BGGN 213

Foundations of Bioinformatics

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Recap From Last Time:

25 Responses:

https://tinyurl.com/bggn213-02-F17

ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

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Basic Idea: Display one sequence above another with spaces (termed **gaps**) inserted in both to reveal **similarity** of nucleotides or amino acids.

```
Seq1: C A T T C A C

Seq2: C T C G C A G C
```

[Screencast Material]

Basic Idea: Display one sequence above another with spaces (termed **gaps**) inserted in both to reveal **similarity** of nucleotides or amino acids.

```
Seq1: C A T T C A C
      |       | |
Seq2: C T C G C A G C
```

mismatch

match

Two types of character correspondence

Basic Idea: Display one sequence above another with spaces (termed **gaps**) inserted in both to reveal **similarity** of nucleotides or amino acids.

```
Seq1: C A T - T C A - C
      |   |     | |   |
Seq2: C - T C G C A G C
```

match

mismatch

Add gaps to increase number of matches

gaps

Basic Idea: Display one sequence above another with spaces (termed **gaps**) inserted in both to reveal **similarity** of nucleotides or amino acids.

```
Seq1: C A T - T C A - C
      |   |     | |   |
Seq2: C - T C G C A G C
```

match

mismatch } **mutation**

insertion
deletion } **indels**

Gaps represent ‘indels’
mismatch represent mutations

Why compare biological sequences?

- To obtain **functional or mechanistic insight** about a sequence by inference from another potentially better characterized sequence
- To find whether two (or more) genes or proteins are **evolutionarily related**
- To find **structurally or functionally similar regions** within sequences (e.g. catalytic sites, binding sites for other molecules, etc.)
- Many practical bioinformatics applications…

Practical applications include...

- **Similarity searching of databases**
  - Protein structure prediction, annotation, etc...
- **Assembly of sequence reads** into a longer construct such as a genomic sequence
- **Mapping sequencing reads to a known genome**
  - "Resequencing", looking for differences from reference genome - SNPs, indels (insertions or deletions)
  - Mapping transcription factor binding sites via ChIP-Seq (chromatin immuno-precipitation sequencing)
  - Pretty much all next-gen sequencing data analysis

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- **Similarity searching of databases**
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  construct such
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  - ping transcription factor binding sites via ChIP-Seq (chromatin immuno-precipitation sequencing)
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N.B. Pairwise sequence alignment is arguably the most fundamental operation of bioinformatics!

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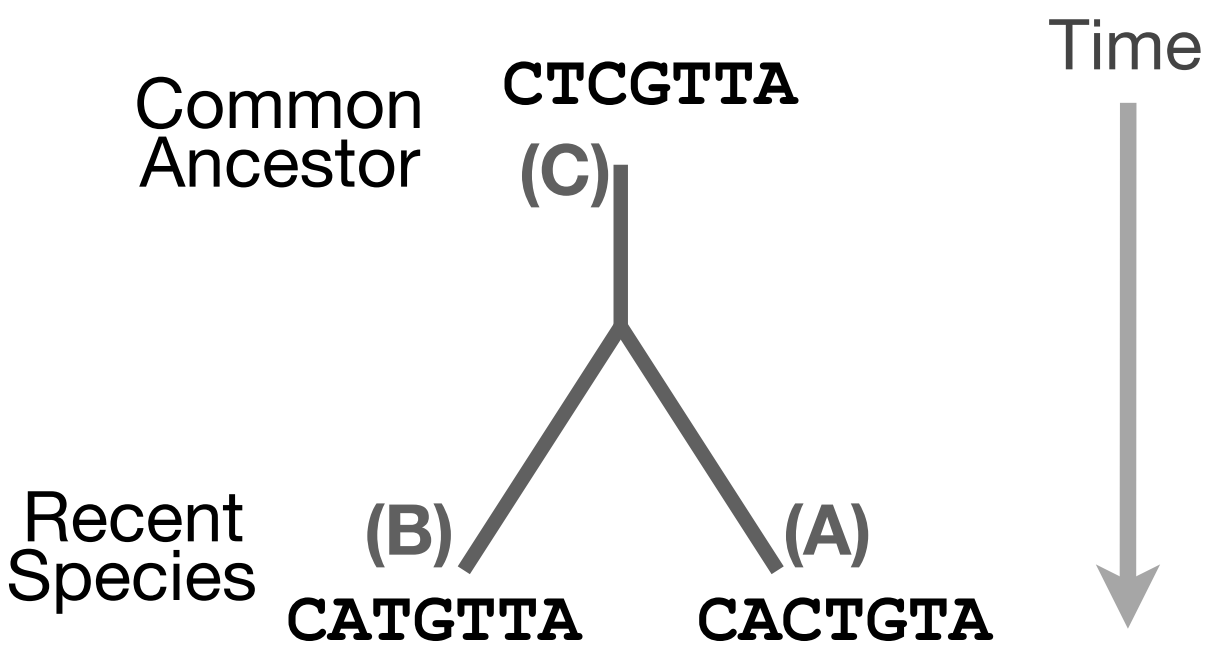
Sequence changes during evolution

There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
- Deletions
- Insertions

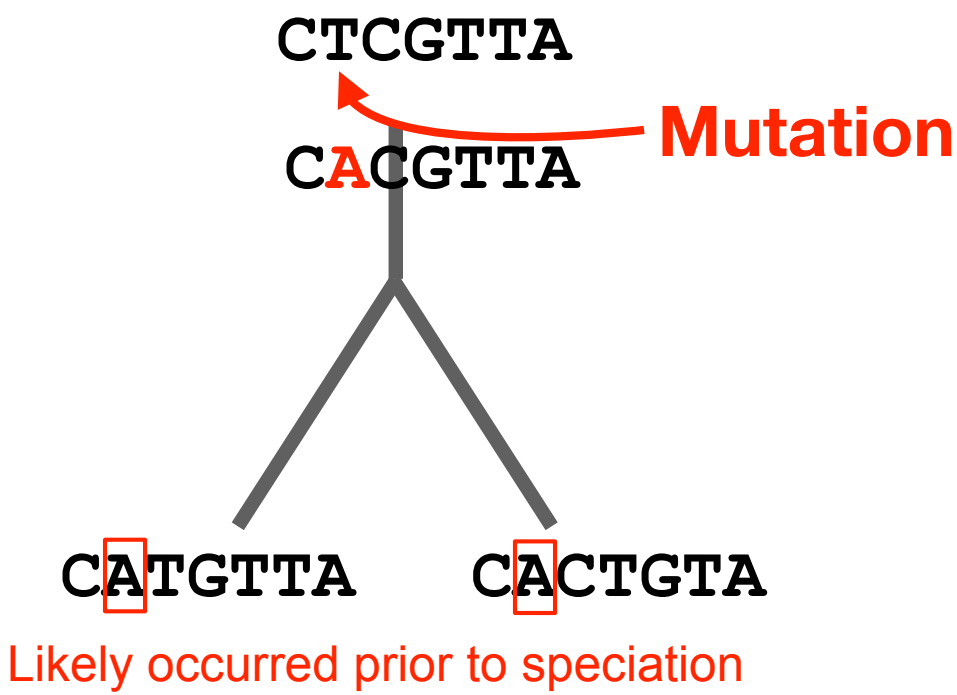


Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

- **Mutations/Substitutions**
- Deletions
- Insertions

CTCGTTA → CACGTTA



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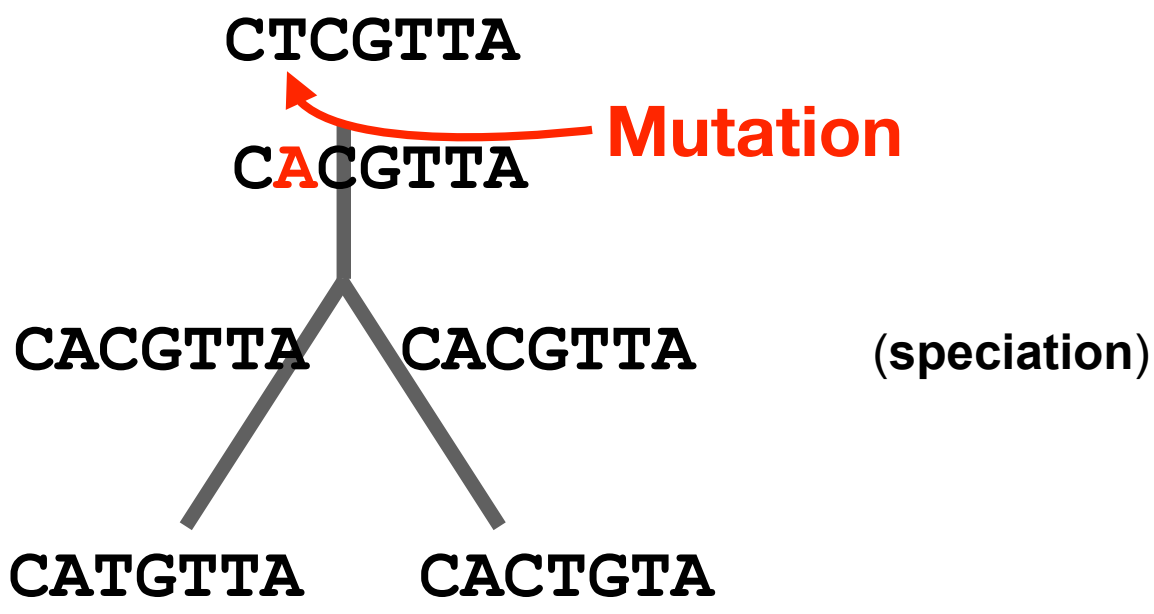


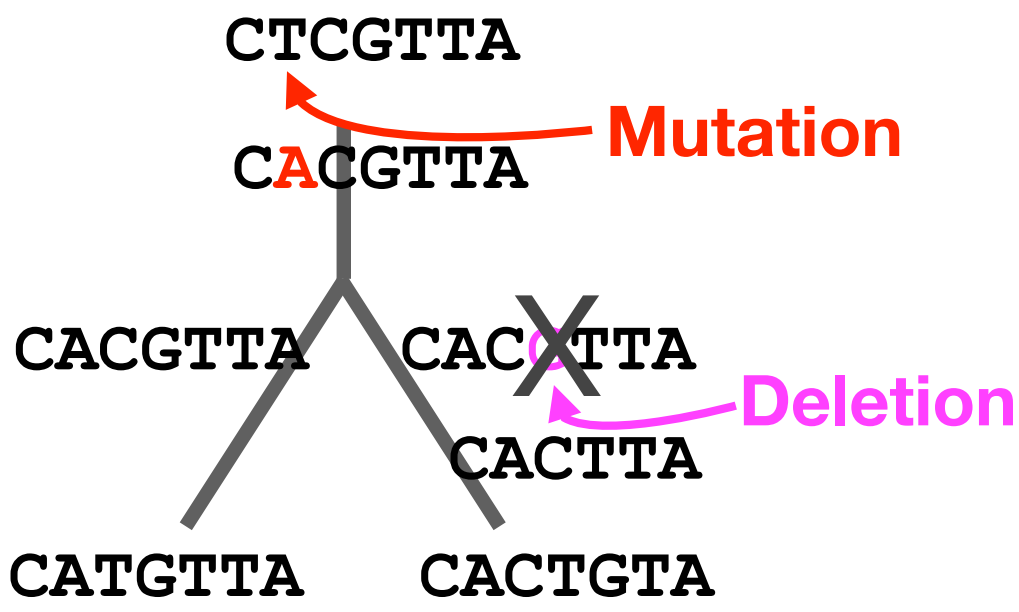
Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
- **Deletions**
- Insertions

CTCGTTA → CACGTTA

CACGTTA → CACTTA



Mutations, deletions and insertions

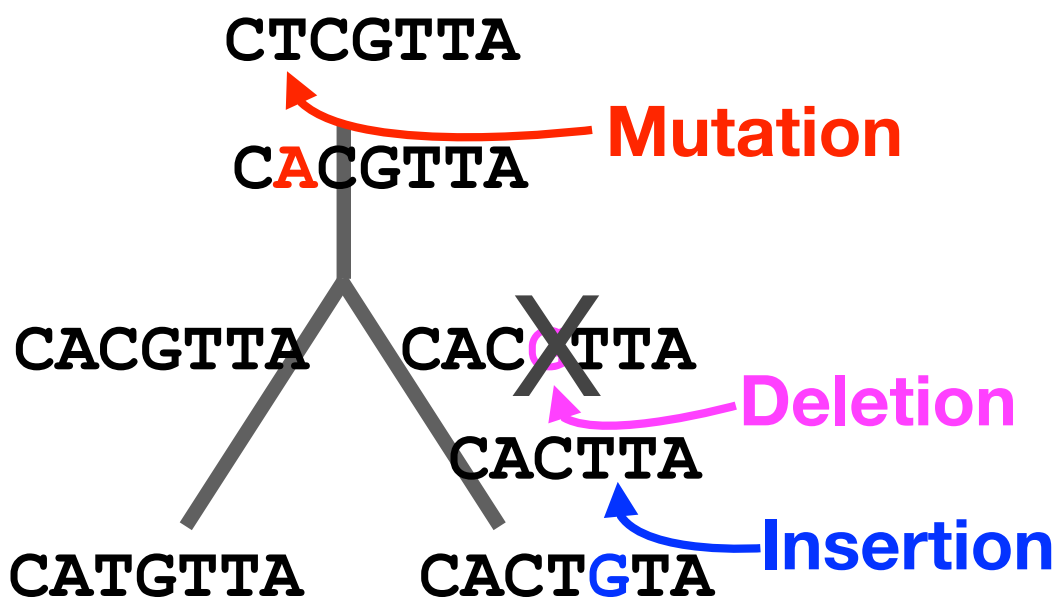
There are three major types of sequence change that can occur during evolution.

- Mutations/Substitutions
- Deletions
- **Insertions**

CTCGTTA → CACGTTA

CACGTTA → CACTTA

CACTTA → CACTGTA



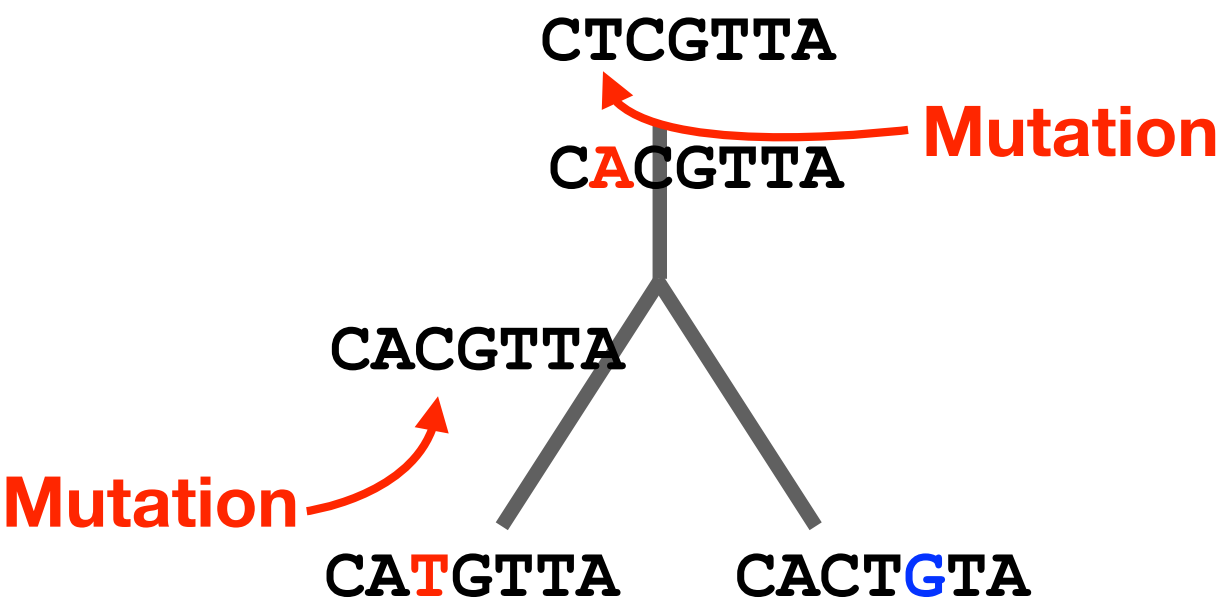
Mutations, deletions and insertions

There are three major types of sequence change that can occur during evolution.

- **Mutations/Substitutions**
- Deletions
- Insertions

CTCGTTA → CACGTTA

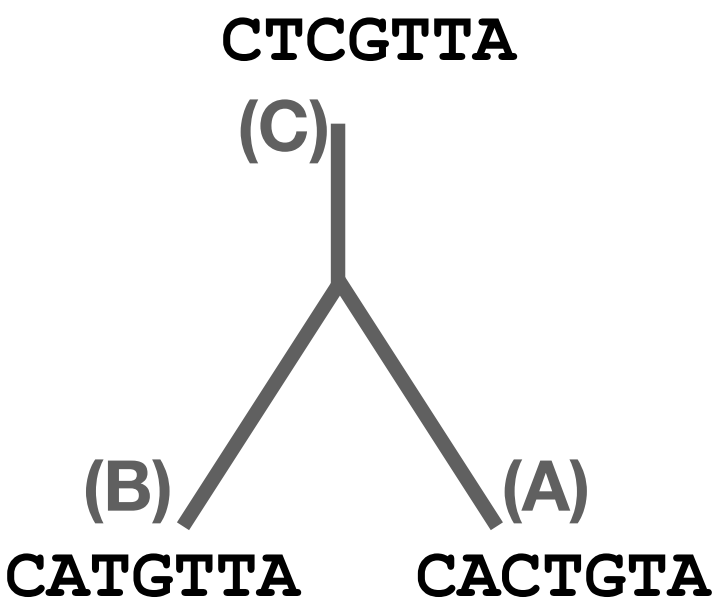
CACGTTA → CATGTTA

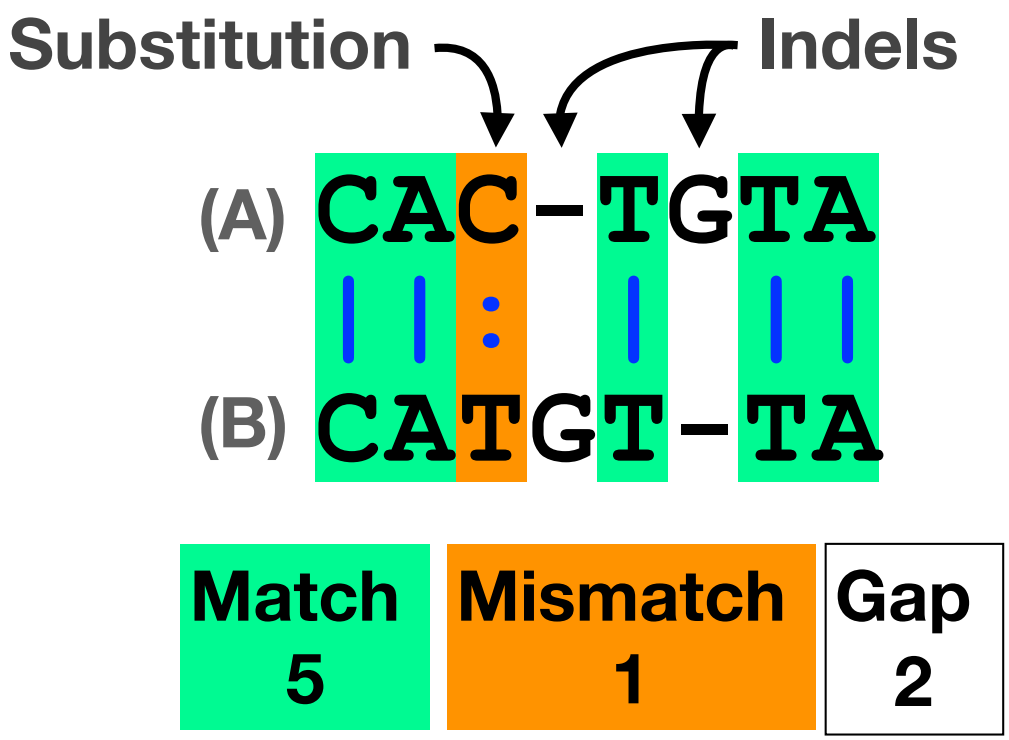


Alignment view

Alignments are great tools to visualize sequence similarity and evolutionary changes in homologous sequences.

- **Mismatches** represent mutations/substitutions
- **Gaps** represent insertions and deletions (indels)





Alternative alignments

- Unfortunately, finding the correct alignment is difficult if we do not know the evolutionary history of the two sequences

Q. Which of these 3 possible alignments is best?

1.

```
CACTGTA
| | : : : | |
CATGTTA
```

2.

```
CACTGT-A
| |  | | |  |
CA-TGTTA
```

3.

```
CAC-TGTA
| | :  |  | |
CATGT-TA
```

Alternative alignments

- One way to judge alignments is to compare their number of matches, insertions, deletions and mutations

4 matches	6 matches	5 matches
3 mismatches	0 mismatches	1 mismatches
0 gaps	2 gaps	2 gaps

```
CACTGTA
| | : : : | |
CATGTTA
```

```
CACTGT-A
| |  | | |  |
CA-TGTTA
```

```
CAC-TGTA
| | :  |  | |
CATGT-TA
```

Scoring alignments

- We can assign a score for each match (+3), mismatch (+1) and indel (-1) to identify the **optimal alignment** *for this scoring scheme*

4 (+3)	6 (+3)	5 (+3)
3 (+1)	0 (+1)	1 (+1)
0 (-1) = 15	2 (-1) = 16	2 (-1) = 14

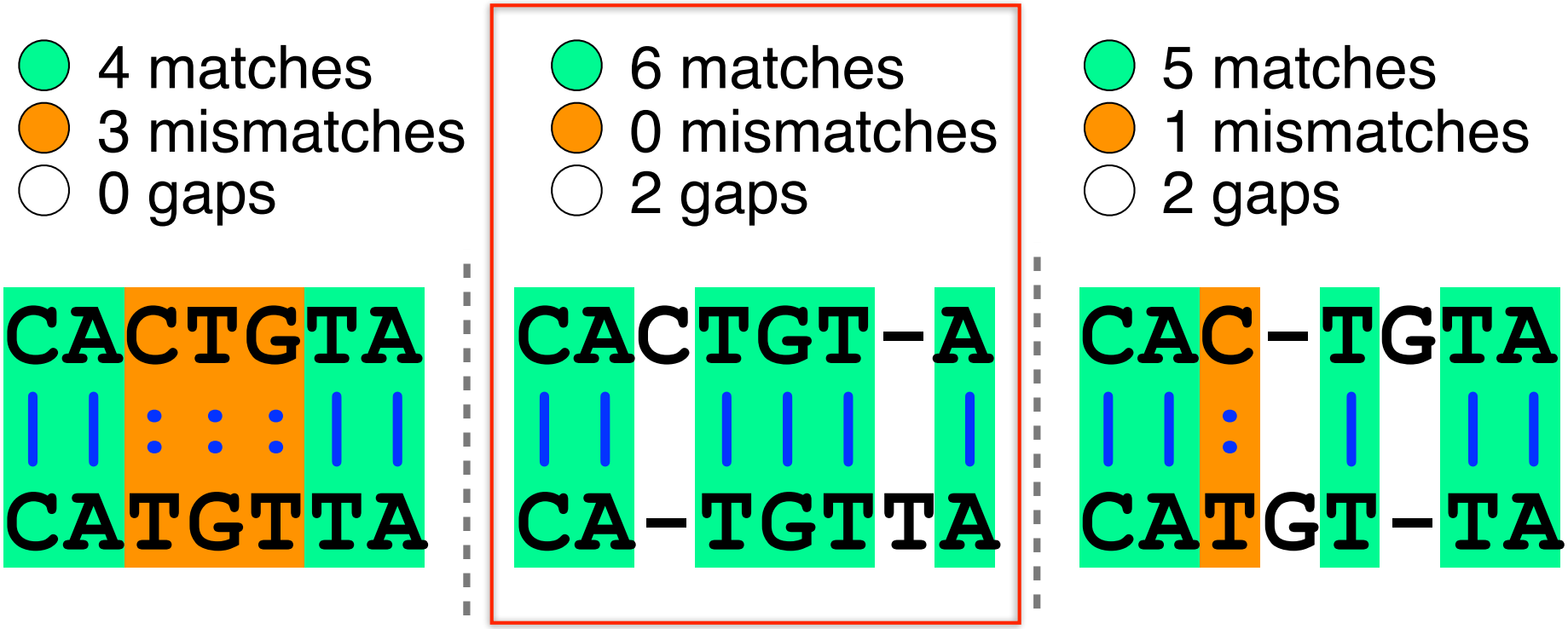
```
CACTGTA
| | : : : | |
CATGTTA
```

```
CACTGT-A
| |  | | |  |
CA-TGTTA
```

```
CAC-TGTA
| | :  |  | |
CATGT-TA
```

Optimal alignments

- Biologists often prefer **parsimonious alignments**, where the number of postulated sequence changes is minimized.



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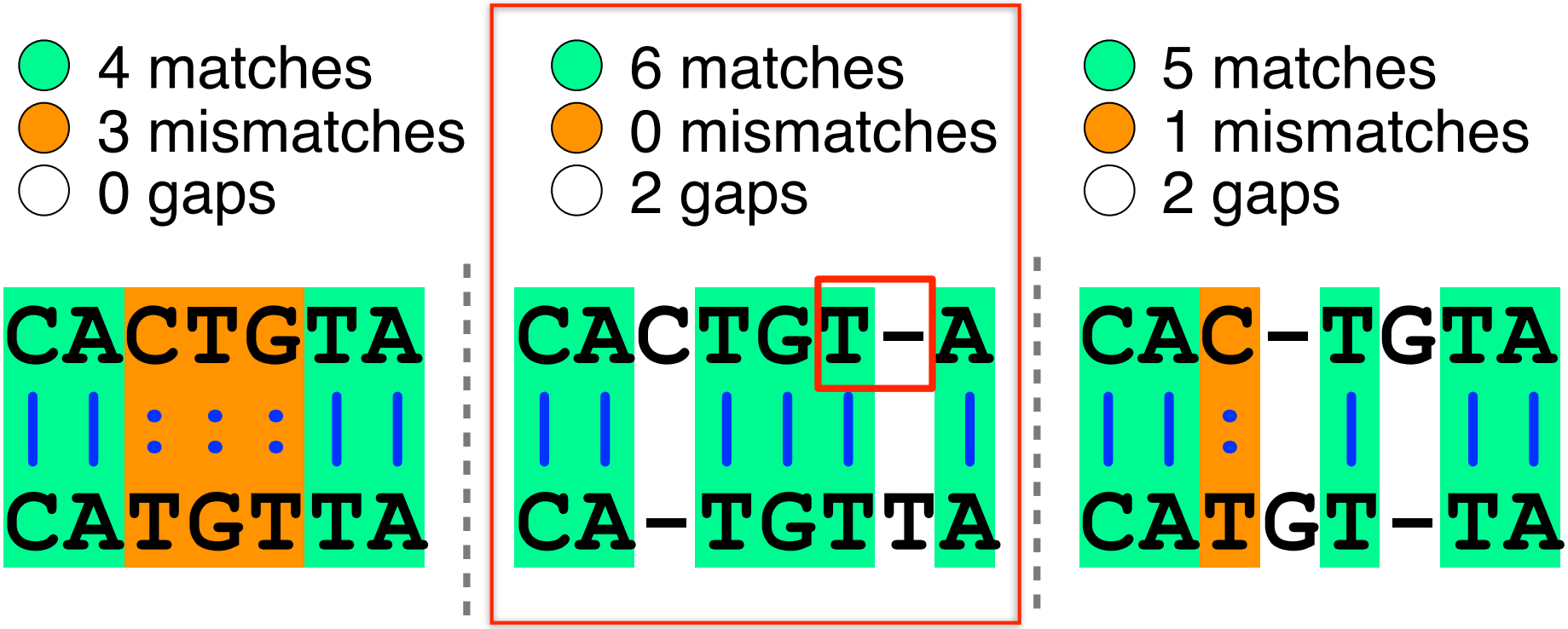

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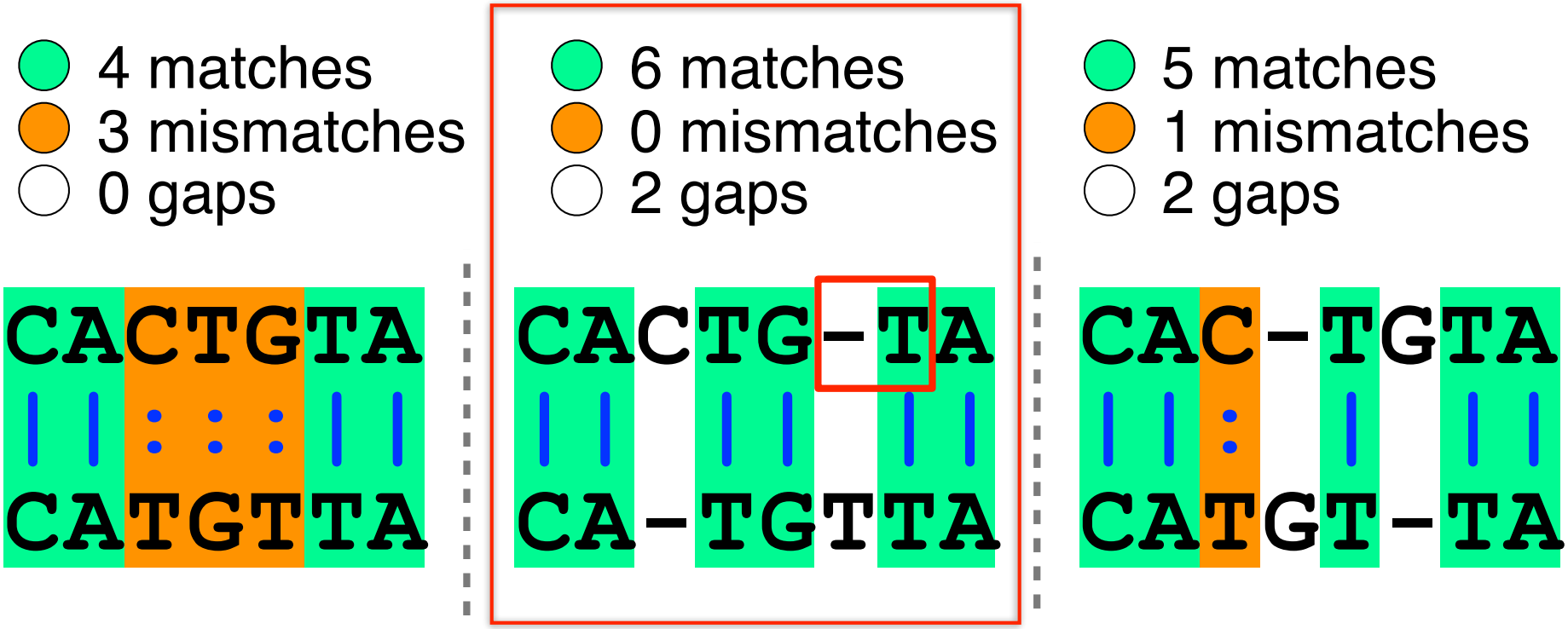

Optimal alignments

- Biologists often prefer **parsimonious alignments**, where the numb sequence changes is m

Warning: There may be more than one *optimal alignment* and these may not reflect the true evolutionary history of our sequences!

4 matches
3 mis
matches
1 mismatches
2 gaps

```
CATGTTA
```

```
CACTGT-A
| |  | | |  |
CA-TGTTA
```

```
CAC-TGTA
| | : |  | |
CATGT-TA
```

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  - Dynamic programing
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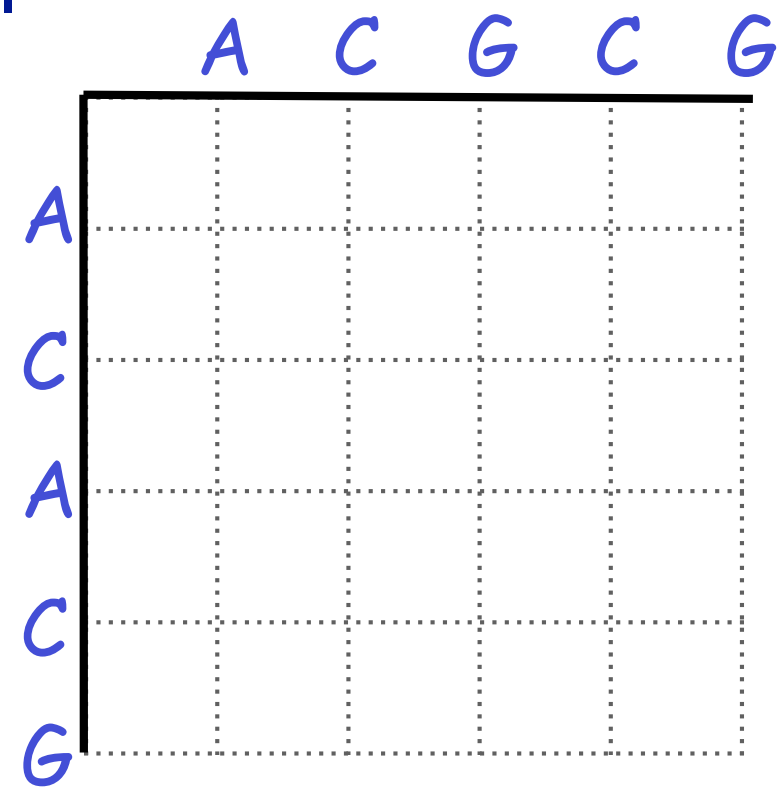
  - D
  - B

How do we compute the optimal alignment between two sequences?

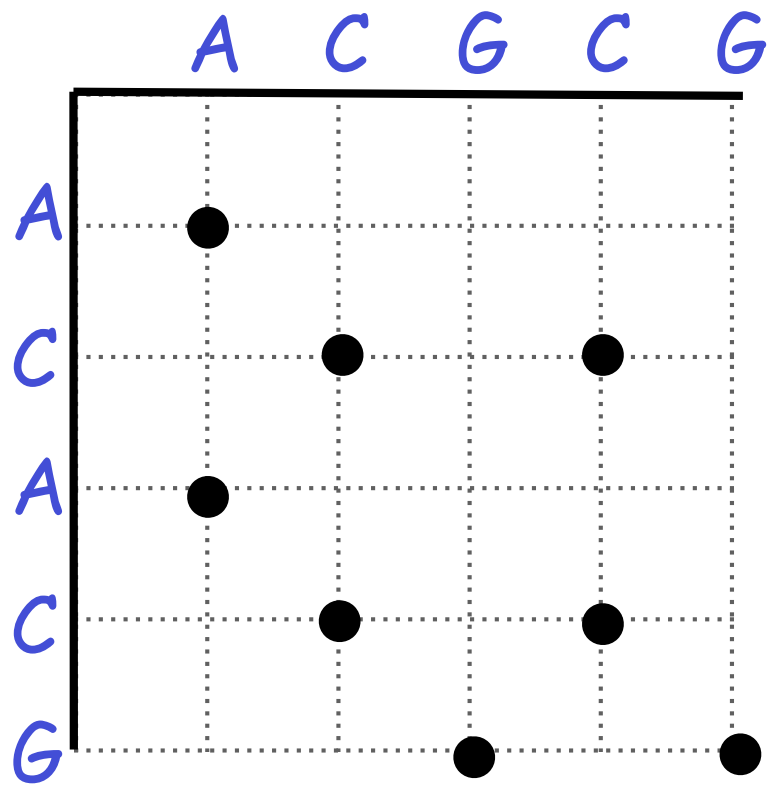
Dot plots: simple graphical approach

- Place one sequence on the vertical axis of a 2D grid (or matrix) and the other on the horizontal



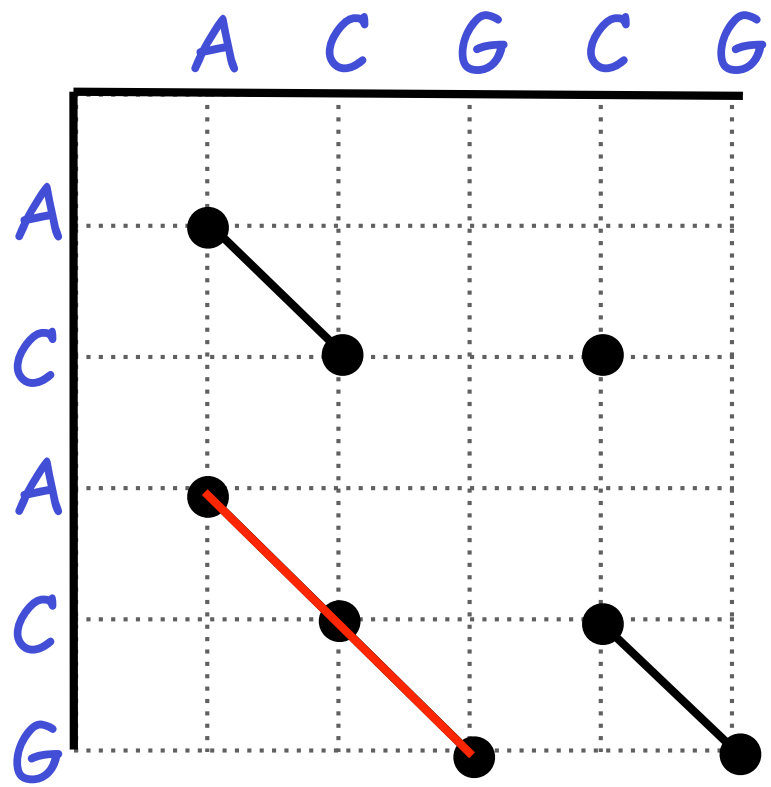
Dot plots: simple graphical approach

- Now simply put dots where the horizontal and vertical sequence values match



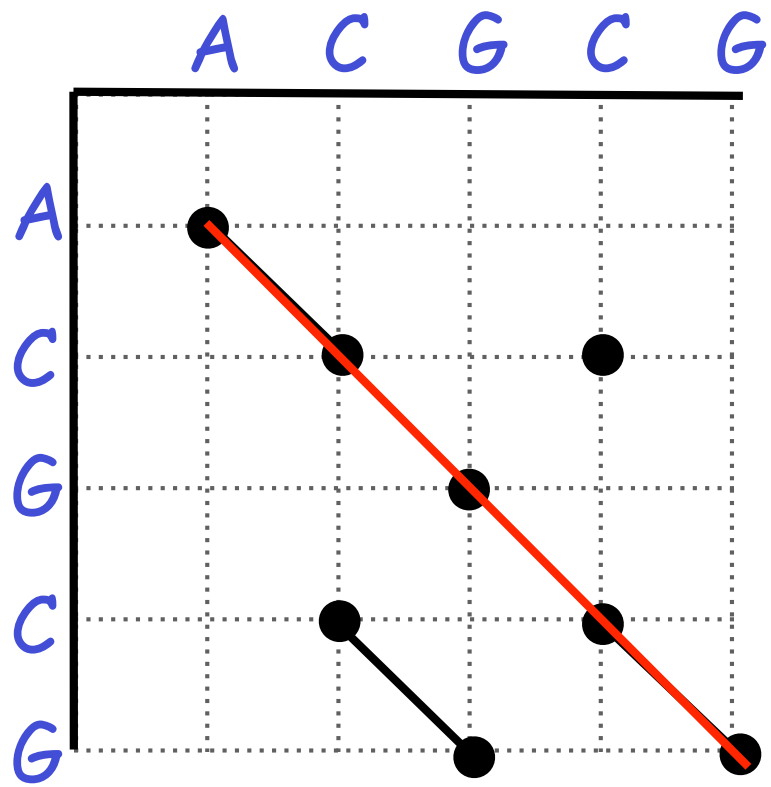
Dot plots: simple graphical approach

- Diagonal runs of dots indicate matched segments of sequence



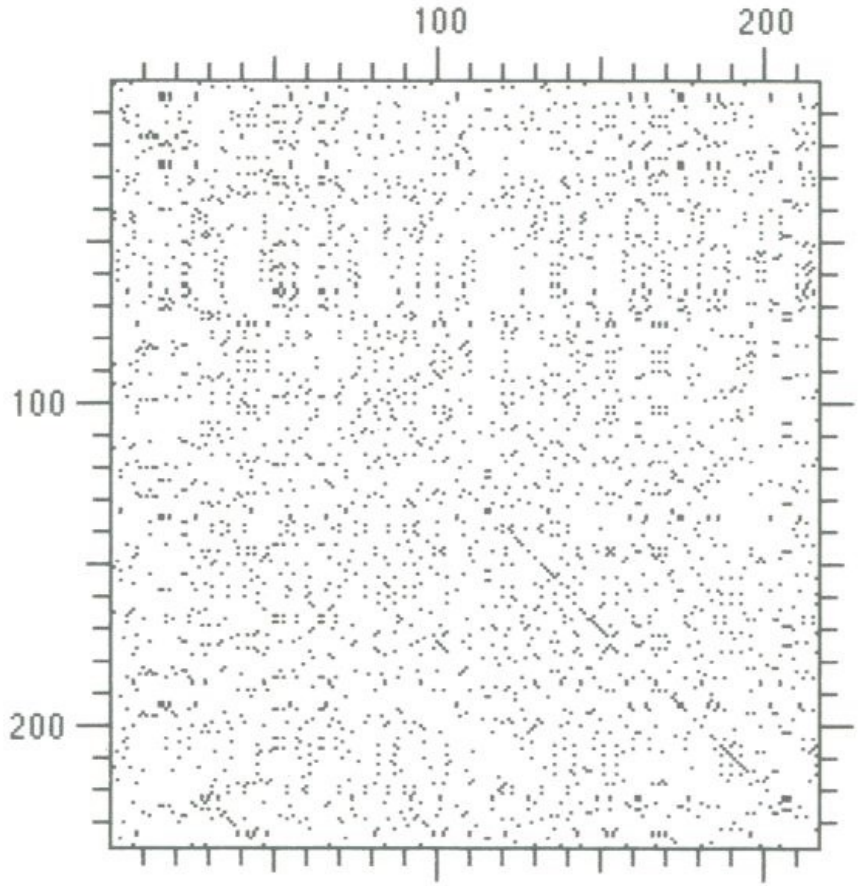
Dot plots: simple graphical approach

Q. What would the dot matrix of a two identical sequences look like?



Dot plots: simple graphical approach

- Dot matrices for long sequences can be noisy



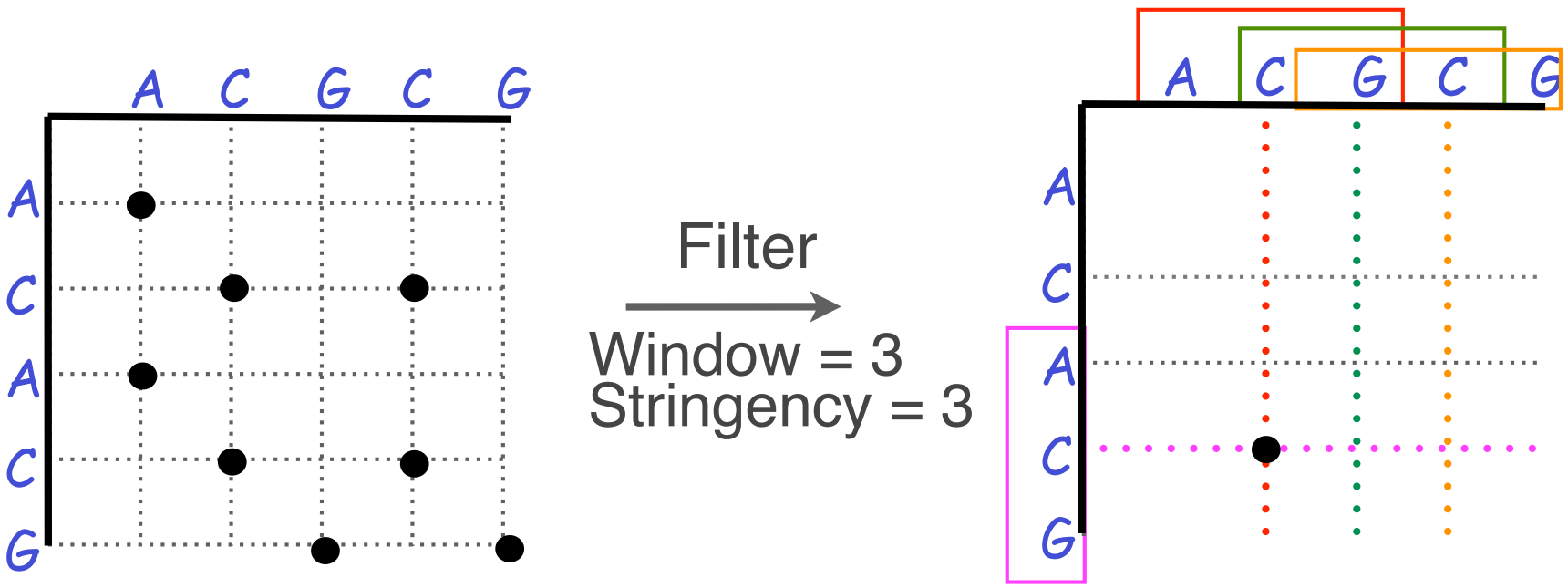
Dot plots: window size and match stringency

Solution: use a window and a threshold

– compare character by character within a window

– require certain fraction of matches within window in order to display it with a dot.

- You have to choose window size and stringency



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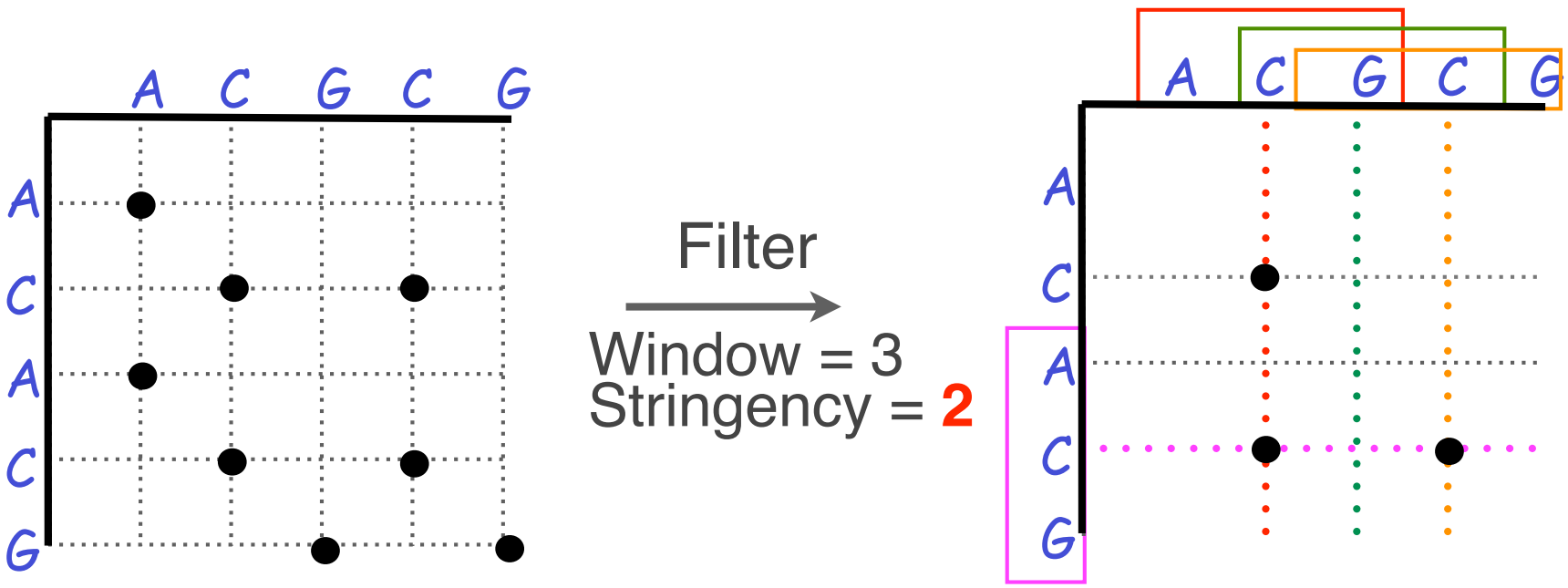
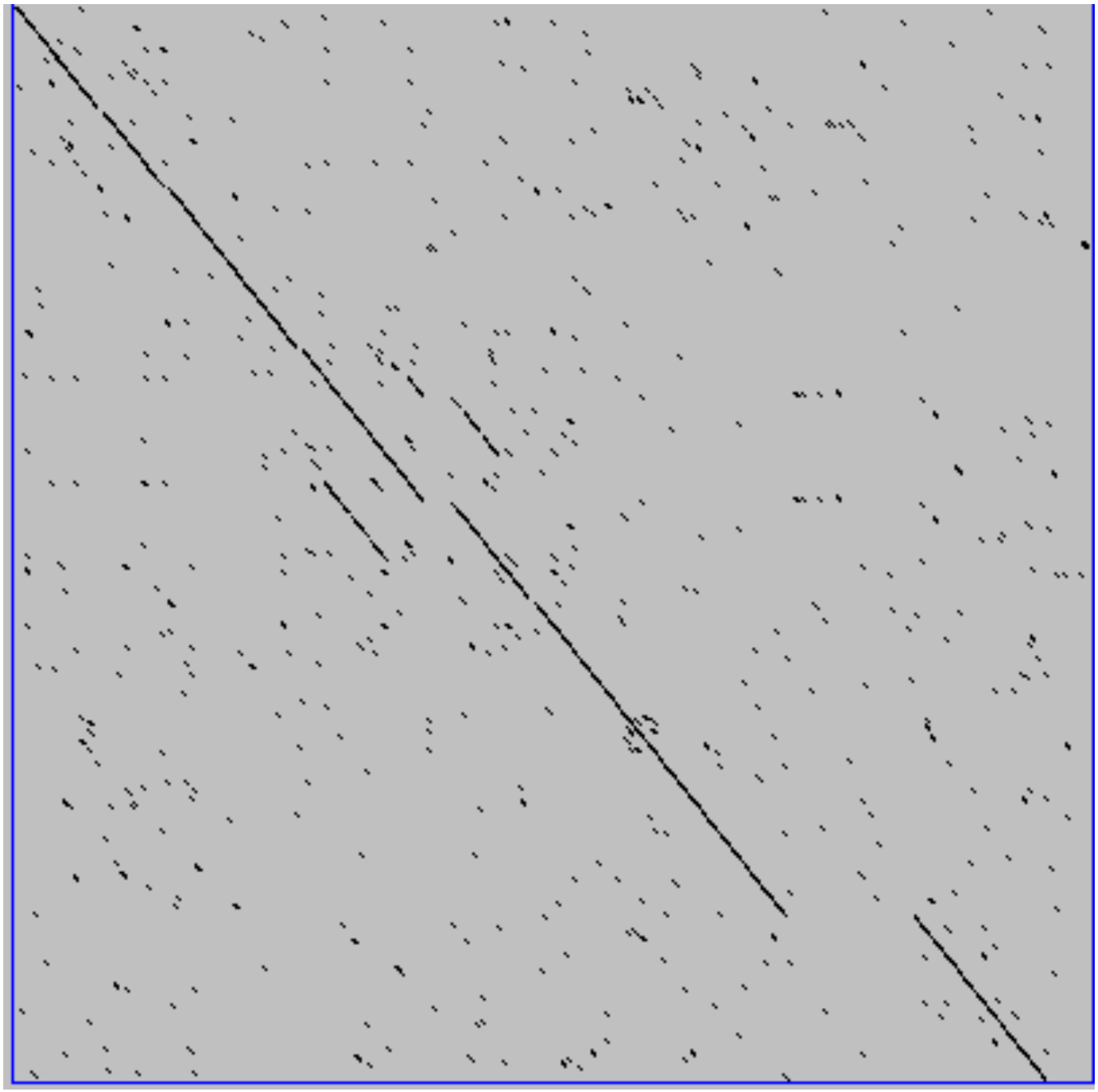


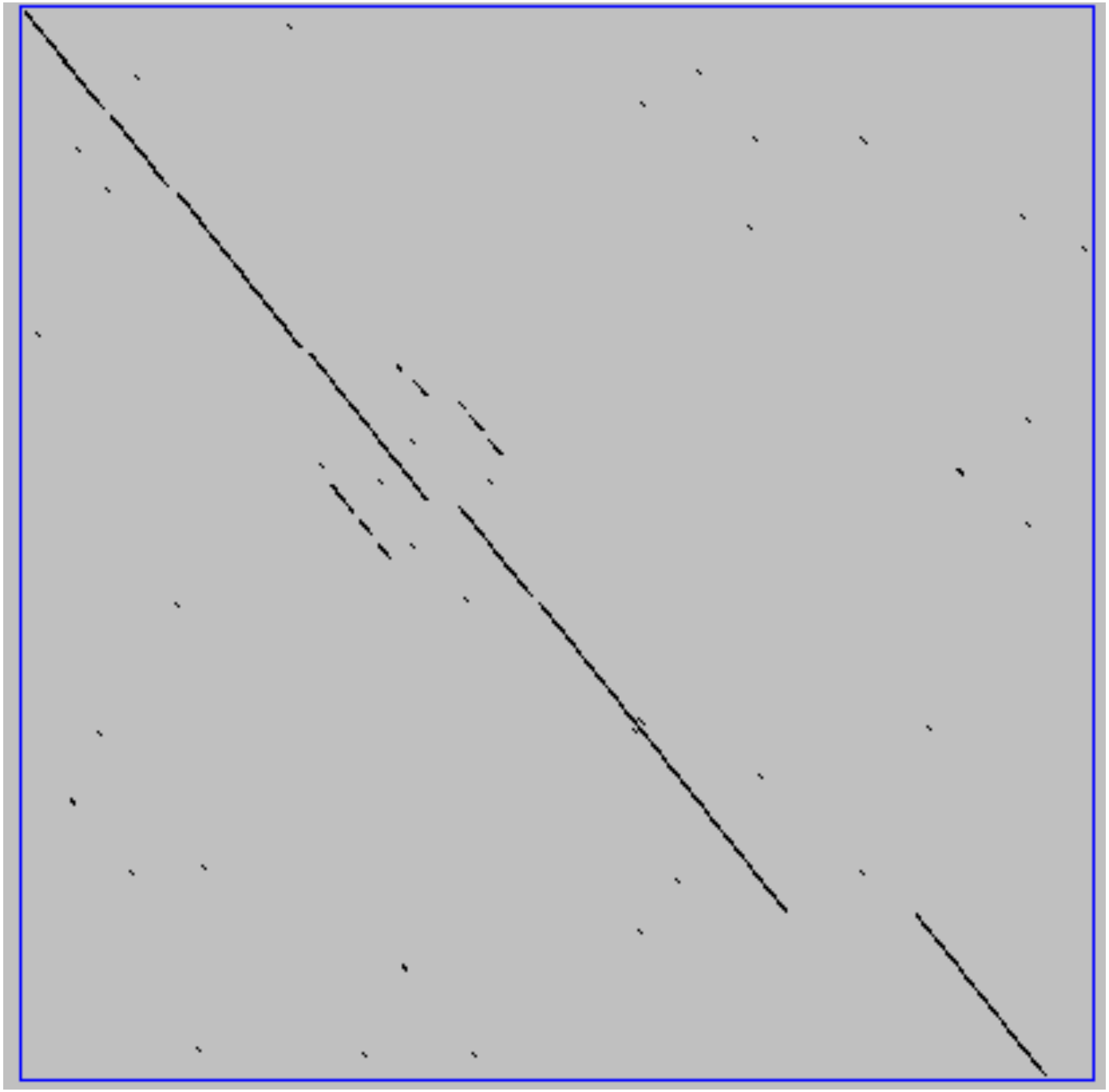
Window size = 5 bases



A dot plot simply puts a dot where two sequences match. In this example, dots are placed in the plot if 5 bases in a row match perfectly. Requiring a 5 base perfect match is a **heuristic** – only look at regions that have a certain degree of identity.

Do you expect evolutionarily related sequences to have more word matches (matches in a row over a certain length) than random or unrelated sequences?

Window size = 7 bases



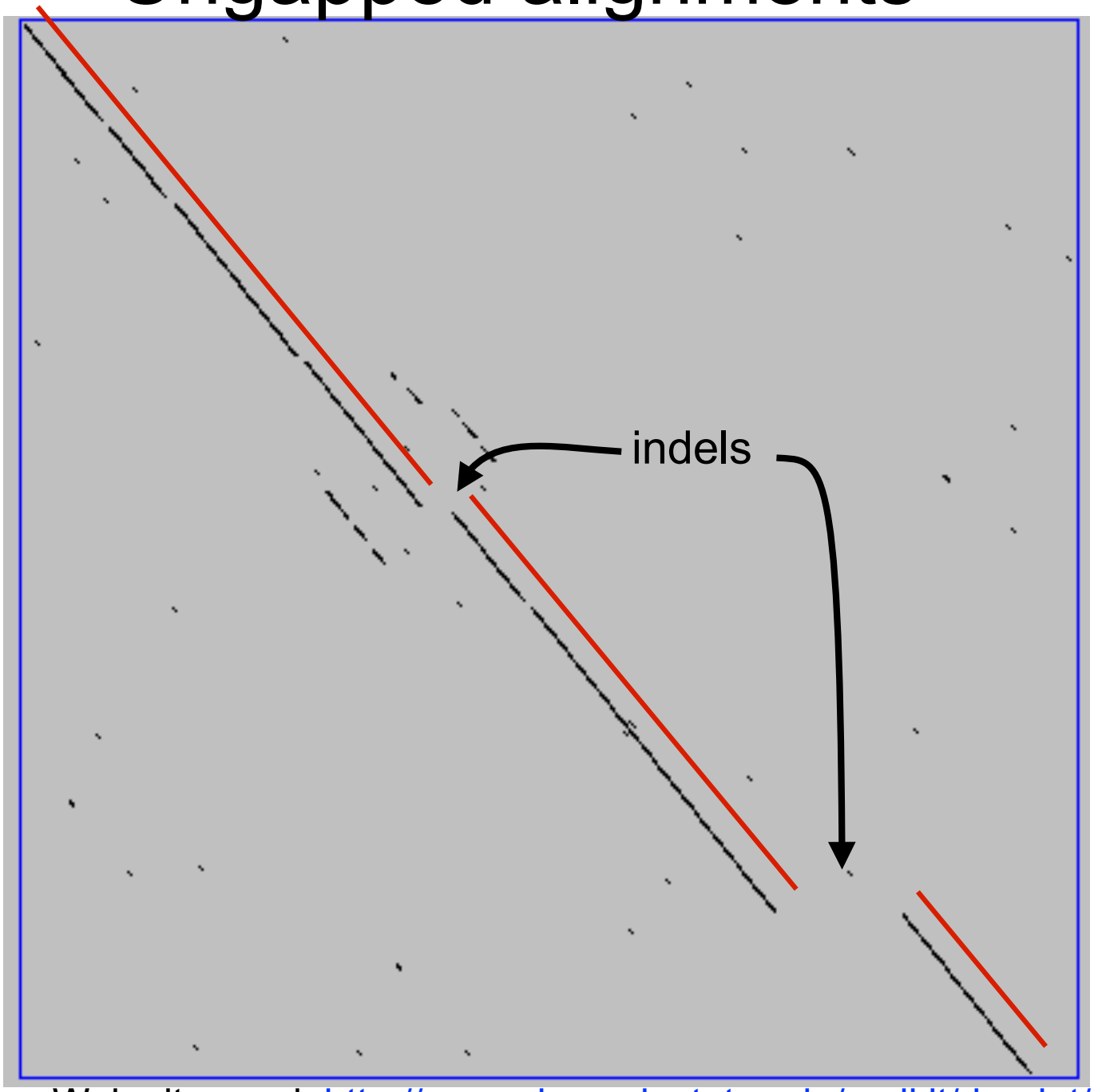
Web site used: http://www.vivo.colostate.edu/molkit/dnadot/

This is a dot plot of the same sequence pair. Now 7 bases in a row must match for a dot to be place. Noise is reduced.

Using windows of a certain length is very similar to using words (kmers) of N characters in the heuristic alignment search tools

Bigger window (kmer) fewer matches to consider

Ungapped alignments



Only **diagonals** can be followed.

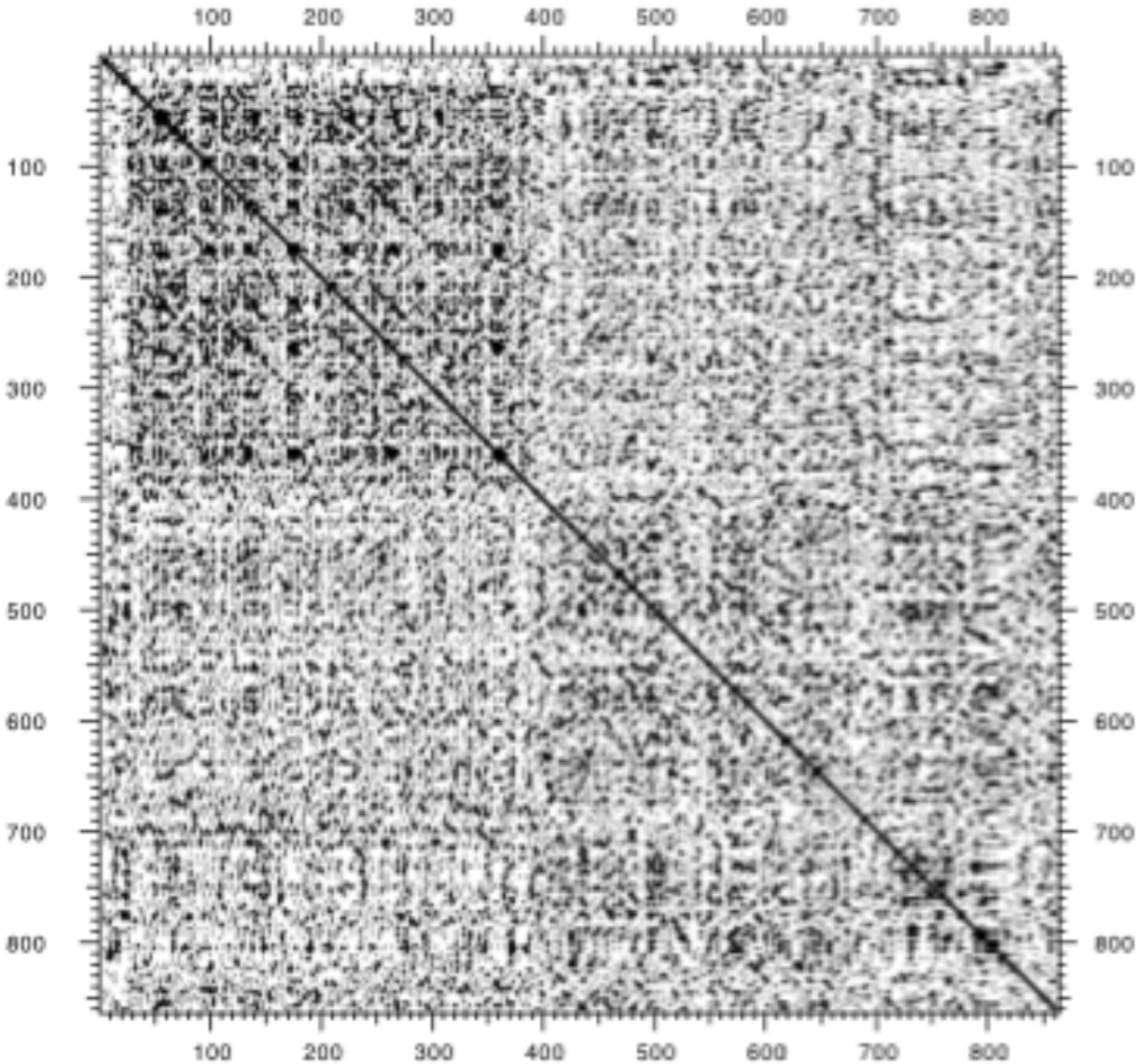
Downward or rightward paths represent **insertion** or **deletions** (gaps in one sequence or the other).

Web site used: http://www.vivo.colostate.edu/molkit/dnadot/

Uses for dot matrices

- Visually assessing the similarity of two protein or two nucleic acid sequences
- Finding local repeat sequences within a larger sequence by comparing a sequence to itself
  - Repeats appear as a set of diagonal runs stacked vertically and/or horizontally

Repeats

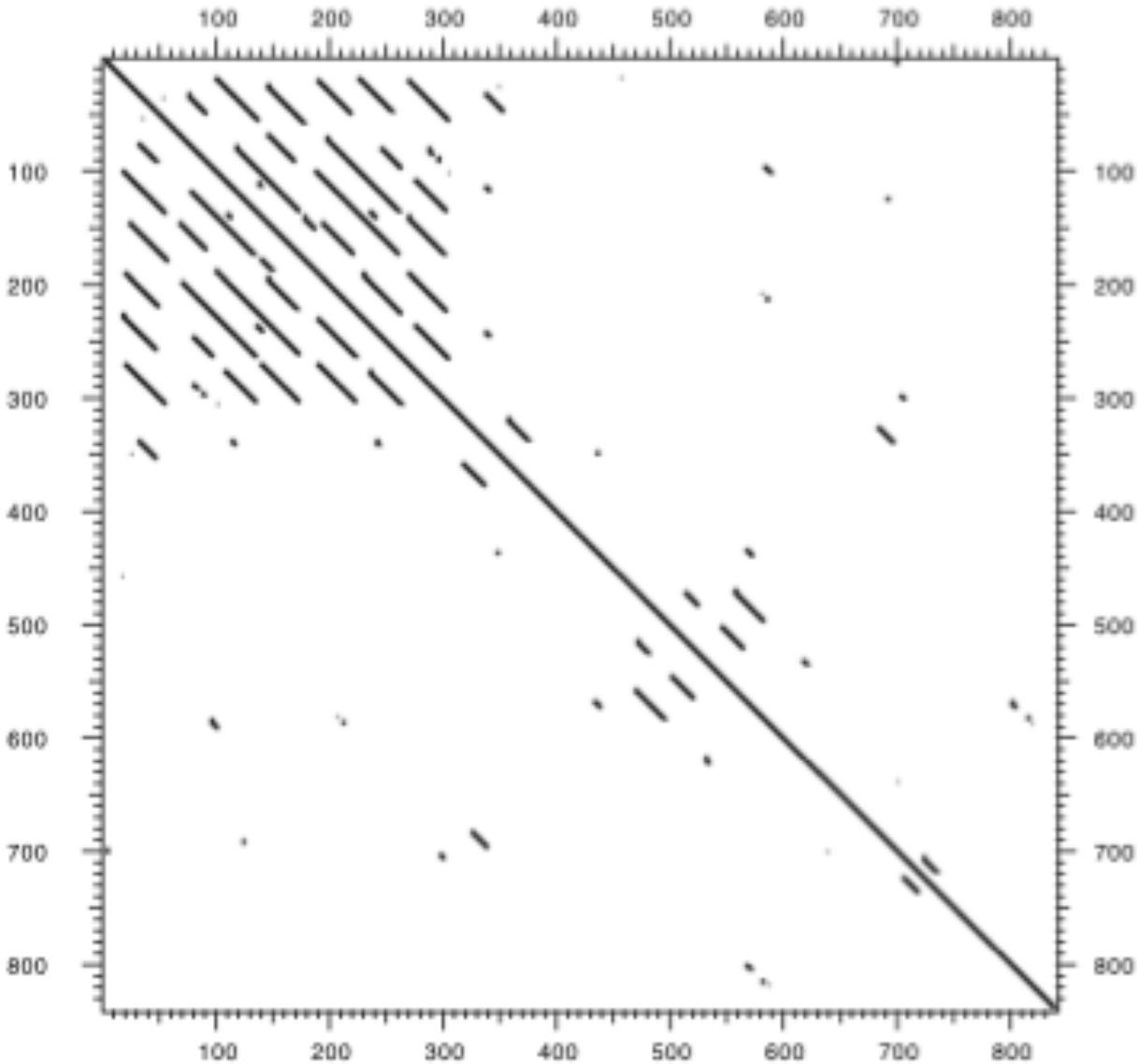


Human LDL receptor protein sequence (Genbank P01130)

W = 1
S = 1

(Figure from Mount, “Bioinformatics sequence and genome analysis”)

Repeats



Human LDL receptor protein sequence (Genbank P01130)

W = 23
S = 7

(Figure from Mount, “Bioinformatics sequence and genome analysis”)

Your Turn!

Exploration of dot plot parameters (hands-on worksheet **Section 1**)

http://bio3d.ucsd.edu/dotplot/ https://bioboot.shinyapps.io/dotplot/

bio3d.ucsd.edu/dotplot/

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BGGN-213: Dot Plot Comparison of Two Sequences

Dot plots are a simple graphical approach for the visual comparison of two sequences. They have a long history (see Maizel and Lenk 1981 and references therein) and entail placing one sequence on the vertical axis of a 2D grid (or matrix) and the other on the horizontal. In its simplest form, a dot is placed where the horizontal and vertical sequence values match. That is a dot is produced at position (*i*,*j*) if character number *i* in the first sequence is the same as character number *j* in the second sequence. More elaborate forms use 'sliding windows' composed of multiple characters and a threshold value, or 'match stringency' for two windows to be considered as matched.

Dot Plot Parameters

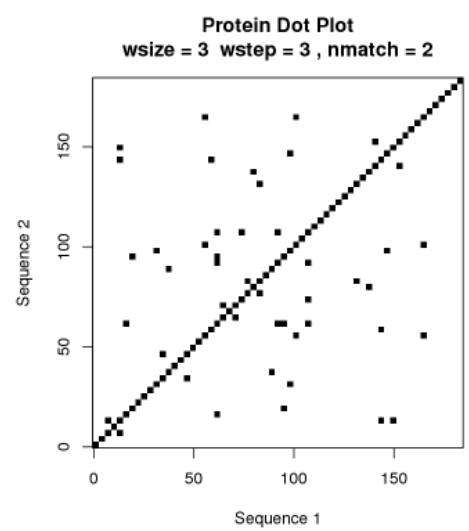
Alter the parameters below to change the displayed protein and DNA dot plots. It is important to have a good feel for these parameters when we get to alignment heuristic approaches later.

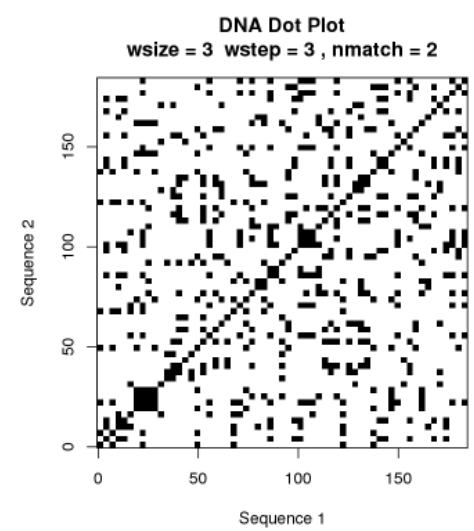
Window Size: 1 3 10

Moving window step size: 1 3 10

Match stringency: 1 2 10

Match stringency specifies the number of match characters required per window. It should not be larger than your window size!





Questions for discussion:

- Why does the DNA sequence have more dots than the protein sequence plot?
- How can we increase the signal to noise ratio?
- What does a 'Match stringency' larger than 'Window size' yield and why?

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The Dynamic Programming Algorithm

- The dynamic programming algorithm can be thought of an extension to the dot plot approach
  - One sequence is placed down the side of a grid and another across the top
  - Instead of placing a dot in the grid, we **compute a score** for each position
  - Finding the optimal alignment corresponds to finding the path through the grid with the **best possible score**

(1) →

	D	P	L	E
D				
P				
M				
E				

(2) →

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

(3) →

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." J. Mol. Biol. 48:443-453.

Algorithm of **Needleman and Wunsch**

- The Needleman–Wunsch approach to global sequence alignment has three basic steps:
  (1) setting up a 2D-grid (or **alignment matrix**),
  (2) **scoring the matrix**, and
  (3) identifying the **optimal path** through the matrix

(1) →

	D	P	L	E
D				
P				
M				
E				

(2) →

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

(3) →

	D	P	L	E
D	6	-1	-4	2
P	-1	7	-3	-1
M	-3	-2	2	-2
E	-2	-1	-3	5

Needleman, S.B. & Wunsch, C.D. (1970) "A general method applicable to the search for similarities in the amino acid sequences of two proteins." J. Mol. Biol. 48:443-453.

Scoring the alignment matrix

- Start by filling in the first row and column – these are all indels (gaps).
  - Each step you take you will add the **gap penalty** to the score ($S_{i,j}$) accumulated in the previous cell

Scores: match = +1, mismatch = -1, gap = -2

		j	Sequence 2			
		-	**D**	**P**	**L**	**E**
i	**-**	0	-2	-4	-6	-8
Sequence 1	**D**	-2				
	P	-4				
	M	-6				
	E	-8				

Scoring the alignment matrix

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Scores: match = +1, mismatch = -1, gap = -2

		j	Sequence 2			
		-	D	P	L	E
i	-	0	-2	-4	-6	-8
Sequence 1	D	-2				
	P	-4				
	M	-6				
	E	-8				

$$S_{i+4} = (-2) + (-2) + (-2) + (-2)$$

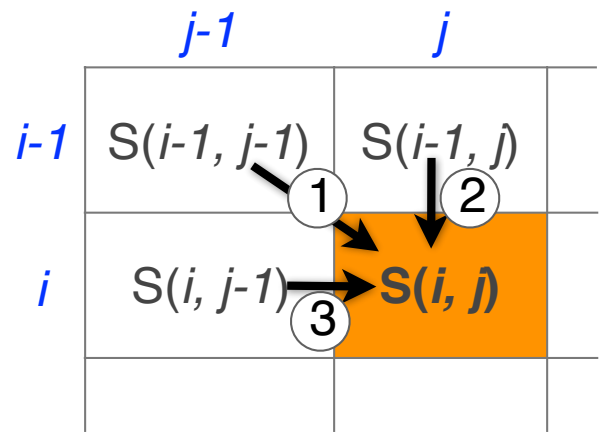
```
Seq1:  DPME
Seq2:  ----
```

Scoring the alignment matrix

- Then go to the empty corner cell (upper left). It has filled in values in up, left and diagonal directions
  - Now can ask which of the three directions gives the highest score?
  - keep track of this score and direction

		j			
	-	**D**	**P**	**L**	**E**
-	0	-2	-4	-6	-8
i **D**	-2	?			
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2



Scoring the alignment matrix

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  - Now can ask which of the three directions gives the highest score?
  - keep track of this score and direction

		j			
	-	**D**	**P**	**L**	**E**
-	0	-2	-4	-6	-8
i **D**	-2	?			
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

$$S(i, j) = \text{Max}\begin{cases} S(i\text{-}1, j\text{-}1) + \text{(mis)match} & \searrow \ (1) \\ S(i\text{-}1, j) + \text{gap penalty} & \downarrow \ (2) \\ S(i, j\text{-}1) + \text{gap penalty} & \rightarrow \ (3) \end{cases}$$

Scoring the alignment matrix

- Then go to the empty corner cell (upper left). It has filled in values in up, left and diagonal directions
  - Now can ask which direction gives the highest score
  - keep track of direction and score

		j			
	-	D	P	L	E
-	0	-2	-4	-6	-8
i D	-2	1			
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (0)+(+1) = +1 <= (D-D) match!

↓ ② (-2)+(-2) = -4

→ ③ (-2)+(-2) = -4

Alignment

D
D

Scoring the alignment matrix

- At each step, the score in the current cell is determine by the scores in the neighboring cells
  - The maximal score and the direction that gave that score is stored (we will use these later to determine the optimal alignment)

			j		
	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1		
P	-4				
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (-2)+(-1) = -3 <= (D-P) mismatch!

↓ ② (-4)+(-2) = -6

→ ③ (1)+(-2) = -1

Alignment

```
D-
DP
```

Scoring the alignment matrix

- We will continue to store the alignment score ($S_{i,j}$) for all possible alignments in the alignment matrix.

		-	D	P	L	E
	-	0	-2	-4	-6	-8
i	D	-2	1	-1	-3	
	P	-4				
	M	-6				
	E	-8				

Scores: match = +1, mismatch = -1, gap = -2

↘ ① (-4)+(-1) = -5 <= (D-L) mismatch

↓ ② (-6)+(-2) = -8

→ ③ (-1)+(-2) = -3

Alignment

```
D--
DPL
```

Scoring the alignment matrix

- For the highlighted cell, the corresponding score ($S_{i,j}$) refers to the score of the optimal alignment of the first *i* characters from sequence1, and the first *j* characters from sequence2.

				j	
	-	**D**	**P**	**L**	**E**
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
i **P**	-4	-1	2	0	
M	-6				
E	-8				

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (-1)+(-1) = -2

↓ ② (-3)+(-2) = -5

→ ③ (2)+(-2) = 0

Alignment

```
DP-
DPL
```

Scoring the alignment matrix

- At each step, the score in the current cell is determine by the scores in the neighboring cells
  - The maximal score and the direction that gave that score is stored

					j	
		-	D	P	L	E
	-	0	-2	-4	-6	-8
	D	-2	1	-1	-3	-5
	P	-4	-1	2	0	-2
i	M	-6	-3	0	1	
	E	-8				

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (2)+(**-1**) = 0 <= mismatch

↓ ② (0)+(-2) = -2

→ ③ (0)+(-2) = -2

Alignment

DPM
DPL

Scoring the alignment matrix

- The score of the best alignment of the entire sequences corresponds to $S_{n,m}$
  - (where *n* and *m* are the length of the sequences)

		-	D	P	L	E (j=m)
	-	0	-2	-4	-6	-8
	D	-2	1	-1	-3	-5
	P	-4	-1	2	0	-2
	M	-6	-3	0	1	-1
i=n	E	-8	-5	-2	-1	2

Scores: match = +1, mismatch = -1, indel = -2

↘ ① (+1)+(+1) = +2

↓ ② (-1)+(-2) = -3

→ ③ (-1)+(-2) = -3

Alignment

```
DPME
DPLE
```

Scoring the alignment matrix

- To find the best alignment, we retrace the arrows starting from the bottom right cell
  - N.B. The optimal alignment score and alignment are dependent on the chosen scoring system

Scores: match = +1, mismatch = -1, indel = -2

	-	D	P	L	E
-	0	-2	-4	-6	-8
D	-2	1	-1	-3	-5
P	-4	-1	2	0	-2
M	-6	-3	0	1	-1
E	-8	-5	-2	-1	2

Alignment

```
DPME
DPLE
```

Questions:

- What is the optimal score for the alignment of these sequences and how do we find the optimal alignment?

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

Questions:

- What is the optimal score for the alignment of these sequences and how do we find the optimal alignment?

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

Questions:

- To find the best alignment we retrace the arrows starting from the bottom right cell

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

More than one alignment possible

- Sometimes more than one alignment can result in the same optimal score

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

Alignment

```
CACTGT-A
CA-TGTTA

CACTG-TA
CA-TGTTA
```

The alignment and score are dependent on the scoring system

- Here we increase the gap penalty from -2 to -3

	-	C	A	T	G	T	T	A
-	0	-3	-6	-9	-12	-15	-18	-21
C	-3	1	-2	-5	-8	-11	14	-17
A	-6	-2	2	-1	-4	-7	-10	13
C	-9	-5	-1	1	-2	-5	-8	-11
T	-12	-8	-4	0	0	-1	-4	-7
G	-15	-11	-7	-3	1	-1	-2	-5
T	-18	-14	-10	-6	-2	2	0	-3
A	-21	-17	-13	-9	-5	-1	1	1

Alignment

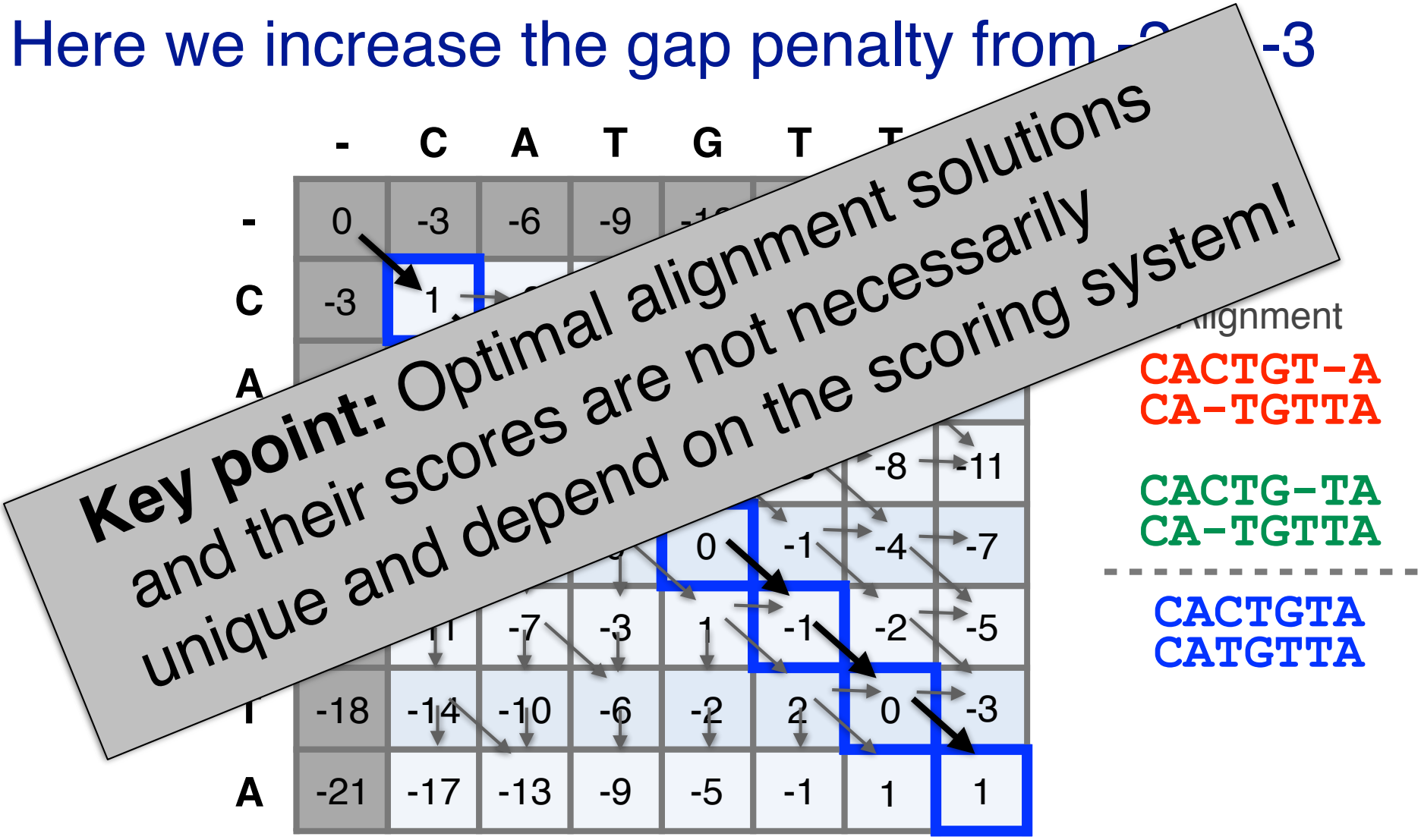
CACTGT-A
CA-TGTTA

CACTG-TA
CA-TGTTA

CACTGTA
CATGTTA

The alignment and score are dependent on the scoring system

- Here we increase the gap penalty from -2 to -3



Your Turn!

Hands-on worksheet **Sections 2** & **3**

Match: +2
Mismatch: -1
Gap: -2

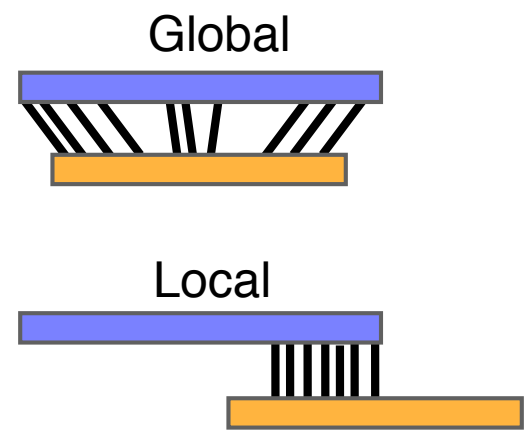
		A	G	T	T	C
	0					
A						
T						
T						
G						
C						

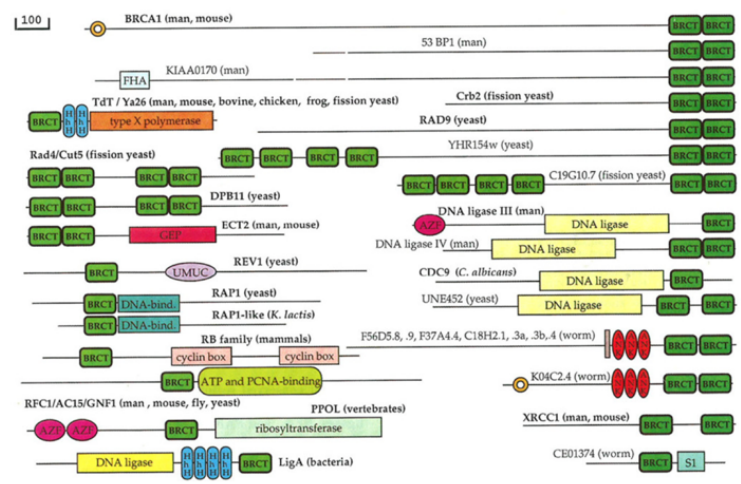
ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

Global *vs* local alignments

- Needleman-Wunsch is a **global alignment** algorithm
  - Resulting alignment spans the complete sequences end to end
  - This is appropriate for closely related sequences that are similar in length
- For many practical applications we require **local alignments**
  - Local alignments highlight sub-regions (*e.g.* protein domains) in the two sequences that align well





Local alignment: Definition

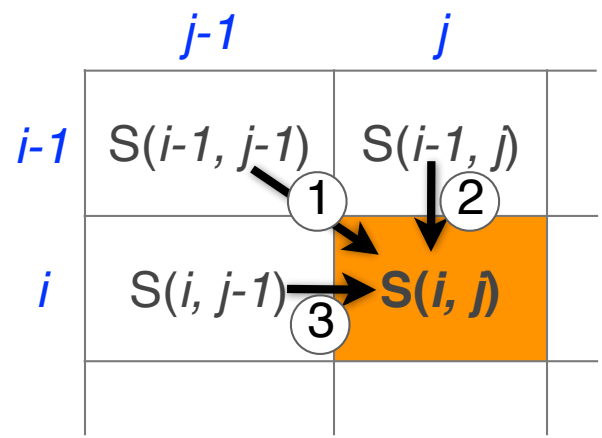
- Smith & Waterman proposed simply that a local alignment of two sequences allow arbitrary-length segments of each sequence to be aligned, with no penalty for the unaligned portions of the sequences. Otherwise, the score for a local alignment is calculated the same way as that for a global alignment

Smith, T.F. & Waterman, M.S. (1981) “Identification of common molecular subsequences.” J. Mol. Biol. 147:195-197.

The Smith-Waterman algorithm

- Three main modifications to Needleman-Wunsch:
  - Allow a node to start at 0
  - The score for a particular cell cannot be negative
    - if all other score options produce a negative value, then a zero must be inserted in the cell
  - Record the highest- scoring node, and trace back from there

$$S(i, j) = \text{Max} \begin{cases} S(i\text{-}1, j\text{-}1) + \text{(mis)match} & \searrow \quad (1) \\ S(i\text{-}1, j) - \text{gap penalty} & \downarrow \quad (2) \\ S(i, j\text{-}1) - \text{gap penalty} & \rightarrow \quad (3) \\ \mathbf{0} & (4) \end{cases}$$



Sequence 1

Sequence 2

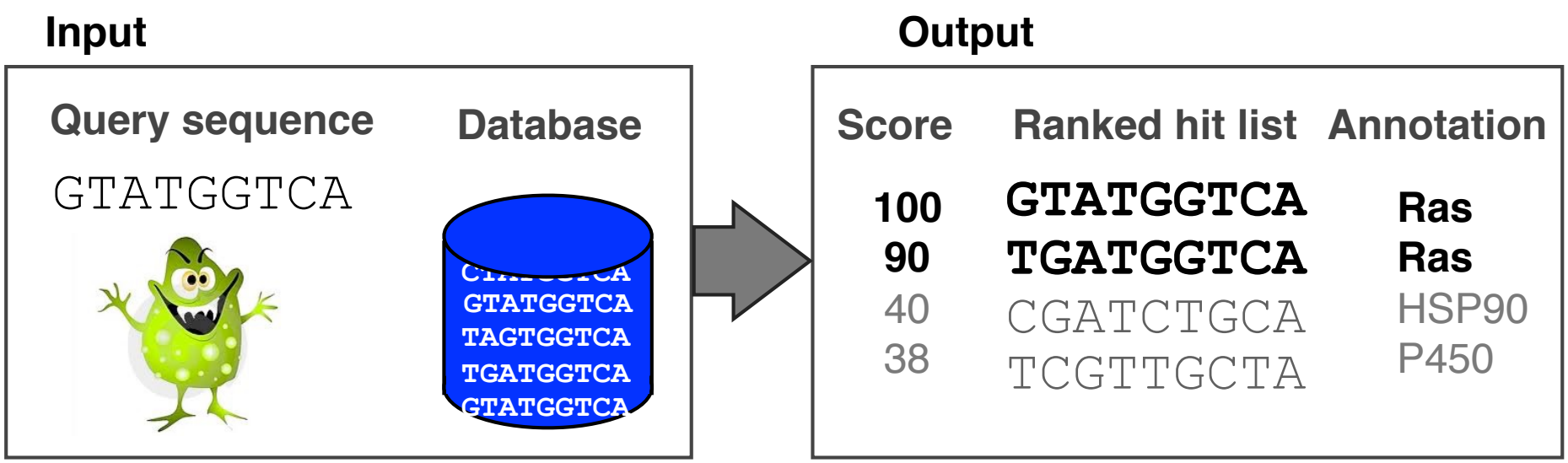
	-	C	A	G	C	C	U	C	G	C	U	U	A	G
-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0
A	0.0	0.0	1.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.7
U	0.0	0.0	0.0	0.7	0.3	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.0	0.7
G	0.0	0.0	0.0	1.0	0.3	0.0	0.0	0.7	1.0	0.0	0.0	0.7	0.7	1.0
C	0.0	1.0	0.0	0.0	2.0	1.3	0.3	1.0	0.3	2.0	0.7	0.3	0.3	0.3
C	0.0	1.0	0.7	0.0	1.0	3.0	1.7	1.3	1.0	1.3	1.7	0.3	0.0	0.0
A	0.0	0.0	2.0	0.7	0.3	1.7	2.7	1.3	1.0	0.7	1.0	1.3	1.3	0.0
U	0.0	0.0	0.7	1.7	0.3	1.3	2.7	2.3	1.0	0.7	1.7	2.0	1.0	1.0
U	0.0	0.0	0.3	0.3	1.3	1.0	2.3	2.3	2.0	0.7	1.7	2.7	1.7	1.0
G	0.0	0.0	0.0	1.3	0.0	1.0	1.0	2.0	3.3	2.0	1.7	1.3	2.3	2.7
A	0.0	0.0	1.0	0.0	1.0	0.3	0.7	0.7	2.0	3.0	1.7	1.3	2.3	2.0
C	0.0	1.0	0.0	0.7	1.0	2.0	0.7	1.7	1.7	3.0	2.7	1.3	1.0	2.0
G	0.0	0.0	0.7	1.0	0.3	0.7	1.7	0.3	2.7	1.7	2.7	2.3	1.0	2.0
G	0.0	0.0	0.0	1.7	0.7	0.3	0.3	1.3	1.3	2.3	1.3	2.3	2.0	2.0

Local alignment

GCC-AUG
GCCUCGC

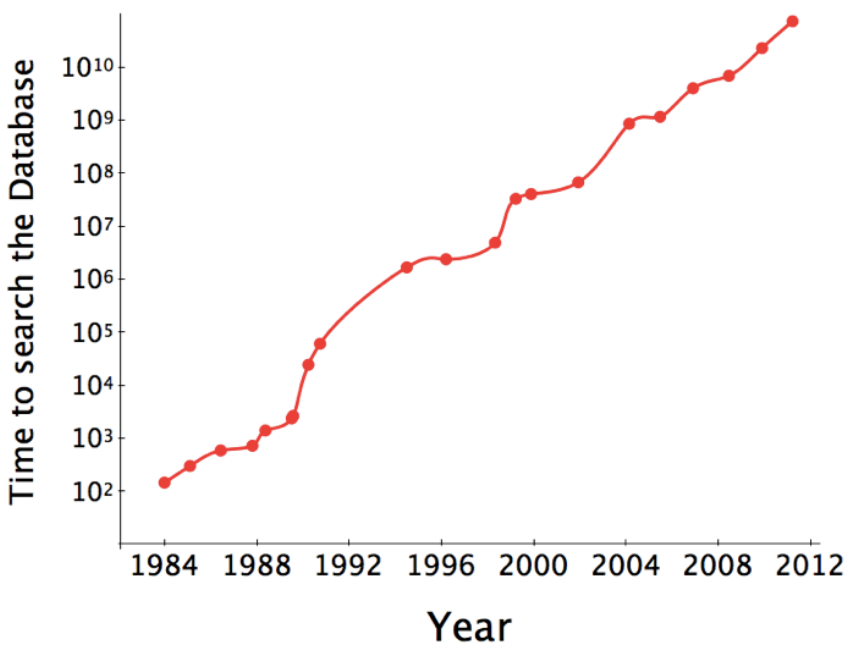
Local alignments can be used for database searching

- **Goal**: Given a query sequence (Q) and a sequence database (D), find a list of sequences from D that are most similar to Q
  - **Input**: Q, D and scoring scheme
  - **Output**: Ranked list of hits



The database search problem

- Due to the rapid growth of sequence databases, search algorithms have to be both efficient and sensitive
  - Time to search with SW is proportional to *m* x *n* (*m* is length of query, n is length of database), **too slow for large databases**!



To reduce search time **heuristic algorithms**, such as BLAST, first remove database sequences without a strong local similarity to the query sequence in a quick initial scan.

The database search problem

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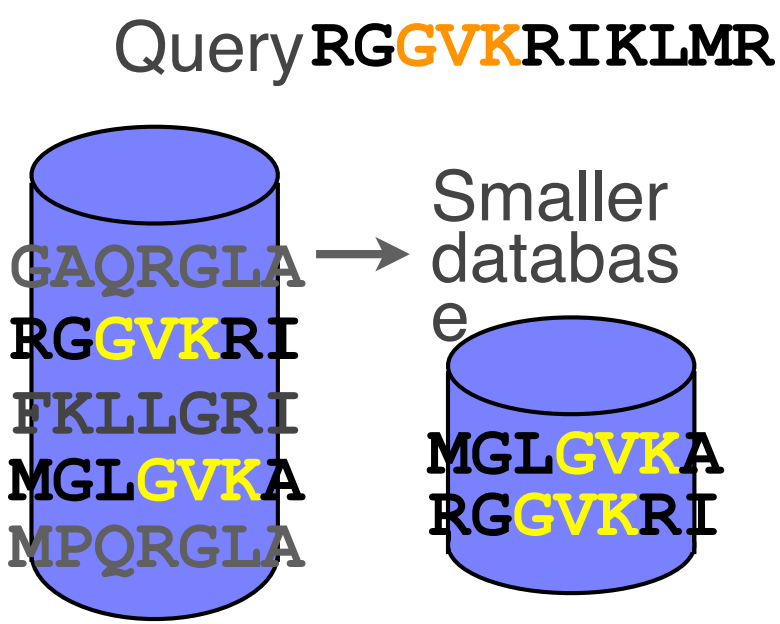

To reduce search time **heuristic algorithms**, such as BLAST, first remove database sequences without a strong local similarity to the query sequence in a quick initial scan.

ALIGNMENT FOUNDATIONS

- **Why…**
  - Why compare biological sequences?
- **What…**
  - Alignment view of sequence changes during evolution (matches, mismatches and gaps)
- **How…**
  - Dot matrices
  - Dynamic programing
    - Global alignment
    - Local alignment
  - BLAST heuristic approach

Rapid, heuristic versions of Smith–Waterman: **BLAST**

- BLAST (Basic Local Alignment Search Tool) is a simplified form of Smith-Waterman (SW) alignment that is popular because it is **fast** and **easily accessible**
  - BLAST is a heuristic approximation to SW - It examines only part of the search space
  - BLAST saves time by restricting the search by scanning database sequences for likely matches before performing more rigorous alignments
  - Sacrifices some sensitivity in exchange for speed
  - In contrast to SW, BLAST is not guaranteed to find optimal alignments

Rapid, heuristic versions of Smith–Waterman: **BLAST**

- BLAST (Basic Local Alignment Search To
  simplified form of Smith-Waterman (S
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    sequences
  - BLAST
    d
    ts
    e sensitivity in exchange for speed
    rast to SW, BLAST is not guaranteed to find optimal
    alignments

hat

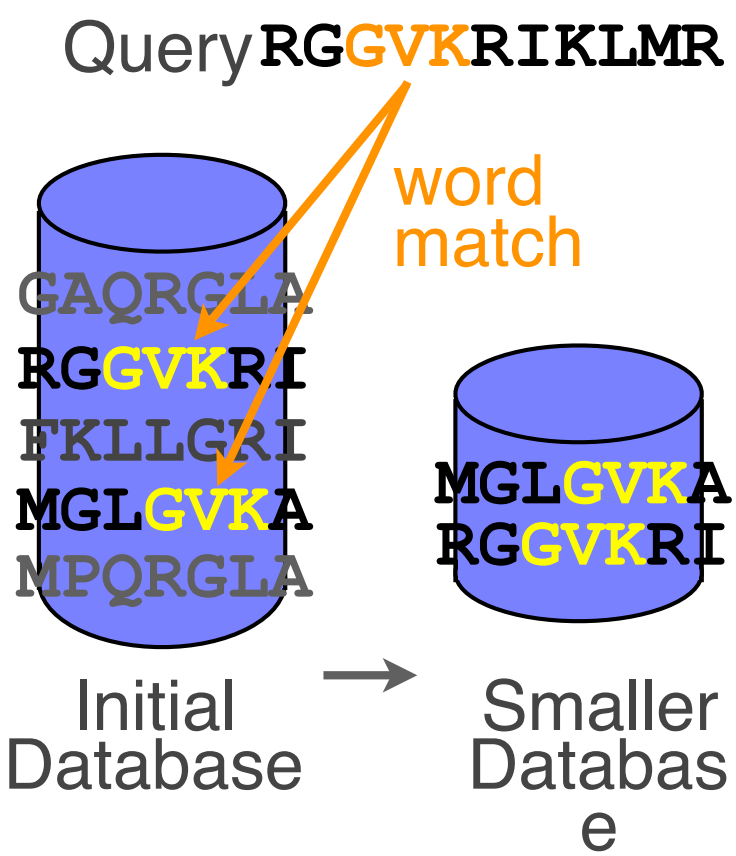
al

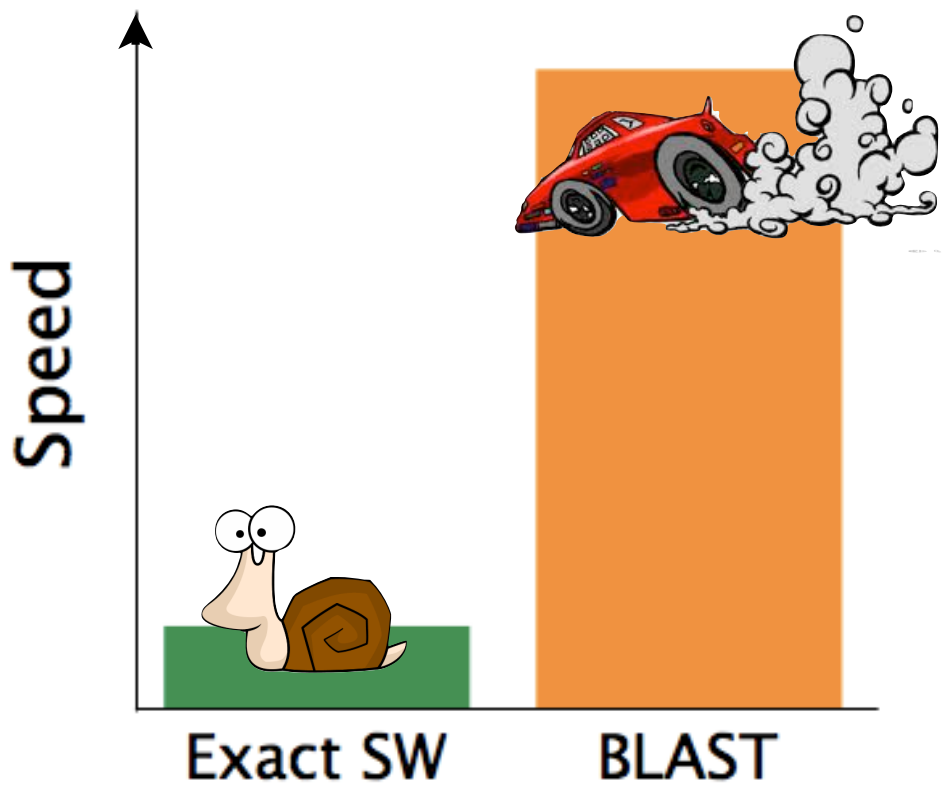
search by scanning

y matches before performing

"The central idea of the BLAST algorithm is to confine attention to sequence pairs that contain an initial **word pair** match"
Altschul et al. (1990)

- BLAST uses this pre-screening heuristic approximation resulting in an an approach that is about 50 times faster than the Smith-Waterman





How BLAST works

- Four basic phases
  - **Phase 1**: compile a list of query word pairs (w=3)

generate list of **w=3 words** for query

```
RGGVKRI
RGG
  GGV
    GVK
      VKR
        KRI
```

Query sequence

Blast

– **Phase 2**: expand word pairs to include those similar to query (defined as those above a similarity threshold to original word, i.e. match scores in substitution matrix)

RGGVKRI Query sequence

RGG RAG RIG RLG ...

GGV GAV GTV GCV ...

GVK GAK GIK GGK ...

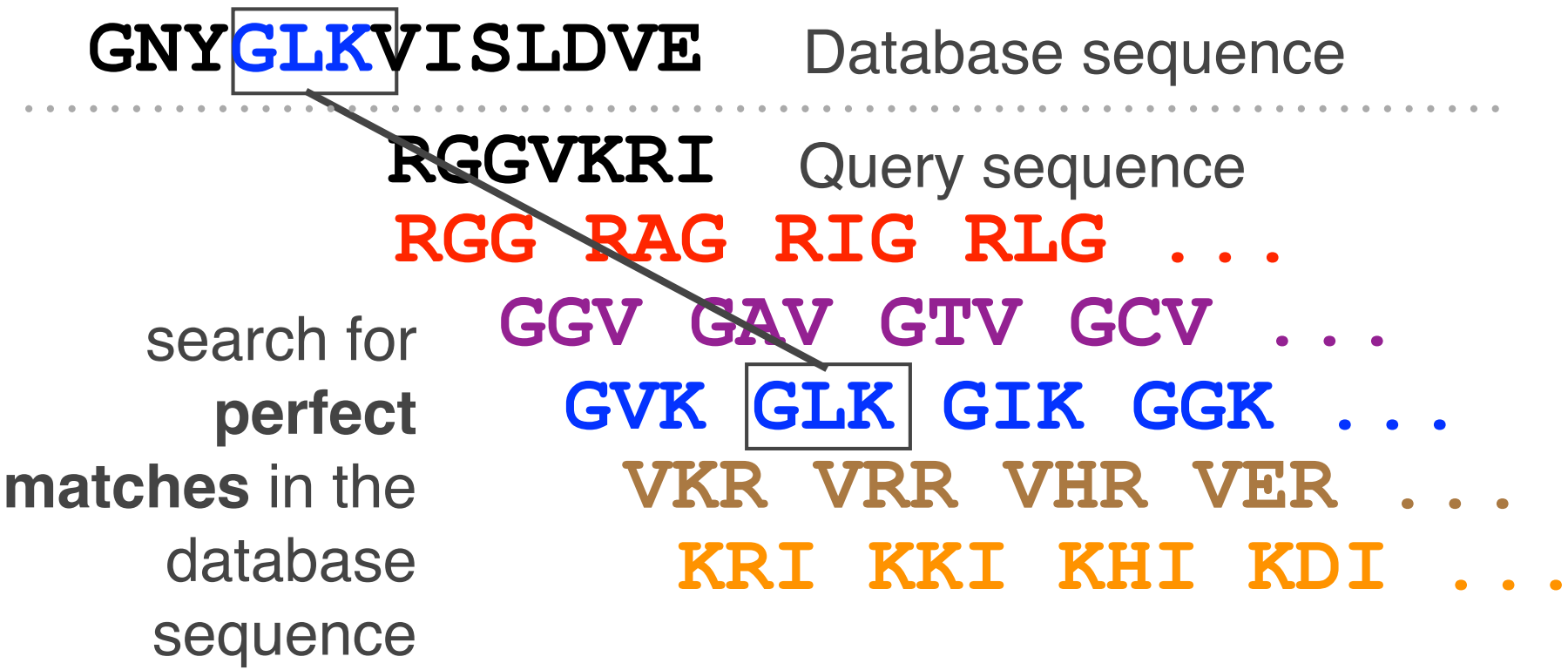
VKR VRR VHR VER ...

KRI KKI KHI KDI ...

extend list of words similar to query

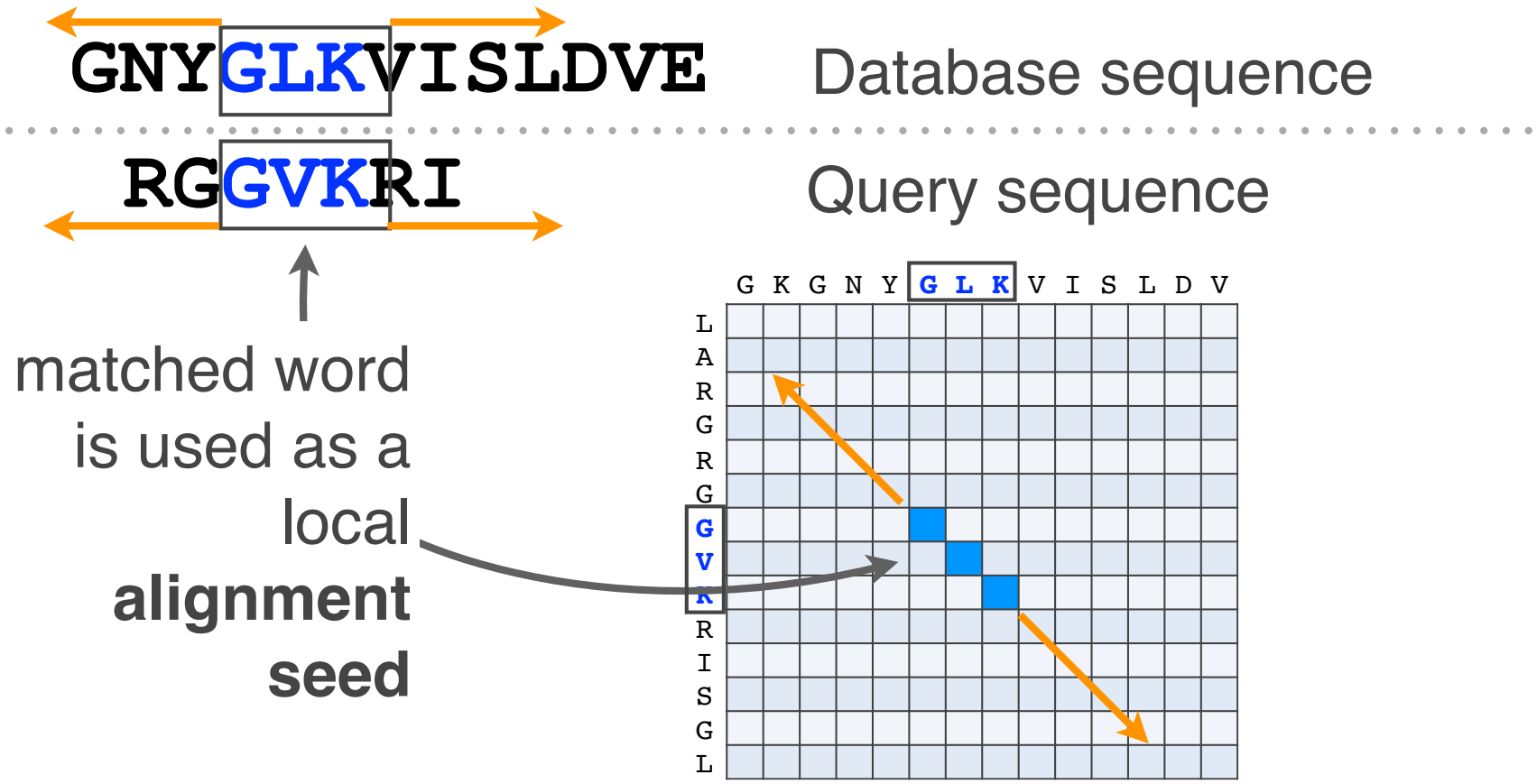
Blast

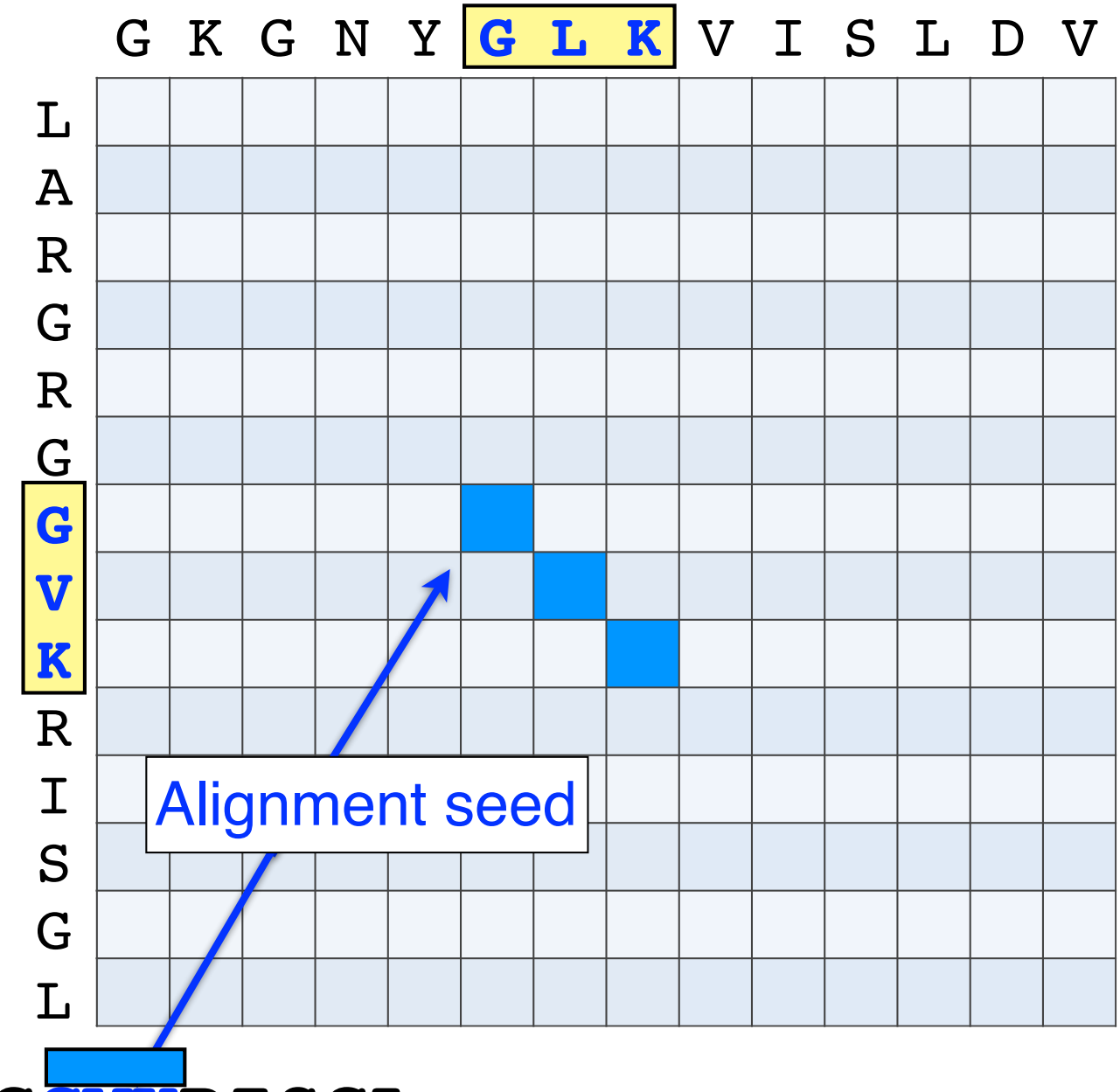
– **Phase 3**: a database is scanned to find sequence entries that match the compiled word list



Blast

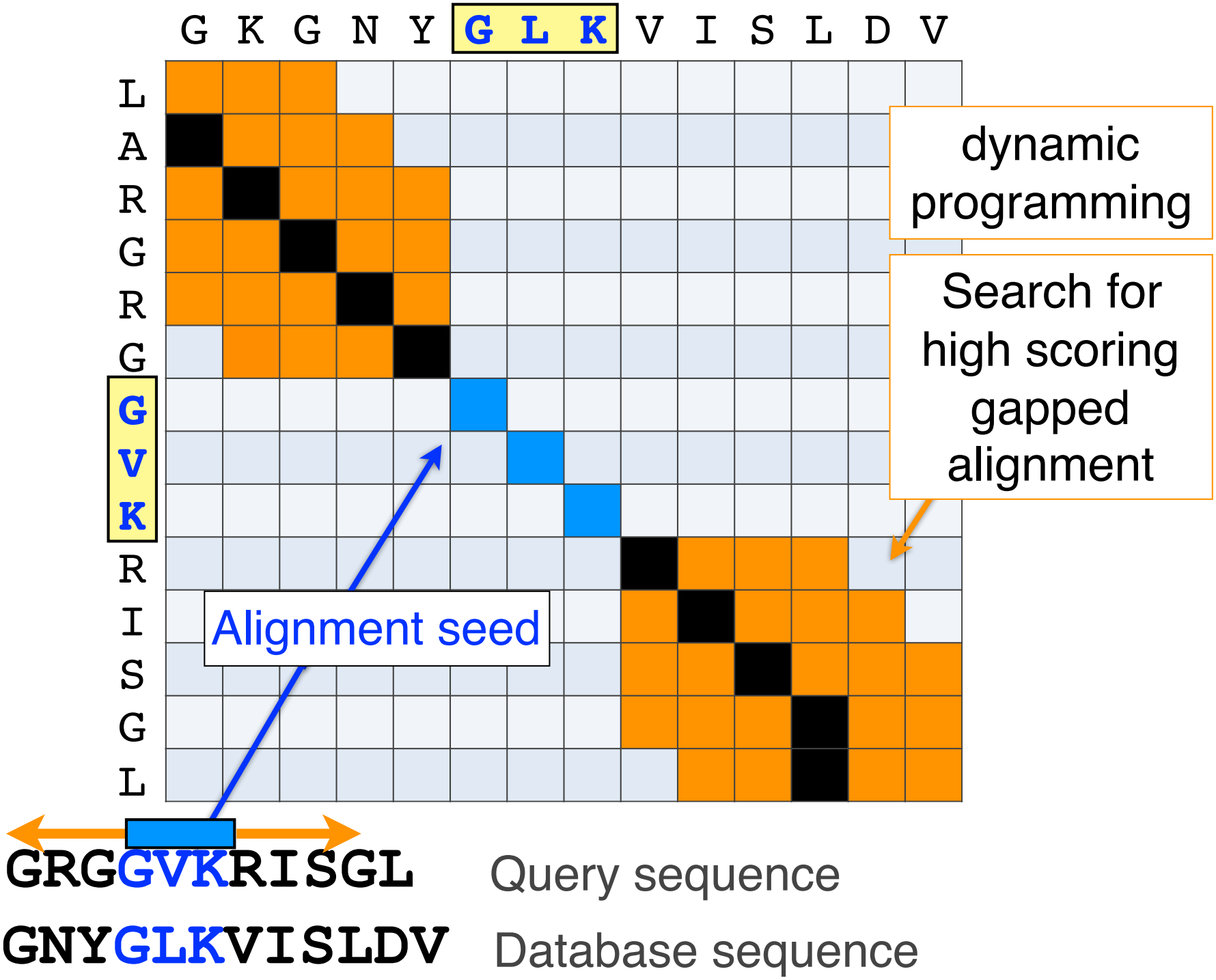
– **Phase 4**: the initial database hits are extended in both directions using dynamic programing

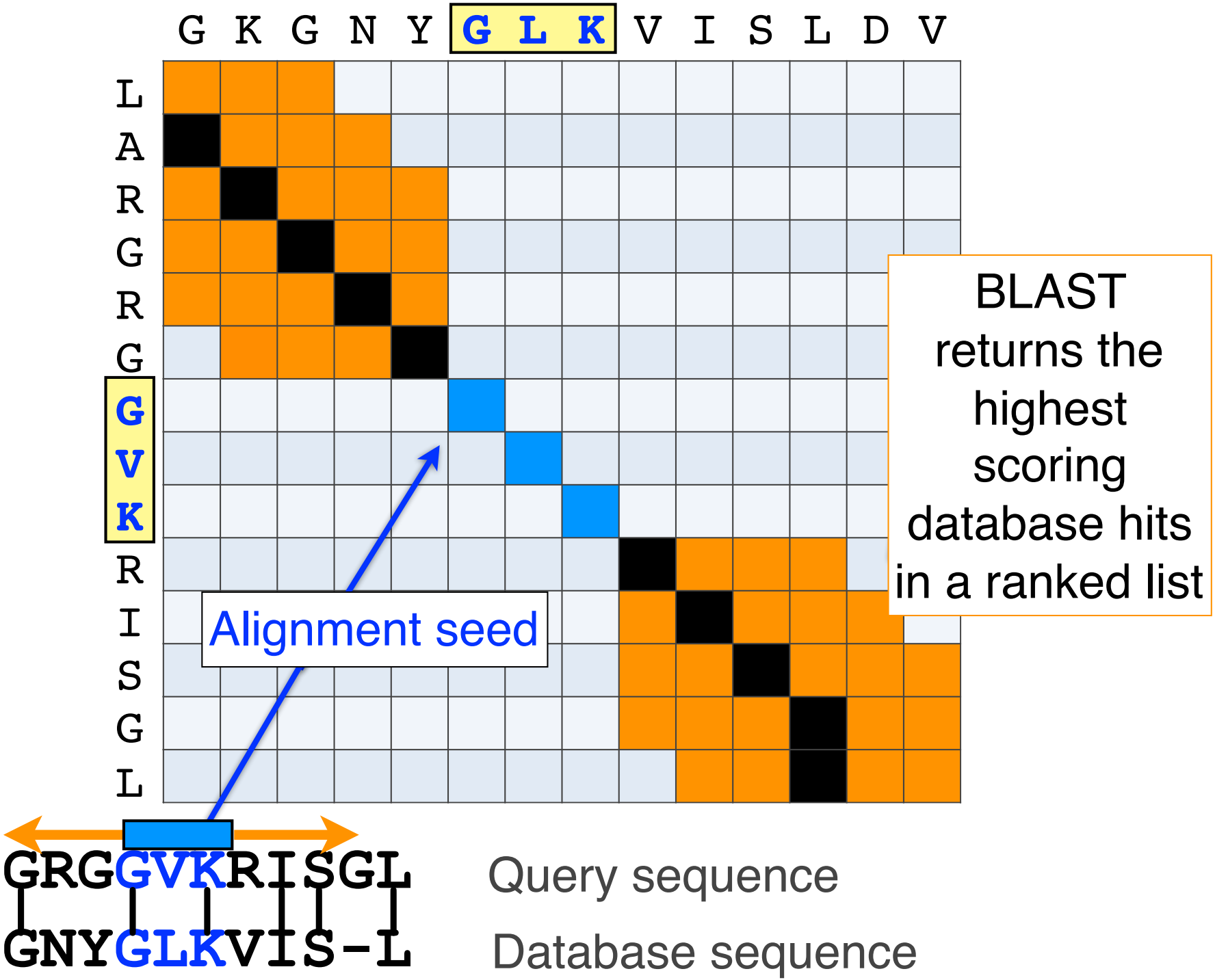




GRGGVKRISGL Query sequence

GNYGLKVISLDV Database sequence


G K G N Y G L K V I S L D V
L A R G R G G V K R I S G L
dynamic programming
Search for high scoring gapped alignment
Alignment seed
GRGGVKRISGL Query sequence
GNYGLKVISLDV Database sequence


G K G N Y G L K V I S L D V
L A R G R G G V K R I S G L
Alignment seed
BLAST returns the highest scoring database hits in a ranked list
GRGGVKRISGL
GNYGLKVIS-L
Query sequence
Database sequence

BLAST output

- BLAST returns the highest scoring database hits in a ranked list along with details about the target sequence and alignment statistics

Description	Max score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo sapiens]	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	100%	0	98%	AAA20133.1
Kinesin-14 heavy chain [Danio rerio]	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	48.2	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	38%	3.02	24%	EHH28205.1

Statistical significance of results

- An important feature of BLAST is the computation of statistical significance for each hit. This is described by the **E value** (expect value)

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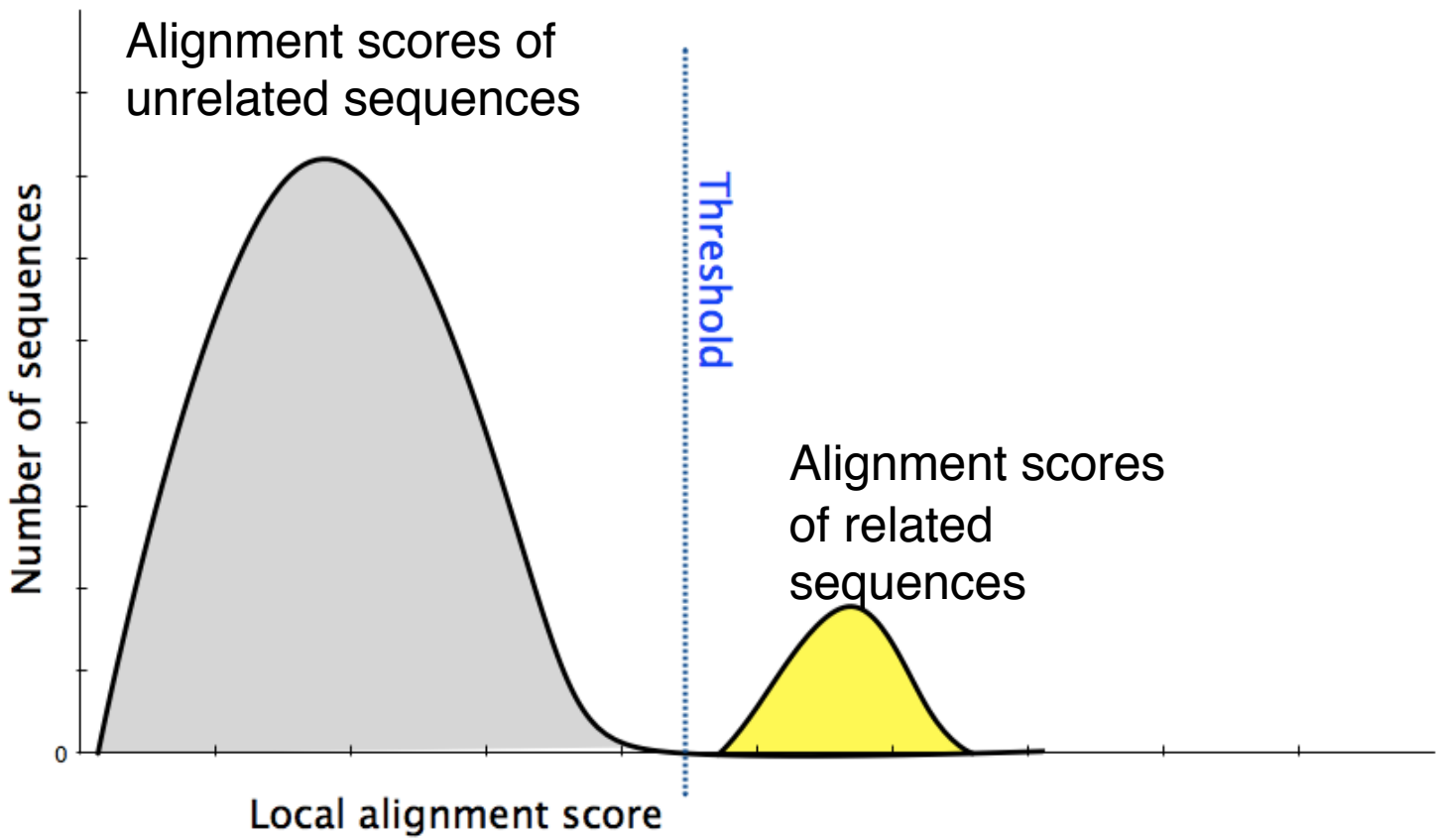
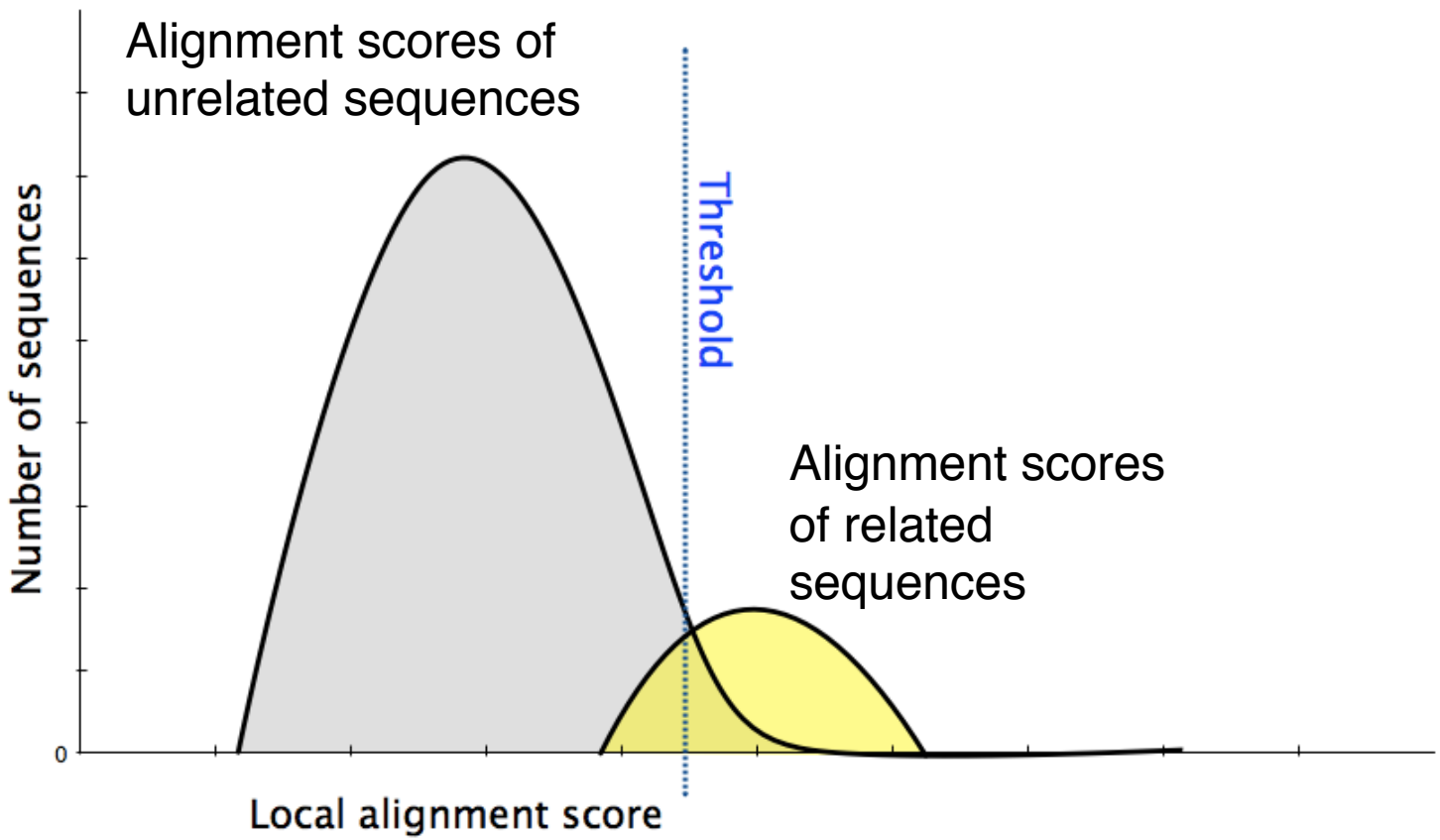
BLAST scores and E-values

- The **E value** is the **expected** number of hits that are as good or better than the observed local alignment score (with this score or better) if the query and database are **random** with respect to each other
  - *i.e.* the number of alignments expected to occur by chance with equivalent or better scores
- Typically, only hits with E value **below** a significance threshold are reported
  - This is equivalent to selecting alignments with score above a certain score threshold

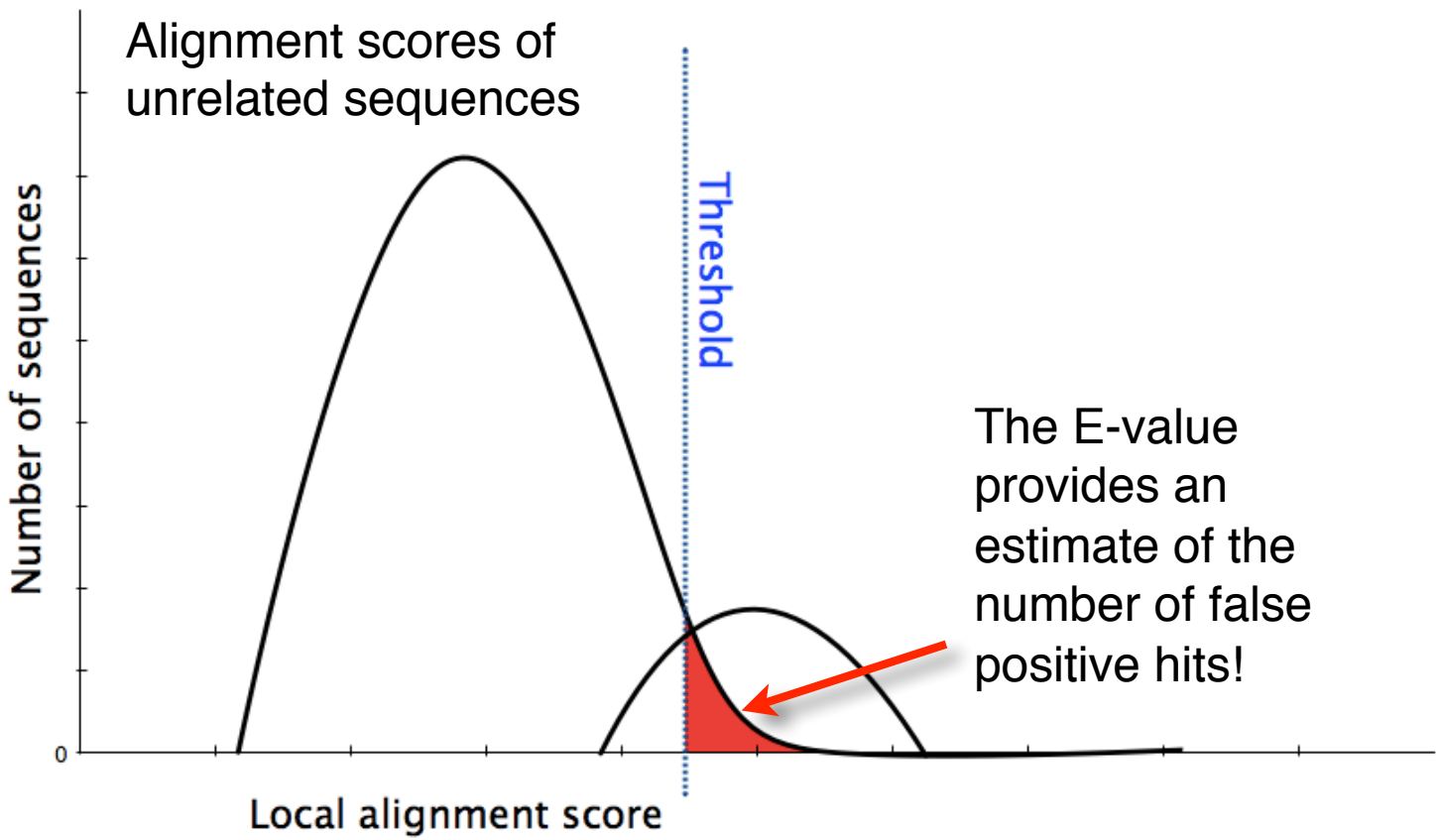
- Ideally, a threshold separates all query related sequences (yellow) from all unrelated sequences (gray)



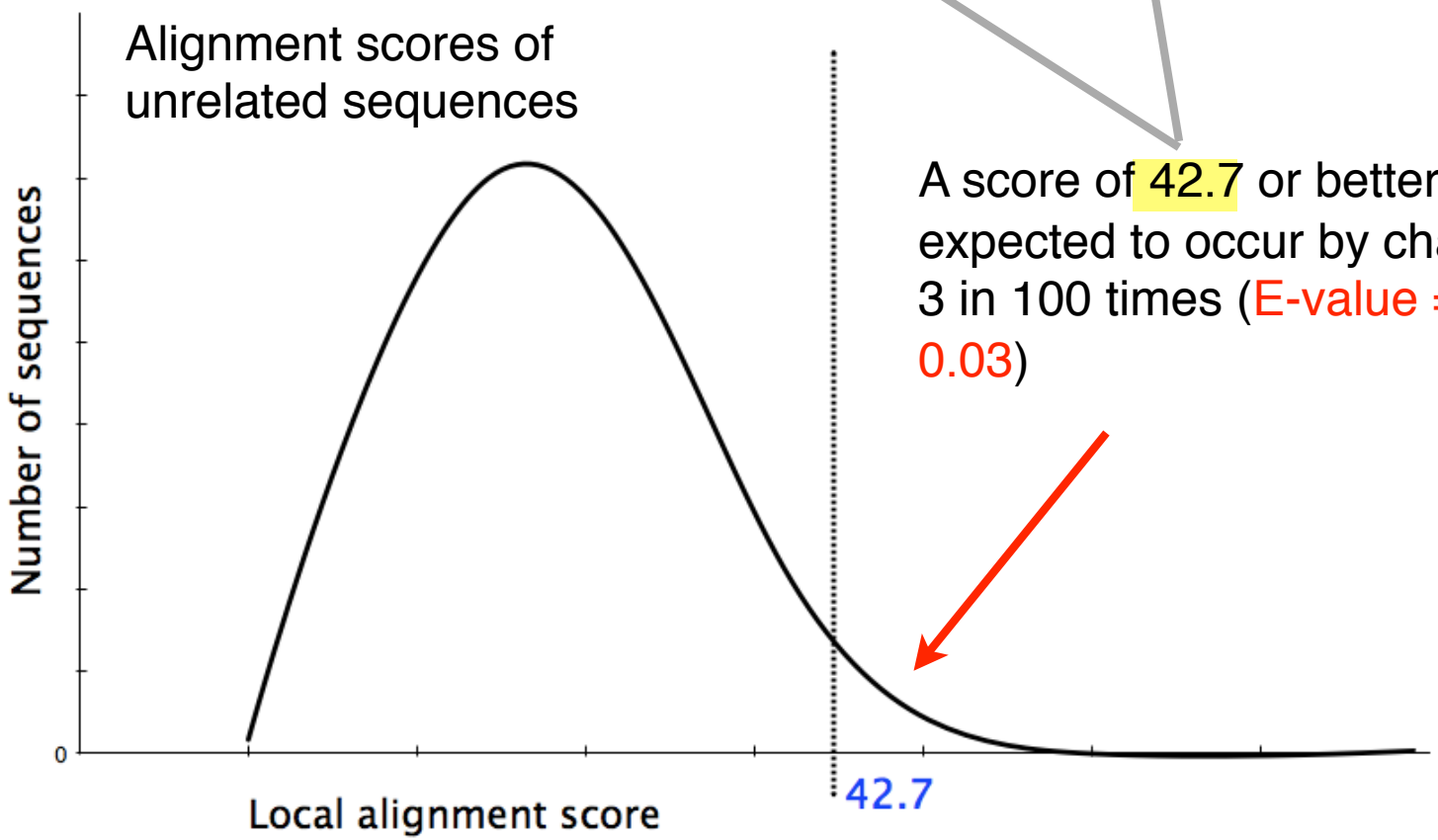
- Unfortunately, often both score distributions overlap
  - The E value describes the expected number of hits with a score above the threshold if the query and database are unrelated



- Unfortunately, often both score distributions overlap
  - The E value describes the expected number of hits with a score above the threshold if the query and database are unrelated



Description	Max score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo sapiens]	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	100%	0	98%	AAA20133.1
Kinesin-14 heavy chain [Danio rerio]	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	42.7	40%	**0.03**	32%	ELK35081.1



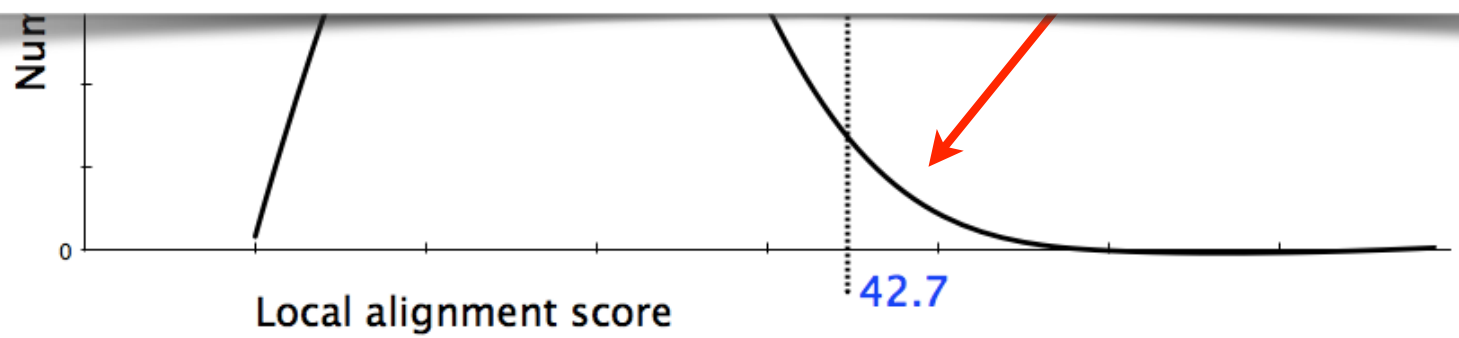
A score of 42.7 or better is expected to occur by chance 3 in 100 times (E-value = 0.03)

Description	Max score	Total score	Query cover	E value	Max ident	Accession
kinesin-1 heavy chain [Homo	677	677	100%	0	100%	NP_004512.1
Kif5b protein [Mus musculus]	676	676	100%	0	98%	AAA20133.1

In general *E* values < 0.005 are usually significant.

To find out more about *E* values see: "*The Statistics of Sequence Similarity Scores*" available in the help section of the NCBI BLAST site:

http://www.ncbi.nlm.nih.gov/blast/tutorial/Altschul-1.html

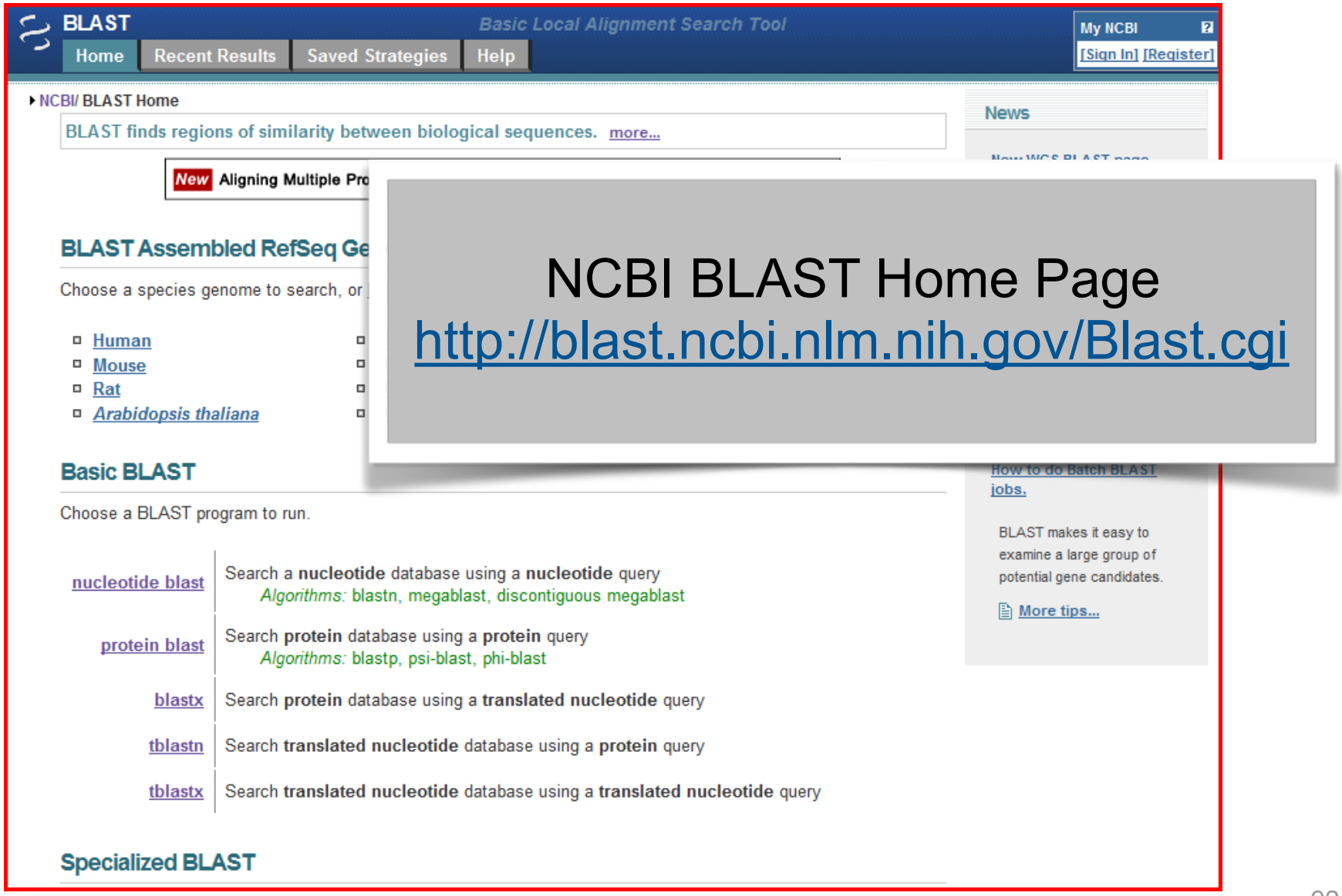


Your Turn!

Hands-on worksheet **Sections 4** & **5**

- Please do answer the last lab review question (**Q19**).
- We encourage discussion and exploration!

Practical database searching with BLAST



Practical database searching with BLAST

- There are four basic components to a traditional BLAST search
  - (1) Choose the sequence (query)
  - (2) Select the BLAST program
  - (3) Choose the database to search
  - (4) Choose optional parameters
- Then click “BLAST”

Step 1: Choose your sequence

- Sequence can be input in FASTA format or as accession number

NCBI Resources How To My N

Protein
Translations of Life

Search: Protein Limits Advanced search Help

Search Clear

Display Settings: FASTA Send to: Change region shown

hemoglobin subunit beta [Homo sapiens]

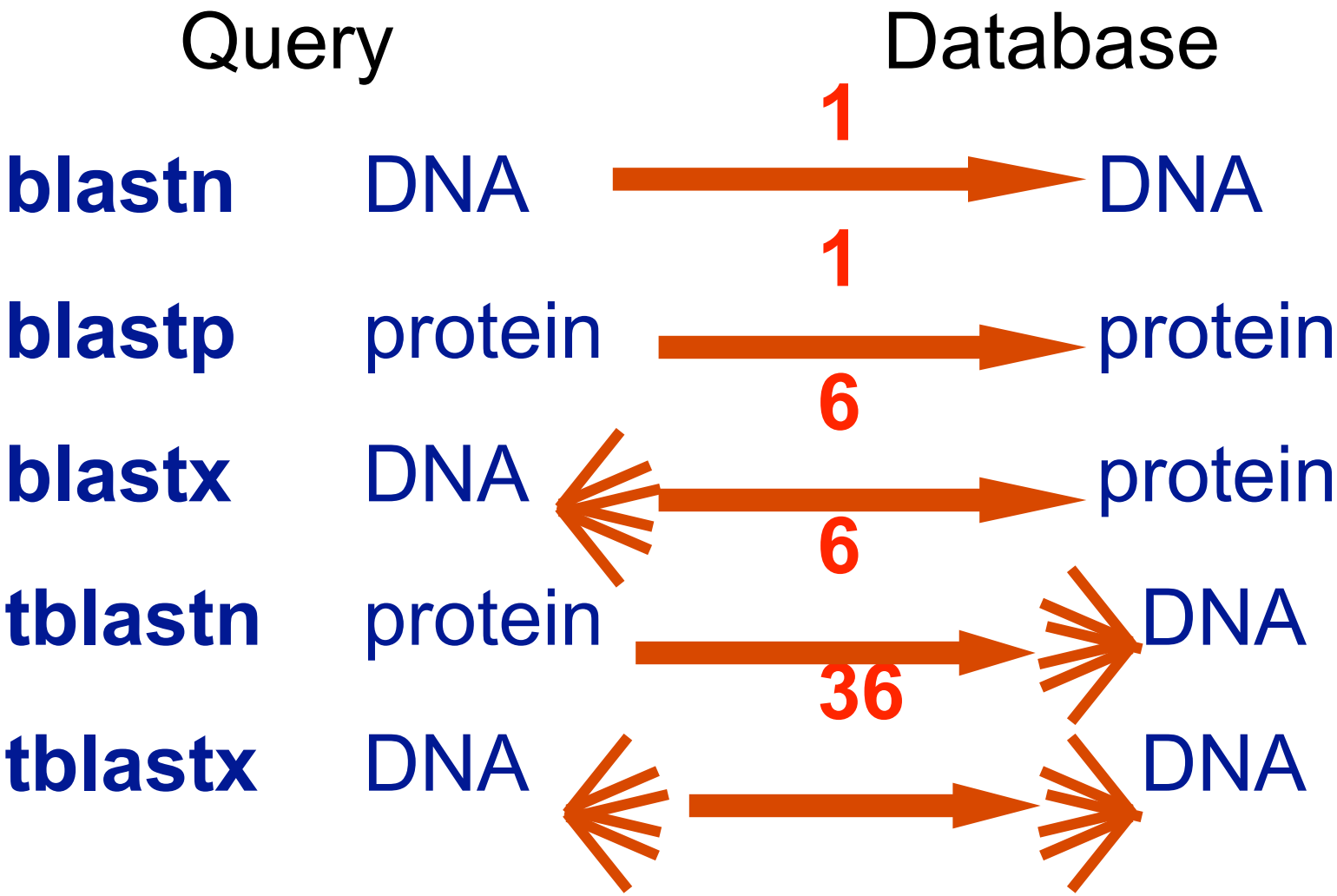
NCBI Reference Sequence: NP_000509.1

GenPept Graphics

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLG
AFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH
```

Analyze this sequence
Run BLAST
Identify Conserved Domains
Find in this Sequence

Step 2: Choose the BLAST program



DNA potentially encodes six proteins

```
     5′ CAT CAA
      5′ ATC AAC
       5′ TCA ACT

5′ CATCAACTACAACTCCAAAGACACCCTTACACATCAACAAACCTACCCAC 3′
3′ GTAGTTGATGTTGAGGTTTCTGTGGGAATGTGTAGTTGTTTGGATGGGTG 5′

                                                 5′ GTG GGT
                                                  5′ TGG GTA
                                                   5′ GGG TAG
```

Protein BLAST: search protein databases using a protein query

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAC

Reader

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

Query subrange

From

To

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGK
KVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQK
VVAGVANALAHKYH
```

Or, upload file Choose File no file selected

Job Title

Enter a descriptive title for your BLAST search

☐ **Align two or more sequences**

Choose Search Set

Database Non-redundant protein sequences (nr)

Organism Optional

☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude Optional

☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Entrez Query Optional

Enter an Entrez query to limit search

Program Selection

Algorithm

- (•) blastp (protein-protein BLAST)
- ( ) PSI-BLAST (Position-Specific Iterated BLAST)
- ( ) PHI-BLAST (Pattern Hit Initiated BLAST)
- ( ) DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

Choose a BLAST algorithm

BLAST

Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

☐ **Show results in a new window**

+ Algorithm parameters

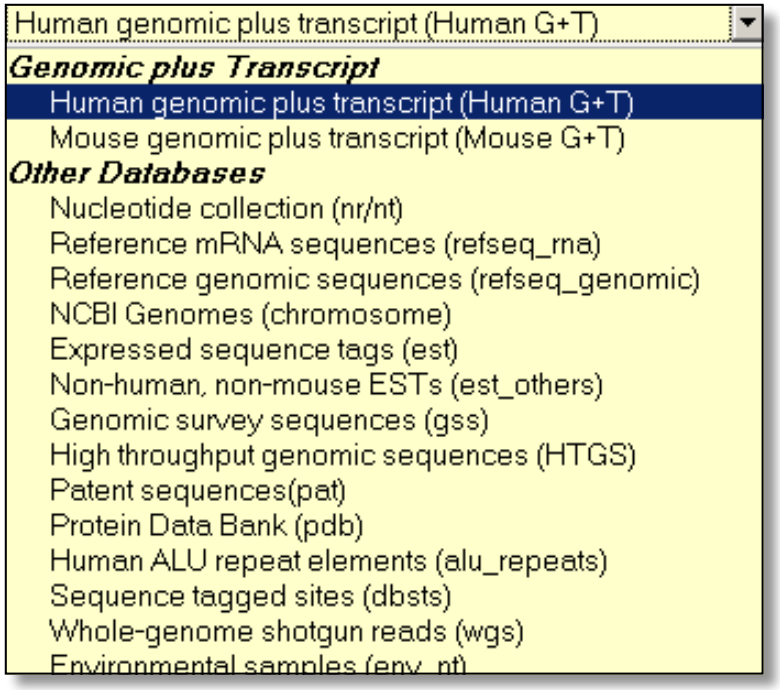
Step 3: Choose the database

nr = non-redundant (most general database)

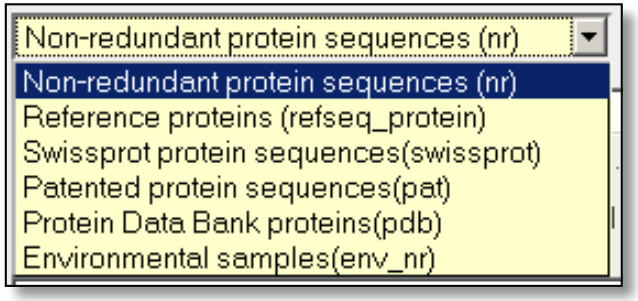
dbest = database of expressed sequence tags

dbsts = database of sequence tag sites

gss = genomic survey sequences



nucleotide databases



protein databases

Organism

Entrez

Settings!

Protein BLAST: search protein databases using a protein query

blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&BLAST_PROGRAMS=blastp&PAC

Reader

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s) Clear

Query subrange

From

To

```
>gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGK
KVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFGKEFTPPVQAAYQK
VVAGVANALAHKYH
```

Or, upload file Choose File no file selected

Job Title

Enter a descriptive title for your BLAST search

☐ **Align two or more sequences**

Choose Search Set

Database Non-redundant protein sequences (nr)

Organism Optional ☐ Exclude +

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Exclude Optional ☐ Models (XM/XP) ☐ Uncultured/environmental sample sequences

Entrez Query Optional

Enter an Entrez query to limit search

Program Selection

Algorithm

- (•) blastp (protein-protein BLAST)
- ( ) PSI-BLAST (Position-Specific Iterated BLAST)
- ( ) PHI-BLAST (Pattern Hit Initiated BLAST)
- ( ) DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)

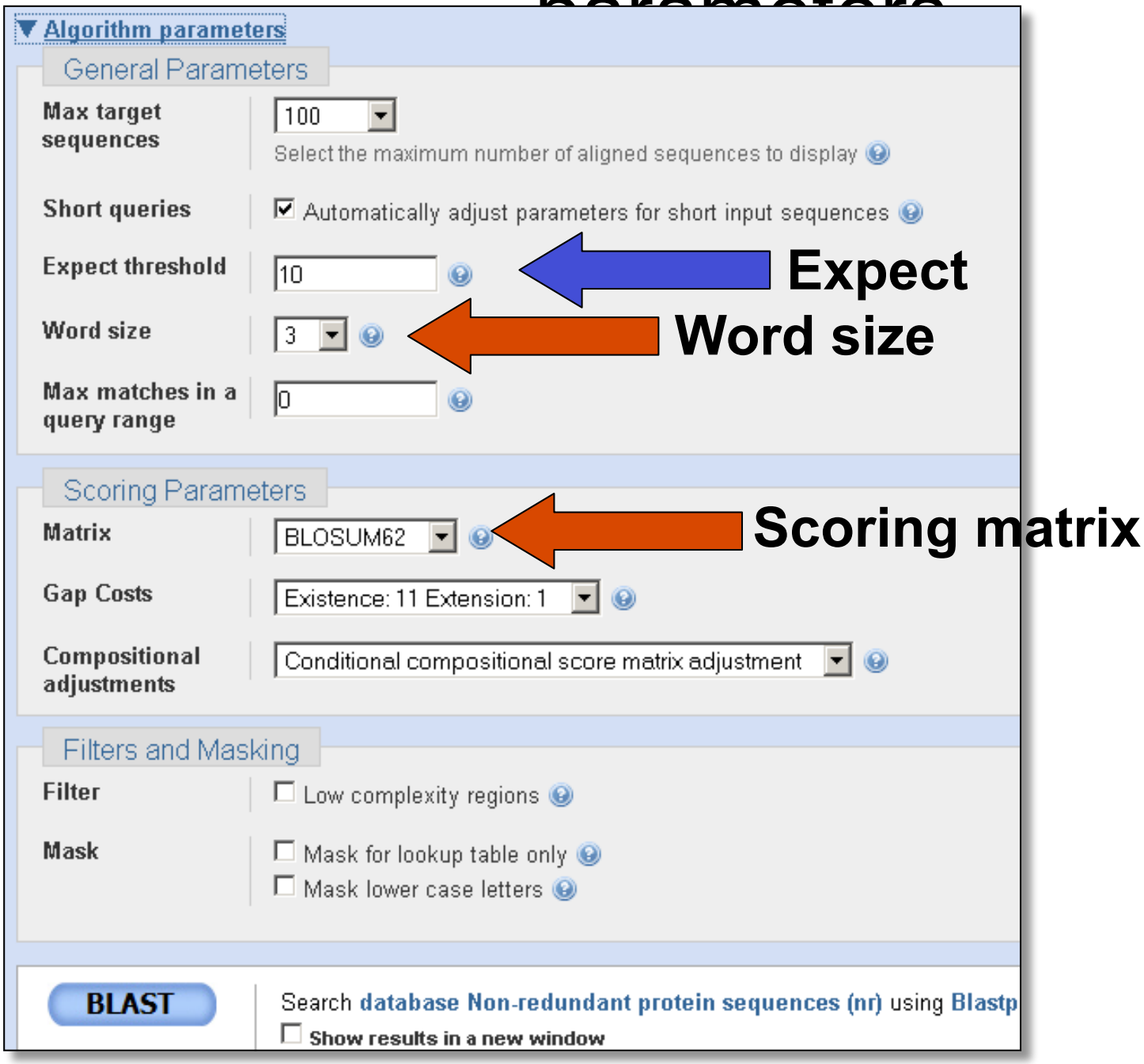
Choose a BLAST algorithm

BLAST Search database Non-redundant protein sequences (nr) using Blastp (protein-protein BLAST)

☐ Show results in a new window

+ Algorithm parameters

Step 4a: Select optional search parameters



Step 4: Optional parameters

- You can...
  - choose the organism to search
  - change the substitution matrix
  - change the expect (E) value
  - change the word size
  - change the output format

Results page

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

BLAST® Basic Local Alignment Search Tool

Home | Recent Results | Saved Strategies | Help

My NCBI [Sign In] [Register]

NCBI/ BLAST/ blastp suite/ Formatting Results - FVGUTMRZ013

Edit and Resubmit | Save Search Strategies | Formatting options | Download | Change the result display back to traditional format

YouTube Learn about the enhanced report | Blast report description

gi|4504349|ref|NP_000509.1| hemoglobin

Query ID lcl|84677
Description gi|4504349|ref|NP_000509.1| hemoglobin subunit beta [Homo sapiens]
Molecule type amino acid
Query Length 147

Database Name nr
Description All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF excluding environmental samples from WGS projects
Program BLASTP 2.2.27+ Citation

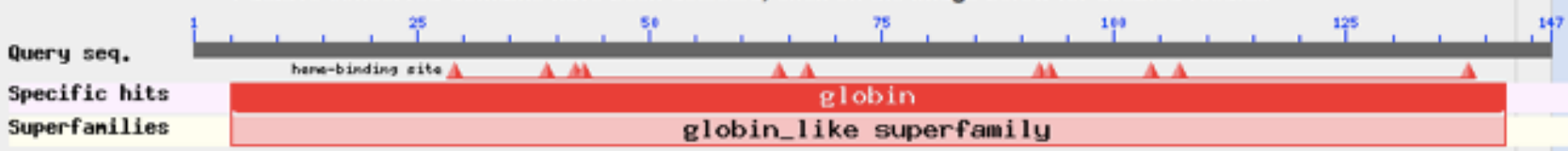
Other reports: Search Summary [Taxonomy reports] [Distance tree of results] [Related Structures] [Multiple alignment]

New DELTA-BLAST, a more sensitive protein-protein search Go

Graphic Summary

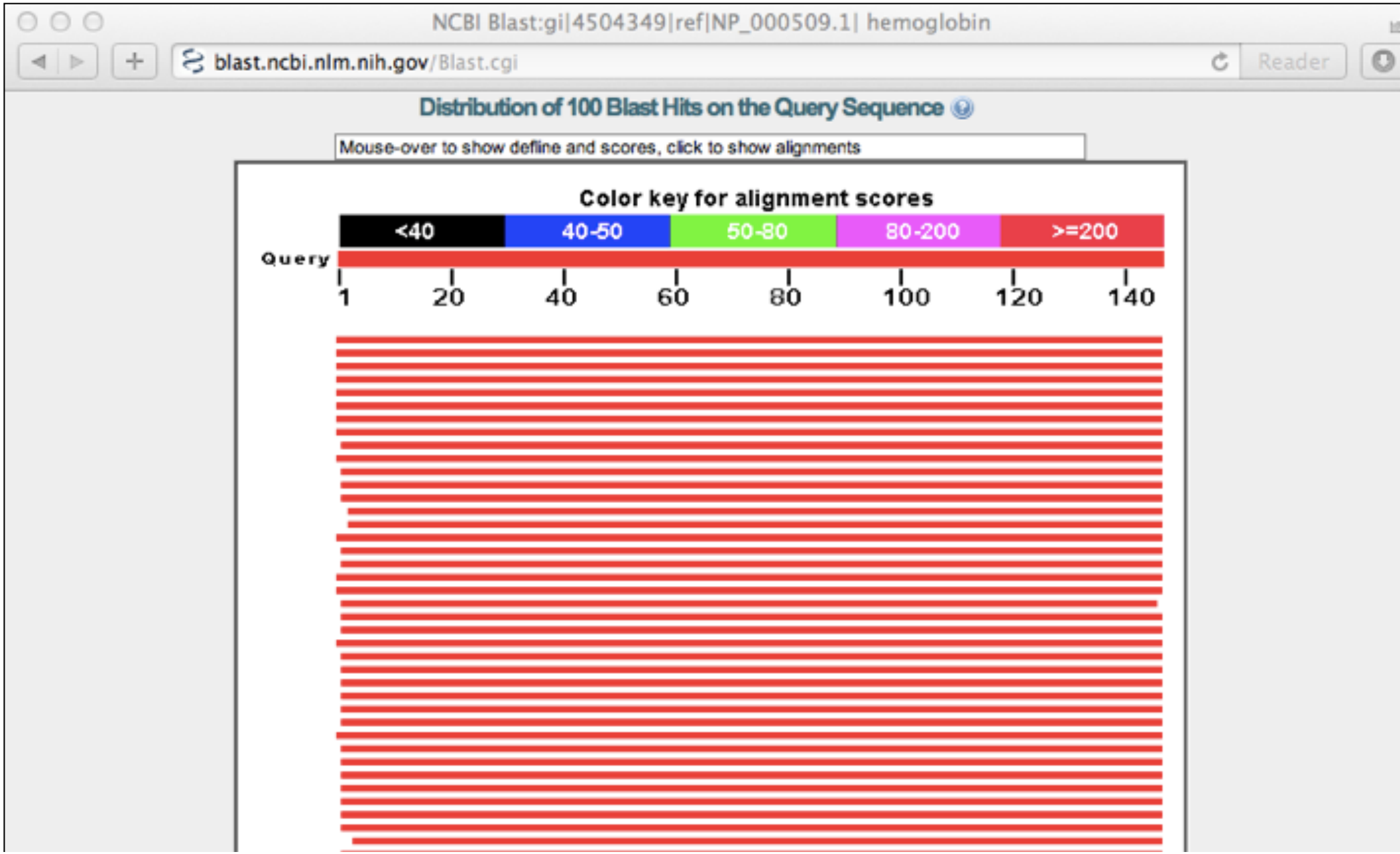
Show Conserved Domains

Putative conserved domains have been detected, click on the image below for detailed results.



Distribution of 100 Blast Hits on the Query Sequence

Further down the results page...



Further down the results page...

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenPept Graphics Distance tree of results Multiple alignment

Description	Max score	Total score	Query cover	E value	Max ident	Accession
hemoglobin beta [synthetic construct]	301	301	100%	9e-103	100%	AAX37051.1
hemoglobin beta [synthetic construct]	301	301	100%	1e-102	100%	AAX29557.1
hemoglobin subunit beta [Homo sapiens] >ref|XP_508242.1| PREDICTED: hemoglobin s	301	301	100%	1e-102	100%	NP_000509.1
RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Her	300	300	100%	4e-102	99%	P02024.2
beta globin chain variant [Homo sapiens]	299	299	100%	5e-102	99%	AAN84548.1
beta globin [Homo sapiens] >gb|AAZ39781.1| beta globin [Homo sapiens] >gb|AAZ39782	299	299	100%	5e-102	99%	AAZ39780.1
beta-globin [Homo sapiens]	299	299	100%	5e-102	99%	ACU56984.1
hemoglobin beta chain [Homo sapiens]	299	299	100%	6e-102	99%	AAD19696.1
Chain B, Structure Of Haemoglobin In The Deoxy Quaternary State With Ligand Bound At	298	298	99%	9e-102	100%	1COH_B
hemoglobin beta subunit variant [Homo sapiens] >gb|AAA88054.1| beta-globin [Homo sa	298	298	100%	1e-101	99%	AAF00489.1
Chain B, Human Hemoglobin D Los Angeles: Crystal Structure >pdb|2YRS|D Chain D, H	298	298	99%	2e-101	99%	2YRS_B
Chain B, High-Resolution X-Ray Study Of Deoxy Recombinant Human Hemoglobins Syn	297	297	99%	3e-101	99%	1DXU_B
Chain B, Analysis Of The Crystal Structure, Molecular Modeling And Infrared Spectroscop	297	297	99%	3e-101	99%	1HDB_B

Further down the results page...

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

Download GenPept Graphics ▼ Next ▲ Previous Descriptions

hemoglobin subunit beta [Homo sapiens]

Sequence ID: ref|NP_000509.1| Length: 147 Number of Matches: 1

See 84 more title(s)

Range 1: 1 to 147 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
301 bits(770)	1e-102	Compositional matrix adjust.	147/147(100%)	147/147(100%)	0/147(0%)

```
Query  1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
            MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK
Sbjct  1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60

Query  61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  120
            VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG
Sbjct  61   VKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHHFG  120

Query  121  KEFTPPVQAAYQKVVAGVANALAHKYH  147
            KEFTPPVQAAYQKVVAGVANALAHKYH
Sbjct  121  KEFTPPVQAAYQKVVAGVANALAHKYH  147
```

Related Information

Gene - associated gene details
UniGene - clustered expressed sequence tags
Map Viewer - aligned genomic context
Structure - 3D structure displays
PubChem Bio
Assay - bioactivity screening

Download GenPept Graphics ▼ Next ▲ Previous Descriptions

RecName: Full=Hemoglobin subunit beta; AltName: Full=Beta-globin; AltName: Full=Hemoglobin beta chain

Sequence ID: sp|P02024.2|HBB_GORGO Length: 147 Number of Matches: 1

Range 1: 1 to 147 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
300 bits(767)	4e-102	Compositional matrix adjust.	146/147(99%)	147/147(100%)	0/147(0%)

Related Information

Different output formats are available

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

BLAST®

Basic Local Alignment Search Tool

Home | Recent Results | Saved Strategies | Help

My NCBI [Sign In] [Register]

NCBI/ BLAST/ blastp suite/ Formatting Results - FVGUTMRZ013

Edit and Resubmit | Save Search Strategies | Formatting options | Download | Change the result display back

YouTube Learn about the enhanced report | Blast

Formatting options | Reforma

Show	Alignment as HTML; Old View; Reset form to defaults
Alignment View	Query-anchored with letters for identities
Display	Graphical Overview; Sequence Retrieval; NCBI-gi
Masking	Character: Lower Case; Color: Grey
Limit results	Descriptions: 50; Graphical overview: 50; Alignments: 50
	Organism Type common name, binomial, taxid, or group name. Only 20 top taxa will be shown. Enter organism name or id--completions will be suggested; Exclude +
	Entrez query:
	Expect Min: ; Expect Max:
	Percent Identity Min: ; Percent Identity Max:
Format for	PSI-BLAST; with inclusion threshold:

gi|4504349|ref|NP_000509.1| hemoglobin

E.g. Query anchored alignments

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

```
Query          1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
AAX37051       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
AAX29557       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
NP_000509      1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
P02024         1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
AAN84548       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
AAZ39780       1    MVHLTPKEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
ACU56984       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFKSFGDLSTPDAVMGNPK  60
AAD19696       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFLESFGDLSTPDAVMGNPK  60
1COH_B         1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
AAF00489       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
2YRS_B         1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1DXU_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1HDB_B         1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1DXV_B         2      HLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
3KMF_C         2      HLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
AAL68978       1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
1NQP_B         1     VHLTPEEKSAVTALWGKVNVDEVGGKALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1K1K_B         1     VHLTPKEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
AAN11320       1    MVHLTPVEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
XP_002822173   1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
1Y85_B         1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1YE0_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLAVYPWTQRFFESFGDLSTPDAVMGNPK  59
1O1O_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
CAA23759       1    MVHLTPVEKSAVTAXWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
1YE2_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVFPWTQRFFESFGDLSTPDAVMGNPK  59
1Y5F_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1A00_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPYTQRFFESFGDLSTPDAVMGNPK  59
1HBS_B         1     VHLTPVEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1ABY_B         1     MHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
1CMY_B         1     VHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  59
```

... and alignments with dots for identities

NCBI Blast:gi|4504349|ref|NP_000509.1| hemoglobin

blast.ncbi.nlm.nih.gov/Blast.cgi

```
Query          1    MVHLTPEEKSAVTALWGKVNVDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPK  60
AAX37051       1    ............................................................  60
AAX29557       1    ............................................................  60
NP_000509      1    ............................................................  60
P02024         1    ............................................................  60
AAN84548       1    ............................................................  60
AAZ39780       1    ......K.....................................................  60
ACU56984       1    ...........................................K................  60
AAD19696       1    ..........................................L.................  60
1COH_B         1     ...........................................................  59
AAF00489       1    ............................................................  60
2YRS_B         1     ...........................................................  59
1DXU_B         1     M..........................................................  59
1HDB_B         1     ...........................................................  59
1DXV_B         2      ..........................................................  59
3KMF_C         2      ..........................................................  59
AAL68978       1    ............................................................  60
1NQP_B         1     ........................K..................................  59
1K1K_B         1     .....K.....................................................  59
AAN11320       1    ......V.....................................................  60
XP_002822173   1    ............................................................  60
1Y85_B         1     ...........................................................  59
1YE0_B         1     M..............................A...........................  59
1O1O_B         1     M..........................................................  59
CAA23759       1    ......V.......X.............................................  60
1YE2_B         1     M...............................F..........................  59
1Y5F_B         1     M..........................................................  59
1A00_B         1     M...................................Y......................  59
```

Common problems

- Selecting the wrong version of BLAST
- Selecting the wrong database
- Too many hits returned
- Too few hits returned
- Unclear about the significance of a particular result - are these sequences homologous?

How to handle too many results

- Focus on the question you are trying to answer
  - select “refseq” database to eliminate redundant matches from “nr”
  - Limit hits by organism
  - Use just a portion of the query sequence, when appropriate
  - Adjust the expect value; lowering *E* will reduce the number of matches returned

How to handle too few results

- Many genes and proteins have no significant database matches
  - remove Entrez limits
  - raise E-value threshold
  - search different databases
  - try scoring matrices with lower BLOSUM values (or higher PAM values)
  - use a search algorithm that is more sensitive than BLAST (*e.g.* PSI-BLAST or HMMer)

Summary of key points

- Sequence alignment is a fundamental operation underlying much of bioinformatics.
- Even when optimal solutions can be obtained they are not necessarily unique or reflective of the biologically correct alignment.
- Dynamic programming is a classic approach for solving the pairwise alignment problem.
- Global and local alignment, and their major application areas.
- Heuristic approaches are necessary for large database searches and many genomic applications.

FOR NEXT CLASS…

Check out the online:

- ☑ **Reading**: Sean Eddy's "What is dynamic programming?"
- ☑ **Homework**: (1) **Quiz**, (2) **Alignment Exercise**.

To Update!

Homework Grading

Both (1) quiz questions and (2) alignment exercise carry equal weights (*i.e.* 50% each).

(Homework 2) Assessment Criteria	Points	
Setup labeled alignment matrix	1	
Include initial column and row for GAPs	1	
All alignment matrix elements scored (i.e. filled in)	1	
Evidence for correct use of scoring scheme	1	
Direction arrows drawn between all cells	1	
Evidence of multiple arrows to a given cell if appropriate	1	D
Correct optimal score position in matrix used	1	C
Correct optimal score obtained for given scoring scheme	1	B
Traceback path(s) clearly highlighted	1	A
Correct *alignment(s)* yielding optimal score listed	1	A+