

## Phase Lock Gel (PLG)

### Maxi-preparation of genomic DNA from leaf tissue

**Note:** Please read the *Phase Lock Gel Manual* before starting.

#### Equipment and reagents to be supplied by user

- Extraction buffer (100 mM Tris-HCl at pH 8.0, 10 mM EDTA at pH 8.0, 100 mM NaCl and 1% n-lauryl-sarcosin)
- RNase A (10 mg/ml)
- Phenol:chloroform:isoamyl alcohol (25:24:1)
- Chloroform:isoamyl alcohol (24:1)
- TE buffer (10 mM Tris-HCl and 1 mM EDTA at pH 8.0)
- 3 M ammonium acetate (pH 5.2)
- Isopropanol
- 70% ethanol
- Phase Lock Gel Heavy tubes (25 x 50 ml) (Ref. No. 2302870)

#### Procedure

This protocol was used to isolate genomic DNA from plant tissue (*Zea mays* Line A188 and *Nicotiana tabaccum* Line SR1).

1. Grind 1 g fresh weight leaf tissue in a mortar with liquid nitrogen. Add 10 ml extraction buffer to the frozen powder.
2. Transfer the samples into pre-spun 50 ml Phase Lock Gel Heavy tubes.
3. Add 10 µl RNase A to the sample to degrade the RNA. Incubate for 10 min at 65°C.
4. Extract the DNA with 10 ml of phenol:chloroform:isoamyl alcohol. Gently mix until a homogeneous suspension is formed. **Do not vortex!** Separate the phases by centrifuging at 1,500 x g for 5 min at room temperature.
5. Transfer the aqueous phase to a fresh 50 ml Phase Lock Gel Heavy tube. Repeat the extraction with 10 ml chloroform:isoamyl alcohol.
6. Transfer the aqueous phase to a fresh 50 ml centrifuge tube and precipitate the genomic DNA by adding 1 ml 3 M ammonium acetate and 700 µl isopropanol. Incubate for a minimum of 20 min on ice, and then pellet the DNA by centrifugation at 5,000 x g for 10 min at 4°C.
7. Decant the supernatant and wash the pellet with 10 ml 70% cold ethanol. After removal of the ethanol, allow the DNA pellet to dry partially before resuspending in 1 ml TE buffer.

#### Ordering Information

Ref. No.	Product Name	Quantity	Sample Volume	Tube Color
2302810	Phase Lock Gel Heavy 1.5 ml – 200 Tubes	200	100 – 500 µl	green
2302830	Phase Lock Gel Heavy 2 ml – 200 Tubes	200	100 – 750 µl	yellow
2302850	Phase Lock Gel Heavy 15 ml – 100 Tubes	100	1 – 6 ml	† clear
2302870	Phase Lock Gel Heavy 50 ml – 25 Tubes	25	5 – 20 ml	† clear

† PLG Heavy is opaque while PLG Light is translucent.

Phase Lock Gel tubes are also available in different sizes and formulations for handling of different sample volumes and using different organic extraction solvents.

[Please find all our Phase Lock Gel products on our website.](#)