BGGN 213

Advanced Database Searching

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http://thegrantlab.org/bggn213

Recap From Last Time:

- Sequence alignment is a fundamental operation underlying much of bioinformatics.
- Introduced dot matrices, dynamic programing and the BLAST heuristic approaches.
- Key point: Even when optimal solutions can be obtained they are not necessarily unique or reflective of the biologically correct alignment.
- Introduced classic global and local alignment algorithms (Needleman–Wunsch and Smith–Waterman) and their major application areas.
- Heuristic approaches are necessary for large database searches and many genomic applications.

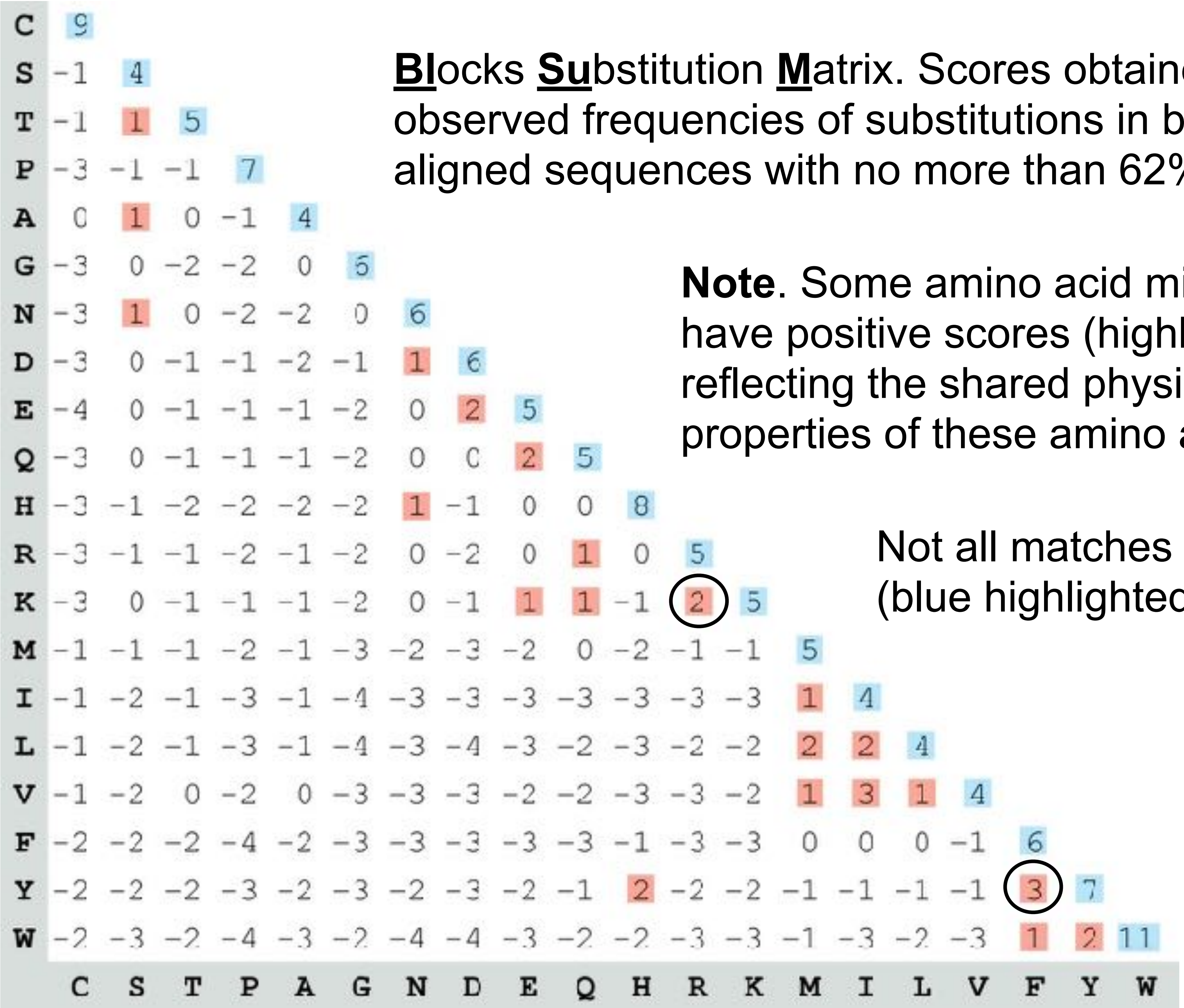
Todays Menu

- Sequence motifs and patterns: Simple approaches for finding functional cues from conservation patterns
- Sequence profiles and position specific scoring matrices (PSSMs): Building and searching with profiles, Their advantages and limitations
- PSI-BLAST algorithm: Application of iterative PSSM searching to improve BLAST sensitivity
- Hidden Markov models (HMMs): More versatile probabilistic model for detection of remote similarities

Side Note:

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

By default BLASTp match scores come from the BLOSUM62 matrix



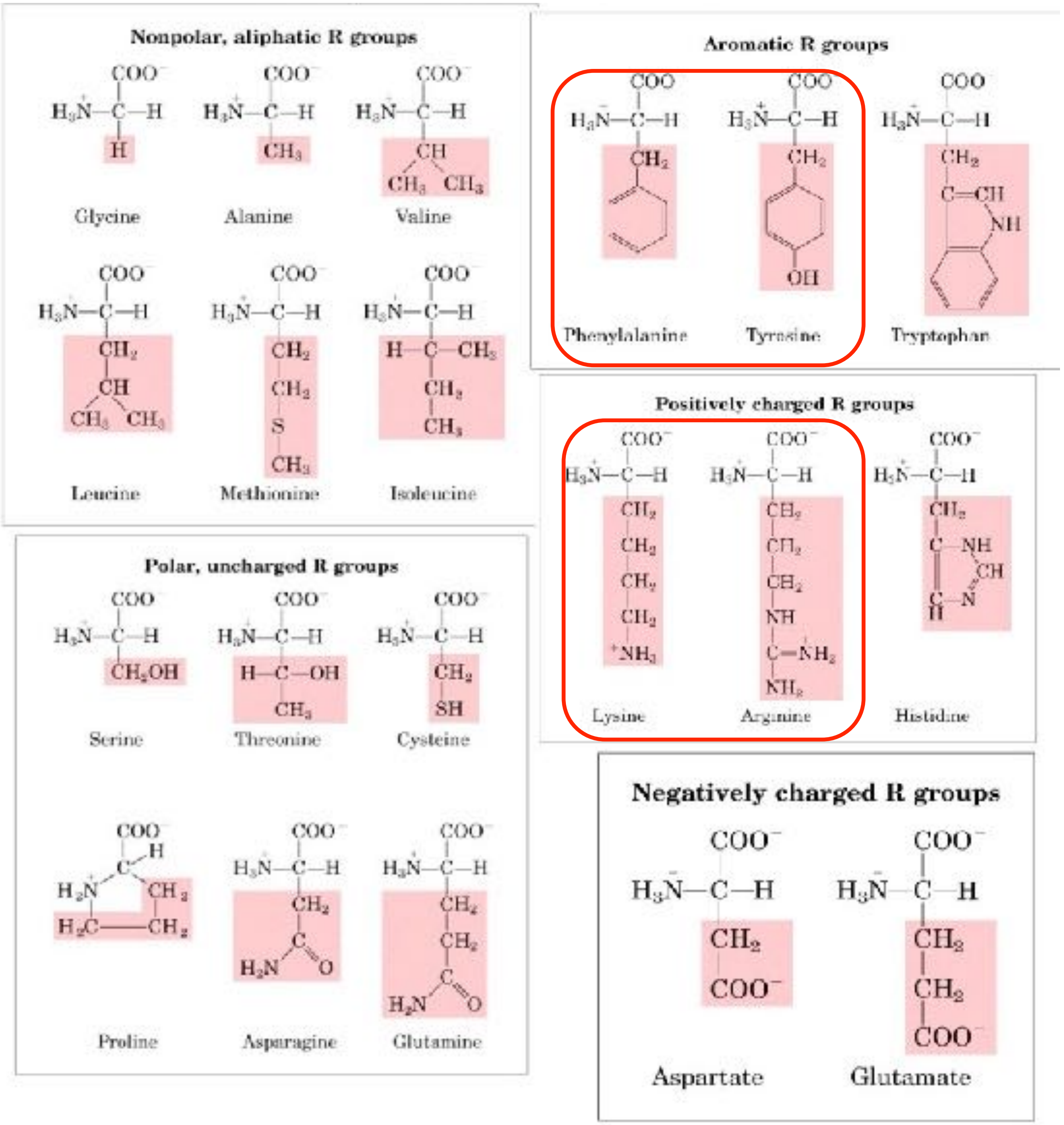
C	9																			
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4															
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6													
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
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 **Su**bstitution **M**atrix. Scores obtained from observed frequencies of substitutions in blocks of aligned sequences with no more than 62% identity.

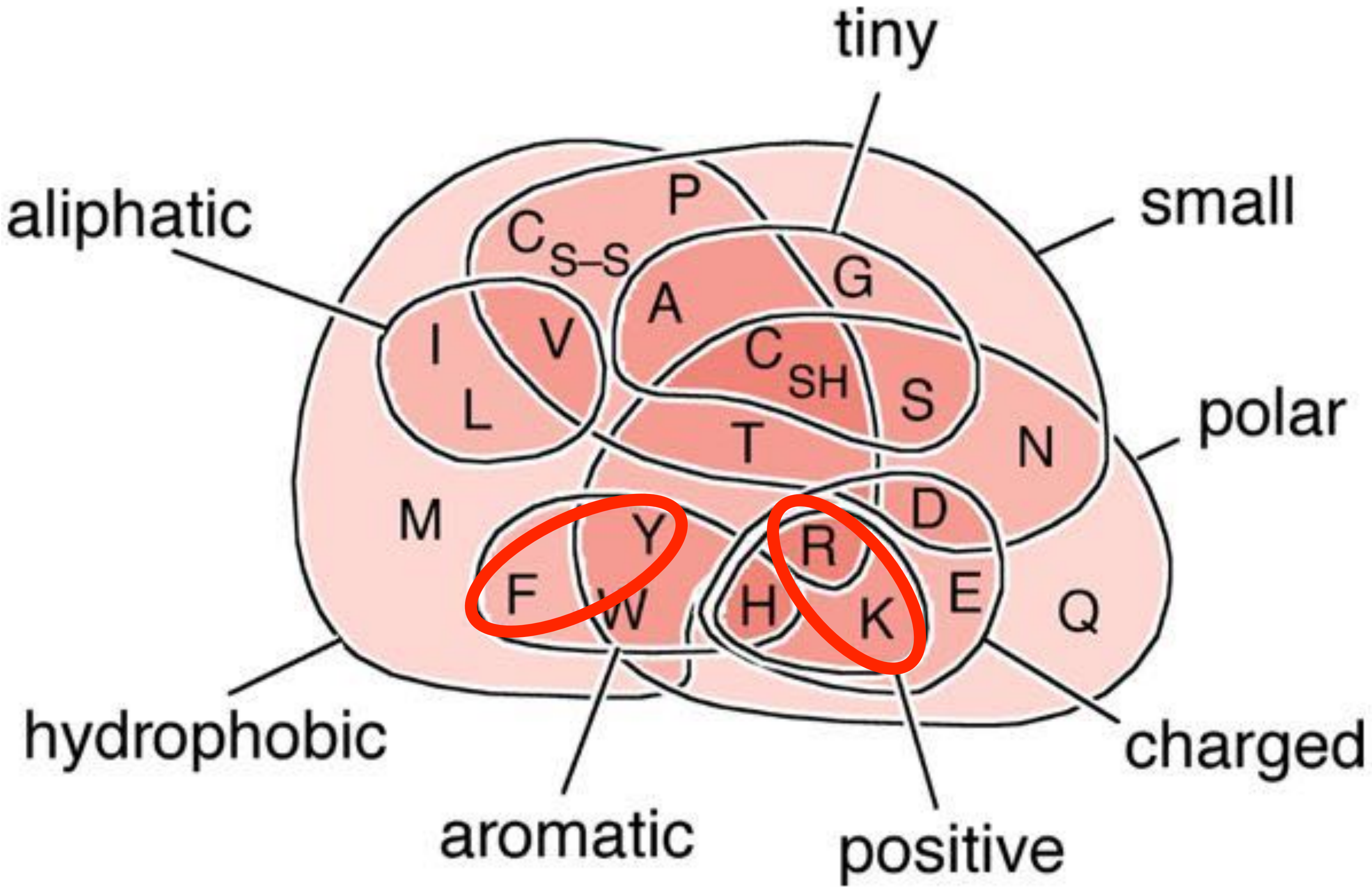
Note. Some amino acid mismatches have positive scores (highlighted in red) reflecting the shared physicochemical properties of these amino acids

Not all matches score equally (blue highlighted values)

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…

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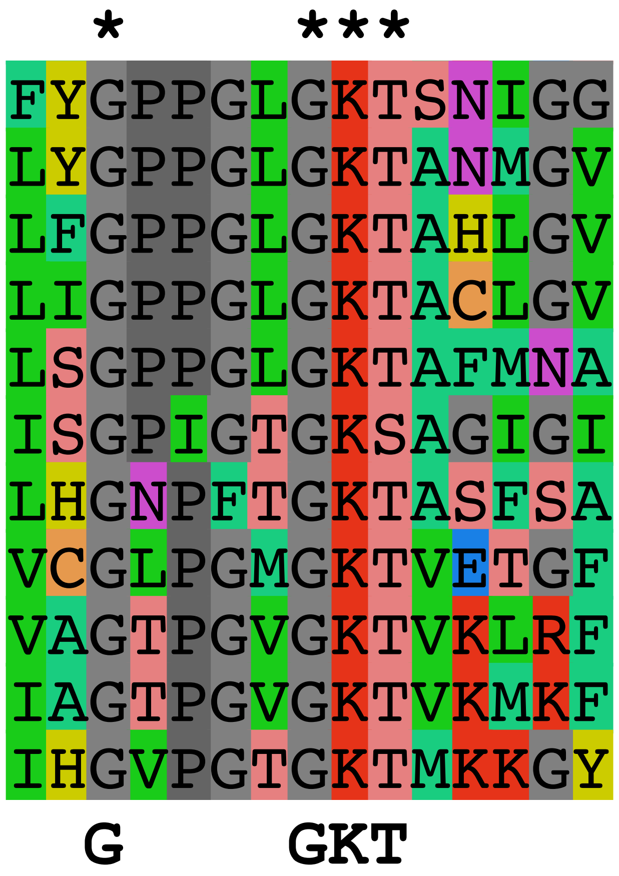
Functional cues from conservation patterns

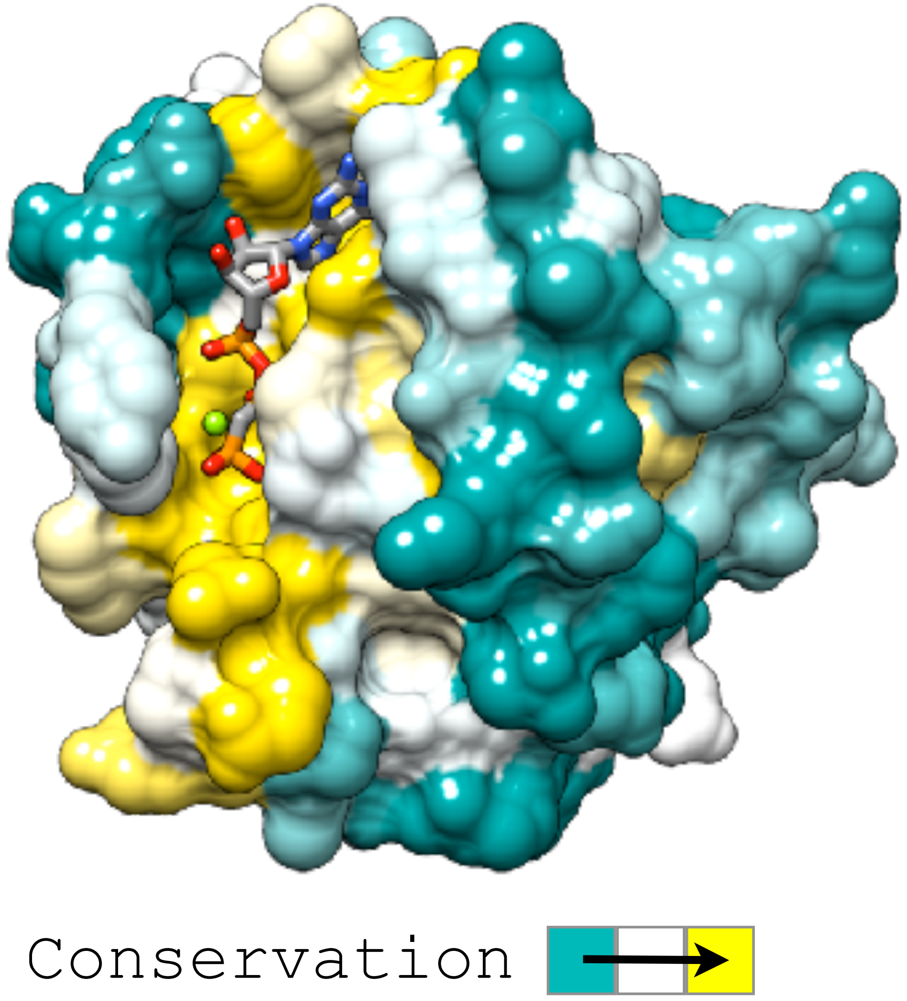
Within a protein or nucleic acid sequence there may be a small number of characteristic residues that occur consistently. These conserved “sequence fingerprints” (or **motifs**) usually contain functionally important elements

- E.g., the amino acids that are consistently found at enzyme active sites or the nucleotides that are associated with transcription factor binding sites.

ATP/GTP-binding proteins: G-x(4)-G-K-T



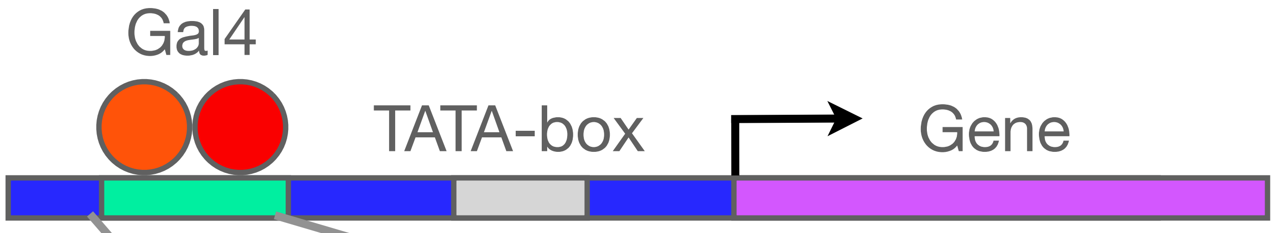
```
  *    ***
FYGPPGLGKTSNIGG
LYGPPGLGKTANMGV
LFGPPGLGKTAHLGV
LIGPPGLGKTACLGV
LSGPPGLGKTAFMNA
ISGPIGTGKSAGIGI
LHGNPFTGKTASFSA
VCGLPGMGKTVETGF
VAGTPGVGKTVKLRF
IAGTPGVGKTVKMKF
IHGVPGTGKTMKKGY
  G    GKT
```



Functional cues from conservation patterns...

Many DNA patterns are binding sites for Transcription Factors.

- E.g., The Gal4 binding sequence
  C-G-G-N(11)-C-C-G



```
        ***           ***
GAL3    CGGTCCACTGTGTGCCG
GAL7    CGGAGCACTGTTGAGCG
GCY1    CGGGGCAGACTATTCCG
GAL1    CGGATTAGAAGCCGCCG
GAL10   CGGAGGAGAGTCTTCCG
GAL2    CGGAAAGCTTCCTTCCG
PCL10   CGGAGTATATTGCACCG
        CGG           CCG
```

Representing recurrent sequence patterns

Beyond knowledge of invariant residues we can define **position-based** representations that highlight the range of permissible residues per position.

- **Pattern**: Describes a motif using a qualitative consensus sequence
  (e.g., IUPAC or regular expression). N.B. Mismatches are not tolerated!

  [LFI]-x-G-[PT]-P-G-x-G-K-[TS]-[AGSI]

- **Profile**: Describes a motif using quantitative information captured in a position specific scoring matrix (weight matrix).
  Profiles quantify similarity and often span larger stretches of sequence.

- **Logos**: A useful visual representation of sequence motifs.

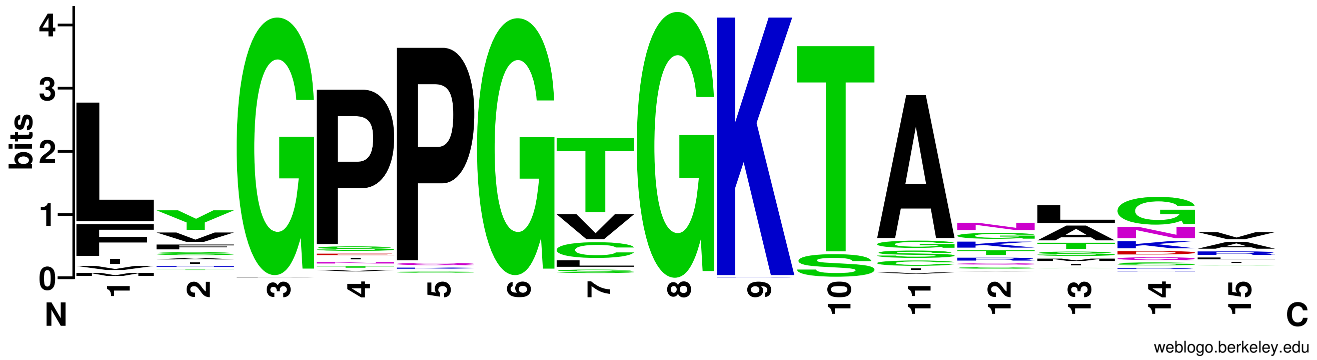


Image generated by:
weblogo.berkeley.edu

PROSITE is a protein pattern and profile database

Currently contains > 1790 patterns and profiles: http://prosite.expasy.org/
Example PROSITE patterns:

PS00087; SOD_CU_ZN_1
[GA]-[IMFAT]-H-[LIVF]-H-{S}-x-[GP]-[SDG]-x-[STAGDE]
The two Histidines are copper ligands

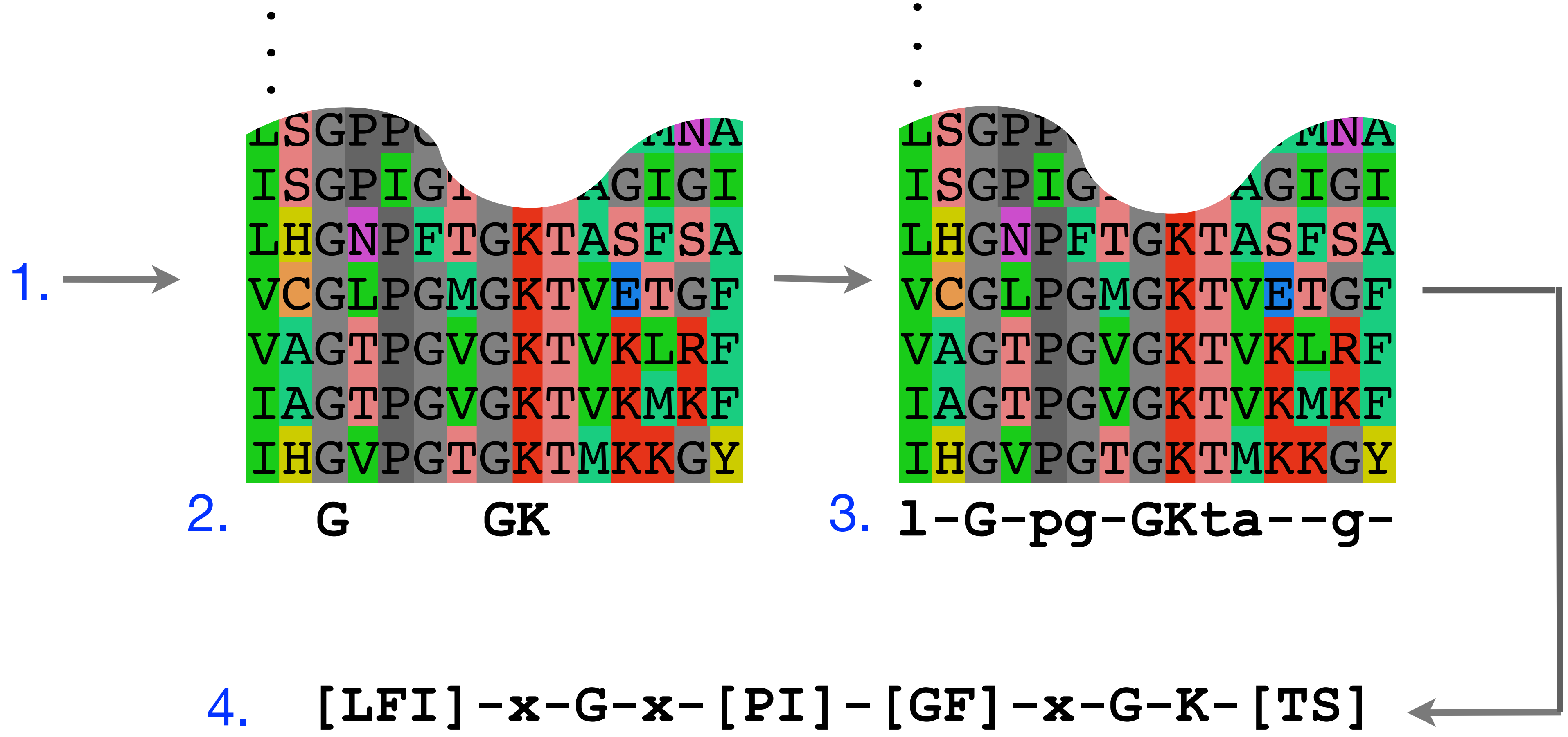
- Each position in the pattern is separated with a hyphen
- x can match any residue
- [ ] are used to indicate ambiguous positions in the pattern
  e.g., [SDG] means the pattern can match S, D, or G at this position
- { } are used to indicate residues that are not allowed at this position
  e.g., {S} means NOT S (not Serine)
- ( ) surround repeated residues, e.g., A(3) means AAA

Information from http://ca.expasy.org/prosite/prosuser.html

Defining sequence patterns

There are four basic steps involved in defining a new PROSITE style pattern:

1. Construct a multiple sequence alignment (MSA)
2. Identify conserved residues
3. Create a core sequence pattern (i.e. *consensus sequence*)
4. Expand the pattern to improve **sensitivity** and **specificity** for detecting desired sequences - more on this shortly...



2. G GK

3. l-G-pg-GKta--g-

4. [LFI]-x-G-x-[PI]-[GF]-x-G-K-[TS]

Pattern advantages and disadvantages

Advantages:

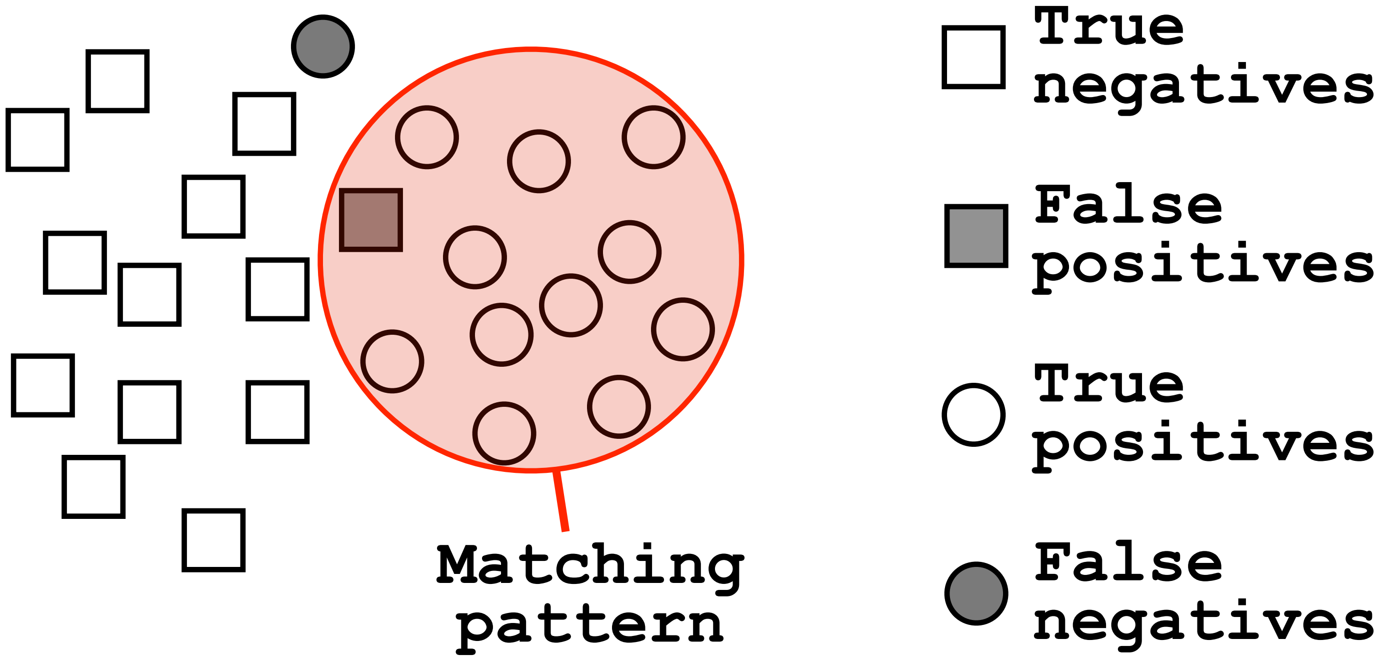
- Relatively straightforward to identify (exact pattern matching is fast)
- Patterns are intuitive to read and understand
- Databases with large numbers of protein (e.g., PROSITE) and DNA sequence (e.g., JASPER and TRANSFAC) patterns are available.

Disadvantages:

- Patterns are qualitative and *deterministic*
  (i.e., either matching or not!)
- We lose information about relative frequency of each residue at a position
  E.g., [GAC] *vs* 0.6 G, 0.28 A, and 0.12 C
- Can be difficult to write complex motifs using regular expression notation
- Cannot represent subtle sequence motifs

Side note: pattern sensitivity, specificity, and PPV

In practice it is not always possible to define one single regular expression type pattern which matches all family sequences (*true positives*) while avoiding matches in unrelated sequences (*true negatives*).

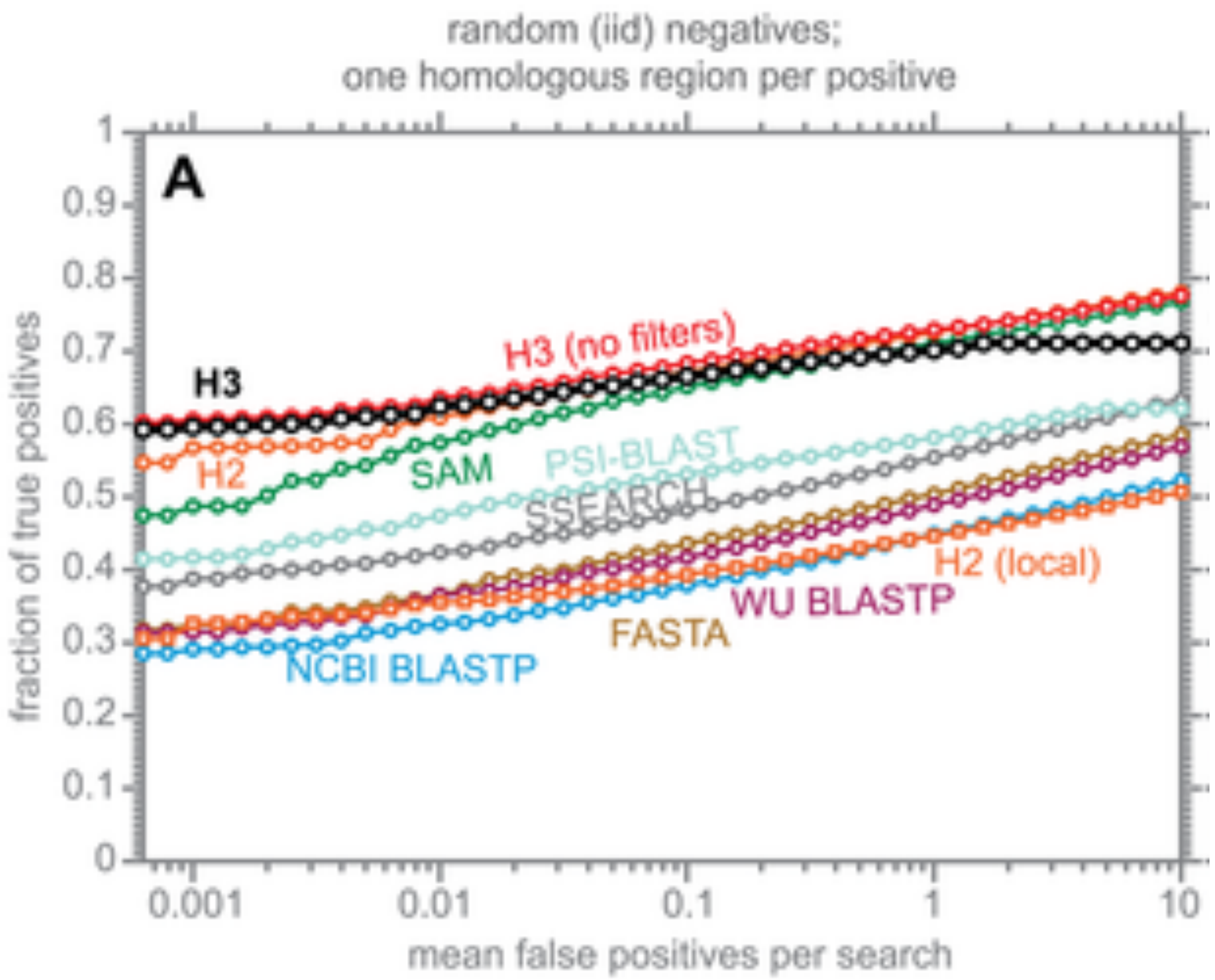


```
Sensitivity = TP/(TP+FN)
Specificity = TN/(TN+FP)      PPV = TP/(TP+FP)
```

The positive predictive value (or PPV) assesses how big a proportion of the sequences matching the pattern are actually in the family of interest.
(i.e., the probability that a positive result is truly positive!)

ROC plot example

ROC plot of sequence searching performance...



H3 (HMMER3) has a much higher search sensitivity and specificity than BLASTp

In each benchmark, true positive subsequences have been selected to be no more than 25% identical to any sequence in the query alignment ... (see paper for details).

See: Eddy (2011) PLoS Comp Biol 7(10): e1002195

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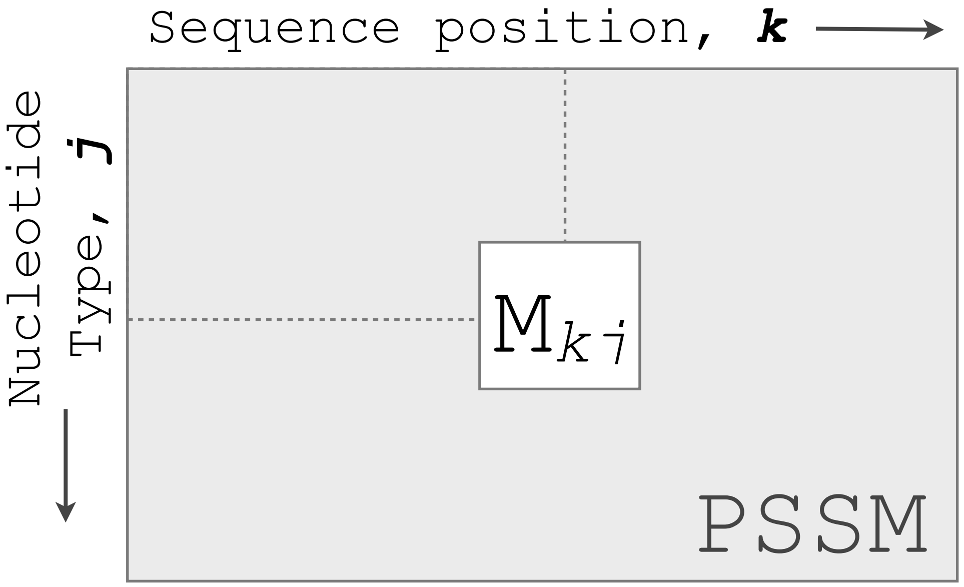
Sequence profiles

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

Unlike deterministic patterns, profiles 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).



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

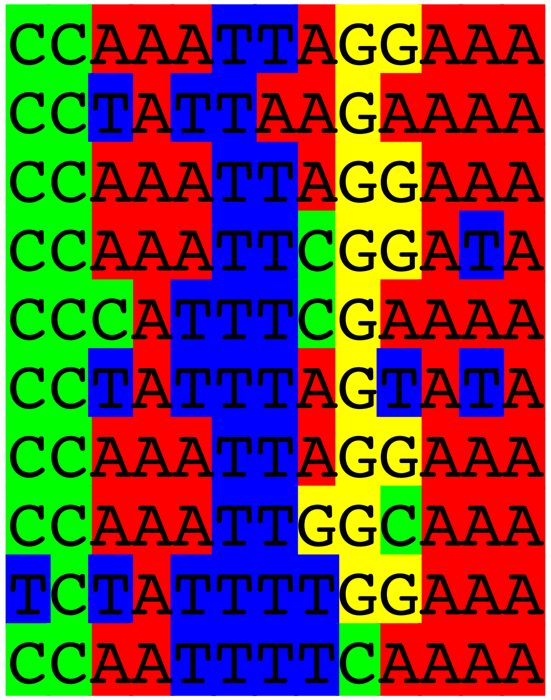
M_{kj} score for the *j*th nucleotide at position *k*

p_{kj} probability of nucleotide *j* at position *k*

p_j "background" probability of nucleotide *j*

See Gibskov *et al.* (1987) PNAS 84, 4355

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	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**	**[ACT]**	**A**	**[AT]**	**T**	**T**	**N**	**G**	**N**	**A**	**[AT]**	**A**

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

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

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*

Adapted from Hertz and Stormo,
Bioinformatics 15:563-577

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

CCTATTTAGGATA

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

Query Score = 1.2 + 1.3 + 0.2 + 1.3 + 0.6 + 1.3 + 1.2
+ 0.6 + 1.2 + 0.6 + 1.3 + -0.2 + 1.3
= 11.9

Scoring a test sequence

Query Sequence

CCTATTTAGGATA

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**

Query Score = 1.2 + 1.3 + 0.2 + 1.3 + 0.6 + 1.3 + 1.2
+ 0.6 + 1.2 + 0.6 + 1.3 + -0.2 + 1.3
= 11.9

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

Scoring a test sequence...

Query Sequence

CCTATTTAGGATA

Best Possible Sequence

CCAATTTAGGAAA

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**

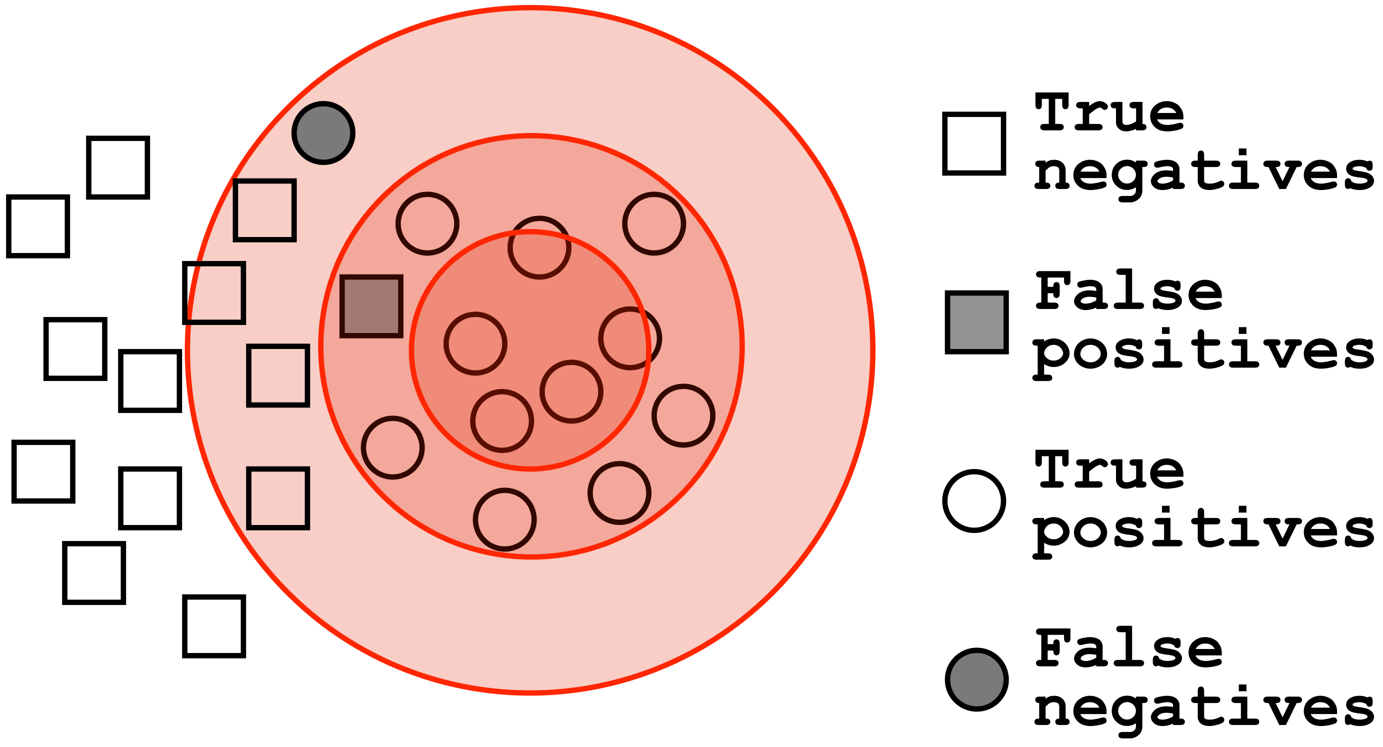
Max Score = 1.2 + 1.3 + 0.8 + 1.3 + 0.6 + 1.3 + 1.2
+ 0.6 + 1.2 + 0.6 + 1.3 + 1.1 + 1.3
= 13.8

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!

Picking a threshold for PSSM matching

Again, you want to select a threshold that **minimizes FPs** (e.g., how many shuffled or random sequences does the PSSM match with that score) and **minimizes FNs** (e.g., how many of the ‘real’ sequences are missed with that score).



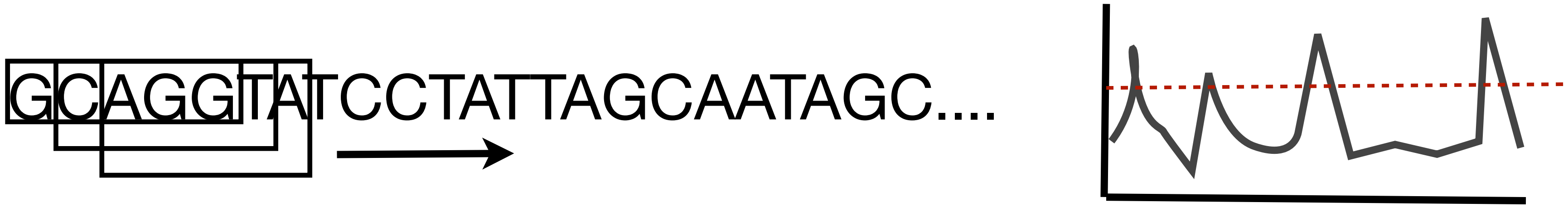
FP=0, FN=7, TP=5
FP=1, FN=1, TP=11
FP=5, FN=0, TP=12

Q. Which threshold has the best PPV (TP/(TP+FP)) ?

Searching for PSSM matches

If we do not allow gaps (i.e., no insertions or deletions):

- Perform a linear scan, scoring the match to the PSSM at each position in the sequence - the “sliding window” method



GCAGGTATCCTATTAGCAATAGC....

See example at http://coding.plantpath.ksu.edu/profile/

If we allow gaps:

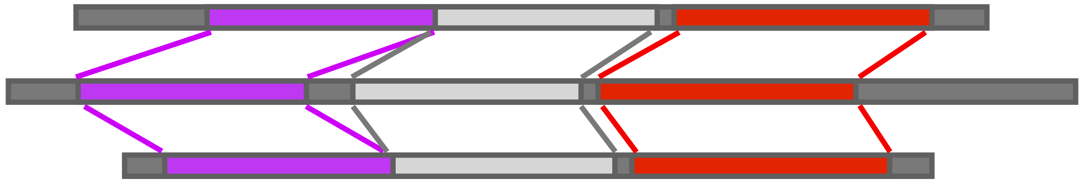
- Can use dynamic programming to align the profile to the protein sequence(s) (with gap penalties)
  We will discuss PSI-BLAST shortly...
  see Mount, Bioinformatics: sequence and genome analysis (2004)

- Can use hidden Markov Model-based methods
  We will cover HMMs in the next lecture...
  see Durbin et al., Biological Sequence Analysis (1998)

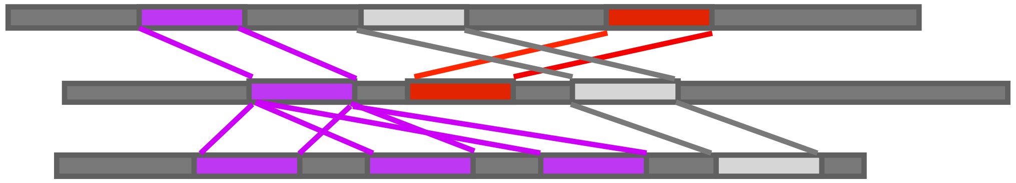
Side note: Building PSSMs from unaligned sequences

Patterns and profiles are most often built on the basis of known site equivalences (i.e. from a pre-calculated MSA).

However, a number of programs have been developed that employ local multiple alignments to search for common sequence elements in unaligned sequences.



Global similarity



Local non-consistent similarity

Gibbs *sampling* methods:
Motif Sampler - http://bayesweb.wadsworth.org/gibbs/gibbs.html
AlignAce - http://atlas.med.harvard.edu/cgi-bin/alignace.pl

Expectation maximization method:
MEME - http://meme.sdsc.edu/

See: Lawrence et al. (1993) Science. 262, 208-14

Profiles software and databases

Pftools is a package to build and search with profiles,
http://www.isrec.isb-sib.ch/ftp-server/pftools/

The package contains (among other programs):

- **pfmake** for building a profile starting from multiple alignments
- **pfsearch** to search a protein database with a profile
- **pfscan** to search a profile database with a protein

PRINTS database of PSSMs
http://bioinf.man.ac.uk/dbbrowser/PRINTS

Collection of conserved motifs used to characterize a protein

- Uses fingerprints (conserved motif groups).
- Very good to describe sub-families.

BLOCKS is another PSSMs database similar to prints
http://www.blocks.fhcrc.org

ProDom is collection of protein motifs obtained automatically using PSI-BLAST
http://prodes.toulouse.inra.fr/prodom/doc/prodom.html

Profiles software and databases...

InterPro is an attempt to group a number of protein domain databases.
http://www.ebi.ac.uk/interpro

It currently includes:

- Pfam
- PROSITE
- PRINTS
- ProDom
- SMART
- TIGRFAMs

- InterPro tries to have and maintain a high quality of annotation
- The database and a stand-alone package (**iprscan**) are available for UNIX platforms, see:
  ftp://ftp.ebi.ac.uk/pub/databases/interpro

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- PSI-BLAST algorithm: Application of iterative PSSM searching to improve BLAST sensitivity
- Hidden Markov models (HMMs): More versatile probabilistic model for detection of remote similarities

Your Turn!

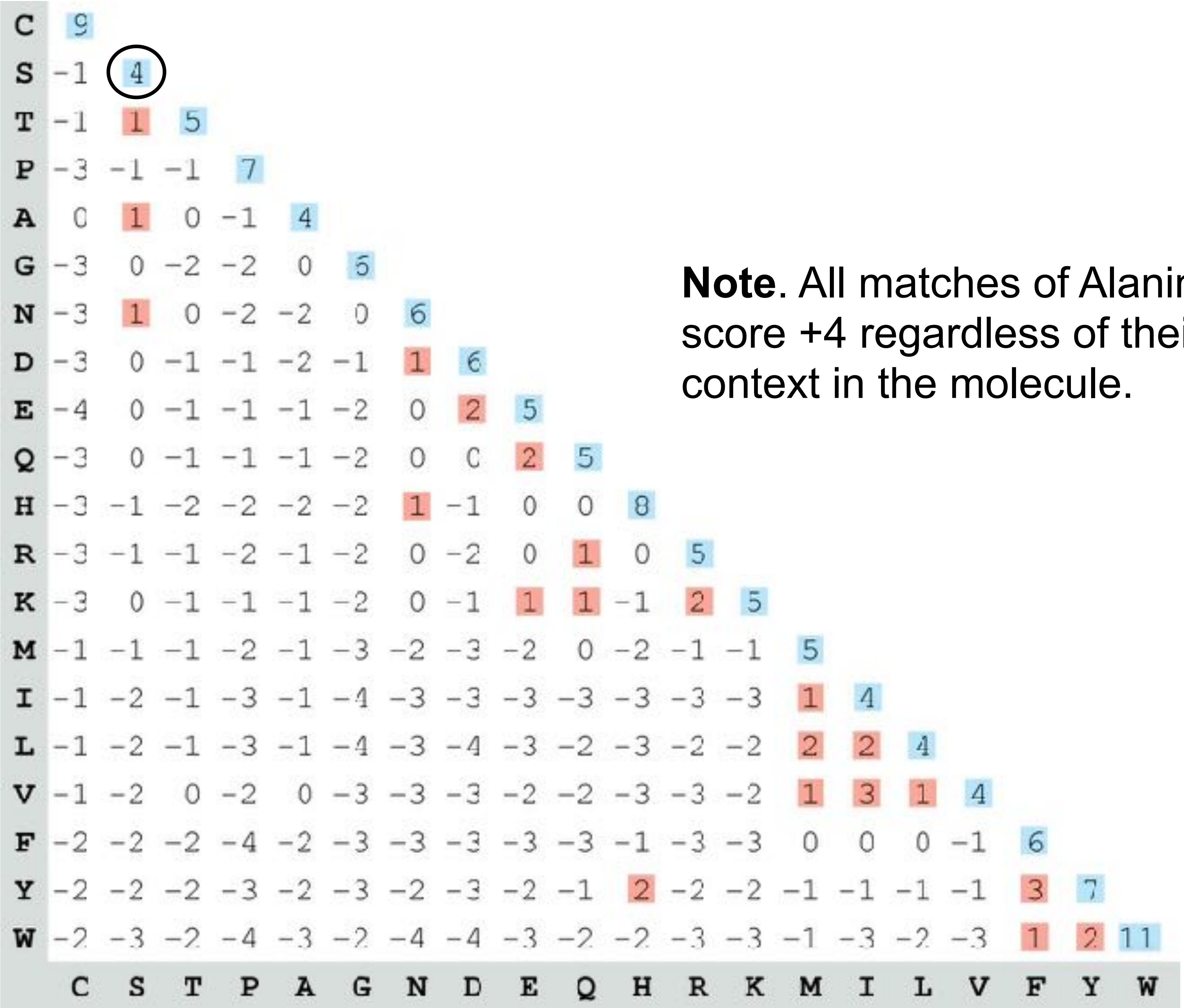
Hands-on sections 1 & 2:
Comparing methods and the trade-off between sensitivity, selectivity and performance

~50 mins

Recall: 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).

By default BLASTp match scores come from the BLOSUM62 matrix



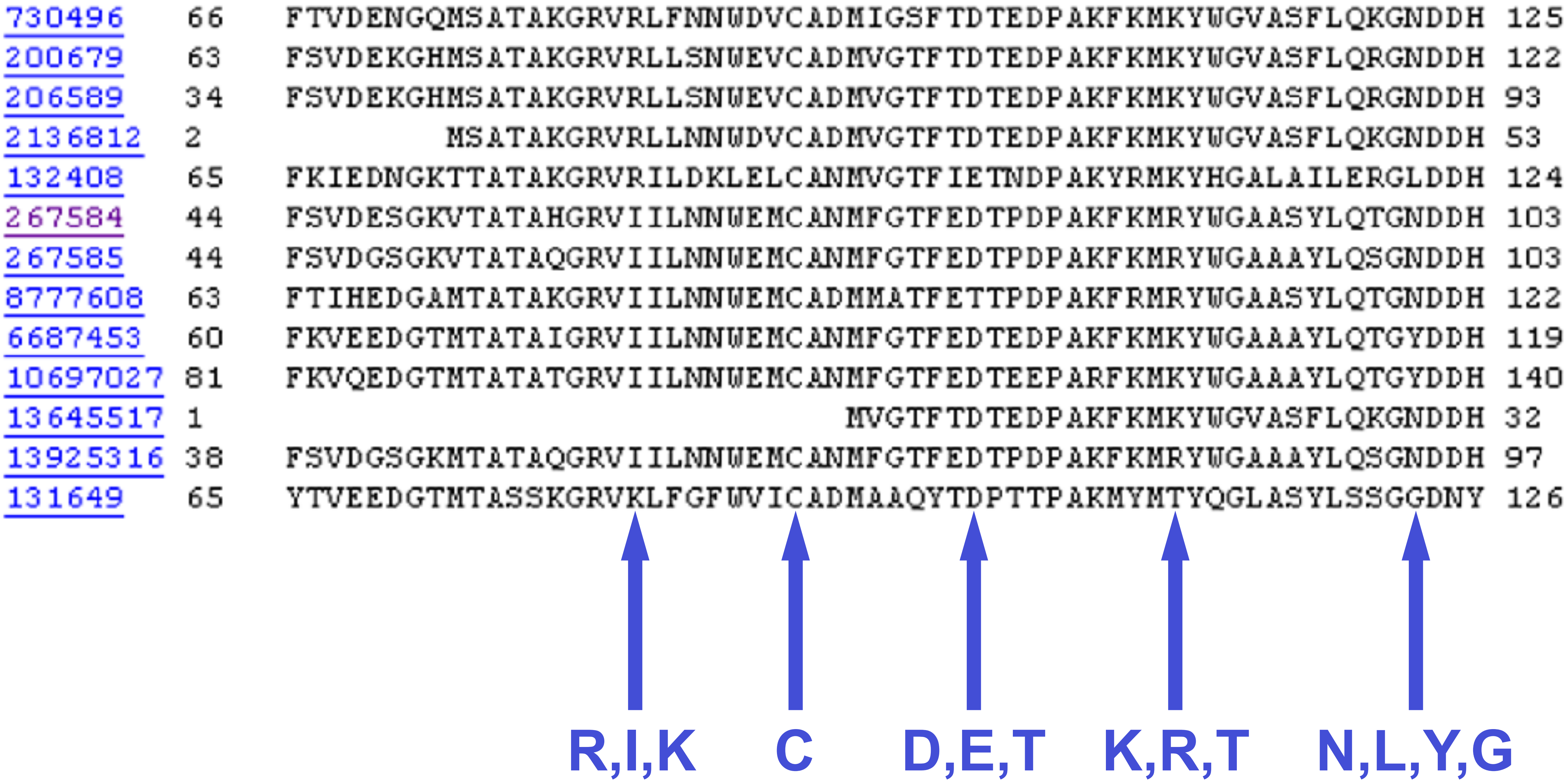
C	9																			
S	-1	4																		
T	-1	1	5																	
P	-3	-1	-1	7																
A	0	1	0	-1	4															
G	-3	0	-2	-2	0	6														
N	-3	1	0	-2	-2	0	6													
D	-3	0	-1	-1	-2	-1	1	6												
E	-4	0	-1	-1	-1	-2	0	2	5											
Q	-3	0	-1	-1	-1	-2	0	0	2	5										
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 *s*pecific 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

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



```
730496    66  FTVDENGQMSATAKGRVRLFNNWDVCADMIGSFTDTEDPAKFKMKYWGVASFLQKGNDDH 125
200679    63  FSVDEKGHMSATAKGRVRLLSNWEVCADMVGTFTDTEDPAKFKMKYWGVASFLQRGNDDH 122
206589    34  FSVDEKGHMSATAKGRVRLLSNWEVCADMVGTFTDTEDPAKFKMKYWGVASFLQRGNDDH 93
2136812   2           MSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDH 53
132408    65  FKIEDNGKTTATAKGRVRILDKLELCANMVGTFIETNDPAKYRMKYHGALAILERGLDDH 124
267584    44  FSVDESGKVTATAHGRVIILNNWEMCANMFGTFEDTPDPAKFKMRYWGAASYLQTGNDDH 103
267585    44  FSVDGSGKVTATAQGRVIILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAAYLQSGNDDH 103
8777608   63  FTIHEDGAMTATAKGRVIILNNWEMCADMMATFETTPDPAKFRMRYWGAASYLQTGNDDH 122
6687453   60  FKVEEDGTMTATAIGRVIILNNWEMCANMFGTFEDTEDPAKFKMKYWGAAAYLQTGYDDH 119
10697027  81  FKVQEDGTMTATATGRVIILNNWEMCANMFGTFEDTEEPARFKMKYWGAAAYLQTGYDDH 140
13645517  1                               MVGTFTDTEDPAKFKMKYWGVASFLQKGNDDH 32
13925316  38  FSVDGSGKMTATAQGRVIILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAAYLQSGNDDH 97
131649    65  YTVEEDGTMTASSKGRVKLFGFWVICADMAAQYTDPTTPAKMYMTYQGLASYLSSGGDNY 126
```

R,I,K C D,E,T K,R,T N,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	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-3	-3	12	2	-3
4	V	0	-3	-3	-4	-1	-3	-3	-4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-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	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-3	2	1	-3	-3	-2	7	0	0
14	A	3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-1	-2	-3	-1	1	-1	-3	-3	-1
15	A	2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0	-2	-3	-1	3	0	-3	-2	-2
16	A	4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-1	-1	-3	-1	1	0	-3	-2	-1
...																					
37	S	2	-1	0	-1	-1	0	0	0	-1	-2	-3	0	-2	-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
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 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											-3	-3	-1	-2	-1	2
10	L	-2	-2	-4	-4											-3	-3	-1	-2	-1	1
11	A	5	-2	-2	-2											-1	1	0	-3	-2	0
12	A	5	-2	-2	-2											-1	1	0	-3	-2	0
13	W	-2	-3	-4	-4											-3	-3	-2	7	0	0
14	A	3	-2	-1	-2											-1	1	-1	-3	-3	-1
15	A	2	-1	0	1											-1	3	0	-3	-2	-2
16	A	4	-2	-1												-1	1	0	-3	-2	-1
...																					
37	S	2	-1	0	-1											-1	4	1	-3	-2	-2
38	G	0	-3	-1	-2											-2	0	-2	-3	-3	-4
39	T	0	-1	0	-1											-1	1	5	-3	-2	0
40	W	-3	-3	-4	-5											-4	-3	-3	9	2	-3
41	Y	-2	-2	-2	-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

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)

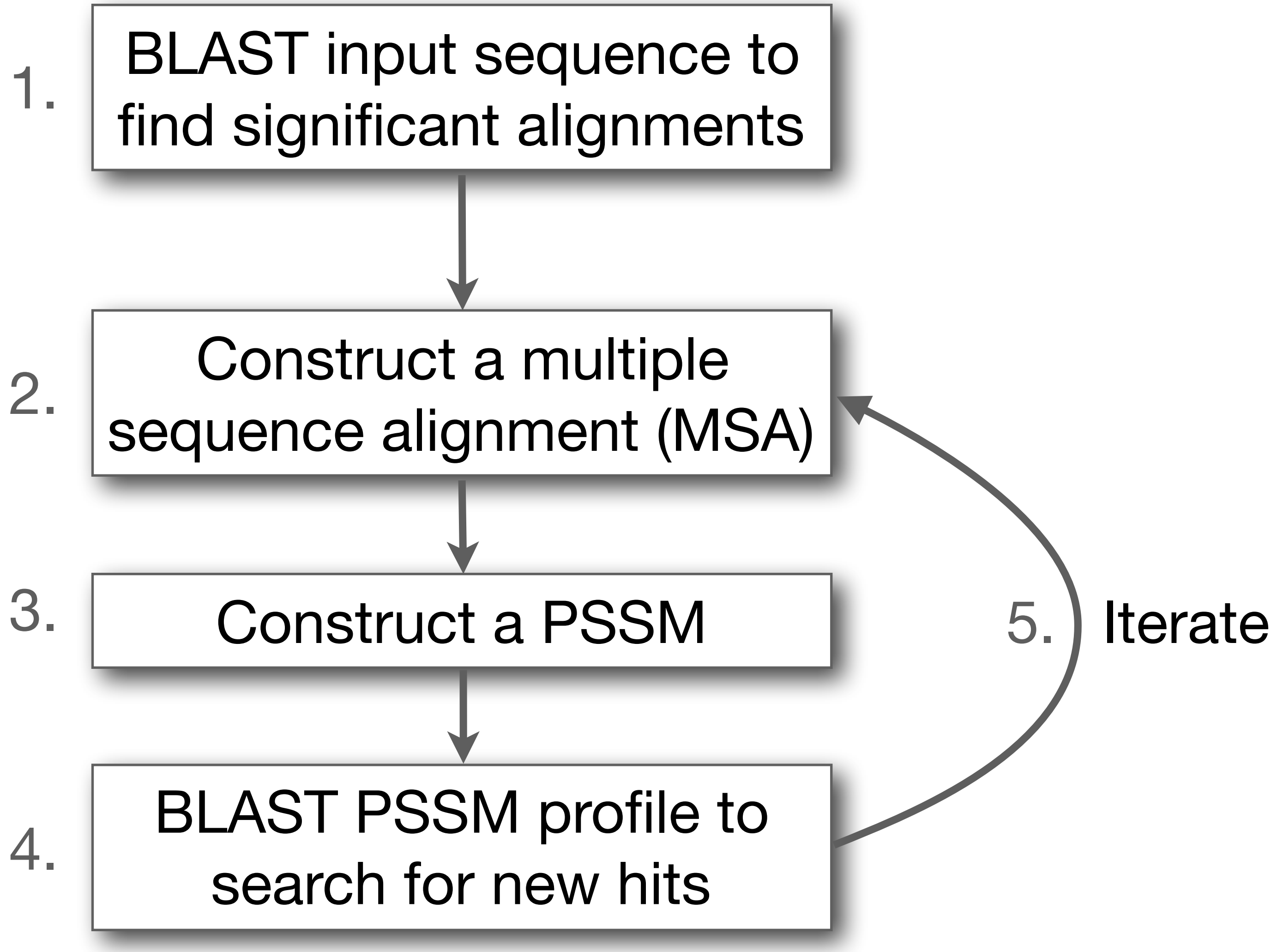
The PSI-BLAST PSSM is essentially a query customized scoring matrix that is more sensitive than BLOSUM.

		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											-3	-3	-1	-2	-1	2
10	L	-2	-2	-4	-4											-3	-3	-1	-2	-1	1
11	A	5	-2	-2	-2											-1	1	0	-3	-2	0
12	A	5	-2	-2	-2											-1	1	0	-3	-2	0
13	W	-2	-3	-4	-4											-3	-3	-2	7	0	0
14	A	3	-2	-1	-2											-1	1	-1	-3	-3	-1
15	A	2	-1	0	1											-1	3	0	-3	-2	-2
16	A	4	-2	-1												-1	1	0	-3	-2	-1
...																					
37	S	2	-1	0	-1											-1	4	1	-3	-2	-2
38	G	0	-3	-1	-2											-2	0	-2	-3	-3	-4
39	T	0	-1	0	-1											-1	1	5	-3	-2	0
40	W	-3	-3	-4	-5											-4	-3	-3	9	2	-3
41	Y	-2	-2	-2	-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

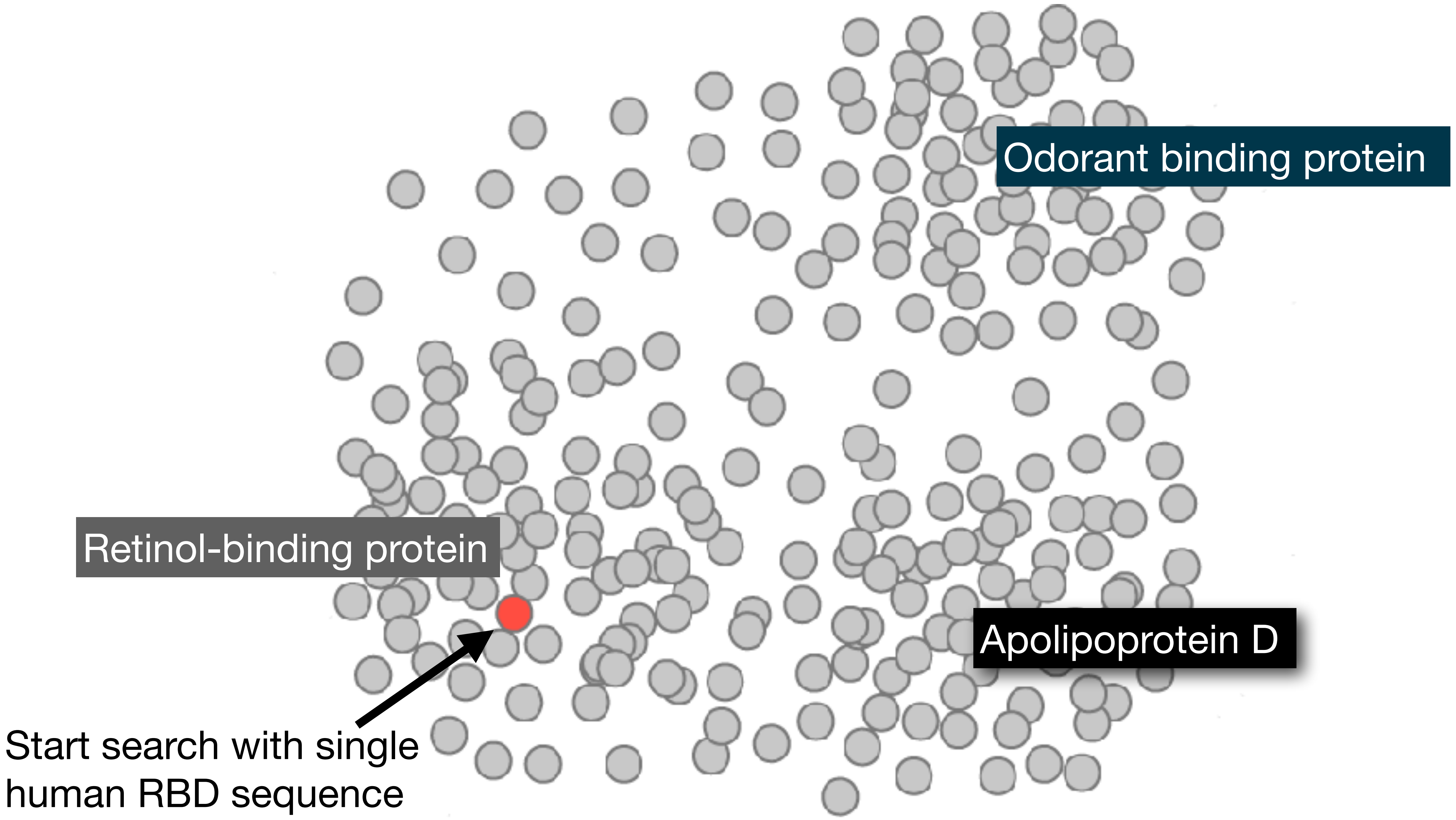
Note: A given amino acid (such as alanine) in your query protein can receive different scores for matching alanine depending on the position in the protein (BLOSUM S_{AA} = +4)

PSI-BLAST: Position-Specific Iterated BLAST

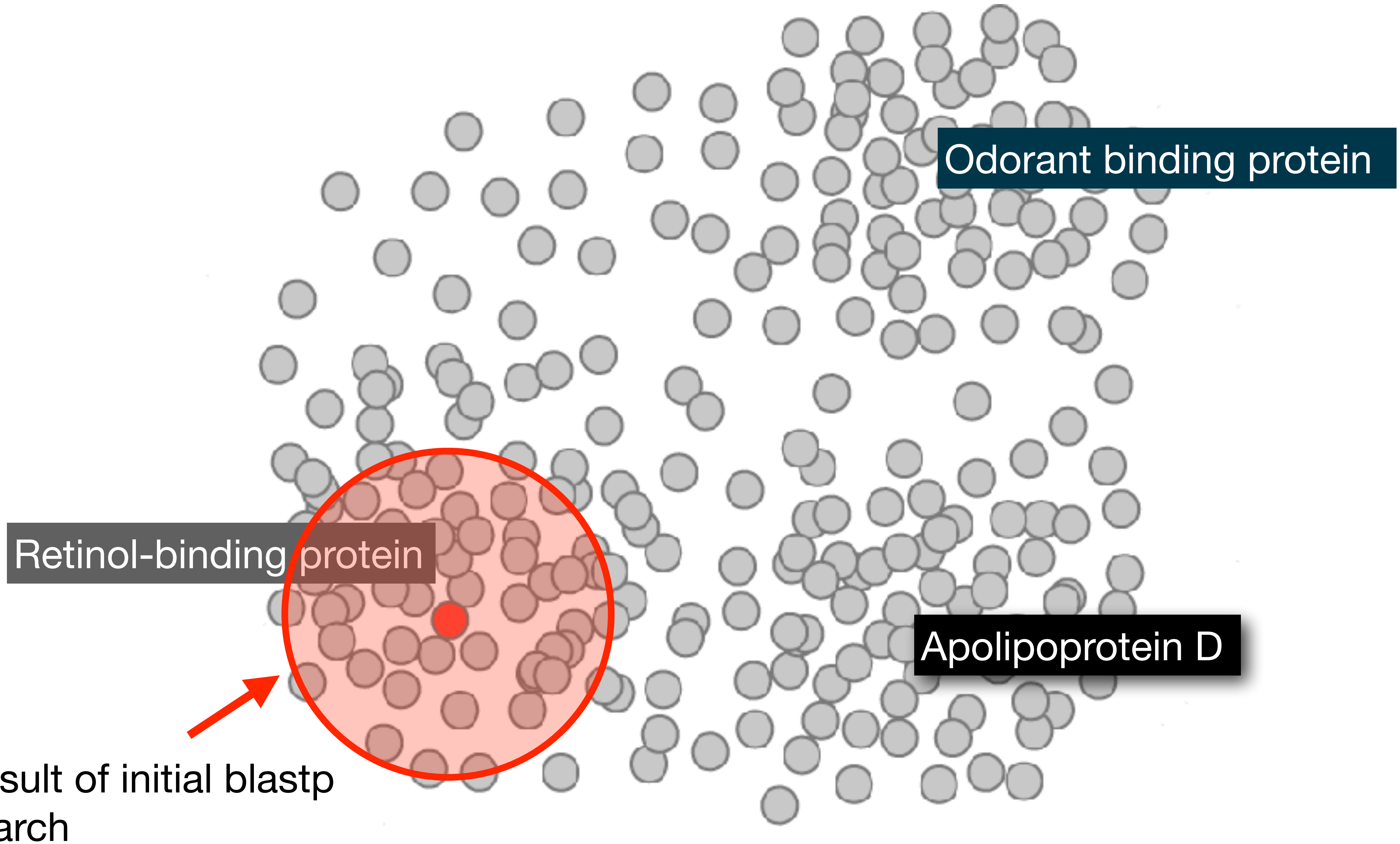
Many proteins in a database are too distantly related to a query to be detected using standard BLAST. In many other cases matches are detected but are so distant that the inference of homology is unclear. Enter the more sensitive PSI-BLAST


1.
BLAST input sequence to find significant alignments
2.
Construct a multiple sequence alignment (MSA)
3.
Construct a PSSM
4.
BLAST PSSM profile to search for new hits
5.
Iterate

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



Start search with single human RBD sequence

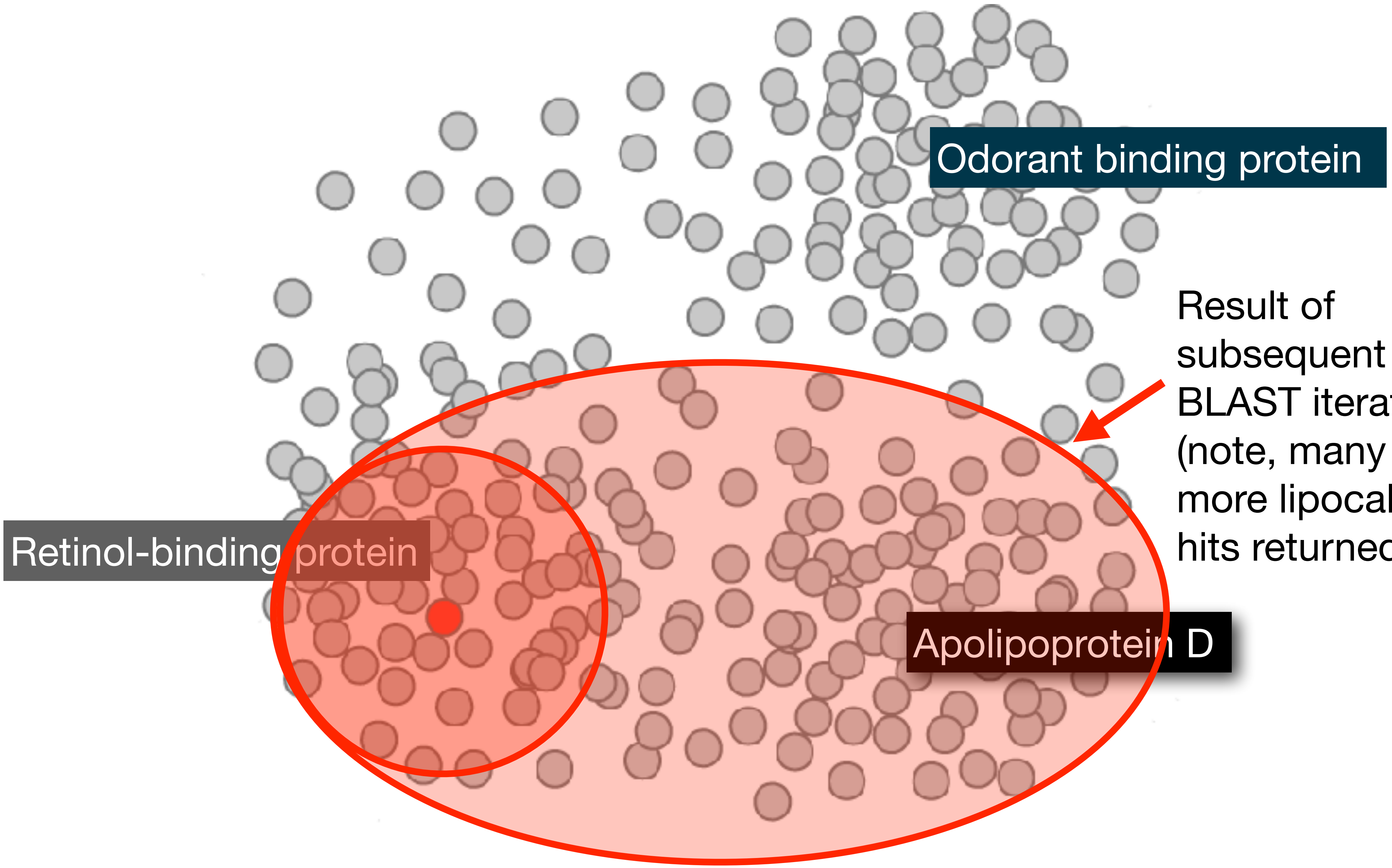


Odorant binding protein

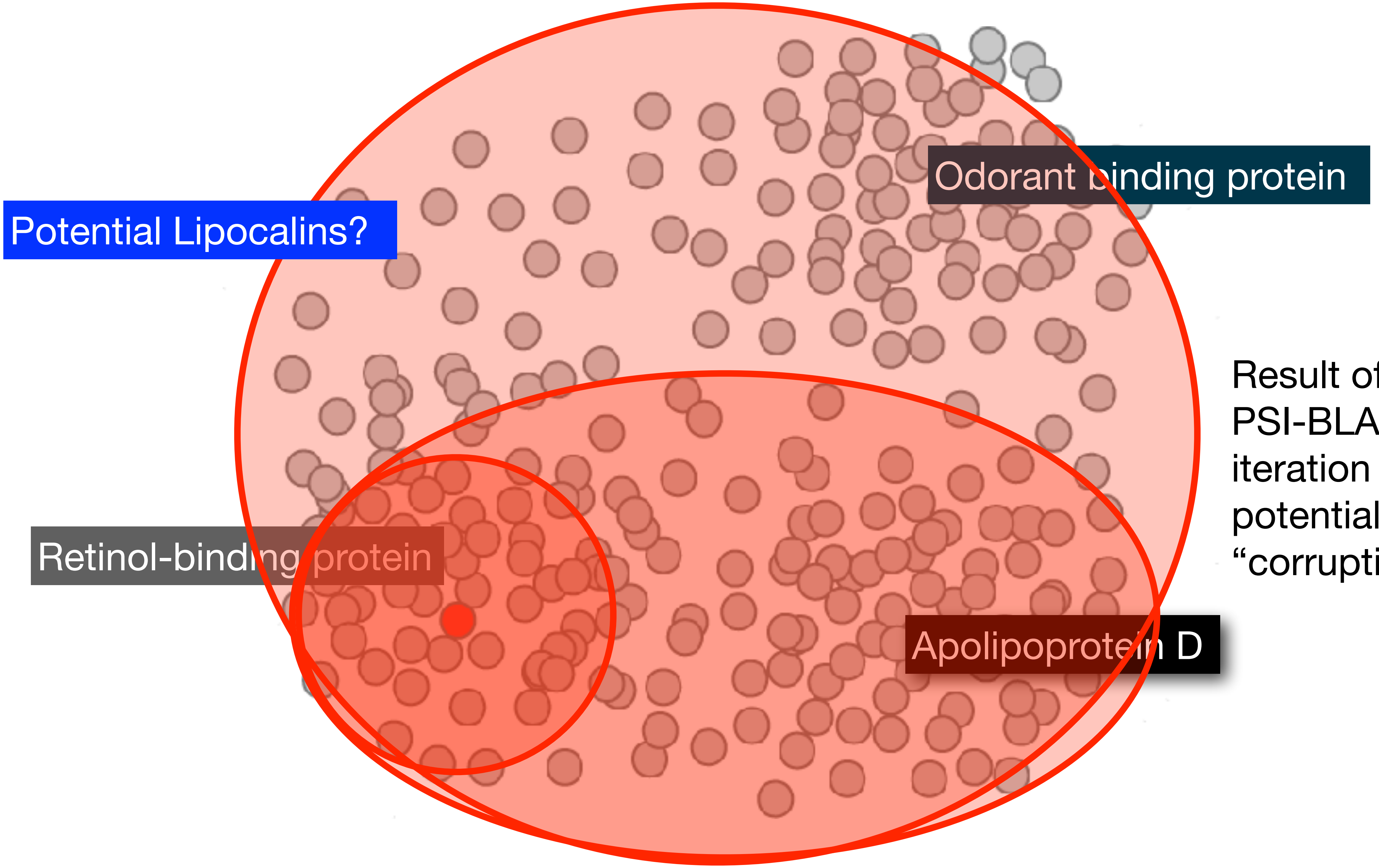
Retinol-binding protein

Apolipoprotein D

Result of initial blastp search



Result of subsequent PSI-BLAST iteration (note, many more lipocalin hits returned!)



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

PSI-BLAST returns dramatically more hits

PSI-BLAST frequently returns many more hits with significant E-values than blastp

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.

(**a**) Iteration 1

```
>ref|NP_001638.1| apolipoprotein D precursor [Homo sapiens]
Length=189

 Score = 57.4 bits (137),  Expect = 3e-07, Method: Composition-based stats.
 Identities = 47/151 (31%), Positives = 78/151 (51%), Gaps = 39/151 (25%)

Query  29   VKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETGQMSATAKGRVRLLNNWDVC  88
            V+ENFD  ++ G WY + +K P        I A +S+ E G        ++++LN  ++
Sbjct  33   VQENFDVNKYLGRWYEI-EKIPTTFENGRCIQANYSLMENG--------KIKVLNQ-ELR  82

Query  89   ADMVGTFTDTE---------DPAKFKMKY-WGVASFLQKGNDDHWIVDTDYDTYAVQYSC  138
            AD  GT    E         +PAK ++K+ W + S        +WI+ TDY+ YA+ YSC
Sbjct  83   AD--GTVNQIEGEATPVNLTEPAKLEVKFSWFMPS------APYWILATDYENYALVYSC  134

Query  139  ----RLLNLDGTCADSYSFVFSRDPNGLPPE  165
                +L ++D      ++++ +R+PN LPPE
Sbjct  135  TCIIQLFHVD------FAWILARNPN-LPPE  158
```

(**b**) Iteration 2

```
>ref|NP_001638.1| apolipoprotein D precursor [Homo sapiens]
Length=189

 Score =  175 bits (443),  Expect = 1e-42, Method: Composition-based stats.
 Identities = 45/163 (27%), Positives = 77/163 (47%), Gaps = 31/163 (19%)

Query  14   GSGRAERDCRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETGQMSA  73
              G+A    +  +  V+ENFD  ++ G WY + +K P        I A +S+ E G++
Sbjct  18   AEGQAFHLGKCPNPPVQENFDVNKYLGRWYEI-EKIPTTFENGRCIQANYSLMENGKIKV  76

Query  74   TAK-----GRVRLLNNWDVCADMVGTFTDTEDPAKFKMKY-WGVASFLQKGNDDHWIVDT  127
              +     G V  +           T  +  +PAK ++K+ W + S        +WI+ T
Sbjct  77   LNQELRADGTVNQIEG-------EATPVNLTEPAKLEVKFSWFMPS------APYWILAT  123

Query  128  DYDTYAVQYSCR----LLNLDGTCADSYSFVFSRDPNGLPPEA  166
            DY+ YA+ YSC     L ++D      ++++ +R+PN LPPE
Sbjct  124  DYENYALVYSCTCIIQLFHVD------FAWILARNPN-LPPET  159
```

(**c**) Iteration 3

```
>ref|NP_000597.1| complement component 8, gamma polypeptide [Homo sapiens]
Length=202

 Score =  104 bits (260),  Expect = 2e-21, Method: Composition-based stats.
 Identities = 40/186 (21%), Positives = 74/186 (39%), Gaps = 29/186 (15%)

Query  24   VSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETG-QMSATAKGRVRLL  82
            +S+ + K NFD  +F+GTW  +A         +    AE +      Q +A A    R L
Sbjct  33   ISTIQPKANFDAQQFAGTWLLVAVGSACRFLQEQGHRAEATTLHVAPQGTAMAVSTFRKL  92

Query  83   NNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQKGNDDHWIVDTDYDTYAVQY------  136
            +   +C  +   + DT    +F ++  G      +G     + +TDY ++AV Y
Sbjct  93   DG--ICWQVRQLYGDTGVLGRFLLQARGA-----RGAVHVVVAETDYQSFAVLYLERAGQ  145

Query  137  -SCRLLNLDGTCADSYSFVFSRDPNGLPPEAQKIVRQRQEELCLARQYRLIVHNGYCDGR  195
             S +L       +DS    F +       EA       ++++    +Y      G+C+
Sbjct  146  LSVKLYARSLPVSDSVLSGFEQRVQ----EA----HLTEDQIFYFPKY------GFCEAA  191

Query  196  SERNLL  201
             + ++L
Sbjct  192  DQFHVL  197
```

blastp E-value for this hit was 0.27

PSI-BLAST errors: the corruption problem

The main source of error in PSI-BLAST searches is the spurious amplification of sequences that are unrelated to the query.

There are three main approaches to stopping corruption of PSI-BLAST queries:

- Perform multi-domain splitting of your query sequence

  If a query protein has several different domains PSI-BLAST may find database matches related to both individually. One should not conclude that these hits with different domains are related.

  - Often best to search using just one domain of interest.

- Inspect each PSI-BLAST iteration removing suspicious hits.

  E.g., your query protein may have a generic coiled-coil domain, and this may cause other proteins sharing this motif (such as myosin) to score better than the inclusion threshold even though they are not related.

  - Use your biological knowledge!

- Lower the default expect level (e.g., E = 0.005 to E = 0.0001).

  This may suppress appearance of FPs (but also TPs)

Profile advantages and disadvantages

Advantages:

- Quantitate with a good scoring system
- Weights sequences according to observed diversity
  Profile is specific to input sequence set
- Very sensitive
  Can detect weak similarity
- Relatively easy to compute
  Automatic profile building tools available

Disadvantages:

- If a mistake enters the profile, you may end up with irrelevant data
  The corruption problem!
- Ignores higher order dependencies between positions
  i.e., correlations between the residue found at a given position and those found at other positions (e.g. salt-bridges, structural constraints on RNA etc...)
- Requires some expertise and oversight to use proficiently

Todays Menu

- Sequence motifs and patterns: Simple approaches for finding functional cues from conservation patterns
- Sequence profiles and position specific scoring matrices (PSSMs): Building and searching with profiles, Their advantages and limitations
- PSI-BLAST algorithm: Application of iterative PSSM searching to improve BLAST sensitivity
- Hidden Markov models (HMMs): More versatile probabilistic model for detection of remote similarities

Your Turn!

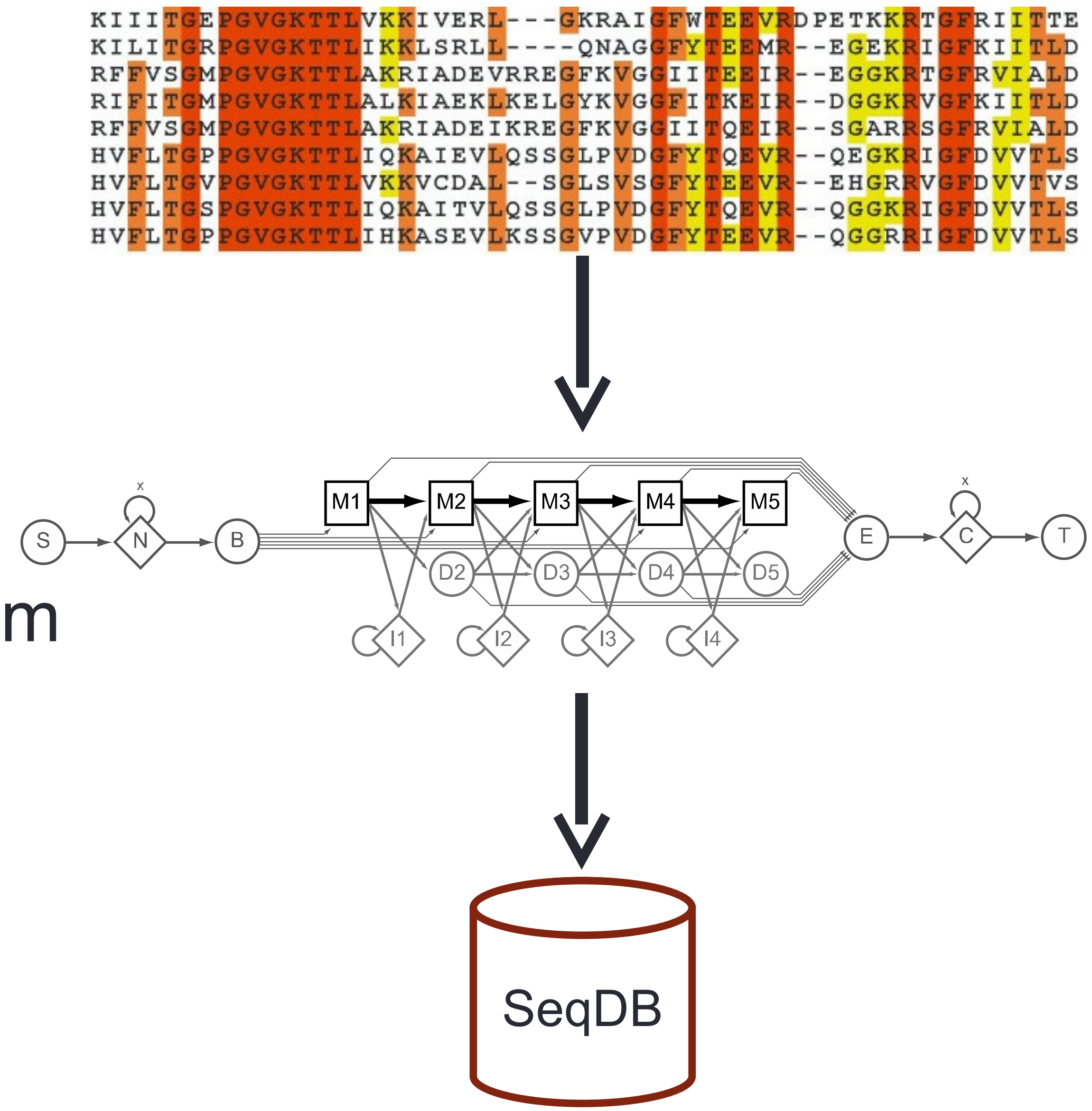
Hands-on sections 3 & 4:

Comparing methods and the trade-off between sensitivity, selectivity and performance

~30 mins

Use of HMMER

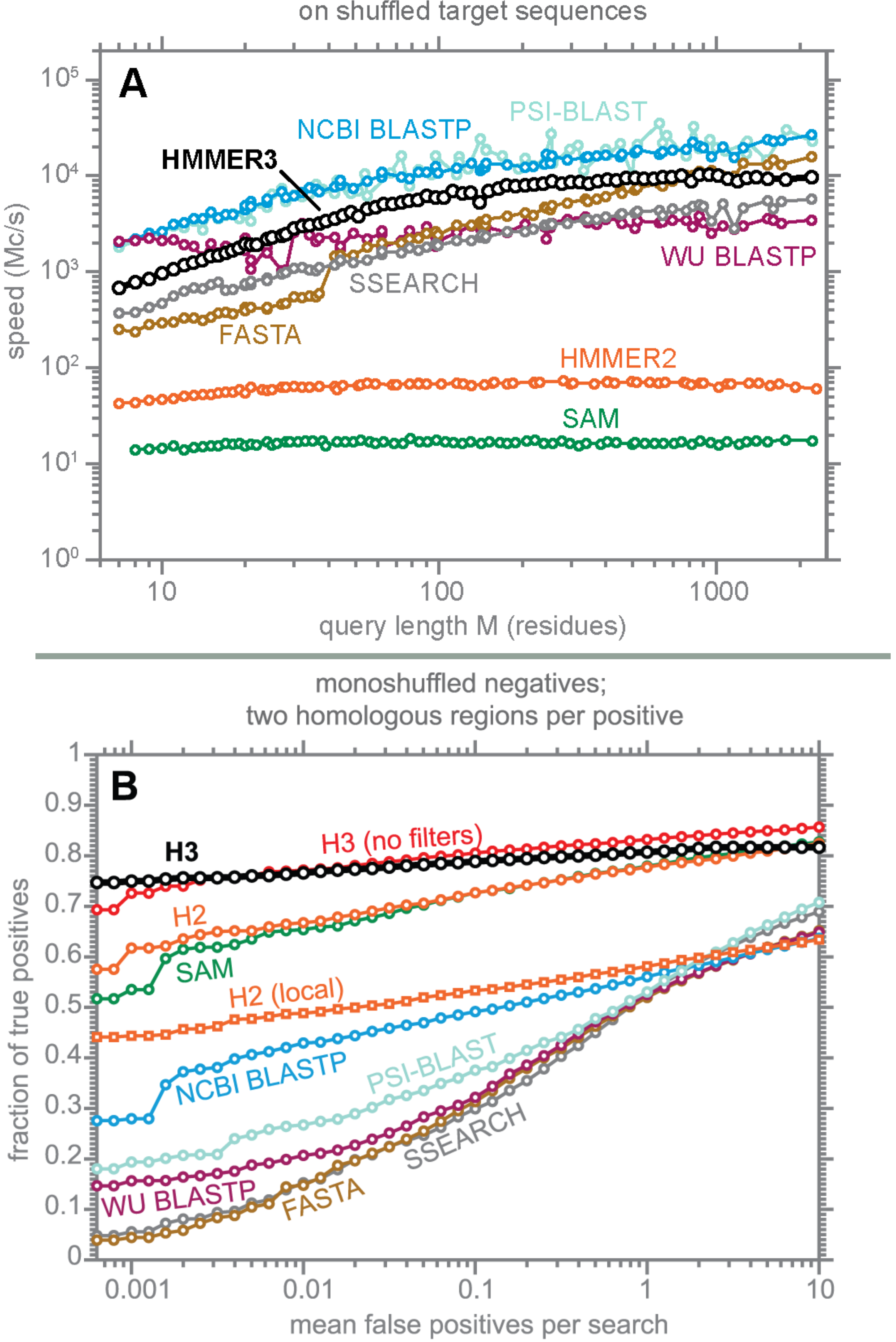
- Widely used by protein family databases
  - Use ‘seed’ alignments
- Until 2010
  - Computationally expensive
  - Restricted to HMMs constructed from multiple sequence alignments
- Command line application



```
KIIITGEPGVGKTTLVKKIVERL---GKRAIGFWTEEVRDPETKKRTGFRIITTE
KILITGRPGVGKTTLIKKLSRLL----QNAGGFYTEEMR--EGEKRIGFKIITLD
RFFVSGMPGVGKTTLAKRIADEVRREGFKVGGIITEEIR--EGGKRTGFRVIALD
RIFITGMPGVGKTTLALKIAEKLKELGYKVGGFITKEIR--DGGKRVGFKIITLD
RFFVSGMPGVGKTTLAKRIADEIKREGFKVGGIITQEIR--SGARRSGFRVIALD
HVFLTGPPGVGKTTLIQKAIEVLQSSGLPVDGFYTQEVR--QEGKRIGFDVVTLS
HVFLTGVPGVGKTTLVKKVCDAL--SGLSVSGFYTEEVR--EHGRRVGFDVVTVS
HVFLTGSPGVGKTTLIQKAITVLQSSGLPVDGFYTQEVR--QGGKRIGFDVVTLS
HVFLTGPPGVGKTTLIHKASEVLKSSGVPVDGFYTEEVR--QGGRRIGFDVVTLS
```

HMMER vs BLAST

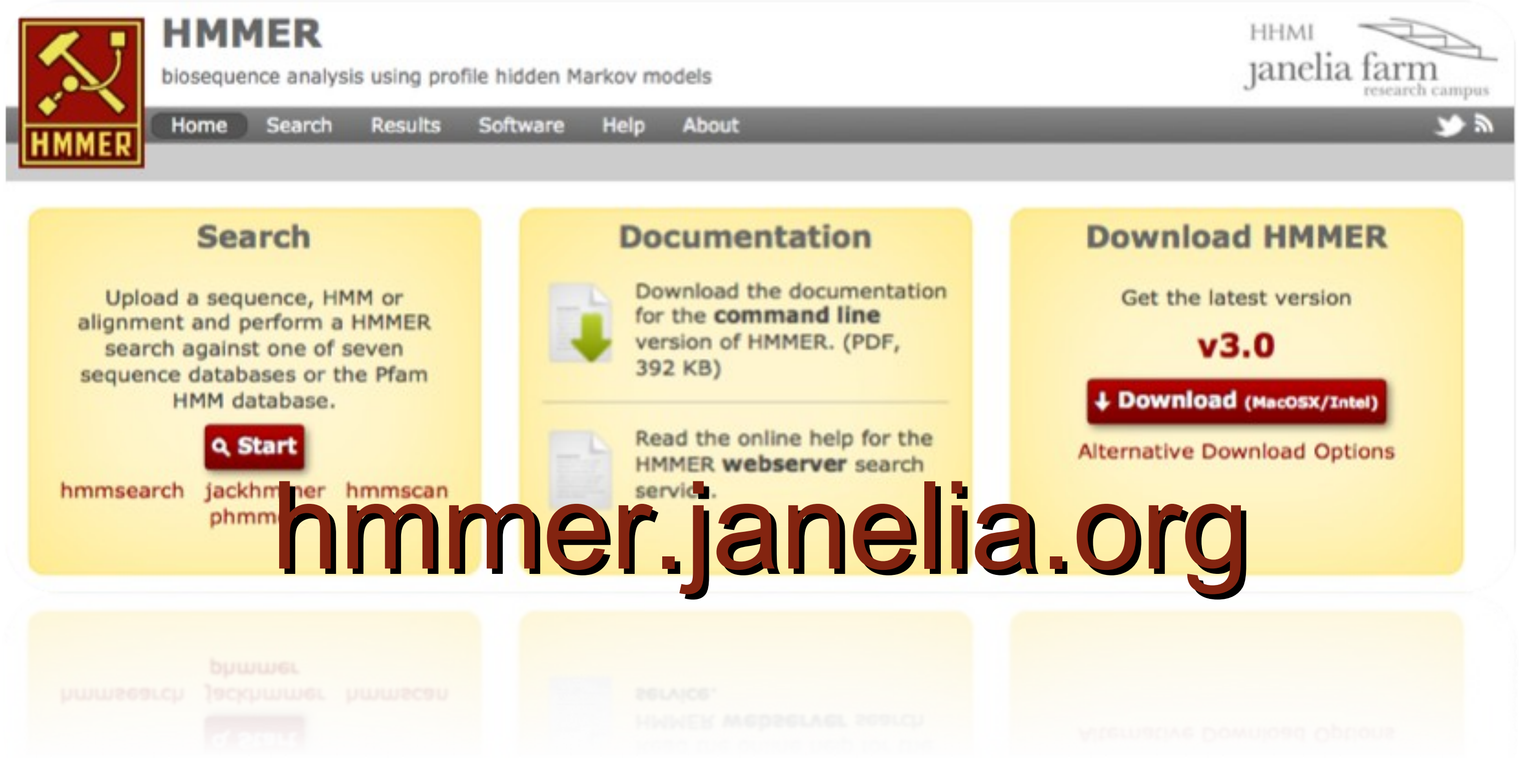
	HMMER	BLAST
Program	*PHMMER*	*BLASTP*
Query	Single sequence	
Target Database	Sequence database	
Program	*HMMSCAN*	*RPSBLAST*
Query	Single sequence	
Target Database	Profile HMM database, e.g. Pfam	PSSM database, e.g. CDD
Program	*HMMSEARCH*	*PSI-BLAST*
Query	Profile HMM	PSSM
Target Database	Sequence database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



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

Fast Web Searches

- Parallelized searches across compute farm
  - Average query returns ~1 sec
- Range of sequence databases
  - Large Comprehensive
  - Curated / Structure
  - Metagenomics
  - Representative Proteomes
- Family Annotations
  - Pfam
- Batch and RESTful API
  - Automatic and Human interface



Search

Upload a sequence, HMM or alignment and perform a HMMER search against one of seven sequence databases or the Pfam HMM database.

Documentation

Download the documentation for the **command line** version of HMMER. (PDF, 392 KB)

Read the online help for the HMMER **webserver** search service.

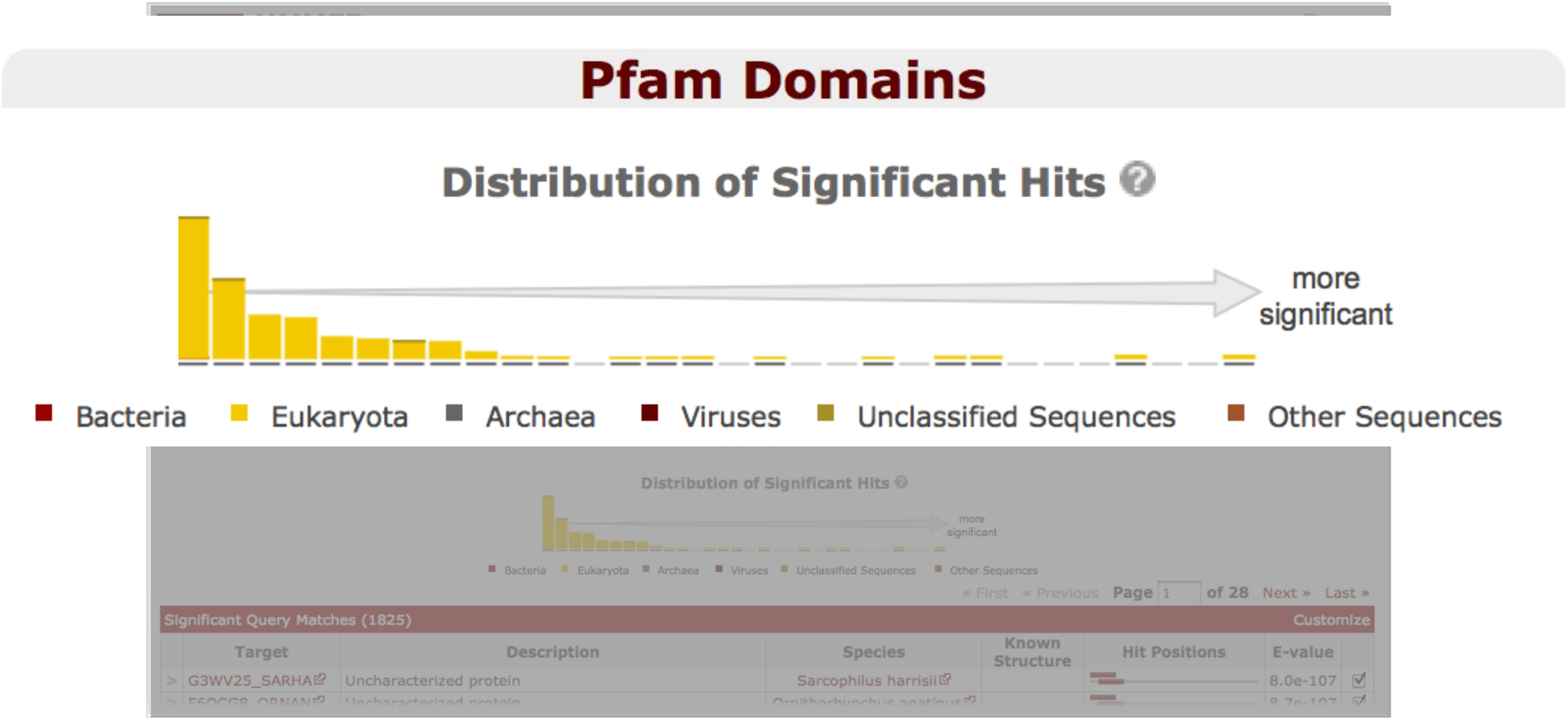
Download HMMER

Get the latest version

v3.0

hmmer.janelia.org

Visualization of Results – By Score

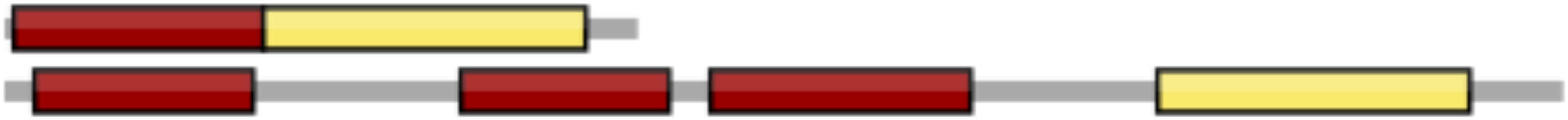


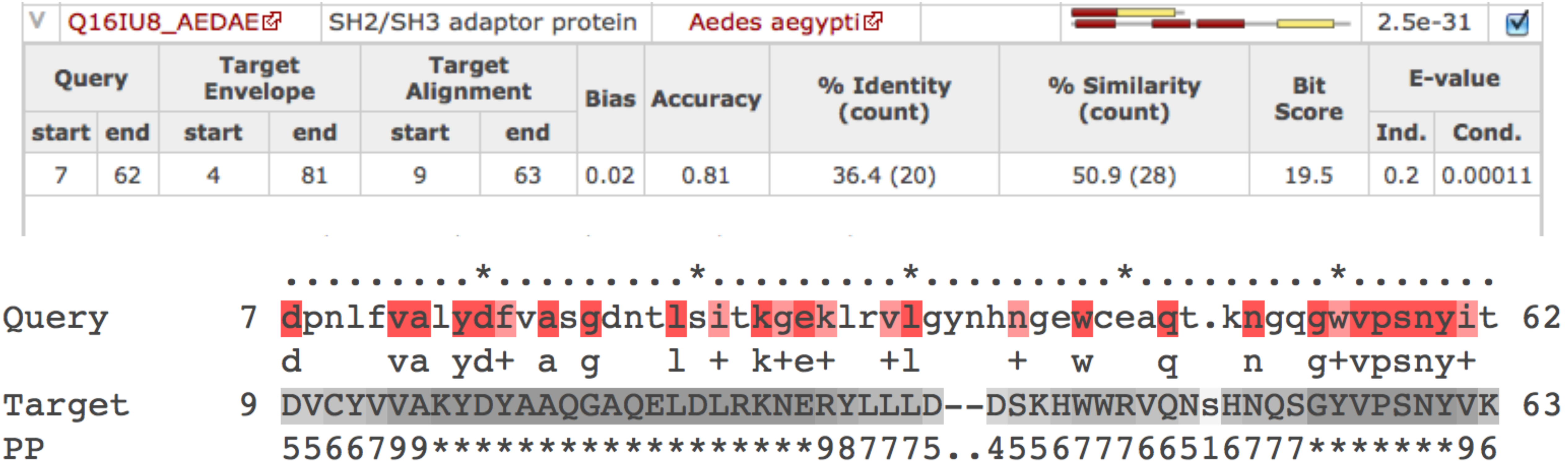
« First « Previous Page 1 of 28 Next » Last »

Significant Query Matches (1825) Customize

	Target	Description	Species	Known Structure	Hit Positions	E-value	
>	G3WV25_SARHA	Uncharacterized protein	Sarcophilus harrisii			8.0e-107	☑
>	F6QCG8_ORNAN	Uncharacterized protein	Ornithorhynchus anatinus			8.7e-107	☑

Visualization of Results – By Score



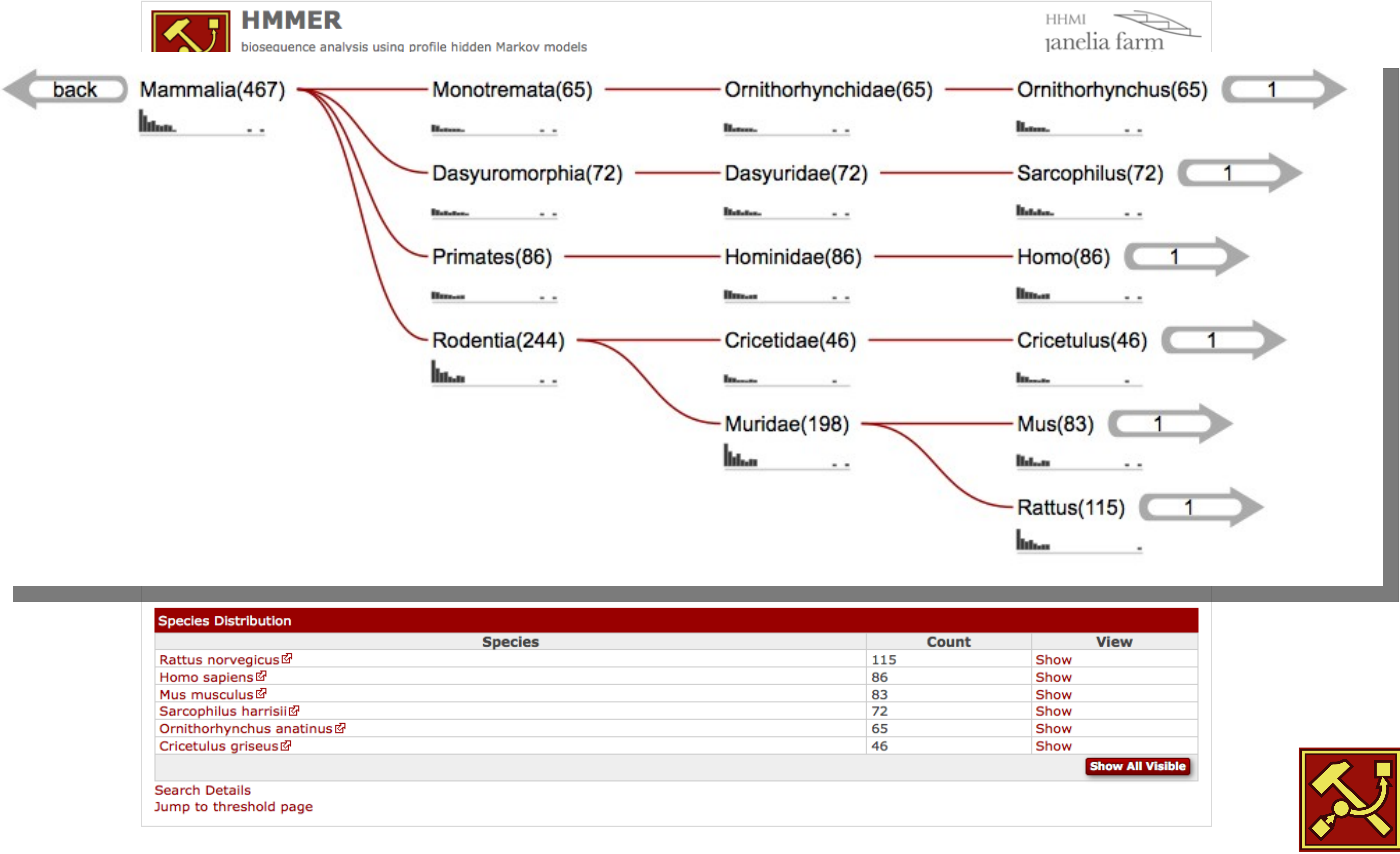


V	Q16IU8_AEDAE	SH2/SH3 adaptor protein	Aedes aegypti		2.5e-31	☑

Query		Target Envelope		Target Alignment		Bias	Accuracy	% Identity (count)	% Similarity (count)	Bit Score	E-value	
start	end	start	end	start	end						Ind.	Cond.
7	62	4	81	9	63	0.02	0.81	36.4 (20)	50.9 (28)	19.5	0.2	0.00011

```
                  .........*.........*.........*.........*.........*.......
Query          7  dpnlfvalydfvasgdntlsitkgeklrvlgynhngewceaqt.kngqgwvpsnyit  62
                  d    va yd+ a g   l + k+e+  +l    +  w   q   n  g+vpsny+
Target         9  DVCYVVAKYDYAAQGAQELDLRKNERYLLLD--DSKHWWRVQNsHNQSGYVPSNYVK  63
PP                5566799******************987775..455677766516777*******96
```

Visualization of Results – By Taxonomy

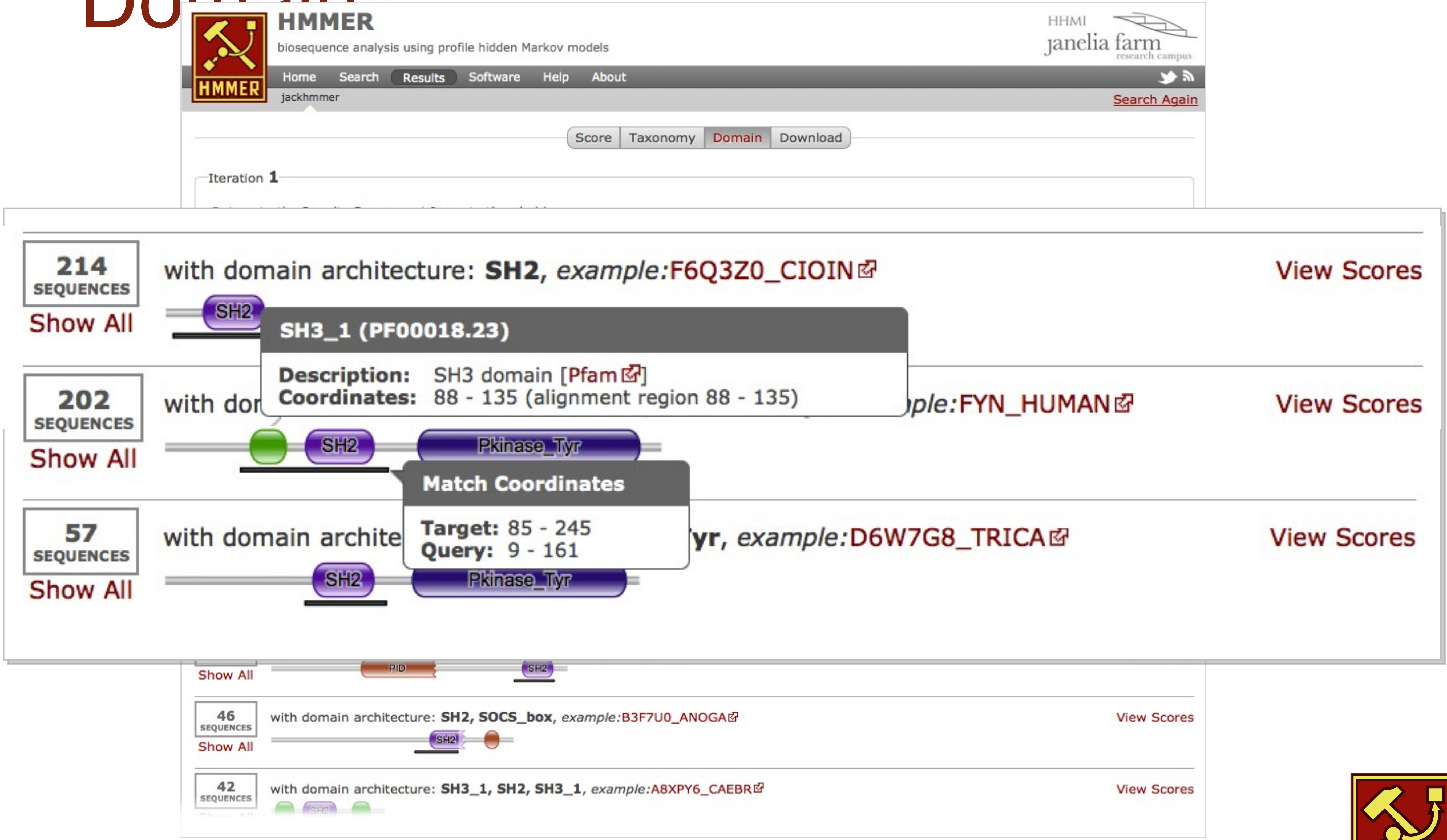


Species Distribution

Species	Count	View
Rattus norvegicus	115	Show
Homo sapiens	86	Show
Mus musculus	83	Show
Sarcophilus harrisii	72	Show
Ornithorhynchus anatinus	65	Show
Cricetulus griseus	46	Show

Search Details
Jump to threshold page

Visualization of Results – By Domain



Iteration 1

214 SEQUENCES Show All — with domain architecture: **SH2**, *example:*F6Q3Z0_CIOIN — View Scores

SH3_1 (PF00018.23)

Description: SH3 domain [Pfam]
Coordinates: 88 - 135 (alignment region 88 - 135)

202 SEQUENCES Show All — with dor … *ple:*FYN_HUMAN — View Scores

Match Coordinates

Target: 85 - 245
Query: 9 - 161

57 SEQUENCES Show All — with domain archite … **yr**, *example:*D6W7G8_TRICA — View Scores

46 SEQUENCES Show All — with domain architecture: **SH2, SOCS_box**, *example:*B3F7U0_ANOGA — View Scores

42 SEQUENCES — with domain architecture: **SH3_1, SH2, SH3_1**, *example:*A8XPY6_CAEBR — View Scores

PFAM: Protein Family Database of Profile HMMs

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

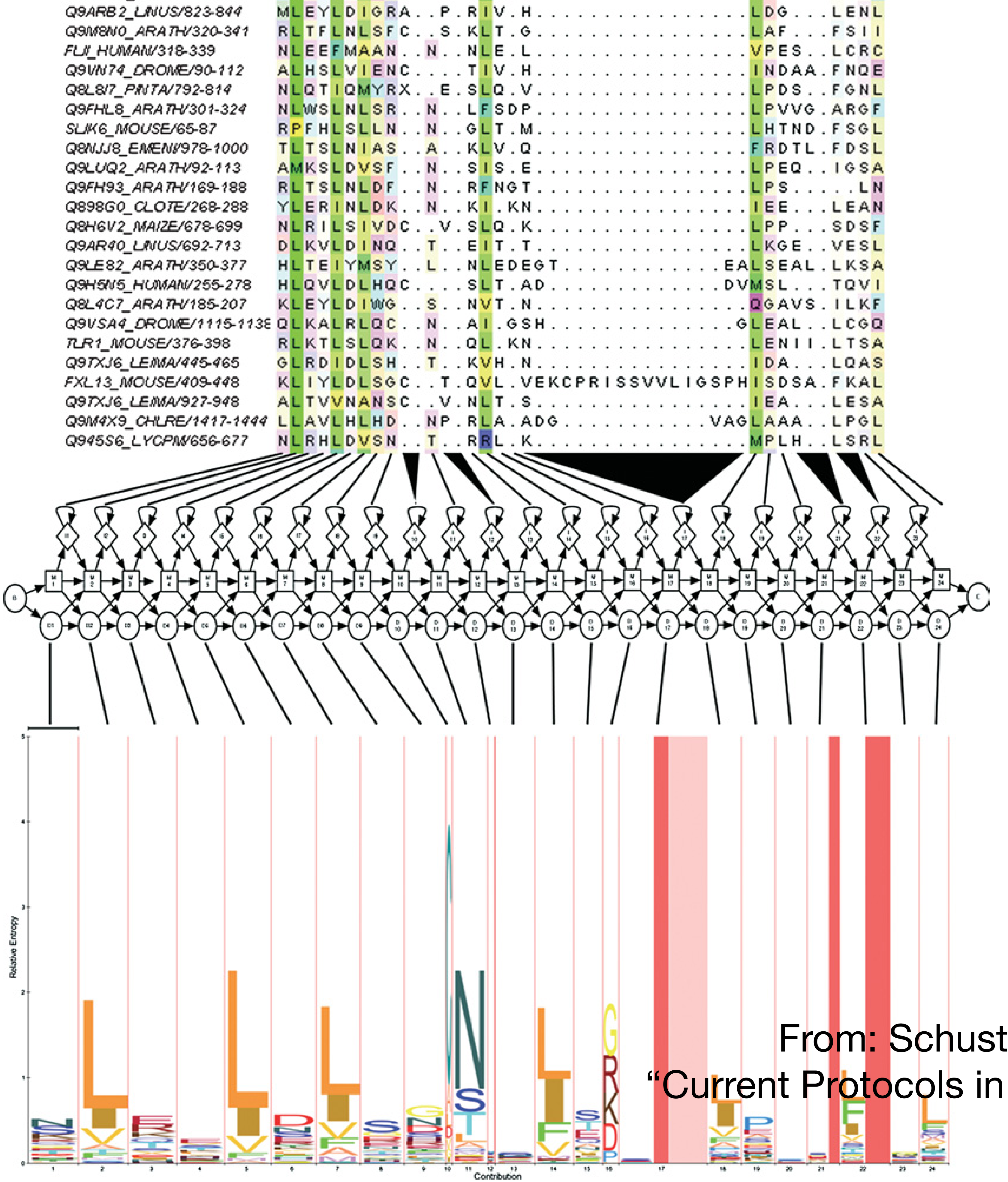
http://pfam.sanger.ac.uk/

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
FLII_HUMAN/318-339     NLEEFMAAN..N..NLE.L................VPES..LCRC
Q9VN74_DROME/90-112    ALHSLVIENC....TIV.H................INDAA.FNQE
Q8L8I7_PINTA/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
Q8NJJ8_EMENI/978-1000  TLTSLNIAS..A..KLV.Q................FRDTL.FDSL
Q9LUQ2_ARATH/92-113    AMKSLDVSF..N..SIS.E................LPEQ..IGSA
Q9FH93_ARATH/169-188   RLTSLNLDF..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   DLKVLDINQ..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/1115-113  QLKALRLQC..N..AI.GSH..............GLEAL..LCGQ
TLR1_MOUSE/376-398     RLKTLSLQK..N..QL.KN................LENII.LTSA
Q9TXJ6_LEIMA/445-465   GLRDIDLSH..T..KVH.N................IDA...LQAS
FXL13_MOUSE/409-448    KLIYLDLSGC..T.QVL.VEKCPRISSVVLIGSPHISDSA.FKAL
Q9TXJ6_LEIMA/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
```

B
M1 M2 M3 M4 M5 M6 M7 M8 M9 M10 M11 M12 M13 M14 M15 M16 M17 M18 M19 M20 M21 M22 M23 M24
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14 D15 D16 D17 D18 D19 D20 D21 D22 D23 D24
E
Relative Entropy
0
1
2
3
4
5
Contribution
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

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

HMM limitations

HMMs are linear models and are thus **unable to capture higher order correlations** among positions (e.g. distant cysteins in a disulfide bridge, RNA secondary structure pairs, etc).

Another flaw of HMMs lies at the very heart of the mathematical theory behind these models. Namely, that the probability of a sequence can be found from the product of the probabilities of its individual residues.

This claim is only valid if the probability of a residue is independent of the probabilities of its neighbors. In biology, there are frequently **strong dependencies between these probabilities** (e.g. hydrophobic residues clustering at the core of protein domains).

These biological realities have motivated research into new kinds of statistical models. These include hybrids of HMMs and neural nets, dynamic Bayesian nets, factorial HMMs, Boltzmann trees and stochastic context-free grammars.

See: Durbin et al. “Biological Sequence Analysis”

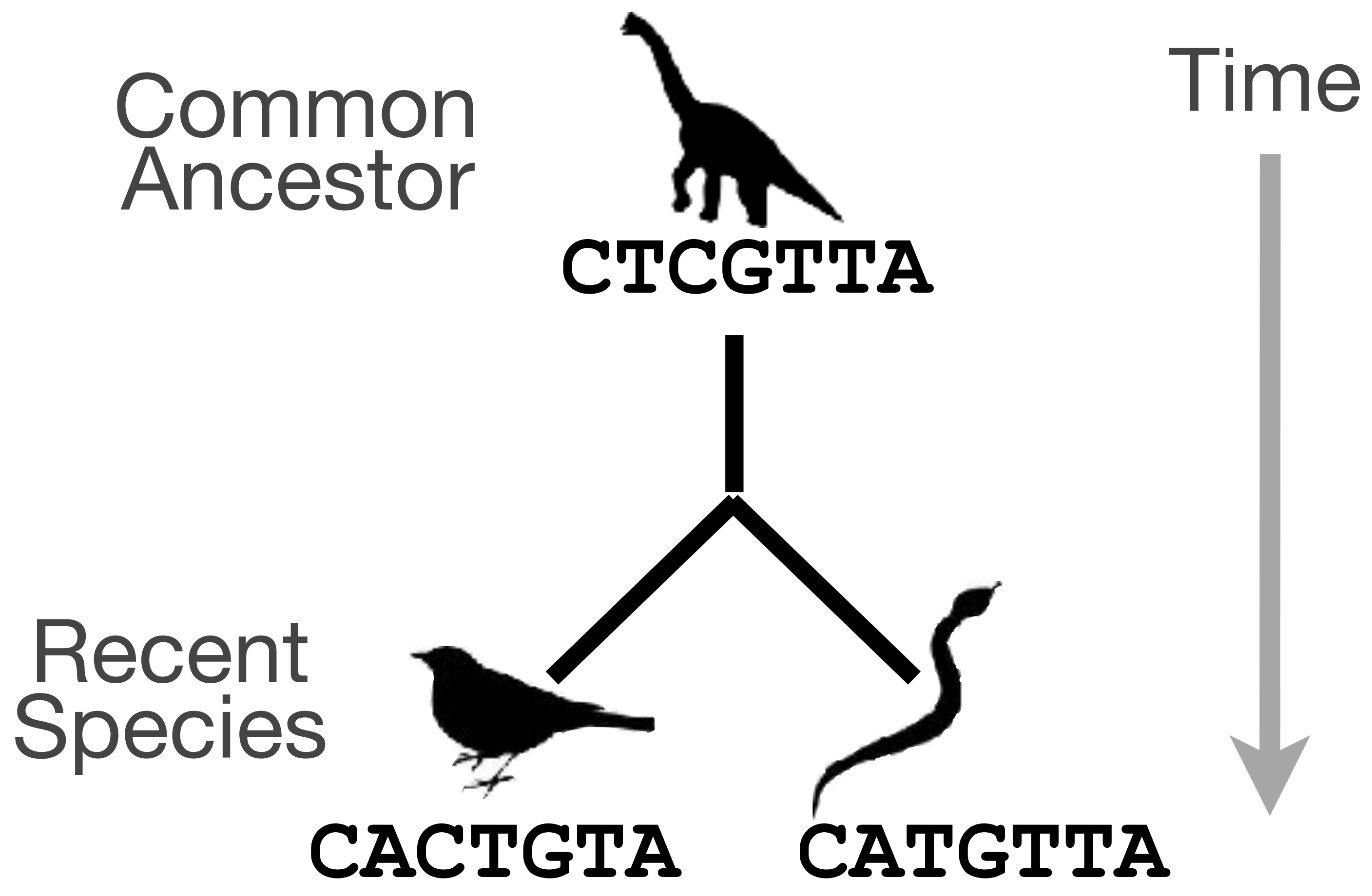
That’s it!

Side Note: Orthologs *vs* Paralogs

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



Can be used to establish evolutionary relationships

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:

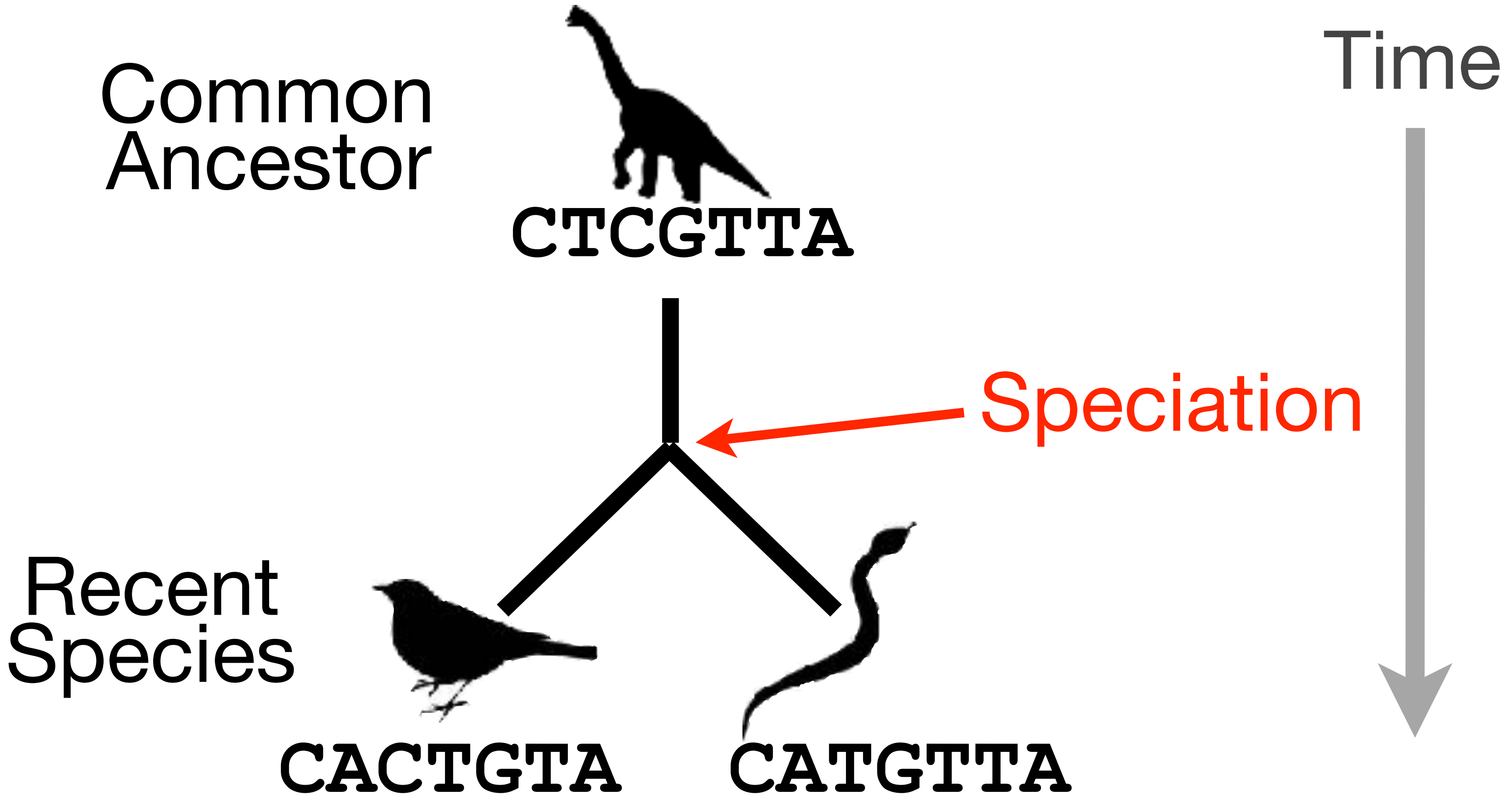
– **Identity** and/or **Similarity**

(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.

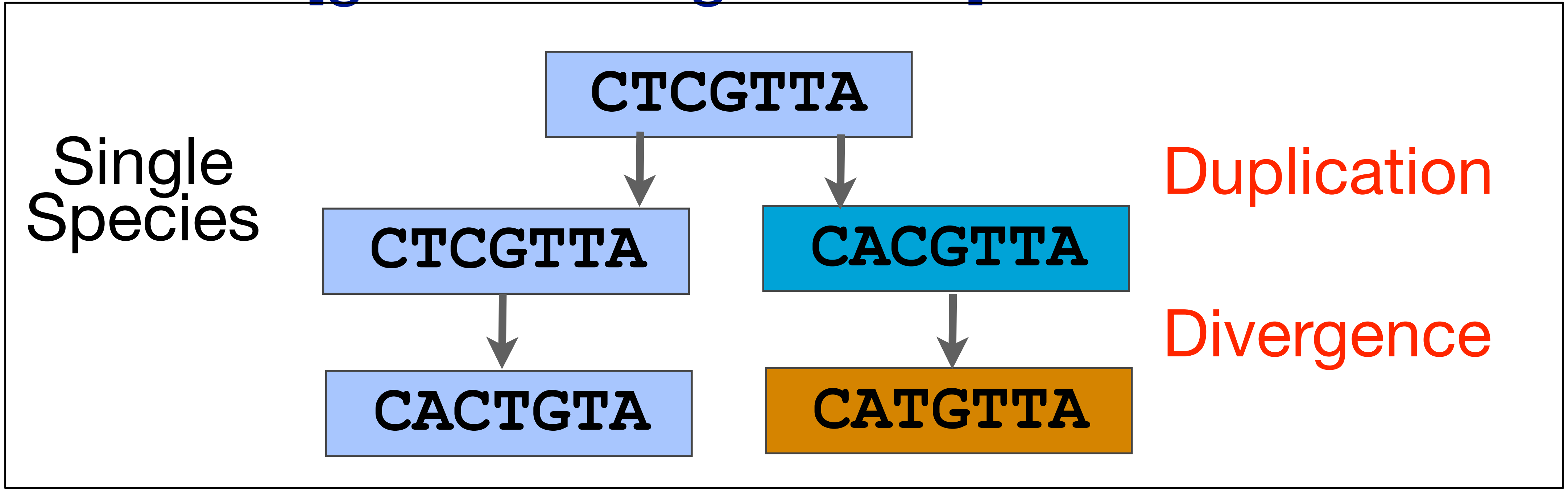
- Ortho = [greek: straight] ... implies direct descent



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