Sequence profiles, Hidden Markov models and homology modeling

Morten Nielsen,
CBS, Department of Health Technology, DTU
and
Instituto de Investigaciones Biotecnológicas,
Universidad de San Martín, Argentina

Identification of essential residues in protein sequences


```
TVNGQ--FPGPRLAGVAREGDQVLVKVVNHVAENITIHWHGVQLGTGWADPPAYVTQCPI

TKAVVLTFNTSVEICLVMQGTSIV----AAESHPLHLHGFNFPSNFNLVDGMERNTAGVP
```

Summary

- Sequence logo is a power tool to visualize (binding) motifs
  - Information content identifies essential residues for function and/or structural stability
- Weight matrices can be derived from very limited number of data using the techniques of
  - Sequence weighting
  - Pseudo counts

Sequence Profiles and Weight matrices

- Alignments based on conventional scoring matrices (BLOSUM62) scores all positions in a sequence in an equal manner
- Some positions are highly conserved, some are highly variable (more than what is described in the BLOSUM matrix)
- Sequence profile are ideal suited to describe such position specific variations

Sequence alignment

- Conventional sequence alignment uses a (Blosum) scoring matrix to identify amino acids matches in the two protein sequences

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

Alignment scoring matrices

- Blosum62 score matrix. Fg=1. Ng=0?

	L	A	G	D	S	D
F						
I						
G						
D						
S						
L						

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

Alignment scoring matrices

- Blosum62 score matrix. Fg=1. Ng=0?

	L	A	G	D	S	D
F	0	-2	-3	-3	-2	-3
I	2	-1	-4	-3	-2	-3
G	-4	0	6	-1	0	-1
D	-4	-2	-1	6	0	6
S	-2	1	0	0	4	0
L	4	-1	-4	-4	-2	-4

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

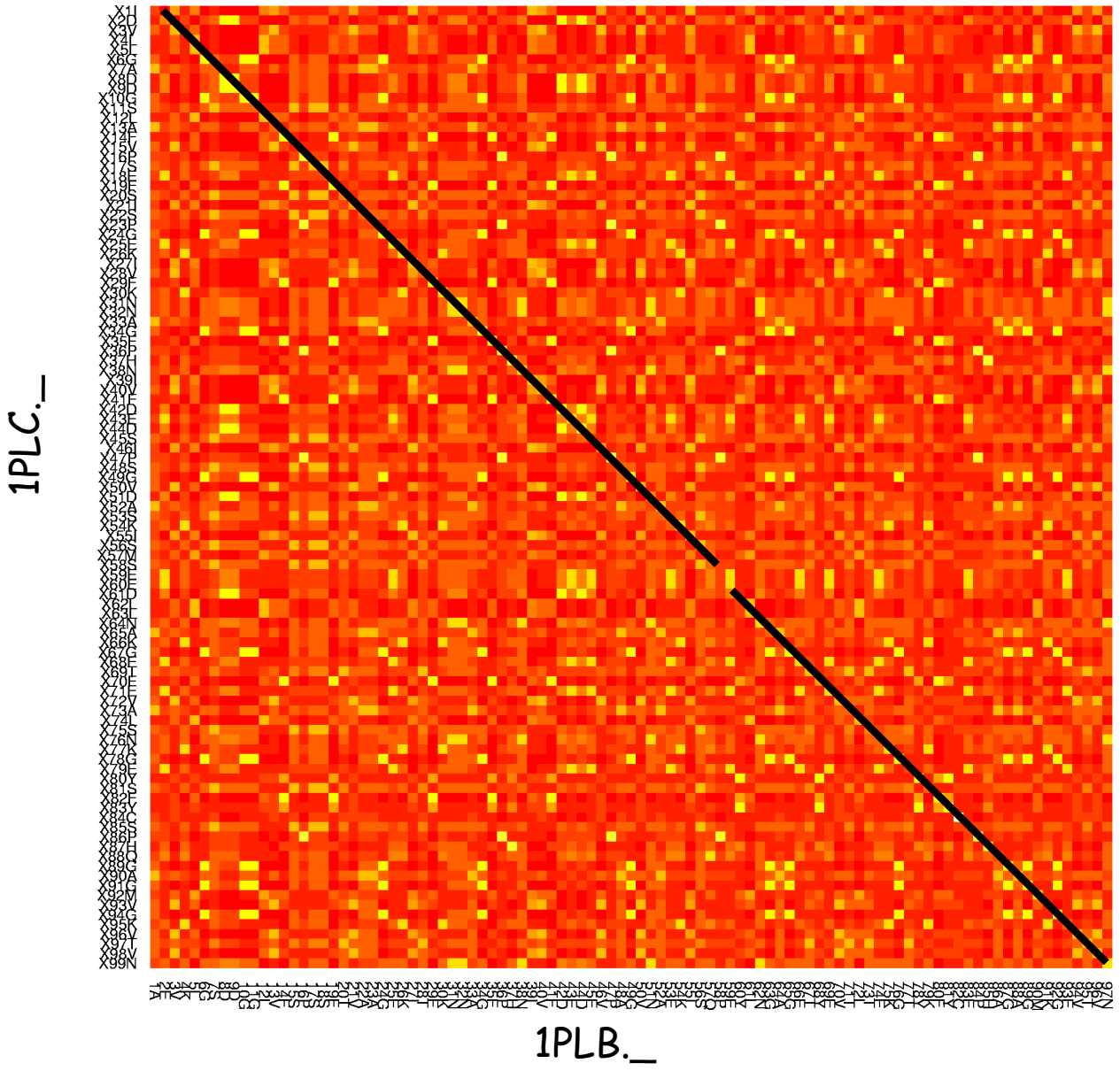
- Score =2-1+6+6+4=17

LAGDS

I-GDS

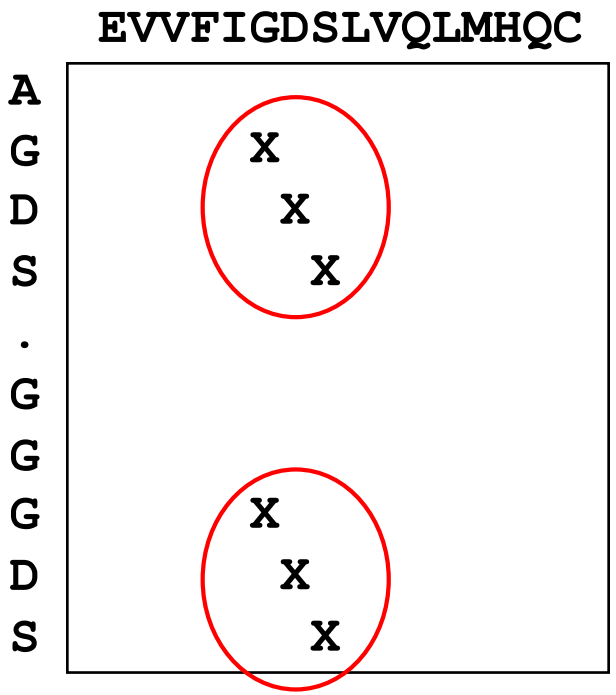
When Blast works!

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS **CBS**

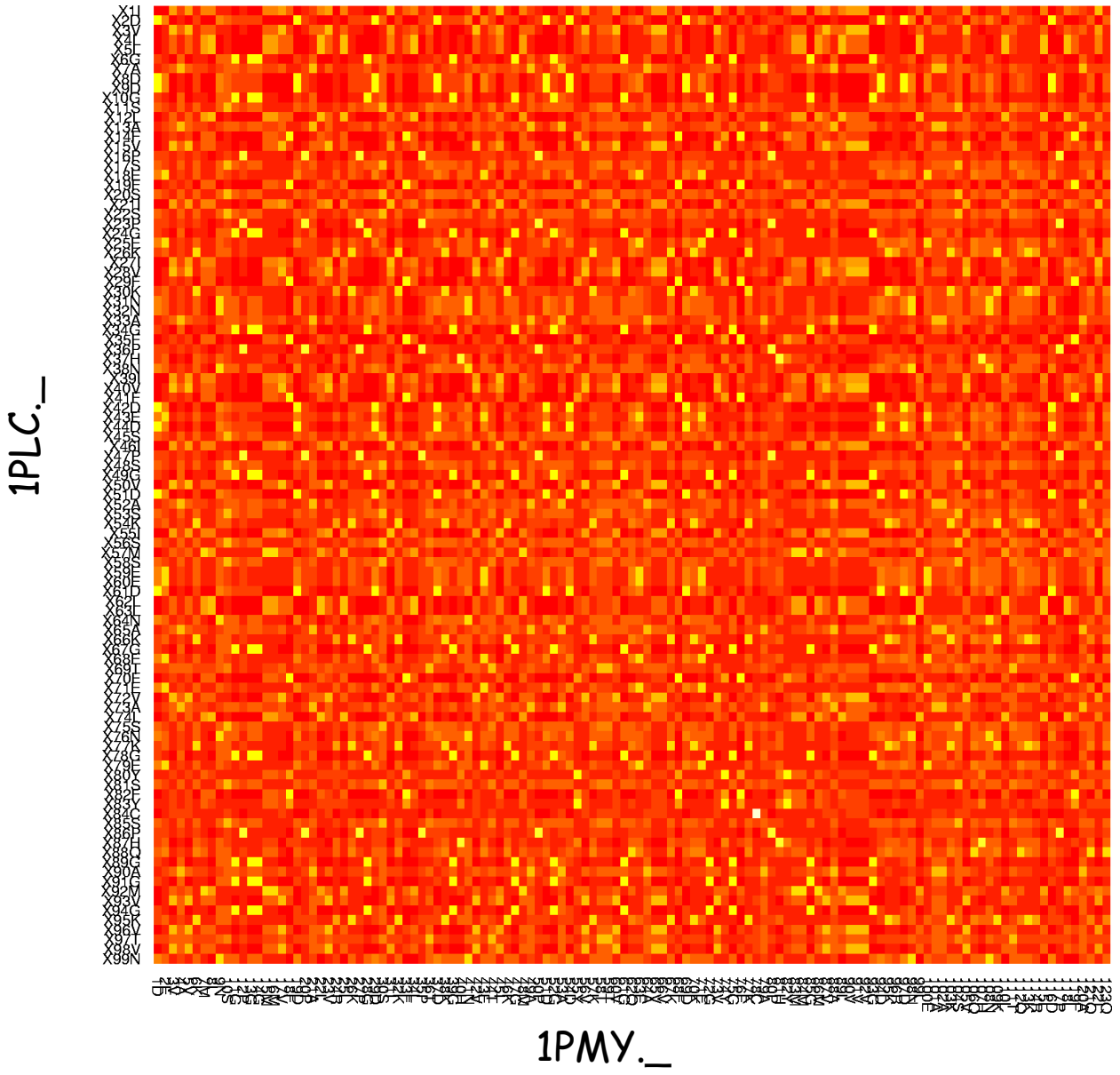


What goes wrong when Blast fails?

- Conventional sequence alignment uses a (Blosum) scoring matrix to identify amino acids matches in the two protein sequences
- This scoring matrix is identical at all positions in the protein sequence!

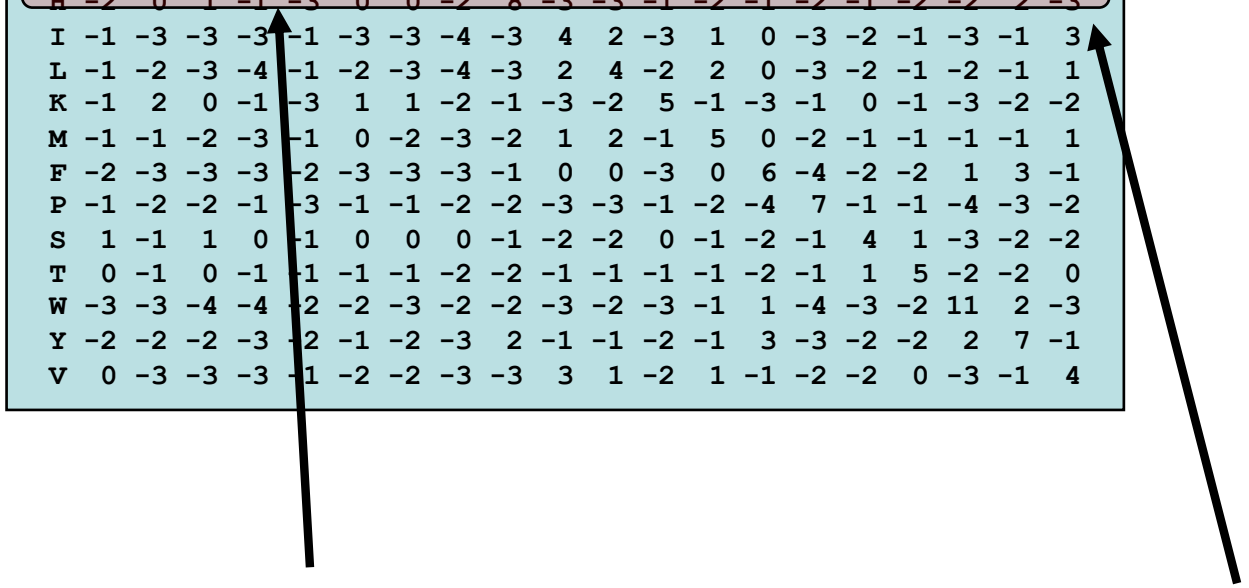


When Blast fails!



Alignment match scores

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

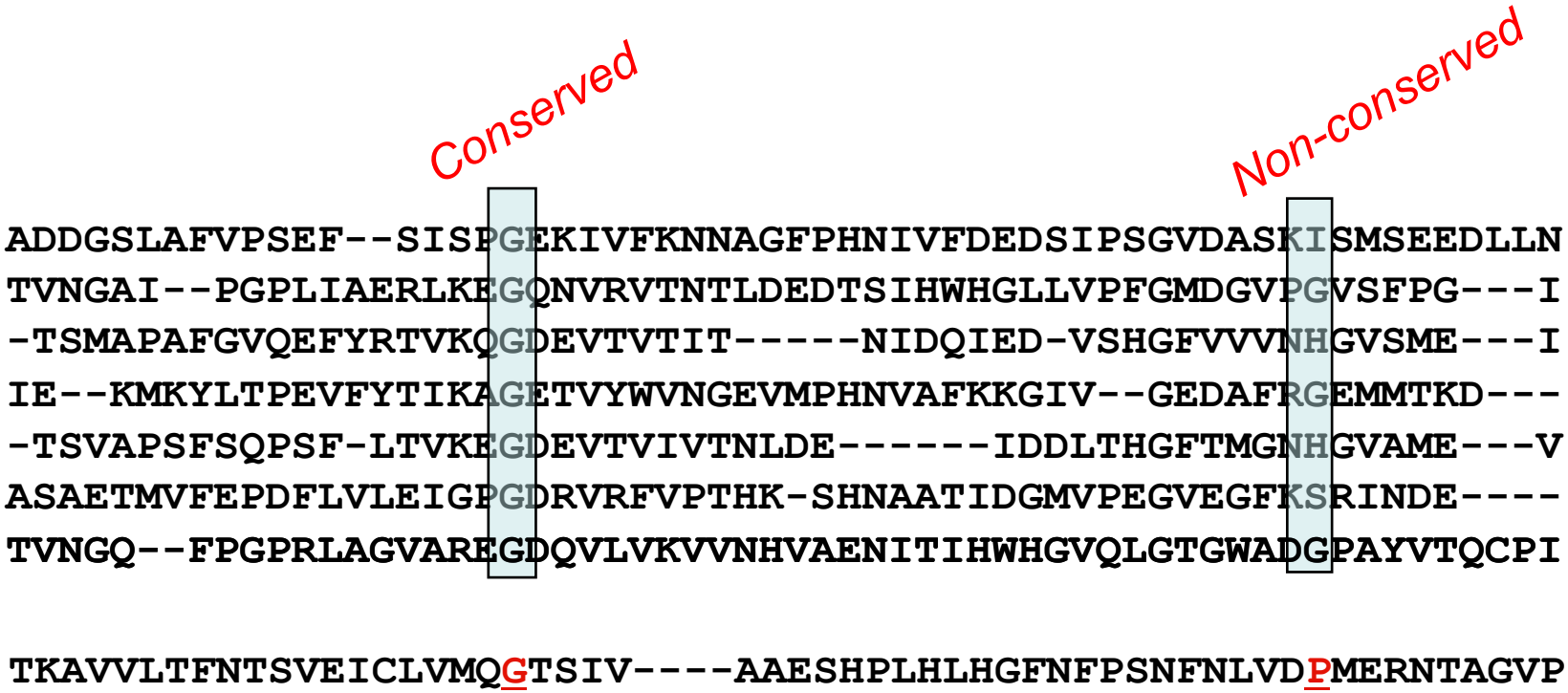


TVNGQ--FPGPRLAGVAREGDQVLVKVVNHVAENITIHWHGVQLGTGWADGPAYVTQCPI

Sequence profiles

- In reality not all positions in a protein are equally likely to mutate
  - Some amino acids (active cites) are highly conserved, and the score for mismatch must be very high
  - Other amino acids can mutate almost for free, and the score for mismatch should be lower than the BLOSUM score
- Sequence profiles can capture these differences

Sequence profiles



Matching any thing
but G => large
negative score

Any thing can match

How to make sequence profiles

1. Align (BLAST) sequence against large sequence database (Swiss-Prot)
2. Select significant alignments and make sequence profile
3. Use profile to align against sequence database to find new significant hits
4. Repeat 2 and 3 (normally 3 times!)

Sequence logos.
Visualization of sequence profiles

$$I = \sum_a p_a \log(\frac{p_a}{q_a})$$

$P_A = 6/10 = 0.6$
$P_G = 2/10 = 0.2$
$P_T = P_K = 1/10 = 0.1$
$P_C = P_D = \ldots P_V = 0.0$

$q_A = 0.07$
$q_G = 0.07$
$q_T = 0.05$
$q_K = 0.06$

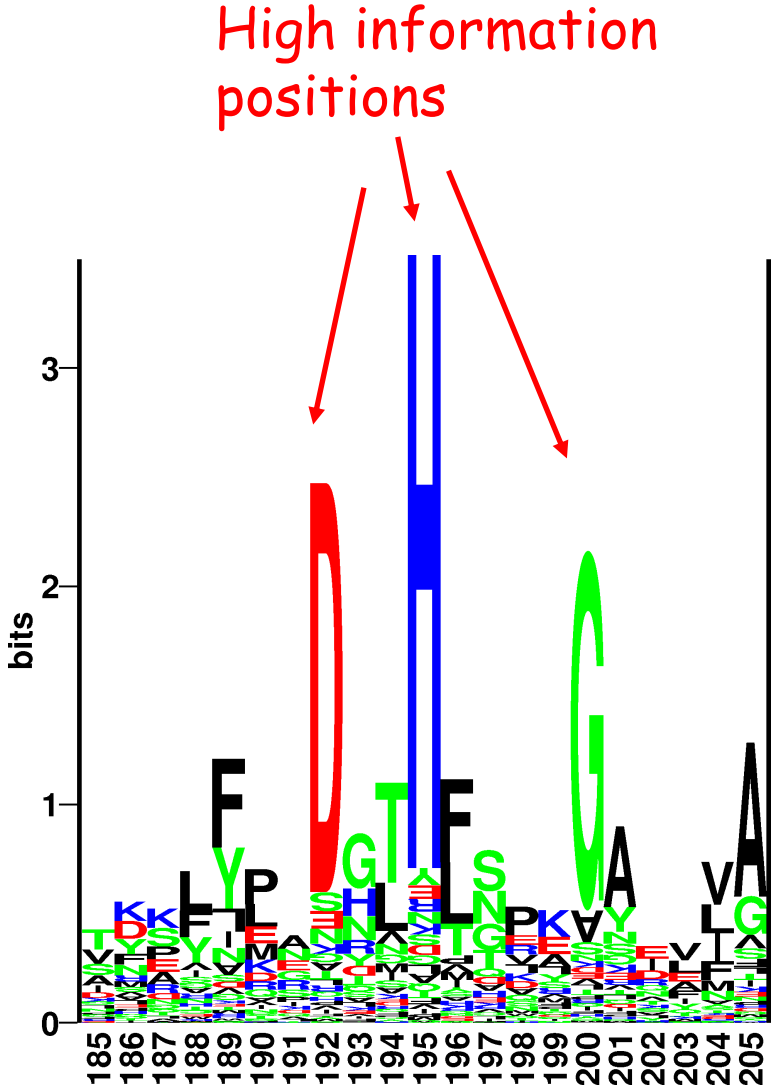
ALAKAAAAM
ALAKAAAAN
ALAKAAAAR
ALAKAAAAT
ALAKAAAAV
GMNERPILT
GILGFVFTM
TLNAWVKVV
KLNEPVLLL
AVVPFIVSV

Sequence logos

$$I = \sum_a p_a \log(\frac{p_a}{q_a})$$

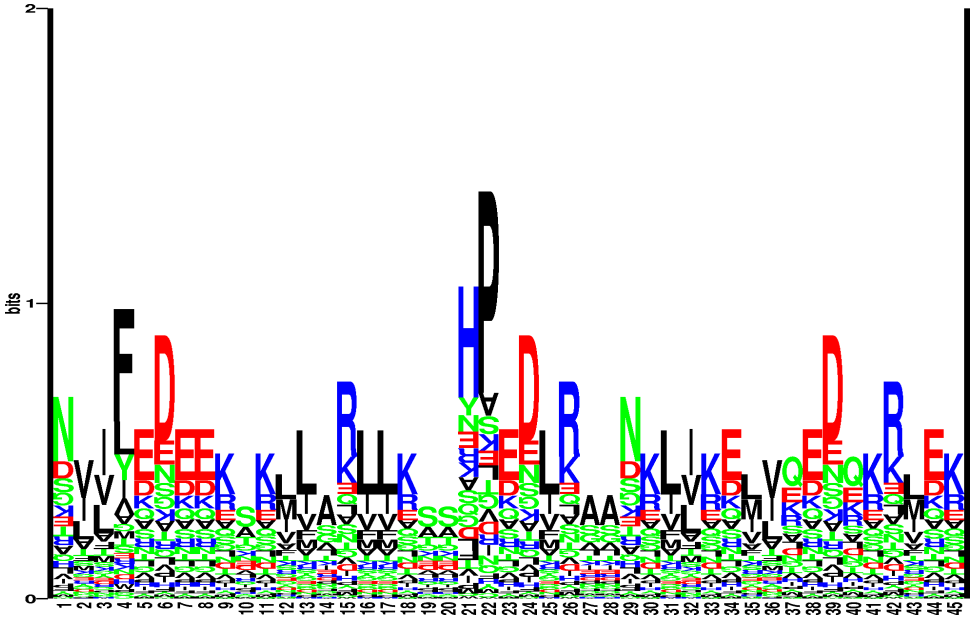
- Height of a column equal to I
- Relative height of a letter is p

(letters are upside down if q>p)



Sequence profiles (1J2J.B)

IFEDEEKSKMLARLLKSSHPEDLRAANKLIKELVQEDQKRLEK



Blosum62

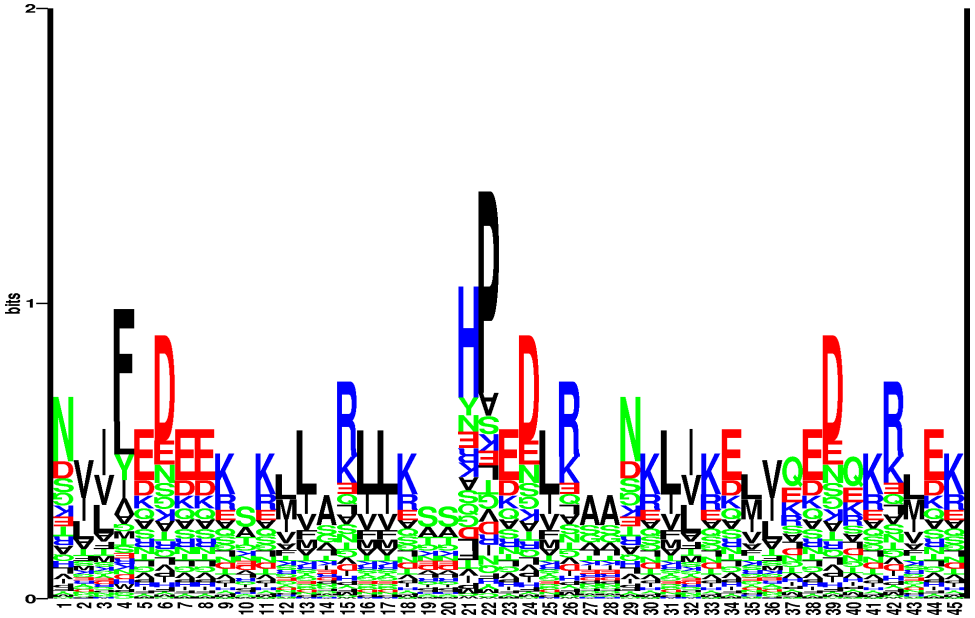
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	1	0	-3	-2	0
R	-1	5	0	-2	-3	1	0	-2	0	-3	-2	2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	6	1	-3	0	0	0	1	-3	-3	0	-2	-3	-2	1	0	-4	-2	-3
D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	9	-3	-4	-3	-3	-1	-1	-3	-1	-2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

$$W_{ij} = \log(p_{ij}/q_j)$$

Sequence profiles (1J2J.B)

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

IFEDEEKSKMLARLLKSSHPEDLRAANKLIKELVQEDQKRLEK

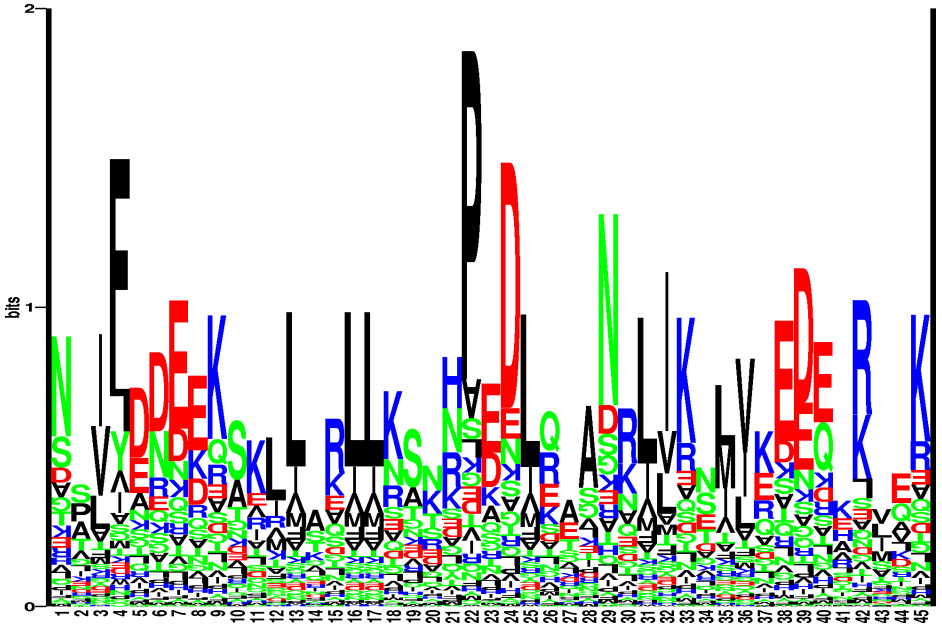


		A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
0	I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4	2	-3	1	0	-3	-2	-1	-3	-1	3
1	F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6	-4	-2	-2	1	3	-1
2	E	-1	0	0	2	-4	2	5	-2	0	-3	-3	1	-2	-3	-1	0	-1	-3	-2	-2
3	D	-2	-2	1	6	-3	0	2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
4	E	-1	0	0	2	-4	2	5	-2	0	-3	-3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

Sequence profiles (1J2J.B)

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

IFEDEEKSKMLARLLKSSHPEDLRAANKLIKELVQEDQKRLEK



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

Blast2logo

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Blast2logo 1.0 Server

Instructions | Output format

SUBMISSION

Paste a single sequence in FASTA format into the field below:

```
>Ex
VALAELYIPEVARRLGQGWHEDECTFAEVTIGTARLQAILRDIATSWSADEGGMRDGPAVLVLLPPG
EQHTLGAMVAVAKLRRLGVSVCLRMSTGPAELRELFGKRRFDAIMISLAHAEMLEVGRKLVKTLKD
MTGGRIPVAMGGALFLDGTEAASIPEADIVTNDIEAALQ
```

Submit a file in FASTA format directly from your local disk:

Choose File no file selected

Upload a file in BLAST PROFILE format:

Choose File no file selected

Blast Database SP

Number of Blast iterations 1

Blast E-value cutoff 0.00001

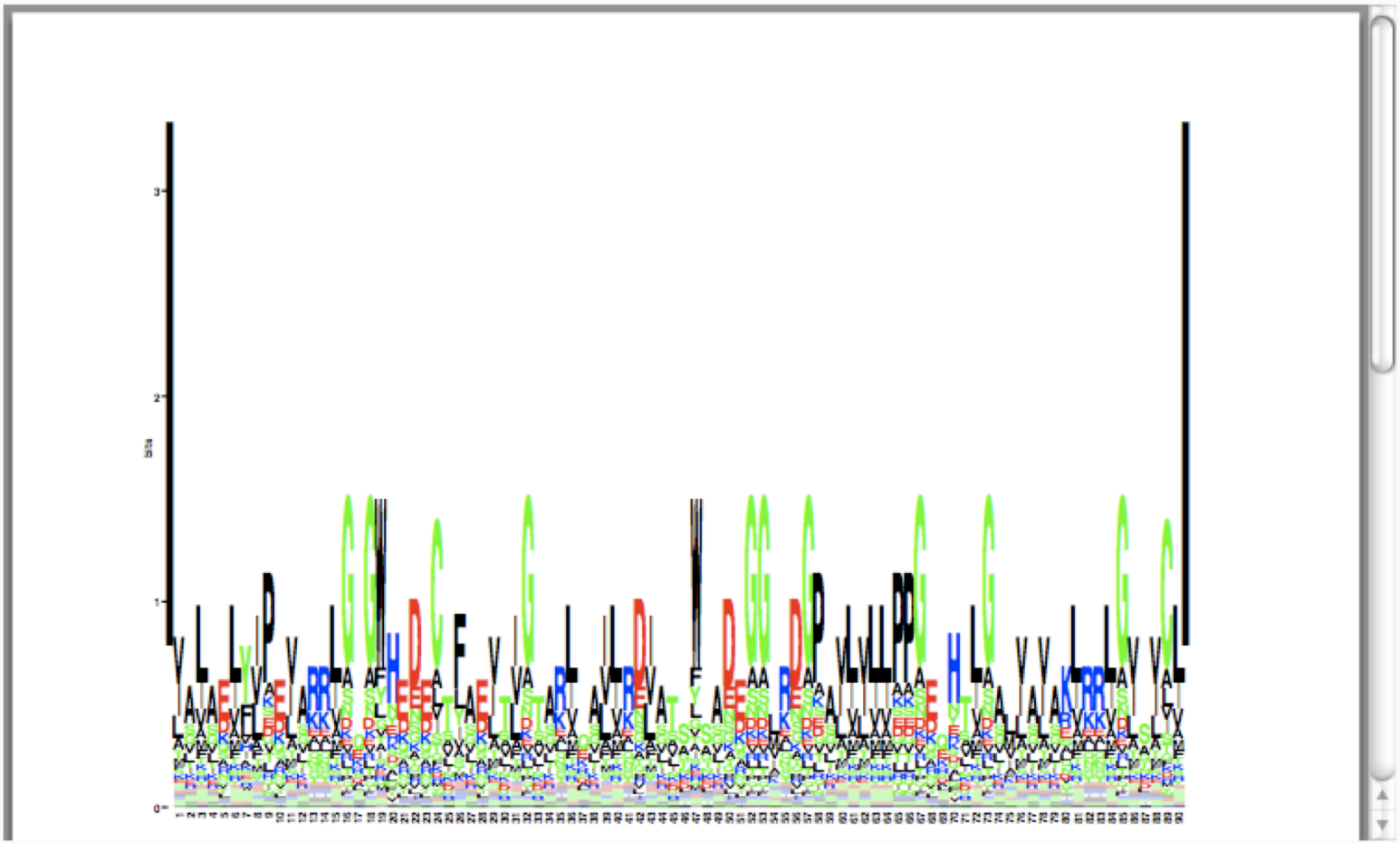
Stack Linesize 90

Plot Kullback-Leibler logo ☐

File format for logo file PDF

Submit Clear fields

Blast2logo



Download logo file Logo

Link to Blastprofile output file Blast.prof

Blast2logo

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Last position-specific scoring matrix computed

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

.
.

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

Blast2logo

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Blast2logo 1.0 Server

Instructions | Output format

SUBMISSION

Paste a single sequence in FASTA format into the field below:

```
>Ex
VALAELYIPEVARRLGQGWHEDECTFAEVTIGTARLQAILRDIATSWSADEGGMRDGPAVLVLLPPG
EQHTLGAMVAVAKLRRLGVSVCLRMSTGPAELRELFGKRRFDAIMISLAHAEMLEVGRKLVKTLKD
MTGGRIPVAMGGALFLDGTEAASIPEADIVTNDIEAALQ
```

Submit a file in FASTA format directly from your local disk:

Choose File no file selected

Upload a file in BLAST PROFILE format:

Choose File no file selected

Blast Database NR70

Number of Blast iterations 3

Blast E-value cutoff 0.00001

Stack Linesize 90

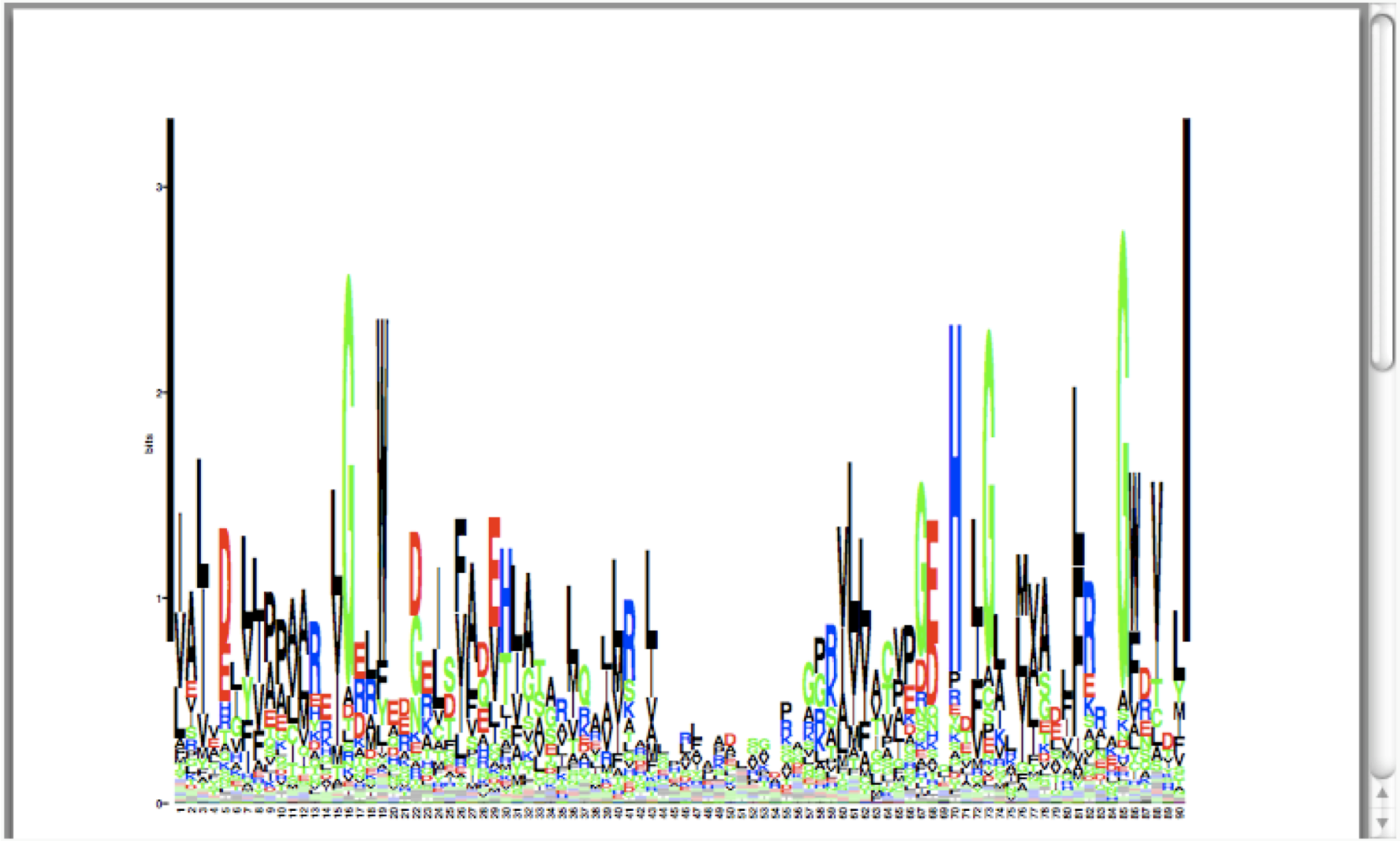
Plot Kullback-Leibler logo ☐

File format for logo file PDF

Submit Clear fields

Restrictions:
At most 1 sequences per submission; each sequence not more than 20,000 amino acids.

Blast2logo



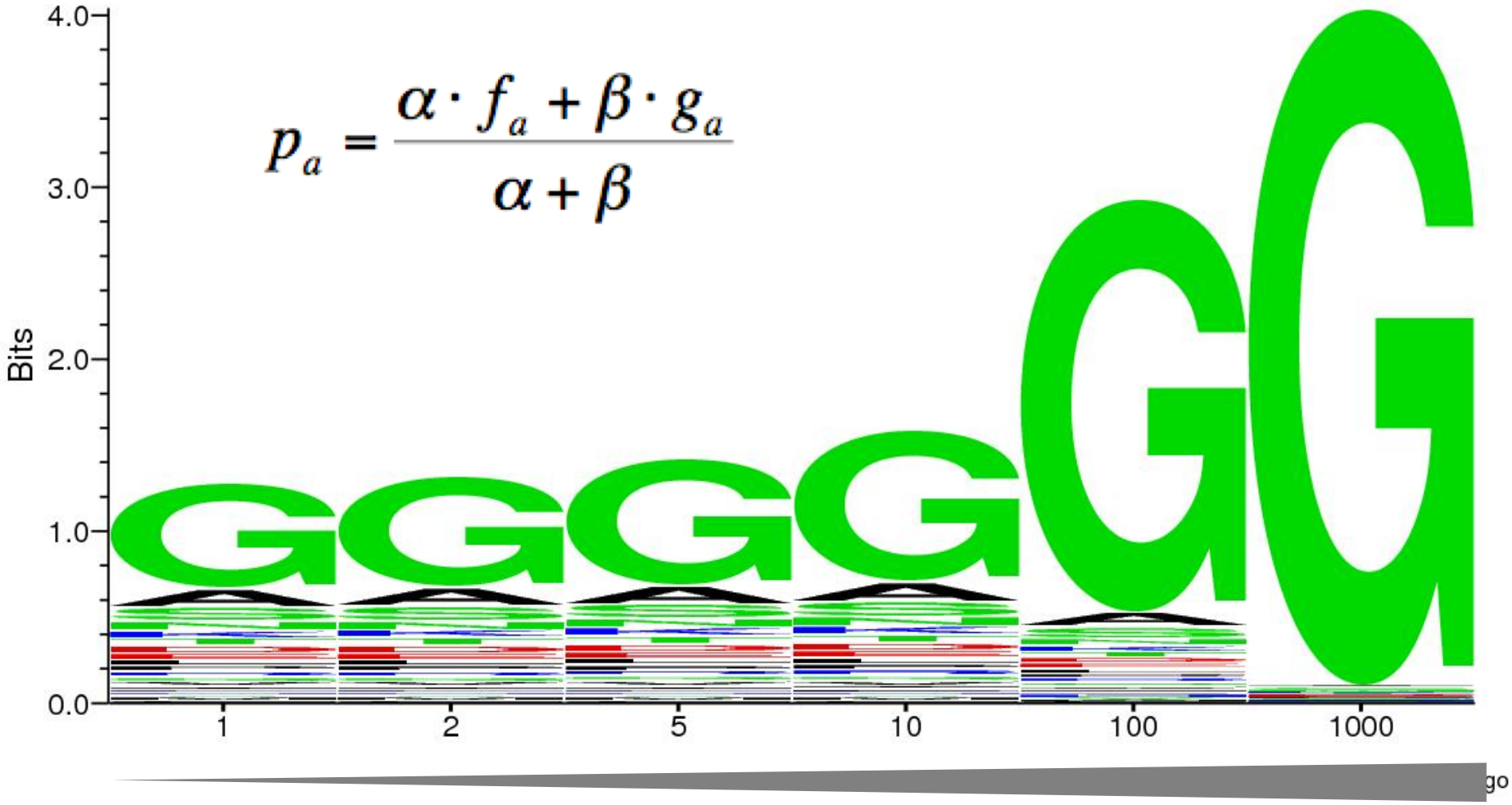
Blast2logo

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Last position-specific scoring matrix computed,

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

Sequence profiles or Gaining confidence



α

Example.

>1K7C.A
TTVYLAGDSTMAKNGGGSGTNGWGEYLASYLSATVVNDAVAGRSARSYTREGRFENIADV
VTAGDYVIVEFGHNDGGSLSTDNGRTDCSGTGAEVCYSVYDGVNETILTFPAYLENAAKL
FTAKGAKVILSSQTPNNPWETGTFVNSPTRFVEYAELAAEVAGVEYVDHWSYVDSIYETL
GNATVNSYFPIDHTHTSPAGAEVVAEAFLKAVVCTGTSLKSVLTTTSFEGTCL

- What is the function
- Where is the active site?

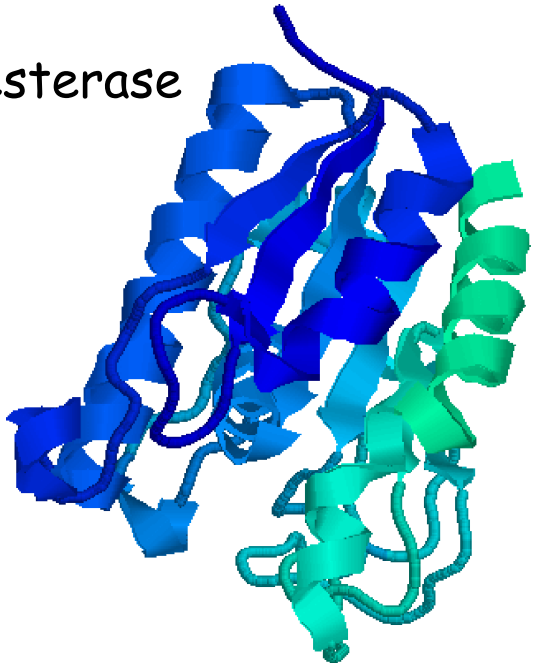
What would you do?

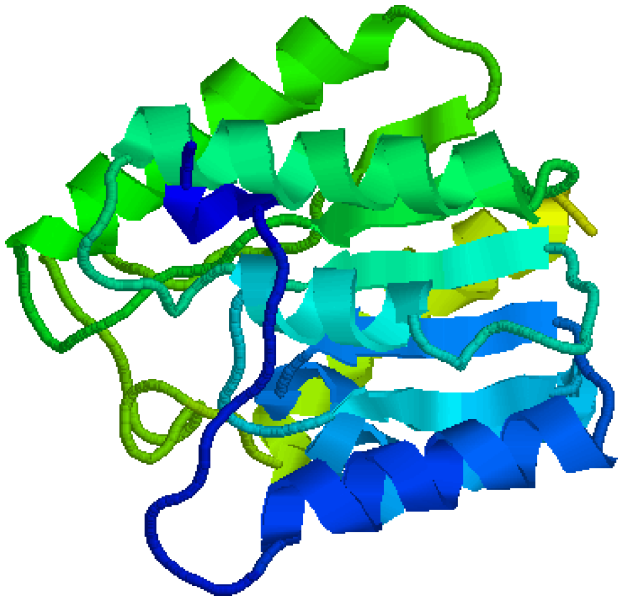
- Function
  - Run Blast against PDB
    - No significant hits
  - Run Blast against NR (Sequence database)
    - Function is Acetylesterase?
- Where is the active site?

Example. Where is the active site?

1USW Hydrolase

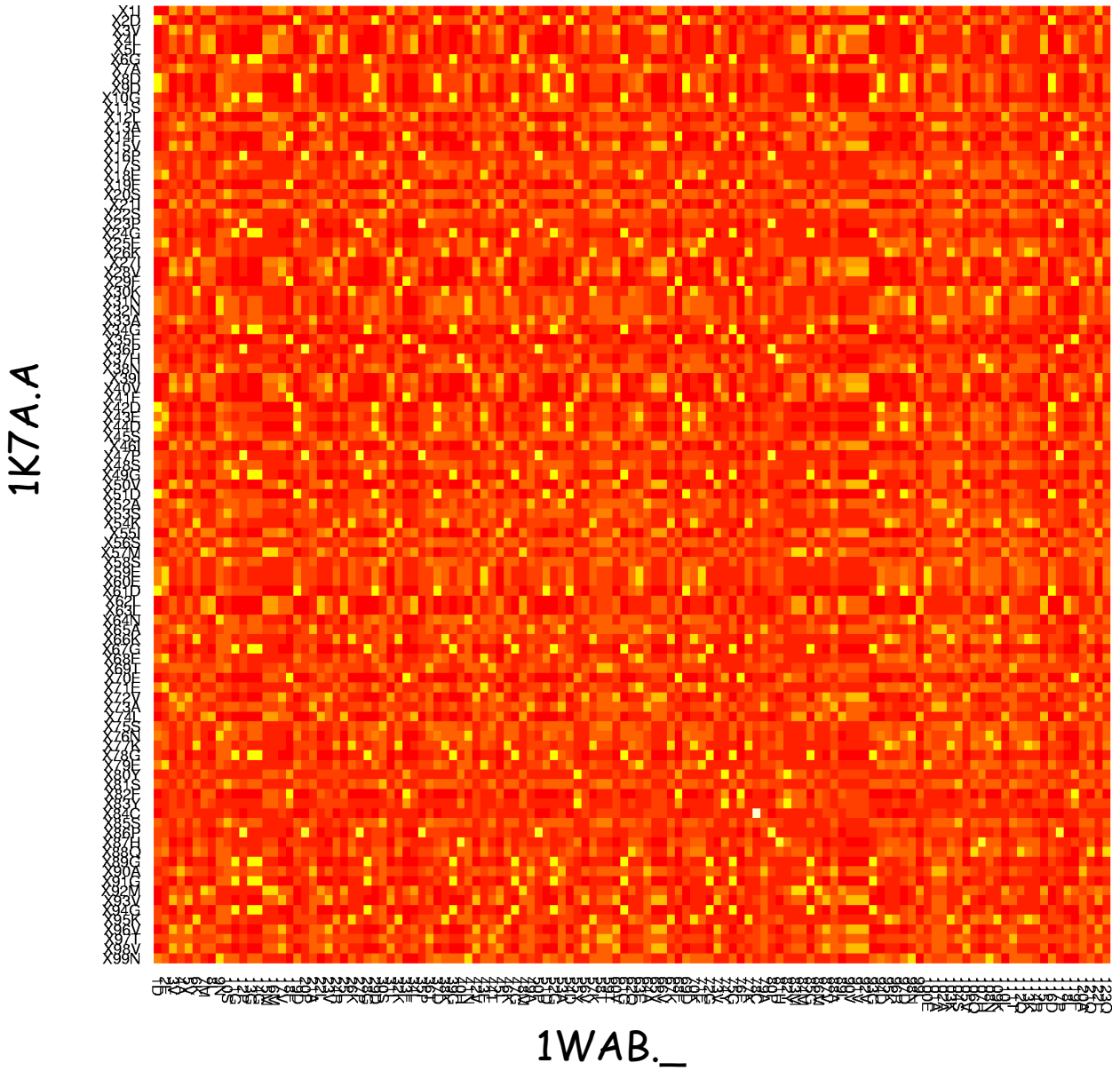
1G66 Acetylxylan esterase



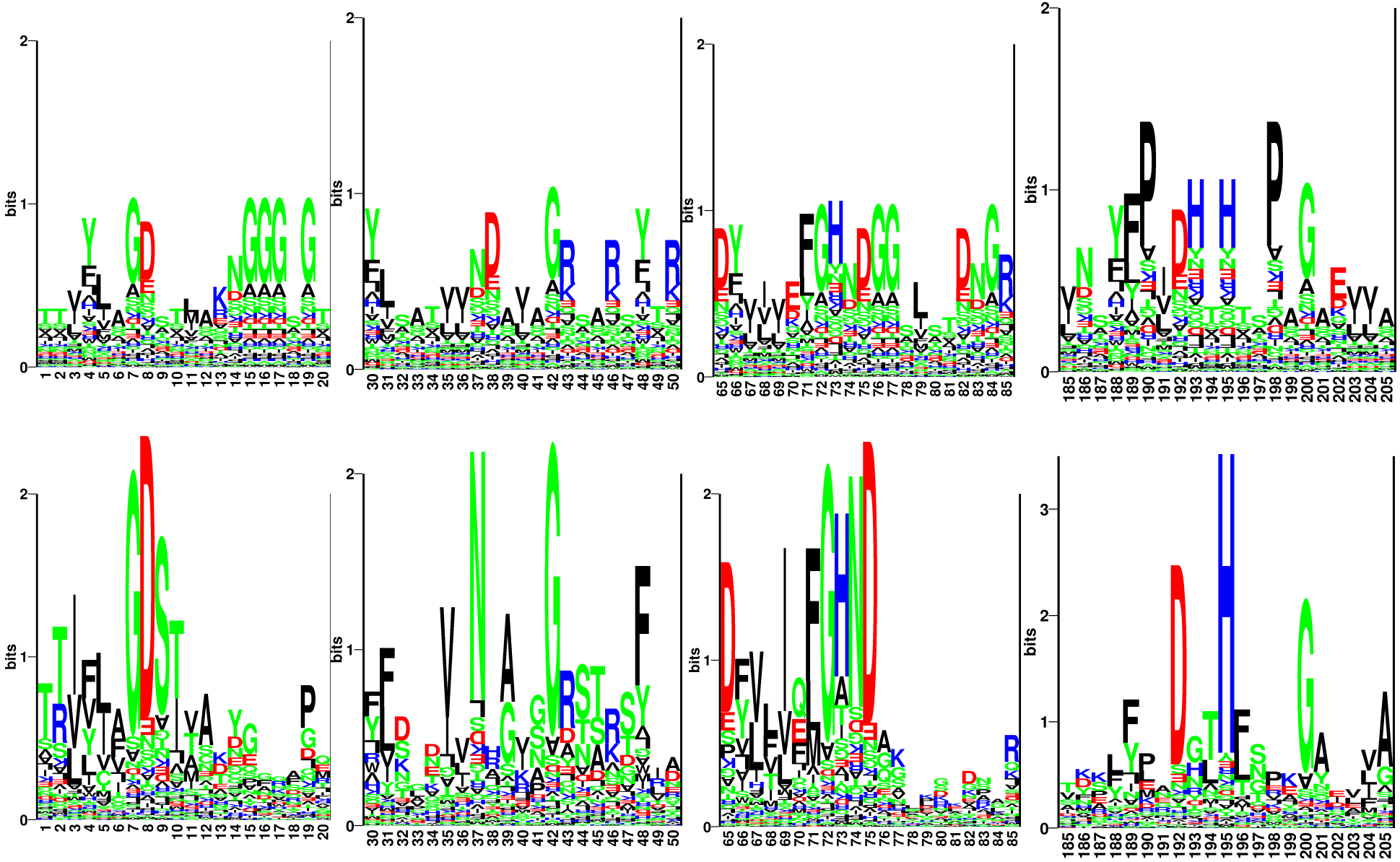


1WAB Acetylhydrolase

When Blast fails!

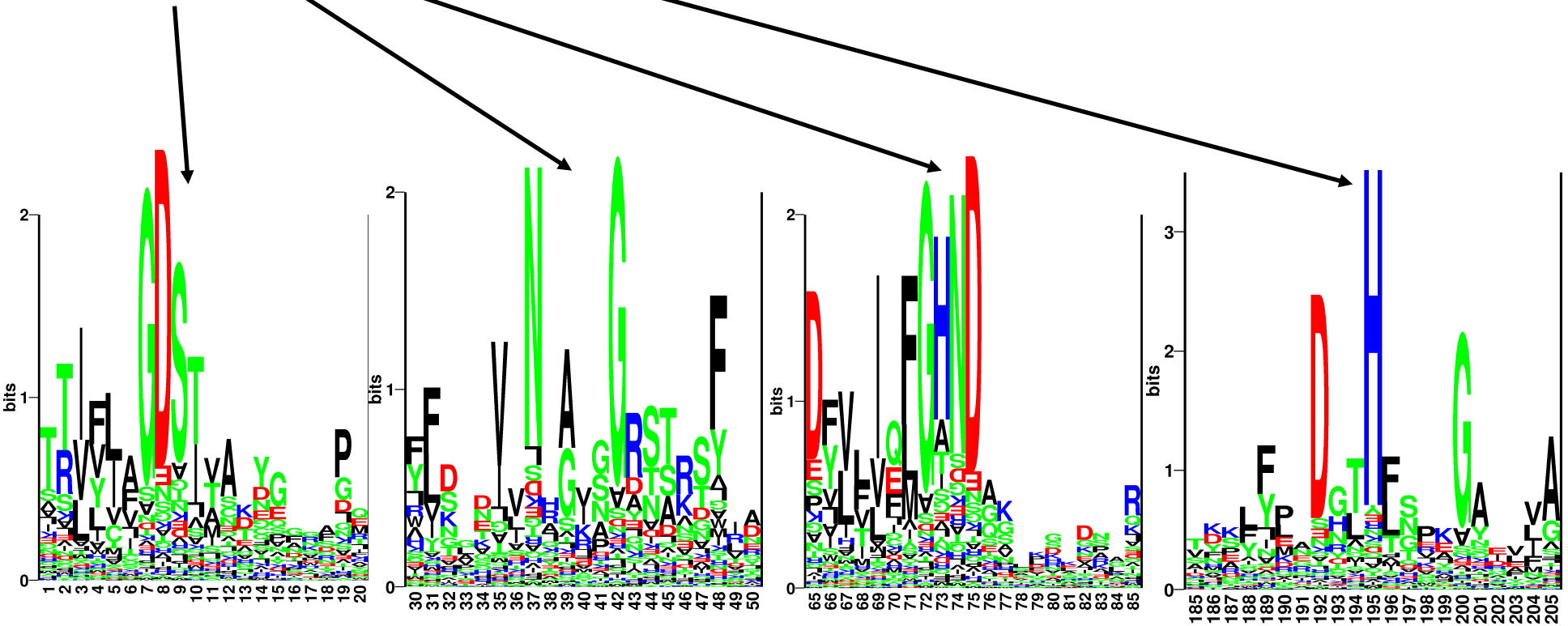


Example. (SGNH active site)

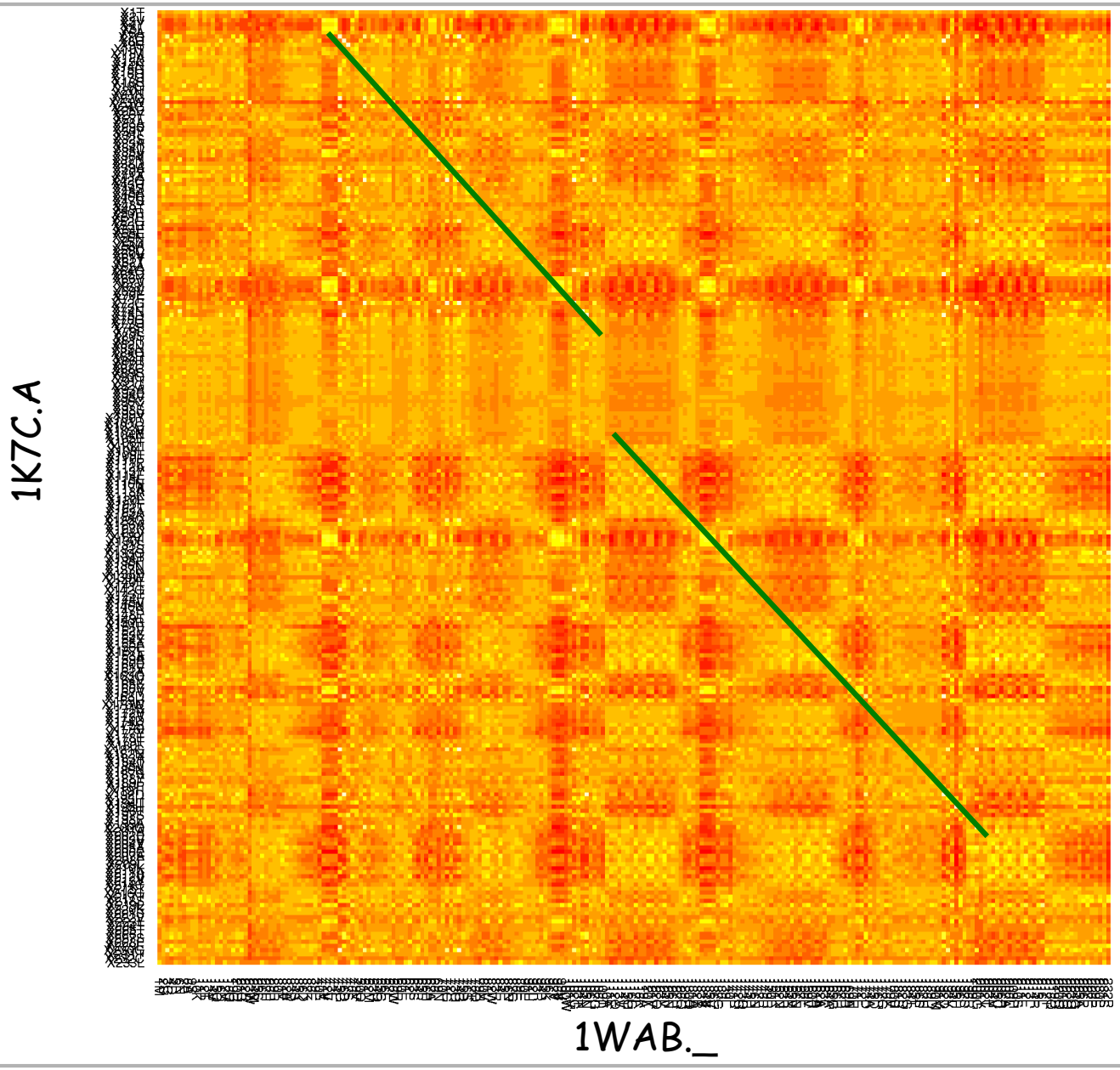


Example. Where is the active site?

- Sequence profiles might show you where to look!
- The active site could be around
  - S9, G42, N74, and H195



Profile-profile scoring matrix



Example. Where is the active site?

CENTERFO
RBIOLOGI
CALSEQU
ENCEANA
LYSIS CBS

Align using sequence profiles

ALN 1K7C.A 1WAB._ RMSD = 5.29522. 14% ID

```
1K7C.A TVYLAGDSTMAKNGGGSGTNGWGEYLASYLSATVVNDAVAGRSARSYTREGRFENIADVVTAGDYVIVEFGHNDGGSLSTDN
              S                                 G                                 N
1WAB._ EVVFIGDSLVQLMHQCE---IWRELFS---PLHALNFGIGGDSTQHVLW--RLENGELEHIRPKIVVVWVGTNNHG------

1K7C.A GRTDCSGTGAEVCYSVYDGVNETILTFPAYLENAAKLFTAK--GAKVILSSQTPNNPWETGTFVNSPTRFVEYAEL-AAEVA
1WAB._ ---------------------HTAEQVTGGIKAIVQLVNERQPQARVVVLGLLPRGQ-HPNPLREKNRRVNELVRAALAGHP

1K7C.A GVEYVDHWSYVDSIYETLGNATVNSYFPIDHTHTSPAGAEVVAEAFLKAVVCTGTSL
                                        H
1WAB._ RAHFLDADPG---FVHSDG--TISHHDMYDYLHLSRLGYTPVCRALHSLLLRL---L
```

Handout exercise

Using Psi-Blast Profiles

Sequence profiles take home message

- Blast will often fail to recognize sequence relationships for low homology sequence pairs
- Sequence profiles contain information on conserved/variable residues in a protein sequence
- Sequence profiles are calculated from (multiple) sequence alignments
- Iterative Blast enables homology recognition also for low sequence similarity
- Sequence profiles give information on residues essential for protein function and protein structure
- Can be used to predict impact of SNP's on protein function
  - This is often done using the Blosum matrix, but profiles are much more precise