

14. gYb2a-PpobA*2-mCherry-SacB-Cmr Qianwen Jin

14.1 Obtain gYb2a fragment by PCR

The target fragment is located on the gYb2a-PpobA2-PobR-mCherry-SacB plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
gYb2a-PpobA2-PobR-mCherry-SacB	10ng
Ori-ti-0401-F	2μL
Ori-ti-0401-R	2μL
2 x Mix	25μL
DDW	To 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the results are as follows. The correct target fragment is 850bp. We obtained the correct target fragment, and the sample is purified.

14.2 Enzyme digestion

Use two enzymes SacI and SpeI to cut the plasmid . The following is the system of digestion system.

Enzyme digestion system (50μL)	
glb2a-PpobA*2-mCherry-SacB-Cmr	2000ng
SacI	1μL
SpeI	1μL
Cutsmart	5μL
DDW	to50μL

Table 2

The correct target fragment is 5200bp. We obtained the correct target fragment, and the sample is purified.

14.3 Gibson connection

The gYb2a fragments and PpobA*2-mCherry-SacB-Cmr fragments are connected by Gibson connection method, and the connection system is as follows.

Connection system (10μL)	
PpobA*2-mCherry-SacB-Cmr	1μL

gYb2a	2.4μL
5 x Cell Buffer	2μL
Exnase II	1μL
DDW	to10μL

Table 3

14.4 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)	
gYb2a-PpobA*2-mCherry-SacB-Cmr	1μL
Ori-ti-0401-F	0.4μL
Ori-ti-0401-R	0.4μL
2 x Mix	5μL
DDW	to 10μL

Table 4

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

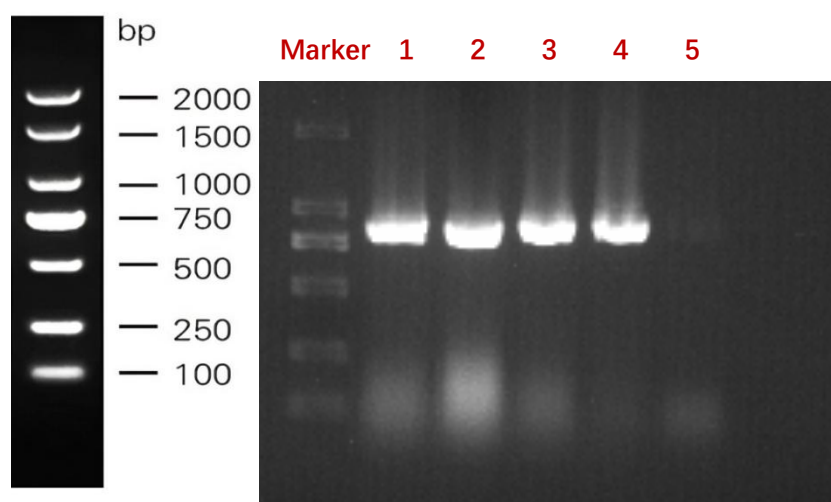


Figure 1

The correct target fragment is 850bp, and the length of the colony PCR sample in lanes 1,2,3 and 4 is inferred from the gel electrophoresis image is correct. The above 2 strains were expanded and the plasmids were put forward.

14.5 Enzyme digestion verification

Use enzymes KpnI to cut the plasmid gYb2a-PpobA*2-mCherry-SacB-Cmr at the same time to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10μL)	
gYb2a-PpobA*2-mCherry-SacB-Cmr	100ng
KpnI	0.2μL
custsmart	1μL
DDW	To 10μL

Table 5

Digested gYb2a-PpobA*2-mCherry-SacB-Cmr is verified by electrophoresis which is showed below.

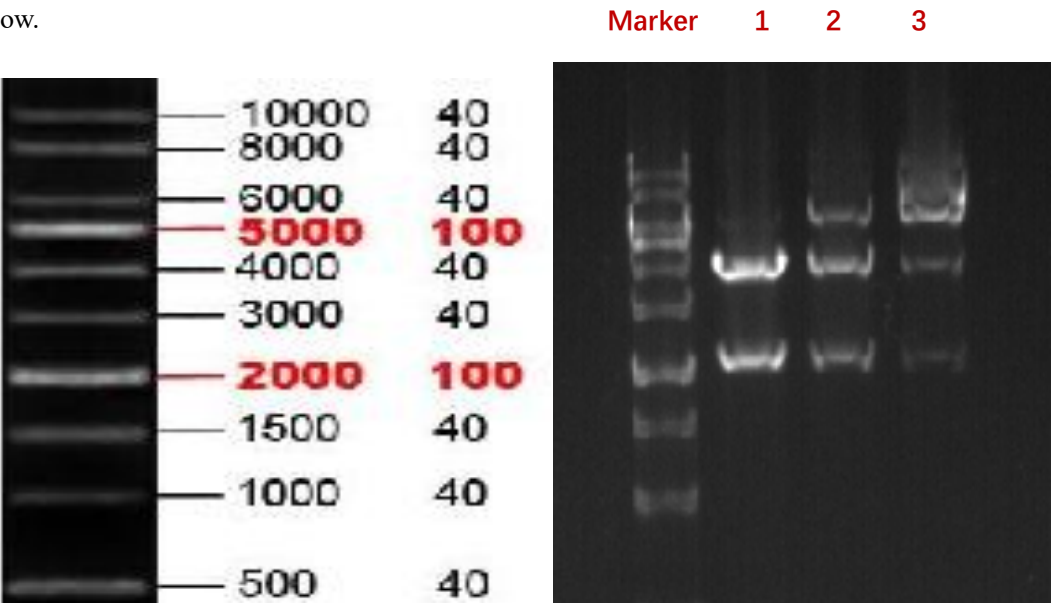


Figure 11

The correct target fragment is 4000 and 2000bp. It is inferred from the gel electrophoresis that the length of the sample in lanes 1, 2 and 3 is as expected. The construction is preliminarily correct.