Multiplexing and demultiplexing signals by E. coli

Pasotti L[(1)], Quattrocelli M[(2)], Galli D[(2)], Cusella De Angelis MG[(2)], Magni P[(1)]

[(1)] Dipartimento di Informatica e Sistemistica, Università degli Studi di Pavia, Pavia, Italy
[(2)] Centro di Ingegneria Tissutale, Università degli Studi di Pavia, Pavia, Italy

Motivation

This abstract describes the project developed by UNIPV-Pavia team and awarded a Silver Medal at iGEM 2008 Synthetic Biology competition at MIT. Two biological systems which mimic multiplexer (mux) and demultiplexer (demux) logic functions have been designed. Mux and demux devices have a remarkable importance in electronic and telecommunication systems, like Central Processing Units (CPUs). Our challenge was to provide multiplexing and demultiplexing capabilities in a living organism such as Escherichia coli. To reach our goal, two genetic circuits have been designed, built up and tested, using MIT's BioBrick standard parts. An interesting feature of these circuits is that they are general and can be specialized to recognize different input signals and to express different genes as output, so that they can be used in several application fields, such as signal processing or bioprocess regulation.

Methods

Mux and demux logic functions can be expressed using AND, OR, and NOT logic gates. In order to design biological mux and demux, a modular approach has been followed:
i) the three basic logic gates have been designed in a biological framework;
ii) the genetic elements composing these gates have been interconnected to give life to the final circuits.
AND gates have been implemented using quorum sensing complexes, such as lux and las operons. They are based on promoters (Plux and Plas) that can only be activated by the simultaneous expression of two specific genes (luxI-luxR and lasI-lasR). OR gate has been implemented replicating a gene of interest under the control of two independent promoters. So, it is sufficient that one of the two promoters is on to express the gene of interest. NOT gate has been implemented using P. repressible promoter, which is normally on, but can be turned off by cI repressor protein binding. In this way, the expression of cI gene determines P. inhibition, while the absence of cI expression determines P. activation, thus mimicking the behaviour of a logic inverter. Interconnecting the genetic elements of the designed logic gates, the final devices implementing mux and demux have been obtained. The designed circuits have no specific input or output elements and they both conform to the Polymerase Per Second (PoPS) boundary standard in a multi-input multi-output framework. This feature makes our mux and demux "universal" systems and allows users to specialize them by assembling the desired biological input and output devices, which must conform to PoPS functional standard too.

Results

Both final circuits have been built up, sequenced and submitted to the MIT's Registry of Standard Biological Parts. Six circuit subparts containing biological logic gates elements have been tested. Genes which encode for GFP (Green Fluorescent Protein) and RFP (Red Fluorescent Protein) have been used to measure promoters activity during tests and fluorescence has been detected using NIKON Eclipse 80i microscope. Assuming that fluorescence presence corresponds to logic 1 and its absence to logic 0, the results of the tests allowed to validate five input combinations of biological logic gates truth tables. Tests also allowed to measure the reporter-inducer steady state characteristic of two test parts containing a reporter gene under Plux promoter and a constitutively expressed luxR gene. These two parts have been induced with different concentrations of 3OC6HSL inducer, which simulated the presence of LuxI protein, and fluorescence intensity at steady state has been computed. The experimental static characteristics of these two parts have been useful to estimate the cut-off concentration of the inducer that discriminates logic 0 state from logic 1 state. To complete devices characterization, validation of the remaining truth table rows should be provided and selector switching dynamics in both devices should be estimated.

a) AND (lux system)

luxI	luxR	P_{lux}
No	No	Off
No	Yes	Off
Yes	No	Off
Yes	Yes	On

b) AND (las system)

lasI	lasR	P_{las}
No	No	Off
No	Yes	Off
Yes	No	Off
Yes	Yes	On

c) NOT (λ-cI system)

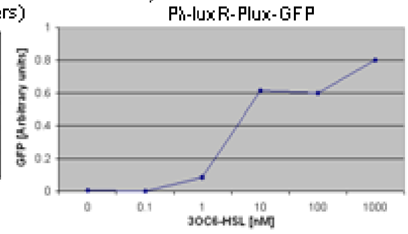
cI	P_{λ}
No	On
Yes	Off

Figure: a-d) truth tables of AND (lux), AND (las), NOT and OR gates; validated rows of truth tables are highlighted. e-f) estimated static characteristics.

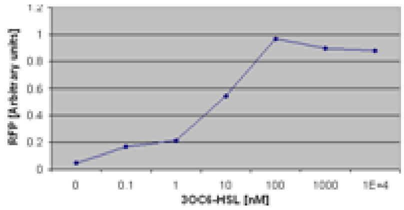
d) Example of OR gate (gene under two independent promoters)

P_{lux}	P_{las}	GFP
Off	Off	No
Off	On	Yes
On	Off	Yes
On	On	Yes

e) Static characteristic of Pλ-luxR-Plux-GFP



f) Static characteristic of cI-lasR-Pλ-luxR-Plux-RFP



Contact : lorenzo.pasotti@unipv.it