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Isolation of DNA fragments from agarose gel by centrifugation

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Although there are a number of protocols and commercial products available for isolating DNA fragments from agarose gels (1), this technique is still troublesome, especially for inexperienced researchers. Disadvantages of some commonly used methods include low recovery rates, insufficient removal of inhibitory substances present in agarose, and coprecipitation of agarose with DNA causing difficulty in redissolving the DNA pellets. Some protocols require special apparatus or gels (e.g. low-melting-temperature agarose), which can be expensive. We describe here a very simple method for isolating DNA fragments from agarose gels with high yield, high quality and low cost. Because the method is extremely simple, the protocol is easy to follow and the results are highly reproducible. The purified DNA is satisfactory for any subsequent protocols. The technique is based on the fact that DNA fragments in a relatively low percentage (0.3–0.6%) agarose gel will easily migrate out of the gel under centrifugal force if the gel is mechanically blocked by some material that allows only DNA molecules to go through. We use Sephadex G-10 spin columns for this purpose. The home-made Sephadex G-10 spin columns consist of one 2 ml and one 0.5 ml microcentrifuge tube without caps, a tiny bit of sterilized glass wool and 150 μl of swollen Sephadex G-10 beads. All of these materials are commonly found in most molecular biology laboratories and one can easily make as many as several dozen spin columns in half an hour. To make such a spin column, heat a needle or a pin with a Bunsen burner in a ventilation hood, lightly stab the heated needle into the center of the bottom of a 0.5 μl microcentrifuge tube to make a small hole, no bigger than 1 mm. Put a tiny bit of glass wool into the tube and push the glass wool to the bottom, using a pipetter tip, to block the hole. Lay 150 μl of swollen Sephadex G-10 beads on top of the glass wool with a large orifice pipetter tip. Put the 0.5 ml tube into a 2 ml tube to form a home-made Sephadex G-10 spin column. The spin column is similar to those commonly used in molecular biology protocols except that a smaller volume of Sephadex beads is used in order to leave some room for the agarose gels. Figure 1 is a schematic picture of a Sephadex G-10 column for separating DNA fragments from gel. Centrifuge the column for 2 minutes at the maximum speed of a bench top microcentrifuge to separate the water from the Sephadex beads. Put the 0.5 ml tube into a clean 2 ml capless tube. Now the column is ready for the agarose gel. As long as the separating resolution permits, use a mini gel with 0.3–0.6% agarose, higher DNA concentration (greater than 1 μg/μl) in loading buffer and shorter electrophoresis time (less than 2 hours). This will substantially improve the recovery rate and the quality of the recovered DNA. The band(s) of interest are cut out with a clean razor blade under long wavelength UV illumination. Be sure to discard as much gel as possible to minimize the size of the DNA-containing gel slice for centrifugation. Transfer the gel slice into the small tube and smash the gel slice against the tube. A

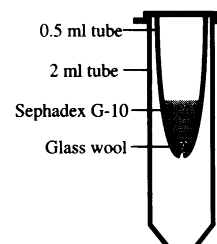

Figure 1. Home-made Sephadex G-10 spin column for isolation of DNA fragments from agarose gel by centrifugation.

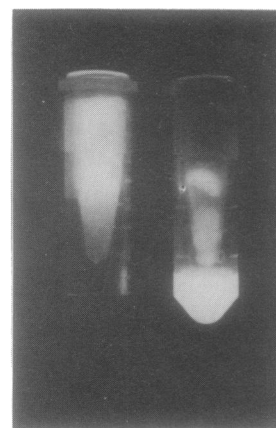

Figure 2. Photograph of the home-made Sephadex G-10 columns with smashed DNA-containing agarose gel in it before (left) and after (right) centrifugation.

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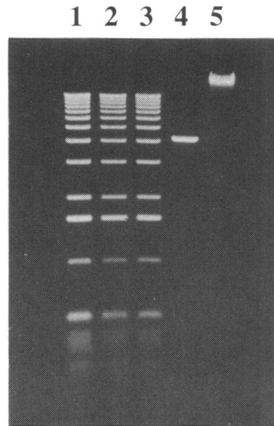

Figure 3. Electrophoresis of gel-recovered DNA and its blunt-end ligation products. Lane 1, 2 μg of the 1 kb DNA ladder (BRL) with molecular weights of 200 bp−12 kb. Lane 2, 1 μg of DNA ladder. Lane 3, DNA recovered from 0.4% agarose gel slices of every band containing the same amount of DNA as in lane 1. Lane 4, *Pvu*II digested pBR322. Lane 5, blunt-end ligation products of *Pvu*II digested pBR322 recovered from the gel.

Millipore MF filter forceps (Catalog number xx62 000 06, Millipore, Bedford, MA, USA) is an excellent tool for both gel transferring and smashing. Centrifuge the column for 10 minutes at the maximum speed of the bench-top microcentrifuge (about 12,000−14,000×g). Transfer the DNA solution to a clean 1.5 ml tube and precipitate the DNA with 1/10 volume of 3 M sodium acetate (pH 5.2) and 0.7 volume of ice-cold isopropanol. Gently wash the DNA pellets twice with ice-cold 70% ethanol and dissolve the DNA in TE. Figure 2 shows the Sephadex G-10 column before (left) and after (right) centrifugation. After centrifugation, a fraction of DNA (usually 30−70%) migrates out of the gel while the gel fragments are blocked by the Sephadex G-10 beads. Figure 3 shows electrophoresis of DNA fragments with different sizes ranging from 200 bp to 12 kb (1 kb DNA ladder, BRL, Gaitherburg, MD 20877, USA) before and after gel recovery. Since lane 2 contains 50% of the amount of DNA loaded in lane 1, and lane 3 contains the DNA recovered from the gel slices of every band containing the same amount of DNA as in lane 1, the recovery rate appears to be about 50%. By comparing the density of each band in lane 3 to the corresponding band of the same molecular weight in lane 2, it is clear that the recovery is equally efficient for all sizes. Lane 4 contains *Pvu*II-cut pBR322 and lane 5 contains the blunt-end ligation products of *Pvu*II-cut pBR322 recovered from the gel. Almost all of the blunt end fragments are ligated to form higher molecular weight concatemers. Although we did not check all possible subsequent uses of the recovered DNA, we did not find any difficulties in all protocols we routinely performs, including sticky-end or blunt-end ligation, restriction enzyme digestion, blunt end generation with Klenow fragment, linker addition to the blunt end fragment, or labeling by either random priming or nick translation. Use of relatively low percentage agarose gels facilitates migration of large DNA molecules off the gel during centrifugation. Low percentage agarose gels are also easier to smash by cutting the gel with the sharp edges of the Millipore forceps and squeezing the gel against the inside wall of the 0.5 ml centrifuge tube with the flat sides of the forceps. High concentrations of DNA in smaller gel slices shortens the migration distance of DNA molecules from the gel during centrifugation and therefore increase the yield. High DNA concentrations in a small volume also facilitates precipitation of the DNA with isopropanol, avoiding the necessity of lyophilization to concentrate the DNA or of addition of carrier tRNA. Moreover, the smaller gel slice decreases contamination by inhibitory substances from agarose. Sephadex G-10 beads effectively block all gel fragments, and probably some of the inhibitory substances as well, and allow DNA molecules of all sizes to penetrate with equal efficiency. We even used this method to isolate DNA fragment as big as 45 kb for cosmid cloning.

REFERENCE

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