Plant Genomic DNA Extraction Mini Kit - PROTOCOL

General Protocol:

HINT: Prepare a ice box and a 65 °C bath for step 2 and 3. Preheat Elution Buffer to 65 °C for step 13 (elutionstep).

Grind 50 mg (up to 100 mg) plant tissue sample or 20 mg dry weight of plant tissue under liquid nitrogen to a fine powder and transfer to a new microcentrifuge tube (not provided).

- Do not allow the sample to thaw, and continue immediately to step 2.

Add 400 µl of FSPG1 Buffer and 8 µl of RNase A stock solution (50 mg/ml) to the tissue powder and vortex vigorously. Incubate the mixture at room temperature for 2 minutes then at 65°C for 10~20 minutes and invert 2-3 times during incubation.

Add 130 µl of FSPG2 Buffer to the mixture. Vortex to mix well and incubate the mixture on ice for 5 min.

Place a Filter Column to a Collection Tube and transfer the entire mixture from previous step to the Filter Column. Centrifuge the Filter Column at full speed (~ 18,000 x g) for 3 min.

Transfer the clarified lysate (supernatant) from the Collection Tube to a new microcentrifuge tube (not provided). Discard used Filter Column and Collection Tube. And adjust the volume of clarified lysate.

Note! Do not aspirate any debris when transfering the clarified lysate.

Add 1.5 volume of FSPG3 Buffer (ethanol added) to the clarified lysate and mix well by pipetting.

- Make sure that ethanol (96~100%) has been added to FSPG3 Buffer when first use.

Place a FSPG Column to a new Collection Tube and transfer up to 750 µl of the sample mixture from previous step carefully to the FSPG Column. Centrifuge at at full speed (18,000 x g or 14,000 rpm) for 1 min. Discard the flow-through and place the FSPG Column back to the Collection Tube.

Repeat step 7 for the rest of the sample mixture.

Add 400 µl of Wash Buffer W1 (ethanol added) to the FSPG Column. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 30 seconds. Discard the flow-through and place the FSPG Column back to the Collection Tube. --Make sure that ethanol (96~100%) has been added into Wash Buffer W1 when first use.

Add 650 µl of Wash Buffer W2 (ethanol added) to FSPG Column. Centrifuge at full speed (18,000 x g or 14,000 rpm) for 30 seconds. Discard the flow-through and place the FSPG Column back to the Collection Tube. --Make sure that ethanol (96~100%) has been added into Wash Buffer W2 when first use.

Repeat step 10 for one more washing.

Centrifuge at full speed (18,000 x g or 14,000 rpm) for an additional 3 min to dry the FSPG column completely. --Important step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

Combine the FSPG Column with a Elution Tube, Add 50~200 µl of preheated Elution Buffer to the membrane center of the FSPG Column. Stand the FSPG Column for 1 minute at room temperature. --Important step! For effective elution, make sure that the elution buffer is dispensed onto the membrane center and is absorbed completely.

Centrifuge at full speed (18,000 x g or 14,000 rpm) for 1 min to elute purified DNA.

For Research Use Only

FISHER MOLECULAR BIOLOGY
36 Terry Drive
Trevose, PA 19048 - USA

Plant Genomic DNA Extraction Mini Kit - Troubleshooting

Low or no yield of genomic DNA

Problems	Possible reasons	Solutions
	Incorrect preparation of FSPG3 Buffer or Wash Buffer	
	FSPG3 Buffer is not mixed with ethanol before use	Repeat the extraction procedure with a new sample.
	Wash Buffer W1 and Wash Buffer W2 is not mixed with ethanol before use	Make sure that the correct volumes of ethanol (96-100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first open. Repeat the extraction procedure with a new sample.
	The volume or the percentage of ethanol is not correct before adding into Wash Buffer W1 and Wash Buffer W2	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer W1 and Wash Buffer W2 when first use. Repeat the extraction procedure with a new sample.
	Elution of genomic DNA is not efficient	
	pH of water (ddH2O) for elution is acidic	Make sure the pH of ddH2O is between 7.5- 9.0.
		Use Elution Buffer (provided) for elution.
	Elution Buffer or ddH2O is not completely absorbed by column membrane	After Elution Buffer or ddH2O is added, stand the PGDE Column for 5 min before centrifugation.
Column is clogged		
	Sample is too viscous	Reduce the sample volume.
Degradation of elutated DNA		
	Sample is old	Always use fresh or well-stored sample for genomic DNA extraction.
	Buffer for gel electrophoresis contaminated with DNase	Use fresh running buffer for gel electrophoresis.

For Research Use Only