

Annotation of the *Haemophilus influenzae* F3031 Genome at Locus Tag HIBPF_14120

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Abstract

The purpose of the Geni-Act bioinformatics research was to prove the computer programs used correctly analyzed genes from *Haemophilus influenzae* F3031. The gene researched was at locus tag HIBPF_14120. Geni-Act was connected to different modules designed to analyze the genes using the basic amino acid sequences, sequence similarities, structure-based evidence, cellular localization and open reading frame analysis. Results for the gene product of this gene was as expected from the Genbank. There was one result which contradicted the information from what was predicted by the databases and computer programs indicating the importance of manual annotation and analysis.

Introduction

Haemophilus influenzae F3031 is a gram negative organism that is a clone of *Haemophilus influenzae* biogroup *aegyptius* which causes Brazilian Purpuric Fever. It was discovered in 1984 during an outbreak in Promissao, Sao Paulo, Brazil. The complete genome of *H. influenzae* F3031 has not been previously manually annotated. The purpose of this study was to manually annotate the genome of the bacterium and to describe the function of the gene products of *H. influenzae* F3031. *Haemophilus influenzae* is best grown at temperatures of 35-37 degrees C. Warmer geographic locations are more at risk for having a presence of this gene. It can be located in the upper respiratory tract of humans, since it cannot survive outside the body. The organism is also seen to be some strand of *H. influenzae aegyptius*, based on studies of the Purpuric Fever epidemic. The Purpuric Fever that this gene is known for is a follow up of conjunctivitis that occurs about a month prior to the fever taking affect. This particular gene is of interest because of its life-threatening abilities. Children under the age of ten are more susceptible, since they have weaker immune systems and can cause leukopenia, which is a decrease in the amount of white blood cells present. The result of this is often fatal and a cure is hard to find since it works so quickly.



Figure 1: Symptoms of Brazilian Purpuric Fever (Healthtool, 2017)



Figure 2: Distribution of Brazilian Purpuric Fever between 1984 and 1993.

(Center for Epidemiologic Surveillance, São Paulo Secretariat of Health and Brazilian Ministry of Health.)

Methods

Modules of the GENI-ACT (<http://www.geni-act.org/>) were used to complete *Haemophilus influenzae* F3031 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?

Results

HIBPF_14120:

The initial proposed result of the gene product from the research done through GENI-ACT was gamma glutamate kinase/ glutamate 5 kinase. This initial proposal was supported by the BLAST results from the well curated database, Swiss-Prot.

In comparison to the orthologs, the gene shows a high degree of alignment and conservation. This was evidenced by BLAST results from Swiss-Prot and Non-Redundant databases as well as Weblogo.

After further analysis using TIGRFAM, PFAM and PDB it confirmed this protein to be glutamate 5 kinase in a group of amino acid kinases. The protein glutamate 5 kinase is an enzyme that is involved in the transformation of glutamate to the amino acid, proline.

This protein is cytoplasmic based on the results from TMHMM, SignalP, PSORTB and LipoP with only results from Phobius contradicting this result.

Based on the KEGG pathway result, it appears this gene is somehow involved in carbapenem biosynthesis. Carbapenem is a class of antibiotics which works against several pathogens.

Based on these results, the gene product of HIBPF_14120 was confirmed as glutamate 5 kinase, an enzyme protein in the cytoplasm of *Haemophilus influenzae* F3031.

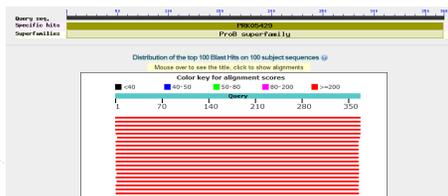


Figure 3: HIBPF_14120 Swiss-Prot BLAST Results showing a high degree of alignment between the target protein and those from other species.

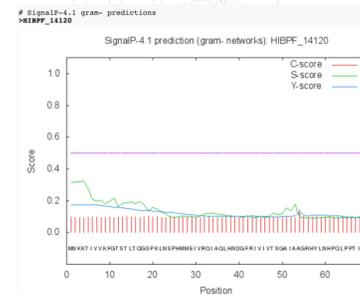


Figure 4: HIBPF_14120 result from Signal P showing the protein is cytoplasmic

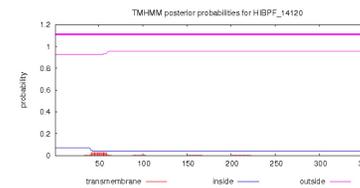


Figure 5: HIBPF_14120 result from TMHMM showing the protein is cytoplasmic

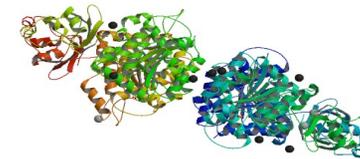


Figure 6: HIBPF_14120 crystalline structure of glutamate 5 kinase from PDB.

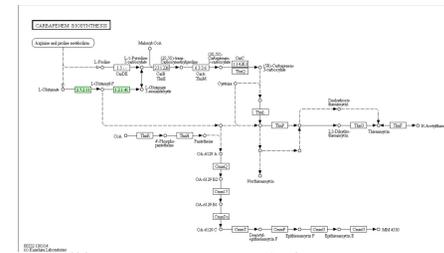


Figure 7: HIBPF_14120 Carbapenem biosynthesis KEGG pathway map. Green indicates the location of the enzyme glutamate 5 kinase.

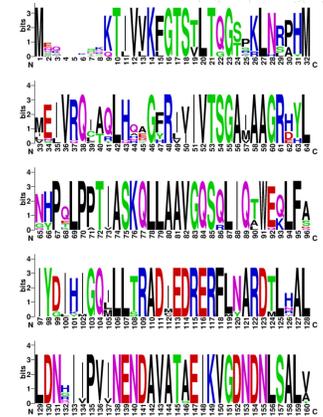


Figure 8: HIBPF_14120 partial Weblogo indicating the protein glutamate 5 kinase is well conserved across a variety of species

Conclusion

The GENI-ACT proposed gene product did not differ significantly from the proposed gene annotation from GENBANK.

Gene Locus

HIBPF_14120

Proposed Gene Products

Glutamate 5 kinase

References

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