Transformation of *E. coli* with plasmid DNA

Grow *E. coli* culture overnight (18-19 hours). Dilute fresh culture 20 times and incubate in shaker for 2 hours to the OD=0.3 – 0.4 (for the cotransformation use LB with the antibiotics).

1. Take 2 ml of culture to the centrifuge tube.
2. Keep on ice for 10 minutes.
3. Centrifuge for 3 min at 6 500 g at +4ºC. Discard supernatant.
4. Resuspend the cell pellet in 0.8 ml of cold 0.1 M $CaCl_2$.
5. Keep the cells on ice for 15 min.
6. Centrifuge for 2 min at 6 500 g at +4ºC. Discard supernatant.
7. Resuspend the cell pellet in 100 µL of 0.1 M $CaCl_2$.
8. Keep the tubes on ice for 4-5 min.
9. Add 10 µL (50ng) of plasmid DNA to the cells.
10. Keep on ice for 15 min.
11. Heat shock (put the tubes in water bath heated to 42 ºC for 2 min).
12. Immediately put the tubes on ice and keep for 30 seconds.
13. Add 1 ml of LB to the cells.
14. Incubate at 37 ºC for 1-2 hours (more than 1 hours for the cells, resistant to 2 or more antibiotics).
15. Plant the cells on plates with LB and corresponding antibiotics.