SESSION 8. EVOLUTION

Resolving a criminal case

A fatal injection with HIV and C-Hepatitis

- *Maria consulted a physician with a symptom of her lymph nodes were swollen with a viral infection.*
- *In Dec. She got annual check-up including HIV test and was found HIV positive and Hepatitis C positive.*
- *She turned to Police in Jan. 1995 and did not deal the accusations seriously.*
- *It turned out all seven men Maria reported as sexual contacts for the period 1984-1995 were tested and found to be HIV negative.*
- *During examining Robert's patients' records, Police found a blood sample from a patient with AIDS and another sample from a patient with Hepatitis C.*
- *Prosecution wanted evidence that the HIV carried by Maria was the same as – or very closely related to – the AIDS patient's HIV. In other word, it was important to exclude the possibility that Maria received the infection from some other sources.*

Molecular phylogeny

Computational methods of molecular phylogeny

- This criminal case was that the **molecular phylogeny was first used in a court in USA**.
- Molecular phylogeny – the science of constructing phylogenetic trees using molecular sequence data.
- Tree construction methods rely on the analysis of multiple sequence alignment (MSA).
- Two major approaches: distance-based and character-based

Molecular phylogeny methods

Distance-based approach (computationally simple)

- Neighbor-joining : based on all pairwise comparison of seqs and minimization of the total branch length

Character-based approach (more biologically relevant)

- Maximum-parsimony: an optimality criterion under which the phylogenetic **tree** that minimizes the total number of character-state changes is preferred.
- Maximum-likelihood: maximizes tree likelihood given specific parameter values

Neighbor-joining (NJ)

- **Building Phylogenetic Trees by Neighbor-Joining:**
  - Algorithm (Given a distance matrix):

    Iterate Until 2 Nodes are left:
    - For each node find $U_i = \sum_{k=1}^{N} d_{i,k}$
    - Choose pair (i, j) with smallest $\delta_{ij} = d_{ij} - \frac{U_i + U_j}{N-2}$
    - Mege two nodes i and j with a new internal node Y, and find branch lengths by $b_{iY} = \frac{1}{2}(d_{ij} + \frac{U_i - U_j}{N-2})$ $b_{jY} = d_{ij} - b_{iY}$
    - Update the distance matrix using $d_{Yk} = \frac{1}{2}(d_{ik} + d_{jk} - d_{ij})$

Neighbor-joining (NJ)

STEP 1 ($N = 5$)

d_{ij}

	B	C	D	E	U_i
A	5	4	9	8	26
B		5	10	9	29
C			7	6	22
D				7	33
E					30

$3\delta_{ij}$

	B	C	D	E	
A	−40	−36	−32	−32	A
B		−36	−32	−32	B
C			−34	−34	C
D				−42	D
E					E

$$U_i = \sum_{k=1}^{N} d_{i,k}$$

$$\delta_{ij} = d_{ij} - \frac{U_i + U_j}{N-2}$$

$$b_{iY} = \frac{1}{2}(d_{ij} + \frac{U_i - U_j}{N-2}) \qquad b_{jY} = d_{ij} - b_{iY}$$

$$d_{Yk} = \frac{1}{2}(d_{ik} + d_{jk} - d_{ij})$$

Ua = 26 Ub=29 Uc=22 Ud=33 Ue=30

Delta(a,b) = 5- (26+29)/(5-2) = 5-18.3=-13.3
Delta(a,c)=4- (26+22)/3 = 12
Delta(a,d) = 9 – (26+33)/3 = 9-19.67= 10.67
Delta(a,e) = 8- (26+30)/3=8 – 18.67 = 10.67
Delta(b,c) = 5 – (29 +22)/3 = 5 – 17 = -12
Delta(b,d) = 10 – (29 + 33)/3 = 10 – 20.67= – 10.67
Delta(b,e)= 9 – (29 + 30)/3 = 9 – 19.67 = -10.67
Delta(c,d) = 7 – (22 + 33)/3 = 7 – 18.33 = -11.33
Delta(c,e) = 6 – (22 + 30)/3 = 6 – 17.33 = -11.33
Delta(d,e) = 7 – (33 + 30)/3 = 7 – 21 = -14

D(dw) = ½(7 + (33-30)/3) = 4; D(ew) = 7 – 4 = 3

D(wa) = ½(9 + 8 – 7) = 5
D(wb) = ½(10 + 9 – 7) = 6
D(wc) = ½(7 + 6 – 7) = 3

Neighbor-joining (NJ)

STEP 2 ($N = 4$)

d_{ij}

	B	C	W
A	5	4	5
B		5	6
C			3
W			

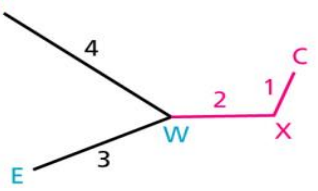
U_i

A	14
B	16
C	12
W	14

$2\delta_{ij}$

	B	C	W	
A	−20	−18	−18	A
B		−18	−18	B
C			−20	C
W				W

C and W are neighbors through internal node X with $d_{CX} = \frac{1}{2}\left(3 + \frac{12-14}{2}\right) = 1$ and $d_{WX} = 3 - 1 = 2$.



$$U_i = \sum_{k=1}^{N} d_{i,k}$$

$$\delta_{ij} = d_{ij} - \frac{U_i + U_j}{N - 2}$$

$$b_{iY} = \frac{1}{2}\left(d_{ij} + \frac{U_i - U_j}{N - 2}\right) \qquad b_{jY} = d_{ij} - b_{iY}$$

$$d_{Yk} = \frac{1}{2}(d_{ik} + d_{jk} - d_{ij})$$

Neighbor-joining (NJ)

STEP 3 ($N = 3$)

d_{ij}

	B	X
A	5	3
B		4
X		

U_i

A	8
B	9
X	7

δ_{ij}

	B	X	
A	−12	−12	A
B		−12	B
X			X

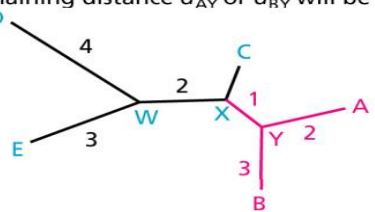
Three alternatives (of which here we choose one of the two with an internal node):
A and X are neighbors through internal node Y with $d_{AY} = 2$ and $d_{XY} = 1$ or
B and X are neighbors through internal node Y with $d_{BY} = 3$ and $d_{XY} = 1$.
Whichever is chosen, the remaining distance d_{AY} or d_{BY} will be found in the next d_{ij} matrix.

$$U_i = \sum_{k=1}^{N} d_{i,k}$$

$$\delta_{ij} = d_{ij} - \frac{U_i + U_j}{N-2}$$

$$b_{iY} = \frac{1}{2}(d_{ij} + \frac{U_i - U_j}{N-2}) \quad b_{jY} = d_{ij} - b_{iY}$$

$$d_{Yk} = \frac{1}{2}(d_{ik} + d_{jk} - d_{ij})$$



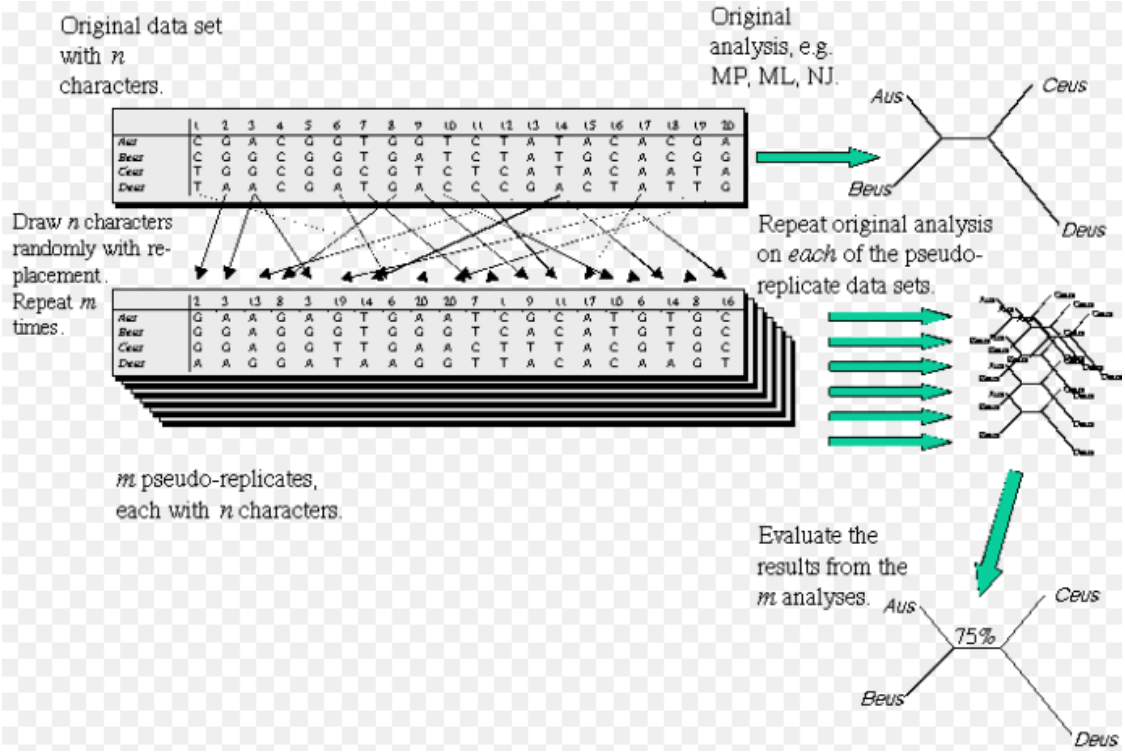
Bootstrapping (Accuracy and Robustness of tree)

Bootstrapping refers to a test that relies on random sampling with replacement that allows us to measure the significance.

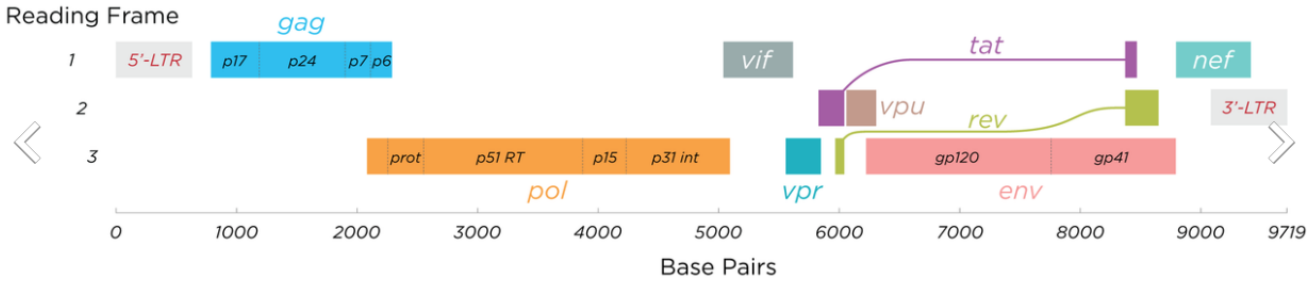
Sampling from the original sample of size N to form a new sample (called a 'resample' or bootstrap sample) that is also of size N.

Repeats 1000-10000 times of resampling and makes a histogram of means.

Bootstrapping (Accuracy and Robustness of tree)



HIV has RNA genome encoding 20 genes



- HIV RNA genome comprises 9749 nt
- Two genes, **env** and **RT** are often used for phylogenetic analysis
- **Env** encodes gp160 -> (gp120 + gp41) presenting in a surface
- **RT** encodes reverse transcriptase that makes dsDNA from RNA.

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

Phylogenetic analysis of HIVs

clustalw2 rt.fa

```
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]
```

clustalw2 rt.aln -tree

```
Sequence 34: gi|24209997|gb|AY156791.1|   805 bp
Sequence 35: gi|24209965|gb|AY156775.1|   805 bp
Sequence 36: gi|24209991|gb|AY156788.1|   805 bp
Sequence 37: gi|24209987|gb|AY156786.1|   805 bp
Sequence 38: gi|24210001|gb|AY156793.1|   805 bp
Sequence 39: gi|24209963|gb|AY156774.1|   805 bp
Sequence 40: gi|24209981|gb|AY156783.1|   805 bp
Sequence 41: gi|24209971|gb|AY156778.1|   805 bp
Sequence 42: gi|24209957|gb|AY156771.1|   805 bp

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

Phylogenetic analysis of HIVs

vi rt.ph

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gi|24209945|gb|AY156737.1|:0.00000,
gi|24209939|gb|AY156734.1|:0.00146)
:0.00000,
gi|24209951|gb|AY156740.1|:0.00000)
:0.00162,
gi|24210015|gb|AY156802.1|:0.00128)
:0.00077,
(
gi|24210009|gb|AY156799.1|:0.00845,
gi|24210011|gb|AY156800.1|:0.00149)
:0.00115)
:0.00080,
gi|24209941|gb|AY156735.1|:0.00338)
:0.00142,
gi|24210007|gb|AY156797.1|:0.00327)
:0.00151,
(
```

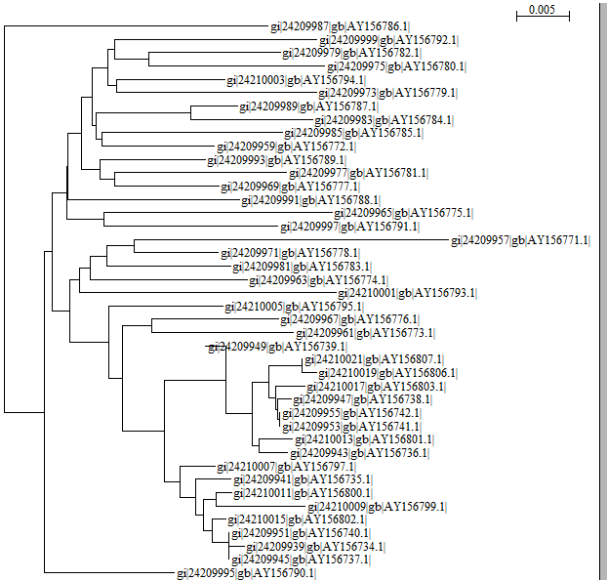
Phylogenetic analysis of HIVs

clustalw2 rt.aln -bootstrap=1000

```
Sequence 40: gi|24209981|gb|AY156783.1|    805 bp
Sequence 41: gi|24209971|gb|AY156778.1|    805 bp
Sequence 42: gi|24209957|gb|AY156771.1|    805 bp

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

Njplot → rt.phb



Reformat_giline.py

>gi|24209939|gb|AY156734.1| HIV-1 clone P1.BCM.RT from USA reverse transcriptase (pol) gene, partial cds

P = patient, V=Victim, LA = Lafayette area control

>P1.BCM.RT

Reformat_giline.py

```
#!/usr/bin/python

import re
import sys

for line in open('rt.fa'):
    line = line.rstrip()
    match = re.search('>.*clone (\S+) ', line)
    if match:
        sys.stdout.write('>')
        print match.group(1)
    else:
        print line
```

\S : non-white space

python reformat_giline.py rt.fa >rt_reformated.fa

Phylogenetic analysis with a new format

clustalw2 rt_reformat.aln -bootstrap=1000