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Plant Biotechnology, 15(1), 45-48(1998)

[*Plant BioTechniques Series* (7)]

A Rapid and Easy-handling Procedure for Isolation of DNA from Rice, *Arabidopsis* and Tobacco

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Received 4 December 1997; accepted 8 December 1997

Introduction

Genetic studies require the isolation of DNA from a large number of plants. In the process of positional cloning, one needs to analyze DNAs from hundreds of F_2 or F_3 plants. To map a gene or DNA fragment on the chromosome, it is usually required to analyze DNAs from several dozen plants of the recombinant inbred lines. Not only in genetics but also in molecular biology, a rapid and easy method to isolate DNA is a powerful tool to reveal the genomic organization of a gene of interest.

The method using cetyltrimethylammonium bromide (CTAB) [1] has been developed to isolate partially purified plant DNA that contains fewer polysaccharides, which are rich in plant cells and inhibit enzyme reaction such as digestion or ligation of DNA. Although the CTAB method has been applied to various plant species, it is not practical to isolate DNA from a large number of plant materials because it requires many steps and is time-consuming. Moreover, the yield with the CTAB method is sometimes very low when DNAs are extracted from starch storage tissues.

We describe here rapid and easily-handled procedures for the isolation of DNA from rice, Arabidopsis and tobacco. These methods do not require careful cautions, which are usually needed in the experiments with CTAB. Particular equipment is not needed. The DNA obtained by these methods can be subjected to enzymatic reactions, such as digestion with restriction enzymes, ligation, and PCR amplification.

1. *Isolation of DNA from rice plants*

Reagents

Lysis buffer

400 mM Tris-HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl, 10 mM β-mercaptoethanol (add 14.4 M soln. just before use), 1% polyvinylpyrrolidone

5 M potassium acetate

20% sodium dodecyl sulfate (SDS)

Chloroform

iso-propanol

70% ethanol

TE (10 mM Tris-HCl (pH 8.0), 1 mM EDTA)

Procedure

Here we describe the standard procedure to isolate DNA from 1 g (wet weight) of plant material, expanded mature leaves of rice. Depending on the volume of the starting material, you can change handling scales.

1. Prepare the disposable centrifuge tubes (15 m*l*) containing a mixture of 3 m*l* of lysis buffer and 200 μl of 20% SDS.
2. Pulverize plant tissues in liquid nitrogen with a motor and pestle.
3. Transfer the powder of plant tissues into the prepared tube and mix well.
4. Add 1 m*l* of 5 M potassium acetate to each tube and gently invert several times.
5. Incubate the mixture at 60°C for 20 min gently inverting occasionally. Place the mixture on ice for 20 min.
6. Add 4.2 m*l* of chloroform (−20°C) to the mixture and shake the tubes at room temperature for 10 min.
7. Centrifuge the tube at 4,000 rpm for 10 min (Swing-type rotor is preferable).
8. Transfer the aqueous phase (DNA extract) into a new tube.
9. Add 2.5 m*l* of iso-propanol to the DNA extract and place the tube at room temperature for 10 min.
10. Centrifuge the tube at 4,000 rpm for 5 min.
11. Discard the supernatant and rinse the resulting DNA pellet with 70% ethanol.
12. Dry the pellet briefly under vacuum.
13. Dissolve the pellet in TE (desired volume). The DNA solutions obtained can be subjected to the RNase treatment of standard procedure.

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Results and Comments

We usually obtained about 40-60 μg of DNA from 1g of expanded mature leaves of rice by the method described here. This yield is as high as that obtained by the CTAB method. The electrophoretic pattern of the isolated DNA showed a clear single band, suggesting that fragmentation of DNA into small sizes rarely occurred during isolation (**Fig. 1**). The sizes of the DNA bands were about 20 kb, the length of which is sufficient for Southern blot analysis or PCR amplification. **Figure 2** shows the digested pattern of rice DNA with *Bam*H I. Several bands were detected on the smear background. The bands may be derived from repetitive sequence of rice genome and the presence of these bands indicates that the DNA was completely digested (**Fig. 2**, lanes 2 and 3).

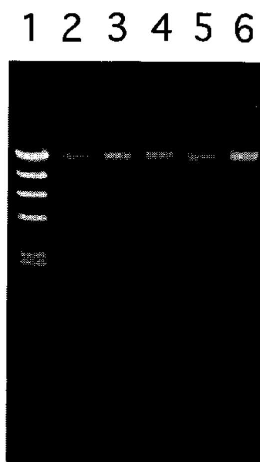

Fig. 1 Isolated DNA from rice leaves.
DNAs were fractionated on 0.8% agarose and stained with ethidium bromide (0.5 μg/m*l*). Lane 1, *Hind* III digests of λDNA as a molecular weight maker. The largest band is 23 kb in length. Lane 2-6, DNAs isolated independently from rice leaves.

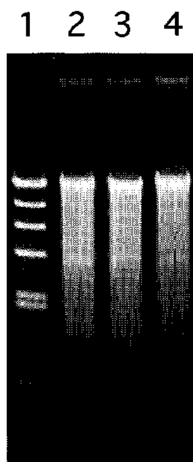

Fig. 2 Digestibility of isolated DNA with restriction enzyme.
Lane 1, *Hind* III digests of λ DNA. Lane. 2-4, Rice DNAs (0.4 μg) were digested with *Bam*HI (8, 4, 2 units, respectively) at 30°C for 1 hour.

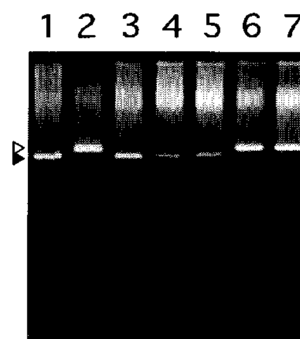

Fig. 3 An example of PCR amplification of a part of *OsNAC6* gene to locate it on rice chromosomes.
DNAs from recombinant inbred lines were used for templates and subjected to PCR amplification. Open and closed triangles indicate the bands for Japonica and Indica type, respectively.

We isolated DNA from seventy strains of the recombinant inbred lines with Japonica and Indica type of rice by the procedure described here. The DNAs obtained have been used for the templates of PCR amplification to map the genes that we focus on by the polymorphism of length or cleavage pattern of amplified fragments. **Figure 3** shows an example of amplified DNA fragments to map the *OsNAC6* gene by the polymorphism of the intron length between Japonica and Indica type ([2]; Kikuchi *et al.*, manuscript in preparation). The clear bands indicate that the DNAs isolated by this procedure were efficiently served as templates for amplification. Thus, the DNAs isolated by our method is sufficient for yields and DNA sizes. Quality of DNA also satisfies us because of the high digestibility with restriction enzyme and template activity for PCR.

The handling in this procedure is very simple and does not require particular cautions. In the CTAB method [1], the removal of CTAB from DNA solution is essential. If CTAB remains in DNA samples, less DNA is recovered by the subsequent treatment of phenol extraction after RNase digestion and the ethanol precipitation. In addition, since CTAB is precipitated at low temperatures, we must pay attention to temperature during experiments, especially in cold seasons. Thus, careful caution is required for isolating DNA by the CTAB method. On the contrary, in the procedure described here, such cautions are not required. Furthermore, the procedure *per se* is simple and includes a small number of steps. Thus, these advantages enable us to isolate plant DNA from a large number of materials at the same time. We are expecting that this procedure will be applied for

isolating DNA from many other plant species.

2. *Isolation of DNA from* Arabidopsis

DNA is usually isolated from 1 to 3-week-old *Arabidopsis* plants. Two methods, Benzyl Chloride (ISOPLANT) and Urea-Phenol method, are presented for the rapid isolation of high molecular weight DNA, which can be used for PCR analysis and complete digestion with restriction endonucleases. These techniques are ideal for isolation of small amounts of DNA from many samples. Urea-Phenol Method is also useful for large-scale isolations, and can be applied to various plant species including rice and tobacco [3].

1) Benzyl Chloride Method (ISOPLANT)

Reagents

All solutions except 70% and 100% ethanol are purchased from Wako Co. as a ISOPLANT kit.

Procedure

1. Transfer 10 to 100 mg plant material into a 1.5 m*l* tube, and add 300 μl of a Extraction Buffer (Solution I) and squash well with a blue tip or a pestle for a 1.5 m*l* tube.
2. Add 150 μl of Lysis Buffer (Solution II) and vortex well.
3. Incubate the mixture for 15 min at 50°C.
4. Add 150 μl of Sodium Acetate (Solution III) and mix.
5. Incubate the mixture for 15 min on ice.
6. Separate the phases by centrifugation (15,000 rpm for 15 min at 4°C), and gently remove the aqueous phase with a blue tip.
7. Recover the DNA by centrifugation (15,000 rpm for 10 min at 4°C) after addition of 2 volumes of ethanol.
8. Rinse the precipitate with 70% ethanol and dry briefly.
9. Immediately and gently redissolve the DNA in approximately 20 μl of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (and 20 μg/m*l* RNase, if necessary).

2) Urea-Phenol Method

Reagents

Stock lysis buffer

8 M urea, 0.35 M NaCl, 50 mM Tris-HCl (pH 7.5), 20 mM EDTA, 2% sarcosyl (prior to use, phenol and sodium dodecyl sulfate are added to 5 and 0.5%, respectively)

phenol/chloroform/isoamyl alcohol (25: 24: 1)

70% and 100% ethanol

10 mM Tris-HCl (pH 8.0), 1 mM EDTA (20 μg/m*l* RNase, if necessary)

Procedure

1. (Frozen, if necessary) Plant material is transferred into a 1.5 m*l* tube, and 600 μl/0.1 mg of a lysis buffer is added and squashed well with a blue tip or a pestle for a 1.5 m*l* tube. (For higher yield, frozen plant tissue is pulverized using a mortar and pestle in the presence of liquid nitrogen.)
2. The mixture is incubated for 30 min at 65°C with occasional gentle mixing.
3. The extract is emulsified by gentle inversion with an equal volume of phenol/chloroform/isoamyl alcohol (25: 24: 1).
4. After centrifugation (*e.g.* 13,000×g for 5 min at room temperature), the aqueous phase is gently removed with a blue tip.
5. If possible, the DNA is spooled out with a yellow tip after addition of 2 volume of ethanol, or is recovered by low speed centrifugation (*e.g.* 2,000×g for 3 min at room temperature).
6. The DNA is rinsed with 70% ethanol and recovered by low speed centrifugation (*e.g.* 2,000×g for 3 min at room temperature).
7. The precipitate is dried briefly. (Excessive centrifugation at forces higher than necessary or complete drying should be avoided because tight or dried pellets may be difficult to redissolve.)
8. The DNA is immediately and gently redissolved in approximately 1/10 of the original lysis volume of 10 mM Tris-HCl (pH 8.0), 1 mM EDTA (and 20 μg/m*l* RNase, if necessary).

3. *Isolation of DNA from tobacco*

A new DNA isolation method using a boric-polymerized silica gel made it possible to eliminate polysaccharides from the DNA sample. The method is the same as that for the isolation of rice DNA with the modification described below. One can isolate 100 μg of DNA from 1 g of tobacco leaves within one hour. A plant DNA isolation kit "PhytoPure" using this method is available from Scotlab (UK), and can be applied to various plants.

Reagents

Reagents used for the isolation of rice DNA (see Section 1)

Boric-polymerized silica suspension

Procedure

1. Follow the protocol for the isolation of rice DNA from step 1 to 5.
2. Add 400 μl of boric-polymerized silica suspension (shaking vigorously before adding), and shake at room temperature for 10 min.
3. Centrifuge at 1,300×g at room temperature for 10 min.

4. Transfer upper phase (containing DNA) into a fresh tube.
5. Add one volume of isopropanol, and invert the tube gently several times.
6. Centrifuge at 4,000×g for 5 min.
7. Wash the DNA pellet with 70% ethanol.
8. Discard the supernatant and dry.
9. Suspend the DNA in a suitable volume of TE. (If the DNA is difficult to dissolve, use slightly alkaline buffer.)

Acknowledgments

We thank Dr. Yohsuke Takahashi for his helpful suggestions on the section of "Isolation of DNA from tobacco".

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