

5. pYT1a-responsive-ttgR Bohui Yangyang

5.1 Obtain pYT1a-responsive-ttgR fragment by PCR

The target fragment is located on the pYT1a-ttgmut-mCherry-kana, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
pYT1a-ttgmut-mCherry-kana	10ng
mCherry-40 bp promoter-F	2μL
ttg-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
ttg-40bp ce-F	0.4μL
ttg-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

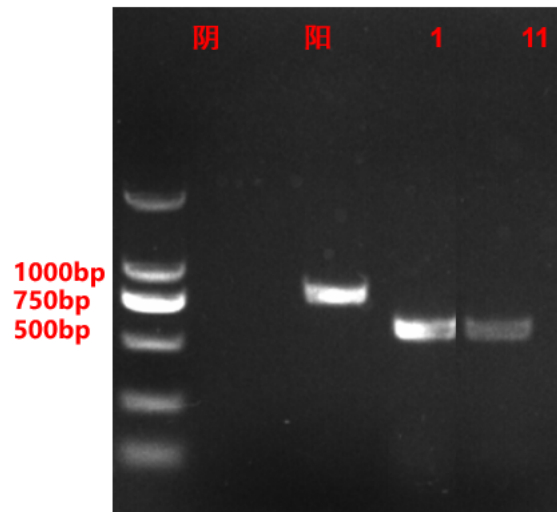


Figure 1

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.