Utilizing Synthetic Biology to Detect Iron in Water

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Biological Systems Engineering

Virginia Tech Invent the Future

Motivation

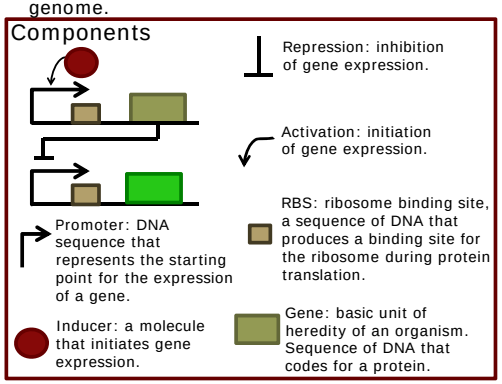
In Virginia, more than 1.7 million homeowners rely on private water supplies. The Virginia Household Water Quality Program (VAHWQP) aims to improve the water quality and health of Virginians using private water supplies. At high concentrations (>0.3mg/L), iron is considered a nuisance chemical and can cause significant aesthetic problems. However, it is difficult and expensive to test for iron levels with today's technology and is especially hard to do so in the field. *E. coli* bacteria already have the natural ability to sense and bind iron. By utilizing this natural pathway, we can reprogram the plasmid DNA of these bacteria to synthetically report the iron levels using a measurable fluorescent output. Our objective is to utilize synthetic biology to reprogram *E. coli* to serve as a robust reporter of iron.

Methodology and Approach

Design → Build → Test

We designed, built and tested three individual constructs before combining them into the final system.

1. **Plasmid 1 (pDW001)**: a circuit that has a reporter gene.
2. **Plasmid 2 (pDW003)**: a full inverter with known inputs and outputs.
3. **Plasmid 3 (pDW005)** : a full inverter with untested promoter and responds to the endogenous pathway of iron in the *E. coli* genome.

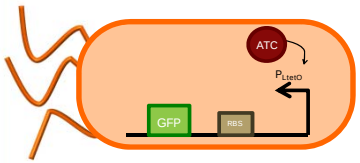


Results

Table 1. List of components with a short description.

Component		Description
PLteto	PLteto	A tetracycline operated promoter
ATC	ATC	A tetracycline analog
GFP	GFP	Green Florescent Protein
Ptrc2	Ptrc2	A lactose operated promoter
TetR	TetR	A repressor for tetracycline operated promoters
$P_{ACE}B$	$P_{ACE}B$	An iron operated promoter

Tetracycline Operated Fluorescent Plasmid (pDW001)



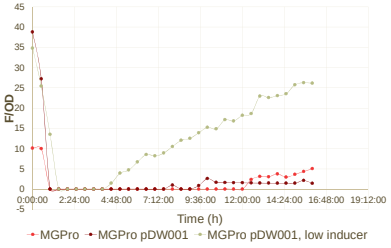
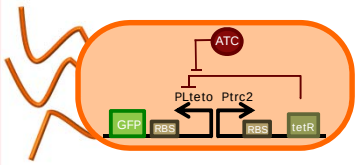


Figure 1. Florescence (GFP)/Optical Density versus time of the tetracycline activated plasmid. The curves describe the average florescence per cell, with and without inducer.

pDW001 was constructed by adding the florescence sequence into a plasmid with many multiple cloning sites. The sequence includes a tetracycline operated promoter driving a green florescent protein, thus, this plasmid expresses a green florescent output in the presence of tetracycline (in this case, ATC). The *E. coli* strain, MGPro, was successfully transformed with pDW001 and was able to grow and fluoresce in the presence of ATC.

Tetracycline Operated Inverter Plasmid (pDW003)



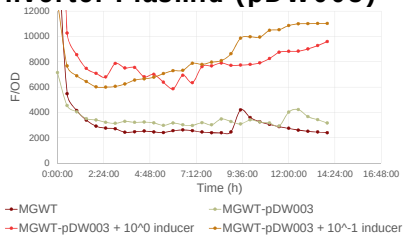
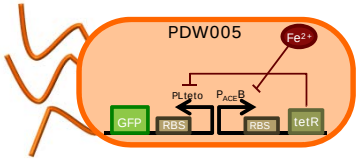


Figure 2. Florescence (GFP)/Optical Density versus time of the tetracycline operated inverter. The curves describe the average florescence per cell with and without inducer.

pDW003 was constructed by the addition of the pDW001 sequence into a multiple cloning site plasmid with a repression sequence using tetR to invert the signal. The *E. coli* wild type strain was successfully transformed with pDW003 and was able to grow and fluoresce in the presence of tetracycline. In the absence of tetracycline, no fluorescence occurred.

Iron Operated Inverter Plasmid (pDW005)



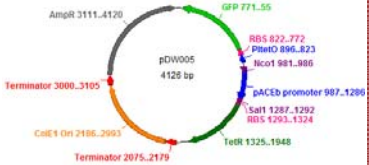


Figure 3. Final Plasmid Design. Schematic of pDW005 with all components.

pDW005 was constructed by replacing the tetracycline operated promoter (PLtetO) in pDW003 with an iron operated promoter (PACEB). The *E. coli* wild type strain was transformed with pDW005 and was able to grow and fluoresce in the presence of highly concentrated iron. Further testing is necessary to refine the sensitivity of the promoter to bind and respond to less concentrated amounts of iron.

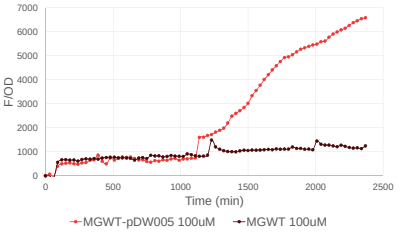


Figure 4. Florescence (GFP)/Optical Density versus time of the iron operated plasmid. The curves describe the average florescence per cell, with and without inducer.

Standards

iGEM Registry of Parts
A growing collection of standard genetic parts that can be mixed and matched to build synthetic biology devices and systems.

EPA 816-F-09-0004
National Secondary Drinking Water Regulations: guidelines regarding contaminants that may have cosmetic aesthetic effects in drinking water.

Outlook

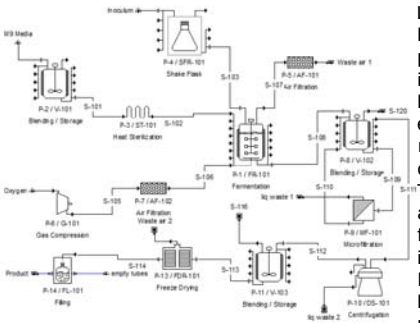


Figure 5. SuperPro Design. Process flow diagram for industrial processing.

Looking forward, the synthetic biology design team has proposed plans for a full scale industrial production of our biosensor. The genetically engineered bacteria would be mass produced using the unit operations shown in Figure 5. The bioreactor would be sold along with one package of freeze-dried bacteria to be installed underneath a household sink. Also, the bacteria would be available to purchase separately as refill packages.

Acknowledgements

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