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**Rapid isolation of high molecular weight plant DNA**

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ABSTRACT

A method is presented for the rapid isolation of high molecular weight plant DNA (50,000 base pairs or more in length) which is free of contaminants which interfere with complete digestion by restriction endonucleases. The procedure yields total cellular DNA (i.e. nuclear, chloroplast, and mitochondrial DNA). The technique is ideal for the rapid isolation of small amounts of DNA from many different species and is also useful for large scale isolations.

INTRODUCTION

With the increasing use of recombinant DNA techniques in plant research the preparation of long, pure DNA has become a major concern. Since the same forces which are required to break cell walls can also shear DNA, considerable care must be taken and one must often reach a compromise between DNA yields and length. While intact nuclei are a good starting source for long plant DNA, the nuclei may be difficult to isolate in quantity and the techniques required are often quite involved. Polysaccharides and tannins pose a major problem in plants because they are difficult to separate from DNA. Polysaccharide-like contaminants which are undetectable by most criteria can cause anomalous reassociation kinetics (1,2). Many of the initial problems encountered in the cloning of plant DNA have been attributed to contaminants which interfere with restriction nucleases as well as to DNA of insufficient length (numerous personal communications). With the following procedure restriction analysis of the DNA can proceed on the day following the start of DNA isolation. Electrophoretic analysis after restriction shows very sharp bands even for high molecular weight fragments.

MATERIALS AND METHODS

Cetyltrimethylammonium bromide (CTAB)<sup>(1)</sup> was purchased from Sigma (cat. # H-5882), CsCl from Kawecki Berylco Industries, and restriction

endonuclease Eco RI from Miles.

The following procedure was designed for use with a small capacity ultracentrifuge rotor such as a Dupont-Sorval TV-865. Other equipment may require some increases in centrifugation times.

Lyophilized tissue is ground to a fine powder prior to extraction. The simplest approach for small samples is to use a mortar and pestle and small (<1mm) glass beads. For fibrous tissues grinding in the presence of liquid N<sub>2</sub> is often helpful. Hard tissues such as leguminous embryos can be reduced by mechanical milling.

Dry powder is gently dispersed in extraction buffer [0.7 M NaCl, 1% CTAB, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 1% 2-mercaptoethanol]. The tissue to buffer ratio varies with the species and tissue. Enough extraction buffer should be added that clumps can easily be dispersed but the solution remains somewhat viscous. Typically this requires 1.0 ml per 30-100 mg d.w. of powder. The mixture is incubated for 20-30 min at 50-60°C with occasional gentle mixing. Temperatures throughout should be kept above 15°C to prevent CTAB precipitation. Glass beads, cell wall debris, denatured protein, and most polysaccharides are simultaneously removed during extraction with chloroform/octanol. The extract is emulsified by gentle inversion with an equal volume of chloroform/octanol (24:1) and, after centrifugation (13,000 x g, 10 min), the aqueous phase is removed with a large bore pipette. One tenth volume of 10% CTAB, 0.7 M NaCl is added and the chloroform/octanol treatment is repeated. The final aqueous phase may be slightly colored but should be clear.

A CTAB-nucleic acid precipitate is formed when the NaCl concentration is reduced from 0.7 to 0.35 M by the addition of one volume of 1% CTAB, 50 mM Tris-HCl (pH 8.0), and 10 mM EDTA. After 30 min at room temperature the precipitate is recovered by low speed centrifugation (e.g. 2000 x g, 5 min). Excessive centrifugation at forces higher than necessary are to be avoided because tight pellets may be difficult to redissolve. We have also noted considerable variation in the ease with which CTAB-nucleic acid precipitates redissolve. Some older CTAB preparations may be mixtures of other alkylammonium bromides and form complexes that require homogenization and/or heating to dissolve.

The CTAB-nucleic acid precipitate is dissolved in 2 ml of 1.0 M CsCl (density= 1.12 gm/ml), 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 200 ug/ml ethidium bromide. The solution may be briefly heated to 60°C to speed dissolution. The solution is placed in the ultracentrifuge tube and 2.4 ml

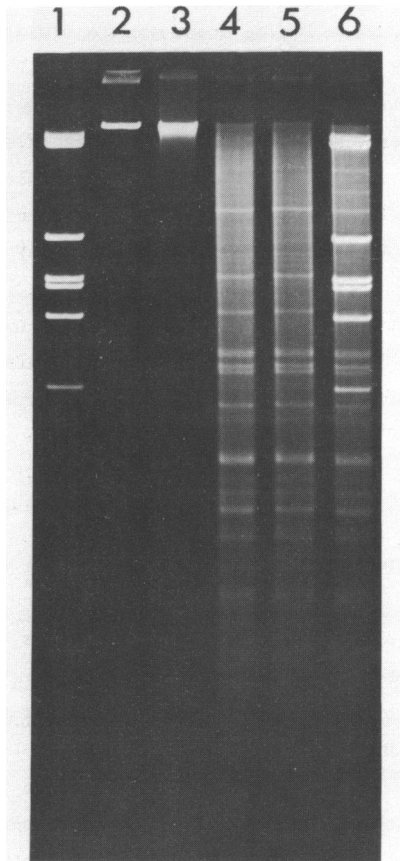
of 6.6 M CsCl (density= 1.82 gm/ml), 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 0.1% sarkosyl are added allowing considerable mixing to preform a crude density gradient. After centrifugation (2-3 hr. 58,000 rpm in a TV-865 rotor) the DNA is visualized under long wave UV illumination, removed with a large bore syringe needle, and transferred to a fresh centrifuge tube. The tube is filled with 4.5 M CsCl (density= 1.55 gm/ml), 50 mM Tris-HCl (pH 8.0), 10 mM EDTA, 0.1% sarkosyl and 100 ug/ml ethidium bromide. After a second centrifugation (6 hr to overnight, 58,000 rpm) the DNA is removed as above.

Ethidium bromide is removed by repeated partitioning against NaCl-saturated isopropanol. CsCl is removed by extensive dialysis against 10 mM Na acetate. Alternatively the DNA can be precipitated by the addition of 2 volumes of H<sub>2</sub>O and 7.5 volumes of ethanol.

#### COMMENTS

Typically from 20-70 ug of DNA can be expected per 100 mg d.w. of powder depending on the species and tissue. We have used this approach to isolate DNA from wheat, peas, oats, carrots, mung beans, tobacco, Mimulus, and various species of Atriplex. We have found it very difficult to isolate long, restrictable mung bean and Atriplex DNAs by other techniques. After CsCl centrifugation the DNA typically shows A<sub>260/230</sub> ratios of 2.2-2.5 and A<sub>260/280</sub> ratios of 1.8-1.9. Some residual RNA is often present which can be removed by RNase treatment. However we have not found the residual RNA to present any problems in restriction analysis or cloning and thus in many cases RNase treatment is unnecessary. With suitable care in handling, DNA preparations generally comigrate with intact lambda DNA indicating a length on the order of 50 kbp or better. An example of mung bean DNA before and after digestion with either 1 or 5 units of Eco RI per ug of DNA is shown in Figure 1. Since the patterns are identical in both cases and since lambda DNA included during digestion of mung bean DNA with 1 unit per ug of DNA shows complete digestion, the DNA appears to be free of contaminants which interfere with this restriction enzyme.

The use of lyophilized tissue offers several advantages. Dry tissue can be efficiently disrupted while the DNA is unhydrated and thus less susceptible to shear. Since the DNA is hydrated immediately in the presence of detergent and EDTA, nucleolytic degradation is minimized. Finally dry tissue can be stored for several years with little loss in DNA quality.



**Figure 1. Electrophoretic analysis of mung bean Eco RI restriction nuclease fragments.** Where indicated, DNA was digested with Eco RI for 1 hr at 37°C using the conditions supplied by the manufacturer (Miles). Samples were analyzed on a 1% agarose gel containing 0.1 M Tris-acetate (pH 8.1) and 1 mM EDTA. Electrophoresis was for 14 hr at 2 volts/cm. 1)  $\lambda$  DNA digested with 1 unit/ $\mu$ g of DNA. 2) undigested  $\lambda$  DNA. 3) undigested mung bean DNA. 4) mung bean DNA digested with 1 unit/ $\mu$ g DNA. 5) mung bean DNA digested with 5 units/ $\mu$ g DNA. 6) mung bean and  $\lambda$  DNA mixture (5:1) digested with 1 unit/ $\mu$ g DNA.

The technique capitalizes on the previous observations that nucleic acids can be selectively precipitated with CTAB (e.g. 3,4). RNA and DNA are soluble in CTAB and 0.7 M NaCl but precipitate when the salt is reduced below 0.4 M. However, many polysaccharides are insoluble over this salt range and are thus not solubilized. Because we see no evidence of poly-

phenol formation we infer that polyphenoloxidase activity is inhibited. Since the RNA recovered after CsCl-ethidium bromide centrifugation appears to be undegraded when analyzed on formaldehyde-agarose gels RNAse activity also appears to be minimal (data not shown). Thus the initial parts of this procedure (i.e. through CTAB precipitation) may be useful for the isolation of total RNA.

Because this procedure yields total DNA some caution is required when interpreting restriction profiles. For example, all of the bright bands in the mung bean restriction profile correspond to the Eco RI fragments of purified mung bean chloroplast DNA (J. D. Palmer, unpublished). We have shown previously that chloroplast DNA is a significant fraction of the total leaf DNA in mung beans (5). While the problem of chloroplast DNA is perhaps more acute because of the small size of the mung bean genome prior knowledge of the chloroplast DNA restriction profile is desirable when comparing restriction profiles for closely related plants with small nuclear genomes.

#### FOOTNOTES

(1) Abbreviations used are; CTAB, cetyltrimethylammonium bromide; kbp, thousand base pairs; EDTA, ethylenediaminetetracetic acid.

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