GENELUTE™ PLANT GENOMIC DNA KIT

Product Information

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Product Description

Sigma's GenElute™ Plant Genomic DNA Kit provides a simple and convenient way to isolate pure DNA from a variety of plant species. The GenElute kit combines the advantages of a silica-based system with a microspin format and eliminates the need for expensive resins, RNase treatment, and dangerous organic compounds such as phenol and chloroform. Several micrograms of DNA can be obtained from up to 100 mg of fresh tissue or 20 mg of freeze-dried material in less than an hour. The DNA obtained with this kit is greater than 20 kb in length and can be used in sensitive downstream applications such as restriction endonuclease digests and PCR† amplification.

Reagents Included

Sufficient for 50 isolations

- Lysis Buffer [Part A], Product No. L7910 — 18 ml
- Lysis Buffer [Part B], Product No. L8035 — 3 ml
- Precipitation Solution, Product No. P9727 — 7 ml
- Binding Solution, Product No. B2177 — 37 ml
- Wash Solution, Product No. W3011 — 17 ml
- TE Buffer, Product No. T7688 — 20 ml
  10 mM Tris-HCl, 1 mM EDTA, pH 8.0
- Filtration Columns, Product No. C9346 — 50 each
- DNA Binding Columns, Product No. C9471 — 50 each
- 2-ml Collection Tubes, Product No. T7813 — 250 each

Reagents and Equipment Required But Not Provided

(Sigma product numbers are given where appropriate)

- Small mortar and pestle
- Liquid nitrogen
- Microcentrifuge tubes
- Ice
- 65°C water bath
- Microcentrifuge
- Ethanol, 95+%, Product No. E7148
- Molecular biology grade water, Product No. W4502

Precautions and Disclaimer

The GenElute™ Plant Genomic DNA Kit is for laboratory use only. Not for drug, household or other uses. Lysis Buffer [Part A] and Binding Solution contain guanidine thiocyanate which is harmful. Wear gloves, safety glasses, and suitable protective clothing when handling these solutions or any reagent provided with the kit. See the Materials Safety Data Sheet (MSDS).

Storage

Store the kit at room temperature. If any kit reagent forms a precipitate upon storage, warm at 65°C until the precipitate dissolves.

Procedure

Before beginning the procedure, do the following:

- Preheat a water bath to 65°C.
- Preheat the TE buffer to 65°C.
- Preheat the binding solution to 65°C to bring any insolubles into solution. Cool to room temperature before use.
- Wash Solution is supplied as a concentrate. Dilute the concentrate with 40 ml of 95+% ethanol. After each use tightly cap the diluted wash solution to prevent the evaporation of ethanol.

All steps are carried out at room temperature unless otherwise noted.

1. Grind plant tissue into a fine powder in liquid nitrogen using a mortar and pestle. Transfer up to 100 mg of the powder to a microcentrifuge tube. Keep the sample on ice until the addition of lysis buffers.

2. Add 350 µl of lysis buffer [Part A] and 50 µl of lysis buffer [Part B] to the tube, then thoroughly mix by vortexing and inverting. Incubate the mixture at 65°C for 10 minutes with occasional inversion. Note: A white precipitate will form upon the addition of lysis buffer [Part B]. The precipitate will dissolve at 65°C.

   Optional RNase digest: This kit is designed to selectively isolate large DNA. In the event that preparations are found to be contaminated with RNA, RNase A (Product No. R4642) can be used to digest the contaminating RNA. Add 50 units of RNase A to the lysis mixture just prior to incubation at 65°C.

3. Add 130 µl of precipitation solution to the mixture, mix completely by inversion, and place the sample on ice for 5 minutes.

4. Centrifuge the sample at maximum speed (12,000-16,000 x g) for 5 minutes to pellet the cellular debris, proteins and polysaccharides.

5. Place a filtration column (blue) into a 2 ml collection tube and carefully pipet the supernatant from step 4 onto the column. Centrifuge at maximum speed for 1 minute. This step removes any cellular debris not removed in step 4.

6. Discard the filtration column and add 700 µl of binding solution directly to the eluent from step 5. Mix thoroughly by inversion.

7. Place a DNA binding column (colorless) into a fresh 2 ml collection tube and carefully pipet 700 µl of the mixture from step 6 onto the column. Centrifuge at maximum speed for 1 minute. Discard the eluent but retain the collection tube.

8. Return the DNA binding column to the collection tube and apply the remaining sample to the column. Repeat the centrifugation as described in step 7 and then discard the eluent and collection tube.

9. Place the DNA binding column into a fresh 2 ml collection tube, apply 500 µl of diluted wash solution to the column, and centrifuge at maximum speed for 1 minute. Discard the eluent but retain the collection tube.

10. Apply another 500 µl of diluted wash solution to the DNA binding column and centrifuge at maximum speed for 1 minute. Discard the eluent and collection tube. Do not allow the wash solution to contact the column, and wipe off any fluid that may be adhering to the outside of the column.

11. Transfer the DNA binding column to a fresh 2 ml collection tube. Apply 100 µl of pre-warmed (65°C) TE buffer to the column and centrifuge at maximum speed for 1 minute.
    Note: Molecular biology grade water can be substituted for TE buffer if desired.

12. Repeat the elution as described in step 11. Eluates may be collected in the same collection tube. Alternatively, another collection tube may be used for the second elution to prevent the dilution of the first eluate.

For short-term storage, maintain the DNA preparations at 4°C and avoid freezing if possible.

Precipitation of DNA

The GenElute Plant Genomic DNA Kit is designed so that the DNA always remains in solution, thus avoiding resuspension problems. However, if it is necessary to concentrate the DNA, ethanol precipitation in the presence of sodium acetate is recommended[1].

Alternative Disruption Procedures

The extraction of nucleic acid from plant tissue is complicated by the tough cell wall that surrounds most plant cells as well as the fibrous nature of many species. Several methods exist for the disruption of plant material. One of the most effective and commonly used methods is to grind the tissue in liquid nitrogen with a mortar and pestle. The Plant Genomic DNA Kit was developed based on this efficient method of disruption. However, other disruption techniques can be substituted for step 1 of the Procedure.

Good yields of high molecular weight DNA can also be obtained from freeze-dried tissue. Dried tissue should be ground into a fine powder with a mortar and pestle, and up to 20 mg of this powder can be used in a single DNA preparation. Liquid nitrogen is not necessary during the grinding of freeze-dried tissue. After grinding the tissue into a powder, follow the Procedure beginning with step 2.

Results

Determine the concentration and purity of the plant DNA by spectrophotometric analysis and agarose gel electrophoresis. If possible, directly measure the absorbance (A) of the DNA preparation at 260 and 280 nm using a quartz microcuvette with an appropriate blank. It may be necessary to dilute a portion of the sample to obtain an accurate absorbance measurement. The A_{260} should be between 0.1 and 1.0 (or within the linear range of your spectrophotometer). An absorbance of 1.0 at 260 nm corresponds to approximately 50 µg/ml of double-stranded DNA. The A_{260}/A_{280} ratio should be in the range of 1.7 to 1.9.

The size and quality of the DNA can be determined by agarose gel electrophoresis[1]. A gel containing 0.8% agarose in 0.5X TBE buffer works well for the resolution of genomic DNA. The DNA can be visualized by staining with an intercalating dye such as ethidium bromide. Genomic DNA should migrate as a single, high molecular weight band with very little evidence of shearing. A more precise determination of the size of the DNA can be made by pulsed-field gel electro-phoresis (Birren and Lai, 1993).

Typical yields of DNA from various plant species per 100 mg of tissue

Corn	7.5 µg
Dianthus tissue culture	3.3 µg
Pepper	3.1 µg
Rice	5.9 µg
Soybean	5.7 µg
Tobacco	5.2 µg
Tomato	6.2 µg
Tomato (20 mg freeze dried leaf tissue)	5.7 µg
Wheat	11.5 µg

Troubleshooting

1. Low Yield
   Yields will vary greatly between plant species and tissues. The age of the starting material is also an important factor. Use the youngest leaves or tissues whenever possible. Also, be sure to thoroughly disrupt the starting material (see step 1 of the Procedure and the discussion under Alternative Disruption Procedures).

2. Sheared DNA
   Agarose gel electrophoresis will give a good indication of the quality of the DNA preparation. Excessive manipulation of genomic DNA will result in shearing. This kit was designed to eliminate precipitation and resuspension of the DNA, common steps found in other genomic DNA kits that can lead to shearing. All pipeting steps should be done as gently as possible. Wide-orifice pipet tips are available (Product No. P1678) to help eliminate potential shearing.

   Sheared DNA can also result from poor starting material. Fresh plant tissue should be frozen in liquid nitrogen and used immediately, stored at -80°C, or freeze-dried to avoid the degradation of its DNA.

3. Inhibition of Downstream Applications
   Ethanol carryover in the DNA preparations can lead to inhibition of enzymes used in downstream applications. After the second wash of the DNA binding column (step 10) do not allow the wash solution to contact the column. Wipe off any fluid that may be adhering to the outside of the column.

References

1. Sambrook, J., *et al*. Molecular Cloning: A Laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, pp. 6.2-6.19, E.10-E.14. 1989)

2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A Practical Guide, (Academic Press, San Diego, CA, 1993)

Related Products

Agarose, Product No. A9539
Ethidium bromide, 10 mg/ml, Product No. E1510
Microcentrifuge tubes, 1.5 ml, Product No. T9661
PCR Core kit with *Taq* DNA polymerase, Product No. CORE-T
Pipet tips, 200 µl, wide orifice, Product No. P1678
RNase A, Product No. R4642
Sodium acetate, 3 M, Product No. S7899
Taq DNA polymerase, Product No. D1806
TBE Buffer, 5X concentrate, Product No. T6400

Related Books

Methods in Plant Molecular Biology: A Laboratory Course Manual, Maliga, P. *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1995 (Product No. Z37,375-3)
Plant Molecular Biology: A Laboratory Manual, M.S. Clark, Ed., Springer-Verlag, New York, NY, 1997 (Product No. Z37,550-0)
Plant Molecular Biology: Labfax, R.R.D. Croy, Ed., Bios Scientific Publishers, Oxford, England, 1994 (Product No. Z35,746-4)

[†]The PCR process is covered by patents owned by Hoffman-LaRoche, Inc..