**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 ℃ oscillating incubator, etc.

2. Experimental steps

①Strain contained target plasmid was cultured in the test tubes with liquid minimal medium. And the concentration gradients of arabinose were set in these tubes (0M,$2\times10^{-5}$M, $8\times10^{-5}$M) .

| Number | 20mM fructose/ μl | 20mM arabinose/μl | Amp/μl |
|---|---|---|---|
| 0 | 80 | 0 | 5 |
| 1 | 80 | 10 | 5 |
| 2 | 80 | 40 | 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.

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

3. Experimental result

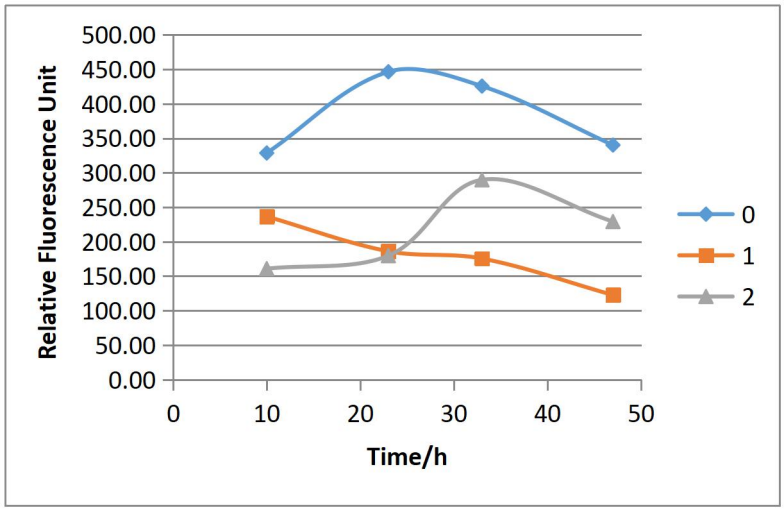


Fig.1 The Relative Fluorescence Unit of the objective strain

4. Details

| 7.31 21：00（10h） | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| classification | ara(10mM) /μL | $OD_{600}$ | | | Fluorescence when $OD_{600}$ is 0.4 | | | Relative Fluorescence Unit | | |
| | | 1 | 2 | Average | 1 | 2 | Average | | | |
| pGLO | 0 | 0.611 | 0.601 | 0.606 | 532.37 | 386.37 | 459.37 | 155.19 | | |
| | 10 | 0.542 | 0.546 | 0.544 | 1354.00 | 1346.80 | 1350.40 | 1046.22 | | |
| | 40 | 0.230 | 0.328 | 0.279 | 1625.40 | 1656.50 | 1640.95 | 1336.77 | | |
| pGLO+lacI | 0 | 0.408 | 0.411 | 0.410 | 680.81 | 584.81 | 632.81 | 328.63 | | |
| | 10 | 0.393 | 0.407 | 0.400 | 592.98 | 488.53 | 540.76 | 236.58 | 92.06 | 0.2801 |
| | 40 | 0.515 | 0.516 | 0.516 | 485.11 | 444.93 | 465.02 | 160.84 | 167.79 | 0.5106 |
| PUC18 | 0 | 0.245 | 0.259 | 0.252 | 283.89 | 324.46 | 304.18 | 0.00 | | |
| | 10 | | | | | | | | | |

| 8.1 10：00（23h） | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| classification | ara(10mM) /μL | $OD_{600}$ | | | Fluorescence when $OD_{600}$ is 0.4 | | | Relative Fluorescence Unit | | |
| | | 1 | 2 | Average | 1 | 2 | Average | | | |
| pGLO | 0 | 0.525 | 0.557 | 0.541 | 781.37 | 787.10 | 784.24 | 269.32 | | |
| | 10 | 0.432 | 0.451 | 0.442 | 1856.90 | 1912.80 | 1884.85 | 1369.93 | | |
| | 40 | 0.472 | 0.475 | 0.474 | 1997.50 | 2058.10 | 2027.80 | 1512.88 | | |
| pGLO+lacI | 0 | 0.554 | 0.573 | 0.564 | 1001.10 | 921.73 | 961.42 | 446.50 | | |
| | 10 | 0.474 | 0.493 | 0.484 | 681.36 | 720.71 | 701.04 | 186.12 | 260.39 | 0.3962 |
| | 40 | 0.399 | 0.411 | 0.405 | 686.13 | 703.14 | 694.64 | 179.72 | 266.79 | 0.4059 |
| PUC18 | 0 | 0.498 | 0.528 | 0.513 | 508.97 | 520.87 | 514.92 | 0.00 | | |
| | 10 | 0.437 | 0.450 | 0.444 | 498.10 | 538.13 | 518.12 | / | | |

| 8.1 20：00（33h） | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| classification | ara(10mM) /μL | $OD_{600}$ | | | Fluorescence when $OD_{600}$ is 0.4 | | | Relative Fluorescence Unit | | |
| | | 1 | 2 | Average | 1 | 2 | Average | | | |
| pGLO | 0 | 0.540 | 0.564 | 0.552 | 413.33 | 426.95 | 420.14 | 128.64 | | |
| | 10 | 0.613 | 0.630 | 0.622 | 1836.00 | 1901.70 | 1868.85 | 1577.35 | | |
| | 40 | 0.663 | 0.644 | 0.654 | 2610.60 | 2868.10 | 2739.35 | 2447.85 | | |
| pGLO+lacI | 0 | 0.544 | 0.563 | 0.554 | 692.83 | 741.50 | 717.17 | 425.67 | | |
| | 10 | 0.533 | 0.530 | 0.532 | 460.62 | 473.50 | 467.06 | 175.56 | 250.11 | 0.5876 |
| | 40 | 0.522 | 0.552 | 0.537 | 566.63 | 595.88 | 581.26 | 289.76 | 135.92 | 0.3193 |
| PUC18 | 0 | 0.610 | 0.637 | 0.624 | 275.87 | 307.12 | 291.50 | (0.00) | | |
| | 10 | | | | | | | / | | |

| 8.2 10：00（47h） | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| classification | ara(10mM) /μL | $OD_{600}$ | | | Fluorescence when $OD_{600}$ is 0.4 | | | Relative Fluorescence Unit | | |
| | | 1 | 2 | Average | 1 | 2 | Average | | | |
| pGLO | 0 | 0.573 | 0.566 | 0.570 | 402.02 | 467.43 | 434.73 | 67.03 | | |
| | 10 | 0.642 | 0.630 | 0.636 | 1725.90 | 1759.30 | 1742.60 | 1374.90 | | |
| | 40 | 0.701 | 0.644 | 0.673 | 2400.20 | 2392.50 | 2396.35 | 2028.65 | | |
| pGLO+lacI | 0 | 0.573 | 0.571 | 0.572 | 684.35 | 731.42 | 707.89 | 340.19 | | |
| | 10 | 0.528 | 0.547 | 0.538 | 492.63 | 488.11 | 490.37 | 122.67 | 217.52 | 0.6394 |
| | 40 | 0.573 | 0.520 | 0.547 | 601.38 | 592.43 | 596.91 | 229.21 | 110.99 | 0.3262 |
| PUC18 | 0 | 0.629 | 0.611 | 0.620 | 373.93 | 361.46 | 367.70 | (0.00) | | |
| | 10 | | | | | | | / | | |