Bacterial transformation Version 2

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Abstract

Standard protocol to transform bacteria with a plasmid using chemically competent E. coli and antibiotic resistance.

Citation: Stephen Floor Bacterial transformation. **protocols.io**
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Materials

MACH1 or DH5a or TOP10 or NEB stable or etc for DNA purification/miniprep by Contributed by users

BL21(DE3) or BL21-Star(DE3) or Rosetta2(DE3) or etc for protein purification by Contributed by users

Protocol

Step 1.

Count the number of transformations to perform and remove the appropriate number of aliquots from the -80 to an ice bucket. Our aliquots are 100 ul each.

NOTES
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If transformating a plasmid with repeats (such as a lentiviral plasmid), be sure to use NEB Stable or Stbl3 cells or similar.

Step 2.

Thaw cells on ice.

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All steps are in a microcentrifuge tube.

Step 3.

Label microcentrifuge tubes and add 20 ul cells to each tube.

Step 4.

Add 10-500 ng DNA (typically just use 1 uL) to each tube of cells.

Step 5.

Incubate for 25 minutes on ice. Anywhere from 5 to 25 minutes usually works.

Step 6.
Heat shock at 42 degrees for one minute in water bath.

TEMPERATURE
42 °C Additional info:

Step 7.
Recover for two minutes on ice.

Step 8.
Add 180 µL LB or SOC media, mix.

AMOUNT
180 µl Additional info: LB or SOC media

Step 9.

Grow at 37 degrees for one hour with shaking. Warm the plates during this step.

TEMPERATURE
37 °C Additional info:

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If a faster transformation is desired the growth step can be skipped entirely for ampicillin resistant plasmids, or shortened to ~15 minutes for kanamycin resistant plasmids. Growth may increase the number of colonies though.

Step 10.

Plate 75 µL of the transformation on plate with appropriate antibiotic.

ANNOTATIONS
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If the transformation is low efficiency (cloning), plate as much as 200 ul.

Step 11.
Leave at 37 degrees overnight or room temperature for two days.