

6.pYB1a-mutator-T7(wt promoter) Qianwen Jin

6.1 Obtain pYB1a-CDA-TadA-UGI fragment by Enzyme digestion

The target fragment is located on the pYB1a-CDA-TadA-UGI plasmid, and use the enzyme SalI-HF to cut the plasmid pYB1a-CDA-TadA-UGI to Obtain pYB1a-CDA-TadA-UGI fragment. The following is the system of digestion.

Digestion system (50μL)	
pYB1a-CDA-TadA-UGI	1000ng
SalI-HF	1μL
custsmart	5μL
DDW	to 50μL

Table 1

We obtained the correct target fragment, and the sample is purified.

6.2 Obtain T7(wt promoter) fragment by PCR

The target fragment is located on the BL21(DE3), and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
BL21(DE3)	1μL
WT T7-G-F	2μL
terminator-overlap-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

The correct target fragment is 3145bp. We obtained the correct target fragment, and the sample is purified.

6.3 Obtain SH₃ fragment by PCR

The target fragment is located on the pYB1a-Phi29-SH₃ plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pYB1a-Phi29-SH ₃	10ng
linker-overlap-F	2μL
anderson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 3

The correct target fragment is 662bp. We obtained the correct target fragment, and the sample is purified.

6.4 Obtain T7(wt promoter)-SH₃ fragment by Overlap

The Overlap system and procedure are showed below.

Overlap system (50μL)	
T7(wt promoter)	1μL
SH ₃	1μL
WT T7-G-F	2μL
anderson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 4

The correct target fragment is 3807bp. We obtained the correct target fragment, and the sample is purified.

6.5 Gibson connection

The T7(wt promoter)-SH₃ fragment and pYB1a-CDA-TadA-UGI fragment are connected by Gibson connection method, and the connection system is as follows.

Connection system (20μL)	
T7(wt promoter)-SH ₃	9μL
pYB1a-CDA-TadA-UGI	5μL
5 x Cell Buffer	2μL
Exnase II	1μL
DDW	To 20μL

Table 5

6.6 Gibson connection

The T7(wt promoter) fragment, SH₃ fragment and pYB1a-CDA-TadA-UGI fragment are connected by Gibson connection method, and the connection system is as follows.

Connection system (20μL)	
T7(wt promoter)	1μL
SH ₃	1μL
pYB1a-CDA-TadA-UGI	5μL
2xClonExpress	10μL
DDW	3μL

Table 6

6.7 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 10 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μ L)	
pYB1a-mutator-T7(wt promoter)	1 μ L
T7RNAP-cexu-F	0.4 μ L
Ori-cexu-R2 x Mix	0.4 μ L
2 x Mix	5 μ L
DDW	3.2 μ L

Table 7

The PCR products were detected by agarose gel electrophoresis, and the results were as follows:

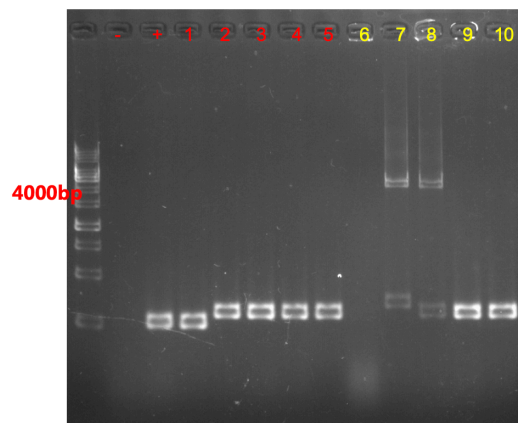


Figure 1

The correct target fragment is about 4188bp, and the length of the colony PCR sample in lanes 7 and 8 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

6.8 Enzyme digestion verification

Use the enzyme BglII to cut the plasmid pYB1a-mutator-T7(wt promoter) at the same time to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10 μ L)	
pYB1a-mutator-T7(wt promoter)	100ng
Bgl II	0.2 μ L
custsmart	1 μ L
DDW	to 10 μ L

Table 5

Digested pYB1a-mutator-T7(wt promoter) is verified by electrophoresis which is showed below.

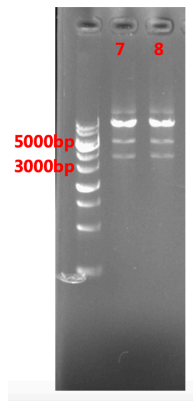


Figure 2

The correct target fragment is about 3591bp and 5117bp. It is inferred from the gel electrophoresis that the length of the sample is as expected. The construction is preliminarily correct. We sequenced the constructed plasmid. The result shows that the sequence is the same as expected, indicating that our target plasmid has been constructed successfully.