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HYDROXYLAPATITE-POLYETHYLENE GLYCOL (HA-PEG) PROCEDURE: AN EFFECTIVE METHOD FOR RECOVERING DNA FROM AGAROSE GEL

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Abstract

A convenient and quantitative procedure for recovering DNA from agarose gel is described. The procedure includes hydroxylapatite absorption, elution and PEG precipitation of DNA. The recovery of a band containing 0.25 μg DNA can be 75%. 90% recovery can be achieved if the amount of DNA is over 0.5 μg. The recovered DNA is suitable for molecular cloning and nick-translation. It can also be used for physical mapping.

Key words: Recombinant DNA techniques; DNA recovery; DNA fragments isolation.

Introduction

Agarose gel electrophoresis is widely used as a high resolution technique for fractionation of DNA fragments by size. It is valuable both on analytical and preparative procedures for isolation individual species of DNA molecules from a mixture. Techniques for the recovery of DNA fragments including gel compression, gel dissolution and electroelution were described (Strongin *et al.*, 1977; Wieslander, 1979; Smith, 1980), but none of them are satisfactory. The major problems for that are the low yield and the contamination of agarose in the preparation (Strongin *et al.*, 1977). To improve the described disadvantages we have developed a simple, convenient and quantitative procedure by absorbing DNA fragments onto hydroxylapatite during electrophoresis. and followed by PEG precipitation of the DNA eluted out from the hydroxylapatite (HA-PEG procedure). This method requires no sophisticated equipment and expensive chemicals. It can be used to isolate DNA fragments in analytical amounts. The recovered DNA is suitable for most of the enzymatic treatments.

Materials and Methods

Preparation of Plasmid DNA

The Triton X-100 lysis method described by Haseltine *et al.* (1980) was routinely used to prepare plasmid DNA. After CsCl density gradient centrifugation, plasmid DNA was extracted with isopropanol to remove ethidium bromide and dialyzed against TEN buffer (10 mM Tris-1 mM EDTA-20 mM NaCl, pH 7.5).

Analytical Agarose Gel Electrophoresis

DNA fragments were fractionated in horizontal agarose gel slabs. All electrophoresis was carried out in Tris-phosphate buffer system (McDonell *et al.*, 1977). DNA fragments larger than 500 bp were fractionated in 1% agarose gel (Sigma Chemical Co.), Small DNA fragments (40-1,000 bp) were fractionated in 4% NuSieve agarose gel (FMC BioProducts).

Recovery of DNA Fragments from Agarose

Hydroxylapatite Absorption: In the presence of ethidium bromide (0.5 μg/ml), DNA bands were visualized during electrophoresis under a UV lamp. A well was cut in the gel just in front of the band and filled with hydroxylapatite (BRL, suspension in 20 mM sodium phosphate buffer, pH 6.8). By continuing the electrophoresis, the DNA band migrated into the well and was absorbed onto hydroxylapatite. The DNA absorbing hydroxylapatite was then soaked out using a Pasteur pipette and transferred to an Eppendorf pipette tip which a small wad of autoclaved cotton was stuffed at the tip. The mini-column was washed and eluted with 20mM and then 0.3 M sodium phosphate buffer, pH 6.8, respectively.

PEG Precipitation: To the elute, NaCl and an appreciate amount of PEG 6000 (BDH Chemicals Ltd.) were added. The final concentration of NaCl was 0.5 M. NaCl and PEG were added as 2 M and 50% (w/v) solution, respectively. The mixture was incubated at 0°C, normally for overnight. The precipitated DNA was collected by 10-min centrifugation at 12,000 rpm in cold and washed once with 70% ethanol at room temperature. The precipitate was then dried and dissolved in TEN buffer.

Quantification of DNA in Agarose Gel

The method described by Hörz *et al.* (1981) was used to estimate the amounts of DNA in agarose gel. After electrophoresis, gel was stained and then photographed under UV light. The negative film (Ilford FP4) was scanned in a gel scanner (Biomed, SL-TLFF). The amount of DNA was calibrated against the quantity standards.

Determination of the Relative Enzyme Activity

The methods descried by Maniatis *et al.* (1982) were used for restriction enzyme digestion, DNA ligation and nick-translation. Both of supercoiled and *Ava* I-linearized pBR 322 DNA were recovered from agarose gel and subsequently treated with restriction enzymes (Boehringer Meinheim) or T4 DNA ligase (New England Biolabs), or used as a probe for nick-translation. Supercoiled pBR322 DNA purified by CsCl density gradient centrifugation or phenolized *Ava* I-linearized pBR322 DNA were used as the standards. The relative enzyme activity is expressed by the comparison of the enzyme activity on the recoverd DNA to that on the standard. For T4 DNA ligase both of the ligation and tranformation efficiencies were accounted. The relative activity of nick-translation was determined by the incorporation of [α-^{32}P] dTTP (NEN Research Products).

Results

PEG Precipitation

Preliminary experiments of PEG precipitation including PEG concentration, incubation time and DNA concentration were performed by the direct addition of PEG into DNA solution.

Effect of PEG Concentration and Incubation Time: *Hae* III-digested pBR 322 DNA was precipitated with each of 7.5, 10 and 15% PEG for 6, 12, 18 and 24 h under the standard condition. The DNA concentration was 20 μg/ml. The precipitates were analyzed in 4% NuSieve agarose gel. An aliquot of the digest (2 μg) was used as the standard. Figure 1 shows the result. The precipitation of the DNA

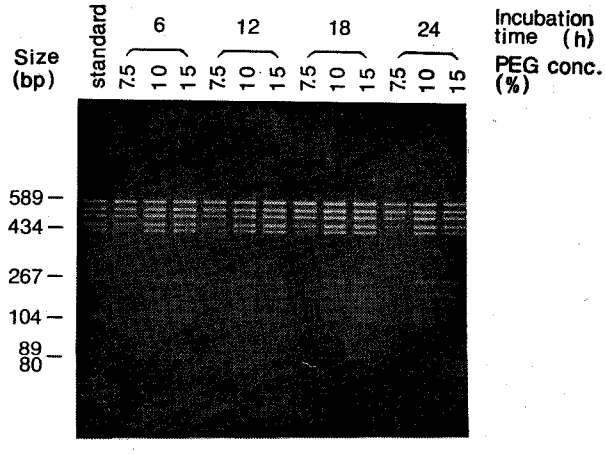

Fig. 1. Precipitation of *Hae* III-digested pBR322 DNA with PEG. *Hae* III-digested pBR322 DNA was precipitated with 7.5, 10 and 15% PEG for 6, 12, 18 and 24h respectively under the standard condition. The precipitates were analysed in 4% agarose gel.

fragments was more dependent on the PEG concentration than the incubation time. In 6 h, DNA fragments with the size around 500, 200 and 100 bp were precipitated by 7.5, 10, and 15% PEG, respectively. A 6-h incubation was enough for the precipitation of the DNA fragments in these size ranges. The precipitation of the DNA fragments smaller than that range was not improved by prolonged incubation.

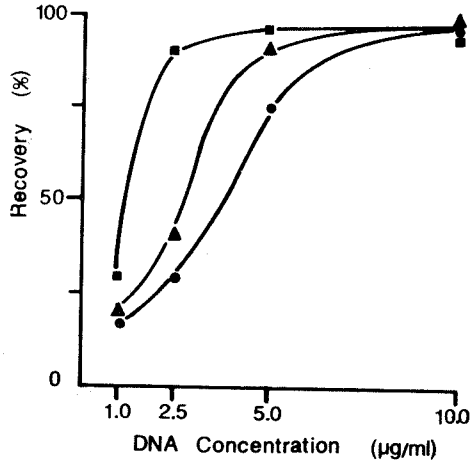

Fig. 2. PEG precipitation on DNA with different concentration. DNA probes with the size of 2,700 bp (—■—■), 500 bp (—▲—▲—) and 220 bp (—●—●—) were precipitated with 10, 10 and 15% PEG, respectively. The precipitation was carried out under the standard condition.

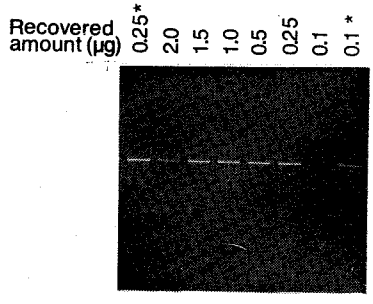

Fig. 3. Recovery of linearized pUC9 DNA from agarose gel by HA-PEG procedure. 0.1–2.0 μg of *Eco* RI-linearized pUC9 DNA were electrophoresed through agarose gel and recovered. Aliquots of recovered DNA which correspond to 0.25 μg were analysed in 1% agarose gel. 0.1 and 0.25 μg of linearized pUC9 DNA (*) were used as the internal standard for the 40% and 100% recovery, respectively.

Effect of DNA Concentration: DNA fragments with three different sizes were used independently, namaly, *Eco* RI-linearized pUC9 (2,700 bp) and fragments (500 and 220 bp) from *Hinf* I-digested pBR322 DNA. The fragments larger than 500 bp were precipitated with 10% PEG, whereas the fragments around 200 bp with 15%. Figure 2 shows that satisfied precipitation was given at the DNA concentration of 5 μg/ml (100, 92 and 75% for the fragments with the size of 2,700, 500 and 220 bp, respectively). Complete precipitation was achieved when the DNA concentration was higher than 10 μg/ml.

Recovery of DNA Fragment from Agarose Gel

0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 μg of *Eco* RI-linearized pUC9 DNA were electrophoresed through a 1% agarose gel and recovered. The recovered DNA was then resuspended in 20, 20, 40, 80, 120 and 160 μl of TEN buffer, respectively. 20 μl of each, theoretically 0.25 μg of DNA except the experiment with 0.1 μg of DNA, were analyzed again in 1% agarose gel. 0.1 and 0.25 μg of the linearized pUC9 were directly used as the standard. Table 1 and Fig. 3 show the result. DNA bands containing more than 0.5 μg of linearized DNA could be 90% recovered in average. The recovery of a band containing 0.25 μg of DNA was around 75%.

Table 1. *Recovery of Eco RI-linearized pUC9 DNA from agarose gel by HA-PEG procedure*

Amount of DNA (μg)	Recovery (%)		
	I*	II*	Average
0.1 (standard)	40.2	41.1	40.7
0.1	29.8	14.0	21.9
0.25	59.2	93.1	76.2
0.5	87.3	95.0	91.2
1.0	85.0	94.0	89.5
1.5	80.0	90.5	85.3
2.0	86.4	89.8	88.1
0.25 (standard)	100.0	100.0	100.0

0.1 and 0.25 μg of DNA were used as the internal standard for the 40% and 100% recovery, respectively. *I and II were two parallel, independent experiments.

Table 2. *Relative enzyme activity (%) on DNA recovered by HA-PEG procedure*

Enzyme or Reaction	Substrate DNA	
	Linearized pBR322	Supercoiled pBR322
Bam HI	91.4	89.5
Eco RI	23.9	38.6
Hind III	5.0	10.0
Pst I	66.3	100.0
Pvu II	86.7	100.0
Sal I	85.7	97.5
T4 DNA ligase	120 (DNA ligation)	
	165 (cell transformation)	
Nick-translation	—	106

Enzyme Activity on Recovered DNA

Both supercoiled and *Ava* I-linearized pBR322 DNA were recovered from agarose gel, then used to test the enzyme activities. The relative enzyme activities are shown in Table 2. There was no significant difference between the enzyme activities on the recovered supercoiled DNA and the recovered linearized DNA. The activities of restriction enzymes *Bam* HI, *Pst* I, *Pvu* II and *Sal* I were slightly affected by the recovered DNA, if any. However, the activities of *Eco* RI and *Hind* III were 70% and and 90-95% inhibited respectively. The activity of T4 DNA ligase was 50% stimulated. By using the recovered DNA as a probe for nick-translation, the incorporation of [α-^{32}P] dTTP was comparable to that of the standard.

Discussion

Hydroxylapatite is one of the excellent chromatographic matrixes for DNA purification. The elution of double-stranded DNA by 0.2-0.3 M phosphate buffer has been reported (Bernardi, 1971). To avoid the precipitation of phosphate, dialysis or Sephadex gel filtration has been carried out generally before the addition of ethanol into the elute. Those methods either are inconvenient or result in poor recovery. Therefore, PEG has been chosen to precipitate the DNA directly from the high salt containing elute.

DNA precipitation by PEG has been included in a plasmid purification procedure (Rambach and Hogness, 1977) and also used for size fractionation of double-stranded DNA (Lis and Schleif, 1975). However, we find that the threshold concentration of DNA for efficient precipition was 5-fold lower than described (Lis and Schleif, 1975). The different results may be caused by the DNA probes to be precipitated. Instead of a mixture, DNA fragments with definite sizes were used in our experiments. The precipitation efficiencies of individual fragments were calculated.

With the exception of *Hind* III and *Eco* RI, the activities of the enzymes tested were not significantly inhibited by using the recovered DNA as substrates. The activity of T4 DNA ligase was even stimulated. This result confirms the reports of polymer-stimulated ligation (Zimmerman and Pheiffer, 1983; Pheiffer and Zimmermann, 1983). The data suggest that the HA-PEG procedure is very useful when the isolated DNA fragment(s) is a valuable probe to be cloned or, for some reasons, a probe to be nick-translated or terminally labeled (gap filling). It can also be used in restriction mapping of long stretches of DNA.

We have successfully isolated the Cm^R determinant containing *Hae* II-fragment (1.3 Kb) from pACYC184 and inserted it into the plasmid RSF1010. For nick-translation, we have also routinely isolated the RF DNA of a filamentous phage Cf

with this method, in order to get rid of the copurified DNA of an indigenous plasmid after CsCl density gradient centrifugation.

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HA-PEG 程序：由洋菜凝膠中回收 DNA 的有效方法

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本研究發展一種由洋菜凝膠中回收 DNA 的簡便方法，此法包括 hydroxylapatite 吸附，磷酸鈉緩衝液冲洗及 PEG 沈澱。0.25 μg DNA 之回收率約 75%，DNA 量超過 0.5 μg 時回收率可達90%。回收 DNA 之純度足以做選殖和缺口轉譯等目的，亦可做物理拼圖。