Remember
Our UT-ToKyo Team
Resettable Biological Counter

- Remember
- Forget

01
The Ability to Forget

Human Being

I DON'T FORGET
The Ability to Forget

I CAN FORGET

E.coli
I CAN’T REMEMBER ANY MORE.
I CAN FORGET!
I CAN FORGET!
Remember and Shift

0 ➔ 1 ➔ 2 ➔ 3
Shift in the Various Direction

0 → Remember → 1 → Remember → 2 → Remember → 3

Forget → Forget → Forget
Sigma Re-Counter System
Counter in Our Daily Life

System
State
Memory
State
Transition
State
Reset
Modeling
Result

Switch
0
Counter in Our Daily Life
Counter in Our Daily Life

System

State Memory

State Transition

State

Reset

Modeling

Result

Switch

0

Switch

0→1
Counter in Our Daily Life
Counter in Our Daily Life

System

State

Memory

State

Transition

State

Reset

Modeling

Result

Switch

0 → 1
Counter in Our Daily Life

Switch

0 → 1

Switch

0 → 1 → 2
Three Elements of the Counter

State Memory
State Transition
State Reset
Count the number
Many positive feedback
Many positive feedback

System

State Memory

State Transition

State Reset

Modeling Result
Many positive feedback
Many positive feedback
Many positive feedback
Many positive feedback

We can express a specific output at a certain number!

Switch

0

Switch

0→1

Switch

0→1→2

Switch

Switch

Switch

PA A PB B PC C PA A PB B PC C PA A PB B PC C PA A PB B PC C
Express GFP at Count One

Count is Expressed by GFP
Count is Expressed by Death
What should be used for A?
1. Various Types

2. One to One Correspondence

What should be used for A?
One to one Correspondence

No Crosstalk
One to one Correspondence

Virgil et al. Mol Syst Biol 2013; 9:702
State Memory with sigma factors

Switch

State Transition
State Reset
Modeling Result
System
State Memory
State Transition
State Reset
Expression in Order
Q. How can we express in order?

A. Ari et al.
Genetic circuit that count (2009)
Riboregulator

P_{BAD} → taRNA → cr → RBS
Riboregulator

PBAD

tRNA

tRNA

cr

RBS
Transition without Riboregulator
Transition without Riboregulator

System
State
Memory
State
Transition
State
Reset
Modeling
Result

Switch
Sigma Factors expressed
Without control
Transition without Riboregulator

Sigma Factors expressed Without control

Switch
Transition with Riboregulator

System
State Memory
State Transition
State Reset
Modeling Result

Arabinose

$P_{BAD}$ taRNA

$cr$ RBS $\sigma_1$

$P_{Const}$ $cr$ RBS $\sigma_1$

$P_{o1}$ $cr$ RBS $\sigma_2$

$P_{o2}$ $cr$ RBS $\sigma_3$
Transition with Riboregulator

System
State Memory
State Transition
State Reset
Modeling Result

Arabinose

PBAD \rightarrow taRNA

P_{\text{const}} \rightarrow RBS_{\alpha 1}
P_{\text{o1}} \rightarrow cr \rightarrow RBS_{\alpha 2}
P_{\text{o2}} \rightarrow cr \rightarrow RBS_{\alpha 3}
Transition with Riboregulator

Arabinose

PBAD → taRNA

taRNA → cr RBS σ1

PConst → cr RBS σ1

Po1 → cr RBS σ2

Po2 → cr RBS σ3
Transition with Riboregulator
Transition with Riboregulator

System
State
Memory
State
Transition
State
Reset
Modeling
Result

Arabinose

\( P_{BAD} \) taRNA

\( P_{const} \) RBS \( \sigma_1 \)

\( P_01 \) RBS \( \sigma_2 \)

\( P_02 \) RBS \( \sigma_3 \)
Transition with Riboregulator

Arabinose

- $P_{BAD}$
- taRNA

- cr
- RBS $\sigma_1$
- cr
- RBS $\sigma_2$

- $P_{Const}$
- cr
- RBS $\sigma_1$

- $P_{o1}$
- cr
- RBS $\sigma_2$

- $P_{o2}$
- cr
- RBS $\sigma_3$
Transition with Riboregulator
Transition with Riboregulator

Transition is Controlled by Arabinose Induction
System
State Memory
State Transition
State Reset
State Memory
State Transition
State Reset

System
Reset of Counter

Reset = Back to initial State
Reset of Cellular Counter

Reset = Stop positive feedback
Anti-sigma inhibits transcription
Reset Function by anti-sigma

System
State
Memory
State
Transition
State
Reset
Modeling
Result

Arabinose

IPTG

$P_{BAD}$
$taRNA$

$P_{o1}$
$RBS$
$\sigma_1$

$P_{o2}$
$RBS$
$\sigma_2$

$P_{o3}$
$RBS$
$\sigma_3$

$Plac$
$\text{anti-}\sigma_1$
Reset Function by anti-sigma

System
State
Memory
State
Transition
State
Reset
Modeling
Result
Reset Function by anti-sigma
Reset Function by anti-\( \sigma \)-\( \rho \)
Reset Function by anti-σ1

System
State
Memory
State
Transition
State
Reset
Modeling
Result
Reset Function by anti-sigma

System
State Memory State Transition
State Reset
Modeling Result

Arabinose

IPTG

PBAD taRNA
Plac anti-σ1

Pσ1 RBS σ1
Pσ2 RBS σ2
Pσ3 RBS σ3
Modeling
Three Counter Construction

- $P_{BAD}$
- $taRNA$
- $P_{Const}$
- $RBS$
- $\sigma_1$
- $P\sigma_1$
- $RBS$
- $\sigma_1$
- $P\sigma_1$
- $cr$
- $RBS$
- $\sigma_2$
Pulse Length: 120 sec

System Modeling Result

Amount of Expression [μM]

σ1
σ2

Arabinose Induction

Time [×10³ sec]
Pulse Length: 900 sec

Amount of Expression [μM]

Time [×10^3 sec]

σ1

σ2

Arabinose Induction
Independence of Pulse Length

System Modeling Result

PBAD \rightarrow PBAD
\text{RBS} \rightarrow \text{RBS} \rightarrow \text{TetR} \rightarrow \text{RBS} \rightarrow X \rightarrow P_{X&Y} \rightarrow \text{taRNA}

PTet \rightarrow PTet
\text{RBS} \rightarrow \text{Y} \rightarrow P_{X&Y} \rightarrow \text{taRNA}

PConst \rightarrow PConst
\text{cr} \rightarrow \text{RBS} \rightarrow \text{cr} \rightarrow \text{RBS} \rightarrow \text{P}_{\text{const}} \rightarrow \text{P}_{\text{const}} \rightarrow \text{P}_{\text{const}} \rightarrow \text{P}_{\text{const}}

P_{\sigma 1} \rightarrow P_{\sigma 1}
\text{RBS} \rightarrow \text{RBS} \rightarrow \text{P}_{\sigma 1} \rightarrow \text{P}_{\sigma 1} \rightarrow \text{P}_{\sigma 1} \rightarrow \text{P}_{\sigma 1}

P_{\sigma 2} \rightarrow P_{\sigma 2}
\text{RBS} \rightarrow \text{RBS} \rightarrow \text{P}_{\sigma 2} \rightarrow \text{P}_{\sigma 2} \rightarrow \text{P}_{\sigma 2} \rightarrow \text{P}_{\sigma 2}

\text{σ1} \rightarrow \text{σ1} \rightarrow \text{σ1} \rightarrow \text{σ1} \rightarrow \text{σ1} \rightarrow \text{σ1}

\text{σ2} \rightarrow \text{σ2} \rightarrow \text{σ2} \rightarrow \text{σ2} \rightarrow \text{σ2} \rightarrow \text{σ2}
Independence of Pulse Length
Independence of Pulse Length
Independence of Pulse Length
Result of New Counter

System Modeling Result

Amount of Expression [μM]

- σ1
- σ2
- X&Y

Time [×10^4 sec]

Arabinose Induction

60

0
Result
State Memory
State Transition
State Reset
Sigma Factor for Our Device

**System**
- Modeling
- Result

**State Memory**
- State Transition
- State Reset

**ecf11**
*Pseudomonas syringae*

**ecf20**
*Pseudomonas fluorescence*
Repressive Influence on growth

Arabinose Induction

Growth Repression
Sigma’s Performance in BioBrick

System Modeling Result
State Memory
State Transition
State Reset
Sigma's Performance in BioBrick

GFP Fluorescence Emission [AU]
Sigma’s Performance in BioBrick

- **GFP Fluorescence Emission (AU)**
  - **Promoter Activated**
  - Sigma: ecf20, ecf11, ecf11, ecf20, None, None, None
  - Promoter: Pecf20, Pecf11, Pecf20, Pecf11, Pecf20, Pecf11, None
Sigma’s Performance in BioBrick

![Graph showing GFP fluorescence emission with bars for different promoters and sigmas. The graph indicates that Promoter NOT Activated for some conditions.]

- **GFP Fluorescence Emission [AU]**
  - 0
  - 30

- **Sigmas**:
  - ecf20
  - ecf11
  - ecf11
  - ecf20

- **Promoters**:
  - Pecf20
  - Pecf11
  - Pecf20
  - Pecf11
  - Pecf20
  - Pecf11
  - None

**Note**: Promoter NOT Activated for some conditions.
Sigma’s Performance in BioBrick

![Graph showing GFP Fluorescence Emission for different conditions.](image)

- **Sigma**: ecf20, ecf11, ecf20, None
- **Promoter**: Pecf20, Pecf11, Pecf20, Pecf11, Pecf20, None

No Leak
Result
State Memory
State Transition
State Reset
Check Our Riboregulator system
Riboregulator

<table>
<thead>
<tr>
<th>GFP RNA</th>
<th>+</th>
<th>cr</th>
<th>cr</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>taRNA</td>
<td>none</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Result
State Memory
State Transition
State Reset
Result
State Memory
State Transition
State Reset
Anti-sigma inhibits transcription
Check the Inhabitance by anti-sigma

System
Modeling
Result
State
Memory
State
Transition

State
Reset
Repression Takes No Effect
Repression Takes No Effect

![Graph showing GFP fluorescence emission levels for ecf20 and ecf11 with and without sigma and anti-sigma factors. The graph indicates no significant difference in fluorescence levels between the conditions.](image-url)
Result
State Memory
State Transition
State Reset
Early detection is important, especially in situ cancer!
Method Using CTC as a Biomarker

CTC

Easy
Less burden
Method Using CTC as a Biomarker

CTCs
Circulating Tumor Cells
What is CTC?
Synthetic Biological Approach
CTCD Method

Blood Sample

DNA Circuit
CTCD System
CTC

EpCAM (Promoter:pEGP-2)

Blood Cell

Specific miRNA
EpCAM and EGP-2

pEGP-2 (Promoter of EpCAM) is highly activated in CTC.
Specific miRNA

Motivation System

miRNA-142-3p miRNA-142-5p

Expressed in blood cell

Repres translation
Construction
Mechanism

CTC

Blood Cell

Activated

Motivation System
Mechanism

CTC

- GFP
- Reporter mRNA
- pEFGP-2
- EGFP
- miRNA target

Blood Cell

- pEFGP-2
- EGFP
- miRNA target

Not Activated
Mechanism

CTC

GFP

Reporter mRNA

pEGP-2

EGFP

miRNA target

Blood Cell

Reporter mRNA

pEGP-2

EGFP

miRNA target

Leak
Mechanism

CTC

- GFP
- Reporter mRNA
- pEGP-2
- EGFP
- miRNA target

Blood Cell

- miRNA
- Reporter mRNA
- pEGP-2
- EGFP
- miRNA target

Leak
Mechanism

CTC

Blood Cell

Motivation System

CTCD (Circulating Tumor Cell Detector)
Achievement
Achievement

Characterization of BBa_K747096

Helping Nagahama Team by modeling

Summarization of Complex Regulations about Biological Research for all the iGEMers