The TUDelft iGEM team presents:

Bacterial Relay Race ‘09

An illustration of a cell-to-cell communication device

Calin, Daniel, Saeed, Sriram, Tim and Tim
The TUDelft iGEM team
Cell-to-cell communication

Overview
- Principles of cellular communication
- Propagation of a set of instructions
- Bacterial relay race
- Conjugation system
- Self-destructive plasmid
- Time-delay device
- Quorum sensing is limited to binary signals

Conjugation
Self-destruction
Time-delay
Ethics
Achievements
System Overview

Overview

Conjugation
Self-destruction
Time-delay
Ethics
Achievements

Cell Culture

Initiator Cell Added

LEGEND

entry exclusion protein
endonuclease
R751* plasmid
signal plasmid

Bacterial Relay Race ‘09
Cell-to-cell communication
Conjugation

- Stop entry exclusion
  - Knockout trbK
- Stop conjugative transfer of helper plasmid
  - Knockout oriTR
- Address lethal zygosis
  - Knockout trbC
- $\lambda$-red knockouts
Conjugation

Conjugation testing plasmids

Overview

Conjugation
Self-destruction
Time-delay
Ethics
Achievements
Conjugation

Entry exclusion expression blocks incoming transfers

Overview

Conjugation

Self-destruction

Time-delay

Ethics

Achievements
Modeling

Overview

Conjugation

Self-destruction

Time-delay

Ethics

Achievements

Time = 00min
DONORS

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Cell-to-cell communication
Modeling

Overview

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Time = 30min

Two modes of propagation

1. Conjugation
   - 8 to 20 μm / hr
2. Cell division
   - > 20 μm / hr
Self-destructive plasmid

- IPTG inducible destruction
- I-SceI homing endonuclease
- 30 bp cut site
- Induction stops GFP expression
Self-destructive plasmid

• Cut site biobricked
• Successful assembly
• *In vivo* testing unsuccessful
  • Undocumented degradation tag

Overview

Conjugation

Self-destruction

Time-delay

Ethics

Achievements
Time-delay device

- Two approaches:
  - synthetic transcriptional cascade
  - biosynthetic AND gate

```
IPTG  Time Delay Device  GFP
|
```

```
Reception (conjugation)  Delivery and self destruction
```

"Message"
Time-delay device

Two level negative cascade

Overview
Conjugation
Self-destruction
Time-delay
Ethics
Achievements

Bacterial Relay Race ‘09
Cell-to-cell communication
Modeling the Time-delay

- Stability
- Sensitivity
- Design

Recommendations

Overview
Conjugation
Self-destruction
Time-delay
Ethics
Achievements
Time-delay device

- Plasmids assembled successfully
- Tunable delay for future teams
- Delay time agrees with predicted value
Time-delay device

Overview
Conjugation
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Bacterial Relay Race ‘09
Cell-to-cell communication
Issues in Synthetic Biology

Overview
Conjugation
Self-destruction
Time-delay
Ethics
Achievements

Bacterial Relay Race ‘09
Cell-to-cell communication
Concepts of life

Overview
Conjugation
Self-destruction
Time-delay
Ethics
Achievements

Overview

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Ethics
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Bacterial Relay Race ‘09
Cell-to-cell communication
Methods

- Literature review
- Survey
  - 242 participants from iGEM community
- Analysis
  - Quantitative
  - Qualitative
- Raise awareness on ethical issues
- Gain insight in opinions and perceptions
Survey Results

Will the reductionist approach lead to an understanding of life?

- **No, living systems are too complex.** (30%)
- **No, biological phenomena are fundamentally special.** (9%)
- **Yes, we will eventually fully understand life.** (47%)
- Other (13%)
- D (1%)

**Overview**
- Conjugation
- Self-destruction
- Time-delay

**Ethics**

**Achievements**
Survey results

A complex living system is more than the sum of its biological components

<table>
<thead>
<tr>
<th>Not Agree</th>
<th>Agree</th>
</tr>
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<tbody>
<tr>
<td>13%</td>
<td>65%</td>
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</table>

Life is physical and can be explained materialistically

<table>
<thead>
<tr>
<th>Not Agree</th>
<th>Agree</th>
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</thead>
<tbody>
<tr>
<td>19%</td>
<td>40%</td>
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</table>

For all results see: http://2009.igem.org/Team:TUDelft/Ethics
Major Achievements

- Engineered a tunable delay device
- Characterized and submitted 21 new BioBricks
- Characterized several existing BioBricks
- New toolbox for modeling conjugation systems
- Lock and Key generation algorithm
- An extensive study on Human Practice
Acknowledgements

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Department of Biotechnology

Department of Bionanoscience

BASECLEAR
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For more information:
http://2009.igem.org/Team:TUDelft