

15. gYb2a-PpobA*2-mCherry-CD-Cmr Qianwen Jin

15.1 Obtain CD fragment by PCR

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

PCR system (50μL)	
pUAM-DE-CD	10ng
CD-Gibson-0425 F	2μL
CD-Gibson-0425 R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

The PCR products are detected by agarose gel electrophoresis, and the correct target fragment is 1332bp. We obtained the correct target fragment, and the sample is purified.

15.2 Obtain gYb2a-PpobA*2-mCherry-Cmr fragment by PCR

The target fragment is located on the gYb2a-PpobA*2-mCherry-SacB-Cmr plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50μL)	
gYb2a-PpobA*2-mCherry-SacB-Cmr	10ng
Mc-Gibson-F	2μL
Mc-Gibson-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 2

The correct target fragment is 4572bp. We obtained the correct target fragment, and the sample is purified.

15.3 Gibson connection

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

Connection system (10μL)	
CD	2.4μL
gYb2a-PpobA*2-mCherry-XX-Cmr	1μL

5 x Cell Buffer	2 μ L
Exnase II	1 μ L
DDW	3.6 μ L

Table 3

15.4 Colony PCR

After the petri dish is incubated at 37°C for 12 hours, 5 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 μ L)	
gYb2a-PpobA*2-mCherry- CD-Cmr	1 μ L
CD-Gibson-0420-F	0.4 μ L
CD-Gibson-0420-R	0.4 μ L
2 x Mix	5 μ L
DDW	3.2 μ L

Table 4

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

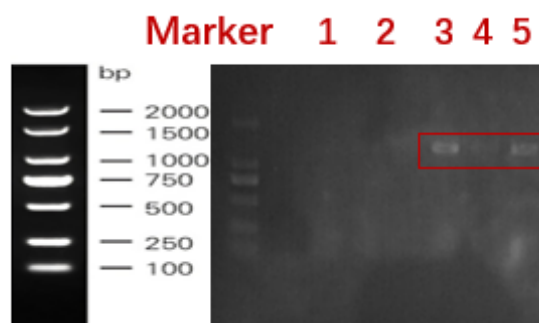


Figure 1

The correct target fragment is about 1300bp, and the length of the colony PCR sample in lanes 3,4 and 5 is inferred from the gel electrophoresis image is correct. The above 3 strains were expanded and the plasmids were put forward.

15.5 Enzyme digestion verification

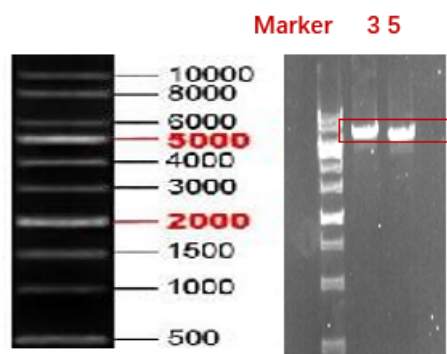
Use the enzyme KpnI to cut the plasmid gYb2a-PpobA*2-mCherry-CD-Cmr at the same time to verify whether the plasmid is constructed correctly. The following is the system of digestion.

Digestion system (10 μ L)	
gYb2a-PpobA*2-mCherry- CD-Cmr	100ng

KpnI	0.2μL
custsmart	1μL
DDW	to 10μL

Table 5

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



It is inferred from the gel electrophoresis that the length of the sample is as expected. The construction is preliminarily correct.