

Programming for Bioinformatics | BIOL 7200

Week 4 Exercise

September 12, 2023

Instructions for submission

- Run a Linux or Mac terminal on your computer
- You may want to create a directory to work in (e.g., "~/biol7200/class4/ex4")
- Download "week4_data.tar.gz" and extract the contents into your working directory (e.g., "~/biol7200/class4/ex4")
- You will need some of the files from week 3's data. Copy needed files to your week 4 directory.
- Prepare a file containing answers to the below questions. Copy the question and either paste the body of a script, write the correct answers, or provide a screenshot of your working below the question as directed in the instructions provided in the question.
- In cases where you need to paste a screenshot of your terminal, you can do that as follows. Screenshot a selected area with Windows: win+shift+S, Mac cmd+shift+4 and then paste in your submission sheet
- Use the `clear` command to tidy up your terminal so you show only relevant commands and outputs
- Name your submission sheet: "gtusername.pdf", or "gtusername.docx"
- Name your script file: "gtusername.sh"

NOTE this week only pure Bash solutions will be accepted. No Python scripts!

Grading Rubric

This assignment will be graded out of 100.

You only need to write one script for this assignment. **No Python code allowed this week!!!** Your solution must be all Bash and Unix built-in tools (i.e., `sed` and `awk` allowed)

Partial credit will be awarded for scripts that do not produce the correct output if the approach taken is on the right track. A total of+ 60 points will be awarded based on approach alone, while the remaining 40 points will be awarded if the correct output is produced,

Question

1. (100 points)

In this weeks data, there are BED files which correspond to the assemblies you were working with last week. The BED files each describe the location of genes in one of the assemblies. Each organism has an assembly file (.fna) and a BED file (.bed). e.g., *Wolbachia*.fna is the *Wolbachia* assembly and *Wolbachia*.bed is the bed file describing the locations of genes in the *Wolbachia* assembly.

Your task is to extend the `find_homologs.sh` script you wrote previously. You may also use one of the example scripts demonstrated in class as the foundation for this assignment.

Below is some background about the task. See the "Instructions" section below for the rest of your instructions.

Background info

More about BED format

This information will help you understand the data you are working with and may be useful in writing your script.

We used a BED file in the first assignment, but didn't discuss the information it encodes in much detail. We saw that BED format is used to encode the location of features in a sequence. Each line of the BED file refers to a different sequence, and the three mandatory columns of BED format are sufficient to describe exactly where the feature is located. The BED files you will work with today have more than 3 columns as they include information from some of the optional BED format fields. [See this website for a reminder of BED format](#)

The columns in the files you are working with today contain the following information:

- The sequence IDs in column 1 of each BED file correspond to the FASTA headers in the associated assembly. Typically, the first set of non-whitespace characters after the ">" symbol of a FASTA header are taken to be the sequence ID. After the first whitespace character, there is sometimes additional metadata, but you will see that BLAST and BED format do not pay attention to anything after the first whitespace. (For example, in a header line ">seq1 species_name something else", only "seq1" is considered to be the sequence ID.)
- The second and third column of the BED file gives the start and stop position in the sequence where the gene is encoded. Each line of the BED file refers to the location of a different gene. Note that start and stop position are misnomers in that they imply direction. Instead, the "start" column simply contains the location of the boundary of the feature that is closest to the first base of the FASTA file. This will become significant in column 6...
- Column 4 contains a name or label for the feature. This can be anything and does not necessarily need to be a gene name.
- Column 5 contains a score. In the files provided here, no score is used. Instead, as BED format forbids empty columns, a "." character is used to populate the unused column. If we did not have data in column 6, this column could be absent and we could have stopped at 4 columns. However, as we want to include the data in column 6, all prior columns need either data or ".".
- Column 6 indicates the strand on which the feature is present. Strand here refers to which of the two DNA molecules contains the feature. In the case of genes, while both DNA strands contain sequence that corresponds to the gene, only one is used during transcription. The strand column of a BED file indicates directionality of the feature. In the case of genes, this directionality indicates whether transcription proceeds left to right through the sequence (+) or right to left (-).

Note that for genes encoded on the "-" strand, transcription *begins* at the "stop" end of the BED coordinates and *ends* at the "start" end. This is not relevant to the assignment, but it is always important to keep in mind the biology being represented in the data you work with as bioinformaticians! Sometimes you must be careful when interpreting words like "start" and "stop" or other decisions in the representation of biological data.

More info about protein domains

This information is solely for those interested and can be safely skipped without impacting your ability to complete the assignment.

Many genes encode proteins. Proteins perform most of the functions that result in life. Proteins are made up of a linear strand of amino acids that typically folds into a globular structure (although linear or unstructured sections can remain). The folded protein can contain multiple regions that perform different functions. These functional regions are called domains.

Different protein domains are associated with different functions. For example, [zinc finger domains](#) bind DNA (and other things) and are often found in proteins that act as transcription factors (gene expression regulators). Histidine kinase domains are involved in transducing a signal (usually an environmental signal) sensed by another domain. However, it is the other domains of histidine kinase proteins that do the sensing. It is the combination of multiple different domains with different functions that produces the overall function of a specific protein.

Many protein domains can be found in combination with a diverse range of other domains. For example, while histidine kinases are not the most diverse, you can see in these two examples ([1](#) and [2](#) - scroll down and look at the Domain architecture section) that HK domains are found in combination with different domains. Note the different domain IDs and different length of the proteins.

Without knowing what the other domains do, we can infer the function of HK genes simply by the presence of the HK domains. If you were a researcher interested in bacterial environmental responses (such as how a pathogen senses it is infecting a person and not in a pond), you might want to identify HK domain-containing proteins as likely candidates of how the organism senses the environmental signal of interest. You could then make gene deletions of each of the HK-domain genes and test the phenotype of each mutant to see if one of the genes is important.

In a study like the one described above, what we are doing here is the first step. However, the process of finding sequence matches to a query of interest in a sequence database is useful in many more situations.

Instructions

Modify the `find_homologs.sh` script you wrote last week to carry out the following steps:

1. Find putative homologs of query amino acid sequences in FASTA format (same as last week's instruction - i.e., what your current script does)
2. Use Bash loops and Bash conditional statements (no Python!) to identify which genes in a BED file contain the identified homologous histidine kinase domains. (Hint: if a gene contains a domain, then the location of the domain will be entirely within the boundaries of the gene)
3. Write an output file containing the unique gene names which your script identified as containing predicted HK domains.

Usage of your script should be: `homolog_identify.sh <query.faa> <subject.fna> <bedfile> <outfile>`

Your submission must contain the following:

1. Upload your script as a .sh file named "gtusername.sh" where gtusername is your Georgia Tech username.

2. Upload a submission sheet showing the following:

1. A screenshot showing the execution of your script in a loop on the 4 genomes provided. Your loop should run your script on each genome and its associated BED file and output the result to a file with a distinct and informative name.
2. A screenshot of your terminal showing the number of genes identified for each organism. It must be clear in the screenshot which organism each file refers to (i.e., name them informatively in your loop).

Note: All of the sequences in HK_domain.faa are Histidine Kinase domains. i.e., many of your query sequences are homologous to one another. That means it is likely that some of your BLAST hits will be overlapping. Therefore, you might need to check for duplicate genes in your results

Format of output file: 1 gene name per line, no other information. Below is the output I got for *Wolbachia* with the script I wrote

```
GQX67_RS05910
GQX67_RS06230
```

Note: not all bacterial gene IDs are nice gene names. Some are locus IDs referring to a place in the genome (e.g., "N16961_RS00025"). This often means that the gene at that locus has not been characterized in a publication, but does not necessarily mean nobody knows what sort of thing it does.

Ungraded extra challenges

To be clear: these are challenges you can set yourself as a learning exercise, but will not impact your grade. These challenges involve concepts which will likely be useful to you in the future and are only not included in the graded portion of the assignment due to their difficulty.

1. Think about how you can improve the efficiency of your script. While each command takes very little time to execute, if you use certain coding constructs like loops within loops, run times can become quite long. Sometime it is necessary to use constructs like that, but there may still be approaches you can take to minimize run times without compromising your output.

For example, on my computer, an unoptimized version of script took 3 minutes to run on the 4 provided genomes. After tweaking the script to make it more efficient, my run time reduced to 90 seconds. Run times are hard to compare between different computers, but perhaps see if you can get your run time below 2 minutes or at least make a change that results in a noticeable improvement.

If you are only going to run a script once, it is usually not worth optimizing run times. However, it is good to learn some tricks that you can commonly employ to optimize speed when you do need to. It can also be a fun puzzle.

You can time how long a command takes to run using `time`. i.e., `time <pipeline or loop etc>`. The "real" result is the total run time.

2. As described in the BED format description above, genes are transcribed in a directional manner. [The wikipedia page linked here](#) has a good description of the relationship between DNA sequence and RNA sequence orientation.

Transcription can either proceed in the same direction that the sequence is represented in a FASTA file, or in the opposite direction. This is represented in BED format using + or -, respectively. A plus simply means that the coding strand of the gene is the sequence shown in the FASTA file. - means that the coding strand is the one not being shown in the FASTA file.

Following transcription of the gene from DNA into RNA, the RNA is then translated into amino acid sequence. Translation occurs 5' to 3' (5 prime to 3 prime) along the RNA strand, which is the same direction the RNA was transcribed.

That means that a gene whose orientation is "+" in a BED file produces an RNA strand with sequence with the same orientation as the DNA in the file. That same gene encodes a protein whose first amino acid was encoded by the first 3 bases at the left-most end of the gene sequence. i.e., + strand genes read left to right for the DNA, RNA, and protein sequence.

The consequence of the relationships between directionality of the 3 sequences involved (DNA, RNA, and protein) is that there is a fixed relationship between the DNA encoding a gene and the sequence of amino acids it will produce. It can only produce a protein in one direction, not both.

As we are performing a tBLASTn of protein sequence against DNA, we end up with a start and stop position for our match which indicates the direction in the DNA that the protein would have to be encoded. We also have a BED file indicating the direction in which we *know* genes are encoded in each genome. If any of our protein matches are in the opposite direction to the gene that contains the matched region, then we know that the gene could not make that protein.

Such a situation is unlikely to occur here, but if it did occur it would be a false-positive result. i.e., we would have a match between our protein of interest and a gene in the genome that is not actually an indication that the gene encodes a HK domain. It is always important to be mindful of ways that your analyses may produce misleading results and to attempt to remove those results.

Think about how you could modify your script to use the data in your BLAST results and in your BED file to filter protein matches that cannot actually be encoded by the matched gene due to mismatching orientation.