

4 月

4.1

1. pYB1a-PobR-mCherry-cmr Xinyao Yuan

1.1 Obtain mcherry by PCR

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

PCR system (50μL)	
pYB1a-PobR-mcherry-SacB	10ng
MCHERRY-F	2μL
MCHERRY-R	2μL
2 x Mix	25μL
DDW	To 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 747bp. We obtained the correct target fragment, and the sample is purified.

1.2 Obtain pYB1a-PobR-Cmr fragment by PCR

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

PCR system (50μL)	
pYB1a-PobR-eGFP-Cmr	10ng
zaitiyinwu-F	2μL
zaitiyinwu -R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

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

1.3 Gibson connection

The glb2a-mcherry fragments and pYB1a-PobR-mcherry-cmr fragments are connected by Gibson connection method, and the connection system is as follows.

Connection system (10μL)	
pYB1a-PobR-cmr	69.79ng
mcherry	27.85ng
5 x Cell Buffer	2μL
Exase II	1μL

DDW	to 10 μ L
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Table 3

1.4 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-PobR-mcherry-cmr	1 μ L
T7-SH3-cexu-F	0.2 μ L
T7 poly-jp-R	0.2 μ L
2 x Mix	5 μ L
DDW	to 10 μ L

Table 4

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

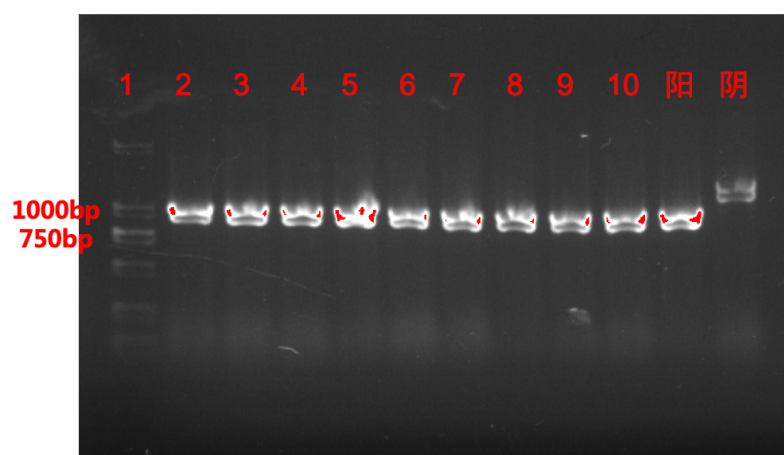


Figure 1

The length of the colony PCR sample in lanes 4 and 10 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

1.5 Enzyme digestion verification

Use EcoR I to cut the plasmid pYB1a-PobR-mCherry-cmr to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10 μ L)	
pYB1a-PobR-mCherry-cmr	100ng
EcoR I	0.2 μ L
Buffer	01 μ L
DDW	to 10 μ L

Table 5

Digested pYB1a-PobR-mCherry-cmr is verified by electrophoresis which is showed below.

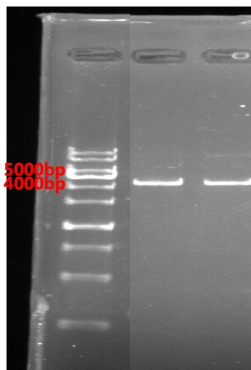


Figure 2

It is inferred from the gel electrophoresis that the length of the sample in lanes 4 and 10 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.

2. pYT1a-ttgR-mCherry-Cmr Qianwen Jin

2.1 Obtain ttgR fragment by PCR

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

PCR system (50μL)	
Plasmid(ttgR)	10ng
TtgR-Gibson-F	2μL
TtgR-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 633bp. We obtained the correct target fragment, and the sample is purified.

2.2 Obtain pYB1a-mcherry-cmr fragment by PCR

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

PCR system (50μL)	
pYB1a-PobR-mCherry-	10ng

cmr	
PYBla-Gibson-F	2 μ L
PYBla-Gibson-R	2 μ L
2 x Mix	25 μ L
DDW	to 50 μ L

Table 2

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

2.3 Gibson connection

The ttgR fragment and pYB1a-mcherry-cmr fragment are connected by Gibson connection method, and the connection system is as follows.

Connection system (10μL)	
ttgR	0.25 μ L
pYB1a-mCherry-cmr	1 μ L
5 x Cell Buffer	2 μ L
Exnase II	1 μ L
DDW	5.75 μ L

Table 3

2.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 9 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10μL)	
pYT1a-ttgR-mCherry-Cmr	1 μ L
TTGR-JUNP-F	0.4 μ L
pYB1a-JUNP-R	0.4 μ L
2 x Mix	5 μ L
DDW	3.2 μ L

Table 4

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

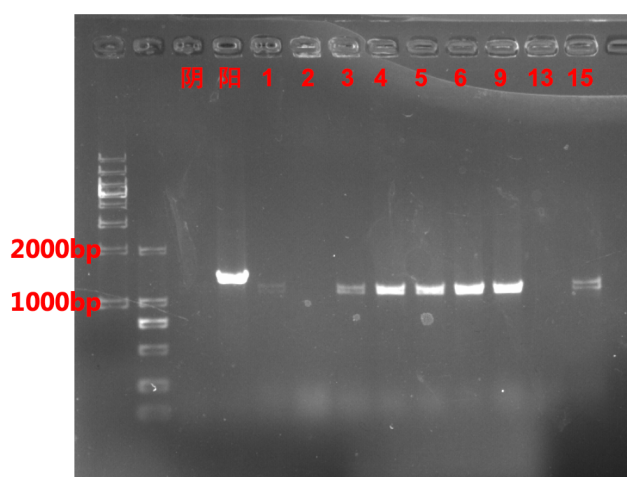


Figure 1

The correct target fragment is about 1182bp, and the length of the colony PCR sample in lanes 9, and 15 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

3.1 Enzyme digestion verification

Use the enzyme XhoI and XbaI to cut the plasmid pYT1a-ttgR-mcherry-Cmr at the same time to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10μL)	
pYT1a-ttgR-mCherry-Cmr	100ng
XhoI	0.2μL
XbaI	0.2μL
custsmart	1μL
DDW	to 10μL

Table 5

Digested pYT1a-ttgR-mCherry-Cmr is verified by electrophoresis which is showed below.

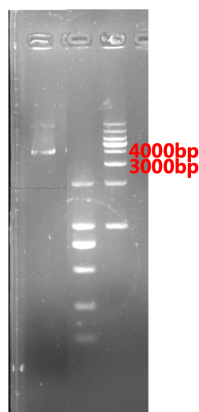


Figure 2

It is inferred from the gel electrophoresis that the length of the sample is not as expected. But 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.

3.2 Obtain kana fragment by PCR

The target fragment is located on the gld2k, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
gld2k	10ng
kana-Gibson-F	2μL
kana-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 816bp. We obtained the correct target fragment, and the sample is purified.

3.3 Obtain pYT1a-ttgR-mcherryfragment by PCR

The target fragment is located on the pYT1a-ttgR-mcherry-cmr plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pYT1a-ttgR-mcherry-cmr	10ng
pYT1a-gibson-F	2μL
pYT1a-gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 3595bp. We obtained the correct target fragment, and the sample is purified.

3.4 Gibson connection

The ttgR fragment and pYB1a-mCherry-cmr fragment are connected by Gibson connection method, and the connection system is as follows.

Connection system (10 μ L)	
Kana	2.7 μ L
pYB1a-ttgR-mCherry-cmr	1.6 μ L
5 x Cell Buffer	2 μ L
Exnase II	1 μ L
DDW	To 10 μ L

Table 3

3.5 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 5 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μ L)	
pYT1a-ttgR-mCherry-kana	1 μ L
kana-JUNP-F	0.4 μ L
kana-JUNP-R	0.4 μ L
2 x Mix	5 μ L
DDW	3.2 μ L

Table 4

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

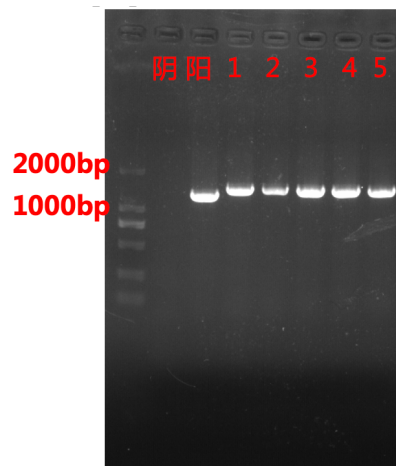


Figure 1

The correct target fragment is about 1381bp, and the length of the colony PCR sample in lanes1, 2, 3,4 and 5 is inferred from the gel electrophoresis image is correct. The above 5 strains were expanded and the plasmids were put forward.

3.6 DNA sequencing

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.

4. pYT1a-ttgRmut-mCherry-Kana Jiale Li

4.1 Obtain ttgRmut fragment by PCR

The target fragment is located on the PYT1a-ttgR-mCherry-kana, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
PYT1a-ttgR-mCherry-kana	10ng
spm-38-F-new-2	2μL
spm-38-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 4862bp. We obtained the correct target fragment, and the sample is purified.

4.2 DNA sequencing

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.

5. pYT1a-responsive-ttgR Bohui Yangyang

5.1 Obtain pYT1a-responsive-ttgR fragment by PCR

The target fragment is located on the PYT1a-ttgrmut-mCherry-kana, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pYT1a-ttgrmut-mCherry-kana	10ng
mCherry-40 bp promoter-F	2μL
ttgr-40 bp promoter-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 4645bp. We obtained the correct target fragment, and the sample is purified.

5.2 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 2 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10μL)	
PYT1a-responsive-ttgR	1μL
ttgr-40bp ce-F	0.4μL
ttgr-40bp ce-R	0.4μL
2 x Mix	5μL
DDW	3.2μL

Table 2

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

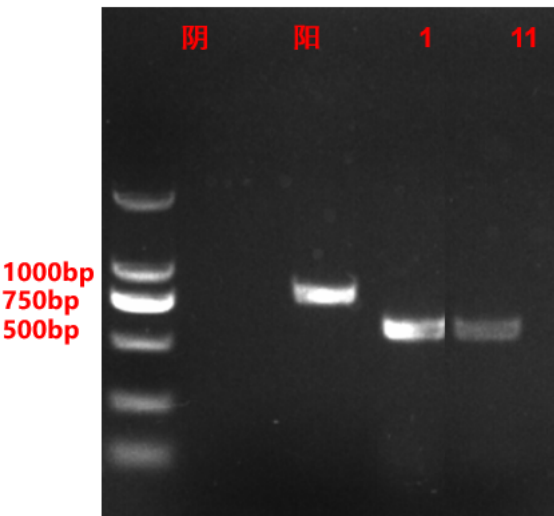


Figure 1

The correct target fragment is about 590bp, and the length of the colony PCR sample in lanes 1, and 11 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

5.3 DNA sequencing

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.

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 Sall-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
Sall-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 (10μL)	
pYB1a-mutator-T7(wt promoter)	1μL
T7RNAP-cexu-F	0.4μL
Ori-cexu-R2 x Mix	0.4μL
2 x Mix	5μL

DDW	3.2 μ L
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Table 7

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

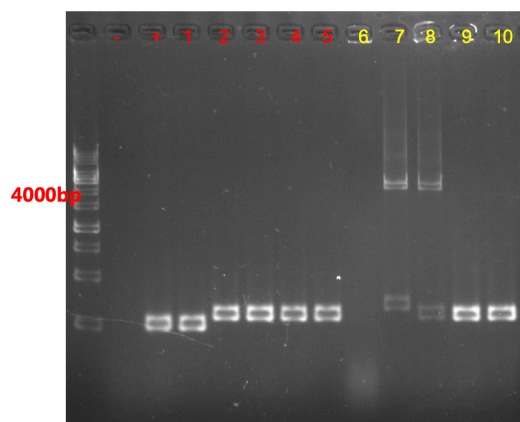


Figure 1

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.

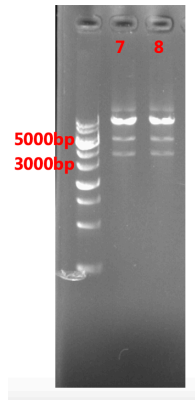


Figure 2

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.

7. pYB1a-mutator(mut) Xinyao Yuan

7.1 Obtain pYB1a-mutator(mut) by PCR

The target fragment is located on the pYB1a-mutator-wt T7 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pYB1a- mutator-wt T7	10ng
T7 pol R==0629	2μL
Linker-SH3-F==0629	2μL
2 x Mix	25μL
DDW	To 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 8498bp. We obtained the correct target fragment, and the sample is purified.

7.2 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 15 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10μL)	
pYB1a-mutator(mut)	1μL
SH3-cexu-F	0.2μL
ORI-cexu-R	0.2μL
2 x Mix	5μL

DDW	to 10 μ L
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Table 2

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

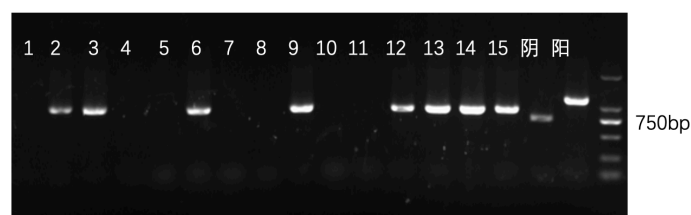


Figure 1

The length of the colony PCR sample in lanes 2、3、6、9、12、13、14、15 are inferred from the gel electrophoresis image are correct. The 9、13 and 14 strains were expanded and the plasmids were put forward.

7.3 Enzyme digestion verification

Use EcoR I to cut the plasmid pYB1a-mutator(mut) to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10 μ L)	
pYB1a-PobR-mcherry-cmr	100ng
EcoR I	0.2 μ L
Buffer	01 μ L
DDW	to 10 μ L

Table 3

Digested pYB1a-mutator(mut) is verified by electrophoresis which is showed below.

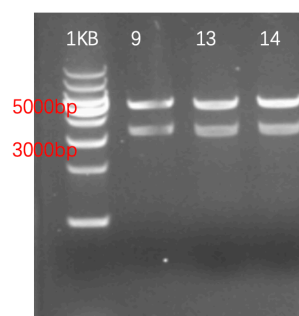


Figure 2

The correct target fragment is 3371bp and 5117bp. It is inferred from the gel electrophoresis that the length of the sample in lanes 9、13 and 14 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.

8. pRBIS-single-T7 Jianing Li

8.1 Obtain Part1 by PCR

The target fragment is located on the pRB1s-hmaS plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pRB1s-hmaS	10ng
Part1-F	2 μ L
str-overlap-R	2 μ L
2 x Mix	25 μ L
DDW	To 50 μ L

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1900bp. We obtained the correct target fragment, and the sample is purified.

8.2 Obtain Part2 by PCR

The target fragment is located on the pRB1s-hmaS plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pRB1s-hmaS	10ng
LacI-overlap-F	2 μ L
part2-R	2 μ L
2 x Mix	25 μ L
DDW	To 50 μ L

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1600bp. We obtained the correct target fragment, and the sample is purified.

8.3 Obtain Part3 by PCR

The target fragment is located on the Peasy-phi29-linker-nCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
Peasy-phi29-linker-nCas9	10ng
part3-F	2 μ L
Part3-R	2 μ L
2 x Mix	25 μ L
DDW	To 50 μ L

Table 3

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 150bp. We obtained the correct target fragment, and the sample is purified.

8.4 overlap

The part1 fragments and part2 fragments are connected by overlap method, and the overlap system is as follows.

Overlap system (50 μ L)	
part1	1 μ L
part2	1 μ L
part1-F	1 μ L
Part2-R	1 μ L
2x Mix	25 μ L
DDW	21 μ L

Table 4

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 3500bp. We obtained the correct target fragment, and the sample is purified.

8.5 goldengate connection

The part1-2 fragments and part3 fragments are connected by Gibson goldengate method, and the connection system is as follows.

Connection system (20 μ L)	
Part1-2	1 μ L
part3	1 μ L
BsaI	0.5 μ L
T4 ligase	0.2 μ L
10 \times BSA buffer	1 μ L
T4 buffer	1 μ L
DDW	5.3 μ L

Table 5

8.6 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 6 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μ L)	
pYB1a-PobR-mcherry-cmr	1 μ L
Part1-F	0.2 μ L
Str-overlap-R	0.2 μ L
2 x Mix	5 μ L
DDW	to 10 μ L

Table 6

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

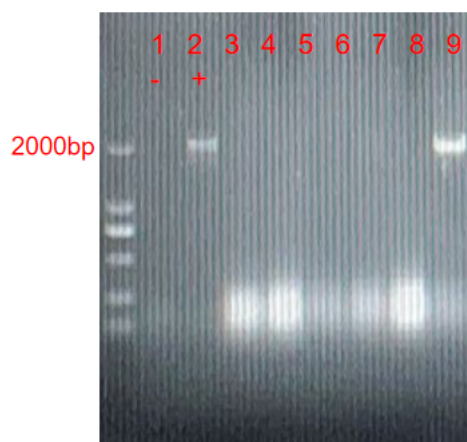


Figure 1

The length of the colony PCR sample in lanes 9 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

8.7 Enzyme digestion verification

Use EcoR I and Xho I to cut the plasmid pRBIS-dualT7 to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10μL)	
pRB12S-dualT7	100ng
EcoR I	0.2μL
Xho I	0.2μL
cutsmart	1μL
DDW	to 10μL

Table 5

Digested pRBIS-dual-T7 is verified by electrophoresis which is showed below.

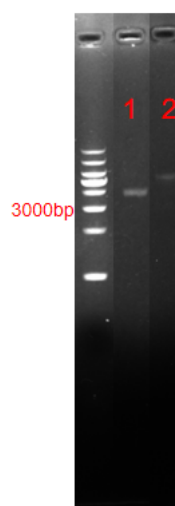


Figure 2

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.

9.pRBI2s-single-T7-STS Xinyao Yuan

9.1 Enzyme digestion

Use two enzymes Xho I to cut the plasmid pRBI2s-single-T7 The following is the system of digestion system.

Enzyme digestion system (50μL)	
pRBI2s-single-T7	10ng
Xho I	1μL
Cutsmart	5μL
DDW	To 50μL

Table 1

The Enzyme digestion products are detected by agarose gel electrophoresis, and the correct target fragment is 3500bp. We obtained the correct target fragment, and the sample is purified.

9.2 Obtain STS by PCR

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

PCR system (50μL)	
STS plasmid	10ng
STS-G-F	2μL
STS-G-R	2μL
2 x Mix	25μL
DDW	To 50μL

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1179bp. We obtained the correct target fragment, and the sample is purified.

9.3 Gibson connection

The STS fragments and pRBI2s-single-T7 are connected by Gibson connection method, and the connection system is as follows.

Connection system (20μL)	
pRBI2S-single-T7	1.2μL
STS	1.4μL
5×CE Buffer	2μL
ExhaseII	1μL
DDW	4.4μL

Table 3

9.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 11 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10μL)	
pRBI2s-single-T7-STS	1μL
STS-JUNP-F	0.2μL
RSF-JUNP-R	0.2μL
2 x Mix	5μL
DDW	to 10μL

Table 4

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

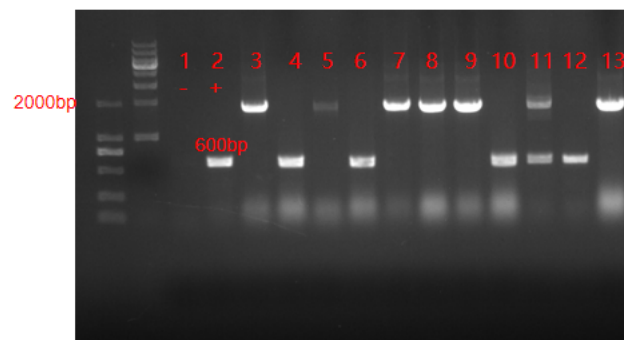


Figure 1

The length of the colony PCR sample in lanes 3、5、7、8、9、13 are inferred from the gel electrophoresis image are correct. The 7 and 8 strains were expanded and the plasmids were put forward.

9.5 Enzyme digestion verification

Use Xho I to cut the plasmid pRBI2s-single-T7-STS to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10μL)	
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pRBI2s-single-T7-STS	100ng
Xho I	0.2μL
cutsmart	1μL
DDW	to 10μL

Table 5

Digested pRBI2s-single-T7-STS is verified by electrophoresis which is showed below.

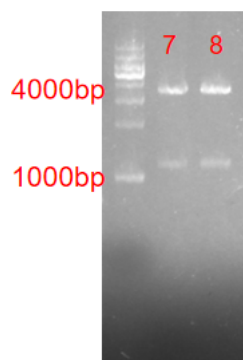


Figure 2

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.