(1) The the linearized carrier were prepared by PCR

1. experimental objective ： preparation of linearized plasmid (dislodge eGFP)

2.experimental equipment

（1）reagents:ddH2O,2xPhanta Max Master Mix,dusk-eGFP-pUC57 plasmid ,10mM pUC57-F,10mM pUC57-R

（2）instrument：Liquid transfer gun and head, PCR tube, ice box, gradient PCR instrument, etc.

3.experimental procedure

Each solution was added to the PCR tubes with the liquid transfer gun according to the following requirements. After rocking, the solution was put into the gradient PCR instrument to carry out the PCR.

PCR/μl	μl
2xPhanta Max Master Mix	25
10mM pUC57-F	2
10mM pUC57-R	2
dusk-egfp-pUC57 Plasmid template	0.5
ddH_2O	20.5

PCR condition setting		dusk-pUC57
Step one		95℃ 180s
30 cycle	Step two	95℃ 15s
	Step three	51℃ 15s
	Step four	72℃ 360s
Step five		72℃ 300s

(2)detection of PCR by the electrophoresis gel

1.experimental objective ： Test for amplification of linearized dusk-pUC57 fragment

2.experimental equipment

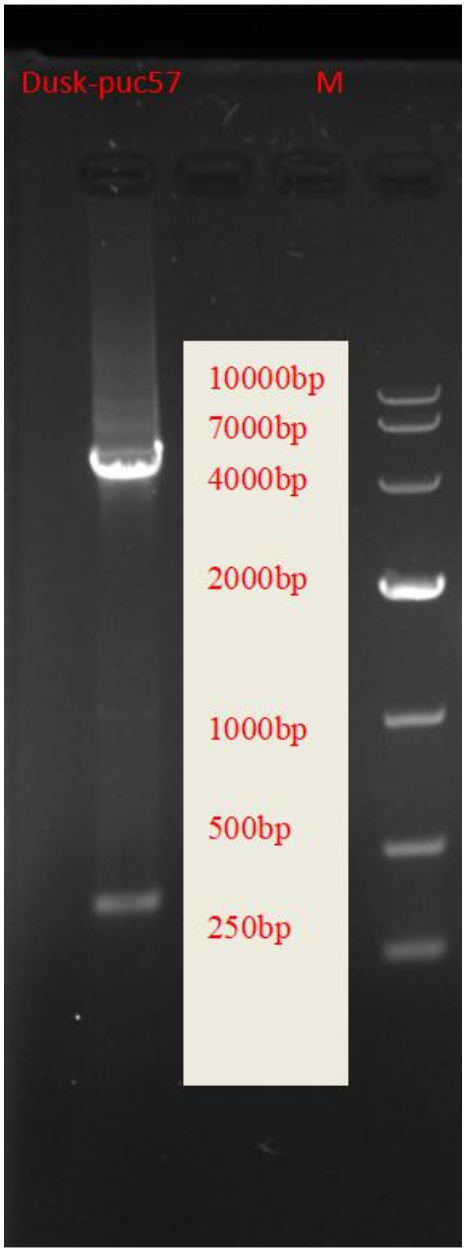
(1)reagents:the DNA sample solution after PCR, 1TAE, agarose, 4S Red Plus Nucleic Acid, DNA buffer, 10000bp DNA Marker.

(2)instrument： liquid transfer guns and gun heads, conical bottle, cylinder, electronic balance, microwave oven, gel plate, electrophoresis apparatus, ultraviolet analyzer, etc.

3.experimental procedure

20ml 1 × TAE was poured into the conical bottle, 0.2g agarose was added to the conical bottle, then heated in the microwave oven to dissolve. When the temperature was reduced to could be touched by hand, 1μl 4S Red Plus Nucleic Acid was added and shake well. Then pour it into the gel plate to be cooled and solidified, then put it into the electrophoresis apparatus, mixed 1μl DNA Buffer and 3μl the liquid to be tested, and then added it to the corresponding hole in the gel plate. After all the samples had been added, added 4μl 10000bp DNA Marker in the hole without the sample. Then turned on the power, and when the samples ran to the back half of the block and was close to the end, turned off the power, put the block into the ultraviolet analyzer, and observe the gel electrophoresis results.

4.experimental results:



It can be seen from the diagram that the linearized plasmid of dusk-puc57 was successfully prepared.

(3)De-template by DpnI

1.experimental objective : to purify cas9 linearized fragment and linearized vector (dusk-pUC57).

2.experimental equipment

(1)reagents:Pcas (experimental group),pUC57-dusk-eGFP (template DNA),dusk-pUC57 (experimental group), 10× Q. Cut buffer, Q. Cut DpnI, ddH_2O.

(2)instrument： liquid transfer guns and gun heads, metal incubator, EP tubes, ice box, etc.

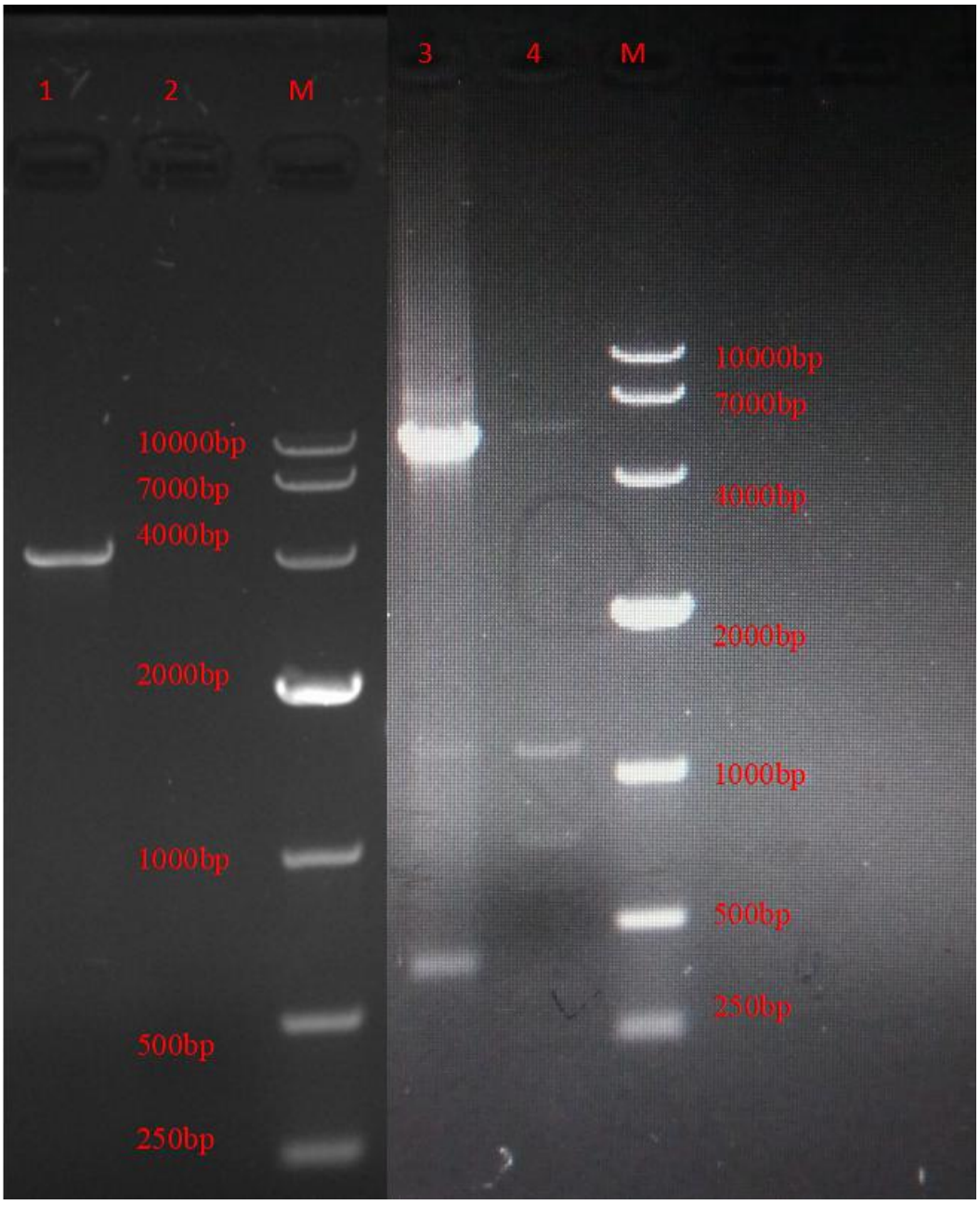
3.experimental procedure

In the ice box, the solution was added to EP tubes according to the following requirements, and shook well, then put into the metal incubator for 2h. The temperature was set at 37 ℃.

De-template(μl)	Linearization carrier		insert	
	experimental group	template DNA	(template DNA	template DNA

Cas9（experimental group ）	0	0	PCR complete product	0
pcas（template DNA）	0	0	0	10
dusk-pUC57（experimental group ）	PCR complete product	0	0	0
pUC57-dusk-eGFP（template DNA）	0	10	0	0
10×Q.Cut Buffer	5	5	5	5
Q.Cut Dpn Ⅰ	1	1	1	1
ddH_2O	0	34	0	34
bulk volume	50	50	50	50

4. Experimental results:



Cas9 linearized fragments and pcas plasmids were found in 1 and 2 holes, respectively;

Dusk-puc57 linearized vectors and dusk-egfp-puc57 plasmids were found in 3 and 4 holes, respectively.

The figure shows that the cas9 template has been eliminated, but the dusk-puc57 template has not been removed. So plan on dusk-puc57 in the elimination of a template.