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SYNTHETIC DNA PROMOTERS TO CONTROL GENE EXPRESSION BASED ON THE PRESENCE OF OXYGEN IN *E. COLI*

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ABSTRACT

A catalogue of synthetic σ70 promoters incorporating oxygen-sensitive transcription factors was designed, and the promoters were tested in *E. coli*. Two known oxygen-dependent transcription factors were utilized in the design of the synthetic promoters: fumarate-nitrate-reduction (FNR), and ArcA (1). The synthetic promoters were based on four distinct promoter templates: the dicarboxylate carrier (dcuC) gene that contains FNR naturally in *E. coli* (2), a synthetic promoter called J23113 from the Registry of Standard Biological Parts (3), the thiol peroxidase (tpx) gene that naturally contains ArcA in *E. coli* (4), and the tpx gene with a modified consensus Pribnow box at the -10 region of the σ70 promoter. The FNR promoters based on the natural dcuC gene were found to induce protein expression the most under anaerobic conditions; however an FNR promoter based on the entirely synthetic promoter J23113 was found to have a response within a similar range of values. The ArcA promoters based on the natural tpx gene were found to have a very low induction and even exhibited some repression in anaerobic conditions, while the ArcA promoters based on a tpx gene modified to incorporate the consensus Pribnow box at the -10 of the σ70 site were found to exhibit very low induction but no repression.

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Chapter 1

Introduction

Knowledge of the presence or absence of atmospheric oxygen is important to many industries. In particular, it is a crucial factor in many microbiological engineering applications, because microbes are often utilized to make useful products through their fermentation pathways, which require an absence of ambient oxygen. For example, the production of beer, wine, silage, yogurt, cheese, corn ethanol, leavened bread, sauerkraut, and other types of food and fuel involve a fermentation step. The products of certain microorganisms' fermentation activity, including ethanol and vinegar, are desired for their flavor, energy content, health benefits, and food preservation properties (5).

Microorganisms such as *E. coli* are able to turn off their fermentation pathways when oxygen is present and turn on those pathways when oxygen is absent (6). This allows the cell to be as efficient as possible with its resources and not waste resources on fermentation pathways unless the much-preferred aerobic respiration using the terminal electron acceptor, oxygen, is impossible.

Proteins called transcription factors regulate gene expression. Transcription factors bind to the DNA of the organism and either promote or prevent RNA polymerase from producing mRNA, necessary for protein formation. Late 20th century technological advances allowed for the discovery of the exact mode of gene expression control in response to changing environmental oxygen levels in *E. coli* (7).

It is the purpose of this project to create a new DNA sequence, called a promoter, which has an even stronger response to the existing transcription factors in order to better control oxygen-dependent gene expression. This would be useful as an added level of control for applications of microbial fermentation, where the goal is to maximize the fermentation product output from the microorganism and reduce the aerobic respiration that occurs.

Also, a strong oxygen-dependent promoter could be applied to microorganisms that are vegetative in the final agricultural or biological product, for example, yogurt. The promoter could be implemented in a system that caused the microbes to change color over time in the event that oxygen was present in the atmosphere. This would alert consumers that the airtight seal had been damaged before reaching its final destination, and the product could be contaminated.

Commercial applications aside, this project is important simply because regulation of cellular processes depending on the presence or absence of oxygen is a fundamental topic in biochemistry and molecular biology, and understanding what works and what does not work when attempting to create a synthetic gene to mirror natural phenomena can provide deeper insight into the workings of the cell.

Chapter 2

Materials and Methods

Two sets of promoters were designed based on known mechanisms for detecting oxygen in *E. coli*. One set of 9 promoters implemented the bacterial transcription factor fumarate-nitrate-reduction (FNR). The other set of 8 promoters was based on ArcA, a global regulatory gene in *E. coli* mediating repression of enzymes in aerobic pathways (1). In total, 17 unique promoters were synthesized, each utilizing one of the two oxygen-detection methods mentioned above.

The transcription factor FNR is present in cells in three forms – [4Fe-4S]FNR, [2Fe-2S]FNR, and apoFNR (8). Only the first form, which contains an iron-sulfur cluster, is active in gene regulation. In the presence of oxygen, [4Fe-4S]FNR loses part of its iron-sulfur cluster, and becomes inactive [2Fe-2S]FNR, causing genes deactivated by FNR to become active, and vice versa. As transcription of the FNR gene is negatively auto-regulated by FNR itself, [4Fe-4S]FNR is continually produced and destroyed under aerobic conditions until oxygen is no longer present, at which point normal anaerobic levels of [4Fe-4S]FNR are restored (9). By designing promoters that take advantage of the action of FNR, it was possible to test whether synthetic sequences were able to harness and even improve the ability of these transcription factors to switch genes "on" and "off" based on the ambient concentrations of oxygen to which the *E. coli* sample was exposed.

The FNR-based promoters created for this project can be separated into two classes. FNR promoters 1 through 5 are based on the C_4-dicarboxylate carrier (dcuC) promoter, which is naturally-occurring in *E. coli*. It has been modified to contain one or two consensus FNR sequences (2). FNR promoters 6 through 9 contain one or two consensus FNR promoters added to the synthetic constitutive promoter J23113 (3).

As described by Melville and Gunsalus, each FNR binding site is actually composed of two specific FNR152 recognition sequences, TTGAT and ATCAA, which are separated by four base pairs (10). The two FNR152 recognition sequences were incorporated in into four distinct FNR consensus sequences. Each of the 9 synthesized FNR-based promoters is shown in Table 2-1.

Table 2-1: The DNA Sequence of FNR Promoters 1-9 containing either one or two out of the consensus FNR binding sites

key	FNR Site 1 FNR Site 2 Segment of Synthetic Promoter J23113 Alpha subunit of dcuC (or portion thereof)
F1	ACACATGGATCCTATTATTTGATCTTTATCAACCGCTAAATATGCGTTTTGATCTTCATCAATAAAAA CAGCAACTTCAATGTCTTAATTCTAGAATAT
F2	ACACATGGATCCTATTATTTGATCTTTATCAACCGCTAAATATGCGTTTAATGACTACGACTTAAAA ACAGCAACTTCAATGTCTTAATTCTAGAATAT
F3	ACACATGGATCCTATTATTAATGACTACGACTCCGCTAAATATGCGTTTTGATCTTCATCAATAAAA ACAGCAACTTCAATGTCTTAATTCTAGAATAT
F4	ACACATGGATCCTATTATTTGATCTTTATCAACGCTAAATATGCGTTTTGATCTTCATCAATAAAAAA CAGCAACTTCAATGTCTTAATTCTAGAATAT
F5	ACACATGGATCCTATTATTTGATCTTTATCAACCGCTAAAATATGCGTTTTGATCTTCATCAATAAAA ACAGCAACTTCAATGTCTTAATTCTAGAATAT
F6	CCGCGGATCCTATATTGATAAAGATCAACCGCTAAATATGCGTTTTGATGAAGATCAACTGATGGCT AGCTCAGTCCTAGGGATTATGCTAGCTCTAGAATAT
F7	CCGCGGATCCtTATATTGATAAAGATCAACCGCTAAATATGCGTTGTTAGTATTATGGCCTGATGGCT AGCTCAGTCCTAGGGATTATGCTAGCTCTAGAATAT
F8	CCGCGGATCCTATAGTTAGTATTATGGCCCGCTAAATATGCGTTTTGATGAAGATCAACTGATGGCT AGCTCAGTCCTAGGGATTATGCTAGCTCTAGAATAT
F9	CCGCGGATCCTATATTGATAAAGATCAACCGCTAAATATGCGTTGATGAAGATCAACACTGATGGCT AGCTCAGTCCTAGGGATTATGCTAGCTCTAGAATAT

The ArcA transcription factor has been studied as far back as 1988 (4). Synthetic promoters incorporating the ArcA transcription factor in this project were created using the naturally-occurring tpx gene as a template (11). The eight promoter sequences use a

consensus binding sequence for ArcA and vary in number of binding sites and binding site locations with the goal of empirically finding a configuration that provides a high magnitude of induction or repression. Each ArcA promoter name contains a number from 1 to 8 followed by “n” or “c”. “N” stands for natural, meaning that the -35 and -10 motifs of the σ70 region of the promoter were identical to those of the native tpx sequence described by Lynch (4). The synthetic promoters denoted “c” have -10 and -35 sequences that have been modified, replacing the naturally-occurring motifs with consensus -10 and -35 sequences. ArcA promoters 1, 3, and 6 were based on the natural configuration. ArcA promoters 2, 4, 7, and 8 were based on the consensus sequence. Each promoter sequence is shown in Table 2-2.

Table 2-2: DNA Sequence of each of the ArcA promoters, containing either one, two or three ArcA consensus binding sites

	ArcA Site Consensus Pribnow Box Natural Pribnow Box
1 n	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGGATTAAGCGCAAATAGCGTTTGCTGTGTTAATTAAATGTTAAATAAACTAGGTGTGACGTTCTAGATATA
2 c	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGGATTAAGCGCAAATAGCTTGACATGTGTTAATTAAATGTTAATATAATTAGGTGTGACGTTCTAGATATA
3 n	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCGTTTGCTGTGTTAATTAAATGTTAAATAAACTAGGTGTGACGTTCTAGATATA
4 c	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCTTGACATGTGTTAATTAAATGTTAATATAATTAGGTGTGACGTTCTAGATATA
5 c	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCTTGACACGAATCGAGGATCGGCAAGTATAATTAGGTGTGACGTTCTAGATATA
6 n	ATATGGATCCCTTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCGTTTGCCGAATCGAGGATCGGCAAGATAAACTAGGTGTGACGTTCTAGATATA
7 c	ATATGGATCCATGGTGTTAATTAAATGTTACTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCTTGACACGAATCGAGGATCGGCAAGTATAATTAGGTGTGACGTTCTAGATATA
8 c	ATATGGATCCATGGTGTTAATTAAATGTTACTTACTCCTTGTTGTCGCGGTGGTCTGCGTTAATTAAATGTTAAGCTTGACATGTGTTAATTAAATGTTAATATAATTAGGTGTGACGTTCTAGATATA

The promoters were created by PCR assembly of 4 oligonucleotide fragments as shown in Figure 2-1. The fragments were then inserted into a modified version of the flexible test vector (FTV). The default reporter protein in pFTV is red fluorescent protein. However, due to the fact that red fluorescent protein does not express adequately in anaerobic conditions, an alternative fluorescent protein was substituted into pFTV via a restriction digest and ligation. This so-called anaerobic fluorescent protein (AFP) was described by Drepper et. al in 2007 and serves the purpose of expressing in both aerobic and anaerobic conditions (12). The new vector containing the sample promoter was then purified and then transformed into *E. coli* by heat shock. The transformed *E. coli* were plated and incubated overnight at 37° C.

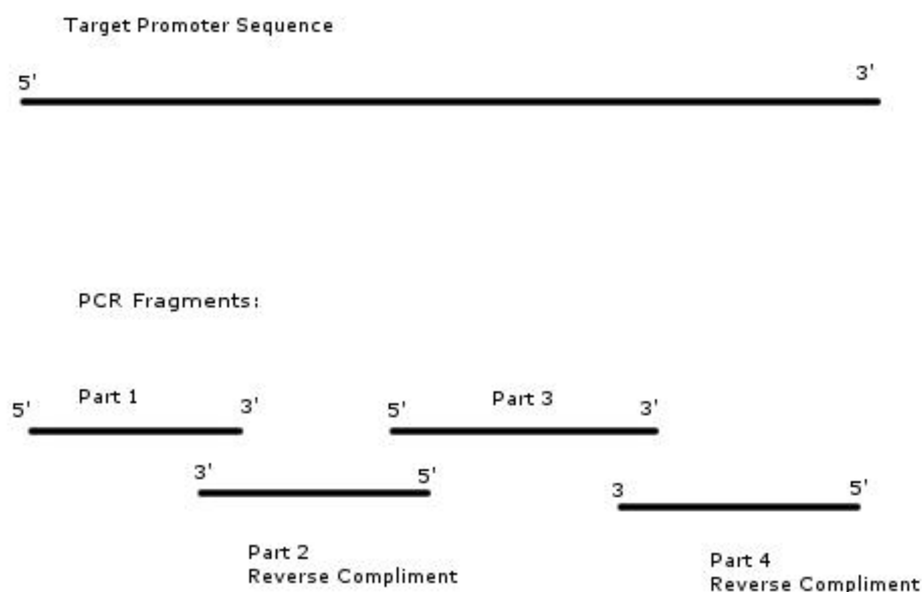

Figure 2-1: A diagram of the PCR assembly of each promoter using 4 separate oligonucleotide templates

Following overnight incubation, colonies were chosen from each plate and inoculated into 10 mL of LB for overnight growth at 37° C while shaking. A portion of the media from each sample was then used to create purified, highly-concentrated DNA in DI, distilled water, which was sequenced to ensure that each sample contained the promoter sequence expected. The rest of the media was used to make a pellet of vegetative E. coli which was mixed with a 50% glyrcerol solution and stored at -80^{0} C.

In preparation for experiments with the TECAN spectrophotometer, the samples were removed from the freezer and used to streak fresh plates of LB agar in order to isolate single colonies of *E. coli* containing the test sample. The plates were incubated overnight. The colonies were used to inoculate wells of a 96-well deep well plate, which was incubated at 37° C and shaken for 10 hours. Samples were then transmitted from the deep well plate to a shallower microtiter plate which was analyzed by the spectrophotometer. Each sample was duplicated in the microtiter plate. The first sample was grown in a well exposed to the atmosphere while shaking, and the other identical sample was covered with 100 µL of mineral oil. When oil was added to the microtiter plates, it did not mix with the media, but formed a layer on top of the media in each well. The oil layer was meant to prevent atmospheric oxygen from being directly exposed to atmospheric oxygen.

Although the tests of the duplicate samples (one with oil, one without) were run in parallel during the same experiment, it was meant the effect one sample sample would elicit when switching one sample from an oxygen-rich environment to an environment devoid of oxygen or vice-versa. In this thesis, the phrase “changed from an aerobic to an

anaerobic environment" will actually mean comparing the results from the two identical constructs, one tested aerobically and the other tested anaerobically.

Chapter 3

Results and Discussion

The efficacy of the oil in creating an anaerobic environment for the microorganisms was explored both theoretically and empirically. A theoretical analysis of oxygen diffusion through the mineral oil was performed. It was assumed that mixing of the oil with the media was minimal. The diameter of each well in the 96-well plate is known to be 6.96 mm (13). Since 100 µL of mineral oil was used, the thickness of the mineral oil layer could be calculated assuming each well in the microtiter plate is a perfect cylinder.

$$V_{well} = \frac{\pi D^2}{4} * L \qquad \text{Eqn. (1)}$$

From this equation, the thickness of the mineral oil layer was calculated to be 2.63 mm. Existing literature indicates that this amount of oil adequately prevents most ambient oxygen from contacting the media below the oil layer (14), (15). However, for the experiments in this study, efforts were not taken to completely remove all dissolved oxygen from either the media or the mineral oil. Moreover, the diffusivity of oxygen into mineral oil, while low, is not zero. Therefore, it is assumed that the microorganisms in the wells under the mineral oil layers did have access to some oxygen, but it was much less than their optimal uptake rate.

In this thesis, when the term anaerobic is used, it is meant to stand for a micro-aerobic environment in which the oxygen concentration is nearly zero. Because both the

FNR and ArcA transcription factors are active both in anaerobic and micro-aerobic conditions, the effects observed in either case would likely be similar.

The optical density (O.D.) and fluorescence of the samples was recorded at constant intervals. O.D., or absorbance, is a measurement performed by a spectrophotometer that is proportional to cell biomass concentration. For each experiment, a total of four plates were serially diluted with inoculations coming from the previous plate with the purpose of keeping the cells in exponential growth phase. Figure 3-1 and Figure 3-2 depict graphs of O.D. vs. time and fluorescence vs. time for one of the synthetic FNR promoters tested in an anaerobic environment, F3. The jumps in each curve correspond with times when one plate was replaced with a second plate containing a diluted sample from the first plate.

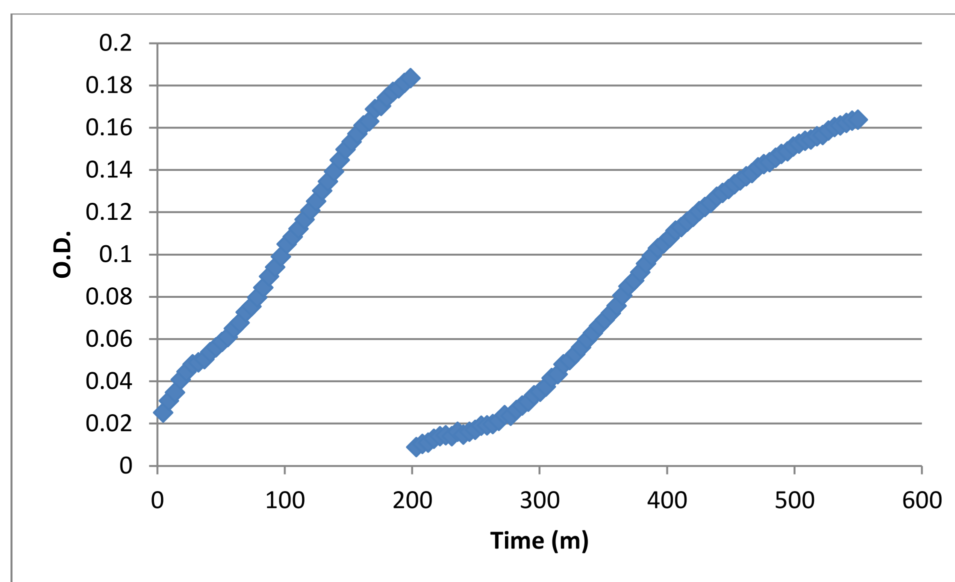

Figure 3-1: O.D. vs. time for FNR promoter F3 in anaerobic environment

In Figure 3-1 the sample in the first plate can be observed to be growing in the exponential phase for almost the entire duration of the time that the plate was in the spectrophotometer. However, towards the end of the first plate's run, the slope of the curve becomes less steep, indicating that it was beginning to reach the stationary phase. This is in corroborated by the fact that the microbial growth corresponding to the second plate in Figure 3-1 begins in lag phase and is not in exponential phase throughout the experiment as in the first case. This is not ideal. The purpose of the serial dilutions is to keep the cell in exponential growth for as long as possible in order to reach a steady-state quantity of fluorescence over O.D. The data in this thesis was selected to reflect steady-state quantities such as that observed in plate one of Figure 3-1 and not that of plate two.

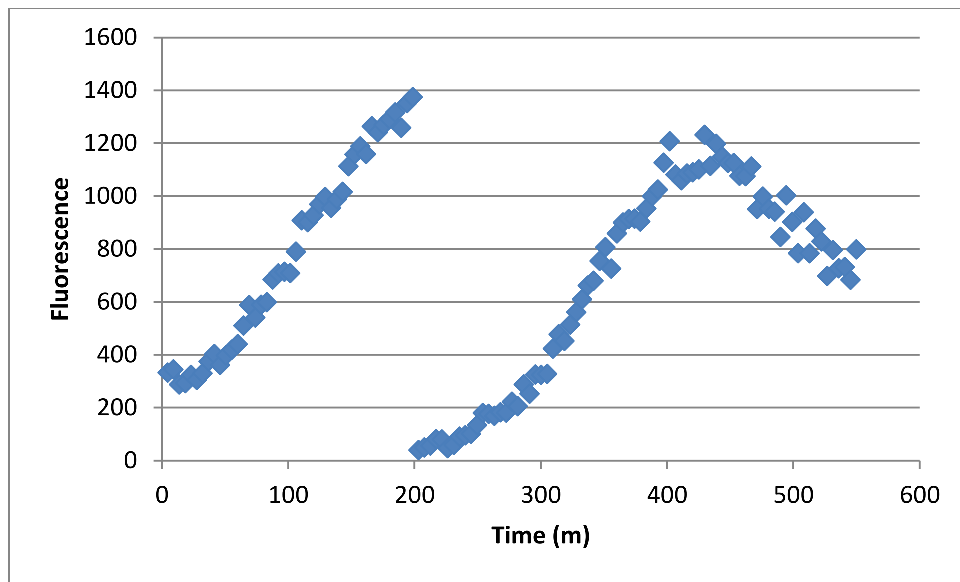

Figure 3-2: Fluorescence vs. time for FNR promoter F3 in anaerobic environment

The effect of the microbial growth going to stationary phase at the end of plate one and lag phase at the beginning of plate two for this example scenario is also reflected in the fluorescence recorded in Figure 3-2. While the fluorescence mirrors the microbial growth for plate one, the fluorescence actually decreases when the cells reach stationary phase at the end of plate two's run.

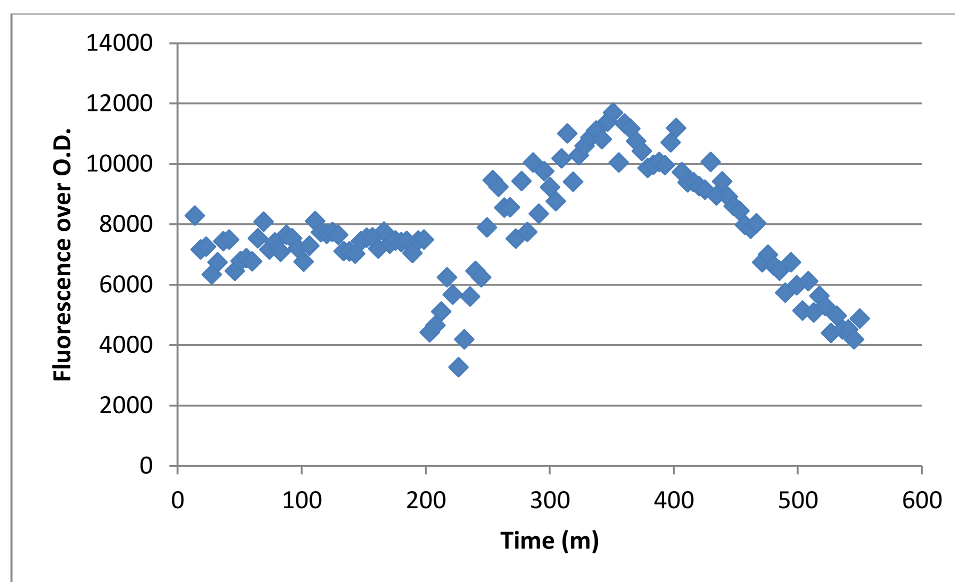

Figure 3-3: Fluorescence over O.D. vs. time for FNR promoter F3 in anaerobic environment

The results in Figure 3-1 and Figure 3-2 can be combined visually into one graph, Figure 3-3, fluorescence over O.D. vs. time for an FNR promoter. The fluorescence over O.D. is directly proportional to fluorescence per cell. It can be observed that, for plate one, which was in exponential growth for almost the entire duration of the experiment, the fluorescence per O.D. is relatively constant. This is a precise steady-state value. The results for plate two, however, rise and fall over time because it reflects the sample going from lag phase to exponential phase to stationary phase. For this thesis, the steady-state quantities were most valuable in order to eventually attain a single value for each promoter's steady-state fluorescence per cell in aerobic and anaerobic conditions.

From the raw data, an average fluorescence per O.D. during steady-state was calculated, and the results were compared to a constitutive promoter that, as a control, contained neither the FNR nor the ArcA transcription factors in its FTV promoter. The

exact formula used to determine the steady-state fluorescence per O.D. (proportional to fluorescence per cell) is Eqn. 2.

$$Sample\ Fluor.per\ O.D. =$$

$$\frac{Sample\ Fluor. - Blank\ Fluor.}{Sample\ O.D. - Blank\ O.D.} - \frac{DH10B\ Fluor. - Blank\ Fluor.}{DH10B\ O.D. - Blank\ O.D.} \qquad \text{Eqn. (2)}$$

The blank fluorescence and blank O.D. are measurements taken from wells in a similar location on the 96-well plate to the test samples, but containing only media and no *E. coli* cultures. This accounts for and negates the background fluorescence and O.D. detected by the spectrophotometer in the absence of any microbial growth.

The DH10B fluorescence and DH10B fluorescence measurements come from wells inoculated with the same volume of cells as the test cultures, but containing cultures from the common DH10B strain without a synthetic promoter. This accounts for and negates the auto-fluorescence of the bacterial cells themselves in the absence test promoter sequences.

A graph of the steady-state fluorescence over O.D. was created for each sample containing one of the synthetic promoters designed for this project, an example of which is shown in Figure 3-4. The first two bars in each graph depict the response of the constitutive promoter, J23100, which was used as a positive control. In each experiment, constitutive promoter J23100 expressed more fluorescence than the test promoter during aerobic conditions. The amount of fluorescence produced by J23100 dropped dramatically in the samples that had mineral oil added to the top of the well. The fluorescence expression changed from 33,335 units of fluorescence /O.D. in aerobic

conditions to 5893 units of fluorescence/O.D. in anaerobic conditions, a decrease of 82.33%.

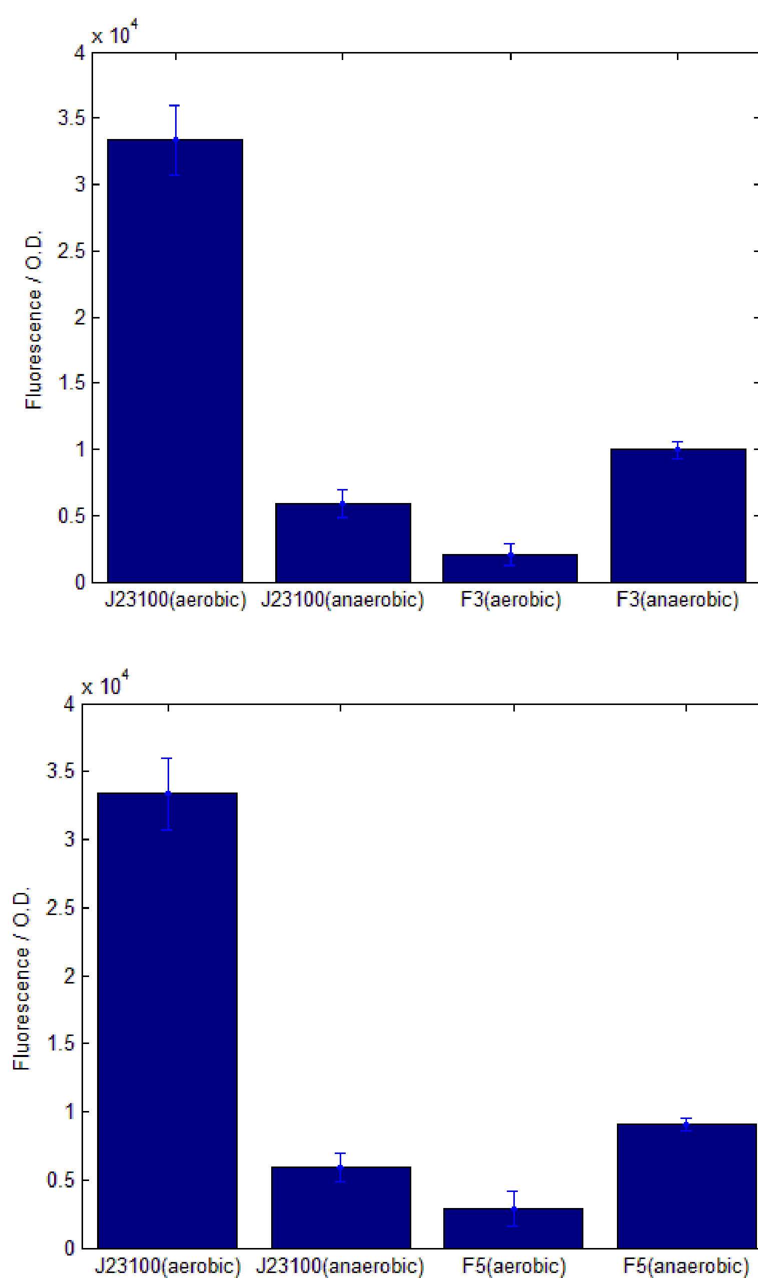

Figure 3-4: The fluorescence per O.D. for FNR promoters F3 and F5 in aerobic and anaerobic conditions compared to a J23100, the control promoter

In contrast, test promoters exhibited a range of responses to the addition of oil to the sample. Some samples responded by producing more fluorescence, some produced less, and some produced roughly the same. The anaerobic environment created in the media below the oil film was likely the cause for these changes.

Most notably, expression of fluorescence increased significantly when conditions were changed from aerobic to anaerobic in samples controlled by promoters F3 and F5, shown in Figure 3-4. More specifically, fluorescence expression in the sample controlled by promoter F3 was 2041 units/O.D. during aerobic conditions and 9948 units/O.D. during anaerobic conditions, an increase of 387.5%. Similarly, fluorescence expression in the sample controlled by promoter F5 was 2862 units fluor./O.D. during aerobic conditions and 9074 units fluor./O.D. during anaerobic conditions, an increase of 217.0%.

The full results showing the percent increase in fluorescence when comparing the anaerobic samples to the aerobic samples in shown in Table 3-1. Note that a positive value means the anaerobic sample had more fluorescence per cell than the aerobic sample for the corresponding promoter. A negative value means the anaerobic sample had less fluorescence per cell than the aerobic sample for the corresponding promoter. Similarly, a promoter is said to induce protein expression under anaerobic conditions if the percent increase in protein expression is positive when going from aerobic to anaerobic conditions. A promoter is said to repress protein expression under anaerobic conditions if the percent increase in protein expression is negative when going from aerobic to anaerobic conditions.

Table 3-1: Each FNR and ArcA Promoter shown in order of percent increase in fluorescence per cell when changing the environment from aerobic to anaerobic

Promoter	Percent Increase When Anaerobic	O2-Dependent Transcription Factor	Promoter Modified
F3	387.52	FNR	dcuC
F5	217.01	FNR	dcuC
F7	89.45	FNR	J23113
F2	68.52	FNR	dcuC
F1	41.70	FNR	dcuC
A5	40.05	ArcA	tpx-consen
F4	-18.58	FNR	dcuC
A8	-24.62	ArcA	tpx-consen
F6	-34.71	FNR	J23113
F8	-61.04	FNR	J23113
A2	-73.99	ArcA	tpx-consen
A3	-76.68	ArcA	tpx-nat
J23100	-82.32	None	J23100
F9	-86.26	FNR	J23113
A1	-100.65	ArcA	tpx-nat
A6	-105.14	ArcA	tpx-nat

It can be seen that all five of the promoters that experienced the largest increase in fluorescence per cell when going from aerobic to anaerobic conditions were FNR-based promoters. Of those five, four were based on the dcuC gene. However, it is notable that one FNR promoter, F7, was based on the synthetic promoter J23113 and still had the third-highest positive response to changing oxygen condition from aerobic to anaerobic. This is evidence that an entirely synthetic promoter implementing the FNR transcription factor can produce a response to changing oxygen levels that is in the same range of values as those of its natural counterparts.

The promoters based on the ArcA transcription factor and tpx gene recorded much lower change in fluorescence per cell overall when going from aerobic to anaerobic conditions. Interestingly, all of the tpx-based promoters with the -35 to -10 Pribnow box

that had been modified with the consensus Pribnow sequence reported a higher positive response to the oxygen change than did the tpx-based promoters containing their original, natural -10 sequence. That is, the percent change in fluorescence per cell going from aerobic to anaerobic in the ArcA-tpx promoters with consensus -10 sequences was 40.0%, -24.6%, and -74.0%. Meanwhile, the percent change in fluorescence per cell going from aerobic to anaerobic in the ArcA-tpx promoters with the natural, original -10 sequences was much lower at -76.7%, -100.6%, and -105.1%.

It is expected that the percent increase for the natural tpx promoters was negative when going from aerobic to anaerobic conditions, because the ArcA-tpx gene is known to repress protein expression when oxygen is absent. However, it is not clear why simply changing the -10 sequence of the Pribnow box to a consensus sequence would cause the promoters in some case to induce instead of repress under anaerobic conditions.

Chapter 4

Conclusion

A catalogue of *E. coli* σ70 synthetic promoters incorporating oxygen-sensitive transcription factors was designed, and the promoters were tested. Two known oxygen-dependent transcription factors were utilized in the design of the synthetic promoters: FNR, and ArcA. The synthetic promoters were based on four distinct templates: The dcuC gene that contains FNR naturally in *E. coli*, a synthetic promoter called J23113 from the Registry of Standard Biological Parts, the tpx gene that naturally contains ArcA in *E. coli*, and the tpx gene with a modified consensus Pribnow box at the -10 of the σ70 site.

When the reporter protein fluorescence of each sample was detected using a spectrophotometer for both aerobic and anaerobic conditions, a determination could be made as to how well the promoter was at regulating gene expression when oxygen conditions changed. Of the 15 synthetic oxygen-dependent promoters tested, 12 increased fluorescence per cell when going from aerobic to anaerobic conditions compared to the control, which was a constitutive “always-on” promoter.

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Appendix A

Figures Excluded from Results and Discussion

The Results and Discussion section provided a selection of the total promoter data in the form of fluorescence per O.D. in anaerobic and aerobic conditions for two of the promoters tested. In actuality, a total of 16 promoters were created and tested using the methods and analyses described in the preceding sections of this thesis. The results for all 16 synthetic promoters are shown in this section.

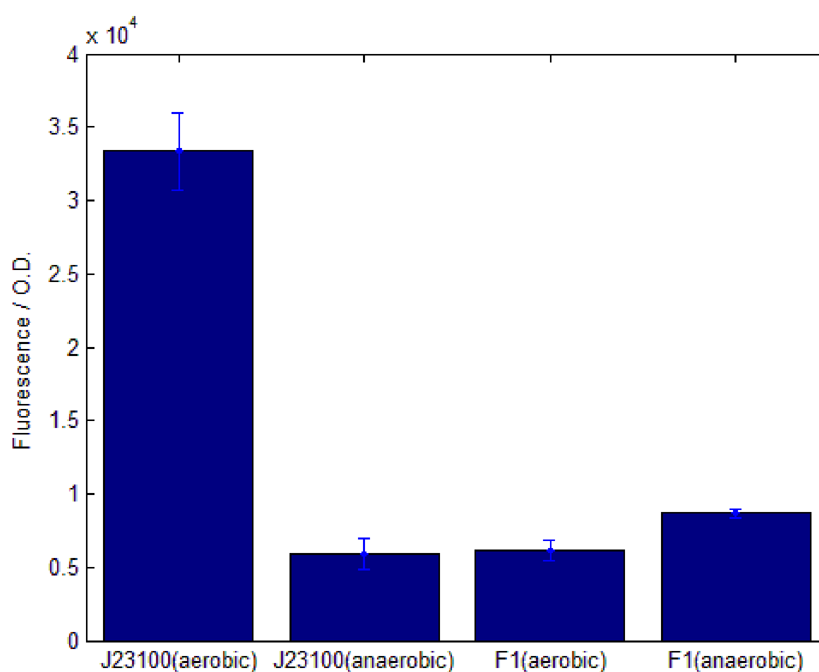

Figure A-1: The fluorescence per O.D. for FNR promoter F1 in aerobic and anaerobic conditions compared to a J23100, the control promoter

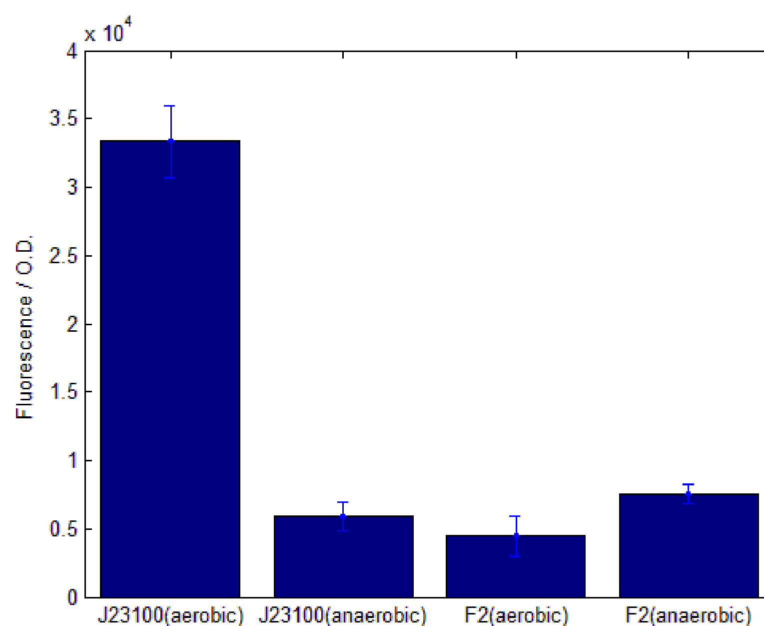

Figure A-2: The fluorescence per O.D. for FNR promoter F2 in aerobic and anaerobic conditions compared to a J23100, the control promoter

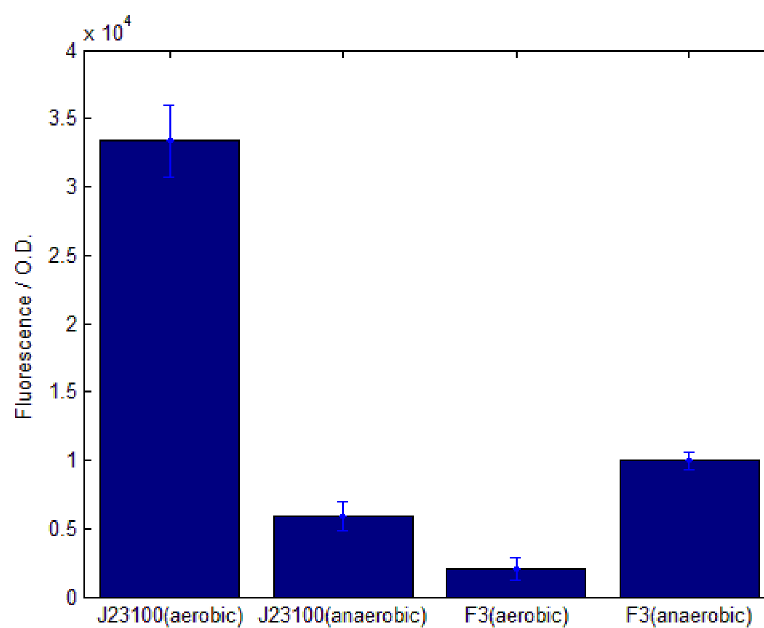

Figure A-3: The fluorescence per O.D. for FNR promoter F3 in aerobic and anaerobic conditions compared to a J23100, the control promoter

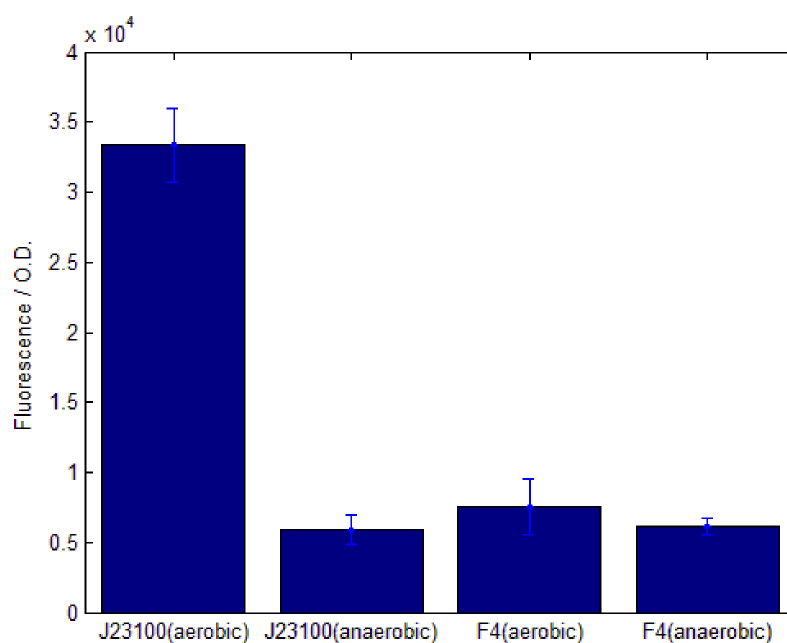

Figure A-4: The fluorescence per O.D. for FNR promoter F4 in aerobic and anaerobic conditions compared to a J23100, the control promoter

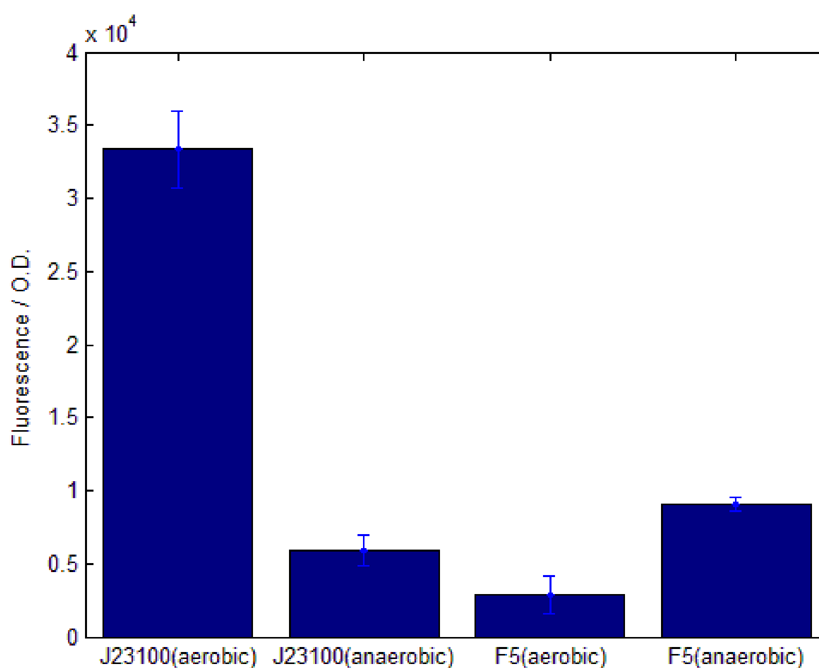

Figure A-5: The fluorescence per O.D. for FNR promoter F5 in aerobic and anaerobic conditions compared to a J23100, the control promoter

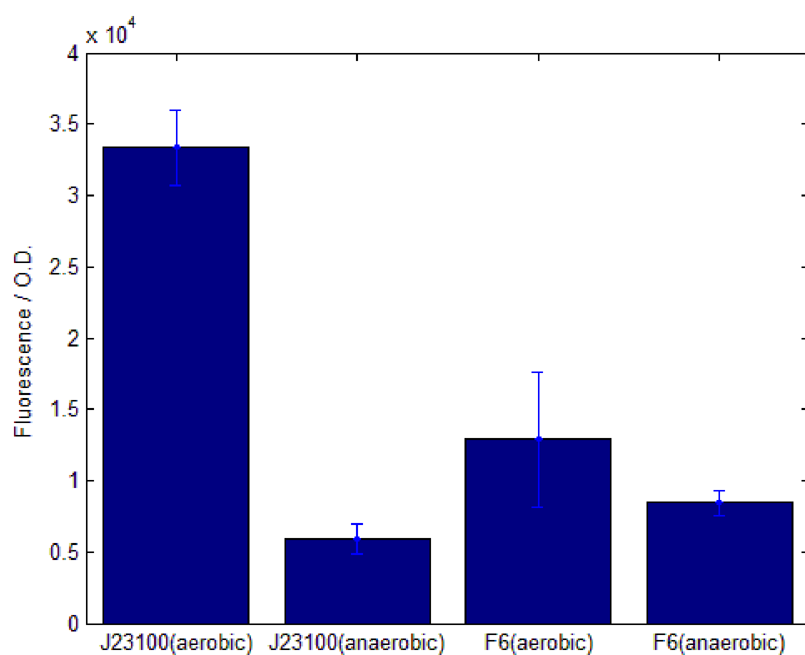

Figure A-6: The fluorescence per O.D. for FNR promoter F6 in aerobic and anaerobic conditions compared to a J23100, the control promoter

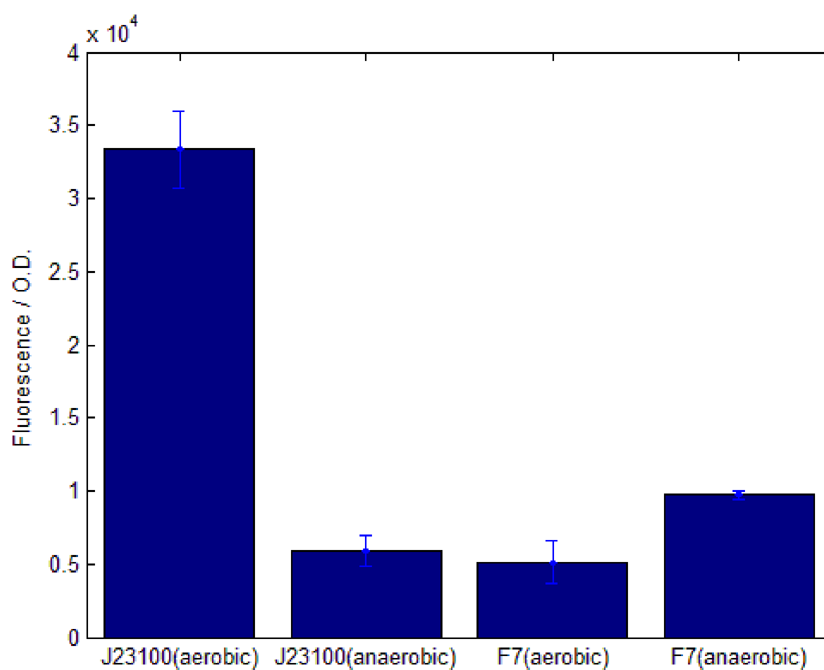

Figure A-7: The fluorescence per O.D. for FNR promoter F7 in aerobic and anaerobic conditions compared to a J23100, the control promoter

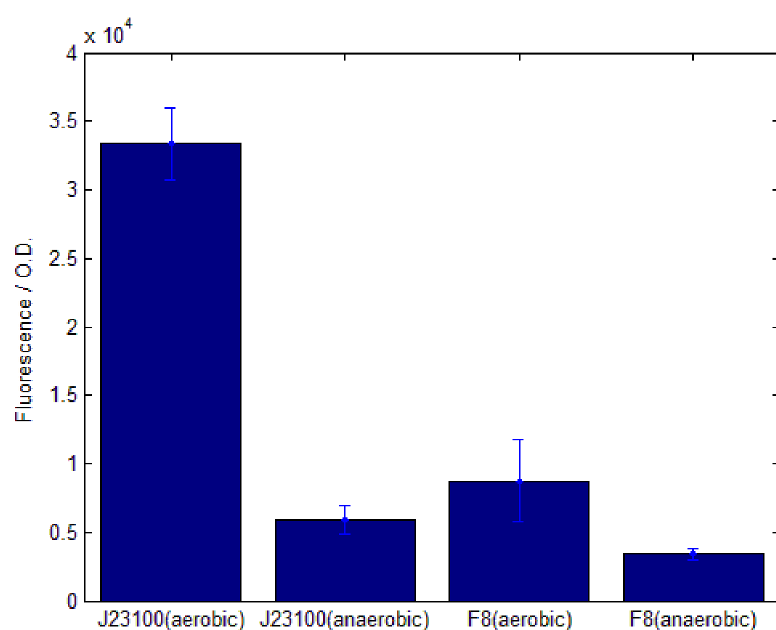

Figure A-8: The fluorescence per O.D. for FNR promoter F8 in aerobic and anaerobic conditions compared to a J23100, the control promoter

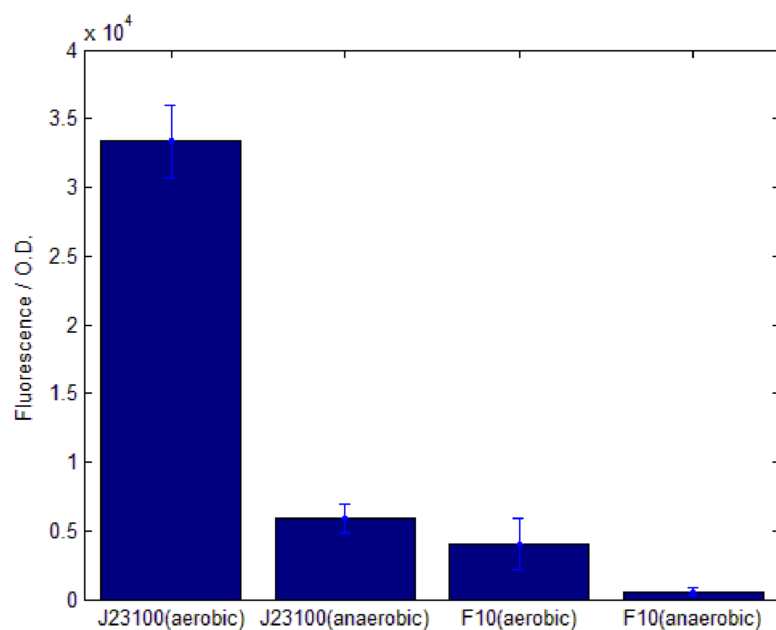

Figure A-9: The fluorescence per O.D. for FNR promoter F10 in aerobic and anaerobic conditions compared to a J23100, the control promoter

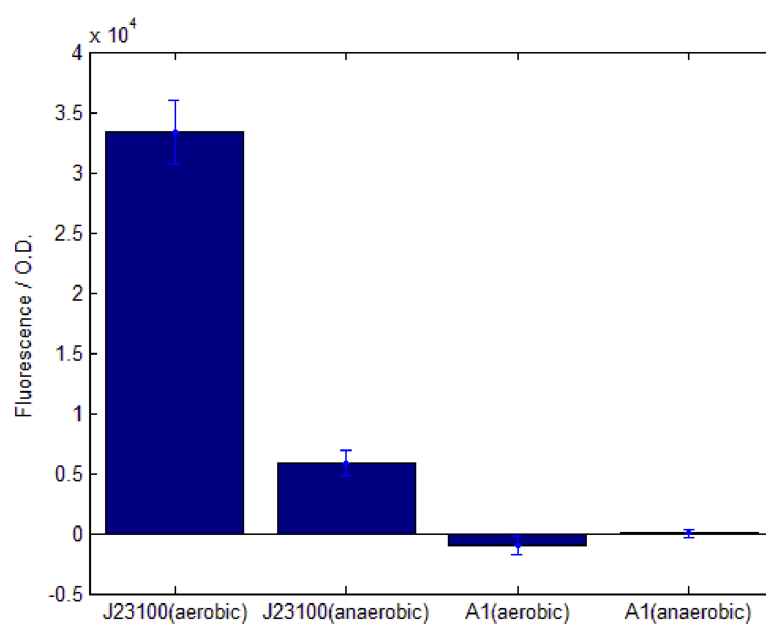

Figure A-11: The fluorescence per O.D. for ArcA promoter A1 in aerobic and anaerobic conditions compared to a J23100, the control promoter

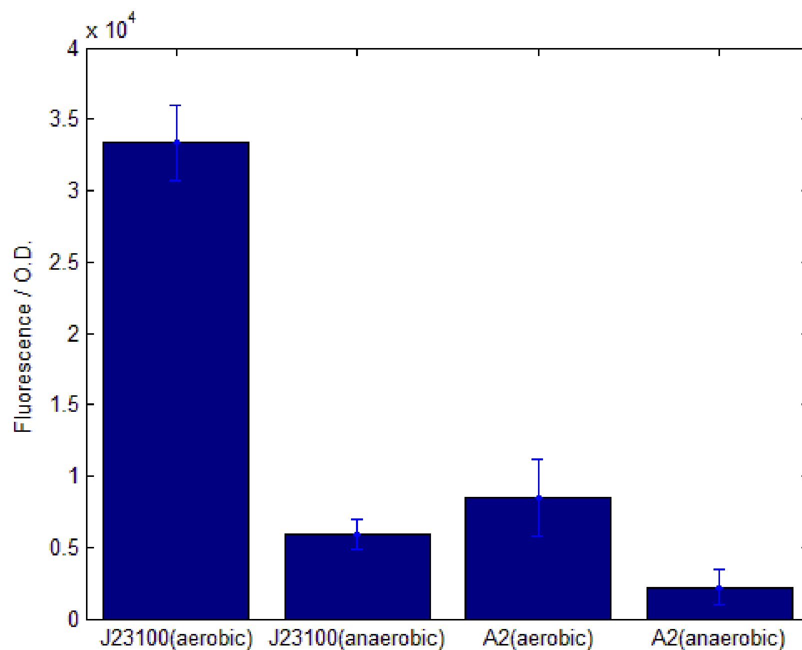

Figure A-12: The fluorescence per O.D. for ArcA promoter A2 in aerobic and anaerobic conditions compared to a J23100, the control promoter

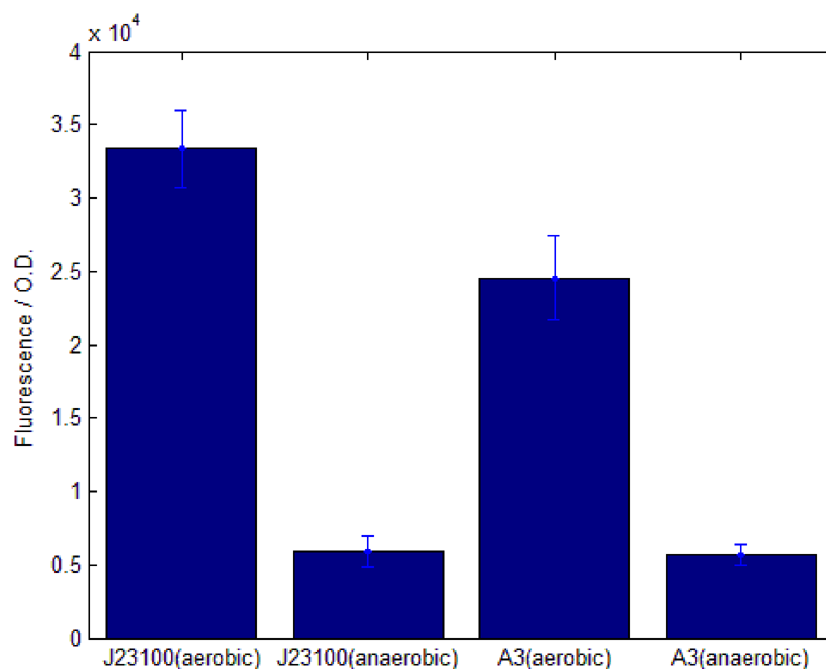

Figure A-13: The fluorescence per O.D. for ArcA promoter A3 in aerobic and anaerobic conditions compared to a J23100, the control promoter

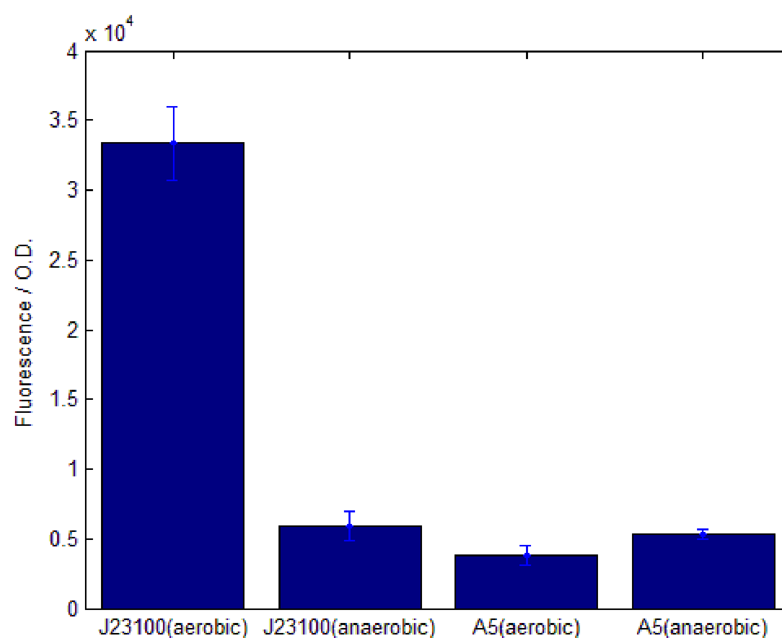

Figure A-14: The fluorescence per O.D. for ArcA promoter A5 in aerobic and anaerobic conditions compared to a J23100, the control promoter

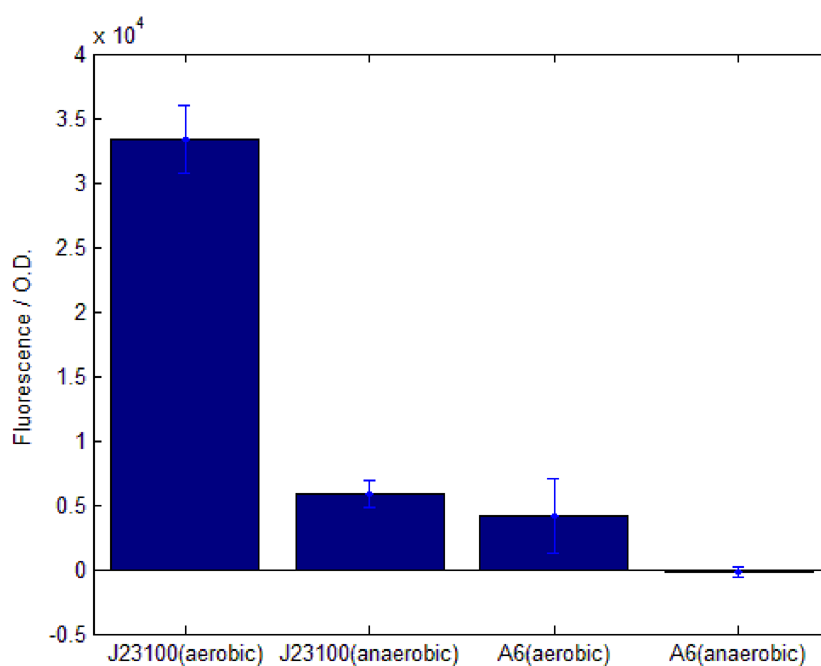

Figure A-15: The fluorescence per O.D. for ArcA promoter A6 in aerobic and anaerobic conditions compared to a J23100, the control promoter

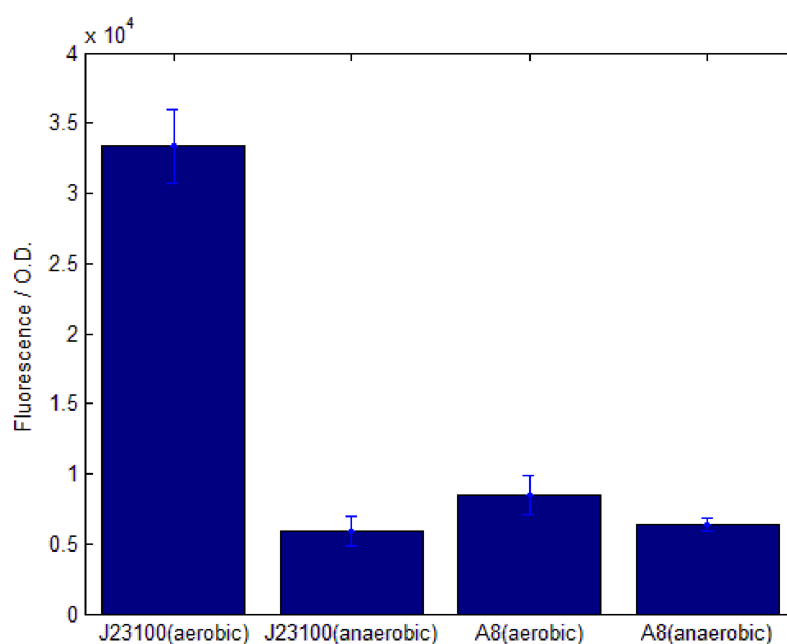

Figure A-16: The fluorescence per O.D. for ArcA promoter A8 in aerobic and anaerobic conditions compared to a J23100, the control promoter.

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Honors and Awards

- Frank and Jeannette S. Peikert Memorial Scholarship for leadership in American Society of Agricultural and Biological Engineers
- Dean's List for 7 semesters
- Member of Phi Kappa Phi honor society
- Penn State Schreyer Honors College Academic Scholarship

Association Memberships/Activities

- Scribe for Penn State branch of American Society of Agricultural and Biological Engineers
- Schreyer Student Ambassador

Professional Experience

- Undergraduate Researcher for the International Genetically Engineered Machines competition Penn State Team, Summer 2010
- Undergraduate Researcher for the Northeast Sustainable Agriculture Research and Education Dairy Cropping Systems project, Summer 2011
- Undergraduate Research for the United States Department of Agriculture, Agricultural Research and Extension, Integrated Farm System Model project, Fall 2011 – Spring 2012
- Research Fellow for the Auburn University NSF Research Experience for Undergraduates Program in Micro/Nano-Structured Materials, Therapeutics, and Devices, Summer 2012

- Teaching Assistant for Introduction to Engineering Design at Penn State, Spring 2011
- Teaching Intern for Heat and Mass Transfer at Penn State, Spring 2013

Research Interests

I am interested in research pertaining to food processing and agricultural production from an engineering perspective, particularly with regards to sustainable processing and production techniques. My research experiences have allowed me to work in large, multidisciplinary projects. This is fitting to my research interests and capabilities because biological engineering itself is interdisciplinary: it draws on diverse principles from engineering mechanics, mechanical engineering, chemical engineering, food science, and more.

Professional Presentations

- International Genetically Engineered Machines competition Jamboree, 2010
- Institute of Biological Engineering Annual Conference, 2011
- Penn State Ag Research Exposition, 2012

Publications and Papers

Hennessey R, Katyal A, Kirk A, McCann E, Rossi L. Bacterial Fireworks: Oxygen-Sensing BioBrick Promoters. International Genetically Engineered Machines Competition, Massachusetts Institute of Technology. Oct. 2010.

Kirk AJ, Karsten HD, Malcolm GM. The effect of crop production practices and seed moisture content on oil yield and meal quality of expeller-pressed canola. Agricultural Research Exposition, Penn State University. Mar. 2012.

Kirk AJ, Sullivan M, Prorok BC. Measuring Pile-Up from Nanoindentation of Thin Films. Auburn University NSF Research Experience for Undergraduates (REU) Program in Micro/Nano-Structured Materials, Therapeutics, and Devices. July 2012.

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Kirk AJ, Salis HM. Synthetic DNA Promoters to Control Gene Expression Based on the Presence of Oxygen in *E. coli.* Penn State University. April 2013.