



BIMM 143

Hands-on Lab Session

Class 03

Barry Grant

UC San Diego

<http://thegrantlab.org/bimm143>

Class 3: Hands-on section

Week 2

<http://thegrantlab.org/bimm143/>

UC San Diego

BIMM 143

A hands-on introduction to the computer-based analysis of genomic and biomolecular data from the [Division of Biological Sciences, UCSD](#).

Overview

Schedule

Computer Setup

Learning Goals

Session	Date	Topic
2	09/30/21	Needleman-Wunsch, Smith-Waterman and BLAST heuristic approaches, Hands on with dot plots, Needleman-Wunsch and BLAST algorithms highlighting their utility and limitations.
3	Tue 10/05/21	Project: Find a gene project assignment (Part 1) Principles of database searching, due in 2 weeks. (Part 2) Sequence analysis, structure analysis and general data analysis with R due at the end of the quarter.
*	Tue 10/05/21	Optional: Advanced sequence alignment and database searching Detecting remote sequence similarity, Database searching beyond BLAST, Substitution matrices, Using PSI-BLAST, Profiles and HMMs, Protein structure comparisons as a gold standard.
4	Thu 10/07/21	Bioinformatics data analysis with R Why do we use R for bioinformatics? R language basics and the RStudio IDE, Major R data structures and functions, Using R interactively from the RStudio console. Introducing Rmarkdown documents.
		Data exploration and visualization in R

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 - ➔ The complete assignment, including responses to **all questions**, is due 12pm Monday of week 10 (**Dec 4th**, 12/03/23).

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Questions:

[Q1] Tell me the name of a protein you are interested in. Include the species and the accession number. This can be a human protein or a protein from any other species as long as its function is known.

If you do not have a favorite protein, select human RBP4 or KIF11. Do not use beta globin as this is in the worked example report that I provide you with online.

[Q2] Perform a BLAST search against a DNA database, such as a database consisting of genomic DNA or ESTs. The BLAST server can be at NCBI or elsewhere. Include details of the BLAST method used, database searched and any limits applied (e.g. Organism).

Also include the output of that BLAST search in your document. If appropriate, change the font to Courier size 10 so that the results are displayed neatly. You can also screen capture a BLAST output (e.g. alt print screen on a PC or on a MAC press ⌘-shift-4. The pointer becomes a bulls eye. Select the area you wish to capture and release. The image is saved as a file called Screen Shot [].png in your Desktop directory). It is **not** necessary to print out all of the blast results if there are many pages.

On the BLAST results, clearly indicate a match that represents a protein sequence, encoded from some DNA sequence, that is homologous to your query protein. I need to be able to inspect the pairwise alignment you have selected, including the E value and score. It should be labeled a "genomic clone" or "mRNA sequence", etc. - but include no functional annotation.

In general, [Q2] is the most difficult for students because it requires you to have a "feel" for how to interpret BLAST results. You need to distinguish between a perfect match to your query (i.e. a sequence that is not "novel"), a near match (something that might be "novel", depending on the results of [Q4]), and a non-homologous result.

If you are having trouble finding a novel gene try restricting your search to an organism that is poorly annotated.

[Q3] Gather information about this "novel" **protein**. At a minimum, show me the protein sequence of the "novel" protein as displayed in your BLAST results from [Q2] as FASTA format (you can copy and paste the aligned sequence subject lines from your BLAST result page if necessary) or translate your novel DNA sequence using a tool called EMBOSS Transeq at the EBI. Don't forget to translate all six reading frames; the ORF (open reading frame) is likely to be the longest sequence without a stop codon. It may not start with a methionine if you don't have the complete coding region. Make sure the sequence you provide includes a header/subject line and is in traditional FASTA format.

Here, tell me the name of the novel protein, and the species from which it derives. It is very unlikely (but still definitely possible) that you will find a novel gene from an organism such as *S. cerevisiae*, human or mouse, because those genomes have already been thoroughly annotated. It is more likely that you will discover a new gene in a genome that is currently being sequenced, such as bacteria or plants or protozoa.

[Q4] Prove that this gene, and its corresponding protein, are novel. For the purposes of this project, "novel" is defined as follows. Take the protein sequence (your answer to [Q3]), and use it as a query in a blastp search of the nr database at NCBI.

- If there is a match with 100% amino acid identity to a protein in the database, from the same species, then your protein is NOT novel (even if the match is to a protein with a name such as "unknown"). Someone has already found and annotated this sequence, and assigned it an accession number.
- If the top match reported has less than 100% identity, then it is likely that your protein is novel, and you have succeeded.
- If there is a match with 100% identity, but to a different species than the one you started with, then you have likely succeeded in finding a novel gene.
- If there are no database matches to the original query from [Q1], this indicates that you have partially succeeded: yes, you may have found a new gene, but no, it is not actually homologous to the original query. You should probably start over.

[Q5] Generate a multiple sequence alignment with your novel protein, your original query protein, and a group of other members of this family from different species. A typical number of proteins to use in a multiple sequence alignment for this assignment purpose is a minimum of 5 and a maximum of 20 - although the exact number is up to you. Include the multiple sequence alignment in your report. Use Courier font with a size appropriate to fit page width.

Side-note: Indicate your sequence in the alignment by choosing an appropriate name for each sequence in the input unaligned sequence file (i.e. edit the sequence file so that the species, or short common, names (rather than accession numbers) display in the output alignment and in the subsequent answers below). The goal in this step is to create an interesting alignment for building a phylogenetic tree that illustrates species divergence.

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Assignments & Grading

Ethics Code

3: (Project) Find a Gene Assignment Part 1

The [find-a-gene project](#) is a required assignment for BIMM-143. The objective with this assignment is for you to demonstrate your grasp of database searching, sequence analysis, structure analysis and the R environment that we have covered to date in class.

You may wish to consult the scoring rubric at the end of the above linked project description and the [example report](#) for format and content guidance.

- Your responses to questions Q1-Q4 are due **Tuesday Oct 19th** (10/19/21) at 12pm San Diego time.
- The complete assignment, including responses to all questions, is due **Thursday Dec 2nd** (12/02/21) at 12pm San Diego time.
- In both instances your PDF format report should be submitted to GradeScope. Late responses will not be accepted under any circumstances.

Videos:

- 3.1 - [Project introduction](#) Please note: due dates may differ from those in video.

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BIMM 143

A hands-on introduction to

Home Gmail Gcal GitHub BIMM143 BGGN213 GDrive Atmosphere CloudLaunch BIMM194 Blink News +

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► Details:

Sequence 1

Sequence 2

Match Score Mismatch Score Gap Score

G T C G A C G C
 G A T T A C - -
 Score = -4

		G	T	C	G	A	C	G	C
	0	-2	-4	-6	-8	-10	-12	-14	-16
G	-2	1	-1	-3	-5				
A	-4	-1	0	-2	-4				
T	-6	-3	0	-1	-3	-5	-5	-7	-9
T	-8	-5	-2	-1	-2	-4	-6	-6	-8
A	-10	-7	-4	-3	-2	-1	-3	-5	-7
C	-12	-9	-6	-3	-4	-3	0	-2	-4

Score from Diagonal cell $-6 + 1$ (Due to a match between G & G) = -5	Score from Upper cell $-8 + -2$ (The Gap score) = -10
Score from Side cell $-3 + -2$ (The Gap score) = -5	Winning (max) score is -5

▼ Reference:

See the lecture and hands-on session for class 2 for a full discussion of Global, Local, and various Heuristic approaches to biomolecular sequence alignment.

[Barry J Grant.](#)

Key Question:

Q. Where do our alignment match and mis-match scores typically come from?

Algorithm parameters

Protein BLAST (BLASTp)

General Parameters

Max target sequences	<input type="text" value="100"/>	Select the maximum number of aligned sequences to display
Short queries	<input checked="" type="checkbox"/>	Automatically adjust parameters for short input sequences
Expect threshold	<input type="text" value="10"/>	
Word size	<input type="text" value="3"/>	
Max matches in a query range	<input type="text" value="0"/>	

Scoring Parameters

Matrix	<input type="text" value="BLOSUM62"/>	
Gap Costs	<input type="text" value="Existence: 11 Extension: 1"/>	
Compositional adjustments	<input type="text" value="Conditional compositional score matrix adjust"/>	

Scoring matrix
For match & mis-match scores

Filters and Masking

Filter	<input type="checkbox"/>	Low complexity regions
Mask	<input type="checkbox"/>	Mask for lookup table only
	<input type="checkbox"/>	Mask lower case letters

BLAST

Search **database Non-redundant protein sequences (nr)** using **Blastp**
 Show results in a new window

By default BLASTp match scores come from the BLOSUM62 matrix

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
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

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R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	5								
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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	
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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	
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C : C scores +9

V : V scores +4

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F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	6		
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Blocks **S**ubstitution **M**atrix. Scores obtained from observed frequencies of substitutions in blocks of aligned sequences with no more than 62% identity.

Note. Some amino acid **mismatches** have positive scores (highlighted in red) reflecting the shared physicochemical properties of these amino acids

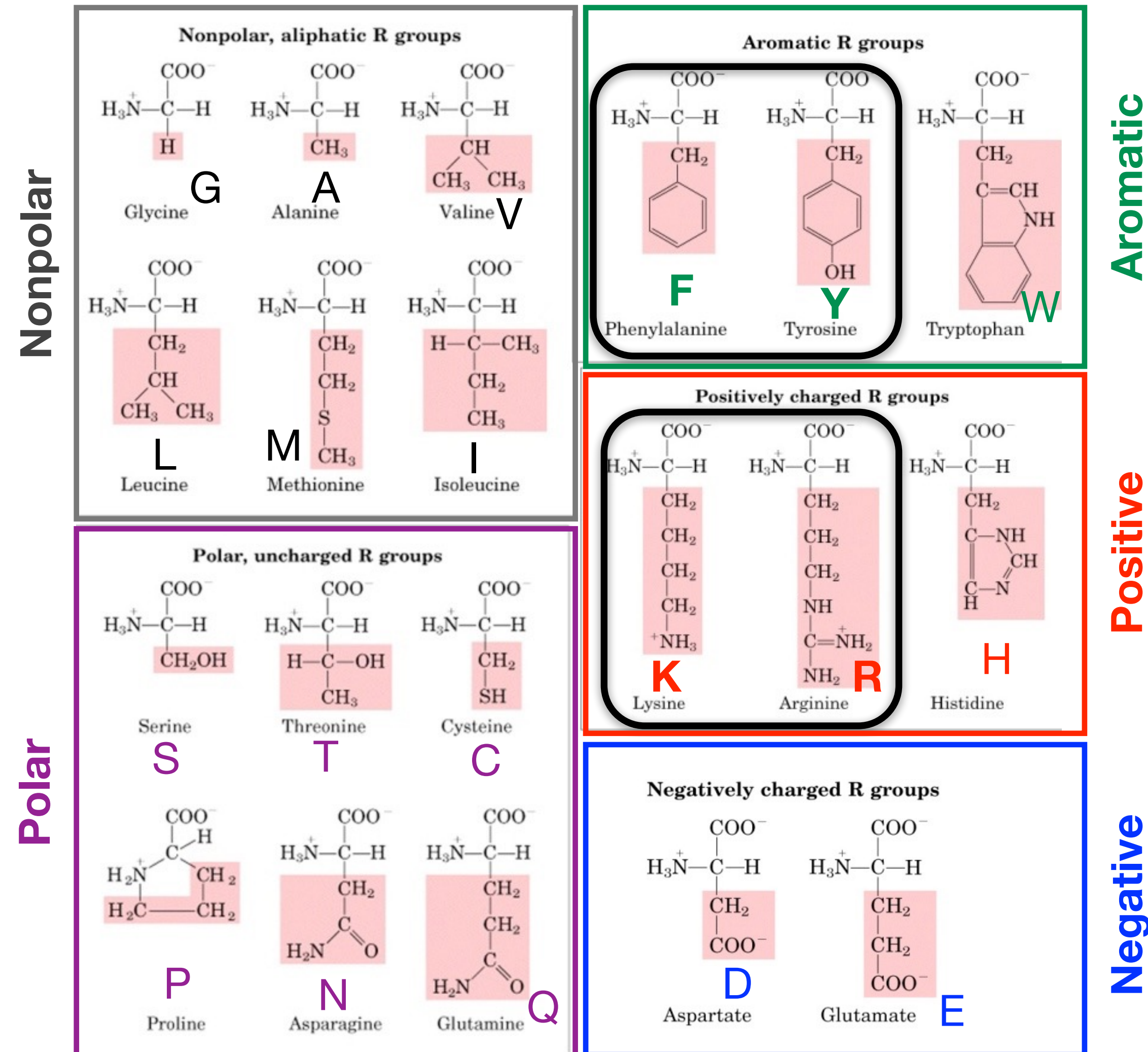
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C : C scores +9

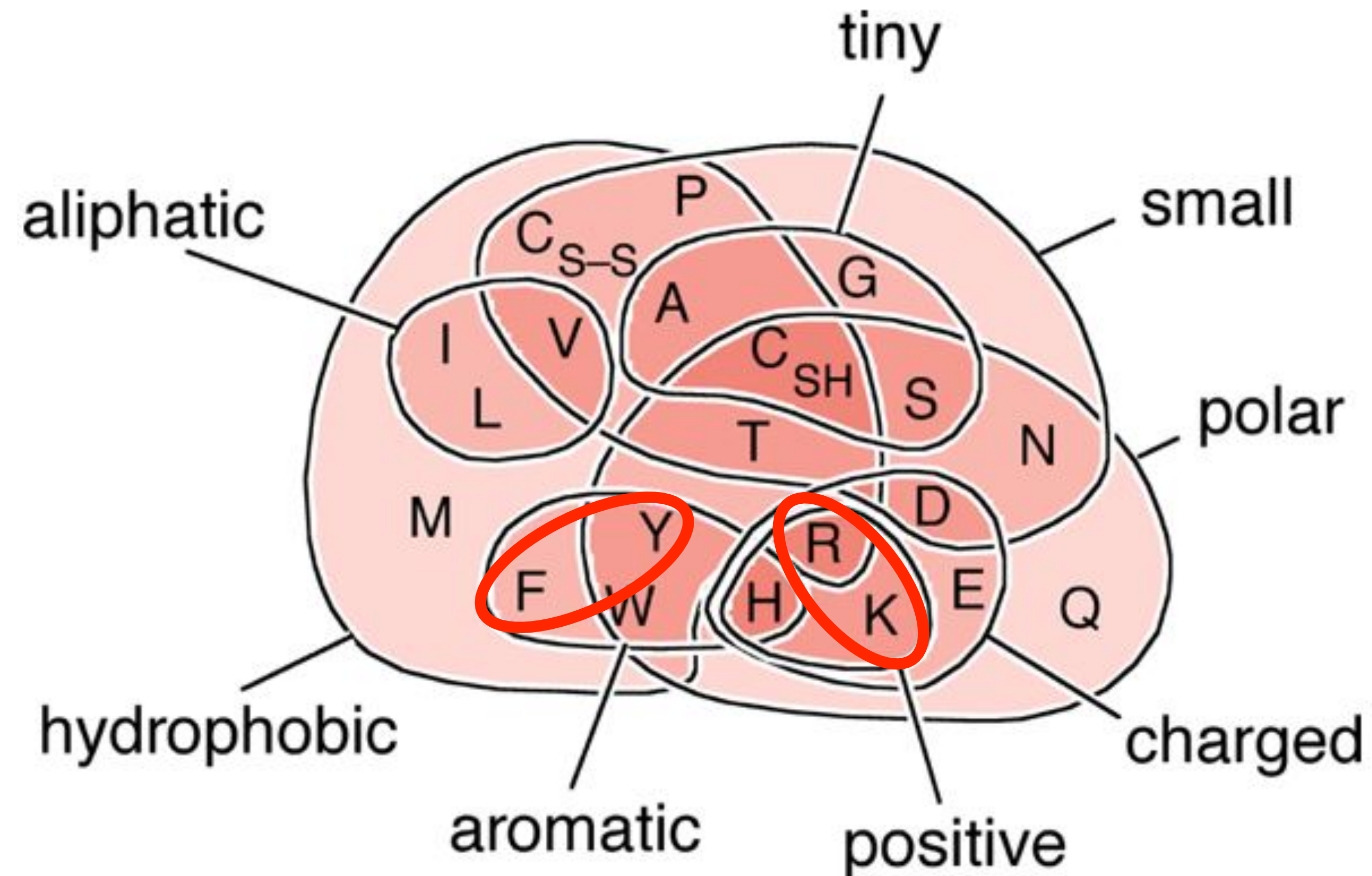
V : V scores +4

F : Y scores +3

Protein scoring matrices reflect the properties of amino acids



Protein scoring matrices reflect the properties of amino acids



Key Trend: High scores for amino acids in the same “biochemical group” and low scores for amino acids from different groups.

N.B. BLOUSM62 does not take the local context of a particular position into account (*i.e.* all like substitutions are scored the same regardless of their location in the molecules).

We will revisit this later...

YOUR TURN!

- There are **four required** and **one optional** hands-on sections including:

1. Limits of using BLAST [~10 mins]
2. Using PSI-BLAST [~30 mins]
3. Examining conservation patterns [~20 mins]
— BREAK [15 mins]—
4. [Optional] Using HMMER [~10 mins]
5. Divergence of protein sequence and structure [~25 mins]

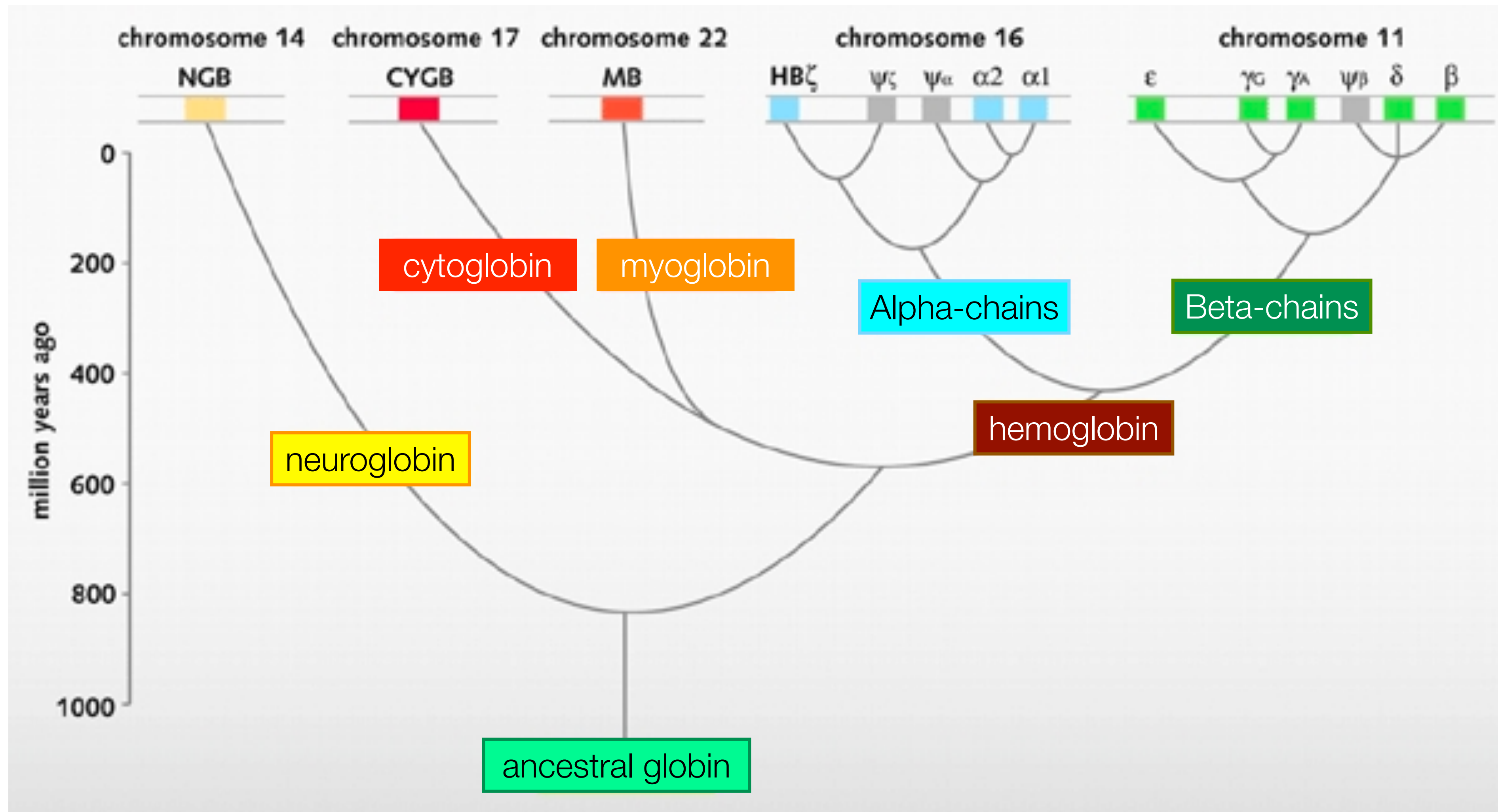
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- ▶ We encourage discussion at your **Table** and on **Piazza!**

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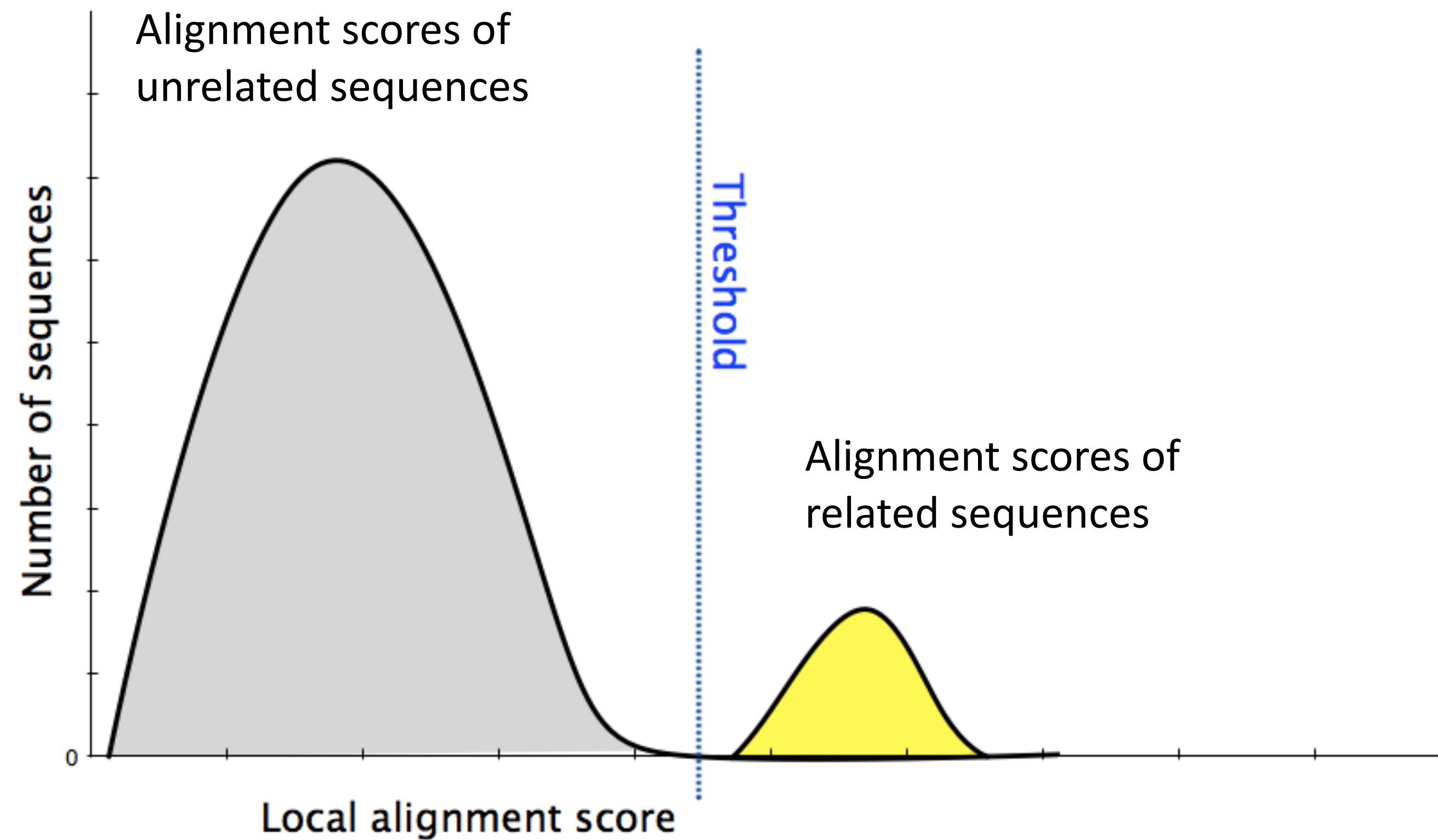
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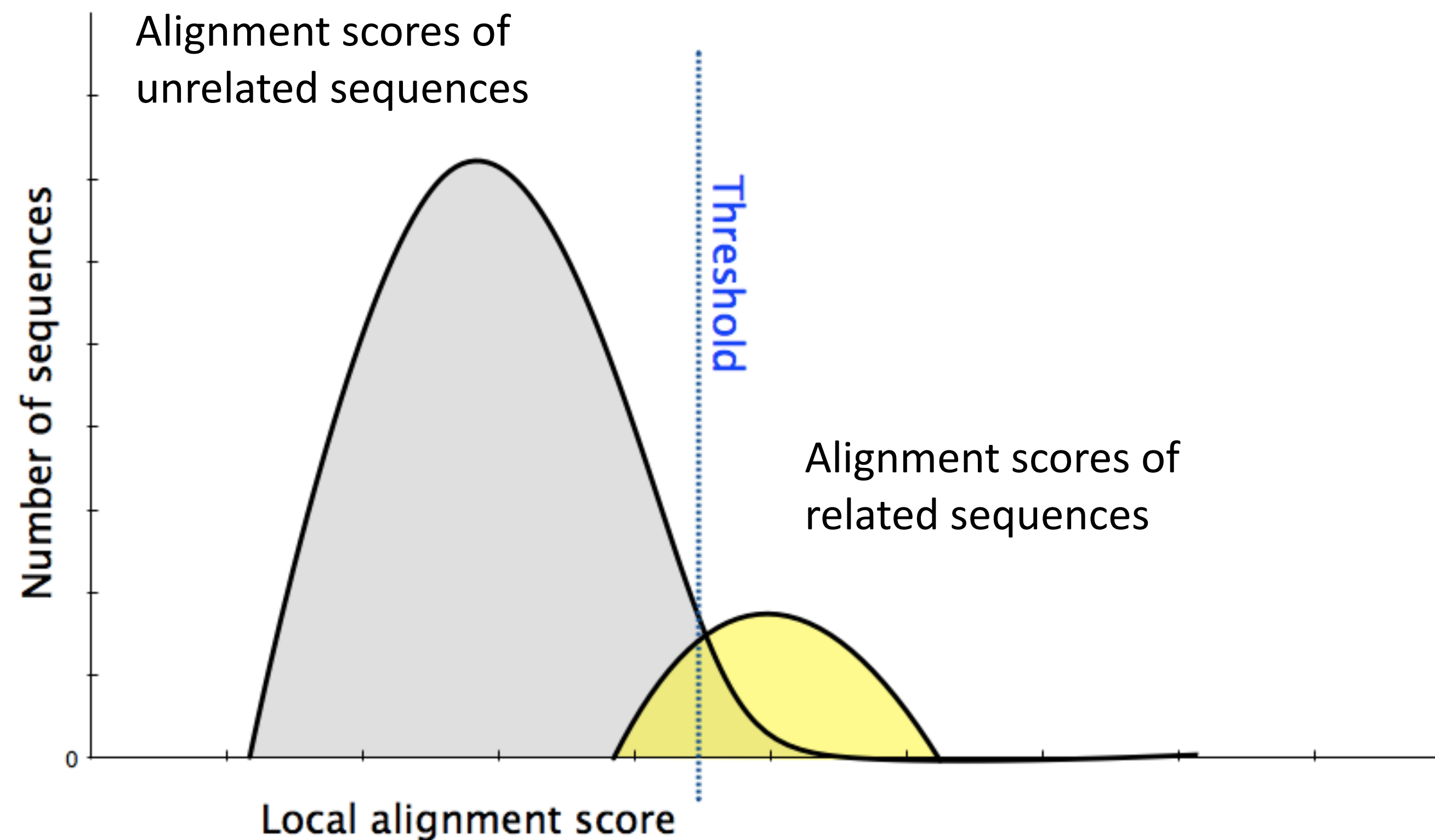
An evolutionary model of human globins.

The different locations of globin genes in human chromosomes are reported at the top of the figure, distinguishing between the functional genes (in color) and the pseudogenes (in grey).

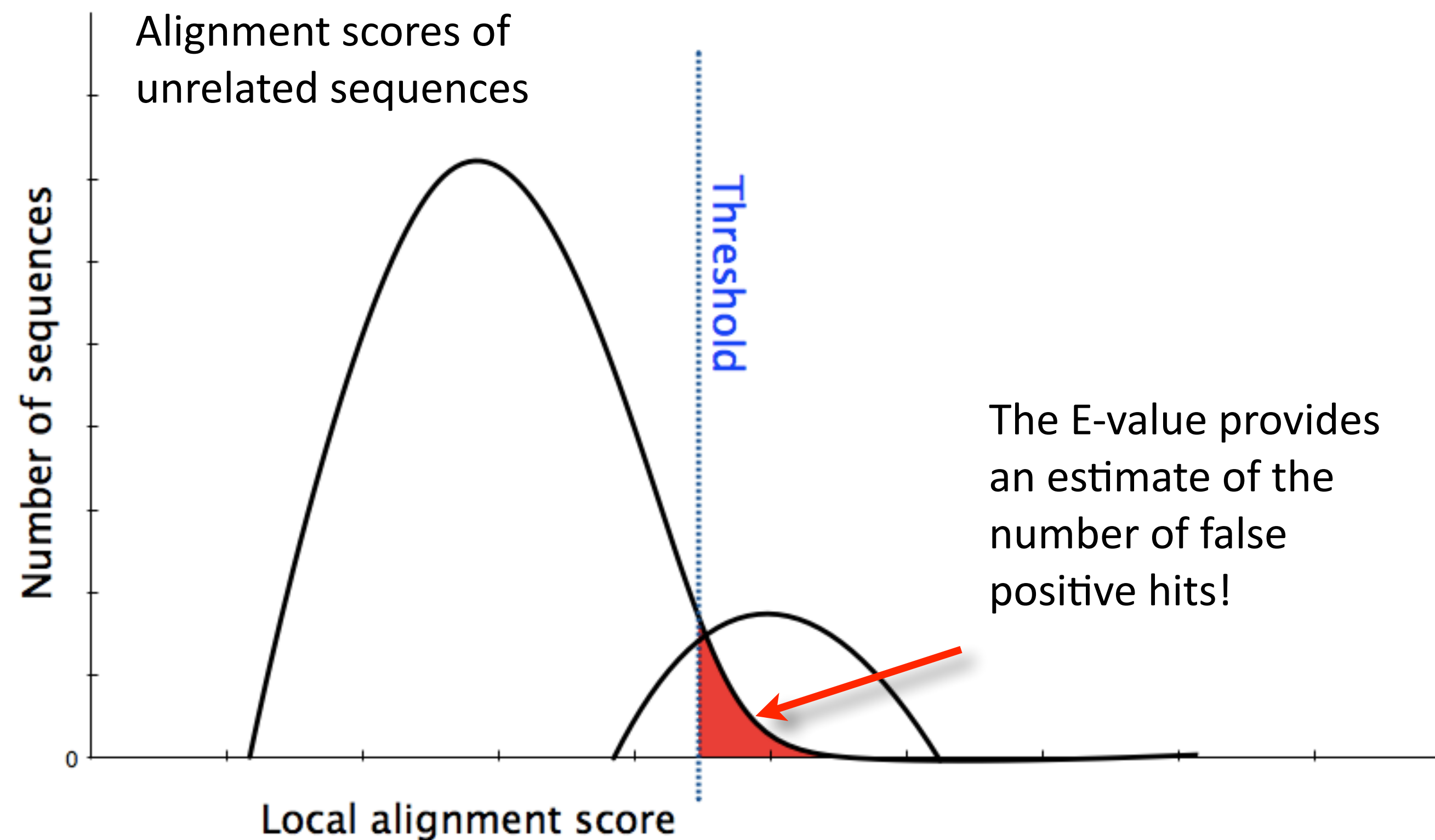
- Ideally, a threshold separates all query related sequences (yellow) from all unrelated sequences (gray)



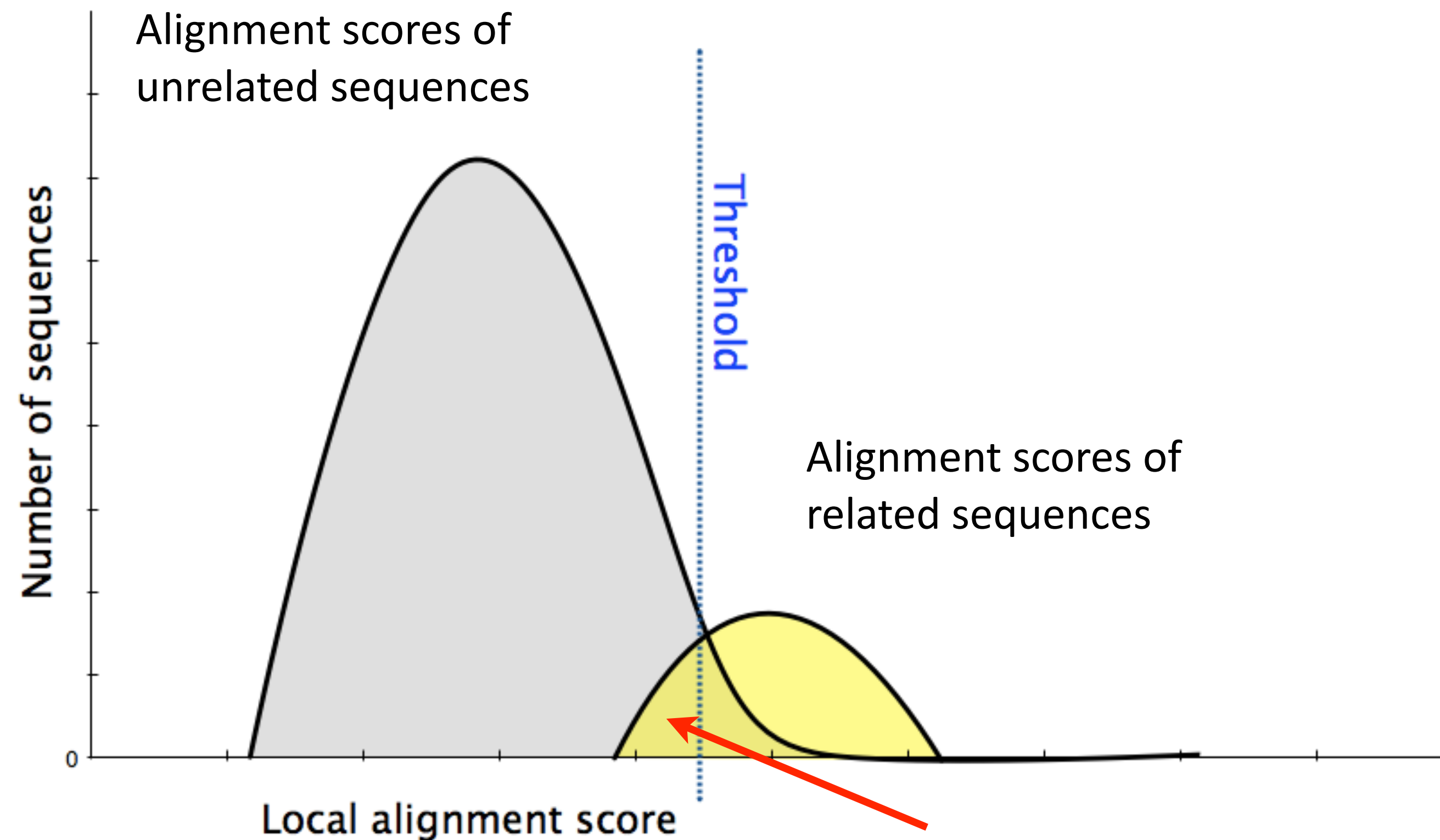
- Unfortunately, often both score distributions overlap
 - The E value describes the expected number of hits with a score above the threshold if the query and database are unrelated



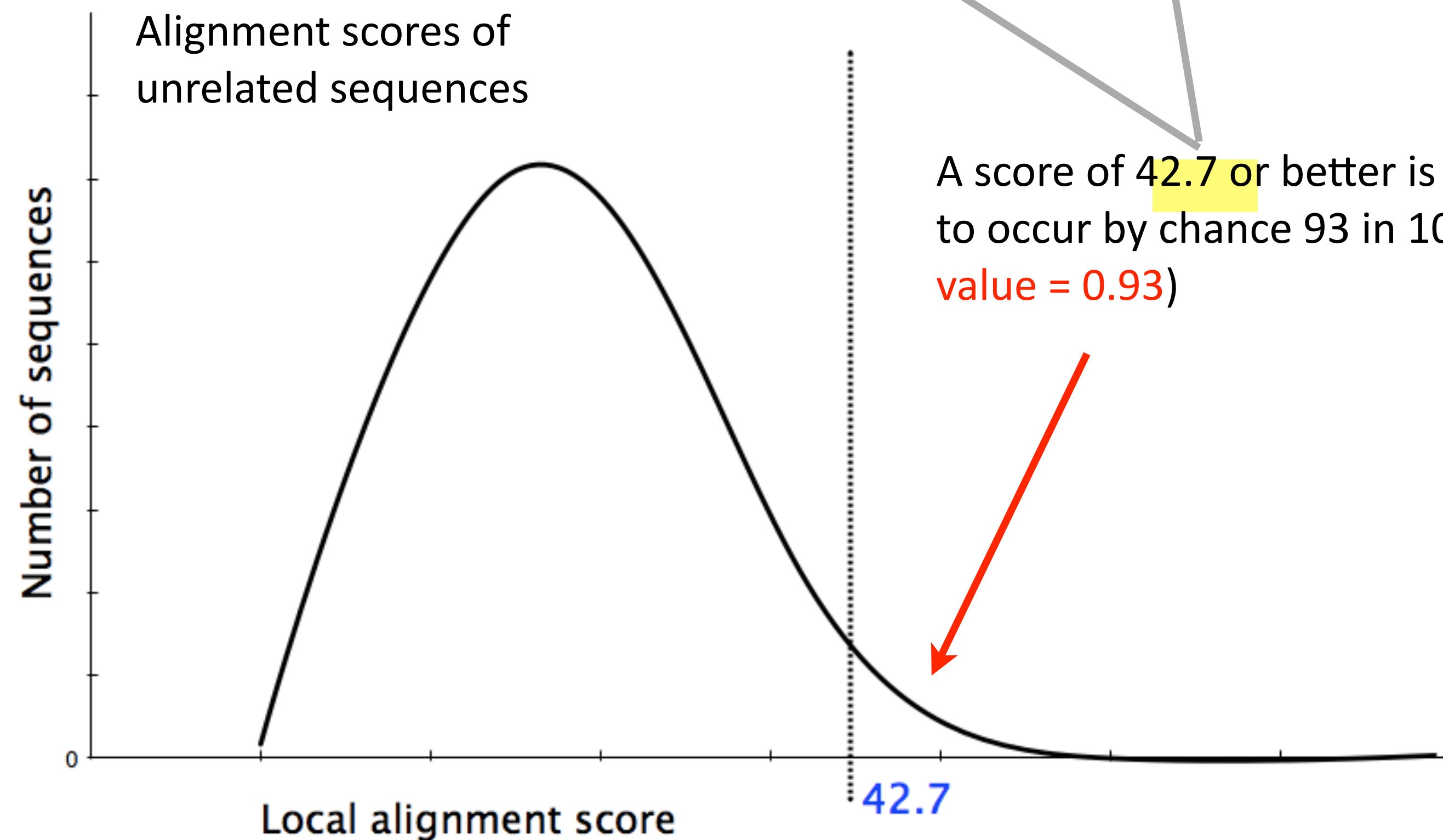
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- Maybe myoglobin, cytoglobin, neuroglobin etc. are found but not reported because of our E-value cutoff?
 - Lets change the cutoff and see...



Description	Max score	Query cover	E value	Max ident	Accession
hemoglobin subunit beta	284	100%	0	100%	NP_000510.1
hemoglobin subunit delta	240	100%	0	75.5%	NP_005321.1
hemoglobin subunit alpha	114	97%	0	43.45%	NP_000508.1
probable ATP-dependent RNA helicase	42.7	10%	0.93	32%	XP_011530405.1



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4. [Optional] Using HMMER [~10 mins]
5. Divergence of protein sequence and structure [~25 mins]

- ▶ Please do answer the last review question (**Q20**).
- ▶ We encourage discussion at your **Table** and on **Piazza!**

Recall: BLOSUM62 does not take the local context of a particular position into account
(*i.e.* all like substitutions are scored the same regardless of their location in the molecules).

Algorithm parameters

Protein BLAST (BLASTp)

General Parameters

Max target sequences | 100 | Select the maximum number of aligned sequences to display

Short queries | Automatically adjust parameters for short input sequences

Expect threshold | 10

Word size | 3

Max matches in a query range | 0

Scoring Parameters

Matrix | BLOSUM62

Gap Costs | Existence: 11 Extension: 1

Compositional adjustments | Conditional compositional score matrix adjustment

Scoring matrix
For match & mis-match scores

Filters and Masking

Filter | Low complexity regions

Mask | Mask for lookup table only
 Mask lower case letters

BLAST

Search **database Non-redundant protein sequences (nr)** using **Blastp**
 Show results in a new window

By default BLASTp match scores come from the BLOSUM62 matrix

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	
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	

Blocks **S**ubstitution **M**atrix. Scores obtained from observed frequencies of substitutions in blocks of aligned sequences with no more than 62% identity.

By default BLASTp match scores come from the BLOSUM62 matrix

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
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

Note. All matches of Alanine for Alanine score +4 regardless of their position or context in the molecule.

PSI-BLAST: Position specific iterated BLAST

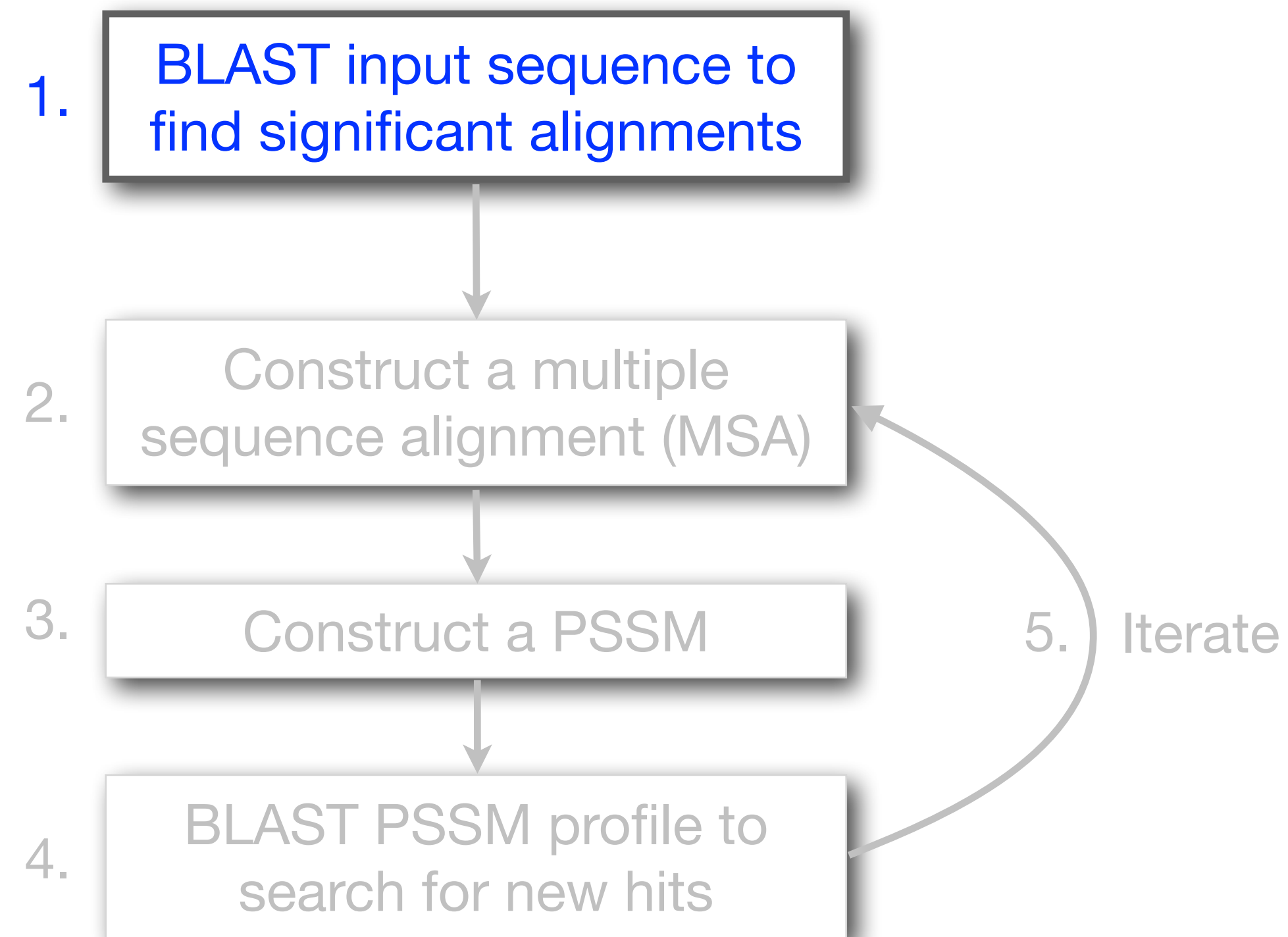
- The purpose of PSI-BLAST is to look deeper into the database for matches to your query protein sequence by employing a scoring matrix that is customized to your query

PSI-BLAST: 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

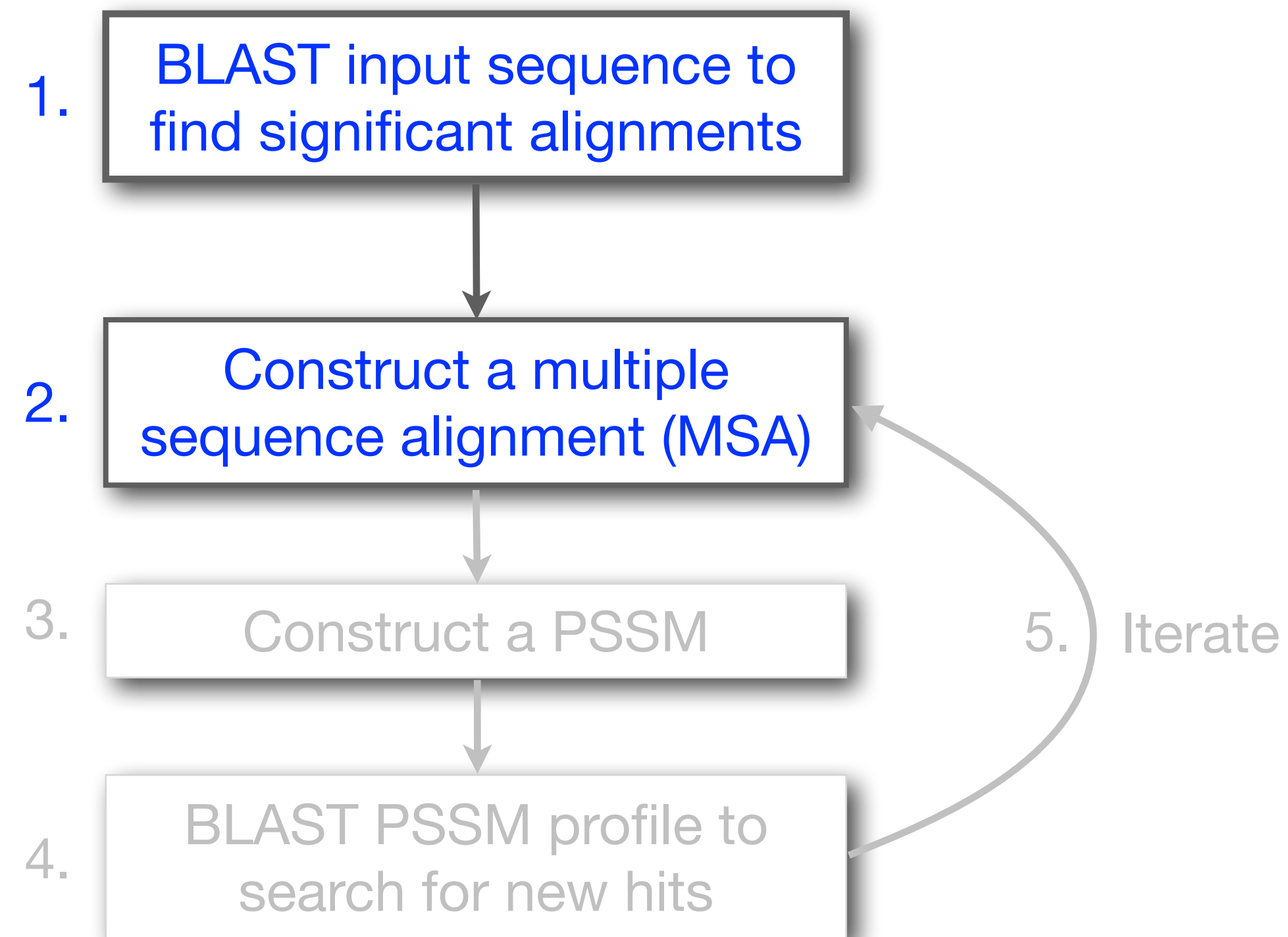
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST



(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

PSI-BLAST: Position-Specific Iterated BLAST

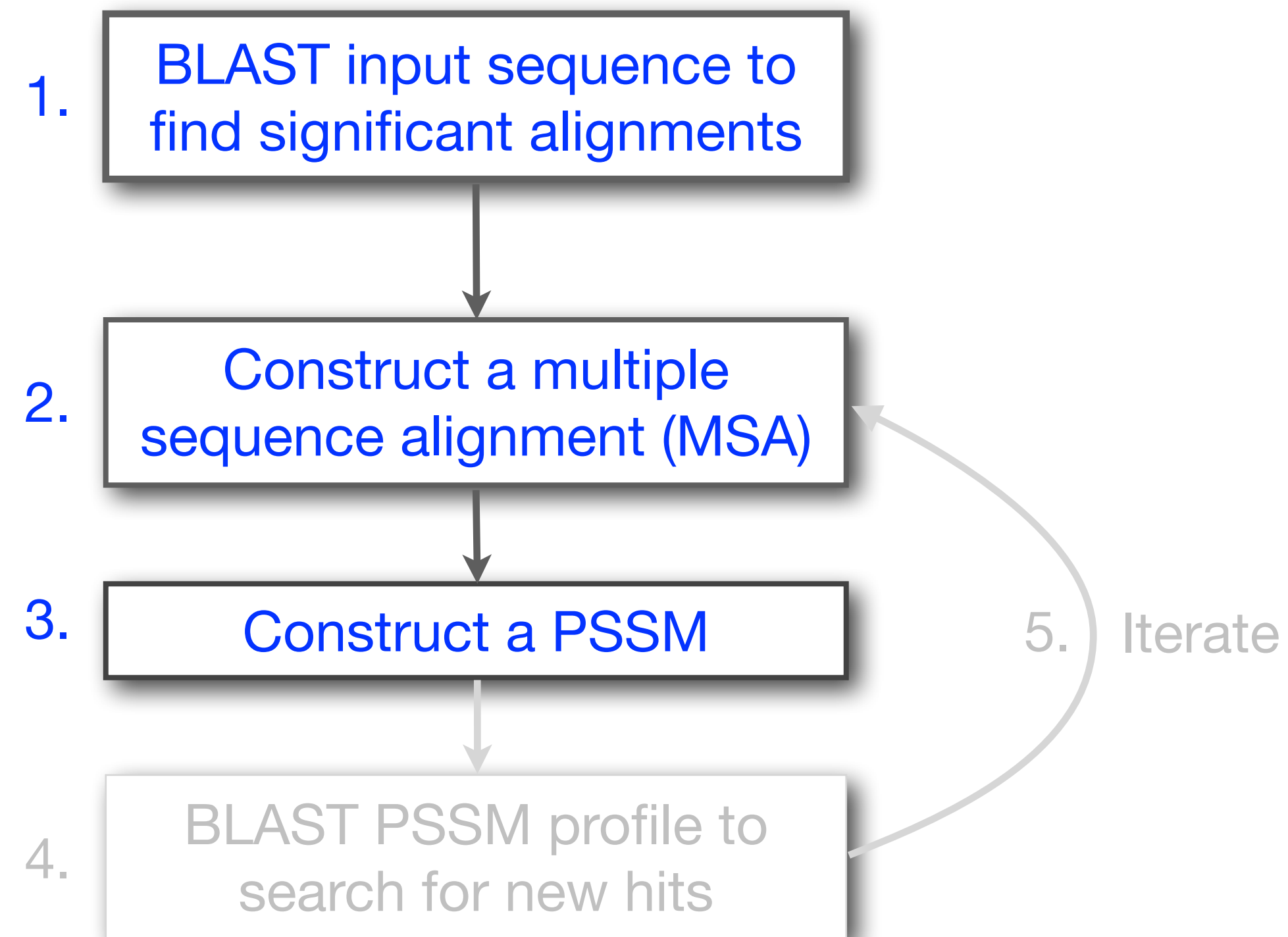
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST



(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

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



(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

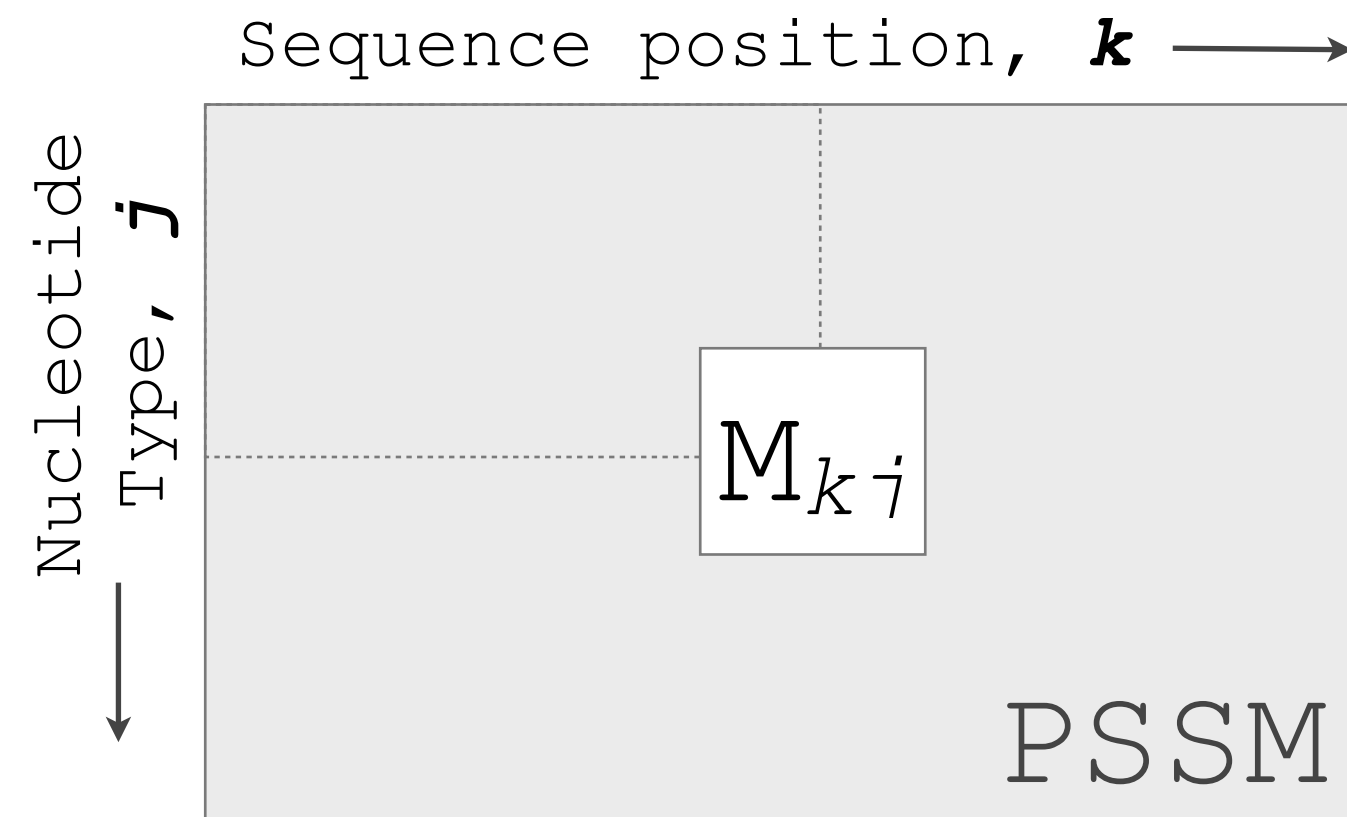
What is a **PSSM**?

What are PSSM sequence profiles?

A sequence profile is a **position-specific scoring matrix** (or **PSSM**, often pronounced 'possum') that gives a *quantitative* description of a set of aligned sequences.

PSSMs assign a score to a query sequence and are widely used for database searching.

A simple PSSM has as many columns as there are positions in the alignment, and either 4 rows (one for each DNA nucleotide) or 20 rows (one for each amino acid).



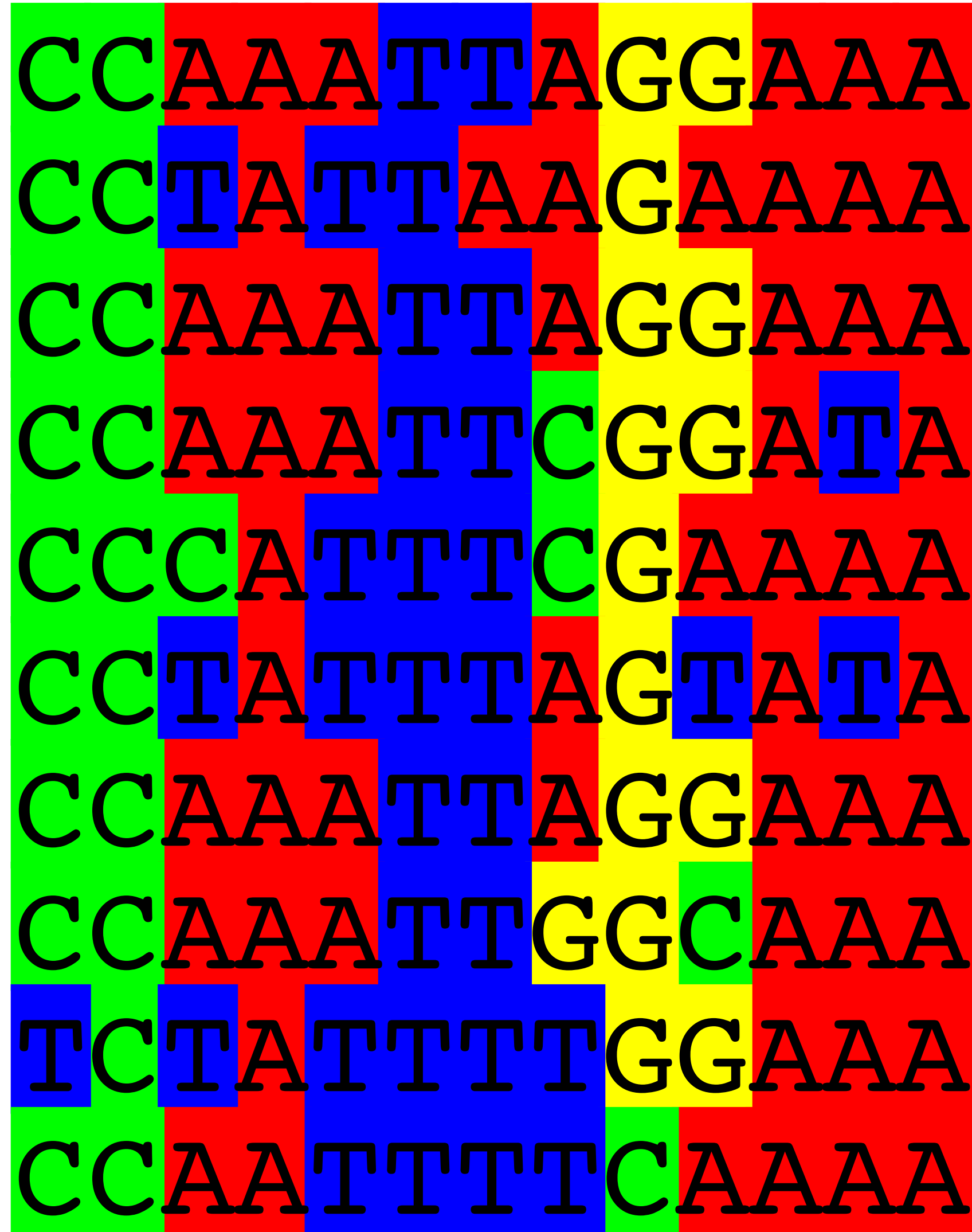
$$M_{kj} = \log \left(\frac{p_{kj}}{p_j} \right)$$

M_{kj} score for the j th nucleotide at position k

p_{kj} probability of nucleotide j at position k

p_j "background" probability of nucleotide j

Example: Computing a transcription factor bind site PSSM



CCAAATTAGGAAA
CCTATTAAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAAA
TCTATTTTGGAAA
CCAAATTTCAAAA

The image shows a 10x13 grid of nucleotide sequences. Each row represents a sequence, and each column represents a position. The sequences are: Row 1: CCAAATTAGGAAA; Row 2: CCTATTAAAGAAAA; Row 3: CCAAATTAGGAAA; Row 4: CCAAATTCGGATA; Row 5: CCCATTTCGAAAA; Row 6: CCTATTTAGTATA; Row 7: CCAAATTAGGAAA; Row 8: CCAAATTGGCAAAA; Row 9: TCTATTTTGGAAA; Row 10: CCAAATTTCAAAA. The background is color-coded by column: Column 1 is green; Column 2 is red; Column 3 is blue; Column 4 is red; Column 5 is yellow; Column 6 is red; Column 7 is green; Column 8 is yellow; Column 9 is red; Column 10 is blue; Column 11 is red; Column 12 is red; Column 13 is red.

Here we have **10 aligned** transcription factor binding site nucleotide sequences

That span **13 positions** (i.e. columns of nucleotides).

We will build a 13 x 4 **PSSM** ($k=13, j=4$).

Computing a transcription factor bind site PSSM

CCAAATTAGGAAA
CCTATTAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:													
C:													
G:													
T:													

Position k = 1



Computing a transcription factor bind site PSSM

CCAAATTAGGAAA
CCTATTAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA

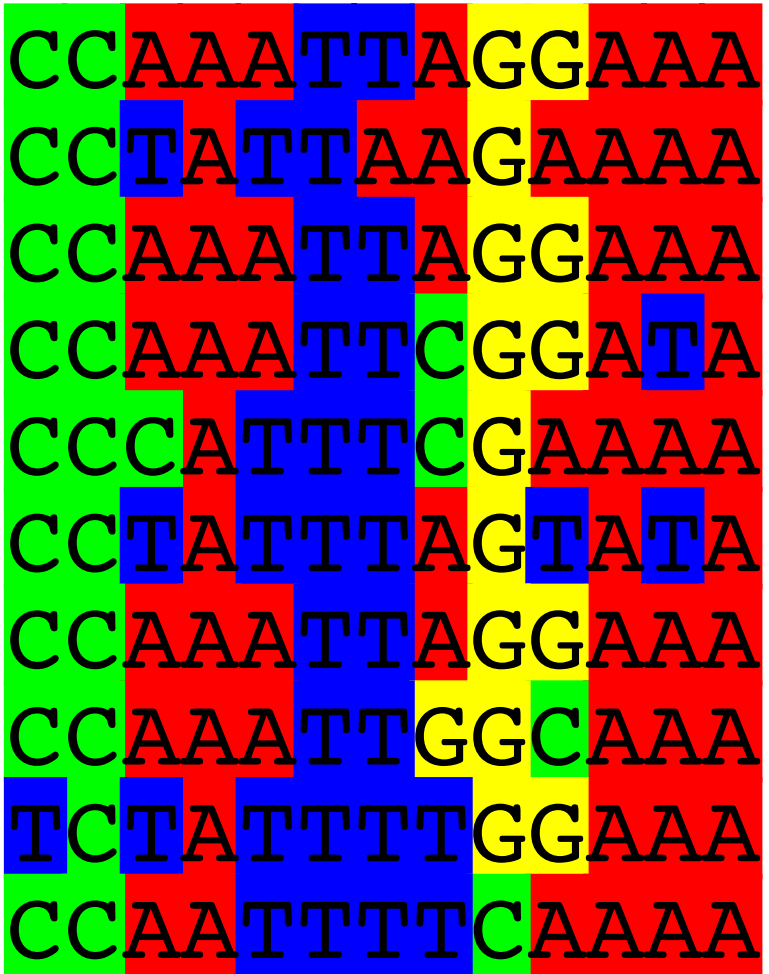
Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0												
C:	9												
G:	0												
T:	1												

Position k = 1



Computing a transcription factor bind site PSSM



Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0												
C:	9												
G:	0												
T:	1												
Consensus	C												



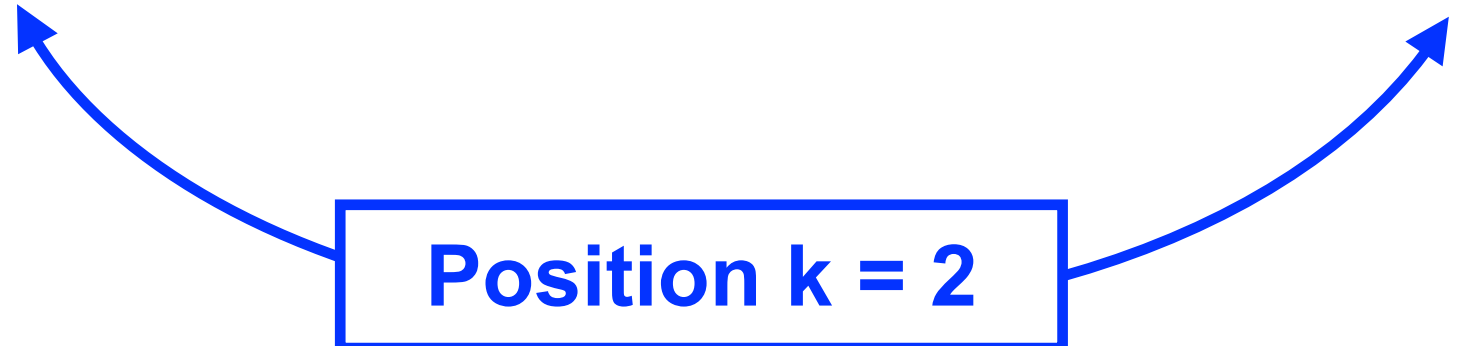
Computing a transcription factor bind site PSSM

CCAAATTAGGAAA
CCTATTAAGAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0											
C:	9	10											
G:	0	0											
T:	1	0											
Consensus	C	C											

Position k = 2

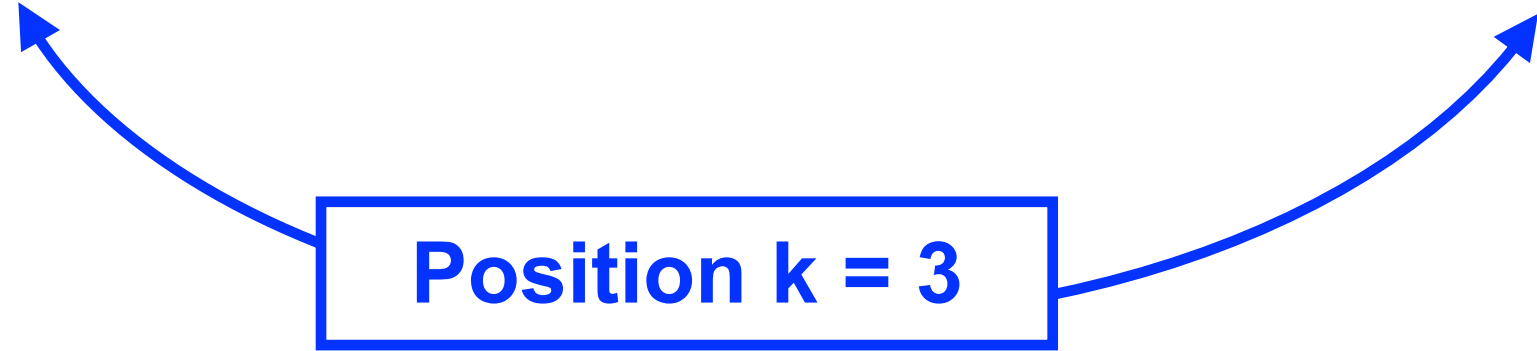


Computing a transcription factor bind site PSSM

CCAAATTAGGAAA
CCTATTAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	6										
C:	9	10	1										
G:	0	0	0										
T:	1	0	3										
Consensus	C	C	[AT]										



Computing a transcription factor bind site PSSM

CCAAATTAGGAAA
 CCTATTAAGAAA
 CCAAATTAGGAAA
 CCAAATTCGGATA
 CCCATTCGAAAA
 CCTATTTAGTATA
 CCAAATTAGGAAA
 CCAAATTGGCAA
 TCTATTTTGGAAA
 CCAATTTTCAAAA

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	6	10	5	0	1	5	0	3	10	8	10
C:	9	10	1	0	0	0	0	2	1	1	0	0	0
G:	0	0	0	0	0	0	0	1	9	5	0	0	0
T:	1	0	3	0	5	10	9	2	0	1	0	2	0
Consensus	C	C	[AT]	A	[AT]	T	T	[ACT]	G	[GA]	A	[AT]	A

Computing a transcription factor bind site PSSM

```

CCAAATTAGGAAA
CCATTAAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA
    
```

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	6	10	5	0	1	5	0	3	10	8	10
C:	9	10	1	0	0	0	0	2	1	1	0	0	0
G:	0	0	0	0	0	0	0	1	9	5	0	0	0
T:	1	0	3	0	5	10	9	2	0	1	0	2	0
Consensus	C	C	[AT]	A	[AT]	T	T	[ACT]	G	[GA]	A	[AT]	A

Average Profile (Frequency) matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	0.6	1	0.5	0	0.1	0.5	0	0.3	1	0.8	1
C:	0.9	1	0.1	0	0	0	0	0.2	0.1	0.1	0	0	0
G:	0	0	0	0	0	0	0	0.1	0.9	0.5	0	0	0
T:	0.1	0	0.3	0	0.5	1	0.9	0.2	0	0.1	0	0.2	0
Consensus	C	C	[AT]	A	[AT]	T	T	[ACT]	G	[GA]	A	[AT]	A

Often we will not communicate with the count matrix but rather the derived **average profile** (a.k.a. frequency matrix).

Computing a transcription factor bind site PSSM

```

CCAAATTAGGAAA
CC TATTAAGAAAA
CCAAATTAGGAAA
CCAAATTCGGATA
CCCATTTCGAAAA
CCTATTTAGTATA
CCAAATTAGGAAA
CCAAATTGGCAAA
TCTATTTTGGAAA
CCAATTTTCAAAA
    
```

Alignment Counts matrix:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	6	10	5	0	1	5	0	3	10	8	10
C:	9	10	1	0	0	0	0	2	1	1	0	0	0
G:	0	0	0	0	0	0	0	1	9	5	0	0	0
T:	1	0	3	0	5	10	9	2	0	1	0	2	0
Consensus	C	C	[AT]	A	[AT]	T	T	[ACT]	G	[GA]	A	[AT]	A

Or the "score (M_{kj}) matrix" = PSSM

C_{kj} Number of j th type nucleotide at position k

Z Total number of aligned sequences

p_j "background" probability of nucleotide j

p_{kj} probability of nucleotide j at position k

$$M_{kj} = \log \left(\frac{p_{kj}}{p_j} \right) \quad p_{kj} = \frac{C_{kj} + p_j}{Z + 1}$$

$$M_{kj} = \log \left(\frac{C_{kj} + p_j / Z + 1}{p_j} \right)$$

Computing a transcription factor bind site PSSM...

Alignment Matrix: C_{kj}

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	0	0	6	10	5	0	1	5	0	3	10	8	10
C:	9	10	1	0	0	0	0	2	1	1	0	0	0
G:	0	0	0	0	0	0	0	1	9	5	0	0	0
T:	1	0	3	0	5	10	9	2	0	1	0	2	0

$$k=1, j=A: M_{kj} = \log\left(\frac{C_{kj} + p_j / Z + 1}{p_j}\right) = \log\left(\frac{0 + 0.25 / 10 + 1}{0.25}\right) = -2.4$$

$$k=1, j=C: M_{kj} = \log\left(\frac{C_{kj} + p_j / Z + 1}{p_j}\right) = \log\left(\frac{9 + 0.25 / 10 + 1}{0.25}\right) = 1.2$$

$$k=1, j=T: M_{kj} = \log\left(\frac{C_{kj} + p_j / Z + 1}{p_j}\right) = \log\left(\frac{1 + 0.25 / 10 + 1}{0.25}\right) = -0.8$$

PSSM: M_{kj}

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	-2.4	-2.4	0.8	1.3	0.6	-2.4	-0.8	0.6	-2.4	0.2	1.3	1.1	1.3
C:	1.2	1.3	-0.8	-2.4	-2.4	-2.4	-2.4	-0.2	-0.8	-0.8	-2.4	-2.4	-2.4
G:	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-0.8	1.2	0.6	-2.4	-2.4	-2.4
T:	-0.8	-2.4	0.2	-2.4	0.6	1.3	1.2	-0.2	-2.4	-0.8	-2.4	-0.2	-2.4

Scoring a test sequence

Query Sequence

CCTATTAGGATA

PSSM:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	-2.4	-2.4	0.8	1.3	0.6	-2.4	-0.8	0.6	-2.4	0.2	1.3	1.1	1.3
C:	1.2	1.3	-0.8	-2.4	-2.4	-2.4	-2.4	-0.2	-0.8	-0.8	-2.4	-2.4	-2.4
G:	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-0.8	1.2	0.6	-2.4	-2.4	-2.4
T:	-0.8	-2.4	0.2	-2.4	0.6	1.3	1.2	-0.2	-2.4	-0.8	-2.4	-0.2	-2.4
Test seq:	C	C	T	A	T	T	T	A	G	G	A	T	A

$$\begin{aligned}\text{Query Score} &= 1.2 + 1.3 + 0.2 + 1.3 + 0.6 + 1.3 + 1.2 \\ &\quad + 0.6 + 1.2 + 0.6 + 1.3 + -0.2 + 1.3 \\ &= \mathbf{11.9}\end{aligned}$$

Scoring a test sequence

Query Sequence

CCTATTAGGATA

PSSM:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	-2.4	-2.4	0.8	1.3	0.6	-2.4	-0.8	0.6	-2.4	0.2	1.3	1.1	1.3
C:	1.2	1.3	-0.8	-2.4	-2.4	-2.4	-2.4	-0.2	-0.8	-0.8	-2.4	-2.4	-2.4
G:	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-0.8	1.2	0.6	-2.4	-2.4	-2.4
T:	-0.8	-2.4	0.2	-2.4	0.6	1.3	1.2	-0.2	-2.4	-0.8	-2.4	-0.2	-2.4
Test seq:	C	C	T	A	T	T	T	A	G	G	A	T	A

$$\begin{aligned}\text{Query Score} &= 1.2 + 1.3 + 0.2 + 1.3 + 0.6 + 1.3 + 1.2 \\ &\quad + 0.6 + 1.2 + 0.6 + 1.3 + -0.2 + 1.3 \\ &= \mathbf{11.9}\end{aligned}$$

Q. Does the query sequence match the DNA sequence profile?

Scoring a test sequence...

Query Sequence

CCTATTAGGATA

Best Possible Sequence

CCAATTAGGAAA

PSSM:

Position k =	1	2	3	4	5	6	7	8	9	10	11	12	13
A:	-2.4	-2.4	0.8	1.3	0.6	-2.4	-0.8	0.6	-2.4	0.2	1.3	1.1	1.3
C:	1.2	1.3	-0.8	-2.4	-2.4	-2.4	-2.4	-0.2	-0.8	-0.8	-2.4	-2.4	-2.4
G:	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-2.4	-0.8	1.2	0.6	-2.4	-2.4	-2.4
T:	-0.8	-2.4	0.2	-2.4	0.6	1.3	1.2	-0.2	-2.4	-0.8	-2.4	-0.2	-2.4
Max Score:	C	C	A	A	T	T	T	A	G	G	A	A	A

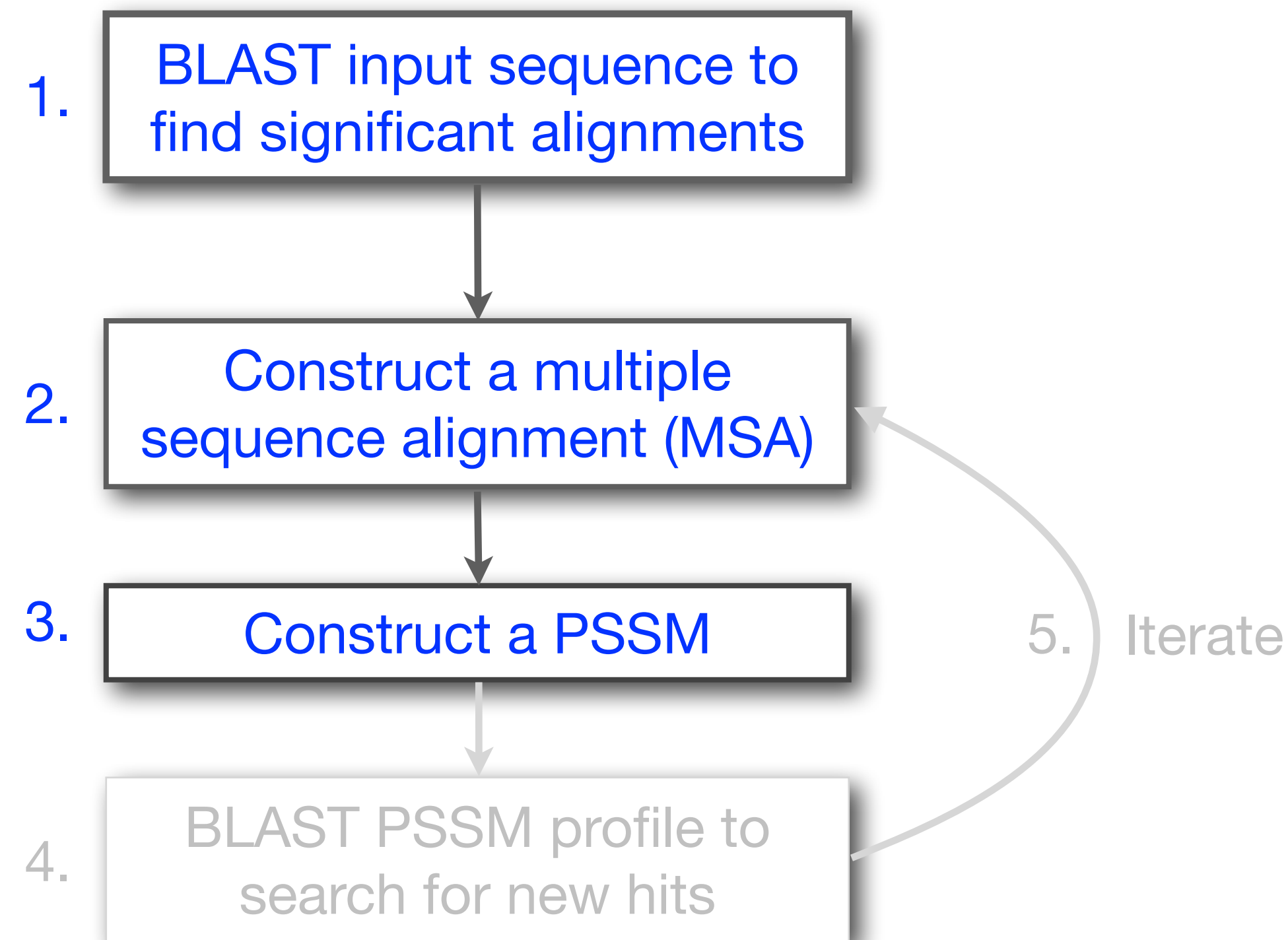
$$\begin{aligned}\text{Max Score} &= 1.2 + 1.3 + 0.8 + 1.3 + 0.6 + 1.3 + 1.2 \\ &\quad + 0.6 + 1.2 + 0.6 + 1.3 + 1.1 + 1.3 \\ &= 13.8\end{aligned}$$

A. Following method in Harbison *et al.* (2004) Nature 431:99-104

Heuristic threshold for match = 60% x Max Score = (0.6 x 13.8 = 8.28);
11.9 > 8.28; Therefore our query is a potential TFBS!

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



(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

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

730496	66	FTVDENGQMSATAKGRVRLFNNWDVCADMIGSFTDTEDEPAKFKMKYWGVASFLQKGNDDH	125
200679	63	FSVDEKGHMSATAKGRVRLLSNWEVCADMVGTFDTEDPAKFKMKYWGVASFLQKGNDDH	122
206589	34	FSVDEKGHMSATAKGRVRLLSNWEVCADMVGTFDTEDPAKFKMKYWGVASFLQKGNDDH	93
2136812	2	MSATAKGRVRLLNWDVCADMVGTFDTEDPAKFKMKYWGVASFLQKGNDDH	53
132408	65	FKIEDNGKTTATAKGRVRILDKLELCANMVGTFIETNDPAKYRMKYHGALAILERGLDDH	124
267584	44	FSVDESGKVTATAHGRVILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAASYLQKGNDDH	103
267585	44	FSVDGSGKVTATAQGRVILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAASYLQKGNDDH	103
8777608	63	FTIHEDGAMTATAKGRVILNNWEMCADMMATFETTPDPAKFRMRYWGAAASYLQKGNDDH	122
6687453	60	FKVEEDGTMTATAIGRVILNNWEMCANMFGTFEDTEDEPAKFKMKYWGAAASYLQKGNDDH	119
10697027	81	FKVQEDGTMTATATGRVILNNWEMCANMFGTFEDTEEPARFKMKYWGAAASYLQKGNDDH	140
13645517	1	MVGTFDTEDPAKFKMKYWGVASFLQKGNDDH	32
13925316	38	FSVDGSGKMTATAQGRVILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAASYLQKGNDDH	97
131649	65	YTVEEDGTMTASSKGRVKLFGFWVICADMAAQYTDPTTPAKMYMTYQGLASYLSSGGDNY	126

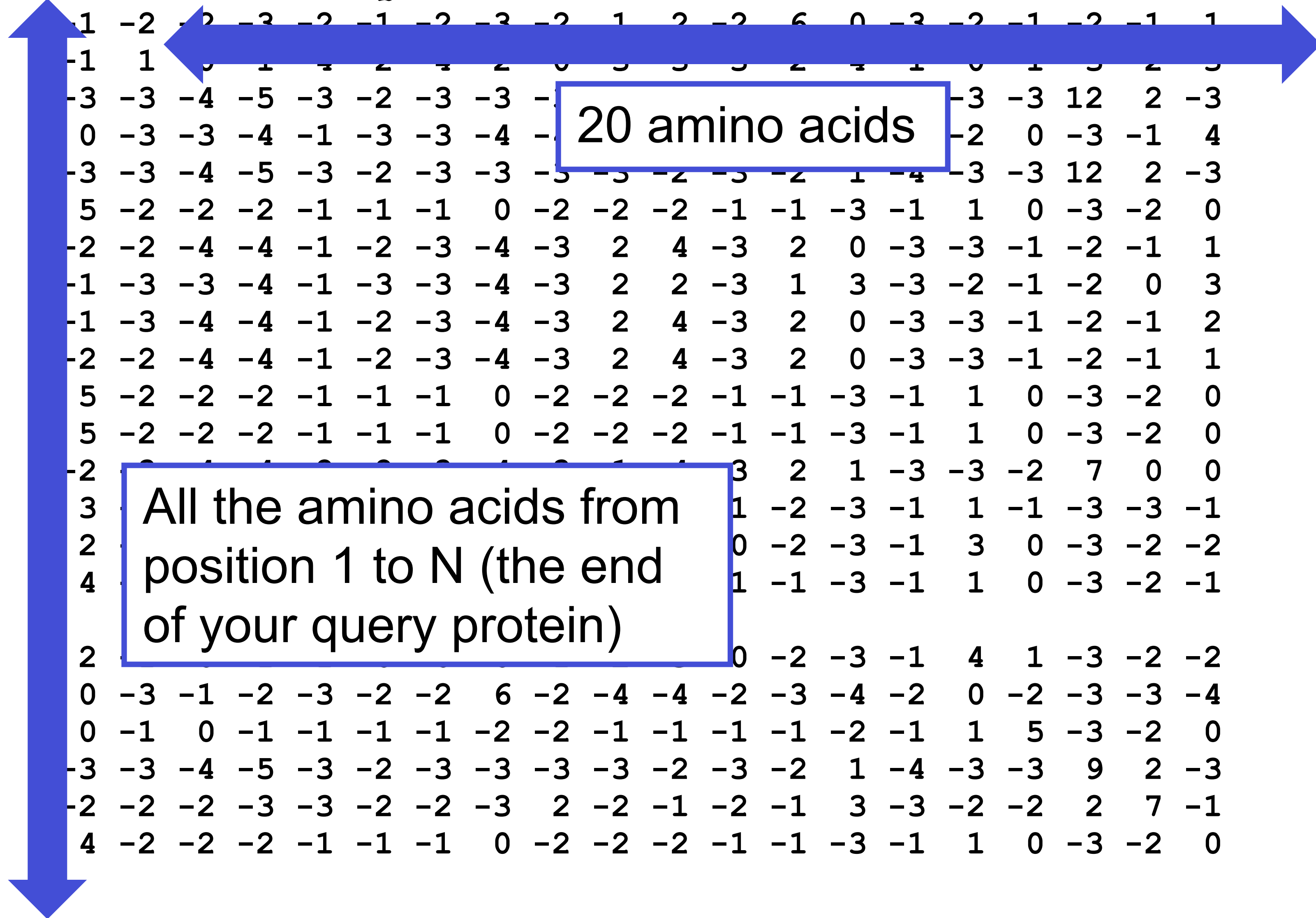
M

N,M,L,Y,G

		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	-2	-1	-2	-2	-2	-2	-2	3	3	3	2	-1	1	0	1	3	2	3
3	W	-3	-3	-4	-5	-3	-2	-3	-3	-3	-3	-3	-3	-2	-4	-3	-3	12	2	-3	
4	V	0	-3	-3	-4	-1	-3	-3	-4	-3	-3	-3	-3	-2	-4	-3	-3	-3	-1	4	
5	W	-3	-3	-4	-5	-3	-2	-3	-3	-3	-3	-3	-3	-2	-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	-2	-3	-3	-1	-2	-3	-4	-3	2	4	-3	2	1	-3	-3	-2	7	0	0
14	A	3	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	1	-1	-3	-3	-1
15	A	2	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	3	0	-3	-2	-2
16	A	4	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	1	0	-3	-2	-1
...																					
37	S	2	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	4	1	-3	-2	-2
38	G	0	-3	-1	-2	-3	-2	-2	6	-2	-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	9	2	-3
41	Y	-2	-2	-2	-3	-3	-2	-2	-3	2	-2	-1	-2	-1	3	-3	-2	-2	2	7	-1
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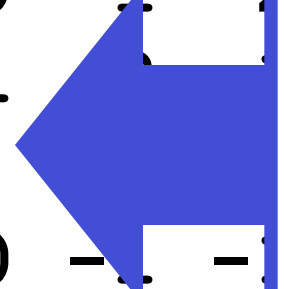
20 amino acids

All the amino acids from position 1 to N (the end of your 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	-1	-2	-3	-4	-3	2	4	-3	2	0	-3	-3	-1	-2	-1	2
10	L	-2	-2	-4	-4	-	-	-	-	-	-	-	-	-	-	-	-3	-1	-2	-1	1
11	A	5	-2	-2	-2	-	-	-	-	-	-	-	-	-	-	-	1	0	-3	-2	0
12	A	5	-2	-2	-2	-	-	-	-	-	-	-	-	-	-	-	1	0	-3	-2	0
13	W	-2	-3	-4	-4	-	-	-	-	-	-	-	-	-	-	-	-3	-2	7	0	0
14	A	3	-2	-1	-2	-	-	-	-	-	-	-	-	-	-	-	1	-1	-3	-3	-1
15	A	2	-1	0	-1	-	-	-	-	-	-	-	-	-	-	-	3	0	-3	-2	-2
16	A	4	-2	-1	-	-	-	-	-	-	-	-	-	-	-	-	1	0	-3	-2	-1
...																					
37	S	2	-1	0	-	-	-	-	-	-	-	-	-	-	-	-	4	1	-3	-2	-2
38	G	0	-3	-1	-2	-	-	-	-	-	-	-	-	-	-	-	0	-2	-3	-3	-4
39	T	0	-1	0	-1	-	-	-	-	-	-	-	-	-	-	-	1	5	-3	-2	0
40	W	-3	-3	-4	-5	-	-	-	-	-	-	-	-	-	-	-	-3	-3	9	2	-3
41	Y	-2	-2	-2	-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

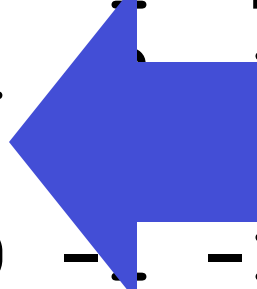
Note: A given amino acid (such as alanine) in your query protein can receive different scores for matching alanine depending on the position in the protein (BLOSUM $S_{AA} = +4$)



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

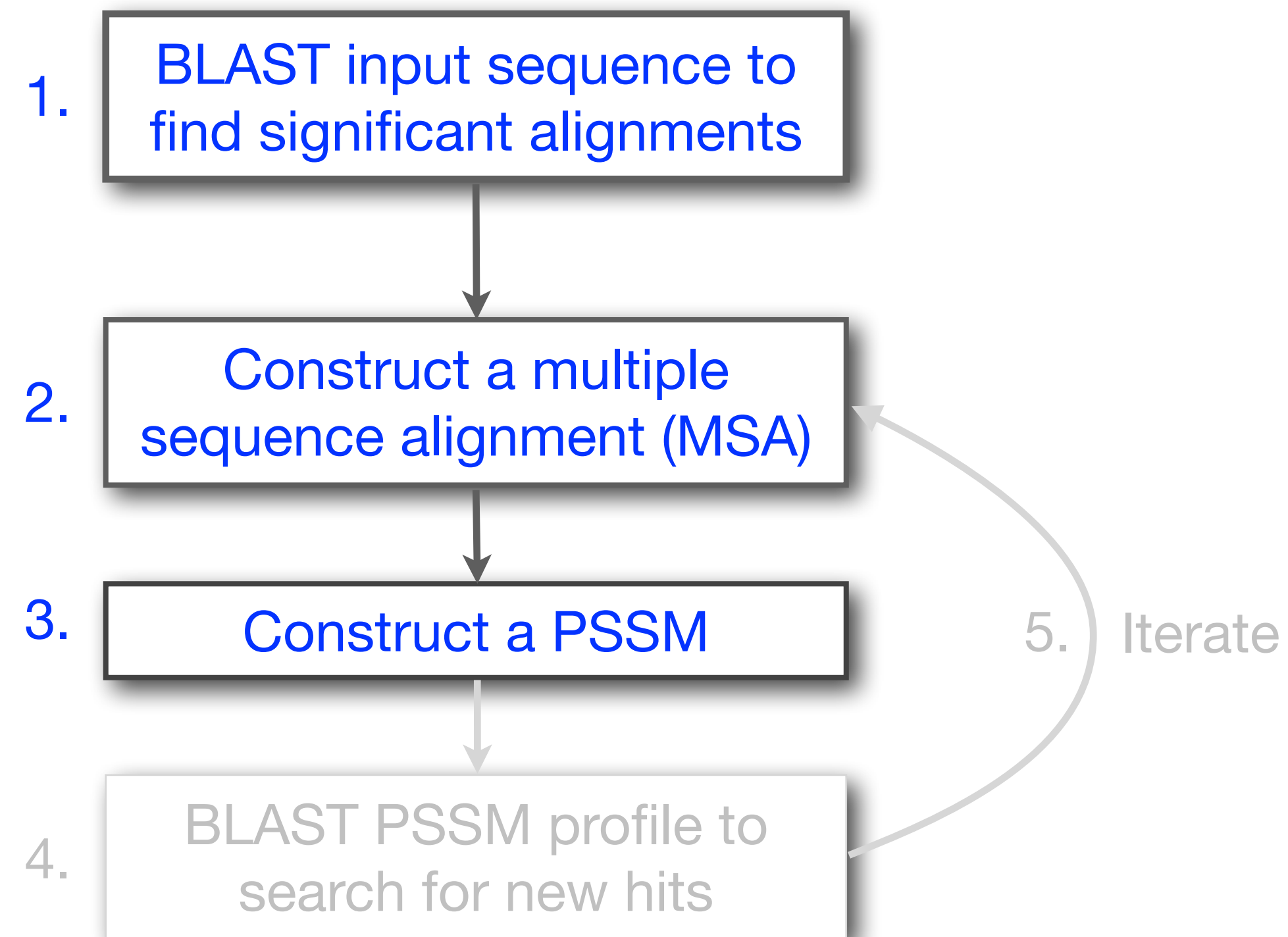
The PSI-BLAST PSSM is essentially a query customized scoring matrix that is more sensitive than BLOSUM.

Note: 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 (BLOSUM $S_{AA} = +4$)



PSI-BLAST: Position-Specific Iterated BLAST

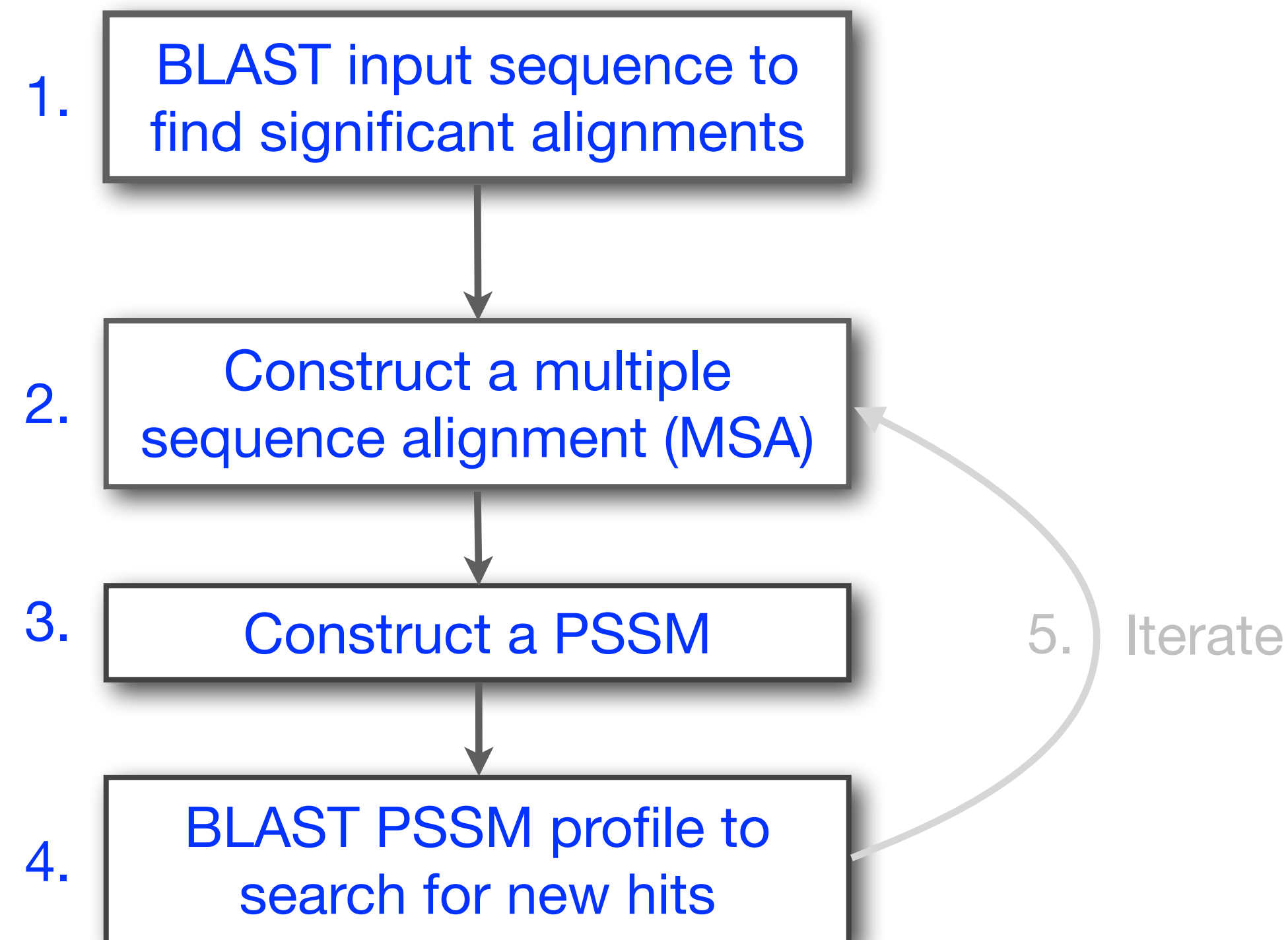
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST



(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

PSI-BLAST: Position-Specific Iterated BLAST

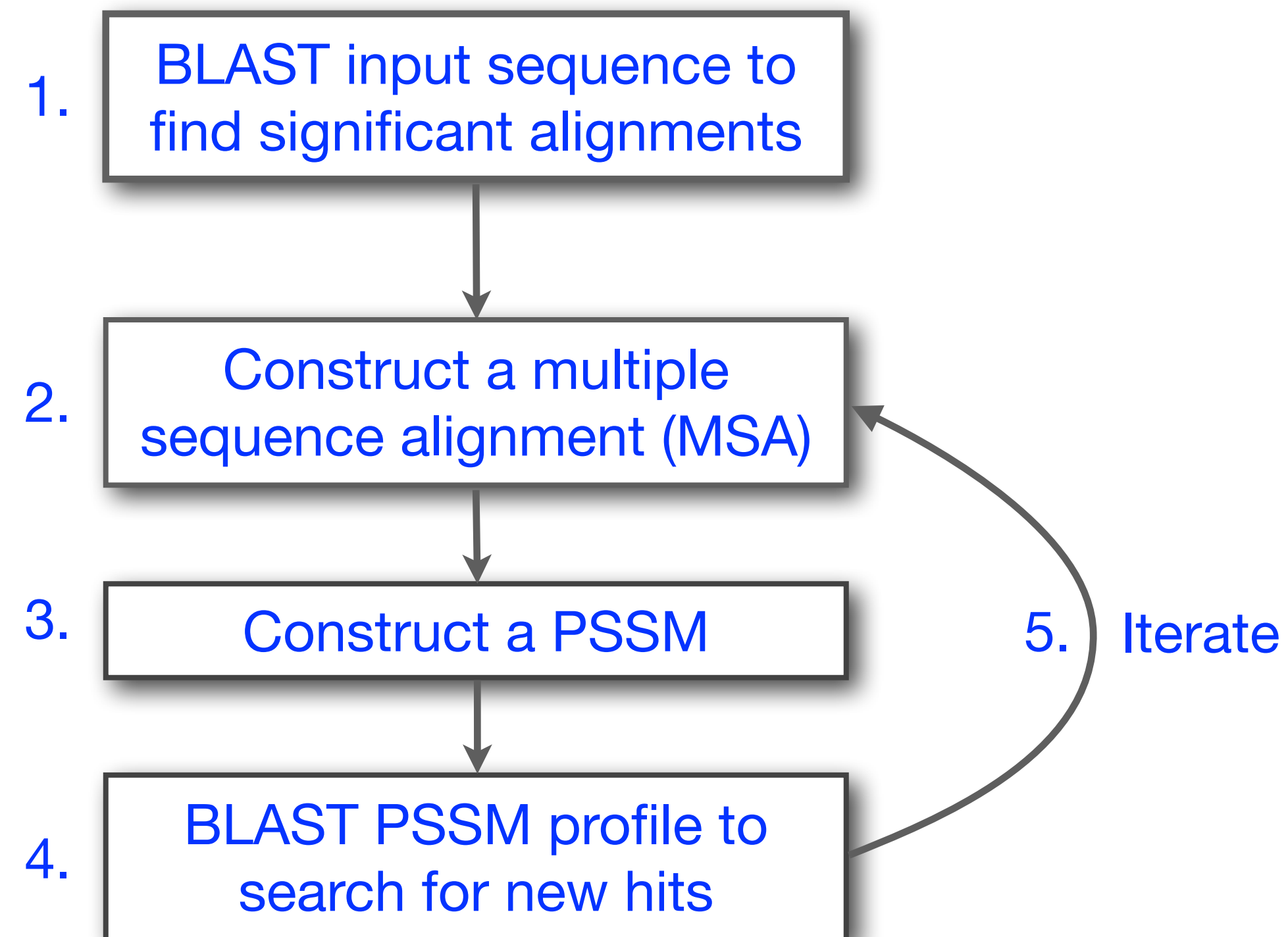
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST



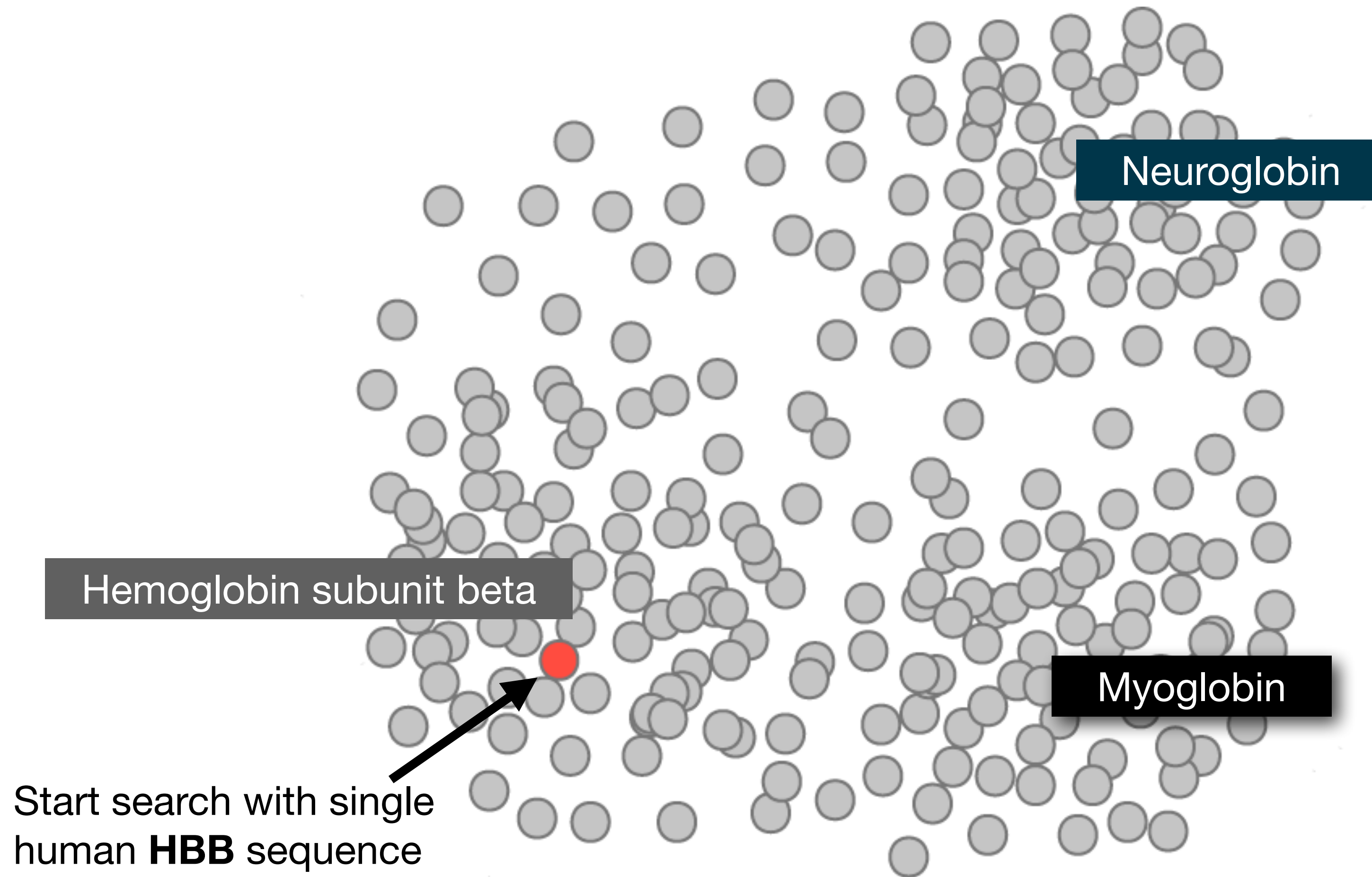
(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)

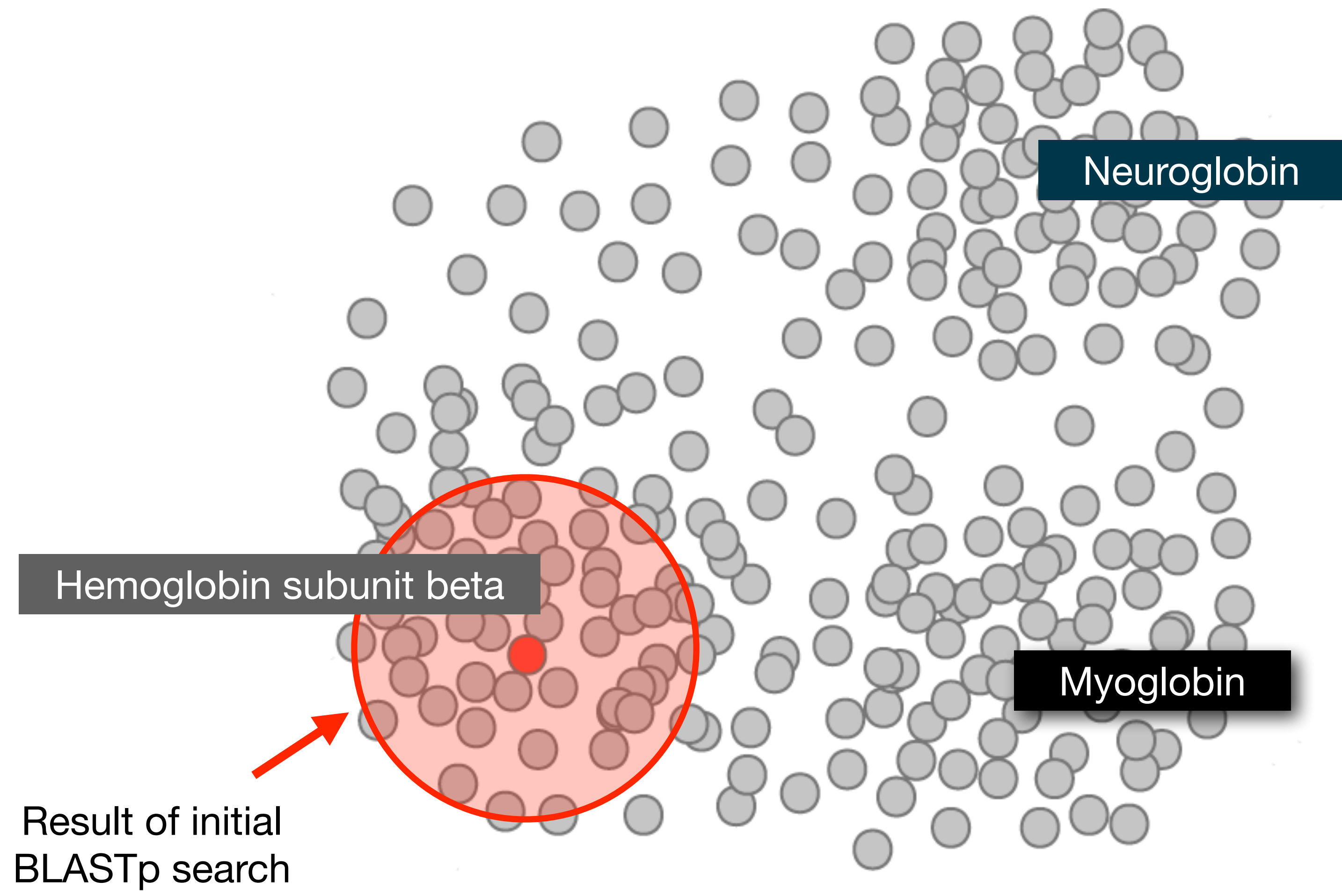
PSI-BLAST: Position-Specific Iterated BLAST

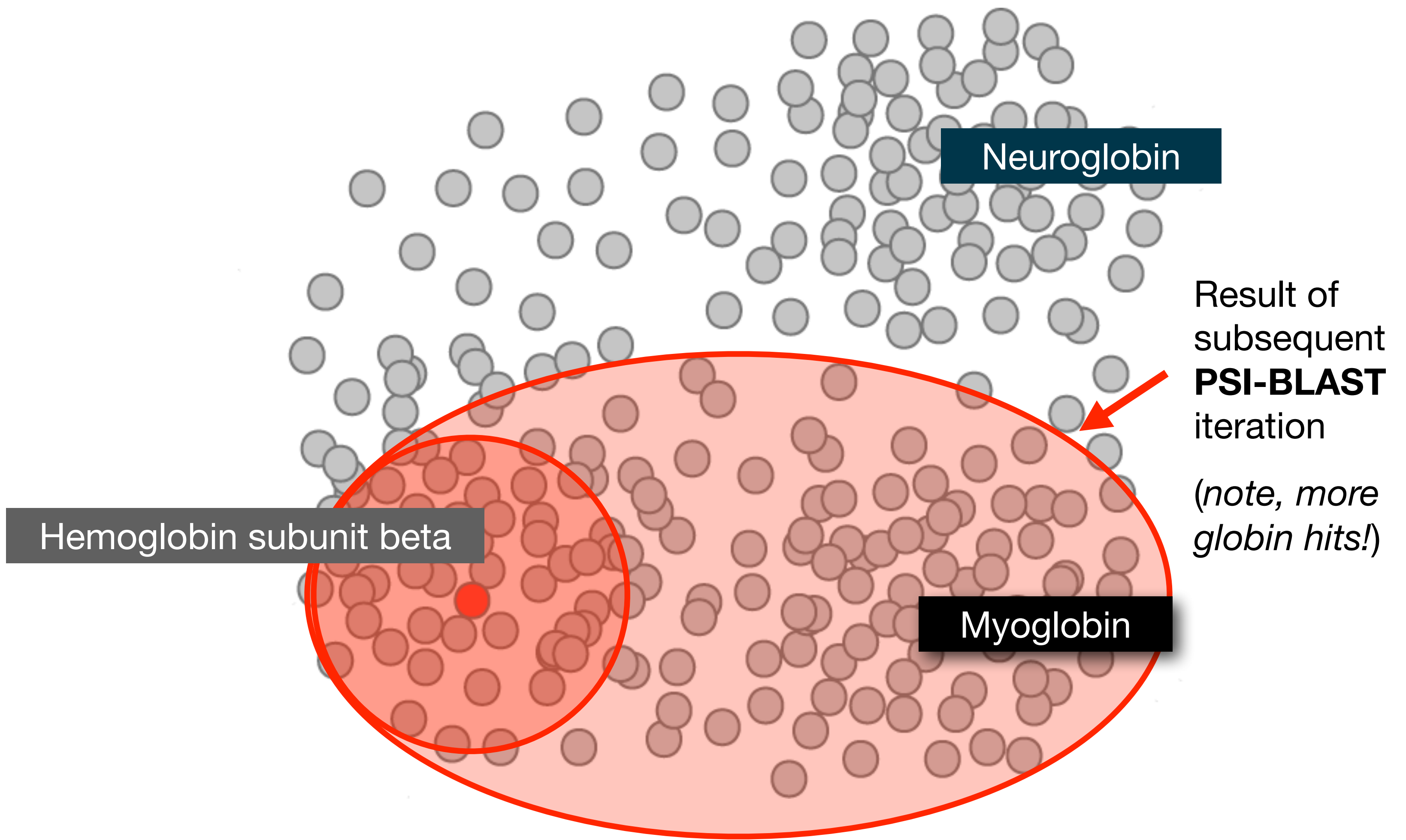
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST

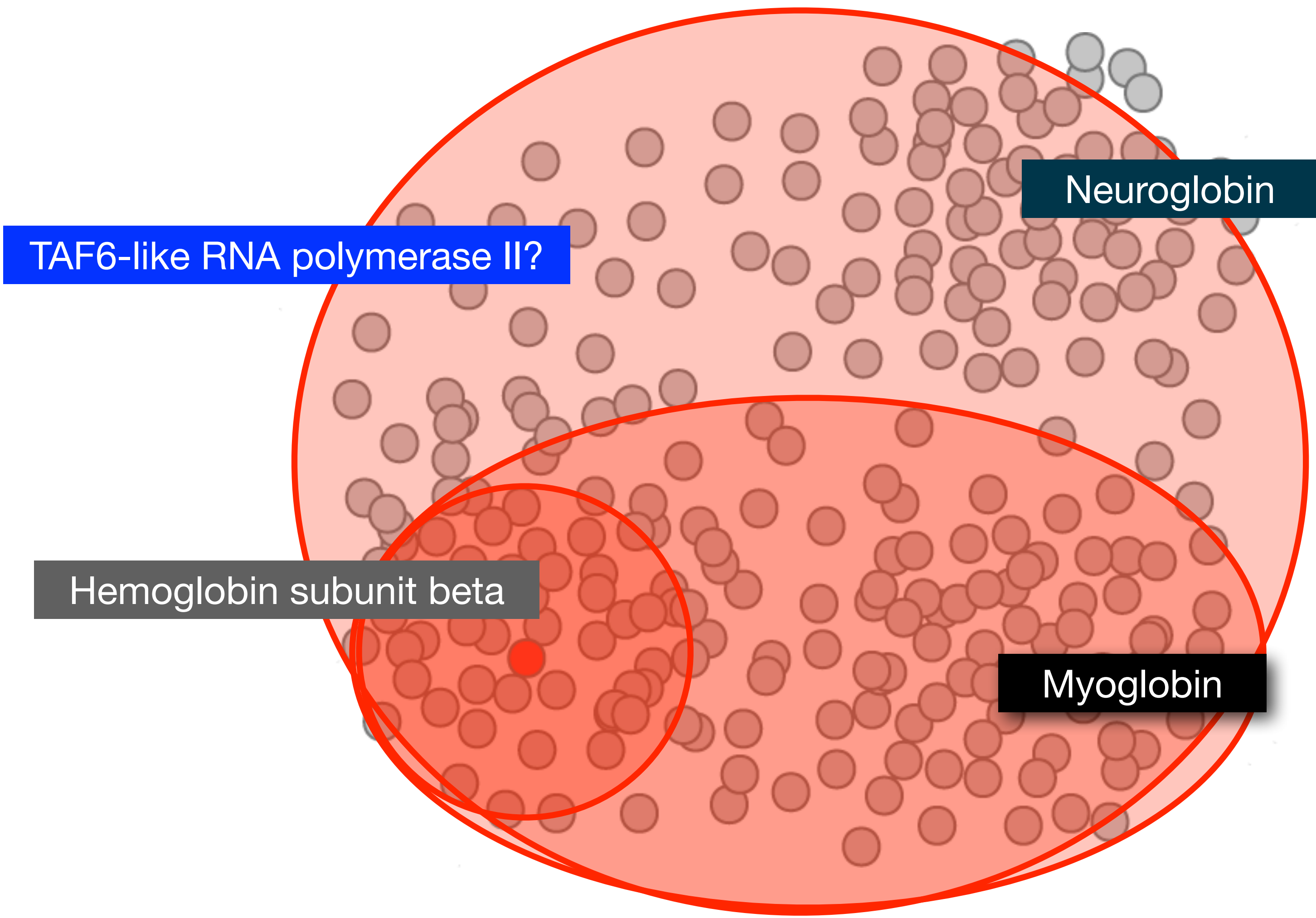


(see Altschul *et al.*, Nuc. Acids Res. (1997) 25:3389-3402)









TAF6-like RNA polymerase II?

Neuroglobin

Hemoglobin subunit beta

Myoglobin

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

Description	Max score	Total score	Query cover	E value	Ident	Accession
hemoglobin subunit beta [Homo sapiens]	301	301	100%	2e-106	100%	NP_000509.1
hemoglobin subunit delta [Homo sapiens]	284	284	100%	7e-100	93%	NP_000510.1
hemoglobin subunit epsilon [Homo sapiens]	240	240	100%	2e-82	76%	NP_005321.1
hemoglobin subunit gamma-2 [Homo sapiens]	235	235	100%	2e-80	73%	NP_000175.1
hemoglobin subunit gamma-1 [Homo sapiens]	232	232	100%	3e-79	73%	NP_000550.2
hemoglobin subunit alpha [Homo sapiens]	114	114	97%	7e-33	43%	NP_000508.1
hemoglobin subunit zeta [Homo sapiens]	100	100	97%	3e-27	36%	NP_005323.1

Description	Max score	Total score	Query cover	E value	Ident	Accession
hemoglobin subunit beta [Homo sapiens]	301	301	100%	2e-106	100%	NP_000509.1
hemoglobin subunit delta [Homo sapiens]	284	284	100%	7e-100	93%	NP_000510.1
hemoglobin subunit epsilon [Homo sapiens]	240	240	100%	2e-82	76%	NP_005321.1
hemoglobin subunit gamma-2 [Homo sapiens]	235	235	100%	2e-80	73%	NP_000175.1
hemoglobin subunit gamma-1 [Homo sapiens]	232	232	100%	3e-79	73%	NP_000550.2
hemoglobin subunit alpha [Homo sapiens]	114	114	97%	7e-33	43%	NP_000508.1
hemoglobin subunit zeta [Homo sapiens]	100	100	97%	3e-27	36%	NP_005323.1
myoglobin [Homo sapiens]	80.5	80.5	97%	2e-19	26%	NP_005359.1
neuroglobin [Homo sapiens]	54.7	54.7	92%	2e-09	23%	NP_067080.1

1

2

New relevant globins found only by PSI-BLAST

Description	Max score	Total score	Query cover	E value	Ident	Accession
hemoglobin subunit beta [Homo sapiens]	301	301	100%	2e-106	100%	NP_000509.1
hemoglobin subunit delta [Homo sapiens]	284	284	100%	7e-100	93%	NP_000510.1
hemoglobin subunit epsilon [Homo sapiens]	240	240	100%	2e-82	76%	NP_005321.1
hemoglobin subunit gamma-2 [Homo sapiens]	235	235	100%	2e-80	73%	NP_000175.1
hemoglobin subunit gamma-1 [Homo sapiens]	232	232	100%	3e-79	73%	NP_000550.2
hemoglobin subunit alpha [Homo sapiens]	114	114	97%	7e-33	43%	NP_000508.1
hemoglobin subunit zeta [Homo sapiens]	100	100	97%	3e-27	36%	NP_005323.1
myoglobin [Homo sapiens]	80.5	80.5	97%	2e-19	26%	NP_005359.1
neuroglobin [Homo sapiens]	54.7	54.7	92%	2e-09	23%	NP_067080.1
myoglobin [Homo sapiens]	159	159	97%	3e-50	26%	NP_005359.1
hemoglobin subunit alpha [Homo sapiens]	151	151	97%	3e-47	42%	NP_000508.1
hemoglobin subunit mu [Homo sapiens]	147	147	97%	6e-46	35%	NP_001003938.1
hemoglobin subunit theta-1 [Homo sapiens]	147	147	97%	2e-45	37%	NP_005322.1
neuroglobin [Homo sapiens]	134	134	92%	3e-40	23%	NP_067080.1
PREDICTED: cytoglobin isoform X2 [Homo sapiens]	115	115	66%	3e-33	25%	XP_016879605.1
PREDICTED: microtubule cross-linking factor 1 isoform X1 [Homo sapie	46.3	46.3	27%	7e-06	39%	XP_011523942.1
PREDICTED: microtubule cross-linking factor 1 isoform X4 [Homo sapie	46.3	46.3	27%	7e-06	39%	XP_005258156.1

1

2

3

?

Inclusion of irrelevant hits can lead to PSSM corruption

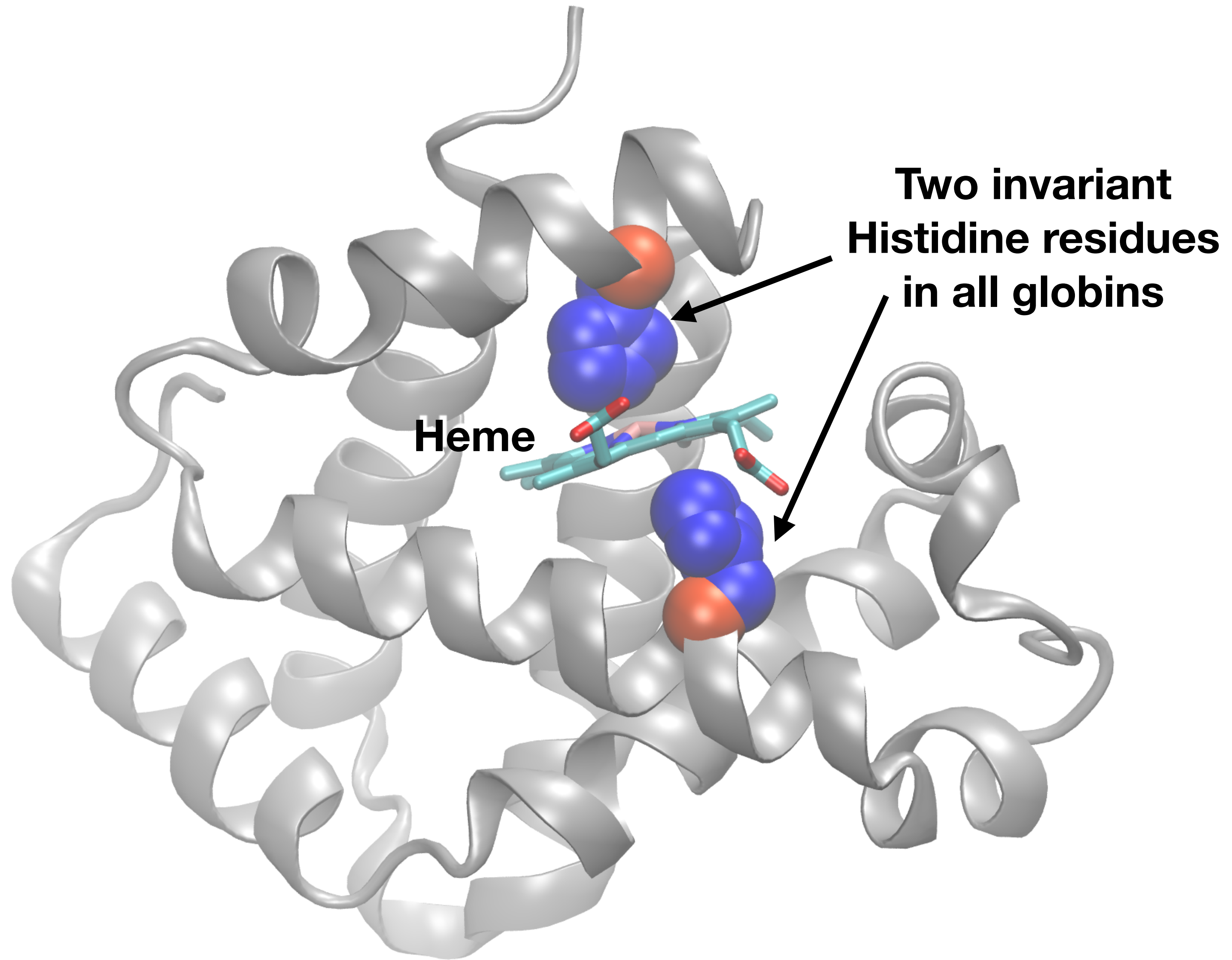
YOUR TURN!

- There are **four required** and **one optional** hands-on sections including:

1. Limits of using BLAST [~10 mins]
2. Using PSI-BLAST [~30 mins]
3. **Examining conservation patterns** [~20 mins]
— BREAK [15 mins]—
4. [Optional] Using HMMER [~10 mins]
5. Divergence of protein sequence and structure [~25 mins]

- ▶ Please do answer the last review question (**Q20**).
- ▶ We encourage discussion at your **Table** and on **Piazza!**

✓ Query_73613	1	MVHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFE-SFGDLSTPDAVM-GNPKVKAHGKKVLGAF	72
✓ NP_000510.1	1	MVHLTPEEKTAVNALWGKV--NVDAVGGEALGRLLVVYPWTQRFFE-SFGDLSSPDAVM-GNPKVKAHGKKVLGAF	72
✓ NP_000175.1	1	MGHFTEEDKATITSLWGKV--NVEDAGGETLGRLLVVYPWTQRFFD-SFGNLSSASAIM-GNPKVKAHGKKVLTSL	72
✓ NP_000509.1	1	MVHLTPEEKSAVTALWGKV--NVDEVGGEALGRLLVVYPWTQRFFE-SFGDLSTPDAVM-GNPKVKAHGKKVLGAF	72
✓ NP_005321.1	1	MVHFTAEEKAAVTSLWSKM--NVEEAGGEALGRLLVVYPWTQRFFD-SFGNLSSPSAIL-GNPKVKAHGKKVLTSL	72
✓ NP_000550.2	1	MGHFTEEDKATITSLWGKV--NVEDAGGETLGRLLVVYPWTQRFFD-SFGNLSSASAIM-GNPKVKAHGKKVLTSL	72
✓ NP_005323.1	1	-MSLTKTERTIIVSMWAKISTQADTIGTETLERLFLSHPQTKTYFP-HF-----DLHpGSAQLRAHGSKVVA	67
✓ NP_000508.1	1	-MVLSPADKTNVKAAWGKVGAGHAGEYGAEALERMFSLFPTTKTYFP-HF-----DLShGSAQVKGHGKKVADAL	67
✓ XP_005257062.1	1	[15] SEELSEAERKAVQAMWARLYANCEDVGVAILVRFFVNFPSAKQYFS-QFKHMEDPLEME-RSPQLRKHACRVMGAL	89
✓ NP_001003938.1	1	--MLSAQERAQIAQVWDLIAGHEAQFGAELLRLFTVYPSTKVYFP-HL-----SACQ-DATQLLSHGQRM	66
✓ NP_005322.1	1	-MALSAEDRALVRALWKKLGSNVGVYTTEALERTFLAFPATKTYFS-H-----LDLSpGSSQVRAHGQKVADAL	67
✓ NP_599030.1	1	[15] SEELSEAERKAVQAMWARLYANCEDVGVAILVRFFVNFPSAKQYFS-QFKHMEDPLEME-RSPQLRKHACRVMGAL	89
✓ XP_016879605.1	1	-----MEDPLEME-RSPQLRKHACRVMGAL	24
✓ NP_001349775.1	1	-MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIIRLFKGHPEKLEKFD-KFKHLKSEDEMK-ASEDLKKGATVLTAL	73
✓ NP_067080.1	1	---MERPEPELIRQSWRAVSRSPLEHGTVLFARLFALPDLLPLFQyNCRQFSSPEDCL-SSPEFLDHIRKVMLVI	72
✓ NP_001369741.1	1	-----MK-ASEDLKKGATVLTAL	18
✓ Query_73613	73	SDGLAHLDNLKGT---FATLSELHCDKLHVDPENFRLGNVLCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH	147
✓ NP_000510.1	73	SDGLAHLDNLKGT---FSQLSELHCDKLHVDPENFRLGNVLCVLAHNFNGKEFTPQMQAAYQKVVAGVANALAHKYH	147
✓ NP_000175.1	73	GDAIKHLDDLKGT---FAQLSELHCDKLHVDPENFKLLGNVLTVLAIHFGKEFTPEVQASWQKMTGVASALSSRYH	147
✓ NP_000509.1	73	SDGLAHLDNLKGT---FATLSELHCDKLHVDPENFRLGNVLCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH	147
✓ NP_005321.1	73	GDAIKNMDNLKPA---FAKLSELHCDKLHVDPENFKLLGNVMVILATHFGKEFTPEVQAAWQKLVSAVAIALAHKYH	147
✓ NP_000550.2	73	GDAIKHLDDLKGT---FAQLSELHCDKLHVDPENFKLLGNVLTVLAIHFGKEFTPEVQASWQKMTAVASALSSRYH	147
✓ NP_005323.1	68	GDAVKSIDDIGGA---LSKLSELHAYILRVDPVNFKLLSHCLLVTLAARFPADFTAEEAHAWDKFLSVVSSVLTEKYR	142
✓ NP_000508.1	68	TNAVAHVDDMPNA---LSALSDLHAHKLVRDPVNFKLLSHCLLVTLAHLPAEFTPAVHASLDKFLASVSTVLT	142
✓ XP_005257062.1	90	NTVVENLHDPDKVssvLALVGKAHALKHKVEPVYFKILSGVILEVVAEEFASDFPPETQRAWAKLRGLIYSHVTAAYK [35]	202
✓ NP_001003938.1	67	GAAVQHVDNLRAA---LSPLADLHALVLRVDPANFPLLIQCFHVVLASHLQDEFVQMQAAWDKFLTGVAVVLTEKYR	141
✓ NP_005322.1	68	SLAVERLDDLPHA---LSALSHLHACQLRVDPASFQLLGHCLLVTLARHYPGDFSPALQASLDKFLSHVISALVSEYR	142
✓ NP_599030.1	90	NTVVENLHDPDKVssvLALVGKAHALKHKVEPVYFKILSGVILEVVAEEFASDFPPETQRAWAKLRGLIYSHVTAAYK [23]	190
✓ XP_016879605.1	25	NTVVENLHDPDKVssvLALVGKAHALKHKVEPVYFKILSGVILEVVAEEFASDFPPETQRAWAKLRGLIYSHVTAAYK [35]	137
✓ NP_001349775.1	74	GGILKKKGHHEAE---IKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNYK [6]	154
✓ NP_067080.1	73	DAAVTNVEDLSSLeeyLASLGRKHRA-VGVKLSFSTVGESLLYMLEKCLGPAFTPATRAAWSQLYGAVVQAMSRGWD [2]	151
✓ NP_001369741.1	19	GGILKKKGHHEAE---IKPLAQSHATKHKIPVKYLEFISECIIQVLQSKHPGDFGADAQGAMNKALELFRKDMASNYK [6]	99



YOUR TURN!

- There are **four required** and **one optional** hands-on sections including:

1. Limits of using BLAST [~10 mins]
2. Using PSI-BLAST [~30 mins]
3. Examining conservation patterns [~20 mins]
— BREAK [15 mins]—
4. **[Optional] Using HMMER** [~10 mins]
5. Divergence of protein sequence and structure [~25 mins]

- ▶ Please do answer the last review question (**Q20**).
- ▶ We encourage discussion at your **Table** and on **Piazza!**

Problems with PSSMs: Positional dependencies

Do not capture positional dependencies

WEIRD
WEIRD
WEIQH
WEIRD
WEIQH

D					0.6
E		I			
H					0.4
I			I		
Q				0.4	
R				0.6	
W	I				

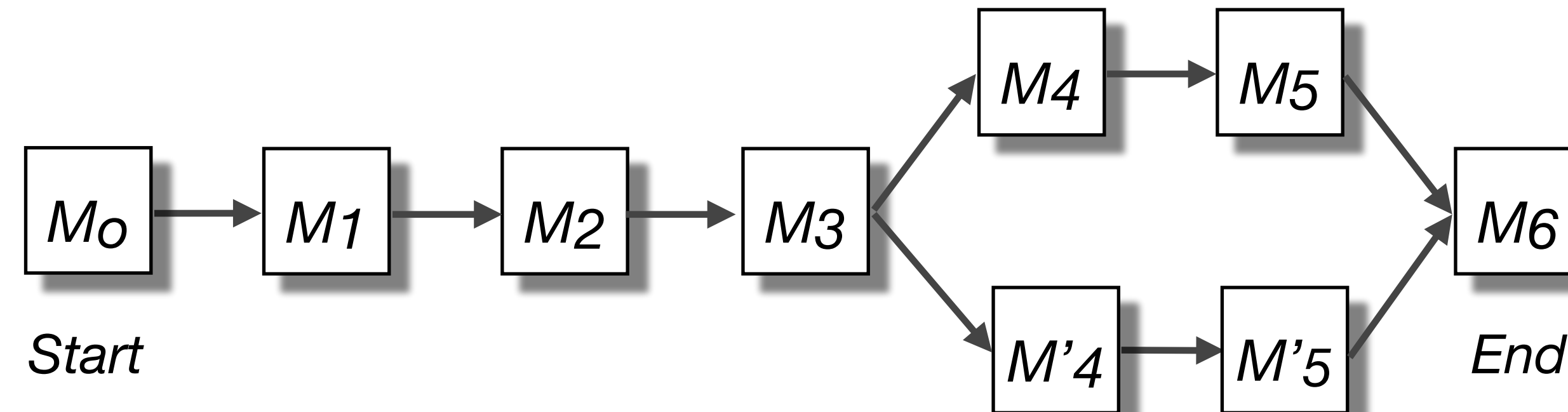
Note: We never see **QD** or **RH**, we only see **RD** and **QH**.
However, $P(RH)=0.24$, $P(QD)=0.24$, while $P(QH)=0.16$

Markov chains: Positional dependencies



The connectivity or **topology** of a Markov chain can easily be designed to capture dependencies and variable length motifs.

WEIRD
WEIRD
WEIQH
WEIRD
WEIQH

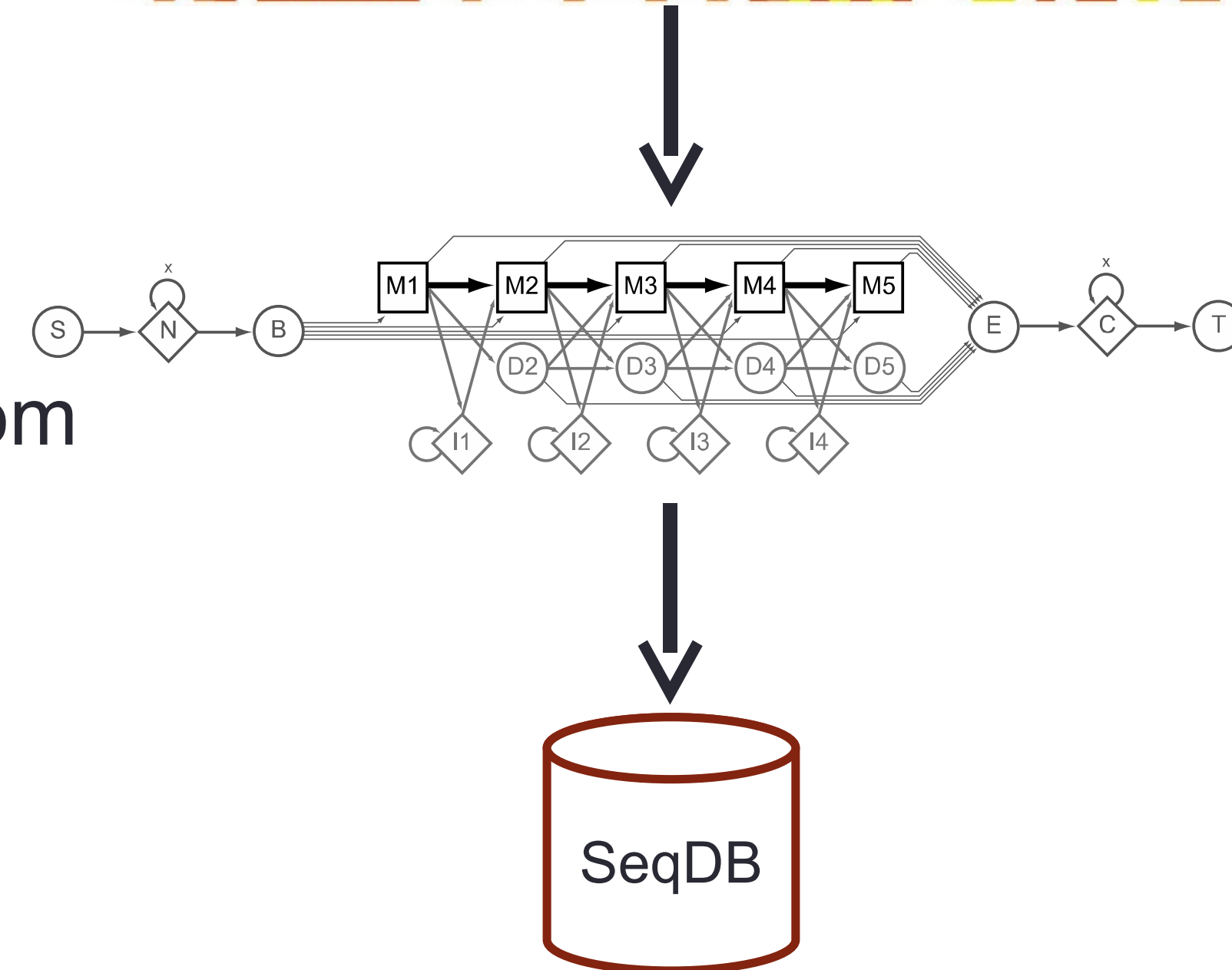


Recall that a PSSM for this motif would give the sequences **WEIRD** and **WEIRH** equally good scores even though the **RH** and **QR** combinations were not observed

Use of HMMER

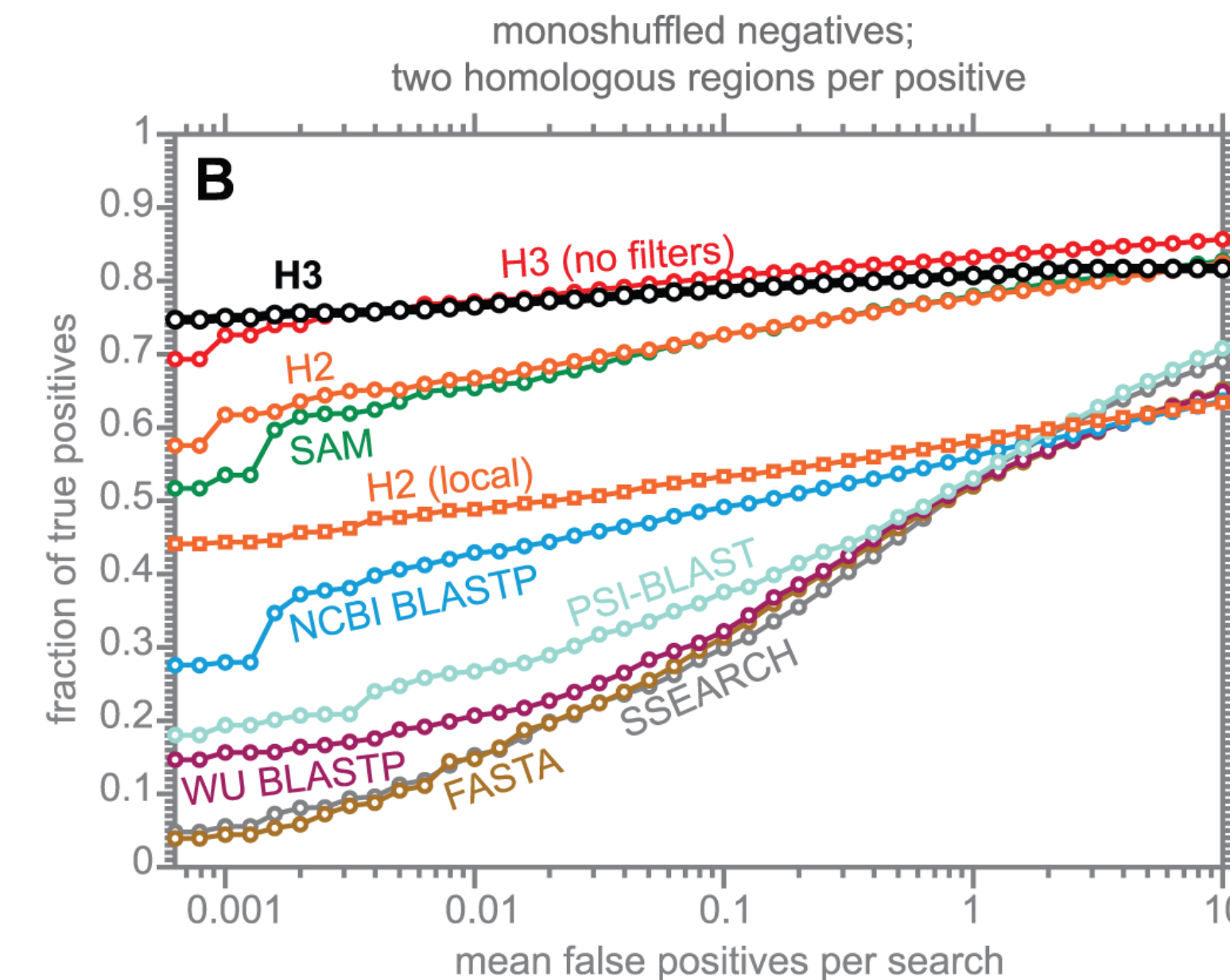
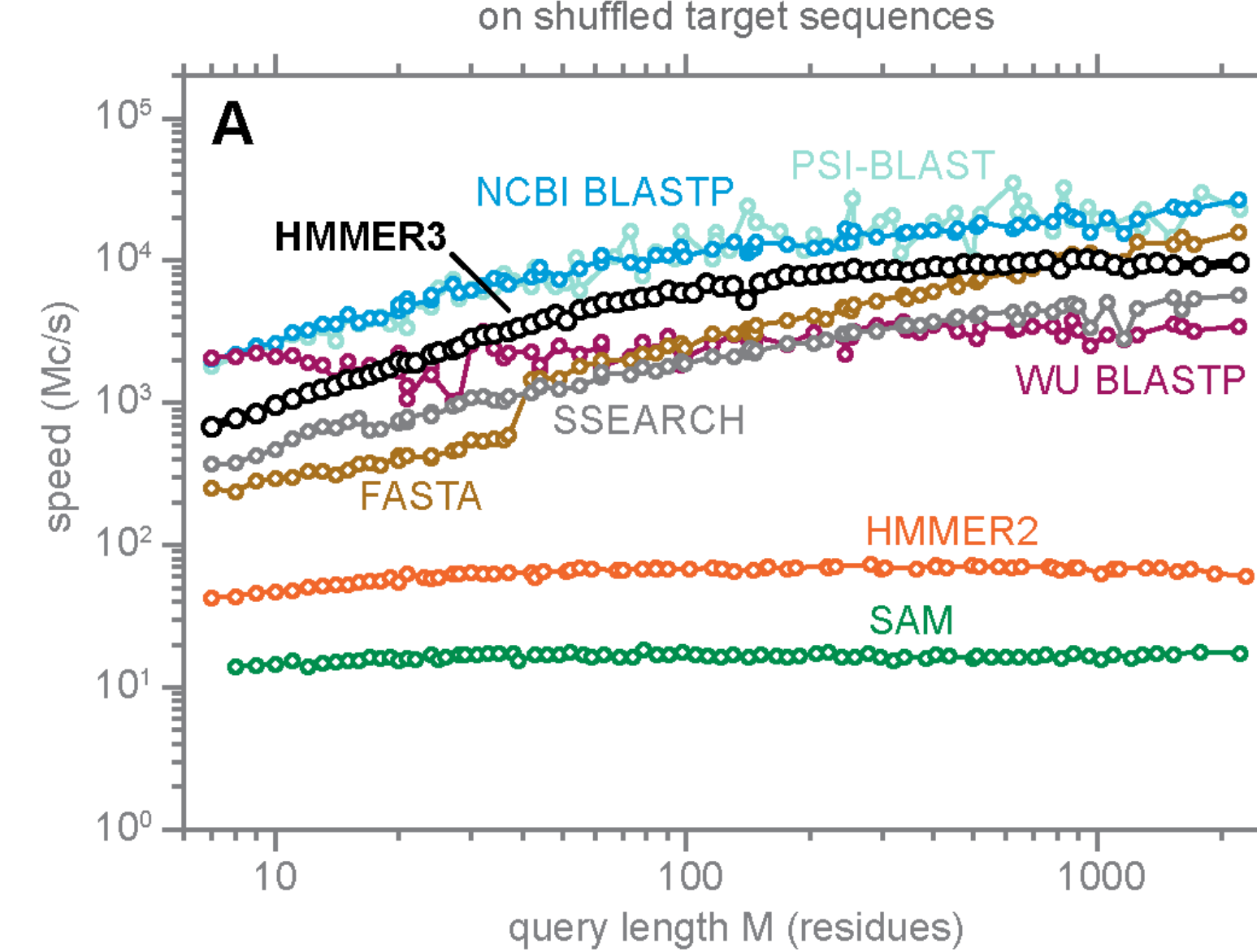
- Widely used by protein family databases
 - Use 'seed' alignments
- Until 2010
 - Computationally expensive
 - Restricted to HMMs constructed from multiple sequence alignments
- Command line application

```
K I I I T G E P G V G K T T L V K K I V E R L - - - G K R A I G F W T E E V R D P E T K K R T G F R I I T T E
K I L I T G R P G V G K T T L I K K L S R L L - - - - Q N A G G F Y T E E M R - - - E G E K R I G F K I I T L D
R F F V S G M P G V G K T T L A K R I A D E V R R E G F K V G G I I T E E I R - - - E G G K R T G F R V I A L D
R I F I T G M P G V G K T T L A L K I A E K L K E L G Y K V G G E I T K E I R - - - D G G K R V G F K I I T L D
R F F V S G M P G V G K T T L A K R I A D E I K R E G F K V G G I I T Q E I R - - - S G A R S G F R V I A L D
H V F L T G P P G V G K T T L I Q K A I E V L Q S S G L P V D G F Y T Q E V R - - - Q E G K R I G F D V V T L S
H V F L T G V P G V G K T T L V K K V C D A L - - - S G L S V S G F Y T E E V R - - - E H G R V G F D V V T V S
H V F L T G S P G V G K T T L I Q K A I T V L Q S S G L P V D G F Y T Q E V R - - - Q G G K R I G F D V V T L S
H V F L T G P P G V G K T T L I H K A S E V L K S S G V P V D G F Y T E E V R - - - Q G G R R I G F D V V T L S
```



HMMER vs BLAST

	HMMER	BLAST
Program	<i>PHMMER</i>	<i>BLASTP</i>
Query	Single sequence	
Target Database	Sequence database	
Program	<i>HMMSCAN</i>	<i>RPSBLAST</i>
Query	Single sequence	
Target Database	Profile HMM database, e.g. Pfam	PSSM database, e.g. CDD
Program	<i>HMMSEARCH</i>	<i>PSI-BLAST</i>
Query	Profile HMM	PSSM
Target Database	Sequence database	
Program	<i>JACKHMMER</i>	<i>PSI-BLAST</i>
Query	Single sequence	
Target Database	Sequence database	



Modified from: S. R. Eddy
PLoS Comp. Biol., 7:e1002195, 2011.





HMMER

Biosequence analysis using profile hidden Markov Models

phmmer

hmmscan

hmmsearch

jackhmmmer

protein sequence vs protein sequence database

Paste a Sequence | Upload a File | Accession Search

Paste in your sequence or use the [example](#)

```
>NP_000509.1 hemoglobin subunit beta [Homo sapiens]
MVHLTPEEKSAVTALWGKVNVDVEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNPKVKAHGKKVLG
AFSDGLAHLNLDLKGTFATLSELHCDKLHVDPENFRLLGNVLCVLAHFGKEFTPPVQAAYQKVVAGVAN
ALAHKYH
```

Submit

Reset

Sequence Database

Frequently used databases:

Current database selection:

SwissProt

Restrict by Taxonomy

Taxon search

Pre-defined representatives

Organism:

Significant Query Matches (12) in *swissprot* (v.2018_11)

Customise Customise

	Target	Description	Species	Cross-references	E-value
>	HBB_HUMAN	Hemoglobin subunit beta	Homo sapiens		6.8e-99
>	HBD_HUMAN	Hemoglobin subunit delta	Homo sapiens		1.6e-91
>	HBE_HUMAN	Hemoglobin subunit epsilon	Homo sapiens		1.5e-74
>	HBG2_HUMAN	Hemoglobin subunit gamma-2	Homo sapiens		8.8e-73
>	HBG1_HUMAN	Hemoglobin subunit gamma-1	Homo sapiens		6.2e-72
>	HBA_HUMAN	Hemoglobin subunit alpha	Homo sapiens		3.8e-29
>	HBAZ_HUMAN	Hemoglobin subunit zeta	Homo sapiens		4.5e-23
>	HBAT_HUMAN	Hemoglobin subunit theta-1	Homo sapiens		5.2e-22
>	HBM_HUMAN	Hemoglobin subunit mu	Homo sapiens		3.4e-19
>	CYGB_HUMAN	Cytoglobin	Homo sapiens		3.1e-14
>	MYG_HUMAN	Myoglobin	Homo sapiens		2.3e-06
>	NGB_HUMAN	Neuroglobin	Homo sapiens		0.0017

(show all) alignments

Your search took: 0.06 secs

showing rows 1 - 12 of 12

[Local Link](#)

PFAM: Protein Family Database of Profile HMMs

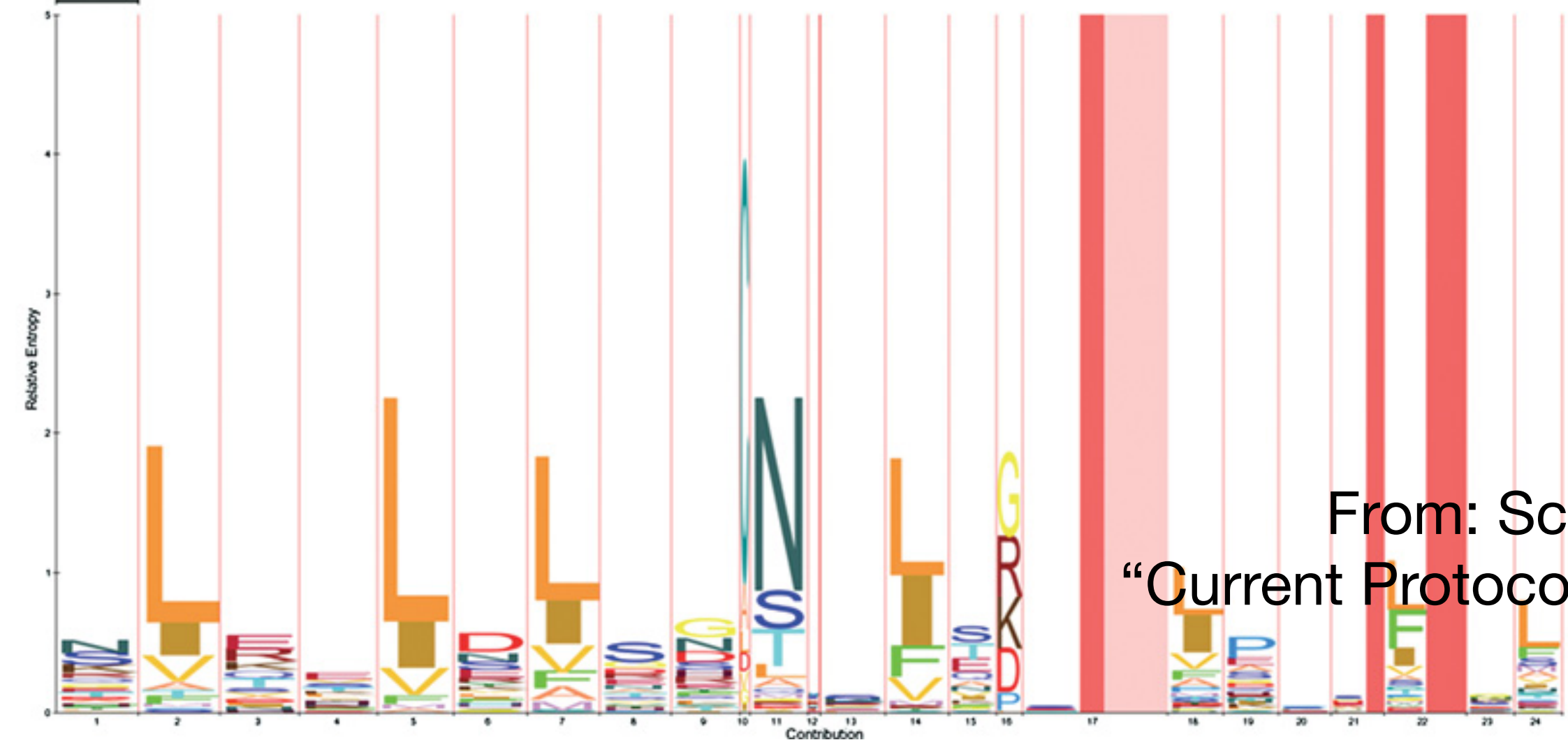
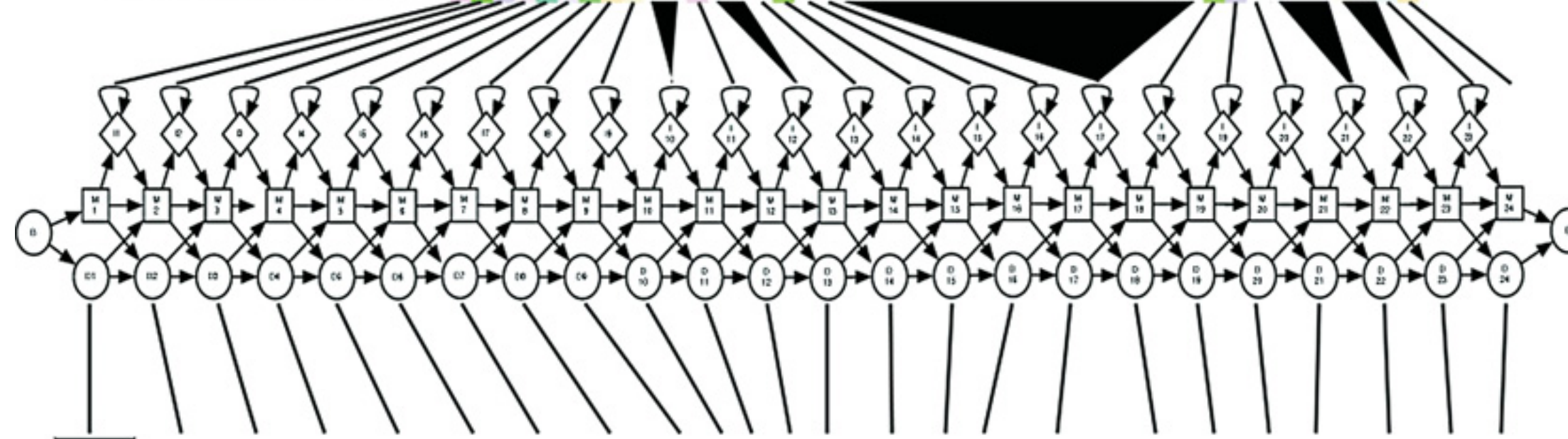
Comprehensive compilation of both multiple sequence alignments and profile HMMs of protein families.

<http://pfam.sanger.ac.uk/>

PFAM consists of two databases:

- **Pfam-A** is a manually curated collection of protein families in the form of multiple sequence alignments and profile HMMs. HMMER software is used to perform searches.
- **Pfam-B** contains additional protein sequences that are automatically aligned. Pfam-B serves as a useful supplement that makes the database more comprehensive.
- Pfam-A also contains higher-level groupings of related families, known as **clans**

Q9ARB2_LINUS/823-844 MLEYLDIGRA..P.RIV.H.....LDG...LENL
 Q9M8N0_ARATH/320-341 RLTFLNLSFC..S.KLT.G.....LAF...FSII
 FLJ_HUMAN/318-339 NLEEFMAAN..N..NLE.L.....VPES..LCRC
 Q9VN74_DROME/90-112 ALHSLVIENC...TIV.H.....INDAA.FNQE
 Q8L8I7_PNTA/792-814 NLQTIQMYRX..E.SLQ.V.....LPDS..FGNL
 Q9FHL8_ARATH/301-324 NLWSLNLSR..N..LFSDP.....LPVVG.ARGF
 SLIK6_MOUSE/65-87 RPFHLSLLN..N..GLT.M.....LHTND.FSGL
 Q8NIJ8_EMEN/978-1000 TLTSLNIAS..A..KLV.Q.....FRDTL.FDSL
 Q9LUQ2_ARATH/92-113 AMKSLDVSF..N..SISE.....LPEQ..IGSA
 Q9FH93_ARATH/169-188 RLTSLNLDL..N..RFNGT.....LPS....LN
 Q898G0_CLOTE/268-288 YLERINLDK..N..KI.KN.....IEE...LEAN
 Q8H6V2_MAIZE/678-699 NLRILSIVDC..V.SLQ.K.....LPP...SDSF
 Q9AR40_LINUS/692-713 DLKVLINQ..T..EIT.T.....LKGE..VESL
 Q9LE82_ARATH/350-377 HLTEIYMSY..L..NLEDEGT.....EALSEAL.LKSA
 Q9H5N5_HUMAN/255-278 HLQVLDLHQC...SLT.AD.....DVMSL...TQVI
 Q8L4C7_ARATH/185-207 KLEYLDIWG..S..NVT.N.....QGAVS..ILKF
 Q9VSA4_DROME/1115-113E QLKALRLQC..N..AIGSH.....GLEAL..LCGQ
 TLR1_MOUSE/376-398 RLKTLSLQK..N..QL.KN.....LENII.LTSA
 Q9TXJ6_LEMA/445-465 GLRDIDLSH..T..KVH.N.....IDA...LQAS
 FXL13_MOUSE/409-448 KLIYLDLSGC..T.QVL.VEKCPRISSVVLIGSPHISDSA.FKAL
 Q9TXJ6_LEMA/927-948 ALTVVNANSC..V.NLT.S.....IEA...LESA
 Q9M4X9_CHLRE/1417-1444 LLAVLHLHD..NP.RLA.ADG.....VAGLAAA..LPGL
 Q945S6_LYCPM/656-677 NLRHLDVSN..T..RRL.K.....MPLH..LSRL



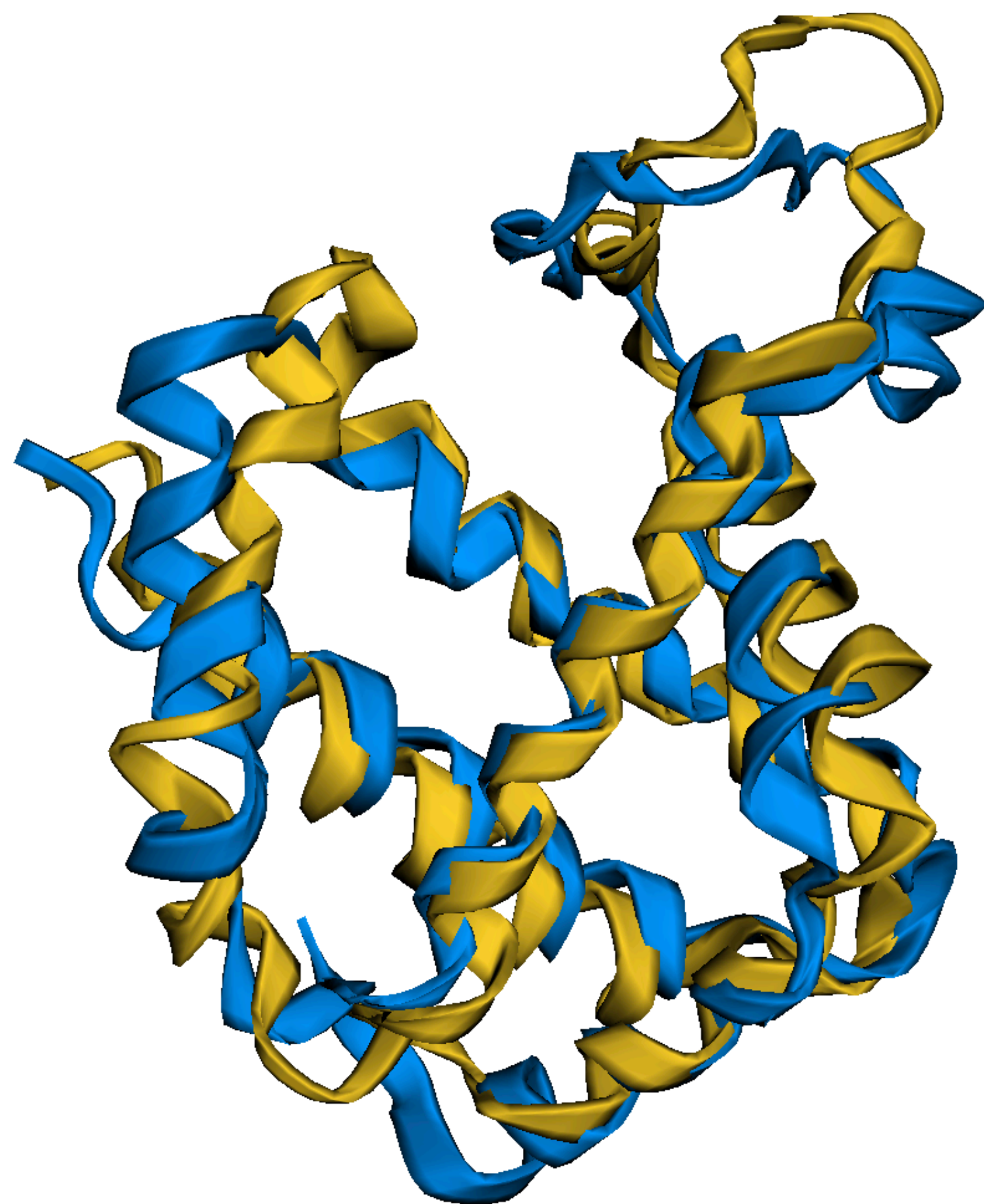
From: Schuster-Bockler *et al.*
 "Current Protocols in Bioinformatics"
 Supplement 18.

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ALIGNMENT		CONTACT MAP	
Align 2hbsB.pdb 146 with 4mpmB.pdb 148			
Twists 0 ini-len 136 ini-rmsd 3.05 opt-equ 143 opt-rmsd 2.65 chain-rmsd 3.05			
Score 318.72 align-len 150 gaps 7 (4.67%)			
P-value 3.26e-14 Afp-num 14073 Identity 20.67% Similarity 40.00%			
Block 0 afp 17 score 318.72 rmsd 3.05 gap 9 (0.06%)			
		.	:
Chain 1	2	HLTPVEKSAVTALWGKVN--VDEVGGEALGRLLVVYPWTQRFFESFG--DLSTPDAVMGNPKVKAHGKKVL	:
		111 11111111111111 1111111111111111111111111111111111 111111111111111111111111	:
Chain 2	2	ERP--EPELIRQSWRAVSRSPLEHGTVLFARLFALEPDLLPLFQYNCRQFSSPEDCLSSPEFLDHIRKVM	:
Chain 1	69	GAFSDGLAHLNPKGTFATLSELHCD--KLHVDPENFRLLGNVLVCVLAHFGKEFTPPVQAAYQKVVAG	:
		11111111111111111111111111111111 11	:
Chain 2	70	LVIDAAVTNVEDLSSLEEYLASLGRKHRVGVKLSFSTVGESLLYMLEKCLGPAFTPATRAAWSQLYGA	:
Chain 1	137	VANALAHKYH	:
		1111111111	:
Chain 2	140	VVQAMSRGWD	:

Summary

- **Find a gene project:** You can start working on this now. Submit your responses to Q1-Q4 to get feedback.
- **PSI-BLAST algorithm:** Application of iterative position specific scoring matrices (PSSMs) to improve BLAST sensitivity
- **Hidden Markov models (HMMs):** More versatile probabilistic model for detection of remote similarities
- **Structure comparisons as gold standards:** Structure is more conserved than sequence

Homework: DataCamp!

Install **R** and **RStudio** (see website)

Complete the **Introduction to R** course on **DataCamp**
(Check Piazza for your DataCamp invite and sign up with your UCSD email (i.e. first part of your email address) please.)

Let me know **NOW** if you don't have access to DataCamp!