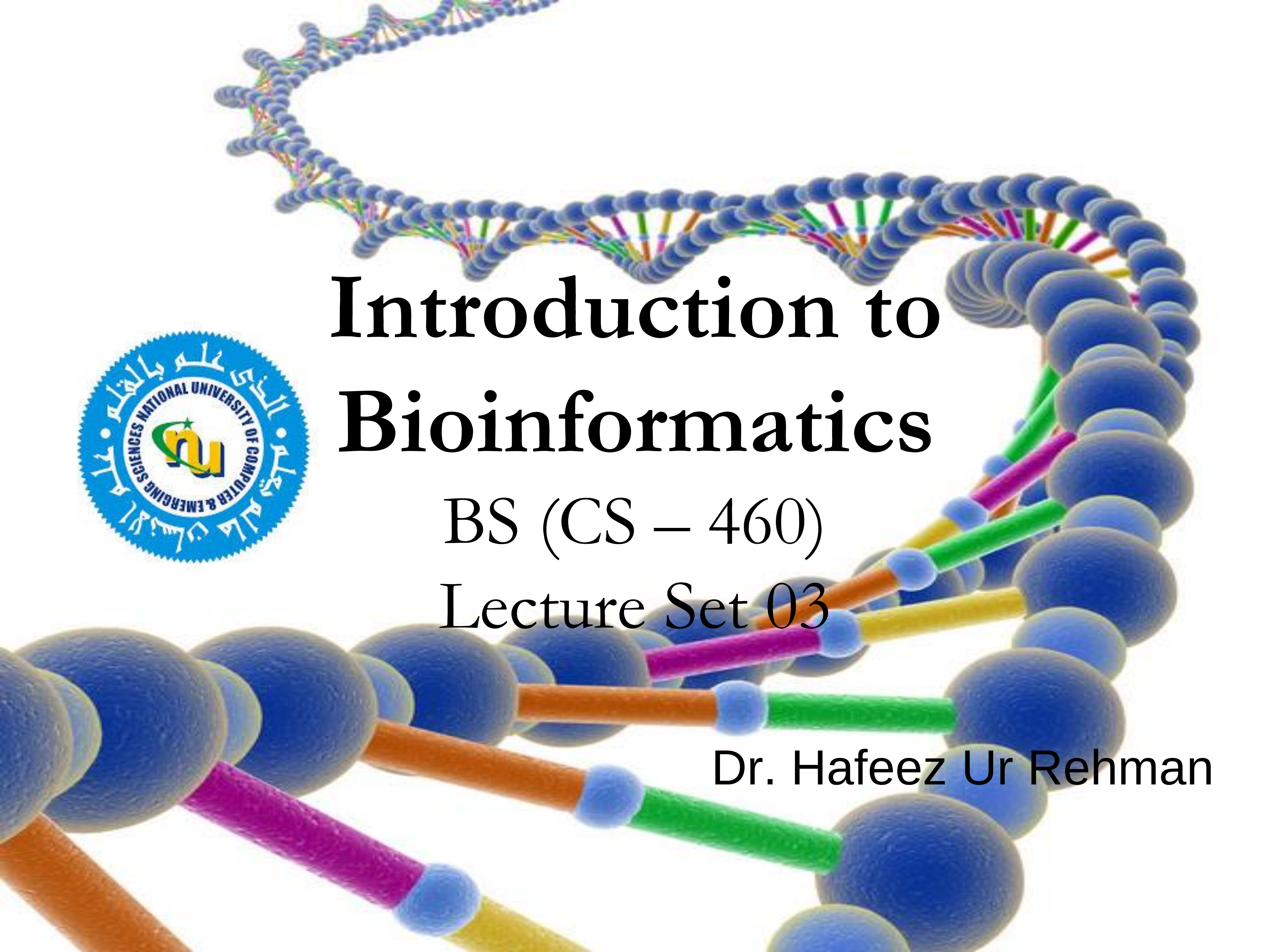


Introduction to Bioinformatics

BS (CS – 460)

Lecture Set 03

Dr. Hafeez Ur Rehman

Assessment of Sequence Alignment

Methods of Sequence Alignment

- Dot Plots
- Dynamic Programming
- Heuristics Methods

The Dot plot

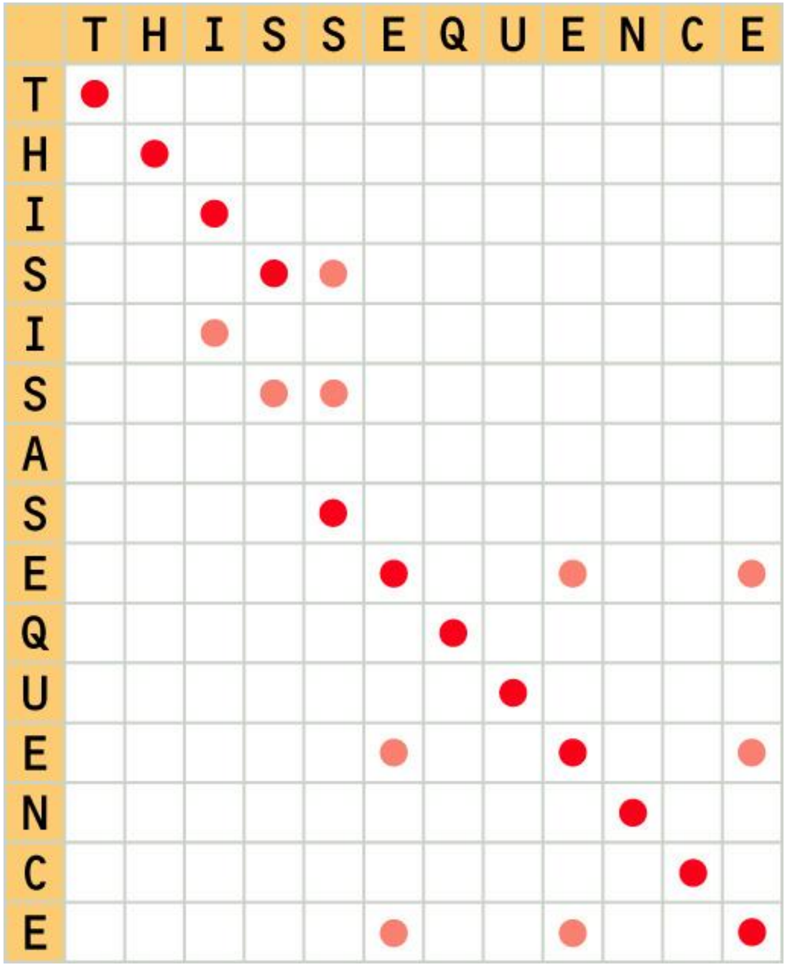
- A "better" way of doing alignment is to represent each sequence as a table or matrix, where one sequence represents the rows and the other the columns. The Dot plot Matrix is a **visual** way of seeing the alignment between two sequences:
  - The first sequence (query sequence) represents the rows and the other sequence (subject sequence) represents the columns.
  - All elements (row/column) are checked for a match and if there is one, then the cell is marked.
  - This will show all areas of both sequences where matches occur.

Dot plot

- Consider the following:
  - Diagonal lines represent an alignments (match)
  - Horizontal lines between aligned sequences indicate gaps are required (where the gaps indicate a deletion/insertion)

- Longest sequence of alignments are:
  - *“THIS” ; and “SEQUENCE“;*
  - *“IS” would be considered as gaps*

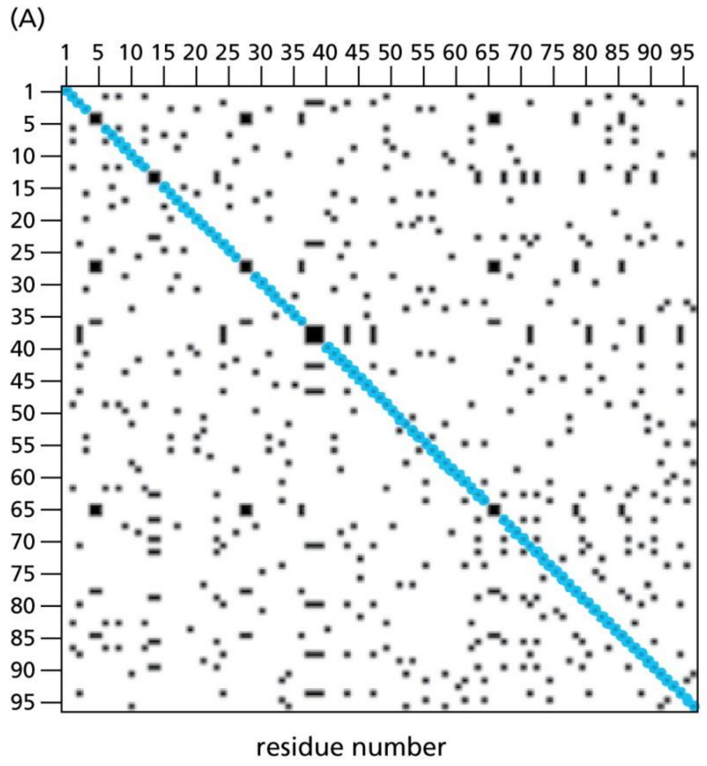
- *The pink dots: they can represent noise (spurious alignments)*

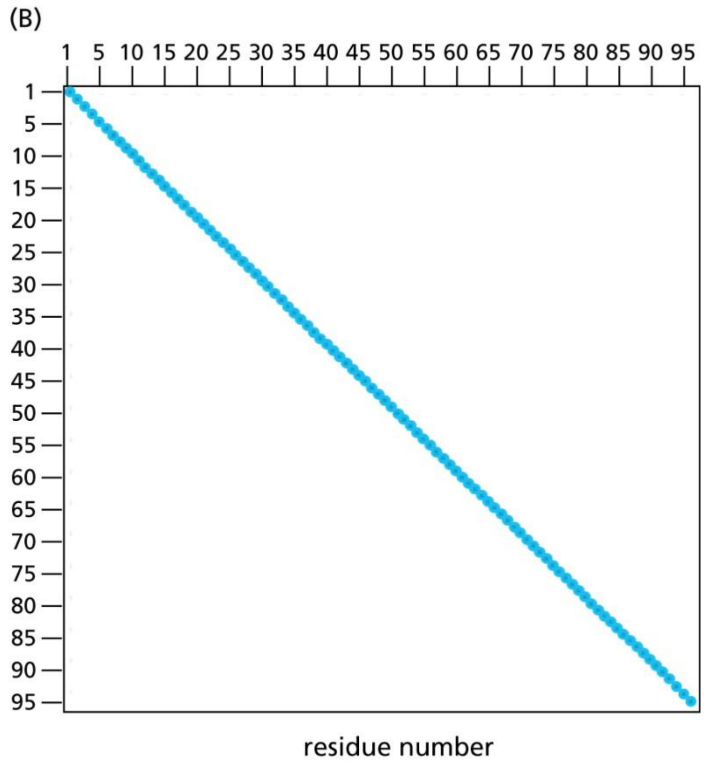


Dot plot Matrix: purpose

- This allows us to **visualise** areas of "**local alignment**" as opposed to global alignment.
- One of the main purpose to find domains / motifs that match . This could be useful for many reasons; e.g. promoter factor binding site, finding exons….
- For visualisation of pair-wise alignment you have one query on the x-axis and the other on the y-axis.

Dot Plot noise





This shows the effect of noise (blue line has been inserted to highlight alignment of interest. The figure on the left represents **SH2** sequence plotted against inself. The one on the right has been filter; in this case an alignment must be at least 10 residues long with a score of 3.

Applying Dot Plot Filter

- Evaluating similarity between 2 sequences
- Window size – number of nucleotides compare each time (usually odd number)*
- Stringency – the minimum number of nucleotides in the window must be "match", so that a dot can be placed
- Mismatch Limit – the maximum number of nucleotides in the window can be "*not* match", so that a dot can still be placed
- **Mismatch Limit = Window size - Stringency**

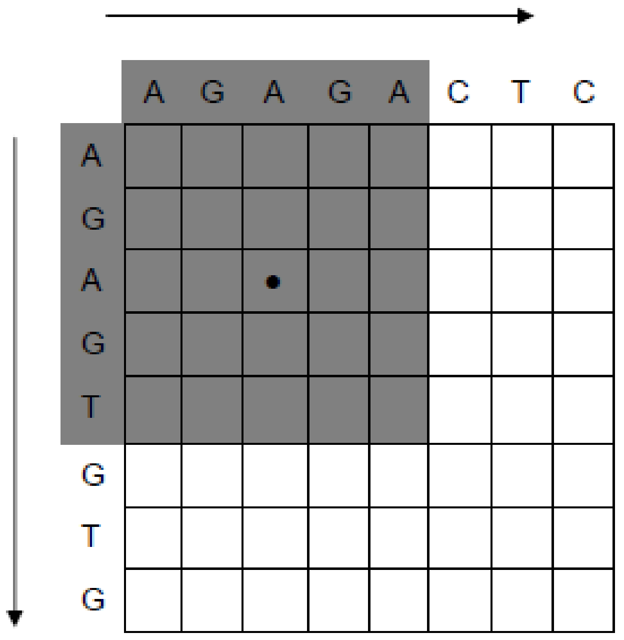
* Because we put dot in the center of the window

Example

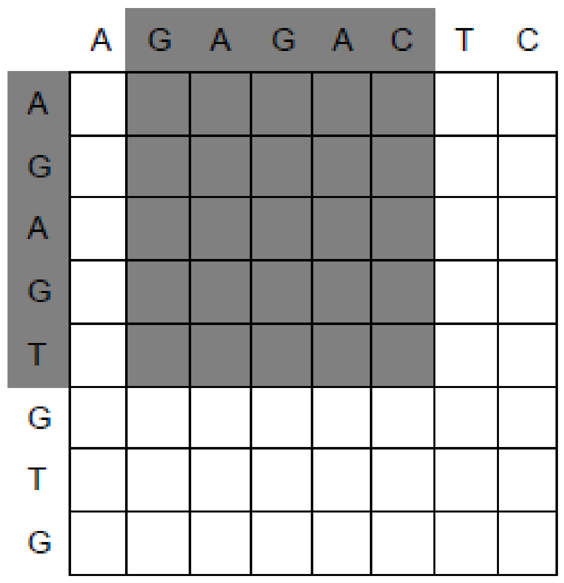
- Example 1: Compare the following sequences, with window size = 5, stringency = 3

AGAGACTC

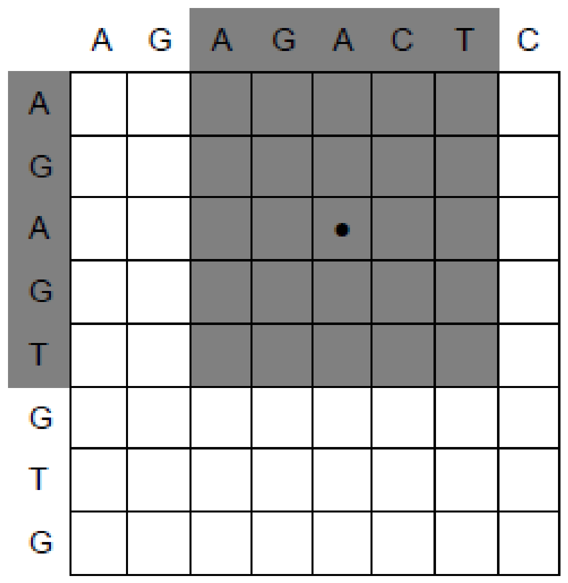
AGAGTGTG



4 matches

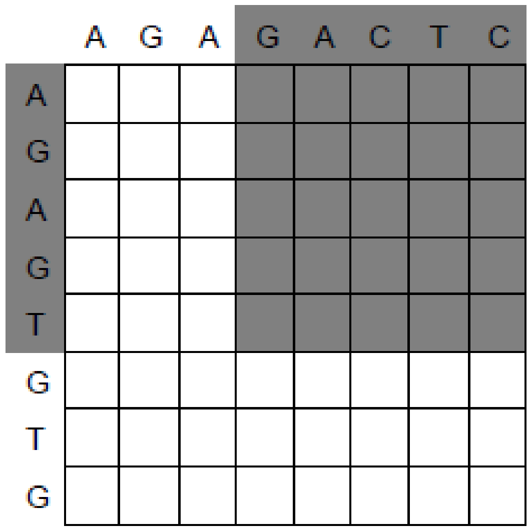


0 match

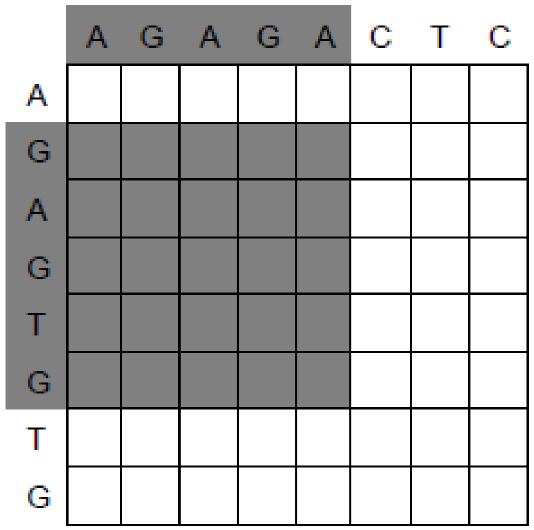


4 matches

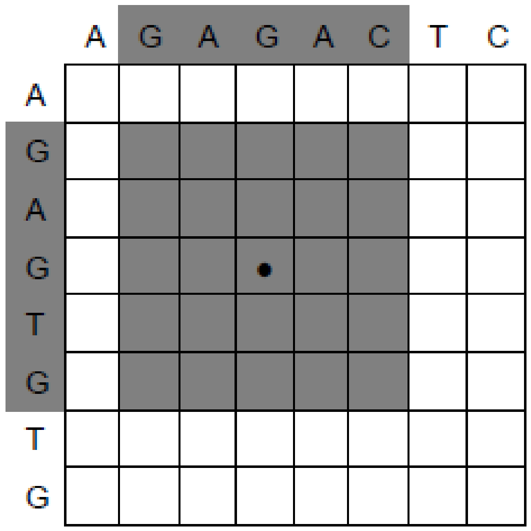
Example (contd…)



0 match

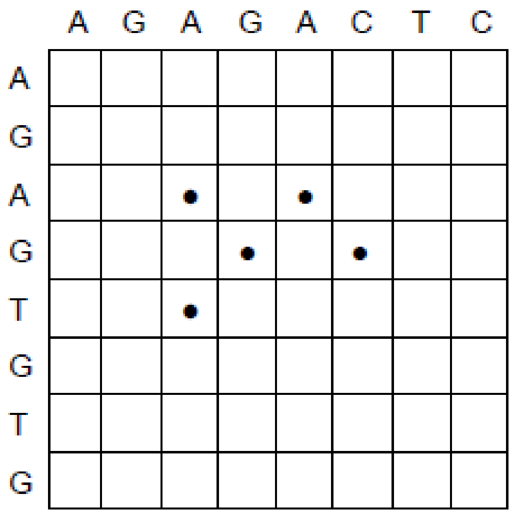


0 match



3 matches

Final answer →



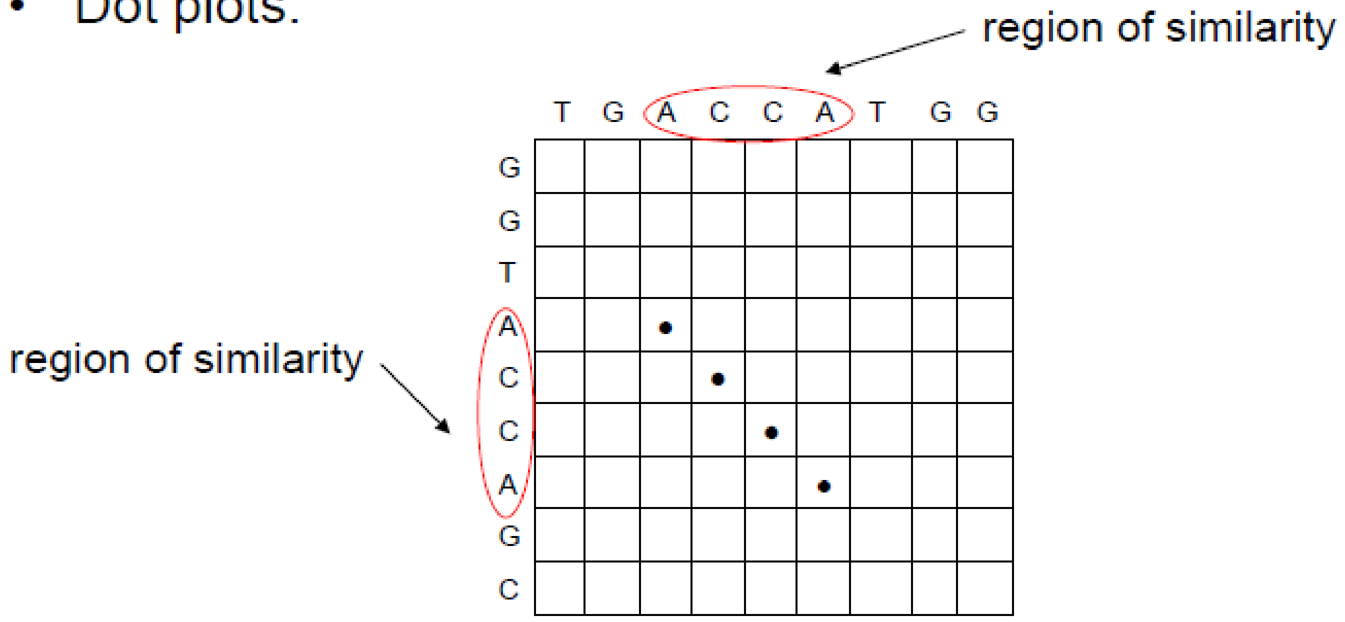
Example 02

- Example 2: Compare the following sequences and find the regions of similarity between two sequences. (window size = 5, stringency = 3)

  TGACCATGG

  GGTACCAGC

- Dot plots:

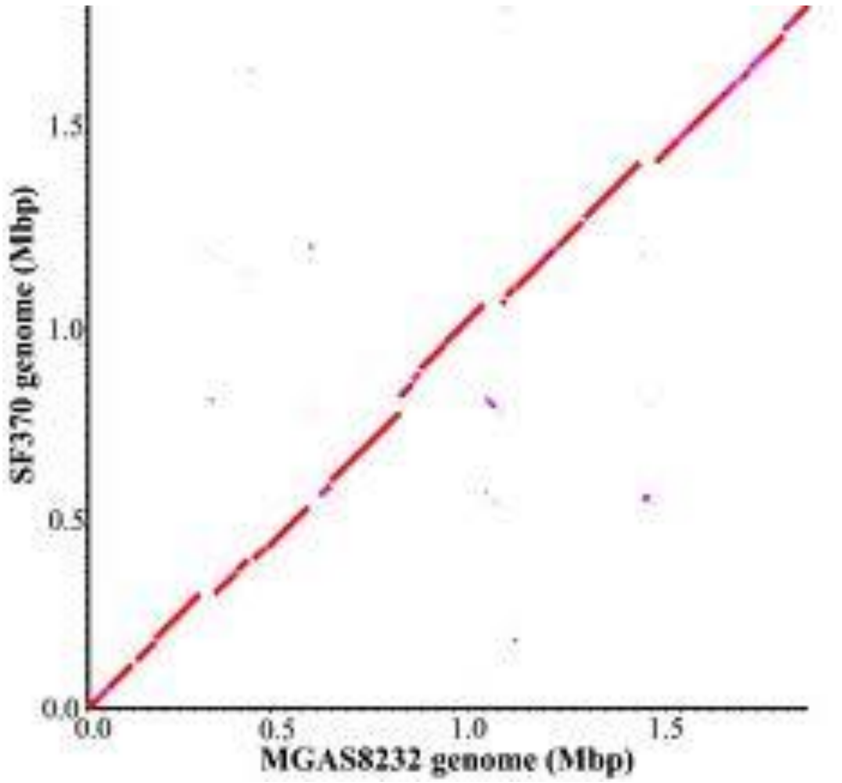


Which Window Size to Choose?

- Long window
  - Clean dot plots
  - Little sensitivity
- Short window
  - Noisy dot plots
  - Very sensitive
- The size of the window should be in the range of the elements you are looking for
  - Conserved domains: 50 amino acids
  - Transmembrane segments: 20 amino acids
- Shorten the window to compare distantly related sequences

Dot plot Matrix: imperfect match

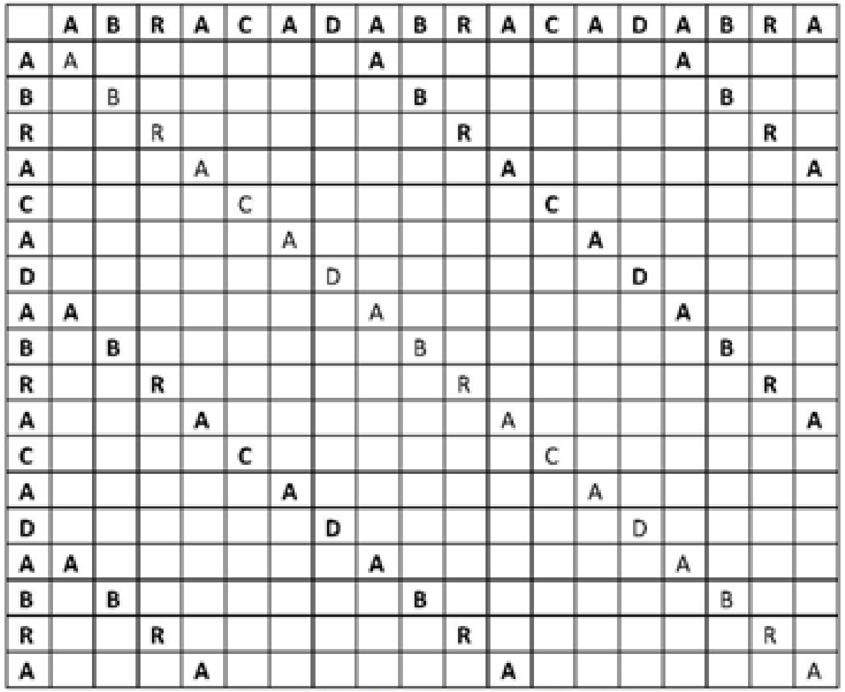
- Some alignments **require gaps to increase the matching score**; the gaps are used to represent inclusion/deletion mutations
- The diagram shows that most of the 2 sequences are aligned.
- Gaps indicate areas of non-alignment i.e., gaps or substitutions.



Tandem repeat dot plot

- To determine if there is tandem repeats the sequence is compared with itself (see table 1 in next slide)
- The more diagonals the more repeats
- The **diagonals at the bottom** left compare the start with the finish
- The fact the main diagonal means the both sequences are the same .
- The lines are symmetrical around the main diagonal (next slide).

Dot plot of tandem repeats



	A	B	R	A	C	A	D	A	B	R	A	C	A	D	A	B	R	A
A	A							A							A			
B		B							B							B		
R			R							R							R	
A				A							A							A
C					C							C						
A						A							A					
D							D							D				
A	A							A							A			
B		B							B							B		
R			R							R							R	
A				A							A							A
C					C							C						
A						A							A					
D							D							D				
A	A							A							A			
B		B							B							B		
R			R							R							R	
A				A							A							A

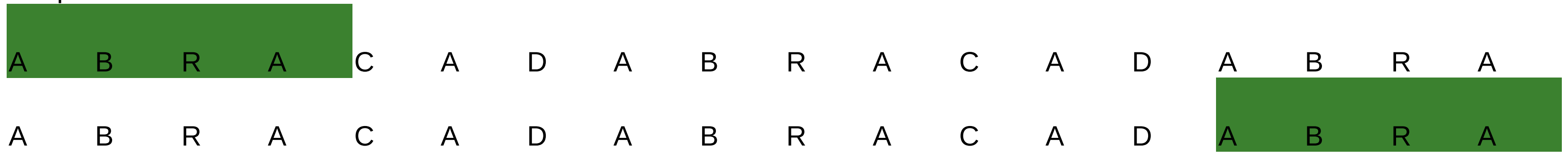
Table 1: Dot plot of tandem repeats, adapted form lesk 2008

The above dotplot illustrates the presence of **Tandem repeats** (there are 2TRs in the above dot plot: the main diagonal is not one)

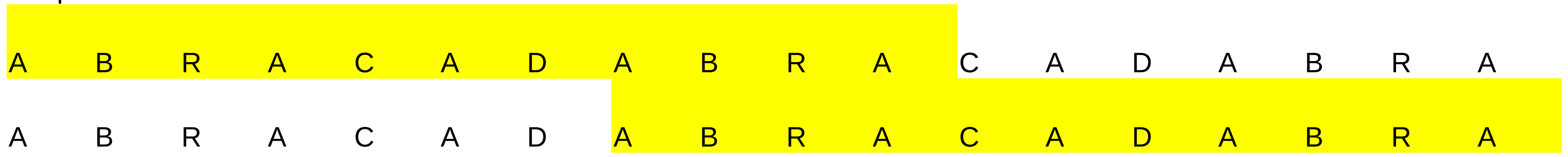
A tandem repeat is a sequence that is repeat: consider the lower left hand diagonal, the sequences that cause this are: ABRA and ABRA Tandem repeats can be short tandem repeats (STR) 2 to 5 bp or tandem repeats (10 to 60 bp); the number of repeating segments can vary from 2 upwards and they can be used in area's such as genetic testing and intron detection. A comprehensive review is given by Gemayel et al 2010.

Tandem repeat as a sequence

Tandem repeat 1

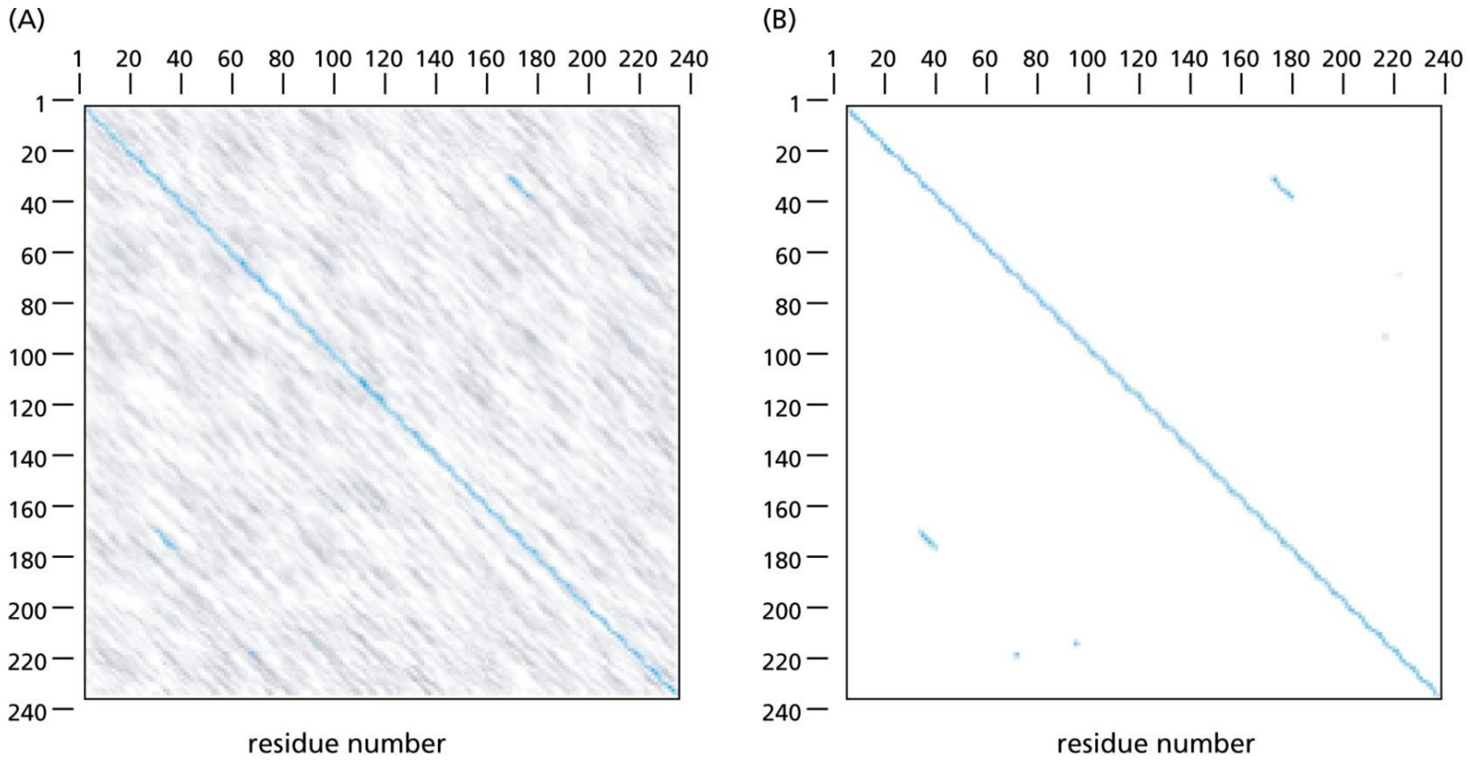

A B R A C A D A B R A C A D A B R A
A B R A C A D A B R A C A D A B R A

Tandem repeat 2


A B R A C A D A B R A C A D A B R A
A B R A C A D A B R A C A D A B R A

Tandem repeats (Example)

- BRCA2 gene has a number of BRC repeats (39 residues long. The diagram shows two plots: one with noise (unfiltered) and the other showing two repeating sequences.



Dynamic Programming

Pair-wise sequence alignments

Idea: Display one sequence above another with spaces inserted in both to reveal similarity

```
A: C A T - T C A - C
   |   |     | |   |
B: C - T C G C A G C
```

Two types of alignment

```
S =  CTGTCGCTGCACG
T =  TGCCGTG
```

Global alignment

```
CTGTCG-CTGCACG
-TGC-CG-TG----
```

Local alignment

```
CTGTCGCTGCACG--
-------TGC-CGTG
```

Global alignment: Scoring

```
CTGTCG-CTGCACG
-TGC-CG-TG----
```

Reward for matches:	α
Mismatch penalty:	β
Space penalty (indel):	γ

$$\text{score}(A) = \alpha w - \beta x - \gamma y$$

w = #matches x = #mismatches y = #spaces

Global alignment: Scoring

Reward for matches:	10
Mismatch penalty:	2
Space penalty:	5

```
 C   T   G   T   C   G   -   C   T   G   C
 -   T   G   C   -   C   G   -   T   G   -

-5  10  10  -2  -5  -2  -5  -5  10  10  -5
```

Total = 11

Optimum Alignment

- The score of an alignment is a measure of its quality
- **Optimum alignment problem:** Given a pair of sequences *X* and *Y*, find an alignment (global or local) with maximum score
- The **similarity** between *X* and *Y*, denoted *sim*(*X*,*Y*), is the maximum score of an alignment of *X* and *Y*

Alignment algorithms

- Global: Needleman-Wunsch
- Local: Smith-Waterman
- Both use ***dynamic programming***
- Variations:
  - Gap penalty functions (Affine Penality)
  - Scoring matrices

1. Global Alignment: Needleman-Wunsch

$$S_{1..i} = \text{Prefix of length } i \text{ of } S$$
$$T_{1..j} = \text{Prefix of length } j \text{ of } T$$

$$C(i, j) = \text{Score of optimum alignment of } \mathrm{S}_{1..i} \text{ and } \mathrm{T}_{1..j}$$

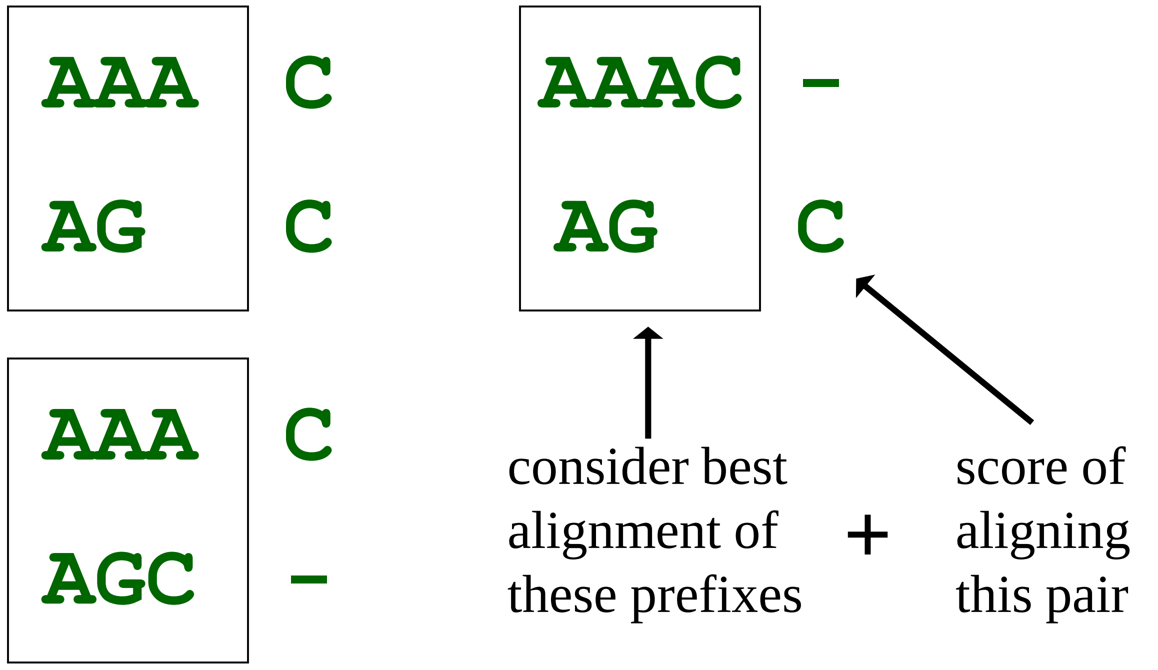
$$w(a,b) = \begin{cases} +\alpha & \text{if } a = b \\ -\beta & \text{if } a \neq b \end{cases}$$

Match Reward

Mismatch Penalty

Dynamic Programming Idea

- consider last step in computing alignment of **`AAAC`** with **`AGC`**
- three possible options; in each we'll choose a different pairing for end of alignment, and add this to the best alignment of previous characters


AAA C
AG C
AAAC –
AG C
AAA C
AGC –
consider best alignment of these prefixes
+
score of aligning this pair

Justification

$$\underbrace{\boxed{\begin{array}{l} S_1\ S_2\ \ldots\ S_{i-1} \\ T_1\ T_2\ \ldots\ T_{j-1} \end{array}}}_{C(i-1,j-1)}\ \underbrace{\begin{array}{c} S_i \\ T_j \end{array}}_{+\ w(S_i,T_j)} \qquad \underbrace{\boxed{\begin{array}{l} S_1\ S_2\ \ldots\ S_{i-1} \\ T_1\ T_2\ \ldots\ T_j \end{array}}}_{C(i-1,j)}\ \underbrace{\begin{array}{c} S_i \\ - \end{array}}_{-\gamma}$$

$$\underbrace{\boxed{\begin{array}{l} S_1\ S_2\ \ldots\ S_i \\ T_1\ T_2\ \ldots\ T_{j-1} \end{array}}}_{C(i,j-1)}\ \underbrace{\begin{array}{c} - \\ T_j \end{array}}_{-\gamma}$$

Example

Case 1: Line up S_i with T_j

```
                   i-1  i
S:  C   A   T   T   C | A   C
T:  C   -   T   T   C | A   G
                   j-1  j
```

Case 2: Line up S_i with space

```
                       i-1  i
S:  C   A   T   T   C   A | -   C
T:  C   -   T   T   C   A | G   -
                        j
```

Case 3: Line up T_j with space

```
                        i
S:  C   A   T   T   C   A | C   -
T:  C   -   T   T   C   A | -   G
                       j-1  j
```

Theorem. C(i,j) satisfies the following relationships:

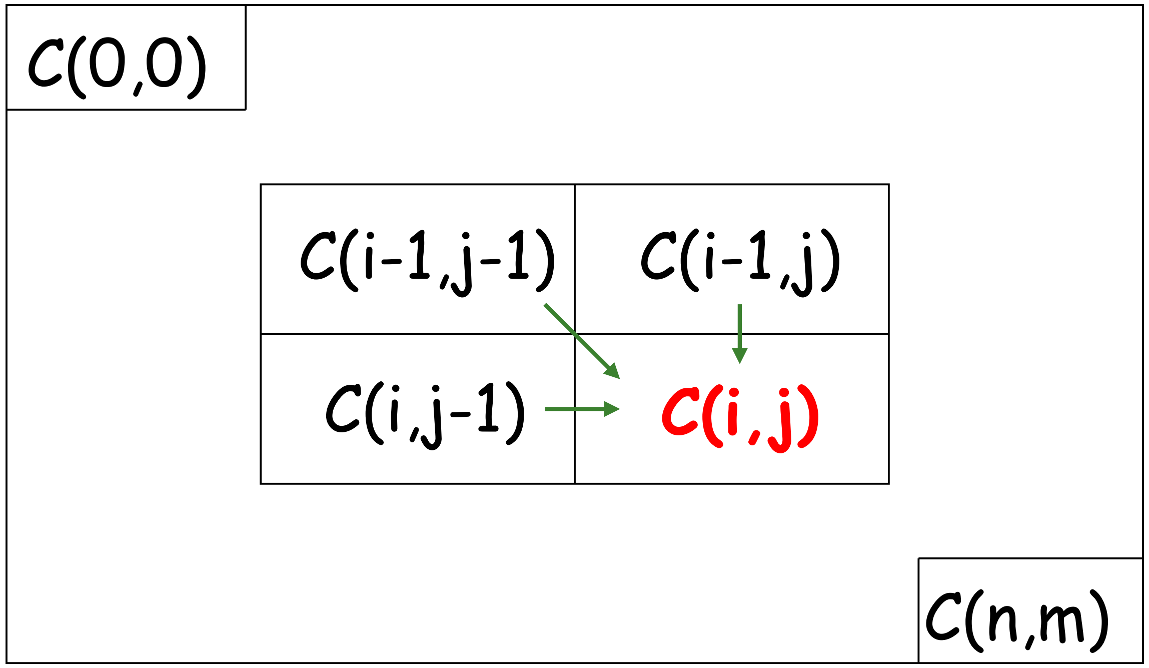
Initial conditions:

$$C(i,0) = -i \cdot \gamma \qquad C(0,j) = -j \cdot \gamma$$

Recurrence relation: For $1 \le i \le n$, $1 \le j \le m$:

$$C(i,j) = \max \begin{cases} C(i-1, j-1) + w(S_i, T_j) \\ C(i-1, j) - \gamma \\ C(i, j-1) - \gamma \end{cases}$$

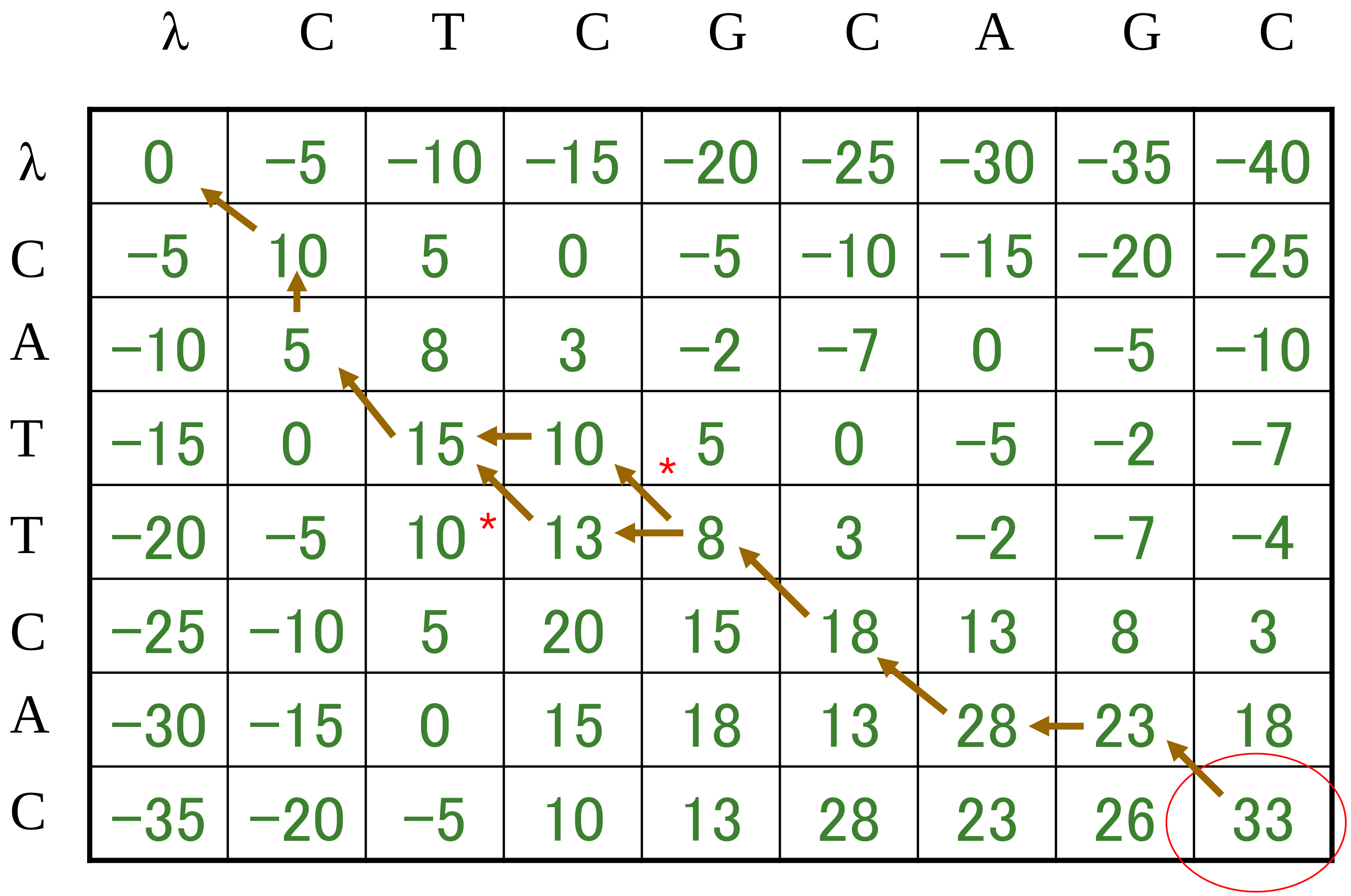
Computation Procedure



$$C(i,j) = \max\left\{C(i-1,j-1) + w(S_i, T_j),\ C(i-1,j) - \gamma,\ C(i,j-1) - \gamma\right\}$$

	λ	C	T	C	G	C	A	G	C
λ	0	-5	-10	-15	-20	-25	-30	-35	-40
C	-5	10	5						
A	-10								
T	-15								
T	-20								
C	-25								
A	-30								
C	-35								

+10 for match, -2 for mismatch, -5 for space



	λ	C	T	C	G	C	A	G	C
λ	0	−5	−10	−15	−20	−25	−30	−35	−40
C	−5	10	5	0	−5	−10	−15	−20	−25
A	−10	5	8	3	−2	−7	0	−5	−10
T	−15	0	15	10	* 5	0	−5	−2	−7
T	−20	−5	10 *	13	8	3	−2	−7	−4
C	−25	−10	5	20	15	18	13	8	3
A	−30	−15	0	15	18	13	28	23	18
C	−35	−20	−5	10	13	28	23	26	33

Traceback can yield both optimum alignments

Traceback

- Horizontal arrow means gap in Y axix sequence.
- Vertical arrow means gap in X axix sequence.
- Diagonal arrow means insert corresponding characters.

Example Exercise:

- Align the following protein sequences globally using Needleman algorithm:

V L S P A V

S V L S A V

2. End-gap free alignment

- Gaps at the start or end of alignment are not penalized

S = c a c t g t a c
T = g a c a c t t g

Match: +2

Mismatch and space: -1

Best global

```
c a c - - t - g t a c
g a c a c t t g - - -
```

Score = 1

Best end-gap free

```
- - c a c - t g t a c
g a c a c t t g - - -
```

Score = 9

Motivation: Shotgun assembly

- **Shotgun assembly** experiment produces large set of partially **overlapping subsequences** from many copies of one **unknown DNA sequence**.
- Problem: Use the overlapping sections to “paste” the subsequences together.
- Overlapping pairs will have low global alignment score, but high end-space free score because of overlap.

Motivation: Shotgun assembly

Strand	Sequence	
Original	AGCATGCTGCAGTCATGCTTAGGCTA	
First shotgun sequence	AGCATGCTGCAGTCATGCT-------	1
	-------------------TAGGCTA	
Second shotgun sequence	AGCATG--------------------	
	------CTGCAGTCATGCTTAGGCTA	2
Reconstruction	AGCATGCTGCAGTCATGCTTAGGCTA	

Algorithm

- Same as global alignment, except:
  - Initialize with zeros (free gaps at start)
  - Locate max in the last row/column (free gaps at end)

	λ	C	T	C	G	C	A	G	C
λ	0	0	0	0	0	0	0	0	0
C	0	10	5	10	5	10	5	0	10
A	0	5	8	5	8	5	20	15	10
T	0	0	15	10	5	6	15	18	13
T	0	-2	10	13	8	3	10	13	16
C	0	10	5	20	15	18	13	8	23
A	0	5	8	15	18	13	28	23	18
G	0	0	3	10	25	20	23	38	33

+10 for match, -2 for mismatch, -5 for gap

3. Local Alignment: Motivation

- **Ignoring stretches of non-coding DNA:**
  - Non-coding regions are more likely to be subjected to mutations than coding regions.
  - Local alignment between two sequences is likely to be between two exons.
- **Locating protein domains:**
  - Proteins of different kind and of different species often exhibit local similarities
  - Local similarities may indicate “functional subunits”.

Local alignment: Example

$S =$ `g g t c t g a g`

$T =$ `a a a c g a`

Match: +2 Mismatch and space: -1

Best local alignment:

```
g g t c t g a g
a a a c - g a -
```

Score = 5

3. Local Alignment: Smith-Waterman Algorithm

$C[i, j]$ = Score of optimally aligning a suffix of *s* with a suffix of *t*.

$$C[i,j] = \max \begin{cases} C[i-1, j-1] + score(s[i], t[j]) \\ C[i-1, j] - \gamma \\ C[i, j-1] - \gamma \\ 0 \end{cases}$$

Initialize top row and leftmost column to zero.

	λ	C	T	C	G	C	A	G	C
λ	0	0	0	0	0	0	0	0	0
C	0	1	0	1	0	1	0	0	1
A	0	0	0	0	0	0	2	0	0
T	0	0	1	0	0	0	0	1	0
T	0	0	1	0	0	0	0	0	0
C	0	1	0	2	0	1	0	0	1
A	0	0	0	0	1	0	2	0	0
C	0	1	0	1	0	2	0	1	1

+1 for a match, -1 for a mismatch, -5 for a space

Backtracking starts at the highest scoring matrix cell and proceeds backwards until a cell with score zero is encountered, yielding the highest scoring local alignment.

Finally Computational Complexity

- initialization: $O(m)$, $O(n)$ where sequence lengths are m, n
- filling in rest of matrix: $O(mn)$
- traceback: $O(m + n)$
- hence, if sequences have nearly same length, the computational complexity is

$$O(n^2)$$

Thank you for your attention!

Questions?