A rapid and reliable one-step method for isolating DNA fragments from agarose gels

J. Errington
Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

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Various methods are available for isolating DNA fragments from agarose gels. For many purposes, particularly sub-cloning, quantitative recovery is not necessary as the amount of DNA needed for ligation is usually a small fraction of the amount that would be visible as an ethidium bromide stained band on the gel. The most commonly used methods suffer from one or more of a variety of disadvantages. They require specialized (and relatively expensive) apparatus, expensive reagents, special gels (e.g. low-melting-point agarose), extractions with organic solvents, or multiple washing or precipitation steps. Here I describe a reliable single-step method that requires no special gel or equipment and has essentially zero cost.

The method is similar to that of Girvitz et al. (1980) but has been greatly simplified by removing several steps that turned out not to be essential. Samples are run on ordinary agarose gels, such as are used for routine restriction mapping. Thus, fragments can be taken from the gel used to map a primary clone, for example. A slit is made in front of the band to be removed and a strip of sterile Whatman 3MM paper inserted. The strip should be about the same width as the band but may protrude from the gel by a few mm. The gel is then returned to the tank and a short period of electrophoresis used to move the DNA into the paper. Some practice may initially be needed to achieve the correct time because prolonged electrophoresis used to move the DNA into the paper. Mobility through the paper seems to be similar to that of the 0.7% gels that we use routinely, so that the timing can be estimated if the rate of mobility of the fragment is known (from the distance travelled and the time of electrophoresis). If the band moves right through the paper the procedure can be repeated with a second strip of paper.

When the band lies at least partially inside the paper, the paper is trimmed. The paper is placed in a 500 μl micro-centrifuge tube with a small hole in the bottom (use a red-hot wire). This tube is then placed inside a 1.5 ml tube and microfuged for about 20 s to recover the DNA in a small volume of electrophoresis buffer. From a band containing about 100 ng, it is usual to recover about 30 to 40 ng of DNA (total volume about 10 μl), which is sufficient for most cloning purposes. The recovery is not improved by loading more DNA unless several strips of paper are inserted together in parallel. To sample a range of sizes of fragment from a partial digest of chromosomal DNA, for example, the paper can be inserted at an angle to the direction of fragment migration.

I have found that it is not necessary to further purify the DNA. The electrophoresis buffer (Tris-acetate-EDTA; Maniatis et al., 1982) does not seem to inhibit ligation significantly, and it probably has little effect on other enzymes such as T4 DNA kinase (although we have not tested this thoroughly). Avoiding the phenol extraction and precipitation steps improves the yield, reliability and speed of the method. We have used the method for the last two years in all of our sub-cloning experiments and find it very convenient and reliable.

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REFERENCES