Controlling the lifetime of GMOs using ColiClock

Vilnius-Lithuania iGEM
Various, usually considering the lack of information
The spread of GMOs to the environment/outrival of natural species
Unknown long lasting effect on offspring
Harm on human health: cancer, gene mutations, etc.
Do you know any kind of mechanisms to control GMOs?

- Do not know any: 39%
- Did not respond: 18%
- Political solutions and labeling: 29%
- Technical solutions (such as sterilization): 14%
If there was a system to control GMOs in the environment, would you agree to use GMOs?

- Yes: 59%
- No: 26%
- Have no opinion: 15%
THE IDEA!
Boston, we have a problem...

- Uncontrollable spread of GMOs
- Kill-switches require an external input
We have a solution…
The ColiClock!

- Effectively destroys modified bacteria
- Targeted against genome
- Responds in a time dependent manner
ColiClock: 2 subunits

Regulatory subunit

Functional subunit
ColiClock
How ColiClock works?

IPTG → cl → No CRISPR/Cas
ColiClock: 2 subunits

Regulatory subunit

Functional subunit
ColiClock: 2 subunits
How the functional subunit works?

- IPTG
- cl
- pLac-Cas
How the functional subunit works?

IPTG

pLac-Cas
How the functional subunit works?

IPTG

pLac-Cas
How the functional subunit works?

- IPTG
- pLac-Cas

Dead
Results:

**Western Blot**

<table>
<thead>
<tr>
<th>Incubation</th>
<th>M</th>
<th>K</th>
<th>16°C/16h</th>
<th>37°C/3h</th>
<th>16°C/16h</th>
<th>37°C/3h</th>
<th>16°C/16h</th>
<th>37°C/3h</th>
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<tbody>
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<td>S</td>
<td>S</td>
<td>M</td>
<td>W</td>
<td>W</td>
<td>BL</td>
<td>BL</td>
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</tbody>
</table>

- Efficient expression of Cascade protein
Results:  

- A significant difference with functional subunit

In vivo
Characterizing λ phage promoter
Characterizing λ phage promoter

- λ phage promoter depends on amount of IPTG
- Collaboration with Toulouse team
Achievements

- HP:
  - connections with ministries
  - influenced law corrections
  - meetings with communities, which navigated us to the idea

- 13 new biobricks at iGEM Parts Registry
  - 3 of these biobricks were characterised
  - they were active \textit{in vivo}

- Developed characterisation of a biobrick (BBa_R1051)
- Collaboration with other teams
- Participated in Interlab Measurement Study
To make this happen: Thank you!

- PI: prof. Virginijus Šikšnys
- Instructor: MSc Dainius Tautvaišas
- Sponsors:
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