## 1. pSC101-dCas9-eGFP

### 1.1 Obtain pSC101-vec fragment by PCR

The target fragment is located on the pCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| **PCR system（50 μL）** | |
|---|---|
| pCas9 | 10 ng |
| pCas-vec-GG-F=0615 | 2 μL |
| pCas-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 4842bp. We obtained the correct target fragment, and the sample is purified.

### 1.2 Obtain dCas9-m fragment by PCR

The target fragment is located on the pCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| **PCR system（50 μL）** | |
|---|---|
| pCas9 | 10 ng |
| dCas9-m1-GG-F=0615 | 2 μL |
| dCas9-m2-GG-R=0615 | 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 2503bp. We obtained the correct target fragment, and the sample is purified.

### 1.3 Obtain link-dCas-sg fragment by PCR

The target fragment is located on the pCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| **PCR system（50 μL）** | |
|---|---|
| pCas9 | 10 ng |
| link-GG-F=0615 | 2 μL |
| link-GG-R=0615 | 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 1711bp. We obtained the correct target fragment, and the sample is purified.

## 1.4 Obtain sgRNA-eGFP fragment by PCR

The target fragment is located on the pCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| **PCR system（50 μL）** | |
|---|---|
| pCas9 | 10 ng |
| sgRNA-GG-F=0615 | 2 μL |
| sgRNA-GG-R=0615 | 2 μL |
| 2 x Mix | 25 μL |
| DDW | To 50 μL |

Table 4

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 143bp. We obtained the correct target fragment, and the sample is purified.

## 1.5 Goldengate connection

The pSC101-vec fragment, dCas9-m fragment, link-dCas-sg fragment and sgRNA-eGFP fragment are connected by Goldengate connection method, and the connection system is as follows.

| **Connection system（10 μL）** | |
|---|---|
| pSC101-vec | 1 μL |
| dCas9-m | 1 μL |
| link-dCas-sg | 1 μL |
| sgRNA-eGFP | 1 μL |
| Cutsmart | 1 μL |
| T4 Buffer | 1 μL |
| BsaI | 0.5 μL |
| T4 ligase | 0.2 μL |
| DDW | 3.3 μL |

Table 5

## 1.6 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 1 colonies were selected on the plate. The colony PCR system and procedure were as follows.

| **PCR system（10 μL）** | |
|---|---|
| pSC-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 6

The correct target fragment is about 1711bp, 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.

## 1.7 Enzyme digestion verification

Use the enzyme SalI and EcoRI to cut the plasmid pSC101-dCas9-eGFP at the same time to verify whether the plasmid is constructed correctly. The following is the system of digestion.

| **Digestion system（10 μL）** | |
|---|---|
| pSC-dCas9-eGFP | 100 ng |
| SalI | 0.2 μL |
| EcoRI | 0.2 μL |
| Cutsmart | 1 μL |
| DDW | To 10 μL |

Table 7

It is inferred from the gel electrophoresis that the length of the sample is as expected. The construction is preliminarily correct. 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.