

Time : 8.14-8.15

1、Experimental purpose: Prepare competent cells of *E.coli* K-12 MG1655, *E.coli* K-12 MG1655 panD⁻; transform

2、Material

E.coli K-12 MG1655、*E.coli* K-12 MG1655 panD⁻、pGLO-Cas9 (428ng/ul)、pTF-p15A (444ng/ul)、50 mM CaCl₂

3、Experimental procedure

(1) Prepare competent cells according to our protocol;

(2)transform according to our protocol;

Add the following volume plasmid to the competent cells:

1		2		3		4	
100ul E.coli K-12 MG1655				100ul E.coli K-12 MG1655 panD-			
pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9	pTF-p15A	pGLO-Cas9
1ul	1ul	0	0	1ul	1ul	0	0

(3)Spread plate as shown below:

LB:

	1		2	3		4
Plate number	A1	A2	A3	B1	B2	B3
Amp	+	+	+	+	+	+
Spec	+	+	+	+	+	+
Ara	+	-	-	+	-	-

Minimal medium

	1		2	3		4
No.	C1	C2	C3	D1	D2	D3
Amp	+	+	+	+	+	+
Spec	+	+	+	+	+	+
Ara	+	-	-	+	-	-

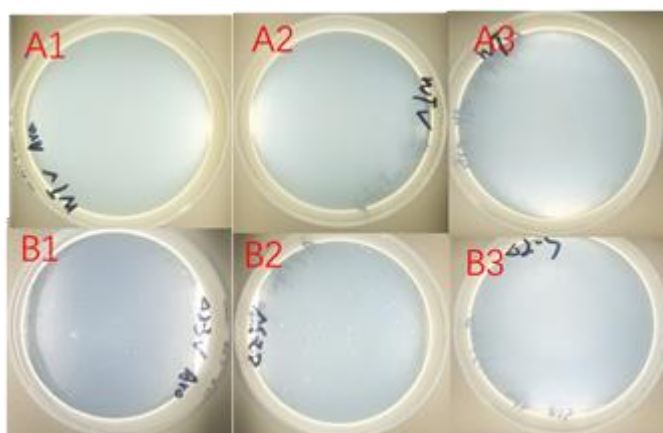
Solid minimal medium

K ₂ HPO ₄ •3H ₂ O	14 g/L
KH ₂ PO ₄	5.2 g/L
(NH ₄) ₂ SO ₄	2 g/L
MgSO ₄	0.3 g/L
Peptone	1 g/L
glucose	10 g/L
Agar powder	20 g/L

4、Results

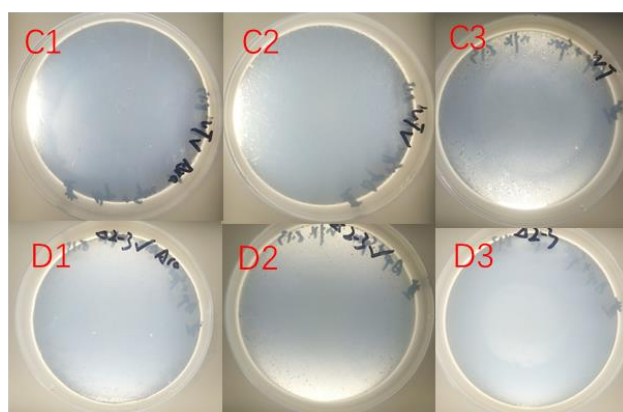
LB plate:

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	A1	A2	A3	B1	B2	B3
CFU 数	0	0	0	22	45	0

Solid minimal medium



	C1	C2	C3	D1	D2	D3
CFU 数	0	0	0	0	0	0