Sterilize all materials before use.
Only open your flasks, tubes or plates in a flow bench.

Nr.	Hoe
1.	Prepare an overnight 5 ml starter culture of the cells in the prescribed medium[1].
2.	Incubate the culture during 16-24h in the shaker at the required cultivation temperature. This incubation period is standard for the most currently used *E. Coli* strains. If necessary this period can be changed for other strains or specific *E. Coli* strains.
3.	Dilute the saturated culture 1/100 in 20 ml of the prescribed medium[1].
4.	Incubate the cells at the prescribed cultivation temperature[2] until the OD_{600} lies between 0,2 and 0,3. Do not let the OD get any higher than 0.4. The OD should be carefully monitored and checked often, especially when it gets above 0.2, as the cells grow exponentially. It usually takes about 3 hours to reach an OD of 0.35 when using a 1/100 diluted starter culture.
5.	Keep the culture on ice for at least 10 minutes. It is also very important to keep the cells at 4°C or on ice for the remainder of the procedure. Any bottles, tubes or solutions used after this point, must be pre-chilled to 4°C.
6.	Transfer the culture into cooled Falcon-tubes and centrifuge them for 5 minutes at 4 °C and 5000 rpm.
7.	Remove the supernatant and wash the cells with 10 ml of cold 0,1M $MgCl_2$.
8.	Centrifuge for 5 minutes at 4 °C and 5000 rpm.
9.	Remove the supernatant and wash the cells with 10 ml of cold 0,1 M $CaCl_2$. Keep the cells on ice for 30 minutes.
10.	Centrifuge for 5 minutes at 4 °C and 5000 rpm.
11.	Remove the supernatant and add 2 ml of cold 0,1 M $CaCl_2$ to the pellet. Keep the cells on ice for 30 minutes.
12.	Per tube containing 2 ml 0,1 M $CaCl_2$ + pellet, 0,3 ml of cold glycerol is needed. Pool the content of the Falcon-tubes and add it to the correct amount of glycerol (on ice). Divide the cell mixture in sterile pre-chilled tubes (100 µl per tube).
13.	Freeze the tubes through snap freezing by immersing the tubes into liquid nitrogen. This increases the transformation efficiency.
14.	Store the tubes at -80 °C.

[1] Use one of the rich media mentioned below to increase the transformation efficiency later on.

SOB broth:
- Tryptone 20 g
- Yeast extract 5 g
- NaCl 0.5 g
- Purified water 950 ml
- KCl 250 mM 10 ml
- Set to pH 7.0 and add purified water to a total volume of 1 liter
- Sterilize
- Add 5 ml of a sterile 2 M $MgCl_2$ solution

SOC broth:
- Prepare 1 liter SOB broth (including MgCl2 solution)
- Add 20 ml of a sterile 1 M glucose solution

[2] Incubation at a lower temperature (18 °C to 20 °C for E. coli) up to an OD_{600} of 0,45 can increase the transformation efficiency later on.