Relative Fluorescence and GFP Synthesis Rate Measurements

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

construct	plasmid	*E. coli* strain
BBa_E0240*	pSB3K3	DH10B
P_{kdpF} - BBa_E0240	pSB3K3	DH10B
P_{kdpF} [-15, T>G] - BBa_E0240	pSB3K3	DH10B
P_{kdpF} [-15, T>C] - BBa_E0240	pSB3K3	DH10B
P_{kdpF} [-15, T>A] - BBa_E0240	pSB3K3	DH10B
BBa_E0240*	pSB3K3	TK2240
P_{kdpF} [-15, T>G] - BBa_E0240	pSB3K3	TK2240

* 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 and put into a micro test plate 96 well flat-bottom. Measure GFP intensity and optical density using Envision Multilabel Reader
    a. For GFP synthesis rate measurement: Measure every 15 minutes for 30 min in total. In between measurements, keep incubating the cells in 37°C, while shaking at 220 rpm.

Filter used on Envision Multilabel Reader:

- Absorbance: Photometric 595nm,
- Excitation: 485nm FITC,
- Emission: 535nm FITC,
- Mirror module: FITC (403) on top.

Data Processing for Relative Fluorescence Measurement

1. Subtract optical density reading of blank media from the optical density of samples.
2. Normalize the fluorescence reading by obtaining $\frac{\text{Fluorescence}}{\text{Optical Density}}$ (F/OD) value for each sample.
3. Subtract the background fluorescence of cells (BBa_E0240) from the normalized fluorescence of the samples.

Data Processing for GFP Synthesis Rate Measurement

1. Calculate the F/OD value for each time point as stated previously.
2. Take the slope of the F/OD over time.
3. Subtracting the slope of blank cell (BBa_E0240) from the slope of sample to obtain GFP synthesis rate.

References:

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