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E.coli DH10B Competent Cell Preparation

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We use this protocol and it's working

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Abstract

Preparation of *E. coli* competent cells suitable for electroporation, to serve as hosts for subsequent plasmid introduction.

Materials

- Cultured log-phase DH10B cells (200 mL culture, OD600 ≈ 0.6–0.8)
- Ice-cold sterile 10% glycerol solution (filter-sterilized, pre-chilled at 4 °C or on ice, prepare at least 100 mL)
- Sterile centrifuge bottles (250–500 mL)
- Sterile microcentrifuge tubes (1.5 mL, 10 tubes)
- Sterile pipette tips and micropipettes
- Ice bucket and crushed ice bath (all procedures must be kept cold)
- Refrigerated centrifuge (set to 4 °C, 4500 × g)
- Shaking incubator (37 °C, 220 rpm)

E.coli DH10B Competent Cell Preparation

1h 10m

1 Obtain log-phase culture (OD600 ≈ 0.6–0.8) and immediately place it on ice to cool for 10 minutes (gently swirling during cooling is recommended). 10m

2 Transfer 200 mL of culture into pre-chilled centrifuge bottles and centrifuge at 4500 × g for 10 minutes at 4 °C. 10m

3 Carefully discard the supernatant without disturbing the cell pellet. 5m

4 Add 50 mL of ice-cold 10% glycerol. Mix by repeatedly pipetting up and down until the suspension is evenly turbid. Centrifuge again at 4500 × g for 10 minutes at 4 °C. 10m

5 Repeat steps 3–4 for a total of two washes. 30m

6 After the final centrifugation, discard the supernatant and resuspend the entire pellet in 1 mL of ice-cold 10% glycerol. Transfer the suspension into sterile 1.5 mL tubes. 5m

7 Aliquot 50–100 µL per tube. Keep on ice for immediate use, or snap-freeze in liquid nitrogen and store at −80 °C (stable for 1–2 weeks).