Nov 28, 2023

Transformation of Plasmid into Competent E. coli Cells (e.g., DH5α)

DOI
dx.doi.org/10.17504/protocols.io.eq2lyj2ywlx9/v1

Hussain Zubair[1]

[1]ZZU

Hussain Zubair
zzu

DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyj2ywlx9/v1

Protocol Citation: Hussain Zubair 2023. Transformation of Plasmid into Competent E. coli Cells (e.g., DH5α). **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2lyj2ywlx9/v1**

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

Created: November 28, 2023

Last Modified: December 02, 2023

Protocol Integer ID: 91495

Keywords: plasmid dna, transformation of plasmid, enhanced plasmid uptake, plasmid, gene cloning, transformed cell, plating of the cell, careful thawing of competent cell, such as gene cloning, crucial for subsequent molecular biology experiment, detailed experimental protocol for the transformation, dh5α strain, subsequent molecular biology experiment, microbiology, molecular biology, dna, cells in lb broth, ampicillin to the final inoculation, cell

Abstract

This is a detailed experimental protocol for the transformation of plasmid DNA into competent E. coli cells, specifically using the DH5α strain. The protocol encompasses the entire process, from the preparation of LB agar plates supplemented with ampicillin to the final inoculation of transformed cells in LB broth. Key steps include the careful thawing of competent cells, the addition of plasmid DNA, a precise heat shock treatment at 42°C for enhanced plasmid uptake, and subsequent recovery and plating of the cells. The methodology is designed to maximize the efficiency of transformation, crucial for subsequent molecular biology experiments such as gene cloning and expression analysis. This protocol serves as a fundamental guide for researchers in the field of molecular genetics and microbiology.

Troubleshooting

Preparation of LB Agar Plates with Antibiotics

1
- Prepare LB (Luria-Bertani) agar plates.
- Once the agar has cooled but not solidified, add ampicillin at a ratio of 1:1000 to the agar.
- Pour the LB agar into 90 mm Petri dishes and allow to solidify.

Thawing of Competent Cells

10m

2
- Retrieve the competent E. coli cells (e.g., DH5α strain) from the -80 °C freezer.
- Thaw the cells slowly on ice to ensure they remain in liquid form. 00:10:00

10m

Addition of Plasmid

30m

3
- Add 1 µL of the plasmid solution to the competent cells. The volume may vary depending on the plasmid's concentration.
- Incubate the mixture on ice for 00:30:00 min to allow the cells to fully absorb the plasmid.

30m

Heat Shock Treatment

1m 10s

4
- Subject the cell-plasmid mixture to heat shock in a water bath set at 42 °C for exactly 00:01:10 min .
- This step facilitates the uptake of the plasmid by the cells.

1m 10s

Cooling Period (Stabilizing the Cells)

5 Immediately after the heat shock, place the mixture on ice for 00:02:00 min to stabilize the cells.

2m

Plating on LB Agar

18h

6

18h

- Gently spread the competent cell-plasmid mixture onto the prepared LB agar plates with ampicillin.
- Incubate the plates at 37 °C Overnight to allow for bacterial growth and colony formation.

Further Inoculation

7

- The following day, select a single colony from the agar plate.
- Inoculate the colony in LB broth for further growth and experimentation as required