Sequence alignment

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Content

➡ 1. Why to make sequence alignment?
2. What is a sequence alignment?
3. How to derive a mutation matrix-PAM
4. How to derive a mutation matrix-BLOSUM
5. Gap penalty
6. Dynamic programming
    a. Global alignment: Needleman-Wunsch
    b. Local alignment: Smith-Waterman
7. Heuristic algorithms

Why to make sequence alignment?

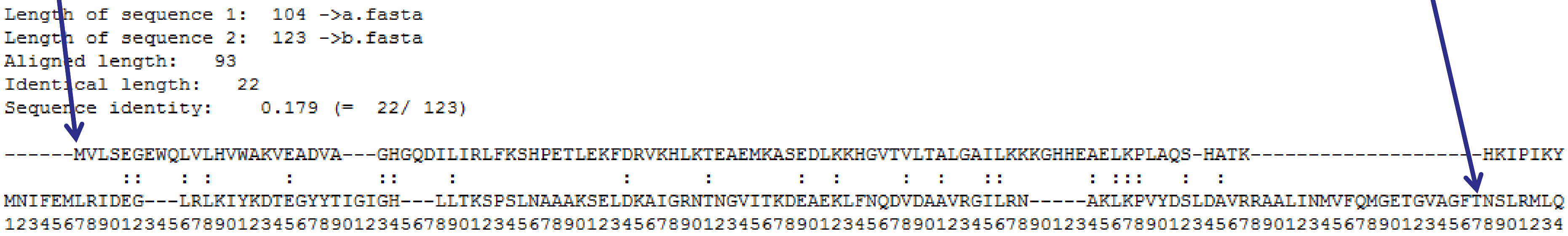
>Protein a

MVLSEGEWQLVLHVWAKVEADVAGHGQD
ILIRLFKSHPETLEKFDRVKHLKTEAEMKAS
EDLKKHGVTVLTALGAILKKKGHHEAELKP
LAQSHATKHKIPIKY

>Protein b

MNIFEMLRIDEGLRLKIYKDTEGYYTIGIGHLLTKSPS
LNAAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDA
AVRGILRNAKLKPVYDSLDAVRRAALINMVFQMGET
GVAGFTNSLRMLQ

Do they have similar structure and function?



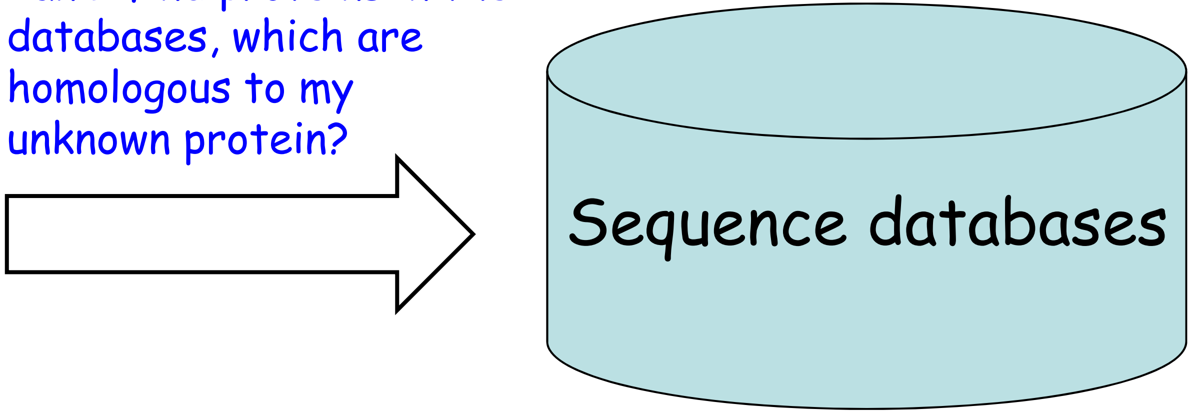
```
Length of sequence 1:  104 ->a.fasta
Length of sequence 2:  123 ->b.fasta
Aligned length:   93
Identical length:   22
Sequence identity:    0.179 (=  22/ 123)

------MVLSEGEWQLVLHVWAKVEADVA---GHGQDILIRLFKSHPETLEKFDRVKHLKTEAEMKASEDLKKHGVTVLTALGAILKKKGHHEAELKPLAQS-HATK--------------------HKIPIKY
          ::   : :      :       ::    :              :      :       :  :     :  :   ::       : :::   :  :
MNIFEMLRIDEG---LRLKIYKDTEGYYTIGIGH---LLTKSPSLNAAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDAAVRGILRN-----AKLKPVYDSLDAVRRAALINMVFQMGETGVAGFTNSLRMLQ
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901234
```

I. Sequence alignment can help establish relationship of two proteins (roughly speaking, sequences having higher sequence identity usually come from the same ancestor and therefore have similar structure and function).
These proteins are called homology.

Why to make sequence alignment?

>Query sequence

MVLSEGEWQLVLHVWAKVEADVAGHGQD
ILIRLFKSHPETLEKFDRVKHLKTEAEMKAS
EDLKKHGVTVLTALGAILKKKGHHEAELKP
LAQSHATKHKIPIKY



Can I find proteins in the databases, which are homologous to my unknown protein?

Sequence databases

(GeneBank for DNA sequences)
(UniProt for protein sequences)
(PDB for protein structures)

II. Sequence alignment can help identify homologies from known databases, to generate structure and function predictions for the unknown proteins.

Why to make sequence alignment?

Many bioinformatics databases:

1. **GeneBank**: contains ~950M DNA sequences
2. **UniProt Swiss-Prot/trEMBL**: ~100M protein sequences (~550K with known function)
3. **Protein Data Bank (PDB)**: contains ~140k protein structures

Summary

Purposes:

- Study the relationship between two proteins
- Scan a database with a query sequence and identify possible structure and function of the query protein

If two sequences are simiar, the following may be true

- The proteins may share a common evolutionary origin
- The proteins may have a similar 3-dimensional structure
- The proteins may have the same or related function

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What is a sequence alignment?

Example 1: Sequence identity=78%

Identical residue pair

```
-MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRVKHLKTEAEMKASEDLKKHGVTVL
   :: :::: :: :: ::::: :::::  :::::  :::::::::  ::::::::::::::::: :  ::
G--LSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEMKASEDLKKTGTVVL
```

Insertions

Example 2: Sequence identity=22%

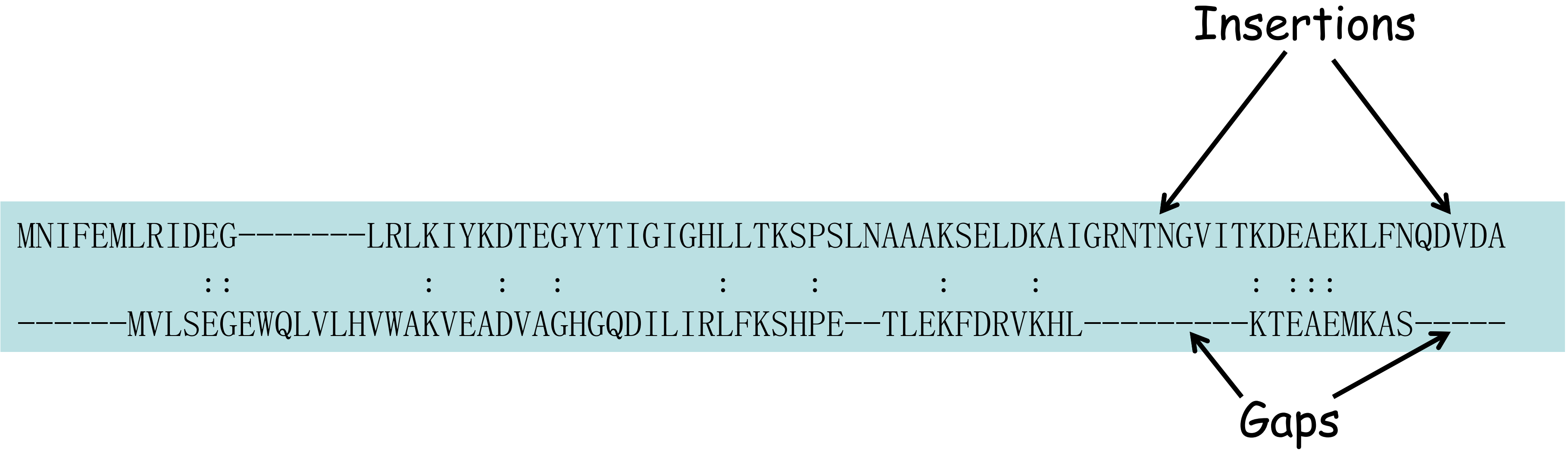
```
MNIFEMLRIDEG-------LRLKIYKDTEGYYTIGIGHLLTKSPSLNAAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDA
          ::          :   :  :        :    :      :    :           : :::
------MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPE--TLEKFDRVKHL---------KTEAEMKAS-----
```

Gaps

Sequence identity = Number of identical residue pairs/Length of query sequence

The principle of an alignment

- We want to align as many as possible THE SAME or THE SIMILAR residues
- We do not want gaps/insertions



```
MNIFEMLRIDEG-------LRLKIYKDTEGYYTIGIGHLLTKSPSLNAAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDA
          ::          :   :  :        :    :      :    :           : :::
------MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPE--TLEKFDRVKHL---------KTEAEMKAS-----
```

The principle of an alignment

Mathematically, the goal is to maximize the following score:

$$Score = \sum_{i=1}^{N_{ali}} M(A_i, B_i) - GapPenalty$$

Residues of similar property should match together

Score for adding gap is always negative

A_i

```
MNIFEMLRIDEG-------LRLKIYKDTEGYYTIGIGHLLTKSPSLNAAAKSELDKAIGRNTNGVITKDEAEKLFNQDVDA
          ::          :   :  :        :    :      :    :           : :::
------MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPE--TLEKFDRVKHL---------KTEAEMKAS-----
```

B_i

Gaps

N_{ali}: number of aligned residue pairs

A_i: amino acid identity of the i-th aligned resideu at the first sequence

B_i: amino acid identity of the i-th aligned resideu at the second sequence

$M(A_{i,} B_i)$: preference score of matching between amino acids A_i and B_i

Scoring matrix

$$Score = \sum_{i=1}^{N_{ali}} M(A_i, B_i) - GapPenalty$$

The simplest scoring matrix is the unit matrix:

$$M = \begin{bmatrix} 1 & 0 & \cdots & 0 \\ 0 & 1 & \cdots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \cdots & 1 \end{bmatrix}_{20\times 20}$$

Question: What will be the problem if we use this simple solution?

Answer: All the similarity due to the evolutionary mutation has been neglected.

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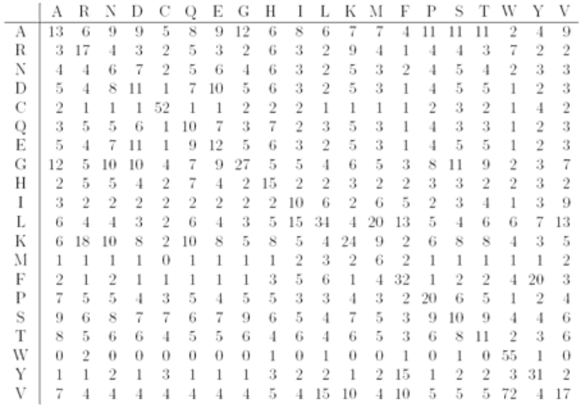
The most often-used scoring matrices

PAM250

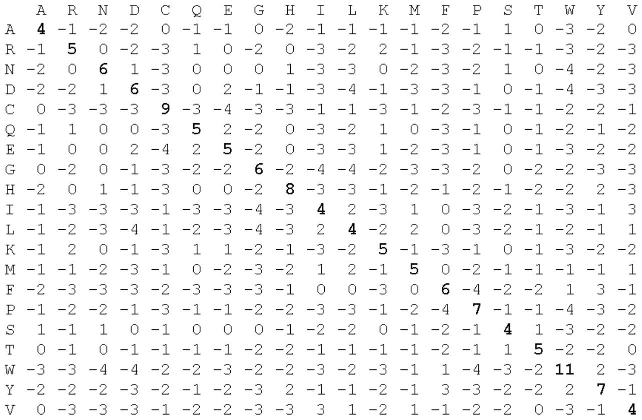
DAYHOFF et al, 1978



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	13	6	9	9	5	8	9	12	6	8	6	7	7	4	11	11	11	2	4	9
R	3	17	4	3	2	5	3	2	6	3	2	9	4	1	4	4	3	7	2	2
N	4	4	6	7	2	5	6	4	6	3	2	5	3	2	4	5	4	2	3	3
D	5	4	8	11	1	7	10	5	6	3	2	5	3	1	4	5	5	1	2	3
C	2	1	1	1	52	1	1	2	2	2	1	1	1	1	2	3	2	1	4	2
Q	3	5	5	6	1	10	7	3	7	2	3	5	3	1	4	3	3	1	2	3
E	5	4	7	11	1	9	12	5	6	3	2	5	3	1	4	5	5	1	2	3
G	12	5	10	10	4	7	9	27	5	5	4	6	5	3	8	11	9	2	3	7
H	2	5	5	4	2	7	4	2	15	2	2	3	2	2	3	3	2	2	3	2
I	3	2	2	2	2	2	2	2	2	10	6	2	6	5	2	3	4	1	3	9
L	6	4	4	3	2	6	4	3	5	15	34	4	20	13	5	4	6	6	7	13
K	6	18	10	8	2	10	8	5	8	5	4	24	9	2	6	8	8	4	3	5
M	1	1	1	1	0	1	1	1	1	2	3	2	6	2	1	1	1	1	1	2
F	2	1	2	1	1	1	1	1	3	5	6	1	4	32	1	2	2	4	20	3
P	7	5	5	4	3	5	4	5	5	3	3	4	3	2	20	6	5	1	2	4
S	9	6	8	7	7	6	7	9	6	5	4	7	5	3	9	10	9	4	4	6
T	8	5	6	6	4	5	5	6	4	6	4	6	5	3	6	8	11	2	3	6
W	0	2	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	55	1	0
Y	1	1	2	1	3	1	1	1	3	2	2	1	2	15	1	2	2	3	31	2
V	7	4	4	4	4	4	4	4	5	4	15	10	4	10	5	5	5	72	4	17

BLOSUM62

Henikoff and Henikoff, PNAS, 1992



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**4**	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	**5**	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	**6**	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	**6**	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	**9**	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	**5**	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	**5**	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	**6**	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	**8**	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	**4**	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	**4**	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	**5**	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	**5**	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	**6**	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	**7**	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	**4**	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	**5**	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	**11**	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	**7**	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	**4**

Questions:

1. How these matrices are obtained?
2. What are the differences between PAM and BLOSUM?

Margaret Dayhoff (1925 - 1983, US)

1945 - BA in Mathematics at NYU
1948 - PhD in Quantum Chemistry

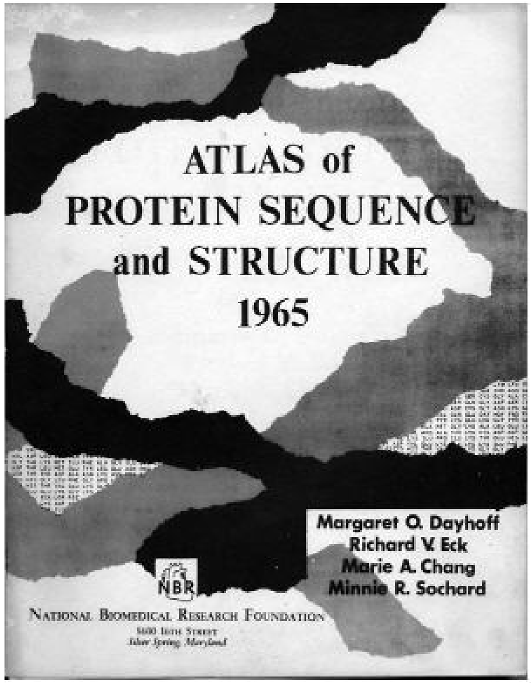
1965 - Protein Atlas (65 proteins) (PIR)

the first public comprehensive, computerised and publicly available database of protein sequences. It is the model for GenBank and many other molecular databases.

1980 - President of Biophysical Society

one of the founders in the field of Bioinformatics

Margaret Oakley Dayhoff Award

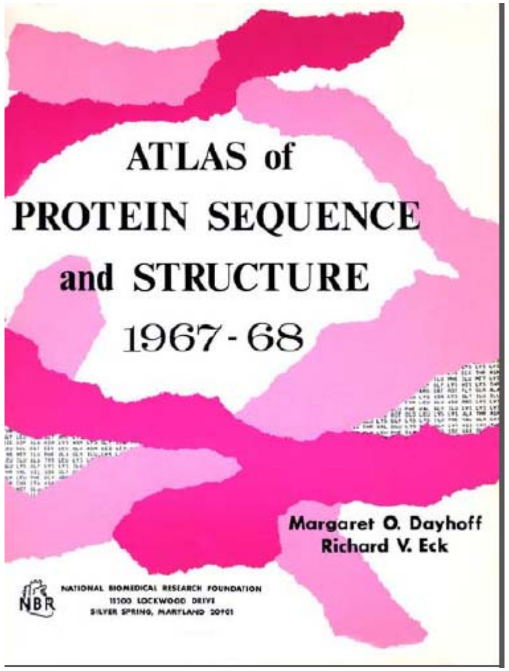


ATLAS of
PROTEIN SEQUENCE
and STRUCTURE
1965

Margaret O. Dayhoff
Richard V. Eck
Marie A. Chang
Minnie R. Sochard

NBR

National Biomedical Research Foundation
8600 16th Street
Silver Spring, Maryland



ATLAS of
PROTEIN SEQUENCE
and STRUCTURE
1967-68

Margaret O. Dayhoff
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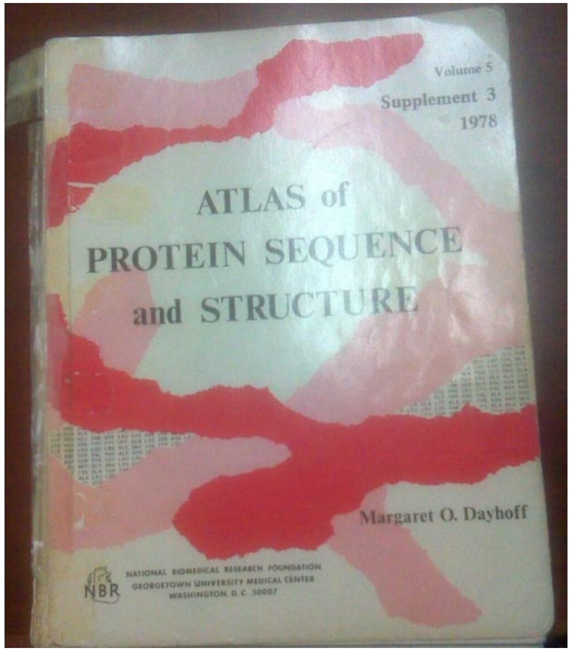
SILVER SPRING, MARYLAND 20901



Volume 5
Supplement 3
1978

ATLAS of
PROTEIN SEQUENCE
and STRUCTURE

Margaret O. Dayhoff

NBR

NATIONAL BIOMEDICAL RESEARCH FOUNDATION
GEORGETOWN UNIVERSITY MEDICAL CENTER
WASHINGTON, D. C. 20007

Scoring matrix PAM

PAM (Percent Accepted Mutation) Matrix (by Dayhoff et al 1978):

- **Reference:** DAYHOFF, M., R. SCHWARTZ, AND B. ORCUTT. 1978. A model of evolutionary change in proteins. Pages 345--352 in Atlas of protein sequence and structure, Volume 5 (M. Dayhoff, ed.). National Biomedical Research Foundation, Washington, D.C.

- **Database**: 1,572 mutations, 71 homologous sequence groups (trees), 34 superfamilies, minimum sequence identity is 85%

- **Purpose:** to derive the mutation probability between amino acids

Scoring matrix PAM

Three steps for building the PAM matrix:

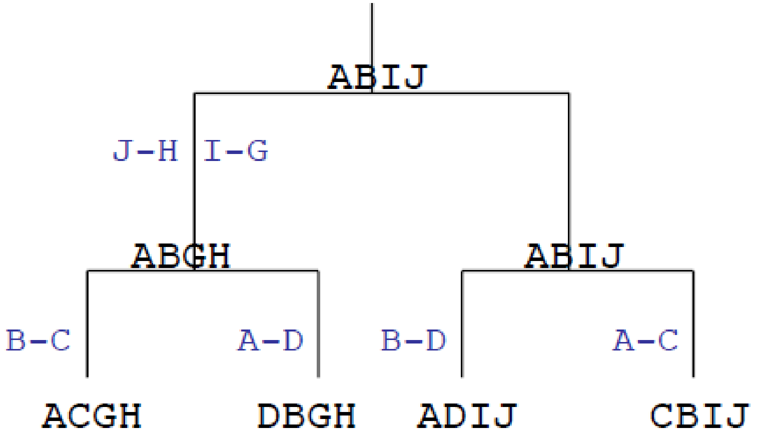
Step 1: Counting the number of mutations

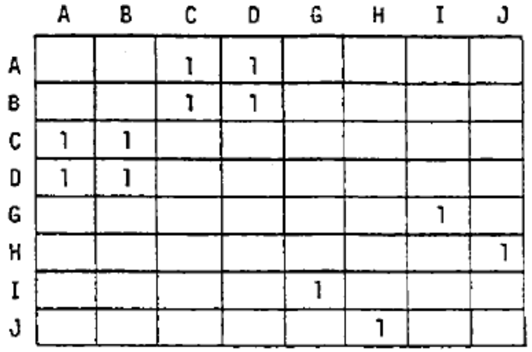
Step 2: Relative mutability of amino acid

Step 3: Probability of mutations between amino acids (M_{ij})

Scoring matrix PAM

Step 1: Counting the number of mutations



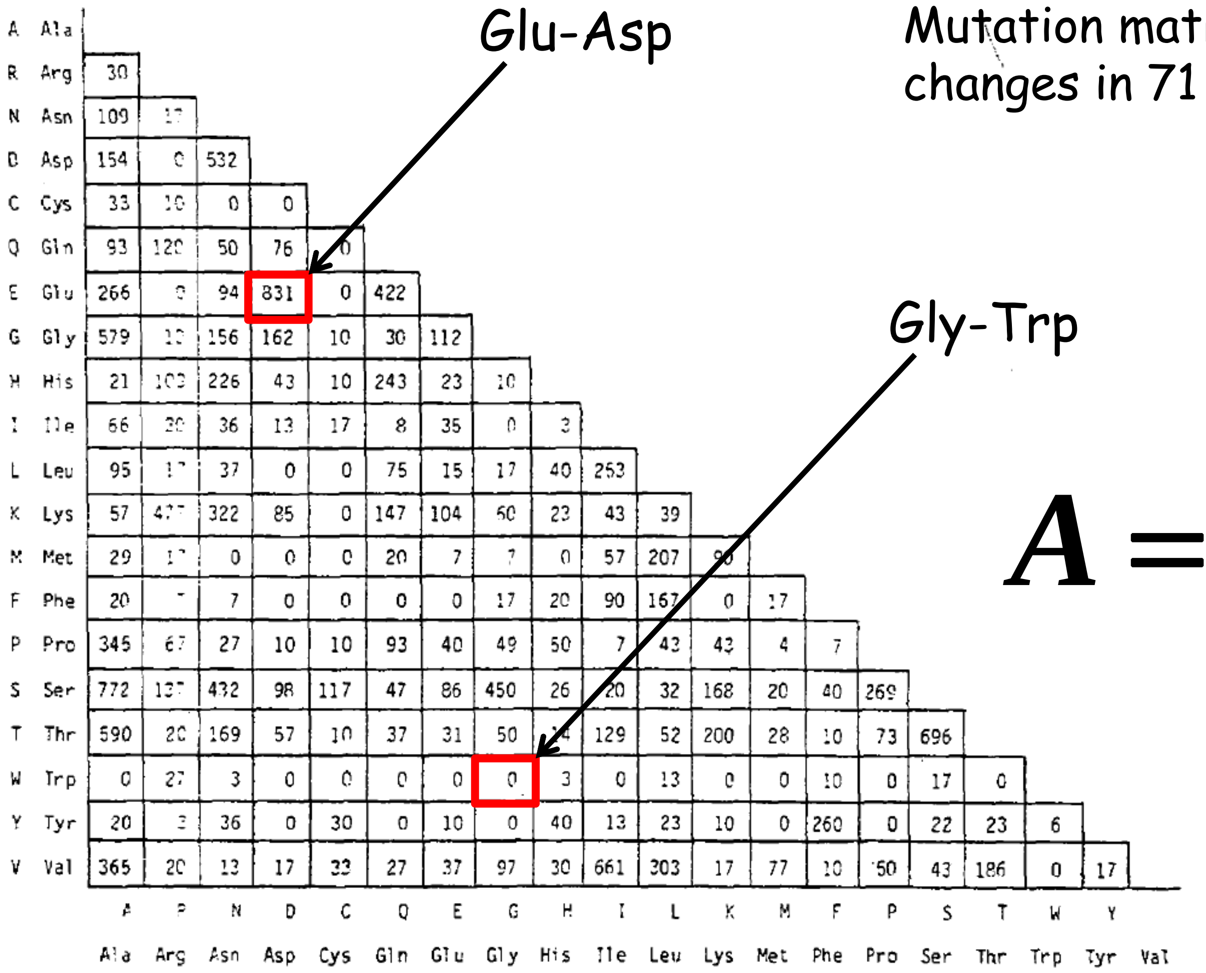


	A	B	C	D	G	H	I	J
A			1	1				
B			1	1				
C	1	1						
D	1	1						
G							1	
H								1
I					1			
J						1		

Figure 79. Matrix of accepted point mutations derived from the tree of Figure 78.

Scoring matrix PAM

Mutation matrix from 1572 changes in 71 groups (id>85%)



Glu-Asp

Gly-Trp

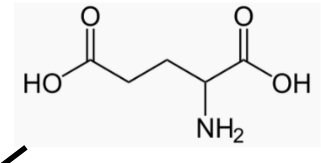
A	Ala																				
R	Arg	30																			
N	Asn	109	17																		
D	Asp	154	0	532																	
C	Cys	33	10	0	0																
Q	Gln	93	120	50	76	0															
E	Glu	266	0	94	831	0	422														
G	Gly	579	10	156	162	10	30	112													
H	His	21	103	226	43	10	243	23	10												
I	Ile	66	30	36	13	17	8	35	0	3											
L	Leu	95	17	37	0	0	75	15	17	40	253										
K	Lys	57	477	322	85	0	147	104	60	23	43	39									
M	Met	29	17	0	0	0	20	7	7	0	57	207	90								
F	Phe	20	[illegible]	7	0	0	0	0	17	20	90	167	0	17							
P	Pro	345	67	27	10	10	93	40	49	50	7	43	43	4	7						
S	Ser	772	137	432	98	117	47	86	450	26	20	32	168	20	40	269					
T	Thr	590	20	169	57	10	37	31	50	14	129	52	200	28	10	73	696				
W	Trp	0	27	3	0	0	0	0	0	3	0	13	0	0	10	0	17	0			
Y	Tyr	20	3	36	0	30	0	10	0	40	13	23	10	0	260	0	22	23	6		
V	Val	365	20	13	17	33	27	37	97	30	661	303	17	77	10	50	43	186	0	17	
		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	
		Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val

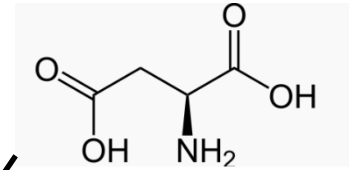
Figure 80. Numbers of accepted point mutations (X 10) accumulated from closely related sequences. Fifteen hundred and seventy-two exchanges are shown. Fractional exchanges result when ancestral sequences are ambiguous.

$$A = (A_{ij})$$

Scoring matrix PAM

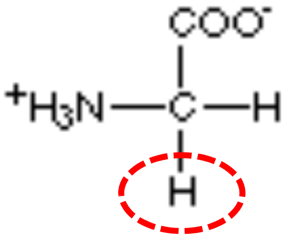
Two factors may influence the mutation numbers:



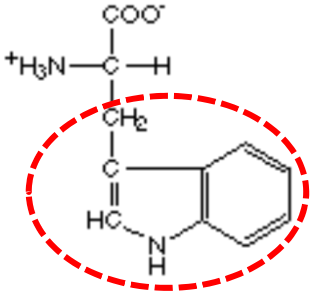


- Codon reason: mutation between Glu (=GAA, GAG) and Asp (=GAC, GAU) is the most frequent

- Physical reason: due to the volume difference, mutation between Gly (=GGG) and Trp (=UGG) never happens



Glycine
(gly)



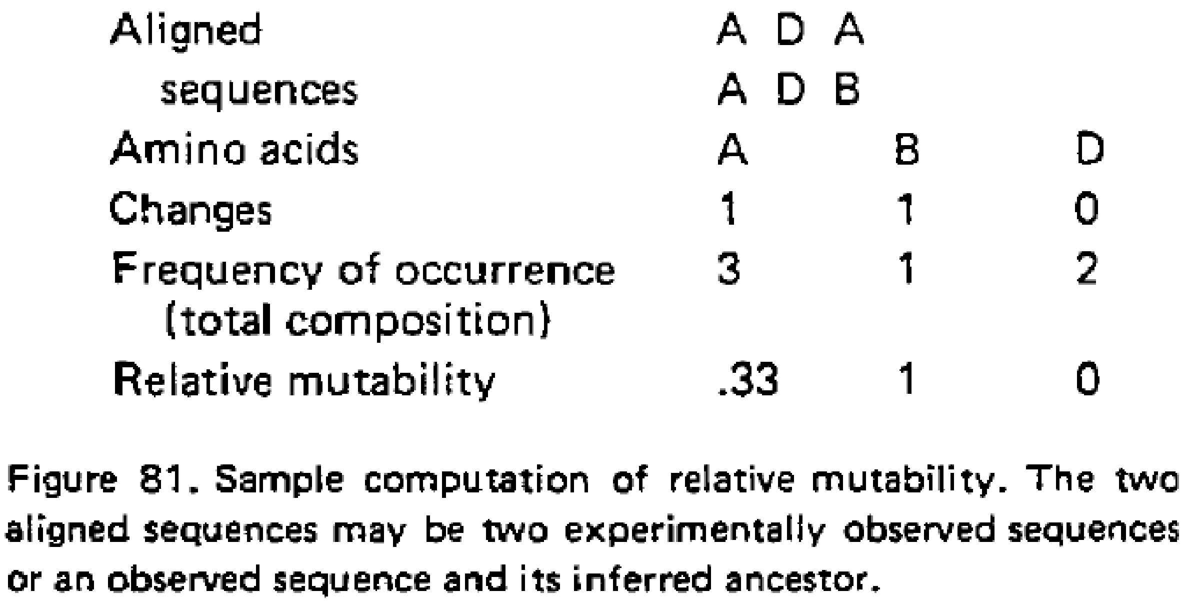
Tryptophan
(trp)

Scoring matrix PAM

Step 2: Relative mutability of amino acid

$$m_i = \frac{N_{mut}(i)}{N_{comp}(i)}, i = 1, 2, \cdots, 20$$

Example:



Aligned sequences	A D A A D B		
Amino acids	A	B	D
Changes	1	1	0
Frequency of occurrence (total composition)	3	1	2
Relative mutability	.33	1	0

Figure 81. Sample computation of relative mutability. The two aligned sequences may be two experimentally observed sequences or an observed sequence and its inferred ancestor.

Scoring matrix PAM

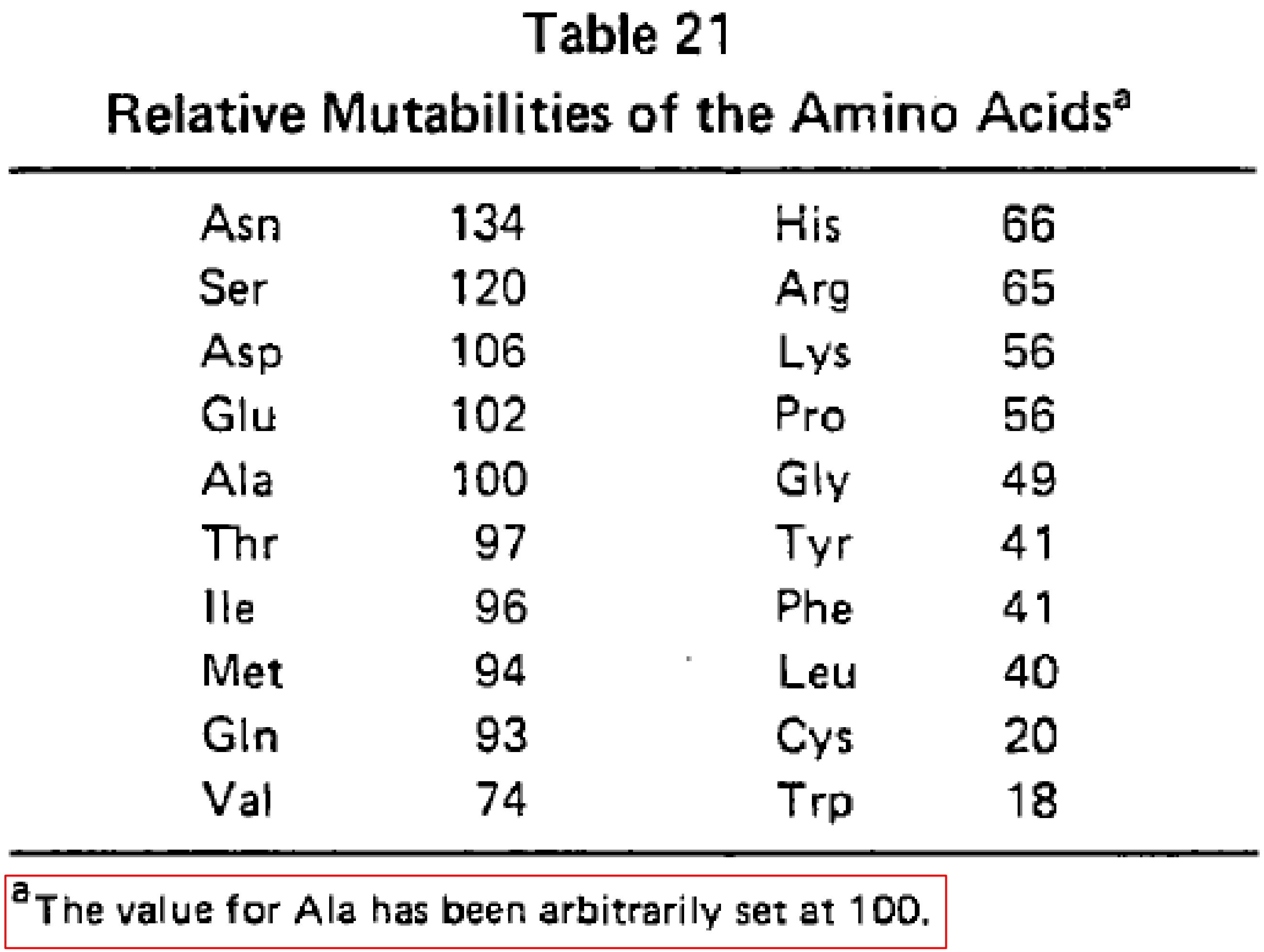


Table 21

Relative Mutabilities of the Amino Acids[a]

Asn	134	His	66
Ser	120	Arg	65
Asp	106	Lys	56
Glu	102	Pro	56
Ala	100	Gly	49
Thr	97	Tyr	41
Ile	96	Phe	41
Met	94	Leu	40
Gln	93	Cys	20
Val	74	Trp	18

[a] The value for Ala has been arbitrarily set at 100.

Scoring matrix PAM

Step 3: Probability of mutations between amino acids (M_{ij})
: probability of *j* being replaced by *i*

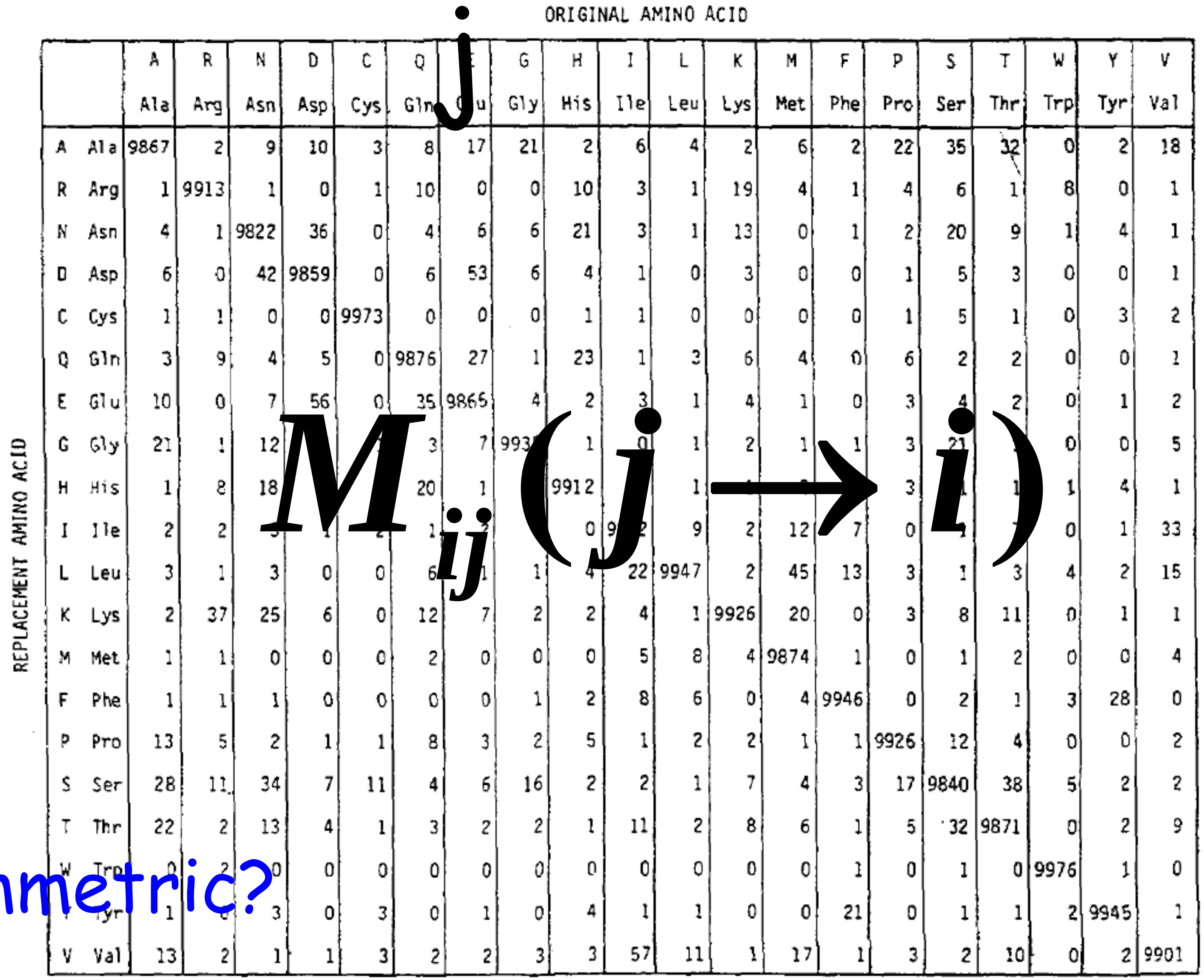
$$M_{ij} = \begin{cases} \lambda \dfrac{m_j A_{ij}}{\sum_{k \neq i} A_{kj}}, & 1 \le i, j \le 20; \quad i \neq j \\ 1 - \lambda m_j, & i = j \end{cases}$$

A_{ij}: Observed number of mutations between a_i and a_j

m_j: Relative mutate probability of a_j to all other amino acids

λ: A constant to decide the evolution distance

PAM1



j

ORIGINAL AMINO ACID

i

REPLACEMENT AMINO ACID

		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
		Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
A	Ala	9867	2	9	10	3	8	17	21	2	6	4	2	6	2	22	35	32	0	2	18
R	Arg	1	9913	1	0	1	10	0	0	10	3	1	19	4	1	4	6	1	8	0	1
N	Asn	4	1	9822	36	0	4	6	6	21	3	1	13	0	1	2	20	9	1	4	1
D	Asp	6	0	42	9859	0	6	53	6	4	1	0	3	0	0	1	5	3	0	0	1
C	Cys	1	1	0	0	9973	0	0	0	1	1	0	0	0	0	1	5	1	0	3	2
Q	Gln	3	9	4	5	0	9876	27	1	23	1	3	6	4	0	6	2	2	0	0	1
E	Glu	10	0	7	56	0	35	9865	4	2	3	1	4	1	0	3	4	2	0	1	2
G	Gly	21	1	12	[illegible]	[illegible]	3	7	9935	1	0	1	2	1	1	3	21	[illegible]	0	0	5
H	His	1	8	18	[illegible]	[illegible]	20	1	[illegible]	9912	[illegible]	1	[illegible]	[illegible]	[illegible]	3	1	1	1	4	1
I	Ile	2	2	3	1	2	1	2	[illegible]	0	[illegible]	9	2	12	7	0	[illegible]	[illegible]	0	1	33
L	Leu	3	1	3	0	0	6	1	1	4	22	9947	2	45	13	3	1	3	4	2	15
K	Lys	2	37	25	6	0	12	7	2	2	4	1	9926	20	0	3	8	11	0	1	1
M	Met	1	1	0	0	0	2	0	0	0	5	8	4	9874	1	0	1	2	0	0	4
F	Phe	1	1	1	0	0	0	0	1	2	8	6	0	4	9946	0	2	1	3	28	0
P	Pro	13	5	2	1	1	8	3	2	5	1	2	2	1	1	9926	12	4	0	0	2
S	Ser	28	11	34	7	11	4	6	16	2	2	1	7	4	3	17	9840	38	5	2	2
T	Thr	22	2	13	4	1	3	2	2	1	11	2	8	6	1	5	32	9871	0	2	9
W	Trp	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	9976	1	0
Y	Tyr	1	0	3	0	3	0	1	0	4	1	1	0	0	21	0	1	1	2	9945	1
V	Val	13	2	1	1	3	2	2	3	3	57	11	1	17	1	3	2	10	0	2	9901

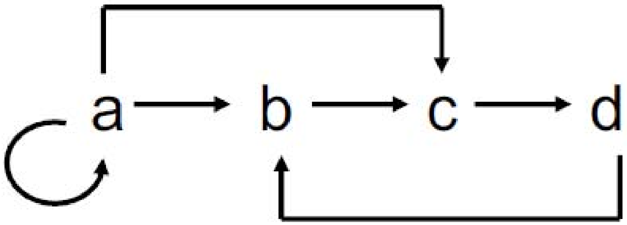
$$M_{ij}(j \rightarrow i)$$

Asymmetric?

For clarity, the values have been multiplied by 10000

From PAM1 to PAM2, PAM100, PAM250, etc…

Remark (from graph theory)



	a	b	c	d
a	1	1	1	0
b	0	0	1	0
c	0	0	0	1
d	0	1	0	0

Matrix **Q** indicates the number of paths going from one node to another in 1 step

	a	b	c	d
a	1	1	2	1
b	0	0	0	1
c	0	1	0	1
d	0	1	1	1

Matrix **Q**^{2} indicates the number of paths going from one node to another in 2 steps

	a	b	c	d
a	…	…	…	…
b	…	…	…	…
c	…	…	…	…
d	…	…	…	…

Matrix **Q**^{n} indicates the number of paths going from one node to another in *n* steps

Source: J. van Helden

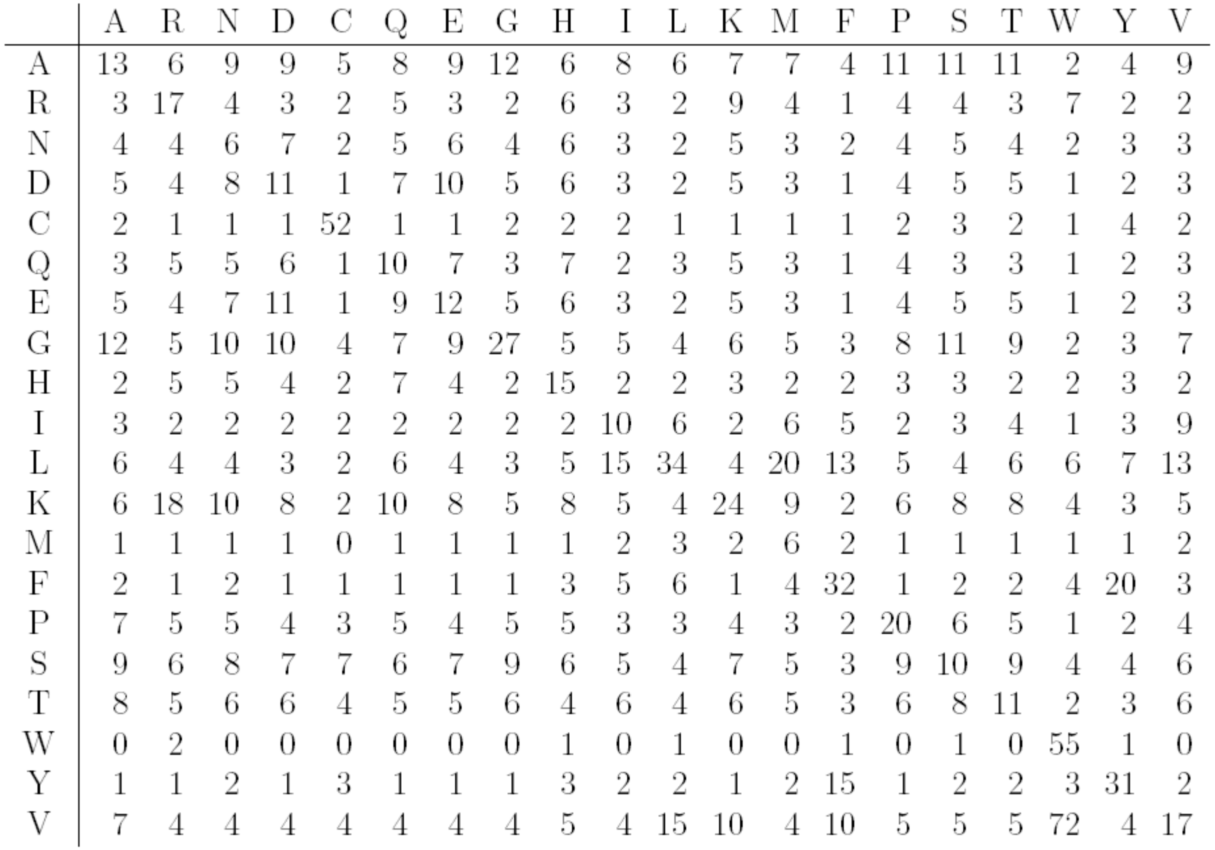
From PAM1 to PAM2, PAM100, PAM250, etc...

$\text{PAM2} = \text{PAM1}^{2}$

$\text{PAM100} = \text{PAM1}^{100}$

$\text{PAM200} = \text{PAM1}^{250}$

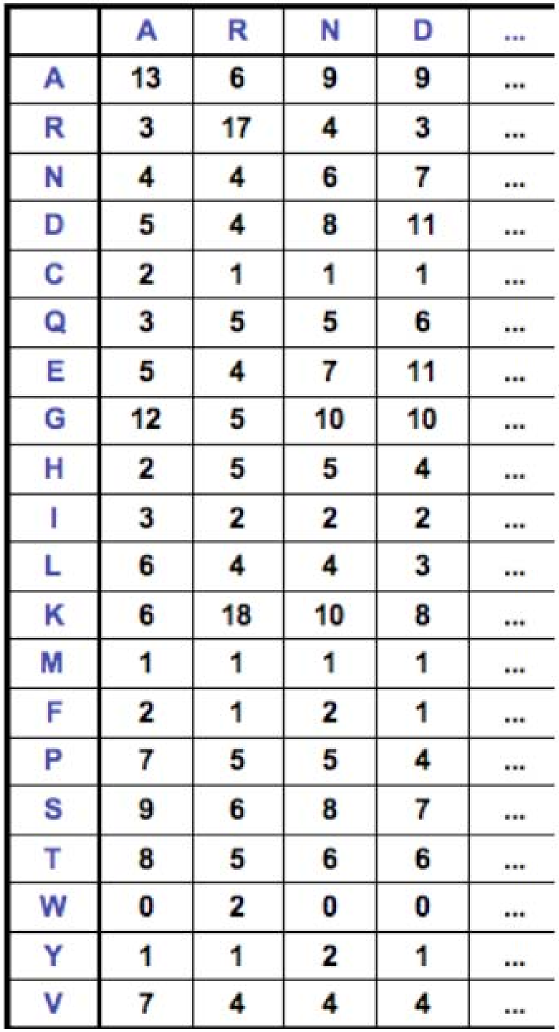
PAM250



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	13	6	9	9	5	8	9	12	6	8	6	7	7	4	11	11	11	2	4	9
R	3	17	4	3	2	5	3	2	6	3	2	9	4	1	4	4	3	7	2	2
N	4	4	6	7	2	5	6	4	6	3	2	5	3	2	4	5	4	2	3	3
D	5	4	8	11	1	7	10	5	6	3	2	5	3	1	4	5	5	1	2	3
C	2	1	1	1	52	1	1	2	2	2	1	1	1	1	2	3	2	1	4	2
Q	3	5	5	6	1	10	7	3	7	2	3	5	3	1	4	3	3	1	2	3
E	5	4	7	11	1	9	12	5	6	3	2	5	3	1	4	5	5	1	2	3
G	12	5	10	10	4	7	9	27	5	5	4	6	5	3	8	11	9	2	3	7
H	2	5	5	4	2	7	4	2	15	2	2	3	2	2	3	3	2	2	3	2
I	3	2	2	2	2	2	2	2	2	10	6	2	6	5	2	3	4	1	3	9
L	6	4	4	3	2	6	4	3	5	15	34	4	20	13	5	4	6	6	7	13
K	6	18	10	8	2	10	8	5	8	5	4	24	9	2	6	8	8	4	3	5
M	1	1	1	1	0	1	1	1	1	2	3	2	6	2	1	1	1	1	1	2
F	2	1	2	1	1	1	1	1	3	5	6	1	4	32	1	2	2	4	20	3
P	7	5	5	4	3	5	4	5	5	3	3	4	3	2	20	6	5	1	2	4
S	9	6	8	7	7	6	7	9	6	5	4	7	5	3	9	10	9	4	4	6
T	8	5	6	6	4	5	5	6	4	6	4	6	5	3	6	8	11	2	3	6
W	0	2	0	0	0	0	0	0	1	0	1	0	0	1	0	1	0	55	1	0
Y	1	1	2	1	3	1	1	1	3	2	2	1	2	15	1	2	2	3	31	2
V	7	4	4	4	4	4	4	4	5	4	15	10	4	10	5	5	5	72	4	17

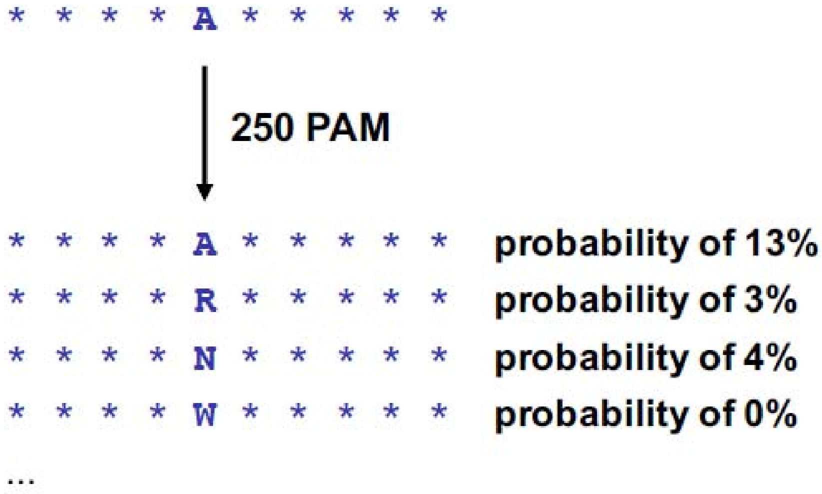
For clarity, the values have been multiplied by 100

Interpretation of the PAM250 matrix



	A	R	N	D	...
A	13	6	9	9	...
R	3	17	4	3	...
N	4	4	6	7	...
D	5	4	8	11	...
C	2	1	1	1	...
Q	3	5	5	6	...
E	5	4	7	11	...
G	12	5	10	10	...
H	2	5	5	4	...
I	3	2	2	2	...
L	6	4	4	3	...
K	6	18	10	8	...
M	1	1	1	1	...
F	2	1	2	1	...
P	7	5	5	4	...
S	9	6	8	7	...
T	8	5	6	6	...
W	0	2	0	0	...
Y	1	1	2	1	...
V	7	4	4	4	...

In comparing 2 sequences at this evolutionary distance (250 PAM), there is:

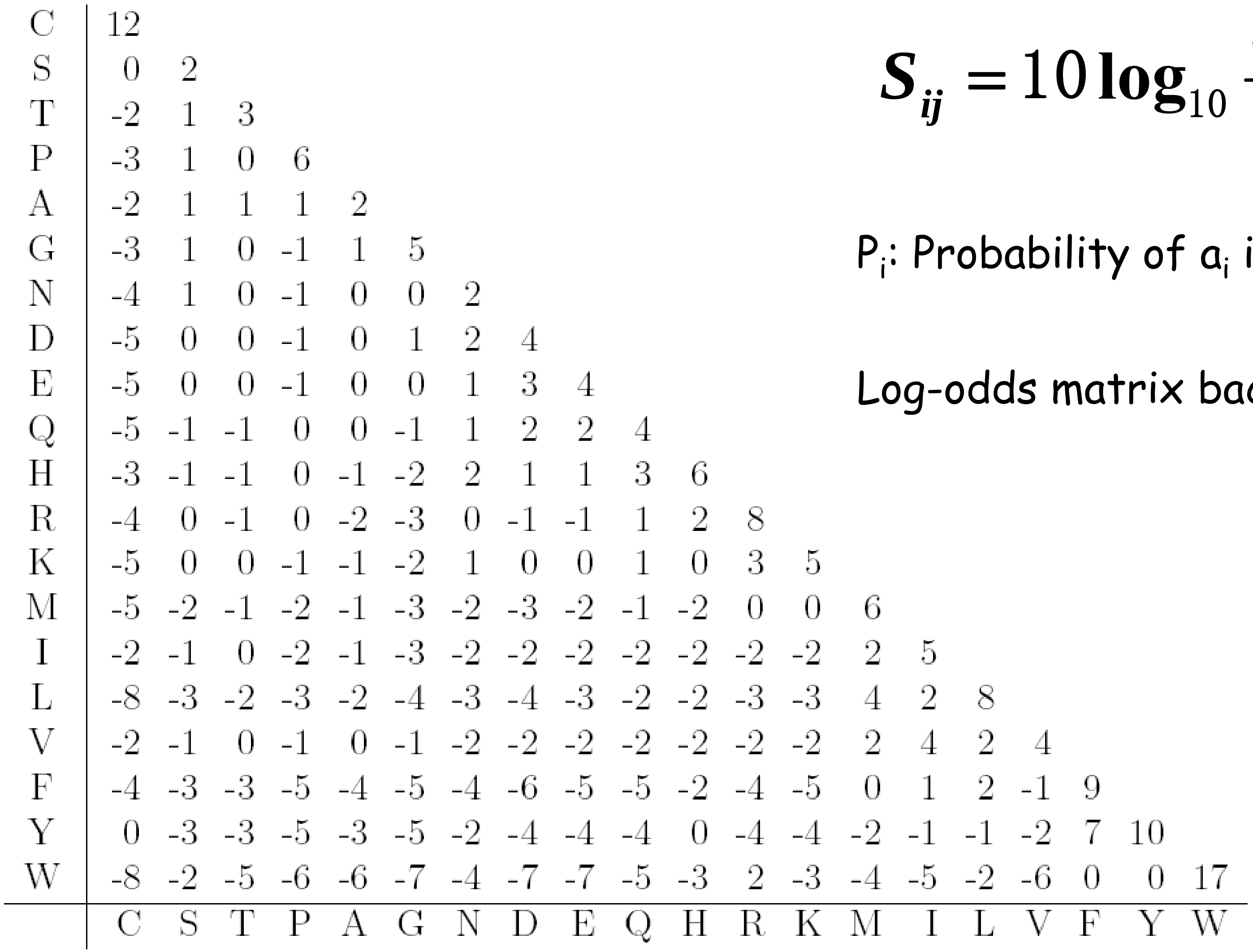


```
* * * * A * * * * *

        250 PAM

* * * * A * * * * *   probability of 13%
* * * * R * * * * *   probability of 3%
* * * * N * * * * *   probability of 4%
* * * * W * * * * *   probability of 0%
...
```

Log-odds of PAM250



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	8								
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	-8	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	8				
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

$$S_{ij} = 10\log_{10}\frac{M_{ij}}{P_i}$$

P_i: Probability of a_i in sequences

Log-odds matrix backs to symmetric

Content

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    a. Global alignment: Needleman-Wunsch
    b. Local alignment: Smith-Waterman
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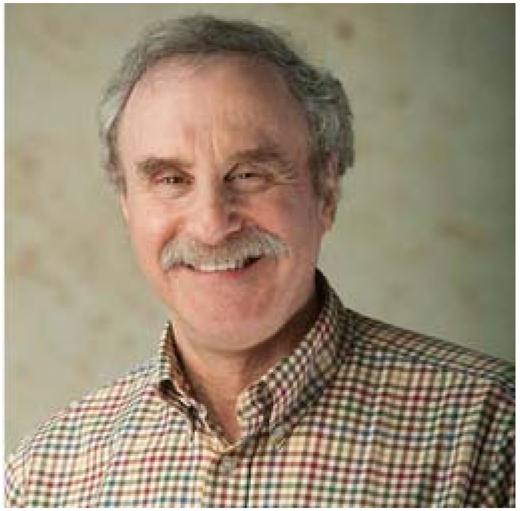
Scoring matrix BLOSUM

Henikoff S, Henikoff JG. Amino acid substitution matrices from protein blocks. Proc Natl Acad Sci U S A. 1992 Nov 15;89(22):10915-9

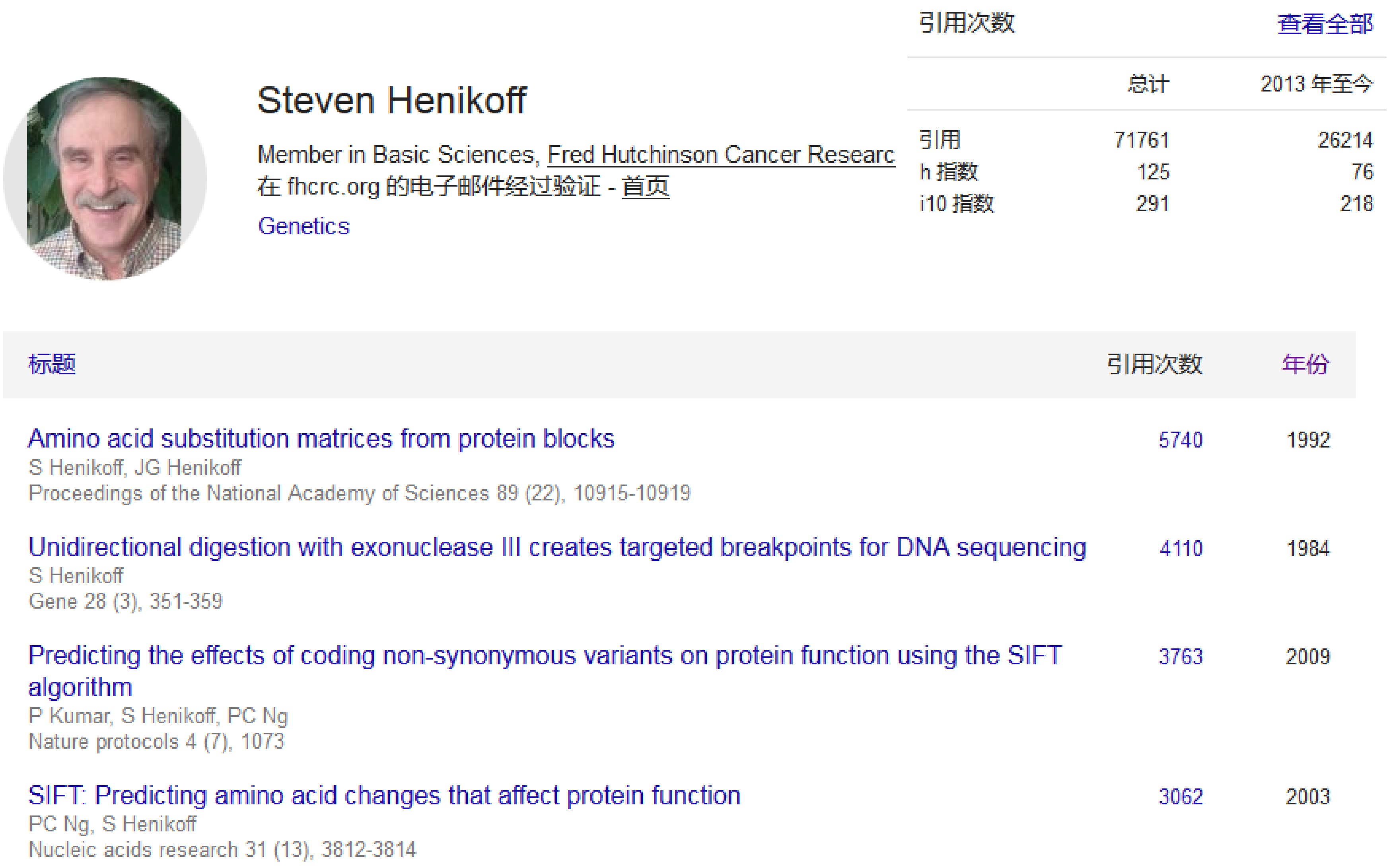


Steve Henikoff

Jorja G. Henikoff

HHMI Investigator
NAS member

Henikoff



Steven Henikoff

Member in Basic Sciences, Fred Hutchinson Cancer Researc
在 fhcrc.org 的电子邮件经过验证 - 首页
Genetics

引用次数 查看全部

	总计	2013 年至今
引用	71761	26214
h 指数	125	76
i10 指数	291	218

标题	引用次数	年份
Amino acid substitution matrices from protein blocks S Henikoff, JG Henikoff Proceedings of the National Academy of Sciences 89 (22), 10915-10919	5740	1992
Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing S Henikoff Gene 28 (3), 351-359	4110	1984
Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm P Kumar, S Henikoff, PC Ng Nature protocols 4 (7), 1073	3763	2009
SIFT: Predicting amino acid changes that affect protein function PC Ng, S Henikoff Nucleic acids research 31 (13), 3812-3814	3062	2003

Scoring matrix BLOSUM

Dataset: >2000 blocks

Four steps for building the BLOSUM matrix:

Step 1: Count frequency table f_{ij}

Step 2: Calculate the observed occurrence probability q_{ij}

Step 3: Calculate the expected occurrence probability e_{ij}

Step 4: Calculate the log-odds matrix S_{ij}

Scoring matrix BLOSUM

Step 1: Count frequency table f_{ij}

A block of known conserved sequences (gapless):

```
LVLHVWAKVEADVAGHGQDILIRLFKSHPETLE
LVLWDWAKVEADVAGHGQDILIRLFKSHPETLE
LDLHVWAKVGGDVAGHGQAALIRLFKSHPETLE
LCLHVWAKVEADVAGGGQGGLIRLFKSHPETLE
DVLHVWAKVEADVAGHGQDILIRLFKSHPETLE
LVLHVWAKVEADVAGHGQDILIRLFKSHPETLE
```

DD pairs: 6
DA pairs: 4
DG pairs: 4
AG pairs: 1
Total pairs at this column: 6x5/2=15

Total pairs in all columns:

$$w \times s(s-1)/2$$

s: number of sequences,
w: number of columns

Scoring matrix BLOSUM

Step 2: Calculate the observed occurrence probability q_{ij}

Probability of occurrence of each i-j pairs:

$$\boldsymbol{q_{ij}} = \frac{\boldsymbol{f_{ij}}}{\sum_{\boldsymbol{i}=1}^{20} \sum_{\boldsymbol{j}=1}^{\boldsymbol{i}} \boldsymbol{f_{ij}}}, \quad 1 \le \boldsymbol{j} \le \boldsymbol{i} \le 20$$

Comparison with PAM

$$\boldsymbol{M_{ij}} = \begin{cases} \lambda \dfrac{\boldsymbol{m_j A_{ij}}}{\sum_{\boldsymbol{k \ne i}} \boldsymbol{A_{kj}}}, & \mathbf{1} \le \boldsymbol{i}, \boldsymbol{j} \le 20; \quad \boldsymbol{i} \ne \boldsymbol{j} \\ 1 - \lambda \boldsymbol{m_j}, & \boldsymbol{i} = \boldsymbol{j} \end{cases}$$

Scoring matrix BLOSUM

Step 3: Calculate the expected occurrence probability e_{ij}

1. Probability of occurrence of the i-th amino acid:

$$p_i = q_{ii} + \frac{1}{2}\sum_{j \neq i} q_{ij}, \quad 1 \leq i \leq 20$$

2. Expected probability of i-j pairs:

$$e_{ij} = \begin{cases} p_i^2, & \textbf{if } i = j \\ 2p_i p_j, & \textbf{otherwise} \end{cases}$$

Scoring matrix BLOSUM

Step 4: Calculate the log-odds matrix S_{ij}

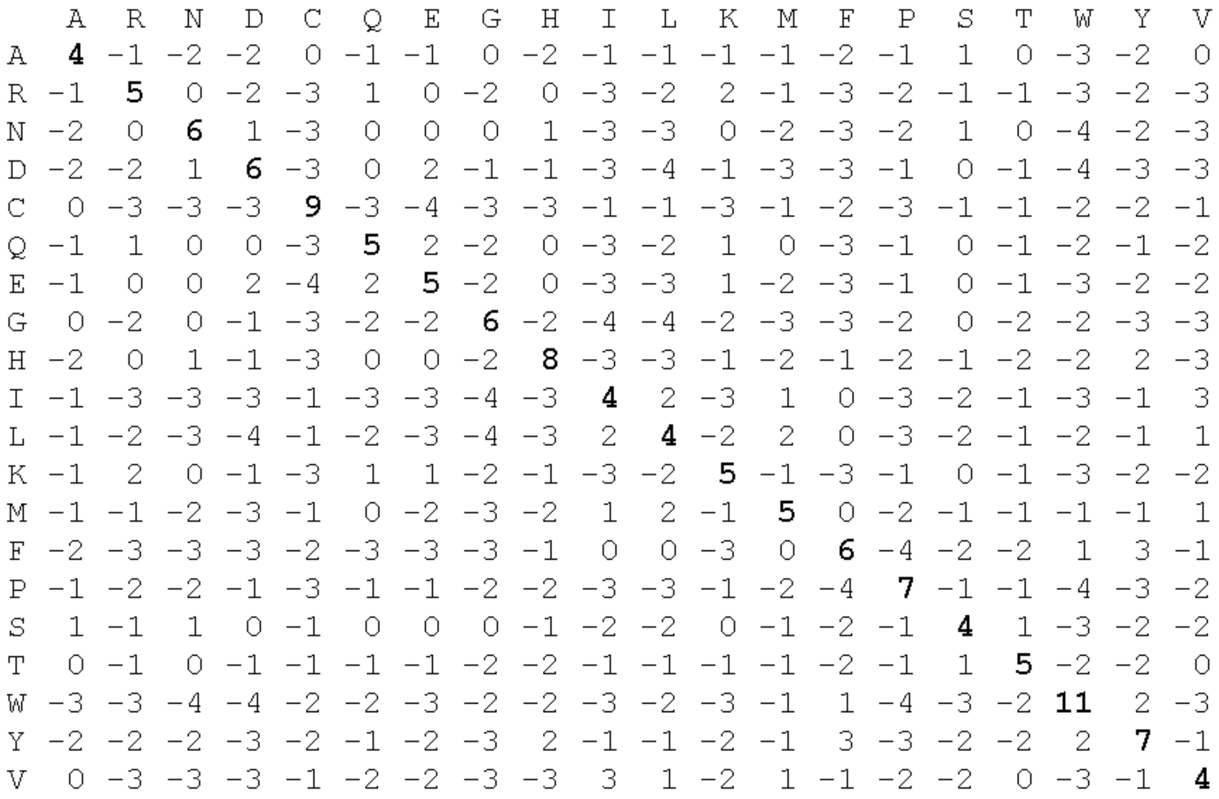
$$S_{ij} = 2\log_2 \frac{q_{ij}}{e_{ij}}, \quad 1 \le j \le i \le 20$$

Comparison with PAM

$$S_{ij} = 10\log_{10} \frac{M_{ij}}{P_i}$$

Scoring matrix BLOSUM62

Sequence identity of the blocks is at least 62%



	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	**4**	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	**5**	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	**6**	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	**6**	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	**9**	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	1	0	0	-3	**5**	2	-2	0	-3	-2	1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	2	-4	2	**5**	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	**6**	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	1	-1	-3	0	0	-2	**8**	-3	-3	-1	-2	-1	-2	-1	-2	-2	2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	**4**	2	-3	1	0	-3	-2	-1	-3	-1	3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	**4**	-2	2	0	-3	-2	-1	-2	-1	1
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	**5**	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	**5**	0	-2	-1	-1	-1	-1	1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	**6**	-4	-2	-2	1	3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	**7**	-1	-1	-4	-3	-2
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	**4**	1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	**5**	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	**11**	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	**7**	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	**4**

S_{ij}<0, probability is less than expected

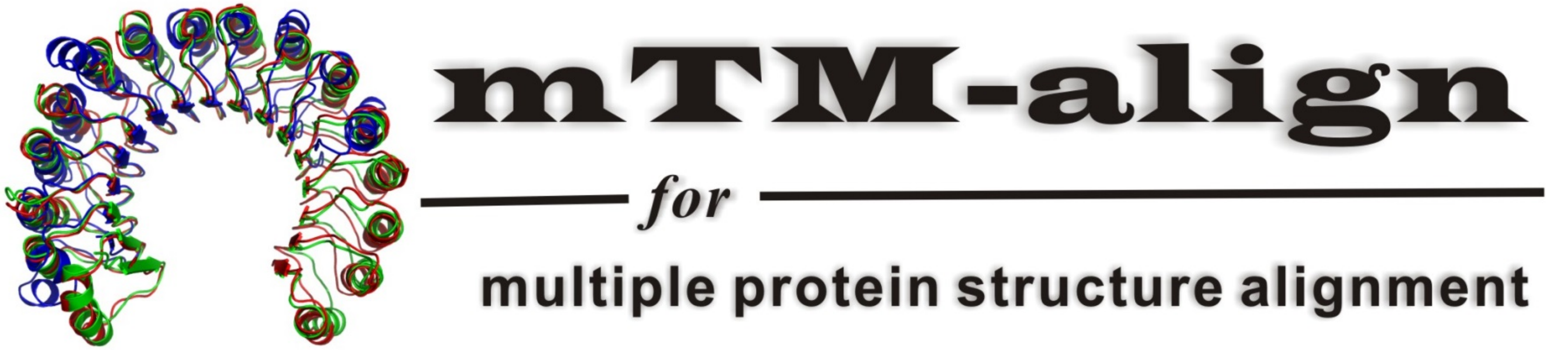
S_{ij}>0, probability is more than expected

A potential research project

One of the major difficulty in the field is to detect remote-homology proteins.

How can we derive a matrix that is more suitable for aligning remote-homology proteins?

One way is probably to use structure alignment to construct blocks for the mutation matrix construction.



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    b. Local alignment: Smith-Waterman
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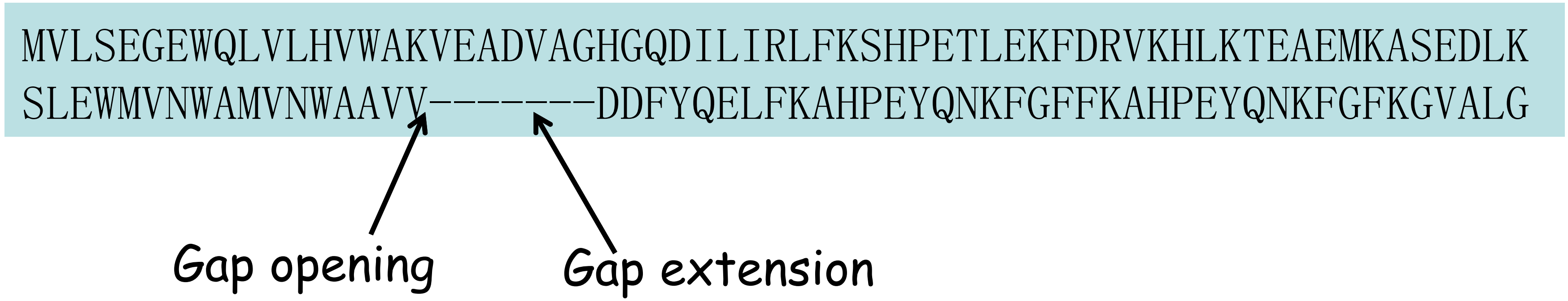
Gap penalty

- What is alignment gap?



```
MVLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRVKHLKTEAEMKASEDLK
SLEWMVNWAMVNWAAVV-------DDFYQELFKAHPEYQNKFGFFKAHPEYQNKFGFKGVALG
```

Gap opening

Gap extension

- Gap penalty:

$$w(k) = a + b(k-1)$$

- a: gap-opening penalty
- b: gap-entension penalty (usually b≤ a)
- k: length of the gaps

Gap penalty

$$Score = \sum_{i=1}^{N_{ali}} M(A_i, B_i) - GapPenalty$$

Question:

For a given score matrix and gap penalty protocol, how to find the best alignment of two protein sequences?

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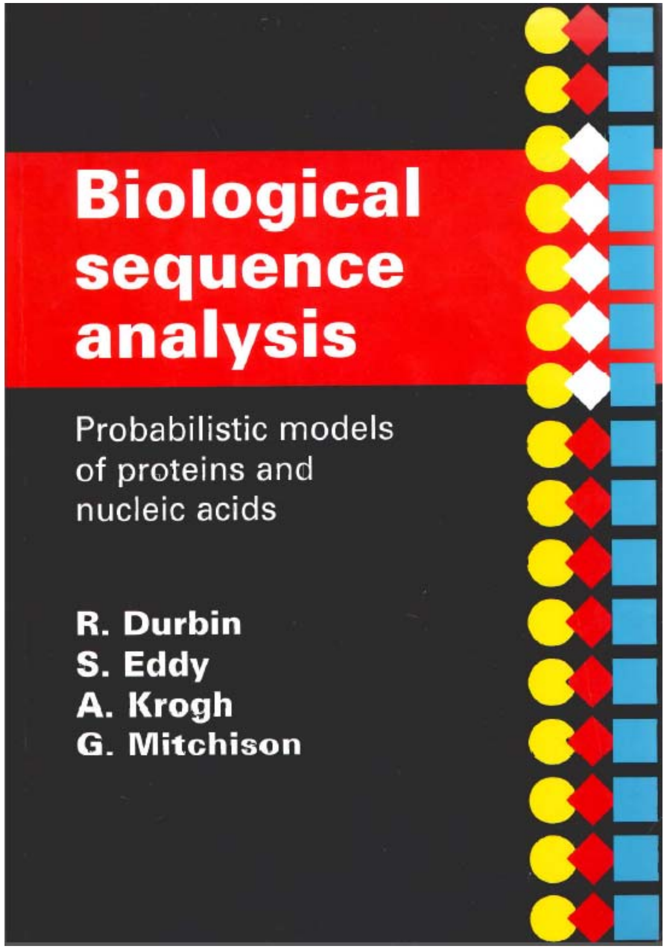
Prepare for next class

Please read P19-P23 of the first textbook



Biological sequence analysis

Probabilistic models of proteins and nucleic acids

R. Durbin
S. Eddy
A. Krogh
G. Mitchison