E. coli as a heavy metal biosensor

Heavy Metal Gang Captured by Capsule Cop

Team: Korea_U_Seoul
iGEM 2010
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Korea University
in Seoul, South Korea

11/7/2010
Team::Korea_U_Seoul@iGEM2010
Meet Korea_U_Seoul team

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Seobeeom Heo

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The initial project from brain-storming

A toggle switch by a electrical signal (redox potential)

But, failed to design a switch controlled by only a single signal... Is it possible?

So, we decided to continue iGEM2009 KU_Seoul team project!
Heavy detecting *E. coli*

1. Preparation
   1) Cell harvest
   2) Store at 4°C

2. Induction
   1) Addition to LB + Cd⁴⁺ for 60 min at 37°C
   2) Cell harvest

3. Detection
   1) Reaction in Tris/HCl (pH 9) + 0.1M AAP for 10 min at 37°C
   2) Quantification of AP

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Why heavy metals?
: Influence of heavy metal

Heavy metal contaminated areas in Korea

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Our project aims to...

- Detect toxic heavy metals in an aquatic environment
- Design a bio-circuit to detect heavy metals
- Construct *E. coli* biosensor having multiple heavy metal detecting circuit
Multiple-heavy metal detector: Zn\textsuperscript{2+}, As\textsuperscript{3+} and Cd\textsuperscript{2+} in a solution

1. Point mutation at two EcoRI sites in the AAA gene → registration of part

2. The problem of plasmid incompatibility for detecting multiple heavy metal ions: two plasmids → merged one plasmid

3. For increasing expression of reporter genes: 1) the removal of degradation tag and 2) the use of the plasmid with the high-copy number origin

4. The characterization of promoters in this project
Bio-circuit Design: heavy metal sensitive promoters

Three promoters from *E. coli*
- Promoter arsenate resistant ($P_{arsR}$):: As
- Promoter znt A ($P_{zntA}$):: Zn
- Promoter yod A ($P_{yodA}$):: Cd

Nature Reviews Microbiology 7, 25-35 (January 2009) doi:10.1038/nrmicro2057 Review
Bio-circuit Design: Reporters

Promoter to reporter genes

- $P_{arsR}$ → GFP
- $P_{zntA}$ → RFP
- $P_{yodA}$ → AAA (aryl acylamidase)
Bio-circuit construction

Reporter part: BBa_K400628

Composite part: BBa_K400000
### Parts used in the circuit

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Description</th>
<th>Designer</th>
<th>Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>BBa_K4000000</td>
<td>Composite</td>
<td>composite heavy metal-detector(Zn, Cd, As)</td>
<td>Jonghwa Kim</td>
<td>5505</td>
</tr>
<tr>
<td>BBa_K400628</td>
<td>Reporter</td>
<td>mAAA (EcoRI site mutated Aryl Acyl-Amidase)</td>
<td>Seoboom Heo</td>
<td>1494</td>
</tr>
</tbody>
</table>

![Chemical Reaction](attachment:chemical_reaction.png)

(R = -CH₃ in p-acetaminophenol)

**Acetaminophen** (colorless)  
**p-aminophenol** (purple)

**Reporter part:** BBa_K400628  
Mutant aryl acylamidase (mAAA)

**Composite part:** BBa_K400000  
Heavy metal detector
Measurements

- **GFP**: Ex=485/Em=530
- **RFP**: Ex=553/Em=585
- **mAAA**: produced p-aminophenol by the activity of mAAA is reacted with o-cresol in reaction mixture → blue color detected spectrophotometrically at OD$_{615}$

OD$_{600}$ = 0.5
Results

Figure 1. The expression of reporter genes (GFP and mAAA) by AsO$^{3-}$ and Cd$^{2+}$.

**gradual increase** according to the concentration of heavy metal ion → **a tunable promoter type** controlled by the concentration of a inducer
Results

Figure 2. The expression of the reporter gene (RFP) by Zn\(^{2+}\).

**Induced** beyond 1mM Zn\(^{2+}\) (turned on at 1mM)

→ turn on/off promoter type (no intermediate expression)

→ similar to T7 promoter
Discussion

To complete our project, we have to upgrade our parts:

1. Amplify reporter proteins for the signals

2. Search for diverse heavy metal sensitive promoters in *other bacteria* (registering of new parts)

3. Simplify the signals by making AND/OR gate with mAAA for detecting many heavy metal ions by one system.

4. Easy handling biosensor kit: freeze-dried cells to make a capsule + cheap chemicals (e.g. acetaminophen)
Application: making a device giving visible signal

cell culture

freeze-dry box

powder-like particles of E. coli

Capsule cop

Zn$^{2+}$ containing water

As$^{2+}$ containing water

Cd$^{2+}$ containing water

E. coli put in a capsule (capsule cop)

When freeze-dried, 50% of the E. coli is available for detection

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Heavy Metals

THE PERIODIC TABLE

[Periodic Table Image]

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Safety

1. Our promoters and linked genes (AAA, GFP, etc.) are considered to cause no harm to the environment.

2. The parts that we made do not raise any safety issues.

3. We have a Bioethics Committee, composed of professors from various departments; Life science, Law, Medical School.

4. First, we think that for making the dealing with safety issues more compatible, it is better that iGEM HQ shows more concrete criteria and guidelines for the safety issues. Next, make it a rule to do the safety-proving experiment when the iGEM team's project shows some safety related problems.

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Supported by CSBL@KU

I'm Hyoek-Jin Ko

HanSeong Roh!

Sorry, HseOn, after me!

I'm SaeYoung Lee, the captain

Sohyun Kim

I will be a captain soon

EunHye Park

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Team:korea_U_Seoul@iGEM2010 11/7/2010