

5.22-6.11

1. Mutation

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1.1 Obtain the strain containing the mutational tool and target gene

The two plasmids, pYB1a-mutator(mut) and pLI2s-single/dual-T7-STs-oripir, were transferred together into *E. coli* DH5a competent cells for obtain the strain containing the mutational tool and target gene. We randomly selected strains in the plates for subsequent mutations.

Each round was 12 hours long, and we ran 21 cycles. In each round, 1% of the bacterial solution of the previous round was absorbed into LB, and Amp and Str antibiotics were added, along with arabinose and IPTG. At the same time, we also carried out negative control without IPTG .

1.2 Obtain the target gene fragment

Fenglin Tao

After every three rounds, the corresponding strains were separated to obtain single colonies, so as to amplify the target gene fragment. The PCR system and procedure are showed below.

PCR system (50μL)	
PYT1a-ttgR-mCherry-kana	1μL
STS-JUNP-F	2μL
ORI-R	2μL
2 x Mix	25μL
DDW	to 50μL

Table 1

We obtained the correct target fragment, and the sample is purified. We sequenced the target fragment. The result shows that the sequences are unexpected no matter the pLI2s-single-STs-oripir (Figure 1) or pLI2s-dual-T7-STs-oripir (Figure 2).

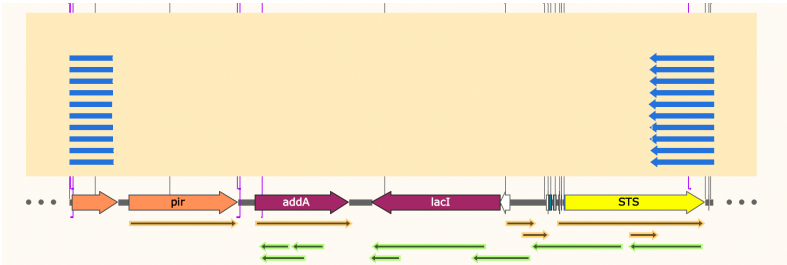


Figure 1

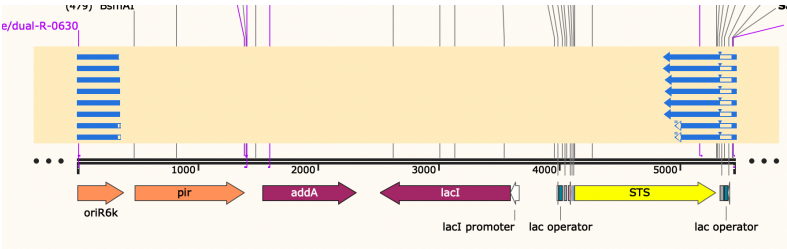


Figure 2