

Genomic DNA Mini Kit (Plant)

For research use only

Sample	: up to 100 mg of fresh or 25 mg of dry plant tissue
Yield	: up to 50 µg
Format	: spin column
Operation time	: within 60 minutes

Geneaid



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Introduction

The Genomic DNA Mini Kit (Plant) provides a quick and easy method for purifying total DNA (including genomic DNA, mitochondrial and chloroplast DNA) from plant tissue. Samples are disrupted by both grinding in liquid nitrogen and lysis buffer incubation. The lysate is treated with RNase A to degrade RNA and then filtered to remove cell debris and salt precipitates. In the presence of the binding buffer, coupled with chaotropic salt, genomic DNA in the lysate binds to the glass fiber matrix of the spin column (1). Contaminants are removed using a Wash Buffer (containing ethanol) and the purified genomic DNA is eluted by a low salt Elution Buffer or TE. The procedure does not require DNA phenol extraction or alcohol precipitation, and can be completed in less than 1 hour. The purified genomic DNA is ready for use in PCR, Real-time PCR, Southern Blotting and RFLP.

Quality Control

The quality of the Genomic DNA Mini Kit (Plant) is tested on a lot-to-lot basis by isolating genomic DNA from 50 mg young leaf samples. More than 10 µg of genomic DNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	GP004	GP100
GP1 Buffer	2 ml	50 ml
GPX1 Buffer	2 ml	50 ml
GP2 Buffer	1 ml	15 ml
GP3 Buffer* (Add Isopropanol)	1.5 ml (3 ml)	30 ml (60 ml)
W1 Buffer	2 ml	45 ml
Wash Buffer** (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)
Elution Buffer	1 ml	30 ml
RNase A (10 mg/ml)	25 µl	550 µl
Filter Column	4 pcs	100 pcs
GD Column	4 pcs	100 pcs
2 ml Collection Tube	8 pcs	200 pcs

Order Information

Product Name	Package Size	Cat. No.
Genomic DNA Mini Kit (Blood/Cultured Cell)	100/300 preps	GB100/300
Genomic DNA Midi Kit (Blood/Cultured Cell)	25 preps	GDI25
Genomic DNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	GDM10/25
Genomic DNA Mini Kit (Tissue)	50/100/300 preps	GT050/100/300
Genomic DNA Mini Kit (Plant)	100 preps	GP100
Genomic DNA Maxi Kit (Plant)	10/25 preps	GPM10/25
96-Well Genomic DNA Kit	2/4/10 x 96 Wells	GBP02/04/10
96-Well Genomic DNA Kit (Plant)	2/4/10 x 96 Wells	GPP02/04/10
Vacuum Manifold (Accessories)	1 set	ZFV04

* Add Isopropanol to the GP3 Buffer prior to initial use (see the bottle label for volume).

** Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

Caution

The components contain irritants. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

- (1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Genomic DNA Mini Kit (Plant) Protocol

Due to various plant species containing different metabolites such as polysaccharides, polyphenols, and proteins, we provide two different lysis buffers to satisfy the various plant samples. The standard protocol uses GP1 Buffer for lysis of plant samples. For most common plant species, the buffer system ensures purified DNA with high yields and high quality. Alternatively, GPX1 Buffer is provided with the kit. The different detergent in this lysis buffer is suitable for some plant samples with high polysaccharide content.

- Add Isopropanol to the GP3 Buffer prior to initial use (see the bottle label for volume).
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
- Additional Requirements: microcentrifuge tubes, Isopropanol, absolute ethanol, ddH₂O

Step 1 Tissue Dissociation	<ul style="list-style-type: none"> ● Cut off 50 mg (up to 100 mg) of fresh or frozen plant tissue or 10 mg (up to 25 mg) of dried sample. ● Grind the sample under liquid nitrogen to a fine powder and transfer it to a 1.5 ml microcentrifuge tube (some plant samples can be disrupted without liquid nitrogen).
Step 2 Lysis	<ul style="list-style-type: none"> ● Add 400 µl of GP1 Buffer (or GPX1 Buffer) and 5 µl of RNase A into the sample tube and mix by vortex. Do not mix GP1 Buffer (GPX1 Buffer) and RNase A before use. ● Incubate at 65°C for 10 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat the required Elution Buffer (200 µl per sample) to 65°C (for Step 5 DNA Elution). ● Add 100 µl of GP2 Buffer and mix by vortex. ● Incubate on ice for 3 minutes. ● Place a Filter Column in a 2 ml Collection Tube. ● Transfer the mixture to the Filter Column. Centrifuge for 1 minute at 1,000 x g. ● Discard the Filter Column and carefully transfer the supernatant in the 2 ml Collection Tube to a new 1.5 ml microcentrifuge tube.
Step 3 DNA Binding	<ul style="list-style-type: none"> ● Add 1.5 volumes of GP3 Buffer (Isopropanol added) to the lysate and vortex immediately for 5 seconds (eg. add 750 µl GP3 Buffer to 500 µl lysate). ● Place a GD Column in a 2 ml Collection Tube. ● Transfer 700 µl of the mixture (including any precipitate) to the GD Column. ● Centrifuge at 14-16,000 x g for 2 minutes. ● Discard the flow-through from the 2 ml Collection Tube and add the remaining mixture to the GD Column. ● Centrifuge at 14-16,000 x g for 2 minutes. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube.
Step 4 Wash	<ul style="list-style-type: none"> ● Add 400 µl of W1 Buffer to the GD Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube. ● Add 600 µl of Wash Buffer (ethanol added) to the GD Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube. ● Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix. <hr/> <p>Optional Step: Residue Pigment Removal If a few pigments remain on the column matrix, perform this optional step.</p> <ul style="list-style-type: none"> ● Following the Wash Buffer addition, add 400 µl of absolute ethanol to the GD Column. ● Centrifuge at 14-16,000 x g for 30 seconds. ● Discard the flow-through and place the GD Column back in the 2 ml Collection Tube. ● Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.
Step 5 DNA Elution	<p>Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution Step to increase DNA recovery and the total elution volume to approximately 200 µl.</p> <ul style="list-style-type: none"> ● Transfer the dried GD Column to a clean 1.5 ml microcentrifuge tube. ● Add 100 µl of preheated Elution Buffer or TE to the center of the column matrix. ● Let stand for 3-5 minutes or until the Elution Buffer or TE is absorbed by the matrix. ● Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

Troubleshooting

Problem	Possible Reasons/Solution
Column Clogged	Too much sample was used. <ul style="list-style-type: none">• Reduce the sample volume or separate it into multiple tubes.
Low Yield	Precipitate was formed at the DNA Binding Step <ul style="list-style-type: none">• Reduce the sample material.• Prior to loading the column, break up the precipitate in the ethanol-added lysate. Incorrect DNA Elution Step <ul style="list-style-type: none">• Ensure that the Elution Buffer or TE is added to the center of the GD Column matrix and is absorbed completely.
Eluted DNA does not perform well in downstream applications	Incomplete DNA Elution <ul style="list-style-type: none">• Elute twice to increase yield. Residual ethanol contamination <ul style="list-style-type: none">• Following the Wash Step, dry the GD Column with additional centrifugation at 14-16,000 x g for 5 minutes or incubate at 60°C for 5 minutes.