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

**Group Number:\_\_\_\_\_1\_\_\_\_\_\_\_\_**

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

**Mystery bacterial operon #: Operon 1**

**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 | 3147 | 1048 | 0.0 | 100 | 100 | [AcrB protein [Enterobacter cloacae]](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_112182849) – multidrug transporter |
| 2 | 1194 | 397 | 0.0 | 100 | 100 | [AcrA protein [Enterobacter cloacae]](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_112182847) - multidrug transporter |
| 3 | 654 | 217 | 1e-156 | 100 | 100 | [AcrR [Enterobacter cloacae]](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_148807118) – transcriptional regulator |
| 4 | 605 | 201 | 3e-107 | 82 | 100 | [AefA [Enterobacter cloacae]](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_148807117) – potassium efflux system |
| 5 | 570 | 189 |  |  |  | No protein found - <100% identity |
| 6 | 324 | 107 |  |  |  | No protein found - <100% identity |
| 7 | 315 | 104 |  |  |  | No protein found - <100% identity |
| 8 | 282 | 93 |  |  |  | No protein found - <100% identity |

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

[**Enterobacter cloacae**](http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=550) **– (**[**http://www.ncbi.nlm.nih.gov/pmc/articles/PMC113501/**](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC113501/)**)**

**“Enterobacter cloacae is part of the normal flora of the gastrointestinal tract of 40 to 80% of people and is widely distributed in the environment” (Keller et al 1998).**

**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?

**By visiting the actual site describing the operon itself (**[**http://www.ncbi.nlm.nih.gov/nuccore/EF627524.1**](http://www.ncbi.nlm.nih.gov/nuccore/EF627524.1)**) and scrolling through the genes in the operon, they are labeled as “transmembrane protein” “component of an efflux pump” and “Inner membrane component of efflux pump” and “acriflavine resistance protein”, all indicating that this operon is a transmembrane pump for moving materials across, and since acriflavine is an antibiotic, this might be involved in antibiotic resistance.**

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.

[**http://www.rcsb.org/pdb/education\_discussion/molecule\_of\_the\_month/download/MultidrugResistanceTransporters.pdf**](http://www.rcsb.org/pdb/education_discussion/molecule_of_the_month/download/MultidrugResistanceTransporters.pdf) **- this link provides a great resource about what these transporters do. Basically, an efflux pump moves the antibiotic out of the bacterial cell as fast as it comes in, before it has a chance to kill the cell.**

**Hypothesis/Prediction: If this protein was mutated or broken, then the bacterium would be vulnerable to being killed by an antibiotic, because its antibiotic defense would be broken.**

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. First, view the **Summary** tab. Count the number of beta pleatings and alpha helixes (can also be found under the **Sequence tab**). 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:\_\_ [AcrB protein [Enterobacter cloacae]](http://www.ncbi.nlm.nih.gov/blast/Blast.cgi#alnHdr_112182849) – \_\_\_\_\_\_\_\_\_\_\_\_\_

Number of Beta-pleated sheets:\_\_\_\_ 16% beta sheet (41 strands; 173 residues)\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Number of alpha helix sections:\_\_\_ 52% helical (43 helices; 549 residues)\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Associated ligands?\_\_\_ DODECYL-BETA-D-MALTOSIDE NICKEL (II) ION\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Additional subunits for protein?\_\_\_3 polypeptide chains in complete protein\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Provide a rough 3-D sketch of your protein: <http://www.rcsb.org/pdb/education_discussion/molecule_of_the_month/download/MultidrugResistanceTransporters.pdf>