Ecoli.PROM: an Erasable and Programmable Genetic Memory with E. coli
What’s a PROM?

PROM: Programmable Read Only Memory

1 0 1 1
0 1 0 0
0 0 1 1
1 1 0 1
From an EPROM to a bacterial pixel

Gel matrix
Flip Flop SR

Flip-Flop is a an electronic circuit with two stable states, which works as 1 bit of memory.

<table>
<thead>
<tr>
<th>INPUT</th>
<th>OUTPUT</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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The genetic flip flop

<table>
<thead>
<tr>
<th>S</th>
<th>R</th>
<th>Q_{n+1}</th>
<th>Q_{n+1}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>LacI OFF</td>
<td>TetR ON</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>LacI ON</td>
<td>TetR OFF</td>
</tr>
<tr>
<td>UVc</td>
<td>0</td>
<td>LacI ON</td>
<td>TetR OFF</td>
</tr>
<tr>
<td>0</td>
<td>IPTG</td>
<td>LacI OFF</td>
<td>TetR ON</td>
</tr>
</tbody>
</table>

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Mathematical Model

Genetic Flip-Flop

Model Equations

\[
\begin{align*}
\frac{dl}{dt} &= \frac{\alpha_l}{1 + \left(\frac{R}{R_{50}}\right)^{\mu_R}} - \frac{\alpha_A}{1 + \left(\frac{A}{A_{50}}\right)^{\mu_A}} - \delta \cdot l \\
\frac{dR}{dt} &= \frac{\alpha_R}{1 + \left(\frac{I^F}{I_{50}}\right)^{\mu_I}} - \delta \cdot R \\
I^F &= \frac{IPTG^2}{1 + \left(\frac{IPTG}{IPTG_{50}}\right)^2}
\end{align*}
\]
**Equilibrium conditions**

In the absence of stimuli, the concentrations of LacI and TetR at equilibrium are related by the following equations:

\[
\begin{cases}
  \frac{I}{I_{E0}} = \frac{K_I}{1 + \frac{R}{R_{E0}}^2} \\
  \frac{R}{R_{E0}} = \frac{K_R}{1 + \frac{I}{I_{E0}}^2}
\end{cases}
\]

\[
\begin{align*}
  K_I &= \frac{\alpha_I}{I_{E0}} \cdot \frac{1}{\delta} \\
  K_R &= \frac{\alpha_R}{R_{E0}} \cdot \frac{1}{\delta}
\end{align*}
\]
Saddle-Node Bifurcation

If $K_i$ or $K_r$ decrease a saddle-node bifurcation can occur and the circuit loses a stable equilibrium.
The Bistability Region

\[ \begin{align*}
K_r &< \frac{3 \sqrt{3}}{4^2} \cdot K_t^2 \\
K_r &> \frac{4}{\sqrt{3 \sqrt{3}}} \cdot \sqrt{K_t}
\end{align*} \]
### Procedure for $K_i$-index identification

<table>
<thead>
<tr>
<th>GENETICAL CIRCUITS</th>
<th>DIFFERENTIAL EQUATIONS</th>
<th>EQUILIBRIUM CONDITIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OPEN LOOP</strong></td>
<td>$\frac{dl}{dt} = \alpha - \delta \cdot l$</td>
<td>$I_Q = \frac{\alpha}{\delta}$</td>
</tr>
<tr>
<td><strong>CLOSED LOOP</strong></td>
<td>$\frac{dl}{dt} = \frac{\alpha}{1 + \left(\frac{l}{l_{SO}}\right)^2} - \delta \cdot l$</td>
<td>$I_Q = \frac{\alpha}{\delta} \cdot \frac{1}{1 + \left(\frac{l}{l_{SO}}\right)^2}$</td>
</tr>
</tbody>
</table>

Assuming that GFP is proportional to $l$ we obtain:

$$K_i = \frac{\alpha}{l_{SO}} \cdot \frac{1}{\delta} = \frac{I_O}{I_{SO}} = \frac{I_O}{I_C} \cdot \sqrt{\frac{I_O - I_C}{I_C}}$$

$$K_i = \frac{GFP_O}{GFP_C} \cdot \sqrt{\frac{GFP_O - GFP_C}{GFP_C}}$$
Numerical Simulations

$K_i = K_r = 10$ Bistable system
Response to IPTG pulse

LacI response to IPTG pulse
- LacI ON
- LacI OFF

TetR response to IPTG pulse
- TetR OFF
- TetR ON

IPTG = 1.2 IPTG_{50}
IPTG = 1.3 IPTG_{50}
IPTG = 3.0 IPTG_{50}

GFP [a.u.f.]
RFP [a.u.f.]

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Does Your System Need a Tune-Up?

- Our Mathematical model points out a defined $K_i$ index for optimum memory performances.
- In the Registry each promoter, however complex, is treated as a “standalone” monolithic element.
- If we want a promoter responsive to a given stimulus or Transcription Factor we're restricted to a fixed strength and repression dynamics.

**Limited control degree on $K_i$ and $K_r$ parameters**

- We wanted a better capability to probe this parameter space.
Another Biobrick in the Wall

We Defined “Operator Sites” as a new Biobrick Part Type

It’s a complex element. Regulation is sensitive both to Sequence and Position (Programming gene expression with combinatorial promoters. Cox 2007)

Can be assembled with Berkeley’s constitutive promoter family:
- Allow to independently fix $\alpha$ and $I_{50}$
- Could establish a basis for modular building of multiple specificity promoters

As Synthetic Biology moves from the proof of concept towards an application-oriented prospective, maturing as an applied science, the need arises to suit the transcription dynamical behavior to the specific demands of the problem.
Partner in Crimes: Concurrent Interests

The benefit of independent promoter elements has been sensed as well by other Igem Groups. Their works gave us some nice inspiration and further direction for our works.

- Building Eukaryotic Promoter regulated by exogenous elements
- Design of control circuit with different activation levels
Building Promoter Libraries

Since Operators sequences flanked by Biobrick ends are unpractical and costly to obtain, both by Dna Sinthesys and Phosphorylated Oligos, we designed a "Standard Collection Vector".

Specifically enable the extraction of one or a combination of operators using exclusively Biobrick prefix and suffix restriction enzymes and allows to get multiple small sequences with a single synthesis process.
An Orientation Chart:

**LacI**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Off/On LacZ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac SymL</td>
<td>aattgtagcgctacaatt</td>
<td>940</td>
</tr>
<tr>
<td>Lac 01</td>
<td>aattgtagcggataacaatt</td>
<td>200</td>
</tr>
<tr>
<td>Lac 02</td>
<td>aattgtagcggatacaacc</td>
<td>21</td>
</tr>
</tbody>
</table>

**Tet**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Beta Gal Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tet O</td>
<td>cctatcaatgataga</td>
<td>0.6%</td>
</tr>
<tr>
<td>TetO-4C</td>
<td>cctgtaatgacgaaga</td>
<td>8.2%</td>
</tr>
<tr>
<td>TetO-wt/4C5G</td>
<td>cctatcaatgagcga</td>
<td>101%</td>
</tr>
</tbody>
</table>

**Lambda**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Binding Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda OR1</td>
<td>tataccggcagaggtga</td>
<td>-10.4 Kcal/mol</td>
</tr>
<tr>
<td>Lambda OR2</td>
<td>tataccggcaggggtga</td>
<td>-7.9 Kcal/mol</td>
</tr>
<tr>
<td>Lambda OR3</td>
<td>tataccggcaggggatt</td>
<td>-7.4 Kcal/mol</td>
</tr>
</tbody>
</table>

**Lex**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LexA 1</td>
<td>ctgtatatatacag</td>
<td>Strong</td>
</tr>
<tr>
<td>LexA 2</td>
<td>ctgtatgacatacag</td>
<td>Weak</td>
</tr>
</tbody>
</table>

Since Operators are complex biological elements, we tried to gather from literature and publish into the registry as much information as possible. We defined an experimental protocol and we're working to characterize all the operators and produce an extensive set of homogeneous comparable data for the [Registry](http://partsregistry.org/wiki/index.php?title=Part:BBa_K079045).

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LacI_2 Operator – Functionality Test

**Open-Loop Configuration** lacking the operator site

K079026

**Closed-Loop Configuration**: GFP regulated by the synthesis of LacI repressor protein

K079020

Protocol

- Inoculation of one colony in 5ml LB medium with antibiotic
- Measurement of O/N samples in fluorescence
Fluorescence Imaging Analysis: SW Tool

Five slides for each O/N culture was prepared and analyzed by VISUAL FLUO BACTERIA Vers. 2.0

- Mean
- Standard deviation
- Median
- Minimum value
- Maximum value
- Number of Clusterized Bacteria

Control parameters

- STD/Mean efficiency
- Area Check
Results: LacI_2 Operator Test – Model Parameter Determination

Open Loop Vs Closed Loop

<table>
<thead>
<tr>
<th></th>
<th>Bacteria Number</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open- Loop (BBa_K079026)</td>
<td>683</td>
<td>116</td>
<td>29</td>
<td>117</td>
</tr>
<tr>
<td>Closed- Loop (BBa_K079020)</td>
<td>683</td>
<td>38</td>
<td>12</td>
<td>35</td>
</tr>
</tbody>
</table>

**K_i = 4.4**

The $K_r$- range for the bi-stability results from 4 to 6

4 < $K_r$ < 6
LexA_2 Operator – Functionality Test (I)

- Box with UV lamp (253.7nm) was realized
- $E < 5 \text{ J/m}^2$

- Time / Distance UV irradiation not optimal
- Uniform irradiation not performed

**UV Homemade Box**

**LexA_2 view induced by UV**
LexA_2 Operator – Functionality Test (II)

Induction by 2.2mM H₂O₂

LexA construct responds uniformly to H₂O₂ induction

SOS system doesn’t interact with IPTG
**IPTG - H₂O₂ CrossTalk**

In order to verify Lac operator with H₂O₂ induction, test was done with the following configuration:

![Diagram of genetic components](image)

LexA and LacI transcription factors can be used in our genetic Flip-Flop.
Conclusions: What has been done . . .

- Genetic circuits closed-loop configuration
  - the constitutive promoter and LacI operator 2 were auto-regulated by the LacI repressor

- UV sensitive trigger
  - GFP reporter protein under the control of the constitutive promoter and LexA 2 operator

- Testing circuit
  - GFP expression controlled by LacI repressor under constitutive promoter

- Operator Library
  - Op3, Op2, Op1

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Conclusions: What remains to do . . .

- Test remaining operator sites with different repressor binding affinity

- Check the use of operator sequences, as independent parts in the assembling of regulated promoters

- Clone each operator site into BioBrick standard assembly plasmids
Check List

Submit DNA for new BioBrick and enter information detailing to the Registry of Parts

- We submitted our part and enter detailing information (wiki)

Demonstrate that new BioBrick Part of your own design and construction works as expected.

- We obtained results as expected by testing our circuits

Help another iGEM team by, for example, charactering a part, debugging a construct, or modeling or simulating their system.

- Collaboration with the Pavia Igem Team

Develop and document a new technical standard


Characterize an existing BioBrick Part distributed via the 2008 iGEM BioBrick Parts collection, and enter this information back on the Registry

- RecA SOS promoter & lacY construct
IGEM Experience

- Discover the world of Synthetic Biology
- Teamwork and confrontation of ideas
- Come to the USA and show our work at IGEM

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Thanks to . . .

University of Bologna

Instructors: Francesca Ceroni, Simone Furini, Silvio Cavalcanti

Advisors: Emanuele Giordano, Marco Caprini

Presented by:
Bologna Team Igem 2008
Questions
Leafing Through...

**Xba**

**PstI**

**Eco RI**

Composite Operator Site

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