REGULATION OF ANTIBIOTIC RESISTANCE GENES IN RESPONSE TO ANTIBIOTICS

An Undergraduate Research Scholars Thesis

by

DUHA ELDOW

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ABSTRACT

Regulation of Antibiotic Resistance Genes in Response to Antibiotics

Duha Eldow
Department of Biochemistry and Biophysics
Texas A&M University

Research Advisor: Dr. Paul Straight
Department of Biochemistry and Biophysics
Texas A&M University

Antibiotic resistance emerged shortly after the introduction of antibiotics into the field of medicine, bringing about a challenging concern. Resistance to antibiotics is encoded by antibiotic resistance genes, among other mechanisms. Antibiotic resistance genes are highly regulated genes that are expressed when antibiotics are introduced. The current research focuses on one resistance gene *ytbD*, encoded by *Bacillus subtilis*. This research will give an insight into *ytbD* regulation by observing the spatial and temporal expression of a luciferase reporter gene fused to the *ytbD* promoters. The expression pattern is observed under the control of different lengths of *ytbD* promoters when ribosome-targeting, including chloramphenicol, and nonribosom-targeting antibiotics are introduced. To build the different lengths of the promoters, we designed primers that will include or exclude predicted regulatory sequences during the engineering of the reporter strains. In doing so, we are trying to test if these upstream sequences play a role in the regulation of *ytbD* and whether antibiotics will affect the regulation pattern. Table A2 has all designed primers listed.

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Thanks also go to my friends and the undergraduates for providing a friendly welcome in the lab. I also want to extend my gratitude to the Undergraduate Research Scholars Program, which provided the guidelines, and to the University Writing Center for editing and polishing my paper.

Finally, thanks to my mother and father for their encouragement and to my coach, Katherine Waskom, for keeping me always aspiring for the best result.

CHAPTER I

INTRODUCTION

Antibiotic resistance emerged shortly after the introduction of antibiotics into the field of medicine [1]. Antibiotic use creates a strong selective pressure that results in the emergence of bacteria that can survive that pressure. In many cases, the genome of the surviving bacteria contains genes known as antibiotic-resistant genes. These genes translate to special metabolic, adaptive responses that help the bacteria in overcoming the inhibitory effects of antibiotics. Previous research showed that there are five main ways antibiotic-resistant bacteria can overcome the inhibitory effects: alteration or modification of the target of the antibiotic, leading to a loss or reduction of the interaction; acquisition of impermeability or increased efflux of antibiotic, decreasing its intracellular concentration; enzymatic detoxification of antibiotic and target [1,2]. There are two major ways that lead to observing antibiotics resistance: acquired and induced. Acquired resistance is obtained through gene transfer. In this form of resistance, the resistant gene can then be transferred vertically or horizontally [9]. The second type of resistance is induced resistance. From the name itself, induced resistance defines the process of activating the expression of antibiotic resistance genes by exposing microbes to antibiotics. Induced resistance is due to an already existing antibiotic resistance gene and in contrast to acquired resistance that is done through gene transfer.

This research is based on resistance genes *B. subtilis,* the model organism in this study. Table 1 lists five antibiotic resistance genes found in *B. subtilis* that are induced by chloramphenicol. The functions and main families of each gene are also listed. The genes *bmrCD, vmlR* and *mdr* are all ABC transporters. ABC transporters require ATP for them to function in effluxing antibiotics [6]. *yxjB* belongs to the methyltransferase family and *ytbD* is from

major facilitator superfamilies [6,7]. The regulation mechanisms of *bmrC*and *yxjB* are known while *vmlR, mdr* and *ytbD* regulation mechanisms are not clearly understood. The current study will provide an insight into both *ytbD* regulation mechanisms.

Table 1: Functions and Regulation Information

Gene	Family	Function	Regulation
yxjB	Methyltransferase superfamily	Code for putative 235 rRNA	Transcriptional attenuation(5)
bmrC	ABC transporter (ATP- binding protein)	Multidrug antibiotic [5]	Repressed by AbrB and through attenuation by bmrB
vmlR	ABC transporter (ATP- binding protein)	Dissociation of antibiotic virginiamycin and lincomycin from ribosome.	Suspected to be sigma regulation by RNA switch regulon.
ytbD	Major facilitator superfamily according to sequence similarity.	Drug resistance against toxic antibiotics	Not Known
mdr	Major facilitator superfamily according to sequence similarity. ABC transporter (ATP- binding protein)	Multidrug antibiotic resistance	Not Known

Note: This information was collected from Subwiki genes' data sheet [10].

Antibiotic resistance genes are highly regulated genes [3]. They are expressed either when associated antibiotics are introduced or when certain conditions are reached, for example, a high population of bacteria. Antibiotics can be either ribosome-targeting or nonribosome-targeting. Ribosome-targeting antibiotics can be specific for either subunit, the 50S or 30S [7]. In general, antibiotics that target the 50S perturbs the binding of aminoacylated-tRNAs at the A- or P-sites while the latter halts the binding or movement of tRNA through the ribosome [7]. Chloramphenicol is a ribosome- targeting antibiotic along with lincomycin and tetracycline. In a previous experiment, the transcriptional analysis showed that the introduction of

chloramphenicol to a *B. subtilis* population increased the expression of certain genes including *bmrCD*, *mdr*, *vmlR*, *ytbD*, and *yxjB*. *bmrCD* and *yxjB* are regulated via transcriptional attenuation, a type of ribo- regulation [8,9]. Ribo regulation is done through untranslated RNA elements that act as terminators or antiterminator [11].

Both *bmrCD* and *yxjB*are regulated via transcriptional attenuation. Attenuators and riboswitches are 5' untranslated RNA elements that are usually located near the promoter of the genes [11]. Antibiotics interfere with this regulation mechanism by allowing the RNA element to switch to the antiterminator form, allowing translation or transcription to proceed. In a previous study by Reilma E et al, it was demonstrated that *bmrCD* is regulated via ribosome-mediated transcription attenuation[8]. The gene *bmrCD* is also encoded with *bmrB,* a small regulatory gene located a few nucleotides upstream from *bmrC*. In the regulatory mechanism, *bmrB*encodes leader peptide. Figure1 illustrates the location of*bmrB*relative to *bmrCD* and demonstrates the steps of *bmrCD* regulation. A finding in the same study shows that chloramphenicol and erythromycin increase *bmrCD* expression by targeting the ribosome. The presence of these two antibiotics enhances the translation of *bmrCD* by preventing *bmrB* from interfering with the ribosome. Figure 1 illustrates this role of antibiotics. As seen by Figure 1, antibiotic molecules bind to the ribosome and slow it down. This gives *bmrB* a chance to switch to the antiterminator form. Another paper by Reiko et al. also studied he function of a small regulatory RNA found within the intergenic region of *vmlR* [9]. In this paper, the disturbance of this regulatory RNA sequence resulted in the constitutive expression of *vmlR* regardless of the presence of chloramphenicol. Besides *bmrC, yxjB*and *vmlR,* we have found that *mdr* and *ytbD* also have small RNA sequences positioned few nucleotides upstream.

Here, we study themechanism for *ytbD* regulation by observing the effect of chloramphenicol on the expression of these two genes with various promoters' lengths and comparing them to *bmrC*, the positive control. By doing this we will try to examine if *ytbD* is regulated via the terminator/antiterminator transcriptional attenuation mechanism. We also built strains of *vmlR* with different portions of RNA regulatory sequence amplified. These strains will be used for future similar experiment.

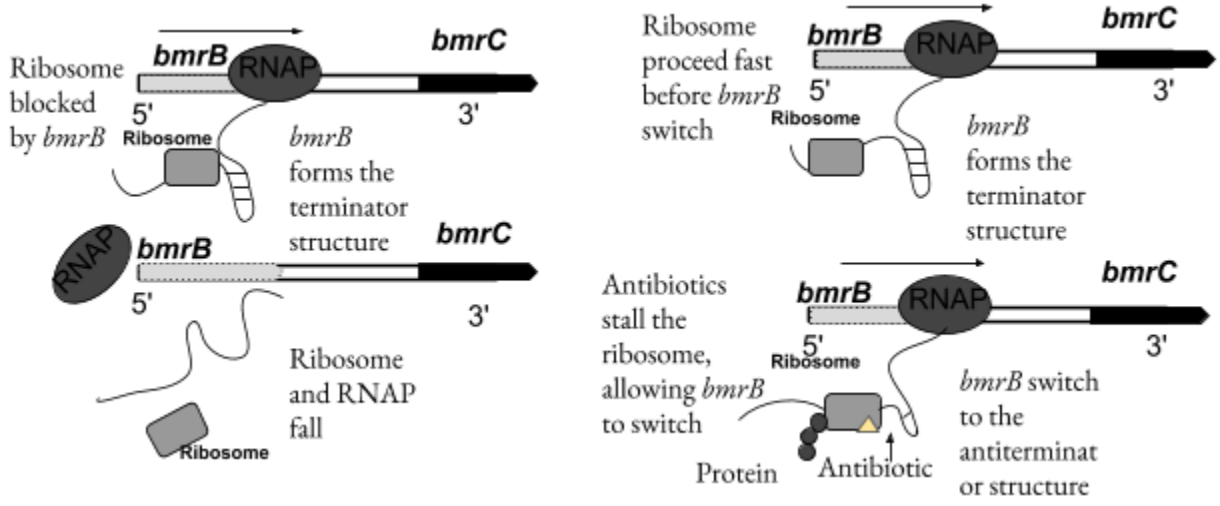


Figure 1: Model of transcriptional attenuation in bmrCD. The Figure demonstrates the regulation mechanism of *bmrCD* (*bmrD*is not shown). In the mechanism, *bmrB* encodes a leader peptide. Soon after *bmrB* is transcribed, it forms a hairpin and blocks the ribosome from continuing translation. Because *bmrCD* and *bmrB* are on the same operon, RNA polymerase will not transcribe *bmrCD*before it transcribes *bmrB*. The stalled ribosome promotes transcription of the downstream *bmrCD* genes.

CHAPTER II

METHODS

Predicting Promoters

Four websites, BROMO, Pepper, CNNPromoter and NNPP, were used to predict the promoters location. Because the promoter of *vmlR*was known from a previous experiment, it was used as a positive control to test the four websites' validity. From this step, we concluded that two out of the four websites, CNNPromoter and NNPP, produce a more valid prediction result. Both websites predict promoters using common markers of the -35 and -10 position, including the sequence of TATA and GA. Table A1 shows each promoter sequence and the specific sequence that helped to predict the location of the promoter. From each website, we had more than one predicted promoter each has a score ranging from 0 to 1, 1 is accurate and 0 is inaccurate. Comparing promoters' results from each website, we picked the ones that appeared on both websites and had the highest scoring. Websites URLs are provided on supplementary pages.

Designing Primers

The plasmid shown in Figure 2 was designed on Benchling, a cloud-based software for digital DNA sequence editing. From Subtiwiki genomic map of *B. subtilis*, we were able to locate the suspected RNA elements upstream to our five genes of interest. After the RNA elements positions were confirmed, we designed two pairs of primers for each of *vmlR* and *ytbD* using Benchling. Two pairs will help amplify only the predicted promoter of both genes while avoiding RNA elements. The other pair of *vmlR* will amplify the whole intergenic region including both RNA elements and promoters. The other pair of *yxjB* and *ytbD* will amplify only

a specific area of the intergenic region. Figure 3A illustrates the location of the deleted portion relative to each desired gene and Figure 3B illustrates the position of attachment of each primer designed. For all three genes of interest, the copied region was then added to the plasmid on Figure 2 as a fragment named promoter. By using Gibson Assembly, the plasmid (derived from pDR111) illustrated in Figure 2 was assembled.

Building Strains

Built plasmids were incorporated into NCIB3610 *B. subtilis*genome through both transformation and SPP1 transduction. Heat shock transformation was performed on E. coli for replication. Plasmids from *E. coli*were then transformed to *B. subtilis* PY79 by one step transformation. SPP1 phage transduction was performed to transfer the designed plasmid from PY79 genome to NCIB3610 genome. To check for the position of the designed plasmid within the NCIB3610 genome, amyE-up655-forward and luxA-Rev-200 were used for the preceding PCR reaction. Table A3 has all strains designed listed.

Plate Assay

GYM plate adjusted to pH 7 was used for plate reader assay. For each strain two plates were prepared, a control plate with no antibiotics added and an experimental plate with 0.312 µg/ml of chloramphenicol. 1.5 µl from an OD_{600} 1 culture for each strain was spotted on the middle of the plates and allowed to dry before incubation at $30^{o}C$. Plates were viewed using chemiluminescence imager for luciferase signal detection at 6, 24 and 48 hours of incubation.

Plate Reader Assay

Expressions of the *bmrC* and the two *ytbD* strains were viewed via Pro200 Tecan plate reader for growth curve and luminescence determination. Each strain had three duplicates for

each antibiotic used: chloramphenicol, spectinomycin, chloramphenicol and phleomycin. These antibiotics include both ribosome targeting, chloramphenicol, spectinomycin and lincomycin; and nonribosome-targeting, phleomycin. Both chloramphenicol and spectinomycin induce *B. subtilis* sliding motility. The antibiotics were applied with their subinhibitory concentrations: 0.312 μg/ml for chloramphenicol; 12.5 μg/ml for spectinomycin; 1.56 μg/ml for lincomycin; 0.005 μg/ml for phleomycin. 10 μL OD $_{600}$ 1 strain culture and 10 μL of each antibiotic was added to the 96-well plate. For the control only 100 μL OD $_{600}$ cultures were added. The plate reader will receive signals of various wavelengths from the *lux* gene that is fused to the promoter of the gene of interest in each strain. The light signal emitted due to the presence of *lux* operon is done through an oxidation reaction of reduced riboflavin phosphate (FMNH2) and a long chain fatty aldehyde [11]. *Lux* operon has 5 genes: *luxA,luxB,luxC,luxD,* and *luxE* [11, 12]. luxA and luxB are responsible for the expression of luciferase, the oxidation enzyme for the reaction [12]. *luxC, luxD* and *luxE* encode for reductase and transferase polypeptides of the fatty acid reductase, the enzymes responsible for FMNH2 production [12]. The light emitted from the oxidation reaction can be detected by the plate reader and recording automatically and immediately. The recording period lasted 16 hours.

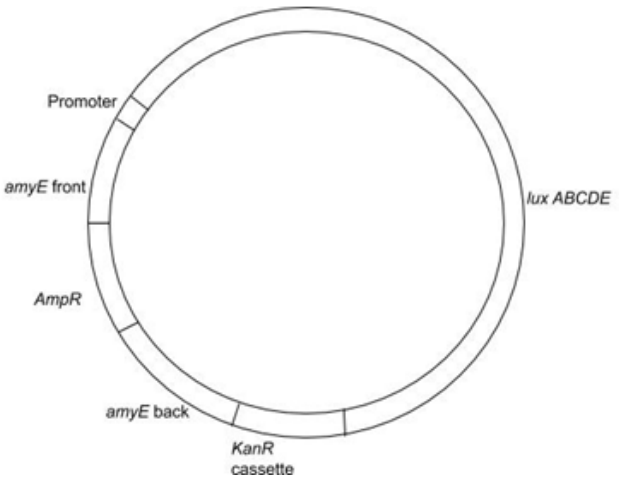
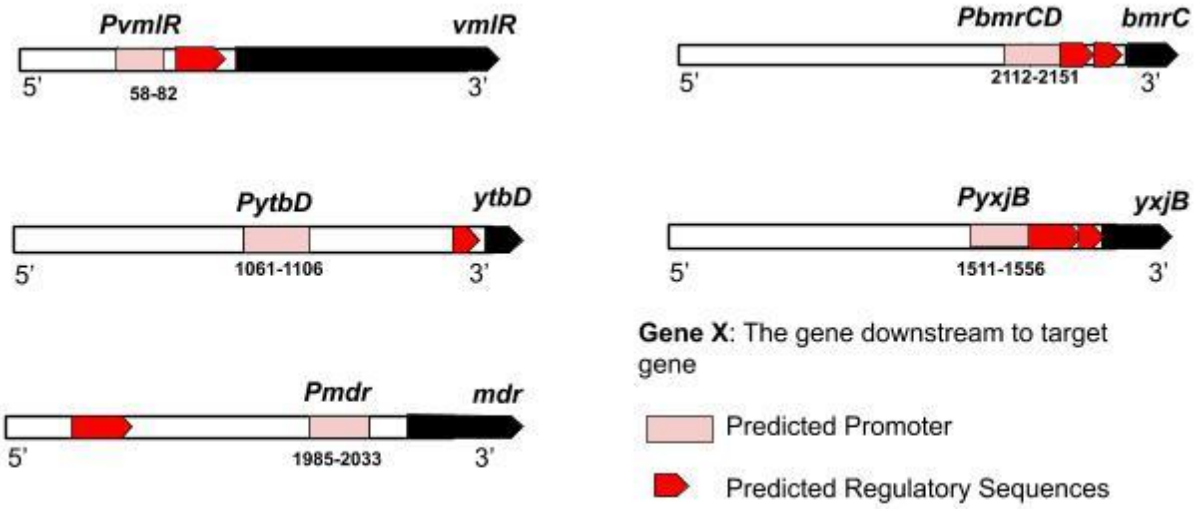


Figure 2: Plasmid construct used for all 5 genes.

A



B

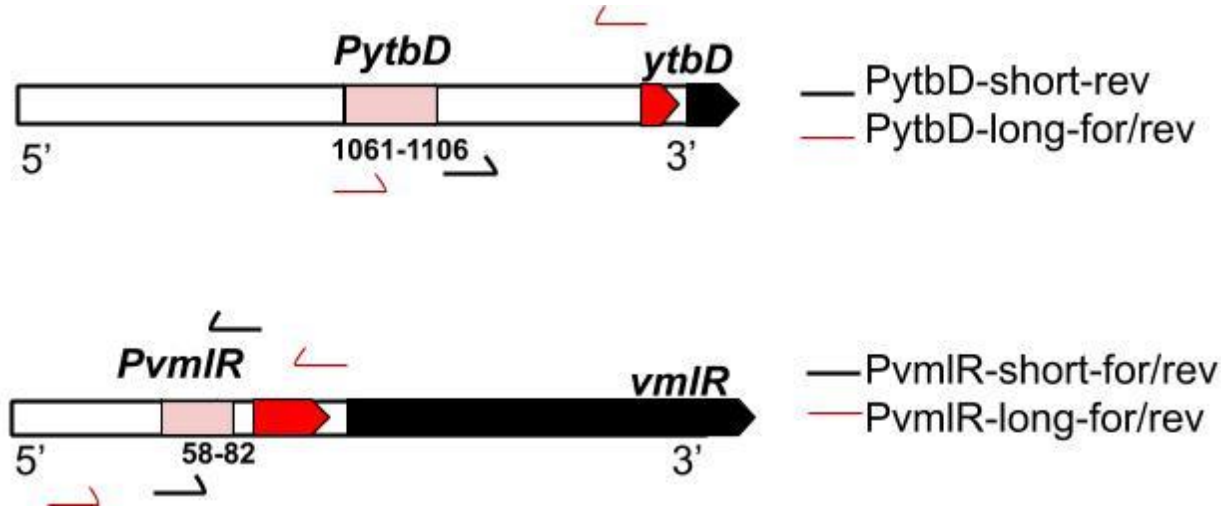


Figure 3 A-B: Relative location of the promoters and the RNA regulatory sequence of each gene. A. This image illustrates the analysis of the intergenic region of each gene. Gene X indicates the gene that precedes our gene of interest. The location of promoters for each gene is shown above by the pink squares and the number underneath the promoters indicates the exact location of the promoter within the intergenic region. The red pointed squares represent the location of the suspected RNA element relative to the promoter location. B. includes the primer designed and the location of their attachment. The grey rectangles are the predicted promoter and the red pointed box is the predicted RNA regulatory sequences for each gene

CHAPTER III

RESULTS

When chloramphenicol was introduced to*B. subtilis*, the expression of five genes, *bmrCD*,*mdr*,*ytbD*,*yxjB* and *vmlR*,increased. These genes are highly regulated and chloramphenicol seems to play an important role in halting down the regulation mechanism. For *bmrCD* and *yxjB* the regulation is found to be transcription attenuation and chloramphenicol bind to the ribosome to affect the attenuation mechanism. This study will focus on *ytbD*, trying to understand its regulation mechanism by assessing their temporal and spatial expression using a plate reader and plate assay, respectively. Different strains with *ytbD* predicted regulatory RNA sequences removed will be designed with *lux* reported genes fused to their promoters. Two questions were expected to be answered from this study: how gene expression patterns are affected with the removal of this regulatory RNA sequence and do this pattern differs from one antibiotic to another, namely ribosome-targeting and nonribosome-targeting antibiotics.

Small regulatory RNA elements were found near the predicted promoters of *mdr*, *yxjB*, and *ytbD*. To identify promoters for the four genes, CNNPromoter and NNPP websites were used. The *vmlR* promoter was previously known from research by Reiko et al, and was used as a positive control. To locate the positions of RNA elements, we used Subtiwiki. Figure 1 demonstrates the relative positions of RNA elements to promoters along the intergenic regions. For each gene, we found out that the RNA elements were upstream from its promoter except for *mdr*. As Figure 1 shows, a small portion of the RNA elements within *ytbD* and *vmlR* intergenic regions were part of their predicted promoters. We designed primers to amplify the promoters

while avoiding the amplification of RNA elements with it or trying to not amplify all of them. In doing so, our goal is to have removed all RNA elements that might have been regulating the five genes expression.

Plate assay showed that *PytbD-lux*-S and *PbmrC-lux* are expressed in similar locations across the sliding population. The purpose of the plate assay illustrated in Figure 4 is to observe the spatial expression of the genes and also the relative intensity of *lux* expression for both*PytbD-lux*-S, *PytbD-lux*-L, and *Pbmrc-lux*. This assay will help locate the areas of the highest expression of the gene across the sliding population. Due to the fusion of the *lux* reporter gene to the promoters of the genes, we could observe for each gene across the sliding population. Based on Figure 4, *ytbD* and *bmrC* are mostly expressed around the edges. Also, *PytbD-lux*-S and *PytbD-lux*-L show no difference in the *lux* gene expression (both of them had very low expression) indicating either *PytbD-lux*-S strain is not constitutive or its promoter is not present. The result of the plate assays is summarized in Table 2.

Table 2: Summarized Result of Plate Assay

Strain	**Result of *lux*expression**	**Locality of *luminescence***
PbmrC-lux	Signals were observed at 6hrs interval	Signals are more concentrated around the edge
PytbD-lux-L	Signals were observed at 6hrs interval but decreased afterword	Signals are more concentrated around the edge
PytbD-lux-S	Signals were observed at 24hrs interval but decreased afterword	Signals are more concentrated around the edge

Plate assay and plate reader assay showed that different antibiotics have different effects on *ytbD*. Based on the result of plate reader we decided to perform plate reader assay which is more sensitive to lux reporter gene signals. The plate reader assay measures the pattern

of temporal signals. From this assay, we hoped to observe the relative effectiveness of each antibiotic on the expression of the targeted genes. Moreover, we wanted to observe if the removal of the RNA regulatory sequence will change the pattern of gene expression. For this assay, absorbance was measured by subtracting blank absorbance from the sample absorbance and a growth curve of absorbance against time was generated and illustrated in Figure 5A. Figure 5A shows the absorbance of each sample against time elapsed to test whether there is growth inhibition done by the subinhibitory concentration of the antibiotics used. For all strains the absorbance values began to decrease beyond eight hours, regardless of what type of antibiotics was used. All antibiotics inhibit growth since the blue line (no antibiotics) has more absorbance during the exponential phase (from four hours to eight hours roughly) than other lines. Comparing the exponential phase of all antibiotics, for all strains, spectinomycin seems to be causing the most detrimental effect on cell growth followed by phleomycin and lincomycin, which have almost similar effects, and finally chloramphenicol. The effect of spectinomycin among three strains does not significantly vary. However, For *PytbD-lux*-S and*PytbD-lux*-L, spectinomycin seemsto be slightly more effective with *PytbD-lux*-S. For other antibiotics, no obvious difference is observed.

Normalized luminescence signals were then calculated by dividing the luminescence signal measured by the plate reader with the associated absorbance and illustrated in Figure 5B. The luminescence signal charts use trendline (not shown on graphs) slope as an indication for the level of the effect of the antibiotics on the gene expression. For *bmrC* chloramphenicol is the most effective as expected. Phleomycin effect is almost similar to the control since it's a nonribosome-targeting antibiotic. Since *bmrC* is regulated vai attenuation, it requires antibiotics to bind to the ribosome to affect its expression. *PytbD-lux*-L seems to be slightly more affected

by spectinomycin. However, the difference in the presence of RNA regulatory sequences in both strains indicates that lincomycin seems to be equally effective to spectinomycin when RNA regulatory sequences are removed. The effectiveness of spectinomycin versus lincomycin and chloramphenicol in *ytbD* cannot be concluded since *PytbD-lux*-S luminescence are very low to state this comparison.

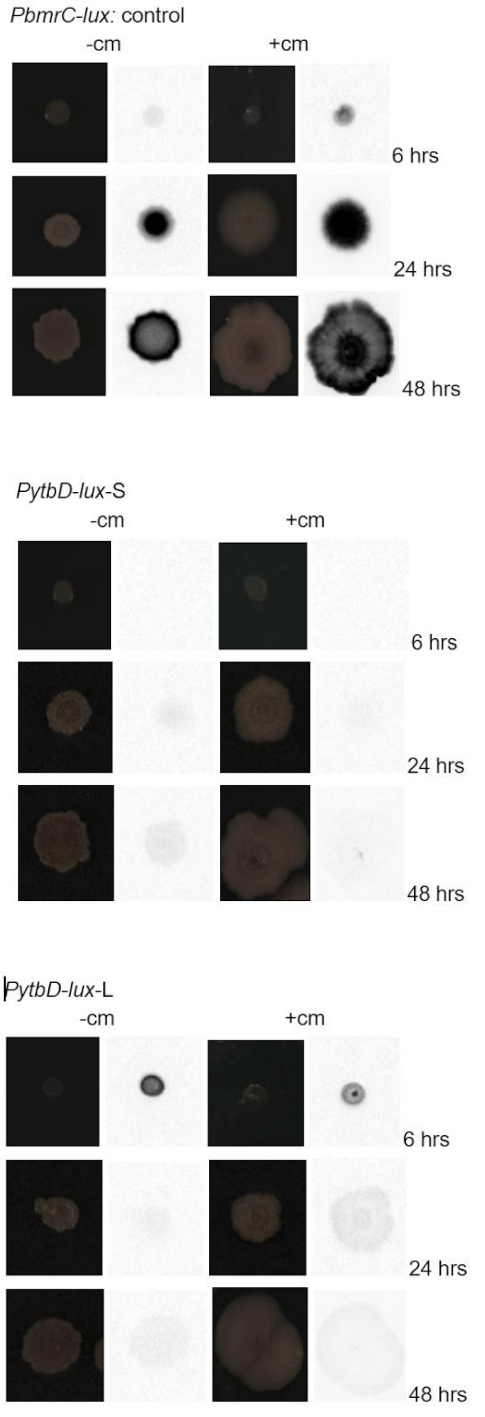


Figure 4: Plate Assay.The plate assay views the spatial expression of *lux* gene which fused to the target genes. For both*PytbD*-S-lux and *PytbD-lux* -L-luminescences are observed as clearly as expected, but the location of the highest intensity can still be viewed. *PytbD-lux*-S expressions seem to be more concentrated at the center.

A

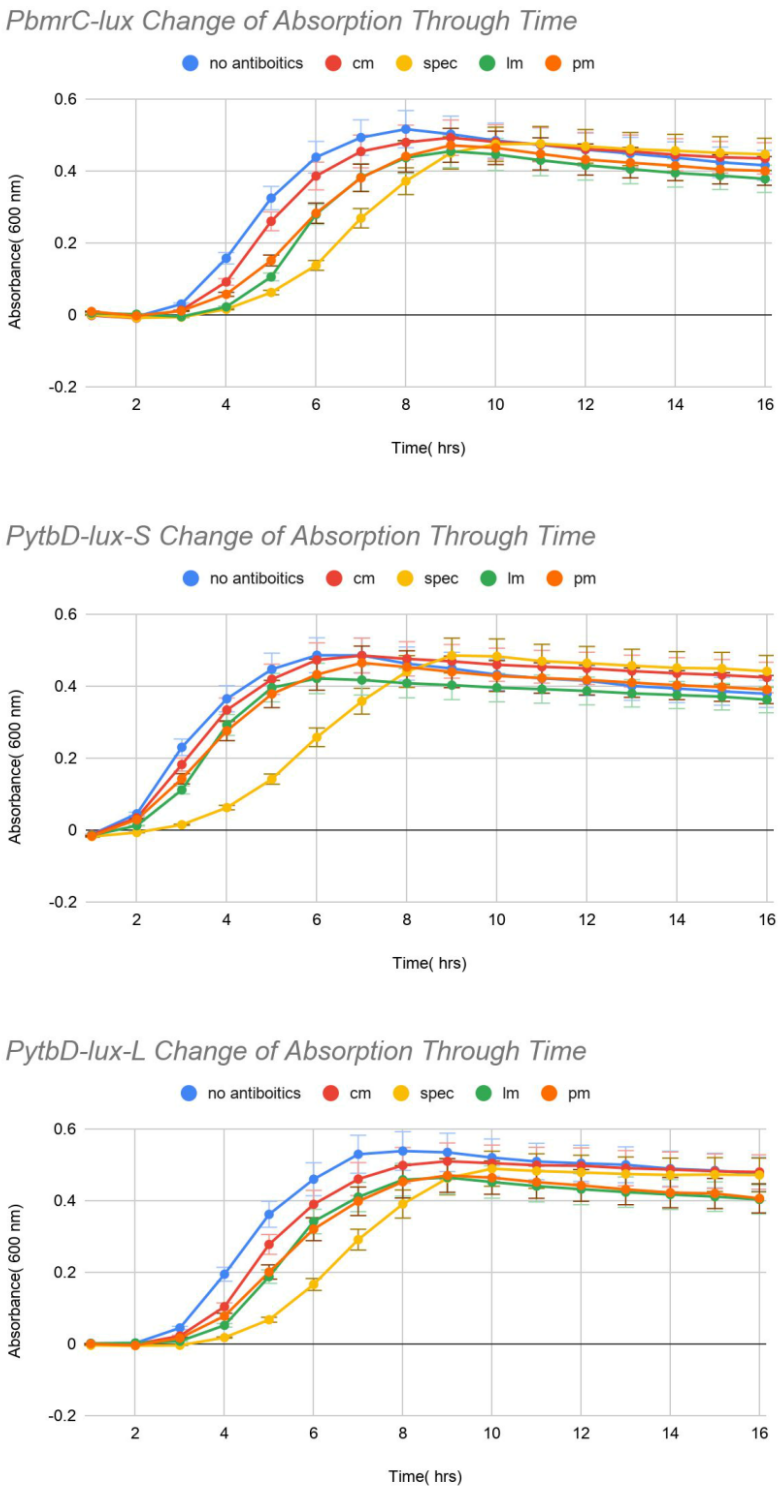


Figure 5A: The graphs of plate readers result. A. The absorbance at 600 nm is measured to calculate for growth.

B

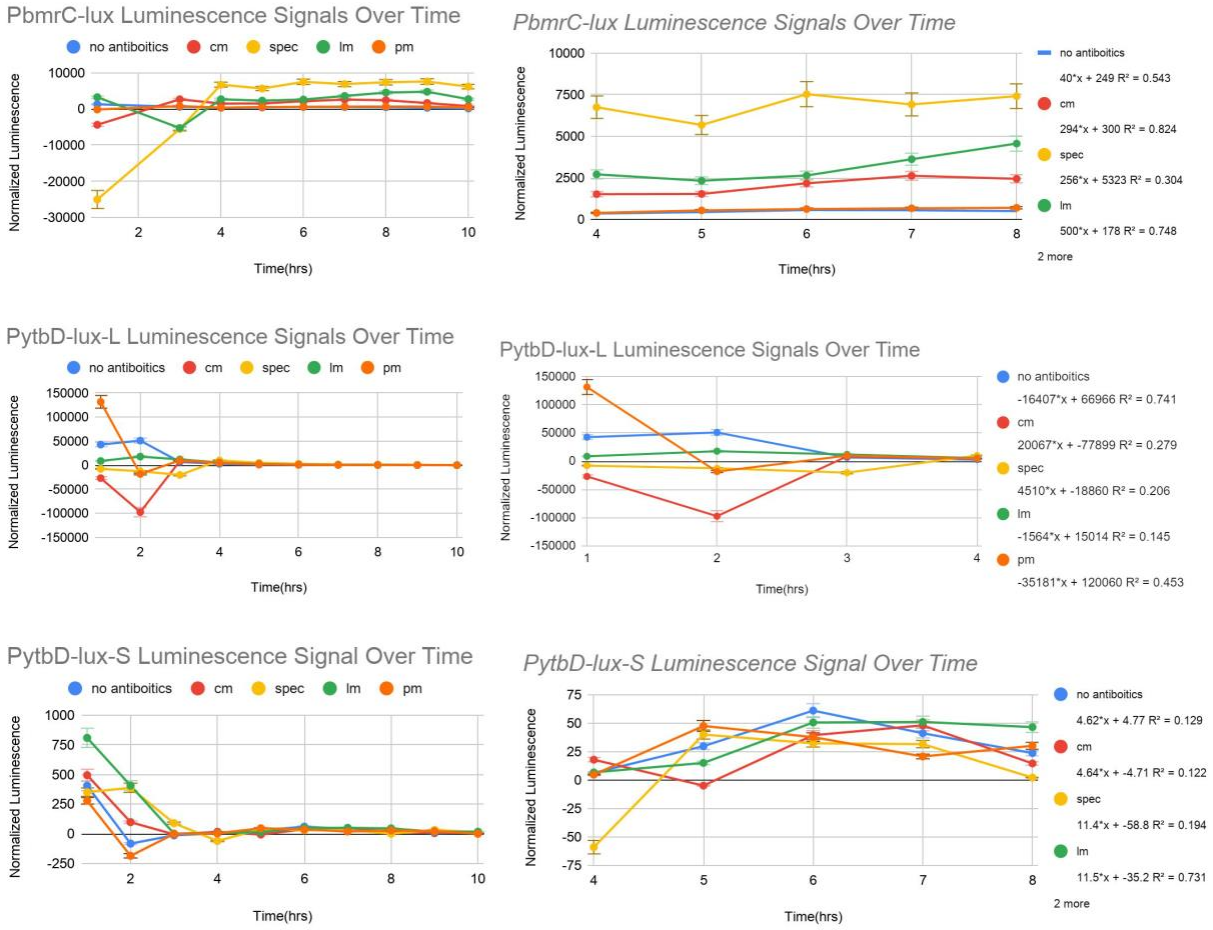


Figure 5B: The graphs of plate readers result. B. The graph to the left illustrates the overall trend of luminescence versus time. The graphs to the right illustrate the trend of only selected time frames that have obvious measurable data. These figures use the slopes of the trendlines to measure the intensity of the effect of each antibiotic on the expression of the lux gene fused to the represented genes.

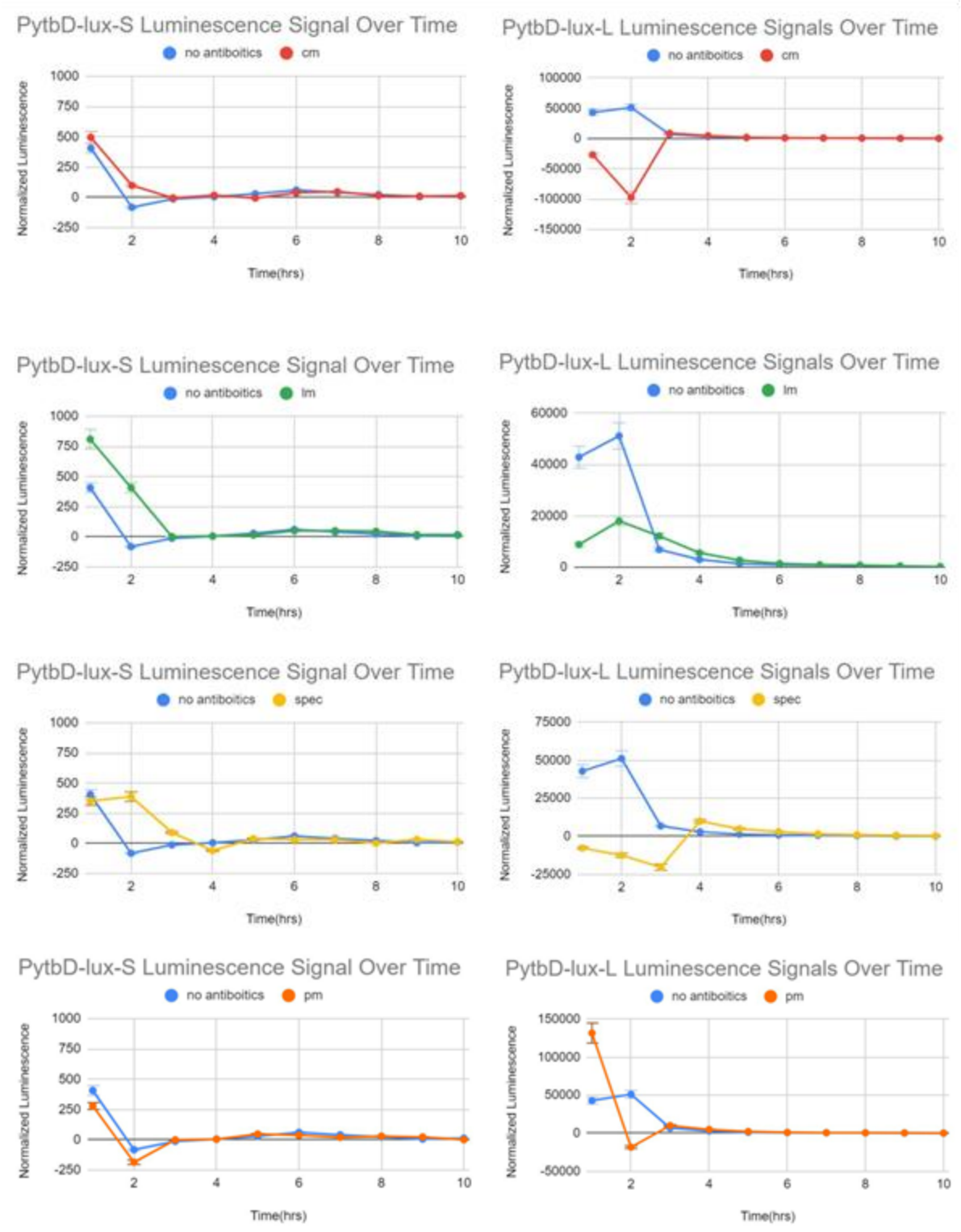


Figure 5C: The graphs of plate readers result. Individual antibiotics luminescence pattern. The graphs separate the antibiotics treatments results for easier comparison.

CHAPTER IV

DISCUSSION

Regulation of antibiotic resistance genes is by far a crucial process of a normal resistance cell. For our model organism in this study, *B. subtilis*, we have found five antibiotics resistance genes: *bmrCD, mdr*, *ytbD, yxjB, and vmlR*. Because *yxjB* and *bmrC* are well- studied, we picked *ytbD* as the main focus of this study.

For *B. subtilis*, chloramphenicol was associated with all five genes mentioned above based on a previous study done by our lab. As noted from plate assay, chloramphenicol induces sliding motility which is a phenotype indicating that bacteria is responding to the subinhibitory concentration of the antibiotic. From this assay, we can observe the location of where each of the genes has the highest expression, which is observable due to the presence of the *lux* reporter gene. Both *bmrC* and *ytbD*are highly expressed at almost the same location across the sliding population. The expression of both genes is mostly at the edge, assuming that it is blocking antibiotics from reaching the inner population. Based on Subtiwiki [10], the function of *bmrC* is an ABC transporter. Even though a conclusion of *ytbD* as an ABC transporter can't be drawn from this result, it supports that *ytbD* might be a transporter protein as well.

The temporal expression was assessed using the plate reader assay described earlier. This assay measures the luminescencesignal and absorbance under a 600 nm wavelength. The luminescence signalsfrom *PytbD*-*lux*-S were not very strong. For *PytbD*-S, since the amplified promoter fragment did not include the same promoter of *PytbD*-*lux*-L, the amplified promoter fragment sequence was run through CNNPromoter and NNPP website to look for any strong predictable promoter present. Both websites predicted two promoters at different locations. The

promoters that were predicted had a score of 0.9 and 0.995. These promoters are at different locations than the promoter illustrated in Figure 3A. The promoter position in Figure 3A was based on the previous promoter prediction trial and has a score of 0.98. A third strain shall be designed that has a promoter amplified from the area between the 0.9 and 0.98 predicted promoters. A comparison between this strain and the other 2 strains used in this study can help finalize the location of the promoter. Table A1 provides more detailed information on all the promoters predicted.

Comparing the plate reader expected result of *bmrC*to both strains of *ytbD*, two conclusions can be made. First, *ytbD* is not very effectively controlled via phleomycin which indicates a possibility for its regulation by attenuation. Second, from the trendline slopes in *PytbD-lux*-L, spectinomycin affects *ytbD* regulation more than chloramphenicol. This conclusion can also be drawn from the absorption graphs shown in Figure 5A. For*PytbD-lux*-S and*PytbD-lux*-L, spectinomycin seems to inhibit growth more effectively with *PytbD-lux*-S. For other antibiotics, no obvious difference is observed. When the RNA regulatory sequences were removed spectinomycin inhibited growth more, indicating that the removed RNA regulatory sequences might be needed for *ytbD* regulation and that spectinomycin plays a direct role in regulation. However, the very low signal observed in *PytbD-lux*-S indicates either the promoter of *ytbD* might have been distorted during amplification or the removed RNA sequences do not play a role in *ytbD* regulation. From the bioinformatics data that analyzed the promoter location (Figure 3B), we can rule out the first assumption since the promoter was fully amplified. Shall the predicted promoter be the wrong promoter is a third assumption that also needs to be further tested.

Can the two questions asked by this study be answered by this result only? For the question regarding whether ribosome-targeting and nonribosome-targeting antibiotics affect the expression differently, it can be answered with yes by this study result, however, there is no obvious difference among the effects of the three ribosome-targeting antibiotics used. On the other hand, the question of whether regulatory sequences of *vmlR* and *ytbD* are responsible for the change in expression and regulation of antibiotic resistance genes, including if antibiotics are associated with the regulatory sequence, cannot be answered fully. The very low signal of *PytbD-lux*-S does not allow for us to compare it accurately with *PytbD-lux*-L. Furthermore, the NCIB3610 *vmlR* strains produced with and without the RNA regulatory are not tested yet.

CHAPTER V

CONCLUSION

This research was done to study the regulation of *ytbD* through the use of both plate reader and plate assays as a method to measure the spatial and temporal luciferase signals, respectively. Although not all questions asked were answered, future recommendations from the result can be given. For *ytbD,* a new promoter introduced in the discussion part of this paper shall be amplified with and without the RNA regulatory sequences. Furthermore, designing strains that will amplify different portions of RNA sequence will help to know which part has the highest effect. Although the question regarding the effect of RNA regulatory sequence on regulation was not answered, it can still be answered if *PytbD*-*lux*-S can be redesigned to produce stronger luminescencesignals with the new promoter. A strain with *lux* reporter gene can be used as a control to roll out for non-functionality of the *PytbD*-*lux*-S strain.

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APPENDIX

Table A1: Promoters Sequences for Figure 3A

Gene	Promoter Sequences
yxjB	5'-GAAACAATCAGACCATATTACTGATCATATACAAGAATGTCTTTTTCTAAGATGATAGCAGTATTTTATCCTTTCACTTTTTGAAAATCAACCTTTTTAACTATTTTCACAATAATAAAATTTATATTCTAAAATGAGAAAATAAAGGAAAAACATGCTGGATTGCTATGCTGATATGATGTTGGCGGGATACCAG-3'
bmrC	5'-ATACGGTCAGCATGGAACATCCTTCTCACCCTTTCAAAGCGTCTTACAACACTACTACCCGAATTTGAGCACAATTAACCTTTTCCTATTCAAGTAATGATTGACAATAAAAGGTTTTGTTTATATGATGATAAAAAGATGAAATCAGAATGGAAGGAGGGTTTGCTATGCCAAGGAATTTGCGTGTTTATCAA-3'
vmlR	5'-GACTCAAAACTCCTGCCTCAAAAATGAGAGCAGGAGTTTTTTTGATGAAAATGACCTTTGCTTTTGAATTTAAAGGTATGCTATAGTGTTTGTAATCAAA-3'
ytbD	5'-CAATATCATATTCCCGCCAAGGCAGCTGTTTTGGATCACGGCTGTTTAACAAAAGCACTTTCTTTCCATTTACGATCAGGCTATCTTCACCAGCCACAACCTCTTTGTCGTATCTGCCGTGAATTGTGTCATACTTTATTAAATGAGCCAGCGTTTCTGCGGAATAGCTGGCGTTAATGGCCACTACTTGAATTTG-3'
mdr	5'-AACAGACCGAGAGCTTGAGCGGCTGCCTGATGAAACAGAACCGTTCTTGTGCCCAGTTTTCGAAAAAGCTGAACAGCAGTCTTTTCCAACTCACTTCTGGAATTTCTTGTTGACAACTGACAGTCCTCCCTATAAAATCGAGTTTATCTTGATAATCGAGATATTCAACAATCAAACTATTATCATGATATCAGAACTT-3'

Table A2: Primers Sequences.

Promoters	*lux sequence- Primer For*	*amyE sequence -Primer Rev*
PvmlR-lux- S1	5’GGTCTGATCGAAATAGTACAGACTCAAAACTCCTGCCTCA3’	5’CTTTAAAGCGAGGGATATGG3’
PvmlR-lux- S2	5’GACTCAAAACTCCTGCCTCA3’	5’ATTTCATAGGCTAGCCTCCTTTTGATTACAAACACTATAG3’
PyxjB-lux-S	5’GGTCTGATCGAAATAGTACAGAAACAATCAGACCATATTAC3’	5’ATTTCATAGGCTAGCCTCCTCTGGTATCCCGCCAAC3’
PytbD-lux-S	5'TACACTCTGTTAATATGATTCG3'	5'ATTTCATAGGCTAGCCTCCTCAAATTCAAGTAGTGGCCAT 3'
PytbD-lux-L	5'TACACTCTGTTAATATGATTCG3'	5’ATTTCATAGGCTAGCCTCCTATATCGTTAAGAAATAAAAGTAC3’
PbmrC-lux-S	5'GTCTGATCGAAATAGTACAATACGGTCAGCATGGAACAT 3'	5'ATTTCATAGGCTAGCCTCCTTTGATAAACACGCAAATTCC 3'
Pmdr-lux-S	5'GGTCTGATCGAAATAGTACAAACAGACCGAGAGCTTG 3'	5'ATTTCATAGGCTAGCCTCCTAAGTTCTGATATCATGATAATAG 3'

Shorter primers don't have the *lux/ amyE* sequence overhang

Table A3: Strains information

Name	luminescence in ecoli	luminescence in PY79	luminescence in NCIB3610
PvmlR-lux-S1	p	p	p
PvmlR-lux-S2	p	p	n
PvmlR-lux-L	p	p	n
PyxjB-lux-L	p	p	p
PytbD-lux-S	n	n	n

Promoter Prediction Websites URL
NNPP:https://fruitfly.org/seq_tools/promoter.html
CNNPromoter:http://www.softberry.com/berry.p