

## 7. pYB1a-mutator(mut) Xinyao Yuan

### 7.1 Obtain pYB1a-mutator(mut) by PCR

The target fragment is located on the pYB1a-mutator-wt T7 plasmid, and the amplified target fragment is obtained by PCR. The PCR system and procedure are showed below.

PCR system (50 $\mu$ L)	
pYB1a- mutator-wt T7	10ng
T7 pol R==0629	2 $\mu$ L
Linker-SH3-F==0629	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 8498bp. We obtained the correct target fragment, and the sample is purified.

### 7.2 Colony PCR

After the petri dish is incubated at 37 $^{\circ}$ C for 12 hours, 15 colonies were selected on the plate. The colony PCR system and procedure were as follows.

PCR system (10 $\mu$ L)	
pYB1a-mutator(mut)	1 $\mu$ L
SH3-cexu-F	0.2 $\mu$ L
ORI-cexu-R	0.2 $\mu$ L
2 x Mix	5 $\mu$ L
DDW	to 10 $\mu$ L

Table 2

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



Figure 1

The length of the colony PCR sample in lanes 2、3、6、9、12、13、14、15 are inferred from the gel electrophoresis image are correct. The 9、13 and 14 strains were expanded and the plasmids were put forward.

### 7.3 Enzyme digestion verification

Use EcoR I to cut the plasmid pYB1a-mutator(mut) to verify whether the plasmid is constructed correctly. The following is the system of digestion.

<b>Digestion system (10<math>\mu</math>L)</b>	
pYB1a-PobR-mcherry-cmr	100ng
EcoR I	0.2 $\mu$ L
Buffer	01 $\mu$ L
DDW	to 10 $\mu$ L

Table 3

Digested pYB1a-mutator(mut) is verified by electrophoresis which is showed below.

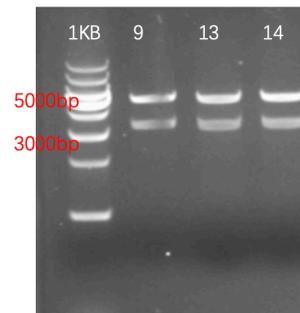


Figure 2

The correct target fragment is 3371bp and 5117bp. It is inferred from the gel electrophoresis that the length of the sample in lanes 9, 13 and 14 is as expected. The construction is preliminarily correct. We sequenced the constructed plasmid. The result shows that the sequence is the same as expected, indicating that our target plasmid has been constructed successfully.