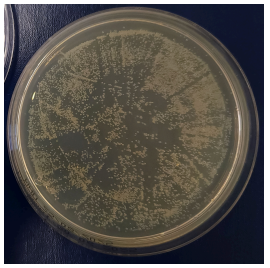
Oct 23, 2025 Version 3

Knockout of bla from pBlue in E. coli V.3



DOI
https://dx.doi.org/10.17504/protocols.io.n92ld62n9g5b/v3

Felix Hausmann[1], Karin Mink[2]

[1]Staffelsee Gymnasium Murnau; [2]TU Munic - Institute for Medical Microbiology and Immunology

W-Seminar AR

F Felix Hausmann

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld62n9g5b/v3

Protocol Citation: Felix Hausmann, Karin Mink 2025. Knockout of bla from pBlue in E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld62n9g5b/v3Version created by **Felix Hausmann**

Protocol status: Working
We use this protocol and it's working

Created: October 22, 2025

Last Modified: October 23, 2025

Protocol Integer ID: 230508

Keywords: CRISPR/Cas, CRISPR, anbitiotic resistance, e. coli, XL1-Blue, Cas9, pBlueSkriptII, bla gene from the pbluescript plasmid, disrupted bla gene, based crispr delivery, demonstrating efficient crispr, efficient crispr, bla gene, escherichia coli xl1, pbluescript plasmid, generation of bacterial colony, removal of antibiotic resistance marker, antibiotic resistance marker, preparation of crrna, bacteria, gene, bacterial colony, knockout of bla, complex formation with cas9

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

This protocol details the knockout of the bla gene from the pBluescript plasmid in Escherichia coli XL1-Blue by delivering a pre-assembled Cas9 ribonucleoprotein complex via electroporation. The method includes preparation of crRNA and tracrRNA, complex formation with Cas9, and subsequent transformation of electrocompetent cells. Expected results are the generation of bacterial colonies with a disrupted bla gene and loss of ampicillin resistance, demonstrating efficient CRISPR/Cas9-mediated plasmid editing. This outcome confirms the possibilites of RNP-based CRISPR delivery as a strategy for targeted removal of antibiotic resistance markers in bacteria.

Image Attribution

Guidelines

Make sure to stick to the instructions very closely, especially if you are at the CRISPR part. There you'll need to work with extreme caution to prevent cross-contamination and eventually invalidate your results.

Materials

Plasmid

- pBluescript SK II (+) (*c = 802 µg/µl*)

CRISPR-components

- crRNA targeting *bla*: 5'-GTATTATCCCGTATTGACGC-3'
- tracrRNA
- crRNA buffer
- Cas9-Protein

Bacteria

- *E. coli* XL1-Blue electrocompetent (*c = 1000 µg/µl*)

Electroporation

- Electroporation cuvettes (0.1 cm gap / 0.2 cm optional)
- Electroporation media

Note

Following EPM was used:

- [M] 2.43 millimolar (mM) K_2HPO_4
- [M] 0.57 nanomolar (nM) KH_2PO4
- [M] 272 nanomolar (nM) Saccharose
- [M] 15 Mass Percent Glycerin

- Electroporator

Culture

- LB-media

Note

Recipe for LB-media:

- [M] 171.1 millimolar (mM) NaCl
- [M] 1 Mass / % volume Tryptone
- [M] 0.5 Mass / % volume Yeast Extract
- [M] 1.5 Mass / % volume Agar

- SOC-media

Note

SOC-media with the following recipe was used:

- [M] 10 millimolar (mM) NaCl
- [M] 2.5 millimolar (mM) KCl
- [M] 2 Mass / % volume Tryptone
- [M] 0.5 Mass / % volume Yeast extract

- LB-agar/Ampicillin-Platte
- Plating spatulas

Equipment

- Thermoshaker
- Centrifuge
- Pipettes
- Ice
- Incubator: stationary and shaker

Software

- Benchling

Safety warnings

The components of this protocol are not designated as dangerous, however since you will be working with antibiotic resistant GMOs, the usage of safety gloves and a lab coat is highly recommended to prevent any contamination or similar.

Furthermore ensure that you plan your experiment according to the safety standards of your lab.

Inform yourself about:

- local waste disposal regulations
- lab specific operating instructions
- lab specific safety procedures
- more local restrictions when working with GMOs

Preparation of electrocompetent E.coli

1d 2h 5m

1 Retrieve XL-1 from the -80 °C freezer and let them thaw On ice

2 After thawing centrifugate 4500 rpm, 37°C, 00:05:00 5m

3 Seperate LB-media using pipettes

4 Resuspend XL1 pellets in [M] 50 µg/µL electroporation buffer

5 Add 1 µL PBSII+

6 Incubate cells in a shaker at 200 rpm, 37°C, 01:00:00 1h

7 Transfer 50 µL of the XL-1/buffer into a 0.1 cm electroporation cuvette

> Note
>
> You can also use a 0.2 cm cuvette for which you'll need to adapt the ep settings depending on your machine

7.1 Electroporate at 1.8-2.5 kV

8 Recover in 950 µL SOC at 37 °C for 01:00:00 1h

9 Plate on selective LB-Amp plates using a sterile plating spatula

Safety information

Make sure to not re-use the spatula to avoid cross-contamination and dispose in the right waste

10 Incubate **upside down** at 37 °C Overnight 1d

Note

Don't incubate longer than 18-24 hours

Expected result

When successful transformed, you should have multiple colonies growing on the plate

Picking transformed XL-1

11 Pick one bacterial colony from the plate using the tip of the pipette

12 Resuspend in 450 µL LBM

Note

To further increase validity of the results, you can also pick three more colonies. Resuspend the first again in 450 µL LMB to serve as control colony. The other two are resuspended in 950 µL LBM, for which one again is used as control colony.

12.1 Incubate in a shaker 200 rpm, 37°C, 00:10:00

Reconstitute lyophilized crRNA in buffer

13 Adjust crRNA concentration to 100 µL for RNP preparation

14 Dilute lyophylized crRNA in buffer depending on n(crRNA)

Note

Aliquots can be stored @ -20 °C for 1 month or at -80 °C for longer periods

Note

Use the following formula to determine the required volume of buffer for 100 µL

$$c = \frac{n}{V} \rightarrow V = \frac{n}{c}$$

Example: n(crRNA) = 2 µL

$$\frac{2 \cdot 10^{-9}\,\mathrm{mol}}{100 \cdot 10^{-6}\,\mathrm{mol\,L^{-1}}} = 2 \cdot 10^{-5}\,\mathrm{L} = 20\,\mu\mathrm{L}$$

RNP complex generation

15m

15 Mix tracrRNA with crRNA in a ratio of **1.2:1.2**

Safety information

- Work with **extreme caution** for the following section, since every µL can make a difference in the end result
- Make sure you're using separate tips for every step that follows to minimize the risk of unwanted dilution.
- Also ensure that you work according to lab specific genetic editing guidelines

Note

RNP complex consists of **tracrRNA:crRNA:Cas9** in a ratio of **1.2:1.2:2.6**

Note

tracrRNA & crRNA were reconstitued in [M] 100 micromolar (µM) each while the Cas9 is available in [M] 40 micromolar (µM)

16 Incubate at 96 °C for 00:05:00 5m

16.1 Cool down to Room temperature for around 00:10:00 10m
Spin down at 13000 rpm, 37°C, 00:03:00

17 In the meantime: bring Cas9 to Room temperature

18 Mix Cas9 to the sgRNA under continuous mixing

19 Incubate RNP complex at 500 rpm, 37°C, 00:15:00 approx. 15 min

Note

Final RNP complex can be stored @ 4 °C for around 2 weeks and at -80 °C for longer periods

Electroporation with Bio-Rad 1d 2h

20 Mix
- 50 µL XL-1/pBSII+
- 2-3 µL Cas9 RNP

21 Transfer to 0.1 cm cuvette

Note

It is also possible to use a 0.2 cm cuvette, however 0.1 cm cuvettes are preferred

21.1 Electroporate at 1.8-2.5 kV

22 Recover in 950 µL SOC at 400 rpm, 37°C, 02:00:00 2h

23 Plate on selective LB-Amp plates

Note

It's recommended that you'll plate on a LB-agar plate as a control plate to validate your results.
Furthermore it'll be helpful if you plate more than one plate

24 Incubate **upside down** at 37 °C Overnight 1d

Note

Don't incubate longer than **18-24** hours

Expected result

If everything has been successful you should see a reduction of the colonies growing on the LB-Agar plate compared to the control plate.
In the best case you shouldn't see any growing colonies on the LB-Amp plate