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in vitro assembly and transformation

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[1]iGEM Gifu

protocol .

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For the in vitro assembly of the DNA fragments, we decided to use the NEBuilder®, an assembly kit based on the Gibson Assembly. In NEBuilder®, exonucleases break down the ends of the DNA fragments and hybridize the cohesive ends, followed by DNA polymerases that synthesize the broken strands. Finally, DNA ligase repairs the nick and completes the DNA assembly. it is expected that in vitro assembly will reliably transform the cyclized plasmid.

For the subsequent transformation, we used the NEB® 10-beta competent cell because NEB® 10-beta can be largely maintained the cloning efficiency even with high molecular weight plasmids, which we considered to be an advantage over other strains when transforming our team's plasmids.

Yuichiroh Ikagawa 2021. in vitro assembly and transformation. **protocols.io** https://protocols.io/view/in-vitro-assembly-and-transformation-bzc9p2z6

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Reagents

DNA samples

- Cas12a fragment 1
- Cas12a fragment 2
- Cas12a fragment 3
- pSB1A3

NEBuilder® Assembly Master Mix (New England Biolabs)

NEB® 10-beta competent cell (New England Biolabs)

SOC medium

LB agar plate

in vitro assembly

1 Thaw the DNA solution and NEBuilder® Assembly Master Mix on ice.

2 Mix the DNA solution by vortexing, and centrifuge to collect the solution to the bottom of the tube.

3 DNA solutions and reagents were mixed according to the compositions in the table below.

A	B	C
COMPONENT	VOLUME(µl)	CONCENTRATION
NEBuilder Assembly Master Mix	10.0	×2
Cas12a fragment 1	0.75	0.34 pmol
Cas12a fragment 2	0.90	0.35 pmol
Cas12a fragment 3	1.00	0.33 pmol
pSB1A3	0.60	0.36 pmol
Nuclease Free water	6.75	
Total Volume	20.0	

4 Incubate at 37°C for 1 hour.

transformation

5 Thaw *Escherichia coli* NEB® 10-beta competent cell on ice.

6 Add 2 µl assembled sample into competent cell tube.

7 Mix gently pipetting 4~5 times.

8 Incubate on ice for 30 minutes.

9 Heat shock at 42°C for 30 seconds on heat block.

10 Incubate on ice for 2 minutes.

11 Add 950 µl of SOC medium and mix gently pipetting.

12 Incubate the tube at 37°C for 1 hour.

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

14 Discard 900 µl of the supernatant.

15 Resuspend cells with the remaining supernatant by Voltex

16 Spread the whole culture on agar plates containing ampicillin.

17 Incubate overnight at 37°C