Genomic DNA Purification From Plant Tissue Using Pall Acroprep™ Advance 96-well Long Tip Filter Plate For Nucleic Acid Binding And Commercially Available Buffers

1. Consumables and Reagents

Table 1

Consumables for gDNA Purification

Supplier	Product Description	Part Number
Pall Laboratory	Pall AcroPrep Advance 96-well Long Tip Filter Plate for Nucleic Acid Binding (NAB)	8133
Pall Laboratory	Cap Mat for Incubation	5230
Corning Axygen◆	96-well Polypropylene Storage Block	3958
Corning Axygen	Corning Universal Fit 200 µL and 1000 µL Pipet Tips	4710; 4713
Corning	Corning 96-well Clear Polystyrene Microplates	3366
Axygen	Sealing Tape	PCR-SP-S

Table 2

Reagents for gDNA Purification

Supplier	Product Description	Part Number
Qiagen	Buffer AP1	1014630
Qiagen	Buffer P3	19053
Qiagen	Buffer AW1	19081
Qiagen	Buffer AW2	19072
Amresco	Ethanol, Anhydrous	E193-4L
Amresco	PBS (500 mL)	K812-500ML
Sigma-Aldrich	Ribonuclease A from Bovine Pancreas	R4875-500MG
Sigma-Aldrich	Lysis Solution Part A	L7910
Sigma-Aldrich	Lysis Solution Part B	L8035
Sigma-Aldrich	Precipitation Solution	P7927
Sigma-Aldrich	Binding Solution	B2177
Sigma-Aldrich	Wash Solution Concentrate	W3011

Note: Sigma-Aldrich reagents are components of GenElute◆ Plant Genomic DNA Miniprep Kit PN G2N70

2. Instruments

Supplier	Product Description
Pall Laboratory	Plate Vacuum Manifold
Pall Laboratory	Vacuum Pump
Eppendorf	Centrifuge with Plate Holders (Maximum 1500 g)

3. Important Points Before Starting

- Pre-heat a water bath or heating block to 65 °C.
- All buffers must be examined for visible precipitation. If precipitation is detected, the buffer must be heated to 55 – 65 °C to dissolve the precipitate.
- Centrifugation steps should be performed at room temperature.
- Add Ethanol to AW1 and AW2 Qiagen buffers and wash solution concentrate of Sigma-Aldrich as per manufacturer's instruction.
- Vacuum pump must be connected to the manifold via a trap kit fitted with vent filter.

4. Protocol

- **Using Qiagen Buffer**

1. Grind small section of leaf (< 100 mg wet weight) using a mortar and pestle.
2. Add 400 µL of buffer AP1 and 4 µL of RNase A to the ground tissue sample. Incubate at 60 °C for 1 hour.
3. Add 150 µL of buffer P3 and incubate on ice for 5 minutes.
4. Centrifuge lysate at 1000 rpm for 5 minutes.
5. Add 1.5 volume of AW1 buffer to the collected supernatant.
6. Place Pall NAB plate on plate vacuum manifold and place 1 mL storage block plate underneath.
7. Add 700 µL of the mixture to the column. Let the solution sit for 1 min. Cover the plate with Pall Cap Mat for incubation.
8. Start vacuum filtration at 85 kPa (25 in. Hg).

   Note: Alternatively, plates can be centrifuged 4 minutes at 1500 × g. Care must be taken to seal filter plate with adhesive tapes prior to centrifugation to prevent cross contamination.
9. Add 500 µL of AW2 buffer. Apply vacuum and filter. Repeat this step twice.

   Note: Alternatively, plates can be centrifuged 4 minutes at 1500 × g.
10. Add 50 µL of ultrapure water
11. Place 350 µL 96-well polystyrene microplates underneath Pall NAB plate and start vacuum filtration.

    Note: Alternatively, plates can be centrifuged 4 minutes at 1500×g.
12. Collect eluted DNA for downstream analysis.

- **Using Sigma-Aldrich Buffer**

1. Grind small section of leaf (< 100 mg wet weight) using a mortar and pestle.
2. Add 350 µL of lysis solution part A and 50 µL of lysis solution part B to the ground tissue sample. Incubate at 60 °C for 1 hour.
3. Add 130 µL of precipitation buffer and incubate on ice for 5 minutes.
4. Centrifuge lysate at 1000 rpm for 5 minutes.
5. Add 700 µL of binding solution to the collected supernatant.
6. Place Pall NAB plate on plate vacuum manifold and place 1 mL storage block plate underneath.
7. Add 700 µL of the mixture to the column. Let the solution sit for 1 min. Cover the plate with Pall cap mat for incubation.
8. Start vacuum filtration at 85 kPa (25 in. Hg).

   Note: Alternatively, plates can be centrifuged 4 minutes at 1500 × g. Care must be taken to seal filter plate with adhesive tapes prior centrifugation to prevent cross contamination.
9. Add 500 µL of wash buffer. Apply vacuum and filter. Repeat this step twice.

   Note: Alternatively, plates can be centrifuged 4 minutes at 1500 × g.
10. Add 50 µL of ultrapure water
11. Place 350 µL 96-well polystyrene microplates underneath Pall NAB plate and start vacuum filtration.

    Note: Alternatively, plates can be centrifuged 4 minutes at 1500 × g.
12. Collect eluted DNA for downstream analysis.

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10/19, PDF, GN19.0418