Winter 2-2016

Riboflavin Metabolism: A study to see if Mrub_1256 is Orthologous to *E. coli* b0415, and if Mrub_1254 is Orthologous to *E. coli* b1662

Anish Sora Reddy
Augustana College, Rock Island Illinois

Dr. Lori Scott
Augustana College, Rock Island Illinois

Anish Reddy
Bio 375
Dr. Scott
2/6/16

Riboflavin Metabolism: A study to see if *Mrub*_1256 is Orthologous to *E. coli* b0415, and if *Mrub*_1254 is Orthologous to *E. coli* b1662

Introduction:

In order to take part in a molecular genetics project, it was necessary to learn about *Meiothermus ruber*, Riboflavin metabolism, and evolutionary descent. In general, bacteria are defined as a cluster of "single-cell microorganisms" that are prokaryotic (Todar 2015). They lack a cell membrane and can also live in extreme environments compared to eukaryotes (Todar 2015). *Meiothermus ruber* is one such bacterium that is considered thermophilic and grows at temperatures close to 55-60^{0}C (Tindall et al. 2010).

For my particular authentic research project, I chose to study Riboflavin metabolism, specifically the genes *E. coli* b0415, *E. coli* b1662, *Mrub*_1256, and *Mrub*_1254. In order to determine if any *Meiothermus ruber* genes were orthologous to the *E. coli* genes, we first need to take an in depth look into what Riboflavin is and functional evidence regarding *E. coli*: b0415 and *E. coli* b1662. Essentially, I am checking *Mruber*_1256 and *Mruber*_1254 against *E. coli* genomes listed in the databases to look for any similarities. This project uses *E. coli* K12 MG1655 as the positive control. *E. coli* is used as the positive control because there is so much research that has been done on it. For example, the graph on Geni-science indicates that there have been 35,000 published studies regarding *E. coli* (Geni-Science). Therefore, it would be beneficial to study organisms that have not been studied that well against *E. coli* as reference point. Once a similarity is found with another species, we refine the search and compare that

species with *Mruber*_1256 and *Mruber*_1254 to see if there are evolutionary connections (Bioinformatics 2015).

Knowledge of Riboflavin metabolism is essential to understanding the use of alternative pathways. Looking at the pathways of *Mrub*_1256 and *Mrub*_1254 DSM 1279 and comparing them to *E. coli* may show that they share similar pathways and enzymes. Also known as Vitamin B_2, Riboflavin is important biologically because it is a big component of cofactors FAD and FMN within the human body (Belinda 2014). These cofactors make up most of the Riboflavin within blood plasma (Belinda 2014). The two most prevalent steps within Riboflavin metabolism are the enzymes 6,7-dimethyl-8-ribityllumazine synthase and riboflavin synthase. 6,7-dimethyl-8-ribityllumazine synthase dephosphorylates 5-amino-6-(D-ribitylamino)uracil and 1-deoxy-L-glycero-tetrulose 4-phosphate via a condensation reaction to produce 6,7-dimethyl-8-(1-D-ribityl)lumazine. Riboflavin synthase dismutates 6,7-dimethyl-8-ribityllumazine to produce Riboflavin (Belinda 2014). Riboflavin deficiency can cause a multitude of problems. Cleft lip-palate, neurogeneration, peripheral neuropathy, and the increase in cancer have been linked to Riboflavin deficiency. Heart disease can also arise from Riboflavin deficiency as this increases the amount of homocycteine within the blood plasma. Thus, Riboflavin is vital for metabolism in humans (Belinda 2014). I had heard of Riboflavin before when talking about vitamins, but I had never studied its physiological properties. Learning about these properties gave me a greater appreciation for the necessity of Riboflavin in living organisms. The figure below shows the two prominent pathways in Riboflavin metabolism:

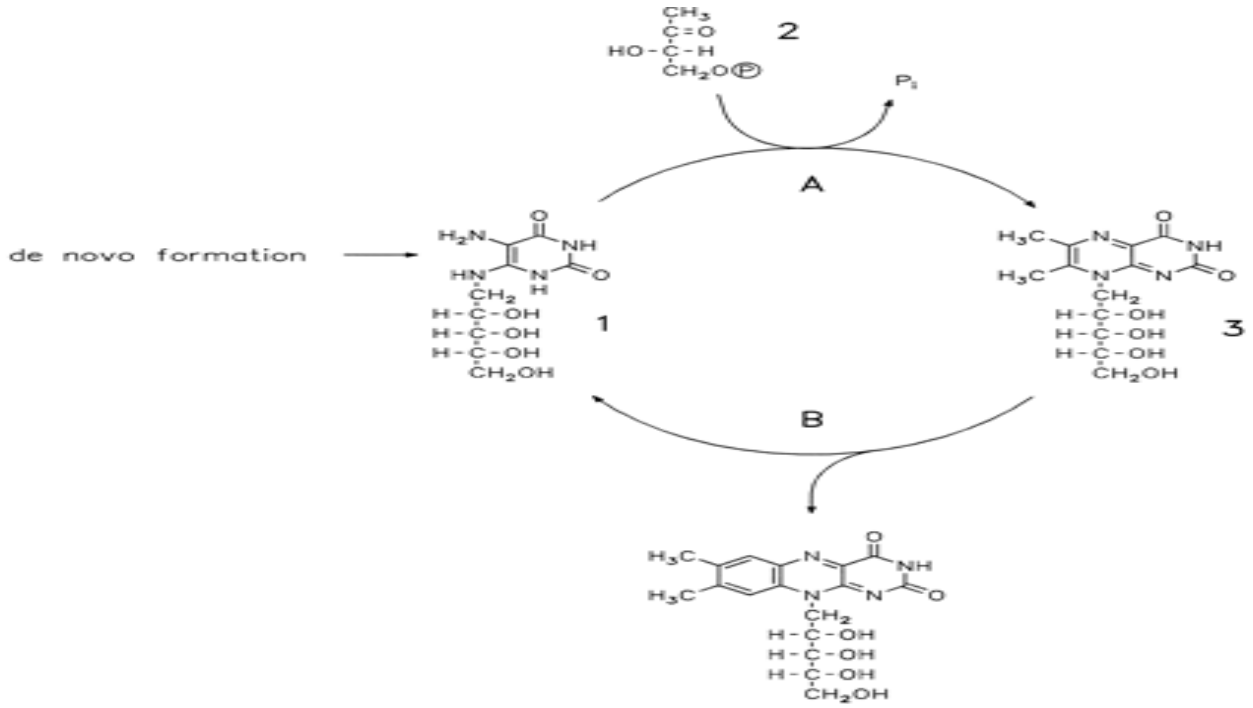


Figure 1.Terminal reactions in the pathway of riboflavin biosynthesis. A, lumazine synthase; B, riboflavin synthase. Diagram and figure legend obtained from Mortl et al. 1996

The first gene of study, *E. coli* b0415, is very important in the production of Riboflavin. This gene is found 443 kilobases on the *Escherichia Coli* chromosome. Also called RibE, this gene catalyzes 6,7-dimehtyl-8-ribityllumazine via a condensation reaction of 5-amino-6-ribityl-amino-2,4(1H,3H)-pyrimidinedione with 3,4-dihydroxy-2-butanone. This specific reaction is followed by the dismutation of Lumazine, which in turn makes Riboflavin. It acts in a trimer protein fashion (Mortl et al., 1996). RibE consists of 471 nucleotides and 156 amino acids (Geni-act). It is also part of a three-operon system, *E. coli* b0415, *E. coli* b0416, and *E. coli* b0417 (IMG/EDU). The second gene of interest, *E. coli* b1662, catalyzes the final step in the production of Riboflavin. Widely know as RibC, or Riboflavin synthase, it catalyzes the last step of dismutation of 6,7-dimehtyl-8-ribityllumazine and forms 5-amino-6-ribityl-amino-2,4(1H,3H)-pyrimidinedione. It codes for a 213 amino acid protein with a mass of 23.4 kDa and can later be

recycled back into the Riboflavin metabolism pathway with the use of the enzyme 6,7-dimehtyl-8-ribityllumazine synthase (Eberhardt et al., 1996). *E. coli b1662* transcribes in the reverse direction and is not a part of an extended operon (IMG/EDU).

The purpose of this research is to discover the genomic organization of *Meiothermus ruber* by comparing it to *E. coli*, which is considered a model organism and is also used as our positive control in this project. To do this, one must check for evolutionary descent by comparing the E-values of the two organisms. E-values show whether the organisms' genetic codes match up by chance or because of an evolutionary link. Lower E-values indicate similarity. We also used bit-scores to analyze the probability of evolutionary descent. A high bit-score predicts that the sequence alignment did not happen by chance (VRIJE Universiteit Brussel). This material was completely new to me, as I have never searched for evolutionary links before.

Because little is known about *Meiothermus Ruber*, any research done will be beneficial to increasing bioinformatics and could be added to the GEBA project (Wu *et al.,* 2009). Future researchers will greatly benefit from added information that will save them time in their own research. It is also possible that a medical breakthrough could occur with greater research on obscure organisms like *M. ruber*. My part in the research is to choose a system in *Meiothermus Ruber* that has not been studied and investigate its similarities compared to *E. coli*. I hypothesize that *Mrub_1256* is orthologous to *E. coli* b0415 and *Mruber_1254* is orthologous to *E. coli* b1662.

Methods:

To begin the research, I used the bioinformatics programs on *E. coli* and *M. ruber* available on GENI-ACT. There were several deviations implemented during the process. The deviations included using a color-coded KEGG map to indicate the origin of transcription in order to make reading the data easier. It also aided in determining the function of the operon. Finally, before even starting the bioinformatics portion of the research, I performed protein BLASTS using the *E. coli* amino acid sequences of *E. coli* b0415 against *Mrub*_1256 and *E. coli* b1662 against *Mrub*_1254. Once the results were tabulated in the database, I wanted to see if it was reasonable to hypothesize that *Mrub_1256* is orthologous to *E. coli* b0415 and *Mrub_1254* is orthologous to *E. coli* b1662 (NCBI). The findings were promising as the E-values were very low, indicating that both *Meiothermus Ruber* genes have a likely candidate for *E. coli*.

Results:

From the procedures supplied by GENI-ACT.org, I was able to tabulate bioinformatics results on the *E. coli* b0415, *E. coli* b1662, *Mrub*_1256, and *Mrub*_1254. Table 1 presents an overview of the pertinent tests performed on *Mrub_1256* and *E. coli b0415*, while table 2 shows the pertinent tests for *Mrub_1254* and *E. coli b1662*. I will first examine table 1 in more detail and later describe the findings of table 2. The figures go into more detail on each of those tests.

Table 1: *E. coli b0415* and *Mrub_1256* are Orthologs

Description of Evidence Collected	***E. coli* (b0415)**	***M. ruber* (*Mrub*_1256)**
Cellular localization	Cytoplasmic	
Blast *E. coli* against *M. ruber*	Score: 151 bits; E-Value: 3e-51	
KEGG Pathway	Riboflavin Metabolism	
Pfam- protein family	PF00885 6,7-dimethyl-8-ribityllumazine synthase	
E-value Pfam	2.2e-54	1.1e-51
CDD (COG category)	COG0054- RibE	

E-value COG	5.84e-75	1.68e-67
TIGRfam- protein family	TIGR00114- lumazine-synthase: 6,7-dimethyl-8-ribityllumaz	
E-value TIGRfam	8.1e-91	4e-68
E.C. number	E.C: 2.5.1.78- 6,7-dimethyl-8-ribityllumazine synthase	
PDB	3MK3- Lumazine Synthase	
E-Value PDB	4.45e-77	1.11e-37

Table 1 displays the outcomes from the bioinformatics tools performed on *E.coli* b0415 and *Mrub*_1256. PSORT-B was done with the use of the amino acid sequences of *E. coli* b0415 and *Mrub*_1256. *Mrub*_1256 had a cytoplasmic score of 9.26, and *E. coli* b0415 also had a cytoplasmic score of 9.26. This indicates that *Mrub*_1256 and *E. coli* b0415 are likely found within the cytoplasm.

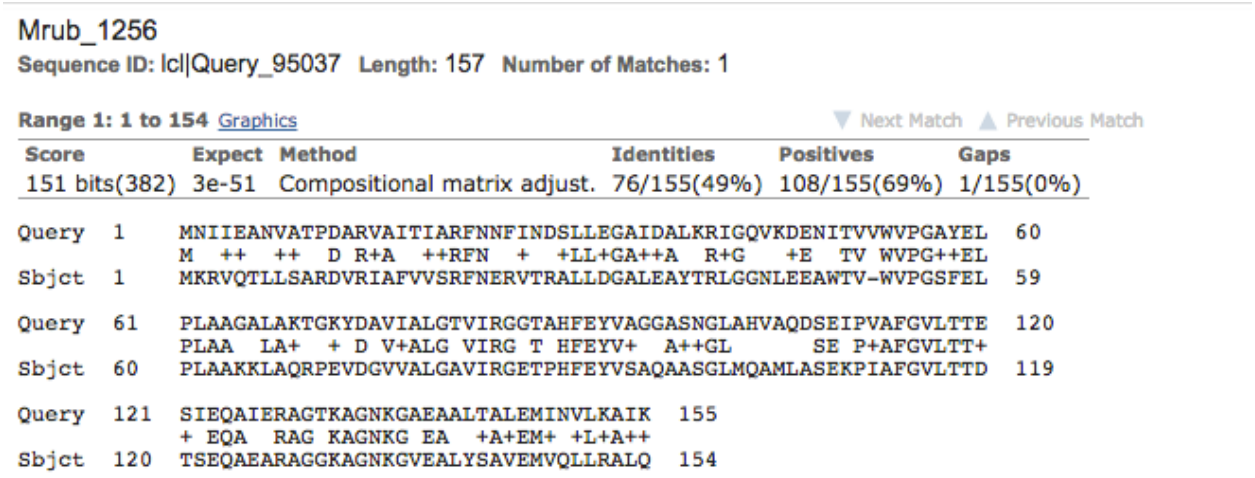
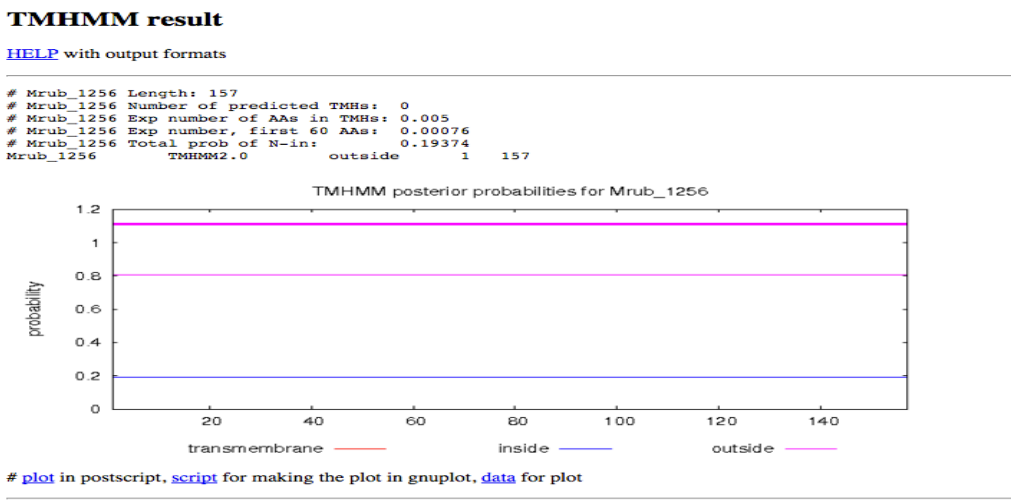


Figure 2. *E.coli* b0415 amino acid sequence BLASTed against *Mrub*_1256; low e-score indicates sequence similarity not due to chance alone. NCBI protein blast was used to generate this result (NCBI BLAST).

When the *E. coli* b0415 was compared against *Mrub*_1256 amino acid sequence, with the use of Protein BLAST, a bit score of 151 was observed which tells us how similar the two amino acid sequences are to each other. The E-value was very low at 3e-51. This indicates that the

*Mrub*_1256 amino acids sequence compared to the *E. coli* b0415 gene sequences are similar and not by chance. Both KEGG pathways contained the Riboflavin metabolism (table 1). Pfam numbers were both PF00885 and a part of the 6,7-dimethyl-8-ribityllumazine synthase family. Since they had the same Pfam name, they are most probably located within the same domain. The Pfam E-values varied slightly with *E. coli* b0415 showing 2.2e-54 and *Mrub*_1256 having 1.1e-51, but these values are very close. Both CDD names were COG0054- RibE, indicating that both genes could be orthologous to each other. Again, their E-values differed slightly as *E. coli* b0415 had 5.84e-75 and *Mrub*_1256 had 1.68e-67. The two shared the same TIGRfam name, TIGR00114- lumazine-synthase: 6,7-dimethyl-8-ribityllumaz, and had very similar E-values- 8.1e-91 for *E. coli* b0415 and 4e-68 for *Mrub*_1256. Because the two genes had the same TIGRfam name and number, it can be concluded that genes could be performing the same function, and the *E. coli* b0415 is a good match to the consensus sequence that falls within the *Mrub*_1256 domain. The E.C. number and the gene name were the exact same at 2.5.1.78- 6,7-dimethyl-8-ribityllumazine synthase. Finally, both PDB numbers and names were the same- 3MK3-Lumazine Synthase, predicting that both of their protein structures were similar. Their PDB E-values were different, *E. coli* at 4.45e-77 and *Mrub*_1256 with 1.11e-37, which is still very close to 0.0 (table 1).

Panel A



Panel B

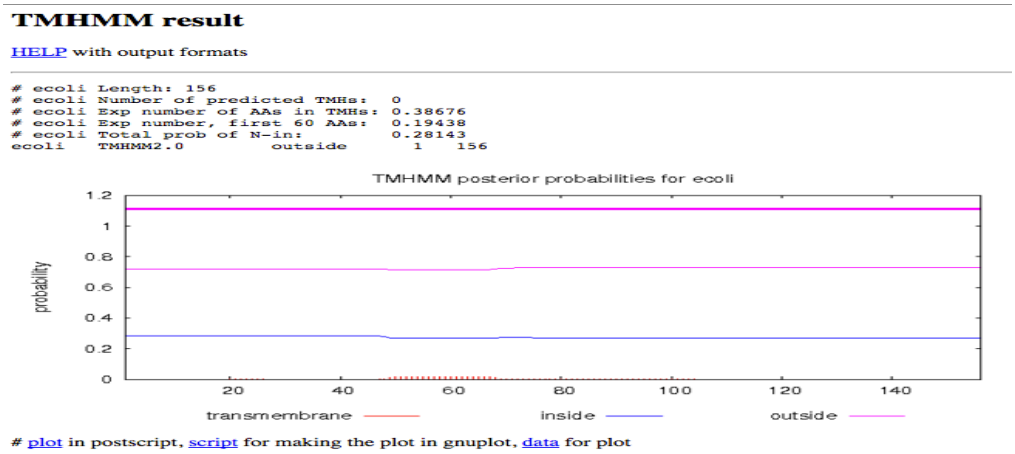
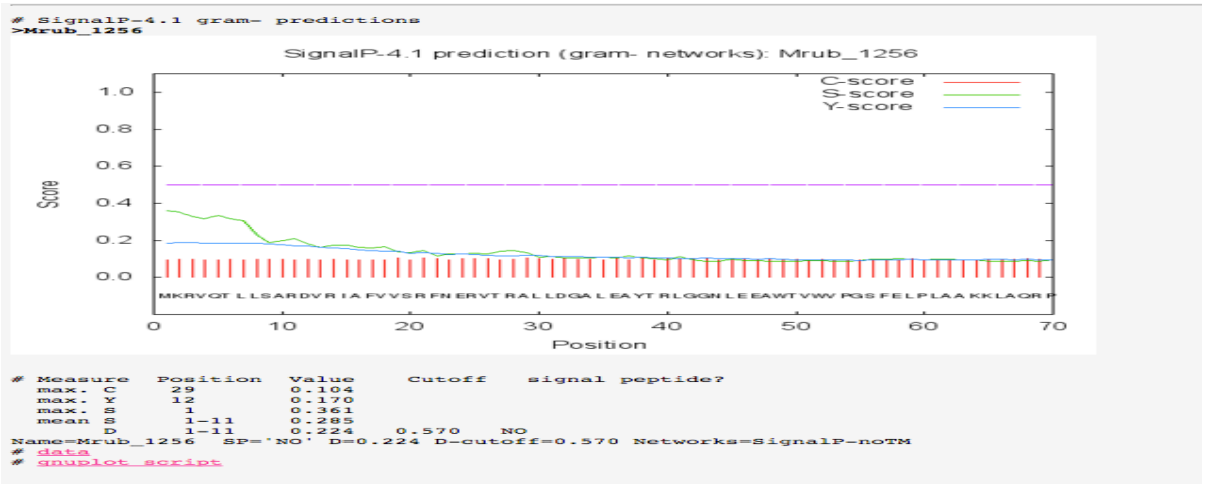


Figure 3. *Mrub*_1256 and *E. coli* b0415 do not contain TMHMM regions; evidence predicted a cytoplasmic position. Panel A is *Mrub*_1256; Panel B is *E. coli* b0415. TMHMM server v. 2.0 created these hydropathy plots TMHMM.

TMHMM developed hydropathy plots on *Mrub*_1256 and *E. coli* b0415. A red line indicates the presence of a transmembrane helix (figure 3). Both *Mrub*_1256 and *E. coli* b0415 have red lines displaying low probability values for finding this gene outside the cytoplasm. Both panels were nearly identical, suggesting that both *Mrub*_1256 and *E. coli* b0415 are located within the cytoplasm.

Panel A



Panel B

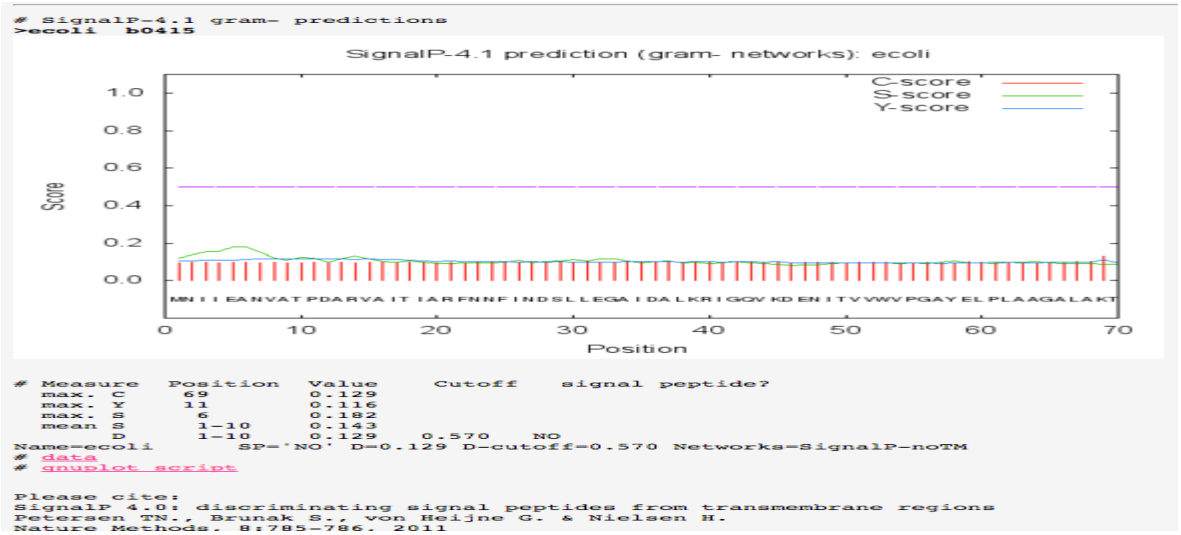


Figure 4. *Mrub*_1256 and *E. coli* b0415 do not contain any cleavage sites; a cytoplasmic location is likely. Panel A=*Mrub*_1256; Panel B=*E. coli* b0415. SignalP 4.1 server created these cleavage site plots (Signal IP 4.1 server).

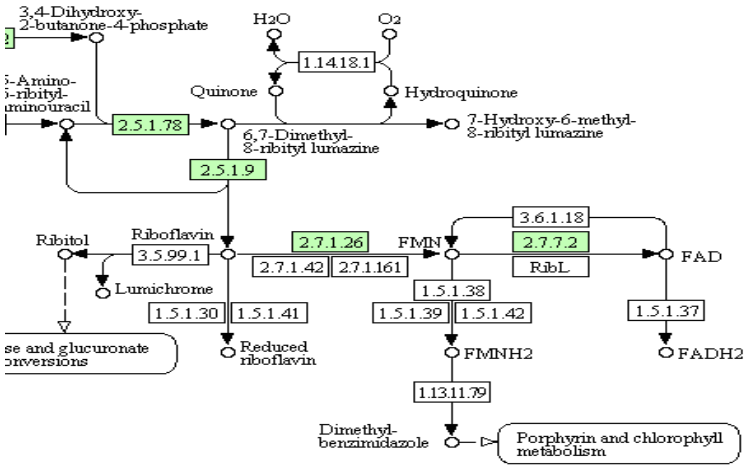
Both *Mrub*_1256 and *E. coli* b0415 lacked evidence of any cleavage sites (Figure 4).

*Mrub*_1256 had a D value of 0.224, which was below the cutoff value of 0.570 (purple line).

This shows that the probability of a cleavage site is very low. It also indicates that there is no

presence of a signal peptide sequence or transmembrane domains within the *Mrub*_1256 gene. *E. coli* b0415 had a D value of 0.129, which is also below the cutoff line of 0.570.

Panel A



Panel B

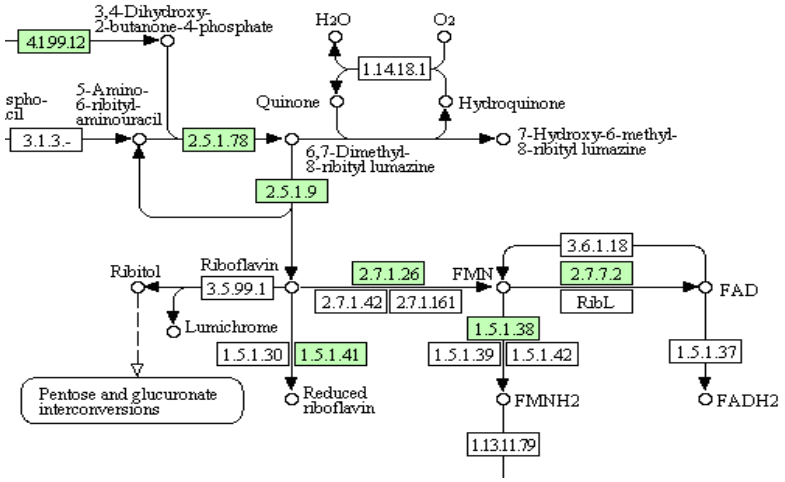


Figure 5. *Mrub*_1256 and *E. coli* b0415 both use the Riboflavin metabolism for creating Riboflavin; both substances are able to make Riboflavin from either 3,4-Dihydroxy-2-butanone-4-phosphate or 5-Amino-6-ribityl-aminouracil. Panel A=*Mrub*_1256; Panel B= *E. coli* b0415. KEGG Pathway Database created these pathway diagrams (KEGG Pathway).

Figure 5 shows the results of a KEGG pathway comparison between *Mrub*_1256 and *E. coli* b0415 and their synthesis of Riboflavin. The green boxes indicate the presence of the enzyme

necessary to produce Riboflavin. *Mruber*_1256 and *E. coli* b0415 contain only one pathway to Riboflavin beginning either 3,4-Dihydroxy-2-butanone-4-phosphate or 5-Amino-6-ribityl-aminouracil. While not identical, the *Mruber*_1256 and *E. coli* b0415 pathways from either 3,4-Dihydroxy-2-butanone-4-phosphate or 5-Amino-6-ribityl-aminouracil to Riboflavin are very similar.

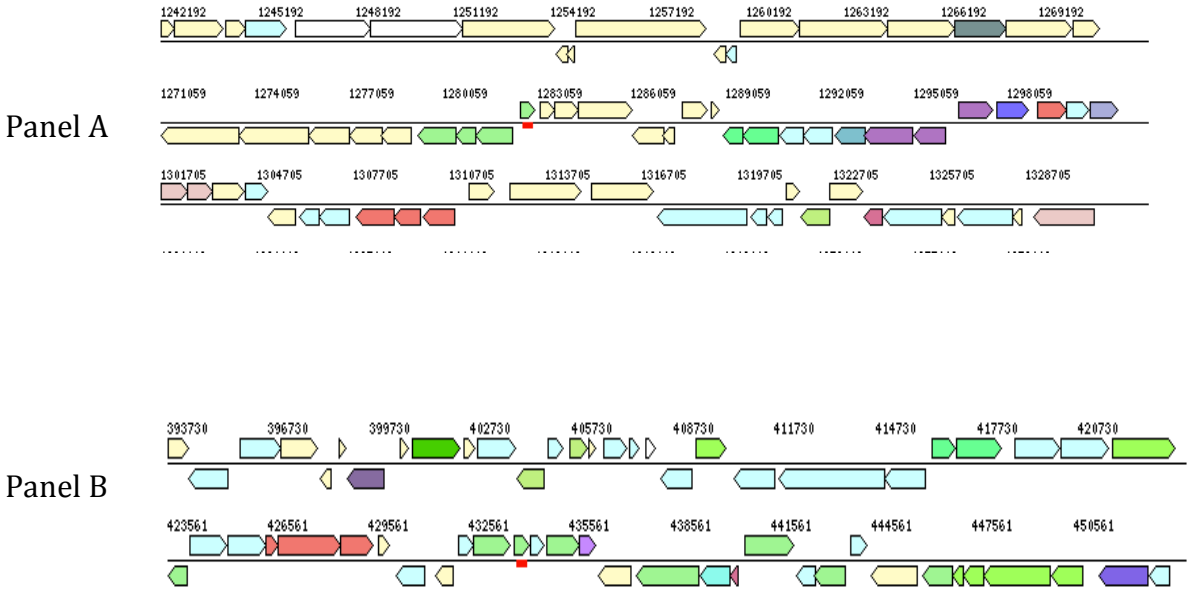


Figure 6. *Mrub*_1256 and *E. coli* b0415 indicate operons with similar functions; transcription occurs in the same direction. Panel A=*Mrub*_1256; Panel B=*E. coli* b0415. Joint Genome Institute created these chromosome viewers (Joint Genome Institute).

Figure 6 shows the chromosomal alignment between *Mrub*_1256 and *E. coli* b0415. *E. coli* b0415 has an operon with two genes. The red line indicates the specified gene of interest.. *Mrub*_1256 has an operon with one gene. Transcription occurs in the same direction as the *E. coli* 0415 gene. The *Mrub*_1256 gene is predicted to perform the same function as the *E. coli* b0415 gene as they have the same color on the KEGG map. Even though both genes transcribe for the same enzymes, they start transcription in different regions of the chromosome.

Table 2: *E. coli b1662* and *Mrub*_1254 are orthologs

Description of Evidence Collected	*E. coli* (b1662)	*M. ruber* (*Mrub*_1254)
Cellular localization	Cytoplasmic	
Blast *E. coli* against *M. ruber*	Score: 122 bits; E-Value: 1e-38	
KEGG Pathway	Riboflavin Metabolism	
Pfam- protein family	PF00677 Lumazine Binding Domain	
E-value Pfam	2.3e-21	9.1e-19
CDD (COG category)	COG0307- RibC	
E-value COG	1.16e-107	2.36e-93
TIGRfam- protein family	TIGR00187-Riboflavin Synthase, alpha subunit	
E-value TIGRfam	1.3e-134	1.9e-75
E.C. number	E.C.2.5.1.9- Riboflavin Synthase alpha subunit	
PDB	1l8D- Riboflavin Synthase	
E-Value PDB	5.86e-124	3.39e-28

Table 2 displays the outcomes from the bioinformatics tools performed on *E.coli* b1662 and *Mrub*_1254. PSORT-B was done with the use of the amino acid sequences of *E. coli* b1662 and *Mrub*_1254. *Mrub*_1254 had a cytoplasmic score of 9.97, and *E. coli* b1662 also had a cytoplasmic score of 9.97. *Mrub*_1254 and *E. coli* b1662 are likely found within the cytoplasm.

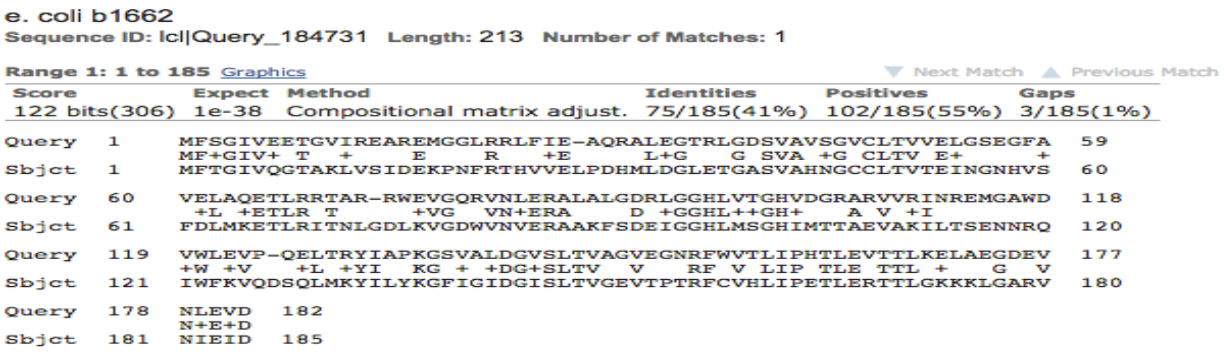


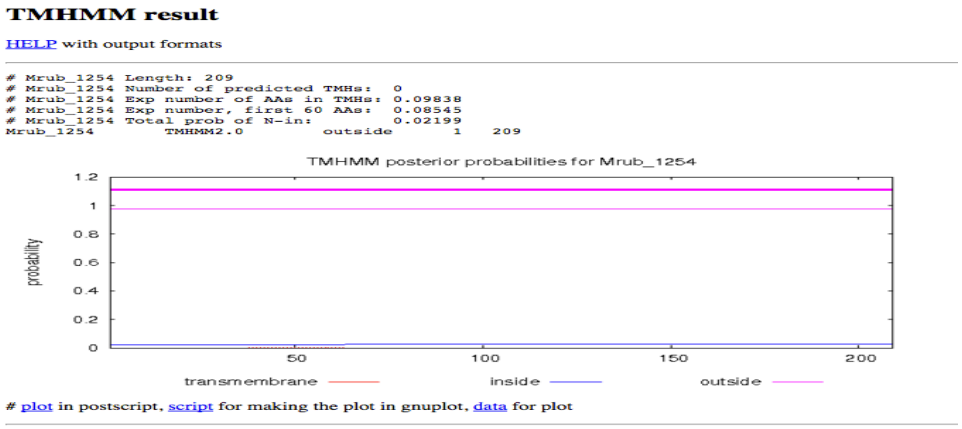
Figure 7. *E.coli* b1662 amino acid sequence run against *Mrub*_1254; low e-score indicates evolutionary descent. NCBI protein blast was used to generate this result (NCBI BLAST).

When the *E. coli* b1662 was run compared to the *Mrub*_1254 (figure 7) amino acid sequence with the use of a protein BLAST, a bit score of 122 was observed which tells us how

similar the two amino acid sequences are to each other. The E-value was very low at 1e-38. This indicates that the *Mrub*_1254 amino acid sequence compared to the *E. coli* b1662 gene is likely related by evolutionary decent, and not by chance.

Both KEGG pathways contained the Riboflavin metabolism (table 2). Pfam numbers were both PF00677 and a part of the Lumazine binding domain. Since they had the same Pfam name, they most probably originate within the same domain. The Pfam E-values varied slightly with *E. coli* b1662 showing 2.3e-21 and *Mrub*_1254 having 9.1e-19. Both CDD names were COG0307-RibC, indicating that both genes could be orthologous to each other. Again, their E-values differed slightly as *E. coli* b1662 had 1.16e-107 and *Mrub*_1254 had 2.36e-93. The two shared the same TIGRfam name, TIGR00187- Riboflavin synthase alpha unit and had very similar E-values: 1.3e-134 for *E. coli* b1662 and 1.9e-75 for *Mrub*_1254. Because the two genes had the same TIGRfam name and number, it can be concluded that genes could be performing the same function and the *E. coli* b1662 is a good match to the consensus sequence that falls within the *Mrub*_1254 domain. The E.C. numbers and names were the exact same at 2.5.1.9- Riboflavin synthase alpha unit. Finally, both PDB number and names were the same- 1l8D- Riboflavin synthase, predicting that both of their protein structures were the same. Their PDB E-values were different, *E. coli* b1662 at 5.86e-124 and *Mrub*_1254 with 3.39e-28, which is still very close to 0 (table 2).

Panel A



Panel B

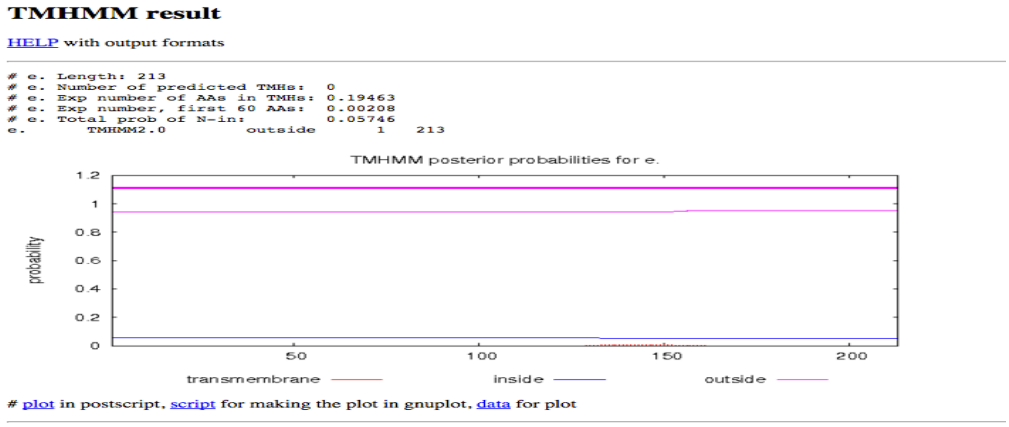


Figure 8. *Mrub*_1254 and *E. coli* b1662 do not contain TMHMM regions; evidence predicted a cytoplasmic position. Panel A is *Mrub*_1254; Panel B is *E. coli* b1662. TMHMM server v. 2.0 created these hydropathy plots TMHMM.

TMHMM developed hydropathy plots on *Mrub*_1254 and *E. coli* b1662. A red line indicates the presence of a transmembrane helix (figure 8). Both *Mrub*_1254 and *E. coli* b1662 have red lines displaying low probability values for finding this gene outside the cytoplasm. Both panels were nearly identical, suggesting that both *Mrub*_1254 and *E. coli* b1662 are located within the cytoplasm.

Panel A

Panel B

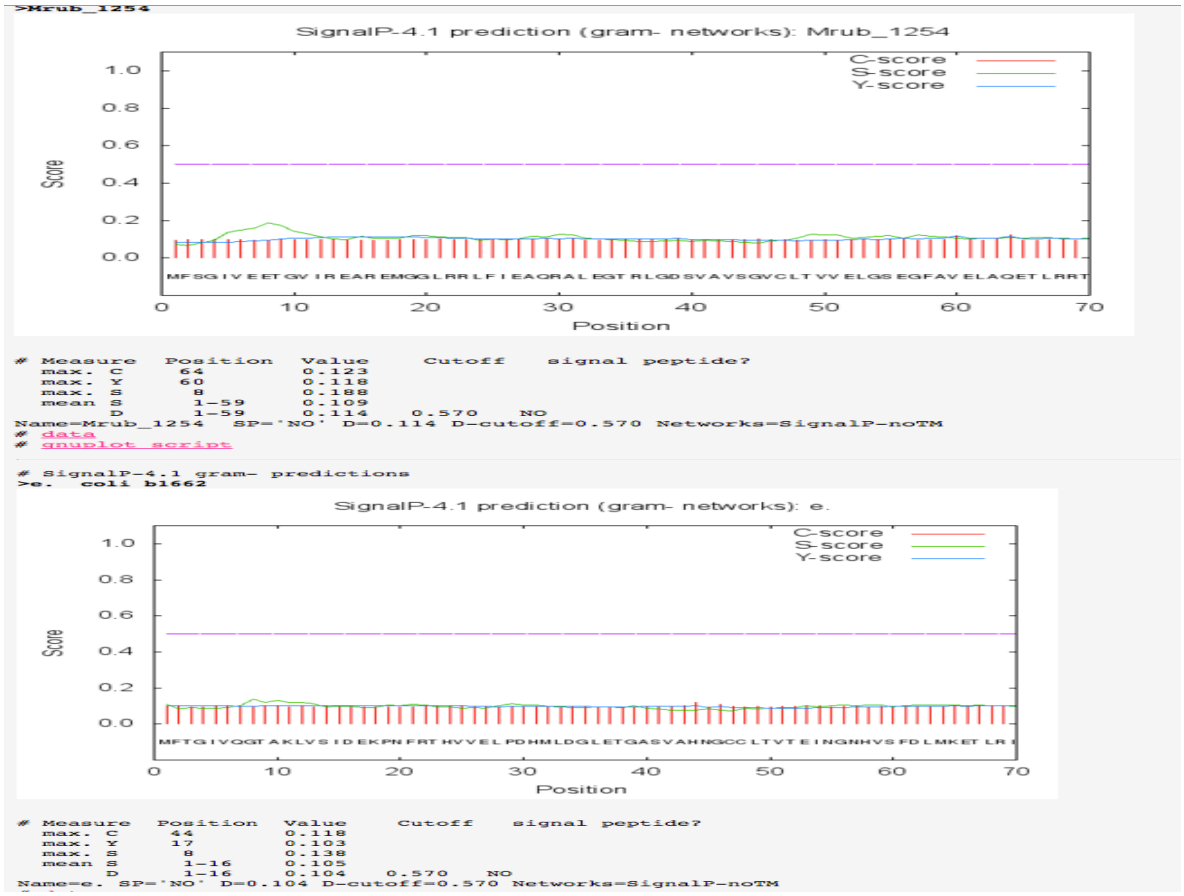
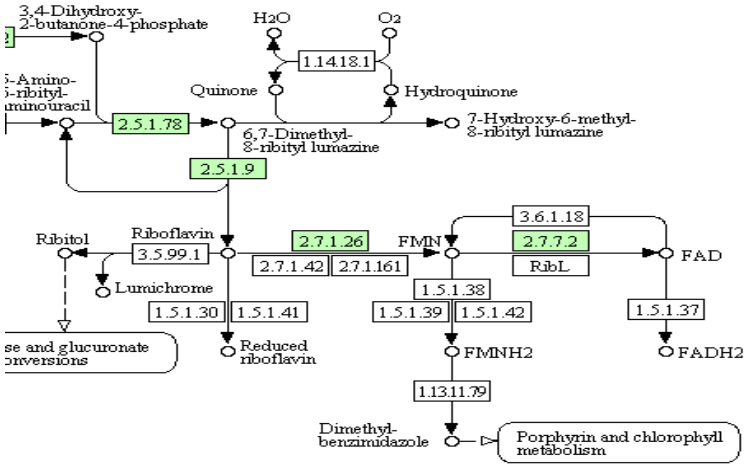


Figure 9. *Mrub*_1254 and *E. coli* b1662 do not contain any cleavage sites; a cytoplasmic location is likely. Panel A=*Mrub*_1254; Panel B=*E. coli* b1662. SignalP 4.1 server created these cleavage site plots (Signal IP 4.1 server).

Both *Mrub*_1254 and *E. coli* b1662 lacked evidence of any cleavage sites (Figure 9). *Mrub*_1254 had a D value of 0.114, which was below the cutoff value of 0.570 (purple line). This shows that the probability of a cleavage site is very low. It also indicates that there is no presence of a signal peptide sequence or transmembrane domains within the *Mrub*_1254 gene. *E. coli* b1662 had a D value of 0.104. This was also below the cutoff 0.570 making this finding insignificant.

Panel A



Panel B

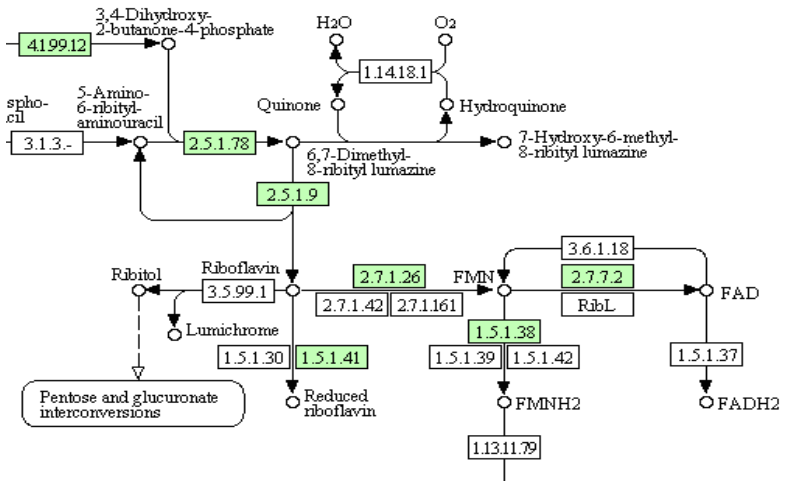
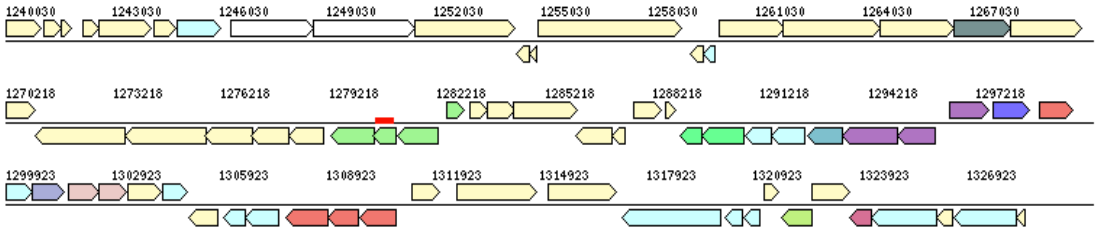


Figure 10. *Mrub*_1254 and *E. coli* b1662 both use the Riboflavin metabolism for creating Riboflavin; both substances are able to make Riboflavin from 6,7-Dimethyl-8-ribityl lumazine. Panel A=*Mrub*_1254; Panel B= *E. coli* b1662. KEGG Pathway Database created these pathway diagrams (KEGG Pathway).

Figure 10 shows the results of a KEGG pathway comparison between *Mrub*_1254 and *E. coli* b1662 and their synthesis of Riboflavin. The green boxes indicate the presence of the enzyme necessary to produce Riboflavin. *Mruber*_1254 and *E. coli* b1662 contain only one pathway to Riboflavin beginning at 3,4-Dihydroxy-2-butanone-4-phosphate. While not identical, the *Mruber*_1254 and *E. coli* b1662 pathways from 6,7-Dimethyl-8-ribityl lumazine to Riboflavin are very similar.

Panel A



Panel B

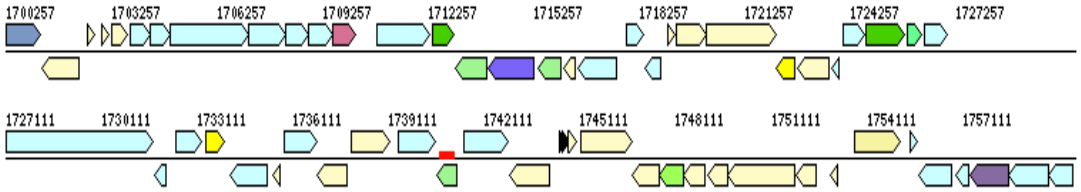


Figure 11. *Mrub*_1254 and *E. coli* b1662 indicate operons with similar functions; transcription occurs in the same direction. Panel A=*Mrub*_1254; Panel B=*E. coli* b1662. Joint Genome Institute created these chromosome viewers (Joint Genome Institute).

Figure 11 shows the chromosomal alignment between *Mrub*_1254 and *E. coli* b1662. The red line indicates the specified gene of interest. *E. coli* b1662 has an operon with one gene. *Mrub*_1254 has an operon with three genes. Transcription occurs in the same direction compared to the *E. coli* b1662. The first three genes are predicted to perform the same function as the *Mrub*_1254 gene as the other genes in the operon have the same color on the KEGG map. Even though both the *E. coli* b1662 and *Mrub*_1254 genes transcribe for the same enzymes, they start transcription in different regions of the chromosome.

Conclusion:

Based upon the evidence, the conclusion can be drawn that *Mrub*_1256 shares a common evolutionary ancestor with *E. coli* b0415 and *Mrub*_1254 shares a common evolutionary ancestor with *E. coli* b1662. All four genes were predicted to be located within the cytoplasm based on the TMHMM hydropathy plots and SignalP results. Several tests showed the function

of *E. coli* b4015 and *Mrub*_1256, as well as *E. coli* b1662 and *Mrub*_1254 were the same, further indicating that the *Meiothermus Ruber* genes are responsible for the production of Riboflavin. The PFam findings had the same domain name, 6,7-dimethyl-8-ribityllumazine synthase for *E. coli* b0415/*Mrub*_1256 and Lumazine Binding Domain for *E. coli* b1662/*Mrub*_1254; TIGRfam numbers were identical for *E.coli* b0415/*Mrub*_1256 (TIGR00114) and *E. coli* b1662/*Mrub*_1254 (TIGR00187); KEGG pathway predicted the same E.C. number of 2.5.1.78 for *E. coli* b0415/*Mrub*_1256 and 2.5.1.9 for *E. coli* b1662/*Mrub*_1254. In addition, the PDB databank pulled the same crystalized protein structure for *E. coli* b0415/*Mrub*_1256 and the same for *E. coli* b1662/*Mrub*_1254. From the Gene Context maps obtained from IMG/EDU, it looked like *Mrub*_1254 was in a three-operon system while *E. coli* b1662 was not. I disregarded these results as both genes, according to the map, were predicted to perform the same function and transcribed in the same direction. The same can be said of *E.coli* b0415. The map indicates that it is part of a two-operon system while *Mrub*_1256 is not. Both transcribed their proteins in the same direction and also were predicted to perform the same function, so I ignored the result. With so many similar or identical findings, it is likely that *Mrub*_1256 has the same function as *E. coli* b0415 and *Mrub*_1254 has the same function as E. coli b1662. It can be concluded that these pairs of orthologous genes share similar functions in the production of Riboflavin.

Literature Cited

2015. Joint Genome Institute; [accessed 2015 Dec 11]. http://jgi.doe.gov/

Available Modules. Geni-Act; [accessed 2015 Dec 11]. http://www.geni-act.org/education/main/

Belinda TJ. 2014. Significance of Riboflavin (Vitamin-B2) for Health.Journal of Pharmaceutical Sciences and Research 6:285–287.

Bioinformatics: Finding Functions. 2015 Mar 5. National Human Genome Research Institute; [accessed 2015 Dec 11]. http://www.genome.gov/25020002

Eberhardt S, Richter G, Gimbel W, Werner T, Bacher A. 1996. Cloning, Sequencing, Mapping and Hyperexpression of the ribC Gene Coding for Riboflavin Synthase of Escherichia coli.The FEBS Journal 242:712–719.

Mortl S, Fischer M, Richter G, Tack J, Weinkauf S, Bacher A. 1996. Biosynthesis of Riboflavin LUMAZINE SYNTHASE OF ESCHERICHIA COLI.The Journal of Biological Chemistry :33201–33207.

NCBI BLAST; [accessed 2015 Nov 25]. http://blast.ncbi.nlm.nih.gov/Blast.cgi

Pathway: flavin biosynthesis I (bacteria and plants). 2016. Metacyc; [accessed 2016 Jan 20]. http://www.metacyc.org/META/new-image?type=PATHWAY&object=RIBOSYN2-PWY&detail-level=2&ENZORG=TAX-511145

Score, Bit-score, P-value, E-value. VRIJE Universiteit Brussel; [accessed 2015 Dec 11]. http://homepages.ulb.ac.be/~dgonze/TEACHING/stat_scores.pdf

Scott L. 2015. Meiothermus ruber Genome Analysis Project. Geni-Science; [accessed 2015 Dec 17]. http://geni-science.org/secure/projects/view/

Scott L. Project Details. Microbial Genome Annotation Network; [accessed 2015 Dec 11]. https://sites.google.com/a/augustana.edu/meiothermus_ruber/project-introduction

SignalP 4.1 Server. 2013 Jun 4. CBS Prediction Servers; [accessed 2015 Dec 11]. http://www.cbs.dtu.dk/services/SignalP/

Tindall, Brian J, Johannes Sikorski, Susan Lucas, Eugene Goltsman, Alex Copeland, Tijana Glavina Del Rio, Matt Nolan, et al. 2010. Complete genome sequence of *Meiothermus ruber* type strain. Standards in Genomic science. (serial online). (cited 2/4/16). 3:26-36. Available from: Ebscohost

TMHMM Server v. 2.0: Prediction of transmembrane helices in proteins. 2015 Aug 4. CBS Prediction Servers; [accessed 2015 Dec 11]. http://www.cbs.dtu.dk/services/TMHMM/

Todar K. 2015. Overview of Bacteriology . Online Textbook of Bacteriology ; [accessed 2015 Dec 17]. http://textbookofbacteriology.net/bacteriology.html

U.S. Department of Energy Joint Genome Institute. [Internet: http://jgi.doe.gov/our-science/science-programs/microbial-genomics/phylogenetic-diversity/ accessed 7-29-14]

Wiring diagrams of molecular interactions, reactions, and relations. 2015 Dec 1. KEGG PATHWAY Database; [accessed 2015 Dec 11]. http://www.genome.jp/kegg/pathway.html

Wu, D et al. 2009. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature 462(7276): 1056-60.