[Sen-TIC] AHL Sender Device

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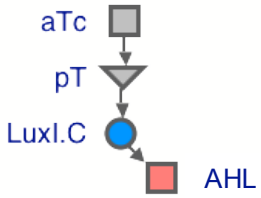
Schematic



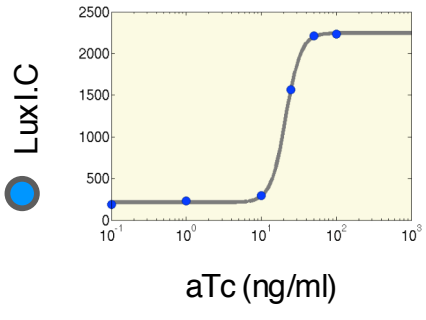
Characteristics

AHL output can be tested in conjunction with any AHL receiver system (see data for [Sen-TIC+Rec-LRY.RC] and [Sen-TIC+Rec-RRY]).

Protocol: We grew cells overnight in LB. We then transferred them to Glu-M9 minimal medium containing the desired final concentration of aTc, 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 aTc mesh was [0 1 10 20 50 100] ng/ml.

Sigmoidal fit:

$$y = a_0 + a_1 \frac{x^n}{K^n + x^n} \qquad a_0 = 212;\ a_1 = 2027;\ K = 21.5;\ n = 4.3;$$



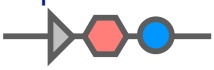
Measurements and analysis were carried out by members of the NCBS iGEM 2007 team:
Kiran, Krishna, Mukund, Navneet, Nilesh, Senthil, Shashanka, Sugat, Sushant, Varun, Vini, Vivek

Host: Ecoli K12Z1

p1.lacI::p2.tetR

Part: [Sen-TIC]

pT.luxI.C



Small molecules

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

Promoters

- p1: Constitutive LacIq promoter.
- p2: Constitutive N25 promoter.
- pT: Tet promoter.

Proteins

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

Description

aTc drives the expression of the LuxI protein (monitored using polycistronic CFP). This in turn drives the synthesis of AHL, which is exported.

Usage and compatibility

This device can be used to drive expression from the promoter LuxpR in any AHL receiver system.
It has been tested with [Rec-LRY.RC] and [Rec-RRY].

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