14 Minute Transformation Protocol (C2987H/C2987I)

Overview

The following protocol results in only 25% efficiency compared to the High Efficiency Transformation Protocol. For C2987H, perform steps 1-6 in the tube provided.

Protocol

1. For C2987H: Remove cells from -80°C freezer and thaw in your hand.
   For C2987I: Thaw a tube of NEB 5-alpha Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
2. Add 1-5 µl 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 10 minutes. Do not mix.
4. Heat shock at exactly 42°C for exactly 30 seconds. Do not mix.
5. Place on ice for 3 minutes. Do not mix.
6. Pipette 200 µl of room temperature SOC into the mixture. Immediately spread 50-100 µl onto a selection plate and incubate overnight at 37-42°C. NOTE: Selection using antibiotics other than ampicillin may require some outgrowth before plating on selective media. Colonies develop faster at temperatures above 37°C, however some constructs may be unstable at elevated temperatures.