Phosphate ABC transporter substrate-binding Protein

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ABSTRACT

In my cell biology class there is an ongoing project to annotate the *Halogeometricum borinquense* genome (3,944,467bp). Each semester we map a 50,000bp section of the genome using an Open Reading Frame (ORF) Finder to find potential genes then assign a few to each student to annotate. I used the Geni-ACT.org platform to access a variety of bioinformatics databases to test my hypothesis that Hbor_08060 locus actually codes for a real gene. To test my hypothesis I looked for homology with other proteins, where the protein could be located and its possible function. Finding homology in other species is strong evidence that the proposed gene is a real gene and suggests similar function and location. Using the databases I concluded Hbor_08060 is a Phosphate ABC transporter substrate-binding protein. Here I present how I came to this conclusion and propose a follow-up experiment to test my annotation of Hbor_08060.

OBJECTIVES

- Determine if Hbor_08060 is a gene by running it through bioinformatics databases looking for homology between already known proteins. Using that information we can determine the function of the protein.

METHODS

Used the following bioinformatics databases (via geni-ACT.org platform)

- BLAST; is a database used to perform pairwise comparisons between my protein and a protein from a different species.
- T-Coffee; performs multiple sequence comparison similar to BLAST but comparing the amino acid sequence of ten proteins simultaneously.
- WebLogo; used to visualize the results from T-Coffee.
- TMHMM; used to find transmembrane regions of the protein.
- SignalP; used to find signal peptides and cleavage regions.
- PSORT-b; used to find out where the protein could be located.
- Phobius; used to finds both transmembrane regions and signal peptides
- Alternative ORF; used to predict alternative start sites.

BASIC INFORMATION

GGTGGCTCGCAATCGATTGATGACGCGGAACTCATCGGATGTGTCGAACAGTGTGTCGAGGCGG
AAATTCGTCGTCGCCGCCGGGGCGACGGGACTTGCGGGCCTCGCGGGTTGTAGTGGAACGGGGG
GAAGTCAGACCCCCCTCCACGTCGGGCGGTAACGACGGTTCAGACGGGCAGACAGAACAGACCAC
CTCATCGTCAACCAGCAGCGACAGCGGATCGGTGACACCGTTGACGGCTGACGGATCTTCGACG
GTGTACCCCGTGACGAACCTCGCCGGGTCGTACTGGAACTCCAACCCGCCCGCAGACGACAAAG
AGTACTGGGGACCGGGACAGTACGGTATTGAAACCGACAAGAGTCTTGCAGACTACTGGGCCGG
ACTCTACGGATTCGAGGGCAATGATGACGGTACGCCCCCGTTCCGAACCAGCATCGGTCTCAGT
CACAGCGGTGTCGGTCTAGAGAAACTCAAGAAGGGACAGGTCGATATCGGCGACTCGAGCGCAC
CGGTCGGTGCGGAGTTCCCCGATGCGTCCGACGACGAACTCGAACCGTTCACGGACCACGTCGT
CGCTGTTGACGCCCAGCCCGTTGTCGTCAGCAAGGAGATTTACGACGCTGGCGTGACGAAGCTC
ACCCCTCGAACAACTCCAAGACATCTATCGTGGAAGAGATCACGAAGTGGTCGGAAATCGATGGTT
ACGATGGTCCTGACAAGGAGATTCAGGCTGTCGGGCGTGCTGAGGGGTCCGGTACGGACACCGC
ATTCCGACTGAAGCGTCCTCGGCGACCCCGACGCGTCGATGTCGGGTGTCGATATTCGGAAAGGG
CAGAACCAACAGGTCAAGACGCTGGTCTCGAAGTCGAACAACGCCGTCGCGTACCTCGCACTGG
CGTTCGTCGATGAGGAGGCAGTCCCGCCCGTCTCCTTGGAGATCGAAGGCACGACCTACACATA
CGGCGAAAACCTCGGTGCCAAGGAGTACCCCCTTTCCCGTGATCTCCACGCGTACACGTGGGAC
GGGACCTCGAAGAAGGAAGCCGCGTTCCTCCGGATGATTCTCACGGAGTTCGGACAGAAGAACT
TCGTCGCGACGAACAACTACGTAACGTTGACGGAGAAACGACTATCGAAAGAACGCTCCAAGCT
CCCTGAACCGGAAGCGTGA

Nucleotides 1119
Amino Acids 372

MSRRKFVVAAGATGLAGLAGCSGTGGSQTPSTSGGNDGSDGQTEQTTSSSTSSDSGSVTPLTAD
GSSTVYPVTNLAGSYWNSNPPADDKEYWGPGQYGIETDKSLADYWAGLYGFEGNDDGTPPFRTS
IGLSHSGVGLEKLKKGQVDIGDSSAPVGAEFPDASDDELEPFTDHVVAVDAQPVVVSKEIYDAG
VTKLTLEQLQDIYRGEITKWSEIDGYDGPDKEIQAVGRAEGSGTDTAFRLNVLGDPDASMSGVD
IRKGQNQQVKTLVSKSNNAVAYLALAFVDEEAVPPVSLEIEGTTYTYGENLGAKEYPLSRDLHA
YTWDGTSKKEAAFLRMILTEFGQKNFVATNNYVTLTEKRLSKERSKLPEPEA

RESULTS

BLAST

phosphate ABC transporter substrate-binding protein, phot family [Halogeometricum pallidum JCM 14848]

Sequence ID: ELZ33455.1 Length: 371 Number of Matches: 1

Range 1: 1 to 370 GenPept Graphics

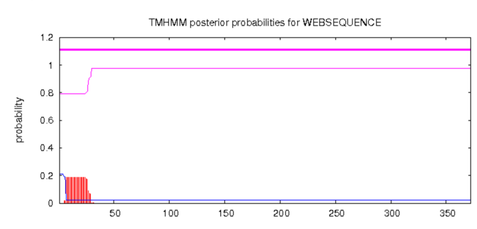
Score	Expect	Method	Identities	Positives	Gaps
588 bits(1516)	0.0	Compositional matrix adjust.	303/372(81%)	340/372(91%)	3/372(0%)

The BLAST results show that the top two proteins that are similar are Phosphate ABC transporter substrate binding protein.

T-Coffee

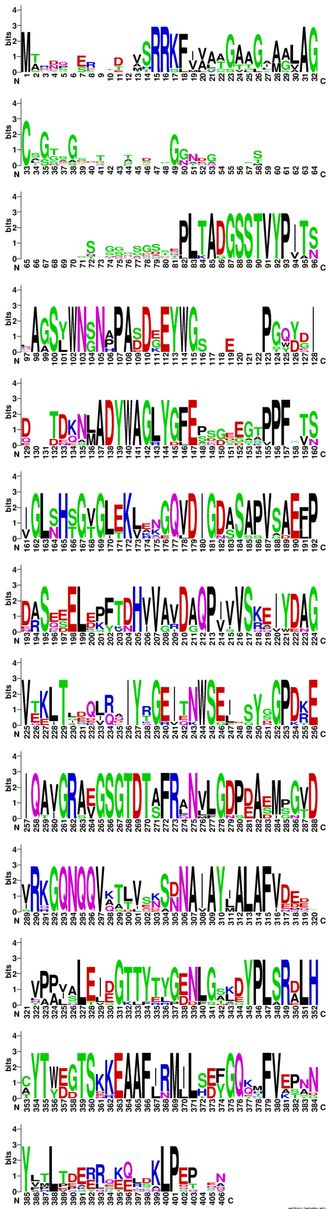
T-Coffee is comparing my protein against nine others simultaneously.

TMHMM



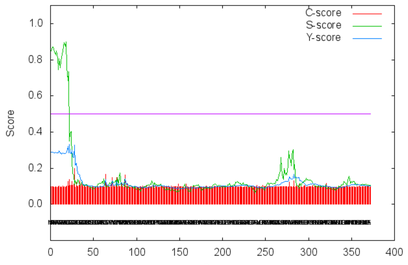
TMHMM shows that there is a small region at the beginning of the protein that could be a transmembrane region that may interact with a membrane but it is not a membrane protein.

WebLogo



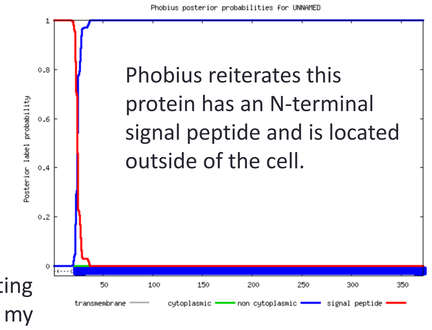
RESULTS

SignalP



The SignalP shows a high S-score indicating there is a signal peptide which suggests my protein is extracellular.

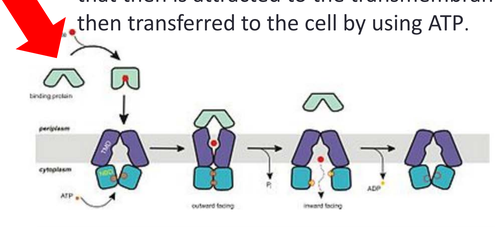
Phobius

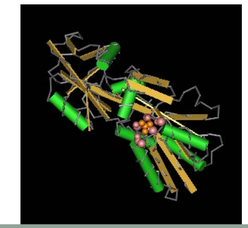


Phobius reiterates this protein has an N-terminal signal peptide and is located outside of the cell.

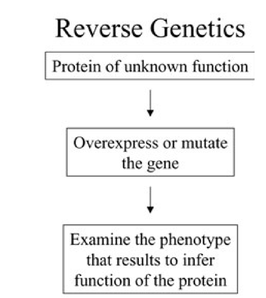
CONCLUSION

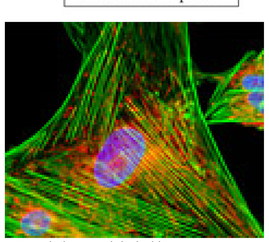
I have concluded that Hbor_08060 is a Phosphate ABC transporter substrate-binding Protein because of the BLAST results and that it is a extracellular protein. The substrate-binding Protein binds to phosphate that then is attracted to the transmembrane protein and the substrate is then transferred to the cell by using ATP.





EXPERIMENT





Cytoskeleton is labeled here.

- My Hypothesis is that this protein is vital in the transportation of phosphate
- I would knockout the Hbor_08060 and have normal *H. borinquense as a control*.
- I would then fluorescently label phosphate in both cells, mutated and not.
- Then using a Fluorescent Microscope I would use it to see if the KO cells have less phosphate transferred into the cell
- If so then the gene Hbor_08060's function is used in phosphate transportation

Resources

- Slccgap.org. (n.d.). Retrieved April 06, 2017, from http://slccgap.org/
- Geni-act. (n.d.). Retrieved April 06, 2017, from http://www.geni-act.org/
- B. (2016, May 26). ABC Transporters - Boundless Open Textbook. Retrieved April 06, 2017, from https://www.boundless.com/microbiology/textbooks/boundless-microbiology-textbook/cell-structure-of-bacteria-archaea-and-eukaryotes-4/transport-across-the-cell-membrane-33/abc-transporters-256-1009/
- Fibroblast Cells: Fluorescence Digital Image Gallery. (n.d.). Retrieved April 06, 2017, from http://www.olympusmicro.com/primer/techniques/fluorescence/gallery/cells/fibroblastindex.html