

#### 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 (50 $\mu$ L)	
PYT1a-ttgR-mCherry-kana	10ng
spm-38-F-new-2	2 $\mu$ L
spm-38-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 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.