Motifs, Patterns and Profiles

INDRAPRASTHA INSTITUTE *of* INFORMATION TECHNOLOGY **DELHI**

Dr. Jaspreet Kaur Dhanjal

Assistant Professor, Department of Computational Biology

Email ID: jaspreet@iiitd.ac.in

September 30, 2025

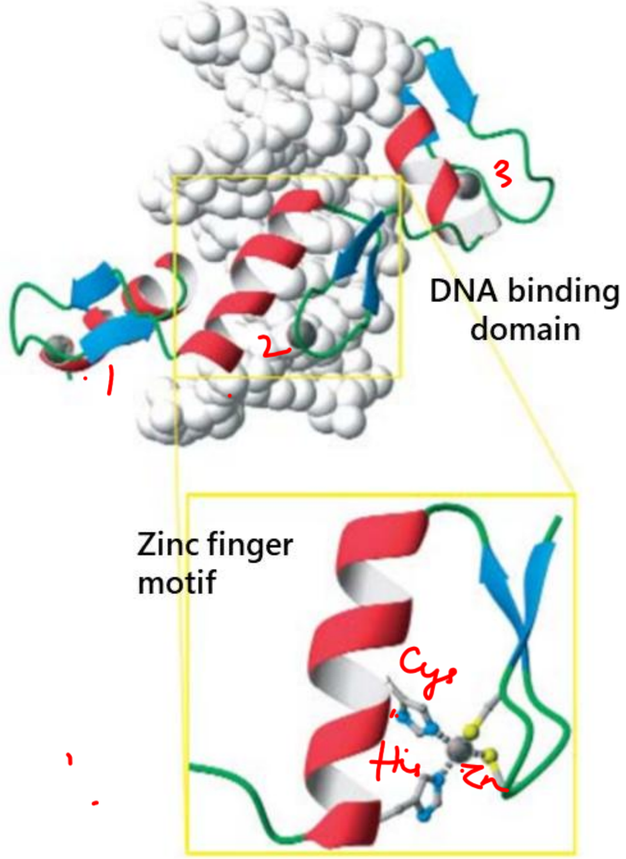
Motifs and Domains

Motifs (supersecondary structure)

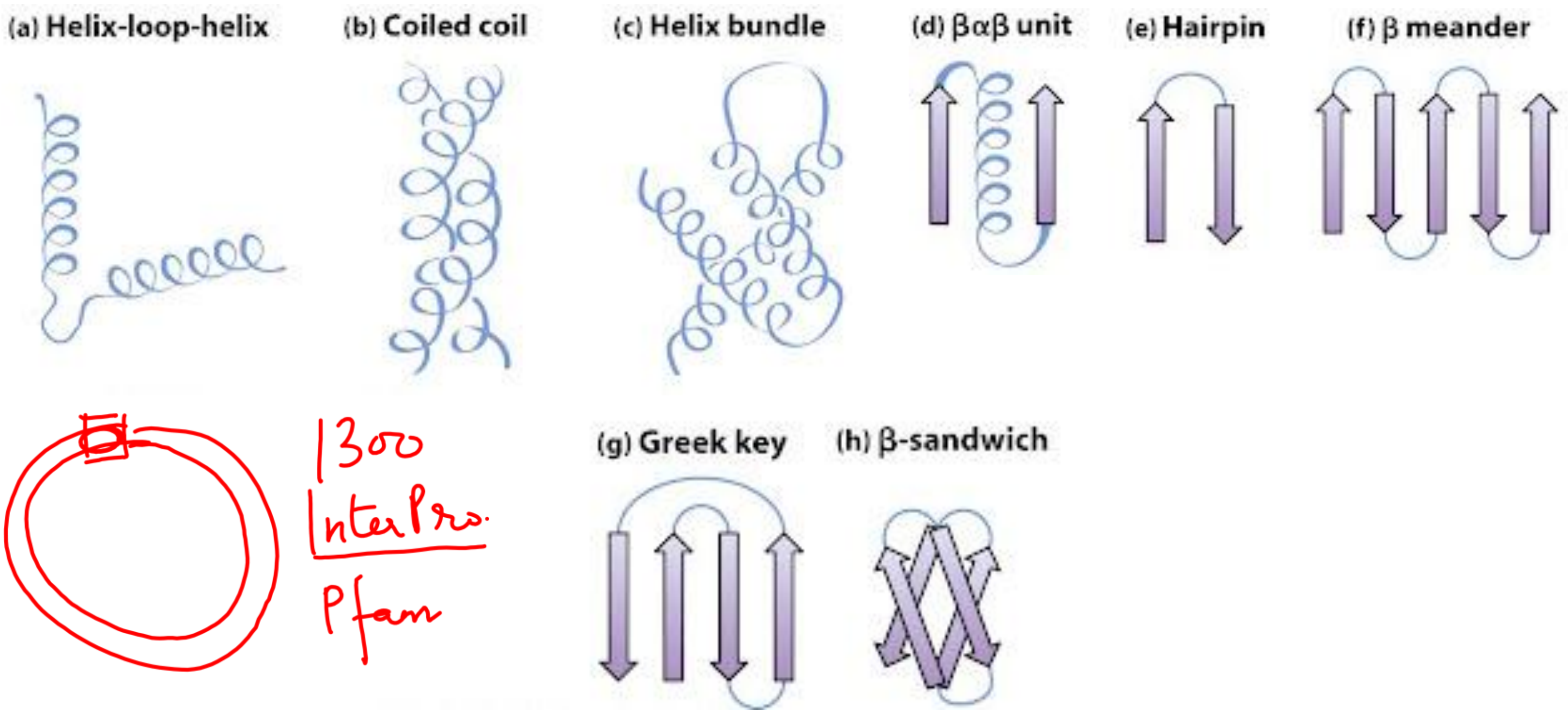
- Simple combinations of secondary structure
- Formed from consecutive sequences of primary structure
- Examples: helix-loop helix (EF hand), beta-alpha-beta, Greek key

Domains

- Stable, independently folded, globular units, often consisting of combinations of motifs
- Vary from 25 to 300 amino acids, average length – 100
- Large globular proteins may consist of several domains linked by stretches of polypeptide
- Separate domain may have distinct functions
- In many cases binding site formed by cleft between 2 domains



Common Motifs

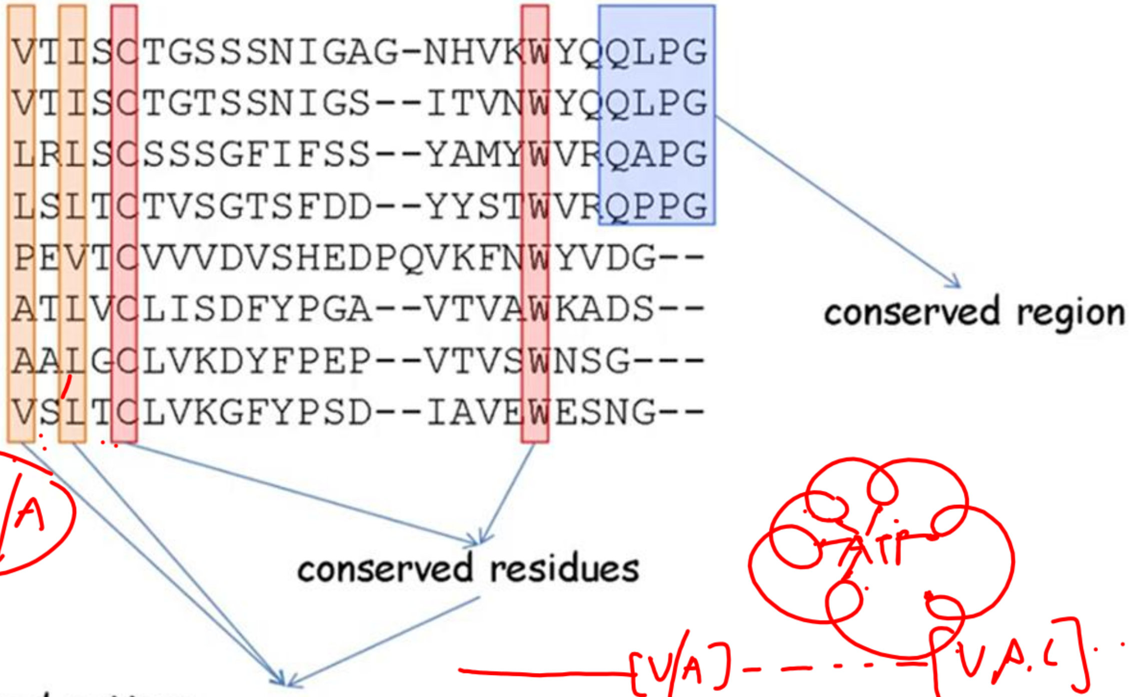


1300

InterPro

Pfam

Multiple sequence alignment



```
VTISCTGSSSNIGAG-NHVKWYQQLPG
VTISCTGTSSNIGS--ITVNWYQQLPG
LRLSCSSSGFIFSS--YAMYWVRQAPG
LSLTCTVSGTSFDD--YYSTWVRQPPG
PEVTCVVVDVSHEDPQVKFNWYVDG--
ATLVCLISDFYPGA--VTVAWKADS--
AALGCLVKDYFPEP--VTVSWNSG---
VSLTCLVKGFYPSD--IAVEWESNG--
```

conserved region

conserved residues

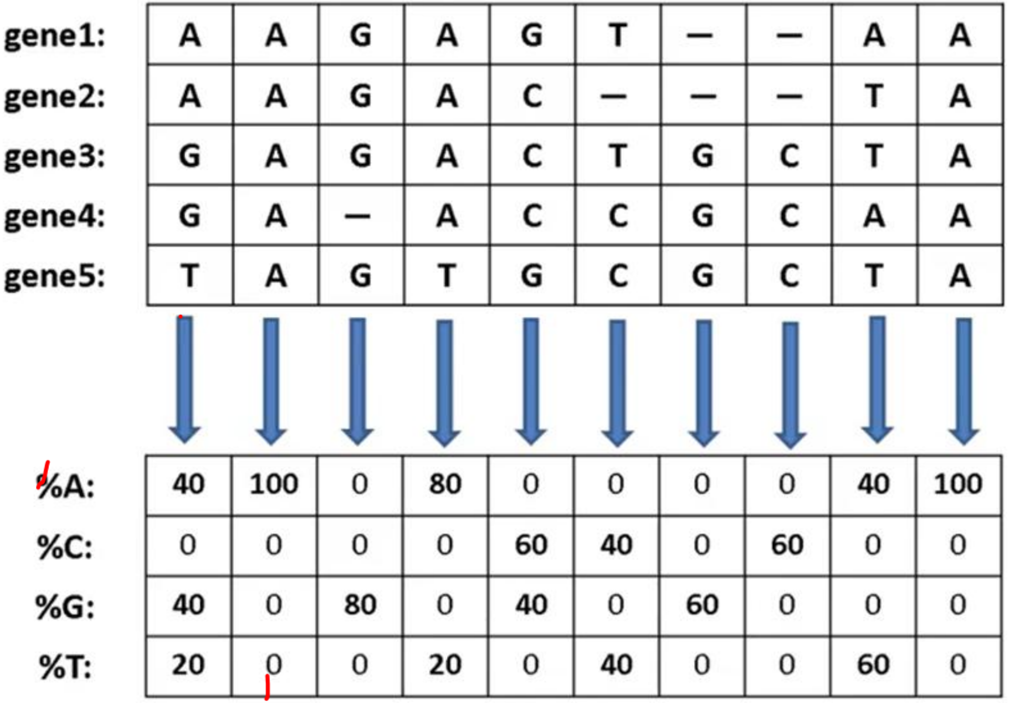
V/L/I/P/A

ATP

[V/A]- - - - -[V,A,L]· · · ·[H

conserved pattern
hydrophobic - x - hydrophobic - x - C - ... - W

Position specific scoring matrices (PSSM)



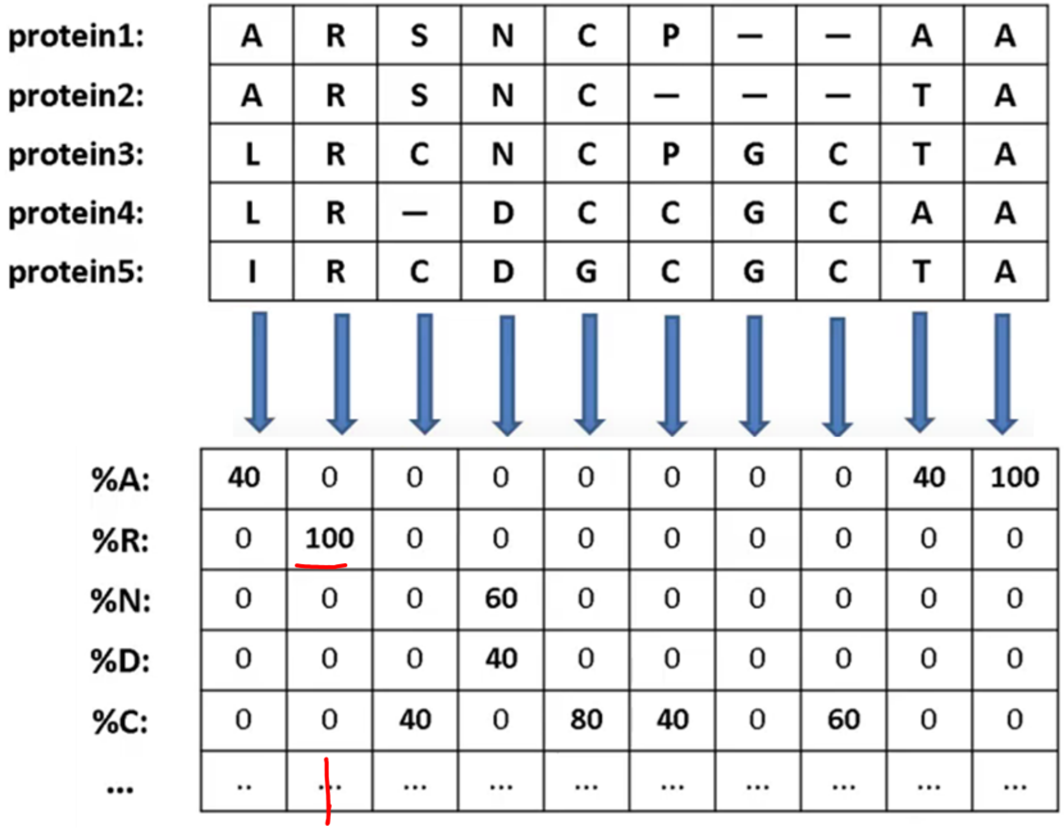
gene1:	A	A	G	A	G	T	–	–	A	A
gene2:	A	A	G	A	C	–	–	–	T	A
gene3:	G	A	G	A	C	T	G	C	T	A
gene4:	G	A	–	A	C	C	G	C	A	A
gene5:	T	A	G	T	G	C	G	C	T	A

%A:	40	100	0	80	0	0	0	0	40	100
%C:	0	0	0	0	60	40	0	60	0	0
%G:	40	0	80	0	40	0	60	0	0	0
%T:	20	0	0	20	0	40	0	0	60	0

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

- $n_{u,a}$: number of residues of type a at column u
- N_{seq}: number of sequences

Position specific scoring matrices (PSSM)

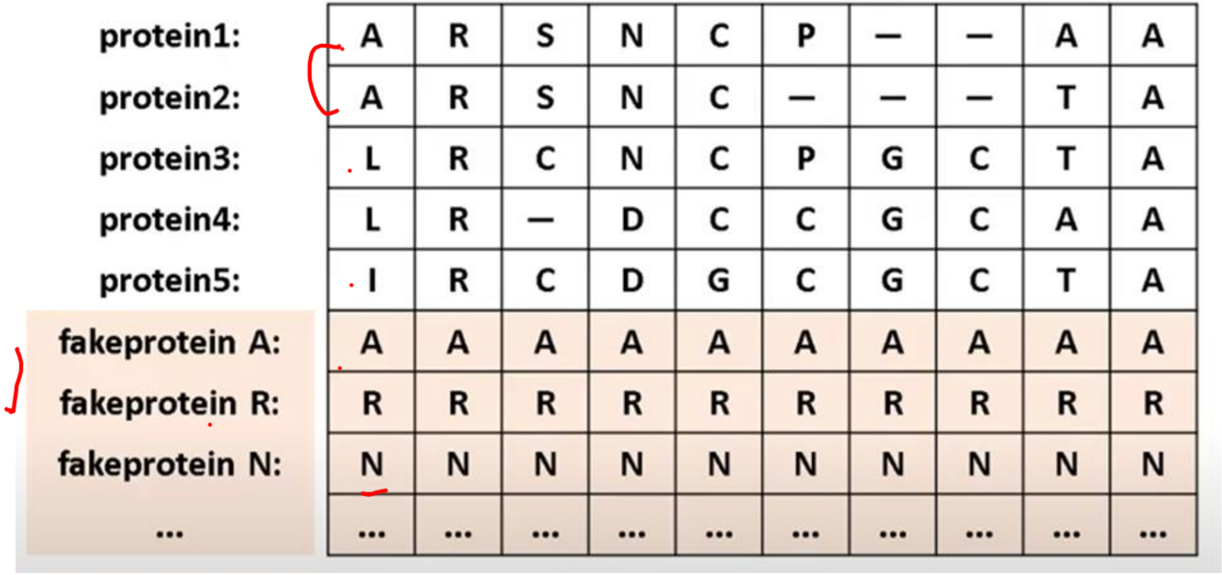


protein1:	A	R	S	N	C	P	—	—	A	A
protein2:	A	R	S	N	C	—	—	—	T	A
protein3:	L	R	C	N	C	P	G	C	T	A
protein4:	L	R	—	D	C	C	G	C	A	A
protein5:	I	R	C	D	G	C	G	C	T	A

%A:	40	0	0	0	0	0	0	0	40	100
%R:	0	100	0	0	0	0	0	0	0	0
%N:	0	0	0	60	0	0	0	0	0	0
%D:	0	0	0	40	0	0	0	0	0	0
%C:	0	0	40	0	80	40	0	60	0	0
...	..	...	...	...	...	...	...	...	...	...

Position specific scoring matrices (PSSM)

Pseudo-counts



protein1:	A	R	S	N	C	P	—	—	A	A
protein2:	A	R	S	N	C	—	—	—	T	A
protein3:	L	R	C	N	C	P	G	C	T	A
protein4:	L	R	—	D	C	C	G	C	A	A
protein5:	I	R	C	D	G	C	G	C	T	A
fakeprotein A:	A	A	A	A	A	A	A	A	A	A
fakeprotein R:	R	R	R	R	R	R	R	R	R	R
fakeprotein N:	N	N	N	N	N	N	N	N	N	N
...	...	...	...	...	...	...	...	...	...	...

Position specific scoring matrices (PSSM)

Correction for lack of or bias in data

- Use pseudocounts: $f'_{u,a} = \dfrac{n_{u,a} + 1}{N_{seq} + 20}$

- Use pseudocounts by background frequencies: $f'_{u,a} = \dfrac{n_{u,a} + \beta p_a}{N_{seq} + \beta}$

  - *Lower the contribution of pseudocounts or substitution matrix if there is enough data*

- Use of substitution matrix: $f'_{u,a} = \sum_b f_{u,b} s_{a,b}$

- Weight the sequence contributions
  - *Lower the weights of highly similar sequences*

Representing profiles using logos

- Entropy (uncertainty) in a column: $H_u = -\sum_a f_{u,a} \log_2(f_{u,a})$

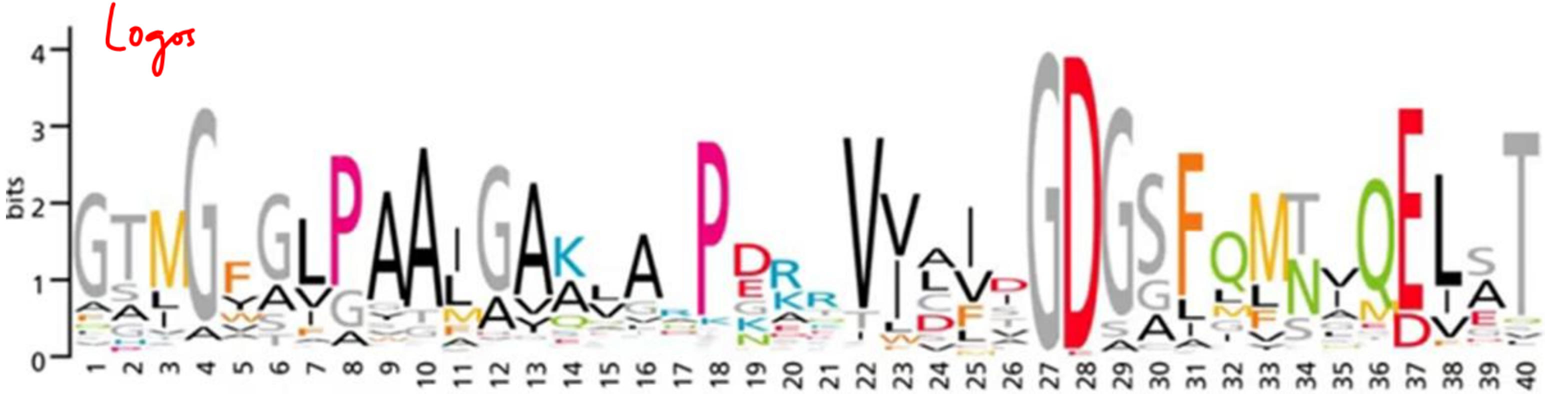
Protein

- Information: $I_u = \log_2 20 - H_u$

DNA: $\log_2 4$

- Contribution of a residue a: $I_{u,a} = f_{u,a} I_u$

Logos



Sequence Logo example

protein1:	A	R	S	N	C	P	–	–	A	A
protein2:	A	R	S	N	C	–	–	–	T	A
protein3:	L	R	C	N	C	P	G	C	T	A
protein4:	L	R	–	D	C	C	G	C	A	A
protein5:	I	R	C	D	G	C	G	C	T	A

%A:	40	0	0	0	0	0	0	0	40	100
%R:	0	100	0	0	0	0	0	0	0	0
%N:	0	0	0	60	0	0	0	0	0	0
%D:	0	0	0	40	0	0	0	0	0	0
%C:	0	0	40	0	80	40	0	60	0	0
...	..	...	...	...	...	...	...	...	...	...

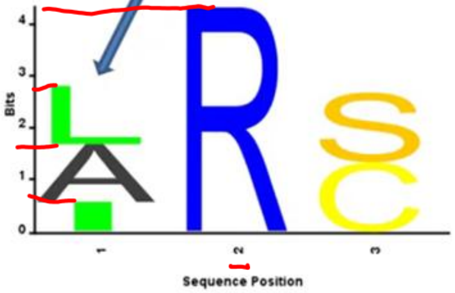
%L
I

20%

$$I_{1,A} = 0.4 * 2.8 = 1.12$$
$$I_{1,L} = 0.4 * 2.8 = 1.12$$
$$I_{1,I} = 0.2 * 2.8 = 0.56$$

$$\begin{aligned} H_1 &= -(\ f_A * \log_2 f_A\ +\ f_L * \log_2 f_L\ +\ f_I * \log_2 f_I\ ) \\ &= -(\ 0.4 * \log_2 0.4\ +\ 0.4 * \log_2 0.4\ +\ 0.2 * \log_2 0.2\ ) \\ &= 1.52 \end{aligned}$$

$$I_1 = \log_2 \underline{20} - H_1 = \boxed{\mathbf{2.8}}$$



Prosite Patterns

- [AC]-x-V-x(4)-{ED}
  - [Ala or Cys]-any-Val-any-any-any-any-{any but Glu or Asp}
- <A-x-[ST](2)-x(0,1)-V
  - Nterminal Ala-any-[Ser or Thr]-[Ser or Thr]-(any or none)-Val

<{C}* >

V/P/A/L/W

[VPALW]

Prosite	Regular Expression	
x	.	any character
[ALT]	[ALT]	any of A, L, or T
{AM}	[^AM]	anything but A or M
A(3)	A{3}	AAA
A(2,4)	A{2,4}	AA, or AAA, or AAAA
<A	^A	A at the N-terminus
A>	A$	A at the C-terminus

Φ

4

VLWAPR

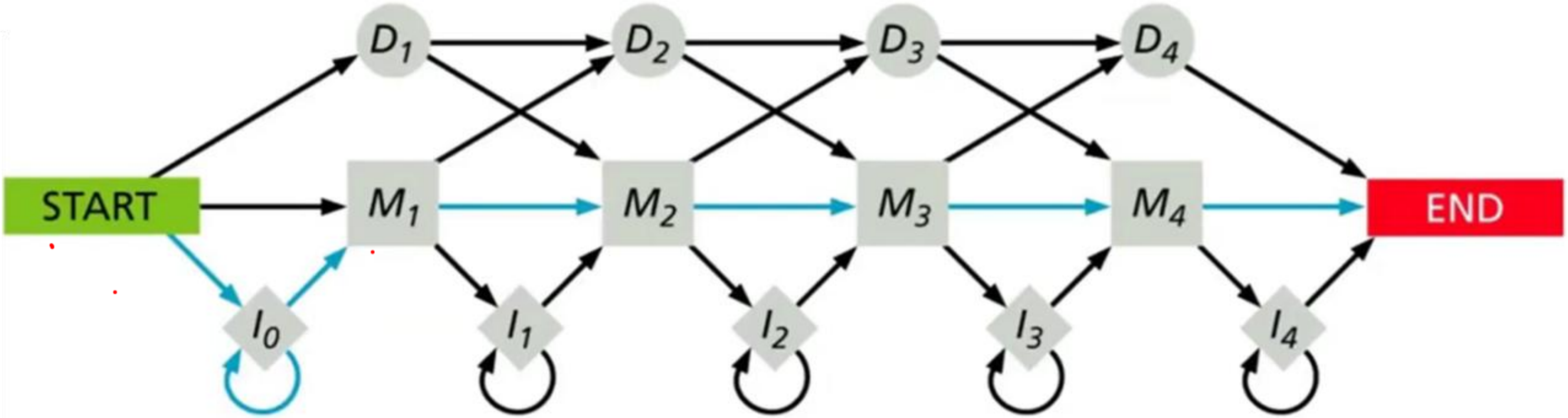
VLWAA.PR

VLW~~AAA~~PR

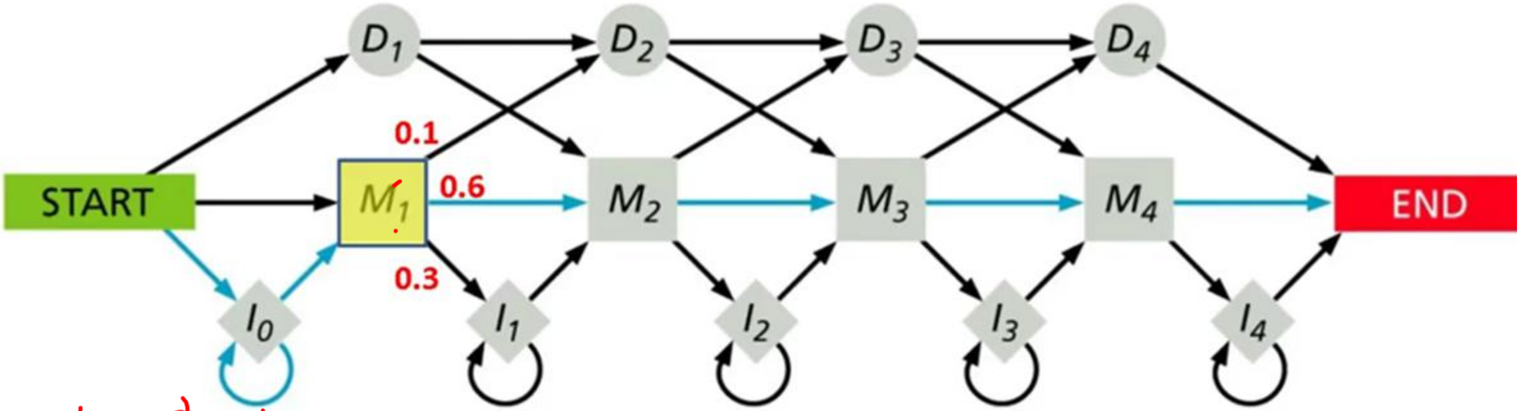
VLW~~AAAAPR~~

H
NH_2
C
COOH
R
NH_2
H_2O

Hidden Markov Model (HMM)

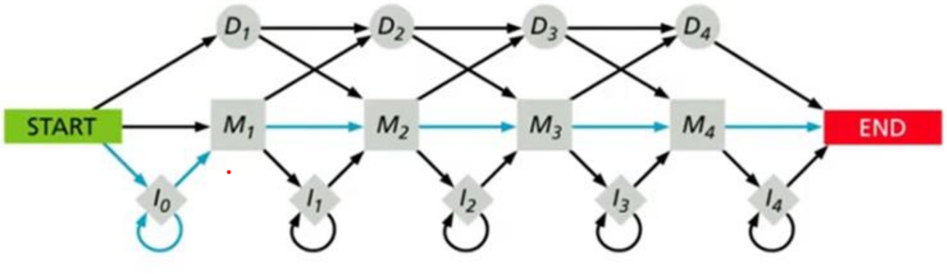


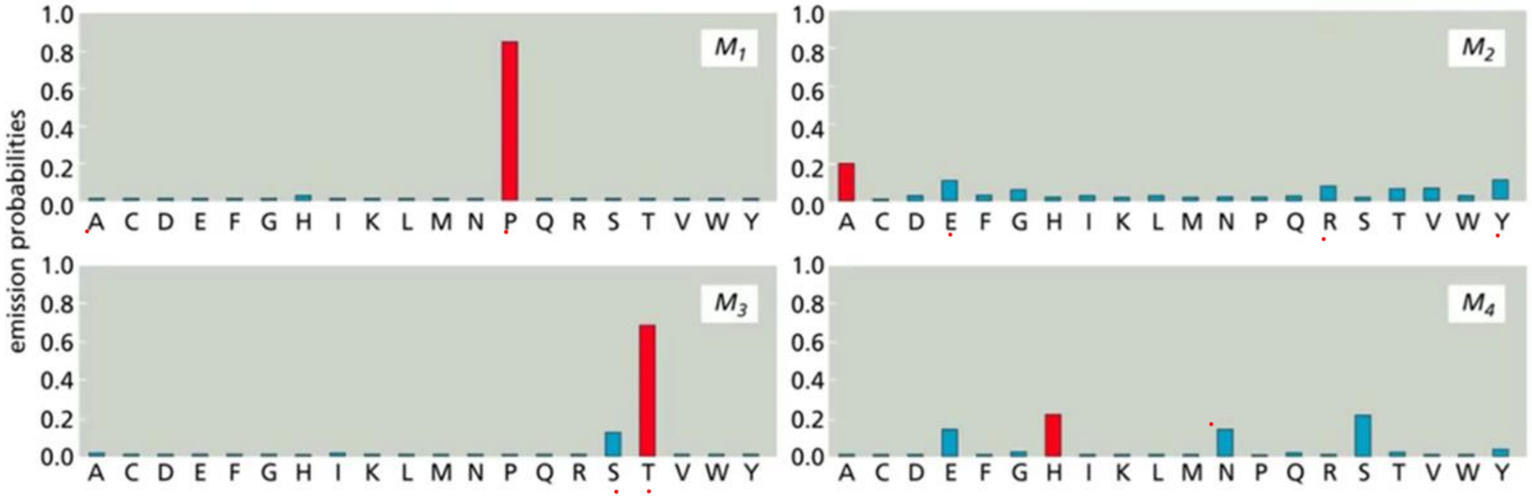
Hidden Markov Model (HMM)



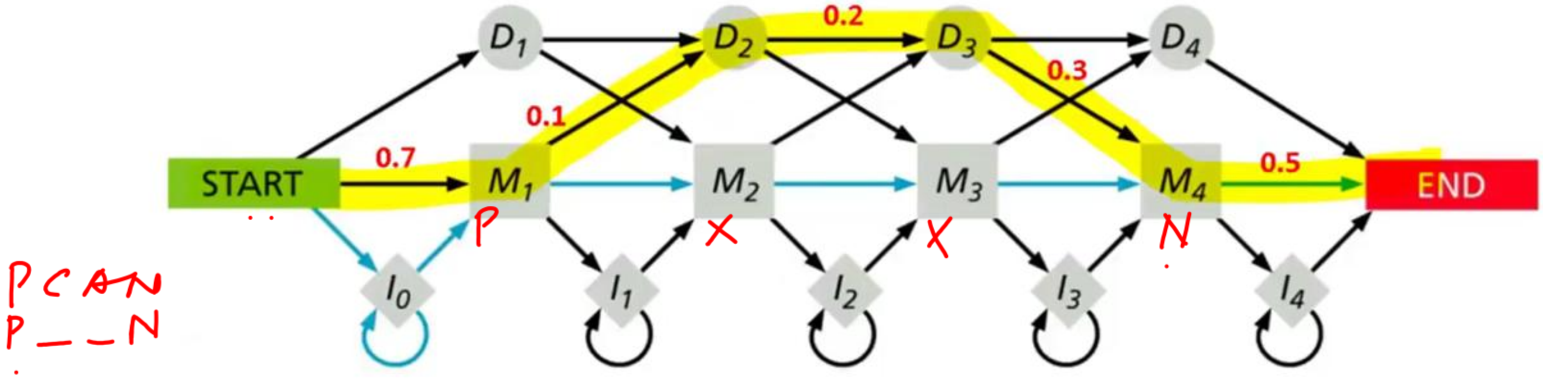
1	2	3		4
C	T	G	A	G
C	T	–	–	G
C	T	G	–	G

Hidden Markov Model (HMM)





Hidden Markov Model (HMM)



- $M_{1,P} = 0.9$, $M_{4,N} = 0.2$
- Path probability = $\underline{0.7}$*$\underline{0.9}$*$\underline{0.1}$*$\underline{0.2}$*$\underline{0.3}$*$\underline{0.2}$*$\underline{0.5}$ = 3.8e-04
- Sequence produced: PN

HMM questions

- What is the most likely path?
- What is the probability of a sequence being produced?
- How do we construct the HMM and identify its parameters?

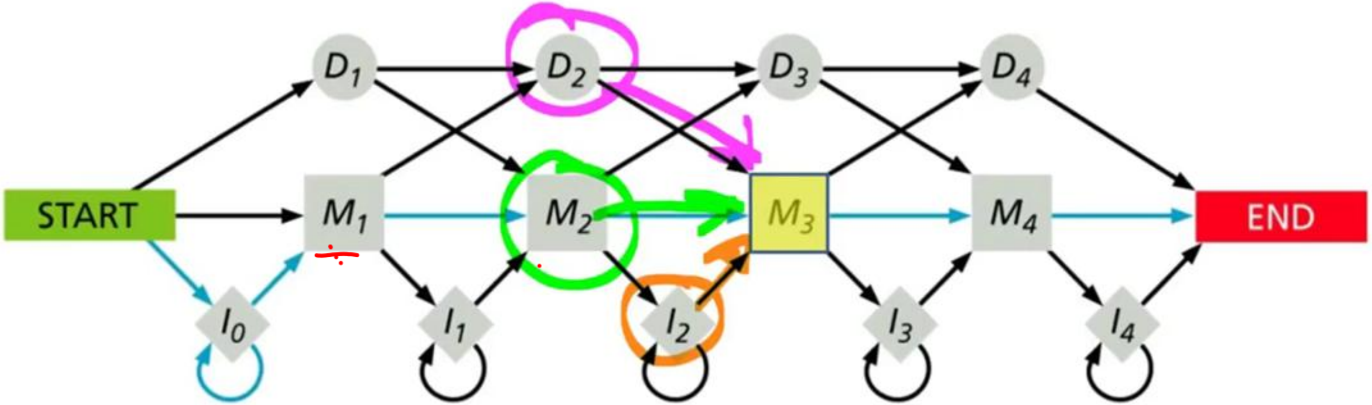
HMM answers

- Dynamic programming: Probability of a node can be decomposed into probabilities of transitioning into it from previous states.
- The most likely path

  - Viterbi algorithm

  - max() of each previous path

Viterbi algorithm



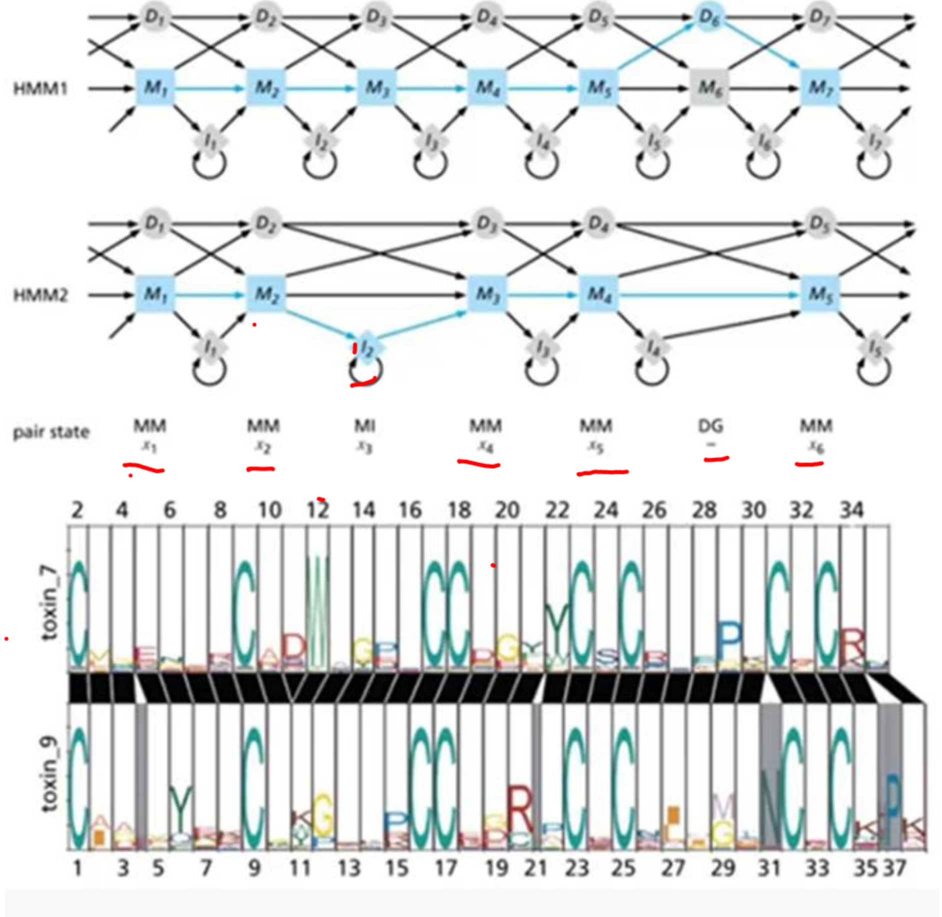
H_{M_3}, most likely path into M3:

$$
max \begin{cases} H_{D_2} * T_{D_2 \to M_3} \\ H_{M_2} * T_{M_2 \to M_3} \\ H_{I_2} * T_{I_2 \to M_3} \end{cases}
$$

Aligning Families

- Two HMMs can be aligned

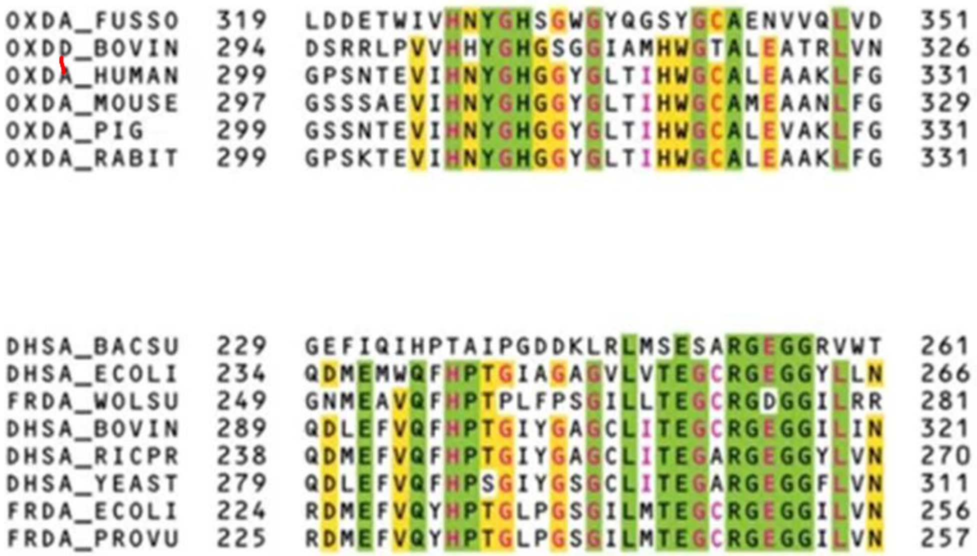
  - COACH, HHSEARCH programs



Aligning Families

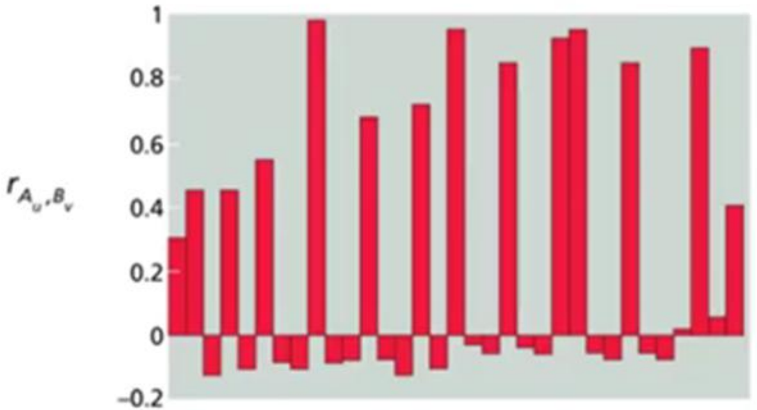
- Two PSSMs can be compared using Pearson correlation coefficient

  - LAMA program



```
OXDA_FUSSO   319   LDDETWIVHNYGHSGWGYQGSYGCAENVVQLVD   351
OXDD_BOVIN   294   DSRRLPVVHHYGHGSGGIAMHWGTALEATRLVN   326
OXDA_HUMAN   299   GPSNTEVIHNYGHGGYGLTIHWGCALEAAKLFG   331
OXDA_MOUSE   297   GSSSAEVIHNYGHGGYGLTIHWGCAMEAANLFG   329
OXDA_PIG     299   GSSNTEVIHNYGHGGYGLTIHWGCALEVAKLFG   331
OXDA_RABIT   299   GPSKTEVIHNYGHGGYGLTIHWGCALEAAKLFG   331

DHSA_BACSU   229   GEFIQIHPTAIPGDDKLRLMSESARGEGGRVWT   261
DHSA_ECOLI   234   QDMEMWQFHPTGIAGAGVLVTEGCRGEGGYLLN   266
FRDA_WOLSU   249   GNMEAVQFHPTPLFPSGILLTEGCRGDGGILRR   281
DHSA_BOVIN   289   QDLEFVQFHPTGIYGAGCLITEGCRGEGGILIN   321
DHSA_RICPR   238   QDMEFVQFHPTGIYGAGCLITEGARGEGGYLVN   270
DHSA_YEAST   279   QDLEFVQFHPSGIYGSGCLITEGARGEGGFLVN   311
FRDA_ECOLI   224   RDMEFVQYHPTGLPGSGILMTEGCRGEGGILVN   256
FRDA_PROVU   225   RDMEFVQYHPTGLPGSGILMTEGCRGEGGILVN   257
```

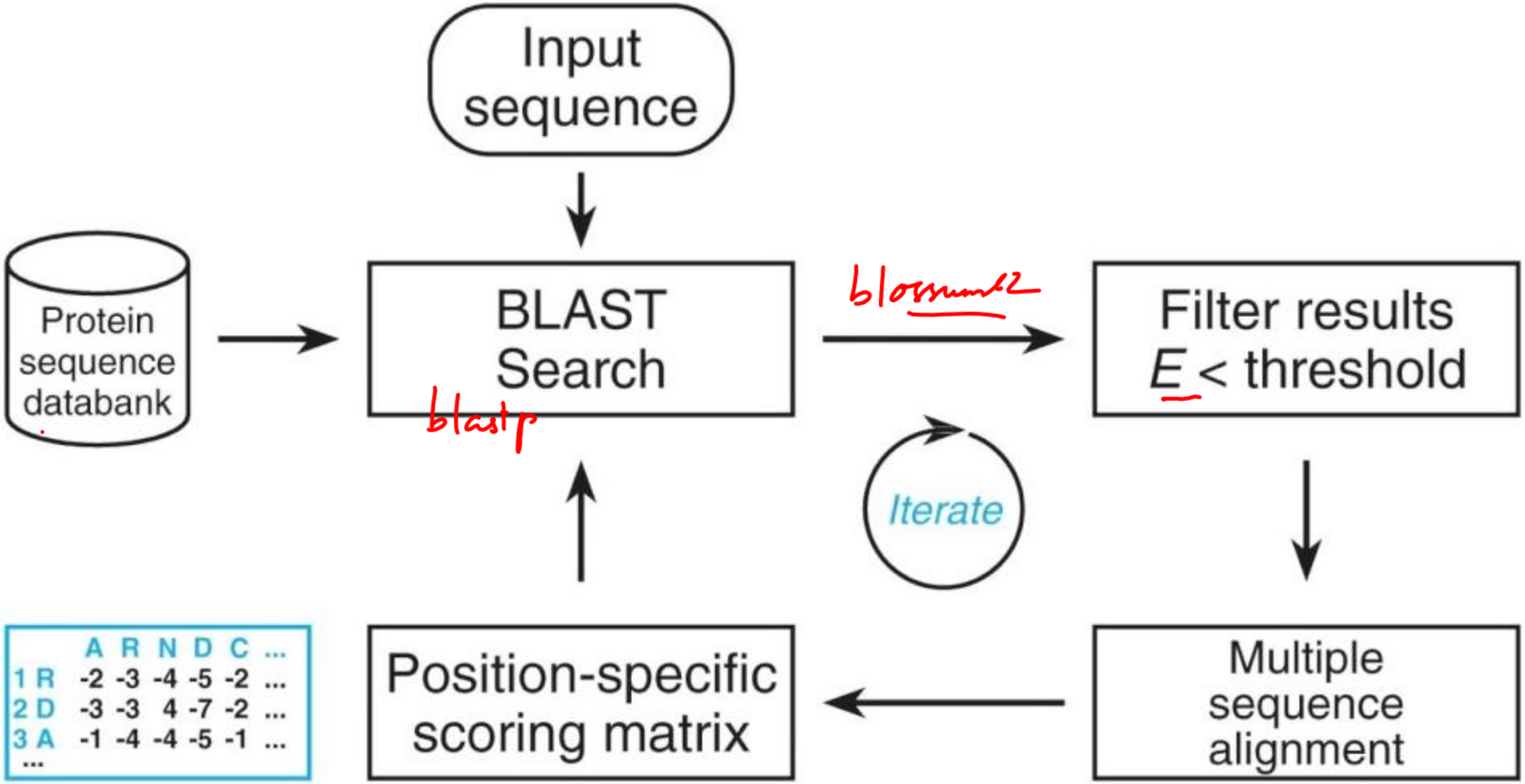


Position-Specific Iterative BLAST (PSI-BLAST)

PSI-BLAST principle

1. A standard BLAST search is performed against a database using a substitution matrix (e.g. BLOSUM62).
2. A PSSM (checkpoint) is constructed automatically from a multiple alignment of the highest scoring hits of the initial BLAST search. High conserved positions receive high scores and weakly conserved positions receive low scores.
3. The PSSM replaces the initial matrix (e.g. BLOSUM62) to perform a second BLAST search.
4. Steps 3 and 4 can be repeated and the new found sequences included to build a new PSSM.
5. We say that the PSI-BLAST has converged if no new sequences are included in the last cycle.

Position-Specific Iterative BLAST (PSI-BLAST)



Input sequence

Protein sequence databank

BLAST Search

blastp

blosum62

Filter results

E < threshold

Iterate

Multiple sequence alignment

Position-specific scoring matrix

		A	R	N	D	C	...
1	R	-2	-3	-4	-5	-2	...
2	D	-3	-3	4	-7	-2	...
3	A	-1	-4	-4	-5	-1	...
...							

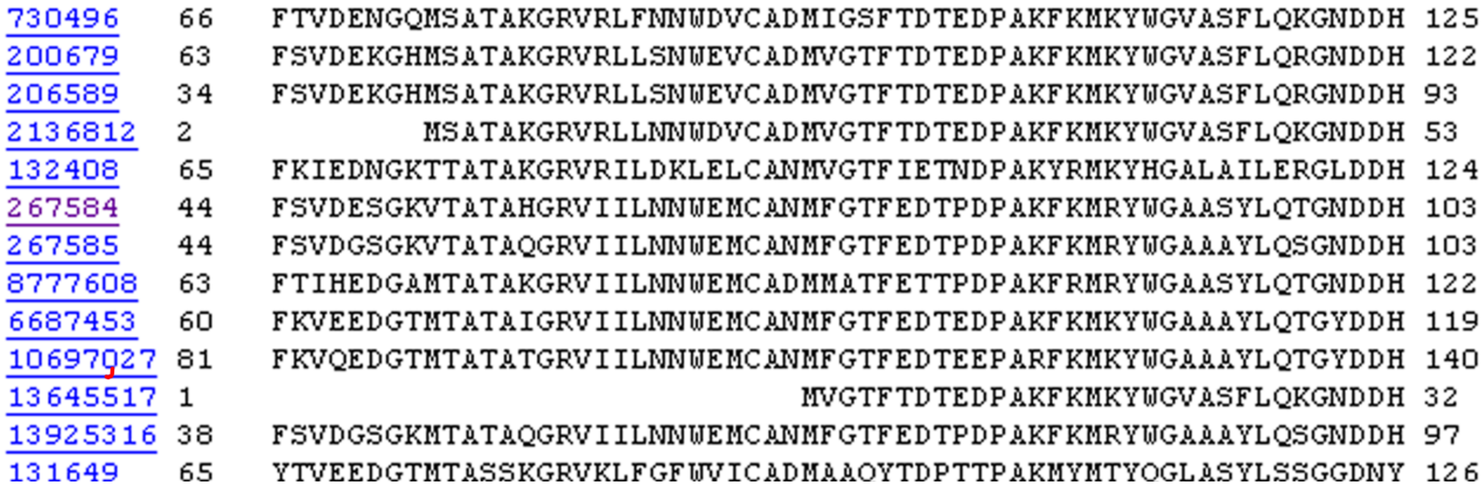
PSI-BLAST

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 is performed in five steps

[1] Select a query and search it against a protein database

PSI-BLAST



```
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  YTVEEDGTMTASSKGRVKLFGFWVICADMAAOYTDPTTPAKMYMTYOGLASYLSSGGDNY 126
```

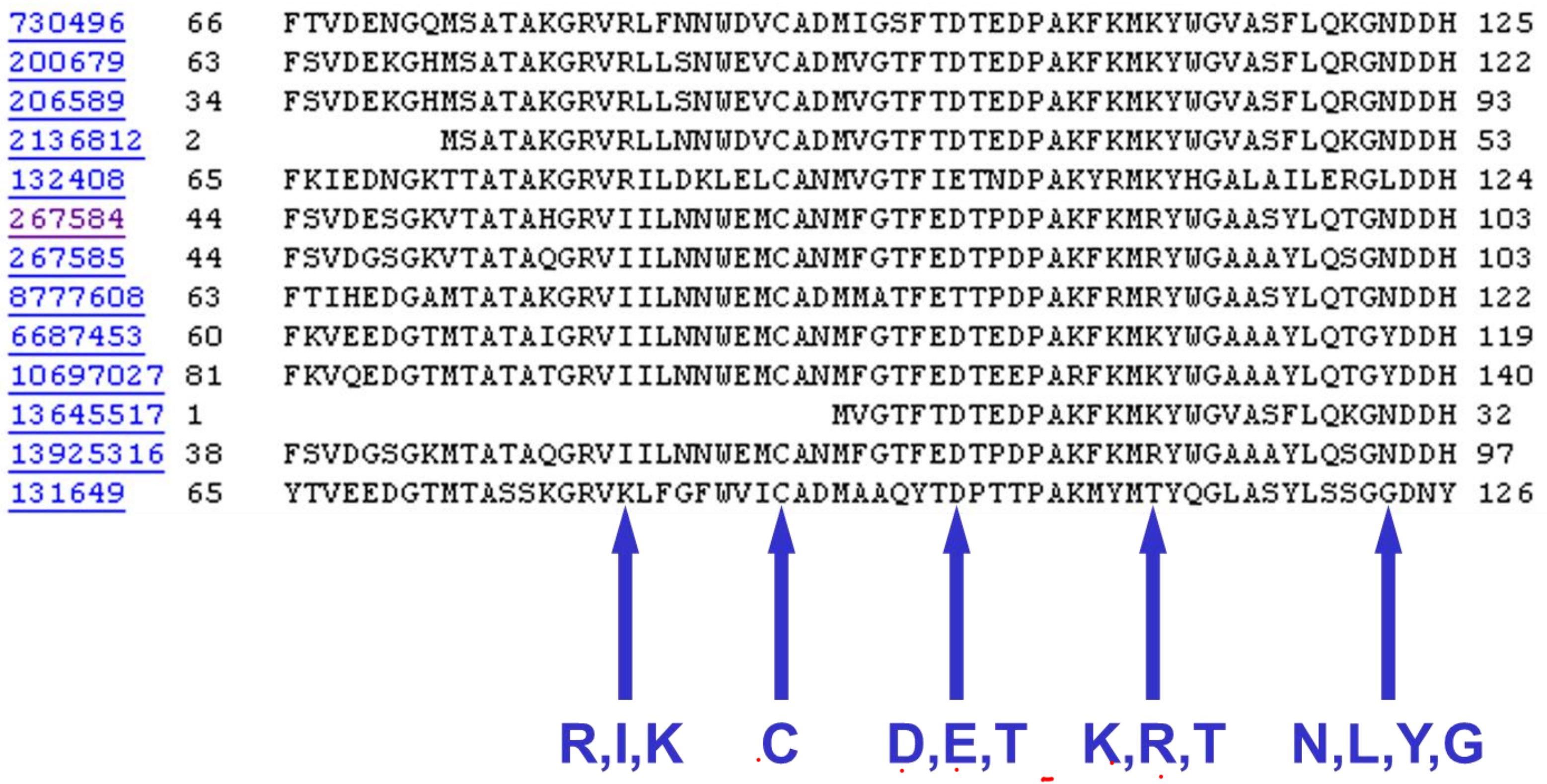
PSI-BLAST

PSI-BLAST is performed in five steps

[1] Select a query and search it against a protein database

[2] PSI-BLAST constructs a multiple sequence alignment then creates a “profile” or specialized position-specific scoring matrix (PSSM)

PSI-BLAST



```
730496   66  FTVDENGQMSATAKGRVRLFNNWDVCADMIGSFTDTEDPAKFKMKYWGVASFLQKGNDDH 125
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8777608  63  FTIHEDGAMTATAKGRVIILNNWEMCADMMATFETTPDPAKFRMRYWGAASYLQTGNDDH 122
6687453  60  FKVEEDGTMTATAIGRVIILNNWEMCANMFGTFEDTEDPAKFKMKYWGAAAYLQTGYDDH 119
10697027 81  FKVQEDGTMTATATGRVIILNNWEMCANMFGTFEDTEEPARFKMKYWGAAAYLQTGYDDH 140
13645517 1                               MVGTFTDTEDPAKFKMKYWGVASFLQKGNDDH 32
13925316 38  FSVDGSGKMTATAQGRVIILNNWEMCANMFGTFEDTPDPAKFKMRYWGAAAYLQSGNDDH 97
131649   65  YTVEEDGTMTASSKGRVKLFGFWVICADMAAQYTDPTTPAKMYMTYQGLASYLSSGGDNY 126
```
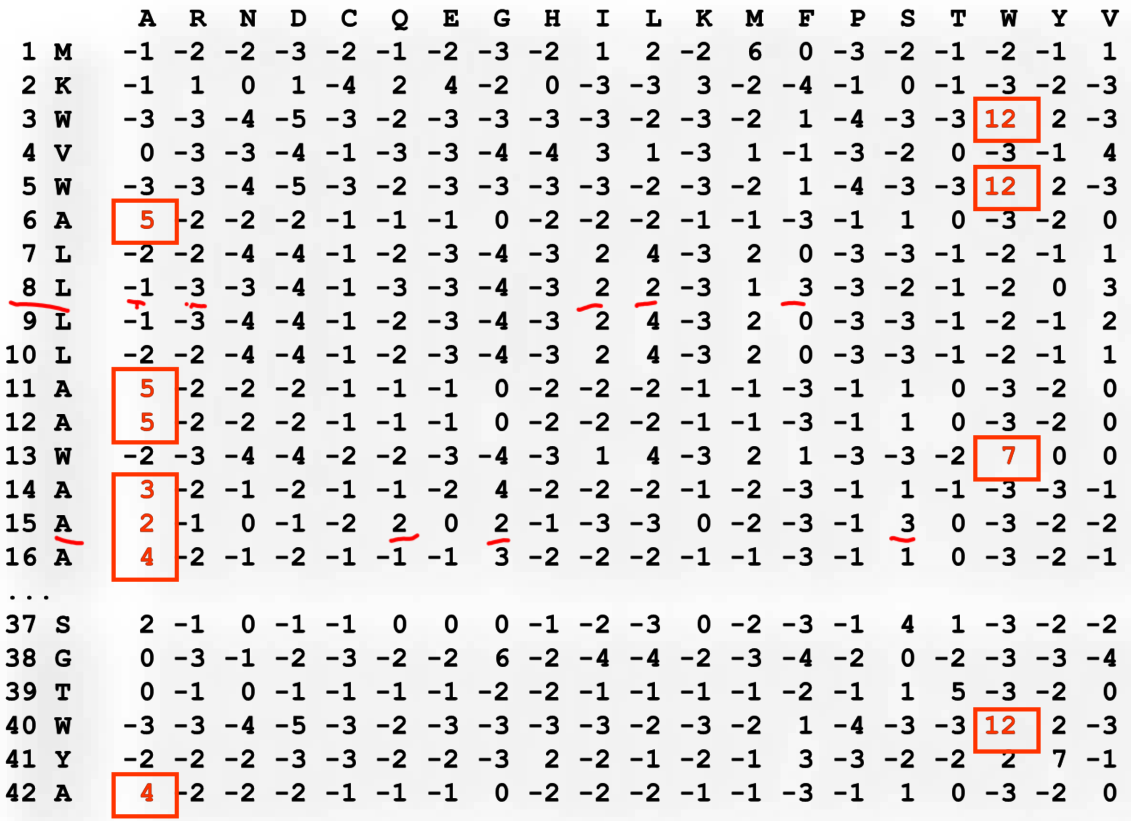

PSI-BLAST



		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	-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	-3	-4	-4	-2	-2	-3	-4	-3	1	4	-3	2	1	-3	-3	-2	7	0	0
14	A	3	-2	-1	-2	-1	-1	-2	4	-2	-2	-2	-1	-2	-3	-1	1	-1	-3	-3	-1
15	A	2	-1	0	-1	-2	2	0	2	-1	-3	-3	0	-2	-3	-1	3	0	-3	-2	-2
16	A	4	-2	-1	-2	-1	-1	-1	3	-2	-2	-2	-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	12	2	-3
41	Y	-2	-2	-2	-3	-3	-2	-2	-3	2	-2	-1	-2	-1	3	-3	-2	-2	2	7	-1
42	A	4	-2	-2	-2	-1	-1	-1	0	-2	-2	-2	-1	-1	-3	-1	1	0	-3	-2	0

PSI-BLAST

PSI-BLAST is performed in five steps

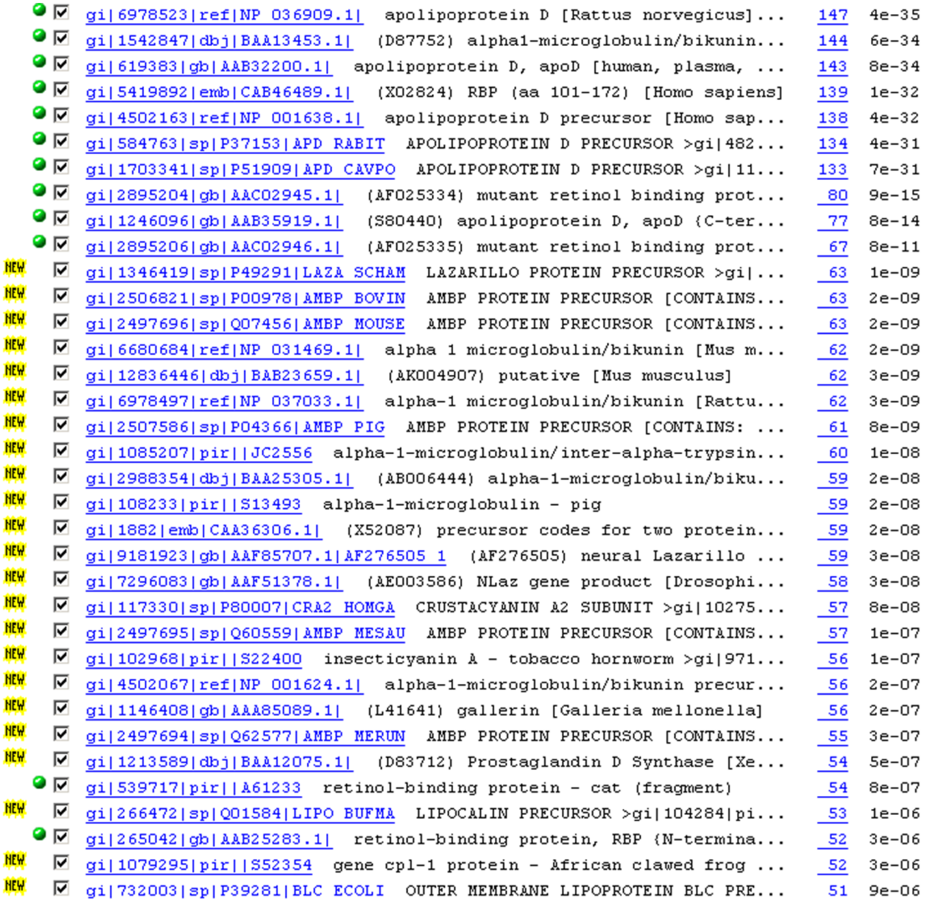
[1] Select a query and search it against a protein database

[2] PSI-BLAST constructs a multiple sequence alignment then creates a "profile" or specialized position-specific scoring matrix (PSSM)

[3] The PSSM is used as a query against the database

[4] PSI-BLAST estimates statistical significance (E values)

PSI-BLAST



```
●   ☑ gi|6978523|ref|NP_036909.1|   apolipoprotein D [Rattus norvegicus]...   147  4e-35
●   ☑ gi|1542847|dbj|BAA13453.1|   (D87752) alpha1-microglobulin/bikunin...   144  6e-34
●   ☑ gi|619383|gb|AAB32200.1|   apolipoprotein D, apoD [human, plasma, ...   143  8e-34
●   ☑ gi|5419892|emb|CAB46489.1|   (X02824) RBP (aa 101-172) [Homo sapiens]   139  1e-32
●   ☑ gi|4502163|ref|NP_001638.1|   apolipoprotein D precursor [Homo sap...   138  4e-32
●   ☑ gi|584763|sp|P37153|APD_RABIT   APOLIPOPROTEIN D PRECURSOR >gi|482...   134  4e-31
●   ☑ gi|1703341|sp|P51909|APD_CAVPO   APOLIPOPROTEIN D PRECURSOR >gi|11...   133  7e-31
●   ☑ gi|2895204|gb|AAC02945.1|   (AF025334) mutant retinol binding prot...    80  9e-15
●   ☑ gi|1246096|gb|AAB35919.1|   (S80440) apolipoprotein D, apoD (C-ter...    77  8e-14
●   ☑ gi|2895206|gb|AAC02946.1|   (AF025335) mutant retinol binding prot...    67  8e-11
NEW ☑ gi|1346419|sp|P49291|LAZA_SCHAM   LAZARILLO PROTEIN PRECURSOR >gi|...    63  1e-09
NEW ☑ gi|2506821|sp|P00978|AMBP_BOVIN   AMBP PROTEIN PRECURSOR [CONTAINS...    63  2e-09
NEW ☑ gi|2497696|sp|Q07456|AMBP_MOUSE   AMBP PROTEIN PRECURSOR [CONTAINS...    63  2e-09
NEW ☑ gi|6680684|ref|NP_031469.1|   alpha 1 microglobulin/bikunin [Mus m...    62  2e-09
NEW ☑ gi|12836446|dbj|BAB23659.1|   (AK004907) putative [Mus musculus]         62  3e-09
NEW ☑ gi|6978497|ref|NP_037033.1|   alpha-1 microglobulin/bikunin [Rattu...    62  3e-09
NEW ☑ gi|2507586|sp|P04366|AMBP_PIG   AMBP PROTEIN PRECURSOR [CONTAINS: ...    61  8e-09
NEW ☑ gi|1085207|pir||JC2556   alpha-1-microglobulin/inter-alpha-trypsin...    60  1e-08
NEW ☑ gi|2988354|dbj|BAA25305.1|   (AB006444) alpha-1-microglobulin/biku...    59  2e-08
NEW ☑ gi|108233|pir||S13493   alpha-1-microglobulin - pig                      59  2e-08
NEW ☑ gi|1882|emb|CAA36306.1|   (X52087) precursor codes for two protein...    59  2e-08
NEW ☑ gi|9181923|gb|AAF85707.1|AF276505_1   (AF276505) neural Lazarillo ...    59  3e-08
NEW ☑ gi|7296083|gb|AAF51378.1|   (AE003586) NLaz gene product [Drosophi...    58  3e-08
NEW ☑ gi|117330|sp|P80007|CRA2_HOMGA   CRUSTACYANIN A2 SUBUNIT >gi|10275...    57  8e-08
NEW ☑ gi|2497695|sp|Q60559|AMBP_MESAU   AMBP PROTEIN PRECURSOR [CONTAINS...    57  1e-07
NEW ☑ gi|102968|pir||S22400   insecticyanin A - tobacco hornworm >gi|971...    56  1e-07
NEW ☑ gi|4502067|ref|NP_001624.1|   alpha-1-microglobulin/bikunin precur...    56  2e-07
NEW ☑ gi|1146408|gb|AAA85089.1|   (L41641) gallerin [Galleria mellonella]      56  2e-07
NEW ☑ gi|2497694|sp|Q62577|AMBP_MERUN   AMBP PROTEIN PRECURSOR [CONTAINS...    55  3e-07
NEW ☑ gi|1213589|dbj|BAA12075.1|   (D83712) Prostaglandin D Synthase [Xe...    54  5e-07
●   ☑ gi|539717|pir||A61233   retinol-binding protein - cat (fragment)         54  8e-07
NEW ☑ gi|266472|sp|Q01584|LIPO_BUFMA   LIPOCALIN PRECURSOR >gi|104284|pi...    53  1e-06
●   ☑ gi|265042|gb|AAB25283.1|   retinol-binding protein, RBP (N-termina...    52  3e-06
NEW ☑ gi|1079295|pir||S52354   gene cpl-1 protein - African clawed frog ...    52  3e-06
NEW ☑ gi|732003|sp|P39281|BLC_ECOLI   OUTER MEMBRANE LIPOPROTEIN BLC PRE...    51  9e-06
```

PSI-BLAST

PSI-BLAST is performed in five steps

[1] Select a query and search it against a protein database

[2] PSI-BLAST constructs a multiple sequence alignment then creates a “profile” or specialized position-specific scoring matrix (PSSM)

[3] The PSSM is used as a query against the database

[4] PSI-BLAST estimates statistical significance (E values)

[5] Repeat steps [3] and [4] iteratively, typically 5 times. At each new search, a new profile is used as the query.

PSI-BLAST

Results of a PSI-BLAST search

Iteration	# hits	# hits > threshold
1	104	49
2	173	96
3	236	178
4	301	240
5	344	283
6	342	298
7	378	310
8	382	320

PSI-BLAST

PSI-BLAST alignment of RBP and b-lactoglobulin: iteration 1

```
Score = 46.2 bits (108), Expect = 2e-04
Identities = 40/150 (26%), Positives = 70/150 (46%), Gaps = 37/150 (24%)

Query: 27  VKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETGQMSATAKGRVRLLNNWDVC 86
           V+ENFD  ++ G WY + +K P      + I A +S+ E G +    K         ++
Sbjct: 33  VQENFDVKKYLGRWYEI-EKIPASFEKGNCIQANYSLMENGNIEVLNK---------ELS 82

Query: 87  ADMVGTF---------TDTEDPAKFKMKYWGVASFLQKGNDDHWIVDTDYDTYAVQYSCR 137
            D  GT          ++  +PAK +++++ +          +WI+ TDY+ YA+ YSC
Sbjct: 83  PD--GTMNQVKGEAKQSNVSEPAKLEVQFFPLMP-----PAPYWILATDYENYALVYSCT 135

Query: 138 ----LLNLDGTCADSYSFVFSRDPNGLPPE 163
               L ++D      + ++  R+P  LPPE
Sbjct: 136 TFFWLFHVD------FFWILGRNPY-LPPE 158
```

PSI-BLAST

PSI-BLAST alignment of RBP and b-lactoglobulin: iteration 2

```
Score =  140 bits (353), Expect = 1e-32
Identities = 45/176 (25%), Positives = 78/176 (43%), Gaps = 33/176 (18%)

Query: 4   VWALLLLAAWAAAERDCRVSSF--------RVKENFDKARFSGTWYAMAKKDPEGLFLQD 55
           V  L+ LA  A      +  +F         V+ENFD  ++ G WY + +K P      +
Sbjct: 2   VTMLMFLATLAGLFTTAKGQNFHLGKCPSPPVQENFDVKKYLGRWYEI-EKIPASFEKGN 60

Query: 56  NIVAEFSVDETGQMSATAKGRVRLLNNWDVCADMV---GTFTDTEDPAKFKMKYWGVASF 112
            I A +S+ E G +    K       + D   + V      ++  +PAK +++++ +
Sbjct: 61  CIQANYSLMENGNIEVLNKEL-----SPDGTMNQVKGEAKQSNVSEPAKLEVQFFPL--- 112

Query: 113 LQKGNDDHWIVDTDYDTYAVQYSCR----LLNLDGTCADSYSFVFSRDPNGLPPEA 164
                  +WI+ TDY+ YA+ YSC     L ++D      + ++  R+P  LPPE
Sbjct: 113 --MPPAPYWILATDYENYALVYSCTTFFWLFHVD------FFWILGRNPY-LPPET 159
```

PSI-BLAST

PSI-BLAST alignment of RBP and b-lactoglobulin: iteration 3

```
Score =  159 bits (404), Expect = 1e-38
Identities = 41/170 (24%), Positives = 69/170 (40%), Gaps = 19/170 (11%)

Query: 3   WVWALLLLAAWAAAERD--------CRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQ 54
             V  L+ LA  A              +  S  V+ENFD  ++ G WY + K
Sbjct: 1   MVTMLMFLATLAGLFTTAKGQNFHLGKCPSPPVQENFDVKKYLGRWYEIEKIPASFE-KG 59

Query: 55  DNIVAEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQ 114
           + I A +S+ E G +    K          V  +     ++  +PAK +++++ +
Sbjct: 60  NCIQANYSLMENGNIEVLNKELSPDGTMNQVKGE--AKQSNVSEPAKLEVQFFPL----- 112

Query: 115 KGNDDHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEA 164
                +WI+ TDY+ YA+ YSC            + ++  R+P  LPPE
Sbjct: 113 MPPAPYWILATDYENYALVYSCTTFFWL--FHVDFFWILGRNPY-LPPET 159
```

PSI-BLAST

1

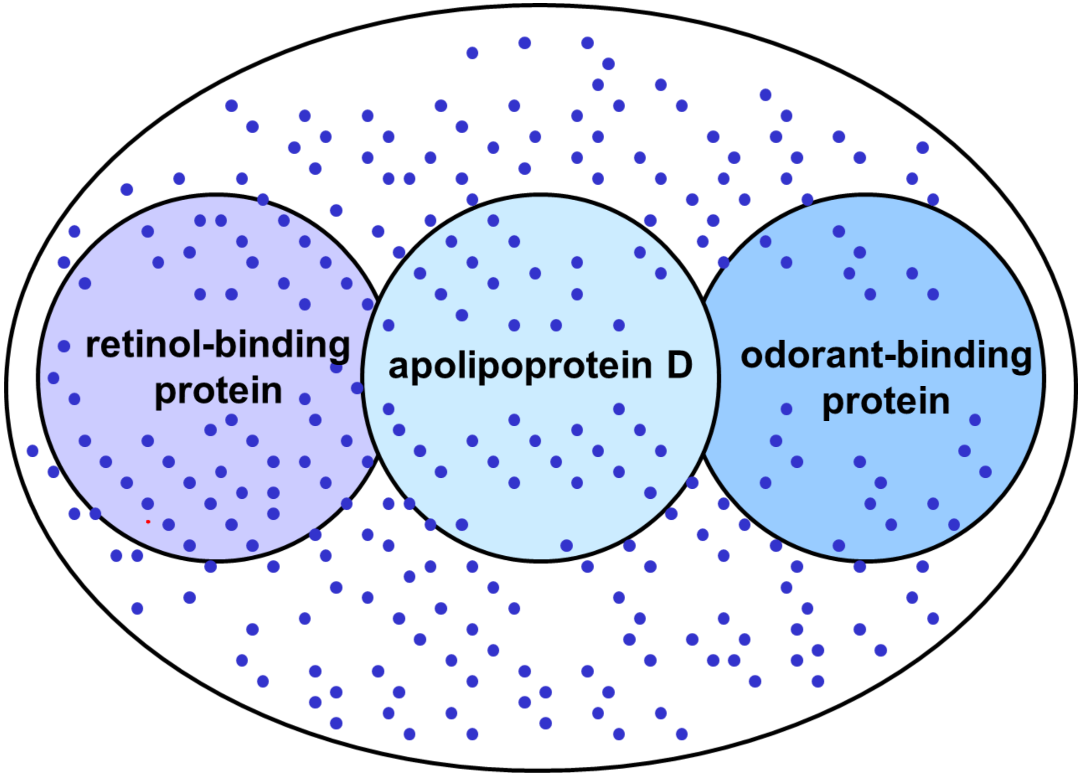
```
Score = 46.2 bits (108), Expect = 2e-04
Identities = 40/150 (26%), Positives = 70/150 (46%), Gaps = 37/150 (24%)

Query: 27  VKENFDKARFSGTWYAMAKKDPEGLFLQDNIVAEFSVDETGQMSATAKGRVRLLNNWDVC 86
           V+ENFD  ++ G WY + +K P      + I A +S+ E G +    K         ++
Sbjct: 33  VQENFDVKKYLGRWYEI-EKIPASFEKGNCIQANYSLMENGNIEVLNK---------ELS 82

Query: 87  ADMVGTF---------TDTEDPAKFKMKYWGVASFLQKGNDDHWIVDTDYDTYAVQYSCR 137
            D  GT          ++  +PAK +++++ +          +WI+ TDY+ YA+ YSC
Sbjct: 83  PD--GTMNQVKGEAKQSNVSEPAKLEVQFFPLMP-----PAPYWILATDYENYALVYSCT 135

Query: 138 ----LLNLDGTCADSYSFVFSRDPNGLPPE 163
               L ++D      + ++  R+P  LPPE
Sbjct: 136 TFFWLFHVD------FFWILGRNPY-LPPE 158
```

3

```
Score =  159 bits (404), Expect = 1e-38
Identities = 41/170 (24%), Positives = 69/170 (40%), Gaps = 19/170 (11%)

Query: 3   WVWALLLLAAWAAAERD--------CRVSSFRVKENFDKARFSGTWYAMAKKDPEGLFLQ 54
            V  L+ LA  A              +  S  V+ENFD  ++ G WY + K
Sbjct: 1   MVTMLMFLATLAGLFTTAKGQNFHLGKCPSPPVQENFDVKKYLGRWYEIEKIPASFE-KG 59

Query: 55  DNIVAEFSVDETGQMSATAKGRVRLLNNWDVCADMVGTFTDTEDPAKFKMKYWGVASFLQ 114
           + I A +S+ E G +    K          V  +     ++  +PAK +++++ +
Sbjct: 60  NCIQANYSLMENGNIEVLNKELSPDGTMNQVKGE--AKQSNVSEPAKLEVQFFPL----- 112

Query: 115 KGNDDHWIVDTDYDTYAVQYSCRLLNLDGTCADSYSFVFSRDPNGLPPEA 164
                +WI+ TDY+ YA+ YSC            + ++  R+P  LPPE
Sbjct: 113 MPPAPYWILATDYENYALVYSCTTFFWL--FHVDFFWILGRNPY-LPPET 159
```

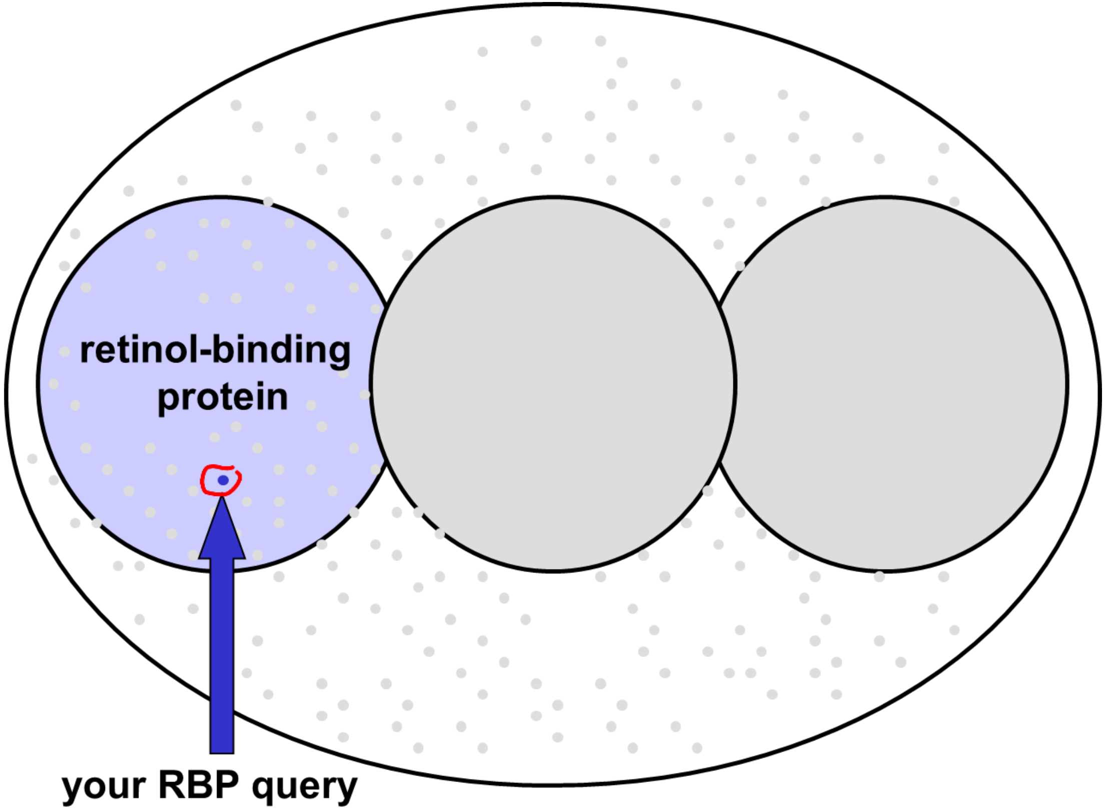
PSI-BLAST

The universe of lipocalins (each dot is a protein)



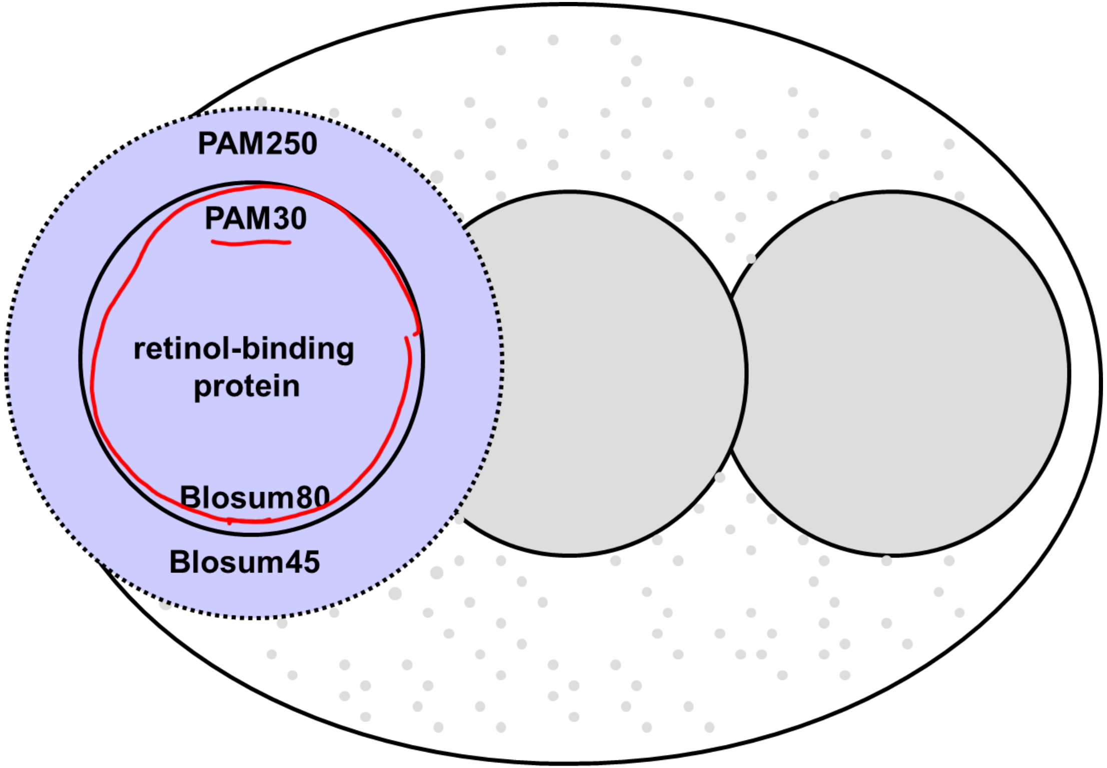
PSI-BLAST

Scoring matrices let you focus on the big (or small) picture



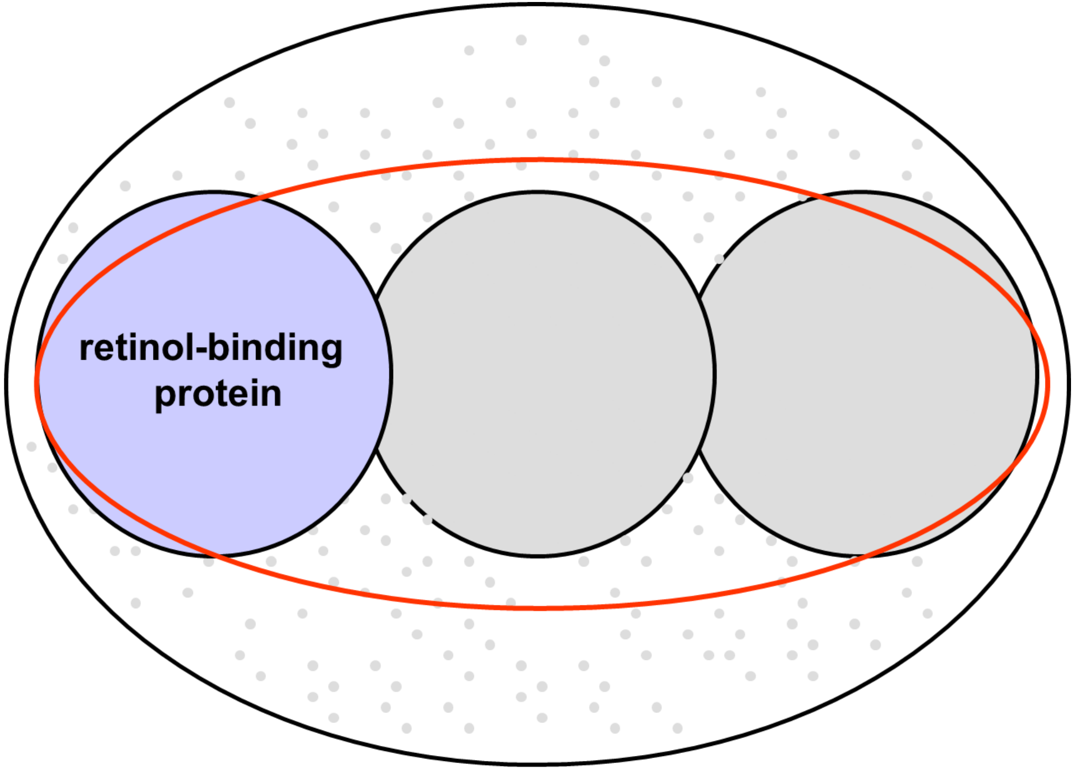
PSI-BLAST

Scoring matrices let you focus on the big (or small) picture



PSI-BLAST

PSI-BLAST generates scoring matrices more powerful than PAM or BLOSUM



Advantages and Disadvantages of PSI-BLAST

Advantages

[1] Fast (40 times faster than DP)

[2] Good E-value estimates

[3] Useful to detect weak but biologically meaningful relationships between proteins

Disadvantages

[1] Not optimal alignments

PSI-BLAST: the problem of corruption

- The main source of false positives is the spurious amplification of sequences not related to the query. For instance, a query with a coiled-coil motif may detect thousands of other proteins with this motif that are not homologous.

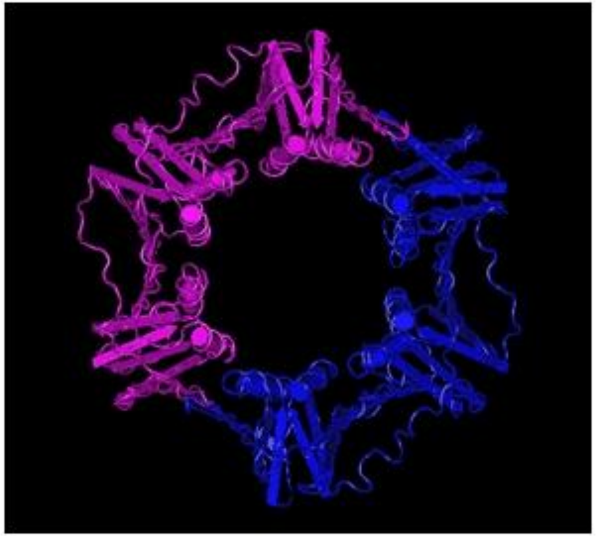
- Once even a single spurious protein is included in a PSI-BLAST search above threshold, it will not go away.

Example -1

Cellular DNA polymerase enzymes tend to dissociate from DNA after adding a few nucleotides and require an accessory factor to tether them to DNA while elongating the growing DNA chain.

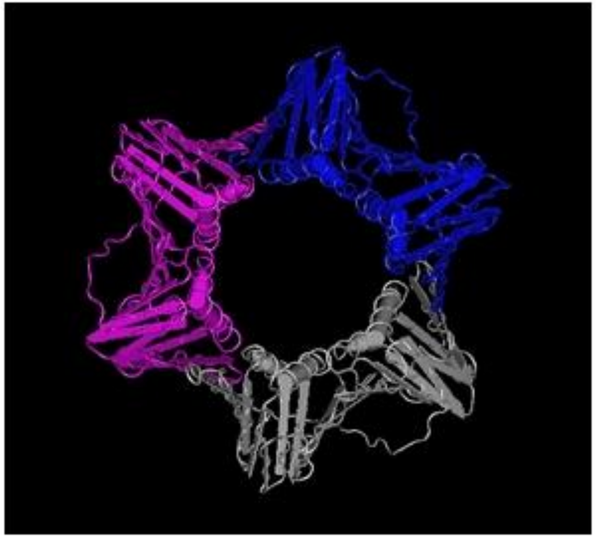
Ring structure called β-clamp

PCNA



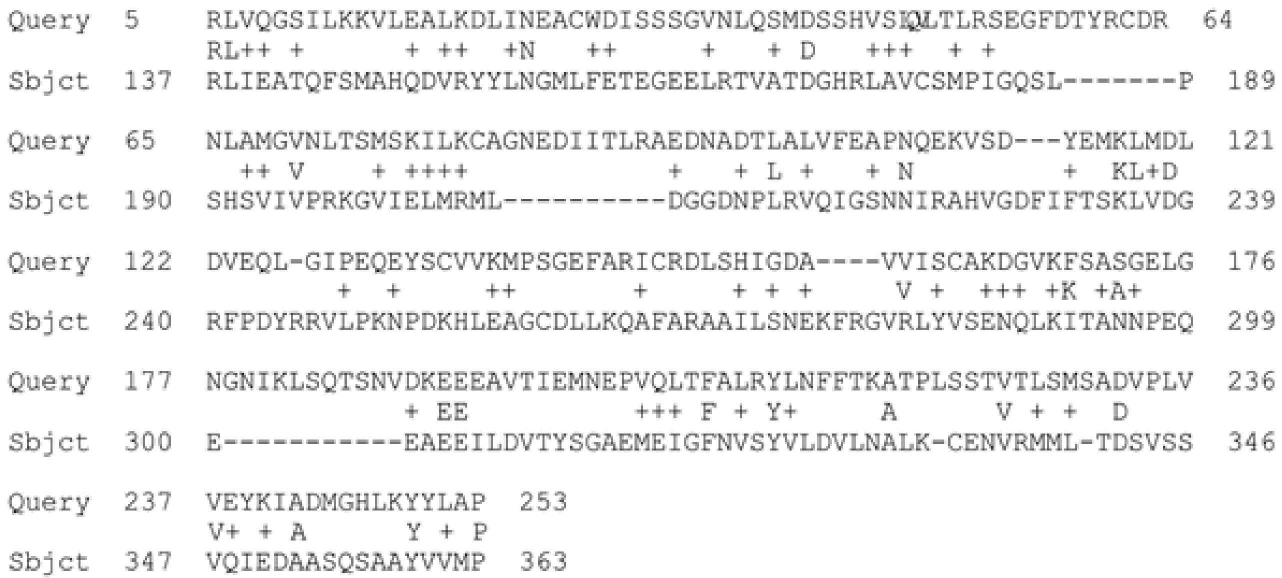
Eukaryotes and Archaea

β-subunit of DNA polymerase



Prokaryotes

Example -1



```
Query  5    RLVQGSILKKVLEALKDLINEACWDISSSGVNLQSMDSSHVSIQLTLRSEGFDTYRCDR  64
            RL++ +      + ++  +N   ++     +   + D   +++  + +
Sbjct  137  RLIEATQFSMAHQDVRYYLNGMLFETEGEELRTVATDGHRLAVCSMPIGQSL-------P  189

Query  65   NLAMGVNLTSMSKILKCAGNEDIITLRAEDNADTLALVFEAPNQEKVSD---YEMKLMDL  121
              ++ V    + ++++            +   + L +   + N         +  KL+D
Sbjct  190  SHSVIVPRKGVIELMRML----------DGGDNPLRVQIGSNNIRAHVGDFIFTSKLVDG  239

Query  122  DVEQL-GIPEQEYSCVVKMPSGEFARICRDLSHIGDA----VVISCAKDGVKFSASGELG  176
                   +  +     ++       +     + + +     V +  +++ +K +A+
Sbjct  240  RFPDYRRVLPKNPDKHLEAGCDLLKQAFARAAILSNEKFRGVRLYVSENQLKITANNPEQ  299

Query  177  NGNIKLSQTSNVDKEEEAVTIEMNEPVQLTFALRYLNFFTKATPLSSTVTLSMSADVPLV  236
                        + EE          +++ F + Y+     A      V + +  D
Sbjct  300  E-----------EAEEILDVTYSGAEMEIGFNVSYVLDVLNALK-CENVRMML-TDSVSS  346

Query  237  VEYKIADMGHLKYYLAP  253
            V+ + A      Y + P
Sbjct  347  VQIEDAASQSAAYVVMP  363
```

Hydrophobic amino acids

- Alanine - Ala - A
- Isoleucine - Ile - I
- Leucine - Leu - L
- Methionine - Met - M
- Phenylalanine - Phe - F
- Valine - Val - V
- Proline - Pro - P
- Glycine - Gly - G

Charged amino acids

- Arginine - Arg - R
- Lysine - Lys - K
- Aspartic acid - Asp - D
- Glutamic acid - Glu - E