Huiming Zhou

1327380

CIS 6060 — Assignment #3

Fall 2025

Due: *Sunday, March 30, 2025 at 23:59*
Total Points: 50

Please submit your assignment solutions as **one PDF** file (named as YOUR/UoG/ID_a3.pdf, e.g. 1234567_a3.pdf) to Dropbox under Assignment 3 before the due date.

Exercise 1 ClustalW, Phylogeny and BLAST (30 pts)

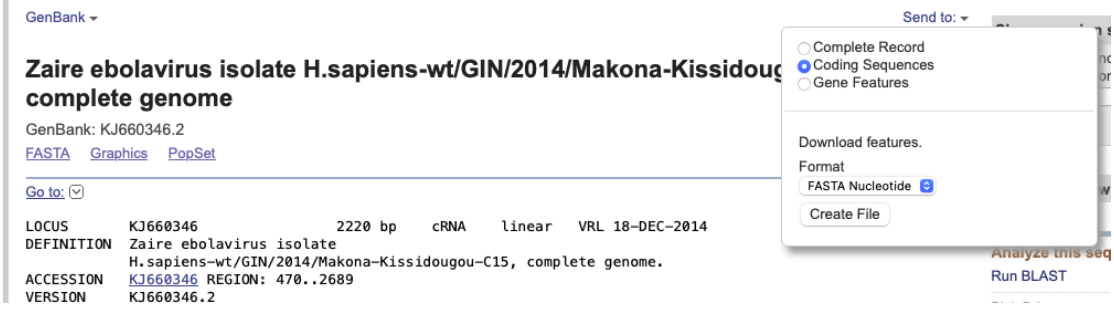
After the ebola outbreak years ago, there has been some work done in trying to construct a phylogeny of the Ebolavirus genus
http://currents.plos.org/outbreaks/article/phylogenetic-analysis-of-guinea-2014-ebov-ebolavirus-outbreak-2/.
Our goal is to construct such phylogeny and see how closely it matches the published one.

The phylogeny of the paper calculates is on the following webpage:
http://currents.plos.org/outbreaks/files/2014/04/EBOV_cdsScaleBar.mb_.tree_.png.
(They corrected it with further analysis later in the paper.)
To construct this phylogeny one may start with performing multiple sequence alignment between all the coding segments concatenated from each of the genomes as provided later in this assignment.

We are only going to build a phylogeny for a subset of these taxa (the published tree is too big for us to deal with). Go to the NCBI Nucleotide website and find the accession numbers in the list below to construct the phylogeny:

- KJ660346.2
- KJ660347.2
- KJ660348.2
- KC242785.1
- KC242800.1
- KC242801.1
- KC242796.1
- KC242794.1
- KC242793.1

Then, find the **FIRST** coding segment for each of these accessions (for all of them, **it should stretch from about 470–2689**) and use **ONLY** these first coding segments for this exercise. From the NCBI GenBank page of each sequence, you can download all the code sequences and just select the first one from the file. Here is a screenshot of the first sequence KJ660346.2.



Create a multi-FASTA file (multiple FASTA sequences in one file) of these coding segment sequences, and shorten the header line for each sequence by deleting information starting from the square brackets.

- For example, for the first one, KJ660346.2,
  change the header from
  ">lcl|KJ660346.2_cds_AHX24646.1_1 [gene=NP] [protein=nucleoprotein] [protein_id=AHX24646.1] [location=470..2689] [gbkey=CDS]"
  (to) ⇒
  ">lcl|KJ660346.2_cds_AHX24646.1_1".

After that, perform a multiple sequence alignment using ClustalW (http://www.genome.jp/tools/clustalw/) with the multi FASTA file. You can upload the created file, and select DNA as the type of sequences. (Do **NOT** change any other parameters, just use the default).

Then, do the following

1. (8 marks) Hand in the multiple sequence alignment result before the actual alignment. You can just copy and paste the output of the result page.

   The actual alignment starts as something like

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clustalw.aln

CLUSTAL 2.1 multiple sequence alignment

lcl|KC242794.1_cds_AGB56773.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50

lcl|KC242793.1_cds_AGB56764.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50

lcl|KC242796.1_cds_AGB56791.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50

lcl|KC242801.1_cds_AGB56836.1_
ATGGATTCTCGTCCTCAGAAAATCTGGATGGCGCCGAGTCTCACTGAATC 50

lcl|KC242800.1_cds_AGB56827.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTTACTGAATC 50

lcl|KC242785.1_cds_AGB56692.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50

lcl|KJ660346.2_cds_AHX24646.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50

lcl|KJ660348.2_cds_AHX24664.1_
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lcl|KJ660347.2_cds_AHX24655.1_
ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC 50
********** ******** ********** ********

   So please hand in the result **before** this part.

2. (6 marks) Then, build 2 separate phylogenies with the ClustalW Phylogeny program (https://www.ebi.ac.uk/jdispatcher/phylogeny/simple_phylogeny); one with UPGMA with both 'distance correction' and 'exclude gaps' **off**, and one with both parameters **on**. Take screenshots of the resulting trees (Phylogram are fine; also the accession number should be written in each label of the phylogram if the header was shrunken correctly in the previous question; this makes it easier to compare to the published phylogeny). (*Hint: You may need to download the multiple sequence alignment from the previous step*)

3. (8 marks) What are the main similarities, and what are the differences between the published phylogeny and yours? (*Hint: You need to specify which groups of accessions' relative relationships are the same as the published one. Also, which ones are different (closer or distant) than the published one, and how the clades are different.*)

4. (4 marks) According to the calculation with 'distance correction' and 'exclude gaps' on (with UPGMA), which pairs of two sequences are the closest, and which are the furthest apart in terms of distance? (*Hint: Inspect the* ***distance matrix****, which can been seen from the results by changing one parameter.*)

5. (4 marks) How much of a difference is the 'distance correction' and 'exclude gaps' having on the topology of the phylogeny, and the distance matrices used to build the phylogeny?

Exercise 2 UPGMA (12 pts)

Consider the following pairwise distances calculated from the number of mismatches after doing a multiple sequence alignment, between taxa a through f:

	a	b	c	d	e	f
a	-	-	-	-	-	-
b	4	-	-	-	-	-
c	12	12	-	-	-	-
d	12	12	6	-	-	-
e	22	22	22	22	-	-
f	22	22	22	22	16	-

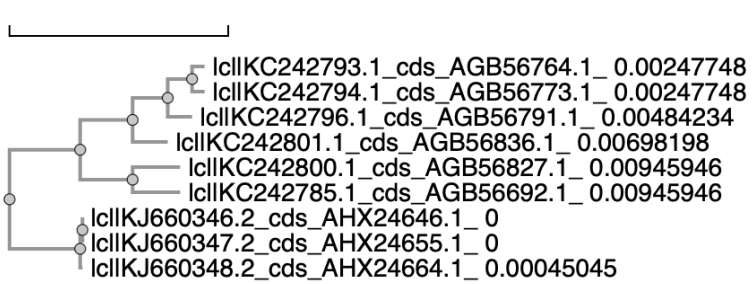
Build a scaled phylogenetic tree using UPGMA (you don't have to actually draw it to scale, but **label the branches with lengths**). You need to demonstrate **how you've created the tree step by step. Calculation process for branch lengths is also needed.**

Exercise 3 De Novo Peptide Sequencing (8 pts)

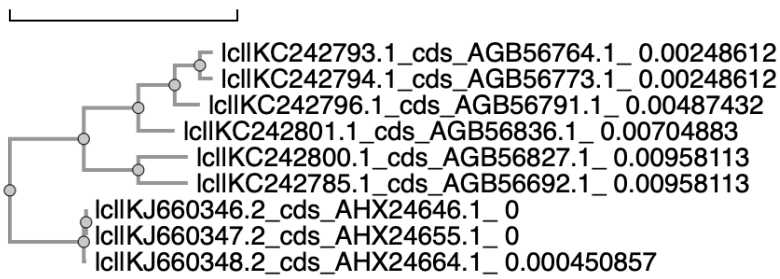
Assume in an MS/MS spectrum, you have a complete series of b-ions, and their residue mass values are 71, 158, 215, and 343. You also know the residue mass values of the common 20 amino acids. What partial peptide sequence can you infer from the spectrum? Please include your calculation steps.

2. (6 marks) Then, build 2 separate phylogenies with the ClustalW Phylogeny program (https://www.ebi.ac.uk/jdispatcher/phylogeny/simple_phylogeny); one with UPGMA with both 'distance correction' and 'exclude gaps' **off**, and one with both parameters **on**. Take screenshots of the resulting trees (Phylogram are fine; also the accession number should be written in each label of the phylogram if the header was shrunken correctly in the previous question; this makes it easier to compare to the published phylogeny). (*Hint: You may need to download the multiple sequence alignment from the previous step*)

both off:

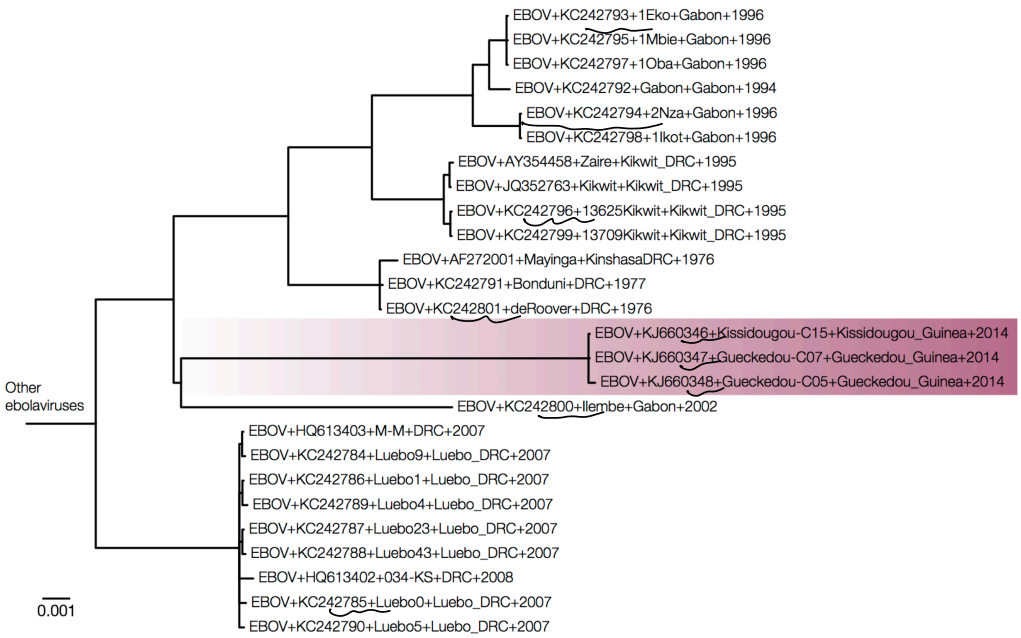


both on:



3. (8 marks) What are the main similarities, and what are the differences between the published phylogeny and yours? (*Hint: You need to specify which groups of accessions' relative relationships are the same as the published one. Also, which ones are different (closer or distant) than the published one, and how the clades are different.*)

same: the 346, 347, 348 are the same,
793, 794, 796, 801, 800 are mostly the same,
but 785 is the different one
785 is set close to 800 in my tree, but it is
distant from all others in published.



4. (4 marks) According to the calculation with 'distance correction' and 'exclude gaps' on (with UPGMA), which pairs of two sequences are the closest, and which are the furthest apart in terms of distance? (*Hint: Inspect the* ***distance matrix****, which can been seen from the results by changing one parameter.*)

The closet pair is the 346, 347 and 793, 348 are the furthese part.

lcl|KC242793.1_cds_AGB56764.1_	0.000	0.005	0.010	0.015	0.023	0.023	0.030	0.031
0.030								
lcl|KC242794.1_cds_AGB56773.1_	0.005	0.000	0.009	0.014	0.021	0.021	0.028	0.029
0.028								
lcl|KC242796.1_cds_AGB56791.1_	0.010	0.009	0.000	0.014	0.021	0.021	0.029	0.030
0.029								
lcl|KC242801.1_cds_AGB56836.1_	0.015	0.014	0.014	0.000	0.020	0.020	0.028	0.029
0.028								
lcl|KC242800.1_cds_AGB56827.1_	0.023	0.021	0.021	0.020	0.000	0.019	0.028	0.028
0.028								
lcl|KC242785.1_cds_AGB56692.1_	0.023	0.021	0.021	0.020	0.019	0.000	0.027	0.028
0.027								
lcl|KJ660346.2_cds_AHX24646.1_	0.030	0.028	0.029	0.028	0.028	0.027	0.000	0.001
0.000								
lcl|KJ660348.2_cds_AHX24664.1_	0.031	0.029	0.030	0.029	0.028	0.028	0.001	0.000
0.001								
lcl|KJ660347.2_cds_AHX24655.1_	0.030	0.028	0.029	0.028	0.028	0.027	0.000	0.001
0.000								

5. (4 marks) How much of a difference is the 'distance correction' and 'exclude gaps' having on the topology of the phylogeny, and the distance matrices used to build the phylogeny?

Distance correction and exclude gaps increase the distance of both phylogeny and the distance matrices.

Exercise 2 UPGMA (12 pts)

Consider the following pairwise distances calculated from the number of mismatches after doing a multiple sequence alignment, between taxa a through f:

	a	b	c	d	e	f
a	-	-	-	-	-	-
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c	12	12	-	-	-	-
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e	22	22	22	22	-	-
f	22	22	22	22	16	-

Build a scaled phylogenetic tree using UPGMA (you don't have to actually draw it to scale, but **label the branches with lengths**). You need to demonstrate **how you've created the tree step by step. Calculation process for branch lengths is also needed.**

as we can tell from the matrix a, b only distant 4.

Thus combine a and b together

then I think is c and d as they only differ by 6.

Thus $\overbrace{a\ b}\ \overbrace{c\ d}$

I want to check distance btw (a b) and (c d).

$$d_{(ab)(cd)} = \frac{1}{|ab||cd|} \cdot \sum_{y \in a,b} \sum_{z \in c,d} d_{y,z}$$

$$= \frac{1}{2 \cdot 2} \cdot (d_{ac} + d_{ad} + d_{bc} + d_{bd})$$

$$= \frac{1}{4} \cdot (12 + 12 + 12 + 12) = 12$$

$$d_{(ab)e} = \frac{1}{2}(22 + 22) = 22$$

$d(ab)f = \frac{1}{2}(22+22) = 22$

$d(cd)e = 22 \qquad d(cd)f = 22$

$\Rightarrow$ ab
cd 12 ← smallest
e 22 22
f 22 22 1b

$\rightarrow \quad ab = x, \quad cd = y$

$\Rightarrow d\,xy, e = \frac{1}{2}(d(xe) + d(ye)) = \frac{1}{2}(22+22)$
$= 22$

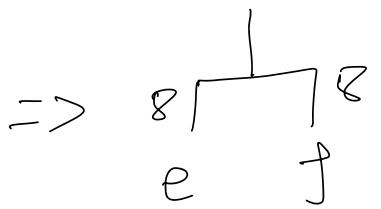
$d\,xy, f = \frac{1}{2}(d(af) + d(yf)) = \frac{1}{2}(22+22)$
$= 22$

$\Rightarrow$ xy
e 22
f 22 1b ← smaller.

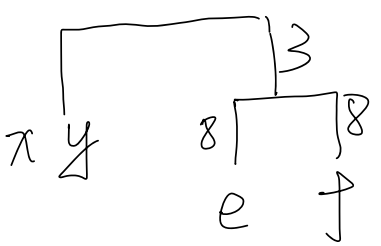
$d\,xy, ef = \frac{1}{2}(22+22) = 22$

$\rightarrow$ xy
ef 22

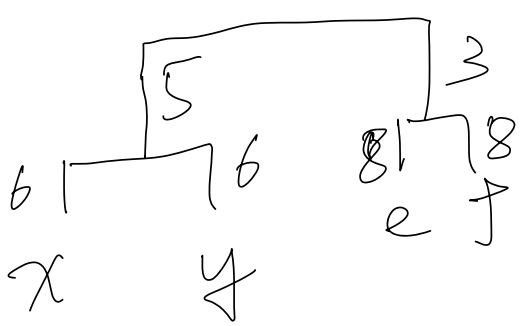
=> first def = 16



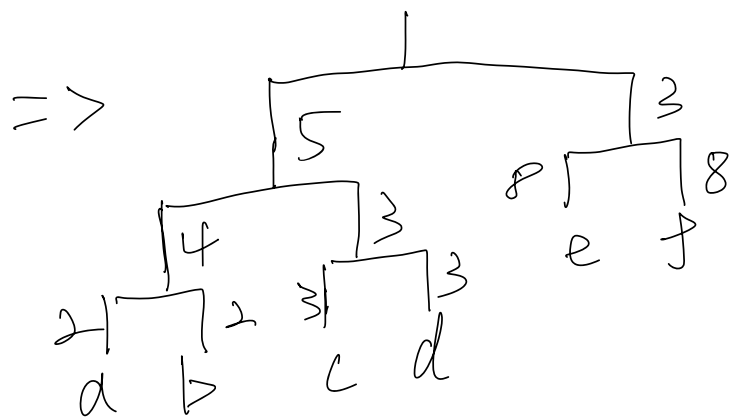
dxy, ef = 22



dxy = 12



dab = 4 dcd = 6



Exercise 3 De Novo Peptide Sequencing (8 pts)

Assume in an MS/MS spectrum, you have a complete series of b-ions, and their residue mass values are 71, 158, 215, and 343. You also know the residue mass values of the common 20 amino acids. What partial peptide sequence can you infer from the spectrum? Please include your calculation steps.

b-ions. 71 -> A -> C_3H_5NO

158-71 = 87 -> S -> $C_3H_5NO_2$

215-158 = 57 -> G -> C_2H_3NO

343-215 = 128 -> Q -> $C_5H_8N_2O_2$

=> A | S | G | Q |

71 158 215 343

clustal w. aln

CLUSTAL 2.1 multiple sequence alignment

```
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ATGGATTCTCGTCCTCAGAAAGTCTGGATGACGCCGAGTCTCACTGAATC
lcl|KC242801.1_cds_AGB56836.1_
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lcl|KJ660348.2_cds_AHX24664.1_
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TGACATGGATTACCACAAGATCTTGACAGCAGGTCTGTCCGTTCAACAGG
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lcl|KC242800.1_cds_AGB56827.1_
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** ********** **********

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                              * ******  ********************** ******************

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lcl|KC242801.1_cds_AGB56836.1_
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lcl|KJ660348.2_cds_AHX24664.1_
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GGGCACGGGTTCCGTTTTGAAGTCAAGAAGCGTGATGGAGTGAAGCGCCT
                              ****************************** ******** ***********

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lcl|KC242801.1_cds_AGB56836.1_
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TGAGGAATTGCTGCCAGCAGTATCTAGTGGAAAAAACATTAAGAGAACAC
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