

LacI-P_{lac}

●Maximize the repression effect

1. Purpose of the experiment: Maximize the repression effect of LacI protein.

1.1. Reagent: The objective strain(pGLO-LacI), Amp, liquid minimal medium, arabinose, fructose.

1.2. Instrument: liquid transfer guns and gun heads, tubes (each containing 5mL liquid minimal medium), EP tube, 37 °C oscillating incubator, etc.

2. Experimental steps

① Strain contained target plasmid was cultured in the test tubes with liquid minimal medium. And the concentration gradients of arabinose were set in these tubes ($2 \times 10^{-5} \text{M}$, $4 \times 10^{-5} \text{M}$, $8 \times 10^{-5} \text{M}$, $1.6 \times 10^{-4} \text{M}$, $3.2 \times 10^{-4} \text{M}$) .

Number	20mM fructose/ μl	20mM arabinose/ μl	Amp/ μl
1	80	0	5
2	80	10	5
3	80	20	5
4	80	40	5
5	80	80	5

② OD_{600} and GFP values of the bacterial culture were measured periodically with the automatic microplate reader. For green fluorescence, excitation and emission wavelengths were 395 and 509 nm.

③ Draw charts using measured data to observe the trends of repression.

3. Experimental result

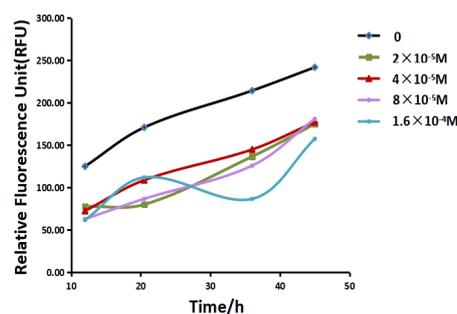


Fig.1 The Relative Fluorescence Unit of the objective strain

4.Details

8.6 10: 00 (12h)									
classification	ara(10mM) / μ L	OD ₆₀₀			Fluorescence when OD ₆₀₀ is 0.45		Relative Fluorescence Unit		
		1	2	Average	1	2	1	2	Average
pGLO+lacI	0	0.505	0.518	0.512	177.80	185.70	120.95	128.85	124.90
	5	0.529	0.521	0.525	133.43	135.77	76.58	78.92	77.75
	10	0.547	0.568	0.558	128.26	130.24	71.41	73.39	72.40
	20	0.531	0.548	0.540	121.28	117.92	64.43	61.07	62.75
	40	0.447	0.449	0.448	118.63	118.44	61.78	61.59	61.69
PUC18	0	0.521	0.525	0.523	58.08	55.62			
						56.85			

8.6 18: 30 (20.5h)									
classification	ara(10mM) / μ L	OD ₆₀₀			Fluorescence when OD ₆₀₀ is 0.45		Relative Fluorescence Unit		
		1	2	Average	1	2	1	2	Average
pGLO+lacI	0	0.575	0.587	0.581	275.33	238.27	189.32	152.26	170.79
	5	0.516	0.536	0.526	157.00	174.93	70.99	88.92	79.95
	10	0.614	0.624	0.619	193.68	195.62	107.67	109.61	108.64
	20	0.614	0.631	0.623	169.70	175.51	83.69	89.50	86.59
	40	0.554	0.547	0.551	221.10	174.57	135.09	88.56	111.82
PUC18	0	0.561	0.605	0.583	82.99	89.04			
						86.014			

8.7 10: 00 (36h)									
classification	ara(10mM) / μ L	OD ₆₀₀			Fluorescence when OD ₆₀₀ is 0.45		Relative Fluorescence Unit		
		1	2	Average	1	2	1	2	Average
pGLO+lacI	0	0.482	0.552	0.517	325.97	336.50	208.91	219.44	214.18
	5	0.595	0.602	0.599	253.16	253.84	136.10	136.78	136.44
	10	0.603	0.628	0.616	265.81	257.95	148.75	140.89	144.82
	20	0.604	0.627	0.616	247.73	237.91	130.67	120.85	125.76
	40	0.725	0.732	0.729	201.96	205.33	84.90	88.27	86.59
PUC18	0	0.610	0.642	0.626	116.93	117.19			
						117.06			

8.7 19: 00 (45h)									
classification	ara(10mM) / μ L	OD ₆₀₀			Fluorescence when OD ₆₀₀ is 0.45		Relative Fluorescence Unit		
		1	2	Average	1	2	1	2	Average
pGLO+lacI	0	0.559	0.582	0.571	385.99	393.95	237.68	245.64	241.66
	5	0.574	0.608	0.591	316.81	329.66	168.50	181.35	174.93
	10	0.600	0.611	0.606	314.16	336.70	165.85	188.39	177.12
	20	0.601	0.623	0.612	309.87	348.54	161.56	200.23	180.90
	40	0.767	0.793	0.780	308.07	303.22	159.76	154.91	157.34
PUC18	0	0.631	0.633	0.632	159.87	136.75			
						148.31			