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F0985K

MagExtractor -*Plant Genome*-

NPK-501 100 preparations
Store at 4 °C

Contents

CAUTION
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precautions, and follow safety guidelines while using this kit.

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[1] Introduction

Description

MagExtractor *-Plant Genome-* provides a simple and reliable method for the rapid purification of genomic DNA from various plant specimens, e.g. leaf and cultured cells, etc, using magnetic silica beads. This kit is based on binding properties of DNA to a silica surface in the presence of chaotropic agents[1)]. Purified genomic DNA can be used directly for PCR experiments.

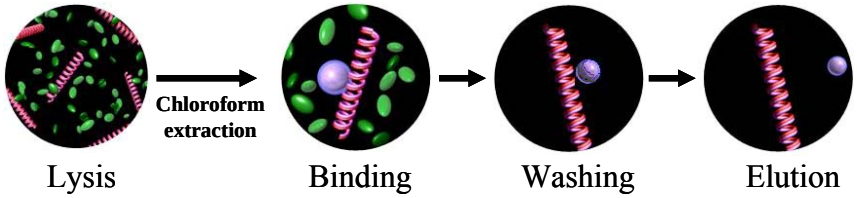

Fig. 1. Principles of the purification process.

Features

- Purified genomic DNA can be used directly for PCR.
- The pretreatment step of this kit is effective in removing polysaccharides.
- This kit is suitable for the high-throughput extraction of genomic DNA from various plant specimens. The following tables show typical sample amounts, yields, and applications for purified genomic DNA.

Table 1. Typical sample amounts and applications

Sample	Sample amount	Application
Plant specimens (leaf, cultured cells, etc)	0.01 - 0.1g	PCR

Table 2. Typical yields from various types of plant tissues (0.1 g).

Sample	Yield (µg)
Tabacco (leaf)	3 - 5
Tabacco (cultured cells)	2 - 3
Rice (leaf)	3 - 5
Arabidopsis (leaf)	1
Tomato (leaf)	3
Pumpkin (leaf)	3
Maize (leaf)	3 - 4

Notes

The yield depends on the types of species, tissues, and sample conditions that are used. Table 2 shows typical yields from various plant tissues.

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[2] Components

This kit includes the following components for 100 preparations. All reagents, except for the lysis solution should be stored at 4 °C. The lysis solution should be stored at room temperature (20 - 30 °C) once dissolved.

Lysis Solution	40 ml
Binding Solution	90 ml
Washing Solution	200 ml
Magnetic Beads	6 ml

Caution:

- The “Binding Solution” and “Washing Solution” contain chaotropic salts, which are irritant. Follow appropriate laboratory safety guidelines, and wear gloves when handling the reagents. If contact with skin occurs, wash thoroughly with water. If the eyes get affected, flush thoroughly for 15 min with cool water, and consult a physician.

- The “lysis solution” generates precipitates consisting of detergents at low temperature. If precipitates are formed, dissolve the precipitates according to the following method. Be cautious as this solution tends to generate transparent precipitates.

1. Incubate the bottle in a hot water bath at 55 °C for 10 min.
2. Mix the contents of the bottle gently by inverting.
3. Incubate the bottle in a hot water bath at 55 °C for 5 min.
4. Mix the contents of the bottle gently by inverting until the precipitates disappear.

Notes

- All reagents at room temperature should be used for extraction.
- For short-term storage (≤ 1 month), all components can be kept at room temperature.

[3] Materials required

The following materials are required for purification.

1. Reagents

- Chloroform / Isoamylalcohol (24:1)
- TE Buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)
- 70% ethanol
- Sterile water
- Liquid nitrogen

2. Instruments

- Tube mixer
- Magnetic stand
- Microcentrifuge
- Heating block (65 °C)

Fig.2
Magnetic stand
Magical Trapper (Code No.MGS-101)

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[4] Protocol

1. Pretreatment of plant specimens

The following pretreatment steps must be performed prior to purification.

(1) Disrupt plant specimens thoroughly using liquid nitrogen.

Notes
Plant specimens should be disrupted thoroughly until the specimens are powdery.

(2) Transfer the specimen (0.01 - 0.1 g) into a chilled 1.5-ml microtube using a chilled spatula.

(3) Add 300 µl **lysis solution**.

Notes
If precipitates have formed in the lysis solution, dissolve the precipitates according to the method described in [2].

(4) Mix thoroughly using a vortex mixer for 10 sec.

(5) Incubate at 65 °C for 10 min (vortex the tube every 3 - 4 min).

(6) Add 300 µl chloroform/isoamylalcohol (24:1) to the tube.

(7) Mix thoroughly by inverting the tube 3 - 5 times.

(8) Centrifuge at 12,000 rpm for 1 min.

Notes
Incomplete separation may be improved by an extra 2 - 3 min centrifugation.

(8) Carefully transfer the upper solution (250 µl) to a fresh 1.5-ml microtube.

(9) Add 600 µl **Binding Solution**.

Notes
If the volume of the upper solution is less than 100 µl, add 750 µl **Binding Solution**.

2. Purification

(1) **Binding.** Add 40µl **Magnetic Beads** to the pretreated solution (see above section) and mix thoroughly for 1 min using a vortex mixer.

Notes
Suspend the magnetic beads completely prior to use.

(2) Place each tube on a magnetic stand. The magnet will attract the magnetic beads, separating them from the specimen solution.

(3) After magnetic capture, carefully remove the supernatant.

Fig. 3.
Magnetic separation

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(4) **Washing.** Add 900 µl **Washing Solution** to the beads, and mix thoroughly for 5 sec using the vortex mixture.

(5) Place each tube on a magnetic stand, and collect the beads with the magnet.

(6) After magnetic capture, carefully remove the supernatant.

(7) **Washing.** Repeat (4) - (6)

(8) **Washing.** Add 900 µl **70% ethanol,** and mix thoroughly for 5 sec using the vortex mixture.

(9) Place each tube on a magnetic stand, and collect the beads with the magnet.

(10) After magnetic capture, carefully remove the supernatant.

(11) **Washing.** Repeat (8) - (10)

(12) **Elution.** Add 100 µl **TE Buffer** and mix thoroughly for 1 min.

(13) Place the tube on a magnetic stand.

(14) Collect the supernatant into a fresh tube.

Notes

Purified DNA samples contain small amounts of ethanol from the washing steps. Ethanol can be evaporated by heating at 75 °C for 5 min, and leaving the lid of the microtube opened.

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[5] Troubleshooting

Symptom	Cause	Solution
Low yield	Excess specimen	Excess samples decrease DNA yields. Use appropriate amounts of plant tissues.
	Insufficient disruption	Plant specimens should be disrupted thoroughly until the specimens are powdery. Be cautious that the specimens do not thaw.
	Incomplete separation at the centrifugation step	-Thoroughly reconstitute disrupted specimens and lysis solution. Prolong the vortexing time from 10 s to 1 min. - Increase the amount of lysis solution up to 350 - 450 µl.
Poor amplification at PCR	Inhibition by ethanol	Decrease the amount of DNA solution for PCR using a decrement up to 20% of the reaction volume. Purified DNA samples contain small amounts of ethanol from the washing steps. Ethanol can be evaporated by heating at 75 °C for 5 min, and leaving the lid of the microtube opened.

[6] Reference

1) B. Vogelstein and D. Gillespie, *Proc. Natl. Acad. Sci. USA*. 76: 615-619 (1979)

[7] Related products

Product name	Package	Code No.
Magnetic standa **Mag*ical Trapper***	1 piece	MGS-101

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