Detecting Metals in Water Using *E. coli* as Biosensors

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Honors Genetics 324-50, Dr. Alvarez

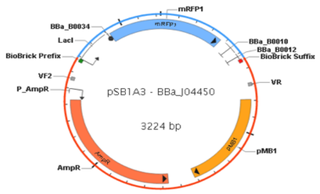


Fig. 1: **Synthetic Biology plasmid.** pSB1A3 is one of the 4 available plasmids used for cloning. Besides the main BioBrick components, it has an origin of replication, a gene that encodes a resistance to an antibiotic (here ampicillin=Amp^R), and the 2 primers used in PCR: VF2 and VR.

GOAL

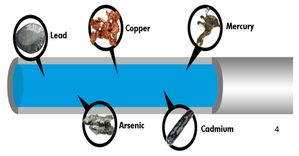
This project began with the question of finding practical applications of synthetic biology and the techniques learned in lab.

BACKGROUND

Water pollution occurs when runoff, fertilizers, chemicals, or any product changes chemical or biological equilibrium in a water system negatively affecting the biodiversity that live and/or use that water source. In America, over 2100 toxic chemicals are found within our everyday drinking water. Laws and regulations have been established to decrease the number of people who lack clean drinking water; however, toxins and water contamination still exists.

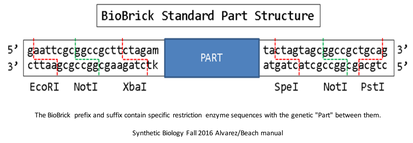
3

In order to detect metal contamination, our study has created biosynthetically modified E. coli cells to sense when water systems have various harmful metals such as mercury, cobalt, and arsenic. After further research of previous studies, the group decided to focus on design functional devices that, if working properly, once each one is introduced into E. coli cells, each will fluoresce in the presence of the respective metal contaminant in water samples.

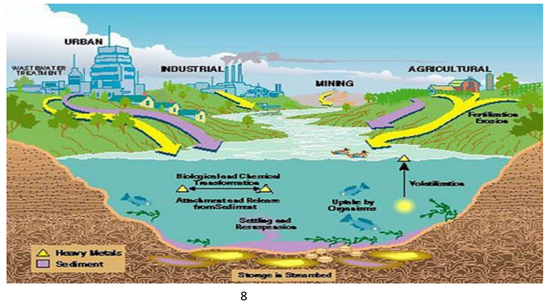


4

Using DNA Biobricks from iGEM (International Genetically Engineered Machine) that match the specific metal promoters mercury, cobalt, and arsenic and using the Biol324-50 (Honors Genetics) laboratory techniques learned during this semester, the three main Biobricks: Promoter, Ribosomal Binding Site, and Reporter Genes were combined and transformed into E. coli cells.



The transformants of the new recombinant DNA, once checked for correct DNA sequences of its Biobricks components, will be put to test in specified water systems. With this project, we hope to detect contamination and then educate the community in order to decrease water pollution in our surrounding ecosystem.



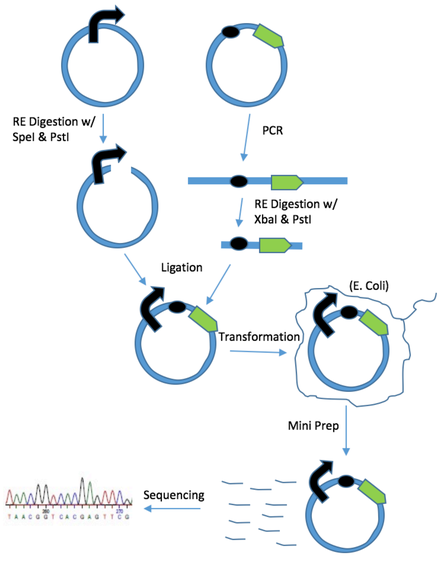
8

METHODS AND MATERIALS

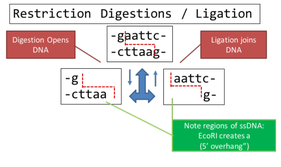
Regulatory Promoters

Metal	Bio Brick ID	Well	Plate (year 2015)	Size (bp)
Arsenic	BB_J33201	10o	3	518
Cobalt	BBa_k540001	13i	1	426
Mercury	BBa_k346002	1o	1	57
Green Fluorescent Protein	BBa_E0040	14k	1	706
Red Fluorescent Protein	BBa_J04450	1c	1	720

Vector used	Insert used	Insert used
Arsenic	RFP	GFP
Cobalt	RFP	GFP
Mercury	RFP	GFP



1. Determine insert and vector parts and sizes.
2. PCR the insert to amplify the DNA.
3. Cut the insert and vector with the appropriate Restriction Enzymes.



1. Ligase the vector and insert with a backend approach with Xba1 and Pst1.
2. Transform the new Plasmid into the *E. coli* on an petri dish with Luria broth and antibiotics.
3. Take the newly transformed *E. coli* and mini prep to destroy the cell wall and send for sequencing.

RESULTS

- Heavy metals can enter our water supplies via industrial and consumer waste, or even come from the Earth's soil, and can contaminate any body of water it is released into.
- Our experiment has resulted in biosensors to detect heavy metals in water, such as cadmium, mercury, and arsenic. We have created new recombinant DNA, that have been sequenced and checked, that will be place in different bodies of water to detects these heavy metals.
- In creating this data, we can now work to determine how to rid our waters of heavy metal contamination.

The BioBrick prefix and suffix contain specific restriction enzyme sequences with the genetic "Part" between them.

Synthetic Biology Fall 2016 Alvarez/Beach manual

Device for Mercury (1o): Detection with Red Fluorescent Protein
>Contig1
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTNNNTNNN
NNNGNCCCNGGGNGNNGNNNNGSGTANTTNNNNNNTTACNATNNAAANNNGGTTCAC
NNGGCAGAATTCAGATAAAAAAAAATCCTTANNNTTTCGCTAAGGATGANNTTNGAATTC
GCGGCCGCTTCTAGATTCCATATCGCTTGACTCCGTACATGAGTACGGAAGTAAGGTTAC
GCTATCCAATCCACTAGAGAAAGAGGAGAAATACTAGATGGCTTCCTCCGAAGACGTTAT
CAAAGAGTTCATGCGTTTCAAAGTTCGTATGGAAGGTTCCGTTAACGGTCACGAGTTCGA
AATCGAAGGTGAAGGTGAAGGTCGTCCGTACGAAGGCACCCAGACCGCTAAACTGAAAGT
TACCAAAGGTGGTCCGCTGCCGTTCGCTTGGGACATCCTGTCCCCGCAGTTCCAGTACGG
TTCCAAAGCTTACGTTAAACACCCGGCTGACATCCCGGACTACCTGAAACTGTCCTTCCC
GGAAGGTTTCAAATGGGAACGTGTTATGAACTTCGAAGACGGTGGTGTTGTTACCGTTAC
CAGGACTCCTCCCTGCAAGACGGTGAGTTCATCTACAAAGTTAAACTGCGTGGTACCAA
CTTCCCGTCCGACGGTCCGGTTATGCAGAAAAAAACCATGGGTTGGGAAGCTTCCACCGA
ACGTATGTACCCGGAAGACGGTGCTCTGAAAGGTGAAATCAAAATGCGTCTGAAACTGAA
AGACGGTGGTCACTACGACGCTGAAGTTAAAACCACCTACATGGCTAAAAAACCGGTTCA
GCTGCCGGGTGCTTACAAAACCGACATCAACTGGACATCACCTCCCACAACGAAGACTA
CACCATCGTTGAACAGTACGAACGTGCTGAAGGTCGTCACTCCACCGGTGCTTAATAACG
CTGATAGTGCTAGTGTAGATCGCTACTAGTAGCGGCCGCTGCAGTCCGGCAAANAAAGGG
CAAGGTGTCACCACCCTGCCCTTTTCTTTAAAACCGAAAAGATTACTTCGCGTTATGCA
GGCTTCCTCGCNNCTGACTCGCTGCGCTGNNNGTCGNNGGNNAGNTNNANNNNNNNNNNG
GGGGGGNNNNNTATNNNANANNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNN

Underline: Prefix/Suffix
Yellow: Vector
Red: Red Florescent Protein (RFP)
Blue: Ribosomal Binding Site (RBS)

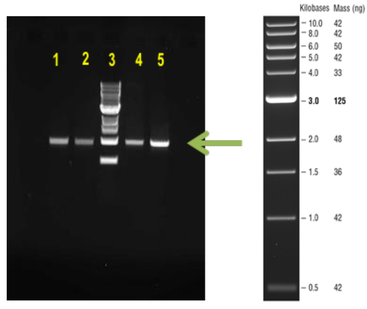


Fig. 5: Amplicon DNA check. A 1% agarose gel prepared in TAE buffer (stained with Ethidium Bromide) is shown here. The green arrows indicate the size of the expected fragment(s), 1040bp. Lane 1-2: composite BioBricks RBS-GFP. Lane 3: markers used, 1Kb DNA ladder. For fragments sizes refer to gel on the right which is the picture provided by the manufacture NEB (N0468S). Lane 4-5: composite BioBricks RBS-RFP.

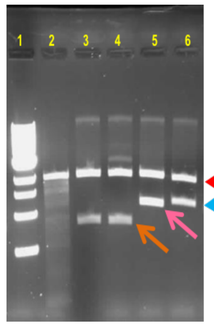


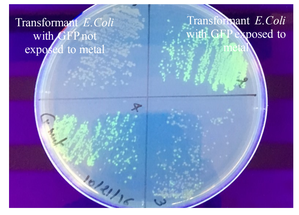
Fig.6: **Plasmid DNA check**. A 1% agarose gel prepared in TAE buffer (stained with Ethidium Bromide) shows the result of DNA plasmid from putative positive transformants cut with NotI. The red arrow indicate the size of the expected plasmid backbone (~2140bp), while the blue (~1300bp), pink (~1200bp) and orange (~800bp) arrows are the expected size of the BioBrick devices engineered. Lane 1is the markers used, 1Kb DNA ladder from NEB (**N0468S**). Lane 2 is a control of the mercury promoter vector alone (57bp) cut with NotI also. Lanes 3-4 are the devices with mercury promoter. Lane 5 is the device with cobalt promoter. Lane 6 is the device with arsenic promoter.

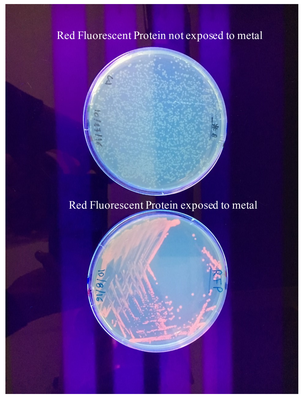
Conclusion

- Although our constructs have yet to be completed, we theorize that they will be able to detect the presents of metals in water sources.
- We applied our techniques from lab in an attempt to create a useful biosensor from a vector and insert.
- Hopefully, our experiment will be continued and the goal of creating biosensors will be reached.

Future Work

- Test finished devices in specified metal (cobalt, mercury, arsenic) contaminated sources for fluorescent results.
- Preserve finished devices for future classes to use for testing/ educational purposes.





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