

3. pYT1a-ttgR-mCherry-kana Qianwen Jin

3.1 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.

3.2 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.

3.3 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)	
Kana	2.7μL
pYB1a-ttgR-mCherry-cmr	1.6μL
5 x Cell Buffer	2μL

Exnase II	1 μ L
DDW	To 10 μ L

Table 3

3.4 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

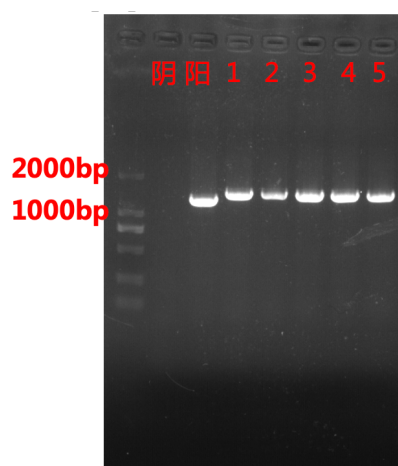


Figure 1

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.

3.5 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.

