Transformation of Bacterial Cultures Using The Calcium Chloride Procedure

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Abstract

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Guidelines

Materials

1) L broth

- 1.0% Bactro-tryptone
- 0.5% Bacto-yeast extract
- 0.5% NaCl

After autoclaving, add 1.0 mL of 20% glucose per 100 mL of L broth

2) 10mM Tris-HCl, pH 8.0, 50 mM $CaCl_2$

3) L broth + antibiotic (of the plasmid transformed)

Reference

Maniatis, E.F. Ftitsch, and J. Sambrook. (1982). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. Pages 250-251.

Protocol

Step 1.
Grow 5 mL of the host cells overnight in L broth at 37°C.

NOTES
Irina Agarkova 14 Apr 2016
See guidelines for L broth recipe.

Step 2.
Inoculate 100 mL of L broth with 1 mL of the overnight culture.

Step 3.

Grow the cells with shaking at 37°C to a density of approximately 5 X 10^7 cells/ml.

NOTES

Irina Agarkova 14 Apr 2016

This usually takes 2-4 hours. For each transformation assay, 3 mL of cells will be needed.

Step 4.

Chill the culture on ice for 10 min.

DURATION

00:10:00

Step 5.

Centrifuge the cell suspension in the Sorvall SS34 rotor at 4,000g, 5 min, 4°C.

DURATION

00:05:00

Step 6.

Discard the supernatant.

Step 7.

Resuspend the cells with 1/2 the original volume with an ice cold, sterile solution of 10 mM Tris-HCl, pH 8.0, 50 mM $CaCl_2$.

Step 8.

Place the cell suspension in an ice bath for 15 min.

DURATION

00:15:00

Step 9.

Centrifuge the suspension in the Sorvall SS34 rotor at 4,000g, 5 min, 4°C.

DURATION

00:05:00

Step 10.

Discard the supernatant.

Step 11.

Resuspend the cells with 1/15 of the original volume with an ice cold, sterile solution of 10 mM Tris-HCl, pH 8.0, 50 mM $CaCl_2$.

Step 12.

Dispense 0.2 mL aliquots into prechilled microfuge tubes.

Step 13.

Store the cells at 4°C for 12-24 hours.

DURATION

24:00:00

Step 14.

Add the DNA in as small a volume as possible (1-2 µL/plate).

Step 15.

Mix and store on ice for 30 min.

DURATION

00:30:00

NOTES

Irina Agarkova 22 Mar 2016

Up to 40 ng of DNA can be used for each transformation reaction. Addition of more DNA or a greater volume of buffer leads to a reduction in transformation efficiency.

Step 16.
Transfer the mixtures to 42°C for 2 min.

DURATION
00:02:00

Step 17.
Add 1.0 mL of L broth to each tube and incubate at 37°C for 30 min (tetracycline selection) or 60 min (ampicillin or kanamycin selection) without shaking.

DURATION
01:00:00

NOTES
Irina Agarkova 22 Mar 2016
This period allows the bacteria to recover and begin to express their antibiotic resistance.

Step 18.
Spread an appropriate volume (usually 100-200 µL) of cells onto selective media by using either the spread plate method or the agar overlay method.

NOTES
Irina Agarkova 14 Apr 2016
Usually the agar overlay method yields slightly more transformants.

Step 19.
Leave the plates at room temperature until the agar overlay has solidified or the liquid has been absorbed into the plate.

Step 20.
Invert the plates and incubate at 37°C. Colonies should appear in 12-16 hours.