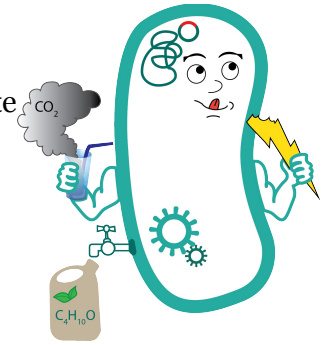
Generating electrocompetent cells

- Material:
  - 550 ml LB-Medium
  - 1 l cooled bidest. H_2O
  - 50 ml cooled 10% glycerine
  - 10 pre-cooled 50 ml Falcons
- Protocol:
  - Inoculate 2x3 ml LB with bacterial stock; incubate over night at 37 °C and 200 rpm
  - Inoculate 2x250 ml LB with the over night cultures in 1-litre-flask at 37 °C and 140 rpm
  - Incubate until OD_{600} 0.4-0.6
  - Cool the culture 15-30 minutes on ice
  - Onwards all steps at 4 °C
  - Divide the cultures into cooled 50 ml Falcons and centrifugate at 4000 rpm, 4 °C for 15 minutes, make sure to slowly accelerate and deccelerate
  - Discard supernatant
  - Resuspend pellet in 5 ml cooled bidest H_2O (and don't get frustrated while doing it, keep shaking gently)
  - Pool two suspensions each, add bidest H_2O up to 50 ml and centrifugate again (see centrifugation above)
  - Discard supernatant
  - Resuspend pellet in 5 ml cooled bidest H_2O
  - Add bidest H_2O up to 50 ml and centrifugate again (see centrifugation above)
  - Discard supernatant
  - Resuspend pellet in 5 ml cooled 10% glycerine
  - Transfer suspensions in two 50 ml Falcons and centrifugate again (see centrifugation above)
  - Discard supernatant



- Add volume of 10% glycerine that is approximately equal to the volume of the pellet and resuspend
- Divide cells in 50 µl aliquots and freeze in liquid nitrogen immediately
- Store at -80 °C