

Time : 8.19

1、 Experimental purpose: Construction of pTF-p15A-cm plasmid transcribing sgRNA targeting at chloramphenicol gene

2、 Material

pTF-p15Aplasmid (target at *panD*) , 2X Phanta Mix (Vazyme)

3、 Experimental procedure

(1) Prepare the PCR system:

	Experimental group 1-4/ ul	Control group 5/ ul
2x Phanta Mix	25	0
template	1	1
Primer F+R	2+2	2+2
ddH ₂ O	20	45

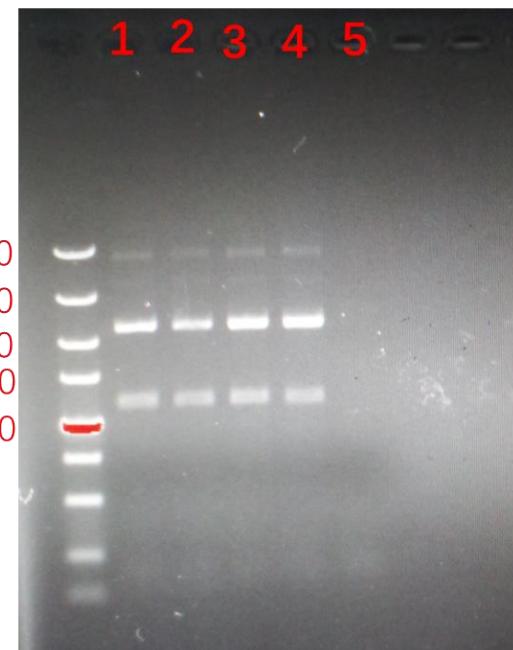
The template is a 10⁻¹ dilution of the pTF-p15A plasmid (470 ng/ul)

(2) PCR program

95°C	5min	
95°C	15s	
60°C	15s	
72°C	2min30s	X30 circle
72°C	5min	

(3) PCR product was analyzed by agarose gel electrophoresis

4、 Results



The target band is 2242 bp, and there are bands of 5000 bp, 4000 bp and 1200 bp in the electrophoresis. The

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reason may be that the template plasmid is impure. The next step is to recover the purified product using purification kit.