An Engineered Bacterium
with Adjustable Timer and Counter Functions

國立交通大學
National Chiao Tung University

NCTU_FORMOSA
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Motivation

Food preservation
Introduction

• *E. coli* with these functions

Self-annihilation
Several hours later.................
Several hours later....................
Several hours later..........................
When outer bacteria invasion happened
When outer bacteria invasion happened
OR Gate

Timer: [Lactose]

Counter: [AHL]

GFP‡ RFP
Time Pass by + Outer Bacteria Invade = GFP†† RFP
Constitutive Promoter

RBS → lacI → RBS → luxR → STOP

Plac

RBS → CI434 → RBS → tetR → RBS → DFP4-LVA → STOP

Pcilp22

RBS → CI434-LVA → STOP

Pci434

RBS → tetR → RBS → CIlp22 → STOP

Ptet

RBS → lux → STOP

Plux/cilp22

RBS → ccdB → RBS → mRFP1 → STOP
\begin{align*}
A\text{-line:} & \quad \frac{d}{dt}[lacI] = \alpha_A - \gamma_{lacI}[lacI] \\
B\text{-line:} & \quad \frac{d}{dt}[GFP] = \alpha_B \left( \frac{[lacI]}{1 + \frac{[lact]}{[lact]}} \right)^m - \gamma_{GFP}[GFP] \\
C\text{-line:} & \quad \frac{d}{dt}[luxI] = \frac{\alpha_C}{1 + [tetR]^m} + \alpha_{out} - \gamma_{luxI}[luxI] \\
D\text{-line:} & \quad \frac{d}{dt}[AHL] = k_2 [luxI] \\
E\text{-line:} & \quad \frac{d}{dt}[RFP] = \frac{\alpha_F [AHL][luxR]^n}{K + [AHL][luxR]^n} - \gamma_{RFP}[RFP]
\end{align*}
\[ \frac{d}{dt} [lacI] = \alpha_A - \gamma_{lacI} [lacI] \]

\( \alpha \) : production rate of the corresponding promoter

\( \gamma \) : decay rate of the corresponding protein
Repressor

\[ \frac{d}{dt} X = \frac{\alpha}{1 + X^n} - \gamma X \]

\[ \frac{d}{dt} [lacI] = \alpha_A - \gamma_{lacI} [lacI] \]
\[
\frac{d}{dt} X = \frac{\alpha X^n}{K + X^n} - \gamma X
\]

\[
\frac{d}{dt} [RFP] = \frac{\alpha_F ([AHL][\text{luxR}])^{n_3}}{K + ([AHL][\text{luxR}])^{n_3}} - \gamma_{RFP} [RFP]
\]
• Goal
  - Design a controllable bio-timer counting variable time duration.
  - Make the longer timing function up to 8-20 hours.
  - Use the lactose as timer switch to turn on and down the counting function.
Three phases

I. Standby phase:
II. Lactose-accession phase:

Three phases

A

Constitutive Promoter

RBS lacI RBS LuxR STOP

Lactose LacI LuxR

B

RBS CI434 RBS tetR RBS GFP-LVA STOP

CI434 tetR GFP
III. Lactose-consumption phase:
The spatiotemporal behavior of the bacterial referees is (A) colorless (B) green (C) yellow (D) red.
Different concentrations of lactose make timing function up to 8-20 hours!
Counter

Bacterial invasion

Complex

Constitutive Promoter

RBS IacI RBS luxR STOP A

Active

LuxR

AHL

RFP

Bacterial invasion
New Idea

• Design a simple and rapid protocol to generate a promoter library
• Built the specific parameter values that model equations indicated
• A library represent many variations in promoter strength.

The library of promoters is created where the -35 or -10 sequences
Protocol

Degenerated primers design → PCR Product

Measurement → Transformation
Generating a promoter library of GFP reporter device

Variety of promoter strength about one decade
Future Work

• Improve the experimental results.

• Finish the implementation in the host cell and develop control schemes.

• Find the application of ‘Bacterial Referee’.
Designed product

Breakable lactose vesicle

Transformed *E. coli*

Glycerol-containing medium

Semi-permeable membrane

Lactose
Application
Application 1

• Contact lenses
Several hours later.............
When outer bacteria invasion happened
Application 2

- Contact lenses
- Wound dressings
Several hours later...
When outer bacteria invasion happened
Acknowledgment
WE’RE
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