ELUTION OF DNA FROM AGAROSE GELS - GLASSMILK METHOD

1. Excise DNA band from ethidium bromide stained agarose gel (run in TAE). Remember ethidium bromide is a mutagen-wear gloves, lab coat and safety glasses. When visualizing bands with UV light, wear protective goggles. Read the MSDS before using this material
2. Weigh gel slice in microfuge tube and add 200 ul/0.1 gm gel sodium iodide solution. Sodium Iodide is corrosive- although we use small amounts wear gloves and safety glasses. If you spill some on yourself, wash it off thouroughly. Read the MSDS before using this material.
3. Heat at 50-55C, 2-5 mins to dissolve agarose, heat longer if not completely dissolved, vortex to mix.
4. Add 2 ul Glassmilk suspension for 1-10 ug DNA (don't use less than 1 ul, and be sure to resuspend the glassmilk THOROUGHLY before using). Vortex + again after 2-3 min.
5. Place on ice 5 mins (or room temperature). (RT gives better recovery of DNA) note: for small DNA fragments (<500bp)heating the glass milk to 55 C improves yeild- see Biotechniques June 1995, p790.
6. Spin down 1 min in microfuge, aspirate off supernatant.
7. Resuspend pellet in 200 ul Nal solution and spin down again.
8. Aspirate off supernatant, add 200 ul NEW Wash, resuspend and spin down.
9. Repeat once more and remove all excess liquid.

ELUTION

- Add 10 ul TE, resuspend, heat 50C 5 mins, spin down, remove TE - save.
- Repeat once more.
- Spin DNA sample prior to use to remove last few glass grains.

All reagents are purchased from B10 101 under the name Geneclean. However, here are recipes for the Nal solution and the Wash.

- Nal solution: 90.8 gm Nal
- 1.5 gm Na2SO3 (sulphite!)
- dissolve in 100 ml water, filter through 0.45 um filter then add another 0.5 gm sodium sulphite and store at +4C.
- It is light sensitive so use dark bottles.

Wash solution:

- 50% ethanol in: (FLAMMABLE)
  - 0.1 M NaCl,
  - 10 mM Tris pH 7.5,

- 1 mM EDTA.

Store at -20C.