Chemicompetent DH5α Cell Transformation

Introduction

This protocol yields transformed DH5α chemicompetent cells.

Reagents

- BL21 electrocompetent cells
- Plasmid DNA
- SOB liquid media
- LB/Kanamycin plates
- H_20

Equipment

- Electroporator
- Electroporation cuvette
- Eppendorf tubes
- 250 mL beaker
- Hot plate
- Thermometer
- Ice in a bucket
- Pipette and tips
- Incubation cabinet

Procedure

1. Thaw a tube of 50 µL chemicompetent cells on ice.
2. Add 1-5 µL of plasmid DNA to the thawed cells and mix them gently by pipetting the mix up and down. Place them on ice for 30 minutes. Incubate LB/Kanamycin plates at 37°C to warm them.
3. Heat shock cells by placing them into a 42°C bath for 30 seconds. Place on ice for 5 minutes.
4. Transfer the sample to an Eppendorf tube and add 950 µL of SOB media. Incubate the media and sample at 37°C for 60 minutes. Shake vigorously at 250 rpm.
5. Mix cells without vortexing and spread 50-100 µL of cells onto pre-warmed LB/Kanamycin plates. Incubate at 37°C overnight.