Yan Lab Protocol by Zhen Yan from Janet Mar 9/2/2009

DNA isolation from acrylamide gel.v1

Procedures

1. Stain acrylamide gel (4-10%, 1.5 mm thick) by immersing it in ethidium bromide for 20-30 minutes.
2. Visualize DNA using the preparative wavelength on the UV box, cut out a piece of acrylamide (as little as possible without losing the DNA) containing the DNA band of interest.
3. Place on parafilm and rinse gel with 0.5 ml of 1X TE, drain off all liquid, cut gel into ~1 mm pieces, put into a 1.5-ml eppendorf tube.
4. Add 400 µl Elution Buffer, make sure all fragments submerged, incubate in 37˚C shaking incubator overnight.
5. Spin for 5 min, transfer the supernatant (keep out gel fragments) into a fresh 1.5-ml eppendorf tube. Add 16 µl 5 M NaCl and 1 ml 100% ethanol. Vortex, incubated on dry ice for 10 min and spin for 20 min.
6. Wash the pellet with 70% ethanol, drain well and vacuum dry. Resuspend the DNA in 15-20 µl of TEE.

Reagents preparation

Elution buffer

5 M NH_4OAc	1.0 ml	(0.5 M)
0.5 M EDTA	0.2 ml	(10 mM)

Notes

1. This procedure works well for fragments below 700 bp (down to 24 bp on 10% gel has worked well) but not in the kb range.
2. Check recovery and approximate concentration of isolated DNA on a gel.
3. Gel slice contains ethidium bromide, so do not cut out gel slice and store for isolation late otherwise you risk having nicks in the DNA.