TIANGEN®

Magnetic Plant Genomic DNA Kit

Isolation and purification of high quality genomic DNA from plant tissue samples

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Cat.no. 4992406/4992407

Kit Contents

Contents	4992409 (50 preps)	4992407 (200 preps)
Buffer GPM	25 ml	100 ml
Buffer GPM plus	25 ml	100 ml
Buffer GHB	20 ml	80 ml
Buffer RD	12 ml	48 ml
Buffer PWB	15 ml	50 ml
RNase A (10 mg/ml)	300 µl	1.25 ml
MagAttract Suspension G	1 ml	3 × 1 ml
Buffer TB	15 ml	60 ml
Handbook	1	1

Storage

This kit can be stored at room temperature (15-25°C) for 12 months. For longer storage, please store at 2-8°C . If a precipitate has formed in Buffer under 2-8°C, please place the buffer at room temperature or warm at 37°C for 10 min to dissolve the precipitate.

Introduction

The kit adopts magnetic beads with unique separation function and a unique buffer system. It can separate and purify high-quality genomic DNA from various plant tissues. The unique embedded magnetic beads have strong affinity for nucleic acid under certain conditions, and when the conditions change, the magnetic beads will release adsorbed nucleic acid, thus achieving the purpose of fast separation and purification of nucleic acid. The whole process is safe and convenient. The extracted genomic DNA fragments are large, with high purity, stable and reliable quality. The method is especially suitable for automatic extraction of high-throughput workstations.

The DNA purified by the kit is suitable for various conventional operations, including enzyme digestion, PCR, library construction, Southern blot, chip detection, high-throughput sequencing and other experiments.

Features

- **Easy and fast:** Ultrapure genomic DNA can be obtained within 1 h.
- **High throughput:** It can integrate the automated instruments of pipetting-based method and magnetic rod method for high throughput extraction experiments.
- **Widely applicable:** Applicable to various plant tissues.
- **Safe and non-toxic:** No toxic organic reagents such as phenol/chloroform are needed.
- **High purity:** The obtained DNA has high purity and can be directly used in chip detection, high-throughput sequencing and other experiments.

Extraction yield

Plant material	Sample amount (mg)	Average DNA yield (μg)	OD_{260}/OD_{280}
Wheat	100	18-25	1.7-1.9
Pine tree	100	25-30	1.7-1.9
Potato	100	4-6	1.7-1.9
Tomato	100	10-15	1.7-1.9
Tobacco	100	10-15	1.7-1.9
Rice	100	10-25	1.7-1.9
Soybean	100	10-25	1.7-1.9
Corn	100	20-30	1.7-1.9
Cotton	100	10-25	1.7-1.9

Note: The genomes of plant materials from different sources will be different. All the above materials are tender leaves.

Important Notes Before Using

1. This product is suitable for manual extraction or automatic instrument integration.
2. Self-prepared reagents: Isopropanol, 96%-100% ethanol.
3. Avoid repeated freezing and thawing of the sample, otherwise the extracted DNA fragments will be smaller and the extraction yield will be decreased.
4. If there is precipitation in Buffer GHB, it can be redissolved in 37°C water bath and used after thoroughly mixing by shaking.

Protocol

Before using, please add 96%-100% ethanol into Buffer RD and Buffer PWB according to the label on the bottle.

I. Manual operation:

1. Use liquid nitrogen to fully grind about 100 mg of fresh plant tissue or about 30 mg of dry weight tissue.
2. Quickly transfer the ground powder to a centrifuge tube added with 400 µl Buffer GPM and 5 µl RNase A (10 mg/ml) in advance. After quickly inverting and mixing, place the centrifuge tube in a 70°C water bath for 10 min. Invert the centrifuge tube during the water bath to mix the samples several times.

   Note: When extracting plant tissue rich in polysaccharide polyphenols, use Buffer GPM plus.
3. Centrifugate at 12,000 rpm (~13,400 × g) for 4 min and transfer 300 µl to a new centrifuge tube.
4. Add 300 µl Buffer GHB, 300 µl isopropanol and 15 µl MagAttract Suspension G and vortex to mix.
5. Place at room temperature for 5 min.
6. Place the centrifuge tube on the magnetic stand and let it stand for 1 min. Carefully remove the liquid when the magnetic beads are completely attached.

7. Remove the centrifuge tube from the magnetic stand, add 500 µl Buffer RD **(ensure that 96%-100% ethanol has been added before use)**, and mix evenly for 30 sec.
8. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely attached, carefully remove the liquid.
9. Add 600 µl of Buffer PWB **(ensure that 96%-100% ethanol has been added before use)** and mix well for 30 sec.
10. Place the centrifuge tube on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely attached, carefully remove the liquid.
11. Repeat steps 9 and 10 once.
12. Place the centrifuge tube on a magnetic stand and air dry at room temperature for 10-15 min.

    Note: The ethanol residue will inhibit the subsequent enzyme reaction, so make sure the ethanol volatilizes completely when drying. However, do not dry for too long, for over-drying will lead to low DNA yield.
13. Remove the centrifuge tube from the magnetic stand, add 50-100 µl Buffer TB, shake and mix evenly, and incubate at 65°C for 3 min.
14. Place the centrifuge tube on a magnetic stand and let it stand for 1 min. After the magnetic beads are completely attached, carefully transfer the DNA solution to a new centrifuge tube and store it under appropriate conditions.

II. Automamted purification (Pipetting-based method)

Preperation and precausions

1. This product can be adapted with pipetting-based automatic instruments such as Hamilton Microlab STAR, Beckman Coulter Biomek® FX and Capitalbio LabKeeper for high throughput genome extraction.
2. Plant samples treatment: The same as steps 1-3 of Manual Operation. Transfer 300 µl of supernatant to a 96-well deep-well plate after completion of lysis.
3. Preparation of magnetic beads diluent: Mix according to the ratio of 15 µl MagAttract Suspension G to 85 µl isopropanol. The dosage of each sample after mixing is 100 µl.

4. For Hamilton Microlab STAR instruments, there is a plate position for placing a 2 ml centrifuge tube. The magnetic beads can be diluted without isopropanol. The volume of isopropanol added is also 300 µl. Add about 1 ml of magnetic beads to each centrifuge tube. Pipette up and down to mix the magnetic beads 5 times before aspirating the magnetic beads, and directly aliquot 15 µl magnetic beads to the plate. After the aliquot, tighten the magnetic bead tube and store it.
5. Considering that there is a certain deviation between the set temperature of the instrument and the actual temperature in the 96-well plate, it is recommended to set the temperature of the instrument 10°C higher than the actual temperature during lysis and elution.

Extraction steps:

1. Add 300 µl of the treated plant sample supernatant into a 96-well plate (self-provided).
2. Add 300 µl of Buffer GHB to each well, pipette up and down to mix for 6 times.
3. Add 215 µl isopropanol to each well, pipette up and down to mix for 6 times.
4. Add 100 µl diluted magnetic bead buffer to each well, pipette up and down to mix for 6 times, and then shake to mix evenly for 10 min.
5. Place the 96-deep-well plate on a magnetic stand and let it stand for 2 min. After the magnetic beads are completely attached, remove the supernatant.
6. Remove the 96-deep-well plate from the magnetic stand, add 500 µl of Buffer RD, pipette up and down to mix for 6 times, and shake to mix evenly for 2 min.
7. Place the 96-deep-well plate on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely attached, remove the supernatant.
8. Remove the 96-deep-well plate from the magnetic stand, add 600 µl of Buffer PWB, pipette up and down to mix for 6 times, and then shake to mix evenly for 2 min.
9. Place the 96-deep-well plate on a magnetic stand and let it stand for 30 sec. After the magnetic beads are completely attached, remove the supernatant.
10. Repeat steps 8 and 9 once.

11. Place the 96-deep-well plate on a magnetic stand and dry it at 37°C for 5 min.
12. Remove the 96-deep-well plate from the magnetic stand, add 50-100 µl Buffer TB, place it at 65°C and mix it evenly for 10 min.
13. Place the 96-deep-well plate on a magnetic stand and stand for 2 min. After the magnetic beads are completely attached, carefully transfer the DNA solution to the collection plate and store it under appropriate conditions.

III. Automamted purification (Magnetic rod method)

Preperation and precausions

1. This product has been successfully adapted on automatic instruments such as Thermo KingFisher Flex.
2. Plant samples treatment: The same as steps 1-3 of Manual Operation. Transfer 290 µl of supernatant to a 96-deep-well plate containing 290 µl of Buffer GHB and 290 µl of isopropanol after lysis is completed.
3. Add the supernatant containing plant sample, Buffer GHB and isopropanol, 500 µl Buffer RD, 600 µl Buffer PWB and 50-100 µl Buffer TB to the corresponding positions of a 96-well plate respectively, and add 15 µl MagAttract Suspension G to 500 µl Buffer RD.

Extraction steps:

1. Add the treated plant sample supernatant to the 96-well plate containing Buffer GHB and isopropanol.
2. Place the 96-well plate in an automatic extractor and incubate at room temperature for 5 min. During this period, slap and mix it evenly at medium and fast speed.
3. Use the magnetic rod comb to penetrate into the wells of Buffer MRD containing magnetic beads, slap and mix for 1 min to resuspend the magnetic beads.
4. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 20 sec.
5. Transfer the magnetic beads to the wells containing plant sample supernatant, Buffer GHB and isopropanol, release the magnetic beads, and slap and mix at medium and fast intervals for 10 min.
6. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 20 sec.

7. Transfer the magnetic beads to the well containing the Buffer RD from the first step, release the magnetic beads, and slap and mix them for 3 min.
8. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 20 sec.
9. Transfer the magnetic beads to the wells containing the 1st time washing Buffer PWB, release the magnetic beads, and slap and mix them for 3 min.
10. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 20 sec.
11. Transfer the magnetic beads to the wells containing the 2nd time washing Buffer PWB, release the magnetic beads, and slap and mix them for 3 min.
12. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 20 sec.
13. Hang the magnetic rod adsorbed with magnetic beads in the air to dry for 5 min.
14. Transfer the magnetic beads to the wells containing Buffer TB, incubate at 75°C and rapidly slap and mix for 10 min.
15. Penetrate the magnetic rod into the magnetic comb and adsorb magnetic beads for 3 times, each time for 30 sec.
16. Transfer the adsorbed waste magnetic beads to wells containing Buffer RD, and slap and mix well for 1 min.
17. After the procedure is completed, carefully transfer the DNA solution to the collection plate and store it under appropriate conditions.

Determination of DNA Concentration and Purity

The size of the obtained genomic DNA fragment is related to factors such as sample storage time and shearing force during operation. The obtained DNA fragments can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer for concentration and purity.

DNA should have a significant absorption peak at OD_{260}, with OD_{260} value of 1 equivalent to about 50 μg/ml double stranded DNA and 40 μg/ml single stranded DNA.

The ratio of OD_{260}/OD_{280} should be 1.7-1.9. If using deionized water instead of elution buffer, the ratio will be low, because the pH value and the presence of ions will affect the light absorption value, but it does not mean the purity is low.