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Purification of DNA fragments from lyophilized agarose gels

Marilee Benore-Parsons* and Lona Anderson

Department of Natural Sciences, University of Michigan-Dearborn, Dearborn, MI 48128, USA

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We have developed a method to rapidly recover DNA from lyophilized agarose gels. We found that freeze drying agarose gels forms an open-pore crystalline lattice structure, with a texture and appearance resembling styrofoam, retaining the size and shape of the gel. In contrast, air dried gels collapse into flat amorphous structures (Fig. 1). The open pore structure of the freeze dried gels allows movement of liquid into the lattice solubilizing and extracting the DNA.

Pure DNA fragments must be obtained for many molecular biology applications, including cloning, hybridization and screening assays. DNA fragments are usually obtained by cutting DNA with restriction enzymes, followed by electrophoresis to separate the fragments by size. The DNA is then extracted from the gel using one of various methods (1). Most procedures require the use of 'low-melt' agarose, which is then degraded by enzymes or damaged by physical manipulation, and the DNA recovered by binding to glass, entrapment, differential precipitation or other methods (1–10). DNA can also be eluted from the gel using an electroeluter (1). All of these methods require expensive kits, enzymes, low-melt agarose or special equipment, and in our hands often result in low recoveries of DNA or protein contamination. Purification of intact large DNA fragments, and large scale isolation of DNA, are particularly difficult.

In our procedure, sections of agarose gel containing DNA are freeze dried by lyophilization and the DNA is eluted from the agarose by a mixture of buffer and ethanol. The DNA is then precipitated from the eluant with salt (1). This method is routinely performed by undergraduates with good results.

The method was tested on two types of DNA. Plasmid DNA was digested with restriction enzymes into smaller fragments. Lambda DNA digested with *Hin*dIII was purchased (Boehringer Mannheim). Aliquots of the DNA samples were stored as controls. The rest of the DNA fragments were separated by electrophoresis on 1% agarose gels in 1× TAE buffer (40 mM Tris acetate pH 8, 1 mM EDTA). The gels were run at 7–20 V overnight to optimize band separation. DNA was visualized by ethidium bromide staining using a Fotodyne transilluminator, and the bands of interest excised. Each agarose slice was placed in a polypropylene tube and lyophilized overnight at 10 mTorr and –60°C in a Virtis Freezemobile. A 1:1 ethanol:TE mixture (10 mM Tris, pH 8, 1 mM EDTA) was added to the lyophilized gels in volume equal to 1.5–2 volumes of the gel slice. The samples were gently mixed for 1 h, the agarose was compressed with a spatula, and the flattened gel slice removed. To compress the DNA the flat side of a small metal spatula was pressed against the top or bottom side of the slice. For long slices, the slice was compressed by repetitive movements, forcing the eluant downward through the gel. The tubes were spun to pellet any agarose particles, the supernatant transferred to a microcentrifuge tube and the volume measured. The DNA was recovered from the eluant by ethanol precipitation using sodium acetate: 1/2 volume of ethanol and 1/20 volume of 3 M sodium acetate was added to the eluant. The samples were placed on ice for 20 min or longer, and the tubes spun at 14 000 *g*. DNA pellets were resuspended in TE.

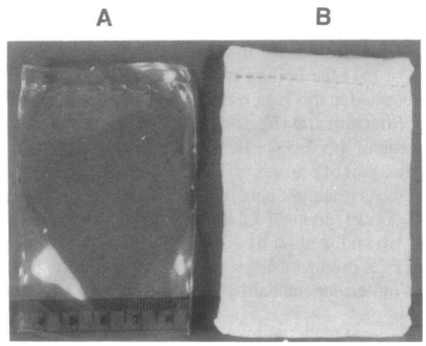

Figure 1. Comparison of (**A**) air dried (amorphous structure) and (**B**) lyophilized (crystalline) gels. Agarose gels (1%) were run at 75 V in 1× TAE for 1 h, and then dried. (A) was dried at room temperature exposed to air. (B) was placed in a lyophilizer and dried under vacuum at –60°C and 10 mTorr until no water remained present.

Yields were determined by two methods. For visual estimations of yields and to determine the integrity of the sample, equivalent aliquots of control and recovered DNA fragments were run on gels, stained and photographed as described. Yields were also determined by fluorescence using Hoescht dye 33358 and the Hoefer TKO 100 Fluorometer (11).

To compare recovery of DNA of various sizes, aliquots (2–4 μg) of lambda DNA digested by *Hin*dIII (Boehringer Mannheim) were electrophoresed on 1% agarose gels in 1× TAE. Vertical slices of each lane from the well to the 2000 base pair (bp) fragment were excised, and the DNA extracted as described. The DNA was resuspended in TE equivalent to the original volume, and recovery determined visually and by fluorescence.

* To whom correspondence should be addressed

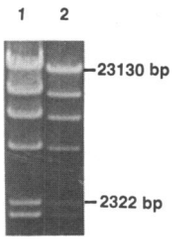

Figure 2. Comparison of control and recovered DNA after extraction from lyophilized gels. Lambda DNA (2.5 μg) digested with *Hin*dIII was separated on 1% agarose in 1× TAE. A slice of the well containing bands from 23 000 to 2000 bp was excised and lyophilized. The DNA was extracted as described, and the recovered DNA (lane 2) run next to an equivalent control (lane 1) sample on a 1% agarose gel in 1× TAE.

Recovery of small fragments was determined using recombinant plasmids containing small fragments cloned into pBR322. Plasmids were digested with restriction enzymes, and the bands separated on a 1% agarose in 1× TAE gel run overnight. Bands were visualized by ethidium bromide staining and the small fragments (~500 bp) were cut out, and the DNA recovered and resuspended into TE for comparison to controls. Recovery was determined visually and by fluorescence.

Lambda DNA/*Hin*dIII fragments (Fig. 2) demonstrated even distribution of recovered fragments over the range of 2000–23 000 bp. Visual inspection indicated recovery of 30–50%. Fluorometric data indicated 30% of the DNA was recovered. Recoveries of smaller fragments (500 bp) from plasmids are more variable; 10–45% of the DNA is recovered, as determined by fluorometric data and confirmed by visual inspection of gels (data not shown).

This method can successfully be used to recover DNA from gels, in yields near 50% recovery. This method uses regular agarose, and requires only electrophoresis and lyophilization equipment, standard in most biochemistry and molecular biology laboratories. The results show that recoveries are equivalent for DNA fragment sizes from 2000 to 23 000 bp. This is significant as most procedures report much lower recoveries or damage of large DNA fragments.

DNA recovered by this method has been successfully used in DNA fingerprinting and gel shift experiments, can be labelled with biotin and radioactive nucleotides, and appears intact when characterized by gel electrophoresis. We have not observed anomalous migration behavior in agarose gels as a result of the lyophilization (12).

The success of this method is due to the open porous structure of the lyophilized gels. The open agarose matrix allows solvent to enter the gel matrix, and large DNA fragments are solubilized and extracted out of the matrix through the large pores with no apparent damage.

Our initial experimental attempts were to reduce the agarose to a powder, similar to the original form, from which we hoped to resuspend and extract DNA. However, once heated in solution agarose undergoes a permanent phase change, and it is not possible to recover the dry powdery agarose form. Several different solutions were tested to extract the DNA from the freeze-dried gel. Extractions into TE, chloroform or isopropanol were not successful. TE:ethanol ratios of 1:0, 1:1 and 1:2 were analyzed; a 1:1 ratio proved optimal (data not shown). We also compared DNA recovery by evaporation of the liquid using a Savant rotoevaporator or ethanol precipitation of the DNA and found the latter method was faster.

DNA has been successfully recovered from dried gel slices stored for one year. Thus, this method provides an alternative method for storing, and perhaps even shipping DNA samples.

We have tried many methods in our lab to recover DNA fragments, including electroelution, filtration through membranes, HPLC, commercial kits and compression of low melt agarose by several methods (1–10). Although the yields claimed by some of these procedures are high, in our hands the only method which gave larger recoveries of DNA than this approach was using the enzyme agarase to recover DNA from low melt agarose. However, agarase can only be used with low melt agarose, rendering it more expensive and less convenient than the method described here. Furthermore, agarase may not completely digest all the agarose, leaving small gummy pieces of gel as well as the agarase enzyme itself in the sample, which may compromise the purity and subsequent use of the DNA.

This method is useful for small and large scale recovery, as the only difference is in the size of the tube needed for lyophilization and extraction. The technique is very useful for researchers who need to purify large amounts of DNA fragments, such as for use in screening, RFLP analysis, binding experiments or commercial enterprise.

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