EasyPrep Plant and Fungi DNA kit

For isolation of genomic DNA from plants and fungi

Contents

Introduction

EasyPrep Plant and Fungi DNA kit adopts a unique buffer system and is especially suitable for the purification of DNA from lyophilized or fresh plant tissues. The kit avoids the use of hazardous organic solvents such as phenol and removes the vast majority of proteins and other organic purities. There is no limit to the starting amount of samples; the experimenter can adjust that according to the actual demand. DNA purified with this kit is ready to use in downstream applications, including enzyme digestion reaction, PCR, library construction, and Southern blotting.

Important Notes

1. Avoid repeated thawing as this causes smaller fragments of DNA and lower yield.
2. Buffer FBP1 may turn yellow upon storage. This does not affect performance.
3. If a precipitate is formed in Buffer FBP1 or Buffer FBP2, please heat to 37 °C to redissolve it, and mix well before use.
4. All centrifugation steps should be carried out at room temperature (15−25 °C) in a microcentrifuge.

Kit Contents

Contents	DPT-BC21 50 preps	DPT-BC21-2 200 preps
FBP1 Buffer	25 mL	100 mL
FBP2 Buffer	10 mL	40 mL
TE Buffer	15 mL	60 mL
RNase A (10 mg/ml)	300 µL	1.25 mL

Storage

EasyPrep Plant and Fungi DNA kit should be stored in a dry place at room temperature (15–25 °C) and is stable for 12 months.

Protocol

All the procedures as below are used for DNA extraction from 100 mg of fresh plant tissue or 20 mg of lyophilized tissue or fungi. For a higher quantity of tissue, the usage amount of buffer should be increased proportionally.

1. Disruption

Place the sample material (100 mg of wet weight or 20 mg of lyophilized tissue or fungi) into a mortar, add liquid nitrogen to the mortar, and grind the sample thoroughly. Add 400 µL of Buffer FBP1 and 6 µL of RNase A (10 mg/mL), vortex vigorously for 1 min, and let the sample stand at room temperature for 10 min.

Note: Because of plant diversity, the starting amount of plant tissue should be adjusted according to the species and part of the plant.

2. Add 130 µL of Buffer FBP2 to the lysate, and mix on a vortex mixer for 1 min.
3. Centrifuge at 12,000 rpm (~13,400 ×g) for 5 min, and transfer the supernatant into a new centrifuge tube.
4. Optional: Centrifuge the lysate for 5 min at 12,000 rpm (~13,400 ×g) again and transfer the supernatant into a new centrifuge tube.

Note: Some plant materials can generate very viscous lysates and numerous precipitates during this step. This can result in the shearing of the DNA in the next step. In this case, optimal results are obtained if most of these precipitates are removed by centrifugation.

5. Add 0.7 volume of isopropanol, and mix on a vortex mixer. The supernatant will form a precipitate (for example, add 350 µL of isopropanol in 500 µL of supernatant). Centrifuge at 12,000 rpm (~13,400 ×g) for 2 min, discard the supernatant, and keep the precipitate.
6. Add 600 µL 70% alcohol and vortex for 5s. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 min and discard the supernatant.
7. Repeat step 6.
8. Open the lid, and incubate at room temperature (15–25 °C) for 5–10 min to dry the precipitate and remove the residual alcohol.

Note: Ensure that no ethanol is carried over. Residual ethanol may interfere with downstream reactions (enzyme digestion reaction, PCR, etc.).

9. Pipette an appropriate volume of Buffer TE, and incubate at 65 °C for 10–60 min in a water bath. Turn upside down during the incubation. The purified DNA will dissolve in Buffer TE.