## Two widely used families of Amino Acid Substitution Matrices Parameterized for evolutionary divergence (*N*)

- PAM matrices, Dayhoff *et al*, 1978
- BLOSUM (Block Sum) matrices, Hennikoff &Hennikoff, 1991

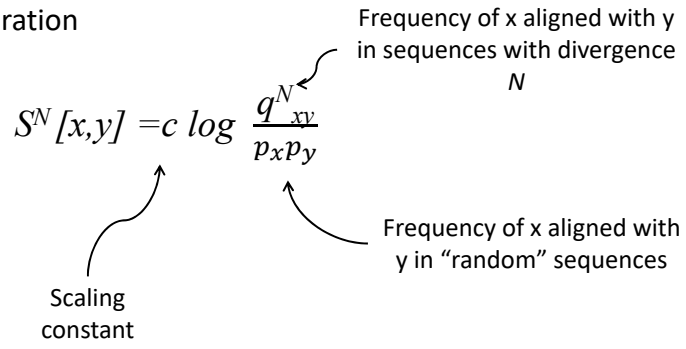
## Amino Acid Substitution Matrices Parameterized for evolutionary divergence (*N*)

Overall strategy for both PAM and BLOSUM

1. Trusted amino acid alignments
2. Obtain amino acid pair counts $\left(A_{xy}^{N}\right)$with corrections for
   - Evolutionary divergence
   - Sample biases
3. Estimate substitution frequencies, $q_{xy}^{N}$, from pair counts, $A_{xy}^{N}$
4. Log odds substitution matrix: $S^{N}[x,y] = c \log \frac{q^{N}_{xy}}{p_x p_y}$

## Log odds substitution matrices

Two sequences have *N* PAMs divergence , if, on average, *N* amino acid replacements per 100 residues occurred since their separation

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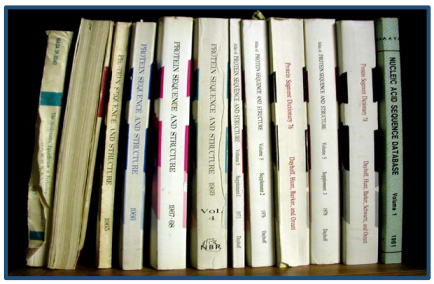
# PAM Matrices

Atlas of Protein Sequence & Structure
1965 - 1978




Examined 1572 changes in 71 groups of closely related proteins

Margaret Dayhoff
PhD in Chemistry, 47
Watson Computing Lab
Fellow 47 - 48

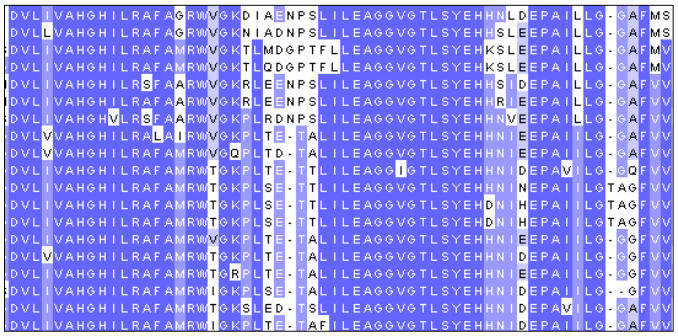
# Evolutionary divergence (amino acids)

- PAM: Percent Accepted Mutation
  - *Accepted Mutations* are mutations that are retained and passed on to future generations
- We say the divergence between two sequences is *N* PAMs, if, on average, *N* amino acid replacements per 100 residues (including multiple substitutions) occurred since their separation.

# 1. Trusted multiple sequence alignments

Examined 1572 changes in 71 groups of closely related proteins

At least 85% identical



# Amino Acid Substitution Matrices
## Parameterized for evolutionary divergence (*N*)

Overall strategy for both PAM and BLOSUM

1. Trusted amino acid alignments
2. **Obtain amino acid pair counts** $\left(A_{xy}^{N}\right)$with corrections for
   - Evolutionary divergence
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3. Estimate substitution frequencies, $q_{xy}^{N}$, from pair counts, $A_{xy}^{N}$
4. Log odds substitution matrix: $S^{N}[x,y] = c\ log\ \frac{q^{N}_{xy}}{p_x p_y}$

## 2. Obtain amino acid pair counts $(A_{xy})$ with corrections for evolutionary divergence and sample biases

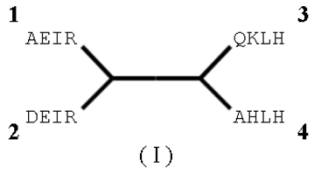
Counting amino acid pairs on a tree:

For each unrooted tree with *k* leaves

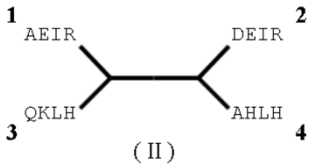
- ➢ Select the tree(s) that require the fewest substitutions to explain the data
- Count amino acid pairs on the branches of the tree

Suppose we have an alignment of four sequences. There are 3 hypotheses (i.e., 3 unrooted trees) for their evolutionary relationships

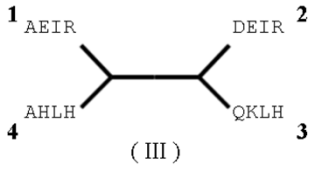
1. AEIR
2. DEIR
3. QKLH
4. AHLH



(I)



(II)

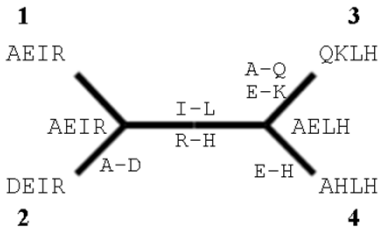


(III)

How to select the tree(s) that require the fewest substitutions to explain the data...

For a given a tree, assign labels to internal nodes that minimize the number of changes required to explain the data

1. AEIR
2. DEIR
3. QKLH
4. AHLH



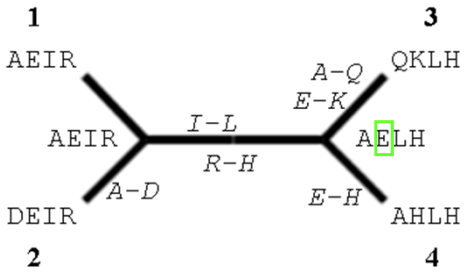

There may be more than one set of labels that satisfies this criterion

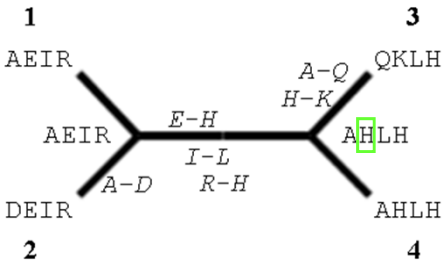
Tree I requires six substitutions

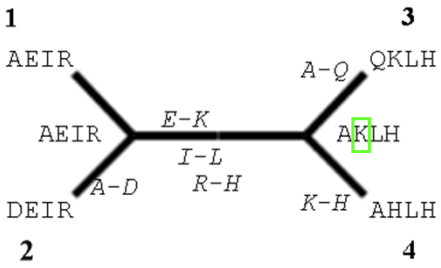
Three most parsimonious ways to assign internal labels to Tree (I)

In each case, six substitutions are required to explain the data.

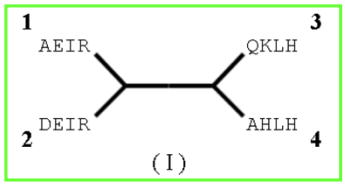
1. **AEIR**
2. **DEIR**
3. **QKLH**
4. **AHLH**



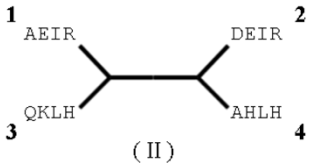



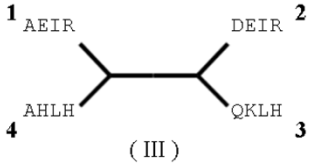



Select the most parsimonious tree; i.e., the tree that requires the fewest substitutions to explain the data.




Tree I requires six substitutions




Convince your self that Trees II and III require more than six substitutions to explain the data

## 2. Obtain amino acid pair counts $(A_{xy})$ with corrections for evolutionary divergence and sample biases

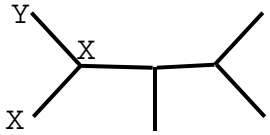
Counting amino acid pairs on a tree:

For each unrooted tree with *k* leaves

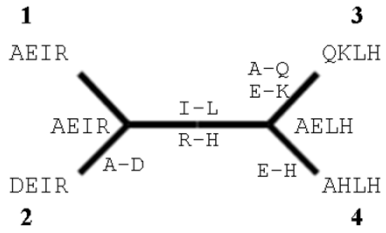
- Select the tree(s) that require the fewest substitutions to explain the data

➢ Count amino acid pairs on the branches of the tree

- For each branch,
  - if labeled x ——— y, $A^N_{xy} = A^N_{xy}+1$ and $A^N_{yx} = A^N_{yx}+1$
  - if labeled x ——— x, $A^N_{xx} = A^N_{xx}+2$



## Impact of counting pairs on a tree: some examples




1. AEIR
2. DEIR
3. QKLH
4. AHLH

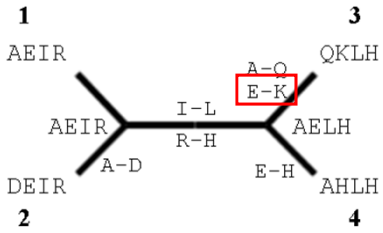
Counts along tree branches

$A_{DQ} = 0$

Pairwise counts in the MSA

$A_{DQ} = 1$

## Impact of counting pairs on a tree: some examples




1. AEIR
2. DEIR
3. QKLH
4. AHLH

Counts along tree branches

$A_{DQ} = 0$

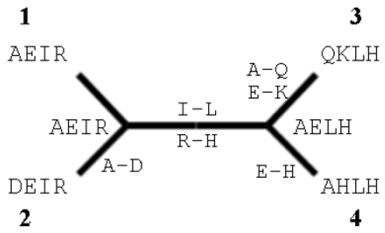
$A_{EK} = 1$

Pairwise counts in the MSA

$A_{DQ} = 1$

$A_{EK} = 2$

## Impact of counting pairs on a tree: some examples




```
1. AEIR
2. DEIR
3. QKLH
4. AHLH
```

Counts along tree branches

$A_{DQ} = 0$

$A_{EK} = 1$

$A_{IL} = 1$

Pairwise counts in the MSA

$A_{DQ} = 1$

$A_{EK} = 2$

$A_{IL} = 4$

## Amino Acid Substitution Matrices
## Parameterized for evolutionary divergence (*N*)

Overall strategy for both PAM and BLOSUM

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4. Log odds substitution matrix: $S^N[x,y] = c \log \frac{q^N_{xy}}{p_x p_y}$

3. Estimate substitution frequencies, $q_{xy}^N$, from pair counts, $A_{xy}$

- Markov model with 20 states (A, C, D, E ... Y)
- Estimate 1 PAM transition matrix $P^1$ from $A_{xy}$
- N-PAM transition matrix: $P^N = (P^1)^N$
- $q_{xy}^N = p_x P_{xy}^N$
- $S^N[x,y] = c \log \frac{q^N_{xy}}{p_x p_y}$

Is $P_{xy}^N$ a symmetric matrix?

Is $S^N[x,y]$ a symmetric matrix?

## Two widely used families of Amino Acid Substitution Matrices Parameterized for evolutionary divergence (*N*)

- PAM matrices, Dayhoff *et al*, 1978

➢BLOSUM (Block Sum) matrices, Hennikoff &Hennikoff, 1991

# BLOSUM Matrices

- Trusted data
  - 2000 blocks of conserved regions in ~500 groups of proteins
- Count amino acid pairs: $A_{xy}^N$
  - **Parameterize by evolutionary distance, *N***
  - **Correct for sample bias**
- Calculate amino acid frequencies:
  - Related pairs: $q_{xy}^N$
  - Background pair frequencies calculated from blocks: $E_{xy}$
- Log likelihood scoring matrix
  - $S^N = 2\ \log_2 \frac{q_{xy}^N}{E_{xy}}$

# 1. Trusted multiple sequence alignments

```
                **BLOCKS from MOTIF**
Tc1
9 sequences are included in 4 blocks

                                  Tc1 A, width = 46
   DmBari-1  120 KTIEITPTNKTKRLRFALEYVKKPLDFWFNILWIDESAFQYQGSYS
     DmHB1    98 KVPLPSPRHIKARLSLAKTYLNWPVSKWRNILWTDGSKIMLPGGTG
    DhMinos  156 EKPLLTLRQKKKRLQWARERMSWTQRQWDTIIFSDEAKFDVSVGDT
  TC1A_CAEBR  53 KKPLVSLKNRKARVEWAKQHLSWGPREWANHIWSDESKFNMFGTDG
  TC1A_CAEEL  53 KKPFISKKNRMARVAWAKAHLRWGRQEWAKHIWSDESKFNLPGSDG
  TC2A_CAEBR  53 KKPSISKKNRIARVAWARAHLHWGRQDWANHVFSDESKFNLPGTDG
  TC3A_CAEEL 110 KLRPAPLLSADHKLKRLEFAKNNMGTNWSKVVFSDEKKFNLDGPDG
     EsTes1  146 QKTIRRWQNKKKRFAWAMKHRQWITENWKKALWTDESKFEIFVSSR
     DhUhu    82 KKPFISTKNKGTRMTFAKTHLDKDLRFWNTIIFRDESKFIIPGSDG

                Tc1 B, width = 11          Tc1 C, width = 11
   DmBari-1  (  21)  187 GGGTVMVWGCL  (  40)  238 WILQQDNAPCH
     DmHB1   (  24)  168 GGPKIMVWACF  (  39)  218 WTPQQDNDQKR
    DhMinos  (  22)  224 FPASTMVWGCM  (  40)  275 PTPQQDGASSH
  TC1A_CAEBR (  23)  122 GGGSVMVWGCF  (  39)  172 WVPQQDNDPKH
  TC1A_CAEEL (  23)  122 GGGSVMVWGCF  (  39)  172 FVPQQDNDPKH
  TC2A_CAEBR (  23)  122 GGGSVMVWGCF  (  39)  172 FVPQQDNDPKH
  TC3A_CAEEL (  20)  176 GGGTVMVWGAF  (  39)  226 FRPQQDNATIH
     EsTes1  (  22)  214 GGGSLMIWGSF  (  39)  264 FILQQDNDPKH
     DhUhu   (  22)  150 HGGSVMVWACI  (  39)  200 FRPYQDNDQKH

                Tc1 D, width = 15
   DmBari-1  (  18)  267 WPPQSPDLNIIENVW
     DmHB1   (  18)  247 WQAPPSHLNPIENLY
    DhMinos  (  18)  304 WPSNSPDLSPIENIW
  TC1A_CAEBR (  18)  201 WPSQSPDLNPIEHMW
  TC1A_CAEEL (  18)  201 WPSQSPDLNPIEHLW
  TC2A_CAEBR (  18)  201 WPSQSPDLNPIEHLW
  TC3A_CAEEL (  18)  255 WPARSPDLNPIENLW
     EsTes1  (  22)  297 WPAQSPDLNPIELVW
     DhUhu   (  18)  229 XPAQSPDVNVIXNLW
```

~2000 blocks representing
500+ groups of proteins

Automated construction and graphical presentation of protein blocks from unaligned sequences

Steven Henikoff [a,b,*], Jorja G. Henikoff [a,1], William J. Alford [a,2], Shmuel Pietrokovski [a,3]

[a] Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, USA
[b] Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, USA

Received 10 May 1995; revised 23 June 1995; accepted 1 July 1995; published 1 August 1995

# 2. Count amino acid pairs: $A_{xy}^{N}$

**Parameterize by evolutionary distance, *N***

**Correct for sample bias**

- Cluster sequences such that if *s1* and *s2* are in different clusters, then *identity(s1, s2) < N%*
- Count amino acid pairs in *s1* aligned with *s2* *only* if *s1* and *s2* are in different clusters
- Normalize for cluster size

An example...

## BLOSUM clustering example

1: KKRK
2: KKKK
3: KNRN
4: NRNR
5: KNKN
6: KRNR

Percent Sequence Identity

| | [2] | [3] | [4] | [5] | [6] |
|---|---|---|---|---|---|
| [1] | 0.75 | 0.5 | 0 | 0.25 | 0.25 |
| [2] | | 0.25 | 0 | 0.5 | 0.25 |
| [3] | | | 0 | 0.75 | 0.25 |
| [4] | | | | 0 | 0.75 |
| [5] | | | | | 0.25 |

Unclustered sequences: Every sequence is at least 25% identical

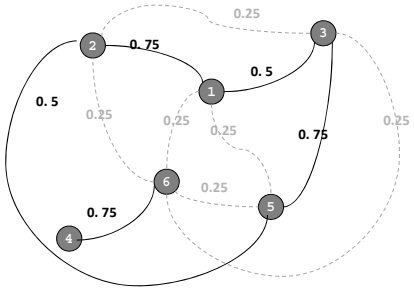
Percent Sequence Identity

| | [2] | [3] | [4] | [5] | [6] |
|---|---|---|---|---|---|
| [1] | **0.75** | **0.5** | 0 | 0.25 | 0.25 |
| [2] | | 0.25 | 0 | **0.5** | 0.25 |
| [3] | | | 0 | **0.75** | 0.25 |
| [4] | | | | 0 | **0.75** |
| [5] | | | | | 0.25 |

1: KKRK
2: KKKK
3: KNRN
5: KNKN

< 45% identical

4: NRNR
6: KRNR




Percent Sequence Identity

| | [2] | [3] | [4] | [5] | [6] |
|---|---|---|---|---|---|
| [1] | **0.75** | 0.5 | 0 | 0.25 | 0.25 |
| [2] | | 0.25 | 0 | 0.5 | 0.25 |
| [3] | | | 0 | **0.75** | 0.25 |
| [4] | | | | 0 | **0.75** |
| [5] | | | | | 0.25 |

< 65% id

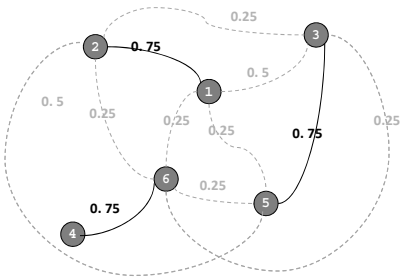
1: KKRK
2: KKKK

< 65% id

3: KNRN
5: KNKN

4: NRNR
6: KRNR

< 65% id



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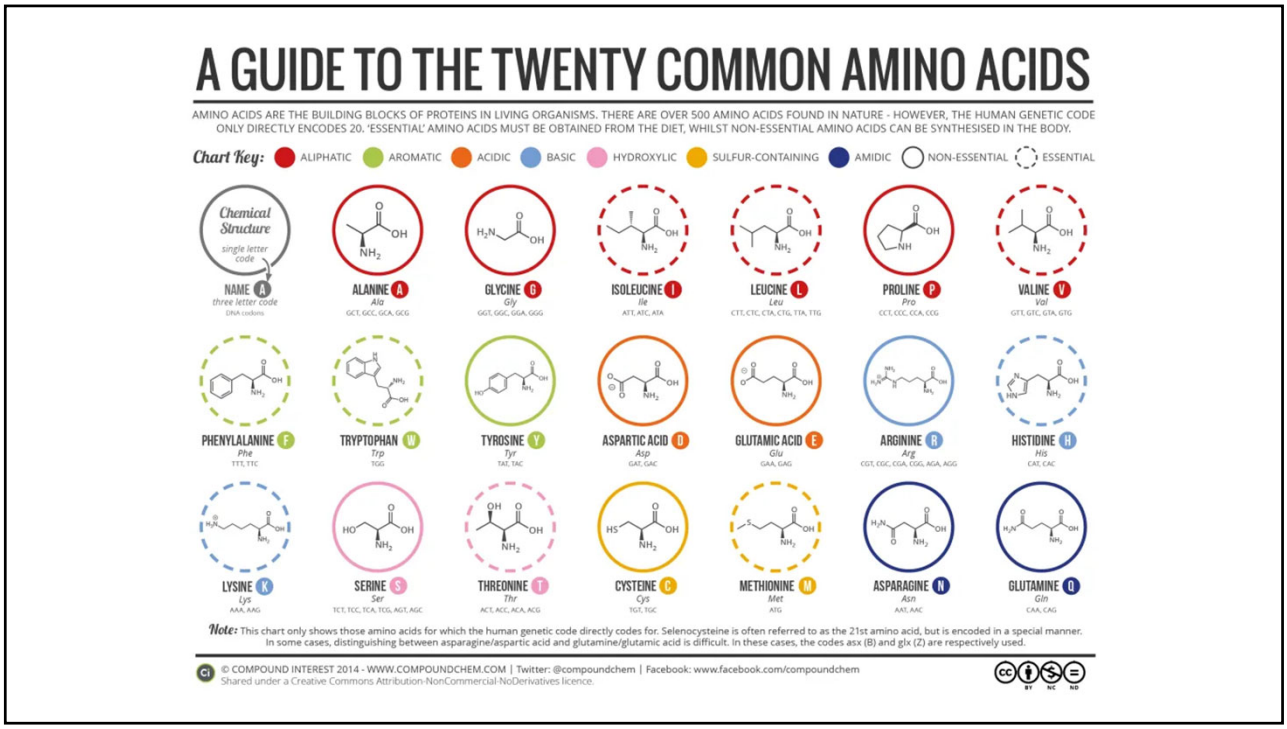
Similarities and differences between PAM and BLOSUM

| | **PAM** | **BLOSUM** |
|---|---|---|
| **Evolutionary model** | Explicit evolutionary model | None |
| **Data** | Full length MSAs of closely related sequences. | Conserved blocks. i.e., ungapped local MSAs |
| **Bias correction** | Trees | Clustering |
| **Multiple substitutions** | Markov model: $P^n = (P^1)^n$ | Implicitly represented in data (clustering) |
| **Evolutionary distance** | Markov model: $P^n = (P^1)^n$ | Clustering |
| **Matrices** | Transition and log odds scoring matrices | Log odds scoring matrix only. |
| **Parameter *n*** | Distance increases with *n* | Distance decreases with *n* |
| **Biophysical properties** | Derived indirectly from data | Derived indirectly from data |




## Comparing PAM and BLOSUM matrices

Less divergent

| PAM | Sequence identity | BLO SUM |
|---|---|---|
| 20 | 83% | |
| 30 | | |
| 60 | 63% | |
| 70 | | |
| 100 | 43% | 90 |
| 120 | 38% | 80 |
| 160 | 30% | 60 |
| 200 | 25% | 50 |
| 250 | 20% | 45 |

More divergent