

13. glb2a-PpobA*2-mCherry-SacB-Cmr Qianwen Jin

13.1 Obtain cmr fragment by PCR

The target fragment is located on the PYB1a-eGfp-Cmr plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
cmr-gibson-0317-F	2 μ L
cmr-gibson-0317-R	2 μ L
PYB1a-eGfp-Cmr	10ng
2 x Mix	25 μ L
DDW	to 50 μ L

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is about 700bp. We obtained the correct target fragment, and the sample is purified.

13.2 Enzyme digestion

Use one enzyme EcoRI to cut the plasmid . The following is the system of digestion system.

Enzyme digestion system (50μL)	
gLB2a-PpobA2-mCherry-SacB	2000ng
EcoR I	1 μ L
buffer	5 μ L
DDW	to 50 μ L

Table 2

The correct target fragment is about 6000bp. We obtained the correct target fragment, and the sample is purified.

13.3 Gibson connection

The glb2a-PpobA2-mCherry-SacB fragments and cmr fragments are connected by Gibson connection method, and the connection system is as follows.

Connection system (10μL)	
gLB2a-PpobA2-mCherry-SacB	4.6 μ L
cmr	2 μ L
5 x Cell Buffer	2 μ L

Exnasell	1 μ L
DDW	to 10 μ L

Table 3

13.4 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 (10μL)	
cmr-gibson-0317-F	0.4 μ L
cmr-gibson-0317-R	0.4 μ L
I5 2X 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.

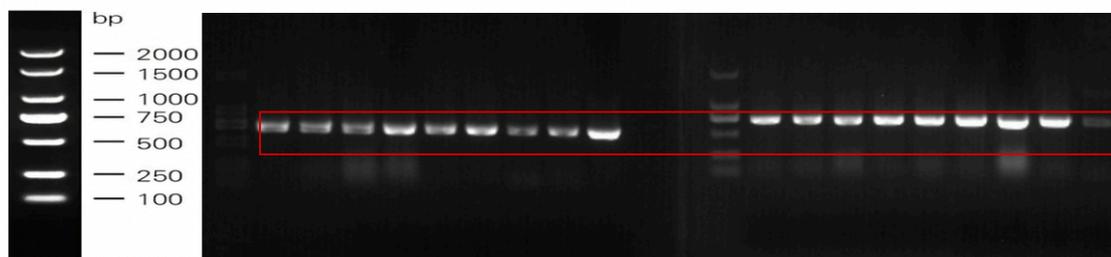


Figure 1

The correct target fragment is about 700bp, and the length of the colony PCR sample in all lanes inferred from the gel electrophoresis image is correct. The above 4 strains were expanded and the plasmids were put forward.

13.5 Enzyme digestion verification

Use one enzyme KpnI to cut the plasmid glb2a-PpobA*2-mCherry-SacB-Cmr to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10μL)	
glb2a-PpobA*2-mCherry-	100ng

SacB-Cmr	
NcoI	0.2 μ L
buffer	1 μ L
DDW	to 10 μ L

Table 5

Digested glb2a-PpobA*2-mCherry-SacB-Cmr is verified by electrophoresis which is showed below.

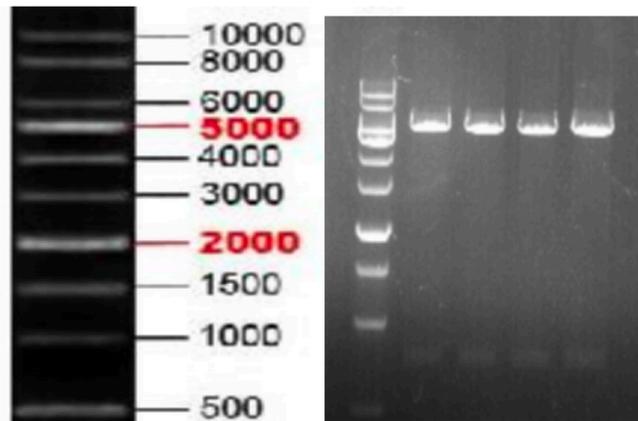


Figure 2

The correct target fragment is 6000 and 760bp. It is inferred from the gel electrophoresis that the length of the sample in lanes is as expected. The construction is preliminarily correct.