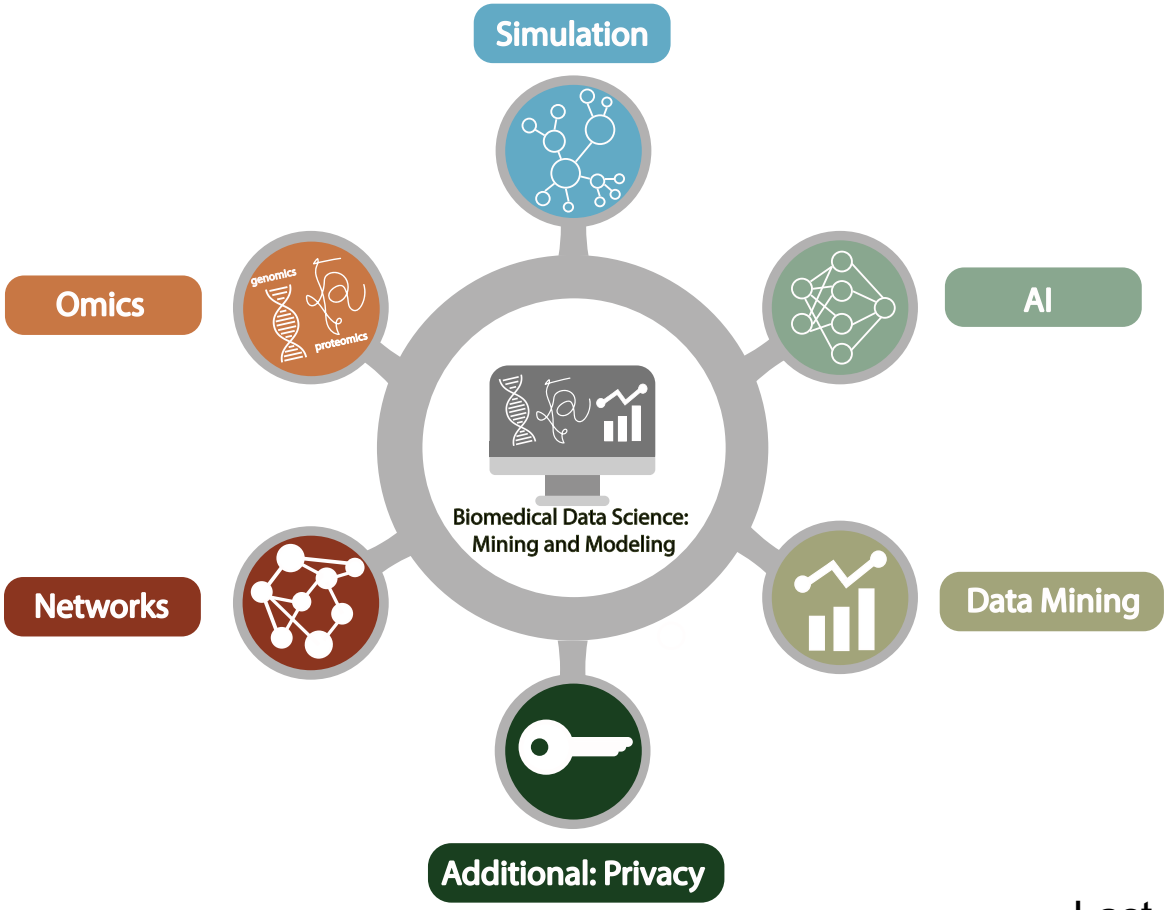
Biomedical Data Science (GersteinLab.org/courses/452)
Sequence Comparison (25m3)



Mark Gerstein
Yale U.

Last edit in spring ‘25,
very similar to 22m3
& 2021’s M3
[which has a video]

Sequence Topics (Contents)

- Basic Alignment via Dynamic Programming
- Suboptimal Alignment
- Gap Penalties
- Similarity (PAM) Matrices
- Local Alignment

Aligning Text Strings

```
Raw Data ???
  T  C  A  T  G
    C  A  T  T  G
```

```
2 matches, 0 gaps
  T  C  A  T  G
           |  |
  C  A  T  T  G
```

```
3 matches (2 end gaps)
  T  C  A  T  G  .
     |  |  |
  .  C  A  T  T  G
```

```
4 matches, 1 insertion
  T  C  A  -  T  G
     |  |     |  |
  .  C  A  T  T  G
```

```
4 matches, 1 insertion
  T  C  A  T  -  G
     |  |  |     |
  .  C  A  T  T  G
```

Dynamic Programming

- What to do for a bigger string?

SSDSEREEHVKRFRQALDDTGMKVPMATTNLFTHPVFKDGGFTANDRDVRRYALRKTIRNIDLAVELGAETYVAWGGREGAESGGAKDVRDALDRMKEAFDLLGEYVTSQGYDIRFAIEP
KPNEPRGDILLPTVGHALAFIERLERPELYGVNPEVGHEQMAGLNFPHGIAQALWAGKLFHIDLNGQNGIKYDQDLRFGAGDLRAAFWLVDLLESAGYSGPRHFDFKPPRTEDFDGVWAS

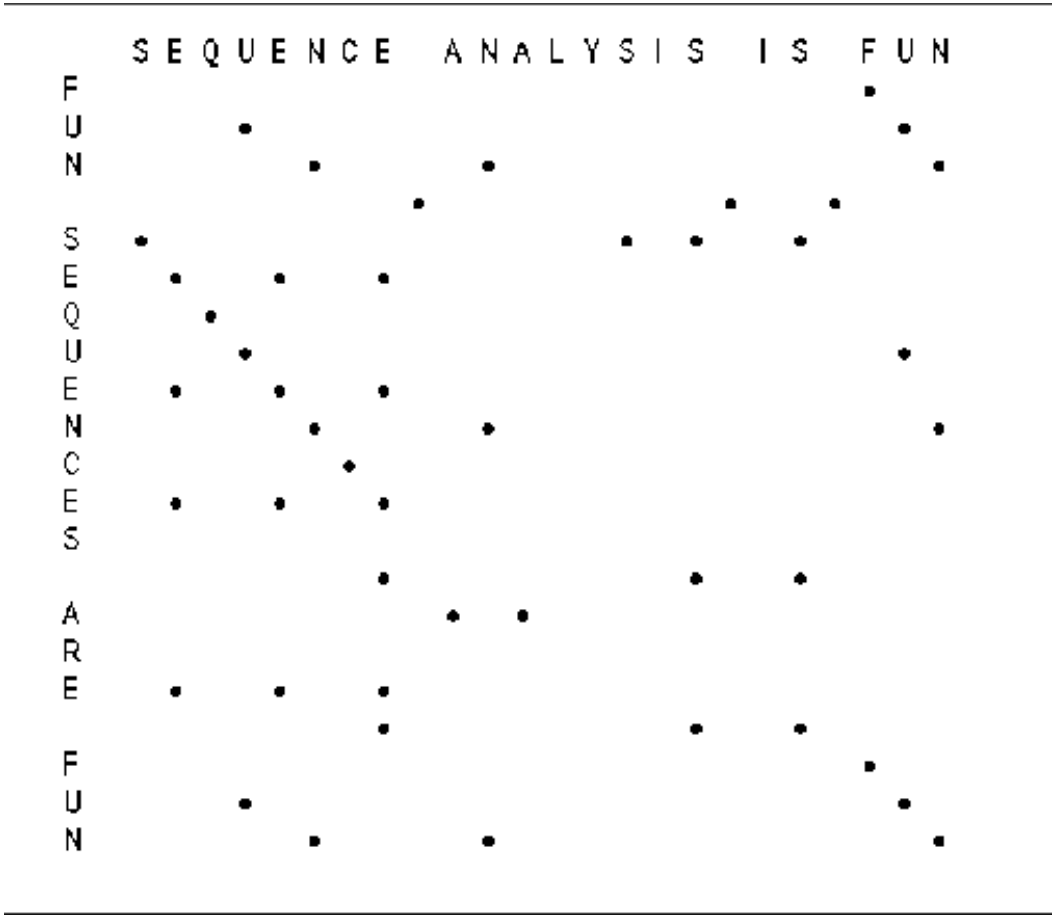
- Needleman-Wunsch (1970) provided first automatic method
  - ◊ Dynamic Programming to Find Global Alignment
- Their Test Data (J->Y)
  - ◊ ABCNYRQCLCRPM
    AYCYNRCKCRBP

Step 1 -- Make a Dot Plot (Similarity Matrix)

Put 1's where characters are identical.

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	**1**												
Y					**1**								
C			**1**					**1**		**1**			
Y					**1**								
N				**1**									
R						**1**					**1**		
C			**1**					**1**		**1**			
K													
C			**1**					**1**		**1**			
R						**1**					**1**		
B		**1**											
P												**1**	

A More Interesting Dot Matrix



(adapted from R Altman)

Step 2 -- Start Computing the Sum Matrix

```
new_value_cell(R,C) <=
  cell(R,C)                          { Old value, either 1 or 0    }
  + Max[
        cell (R+1, C+1),             { Diagonally Down, no gaps    }
        cells(R+1, C+2 to C_max),{ Down a row, making col. gap }
        cells(R+2 to R_max, C+1) { Down a col., making row gap }
       ]
```

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	**1**												
Y					**1**								
C			**1**					**1**		**1**			
Y					**1**								
N				**1**									
R						**1**					**1**		
C			**1**					**1**		**1**			
K													
C			**1**					**1**		**1**			
R						**1**					**1**		
B		**1**											
P												**1**	

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					*2*	**0**	**0**
B	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**
P	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**

Step 2 -- Start Computing the Sum Matrix

```
new_value_cell(R,C) <=
  cell(R,C)                          { Old value, either 1 or 0    }
  + Max[
        cell (R+1, C+1),             { Diagonally Down, no gaps    }
        cells(R+1, C+2 to C_max),{ Down a row, making col. gap }
        cells(R+2 to R_max, C+1) { Down a col., making row gap }
       ]
```

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	**1**												
Y					**1**								
C			**1**					**1**		**1**			
Y					**1**								
N				**1**									
R						**1**					**1**		
C			**1**					**1**		**1**			
K													
C			**1**					**1**		**1**			
R						**1**					**1**		
B		**1**											
P												**1**	

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					***2***	**0**	**0**
B	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**
P	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**

Step 3 -- Keep Going

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					*2*	0	0
B	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**
P	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						*5*	**4**	**3**	**3**	**2**	**2**	**0**	**0**
C	**3**	**3**	**4**	**3**	**3**	**3**	**3**	**4**	**3**	**3**	**1**	**0**	**0**
K	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**2**	**1**	**0**	**0**
C	**2**	**2**	**3**	**2**	**2**	**2**	**2**	**3**	**2**	**3**	**1**	**0**	**0**
R	**2**	**1**	**1**	**1**	**1**	**2**	**1**	**1**	**1**	**1**	**2**	**0**	**0**
B	**1**	**2**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**0**	**0**
P	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**0**

Step 4 -- Sum Matrix All Done

Alignment Score is 8 matches.

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						*5*	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	2	1	0	0
C	2	2	3	2	2	2	2	3	2	3	1	0	0
R	2	1	1	1	1	2	1	1	1	1	2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	*8*	7	6	6	5	4	4	3	3	2	1	0	0
Y	7	7	6	6	6	4	4	3	3	2	1	0	0
C	6	6	7	6	5	4	4	4	3	3	1	0	0
Y	6	6	6	5	6	4	4	3	3	2	1	0	0
N	5	5	5	6	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	5	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	4	3	3	1	0	0
K	3	3	3	3	3	3	3	3	3	2	1	0	0
C	2	2	3	2	2	2	2	3	2	3	1	0	0
R	2	1	1	1	1	2	1	1	1	1	2	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

Step 5 -- Traceback

Find Best Score (8) and Trace Back

A B C N Y - R Q C L C R - P M

A Y C - Y N R - C K C R B P

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	**8**	7	6	6	5	4	4	3	3	2	1	0	0
Y	7	**7**	6	6	6	4	4	3	3	2	1	0	0
C	6	6	**7**	6	5	4	4	4	3	3	1	0	0
Y	6	6	6	5	**6**	4	4	3	3	2	1	0	0
N	5	5	5	6	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	**5**	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	**4**	3	3	1	0	0
K	3	3	3	3	3	3	3	3	**3**	2	1	0	0
C	2	2	3	2	2	2	2	3	2	**3**	1	0	0
R	2	1	1	1	1	2	1	1	1	1	**2**	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	**1**	0

Hansel & Gretel

Step 6 -- Alternate Tracebacks

A B C - N Y R Q C L C R - P M
A Y C Y N - R - C K C R B P

Also, Suboptimal Aligments

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	**8**	7	6	6	5	4	4	3	3	2	1	0	0
Y	7	**7**	6	6	6	4	4	3	3	2	1	0	0
C	6	6	**7**	6	5	4	4	4	3	3	1	0	0
Y	6	6	6	5	*6*	4	4	3	3	2	1	0	0
N	5	5	5	**6**	5	4	4	3	3	2	1	0	0
R	4	4	4	4	4	**5**	4	3	3	2	2	0	0
C	3	3	4	3	3	3	3	**4**	3	3	1	0	0
K	3	3	3	3	3	3	3	3	**3**	2	1	0	0
C	2	2	3	2	2	2	2	3	2	**3**	1	0	0
R	2	1	1	1	1	2	1	1	1	1	**2**	0	0
B	1	2	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	**1**	0

Gap Penalties

The score at a position can also factor in a penalty for introducing gaps (i. e., not going from i, j to i- 1, j- 1).

Gap penalties are often of linear form:

$$GAP = a + bN$$

GAP is the gap penalty

a = cost of opening a gap

b = cost of extending the gap by one (affine)

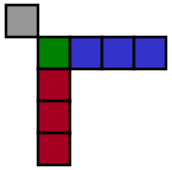
N = length of the gap

(Here assume b=0, a=1/2, so GAP = 1/2 regardless of length.)

```
ATGCAAAAT
ATG-AAAAT .5
ATG--AAAT .5 + (1)b  [b=.1]
ATG---AAT .5 + (2)(.1) =.7
```

Step 2 -- Computing the Sum Matrix **with Gaps**

```
new_value_cell(R,C) <=
  cell(R,C)                                  { Old value, either 1 or 0     }
  + Max[
       cell (R+1, C+1),                      { Diagonally Down, no gaps     }
       cells(R+1, C+2 to C_max) - GAP ,{ Down a row, making col. gap }
       cells(R+2 to R_max, C+1) - GAP  { Down a col., making row gap }
      ]
```



	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					1		
B		1											
P												1	

	A	B	C	N	Y	R	Q	C	L	C	R	P	M
A	1												
Y					1								
C			1					1		1			
Y					1								
N				1									
R						1					1		
C			1					1		1			
K													
C			1					1		1			
R						1					1.5	0	0
B	1	1.5	1	1	1	1	1	1	1	1	1	0	0
P	0	0	0	0	0	0	0	0	0	0	0	1	0

GAP =1/2

All Steps in Aligning a 4-mer

C R B P

C R P M

- C R P M

C R - P M

	C	R	P	M
C	**1**			
R		**1**		
B				
P			**1**	

	C	R	P	M
C	1			
R		***2***	**0**	**0**
B	**1**	**1**	**0**	**0**
P	**0**	**0**	**1**	**0**

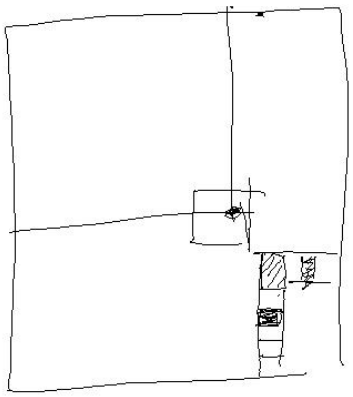
	C	R	P	M
C	**3**	**1**	**0**	**0**
R	**1**	**2**	**0**	**0**
B	**1**	**1**	**0**	**0**
P	**0**	**0**	**1**	**0**

	C	R	P	M
C	**3**	1	0	0
R	1	**2**	0	0
B	1	**1**	0	0
P	0	0	**1**	0

Bottom right hand corner of previous matrices

Key Idea in Dynamic Programming

- ◊ The best alignment that ends at a given pair of positions (i and j) in the 2 sequences is the score of the best alignment previous to this position PLUS the score for aligning those two positions.
- ◊ An Example Below
  - Aligning R to K does not affect alignment of previous N-terminal residues. Once this is done it is **fixed**. Then go on to align D to E.
  - How could this be violated?
    Aligning R to K changes best alignment in box.



```
ACSQRP--LRV-SH  RSENCV
A-SNKPQLVKLMTH  VKDFCV

ACSQRP--LRV-SH  -R  SENCV
A-SNKPQLVKLMTH  VK  DFCV
```

Similarity (Substitution) Matrix

- Identity Matrix
  - ◊ Match L with L => 1
    Match L with D => 0
    Match L with V => 0??
- S(aa-1,aa-2)
  - ◊ Match L with L => 1
    Match L with D => 0
    Match L with V => .5
- Number of Common Ones
  - ◊ PAM
  - ◊ Blossum
  - ◊ Gonnet

	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	8	-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	7	-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	6	-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	10	2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	6	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Where do matrices come from?

+ —> More likely than random
0 —> At random base rate
- —> Less likely than random

1 Manually align protein structures (or, more risky, sequences)

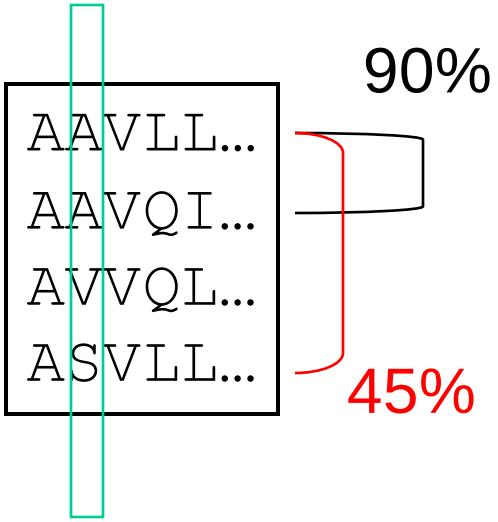
2 Look at frequency of a.a. substitutions at structurally constant sites. -- i.e. pair i-j exchanges

3 Compute log-odds

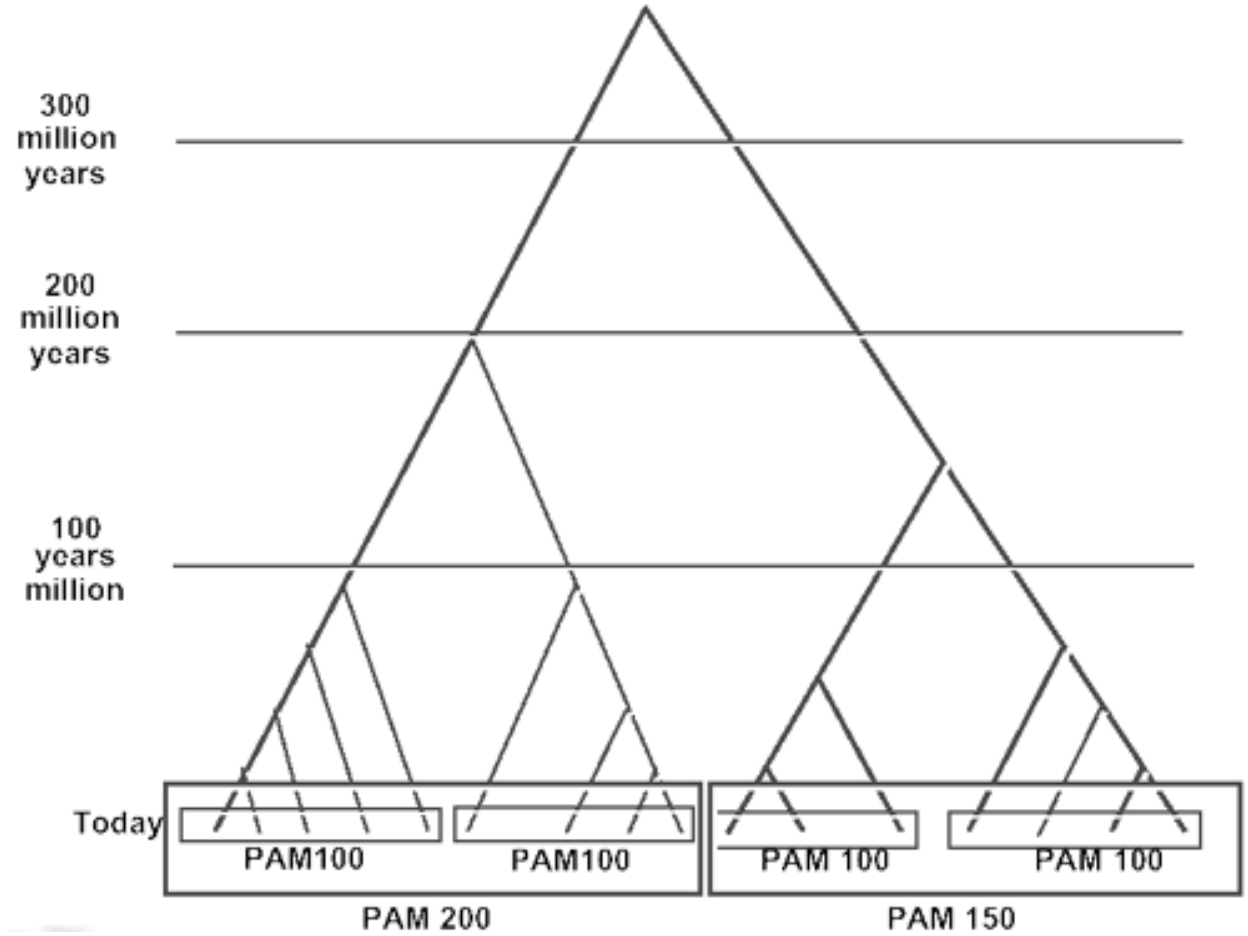
$S(aa\text{-}1,aa\text{-}2) = \log_2 (freq(O) / freq(E))$

O = observed exchanges,
E = expected exchanges

- odds = freq(observed) / freq(expected)
- Sij = log odds
- freq(expected) = f(i)*f(j)
  = is the chance of getting amino acid i in a column and then having it change to j
- e.g. A-R pair observed only a tenth as often as expected



Different Matrices are Appropriate at Different Evolutionary Distances



Different gold std. sets of seq at diff ev. dist. --> matrices

Ev. Equiv. seq. (ortholog) [hb and mb]

(Adapted from D Brutlag, Stanford)

Change in Matrix with Ev. Dist.

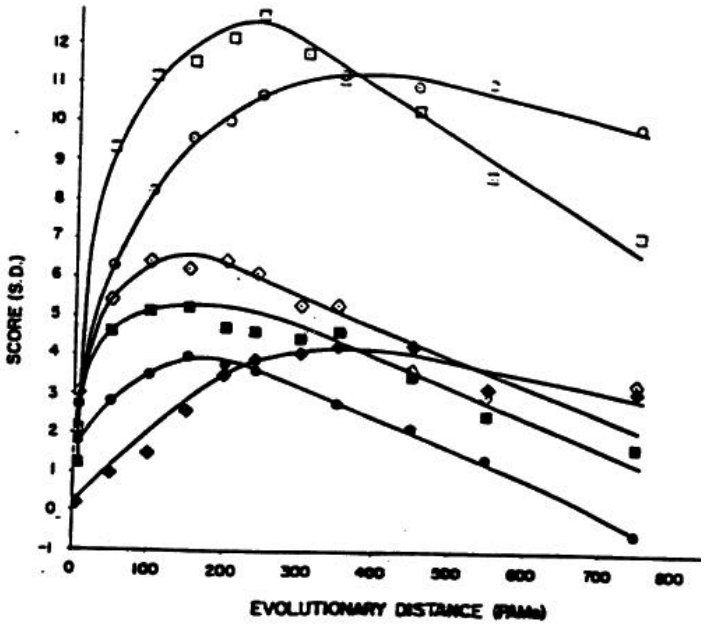
PAM-250 (distant)

C Cys	12																			
S Ser	0	2																		
T Thr	-2	1	3																	
P Pro	-3	1	0	6																
A Ala	-2	1	1	1	2															
G Gly	-3	1	0	-1	1	5														
N Asn	-4	1	0	-1	0	0	2													
D Asp	-5	0	0	-1	0	1	2	4												
E Glu	-5	0	0	-1	0	0	1	3	4											
Q Gln	-5	-1	-1	0	0	-1	1	2	2	4										
H His	-3	-1	-1	0	-1	-2	2	1	1	3	6									
R Arg	-4	0	-1	0	-2	-3	0	-1	-1	1	2	6								
K Lys	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5							
M Met	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6						
I Ile	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	5					
L Leu	-6	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	6				
V Val	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4			
F Phe	-4	-3	-3	-5	-4	-5	-4	-6	-5	-5	-2	-4	-5	0	1	2	-1	9		
Y Tyr	0	-3	-3	-5	-3	-5	-2	-4	-4	-4	0	-4	-4	-2	-1	-1	-2	7	10	
W Trp	-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	M

PAM-70

C Cys	9																			
S Ser	-1	5																		
T Thr	-5	2	6																	
P Pro	-5	0	-2	7																
A Ala	-4	1	1	0	5															
G Gly	-6	0	-3	-3	0	6														
N Asn	-7	1	0	-3	-2	-1	6													
D Asp	-9	-1	-2	-4	-1	-1	3	6												
E Glu	-9	-2	-3	-3	-1	-2	0	3	6											
Q Gln	-9	-3	-3	-1	-2	-4	-1	0	2	7										
H His	-5	-3	-4	-2	-4	-6	1	-1	-2	2	8									
R Arg	-5	-1	-4	-2	-4	-6	-3	-6	-5	0	0	8								
K Lys	-9	-2	1	-4	-4	-5	0	-2	2	-1	-3	2	6							
M Met	-9	-3	-2	-5	-3	-6	-5	-7	-4	-2	-6	-2	0	10						
I Ile	-4	-4	-1	-5	-2	-6	-3	-5	-4	-5	-6	-3	-4	1	7					
L Leu	-10	-6	-4	-5	-4	-7	-5	-8	-6	-3	-4	-6	-5	2	1	6				
V Val	-4	-3	-1	-3	-1	-3	-5	-5	-4	-4	-4	-5	-6	0	3	0	6			
F Phe	-8	-3	-6	-7	-6	-3	-6	-10	-9	-9	-4	-7	-9	-2	0	-1	-5	8		
Y Tyr	2	-5	-4	-9	-5	-9	-3	-7	-6	-8	1	-7	-7	-7	-4	-4	-5	4	9	
W Trp	-11	-3	-8	-9	-9	-10	-6	-10	-11	-8	-5	0	-7	-8	-9	-4	-10	-2	-3	13
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	M

Chemistry (far) Vs. genetic code (near)



(Adapted from D Brutlag, Stanford)

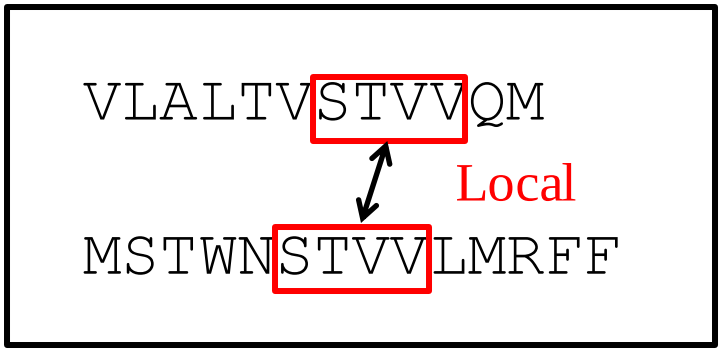
Modifications for Local Alignment

1 The scoring system uses negative scores for mismatches

2 The minimum score for at a matrix element is zero

3 Find the best score anywhere in the matrix (not just last column or row)

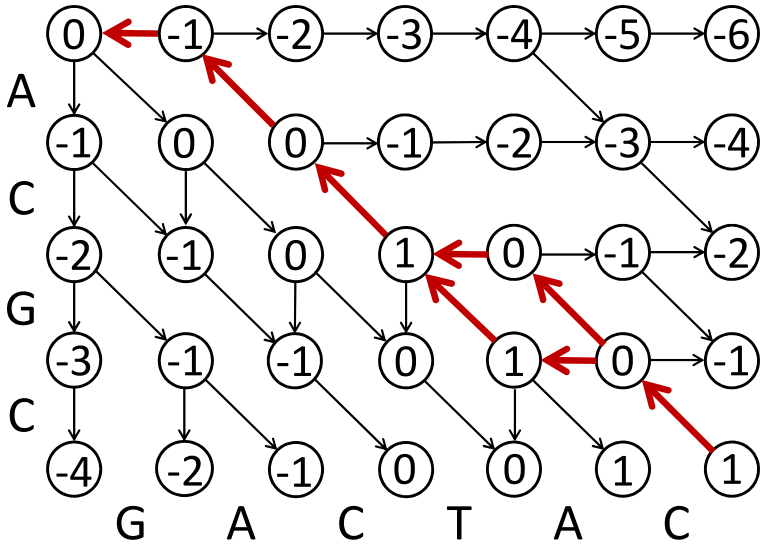
- These three changes cause the algorithm to seek high scoring subsequences, which are not penalized for their global effects (mod. 1), which don't include areas of poor match (mod. 2), and which can occur anywhere (mod. 3)

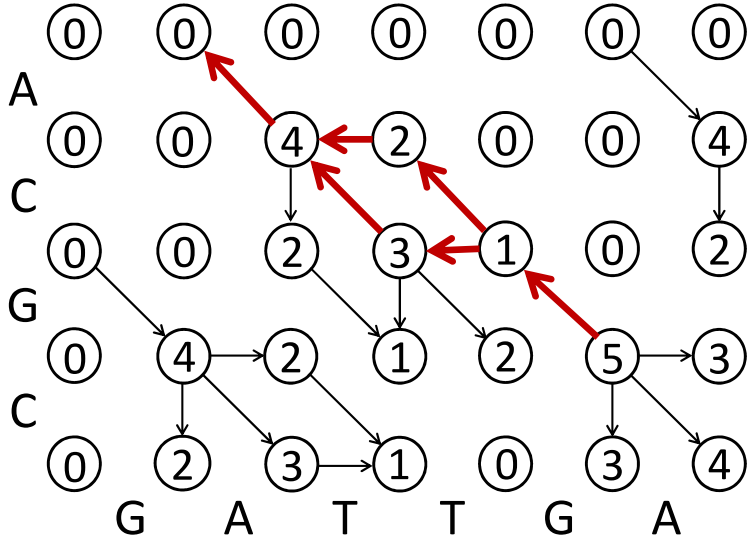


Global

(Adapted from R Altman)

Global (NW) Vs. Local (SW) Alignments






```
-ACG-C      -AC-GC
 ||   |  or  ||   |
GACTAC      GACTAC
```

```
AC-G      A-CG
|  |  or  |  |
GATTGA    GATTGA
```

Global : Match: +1 Mismatch: 0 Gap: -1

Local : Match: +4 Mismatch: -1 Gap: -2

[Slightly different sum matrix layout than in preceding slides]

(Adapted from Stephen F. Altschul)

References

- Smith, T., & Waterman. (**1981**).
  Journal of Molecular Biology, 147(1), 195–197.
  Identification of common molecular subsequences.
  https://doi.org/10.1016/0022-2836(81)90087-5
  (http://www.gersteinlab.org/courses/452/10-spring/pdf/sw.pdf)
  (Just Algorithm Section)