RECOVERY OF DNA FROM LOW-MELTING-TEMPERATURE AGAROSE

A number of grades of agarose are available in which hydroxyethyl groups have been introduced into the agarose molecule. This substitution causes the agarose to gel at 30°C and to melt at 65°C - well below the melting temperature for most DNAs. These properties have been exploited to develop a simple technique for the recovery of DNA from gels (Weislander 1979).

1. Dissolve the low-melting-temperature agarose by heating in electrophoresis buffer to 70°C. Cool to 37°C. Add ethidium bromide to a final concentration of 0.5 µg/ml. Pour the gel at 4°C to ensure that it sets properly.

2. Load the samples of DNA and carry out electrophoresis at 4°C to ensure that the gel does not melt during the run. The electrophoretic characteristics of low-melting-temperature agarose gels are similar to those of conventional agarose gels.

3. Cut out the desired segment(s) of gel and add about 5 volumes of 20 mM Tris ·Cl (pH 8.0) and 1 mM EDTA.

4. Heat for 5 minutes at 65°C to melt the gel.

5. At room temperature, extract the melted gel slice with an equal volume of phenol. Recover the aqueous phase by centrifugation at 20°C and re-extract with phenol/chloroform and then with chloroform.

6. Recover the DNA by ethanol precipitation. Usually, the DNA is now pure enough to serve as a substrate for restriction enzymes, ligases, etc. If necessary, the DNA can be further purified by chromatography on DEAE-Sephacel.

Procedure from Maniatis cloning manual

Reference: Weislander, L. 1979. Anal. Biochem. **98**, 305.