CS481/CS583: Bioinformatics Algorithms

Can Alkan
EA509
calkan@cs.bilkent.edu.tr

http://www.cs.bilkent.edu.tr/~calkan/teaching/cs481/

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

x coordinate

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

y coordinate

	--	A	T	G	C

-

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

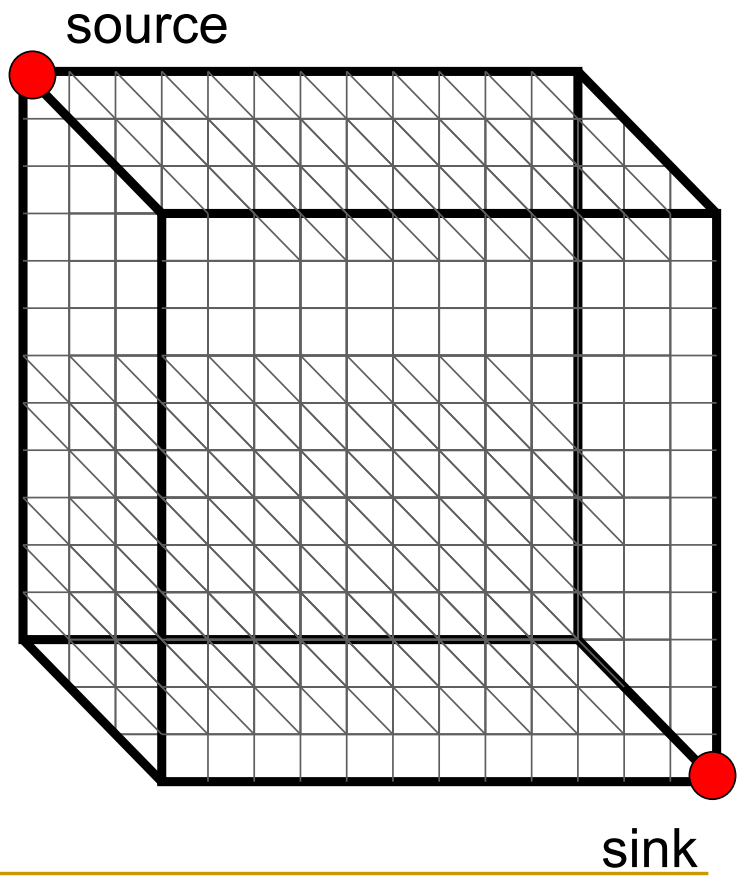
z coordinate

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

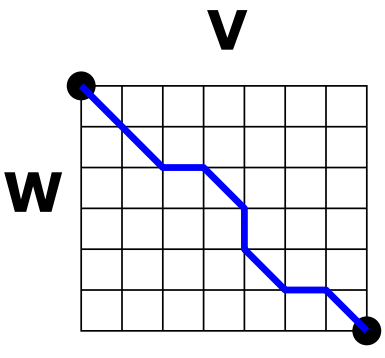
(0,0,0)→(1,1,0)→(1,2,1) →(2,3,2) →(3,3,3) →(4,4,4)

Aligning Three Sequences

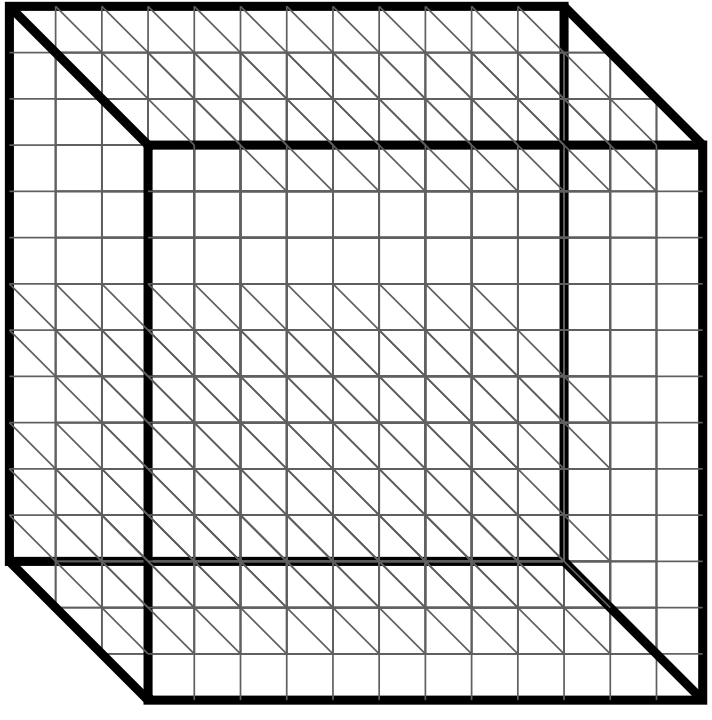
- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink



2-D vs 3-D Alignment Grid

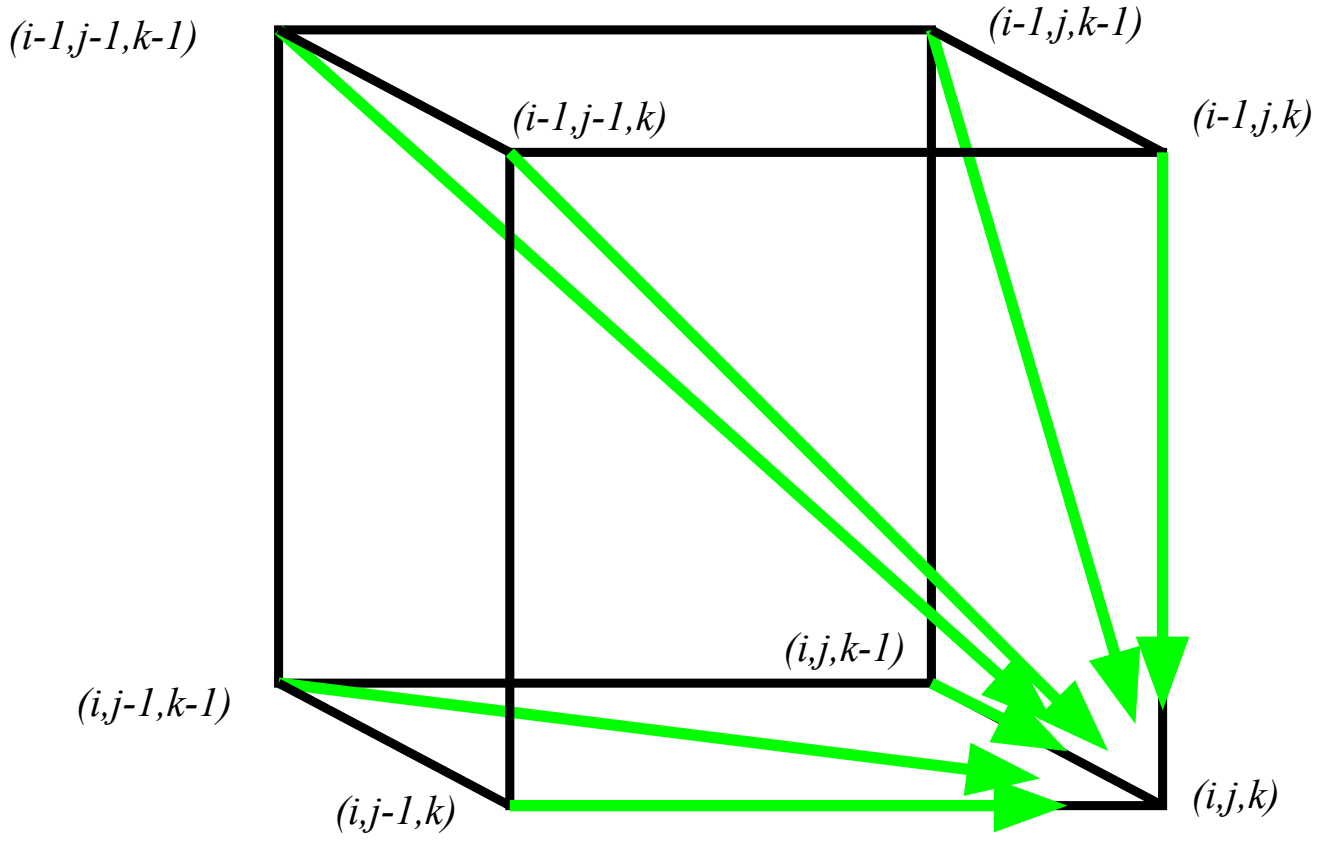


2-D edit graph



3-D edit graph

Architecture of 3-D Alignment Cell



Multiple Alignment: Dynamic Programming

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

  cube diagonal: no indels

  face diagonal: one indel

  edge diagonal: two indels

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

Multiple Alignment: Running Time

- For 3 sequences of length ***n***, the run time is $7n^3$; $O(n^3)$
- For ***k*** sequences, build a ***k***-dimensional Manhattan, with run time $(2^k-1)(n^k)$; $O(2^k n^k)$
- Conclusion: dynamic programming approach for alignment between two sequences is easily extended to ***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

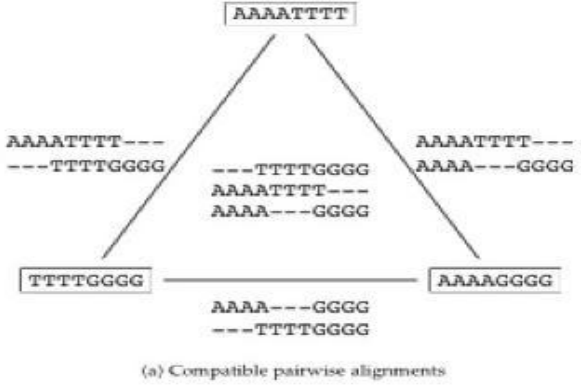
Pairwise alignments may be inconsistent

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

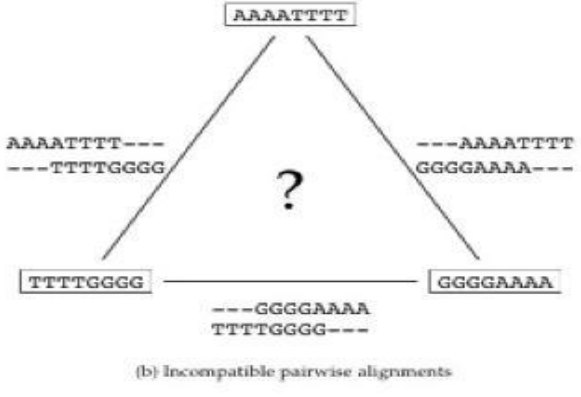
Combining Optimal Pairwise Alignments into Multiple Alignment

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-----
```

Sequence to profile alignment

Sequence S_1 to Profile C

- Let:
  - p(y,j): frequency that character y appears in column j of the profile C --- $y \in \Sigma \cup \{'-'\}$
  - S(x,j): score for aligning x with column j
    - $\sum_y [\delta(x,y) \times p(y,j)]$ -- δ(x,y) is the "regular" scoring func.
  - V(i, j): value of optimal alignment of substring S[1..i] with the first j columns of profile C

Col	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
C	.6	0	0	0	1	0	0	.4	1	0	0	.6	.2	0	0

p(C, 1) = 0.6, p(C,2) = 0, p(C, 3) = 0, …. p(C, 9) = 1 …

Sequence to profile alignment

- $V(0,j) = \sum_{k \le j} S('-', k)$
- $V(i,0) = \sum_{k \le i} \delta(S_1(k), '-')$ where $S_1(k)$ is the k^{th} character of S_1

- $$V(i,j) = max \begin{cases} V[i-1, j-1] + S(S_1(i), j) \\ V[i-1, j] + \delta(S_1(i), '-') \\ V[i, j-1] + S('-', j) \end{cases}$$

O(|∑|nm). |∑|: alphabet size, n: sequence length, m: profile length

Sequence to profile

Match = 2
Mismatch = -1
Gap = -1

	Profile														
	0	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11	-12	-13	-14
C	-1	0.8	-0.2	-1.2	-2.2	-2	-3	-4	-5	-6	-7	-8	-9	-10	-11
A	-2	-0.2	2.8	1.8	0.8	-0.2	-1.2	-1	-2	-3	-4	-5	-6	-7	-8
G	-3	-1.2	1.8	4.8	3.8	2.8	1.8	0.8	-0.2	-1.2	-2.2	-3.2	-4.2	-5.2	-5
G	-4	-2.2	0.8	3.8	4.4	3.4	2.4	1.4	0.4	-0.6	-1.6	-2.6	-3.6	-4	-3.2
T	-5	-3.2	-0.2	2.8	3.4	3.4	5.4	4.4	3.4	2.4	1.4	0.4	-0.6	-1.6	-2.6
A	-6	-4.2	-1.2	1.8	2.4	2.4	4.4	7.4	6.4	5.4	4.4	3.4	2.4	1.4	0.4
C	-7	-5.2	-2.2	0.8	1.4	4.4	3.4	6.4	7.6	8.4	7.4	6.4	5.4	4.4	3.4
C	-8	-6.2	-3.2	-0.2	0.4	3.4	3.4	5.4	6.6	9.6	8.6	8.2	7.2	6.2	5.2
A	-9	-7.2	-4.2	-1.2	-0.6	2.4	2.4	5.4	5.6	8.6	11	10	9	8	7
C	-10	-8.2	-5.2	-2.2	-1.6	1.4	1.4	4.4	5.6	7.6	10	11.8	10.8	9.8	8.8
G	-11	-9.2	-6.2	-3.2	-2.6	0.4	0.4	3.4	4.6	6.6	9	10.8	10.8	11	11.8
G	-12	-10.2	-7.2	-4.2	-3.6	-0.6	-0.6	2.4	3.6	5.6	8	9.8	9.8	11	13

C A G G - T A C C A C – G G

Sequence to profile alignment

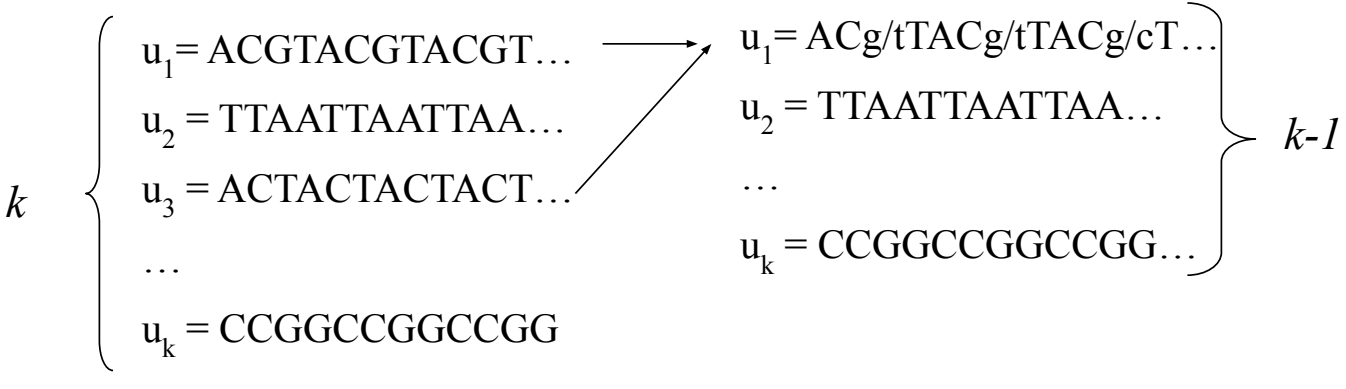
```
New Profile C:
     -  A  G  G    C   T  A  T  C    A  C  C     T     G
     T  A  G  -    C   T  A  C  C    A  -  -     -     G
     C  A  G  -    C   T  A  C  C    A  -  -     -     G
     C  A  G  -    C   T  A  T  C    A  C  -     G     G
     C  A  G  -    C   T  A  T  C    G  C  -     G     G

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


A       1                    1          .83
C   .67             .83         .5  1     .67 .17
G          1  .33                          .17          .50   1
T   .16                     1     .5                    .17
-   .17         .67 .17                      .33  .83 .33
```

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



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
- ‘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	–

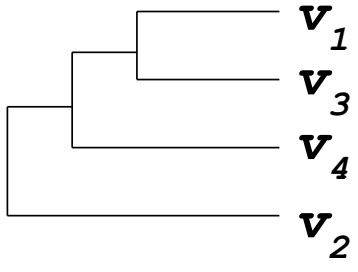
(.17 means 17 % identical)

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

$$entropy\begin{pmatrix} A \\ A \\ A \\ A \end{pmatrix} = 0$$ Best case

Worst case $$entropy\begin{pmatrix} A \\ T \\ G \\ C \end{pmatrix} = -\sum \frac{1}{4}\log\frac{1}{4} = -4(\frac{1}{4} * -2) = 2$$

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:

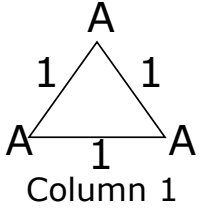
$$s(a_1 \ldots a_4) = \Sigma s^*(a_i, a_j) = s^*(a_1, a_2) + s^*(a_1, a_3) + s^*(a_1, a_4) + s^*(a_2, a_3) + s^*(a_2, a_4) + s^*(a_3, a_4)$$

SP-Score: Example

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

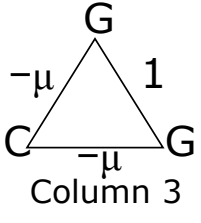
To calculate each column:

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



Column 1

Score=3



Column 3

Score = 1 − 2μ

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_1 AATGCC--

S_c A-ACC-TT

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