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
P_{kdpF} [-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 and dilute all samples to the same optical density.
5. Take out 25 µL of washed cells to mix with K minimal medium of different K concentration in the 96-well storage block.
6. Incubate the culture in 37°C until it reaches the mid-exponential phase. ($OD_{600} \approx 0.4$)
7. 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.
8. Mix the measured sample with 200 µL of fixation solution.
9. 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:

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

$[F]_{\text{cell},\phi}$ 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 optical density over time. ϕ

refers to the experimental construct while J23101 refers to BBa_J32101 with GFP.

<u>References:</u>

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