Preparation of Chemically Competent Cells

TBF I Buffer (pH 5.8)*
30 mM KOAc
100 mM RbCl
50 mM $MnCl_2$
10 mM $CaCl_2$
→ sterile filtered

TBF II Buffer (pH 7.0)
10 mM MOPS
10 mM RbCl
75 mM $CaCl_2$
15 % Glycerol
→ sterile filtered

STERILE WORKING TECHNIQUES REQUIRED THROUGHOUT!!!

- 1 colony of *E. coli* into 4 ml LB medium (+ appropriate antibiotics)
  → 37°C overnight
- 500 µl pre-culture into 200 ml LB medium (+ appropriate antibiotics)
  → 37°C to OD_{600} = 0.45-0.55

FROM NOW ON WORK ON ICE OR AT 4°C!!!

- collect culture in 4x50mL sterile conical centrifugation tubes (Falcon™ tubes)
- centrifuge at 3000 rpm for 10 min, discard supernatant
- gently (!) resuspend each pellet in 1 ml chilled TBF I and combine in one tube
- fill up to 15mL with TBF I and incubate on ice for 60 min
- centrifuge at 3000 rpm for 10 min, discard supernatant
- resuspend pellet in 4 ml ($^1/_{50}$ of original culture volume) TBF II
- aliquot competent cells (50 or 100 µL) and freeze immediately in liquid nitrogen
- store at -70°C

* To avoid precipitation of MnO_2 (brown colored solution or precipitate), dissolve everything except $MnCl_2$ in $^3/_4$ of the volume, abjust pH to 6.0 with acetic acid, add $MnCl_2$ (dissolved in water), fill up with water nearly to the required total volume and then further adjust the pH to 5.8 if required. Avoid adding KOH (or any base) to a solution containing a Mn^{2+} salt!!!