

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.