Preparation of chemically competent cells (E.coli k-12):

Materials and solutions:

- LB medium
- Glycerol stock of E.coli k-12
- LB-agar plate
- PEG
- DMSO
- $MgCl_2$

Equipment:

- Rotating incubator
- Spectrophotometer
- Ice bucket
- Centrifuge
- Liquid nitrogen container

Procedure:

- Streak E.coli k-12 over an LB-agar plate and incubate overnight at 37°C.
- Grow starter: pick a single colony and transfer it into 2ml LB in a 14ml falcon tube.
- Grown in an incubator at 37°C and 200rpm overnight.
- Dilute the bacteria to $OD600_{nm}$=0.1 with LB in a small Erlenmeyer.
- Grow the bacteria up to $OD600_{nm}$ =0.3-0.6 OD.

- Transfer the culture into a 50ml tube and incubate on ice for 30 min.
- Prepare TSS (see below).
- Centrifuge at 4,500 rpm and 4°C, for 5 min.
- Put the tubes on ice.
- Discard the supernatant.
- Resuspend the pellet with TSS (1/10 of the starting volume).
- Incubate the tube for at least 15min on ice (time for bringing liquid nitrogen).
- Dispense with pipet 50 µl into 1.5 ml sterile tubes.
- Throw the tubes immediately to liquid nitrogen.
- Put all the tubes in a box and store it at -80◦.

TSS for Chemo competent (5ml): work sterile

Mix the following components in a 15ml tube:

- 0.5 gr PEG (10%)
- 250 µl DMSO (5%)
- 250µl $MgCl_2$ -1M
- LB to make 5ml (always use unopened media for TSS)
- Filter the solution and keep it in 4 C for 1 week