Chemically Competent Cells Preparation

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, 1.5mL 1M MgCl2 (or 0.30g MgCL2*6H_2O), 2,5mL 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

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 3000
- Remove the supernatant
- Resuspend the cells in 10% volume of TSS buffer
- Aliquot the cells in 100µl in 1.5mL tubes with Multipipette
- 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 900µl 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