## ***E. coli* competent cells and transformation** (Inoue et al. 1990 Gene 96, 23-28)

**How to prepare TB buffer?**

1. Prepare 10 mM Hepes (or Pipes)/15 mM $CaCl_2$/250 mM KCl
2. Adjust pH to 6.7 with KOH.
3. Dissolve $MnCl_2$ to have final concentration of 55 mM in TB buffer.
4. Sterilize solution by filtration through 45 µm filter and store at +4$^0$C.

Note: All salts should be added as solids.

**How to prepare competent *E. coli* (*e.g.* DH5) cells?**

1. Inoculate 250 ml of SOB medium in a 2 liter flask and grow to $OD_{600}$ =0.6 at 18$^0$C (or at room temperature) with vigorous shaking (200-250 rpm).
2. Put flask on ice for 10 min.
3. Spin down cells at 2,500 x g (*i.e.* 3K rpm Beckman J-6B centrifuge) for 10 min at +4$^0$C.
4. Resuspend pellet in 80 ml of ice-cold TB and incubate for 10 min, then spin down cells.
5. Resuspend pellet gently in 20 ml of TB and add DMSO to final concentration of 7% with gentle swirling.
6. Incubate on ice for 10 min.
7. Dispense cell suspension in 0.5 ml aliquots and immediately flash-freeze by immersion in liquid $N_2$.
8. Store frozen cells at -70/80$^0$C.

**How to perform a transformation?**

1. Thaw competent cells on ice.
2. Dispense 100 µl into 1.5 ml microcentrifuge tubes.
3. Add 0.5-5 µl of plasmid DNA in each tube and incubate on ice for 30 minutes.
4. Heat shock cells without agitation at 42°C for 90 seconds and transfer to ice bath.
5. Add 1 ml of SOC medium (or LB+) and shake vigorously at 37°C for 1 hour (tape tubes to rotating wheel).
6. Plate cells onto LB+Amp plates (50 µg/ml Ampicillin) and incubate them at 37°C over night.

SOB
0.5 % yeast extract
2 % tryptone
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$

SOC
0.5 % yeast extract
2 % tryptone
10 mM NaCl
2.5 mM KCl
10 mM $MgCl_2$
10 mM $MgSO_4$
20 mM glucose

LB+
5 ml LB
50 µl 40% glucose
100 µl 1M $MgCl_2$

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