IGEM-ZJUT 2018 WetLab

recorder：Yankang

Time：8月2日

1、Experimental purpose: pTF-p15A transformant colony PCR

2、Material

Green Taq Mix（Vazyme）

Primer:

F: **5'**— cacgatcgacattgatctgg—3'

R: 5'— GTGTCATTCCGCTGTTATGG—3'

3、Experimental procedure

(1) Pick 8 transformants for colony PCR verification;

(2) After picking the selected single colonies and spotting on another spectinomycin-resistant plate, the colonies were dissolved in 20 ul of ddH2O, and a single colony containing the pTargetF plasmid was picked as a control, a total of 9 samples;

(3) The bacterial suspension was boiled in boiling water for 5 min, and the resulting solution was used as a template;

(4) Prepare the PCR system:

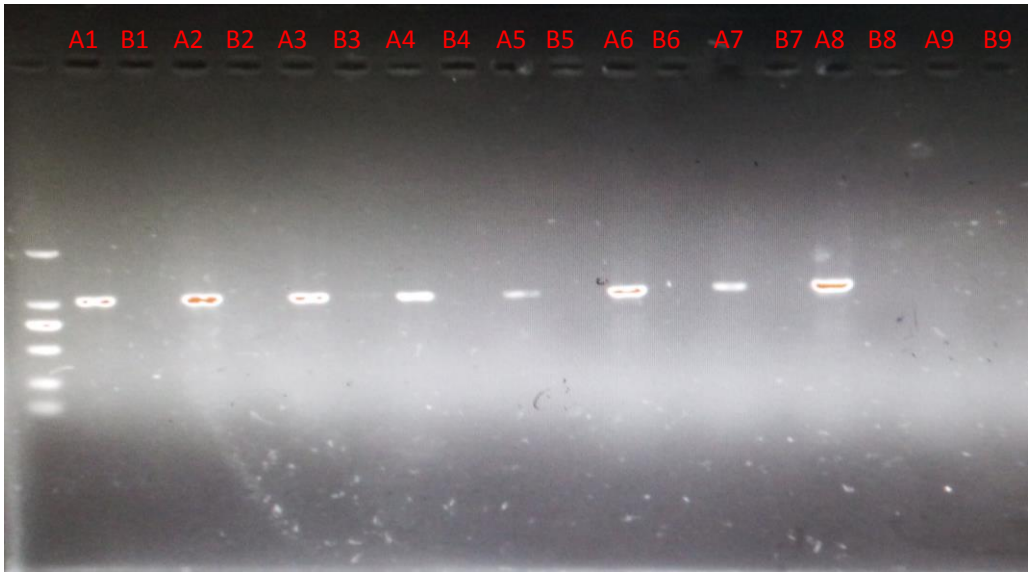
	Experimental group A1-A9/ ul	Control group B1-B9/ ul
2x Green Taq Mix	25	0
template	1	1
Perimer F+R	2+2	2+2
ddH_2O	20	45

(5)PCR program

94℃	5min	
94℃	30s	X30 circle
50℃	30s	
72℃	70s	
72℃	5min	

(6) PCR product analysis by agarose gel electrophoresis

4、Results



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The target product of colony PCR was 1033 bp. The electrophoresis results showed that the PCR products of the experimental group 1-8 were slightly larger than 1000 bp, and there was no band in the control group. There were no bands in the pTargetF control group A9 and B9, so 1-8 were non-empty carriers. This can be used to preliminarily demonstrate that pTF-p15A was successfully constructed. The next step will be to verify the function.