EECS 4425:
Introductory Computational Bioinformatics

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Course page: http://www.cse.yorku.ca/course/4425

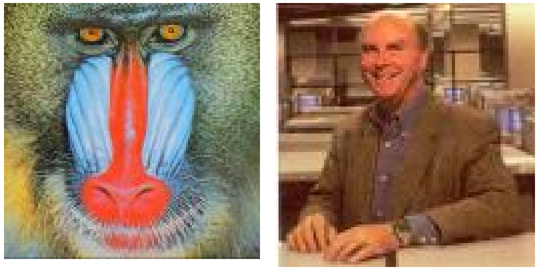
Many of the slides are taken from www.bioalgorithms.info

Next

- Multiple Alignments

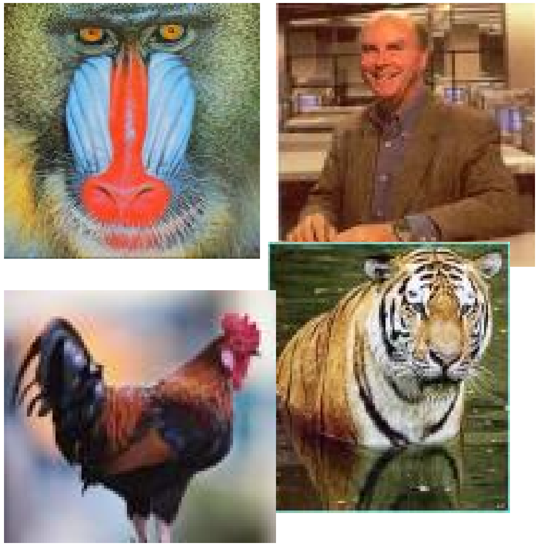
Multiple Alignment versus Pairwise Alignment

- **Up until now we have only tried to align two sequences.**



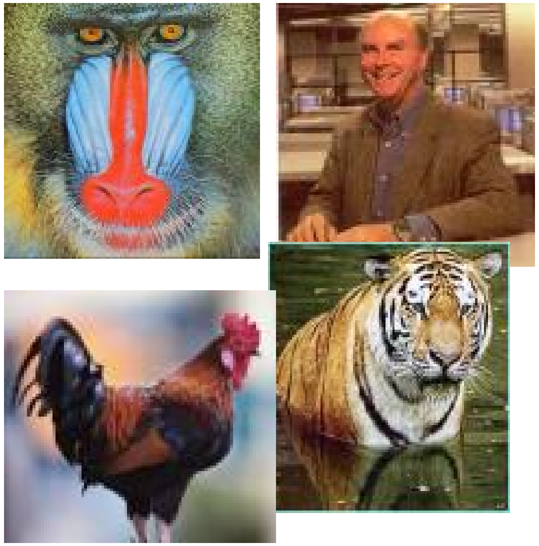
Multiple Alignment versus Pairwise Alignment

- Up until now we have only tried to align two sequences.
- What about more than two? And what for?



Multiple Alignment versus Pairwise Alignment

- Up until now we have only tried to align two sequences.
- What about more than two? And what for?
- 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

x coordinate

	A	A	T	--	C

	--	A	T	G	C

Alignment Paths

- Align the following 3 sequences:

ATGC, AATC,ATGC

0	1	1	2	3	4	*x* coordinate
	A	--	T	G	C	
0	1	2	3	3	4	*y* coordinate
	A	A	T	--	C	
	--	A	T	G	C	

-

Alignment Paths

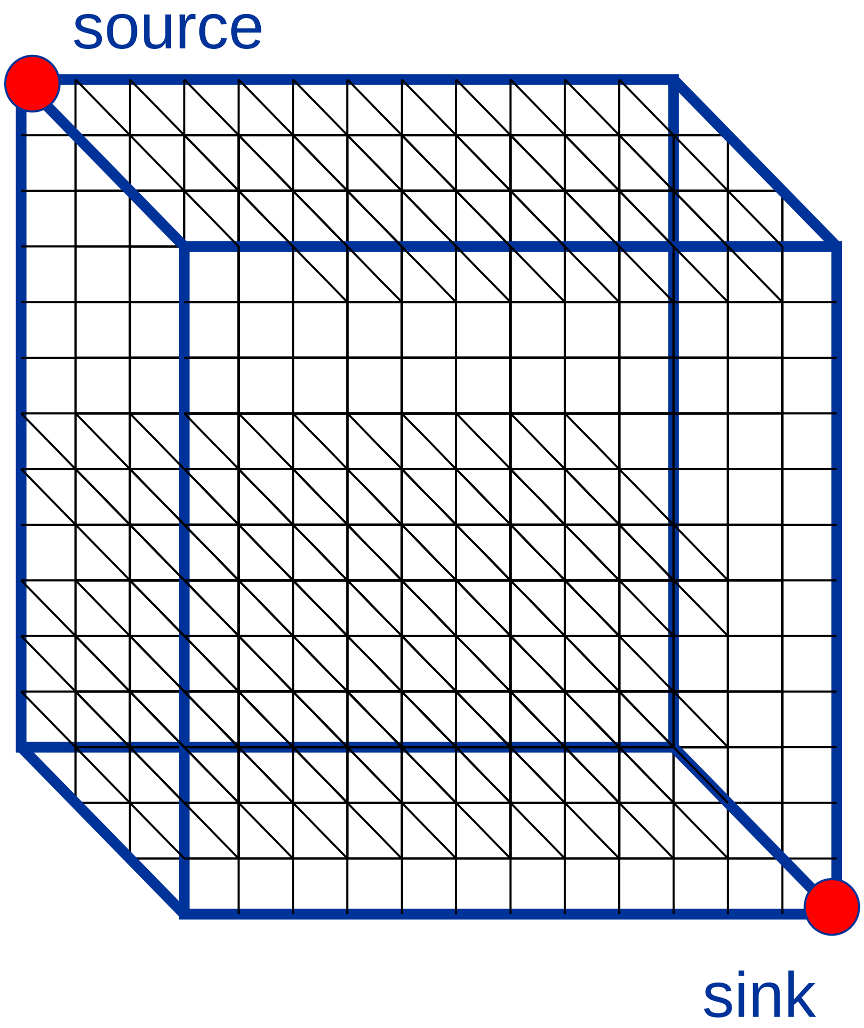
0	1	1	2	3	4	*x* coordinate
	A	--	T	G	C	
0	1	2	3	3	4	*y* coordinate
	A	A	T	--	C	
0	0	1	2	3	4	*z* coordinate
	--	A	T	G	C	

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

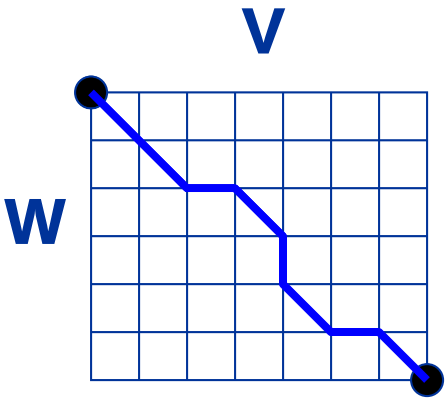
(0,0,0)→(1,1,0)→(1,2,1) →(2,3,2) →(3,3,3) →(4,4,4)

Aligning Three Sequences

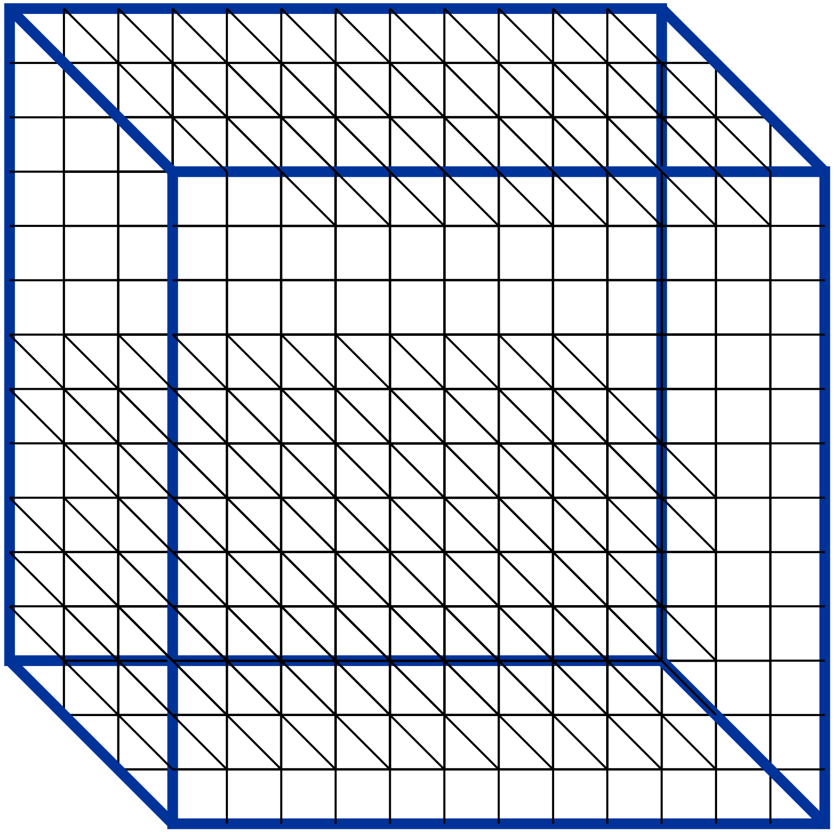
- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink



2-D vs 3-D Alignment Grid

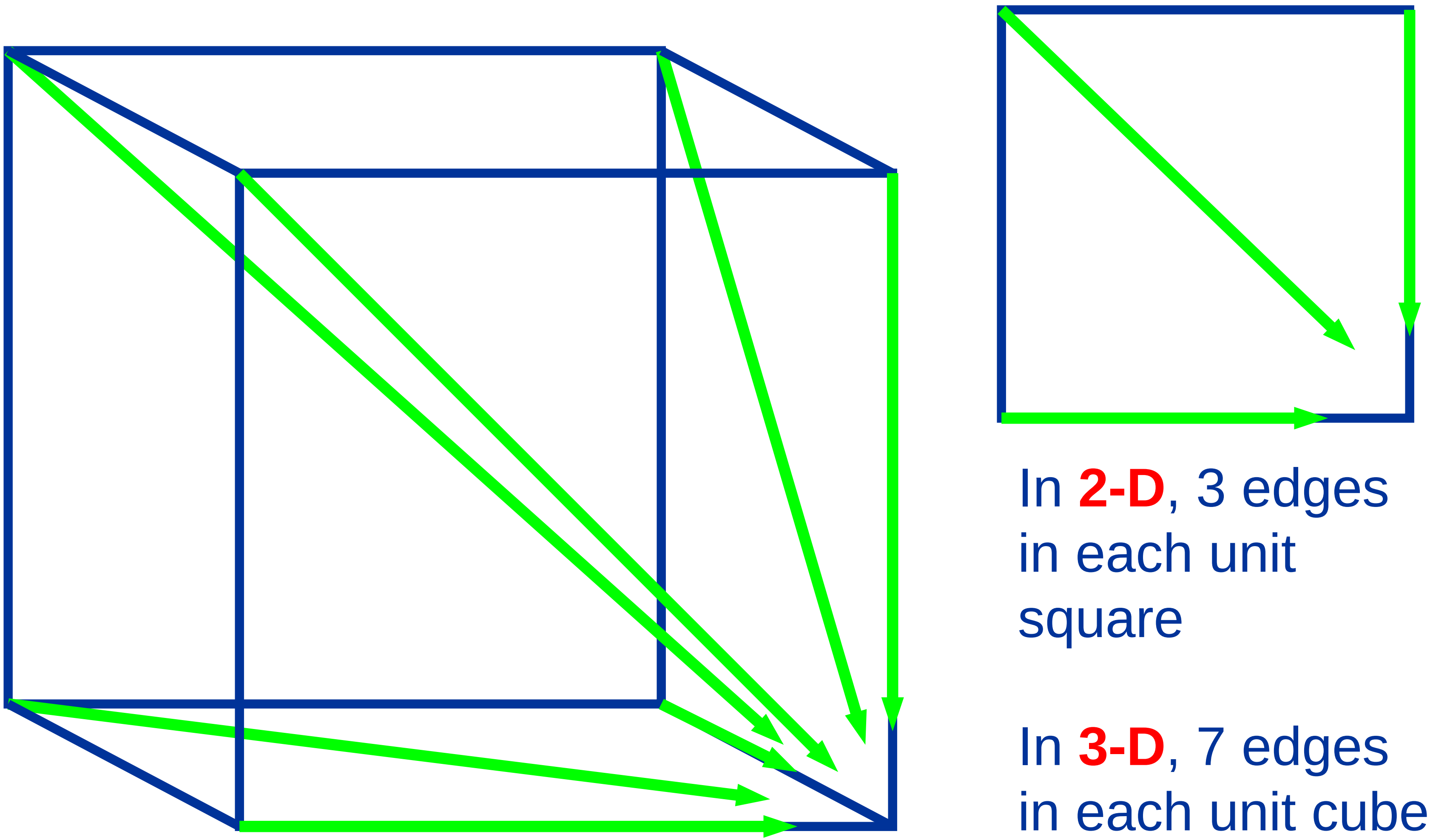


2-D edit graph



3-D edit graph

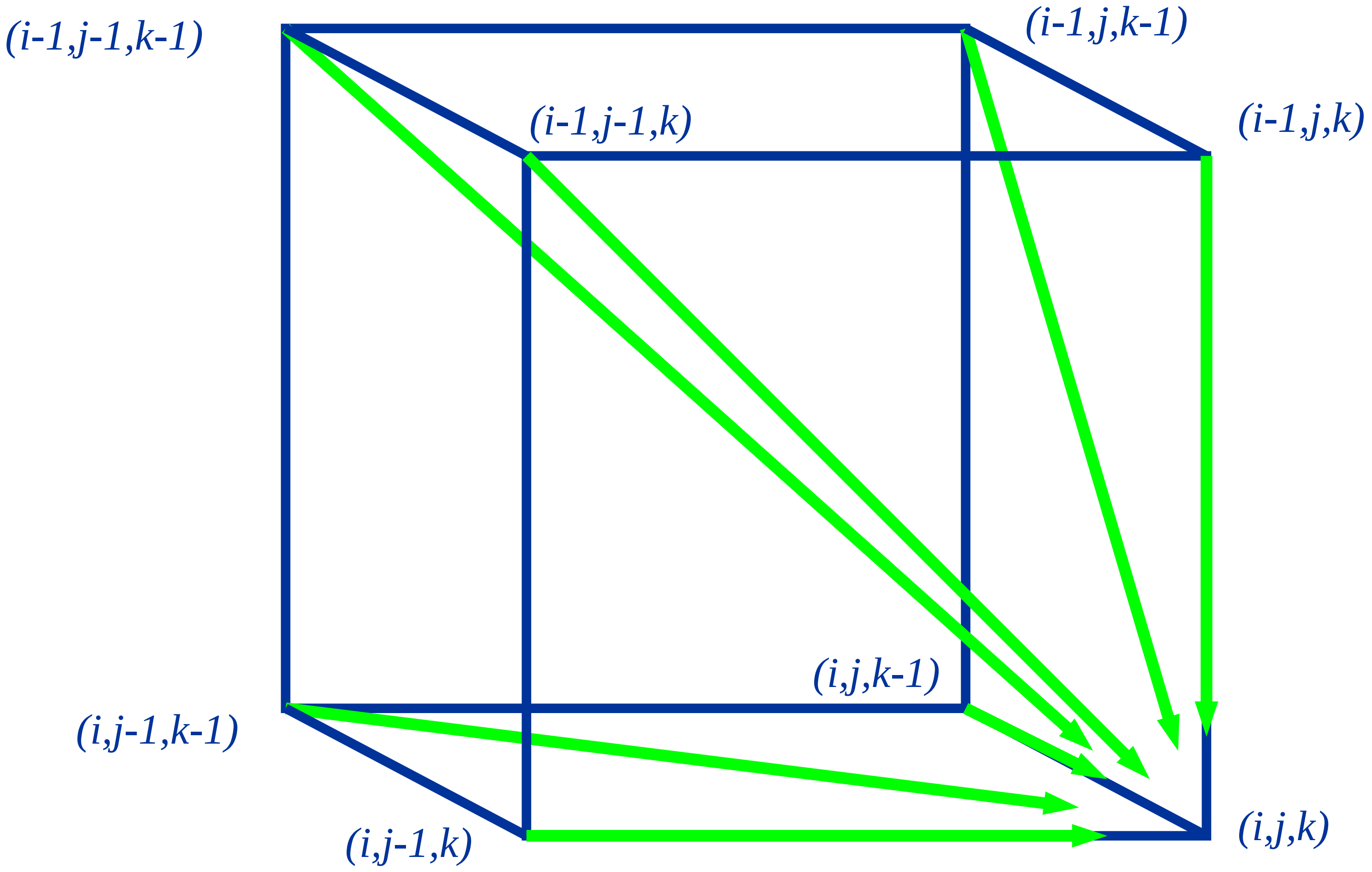
2-D cell versus 2-D Alignment Cell



In **2-D**, 3 edges in each unit square

In **3-D**, 7 edges in each unit cube

Architecture of 3-D Alignment Cell



Multiple Alignment: Dynamic Programming

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

- $\delta(x, y, z)$ 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
```

Q: can we construct a multiple alignment that induces them?

A: NOT ALWAYS

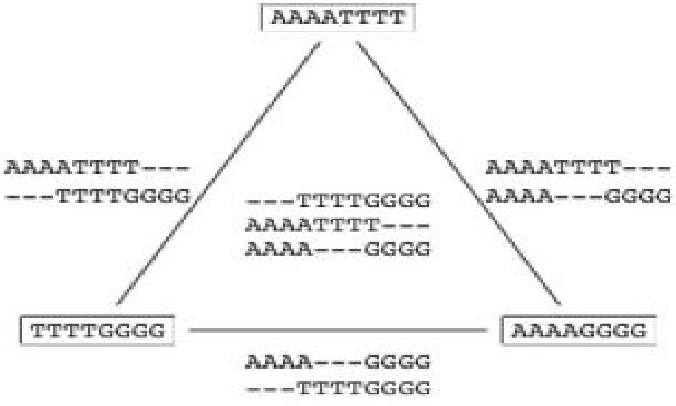
Pairwise alignments may be inconsistent

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

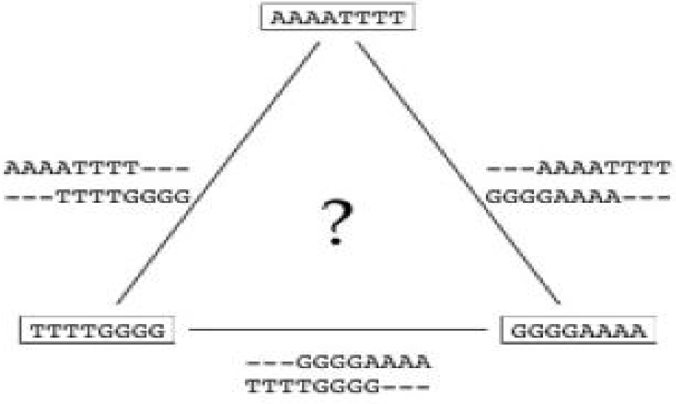
Combining Optimal Pairwise Alignments into Multiple Alignment

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	

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-----
```

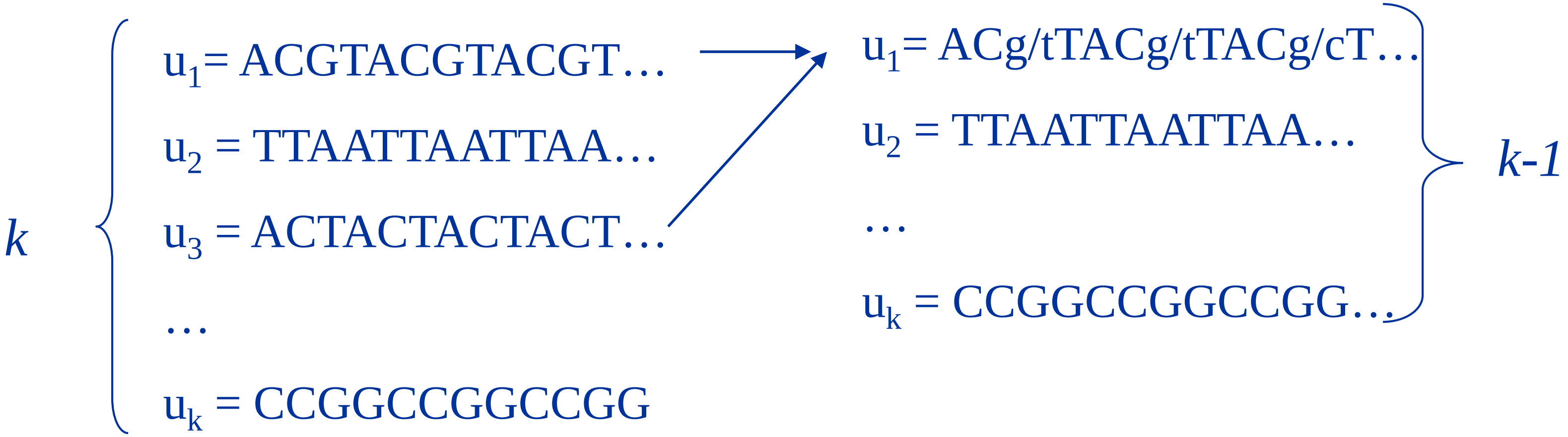
Aligning alignments

- 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

u_1= ACGTACGTACGT…

u_2 = TTAATTAATTAA…

u_3 = ACTACTACTACT…

…

u_k = CCGGCCGGCCGG

u_1= ACg/tTACg/tTACg/cT…

u_2 = TTAATTAATTAA…

…

u_k = CCGGCCGGCCGG…

k-*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
s4   GTCAGC (score = 2)
```

```
s1   GAT-TCA
s2   G-TCTGA (score = 1)
```

```
s1   GAT-TCA
s3   GATAT-T (score  = 1)
```

```
s1   GATTCA--
s4   G—T-CAGC(score = 0)
```

```
s2   G-TCTGA
s3   GATAT-T (score = -1)
```

```
s3   GAT-ATT
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)

$$\begin{array}{c|cccc} & v_1 & v_2 & v_3 & v_4 \\ \hline v_1 & - & & & \\ v_2 & .17 & - & & \\ v_3 & .87 & .28 & - & \\ v_4 & .59 & .33 & .62 & - \end{array}$$

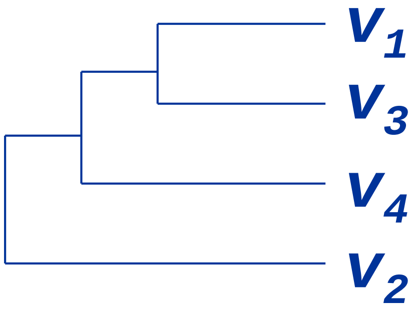
(.17 means 17 % identical)

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.

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 = $-[1*\log(1) + 0*\log 0 + 0*\log 0 + 0*\log 0]$
  $= 0$

- Column 2 = $-[(^1/_4)*\log(^1/_4) + (^3/_4)*\log(^3/_4) + 0*\log 0 + 0*\log 0]$
  $= -[\ (^1/_4)*(-2) + (^3/_4)*(-.415)\ ] = +0.811$

- 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:

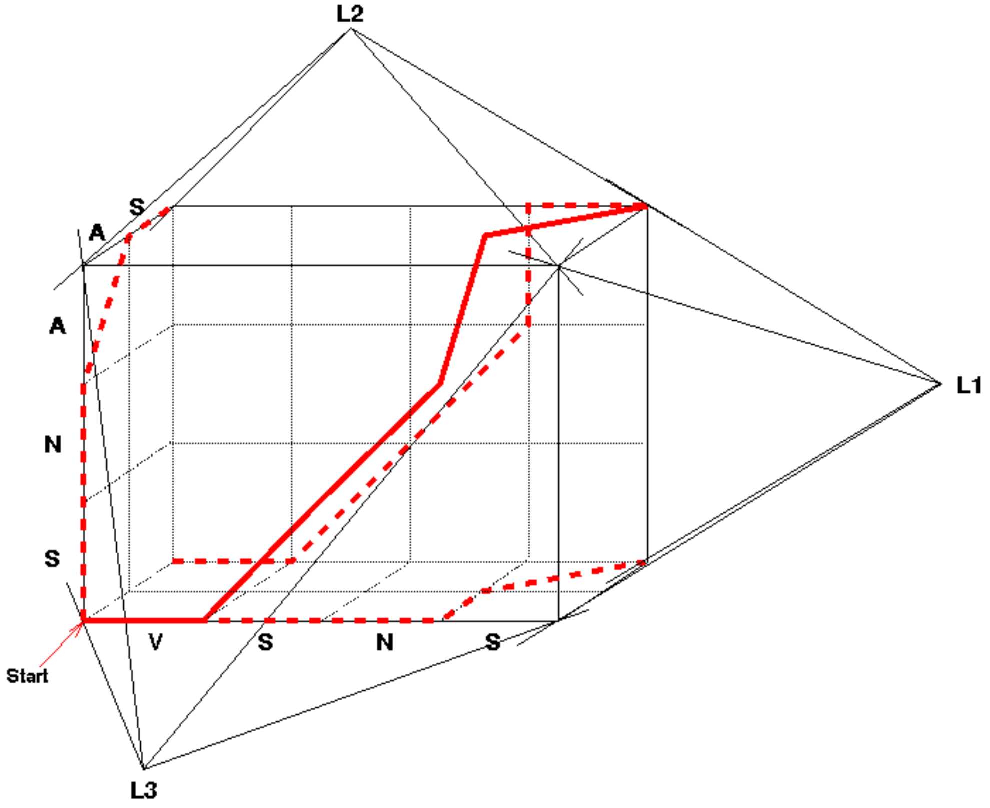
```
    x: ACGCGG-C;  x: AC-GCGG-C;  y: AC-
GCGAG
    y: ACGC-GAC;  z: GCCGC-GAG;  z:
GCCGCGAG
```

Inferring Pairwise Alignments from Multiple Alignments

- From a multiple alignment, we can infer pairwise alignments between all sequences, but they are not necessarily optimal

- This is like projecting a 3-D multiple alignment path on to a 2-D face of the cube

Multiple Alignment Projections



All 3 Pairwise Projections of the Multiple Alignment

A 3-D alignment can be projected onto the 2-D plane to represent an alignment between a pair of sequences.

Sum of Pairs Score(SP-Score)

- Consider pairwise alignment of sequences

  $$a_i \text{ and } a_j$$

  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:

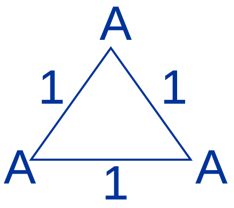
$$\begin{aligned} s(a_1 \ldots a_4) = \Sigma s^*(a_i, a_j) &= s^*(a_1, a_2) + s^*(a_1, a_3) \\ &+ s^*(a_1, a_4) + s^*(a_2, a_3) \\ &+ s^*(a_2, a_4) + s^*(a_3, a_4) \end{aligned}$$

SP-Score: Example

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

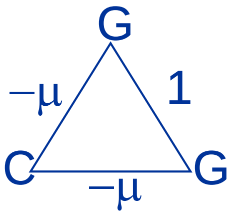
To calculate each column:

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



Column 1

Score=3



Column 3

Score = $1 - 2\mu$

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

What’s next?