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Protocol status: Working
We use this protocol and it's working

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CportucalensisElectrocompetentCells

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ABSTRACT

Protocol to prepare electrocompetent *Citrobacter portucalensis* MBL cells.

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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 ºC.

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 ºC 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 ºC.

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 ºC; 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 ºC 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 µL overnight culture into 5 mL LB and incubate slanted, shaking at 250 rpm at 30 ºC 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 µL (can also spin full 5 mL culture in conical tube directly).