Competent cell preparation

Introduction

The competent cell preparation is the initial step in the production of transformants. The delivery of DNA into the host cell occurs in three stages- DNA adhering to the host cell, internalization and release into the host cell. The successful transformation relays on 3 parameters- surface charge of host, presence or absence of cell wall and charge of DNA. Host cell surface charges either will attract or repel DNA as a result of opposite or similar charges. By nature, the cell wall of the bacteria is rigid and does not allow the easy transformation. Both cloning host (DH5α) and expression host (BL21) are bacteria so they need to be made competent to get effective transformants. Competent cell preparation is the procedure of making the cell wall of the bacteria fragile and stabilizes the surface charge of the cell wall in order to achieve flexible and effective transformation. Most types of cells cannot take up DNA efficiently unless they have been exposed to special chemical or electrical treatments to make them competent. The standard method for making the bacteria permeable to DNA involves treatment with calcium ions. Brief exposure of cells to an electric field can also allow the bacteria to take up DNA and this process is called as electroporation. The exact mechanisms involved in artificial competence are not yet known well. In $CaCl_2$ method, the competency can be obtained by creating pores in bacterial cells by suspending them in a solution containing the high concentration of calcium. DNA is then forced into the host cell by heat shock treatment at 42^{o}C for the process of transformation.

Materials

- DH5α and BL21 cultures glycerol stocks
- LB agar
- *E. coli BL21*
- Falcons
- Calcium Chloride

Procedure

Cell preparation

1. Both DH5α and BL21 cultures glycerol stocks were taken out from -80^{o}C freezer and thawed at 4^{o}C in the refrigerator.
2. A loop full of DH5α culture was taken and streaked on normal LBA (Luria-Bertani agar without antibiotic) medium and the same procedure was followed with BL21 to get the colonies.
3. Incubated it for overnight at 37^{o}C.
4. Once colonies were prominently observed. A single colony was transferred to 10mL of autoclaved LB (Luria-Bertani) medium.
5. The culture was incubated for overnight at 37^{o}C in a shaker incubator at 120rpm.

Competent preparation

6. 1% of stock culture was transferred to 20mL of LB in autoclaved falcon tube.
7. The culture was incubated at 37^{o}C in a shaker incubator (120rpm) for approximately 2hours until the culture attained the desired optical density of 0.5-0.6.
8. The culture was then placed on ice to ensure the inhibition of further growth
9. The culture was centrifuged at 6500rpm for 10 minutes at 4^{o}C.
10. The supernatant was discarded and pellet was re-suspended in chilled 100mM calcium chloride and mixed properly
11. The culture was incubated on ice for 10minutes
12. Again centrifuged for 10minutes at 5,000rpm, 4^{o}C.
13. The resulted supernatant was discarded and pellet was re-suspended in 5mL of 100mM chilled calcium chloride.
14. Again centrifuged for 5000rpm at 4^{o}C for 10minutes.
15. The cell pellet was obtained by carefully discarding the supernatant.
16. The cell pellet was re-suspended in 0.5mL of 100mM chilled calcium chloride
17. The competent cell solution was left overnight for approximately 14 to 18 hours.