Worcester Polytechnic Institute

Inducibility of Synthetic Genetic Circuits in *Pseudomonas putida* under Simulated Soil Conditions

A Major Qualifying Project

Submitted to the Faculty of

Worcester Polytechnic Institute in partial

fulfillment of the requirements for the Degree

of Bachelor of Science in

Biology and Biotechnology

by

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Abstract

This research tests the efficacy of an inducible system present in genetically engineered bacteria *Pseudomonas putida* (*P. putida*), a widespread soil bacterium. Inducible systems in bacteria are used for a variety of functions in biotechnology, including bioremediation and working as cell factories to produce natural products and small molecules. The inducible system used in this research responds to the addition of an anhydrotetracycline (aTc) with the synthesis of the far-red fluorescent protein mCardinal to report the presence of aTc. To further research and address how the biosensor would respond under real-world environmental conditions, this Major Qualifying Project expanded upon biosensor testing under simulated soil conditions using a liquid soil extract in the lab for testing. The goal of this research project was to observe the effects, if any, of addition of an aTc inducer to the inducible system in liquid soil extract. While we were able to confirm inducibility in laboratory growth conditions, we were unable to induce the circuit in liquid soil extract conditions. We believe that the promoters that drive the system may not be functional under soil-like conditions and will need to be further optimized in order to apply inducible genetic circuits within real-world environmental conditions. These discoveries motivate further research into the potential use of *P. putida* as a biosensor for antibiotics and other environmental contaminants in soil environments.

Acknowledgements

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To Ally, Nicole, and Christine, thank you for your assistance with data analysis and for always giving me a smile at the lab as I ran my experiments.

To my family, thank you for all your love, encouragement, and unfailing good humor. I would not have been able to complete any of this research without your constant and unwavering support as I conducted experiment after experiment and worked through drafting my project paper.

To my friends, thank you for being there and cheering me on. You have each been a fantastic source of love and laughter.

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Background

Genetically engineered microbes (GEMs) are being harnessed for use in detecting antibiotics, heavy metals, and other contaminants in soil environments. This detective work by a GEM can be effectively accomplished with the use of a biosensor in the form of an inducible genetic circuit within the GEM that senses the presence of these chemical materials or contaminants. An inducible gene is a gene that is expressed, or turned on, when a substance (an inducer) is introduced into the GEM environment. The inducer controls the expression of the gene. The microbial biosensor is a tool which integrates the microbe with a transducer in order to generate a measurable response output (e.g. fluorescence) from a certain input - the inducer (e.g. antibiotic, heavy metals) (Su et. al, 2011)**.** Once the GEM senses one or more of these materials, it produces a signal to flag the presence of the contaminant.

For this Major Qualifying Project, extensive research was conducted to test the fluorescent protein production response of an inducible genetic circuit to the presence of an antibiotic inducer. We selected the soil bacteria *Pseudomonas putida (P. putida*) for testing. *P. putida* is a rod-shaped, flagellated, gram-negative bacterium that is found in most soil and water habitats where there is oxygen. It grows optimally at 25-30°C. *P. putida* is amenable to genetic engineering and is frequently used in research and bioremediation work (Sayler & Ripp, 2000). This is due to *P. putida*'s flexible metabolism, ability to withstand physical and chemical stresses, and ability to thrive in diverse and difficult environments. *P. putida* is found in contaminated environments as seen in Figure 1 and has become a microbe of choice for bioremediation research (Weimer et. al, 2020).

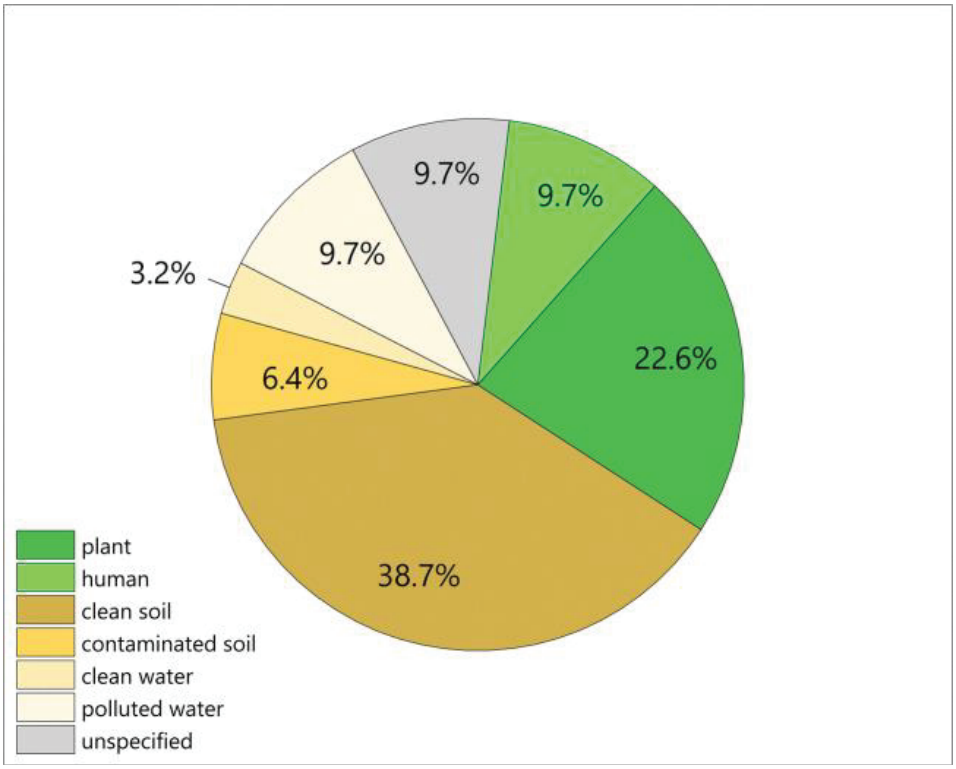

Figure 1. Image Adapted from Weimer et. al, 2020. "Isolation, source, and distribution of *P. putida* strains with full genome sequence available. The *P. putida* strains have been isolated from soil, polluted soil, water, polluted water, and/or wastewater and other unspecified sources (Data from Pseudomonas Genome DB and NCBI BioSamples Database, accessed: 05/20/2020; see Tables S2 for further information.)"

This Major Qualifying Project expands upon the research conducted by Dr. Andres Felipe Carrillo Rincón. Dr. Carrillo Rincón created a plasmid containing a biosensor for use in *Escherichia coli*, *Vibrio natriegens*, and *P. putida* that produced mCardinal, a fluorescent protein, in response to the addition of an antibiotic inducer (Carrillo Rincón & Farny, 2023). Dr. Carrillo Rincón's research results indicated that the biosensor was functional in Luria Broth (LB). The research conducted for this Major Qualifying Project looked to expand into other environmental conditions beyond LB to test if the biosensor would retain its functionality. These environmental conditions were multiple types of liquid soil extract, also known as solubilized extracted soil organic matter (SESOM). Liquid soil experiments can act as a precursor to solid soil experiments as soil synthetic biology can encounter challenges in the GEM's ability to

survive, persist, and maintain its function over a period of time within the soil environment. Validating *P. putida* as a model specimen in soil environments as well as understanding the strengths and limitations of *P. putida* was the overarching goal of our research work. It can be easier to grow bacteria and measure such growth by flow cytometry or plate reader assays when bacteria is grown in liquid media. Different genes are expressed when bacteria are on a solid matrix in a solid soil environment compared with when bacteria are floating in a planktonic state in a liquid soil media. There is precedent for bacterial growth differences in SESOM in comparison with solid soil models using SESOM agar for *Bacillus* species (Vilain et. al, 2006). No research has yet been conducted to understand the behavior and viability of *P. putida* in liquid versus solid soil conditions.

The biosensor mentioned above takes the form of an inducible genetic circuit made up of multiple genetic elements that work together to report the presence of the inducer. A visual representation of the architecture of the inducible circuit used in this research can be seen in Figure 2. The yellow circles represent the inducer, or "on" switch, used in each experiment. The inducer is the antibiotic anhydrotetracycline (aTc), a derivative of tetracycline. The purple ovals in Figure 2 represent the Tet repressor protein (TetR). In the "off" state, TetR proteins inhibit the *tet* promoter, and, therefore, also inhibit transcription of the mCardinal (a fluorescent protein) open reading frame, represented by the red arrow shape, by binding to the *tet* promoter. The *tet* promoter regulates the transcription of fluorescent mCardinal. The top section of Figure 2 illustrates the successful repression of the *tet* promoter because the mCardinal open reading frame (ORF) does not produce any fluorescent proteins. In the lower section of Figure 2, aTc is added. The aTc binds to the TetR proteins, which prevent the TetR proteins from binding to the *tet* promoter. With the addition of the inducer, the *tet* promoter is functional and transcribes the mCardinal ORF, leading to production of mCardinal fluorescent proteins and, therefore, quantifiable fluorescence.

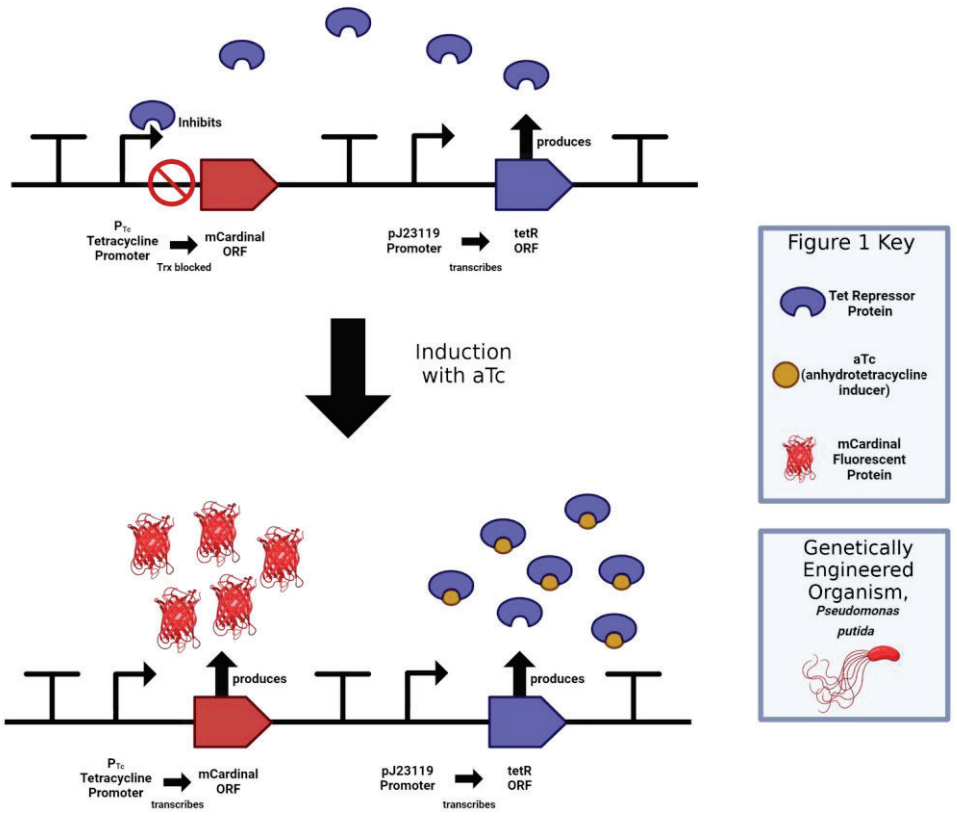

Figure 2. The aTc Inducible Circuit used in *P. putida* strains P38 and P52. Created in Biorender.com

The goal of this Major Qualifying Project was to conduct experiments and statistical analysis to determine if the inducible genetic circuit in bioengineered *P. putida* elicits a response output in both a nutrient-rich LB environment and a nutrient-poor liquid soil environment. If such a response output is generated in a nutrient-poor liquid soil environment, further experiments in solid soil environments could bring the biosensor closer to use in real world applications.

In this work, we show how the biosensor responds under simulated soil conditions. We found that while we were able to confirm inducibility in laboratory growth conditions, we were unable to induce the circuit in liquid soil extract conditions. These results did not support our

hypothesis that the inducible circuit was functional in liquid soil extract. We believe that the promoters that drive the system may not be functional under soil-like conditions and will need to be further optimized in order to apply inducible genetic circuits within real-world environmental conditions.

Materials and Methods

A. Bacterial Strains and Plasmids

Pseudomonas putida belongs to the Gammaproteobacteria phylum and the Pseudomonadaceae family. The *P. putida* strain KT2440 and plasmids for gene expression and genomic integration were gifts from Dr. Adam Guss of Oak Ridge National Laboratory to the Farny Lab, Dr. Natalie Farny, Principal Investigator. Using the *P. putida* strain KT2440, plasmids for *P. putida* strains P52 and P38 were created by Dr. Carrillo Rincón of the Farny Lab at Worcester Polytechnic Institute for use in *Escherichia coli*. The plasmids were subsequently integrated into *P. putida* by Dr. Carrillo Rincón.

Three strains of *P. putida* were used in the experiments detailed in this report: a wild-type *P. putida* strain not impacted by the addition of an inducer, a *P. putida* strain with constitutive fluorescence response, and a *P. putida* strain with an inducible response. The full names of each plasmid found within each strain can be seen below in Table 1.

Table 1: Bacterial Strains and Plasmids

Code	Strain	Plasmid	Features	Selective Marker	Predicted Fluorescence
P38	*P. putida* AG4775	pJH0204 V2TC tetR-Cardinal	Integrated with pJH0204	Kanamycin	Inducible Response
P52	*P. putida* AG4775	pJH0204 V2TC Cardinal	Integrated with pJH0204	Kanamycin	Constitutively “on”
WT	*P. putida* AG4775	pJH0204	N/A	None	None

B. Soil Collection

Two types of soil-extracted solubilized organic matter (SESOM) were used for inducible circuit testing. The first type of SESOM, black bag (“BB") SESOM, was used in the preliminary testing of the inducible circuit. Soil for BB SESOM was purchased online: “Miracle-Gro

Performance Organics All Purpose Container Mix - Organic and Natural Plant Soil". After initial results indicated that the inducible genetic circuit was nonfunctional in BB SESOM due to low levels of bacterial growth, oak soil was collected with permission from (Lat. 42.491926, Lon. -71.247929) in Bedford, Massachusetts, beneath a Pin Oak, *Quercus palustris.* Tree genus was determined using iNaturalist. The temperature outside during soil collection was 55.4°F. The soil was collected between 6 and 8 inches of depth, and contained a mixture of loam and yellow-gold clay. A visual representation of a leaf from the oak tree, soil collection method, and the color difference observed between BB and Oak SESOM can be seen in Figure 3. Examples of bacterial growth in Oak SESOM include Liebeke et. al, 2009 and Luo et. al, 2007.

Figure 3. Oak Soil Collection. Rightmost Image: Oak SESOM (left), BB SESOM (right).

C. Soil-Extracted Solubilized Organic Matter (SESOM) Production

One hundred grams of Miracle-Gro Performance Organics Potting Soil Mix (purchased online) or one hundred grams of oak soil (collection details for oak soil can be found in Materials and Methods Section B) were combined with 500mL of phosphate buffered saline (PBS) in a 1L Erlenmeyer flask and the mouth of the flask was covered with tinfoil. The flask containing the soil-PBS mixture was placed into a shaking incubator at 220 rpm at 37°C for two hours. After the incubation period, the soil-PBS mixture was poured into a French Press and the large particulates of the soil were filtered out. Next, a Büchner funnel vacuum filtration system was set

up and lined with two pieces of Whatman filter paper. A grade one piece of Whatman paper was placed first into the Büchner funnel vacuum filtration system and a grade four piece of Whatman paper was placed on top. Both pieces of filter paper were wetted with PBS prior to addition of the filtered soil-PBS mixture. After filtration through the Büchner funnel vacuum filtration system, the soil extract was filtered through a 0.2-micron sterilizing filter unit. The resulting sterile soil liquid extract was stored at 4°C until use in experiments. A visual representation of this process can be found in Figure 4.

Figure 4. Soil-Extracted Solubilized Organic Matter (SESOM) Production.

D. Growth and Induction of Bacterial Strains in Differential Conditions

The growth and induction phases of experiments took place over two consecutive days. During Day 1, a freezer stock of kanamycin (KAN) antibiotic was thawed and mixed with LB at a final concentration of 50 ug/mL. Three colonies each of P52 and P38 were picked from agar plates to be grown overnight in 5mL LB + KAN media. The P52 and P38 strains each contain a KAN resistance gene as a selectable marker. The inclusion of the selectable marker ensures that any P52 or P38 bacteria only survive if they contain the biosensor. If a bacteria within the P52 or P38 strain mutates and no longer contains the biosensor, the mutated bacteria would also no longer contain the KAN resistance gene and, therefore, be killed by the antibiotic. Three colonies were picked from an agar plate of the wild-type (WT) strain to be grown overnight in 5mL LB media. The WT strain does not contain an antibiotic resistance selectable marker or biosensor

and, therefore, is not grown in media with antibiotics. Nine tubes total, three of each strain, were then incubated in a shaking incubator at 30°C overnight.

Day 2 of growth and induction was comprised of multiple stages: (a) creating undiluted SESOM cultures, (b) creating 1:10 dilutions of undiluted SESOM and LB cultures, (c) measuring optical density of 1:10 SESOM and LB cultures, (d) creating +/- aTc cultures with diluted SESOM and LB cultures, (e) creating SESOM controls, and (f) incubation and induction.

a. Creating undiluted SESOM cultures

To create the undiluted SESOM cultures, 2mL of each LB culture from Day 1 was pipetted into a 15mL snap cap tube. Samples were centrifuged at 3900 rcf for 5 minutes. LB supernatant was decanted and 2mL of SESOM was pipetted into each tube and the bacterial pellet was resuspended in the SESOM.

b. Creating 1:10 dilutions of undiluted SESOM and LB cultures

To make 1:10 dilutions of the undiluted LB cultures from Day 1 and the undiluted SESOM cultures created in section (a) above, 9mL of media (SESOM or LB) was pipetted into 15mL snap cap tubes. Using the newly created undiluted SESOM cultures and the undiluted LB cultures from Day 1, 1mL of each undiluted culture was pipetted into each tube with 9mL of the appropriate media (LB, LB + KAN, SESOM, SESOM + KAN).

c. Measuring Optical Density (OD) of 1:10 dilutions of undiluted SESOM and LB cultures

A sample dilution equations template (seen below in Table 2) was used for each condition (e.g. LB and SESOM) to ensure that the final culture density across and within each culture were identical before the addition of the inducer to the designated cultures. The optical density (OD600) of each 1:10 diluted culture of *P. putida* was measured and recorded using the Eppendorf BioPhotometer Model #6131 on the dilution equations template (see Table 2). Values associated with LB cultures were listed in columns labeled in red and values associated with SESOM cultures were listed in columns labeled in green.

The process of determining culture density was as follows: the spectrophotometer was first blanked with the appropriate media. The use of a "blank" calibrates the spectrophotometer to the optical density of the media in which the sample is located. For WT cultures, SESOM or LB without KAN added were used as the blank. For P52 and P38 cultures, SESOM or LB with KAN added were used as the blank. One milliliter of media was micropipetted into a cuvette before insertion into the spectrophotometer and the blank value was set for each media..

Next, 1mL of each biological replicate culture (e.g. WT-1, WT-2, WT-3) was micropipetted into a cuvette and OD600 values were collected in the second column. After the OD600 values for the LB and the SESOM cultures were collected, the OD600 was divided by 0.05. After multiple trials were conducted, visual observation of the SESOM cultures showed that the turbidity of the cultures grown in SESOM was low compared to the turbidity of the LB cultures. To account for this difference in growth, 0.2 divided by the OD600 was used in the third column of the SESOM cultures. The number in the third column is how much 1:10 diluted culture (in milliliters) would be needed to create a final culture with a volume of 1mL. In the fourth column (labeled as "1000-x=y"), the value from the third column is subtracted from 1000 to give the amount of media (in microliters) that would be needed to create a final culture with a volume of 1mL. Because 1mL would be too small a volume to use for testing, both numbers were multiplied by five before creation of the final cultures. The 5x column shows how much 1:10 diluted culture would be added to each set of two tubes in microliters (one tube to be induced and one tube to remain without the inducer) before incubation and induction. The 5y column shows how much media in milliliters would be added to each set of empty tubes before incubation and induction.

Strain	OD600 val	0.05/val = x	1000-x=y	5x	5y
P52-1	0.274	0.182	818	0.910	4.090
P52-2	0.282	0.177	823	0.885	4.115
P52-3	0.414	0.120	880	0.600	4.400
P38-1	0.450	0.111	889	0.555	4.445
P38-2	0.295	0.169	831	0.845	4.155
P38-3	0.474	0.105	895	0.525	4.475
WT-1	0.466	0.107	893	0.535	4.465
WT-2	0.407	0.122	878	0.610	4.390
WT-3	0.353	0.141	859	0.705	4.295

Table 2a. LB Dilution Equations Template for creating properly diluted cultures.

Strain	OD600 val	0.2/val = x	1000-x=y	5x	5y
P52-1	0.296	0.675	325	3.375	1.625
P52-2	0.306	0.653	347	3.265	1.735
P52-3	0.390	0.512	488	2.560	2.440
P38-1	0.377	0.530	470	2.650	2.350
P38-2	0.280	0.714	286	3.570	1.430
P38-3	0.404	0.495	505	2.475	2.525
WT-1	0.437	0.457	543	2.285	2.715
WT-2	0.424	0.471	529	2.355	2.645
WT-3	0.350	0.571	429	2.855	2.145

Table 2b. SESOM Dilution Equations Template for creating properly diluted cultures.

d. Creating "+/-" aTc cultures with diluted SESOM and LB cultures

After the dilution equation templates were filled out for LB and for SESOM, the final set of cultures were then created. The proper amount of 1:10 diluted culture and media were mixed in 2 tubes per sample, such that each colony isolated had a tube with aTc at a final concentration of 0.1 ug/mL (a 50mL ``+" tube marked with a red sticker) as well as a tube without aTc (a 50mL "-" tube marked with a blue sticker). A 5mL micropipette was used to pipette the proper amount of media into each set of tubes, A p1000 micropipette was used to pipette the proper amount of 1:10 diluted culture into each set of tubes. LB cultures are denoted by a yellow sticker on the cap and SESOM cultures are denoted by a green sticker on the cap. An image of what the final set of tubes might look like before incubation and induction can be seen in Figure 5.

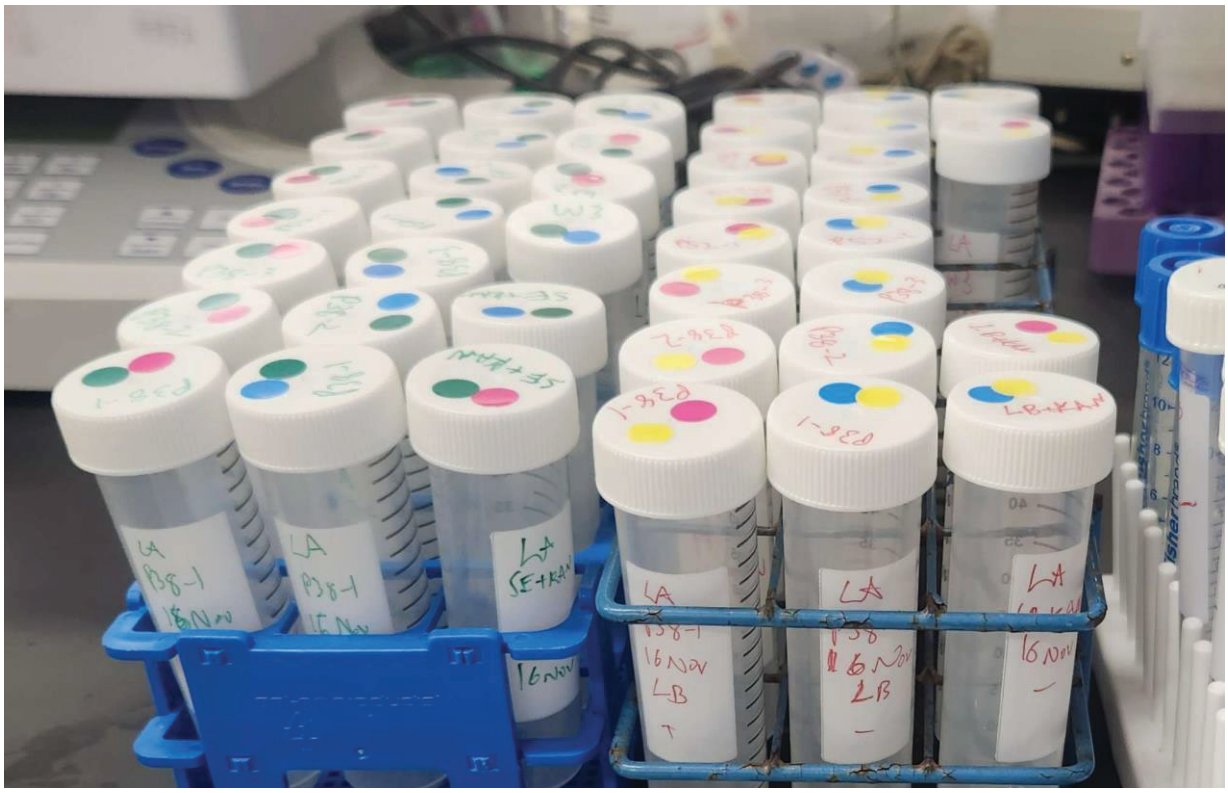

Figure 5. Sample Final Set of Tubes Image before Incubation and Induction.

e. Creating Controls

To create controls for the LB and SESOM set of tubes, four total 50mL conical tubes were filled with 5mL SESOM + KAN + aTc, 5mL SESOM + KAN, 5mL LB + KAN + aTc, and 5mL LB + KAN, respectively, to determine if the LB or SESOM media was contaminated. These tubes did not have any bacteria added and functioned as a test to determine if the LB or SESOM media contained contaminating bacteria or fungi. Only the *P. putida* strains that were being tested should have experienced growth during the incubation period, not bacterial or fungal contaminants in tubes that contained only media. In the case of contamination, the control tubes would be visibly cloudy or contain spheres of fungi and the experiment results would be discarded due to the lack of a functional control.

f. Incubation and Induction

All cultures, LB controls, and SESOM controls were incubated at 30°C in a shaking incubator for four hours. After four hours, the "+" aTc cultures, tubes labeled with red stickers, were induced with 5uL of aTc. All cultures and controls were then grown overnight to be measured the following day.

E. Testing of Bacterial Strains in Differential Conditions

On Day 3 of the experiment series, one hundred microliters of each sample were pipetted into a black-walled 96 well plate to capture fluorescence. One plate was made for the SESOM cultures and one plate was made for the LB cultures. The plate map in Figure 6 shows three biological replicates for each strain (three colonies of each strain) and four technical replicates per strain. Two sets of the three-day experiment series were conducted on two different plate readers. In the first set of experiments, the VICTOR Nivo Multimode Microplate Reader was utilized for measuring fluorescence of the cultures. It is worth noting that there was a filter error during these trials that stated that the filter was not optimal for 615/8nm, which is the excitation and emission range used for measuring mCardinal. In the second set of experiments, the Agilent BioTek Synergy H1 Microplate Reader H1M Unit 2 was used. No such filter error was reported when measuring mCardinal because this plate reader is customizable for specific excitation and

emission wavelengths. After all raw data was collected, the four technical replicates were averaged, and the resulting values were normalized by dividing the sample's RFUs by the RFU of the media control from that experiment (e.g. LB + KAN or SESOM + KAN).

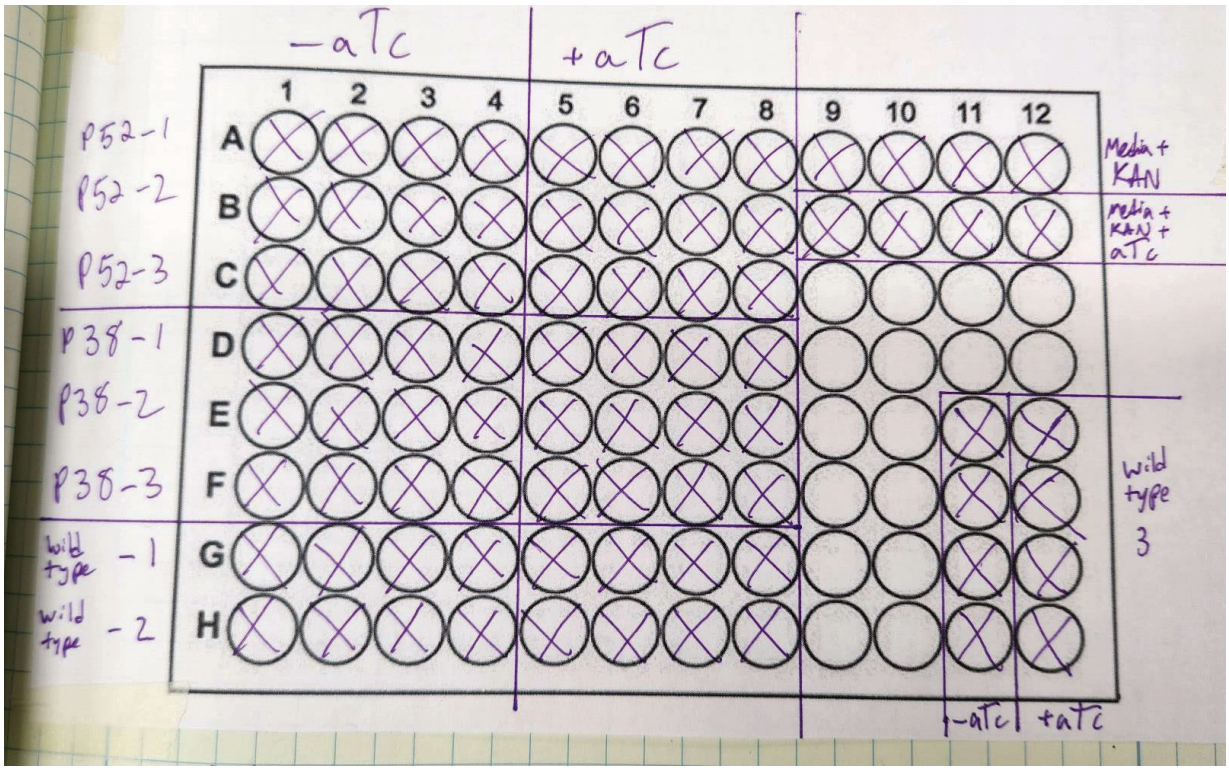

Figure 6. Plate Map for Testing of Bacterial Strains in Differential Conditions.

F. Methods for Biosafety and Biocontainment

Soils used for the experiments and testing were from a commercial source (Black Bag SESOM) and from private property with permission (Oak SESOM). As engineered organisms, from one of the three strains of *P. putida,* were introduced to LB or SESOM, the resulting liquid cultures and media were bleached and disposed of. Personal protective equipment including gloves, safety goggles, and a lab coat were used during all experiments and creation of SESOM. Requirements of a BSL-2 workspace, as well as all Farny Lab lab protocols, were complied with during all testing and work in the lab environment in order to prevent unintentional exposure to

biohazardous materials or their accidental release. During the course of this MQP project, there were no issues or accidental releases of any engineered organisms. Once soil was brought into the lab environment, it was not placed back into the environment. Used soil was discarded in the general building waste stream if it did not come into contact with engineered organisms. If soil came into contact with the bacterial strains, it was disposed of in a biohazardous waste container.

G. Data Processing and Statistical Analysis

Data were processed via normalization of the media that they were grown in using values collected from plate reader experiments. All data points were recorded and normalized using Microsoft Excel and measured in relative fluorescence units (RFUs). Grouped bar graphs were used to visualize data points collected from multiple trials of plate reader experiments using GraphPad Prism. The graphs were generated for three strains of *P. putida* in varying media conditions including LB as a positive control, Black Bag (BB) SESOM, and Oak SESOM. Figures 7-15 were generated with data collected from the VICTOR Nivo Multimode Microplate Reader. Figures 16-18 were generated with data collected from the Agilent BioTek Synergy H1 Microplate Reader H1M Unit 2. One-way ANOVA was performed in GraphPad Prism.

Results

In order to confirm the prior results of Dr. Carrillo Rincón, I first sought to demonstrate the inducibility of the genetic circuit by aTc in LB growth conditions. The results depicted in Figure 7 below indicate that the inducible circuit is functional in LB. The bar graph for the wild-type shows a low level of likely background fluorescence as the wild-type bacteria does not possess the genetic circuitry needed to produce mCardinal. Both the uninduced and induced bars for P52, the constitutively "on" bacteria, are equally high and indicate functionality of the constitutive bacteria. The uninduced bar for P38, the bacteria that possesses the inducible genetic circuit, is lower than the induced bar for P38, indicating that when the inducer is present the genetic circuit turns on. These trials therefore successfully confirm the prior results of Dr. Carrillo Rincón and show that all bacteria are functioning as expected in LB growth conditions.

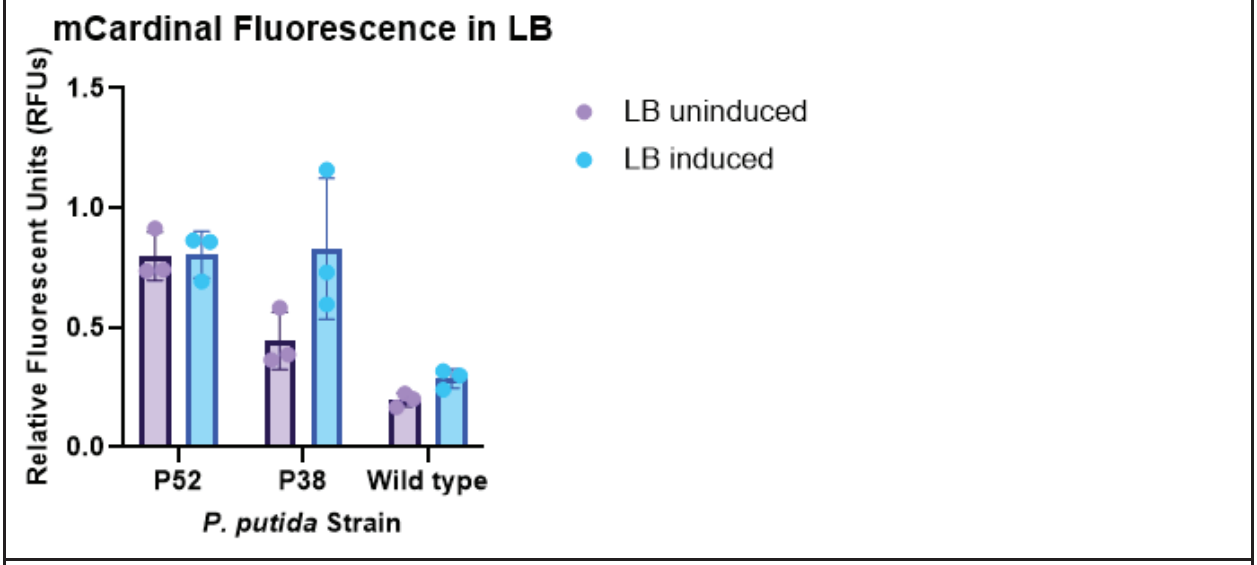

Figure 7. A Term LB (3 trials)

I further wanted to determine whether this same circuit would be inducible under SESOM growth conditions. Figure 8 shows only SESOM RFU values and indicates that high levels of background fluorescence innate to BB SESOM are being picked up by the plate reader. It is clear in Figure 8 that this is a level of background fluorescence because it is in the same high range regardless of the strain of bacteria or the status of inducibility. This makes it difficult to discern between fluorescence that is innately part of BB SESOM and fluorescence that is being

produced by the bacteria. During the collection of data during A term, visual observations showed that turbidity of the cultures grown in BB SESOM was very low compared to the turbidity of the LB cultures. There were no experiments conducted to measure this turbidity difference but the substantially less growth was visibly obvious. This observation led to the conclusion that the bacteria were not growing well in BB SESOM and therefore the plasmid was non-functional.

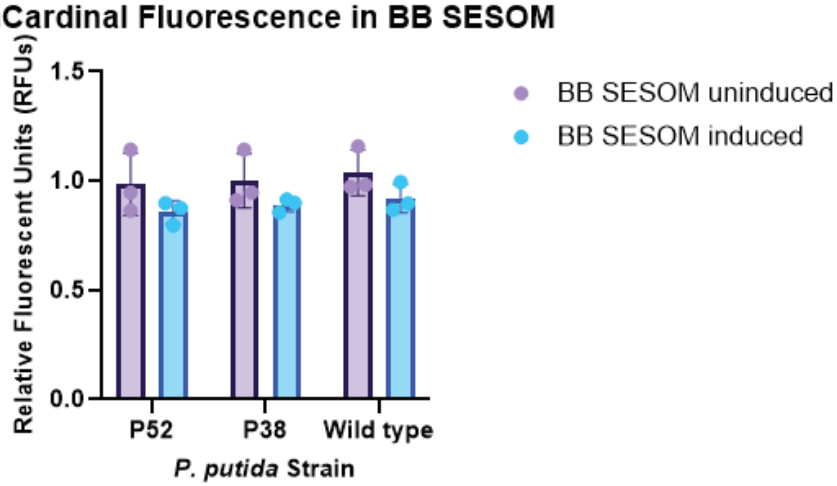

Figure 8. A Term Black Bag SESOM (3 trials)

Figure 9 includes both LB and SESOM relative fluorescence unit (RFU) values on the same graph and shows the visible difference between fluorescence produced by the bacteria in LB conditions compared to the fluorescence of the SESOM media.

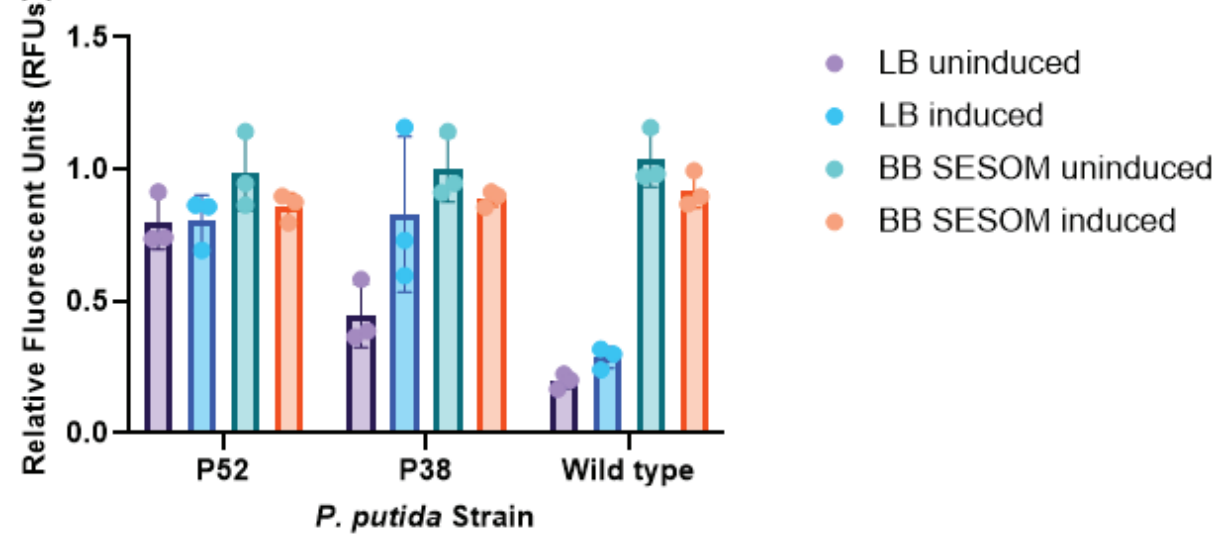

Figure 9. A Term LB and Black Bag SESOM Total (3 trials)

In an attempt to change the media type to determine if the bacteria might grow in an alternative soil environment, further testing was conducted using Oak SESOM instead of BB SESOM. Figure 10 below indicates, with less robust results (lower RFUs despite the same LB conditions), that the inducible circuit is functional in LB. The test results seen in Figures 10-12 show data from two trials referred to as the "no centrifugation" trials. We ran trials without centrifugation in order to test bacteria in the media in which the bacteria were grown. Figures 10-12 are therefore referred to as the "no centrifugation" results. The no centrifugation trials showed similar background fluorescence in Oak SESOM at higher RFUs than seen in LB.

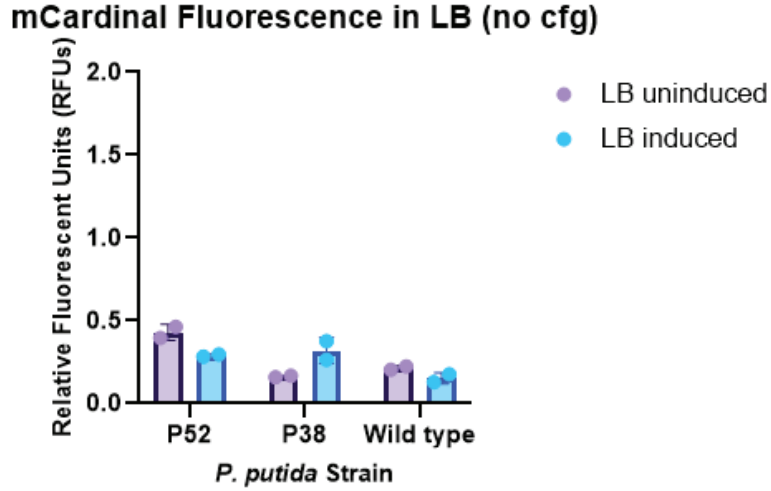

Figure 10. No centrifugation for LB (2 trials)

Figure 11 shows similarly high bars with only Oak SESOM values, indicating that Oak SESOM exhibits a high level of background fluorescence similar to BB SESOM.

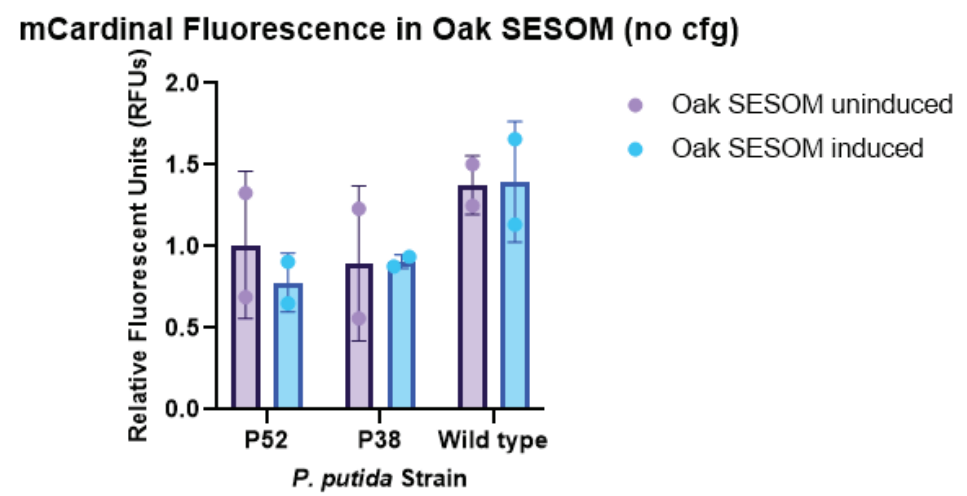

Figure 11. No centrifugation for Oak SESOM (2 trials)

Figure 12 shows the stark contrast between low levels of bacterially produced fluorescence and high levels of SESOM background fluorescence.

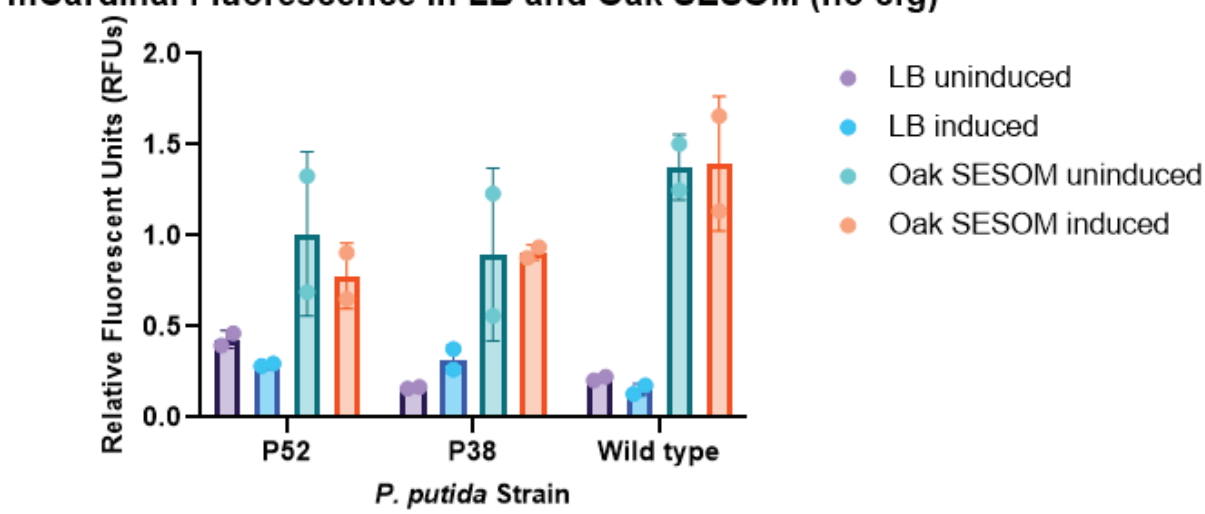

Figure 12. No Centrifugation for LB and SESOM (2 trials)

I observed that there was a significant fluorescent signal from SESOM alone in prior experiments (shown in Figures 8 and 11). Therefore, I next attempted to isolate the bacteria from the soil extract by centrifugation prior to plate reader measurement, to eliminate the background fluorescence caused by the SESOM. Thus, one trial, shown in Figures 13-15, was completed where the cultures were centrifuged in an attempt to concentrate the bacteria that were in the sample. In this trial, the cultures were centrifuged and resuspended in phosphate buffered saline (PBS) before measuring on the plate reader. This experiment was conducted to evaluate if there would no longer be any background fluorescence if the SESOM was removed from the tube containing the bacteria. By pelleting the bacteria, resuspending the pellet in PBS, centrifuging the bacteria, and then resuspending the pellet again, the fluorescent SESOM would be removed and only the fluorescence of the bacterial pellet would be measured by a plate reader. Unfortunately, after centrifugation, no bacterial pellets were visible from the centrifuged SESOM cultures and therefore the data collected only measured the low fluorescence of PBS during the centrifugation trial. With this testing approach, we were unable to draw any conclusions regarding the efficacy of the biosensor or level of bacterial growth in SESOM due to the lack of a bacterial pellet.

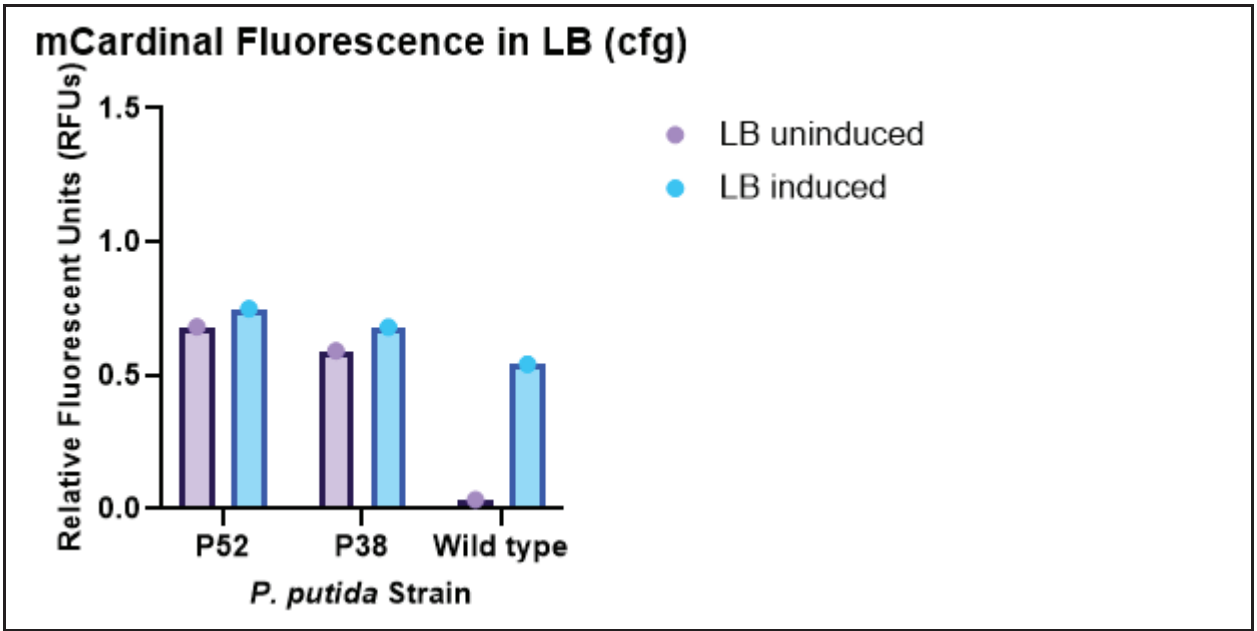

Figure 13. Centrifugation for LB (1 trial)

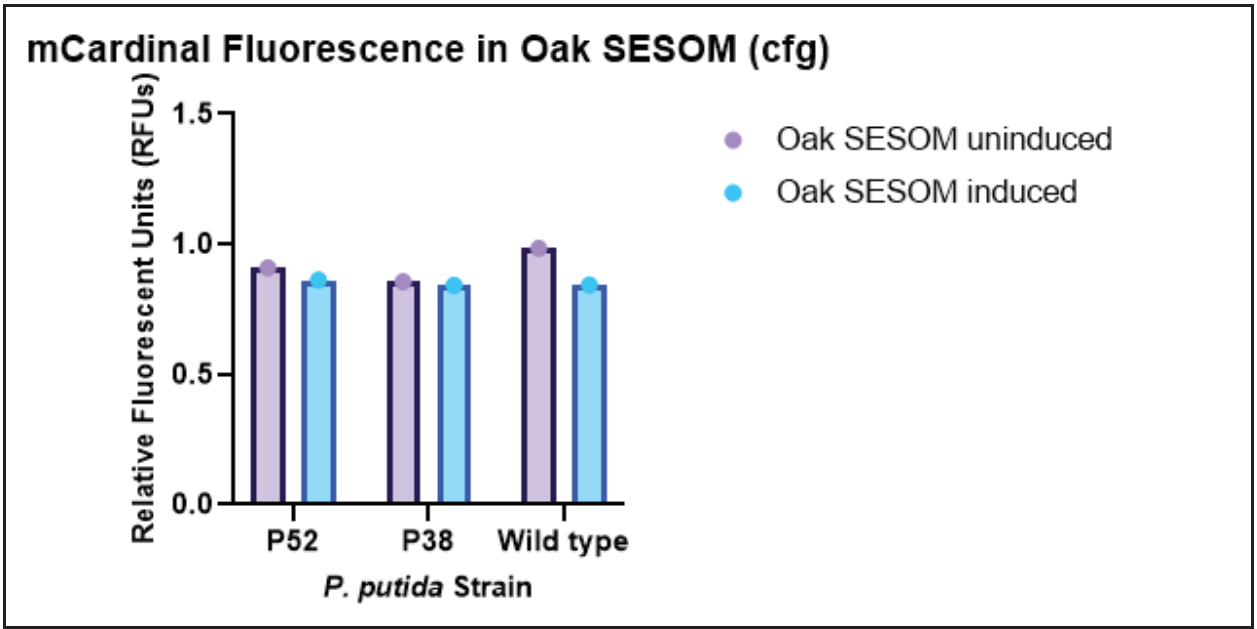

Figure 14. Centrifugation for Oak SESOM (1 trial)

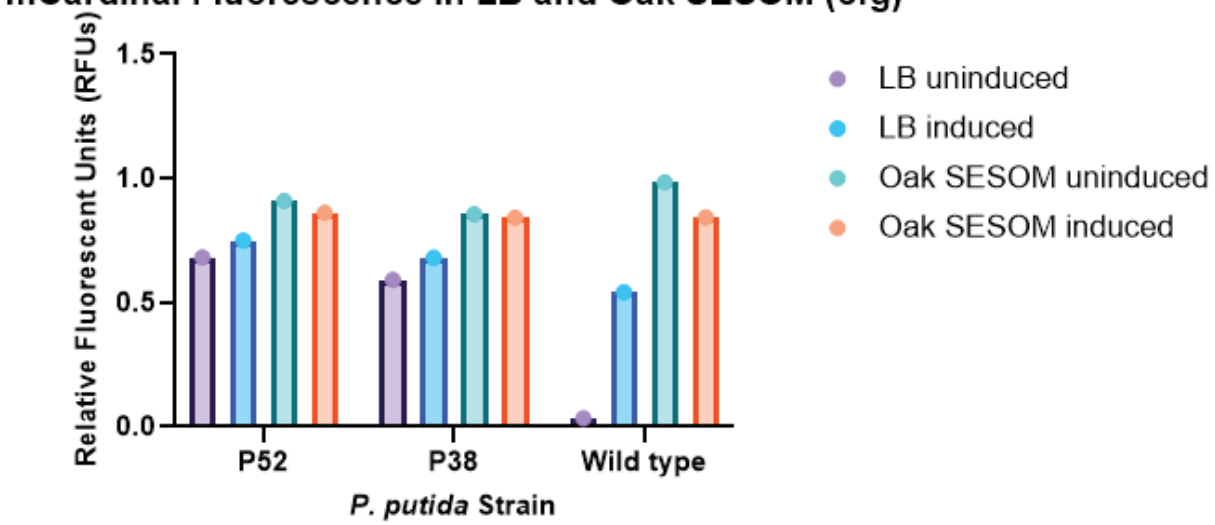

Figure 15. Centrifugation for LB and SESOM (1 trial)

After multiple unsuccessful trials with the VICTOR Nivo Multimode Microplate Reader due to high levels of background fluorescence, two trials were run on the Agilent BioTek Synergy H1 Microplate Reader H1M Unit 2 (“Agilent"). The Agilent plate reader was able to successfully eliminate background fluorescence from the Oak SESOM. Data collected on the Agilent plate reader can be seen in Figures 16-18. Figure 16 shows the clearest display of functionality from the LB biosensor. Visible fluorescence can be seen from the constitutive and inducible bacteria when it was switched “on" (P52 and P38 columns respectively).

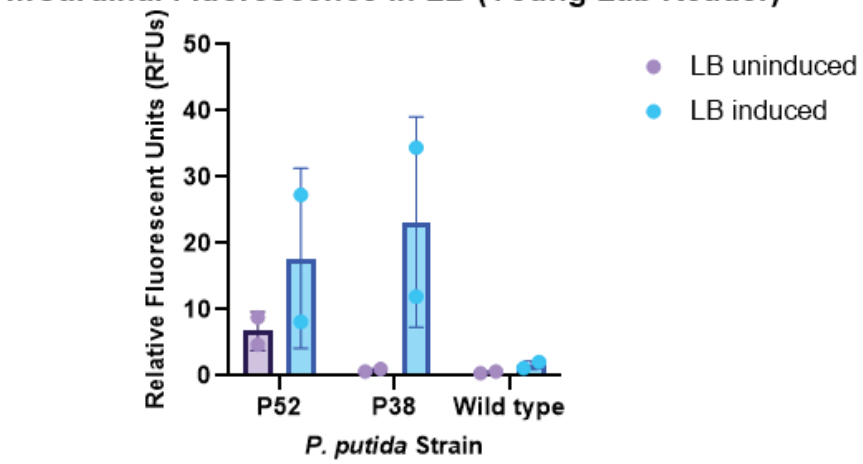

Figure 16. Young Lab Reader results for LB (2 trials)

Figure 17 shows the results from Oak SESOM, with one version matching the Y-axis of Figure 16, and one version with a Y-axis that shows off the RFU differences between each *P. putida* strain. The version of Figure 17 that is Y-axis corrected to match Figure 16 shows consistently low RFU values regardless of the *P. putida* strain. The low RFU values indicate that *P. putida* is not living in Oak SESOM and, therefore, the biosensor is non-functional in a liquid soil environment.

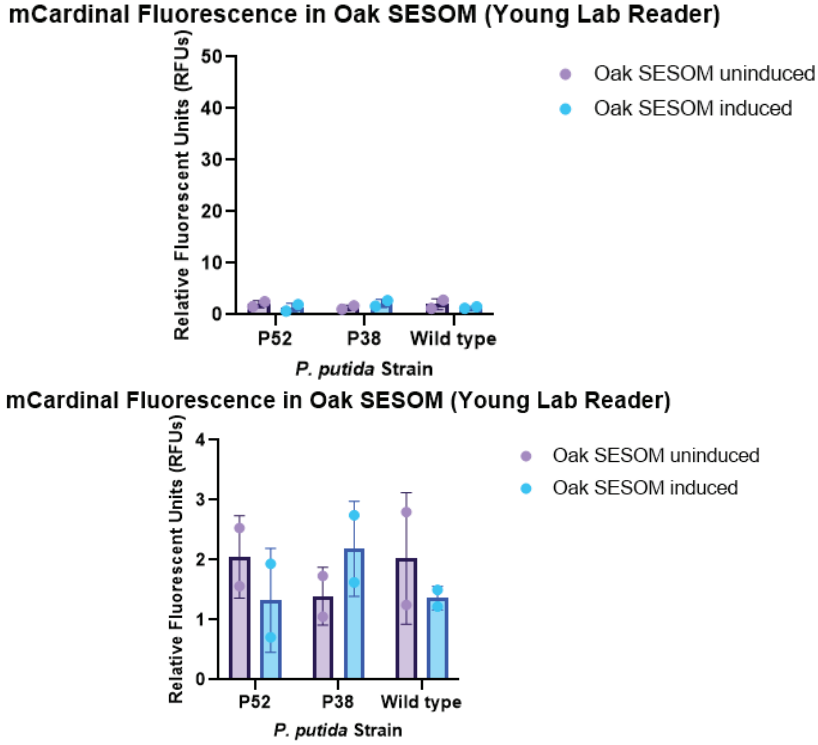

Figure 17. Young Lab Reader results for Oak SESOM (2 trials)

Figure 18 shows the combination of the results from Figure 16 and Figure 17. The visual difference between genuine fluorescence production and the lack of background fluorescence from the Oak SESOM clearly shows that the biosensor works well in LB and that no fluorescent proteins are being produced in Oak SESOM.

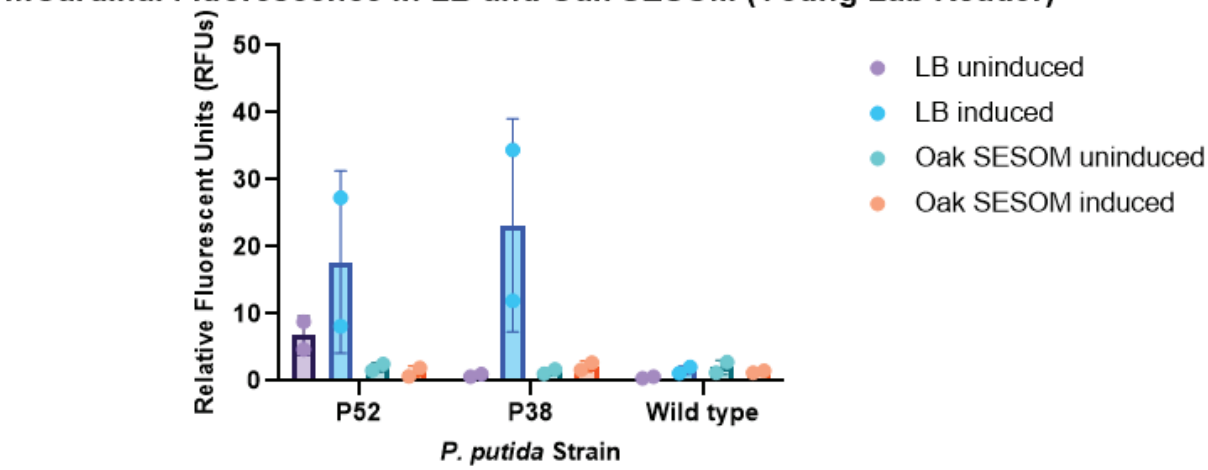

Figure 18. Young Lab Reader Results for LB and SESOM (2 trials)

Discussion

The goal of this research was to test a previously constructed aTc biosensor in *P. putida* in liquid soil conditions. As the test results above show, the biosensor in its current state of genetic architecture is not functional in BB or Oak SESOM. Despite the number of replicates, no inducibility was observed in BB or Oak SESOM growth conditions.

Analysis of the test results leads to the conclusion that the underlying causes of the lack of inducibility in BB and Oak SESOM could be attributed to one of two factors: (1) Repression of *tet* is being relieved when aTc is added, but the promoter that drives mCardinal is not active under these conditions and/or (2) Under these conditions, aTc is not binding to TetR and so repression of *tet* is not being relieved.

In the first scenario, where lack of inducibility is due to an inactive promoter, bacterial growth and production of fluorescence would be limited. Since the results indicate low levels of both bacterial growth and production of fluorescence in SESOM, it is possible that the proper sigma factor needed for bacterial growth is not present in BB or Oak SESOM conditions. A visual representation of this idea can be seen in Figure 19. We infer that the sigma factors that are most dominant in liquid soil conditions are not the sigma factors being used by the *tet* promoter. A sigma factor is a protein involved in the recognition of promoters and RNA synthesis (Paget 2015). It is probable that even when TetR is not bound to *tet*, there is still no transcription from the promoter due to the lack of the proper sigma factors in SESOM. In the event that the proper sigma factor is not present, bacterial growth is limited or prevented and this would thus limit the ability to test the biosensor.

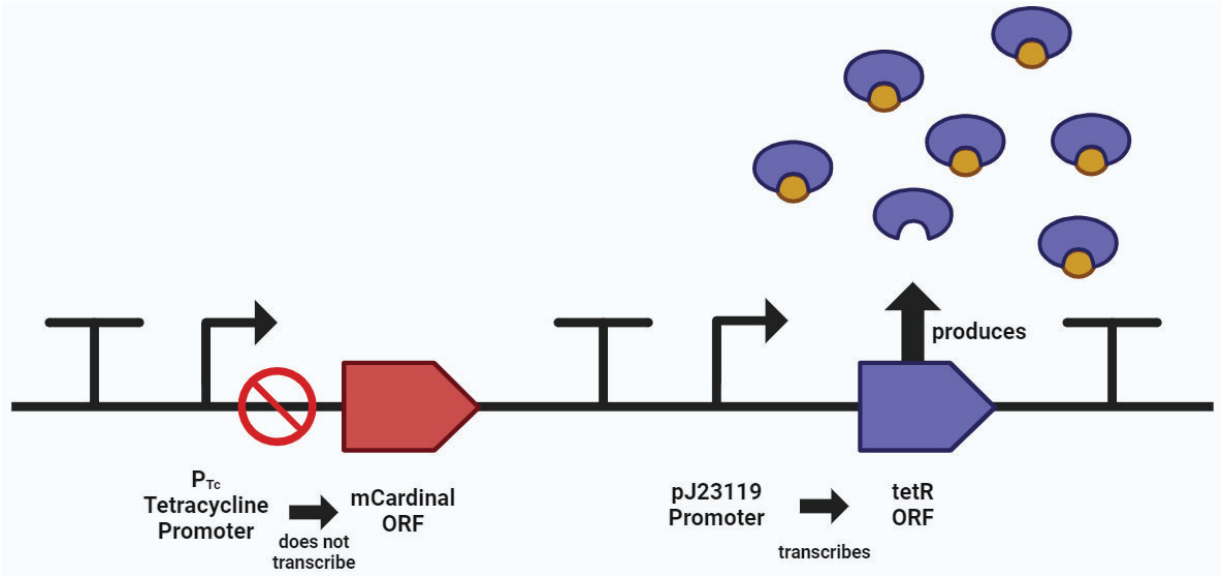

Figure 19. *tet* Promoter Inactivity.
Created in Biorender.com

With the second alternative reasoning for lack of inducibility, we surmise that even when aTc is added, TetR does not bind to aTc and *tet* is not relieved of repression. In this event, that TetR is able to bind to *tet*, we anticipate that TetR is not responsive to the presence of aTc and that repression of *tet* remains constant and unchanged and, therefore, repression cannot be relieved. A visual representation of this hypothesis can be seen in Figure 20. It is also possible that aTc is destroyed or bound by an unknown molecule and thus does not bind to TetR in SESOM.

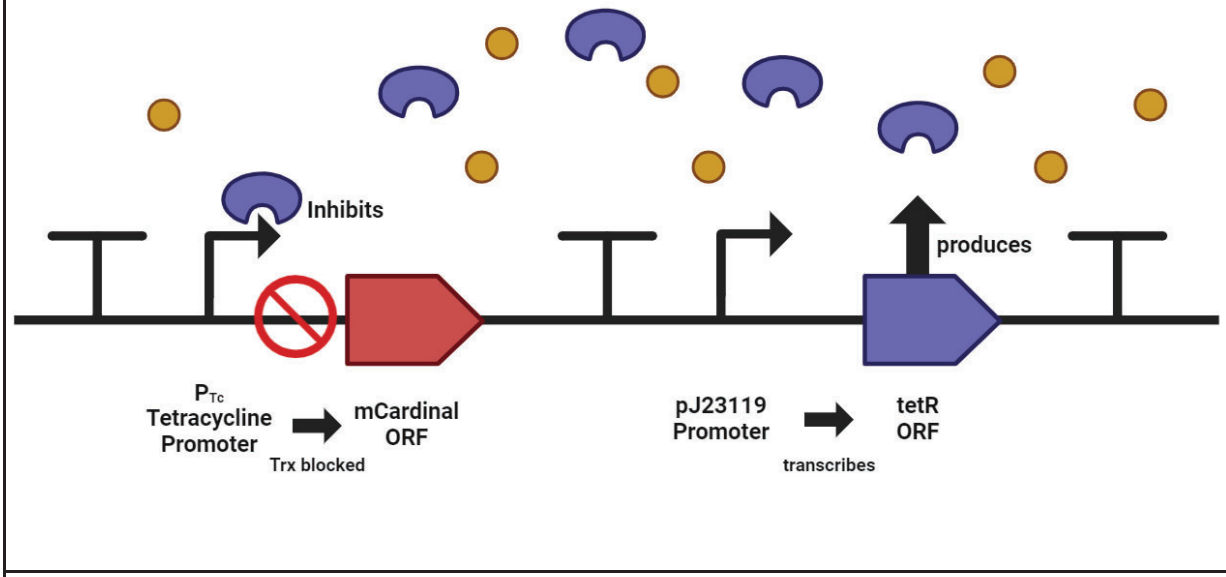

Figure 20. Repression is Not Relieved.
Created in Biorender.com

The two differing ideas presented above on possible causes for lack of inducibility indicate that the inactivation of the biosensor merits further investigation to figure out if such inactivation is caused by nonfunctional *tet* or a lack of aTc binding to TetR.

The experiment that could be conducted in order to determine which idea may be correct would be chromatin immunoprecipitation combined with high throughput sequencing, or ChIP-Seq. ChIP-Seq "identifies the locations in the genome bound by proteins" (Statquest with Josh Starmer, 2018). This process is completed by gluing the proteins that are bound to the DNA to the DNA strand, cutting the DNA up into fragments, adding antibodies, isolating the proteins bound to those antibodies, and isolating the DNA attached to the isolated proteins (Statquest with Josh Starmer, 2018). In the context of this research, the added antibodies would bind to TetR.

If TetR does not bind to *tet* regardless of the presence of aTc, and the promoter is nonfunctional like in the first scenario presented above, a weak signal from ChIP-Seq would be produced. If TetR is found to bind to *tet* regardless of the presence of aTc as seen in the second

scenario presented above, the result of ChIP-Seq would be a strong signal. This strong signal would be produced in both conditions where aTc is absent and where aTc is present.

A visual representation of a hypothetical antibody bound to TetR as well as a potential predicted result of ChIP-Seq can be seen in Figure 21.

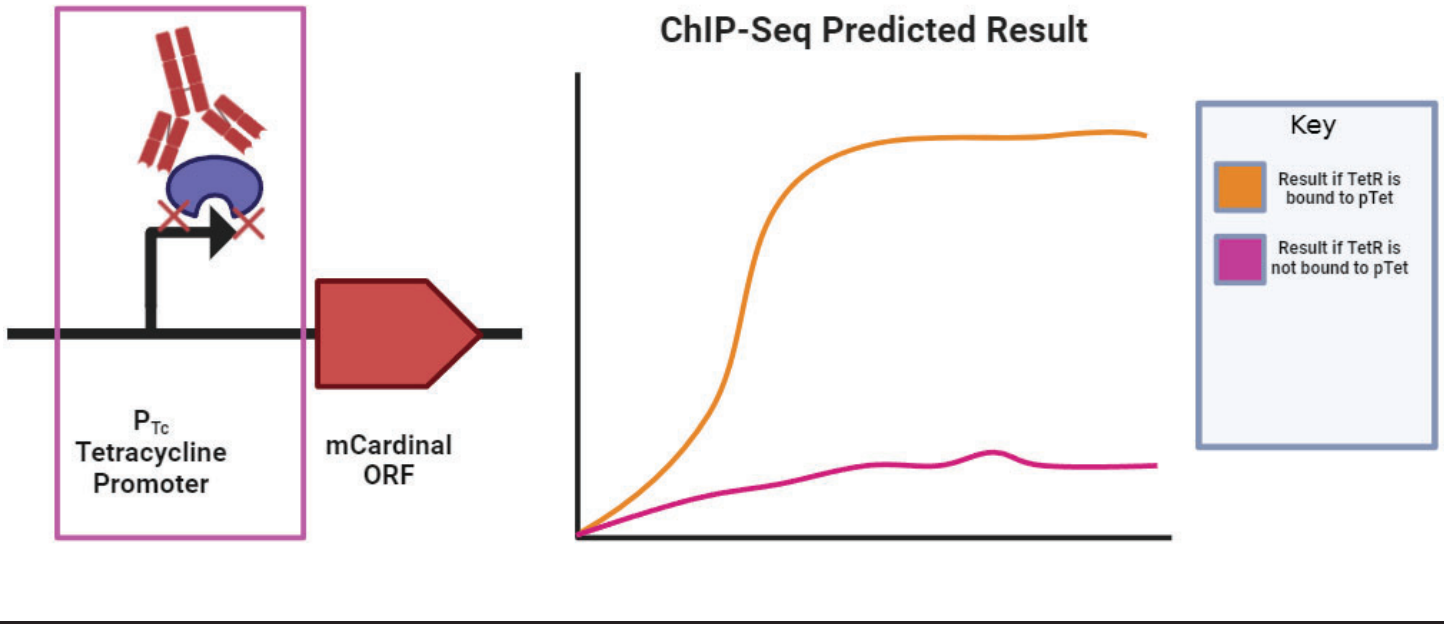

Figure 21. ChIP-Seq Visualization.
Created in Biorender.com

Thus, we anticipate that continued research could provide a deeper understanding of the status of *tet* which will bring the synthetic biology and environmental remediation fields one step closer to a functional biosensor in *P. putida.*

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Appendix

Appendix A: Raw Data from Experiments

A Term Raw Data

Trial 1, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	113936	79674	77911	171048	110642.3	134584	186235	167704	138790	156828.3	146613	160140	154356	150949	153014.5
B	138282	84092	96779	137861	114253.5	82138	104663	116813	89361	98243.75	146516	162530	151964	157220	154557.5
C	128026	105157	110190	118261	115408.5	54007	83780	75462	52038	66321.75					
D	23495	33032	32244	73695	40616.5	155174	60771	125481	60557	100495.8					
E	89968	60933	104016	73963	82220	102439	102010	59032	96382	89965.75			13935	21498	
F	42211	31830	41597	102727	54591.25	62335	148274	73272	60445	86081.5			19911	28320	
G	23951	19924	20206	16709	20197.5	53592	15387	129346	16647	53743			17365	35055	
H	41583	19523	16881	40847	29708.5	17735	73331	17317	18602	31746.25			55995	19842	
					26801.5					26178.75			26801.5	26178.75	
													AVG W3 ^		

Trial 1, BB SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	165990	171961	169398	160170	166879.8	156086	141484	142795	134628	143748.3	150742	118778	146559	146335	140603.5
B	164657	159668	168355	155571	162062.8	138816	148586	140466	133819	140421.8	138077	154603	186513	161526	160179.8
C	158588	149472	152009	152234	153075.8	122567	147166	157816	162179	147432					
D	169140	164766	171058	154922	164971.5	151959	131049	141263	150206	143619.3					
E	163149	158935	154499	153280	157465.8	150285	151235	133991	129161	141168			160691	171907	
F	155602	162931	164012	155571	159529	153992	154418	156842	153630	154720.5			169108	165450	
G	158705	157271	156624	152655	156313.8	145736	140353	146777	155668	147133.5			173978	168650	
H	166480	161329	168055	159611	163868.8	158005	164842	159975	168426	162812			169066	165640	
					168210.8					167911.8			168210.8	167911.8	
													AVG W3^		

Trial 2, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	116777	145071	130760	150915	135880.8	69422	185783	171549	73906	125165	154970	144529	143335	95973	134701.8
B	166684	169684	145546	61518	135858	117738	140689	110859	192427	140428.3	171689	111580	119924	157593	140196.5
C	108440	108595	73955	99152	97535.5	88101	125382	82084	97156	98180.75					
D	123684	78361	93357	52404	86951.5	113337	169899	153724	112909	137467.3					
E	77290	78505	100406	118573	93693.5	99164	177702	165934	129107	142976.8			71403	68121	
F	80869	23927	21515	92810	54780.25	200699	238845	187094	201791	207107.3			20953	61973	
G	32654	26495	20281	26547	26494.25	71220	37814	36777	18995	41201.5			28214	48029	
H	22633	25341	20324	23957	23063.75	23987	22505	19899	19715	21526.5			44485	75255	
					41263.75					63344.5			41263.75	63344.5	
													AVG W3 ^		

Trial 2, BB SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	175062	174508	162084	152365	166004.8	145926	142135	139961	140065	142021.8	189894	187195	194701	189539	190332.3
B	182172	167974	140906	175539	166647.8	156066	140111	165601	176977	159688.8	198307	192255	186328	186436	190831.5
C	175607	174223	146983	145967	160695	146423	164486	173289	134807	154751.3					
D	177860	179193	193116	161423	177898	154450	158360	175334	171094	164809.5					
E	190838	169096	130818	180648	167850	168148	176356	169803	169775	171020.5			183114	166777	
F	182128	186795	184633	144807	174590.8	177481	189169	175250	174880	179195			184521	181170	
G	196001	189953	177080	171641	183668.8	173881	166170	169123	164011	168296.3			181487	193818	
H	184508	186866	199431	186980	189446.3	173100	166266	161161	160464	165247.8			202414	178340	
					187884					180026.3			187884	180026.3	
													AVG W3^		

Trial 3, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	135773	131258	105912	118737	122920	121268	130428	158602	120993	132822.8	182480	188798	179377	201428	188020.8
B	137149	155985	152400	158380	150978.5	158066	167062	130036	131013	146544.3	179975	153642	154699	165783	163524.8
C	162440	173959	130941	99385	141681.3	141975	134037	135516	154669	141549.3					
D	106184	118316	62082	23618	77550	146277	145835	138390	103079	133395.3					
E	137603	104001	20036	20920	70640	166336	142438	170735	61367	135219			20133	70683	
F	69527	52365	20335	87616	57460.75	81543	62858	61549	152535	89621.25			73964	19024	
G	34897	23300	19832	18477	24126.5	113644	92199	81461	22501	77451.25			112881	89480	
H	24203	51086	19397	36985	32917.75	34881	20818	17692	24058	24362.25			19513	38199	
					56622.75					54346.5			56622.75	54346.5	
													AVG W3 ^		

Trial 3, BB SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	166543	181991	184034	179655	178055.8	169400	170826	172607	175451	172071	170284	191073	185503	200066	186731.5
B	170342	179527	189527	178023	179354.8	154237	159129	162176	177127	163167.3	191658	199609	186634	187559	191365
C	170655	181278	174947	165641	173130.3	154441	173140	174220	167349	167287.5					
D	172703	184276	176915	175234	177282	172807	172884	164260	186530	174120.3					
E	165706	179039	165099	173152	170749	165866	162695	165589	169658	165952			188743	179685	
F	186155	188859	173481	181704	182549.8	183536	154289	135512	132391	151432			183238	176296	
G	174216	172131	170962	167461	171192.5	170575	156630	156625	122254	151521			196036	185476	
H	183871	181874	190656	175287	182922	173318	172756	143250	170215	164884.8			195646	186178	
					190915.8					181908.8			190915.8	181908.8	
													AVG W3^		

Averages of three colonies, not normalized

Trial 1, LB

	no aTc	yes aTc
P52	113434.8	107131.3
P38	59142.58	92181
Wild Type	25569.17	37222.67

Trial 1, BB SESOM

	no aTc	yes aTc
P52	160672.8	143867.3
P38	160655.4	146502.6
Wild Type	162797.8	159285.8

-->

Trial 2, LB

	no aTc	yes aTc
P52	123091.4	121258
P38	78475.08	162517.1
Wild Type	30273.92	42024.17

Trial 2, BB SESOM

	no aTc	yes aTc
P52	164449.2	152153.9
P38	173446.3	171675
Wild Type	186999.7	171190.1

Trial 3, LB

	no aTc	yes aTc
P52	138526.6	140305.4
P38	68550.25	119411.8
Wild Type	37889	52053.33

Trial 3, BB SESOM

	no aTc	yes aTc
P52	176846.9	167508.6
P38	176860.3	163834.8
Wild Type	181676.8	166104.8

Averages of three colonies, normalized

Trial 1, LB

	no aTc	yes aTc
P52	0.741333	0.693148
P38	0.386516	0.596419
Wild Type	0.167103	0.240834

Trial 1, BB SESOM

	no aTc	yes aTc
P52	1.142736	0.898162
P38	1.142613	0.914614
Wild Type	1.15785	0.994419

Trial 2, LB

	no aTc	yes aTc
P52	0.913807	0.864915
P38	0.582584	1.159209
Wild Type	0.224748	0.299752

Trial 2, BB SESOM

	no aTc	yes aTc
P52	0.864011	0.797321
P38	0.911281	0.899616
Wild Type	0.982491	0.897075

Trial 3, LB

	no aTc	yes aTc
P52	0.736762	0.858007
P38	0.364589	0.730237
Wild Type	0.201515	0.318321

Trial 3, BB SESOM

	no aTc	yes aTc
P52	0.947065	0.875336
P38	0.947137	0.856137
Wild Type	0.97293	0.868

no aTc, trial 1 LB:	153014.5
yes aTc, trial 1 LB:	154557.5
no aTc, trial 1 BB SESOM:	140603.5
yes aTc, trial 1 BB SESOM:	160179.8
no aTc, trial 2 LB:	134701.8
yes aTc, trial 2 LB:	140196.5
no aTc, trial 2 BB SESOM:	190332.3
yes aTc, trial 2 BB SESOM:	190831.5
no aTc, trial 3 LB:	188020.8
yes aTc, trial 3 LB:	163524.8
no aTc, trial 3 BB SESOM:	186731.5
yes aTc, trial 3 BB SESOM:	191365

graphpad input for LB
3 trials so 3 points plotted

	no aTc t1	yes aTc 1		no aTc t2	yes aTc 2		no aTc t3	yes aTc 3
P52	0.741333	0.693148	P52	0.913807	0.864915	P52	0.736762	0.858007
P38	0.386516	0.596419	P38	0.582584	1.159209	P38	0.364589	0.730237
Wild Type	0.167103	0.240834	Wild Type	0.224748	0.299752	Wild Type	0.201515	0.318321

graphpad input for BB sesom
3 trials so 3 points plotted

	no aTc t1	yes aTc 1		no aTc t2	yes aTc 2		no aTc t3	yes aTc 3
P52	1.142736	0.898162	P52	0.864011	0.797321	P52	0.947065	0.875336
P38	1.142613	0.914614	P38	0.911281	0.899616	P38	0.947137	0.856137
Wild Type	1.15785	0.994419	Wild Type	0.982491	0.897075	Wild Type	0.97293	0.868

Data Normalization Before Plotting

1. Average technical replicates (ex. 4 wells of one colony of P52 across a row), seen in yellow
2. Averate three colonies of each bacteria (ex. P52-1, P52-2, P52-3 in rows A, B and C)
3. Divide that number by the RFUs of the media (normalization)
4. Plot on GraphPad

Final GraphPad Table

Table format: Grouped	Group A: LB unreduced			Group B: LB reduced			Group C: BB SESOM unreduced			Group D: BB SESOM reduced		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1 P52	0.741333333	0.913807108	0.736762210	0.693148181	0.864915602	0.858007223	1.142736409	0.864011047	0.947065360	0.898161885	0.797320760	0.875336528
2 P38	0.386516202	0.582583993	0.364588745	0.596418865	1.159209277	0.730237064	1.142613211	0.911281457	0.947136664	0.914613635	0.899615929	0.856137486
3 Wild type	0.167102900	0.224747761	0.201514962	0.240833778	0.299751896	0.318320825	1.157849911	0.982450706	0.972930384	0.994419770	0.897074557	0.868000070

B Term no cfg Raw Data

Trial 1, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	113298	52294	129425	53463	87120	53544	54093	53400	83523	61140	153026	192242	143837	168589	164423.5
B	56465	53954	53306	54393	54529.5	53350	55098	53406	63970	56456	201592	191317	183908	187049	190966.5
C	62405	51456	49922	49478	53315.25	52066	52398	50839	51211	51628.5					
D	41830	20588	19996	19875	25572.25	42254	42407	41464	44257	42595.5					
E	49599	35551	21123	20919	31798	57018	56587	54062	58254	56480.25			44452	75960	
F	21658	20595	21009	21372	21158.5	49727	50777	51769	57051	52331			46679	38408	
G	19936	19586	18937	19242	19425.25	18708	17360	17175	17949	17798			52492	63704	
H	22024	20931	21409	50443	28701.75	17807	18253	17974	17898	17983			66099	81633	
					52430.5					64926.25			52430.5	64926.25	
													AVG W3 ^		

Trial 1, Oak SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	98279	126928	142543	93403	115288.3	111446	82582	65865	88422	87078.75	85441	105243	90591	129253	102632
B	197303	185635	144955	134221	165528.5	87204	96178	86587	86844	89203.25	184023	199470	142335	82329	152039.3
C	185223	141845	104297	80778	128035.8	97457	189728	66056	129435	120669					
D	169421	108698	92405	185116	138910	76252	198505	173504	194823	160771					
E	97223	91228	88030	132841	102330.5	106061	179887	105448	83278	118668.5			196410	180633	
F	181281	113401	79721	177071	137868.5	106330	104558	191338	188549	147693.8			204950	152871	
G	195677	112888	97869	111432	129466.5	184142	109974	177231	186452	164449.8			211165	198666	
H	198128	97038	130040	91442	129162	188610	173517	177248	171632	177751.8			205048	168159	
					204393.3					175082.3			204393.3	175082.3	
													AVG W3^		

Trial 2, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	62719	56172	56532	58692	58528.75	51188	52251	62443	51794	54419	179796	110199	99190	117525	126677.5
B	53152	51666	49936	49897	51162.75	53686	73257	51267	61059	59817.25	187696	196832	203387	206059	198493.5
C	76065	57355	74955	56778	66288.25	57187	51915	54164	52108	53843.5					
D	21648	20210	19097	19107	20015.5	50142	55222	57540	49257	53040.25					
E	26482	19808	20401	19909	21650	89662	124483	101953	100308	104101.5			35513	36438	
F	22899	22665	19894	21287	21686.25	69599	61648	62954	71180	66345.25			45678	45539	
G	21009	21452	22381	24255	22274.25	20846	19122	18013	18658	19159.75			48446	31742	
H	25117	22178	21992	21378	22666.25	19576	19846	19758	19736	19729			32270	38850	
					40476.75					38142.25			40476.75	38142.25	
													AVG W3 ^		

Trial 2, Oak SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	79031	108336	85815	107310	95123	130657	78602	70904	80591	90188.5	137371	71518	84974	173975	116959.5
B	81205	68865	70817	70744	72907.75	66282	60965	74805	60632	65671	61829	67087	76136	110833	78971.25
C	80486	65945	77031	72620	74020.5	68740	53313	64680	48796	58882.25					
D	84437	77073	68492	49122	69781	71175	55953	65460	75343	66982.75					
E	66362	59886	63188	70588	65006	78127	71058	76614	82268	77016.75			141632	199618	
F	70901	61976	53189	60693	61689.75	58858	60434	74794	61474	63890			124370	191485	
G	173427	184164	185539	155057	174546.8	71209	75445	77838	82251	76685.75			159121	211453	
H	176648	97453	84229	166717	131261.8	148041	96298	77762	147414	117378.8			104530	192440	
					132413.3					198749			132413.3	198749	
													AVG W3^		

Data Normalization Before Plotting

1. Average technical replicates (ex. 4 wells of one colony of P52 across a row) seen in yellow
2. Averate three colonies of each bacteria (ex. P52-1, P52-2, P52-3 in rows A, B and C)
3. Divide that number by the RFUs of the media (normalization)
4. Plot on GraphPad

Averages of three colonies, not normalized

Trial 1, LB

	no aTc	yes aTc
P52	64988.25	56408.17
P38	26176.25	50468.92
Wild Type	33519.17	33569.08

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	136284.2	98983.67
P38	126369.7	142377.8
Wild Type	154340.6	172427.9

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Trial 2, LB

	no aTc	yes aTc
P52	58659.92	56026.58
P38	21117.25	74495.67
Wild Type	28472.42	25677

Trial 2, Oak SESOM

	no aTc	yes aTc
P52	80683.75	71580.58
P38	65492.25	69296.5
Wild Type	146073.9	130937.8

Averages of three colonies, normalized

Trial 1, LB

	no aTc	yes aTc
P52	0.395249	0.295383
P38	0.1592	0.264282
Wild Type	0.203859	0.175785

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	1.327892	0.65104
P38	1.231289	0.936454
Wild Type	1.503825	1.134101

no aTc, trial 1 LB:	164423.5
yes aTc, trial 1 LB:	190966.5
no aTc, trial 1 Oak SESOM:	102632
yes aTc, trial 1 Oak SESOM:	152039.3

Trial 2, LB

	no aTc	yes aTc
P52	0.463065	0.282259
P38	0.166701	0.375305
Wild Type	0.224763	0.129359

Trial 2, Oak SESOM

	no aTc	yes aTc
P52	0.689843	0.906413
P38	0.559957	0.87749
Wild Type	1.248927	1.658044

no aTc, trial 2 LB:	126677.5
yes aTc, trial 2 LB:	198493.5
no aTc, trial 2 Oak SESOM:	116959.5
yes aTc, trial 2 Oak SESOM:	78971.25

B term no cfg

graphpad input for LB
only 2 trial so 2 point plotted

	no aTc t1	yes aTc 1
mCard constit (52)	0.395249	0.295383
mCard induc (38)	0.1592	0.264282
WildType	0.203859	0.175785

	no aTc t2	yes aTc 2
mCard constit (52)	0.463065	0.282259
mCard induc (38)	0.166701	0.375305
WildType	0.224763	0.129359

graphpad input for sesom
only 2 trial so 2 point plotted

	no aTc t1	yes aTc 1
mCard constit (52)	1.327892	0.65104
mCard induc (38)	1.231289	0.936454
WildType	1.503825	1.134101

	no aTc t2	yes aTc 2
mCard constit (52)	0.689843	0.906413
mCard induc (38)	0.559957	0.87749
WildType	1.248927	1.658044

Final GraphPad Table

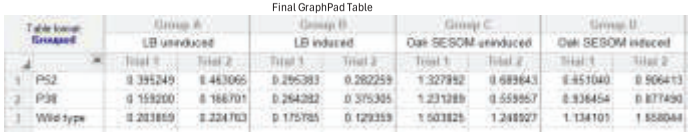

Table format: Grouped	Group A: LB uninduced		Group B: LB induced		Group C: Oak SESOM uninduced		Group D: Oak SESOM induced	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1 P52	0.395249	0.463066	0.295383	0.282259	1.327892	0.689843	0.651040	0.906413
2 P38	0.159200	0.166701	0.264282	0.375305	1.231289	0.559957	0.936454	0.877490
3 Wild type	0.203859	0.224763	0.175785	0.129359	1.503825	1.248927	1.134101	1.658044

B Term cfg Raw Data

Trial 1, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	134845	143511	125649	146016	137505.3	132931	153089	158269	156090	150094.8	185958	198007	192042	197616	193405.8
B	130621	129976	129906	126368	129217.8	143581	148316	142279	141667	143960.8	183068	190838	190683	197482	190517.8
C	125827	131895	127203	129456	128595.3	139274	132443	142608	124021	134586.5					
D	102397	113196	112030	110725	109587	132853	110570	128775	147524	129930.5					
E	116416	129426	123597	106547	118996.5	65188	150198	148032	137063	125120.3			82565	78954	
F	110556	111420	117870	120620	115116.5	136230	127877	137961	133599	133916.8			97790	104883	
G	118804	108484	115176	64859	101830.8	110304	106869	99855	110645	106918.3			95267	110951	
H	106305	116203	118375	111444	113081.8	113748	105932	110733	88075	104622			103317	97213	
					94734.75					98000.25			94734.75	98000.25	
													AVG W3 ^		

Trial 1, Oak SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	159708	185350	192080	188459	181399.3	183122	118579	176616	178605	164230.5	180194	197436	174377	202392	188599.8
B	181244	186374	177141	135517	170069	171294	174210	158991	182830	171831.3	186616	188097	184867	199618	189799.5
C	178984	171307	179194	122397	162970.5	155374	148139	167350	149034	154974.3					
D	132061	184931	187598	140521	161277.8	126268	161256	136363	182271	151539.5					
E	158127	157224	160497	182183	164507.8	162876	155788	176642	153908	162303.5			182353	167130	
F	122600	177930	174316	158399	158311.3	170066	162608	166121	162146	165235.3			174625	184455	
G	193682	181368	160864	193360	182318.5	104334	156264	127637	165119	138338.5			188342	195651	
H	197410	185475	187294	182314	188123.3	183176	154504	138085	163363	159782			201539	181268	
					186714.8					182126			186714.8	182126	
													AVG W3^		

Data Normalization Before Plotting

1. Average technical replicates (ex. 4 wells of one colony of P52 across a row) seen in yellow
2. Averate three colonies of each bacteria (ex. P52-1, P52-2, P52-3 in rows A, B and C)
3. Divide that number by the RFUs of the media (normalization)
4. Plot on GraphPad

Averages of three colonies, not normalized

Trial 1, LB

	no aTc	yes aTc
P52	131772.8	142880.7
P38	114566.7	129655.8
Wild Type	6577.333	103180.2

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	171479.6	163678.7
P38	161365.6	159692.8
Wild Type	185718.8	160082.2

-->

Averages of three colonies, normalized

Trial 1, LB

	no aTc	yes aTc
P52	0.681328	0.74996
P38	0.592364	0.680545
Wild Type	0.034008	0.541578

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	0.909225	0.862377
P38	0.855598	0.841376
Wild Type	0.984725	0.843428

no aTc, trial 1 LB:	193405.8
yes aTc, trial 1 LB:	190517.8
no aTc, trial 1 Oak SESOM:	188599.8
yes aTc, trial 1 Oak SESOM:	189799.5

B Term cfg

graphpad input for LB
only 1 trial so only 1 point plotted for each. and no need for averages since it's only 1 point

	no aTc t1	yes aTc 1
mCard constit (52)	0.681328	0.74996
mCard induc (38)	0.592364	0.680545
WildType	0.034008	0.541578

Final GraphPad Table

Table format: Grouped	Group A		Group B		Group C		Group D	
	LB uninduced		LB induced		Oak SESOM uninduced		Oak SESOM induced	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1	P52		0.681328		0.749960		0.909225		0.862377	
2	P38		0.592364		0.680545		0.855598		0.841376	
3	Wild type		0.034008		0.541578		0.984725		0.843428	

graphpad input for oak
only 1 trial so only 1 point plotted for each. and no need for averages since it's only 1 point

	no aTc t1	yes aTc 1
mCard constit (52)	0.909225	0.862377
mCard induc (38)	0.855598	0.841376
WildType	0.984725	0.843428

B Term Agilent Plate Reader Raw Data

Trial 1, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	36	46	33	35	37.5	39	38	27	32	34	4	4	4	3	3.75
B	26	27	27	26	26.5	28	43	38	41	37.5	0	0	0	6	1.5
C	30	35	37	38	35	50	47	57	52	51.5					
D	5	4	7	0	4	67	81	54	62	66					
E	0	7	3	4	3.5	58	58	62	51	57.25			0	4	
F	6	4	0	6	4	32	38	31	26	31.75			2	5	
G	0	6	0	0	1.5	4	2	0	6	3			1	9	
H	2	2	4	1	2.25	0	1	0	2	0.75			0	4	
					0.75					5.5			0.75	5.5	
													AVG W3 ^		

Trial 1, Oak SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	3	13	1	1	4.5	0	0	2	1	0.75	0	0	0	5	1.25
B	1	6	1	4	3	1	4	0	2	1.75	8	0	0	0	2
C	3	2	0	3	2	0	3	4	0	1.75					
D	0	8	1	0	2.25	6	0	8	5	4.75					
E	6	0	4	3	3.25	0	5	1	3	2.25			4	2	
F	1	0	0	3	1	0	3	5	3	2.75			1	4	
G	0	4	5	6	3.75	0	1	7	1	2.25			0	1	
H	1	3	6	8	4.5	0	9	0	2	2.75			4	9	
					2.25					4			2.25	4	
													AVG W3^		

Trial 2, LB

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	45	51	46	50	48	46	53	40	54	48.25	13	10	12	6	10.25
B	39	60	55	49	50.75	42	52	47	42	45.75	0	9	8	6	5.75
C	54	40	48	42	46	49	40	47	49	46.25					
D	11	12	1	5	7.25	61	71	66	53	62.75					
E	6	0	7	11	6	32	39	34	43	37			3	9	
F	7	8	3	6	6	100	111	114	101	106.5			10	7	
G	6	0	9	9	6	9	8	6	3	6.5			11	7	
H	7	1	9	6	5.75	7	6	3	8	6			6	5	
					7.5					7			7.5	7	
													AVG W3 ^		

Trial 2, Oak SESOM

	1	2	3	4	AVG	5	6	7	8	AVG	9	10	11	12	AVG
A	25	17	15	16	18.25	16	24	21	23	21	17	14	7	13	12.75
B	27	22	23	14	21.5	15	16	26	17	18.5	15	13	6	8	10.5
C	24	21	19	16	20	24	21	20	21	21.5					
D	7	20	12	17	14	26	23	31	33	28.25					
E	23	19	11	12	16.25	30	26	33	31	30			20	15	
F	17	11	2	10	10	24	30	36	23	28.25			9	12	
G	10	15	24	12	15.25	8	5	17	19	12.25			23	6	
H	14	13	18	16	15.25	13	12	19	14	14.5			17	14	
					17.25					11.75			17.25	11.75	
													AVG W3^		

Data Normalization Before Plotting

1. Average technical replicates (ex. 4 wells of one colony of P52 across a row) seen in yellow
2. Averate three colonies of each bacteria (ex. P52-1, P52-2, P52-3 in rows A, B and C)
3. Divide that number by the RFUs of the media (normalization)
4. Plot on GraphPad

Averages of three colonies, not normalized

Trial 1, LB

	no aTc	yes aTc
P52	33	41
P38	3.833333	51.66667
Wild Type	1.5	3.083333

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	3.166667	1.416667
P38	2.166667	3.25
Wild Type	3.5	3

-->

Trial 2, LB

	no aTc	yes aTc
P52	48.25	46.75
P38	6.416667	68.75
Wild Type	6.416667	6.5

Trial 2, Oak SESOM

	no aTc	yes aTc
P52	19.91667	20.33333
P38	13.41667	28.83333
Wild Type	15.91667	12.83333

Averages of three colonies, normalized

Trial 1, LB

	no aTc	yes aTc
P52	8.8	27.33333
P38	1.022222	34.44444
Wild Type	0.4	2.055556

Trial 1, Oak SESOM

	no aTc	yes aTc
P52	2.533333	0.708333
P38	1.733333	1.625
Wild Type	2.8	1.5

no aTc, trial 1 LB:	3.75
yes aTc, trial 1 LB:	1.5
no aTc, trial 1 Oak SESOM:	1.25
yes aTc, trial 1 Oak SESOM:	2

Trial 2, LB

	no aTc	yes aTc
P52	4.707317	8.130435
P38	0.626016	11.95652
Wild Type	0.626016	1.130435

Trial 2, Oak SESOM

	no aTc	yes aTc
P52	1.562092	1.936508
P38	1.052288	2.746032
Wild Type	1.248366	1.222222

no aTc, trial 2 LB:	10.25
yes aTc, trial 2 LB:	5.75
no aTc, trial 2 Oak SESOM:	12.75
yes aTc, trial 2 Oak SESOM:	10.5

B Term Agilent reader

graphpad input for LB
2 trials so 2 point plotted

	no aTc t1	yes aTc 1
mCard constit (52)	8.8	27.33333
mCard induc (38)	1.022222	34.44444
WildType	0.4	2.055556

	no aTc t2	yes aTc 2
mCard constit (52)	4.707317	8.130435
mCard induc (38)	0.626016	11.95652
WildType	0.626016	1.130435

graphpad input for SESOM
2 trials so 2 point plotted

	no aTc t1	yes aTc 1
mCard constit (52)	2.533333	0.708333
mCard induc (38)	1.733333	1.625
WildType	2.8	1.5

	no aTc t2	yes aTc 2
mCard constit (52)	1.562092	1.936508
mCard induc (38)	1.052288	2.746032
WildType	1.248366	1.222222

Final GraphPad Table

Table format: Grouped	Group A: LB uninduced		Group B: LB induced		Group C: Oak SESOM uninduced		Group D: Oak SESOM induced	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
1 P52	8.800000	4.707317	27.333330	8.130435	2.533333	1.562092	0.708333	1.936508
2 P38	1.022222	0.626016	34.444440	11.956520	1.733333	1.052288	1.625000	2.746032
3 Wild type	0.400000	0.626016	2.055556	1.130435	2.800000	1.248366	1.500000	1.222222