E.coli**RULER**: an application for a biological comparator

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The aim of our project is to design and build a biological system which functions as an electronic comparator. The comparator consists of two constructions, that should be implemented in one single *E. coli*. This device compares two input levels and evolves two output signals, in such a way that slight differences between the inputs are enhanced at the output level. This behaviour comes directly from the design of our device, where two repressor proteins are cross repressed.

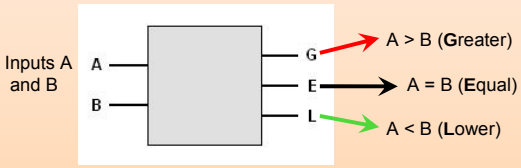
We have decided to build a biological comparator for its modularity, which permits the construction of several useful systems.

The main focus was on a promoter calibrator, the E.coliRuler. We wanted to compare the promoter strengths of different mutants of the osmolarity-sensing promoter for the gene OmpC up to a standard. An effective model of the system was developed to analyze *in silico* the behaviour of the system and study if it could be reliable. In order to further study the modularity of the biological comparator, we modelled and simulated a proportional controller.

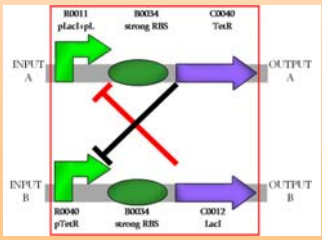
We present this simple, useful device that can be implemented in a variety of systems and constructions. Examples of its usefulness could be found on an analogic to digital converter (ADC), high band and low pass filters or a discrete level detector.

COMPARATOR: compare sensibly differences between two inputs.

· **ELECTRONIC comparator:**



· **BIOLOGICAL comparator**, like an electronic comparator**:**



equal amount of input A and B → equal amounts of outputs.

input A sensibly higher than B → less output B

input B sensibly higher than A → less output A

Dynamical process:

Equations:

$$\frac{dx}{dt} = \alpha_x \frac{\left(\frac{p_1}{k_1}\right)^{n_1}}{1+\left(\frac{p_1}{k_1}\right)^{n_1}} \frac{1}{1+\left(\frac{y}{k_y}\right)^{n_y}} - \beta_x x + \gamma_x$$

$$\frac{dy}{dt} = \alpha_y \frac{\left(\frac{p_2}{k_2}\right)^{n_2}}{1+\left(\frac{p_2}{k_2}\right)^{n_2}} \frac{1}{1+\left(\frac{x}{k_x}\right)^{n_x}} - \beta_y y + \gamma_y$$

Constants:
K : the threshold to protein concentration.
n : Hill coefficient.
α : information of transcription and translation.
β : decay constant.
γ : basal production of the protein Y.

Final result of the system; depend of the initial concentration of p1 and p2:

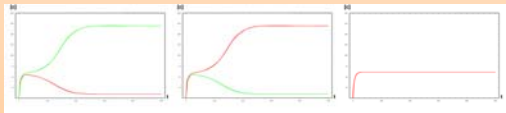


Fig.: Numerical simulations depending of p1 and p2.

PROMOTER CALIBRATOR: compare promoters strenghts, quantified output levels.

With the same principle that biological comparator but an opposite formulation, both constructions have different promoters for the same input.

Our example:
- Inputs: different osmolarity promoters, pOMPR (wild type) and pOMPRm (mutant).
- Outputs: fluorescence proteins (CFP and YFP)
- Comparator: pLac-tet and pTet-lac.

pOMPR-pLacI-strongRBS-tetR(ssrA+)-strongRBS-CFP-term-term
pOMPRm-ptetR-strongRBS-LacI(LVA+)-strongRBS-YFP-term-term

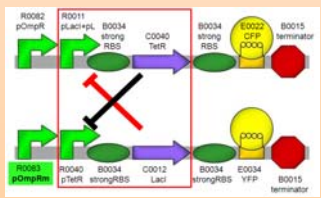
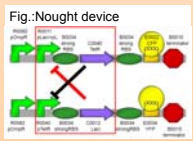


Fig.:Nought device

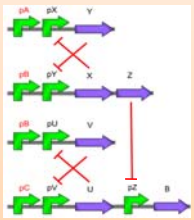


New parts:
pLacI-strong RBS-tetR(ssrA+)
ptetR-strong RBS-LacI(LVA+)
pOMPR-pLacI-strong RBS-tetR(ssrA+)
pLacI-strong RBS-tetR(ssrA+)-strong RBS-CFP-term-term
ptetR-strong RBS-LacI(LVA+)-strong RBS-YFP-term-term

Another application of the comparator

CONTROLLER: control the concentration of certain protein (B) in order to maintain it between certain values.

Similar as a proportional controller in engineering.



Model of the system

$$\frac{\partial x}{\partial t} = \frac{\alpha_x}{1+\left(\frac{v(t)}{K_v}\right)^{n_v}} \frac{\left(\frac{p_1}{K_1}\right)^{n_1}}{1+\left(\frac{p_1}{K_1}\right)^{n_1}} - \beta_x x(t) - \gamma_x$$

$$\frac{\partial y}{\partial t} = \frac{\alpha_y}{1+\left(\frac{v(t)}{K_v}\right)^{n_v}} \frac{\left(\frac{p_2}{K_2}\right)^{n_2}}{1+\left(\frac{p_2}{K_2}\right)^{n_2}} - \beta_y y(t) - \gamma_y$$

$$\frac{\partial z}{\partial t} = \frac{\alpha_z}{1+\left(\frac{v(t)}{K_v}\right)^{n_v}} \frac{\left(\frac{p_3}{K_3}\right)^{n_3}}{1+\left(\frac{p_3}{K_3}\right)^{n_3}} - \beta_z z(t) - \gamma_z$$

$$\frac{\partial v}{\partial t} = \frac{\alpha_v}{1+\left(\frac{u(t)}{K_u}\right)^{n_u}} \frac{\left(\frac{p_4}{K_4}\right)^{n_4}}{1+\left(\frac{p_4}{K_4}\right)^{n_4}} - \beta_v v(t) - \gamma_v$$

$$\frac{\partial u}{\partial t} = \frac{\alpha_u}{1+\left(\frac{u(t)}{K_u}\right)^{n_u}} \frac{\left(\frac{p_5}{K_5}\right)^{n_5}}{1+\left(\frac{p_5}{K_5}\right)^{n_5}} - \beta_u u(t) - \gamma_u$$

$$\frac{\partial p2}{\partial t} = \frac{1}{1+\left(\frac{v(t)}{K_v}\right)^{n_v}} \frac{\alpha_{p2}}{1+\left(\frac{u(t)}{K_u}\right)^{n_u}} \frac{\left(\frac{p_2}{K_2}\right)^{n_2}}{1+\left(\frac{p_2}{K_2}\right)^{n_2}} - \beta_{p2} p2(t) - \gamma_{p2}$$

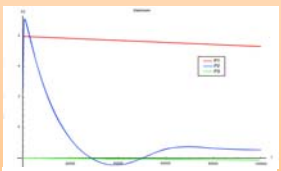


Fig.: Dynamical Solution of the controller. P1 and P3 are the edge of the interval, and P2 represents the stabilized protein.

Other applications:
•Filters.
•Discrete level detector.
•Analogic to digital signal converter

Conclusions:

▪We devise and construct a simple device (few number of genes) which several applications. Comparator module have two pair of genes, one for each construction. The optional outputs (fluorescence, enzyme, etc...) and promoters (to sensed inputs, controlled inputs effect, compare their affinity or to any other new application) serve as a reference of modularity.

▪Built comparator is only an example with a particular promoters, repressors and outputs (pLac-Tet-CFP and pTet-Lac-YFP) and an specific promoters to sense (different pOMPR mutants).

▪We proposed use the comparator to simplify other projects and invite them to look for new applications.

References:

1.Elowitz, Michael B; Leibler, Stanislas, A synthetic oscillatory network of transcriptional regulators, 2000, Nature, 403,6767, p. 335.

2.Forst *et al* (1989). *DNA-binding properties of the transcription activator (OmpR) for the upstream sequences of ompF in Escherichia coli are altered by envZ mutations and medium osmolarity.* **J. Bacteriol.**,171 (6), 2949-2955.

3.http://parts.mit.edu/igem07/index.php/Valencia/Summary

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