The Beastly Yeast

Our Idea

Engineer yeast to be a sensor for arsenic in ground water samples. Then develop yeast to sequester the arsenic for removal from water.

The Problem

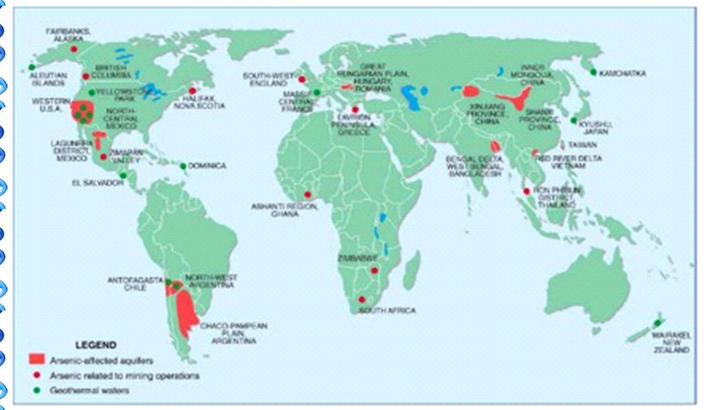
Arsenic is a human carcinogen ubiquitous in the environment. Millions of people worldwide are exposed to this metalloid primarily through drinking water from contaminated ground water. Chronic exposer to As is associated with several adverse effects on humans. These health risks range from skin pigmentation, nonmalignant pulmonary disease, cardiovascular disease and cancer of the skin, lung, bladder, liver, and kidney.

Arsenic can be found in the natural environment. Arsenic can bind to form organic arsenic molecules or inorganic arsenic molecules. The inorganic arsenic molecules tend to be more toxic. Inorganic arsenic has four main chemical valency. The two common forms are As III (arsenite) and As V (arsenate). Arsenite being the more toxic chemical.

A study in 2007 found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning of drinking water. [1]

The UN has estimated that around 1.4 million people are at risk from arsenic contamination in Nepal. [2]

Arsenic in the water posses an extreme health risk to the quality of human life. As seen in the map below, ground water contamination by arsenic is a global issue. In the United States ground water contamination has increased because of abandoned mines and the strict cleaning process for



Current testing technology

Chemical test that takes 12 minutes to complete. It involves multiple steps with different reagents. This testing kits tests for all forms of arsenic.

Current removal technology

Multi step filtration system can be used in the home.

For large quantities of water reverse osmosis can be used.

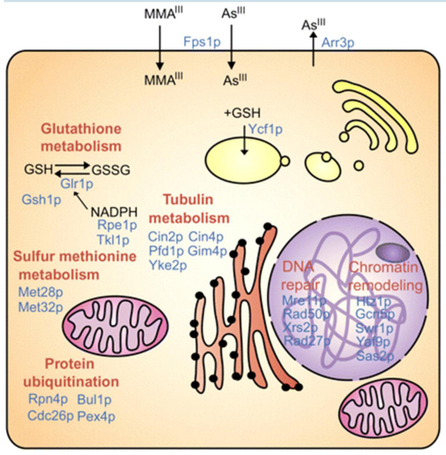
Why Yeast?

We chose yeast because yeast has several types of metal sensing genes. We also discovered that yeast has metal sensing genes that are similar to human genes. Arsenate and Arsenite travels into the yeast cell by using transport system for glucose and other small transport channels. Current research believes that arsenite uses polyol transporters and arsenate using hexose permeases to catalyze the majority of the transport into the cell [7].

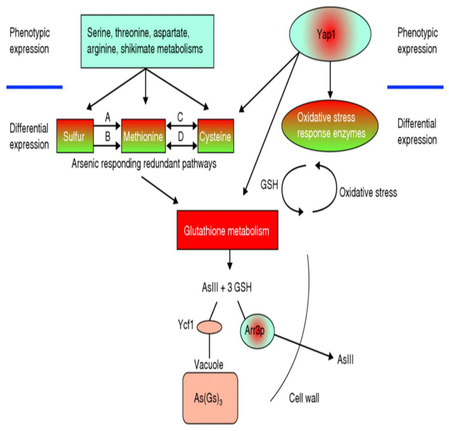
Since arsenates are used for chemotherapy a future project would be to develop a human capable biological system that could identify and sequester arsenate, arsenite, and MMA (monomethylarsonous acid). When humans are exposed to arsenite (AsIII) they methylate the arsenite to for the more toxic MMAIII.

The Gene

In our research we found a system of three genes Arr1, Arr2, and Arr3. Arr1 is a transcription factor for arsenic resistance. Arr2 is an arsenate reductase. Arr3 is the arsenite transporter. The diagrams below show the impact of the Arr series of genes and how they work in the yeast system. Arr1 and Yap1 are structurally similar. Arr3 promoter is turned on by Arr1. Research done on the Arr series by Bobrowicz identified that Arr3 is sensitive to arsenite and arsenate. We chose Arr3 promoter because of the sensitivity to both forms of inorganic arsenic [6], Arr3 has been shown to be turned on by Arr1 by plasmid[5], and the recommendation of our yeast mentor Dr. Goebl.



Toxicol. Sci. (2009) 111 (2): 424-436. doi: 10.1093/toxsci/kfp162
First published online: July 27, 2009



Haugen *et al. Genome Biology* 2004 **5**:R95 doi:10.1186/gb-2004-5-12-r95

Our Results

Step One:
Gel showed extraction and extension of the Arr3 with primers

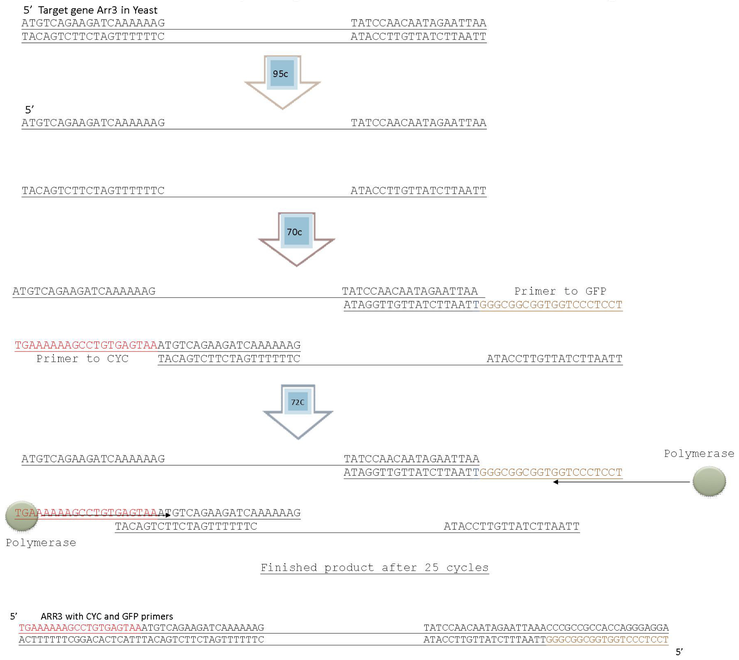
Step Two (iGEM submission):
Primers attached to Arr3 are the prefix and suffix enzyme code.
Gibson our promoter into the iGEM plasmid. Growth on the Amp plates.
Gel confirmed plasmid

Step Two (our machine):
Gibson our promoter, GFP, and plasmid. No growth on the Amp plates

Why no growth?
We believe that our primer for GFP to ADH was incorrect.

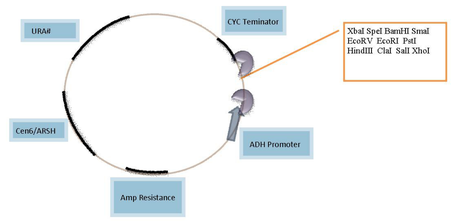
How we did it

Step One: Extraction from yeast genome and extension with Gibson primers



Step Two Gibson Assembly into Plasmid

Linear plasmid 416 with restriction enzyme cut, exposing CYC1 and ADH1



Parts for Gibson Assembly:

5′ ARR3 with CYC and YFP primers
TGAAAAAAGCCTGTGAGTAAATGTCAGAAGATCAAAAAAG TATCCAACAATAGAATTAAACCCGCCGCCACCAGGGAGGA
ACTTTTTTCGGACACTCATTTACAGTCTTCTAGTTTTTTC ATACCTTGTTATCTTTAATTGGGCGGCGGTGGTCCCTCCT

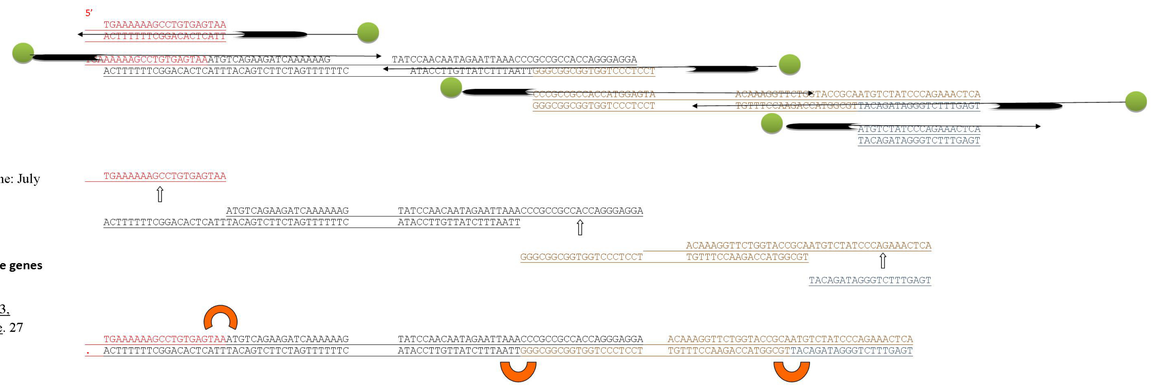
5′ YFP with ADH primer
CCCGCCGCCACCATGGAGTA ACAAAGGTTCTGGTACCGCATACAGATAGGGTCTTTGAGT
GGGCGGCGGTGGTCCCTCCT TGTTTCCAAGACCATGGCGTATGTCTATCCCAGAAACTCA

5′ ADH exposed end from plasmid
ATGTCTATCCCAGAAACTCA
TACAGATAGGGTCTTTGAGT

5′ CYC exposed end from plasmid
TGAAAAAAGCCTGTGAGTAA
ACTTTTTTCGGACACTCATT

Materials:

- Taq Polymerase 5′ to 3′ orientation
- Exonuclease 5′ to 3′ orientation
- Ligase



Transform plasmid into Ecoli (incubate for 24hrs on Amp LB and LB plates)

Transform Amp resistant Ecoli into Yeast after confirmation on gel for the plasmid (Incubate 2-3 days in Uracil + broth and YPB broth)

Citation

1. "Arsenic in drinking water seen as threat". Associated Press. 2007-08-30.
2. UNICEF (2006) Diluting the Pain of Arsenic Poisoning in Nepal
3. *Toxicol. Sci. (2009) 111 (2): 424-436. doi: 10.1093/toxsci/kfp162* First published online: July 27, 2009
4. Haugen *et al. Genome Biology* 2004 **5**:R95 doi:10.1186/gb-2004-5-12-r95
5. Wysocki R, Fortier PK, Maciaszczyk E, Thorsen M, Leduc A, Odhagen A, Owsianik G, Ulaszewski S, Ramotar D, Tamas MJ: **Transcriptional activation of metalloid tolerance genes in *Saccharomyces cerevisiae* requires the AP-1-like proteins Yap1p and Yap8p.**
6. Bobrowicz, Piotr, et al. Isoation of Three Continuous Genes, ACR1, ACR2, and ACR3, Involved in Resistance to Arsenic Compounds in the Yeast Saccharomyces cerevisiae. 27 Jan. 1997, John Wiley and Sons, Ltd. Print.
7. Liu, Z, Boles, E, & Rosen, B. (2004). Arsenic trioxide uptake by hexose permeases in saccharamyces cerevisiae. *The Journal of Biological Chemistry*, *17312-17318*. Retrieved from http://www.jbc.org/content/279/17/17312.full