Constructing gy9s-hmas(Am,scpa1)-bio177 mutant library

1. Obtain *hmaS* mutant fragment by PCR

The target fragment is located on the Pccd-K-scpa1 plasmid, and the amplified target fragment is obtained by PCR. And we use conditions that confirm the appropriate mutation rate by adjusting the concentration of Mn^{2+} and Mg^{2+}.

PCR system (50μL)	
Scpa1-R==0725	2 μL
Hmas-R==0617	2 μL
gy9s(scpa1)-177-hmas3	2 ng
2xMix	25 μL
Mn^{2+}(1 mM)	1 μL
Mg^{2+}(25 mM)	1 μL
dNTP	1 μL
DDW	15.5 μL

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1248bp. We obtained the correct target fragment, and the sample is purified.

2. Obtain Bio177 vector fragment by PCR

The target fragment is located on the pYB1a-Bio177-Str plasmid, and the amplified target fragment is obtained by PCR. The PCR system is showed below.

PCR system(50μL)	
Bio177-F	2 μL
Bio177-R	2 μL
gy9s(100)-177-hmas3	2 ng
2xMix	25 μL
DDW	20 μL

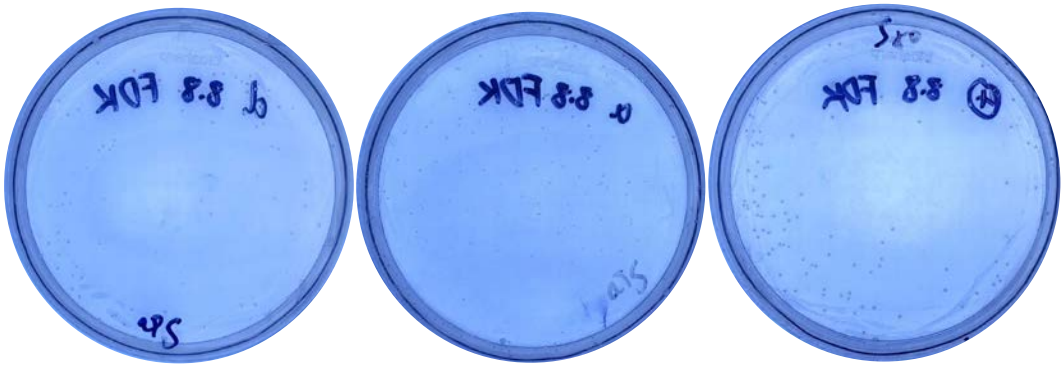
The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 5089bp. We obtained the correct target fragment, and the sample is purified.

3. Golden gate assembly

The *hmaS* mutant fragments and Bio177 vector fragments are connected by Golden gate assembly method, and the system is as follows.

Golden gate system (50 μL)	
Hmas-mut	1 μL
Bio177	1 μL
T4 Buffer	1 μL
rCutsmart	1 μL
T4 ligase	0.2 μL
BsaI	0.5 μL
DDW	5.3 μL

Golden gate	
37 ℃	60 min
55 ℃	15 min
80 ℃	15 min

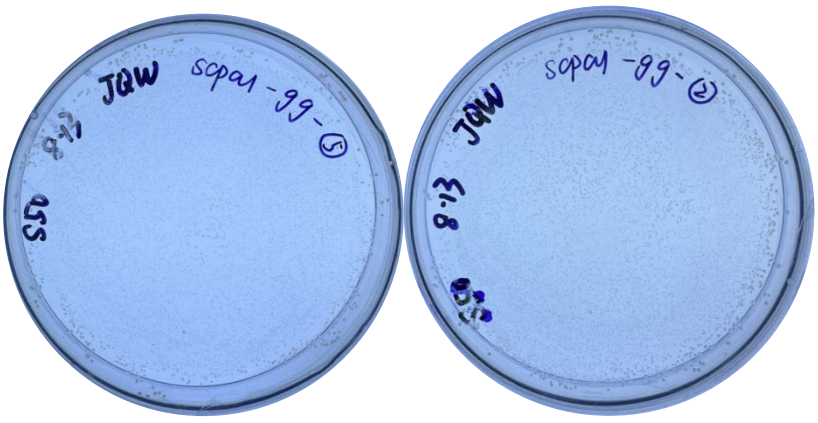


4. Optimization of Golden gate assembly

To optimize the procedure of ligation, we continuously cycled the T4 ligase and restriction endonucleases operating temperatures to alternate their function.

Golden gate system (50 μL)	
Hmas-mut	1 μL
Bio177	1 μL
T4 Buffer	1 μL
rCutsmart	1 μL
T4 ligase	0.2 μL
BsaI	0.5 μL
DDW	5.3 μL

Golden gate		
37 ℃	10 min	
37 ℃	3 min	×25
16 ℃	3 min	
16 ℃	20 min	
37 ℃	20 min	
55 ℃	15 min	
80 ℃	15 min	



5. Colony PCR

After cultured at 37°C for 12 hours, 20 colonies were selected on the plate. The colony PCR system is as follows.

PCR system (50 μL)	
Bio177-F	2 μL
Bio177-R	2 μL
gy9s(scpa1)-177-hmas3	2 ng
2 x Mix	25 μL
DDW	20 μL

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