PAIRWISE SEQUENCE ALIGNMENT AND DATABASE SEARCHING

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MODULE OVERVIEW

Objective: Provide an introduction to the practice of bioinformatics as well as a practical guide to using common bioinformatics databases and algorithms

1.1. ▸ *Introduction to Bioinformatics*

1.2. ▸ *Sequence Alignment and Database Searching*

1.3 ▸ *Structural Bioinformatics*

1.4 ▸ *Genome Informatics: High Throughput Sequencing Applications and Analytical Methods*

WEEK ONE REVIEW

- **Answers to last weeks homework** (19/20)**:**
  Answers week 1

- **Muddy Point Assessment** (14/20)**:**
  Responses

  - NCBI BLAST frustrations
  - Need for FASTA header lines “>example1”
  - More on protein structure viewing and finding
  - “Nice Assignment”.

THIS WEEK'S HOMEWORK

- ☑ Check out the "**Background Reading**" material online:
  Dynamic Programming
  Database Searching

- ☑ Complete the **lecture 1.2 homework questions**:
  http://tinyurl.com/bioinf525-quiz2

TODAYS MENU

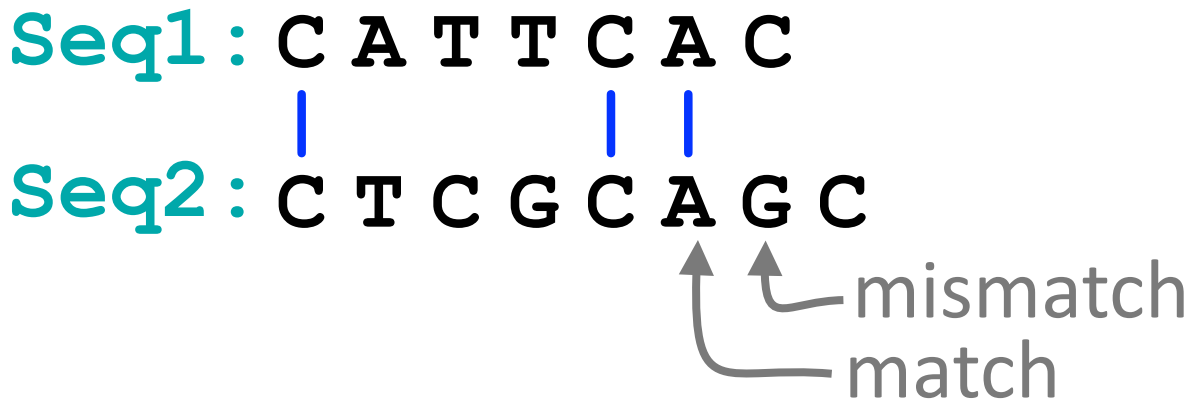
- Alignment basics
  - Why compare biological sequences?
- Homologue detection
  - Orthologs, paralogs, similarity and identity
  - Sequence changes during evolution
  - Alignment view: matches, mismatches and gaps
- Pairwise sequence alignment methods
  - Brute force alignment
  - Dot matrices
  - Dynamic programing

  (global vs local alignment)
- Rapid heuristic approaches
  - BLAST
- Practical database searching
  - PSI-BLAST and HMM approaches

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

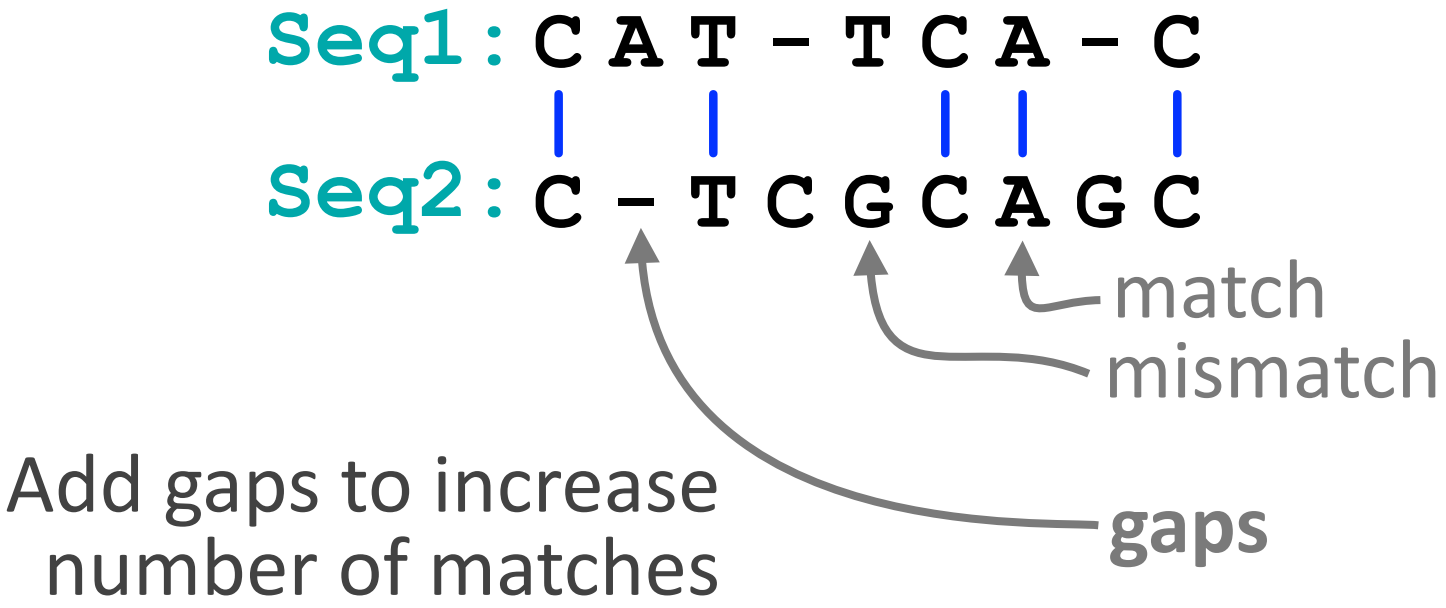
```
Seq1: C A T T C A C
Seq2: C T C G C A G C
```

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



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.



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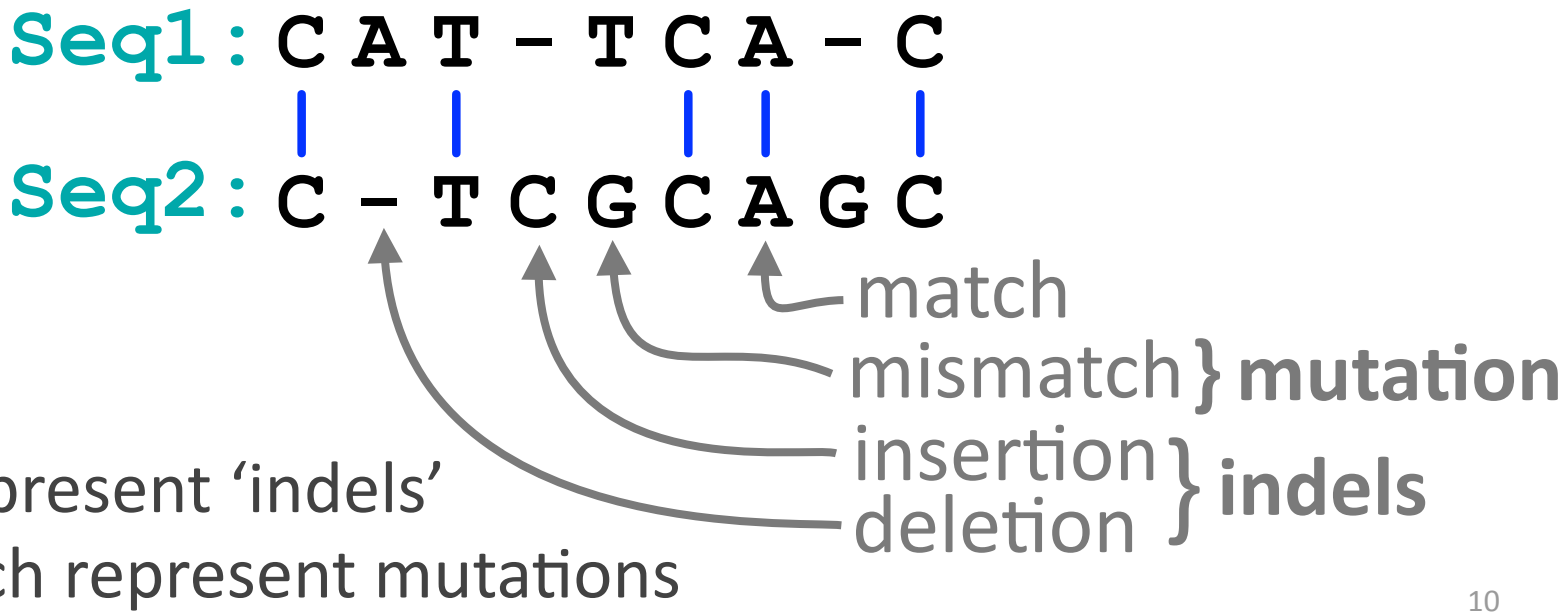

Gaps represent 'indels'
mismatch represent mutations

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 of sequence alignment 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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N.B. Pairwise sequence alignment is arguably the most fundamental operation of bioinformatics!

Outline for today

- Alignment basics
  - Why compare biological sequences?
- Homologue detection
  - Orthologs, paralogs, similarity and identity
  - Sequence changes during evolution
  - Alignment view: matches, mismatches and gaps
- Pairwise sequence alignment methods
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  (global vs local alignment)
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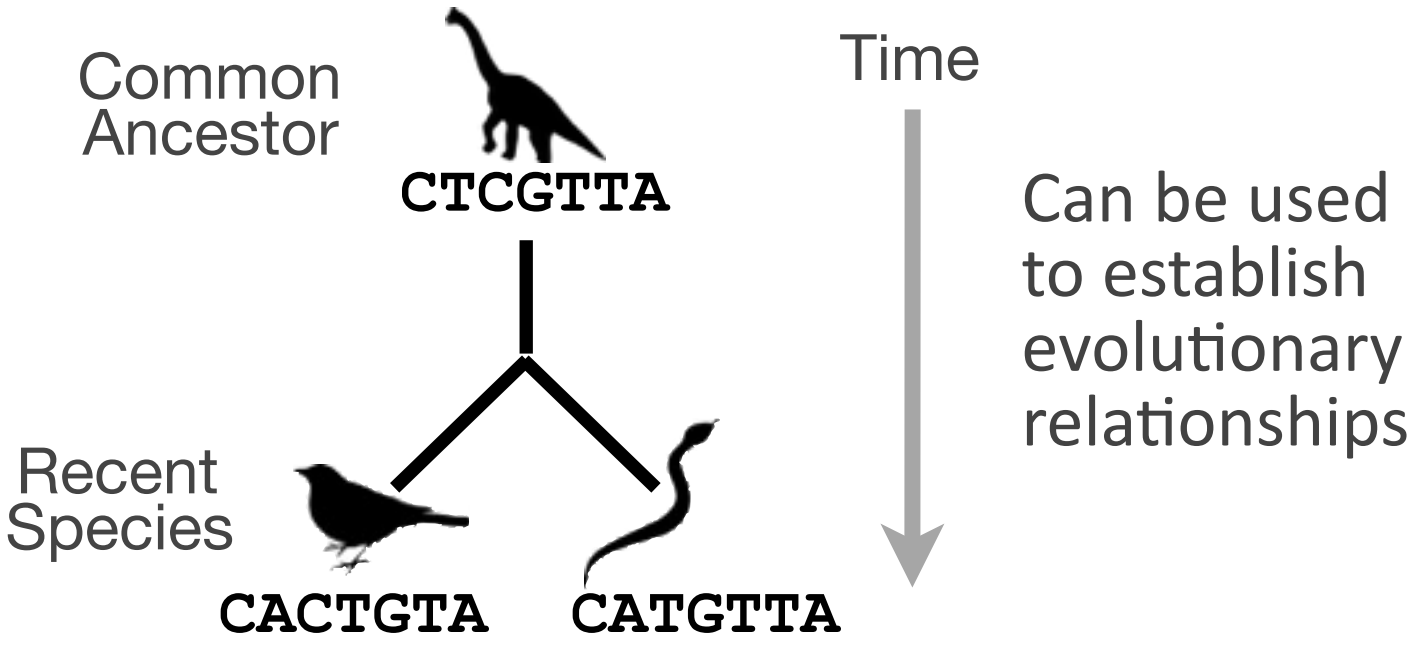
Sequence comparison is most informative when it detects **homologs**

Homologs are sequences that have common origins *i.e.* they share a **common ancestor**

- They may or may not have common activity



Key terms

When we talk about related sequences we use specific terminology.

Homologous sequences may be either:

– **Orthologs** or **Paralogs**

(Note. these are all or nothing relationships!)

Any pair of sequences may share a certain level of:

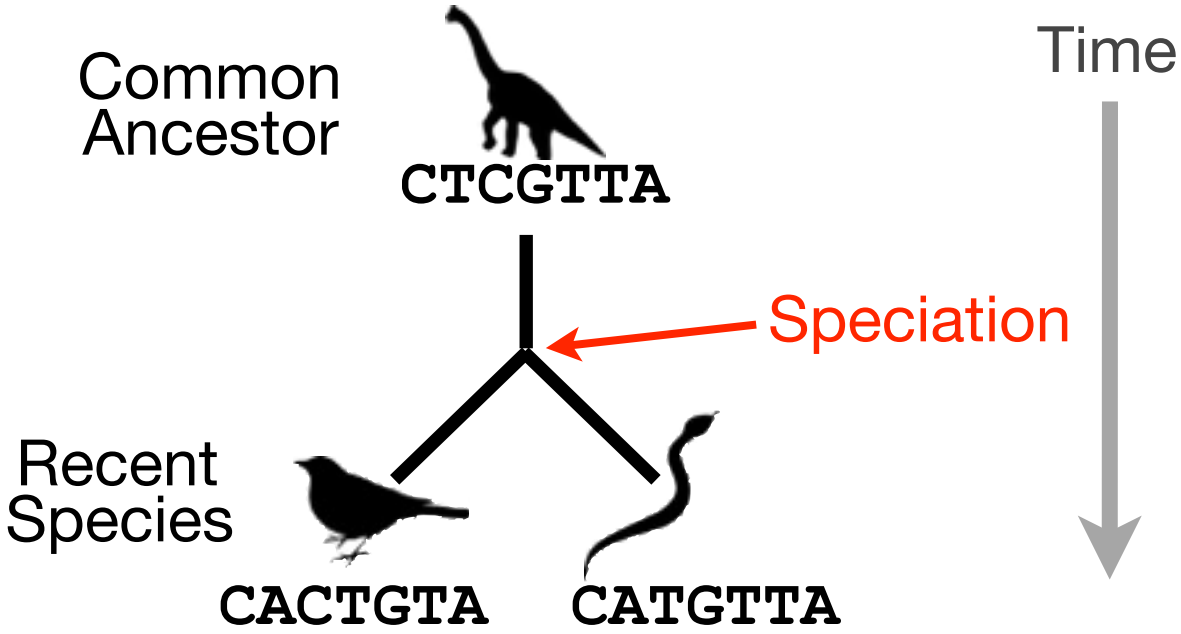
– **Identity** and/or **Similarity**

(Note. if these metrics are above a certain level we often infer homology)

Orthologs tend to have similar function

Orthologs: are homologs produced by speciation that have diverged due to divergence of the organisms they are associated with.

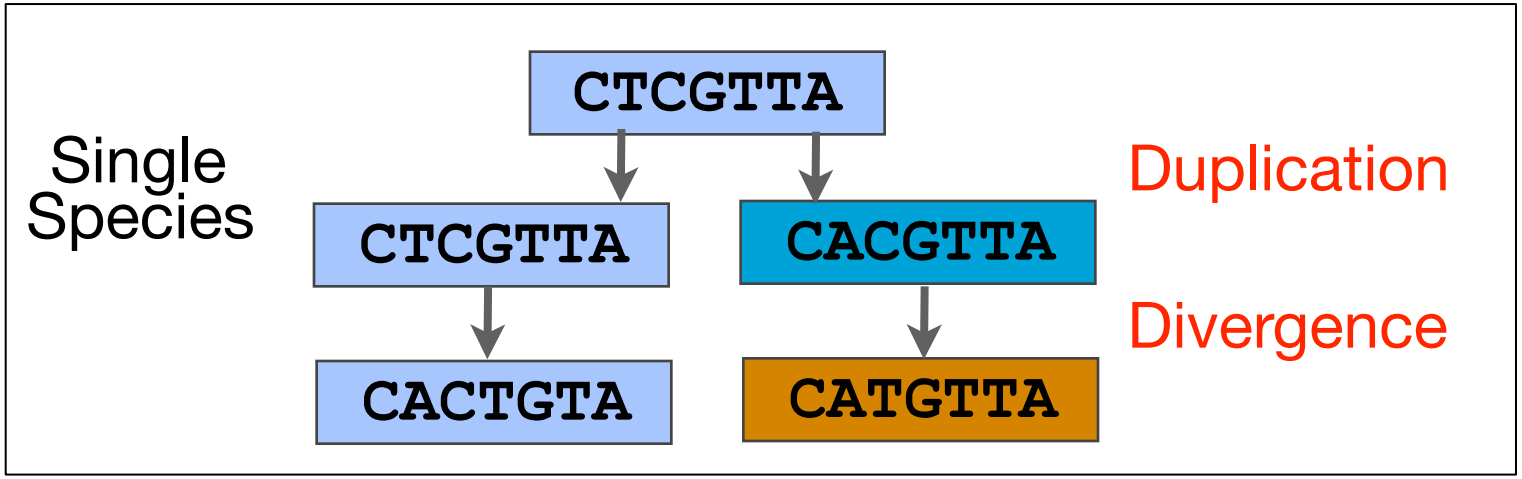
– Ortho = [greek: straight] ... implies direct descent



Paralogs tend to have slightly different functions

Paralogs: are homologs produced by **gene duplication**. They represent genes derived from a common ancestral gene that *duplicated within an organism* and then subsequently *diverged by accumulated mutation*.

– Para = [greek: along side of]



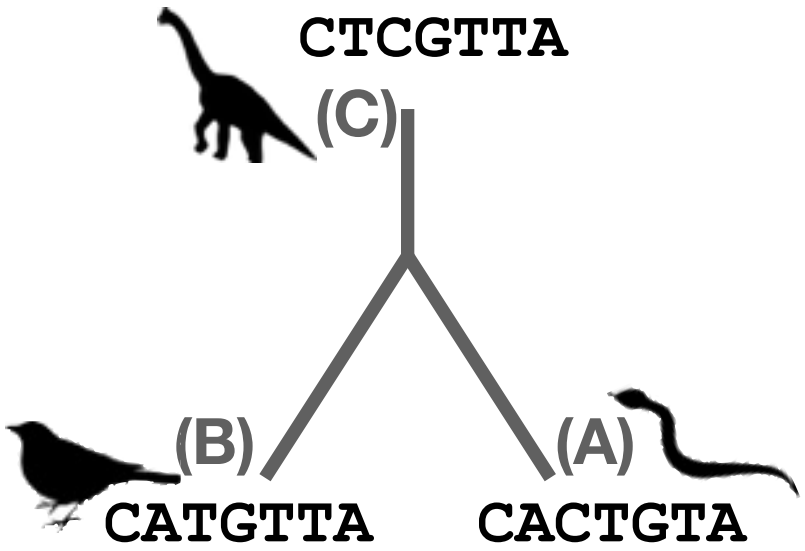
Orthologs *vs* Paralogs

- In practice, determining ortholog *vs* paralog can be a complex problem:
  - gene loss after duplication,
  - lack of knowledge of evolutionary history,
  - weak similarity because of evolutionary distance
- Homology does not necessarily imply exact same function
  - may have similar function at very crude level but play a different physiological role

Sequence changes during evolution

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

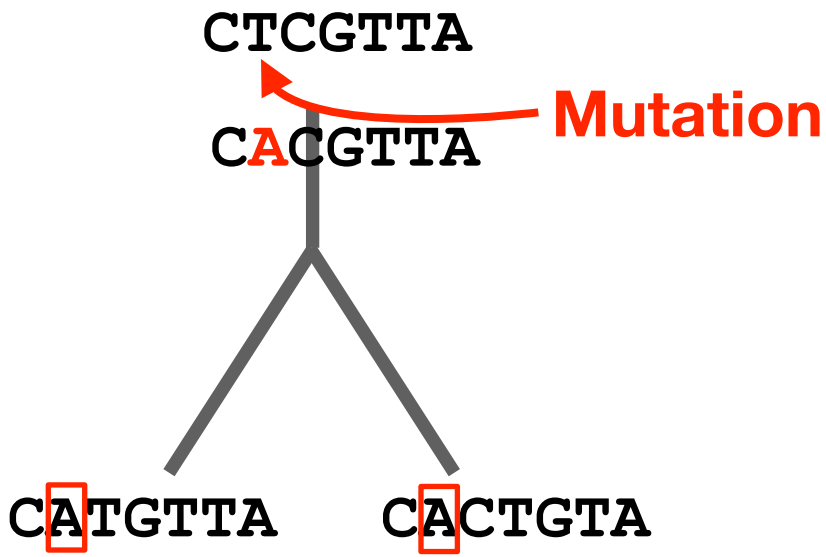
- Mutations/Substitutions
- Deletions
- Insertions



Mutations, deletions and insertions

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

- **Mutations/Substitutions** CTCGTTA → CACGTTA
- Deletions
- Insertions



Likely occurred prior to speciation

Mutations, deletions and insertions

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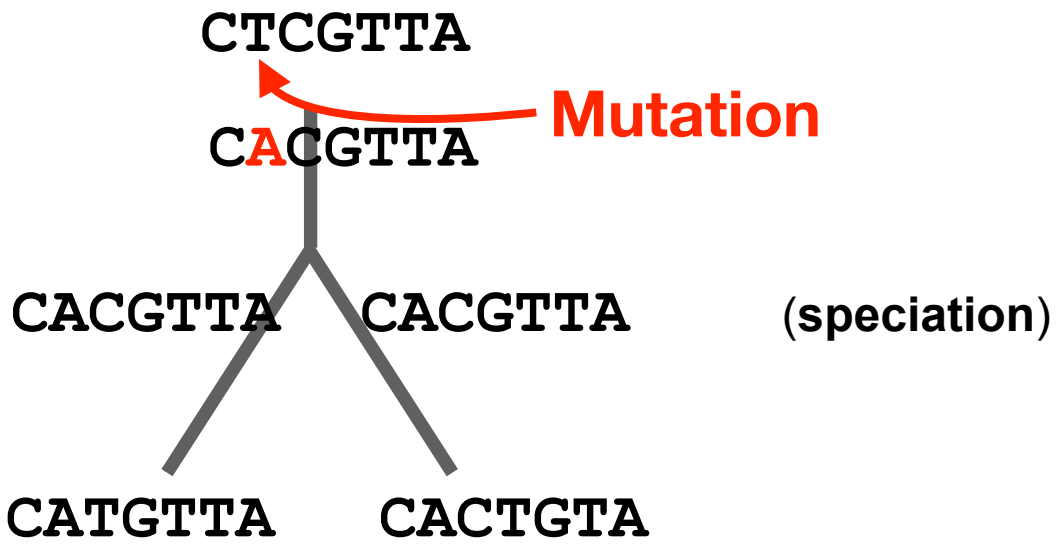
CTCGTTA → CACGTTA



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- **Deletions**
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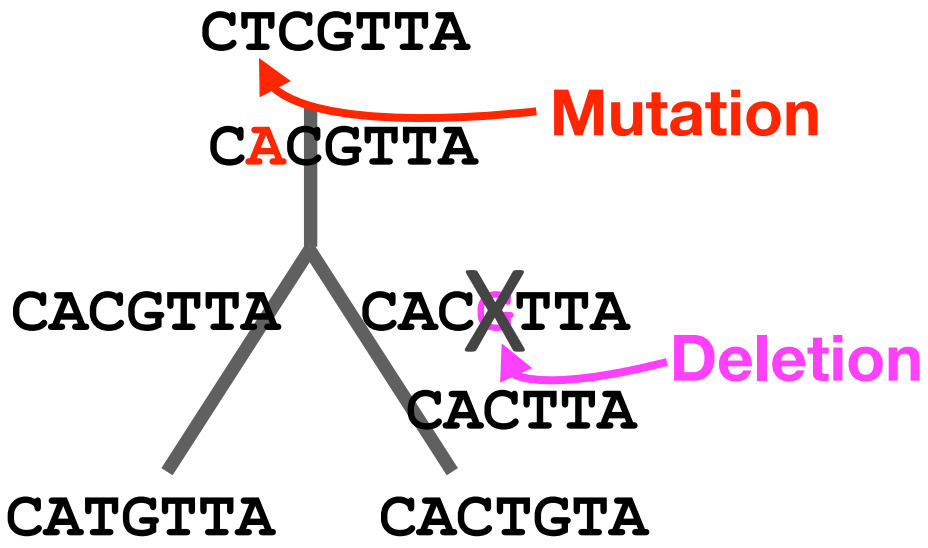
CTCGTTA → CACGTTA

CACGTTA → CACTTA



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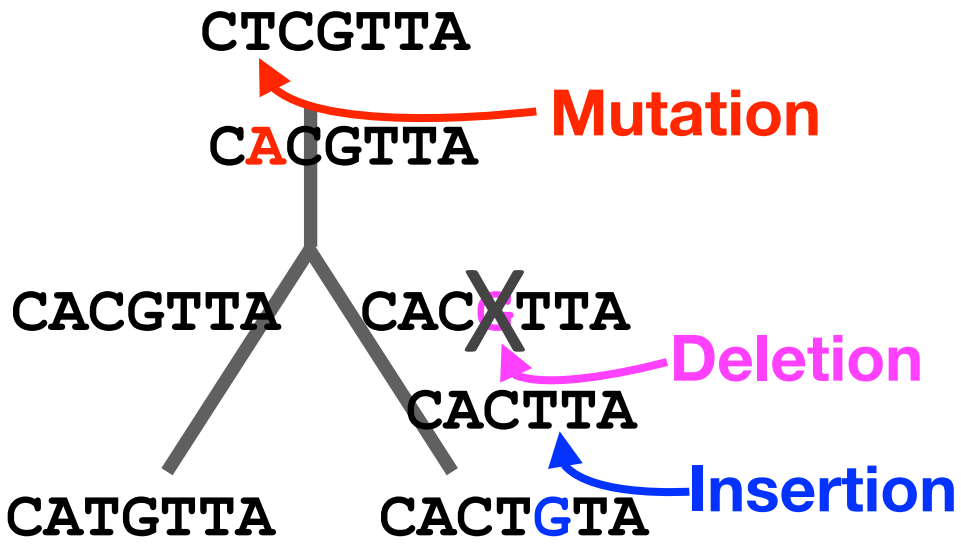
- **Insertions**

CTCGTTA → CACGTTA

CACGTTA → CACTTA

CACTTA → CACTGTA



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- **Mutations/Substitutions**
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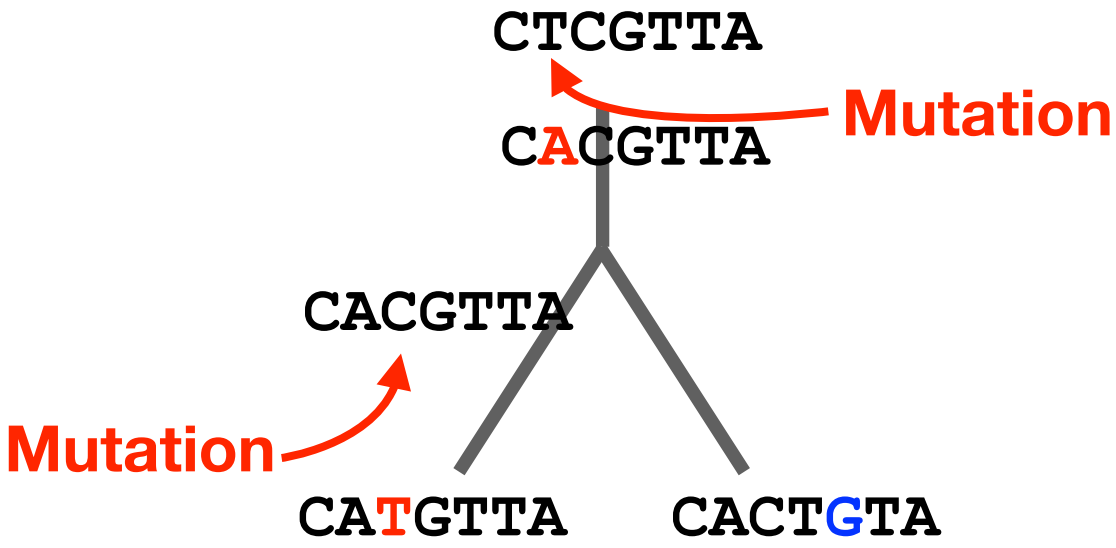
CTCGTTA → CACGTTA

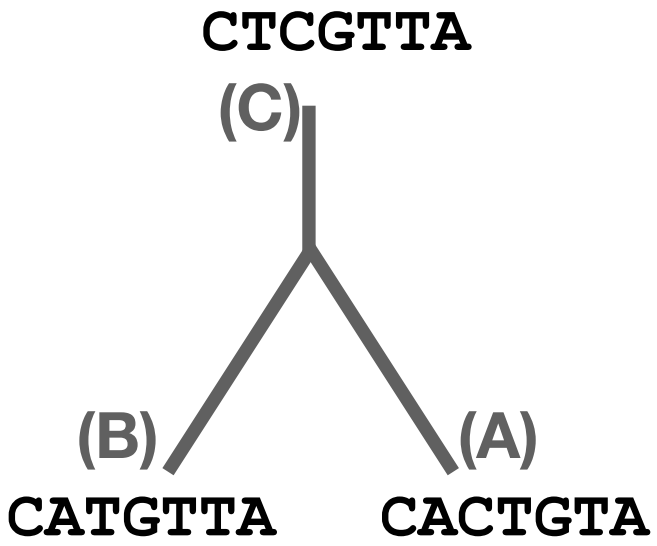
CACGTTA → CATGTTA

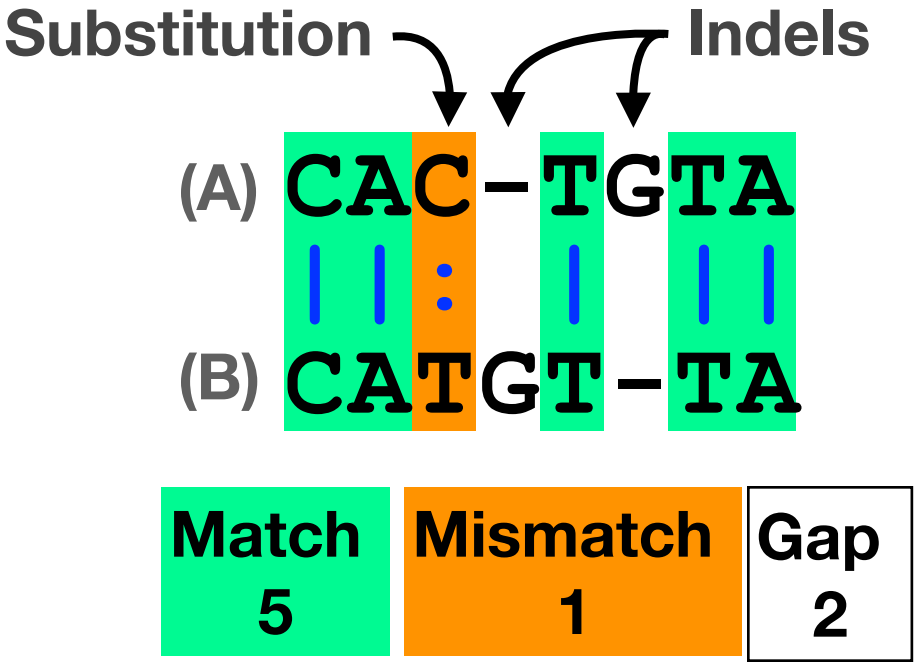


Alignment view

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

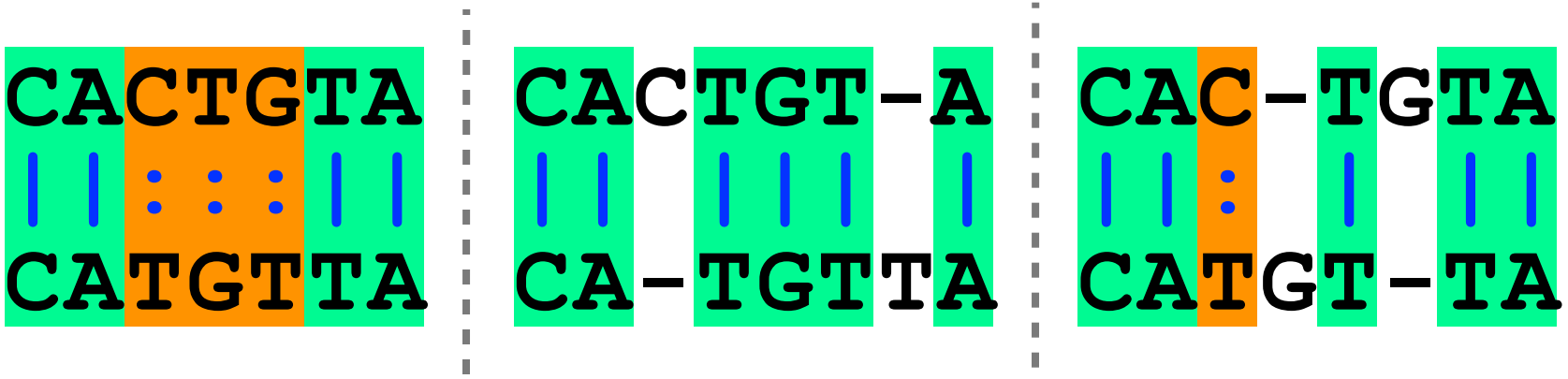
- **Mismatches** represent mutations/substitutions
- **Gaps** represent insertions and deletions (indels)





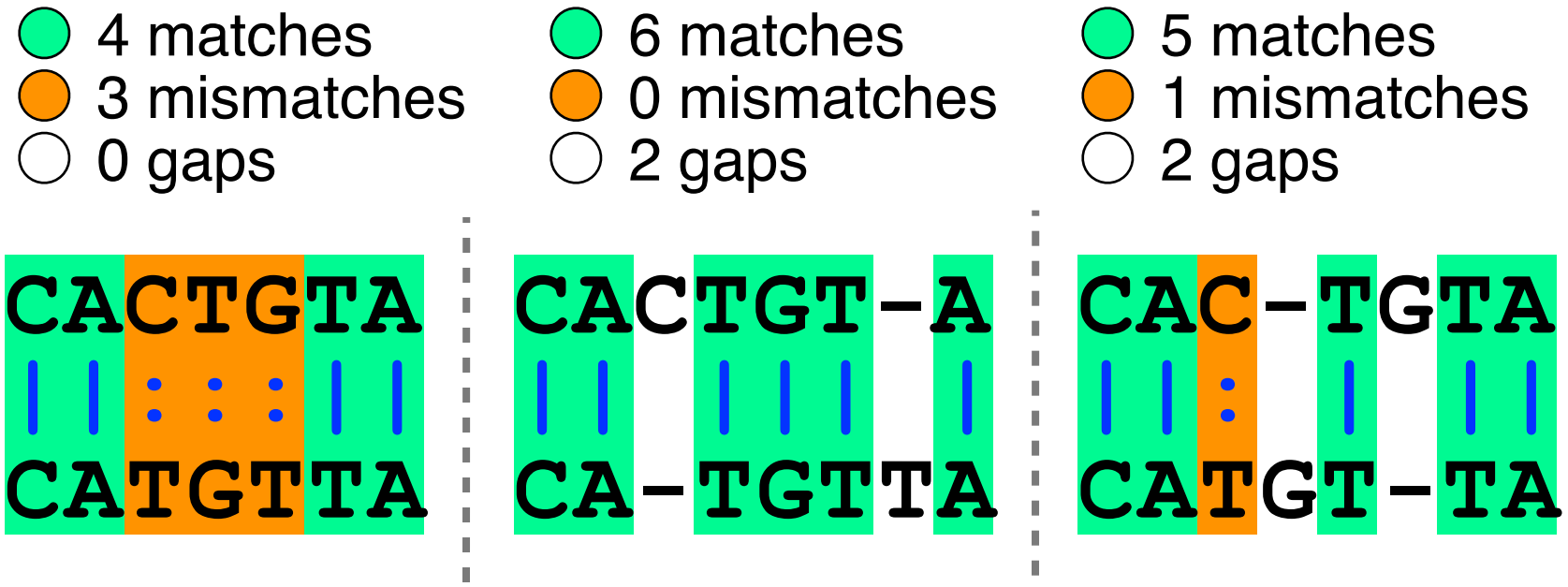
Alternative alignments

- Unfortunately, finding the correct alignment is difficult if we do not know the evolutionary history of the two sequences
  - There are many possible alignments
  - Which alignment is best?



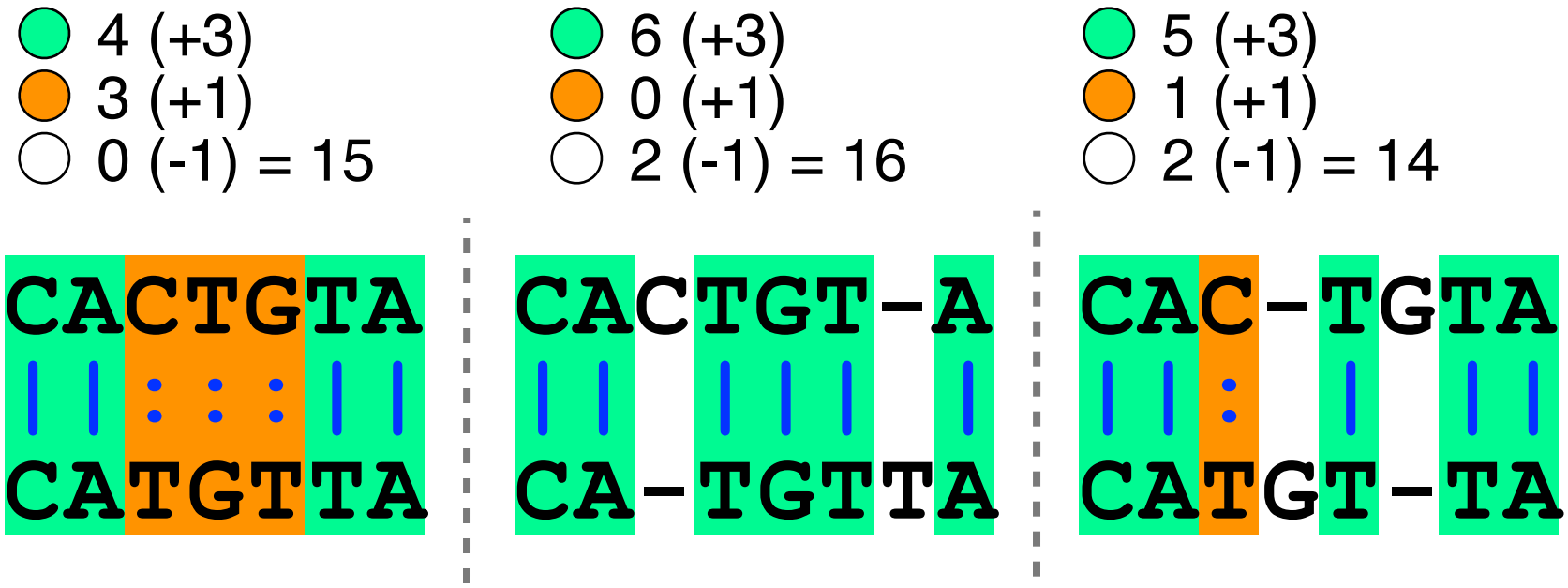
Alternative alignments

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



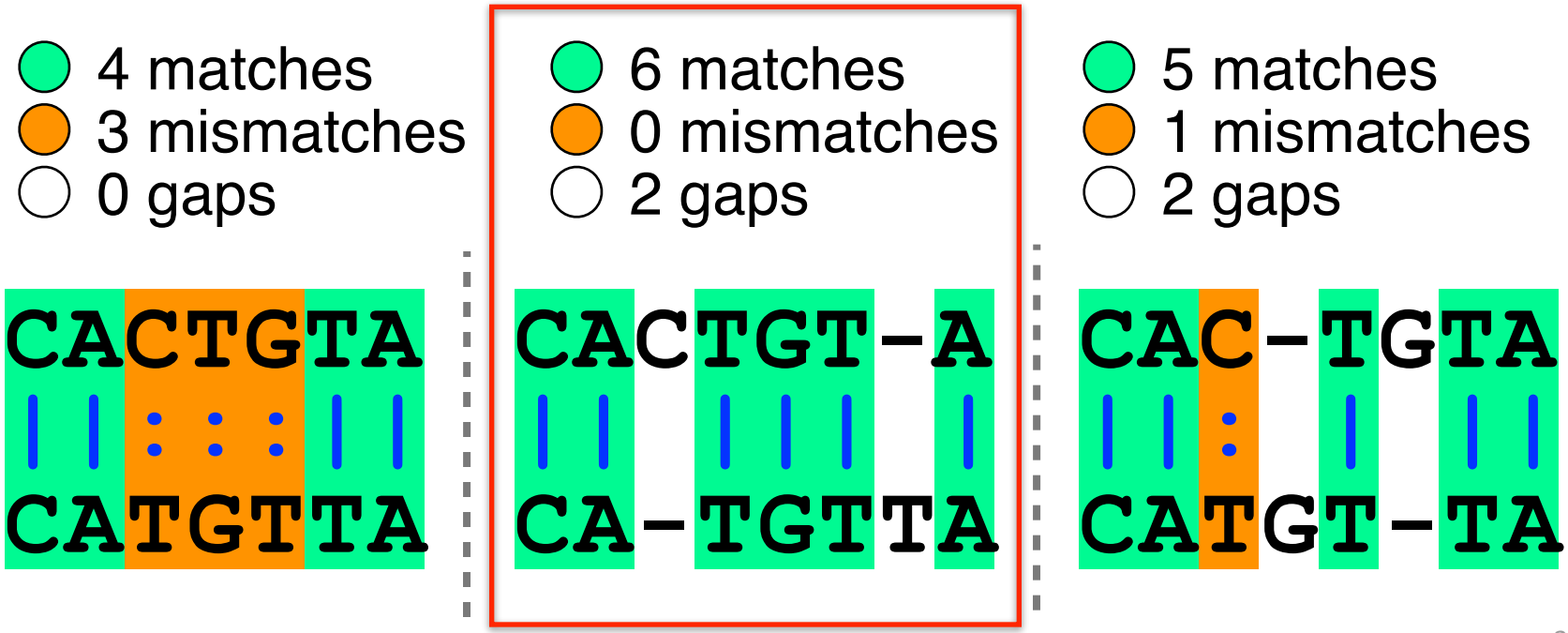
Scoring alignments

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



Optimal alignments

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



Optimal alignments

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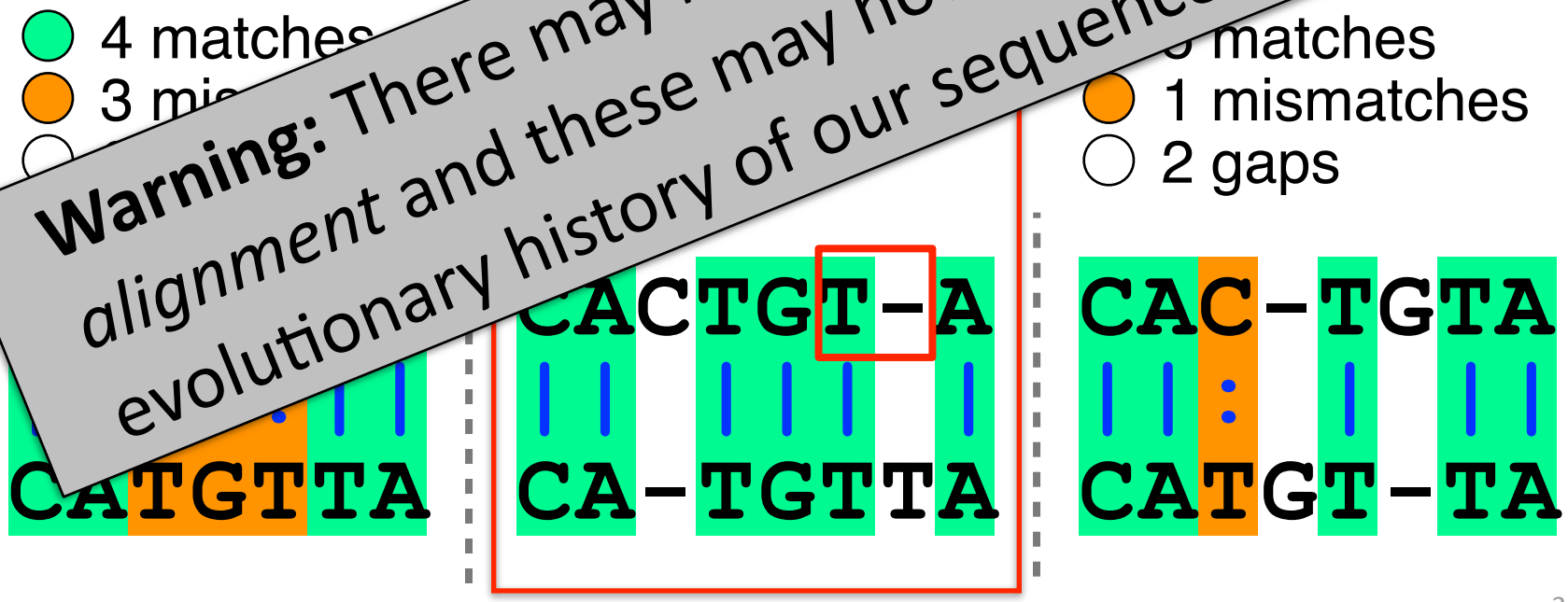
Warning: There may be more than one optimal alignment and these may not reflect the true evolutionary history of our sequences!



Side note: sequence *identity* and *similarity*

- Two commonly quoted metrics for pairs of aligned sequences.
  - **Sequence identity**: typically quotes the percent of identical characters in the aligned region of two sequences
  - **Sequence similarity**: typically the score resulting from optimal pair-wise alignment (note dependence on parameters used: *i.e.* scoring scheme)
- N.B. In contrast, **homology is an all or nothing relationship**, you can not have a percent homology!

Side note: sequence identity and similarity

- High sequence similarity is frequently used as an indicator of homology
  - Use to find genes and/or proteins with potentially similar or identical function
  - Can query a database of sequences by performing a series of pair-wise alignments
- Knowledge of the difference between sequences can also yield valuable functional and mechanistic insights
  - A gene from a normal and an affected subject – possible cause of a heritable disease
  - Similar proteins with different substrate specificities – what amino acid changes might be responsible for this?

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  - PSI-BL

Quiz questions:

http://tinyurl.com/bioinf525-quiz2

Pair-wise Sequence Alignment

- **Objective**: arrange two sequences in such a fashion that pairs of matching characters between the two sequences are maximized
  - Match does not have to be identity, can be defined by a function that ranks or scores the characters being compared (often termed a **substitution matrix**)
  - Ungapped alignment example – bars indicate matching characters

```
Seq1:  GTAATCTG-
         |||  |||
Seq2:  -TAAGCTGA
```

Simplest case – brute force alignments

- In the simplest case we can simply slide one sequence across the other and count matching characters for each possible alignment
  - Chose a scoring scheme and do not allow internal gaps within sequences
  - Algorithmic complexity is linear

    N + M alignments to consider

    (where N and M are the length of each sequence)

Brute Force Alignment, No Gaps

Slide from Jeffery de Wet

Gaps make the brute force method unusable for all but the shortest sequences

- Pairs of related sequences often have insertions or deletions relative to one-another, we therefore require **gapped pair-wise alignment**
  - Need to generate all the possible gap lengths and combinations of gaps at all possible positions in both sequences
  - For two sequences of equal length, the formula is:

$$\binom{2N}{N} = \frac{(2N)!}{(N!)^2} \cong \frac{2^{2N}}{\sqrt{\pi N}}$$

N = 10: 184756
N = 50: ~1.00E29
N = 250: ~1.17E149

Slide from Jeffery de Wet

Three general solutions to the alignment problem

- The **dot plot** or **dot matrix** approach
  - A simple graphical method for pair-wise alignment
  - No scoring, so difficult to compare alternative alignments
  - Can give visual clues to sequence structure but requires human interaction
- **Dynamic programming** algorithms
  - Provides Optimal solutions (but not necessarily unique solutions)
- Heuristic **word** or **k-tuple** approaches
  - Much faster (e.g. **BLAST** and **FASTA**)
  - Widely used for database searches
  - May miss some pairs with low similarity

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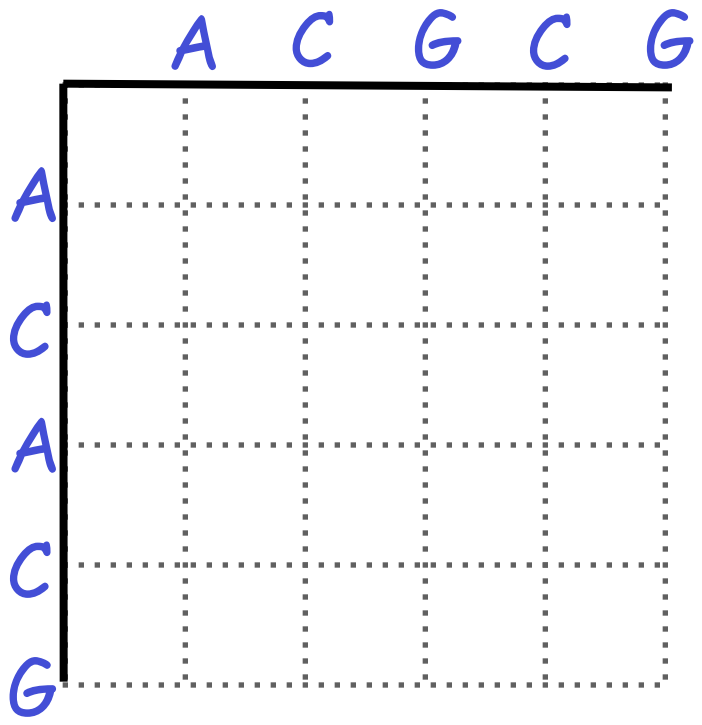
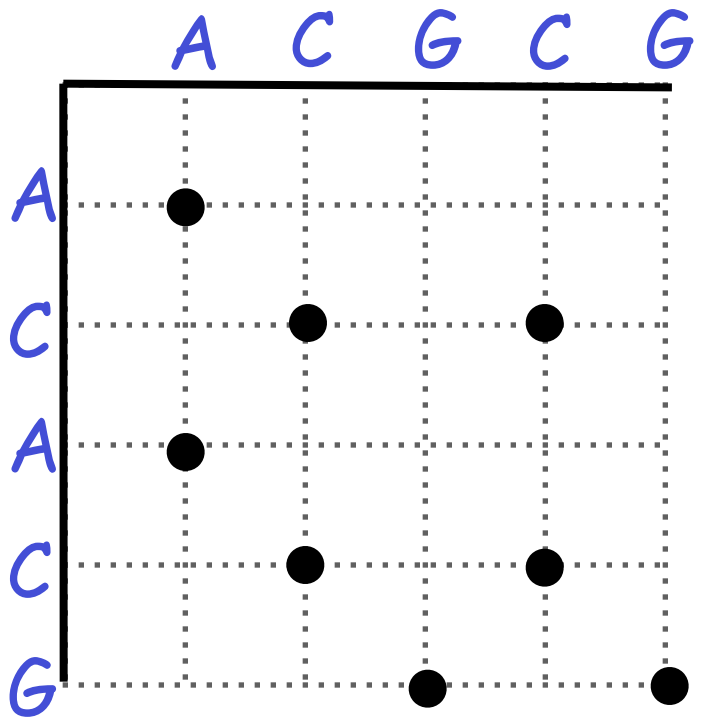
Dot plots: simple graphical approach

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



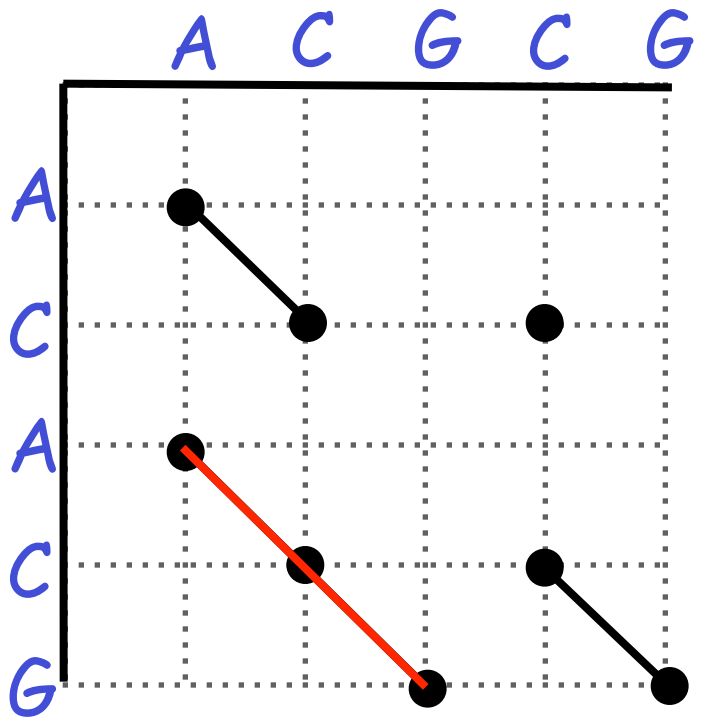
Dot plots: simple graphical approach

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



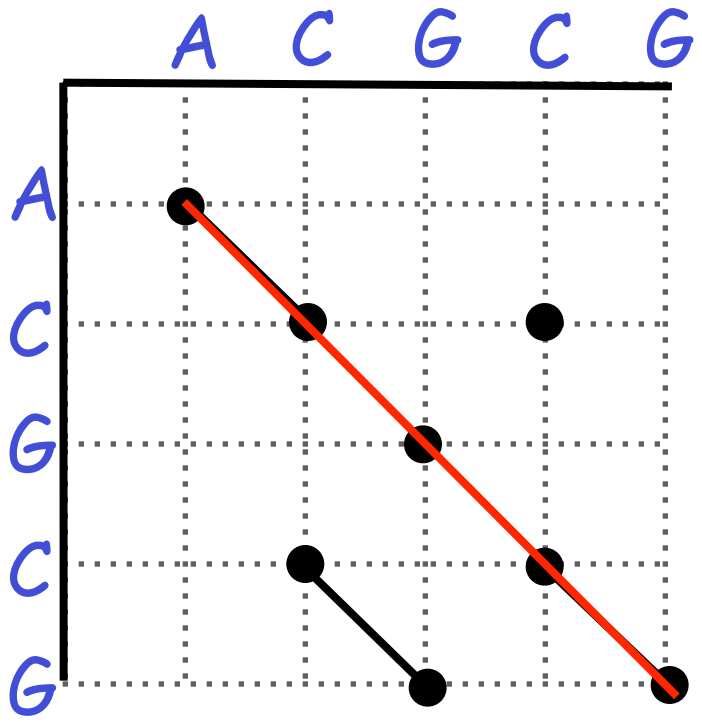
Dot plots: simple graphical approach

- Diagonal runs of dots indicate matched segments of sequence



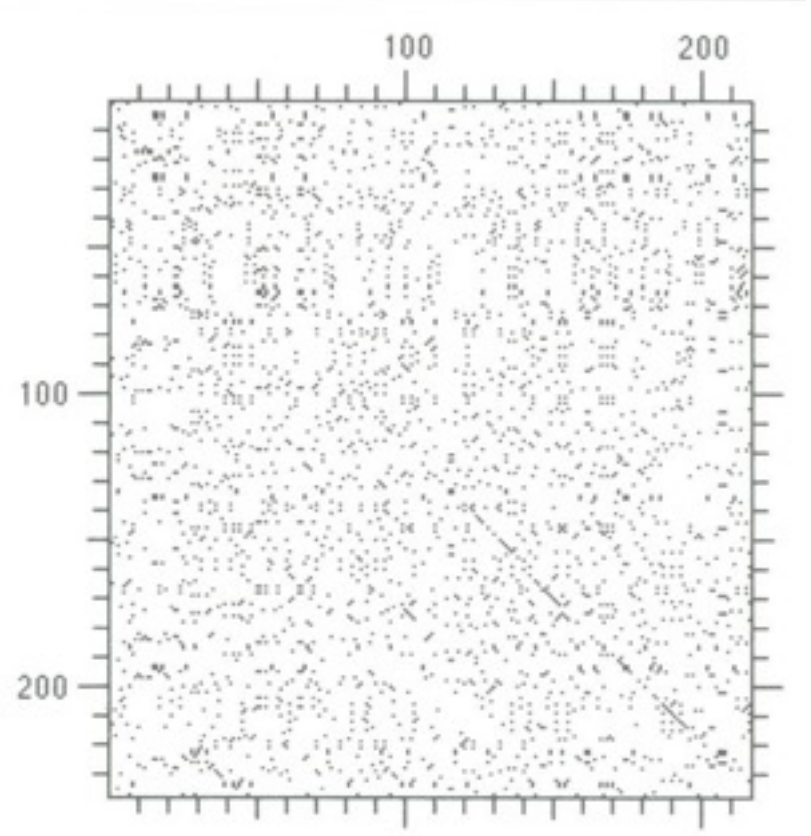
Dot plots: simple graphical approach

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



Dot plots: simple graphical approach

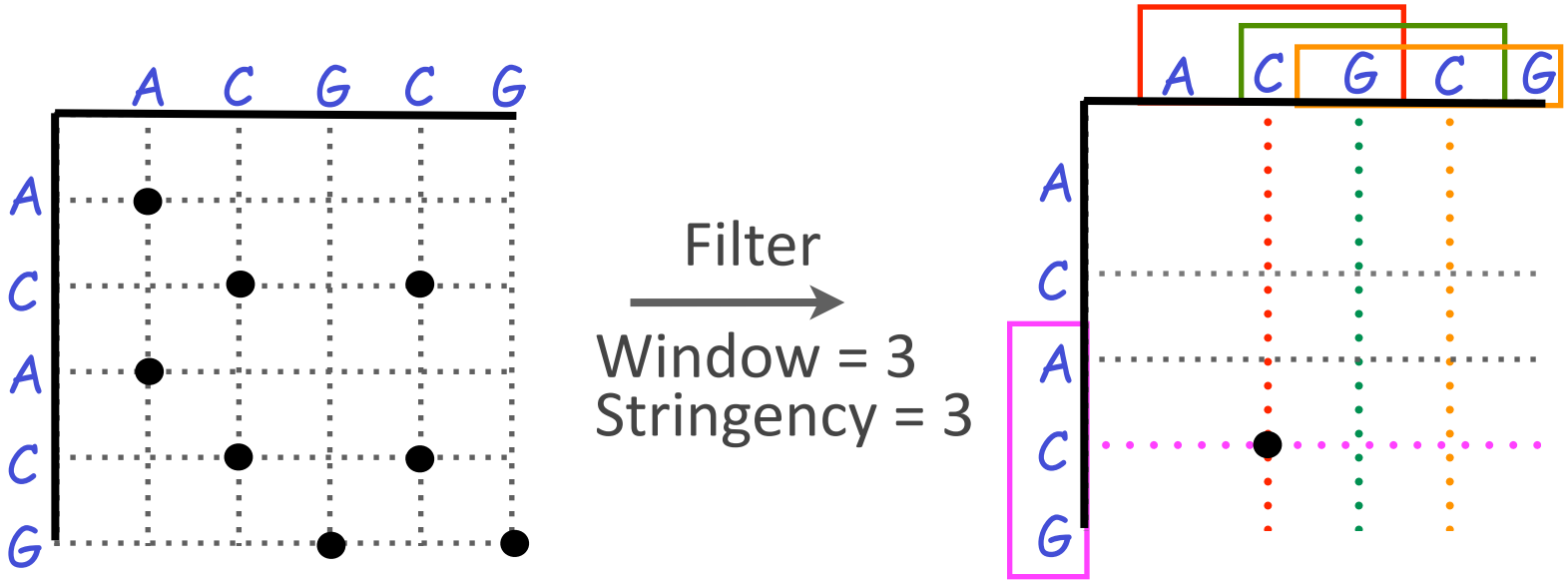
- Dot matrices for long sequences can be noisy



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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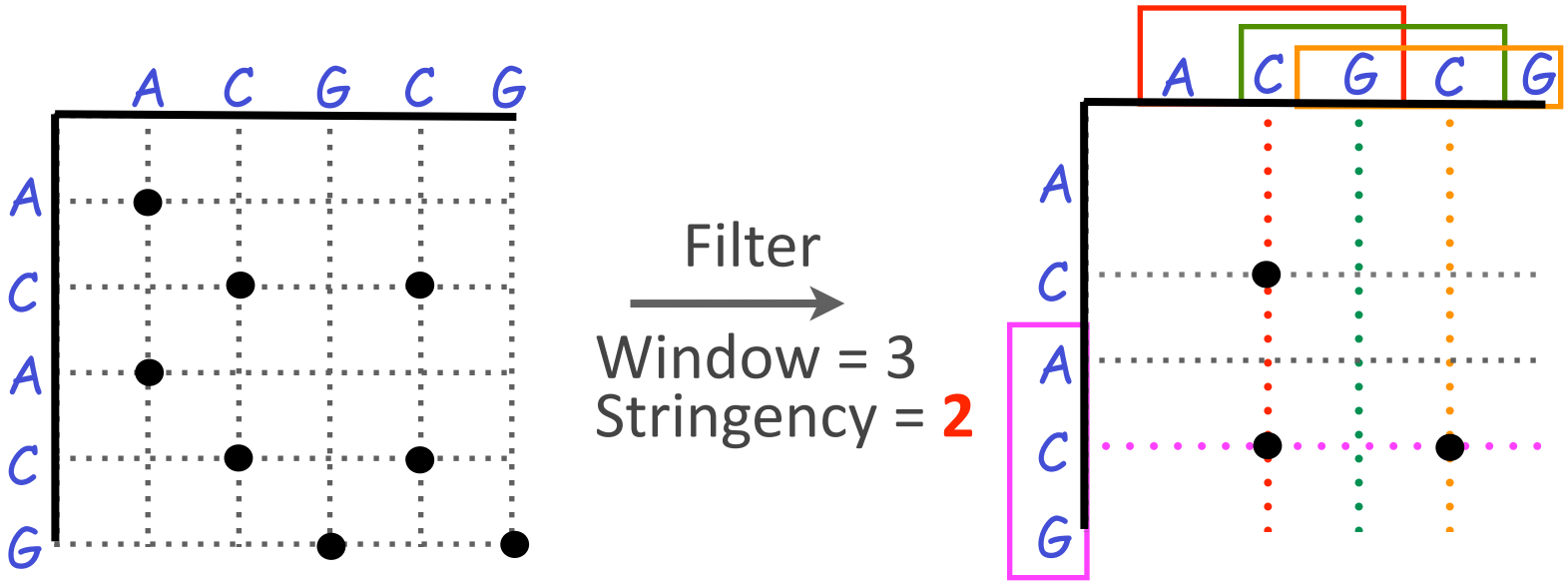
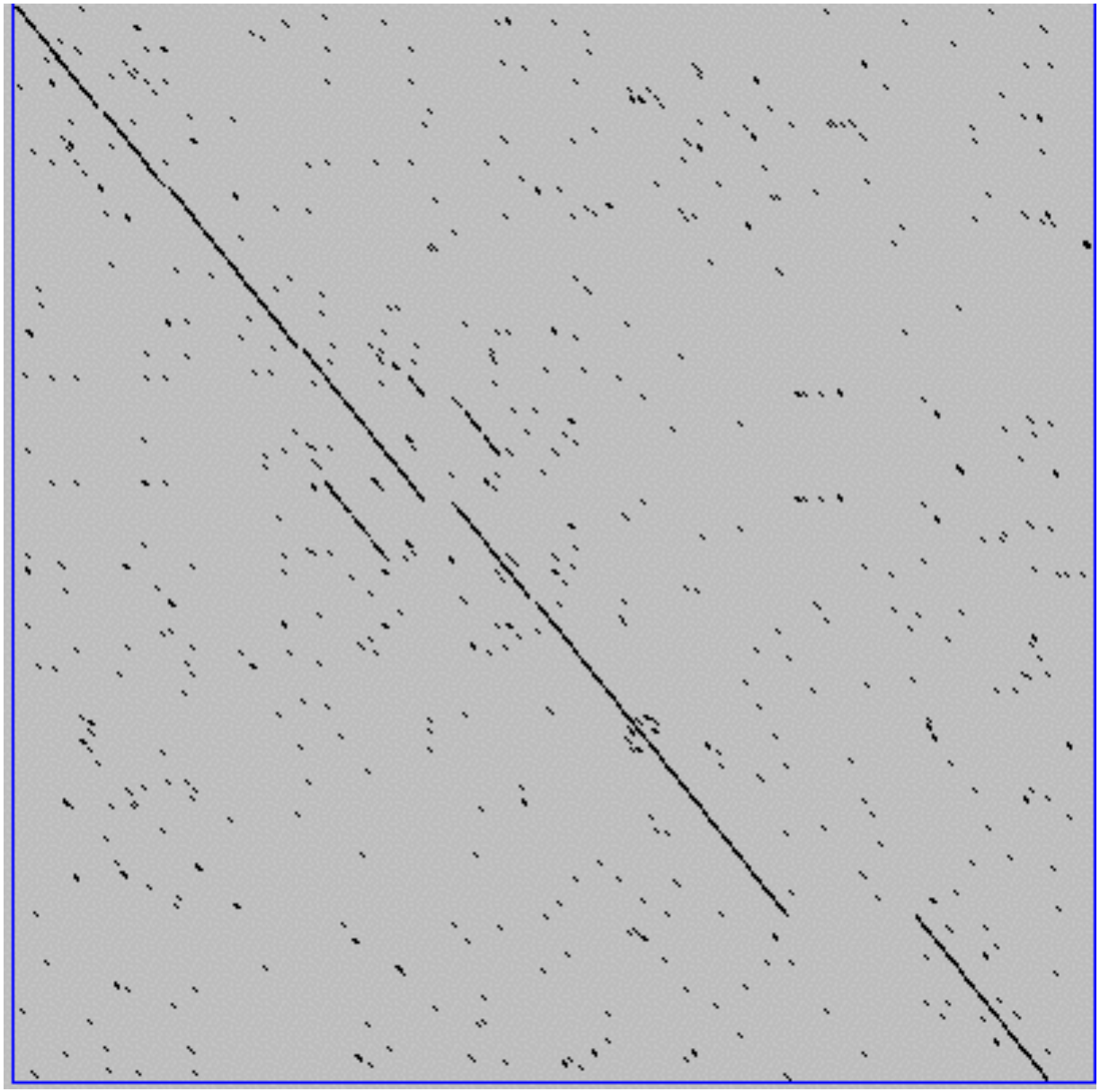


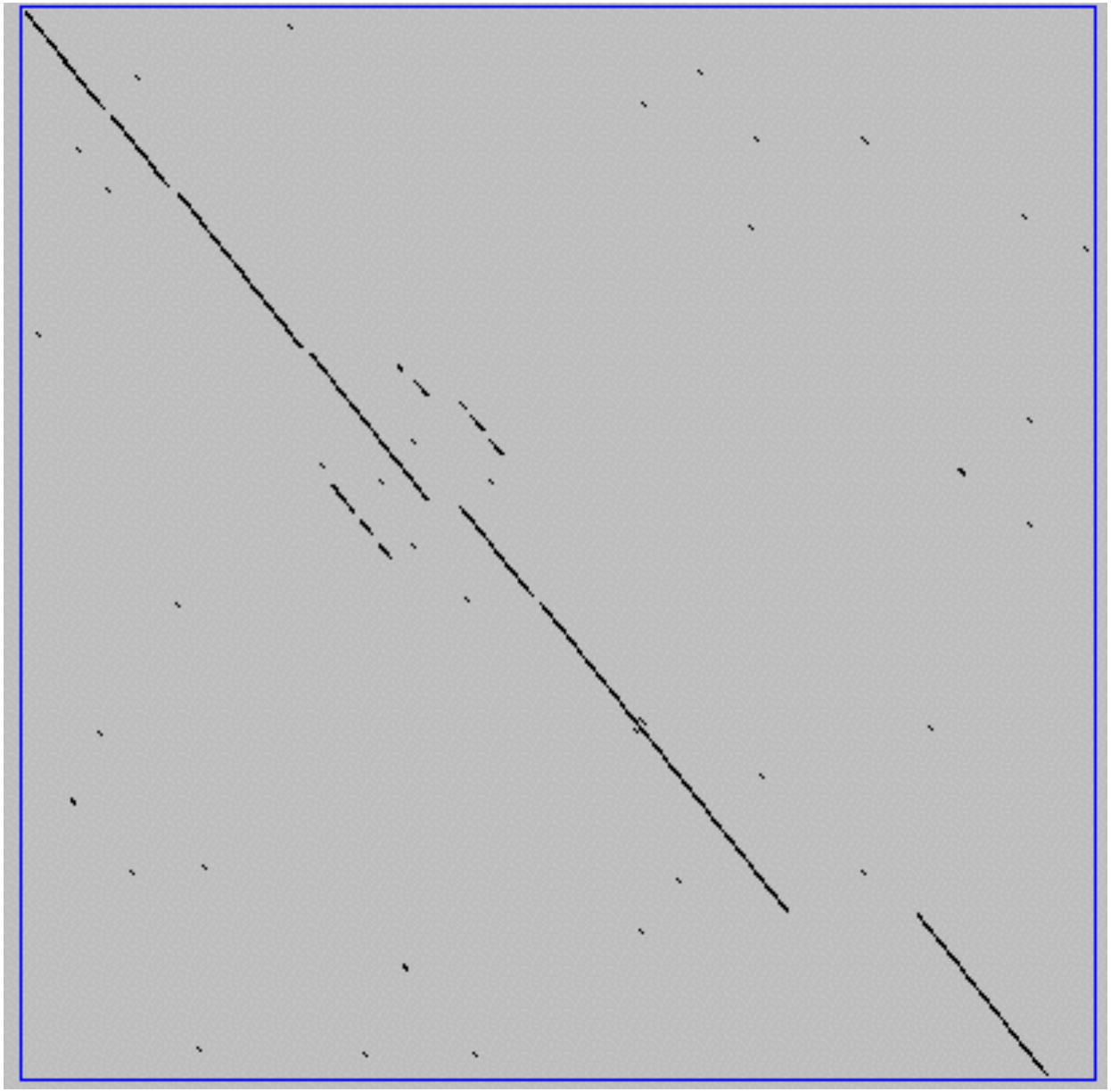
Window size = 5 bases



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



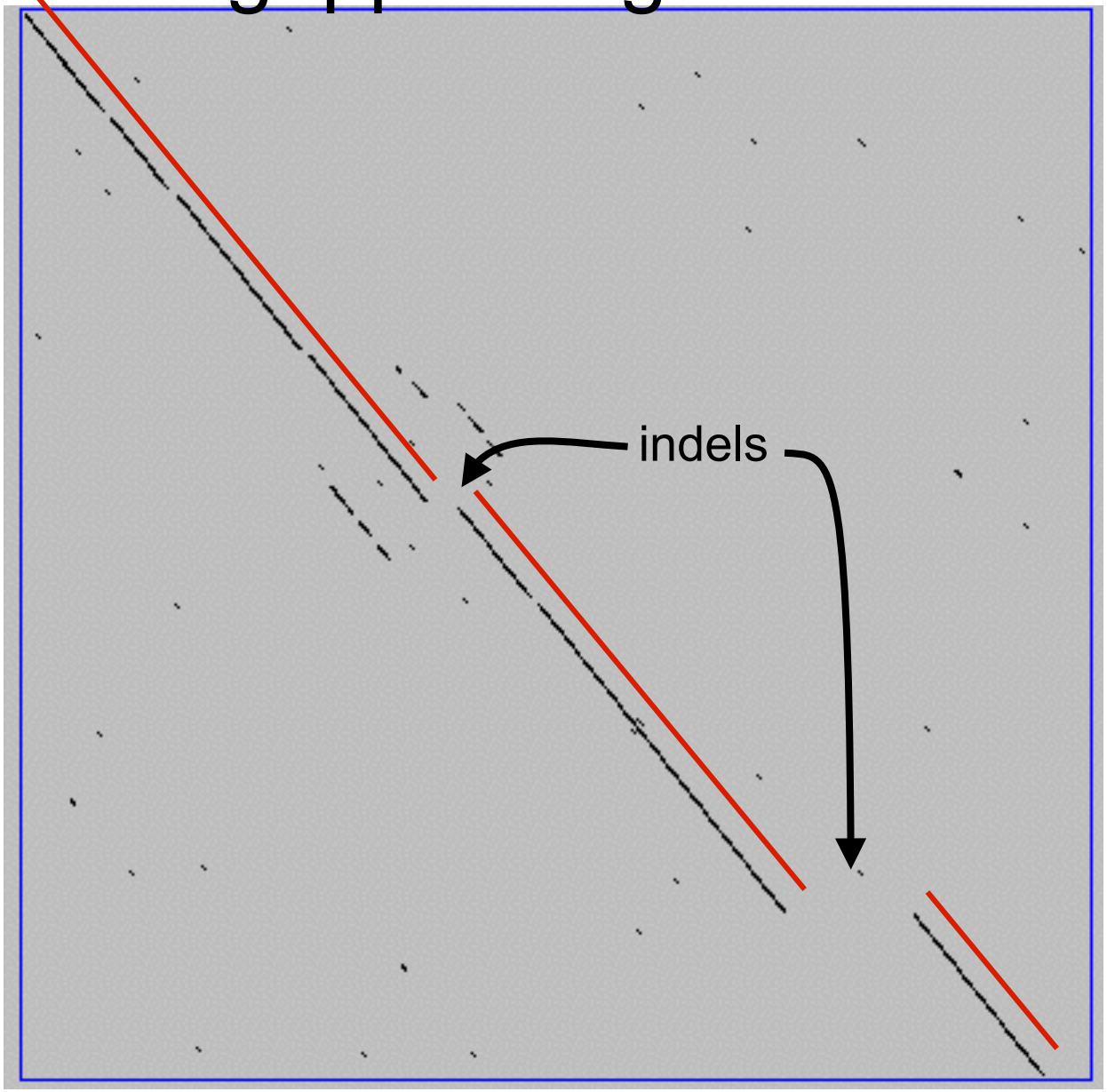
This is a dot plot of the same sequence pair. Now 7 bases in a row must match for a dot to be place. Noise is reduced.

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

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

Ungapped alignments

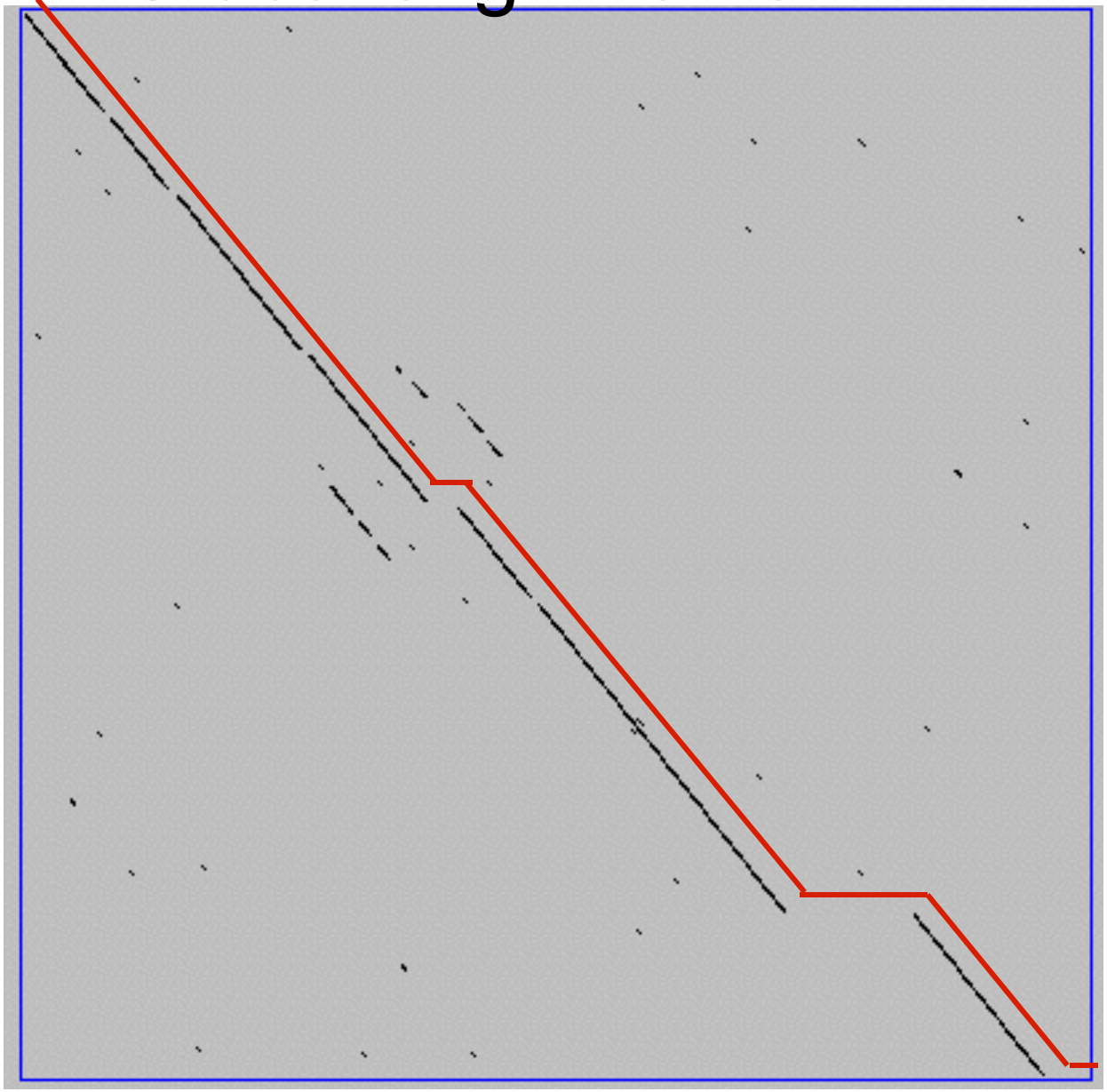


Only **diagonals** can be followed.

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/

Global alignments



Global alignments go from end to end, *i.e.* from the upper left corner to the lower right corner.

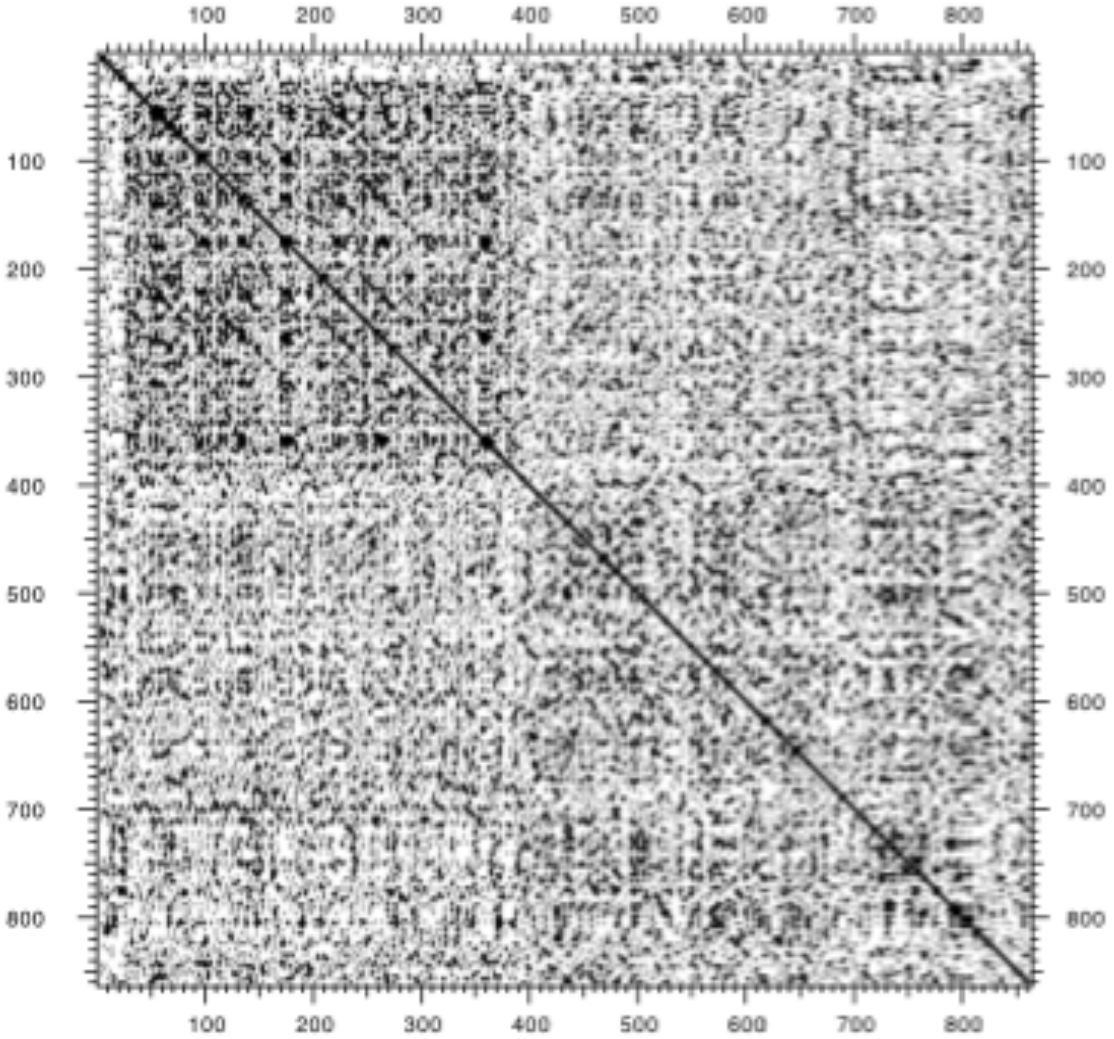
Global alignments do not have good statistical characterization and are **not used for database searches.**

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



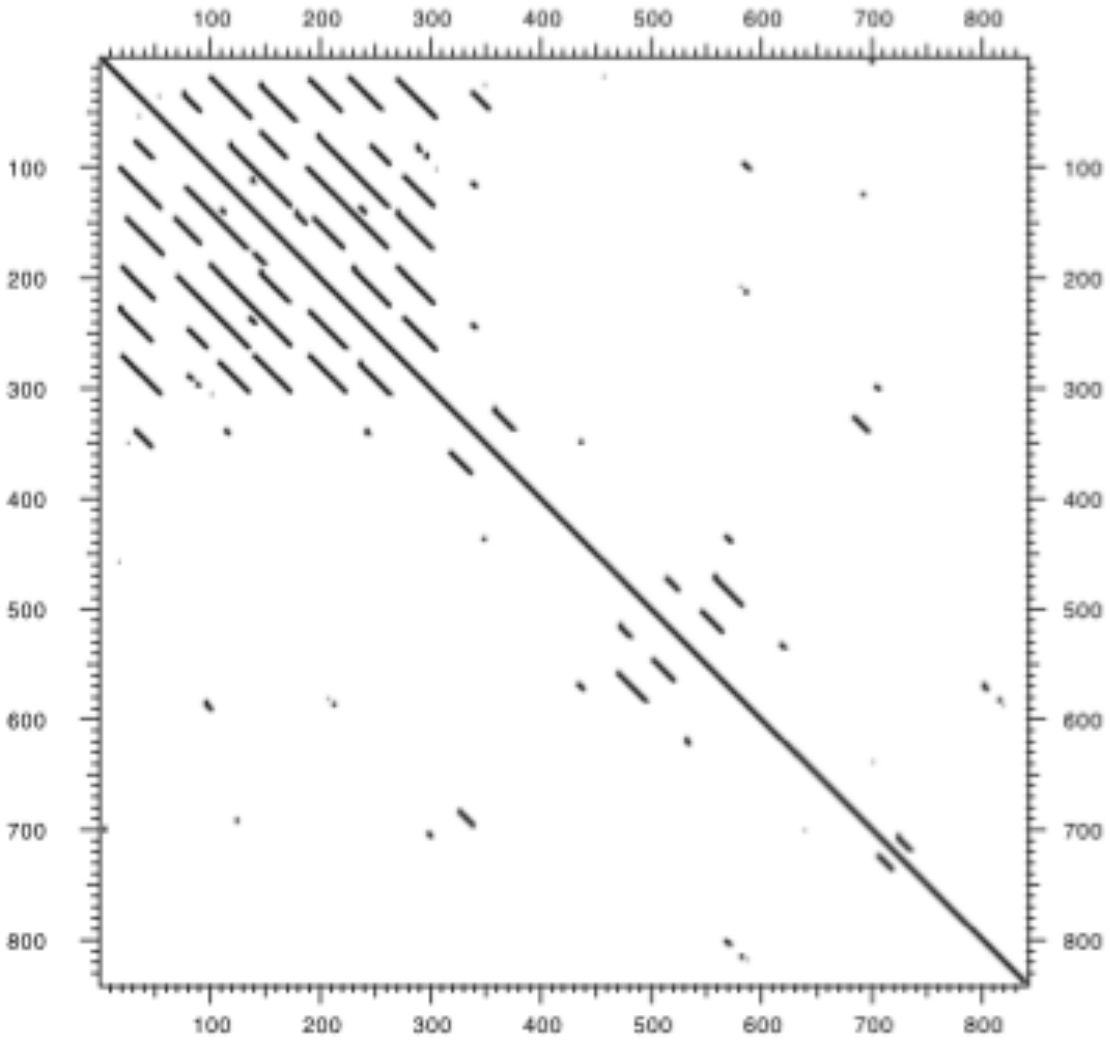
Human LDL receptor protein sequence (Genbank P01130)

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”)

Side note: dots can have "weights"

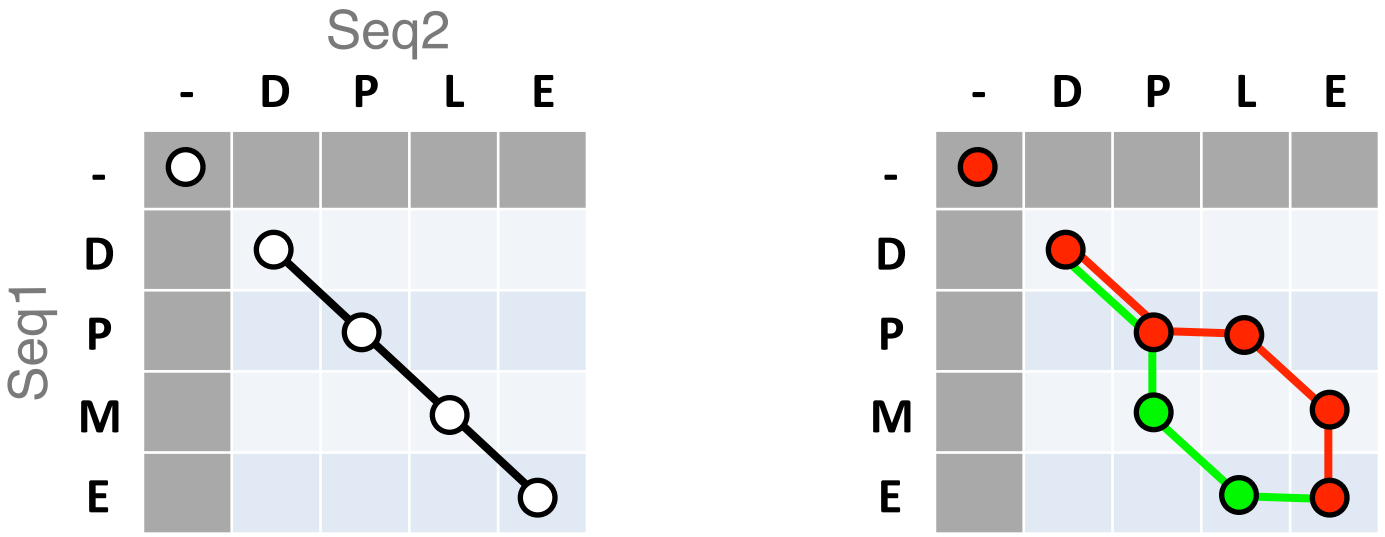
- Some matches can be rewarded more than others, depending on likelihood
- Use PAM or BLOSUM **substitution matrix**
  - (more on these later)
- Put a dot only if a minimum total or average weight is achieved
  - See chapter 3 in *Mount, "Bioinformatics sequence and genome analysis"*.

Three general solutions to the alignment problem

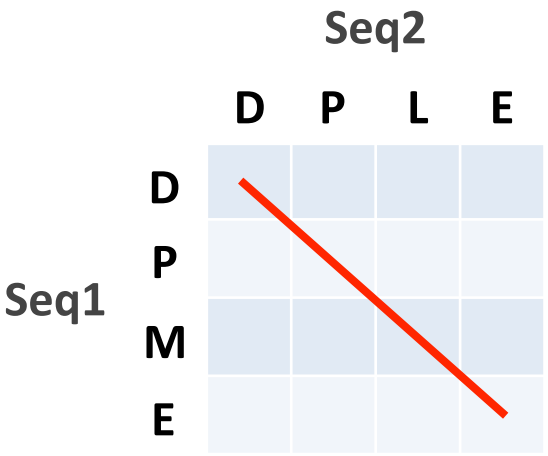
- The **dot plot** or **dot matrix** approach
  - A simple graphical method for pair-wise alignment
  - No scoring, so difficult to compare alternative alignments
  - Can give visual clues to sequence structure but requires human interaction
- **Dynamic programming** algorithms
  - Provides Optimal solutions (but not necessarily unique solutions)
- Heuristic **word** or **k-tuple** approaches
  - Much faster (e.g. **BLAST** and **FASTA**)
  - Widely used for database searches
  - May miss some pairs with low similarity

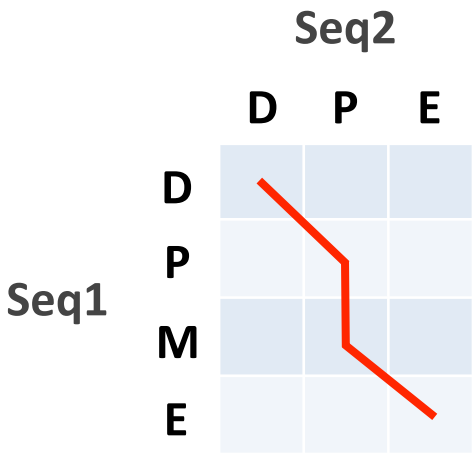
The Dynamic Programming Algorithm

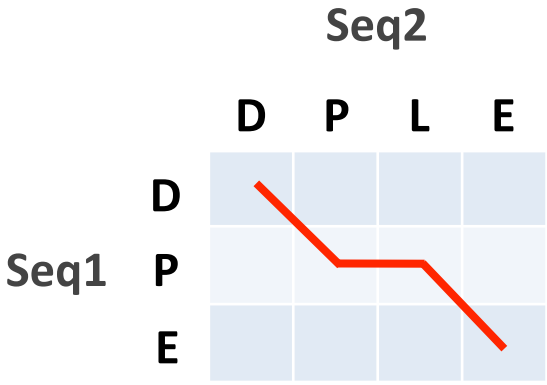
- The dynamic programming algorithm can be thought of an extension to the dot plot approach
  - One sequence is placed down the side of a grid and another across the top
  - Instead of placing a dot in the grid, we **compute a score** for each position
  - Finding the optimal alignment corresponds to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



Different paths represent different alignments








```
Seq1: D P L E
      | | : |
Seq2: D P M E
```

```
Seq1: D P M E
      | |   |
Seq2: D P - E
```

```
Seq1: D P - E
      | |   |
Seq2: D P L E
```

Matches are represented by diagonal paths and indels with horizontal or vertical path segments

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

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

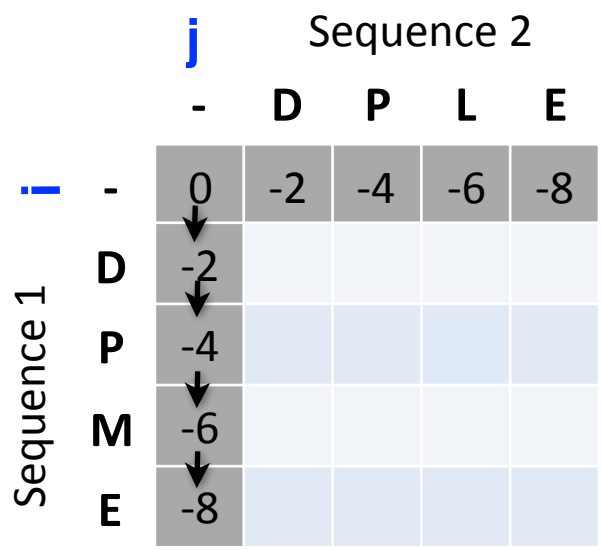
i	j	-	D	P	L	E
	-	0	-2	-4	-6	-8
Sequence 1	D	-2				
	P	-4				
	M	-6				
	E	-8				

Sequence 2

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

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

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

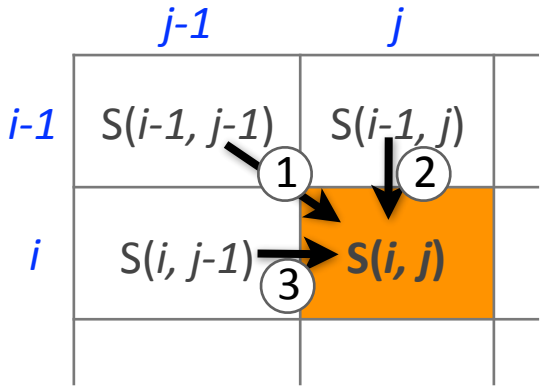
```
Seq1: DPME
Seq2: ----
```

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



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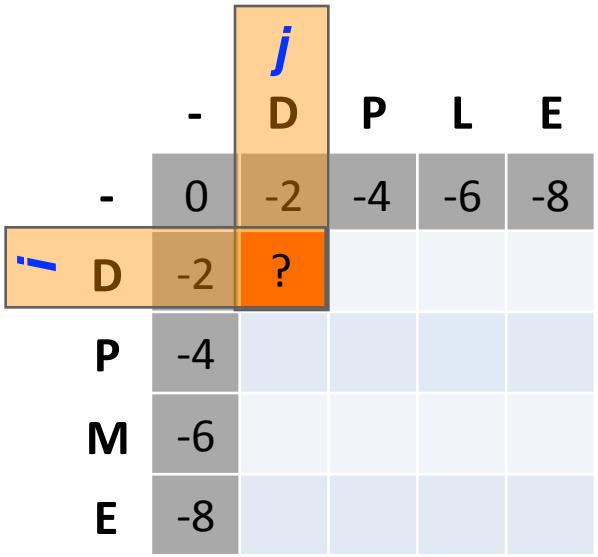
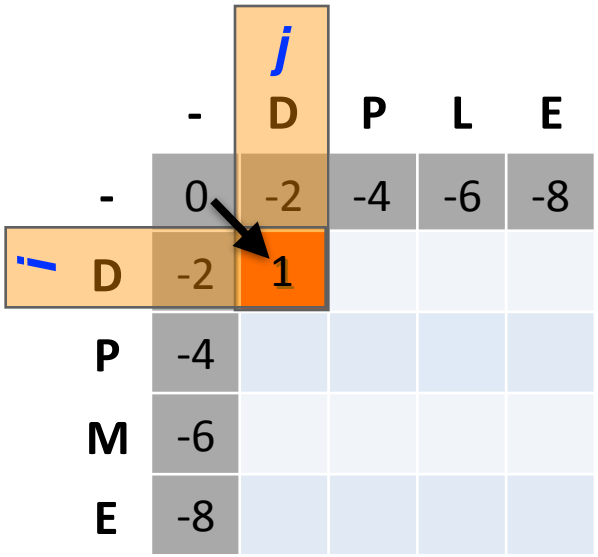


Scores: match = +1, mismatch = -1, gap = -2

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

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



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

① (0)+(+1) = +1 <= (D-D) match!

② (-2)+(-2) = -4

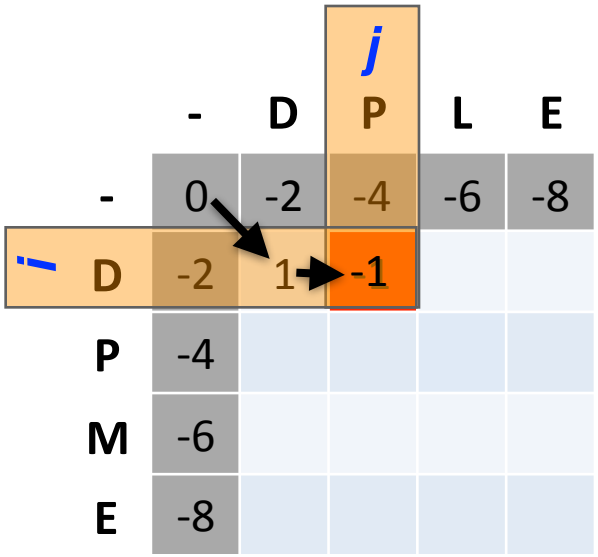
③ (-2)+(-2) = -4

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)



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

① (-2)+(-1) = -3 <= (D-P) mismatch!

② (-4)+(-2) = -6

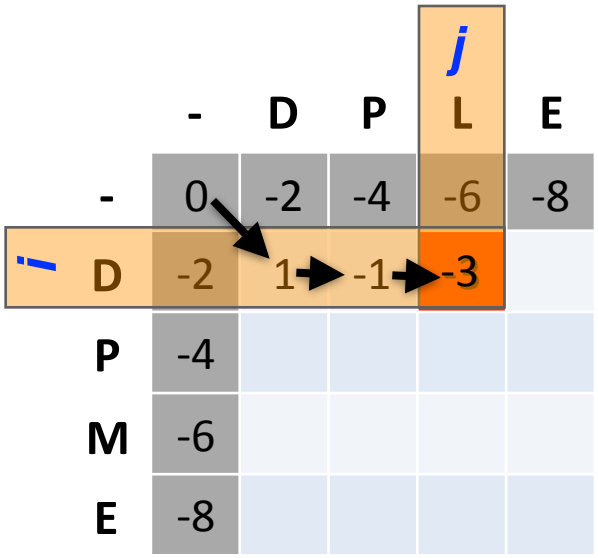
③ (1)+(-2) = -1

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.



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

① (-4)+(-1) = -5 <= (D-L) mismatch

② (-6)+(-2) = -8

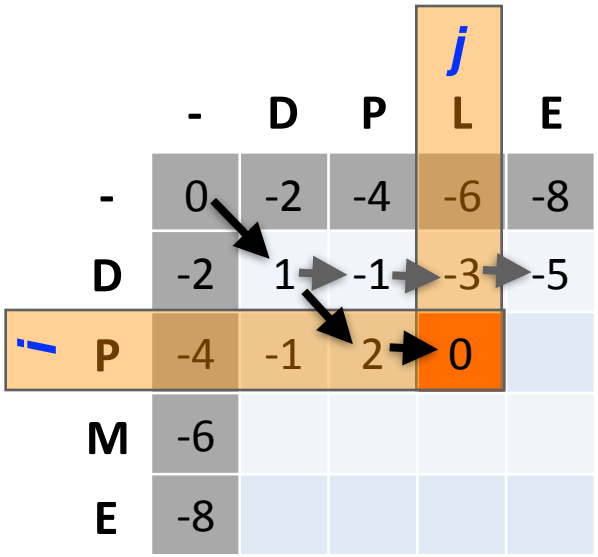
③ (-1)+(-2) = -3

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.



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

① (-1)+(-1) = -2

② (-3)+(-2) = -5

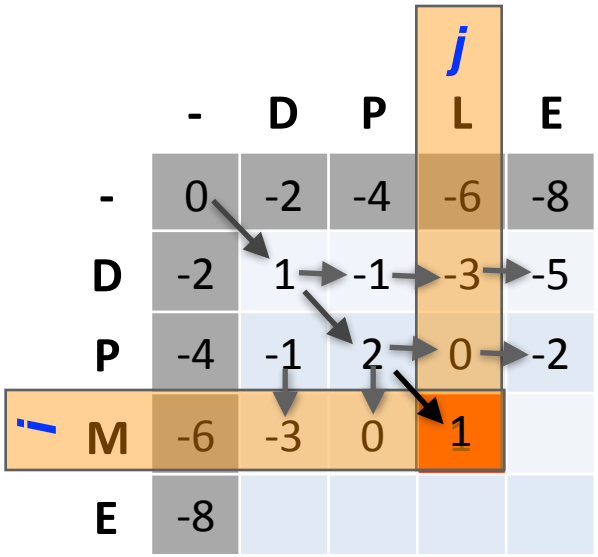
③ (2)+(-2) = 0

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



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

① (2)+(**-1**) = 0 <= mismatch

② (0)+(-2) = -2

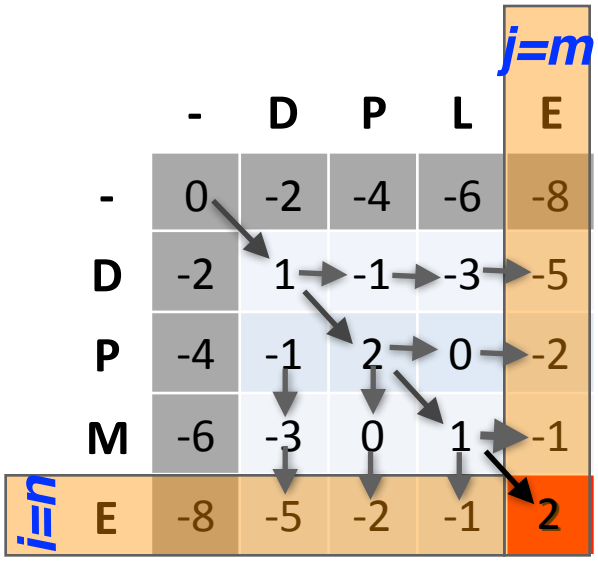
③ (0)+(-2) = -2

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)



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

① (+1)+(+1) = +2

② (-1)+(-2) = -3

③ (-1)+(-2) = -3

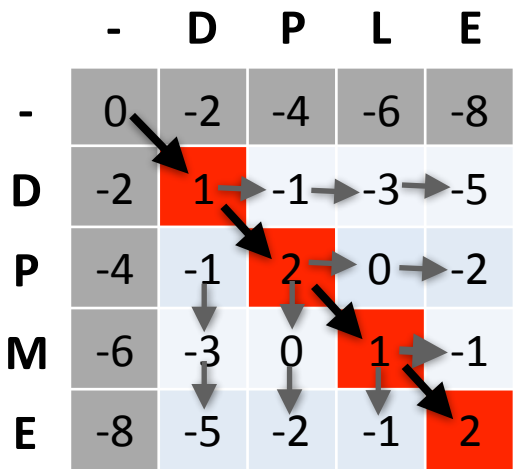
Alignment

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

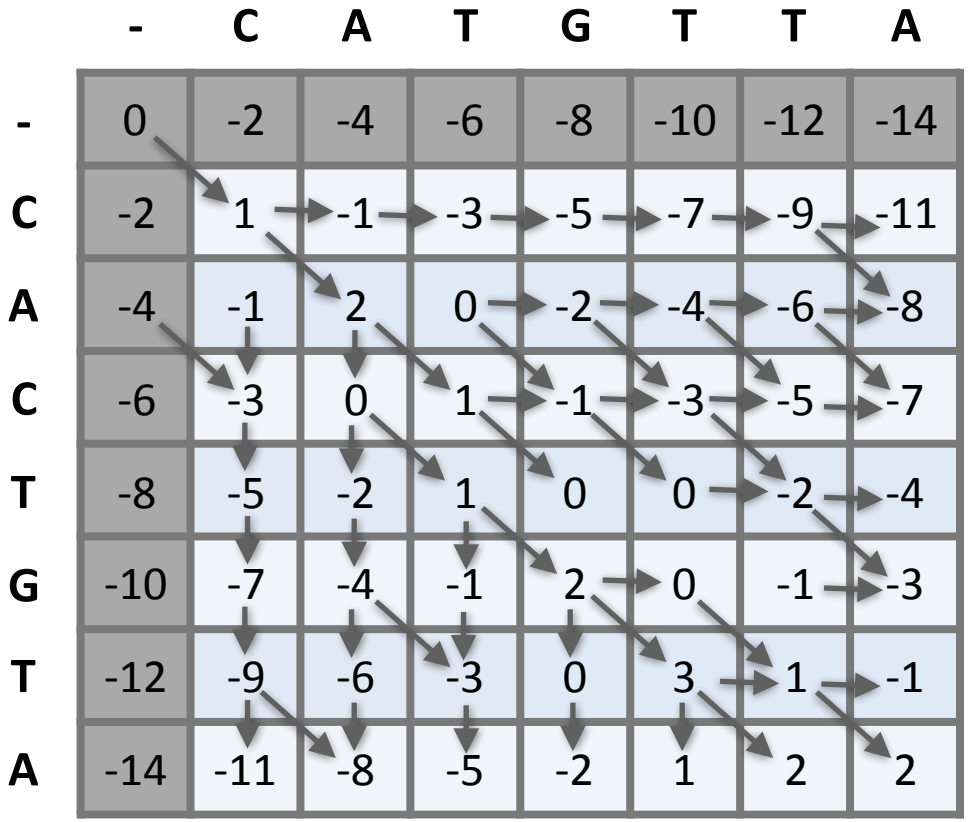


Alignment

DPME
DPLE

Questions:

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



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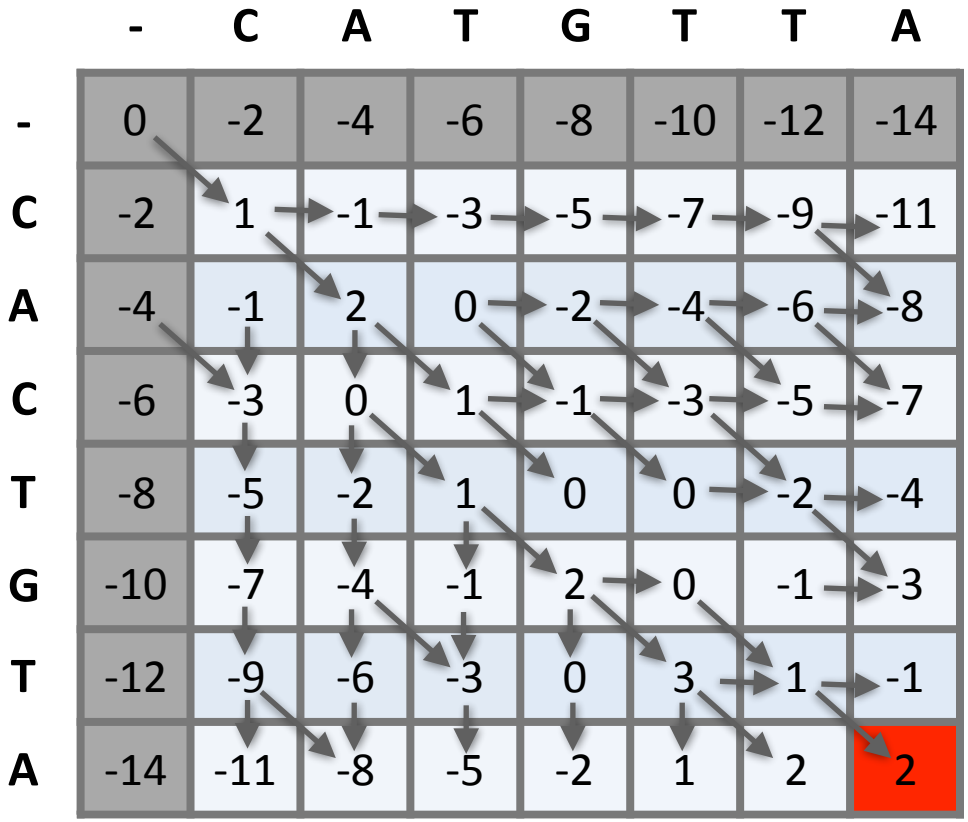

Questions:

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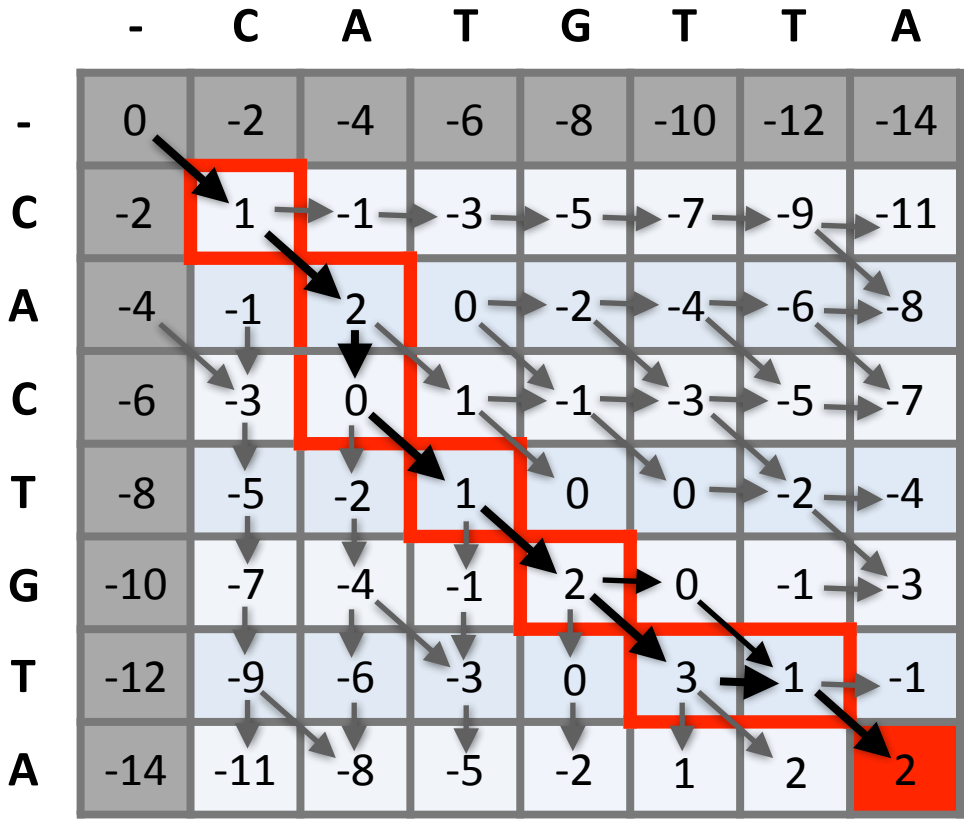
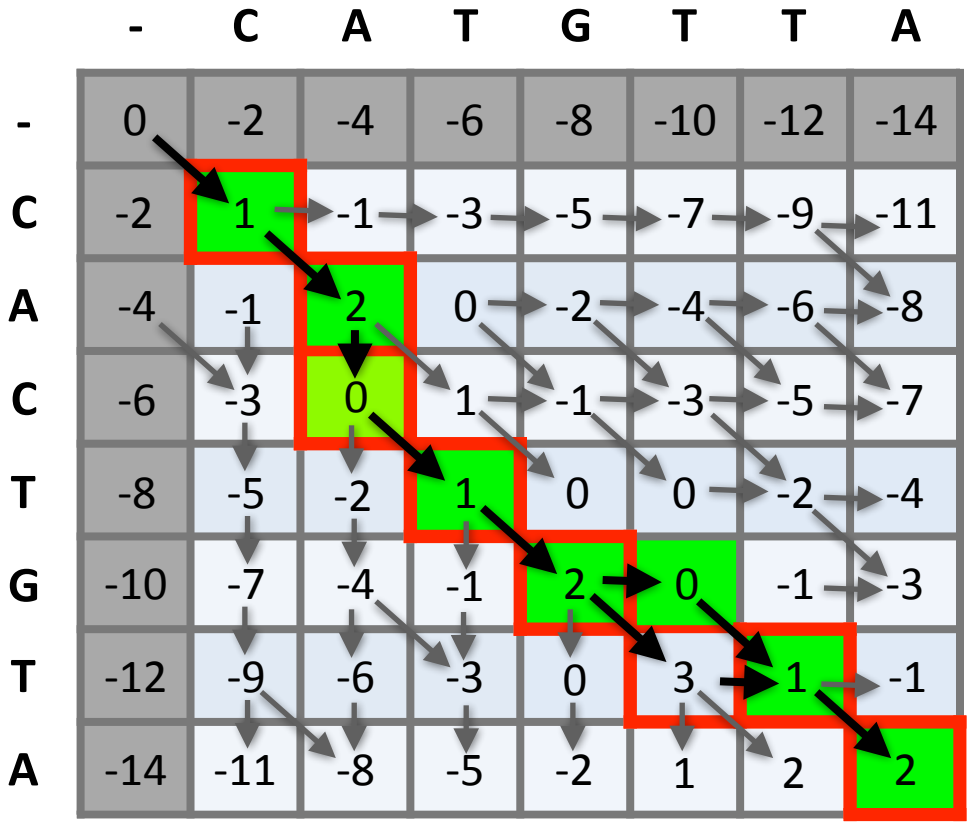


More than one alignment possible

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



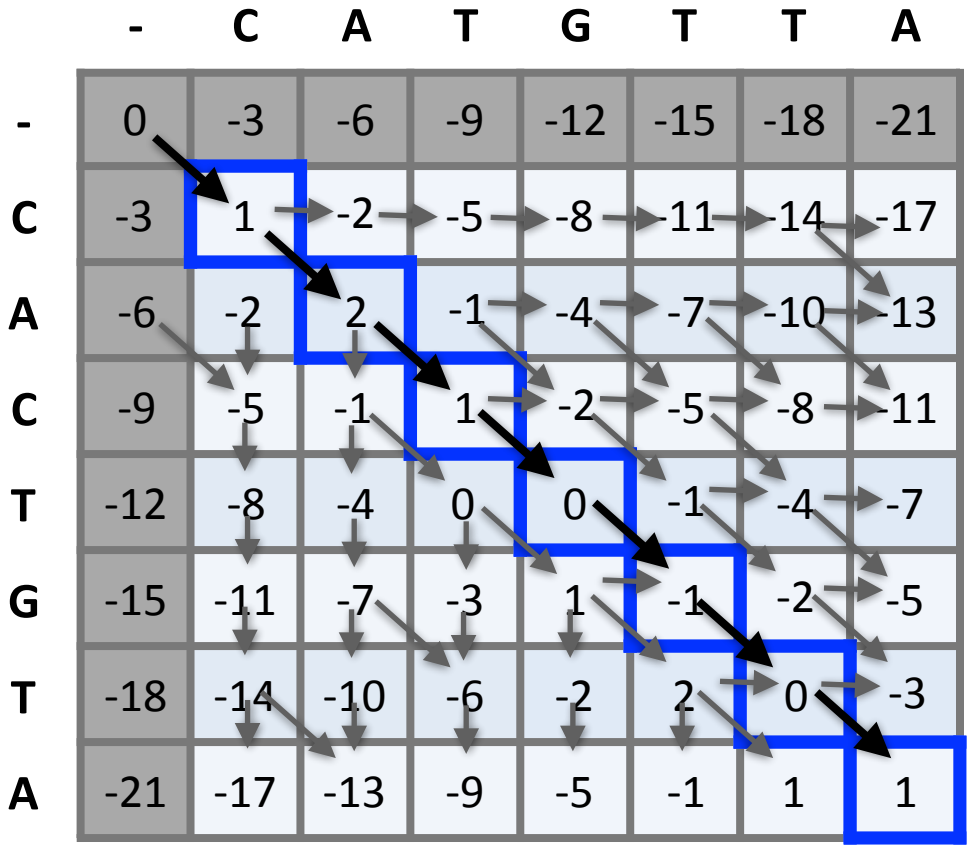
Alignment

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



Alignment

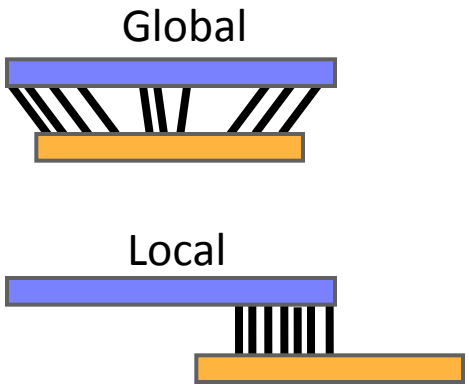
CACTGT-A
CA-TGTTA

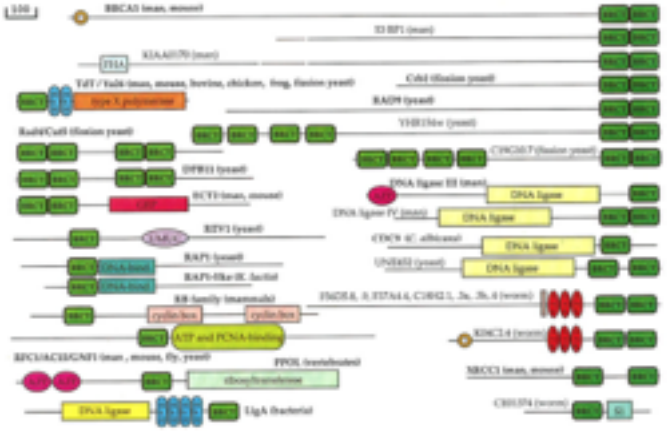
CACTG-TA
CA-TGTTA

CACTGTA
CATGTTA

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

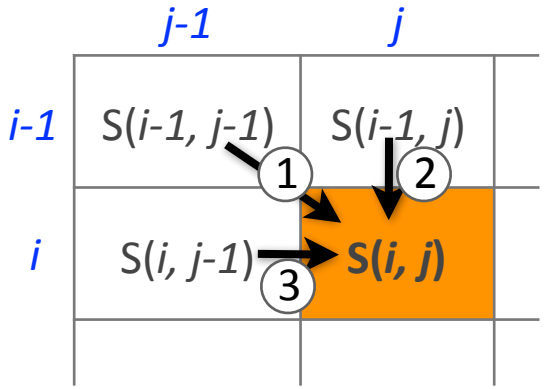
- Smith & Waterman proposed simply that a local alignment of two sequences allow arbitrary-length segments of each sequence to be aligned, with no penalty for the unaligned portions of the sequences. Otherwise, the score for a local alignment is calculated the same way as that for a global alignment

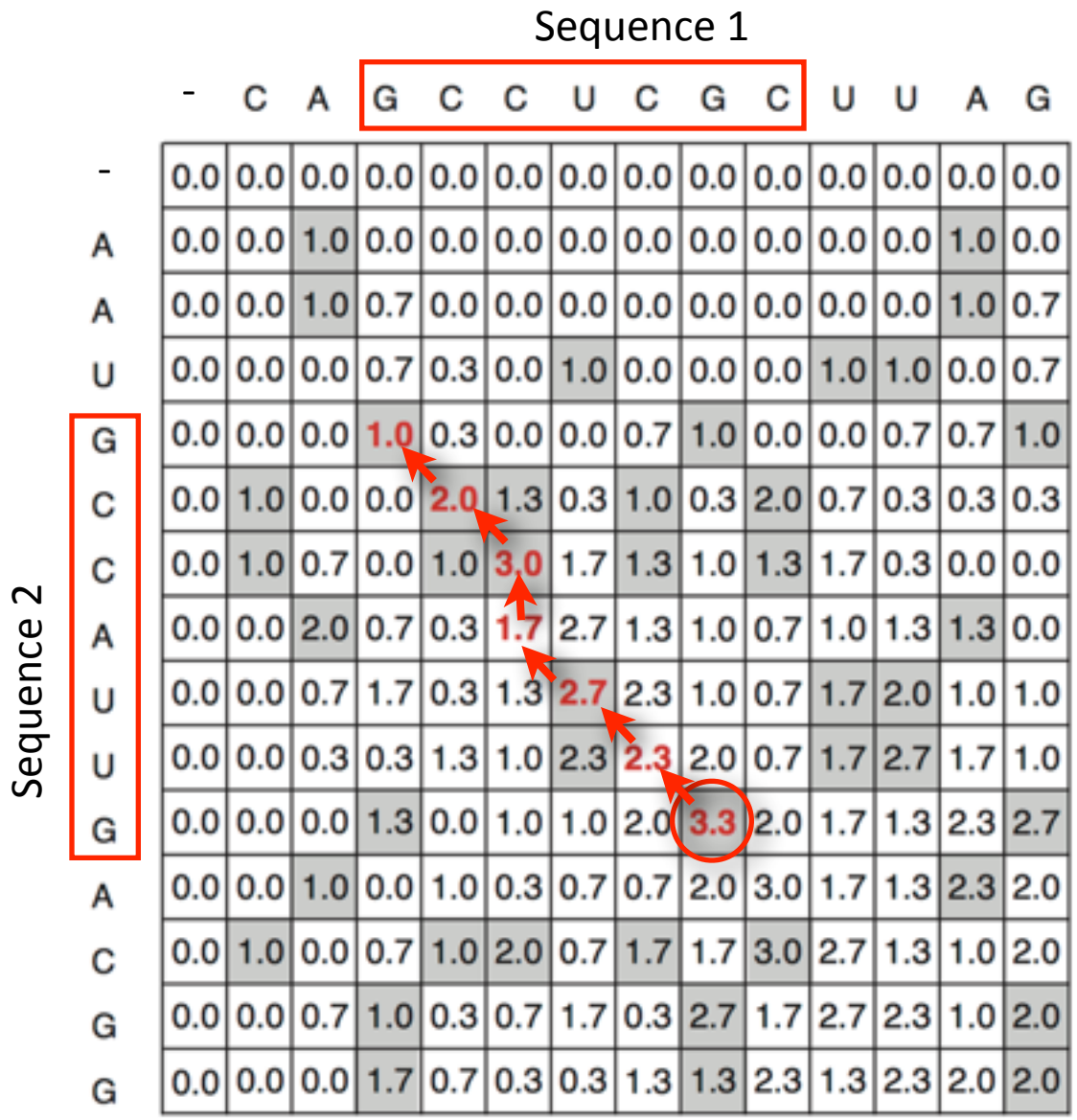
Smith, T.F. & Waterman, M.S. (1981) “Identification of common molecular subsequences.” J. Mol. Biol. 147:195-197.

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

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



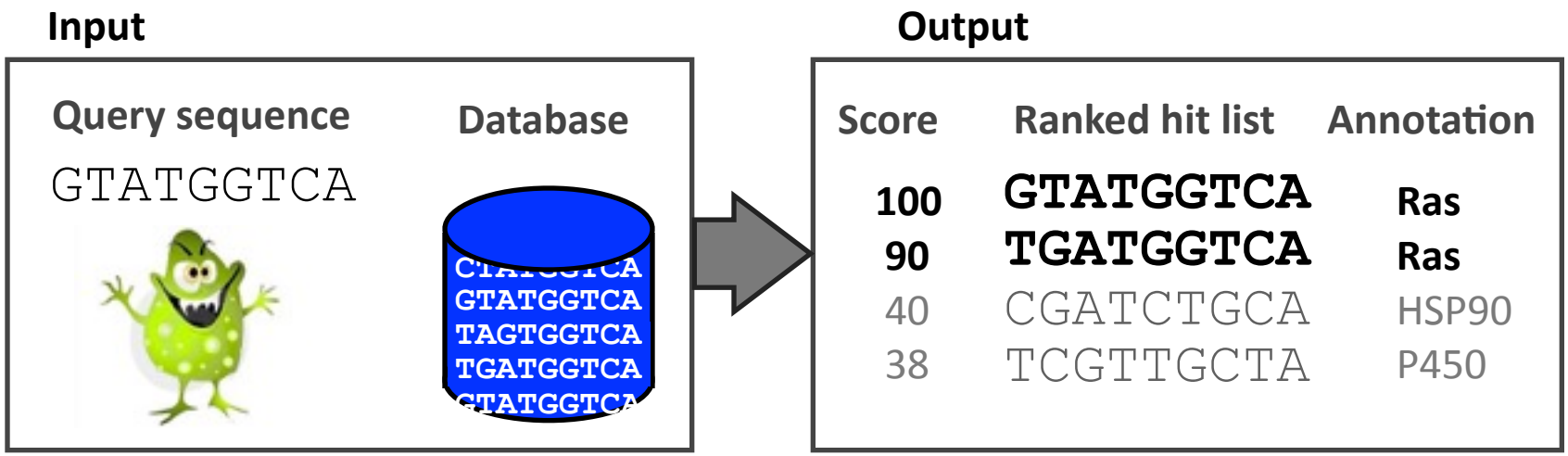


Local alignment

GCC-AUG
GCCUCGC

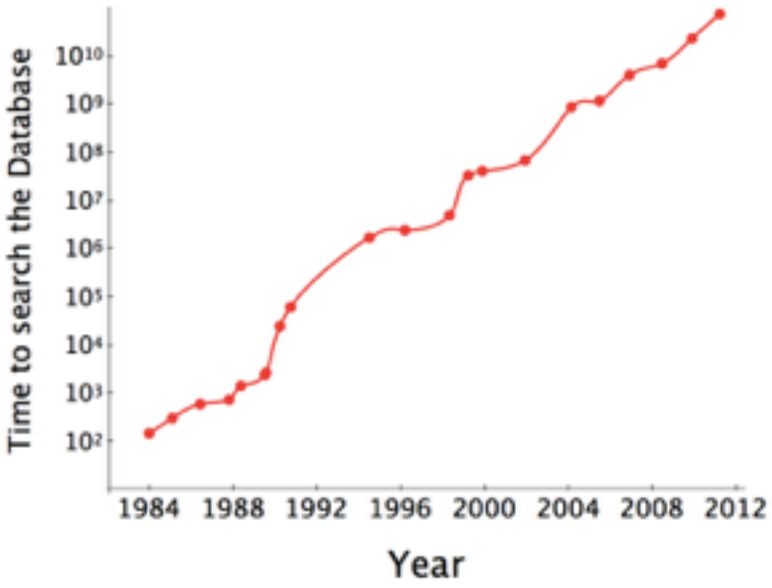
Local alignments can be used for database searching

- **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.

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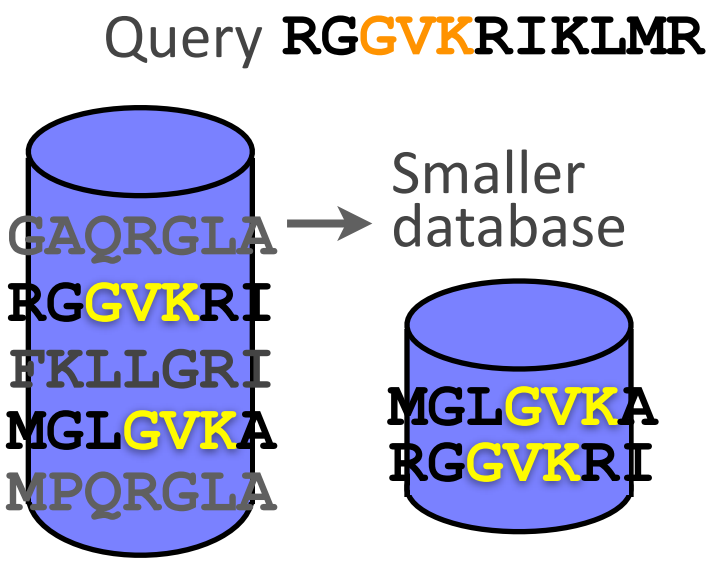


To reduce search time **heuristic algorithms**, such as BLAST, first remove database sequences without a strong local similarity to the query sequence in a quick initial scan.

Outline for today

- Alignment basics
  - Why compare biological sequences?
- Homologue detection
  - Orthologs, paralogs, similarity and identity
  - Sequence changes during evolution
  - Alignment view: matches, mismatches and gaps
- Pairwise sequence alignment methods
  - Brute force alignment
  - Dot matrices
  - Dynamic programing

  (global vs local alignment)
- Rapid heuristic approaches
  - BLAST
- Practical database searching
  - PSI-BLAST and HMM approaches

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

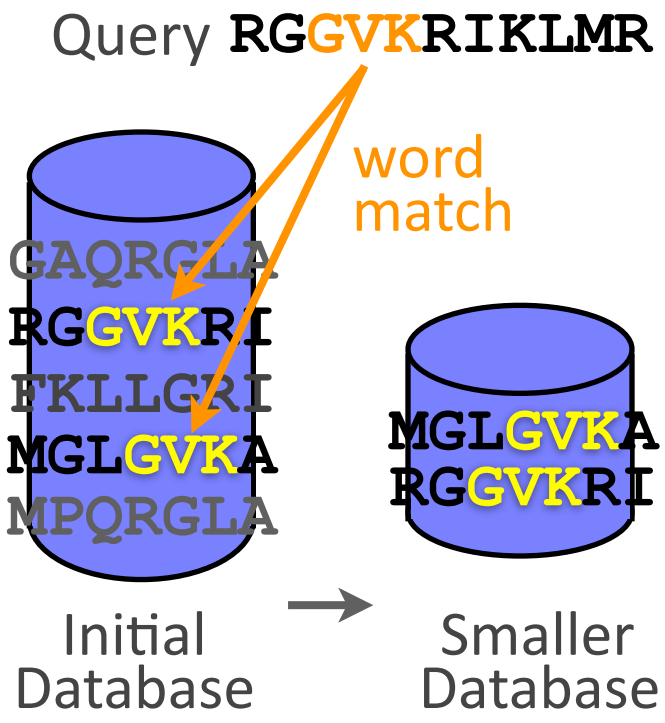
- BLAST (Basic Local Alignment Search Tool) is a simplified form of Smith-Waterman (SW) alignment that is popular because it is **fast** and **easily accessible**
  - BLAST is a heuristic approximation to SW - It examines only part of the search space
  - BLAST saves time by restricting the search by 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

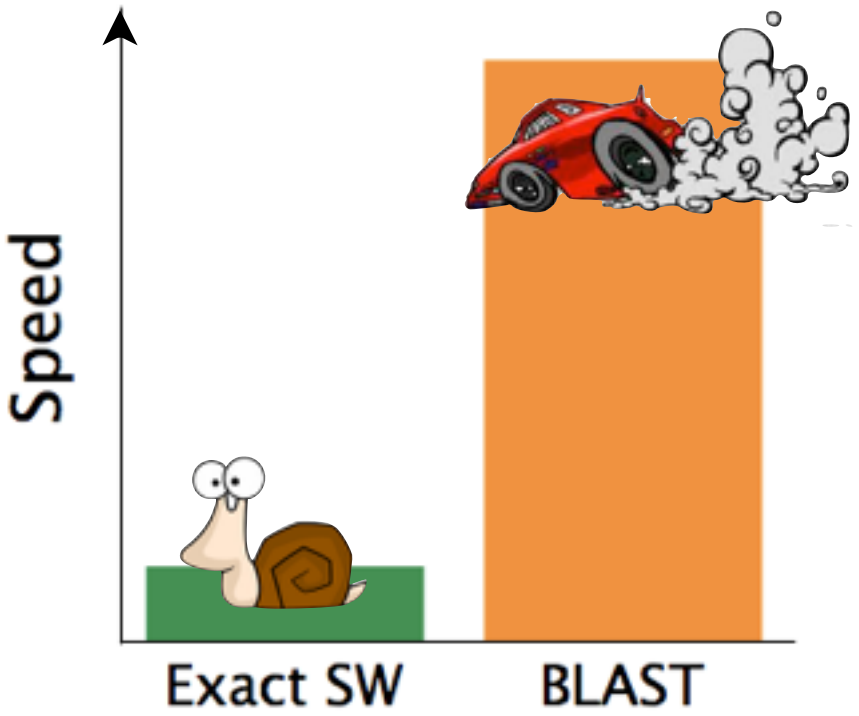
Rapid, heuristic versions of Smith–Waterman: **BLAST**

- BLAST (Basic Local Alignment Search Tool) i… ied form of Smith-Waterman (SW) alig… ar because it is **fast** and **easily**
  - BLAST finds regions… gical sequences
  - BL… the search by scanning …ikely matches before performing …gnments
  - … some sensitivity in exchange for speed
  - In contrast to SW, BLAST is not guaranteed to find optimal alignments

"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 algorithm





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    Query sequence
RGG
 GGV
  GVK
   VKR
    KRI
```

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 . . .

extend list of words similar to query

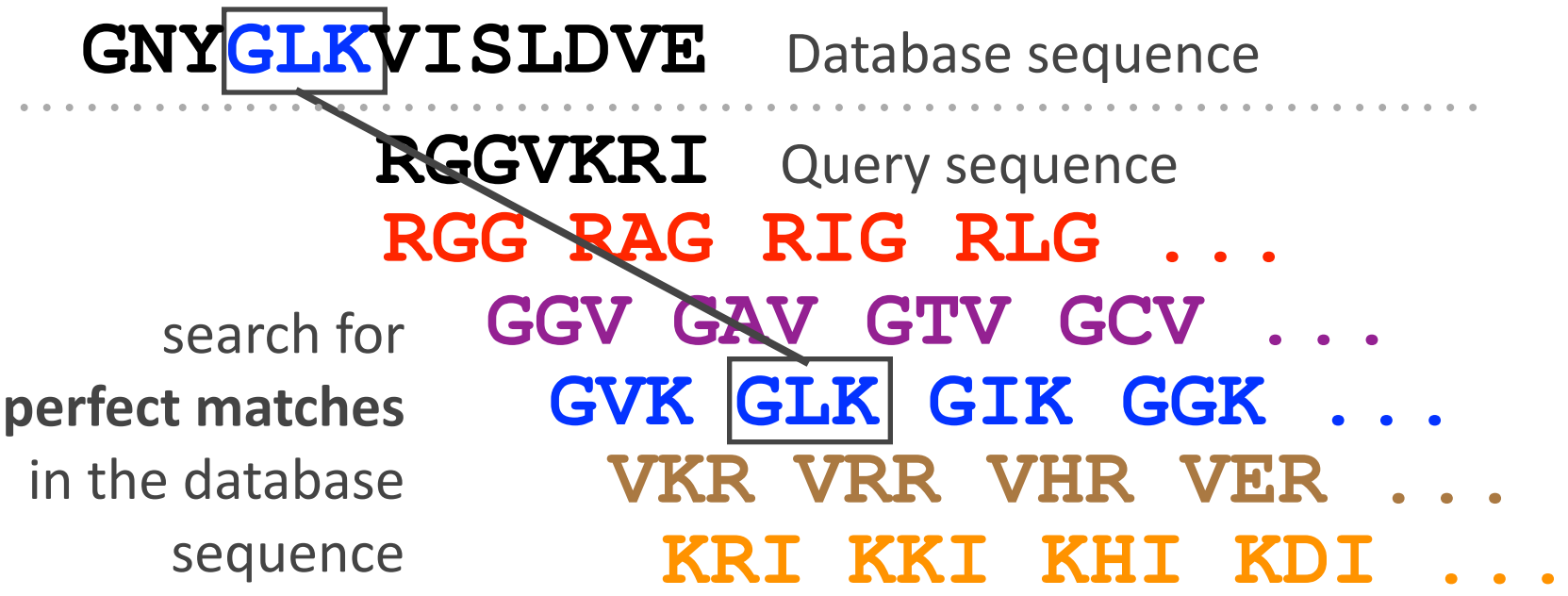
GVK GAK GIK GGK . . .

VKR VRR VHR VER . . .

KRI KKI KHI KDI . . .

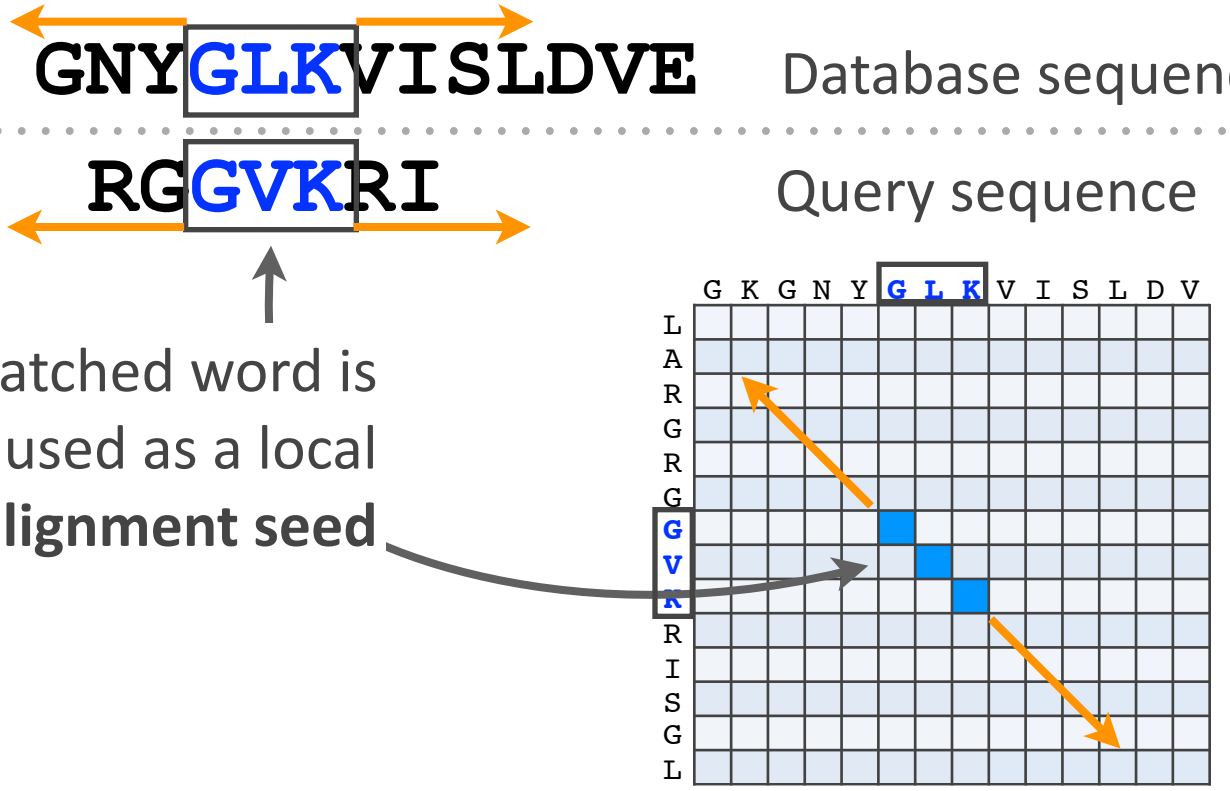
Blast

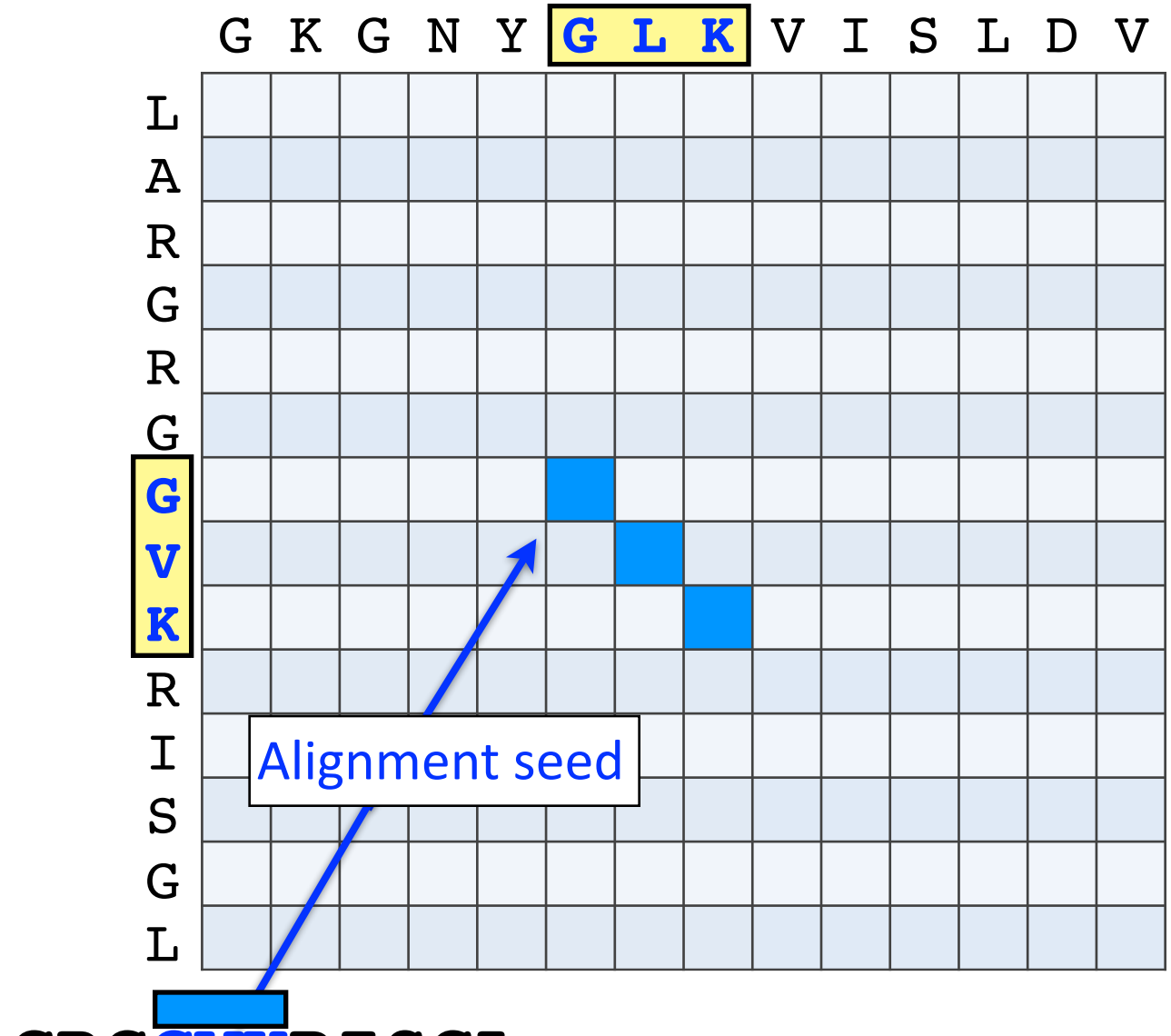
– **Phase 3**: a database is scanned to find sequence entries that match the compiled word list



Blast

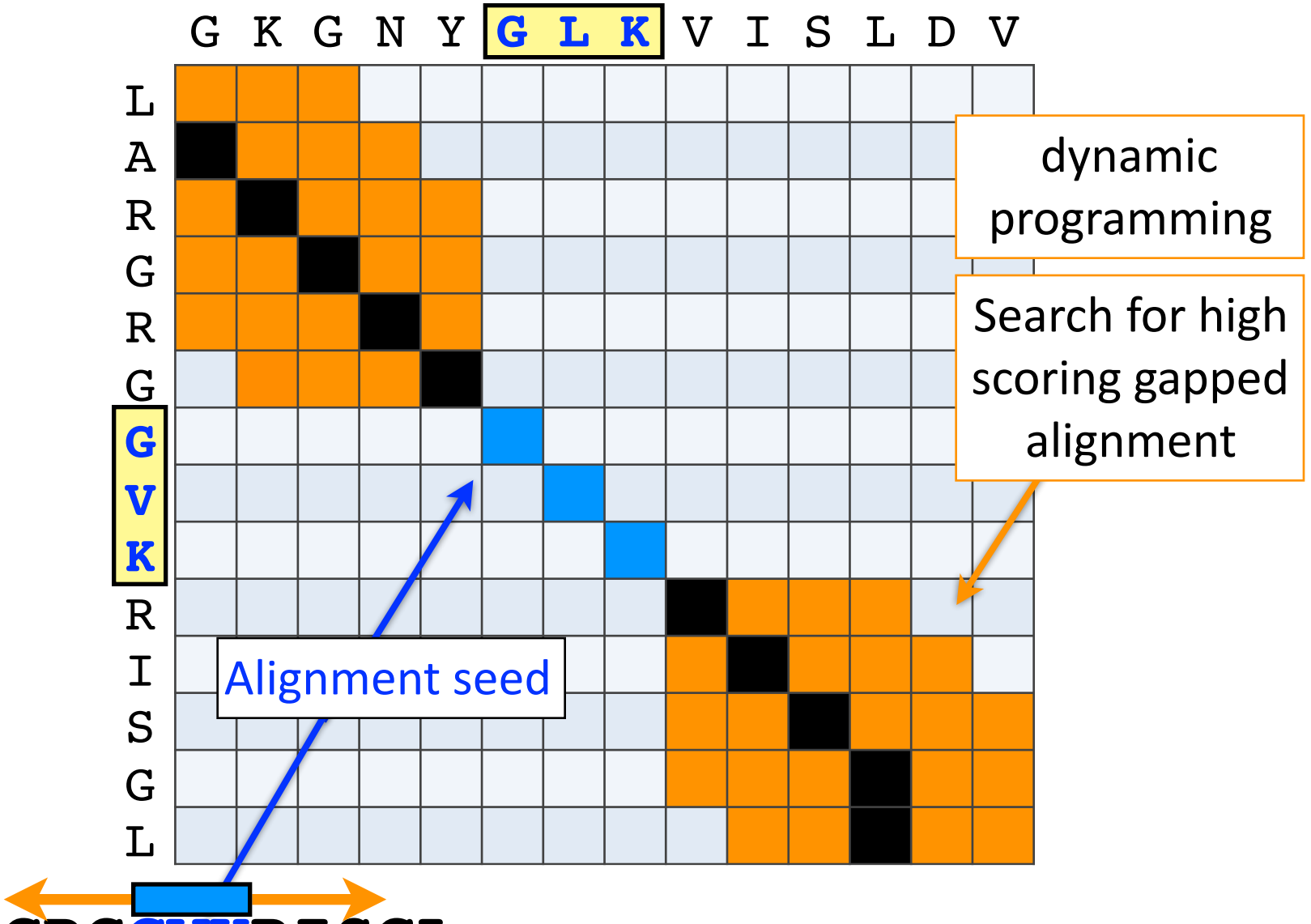
– **Phase 4**: the initial database hits are extended in both directions using dynamic programing

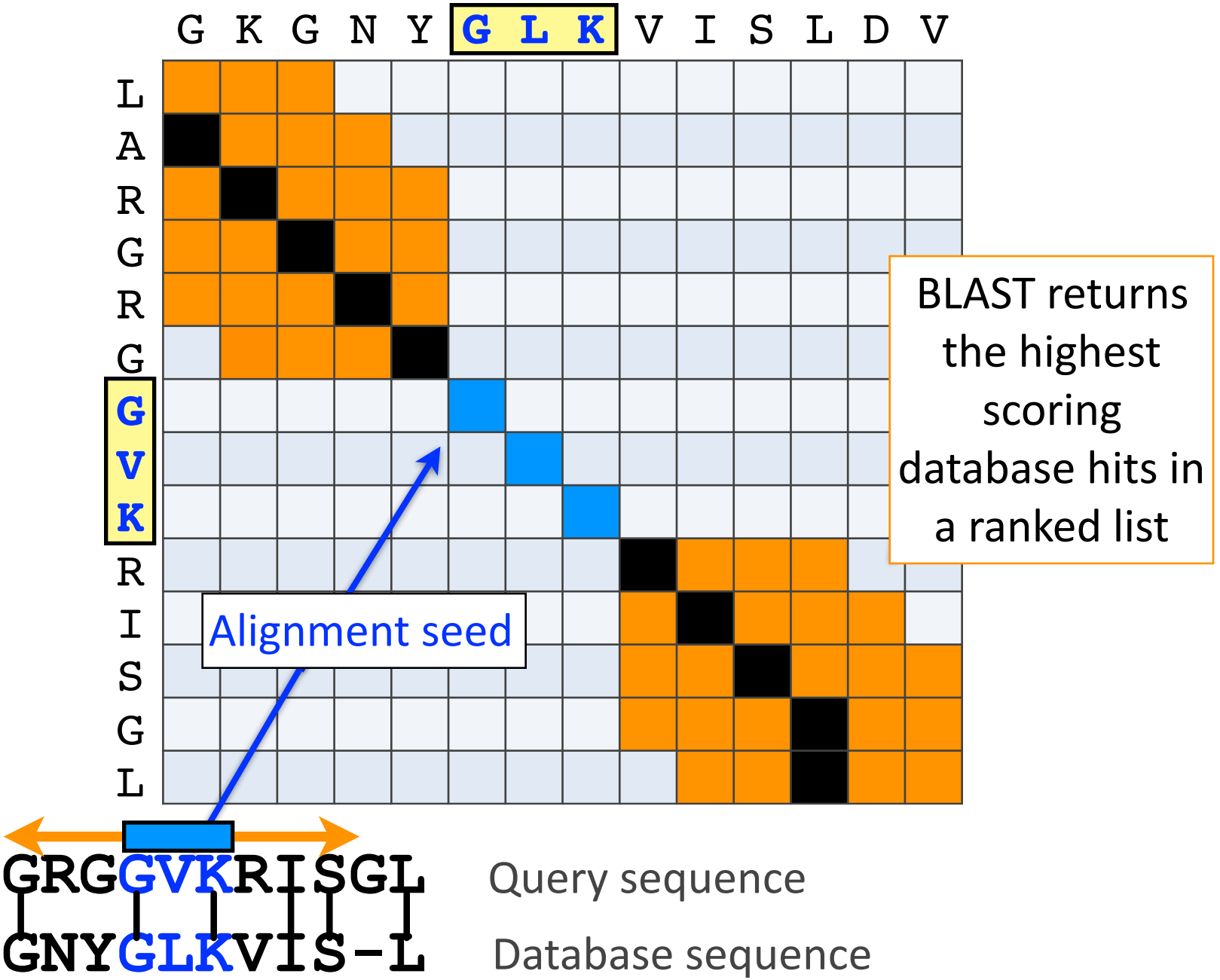




GRGGVKRISGL Query sequence

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
BLAST returns the highest scoring database hits in a ranked list
Alignment seed
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	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
Kinesin-14 heavy chain [Danio rerio]	595	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	48.2	52	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	42.7	38%	3.02	24%	EHH28205.1

Statistical significance of results

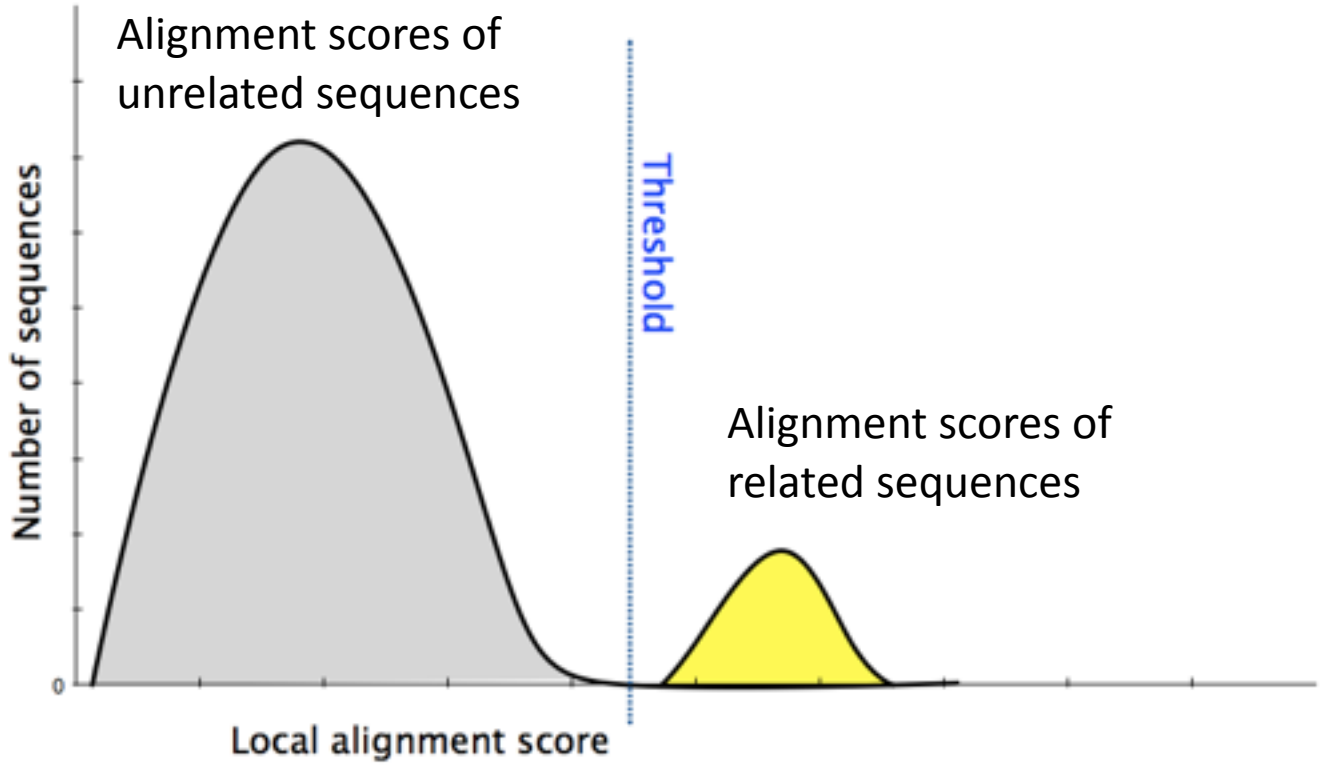
- An important feature of BLAST is the computation of statistical significance for each hit. This is described by the **E value** (expect value)

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
Kinesin-14 heavy chain [Danio rerio]	595	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	48.2	52	40%	0.03	32%	ELK35081.1
mKIAA4102 protein [Mus musculus]	42.7	42.7	38%	3.02	24%	EHH28205.1

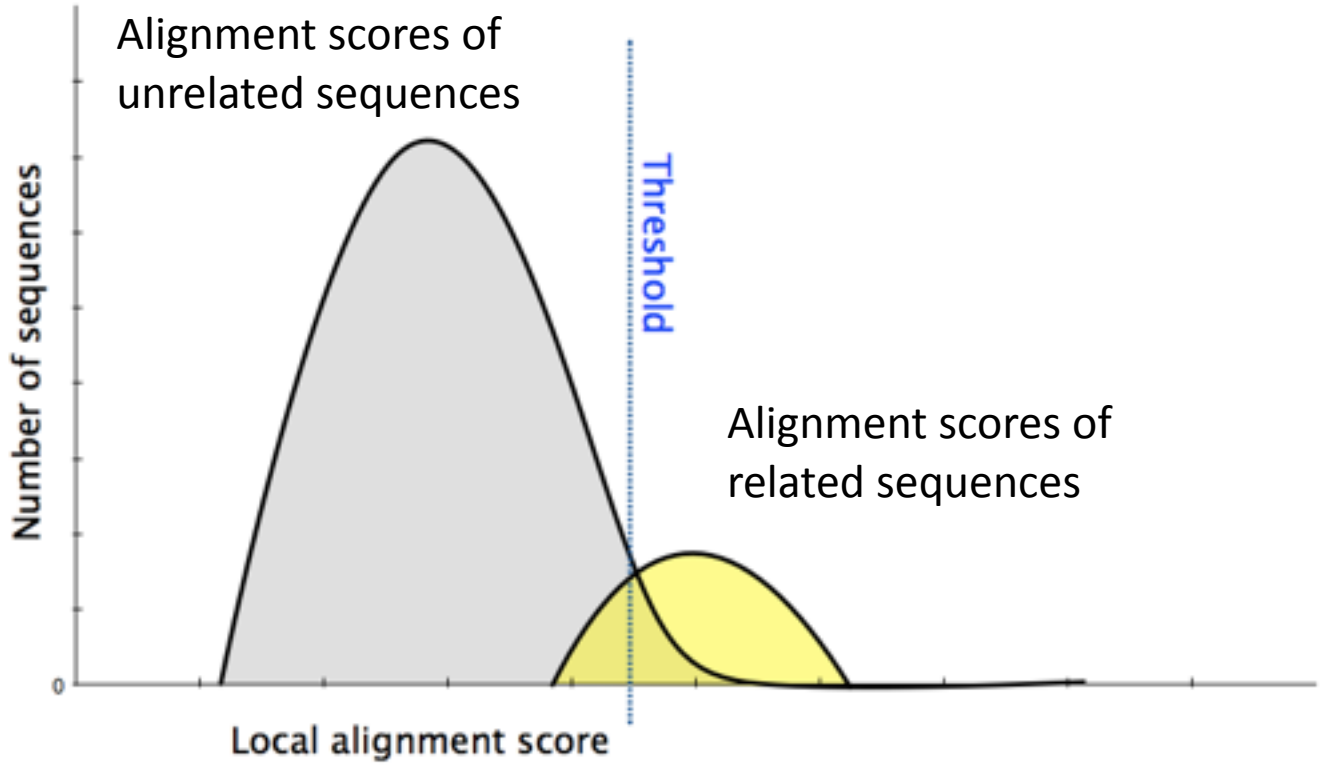
BLAST scores and E-values

- 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

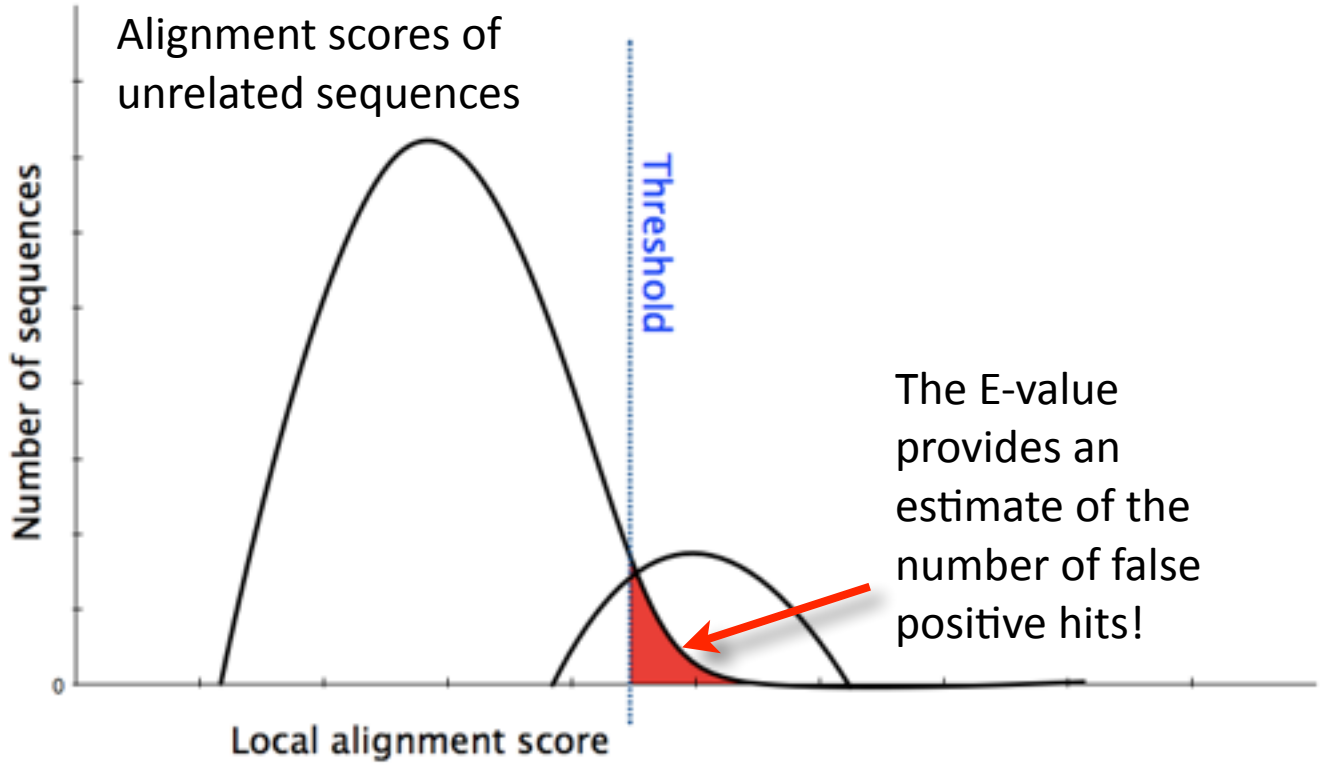
- Ideally, a threshold separates all query related sequences (yellow) from all unrelated sequences (gray)



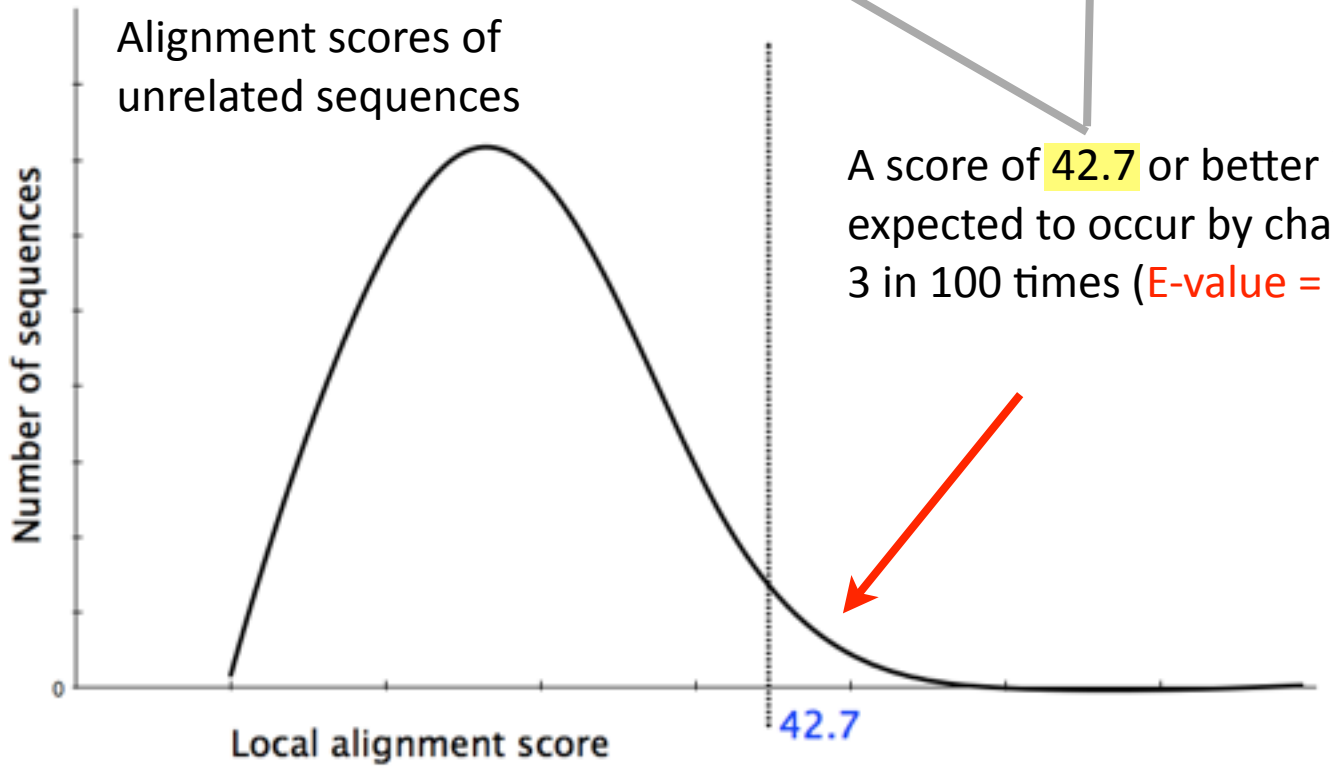
- Unfortunately, often both score distributions overlap
  - The E value describes the expected number of hits with a score above the threshold if the query and database are unrelated



- 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	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
Kinesin-14 heavy chain [Danio rerio]	595	595	88%	0	78%	XP_00320703
hypothetical protein EGK_18589	42.7	52	40%	**0.03**	32%	ELK35081.1



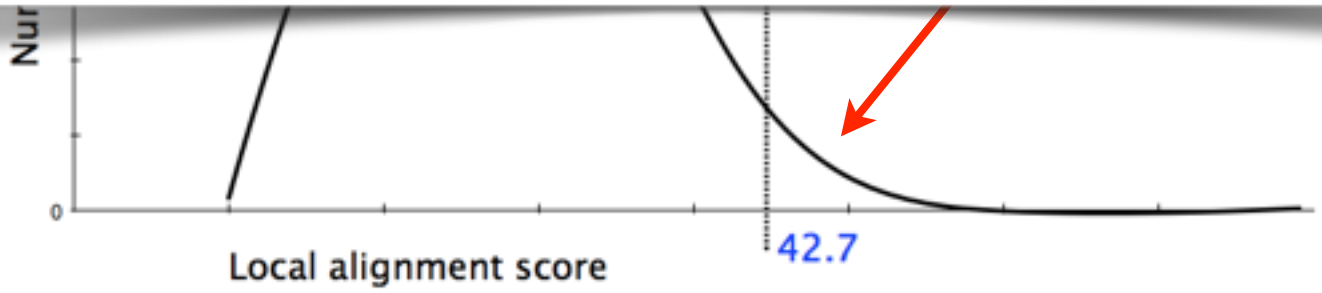
A score of 42.7 or better is expected to occur by chance 3 in 100 times (E-value = 0.03)

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

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

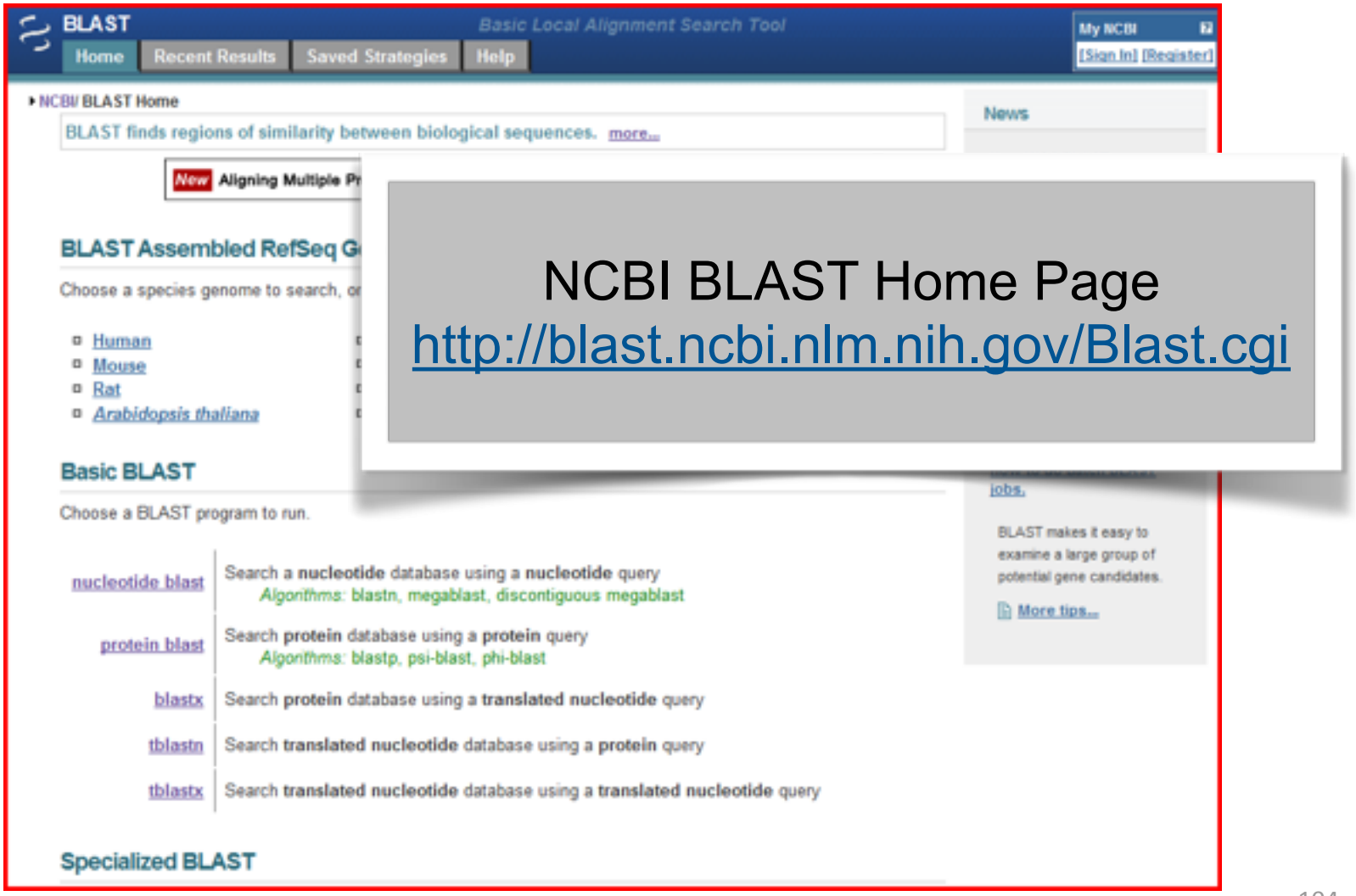


Outline for today

- Alignment basics
  - Why compare biological sequences?
- Homologue detection
  - Orthologs, paralogs, similarity and identity
  - Sequence changes during evolution
  - Alignment view: matches, mismatches and gaps
- Pairwise sequence alignment methods
  - Brute force alignment
  - Dot matrices
  - Dynamic programing

  (global vs local alignment)
- Rapid heuristic approaches
  - BLAST
- Practical database searching
  - BLAST, PSI-BLAST and HMM approaches

Practical database searching with BLAST

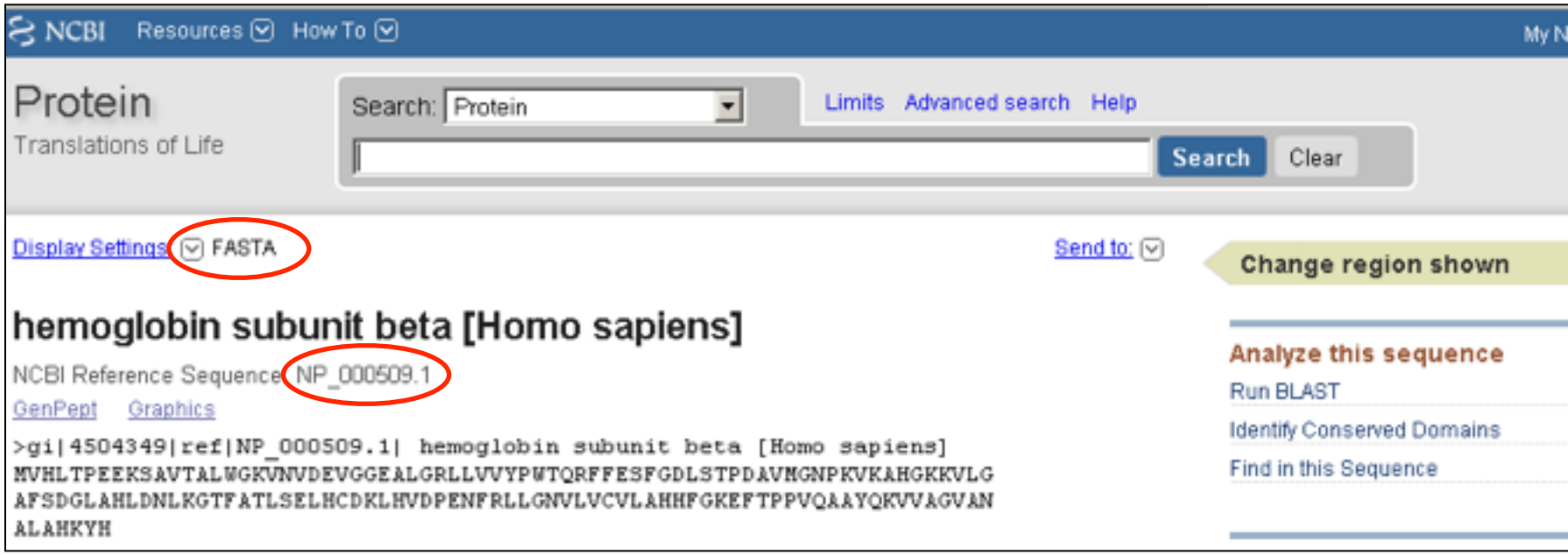


Practical database searching with BLAST

- 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



Step 2: Choose the BLAST program

- Rat
- *Arabidopsis thaliana*
- *Danio rerio*
- *Drosophila melanogaster*
- *Microbes*
- *Apis mellifera*

Basic BLAST

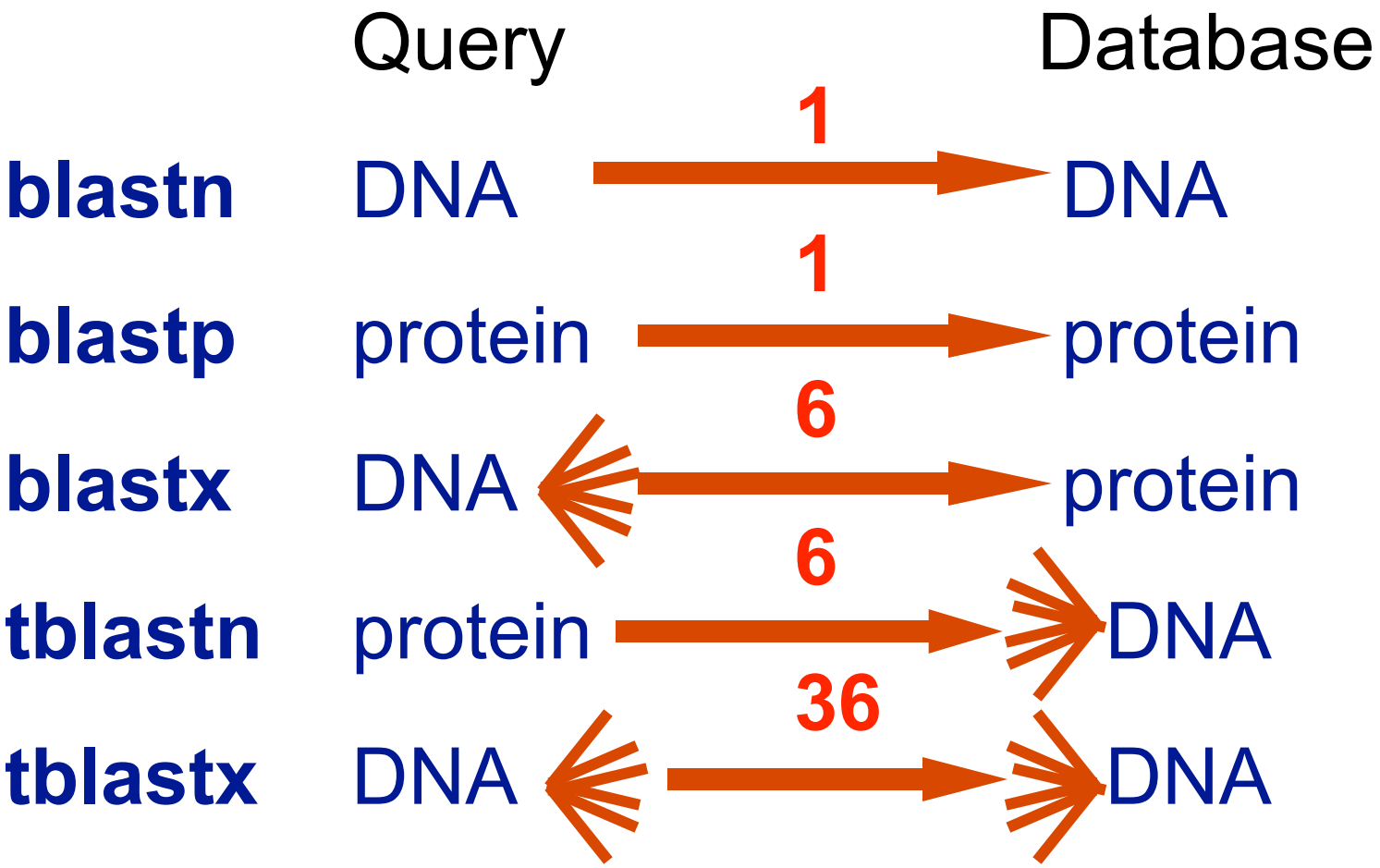
Choose a BLAST program to run.

nucleotide blast	Search a **nucleotide** database using a **nucleotide** query *Algorithms:* blastn, megablast, discontiguous megablast
protein blast	Search **protein** database using a **protein** query *Algorithms:* blastp, psi-blast, phi-blast
blastx	Search **protein** database using a **translated nucleotide** query
tblastn	Search **translated nucleotide** database using a **protein** query
tblastx	Search **translated nucleotide** database using a **translated nucleotide** query

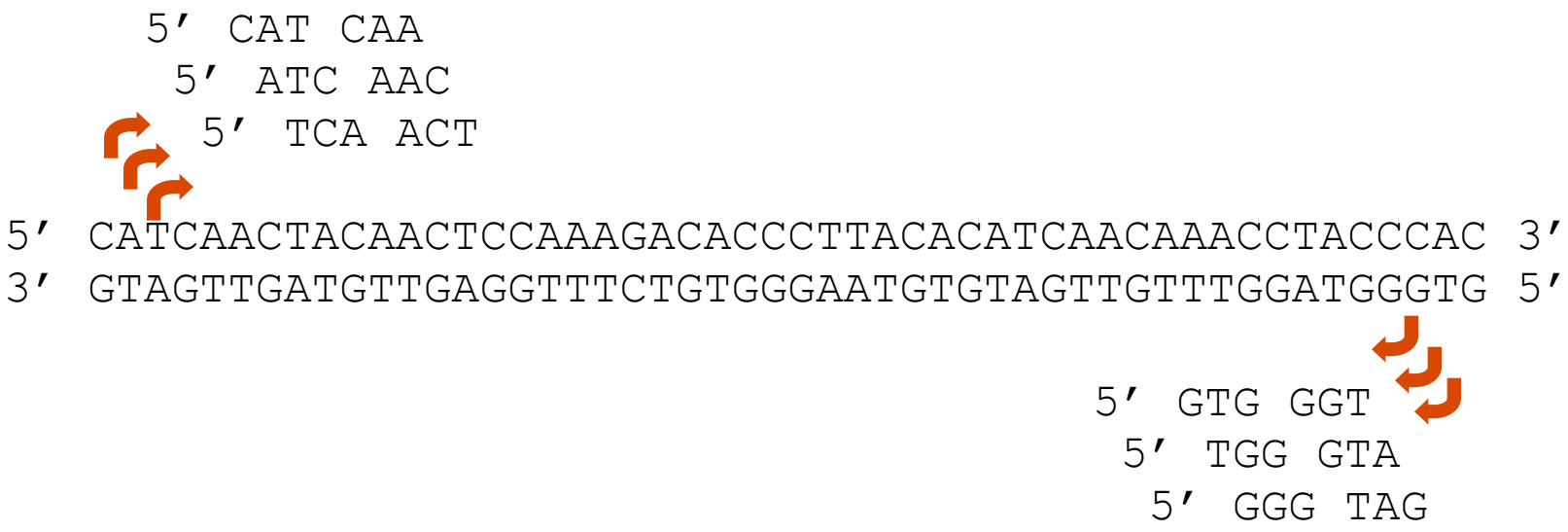
Specialized BLAST

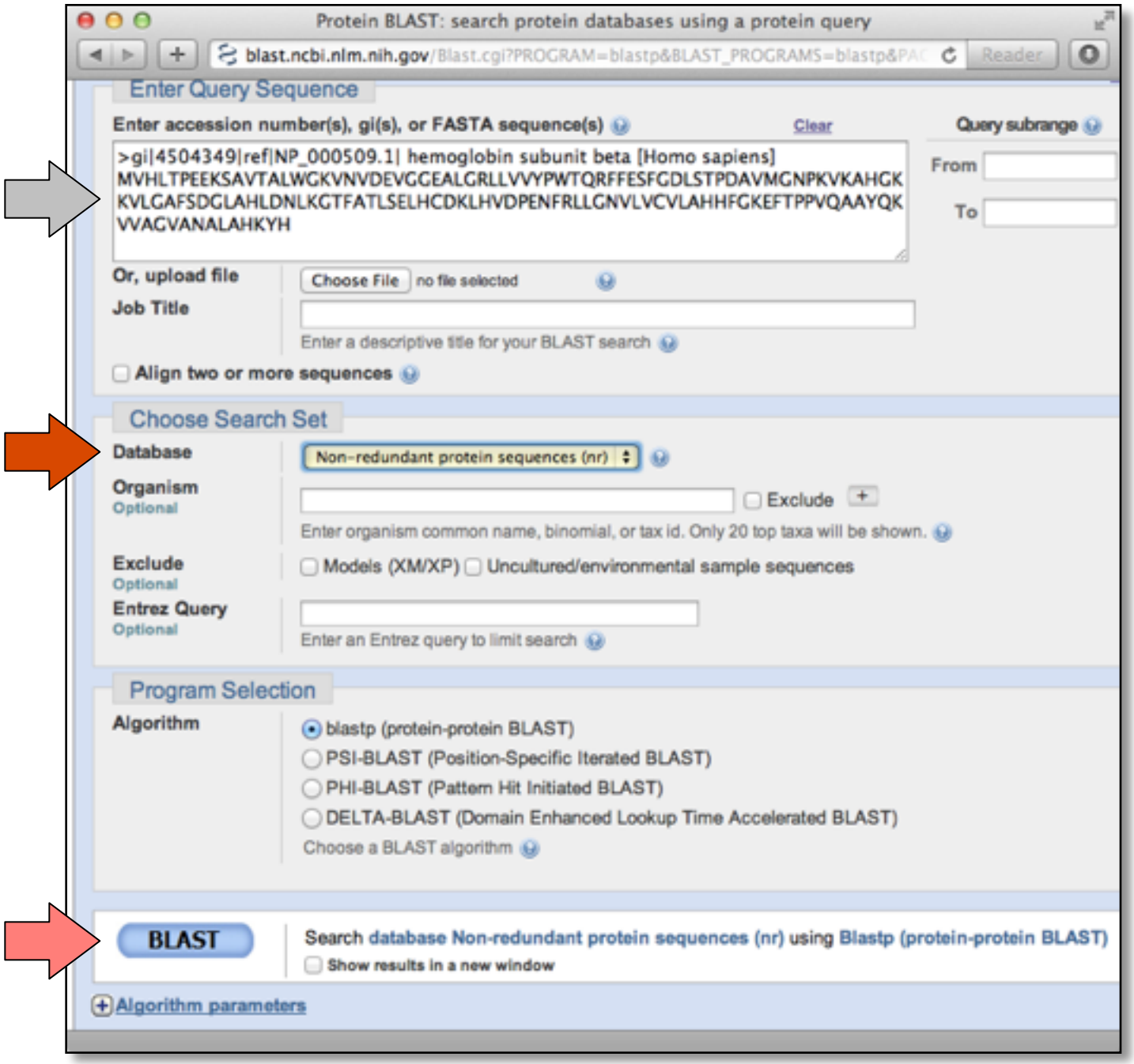
Choose a type of specialized search (or database name in parentheses.)

Step 2: Choose the BLAST program



DNA potentially encodes six proteins




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

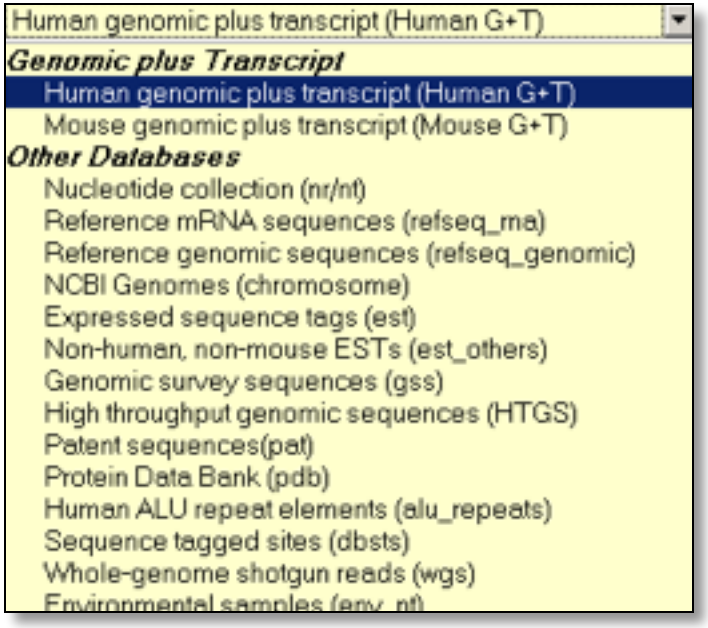
Step 3: Choose the database

nr = non-redundant (most general database)

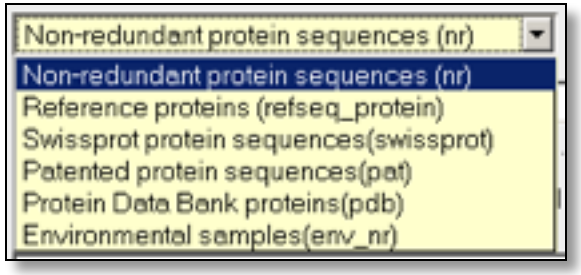
dbest = database of expressed sequence tags

dbsts = database of sequence tag sites

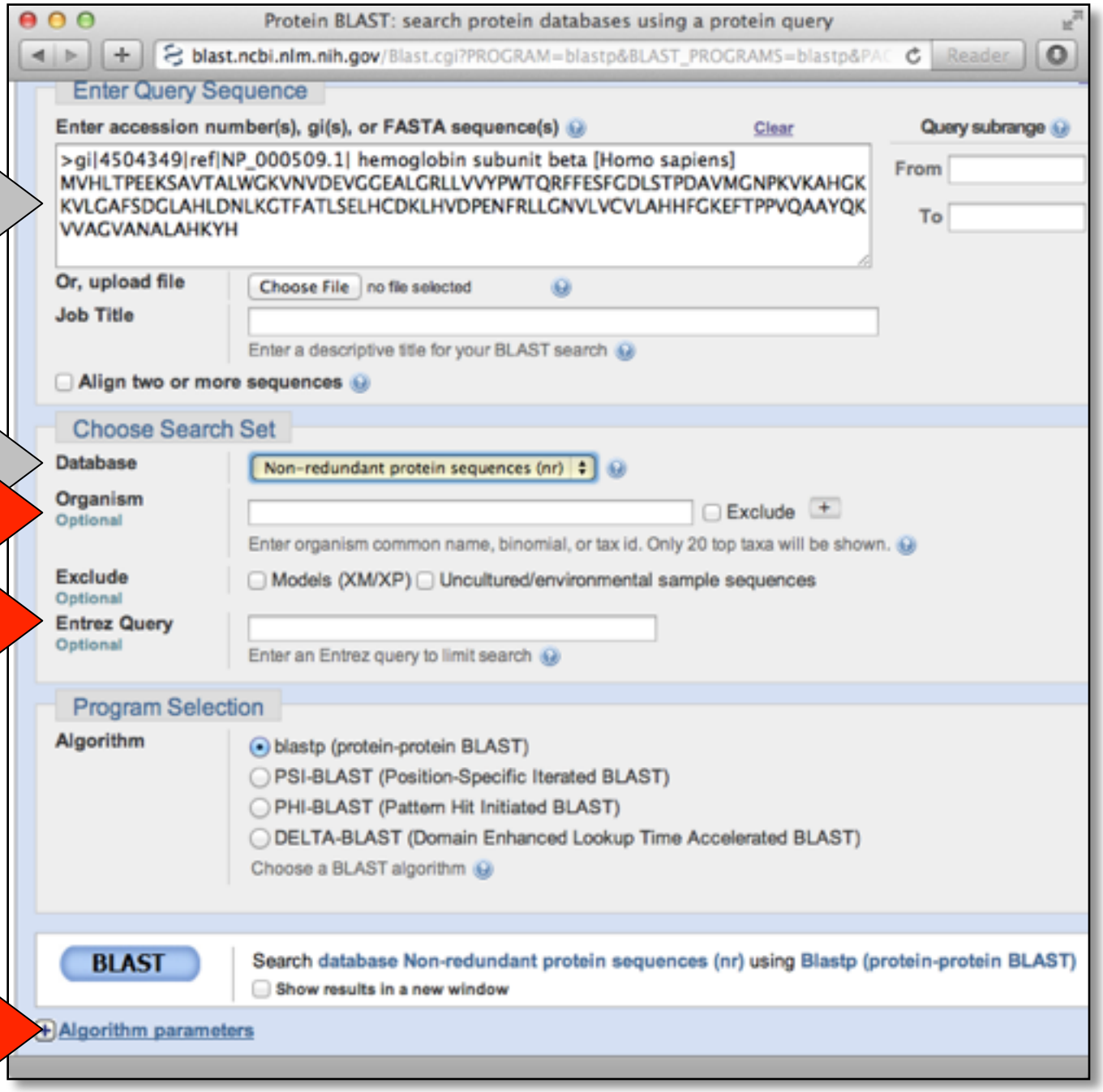
gss = genomic survey sequences



nucleotide databases



protein databases

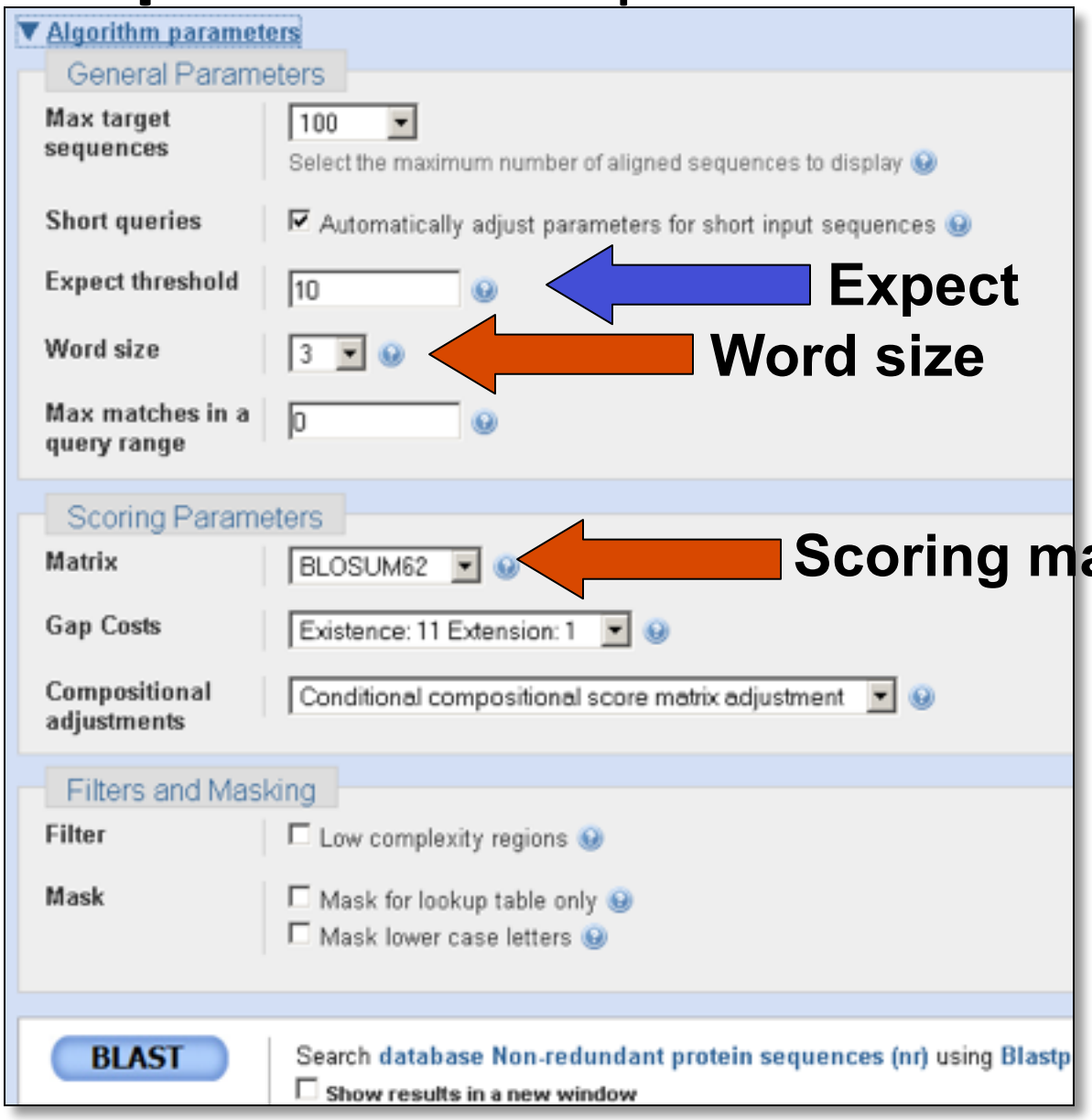


Organism

Entrez

Settings!

Step 4a: Select optional search parameters

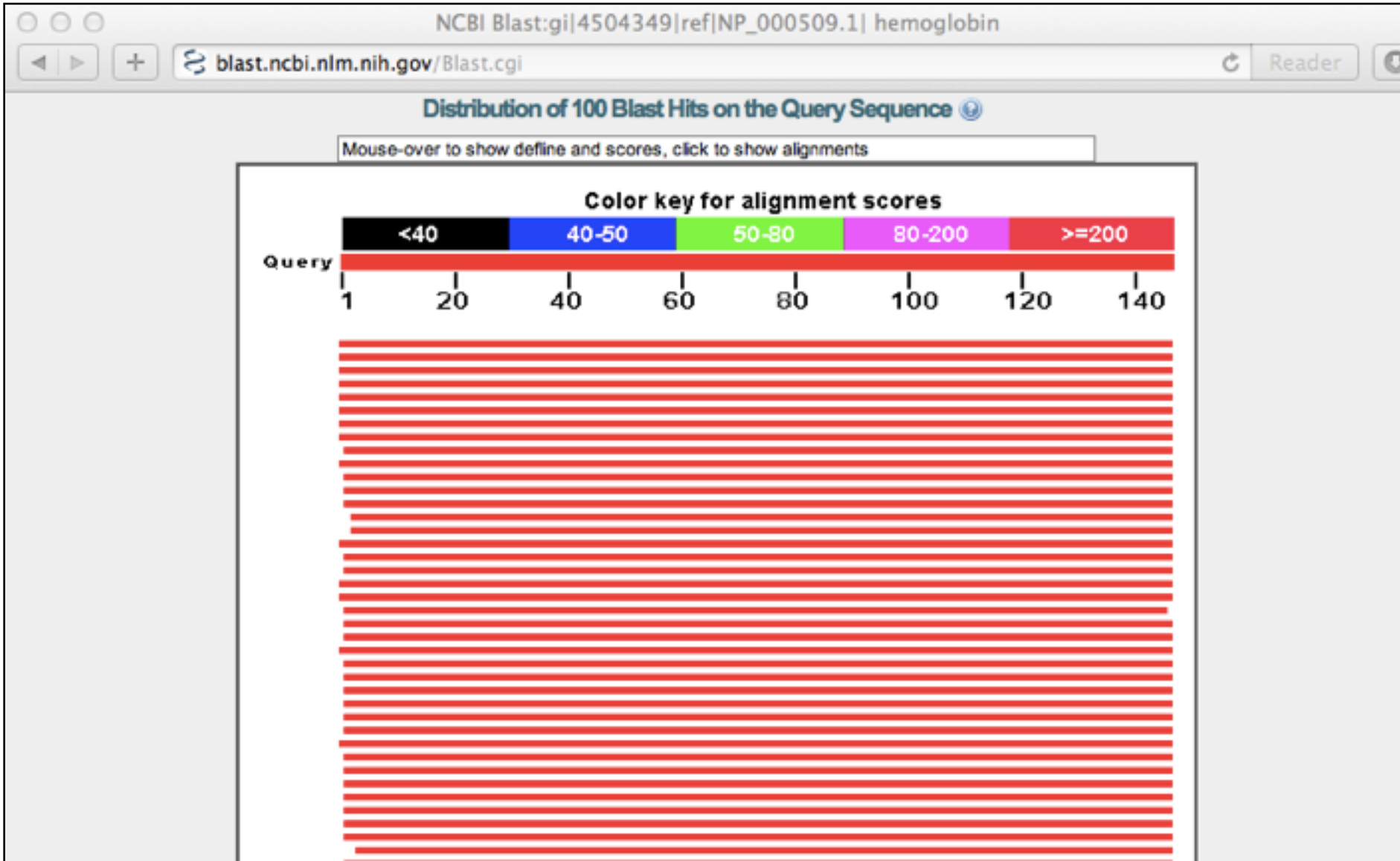


Step 4: Optional parameters

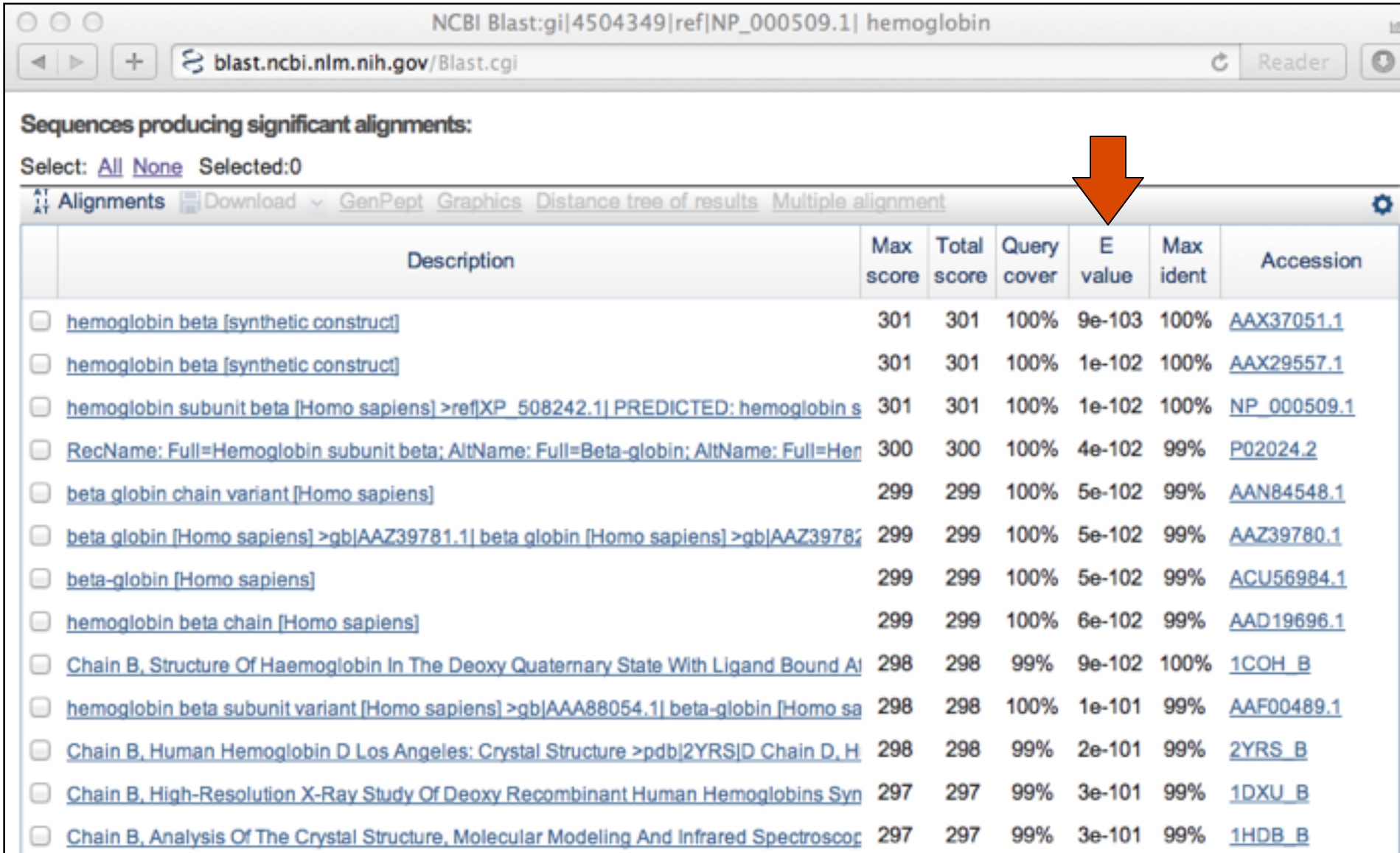
- You can...
  - choose the organism to search
  - change the substitution matrix
  - change the expect (E) value
  - change the word size
  - change the output format

Results page

Further down the results page...

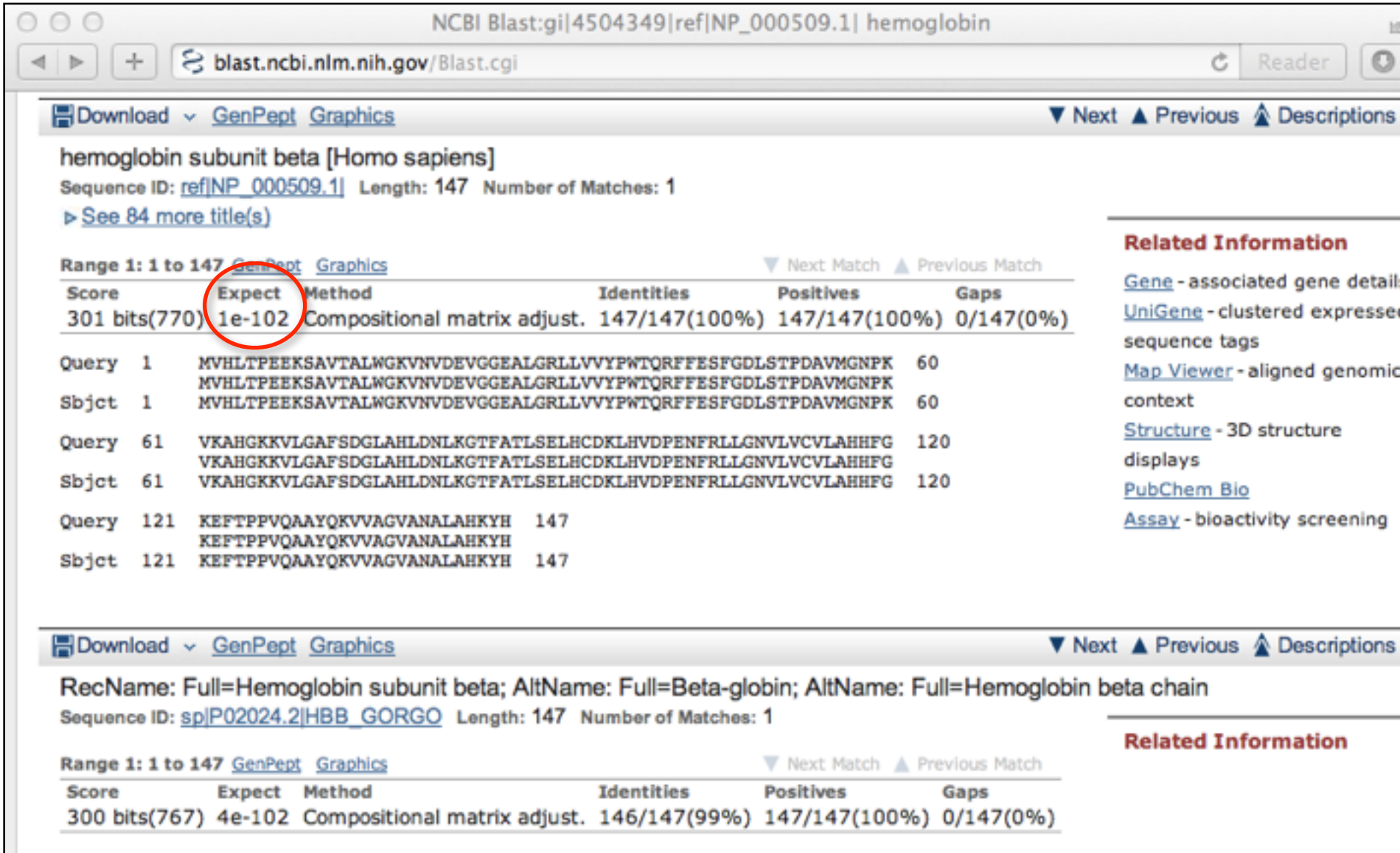


Further down the results page...

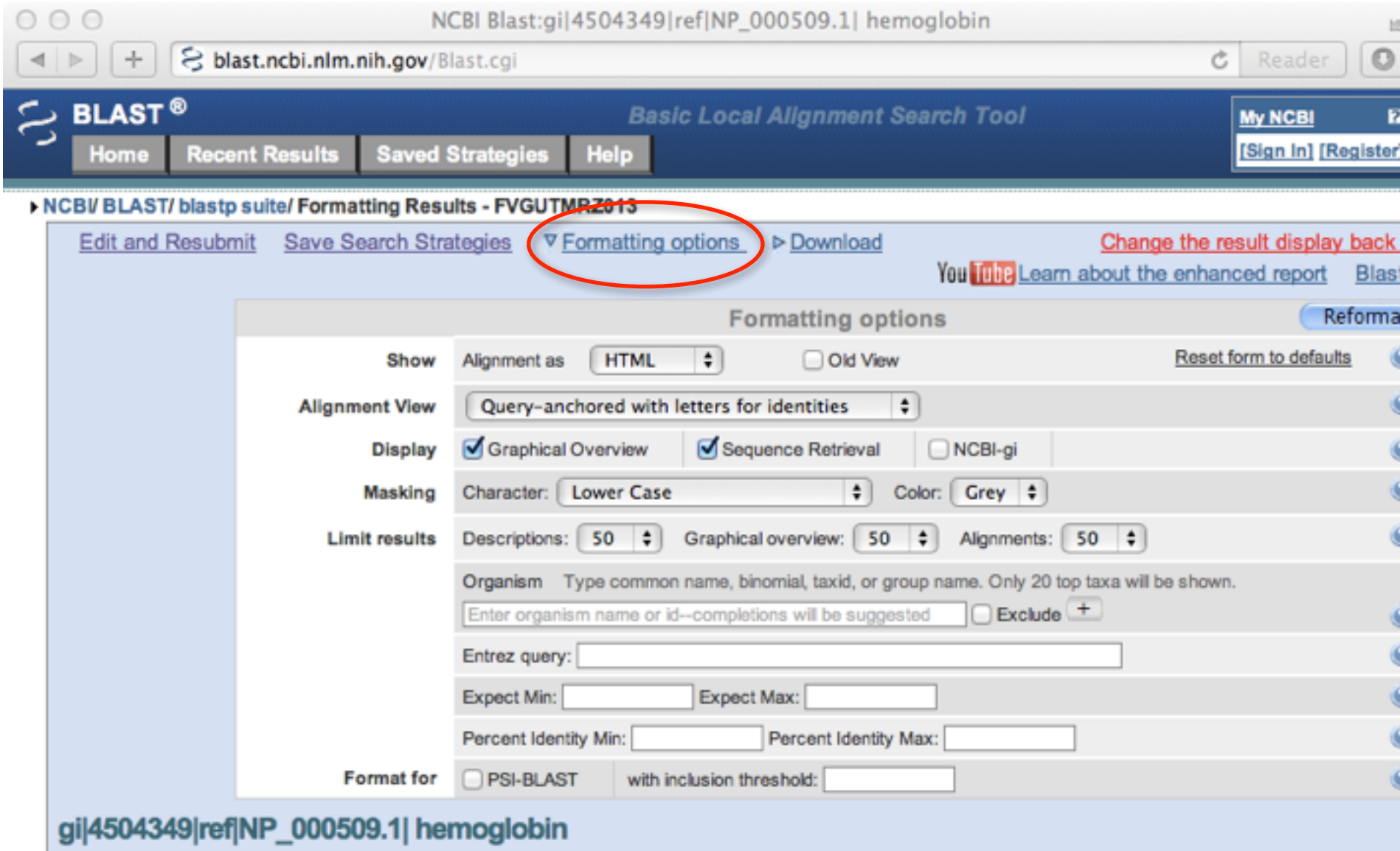


Description	Max score	Total score	Query cover	E value	Max ident	Accession
hemoglobin beta [synthetic construct]	301	301	100%	9e-103	100%	AAX37051.1
hemoglobin beta [synthetic construct]	301	301	100%	1e-102	100%	AAX29557.1
hemoglobin subunit beta [Homo sapiens] >ref|XP_508242.1| PREDICTED: hemoglobin 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|AAZ3978	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

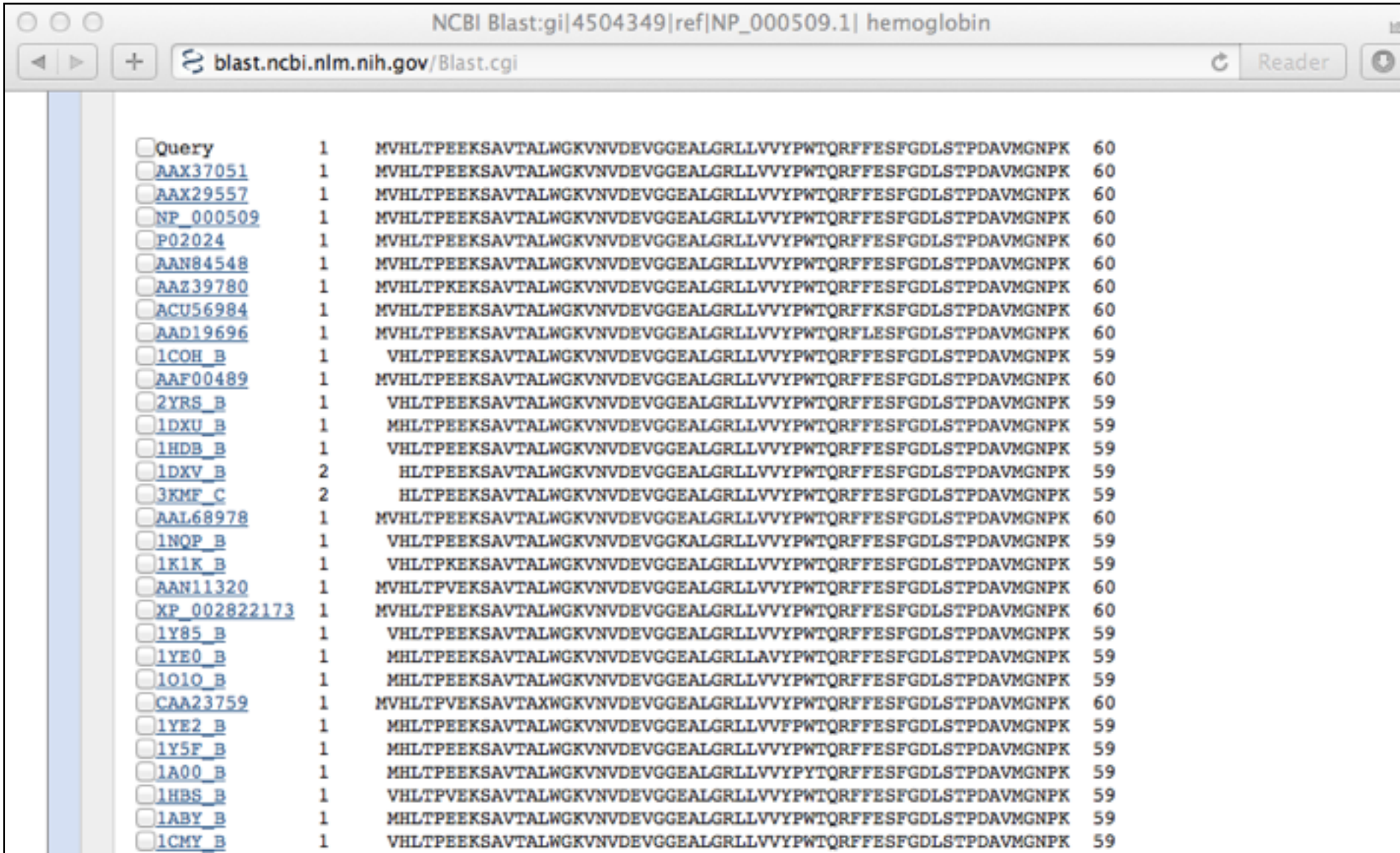
Further down the results page...



Different output formats are available

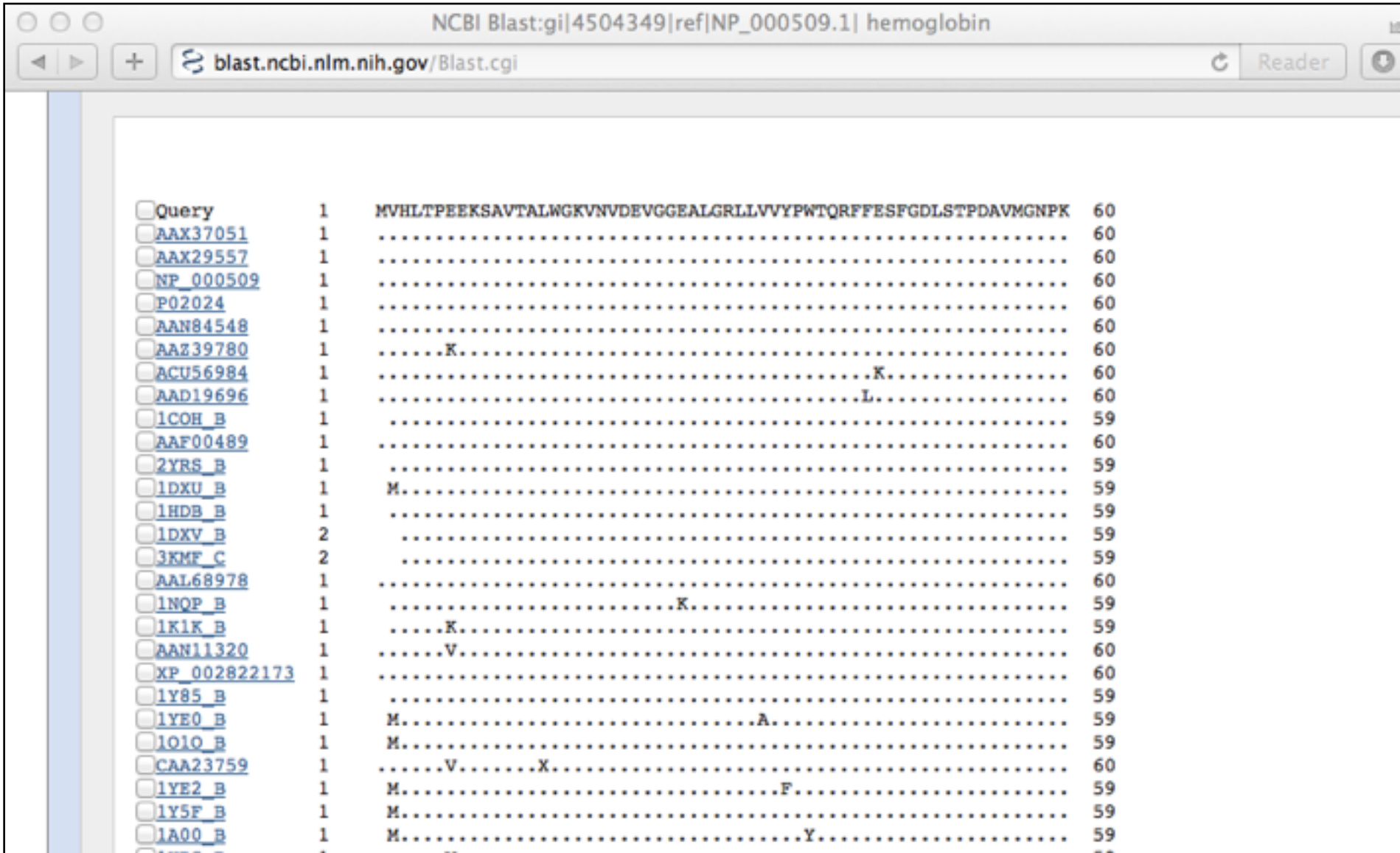


E.g. Query anchored alignments




```
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



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)

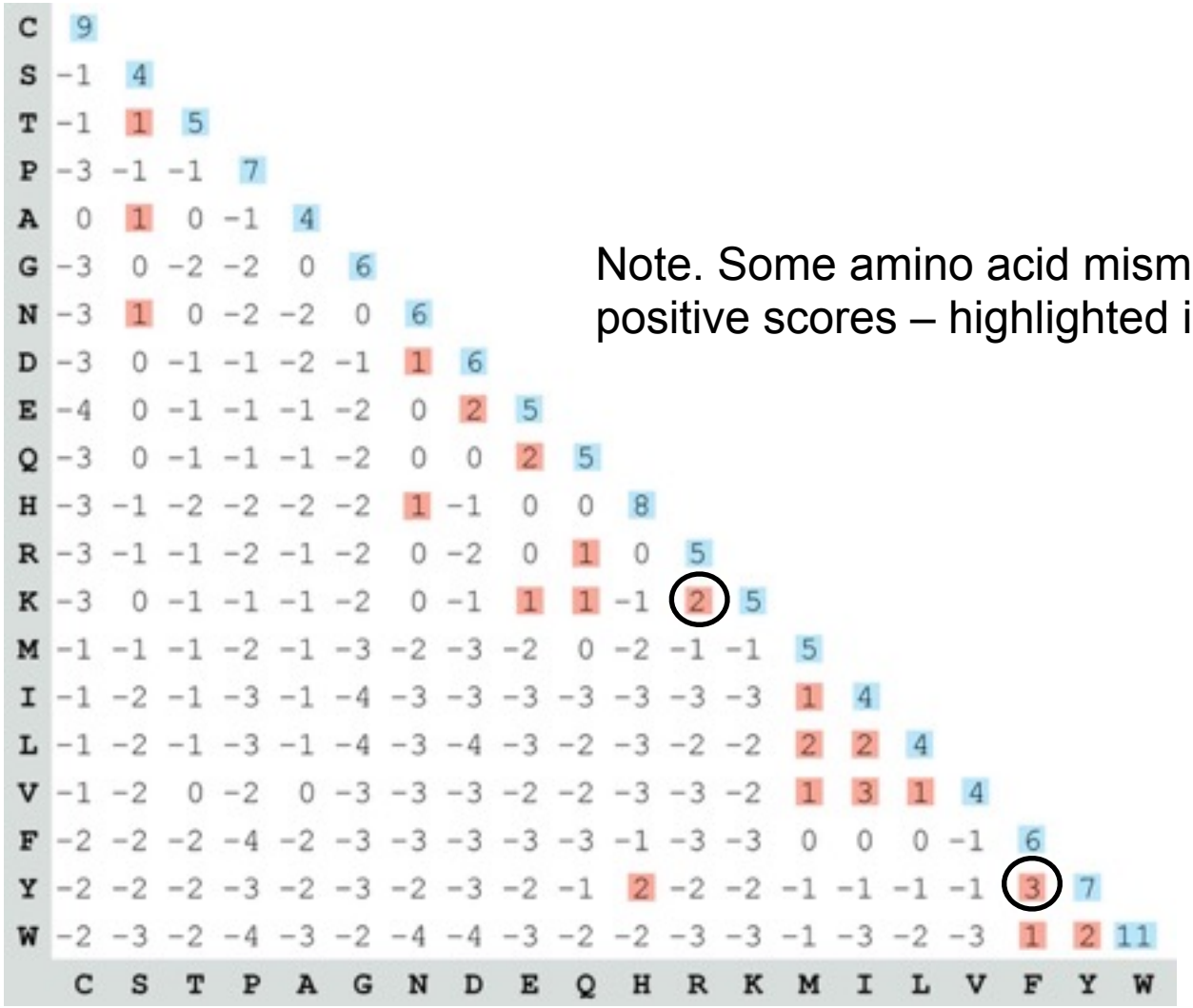
Side note: Scoring matrices

- A substitution matrix contains values proportional to the probability that amino acid *i* mutates into amino acid *j* for all pairs of amino acids
- Substitution matrices are constructed by assembling a large and diverse sample of verified pairwise alignments (or multiple sequence alignments) of amino acids.
- Substitution matrices should reflect the probabilities of mutations occurring through a period of evolution
- The two major types of substitution matrices are **PAM** and **BLOSUM**

BLOSUM62 is the default BLASTp scoring matrix

- BLOSUM matrices are based on short, ungapped blocks of conserved amino acid sequences from multiple alignments
  - members of a block that have a most X percent sequence identity to each other are used to generate a BLOSUM**X** matrix
  - For example, using a cutoff of 62% identity will generate the BLOSUM62 matrix
- PAM matrices are similar but built from multiple alignments where amino acid substitutions are at rate of 1% (PAM 1)
  - Matrix multiplication is used generate higher PAM matrices
  - PAM3 = (PAM1 x PAM1 x PAM1) etc...

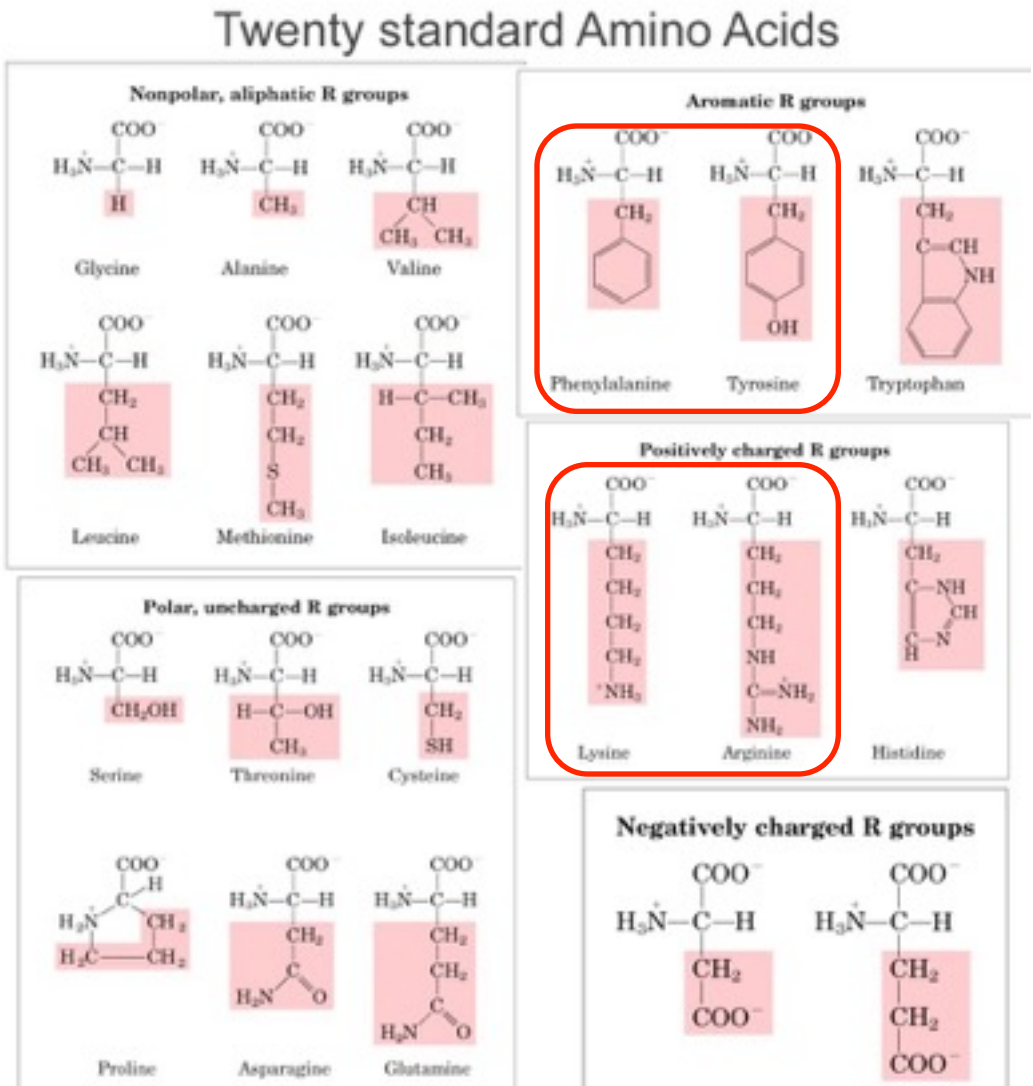
By default BLASTp Match scores come from the BLOSUM62 matrix



	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W
C	9																			
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4															
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6													
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	8									
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	5							
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	5						
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	4					
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	4				
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	4			
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6		
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	7	
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	11

Note. Some amino acid mismatches have positive scores – highlighted in red

Protein scoring matrices reflect the properties of amino acids



Two problems standard BLAST cannot solve

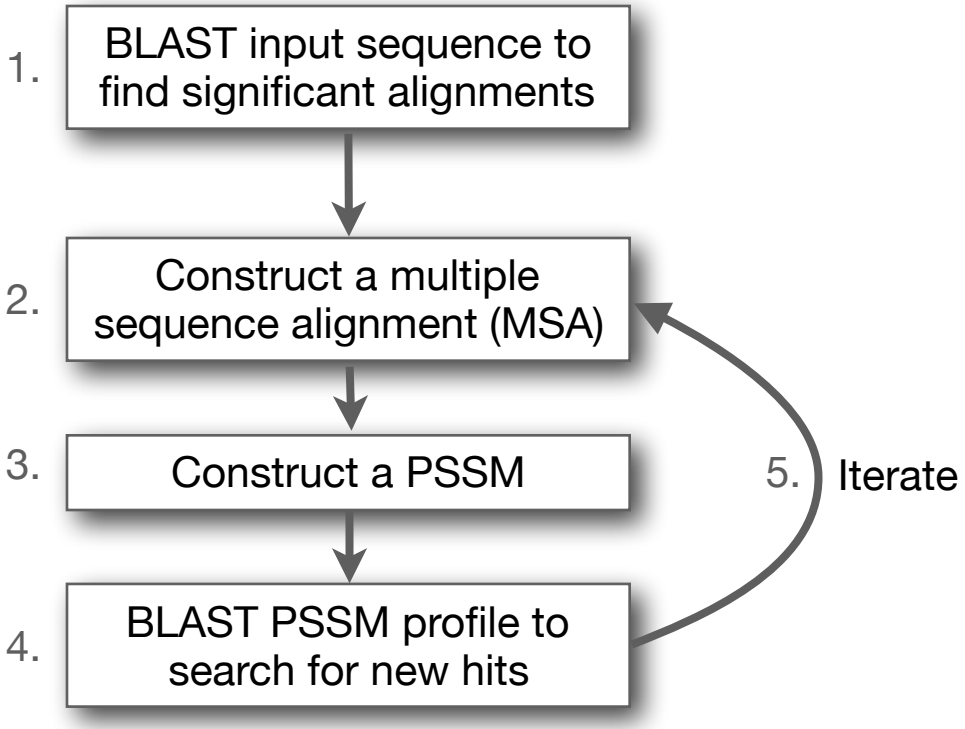
- Use human beta globin as a query against human RefSeq proteins, and blastp does not "find" human myoglobin
  - This is because the two proteins are too distantly related
  - **PSI-BLAST** at NCBI as well as hidden Markov models (HMMs) easily solve this problem
- How can we search using 10,000 base pairs as a query, or even millions of base pairs?
  - Many BLAST-like tools for genomic DNA are now available such as Megablast

PSI-BLAST: Position specific iterated BLAST

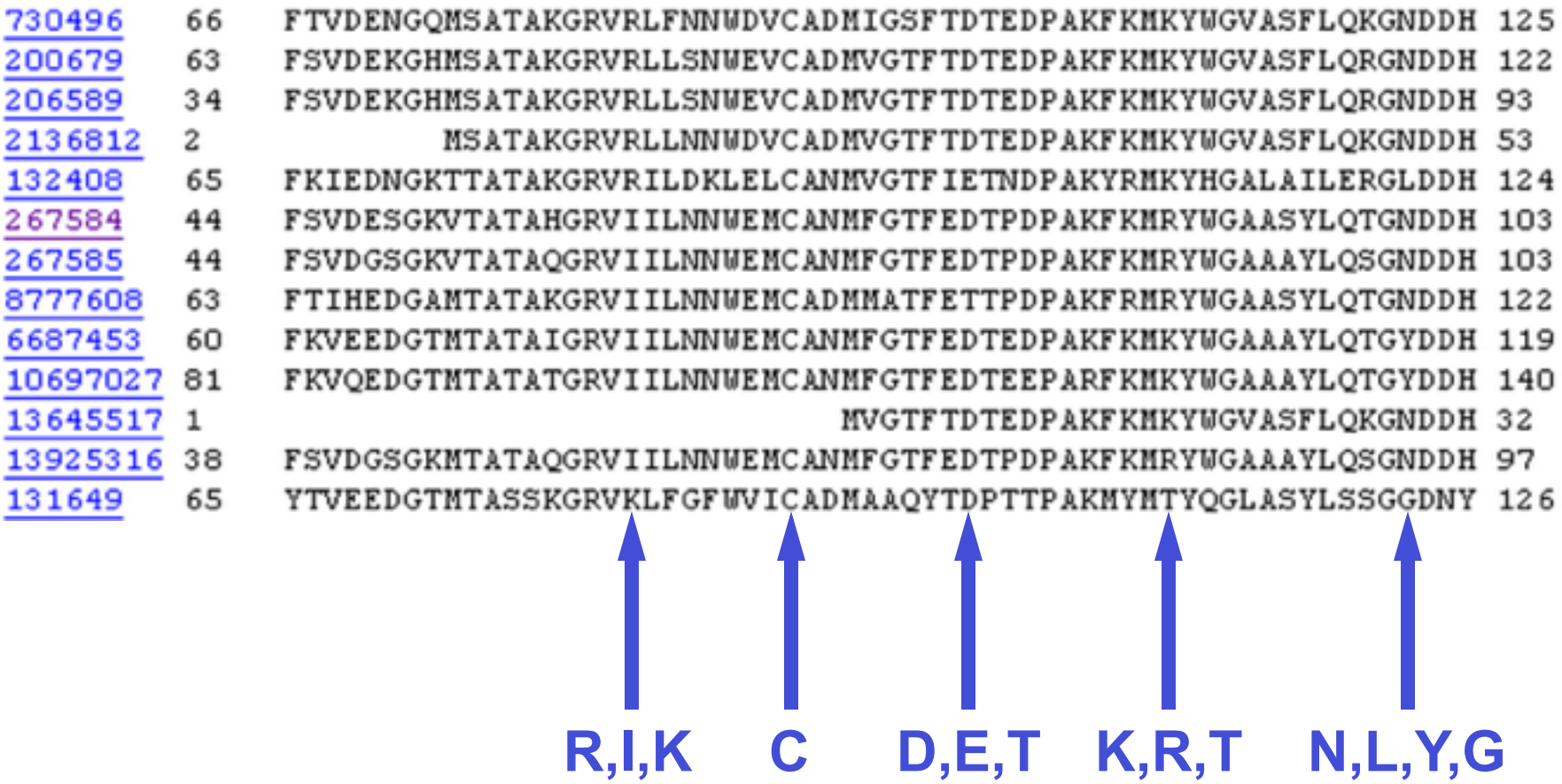
- The purpose of PSI-BLAST is to look deeper into the database for matches to your query protein sequence by employing a scoring matrix that is customized to your query
  - PSI-BLAST constructs a multiple sequence alignment from the results of a first round BLAST search and then creates a "profile" or specialized **position-specific scoring matrix** (**PSSM**) for subsequent search rounds

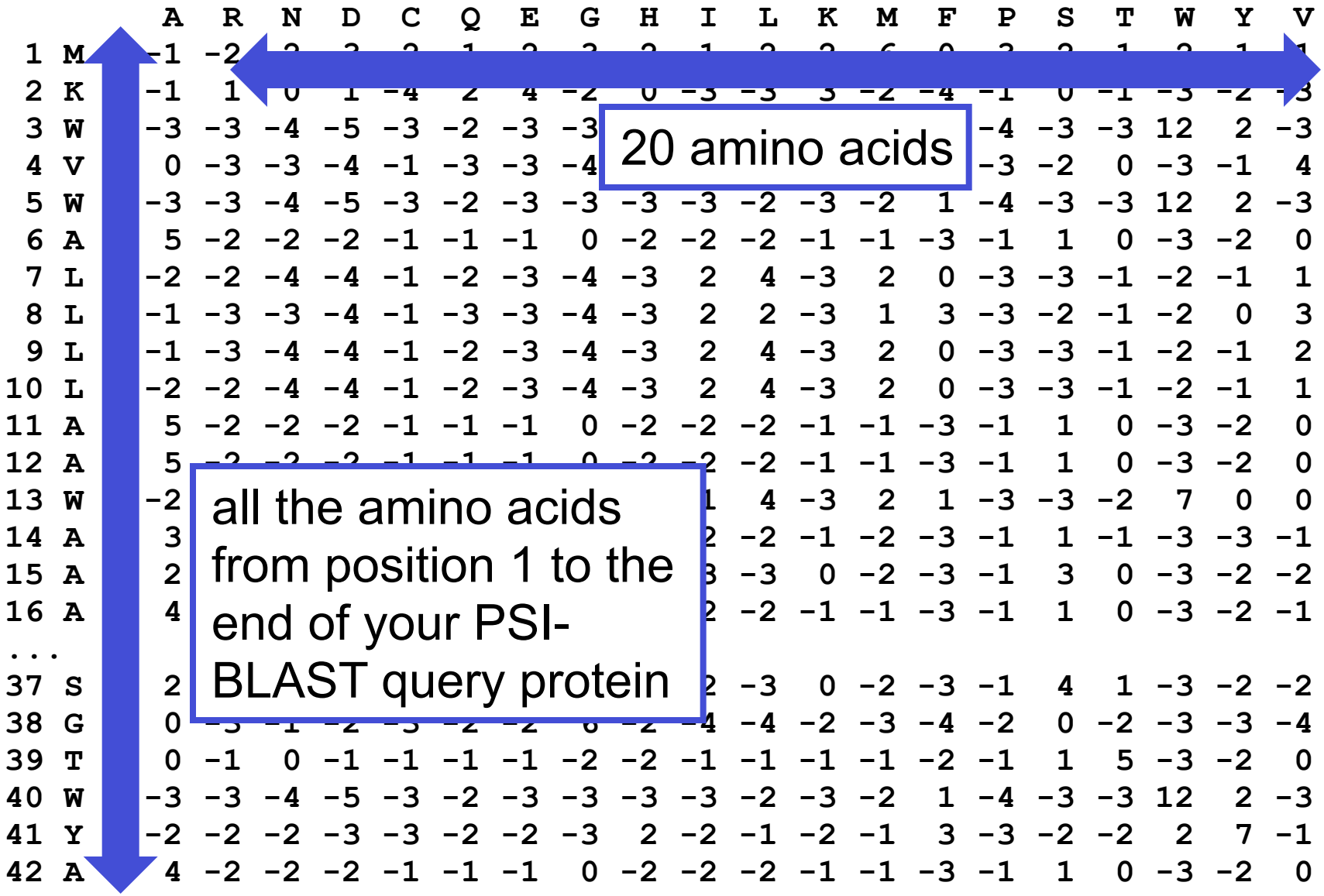
PSI-BLAST: Position-Specific Iterated BLAST

- Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST



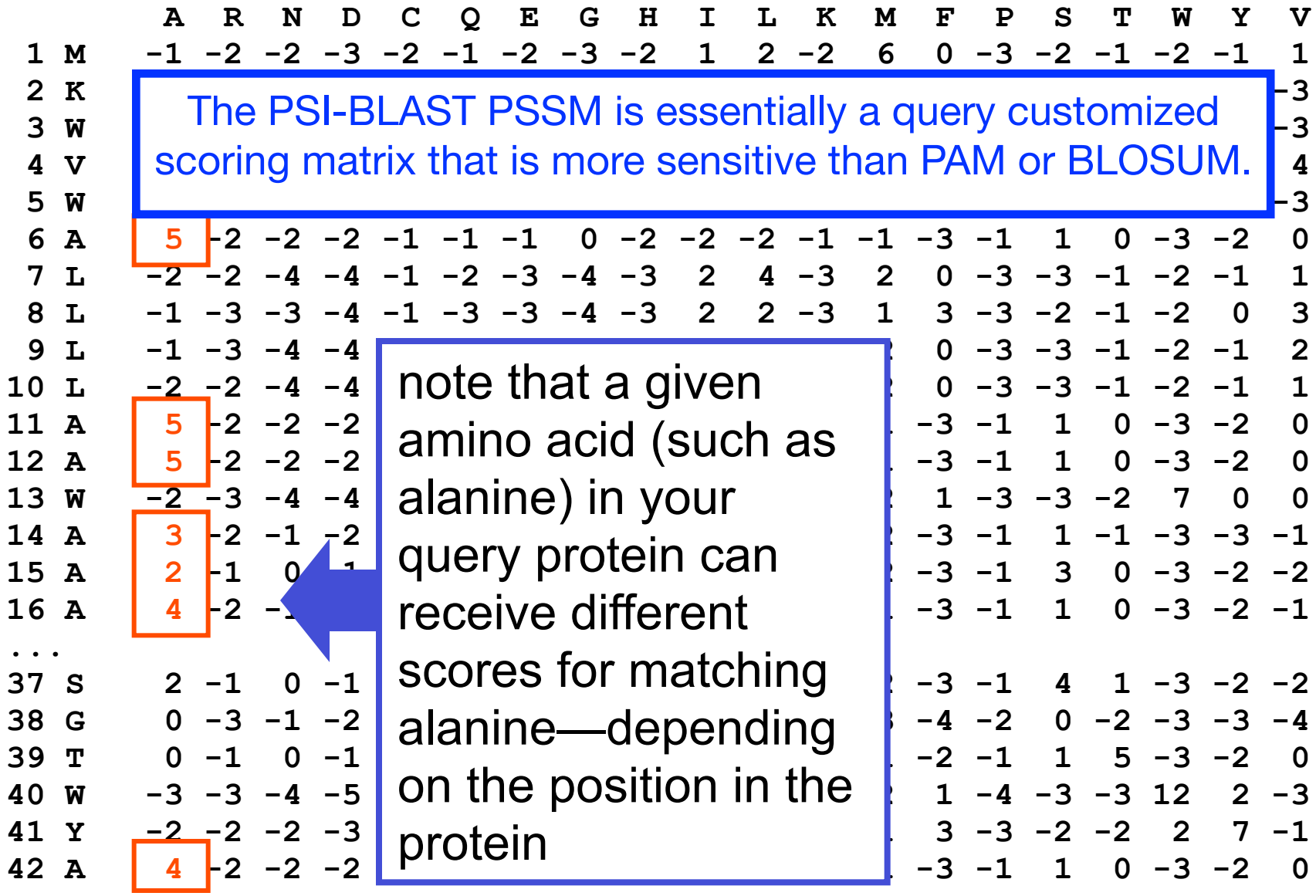
Inspect the blastp output to identify empirical “rules” regarding amino acids tolerated at each position

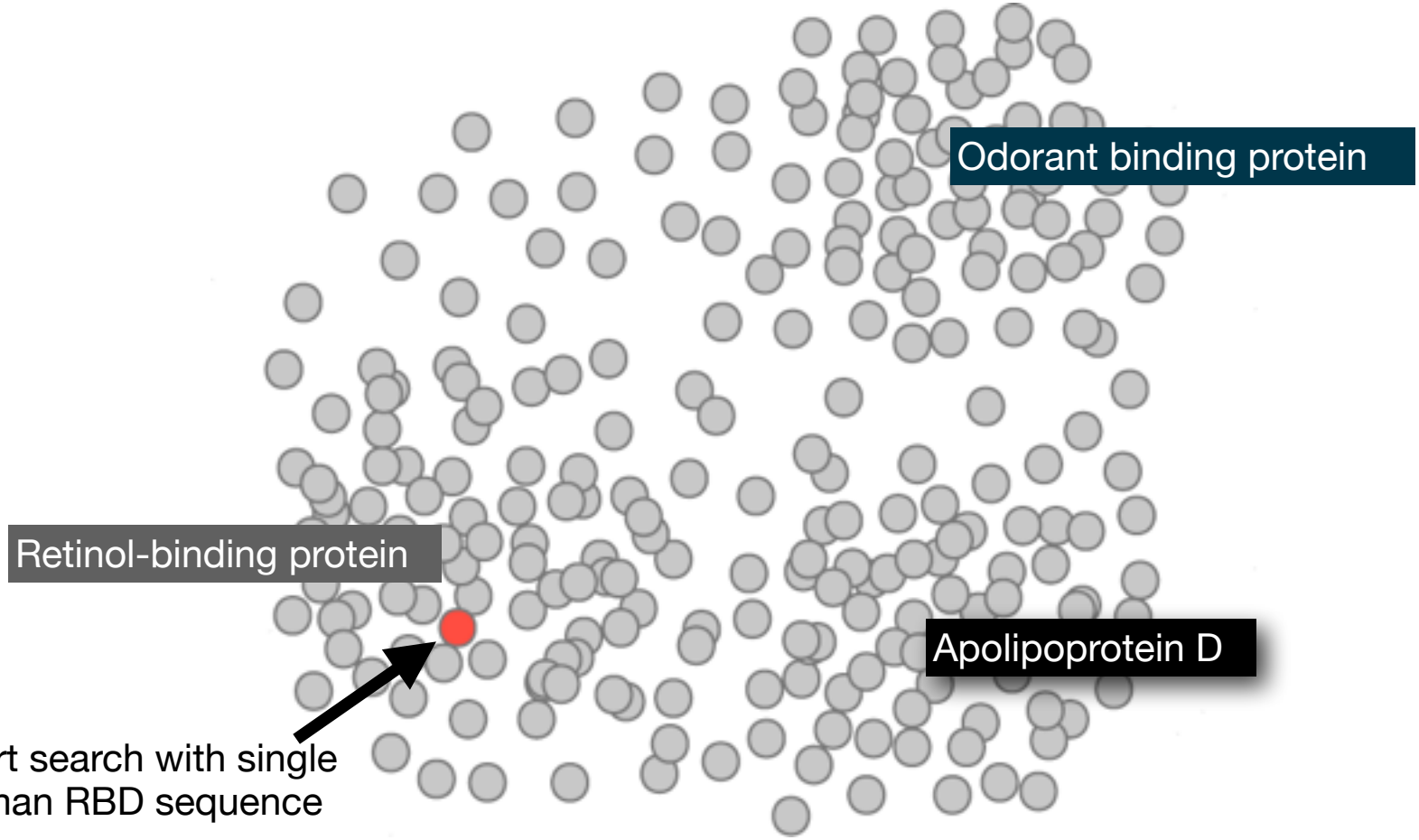


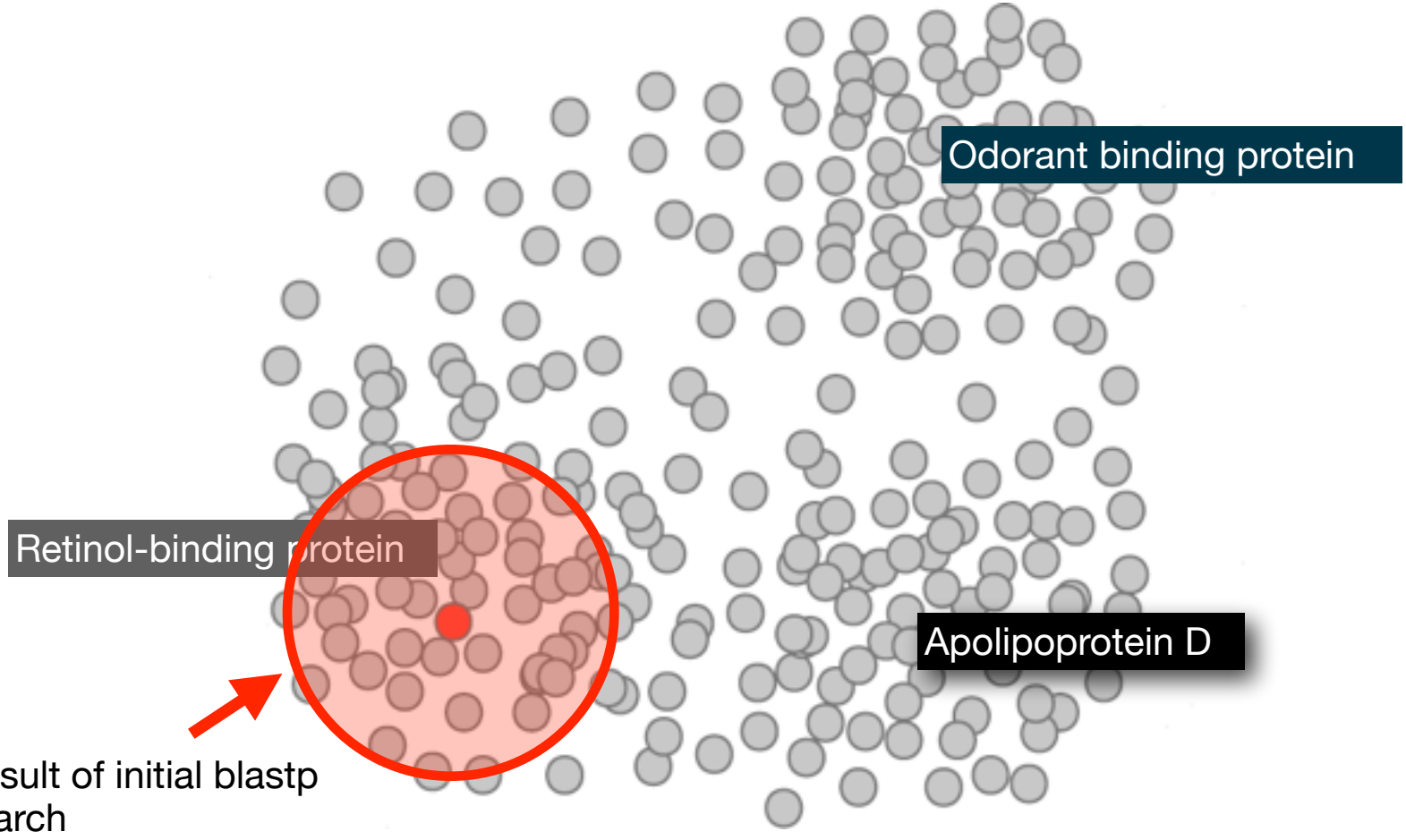

A R N D C Q E G H I L K M F P S T W Y V
1 M -1 -2
2 K -1 1 0 1 -4 2 4 -2 0 -3 -3 3 -2 -4 -1 0 -1 -3 -2 -3
3 W -3 -3 -4 -5 -3 -2 -3 -3 -4 -3 -3 12 2 -3
4 V 0 -3 -3 -4 -1 -3 -3 -4 -3 -2 0 -3 -1 4
5 W -3 -3 -4 -5 -3 -2 -3 -3 -3 -3 -2 -3 -2 1 -4 -3 -3 12 2 -3
6 A 5 -2 -2 -2 -1 -1 -1 0 -2 -2 -2 -1 -1 -3 -1 1 0 -3 -2 0
7 L -2 -2 -4 -4 -1 -2 -3 -4 -3 2 4 -3 2 0 -3 -3 -1 -2 -1 1
8 L -1 -3 -3 -4 -1 -3 -3 -4 -3 2 2 -3 1 3 -3 -2 -1 -2 0 3
9 L -1 -3 -4 -4 -1 -2 -3 -4 -3 2 4 -3 2 0 -3 -3 -1 -2 -1 2
10 L -2 -2 -4 -4 -1 -2 -3 -4 -3 2 4 -3 2 0 -3 -3 -1 -2 -1 1
11 A 5 -2 -2 -2 -1 -1 -1 0 -2 -2 -2 -1 -1 -3 -1 1 0 -3 -2 0
12 A 5 -2 -2 -2 -1 -1 -1 0 -2 -2 -2 -1 -1 -3 -1 1 0 -3 -2 0
13 W -2 4 -3 2 1 -3 -3 -2 7 0 0
14 A 3 -2 -1 -2 -3 -1 1 -1 -3 -3 -1
15 A 2 -3 0 -2 -3 -1 3 0 -3 -2 -2
16 A 4 -2 -1 -1 -3 -1 1 0 -3 -2 -1
...
37 S 2 -3 0 -2 -3 -1 4 1 -3 -2 -2
38 G 0 -4 -4 -2 -3 -4 -2 0 -2 -3 -3 -4
39 T 0 -1 0 -1 -1 -1 -1 -2 -2 -1 -1 -1 -1 -2 -1 1 5 -3 -2 0
40 W -3 -3 -4 -5 -3 -2 -3 -3 -3 -3 -2 -3 -2 1 -4 -3 -3 12 2 -3
41 Y -2 -2 -2 -3 -3 -2 -2 -3 2 -2 -1 -2 -1 3 -3 -2 -2 2 7 -1
42 A 4 -2 -2 -2 -1 -1 -1 0 -2 -2 -2 -1 -1 -3 -1 1 0 -3 -2 0
20 amino acids
all the amino acids from position 1 to the end of your PSI-BLAST query protein

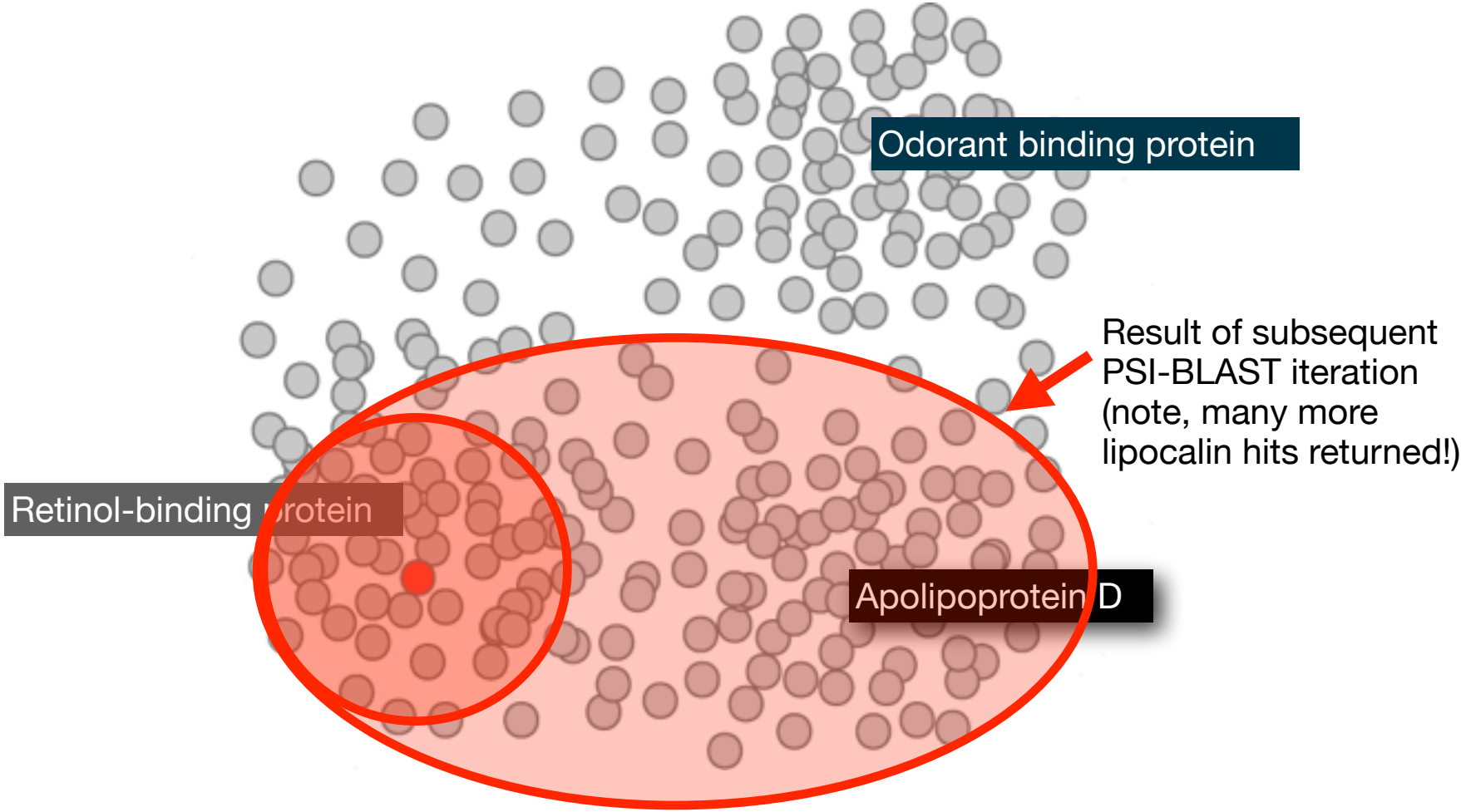
		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
1	M	-1	-2	-2	-3	-2	-1	-2	-3	-2	1	2	-2	6	0	-3	-2	-1	-2	-1	1
2	K	-1	1	0	1	-4	2	4	-2	0	-3	-3	3	-2	-4	-1	0	-1	-3	-2	-3
3	W	-3	-3	-4	-5	-3	-2	-3	-3	-3	-3	-2	-3	-2	1	-4	-3	-3	12	2	-3
4	V	0	-3	-3	-4	-1	-3	-3	-4	-4	3	1	-3	1	-1	-3	-2	0	-3	-1	4
5	W	-3	-3	-4	-5	-3	-2	-3	-3	-3	-3	-2	-3	-2	1	-4	-3	-3	12	2	-3
6	A	5	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	1	0	-3	-2	0
7	L	-2	-2	-4	-4	-1	-2	-3	-4	-3	2	4	-3	2	0	-3	-3	-1	-2	-1	1
8	L	-1	-3	-3	-4	-1	-3	-3	-4	-3	2	2	-3	1	3	-3	-2	-1	-2	0	3
9	L	-1	-3	-4	-4										0	-3	-3	-1	-2	-1	2
10	L	-2	-2	-4	-4										0	-3	-3	-1	-2	-1	1
11	A	5	-2	-2	-2										-3	-1	1	0	-3	-2	0
12	A	5	-2	-2	-2										-3	-1	1	0	-3	-2	0
13	W	-2	-3	-4	-4										1	-3	-3	-2	7	0	0
14	A	3	-2	-1	-2										-3	-1	1	-1	-3	-3	-1
15	A	2	-1	0	1										-3	-1	3	0	-3	-2	-2
16	A	4	-2	-1											-3	-1	1	0	-3	-2	-1
...																					
37	S	2	-1	0	-1										-3	-1	4	1	-3	-2	-2
38	G	0	-3	-1	-2										-4	-2	0	-2	-3	-3	-4
39	T	0	-1	0	-1										-2	-1	1	5	-3	-2	0
40	W	-3	-3	-4	-5										1	-4	-3	-3	12	2	-3
41	Y	-2	-2	-2	-3										3	-3	-2	-2	2	7	-1
42	A	4	-2	-2	-2										-3	-1	1	0	-3	-2	0

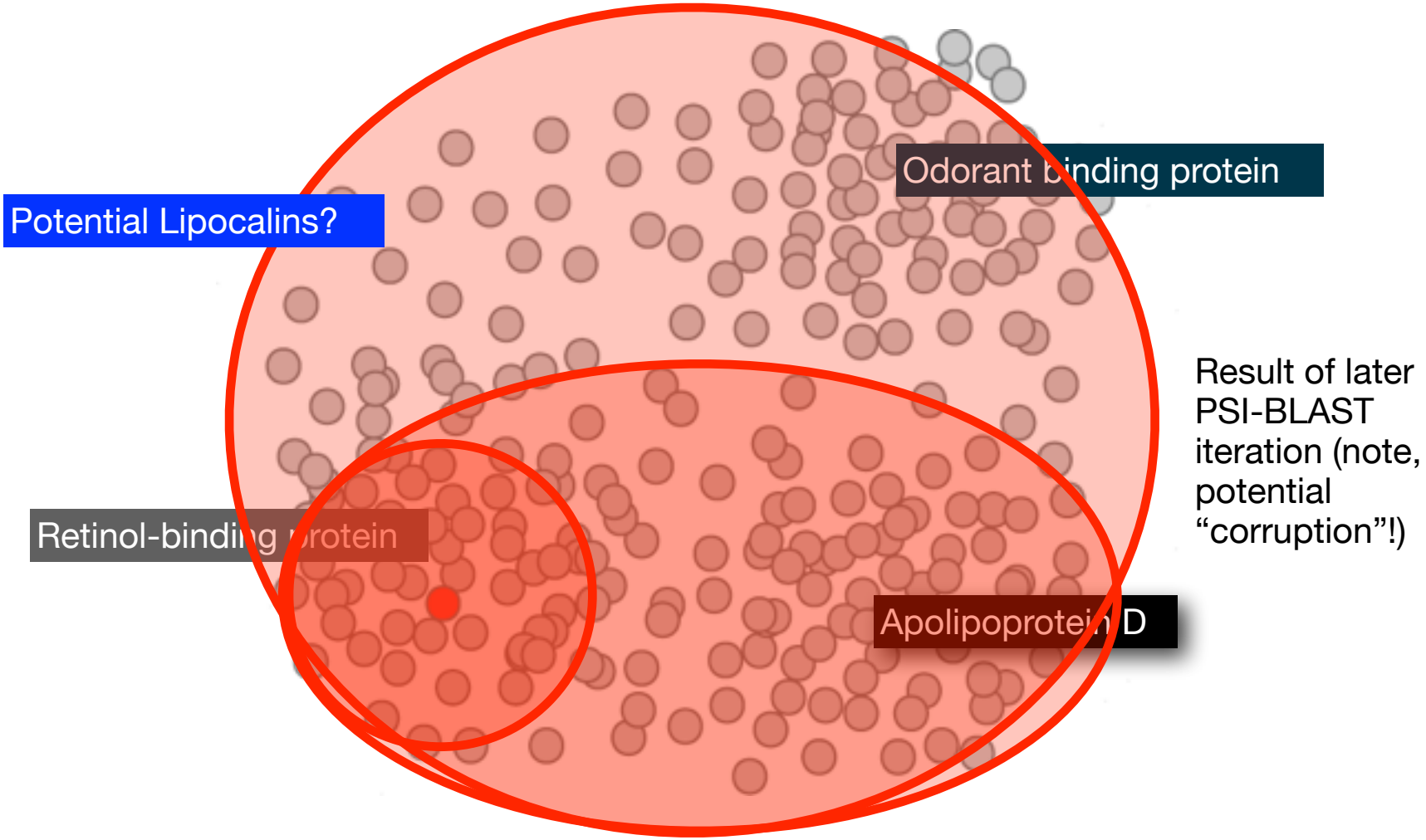
note that a given amino acid (such as alanine) in your query protein can receive different scores for matching alanine—depending on the position in the protein


A R N D C Q E G H I L K M F P S T W Y V
1 M -1 -2 -2 -3 -2 -1 -2 -3 -2 1 2 -2 6 0 -3 -2 -1 -2 -1 1
2 K -3
3 W -3
4 V 4
5 W -3
6 A 5 -2 -2 -2 -1 -1 -1 0 -2 -2 -2 -1 -1 -3 -1 1 0 -3 -2 0
7 L -2 -2 -4 -4 -1 -2 -3 -4 -3 2 4 -3 2 0 -3 -3 -1 -2 -1 1
8 L -1 -3 -3 -4 -1 -3 -3 -4 -3 2 2 -3 1 3 -3 -2 -1 -2 0 3
9 L -1 -3 -4 -4 0 -3 -3 -1 -2 -1 2
10 L -2 -2 -4 -4 0 -3 -3 -1 -2 -1 1
11 A 5 -2 -2 -2 -3 -1 1 0 -3 -2 0
12 A 5 -2 -2 -2 -3 -1 1 0 -3 -2 0
13 W -2 -3 -4 -4 1 -3 -3 -2 7 0 0
14 A 3 -2 -1 -2 -3 -1 1 -1 -3 -3 -1
15 A 2 -1 0 -3 -1 3 0 -3 -2 -2
16 A 4 -2 -3 -1 1 0 -3 -2 -1
...
37 S 2 -1 0 -1 -3 -1 4 1 -3 -2 -2
38 G 0 -3 -1 -2 -4 -2 0 -2 -3 -3 -4
39 T 0 -1 0 -1 -2 -1 1 5 -3 -2 0
40 W -3 -3 -4 -5 1 -4 -3 -3 12 2 -3
41 Y -2 -2 -2 -3 3 -3 -2 -2 2 7 -1
42 A 4 -2 -2 -2 -3 -1 1 0 -3 -2 0
The PSI-BLAST PSSM is essentially a query customized scoring matrix that is more sensitive than PAM or BLOSUM.
note that a given amino acid (such as alanine) in your query protein can receive different scores for matching alanine—depending on the position in the protein


Odorant binding protein
Retinol-binding protein
Apolipoprotein D
Start search with single
human RBD sequence


Odorant binding protein
Retinol-binding protein
Apolipoprotein D
Result of initial blastp search


Odorant binding protein
Result of subsequent PSI-BLAST iteration (note, many more lipocalin hits returned!)
Retinol-binding protein
Apolipoprotein D



Result of later PSI-BLAST iteration (note, potential "corruption"!)

PSI-BLAST returns dramatically more hits

- The search process is continued iteratively, typically about five times, and at each step a new PSSM is built
  - You must decide how many iterations to perform and which sequences to include!
  - You can stop the search process at any point - typically whenever few new results are returned or when no new "sensible" results are found

Iteration	Hits with E < 0.005	Hits with E > 0.005
1	34	61
2	314	79
3	416	57
4	432	50
5	432	50

Human retinol-binding protein 4 (RBP4; P02753) was used as a query in a PSI-BLAST search of the RefSeq database.

HMMER

biosequence analysis using profile hidden Markov models

HHMI janelia farm research campus

HMMER3: a new generation of sequence homology search software

HMMER is used for searching sequence databases for homologs of protein sequences, and for making protein sequence alignments. It implements methods using probabilistic models called **profile hidden Markov models** (profile HMMs).

Compared to BLAST, FASTA, and other sequence alignment and database search tools based on older scoring methodology, HMMER aims to be significantly *more* accurate and *more* able to detect remote homologs because of the strength of its underlying mathematical models. In the past, this strength came at significant computational expense, but in the new HMMER3 project, HMMER is now essentially **as fast as** BLAST.

As part of this evolution in the HMMER software, we are committed to making the software available to as many scientists as possible. Earlier releases of HMMER were restricted to command line use. To make the software more accessible to the wide scientific community, we now provide **servers** that allow sequence searches to be performed interactively via the **Web**.

The current version is **HMMER 3.0** (28 March 2010) and can be downloaded from the software section of the site. Previous versions of the HMMER software can be obtained from the archive section.

If you have used the HMMER website, please consider citing the following reference that describes this work:

HMMER web server: interactive sequence similarity searching
R.D. Finn, J. Clements, S.R. Eddy
Nucleic Acids Research (2011) Web Server Issue 39:W29-W37. PDF

Download HMMER

Get the latest version

v3.0

Release notes (28 March 2010)

TAR.GZ

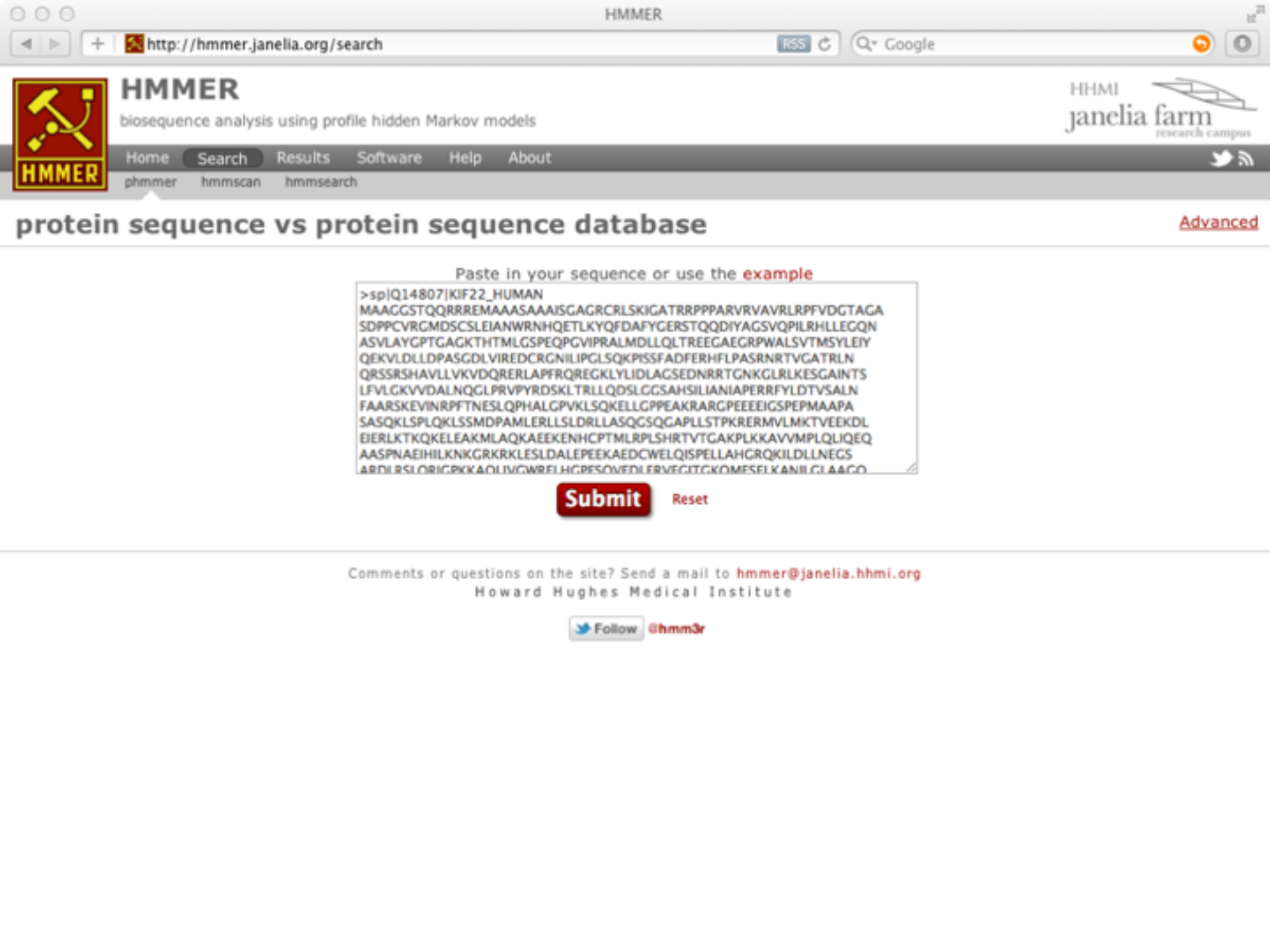
Alternative Download Options

Source

Search

Perform an interactive search now.

Search


HMMER
http://hmmer.janelia.org/search
Google
HMMER
biosequence analysis using profile hidden Markov models
HHMI janelia farm research campus
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Results
Software
Help
About
phmmer
hmmscan
hmmsearch
protein sequence vs protein sequence database
Advanced
Paste in your sequence or use the example
>sp|Q14807|KIF22_HUMAN
MAAGGSTQQRRREMAAASAAAISGAGRCRLSKIGATRRPPPARVRVAVRLRPFVDGTAGA
SDPPCVRGMDSCSLEIANWRNHQETLKYQFDAFYGERSTQQDIYAGSVQPILRHLLEGQN
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ARDLRSLQRIGPKKAQLIVGWREHGPFSQVEDLERVEGITGKQMESLKANILCLAAGQ
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HMMER

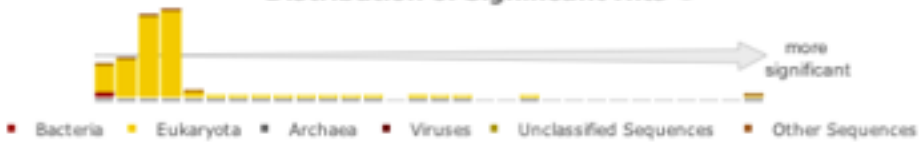
biosequence analysis using profile hidden Markov models

HHMI janelia farm research campus

Pfam Domains

Show hit details

Distribution of Significant Hits



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Query Matches (5100) Customize

Target	Description	Species	E-value	Alignments (show all)
123979736	kinesin family member 22	synthetic construct	0.0e+00	show
6453818	kinesin-like protein KIF22	Homo sapiens	0.0e+00	show
30584615	Homo sapiens kinesin-like 4	synthetic construct	0.0e+00	show
123994513	kinesin family member 22	synthetic construct	0.0e+00	show
189053342	unnamed protein product	Homo sapiens	0.0e+00	show
62898423	kinesin family member 22 variant	Homo sapiens	0.0e+00	show
332845643	PREDICTED: kinesin family member 22 isoform 2	Pan troglodytes	0.0e+00	show
75062021	RecName: Full=Kinesin-like protein KIF22	Pongo abelii	0.0e+00	show
332266048	PREDICTED: kinesin-like protein KIF22-like isoform 1	Nomascus leucogenys	0.0e+00	show
297283748	PREDICTED: hypothetical protein LOC706401 isoform 3	Macaca mulatta	0.0e+00	show
296219941	PREDICTED: LOW QUALITY PROTEIN: kinesin-like protein KIF22-like	Callithrix jacchus	0.0e+00	show
296196456	PREDICTED: kinesin-like protein KIF22-like	Callithrix jacchus	0.0e+00	show
335284407	PREDICTED: kinesin-like protein KIF22-like	Sus scrofa	0.0e+00	show
221046166	unnamed protein product	Homo sapiens	0.0e+00	show
221045488	unnamed protein product	Homo sapiens	0.0e+00	show

Query

Jump to the exact match for your query architecture

« First « Previous Page 1 of 7 Next » Last »

Domain Architectures

3624 SEQUENCES Show All	with domain architecture: **Kinesin**, *example:*148685550 	View Scores
126 SEQUENCES Show All	with domain architecture: **Kinesin, FHA**, *example:*157125836 	View Scores
101 SEQUENCES Show All	with domain architecture: **Kinesin, Kinesin**, *example:*296088325 	View Scores
80 SEQUENCES Show All	with domain architecture: **Kinesin, FHA, KIF1B, DUF3694, PH**, *example:*118101106 	View Scores
69 SEQUENCES Show All	with domain architecture: **HHH_3**, *example:*337289058 	View Scores
62 SEQUENCES Show All	with domain architecture: **CH, Kinesin**, *example:*224061629 	View Scores
60 SEQUENCES Show All	**Exact match with query architecture: Kinesin, HHH_3**, *example:*332266048 	View Scores

HMMER

biosequence analysis using profile hidden Markov models

HHMI janelia farm research campus

- **Job:** 9924F9AC-FEB5-11E0-A304-2B0C998A7913
- **Started:** 2011-10-24 23:01:15
- **Algorithm:** phmmer
- **HMMER Options:** -E 1 --domE 1 --incE 0.01 --incdomE 0.03 --mx BLOSUM62 --pextend 0.4 --popen 0.02 --seqdb nr

▼ Format

FASTA
Download the significant hits from your search as a gzipped FASTA file.

Full length FASTA
A gzipped file containing the full length sequences for significant search hits.

Aligned FASTA
A gzipped file containing aligned significant search hits in FASTA format.

STOCKHOLM
Download an alignment of significant hits as a gzipped STOCKHOLM file.

Text
A plain text file containing the hit alignments and scores.

XML
An XML file formated for machine parssing of the data.

JSON
All the results information encoded as a single json string.

HMM
Profile HMM downloads are not available.

Download Reset

Summary

- Alignment basics
  - Why compare biological sequences?
- Homologue detection
  - Orthologs, paralogs, similarity and identity
  - Sequence changes during evolution
  - Alignment view: matches, mismatches and gaps
- Pairwise sequence alignment methods
  - Brute force alignment
  - Dot matrices
  - Dynamic programing

  (global vs local alignment)
- Rapid heuristic approaches
  - BLAST
- Practical database searching
  - BLAST, PSI-BLAST and HMM approaches