Isolation of DNA from LMP Agarose II

1. Locate band of interest using a hand-held long-wave UV light (this minimizes radiation damage to DNA), and excise the band in the smallest slice possible.

2. Add about 5 volumes of 20mM Tris pH 8.0/ 1mM EDTA to the agarose slice and incubate at 65°C for 5 minutes to melt the gel.

3. Cool the solution to room temperature, and add an equal volume of phenol (equilibrated with 0.1M Tris pH 8.0), vortex 20 seconds, and spin at 4,000g for ten minutes at 20°C recover the aqueous phase by. The white interface is powdered agarose.

4. Re-extract the aqueous phase once with phenol:chloroform, and one with chloroform.

5. Transfer the aqueous phase to a polystyrene tube, and add 0.2 volumes of 10mM ammonium acetate and two volumes of ethanol at 4°C.

6. Store the mixture for 10 minutes at room temperature, and recover the DNA by centrifugation.

NOTE: Because low-melting temperature agarose remains fluid at 37°C, enzymatic manipulations (such as ligations, synthesis of radioactive probes and digestion with restriction enzymes) can be carried out by adding portions of the melted gel slice directly to the reaction mixture. (Parker and Seed 1980; Struhl 1985).

Taken from: Sambrook et.al Molecular Cloning , A Laboratory Manual; second edition; pp. 6.3 - 6.31. Look here for more detailed information.
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