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## Bacterial Transformation [↗](#)

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**1** Works for me [dx.doi.org/10.17504/protocols.io.8irhud6](https://doi.org/10.17504/protocols.io.8irhud6)

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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.

### EXTERNAL LINK

<https://international.neb.com/protocols/0001/01/01/high-efficiency-transformation-protocol-c3019>

### MATERIALS TEXT

#### 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

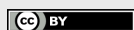
#### DNA Transformation

- 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.  
Click 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



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