Relative Promoter Unit Measurement Using FACS

1. Streak the cells with following construct on a new plate

construct	plasmid	strain
BBa_E0240	pSB3K3	DH10B
BBa_I20260	pSB3K3	DH10B
kdpFp [-15, T>G] - BBa_E0240	pSB3K3	DH10B

* BBa_E0240 = BBa-B0032 - BBa_E0040 - BBa_B0015

2. Inoculate a colony from each plate in 2 mL of K115 minimal medium. Incubate at 37°C.
3. Prepare the stock of media of specific K^+ concentration and then aliquot 1 mL into Corning® 96 well storage system storage block, 2 mL, V-bottom.
4. Wash the cell three times with 2 mL 0.8% NaCl solution. After washing, resuspend the cells in fresh K0 medium.
5. Check OD_{595} of the washed cells and then dilute all of them to the same OD_{595} (~1).
6. Take out 25 μL of washed cells to mix with K minimal medium of different K concentration in the 96-well storage block.
7. Incubate the culture in 37°C until it reaches the mid-exponential phase (OD_{600}: 0.3-0.5)
8. Take out 200 μL of the culture from the storage block, measure and record the OD_{595} value.
    a. Take samples every 15 minutes for 30 mins in total. In between sampling, keep incubating the cells in 37℃ while shaking.
9. Mix the measured sample with 200 μL of fixation solution.
10. Per cell GFP intensity is then measured by fluorescence-activated cell sorting using Becton Dickinson FACSAria IIIu.

Data Processing for Relative Promoter Unit Measurement

RPU is calculated according to the following equation:

$$\mathrm{RPU} = \frac{[\mathrm{G}]_{\mathrm{cell},\phi}}{[\mathrm{G}]_{\mathrm{cell,J23101}}} * \frac{\mu_{\phi}}{\mu_{J23101}} = \frac{[\mathrm{F}]_{\mathrm{cell},\phi}}{[\mathrm{F}]_{\mathrm{cell,J23101}}} * \frac{\mu_{\phi}}{\mu_{J23101}}$$

$[\mathrm{F}]_{\mathrm{cell},\phi}$ stands for the background fluorescence (BBa_E0240) subtracted geometric mean of per cell GFP intensity. μ stands for the growth rate

approximated by the slope of blank-corrected OD_{595} over time. ϕ refers to the experimental construct while J23101 refers to BBa_J32101 with GFP.

References:

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