

## 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.

<b>PCR system (50<math>\mu</math>L)</b>	
pUAM-DE-CD	10ng
CD-Gibson-0425 F	2 $\mu$ L
CD-Gibson-0425 R	2 $\mu$ L
2 x Mix	25 $\mu$ L
DDW	to 50 $\mu$ L

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.

<b>PCR system (50<math>\mu</math>L)</b>	
gYb2a-PpobA*2-mCherry- SacB-Cmr	10ng
Mc-Gibson-F	2 $\mu$ L
Mc-Gibson-R	2 $\mu$ L
2 x Mix	25 $\mu$ L
DDW	to 50 $\mu$ L

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.

<b>Connection system (10<math>\mu</math>L)</b>	
CD	2.4 $\mu$ L
gYb2a-PpobA*2-mCherry- XX-Cmr	1 $\mu$ L

5 x Cell Buffer	2 $\mu$ L
Exnase II	1 $\mu$ L
DDW	3.6 $\mu$ 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 $\mu$ L)	
gYb2a-PpobA*2-mCherry- CD-Cmr	1 $\mu$ L
CD-Gibson-0420-F	0.4 $\mu$ L
CD-Gibson-0420-R	0.4 $\mu$ L
2 x Mix	5 $\mu$ L
DDW	3.2 $\mu$ L

Table 4

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

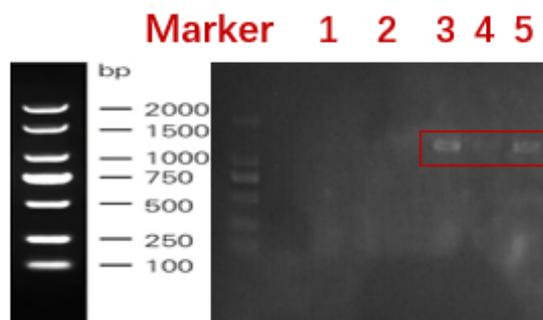


Figure 1

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.

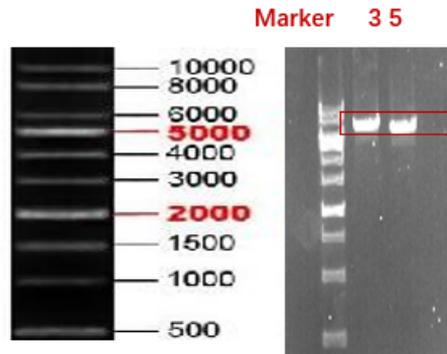
Digestion system (10 $\mu$ L)	
gYb2a-PpobA*2-mCherry- CD-Cmr	100ng

KpnI	0.2 $\mu$ L
custsmart	1 $\mu$ L
DDW	to 10 $\mu$ L

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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.