

E.coli Heat-shock competent preparation

Introduction

preparation of agro heat shock transformation

Materials

- › Liquid nitrogen
- › ice
- › LB with suitable antibiotics
- › 20mM CaCl_2 + 15% glycerol sterile

Procedure

pre-culture

1. Pick single colony into a 4ml LB (antibiotic if needed)
2. Grow at 37°C, 150 rpm overnight

culture

3. Add 0.5ml of pre-culture to 50ml LB, and incubate until OD_{600} 0.5-1
4. Chill culture on ice for 5-10min
5. transfer maximum 30ml of the culture to a 50ml tube
6. Centrifuge for 10min at 5000rpm in 4°C
7. Discard the supernatant and let the tube to dry inverted for 1min
8. Add 0.5ml ice cold 20mM CaCl_2 + 15% glycerol
9. Aliquot 100µl and freeze directly in liquid nitrogen