IGEM 2008

Genetic Network Generating Spatial Patterns Through Cell-Cell Communication
Who are we?
Cells can give rise to complex systems by forming patterns of gene expression and undergoing cellular differentiation.

Cell-cell signaling mechanisms play a key role in pattern generation.

- Early stage of development (gastrulation)
- Pattern formation (drosophila segmentation)
- Advanced skin patterns (clownfish)
- Advanced skin patterns (zebra)
What are the questions we want to address?

- How do cells self-organize to build complexity?
- Can we generate spatial cellular patterns from a genotypically homogenous population using a de novo engineered genetic network?

→ Towards a self-patterning *E.coli* population:
Quorum sensing molecules: AHL molecules from LuxI and RhlI signal the state of the cell.
Cell-Cell communication is complex and can’t be easily studied.

Chamber-chamber communication allows greater control over the system:
• Confinement of a population of cells
• Signals can be spatially and temporally controlled.

This can be achieved using microfluidics.
General mechanism

Initiation

Signal Initiation by quorum sensing molecules

Establishment of pattern

Signal Propagation
Decide on a connectivity schematic

Establish possible rules

Simulate system in Matlab

<table>
<thead>
<tr>
<th>Input</th>
<th>Output</th>
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<tbody>
<tr>
<td>GG</td>
<td>G</td>
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<tr>
<td>RG</td>
<td>R</td>
</tr>
<tr>
<td>RR</td>
<td>R</td>
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</table>
Examples of possible rule sets

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<tbody>
<tr>
<td>GGG</td>
<td>R</td>
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<tr>
<td>RGG</td>
<td>G</td>
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<tr>
<td>RRG</td>
<td>R</td>
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<tr>
<td>RRR</td>
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Ron WEISS’s band detect network.
Our Network

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How it works

Input

Output

\[ = \text{AHL}_{\text{Rhl}} \]
\[ = \text{AHL}_{\text{Lux}} \]

Input | Output
--- | ---
GGG | R
RGG | R
RRG | G
RRR | R

Diagram:

- **R**
- **G**
- **G**

- **luxR**
  - \( P_{\text{LuxR}} \)
  - **lacIm**
  - \( P_{\text{LuxR}} \)
  - **rhIR**
  - \( P_{\text{RhlR}} \)

- **lacI**
  - \( P_{\text{Lac}} \)

- **GFP**
  - **rhl I**
  - \( P_{\text{TetR}} \)

- **RFP**
  - **luxI**
  - \( P_{\text{TetR}} \)
How it works

Input:
- RRG
- RRG

Output:
- G
- G

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Equations:
- \( \text{AHL}_{\text{Rhl}} \)
- \( \text{AHL}_{\text{Lux}} \)
Simulations:
Simulations with Feed-Back:
Building the Synthetic network

Cloning & Parts
The plasmids
Cloning Strategy
2-step PCR: how it works

Primer 1A
Barcode 1 + RBS

Primer 3A
Prefix

Primer 2A
Prefix + Extension + Barcode 1

Primer 1A

Primer 2B
Barcode 2 + Extension + Suffix

Primer 1B
Barcode 2

Primer 3B
Suffix

Prefix | Promoter | Barcode1 | RBS | ORF | Barcode2 | Terminator | Suffix
---|---|---|---|---|---|---|---
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Full BioBrick operon
Example – 2 ORFs operon (8 parts)
- Standard Biobricks: Time ~ 3 * 56 = 168 hours (7 days)
- 2 step PCR: Time ~ 12 + 48 = 60 hours (2.5 days)
Submitted Parts

BBa K092000

BBa K092300

BBa K092800

BBa K092200

BBa K092400

BBa K092100

BBa K092600

BBa K092700

BBa K092900

BBa K092000
Characterization of part BBa_K092600 by varying concentrations of tetracycline.

What we expect is a constitutive expression of RFP with slight leakage of TetR due to the absence of P(Lac) Promoter.

Increase in RFP with tetracycline induction

Shows that construct works, and will work better with Plac promoter attached
Characterization of parts: Response Curve

Induction of part BBa_K092600

Flow cytometry analysis
Producing the physical support of the experiment

Microfluidic Device
Fabrication of microfluidic chips

1. Design of the chip on a computer
2. Soft lithography
3. Device
Characterization of TetR Inducible RhIR promoter

Tetracycline gradient

Response zone?
Results: RFP expression
Conclusion

- We designed a genetic circuit for detecting and reacting to various levels of quorum sensing molecules in a band-pass manner.
- We were able to simulate the results in a semi-quantitative model to prove the concept is feasible.
- We submitted 9 parts to the registry.
- We characterised the transfer function for one part.
- We were able to complete our cloning scheme although after the deadline for whole part submission.
- We successfully implemented a novel PCR-based strategy for Biobrick construction.
- We successfully designed and constructed microfluidic chips for cell culture and tested the growth and RFP expression of cells growing in them.
The Team

igem@epfl.ch
Chip design and functionality

- Matrix that allows cell sequestration in isolated chambers but does not prevent cell growth and proliferation
- Specific spatial connectivity between cells
- Control of the time when two columns are allowed to connect
- Possibility of initiating the signal in multiple ways

All these features needed to be integrated on a relatively small scale (~300μm)