

## User Bulletin

Cat. #: GPG1002

## Plant Genomic DNA Extraction Miniprep System

Isolation of genomic DNA from 100 mg plant material or  $1 \times 10^8$  cells.

### Kit contents:

PX1 Buffer (1), PX2 Buffer (1), PX3 Buffer (1), RNase A(1), WS Buffer (2), Plant Genomic DNA Mini Column (250), Shearing Tube (250), Collection Tube (500) and protocol (1)

### Protocol:

#### <Note>:

- All centrifugation should be done at room temperature.
- Preheat a water bath to 65°C.
- Preheat TE or ddH<sub>2</sub>O to 65°C for DNA elution.
- PX1 Buffer and PX3 Buffer may form a precipitate, warm at 65°C to redissolve.
- Add 180 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.
- Add 1100 µl of ddH<sub>2</sub>O to the RNase A powder tube, vortex to dissolve and store at 4°C.

1. **Grind 100 mg (or less) plant sample under liquid nitrogen to fine powder and transfer to a new tube.**

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

2. **Add 400 µl of PX1 Buffer and 4 µl of RNase A solution (100 mg/ml) to the tissue powder and vortex vigorously, then incubate the mixture at 65°C for 10 minutes.**  
Do not mix PX1 Buffer and RNase A prior to use. Invert 2-3 times during 65°C incubation.
3. **Add 130 µl of PX2 Buffer to the lysate, vortex, and incubate on ice for 5 minutes.**
4. **Apply lysate to the Shearing Tube sitting in a Collection Tube and centrifuge at full speed for 2 minutes. Transfer flow-through sample from the Collection Tube to a new tube.**  
Avoid pipetting any debris or pellet in the collection tube.
5. **Add 0.5 volume of PX3 Buffer and 1 volume of 96-100% ethanol to the clear lysate and invert the tube 3-5 times.**  
For example: If 450 µl clear lysate collected, add 225 µl PX3 Buffer and 450 µl ethanol.
6. **Apply 650 µl of the ethanol added sample (including any precipitate) from step 5 to a Plant Genomic DNA Mini Column sitting in a Collection Tube, close the cap, centrifuge at 8,000 x g (10,000 rpm) for 1 minute, and discard the filtrate.**  
If the solution remains above the membrane, centrifuge again at 13,000 rpm.
7. **Repeat step 6 for rest of the sample.**
8. **Wash the column twice with 0.7 ml of WS Buffer by centrifuging at full speed (13,000 rpm or 10,000 x g) for 30-60 seconds and discard the filtrate.**

Add 180 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

- 9. Centrifuge at full speed for 2 minutes to remove traces of WS Buffer.**
- 10. Transfer the column to a new 1.5 ml tube, add 200  $\mu$ l of 65°C TE or ddH<sub>2</sub>O, and centrifuge at full speed for 1 minute to elute DNA.**
- 11. Store DNA at -20°C.**