

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

Nr.	Hoe
1.	Thaw the competent bacterial host cells on ice.
2.	Add – if the concentration is known – 100 ng plasmid DNA to 100 µl of competent bacteria (= 1 tube). If the concentration is not known, add 1 µl.
3.	Put the mixture on ice for 15 minutes. Keeping the mixture on ice for 30 minutes may increase transformation efficiency of <i>E. coli</i> strains.
4.	Give the mixture a temperature shock by placing it in the thermomixer for 1 minute at 42°C.  For phage-containing host strains (e.g. <i>E. coli</i> MC1061λ) incubate 5 minutes at 28 °C; for <i>E. coli</i> BL21 incubate 45 seconds at 42°C.
5.	Put the mixture on ice for 5 minutes.
6.	Add 900 µl of sterile prescribed non-selective medium to the mixture. The medium can have an influence on the transformation efficiency of <i>E. coli</i> strains. If necessary use SOB (Super Optimal Broth) or SOC (SOB + glucose) <sup>1</sup> .
7.	Keep the mixture at the prescribed cultivation temperature for 45 minutes. The time can have an influence on the transformation efficiency of <i>E. coli</i> strains. If necessary keep the mixture at the prescribed temperature for 60 minutes.
8.	A 4x4 dilution ent is made on a plate with the prescribed selective medium.
9.	The plates are incubated for 16-24h at the prescribed cultivation temperature: <ul style="list-style-type: none"> <li>• aerobic strains: in an incubator;</li> <li>• facultative anaerobic strains: in an airtight closed jar in an incubator. Place an O<sub>2</sub> absorbing sachet in the jar.</li> </ul> This incubation time is standard for the frequently used <i>E. coli</i> strains; if necessary the time can be adjusted for specific <i>E. coli</i> strains and other strains.

- <sup>1</sup> 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<sub>2</sub> solution
- SOC broth: Prepare 1 liter SOB broth (including MgCl<sub>2</sub> solution)  
Add 20 ml of a sterile 1 M glucose solution