3. R6K-dCas9-eGFP

3.1 Obtain R6k-pir fragment by PCR

The target fragment is located on the pLl2s-dual-T7-HmaS plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system（50 μL）	
pLl2s-dual-T7-HmaS	10 ng
pir-F=0719	2 μL
R6K-R=0719	2 μL
2 x Mix	25 μL
DDW	To 50 μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1414bp. We obtained the correct target fragment, and the sample is purified.

3.2 Obtain KanR-dCas fragment by PCR

The target fragment is located on the pSC101-dCas9-eGFP plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system（50 μL）	
pSC101-dCas9-eGFP	10 ng
R6K-F-0719	2 μL
dCas9-m2-R=0709	2 μL
2 x Mix	25 μL
DDW	To 50 μL

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 4535bp. We obtained the correct target fragment, and the sample is purified.

3.3 Obtain dCas-lacI fragment by PCR

The target fragment is located on the pSC101-dCas9-eGFP plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system（50 μL）	
pSC101-dCas9-eGFP	10 ng
dCas9-m2-F=0709	2 μL
pir-R=0719	2 μL
2 x Mix	25 μL
DDW	To 50 μL

Table 3

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 3465bp. We obtained the correct target fragment, and the sample is purified.

3.4 Gibson connection

The R6k-pir fragment, KanR-dCas fragment and dCas-lacI fragment are connected by Gibson

connection method, and the connection system is as follows.

Connection system（10 μL）	
R6k-pir	1 μL
KanR-dCas	1 μL
dCas-lacI	1 μL
2 x ClonExpressMix	5 μL
DDW	2 μL

Table 4

3.5 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 10 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system（10 μL）	
R6K-dCas9-eGFP	1 μL
link-GG-F=0615	0.2 μL
link-GG-F=0615	0.2 μL
2 x Mix	5 μL
DDW	3.6 μL

Table 5

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

The correct target fragment is about 1711bp, and The positive rate was approximately 80%. and the length of the colony PCR sample in lanes 1 is inferred from the gel electrophoresis image is correct. The above strain were expanded and the plasmids were put forward.

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.