Annotation of Locus Tag SAI1T1_2000280 of the *Staphylococcus aureus* Genome

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Abstract

A gene from the microorganism *Staphylococcus aureus* (SAI1T1_2000280) was annotated using the collaborative genome annotation website GENI-ACT. The Genbank proposed gene product name for the gene was assessed in terms of the general genomic information, amino acid sequence-based similarity data, structure-based evidence from the amino acid sequence, cellular localization data, potential alternative open reading frames, structure-based evidence, enzymatic function, gene duplication and degradation, evidence for horizontal gene transfer, and RNA. The Genbank proposed gene product name did not differ significantly from the proposed gene annotation for the gene.

Introduction

Staphylococcus aureus is gram stain positive bacteria. This organism is small and a non-motile cocci bacteria. This round bacteria is usually found in clusters that resemble grapes. As a result, the greek prefix staphylo-, meaning grape like, and the Latin suffix -cocus, meaning round, came together to form the genus name *Staphylococcus.* According to Mandal, surgeon Sir Alexander Ogston first discovered *Staphylococcus aureus* in surgical pus around the year 1800 in Scotland (Mandal, 2009).

Also according to Mandal, *S. aureus* can cause an array of illness ranging skin infections to life threatening food poisonings. One third of the public carries *S. aureus* in or on their noses, pharynx, or skin. Typically, the bacteria does not cause an infection until a wound or other infection weakens the body. Once, weakened *S. aureus* then can cause an infection which can be life threaten. As an example, Methicillin-resistant *Staphylococcus aureus,* commonly referred to as MRSA, is a life threatening infection in many cases (Mandal, 2009).

Methods and Materials

Modules of the GENI-ACT (http://www.geni-act.org/) were used to complete *Staphylococcus aureus* genome annotation . The modules are described below:

Modules	Activities	Questions Investigated
Module 1- Basic Information Module	DNA Coordinates and Sequence, Protein Sequence	What is the sequence of my gene and protein? Where is it located in the genome?
Module 2- Sequence-Based Similarity Data	Blast, CDD, T-Coffee, WebLogo	Is my sequence similar to other sequences in Genbank?
Module 3- Cellular Localization Data	Gram Stain, TMHMM, SignalP, PSORT, Phobius	Is my protein in the cytoplasm, secreted or embedded in the membrane?
Module 4- Alternative Open Reading Frame	IMG Sequence Viewer For Alternate ORF Search	Has the amino acid sequence of my protein been called correctly by the computer?
Module 5- Structure-Based Evidence	TIGRfam, Pfam, PDB	Are there functional domains in my protein?
Module 6- Enzymatic Function	KEGG, MetaCyc, E.C. Number,	In what process does my protein take part?
Module 7- Gene Duplication/ Gene Degradation	Paralog, Pseudogene	Are there other forms of my gene in the bacterium? Is my gene functional?
Module 8- Evidence for Horizontal Gene Transfer	Phylogenetic Tree,	Has my gene co-evolved with other genes in the genome?
Module 9- RNA	RFAM	Does my gene encode a functional RNA?

Results

Staphylococcus aureus 2000280 Annotation:

The initial proposed product for this gene is Methicillin-resistance regulatory protein MecR1 and throughout this investigation and annotation of this gene, all evidence supports this original annotation.

BLAST RESULTS:

The significant BLAST results, depict gene products very similar to Methicillin-resistance regulatory protein MecR1. For example the top two BLAST results are Peptidase M56 family in *Staphylococcus epidermidis W23144* and beta-lactam sensor/signal transducer MecR1 in *Staphylococcus argenteus* These two proteins serve very similar functions in very similar organisms to Methicillin-resistance regulatory protein MecR1 in *Staphylococcus aureus* and therefore support the original annotation that the gene produces Methicillin-resistance regulatory protein MecR1.

Figure 1.1- WebLogo plot of the significant blast results of SAI1T1_2000280.

Moreover, Figure 1.1 is WebLogo plot showing the conservation of the gene relative to the significant BLAST results. The large letters throughout the entire extent of the plot supports the claim that the gene is very well conserved and is very similar to many other genes in the Gene bank. In turn, a very well conserved WebLogo plot further supports the conclusion that the gene was correctly annotated and does in fact produce Methicillin-resistance regulatory protein MecR1 and was also correctly called by computer.

Function of Methicillin-resistance regulatory protein MecR1:

As stated in the introduction, *Staphylococcus aureus* is an infection causing gram positive bacteria that can develop bacterial resistance. The regulatory protein MecR1 plays a critical role in the bacteria developing bacterial resistance and the mechanism by which MecR1 contributes to the resistance will be explained below.

Bacterial Operons:

According to Mader et al., prokaryotes regulate gene expression through a unit of DNA called an operon. An operon has a few general components (Mader et al., 2016).

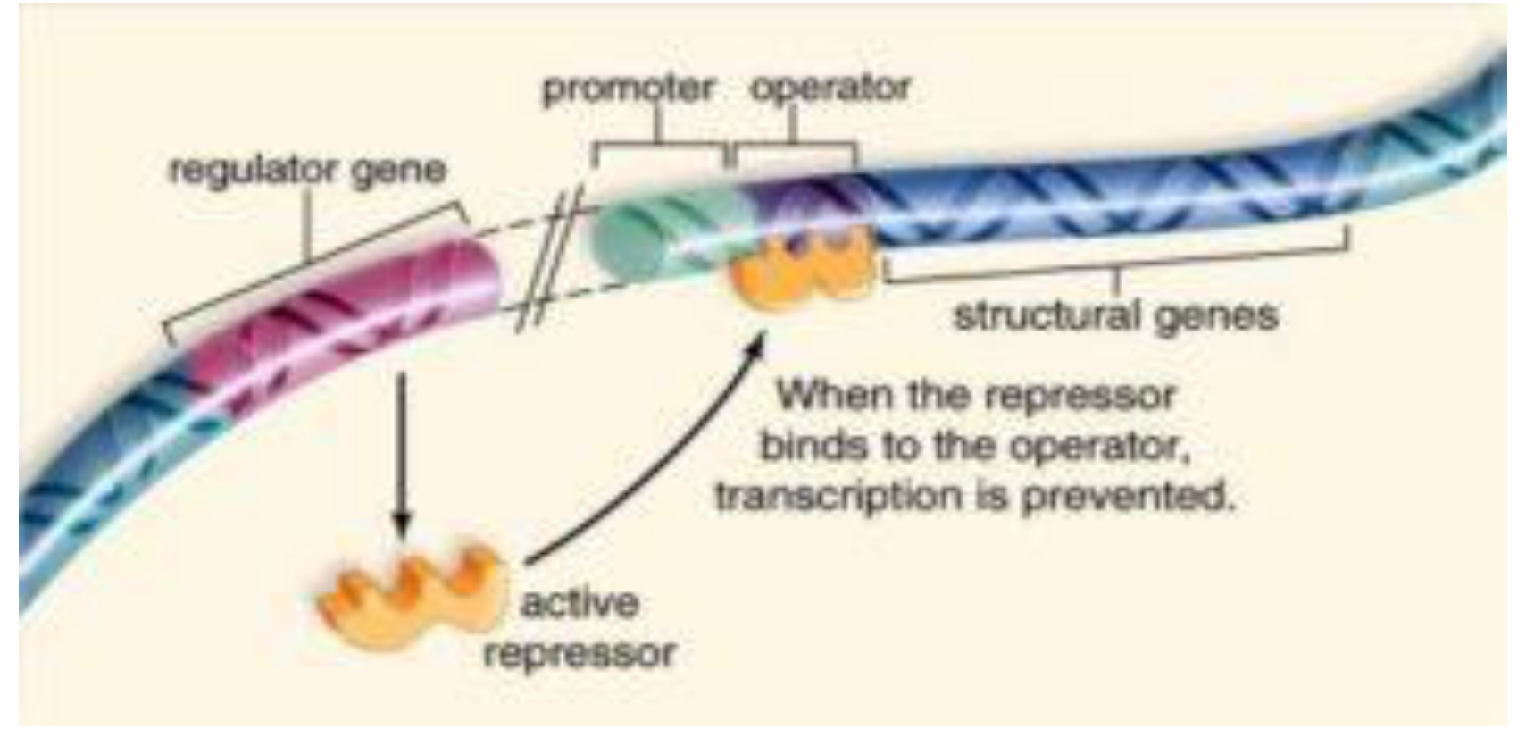

Figure 1.2- Generic Operon

As shown in Figure 1.2, an operon contains structural genes which RNA polymerases transcribes into mRNA. The mRNA then in turn is translated at the ribosome into amino acids that make up proteins. Also present in the operon is the promoter region where the RNA polymerase first attaches to transcribe the structural genes. Lastly, the regulator gene codes for a gene product that can either prohibit or allow the transcription of the structural genes by changing the shape of the repressor prohibiting or allowing it to bind to operator site. If the repressor is allowed to bind to the operator site than RNA polymerase cannot transcribe the structural genes (Mader et al., 2016)

MecA Regulation:

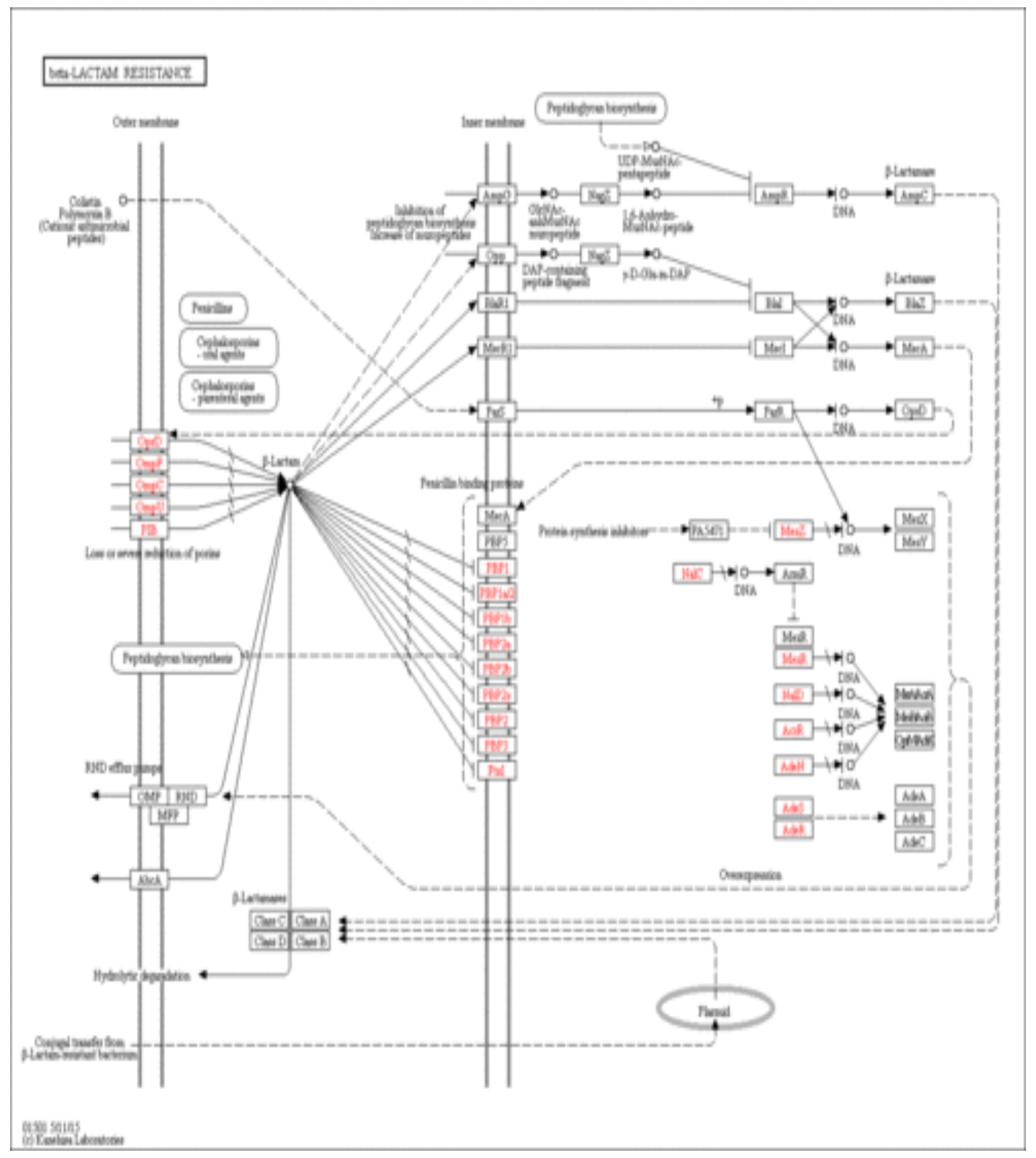

Figure 1.3- The KEGG Pathway Map of Function of MecR1 in regulation of peptidoglycan biosynthesis

As shown in the top right hand side of Figure 1.3, after being produced by the regulator gene SAI1T1_2000280, MecR1 is modified into regulator protein MecI. MecI than stimulates the transcription of the structural genes that code for penicillin binding protein, MecA. As shown in the middle of Figure 1.3, the penicillin binding proteins, such as MecA aid in the biosynthesis of peptidoglycan. Peptidoglycan makes up the cell walls of bacteria and therefore is necessary for the growth and division of the bacteria cells. Without peptidoglycan, *Staphylococcus aureus* is unable to survive. A more complex KEGG Pathway Map of the biosynthesis of peptidoglycan is shown in Figure 1.4.

The Role of MecA in Bacterial Resistance:

As stated above, penicillin binding proteins aid in the biosynthesis of peptidoglycan. Penicillin or other beta lactam antibiotics, such as methicillin, function by binding to the penicillin binding protein, hence the name of the protein. This prevents the protein from synthesizing peptidoglycan. Without peptidoglycan, the bacterial cell ends of dying as stated above.

As shown in figure 1.3, there are many penicillin binding proteins, and MecA one the proteins, has the lowest affinity for beta lactam antibiotic. Consequently, if beta lactam antibiotic is present a bacterial cell can still transcribe MecA and in turn biosynthesize peptidoglycan. As long as peptidoglycan is being produced the beta lactam antibiotic will not kill the *Staphylococcus aureus* bacteria and it will appear antibiotic resistant. Although the pathway from the MecR1 coding gene to bacterial resistance appears complicated, it is truly this gene that is at the root of bacterial resistance in *Staphylococcus aureus* and other bacteria.

A Note Regarding Horizontal Gene Transfer:

Figure 1.3 shows the horizontal gene transfer of a resistance gene via a plasma and is the mechanism that allows bacterial resistance to spread so far and so fast.

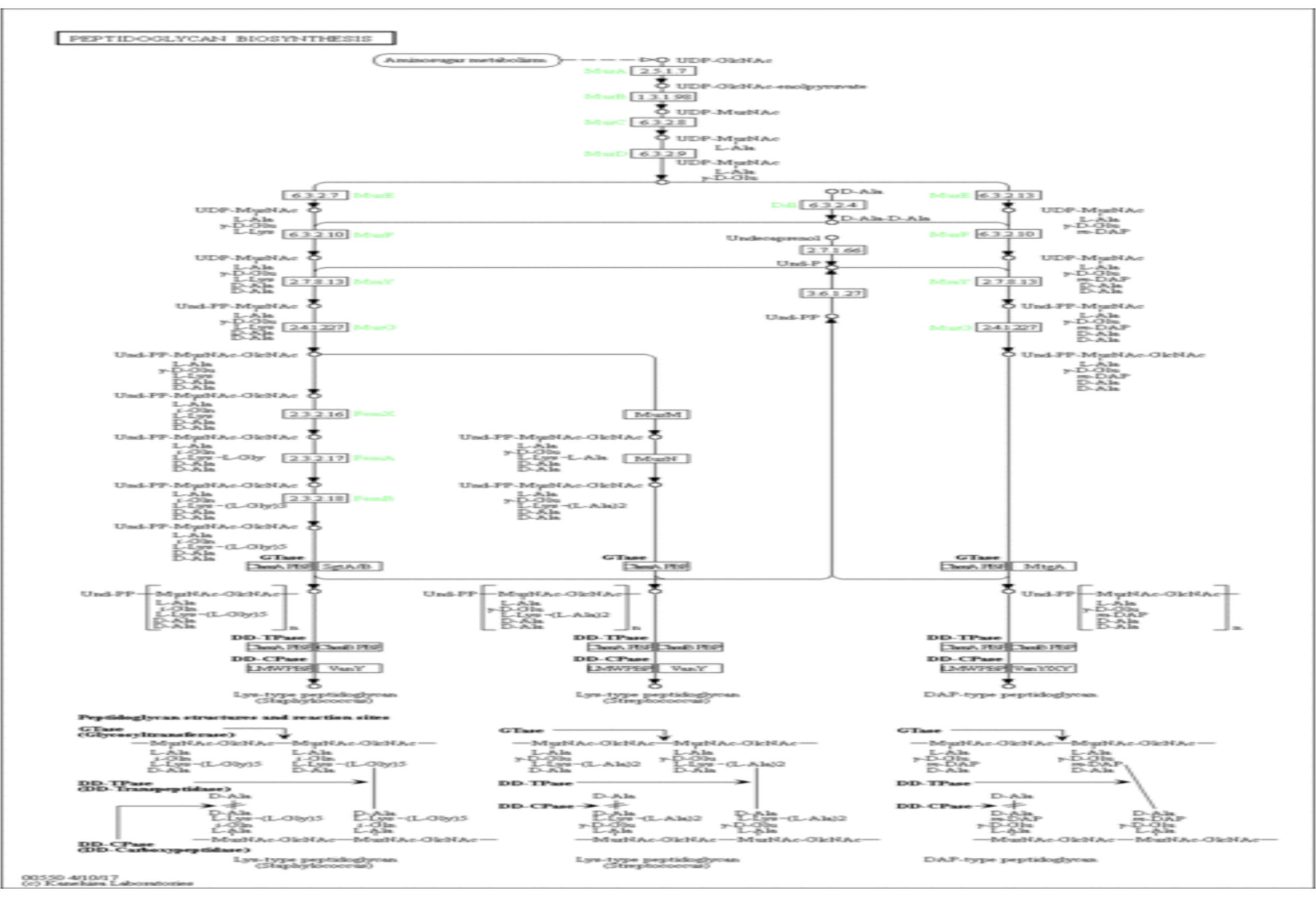

Figure 1.4- A KEGG Pathway Map showing the biosynthesis of peptidoglycan

Conclusion

The Geni-Act proposed gene product did not differ significantly from the proposed gene annotation for the gene and as such, the genes appear to be correctly annotated by the computer database. These results support that SAI1T1_2000280 forms Methicillin-resistance regulatory protein MecR1 which aids in the bacterial resistant nature of *Staphylococcus aureus.*

Gene Locus	Proposed Annotation
2000280	Methicillin-resistance regulatory protein MecR1

References

Mader, S. S., & Windelspecht, M. (2016). *AP Edition: Biology Textbook* (Twelfth ed.). New York, NY: McGraw Hill Education.
Mandal, A., Dr,. (2009, December 9). What is Staphylococcus Aureus? Retrieved May 17, 2017.

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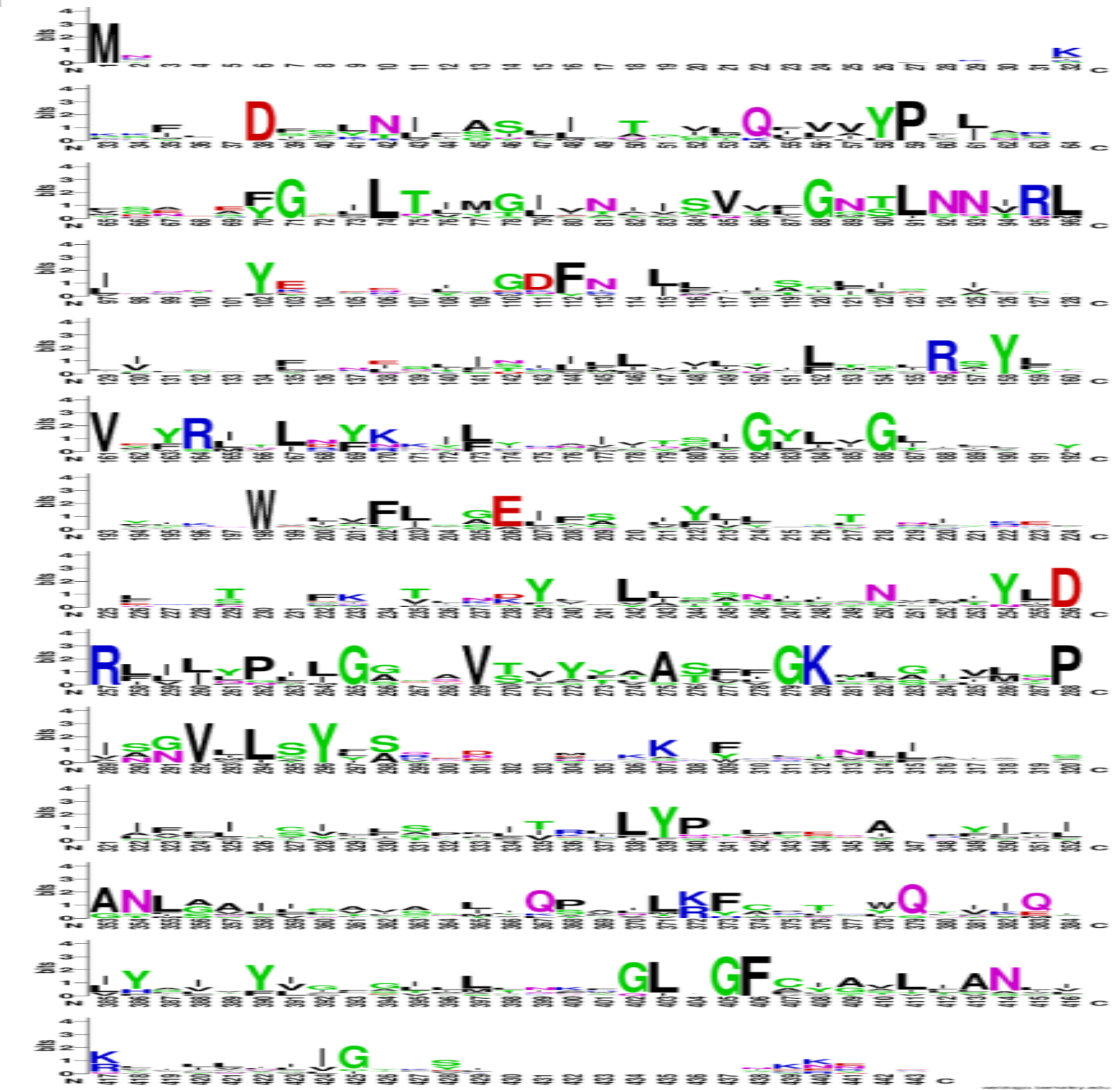
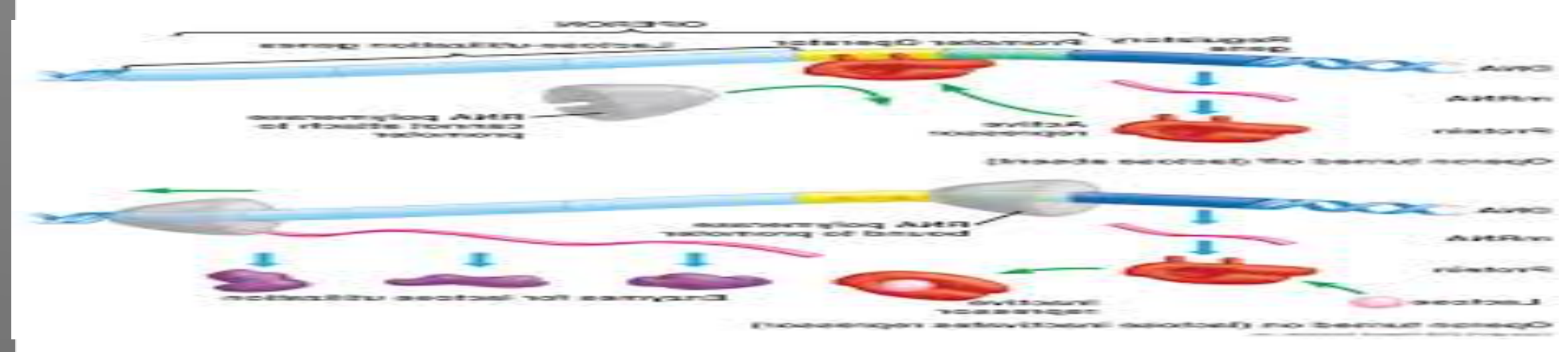


The WebLogi diagram shows that the blast results we got were not very well conserved. The different sized letters throughout clarify that the blast results we got were not well conserved.



Generic operon