

Generating chemocompetent cells

- ◆ Protocol:
 - ◇ Inoculate 50 mL LB with bacterial stock.
 - ◇ Incubate until $OD_{600} = 0.6$.
 - ◇ Centrifuge at 5000 rpm, 4 °C for 5 minutes.
 - ◇ Discard supernatant.
 - ◇ Resuspend pellet in 25 mL 50mM $CaCl_2$.
 - ◇ Incubate on ice for 30 minutes.
 - ◇ Centrifuge at 5000 rpm, 4 °C for 5 minutes.
 - ◇ Discard supernatant.
 - ◇ Resuspend pellet in 5 mL 50mM $CaCl_2$.
 - ◇ Add 1 mL 10% glycerin, invert 4-6 times.
 - ◇ Divide cells in 200 μ L aliquots and freeze in liquid nitrogen immediately.
 - ◇ Store at -80 °C.

From [Bielefeld-CeBiTec 2014](#)