COLISWEEPER

the world’s first bacterial minesweeper game

iGEM ETH Zurich 2013
The Computer Game Minesweeper
From Minesweeper to Colisweeper
From Minesweeper to Colisweeper
From Minesweeper to Colisweeper
From Minesweeper to Colisweeper
From Minesweeper to Colisweeper
Bio-Game Rules

Pipette a solution – player move

Cursor – player move
Bio-Game Rules

Pipette Playing Solution

No mine – empty
Bio-Game Rules

One mine

Pipette Playing Solution
Bio-Game Rules

Two mines

Pipette Playing Solution
Bio-Game Rules

Pipette Playing Solution

Mine

51
Bio-Game Rules

Detonated Mine

Pipette Playing Solution
Bio-Game Rules

Pipette Playing Solution

Detonated Mine

GAME OVER

Detonated Mine

GAME OVER
Bio-Game Rules

Flagging

Pipette Flagging Solution

1

2

Flagging
Lux Signalling System

$\text{LuxR}$

$P_{\text{LuxL}}$

Inactive
Lux Signalling System

luxR

PLuxL

PLuxR

luxI

LuxR

Inactive

LuxI
Lux Signalling System

The Lux signalling system involves the interaction of LuxR and LuxI proteins. LuxR activates the expression of LuxI, which produces AHL (N-acylhomoserine lactone), a quorum-sensing molecule. LuxI then inhibits the expression of LuxR, creating a feedback loop that regulates the system's activity. The diagram illustrates this regulatory mechanism with arrows indicating the activation and inhibition steps.
Overview of Biological Circuit

Mine cell / Sender

\[ P_{\text{const}} \rightarrow \text{luxI} \]

\[ P_{\text{const}} \rightarrow \text{nagZ} \]
Overview of Biological Circuit

Mine cell / Sender

\[ P_{\text{const}} \quad \text{luxI} \quad P_{\text{const}} \quad \text{nagZ} \]

AHL
Overview of Biological Circuit

Mine cell / Sender

Non Mine cell / Receiver

AHL

\[ P_{\text{const}} \]

\[ P_{\text{const}} \]

\[ \text{nagZ} \]

\[ P_{\text{luxl}} \]

\[ P_{\text{luxR}} \]

\[ P_{\text{const}} \]

\[ \text{phoA} \]

\[ P_{\text{LuxR (high sensitivity)}} \]

\[ \text{gusA} \]

\[ P_{\text{LuxR (low sensitivity)}} \]

\[ \text{aes} \]
Biological Game – 0 Mines nearby

Non Mine

P_{\text{const}} \rightarrow \underline{\text{phoA}}

P_{\text{LuxR (high)}} \rightarrow \underline{\text{gusA}}

P_{\text{LuxR (low)}} \rightarrow \underline{\text{aes}}

P_{\text{const}} \rightarrow \underline{\text{luxR}}
Biological Game – 0 Mines nearby

- **phoA**
- **LuxR**
- **gusA**
- **aes**
- **P**

Diagram showing the relationship between the genes and their expressions.
Biological Game – 0 Mines nearby

Non Mine

\[ \text{phoA} \]

\[ \text{luxR} \]

\[ \text{gusA} \]

\[ \text{aes} \]

\[ \text{P}_{\text{const}} \]

\[ \text{P}_{\text{LuxR (high)}} \]

\[ \text{P}_{\text{LuxR (low)}} \]

p-Nitrophenoyl-Phosphate

\[ \text{PhoA} \]

\[ \text{Non Mine} \]
Biological Game – 1 Mine nearby

- \( P_{\text{const}} \) \( luxI \)
- \( P_{\text{const}} \) \( nagZ \)
- Mine
- AHL
- \( P_{\text{const}} \) \( luxR \)
- \( P_{\text{LuxR} \text{ (high)}} \) \( gusA \)
- \( P_{\text{LuxR} \text{ (low)}} \) \( aes \)
- Non Mine
- \( P_{\text{const}} \) \( phoA \)
Biological Game – 1 Mine nearby

- **P**\(_{\text{const}}\) **luxI**
- **P**\(_{\text{const}}\) **nagZ**
- **P**\(_{\text{const}}\) **luxR**
- **P**\(_{\text{LuxR (high)}}\) **phoA**
- **P**\(_{\text{LuxR (low)}}\) **gusA**
- **P**\(_{\text{const}}\) **aes**

**Mine**

**Salmon-Glucouronide**

**GusA**

**Non Mine**
Biological Game – 2 Mines nearby

- **P\text{const}**
- **luxI**
- **nagZ**

2 Mines

- **AHL**

Non Mine

- **P\text{const}**
- **phoA**
- **gusA**

- **P\text{const}**
- **LuxR (high)**
- **aes**

- **P\text{LuxR (low)}**

**ICEM**
**ETH Zürich**
Biological Game – 2 Mines nearby

- **Magenta-Butyrate**
- **Non Mine**

- **luxI**
- **nagZ**
- **2 Mines**
- **AHL**
- **P_{const}**

- **phoA**
- **gusA**
- **aes**

- **P_{const}**
- **P_{LuxR (high)}**
- **P_{LuxR (low)}**

- **Magenta-Butyrate**
- **Aes**
Biological Game – Mine

Mine

\[ P_{\text{const}} \]

\[ \text{luxI} \]

\[ P_{\text{const}} \]

\[ \text{nagZ} \]
Biological Game – Mine

Mine

P_{\text{const}} \rightarrow \text{luxI}

P_{\text{const}} \rightarrow \text{nagZ}

X-GlucNAc

\text{NagZ}
Biological Game – Flagging

Mine/Non mines

Chromosomal

\( lacZ \)
Biological Game – Flagging

Mine/Non mines

Chromosomal

Green-Galactopyranoside

LacZ

Flag
Information Processing
Information Processing

Signal Origin

Sender/Mine cell

AHL

P \text{const}

\text{luxI}
Information Processing

Signal Origin

Sender/Mine cell

AHL

P\text{const}

luxI

Signal Gradient

Non mine

0

Mine

1

2

76
Information Processing

Sender/Mine cell

Signal Origin

Signal Gradient

Signal Processing

P_{const}

phoA

P_{LuxR\ (high)}

gusA

P_{LuxR\ (low)}
aes
Results

- Gradient establishment
- \( P_{\text{LuxR}} \) sensitivities
- Reporter system: Hydrolases
Gradient Establishment

2D Spatiotemporal model

- 1 PDE + 5 ODEs
- 3 domains
- 23 parameters
- Finite element method with Neumann boundary condition
- Stationary solution

Parameters: Basu et al., A synthetic multicellular system for programmed pattern formation. Nature 2005
Gradient Establishment

Parameters:

- Basu et al.,
- *A synthetic multicellular system for programmed pattern formation.*
  - Nature 2005

2D Spatiotemporal model

- 1 PDE + 5 ODEs
- 3 domains
- 23 parameters
- Finite element method with Neumann boundary condition
- Stationary solution
Gradient Establishment

**AHL concentration [μM]**

- **EC$_{50}$**
- **Dist. from center of mine [cm]**
- **Time [h]**

- **0** to **3**
- **0** to **5**
- **0** to **20**

- **0.0036** to **56.6214**
Gradient Establishment

![AHL concentration vs. distance and time graph]

- **AHL concentration [μM]**
- **Time [h]**
- **Dist. from center of mine [cm]**

- **EC₅₀**

Values:
- 56.6214
- 14.843
- 2.6417
- 0.2319
- 0.0036
Gradient Establishment

AHL concentration [\mu M]

Dist. from center of mine [cm]

0 0.5 1 1.5 2 2.5 3

Time [h]

0 5 10 15 20

EC_{50}

AHL Concentration at Receiver Cells at 1.5 cm

AHL concentration [\mu M]

0 0.5 1 1.5

Time [h]

0 5 10 15 20

ETH Zürich
Gradient Establishment

AHL concentration $[\mu M]$

Dist. from center of mine [cm]

Time [h]

$EC_{50}$

AHL Concentration at Receiver Cells at 1.5 cm

AHL concentration $[\mu M]$

Time [h]

0 Mines
Gradient Establishment

![AHL concentration graph](image1)

![AHL Concentration at Receiver Cells at 1.5 cm](image2)
Gradient Establishment

AHL concentration [µM]

Dist. from center of mine [cm]

Time [h]

AHL Concentration at Receiver Cells at 1.5 cm

AHL concentration [µM]

Time [h]

EC_{50}
Gradient Establishment

AHL concentration [μM]

Dist. from center of mine [cm]

Time [h]

0 5 10 15 20

EC\textsubscript{50}

56.6214
14.843
2.6417
0.2319
0.0036

AHL Concentration at Receiver Cells at 1.5 cm

Time [h]

0 5 10 15 20

3 Mines
2 Mines
1 Mine
0 Mines
Gradient Establishment

AHL concentration [µM]

Dist. from center of mine [cm]

Time [h]

AHL Concentration at Receiver Cells at 1.5 cm

Time [h]

EC$_{50}$
Gradient Establishment

**AHL concentration [μM]**

- **EC$_{50}$**

**AHL Concentration at Receiver Cells at 1.5 cm**

- 3 Mines
- 2 Mines
- 1 Mine
- 0 Mines

**Dist. from center of mine [cm]**

**Time [h]**

**AHL concentration [μM]**

0 0.5 1 1.5 2 2.5 3

0 5 10 15 20

0 0.5 1 1.5

56.6214 14.843 2.6417 0.2319 0.0036
Gradient Establishment

**AHL Concentration at Receiver Cells at 1.5 cm**

**Dist. from center of mine [cm]**

**Time [h]**

- **EC$_{50}$**

**AHL concentration [μM]**

- 56.6214
- 14.843
- 2.6417
- 0.2319
- 0.0036

**Time [h]**

- 0
- 5
- 10
- 15
- 20

**0 Mines**

**1 Mine**

**2 Mines**

**3 Mines**
Signalling with $P_{\text{LuxR}}$

MODEL

EXPERIMENT

Time: 5.5 h

$P_{\text{Lac}}$ $P_{\text{LuxR}}$

luxR $\rightarrow$ gfp

AHL

$P_{\text{const}}$

luxI

GFP Concentration (mM)

0.3
0.25
0.2
0.15
0.1
0.05
0

1.5 cm
Signalling with $P_{LuxR}$

MODEL

EXPERIMENT

Time: 6.5 h

$P_{Lac}$  $P_{LuxR}$

luxR  gfp

AHL

$P_{const}$

luxl

GFP Concentration (mM)

0 0.05 0.1 0.15 0.2 0.25 0.3

1.5 cm
Signalling with $P_{LuxR}$

**MODEL**

- $P_{Lac} \rightarrow luxR$
- $P_{LuxR} \rightarrow gfp$
- $P_{const} \rightarrow luxI$
- AHL

**EXPERIMENT**

- GFP Concentration (mM)
  - 0.3
  - 0.25
  - 0.2
  - 0.15
  - 0.1
  - 0.05
  - 0

- 1.5 cm

Time: 11 h
Signalling with $P_{\text{LuxR}}$
Signal Processing: $P_{\text{LuxR}}$ Promoter

$P_{\text{LuxR}}$ (high sensitivity) → reporter

[Diagram showing a curve relating Reporter concentration to [AHL] concentration]
Signal Processing: $P_{\text{LuxR}}$ Promoter

$P_{\text{LuxR}}$ (high sensitivity)

$P_{\text{LuxR}}$ (low sensitivity)
$P_{LuxR}$ Promoter Mutagenesis

Site-saturation mutagenesis

BBa_J09855 and BBa_E0840

$L.Caetano et al.$,
*A Mutational Analysis Defines Vibrio fischeri LuxR Binding Sites*,
Journal of Bacteriology 2008
**P_{LuxR} Promoter Mutagenesis**

**Site-saturation mutagenesis**

BBA_J09855 and BBA_E0840

**Positive selection:**
high [AHL]

**Negative selection:**
no AHL

**R0062 P_{LuxR}

ACCTGTAGGATCGTACAGGTWT
ACNNNTAGGATCGTANNNGT Library

**P_{LuxR} Promoter Mutagenesis**

**Site-saturation mutagenesis**

BBA_J09855 and BBA_E0840

- **Positive selection:** high [AHL]
- **Negative selection:** no AHL

**Characterization by flow cytometry**

<table>
<thead>
<tr>
<th>WT Library</th>
<th>G1 Library</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCTGTAGGATCGTACAGGT</td>
<td>G1</td>
</tr>
<tr>
<td>ACNNNTAGGATCGTANNNGT</td>
<td>G1</td>
</tr>
<tr>
<td>ACCAGTAGGATCGTAGGGT</td>
<td>G1</td>
</tr>
</tbody>
</table>

Analysis: $P_{\text{LuxR}}$ Promoter G1

$P_{\text{LuxR}}$ G1

BBa_K1216007

$P_{\text{Lac}}$ $P_{\text{LuxR}}$

luxR gfp

AHL

$P_{\text{const}}$

luxl
Analysis: $P_{LuxR}$ Promoter G1

**Model**

- $P_{LuxR}$ G1
- $P_{Lac}$
- $luxR$ → $gfp$
- $AHL$
- $P_{const}$
- $luxI$

BBa_K1216007

GFP expression (mM)

$0.06$

$0.05$

$0.04$

$0.03$

$0.02$

$0.01$

$2.0413 \times 10^{-3}$
Analysis: $P_{\text{LuxR}}$ Promoter G1

Experiment

Model

$P_{\text{Lac}}$ $P_{\text{LuxR}}$ G1

BBa_K1216007

luxR gfp

AHL

$P_{\text{const}}$

luxI

GFP expression (mM)
Enzyme-Substrate Reactions

BBa_K1216001
PhoA NagZ LacZ

BBa_K1216000
LacZ GusA Aes

BBa_K1216002

BBa_K1216003
Enzyme-Substrate Reactions

BBa_K1216000
BBa_K1216001
BBa_K1216002
BBa_K1216003

PhoA NagZ LacZ LacZ GusA Aes

Magenta-butyrate
Salmon-Gluc
X-GluNAc
pNPP

Aes GusA NagZ PhoA neg.
Enzyme-Substrate Reactions

Mixed cultures with mixed substrates:
1: PhoA
2: PhoA + GusA
3: PhoA + GusA + Aes

Magenta-butyrate
Salmon-Gluc
X-GluNAc
pNPP

BBa_K1216001
BBa_K1216000
BBa_K1216002
BBa_K1216003

PhoA NagZ LacZ LacZ GusA Aes

Aes GusA NagZ PhoA neg.

The images show various tubes containing different substrates and enzymes, illustrating the reactions and color changes resulting from the enzymatic activities.
Human Practice
Human Practice
Human Practice

Access

Remote Control

Global players

Gamification

Synthetic Biology I
Lab Course

ETH Zürich
Achievements

Proof of Principle with GFP wild type $P_{LuxR}$ promoter
Achievements

Proof of Principle with GFP wild type $P_{\text{LuxR}}$ promoter

Predictive spatio-temporal model of the system
Achievements

Proof of Principle with GFP wild type $P_{\text{LuxR}}$ promoter

Predictive spatio-temporal model of the system

$P_{\text{LuxR}}$ promoter mutagenesis
New biobrick: $P_{\text{LuxR}}$ promoter (low sensitivity) BBa_K1216007
Achievements

Proof of Principle with GFP wild type $P_{\text{LuxR}}$ promoter

Predictive spatio-temporal model of the system

$P_{\text{LuxR}}$ promoter mutagenesis
New biobrick: $P_{\text{LuxR}}$ promoter (low sensitivity) BBa_K1216007

Characterization of a reporter system with five different hydrolases
New biobricks: Aes BBa_K1216002, Aes-His BBa_K1216006, NagZ BBa_K1216003
Improved biobricks: PhoA-His BBa_K1216005, GusA-His BBa_K1216004
Achievements

**Proof of Principle with GFP wild type $P_{\text{LuxR}}$ promoter**

**Predictive spatio-temporal model of the system**

**$P_{\text{LuxR}}$ promoter mutagenesis**
New biobrick: $P_{\text{LuxR}}$ promoter (low sensitivity) BBa_K1216007

**Characterization of a reporter system with five different hydrolases**
New biobricks: Aes BBa_K1216002, Aes-His BBa_K1216006, NagZ BBa_K1216003
Improved biobricks: PhoA-His BBa_K1216005, GusA-His BBa_K1216004

**Human Practice**
Colisweeper as novel, fun and educational tool
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Thank you for your attention!