E. coli transformation v1.0

Introduction

High efficiency *E. coli* transformation protocol. Use this protocol to transform cells prepared by the "Chemically competent *E. coli* cells" protocol. Can both use USER reation of DNA for transformation.

Materials

- 5µL USER reactions or 50 - 200ng purified DNA
- 50µL chemically competent E. coli cells stored in 25% glycerol at -80°C.
- 250µL SOC medium
- Eppendorf tubes

Procedure

Prepare

1. Thaw cells on ice for 15 minutes

00:15:00

2. Add USER reaction or DNA directly to the cells and stirr carefully with the pipett tip
3. Incubate on ice for 30 min

00:30:00

4. In this time it is a good idea to pre-heat the thermoblock to 42°C. It is a good idea to add water to the thermoblock to allow for sufficient heat transfer.

Heat shock

5. Transfer the tubes containing the cells to thermoblock and incubate for **EXACTLY** 45s.

00:00:45

6. Transfer directly to ice and incubate for 2 min

00:02:00

7. While waiting adjust the thermoblock to 37°C

Recovery

8. Add 250µL SOC medium to the tubes containing the transformants

9. Incubate the transformants at 37°C with shaking @ 600 RPM for 1h

Plate

10. Plate transformants on LB plates with appropriate antibiotics (20µL and 200µL of undiluted culture is normally alright. 20µL and 200µL of a 1:100 dilution can also be included)