[Sen-TIC+Rec-RRY] Closed-Loop System

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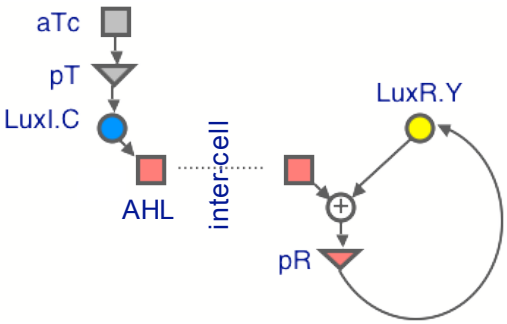
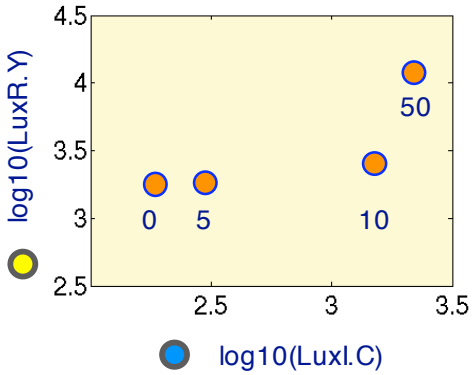
Schematic



Characteristics

Protocol: We grew sender cells overnight in LB, then transferred them to Glu-M9 minimal medium containing the desired final concentration of aTc, and allowed them to grow for ~12h, until they reached OD600=0.2. We then removed the sender cells by filtration, retaining the medium (which now contains AHL) and replenishing it with an equal volume of fresh Glu-M9 with double the standard glucose concentration. Receiver cells, which had previously been grown overnight in LB, were added to this medium. These cells were allowed to grow for ~12h, and harvested at OD600 < 0.1. Both sender and receiver 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 aTc mesh was [0 1 5 10 20 50] ng/ml (shown below the datapoints). *The results shown below are preliminary.*



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

Parts: [Sen-TIC+Rec-RRY]

Small molecules

- aTc: anhydrotetracycline.
- AHL: acyl homoserine lactone.

Promoters

- p1: Constitutive LacIq promoter.
- p2: Constitutive N25 promoter.
- pT: Tet promoter
- pR: *V. fischeri* LuxpR promoter.

Proteins

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

Description

Sender: aTc drives the expression of the LuxI protein (monitored using polycistronic CFP). This in turn drives the synthesis of AHL, which is exported. Receiver: In the presence of AHL, the LuxR protein activates its own expression at promoter pR (monitored using polycistronic YFP).

Usage and compatibility

This is a stand-alone system.