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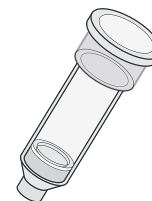
GenCatch™ Plant Genomic DNA Purification Kit



User's Guide for

Plant Genomic DNA Purification

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Overview

GenCatch™ Plant Genomic DNA Purification System provides a simple, fast and cost-effective method to purify genomic DNA from various plant species. It comes with shearing tubes for simple and fast homogenization and filtration of plant tissues. Genomic DNA of predominantly 20-30 kb can be isolated (from 100 mg plant material or 1×10^8 cells) free of contaminants and without phenol/chloroform extraction and ethanol precipitation.

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_2O to 65°C for DNA elution.
- PX1 Buffer and PX3 Buffer may form precipitates, warm at 65°C to dissolve.
- Add 1100 μl of ddH_2O to the RNase A powder tube, vortex to dissolve and store at 4°C.
- Add 180 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

1. **Grind 100 mg (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 400 μl of PX1 Buffer and 4 μl of RNase A stock 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℃ 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 <u>flow-through sample</u> 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 mix by pipetting.**
   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 the rest of the sample.**

8. **Wash the column <u>twice</u> with 0.7 ml of WS Buffer by centrifuging at full speed (13,000 rpm or 10,000 x g) for 30 seconds and discard the filtrate.**
   Remember to add 180 ml of ethanol (96-100%) to the WS Buffer bottle when first open the bottle.

9. **Centrifuge at full speed for another 2 minutes to remove traces of WS Buffer.**

10. **Transfer the column to a new 1.5 ml tube, add 200 μl of preheated (65°C) TE or ddH_2O, and centrifuge at full speed for 1 minute to elute DNA.**

11. **Store DNA at -20°C.**