Based on the results we have achieved, we chose lacI-P_{lac} to carry out the next step. In this system, the arabinose-controlled repressor protein is utilized to regulate the transcription of sgRNA.

2018-8-21

One step cloning and transformation

Materials（20 μl Reaction）:

5×CE Multis Buffer	4μl
pGLO-lacI	2μl
sgRNA	1μl
Exnase Multis	2μl
ddH_2O	11μl

2018-8-23

Colony PCR

Materials（20 μl Reaction）:

Green Taq Mix	25μl
P1	2μl
P2	2μl
Boiled bacteria solution	1μl
ddH_2O	20μl

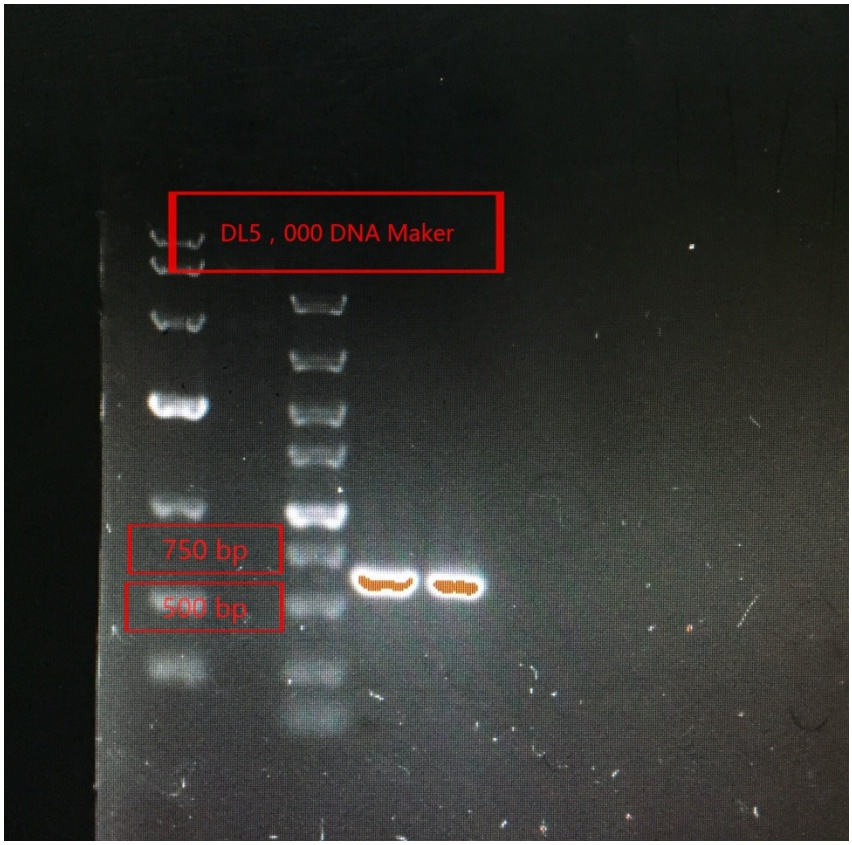
PCR condition		pGLO-lacI-sgRNA
Step one		94℃ 300s
30 cycles	Step two	94℃ 30s
	Step three	48℃ 30s
	Step four	72℃ 60s
Step five		72℃ 420s

Primers:

P1 5’-ATACGACGATACCGAAGACA-3’

P2 5’-CAAGTTGATAACGGACTAGC-3’

Here is the result and the correct is to get a 633bp fragment.


DL5，000 DNA Maker
750 bp
500 bp