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Preparation of calcium competent *Escherichia coli* and heat-shock transformation

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Calcium chloride heat-shock transformation is a powerful molecular biology technique used to introduce foreign DNA into a host cell. The concept of the technique is to render cells competent using $CaCl_2$ to allow for introduction of plasmid. Plasmids usually contain the gene(s) of interest in addition to selection and/or antibiotic resistance markers. The Hanahan or calcium chloride method is used to generate chemically competent cells. Heat-shocking facilitates the transport of plasmid into the competent cell. Transformed cells will allow for downstream applications such as plasmid amplification or protein expression. This methods paper will outline the protocol for the preparation of calcium competent *Escherichia coli* using the Hanahan method and heat-shock transformation of calcium competent *Escherichia coli*.

INTRODUCTION

The process of calcium chloride heat-shock transformation encourages bacterial cells to uptake DNA from the surrounding environment. The exact mechanism of how this process works is still largely unknown, but there are hypotheses on the different aspects of the procedure. The role of calcium ions in the cell suspension is hypothesized to be a cation bridge between the negative charges on phosphorylated lipid A in lipopolysaccharide (LPS), and the phosphate backbone of DNA (1, 2). The ice-cold $CaCl_2$ solution facilitates binding of DNA to the surface of the cell, which then enters the cell after a short period of heat-shock (3). Cells that are successfully transformed are usually identified by selection or screening markers such as drug resistance or fluorescence (4). This technique is commonly used to transform cells with plasmids for various purposes like recombinant protein expression, cloning, and long term storage of the plasmids.

Key words: $CaCl_2$ method, Hanahan's method, heat-shock transformation, competent cell, *E. coli*, plasmid, DNA, molecular biology

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MATERIALS AND EQUIPMENT

Materials:

Competent cell preparation:

- 1mL of overnight *Escherichia coli* (*E. coli*) culture
- 100mL of 0.1M $CaCl_2$ (ice cold)
- 20mL of 0.1M $CaCl_2$ with 15% glycerol solution (ice cold)
- 100mL of fresh lysogeny broth (LB) media

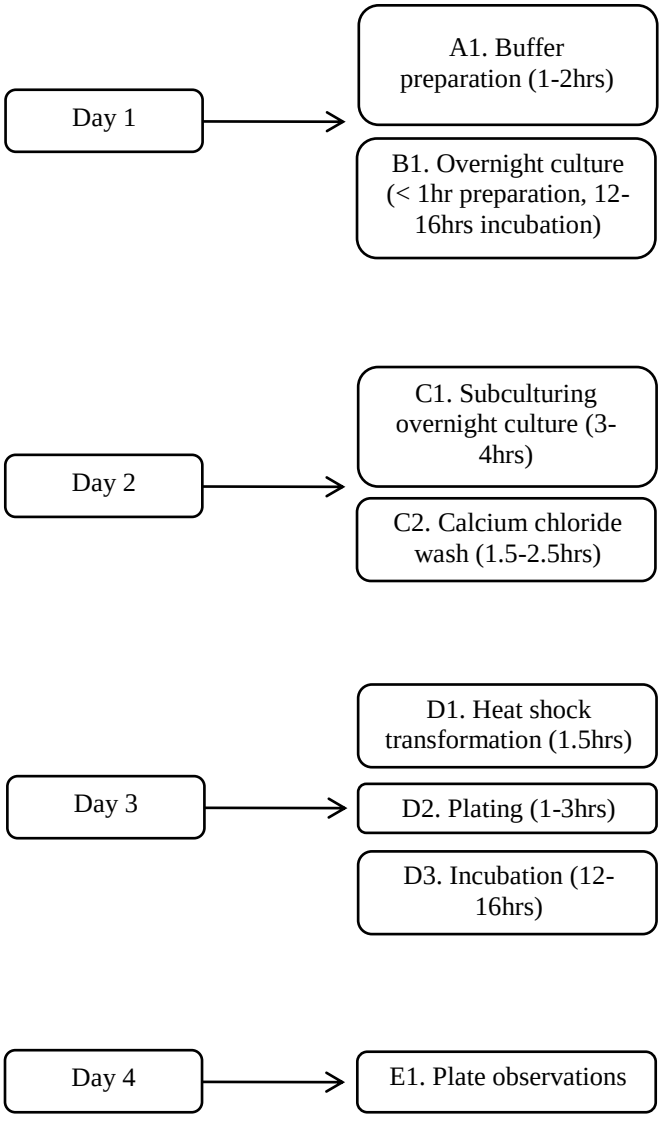
Heat-shock transformation:

- 1pg - 100ng plasmid DNA (1-5uL)
- 1mL of pre-warmed LB media or SOC media at 37°C
- LB agar plates (with appropriate reagent for selective or screening)
- Ice

Equipment:

- 37°C shaking incubator
- 42°C water bath
- Spectrophotometer

PROTOCOL



Buffers and overnight cultures are prepared the day before the experiment. Subculture the overnight the next day and allow for 3-4 hours of growth. Pellet the subculture and wash with $CaCl_2$. Following generation of competent cells, use the heat-shock method to introduce plasmid DNA into cells. Plate the transformed cells on the appropriate LB plates supplemented with the reagent for selection or screening. Incubate the plates overnight and record colony counts the next day.

METHODS

A. $CaCl_2$ Buffers Preparation

1M $CaCl_2$ (stock solution, 10x working concentration)

- Weigh out 11.1g of anhydrous $CaCl_2$
- Add to 80mL of dH2O
- Mix solution until $CaCl_2$ is fully dissolved
- Top up to 100mL
- Filter sterilize through a 0.22µm pore

0.1M $CaCl_2$ (working solution)

- Add 10mL of 1M $CaCl_2$ to 90mL of dH2O for a 1:10 dilution
- Filter sterilize through a 0.22µm pore

0.1M $CaCl_2$ + 15% glycerol (working solution)

- Mix 6mL 1M $CaCl_2$ with 9mL sterile glycerol and 45mL dH2O

B. Overnight Culture(s)

- Inoculate 1mL of LB with *E. coli*
- Place in shaking incubator at 37°C and 200rpm
- Incubate for 12-16 hours

C. Generation of Competent Cells ($CaCl_2$ wash)

Subculturing overnight culture:

- Add 1mL of overnight culture to 99mL of fresh LB (1:100 dilution, no antibiotics)
- Shake incubate at 37°C and 200rpm for 3-4 hours or until OD reaches 0.4

$CaCl_2$ wash:

- Ensure that all reagents ($CaCl_2$ solutions, Oakridge tubes, centrifuge) are ice-cold or at 4°C
- Separate culture into multiple Oakridge tubes
- Place on ice for 20 minutes
- Centrifuge at 4°C at 4000rpm for 10 minutes
- Discard the supernatant by tipping tubes over a discard bin and then aspirating any remaining media

- Resuspend each pellet with 20mL ice-cold 0.1M $CaCl_2$, incubate on ice for 30 minutes
- Centrifuge at 4°C at 4000rpm for 10 minutes
- Discard the supernatant and combine pellets by resuspending in 5mL ice-cold 0.1M $CaCl_2$ with 15% glycerol
- Use for downstream transformation or store in -80°C freezer

D. Heat-shock transformation

Heat-shock:

- Thaw competent cells on ice
- Add 1-5µl (10pg-100ng) of plasmid (do not exceed 5µL for a 50µL cell aliquot)
- Incubate on ice for 30 minutes
- Heat-shock by placing in 42°C water bath for exactly 30 seconds
- Place cells on ice for 2 minutes
- Add 1mL pre-warmed LB or SOC medium
- Shake incubate 37°C, 200rpm, 1 hour for outgrowth

Plating and incubation:

- Spread plate 1:10 and 1:100 dilutions of the outgrowth cultures on warm selective and/or screening plates (e.g. Ampicillin and/or X-gal if required)
- Incubate at 37°C for 12-16 hours

Plate observations:

- Inspect plates for isolated colonies

ANTICIPATED RESULTS AND CONTROLS

Anticipated results:

- No colonies appear on the plate.
- A countable number of colonies (30-300 colonies) appear on the plate.
- Many colonies that are too numerous to count appear on the plate.

Controls:

- Use a DNA preparation that has been shown to give transformants in previous experiments to act as a positive control.
- Perform another transformation to which plasmid DNA is not added to act as a negative control.
- Transform 1ng of target plasmid to check competent cell viability, calculate transformation efficiency, and verify the antibiotic resistance of the plasmid.

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TROUBLESHOOTING

Problem	Explanation	Solution
Few or no transformants present	Incorrect antibiotic or antibiotic concentration was used	Confirm usage of correct antibiotic and antibiotic concentration
	Cells are not competent	Transform a plasmid (e.g. pUC19) and calculate the transformation efficiency of the competent cells. If the transformation efficiency is low, make a new batch of competent cells.
	If using chemically competent cells, the incorrect heat-shock protocol was used.	Follow the manufacturer's specific transformation protocol. For example, heat-shocking at a higher temperature than specified on protocol may result in cell death or drastically reduced cell competence.
Colonies do not contain a plasmid	Satellite colonies were selected	Choose large, individual colonies that are well-established for analysis.
	Antibiotic concentration used was too low	Increase the antibiotic concentration on media plates to the recommended amount. Alternatively, use fresh plates with fresh antibiotics.
Too many colonies/lawn of growth present on plate	Concentration of transformed cells too high	Plate fewer cells for the next transformation to allow for fair distribution and presence of isolated colonies. Dilute the outgrowth culture further prior to plating.
	Adding antibiotic to hot agar breaks down the antibiotic, causing antibiotic plates to be ineffective	Make sure the agar is cool enough for the antibiotic to be added, while still being molten. For example, agar should be warm enough for one to touch comfortably when the antibiotic is added.