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Preparation of chemical competent E. coli cells

transformation buffer (TB)

- 600 mL of milliQ H_2O
- 10 mM PIPES buffer (3.02 g SIGMA no. p6757; MW 302.37)
- 15 mM $CaCl_2$ (2.2 g SIGMA no. c3306; MW 147.01)
- 250 mM KCl (83.3 mL of a 3 M stock solution)
- adjust pH tp 6.7 with 6 N KOH
- 55 mM $MnCl_2$ (10.89 g SIGMA no. m5005; MW 197.91)
- add milliQ H_2O to 1000 mL
- filter sterile and store at RT. Cool to 4 deg C before use.

protocol

- streak-plate cells from glycerol stock onto LB-agar plate with appropriate selection marker.
- Next day (likely after about 24h). Inoculate 250 mL of sterile SOB medium with one streak (10-15 colonies) (2 L flask). **Alternative**: Inoculate 5 ml over-night culture of LB medium with 2 colonies
- grow over-night at 18 deg C and 220 rpm. **Alternative**: grow over-night at 37C with shaking
- Next day, at $OD_{600\ nm}$ = 0.4-0.5 transfer flask to ice and let cool down for 10-20 min. **Alternative**: The day after, inoculate 250 mL of sterile SOB medium with 1 ml of over-night culture and grow at 37C with shaking until $OD_{600\ nm}$ = 0.4-0.5. Then transfer flask to ice and let cool down for 10-20 min.
- transfer to 6 x 50 mL pre-chilled falcon tubes and spin down at 1,500 xg for 10 min at 4 deg C.
- discard supernatant. For each tube, resuspend pellet in 1 mL ice-cold TB. Add 12 mL TB on top. Incubate for 10 min on ice.
- spin down at 1,500 xg for 10 min at 4 deg C.
- discard supernatant, resuspend pellet in 1 mL ice-cold TB. Merge samples to 1 tube and add TB to about 9.5 mL.
- add 7% (v/v) = 0.7 mL room-temperature DMSO and incubate for 10 min on ice.
- snap freeze 0.1 mL aliquots in liquid N_2.
- store at -80 deg C.

comments

- Mach1 cells divide much faster than Dh5a or BL21 cells, keep that in mind when setting up the culture.
- Origami2 cels divide much more slowly. It helps to start from much more cells (one entire plate) and/or to increase the grow temperature to 25 deg C.

*** From 22 July 2015,** Rosetta2 cells from glycerol stock > 25 mL shaking at 300 rpm/37C took 9 hours to have a

culture ready to innoculate. 2 mL / 200 uL > 500 mL non-baffled with SOC instead of SOB. 20C/200 rpm. 2mL culture took 12 hours to reach 0.5. 200 uL culture was only 0.1 after 12 hours. *** From 05 August 2015,** NEB Turbo cells from glycerol stock > 25 mL shaking at 270 rpm/37C took 6 hours to have a culture ready to innoculate. 1 mL / 500 uL > 500 mL non-baffled with SOB. 23C/220 rpm. Cultures in 15 hours were 0.8 and 1.. still used and competent

Preparation of chemical competent E. coli cells Alternative AM

transformation buffer I (TFBI)

- 30 mM KOAc
- 50 mM MnCl2
- 100 mM RbCl
- 10 mM CaCl2
- 15% glycerol
- pH 5.0 (HCl)
- filter sterile and store at RT. Cool to 4 deg C before use.

transformation buffer II (TFBII)

- 10 mM NaMOPS pH 7.0
- 10 mM RbCl
- 75 mM CaCl2
- 15% glycerol
- filter sterile and store at RT. Cool to 4 deg C before use.

protocol

- streak-plate cells from glycerol stock or commercial cells onto LB-agar plate with appropriate selection marker.
- Take 1-2 colonies and make overnight cultures in LB + selection marker
- The day after Inoculate 1 mL of overnight culture in 250 mL of SOB (2L flask)
- Grow the culture at 37 C for ~ 3h until OD600 is 0.4 - 0.5
- Cool down the flask in the cold room for 20 minutes
- Chill on ice 6 falcon tubes and aliquot and centrifuge the culture at 4500g $ c for 10 minutes
- Resuspend each pellet in 15 mL of TFBI and leave them on ice for 1 hour
- After 1 hour centrifuge at 4500g 4 C for 10 minutes
- Resuspend pellets in 2 mL of TFBII
- Aliquot cells ibn the cold room in 100 uL aliquots flash forze them and store at -80C

reference: doi: https://doi.org/10.1101/285866 [https://doi.org/10.1101/285866]
