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Bacterial Transformation

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We use this protocol and it's working

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Abstract

This protocol explains how to transform a plasmid DNA template into competent bacterial cells which are then cultivated. The DNA is then purified from the cells using a miniprep kit.

The competent cells used in this protocol come from the NEB®10-beta Competent *E. coli* kit. This protocol was adapted from the one provided with the cells.

Materials

Materials required

- a tube of NEB 10-beta Competent *E. coli* cells
- 1-5 µl containing 1 pg-100 ng plasmid DNA template
- selection plates with appropriate antibiotic
- DNA miniprep kit

DNA Transformation

1 Thaw a tube of NEB 10-beta Competent *E. coli* cells on ice for 10 minutes

2 Add 1-5 µl containing 1 pg-100 ng of plasmid DNA to the cell mixture.
Flick the tube 4-5 times to mix cells and DNA but 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 µl of room temperature NEB 10-beta/Stable Outgrowth Medium 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 Spread 50-100 µl 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.

DNA Miniprep

10 Purify the DNA from selected cultures using a DNA miniprep kit