KU Seoul Team: Integrated Heavy Metal Detection System

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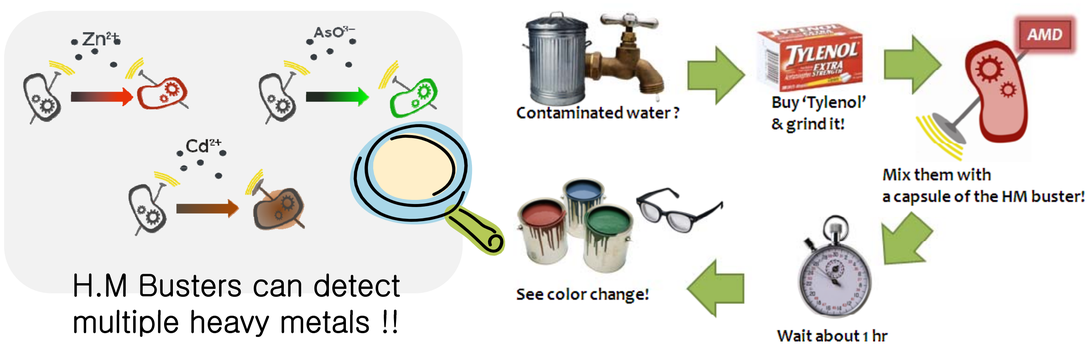
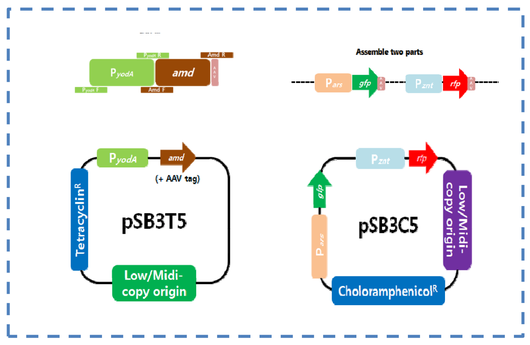
Overview

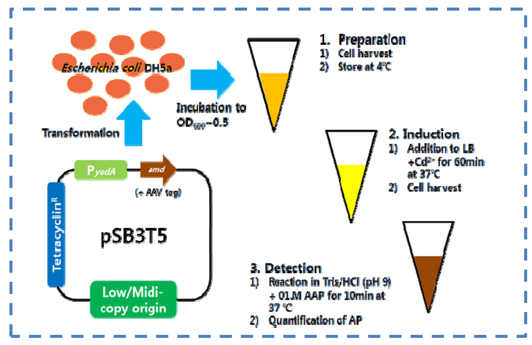
Our team project is designing synthetic modules for simultaneous detection of multiple heavy metals such as arsenic, zinc, and cadmium in *Escherichia coli*. We call this H.M busters! The ultimate goal is to build a micromachine sensing and determining of the concentration of heavy metals in a sample solution (e.g. the waste water). In order to design the system, we will employ two fluorescence proteins (GFP and RFP) and aryl acylamidase as signal reporters. The aryl acylamidase converts a colorless acetaminophen(Tylenol TM) to a brown color substrate. Since H.M busters have three heavy metal promoters, if more than two heavy metals coexist in a solution, the results would be interpreted from the mixed fluorescence and/or color rather than a single signal detection. The successful construction of the synthetic modules in *E. coli* can be utilized in the form of a lyophilized powder, which can be stored in a drug capsule to make it portable.



Materials & Methods



STEP 1. Assembly parts



STEP2. Measurement

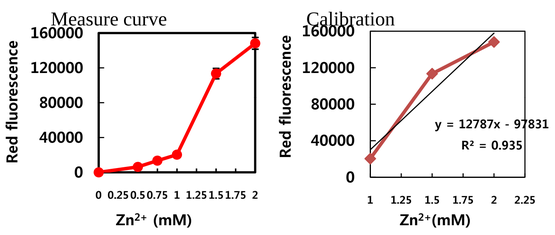
1. Backbone Plasmids
   Plasmid pSB3C5 with part BBa_J04450 : 2009 Kit Plate 1 [5C]
   Plasmid pSB3T5 with part BBa_J04450 : 2009 Kit Plate 1 [9C]

2. Promoters
   Promoter P*arsR*, P*znt* and P*yodA* originated from genomic DNA of *E. coli* XL-1 Blue

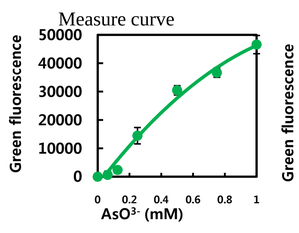
3. Protein Coding Sequences
   Green fluorescent protein [BBa_E0044] : 2009 Kit Plate 1 [14G]
   Red fluorescent protein [BBa_E1010] : 2009 Kit Plate 1 [18F]
   Aryl acylamidase protein : [BBa_K271000] : New biological part

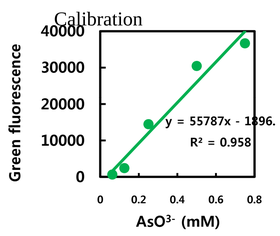
4. Chemicals
   Acetaminophen (Sigma), $CdCl_2 \cdot H_2O$ (Junsei), $ZnCl_2$ (Sigma), $CuSO_4 \cdot 5H_2O$ (Sigma)
   KH_2AsO_3 (Sigma) , Luria-Bertani broth & Bacto agar (Difco) , o-Cresol (Sigma)

Results

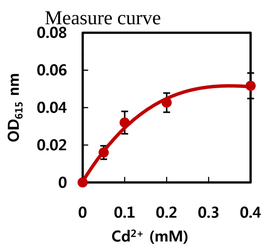


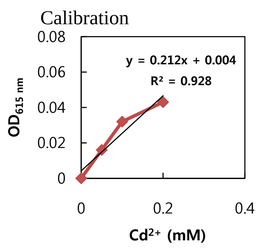
Expression of Red Fluorescent Protein





Expression of Green Fluorescent Protein





Expression of Aryl acylamidase

We successfully constructed metal detector in *E. coli* empolying natural metal detecting promoters found in *E. coli*.

1. Zinc detector using RFP as a reporter was constructed and worked at the range of 1~2mM concentration

2. Arsenic detector using GFP as a reporter was constructed and worked at the range of 0.15~5mM concentration

3. Cadimium detector using AMD as a reporter was constructed and worked at the range of 0.2~0.4mM concentration

Summary

We successfully constructed zinc, arsenic and cadmium detector, and confirmed their operation. We registered new part; aryl acylamidase which is used in cadmium detector. It has shown the possibility of a cheaper and more visible detector. Because Tylenol is very cheap, it will be very economical to use aryl acylamidase in any places where fluorescence proteins are used!

Discussion

1. **Zinc detector** : P_{znt}-*rfp*

The zinc detecting promoter can be induced beyond 1mM concentration of zinc ion. This range from 1mM to 2mM is enough to detect zinc level in common waste water. However, when we need to detect higher concentration of zinc ion, we should use with dilution methods.

2. **Arsenic detector** : P_{ars}-*gfp*

It seems that the cost of the expression of arsenic ion binder is very expensive for microbial cells. In lower levels of concentration, the fluorescence was not enough to be distinguished from the control. A strong inhibitor might exist at the imitation of expression. Compared to zinc ion, the arsenic ion has more harmful effect on the growth of bacteria. This is a drawback of our detecting system considering that the bacteria can be killed while measuring concentrations.

3. **Cadmium detector** : Synthetic circuit – II : P_{yodA}-*amd*

Cadmium detector has a similar problem related to toxicity, as discussed above. Like arsenic ion, cadmium has harmful effects on bacteria. 0.2mM of cadmium is enough to inhibit the growth of E. coli. The growth of E. coli did not show big difference between 0.2mM and 0.4mM concentration of cadmium.

We wanted to detect the proportional change according to concentration with our bare eyes, but the difference was too small to be detected. Because of low fluorescence of GFP and RFP, we considered using a stronger promoter to detect heavy metals. However we did not use it due to standards issues. Some parts registered on the registry did not work, not to mention inaccurate sequencing. The fluorescence GFP and RFP can be seen only with UV irritation.

However, it is possible to see the changes of the color which stimulated in good contrast by metal ion, using aryl acylamidase. The amd gene, which induces brown color by cleaving aceteaminopen (Tylenol TM).

References

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2. Regulation of *yodA* encoding a novel cadmium-induced protein in *Escherichia coli. A. Puskarova, P. Ferianc, J. Kormanec, D. Homerova, A. Farewell*
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