Novel Plant Genomic DNA Extraction Kit

Project number: N667427

Storage conditions: room temperature.

Products

individual parts making up a compound	50T	200T
Buffer LP1	25mL	100mL
Buffer LP2	10mL	40mL
Buffer LP3 (concentrate)	21ml	84ml
Buffer GW2 (concentrate)	15mL	75ml
Buffer GE	15mL	60mL
RNase A (10 mg/ml)	300μl	1.25mL
Spin Columns DM with Collection Tubes	50	200

Products

This kit uses centrifugal adsorption columns with high efficiency and specific binding of nucleic acids and a unique buffer system, which is suitable for extracting genomic DNA from a wide variety of different fresh or frozen plant tissues with maximum removal of impurities from the plant tissues. The kit eliminates the need for phenol/chloroform extraction and is safe to handle. The extracted genomic DNA fragments are large, high purity, stable and reliable quality, suitable for PCR, fluorescence quantitative PCR, molecular labeling, library construction and other experiments.

Self-contained reagent: anhydrous ethanol

Pre-experiment Preparation and Important Notes

1. Repeated freezing and thawing of the sample should be avoided, as this may result in smaller fragments of extracted DNA and a decrease in the amount extracted.
2. Anhydrous ethanol should be added to Buffer LP3 and Buffer GW2 according to the instructions on the label of the reagent bottle before first use. Check Buffer LP1 and Buffer LP2 for crystallization or precipitation before use. If crystallization or precipitation occurs, re-dissolve Buffer LP1 and Buffer LP2 in a 56° C water bath.

procedure

1. Take about 100mg of fresh plant tissue or about 20mg of dry weight tissue and add liquid nitrogen to grind it fully.
2. Collect the ground powder into a centrifuge tube (self-provided), add 400 μl Buffer LP1 and 6 μl RNase A (10 mg/ml), vortex and oscillate for 1 minute, and leave it at room temperature for 10 minutes to allow for full cleavage.
Note: 1) Use vortex shaking or pipette blowing to fully lyses the tissue, incomplete tissue lysis will affect the final DNA yield. 2) Do not mix Buffer LP1 with RNase A prior to use.
3. Add 130 μl Buffer LP2, mix well and vortex for 1 minute.
4. Centrifuge at 12,000 rpm (~13,400 x g) for 5 minutes and transfer the supernatant to a new centrifuge tube (supplied).
5. Add 1.5 times the volume of Buffer LP3 (check that anhydrous ethanol has been added before use) and mix thoroughly (e.g., 500 μl filtrate to 750 μl Buffer LP3).
Note: Buffer LP3 should be mixed immediately after addition; precipitation may occur but will not affect subsequent experiments.
6. Add all of the solution and precipitate obtained in the previous step to the adsorption columns (Spin Columns DM) that have been loaded into the collection tubes, if the solution cannot be added all at once, it can be transferred in several times. centrifuge the columns at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tubes, and put the columns back into the collection tubes.
7. Add 500 μl of Buffer GW2 to the adsorption column (check that anhydrous ethanol has been added before use), centrifuge at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorption column back into the collection tube.
Note: If the adsorbent membrane appears green, add 500 μl of anhydrous ethanol to the adsorbent column, centrifuge the column at 12,000 rpm for 1 minute, pour off the waste liquid in the collection tube, and put the adsorbent column back into the collection tube.
8. Repeat step 7.
9. Centrifuge at 12,000 rpm for 2 minutes and pour off the waste liquid in the collection tube. Leave the adsorption column at room temperature for several minutes to dry thoroughly.
Note: The purpose of this step is to remove residual ethanol from the adsorption column, which can interfere with subsequent enzymatic reactions (digestion, PCR, etc.).
10. Place the adsorption column in a new centrifuge tube (supplied), add 50-100 μl of Buffer GE or sterilized water dropwise to the middle of the adsorbent membrane, leave it at room temperature for 2-5 minutes, and centrifuge it at 12,000 rpm for 1 minute to collect the DNA solution. -The DNA solution was collected by centrifugation at 12,000 rpm for 1 min.
Note: 1) If the downstream experiment is sensitive to pH or EDTA, you can use sterilized water for elution. The pH value of the eluent has a great influence on the elution efficiency, if you use water as the eluent, you should ensure that the

pH value is 7.0-8.5 (you can use NaOH to adjust the pH value of the water to this range), and when the pH value is lower than 7.0, the elution efficiency is not high.

2) Incubation at room temperature for 5 minutes prior to centrifugation increases yield.

(3) If the final concentration of DNA is to be increased, the DNA eluate obtained in step 10 can be re-added to the adsorbent membrane and repeat step 10; if the elution volume is less than 100μl, the final concentration of DNA can be increased, but it may reduce the total DNA yield. If the amount of DNA obtained is less than 1μg, 50μl Buffer GE is recommended for elution.

4) Because DNA stored in water is subject to acidic hydrolysis, for long-term storage, elution with Buffer GE and storage at -20° C are recommended.