

CSC 360, Bioinformatics Lab #10:
BLAST and Conserved Domain Identification

Name: _____

Escherichia coli (*E. coli*) is a rod-shaped bacterium that is prevalent in the human gut. Although most strains are harmless, some strains cause food poisoning in their hosts. One such strain is EDL933, which was isolated from contaminated ground beef from a McDonald's in Michigan. An important biological question is why does the EDL933 strain cause disease, while harmless strains such as MG1655 do not. *Bioinformatics* can be used to help answer this question, by identifying *virulent* proteins found in the disease-causing strain but not in the harmless strains.

Through gene sequencing and a bioinformatics analysis, approximately 1000 genes have been identified that are present in the pathogenic EDL933 strain but that are not present in the harmless MG1655 strain.

In this lab, you will use the protein Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) along with the Conserved Domain Identification search to identify potential virulence factors in the EDL933 strain. For a quick overview of BLAST, see: ftp://ftp.ncbi.nlm.nih.gov/pub/factsheets/HowTo_BLASTGuide.pdf

BLAST works as follows:

1. All sequences are identified that contain matches of a fixed window size.
2. The alignment is then extended in both directions, using local alignment.

Several versions of BLAST are available:

- *blastp* takes a protein sequence and BLASTS it against a protein database
- *blastn* takes a nucleotide sequence and BLASTS it against a nucleotide database
- *blastx* takes a nucleotide sequence, translates it, and BLASTS it against a protein database
- *tblastn* takes a protein sequence and BLASTS it against a translated nucleotide database

Potential virulence factors can be identified by considering proteins that have either (a) similar proteins (potential homologs) in strains of bacteria known to cause disease, and/or (b) conserved domains with functions known to be associated with disease. Functions associated with disease include Type III or Type IV secretion systems, which deliver toxins; enzymes that break down host proteins; and features that allow attachment to host cells, among others. Pathogenic strains include *Salmonella enterica*, a bacterium that causes Salmonellosis, a disease associated with vomiting, diarrhea, fever, and abdominal cramps; and the *Shigella* bacteria, which can cause dysentery. You may assume that other strains are not pathogenic.

Assignment

The sequences of 3 proteins are available in the file sequences.txt.

1. Answer the questions below for each sequence:
 - A. Perform a protein-protein BLAST search against the *reference proteins* database, limiting your search to *enterobacteria (taxid:91347)* and excluding all *Escherichia (taxid:561)* bacteria. For the top hit, identify the protein name, the species, and accession number.
 - B. For the top hit, how many matches (regions of the protein) were identified? For each match, specify the E-value, its percent identity, and percent similarity score.
 - C. Based on your answer to (B) above, do you think the matches may be due to chance? Why or why not?
 - D. Based on the species of the top hit, do you believe that the protein may be a virulence factor in EDL933? Why or why not?
 - E. Look at the conserved domains, and answer the following for the first pfam match (You should only look at the first pfam match for this question) What is *pfam* accession # and domain name? Does this domain indicate that the protein may be a virulence factor in EDL933? Why or why not?

Part II. Based on the above analysis for each protein, which protein is most likely to be a virulence factor, and why?

Note: The top matches for the three sequences are listed below. If your top matches are different, then your results are incorrect, and you should see me if you are unable to get the correct ones.

1. Unknown protein #1: [NP_709290.2](#)
2. Unknown protein #2: [WP_005113712.1](#)
3. Unknown protein #3: [WP_014657490.1](#)