Lecture 10:
Local Alignments

Study Chapter 6.8-6.10

Outline

- Edit Distances
- Longest Common Subsequence
- Global Sequence Alignment
- Scoring Matrices
- Local Sequence Alignment
- Alignment with Affine Gap Penalties
- Multiple Alignment problem

Local vs. Global Alignment

- The Global Alignment Problem tries to find the longest path between vertices *(0,0)* and (*n*,*m*) in the edit graph.

- The Local Alignment Problem tries to find the longest path among paths between **arbitrary vertices** (*i*,*j*) and (*i′*, *j′*) in the edit graph.

- In the edit graph with negatively-scored edges, Local Alignment may score higher than Global Alignment

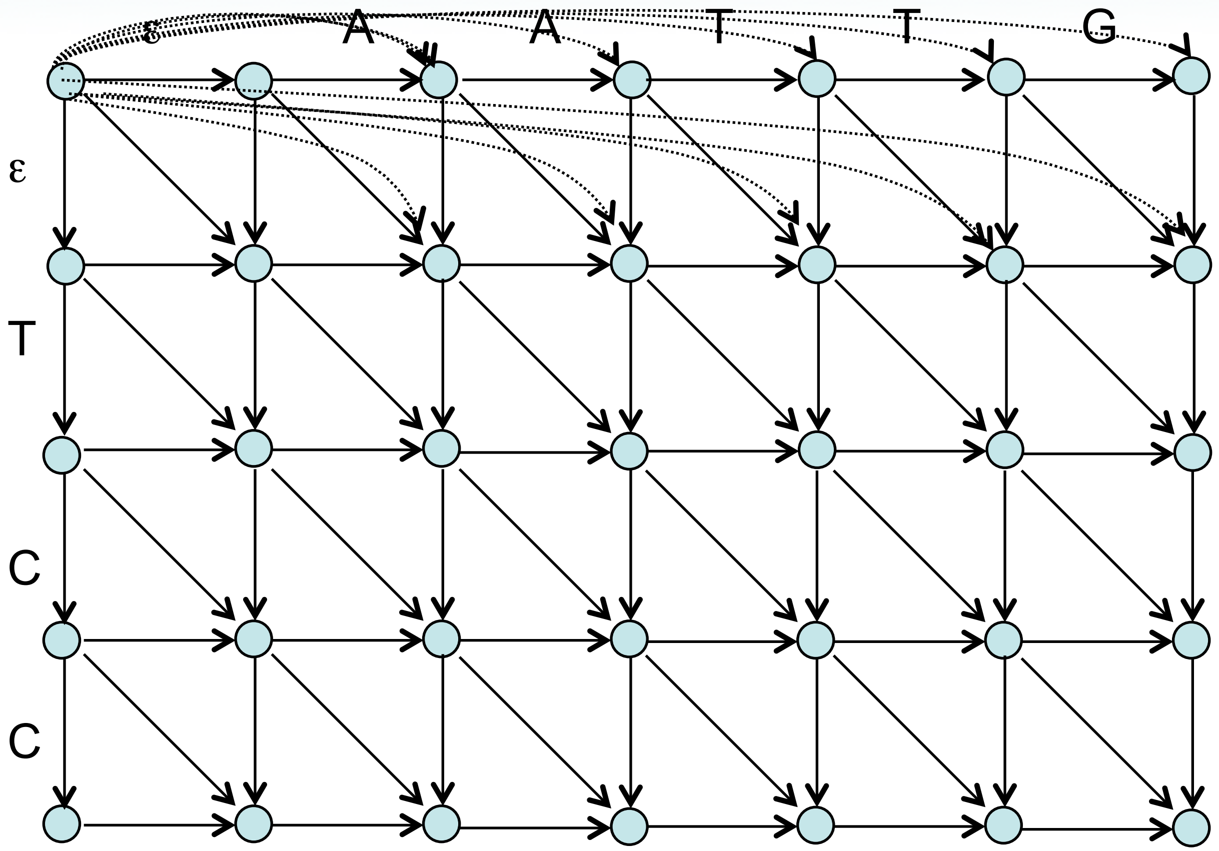
The Local Alignment Recurrence

- The largest value of $s_{i,j}$ over the whole edit graph is the score of the best local alignment.
- Smith-Waterman local alignment
- The recurrence:

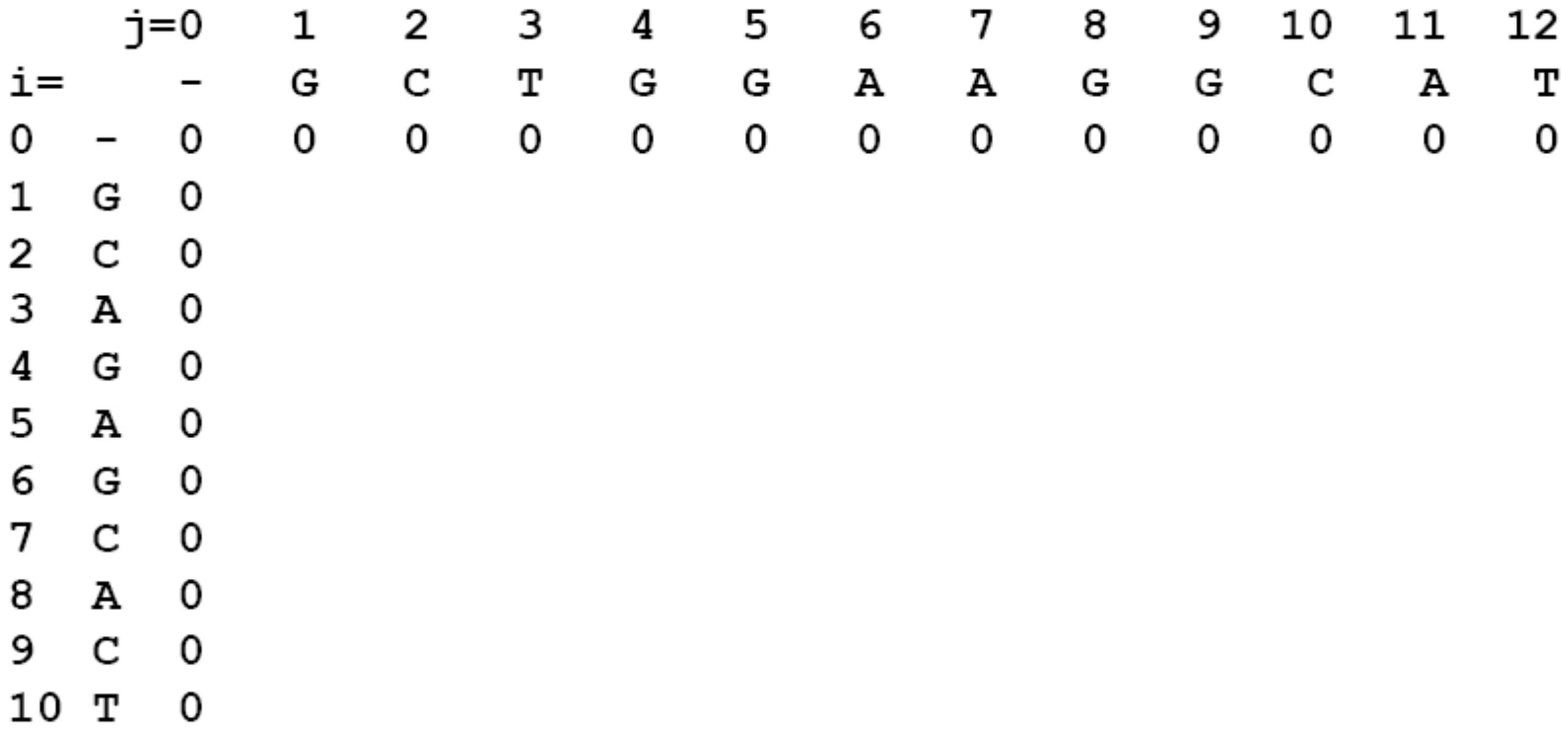
$$s_{i,j} = max \begin{cases} 0 \\ s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, -) \\ s_{i,j-1} + \delta(-, w_j) \end{cases}$$

Power of ZERO: 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

Smith-Waterman Local Alignment



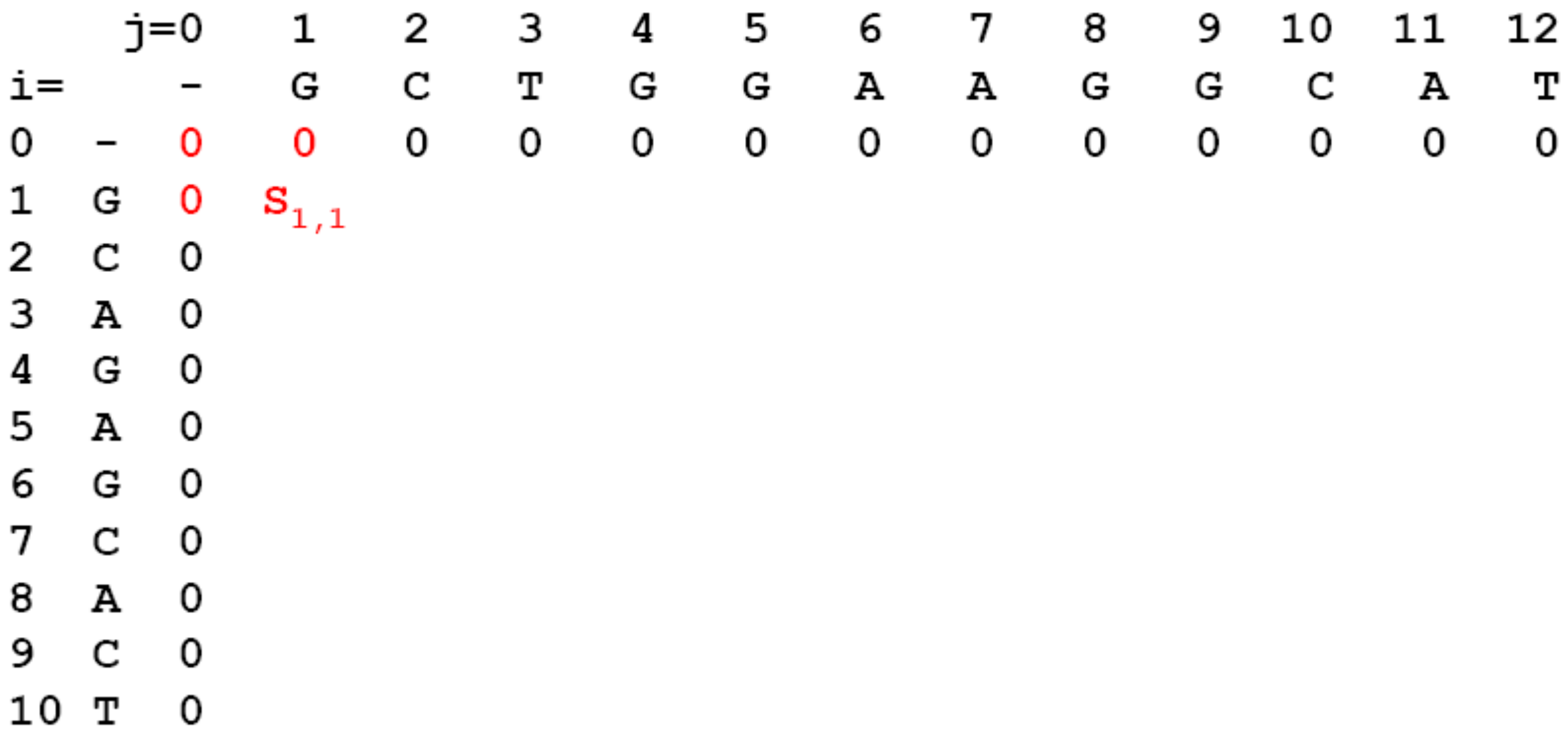
An Example



		j=0	1	2	3	4	5	6	7	8	9	10	11	12
i=		-	G	C	T	G	G	A	A	G	G	C	A	T
0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1	G	0												
2	C	0												
3	A	0												
4	G	0												
5	A	0												
6	G	0												
7	C	0												
8	A	0												
9	C	0												
10	T	0												

Match = 5, Mismatch = -4, Indel = -7

Local Alignment



		j=0	1	2	3	4	5	6	7	8	9	10	11	12
i=		-	G	C	T	G	G	A	A	G	G	C	A	T
0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1	G	0	$S_{1,1}$											
2	C	0												
3	A	0												
4	G	0												
5	A	0												
6	G	0												
7	C	0												
8	A	0												
9	C	0												
10	T	0												

Match = 5, Mismatch = -4, Indel = -7

Local Alignment

		j=0	1	2	3	4	5	6	7	8	9	10	11	12
i=		-	G	C	T	G	G	A	A	G	G	C	A	T
0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1	G	0	5	$S_{1,2}$										
2	C	0												
3	A	0												
4	G	0												
5	A	0												
6	G	0												
7	C	0												
8	A	0												
9	C	0												
10	T	0												

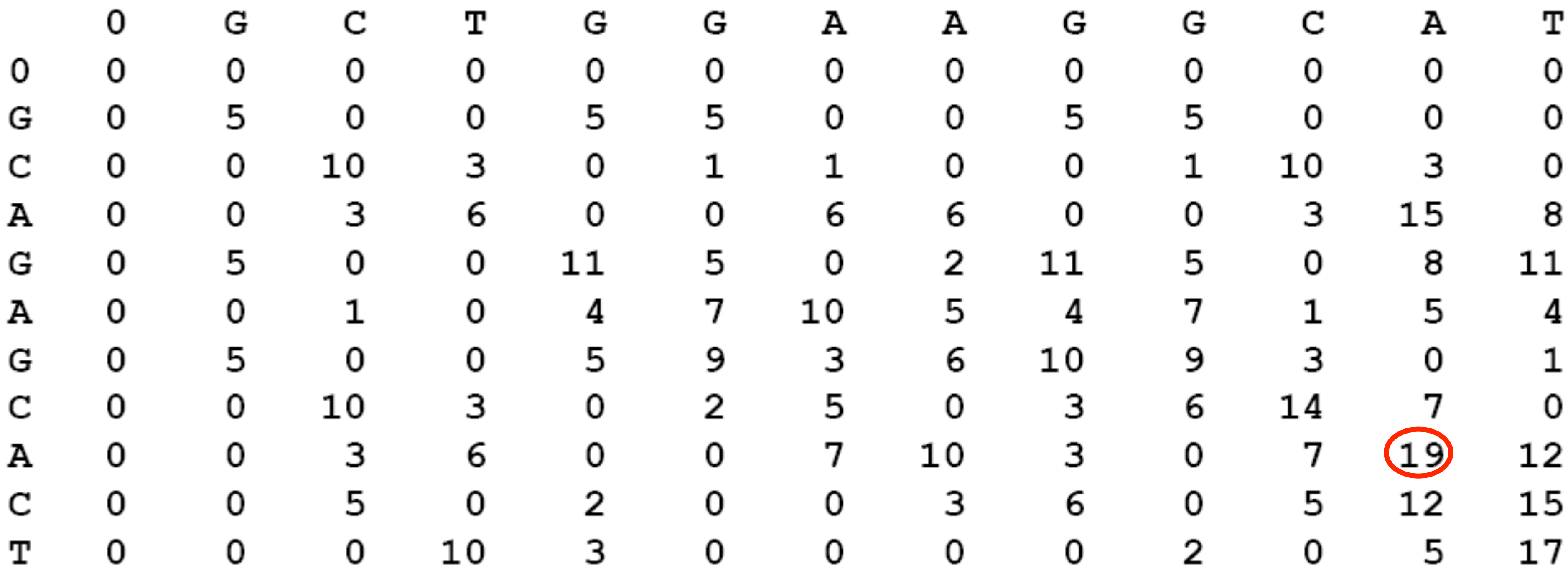
Match = 5, Mismatch = -4, Indel = -7

Local Alignment

		j=0	1	2	3	4	5	6	7	8	9	10	11	12
i=		-	G	C	T	G	G	A	A	G	G	C	A	T
0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1	G	0	5	0										
2	C	0	0	$S_{2,2}$										
3	A	0												
4	G	0												
5	A	0												
6	G	0												
7	C	0												
8	A	0												
9	C	0												
10	T	0												

Match = 5, Mismatch = -4, Indel = -7

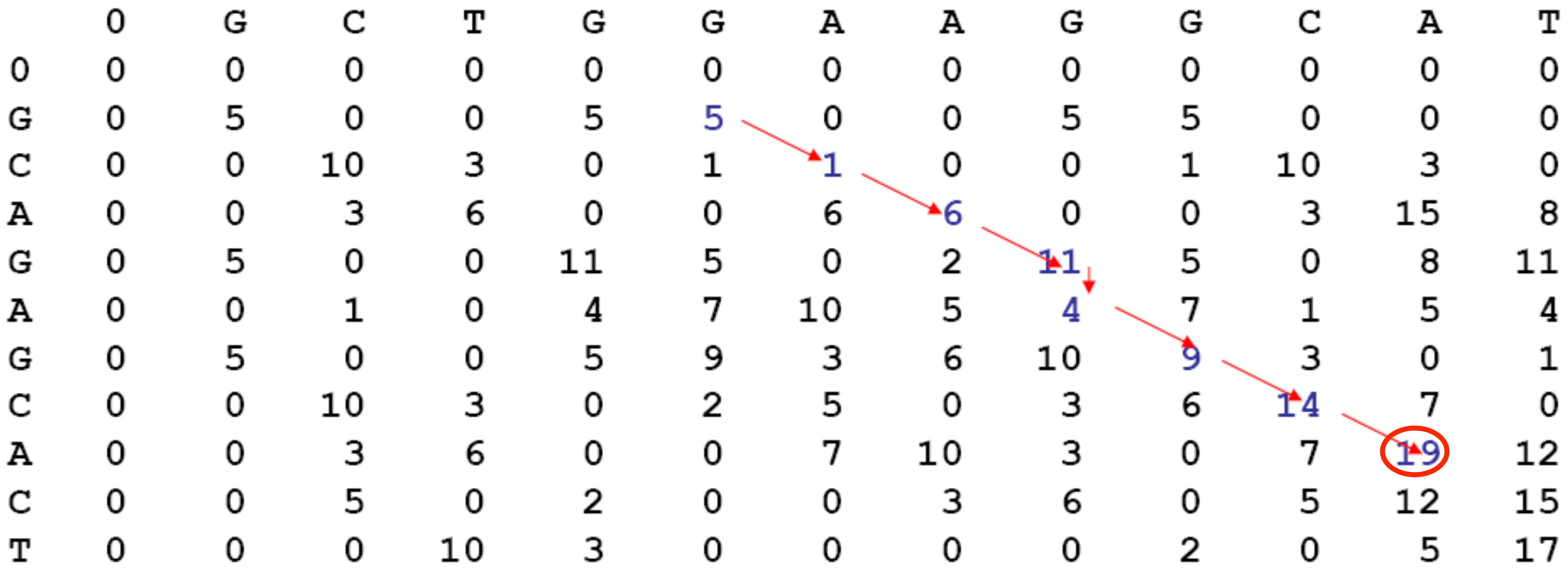
Local Alignment



	0	G	C	T	G	G	A	A	G	G	C	A	T
0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	0	0	5	5	0	0	5	5	0	0	0
C	0	0	10	3	0	1	1	0	0	1	10	3	0
A	0	0	3	6	0	0	6	6	0	0	3	15	8
G	0	5	0	0	11	5	0	2	11	5	0	8	11
A	0	0	1	0	4	7	10	5	4	7	1	5	4
G	0	5	0	0	5	9	3	6	10	9	3	0	1
C	0	0	10	3	0	2	5	0	3	6	14	7	0
A	0	0	3	6	0	0	7	10	3	0	7	19	12
C	0	0	5	0	2	0	0	3	6	0	5	12	15
T	0	0	0	10	3	0	0	0	0	2	0	5	17

Match = 5, Mismatch = -4, Indel = -7

Local Alignment



	0	G	C	T	G	G	A	A	G	G	C	A	T
0	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	0	0	5	5	0	0	5	5	0	0	0
C	0	0	10	3	0	1	1	0	0	1	10	3	0
A	0	0	3	6	0	0	6	6	0	0	3	15	8
G	0	5	0	0	11	5	0	2	11	5	0	8	11
A	0	0	1	0	4	7	10	5	4	7	1	5	4
G	0	5	0	0	5	9	3	6	10	9	3	0	1
C	0	0	10	3	0	2	5	0	3	6	14	7	0
A	0	0	3	6	0	0	7	10	3	0	7	19	12
C	0	0	5	0	2	0	0	3	6	0	5	12	15
T	0	0	0	10	3	0	0	0	0	2	0	5	17

Match = 5, Mismatch = -4, Indel = -7

Local Alignment

```
G   A   A   G   -   G   C   A
|       |   |       |   |   |
G   C   A   G   A   G   C   A
```

6 matches: 6 × 5 = 30

1 mismatch: -4

1 indel: -7

Total: 19

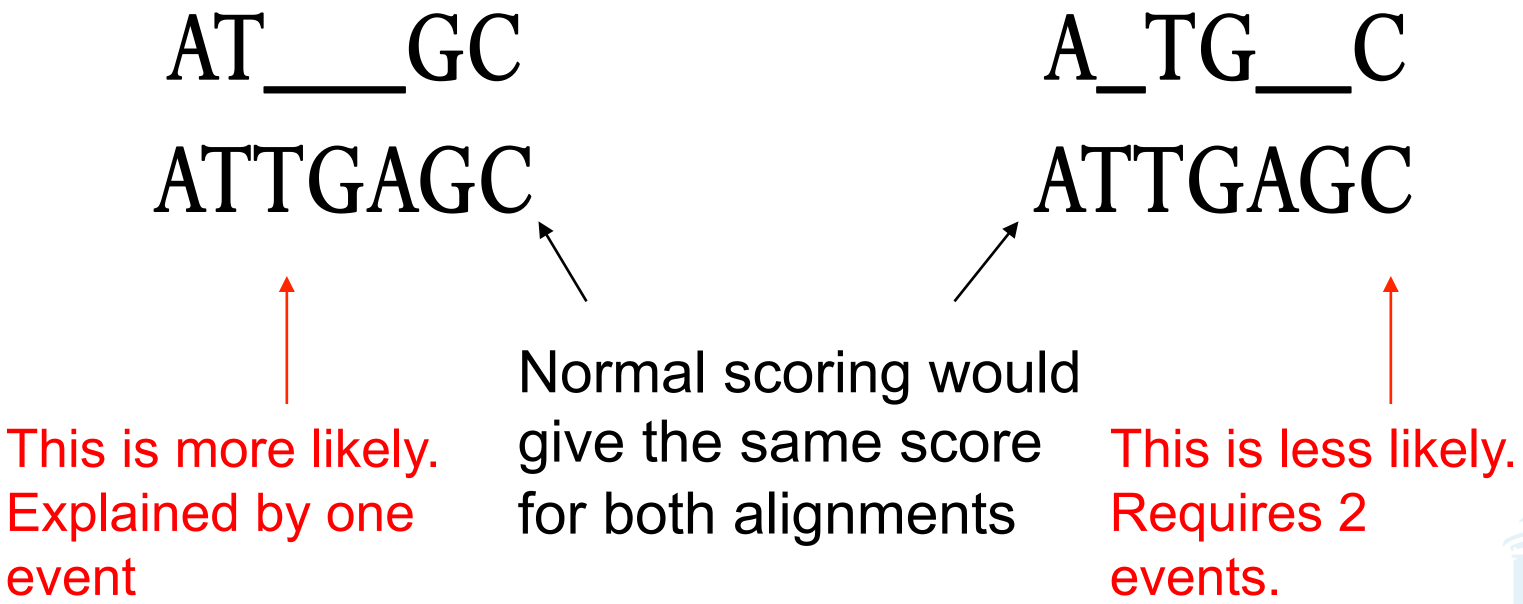
Scoring Indels: Naive Approach

- A fixed penalty σ is given to every indel:
  - $-\sigma$ for 1 indel,
  - -2σ for 2 consecutive indels
  - -3σ for 3 consecutive indels, etc.

Can be too severe penalty for a series of consecutive indels

Affine Gap Penalties

- In nature, a series of *k* indels often come as a single event rather than a series of *k* single nucleotide events:



```
AT___GC
ATTGAGC
```

```
A_TG__C
ATTGAGC
```

This is more likely. Explained by one event

Normal scoring would give the same score for both alignments

This is less likely. Requires 2 events.

Accounting for Gaps

- *Gaps*- contiguous sequence of indels in one of the rows

- Modify the scoring for a gap of length x to be:

$$-(\rho + \sigma x)$$

where $\rho + \sigma > 0$ is the penalty for introducing a gap:

gap opening penalty

and σ is the cost of extending it further ($\rho + \sigma >> \sigma$):

gap extension penalty

because you do not want to add too much of a penalty for further extending the gap, once it is opened.

Affine Gap Penalties

- Gap penalties:

  $-\rho - \sigma$ when there is 1 indel

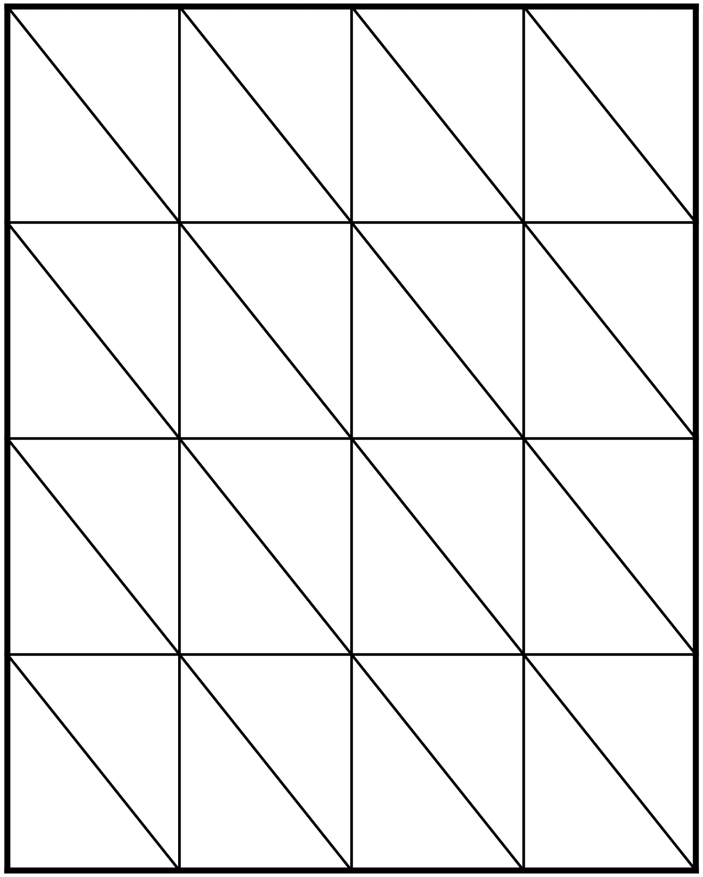
  $-\rho - 2\sigma$ when there are 2 indels

  $-\rho - 3\sigma$ when there are 3 indels, etc.

  $-\rho - x \cdot \sigma$ (-gap opening - x gap extensions)

- Somehow reduced penalties (as compared to naïve scoring) are given to runs of horizontal and vertical edges

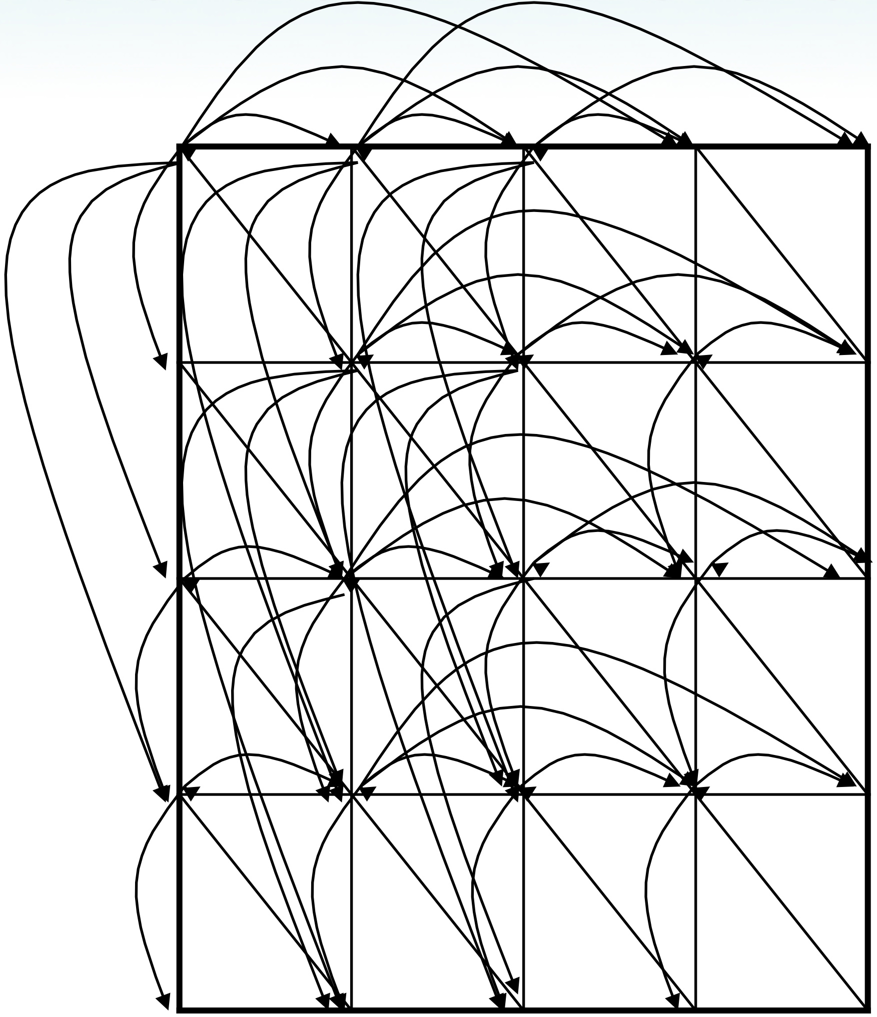
Affine Gap Penalties and Edit Graph



To reflect affine gap penalties we have to add "long" horizontal and vertical edges to the edit graph. Each such edge of length *x* should have weight

$$-\rho - x * \sigma$$

Adding "Affine Penalty" Edges to the Edit Graph



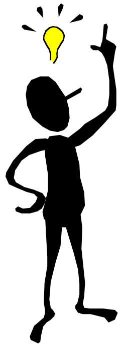
There are many such edges!

Adding them to the graph increases the running time of the alignment algorithm by a factor of ***n*** (where ***n*** is the number of vertices)

So the complexity increases from O(n^2) to O(n^3)

Affine Gap Penalty Recurrences

Keep track of these intermediate values in two new tables



$$t_{i,j} = \max \begin{cases} t_{i-1,j} - \sigma \\ s_{i-1,j} - (\rho + \sigma) \end{cases}$$

Continue Gap in *w* (deletion)

Start Gap in *w* (deletion): from middle

$$u_{i,j} = \max \begin{cases} u_{i,j-1} - \sigma \\ s_{i,j-1} - (\rho + \sigma) \end{cases}$$

Continue Gap in *v* (insertion)

Start Gap in *v* (insertion):from middle

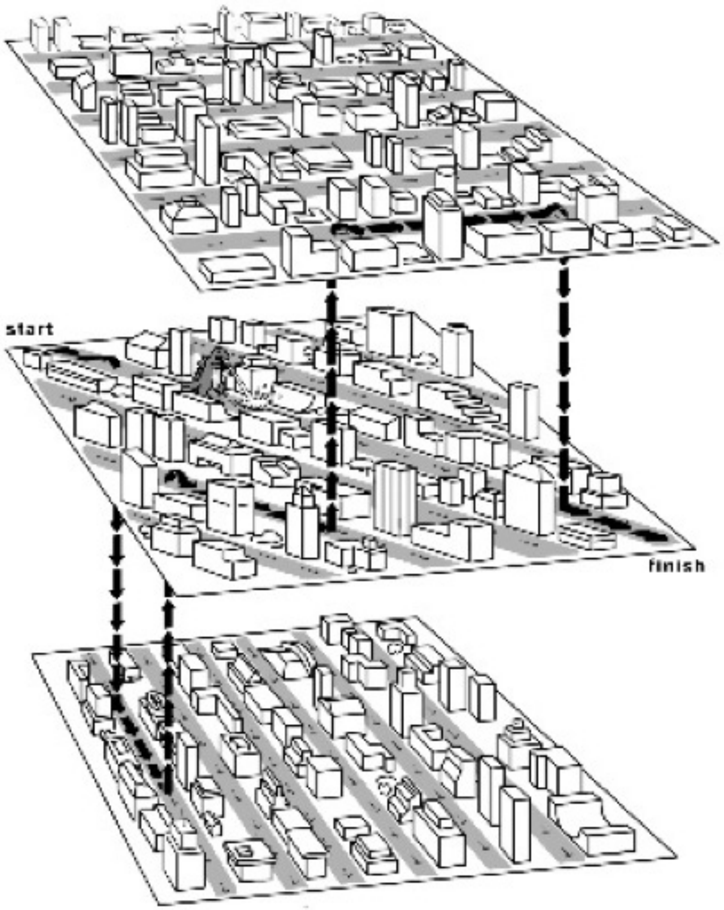
$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) \\ t_{i,j} \\ u_{i,j} \end{cases}$$

Match or Mismatch

End deletion: from top

End insertion: from left

The 3-leveled Manhattan Grid



Gaps in w (t-table)

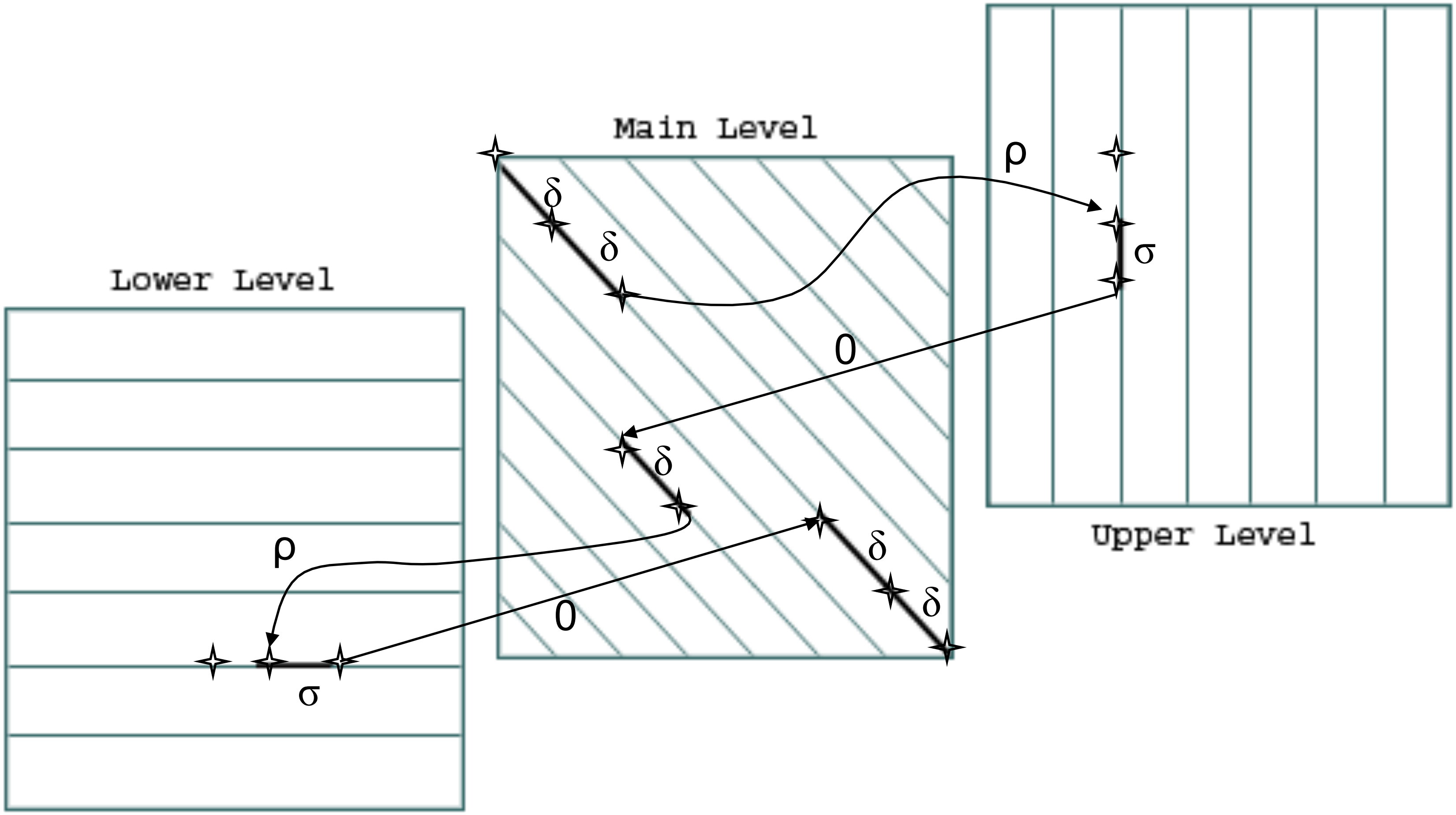
Matches/Mismatches (s-table)

Gaps in v (u-table)

Affine Gap Penalties and 3 Layer Manhattan Grid

- The three recurrences for the scoring algorithm creates a 3-layered graph.
- The top level creates/extends gaps in the sequence *w*.
- The bottom level creates/extends gaps in sequence *v*.
- The middle level extends matches and mismatches.

Manhattan in 3 Layers



Switching between 3 Layers

- Levels:
  - The **main level** is for diagonal edges
  - The **lower level** is for horizontal edges
  - The **upper level** is for vertical edges
- A jumping penalty is assigned to moving from the main level to either the upper level or the lower level ($-\rho - \sigma$)
- There is a gap extension penalty for each continuation on a level other than the main level ($-\sigma$)

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

Alignment Paths

- Align 3 sequences: ATGC, AATC,ATGC

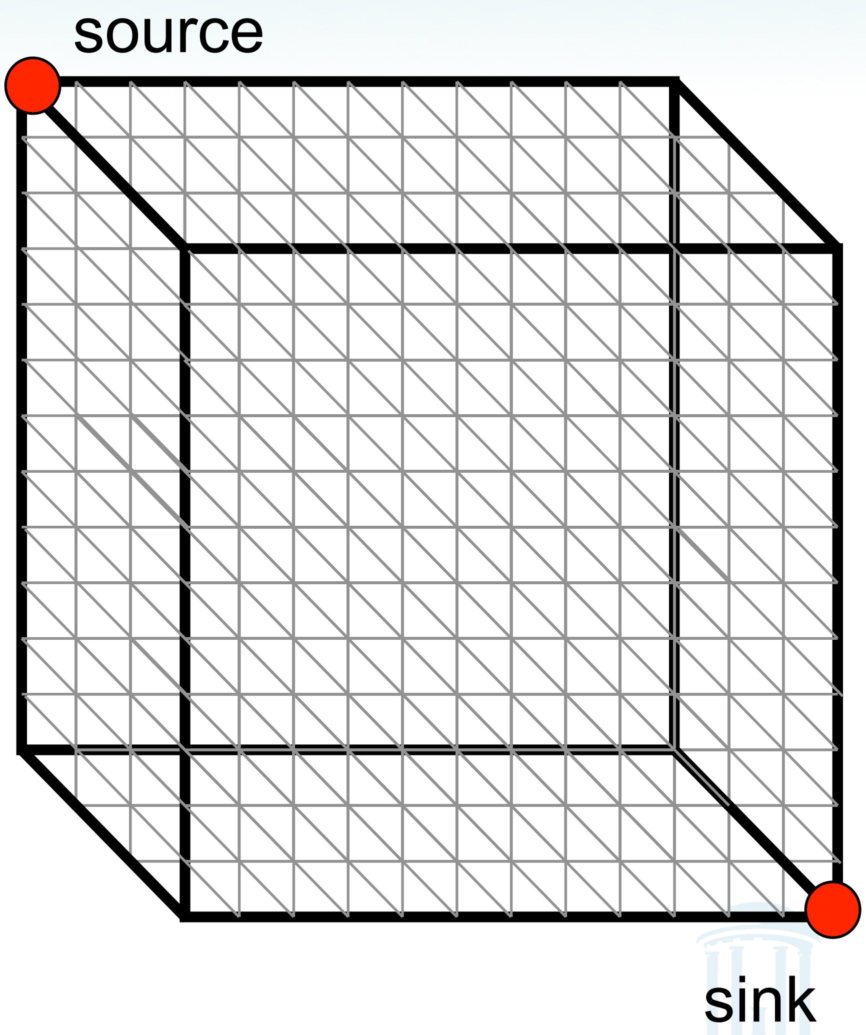
0	1	1	2	3	4	*x* coordinate
	A	--	T	G	C	
0	1	2	3	3	4	*y* coordinate
	A	A	T	--	C	
0	0	1	2	3	4	*z* coordinate
	--	A	T	G	C	

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

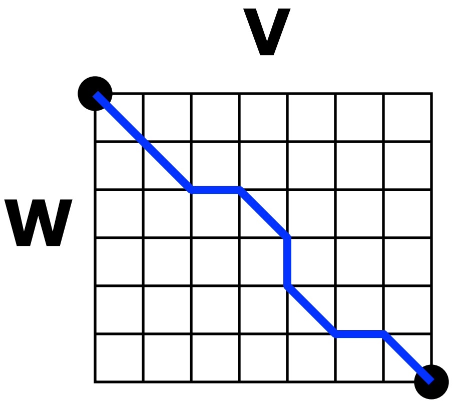
$$(0,0,0)\rightarrow(1,1,0)\rightarrow(1,2,1)\rightarrow(2,3,2)\rightarrow(3,3,3)\rightarrow(4,4,4)$$

Aligning Three Sequences

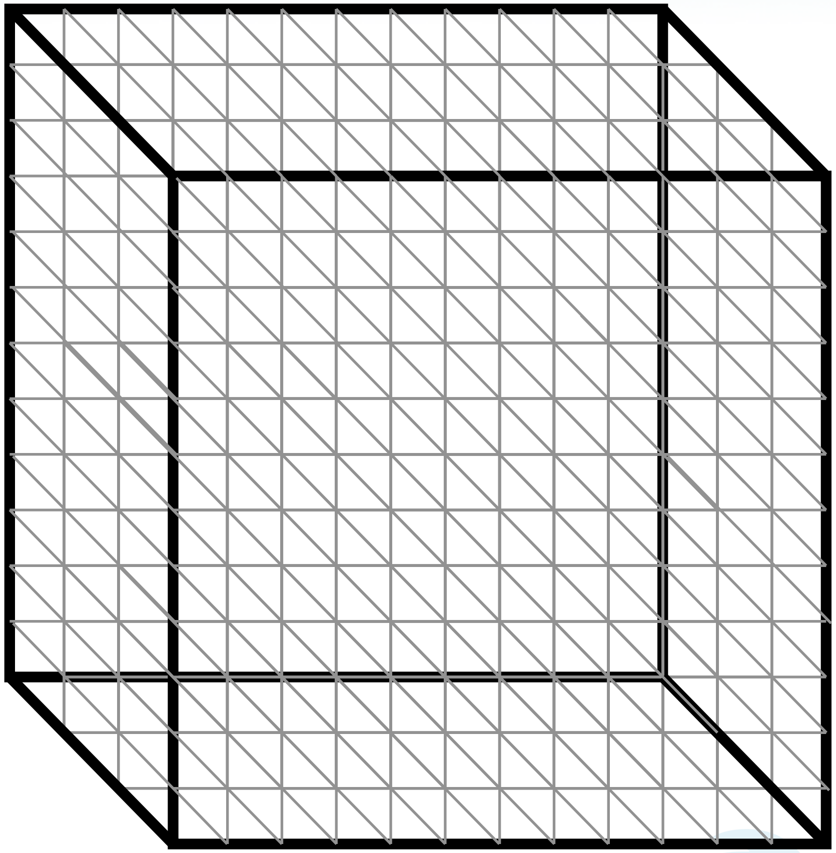
- Same strategy as aligning two sequences
- Use a 3-D "Manhattan Cube", with each axis representing a sequence to align
- For global alignments, go from source to sink



2-D vs 3-D Alignment Grid

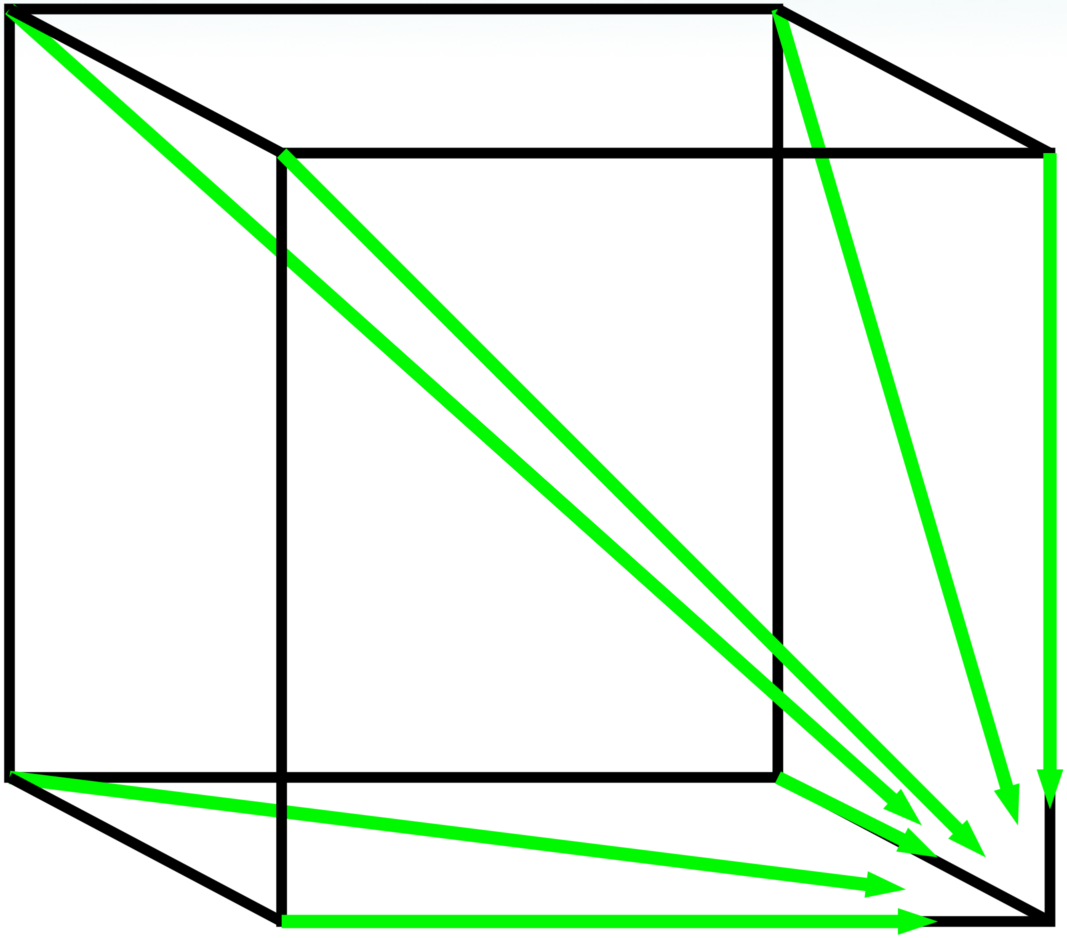


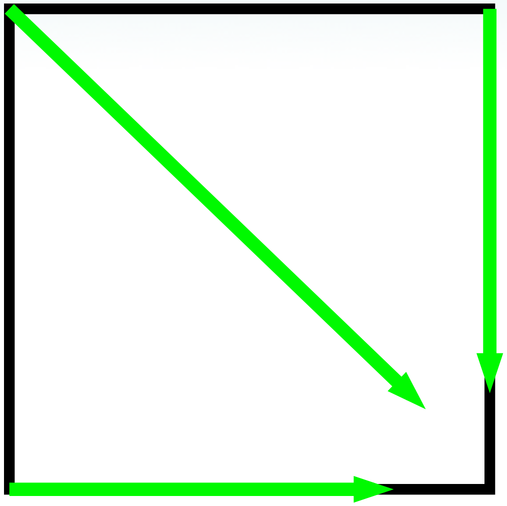
2-D edit graph



3-D edit graph

2-D cell versus 2-D Alignment Cell





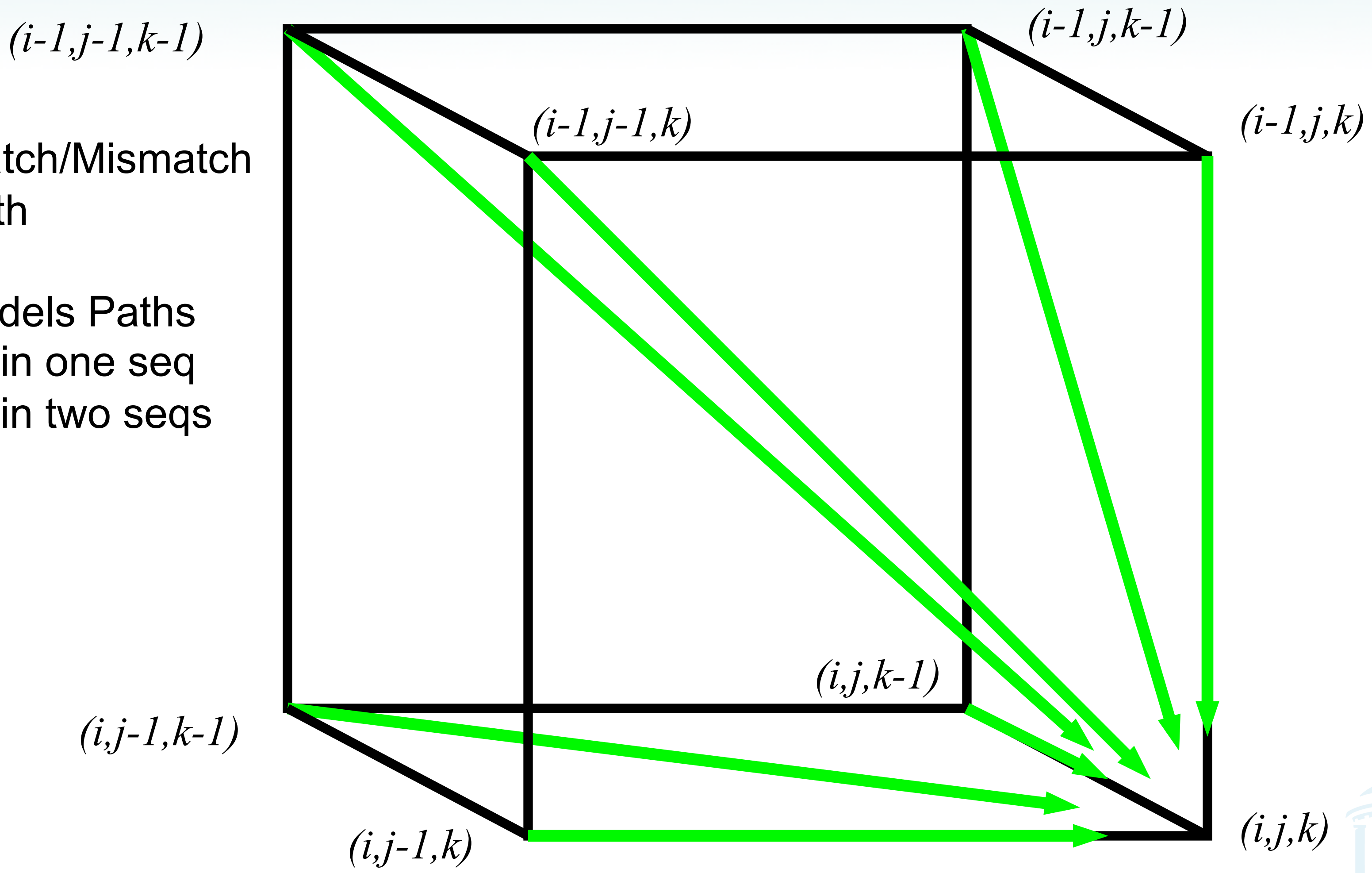
In **2-D**, 3 edges lead to each interior vertex

In **3-D**, 7 edges lead to each interior vertex

Architecture of 3-D Alignment Cell

1- Match/Mismatch Path

6 – indels Paths
3 in one seq
3 in two seqs



Multiple Alignment: Dynamic Programming

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

- $\delta(x, y, z)$ 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 $(2^k-1)(\boldsymbol{n}^k)$; $O(2^k\boldsymbol{n}^k)$
- 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
```

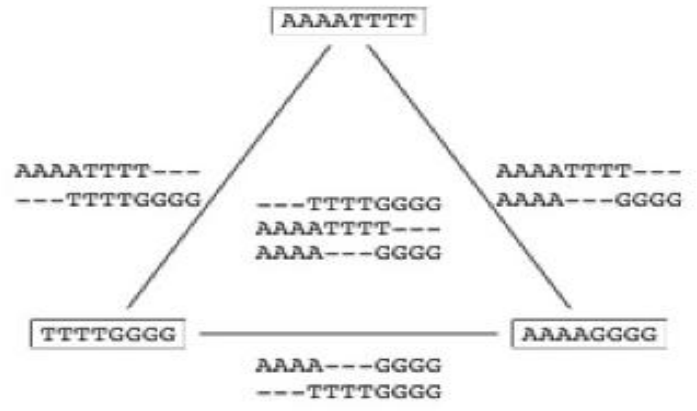
Can we construct a multiple alignment that induces them?

NOT ALWAYS

Why? Because pairwise alignments may be arbitraily inconsistent

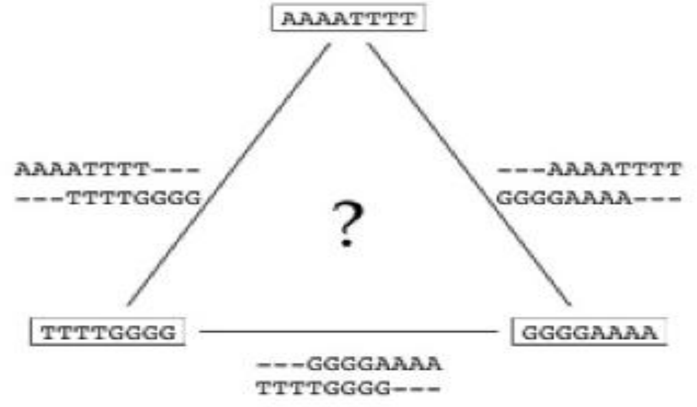
Combining Optimal Pairwise Alignments into Multiple Alignment

Can combine pairwise alignments into multiple alignment



(a) Compatible pairwise alignments

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



(b) Incompatible pairwise alignments

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
- Are we stuck, or is there some other trick?

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	

Thus far we have aligned 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-----
```

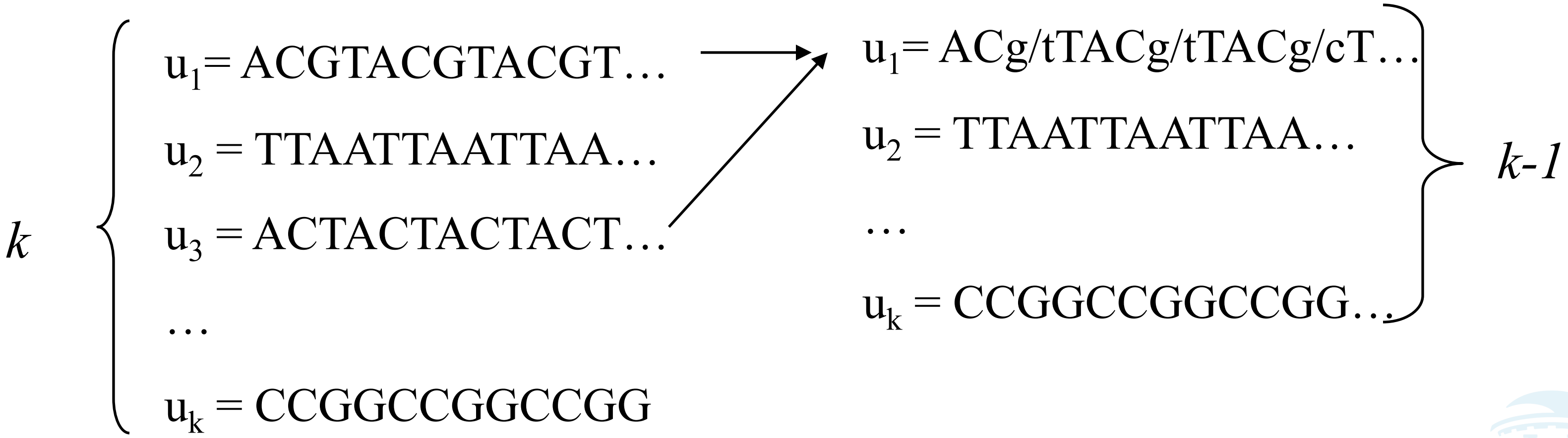
Aligning alignments

- Given two alignments, can we align them?
- Hint: don’t use the sequences…
  alignment 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

w/Scoring Matrix:
Match = 1
Mismatch = -1
Indel = -1

Greedy Approach: Example

- 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

s_2 *and* s_4 are closest; combine:

$s2$ GTCTGA

$s4$ GTCAGC

$s_{2,4}$ GTCt/aGa/c

(profile)

new set of 3 sequences:

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

Repeat

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
- CLUSTAL

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	–

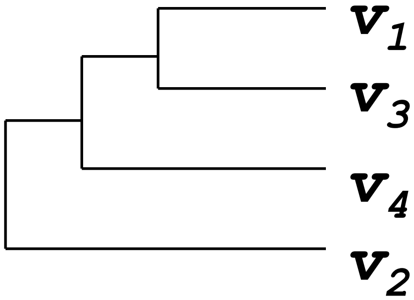
(.17 means 17 % identical)

Step 2: Guide Tree

- Create Guide Tree using the similarity matrix
  - ClustalW uses the neighbor-joining method (we will discuss this later in the course, in the section on clustering)
  - 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
```

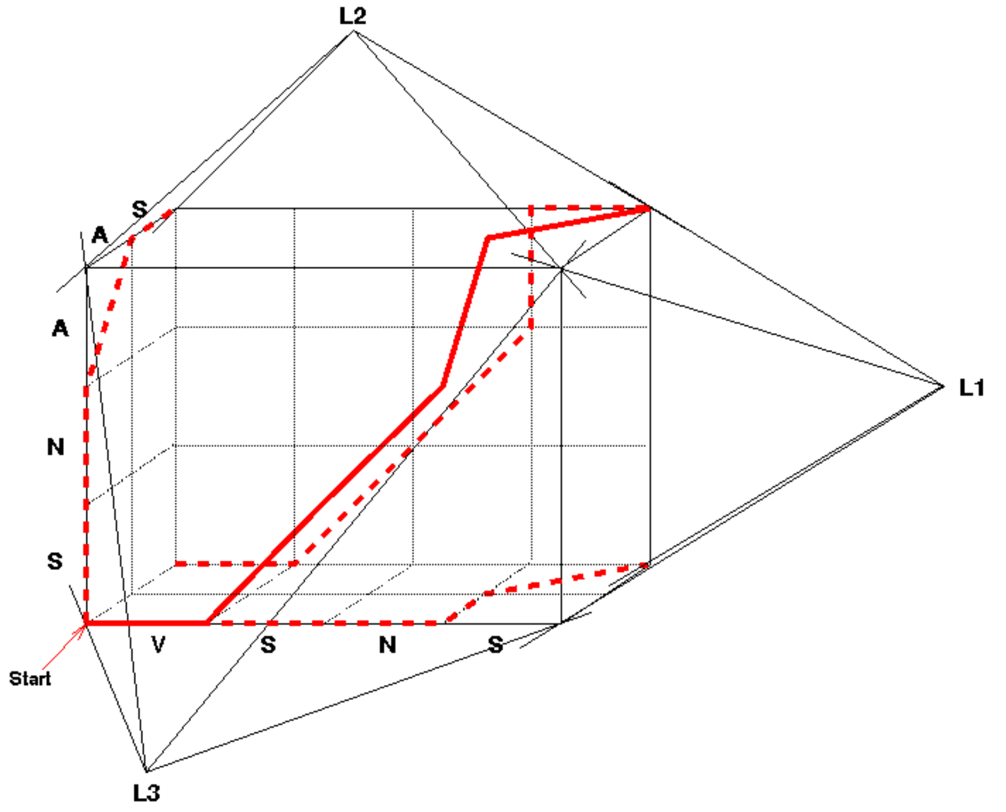
Induces:

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

Inferring Pairwise Alignments from Multiple Alignments

- This is the inverse of the problem described on slides 34 and 35
- 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 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:

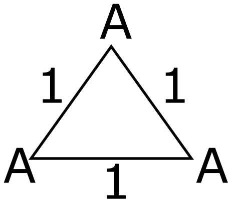
$$\begin{aligned} s(a_1 \ldots a_4) = \Sigma s^*(a_i,a_j) &= s^*(a_1,a_2) + s^*(a_1,a_3) \\ &+ s^*(a_1,a_4) + s^*(a_2,a_3) \\ &+ s^*(a_2,a_4) + s^*(a_3,a_4) \end{aligned}$$

SP-Score: Example

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

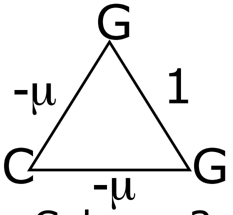
To calculate each column:

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



Column 1

Score=3



Column 3

Score = 1 – 2μ

Next Time

- Gene Prediction