CISC Computational Biology & Bioinformatics (Fall 2016)

Pairwise sequence alignment

Sequence Alignment

Motivation

- Sequence assembly: reconstructing long DNA sequences from overlapping sequence fragments
- Annotation: assign functions to newly discovered genes
  - Raw genomic (DNA) sequences → coding sequences (CDS), candidate for genes → protein sequence → function
  - Evolution: mutation → sequence diversity (versus homology) → (new) phenotype ?
  - Basis for annotation: sequence similarity → sequence homology → same function
    - Caveat: homology can only be inferred, not affirmed, since we can not rewind to see how evolution actually happened.

Ancestral sequence: `ACGTACGT`

After 9540 generations (del: 0.0001, ins: 0.001, trans_mut: 0.00008, transv_mut: 0.00002)

Sequence1: `ACACGGTCCTAATAATGGCC`

Sequence2: `CAGGAAGATCTTAGTTC`

True history:

```
--ACG-T-A---CG-T----
ACACGGTCCTAATAATGGCC

---AC-GTA-C--G-T--
CAG-GAAGATCTTAGTTC
```

Alignment that reflects the true history:

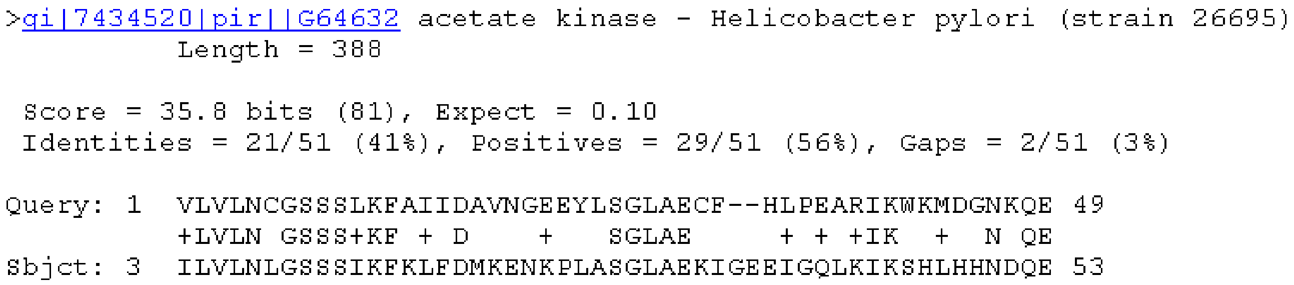
Seq1: -AC**AC**-**G**G**T**CCTAAT--AA**T**GGCC

Seq2: CAG-GAA-G-AT--CTTAGTTC--

Alignment algorithms

- What is an alignment?

  A one-to-one matching of two sequences so that each character in a pair of sequences is associated with a single character of the other sequence or with a null character (gap). Alignments are often displayed as two rows with an optional third row in between pointing out regions of similarity.
- Example:



```
>gi|7434520|pir||G64632 acetate kinase - Helicobacter pylori (strain 26695)
          Length = 388

 Score = 35.8 bits (81), Expect = 0.10
 Identities = 21/51 (41%), Positives = 29/51 (56%), Gaps = 2/51 (3%)

Query: 1  VLVLNCGSSSLKFAIIDAVNGEEYLSGLAECF--HLPEARIKWKMDGNKQE 49
          +LVLN GSSS+KF + D    +   SGLAE     + + +IK  +  N QE
Sbjct: 3  ILVLNLGSSSIKFKLFDMKENKPLASGLAEKIGEEIGQLKIKSHLHHNDQE 53
```

- Types of alignment:
  - pairwise vs multiple;
  - global vs local
- Algorithms
  - Rigorous
  - heuristic

Substitution Score matrix

- Alignments are used to reveal homologous proteins/genes
- Substitution scores are used to assess how *good* the alignments of a pair of residues are.
- Under the assumption that each mutation (i.e., *del*etion, *ins*ertion, and *sub*stitution) is independent, the total score of an alignment is the sum of scores at each position.
- Substitution score matrix is a 20 x 20 matrix that gives the score for every pair of amino acids.
- The ways to derive a substitution score matrix.
  - *Ad hoc*
  - Physical/chemical properties of amino acids
  - Statistical

PAM matrices (Margaret Dayhoff, 1978)

- point accepted mutation or percent accepted mutation
- unit of measurement of evolutionary divergence between two amino acid sequences
- substitute matrices (scoring matrices)

1 PAM = one accepted point-mutation event per one-hundred amino acids

PAM matrix is a 20 by 20 matrix, and each element p_{ij} represents the expected evolutionary exchange between the two corresponding amino acids for sequences that are a specific number of PAM units diverged. That is,

$$p_{ij} = \log[f(i,j)/f(i)f(j)]$$

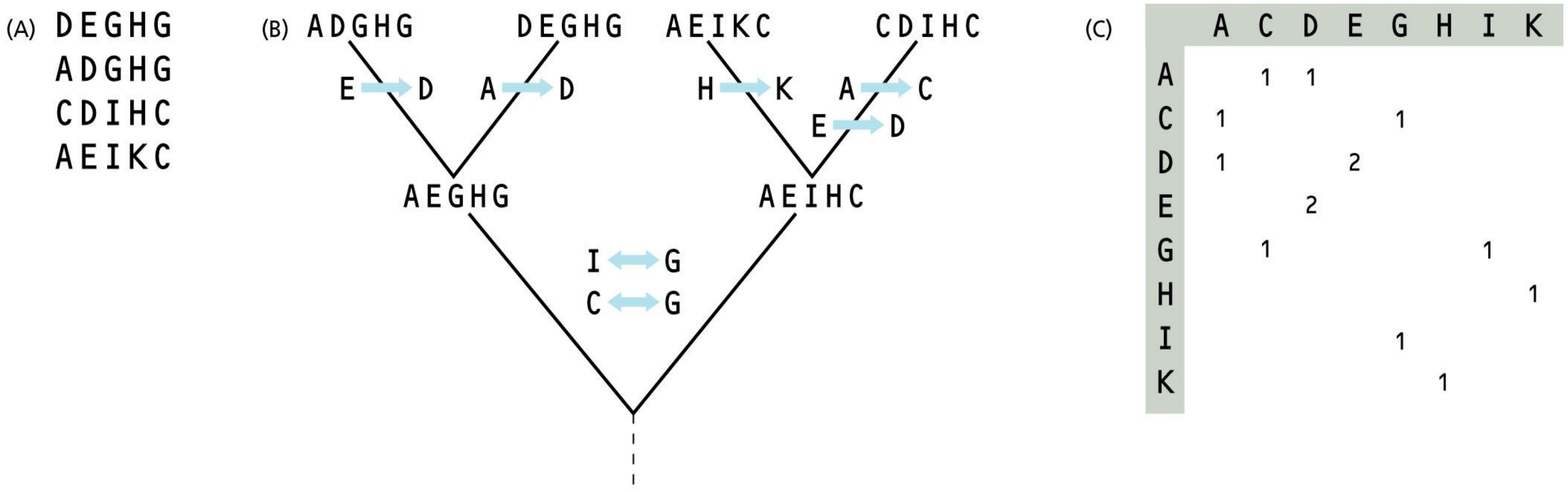
where f(i) and f(j) are the frequencies that amino acids A_i and A_j appear in the sequences, and f(i,j) the frequency that A_i and A_j are aligned.

PAM1 was manually constructed from sequences that are highly similar (one mutation per 100 amino acids, to be exact) and therefore are easily aligned.

Assuming constant mutation rate, PAMn is constructed by multiplying PAM1 to itself n times. E.g.,

$$\text{PAM50} = \underbrace{\text{PAM1 x PAM1 x} \ldots \text{x PAM1}}_{\text{50 times}}.$$

Schematic illustration of constructing substitution score matrix


(A)

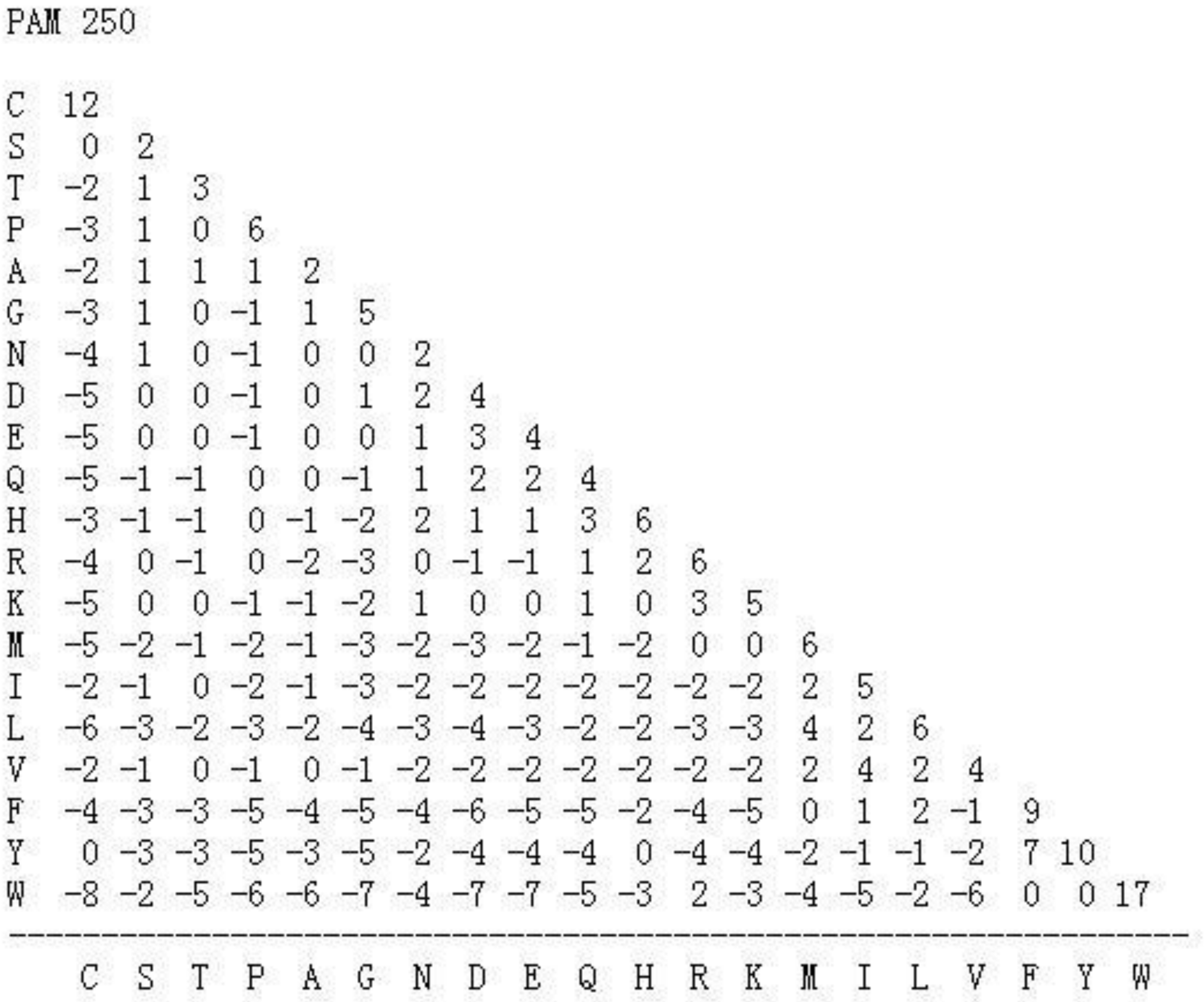
DEGHG
ADGHG
CDIHC
AEIKC

(B)

(C)

	A	C	D	E	G	H	I	K
A		1	1					
C	1				1			
D	1			2				
E			2					
G		1					1	
H								1
I					1			
K						1		

$$p_{ij} = \log[f(i,j)/f(i)f(j)]$$



PAM 250

C	12																			
S	0	2																		
T	-2	1	3																	
P	-3	1	0	6																
A	-2	1	1	1	2															
G	-3	1	0	-1	1	5														
N	-4	1	0	-1	0	0	2													
D	-5	0	0	-1	0	1	2	4												
E	-5	0	0	-1	0	0	1	3	4											
Q	-5	-1	-1	0	0	-1	1	2	2	4										
H	-3	-1	-1	0	-1	-2	2	1	1	3	6									
R	-4	0	-1	0	-2	-3	0	-1	-1	1	2	6								
K	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5							
M	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6						
I	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	5					
L	-6	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	6				
V	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4			
F	-4	-3	-3	-5	-4	-5	-4	-6	-5	-5	-2	-4	-5	0	1	2	-1	9		
Y	0	-3	-3	-5	-3	-5	-2	-4	-4	-4	0	-4	-4	-2	-1	-1	-2	7	10	
W	-8	-2	-5	-6	-6	-7	-4	-7	-7	-5	-3	2	-3	-4	-5	-2	-6	0	0	17
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

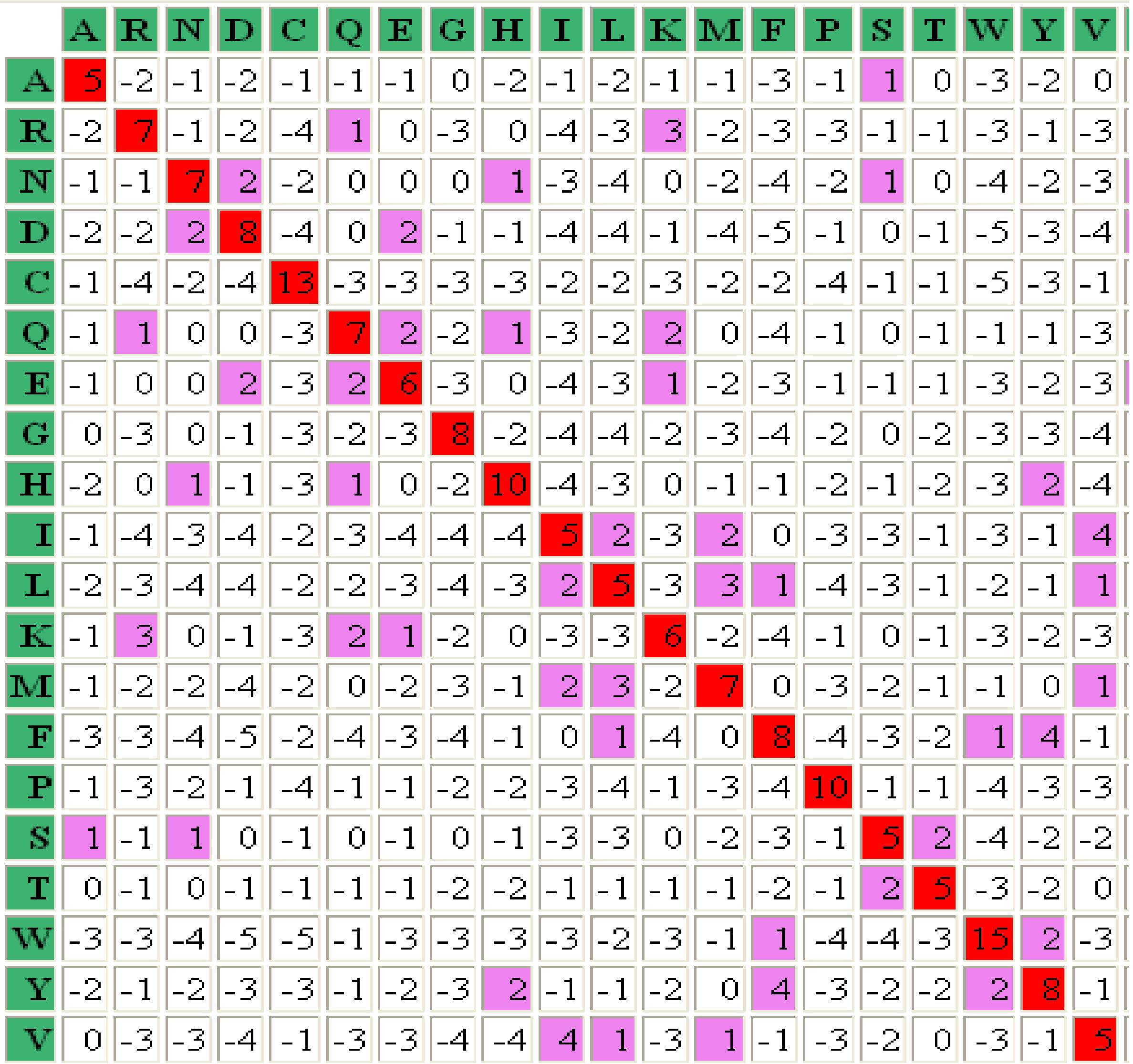
BLOSUM matrices [Steven and Jorja Henikoff]

- BLOSUM x matrix is a 20 by 20 matrix. Its elements are defined like those of PAM matrices but the frequencies are collected from sequences in BLOCKs database that are less than x percent identical (generally x is between 50 and 80).

- By their construction, BLOSUM matrices are believed to be more effectively detect distant homology.

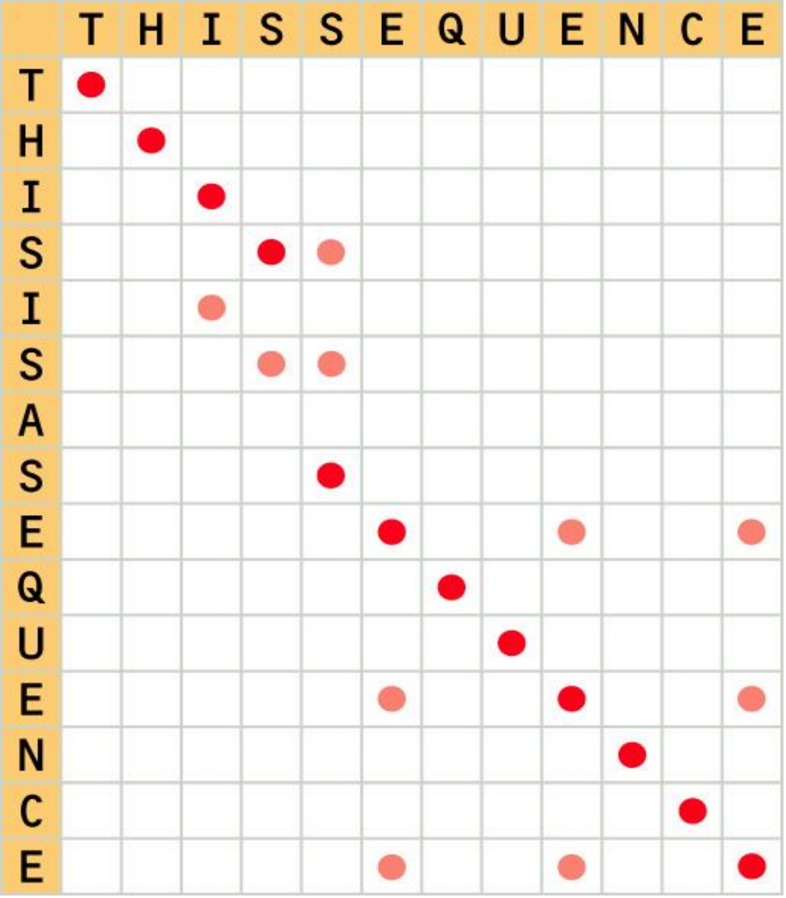
- Taking the place of PAM 250, BLOSUM 62 is now the default matrix used in database search.

BLOSUM50

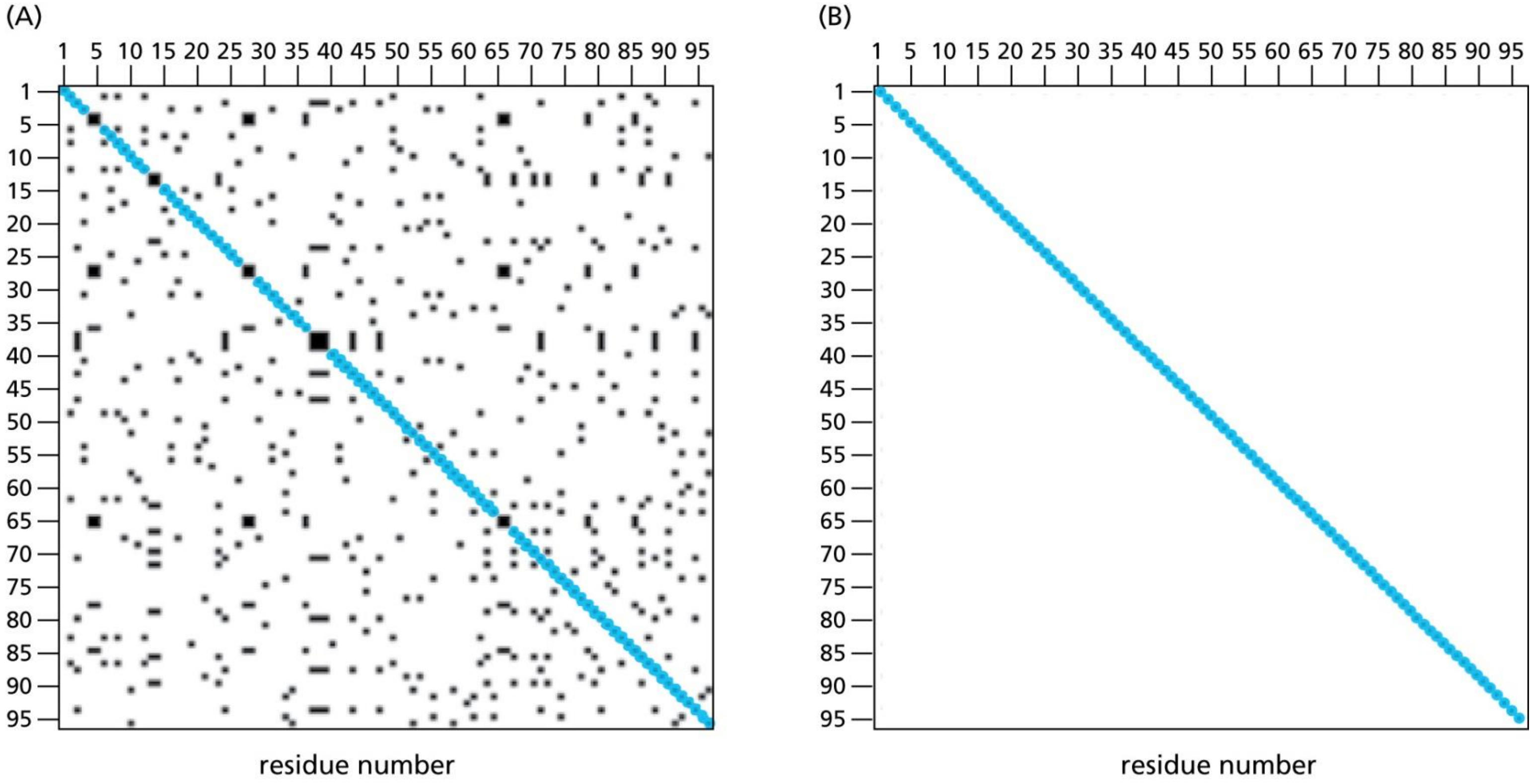


	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	2	5	-3	-2	0
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

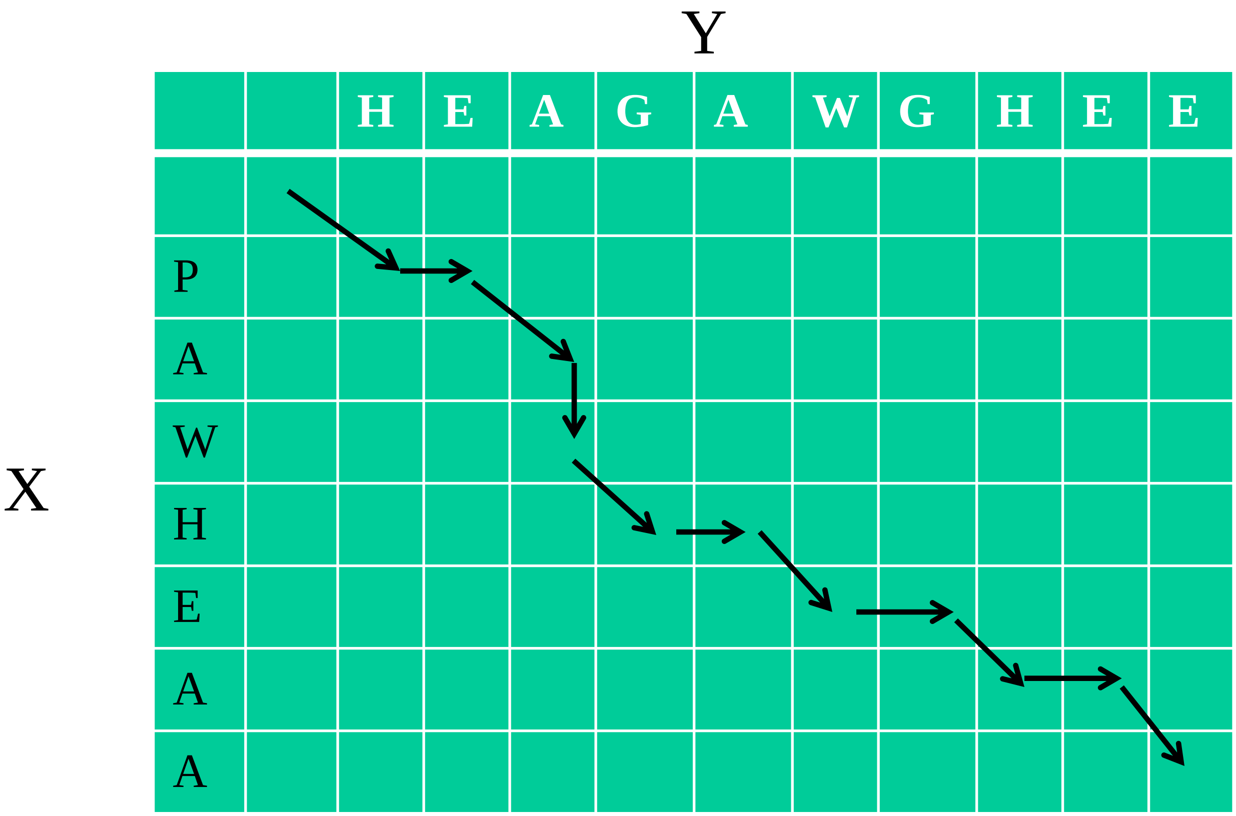
Dot plot



	T	H	I	S	S	E	Q	U	E	N	C	E
T	●											
H		●										
I			●									
S				●	○							
I			○									
S				○	○							
A												
S					●							
E						●			○			○
Q							●					
U								●				
E						○			●			○
N										●		
C											●	
E						○			○			●


(A)
1 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
residue number
(B)
1 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
1
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
residue number

Example: Align HEAGAWGHEE and PAWHEAE.



Any path from upper left corner to lower right corner gives rise to an alignment: diagonal step → align two letters; vertical step → align letter in sequence X to “-”; horizontal step → align letter in sequence Y to “-”

```
HEA-GAWGHEE
P-AWH-E-A-A
```

Example: Align HEAGAWGHEE and PAWHEAE.

```
HEA-GAWGHEE
P-AWH-E-A-A
```

Similarity measured using BLOSUM50 and gap penalty -8:

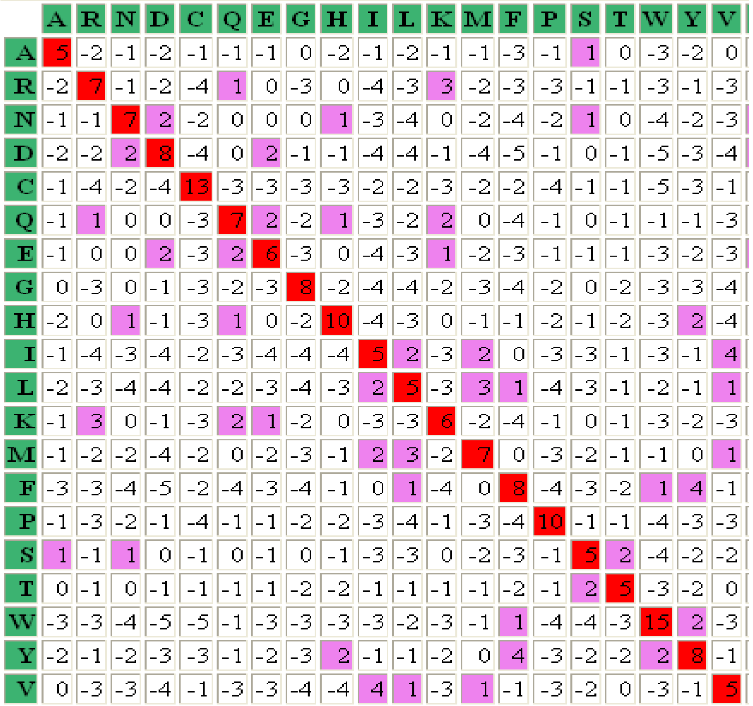
Score = S(H,P) + S(E,-) + S(A,A) + S(-,W) + S(G,H) + S(A, -) + S(W, E) + S(G,-)
+S(H,A) + S(E,-) + S(E,A)

= -2 -8 +5 -8 -2 -8 -3 -8 -2 -8 -1
= -46

How many possible alignments?

How to find the best alignment?

- brute-force
- Dynamic Programming



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	2	5	-3	-2	0
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

Needleman-Wunsch algorithm (Global Pairwise optimal alignment, 1970)

To align two sequences x[1...n] and y[1...m],

i) if x at *i* aligns with y at *j*, a score $s(x_i, y_j)$ is added; if either x_i or y_j is a gap, a score of d is subtracted (penalty).

ii) The ***best*** score up to (i,j) will be

$$F(i,j) = \max \{ F(i-1, j-1) + s(x_i, y_j),$$
$$F(i-1,j) - d, \quad // \text{ gap in y}$$
$$F(i, j-1) - d \quad // \text{ gap in x}$$
$$\}$$

Needleman-Wunsch (cont’d)

iii) Tabular computing to get F(i,j) for all 1<i<n and i<j<m

Draw a diagram:

$$\begin{tikzcd}
F(i-1, j-1) \arrow[dr, "s(x_i,y_j)"'] & F(i-1, j) \arrow[d, "-d"] \\
F(i, j-1) \arrow[r, "-d"'] & F(i,j)
\end{tikzcd}$$

By definition, F(n,m) gives the best score for an alignment of x[1...n] and y[1...m].

iv) Trace-back

To find the alignment itself, we must find the path of choices (in applying the formulae of ii) when tabular computing that led to this final value.

> Vertical move is gap in the column sequence.
> Horizontal move is gap in the row sequence.
> Diagonal move is a match.

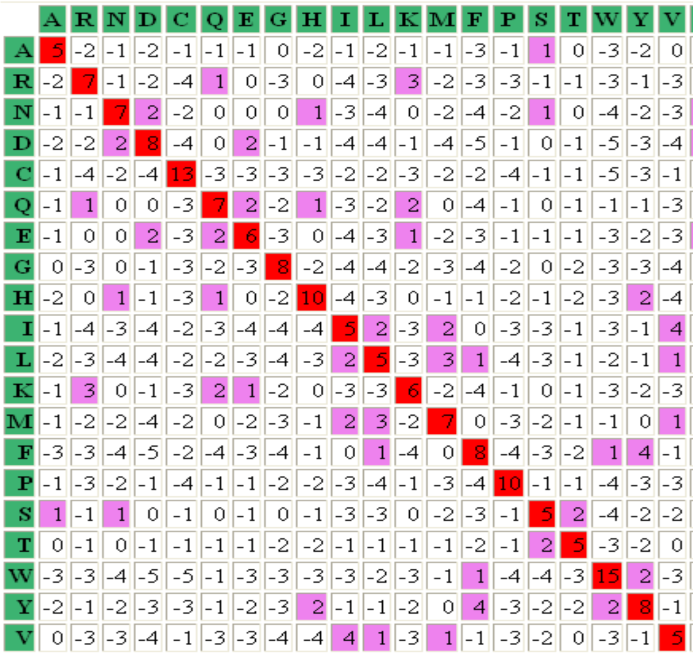
Example: Align HEAGAWGHEE and PAWHEAE.

Use BLOSUM 50 for substitution matrix and d=-8 for gap penalty.

		H	E	A	G	A	W	G	H	E	E
	0	**-8**	**-16**	-24	-32	-40	-48	-56	-64	-72	-80
P	-8	-2	-9	**-17**	**-25**	-33	-42	-49	-57	-65	-73
A	-16	-10	-3	-4	-12	**-20**	-28	-36	-44	-52	-60
W	-24	-18	-11	-6	-7	-15	**-5**	**-13**	-21	-29	-37
H	-32	-14	-18	-13	-8	-9	-13	-7	**-3**	-11	-19
E	-40	-22	-8	-16	-16	-9	-12	-15	-7	**3**	-5
A	-48	-30	-16	-3	-11	-11	-12	-12	-15	**-5**	2
E	-56	-38	-24	-11	-6	-12	-14	-15	-12	-9	**1**

HEAGAWGHE-E

--P-AW-HEAE



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	2	5	-3	-2	0
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

Time complexity: $O(nm)$

Space complexity: $O(nm)$

Big-O notation:

$f(x) = O(g(x))$ => f is upper bound by g

$f(x) = \Omega(g(x))$ => f is lower bound by g

$f(x) = \Theta(g(x))$ => f is bound to g within constant factors