
# Ivy Tech Undergraduate Research Fellowship

## 2012

## South Bend Campus

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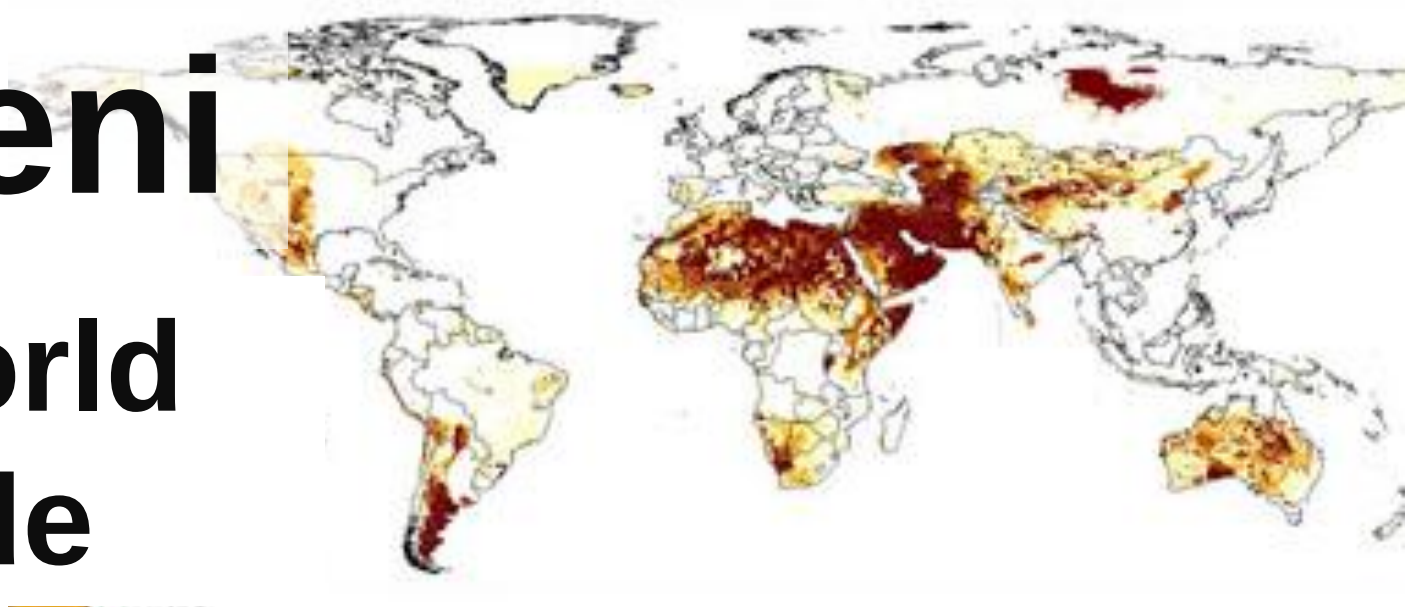
# Arseni

## A world wide problem



- Mortality rates appear  at levels above 150 ppb
- In Bangladesh of the 50,000 tubewells tested, 40% were too contaminated to drink
- Tubewells provide water to 90% of the population in Bangladesh
- In the US, over 20% of wells may contain levels that exceed established limits of 10ppb
- Low-level exposure to arsenic may increased the risk for more serious illness or death in response to infection from the H1N1 virus.

# Ramifications of Arsenic exposure

Arsenicosis is the effect of arsenic poisoning

Symptoms:

- skin problems such as color changes,
- hard patches on the palms and soles of the feet
- skin cancer
- cancers of the bladder, kidney and lung
- diseases of the blood vessels of the legs and feet,
- Diabetes
- high blood pressure
- reproductive disorders

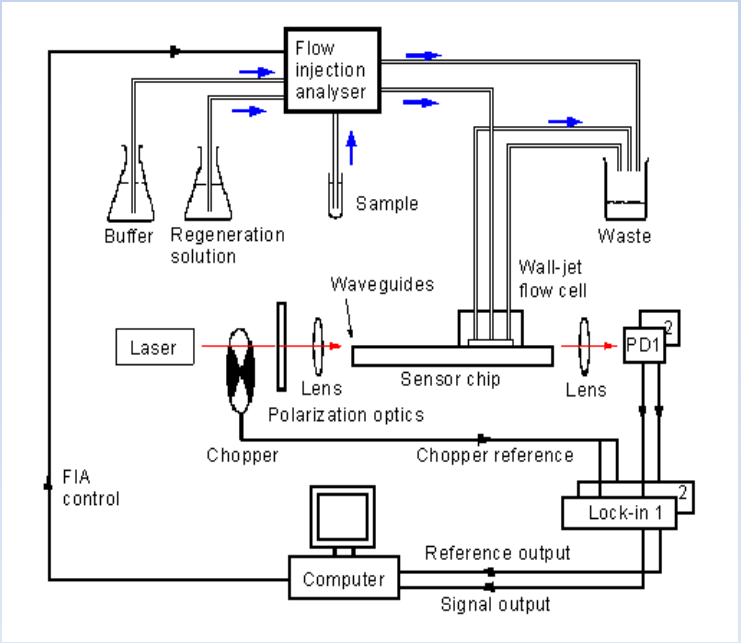
Approximately 1 in 100 people who drink water containing 0.05 mg arsenic per litre or more for a long period may eventually die from arsenic related cancers

# Solutions for prevention of arsenicosis include.

- Deeper wells are often less likely to be contaminated.
- Rain water harvesting in areas of high rainfall such as in Bangladesh.
- Use of arsenic removal systems in households (generally for shorter periods) and before water distribution in piped systems.
- Testing of water for levels of arsenic and informing users.

# Current detection methods available

Current devices available to detect for Arsenic can be accurate, however; the cost and technicality of these devises made them unavailable for use in poverty stricken countries and in remote areas.




# Synthetic Biology offers a simple, rapid detect alternative
# Inducible operons can be turned into biosensors

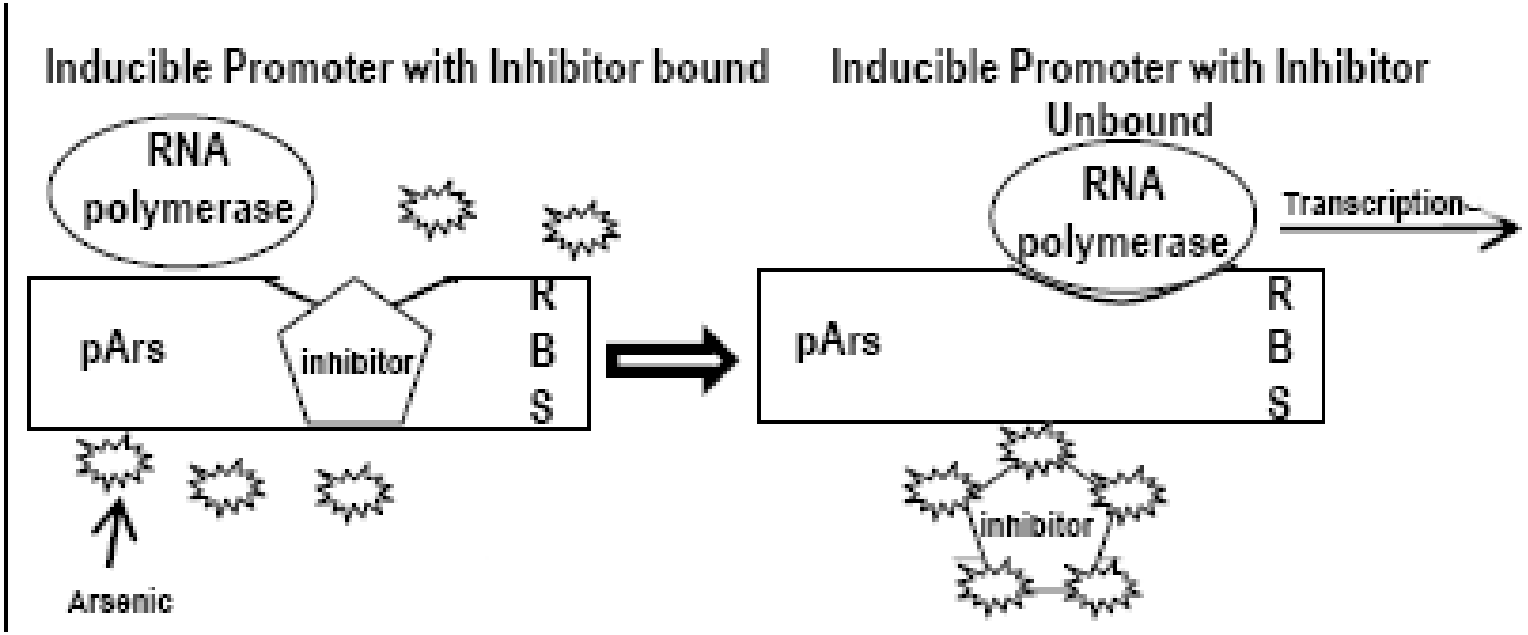
A number of arsenic detection or clearance devices have been engineered for the iGEM competition.

Some of the  major ones are:

Gronigen 2009
Edinburgh 2010
Brown 2007

# Function and Structure of ArsR Inhibitor

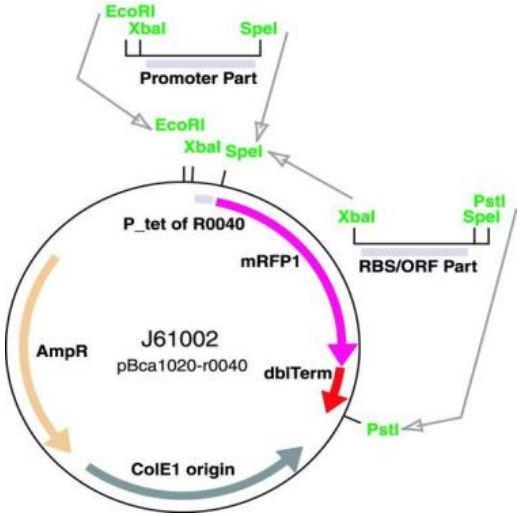
- ArsR negatively controls expression
- The binding of arsenic or antimonite to ArsR causes a conformational change in it, leading to dissociation from DNA and hence depression
- The structural and thermodynamic underpinnings of metalloregulation for any member of the ubiquitous ArsR family remains poorly understood due to a lack of detailed insight for the DNA operator-bound state

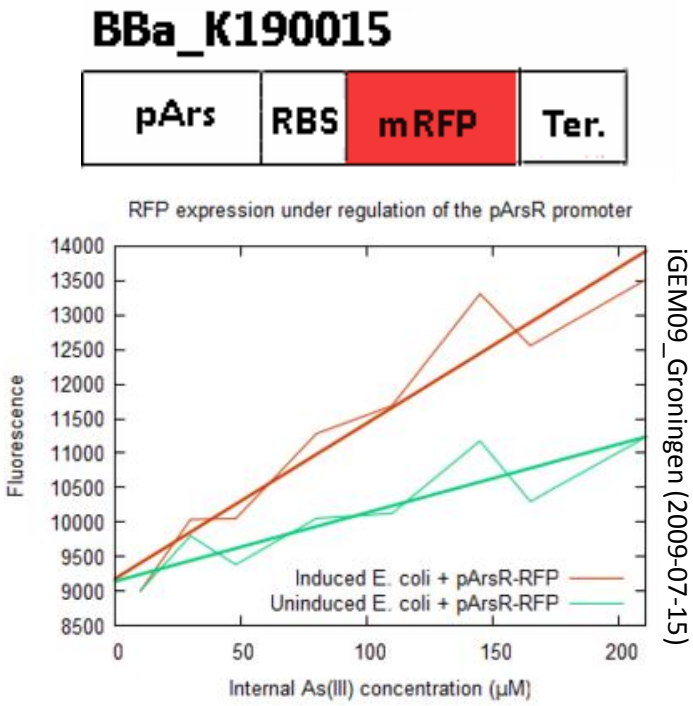


# pArs “Leakiness”

E. coli pArs as a basis of a biosensor is its high rate of basal transcription

2010 Gronigen Team’s BBa_K190015




Even without induction (exposure to arsenic) transformants of K190015 Are visibly “red” and the redness increases with time in culture.

An arsenic sensitive device based on the *E. coli* pArs will require calibration but as we propose: genetically tuning the expression system to raise the visual threshold of the response.

# Our Goal

To engineer a genetically tunable biosensor that has a low cost, simplified read out that can be taken in to remote areas, easily used and read without worry about shelf life or storage conditions.

**To counter Leakiness and raise the visual threshold of the pArs-RFP system:**

Site directed mutagenesis of the pArs between -35 and -11 to lower the affinity of the sigma subunit of RNA pol for the promoter region

Adding pArs binding sites to the intervening DNA between pArs and RFP gene

Increasing the expression of the ArsR inhibitor in the cell

# Our Hypothesis

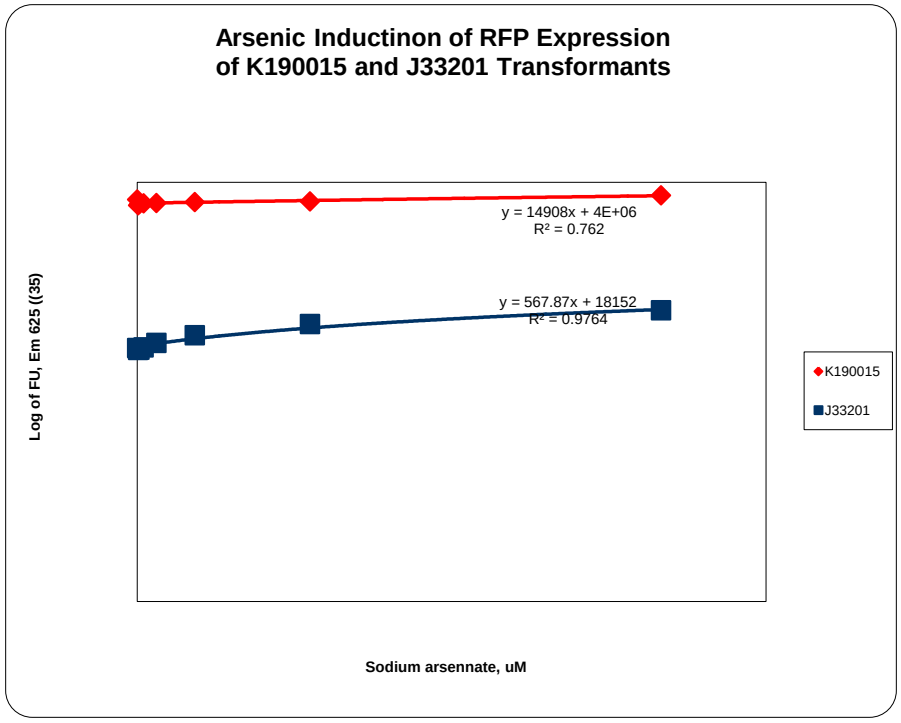
The high background of arsenic responsive element K190015 is based either on the low affinity of the ArsR inhibitor for the control region of the promoter or the Trace levels of arsenic (arsenite) in our water.

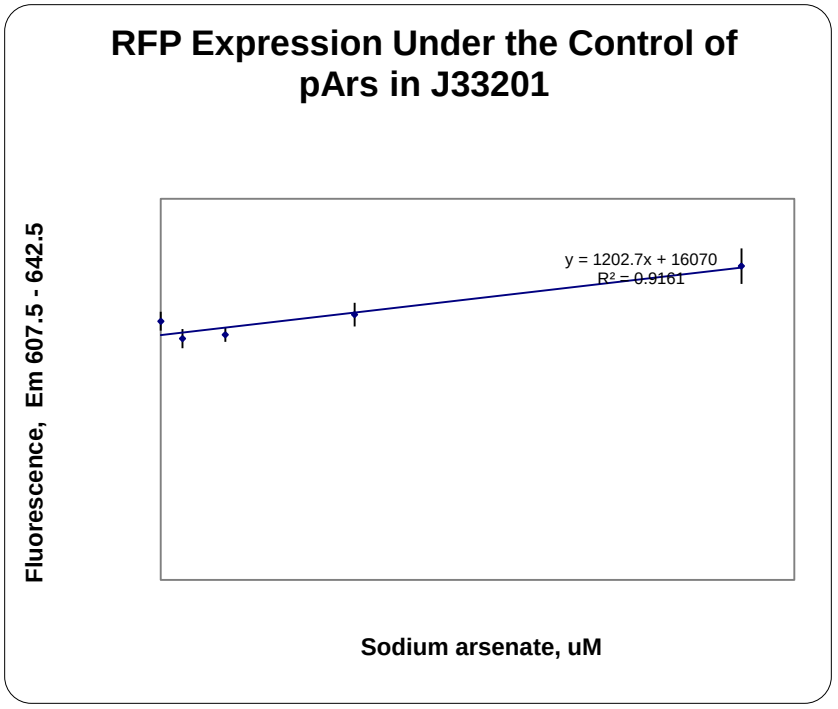
Increasing the gene quantity of the ArsR inhibitor will increase the threshold of the arsenic response element

# Increased expression of ArsR effect on the basal transcription rate of pArs

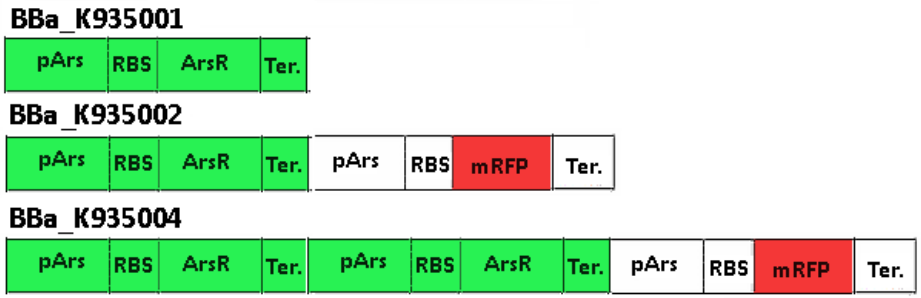
**BBa_K190015**

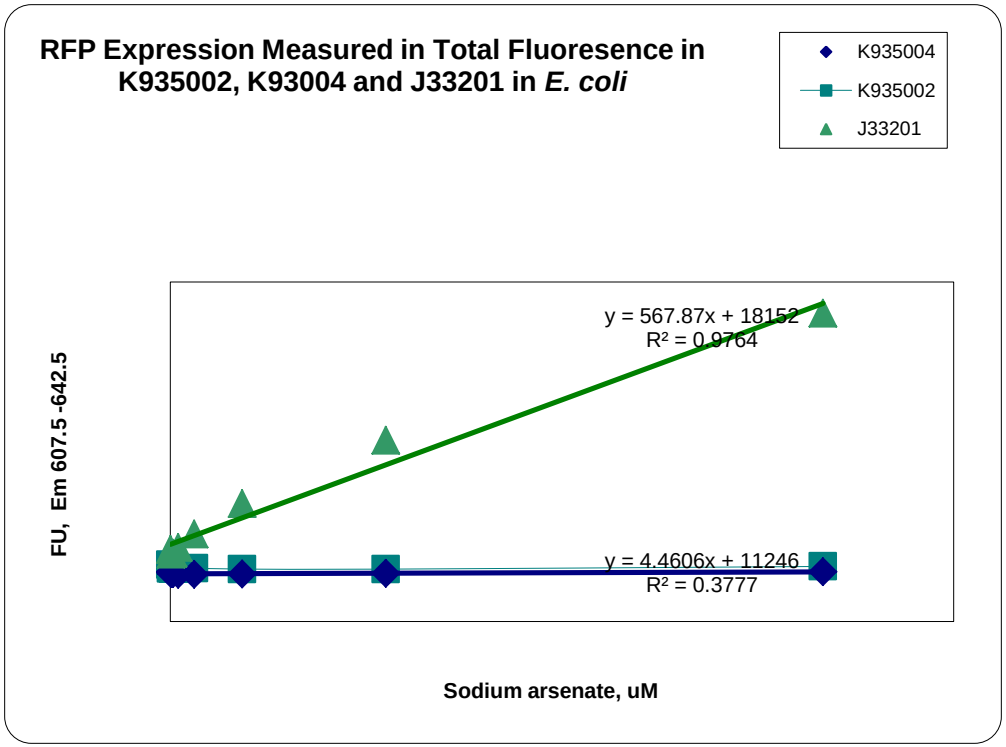

**BBa_J33201**

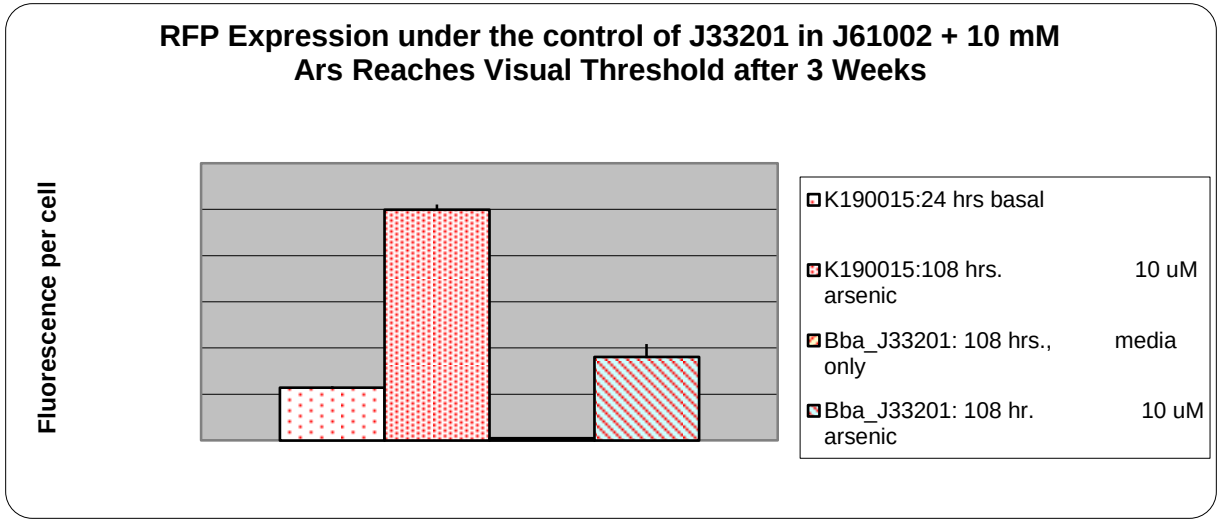



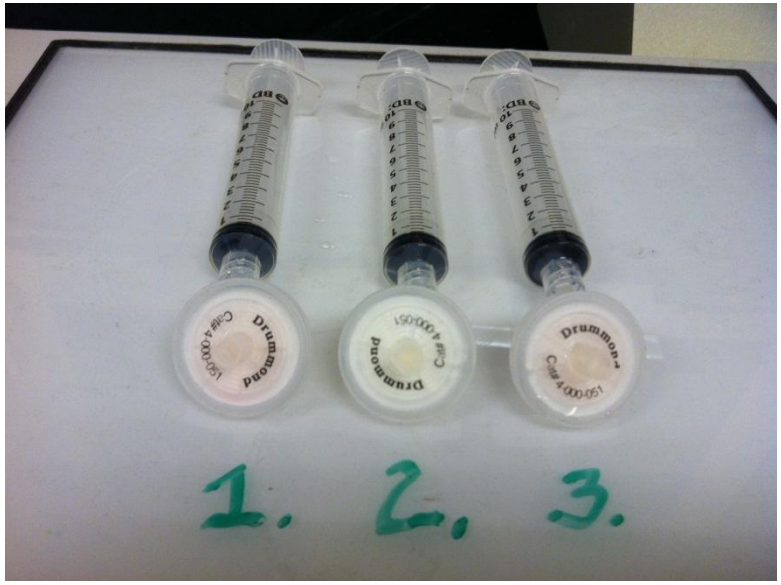
# Further Testing demonstrates the effect of increased ArsR expression on pArs Function






Prototype:
Syringe and
Filter Assembly




Simple procedure

1. Lyophilized GMO
2. Powdered media
3. Water drawn in
4. Incubation
5. Water expelled
6. Bacteria concentrated on the filter

Accomplishments

Working with the parts developed by the Gronigen 2009 and Edinburgh 2010 teams, we imporved on their parts and include our results in the parts experience pages.

We have been able to submit 4 parts into the registry

Through stimulation testing, we have characterized and developed sensitivity readouts

We have been able to show how the increased expression of the arsR inhibitor has raised the threshold of the reporter without decreasing the sensitivity.

# Our Future Direction…

To increase the reliability and safety of our device, the following features have also been taken into consideration

(1) Inclusion of a second test chamber with a transformant that makes a visual chromophore of a different color at the same kinetics as pArs-RFP detection system.

(2) A kill switch under the control of reporter in both the positive and test transformants that would result in the death of both AFTER the signals is produced.

# Conclusion

THE WORLD'S POOREST POPULATIONS ARE SUFFERING FROM ARSENIC POISONING, EVEN HERE IN THE UNITED STATES, THE PROBLEM PERSISTS AND PROVES TO HAVE A SIGNIGICANT IMPACT ON OUR HEALTH.  PROPER TESTING AND MANAGEMENT IS NESSESARY TO PREVENT FURTHER HEALTH PROBLEMS.

# Material Cited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