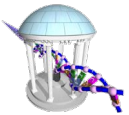
Comp 555 - BioAlgorithms - Spring 2020



Welcome back!
To the new uncharted world that we now live in

```
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
             *             *.**  ***.  **********:*:*******.***
```

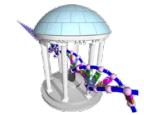
- Midterm Results
- Back to business
- Review of Sequence Alignment
- Scoring matrices
- Global vs. Local

Advanced Sequence Alignment

How does the rest of the semester play out?

- We will continue to meet at our regular time
  T-Th 9:30-10:45
  (I will linger around until 11am)
- My office hours will also move to Zoom
  at the same time W 2-4pm
- We will continue to have "in-class" exercises
- The two remaining problem sets and the final exam will be downloaded and submitted online as before.
- Everyone can take the course as either a letter grade or P/F, and you can wait until the end of the course to decide.

Midterm Results



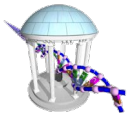
Average: 71.91
Q1: 83.00
Median: 75.00
Q3: 65.00

All curves will be applied to the combined weighted course average

The median course grade will be a "B", "B+", or "B-"

At least as good as it would on a 10-point scale

A Refresher



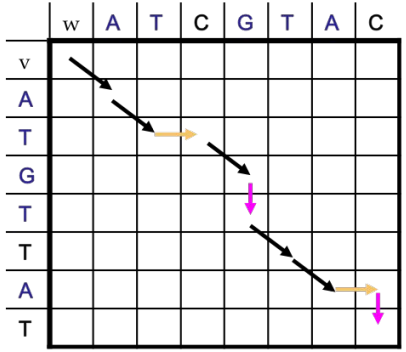
Longest Common Subsequence (LCS) is a special case of alignment

1. Construct a graph
2. Define a recurrence relation
3. Solve it for all paths from (0,0) to (n,m)
4. Used a dynamic program where each step relies only on solutions already computed and saved in our tableau

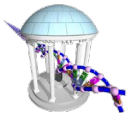
$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + 1 & if\ v_i = w_i \quad \searrow \\ s_{i-1,j} & \downarrow \\ s_{i,j-1} & \rightarrow \end{cases}$$

How about alternate recurrence relations?

$$S_{ij} = max \begin{cases} S_{i-1,j-1} + 1 & \text{if } v_i = w_j \\ S_{i-1,j-1} - 1 & \text{if } v_i \neq w_j \\ S_{i-1,j} - 2 \\ S_{i,j-1} - 2 \end{cases}$$



A more "*general purpose*" alignment graph



Now consider a more uniform "Manhatten"

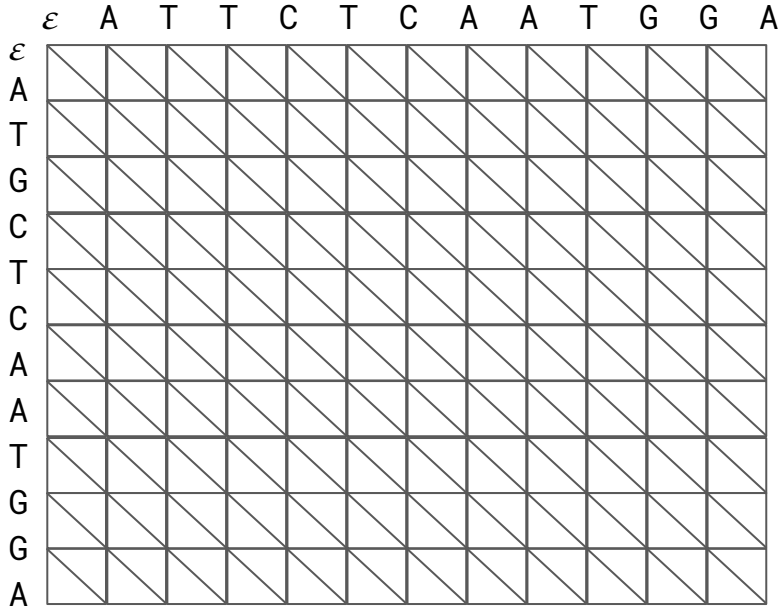
There are four ways to reach an intersection

From the north,

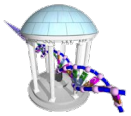
Fom the east,

from a diagonal with a "match",

from a diagonal with a "mismatch"



Alignment using a Scoring Matrix

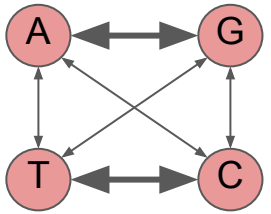


- Rather *edit distance* one can use a table with costs for every symbol aligned to any other
- Scoring matrices allow alignments to consider biological constraints
- Alignments can be thought of as two sequences that differ due to mutations.
- Some types of mutations are more common, or have little or no effect on function, therefore some mismatch penalties, $\delta(v_i, w_j)$, should be less harsh than others.

δ	A	C	G	T	_
A	1	-2	-1	-2	-3
C	-2	1	-2	-1	-3
G	-1	-2	1	-2	-3
T	-2	-1	-2	1	-3
_	-3	-3	-3	-3	

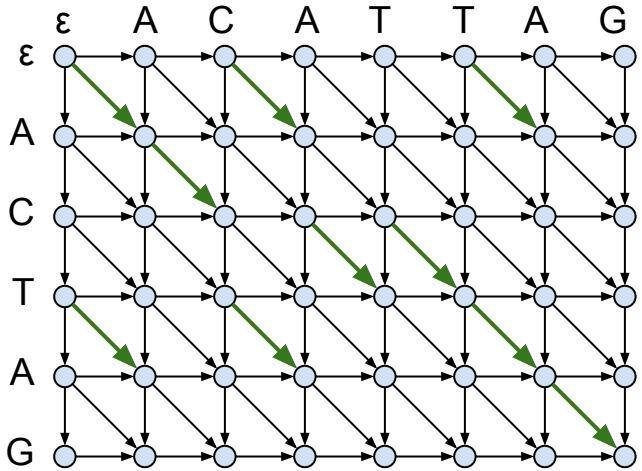
Example: ***DNA transitions and transversions***

- Like LCS, we want to maximize sequence matches, so each should have a positive score (diagonal of scoring matrix)
- Unlike LCS, we need to allow for occasional mismatches, as well as INDELs.
- The 4 DNA nucleotides come in two types, *purines* (A and G), which have two-rings and *pyrimidines*, (C and T) which have only one.
- Mutations within types are far more common than mutations between types, despite there being twice as many. This higher mutation rate can be encoded as a smaller substitution penalty.
- Insertions and deletions are even less common that any substitution, thus they have even higher penalties.



Impact on Alignment

Graph includes all diagonal edges, but many with negative weights

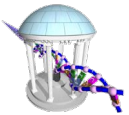


$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) \\ s_{i-1,j} + \delta(v_i, \_) \\ s_{i,j-1} + \delta(\_, w_j) \end{cases}$$

Generalized recurrence relation

Needleman–Wunsch Alignment Algorithm

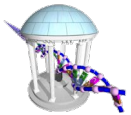
Global Alignment with a scoring matrix



In [9]:
```
import numpy

def GlobalAlign(v, w, scorematrix, indel):
    s = numpy.zeros((len(v)+1,len(w)+1), dtype="int32")
    b = numpy.zeros((len(v)+1,len(w)+1), dtype="int32")
    for i in range(0,len(v)+1):
        for j in range(0,len(w)+1):
            if (j == 0):
                if (i > 0):
                    s[i,j] = s[i-1,j] + indel
                    b[i,j] = 1
                continue
            if (i == 0):
                s[i,j] = s[i,j-1] + indel
                b[i,j] = 2
                continue
            score = s[i-1,j-1] + scorematrix[v[i-1],w[j-1]]
            vskip = s[i-1,j] + indel
            wskip = s[i,j-1] + indel
            s[i,j] = max(vskip, wskip, score)
            if (s[i,j] == vskip):
                b[i,j] = 1
            elif (s[i,j] == wskip):
                b[i,j] = 2
            else:
                b[i,j] = 3
    return (s, b)

match = {('A','A'):  1, ('A','C'): -2, ('A','G'): -1, ('A','T'): -2,
         ('C','A'): -2, ('C','C'):  1, ('C','G'): -2, ('C','T'): -1,
         ('G','A'): -1, ('G','C'): -2, ('G','G'):  1, ('G','T'): -2,
         ('T','A'): -2, ('T','C'): -1, ('T','G'): -2, ('T','T'):  1}

v = "TTCCGAGCGTTA"
w = "TTTCAGGTTA"

s, b = GlobalAlign(v,w,match,-3)
print("Best score =", s[-1,-1])
align = Alignment(b,v,w,b.shape[0]-1,b.shape[1]-1)
print("v =", align[0])
print("w =", align[1])
```

```
Best score = 2
v = TTCCGAGCGTTA
w = TTTC_AG_GTTA
```

Local vs. Global Alignment



- The *Global Alignment Problem* tries to find the highest scoring path between vertices (0,0) and (n,m) in the edit graph.
- The *Local Alignment Problem* tries to find the highest scoring subpath between *all vertex pairs* (i_1,j_1) and (i_2,j_2) in the edit graph where $i_2>i_1$ and $j_2>j_1$.
- In an edit graph with negatively-weighted scores, a Local Alignment may score higher than a Global Alignment

Example:

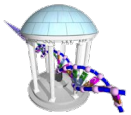
- Global Alignment finds a match for the entire sequence

```
--T--CC-C-AGT--TATGT-CAGGGGACACG—A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || | | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG—T-CAGAT--C
```

- Local Alignment finds a long conserved subsequence

```
                        tccCAGTTATGTCAGgggacacgagcatgcagagac
                           ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Local Alignments: Why?



Two genes in different species may be similar over short conserved regions and dissimilar over remaining regions.

Example:

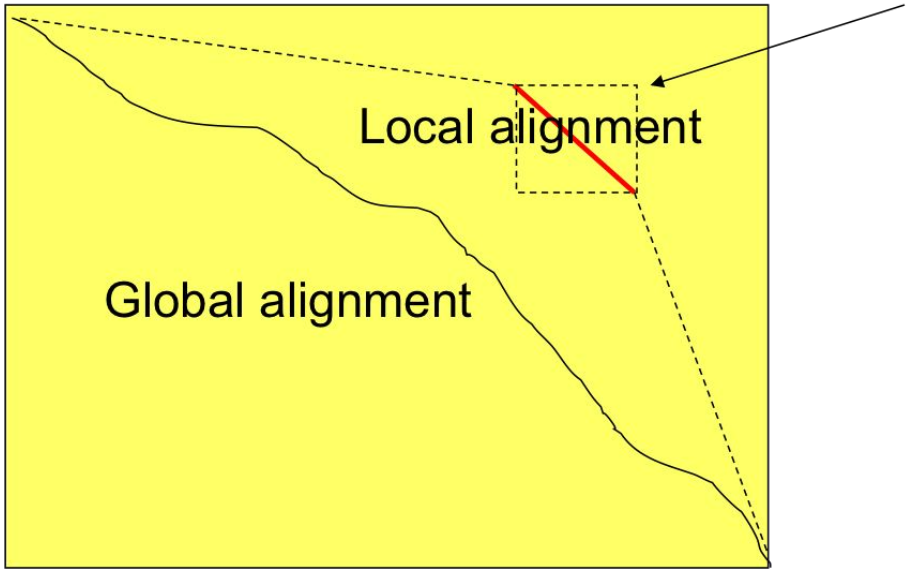
- Homeobox genes have a short region called the homeodomain that is highly conserved between species.
- A global alignment would not find the homeodomain because it would try to align the ENTIRE sequence

Local Alignment Problem:

- **Goal**: Find the best local alignment between two strings
- **Input**: Strings *v, w* and scoring matrix δ
- **Output**: Alignment of substrings of *v and w* whose alignment score is maximum among all possible alignment of all possible substrings

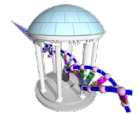
Local Alignment Approach

A local alignment is a subpath in a global alignment

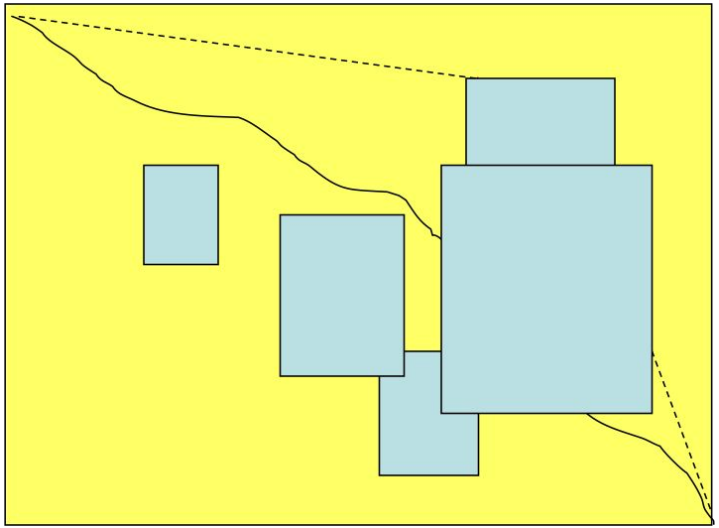


Compute a “mini” Global Alignment to get Local

Brute Force Local Alignment



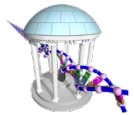
Find the best global alignment among all blocks (i_1,j_1,i_2,j_2)



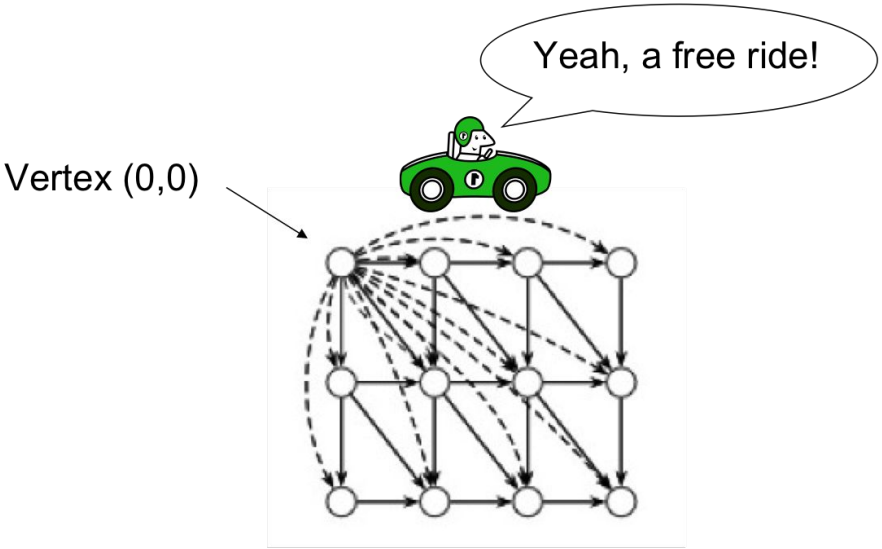
Long run time $O(n^4)$:

- In the grid of size n x n there are $O(n^2)$ vertices (i_1,j_1) that may serve as a source.
- For each such vertex computing alignments from (i_1,j_1) to (i_2,j_2) takes $O(n^2)$ time.

Local Alignment with Free Rides

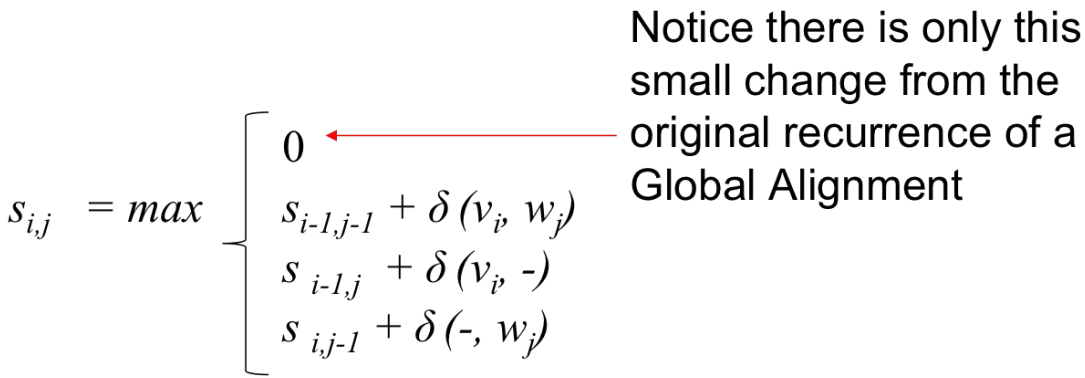


- ***Key Ideas:*** Add extra edges to our graph, consider all scores in matrix



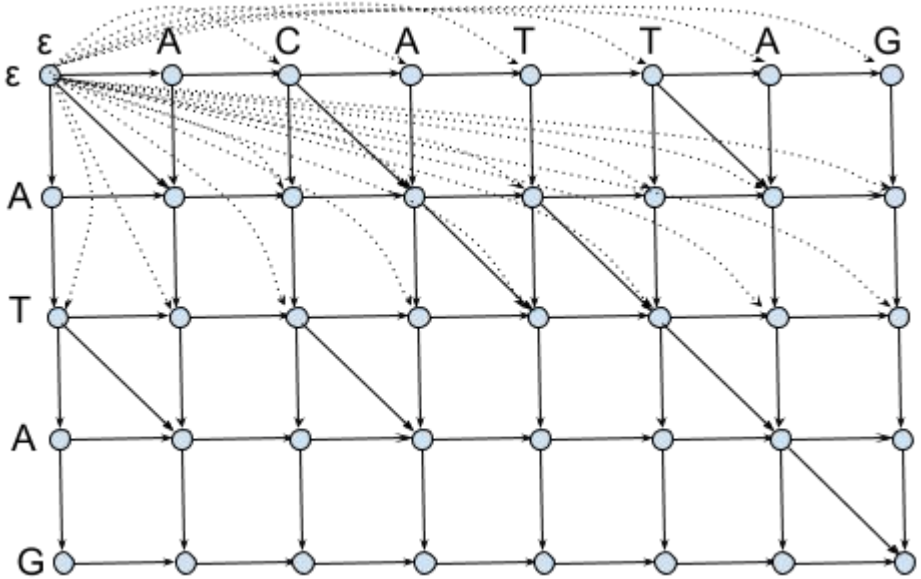
- The dashed edges represent a *free ride* from (0,0) to any other node
- The largest value of $s_{i,j}$ over the *whole score matrix* is the end point of the best local alignment (instead of $s_{n,m}$)

Local Alignment Recurrence



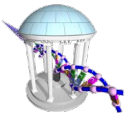
- The *zero* is our *free ride* that allows the node to restart with a score of 0 at any point
  - What does this imply?
- After solving for the entire score matrix, we then search for si,j with the highest score, this is (i_2,j_2)
- We follow our back tracking matrix until we reach a *score* of 0, whose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

Smith-Waterman Local Alignment



Key Idea: Add edges from the source to any intersection. These free rides might be better than any other path reaching an intersection.

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

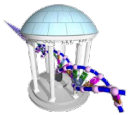
Local Alignment Example - continued

		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												

$$S_{1,1} = \max \begin{cases} S_{0,0} + s_{G,G} = 0 + 5 = 5 \\ S_{1,0} + w = 0 - 7 = -7 \\ S_{0,1} + w = 0 - 7 = -7 \\ 0 \end{cases} = 5$$

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

Local Alignment Example - continued

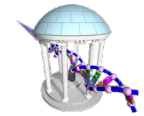


		j=0	1	2	3	4	5	6	7	8	9	10	11	12
i=		-	G	C	T	G	G	A	A	G	G	C	A	T
0	-	0	0	0	0	0	0	0	0	0	0	0	0	0
1	G	0	5	0										
2	C	0	0	$s_{2,2}$										
3	A	0												
4	G	0												
5	A	0												
6	G	0												
7	C	0												
8	A	0												
9	C	0												
10	T	0												

$$S_{2,2} = \max \begin{Bmatrix} S_{1,1} + s_{C,C} = 5 + 5 = 10 \\ S_{2,1} + w = 0 - 7 = -7 \\ S_{1,2} + w = 0 - 7 = -7 \\ 0 \end{Bmatrix} = 10$$

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

Local Alignment Example - continued

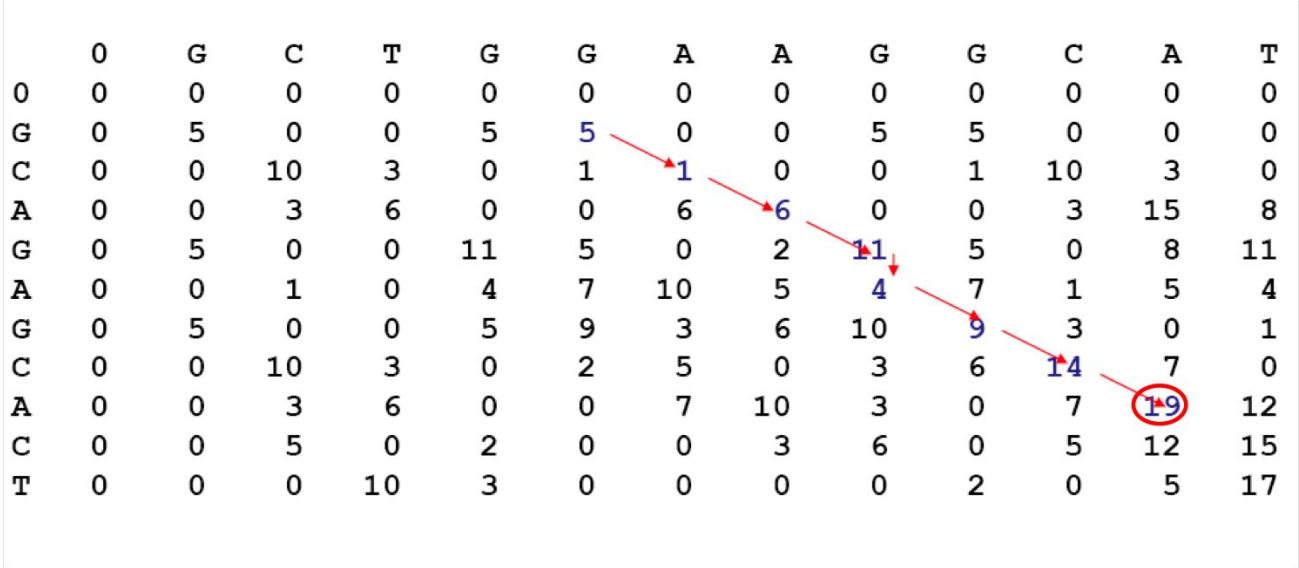


		G	C	T	G	G	A	A	G	G	C	A	T
	0	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	0	0	5	5	0	0	5	5	0	0	0
C	0	0	10	3	0	1	1	0	0	1	10	3	0
A	0	0	3	6	0	0	6	6	0	0	3	15	8
G	0	5	0	0	11	5	0	2	11	5	0	8	11
A	0	0	1	0	4	7	10	5	4	7	1	5	4
G	0	5	0	0	5	9	3	6	10	9	3	0	1
C	0	0	10	3	0	2	5	0	3	6	14	7	0
A	0	0	3	6	0	0	7	10	3	0	7	19	12
C	0	0	5	0	2	0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

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

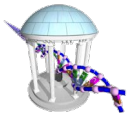
Match = 5, Mismatch = -4, Indel = -7

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

Local Alignment Example - continued

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

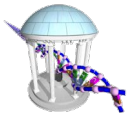
Local Alignment Code



In [11]:
```
import numpy

def LocalAlign(v, w, scorematrix, indel):
    s = numpy.zeros((len(v)+1,len(w)+1), dtype="int32")
    b = numpy.zeros((len(v)+1,len(w)+1), dtype="int32")
    for i in range(1,len(v)+1):
        for j in range(1,len(w)+1):
            if (j == 0):
                if (i > 0):
                    s[i,j] = max(s[i-1,j] + indel, 0)
                    b[i,j] = 1
                continue
            if (i == 0):
                s[i,j] = max(s[i,j-1] + indel, 0)
                b[i,j] = 2
                continue
            score = s[i-1,j-1] + scorematrix[v[i-1],w[j-1]]
            vskip = s[i-1,j] + indel
            wskip = s[i,j-1] + indel
            s[i,j] = max(vskip, wskip, score, 0)
            if (s[i,j] == vskip):
                b[i,j] = 1
            elif (s[i,j] == wskip):
                b[i,j] = 2
            elif (s[i,j] == score):
                b[i,j] = 3
            else:
                b[i,j] = 0
    return (s, b)

match = {('A','A'):  5, ('A','C'): -4, ('A','G'): -4, ('A','T'): -4,
         ('C','A'): -4, ('C','C'):  5, ('C','G'): -4, ('C','T'): -4,
         ('G','A'): -4, ('G','C'): -4, ('G','G'):  5, ('G','T'): -4,
         ('T','A'): -4, ('T','C'): -4, ('T','G'): -4, ('T','T'):  5}

v = "GCTGGAAGGCAT"
w = "GCAGAGCACT"

s, b = LocalAlign(v,w,match,-7)
print(s)
print()
print(b)
```

```
[[ 0  0  0  0  0  0  0  0  0  0  0]
 [ 0  5  0  0  5  0  5  0  0  0  0]
 [ 0  0 10  3  0  1  0 10  3  5  0]
 [ 0  0  3  6  0  0  0  3  6  0 10]
 [ 0  5  0  0 11  4  5  0  0  2  3]
 [ 0  5  1  0  5  7  9  2  0  0  0]
 [ 0  0  1  6  0 10  3  5  7  0  0]
 [ 0  0  0  6  2  5  6  0 10  3  0]
 [ 0  5  0  0 11  4 10  3  3  6  0]
 [ 0  5  1  0  5  7  9  6  0  0  2]
 [ 0  0 10  3  0  1  3 14  7  5  0]
 [ 0  0  3 15  8  5  0  7 19 12  5]
 [ 0  0  0  8 11  4  1  0 12 15 17]]

[[0 0 0 0 0 0 0 0 0 0 0]
 [0 3 0 0 3 0 3 0 0 0 0]
 [0 0 3 2 0 3 0 3 2 3 0]
 [0 0 1 3 0 0 0 1 3 0 3]
 [0 3 0 0 3 2 3 0 0 3 1]
 [0 3 3 0 3 3 3 2 0 0 0]
 [0 0 3 3 0 3 2 3 3 2 0]
 [0 0 0 3 3 3 3 0 3 2 0]
 [0 3 0 0 3 2 3 2 1 3 0]
 [0 3 3 0 3 3 3 3 0 0 3]
 [0 0 3 2 0 3 3 3 2 3 0]
 [0 0 1 3 2 3 0 1 3 2 2]
 [0 0 0 1 3 2 3 1 1 3 3]]
```

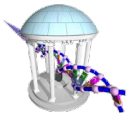
Scoring Indels: Naive Approach



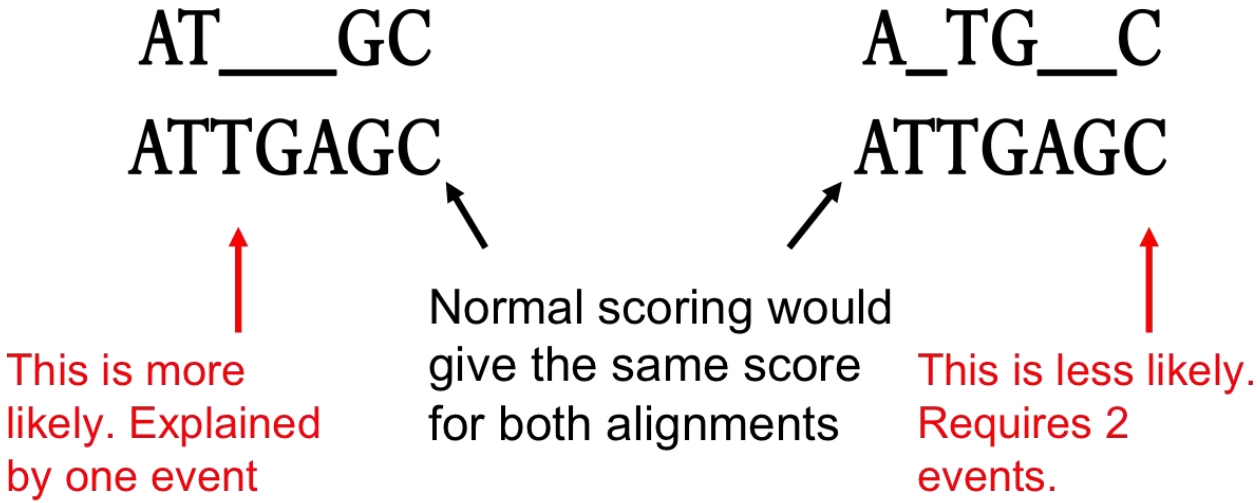
```
ATCTTCAGCCATAAAAGATGAAGTT        Reference
ATCTTCAGCCAAAGATGAAGTT           3 base deletion relative to the reference

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

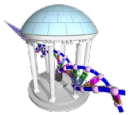
Affine Gap Penalties



In nature, a series of k indels often come as a single, albeit rare, event rather than as a series of muliple events



Accounting for Gaps



- Gaps- contiguous sequence of indels in a row
- 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:

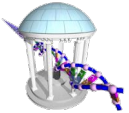
ρ = gap opening penalty

- 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.

Adding Affine Gap Penalties to our Graph

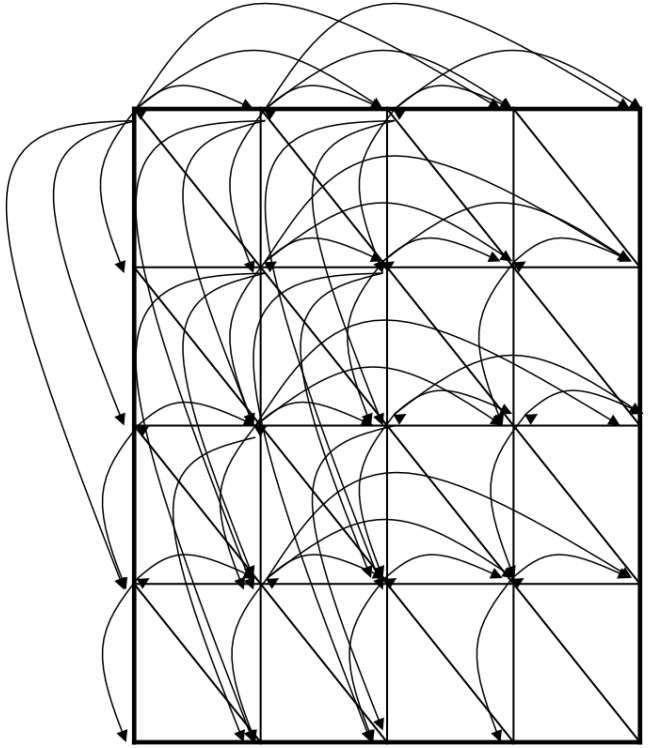


- To reflect affine gap penalties we have to add “long” horizontal and vertical edges to the edit graph.
- Each such edge of length x should have weight

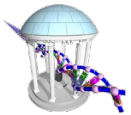
$$-\rho - x \cdot \sigma$$

- 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 $O(n^3)$

Can we do it some other way?



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

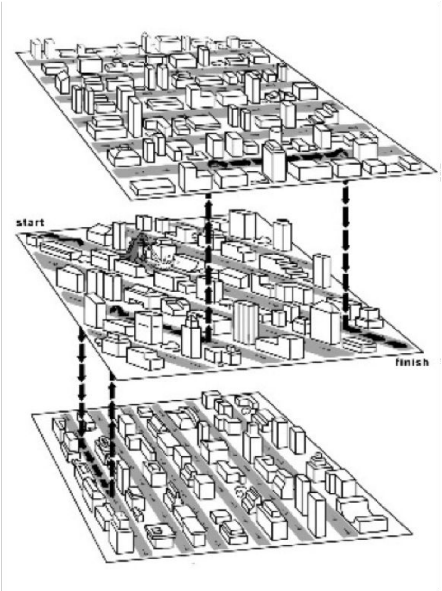
$$s_{i,j} = \max \begin{cases} s_{i-1,j-1} + \delta(v_i, w_j) \\ t_{i,j} \\ u_{i,j} \end{cases}$$

Match or Mismatch

End deletion: from top

End insertion: from left

A 3-level Manhattan Grid



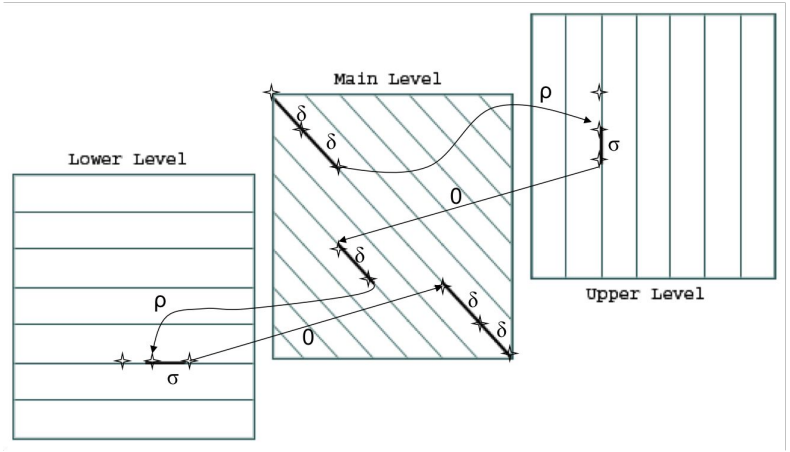
Gaps in w (t-table)

Matches/Mismatches (s-table)

Gaps in v (u-table)

- 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.

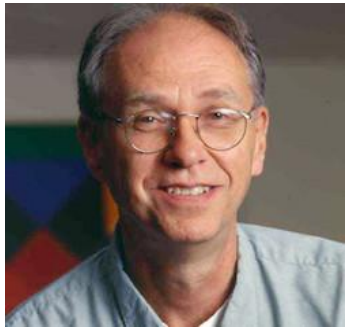
Switching between Layers



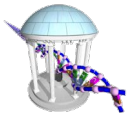
- Levels:
  - The main level is for diagonal edges
  - The lower level is for horizontal edges
  - The upper level is for vertical edges
- A jumping penalty is assigned to moving from the main level to either the upper level or the lower level (-ρ - σ)
- There is a gap extension penalty for each continuation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

Multiple versus Pairwise Alignment

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

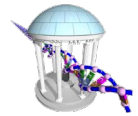


- Alignment of 2 sequences is represented as a 2-row matrix
- In a similar way, we represent alignment of 3 sequences as a 3-row matrix

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

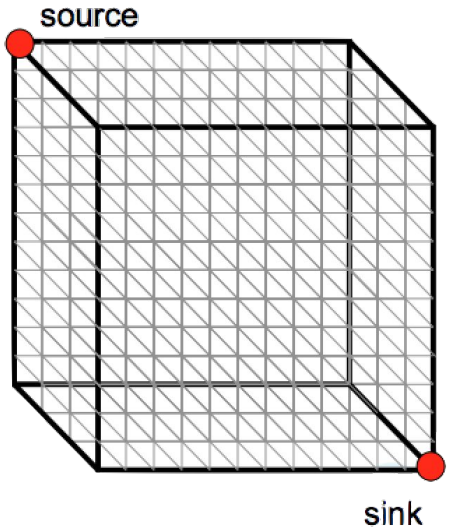
x coordinate

y coordinate

z coordinate

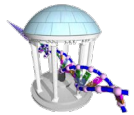
- Resulting path in (x,y,z) space:
  (0,0,0) → (1,1,0) → (1,2,1) → (2,3,2) → (3,3,3) → (4,4,4)
- Is there a better one?

Aligning Three Sequences

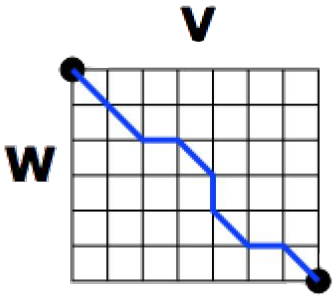


- Same strategy as aligning two sequences
- Use a 3-D “Manhattan Cube”, with each axis representing a sequence to align
- For global alignments, go from source to sink

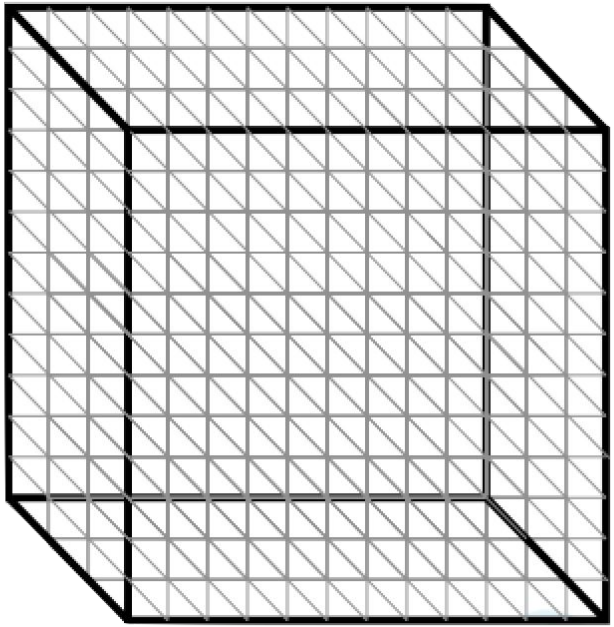
2-sequence vs 3-sequence Alignment



- In a 2-D grid there are 3 approaches to each intersection
- I'm now ignoring
  - Free-passes
  - Affine jumps
- How about 3-D?
- How does this impact our recurrence relations?

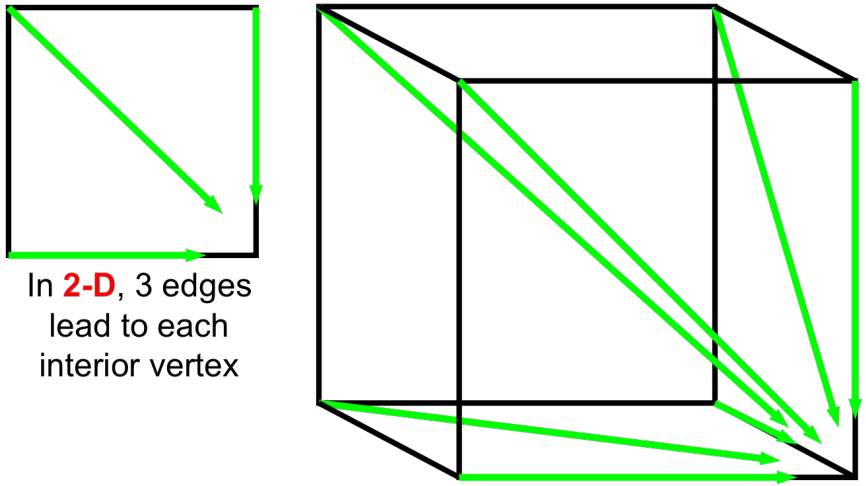


2-D edit graph



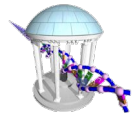
3-D edit graph

A 2-D versus a 3-D neighborhood



- 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



There are three path types

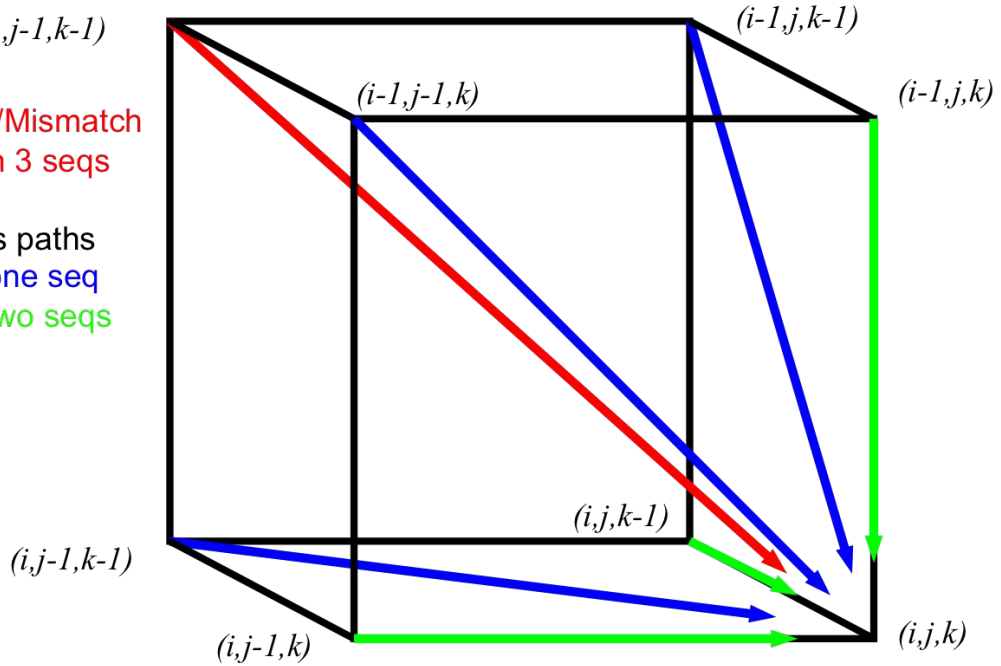
1. Consume a character in all 3 sequences (diagonal "red" edge)

2. Consumes characters in 2 of 3 sequences ("blue" diagonals on faces)

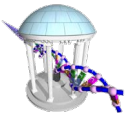
3. Consuumes a character from only one sequence ("green" edges"

1- Match/Mismatch Path in 3 seqs

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



Multiple Alignment: Recursion Relation



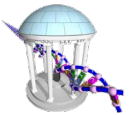
- $s_{i,j,k} = \max$

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

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

Scoring matrix has 5^3 entries

Multiple Alignment: Running Time



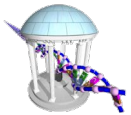
- For 3 sequences of length n, the run time is $7n^3$; $O(n^3)$
- For k sequences, build a k-dimensional Manhattan, with run time $(2^k-1)(n^k)$; $O(2^k n^k)$
- Conclusion: dynamic programming approach for alignment between two sequences is easily extended to k sequences but it is impractical due to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

Example:

To align 6, 100-base sequences, there are 63 directions to consider and 10^{12} cells to compute

Compare to aligning all 6(5-1)/2 = 15 pairs, each with 3 directions and 10,000 cells

Multiple Alignment Induces Pairwise Alignments



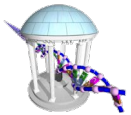
Every multiple alignment induces pairwise alignments

```
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:

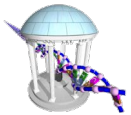
```
x: ACGCTGG-C;      x: AC-GCTGG-C;      y: AC-GC-GAG
y: ACGC--GAC;      z: GCCGCA-GAG;      z: GCCGCAGAG
```

- Can we construct a multiple alignment that induces them?

NOT ALWAYS

- Why? Because pairwise alignments can be arbitrarily inconsistent

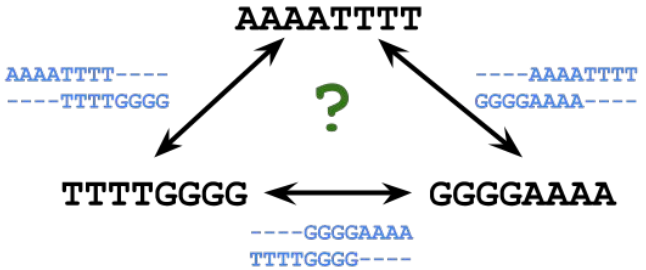
Combining Optimal Pairwise Alignments



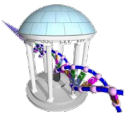
- In some cases we can combine pairwise alignments into a single multiple alignment
- But, in others we cannot because one alignment makes a choice that is inconsistent with the overall best choice

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

- Is there another way?

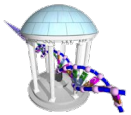


Multiple Alignment from Pairwise Alignments



- 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

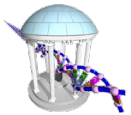


- We used profile scores earlier when we discussed Motif finding

```
      -  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:

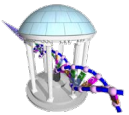
- Given two alignments, can we align them?

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

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

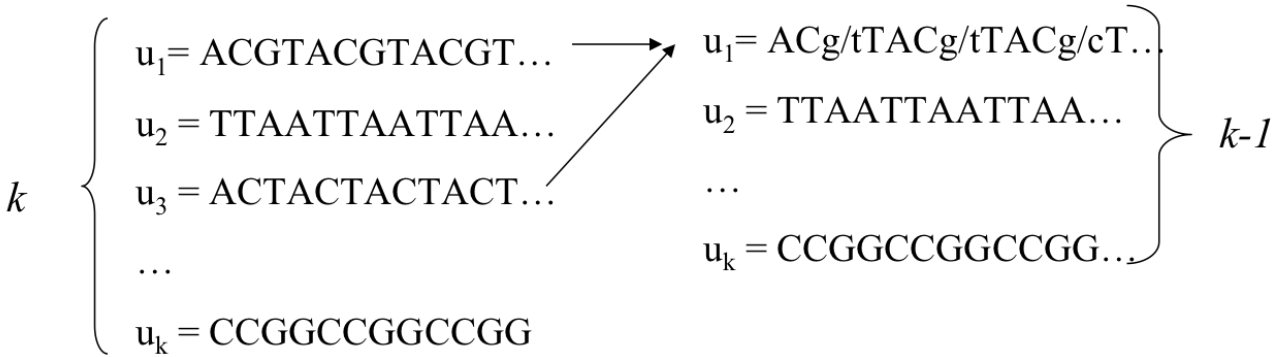
- Idea: don't use the sequences, but align their profiles

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

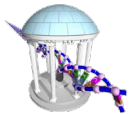
Profile-Based Multiple Alignment: A Greedy Approach



- 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

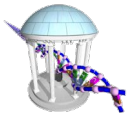


- Consider these 4 sequences

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

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

Example (continued)

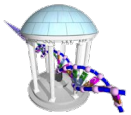


- There are 4 choose 2 = 6 possible pairwise alignments

```
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)
```

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

Example (continued)



- Combine s2 and s4:

```
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 3 choose 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)
```

Next Time

- We'll take a look at proteins
- How the molecular weights of peptide sequences can be used to untangle a protein's sequence