University of Pennsylvania
BIOL4536 Fall 2023

HW#5 (SOLUTIONS)
(Alignment)

Assigned October 4th
Due October 11th, 3:30pm

We're finally getting to some real bioinformatics. In this assignment you will:

(1) Learn how to do Smith-Waterman alignment at the Unix command line.
(2) See alignments change as you vary the alignment parameters.
(3) Practice wrapping commands in a python script.
(4) Practice parsing files with python.
(5) Lern how to run ClustalW multiple sequence alignment.

Log into your UNIX account. Create a subdirectory of your home directory called `HW5` and move down into that directory.

We have installed a program called "`water`" which performs Smith-Waterman alignments. Execute the `water` command with the `--help` option to see its usage page:

```
> water --help
```

The option `-h` also works. These options don't work for every command, but most command authors will implement them. Sometimes all you have to do is run a command with no options in order to see its help page. And of course there's also `man water`.

You do not need to read the man or help pages, I'm going to explain how to run them. But just be aware that those pages are there.

We are going to use `water` to see how sensitive the alignment results are to the scoring scheme.

Make a fastA file called `seq1.fa` with the following sequence. You should be able to copy/paste from the PDF.

```
>NP_005359.1 myoglobin isoform 1 [Homo sapiens]
MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKASEDLKKHGATVL
TALGGILKKKGHHEAEIKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFR
KDMASNYKELGFQG
```

Next make a fastA file called `seq2.fa` with the following sequence.

```
>NP_000509.1 hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLG
AFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH
```

The substitution matrices this program uses are in the `/usr/share/EMBOSS/data` directory. Not everything in this directory is a substitution matrix, but do an `ls` on this directory to see what substitution matrices are available. *Note: they call BLOSUM and PAM EBLOSUM and EPAM for some reason.*

(1) Pipe the `ls` command to the proper `grep` command in order to count how many EBLOSUM and EPAM matrices there are. Give your commands and the answer.

ANSWER:

```
ggrant@workstation:~$ ls /usr/share/EMBOSS/data|grep -c PAM
50
ggrant@workstation:~$ ls /usr/share/EMBOSS/data|grep -c BLOSUM
16
```

Next we are going to fix the gap penalties and see how the alignment varies depending on the substitution matrix. Execute the following command:

```
water -gapopen 10 -gapextend .5 -outfile alignment1.txt -datafile EPAM10 seq1.fa seq2.fa
```

Examine the command carefully, this will align the two sequences with the PAM10 matrix.

(2) Report the alignment. What is its length and score?

ANSWER: The length is 13, the score is 37.0. The alignment is:

```
NP_005359.1       15 WGKV-------EA     20
                        ||||       ||
NP_000509.1       16 WGKVNVDEVGGEA     28
```

(3) Now do the same but using the EPAM100 matrix. Output this time to a file called `alignment2.txt`. Report the alignment, length and score.

ANSWER: The length is 62, the score is 71.5. The alignment is:

```
NP_005359.1       11 VLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKASE     60
                        |...||||:.|  ..|.|.|.||....|.|...|:.|..|.:.|.:.::.
NP_000509.1       12 VTALWGKVNVD--EVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP     59

NP_005359.1       61 DLKKHGATVLTA     72
                        ..|.||..||.|
NP_000509.1       60 KVKAHGKKVLGA     71
```

(4) Now do the same but using the EPAM250 matrix. Output this time to a file called `alignment3.txt`. Report the alignment. What is its length and score?

ANSWER: The length is 145, the score is 172.5. The alignment is:

```
NP_005359.1         3 LSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKS     52
                      |:..|...|..:||||  ::...|.|.|.||:...|.|...|:.|.:|.:
NP_000509.1         4 LTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFESFGDLST     51

NP_005359.1        53 EDEMKASEDLKKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPV    102
                      .|.:.::..:|.||..||.|:::.|...::..:.:..|::.|..|..:..
NP_000509.1        52 PDAVMGNPKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDP    101

NP_005359.1       103 KYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNY    147
                      ..:.::::.::.||......:|.:..|:|.:|.:....:.:|.:|
NP_000509.1       102 ENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKY    146
```

Now let's change the gap penalties.

(5) Align them again using the EPAM250 but this time change the gap open penalty to 1. Report the alignment. What's its score? How many more "gaps" are there than there were with the gap open penalty of 10 with EPAM250?

ANSWER: The score is 304.5. There were 84 gaps out of 192 positions with gap open penalty of 1, versus 2 gaps out of 145 positions with gap open penalty of 10.

```
NP_005359.1         1 MG-L-SDGEWQL--V--LNVWGKVEAD-IPGHGQEVLI-RL---FKGHPE     39
                      |. | :: | :   |  |  ||||:.| : | | |.|  ||   :   |
NP_000509.1         1 MVHLTPE-E-K-SAVTAL--WGKVNVDEV-G-G-EAL-GRLLVVY---P-     37

NP_005359.1        40 -TLEK-FDKFKHLKSEDE-M---KASEDLKKHGATVLTAL-GGILKKKGH     82
                       | :: |:.|.:|.:.|. |   |    :|.||..||.|: :| |   :|
NP_000509.1        38 WT-QRFFESFGDLSTPDAVMGNPK----VKAHGKKVLGAFSDG-L---AH     78

NP_005359.1        83 HEAEIK---P-LAQS-HATK-HKI-PVKY--L-E-FISECIIQVLQSKHP    121
                      .: ::|   : |::  |..| | : |.::  | : ::  |   || : |
NP_000509.1        79 LD-NLKGTFATLSE-LHCDKLH-VDPENFRLLGNVLV--C---VL-A-H-    117

NP_005359.1       122 GDFGADAQGAMNKALELFRKDMASNY-K------E-LG--FQ    153
                       :||        |  | |...:.:.| |      : |:  ::
NP_000509.1       118 -HFG--------K--E-FTPPVQAAYQKVVAGVANALAHKYH    147
```

(6) You'll note from the previous exercise that identities and gaps went way up. Which alignment has a higher score?

ANSWER: With gap open and EPAM250 the score is 172.5, with gap open penalty of 1 and EPAM250 the score is 304.5. So the alignment with gap open of 1 is higher.

(7) Is the alignment from question 6 with higher score better? Could we somehow know which to prefer? Discuss.

ANSWER: We do not have enough information to judge. The one with lower score looks better, but the only way to know which gap penalty to prefer would be to know something the likelihood of real gaps in real data representing sequences of the same evolutionary distance. That could require a literature search or an actual data analysis. Without knowing more, we would probably trust the default gap open of 10.

(8) Create a directory in `HW5` called `alignments`. Write a python or a shell script (you choose) called `align.py` to execute the `water` command on every available EPAM substitution matrix (with the default gap penalties: gap-open = 10, gap-extend = 0.5). Output the results for matrix EPAM*N* to a file called `alignment_EPAM`*N* in the `alignments` directory (*N* will take the values 10, 20, 30, ..., 500 and consequently your script will output 50 files). Your answer to this exercise is your code, include it as an attachment. We may also check your directory for the 50 files, so don't delete them.

ANSWER: Here's a python script to do it.

```
import subprocess

for i in range(1,51):
    N = i * 10
    command = "water -gapopen 10 -gapextend .5 -outfile alignments/alignment"
    command = command + str(i) + ".txt -datafile EPAM" + str(N) + " seq1.fa seq2.fa"
    subprocess.getoutput([command])
```

(9) Write a python program called `parse_alignments.py` to parse the output files from the previous question to extract the percent identities from each one. The program should output tab-delimited text to the screen with two columns, the name of the matrix and the percent identity achieved with that matrix. Then it should output at the end some sort of horizontal line (several dashes will do) followed by which matrix achieved the highest percent identity, and what that highest percent identity is. Run the program and redirect the output to "`hw5_prob9.txt`" Include as attachments the `hw5_prob9.txt` file and your code.

ANSWER: There are many ways to solve this problem, here is one way that calls grep and then uses regular expression replacement function to manipulate the strings.

This problem had a slight wrinkle in that there's a tie for highest percent. Full credit was given for finding either one, but one should typically report ties. The following code will find them all.

```
import subprocess
import re

max_percent = 0
max_matrix = ""
ties = ""
for i in range(1,51):
    N = i * 10
    matrix = "EPAM" + str(N)
    command = "grep Identity alignments/alignment_" + matrix + ".txt"
    line = subprocess.getoutput([command])
    line = re.sub('.*\(', '', line)
    line = re.sub('\)', '', line)
    percent = re.sub('%', '', line)
    percent = float(percent)
    # check for ties
    if(percent == max_percent):
        ties = ties + ", " + matrix
    if(percent > max_percent):
        max_percent = percent
        max_matrix = matrix
        ties = max_matrix
    print(matrix + "\t" + line)
print("--------------")
if(re.search('^[^,]*,[^,]*$', ties)):
    print("both matrices " + ties + " have max percent identity (" + str(max_percent) + "%)")
elif(re.search(',.*,', ties)):
    print("both matrices " + ties + " have max percent identity (" + str(max_percent) + "%)")
else:
    print("matrix " + ties + " had max percent identity (" + str(max_percent) + "%)")
```

The bottom of the output file should look like this:

```
EPAM390 24.3%
EPAM400 24.3%
EPAM410 24.3%
EPAM420 20.8%
EPAM430 24.3%
EPAM440 24.3%
EPAM450 24.3%
EPAM460 25.8%
EPAM470 22.4%
EPAM480 22.4%
EPAM490 20.8%
EPAM500 22.4%
--------------
both matrices EPAM20, EPAM30 have max percent identity (46.7%)
ggrant@workstation:~/files/HW5$
```

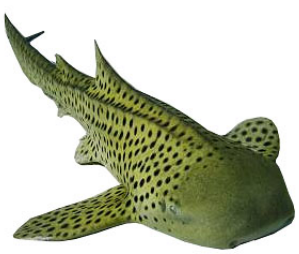
Next we're going to perform a multiple sequence alignment using ClustalW. To prepare, make a fastA file called `hw5.fa` with both of the sequences from the previous exercises. We are going to add three more for a total of five. You already have the first two, we'll get the other three from GenBank.

The following link is to the hemoglobin gene in *Stegostoma fasciatum* (Zebra shark).

`https://www.ncbi.nlm.nih.gov/protein/XP_048408641.1`
Click the "fastA" link to get the protein sequence in fastA format.

Change the ID in the URL to get the following two protein sequences as well:

`XP_033016679.1`
`XP_020922233.1`.



You should now have five sequences in the file `hw5.fa`. Make sure your file is in fastA format, in particular each sequence should have its ID line that starts with ">".

Download the `hw5.fa` file to your local computer. You will need it there to upload to the ClustalW server.

Run ClustalW using the following online server:
`https://www.ebi.ac.uk/Tools/msa/clustalo/`

Under "OUTPUT FORMAT" select option "ClustalW" (*not* "ClustalW with character counts").

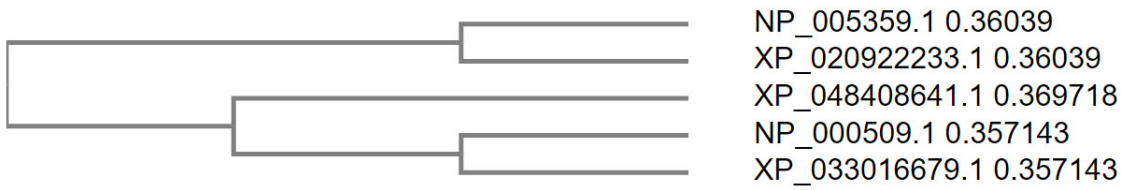
Click on "More options" and set both the following to "no":
`MBED-LIKE CLUSTERING GUIDE-TREE`
`MBED-LIKE CLUSTERING ITERATION`
And set `DISTANCE MATRIX` to "yes".

Point it to your fastA file and hit Submit. It will take a minute to run, wait for it.

(10) Include a screenshot of the alignment and the Guide Tree. Click on "Submission Details" and scroll down to "Command". You'll notice this web page is just running a Unix command for you behind the scenes. It won't run on our machine because the `singularity` command is not installed. But copy this command to your write-up (or give a screen shot).

ANSWER:

```
CLUSTAL O(1.2.4) multiple sequence alignment


NP_005359.1         ----------------MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKF
XP_020922233.1      MEKVPGEMEIERRERSEELSEAERKAVQATWARLYANCEDVGVAILVRFFVNFPSAKQYF
XP_048408641.1      -------------MACELFSQDEKKVIEEFGQLLKSNAQNYGAESLSRLFMTNPGSKTYF
NP_000509.1         ---------------MVHLTPEEKSAVTALWGKVNV--DEVGGEALGRLLVVYPWTQRFF
XP_033016679.1      ----------------MWLTDDDKSHVKAVWGEIQSHARDIGAEPITRRFAAHPTTKTYF
                                       ::  : . :      :       *   : * :   * :   *

NP_005359.1         DKFKHLKSEDEMKASEDLKKHGATVLTALGGILKKKGHH---EAEIKPLAQSHATKHKIP
XP_020922233.1      SQFKHMEDPLEMERSPQLRKHACRVMGALNTVVENLHDPEKVSSVLALVGKAHALKHKVE
XP_048408641.1      DYS--------DFSMPNLKGHGEKVMSALAKAADNVDN---LKGSLCELAALHGKTLLVD
NP_000509.1         ESFGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLDN---LKGTFATLSELHCDKLHVD
XP_033016679.1      VHI------DVSPGSGDIKAYGKKVTAAIGEAVAHIDN---IAGALNKLSNLHTQKLHVD
                                    .:: :.  *  *:     :  .     . :  :.  *  .  :

NP_005359.1         VKYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNYKELGFQG-------
XP_020922233.1      PVYFKILSGVILEVIAEEFANDFPPETQRAWAKLRSLIYSHVTAAYKEVGWPPSHAALFG
XP_048408641.1      PQNFPIFSRCIQVTLANNLS-SFPPGHQLAIDKFLKAVYQNLSSKYR-------------
NP_000509.1         PENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH-------------
XP_033016679.1      PINFALLGHCILVAIAANHPGLLKASTPVSMDKFLGRISAVLLGPKKPKPAAEEPPRCRP
                       : ::.  :  .:  .    :     :  *    .   :    :

NP_005359.1         ------------------------------------------------------------
XP_020922233.1      AVGPSLSSPVPGSTLSRRLSSEDPPPLGAKEAGGIPDSSSRKDATSASATVPLEPPGGGS
XP_048408641.1      ------------------------------------------------------------
NP_000509.1         ------------------------------------------------------------
XP_033016679.1      A--------TPQH--------HNKPALDS---------SKNK------------------


NP_005359.1         ---------------------
XP_020922233.1      GCGPQLGGAAGGLWGWGTLPP
XP_048408641.1      ---------------------
NP_000509.1         ---------------------
XP_033016679.1      ---------------------
```



Command

```
singularity exec $APPBIN/clustalo:1.2.4 clustalo --infile clustalo-I20230812-173518-0373-20482505-p1m.upfile --threads 8 --MAC-RAM 8000 --verbose --guidetree-out clustalo-I20230812-173518-0373-20482505-p1m.dnd --distmat-out clustalo-I20230812-173518-0373-20482505-p1m.matrix --full --full-iter --outfmt clustal --outfile clustalo-I20230812-173518-0373-20482505-p1m.clustal --output-order tree-order --seqtype protein
```