

Preparation of electrocompetent E. coli

Hahn Lab, 2007

Use strain DH10b from Invitrogen (DH10B™-T1R: F- mcrA delta(mrr-hsdRMS-mcrBC) delta 80lacZ_M15 delta lacX74 deoR recA1 endA1 ara delta139 delta(ara, leu)7697 galU galK delta- rpsL(StrR) nupG tonA)

Streak strain on SOB - Mg plate from frozen stock (no antibiotic). Use a single colony to inoculate a 20 ml overnight culture in SOB -Mg media at 37 deg.

Inoculate 5 ml overnight culture per 600 ml prewarmed SOB -Mg media. Usually grow 3 x 600 ml for electrocompetent cell preparation. Grow at 37 deg with shaking for ~2 hr 50 min till A600 ~ 0.75.

Harvest cells by centrifugation in 1 liter bottles at 4000 rpm for 20 min. Drain supernatant well. Keep cells cold from this point on by keeping everything on ice.

Resuspend cell pellet in a volume of cold 10% glycerol equal to the original culture volume (i.e., 3 x 600 ml). Transfer to 500 ml centrifuge bottles and spin in GS3 rotor at 7000 rpm for 10 min (5,200 xg).

(Note: centrifugation in 1 liter bottles at this step will not be hard enough to fully pellet cells and you will lose a significant amount of cells.)

Remove supernatant well and Resuspend pellet by vortexing in 10% cold glycerol equal to the original culture volume and centrifuge as above. Remove supernatant well.

Resuspend cell pellet in residual liquid in the centrifuge bottles by vortex. Expect about 2.5 ml cell suspension from 600 ml culture.

Aliquot cells to 1.5 ml eppendorf tubes, quick freeze on dry ice and store frozen at -75 deg. or colder.

Rumor has it that for best storage of this strain, grow fresh overnight in SOB +Mg media. Add glycerol to 20% final and store in a strain storage vial at -75 deg.

Rumor also has it that cells are more competent when grown on SOB - Mg for preparation of competent cells.

SOB-Mg Medium

30 gm Bacto-tryptone
7.5 gm Bacto yeast extract
0.87 gm NaCl
0.285 gm KCl
dH₂O to 1.5 liters

SOB-Mg Agar

100 ml SOB-Mg
1.5 gm agar

Mix in 250 ml flask, autoclave. Pour 4 plates.

*Used in streaking the -80°C frozen stock

SOB Medium

100 ml SOB-Mg
1 ml 2M MgCl₂-MgSO₄

Mix in 100 ml bottle, autoclave.

*Used for preparation of frozen stock.