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Optimized heat shock transformation protocol for Escherichia coli TOP10, JM109, and BL21 strains

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[1]Reclone (The Reagent Collaboration Network)

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We use this protocol and it's working

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Abstract

This protocol describes the standard method for transforming chemically competent Escherichia coli using a heat shock approach.

Materials

- Chemically competent *Escherichia coli* cells (e.g., TOP10, JM109, BL21)
- Plasmid DNA (10 pg – 100 ng)
- SOC medium
- Ice
- Sterile 1.5 mL microcentrifuge tubes
- Water bath or heat block at 42 °C
- Incubator or water bath at 37 °C
- Sterile LB agar plates with appropriate antibiotic
- Vortex mixer

Protocol

1 Thaw 50 µL of chemically competent Escherichia coli cells (tested with TOP10, JM109, and BL21 strains) on ice for 5–10 minutes.

2 Add 1 µL of plasmid DNA (between 10 pg and 100 ng) and gently mix by tapping or pipetting.

3 Incubate the tube on ice for 30 minutes.

4 Heat shock the cells by placing the tube at 42 °C for 45 seconds in a water bath or incubator.

5 Immediately return the tube to ice and incubate for 2 minutes.

6 Add 400 µL of SOC medium to the tube.

7 Incubate the cells at 37 °C for 1 hour with shaking or static incubation in a water bath or incubator (to allow expression of antibiotic resistance genes).

8 Plate 100 µL on an agar plate containing the corresponding antibiotic for selection, depending on the plasmid used.

Results

9 The image below shows bacterial colonies grown on an LB agar plate supplemented with ampicillin, resulting from the transformation of E. coli BL21 cells.

Figure 1: *Bacterial colonies obtained after transformation of Escherichia coli BL21 cells.*