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in vivo cloning (iVEC)

Yuichiroh Ikagawa[1]

[1]iGEM Gifu

protocol .

iGEM Gifu

Yuichiroh Ikagawa

In vivo cloning is a seamless cloning method in which a DNA fragment, to which a homologous sequence has been added by PCR, is introduced directly into *Escherichia coli* SN1187 for assembly and cloning in the bacterium. The 5' end of the DNA fragment is degraded by Exonuclease III (XthA) from *E. coli* strain SN1187. The degradation generates cohesive ends, which allow the fragments to hybridize to each other and to be repaired by DNA polymerase. Finally, the nick is repaired by DNA ligase and the plasmid is constructed in the bacterium. Here we describe a protocol for the preparation and transformation of a competent cell, based on the paper and materials by Nozaki et al. [1], [2]

[1]Shingo Nozaki, Hironori Nikia. Exonuclease III (XthA) Enforces In Vivo DNA Cloning of Escherichia coli To Create Cohesive Ends. Journal of Bacteriology, Volume 201, Issue 5, (2019)
[2]iVEC3 株の解説及び使用方法 - SHIGEN
https://shigen.nig.ac.jp/ecoli/strain/download/pdf/strainGeneMutant/iVEC3_jp_20170706.pdf;jsessionid=0282CEF77B5F3B6DF187E89A2867CC05

Yuichiroh Ikagawa 2021. in vivo cloning (iVEC). **protocols.io**
https://protocols.io/view/in-vivo-cloning-ivec-bzcmp2u6

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Reagents

Escherichia coli SN1187
LB medium
SOC medium
2x TSS
DMSO
Liquid nitrogen
LB agar plate(includes ampicillin/chloramphenicol)

competent cell preparation

1 Scrape the glycerol stock of *Escherichia coli* strain SN1187 and inoculate it into 3ml of LB medium.

2 Incubate at 37ºC overnight (16-18 hours) with shaking.

3 Inoculate 1 ml of the overnight culture into 60 ml of LB medium warmed to 37 ºC.

4 Incubation at 37 ºC with shaking until reached 0.4-0.5

5 Chill the medium in ice.

6 Centrifuge 5,000 g, at 4º C for 5 min.

7 Discard the supernatant and suspend the pellet in 2 ml of ice-cold LB medium.

8 Add 1.6 ml of ice-cold 2xTSS solution and mix by pipetting

9 Add 400 µl of DMSO and mix by pipetting.

10 Dispense 100 µl each on ice.

11 Freeze in liquid nitrogen and store at -80 ºC.

Transformation

12 Thaw the 100 µl competent cell *E. coli* SN1187 strain on ice.

13 Add 100 ng of DNA samples (vector and insert) into the competent cell.

14 Mix by gently pipetting and incubate for 20 minutes on ice.

15 Add 1 ml SOC medium and mix by carefully inverting

16 Incubate at 37°C for 1 hour.

17 Centrifuge at 5,000 g for 1 minute at room temperature

18 Discard 1 ml of the supernatant.

19 Resuspend cells with the remaining supernatant by Voltex

20 Spread the whole culture on an agar plate with the antibiotics (ampicillin/chloramphenicol) for selection.

21 Incubate overnight at 37°C.