**Abstract:**
- We designed *Bacillus subtilis* to capture metal ions and ferment organic acids to produce bioethanol in order to remediate industrial wastewater important to the Scottish economy.
- We successfully tested a new assembly method called GenBrick and produced higher levels of bioethanol with a protein fusion. We submitted 21 new parts to the Registry.

**Lab Work**
- We decided to use *Bacillus subtilis* 168 because of its known ability to form biofilms and spores.
- Our plan was to engineer the organism to remediate the organic waste (through metal binding) and exploit the organic waste (through bioethanol production).

**Metal Binding**
- We created an assembly using the GenBrick method (see GenBrick) to demonstrate that the Fur box is able to repress gene expression upon exposure to high iron concentrations (Figure 1).
- A Fur transcription factor was cloned out and characterised.
- Nonsensral Feircic binding protein A (FtpA) was cloned as a BioBrick.

**Bioethanol**
- We generated a fusion of pyruvate decarboxylase (Pdc) and alcohol dehydrogenase B (AdhB) (see Devices).
- We characterised it thoroughly and demonstrated that such co-localisation can increase ethanol production (Figure 2).

**Aggregation**
- In order to collect and remove our organism once the metal has been captured, we decided to aggregate the *Bacillus* by forming a biofilm (see Devices).
- Since SinR is the biofilm master regulator, it directly suppresses biofilm production in *B. subtilis*.
- We cloned SinR and analysed biofilm formation in wildtype *B. subtilis*.

**Future Work:**
- Work on the fusion proteins focusing on the metabolic networks necessary to maintain the coenzyme and cofactor balance.
- We aim to link the separate assembled metal sensing and binding parts into one pathway.
- A vast amount of other metal binding entities exist (e.g. non-ribosomal peptide synthases) that we want to investigate.
- Some work needs to be done to obtain our initial goals in aggregation, starting with assembling our cloned SinR parts.

**Computational models**
- Compositional models that can account for integrated function of genes increasingly contribute to how we understand, discover, and design biological systems.
- Models facilitate the prediction of experimental results and improvement on existing cellular systems.

**Computer Model**
- We implemented a whole cell model to capture the interactions between the synthetic circuit and the host cell.
- A modular platform was used, which allows easy insertion of models for synthetic circuits into the whole cell model.
- A simple circuit for ethanol production was inserted into the model.
- We predicted the exponential growth rate of wild-type and ethanol producing bacteria in different nutrient environments (see table below).

<table>
<thead>
<tr>
<th>Nutrient Environment</th>
<th>Growth rate of unmodified cells (hr⁻¹)</th>
<th>Growth rate of ethanol producing cells (hr⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>0.701</td>
<td>0.632</td>
</tr>
<tr>
<td>Medium</td>
<td>0.712</td>
<td>0.650</td>
</tr>
<tr>
<td>Rich</td>
<td>0.753</td>
<td>0.682</td>
</tr>
</tbody>
</table>

**Human Practices**
- As the vote on Scottish Independence grows nearer, a closer look at how the expanding life-sciences industry will affect the economy in comparison to the oil and gas industry already prominent in Scotland.
- We talked to a member of the Scottish parliament about the potential impact of Scottish independence on the life-sciences sector.

**Politics**
- Designed a proposal for wastewater treatment system involving our organism based on existing purification technologies.

**Engineering Model**
- In Scotland, as in other countries, there are already chemical treatments in place, but these can be expensive and do not do anything special with the waste.
- Our system would fit like a puzzle piece into current industrial settings as an add-on to an existing factory.
- The three main Scottish industries we aim to target are: textiles, leather and whisky.

**Industry**
- In Scotland, as in other countries, there are already chemical treatments in place, but these can be expensive and do not do anything special with the waste.
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**GenBrick**
- Anneals specific linkers to both ends of the gene, thus eliminating error in assembly order (see cartoon below).
- It can anneal up to 10 constructs in 1 single reaction.
- Directly compatible with the established BioBrick standard.

**SULSA**
- Scottish Universities Life Sciences Alliance.

**RSC**
- Royal Society of Chemistry.

**SAM**
- Scottish Academic and Medical.