

### 3. pYT1a-ttgR-mCherry-kana Qianwen Jin

#### 3.1 Obtain kana fragment by PCR

The target fragment is located on the glld2k, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

<b>PCR system (50<math>\mu</math>L)</b>	
glld2k	10ng
kana-Gibson-F	2 $\mu$ L
kana-Gibson-R	2 $\mu$ L
2 x Mix	25 $\mu$ L
DDW	to 50 $\mu$ L

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.

<b>PCR system (50<math>\mu</math>L)</b>	
pYT1a-ttgR-mcherry-cmr	10ng
pYT1a-gibson-F	2 $\mu$ L
pYT1a-gibson-R	2 $\mu$ L
2 x Mix	25 $\mu$ L
DDW	to 50 $\mu$ L

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.

<b>Connection system (10<math>\mu</math>L)</b>	
Kana	2.7 $\mu$ L
pYB1a-ttgR-mCherry-cmr	1.6 $\mu$ L
5 x Cell Buffer	2 $\mu$ L

Exnase II	1 $\mu$ L
DDW	To 10 $\mu$ 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 $\mu$ L)	
pYT1a-ttgR-mCherry-kana	1 $\mu$ L
kana-JUNP-F	0.4 $\mu$ L
kana-JUNP-R	0.4 $\mu$ L
2 x Mix	5 $\mu$ L
DDW	3.2 $\mu$ L

Table 4

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

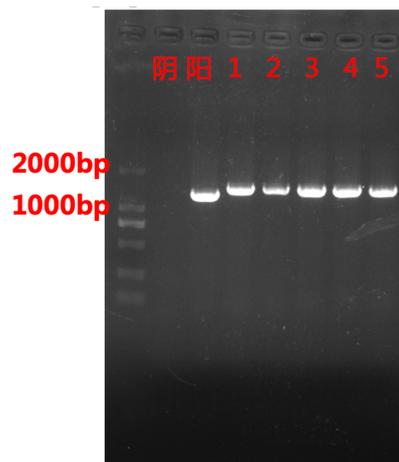


Figure 1

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.

