E. coli plasmid Transformation

Before starting: Book heating block @42°C

Get competent cells from -80°C freezer

1. Thaw cells on ice
2. Pipette 2 µL of solution, containing plasmid, into the cell stocks. Mix gently by pipetting up and down.
3. Leave the tubes on ice for 30 min.
   - **OBS!** Don’t mix/shake the tubes after, since that will disperse the DNA again.
   - **OBS!** Put rack in fridge to prevent the ice from melting.
4. Put the tubes in the heating block @42°C for 90 s.
   - **OBS!** If you have many tubes, be sure to remove them from the heating block in the same order that you put them in.
5. Take the tubes out of the heating block and put them on ice.
6. Pipette 500 µL LB-medium into each tube.
   - **OBS!** Use a sterile fume hood. Make sure to wipe the floor of the hood with ethanol and do the same for everything you put in the hood (like pipette, LB-solution and your hands).
   - **OBS!** Pipette carefully, to avoid splash.
7. Incubate @37°C for 1h.
   - **TIP:** Take the plates, out of the fridge, and put them in 30°C to remove moisture, making it easier to write on them.
8. Transfer 10% (50-100 µL) of the solution to the plastes marked 10% (LB/Amp plates)
   - **OBS!** Use sterile technique.
9. Spin down the solution into a pellet.
10. Resuspend the pellet in ~100 µL MQ.
11. Plate the resuspended cells on the plates marked 90% (LB/Amp plates)
12. Incubate the plates @37°C for 16h (overnight).