Transformation of S. cerevisiae

Steve Biggar
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Crabtree Lab 723 7671

Solutions and Reagents
1. YPD for liquid culture.
   2% Bacto-peptone
   1% Bacto-yeast extract
   2% Dextrose
   Distilled water to 1 liter., Autoclave
2. Selective media nutrient plates
3. Salmon Sperm DNA (Sigma) 10 mg/ml
   Resuspend at 10 mg/ml in distilled H2O. Shear by passage through a 21 gauge needle, denature by boiling.
4. PLATE Solution (must be made and used within 3 months)
   40% PEG 3350
   100 mM LiOAc pH 7.5
   10 mM Tris, 1 mM EDTA pH 7.5
   Prepare 80 ml of 50% PEG (w/v) in distilled H2O.(autoclave to sterile)
   Add 10 ml of sterile filtered 1 M LiOAc pH 7.5
   Add 10 ml of sterile filtered EDTA. (100 mM Tris 10 mM EDTA, pH 7.5)
5. Lithium Sorbitol
   100 mM LiOAc pH 7.5
   10 mM Tris, 1 mM EDTA pH 7.5
   1 M Sorbitol
   Prepare by adding 18.22 gm of Sorbitol to 80 ml Distilled water. Autoclave
   Add 10 ml of 10 sterile filtered 1 M LiOAc pH 7.5
   Add 10 ml of sterile filtered 100 mM Tris, 10 mM EDTA pH 7.5
6. Plasmid DNA, about 2 µg per transformation
7. 100 mM Tris, 10 mM EDTA pH 7.5 (Prepare then sterilize by filtration)
8. 1 M LiOAc pH 7.5 (Prepare then sterilize by filtration)

Procedure
1. Grow overnight culture in YPD
2. Dilute to an O.D. of about 0.1 in YPD (5 ml per transformation)
3. Grow at 30˚C to O.D. 600 of 0.4 to 1.0
4. Spin cells for 3 minutes at 3000 rpm.
5. Aspirate media and resuspend in LiSorb (50 µl/5ml culture)
6. Add the 50 microliters of cells to the plasmid DNA which has been premixed with 10 microliters salmon sperm DNA (100µg). This can be done in an eppendorf tube. Flick the tube to mix the DNA and cells.
7. Add 0.5 ml PLATE Solution, invert to mix
8. Incubate at room temperature for 30 minutes to overnight. There is great variation in the time required here. Three hours appears to work well for most strains including YDF6.
9. Incubate at 42˚C for 10 minutes.
10. Plate 50 µl and 350 µl on selective plates to select transformants.
11. Incubate plates at 30˚C for 3 to 4 days. Colonies will generally appear at about 36 to 48 hours.