

11. pL12s-single/dual-T7-STS-oripir Xiaoya Wei

11.1 Obtain 3-no ori-dual-oripir by PCR

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

| PCR system (50μL) | |
|---------------------|---------|
| pR12s-dual-T7-STS-3 | 10ng |
| single/dual-R-0630 | 2μL |
| single/dual-F-0630 | 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 4063bp. We obtained the correct target fragment, and the sample is purified.

11.2 Gibson connection

The ori-L-oripir fragments and 3-no ori-dual-oripir are connected by Gibson connection method, and the connection system is as follows.

| Connection system (10μL) | |
|--------------------------|-----|
| ori-L-oripir | 1μL |
| 3-no ori-dual-oripir | 2μL |
| 5×CE Buffer | 2μL |
| ExhaseII | 1μL |
| DDW | 4μL |

Table 2

11.3 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 11 colonies were selected on the plate. The colony PCR system and procedure were as follows.

| PCR system (10μL) | |
|---------------------------------|-------|
| pL12s-single/dual-T7-STS-oripir | 1μL |
| ori pir ce-R-0630 | 0.2μL |
| ori pir ce-F-0630 | 0.2μL |

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

Table 3

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

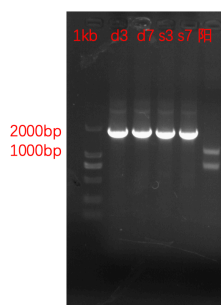


Figure 1

The length of the colony PCR sample in lanes d3、d7、s3、s7 are inferred from the gel electrophoresis image are correct. The four strains were expanded and the plasmids were put forward.

11.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 11 colonies were selected on the plate. The colony PCR system and procedure were as follows.

| PCR system (10 μ L) | |
|----------------------------|---------------|
| pL12s-single-T7-STS-oripir | 1 μ L |
| back-cexu-F | 0.2 μ L |
| T7 back promotor-R | 0.2 μ L |
| 2 x Mix | 5 μ L |
| DDW | to 10 μ L |

Table 4

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

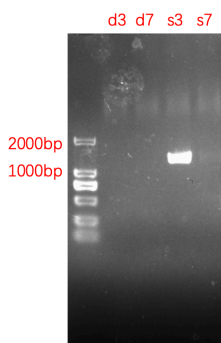


Figure 2

The length of the colony PCR sample in lanes s3、s7 are inferred from the gel electrophoresis

image are correct. The two strains were expanded and the plasmids were put forward.

3.5 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 11 colonies were selected on the plate. The colony PCR system and procedure were as follows.

| PCR system (10μL) | |
|--------------------------|---------|
| pLI2s-dual-T7-STs-oripir | 1μL |
| back-cexu-F | 0.2μL |
| T7 back promotor-R | 0.2μL |
| 2 x Mix | 5μL |
| DDW | to 10μL |

Table 5

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

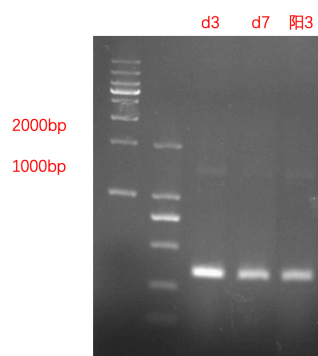


Figure 3

The length of the colony PCR sample in lanes d3、d7 are inferred from the gel electrophoresis image are correct. The two strains were expanded and the plasmids were put forward.

11.6 Enzyme digestion verification

Use Bgl II to cut the plasmid pLI2s-single/dual-T7-STs-oripir to verify whether the plasmid is constructed correctly. The following is the system of digestion.

| Digestion system (10μL) | |
|---------------------------------|---------|
| pLI2s-single/dual-T7-STs-oripir | 100ng |
| Bgl II | 0.2μL |
| cutsmart | 1μL |
| DDW | to 10μL |

Table 6

Digested pLI2s-single/dual-T7-STs-oripir is verified by electrophoresis which is showed below.

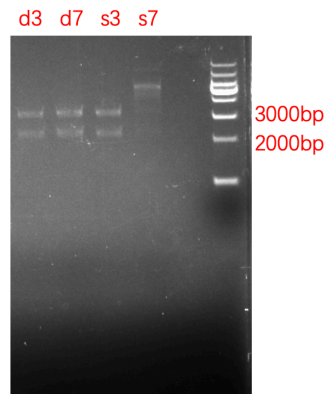


Figure 4

It is inferred from the gel electrophoresis that the length of the sample is as expected expect the s7 strain. 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.