

Position Specific Score Matrix (PSSM) & PSI-BLAST

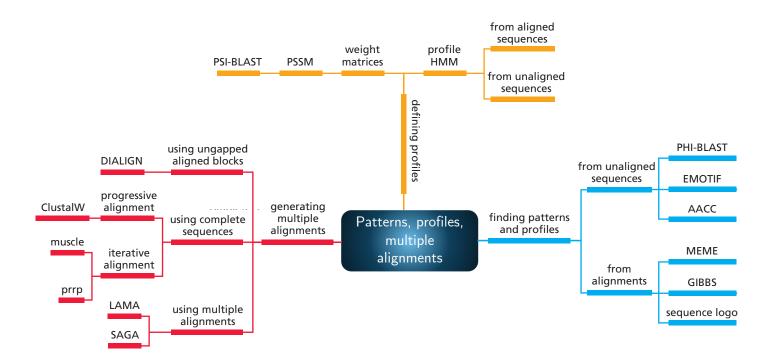
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Sequence Families

 Functional biological sequences typically come in families – these sequences will be expected to have similar properties at equivalent regions

 Sequences in a family have diverged during evolution, but normally maintain the same or a related function

 Thus, identifying that a sequence belongs to a family tells about its function



Sequences from a Globin Family

BBBBBBBBBBBBBBBBCCCCCCCCCCC

-VLSPADKTNVKAAWGKVGA--HAGEYGAEALERMFLSFPTTKTYFPHF

AAAAAAAAAAAAA

Helix

```
HBB HUMAN
          -----VHLTPEEKSAVTALWGKV----NVDEVGGEALGRLLVVYPWTQRFFESF
          -----VLSEGEWQLVLHVWAKVEA--DVAGHGQDILIRLFKSHPETLEKFDRF
          -----DPVGILYAVFKADPSIMAKFTQF
GLB5 PETMA PIVDTGSVAPLSAAEKTKIRSAWAPVYS--TYETSGVDILVKFFTSTPAAQEFFPKF
          -----GALTESQAALVKSSWEEFNA--NIPKHTHRFFILVLEIAPAAKDLFS-F
                  -GLSAAQRQVIAATWKDIAGADNGAGVGKDCLIKFLSAHPQMAAVFG-F
                                            g . L.. f . P .
Consensus
                    Ls.... v a W kv . .
Helix
              DDDDDDDEEEEEEEEEEEEEEEE
HBA HUMAN -DLS----HGSAOVKGHGKKVADALTNAVAHV---D--DMPNALSALSDLHAHKL-
HBB HUMAN
          GDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHL - - - D - NLKGTFATLSELHCDKL -
MYG PHYCA
          AG-KDLESIKGTAPFETHANRIVGFFSKIIGEL--P---NIEADVNTFVASHKPRG-
GLB5 PETMA KGLTTADQLKKSADVRWHAERIINAVNDAVASM--DDTEKMSMKLRDLSGKHAKSF-
LGB2 LUPLU LK-GTSEVPONNPELOAHAGKVFKLVYEAAIOLOVTGVVVTDATLKNLGSVHVSKG-
GLB1 GLYDI SG----AS---DPGVAALGAKVLAQIGVAVSHL--GDEGKMVAQMKAVGVRHKGYGN
                   ... v...Hg kv. a a...l d . a l. l
Consensus
Helix
          FFGGGGGGGGGGGGGGG
HBA HUMAN
          -RVDPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTSKYR
HBB HUMAN
          -HVDPENFRLLGNVLVCVLAHHFGKEFTPPVOAAYOKVVAGVANALAHKYH-
MYG PHYCA
          -KIPIKYLEFISEAIIHVLHSRHPGDFGADAOGAMNKALELFRKDIAAKYKELGYOG
          --VTHDQLNNFRAGFVSYMKAHT--DFA-GAEAAWGATLDTFFGMIFSKM--
          -QVDPQYFKVLAAVIADTVAAG-----DAGFEKLMSMICILLRSAY--
          KHIKAQYFEPLGASLLSAMEHRIGGKMNAAAKDAWAAAYADISGALISGLOS-----
Consensus
```

Alignment of 7 globins

The 8 alpha helices are shown as A-H above the alignment

Helices are more conserved than the loop regions



Position Specific Score Matrix (PSSM)

- Substitution score matrix defines score $s_{a,b}$ for two residues without regard to their environment
- For finding all family members we need to account for known residue preferences at each alignment position.
- Inclusion of these position- specific preferences in the scoring scheme is achieved with the use of a scoring profile in which each alignment position has its own substitution scores.
- Position-specific scoring matrices (PSSMs)
 - frequencies of each residue in a specific position of a multiple alignment.

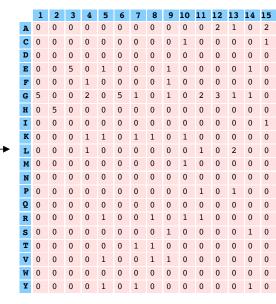


Position Specific Score Matrix (PSSM)

frequencies of each residue in a specific position of a multiple alignment.

$$f_{u,b} = \frac{n_{u,b}}{N_{sea}}$$

- Column 1: $f_{1,A} = \frac{0}{5} = 0, f_{1,G} = \frac{5}{5} = 1, \dots$
- Column 2: $f_{2,A} = \frac{0}{5} = 0, f_{2,H} = \frac{5}{5} = 1, \dots$
- . .
- Column 15: $f_{15,A} = \frac{2}{5} = 0.4, f_{15,C} = \frac{1}{5} = 0.2, \dots$





Position Specific Score Matrix (PSSM)

frequencies of each residue in a specific position of a multiple alignment.

$$f_{u,b} = \frac{n_{u,b}}{N_{seq}}$$

Score Associated with row a and column u will be

$$m_{u,a} = \sum_{\substack{ ext{residue} \ ext{types } b}} f_{u,b} s_{a,b}$$
 This is not a log-odds ratio

Log-odds form for a PSSM element

$$m_{u,a} = \log \frac{q_{u,a}}{p_a} \rightarrow \text{background frequency}$$

PSSM: Sequence Weighting

- PSSM should represent the full range of diversity within the sequence family in an unbiased fashion.
- A partial set of family sequences will most probably be biased toward a certain subgroup
- Different sequences must be weighted, the weighting should be reduced for very similar sequences.
 - More sensitive to distant relationships.



Sequence Weighting Scheme for MSA

- Tree-based weights assume that sequences are related by an evolutionary tree
 - Need not be the case for alignments of short and distantly related sequences,
 where root location can be uncertain.
- In a pairwise distance method, every sequence is assumed to lie some distance away from every other sequence, or from some generalized sequence
- The sequence weights are typically applied to PSSMs in which each position vector is considered independently of all others.
 - Useful sequence weights might be based on the diversity observed at each position in an alignment rather than on the diversity measured for whole sequences.



 PSSM: Useful sequence weights based on the diversity observed at each position in an alignment rather than on the diversity measured for whole sequences.

J. Mol. Biol. (1994) 243, 574-578

Position-based Sequence Weights

Steven Henikoff and Jorja G. Henikoff

Sequence weighting methods have been used to reduce redundancy and emphasize diversity in multiple sequence alignment and searching applications. Each of these methods is based on a notion of distance between a sequence and an ancestral or generalized sequence. We describe a different approach, which bases weights on the diversity observed at each position in the alignment, rather than on a sequence distance measure. These position-based weights make minimal assumptions, are simple to compute, and perform well in comprehensive evaluations.



- In an alignment column, If there are m different residues, each is assigned a weight of 1/m.
- for each residue type, the number of sequences that have this residue at this position is counted.
- If there are n sequences with this residue, the weight becomes 1/mn.
- An overall weight for the whole sequence can be defined by adding the individual column weights and then normalizing by the number of columns



HSAPL

HTADV

HTAEV

HTGLI

HTGVI

Pos = 1

Pos = 2

$$w(S) = \frac{1}{2}$$

$$w(T) = \frac{1}{8}$$

Pos = 3

$$w(A) = \frac{1}{6}$$

$$w(T) = \frac{1}{8}$$
 $w(G) = \frac{1}{4}$ $w(D) = \frac{1}{5}$ $w(V) = \frac{1}{6}$

$$Pos = 4$$

$$w(H) = \frac{1}{5}$$
 $w(S) = \frac{1}{2}$ $w(A) = \frac{1}{6}$ $w(P) = \frac{1}{5}$ $w(L) = \frac{1}{3}$

Pos = 5

$$w(D) = \frac{1}{2}$$

$$w(E) = \frac{1}{5}$$
 $w(I) = \frac{1}{6}$

$$w(L) = \frac{1}{5}$$

$$w(V) = \frac{1}{5}$$



```
12345

HSAPL =(\frac{1}{5}+\frac{1}{2}+\frac{1}{6}+\frac{1}{5}+\frac{1}{3})/5=0.280 0.280

HTADV 0.171

HTAEV 0.171

HTGLI 0.188

HTGVI
```



Overcoming lack of Data: Pseudocounts

- If any residue type is not observed in the column, the score for aligning that residue type in that column will have the value $-\infty$
 - lack of sequence alignment data
 - exclusion of the corresponding residue at this position
- Add a small number to all observed frequencies.

$$q_{u,a} = \frac{n_{u,a} + 1}{N_{seq} + 20} \qquad q_{u,a} = \frac{\alpha f_{u,a} + \beta p_a}{\alpha + \beta}$$

$$q_{u,a}=rac{lpha f_{u,a}+eta g_{u,a}}{lpha+eta}$$
 Pseudocount based on Substitution matrix



PSSM Example

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
GHEGVGKVVKLGAGA	Α	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	1.3	0.7	-0.2	1.3
GHEKKGYFEDRGPSA	C	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	0.7
GHEGYGGRSRGGGYS	D	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
GHEFEGPKGCGALYI	Ε	-0.2	-0.2	2.3	-0.2	0.7	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	0.7	-0.2
GHELRGTTFMPALEC	F	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	G	2.3	-0.2	-0.2	1.3	-0.2	2.3	0.7	-0.2	0.7	-0.2	1.3	1.7	0.7	0.7	-0.2
A 0 0 0 0 0 0 0 0 0 0 0 0 0 2 1 0 2	Н	-0.2	2.3	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
C 0 0 0 0 0 0 0 0 0 0 1 0 0 0 0 1 D 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	- 1	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7
E 0 0 5 0 1 0 0 0 1 0 0 0 1 0	K	-0.2	-0.2	-0.2	0.7	0.7	-0.2	0.7	0.7	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	-0.2
F 0 0 0 1 0 0 0 0 1 0 0 0 0 0 0	L	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	1.3	-0.2	-0.2
H 0 5 0 0 0 0 0 0 0 0 0 0 0 0 0	М	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	-0.2
I 0 0 0 0 0 0 0 0 0 0 0 0 0 0 1	Ν	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
L 0 0 0 1 1 0 1 1 0 1 0 0 0 0 0 0 L 0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Р	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	0.7	-0.2	-0.2
M 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Q	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
N 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	R	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	0.7	-0.2	0.7	0.7	-0.2	-0.2	-0.2	-0.2
Q 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	S	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	0.7	-0.2
R 0 0 0 0 1 0 0 1 0 0 1 0 0 0 0	Т	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	0.7	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
S 0 0 0 0 0 0 0 0 1 0 0 0 1 0 T 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	V	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	-0.2	0.7	0.7	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
V 0 0 0 0 1 0 0 1 1 0 0 0 0 0 0 0	W	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2
W 0	Υ	-0.2	-0.2	-0.2	-0.2	0.7	-0.2	0.7	-0.2	-0.2	-0.2	-0.2	-0.2	-0.2	0.7	-0.2



PSI-BLAST (Position-Specific Iterated BLAST)

 Carefully constructed PSSM can find many distant members of a protein sequence family not easily found by a standard sequence search

 PSI-BLAST enhances the BLAST database searching method to incorporate PSSMs.

Involves series of repeated steps or iterations



PSI-BLAST Algorithm

- 1. Perform standard BLAST search using a substitution matrix with a single query sequence.
- 2. Obtain initial set of related sequences whose BLAST score gives an *E*-value smaller than a predetermined cut-off
- 3. Create a PSSM from alignments of these significant matches with the query sequence
- 4. Scan PSSM against the database using a variant of the BLAST program to identify new sequences with suitably small *E*-values.
- 5. If this second search finds some newly identified related sequences, use them to update the PSSM

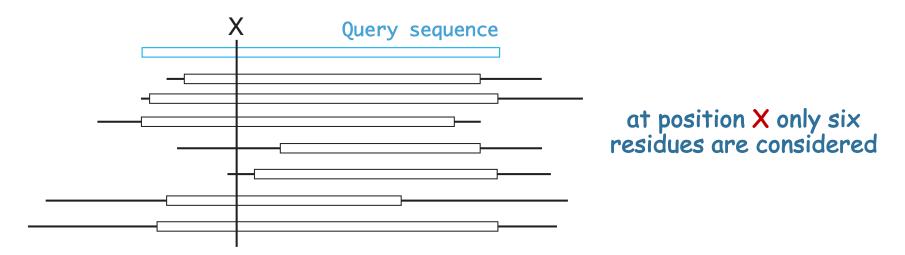


PSI-BLAST: Profile Length

 The profile constructed by PSI-BLAST has exactly the same length as the query sequence. Insertions with respect to the query are simply ignored



PSI-BLAST: PSSM Construction



- PSSM is restricted to those residues that have been aligned to a residue in the query sequence
- At each residue position of the query, a PSSM is constructed using only those sequences whose BLAST alignments involve that position.
- Number of sequences in the alignment changes from column to column



The Corruption of Profiles

- PSI-BLAST *E*-values are calculated for the profiles PSI-BLAST produces, and can not be interpreted as referring to the original query sequence
- Once a sequence unrelated to the query is included in a PSI-BLAST multiple alignment, and thus in the construction of PSI-BLAST's profile, it will bring in many of its "neighbors" on the next iteration, and this process can snowball. Sequence weighting will exacerbate this process.

Profile corruption is a major problem for Iterative approaches such as PSI-BLAST



PSI-BLAST vs BLAST

 Because of its cycling nature, PSI-BLAST allows to find more distant homologs than a simple BLAST search.

- PSI-BLAST uses two *E*-values:
 - the threshold *E*-value for the initial BLAST (-e option). The default is 10 as in the standard BLAST;
 - the inclusion *E*-value to accept sequences (-h option) in the PSSM construction (default is 0.001).



PSI-BLAST Advantages

- Fast because of the BLAST heuristic.
- Allows PSSMs searches on large databases.
- A particularly efficient algorithm for sequence weighting.
 - position-based sequence weight scheme, slightly modified to include gaps as another residue type, and to ignore fully conserved residues.
- A very sophisticated statistical treatment of the match scores.

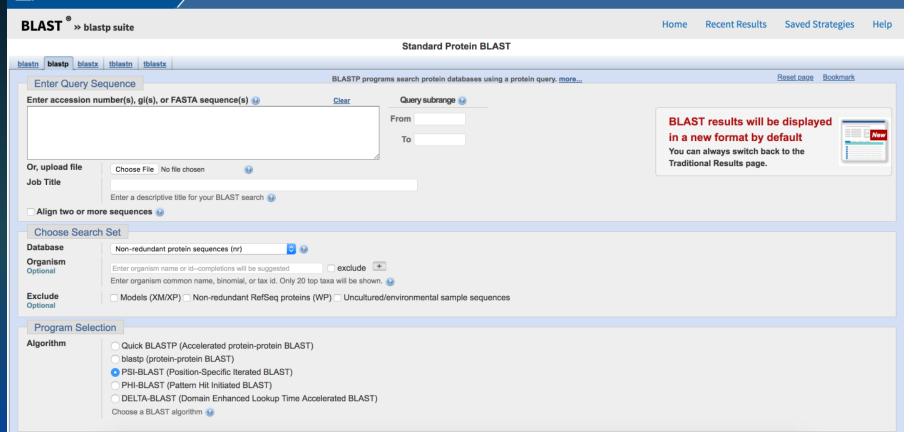


PSI-BLAST Caution

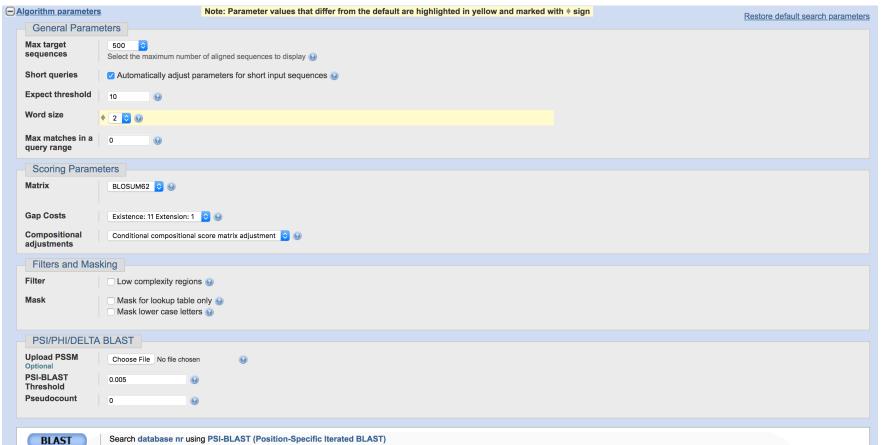
- Avoid too close sequences ⇒ overfit!
- Can include false homologs! Therefore check the matches carefully: include or exclude sequences based on biological knowledge.
- The *E*-value reflects the significance of the match to the previous training set not to the original sequence!



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