A simple and efficient method for isolation of DNA fragments from agarose gel

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We describe a simple method for isolation of purified DNA fragments from agarose gels. There are different methods for isolation of DNA from gels, but the major disadvantage of most of the techniques is that the recovery is often low for the fragments of longer or shorter sizes. Some methods are inefficient at removing impurities that interfere with subsequent enzymatic manipulation of the DNA. The method we describe is quick, efficient, and does not require expensive materials. It enables us to recover a high yield of intact pure DNA fragments of any size.

The procedure involves the electrophoresis of the DNA fragment in 1% low-melting-point agarose (BRL) gel either in TAE or TBE buffer. Sephadex G-50 beads were equilibrated with TE buffer and autoclaved. A Sephadex G-50 column was made in a 10-ml syringe and spun at 3000 rpm for 5 min; dried Sephadex beads were stored in a tube. The bottom of a 1.5-ml microfuge tube was plugged with a small amount of sterile siliconized glass wool. The desired DNA band was excised from the ethidium-bromide-stained gel. A volume of Sephadex G-50 dry beads was placed on top of the glass wool. The gel slice was placed on top of Sephadex beads. The tube was closed and placed in a 55°C to 60°C water bath to melt the gel (5 to 10 min, approximately). As the gel slice melted, it entered into the Sephadex pack. A volume of TE buffer was added and incubated for another 5 min at 55°C. The tube was then placed in ice for 5 min. Two small holes were made, one at the top and another at the very bottom of the tube, with a 21-gauge needle. The tube was placed on top of another microfuge tube and spun at 1000 rpm for 5 min at 4°C in a tabletop centrifuge. About 85% of the DNA came out in the flowthrough. However, if 15 to 20 μ l of TE buffer was added to the tube, further centrifugation would increase the yield to 95%. Since the DNA fragment passes through the Sephadex beads, it is clean and ready for subsequent manipulation. This method is also applicable to standard agarose gels, but the gel piece melts at 80°C, and 2 equivalent volumes of TE buffer must be added during the melting of the gel.

To determine the percentage of recovery, restriction fragments of different sizes of HindIII digested DNA (BRL) were nick translated under mild conditions with α -³²P labeled dCTP. The DNA was run on 1% low melting point agarose gel; ethidium-bromide-stained gel bands were excised and the radioactivity of the gel pieces was counted. The DNA from each gel piece was eluted through the column as described above and the percent radioactivity recovered was calculated as shown on Table 1.

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Figure 1. One microgram of HindIII digested bluescript vector was size fractionated in 1% low-melting-point agarose gel at 80 V for 1 h (a). A linearized single DNA band was excised from the gel and eluted according to the method described. The total eluted DNA was further run on the same type of gel and photographed (b).

Table 1. Recovery of DNA fragments from agarose gels

Gel frag	Size (10 ³ bp)	Radioactivity in gel slice (10 ³ Cpm)	Radioactivity (Total)	Radioactivity eluted from column (10 ⁵ cpm)	Radioactivity (Total)	% Recovery
1	23.1	1.97	(197910)	1.8	(186850)	94.4
2	9.4	1.05	(105270)	0.95	(95753)	90.9
3	6.6	0.75	(75706)	0.69	(69893)	92.3
4	4.4	0.61	(61230)	0.57	(57930)	94.6
5	2.3	0.60	(60975)	0.56	(56645)	92.8
6	2.0	0.43	(43736)	0.41	(41716)	95.3
7	0.6	0.58	(58210)	0.55	(55985)	96.1

The percent recovery of the DNA fragments which ranged from 500 bp to 20 kb. Usually, about 80% to 90% recovery is achieved regardless of fragment size. However, it should be noted that high molecular weight DNA fragments are more efficiently recovered. Frequently, high molecular weight DNAs are difficult to elute from the gel piece; either the recovery is very low or the DNA is broken down during extraction. This method allows an efficient alternative for isolating particularly large DNA fragments.