

Transformation

Aims of the Experiment

To generating desired plasmids for the gene expression analysis of our homemade cell extract.

Materials

- DH5α or Turbo chemically competent cells
- Purified plasmid
- SOC medium
- Electroporation cuvettes
- 1.5-mL micro-centrifuge tubes
- Agar plate
- Respective antibiotics for the selected plasmids

Procedure

Transformation of Chemically Competent cells for Ligation products etc.

1. Thaw the cells on ice.
2. Add 1 – 5µl Plasmid onto the cell.
3. Incubate the cells on ice for 30 minutes.
4. Heat shock the cells at 42°C for 30 – 45 sec.
5. Immediately incubate the cells on ice for 2 minutes.
6. Add 400 – 1000µl SOC medium into the cells.
7. Incubate the cells at 37°C for 1 hour.
8. Spin down the cells and remove supernatant until 200µl is left.
9. Plate 200µl of cells onto agar plate with respective antibiotic(s).
10. Grow cells overnight at 37°C.

Transformation of electrocompetent cells for purified plasmids

1. Thaw the cell on ice.
2. Pre-cool required electroporation cuvettes on ice.
3. Add 1µg plasmid (or adjust depends on the volume) to the cells.
4. Add cells into the cuvette and insert it into the cuvette holder of the electroporator.

5. Start the electroporation and pulse duration around 5ms.
6. Add 500 – 1000µl SOC medium into the cuvette.
7. Transfer the cells into a micro-centrifuge tube.
8. Incubate the cells at 37°C for 1 hour.
9. Spin down the cells and remove supernatant until 200µl is left.
10. Plate 200µl of cells onto agar plate with respective antibiotic(s).
11. Grow cells overnight at 37°C.

Cultivation and Purification

1. After growing cells on agar plate, pick a single colony and prepare an overnight culture in its respective media of 5ml.
2. Obtain the generated plasmids from the cells from the previous prepared overnight culture by the Mini-Prep Kit.