

Oct 24, 2020

Gene knockout

DOI

<https://dx.doi.org/10.17504/protocols.io.bnwzmff6>

Zhujun Wei¹

¹2020 iGEM NEFU China

2020 iGEM NEFU China



Zhujun Wei

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DOI: <https://dx.doi.org/10.17504/protocols.io.bnwzmff6>

Protocol Citation: Zhujun Wei 2020. Gene knockout. protocols.io <https://dx.doi.org/10.17504/protocols.io.bnwzmff6>

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Protocol status: Working

We use this protocol and it's working



Created: October 24, 2020

Last Modified: October 24, 2020

Protocol Integer ID: 43705

Keywords: gene knockout, protocol

Abstract

We use this protocol to knock out yhaK in E.coli BW25113

Materials

E. coli BW25113

pCas9 plasmid

LB medium

Sterilized 10% glycerol

1mM IPTG

Kana

Str

Safety warnings

 We use this protocol to knock out yhaK in E.coli BW25113

Before start

Please read the protocol carefully before doing the experiment.

- 1 Transfer the pCas9 plasmid into the recipient *E. coli* BW25113 (Kana resistance, temperature sensitive).
- 2 Preparation of pCas9 competent cells:
 - 2.1 Add 50 μ L of bacterial solution to 5 mL of LB medium, incubate at 30°C, 200 rpm for about 12 h, Add 2% to 100 mL LB medium, add 0.5% of arabinose at a final concentration after 0.5 hours of culture;
 - 2.2 Incubate at 30°C for about 2~2.5 h, until the OD600 is about 0.55-0.6, then take it out and let it stand on ice for 30 min;
 - 2.3 Collect cells at 4200 rpm for 10 min in a 4°C refrigerated centrifuge;
 - 2.4 Resuspend the cells gently with 10 ml of pre-cooled and sterilized 10% glycerol on ice.
 - 2.5 Repeat steps 2.3 and 2.4 three times, using 10 mL of pre-cooled and sterilized 10% glycerol for the first two washes. The last time it was resuspended with 0.5 mL of glycerol.
 - 2.6 Dispense in 100 μ L/tube into a pre-cooled centrifuge tube, it can be used for electroporation or immediately placed in a -80°C refrigerator. The bacteria can be stored at -80°C for half a year.
- 3 Prepare target segment: Clone upstream and downstream sequences of *yhaK* by PCR to about 500 bp each, then connect upstream and downstream homology arms together by overlap.
- 4 pTarget-*yhaK* vector construction (Str resistance): Download the *yhaK* gene sequence, Find the appropriate gRNA affinity site and replace 1961 to 1980 bases of the pTarget plasmid by digestion and ligation or overlap.
- 5 Electroporation:
 - 5.1 Add >200ng target fragment and >40ng pTarget-*yhaK* plasmid to 100 μ L competent cells on ice for 10~30 min, the plasmid and the ligation product should not exceed 5 μ L;
 - 5.2 UV-sterilize the washed and dried 2 mm electroporation cuvette for 20 min and pre-cooled on ice, then quickly transfer the competent cells to the bottom of the



electroporation cuvette;

- 5.3 Dry the outer wall of the cup. Immediately after the electric shock, add 900~1000 μ L of 37 °C preheated LB medium, mix gently, transfer to a 1.5 mL centrifuge tube, shake at 30 °C, 150 rpm, 45 ~ 60 min, then apply an appropriate amount of bacterial solution to the plate containing Kana and Str.
- 6 Knockout verification: Primers were designed at 700-1000 bp upstream and downstream of the yhaK in the genome to perform colony PCR validation on clones on the plates.
- 7 pTarget-yhaK elimination: Take the correct clone, add 1mM IPTG at 30°C for 12 h, streak to isolate the monoclonal. The clones were tested for the presence of Str resistance, clones with pTarget eliminated is not resistant to str. The pCas9 plasmid can be eliminated by incubation at 37°C.
- 8 Strains that retain the pCas9 plasmid can be used to knock out other genes.