Sep 23, 2019

Removal of kanamycinR gene from Keio collection strain

DOI
dx.doi.org/10.17504/protocols.io.7jqhkmw

Ben Kuipers[1]

[1]Wageningen University

iGEM Wageningen 2019

Ben Kuipers

DOI: https://dx.doi.org/10.17504/protocols.io.7jqhkmw

Protocol Citation: Ben Kuipers 2019. Removal of kanamycinR gene from Keio collection strain. **protocols.io https://dx.doi.org/10.17504/protocols.io.7jqhkmw**

Protocol status: Working
We use this protocol in our group and it is working. (This protocol can also be completed in 3 days, starting after the overnight culture).

Created: September 23, 2019

Last Modified: September 23, 2019

Protocol Integer ID: 27984

Keywords: kanamycinr gene from the jw3367 strain, removal of kanamycinr gene, kanamycinr gene, flp recombination, step inactivation of chromosomal gene, jw3367 strain, chromosomal gene, gene, using pcr product, step inactivation, removal

Abstract

This protocol is used to remove the kanamycinR gene from the JW3367 strain from the Keio Collection.
References Datsenko, K.A., and Wanner, B.L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U.S.A. 97: 6640-6645.
Barrick Lab. (16 May, 2014). FLP Recombination in E. coli. Retrieved 3 September, 2016, from http://barricklab.org/twiki/bin/view/Lab/ProcedureFLPFRTRecombination
T--Hong_Kong_HKUST--Protocol_RemoveKAN.pdf

Troubleshooting

1 Chemical competent cells of E. coli JW0336 were prepared with the mix and go protocol.

2 Day 1: Transformation of the recombinase plasmid pCP20

- The competent cells were transformed with plasmid pCP20. (This plasmid has a temperature-sensitive origin of replication, resistant to ampicillin and chloramphenicol. With the presence of the FLP recombinase, the ampicillin and chloramphenicol resistance genes would be removed under the temperature higher than 43ºC).
- 1 ml LB was added for recovery which lasted for 1 hour.
- The eppendorf was centrifuged for 2.5 minutes with 11000xg. Supernatant was discarded and the pellet was resuspended and plated on the LB + AMP plate.

The plate was then incubated for 1.5 days under 30ºC.

3 Day 2: Induction of the recombination

- Three colonies were picked and inoculated into 5 mL liquid LB cultures.

The tubes were shaken overnight at 45ºC so as to induce FLP recombinase expression and select for the loss of pCP20.

4 Day 3: Plating to get single candidate recombinants

- 100-fold dilution of the overnight culture was made by using fresh LB.
- 50 ul of the diluted overnight culture was plated on LB plate.

The plate was then incubated for 1.5 days under 30ºC to prevent partial loss of plasmid from the colonies which developed from the cells containing pCP20.

5 Day 4: Screening for genomic recombination and plasmid loss

- 6 colonies were picked from each plate and resuspended in MilliQ
- Little amount of the MilliQ with cells was streaked on the LB plate(without antibiotics). The remaining solution was divided over LB + AMP, LB + CHL and LB + KAN plates dropwise for screening.

The plate was then incubated overnight under 37ºC.

6 Day 5: Obtaining successful recombinants.

- The candidate which was sensitive to all antibiotics would be selected and archived.