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CportucalensisElectrocompetentCells

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We use this protocol and it's working

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Abstract

Protocol to prepare electrocompetent *Citrobacter portucalensis* MBL cells.

Large batch electrocompetent cells

- 1 Prepare 100 mL sterile LB in a 500 mL Erlenmeyer flask.
- 2 Prepare ice-cold 10 % glycerol and ultrapure water (both sterile).
- 3 Two days prior to electroporation, streak out strain on LB agar and grow at 30 °C.
- 4 One day prior to electroporation, inoculate a 5 mL LB liquid culture with a patch of cells from the overnight streak and incubate slanted, shaking at 250 rpm at 30 °C overnight.
- 5 Day of the electroporation, inoculate 1 mL of the overnight culture into the 100 mL LB flask and grow to OD600 = 0.4, shaking at 250 rpm at 30 °C.
- 6 Chill flask on ice for 20 minutes.
- 7 Wash two the culture two times into ice-cold water (5000 x g for 10 minutes at 4 °C; I typically divide the 100 mL volume into four 50 mL conical tubes, washing with 25 mL volumes).
- 8 Combine pellets and spin final time.
- 9 Resuspend in 2 mL ice-cold 10% glycerol.
- 10 Aliquot 50 µL volumes into ice-cold microcentrifuge tubes and flash-freeze in liquid nitrogen.
- 11 Store at -80 °C or use immediately.

Small batch electrocompetent cells

- 12 Two days prior, streak out strain as above.



- 13 One day prior, grow 5 mL LB culture overnight as above.
- 14 Day of, inoculate 50 μ L overnight culture into 5 mL LB and incubate slanted, shaking at 250 rpm at 30 °C until OD600 = 0.3.
- 15 Chill on ice for 10 minutes.
- 16 Wash three times into ice-cold water, final resuspension of combined pellets 50 μ L (can also spin full 5 mL culture in conical tube directly).