

## Protocol: Heat Shock Transformation of *E. coli*

This protocol can be used to transform chemically competent cells (e.g. from CaCl<sub>2</sub>) with a purified plasmid.

Material	Amount	Notes
Chemically competent cells	20 µl	
LB medium	200 µl	Another rich medium can substitute
LB agar with antibiotic	1 plate	Antibiotic concentrations are below

**Note: Never vortex competent cells.  
Mix cells with gentle shaking.**

### 1) Thaw competent cells on ice.

These can be prepared using the CaCl<sub>2</sub> protocol.

### 2) Place 20 µl of cells in a pre-chilled Eppendorf tube.

### 3) Add a small amount of purified plasmid to the chilled cells (0.5 µl or less).

### 4) Mix gently by flicking the tube.

### 5) Chill on ice for 10 minutes.

This step is optional, but can improve yields when transforming a ligation product.

### 4) Heat shock at 42 °C for 30 seconds.

### 5) Return to ice for 2 minutes.

### 6) Add 200 µl LB medium and recover the cells by shaking at 37 °C.

Another rich medium can substitute for the recovery.

The recovery time varies with the antibiotic selection.

Ampicillin: 15-30 minutes

Kanamycin or Spectinomycin: 30-60 minutes

Chloramphenicol: 60-120 minutes

### 7) Plate out the cells on selective LB.

Most purified plasmids produce high transformation efficiencies. Plating out 10 µl of cells will produce many colonies. Plasmids with low transformation efficiency may require larger volumes. 200 µl is the largest volume that an LB plate can absorb.

### 8. Incubate at 37 °C. Transformants should appear within 12 hrs.

**Appendix: Suggested antibiotic concentrations for selection**

Antibiotics stocks are maintained at the stock concentration in the indicated solution. They are diluted by the indicated dilution factor into LB agar as it cools, to obtain LB agar plates at the indicated working concentration.

<b>Antibiotic</b>	<b>Working concentration</b>	<b>Dilution factor</b>	<b>Stock concentration</b>	<b>Solution</b>
Ampicillin	100 µg/mL	1000x	100 mg/mL	Water
Kanamycin	100 µg/mL	1000x	100 mg/mL	Water
Spectinomycin	25 µg/mL	1000x	25 mg/mL	Water
Chloramphenicol	30 µg/mL	1000x	30 mg/mL	Ethanol