

Time : 8.9-8.10

1、Experimental purpose: Prepare competent cells of *E.coli* DH5 α (pGLO-Cas9), *E.coli* DH5 α (pTF-p15A) competent cells; transform

2、Material

E.coli DH5 α (pGLO-Cas9)、*E.coli* DH5 α (pTF-p15A)、*E.coli* DH5 α competent cells (Tsingke)、pGLO-Cas9 plasmid (428ng/ μ l)、pTF-p15A plasmid (444ng/ μ l)、50 mM CaCl₂

3、Experimental procedure

Competent cell preparation:

- (1) Single colonies were picked from *E. coli* DH5 α (pGLO-Cas9) and *E. coli* DH5 α (pTF-p15A) plates and cultured in 5 ml LB medium, and cultured to *E. coli* DH5 α (pGLO-Cas9) OD600=0.45 *E. coli* DH5 α (pTF-p15A) OD600=0.42
- (2) The culture and CaCl₂ was pre-cooled for 10 min on ice;
- (3) Take 1.5 ml of the bacterial culture into a 1.5 ml EP tube, centrifuge at 8000 rpm for 5 min at 4°C, and discard the supernatant;
- (4) Repeat step 3;
- (5) The suspension was resuspended by adding 1 ml of pre-cooled 50 mM MgCl₂, centrifuged at 8000 rpm for 5 min at 4°C, and the supernatant was discarded;
- (6) Repeat step 5;
- (7) Add 100 μ l of 50 mM CaCl₂, resuspend and store at 4°C.

Transformation:

- (1) Taking *E.coli* DH5 α (pGLO-Cas9), *E.coli* DH5 α (pTF-p15A), *E.coli* DH5 α competent cells pre-cooled on ice;
- (2) Taking pGLO-Cas9(428 ng/ μ l), pTF-p15A(444 ng/ μ l) pre-cooled on ice;
- (3) Add the following volume plasmid to the competent cells:

1				2				3				4				5				6			
100ul <i>E.coli</i> DH5 α (pGLO-Cas9)				100ul <i>E.coli</i> DH5 α (pTF-p15A)								100ul <i>E.coli</i> DH5 α											
pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9				
1ul	0	0	0	0	1ul	0	0ul	1ul	1ul	0ul	0ul	1ul	1ul	0ul	0ul	0ul	0ul	0ul	0ul				

- (4) Place on ice for 30min;
- (5) Heat shocked in a 42°C metal bath for 90 s;
- (6) Place on ice for 2min;
- (7) Add 1 ml of pre-warmed LB medium at 37°C, and incubate at 37°C for 1 h;
- (8) Centrifuge at 4000 rpm for 5 min, discard 800 μ l of supernatant, and resuspend the cells with the remaining liquid;
- (9) Spread plate with 50ul resuspension, culture overnight.

Spread plate as shown below:

	1		2		3		4		5		6	
	A1	A2	A3	B1	B2	B3	C1	C2	C3			
Amp	+	+	+	+	+	+	+	+	+	+	+	+
Spec	+	+	+	+	+	+	+	+	+	+	+	+
Ara	+	-	-	+	-	-	+	-	-	-	-	-

4、Results

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