Recovery of DNA from Low Melting Point Agarose

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Abstract

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Protocol

Step 1.
Low melting point agarose gels should be poured and allowed to set up in the cold room, but electrophoresed at room temperature.

Step 2.
The gels should be stained at 4°C and kept cold until ready to cut out the DNA bands from the gel.

NOTES
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The gels will be fragile, so handle gently.

Step 3.
Cut out the DNA bands from the gel with new razor blades. Use a new razor blade for each band.

Step 4.
Place the agarose pieces into sterile capped tubes (the size of the tubes depends on the volume of the agarose involved).

Step 5.
Add 1X TE buffer to the tubes of agarose (as small a volume as possible, usually about 5X the volume of the gel pieces).

Step 6.
Heat the tubes at 65°C for 10-15 min.

DURATION
00:15:00

Step 7.
Transfer the tubes to 37°C.

Step 8.
Add an equal volume of buffer-saturated phenol. The phenol should have been warmed to 37°C.

Step 9.
Mix well.

Step 10.
Centrifuge the tubes at room temperature to separate the phases. Centrifuge at 10,000 rpm, 10 min in the Sorvall or 5 min in the microfuge.

DURATION
00:10:00

Step 11.
Transfer the upper aqueous layers to clean tubes.

Step 12.
Add an equal volume of phenol:$CHCl_3$:Isoamyl alcohol (25:24:1) to the tubes.

Step 13.
Mix well and centrifuge at 10,000 rpm, 10 min in the Sorvall or 5 min in the microfuge.

DURATION
00:10:00

Step 14.
Re-extract the aqueous layer 2X with $CHCl_3$:Isoamyl alcohol (24:1) in the centrifuge as before.

Step 15.
Add 3 M Na acetate to a final concentration of 0.3 M and precipitate the DNAs with 2X volumes of 100% EtOH at -20°C overnight.

DURATION
18:00:00

Step 16.
Centrifuge the tubes to pellet the DNAs. Centrifuge in the Sorvall at 10,000 rpm, 10 min, 4°C or 10-15 min at 4°C in the microfuge

DURATION
00:10:00

Step 17.
Wash the DNAs 1X with 70% EtOH in the centrifuge as before and dry the DNA pellets in the vacuum desiccator briefly (10-15 min) to remove the EtOH.

DURATION
00:15:00

Step 18.
Resuspend the DNAs with a small volume of 1X TE buffer.