BISHOP LAB
GLASS MILK RECIPE
GEL PURIFICATION OF DNA WITH GLASS MILK
Adapted from Patterson Protocol (PNAS, 76:615, 1979)

GLASS MILK

Glass milk is made from silica 325 mesh, a powdered pottery glaze. Do not use mesh of other sizes, otherwise the glass milk will contain particles that are too large or too fine for efficient DNA purification.

This protocol requires a heating/stir plate that can be used in a fume hood.

1. In a 1L glass beaker, resuspend 400g glass powder in 800mL ddH_20
2. Stir for 1 hour
3. Turn off the stir plate and allow the slurry to settle for 90 minutes
4. Remove the supernatant, which contains the fine particles used for glass milk, and put into a large centrifuge bottle (250mL or similar)
5. Pellet the glass particles by spinning at 6000rpm for 10 minutes in a Sorvall centrifuge
6. Resuspend the pellet in 250mL ddH_20
7. In FUME HOOD, add concentrated nitric acid to 50%
8. Stir the solution gently and turn on the heat to "high"
9. Bring the temperature of the slurry almost to a boil, then turn off the heat
10. Continue stirring and allow the slurry to return to room temperature
11. Pellet the glass as in step 5
    Note: Solution is highly acidic! Be sure that the centrifuge bottles being used can handle the low pH of the slurry. If not, add a small volume of concentrated NaOH to bring the slurry to a pH that the centrifuge bottles can sustain.
12. Resuspend glass in 250mL ddH_20
13. Spin as in step 5
14. Continue washing glass particles and spinning as above until the pH of the slurry is neutral (if the pH continues to be very low after 5 washes, increase the washing volume to dilute the acid or add NaOH to neutralize the pH)
    Note: Even if the pH was neutralized in step 11, still do at least five washes to clean the glass thoroughly.
15. After slurry is neutralized, spin as in step 5
16. Resuspend glass pellet to make a 50% slurry based on volume
    Note: To determine the volume of the glass, add enough ddH_20 (~1mL) to get the glass into solution and then measure volume using a pipet
17. Make 1mL aliquots in microcentrifuge tubes
18. Store indefinitely at room temperature

GEL PURIFICATION USING GLASS MILK

1. Weigh the gel slice in a 1.5mL microcentrifuge tube
2. Add 3 volumes of NaI solution per gram of gel
3. Incubate at 45-55ºC to melt the gel slice (mix the contents of the tube occasionally until the entire slice is melted – approximately 5 minutes)
4. Vortex glass milk to resuspend
5. Add 1μL glass milk per 1μg DNA and mix the tube well
6. Incubate for 5 minutes at room temperature, mixing occasionally to keep the glass milk in suspension
7. Quick spin the tube in a microfuge to pellet the glass milk (approximately 5 seconds at full speed)
8. Wash pellet 3 times with 500μL wash solution, spinning after each wash step as in step 7
   Note: The glass pellet in the wash solution is more difficult to resuspend than in the NaI solution. If pellet is resistant to resuspension, swirl the pipet tip in the pellet while pipetting up and down to get the pellet into solution.
9. Dry the pellet at room temperature for 5-10 minutes, or for a few minutes at 55ºC, with the cap open
10. To elute the DNA, resuspend the glass pellet in an equal volume of TE or water
11. Spin tube for 30 seconds at top speed in a microfuge to pellet glass milk
12. Carefully remove supernatant with eluted DNA into a fresh tube, making sure to avoid transfer of glass pellet

General Notes:
Borate in TBE (and similar) buffers inhibits DNA binding to glass particles. If purifying DNA from TBE gels, weigh the gel slice and add 1/10 volume of 1M sodium phosphate, pH 6.5 and 1M mannitol to a final concentration of 0.1M. Continue with the protocol as above (include the volume of the sodium phosphate and mannitol in the volume of the gel slice when determining how much NaI solution to add). If using reagents from the GeneClean kit, weigh the TBE gel slice, then add 1/2 volume of TBE modifier and 4.5 volumes of NaI.

RECIPES

If using the GeneClean Kit reagents, NaI solution, NEW wash solution, and TBE modifier are supplied.

NaI Solution (Saturated)
6M NaI
120mM Na_2SO_3 (sodium sulFITE)

Filter sterilize
Store wrapped in foil at room temperature

Wash Solution
100mM NaCl
1mM EDTA
10mM Tris-HC1, pH 7.5
50% EtOH

Filter sterilize
Store at room temperature