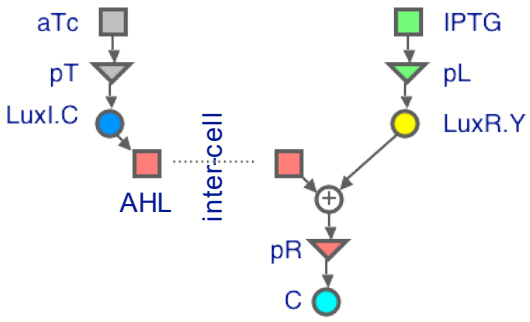
[Sen-TIC+Rec-LRY.RC] Open-Loop System

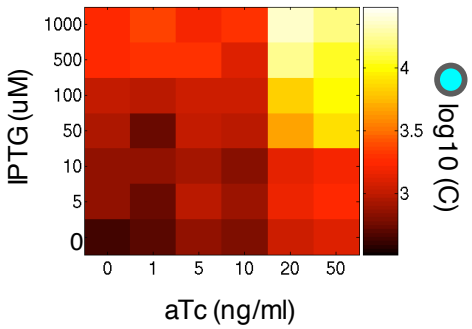
Author: Mukund Thattai (thattai@ncbs.res.in) Date: October 16th, 2007

Schematic



Characteristics

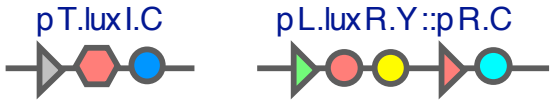
Protocol: We grew sender cells overnight in LB, then transferred them to Glu-M9 minimal medium containing the desired final concentration of aTc, and allowed them to grow for ~12h, until they reached OD600=0.2. We then removed the sender cells by filtration, retaining the medium (which now contained AHL) and replenishing it with an equal volume of fresh Glu-M9 containing double the standard glucose concentration, and the desired final concentration of IPTG. 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, and the IPTG mesh was [0 5 10 50 100 500 1000] uM. The results shown below are the average of two replicates.



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-LRY.RC]



Small molecules

- IPTG: Isopropyl β-D-1-thiogalactopyranoside.
- aTc: anhydrotetracycline.
- AHL: acyl homoserine lactone.

Promoters

- p1: Constitutive LacIq promoter.
- p2: Constitutive N25 promoter.
- pT: Tet promoter
- pL: Lac 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: IPTG drives the expression of the LuxR protein (monitored using polycistronic YFP). LuxR, when bound imported AHL, drives the expression of CFP.

Usage and compatibility

This is a stand-alone system.