BRAINSTORMING
BRAINSTORMING
BRAINSTORMING
CONCERNS

Uncontrolled spread of GE bacteria

Horizontal Gene Transfer

- Conjugation
- Transformation
- Transfection
HUMAN PRACTICE
RELATIVE OCCURRENCE OF BIOSAFETY TERMS

Biosafety page becomes mandatory!
How safe is safe enough?

Aim: Better safety practice in synthetic biology and communication with the larger society
APPROACH - WE LISTEN FIRST

Our inputs:
APPROACH - WE LISTEN FIRST

Our inputs:

• Interviews with sociologists, ethicists, philosophers, geneticists and synthetic biologists
• Public debate
• Workshop with high school students
• Literature research on the state-of-the-art
• Screening through iGEM wikis for safety modules
Experts from different fields

Workshop!
60 highschool students

Debate!
2 teams of 5 students
**Name:** Kill Switch Engage!

**Summary:** Kill Switch

**Idea:** "Our kill switch is designed by inserting an antimicrobial peptide (AMP) gene into E.Coli"

**Efficiency:** No quantitative results in terms of number or proportion of cell death.
Name: Auxin

Summary: Engineer bacteria to accelerate plant root development


Efficiency: Anti-holin was expressed in cells, but no experiments for this system have been made.

WIKI SCREEN : PREVIOUS iGEM BIOSAFETY MODULES

- 32 Teams addressed the question of biosafety up to 2011
- Very limited number of quantitative approaches and results
How safe is safe enough: towards best practices of synthetic biology
How safe is safe enough: towards best practices of synthetic biology

2. Synthetic Biology: Awareness, perceptions, concerns and regulations

We are going to look at the population’s awareness, perceptions and concerns about synthetic biology, as well as how these vary by background, and how they are related to the government’s regulation of the field.

1. Awareness of synthetic biology

In Europe

According to the 2013 Eurobarometer on Biotechnology [2], most Europeans have never heard of synthetic biology (only 1% of respondents at the EU27 level had heard anything about synthetic biology prior to the survey). In France, this number rises to 13%, as shown in the chart below.

- Awareness of synthetic biology

Comparison between Europe and the US

Europeans were more aware of synthetic biology than Americans. In 2013, only 4% had never heard of synthetic biology, whereas the number rose to 8% in Europe.

5. Perception of Synthetic Biology

In Europe

The Eurobarometer International Center for Schmell studied European media coverage on Synthetic Biology [6].

- “Public interest in science should not exist in a public sphere: a broad debate that would involve a broad range of actors, including all stakeholders, could help to shape the public debate on synthetic biology, and thus inform the public of the potential benefits and risks of the technology.”

In the USA

For a majority of Europeans, scientific evidence should weigh more than ethical considerations in the process of decision making.

- 5% of Europeans think the opposite.

Therefore, we can say that there is no consensus on the subject.

40% of Europeans think that the advice of experts is more important than the advice of the general public in the decision-making process. In the USA, the government thinks in the process of decision making.

This shows that the United States is more democratic in its advice processes, while the European Union is more focused on experts’ opinions.

The experts responsible for bioethics may have to take into account the public opinion during the decision-making process. However, we can see that the majority of American experts are right-wing and in favor of stem cell research. However, we can see that the majority of European experts are left-wing and are against stem cell research. This shows that experts tend to share the same political views as the people they are adressing.
CONCLUSIONS

• Horizontal Gene Transfer (HGT) is one of the main safety concerns.

• On an application-by-application basis, discussion with the public should guide the balance between risks and benefits.

• A major efforts is needed in the synthetic biology community in characterization and quantification of safety modules.

• Environmental & health iGEM teams should embed safety devices.
Semantic Containment

Here are parts that would add a semantic containment to genes, or parts that would allow the cell to read semantic containments gene.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BBa_K914000</td>
<td>RNA</td>
<td>pLac-supD-T</td>
<td>241</td>
</tr>
<tr>
<td>W BBa_K914009</td>
<td>Translational_Unit</td>
<td>P1003* Ser133-&gt;Amber Codon</td>
<td>997</td>
</tr>
<tr>
<td></td>
<td>Intermediate</td>
<td>supD-T - intermediate</td>
<td>178</td>
</tr>
<tr>
<td>BBa_K914011</td>
<td>Plasmid_Backbone</td>
<td>pSB1A2 with 1 amber mutation</td>
<td>2079</td>
</tr>
<tr>
<td>W BBa_K914016</td>
<td>Translational_Unit</td>
<td>P1003** Kan resistant gene with 2 Amber Codon</td>
<td>997</td>
</tr>
</tbody>
</table>

Kill switch

Here are parts that could trigger cell death through disruption at the level of the protein and the cell.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BBa_K117000</td>
<td>Coding</td>
<td>Lysis gene (promotes lysis in colicin-producing bacteria strain)</td>
<td>144</td>
</tr>
<tr>
<td>W BBa_K515104</td>
<td>Composite</td>
<td>J23100 promoter - Antiolin</td>
<td>488</td>
</tr>
<tr>
<td>BBa_K628006</td>
<td>Composite</td>
<td>Protegrin-1 Kill Switch</td>
<td>353</td>
</tr>
<tr>
<td>W BBa_K879917</td>
<td>Plasmid_Backbone</td>
<td>LacO ori</td>
<td>2072</td>
</tr>
<tr>
<td>W BBa_K879918</td>
<td>Plasmid_Backbone</td>
<td>TetO ori</td>
<td>2074</td>
</tr>
</tbody>
</table>

XNase

Here are parts that could trigger cell death through disruption at the level of the DNA or RNA.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>W BBa_K131000</td>
<td>Generator</td>
<td>ColE2 Operon</td>
<td>2640</td>
</tr>
<tr>
<td>W BBa_K150009</td>
<td>Generator</td>
<td>ColicinE1 Producer Controlled by 3OC6HSL Receiver Device</td>
<td>3180</td>
</tr>
<tr>
<td>? BBa_K526001</td>
<td>Coding</td>
<td>ptrC*-D spills</td>
<td>1552</td>
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<tr>
<td>W BBa_K914001</td>
<td>Composite</td>
<td>pLac Colicin E2 Immunity protein</td>
<td>345</td>
</tr>
<tr>
<td>W BBa_K914002</td>
<td>Intermediate</td>
<td>repressilator RBS Colicin E2 Immunity protein</td>
<td>282</td>
</tr>
</tbody>
</table>

All biosafety parts
WET LAB!
CONTAINMENT SYSTEMS
REQUIREMENTS

Robust
CONTAINMENT SYSTEMS
REQUIREMENTS

- Robust
- Redundant
- Modular
- Quantified
- Efficient
- Harmless
LIBRARY OF bWARE DEVICES
LIBRARY OF bWARE DEVICES

• Physical containment
LIBRARY OF bWARE DEVICES

- Delay system
LIBRARY OF bWARE DEVICES

• DNA degradation and population-level suicide
LIBRARY OF bWARE DEVICES

• Semantic containment
PHYSICAL CONTAINMENT
SIMPLE AND COVALENTLY STABILIZED BEADS
BACTERIA CAN SURVIVE AND EXPRESS PROTEINS INSIDE THE CAPSULES
BEADS PHYSICALLY PREVENT BACTERIA FROM ESCAPING

- Untreated beads: 70% containment
- Covalently stabilized beads: 99.96% containment

(cells released after 24 hours incubation)
KILL-SWITCH
COLICINS

• DNA degradation and population-level suicide
DELAY SYSTEM

Arabinose

Lac I

RE

DNAse

activity

time

dimanche 4 novembre 12
DELAY SYSTEM

Arabinose

DNAse

activity

time

dimanche 4 novembre 12
DELAY SYSTEM

Arabinose

DNAse

RE

Lac I
DELAY SYSTEM

activity vs. time

Arabinose

Lac I

RE

DNAse

dimanche 4 novembre 12
DELAY SYSTEM

Arabinose

DNAse

activity
time
RESTRICTION ENZYME

activity vs. time

Lac I

DNAse
DNA DEGRADATION

activity
time

DNAse
DNA DEGRADATION

DNAse

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DNA DEGRADATION

DNAse
COLLECTIVE SUICIDE!
COLLECTIVE SUICIDE!

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COLLECTIVE SUICIDE!

No DNA!
CHARACTERIZATION OF THE DELAY SYSTEM

~4 generation genetic delay by LacI dilution

Normalized Fluorescence [A.U.]

-20,0

107,5

150,0

22,5

65,0

107,5

150,0

0 125 250 375 500

time [minutes]

Uninduced
Arabinose 1%
IPTG

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RHAMNOSE PROMOTER

Rhamnose

- +

Rhamnose

Biobricked

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CHARACTERIZATION OF THE I-SCEI MEGANUCLEASE

IPTG

I-SceI

CmR

I-SceI site

BIOBRICKED

AmpR

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CHARACTERIZATION OF THE I-SCEI MEGANUCLEASE

Plating on Kan+Amp

<table>
<thead>
<tr>
<th>CFU Ratio</th>
<th>IPTG</th>
<th>w/o IPTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.09</td>
<td></td>
<td>0.17</td>
</tr>
</tbody>
</table>

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TOXIN KILLS SENSITIVE CELLS, ANTI-TOXIN PROTECTS THEM

Colicin producing cell

Sensitive cell

Immune cell

zone of clearance

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TOXIN KILLS SENSITIVE CELLS, ANTI-TOXIN PROTECTS THEM

**Quantitative characterization of immune cells**

- Colicin+ Immunity-
- Colicin+ Immunity+
- Colicin- Immunity-
- Colicin- Immunity+

**OD600 vs. Time (h)**

*dimanche 4 novembre 12*
SYNTHETIC IMPORT DOMAIN
CAN WE HIJACK COLICIN IMPORT PROPERTIES?
CAN WE HIJACK COLICIN IMPORT PROPERTIES?

**IPTG**

Producer cells:
Synthetic Import Domain + lacZ-α

Receptor cells: lacZ-Ω

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CAN WE HIJACK COLICIN IMPORT PROPERTIES?

producer cells: Synthetic Import Domain + lacZ-α

Receptor cells: lacZ-Ω
CAN WE HIJACK COLICIN IMPORT PROPERTIES?

producer cells:
Synthetic Import Domain+\textit{lacZ-\alpha}

Receptor cells: \textit{lacZ-\Omega}
CAN WE HIJACK COLICIN IMPORT PROPERTIES?
SEMANTIC CONTAINMENT
SEMANTIC CONTAINMENT Encrypts Genetic Information

Serine ➔ Stop codon (TAG)

HGT

WT cells

No Kan resistance

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SEMANTIC CONTAINMENT ENCRYPTS GENETIC INFORMATION

Concentration of [Kan] (µg/mL) at t = 8.37h

OD600 normalized

Resistant strain
Sensitive strain
SEMANTIC CONTAINMENT ENCRYPTS GENETIC INFORMATION

Concentration of [Kan] (µg/mL) at t = 8.37h

- Resistant strain
- 1 mutation, non suppressor strain

Sensitive strain

OD600 normalized
SEMANTIC CONTAINMENT ENCRYPTS GENETIC INFORMATION

Concentration of [Kan] (µg/mL) at t = 8.37h

- Sensitive strain
- 2 mutations, non suppressor strain
- 1 mutation, non suppressor strain
- Resistant strain

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SEMANTIC CONTAINMENT ENCRYPTS GENETIC INFORMATION

Concentration of [Kan] (µg/mL) at t = 8.37h

- Sensitive strain
- 2 mutations, non suppressor strain
- 1 mutation, non suppressor strain
- 1 or 2 mutation(s), suppressor strain
- Resistant strain

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SAFETY ASSESSMENT
"This machine is perfectly safe, as long as you don’t press this button"
SAFETY ASSESSMENT

Hazards Identification

- GE bacteria outcompete natural strains
- Horizontal gene transfer

Control design

Check control
FAULT TREE ANALYSIS

1. Safety system fails
   OR
   GEO outcompete natural strains
   AND
   High fitness mutant
   Cells are not killed
   Physical containment fails
   OR
   No antitoxin degradation
   Toxin production fails
   P1

2. Engineered DNA spreads
   AND
   DNA is uptaken by other strains
   P2

3. Semantic containment fails
   AND
   Horizontal gene transfer
   P3

4. Physical containment fails
   AND
   DNAse fails
   P1

5. Population suicide fails
   Toxin production fails
   P4

6. Delay fails to trigger RE
   RE system fails
   P7

7. RS fails
   RE fails
   P8

8. RE system fails
   P9
FAULT TREE ANALYSIS
Estimation of total failure rate: $10^{-11}$ accidents/generation
ACHIEVEMENTS
OUR ACHIEVEMENTS AND CONTRIBUTIONS

WE GOT

- iGEM distribution plates
- Previously developed containment devices
- Expert interviews
- Public engagement
- iGEM Team collaboration

Paris Bettencourt

WE ARE GIVING

- 17 working biobricks
- 6 containment modules
- GMO debate
- SynBio workshop
- Biosafety report
- Working containment modules
- Biosafety registry page
- Human Practice proposals
- Opportunities for public collaboration
THANKS TO OUR SPONSORS
OUR TEAM

Members
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Dylan Iverson
Zoran Marinkovic
Claire Mayer
Ernest Mordret
Aishah Prastowo
Julianne Rieders
Denis Samuylov
Guillaume Villain

Advisers
Antoine Decrulle
Ariel Lindner
Babak Nichabouri
Aleksandra Nivina
Edwin ‘Jake’ Wintermute
Yifan Yang

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