Paris Bettencourt 2012

bWARE
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:

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

Workshop!

Debate!
<table>
<thead>
<tr>
<th>Team</th>
<th>Year</th>
<th>Project Name</th>
<th>Project Summary</th>
<th>Biosafety Idea</th>
<th>Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Andrews</td>
<td>2011</td>
<td>Kill switch engaged</td>
<td>Kill switch</td>
<td><strong>Kill switch.</strong> &quot;Our kill switch is designed by inserting an antimicrobial peptide (AMP) gene into E. Coli&quot;</td>
<td>Used the LIVE/DEAD BacLight Bacterial Viability kit, but don’t have any quantitative results in terms of number or proportion of cell death. &quot;The relationship between the concentration of arabinose and the amount and rate of cell death seems linear in nature.&quot;</td>
</tr>
<tr>
<td>Imperial</td>
<td>2011</td>
<td>Auxin</td>
<td>Engineer bacteria to accelerate plant root development</td>
<td><strong>Toxin/antitoxin.</strong> Consists of the insertion of the Holin + Endolysine and Anti Holin genes.</td>
<td></td>
</tr>
<tr>
<td>Bristol</td>
<td>2010</td>
<td>AgrEcoi</td>
<td>Bacteria that detects and signals the presence of nitrates</td>
<td><strong>Toxin/antitoxin.</strong> Anti-holin was expressed in cells, but no experiments for this system have been made. &quot;It seems very likely that our GM E. coli have been able to survive in soil and retain their plasmid for six weeks despite competition and selective pressure against the plasmid.&quot;</td>
<td></td>
</tr>
<tr>
<td>Colombia</td>
<td>2011</td>
<td>Defense aid for coffee plantations against fungi (“Detect and alert system”)</td>
<td>Detect chitin and alert the plant by stimulating an early hypersensitive response against infection.</td>
<td><strong>Encapsulation in a gel.</strong> Due to the beads, &quot;bacteria are kept separate from the environment, reducing public safety fears.&quot; No quantification provided.</td>
<td></td>
</tr>
</tbody>
</table>

- 32 iGEM safety modules
How safe is safe enough: towards best practices of synthetic biology

Chapter 1
Synthetic Biology: Assumptions, perceptions, concerns and regulation

1. Assumptions of Synthetic Biology

Introduction
According to the 2011 Eurobarometer on biotechnology, most Europeans have little knowledge about synthetic biology. Only 1% of respondents were aware of synthetic biology, and only 5% were able to explain what synthetic biology is. A graph shows the number of people who opined on the subject.

1.1. Awareness of Synthetic Biology

In Europe
In Europe, awareness of the potential risks of synthetic biology is relatively low. In a survey conducted in 2011, 46% of respondents agreed that synthetic biology could have negative impacts on public health and safety. A pie chart illustrates the distribution of these views.

1.2. Concerns about Synthetic Biology

In the United States of America
In the United States, concerns about synthetic biology are even greater. A survey conducted in 2012 found that 54% of respondents believed that synthetic biology could have negative impacts on public health and safety. A bar chart illustrates the distribution of these views.

In Europe
In Europe, the concern is not limited to synthetic biology. A recent survey found that 56% of respondents believed that synthetic biology could have negative impacts on public health and safety. A pie chart illustrates the distribution of these views.

In the United States of America
In the United States, concern about synthetic biology is even more pronounced. A survey conducted in 2013 found that 62% of respondents believed that synthetic biology could have negative impacts on public health and safety. A bar chart illustrates the distribution of these views.

1.3. Regulation of Synthetic Biology

In the United States of America
In the United States, the regulation of synthetic biology is more stringent. A survey conducted in 2014 found that 58% of respondents believed that synthetic biology should be more regulated. A bar chart illustrates the distribution of these views.

1.4. Conclusion

In conclusion, the concerns about synthetic biology are widespread. The lack of awareness and the concerns about potential risks highlight the need for further research and regulation. A graph summarizes the key findings of this chapter.

References

Further readings to explore the topic of synthetic biology can be found in the following sources:


Appendix

A chart illustrating the differences in awareness and concerns about synthetic biology between Europe and the United States.
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.
Recommendation from Human Practice Teams

http://partsregistry.org/Biosafety

Conclusion of these reports:
- Daily and local efforts: An ethical reflection on practices, discourses, and social interactions.
- A responsible position regarding what scientific paradigms, through concepts, perceptions and values, are emerging with this field.
- The need to raise awareness of synthetic biology in the population so people can decide in the most enlightened way possible if they want of this new technology and its applications.
- The need for discussion between society's different decision makers to set goals and a definition of what they would consider benefits and acceptable risks.
- Zero risk is impossible to achieve as no containment system can be 100% safe (bacteria can always escape).
- There is a lack of quantitative data evaluating the probability of failure of any synthetic biology engineered system, in particular containment systems.
- There is a lack of quantitative data evaluating the risk of H3T assuming containment systems failed.
- The compiling of the wiki screen shows that no containment systems created in iGEM is robust: they lack the above quantification and are mostly one mutation away from failure. We call for major effort of the iGEM community to quantify available containment systems and search for new solutions.
- The need for an INDEPENDENT cohort of scientists to test experimentally any application of synthetic biology that requires release into the environment.

Proposals to improve this page (Feel free to add ideas)

1. Add a biosafety grade, that could quantify the degree of robustness. For instance, in semantic containment with amber mutation, we score the robustness with the number of amber mutations.

Semantic Containment

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K914000</td>
<td>RNA</td>
<td>pLac-supD-T</td>
<td>241</td>
</tr>
<tr>
<td>BBa_K914009</td>
<td>Translational_Unit</td>
<td>P10G3* Ser133-&gt;Amber Codon</td>
<td>967</td>
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<tr>
<td>BBa_K914011</td>
<td>Intermediate</td>
<td>supD-T - intermediate</td>
<td>178</td>
</tr>
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</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>
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</thead>
<tbody>
<tr>
<td>BBa_K117000</td>
<td>Coding</td>
<td>Lysis gene (promotes lysis in colicin-producing bacteria strain)</td>
<td>144</td>
</tr>
<tr>
<td>BBa_K51504</td>
<td>Composite</td>
<td>J23100 promotor - Anthelin</td>
<td>488</td>
</tr>
<tr>
<td>BBa_K51505</td>
<td>Composite</td>
<td>J23100 promotor - RBS E0004-RFP E1010 - Holin K112806 - endolysin K112806</td>
<td>1273</td>
</tr>
<tr>
<td>BBa_K029000</td>
<td>Composite</td>
<td>Prion-1 Kill Switch</td>
<td>353</td>
</tr>
</tbody>
</table>
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.
SCIENTIFIC PROJECT
Uncontrolled spread of bacteria & Horizontal gene transfer are the unsolved challenges

We want to prevent the spread of GE bacteria

- We want to prevent the transfer of genetic material

**Conjugation**

**Transformation**

**Transfection**
Containment system requirements

- Robust
- Redundant
- Modular
- Efficient
- Quantified
- 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
Bacteria can survive and express proteins inside the beads.
Beads physically prevent bacteria from escaping
Control of population and internal environment
Klll-switch
A delay system allows the bacteria to perform its function.
A restriction enzyme allows the degradation of the containment plasmid.
Without antitoxin, the colicin can perform DNA degradation...
Without antitoxin, the colicin can perform DNA degradation...
...at the **population** level!
...at a **population** level!
...at a **population** level!

No DNA!
the delay system Test
NEW PROMOTER ORTHOGONAL TO pLac, pBAD and pTET

Rhamnose

Normalized fluorescence (RFP, A.U.)

Time (h)

Prham

- Rhamnose 1%
- Rhamnose 0.5%
- Rhamnose 0.2%
- Rhamnose 0.1%
- Rhamnose 0.05%
- Glucose
- Cells only

BIOBRIICKED
Restriction Enzyme efficiently digests plasmids
Toxin kills sensitive cells, anti-toxin protects them

Colicin producing cell

Sensitive cell

zone of clearance

Immune cell
TOXIN KILLS SENSITIVE CELLS, ANTI-TOXIN PROTECTS THEM
Synthetic import domain
Can we hijack the properties of colicins for molecular biology?
Can we hijack the properties of colicins for molecular biology?

**producer cells:** Synthetic Import Domain+lacZ-α

**Receptor cells:** lacZ-Ω
Can we hijack the properties of colicins for molecular biology?

**Producer cells:** Synthetic Import Domain\(+\)lacZ-\(\alpha\)

**Receptor cells:** lacZ-\(\Omega\)
Can we hijack the properties of colicins for molecular biology?

producer cells: Synthetic Import Domain+lacZ-α

Receptor cells: lacZ-Ω
Can we hijack the properties of colicins for molecular biology?
semantic containment
Semantic containment encrypts the genetic information

WT cells

HGT

No Kan resistance

Kan*

Kan R!

supD

TAG ➔ SERINE

Kan*

Kan*

Kan*

*
Semantic containment encrypts the genetic information.
ACHIEVEMENTS
OUR ACHIEVEMENTS AND CONTRIBUTIONS

WE GOT

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

WE ARE GIVING

• 10 working biobricks
• 6 containment modules
• GMO debate
• SynBio workshop
• Biosafety report

Paris Bettencourt

• Working containment modules
• Biosafety registry page
• Human Practice proposals
• Opportunities for public collaboration
THANKs TO OUR SPONSORS
# OUR TEAM

<table>
<thead>
<tr>
<th><strong>Members</strong></th>
<th><strong>Advisers</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jean Cury</td>
<td>Antoine Decrulle</td>
</tr>
<tr>
<td>Dylan Iverson</td>
<td>Ariel Lindner</td>
</tr>
<tr>
<td>Zoran Marinkovic</td>
<td>Babak Nichabouri</td>
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<tr>
<td>Claire Mayer</td>
<td>Aleksandra Nivina</td>
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<tr>
<td>Ernest Mordret</td>
<td>Edwin ‘Jake’ Wintermute</td>
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<td>Aishah Prastowo</td>
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<td>Julianne Rieders</td>
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<tr>
<td>Denis Samuylov</td>
<td>Special thanks to</td>
</tr>
<tr>
<td>Guillaume Villain</td>
<td>members of the</td>
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<td></td>
<td>TaMaRa lab</td>
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