Preparing and transforming chemically competent cells using the BacGen protocol

Buffer 1

- 1 M MgSO4
- 1 M MgCl2

Buffer 2

- 100 mM RbCl2
- 50 mM MnCl2
- 10 mM CaCl2
- 15 % glycerol
  pH 5.8

Buffer 3

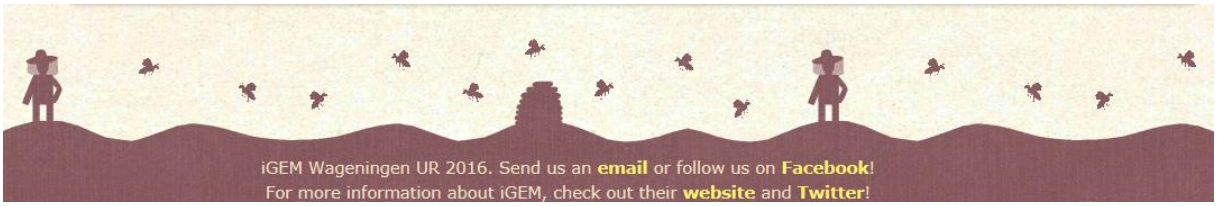
- 10 mM MOPS
- 10 mM RbCl2
- 75 mM CaCl2
- 15 % glycerol
  pH 6.8

Preparation of competent cells

1. Grow ON at 37°C and 180 rpm in 10mL LB medium in a 50mL Greiner tube.
2. Next morning, inoculate49 mL fresh LB medium in 250mL flaskwith 0.5 mL buffer 1 and 0.5 mL ON culture and grow for 2h at 37°C and 180 rpm until OD ≈ 0.4-0.5.
3. Spin down at 4700 rpm for 15 min at 4°C.
4. Resuspend the pellet in 17 mL (1/3 of total volume) buffer 2.
5. Keep on ice for 1 hr.
6. Spin down and resuspend the pellet in 5 ml (1/10 of total volume) buffer 3.
7. Keep on ice for 20 min.
8. Make 100 µL aliquots in 2 mL epps and snap freeze cells, store at -80°C. Note: use strong eppies otherwise they will 'explode'!

Heat shock transformation of competent E. coli

1. Defrost a 100 µL aliquot competent cells on ice.



2. Add 5µL ligation mix and incubate on ice for 30 min.
3. Incubate at 42°C for 45 sec.
4. incubate on ice for 2 min.
5. Add 1mL SOC medium and incubate at 37°C for 1.5h.
6. Plate cells on plates with the appropriate antibiotic, in 1/10 and 9/10 dilutions.