DSMO-Competent E. coli cells

Modified by Gunther Stier from a protocol described in H. Inoue, H. Nojima, and H. Okayama. "High efficiency transformation of Escherichia coli with plasmids" Gene 96 (1):23-28, 1990.

Procedure

1. Grow a 5ml inoculus o/n in LB at 37°C
2. Next day innoculate 100ml SOB with the o/n inoculus and grow it to OD600=0.6 at 30°C in a 500ml or larger flask
3. Chill cells 10-20 minutes on ice
4. Spin cells down at 4°C (10-15min at 4500x g)
5. Gently resuspend in 25ml TB and incubate 10 minutes on ice
6. Spin gently at 4°C (15min at 3500 x g)
7. Resuspend in 4.65ml TB and add 0.35ml DMSO
8. Aliquot as 50-100µl and shock-freeze.

Needed

Solutions:

SOB

1. LB + 10mM $MgSO_4$ + 10mM $MgCl_2$

10x TB

1. 2.38g HEPES + 18.6g KCl + 15ml 1M $CaCl_2$
2. adjust pH to 6.7 with KOH and (only then) add 10.88g $MnCl_2\ 4H_2O$
3. Add water to 100ml
4. Filter sterile.

Bookings

1. shaker 1 day at 30°C
2. centrifuge

Sterilize one day before

1. centrifuge tubes (don't forget to open the lid!!)
2. Eppendorf tubes