Competent cell preparation

Introduction

This protocol describes the preparation of competent bacterial cells for downstream molecular biology applications, including transformation and plasmid introduction experiments. Competent cells are prepared by processing bacterial cultures under controlled laboratory conditions to improve DNA uptake capability. Cell harvesting, washing, buffer exchange, and cold-chain handling are key factors affecting final cell quality and performance.

Reference:
1. Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *Journal of Molecular Biology, 166*(4), 557-580. https://doi.org/10.1016/S0022-2836(83)80284-8
2. Inoue, H., Nojima, H., & Okayama, H. (1990). High efficiency transformation of *Escherichia coli* with plasmids. *Gene, 96*(1), 23-28. https://doi.org/10.1016/0378-1119(90)90336-P
3. Green, M. R., & Sambrook, J. (2012). *Molecular Cloning: A Laboratory Manual* (4th ed.). Cold Spring Harbor Laboratory Press. Seidman, C. E., Struhl, K., Sheen, J., & Jessen, T. (2001). Introduction of plasmid DNA into cells. In *Current Protocols in Molecular Biology*. John Wiley & Sons.

Materials

- Bacterial strain
- SOB medium
- LB agar plate
- 10% Glycerol solution

Procedure

Cell Expansion

1. Streak cells from a frozen glycerol stock onto a fresh LB agar plate and incubate overnight at 37°C (16-18 hours).
2. Take a single well-isolated colony from the plate and inoculate it into 2mL of LB medium and incubate at 37°C overnight with vigorous shaking at 200 rpm.
3. Inoculate 50 mL of freshly prepared SOB broth with 1000 µL of the overnight starter culture and incubate at 37°C (200 rpm) for 1.5hr

Cell Cooling

4. Immediately place the culture on ice for 10 minutes to stop growth.

   Maintain cold-chain handling throughout subsequent processing steps.

Cell Harvesting

5. Centrifuge the cells at 6000rpm, 5min at 4°C.
6. Discard the supernatant.

Cell Washing

7. Gently resuspend the pellet in 1 ml 10% glycerol and aliquot in an eppendorf.
8. Centrifuge the cells at 12000 rpm for 1min at 4°C.
9. Discard the supernatant.
10. Gently resuspend the pellet in 1 ml 10% glycerol.
11. Centrifuge the cells at 12000 rpm for 1min at 4°C.
12. Discard the supernatant.
13. Gently resuspend the final pellet in 250 ul 10% glycerol.
14. store the product at –80°C if not directly proceeding other experiment.