

E.coli transformation

- thaw chemically competent *E.coli* (strain DH10b) on ice.
- pipette 100 μL of the *E.coli* cells into a 1,5 mL tube.
- add 10 μL of the Golden Gate assembly (always transform the negative control too).
- put the mix 10 minutes on ice.
- transfer the tube into a thermoblock: 42°C for 90 seconds.
- recover cells on ice for 2 minutes.
- add 900 μL LB liquid medium without antibiotics.
- transfer the tube into a thermomixer: 37°C for 1h and 700 rpm.
- pipette 1x 50 μL and 1x 10 μL of your sample onto a LB-Agar plate containing the desired antibiotic.
- take a spatula and distribute the sample (don't make a smear, you want to get single colonies in the end)
- incubate the plates over night at 37°C
- the next day compare the plate containing the cells with the plasmid with the negative control: you should count way more cells to know that the transformation was successful.