Conditioned Reflex Mimicking in *E.coli*

**Team PKU_Beijing**

**iGEM 2009**

**Team Member:** Min Lin, Rencheng Gao, Shan Shen, Shuke Wu, Hao Wang, Haoqian Zhang, Guosheng Zhang, Chengzhe Tian, Xiaomeng Zhang, Siheng He, Zhenglin Yang, Tiffany Saw

**Instructor:** Qi Ouyang, Luhua Lai, Yipeng Wang, Chunxiong Luo, Lingli Jiang, Chunbo Lou
Classic Conditioned Reflex experiment was first carried out by Pavlov on dog.

The Ring is linked to Food!

Learning & Memory

Salivation
Logic Circuit

Ring

Food

Learning AND Gate

Memory 0

Recalling AND Gate

OR

Saliva

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Conditioned Reflex Mimicking in *E.coli*, Team PKU_Beijing, iGEM 2009
Ring

Learning AND Gate  Memory 0  Recalling AND Gate

OR

Saliva
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Logic Circuit

Ring

Saliva

Learning AND Gate

Recalling AND Gate

Memory 1

OR

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Logic Circuit

Small Molecule 1

Learning AND Gate  
Memory 1  
Recalling AND Gate

Small Molecule 2

Food  
Saliva  
GFP

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SupD tRNA

T7 polymerase

P2 PhiR73 or T3pol

Amber mutation

PO promoter T3 promoter

Downstream protein

J Christopher Anderson, et. al., Environmental signal integration by a modular AND gate, *Molecular Systems Biology* 3:133,
Memory

Bistable Switch

CI repressor → CI434 repressible Promoter → CI Repressor

CI repressible Promoter → CI434 repressor

Original State
OR Gate

Food signal
Inducible
Promoter

T3/PO
Promoter

GFP (saliva)
Modeling

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Equations
Equations

Transcription

Hill’s Equation

\[
\frac{d[\text{mRNA}]}{dt} = k \frac{([A]^n)}{K^n} - \gamma[\text{mRNA}]
\]

Activate: \( \beta = 1 \)
Repress: \( \beta = 0 \)

Translation

Elementary Reaction

\[
\frac{d[B]}{dt} = k'[\text{mRNA}] - \gamma[B]
\]

Degradation

Elementary Reaction

\[
\frac{d[\text{Pr}]}{dt} = k[\text{mRNA}][\frac{p[Aa - tRNA]}{q + p[Aa - tRNA]}]^n - r[\text{Pr}]
\]

AND Gate

Reference
J Christopher Anderson, et. al., Environmental signal integration by a modular AND gate, Molecular Systems Biology 3:133, supplementary information
Equations

\[
\begin{align*}
\frac{dc_1}{dt} &= \frac{k_1(s_1/K_1)^{n_1}}{1 + (s_1/K_1)^{n_1}} - \gamma_1 c_1 + \gamma_2 c_2 - k_2 c_1 + 2\frac{dc_4}{dt} + 2\frac{dc_{11}}{dt} \\
\frac{dc_2}{dt} &= k_2 c_1 - \gamma_2 c_2 - 2\frac{dc_4}{dt} - 2\frac{dc_{11}}{dt} \\
\frac{dc_3}{dt} &= \frac{k_3(s_3/K_3)^{n_3}}{1 + (s_3/K_3)^{n_3}} - \gamma_3 c_3 \\
\frac{dc_4}{dt} &= k_4 c_3 \left(\frac{k_S c_2}{\gamma_0 + k_S c_2}\right)^2 - \gamma_4 c_4 \\
\frac{dc_5}{dt} &= \frac{k_5(c_4/K_5)^{n_5}}{1 + (c_4/K_5)^{n_5}} - \gamma_5 c_5 \\
\frac{dc_6}{dt} &= \frac{k_6(c_6/K_6)^{n_6} + k_6'}{1 + (c_6/K_6)^{n_6} + (c_6/K_6)^{n_6'}} - \gamma_6 c_6 \\
\frac{dc_7}{dt} &= \frac{k_7(c_8/K_7)^{n_7} + k_7'}{1 + (c_8/K_7)^{n_7} + (c_8/K_7)^{n_7'}} - \gamma_7 c_7 \\
\frac{dc_8}{dt} &= k_8 c_5 + k_8' c_6 - \gamma_8 c_8 \\
\frac{dc_9}{dt} &= \frac{k_9}{1 + (c_8/K_9)^{n_9}} - \gamma_9 c_9 \\
\frac{dc_{10}}{dt} &= k_{10} c_9 - \gamma_{10} c_{10} \\
\frac{dc_{11}}{dt} &= k_{11} c_7 \left(\frac{k_S c_2}{\gamma_0 + k_S c_2}\right)^2 - \gamma_{11} c_{11} \\
\frac{dc_{12}}{dt} &= \frac{k_{12}(s_{12}/K_{12})^{n_{12}}}{1 + (s_{12}/K_{12})^{n_{12}}} + \frac{k_{12}'(c_{12}/K_{12})^{n_{12}'}}{1 + (c_{12}/K_{12})^{n_{12}'}} - \gamma_{12} c_{12} \\
\frac{dc_{13}}{dt} &= k_{13} c_{12} - \gamma_{13} c_{13}
\end{align*}
\]

53 Parameters

From literature: 26

Trial and Error: 27

> Sensitive: 4

> Insensitive: 23
Result

No Memory

<table>
<thead>
<tr>
<th>Food</th>
<th>Ring</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Production (nM) over Time (min)

- CI434
- Cl
- GFP

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## Result

### With Memory

<table>
<thead>
<tr>
<th>Food</th>
<th>Ring</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Production (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>600</td>
<td>1200</td>
</tr>
<tr>
<td>700</td>
<td>1000</td>
</tr>
<tr>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td>900</td>
<td>600</td>
</tr>
<tr>
<td>1000</td>
<td>400</td>
</tr>
<tr>
<td>1100</td>
<td>200</td>
</tr>
<tr>
<td>1200</td>
<td>0</td>
</tr>
</tbody>
</table>

- **Cl434**
- **Cl**
- **GFP**

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Stochastic Model

Food + Ring Stimuli

Training: Every 400 min
Testing: 200 min after training

Memory-Off (CI434)

Memory-On (CI)

Bell Stimulus
Wet lab results

Learning AND gate

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Learning AND gate

<table>
<thead>
<tr>
<th>4 Promoters</th>
<th>9 RBSs</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLac: IPTG</td>
<td>B0030</td>
</tr>
<tr>
<td>pSal: salicylate</td>
<td>B0032</td>
</tr>
<tr>
<td>luxP: homoserine lactone (HSL)</td>
<td>B0034</td>
</tr>
<tr>
<td>pBad: arabinose</td>
<td>J61107</td>
</tr>
</tbody>
</table>

4 × 3 = 12

4x3x9 = 108 AND gate candidates, theoretically
Test inducible promoters

- **pSal promoter**
- **pBad promoter**
- **pLac promoter**
- **luxP promoter**
Characterize pSal promoters

**pSal promoter**

- Experimental Data - 20 min
- Experimental Data - 40 min
- Experimental Data - 60 min
- Experimental Data - 80 min
- Experimental Data - 100 min
- Experimental Data - 120 min
- Fit of Exp Data - 20 min
- Fit of Exp Data - 40 min
- Fit of Exp Data - 60 min
- Fit of Exp Data - 80 min
- Fit of Exp Data - 100 min
- Fit of Exp Data - 120 min

Fluorescence/OD vs. Salicylate (M)
**AND gates constructed and tested**

63/108 AND gate candidates -> parts

<table>
<thead>
<tr>
<th>Family</th>
<th>SupD</th>
<th>T7ptag</th>
<th>Parts (9 RBSs)</th>
<th>who</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>luxP</td>
<td>pLac</td>
<td>K228119~K228127</td>
<td>Shan Shen</td>
</tr>
<tr>
<td>2</td>
<td>pLac</td>
<td>pBad</td>
<td>K228823~K228831</td>
<td>Shuke Wu</td>
</tr>
<tr>
<td>3</td>
<td>pBad</td>
<td>pSal</td>
<td>K228255~K228263</td>
<td>GuoSheng Zhang</td>
</tr>
<tr>
<td>4</td>
<td>pSal</td>
<td>pBad</td>
<td>K228870~K228878</td>
<td>Haoqian Zhang</td>
</tr>
<tr>
<td>5</td>
<td>pSal</td>
<td>pLac</td>
<td>K228852~K228860</td>
<td>Siheng He</td>
</tr>
<tr>
<td>6</td>
<td>pBad</td>
<td>luxP</td>
<td>K228038~K228046</td>
<td>Rencheng Gao</td>
</tr>
<tr>
<td>7</td>
<td>pBad</td>
<td>pLac</td>
<td>K228110~K228118</td>
<td>Shan Shen</td>
</tr>
</tbody>
</table>
An example of AND gate candidate

AND Gate family: luxP-supD, pLac-T7ptag
The best AND gate

AND Gate family: pBad-supD, pSal-T7ptag

GFP measured by microarray reader
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Bistable switch

Plasmid from Peking University iGEM 2007 team

Colonies under fluorescent stereoscope
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Recalling AND gate

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Recalling AND gate

Phi system
inducible promoter
PhiR73
AND
SupD
inducible promoter
Amber mutations
PhiR73 delta

Data after Wiki freeze

Control Arabinose Salicylate Arabinose & Salicylate

EP tubes under GFP excitation light
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**OR gate**

- **Salicylate**
- **PhiR73 delta**
- **GFP**
- **B0015**

**EP tubes under GFP excitation light**

- **control**
- **Salicylate**
- **phiR73 delta**

OR
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One step further

Assemble: learning AND gate & bistable

Learning AND gate (Best)

Bistable switch

Several strategies: on 3 plasmids, 2 plasmids
AND gate & Bistable on pSB4K5, C1 on pSB6A1

Control, no inducing

Salicylate & Arabinose on the plate

Colonies under fluorescent stereoscope
Flow cytometry: count cells on different states

Define the regions of RED cells & GREEN cells

Criterion: number of cell in the RED region & GREEN region
Assembly result (Quantified)

Quantitative data by flow cytometry

Control

Salicylate

Arabinose

Salicylate & Arabinose

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Contribution

Types of Parts | Numbers
---|---
Generator | 74
Device | 63
Transcriptional Factor | 19
Composite | 10
Reporter | 8
Coding | 2
Regulatory | 2
RNA | 1
Plasmid Backbone | 1
Total | 180
Collaboration

- SJTU-BioX-Shanghai
  - Visit in July and Discussion

- UCSF
  - Student Exchange Program

- Paris
  - Discussion

- NTU-Singapore
  - Google Group & Survey

Prof. Ariel Lindner
Tiffany Saw
Yann Le Gunff
Our Team

11 undergraduates, 2 graduate advisors, 2 faculty advisors
Members from 2 countries and 2 universities
Advisors:
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Center for Theoretical Biology
Teaching Center for Experimental Biology
Thank you very much!