IGEM-ZJUT 2018 WetLab recorder：Yankang

Time：8月3日

1、Experimental purpose: pGLO-Cas9 transformant colony PCR

2、Material

Green Taq Mix（Vazyme）

Colony PCR primer: TCas-F: 5'- TCACACTTTGCTATGCCATAGC-3'

TCas-R: 5’- acggcatttagatacgcatcat-3’

3、Experimental procedure

(1) Four transformants were picked from the transformant plate for colony PCR verification;

(2) Dissolving the colonies in 20 ul of ddH2O, and picking a single colony containing the pGLO plasmid as a control, a total of 5 samples;

(3)Boiling the bacterial suspension in boiling water for 5 min, and the obtained solution is used as a template;

(4) Prepare the PCR system:

	Experimental groupA1-A5/ ul	Control group B1-B5/ ul
2x Green Taq Mix	25	0
template	1	1
Primer F+R	2+2	2+2
ddH_2O	20	45

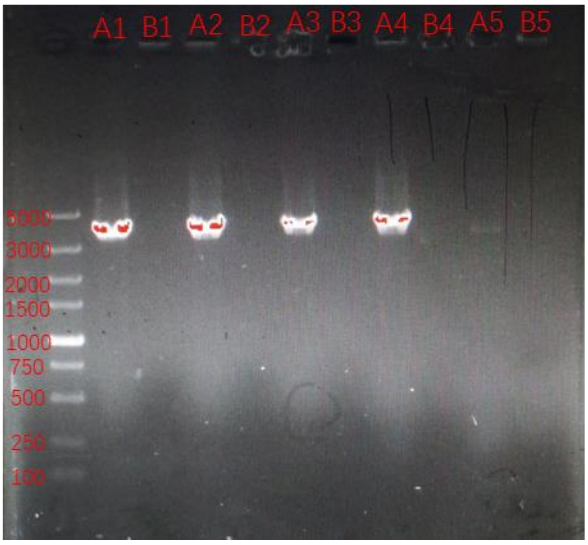
1-4 is pGLO-Cas9 transformant and 5 is pGLO control

(5) PCR Program

94℃	**5min**	
94℃	**30s**	**X30circle**
50℃	**30s**	
72℃	**4min20s**	
72℃	**5min**	

(6) PCR product analysis by agarose gel electrophoresis

4、Results



The target product of the colony PCR was 3866 bp. The experimental group A1-A4 has about 4000 target strips, and the A5 no band indicates that the selected colonies are not false positive transformants containing pGLO. B1-

B4 has no strips, which excludes the effects of empty carrier running. The experimental results indicated that the selected single colonies were positive transformants containing pGLO-Cas9, and the next experiment will verify whether the protein expressed by the Cas9 gene is functional.