PALL Laboratory

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Protocol

Protocol for Genomic DNA Purification from Plant Leaves Using Pall Nucleic Acid Binding Nanosep® Centrifugal Device

1. Consumables and Reagents

Table 1

Consumables for gDNA Purification (nuclease-free consumables are recommended)

Supplier	Product Description	VWR Cat. No.
Pall Laboratory	Nucleic Acid Binding (NAB) Nanosep Centrifugal Device	76360-454, 76360-456
VWR	Ethanol (not denatured)	71001-866
VWR	Spectrophotometer Cuvettes ~100 µL (260/280 nm)	47743-840
VWR	Tubes 15 mL (RNase-DNase free)	89401-574
VWR	Tubes 50 mL (RNase-DNase free)	89401-572
VWR	Microcentrifuge tubes 1.5 mL (RNase-DNase free)	76005-210

Table 2

Reagents for gDNA Purification (nuclease-free reagents are recommended)

Supplier	Product Description	VWR Cat. No.
Various	Lysis Buffer	N/A
Various	Cell Debris Removal Buffer	N/A
Various	Wash Buffer 1	N/A
Various	Wash Buffer 2	N/A
Various	Proteinase K	N/A
VWR	Tris Buffer pH 7.0 (1 M)	89500-584
Various	RNase A (100 mg/mL)	N/A
Various	Nuclease-free water	N/A

2. Instruments

- Microcentrifuge
- Spectrophotometer
- Vortex
- Pestle and mortar
- Liquid nitrogen
- Water bath or heating block

3. Important Points Before Starting

- Some commercially available buffers are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100 %) as indicated on the bottle to obtain a working solution. Before each use, mix these buffers by inverting several times.
- All centrifugation steps are performed at room temperature at 10,000 – 14,000 x g.
- Use only the collection tubes provided in the NAB Nanosep device box. There are enough provided to complete the below process in full.
- All buffers should be allowed to equilibrate to room temperature before use.
- Briefly centrifuge the tubes after vortexing to remove drops from inside the lid.
- Increase volumes of lysis buffers and reagents proportionally for samples >200 µL.
- Ensure that any precipitate formed during any of the below steps is not lost.
- Centrifugation at full speed will not affect yield or purity of the DNA. If, after centrifugation the sample has not completely passed through the membrane, centrifuge again until all the solution has passed through.

4. Harvest and Pre-Lysis of Cells

- The amount and type of material being processed will result in variances in yield
- Determine the amount of tissue to be processed by weighing.
- Place plant leaves in liquid nitrogen and immediately grind to a powder with a pestle and mortar.
- Decant the powdered tissue into a 1.5 mL microcentrifuge tube (not provided) and allow the liquid nitrogen to evaporate. Do not allow the tissue to thaw and add 180 µL of lysis buffer and follow the below steps.

5. Protocol

1. Add 20 µL of proteinase K to the prepared sample.
2. Vortex to mix and then incubate at 56 °C to complete lysis.
3. Vortex occasionally during incubation to homogenise the lysate, or place in a shaking water bath/incubator.
4. Centrifuge the tube for 5 seconds to remove drops from the inside of the lid.
5. Optional RNase digestion steps: *(If you do not wish to perform RNase digestion, move on to step 8)*
   a. Add 4 µL RNase A (100 mg/mL) to the 200 µL sample of Proteinase K in lysis buffer. Pulse vortex to mix for 15 seconds.
   b. Incubate for 2 minutes at room temperature.
   c. Centrifuge for 5 seconds to remove drops from the inside of the lid.
6. Add 200 µL of cell debris removal buffer to the sample and pulse vortex for 15 seconds to mix.
7. Incubate at 70 °C for 10 minutes.
8. Centrifuge again for 5 seconds to remove drops from the lid.
9. Add 200 µL of 100% non-denatured ethanol to the sample and pulse vortex for 15 seconds to mix. Use of alcohols other than ethanol will decrease yields.

10. Centrifuge again for 5 seconds to remove drops from the lid.
    (Be thorough with the mixing at each stage to obtain a homogenous solution).
11. Apply the mixture in ethanol, including any precipitate to the NAB Nanosep device insert inside a receiver tube.
12. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
13. Discard the flow-through and re-use the receiver tube for the next step.
14. Open the NAB Nanosep device and add 500 µL wash buffer 1.
15. Close the cap and centrifuge for 60 seconds at 10,000 – 14,000 x g.
16. Discard the collection tube and flow-through and transfer the NAB Nanosep device insert into a clean collection tube (provided).
17. Add 500 µL wash buffer 2 to the column insert.
18. Close the cap and centrifuge for 3 minutes at 10,000 – 14,000 x g.
19. Discard the flow-through and re-use the receiver tube for the next step.
20. Centrifuge for 60 seconds at 10,000 – 14,000 x g to remove possible buffer carry-over.
21. Discard the receiver tube with the flow-through and transfer the NAB Nanosep device insert to the final, clean collection tube (provided).
22. To elute the DNA, add 100 µL of DNase-free distilled water (or elution buffer) directly onto the filter membrane inside the NAB Nanosep device insert and incubate at room temperature for 5 minutes.
23. Centrifuge for 60 seconds at 10,000 – 14,000 x g.
24. *Optional: For maximum DNA yield, repeat the above elution steps with a further 100 µL of DNase-free distilled water.*

Storage of DNA

If you intend to store the purified DNA, we recommend elution with a commercially available elution buffer and storage at -30 ºC to -15 ºC. As high pH or EDTA can affect sensitive downstream applications, water can be used for elution. However, ensure that the pH of the water is a minimum of 7.0 as acid hydrolysis will occur to DNA stored in water which will result in degradation.

Quantification of DNA

DNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer (See details below). For small quantities of DNA however, it can be difficult to determine these amounts photometrically. Smaller quantities of DNA can be accurately quantified using fluorometric quantification.

Spectrophotometric quantification of DNA

A_{260} readings should be greater than 0.10 and lower than 1.0 to ensure significance. An absorbance reading of 1.0 at 260 nm corresponds to 50 µg of DNA per mL. This is only valid for measurements at neutral pH however. As a result, if it is necessary to dilute the DNA sample, ensure that the dilution buffer is of neutral pH.

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4/20, PDF, GN19.1112