DYS SEE

Chemically Competent Cells.

iGEM Thessaly

Protocols

Chemically Competent Cells (E. coli)

1. Grow primary culture (pre-culture) of desired strain the day before in 5ml LB.
2. Inoculate 100-200ml LB with 1-4ml primary culture (be sure to keep a fre ml of the LB batch to use as a blank in the spectrometry later on)
3. Grow at 37 degrees Celsius 210 RPM for approximately 3h, or until OD_{600}=0.3-0.6
4. Split culture into 50ml falcon tubes or suitable centrifuge tubes of desired volume. Spin at 4000g for 15-20 min at 4 degrees Celsius. Poor off supernatant
5. GENTLY resuspend in 25-30 ml sterile, ice-cold $MgCl_2$ 100mM (on ice or in the cold-room)
6. Spin down, same conditions as 4. for 10 min
7. Remove supernatant, resuspend in 40-50 mL sterile, ice-cold $CaCl_2$ 100mM (note: It's easier to resuspend in ~5mL by gentle pipetting and then adding the rest of the volume)
8. Leave on ice for 20-45 mins (the longer the better)
9. Repeat spin step as in 6., poor off supernatant (remove entirely with pipette)
10. Redissolve in 1-3ml resuspension buffer 15% glycerol, 85mM $CaCl_2$ (note this resuspension should milky white, you don't want it to be too concentrated or too diluted. Aliquot 50-100µL of this into 1.5 ml Eppendorf tubes
11. Flash freeze in liquid nitrogen and store at -80 degrees Celsius for ≤6 months

- After 4., all manipulations on ice or in cold room
- Resuspension should be very gentle, light swirling VERY gentle pipetting
- Test CCs for competence viability, try redo with known resistance and also non-transformed cells on plain media to see if they grow.

Protocols

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