Glassmilk Purification Kit

Making Glass Milk:

1. Take Borosilliacate Pasteur pipettes and break in a paper towel using a hammer.
2. Using a mortar and pistle grind the pipettes into a fine whilte powder under a fume hood. (This takes approximately 20 minutes).
3. Put ground glass into a 50 mL falcon tube and add 15 mL of sterile water and invert several times. Let sit for 5 minutes and take off supernatant and place in 15 mL falcon tube.
4. Set precipitated glass aside and let dry for use in making more glassmilk at a later date.
5. Take 15 ml slurry and aliquot 1.2 mL into 1.5 mL eppendorph tubes.
6. Spin down glass in 1.5 centrifuge tubes and wash with 1 mL of sterile water
7. Repeat step 6 four times.
8. After final wash resuspend glassmilk in 250 μL of sterile water.
9. Store in –20 C until needed. Thaw and resuspend by vortexing. Use 20 μL of slurry for glassmilk purifications.

Low-melt Gel Extraction of DNA Using Glassmilk:

1. Excise DNA band from low-melt agarose gel using a clean scapel and place into a 1.5 mL eppendorph tube.
2. Add 3 vols of 6M NaI solution to gel slice and place at 50 C for 10 minutes to dissolve low-melt agarose (I usually use 500 μL per slice).
3. Place at room temp for 4 minutes and add 20 μL of glassmilk slurry. Let incubate at room temperature for 10 minutes. Invert once or twice during incubation.
4. Spin down for 1 min at max speed and remove supernatant.
5. Add 1 mL of New Wash solution and resuspend glassmilk by pipetting then spin down for 1 min at max speed and remove supernatant.
6. Wash glassmilk 2 more times as above with 1 mL New Wash solution.
7. After last wash. Spin glassmilk down one more time and remove any residual New Wash Solution.
8. Add 7μL of sterile water and resuspend glassmilk and then place at 37 C for 2 minutes.
9. Spin down max speed 1 min and place supernatant into a new eppendorph tube (this elute contains DNA).
10. Repeat step 8 and 9 pooling both ellutants into the new eppendoroph tube.

Solutions:

6 M NaI binding solution:

NaI	90 grams
Sodium sulfite	1 gram
water	up to 100 mL

Filter through Whatmann filter paper and store in a opaque or brown bottle.

New Wash Solution:

50 % Ethanol
20 mM Tris-HCl (pH 7.6-8.0)
1 mM EDTA (pH 8.0)
100 mM NaCl