Info based on questions I've received

- Based on the scoring matrix we've discussed, can we assume that the edge cost for any horizontal or vertical move is -1?
 - In a linear gap penalty framework, yes. Every horizontal or vertical move is a gap, because it only makes progress in one of the sequences, not both – hence the other sequence is a gap.
 - This is not true in an affine gap penalty framework, as we will see today

Info based on questions I've received



- Should we use the linear or circular settings on NCBI Glimmer?
 - Linear. We are not dealing with organisms with circular DNA.
- Regarding the genetic coding, should we use "Bacteria, Archaea" or "Mycoplasma, Spiroplasma"?
 - Mycoplasma. I seem to be getting better results with this, even though neither groups is any closer to viruses than the other.

A general overview on deciphering contigs

A jigsaw puzzle on contigs

- The contigs you get are not assembleable: assume they are the result of some assembly process/software
- Genes from the virus are in the contigs, but the sequencing process does not guarantee that the genes are not spread across a few contigs
- Thus, gene predictions made with Glimmer and Genemark can be inaccurate for a number of reasons:
 - The gene might not be wholly contained on the contig, and thus it's incompleteness might throw off Glimmer
 - Glimmer and Genemark are not 100% accurate, so they could simply be wrong (note that they are not trained on viruses either)

Making gene predictions on this data

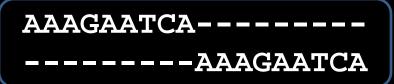
- 1. Use Nano to assemble putative contigs
 - You know the contigs fit together somehow, so there are only so many combinations. Try putting a few contigs together and submitting the concatenated string to see how that changes Glimmer/Genemark's predictions
- 2. Use Blast to search for genes predicted by Glimmer and Genemark
 - Blast can help you find the gene you are looking for, and it's total length – use the length and the length of the contig, to guess if you need another contig added on, and to also guess which contigs connect to which.

You should be able to find all genes and which contigs they are on through this kind of deduction.

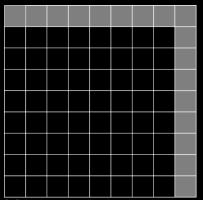
Dynamic Programming with Affine Gap Penalties Part 1

A biological rationale for gap penalties

- The purpose of gap scoring:
 - To bias automated alignment algorithms towards realistic explanations of insertion and deletion
- The grid of alignment possibilities outlines all possible alignments – mostly implausible:

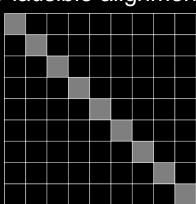


Implausible alignment



AAAGAATCA AAAGAATCA

Plausible alignment



Linear gap penalties are implausible

- We score implausible alignments negatively
 - The more implausible the alignment is, the more negatively it SHOULD score
 - Otherwise our scoring system is bad.
- But large insertions are not much more implausible than small insertions.
 - The same mechanics cause them:
 - Crossovers, viral insertions, etc etc
 - The probability of small insertions is not so different from large insertions, relative to the probability of no insertion at all.
- Affine penalties penalize less for longer deletions or insertions

An example of linear gap failure

AAAGAATTCA

A-A-A-T-CA

An implausible alignment

AAAGAATTCA

AAA---TCA

An plausible alignment

- We score implausible alignments negatively
- Large insertions are not much more implausible than small insertions.
 - The same mechanics cause them:
 - Crossovers, viral insertions, etc
- The probability of small insertions is not so different from large insertions
- Affine penalties penalize less for longer deletions or insertions

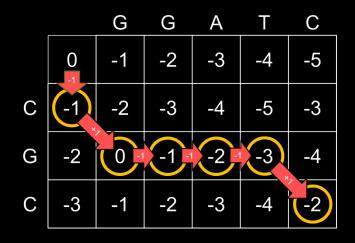
What is an Affine Gap Scoring Penalty?

- The penalty has two parts:
 - S, the Gap Opening Penalty: -10
 - This is the penalty for opening a new gap
 - E, the Gap Extension Penalty: -.5
 - This is the penalty for making a gap bigger

- Gap Scoring Penality = S + d*E
 - For gaps of size d.

Affine Gaps and Dynamic Programming

		G	G	Α	Т	С
	0	-1	-2	-3	-4	-5
С	-1	-2	-3	-4	-5	-3
G	-2	0	-1	-2	-3	-4
С	-3	-1	-2	-3	-4	-2



- Using Dynamic Programming, we could fill out a grid and backtrack to find the optimal alignment
 - Every grid square had an optimal score
 - Adding a new nucleotide to the end of the sequence requires calculation only of the new squares
- With Affine Gaps, the score depends on how long the gap was. But we don't know that from a single grid.

Example: How affine scoring is different

match = 10, mismatch = -2, gap = -7, $gap_start = -15$

Alignment

Scoring

CAGT

CA-T

Linear score: $\frac{30}{15} - \frac{7}{15} = 8$

CAGTA

CA-T-

WRONG: 30 - 15 - 7 - 15 - 7 = -14

This does not correctly implement

Affine Gap Scoring

CAGTA

CAT--

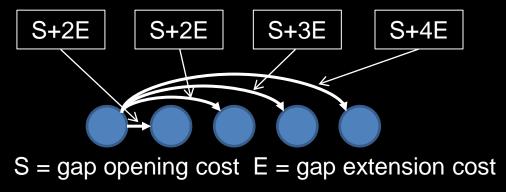
Correct: 20 - 2 - 15 - 14 = -11

Mismatch is outscored by the cost of a second gap opening penalty

 Under affine gap scoring, adding a new nucleotide changes the entire score

A false start on affine gap penalty scoring

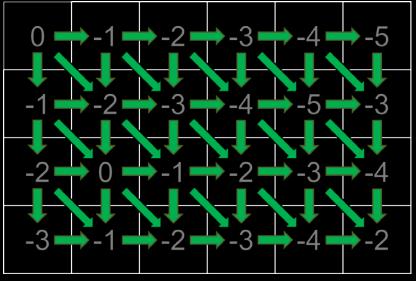
You could add more gap edges in your graph



- This would correctly represent the gap opening and extension costs (if you did it in both dimensions, for every node)
- Unfortunately, you would have to build many more edges: ~ mn² + nm² rather than ~mn
- It would take much longer to update and score such a graph.

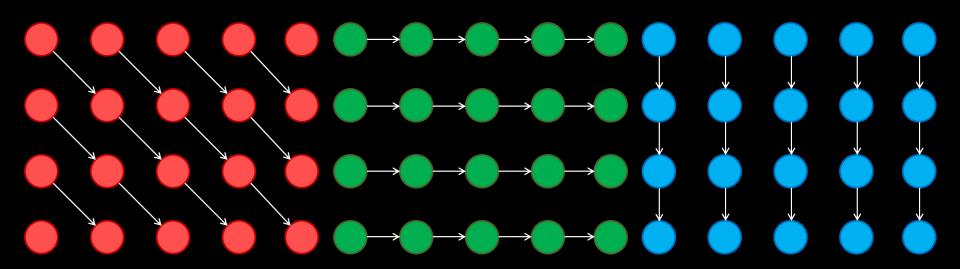
Topology of the Affine Gap Scoring Graph

The Linear Gap scoring graph



The Affine Gap Scoring Graph (Below)

- This graph is just a deconstructed version of the linear graph, with 3 subgraphs: M, X, Y.
- M scores matches, mismatches
- X and Y score gaps
- Edges between them score Gap Extension penalties (not shown)

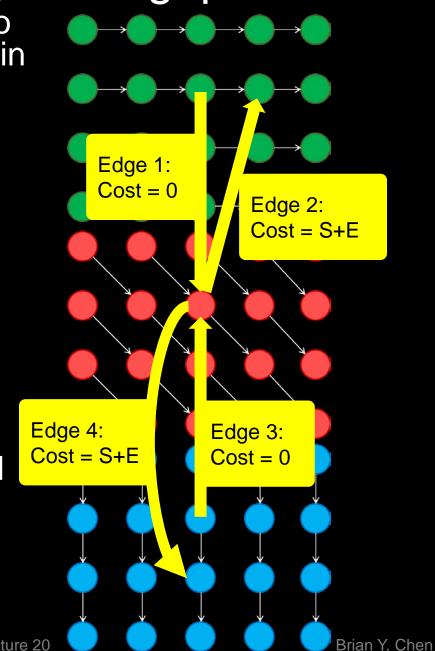


Edges connecting M, X, Y are gap starters

 Every node in M is connected to two nodes in X, and two nodes in Y.

Start cost = S, Extension = E

- Lets look at M[2, 1]:
 - Edge 1 comes from X[2, 1]
 - "done with horizontal gap"
 - Edge 2 goes to X[3, 1]
 - "starting horizontal gap"
 - Edge 3 comes from Y[2, 1]
 - "done with vertical gap"
 - Edge 4 goes to Y[2,2]
 - "starting vertical gap"
- Inter-graph edges start and end gaps.
- Not Shown: Edges from X to Y
 - "cross sequence gaps"



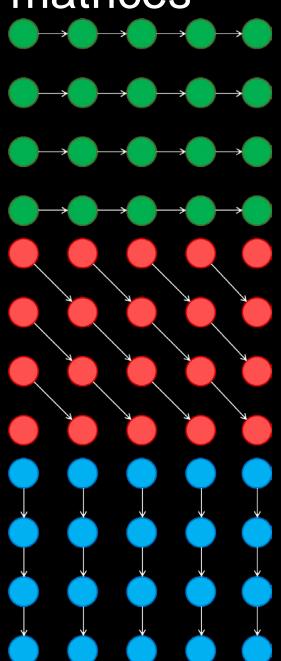
Edges Values inside the 3 matrices

Edges in X have the weight of exactly one gap extension
See previous slide for intersubgraph edges

S = Gap opening, E = Gap extension

Edges in M have the weight exactly one match or mismatch (depending on the row/column), See previous slide for intersubgraph edges

Edges in Y have the weight of exactly one gap extension. See previous slide for intersubgraph edges



Node values in the 3 matrices

M: scores of alignments ending in match, mismatch

X: scores of alignments ending in gaps in seq. x

Y: scores of alignments ending in gaps in seq. y

$$\begin{aligned} & \underbrace{\text{Score of a match}}_{\text{or mismatch}} & \underbrace{\text{M}(i\text{-}1,\,j\text{-}1)}_{\text{X}(i,\,j)} \\ & \underbrace{\text{M}[i,\,j] = \text{match}(i,\,j) + \text{max}}_{\text{Y}(i,\,j)} & \underbrace{\text{X}(i,\,j)}_{\text{Y}(i,\,j)} \\ & \underbrace{\text{X}[i,\,j] = \text{max}}_{\text{S}} & \underbrace{\text{S} + \text{E} + \text{M}(i,\,j\text{-}1)}_{\text{S} + \text{E} + \text{Y}(i,\,j\text{-}1)} \\ & \underbrace{\text{S} + \text{E} + \text{M}(i\text{-}1,\,j)}_{\text{S} + \text{E} + \text{X}(i\text{-}1,\,j)} \\ & \underbrace{\text{S} + \text{E} + \text{X}(i\text{-}1,\,j)}_{\text{E} + \text{Y}(i\text{-}1,\,j)} \end{aligned}$$

Explaining the scores

Score of a match or mismatch

M[i, j] = match(i, j) + max

$$X(i, j)$$

Y(i, j)

- M[i,j] is the score you get from a matching or mismatching pair of bases.
 - The alternate X and Y scores correspond to ending a gap for zero cost.
 - Some gaps end in a match or a mismatch, and this is how it ends – the X and Y scores here are the edges coming back from the other two matrices

Explaining the scores

$$X[i, j] = max$$

$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$

- X[i,j] is the score you get for gapping in X.
 - The S + E cost in the M() component is the cost of starting a new gap (S) from a matched pair, plus the first extension and whatever the score from M was.
 - The E cost alone is simply extending a gap, so you do not incur a new gap opening cost
 - The S + E cost in the Y component is a the cost of starting a new gap on the X sequence whereas before it was a gap on the Y sequence

Explaining the scores

$$Y[i, j] = max$$

$$\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$$

- Y[i,j] is the score you get for gapping in Y.
 - The S + E cost in the M() component is the cost of starting a new gap (S) from a matched pair, plus the first extension and whatever the score from M was.
 - The E cost alone is simply extending a gap, so you do not incur a new gap opening cost
 - The S + E cost in the X component is a the cost of starting a new gap on the Y sequence whereas before it was a gap on the X sequence

Major steps in Alignment with Affine Gaps

Scoring

 Scoring is a little complex, because it does additional inter-subgraph nodes. Otherwise it is the same scoring methods used in linear gap scores

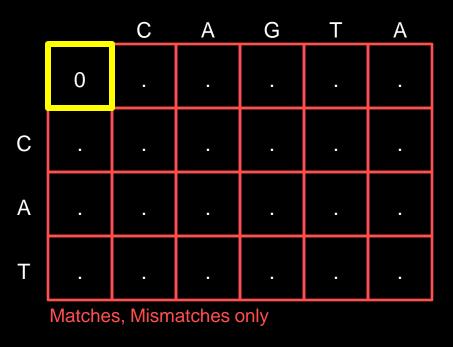
Backtracking

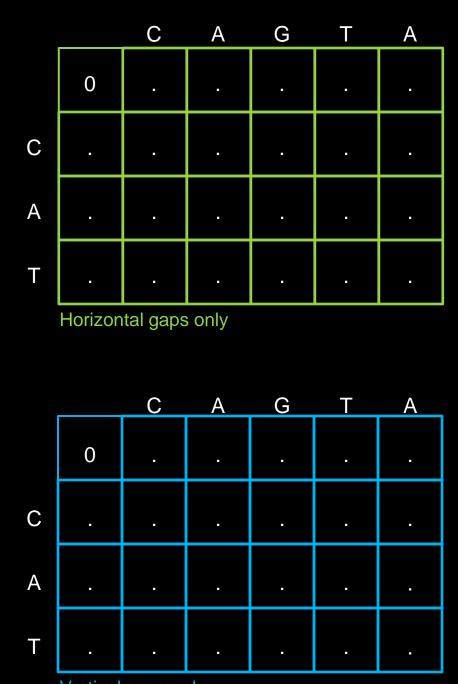
 Backtracking the graph requires us to check all connected graph nodes, but is otherwise exactly the same as before

Reference for the next slides:

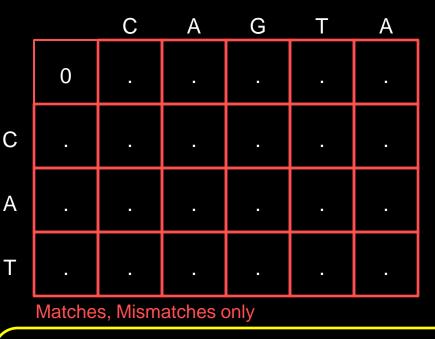
- X is the vertical sequence, Y the horizontal sequence
- i iterates vertically, j iterates horizontally, when both used
- S is the gap start penalty, -10.
- E is the gap extention penality, -.5.

This spot starts as zero





- This means "score of best alignment between 0 bases of x and i bases of y that ends in a gap in x"
- In other words, x is all gaps against y, so score = S + i*E

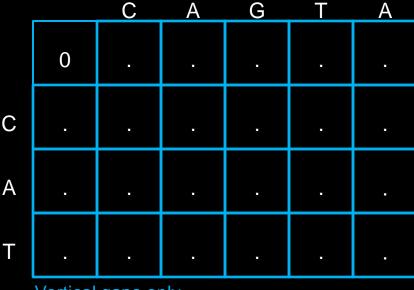


$$X[i, j] = max$$

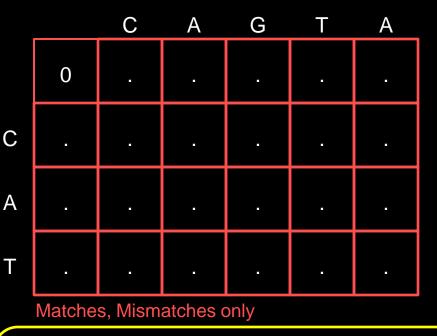
$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$

		С	Α	G	Т	А
	0	-10.5	-11	-11.5	-12	-12.5
;						
\						
-			·			
	Lorizon	tal nans	only			

Horizontai gaps oniy

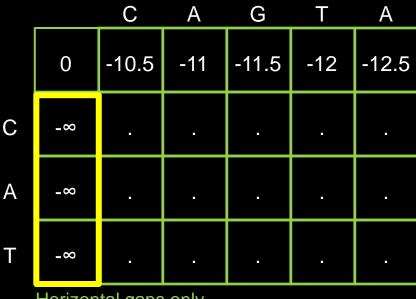


- This row means "score of the best alignment between i bases of x and 0 bases of y, ending in a gap in x".
- This is impossible; the bases of x cannot be a gap.
 - so the score is -∞



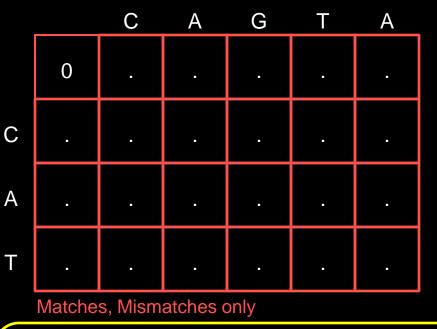
$$X[i, j] = max$$

$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$





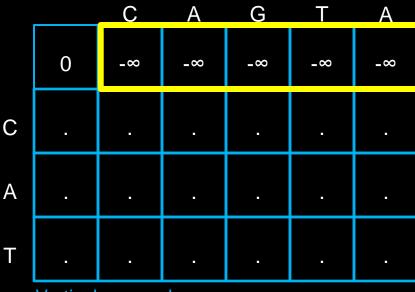
- This row means "score of the best alignment between i bases of y and 0 bases of x, ending in a gap in Y.
- This is impossible; the bases of Y cannot be a gap.
 - so the score is -∞



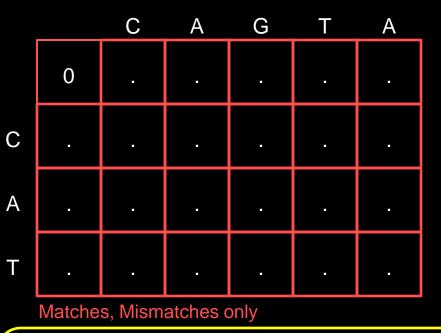
$$Y[i, j] = max$$

$$\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$$

		С	Α	G	T	Α
	O	-10.5	-11	-11.5	-12	-12.5
С	-∞		·			
А	-8	·				
Т	-8		·	·		
	l la diman	(-1	a sa la s			

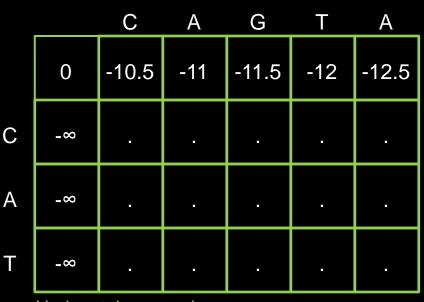


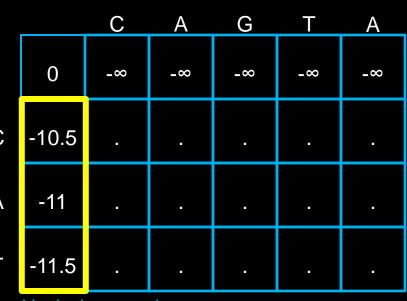
- This column means "score of best alignment between 0 bases of y and i bases of x that ends in a gap in y"
- In other words, y is all gaps against x, so score = S + i*E



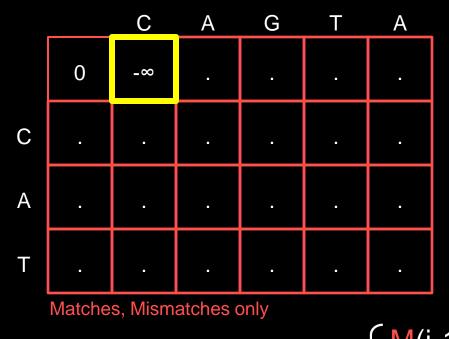
$$Y[i, j] = max$$

 $\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$





- This means: "score of best alignment between 0 bases of x and 1 base of y that ends in a match". Impossible.
- Score: -∞, a special case.

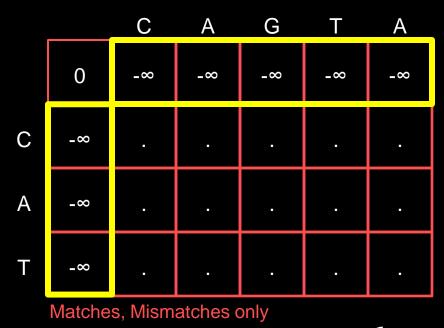


$$M[i, j] = match(i, j) + max \begin{cases} M(i-1, j-1) \\ X(i, j) \\ Y(i, i) \end{cases}$$

		С	Α	G	Т	Α
	O	-10.5	-11	-11.5	-12	-12.5
;	-8					
\	-∞					
	-∞		·			

	С	Α	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5					
-11					
-11.5				·	·

- This point extends to all other values of M[0, i] and M[i, 0].
- No alignment of 0 and i bases can end in a match.



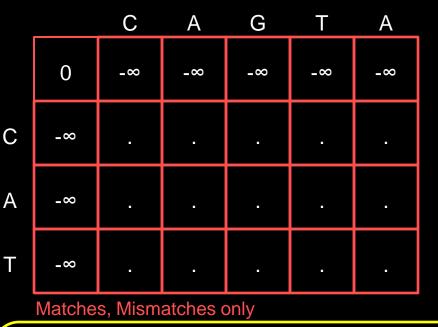
$$M[i, j] = match(i, j) + max \begin{cases} M(i-1, j-1) \\ X(i, j) \\ Y(i, j) \end{cases}$$

	С	Α	G		A
0	-10.5	-11	-11.5	-12	-12.5
-∞					
-∞					
-∞					
	-8	-∞ . -∞ .	-∞	-∞	-∞

	С	А	G	Т	А
0	-∞	-∞	-∞	_∞	-∞
-10.5					
-11					
-11.5					

•
$$S + E + M(i, j-1) = -\infty$$

- $E + X(i, j-1) = -\infty$
- S + E + Y(i, j-1) = -21
- This means you gapped the C in C
 Y, then gapped the C in X.



$$X[i, j] = max$$

$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$

	С	Α	G	T	Α
0	-10.5	-11	-11.5	-12	-12.5
-∞	-21				
-∞	·	·			
-∞		·	·		·

	С	Α	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5					
-11					
-11.5		·	·	·	·

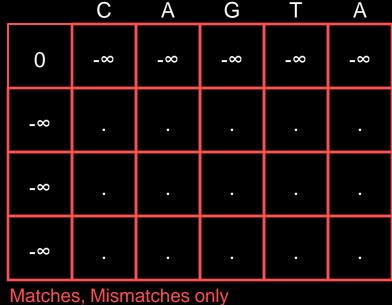
•
$$S + E + M(i-1, j) = -\infty$$

- S + E + X(i-1, j) = -21
- $E + Y(i-1, j) = -\infty$

C

Α

 This means you gapped the C in C X, then gapped the C in Y.



Y[i, j] = max
$$\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$$

	С	Α	G	T	Α
0	-10.5	-11	-11.5	-12	-12.5
-8	-21				
-∞					
-8					·

Horizontal gaps only

	С	Α	G	Ţ	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5	-21				
-11					
-11.5			·		

•
$$M(i-1, j-1) = 0$$

•
$$X(i, j) = -21$$

•
$$Y(i, j) = -21$$

C

A

$$M[i, j] = match(i, j) + max \langle X(i, j) \rangle$$

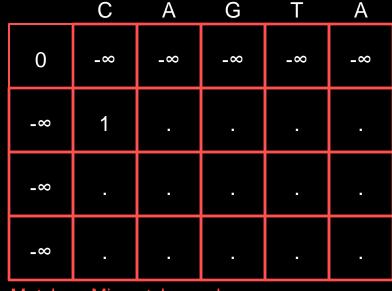
$$\max \begin{cases} M(i-1, j-1) \\ X(i, j) \\ Y(i, j) \end{cases}$$

	С	Α	G	Т	Α
0	-10.5	-11	-11.5	-12	-12.5
-∞	-21				
-∞		·			
-8			·	·	·

Horizontal gaps only

	С	Α	G	Т	Α
0	-∞	-∞	-∞	_∞	-∞
-10.5	-21	·			
-11					
-11.5					

- Now we do X:
 - i.e. suppose we start a new gap on x
- S + E + M(i, j-1) = -9.5
- E + X(i, j-1) = -21.5
- S + E + Y(i, j-1) = -31.5



Matches, Mismatches only

C

A

$$X[i, j] = max$$

$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$

	С	Α	G	Т	Α
0	-10.5	-11	-11.5	-12	-12.5
-∞	-21	-9.5			
-∞					
-∞	·	·			

Horizontal gaps only

	С	Α	G	Т	Α
0	-∞	-∞	_∞	_∞	-∞
-10.5	-21				
-11					
-11.5					

- Now we do Y:
 - i.e. suppose we start a new gap on y
- S + E + M(i-1, j) = $-\infty$
- S + E + X(i-1, j) = -21.5
- E + Y(i-1, j) = $-\infty$

	С	Α	G		Α
0	-∞	-∞	-∞	-∞	-∞
-∞	1				
-∞					
-∞					
Mataba	B 41	otoboo			

Matches, Mismatches only

$$Y[i, j] = max$$

$$\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$$

	С	Α	G	Т	Α
0	-10.5	-11	-11.5	-12	-12.5
-∞	-21	-9.5	·		
-∞			·		
-∞					·

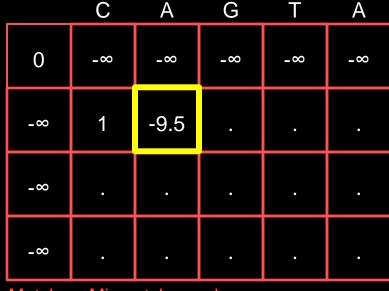
Horizontal gaps only

	С	Α	G	Т	Α
0	-∞	-∞	-∞	_∞	_∞
-10.5	-21	-21.5			
-11	·				
-11.5					

- Now, the next square:
- match(i, j) = -4 (mismatch)
 - $-M(i-1, j-1) = -\infty$
 - X(i, j) = -9.5
 - Y(i, j) = -21.5

C

A



Matches, Mismatches only

$$M[i, j] = match(i, j) + max \begin{cases} M(i-1, j-1) \\ X(i, j) \\ Y(i, j) \end{cases}$$

		С	Α	G	Τ	Α
	O	-10.5	-11	-11.5	-12	-12.5
;	-8	-21	-9.5			
	-8		·			
	-8		·			·

Horizontal gaps only

	С	А	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5	-21	-21.5			
-11				·	
-11.5	·		·	·	·

- Suppose a gap opened on X:
- S + E + M(i, j-1) = -20
- E + X(i, j-1) = -10
- S + E + Y(i, j-1) = -32

	С	Α	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-8	1	-9.5			
-∞					

Matches, Mismatches only

$$X[i, j] = max$$

$$\begin{cases} S + E + M(i, j-1) \\ E + X(i, j-1) \\ S + E + Y(i, j-1) \end{cases}$$

		С	Α	G	Т	Α
	O	-10.5	-11	-11.5	-12	-12.5
;	-∞	-21	-9.5	-10		
\	-∞					
-	-∞		·			·

Horizontal gaps only

	С	Α	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5	-21	-21.5			
-11					
-11.5		·	·	·	

- Suppose a gap opened on Y:
- S + E + M(i-1, j) = $-\infty$
- S + E + X(i-1, j) = -22
- $E + Y(i-1, j) = -\infty$

	С	Α	G	T	Α
0	-∞	-∞	-∞	-∞	-∞
-∞	1	-9.5			
-∞					
-8					

Matches, Mismatches only

$$Y[i, j] = max$$

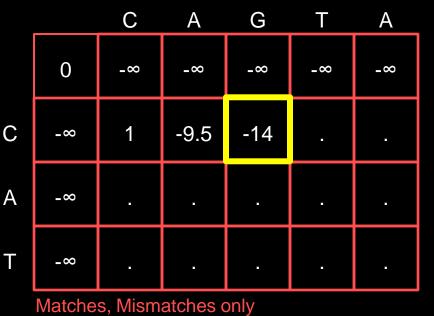
$$\begin{cases} S + E + M(i-1, j) \\ S + E + X(i-1, j) \\ E + Y(i-1, j) \end{cases}$$

		С	Α	G	Т	Α
	0	-10.5	-11	-11.5	-12	-12.5
2	-∞	-21	-9.5	-10		
Д	-∞				·	
Т	-∞		·			

Horizontal gaps only

	С	Α	G	Т	Α
0	-∞	-∞	-∞	-∞	-∞
-10.5	-21	-21.5	-22		
-11					
-11.5	·				·

- Back to M
- match(i, j) = -4
 - M(i-1, j-1) = -∞
 - X(i, j) = -10
 - -Y(i, j) = -22



M[i, j] = match(i, j) + max≺ X(i, j)

$$X = \begin{cases} M(i-1, j-1) \\ X(i, j) \\ Y(i, j) \end{cases}$$

	С	Α	G	Т	Α
0	-10.5	-11	-11.5	-12	-12.5
-∞	-21	-9.5	-10		
-∞					
-∞					

Horizontal gaps only

	С	А	G	Т	А
0	-∞	-∞	-∞	_∞	-∞
-10.5	-21	-21.5	-22		
-11					
-11.5					

I did a few more by hand:

	С	А	G	Т	Α		
0	-∞	-∞	-∞	-∞	-∞		
-∞	1	-13.5	-14	-14.5	-15		
-∞	-13.5	2	-12.5	-13	-8.5		
-∞	-14	-12.5	-2	-11.5	-17		
Matches, Mismatches only							

		С	A	G	T	A
	0	-10.5	-11	-11.5	-12	-12.5
С	-∞	-21	-9.5	-10	-10.5	-11
A	-∞	-21.5	-20	-8.5	-9	-9.5
т [-∞	-22	-20.5	-19	-12.5	-13

Horizontal gaps only

		С	Α	G	Т	Α
	0	-∞	-∞	-∞	_∞	-∞
С	-10.5	-21	-21.5	-22	-22.5	-23
Α	-11	-9.5	-20	-20.5	-21	-21.5
Т	-11.5	-10	-8.5	-19	-19.5	-19

So far:

 We have seen how we can do scoring for affine gap penalties

 Next time we will see how to do backtracking for affine gap penalties

 Also, next time we will have a question and answer session on Project 2