Bacterial transformation

E. coli

Materials and solutions:

- LB medium
- Competent E. coli k-12
- LB-agar plate with antibiotics

Equipment:

- Heating block
- Eppendorf shaker + temp. control
- Ice bucket
- Centrifuge

Procedure:

- Thaw competent cells on ice for 10 min.
- For 50ul competent cells add 50-100ng of the plasmid.
- Flick the tube to mix and incubate on ice for 20min.
- Heat shock the bacteria at 42°C for 45sec.
- Immediately put back on the ice for 2min.
- Add 1ml LB and incubate at 37°C while rotating for 1hr.
- Centrifuge for 2min at 4500 rpm.
- Discard the supernatant (~900 µl) and gently re-suspend the pellet with the remnant (~100 µl).
- Spread bacteria over LB-agar + antibiotics plates by using a cells spreader.
- Incubate the plate overnight at 37°C.

B. subtilis

Materials and solutions:

- LB medium
- B. subtilis PY79
- MCX10 solution
- LB-agar plate with and without antibiotics
- 1M $MgSO_2$

Equipment:

- Rotating incubator
- Centrifuge

Procedure:

- Prepare MCX10 stock solution as follows:

Reagent	Amount
$K_2HPO_4x3H_2O$	14.036gr
KH_2PO_4	5.239gr
Glucose	20gr
Trisodium citrate	10ml 300mM
Ferric ammonium citrate (X1000)	1ml of 22mg/ml solution
Casein Hydrolysate	1gr
Potassium Glutamate	2gr

- Mix with autoclaved DDW and bring to a final volume of 100ml and filter.

- Dispense to 1ml aliquots and store at -20°C.

***Perform all bench steps under a flame**

- Streak B. subtilis (from glycerol stock) over an LB plate lacking antibiotics, and grow overnight at 37°C.
- Pick a single colony from the plate with a pipette tip and transfer it into a 14ml falcon tube containing the following:
  * 900µl sterile DDW
  * 100µl X10MC
  * 10µl 1M $MgSO_2$
- Mix by vortex
- Incubate at 37°C and shake at 200rpm for 3.5hrs.
- Dispense sample to sterile tubes each containing 300µl of the bacterial suspension.
- Add to the tube 300ng plasmid.

*** One tube should contain no DNA for negative control.**

- Incubate again at 37°C and shake at 200rpm for 3hrs.
- Meanwhile, warm LB-agar + antibiotics plates in the incubator.
- Spread ~150 µl of bacterial suspension over the plate and incubate overnight 37°C.