4. R6K-dCas9-sgeGFP-M library

4.1 Obtain R6K-dCas9-eGFP-M-1 fragment by PCR

The target fragment is located on the R6K-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
pCas-vec-GG-F=0615	2 μL
pCas9-vec-GG-R=0615	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 5015bp. We obtained the correct target fragment, and the sample is purified.

4.2 Obtain R6K-dCas9-eGFP-M-2 fragment by PCR

The target fragment is located on the R6K-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
2-F	2 μL
M2-R	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 4348bp. We obtained the correct target fragment, and the sample is purified.

4.3 Gibson connection

The pSC101-dCas9-eGFP-M-1 fragment and R6K-dCas9-eGFP-M-2 fragment are connected by Goldengate connection method, and the connection system is as follows.

Connection system（10 μL）	
R6K-dCas9-eGFP-M-1	1 μL
R6K-dCas9-eGFP-M-2	1 μL
Cutsmart	1 μL
T4 Buffer	1 μL
BsaI	0.5 μL
T4 ligase	0.2 μL
DDW	3.3 μL

Table 3

4.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 48 colonies were selected on the plate. The

colony PCR system and procedure were as follows.

PCR system（10 μL）	
pSC101-dCas9-eGFP-M	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 4

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%. We collectively transferred the positive monoclonal colonies identified through colony PCR into a single LB medium for Extended cultivation, and the plasmids were put forward as a mutation library.