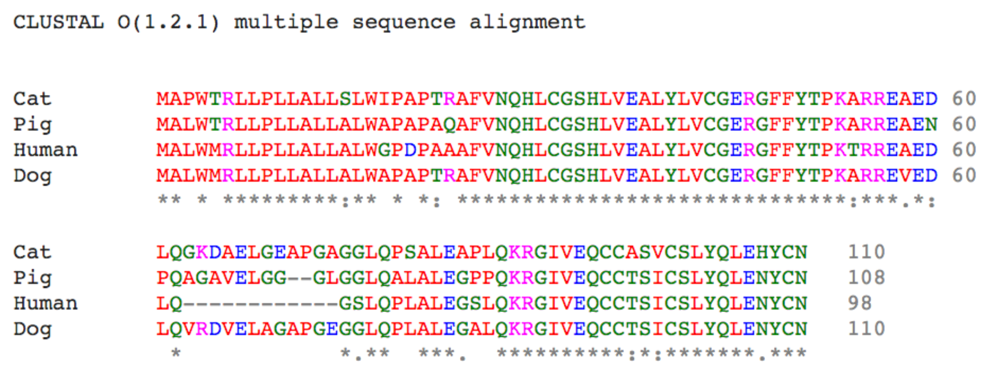
Advanced Sequence Alignment



```
CLUSTAL O(1.2.1) multiple sequence alignment


Cat         MAPWTRLLPLLALLSLWIPAPTRAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREAED 60
Pig         MALWTRLLPLLALLALWAPAPAQAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREAEN 60
Human       MALWMRLLPLLALLALWGPDPAAAFVNQHLCGSHLVEALYLVCGERGFFYTPKTRREAED 60
Dog         MALWMRLLPLLALLALWAPAPTRAFVNQHLCGSHLVEALYLVCGERGFFYTPKARREVED 60
            ** * *********:** * *: ******************************:***.*:

Cat         LQGKDAELGEAPGAGGLQPSALEAPLQKRGIVEQCCASVCSLYQLEHYCN   110
Pig         PQAGAVELGG--GLGGLQALALEGPPQKRGIVEQCCTSICSLYQLENYCN   108
Human       LQ------------GSLQPLALEGSLQKRGIVEQCCTSICSLYQLENYCN   98
Dog         LQVRDVELAGAPGEGGLQPLALEGALQKRGIVEQCCTSICSLYQLENYCN   110
             *            *.**  ***.  **********:*:*******.***
```

- Problem Set #4 is posted.

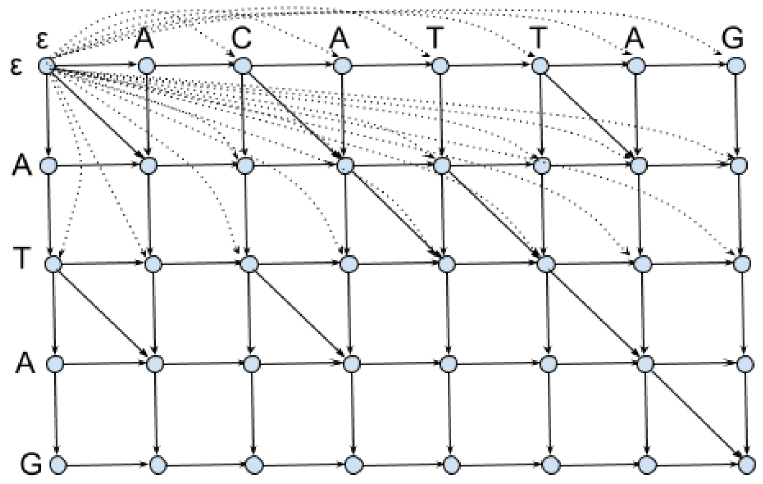
Recall Local Alignment

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

Notice there is only this small change from the original recurrence of a Global Alignment

- The *zero* is our *free ride* that allows the node to restart with a score of 0 at any point
  - What does this imply?
- After solving for the entire score matrix, we then search for $s_{i,j}$ with the highest score, this is (i_2, j_2)
- We follow our back tracking matrix until we reach a *score* of 0, whose coordinate becomes (i_1, j_1)

Smith-Waterman Local Alignment



Key idea: Adding *"free-rides"* from the source to any intersection

A Local Alignment 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

A Local Alignment Example - continued

		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												

$$S_{1,1} = \max\left\{\begin{array}{l} S_{0,0}+s_{G,G} = 0+5=5 \\ S_{1,0}+w = 0-7=-7 \\ S_{0,1}+w = 0-7=-7 \\ \qquad 0 \end{array}\right\} = 5$$

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

A Local Alignment Example - continued

		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												

$$S_{1,2} = \max \begin{Bmatrix} S_{0,1} + s_{G,C} = 0 - 4 = -4 \\ S_{1,2} + w \;= 5 - 7 = -2 \\ S_{0,2} + w \;= 0 - 7 = -7 \\ 0 \end{Bmatrix} = 0$$

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

A Local Alignment Example - continued

		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												

$$S_{2,2} = \max \left\{ \begin{array}{l} S_{1,1}+s_{C,C} = 5+5=10 \\ S_{2,1}+w \ = 0-7=-7 \\ S_{1,2}+w \ = 0-7=-7 \\ \qquad\quad 0 \end{array} \right\} = 10$$

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

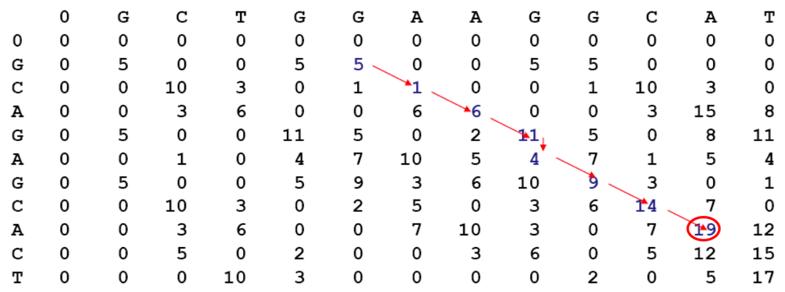
A Local Alignment Example - continued

	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

- Once the matrix is filled in we find the best alignment
- Rather than using the score of the last entry as we did for a global alignment, we search for the entire matrix for the maximum entry (*O(m n)* steps)

A Local Alignment Example - continued



	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

- From the largest score attained, then backtrack from there until a beginning "0" is reached to find the alignment.

A Local Alignment Example - continued

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

6 matches: 6 × 5 = 30
1 mismatch: -4
1 indel: -7
Total: 19

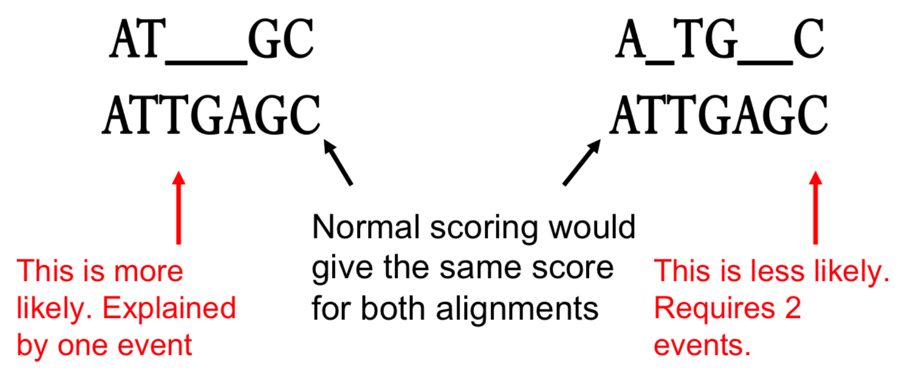
Scoring Indels: Naive Approach

```
ATCTTCAGCCATAAAAGATGAAGTT        Reference
ATCTTCAGCCAAAGATGAAGTT           3 base deletion relative to the reference

ATCTTCAGCC---AAAGATGAAGTT        version 1
ATCTTCAGCCA---AAGATGAAGTT        version 2
ATCTTCAGCCA--A-AGATGAAGTT        version 3
ATCTTCAGCCA-AA--GATGAAGTT        version 4
ATCTTCAGCCA-A-A-GATGAAGTT        version 5

ATCTTCAGCCATATGTGAAAGATGAAGTT    4 base insertion
```

- A fixed penalty σ is given to every indel:
  - -σ for 1 indel,
  - -2σ for 2 consecutive indels
  - -3σ for 3 consecutive indels, etc.
- Can be too severe penalty for a series of 100 consecutive indels
  - large insertions or deletions might result from a single event

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:

ρ = gap opening penalty

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

σ = 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

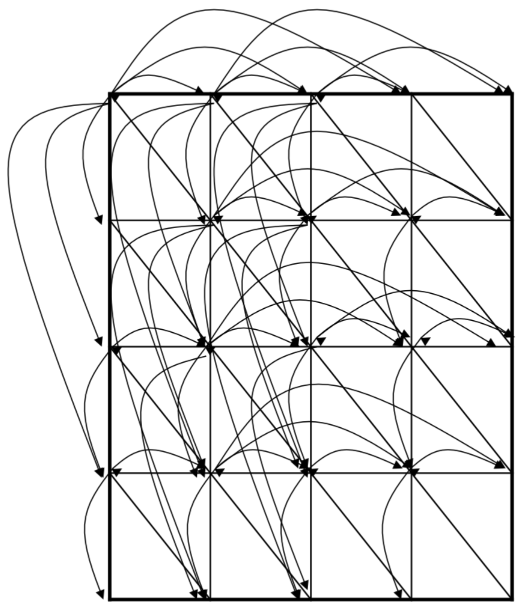
Gap penalties:

- $-\rho - \sigma$ 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

Adding Affine Gap Penalties to our 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 -ρ - x·σ
- 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 $O(n^3)$



Adding Two More Tables

- Affine Gap penalties can be more easily expressed in terms of 3 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

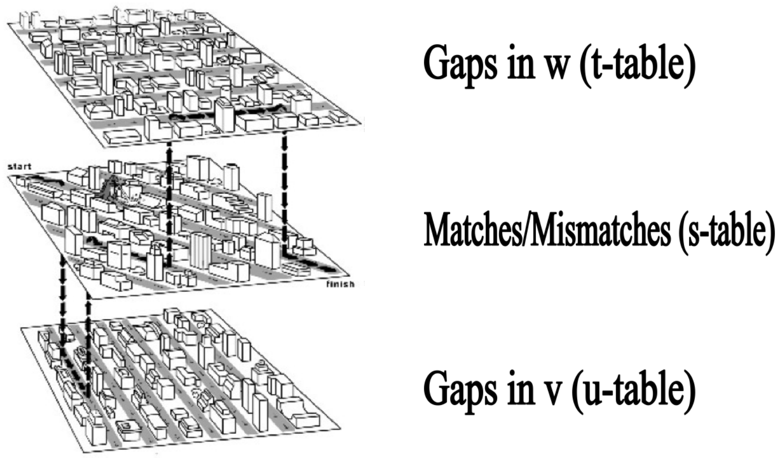
$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) \\ t_{i,j} \\ u_{i,j} \end{cases}$$

Match or Mismatch

End deletion: from top

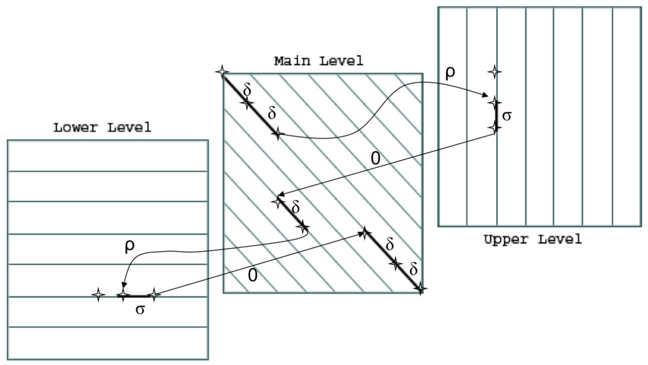
End insertion: from left

A 3-level 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.

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 (-ρ - σ)
- There is a gap extension penalty for each continuation on a level other than the main level (-σ)

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 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

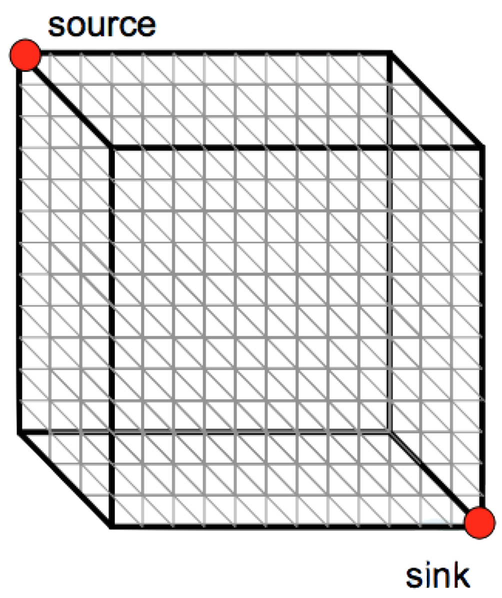
Three-D Alignment Paths

- An alignment of 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	

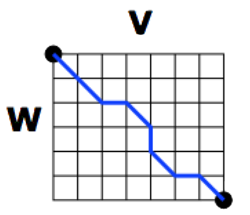
- Resulting path in (x,y,z) space:
  (0,0,0) → (1,1,0) → (1,2,1) → (2,3,2) → (3,3,3) → (4,4,4)
- Is there a better one?

Aligning Three Sequences

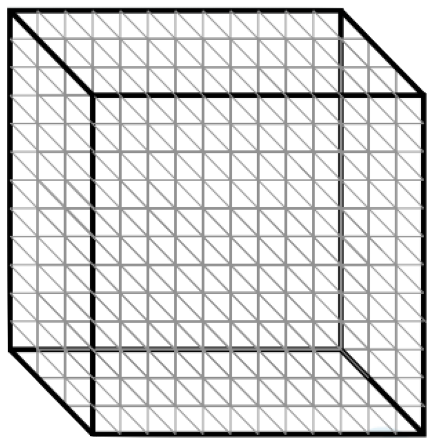


- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink

2-sequence vs 3-sequence Alignment

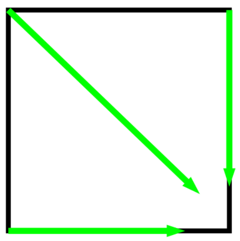


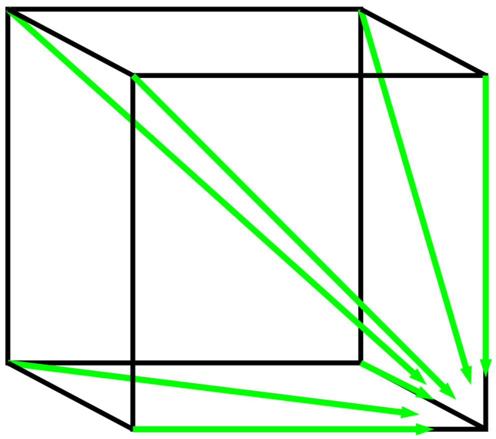
2-D edit graph



3-D edit graph

A 2-D cell versus a 3-D Alignment Cell


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

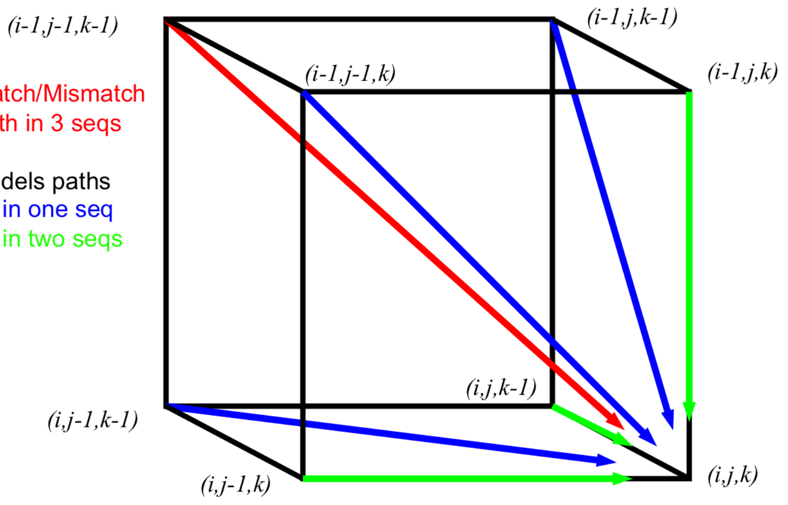

In **3-D**, 7 edges lead to each interior vertex

- 2-D *[(i-1,j-1), (i-1,j), (i,j-1)]* → *(i,j)* (3 directions)
- 3-D *[(i-1,j-1,k-1), (i-1,j,k), (i,j-1,k), (i,j,k-1), (i,j-1,k-1), (i-1,j,k-1), (i-1,j-1,k),]* → *(i,j,k)* (7 directions)
- N-D (2^N -1 directions)

Structure of a 3-D Alignment Cell

1- Match/Mismatch
Path in 3 seqs

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



Multiple Alignment: Recursion Relation

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

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

Multiple Alignment: Running Time

- For 3 sequences of length 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
```

Inverse Problem

Do Pairwise Alignments imply a Multiple Alignment?

- 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

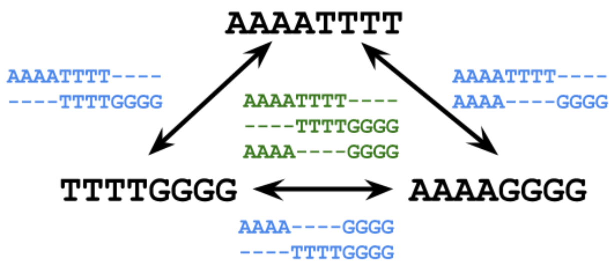
- Why? Because pairwise alignments may be arbitrarily inconsistent

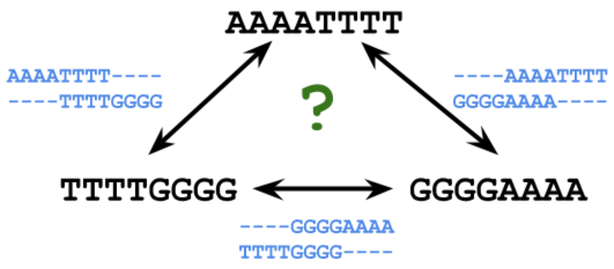
Combining Optimal Pairwise Alignments

- In some cases we can combine pairwie alignments into a single multiple alignment
- But, in others we cannot because one alignment makes a choice that is inconsistent with the overall best choice

```
AAAATTTT--------            ----AAAATTTT----
----TTTTGGGG----    -OR-    --------TTTTGGGG
--------GGGGAAAA            GGGGAAAA--------
```

- Is there another way?





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?

Multiple Alignment using a Profile Scores

- We used profile scores earlier when we discussed Motif finding

```
-  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	0	5	0	0	0	0	5	0	0	4	0	0	0	0
C	3	0	0	0	5	0	0	2	5	0	3	1	0	0
G	0	0	5	1	0	0	0	0	0	1	0	0	2	5
T	1	0	0	0	0	5	0	3	0	0	0	0	1	0
-	1	0	0	4	0	0	0	0	0	0	2	4	2	0

- Thus far we have aligned sequences against other sequences
- Can we align a sequence against a profile?
- Can we align a profile against a profile?

Aligning Alignments

A more general version of the multi-alignment problem:

- Given two alignments, can we align them?

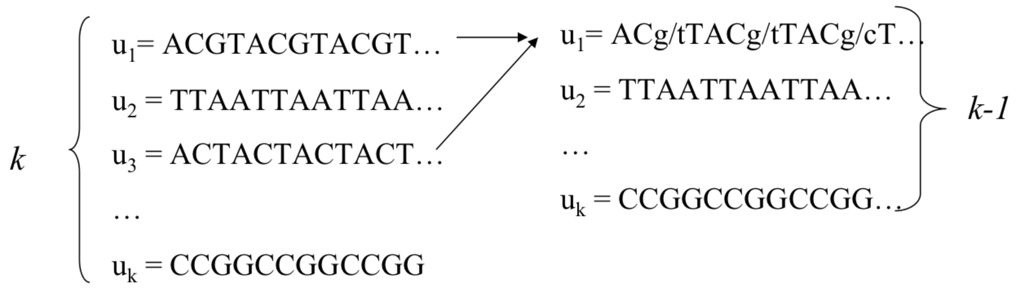
```
x: GGGCACTGCAT
y: GGTTACGTC--    Alignment 1
z: GGGAACTGCAG

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

- Idea: don’t use the sequences, but align their profiles

```
x: GGGCAC=TGCAT
y: GGTTAC=GTC--
z: GGGAAC=TGCAG     Combined Alignment
   ||  || | |
w: GG==ACGTACC--
v: GG==ACCT-----
```

Profile-Based Multiple Alignment: A Greedy Approach

- Choose the most similar pair of strings and combine them 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



Example

- Consider these 4 sequences

```
s1:    GATTCA
s2:    GTCTGA
s3:    GATATT
s4:    GTCAGC
```

- with the scoring matrix: {Match = 1, Mismatch = -1, Indel = -1}

Example (continued)

- There are $\binom{4}{2} = 6$ possible pairwise 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)
```

- The best pairwise score, 2, is between s_2 and s_4

Example (continued)

- Combine s_2 and s_4:

```
s2:  G T C T G A
     | | |   |          →      s2,4:  G T C t/a G a/c
s4:  G T C A G C
```

- Giving a set of three sequences:

```
s1  :   G  A  T  T  C  A
s3  :   G  A  T  A  T  T
s2,4:   G  T  C t/a G a/c
```

- **Repeat** for $\binom{3}{2} = 3$ possible pairwise alignments

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

s1  :  GAT-TCA
s2,4:  G-TCtGa (score  = 2 - 2 + 2 - 2 + ½ - 1 + ½ = 0)

s3  :  GATAT-T
s2,4:  G-TCtGa (score  = 2 - 2 + 2 - 2 + ½ - 1 - 1 = -1½)
```

Progressive Alignment

- Progressive alignment is a variation of a greedy profile alignment 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
  - Once a gap appears in a consensus string it is permanent
  - Uses profiles to compare sequences
- CLUSTAL OMEGA

Clustal Omega

- A popular multiple alignment tool commonly used 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

Clustal Omega's First Step

Pairwise alignment

- Align each sequence against all others giving a similarity matrix
- Similarity = exact matches / sequence length (percent identity)

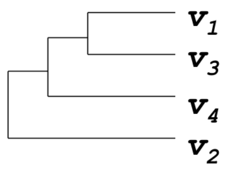
$$
\begin{array}{c|cccc}
 & v_1 & v_2 & v_3 & v_4 \\
\hline
v_1 & - & & & \\
v_2 & .17 & - & & \\
v_3 & .87 & .28 & - & \\
v_4 & .59 & .33 & .62 & -
\end{array}
$$

(.17 means 17 % identical)

ClustalW's Second Step

- 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

	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$((v_{1,3,4}), v_2)$

ClustalW's Third Step

- 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.

Next Time

- Other approaches to sequence alignment
- Divide-and-Conquer Alignment
- Other Dynamic Programming problems

Prologue to Experiment 3
OH, WHAT TO WEAR? WHAT TO WEAR?
SUCH A VAST WARDROBE! HOW WILL I EVER FIND THE RIGHT COMBINATION?
PERHAPS I CAN HELP!
WHO ARE YOU?
MACE
I'M HERE TO TELL YOU ABOUT "DynaMix" — THE NEW "SELECTION OPTIMIZATION SOFTWARE FROM ALGORITHMIC ANOMALY!
HOW DOES IT WORK?
WE USE DYNAMIC PROGRAMMING TO RECURSIVELY CREATE AN OUTFIT THAT YOU'LL LOOK JUST STUNNING IN!
NOW, IF WE WANT TO DO THIS IN O(n²) TIME, WE'LL HAVE TO HURRY!
LET'S BEGIN! THAT SHIRT AND THOSE PANTS YOU'RE HOLDING — HOW GOOD DO THEY LOOK TOGETHER, ON A SCALE FROM 1 TO 10?
UM... SEVEN?
EXCELLENT! I'LL ENTER THAT INTO OUR ARRAY!
THOSE SHOES WITH THAT NECKLACE?
THREE.
THOSE OTHER SHOES WITH THAT SHIRT?
SIX.
THIS NECKLACE WITH THESE PANTS?
EIGHT.
THAT SHIRT WITH THOSE PANTS?
TWO.
THREE HOURS LATER...
THERE! I'VE TOLD YOU EVERYTHING!
AND NOW, IN A FEW SECONDS, THE PARADIGM WILL HAVE WORKED ITS MAGIC!
VOILA! ALL DONE! SOOO...YOU'LL WANT TO WEAR THESE PANTS, THAT SHIRT, THOSE SHOES, AND THIS NECKLACE!
LATER THAT NIGHT...
DARLING, YOU LOOK ABSOLUTELY DYNAMIC!
SOMEHOW, I KNEW HE WAS GOING TO SAY THAT!
BUT HOW DOES IT WORK? TURN THE PAGE TO FIND OUT!