**Bioinformatics Worksheet #2: BLAST Practice - Assignment**

**Group Number:\_\_\_\_\_4\_\_\_\_\_\_\_\_**

**Names of individuals in group:\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Mystery bacterial operon #: Operon 4**

**Table 1: Table of results for 8 largest proteins in operon**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Protein #** | **Length (base pairs)** | **Length (amino acids)** | **E-value** | **Query Cover** | **% Identity** | **Protein name – brief description** |
| 1 | 2748 | 915 | 0.0 | 100 | 100 | Transferrin binding protein A |
| 2 | 1168 | 355 | 0.0 | 100 | 100 | Transferrin binding protein b |
| 3 |  |  |  |  |  | **All others – no protein found - <100 identity** |
| 4 |  |  |  |  |  |  |
| 5 |  |  |  |  |  |  |
| 6 |  |  |  |  |  |  |
| 7 |  |  |  |  |  |  |
| 8 |  |  |  |  |  |  |

1. What organism did this operon come from? What evidence did you use to come to this conclusion?

[**Mannheimia haemolytica]**](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_61743073) **(http://www.ncbi.nlm.nih.gov/nuccore/AY850238.1)**

**Evidence from the fact that all of the best matching proteins were from this organism.**

1. Are the functions of the genes found in this operon related? What cellular system do you think this operon and the genes contained are involved in?

**From the study that found this protein,** [**evidence for a common gene pool and frequent recombinational exchange of the tbpBA operon in Mannheimia haemolytica,Mannheimia glucosida and Bibersteinia trehalosi**](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387554/) **(**[**http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387554/**](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387554/)**), the tbpBA protein binds trasferin to iron for transport across the outer membrane. It is an intergral protein for iron uptake**

1. Do some research using NCBI (<http://www.ncbi.nlm.nih.gov>) and other internet resources to learn about the system. What role does this system have in this organism? Create a hypothesis about what might happen to the organism if these genes were deactivated.

**“The ability to sequester iron is recognised as a key virulence factor of pathogenic bacteria. Bacterial species representing the families Neisseriaceae,Pasteurellaceae and Moraxellaceae have evolved highly specific uptake systems to obtain iron direct from host transferrin ([Cornelissen, 2003](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387554/" \l "R7)). These transferrin-iron uptake systems consist of (i) two receptor proteins, TbpA and TbpB, responsible for binding transferrin and transport of iron across the outer membrane, (ii) a periplasmic-protein dependent transport system, consisting of three proteins, FbpA, FbpB, and FbpC, that is responsible for transport of iron across the periplasm and cytoplasmic membrane, and (iii) the TonB-ExbB-ExbD energy transduction system ([Cornelissen, 2003](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3387554/" \l "R7)). TbpA is a TonB-dependent integral membrane protein that is essential for iron uptake.” From the paper linked above.**

**Hypothesis/Prediction: If this protein was mutated or broken, then the bacterium would be unable to sequester iron, reducing its virulence as a pathogen.**

1. Share what you learned with the other groups and write down what you learned about the other systems.

**Various answers**

**Bonus**: Go to RCSB Protein Data Bank (<http://www.rcsb.org/pdb/home/home.do>) and search for one of the proteins you found by name. You may not be able to find it for your organism, or at all, but here you can see the structure of the protein determined by protein crystallography. Count the number of beta pleatings and alpha helixes. Are there any ligands associated with this protein? Are there any other units for this protein? If so, name them. Be sure to look at the protein in the 3-D view.

Name of protein you investigated:\_\_ tbpB \_\_\_\_\_\_\_\_\_\_\_\_\_

Number of Beta-pleated sections:\_\_\_\_\_\_\_4\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Number of alpha helix sections:\_\_\_\_\_\_45\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Associated ligands?\_\_\_\_\_\_\_\_\_\_\_\_\_\_Glycerol and Cacodylate ion\_\_\_\_\_\_\_\_\_\_\_\_\_

Additional subunits for protein?\_\_\_\_One, tbpA\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Provide a rough 3-D sketch of the protein

<http://www.rcsb.org/pdb/images/3pqs_bio_r_500.jpg> 