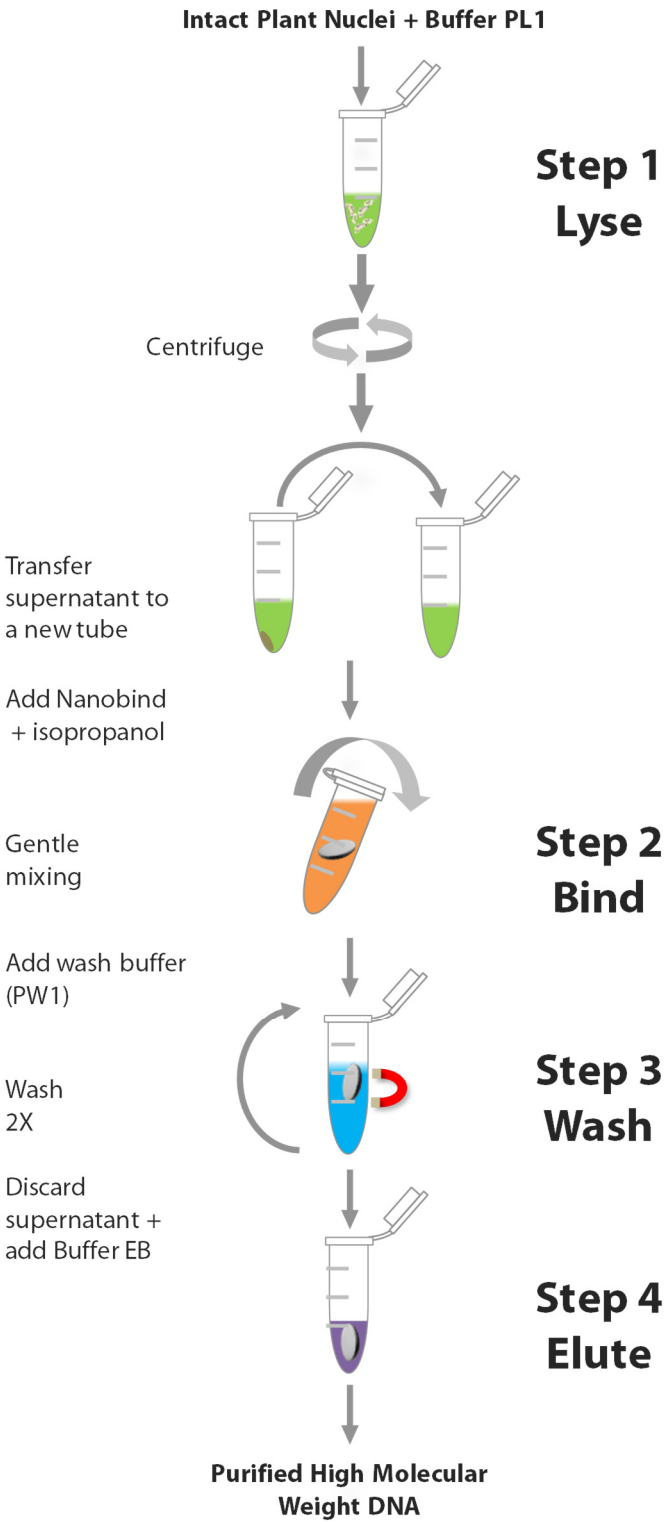
Oxford Nanopore Sequencing

Sequencing validation of HMW DNA extracted from several sample types using the Nanobind Plant Nuclei Big DNA Kit was performed using Oxford Nanopore GridION and MinION long read sequencing. The following tables show typical sequencing results.

Nanobind Plant Nuclei Big DNA Kit – ONT MinION/GridION Sequencing				
Sample	**Library Prep**	**Read Length N50 (bp)**	**Longest Read (bp)**	**Total Data (Gb)**
Giant Sequoia	Megaruptor (8 kb), No size selection	7,039	121,596	7.0
Coastal Redwood	Covaris G-tube (8 kb), No size selection	6,567	78,241	10.1
Coastal Redwood	5X needle shear, No size selection	29,196	227,249	3.3

Typical sequencing metrics from HMW DNA extracted using the Nanobind Plant Nuclei Big DNA Kit. The HMW DNA was prepared using the Oxford Nanopore Ligation Sequencing Kit 1D (SQK-LSK108) and sequenced on either Oxford Nanopore MinION or GridION (FLO-MIN106). Data generated in collaboration with Timp Lab at Johns Hopkins University.

Workflow


Intact Plant Nuclei + Buffer PL1
Step 1
Lyse
Centrifuge
Transfer
supernatant to
a new tube
Add Nanobind
+ isopropanol
Gentle
mixing
Step 2
Bind
Add wash buffer
(PW1)
Wash
2X
Step 3
Wash
Discard
supernatant +
add Buffer EB
Step 4
Elute
Purified High Molecular
Weight DNA

Processing Tips

Magnetic Rack Handling Procedure

To capture the Nanobind disk and enable simple processing, the 1.5 mL 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 disturbances 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 (**Figure 4)** is recommended.

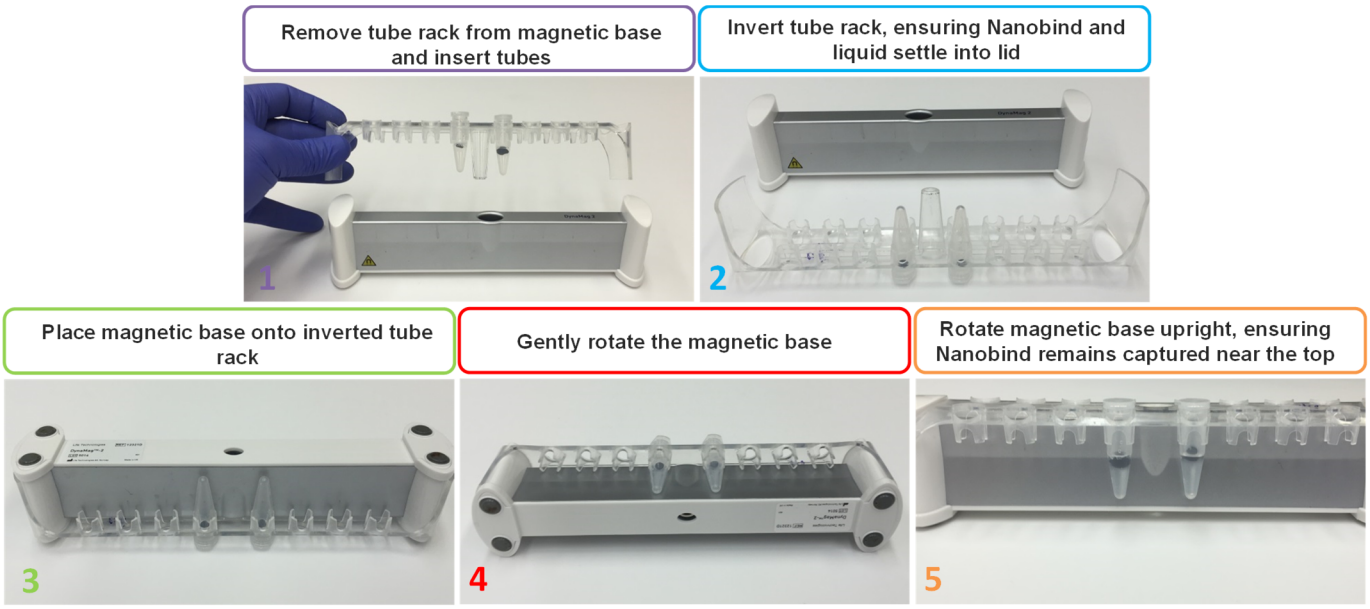


Figure 4 Recommended procedure for capturing Nanobind disk on a tube rack and magnetic base. This procedure ensures that the Nanobind disk is captured near the top of the liquid interface which minimizes the chances for disturbing the bound DNA and facilitates processing.

Pipetting

When removing liquid from the 1.5 mL 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 (**Figure 5**). This will minimize the chances of accidentally pipetting bound DNA. Likewise, when adding liquid, dispense into the bottom of the tube or against the wall opposite the Nanobind disk.

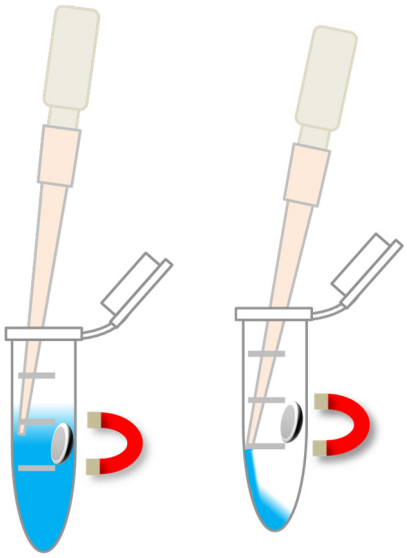


Figure 5. Pipetting procedure for removal (left) and addition (right) of liquid during wash steps. Avoid disrupting the Nanobind disk and

Heterogeneity and Viscosity

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. Our standard protocols provide reliable means to obtain HMW (50 kb – 300+ kb) and UHMW (50 kb – 1+ Mb) DNA. The table below illustrates how various processing steps affect the final DNA size and heterogeneity.

	HMW Protocols	UHMW Protocols
Mixing Steps	Thermomixing Vortexing	Pipetting with wide-bore tips Inversion mixing
General Pipetting	Standard tips	Wide-bore tips
Homogenization of DNA Eluate	Pipette mixing Needle shearing Vortexing	Extended resting at RT
Typical DNA Size	50 kb – 300+ kb	50 kb – 1+ Mb
Sample Heterogeneity	Low Heterogeneity <10% after resting	Higher Heterogeneity <30% CV after resting

If the extracted DNA is very heterogeneous and contains large amounts of unsolubilized "jellies," we first recommend:

More aggressive mixing during lysis – 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. Aggressive mixing during lysis will not significantly impact DNA length. Mixing may be carefully scaled back by skilled users to obtain UHMW DNA, as necessary.

If the extracted DNA is still heterogeneous after more aggressive processing, we recommend:

Pipette mixing the extracted DNA and RT resting – The extracted DNA can be mixed 1–10X using a standard P200 pipette tip to coax the "jellies" into solution. The pipette mixed DNA should then be left to rest at RT for 1 hour to overnight. Moderate amounts of pipette mixing will not significantly impact DNA length. This is our standard DNA homogenization method. It is appropriate for both HMW and UHMW DNA. We routinely use it for all long-read sequencing and optical mapping applications.

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

Needle shearing the extracted DNA – 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. This method is appropriate for HMW DNA. We routinely use this method for long-read sequencing applications.

Vortexing the extracted DNA – The extracted DNA can be vortexed for 10–30 s on high. The vortexed DNA should then be left to rest at RT for a few hours until homogenous. This method is appropriate for HMW DNA

Recommended HulaMixer Settings

The following HulaMixer settings are recommend to ensure efficient recovery of DNA. If a HulaMixer is not available, the tubes can be manually inverted 5X every 2–3 min to facilitate binding. A tube rotator is not recommended as there is not enough fluid volume to allow for adequate mixing in this protocol.

Step	Setting	Time (s)
Rotation	9 rpm	OFF
Tilting	70°	12
Vibration	2°	1

Protocols

Intact Plant Nuclei

The following protocol details extraction of total nucleic acids (combined DNA and RNA) from intact plant nuclei. RNase A treatment is described as an option to obtain pure DNA.

1. **Isolate a plant nuclei pellet with sufficient material for 5–20 µg of isolated DNA.**
    - This typically requires 1–10 g of plant tissue.
    - The size of the pellet will vary based on the plant sample, nuclei isolation method, and amount of starting material. Typical pellets range from 10–100 µL. Smaller pellet volumes are acceptable but will likely result in lower DNA yields; larger pellet volumes may require additional Proteinase K and lysis buffer.
    - The nuclei isolation protocol we used is Workman *et al*. Protocol Exchange (2018) DOI:10.1038/protex.2018.059
    - Another common protocol for nuclei isolation is Zhang *et al*. Nature Protocols (2012) DOI:10.1038/nprot.2011.455

Quick Tip

The Protein LoBind tubes will improve UV 260/230 ratios by up to 0.1 – 0.4 by preventing carryover of contaminants stuck to the tube surfaces.

2. **Add 30 µL of Proteinase K and vortex on high until fully resuspended. Spin tube on mini-centrifuge for 2 s to remove liquid from cap.**
    - Resuspending the nuclei directly in Proteinase K is more effective than resuspending in buffer.
    - Vortexing is critical and will not damage high molecular weight DNA at this step.
    - If removal of RNA is not necessary, proceed directly to step 4.

Quick Tip

The nuclei suspension must be mixed until it appears homogeneous and not chunky. Insufficient mixing will result in low purity and poor yield. Pipette mixing can be used to aid homogenization.

3. **Optional for removal of RNA: add 10 µL of RNase A and pulse vortex 5X. Spin tube on mini-centrifuge for 2 s to remove liquid from cap. Incubate at RT (18–25 °C) for 3 min.**
4. **Add 80 µL Buffer PL1 and pulse vortex 10X. Spin tube on mini-centrifuge for 2 s to remove liquid from cap.**
    - Thorough mixing at this step is necessary to ensure complete lysis of the nuclei. Sample should appear cloudy and very viscous but homogeneous.
    - If sample appears inhomogeneous or chunky vortex continuously on high speed for 10 s or until sample appears homogeneous. Mixing at this stage will only have minor effects on DNA length but is critical for yield and purity.

Quick Tip

Do not skip vortexing steps. Mix aggressively. Even with aggressive vortexing, the DNA will be hundreds of kilobases in length. Once users are familiar with the system, they can carefully reduce mixing to increase DNA size if desired.

5. **Incubate on a ThermoMixer at 55 °C and 900 rpm for 30 min.**
    - Mid-lysis pulse vortex 5X can be used to enhance mixing (pulse vortexing at this stage will still result in very large DNA).
    - If a ThermoMixer is not available, a heat block or water bath can be used instead. Inversion mix 5X every 5 min.
    - If sample does not appear to be lysed, incubation time can be increased up to 2 h.
6. **Centrifuge lysate at 16000 x *g* (max speed) at RT for 5 min.**
7. **Transfer supernatant to new 1.5 mL Protein LoBind tube using a wide-bore pipette.**
    - The DNA should be handled carefully from this point forward to prevent shearing.
8. **Add Nanobind disk to supernatant followed by ~150 µL (1X volume) of isopropanol.**

- If the volume of supernatant is greater than 150 µL, the volume of isopropanol should be adjusted accordingly.
- For best results, the Nanobind disk should always be added before isopropanol.

9. **Carefully mix at RT for 20 min. Verify that the fluid is continuously mixing and that the Nanobind disk remains submerged in the binding solution throughout the binding process for efficient recovery of DNA.**
    - Mixing can be performed by inverting the tubes and placing them cap-side down on a HulaMixer (Thermo Fisher) or similar device. See **Recommended HulaMixer Settings**.
    - If a HulaMixer in not available, manually mix the tubes by inversion (e.g., 5X inversions every 2-3 min) to facilitate binding.
    - A tube rotator is not recommended as there is not enough fluid for adequate mixing while maintaining continuous contact with the Nanobind disk during end-over-end rotation.
10. **Place tubes on the magnetic tube rack using the procedure described in Figure 4.**
11. **Discard the supernatant with a pipette using the procedure described in Figure 5, taking care to avoid pipetting the DNA or contacting the Nanobind disk.**
12. **Add 500 µL of Buffer PW1, inversion mix 4X, replace tube rack on the magnetic base, and discard the supernatant.**
13. **Repeat step 12.**
14. **Remove any residual liquid from cap of 1.5 mL tube.**
15. **Spin the tube on a mini-centrifuge for 2 s and remove the residual liquid.**
    - If the Nanobind disk is blocking the bottom of the tube, gently push it aside with the tip of the pipette. At this stage, DNA is tightly bound to the disk and gently manipulating the disk with a pipette tip should not cause any damage.
16. **Repeat step 15.**
17. **Add 50-200 µL Buffer EB and spin the tube on a mini-centrifuge for 2 s. Incubate at RT for 10 min. Confirm the entire Nanobind disk is fully immersed in Buffer EB during elution.**
18. **Collect DNA by transferring eluate to a new 1.5 mL microcentrifuge tube using a wide bore pipette.**
    - Using a wide-bore pipette helps ensure that the extracted DNA is 50–300+ kb in length.
    - A standard P200 pipette can be used to aid in the removal of residual liquid after the majority of the eluate has been removed with a wide-bore pipette.
19. **Spin the tube containing the Nanobind disk on a mini-centrifuge for 5 s and transfer any additional liquid that comes off of the disk to the previous eluate. Repeat if necessary.**
    - A small amount of white or gel-like material may remain on the Nanobind disk after transferring the eluate in step 18. This is HMW DNA that has not fully solubilized. The spin in step 19 will allow DNA to slide off the Nanobind disk into the bottom of the tube, after which it can be pipetted out and combined with the previously transferred eluate.

Quick Tip

We do not recommend a 2nd elution or heated elution. The spin in step 19 will remove the remaining DNA quickly without diluting the eluate.

20. **Analyze the recovery and purity of the DNA by NanoDrop and Qubit as described in QC Procedure.**
    - The extracted HMW DNA can be heterogeneous. This is normal and is one of the challenges of working with HMW DNA. The bigger the DNA, the more this will be apparent. See **Heterogeneity and Viscosity** section for detailed tips.
    - Heterogeneity can result from insufficient vortexing in step 2 and step 4. Use aggressive mixing until familiar with the protocol.
    - If the extracted HMW DNA has large amounts of unsolubilized “jellies”, pipette mix 1–10X with a standard P200 pipette and then let the sample rest at RT for 1 hour to overnight.
    - Alternatively, the DNA sample can be needle sheared 5X using a 26g needle and used immediately.
    - Vortexing the eluted DNA 10–30 s can also be used with minor effects on DNA size.
    - All of the above suggestions will only have minor to unnoticeable effects on DNA size and sequencing read lengths.

Quick Tip

The DNA will solubilize after resting at RT or by coaxing it into solution using gentle mixing. For samples that need to be used immediately, we recommend pipette mixing or needle shearing.

QC Procedures

It is recommended that QC is performed after samples have been allowed to rest and they appear homogenous under visual examination and when pipetting.

1. **Use NanoDrop UV/VIS 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%.
    - If the DNA is very heterogeneous or contains large amounts of unsolubilized "jellies," see **Troubleshooting Guide** and **Heterogeneity and Viscosity** section.
    - For plant samples, we routinely see A260/A280 in the range of 1.8–2.0 and A260/A230 in the range of 1.2–2.0.
    - Samples with UV purities within the expected range should sequence well. UV purities outside of these ranges may indicate abnormalities in the extraction process. See **Troubleshooting Guide**.
2. **Use Qubit to determine DNA concentration.**
    - We recommend making multiple measurements to ensure an accurate DNA concentration reading.
    - We use the Qubit 3.0 (Thermo Fisher Scientific) with either the dsDNA BR Assay or HS Assay kits.
3. **Run pulsed field gel electrophoresis to determine size.**
    - The size of the extracted genomic DNA can be determined using pulsed field gel electrophoresis (PFGE) (**Figure 6**). We recommend loading 200 ng of DNA per well. For analysis of 50 kb –1000 kb DNA, we recommend the following PFGE conditions:
        - Instrument: Bio-Rad CHEF-DR III Variable Angle System or CHEF Mapper XA System
        - Agarose: 1.0% Certified Megabase Agarose (Bio-Rad #1613109)
        - 30-well comb (Bio-Rad #1703628)
        - Buffer: 0.5X TBE
        - Lambda DNA Ladder: Bio-Rad #1703635, Lonza #50401, or NEB #N0341S
        - 6X gel loading dye (NEB #B7021S)
        - Temperature: 14 °C
        - Initial Switch Time: 35 seconds
        - Final Switch Time: 90 seconds
        - Run Time: 22 hours
        - Angle: 120°
        - Voltage Gradient: 5.5 V/cm

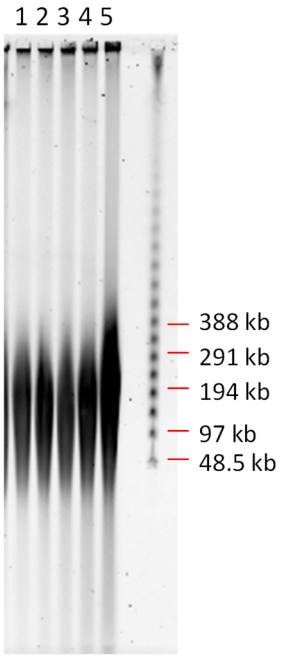


Figure 6. PFGE of replicate extractions of $2x10^6$ MCF7 cells on a Nanobind disk along with a lambda ladder (Lonza).

Storage of DNA

DNA can be stored in Buffer EB at 4 °C for up to a month. Avoid freeze/thaw cycles since this can degrade high molecular weight DNA.

Troubleshooting Guide

1	**Extracted DNA is heterogeneous and/or contains visible insoluble "jellies"**	
a)	Sample contains HMW and UHMW DNA	HMW and UHMW DNA is inherently difficult to work. The bigger it is, the more heterogeneous it tends to be. Homogeneity can be improved by overnight incubation at RT or 4 °C without affecting DNA length. The sample can also be pipette sheared or needle sheared to improve homogeneity in a more time efficient manner, however, this will result in minor reductions of DNA length. See **Heterogeneity and Viscosity** for details. Minor amounts of DNA "jellies" can often be left in the sample without affecting downstream analysis.
b)	Poor mixing during lysis and digestion	Increase the amount of vortexing and pipette mixing during the lysis and digestion steps prior to binding. This will not affect DNA length much but will improve sample homogeneity by improving digestion and lysis efficiency.
c)	Poor sampling of DNA	HMW DNA will be less homogeneous and more viscous than low molecular weight DNA, which can lead to measurement errors that appear as large %CV in NanoDrop or Qubit measurements. In addition, measurement and sampling errors can occur by pipetting an incorrect volume of sample or by pipetting a concentrated glob of DNA gel. For highly viscous samples, the tip of the pipette can be gently scraped along the bottom of the tube to aid in sample pipetting. We recommend allowing the sample to rest before QC and taking at least three measurements, sampling from the top, middle, and bottom of the eluate, to get an accurate concentration reading.
d)	Sample input is too high	HMW DNA that is high concentration can be particularly viscous and challenging to handle. To improve homogeneity, a larger elution volume can be used. Alternatively, a smaller input can be used.
e)	Insufficient elution (time, temperature, volume)	Ensure that the correct temperature and time are used so that DNA completely elutes from the Nanobind disk. If large cell inputs are used, elution volumes greater than 200 µL may be necessary. Increasing the time can also help to improve elution of bound DNA. Elution at temperatures higher than room temperature is not recommended since it can degrade HMW DNA.
f)	Binding time exceeded 20 min	Binding time should not exceed 20 min as this can lead to DNA that is inhomogeneous and difficult to solubilize.

2	**DNA remains on Nanobind after elution or is difficult to remove**	
a)	Sample contains HMW and UHMW DNA	Solubilizing HMW and UHMW DNA can be inherently challenging. In some cases, DNA in the form of a "gel" will remain adhering to the Nanobind after elution. It is important to remove the DNA to ensure a high yield of HMW DNA. The DNA can be eluted by spinning the tube with a mini-centrifuge for 5–10 s. This spin can be repeated multiple times to ensure full elution. We do not recommend 2nd elutions or heated elution as these methods dilute the eluate and can damage DNA.
b)	Extracted DNA is difficult to pipette	HMW DNA is inherently very viscous and difficult to pipette. When pipetting the highly viscous sample, the tip of the pipette can be gently scraped along the bottom of the tube to aide in sample pipetting. Wide-bore pipette tips can also be used.
c)	Binding time exceeded 20 min	Binding time should not exceed 20 min as this can lead to DNA that is inhomogeneous and difficult to elute.
d)	High sample input and/or small elution volume	High sample input and high DNA concentration can result in DNA that is difficult to completely solubilize. For large nuclei inputs, a

		larger elution volume can be used to improve elution of the DNA. Consider smaller inputs if the problem persists.
e)	Wrong volume or concentration of isopropanol used during binding	Ensure that the correct volume of 100% isopropanol was added after inserting the Nanobind into the cell lysate. Addition of too much isopropanol can lead to DNA that is difficult to elute from the Nanobind.
f)	Nanobind disk was overdried	Over-drying the Nanobind disk will lead to DNA that is difficult to elute from the Nanobind. Do not allow bound DNA to over-dry after wash steps.
g)	Buffer PW1 prepared incorrectly	Ensure that the proper amount of 100% ethanol was added to the buffer (page 2).

3 Low yield

a)	Inefficient nuclei isolation	Nuclei are easily lost during nuclei isolation. The two most common areas where nuclei are lost are during the Miracloth filtration step and during the centrifugation step. During Miracloth/cheesecloth filtration, it is important to squeeze the pellet to ensure that nuclei do not remain trapped on the filter. During centrifugation, it is necessary to optimize the centrifugation spin speed based on the expected size of the genome. Using a spin speed that is too slow will result in poor recovery of nuclei. Alternatively, increase the amount of plant material entering nuclei extraction.
b)	Poor mixing during nuclei resuspension and lysis	Increase the amount of vortexing and pipette mixing during the nuclei resuspension and lysis steps prior to binding. This will improve yield by improving digestion and lysis efficiency.
c)	Insufficient lysis time or enzymatic treatment	For large nuclei inputs, increased lysis time may be necessary to ensure complete lysis. Increase lysis time by 10 min increments until complete lysis is observed. Additional Proteinase K may also be added to facilitate lysis.
d)	DNA remains on Nanobind after elution	For maximum recovery of HMW DNA, any DNA remaining on the Nanobind after elution should be removed by spinning on a mini centrifuge. This step can be repeated as necessary until all DNA is removed. Heat elution can be used as a last resort.
e)	DNA lost during removal of binding and wash buffers	DNA can be lost during the binding and wash steps by accidental pipetting of the bound DNA. Use the technique illustrated in **Figure 3** to capture the Nanobind disk near the top of the tube liquid interface when placing the tube rack on the magnetic base to reduce accidental pipetting of DNA. Pipette the near the top of the liquid interface away from the disk to prevent accidental removal of DNA.
f)	DNA does not bind to Nanobind	Ensure that the correct volume of Buffer PL1 was added prior to adding Nanobind disk to lysate.
g)	Extracted DNA sample is inhomogeneous	It is possible that the yield is fine but sample heterogeneity is skewing concentration measurements. For accurate quantification of HMW DNA using NanoDrop or Qubit, take multiple measurements per tube, sampling from the top, middle, and bottom of the liquid. A portion of the sample can also be sheared if necessary.
h)	Buffers PW1 prepared incorrectly	Ensure that the correct volume of 100% ethanol was added to Wash Buffer CW1.

4 Yield too high

a)	Extracted DNA sample is inhomogeneous	For accurate quantification of HMW DNA using NanoDrop or Qubit, take multiple measurements per tube, sampling from the top, middle, and bottom of the liquid.

b)	RNA contamination	Use the optional RNase A addition step to minimize RNA contamination.

5 A_{260}/A_{280} or A_{260}/A_{230} signals are low

a)	Poor mixing during nuclei resuspension and lysis	Mix more aggressively during the nuclei resuspension and lysis steps. This is the primary reason for low purity. Poor mixing will result very large DNA with accompanying high heterogeneity and low purity due to poor digestion efficiency and poor removal of contaminants.
b)	Insufficient mixing during washes	When performing the washes, it is important not only to completely wash the Nanobind disk but also to completely wash all surfaces of the 1.5 mL tubes. Inversion mixing 5 times is typically sufficient, but in some cases more mixing may be necessary. No noticeable decrease in DNA length has been observed from gentle inversion mixing.
c)	Insufficient number of washes	For some samples, an additional PW1 wash may be necessary to remove all contaminants. In some cases, transferring the Nanobind disk to a clean 1.5 mL tube after the PW1 wash may be necessary.
d)	Buffers PW1 prepared incorrectly	Ensure that the proper amount of 100% ethanol was added to Buffer PW1.

6 Large DNA "bundle" interferes with washing

a)	Large nuclei input used	With a large nuclei input, a large DNA bundle can form that hangs from the Nanobind disk. Care should be taken to avoid accidental pipetting of the bound DNA. In some cases, it is not possible to completely remove all of the fluid during one or more of the wash steps without disrupting the bound DNA. This should not dramatically impact the extraction results.
b)	Nanobind not captured near top of tube when placed on magnet	Capturing the Nanobind disk near the top of the 1.5 mL tube when placing the tube rack on the magnetic base will help minimize accidental pipetting of the bound DNA. Follow the procedure outlined in **Figure 3.**

7 Nanobind not held in place by magnet

a)	Large number of cells used	With a large input of cells (>5 x 10^6), a large DNA bundle can form that hangs from the Nanobind disk. The extra weight of the bound DNA can cause the Nanobind disk to slide towards the bottom of the tube. Care should be taken to capture the disk at the top of the tube to minimize this effect and to remove supernatant slowly during the wash steps.
b)	Buffer PW1 prepared incorrectly	Ensure that the proper amount of 100% ethanol was added to Buffer PW1.