Preparation of chemically competent cells

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Abstract

Standard protocol to prepare cells for highly efficient DNA uptake using $CaCl_2$.

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Protocol

Before starting

Step 1.

Required:

Ice, sterile 1.5 mL tubes (label before starting), pre-cooled centrifuge

- 100 mM $CaCl_2$, glycerol, both sterile
- 85 mM $CaCl_2$ with 15% glycerol

!!! Always work under sterile conditions to avoid contamination !!!

Inoculation

Step 2.

Inoculate a 5 ml overnight-culture in LB-medium without antibiotics (for DH5a) or with appropriate antibiotics (for other strains).

Grow over night at 37 ˚C at 250 rpm.

REAGENTS
Luria-Bertani (LB) broth, makes 1L K488 by Amresco

Dilution

Step 3.

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.

Step 4.

Transfer cells to two sterile 50 ml falcon tubes and incubate on ice for 20 min.

From this step on, always keep cells cold.

DURATION
00:20:00

Step 5.

Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

DURATION
00:05:00

CaCl2

Step 6.

Carefully suspend each pellet in 20 ml of ice-cold 100 mM $CaCl_2$ and incubate on ice for 1 h.

DURATION
01:00:00

CaCl2

Step 7.

Centrifuge for 5 min at 4500 rpm and 4 °C (pre-cool centrifuge) and discard the supernatant.

DURATION
00:05:00

CaCl2 + glycerol

Step 8.

Carefully suspend each pellet in 2 ml icecold 85 mM CaCl2 containing 15% Glycerol.

Aliquots

Step 9.

Pipette 50 - 200 µl aliquots in prepared, pre-cooled 1.5 ml tubes (work on ice), shock-freeze in liquid nitrogen or on dry ice and store at -80 °C.

For DNA transformation, thaw competent cells on ice.