Welcome To Our Presentation!

Team:
- Fatih Gu
- Berka Ahlan
- Abdulkadir Karadag
- Ibrahim Yasir Orhan

Alihan Celikcan
Our project is a development project, with a major newly designed circuit and enhanced functionality of the 2013 project. The new project is a 'smart' thermoregulatory system functioning both ways, able to cool and heat. BactoCooler II detects the temperature, cooling and heating as required.
Main Component 1:

**Urease**

Two Urease Structures:
- *S. Pasteurii* – Bacterial
- *Endocarpon Pusillum* – Fungus

*Urease* is a foundational enzyme, first enzyme to be purified and crystallized.

Newly discovered Ni

It breakdowns urea, with a very high kinetic rate and endothermic enthalpy. It is a perfect cooling enzyme.

100x of Soy Bean urease

14x of Jack Bean Urease.
Catalase is a common enzyme present in all oxidative organisms. *E. coli* originated HPI and *H. pylori* originated HPC. Both have high reaction rates and high enthalpy values. The enthalpy value of HPI is +17 kJ mol⁻¹. While, the enthalphy value of HPC is +100.4 kJ mol⁻¹.
Main Component 3:

**RNA Thermometer**

RNA Thermometers are hairpin-like structures acting as ribosome binding sites. Sensitive to temperature, the RNA Thermometers act as on-off switches for translation.

The thermometer structure closes the SD(Shine-Dalgarno) Sequence in lower temperatures and opens it at higher temperatures to function.

We use the IbpB Thermometer of E. Coli.
**Parts**

**BBa_K1806001**: This part is the coding sequence for the Helicobacter pylori catalase, which effectively catalyses the breakdown of hydrogen peroxide.

**BBa_K1806002**: The part has been designed as a backbone part to integrate the T7 lacO pelB + 6xHis system to a targeted sequence.

**BBa_K1806003**: Designed to close lacO present systems in higher temperatures by producing lacI.

**BBa_K1806004**: The part is to act as a backbone for the T7+ibPB RNA Thermometer+pelB+6xHis system.

**BBa_K1806005**: The part is designed to produce RFP in higher temperatures. The RNA Thermometer acts as a heat-shock RBS.

**BBa_K1806006**: The natural urease of the bacteria species *S. pasteurii* Urease catalyses the breakdown of urea at an higher producing lacI.

**BBa_K1806007**: Designed to integrate the T7 lacO lacI pelB RFP 6xHis Ter system.
Our system revolves around the 'smart' reaction of the heat-responsive composite system. The cooling system utilizes urease and the heating system uses catalase. Instead of being regulated through a lac system when cold, our system produces lacI and lacO, regulating catalase heat shock protein. Urease is produced from the urease system, which has an RNA thermometer attached to it, stimulating cooling at higher temperatures. When hot, catalase is produced, and when cold, lactose is not produced. The catalase system is not regulated by an RNA thermometer as it shows an opposite trend.
Cloning

**Results**

**BBa_K1806001**: pSB1C3 ligation, cloned
- Cut with EcoRI and PstI

**BBa_K1806006**: IDT had a base pair cap for each G-Block
- So the parts was synthesized as two segments
- The two segments were tagged as G-Block 10 and 11
- Restriction Site (The RFP in the pSB1C3 ligation cloned registry contains Hind3 sites)
- G-Block 10 was cut with EcoRI and Hind3, G Block 11 was cut with Hind3 and PstI and the two segments were then verified and ligated
- The ligated complete part was cut with EcoRI and PstI

**BBa_K1806002**: pSB3 ligation, cloned
- Cut with EcoRI and PstI

**BBa_K1806005**: pSB1C3 ligation, cloned
- Cut with EcoRI and PstI
Results

Functional Assay

Cultured samples for 13 hours. Functions of IPTG Inducible Promoters in different temperatures. Data on RFP concentration was acquired at 584 nm of emission and 607 nm of excitation. After 13 hours IPTG added (50 mM) every 3 hours and further culturing for 6 hours.

The proteins were isolated and analyzed.
Policy & Practices

Nilufer Aksoz

Serhat Karabulut

Sadrettin Basalan

Yusuf Durmus
A Future

Policy & Practices

A Future
Characterization for ATOMS_Turkiye

Bacteria containing the two parts were cultured for 16 hours, and then periodically added and incubated. The acquired protein containing samples were isolated and measured yielding the necessary data for the part.

The difference rate in between the rates of the two parts were 1 to 68.

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>GFP/TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toehold</td>
<td>0.038099</td>
</tr>
<tr>
<td>Toehold trigger</td>
<td>2.5802</td>
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</tbody>
</table>
We completed all bronze medal requirements including collaborating with a team.

We documented 6 novel standard parts to the registry, characterized and documented 4 parts and met with experts to develop our project.

We have demonstrated a design for future application, helped another team characterize 2 parts, and shown that 2 of our parts work as expected.
We thank:
Our Sponsors

sentegen

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INTEGRATED DNA TECHNOLOGIES

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BioLabs Inc.

and

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Thanks for listening!