Schwer Lab

Chemically competent DH5α bacteria

The day before: Rinse two 1-L Erlenmeyer flasks thoroughly with DI water, then autoclave. Streak *E. coli* DH5α strain directly from frozen stock onto NON-selective 2X LB agar plate and Carbenicillin or Ampicillin plate (control); incubate streaked plates and one unstreaked NON-selective 2X LB plate (control for contamination) at 37°C for 16 h (3 plates total).

Autoclave microcentrifuge tubes and multistep-pipette tips (for 200 μl). Prepare TfbI and TfbII. Store solutions and tubes in 4°C cold room O/N.

0.1 M MOPS, pH 7.0
2.09 g MOPS in 80 mL water, pH with NaOH to pH 7.0 (DO NOT overshoot pH!).
Make volume up to 100 mL.

TfBI *[30mM CH_3COOK (K-acetate, pH 7), 50 mM $MnCl_2$, 100 mM KCl, 10 mM $CaCl_2$, 15% glycerol]*
1.5 mL 1 M KOAc
2.5 mL 1 M $MnCl_2$
5 mL 1 M KCl
0.5 mL 1 M $CaCl_2$
7.5 g glycerol
pH to 7.0 with glacial acetic acid
Add water to 50 mL. Filter-sterlize (0.2-μm) in tissue culture hood. Cool to 4°C O/N.

TfBII *[10mM MOPS (pH 7 with NaOH), 10mM KCl, 75mM $CaCl_2$, 15% glycerol]*
5 mL 0.1 M MOPS
3.75 mL 1 M $CaCl_2$
0.5 mL 1 M KCl
7.5 g glycerol
pH to 7.0 with NaOH
Add water to 50 mL. Filter-sterilize in tissue culture hood. Cool to 4°C O/N.

TYM
20 g Tryptone
5 g Yeast Extract
6.8 g NaCl
Bring to 1 L with DI water, autoclave. Add 5 mL of filter-sterilized 1 M MgSO4 to each 500 mL when solution is below 50°C.

The day of: place six 50-mL conical tubes, sterile multistep pipette tips, and serological pipets (six 25-mL, three 10-mL) on ice.

1. Transfer five well-isolated colonies to 1 mL of TYM (unopened bottle) in sterile 15-mL tube. Vortex at moderate speed; pipette 1 mL culture into 100 mL of TYM in each of two sterile 1-L flasks.

2. Grow cells for ~3.5 h at 37°C (220-250 RPM). OD600 should be ~0.6 (1 OD600 = ~1 billion cells/mL).

3. Near open flame: transfer cells to sterile, ice-cold 50-mL polypropylene tubes. Cool cultures in ice-water slurry for 10 min.

4. Centrifuge at 2700 x g for 10 min at 4°C (swing-out benchtop centrifuge set to 2°C) to pellet cells.

5. Decant the medium; invert tubes for 1 min to drain medium onto paper towels.

6. Resuspend pellets in 20 mL (for each 100-mL culture) of ice-cold TfBI by swirling and VERY GENTLE tapping (like slowly dragging across a tube rack); can also pipette GENTLY with 25-mL pipette; Pool into one tube.

7. Incubate in ice-water slurry for 15-25 min.

8. Recover cells by centrifugation at 2700 x g for 10 min at 4°C (MUST be 4°C).

9. Pour off buffer; invert tubes for 1 min to drain medium from cell pellet.

10. Resuspend pellet by swirling and VERY GENTLY pipetting in 8 mL of ice-cold TfBII.

11. Incubate in ice-water slurry for 5 min.

12. Aliquot 200 µL into ice-cold sterile microcentrifuge tubes that are placed into cardboard box with holes in bottom on wet ice.

13. After aliquoting all, immerse box in dry ice with 95% ethanol for snap freezing. Remove box and place on dry ice to get rid of excess ethanol. Store box at -80°C.