Aug 04, 2021

E. coli CRISPR Barcoding

Leanne Hobbs[1]

[1]University of Newcastle-upon-Tyne

This protocol is published without a DOI.

Leanne Hobbs

ABSTRACT

This is the protocol for barcoding *E. coli* using CRISPR for CellRepo

EXTERNAL LINK

https://identifiers.org/cellrepo:59

PROTOCOL CITATION

Leanne Hobbs 2021. E. coli CRISPR Barcoding. **protocols.io** https://protocols.io/view/e-coli-crispr-barcoding-bw5jpg4n

CREATED

Aug 04, 2021

LAST MODIFIED

Aug 04, 2021

PROTOCOL INTEGER ID

52107

Day 1

1 Start an overnight culture (37 °C) by inoculating LB medium from a single colony. **00:00:00**

Day 2

2 Prepare competent cells following your favorite protocol.

3 Transform E. coli cells with plasmid pREDCas9 and plate the cells at 30 °C in LB supplemented with 50 µg/mL spectinomycin.

Day 3

4 Start an overnight culture at 30 °C in LB/Spec from a single colony.

Day 4

5 Next morning refresh the culture with LB/Spec and grow the cells until OD600 reaches 0.1.

6 Add IPTG to a final concentration of 2 mM and grow the cells to an OD600 =0.6 (recombination proteins are being expressed at this point).

7 Frost cells on ice for 20 minutes and electrocompetent cells are then prepared by washing bacteria with ice-cold milliQ water after spinning aliquots 10 minutes at 5000 rpm in a 4 °C centrifuge. (Heat shock transformation also could be performed)

8 After two washes, resuspend cells in the residual milliQ water and electroporate with pEC-CRISPR2-BC.

9 Add 950 µl of fresh LB without antibiotics and resuspend cells. Incubate cells 1 hour at 30 °C.

10 Then, spread 100 µL of bacterial culture on LB plates supplemented with Spec (50 µg/mL) /Amp (100 µg/mL).

Day 5

11 Check colonies for barcode presence by colony-PCR.

12 Inoculate a positive clone in 2 mL of LB/Spec/Ara (30 mM) (sgRNA targeting pUC origin in pEC-CRISPR2-BC is expressed).

13 Grow for 4-6 hours.

14 Plate on LB/Spec.

Day 6

15 Check some colonies for Ampicillin resistance by restreaking them on LB/Amp.

Day 7

16 Take a sensitive clone and restreak it on LB plates at 37°C.

Day 8

17 Check again by colony PCR the presence of the barcode and restreak on LB/Spec and LB/Amp plates to double check

plasmid curing.