# Bacterial Fireworks: Oxygen-Sensing BioBrick Promoters

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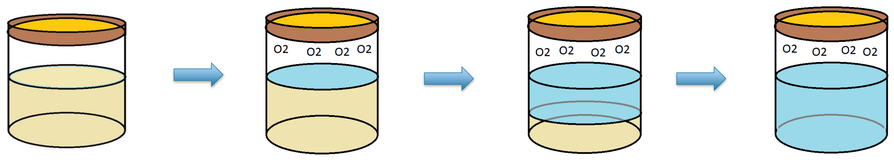
## Project Goals

The Penn State Team combined quorum sensing and oxygen sensing promoters to construct a bacterial fireworks genetic circuit. We characterized the transcriptional regulation of the FNR oxygen sensing protein, which can either repress or activate protein expression in the presence of oxygen. We then combined this oxygen sensing promoter with AHL sensing promoters in a positive and negative feedback loop system.

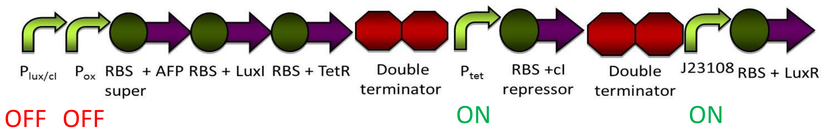



In the example above, bacteria lining a sealed jar will only begin to fluoresce when oxygen is present. After initial contact with oxygen, the exposed cells send a signal to the surrounding (non-oxygenated) cells through quorum sensing. This amplifies the fluorescence expression and results in a stronger, easier-to-detect signal.

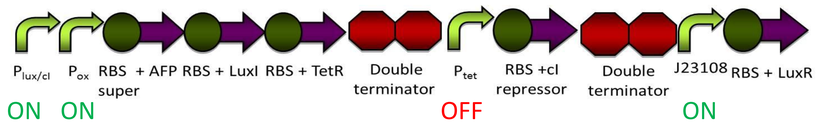
## Genetic Circuit

The reporter in this circuit is an anaerobic fluorescent protein controlled by an oxygen-sensing promoter and a lux promoter. The tet promoter initally turns the lux promoter off via the lambda repressor, and absence of oxygen keeps the oxygen promoter off. When oxygen is present, the oxygen-sensing promoter turns on and represses the Tet promoter and also induces the lux promoter by creating AHL via the LuxI protein. Anaerobic fluorescent protein is then expressed.

Anaerobic




Aerobic




## Oxygen Promoter

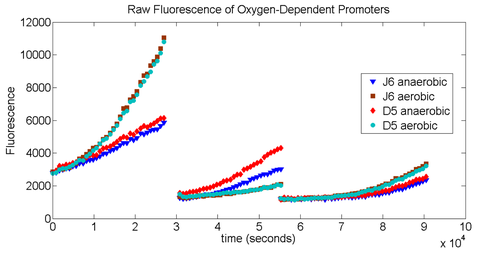
gaattcgcggccgcttctagagtataTTGATAAAGATCAACCGCTAA
ATATGCGTTTTGATGAAGATCAACTGATGGCTAGCTCAGTCC
TAGGGATTATGCTAGCtactagtagcggccgctgcag

FNR binding sites (2X)
α–CTD RNAP binding site from the dcuC promoter
synthetic promoter J23113 from parts registry

The design of the oxygen-sensing promoter was based on the dcuC gene of E. coli. The two binding sites for FNR surrounded the RNAP binding site and were systematically shifted upstream and downstream from this site in multiple constructs, causing overlap of the RNAP binding site in some cases. Using this strategy, five constructs were designed with the constitutive promoter J23113 as the basis for the sequence following the downstream FNR binding site. The construct that worked best as an oxygen-sensing promoter (J6, BBa_J376003) was fully characterized and is shown above.

## Results

1

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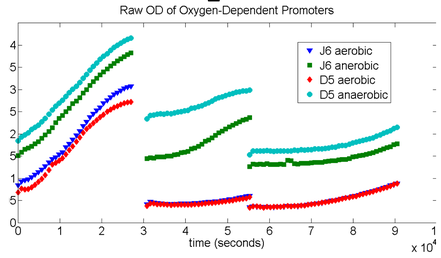


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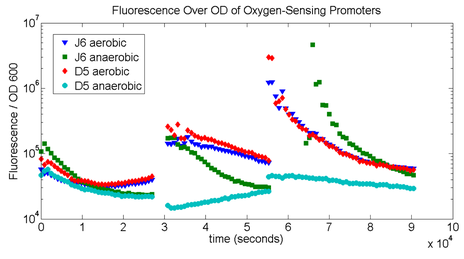


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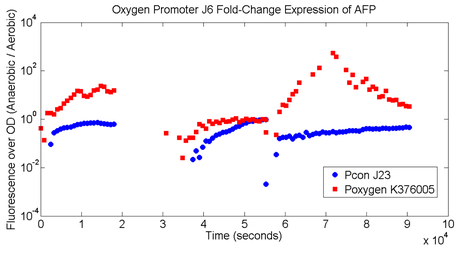


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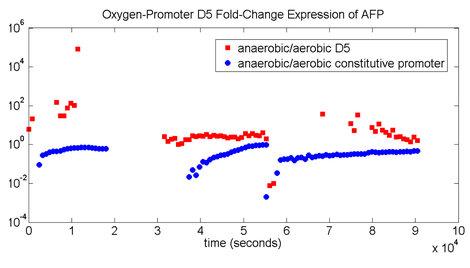
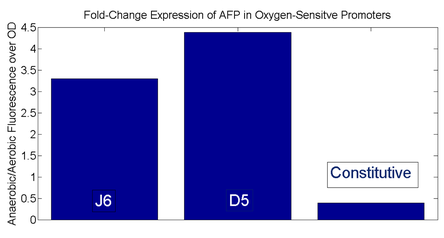



6




Graphs 1-3 show raw fluorescence, raw OD, and the fluorescence over OD for expression of a fluorescent protein controlled by oxygen-dependent promoters under both aerobic and anaerobic conditions. The fold change in expression when conditions were switched from aerobic to anaerobic was then graphed and is displayed in graphs 4-5. Finally, graph 6 shows that the oxygen-dependent promoters were indeed induced by anaerobic conditions, while the constitutive promoter was not.

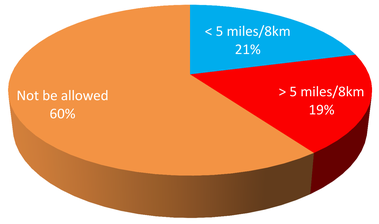
## Human Practices

A survey was distributed to 9 schools around the globe in order to receive a better understanding of the public's perception of Genetically Modified Organisms in different applications such as the use in biotechnology, agriculture, and the implementation of a cell lysis device. The results were analyzed by hemisphere. Significant results in the Northern Hemisphere indicate that people who:

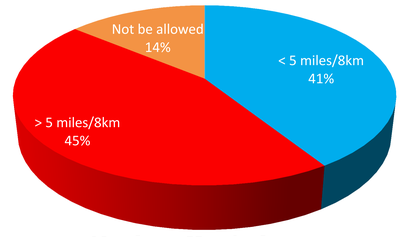
- trust their nation's policies and regulations are more likely to accept GMOs.
- trust scientists are more likely to accept GMOs.
- support the use of GMOs are more likely to support "cell-death" mechanisms.

Significant differences in opinion between the two hemispheres are shown below.

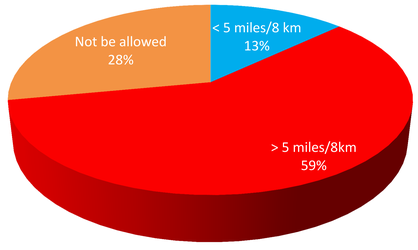
**Southern Hemisphere**
**How close would you be willing to live to a farm, field or stream where GMOs are used?**




**Northern Hemisphere**
**How close would you be willing to live to a farm, field or stream where GMOs are used?**




**Southern Hemisphere**
**How close would you be willing to live to a factory or laboratory where GMOs are used?**




**Northern Hemisphere**
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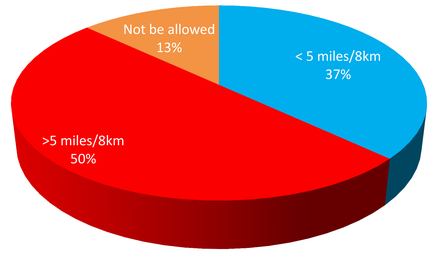


Human Practices Collaborators