2.gY9s-dual T7-HmaS(scpaI)-Bio 177
2.1 Obtain the Bio177 vector fragment by PCR

The target fragment is located on the gY9s-177-HmaS(scpaI) plasmid, and the target fragment was amplified by PCR. The PCR system and procedure are showed below.

PCR system (50 μL)	
gY9s-177-HmaS(scpa1)	1 μL
Bio-F=0811	2μL
Bio-R=0811	2μL
2×Mix	25μL
DDW	20μL

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 5255bp. The correct target fragment was obtained, and the sample was purified.

2.2 Obtain dual T7-HmaS fragment by PCR
The target fragment is located on the gY9s-177-HmaS(scpaI) plasmid, and the target fragment is amplified by PCR. The PCR system and procedure are showed below.

PCR system (50 μL)	
gY9s-177-HmaS(scpa1)	1 μL
AM-gg-F=0709	2 μL =
AM-R=0811	2 μL
2×Mix	25μL
DDW	20μL

The PCR product is detected by agarose gel electrophoresis, the correct target band is 1106bp. The correct target fragment was obtained, and the sample was purified.

2.3 Obtain RE-TrrnB fragment by PCR
The target fragment is located on the gY9s-177-HmaS(scpaI) plasmid, and the target fragment is amplified by PCR. The PCR system and procedure are showed below.

PCR system (50 μL)	
gY9s-177-HmaS(scpa1)	1 μL
RET-F=0811	2μL
RET-R=0811	2μL
2×Mix	25μL
DDW	20μL

The PCR product is detected by agarose gel electrophoresis, the correct target band is 192bp. The correct target fragment was obtained, and the sample was purified.

2.4 Obtain TrrnB fragment by PCR
The target fragment is located on the gY9s-177-HmaS(scpaI) plasmid, and the target fragment is amplified by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
gY9s-177-Hmas(scpa1)	1 μL
T-F=0811	2 μL
T-R=0811	2 μL
2×Mix	25μL
DDW	20μL

The PCR product is detected by agarose gel electrophoresis, the correct target band is 188bp. The correct target fragment was obtained, and the sample was purified.
The detection of Bio177 carrier fragment, dual T7-HmaS fragment, RE-TrrnB fragment and TrrnB fragment by agarose gel electrophoresis is as follows.

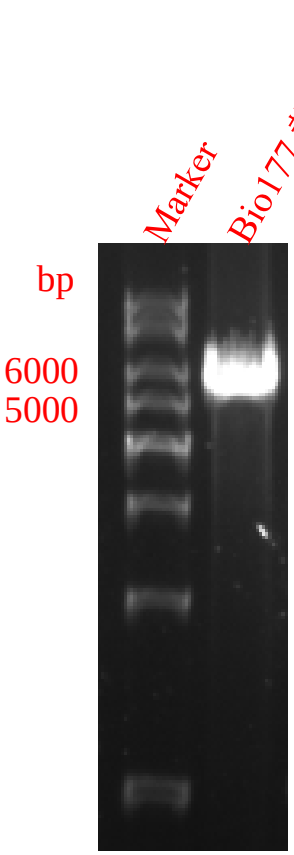


Figure 1

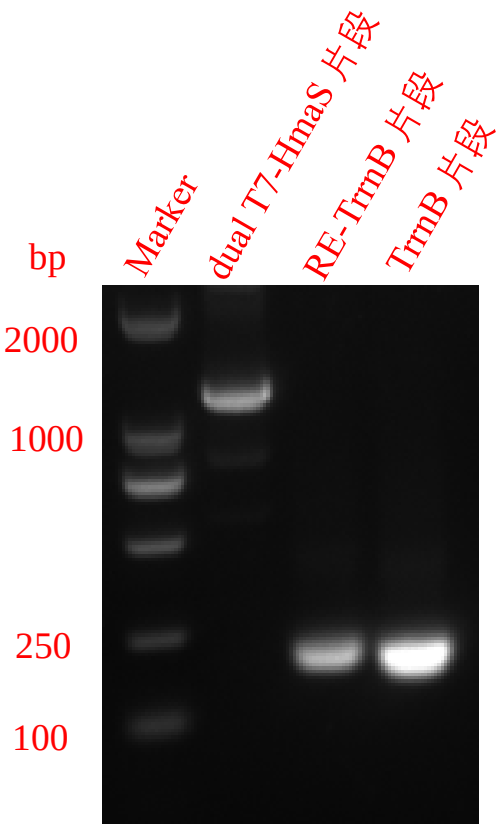


Figure 2

2.5 Golden Gate connection

The Bio177 carrier fragment, dual T7-HmaS fragment, RE-TrrnB fragment and TrrnB fragment are connected by Golden Gate connection method. The connection system is as follows.

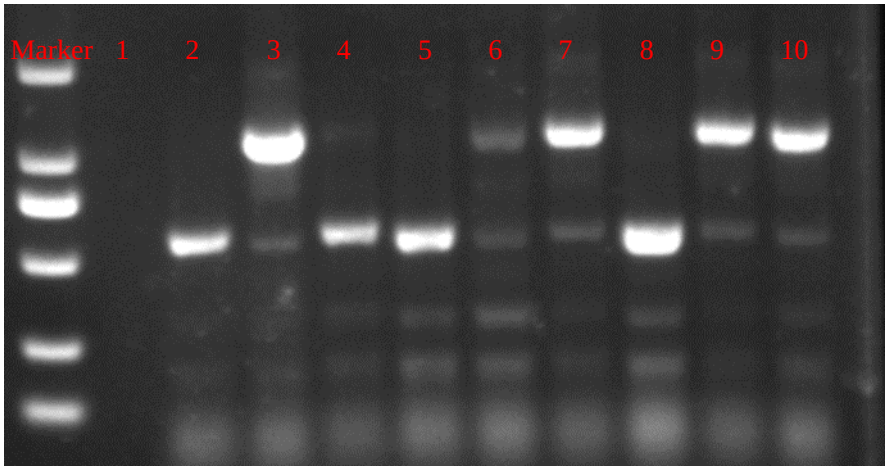
Assembly System (10μL)	
Bio177 carrier fragment	2 μL
dual T7-HmaS fragment	2μL
RE-TrrnB fragment	2 μL
TrrnB fragments	1 μL
Bsa I	0.5μL
rCutsmart	1 μL
T4 ligase	0.2 μL
T4 buffer	1μL
DDW	0.3 μL

2.6 Colony PCR

After the petri dish is incubated at 37 ° C for 12 hours, 10 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μL)	
AM-gg-F=0709	0.4 μL
AM-R=0811	0.4 μL
2×Mix	5μL
DDW	4.2μL

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



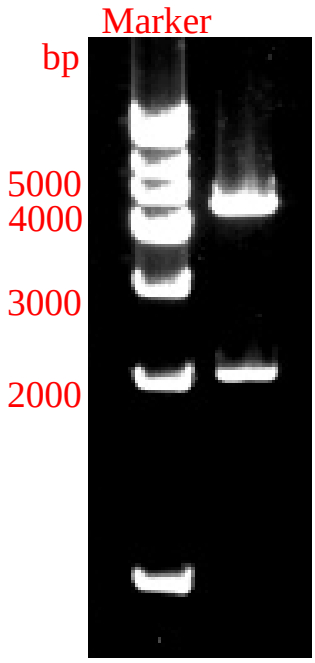
The expected target fragment is about 1106bp, and the length of the colony PCR sample in lanes 3, 7, 9, and 10 is inferred from the gel electrophoresis image is expected. The monoclone of lane 9 was amplified and the plasmid was extracted.

2.7 Enzyme digestion verification

The plasmid gY9s-dual T7-HmaS (Scpa1) -Bio 177 was cut with BglII to verify whether the plasmid was correctly constructed. The enzyme cleavage system is as follows.

Enzyme digestion system (10 μL)	
gY9s-dual T7-HmaS (Scpa1) -Bio 177	200 ng
BglII	0.2 μL
rCutsmart	1 μL
DDW	To 10 μL

Digested gY9s-dual T7-HmaS（Scpa1）-Bio 177 is verified by electrophoresis which is showed below.



The anticipated fragment sizes of 4568bp and 2053bp were observed, and the sample length was consistent with gel electrophoresis results. Sequencing analysis of the constructed plasmid confirmed that the sequence matched expectations, indicating successful construction of the target plasmid.