E. coli heat-shock competent cells preparation

Material:

- *E. coli* strain
- LB medium
- 0.1M $CaCl_2$ solution (ice cold)
- LB plates with proper antibiotic
- 0.1M $CaCl_2$ solution containing 15% glycerol (ice cold)

The day before:

1. Put 0.1M $CaCl_2$ solution and 0.1M $CaCl_2$ solution containing 15% glycerol at +4°C.
2. Inoculate one single colony of the *E. coli* strain in 5mL of LB medium. Incubate at +37°C shaking overnight.

The next day:

1. Put at least 30 eppendorf tubes at -80°C. Keep pipet tips and all the solutions on ice during the procedure.
2. Inoculate 1mL of the overnight culture in 10 mL of LB medium within a 500mL flask.
3. Measure the initial OD_{600} values of the culture and grow at 37°C with shaking until OD_{600} reaches 0.25-0.3.
4. Chill the culture on ice for 15 minutes.
5. Divide the chilled culture into 2 Falcon tubes à 50mL. Centrifuge at +4°C at 1,000 x g for 10 minutes.
6. Discard the supernatant and resuspend the pellet with 40mL ice-cold 0.1M $CaCl_2$ solution.
7. Keep the cells on ice for 30 minutes.
8. Centrifuge at +4°C at 1,000 x g for 10 minutes.
9. Discard the supernatant and resuspend the pellet with 5mL of ice-cold 0.1M $CaCl_2$ solution containing 15% glycerol.
10. Pipet 50µL of cell suspension into -80°C frozen eppendorfs and directly transfer them to -80°C freezer.

E. coli heat-shock transformation

Material:

- 50 µL competent cells
- plasmid DNA
- heating block at 42°C
- LB plates with proper antibiotics

1. Turn on the heating block at +42°C.
2. Take competent cells from -80°C freezer and thaw them on ice.
3. Pipette 10ng of plasmid DNA (or 10µL of ligation mixture) into 50µL of competent cells after thawing.
4. Incubate the tubes on ice for 30 minutes.
5. Heat-shock the cells at +42°C for 45 seconds.
6. Incubate on ice for 2 minutes.
7. Add 450µL of LB in each tube and incubate at +37 °C with shaking for 1 hour.
8. Spread totally 100µL of cells with transformed DNA on an LB plate with proper antibiotic. For cells transformed with a ligation mixture: an additional concentration step by centrifuging may be performed before plating.
9. Keep LB plates at +37°C overnight and pick colonies after more than 12 hours of incubation.

Plasmid isolation

The day before:
1. Inoculate one single colony from the transformation plate in 5mL of LB medium with proper antibiotic. Incubate at +37°C with shaking overnight.

The next day:
1. Isolate plasmids from the culture using the Macherey-Nagel Nucleospin Plasmid kit, following the isolation of high copy number plasmids protocol.

Check-up digestion of the cloned plasmids

1. Restrict the plasmids with the same restriction enzymes used to digest the vector and insert. Prepare a normal restriction mixture with e.g. 3-5µL of plasmid DNA.
2. Run the restricted plasmids on an agarose gel. Image the gel and examine whether bands of the correct size can be observed for the vector and the insert.