**Abstract**

As an common pollutant source of industry, low concentration of copper ions can do harm to many creatures. Whereas, Escherichia coli has high tolerance for copper through specific systems. There are copA promoter, copA promoter (operator of mar locus) and marAB promoter existing in E.Coli, all of which are directly or indirectly regulated by copper ions. The most sensitive and effective one is the copA promoter chosen to trigger the sequential reactions. Glutathione is an intracellular redox-active tripeptide thiol which can effectively detoxify the metal ions by chelation. It can be synthesized by gshf, an encoding sequence derived from Streptococcus thermophilus, which can only bind both copper ions and gshf. In addition, we also utilize E.coli promoter to enhance the efficiency of the other two promoters by repression the expression of copA. With all these designs, an automatically and efficiently copper-preventing guard have been created successfully.

**FUNDAMENTAL MODULE**

A. Sensitivity and specificity experiments to choose the promoter copA.

For the sensor part, we select three genes, copA, cueO and marAB as our candidates of sensor. After the experiments to compare sensitivity and specificity with each other, we choose copA as our sensor. The sensitivity experiment shows that copA promoter is more sensitive, specially copper concentration over 1mg/ml (Fig.3). Moreover, in the higher level of copper, the part displayed higher intensity fluorescence.

B. Extract and amplify the gshf gene in *Streptococcus thermophilus*.

The result on % agarose gel electrophoresis showed that there is a clear band around 2250bp which was predicted. The sequence testing results showed homology of *Streptococcus thermophilus* SBM.

**MODEL**

A. Simulation

Second order Runge-Kutta method with a step of 0.1 min and 1000 times iteration is carried out to simulate this process. Figure 2 shows that the Cu2+ concentration outside is decreased to a comparatively low level with 300 minutes. Figure 3 shows the growth curve of E.coli from 100 CFU/L to 30000 CFU/L along this process.

B. Global Sensitivity Analysis

Global sensitivity analysis is a method to analyze all the parameters at one time to find out the influence on the result for each parameter and the interaction between those parameters. Figure 3 shows that the top 4 influential parameters are K, h, D, k. Those parameters are all controllable by the designer of this equipment, so the effect of Cu2+ elimination can be highly improved by changing those parameters.

**FACILITATING MODULE**

We build sgRNA combining with dCas9 protein activated by promoter vgb which is induced by oxygen concentration.

In vitro evidence for Pvgb induced by O2

We labeled the promoter vgb in standard plasmid pSB1C3 with green florescence protein (Bsa_E640) and test the intensity of florescence under various of anaerobic conditions.

**Practices**

**Acknowledgement**

**System Design**

Will GSH detoxify Cu2+ in water or will GSH become the new pollution?

Cu2+ can cause great damage to many kinds of aquatic organisms especially algae, even 1ppm can affect the growth of it. We choose the most common one, Chlamydomonas reinhardtii as the material. And the trend of relationship among cupric ions, GSH and Chlamydomonas reinhardtii are showed below. As a result, GSH does have significant detoxification effect.

**Facilitating System**

To make our system more efficient, we silenced the copA coding gene, which has been indicate that it’s protein CopA is a Cu2+ translocating efflux pump that is similar to the copper pumps via CRISPRi system. Pvgb, which can maximally induced under anaerobic condition, are also designed into this system to ensure the effect of GSH compound with copper ions.

**Exhibition**

A sgRNA along with its DNA template sequence was designed and the plasmid pSB1C3 was constructed according to the references concerned.

**Communication**

This summer, as being invited, our team established a delegation taking part in the Internationally Genetically Engneered Machine Conference held by iGEM. During the conference, we exchanged our ideas with other teams over several topics ranging from our projects to biotechnology. Nearly all teams pointed out that security methods should be treated as a real part while designing bio-machines. We promted our CAM Bio’s growth along with the projects of ourselves through this communication in Taiwan.

**Collaboration**

Anyway, all experiences participants or freshmen are always new in this synthetic biology field. So basic to an excellent work is the collaboration with other teams. We connected with CAM Bio for gene material and discussed over our project. Moreover, we collaborated with CAM Bio by providing them with the protocol of GFP detection and they taught us the method to synthesize gshf gene.