FavorPrep™ Plant Genomic DNA Extraction Mini Kit

User Manual

Cat. No.: FAPGK 001 (50 Preps)
FAPGK 001-1 (100 Preps)

For Research Use Only

v.0905

Australian distributors:
Fisher Biotec Australia
free call: 1800 066 077
email: info@fisherbiotec.com
web: www.fisherbiotec.com

FavorPrep™ Plant Genomic DNA Extraction Maxi Kit

User Manual

Cat. No.: FAPGK 002 (10 Preps)
FAPGK 002-1 (24 Preps)

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Introduction

Genomic DNA Maxi Kit provides a fast and simple method to isolate total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. In the process, sample is distrusted by grinding in liquid nitrogen and lysis buffer incubation. The Lysate is treated with RNase A to degrade RNA and filtrated by filter column to remove cell debris and salt precipitations. In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to glass fiber matrix in the spin column. The contaminants are washed with an ethanol contained wash buffer and finally, the purified genomic DNA is eluted by low salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes. The purified genomic DNA is ready for PCR, real-time PCR, Southern blotting and RFLP.

Quality Control

The quality of Plant Genomic DNA Maxi Kit is tested on a lot-to-lot basis. The Kits are tested by isolation of genomic DNA from 50 mg young leave. More than 10 μg of genomic DNA could be quantified with spectrophotometer and checked by agarose gel.

Caution

ponent contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Sample: up to 1g of plant tissue
Yield: 50~300μg
Operation time: about 60 min

Kit Contents

	FAPGK 002 (10 preps)	FAPGK 002-1 (24 preps)
FAPG1 Buffer	45 ml	110 ml
FAPGX Buffer	45 ml	110 ml
FAPG2 Buffer	13 ml	30 ml
FAPG3 Buffer*	30 ml	70 ml
W1 Buffer**	33 ml	88 ml
Wash Buffer***	20 ml	45 ml
Elution Buffer	30 ml	60 ml
RNase A (10mg/ml)	550 µl	1300 µl
Filter Column	10 pcs	24 pcs
FAPG-Maxi Column	10 pcs	24 pcs

*Add 60/ 140 ml ethanol(96%~100%) to FAPG3 Buffer When first open.

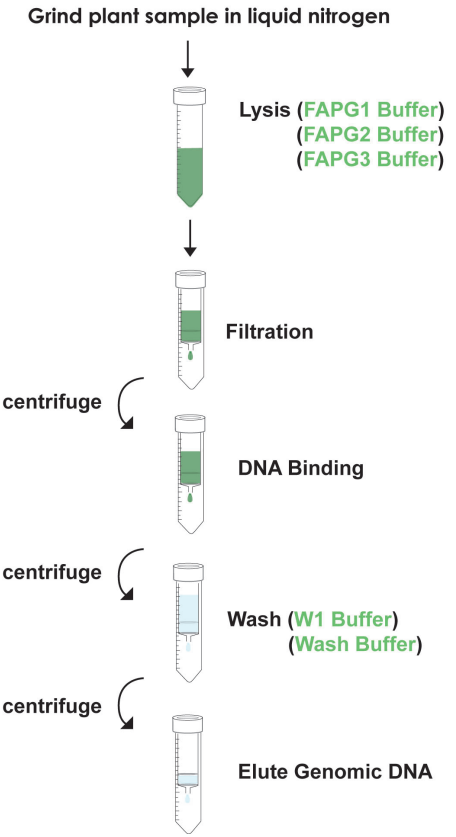
**Add 12/ 32 ml ethanol(96%~100%) to W1 Buffer When first open.

***Add 80/180 ml ethanol(96%~100%) to Wash Buffer When first open.

Protocol Technical Specification

Because of different plant species contain a lot of different metabolites like polysaccharides, polyphenolics, or proteins. Therefore, we provide two different lysis buffers for the various plant samples. The standard protocol uses FAPG1 Buffer for lysis of plant sample. For most of common plant species, the buffer system ensures purified DNA with high yields and a good quality. Alternatively, buffer FAPGX is provided with the kit also. The different detergent in this lysis buffer is suitable for some plant sample with a lot of polysaccharides.

Brief procedure



Protocol

Step 1 Tissue Dissociation	• Cut off up to 1g of fresh or frozen plant tissue or 50 mg (up to 100 mg) of dried sample. • Grind the sample under liquid nitrogen to a fine powder. Transfer it into a 15 ml centrifuge tube (not provided). For some plant sample, we can destruct it without liquid nitrogen.
Step 2 Lysis	• Add 4 ml **FAPG1 Buffer** (or **FAPGX Buffer**) and **50µl Rnase A (10 mg/ml)** into the sample tube and mix by vortexing. (Do not mix FAPG1 buffer and RNase A before use.) • Incubate at 65°C for 20 minutes. During incubation, invert the tube every 5 minutes. At the same time, preheat required Elution Buffer (2 ml per sample) at 65°C. • Add 1 ml **FAPG2 Buffer** and mix by vortexing. • Incubate at ice for 5 minutes. • Place a **Filter Column** in a 50 ml centrifuge tube (not provided). • Apply the mixture from previous step to the **Filter Column**. Centrifuge at 4000 X g for 5 minutes. • Discard the **Filter Column** and carefully transfer clarified supernatant in Collection Tube to a new 50 ml centrifuge tube (not provided).
Step 3 DNA Binding	• Add 1.5 volumes of FAPG3 Buffer (isopropanol added) to the cleared lysate and mix immediately by vortexing for 10 seconds. For example, add 7.5 ml FAPG3 Buffer to 5 ml of lysate. • Place a **FAPG-Maxi Column** in a 50 ml centrifuge tube. • Apply the mixture (including any precipitate) from previous step to the FAPG-Maxi Column. • Centrifuge at 4000 X g for 5 minutes. • Discard the flow-through and place the **FAPG-Maxi Column** back in the Collection Tube.

Step 4 **Wash**	• Add 4 ml of **W1 Buffer** into the column. • Centrifuge at 4000 X g for 3 minutes. • Discard the flow-through and place the **FAPG-Maxi Column** back in the Collection Tube. • Add 6 ml of **Wash Buffer** (ethanol added) into the column. • Centrifuge at 4000 X g for 3 minutes. • Discard the flow-through and place the **FAPG-Maxi Column** back in the Collection Tube. • Centrifuge at 4000 X g for 10 minutes to dry the column matrix.
	Optional Step: Remove residue pigment If a few pigment remain on the column matrix, perform this optional step. • After **Wash Buffer**, add **4 ml of ethanol (96-100%)** in the FAPG-Maxi Golumn. • Centrifuge at 4000 X g for 5 minutes. • Discard the flow-through and place the FAPG-Maxi Golumn back in the Collection Tubes. • Centrifuge again for 10 minutes at 4000 X g to dry the column martix.
Step 5 **DNA Elution**	Standard elution volume is 1 ml. If less sample to be used, reduce the elution volume (200-500µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume is about 2 ml. • Transfer dried **FAPG-Maxi** into a clean 50 ml centrifuge tube (not provided). • Add 1 ml of preheated **Elution Buffer** into the center of the column matrix. • Stand for 5 minutes until Elution Buffer absorbed by the matrix. • Centrifuge at 4,000 x g for 3 minutes to elute purified DNA.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Insufficient Lysis** ・Prolong the incubation time in lysis buffer to obtain higher yields of DNA.
	Insufficient disruption ・For most of species we recommend grinding with liquid nitrogen. Homogenization should be done thoroughly until the plant material is ground to a fine powder.
	DNA still bound to the membrane ・The DNA can be either eluted in higher volumes or by repeating the elution step up to three times. Elution buffer should be preheated to 60℃ prior to elution. ・To ensure correct pH, use supplied elution buffer.
DNA is degraded	**Sample was contaminated with DNase** ・Preheat elution buffer to 60 ℃ for 5 minutes to eliminate any possible Dnase
	Centrifugation speed was too high ・Higher velocities may cause shearing of the DNA. The centrifugation maximum speed is at 11,000xg.
Column clogged	**Too much tissue was used.** ・Too much tissue was used. Reduce the amount of sample material or separate it into multiple tubes.
	Insufficient centrifugation ・Centrifuge again and extend centrifugati on time.
	Precipitate was formed at DNA Binding Step ・Reduce the sample material.

Introduction

Genomic DNA Mini Kit provides a fast and simple method to isolate total DNA (genomic DNA, mitochondrial and chloroplast) from plant tissue and cells. In the process, sample is distrusted by grinding in liquid nitrogen and lysis buffer incubation. The Lysate is treated with RNase A to degrade RNA and filtrated by filter column to remove cell debris and salt precipitations. In the presence of binding buffer with chaotropic salt, the genomic DNA in the lysate binds to glass fiber matrix in the spin column. The contaminants are washed with an ethanol contained wash buffer and finally, the purified genomic DNA is eluted by low salt elution buffer or water. The protocol does not require DNA phenol extraction and alcohol precipitation. The entire procedure can be completed in 60 minutes. The purified genomic DNA is ready for PCR, real-time PCR, Southern blotting and RFLP.

Quality Control

The quality of Plant Genomic DNA Mini Kit is tested on a lot-to-lot basis. The Kits are tested by isolation of genomic DNA from 50 mg young leave. More than 10μg of genomic DNA could be quantified with spectrophotometer and checked by agarose gel.

Sample: 100 mg of plant tissue
Yield: 5~40μg
Operation time: <60 min

Kit Contents

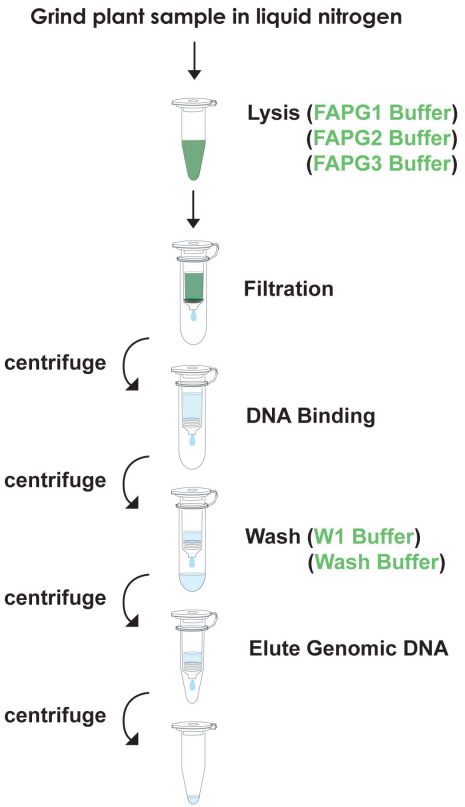
	FAPGK 001 (50 preps)	FAPGK 001-1 (100 preps)
FAPG1 Buffer	24ml	50ml
FAPG2 Buffer	8ml	15ml
FAPG3 Buffer*	15ml	30ml
W1 Buffer**	22ml	44ml
Wash Buffer***	10ml	20ml
Elution Buffer	15ml	30ml
RNase A (50mg/ml)	500µl	840µl
Filter Column	50 pcs	100 pcs
FAPG Column	50 pcs	100 pcs
1.5 ml Elution tube	50 pcs	100 pcs
2ml Collection tube	100 pcs	200 pcs

*Add 30 ml/60 ml ethanol to FAPG3 Buffer before first use.
**Add 8 ml/16ml ethanol to W1 Buffer before first use.
***Add 40 ml/80ml ethanol to Wash Buffer before first use.

Caution

The component contains irritant agent. During operation, always wear a lab coat, disposable gloves, and protective goggles.

Procedure


Grind plant sample in liquid nitrogen
Lysis (FAPG1 Buffer)
(FAPG2 Buffer)
(FAPG3 Buffer)
Filtration
centrifuge
DNA Binding
centrifuge
Wash (W1 Buffer)
(Wash Buffer)
centrifuge
Elute Genomic DNA
centrifuge

Protocol

Step 1 **Tissue Dissociation**	• Cut off 50mg (up to 100mg) of fresh or frozen plant tissue or 5 mg (up to 100 mg) of dried sample. • Grind the sample under liquid nitrogen to a fine powder. Transfer it into a microcentrifuge tube (not provided). For some plant sample, we can destruct it without liquid nitrogen.
Step 2 **Lysis**	• Add 400 µl **FAPG1 Buffer** and 8µl **RNase A (50 mg/ml)** into the sample tube and mix by vortexing. Do not mix FAPG1 Buffer and RNase A before use. • Incubate at 65℃ for 10 minutes. During incubation, invert the tube every 5 minutes. At the same time, preheat required Elution Buffer (200µl per sample) at 65℃. • Add 130µl **FAPG2 Buffer** and mix by vortexing. • Incubate at ice for 5 minutes. • Place a **Filter Column** in a 2 ml Collection Tube. • Apply the mixture from previous step to the **Filter Column**. Centrifuge for 3 minutes at full speed (13,000 rpm). • Discard the **Filter Column** and carefully transfer clarified supernatant in Collection Tube to a new microcentrifuge tube (not provided).
Step 3 **DNA Binding**	• Add 1.5 volumes of **FAPG3 Buffer (ethanol added)** to the cleared lysate and mix immediately by vortexing for 5 seconds. For example, add 750µl FAPG3 Buffer to 500µl lysate. • Place a **FAPG Column** in a 2 ml Collection Tube. • Apply 750µl the mixture (including any precipitate) from previous step to the FAPG Column. • Centrifuge at full speed (13,000 rpm) for 2 minute. • Discard flow-through in Collection Tube and apply remaining mixture to FAPG Column. • Centrifuge at full speed (13,000 rpm) for 2 minute. • Discard flow-through in Collection Tube.

Step 4 **Wash**	• Add 500μl of **W1 Buffer (ethanol added)** into the column. • Add 750μl of **Wash Buffer (ethanol added)** into the column. • Centrifuge at full speed (13,000 rpm) for 30 seconds. • Discard the flow-through and place the FAPG Column back in the Collection Tube. • Centrifuge at full speed for 3 minutes to dry the column matrix. ---**Important Step!** The residual liquid can affect the quallity of DNA and inhibit subsequent enzymatic reactions.
Step 5 **DNA Elution**	• Transfer dried **FAPG Column** into a clean 1.5 ml microcentrifuge tube (not provided). • Add 50-200μl of **preheated Elution Buffer** into the center of the column matrix. • Stand for 3 minutes until Elution Buffer absorbed by the matrix. • Centrifuge full speed (13,000 rpm) for 2 minutes to elute purified DNA. ---**Important Step!** For effective elution, make sure that the elution solution is dispensed on the membrane center and is absorbed completely. ---Standard elution volume is 200μl. If less sample to be used, reduce the elution volume (50-150μl) to increase DNA concentration.

Troubleshooting

Problem	Possible Reasons/ Solution
Low yield	**Insufficient Lysis** ・Prolong the incubation time in lysis buffer to obtain higher yields of DNA.
	Insufficient disruption ・For most of species we recommend grinding with liquid nitrogen. Homogenization should be done thoroughly until the plant material is ground to a fine powder.
	DNA still bound to the membrane ・The DNA can be either eluted in higher volumes or by repeating the elution step up to three times. Elution buffer should be preheated to 60℃ prior to elution. ・To ensure correct pH, use supplied elution buffer.
DNA is degraded	**Sample was contaminated with DNase** ・Preheat elution buffer to 60℃ for 5 minutes to eliminate any possible Dnase
	Centrifugation speed was too high ・Higher velocities may cause shearing of the DNA. The centrifugation maximum speed is at 11,000xg.
Column clogged	**Too much tissue was used.** ・Too much tissue was used. Reduce the amount of sample material or separate it into multiple tubes.
	Insufficient centrifugation ・Centrifuge again and extend centrifugation time.
	Precipitate was formed at DNA Binding Step ・Reduce the sample material. ・Before loading the column, break up the precipitate in ethanol-added lysate by pipetting.