Volume 5 Number 7 July 1978 Nucleic Acids Research

A method for the recovery of DNA from agarose gels

H.F.Tabak and R.A.Flavell

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, PO Box 60.000, 1005 GA Amsterdam, Netherlands

Received 4 May 1978

ABSTRACT

We describe a quick and versatile method for the isolation of DNA from agarose gels. The DNA is electrophoresed into a trough containing hydroxyapatite, where it is bound. The hydroxyapatite is taken out and the DNA eluted with phosphate buffer. By putting the hydroxyapatite on a small column of Sephadex G50, elution and subsequent removal of phosphate can be performed in one step. The DNA recovered can be used equally well in enzymatic incubations as DNA not purified through agarose gel electrophoresis. Several applications of this technique are described.

INTRODUCTION

Agarose gel electrophoresis is an essential technique for the fractionation of DNA fragments according to length (1,2). Sometimes it is necessary to isolate specific DNA fragments from these agarose gels for further study, *e.g.* redigestion with restriction endonucleases for physical mapping, *in vitro* labelling by nick-translation or terminal labelling with polynucleotide kinase for hybridization studies or sequence analysis.

Several techniques for the recovery of DNA from agarose gels have been described (3-10). With most procedures we have encountered difficulties when the isolated DNA fragments were subsequently used in enzymatic assays, probably due to inhibition by residual traces of agarose. We have therefore worked out a technique that can be applied to DNA fragments irrespective of their length. A small trough is cut out in the agarose gel just in front of the fluorescent DNA band and filled with hydroxyapatite. By further electrophoresis the DNA is trapped in the hydroxyapatite. The hydroxyapatite is removed, poured on top of a small column of Sephadex G50 and the DNA eluted with phosphate buffer. The DNA-ethidium complex can be followed by its fluorescence on UV irradiation and it recovered free of phosphate.

METHODS AND MATERIALS

DNA preparations

DNA preparations were isolated according to standard published procedures: phage PM2 DNA (10), phage lambda DNA (11), rabbit DNA (12); PβG1 DNA, a recombinant plasmid containing part of the rabbit β-globin gene (13) was a gift of Prof. C. Weissmann (Institute für Molekularbiologie, University of Zurich, Switzerland). *In vitro* labelling of DNA by nick-translation was carried out as described in ref. 12.

Restriction endonuclease digestions

The source and incubation conditions for the endonucleases PstI, EcoRI, KpnI and HindIII are given in ref. 12. HaeIII was bought from New England Biolabs and used in an incubation mixture consisting of 10 mM $MgCl_2$, 7 mM mercaptoethanol, 90 mM Tris-HCl (pH 7.9).

Gel electrophoresis

Conditions for gel electrophoresis are described in ref. 12. This involves an apparatus for horizontal slab gels, designed by Dr. W. Schaffner (of the Institute für Molekularbiologie II, Universität Zürich; currently at the Cold Spring Harbor Laboratory, USA), in which the gel makes direct contact with the electrophoresis buffer without the use of wicks (details may be obtained from Dr. W. Schaffner). The electrophoresis buffer contained 40 mM Tris-acetate, 20 mM Na-acetate, 1 mM EDTA and 0.5 µg/ml ethidium bromide (pH 7.7). DNA bands were visualised by illumination with an UV lamp (366 nm).

Transfer of DNA to nitrocellulose filters

Transfer of DNA from agarose to nitrocellulose filters was carried out by the 'blotting' procedure of Southern (14) with modifications as described in ref. 12.

Materials

Agarose was from Sigma; hydroxyapatite (Bio-Gel HTP) from Bio-Rad Laboratories.

RESULTS

To illustrate the method, we have reisolated the intact cir-

cular DNA fraction of phage PM2 DNA from a horizontal agarose slab gel of the type described in Methods. Fig. 1a shows the separation of intact and broken circular DNA and linear phage PM2 DNA. In front of the intact circular fraction a narrow trough of 1-2 mm width was cut out of the gel with a sharp scalpel. The troughs were filled with hydroxyapatite suspended in electrophoresis buffer. Excess buffer drains away between glass plate and agarose gel (Fig. 1b). The completely filled troughs can be covered with plastic foil to prevent evaporation. By continuing the electrophoresis, the DNA is electrophoresed into the hydroxyapatite where it is bound (Fig. 1c). The hydroxyapatite-containing DNA is carefully removed with a pasteur pipette (Fig. 1d) and suspended on top of a bed of Sephadex G50, equilibrated with electrophoresis buffer, in a pasteur pipette. The DNA is eluted from the hydroxyapatite with 1 M Na phosphate buffer and can be followed by the fluorescence of the ethidium-DNA complex on illumination with UV light (Fig. 1e). The DNA-containing fractions, free of phosphate used for the elution, were pooled and electrophoresed again (Fig. 1f). Only a small amount of broken circles and linear DNA is found, indicating that the DNA can be isolated intact with this technique. To study the recovery of the procedure we have used pβG1 plasmid DNA, which has been linearized with endonuclease HindIII, and labelled *in vitro* with ^{32}P-deoxynucleotides by nick-translation. Electrophoresis was carried out without carrier PβG1 DNA and with a 10- and 100-fold excess of unlabelled linear PβG1 DNA present. About 80% of the input DNA is recovered in the void volume fractions of the Sephadex columns, irrespective of the amount of carrier PβG1 DNA added (Table I). The remaining 20% is lost, as a result of incomplete recovery of hydroxyapatite from the agarose troughs and incomplete elution from the hydroxyapatite on top of the Sephadex column (Table I). Both experiments indicate that DNA can be isolated from agarose gels with this procedure undegraded and in good yields.

DNA preparations obtained with this procedure are free from contaminants that inhibit further incubations with enzymes. This is demonstrated in Fig. 2 where untreated phage lambda DNA and phage lambda DNA purified through agarose gel electrophoresis

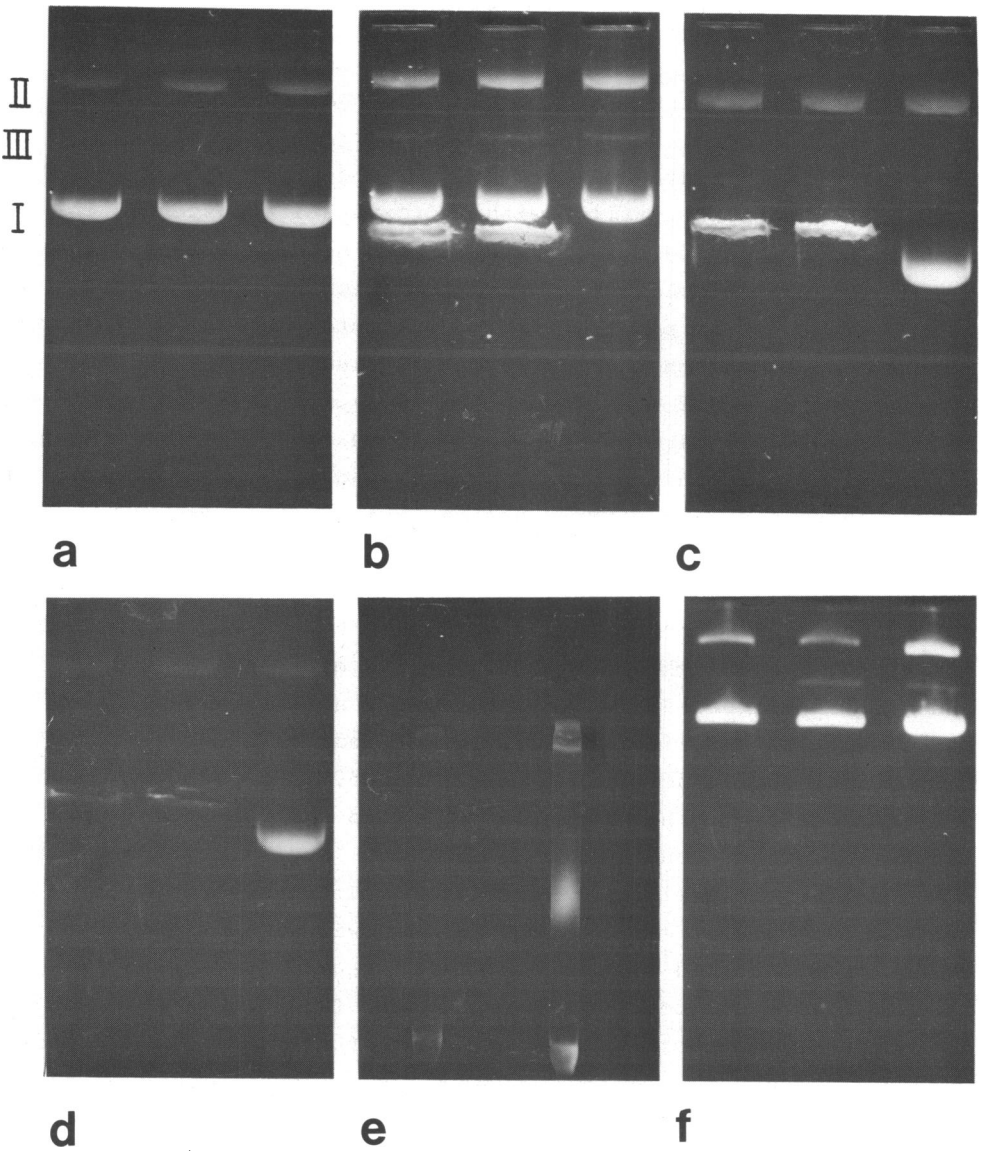

Fig. 1. Isolation of the intact circular phage PM2 DNA fraction from an agarose gel. Phage PM2 DNA was electrophoresed in a 1% agarose gel in three lanes (10 µg/slot). In front of the intact circular DNA fraction in two lanes a small trough was cut out with a scalpel. The trough was filled with hydroxyapatite (b). Electrophoresis was continued till the intact circular DNA fraction in the third lane had passed the trough (c). After removal of the hydroxyapatite, hardly any fluorescence is left in the trough (d). The DNA is eluted with phosphate buffer and the phosphate removed on a Sephadex column. In (e) such an elution is shown where the DNA is in the column. The middle lane in (f) shows the quality of the reisolated intact circular phage PM2 DNA fraction flanked by the original phage PM2 preparation as two concentrations.

TABLE I

RECOVERY OF ^{32}P-LABELLED PβG1 DNA FROM AGAROSE GELS

Fraction	Radioactivity (cpm)			
	Slot 1	Slot 2	Slot 3	Slot 4
1. PβG1 DNA bound to hydroxyapatite taken from the trough	-	8.408	10.070	9.295
2. Agarose gel block containing DNA that would have reached the trough	9.720	-	-	-
3. Agarose boundaries of the trough containing residual hydroxyapatite crystals	-	1.119	896	908
4. Void volume fractions of Sephadex elution	-	6.791	7.556	7.575
5. Hydroxyapatite taken from top of Sephadex column	-	1.215	1.377	1.126
6. Total recovery of radioactivity (summation of lines 3-5)	-	9.125	9.830	9.609
7. % of DNA found in the void volume fractions of the Sephadex column	-	81	75	81

PβG1 plasmid DNA was cut with endonuclease HindIII into linear molecules and *in vitro* labelled with ^{32}P by nick-translation as described in ref. 12. Samples were electrophoresed in a 1% agarose gel. In lane 1: 0.1 µg ^{32}P-labelled PβG1 DNA; lane 2: 0.1 µg ^{32}P-labelled PβG1 DNA; lane 3: 0.1 µg ^{32}P-labelled PβG1 DNA and 1 µg linear PβG1 DNA; lane 4: 0.1 µg ^{32}P-labelled PβG1 DNA and 10 µg linear PβG1 DNA. Radioactivity was measured by Cerenkov counting of the different fractions. Lane 1 was used to measure the input radioactivity. No trough was made in this lane and a piece of agarose was cut out from the gel including the fluorescent PβG1 DNA band and covering the distance migrated from the original position where in the other lanes the troughs were cut out. From the other lanes the DNA was isolated as described for Fig. 1. The recovery was calculated from the radioactivity found in the void volume fractions of the Sephadex column (line 4) and the input radioactivity (line 1).

are fragmented with several restriction endonucleases. With both DNA preparations a limit of digestion is reached with the same amount of restriction enzyme. DNA preparations are also easily labelled *in vitro* to high specific activity by nick-translation (not shown).

Fractionation of the DNA from complex genomes

This method can be successfully applied to the recovery of restricted DNA from organisms which have large genome sizes. In the case of mammalian DNAs, cleavage with a given restriction

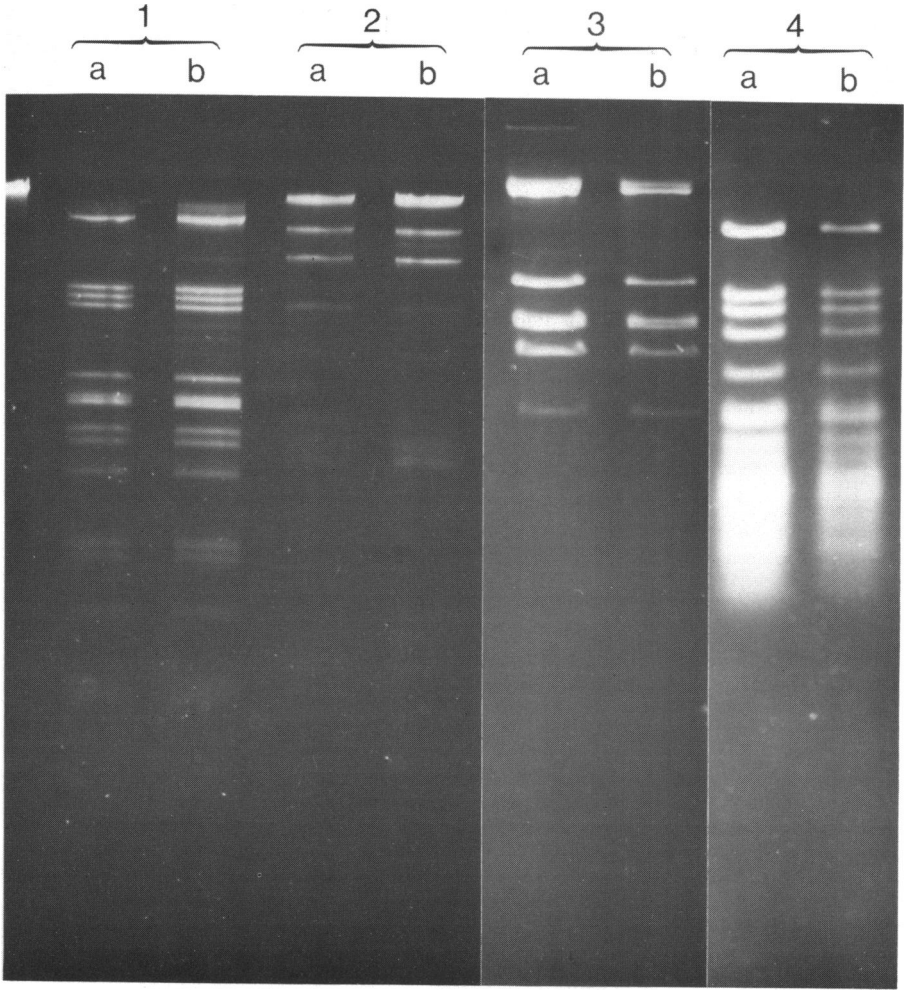

Fig. 2. Fragmentation of phage lambda DNA, isolated from agarose gels, with different restriction endonucleases. In lanes a the control phage lambda DNA is shown. In lanes b the DNA preparation isolated from agarose gels is shown. The restriction endonucleases used are PstI (1), HindIII (2), EcoRI (3) and HaeIII (4).

endonuclease generates about 1 million DNA fragments. Fractionation of these fragments by agarose gel electrophoresis gives therefore a smear of DNA of various molecular weights. In the case of a single-copy gene the band cannot be visualized directly but can be detected by hybridization, either in solution (15) or by use of *in situ* 'blotting' (14) in conjunction with a sensitive hybridization system (16,12). To recover size-fractionated DNA from such complex mixtures either sophisticated con-

tinuous electrophoresis apparatus has been used (Southern, E.M., personal communication (15); or time-consuming extraction of agarose slices (17).

We have applied the hydroxyapatite method to the partial purification of a DNA fragment containing a single-copy mammalian gene, in this case the rabbit β-globin gene. 5 mg of rabbit DNA cleaved with endonuclease KpnI was applied to a 0.7% agarose gel (20 x 20 x 3 cm deep) in a 10-ml solution of 0.3% agarose so that the DNA-agarose formed a gel in the loading slot (12 x 0.4 x 2.6 cm deep). In the 0.7% gel two additional rows of staggered slots (0.2 x 0.5 x 2.8 cm deep) were made by setting up a double comb-type slot former perpendicular to the loading slots (Fig. 3). The rabbit DNA was electrophoresed to give the desired fractionation (Fig. 4a). The perpendicular slots were then filled with hydrox apatite, the gel rotated 90° and the DNA electrophoresed into the hydroxyapatite (Fig. 4b). This step takes from 24-48 h with a gel of these

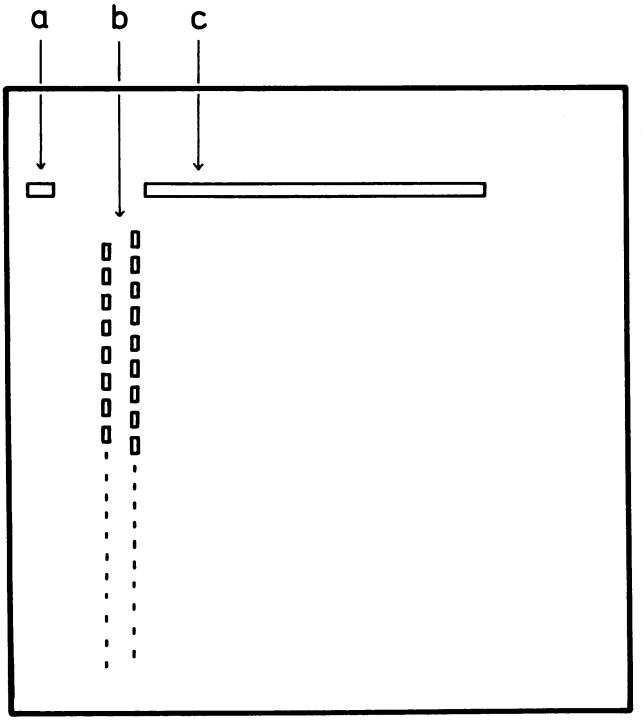

Fig. 3. Diagram of the upper surface of a gel used for two-dimensional electrophoresis-hydroxyapatite recovery of DNA shown in Fig. 4. a) Slot for marker DNA digest; b) double row of slots for hydroxyapatite; c) loading slot.

Figure 4a

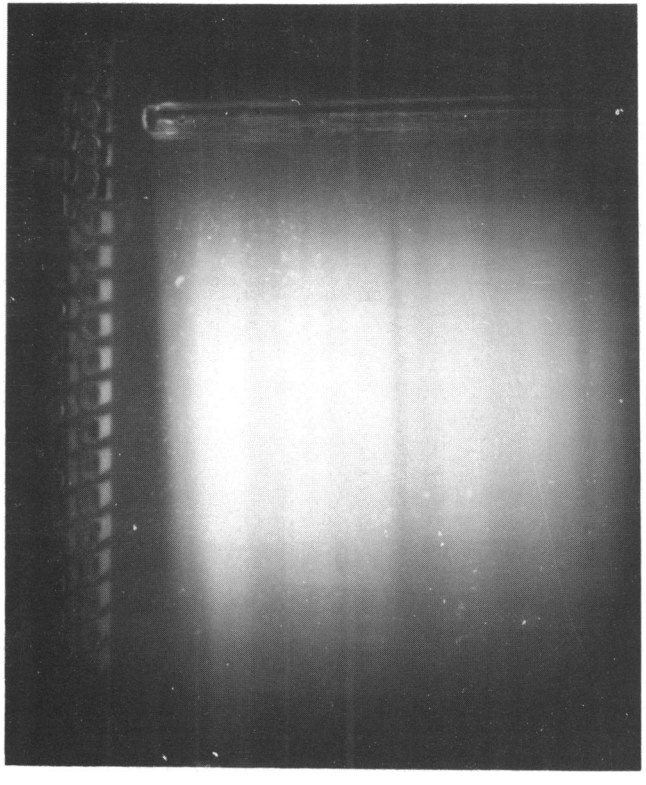

Figure 4b

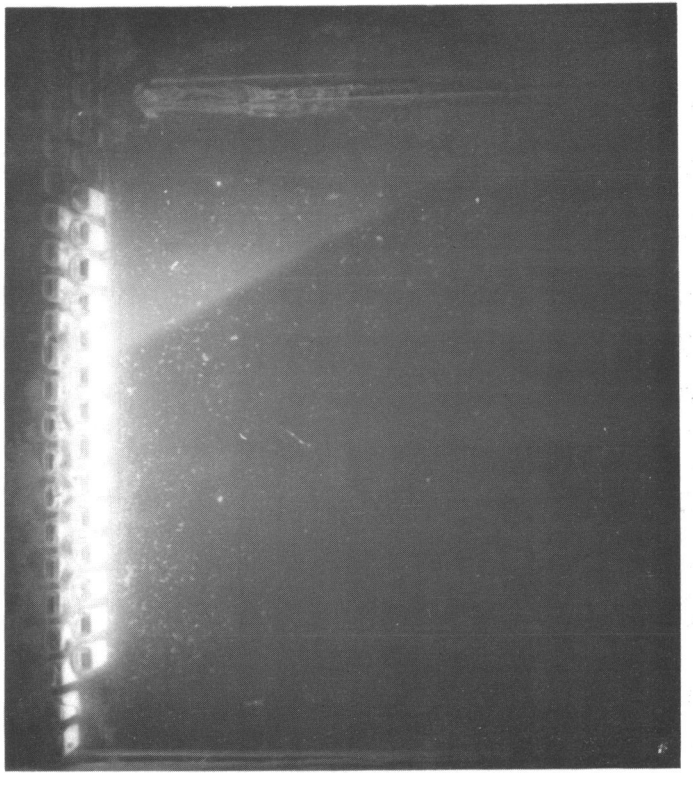

Fig. 4. Electrophoresis of rabbit DNA cleaved with endonuclease KpnI in a preparative agarose gel of the type shown in Fig. 3. a) First dimension: Separation of the DNA fractions according to the molecular weight of the DNA. Electrophoresis was for 12 h at 0.5 V/cm followed by 48 h at 1 V/cm. Hydroxyapatite was then added to the double row of slots, the gel stained with ethidium bromide (0.5 μg/ml) in electrophoresis buffer for 30 min and then photographed under UV light as described (12). b) Electrophoresis in the second dimension: The gel was rotated 90° and the DNA electrophoresed (1.5 V/cm) for 24 h to bind the DNA to the hydroxyapatite. The gel was then photographed under UV light (12). Fluorescence is seen in the hydroxyapatite and of some DNA in the gel which has not yet reached the hydroxyapatite wells.

dimensions at 250 mA, 25 V. After the DNA was adsorbed to the hydroxyapatite, the hydroxyapatite was removed from the perpendicular slots and layered onto columns of Sephadex G50 and the columns were washed with 10 mM Tris-HCl (pH 7.5). The DNA was eluted with 400 µl of either 0.6 M sodium phosphate buffer or 0.5 M EDTA (pH 7.5) and recovery was monitored by following the fluorescence of the ethidium-DNA complex on UV irradation. This simple method makes it possible to fractionate a gel (about 40-50 slots) in a few hours (in practice we elute 20-25 columns at a time). The fractions obtained were analysed by agarose gel electrophoresis (Fig. 5) which shows that the DNA has been fractionated into discrete size classes. The DNA from this gel was then denatured and transferred to a nitrocellulose filter which was hybridized with ^{32}P-labelled rabbit cDNA-plasmid pβG1 to detect the globin genes. Fig. 6 shows an autoradiogram of the filter on which can be seen that the 5.1 kb KpnI β-globin DNA fragment which hybridizes with the probe, is restricted to a few fractions.

Estimation of the amount of DNA recovered in these fractions shows that the β-globin gene is about 20-40-fold purified by this method, which brings the frequency of β-globin gene-containing fragments into a range in which molecular cloning is feasible.

DISCUSSION

A number of methods have been described for isolation of nucleic acids from gels (3-10): salt extraction of homogenized gel slices, electrophoresis into a dialysis bag, preparative gel electrophoresis with continued elution, dissolving agarose in $NaClO_4$ and removal of agarose by hydroxyapatite chromatography, dissolving agarose with agarase, dissolving the agarose slice in KI followed by equilibrium density centrifugation, extrusion of DNA from frozen agarose gel slices. Most of these methods have their limitations: some are time consuming or need special equipment; nucleic acid preparations are sometimes contaminated with traces of gel material or their usefulness is limited to DNA of low molecular weight.

The procedure described here has been successfully used for some time for several purposes. No problems were encountered with subsequent restriction enzyme digestions or *in vitro* labelling

Fig. 5. Analysis of the DNA recovered from the gel in Fig. 4. by agarose gel electrophoresis. DNA eluted from the hydroxyapatite wells in Fig. 4. was recovered as described in Results and Fig. 1. An aliquot of each fraction was then applied to a 0.7% agarose gel and electrophoresed for 3 h at 4 V/cm. The ethidium bromide-stained DNA was then detected by UV fluorescence photography. The left and right lanes contain a marker mixture containing phage lambda DNA (48 kb), phage ϕ29 DNA (17.5 kb) and phage ϕ29 DNA x EcoRI (9.2, 5.4, 1.6, 0.85 and 0.54kb), together with the hybridization markers described in Fig. 6; the middle lanes contain the samples from the hydroxyapatite elution (about 0.3 μg DNA per slot).

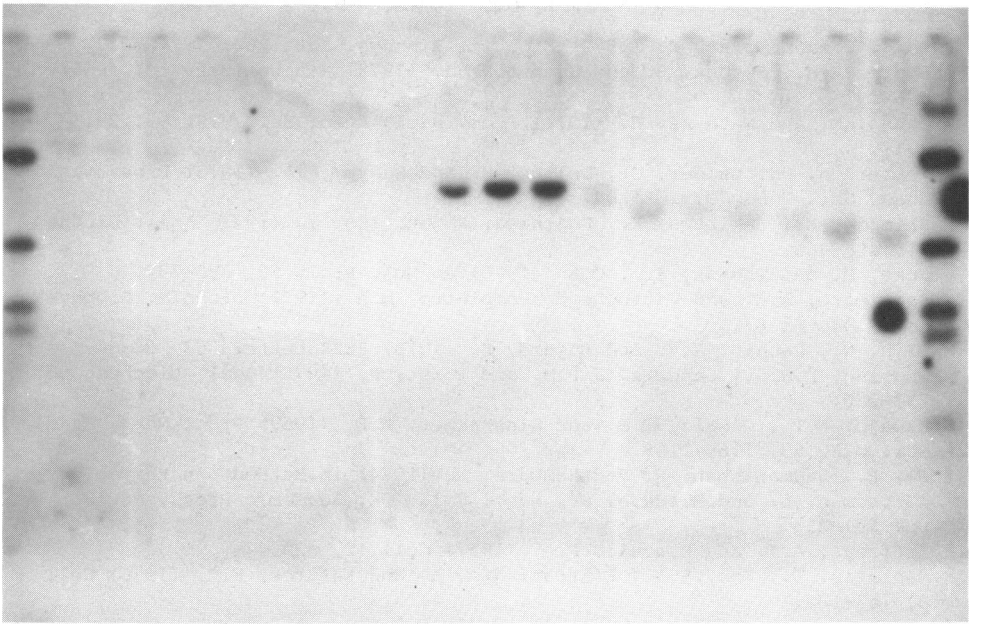

Fig. 6. Detection of DNA fragments containing the rabbit β-globin DNA in the samples analysed in Fig. 5. The gel in Fig. 5 was soaked in alkali to denature the DNA (14) and then the DNA was transferred to a nitrocellulose filter as described in ref. 14. The filter was hybridized with ^{32}P-labelled PβG1 DNA at 65°C in 3 x SSC as described (12). The filter was washed extensively and the remaining labelled components on the filter detected by autoradiography. The two outermost slots show the hybridized marker bands (phage lambda DNA, 48 kb; PβG1 DNA x HindIII, 5.6 kb; PβG1 DNA x EcoRI x AvaI x HindIII, 2.3, 1.5, 1.2 and 0.65 kb; see ref. 12).

techniques and many DNA samples could be processed in a short time. The recovery of the DNA is acceptably high and independent of the amount of nucleic acid processed. The technique may be useful for the isolation of DNA fragments to be sequenced by the Gilbert-Maxam procedure (18) and for the enrichment of DNA fragments for molecular cloning.

ACKNOWLEDGEMENTS

We thank Prof. Piet Borst for his comments and Ms Hilly H. Menke, Mr. Jan M. Kooter and Ms Ernie de Boer for enthusiastic technical assistance. We are also grateful to Ms. Francesca Fase-Fowler for gifts of phage PM2 and lambda DNA and to Prof. Charles Weissman for a generous gift of PβG1 DNA. This work was supported in part by grants to P. Borst and R.A.F. of the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

REFERENCES

1 Aaij, C. and Borst, P. (1972) Biochim. Biophys. Acta 269, 192-200.
2 Sharp, P.A., Sugden, B. and Sambrook, J. (1973) Biochemistry 12, 3055-3063.
3 Danna, K. and Nathans, D. (1971) Proc. Natl. Acad. Sci. U.S. 68, 2913-2917.
4 Petterson, U., Mulder, C., Delius, H. and Sharp, P.A. (1973) Proc. Natl. Acad. Sci. U.S. 70, 200-204.
5 Shimada, K., Sekikawa, K., Fujinaga, K. and Ito, Y. (1973) Anal. Biochem. 51, 456-465.
6 Fuke, M. and Thomas, Jr., C.A. (1970) J. Mol. Biol. 52, 395-397.
7 Sampietro, A.R. and Vattuone de Sampietro, M.A. (1971) Biochim. Biophys. Acta 244, 65-67.
8 Blin, N., Gabain, A.V. and Bujard, H. (1975) FEBS Letters 53, 84-86.
9 Thuring, R.W.J., Sanders, J.P.M. and Borst, P. (1975) Anal. Biochem. 66, 213-220.
10 Espejo, R.T., Canelo, E.S. and Sinsheimer, R.L. (1969) Proc. Natl. Acad. Sci. U.S. 63, 1164-1168.
11 Wu, R., Padmanabhan, R. and Bambare, R. (1974) in Methods in Enzymology (Grossman, L. and Moldave, K., eds), Vol. 29E, Academic Press, New York, pp. 231-253.
12 Jeffreys, A.J. and Flavell, R.A. (1977) Cell 12, 429-439.
13 Maniatis, T., Kee, S.G., Efstratiadis, A. and Kafatos, F.C. (1976) Cell 8, 163-182.
14 Southern, E.M. (1975) J. Mol. Biol. 98, 503-517.
15 Tilgman, S.M., Tiemeier, D.C., Polsky, F., Edgell, M.H., Seidman, J.G., Leder, A., Enquist, L.W., Norman, B. and Leder, P. (1977) Proc. Natl. Acad. Sci. U.S. 74, 4406-4410
16 Botchan, M., Topp, W. and Sambrook, J. (1976) Cell 9, 269-287.
17 Hozumi, N. and Tonegawa, S. (1976) Proc. Natl. Acad. Sci. U.S. 73, 3628-3632.
18 Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. U.S. 74, 560-564.