Plant Genomic DNA Isolation Reagent

Cat No. PDR02-0100/PDR02-0100S
Size: 100 ml/4 ml

Description

The PG Reagent provides an easy 3-step method to isolate a high yield of total DNA (including genomic, mitochondrial, and chloroplast DNA from the plant tissue and cells). This unique reagent is able to lyse the most common plant samples as well as the 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. The 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.

Background on Genomic DNA Isolation and Purification

The goal of the genomic DNA isolation depends on the type(s) of DNA application after isolation. The DNA's purity, source, quantity, and quality are all key issues that need to be addressed prior to the genomic DNA extraction. A whole host of different methods, technologies and kits are available now to researchers to isolate the genomic DNA from cells. Generally, all methods involve the disruption and lysis of cells. This is followed sometimes by the removal of RNA by RNAse, salt or other methods. The DNA is isolated from the proteins by several methods, including the digestion of proteins by the enzyme proteinase K. The proteins are removed subsequently by salting-out, organic extraction, or binding of the DNA to a solid-phase support (such as an anion-exchange column or silica technology). The DNA is finally recovered by ethanol precipitation or isopropanol precipitation.

Feature

- Fast procedure delivering high-quality genomic DNA
- Ready-to-use DNA for high performance in any downstream application
- Consistent DNA yield from the starting material with a small amount
- Provide sufficient reagents and 3 steps to treat samples from 50 mg to 100 mg
- Time flexibility
- Ease of DNA extraction technique or method
- Expense reduction

Application

- Quantity of DNA needed
- Molecular weight and size of the DNA
- Purity of DNA required
- Downstream DNA applications

Required Materials

- Homogenizer (mortar and pestle)
- Microcentrifuge tubes
- 70% EtOH
- Chloroform
- Isopropanol
- RNase A (50 mg/ml)
- TE (Tris-EDTA, pH8.0) or ddH_2O

Buffer Preparation

- TE Buffer (Tris-EDTA, pH8.0): 10 mM Tris-HCl, pH 8.0 with 0.1mM EDTA

Plant Genomic DNA Isolation Protocol

The standard protocol uses the PG Reagent for lysis of plant samples. For most common plant species, the reagent system ensures the isolated plant genomic DNA with a high yield and good quality.

Sample Preparation

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

Step 1 Lysis

1. Add 1 ml of the PG Reagent and 0.5 µl of the RNase A (50 mg/ml) to the sample in the mortar and grind the sample until it is completely dissolved.
2. Transfer the dissolved sample to a 1.5 ml microcentrifuge tube.
3. Incubate at 65ºC for 30~50 minutes. Centrifuge at 14~16,000 x g for 10 minutes. Transfer he supernatant to a new 1.5 ml microcentrifuge tube.

Step 2 Phase Separation

Standard Samples

Add 600 µl of the chloroform to the supernatant from Step 1.

High Polysaccharide Samples

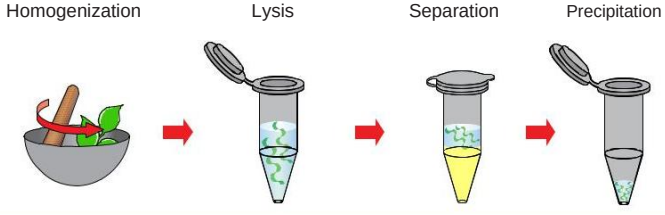
Add a 1/10 volume of the PG Reagent and 600 µl of the chloroform to the supernatant from Step 1.

1. Shake vigorously and then centrifuge at 14~16,000 x g for 10 minutes.
2. Carefully remove the upper phase and transfer it to a new 1.5 ml microcentri fuge tube.
3. Repeat the Phase Separation Step until the interphase becomes clear, then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.

NOTE: The number of repetitions is dependent on the sample type; e.g. dense tissue samples may require a higher number of repeats.

Step 3 DNA Precipitation

1. Add 800 µl of the isopropanol to the 1.5 ml microcentrifuge tube containing the clear upper phase from step2.
2. Mix the sample by inverting gently and letting it stand for 5 minutes at the room tempera ture (DNA precipitation can be increased with the extended standing time).
3. Centrifuge at 14~16,000 x g for 20 minutes.
4. Discard the supernatant and wash the pellet with 1 ml of 70% EtOH.
5. Centrifuge at 14~16,000 x g for 5 minutes.
6. Completely discard the supernatant and re-suspend the pellets in 50~100 µl of the TE buffer (no provided) or ddH_2O.
7. Incubate for 10 minutes at 60ºC to dissolve the pellet.



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Troubleshooting

Refer to the table below to troubleshooting problems that you may encounter when purifying plasmid DNA with the kit.

Problem	Cause	Solution
Difficult to dissolve	Incomplete removal of EtOH	Remove EtOH in the hood briefly.
RNA containment	Incomplete removal of RNA	RNase A treatment.
Low yields of gDNA	Incomplete lysis and homogenization	Complete lysis and homogenization. Use the appropriate method for the lysate preparation based on the amount of the starting materials. Cut tissue samples into smaller pieces and ensure the tissue is completely immersed in the PG Reagent to achieve the optimal lysis.
	Presence of EtOH	Remove EtOH in the hood briefly.
	Incorrect elution conditions	Add TE Buffer or RNase-free H_2O (50~100 µl) and incubate for 10 min at 60°C.
	Incorrect separation phase	Repeat the Phase Separation Phase until the interphase becomes clear, and then transfer the clear upper phase to a new 1.5 ml microcentrifuge tube.
Inhibition of downstream enzymatic reactions	Presence of ethanol in the purified RNA	Remove EtOH in the hood briefly.

Caution

- Check buffers before use for salt precipitation. Re-dissolve any precipitate by warming up to 37°C.
- During the operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses