Who we are…

Kuba
Réka
Oscar
Ellie
Evgeniya
Răzvan
Nelly
Kirsty
Melanie
Tools that make synthetic biology easier and safer

Questioning legacy and friendliness

E. coli

Citrobacter freundii
Chassis characterisation – *Citrobacter freundii*

Start a dialogue on ‘the ideal chassis’

**Who is it friendly to?**

**What would the ‘public’ think about it?**

**Unfriendly disease-causing poo bacterium**

‘Friendly lemon bacterium’

Unfriendly disease-causing poo bacterium

What do scientists think of the public?

Who *is* the public?
Perceptions of the public

What do you think of when you hear the term “the public”?

“As soon as the news says the public then that’s me (…), as soon as science starts talking about the public it’s not.”

Should public opinion of science (and synthetic biology) matter?

“Public opinion matters. (…) But there has to be a dialogue, it has to be a two way process.”

“Ok, so this is a democracy, so (…) if lots of people don’t want something to happen, it shouldn’t happen.”
Future is awesomeness likely.
Chassis characterisation – *Citrobacter freundii*
- Replicon characterisation

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>pSB1C3</th>
<th>pSB3C5</th>
<th>pSB4C5</th>
<th>pSB2K3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicon type</td>
<td>pMB1</td>
<td>p15A</td>
<td>pSC101</td>
<td>F” and P1 lytic</td>
</tr>
</tbody>
</table>

BBa_I742123 – Edinburgh’s multi-host vector
• built for *E. coli* and *Bacillus subtilis*
• *cmlR* from *Lactobacillus* (gram positive bacterium)
Chassis characterisation – *Citrobacter freundii*
- Replicon characterisation
- Lac promoter characterisation

Protein comparison of *E. coli* and *C. freundii* LacI protein sequence

Sequence difference between *E. coli* and *C. freundii* operator region sequences
Chassis characterisation – *Citrobacter freundii*
- Replicon characterisation
- Lac promoter characterisation
- Growth in salt media

Comparison of *Citrobacter* and *E coli* growth without and with sea salts (40g/l) in LB

Growth of *Citrobacter freundii* in M9 minimal medium + varying concentrations of sea salts

Sea salt concentration (g/l)

Absorbance at 600 nm
Chassis characterisation – *Citrobacter freundii*

- Replicon characterisation
- Growth in salt media
- Lac promoter characterisation
- Sugar utilisation

![OD at 600nm vs Sugar Utilisation](image_url)
Chassis characterisation – *Citrobacter freundii*

Genome sequencing

Our two *Citrobacter freundii* genomes got sequenced at Newcastle University’s Centre for Bacterial Cell Biology, using Ion Torrent sequencing.

The raw reads, de novo assemblies and RAST annotation can be downloaded from our wiki page.

We would like to kindly thank Prof. Anil Wipat and Dr. Wendy Smith for their help. A little bird also told us that Prof. Wipat will start a Synthetic Biology masters programme.
Antibiotic-free selection
Antibiotic-free selection
Sucrose hydrolase (CscA)

We designed a theoretical xylitol-based selection system for *C. freundii*.
Alternative counter-selection
Levansucrase (SacB) improvement

Simple old sacB BioBrick
Causes fructose polymers to form, killing the cell

NEW *Citrobacter*-friendly sacB BioBrick, now with pLac and RFP!

- Kanamycin-independent control
- Controlled level of selection

Can distinguish between cells that lost the cassette (white) and cells without SacB function (still red)

We also made a KanR-pLac-RFP-sacB selection/counter-selection cassette
Alternative counter-selection
Nitroreductase – Quoth the plates – nevermore

After E. A. Poe

Streak plates, note all the cells that should not be there
Alternative counter-selection
Nitroreductase (NfsI)

Metronidazole (pro-drug) $\xrightarrow{\text{Nitroreductase}}$ Metronidazole (active form)

$E. coli +$BioBricked nitrorereductase

NfsI works well in aerobic liquid cultures with 150 $\mu$g/ml metronidazole (MTZ)
Blue-sky research

Project types based on region

<table>
<thead>
<tr>
<th>Region</th>
<th>Problem-based</th>
<th>Tools-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>America</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>Asia</td>
<td>11</td>
<td>28</td>
</tr>
<tr>
<td>Europe</td>
<td>15</td>
<td>29</td>
</tr>
</tbody>
</table>

Our Project

Problem-based
Indirectly, helps take other problem-based projects outside

Tools-based
Directly, adds to the pool of already existing tools
Bio-electric interface
Communications between machines and cells
An inducible electricity generator based on the *Shewanella oneidensis* MtrCAB gene cluster
DIY biology

OpenPCR (DIY effort)

iGEM and DIY bio
Bio-electric interface

*Simplified* schematic representation

**Electron transport**

**Genes’ origins**

**Cytochrome maturation**
Need to do sensitivity analyses to pinpoint limiting factors

Modelling work informs lab work and vice versa
Bio-electric interface
Modelling – Simulation results

Increasing NapC in its sub-system increases electron transfer

Modelling work informs lab work and vice versa
Bio-electric interface
Modelling – Simulation results

Increasing [Complex I] increases electron transfer in the whole system

Modelling work informs lab work and vice versa
Bio-electric interface
Half-cells & results
Bio-electric interface
Microbial half fuel cells results

$S.\ oneidensis$ voltage  $E.\ coli$ voltage

Voltage [mV] vs Time [hours]
Bio-electric interface
Growth-based arsenic biosensor

Growth-based arsenic biosensor

Voltage [mV]

Time [hours]

E. coli (J33203) with arsenate
E. coli (J33203), no arsenate
E. coli control with arsenate

No arsenate

Arseinate

Growth
Our project, iGEM and …
The Future

Crowdfunding – the project grant of the future?

Our synthetic biology-centric science-fiction blog

The world with synthetic biology

Where the Petri dish is half full or half empty

iGEMers guide to SynthBioPunk

The aim of this blog is to share snippets from the lives of people who live in a world where Synthetic Biology has integrated seamlessly into their everyday lives. We get to see how everyday things such as food, health, fuel, advertising, pets, entertainment and clothing have been revolutionized due to advances in this field. We also get to see the state of The Registry.

Intertwined with these aspects that apply to the general population come the personal stories of the characters involved:
Tools that make synthbio easier and safer – Questioning legacy and friendliness

*Citrobacter freundii* — sugary, salty, LacI-less, genome sequenced, replicon and new BioBrick compatible

Novel markers — *cscA, nfsI, dhlA, kanR-P_{lac}+ sacB + RFP* (OMG!)

Bio-electric interface — electron transport, half cells, biosensor, modelling

Human practices — Interviews, Tools vs Problem based projects, DIY biology, Failures, Safety & Legislation, Blog, Crowdfunding
Thank you for your attention!
Questions?