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Isolating nuclei from plant tissue using LN2 disruption

Procedure & checklist

For isolation of nuclei from plant tissues for HMW DNA extraction using the Nanobind® plant nuclei big DNA kit.

Equipment and reagent list

Equipment/reagent	Manufacturer (part number)
Liquid nitrogen-cooled mortar	Fisher Scientific (H372600000)
Porcelain pestle	Fisher Scientific (FB961N)
Refrigerated centrifuge	Eppendorf (022625101)
Micro-centrifuge	Eppendorf (5404000413)
Tube rotator, shaker, or platform rocker	Fisher Scientific Mini-Tube Rotator (05-450-127)
Steriflip (20 µm pore size, 50 mL process volume)	Millipore (SCNY00020)
Small nylon or synthetic paintbrush	
1.5 mL Protein LoBind micro-centrifuge tubes	Eppendorf (022431081)
50 mL conical tubes	ThermoFisher (339652)
2-mercaptoethanol, 14 M	Sigma-Aldrich (M3148)
Triton X-100	Sigma-Aldrich (X100)
Trizma base	Sigma-Aldrich (T4661)
Potassium chloride (KCl)	Sigma-Aldrich (P9541)
Ethylenediaminetetraacetic acid (EDTA), 0.5 M, pH 8.0	ThermoFisher (15575020)
Spermidine trihydrochloride	Sigma-Aldrich (S2501)
Spermine tetrahydrochloride	Sigma-Aldrich (S1141)
Sucrose	Sigma-Aldrich (S0389)
Sodium hydroxide (NaOH), 10 M	Sigma-Aldrich (72068)
Polyvinylpyrrolidone (MW ~360 kD) (PVP360)	Sigma-Aldrich (PVP360)
Ultra pure water	
Liquid nitrogen (LN2)	
pH meter	

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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.

Buffer preparation

Prepare the following buffers prior to beginning nuclei isolation.

Buffer 10X HB – homogenization buffer stock (500 mL)

Add the following reagents to a clean beaker and stir until dissolved:

Reagent	Amount	Final concentration
Trizma	6.06 g	0.1 M
KCl	29.8 g	0.8 M
EDTA (0.5 M)	100 mL	0.1 M
Spermidine	1.28 g	17 mM
Spermine	1.74 g	17 mM

Bring to a final volume of 500 mL with ultra pure water and adjust pH to 9 with 10 M NaOH. Store at 4°C in a glass bottle for up to 1 year.

Buffer 1X HB – homogenization buffer (1 L)

Add the following reagents to a clean beaker and stir until dissolved:

Reagent	Amount
Buffer 10X HB	100 mL
Sucrose	171.2 g

Bring to a final volume of 1 L with ultra-pure water. Store at 4°C in a glass bottle for 3 months.

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Buffer TSB – triton-sucrose buffer (100 mL)

Add the following reagents to a clean beaker and stir until dissolved:

Reagent	Amount
Triton X-100	20 mL
Buffer 10X HB	10 mL
Sucrose	17.1 g

Bring to a final volume of 100 mL with ultra-pure water. Store at 4°C in a glass bottle for up to 1 year.

Buffer NIB – nuclei isolation buffer (50 mL)

Note 1: Make 50 mL of Buffer NIB per gram of tissue being processed. Buffer NIB can be stored at 4°C for up to one week prior to the addition of 2-mercaptoethanol.

Note 2: PVP360 dissolves slowly so please allow at least 2 h to prepare Buffer NIB before beginning the nuclei isolation protocol.

Add the following reagents to a clean bottle and stir or use end-over-end mixing until dissolved:

Reagent	Amount
Buffer 1X HB	48.75 mL
Buffer TSB	1.25 mL
PVP360	0.5 g

Use immediately or store at 4°C for up to one week.

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Nuclei isolation protocol

This protocol describes the isolation of nuclei from plant tissues for HMW DNA extraction using the Nanobind plant nuclei kit (102-302-000). This protocol has been specifically optimized for HiFi sequencing. It is based on the protocol described in Workman *et al*. protocol exchange (2018) (DOI:10.1038/protex.2018.059).

Input requirements

Amount: 1-5 g of plant tissue. Input will vary by plant type, age, and growing conditions.

- We recommend initially testing this protocol with 1 g of tissue. After evaluating the outcome from 1 g of tissue, the input mass can be increased or decreased based on the needs of the user.
- The maximum tissue mass we recommend processing at once using liquid nitrogen (LN2) grinding is 5 g.
- The amount of plant tissue required will depend on species, age, and nuclei isolation efficiency. Therefore, the input mass may need to be adjusted to achieve suitable DNA yield and purity.
- Young leaves, preferably grown in a greenhouse or growth chamber, will produce the highest quantity and quality of DNA. Mature plants grown in more natural environments tend to require larger tissue inputs and more wash steps to obtain equivalent DNA yields and purity.
- This protocol has been validated on a wide range of tissues, including leaf tissues from pepper, sugar beet, lavender, banana, arabidopsis, wheat, fescue, tomato, and spinach.
- This protocol is suitable for fresh or frozen plant tissues.

Processing tips

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 (**Procedure & checklist – Isolating nuclei from plant tissue using TissueRuptor disruption**).

Nuclei isolation – LN2 plant tissue

1. Add 125 µL of 2-mercaptoethanol (14 M) to 50 mL of Buffer NIB and store on ice throughout use.
   - This protocol should be performed under a fume hood to avoid inhalation of 2-mercaptoethanol.
   - If >1 g of tissue is being processed, add 125 µL of 2-mercaptoethanol (14 M) per 50 mL Buffer NIB.
2. Using a liquid nitrogen (LN2)-cooled mortar and pestle, grind 1-5 g of frozen plant tissue into a flour-like powder. This requires >25 min of manual grinding.
   - Plant tissue must remain frozen during grinding. If an LN2-cooled mortar and pestle is not available, LN2 must be continuously added throughout grinding to keep the tissue frozen.
   - Tissue should be ground into an extremely fine powder with flour-like consistency.
   - Insufficient grinding will result in decreased DNA quantity and purity. We have observed that grinding for only 15 min results in a 50% decrease in DNA recovery and only 5 min results in a 75% decrease in DNA recovery compared to a 20 min grinding effort.

Quick tip

Grinding in liquid nitrogen for at least 25 min is critical for maximizing DNA recovery and purity for most plant tissues. If in doubt, grind more!

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3. Transfer frozen ground plant tissue into a 50 mL conical tube and add ~10 mL per gram tissue input of the prepared ice-cold Buffer NIB (e.g., 30 mL for 3 g tissue).
   - It is important to work quickly at this step – volumes do not need to be exact.
   - If using a 5 g input, use caution when adding Buffer NIB to avoid letting the tube overflow. Less than 50 mL Buffer NIB will still be sufficient for lysis.
4. Cap the 50 mL conical tube and mix end-over-end at the maximum speed (15 rpm) for 15 min at RT (15–30°C).
   - Alternatively, lay the tube horizontally on a shaker (150 rpm) or a platform rocker (maximum speed) to achieve continuous, thorough mixing.
5. Filter the lysate using a Steriflip (20 µm pore size, 50 mL process volume).
   - Using filtration methods with pore sizes larger than 20–25 µm will allow excess debris to pass through, resulting in larger nuclei pellets that inhibit downstream lysis and compromise DNA yield.
   - If more than 1 g tissue is being processed, more than one Steriflip may be necessary to filter the entire volume if the filter becomes clogged.
6. Centrifuge at the following spin speed for 20 min at 4°C.
   - Use 3,000 x *g* for plants with genome size >1 Gb.
   - Use 7,000 x *g* for plants with genome size <1 Gb, or if genome size is unknown.
   - Note: If centrifuge cannot reach 7,000 x *g*, spin at the fastest speed possible.
7. Decant the supernatant and add 1 mL of ice-cold Buffer NIB. Resuspend the pellet using a small paint brush pre-soaked in Buffer NIB. Mix using a P1000 pipette until the solution is homogeneous.
   - Most plant species result in pellets that are too sticky to be sufficiently resuspended by pipetting alone.
8. Bring volume up to 15 mL with ice-cold Buffer NIB. Centrifuge at 4°C for 10 minutes using the same centrifugation speed used in step 6.
9. Repeat steps 7 and 8 twice. The supernatant should be clear in color after the last centrifugation.
   - If the supernatant is not clear in color after 3 washes in Buffer NIB, repeat steps **7** and **8** until the supernatant is colorless.
   - The number of washes is species-dependent and depends on how well the tissue grinding was performed.
10. Decant the supernatant and add 1 mL of ice-cold Buffer 1X HB per gram of tissue being processed (e.g. 3 mL Buffer 1X HB for 3 g tissue).
11. Resuspend the pellet using a small paint brush pre-soaked in ice-cold Buffer 1X HB (the paint brush from step 7 may be reused). Mix with a P1000 pipette until the solution is homogeneous.
12. Transfer 1 mL of the nuclei suspension per 1.5 mL Protein LoBind microcentrifuge tube (equivalent to 1 g tissue/tube).
13. Centrifuge the 1.5 mL tubes at 7000 x *g* for 5 min at RT, discard the supernatant, and proceed by either snap freezing the nuclei pellet or immediately starting DNA Extraction.

Quick tip

Do not filter the lysate using pore sizes larger than 20-25 µm.

Quick tip

We recommend using a paint brush in step 7 to resuspend the pellet. Pipetting alone is insufficient.

Quick tip

The nuclei pellet must be thoroughly resuspended in Buffer 1X HB to ensure proper amounts of nuclei are distributed to each pellet.

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

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- If snap freezing, place tube in liquid nitrogen to rapidly freeze the nuclei pellet and immediately store at
-80°C.
- If continuing to Nanobind DNA extraction, proceed with step 1 of the **Procedure & checklist – Extracting HMW DNA from plant nuclei using Nanobind kits**.

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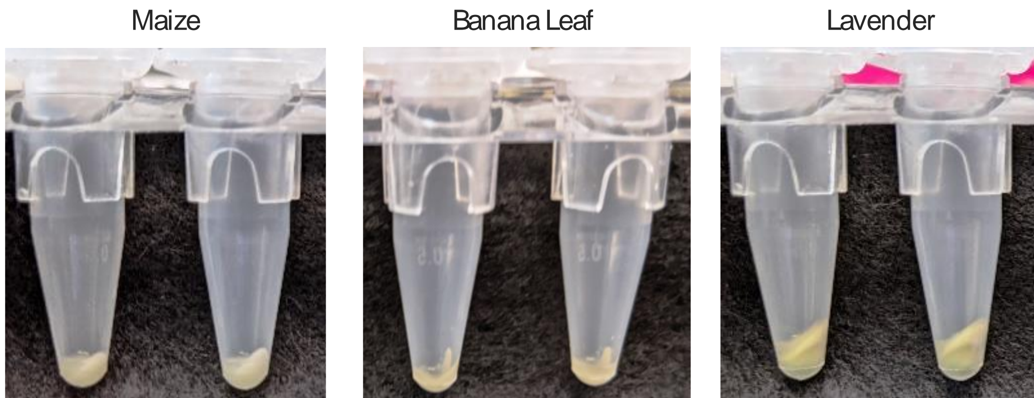
Results

Images of 1 g nuclei pellets are shown below:



Images of nuclei pellets after the final wash in Buffer 1X HB.

Revision history (description)	Version	Date
Initial release	01	July 2022
Minor updates	02	December 2022

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