HiYield Genomic DNA Midi Kit (Plant)

Cat. No.:	YGPI25
Product Name:	HiYield Genomic DNA Midi Kit (Plant)
Reaction:	25
Sample:	500 mg of Fresh Plant Tissue or 125 mg of Dry Plant Tissue
Yield:	Up to 200 ug
Format:	Spin Column
Operation:	Centrifuge
Operation Time:	Within 60 Minutes

Introduction

HiYield Genomic DNA Midi Kit (Plant) provides an efficient method for purifying total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. 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, the 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.

Components

ITEM	YGPI25
GP1 Buffer	60 ml
GPX1 Buffer	60 ml
GP2 Buffer	15 ml
GP3 Buffer*	40 ml
W1 Buffer	60 ml
Wash Buffer**	50 ml
Elution Buffer	30 ml
RNase A (10 mg/ml)	650 ul
Lysate Filter Column	25 pcs
GD Maxi Column	25 pcs

*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).

Features

1. Duo lysis buffer system.
2. High and reproducible recoveries for constant results.
3. Ready-to use highly concentrated DNA within 60 minutes.

Quality Control

The quality of HiYield Genomic DNA Midi Kit (Plant) is tested on a lot-to-lot basis by isolating genomic DNA from 100 mg young leaf samples. More than 50 µg of genomic DNA is quantified with a spectrophotometer and checked by electrophoresis.

Caution

Some components are 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.

Protocol

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

Things to do before starting

1. Add Isopropanol to the GP3 Buffer prior to initial use (see the bottle label for volume).
2. Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).
3. Additional requirements: 15 ml centrifuge tube, 50 ml centrifuge tube, Ethanol (96-100%).

Step 1 Tissue Dissociation	1. Cut off 250 mg (up to 500 mg) of fresh or frozen plant tissue or 25 mg (up to 125 mg) of a dried sample. 2. Grind the sample under liquid nitrogen to a fine powder and transfer it to a 15 ml centrifuge tube (some plant samples can be disrupted without liquid nitrogen).
Step 2 Lysis	3. Add **2 ml of GP1 Buffer (or GPX1 Buffer)** and **25 µl of RNase A** into the sample tube and mix by vortex. **Do not mix GP1 Buffer and RNase A before use**. 4. Incubate at 65ºC for 20 minutes. During incubation, invert the tube every 5 minutes. At this time, preheat the required **Elution Buffer** (1 ml per sample) to 65ºC (for Step 5 DNA Elution). 5. Add **0.5 ml of GP2 Buffer** and mix by vortex. Then incubate on ice for 5 minutes. 6. Place a **Lysate Filter Column** in a 50 ml centrifuge tube. 7. Transfer the mixture to the **Lysate Filter Column** and centrifuge at 4,000 xg for 5 minutes. 8. Discard the **Lysate Filter Column** and carefully transfer the supernatant to a new 15 ml centrifuge tube.

<table>
<tr><td>Step 3
DNA Binding</td><td>9. Add 1.5 volumes of GP3 Buffer (Isopropanol added) to the lysate and vortex immediately for 10 seconds (ex: add 3.75 ml of GP3 Buffer to 2.5 ml of lysate).
10. Place a GPI Column in a 50 ml centrifuge tube.
11.Transfer the mixture (including any precipitate) to the GPI Column.
12. Centrifuge at 4,000 x g for 5 minutes.
13. Discard the flow-through and place the GPI Column back in the 50 ml centrifuge tube.</td></tr>
<tr><td rowspan="2">Step 4
Wash</td><td>14. Add 2 ml of W1 Buffer into the center of the GPI Column.
15. Centrifuge at 4,000 x g for 3 minutes.
16. Discard the flow-through and place the GPI Column back in the 50 ml centrifuge tube.
17. Add 4 ml of Wash Buffer (ethanol added) to the GPI Column.
18. Centrifuge at 4,000 x g for 3 minutes.
19. Discard the flow-through and place the GPI Column back in the 50 ml centrifuge tube.
20. Centrifuge at 4,000 x g for 10 minutes to dry the column matrix.</td></tr>
<tr><td>Optional Step: Residue Pigment Removal
If a few pigments remain on the column matrix, perform this optional step.
1) Following the Wash Buffer addition, add 4 ml of absolute ethanol to the GPI Column.
2) Centrifuge at 4,000 x g for 5 minutes
3) Discard the flow-through and place the GPI Column back in the 50 ml centrifuge tube.
4) Centrifuge again for 10 minutes at 4,000 x g to dry the column matrix.</td></tr>
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Step 5 **DNA Elution**	Standard elution volume is 0.5 ml. If less sample is to be used, reduce the elution volume (150-300µl) 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 about 1 ml. 21. Transfer the dried GPI Column into a clean 50 ml centrifuge tube (not provided). 22. Add 0.5 ml of preheated Elution Buffer or TE into the center of the column matrix. 23. Let stand for 5 minutes until Elution Buffer or TE absorbed by the matrix. 24. Centrifuge at 4,000 x g for 3 minutes to elute purified DNA.

Troubleshooting

Problem	Possible Reason/ Solution
Column clogged	**Too much sample was used.** →Reduce sample volume or separate into multiple tubes.
Low yield	**Incorrect DNA Elution Step.** →Ensure that Elution Buffer was added and absorbed to the center of GPI Column matrix.
	Precipitate was formed at DNA Binding Step. →Reduce the sample material. →Prior to loading the column, break up precipitate in ethanol-added lysate.
Eluted DNA does not perform well in downstream applications.	**Residual ethanol contamination.** →Following the wash step, dry the GPI Column with additional centrifugation at full speed for 5 minutes or incubation at 60℃ for 5 minutes.
	Incomplete DNA Elution. →Elute twice to increase yield.