Procedure

1. Start an o/n culture, by inoculating a single colony in 3ml LB

   In the cold room store the following:

   - MiliQ water STERILE, 2x0.5l
   - Glycerol 10%
   - 20ml STERILE pipette
   - 5ml CombiTips for the Eppendorf Multistepper
   - Eppendorf tubes, 0.5 or 1,5 ml, STERILE

2. In the morning dilute the preculture 1/100 (v/v) in 500ml of fresh LB-<antibiotic> in a 2-3l flask
3. Incubate till OD600 = 0,7
4. Pellet the cells (?4500x g, 15')
5. Wash in 500 ml MilliQ sterile cold
6. Pellet
7. Wash in 200 ml MilliQ sterile cold
8. Pellet
9. Wash with 20 ml 10% (v/v) glycerol
10. Pellet
11. Resuspend in 2 ml 10% glycerol
12. Aliquot as 50μl; snap-freeze in liq. N2 and store at -80°C
13. Test competence: use 2μl of DNA (from a maxi-prep) to transform 50μl of bacteria. Plate less than 1μl of bacteria (dilute after the incubation time)

Needed

1. 1 Liter MiliQ water, sterile
2. 10% or 50% sterile glycerol
3. sterile pipette tips, Eppendorf tubes, centrifuge bottles (everything autoclaved, all the bottles with cap slightly opened)
4. 2 Liter [baffled] flask
5. 50ml Falcon tubes, 20ml pipettes, both sterile
6. 500ml LB-<antibiotic> (the antibiotic is needed only for some special strains