DNA Purification from Agarose Gel.

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Use a LOW MELT specification Agarose.

1. Run an agarose gel as per a normal protocol (do not use excessive voltages) unless you intend to us an agarose percentage less than 1% in which instance the gel will need to be constructed and run in a cold room.
2. Cut the required DNA bands out of the gel with as little excess agarose as possible.
3. Place the gel slice(s) in a waterbath at 65C until the agarose has completely melted – vortex frequently to assist the process.
4. Add an equal volumes of Phenol.
5. Vortex to an emulsion.
6. Centrifuge for 10min RT 13,000rpm.
7. Pipette off and keep the top (aqueous layer) avoiding taking and precipitated protein or phenol.
8. Add and equal volume of phenol chloroform.
9. Vortex to an emulsion.
10. Centrifuge for 10min RT 13,000rpm.
11. Pipette off and keep the top (aqueous layer) avoiding taking and precipitated protein or phenol.
12. Add1/10 volume sodium acetate pH 5.5.
13. Add 2 volumes of 100% ethanol.
14. Vortex or invert to mix well for a few seconds.
15. Centrifuge for 15min RT 13,000rpm.
16. Carefully remove the ethanol.
17. It is advisable to keep this ethanol if you cannot see a pellet – the DNA may not have fully precipitated – see note below.
18. Add 70% ethanol – typically 500µl in 1.5ml microtube.
19. Centrifuge 2min max speed to re-pellet the DNA
20. Remove ALL the ethanol (re pellet again if necessary).
    a. The pellet may now be slippery so it is advisable to pipette off the liquid.
21. Air dry for 5-10 minutes to ensure ALL ethanol has gone BUT do not over dry.
22. Resuspend in dH2O or TE as appropriate

NOTE if your expected DNA yield is small you may need to put the ethanol precipitation stage at -80C for a few hours/overnight.