

DNA Fragment Isolation from LMP Agarose Gels

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1. Digest your DNA sample with desired restriction enzyme. How much DNA you digest depends on how much of the total vector your fragment is. You'll need to do a diagnostic digest first and then scale up.
 2. Make a 0.7% low melting point (LMP) agarose gel. Pour it in the cold room.
 3. Run your sample in all 10 wells of the gel. Overnight at 10-30 volts is best, and you can run it at room temperature. If you need to run it at a higher voltage, it's best to do it in the cold room.
- ☆☆☆ Prepare 65°C water bath; put 100% EtOH in -20°C freezer; get dry ice. ☆☆☆
4. Photograph your gel before you cut out your fragment for later reference. Cut out your fragment over the UV source (use LOW power UV and wear goggles AND a face shield). Turn the fragment on its side to ensure you trim away as much excess gel as possible.
 5. Melt your fragment in a Falcon 2059 tube @ 65°C for 10'.
 6. Add 1% SDS to melted gel; use 70 µl SDS/ml of gel.
 7. Add an equal volume of phenol, vortex, then put at -20°C for 5'.
 8. Spin @ 8000 rpm for 10'. (You may want to transfer to Eppendorf tubes for this step)
 9. Extract 2X with equal volume of phenol:chloroform; do 10' spins @ 14000 rpm.
 10. Add 0.1 volume 3M NaOAc and 2 volumes 100% cold EtOH. Put on dry ice for 10'.
 11. Spin 10'.
 12. Wash 2X with 70% EtOH (2' spins), then airdry 10'.
 13. Resuspend in 30 µl TE.