Bacterial Transformation with Plasmids

Aim:

- Introducing plasmids into competent bacterial cells, such as *E. coli* DH5α or BL21

Timeframe:

- Preparation: 15 minutes
- Wait-time: 30 min, 60 min and overnight incubation
- Overall: 1 day

Materials:

- Agar plates with appropriate antibiotic
- Liquid DNA aliquot of your plasmid of interest
- Competent *E. coli* cells (stored in the -80°C)
- Pipette tips of appropriate volumes (1 ul, 10 ul, 100 ul)
- Water bath
- Shaking incubator
- SOC media
- LB media with 1mM MgSO4

Procedure:

1. Turn on 42°C water bath and 37°C shaking incubator.
2. Fill an ice box.
3. Thaw competent cells on ice for 10 min (dispose of unused competent cells, do not refreeze unused thawed cells).
4. In a separate labelled 1.5 ml eppendorf tube for each plasmid, combine the following:
    - 50 µl of competent cells
    - 1 µl of the plasmid or ligation reaction
5. Incubate on ice for 30 mins.
6. Heat shock tubes at 42°C for 30 seconds in a waterbath.
7. Incubate on ice for 1 min.
8. Pipette 950 µl SOC media (or LB media with sterile 1 mM MgSO4) to each transformation.
9. Incubate at 37°C for 1 hour, shaking at 200-300 rpm.
10. Pipette 100 µl of each transformation onto petri plates, spread with sterilised spreader.
11. Spin down cells at 6800g for 3 mins and discard 800 µL of the supernatant. Resuspend the cells in the remaining 100 µL, and pipette each transformation onto petri plates.
12. Incubate petri plates overnight (14-18 hours) at 37°C.