Introduction

For creating an RPS game between humans and E. coli, we tackled three problems. Firstly, we used two sets of three signaling molecules which correspond each to rock, paper or scissors. Secondly, to know who wins the game, we needed a set of E. coli that acts as judges. We constructed the judge E. coli by using AND-gate promoters. Finally, we must create randomizers which enable E. coli to choose any of its three signaling molecules with the same probability in order to be able to play the RPS game fairly and properly. To do so, we designed two randomizers: Conditional Knockout by Recombination and Survival of One Strain.

Because it was very hot in summer vacation, as cool prizes for winners in RPS game, we also designed E. coli that can make it rain and other E. coli that can allow us to make urea coolers. These E. coli will definitely make the hot summer more fun and refreshing!

Judge
Using AND gate promoter for Judging

The AND gate promoter can sense the two different signaling molecules as inputs and produce one output. We use three different fluorescent proteins as an output to indicate the winner of the game.

<table>
<thead>
<tr>
<th>Human wins: GFP</th>
<th>Tie: CFP</th>
<th>E. coli wins: RFP</th>
</tr>
</thead>
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We need a set of nine judges because each of the players has a set of three different signaling molecules.

Each of the 9 Judges contains one AND gate promoter

We constructed one of the judges

This judge detects human's paper and E. coli's rock, and shows human's winning by green fluorescence.

+Plux-lac-gfp bursting plasmid[1]

We confirmed the expression of gfp gene is dually regulated by IPTG and 3OC6-HSL.

Choosing one Hand
randomizers which enable E. coli to choose any of three signaling molecules with the same probability

-Conditional Knockout

We designed Cre-Lox system, depicted on the left, which allows E. coli to express one of its three signaling molecules by conditional knockout.

To design the randomizer based on Cre-lox recombination, we needed twolox cassettes of different recombination frequencies.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Cre</th>
<th>P&lt;sub&gt;rtol&lt;/sub&gt;</th>
<th>lac-luxR</th>
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</thead>
<tbody>
<tr>
<td>Bba_K546302</td>
<td>-</td>
<td>+</td>
<td>310</td>
<td></td>
</tr>
<tr>
<td>Bba_K546302</td>
<td>-</td>
<td>+</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>Bba_K546302</td>
<td>-</td>
<td>+</td>
<td>2.9</td>
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<td>Bba_K546302</td>
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In the process of constructing the judges, we improved two faulty BioBricks: las promoter and lsrA promoter.

-Plas-gfp (Bba_K546302[e])
-Plas-gfp (Bba_K546302[f])

Our las promoter is activated by 3OC12-HSL.

In the process of constructing the judges, we improved two faulty BioBricks: las promoter and lsrA promoter.

1. The Old Model
2. Our New Model

Survivor
(Producer)
(Sensitive)
(Resistant)
Producer 0.271 0.270 0.270
Sensitive 0.270 0.271 0.270
Resistant 0.270 0.270 0.271

Survival of one Strain

To create a randomizer, we used three rival strains each corresponding to rock, paper, and scissors. We make them compete for survival and take the surviving strain to represent the bacteria's choice for the RPS game.

Durrett & Levin (1996)

Tokyo Tech iGEM 2011

In the old model, the producer strain can't survive, which is a critical limitation as a randomizer for the RPS game. To be able to create a true randomizer, we modified the differential equations of the model. We limited the resistance of the colicin-resistant bacteria. In this case, a type of bacteriocin, produced by colicin bacteria, is toxic to the colicin-sensitive strain as well as itself. With our new differential equations, any of the three types of bacteria can outcompete the other two strains and ultimately survive by very small differences between the initial population densities of the strains.


Cool Prizes
Rain and urea cooler as prizes

To make it rain, we focused on producing isoprene. It can act as condensation nuclei when photooxidized [1]. We introduced the ispS gene encoding isoprene synthase to produce isoprene.

We succeeded in producing isoprene.


We made urea production possible by introducing lacI gene encoding arginase.

Urea concentration (mM)

We also performed flux analysis to increase urea production [2]. By mathematical modeling, we confirmed that our artificial urea cycle is already feasible.


Human Practice

We created the iGEM Card Game: "Manabi Gate", an educational event.

We fascinated our visitors with the card game! (206 participants!)

We taught Synthetic Biology to them and made a survey.

Achievement

20 New working parts
2 New applications
1 New model

Acknowledgements

This work was supported by the Integrated Research Project of the Tokyo Tech Innovation Center. We thank the AMC group members for their valuable comments. 
