CS481: Bioinformatics Algorithms

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Quiz 2: Local alignment

- Scores
  - Match: +3
  - Mismatch: -2
  - Indel: -3 (DO NOT USE AFFINE GAP MODEL)
- Write DP equations for local alignment
- Fill DP matrix with backtracking for:
  - S1 = GACAGC; S2= GCGTCTAGT
- Show the alignment path and write the best local alignment

The Local Alignment Recurrence

- The largest value of $s_{i,j}$ over the whole edit graph is the score of the best local alignment.
- In the traceback, start with the cell that has the highest score and work back until a cell with a score of 0 is reached

- The recurrence:

$$s_{i,j} = \max \begin{cases} 0 \\ s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \end{cases}$$

there is only this change from the original recurrence of a Global Alignment - since there is only one “free ride” edge entering into every vertex

Quiz 2: Local alignment

$$s_{i,j} = \max \begin{cases} 0 \\ s_{i-1,j-1} + 3 \text{ if } S1[i]=S2[j] \\ s_{i-1,j-1} - 2 \text{ if } S1[i]\neq S2[j] \\ s_{i-1,j} - 3 \\ s_{i,j-1} - 3 \end{cases}$$

		G	C	G	T	C	T	A	G	T
	0	0	0	0	0	0	0	0	0	0
G	0	3	0	3	0	0	0	0	3	0
A	0	0	1	0	1	0	0	3	0	1
C	0	0	3	0	0	4	1	0	1	0
A	0	0	0	1	0	1	2	4	1	0
G	0	3	0	3	0	0	0	1	7	4
C	0	0	6	3	1	3	0	0	4	5

Quiz 2: Local alignment

```
G   T   C   T   A   G
|   x   |       |   |
G   A   C   -   A   G
```

		G	C	G	T	C	T	A	G	T
	0	0	0	0	0	0	0	0	0	0
G	0	3	0	3	0	0	0	0	3	0
A	0	0	1	0	1	0	0	3	0	1
C	0	0	3	0	0	4	1	0	1	0
A	0	0	0	1	0	1	2	4	1	0
G	0	3	0	3	0	0	0	1	7	4
C	0	0	6	3	1	3	0	0	4	5

MULTIPLE SEQUENCE ALIGNMENT

Multiple Alignment versus Pairwise Alignment

- Up until now we have only tried to align two sequences.
- What about more than two?
- A faint similarity between two sequences becomes significant if present in many
- Multiple alignments can reveal subtle similarities that pairwise alignments do not reveal

Generalizing the Notion of Pairwise Alignment

- Alignment of 2 sequences is represented as a 2-row matrix
- In a similar way, we represent alignment of 3 sequences as a 3-row matrix

```
A T _ G C G _
A _ C G T _ A
A T C A C _ A
```

- Score: more conserved columns, better alignment

Alignments = Paths in…

- Align 3 sequences: ATGC, AATC,ATGC

	A	--	T	G	C

	A	A	T	--	C

	--	A	T	G	C

Alignment Paths

0	1	1	2	3	4
	A	--	T	G	C

	A	A	T	--	C

	--	A	T	G	C

x coordinate

Alignment Paths

- Align the following 3 sequences:

ATGC, AATC,ATGC

0	1	1	2	3	4
	A	--	T	G	C
0	1	2	3	3	4
	A	A	T	--	C
	--	A	T	G	C

x coordinate

y coordinate

-

Alignment Paths

0	1	1	2	3	4
	A	--	T	G	C
0	1	2	3	3	4
	A	A	T	--	C
0	0	1	2	3	4
	--	A	T	G	C

x coordinate

y coordinate

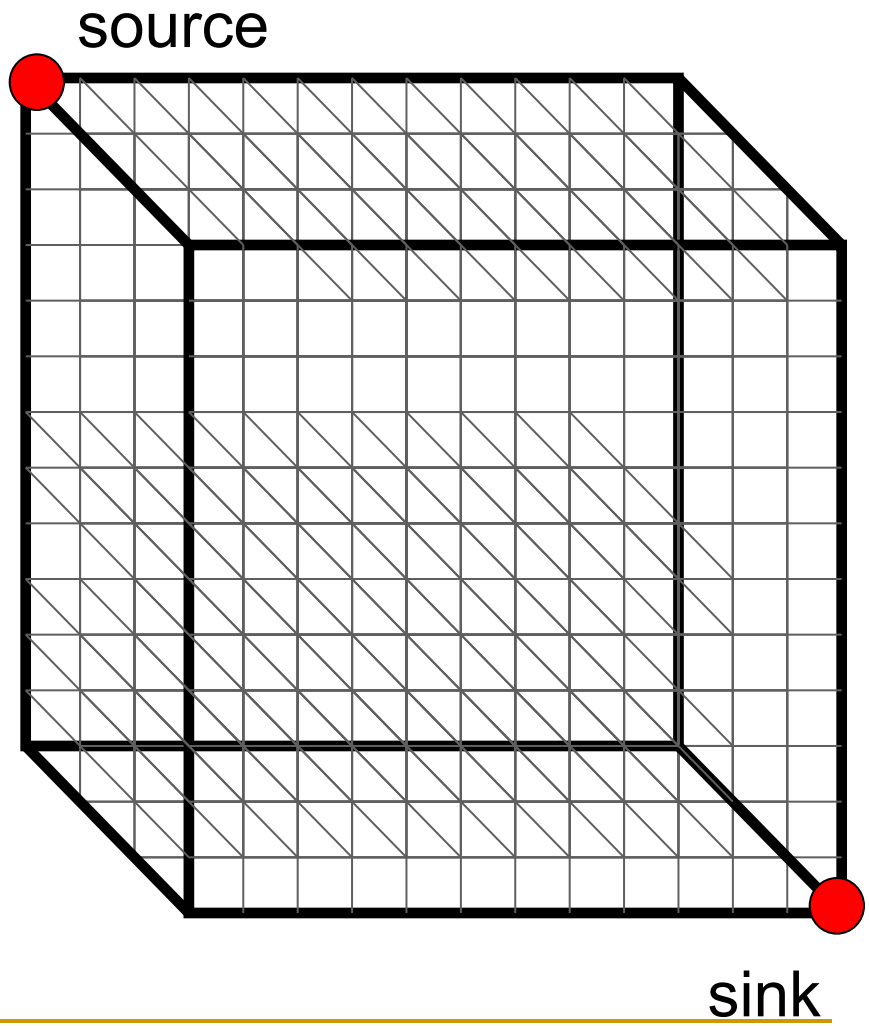
z coordinate

- Resulting path in *(x,y,z)* space:

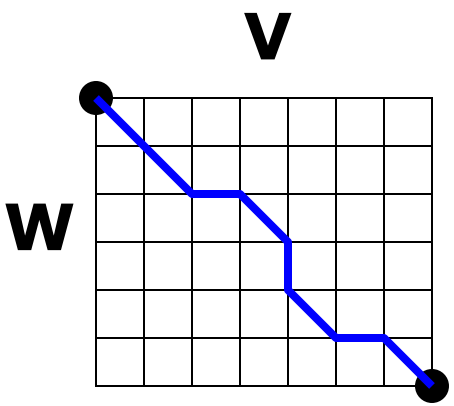
$$(0,0,0)\rightarrow(1,1,0)\rightarrow(1,2,1)\rightarrow(2,3,2)\rightarrow(3,3,3)\rightarrow(4,4,4)$$

Aligning Three Sequences

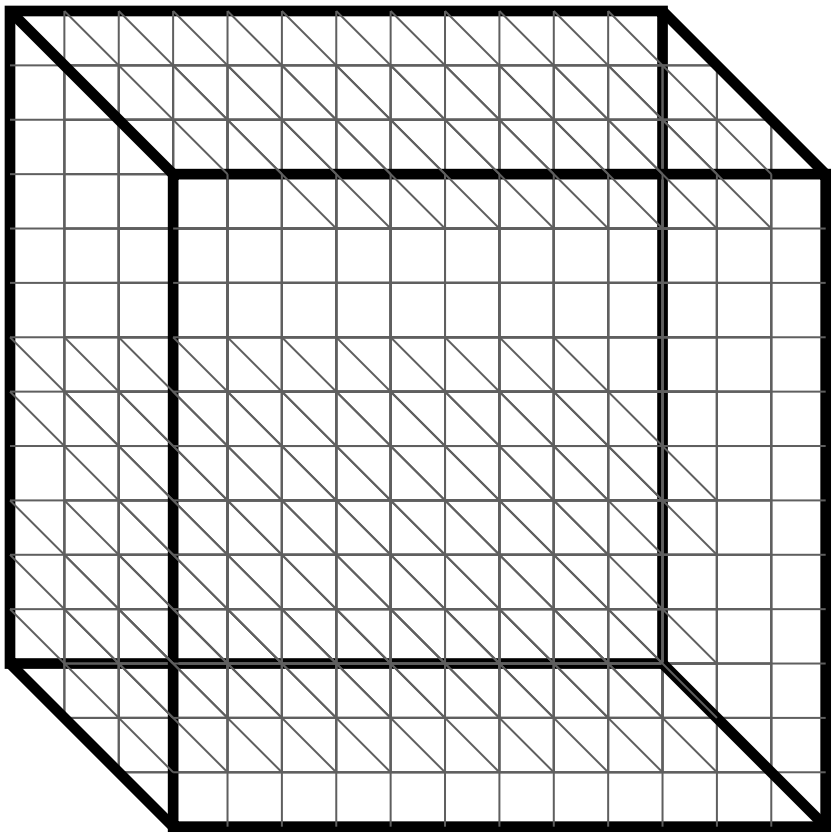
- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink



2-D vs 3-D Alignment Grid

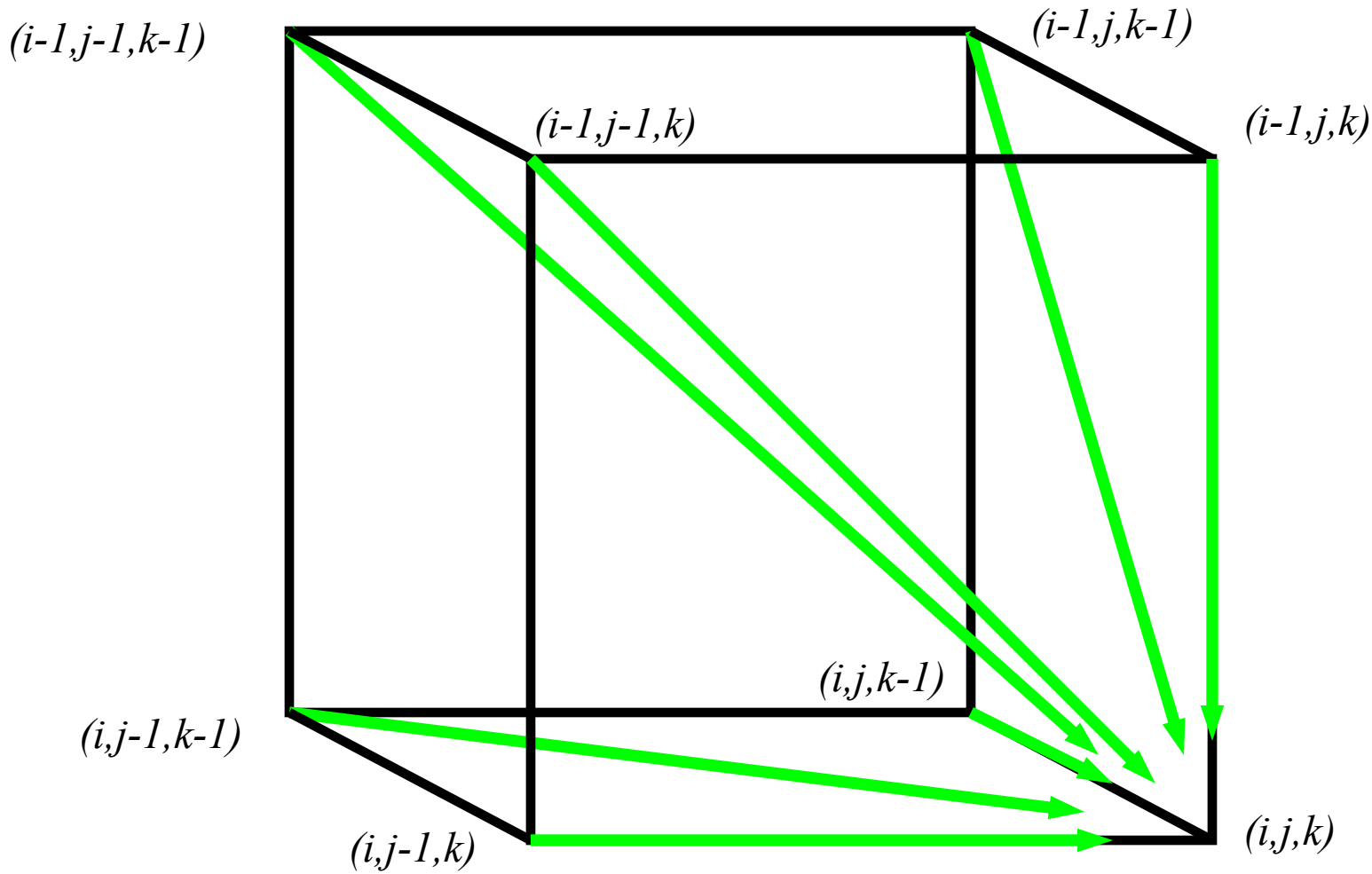


2-D edit graph



3-D edit graph

Architecture of 3-D Alignment Cell



Multiple Alignment: Dynamic Programming

- $$s_{i,j,k} = \max \begin{cases} s_{i-1,j-1,k-1} + \delta(v_i, w_j, u_k) & \text{cube diagonal: no indels} \\ s_{i-1,j-1,k} + \delta(v_i, w_j, \_) & \\ s_{i-1,j,k-1} + \delta(v_i, \_, u_k) & \text{face diagonal: one indel} \\ s_{i,j-1,k-1} + \delta(\_, w_j, u_k) & \\ s_{i-1,j,k} + \delta(v_i, \_, \_) & \\ s_{i,j-1,k} + \delta(\_, w_j, \_) & \text{edge diagonal: two indels} \\ s_{i,j,k-1} + \delta(\_, \_, u_k) & \end{cases}$$

- $\delta(x, y, z)$ is an entry in the 3-D scoring matrix

Multiple Alignment: Running Time

- For 3 sequences of length $\boldsymbol{n}$, the run time is $7\boldsymbol{n}^3$; $O(\boldsymbol{n}^3)$
- For $\boldsymbol{k}$ sequences, build a $\boldsymbol{k}$-dimensional Manhattan, with run time $(2^{\boldsymbol{k}}-1)(\boldsymbol{n}^{\boldsymbol{k}})$; $O(2^{\boldsymbol{k}}\boldsymbol{n}^{\boldsymbol{k}})$
- Conclusion: dynamic programming approach for alignment between two sequences is easily extended to $\boldsymbol{k}$ sequences but it is impractical due to exponential running time

Multiple Alignment Induces Pairwise Alignments

Every multiple alignment induces pairwise alignments

```
x:  AC-GCGG-C
y:  AC-GC-GAG
z:  GCCGC-GAG
```

Induces:

```
x: ACGCGG-C;   x: AC-GCGG-C;   y: AC-GCGAG
y: ACGC-GAC;   z: GCCGC-GAG;   z: GCCGCGAG
```

Reverse Problem: Constructing Multiple Alignment from Pairwise Alignments

Given 3 arbitrary pairwise alignments:

```
x: ACGCTGG-C;   x: AC-GCTGG-C;   y: AC-GC-GAG
y: ACGC--GAC;   z: GCCGCA-GAG;   z: GCCGCAGAG
```

can we construct a multiple alignment that induces them?

Reverse Problem: Constructing Multiple Alignment from Pairwise Alignments

Given 3 arbitrary pairwise alignments:

```
x: ACGCTGG-C;   x: AC-GCTGG-C;   y: AC-GC-GAG
y: ACGC--GAC;   z: GCCGCA-GAG;   z: GCCGCAGAG
```

can we construct a multiple alignment that induces them?

NOT ALWAYS

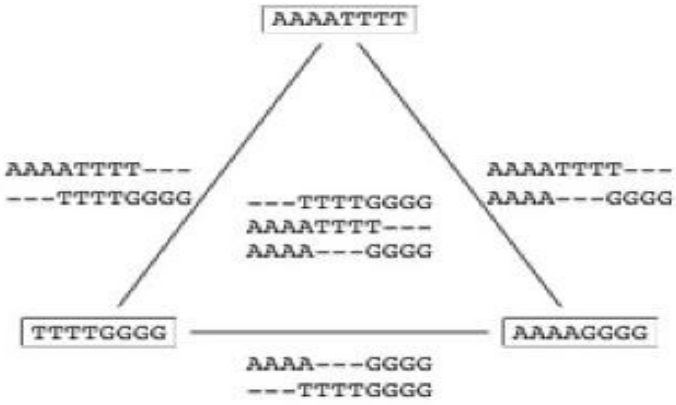
Pairwise alignments may be inconsistent

Inferring Multiple Alignment from Pairwise Alignments

- From an optimal multiple alignment, we can infer pairwise alignments between all pairs of sequences, but they are not necessarily optimal
- It is difficult to infer a “good” multiple alignment from optimal pairwise alignments between all sequences

Combining Optimal Pairwise Alignments into Multiple Alignment

Can combine pairwise alignments into multiple alignment



(a) Compatible pairwise alignments

Can ***not*** combine pairwise alignments into multiple alignment

(b) Incompatible pairwise alignments

Profile Representation of Multiple Alignment

-	A	G	G	C	T	A	T	C	A	C	C	T	G
T	A	G	-	C	T	A	C	C	A	-	-	-	G
C	A	G	-	C	T	A	C	C	A	-	-	-	G
C	A	G	-	C	T	A	T	C	A	C	-	G	G
C	A	G	-	C	T	A	T	C	G	C	-	G	G

A		1				1			.8				
C	.6			1			.4	1		.6	.2		
G			1	.2					.2			.4	1
T	.2				1		.6					.2	
-	.2			.8						.4	.8	.4	

Profile Representation of Multiple Alignment

-	A	G	G	C	T	A	T	C	A	C	C	T	G
T	A	G	-	C	T	A	C	C	A	-	-	-	G
C	A	G	-	C	T	A	C	C	A	-	-	-	G
C	A	G	-	C	T	A	T	C	A	C	-	G	G
C	A	G	-	C	T	A	T	C	G	C	-	G	G

A		1				1			.8				
C	.6			1			.4	1		.6	.2		
G			1	.2					.2			.4	1
T	.2				1		.6					.2	
-	.2			.8						.4	.8	.4	

In the past we were aligning a **sequence against a sequence**

Can we align a **sequence against a profile?**

Can we align a **profile against a profile?**

Aligning alignments

- Given two alignments, can we align them?

```
x GGGCACTGCAT
y GGTTACGTC--     Alignment 1
z GGGAACTGCAG

w GGACGTACC--     Alignment 2
v GGACCT-----
```

Aligning alignments

- Given two alignments, can we align them?
- Hint: use alignment of corresponding profiles

```
x GGGCACTGCAT
y GGTTACGTC--     Combined Alignment
z GGGAACTGCAG
w GGACGTACC--
v GGACCT-----
```

Multiple Alignment: Greedy Approach

- Choose most similar pair of strings and combine into a profile , thereby reducing alignment of *k* sequences to an alignment of of *k-1* sequences/profiles. **Repeat**
- This is a heuristic greedy method

$$k \left\{ \begin{array}{l} u_1 = \text{ACGTACGTACGT}\ldots \\ u_2 = \text{TTAATTAATTAA}\ldots \\ u_3 = \text{ACTACTACTACT}\ldots \\ \ldots \\ u_k = \text{CCGGCCGGCCGG} \end{array} \right. \longrightarrow \left. \begin{array}{l} u_1 = \text{ACg/tTACg/tTACg/cT}\ldots \\ u_2 = \text{TTAATTAATTAA}\ldots \\ \ldots \\ u_k = \text{CCGGCCGGCCGG}\ldots \end{array} \right\} k\text{-}1$$

Greedy Approach: Example

- Consider these 4 sequences

s1	GATTCA
s2	GTCTGA
s3	GATATT
s4	GTCAGC

Greedy Approach: Example (cont'd)

- There are $\binom{4}{2}$ = 6 possible alignments

```
s2  GTCTGA                  s1  GATTCA--
s4  GTCAGC (score = 2)      s4  G–T-CAGC(score = 0)

s1  GAT-TCA                 s2  G-TCTGA
s2  G-TCTGA (score = 1)     s3  GATAT-T (score = -1)

s1  GAT-TCA                 s3  GAT-ATT
s3  GATAT-T (score  = 1)    s4  G-TCAGC (score = -1)
```

Greedy Approach: Example (cont'd)

s_2 *and* s_4 are closest; combine:

s2 GTCTGA
s4 GTCAGC

$s_{2,4}$ (profile) GTCt/aGa/cA

new set of 3 sequences:

s_1 GATTCA
s_3 GATATT
$s_{2,4}$ GTCt/aGa/c

Progressive Alignment

- *Progressive alignment* is a variation of greedy algorithm with a somewhat more intelligent strategy for choosing the order of alignments.
- Progressive alignment works well for close sequences, but deteriorates for distant sequences
  - Gaps in consensus string are permanent
  - Use profiles to compare sequences

ClustalW

- Popular multiple alignment tool today
- ‘W’ stands for ‘weighted’ (different parts of alignment are weighted differently).
- Three-step process

  1.) Construct pairwise alignments

  2.) Build Guide Tree

  3.) Progressive Alignment guided by the tree

Step 1: Pairwise Alignment

- Aligns each sequence again each other giving a similarity matrix
- Similarity = exact matches / sequence length (percent identity)

	v_1	v_2	v_3	v_4
v_1	-			
v_2	.17	-		
v_3	.87	.28	-	
v_4	.59	.33	.62	-

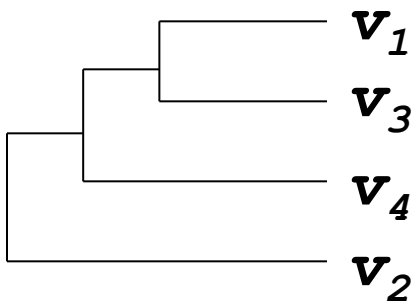
(.17 means 17 % identical)

Step 2: Guide Tree

- Create Guide Tree using the similarity matrix
  - ClustalW uses the neighbor-joining method
  - Guide tree roughly reflects evolutionary relations

Step 2: Guide Tree (cont'd)

	v_1	v_2	v_3	v_4
v_1	-			
v_2	.17	-		
v_3	.87	.28	-	
v_4	.59	.33	.62	-



Calculate:

$V_{1,3}$ = alignment (v_1, v_3)

$V_{1,3,4}$ = alignment$((V_{1,3}), v_4)$

$V_{1,2,3,4}$ = alignment$((V_{1,3,4}), v_2)$

Step 3: Progressive Alignment

- Start by aligning the two most similar sequences
- Following the guide tree, add in the next sequences, aligning to the existing alignment
- Insert gaps as necessary

```
FOS_RAT         PEEMSVTS-LDLTGGLPEATTPESEEAFTLPLLNDPEPK-PSLEPVKNISNMELKAEPFD
FOS_MOUSE       PEEMSVAS-LDLTGGLPEASTPESEEAFTLPLLNDPEPK-PSLEPVKSISNVELKAEPFD
FOS_CHICK       SEELAAATALDLG----APSPAAAEEAFALPLMTEAPPAVPPKEPSG--SGLELKAEPFD
FOSB_MOUSE      PGPGPLAEVRDLPG-----STSAKEDGFGWLLPPPPPPP-----------------LPFQ
FOSB_HUMAN      PGPGPLAEVRDLPG-----SAPAKEDGFSWLLPPPPPPP-----------------LPFQ
                .   . :   ** .     :..  *:.*   *   . *                  **:
```

Dots and stars show how well-conserved a column is.

SCORING ALIGNMENTS

Multiple Alignments: Scoring

- Number of matches (multiple longest common subsequence score)
- Entropy score
- Sum of pairs (SP-Score)

Multiple LCS Score

- A column is a “match” if all the letters in the column are the same

AAA
AAA
AAT
ATC

- Only good for very similar sequences

Entropy

- Define frequencies for the occurrence of each letter in each column of multiple alignment
  - $p_A = 1$, $p_T = p_G = p_C = 0$ (1st column)
  - $p_A = 0.75$, $p_T = 0.25$, $p_G = p_C = 0$ (2nd column)
  - $p_A = 0.50$, $p_T = 0.25$, $p_C = 0.25$ $p_G = 0$ (3rd column)
- Compute entropy of each column

$$-\sum_{X=A,T,G,C} p_X \log p_X$$

AAA
AAA
AAT
ATC

Entropy: Example

$$entropy\begin{pmatrix} A \\ A \\ A \\ A \end{pmatrix} =)$$

Best case

Worst case

$$entropy\begin{pmatrix} A \\ T \\ G \\ C \end{pmatrix} = -\sum_{+} \log\frac{1}{4} = -\left(\frac{1}{4} * \cdot\right) = ?$$

Multiple Alignment: Entropy Score

Entropy for a multiple alignment is the sum of entropies of its columns:

$$\Sigma_{\text{ over all columns }} \Sigma_{X=A,T,G,C}\, p_X \log p_X$$

Entropy of an Alignment: Example

column entropy:

$$-(p_A \log p_A + p_C \log p_C + p_G \log p_G + p_T \log p_T)$$

A	A	A
A	C	C
A	C	G
A	C	T

- Column 1 = -[1*log(1) + 0*log0 + 0*log0 +0*log0]
  = 0
- Column 2 = -[($^1/_4$)*log($^1/_4$) + ($^3/_4$)*log($^3/_4$) + 0*log0 + 0*log0]
  = -[($^1/_4$)*(-2) + ($^3/_4$)*(-.415)] = +0.811
- Column 3 = -[($^1/_4$)*log($^1/_4$)+($^1/_4$)*log($^1/_4$)+($^1/_4$)*log($^1/_4$) +($^1/_4$)*log($^1/_4$)]
  = 4* -[($^1/_4$)*(-2)] = +2.0
- Alignment Entropy = 0 + 0.811 + 2.0 = +2.811

Multiple Alignment Induces Pairwise Alignments

Every multiple alignment induces pairwise alignments

```
x:  AC-GCGG-C
y:  AC-GC-GAG
z:  GCCGC-GAG
```

Induces:

```
x: ACGCGG-C;   x: AC-GCGG-C;   y: AC-GCGAG
y: ACGC-GAC;   z: GCCGC-GAG;   z: GCCGCGAG
```

Not necessarily optimal

Sum of Pairs Score(SP-Score)

- Consider pairwise alignment of sequences

  a_i and a_j

  imposed by a multiple alignment of k sequences
- Denote the score of this suboptimal (not necessarily optimal) pairwise alignment as

  $$s^*(a_i, a_j)$$
- Sum up the pairwise scores for a multiple alignment:

  $$s(a_1, \ldots, a_k) = \Sigma_{i,j}\, s^*(a_i, a_j)$$

Computing SP-Score

Aligning 4 sequences: 6 pairwise alignments

Given a_1, a_2, a_3, a_4:

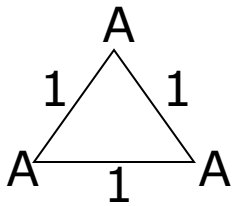
$$s(a_1 \ldots a_4) = \Sigma s^*(a_i, a_j) = s^*(a_1, a_2) + s^*(a_1, a_3) + s^*(a_1, a_4) + s^*(a_2, a_3) + s^*(a_2, a_4) + s^*(a_3, a_4)$$

SP-Score: Example

a_1 ATG–C–AAT
. A–G–CATAT
a_k ATCCCATTT

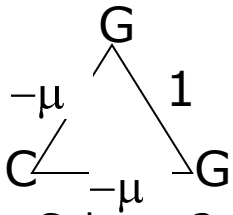
To calculate each column:

$$s'(a_1 \ldots a_k) = \sum_{i,j} s^*(a_i, a_j) \longleftarrow \binom{n}{2} \text{Pairs of Sequences}$$



Column 1

Score=3



Column 3

Score = $1 - 2\mu$

Back to guide trees for MSA

- Guide tree construction
  - UPGMA
  - Neighbor Joining
  - ….
- Easy MSA: Center Star

Star alignments

- Construct multiple alignments using pair-wise alignment relative to a fixed sequence
- Out of a set $S = \{S_1, S_2, \ldots, S_r\}$ of sequences, pick sequence S_c that maximizes

  $$\text{star\_score}(c) = \sum \{\text{sim}(S_c, S_i) : 1 \leq i \leq r, i \neq c\}$$

  where $\text{sim}(S_i, S_j)$ is the optimal score of a pair-wise alignment between S_i and S_j

Star alignment Algorithm

1. Compute sim(S_i, S_j) for every pair (i,j)
2. Compute star_score(i) for every i
3. Choose the index c that minimizes star_score(c) and make it the center of the star
4. Produce a multiple alignment M such that, for every i, the induced pairwise alignment of S_c and S_i is the same as the optimum alignment of S_c and S_i.

Star alignment example

S_c	AA--CCTT	S_c	A-ACC-TT
S_1	AATGCC--	S_2	AGACCGT-

S_c	A-A--CC-TT
S_1	A-ATGCC---
S_2	AGA--CCGT-

Multiple Alignment: History

1975 Sankoff

Formulated multiple alignment problem and gave dynamic programming solution

1988 Carrillo-Lipman

Branch and Bound approach for MSA

1990 Feng-Doolittle

Progressive alignment

1994 Thompson-Higgins-Gibson-ClustalW

Most popular multiple alignment program

1998 Morgenstern et al.-DIALIGN

Segment-based multiple alignment

2000 Notredame-Higgins-Heringa-T-coffee

Using the library of pairwise alignments

2004 MUSCLE

Problems with Multiple Alignment

- Multidomain proteins evolve not only through point mutations but also through domain duplications and domain recombinations
- Although MSA is a 30 year old problem, there were no MSA approaches for aligning **rearranged** sequences (i.e., multi-domain proteins with shuffled domains) prior to 2002
- Often impossible to align all protein sequences throughout their entire length