Psoralen=Guanidine DNA preparation

1. To15 mL meiotic sample (~6 x 10^8 cells) in orange cap tube add Na-azide to 0.1 %. Harvest cells, 4 mins 2.2 Krpm. Drain well.
2. Resuspend well in 1.5 mL psoralen solution. Pipette into petri dish (60mm x 15 mm, Corning 25010).
3. Place on long wave UV box and irradiate with 360nM UV light for 10 minutes. Swirl twice during this time.
4. Pipette cells back into tube, rinse dish with 1 mL 50mM EDTA 50mM Tris pH8.0. Harvest cell as above. Spin again briefly and pull off last of supernatant. Freeze pellet at –20oC.
5. Resuspend cells well in 0.5 mL spheroplasting buffer plus β-mercaptoethanol and 0.25 mg 100-T zymolyase. Incubate at 37oC for 15 mins. Swirl twice during incubation.
6. Harvest spheroplasts 4 mins, 3 Krpm. Carefully pull off SN. Repeat spin if necessary.
7. Add 1.5 mL Guanidine solution and resuspend stringy pellet by finger-vortexing. Place at 65oC for 20 minutes; finger vortex several times during this incubation to completely lyse and resuspend.
8. Cool tubes on ice. Add 1.5 mL EtoH., mix well by inversion and store at –20oC overnight.
9. Pellet for 15 minutes at 3.2 to 4 Krpm in benchtop or Beckman. Drain well. Respin briefly and pull off last traces of SN.
10. Add 0.5 mL RNase solution. Stir with blue tip to break up pellet but DO NOT vortex. Incubate at 50oC for 15 minutes. Finger vortex to break up pellet. Shift to 37oC and incubate for 1 hr.

11. Add 15 µL proteinase-K and incubate at 65oC for 1 hr. Can freeze overnight at this stage.
12. Extract with 0.5 mL phenol/chloroform: shake and invert well, let stand for 3 minutes, shake again and spin at full speed for 10 minutes. Carefully remove SN.
13. Repeat phenol/chloroform extraction if necessary (I usually do!)
14. Ethanol precipitate. I usually add only 1/20th volume NaOAc to avoid salt precipitating, and 2 volumes ethanol. Invert well and let stand for 20 minutes. Spin for 5 minutes at full speed.
15. Pour off SN, rinse in 70% ethanol, repeat spin, drain on spikey rack. Pulse spin and remove traces of ethanol.
16. Air dry for 10 minutes and resuspend in 25-100 µL TE. Resuspend in fridge overnight. Flick tube to mix and store at -20 oC.

Solutions

5 X Psoralen stock is: 0.5mg/ml Trioxalen (SIGMA T-6137) in EtOH (200 proof). Store in dark at 4oC. Dissolve overnight before use by shaking at room temperature.
Psoralen working stock is: 1 X psoralen stock, 50mM Tris-Cl pH8, 50mM EDTA.
Keep on ice, wrapped in aluminum foil.

Spheroplasting buffer:

36.43 g Sorbitol (1M)

10 ml 1 M KPO_4 buffer pH 7.0 (50mM)

4 ml 0.5 M EDTA pH 7.5 (10 mM)

H_20 to 200 ml

Filter sterilize. Store @ 4°C. Add 1/100th volume β-mercaptoethanol to desire volume immediately prior to use.

Guanidine solution:

21.5 g Guanidine-HCl (4.5 M, saturated)

10 mL of 0.5M EDTA (0.1 M)

0.44 g NaCl (0.15 M)

0.25 mL of 10% sarkosyl (0.05%)

Make up to 50 mL and adjust to pH 8.0 with 100-150 µL 50% NaOH.

RNase solution:

10 x TE pH 8.0 plus 50 µg/mL RNase

Proteinase-K Solution:

20mg/mL, in 20mM $CaCl_2$ 10mM Tris-HCl pH7.5 50% glycerol.

Weigh 20 mg Proteinase-K into an ependorf tube. Add 20 µL IM $CaCl_{2,}$ 10 µL 1M Tris-HCl pH7.5, 470 µL dH_2O and 0.5 mL 100% glycerol.

Store at -20^{0}C. Mix before use.