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GENE PREP

Application Guide

Plant (Version 1)

I. **Purpose:** To extract genomic DNA from plant tissue.

II. **Chemical Principal:** Modified CTAB method.

IIIA. Preparation of Tissue Digest (off-line CTAB digestion):

A. Put plant material (50~300mg(wet)* / hole) into AutoGen's tube unit, freeze in liquid nitrogen and grind the frozen samples in AutoGen's automated sample grinder or use a mortar and pestle.
B. Add 0.45ml of PL-M1 (Plant Lysis Solution) to the ground samples, seal and digest at 65C for 30~60 minutes in shaking incubator.

IIIB. Preparation of Tissue Digest (on-board CTAB digestion):

A. Put plant material (50~300mg(wet)* / hole) into AutoGen's tube unit, freeze in liquid nitrogen and grind the frozen samples in AutoGen's automated sample grinder or use a mortar and pestle.
B. Load the tube unit onto GENE PREP

IV. Protocol Parameters:

A. Sample Volume: 0.5ml of CTAB digest.

B. Maximum Number of Samples: 48 samples per run
192 samples per run (with an optional Tube Unit Stacker)

C. Processing Time: 2 hours for 48 samples (including 60 minute drying period).
8 hours for 192 samples (including 60 minute drying period).

D. Yield: 10 ~ 20 µg genomic DNA / 100 mg of young rice plant
10 ~ 20 µg genomic DNA / 300 mg Arabidopis thaliana

E. Quality: OD260/280 values are 1.7-1.9.
The DNA can be used directly in downstream processes such as fluorescence DNA sequencing, PCR and restriction enzyme digestions*.
** The DNA from poly-saccharide rich samples might not be suitable for some downstream applications. Version 2 protocol is available for these types of plant materials.*

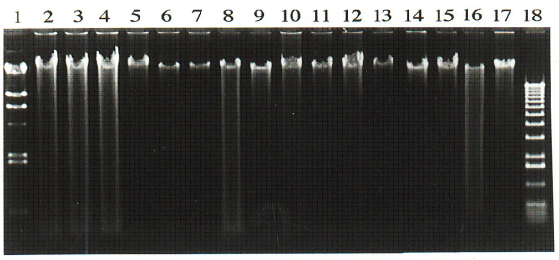
V. Running the Protocol:

- A. Load Reagents and Samples
- B. Select a Protocol
- C. Enter Number of Samples
- D. Start the Run

VI. Example of extracted genomic DNA:

Gel Electrophoresis of the extracted genomic DNA
Gel: 0.7% agarose

Lane 1: DNA mass marker
Lanes 2-3: rice
Lane 4: corn
Lanes 5-7: lily
Lane 8: strawberry
Lane 9: dropwort
Lanes 10-11:chrysanthemum
Lane 12: tobacco
Lane 13: broccoli
Lane 14: Chinese cabbage
Lane 15: kidney bean
Lane 16: gladiolus
Lane 17: taro
Lane 18: DNA mass marker



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Manual	Break plant cell wall	Grind plant in liquid nitrogen.
2. Manual	Digest plant tissue	Add Reagent PL-M1 and incubate.
3. Automated	Denature protein	Add Reagent PL-R1, mix, and Reagent PL-R2 and mix.
4. Automated	Remove protein and cellular debris	Add Reagent PL-R3, mix, centrifuge to pellet debris, transfer supernatant to new tube.
5. Automated	Precipitate DNA	Add Reagent PL-R4, mix, centrifuge to pellet DNA and discard supernatant.
6. Automated	Wash DNA	Add Reagent PL-R5, mix, centrifuge to pellet DNA and discard supernatant. Repeat several times.
5. Automated	Dry DNA	Dry DNA by heating.
6. Automated	Resuspend DNA	Add Reagent PL-R6 and mix.

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