

BIOINFORMATICS SESSION 8. PRACTICE

2023-10-23

Evolution Resolving a criminal case

Phylogenetic analysis of HIVs

DNA samples from Victim, patients, and controls (from Lafayette regions)

PCR DNAs with primers of Env and RT and sequenced them.

Computational analysis of phylogenetic trees with the sequences using ClustalW

Data is publicly available in NCBI Entrez (AY156734-AY156907)

- 132 env sequences
- 42 RT sequences
- clustalw2 rt.fa

How to download Data from NCBI database

The screenshot shows the NCBI website homepage. At the top, there is a navigation bar with 'NCBI Resources' and 'How To' menus, and a 'Sign in to NCBI' link. Below this is a search bar with a dropdown menu set to 'All Databases' and a 'Search' button. The main content area is divided into several sections:

- NCBI Home**: A blue arrow-shaped button.
- Resource List (A-Z)**: A vertical list of categories including All Resources, Chemicals & Bioassays, Data & Software, DNA & RNA, Domains & Structures, Genes & Expression, Genetics & Medicine, Genomes & Maps, Homology, Literature, Proteins, Sequence Analysis, Taxonomy, Training & Tutorials, and Variation.
- Welcome to NCBI**: A central section with the text: 'The National Center for Biotechnology Information advances science and health by providing access to biomedical and genomic information.' Below this are links for 'About the NCBI', 'Mission', 'Organization', 'NCBI News', and 'Blog'.
- Submit**: A tile with an upload icon, text 'Deposit data or manuscripts into NCBI databases'.
- Download**: A tile with a download icon, text 'Transfer NCBI data to your computer'.
- Learn**: A tile with a book icon, text 'Find help documents, attend a class or watch a tutorial'.
- Develop**: A tile with a code icon, text 'Use NCBI APIs and code libraries to build applications'.
- Analyze**: A tile with a network icon, text 'Identify an NCBI tool for your data analysis task'.
- Research**: A tile with a microscope icon, text 'Explore NCBI research and collaborative projects'.
- Popular Resources**: A list of links including PubMed, Bookshelf, PubMed Central, PubMed Health, BLAST, Nucleotide, Genome, SNP, Gene, Protein, and PubChem.
- NCBI Announcements**: A section with two entries: 'May 4th NCBI Minute: Linking PubMed and ClinicalTrials.gov' (dated 26 Apr 2016) and 'Next Wednesday, May 4th, NCBI will present a short tutorial that will teach you'.
- New NCBI video on YouTube: "Sequence Viewer: Display dbVar Supporting Calls"** (dated 25 Apr 2016).

How to download Data from NCBI database (data from the paper in previous slide)

The screenshot shows the NCBI Nucleotide search interface. The search term 'metzker hiv-1 clone USA' is entered in the search box and highlighted with a red rectangle. The search results are displayed in a list format, with the first four items highlighted by red boxes:

- 1. **HIV-1 clone V50 from USA envelope glycoprotein (env) gene, partial cds**
793 bp linear DNA
Accession: AY156907.1 GI: 24210283
[GenBank](#) **FASTA** [Graphics](#) [PopSet](#)
- 2. **HIV-1 clone V49 from USA envelope glycoprotein (env) gene, partial cds**
793 bp linear DNA
Accession: AY156906.1 GI: 24210281
[GenBank](#) **FASTA** [Graphics](#) [PopSet](#)
- 3. **HIV-1 clone V48 from USA envelope glycoprotein (env) gene, partial cds**
793 bp linear DNA
Accession: AY156905.1 GI: 24210279
[GenBank](#) **FASTA** [Graphics](#) [PopSet](#)
- 4. **HIV-1 clone V47 from USA envelope glycoprotein (env) gene, partial cds**
775 bp linear DNA
Accession: AY156904.1 GI: 24210277
[GenBank](#) **FASTA** [Graphics](#) [PopSet](#)

The right sidebar contains several sections: 'Results by taxon' showing 'Human immunodeficiency virus 1 (806)', 'Homo sapiens (23)', and 'Rattus norvegicus (3)'; 'Find related data' with a 'Database: Select' dropdown and a 'Find items' button; and 'Search details' showing the search query: 'metzker[All Fields] AND ("Human immunodeficiency virus 1"[Organism] OR hiv-1[All Fields]) AND clone[All Fields] AND USA[All Fields]'. A 'Search' button and a 'See more...' link are also present.

How to download Data from NCBI database (data from the paper in previous slide)

ational Library of Medicine
nal Center for Biotechnology Information

Nucleotide Search

Advanced

V50 from USA envelope glycoprotein (env) gene, part

7.1

[PopSet](#)

clone V50 from USA envelope glycoprotein (env) gene, partial cds

```
AACTCGATTACTGTTAAATGGCAGTCTAGCAGAAAAAGAGGTAGTAATTAGA
AATGCTAAAAACCATAATAGTACAGCTGACAAACCCCTGTAGAAATTAATTGTA
CAAGAAAAAGTAACTTACCTTAGGACCAAGGAGAGCACTTTATACAACAGGAGA
AAAAGCATATTGTAACATTAGTAAAGCAAAATGGAATGACACTCTAAGCCAG
CAACAATTTAATAAAAACAACAATAGTCTTTAAGCAATCCTCAGBAGGGGACC
GTTTTAACTGTGGAGGGGAATTTTTCTACTGTAATACAACACAACACTGTTAA
TGAAGGAACAAAATTTACTGAAGGAACAAAATAACATGGAAAGGAAATAATACA
AGAATAAAACAAATGATAAACATGTGGCAGBAGGTAGGAAAGCAATGTATG
TAATTAGCTGCTCATCAAAATTTACAGGGCTACTACTAACCAAGAGATGGCCG
AGATAATGAGACCTTCAGACCTGGAGGAGGAGATAGAAAGGCAATTGGAGA
AAAGTAGTAAAAATTTGAACCATTAGGAGTAGCACCCAAGGCCAAGAGAA
AAAAA
```

Send to:

Complete Record
 Coding Sequences
 Gene Features

Choose Destination

File Clipboard
 Collections Analysis Tool

Download 1 item.

Format
FASTA

Show GI

Create File

Related information

Basic Shell Commands

```
$ cd 2023123456_hyunwoo
```

```
$ mkdir session8
```

```
$ cd session8
```

Using clustalw2 for phylogenetic analysis

```
$ ln -s /home/biguser/tutor/session8/rt.fa .  
$ ll
```

```
[biguser@biglab-master session8]$ ll  
total 36  
-r--r--r-- 1 biguser biguser 34710 Apr 27 10:37 rt.fa
```

Using clustalw2 for phylogenetic analysis

```
$ clustalw2 rt.fa  
$ clustalw2 rt.aln -tree  
$ clustalw2 rt.aln -bootstrap=1000
```

```
[biguser@biglab-master session8]$ ll  
total 108  
-rw-rw-r-- 1 biguser biguser 58796 Apr 27 15:10 rt.aln  
-rw-rw-r-- 1 biguser biguser  1983 Apr 27 15:10 rt.dnd  
-r--r--r-- 1 biguser biguser 34710 Apr 27 10:37 rt.fa  
-rw-rw-r-- 1 biguser biguser  1984 Apr 27 15:10 rt.ph  
-rw-rw-r-- 1 biguser biguser  2172 Apr 27 15:10 rt.phb
```


Using clustalw2 for phylogenetic analysis

```
There are 41 groups
Start of Multiple Alignment
```

```
Aligning...
```

```
Group 1: Sequences: 2      Score:13091
Group 2: Sequences: 3      Score:13072
Group 3: Sequences: 4      Score:13065
Group 4: Sequences: 2      Score:15209
Group 5: Sequences: 6      Score:13533
Group 6: Sequences: 7      Score:13004
Group 7: Sequences: 8      Score:14848
Group 8: Sequences: 2      Score:13034
Group 9: Sequences: 2      Score:13091
Group 10: Sequences: 3     Score:13072
Group 11: Sequences: 4     Score:13065
Group 12: Sequences: 2     Score:15285
Group 13: Sequences: 6     Score:13599
Group 14: Sequences: 8     Score:13578
Group 15: Sequences: 9     Score:13050
Group 16: Sequences: 17    Score:13212
Group 17: Sequences: 2     Score:12834
Group 18: Sequences: 3     Score:12853
Group 19: Sequences: 20    Score:12760
Group 20: Sequences: 2     Score:12853
Group 21: Sequences: 2     Score:12802
Group 22: Sequences: 2     Score:12967
Group 23: Sequences: 3     Score:12856
Group 24: Sequences: 5     Score:12827
Group 25: Sequences: 6     Score:12837
Group 26: Sequences: 2     Score:12863
Group 27: Sequences: 3     Score:12882
Group 28: Sequences: 4     Score:12843
Group 29: Sequences: 10    Score:12785
Group 30: Sequences: 12   Score:12787
Group 31: Sequences: 2     Score:12806
Group 32: Sequences: 3     Score:12753
Group 33: Sequences: 4     Score:12774
Group 34: Sequences: 16    Score:12713
Group 35: Sequences: 17    Score:12690
Group 36: Sequences: 37    Score:12479
Group 37: Sequences: 38    Score:12695
Group 38: Sequences: 39    Score:12760
Group 39: Sequences: 40    Score:12754
Group 40: Sequences: 41    Score:12802
Group 41: Sequences: 42    Score:12609
Alignment Score 3678234
```

```
CLUSTAL-Alignment file created [rt.aln]
```

```
[biguser@R440 session8]$ clustalw2 rt.aln -tree
CLUSTAL 2.1 Multiple Sequence Alignments
```

```
Sequence format is CLUSTAL
```

```
Sequence 1: g1|24209945|gb|AY156737.1| 805 bp
Sequence 2: g1|24209951|gb|AY156740.1| 805 bp
Sequence 3: g1|24209939|gb|AY156734.1| 805 bp
Sequence 4: g1|24210015|gb|AY156802.1| 805 bp
Sequence 5: g1|24210009|gb|AY156799.1| 805 bp
Sequence 6: g1|24210011|gb|AY156800.1| 805 bp
Sequence 7: g1|24209941|gb|AY156735.1| 805 bp
Sequence 8: g1|24210007|gb|AY156797.1| 805 bp
Sequence 9: g1|24209943|gb|AY156736.1| 805 bp
Sequence 10: g1|24210013|gb|AY156801.1| 805 bp
Sequence 11: g1|24209953|gb|AY156741.1| 805 bp
Sequence 12: g1|24209955|gb|AY156742.1| 805 bp
Sequence 13: g1|24209947|gb|AY156738.1| 805 bp
Sequence 14: g1|24210017|gb|AY156803.1| 805 bp
Sequence 15: g1|24210019|gb|AY156806.1| 805 bp
Sequence 16: g1|24210021|gb|AY156807.1| 805 bp
Sequence 17: g1|24209949|gb|AY156739.1| 805 bp
Sequence 18: g1|24209961|gb|AY156773.1| 805 bp
Sequence 19: g1|24209967|gb|AY156776.1| 805 bp
Sequence 20: g1|24210005|gb|AY156795.1| 805 bp
Sequence 21: g1|24209959|gb|AY156772.1| 805 bp
Sequence 22: g1|24209995|gb|AY156790.1| 805 bp
Sequence 23: g1|24209969|gb|AY156777.1| 805 bp
Sequence 24: g1|24209973|gb|AY156779.1| 805 bp
Sequence 25: g1|24209983|gb|AY156784.1| 805 bp
Sequence 26: g1|24209989|gb|AY156787.1| 805 bp
Sequence 27: g1|24209931|gb|AY156789.1| 805 bp
Sequence 28: g1|24209985|gb|AY156785.1| 805 bp
Sequence 29: g1|24209975|gb|AY156780.1| 805 bp
Sequence 30: g1|24209979|gb|AY156782.1| 805 bp
Sequence 31: g1|24210003|gb|AY156794.1| 805 bp
Sequence 32: g1|24209999|gb|AY156792.1| 805 bp
Sequence 33: g1|24209977|gb|AY156781.1| 805 bp
Sequence 34: g1|24209987|gb|AY156791.1| 805 bp
Sequence 35: g1|24209965|gb|AY156775.1| 805 bp
Sequence 36: g1|24209963|gb|AY156788.1| 805 bp
Sequence 37: g1|24209987|gb|AY156786.1| 805 bp
Sequence 38: g1|24210001|gb|AY156793.1| 805 bp
Sequence 39: g1|24209963|gb|AY156774.1| 805 bp
Sequence 40: g1|24209981|gb|AY156783.1| 805 bp
Sequence 41: g1|24209971|gb|AY156778.1| 805 bp
Sequence 42: g1|24209957|gb|AY156771.1| 805 bp
```

```
Phylogenetic tree file created: [rt.ph]
```

```
[biguser@R440 session8]$ clustalw2 rt.aln -bootstrap=1000
CLUSTAL 2.1 Multiple Sequence Alignments
```

```
Sequence format is CLUSTAL
```

```
Sequence 1: g1|24209945|gb|AY156737.1| 805 bp
Sequence 2: g1|24209951|gb|AY156740.1| 805 bp
Sequence 3: g1|24209939|gb|AY156734.1| 805 bp
Sequence 4: g1|24210015|gb|AY156802.1| 805 bp
Sequence 5: g1|24210009|gb|AY156799.1| 805 bp
Sequence 6: g1|24210011|gb|AY156800.1| 805 bp
Sequence 7: g1|24209941|gb|AY156735.1| 805 bp
Sequence 8: g1|24210007|gb|AY156797.1| 805 bp
Sequence 9: g1|24209943|gb|AY156736.1| 805 bp
Sequence 10: g1|24210013|gb|AY156801.1| 805 bp
Sequence 11: g1|24209953|gb|AY156741.1| 805 bp
Sequence 12: g1|24209955|gb|AY156742.1| 805 bp
Sequence 13: g1|24209947|gb|AY156738.1| 805 bp
Sequence 14: g1|24210017|gb|AY156803.1| 805 bp
Sequence 15: g1|24210019|gb|AY156806.1| 805 bp
Sequence 16: g1|24210021|gb|AY156807.1| 805 bp
Sequence 17: g1|24209949|gb|AY156739.1| 805 bp
Sequence 18: g1|24209961|gb|AY156773.1| 805 bp
Sequence 19: g1|24209967|gb|AY156776.1| 805 bp
Sequence 20: g1|24210005|gb|AY156795.1| 805 bp
Sequence 21: g1|24209959|gb|AY156772.1| 805 bp
Sequence 22: g1|24209995|gb|AY156790.1| 805 bp
Sequence 23: g1|24209969|gb|AY156777.1| 805 bp
Sequence 24: g1|24209973|gb|AY156779.1| 805 bp
Sequence 25: g1|24209983|gb|AY156784.1| 805 bp
Sequence 26: g1|24209989|gb|AY156787.1| 805 bp
Sequence 27: g1|24209931|gb|AY156789.1| 805 bp
Sequence 28: g1|24209985|gb|AY156785.1| 805 bp
Sequence 29: g1|24209975|gb|AY156780.1| 805 bp
Sequence 30: g1|24209979|gb|AY156782.1| 805 bp
Sequence 31: g1|24210003|gb|AY156794.1| 805 bp
Sequence 32: g1|24209999|gb|AY156792.1| 805 bp
Sequence 33: g1|24209977|gb|AY156781.1| 805 bp
Sequence 34: g1|24209987|gb|AY156791.1| 805 bp
Sequence 35: g1|24209965|gb|AY156775.1| 805 bp
Sequence 36: g1|24209963|gb|AY156788.1| 805 bp
Sequence 37: g1|24209987|gb|AY156786.1| 805 bp
Sequence 38: g1|24210001|gb|AY156793.1| 805 bp
Sequence 39: g1|24209963|gb|AY156774.1| 805 bp
Sequence 40: g1|24209981|gb|AY156783.1| 805 bp
Sequence 41: g1|24209971|gb|AY156778.1| 805 bp
Sequence 42: g1|24209957|gb|AY156771.1| 805 bp
```

```
Bootstrap output file created: [rt.phb]
```



Code 9.1 reformat_giline.ipynb

```
In [4]: import sys
import re

output_open= open("rt_reformat.fa","w")
for line in open("rt.fa", "r"):
    line= line.strip()
    match= re.search(">.*clone (\\S+) ", line)
    if match:
        id= ">" + match.group(1)+ "\\n"
        output_open.write(id)
    else:
        output_open.write(line.rstrip()+ "\\n")
output_open.close()
```

*Regular expression "\\S" - Matches any character which is not a Unicode whitespace character

Code 9.1 reformat_giline.ipynb

 jupyter rt_reformat.fav 2분 전

File Edit View Language

```
1 >P1_.BCM.RT
2 CCCATAAGTCCTATTGAAACTGTACCAGTAAAAATAAGCCAGGAATGGATGGCCAAAAAGTTAAACAAT
3 GGCCACTGACAGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAAT
4 TTCAAAAAATGGGCCGAAAAATCCATACAATACTCCAGTATTGCCATAAAGAAAAAGCAGACTAAA
5 TGGAGAAAAATAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGSAA
6 TACCACATCCTGCAGGGTTAAAAAGAAAAAATCAGTAACAGTGTGGATGTGGGTGATGCATATTTTTTC
7 AGTTCCTTAGATAAAGAGTTCAGGAAGTATACTGCATTACCATAACCTAGTATAAACAATGAGACACCA
8 GGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAGGATCACCAGCAATATCCAAAGTAGCA
9 TGACAAAAATCTTAGAGCCTTTAGAAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGATCT
10 GTATGTAGGATCTGACTTAGAAAATAGGGCAGCATAGAATAAAAAATAGAGGAACTAAGACAACATCTGTG
11 AAGTGGGGACTTACCACACCAGACAAAAAACATAGAAGGAACCCCATCTCCTTTGGAT
12
13 >P2_.BCM.RT
14 CCCATAAGTCCTATTGAAACTGTACCAGTAAAAATAAGCCAGGAATGGATGGCCAAAAAGTTAAGCAAT
15 GGCCACTGACAGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAAT
16 TTCAAAAAATGGGCCGAAAAATCCATACAATACTCCAGTATTGCCATAAAGAAAAAGCAGACTAAA
17 TGGAGAAAAATAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGSAA
18 TACCACATCCTGCAGGGTTAAAAAGAAAAAATCAGTAACAGTGTGGATGTGGGTGATGCATATTTTTTC
19 AGTTCCTTAGATAAAGGAGTTCAGGAAGTATACTGCATTACCATAACCTAGTATAAACAATGAGACACCA
20 GGGATTAGATATCAGTACAATGTGCTTCCACAGGGATGGAAGGATCACCAGCAATATCCAAAGTAGCA
21 TGACAAAAATCTTAGAGCCTTTAGAAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGATTT
22 GTATGTAGGATCTGACTTAGAAAATAGGGCAGCATAGAATAAAAAATAGAGGAACTAAGACAACATCTGTG
23 AAGTGGGGACTTACCACACCAGACAAAAAACATAGAAGGAACCTCCATCTCCTTTGGAT
24
25 >P3_.BCM.RT
26 CCCATAAGTCCTATTGAAACTGTACCAGTAAAAATAAGCCAGGAATGGATGGCCAAAAAGTTAAACAAT
27 GGCCACTGACAGAAGAAAAATAAAGCATTAGTAGAAATTTGTACAGAAATGGAAAAGGAAGAAAAAT
28 TTCAAAAGATGGGCCGAAAAATCCATACAATACTCCAGTATTGCCATAAAGAAAAAAGCAGACTAGA
29 TGGAGAAAAATAGTAGATTTCAGAGAACTTAATAAGAGAACTCAAGACTTCTGGGAAGTTCAATTAGSAA
30 TACCACATCCTGCAGGGTTAAAAAGAAAAAATCAGTAACAGTGTGGATGTGGGTGATGCATATTTTTTC
31 AGTTCCTTAGATAAAGGAGTTCAGGAAGTATACTGCATTACCATAACCTAGTATAAACAATGAGACACCA
32 GGGATTAGATATCAATACAATGTGCTTCCACAGGGATGGAAGGATCACCAGCAATATCCAAAGTAGCA
33 TGACAAAAATCTTAGAGCCTTTAGAAAAACAAAATCCAGACATAGTTATCTATCAATACATGGATGATCT
34 GTATGTAGGATCTGACTTAGAAAATAGGGCAGCATAGAATAAAAAATAGAGGAACTAAGACAACATCTGTG
35 AAGTGGGGATTTATCACACCAGACAAAAACACCAGGAACCTCCATCTCCTTTGGAT
36
```

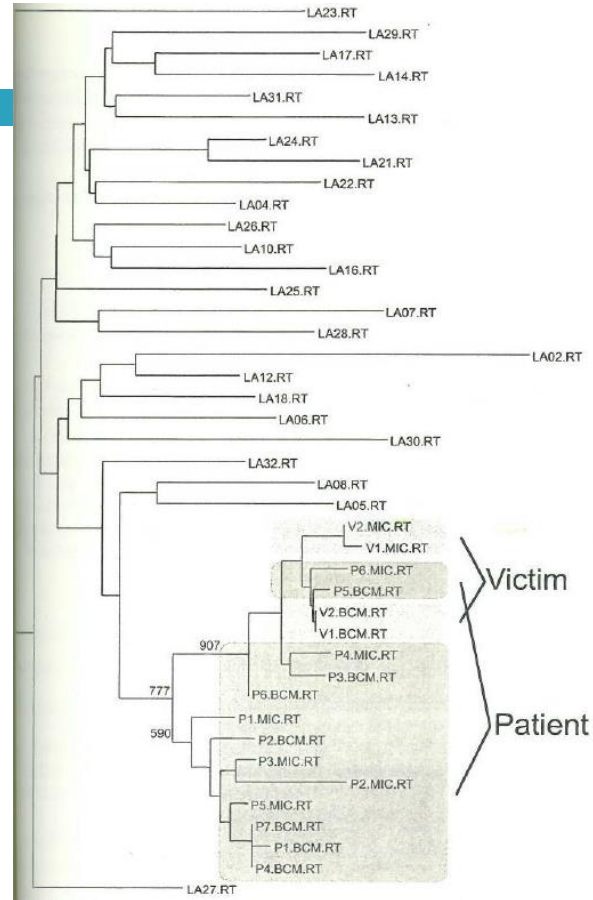
Using clustalw2 for phylogenetic analysis

```
$ clustalw2 rt_reformat.fa  
$ clustalw2 rt_reformat.aln -tree  
$ clustalw2 rt_reformat.aln -bootstrap=1000
```

```
[biguser@biglab-master session8]$ ll  
total 236  
-r--r--r-- 1 biguser biguser 117608 Apr 27 14:00 env.fa  
-r--r--r-- 1 biguser biguser  34710 Apr 27 10:37 rt.fa  
-rw-rw-r-- 1 biguser biguser  44904 Apr 27 15:04 rt_reformat.aln  
-rw-rw-r-- 1 biguser biguser   1219 Apr 27 15:04 rt_reformat.dnd  
-rw-rw-r-- 1 biguser biguser  30762 Apr 27 15:04 rt_reformat.fa  
-rw-rw-r-- 1 biguser biguser   1408 Apr 27 15:05 rt_reformat.phb
```

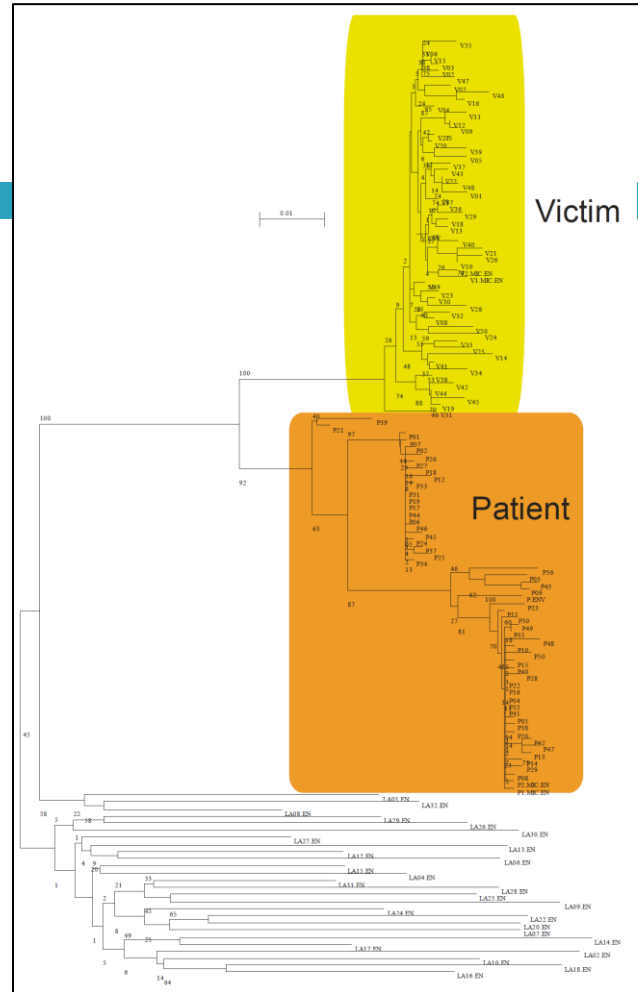
Using clustalw2 for phylogenetic analysis (“rt.fa”)

```
(  
(  
P4.BCM.RT:0.00000,  
P1.BCM.RT:0.00146)  
:0.00000[590],  
P7.BCM.RT:0.00000)  
:0.00162[976],  
P5.MIC.RT:0.00128)  
:0.00077[431],  
(  
P2.MIC.RT:0.00845,  
P3.MIC.RT:0.00149)  
:0.00115[318])  
:0.00080[295],  
P2.BCM.RT:0.00338)  
:0.00142[389],  
P1.MIC.RT:0.00327)  
:0.00151[590],  
(  
(  
(  
P3.BCM.RT:0.00267,  
P4.MIC.RT:0.00313)  
:0.00070[417],  
(  
(  
(  
(  
V1.BCM.RT:0.00000,  
V2.BCM.RT:0.00000)  
:0.00018[961],  
P5.BCM.RT:0.00127)  
:0.00017[697],  
P6.MIC.RT:0.00273)  
:0.00071[400],
```



Using clustalw2 for phylogenetic analysis (“env.fa”)

```
(
(
(
LA16.EN:0.03515,
LA18.EN:0.05200)
:0.00998[68],
LA02.EN:0.06466)
:0.00325[9],
(
(
LA10.EN:0.05145,
LA26.EN:0.04984)
:0.00366[33])
:0.00593[2],
(
LA17.EN:0.02509,
LA14.EN:0.06339)
:0.01176[55])
:0.00212[1],
(
(
LA07.EN:0.05054,
LA24.EN:0.03889)
:0.00531[25],
(
LA20.EN:0.04773,
LA22.EN:0.04938)
:0.00634[49])
:0.00281[4])
:0.00036,
(
(
LA06.EN:0.06420,
LA05.EN:0.04289)
:0.00278[15],
(
(
LA09.EN:0.06125,
LA25.EN:0.04618)
:0.00343[25],
(
LA28.EN:0.05127,
```



Exercise

- Make a Python script to derive one of the distance matrices shown in Fig. 9.1. The starting point for the script is the multiple alignment in the same figure.

```
>A
GGACCACTACGAGCGCCTACGAC
GTA
>B
GGACCCCTACGAGCCCCTACGAC
GTA
>C
GGACCGCTGCGAGCTTCTACGAC
GTA
>D
GGACCTCTCCGGGCAGCTAGGAC
GTA
```



	A	B	C	D
A	0	2	4	6
B	2	0	4	6
C	4	4	0	6
D	6	6	6	0

Answer for Exercise

i : row
j : column

for j in xrange

for i in xrange

	A	B	C	D
A	0	2	4	6
B	2	0	4	6
C	4	4	0	6
D	6	6	6	0

```
1 # exercise
2
3
4 seqs= ["GGACCACTACGAGCGCCTACGACGTA",
5         "GGACCCCTACGAGCCCCTACGACGTA",
6         "GGACCGCTGCGAGCTTCTACGACGTA",
7         "GGACCTCTCCGGGCAGCTAGGACGTA"]
8 for i in range(len(seqs)):
9     rowseq= seqs[i]
10    for j in range(len(seqs)):
11        colseq= seqs[j]
12        diff=0
13        for k in range(len(colseq)):
14            if rowseq[k] != colseq[k]:
15                diff +=1
16        print(diff, end = ' ')
17    print("\n")
```