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PG reagent (Plant Genomic DNA Isolation Kit)

For research use only

Introduction

PG reagent provides an easy 3 step method to isolate high yields of total DNA (including genomic, mitochondrial and chloroplast DNA from plant tissue and cells). This unique reagent is able to lyse most common plant samples and also samples high in polysaccharides. If a large sample is required, the reagent volume can be scaled proportionately, making this reagent not only very user friendly but also highly versatile. DNA phenol extraction is not required and the entire procedure can be completed in 90 minutes. The extracted total DNA is ready for use in PCR, Real-time PCR, Southern Blotting, Mapping and RFLP.

Kit Content

Catalog No.	PG0004	PG0100	PG0500
PG Reagent	4 ml	100 ml	500 ml

Quality Control

In accordance with FairBiotech's ISO-certified Total Quality Management System, the quality of the FB PG Reagent (Plant Genomic DNA Isolation Kit) is tested on a lot-to-lot basis to ensure consistent product quality.

Additional requirements

∗mortar and pestle ∗microcentrifuge tubes ∗70% EtOH ∗chloroform ∗isopropanol ∗RNase A (50 mg/ml) ∗TE or ddH_2O

PG reagent (Plant Genomic DNA Isolation Kit) Protocol

Sample Preparation

- Cut off 100 mg of fresh plant tissue or 50 mg of dry plant tissue. Grind the sample under liquid nitrogen to a fine powder using a mortar and pestle.

Step 1 Lysis

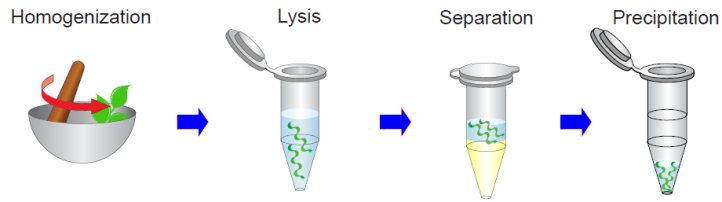
- Add **1 ml of PG reagent and 0.5 µl of RNase A (50 mg/ml)** to the sample in the mortar and grind the sample until it is completely dissolved.
- Transfer the dissolved sample to a 1.5 ml microcentrifuge tube. Incubate at 65ºC for 30-50 minutes.(invert the tube every 10 minutes)
- Centrifuge at 16,000 x g for 10 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube.

Step 2 Phase Separation

- **Standard Samples:** Add 600 µl of chloroform to the supernatant from Step 1. **High Poly- saccharide Samples:** Add a **1/10 volume of PG reagent** and 600 µl of chloroform to the supernatant from Step 1.
- Shake vigorously and then centrifuge at 16,000 x g for 10 minutes. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentrifuge tube.
- **Repeat the Phase Separation Step until the interphase becomes clear then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.(** The number of repetitions is dependent on sample type; e.g. dense tissue samples may require a higher number of repeats.)

Step 3 DNA Precipitation

- Add 800 µl of isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step 2.
- Mix the sample by inverting gently and let stand for 5 minutes at room temperature (DNA precipitation can be increased with extended standing time).
- Centrifuge at 16,000 x g for 15 minutes. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
- Centrifuge at 16,000 x g for 5 minutes. **Completely discard the supernatant** and re-suspend the pellets in 50-100 µl of TE buffer or ddH_2O.
- Incubate for 10 minutes at 60ºC to dissolve the pellet.



Troubleshooting

Problem	Cause	Solution
Low yield of DNA	Incompletely lysed sample	For each different sample type, use only the required range or amount of starting materials to prepare the lysates.
		For tissues, cut the tissue into smaller pieces and Ensure that the tissue is completely immersed in the Lysis step to obtain optimal lysis.
DNA degradation	Sample not fresh	Avoid repeated freeze / thaw cycles of the sample. Use a new sample for DNA isolation. Perform the extraction using fresh materials whenever possible.
	Inappropriate sample storage conditions	Store mammalian tissues at -80°C and bacteria at -20°C until use. The whole blood can be stored at 4°C for no longer than 1-2 days.
	DNase contaminantion	Maintain a sterile environment while working (e.g. wear gloves and use DNase-free reagents).
Inhibition of downstream enzymatic reactions	Purified DNA containing residual ethanol	Remove EtOH in the hood briefly.
Presence of RNA	RNA contamination	Perform RNase A digestion step during the Lysis Step.