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Bacterial transformation

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1 Works for me dx.doi.org/10.17504/protocols.io.6taheie

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ABSTRACT

Transformation of competent *Escherichia coli* cultures with heterologous DNA.

This protocol allows the introduction of external DNA into competent *E. coli* cells. Typically, the piece of DNA consists of a plasmid, either for its cloning or for heterologous protein expression.

EXTERNAL LINK

https://www.addgene.org/protocols/bacterial-transformation/

GUIDELINES

Work at 4°C unless otherwise indicated.
Sterile conditions are needed. Work always next to the flame.

MATERIALS

NAME	CATALOG #	VENDOR
37˚C Incubator		
Shaker incubator		
LB agar plates with the proper antibiotic(s) (eg. Kanamycin)		
Positive transformation control DNA		
Competent Cells		
SOC Media		
Ice & ice bucket		
42°C water bath		
Microcentrifuge tubes		Eppendorf

SAFETY WARNINGS

Be careful about using disposable plastic gloves next to the flame. Burns are really painful.

BEFORE STARTING

Clean all the working surface with ethanol.
Make sure all the samples are placed on ice before start working.
The plates can be prepared and stored in advance.

Transformation

1 Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins). 3m

2 Chill approximately 5 ng (2 µl) of DNA in a 1.5 ml microcentrifuge tube. 1m

** Please note: if draw from MoClo protocol samples, add the whole 20 µl of DNA*

3 Add 50 µl of competent cells to the DNA. 30s
Mix gently by pipetting up and down or flicking the tube 4–5 times to mix the cells and DNA.
Do not vortex.

4 Place the mixture on ice for 30 minutes. 3m
Do not mix.

5 Heat shock at 42°C for 45 seconds. 04s
Do not mix.

6 Add 950 µl of room temperature LB or SOC media to the tube. 30s

7 Place tube at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate. 6m

** Please note: For the duration and temperature of the heat shock step as well as for the media to be used during the recovery period, please follow the recommendations provided by the competent cells' manufacturer.*

Selection of transformants

8 Prepare selection plates:

- LB + ampicillin 100 µg/mL
- LB + kanamycin 100 µg/mL
- LB + spectinomycin 50 µg/mL

9 Warm selection plates to 37°C. 1m

10 Spread 50–100 µl of the cells and ligation mixture onto the plates, and incubate O/N at 37°C. 1h

** Please note: Remember to add always a negative control plate (with medium without cells) to check contamination, and a positive control plate (same cells transformed with pUC) to check the efficiency of the process.*

** Tip: to ensure that all the positive results are plated, it is advisable to pellet the rest of the sample and resuspend ot in a very low volume (50-100 µl), and then plate it.*

11 Check the samples for contamination. If clean, continue the protocol.

1h

12 Choose three positive colonies of each plate.
Pick one with the inoculating loop and draw a strain in a new plate.
Do the same with the other two, using the same plate but leaving a separation between each strain.
Incubate O/N at 37°C.

** This will allow you to maintain and store in a separated plate the single clones chosen from each initial plate, and work with them.*

1h

13 Prepare a liquid inoculum in a 15 mL Falcon tube of each of the three strains: pick a colony with the inoculating loop, resuspend it in 5 mL LB + antibiotic, and incubate 37°C O/N.

** Tip: if the chosen colonies are big enough, you may be able to do both steps 12 and 13 together. Selection of recombinants*

Selection of recombinants

4m

14 Take a 1.5 mL sample of the Falcon tubes into a new eppendorf, and perform a miniprep.

15 Check the plasmids following the Plasmid Verification Protocol, and/or sequence them.