

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-cmr	10ng
PYB1a-Gibson-F	2μL
PYB1a-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

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 (10 μ L)	
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 (10 μ L)	
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

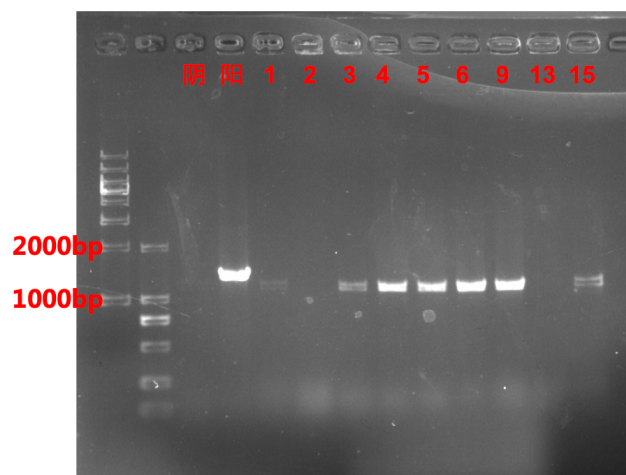


Figure 1

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.

1.5 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	0.2 μ L
XbaI	0.2 μ L
custsmart	1 μ L
DDW	to 10 μ L

Table 5

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

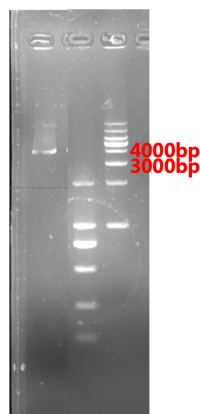


Figure 2

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.