

10. pR12s-reverse-T7 Qianwen Jin

10.1 Enzyme digestion

Use two enzymes EcoR I and Xho I to cut the plasmid pR12s-single-T7 The following is the system of digestion system.

Enzyme digestion system (50μL)	
pR12s-single-T7	10ng
EcoR I	1 μ L
Xho I	1 μ L
Cutsmart	5 μ L
DDW	To 50 μ L

Table 1

The Enzyme digestion products are detected by agarose gel electrophoresis, and the correct target fragment is 3505bp. We obtained the correct target fragment, and the sample is purified.

10.2 Obtain Part3-Gibson 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
XhoI I-Gibson-F	2 μ L
EcoR I-Gibson-R	2 μ L
2 x Mix	25 μ L
DDW	To 50 μ L

Table 2

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 114bp. We obtained the correct target fragment, and the sample is purified.

10.3 Gibson connection

The Part3-Gibson fragments and pR12s-single-T7 are connected by Gibson connection method, and the connection system is as follows.

Connection system (20μL)	
pR12s-single-T7	1.1 μ L
Part3-Gibson	1.2 μ L
Exnase II	1 μ L
5x CEII Buffer	2 μ L

DDW	1.2 μ L
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Table 3

10.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 8 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μ L)	
pRI2s-dual-T7 (reveser)	1 μ L
Part3-F	0.2 μ L
RSF-Junp-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.

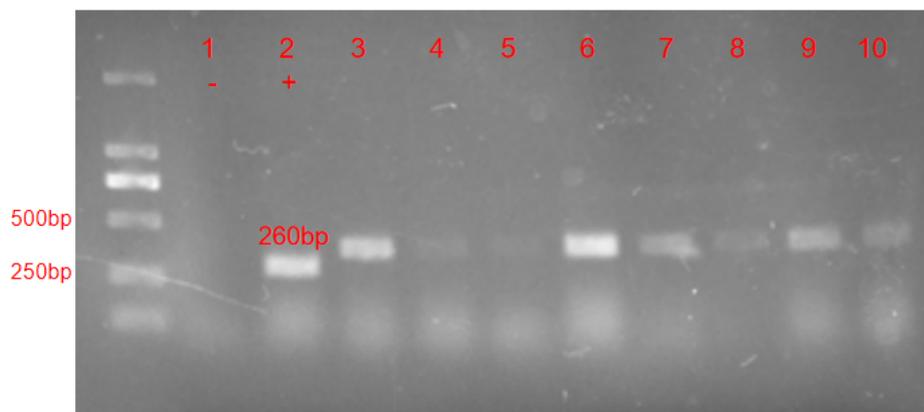


Figure 1

The length of the colony PCR sample in lanes 3, 4, 6, 7, 8, 9, 10 are inferred from the gel electrophoresis image are correct. The 3 and 6 strains were expanded and the plasmids were put forward.

10.5 Enzyme digestion verification

Use Xho I to cut the plasmid pRI2s-dual-T7 (reveser) to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10 μ L)	
pRI2s-dual-T7 (reveser)	100ng
Xho I	0.2 μ L
cutsmart	1 μ L

DDW

to 10 μ L

Table 5

Digested pR12s-reverse-T7is verified by electrophoresis which is showed below.

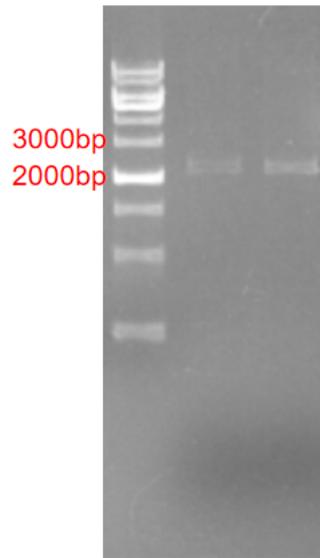


Figure 2

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.