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LacI-P_{lac}

● We prepared the insertion fragment and the linearized carrier（pGLO） by PCR.

1.Purpose of the experiment: to prepare for one step cloning

2.Experimental equipment

2.1. Reagent: 2×Phanta Max Master Mix, 10mM LacI-F, 10mM LacI-R, 10mM pGLO-F, 10mM pGLO-R, 33ng /μl pCAS plasmid DNA, 426ng /μl pGLO plasmid DNA, ddH_2O.

2.2. Instrument: liquid transfer guns and gun heads, PCR tubes, ice box, gradient PCR instrument, etc.

3. Experimental steps

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

Primer

LacI-F:

5’tagaaataattttgtttaacaaagaggagaaaggatcttccagagatGTGAAACCAGTAACGTTATACGATGTC3’

LacI-R:

5’ctttgctagccatatgtataGGTTTCCTGTGTGAACTGCCGTTCGACGATCTGGTAACAGGATTAG CAGATGTGTG3’

pGLO-F:

5’TATACATATGGCTAGCAAAGGAGAAGA3’

pGLO-R:

5’GTTAAACAAAATTATTTCTAGCCCAAA3’

Materials（50 μl Reaction）:

PCR	linearized carrier	insertion fragment
Fast Pfu Buffer	10μl	10μl
P1	1μl	1μl
P2	1μl	1μl
dNTP	4μl	4μl
Template（pGLO&pCAS）	1μl diluent	1μl
Fast Pfu	1μl	1μl
ddH_2O	32μl	32μl

Attention: 10μl diluent was prepared by 1μl pGLO and 9μl ddH_2O.

PCR condition setting		pGLO	pCAS
Step one		94℃ 240s	94℃ 240s
30 cycles	Step two	94℃ 60s	94℃ 60s
	Step three	50℃ 30s	61℃ 30s
	Step four	72℃ 180s	72℃ 60s

Step five	72℃ 600s	72℃ 600s

● The PCR product was de-templated by DpnI.

1.Purpose of the experiment: to purify the inserted fragmentand linearized carrier .

2. Experimental equipment

2.1. Reagent:the linearized carrier（pGLO）,the insertion fragment(LacI-P_{lac}), 10 ×Q. Cut buffer, Q. Cut DpnI, ddH_2O.

2.2. Instrument: liquid transfer guns and gun heads, metal incubator, EP tubes, ice box, etc.

3. Experimental steps

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℃.

Materials（50μl Reaction）:

PCR product	44μl
Q.Cut DpnI	1μl
10×Q.Cut Buffer	5μl
ddH_2O	0μl

●PCR product was purified by TaKaRa kit to prepare for ligation.

1. Purpose of the experiment: purify the linearized carrier（pGLO）and the insertion fragment(LacI-P_{lac})

2. Experimental equipment

2.1. Reagent: the sample solution treated with DpnI, ddH_2O(preheated by oven at 55℃).

2.2. Instrument: liquid transfer guns and gun heads, EP tubes, centrifuge, purification kit, etc.

3. Experimental steps

The linearized carrier （pGLO） after de-template DNA and the insertion fragment(LacI-P_{lac}) sample solution were added with 3 times volume of Buffer DC, and then mixed evenly. The Spin Column in the kit was placed on Collection Tube. The mixture of Buffer DC was transferred to Spin Column and centrifuged at room temperature 12000rpm for 1 minute. Then the filtrate was added to Spin Column, and the filtrate was centrifuged at room temperature 12000rpm for 1 minute. Then the filtrate was discarded. 700μl Buffer WB was added to Spin Column, and put for 5 minutes at room temperature, then centrifuged at room temperature 12000rpm for 30 seconds. The 700μl Buffer WB was added to the Spin Column, and put for 5 minutes at room temperature, and then centrifuged for 30 seconds at room temperature 12000rpm and discarded the filtrate. Spin Column was placed on Collection Tube and

centrifuged at room temperature 12000rpm for 1 minute. Place the Spin Column on the new EP tube and open the lid. Put at room temperature for 2 minutes to volatilize the residual alcohol. 30μl ddH_2O was added in the center of the Spin Column membrane, and centrifuged for 1 minute at room temperature 12000rpm to dissolve the DNA. Then the filtrate was added to the center of the Spin Column membrane and 30μl ddH_2O was added. DNA was dissolved in the filtrate by centrifuging for 1 minute at room temperature 12000rpm.