

12. glb2a-PpobA*2-mCherry-Cmr-SacB Xinyao Yuan

12.1 Obtain glb2a-mcherry fragment by PCR

The target fragment is located on the glb2a-mCherry plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
glb2a-mCherry	10ng
PpobA-Gibson-F	2μL
CmR-Gibson-R	2μL
2 x Mix	25μL
DDW	To 50μL

We obtained the correct target fragment, and the sample is purified.

12.2 Obtain glb2a-PpobA*2-eGFP-cmr-SacB fragment by PCR

The target fragment is located on the glb2a-PpobA*2-eGFP-cmr-SacB plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
glb2a-PpobA*2-eGFP-Cmr-SacB	10ng
PpobA-Gibson-F	2μL
CmR-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

We obtained the correct target fragment, and the sample is purified.

12.3 Gibson connection

The glb2a-mcherry fragments and glb2a-PpobA*2-eGFP-Cmr-SacB fragments are connected by Gibson connection method, and the connection system is as follows.

Connection system (20μL)	
glb2a-mCherry	100ng
glb2a-PpobA*2-eGFP-Cmr-SacB	70ng
5 x Cell Buffer	4μL
Exase II	2μL

DDW	to 20 μ L
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Table 3

12.4 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 (10 μ L)	
glb2a-PpobA*2-mCherry-Cmr-SacB	1 μ L
mcherry-Gibson-F	0.2 μ L
mcherry-Gibson-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.

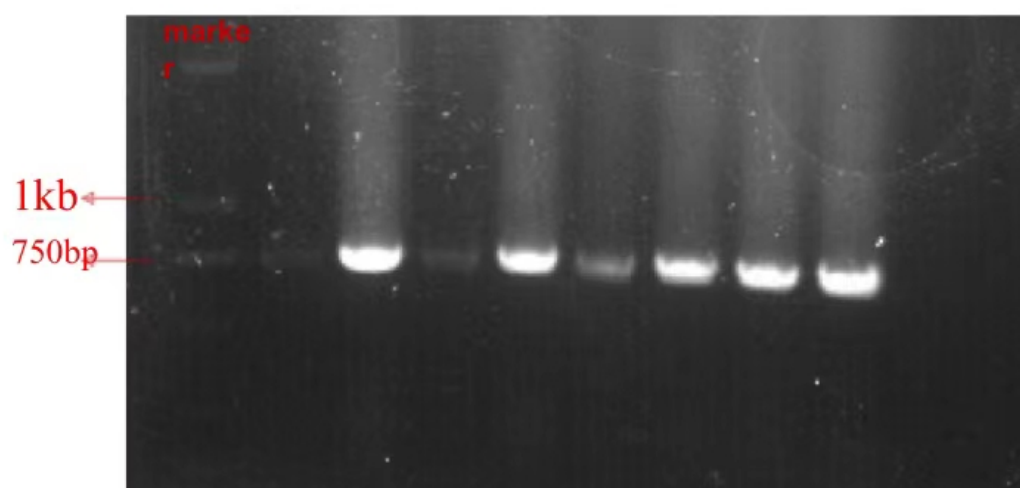


Figure 1

The correct target fragment is about 750bp, and the length of the colony PCR sample in lanes 2-9 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

12.5 Enzyme digestion verification

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

Digestion system (10 μ L)	
glb2a-PpobA*2-mCherry-Cmr-SacB	100ng

Nco1	0.2μL
Buffer	01μL
DDW	to 10μL

Digested glb2a-PpobA*2-mcherry-cmr-sacB is verified by electrophoresis which is showed below.

Figure

12.6 DNA sequencing

[illegible]

The result shows that the sequence is the same as expected, indicating that our target plasmid has been constructed successfully.