Silencing Xylella fastidiosa
Mario
Pugliese Olive Farmer
Europe's olive trees threatened by spread of deadly bacteria

*Xylella* has forced the entire Brazilian citrus industry to go bankrupt

Prof. Eiko Kuramae
*X. fastidiosa* researcher, NIOO
Life-cycle

Solutions must consider the complex Plant-Vector-Microbe interactions

Prof. Rodrigo Almeida
X. fastidiosa researcher, UC Berkeley

infected plant

healthy plant
Model: Current Situation

Diseased

Healthy

3 years and 000 days
Phage Therapy

New solutions should not interfere with other ecosystems

Prof. Eiko Kuramae
X. fastidiosa researcher, NIOO

Xylella phage
Pillars of Xylencer

Delivery

Remediation

Spread

Detection
Phage Delivery

Phages are easily harmed by environmental factors

Prof. Britt Koskella
Phage therapy expert, UC Berkeley

Phage Delivery Bacterium (PDB)
Phage Delivery Bacterium

non-pathogenic

pathogenic

Xanthomonas
Selecting a Safe PDB

104 Xanthomonas genomes

principal component 1 (15.8%)

principal component 2 (11.7%)

X. arboricola CITA 44

Model Prediction
- missed non-pathogenic
- missed pathogenic
- non-pathogenic
- pathogenic
Phage Delivery Bacterium
Phage Repression

Phage Lambda Operator Region

dCas

Prevent Dual-Use by applying a model system

Dick Verduin
Biosecurity Expert

Phage Lambda Operator Region

Relative Fluorescence

dCas Targeting Lambda Operon

n = 3

GFP

RFP

Control
dCas
Sensing *X. fastidiosa*
Relieving Repression

Anti-CRISPR toolbox

Phage Lambda Operator Region

P_L P_RM P_R

Regulation

GFP

iGEM MSP Maastricht

dCas Targeting Lambda Operon

<table>
<thead>
<tr>
<th></th>
<th>GFP</th>
<th>RFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>dCas</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Acr-dCas</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

n = 3
Phage Delivery Bacterium
Good solutions need a built-in fail-safe mechanism.

Dutch National Institute of Safety and Environmental Health (RIVM)
Phage Delivery Bacterium
Phage Delivery Bacterium
Immune Activation

Remediation

Flagellin 22

Immune Response Elicitor
Immune Activation

Flagellin 22
Immune Response Elicitor

Xanthomonas campestris

Infect

Rescue

Measure
Immune Activation

Effect of Flg22 on Lesion Size

- Flg22
- Control

n=6
Immune Activation
Silent Disease
Spread
Protein Modeling

- Predict domains
- Model domains
- Assemble
Yeast Recombination

Protein Ladder
Fusion Protein + Linker
Fusion Protein + frameshift

25 kDa
15 kDa
Cell Free

Functional T7 phage

phage Lambda capsid
Chitin Binding Assay

n = 3

% of proteins bound

<1%

Adhesin

Control
Spread
Detection

Monitoring of *X. fastidiosa* forms a major bottleneck

**Dr. Martijn Schenk**
Policy Maker, Dutch Government

Farmers need a practical solution that saves time

**Jan Veltmans**
Head Dutch tree farmer organization

---

Xylencer, Wageningen UR
Insect Transmission Assay
Real-world Detection Test
Spatial Spread Model

Diseased

Healthy
Conclusion

Dr. Martijn Schenk
Prof. Rene van der Vlugt
Prof. Louise Fresco
Dick Verduin
Jan Veltmans
Education and Public Engagement
Outreach and Collaborations
Contributing to the iGEM community

Wiki HTML

At this point you should be able to put content in the appropriate boxes and they should all be ready for some wiki-specific styling. However, to gain more control over how things look, we need to make use of the &lt;div&gt; and &lt;img&gt; attributes. Let's take a look at a simple example:

```html
&lt;div&gt;
  &lt;p&gt; Co-working on the wiki is so cool. I really love it! &lt;/p&gt;
&lt;/div&gt;
```
Acknowledgements

iGEM Supervisors
Microbiology
Systems and Synthetic Biology

• Virology
• Entomology
• Bionanotechnology
• Biointeractions & Plant Health
• Biosystematics
• Dr. Klaas Bouwmeester
• Wasin Poncheewin
• Prof. Louise Fresco
• Dr. Raoul Bino
• Dr. Ernst van der Ende
• Dr. Anouk Geelen
• All stakeholders
• Crowdfunders

www.patriciakuehfuss.com for providing us with the beautiful picture of Mario