Competent cells (DH5α and BL21).v1

This protocol is modified based on NAR 16(8) 1988 page 3580

Procedures

Preparation

1. Grow an overnight culture of DH5α or BL21 bacteria on an LB plate (WITHOUT antibiotics).
2. Inoculate the bacteria into 3-ml LB medium in a 13-ml test tube and shake at 250 rpm at 37°C O/N.
3. Pipette 2 ml of the overnight culture into 500 ml of LB in a 2-liter flask. Shake at 220 rpm at 37°C to allow to grow to 0.3-0.6 at OD600. It takes about 3 hours. Need to check periodically, especially when the OD reading is getting close to 0.3-0.6 (the exponential growth period).
4. Spin the culture at 1,000 x g for 10 min at 4°C.
5. Resuspend the pellet gently in 50 ml of ice-cold TSB.
6. Incubate on ice for 10 min
7. Aliquot in 1.5-ml Eppendorf tubes, quick freeze in an ethanol-dry ice bath and store at -80°C.

Transformation

1. Thaw the bacteria on ice. Transfer 100 µl to each 13-ml tube on ice.
2. Mix with 100 pg of plasmid DNA or 2 µl of ligation mixture.
3. Incubate on ice for 20 min. Heat shock for 45 seconds at 43°C. Put on ice for 2 min.
4. Add 0.9 ml of TSBG and shake at 37°C for 1 h.
5. Plate the cells on plate with appropriate antibiotics.

Reagents and Solutions

TSB (500 ml)

LB broth (pH 6.1)	465 ml
PEG (MW 3500)	50 g (10%)
1 M MgCl2	5 ml (10 mM)
1 M MgSO4	5 ml (10 mM)

Autoclave and add 25 ml DMSO to a final concentration of 5% and store at 4°C.

TSBG (50 ml)

TSB	50 ml
40% (2.22 M) Glucose	450 µl

Store at 4°C.