

8. pRB1S-single-T7 Jianing Li

8.1 Obtain Part1 by PCR

The target fragment is located on the pRB1S-hmaS plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| PCR system (50μL) | |
|-------------------|---------|
| pRB1S-hmaS | 10ng |
| Part1-F | 2μL |
| str-overlap-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 1900bp. We obtained the correct target fragment, and the sample is purified.

8.2 Obtain Part2 by PCR

The target fragment is located on the pRB1S-hmaS plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| PCR system (50μL) | |
|-------------------|---------|
| pRB1S-hmaS | 10ng |
| LacI-overlap-F | 2μL |
| part2-R | 2μL |
| 2 x Mix | 25μL |
| DDW | To 50μL |

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1600bp. We obtained the correct target fragment, and the sample is purified.

8.3 Obtain Part3 by PCR

The target fragment is located on the Peasy-phi29-linker-nCas9 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

| PCR system (50μL) | |
|--------------------------|---------|
| Peasy-phi29-linker-nCas9 | 10ng |
| part3-F | 2μL |
| Part3-R | 2μL |
| 2 x Mix | 25μL |
| DDW | To 50μL |

Table 3

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 150bp. We obtained the correct target fragment, and the sample is purified.

8.4 overlap

The part1 fragments and part2 fragments are connected by overlap method, and the overlap system is as follows.

| Overlap system (50 μ L) | |
|-----------------------------|------------|
| part1 | 1 μ L |
| part2 | 1 μ L |
| part1-F | 1 μ L |
| Part2-R | 1 μ L |
| 2x Mix | 25 μ L |
| DDW | 21 μ L |

Table 4

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 3500bp. We obtained the correct target fragment, and the sample is purified.

8.5 goldengate connection

The part1-2 fragments and part3 fragments are connected by Gibson goldengate method, and the connection system is as follows.

| Connection system (20 μ L) | |
|--------------------------------|-------------|
| Part1-2 | 1 μ L |
| part3 | 1 μ L |
| BsaI | 0.5 μ L |
| T4 ligase | 0.2 μ L |
| 10 \times BSA buffer | 1 μ L |
| T4 buffer | 1 μ L |
| DDW | 5.3 μ L |

Table 5

8.6 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 6 colonies were selected on the plate. The colony PCR system and procedure were as follows.

| PCR system (10 μ L) | |
|-------------------------|-------------|
| pYB1a-PobR-mcherry-cmr | 1 μ L |
| Part1-F | 0.2 μ L |
| Str-overlap-R | 0.2 μ L |

| | |
|---------|---------------|
| 2 x Mix | 5 μ L |
| DDW | to 10 μ L |

Table 6

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

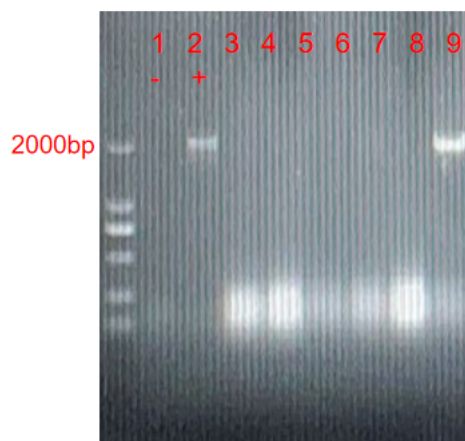


Figure 1

The length of the colony PCR sample in lanes 9 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

8.7 Enzyme digestion verification

Use EcoR I and Xho I to cut the plasmid pRBIS-dualT7 to verify whether the plasmid is constructed correctly. The following is the system of digestion.

| Digestion system (10 μ L) | |
|-------------------------------|---------------|
| pRB12S-dualT7 | 100ng |
| EcoR I | 0.2 μ L |
| Xho I | 0.2 μ L |
| cutsmart | 1 μ L |
| DDW | to 10 μ L |

Table 5

Digested pRBIS-dual-T7 is verified by electrophoresis which is showed below.

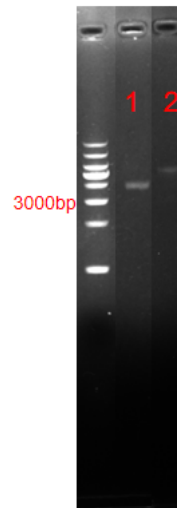


Figure 2

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.