CHEMOCOMPETENT CELLS PREPARATION AND TRANSFORMATION

ADAPTED FROM THE BONNET TEAM PROTOCOL REPOSITORY

MATERIALS:

To prepare 50mL of culture :

- LB medium
- Antibiotics (according to the plasmid used for transformation)
- TSS medium: To make 50 mL : 5g PEG 8000, 1.5 mL 1M MgCl2 (or 0.30g MgCl2*6H20), 2.5 mL DMSO and LB to 50 mL. Filter sterilized (0.22 µm filter).
- LB plates
- 1.5mL tubes
- 50mL falcons
- Ice
- 500mL flask for culture
- Liquid nitrogen

For transformation:

- Chemical competent cells
- Ice
- Clean DNA to transform
- SOC
- Selective agar plates

PROTOCOL:

Cells preparation

Day 1:

- Dilute the overnight culture into 50mL of LB without antibiotic at 1/500 (200µL in 100mL)

- Incubate at 37°C until OD_{600} reaches 0.2-0.3 (3-4h)
- Place 1.5mL tubes, racks, 10mL pipettes at -20°C
- **FROM NOW ON DO EVERYTHING ON ICE**
- Incubate the culture on ice for 10min in 50mL falcon tubes
- Cool down the centrifuge to 4°C
- Centrifuge the culture at 3000rpm 4°C for 10min
- Remove the supernatant
- Resuspend cells in 10% volume of TSS buffer
- Aliquot cells in 100uL in 1.5mL tubes with Multipette
- Flash freeze them in liquid nitrogen
- Store at -80°C

To test them: use pUC19 as positive control and do not forget negative control.

- Thaw gently 3 tubes of cells on ice
- Add 1µL pUC19 in 1 tube, add nothing in the others (negative control) (keep cells on ice)
- Incubate 30 min on ice
- Heat-shock cells at 42°C during 45s (in water bath)
- Put back on ice for 2 minutes after heat-shock
- Add 900 µL pre-warmed SOC (37°C) (rich medium)
- Incubate cells at 37°C with agitation during at least 30min
- Centrifuge cells at 4000rpm for 2min, remove 800µL of supernatant
- Plate the rest from pUC19 positive control on LB Carb plate
- Plate 100µL from negative control respectively on LB Chloramphenicol, LB Kanamycin, LB Carbenicillin and LB Spectinomycin plates
- Incubate at 37°C overnight

Efficiency should be around 10^7 colonies/µg pUC19

Transformation protocol:

- Thaw gently cells on ice
- Add 1µL DNA (keep cells on ice)
- Incubate on ice for 30 min
- Heat-shock cells at 42°C during 45s (in water bath)
- Put back on ice after heat-shock for 2 min
- Add 900 µL pre-warmed SOC (37°C) (rich medium)
- Incubate cells at 37°C with agitation during at least 30min
- Plate 100 µL of transformation on selective LB agar plate or centrifuge cells at 4000rpm during 1min, remove 800µL of supernatant and plate the resuspended pellet
- Incubate at 37°C overnight