Standard Reagent Protocol for Genomic DNA Isolation From Plant Tissue Using Pall Acroprep™ Advanced 96-well Long Tip Filter Plate for Nucleic Acid Binding

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	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

Buffer	Supplier	Product Description	Part Number
2X CTAB	Sigma-Aldrich	CTAB	1102974-1G
	Sigma-Aldrich	Trizma◆ Hydrochloride	T3253-500G
	Sigma-Aldrich	EDTA	E6758
	Sigma-Aldrich	NaCl	S3014
Binding Buffer (Stock)	Calbiochem	GuSCN	368975-500GM
	Sigma-Aldrich	EDTA	E6758
	Sigma-Aldrich	Trizma Hydrochloride	T3253-500G
	Sigma-Aldrich	Triton◆ X-100	T8787-50ML
Wash Buffer	Amresco	Ethanol, Anhydrous	E193-4L
	Sigma-Aldrich	NaCl	S3014
	Sigma-Aldrich	Trizma Hydrochloride	T3253-500G
	Sigma-Aldrich	EDTA	E6758
RNase A	Merck	RNase A solution	70856-3
Proteinase K	Sigma-Aldrich	Proteinase K from Tritirachium Album	P2308-10MG

Table 3

Buffers and their composition for DNA purification using standard reagent protocol

Buffer	Composition
2X CTAB Lysis Buffer	2% CTAB, 100 mM Trizma, pH 8.0, 20 mM EDTA, 1.4 M NaCl
Binding Buffer (Stock)	6M GuSCN, 20 mM EDTA, 10 mM Trizma, pH 6.4, 4% Triton X-100
Binding Buffer (Working Solution)	80% Stock solution in Ultrapure water
Protein Wash Buffer	Binding Buffer and Ultrapure water (1:1)
Wash Buffer	60% Ethanol, 50 mM NaCl, 10 mM Trizma pH 7.4, 0.5 mM EDTA pH 8.0,

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

1. Pre-heat a water bath or heating block to 65 °C.
2. 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.
3. Centrifugation steps should be performed at room temperature.
4. Vacuum pump must be connected to the manifold via a trap kit fitted with vent filter.

4. Protocol

1. Grind small section of fresh leaf (<100 mg weight) using a mortar and pestle.
2. Add 500 µL of CTAB lysis buffer, 5 µL of RNase A and 5 µL of Proteinase K to the ground tissue sample. Grind further into a smooth slurry. Incubate the slurry at 65 °C for 1 hour.
3. Add 150 µL of binding buffer to each sample and mix by pipetting 5 – 10 times.
4. Incubate lysate at room temperature for 5 minutes.
5. Place Pall Nucleic Acid Binding (NAB) plate on plate vacuum manifold. Place 1 mL storage block plate underneath.
6. Load the lysate into the wells of the plate and let the solution sit for 1 min. Cover the plate with Pall Cap Mat for incubation.
7. Start vacuum filtration at 85 kPa (25 in. Hg).

   Note: Alternatively, plates can be centrifuged for 4 minutes at 1500 × g. Care must be taken to seal filter plate with adhesive tapes prior centrifugation to prevent cross contamination.
8. Add 200 µL of protein wash buffer to each well of NAB plate. Seal with the cap mat and apply vacuum at 85 kPa (25 in. Hg)

   Note: Alternatively, plates can be centrifuged for 4 minutes at 1500 × g. Care must be taken to seal filter plate with adhesive tapes prior centrifugation to prevent cross contamination.

9. Add 750 µL of wash buffer to each well of the NAB plate. Apply vacuum and filter.
   Note: Alternatively, plates can be centrifuged for 4 minutes at 1500 × g.
10. Residual ethanol can be eliminated by applying vacuum or by centrifuging at 1500 × g for 1 min.
11. Add 50 µL of ultrapure water directly onto the membrane.
12. Place 350 µL 96-well polystyrene microplates underneath Pall NAB Plate and start vacuum filtration.
    Note: Alternatively, plates can be centrifuged for 4 minutes at 1500 × g.
13. Collect eluted DNA for downstream analysis.

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