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High Efficiency Transformation

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New England Biolabs¹

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for high efficiency heat shock transformation of competent *E. coli* cells.

Source: New England Biolabs

Materials

SOC Outgrowth Medium - 100 ml (New England Biolabs Catalog #B9020S)



- 1 Thaw a tube of competent E. coli cells on ice for 00:10:00 . Mix gently and carefully pipette 50 μL of cells into a transformation tube on ice. 10m
- 2 Add 1 μL containing 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 00:30:00 . Do not mix. 30m
- 4 Heat shock at exactly 42 °C for exactly 00:00:30 . Do not mix. 30s
- 5 Place on ice for 00:05:00 . Do not mix. 5m
- 6 Pipette 950 μL of room temperature SOC into the mixture.
- 7 Place at 37 °C for 01:00:00 . Shake vigorously (250 rpm) or rotate. 1h
- 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 μL of each dilution onto a selection plate and incubate overnight at 37 °C . Alternatively, incubate at 30 °C for 24:00:00 or 25 °C for 48 hours. 1d