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Preparation of chemically competent *E. coli* cells

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Protocol status: Working

We use this protocol and it's working

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Abstract

Standard protocol to prepare cells for highly efficient DNA uptake using CaCl₂ (Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 2001).

Materials

MATERIALS

 LB-Medium (Lennox) vegetal **Carl Roth Catalog #0155.1**

- 10 mM CaCl₂
- sterile glycerole



- 1 Inoculate a 5 ml overnight-culture in LB-medium without antibiotics.
- 2 Inoculate 100 ml LB-medium with 1/100 volume of the overnight culture and incubate to an $OD_{600} = 0.5 - 0.6$ at 37 °C and 230 rpm.
- 3 Transfer cells to two 50 ml sterile falcon tubes and incubate on ice for 20 min.

From this step on, keep cells always cold.

 00:20:00

- 4 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.
 00:05:00
- 5 Carefully suspend each pellet in 20 ml of icecold 100 mM $CaCl_2$ and incubate on ice for 1 h.
 01:00:00
- 6 Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.
 00:05:00
- 7 Carefully suspend each pellet in 2 ml icecold 85 mM $CaCl_2$ containing 15% Glycerol.
- 8 Pipette 100 to 200 μ l aliquots in 1.5 ml tubes (work on ice) and store at -80 °C. (you can shock-freeze the cells in liquid nitrogen or in dry ice prior to storage at -80°C, but it is not required)

For DNA transformation, thaw 100 μ l of the competent cells on ice.