When Life Gives You Lyme: Investigating Repressors Important in the Transmission of Lyme Disease

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Abstract

Lyme disease is caused by *Borrelia burgdorferi*, a spirochete that infects across phyla from ticks to humans. BadR and Hbb are transcriptional repressors that are involved in transitioning the spirochete for survival in mammals, however their targets are largely unknown. We created a reporter system to screen candidate promoters for regulation by BadR and Hbb. We show the reporter system is functional in *E. coli*, and could be used in the future to identify therapeutic targets to inhibit *Borrelia* infections.

Introduction

Lyme disease by infection of Borrelia burgdorferi

An average of 30,000 cases of Lyme disease occur per year, making it the most common vector-borne illness in the United States (Meyerhoff 2017). Lyme disease is an inflammatory disease most commonly caused by infection of *Borrelia burgdorferi*. *B. burgdorferi* is a spirochete that can be transmitted to mammals by the bite of a deer tick, mainly *Ixodes scapularis*. As climate change has worsened, the habitable zones for this species have increased and have become more conducive to greater tick populations (Brownstein et al., 2005). As *I. scapularis* populations have risen, infection rates of Lyme disease have also risen, indicating that there is a direct comparison of the tick population size with the occurrence of Lyme disease. Within the past 10 years, the steady increase in Lyme disease cases equates to about 10% per year (Leland 2014).

One of the earliest signs of infection by *B. burgdorferi* is a skin rash, typically in the shape of a bullseye, that appears at the tick bite site. Lyme disease affects mammals in three stages (Figure 1). The first stage begins soon after the initial infection and the host typically exhibits flu-like symptoms such as fatigue, fever, and headaches. Within a few months of the initial infection, the host will begin to exhibit stage two symptoms including numbness, carditis, and Bell palsy. Months to years after the initial infection, the host may begin to exhibit symptoms connected to the third stage of Lyme disease including damage to cardiac nerves, swelling of the brain, joint pain, and swollen lymph nodes (Hellwage 2000). In order for a mammal to exhibit symptoms of stage three Lyme disease, the spirochete must remain in the host for up to a year after the initial infection (Steere 1983). Recent research indicates that the spirochete has a variety of mechanisms in which it is able to evade the immune system that have yet to be fully researched (Bykowski 2007).

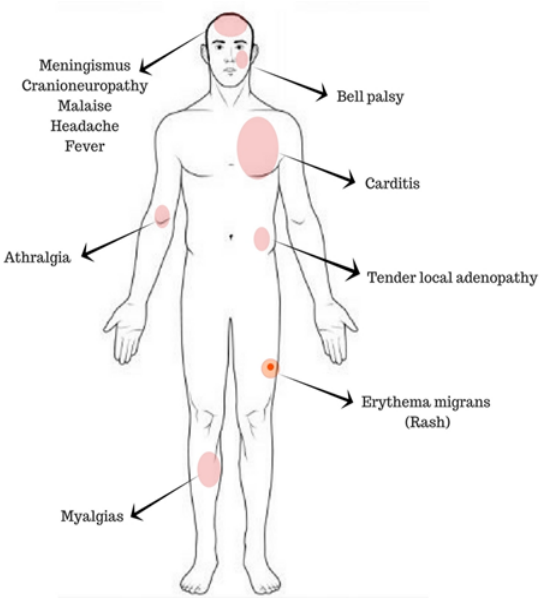

Figure 1: Diagram of symptoms caused by Lyme disease infection (Hellwage 2000).

Borrelia burgdorferi Regulation Factors

B. burgdorferi depends on its host for nutritional needs because it has a small genome that contains few metabolic genes (Fraser et al., 1997). Many of the genes present in the spirochete have functions related to the invasion of and survival in hosts with vastly different internal environments. *B. burgdorferi* must increase its infectivity and change metabolic rates to adapt from the tick vector to the nutrient rich environment of the mammalian host. In order to successfully travel back to the vector to infect more hosts, the spirochete must readjust to the nutrient sparse environment of the tick to survive. This transition between environments is accomplished through the up and down regulation of different genes within *B. burgdorferi* (Caimano et al., 2007).

RpoS, one of three sigma factors encoded in the *B. burgdorferi* genome, is essential for mammalian infection and likely upregulates genes needed to transition between a tick and mammalian host environment. Mutants lacking RpoS are unable to transition out of the tick gut to infect mammals (Dunham-Ems et al., 2012). Among the genes RpoS regulates are outer surface proteins (Osps). The Osps play an important role in the survival of *B. burgdorferi* from host to host. These surface proteins have functions ranging from host colonization to evasion of the host's immune system. Most, if not all, of these Osps are essential for the infection and survival of spirochetes in either arthropod or mammalian hosts (Kenedy et al., 2012).

OspC is an example of an Osp that may be involved in immune system evasion during mammalian infection. There is little detectable OspC expression in mutants lacking RpoS, while constitutively expressed RpoS mutants show high levels of OspC (Huber et al., 2001). OspC evades recognition by antibodies and prevents activation of CD4 cells in the host immune system by binding to Salp15, a protein found in tick salivary glands (Anguita et al., 2002).

Borrelia Host Adaptation Regulator (BadR)

RpoS expression is thought to be regulated by Borrelia host adaptation regulator (BadR). BadR binds around -10 upstream of the gene repressing RpoS expression (Ouyang and Zhou, 2015). Regulation of the RpoS regulon is imperative to *B. burgdorferi* survival as constant RpoS expression results in cell death. Down-regulation of RpoS is necessary for spirochete survival in the low nutrient tick environment because if spirochete metabolism is high when nutrients are lacking, it will die. RpoS can be upregulated in host animals as the host provides plenty of nutrition to maintain a higher spirochete metabolism (Chen et al., 2013). Because of this need for regulation, *B. burgdorferi* developed BadR, a very specialized member of the repressor, open reading frame, kinase (ROK) family of regulators. The regulation of RpoS by BadR can be seen in Figure 2 below.

BadR has many differences from most ROK proteins. In many ROK proteins, DNA binding is facilitated by one distinct inducer or effector, usually some type of sugar. In BadR, it is found that sugar binding is not involved, so an unknown metabolic intermediate acts as an allosteric regulator of BadR (Miller et al., 2013). In a nutrient-deficient environment, the metabolic intermediate is not present to prevent BadR from binding to DNA once again. Another

difference between BadR and most ROK proteins is that the sequence homology between BadR and usual ROK proteins is found to be fairly low. Also, while most ROKs bind to DNA through many specific residues on DNA, BadR only conserves about two of the amino acids that ROKs do, G153 and H243. An important factor learned about BadR DNA binding is the sequence motif critical for binding to: TAAAATAT or ATTTTATA. This sequence, though similar to other sequence motifs for similar regulators, contains key nucleotides for BadR to bind to. The various differences between BadR and many of its ROK family members showcase the specialized role that BadR plays in *B. burgdorferi* (Ouyang and Zhou, 2015).

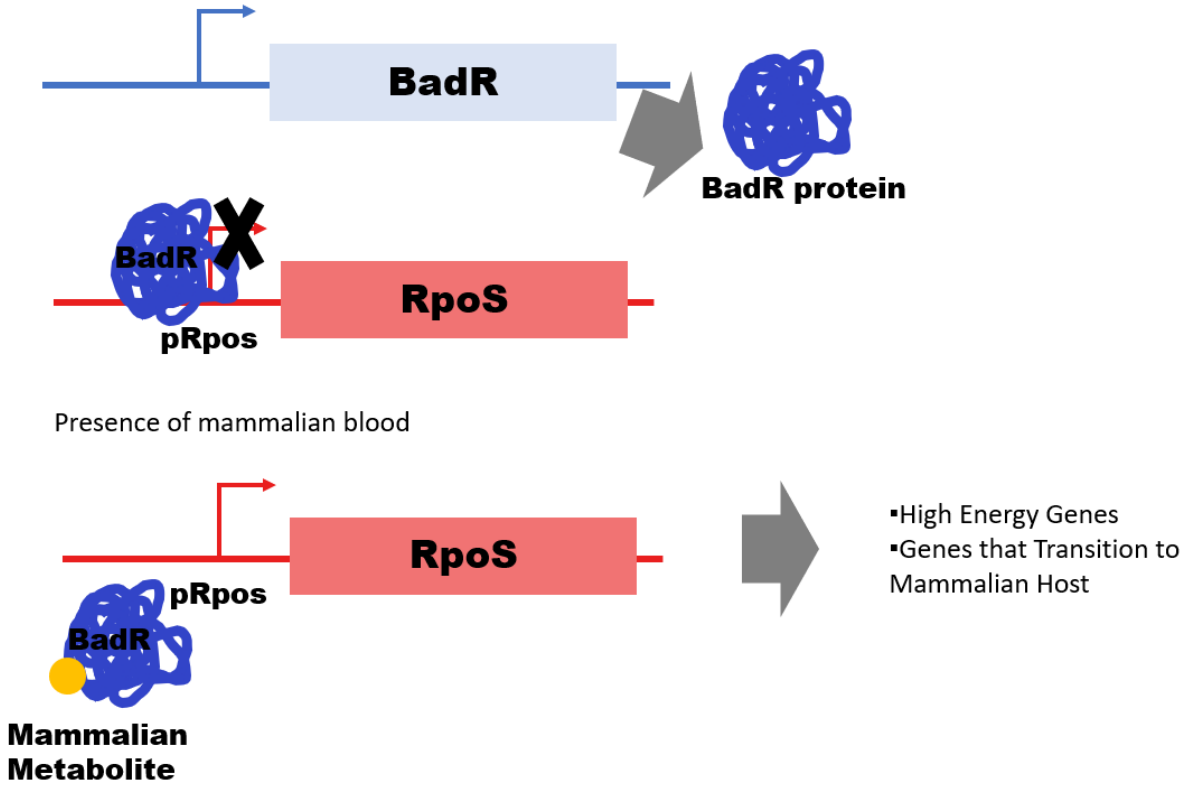

Figure 2: Diagram showing regulation of RpoS by BadR

Histone-like Integration Host Factor Beta (Hbb)

One of the signature aspects of Lyme disease is that it has the ability to infect different species, showing that it can reproduce under various environmental conditions. On the molecular level, this is due to the spirochete's ability to employ a range of secondary and tertiary structures that are integral for survival under various environmental constraints. Though many of these structures are not known, one was discovered to function similar to the integration host factor (IHF) in mammals (Mouw and Rice, 2007). This member of the DNABII protein family is called the Histone-like Integration Host Factor Beta (Hbb). Hbb is thought to function as a part of a signal transduction pathway by bending DNA in order to express or repress different genes depending on environmental constraints.

While the binding site of Hbb is not specific, it has been found to bind to specific DNA sequences throughout the *B. burgdorferi* genome (Mouw and Rice, 2007). An interesting target of Hbb is p66, which is a promoter that is only expressed in mammals, laboratory media, and when *B. burgdorferi* is acquired or transmitted by the tick (Medrano et al., 2009). Hbb was found to act as a transcription factor and decrease the function of p66 in order to regulate the expression of the integrin-binding outer surface protein genes that it turns on. In this way, it is thought that Hbb regulates genes that are essential to *B. burgdorferi*'s survival when returning to a tick host environment where nutrients are scarce and host tissue invasion is not necessary.

Experimental Investigation of BadR and Hbb Function

BadR appears to be an imperative gene for adaptation both to and from the mammalian host (Ouyang and Zhou, 2015). However, there are very few genes that BadR is known to regulate. We hypothesize that BadR represses more than RpoS, and may even upregulate genes that are important for survival in the tick host. Our experiment focuses on setting up a reporter system to screen candidate genes for BadR binding. BadR has been shown to unbind RpoS in the presence of mammalian serum. We hypothesize that it is being allosterically regulated, and will also be investigating metabolites present in mammals to see if any cause the repressor to unbind.

Additionally, the information known about Hbb shows to play a significant role in the survival of the spirochete in a mammalian host due to its regulation of different genes. The similarities between this gene and the BadR gene prompts us to question whether the regulatory activity of Hbb can be tested using the reporter designed for BadR. We hypothesize that, like BadR, Hbb is being allosterically regulated and will cause its repressor to unbind when presented with chemical metabolites.

Due to the genes it upregulates and downregulates, BadR is found to be a very important gene for *B. burgdorferi* to not only adapt from the tick vector to the mammalian host by increasing infectivity and changing metabolic rates, but also to help the spirochete's movement back from the host to the vector. In order to travel back to the vector to infect more hosts, the spirochete must readjust back to the nutrient sparse environment of the tick or they will not survive. Because of the importance of this gene for the survival of the spirochete, and the small amount of knowledge known about the gene, any information we can gain about the allosteric regulator or other genes affected would be beneficial. Information like this could be used to further research into finding new vaccines or new potential ways to create mutant spirochete populations.

Methods

We designed a reporter construct of BadR to experiment the expression of BadR and RpoS. A schematic overview of the construct preparation is shown in Figure 3.

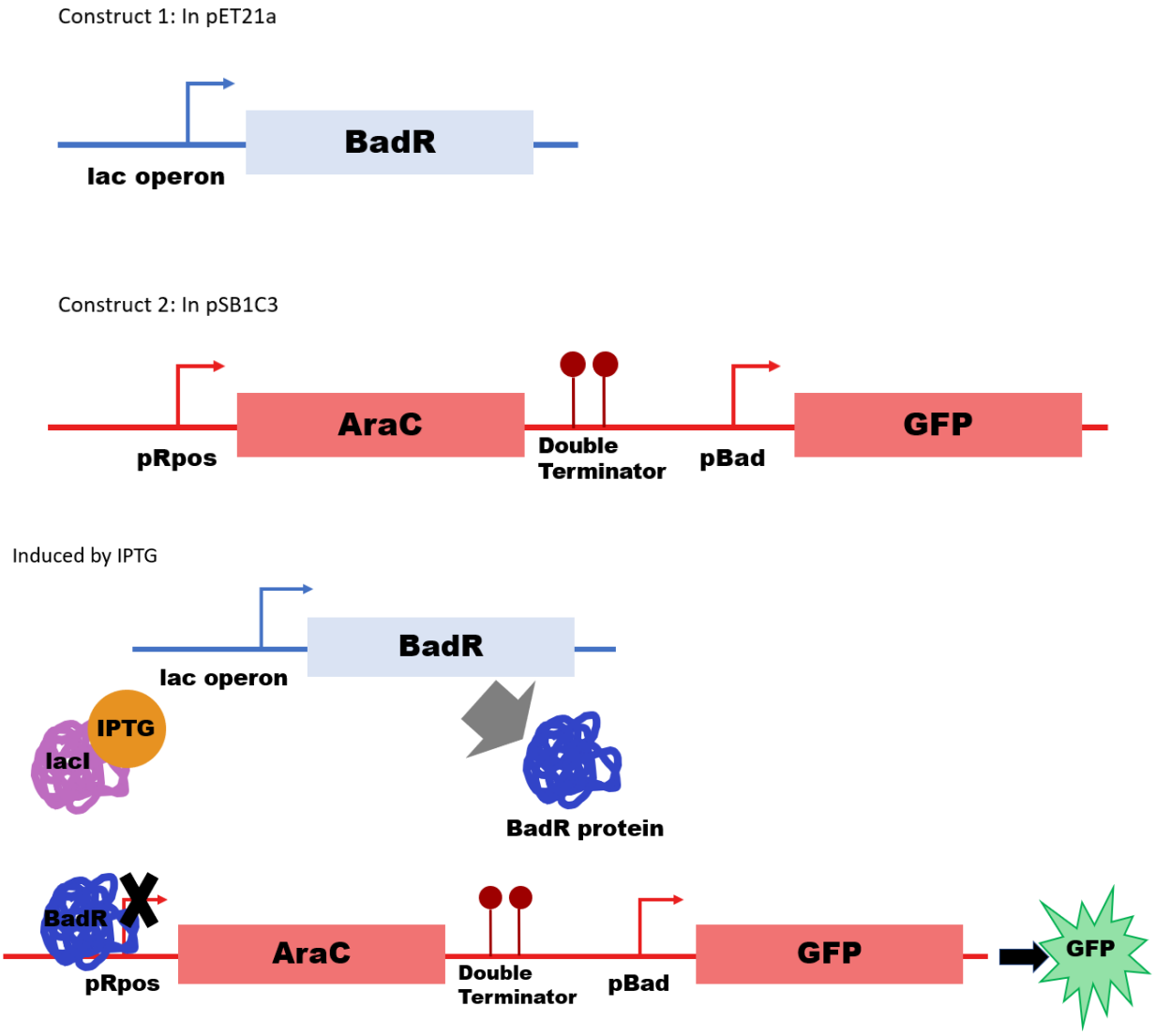

Figure 3: Schematic Overview of constructs

The vector backbones used in these experiments were pET21a and pSB1C3. pET21a was transformed into competent DH5alpha cells. These were grown in liquid LB culture and mini-prepped using a NucleoSpin Plasmid kit from Macherey-Nagel. The resulting DNA was digested with EcoRI and XhoI (New England BioLabs) in CutSmart 10X buffer (New England BioLabs). This was gel purified using a Gel and PCR Clean-up kit from Macherey-Nagel.

pSB1C3 was obtained through the part J23101 from the iGEM registry. This plasmid was transformed into competent DH5alpha cells, grown in liquid culture and mini prepped (kit from

Macherey-Nagel). The DNA was digested with EcoRI and SpeI in CutSmart buffer. This was then gel purified. Once the backbones were prepared and digested, they were ready for adding inserts. The insert sequences and their primers were manufactured by IDT (Appendix A). These went through PCR in Taq Quick Load 2x (New England BioLabs).

The pRpos-AraC fragment was digested with EcoRI and SpeI, the pBad-GFP fragment was digested with XbaI and PstI, and the BadR was digested with EcoRI and XhoI. The inserts and vectors were ligated together using T4 ligase and T4 ligase buffer from New England BioLabs. The ligation mixture was then transformed into DH5alpha cells. Any colonies that grew were inoculated into a liquid culture, mini prepped, test digested with corresponding enzymes, and run on a gel.

The pRpos-AraC in pSB1C3 was next digested with SpeI and PstI, while the pAra-GFP fragment was digested with XbaI and PstI. These fragments were gel purified and went through the same ligation process. The pET21a-Hbb plasmid was a gift from Phoebe Rice (Addgene plasmid # 26639) (Mouw and Rice, 2007).

The BadR in pET21a (referred to from now on as BadR) and pRpos/AraC/pAra/GFP in pSB1C3 (referred to as AraC-GFP) were both transformed into DH5a cells in a double transformation. For test inductions, there were three different experimental conditions: 1). Empty pET21a and AraC-GFP, 2). BadR and empty pSB1C3, and 3). BadR and AraC-GFP. These were grown overnight on plates containing both ampicillin and chloramphenicol. Colonies from these plates were picked and inoculated into liquid cultures containing both antibiotics. For test inductions, the overnight liquid cultures were diluted to an OD of 0.1 (at 600nm). 5mL cultures were made of each condition in triplicate and the cultures were subjected to the following:

Table 1: Culture Conditions

Tube Number (in each culture)	Additions
1	50 uL Arabinose
2	50 uL IPTG
3	Nothing

The conditions outlined in Table 1 were put in the shaking incubator for 4 hours at 37 degrees Celsius and viewed under UV light.

Next, Coomassie staining was used to check if the BadR was being produced. The cells were induced with IPTG and put on the shaker for 4 hours at 37 degrees Celsius. Then the cells were spun down at 11,000 rpm for 5 minutes. The media was removed and 200uL of a SDS and DTT mixture was added (Appendix). These samples were boiled at 95 degrees Celsius for 10 minutes, cooled to room temperature and stored at -80 degrees Celsius until running the gel. The samples

were loaded into an acrylamide gel (Bio-RAD Mini-PROTEAN Precast Gels) and run at 120V until the samples reached the bottom of the gel. The gel was run through three 5-minute water washes followed by a 1 hour soak in coomassie blue stain. The gel was removed from the stain and rinsed with water before being left overnight to soak in water. The gel was imaged the following day.

Results

Analysis of Plasmid Constructs

After cloning our proposed plasmids, we performed several steps to attempt to demonstrate that our constructs were correct and functional. The AraC-GFP and BadR constructs were sequence confirmed, but the Hbb-pet21a construct was not sequence confirmed because it was ordered from Addgene and previously sequence confirmed.

The AraC-GFP construct had two nucleotides that were deleted in the transformed AraC-GFP sequence (highlighted in Appendix B). The peaks at those locations, which are in the open reading frame of the AraC section of the reporter, appear to be cut off, as seen in Figure 4. The gaps in the trace could be artifacts from messy sequence as the reporter functioned as intended, making it unlikely that there was a frameshift mutation.

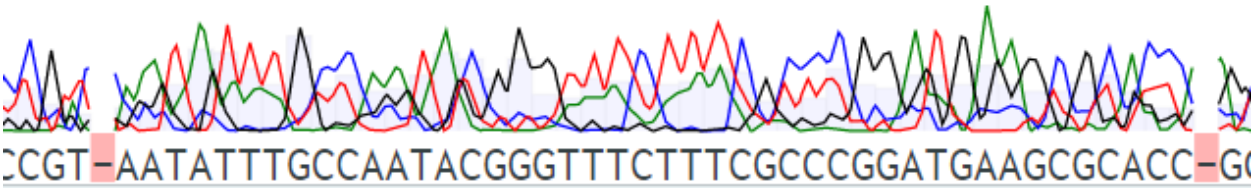

Figure 4: Part of the trace obtained from sequencing RpoS-GFP. This region is in the AraC ORF.

Similar to the Arac-GFP construct sequence, the sequence of BadR showed a few differences from the original, including a silent C to T mutation within codon 20 and a single T insertion within codon 238 (highlighted in Appendix C). The alignments of the translations of the original and cloned BadR beginning at the T is shown in Figure 5. As the translation shows, the single base insertion frameshift causes a functional change in amino acids, resulting in a triple stop codon 12 codons downstream from the mutation.

Figure 5: Translation of the original BadR sequence (A) and sequenced BadR (B) starting from codon 238 where the T was inserted (highlighted).

Protein Confirmation

Once sequences were confirmed, the next step was to see if protein was being made. To know whether or not BadR protein was produced, the cell lysate from IPTG induced and uninduced

cells were Coomassie stained. If BadR was produced, there would be a band that would not be present in uninduced cells. These samples were also compared to GFP, which was known to be inducible. The results of the staining can be seen in Figure 6. BadR does not have any difference between induced and uninduced. This result is likely caused by the truncation of the BadR protein due to the triple stop codon, resulting a truncated and possibly unstable protein. For the GFP positive control in lane 5, a faintly darker band can be seen around 26 kDa. Both induced and uninduced Hbb samples were not visible. This is possibly because the colonies grew slowly and the pellets were small when harvested.

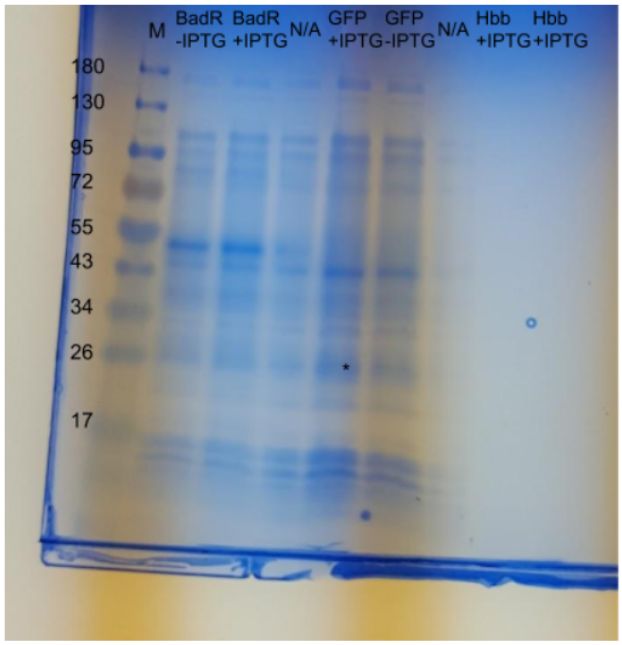

Figure 6: SDS Page Gel of two repressors and a positive control. Lane 2 is uninduced BadR while lane 3 is induced with IPTG. Lane 4 was skipped because of overflow. Lane 5 is GFP induced with IPTG and lane 6 is uninduced GFP. Lane 7 was also skipped because of overflow. Lanes 8 and 9 have no visible protein, but were induced Hbb and uninduced respectively. GFP is expected to be about 26kDa, represented by the asterisk in lane 5. Full length BadR is 46.1kDa, and the truncated version about 27.2kDa. Neither is observed on the gel. Hbb is about 11.88 kDa (Valsangiacomo et al., 1997).

While neither repressor was shown to be produced in *E. coli*, the AraC-GFP reporter was shown to be very robust, only fluorescing when induced with arabinose. Tubes that were not induced or induced with IPTG did not fluoresce. These experiments also showed that the RpoS promoter is functional in *E. coli* and seems to be a strong promoter. The AraC produced under the control of the RpoS promoter was sufficient to prevent transcription of GFP downstream of the pBad promoter.

Test Inductions

During test inductions, the expected results were that 3 of the 9 samples would fluoresce, as seen in Table 2. While the expected tubes did glow in the presence of arabinose, induction with IPTG was not successful, likely due to the stop codons in BadR. As seen in Figure 7, the reporter

was brightly fluorescent when induced compared to a negative control. Both Hbb and BadR had similar results: only tubes induced with arabinose would induce, while IPTG induced tubes were identical to negative controls. None of the uninduced tubes were fluorescent.

Table 2: Expected Fluorescence in Induction Experiments

	Induction Type		
Colony Type	Nothing	IPTG	Arabinose
+Hbb/BadR +AraC-GFP	-	+	+
-Hbb/BadR +AraC-GFP	-	-	+
+Hbb/BadR -AraC-GFP	-	-	-

Expected results for induction experiments. A (+) represents that the cells were expected to fluoresce. A (-) means no GFP was expected.

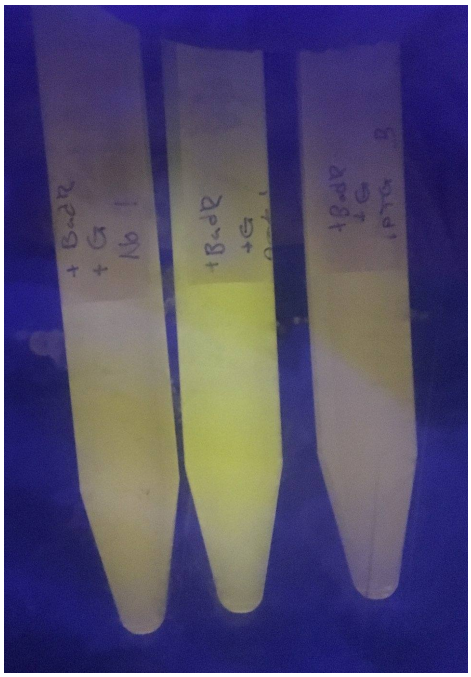

Figure 7: Representative tubes induced not induced (left) induced with arabinose (middle) and IPTG (right). These samples all are from +BadR +AraC-GFP colonies, and show typical results of only the tube induced with arabinose fluorescing.

In addition to viewing the fluorescence of the representative tubes, GFP expression in the samples of each condition were quantified on a plate reader (Appendix D). Three technical replicates of each of three different colonies for both Hbb and BadR were sampled, and each fluorescent reading was normalized by OD reading at 600nm for data analysis (Figures 8 and 9).

Figure 8 shows that only the arabinose induction on Hbb constructs caused fluorescence. The fluorescence is about 3 times as strong when comparing colonies that had Hbb present when compared to colonies with only empty pET21a vector.

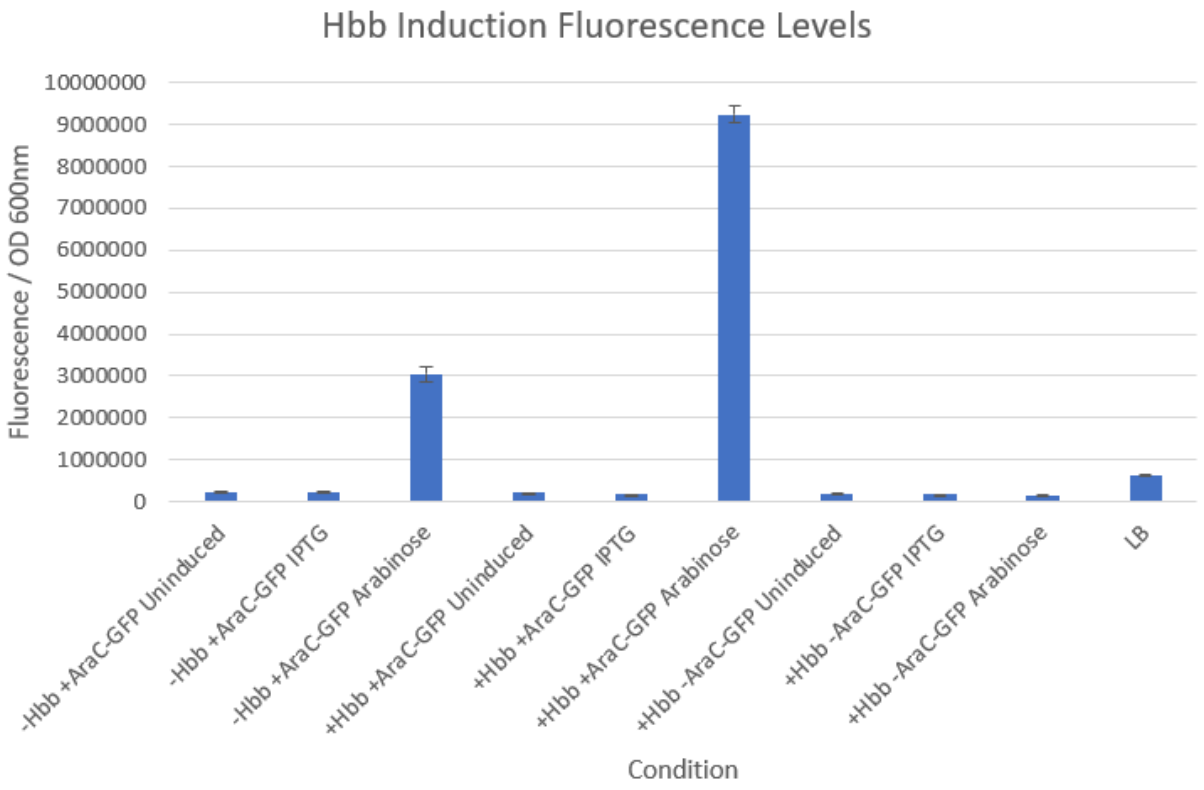

Figure 8: Data obtained from plate reader experiments. The cells that were transformed with both Hbb or pET21a and AraC-GFP or pSB1C3 were either not induced or had IPTG or arabinose added. The standard error is shown in the error bars for each condition (n=3).

The error bars for the BadR experiment (Figure 9) were much higher than the ones observed for Hbb, though the data was analysed in the same way. The same pattern was seen in Hbb and BadR. The colonies that contained BadR and not pET21a had a higher average fluorescence. However, in the BadR experiment the colonies that did not contain AraC-GFP was also fluorescent. It is unknown if this is due to leaky expression or if an experimental error was made.

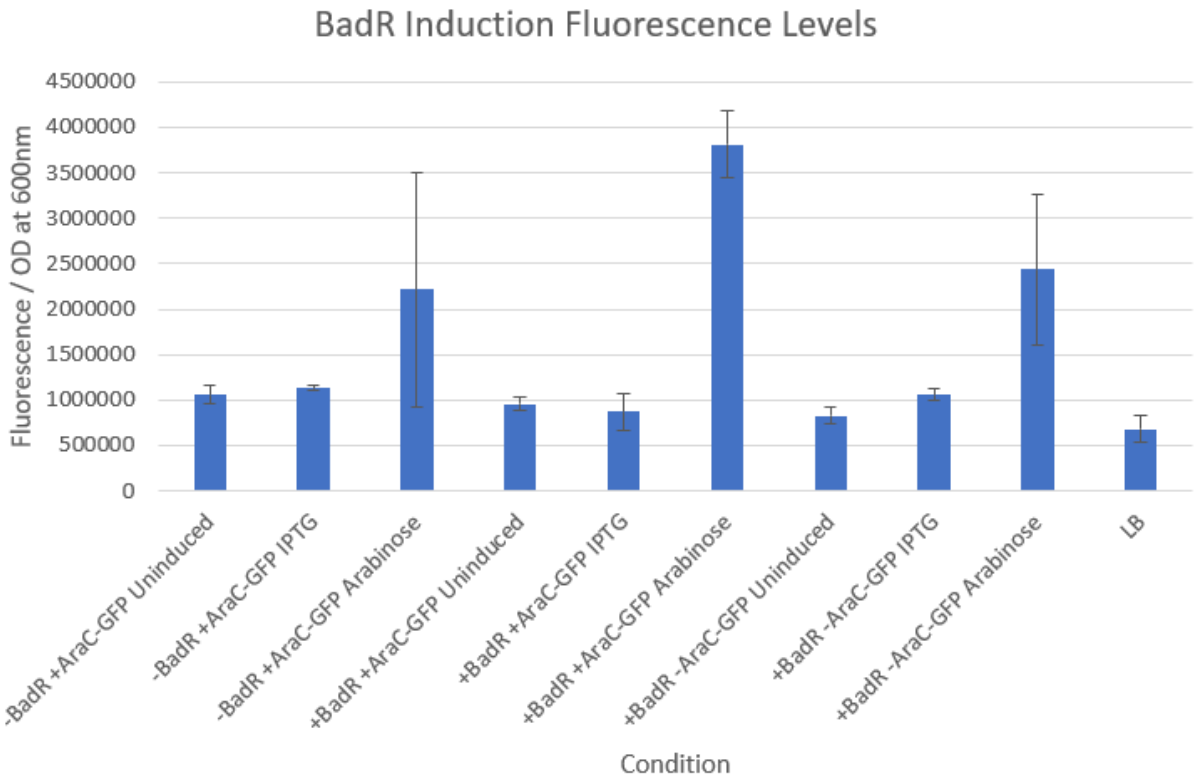

Figure 9: Data obtained from BadR plate reader experiments.

Discussion

The reporter system that we created for BadR has many applications for future Lyme disease research. Using our reporter system, researchers have the ability to test the effects of different promoters and metabolites on genes that are inducible by IPTG or other biological reagents. The simplicity of identifying results from our reporter makes our system a competitive choice for scientists seeking fluorescent reporters.

Additionally, our reporter system can continue being used to test our original hypothesis about the behavior of BadR in *B. burgdorferi* spirochetes. As shown in the *Test Inductions* section, BadR was not induced by IPTG, which indicated that the protein was either not produced or nonfunctional. After final sequence research, it was determined that different mini-preps from our samples had different sequences. However, the one constant between our different mini-preps was the presence of an inserted thymine nucleotide. This insertion created three premature stop codons downstream which explains lack of production of BadR in our experiments. With more transformation and cloning attempts it is possible to successfully clone BadR into PET21a without the creation of premature stop codons. Further research into new methods of cloning is necessary and shows promise for gaining more information about the function of this gene in the future.

Once the correct sequence of BadR has been cloned into a PET21a backbone, there are a multitude of opportunities for experiments that can be run to test the reporter circuit even more. First, the promoters for Arabinose and GFP could be swapped to directly quantitatively test the binding of BadR and/or Hbb to RpoS. Secondly, arabinose and IPTG titrations could be performed to better the results of the system. Finally, RpoS can be swapped out with other promoters that are thought to be repressed by the translation of BadR in organisms. Further experiments using different promoters will also allow researchers in this area to focus in on the function and purpose of BadR in *Borrelia burgdorferi* during the infection Lyme disease.

In addition to the triple stop codon created in BadR, we also believe that in the Hbb experiments, the amount of AraC may be too great to see a down-regulating effect in the presence of arabinose. If AraC is greater in quantity than arabinose, then when the arabinose is added it will bind to some AraC molecules but not all, leaving some AraC still able to bind to and repress pBad. In the presence of the Hbb plasmid, there could be some leaky expression of Hbb that represses AraC. The lower level of AraC would cause a greater percentage of the protein to be bound by arabinose, causing higher GFP levels, like what was observed in Figure 8 in the *Test Inductions* section of this report. We provide a visual for our theory in Figure 10.

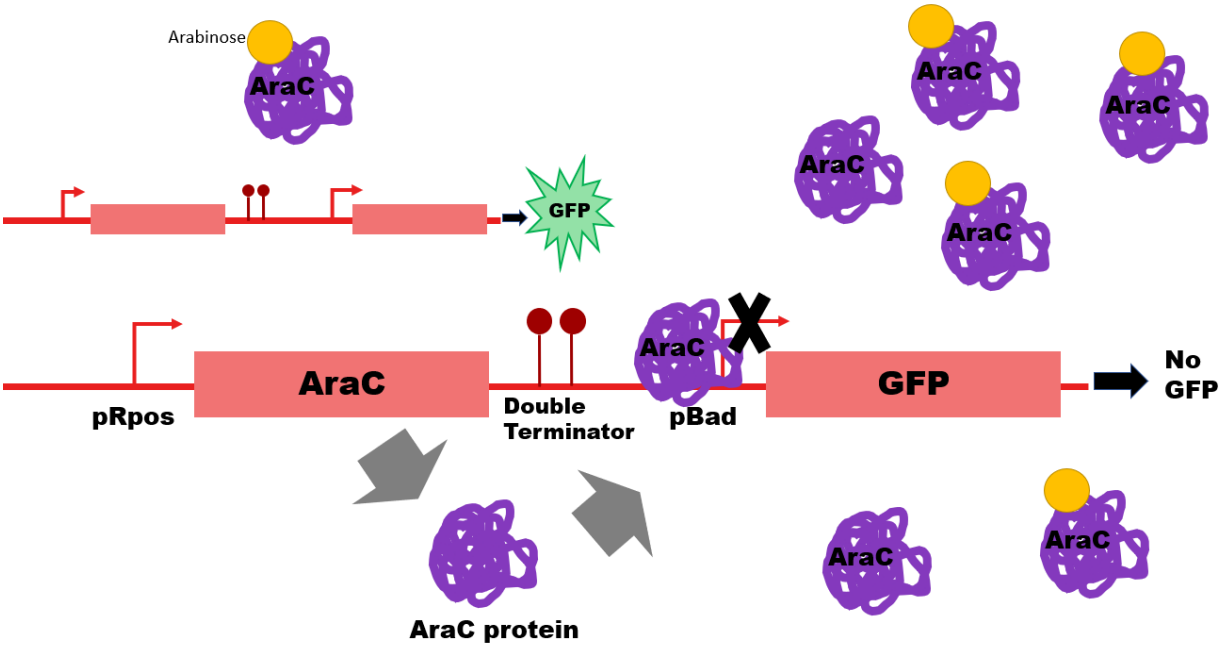

Figure 10: Schematic of hypothesis of the stoichiometry of AraC and Arabinose when regulating circuit function

Our reporter can also be used for research outside of the field of Lyme disease. Many inducible systems can be tested by using our reporter including those put into a genetic circuit using a Lac promoter. While the research done with this reporter is limited to work that can be done in inducible genetic circuits, it lends itself as easy to read and understand due to the fact that the sample will either glow for a positive result or will not glow for a negative result.

The possibilities for future research in the field of Lyme disease are endless. With so much unknown about the *B. burgdorferi* spirochete, researchers have the potential to investigate many different aspects at their choosing. For example, in the past 10 years it has been hypothesized that the outer-surface proteins (Osps) of *B. burgdorferi* are up and down-regulated genes that are important for survival in both a tick and mammalian host environment, including the transition between the two. It has also been hypothesized that OspE is heavily involved in the spirochetes ability to evade the human immune system by cleaving complement proteins of mammals (Ferreira 2010). By further understanding this cleavage action and the genes responsible for it, scientists have the potential to design synthetic plasmids to prevent the cleavage from happening. A success in this area of Lyme disease research could lead to a significant drop in the cases of Type III Lyme disease since the spirochete would not be able to withstand the human immune system and would be eradicated from the body shortly after entering it. There are multiple possibilities and hypotheses surrounding this organism and its interactions with its environment that warrant exploration. The development of new, possible vaccines and eradication efforts is expected as we begin to understand more about how Lyme disease functions in mammals and how to prevent its spread from host to host.

On the other side of Lyme disease research, many labs have begun researching into samples that are taken directly from ticks found in New England. Their work has been focused on sequencing the genome of *B. burgdorferi* and shows promise for obtaining a better understanding of the workings of this spirochete while inside a tick host (Fraser 1997). Continued research in this area may help scientists to discover a way to lessen or eradicate the presence of *B. burgdorferi* from the tick population in New England and, in turn, significantly lessen the number of cases of Lyme disease in the United States.

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Appendix

Appendix A: Tables of Sequences Used in this Study

Table 3: Primers and Inserts Ordered from IDT

Name	Sequence
OspC	ATGAAAAAGAATACATTAAGTGCGATATTAATGACTTTATTTTTATTTATATCTT GTAATAATTCAGGGAAAGATGGGAATACATCTGCAAATTCTGCTGATGAGTCTG TTAAAGGGCCTAATCTTACAGAAATAAGTAAAAAAATTACGGATTCTAATGCGG TTTTACTTGCTGTGAAAGAGGTTGAAGCGTTGCTGTCATCTATAGATGAAATTG CTGCTAAAGCTATTGGTAAAAAAATACACCAAAATAATGGTTTGGATACCGAAA ATAATCACAATGGATCATTGTTAGCGGGAGCTTATGCAATATCAACCCTAATAA AACAAAAATTAGATGGATTGAAAAATGAAGGATTAAAGGAAAAAATTGATGCG GCTAAGAAATGTTCTGAAACATTTACTAATAAATTAAAAGAAAAACACACAGAT CTTGGTAAAGAAGGTGTTACTGATGCTGATGCAAAAGAAGCCATTTTAAAAACA AATGGTACTAAAACTAAAGGTGCTGAAGAACTTGGAAAATTATTTGAATCAGTA GAGGTCTTGTCAAAAGCAGCTAAAGAGATGCTTGCTAATTCAGTTAAAGAGCTT ACAAGCCCTGTTGTGGCAGAAAGTCCAAAAAAACCTTAA
OspC codon optimized	ATGGAGAAGTTCATGAACAAAAAAATGAAAATGTTCATCGTGTGCGCTGTGTTT ATTTTAATCGGGGCATGTAAGATTCATACTTCGTATGATGAGCAGTCTAGCGGC GAAATTAATCATACCTTATATGATGAGCAATCGAATGGAGAGCTGAAATTGAAG AAGATTGAATTTTCGAAATTCACGGTGAAAATCAAGAATAAAGATAATAATTCC AACTGGACGGATTTGGGCGACTTGGTGGTACGCAAAGAAGAGAATGGCATTGA CACCGGGCTTAATGCCGGAGGTCACAGTGCTACGTTTTTTAGTCTTAAAGAATC CGAGGTTAATAATTTTATCAAGGCGATGACAAAAGGGGGGTCATTTAAGACATC CCTGTACTATGGATACAAGTACGAACAGTCCAGTGCAAACGGCATCCAGAATAA GGAGATTATCACCAAGATTGAAAGTATCAATGGCGCTGAACACATCGCTTTTTT GGGAGACAAGATCAACAACTAA
OspC 5' Primer	GCGCCATATGATGAAAAAGAATACATTAAG
OspC 3' Primer	TATACTCGAGAGGTTTTTTTGGACTTTCTGCC
OspE	GTTAATATGTAATAGCTGAATGTAACAAAATTATATATTTAAATCTTTGAAATAT TCGAATTATTAGCTGTTGTGGTATGATTAGGACTTATGGAGAAATTTATGAATA AGAAAATGAAAATGTTTATTGTTTGTGCTGTTTTTATACTTATAGGTGCTTGCAA AATTCATACTTCATATGATGAGCAAAGTAGTGGTGAGATAAACCATACTTTATA TGATGAGCAAAGTAATGGTGAGTTAAAACTTAAAAAAATAGAATTCTCTAAATT TACTGTAAAAATTAAAAATAAAGATAATAATAGTAACTGGACAGACCTAGGAGA TTTAGTTGTAAGAAAAGAAGAAAATGGTATTGATACGGGTTTAAACGCTGGGG GACATTCGGCTACATTCTTTTCATTAAAAGAATCAGAAGTTAATAACTTTATAAA AGCAATGACTAAAGGCGGATCATTTAAAACTAGTTTGTATTATGGATATAAGTA

	CGAACAAAGTAGTGCAAATGGTATCCAAAACAAAGAGATCATAACAAAAATAGA AAGTATTAATGGTGCTGAACATATTGCGTTTTTAGGAGATAAAATTAATAACG
OspE codon optimized	ATGGAGAAGTTCATGAACAAAAAAATGAAAATGTTCATCGTGTGCGCTGTGTTT ATTTTAATCGGGGCATGTAAGATTCATACTTCGTATGATGAGCAGTCTAGCGGC GAAATTAATCATACCTTATATGATGAGCAATCGAATGGAGAGCTGAAATTGAAG AAGATTGAATTTTCGAAATTCACGGTGAAAATCAAGAATAAAGATAATAATTCC AACTGGACGGATTTGGGCGACTTGGTGGTACGCAAAGAAGAGAATGGCATTGA CACCGGGCTTAATGCCGGAGGTCACAGTGCTACGTTTTTTAGTCTTAAAGAATC CGAGGTTAATAATTTTATCAAGGCGATGACAAAAGGGGGGTCATTTAAGACATC CCTGTACTATGGATACAAGTACGAACAGTCCAGTGCAAACGGCATCCAGAATAA GGAGATTATCACCAAGATTGAAAGTATCAATGGCGCTGAACACATCGCTTTTTT GGGAGACAAGATCAACAACTAA
OspE 5' primer	GCGCCATATGATGGAGAAGTTCATGAAC
OspE 3' primer	TGTGCTCGAGGTTGTTGATCTTGTCTCC

Table 4: pAra-sfGFP Sequence

pAra-sfGFP: prefix-pBad-RBS-sfGFP-DTag-DT-suffix
GAATTCGCGGCCGCTTCTAGAGACATTGATTATTTGCACGGCGTCACACTTTGCTA TGCCATAGCATTTTTATCCATAAGATTAGCGGATCCTACCTGACGCTTTTTATCGCA ACTCTCTACTGTTTCTCCATACCGTTTTTTTGGGCTAGCATTAAAGAGGAGAAAAT GCGTAAAGGCGAAGAACTGTTCACGGGCGTAGTTCCGATTCTGGTCGAGCTGGAC GGCGATGTGAACGGTCATAAGTTTAGCGTTCGCGGTGAAGGTGAGGGCGACGCGA CCAACGGCAAACTGACCCTGAAGTTCATCTGCACCACCGGTAAACTGCCGGTGCC TTGGCCGACCTTGGTGACGACGTTGACGTATGGCGTGCAGTGTTTTGCGCGTTATC CGGACCACATGAAACAACACGATTTCTTCAAATCTGCGATGCCGGAGGGTTACGT CCAGGAGCGTACCATTTCCTTCAAGGATGATGGCTACTACAAAACTCGCGCAGAG GTTAAGTTTGAAGGTGACACGCTGGTCAATCGTATCGAATTGAAGGGTATCGACTT TAAAGAGGATGGTAACATTCTGGGCCATAAACTGGAGTATAACTTCAACAGCCAT AATGTTTACATTACGGCAGACAAGCAAAAGAACGGCATCAAGGCCAATTTCAAGA TTCGCCACAATGTTGAGGACGGTAGCGTCCAACTGGCCGACCATTACCAGCAGAA CACCCCAATTGGTGACGGTCCGGTTTTGCTGCCGGATAATCACTATCTGAGCACCC AAAGCGTGCTGAGCAAAGATCCGAACGAAAAACGTGATCACATGGTCCTGCTGGA ATTTGTGACCGCTGCGGGCATCACCCACGGTATGGACGAGCTGTATAAG GCTGCTAACGACGAAAACTACGCTGACGCTTCTTAATGACCAGGCATCAAATAAA ACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGA ACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGGCCTTTCTGCGTTTAT ATACTAGTAGCGGCCGCTGCAG

Table 5: pRpoS-AraC Sequence

pRpoS-AraC: prefix-pRpos-RBS-AraC-Dtag-DT-suffix
GAATTCGCGGCCGCTTCTAGAGAAAGCTTTGGCCTTGCCGATTTAATTTACAATCA ATTACAAAAAAGTAAATAATTCAAAAAATACTCCCCCTAAACTCAAAATTTTATAT CCTATTTAGTTTAAAACCATTTTTAAATTAAATTGGCACAGTTTTTGCATGAAAATT AAGTAGTAACTCGAGATTAAAGAGGAGAAAATGGCTGAAGCGCAAAATGATCCC CTGCTGCCGGGATACTCGTTTAACGCCCATCTGGTGGCGGGTTTAACGCCGATTGA GGCCAACGGTTATCTCGATTTTTTTATCGACCGACCGCTGGGAATGAAAGGTTATA TTCTCAATCTCACCATTCGCGGTCAGGGGGTGGTGAAAAATCAGGGACGAGAATT TGTCTGCCGACCGGGTGATATTTTGCTGTTCCCGCCAGGAGAGATTCATCACTACG GTCGTCATCCGGAGGCTCGCGAATGGTATCACCAGTGGGTTTACTTTCGTCCGCGC GCCTACTGGCATGAATGGCTTAACTGGCCGTCAATATTTGCCAATACGGGTTTCTT TCGCCCGGATGAAGCGCACCAGCCGCATTTCAGCGACCTGTTTGGGCAAATCATT AACGCCGGGCAAGGGGAAGGGCGCTATTCGGAGCTGCTGGCGATAAATCTGCTTG AGCAATTGTTACTGCGGCGCATGGAAGCGATTAACGAGTCGCTCCATCCACCGAT GGATAATCGGGTACGCGAGGCTTGTCAGTACATCAGCGATCACCTGGCAGACAGC AATTTTGATATCGCCAGCGTCGCACAGCATGTTTGCTTGTCGCCGTCGCGTCTGTC ACATCTTTTCCGCCAGCAGTTAGGGATTAGCGTCTTAAGCTGGCGCGAGGACCAAC GCATTAGTCAGGCGAAGCTGCTTTTGAGCACTACCCGGATGCCTATCGCCACCGTC GGTCGCAATGTTGGTTTTGACGATCAACTCTATTTCTCGCGAGTATTTAAAAAATG CACCGGGGCCAGCCCGAGCGAGTTTCGTGCCGGTTGTGAAGAAAAAGTGAATGAT GTAGCCGTCAAGTTGTCAGCTGCTAACGACGAAAACTACGCTGACGCTTCTTAACC AGGCATCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCT GTTGTTTGTCGGTGAACGCTCTCTACTAGAGTCACACTGGCTCACCTTCGGGTGGG CCTTTCTGCGTTTATATACTAGTAGCGGCCGCTGCAG

Table 6: BadR Sequence

BadR Insert
TTCCAAGGAGAAAACATGGTGTCCATTCGTGGGGGGAATCGTCGCAAGATCTTAT TAAGCTTAAAGAACATGCAGTACAGCCGCACAGACCTGGCCCGTAAGTTGACTCT GACGAATGCAGCGGTTACCATCCTGACAAATCAGATGATCAAAGAAAATTTATTG ATCGAAGTTGGCAGTCGCGTGAGCGATATTAAGAAACATGGTCGCAAAGAAATCC TTCTGGATATCAATAAGGATTATGCATACTCCATGGGGGTAATTATTAGCTCGAAT TATTTTCAGATTGGCATCGCCAATTTGAAGTGCGAGGTTCTGATCAGTGAAACTCA CTCTTTTGAGCCTCCAGTGTCGGCCTACGAGATCCTGGAAAAAATCAAAGATCACA TGATCGAAATTATTTGGAAGCACAACTTTAGCCGTGATAAGTTTATTGGTTTGGGT TTCTCAATTACAGGTTTGATCAAGGATAAGGAGTCCGGGATTGTAAACGACAGTT ATGGCGCGTGGATTGAGAAGGATGTCCCAGTGAAGCGCATCCTGGAGGAGTATTT CTCTTTGACTGTTTACTTAGAGTCATACGTGAAGAACTTGTCATTGGCGGAATTTA TGGGTAAAAACATTGACAACATCATGTTCTTCGACTATACAGATACGGCGGAGTT

GTCTATTTGGAGCGATGGCAACGTGTATCCTGGCTTTAATAATAAGTCGGGCATGG
TCTCACACATGATTATCGATTACGAGGGGGAGAAGAACTGCCCCACGTGTGGCAA
CAAAGGATGTGTGAATATGTTAATTTCAAACTTTGCATTACAGCGCTTAATTTCCA
AGGAGTTCATGAATGGTGAAATTCCTGAACTTTACGAAAAGTACGAAGGGCGCCT
TAAAAAGGTTACAATTTACGATGTTTTTTCGTTGTACGAAAAATATGATTTTGTCA
ACAAGATTATGCAGGATACCGTCAAGTACTTGGCTATTATTATTATCAACATCCAG
CGTATGCTTGACTTTAATTATCTTGTGCTTTATGGGCAATCATTCAAATTGAAAGCC
TTCTTCGACAGTCTGAAAGAGGAAATTAAAAAGCGCAACAAGGAGAACATTATTC
TGAAACTGAGCAGTCTTGACACTGAAGTGTCAGTTGTAGGACCTGCATCGTCGGTG
ATCTTCAACAAATTTTATTTAACGGGTGGGGATATCGATGAGCTCCGTCGACAAGC
TTGCGGCCGCACTC

Table 7: BadR Primer Sequences

5' biobrick	TAT AGA ATT CGC GGC CGC TTC TAG AG
3' biobrick	TAT ACT GCA GCG GCC GCT ACT AGT A
5' BadR EcoRI	GCG CGA ATT CCA AGG AGA AAA CAT GGT
3' BadR XhoI	TAT ACT CGA GAT CGA TAT CCC CAC CCG T

Appendix B: BadR Sequence Alignment

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#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1368
# Identity:    1335/1368 (97.6%)
# Similarity:  1335/1368 (97.6%)
# Gaps:          32/1368 ( 2.3%)
# Score: 6626.5
#
#
#=======================================

EMBOSS_001      5174 ttttgtttaactttaagaaggagatatacatatggctagcatgactggtg   5223
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001         1 TTTTGTTTAACTTTAAGAAGGAGATATACATATGGCTAGCATGACTGGTG     50

EMBOSS_001      5224 gacagcaaatgggtcgcggatccgaattcCAAGGAGAAAACATGGTGTCC   5273
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001        51 GACAGCAAATGGGTCGCGGATCCGAATTCCAAGGAGAAAACATGGTGTCC    100

EMBOSS_001      5274 ATTCGTGGGGGGAATCGTCGCAAGATCTTATTAAGCTTAAAGAACATGCA   5323
                     |||||||||||||||||||||||||||||||||||.||||||||||||||||
EMBOSS_001       101 ATTCGTGGGGGGAATCGTCGCAAGATCTTATTAAGTTTAAAGAACATGCA    150

EMBOSS_001      5324 GTACAGCCGCACAGACCTGGCCCGTAAGTTGACTCTGACGAATGCAGCGG   5373
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       151 GTACAGCCGCACAGACCTGGCCCGTAAGTTGACTCTGACGAATGCAGCGG    200

EMBOSS_001      5374 TTACCATCCTGACAAATCAGATGATCAAAGAAAATTTATTGATCGAAGTT   5423
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       201 TTACCATCCTGACAAATCAGATGATCAAAGAAAATTTATTGATCGAAGTT    250

EMBOSS_001      5424 GGCAGTCGCGTGAGCGATATTAAGAAACATGGTCGCAAAGAAATCCTTCT   5473
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       251 GGCAGTCGCGTGAGCGATATTAAGAAACATGGTCGCAAAGAAATCCTTCT    300

EMBOSS_001      5474 GGATATCAATAAGGATTATGCATACTCCATGGGGGTAATTATTAGCTCGA   5523
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       301 GGATATCAATAAGGATTATGCATACTCCATGGGGGTAATTATTAGCTCGA    350

EMBOSS_001      5524 ATTATTTTCAGATTGGCATCGCCAATTTGAAGTGCGAGGTTCTGATCAGT   5573
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       351 ATTATTTTCAGATTGGCATCGCCAATTTGAAGTGCGAGGTTCTGATCAGT    400
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EMBOSS_001      5574 GAAACTCACTCTTTTGAGCCTCCAGTGTCGGCCTACGAGATCCTGGAAAA   5623
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       401 GAAACTCACTCTTTTGAGCCTCCAGTGTCGGCCTACGAGATCCTGGAAAA    450

EMBOSS_001      5624 AATCAAAGATCACATGATCGAAATTATTTGGAAGCACAACTTTAGCCGTG   5673
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       451 AATCAAAGATCACATGATCGAAATTATTTGGAAGCACAACTTTAGCCGTG    500

EMBOSS_001      5674 ATAAGTTTATTGGTTTGGGTTTCTCAATTACAGGTTTGATCAAGGATAAG   5723
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       501 ATAAGTTTATTGGTTTGGGTTTCTCAATTACAGGTTTGATCAAGGATAAG    550

EMBOSS_001      5724 GAGTCCGGGATTGTAAACGACAGTTATGGCGCGTGGATTGAGAAGGATGT   5773
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       551 GAGTCCGGGATTGTAAACGACAGTTATGGCGCGTGGATTGAGAAGGATGT    600

EMBOSS_001      5774 CCCAGTGAAGCGCATCCTGGAGGAGTATTTCTCTTTGACTGTTTACTTAG   5823
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       601 CCCAGTGAAGCGCATCCTGGAGGAGTATTTCTCTTTGACTGTTTACTTAG    650

EMBOSS_001      5824 AGTCATACGTGAAGAACTTGTCATTGGCGGAATTTATGGGTAAAAACATT   5873
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       651 AGTCATACGTGAAGAACTTGTCATTGGCGGAATTTATGGGTAAAAACATT    700

EMBOSS_001      5874 GACAACATCATGTTCTTCGACTATACAGATACGGCGGAGTTGTC-TATTT   5922
                     ||||||||||||||||||||||||||||||||||||||||||||| |||||
EMBOSS_001       701 GACAACATCATGTTCTTCGACTATACAGATACGGCGGAGTTGTCTTATTT    750

EMBOSS_001      5923 GGAGCGATGGCAACGTGTATCCTGGCTTTAATAATAAGTCGGGCATGGTC   5972
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       751 GGAGCGATGGCAACGTGTATCCTGGCTTTAATAATAAGTCGGGCATGGTC    800

EMBOSS_001      5973 TCACACATGATTATCGATTACGAGGGGGAGAAGAACTGCCCCACGTGTGG   6022
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       801 TCACACATGATTATCGATTACGAGGGGGAGAAGAACTGCCCCACGTGTGG    850

EMBOSS_001      6023 CAACAAAGGATGTGTGAATATGTTAATTTCAAACTTTGCATTACAGCGCT   6072
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       851 CAACAAAGGATGTGTGAATATGTTAATTTCAAACTTTGCATTACAGCGCT    900

EMBOSS_001      6073 TAATTTCCAAGGAGTTCATGAATGGTGAAATTCCTGAACTTTACGAAAAG   6122
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       901 TAATTTCCAAGGAGTTCATGAATGGTGAAATTCCTGAACTTTACGAAAAG    950

EMBOSS_001      6123 TACGAAGGGCGCCTTAAAAAGGTTACAATTTACGATGTTTTTTCGTTGTA   6172
                     ||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       951 TACGAAGGGCGCCTTAAAAAGGTTACAATTTACGATGTTTTTTCGTTGTA   1000
```

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EMBOSS_001      6173 CGAAAAATATGATTTTGTCAACAAGATTATGCAGGATACCGTCAAGTACT   6222
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1001 CGAAAAATATGATTTTGTCAACAAGATTATGCAGGATACCGTCAAGTACT   1050

EMBOSS_001      6223 TGGCTATTATTATTATCAACATCCAGCGTATGCTTGACTTTAATTATCTT   6272
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1051 TGGCTATTATTATTATCAACATCCAGCGTATGCTTGACTTTAATTATCTT   1100

EMBOSS_001      6273 GTGCTTTATGGGCAATCATTCAAATTGAAAGCCTTCTTCGACAGTCTGAA   6322
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1101 GTGCTTTATGGGCAATCATTCAAATTGAAAGCCTTCTTCGACAGTCTGAA   1150

EMBOSS_001      6323 AGAGGAAATTAAAAAGCGCAACAAGGAGAACATTATTCTGAAACTGAGCA   6372
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1151 AGAGGAAATTAAAAAGCGCAACAAGGAGAACATTATTCTGAAACTGAGCA   1200

EMBOSS_001      6373 GTCTTGACACTGAAGTGTCAGTTGTAGGACCTGCATCGTCGGTGATCTTC   6422
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001      1201 GTCTTGACACTGAAGTGTCAGTTGTAGGACCTGCATCGTCGGTGATCTTC   1250

EMBOSS_001      6423 AACAAATTTTATTTAACGGGTGGGGATATCGATgagctccgtcgacaagc   6472
                     |||||||||||||||||||||||||||||||||
EMBOSS_001      1251 AACAAATTTTATTTAACGGGTGGGGATATCGAT----------------   1283

EMBOSS_001      6473 ttgcggccgcactcgagcataaccaccaccaccaccactgagatccggct   6522
                           |||||||   |||||||||||||||||||||||||||||
EMBOSS_001      1284 -----------CTCGAGC---ACCACCACCACCACCACTGAGATCCGGCT   1319

EMBOSS_001      6523 gctaacaaagcccgaaag   6540
                     ||||||||||||||||||
EMBOSS_001      1320 GCTAACAAAGCCCGAAAG   1337
```

Appendix C: RpoS Sequence Alignment

```
#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EDNAFULL
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 749
# Identity:     747/749 (99.7%)
# Similarity:   747/749 (99.7%)
# Gaps:           2/749 ( 0.3%)
# Score: 3715.0
#
#
#=======================================

EMBOSS_001      1284 cgaggcagaatttcagataaaaaaaatccttagctttcgctaaggatgat   1333
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001         1 CGAGGCAGAATTTCAGATAAAAAAAATCCTTAGCTTTCGCTAAGGATGAT     50

EMBOSS_001      1334 ttctggAATTCGCGGCCGCTTCTAGAGaaagctttggccttgccgattta   1383
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001        51 TTCTGGAATTCGCGGCCGCTTCTAGAGAAAGCTTTGGCCTTGCCGATTTA    100

EMBOSS_001      1384 atttacaatcaattacaaaaaagtaaataattcaaaaaatactccccta   1433
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       101 ATTTACAATCAATTACAAAAAAGTAAATAATTCAAAAAATACTCCCCCTA    150

EMBOSS_001      1434 aactcaaaattttatatcctatttagtttaaaaccatttttaaattaaat   1483
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       151 AACTCAAAATTTTATATCCTATTTAGTTTAAAACCATTTTTAAATTAAAT    200

EMBOSS_001      1484 tggcacagtttttgcatgaaaattaagtagtaaCTCGAGattaaagagga   1533
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       201 TGGCACAGTTTTTGCATGAAAATTAAGTAGTAACTCGAGATTAAAGAGGA    250

EMBOSS_001      1534 gaaaatgGCTGAAGCGCAAAATGATCCCCTGCTGCCGGGATACTCGTTTA   1583
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       251 GAAAATGGCTGAAGCGCAAAATGATCCCCTGCTGCCGGGATACTCGTTTA    300

EMBOSS_001      1584 ACGCCCATCTGGTGGCGGGTTTAACGCCGATTGAGGCCAACGGTTATCTC   1633
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       301 ACGCCCATCTGGTGGCGGGTTTAACGCCGATTGAGGCCAACGGTTATCTC    350

EMBOSS_001      1634 GATTTTTTTATCGACCGACCGCTGGGAATGAAAGGTTATATTCTCAATCT   1683
                     ||||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       351 GATTTTTTTATCGACCGACCGCTGGGAATGAAAGGTTATATTCTCAATCT    400
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EMBOSS_001      1684 CACCATTCGCGGTCAGGGGGTGGTGAAAAATCAGGGACGAGAATTTGTCT   1733
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       401 CACCATTCGCGGTCAGGGGGTGGTGAAAAATCAGGGACGAGAATTTGTCT    450

EMBOSS_001      1734 GCCGACCGGGTGATATTTTGCTGTTCCCGCCAGGAGAGATTCATCACTAC   1783
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       451 GCCGACCGGGTGATATTTTGCTGTTCCCGCCAGGAGAGATTCATCACTAC    500

EMBOSS_001      1784 GGTCGTCATCCGGAGGCTCGCGAATGGTATCACCAGTGGGTTTACTTTCG   1833
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       501 GGTCGTCATCCGGAGGCTCGCGAATGGTATCACCAGTGGGTTTACTTTCG    550

EMBOSS_001      1834 TCCGCGCGCCTACTGGCATGAATGGCTTAACTGGCCGTCAATATTTGCCA   1883
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       551 TCCGCGCGCCTACTGGCATGAATGGCTTAACTGGCCGT-AATATTTGCCA    599

EMBOSS_001      1884 ATACGGGTTTCTTTCGCCCGGATGAAGCGCACCAGCCGCATTTCAGCGAC   1933
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       600 ATACGGGTTTCTTTCGCCCGGATGAAGCGCACC-GCCGCATTTCAGCGAC    648

EMBOSS_001      1934 CTGTTTGGGCAAATCATTAACGCCGGGCAAGGGGAAGGGCGCTATTCGGA   1983
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       649 CTGTTTGGGCAAATCATTAACGCCGGGCAAGGGGAAGGGCGCTATTCGGA    698

EMBOSS_001      1984 GCTGCTGGCGATAAATCTGCTTGAGCAATTGTTACTGCGGCGCATGGAA   2032
                     |||||||||||||||||||||||||||||||||||||||||||||||||||
EMBOSS_001       699 GCTGCTGGCGATAAATCTGCTTGAGCAATTGTTACTGCGGCGCATGGAA    747
```

Appendix D: Plate Reader Data Table

Table 8: Plate Reader Data for Hbb followed by BadR

Name (Gene and Condition)	Fluorescece /OD 600nm	Standard Error
-Hbb +AraC-GFP Uninduced	233306.2	14617.81
-Hbb +AraC-GFP IPTG	230929.8	10744.27
-Hbb +AraC-GFP Arabinose	3049274	177950.9
+Hbb +AraC-GFP Uninduced	212856.3	3585.021
+Hbb +AraC-GFP IPTG	173330.2	3096.615
+Hbb +AraC-GFP Arabinose	9242504	205588.3
+Hbb -AraC-GFP Uninduced	186031.3	3505.157
+Hbb -AraC-GFP IPTG	171066.3	1509.978
+Hbb -AraC-GFP Arabinose	156888.5	1282.535
LB control	627062.9	7306.062
-BadR +AraC-GFP Uninduced	1055134	104285.4
-BadR +AraC-GFP IPTG	1132903	27916.15
-BadR +AraC-GFP Arabinose	2212774	1291742
+BadR +AraC-GFP Uninduced	955537.5	78918.35
+BadR +AraC-GFP IPTG	872157.2	204839.4
+BadR +AraC-GFP Arabinose	3812431	374972.7
+BadR -AraC-GFP Uninduced	830231.8	99767.87
+BadR -AraC-GFP IPTG	1056219	60562.66
+BadR -AraC-GFP Arabinose	2435628	825660.4
LB control	680008.8	143371.5