

User Bulletin

Cat. #: GPGM1001

Plant Genomic DNA Extraction Maxiprep System

Isolation of genomic DNA from 1 g plant material.

Kit contents:

PX1 Buffer (1), PX2 Buffer (1), PX3 Buffer (1), RNase A (1), WS Buffer (1), Plant Genomic DNA Maxi Column (20), Shearing tube (20), 15ml Collection tube (40) and protocol (1)

Protocol:

<Note>:

- All centrifugation should be done at room temperature with a swing-bucket centrifuge.
- Preheat a water bath to 65°C.
- Preheat TE or ddH₂O to 65°C for DNA elution.
- PX1 Buffer and PX3 Buffer may form a precipitate, warm at 65°C to redissolve.
- Add 850 µl of ddH₂O to the RNase A powder tube, vortex to dissolve and store at 4°C.

1. **Grind 1 g (or less) plant sample under liquid nitrogen to a fine powder and transfer to a new tube.**
Do not allow the sample to thaw, and continue immediately to step 2.

2. **Add 4 ml of PX1 Buffer and 40 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 1.3 ml 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 (about 3000 rpm or 2500 x g) for 2 minutes. Transfer flow-through sample from the Collection tube to a new tube (not provided).**
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 mix by pipetting.**
For example: If 4.5 ml clear lysate collected, add 2.25 ml PX3 Buffer and 4.5 ml ethanol.
6. **Apply 6.5 ml of the ethanol added sample (including any precipitate) from step 5 to a Plant Genomic DNA Maxi Column sitting in a Collection tube, close the cap, centrifuge at full speed for 3 minutes, and discard the filtrate.**
If the solution remains above the membrane, centrifuge again.
7. **Repeat step 6 for rest of the sample.**
8. **Wash the column twice with 5 ml of WS Buffer by centrifuging at full speed for 3 minutes and discard the filtrate.**
Add 200 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

- 9. Centrifuge at full speed for 5 minutes to remove traces of WS Buffer.**
- 10. Transfer the column to a new 15 ml tube (not provided), add 2 ml of 65°C TE or ddH₂O, and centrifuge at full speed for 5 minutes to elute DNA.**
- 11. Store DNA at -20°C.**