

# High Efficiency Transformation Protocol for Competent *E. coli*

## Overview

Perform steps 1-7 in the tube provided.

## Protocol

1. Thaw a tube of Competent *E. coli* cells on ice for 10 minutes.
2. Add 1-5  $\mu\text{l}$  containing 1 pg-100 ng of plasmid DNA to the cell mixture. Carefully flick the tube 4-5 times to mix cells and DNA. **Do not vortex.**
3. Place the mixture on ice for 30 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950  $\mu\text{l}$  of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50-100  $\mu\text{l}$  of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24-36 hours or 25°C for 48 hours.