[Trc-LRY] Inducible LuxR Expression Device

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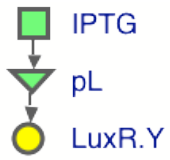
Schematic



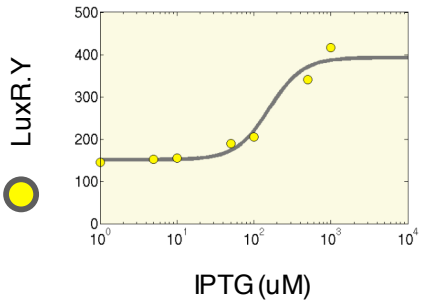
Characteristics

LuxR activity can be tested only when the promoter LuxpR is present, in conjunction with an AHL sender (see data for [Sen-TIC+Rec-LRY.RC]).

Protocol: We grew cells overnight in LB. We then transferred them to Glu-M9 minimal medium containing the desired final concentration of IPTG, and allowed them to grow for 12h. The cell density at transfer was chosen so that the final OD600 was < 0.1. Cells were concentrated by centrifugation, and imaged on an epifluorescence microscope. We calculated the fluorescence per unit area of single cells, obtaining data from ~500 cells for each run. We then averaged these values in log space to obtain the final estimate of protein expression. The IPTG mesh was [0 5 10 50 100 500 1000] uM. The results shown below are the average of four replicates.

Sigmoidal fit:

$$y = a_0 + a_1 \frac{x^n}{K^n + x^n} \qquad a_0 = 151;\ a_1 = 241;\ K = 158;\ n = 2;$$



Measurements and analysis were carried out by members of the NCBS iGEM 2007 team:
Kiran, Krishna, Mukund, Navneet, Nilesh, Senthil, Shashanka, Sugat, Sushant, Varun, Vini, Vivek

Host: Ecoli K12Z1

p1.lacI::p2.tetR

Part: [Trc-LRY]

pL.luxR.Y

Small molecules

IPTG: Isopropyl β-D-1-thiogalactopyranoside.

Promoters

- p1: Constitutive LacIq promoter.
- p2: Constitutive N25 promoter.
- pL: Lac promoter.

Proteins

- LacI: Lac repressor, negative regulator of pL.
- TetR: Tet repressor, negative regulator of pT.
- LuxR: *V. fischeri* LuxR protein, positive regulator of pR.
- Y: Yellow fluorescent protein

Description

IPTG drives the expression of the LuxR protein (monitored using polycistronic YFP).

Usage and compatibility

LuxR can be used to drive expression from the promoter LuxpR in the presence of AHL. This device is a sub-component of the AHL open-loop receiver [Rec-LRY.RC].

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