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VIOGENE
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User Bulletin

Plant Genomic DNA Extraction Maxiprep System

Isolation of genomic DNA from 1 g plant material .

Downstream Application

* Restriction digestion
* Southern Blotting
* RAPD, RFLP
* PCR, Real-Time PCR

Product Contents

Cat. No	GPGM1001
Preps	20
PX1 Buffer	90ml
PX2 Buffer	30ml
PX3 Buffer	110ml
RNase A	85mg
WS Buffer	50ml
Plant Genomic DNA Maxi Column	20
Shearing Tube	20
Collection Tube	40
Protocol	1

All buffers need to be mixed well before use.

Shipping & Storage

Viogene Plant Genomic DNA Extraction Maxiprep is shipping and storage at ambient temperature up to 12 months.
If precipitate form by freezing temperature on any buffer, warm up at 37℃ to redissolve.

Protocol

❖ **Please read the following notes before starting the Procedures.**

Important Notes

- All centrifugation should be done at room temperature with a swing-bucket centrifuge.
- Preheat a water bath to 65℃.
- Preheat TE or ddH_2O to 65℃ for DNA elution.
- PX1 buffer may form unclear upon storage. This does not affect the procedure and efficiency.
- PX1 Buffer may form a precipitate, warm at 65℃ to redissolve.
- Add 200 ml of ethanol (98-100 %) to the WS Buffer bottle when first open the bottle.
- Add 850 µl of ddH_2O to the RNase A powder tube, vortex to dissolve and store at 4℃.

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℃ for 10 minutes basically.**

   Do not mix PX1 Buffer and RNase A prior to use. Invert 2-3 times during 65℃ 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 98-100% 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 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 (98-100%) 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℃ TE or ddH_2O, Stand the column for 5 minutes, and centrifuge for 1-2 minutes to elute DNA.**

11. **Centrifuge at full speed for 5 minute to elute DNA.**

12. **Store DNA at -20℃.**