

AutoGenprep 965 Application Guide

Plant



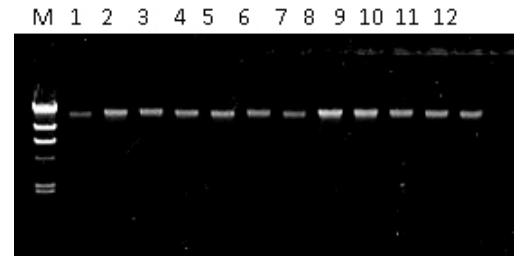
- I. **Purpose:** To extract genomic DNA from plant material.
- II. **Chemical Principal:** Modified CTAB Method.
- III. **Pretreatment of Plant Material:**
 - A. 50mg (wet) of plant material is frozen in liquid nitrogen and ground using a pestle or a commercially available grinder.
 - B. After Plant lysis solution (Reagent M1) is added, the ground sample is digested at 65°C for 30 minutes.
- IV. **Protocol Parameters:**
 - A. **Sample Volume:** 0.3 ml of digested solution.
 - B. **Maximum Number of Samples:** Up to 384 (4 of 96 plates) samples per run.
 - C. **Processing Time:** 2.7 hours for 192 samples
4.0 hours for 384 samples.
This includes a 20 minute DNA drying time
 - D. **Yield:** 5.0 µg of purified genomic DNA from 50mg (wet) of young rice [*Oryza sativa*] leaf.
Note: The actual yield may vary depending on a number of factors, including the type, part and maturity of plant material.
 - E. **Quality:** Typical OD_{260/280} values are 1.75 - 1.90
The DNA can be used directly in downstream applications such as fluorescent DNA sequencing, PCR and restriction endonuclease digestions.
- 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 DNA extracted on the AutoGenprep 965:

Gel electrophoresis of the extracted Plant DNA
(0.7% agarose gel in TAE, 50Vx1hr)

M: Hind III marker

Lanes 1-12: DNA extracted on the AutoGenprep 965/960



VI. Extraction and Purification Process

Process Site	Purpose	System Process
1. Manual	Break plant cell wall	Grind plant in liquid nitrogen.
2. Manual	Lyse plant material	Incubate ground plant in Reagent PL-M1.
3. Automated	Denature protein	Add Reagent PL-R1, mix, and Reagent PL-R2 and mix.
4. Automated	Remove debris	Add Reagent PL-R3, mix, centrifuge to pellet debris and transfer supernatant to a new plate.
5. Automated	Precipitate DNA	Add Reagent PL-R4, mix, centrifuge to pellet DNA and discard supernatant.
6. Automated	Wash DNA	Add Reagent PL-R6/7/8, mix, centrifuge to pellet DNA and discard supernatant. Repeat 2 times.
7. Automated	Dry DNA	Dry DNA on blower station.
8. Automated	Resuspend DNA	Add Reagent PL-R9 and mix.

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