

B.Sc. (Hons.) Biotechnology
Core Course 13:
Basics of Bioinformatics and
Biostatistics (BIOT 3013)

Unit 5:
**Sequence Alignment and
database searching**

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Challenges in bioinformatics

- 1. Obtain the genome of an organism.**
- 2. Identify and annotate genes.**
- 3. Find the sequences, three dimensional structures, and functions of proteins.**
- 4. Find sequences of proteins that have desired three dimensional structures.**
- 5. Compare DNA sequences and proteins sequences for similarity.**
- 6. Study the evolution of sequences and species.**

***Sequence alignments
lie at the heart of all
bioinformatics***

- In global alignment, an attempt is made to align the entire sequences, as many characters as possible.
- In local alignment, stretches of sequence with the highest density of matches are given the highest priority, thus generating one or more islands of matches in the aligned sequences.
- Eg: problem of locating the famous *TATAAT*-box (a bacterial promoter) in a piece of DNA.

Method for pairwise sequence Alignment: Dynamic Programming

- **Global Alignment: Needleman-Wunsch Algorithm**
- **Local Alignment: Smith-Waterman Algorithm**

Needleman & Wunsch algorithm :

Global alignment

- **There are three major phases:
1. initialization 2. Fill 3. Trace back.**
- **Initialization assign values for the first row and column.**
- **The score of each cell is set to the gap score multiplied by the distance from the origin.**
- **Each cell of the matrix contains two values: a score and an arrow that points up, left, or diagonally up.**

Needleman-Wunsch: Global Alignments

Two sequences

COELACANTH
PELICAN

Scoring scheme

- match = 1
- mismatch = -1
- gap penalty = -1

1. Initialization Phase

		C	O	E	L	A	C	A	N	T	H
	0	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
P	↑-1										
E	↑-2										
L	↑-3										
I	↑-4										
C	↑-5										
A	↑-6										
N	↑-7										

Figure 3-2. Initialization of the alignment matrix

Scoring scheme

match = 1

mismatch = -1

gap $g = -1$

- Compute three score for each matrix cell
- Assign max. value to the cell and point the arrow in the direction of the maximum score.
- Make the consistency when two scores are equal (always choose Diagonal vs gap).
- Continue operation until the entire matrix is filled.

2. Fill Phase

$$M_{ij} = \max \begin{cases} M_{i-1,j-1} + S(c_i, c_j) \\ M_{i,j-1} + g \\ M_{i-1,j} + g \end{cases}$$

		C	O	E	L	A	C	A	N	T	H
	0	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
P	↑-1	↖-1	↖-2	↖-3	↖-4	↖-5	↖-6	↖-7	↖-8	↖-9	↖-10
E	↑-2	↖-2	↖-2	↖-1	←-0	←-3	←-4	←-5	←-6	←-7	←-8
L	↑-3	↖-3	↖-3	←-2	←-2	←-1	←-2	←-3	←-4	←-5	←-6
I	↑-4	↖-4	↑-4	↑-3	↑-1	↖-1	↖-2	↖-1	↖-4	↖-5	↖-6
C	↑-5	↖-3	←-4	↑-4	↑-2	↖-2	↖-0	←-1	←-2	←-3	←-4
A	↑-6	↑-4	↖-4	↖-5	↑-3	↖-1	↑-1	↖-1	←-0	←-1	←-2
N	↑-7	↑-5	↖-5	↖-5	↑-4	↑-2	↖-2	←-0	↖-2	←-1	←-0

3.Trace Back

		C	O	E	L	A	C	A	N	T	H
	0	← -1	← -2	← -3	← -4	← -5	← -6	← -7	← -8	← -9	← -10
P	↑ -1	↖ -1	↖ -2	↖ -3	↖ -4	↖ -5	↖ -6	↖ -7	↖ -8	↖ -9	↖ -10
E	↑ -2	↖ -2	↖ -2	↖ -1	← -0	← -3	← -4	← -5	← -6	← -7	← -8
L	↑ -3	↖ -3	↖ -3	← -2	↖ -2	← -1	← -2	← -3	← -4	← -5	← -6
I	↑ -4	↖ -4	↑ -4	↑ -3	↑ -1	↖ -1	↖ -2	↖ -1	↖ -4	↖ -5	↖ -6
C	↑ -5	↖ -3	← -4	↑ -4	↑ -2	↖ -2	↖ -0	← -1	← -2	← -3	← -4
A	↑ -6	↑ -4	↖ -4	↖ -5	↑ -3	↖ -1	↑ -1	↖ -1	← -0	← -1	← -2
N	↑ -7	↑ -5	↖ -5	↖ -5	↑ -4	↑ -2	↖ -2	← -0	↖ -2	← -1	← -0

Globally Aligned Sequence

COELACANTH

- PELICAN - -

Smith-Waterman Algorithm :

Local alignment

- Simple modification of N-W algorithm (Only Four Changes)
- The edges of the matrix are initialized to 0
- The maximum score is never less than 0 and no arrow is recorded unless the score is greater than 0
- Traceback is started at the highest values rather than at the lower right hand corner.
- Traceback is stopped as soon as a zero is encountered.

Trace Back

		C	O	E	L	A	C	A	N	T	H
P	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	1	0	0	0	0	0	0	0
L	0	0	0	0	2	1	0	0	0	0	0
I	0	0	0	0	1	1	0	0	0	0	0
C	0	1	0	0	0	0	2	0	0	0	0
A	0	0	0	0	0	1	0	3	2	1	0
N	0	0	0	0	0	0	0	1	4	3	2

Locally Aligned Sequence

ELACAN

ELICAN

Matrices: Measures of Similarity

- **Every sequence comparison method requires a set of scores.**
- **Thus, the similarity matrices are the basis of sequence analysis methods.**
- **Choice of matrix can influence outcome of analyse.**

Amino acid substitution matrices

Amino acids are **not** equal:

1. Some are easily substituted because they have similar:
 - physico-chemical properties
 - structure
2. Some mutations between amino acids occur more often due to similar codons

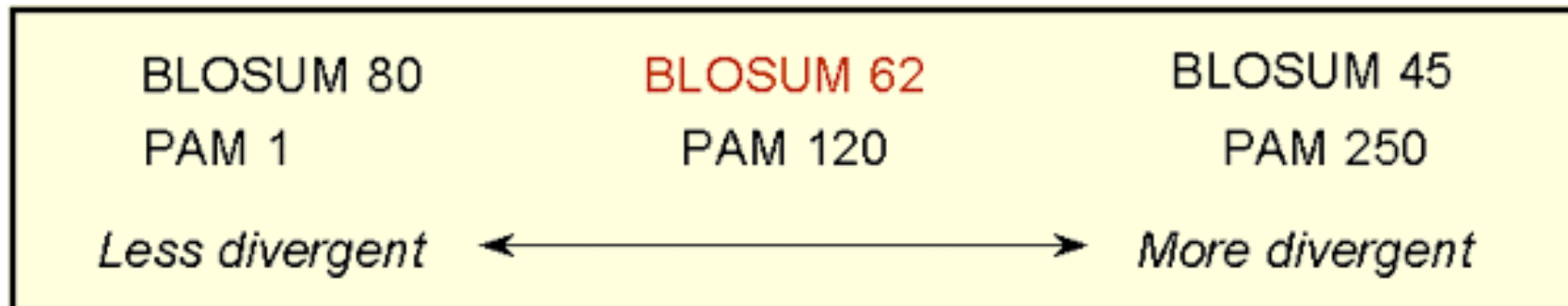
The two above observations give us ways to define *substitution matrices*

Substitution Matrices

- **PAM**
 - Developed by Margaret Dayhoff and published in 1978
- **BLOSUM**
 - Developed by Henikoff and Henikoff and published in 1992

The relationship between BLOSUM and PAM substitution matrices

- BLOSUM matrices with low numbers and PAM matrices with high numbers are designed for comparisons of distantly related proteins.
- ✦ Overall **BLOSUM62** is most effective for local alignment.



BLOSUM62

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

Example

- **1. FGKISESREFDNQNGPSTKDFGKIS**
- **2. FGKINMRLEDALVQNQLERSFGKIN**
 - Matrix: EBLOSUM62
 - Gap penalty: 10.0
 - Extend penalty: 0.5
 - Length: 25
 - Identity: 9/25 (36.0%)
 - Similarity: 12/25 (48.0%)
 - Gaps: 0/25 (0.0%)
 - Score: 32.0

Identity & similarity

- **The %id is the percentage of identical matches between the two sequences over the reported aligned region.**
- **The %similarity is the percentage of matches between the two sequences over the reported aligned region where the scoring matrix value is greater or equal to 0.0.**

Similarity versus Homology

- **Similarity refers to the likeness or % identity between 2 sequences**
- **Similarity means sharing a statistically significant number of bases or amino acids**
- **Similarity does not imply homology**
- **Homology refers to shared ancestry**
- **Two sequences are homologous if they are derived from a common ancestral sequence**
- **Homology usually implies similarity**

Similarity versus Homology

- **Similarity can be quantified**
- **It is correct to say that two sequences are X% identical**
- **It is correct to say that two sequences have a similarity score of Z**
- **It is generally *incorrect* to say that two sequences are X% *similar***

Difference between Homology and Similarity

- **Since homology is a qualitative description of the relationship, the term “% homology” has no meaning.**
- **Supporting data for a homologous relationship may include sequence or structural similarities, which can be described in quantitative terms.**
 - % identities, rmsd

Some Simple Rules

- If two sequence are > 100 residues and $> 25\%$ identical, they are likely related
- If two sequences are 15-25% identical they **may** be related, but more tests are needed
- If two sequences are $< 15\%$ identical they are probably not related
- If you need more than 1 gap for every 20 residues the alignment is suspicious

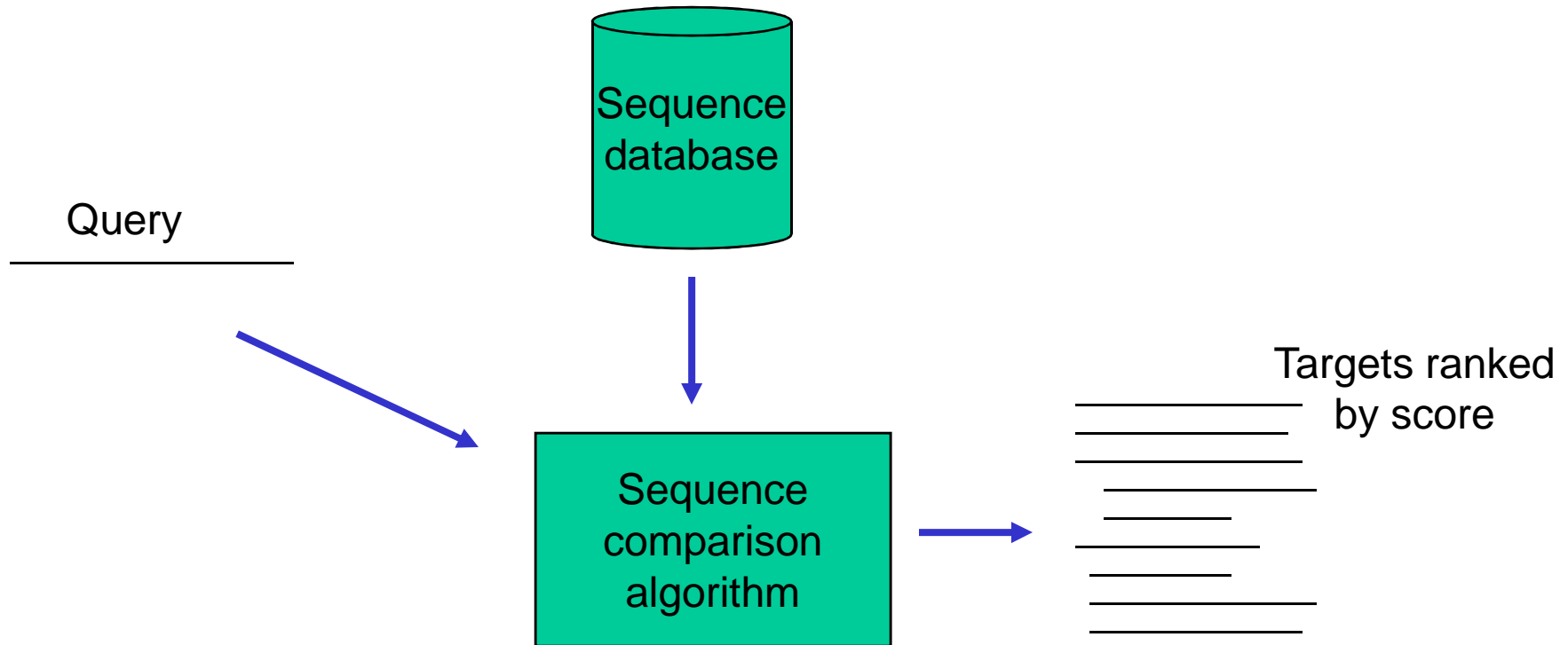
Dynamic Programming

- **Great for doing pairwise global alignments**
- **Produces a quantitative alignment “score”**
- **Problems if one tries to do alignments with very large sequences (memory requirement grows as N^2 or as $N \times M$)**
- **Serious problems if one tries to align one sequence against a database (10's of hours)**
- **Need an alternative Like BLAST....**

Basic Local Sequence Alignment Tool

- Time complexity of dynamic programming algorithm lead to the development of BLAST algorithms which are significantly faster but do not guarantee to find the optimal alignment.
- BLAST does not explore the entire search space between two sequences.
- Minimizing the search space is the key to its speed but at the cost of a loss in sensitivity.

Database searching



BLAST Algorithm

It uses three layers of rules to sequentially find potential high scoring pairs (HSPs)

1. Seeding

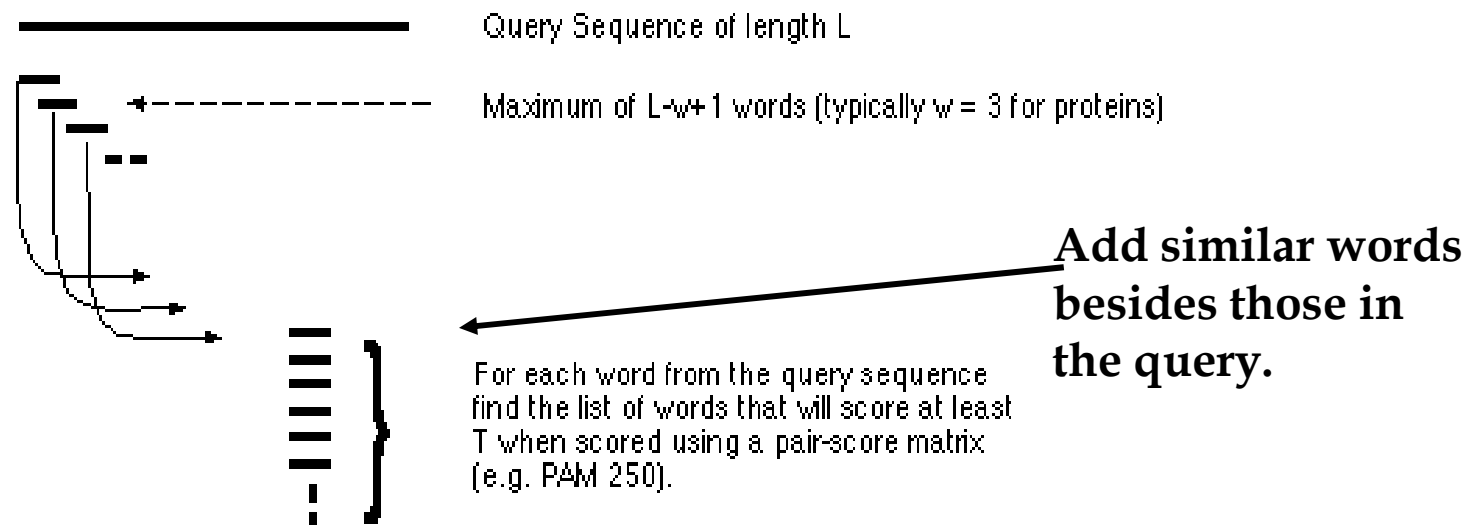
2. Extension

3. Evaluation

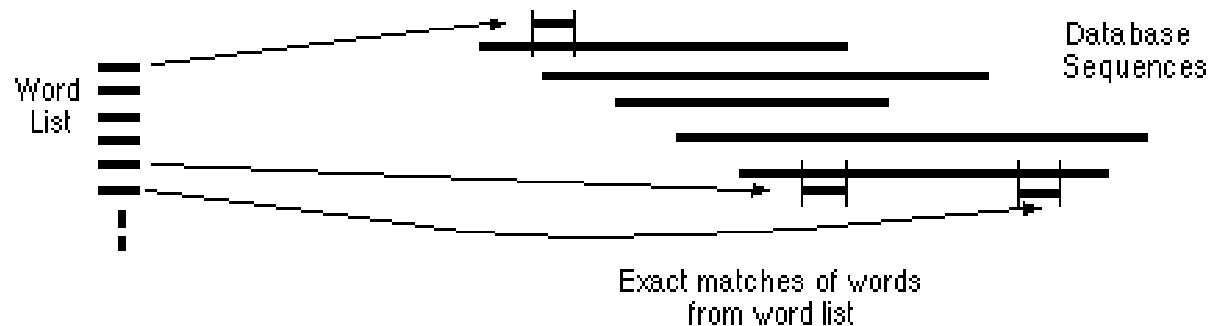
to sample the entire search space without wasting time on dissimilar regions.

BLAST Algorithm

(1) For the query, find the list of high scoring words of length w



(2) Compare the word list to the database and identify exact matches



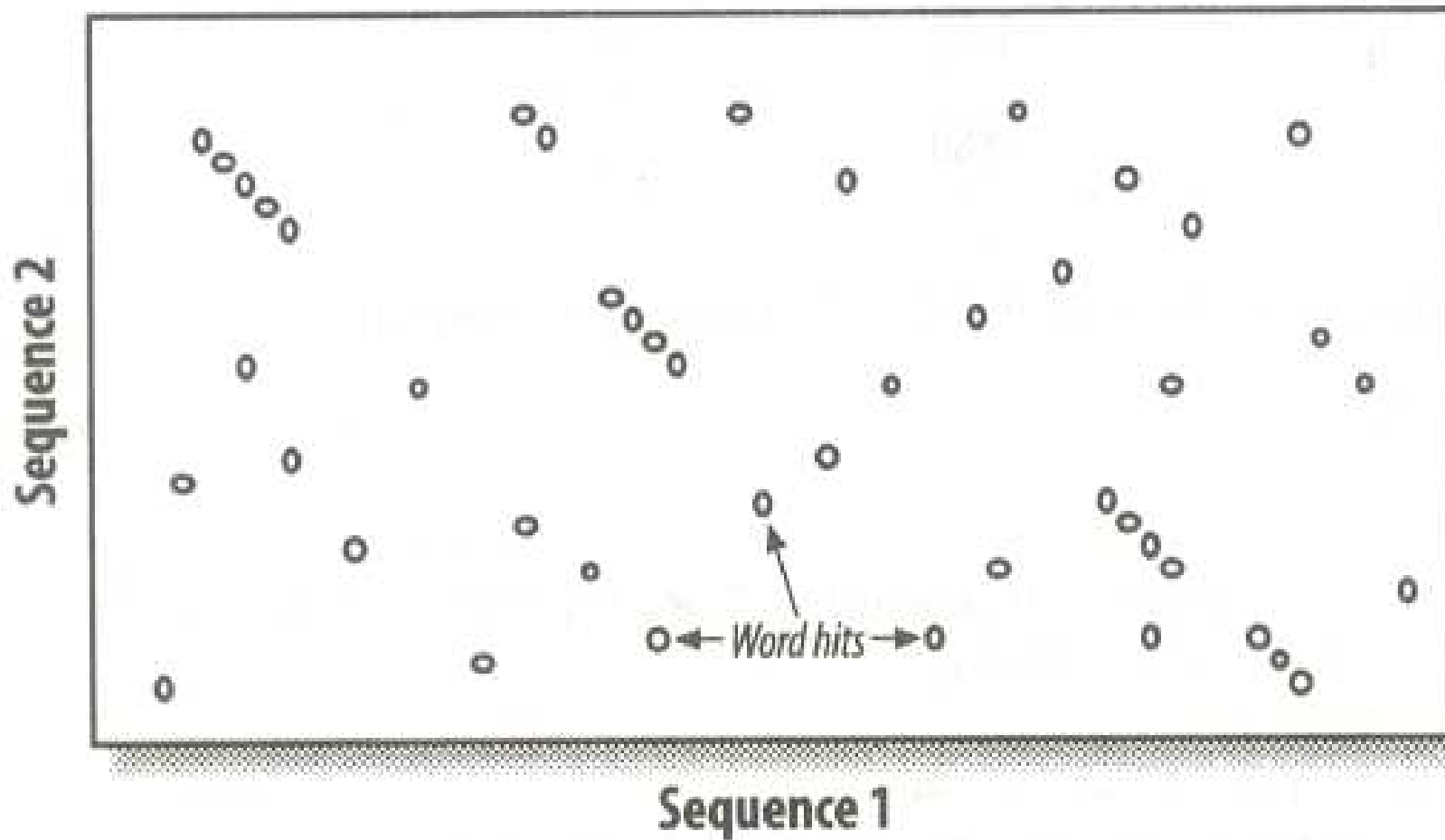
BLOSUM62

Word	Score
RGD	17
KGD	14
QGD	13
RGE	13
EGD	12
HGD	12
NGD	12
RGN	12
AGD	11
MGD	11
RAD	11
RGQ	11
RGS	11
RND	11
RSD	11
SGD	11
TGD	11

PAM200

Word	Score
RGD	18
RGE	17
RGN	16
KGD	15
RGQ	15
KGE	14
HGD	13
KGN	13
RAD	13
RGA	13
RGG	13
RGH	13
RGK	13
RGS	13
RGT	13
RSD	13
WGD	13

1. Seeding



Selection of T and W and Scoring matrix

- **The proper value for T depends on both the values in the scoring matrix and the balance between speed and sensitivity.**
- **Higher values of T progressively remove more hits and reduces the search space (run faster) but increases the chance of missing an alignments**
- **Word size (w) also control the word hits.**
- **Smaller w increases sensitivity but decreases speed.**
- **So interplay between W,T and matrix is critical to control speed and sensitivity of BLAST.**

Effect of threshold T

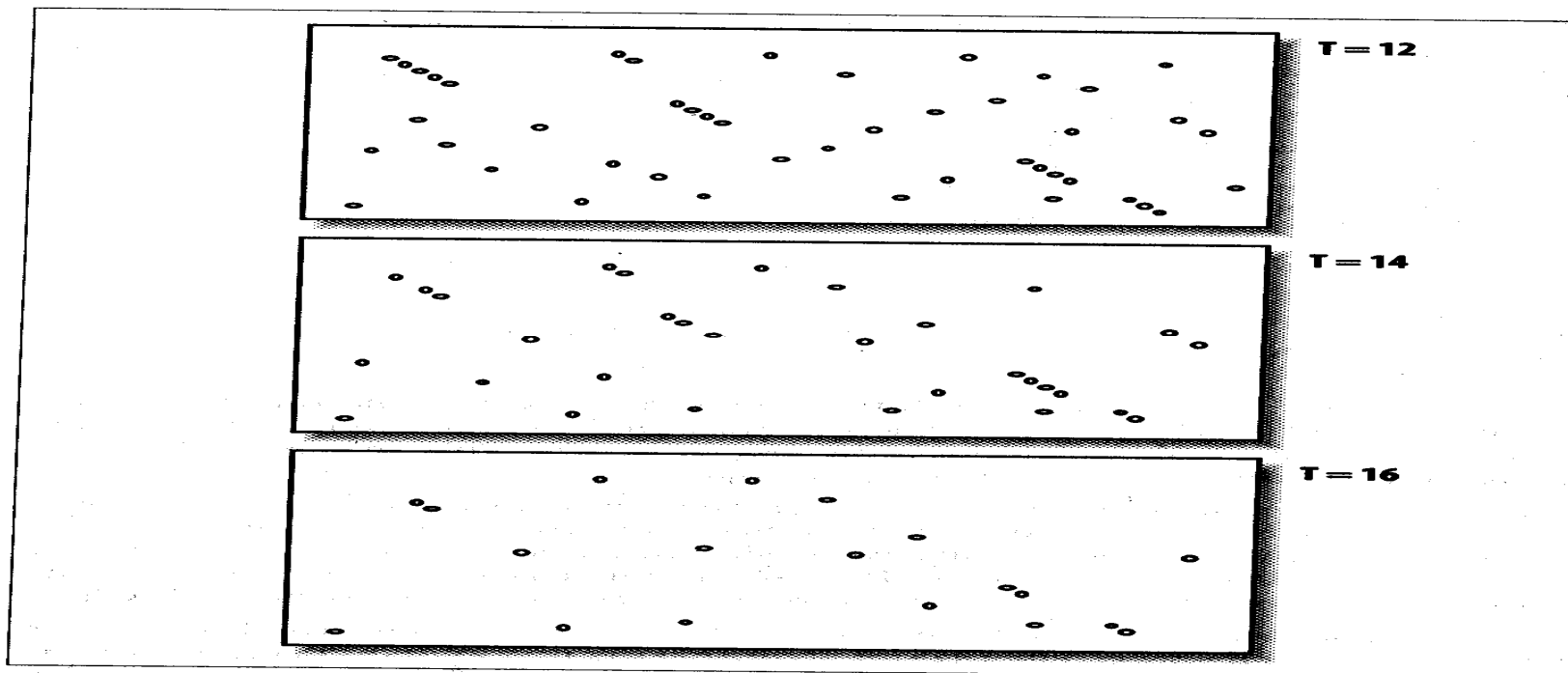


Figure 5-3: How T affects seeding

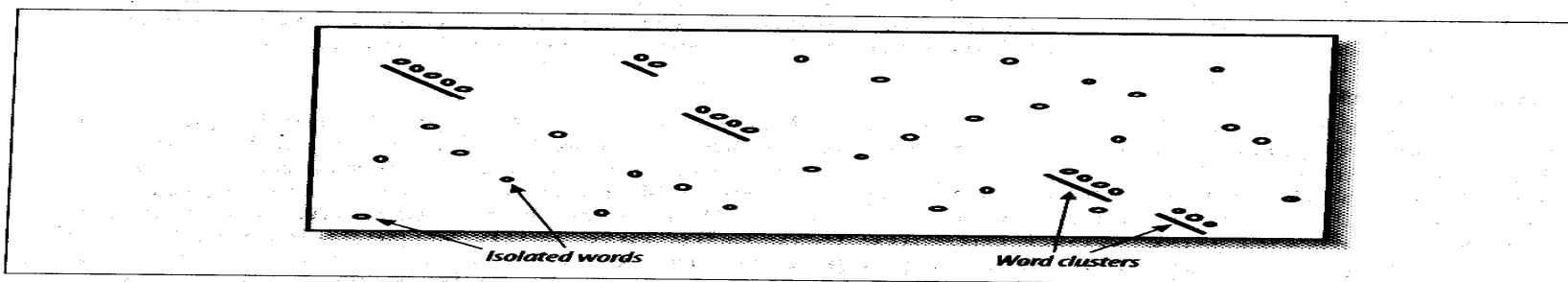
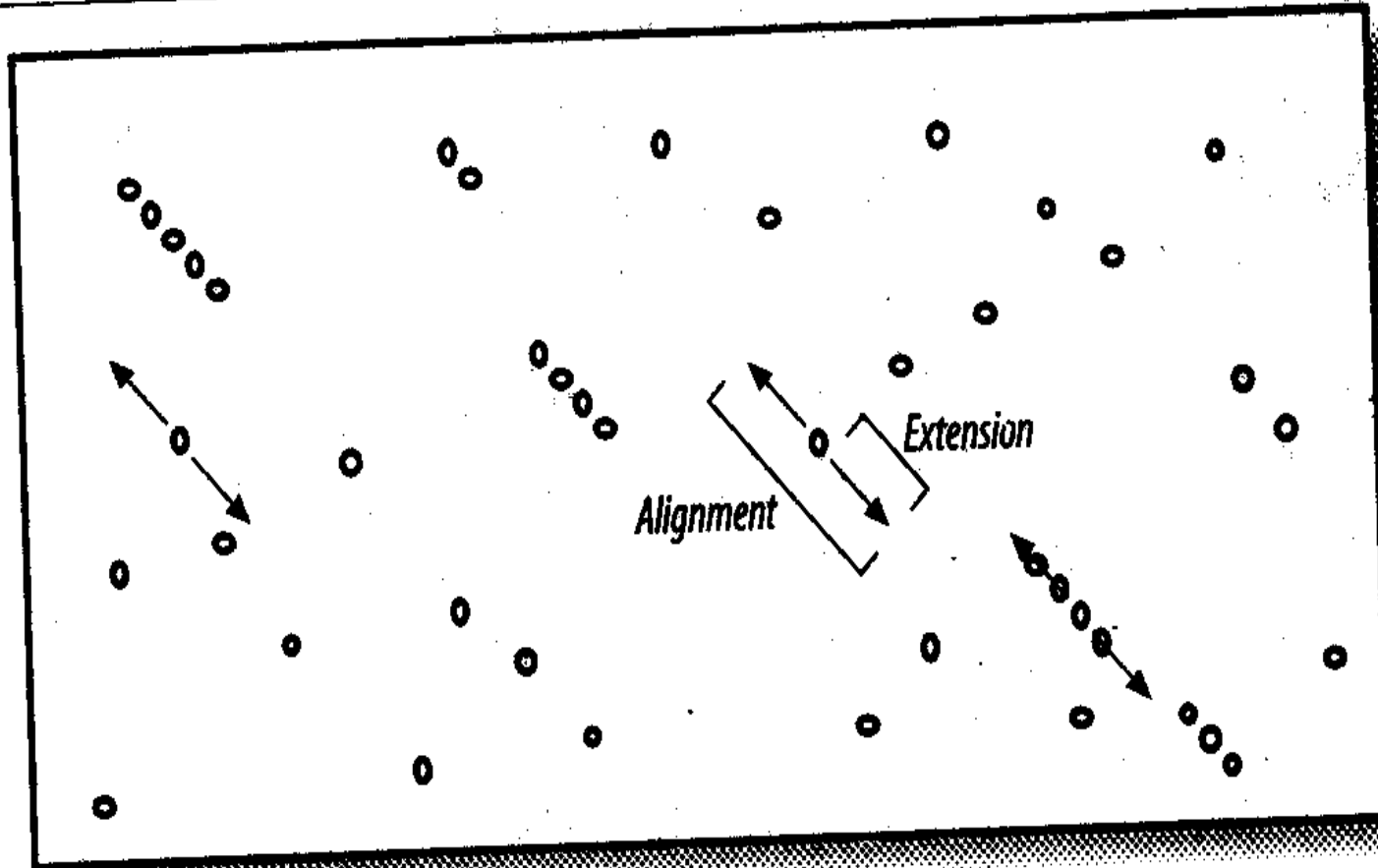


Figure 5-4: Isolated and clustered words

2. Extension

- **Once the search space is seeded, alignments can be generated from individual seeds in both direction.**

2. Extension



2: Extend matches

```
L P P Q G L L Query sequence
M P P E G L L Database sequence
      <word>
      7 2 6 BLOSUM62 scores
              word score = 15

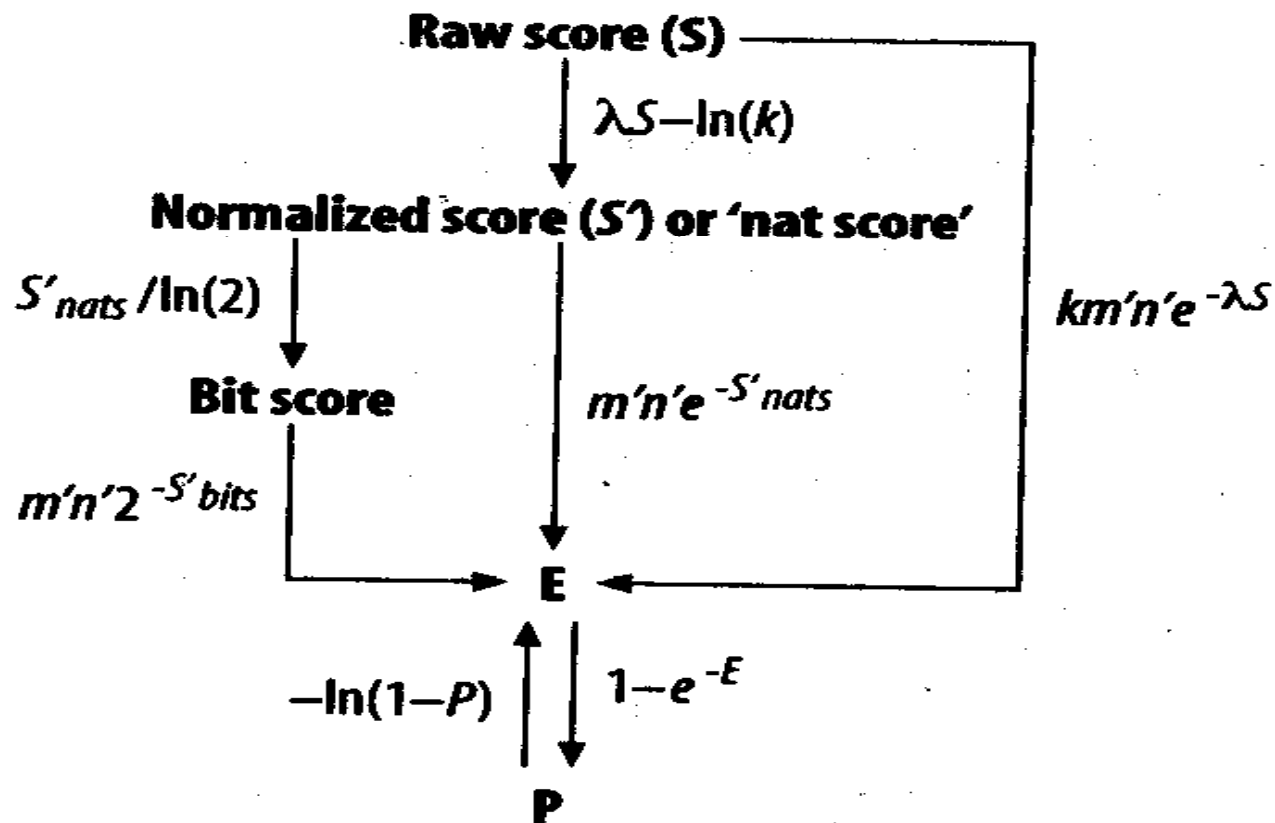
<----          ---->
2 7  7 2 6  4 4 HSP SCORE = 32
```

- **Each match is extended to the left and right until a negative BLOSUM62 score is encountered.**

3. Evaluation

- **Statistical significant of the alignments are evaluated and termed as HSPs.**
- **Because alignment score (S) and Expect(E) are directly related through Karlin-Altschul equation, so S is an synonymous with a statistical threshold.**
- **$E = k m n e^{-\lambda S}$**
- **m=no. of letters in query**
- **n= no. of letters in database**
- **K=minor constant**
- **λS = normalized score**

Statistical parameter of BLAST



P and E-values

- A p-value is the probability of making a mistake.
- The E-value is the expected number of times that the given score would appear in a random database of the given size.
- The E-value is computed by multiplying the p-value times the size of the database.
- Thus, for a p-value of 0.001 and a database of 1,000,000 sequences, the corresponding E-value is $0.001 \times 1,000,000 = 1,000$.



U.S. National Library of Medicine

NCBI National Center for Biotechnology Information

BLAST [®] >> blastn suite



COVID-19 is an emerging, rapidly evolving situation.
Get the latest public health information from CDC: <https://www.coronavirus.gov>.
Get the latest research from NIH: <https://www.nih.gov/coronavirus>.

Align Sequences Nucleotide BLAST

blastn | [blastp](#) | [blastx](#) | [tblastn](#) | [tblastx](#)

BLASTN programs search nucleotide subjects using a nucleotide query. [more...](#)

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

[Clear](#)

Query subrange

From

To

Or, upload file

Choose File

No file chosen

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

Enter Subject Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

[Clear](#)

Subject subrange

From

To

Formatting Results

NCBI Blast - Netscape

NCBI Blast

Open a new tab

NCBI *formatting* **BLAST**

Nucleotide Protein Translations Retrieve results for an RID

Your request has been successfully submitted and put into the Blast Queue.

Query = (77 letters)

No putative conserved domains have been detected

The request ID is

Format! or **Reset all**

The results are estimated to be ready in 43 seconds but may be done sooner.

Please press "FORMAT!" when you wish to check your results. You may change the formatting options for your result via the form below and press "FORMAT!" again. You may also request results of a different search by entering any other valid request ID to see other recent jobs.

Format

Show [Graphical Overview](#) [Linkout](#) [Sequence Retrieval](#) [NCBI-gi](#) Alignment [format](#)

Number of: [Descriptions](#) [Alignments](#)

[Alignment view](#)

Format for

start T-COFFEE serv... NCBI Blast - Ne... postdoc - Inbo... BLAST Query I... Bioinfo3.1 10:24 AM

BLAST Output

RID=1076347219-30071-17917880302.BLASTQ3, - Netscape

http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi

Sequences producing significant alignments:

Sequences producing significant alignments:	Score (bits)	E Value
gi 15678915 ref NP_276032.1 conserved protein [Methanother...	124	5e-28
gi 23111526 ref ZP_00097156.1 COG0526: Thiol-disulfide iso...	71	4e-12
gi 21226839 ref NP_632761.1 conserved protein [Methanosarc...	70	9e-12
gi 20092734 ref NP_618809.1 conserved hypothetical protein...	69	2e-11
gi 15643756 ref NP_228804.1 conserved hypothetical protein...	65	4e-10
gi 21674543 ref NP_662608.1 glutaredoxin family protein [C...	64	5e-10
gi 23054435 ref ZP_00080592.1 COG0526: Thiol-disulfide iso...	62	3e-09
gi 22971774 ref ZP_00018701.1 hypothetical protein [Chloro...	59	2e-08
gi 39998047 ref NP_953998.1 redox-active disulfide protein...	59	2e-08
gi 34557156 ref NP_906971.1 hypothetical protein W30755 [W...	58	3e-08
gi 17229003 ref NP_485551.1 hypothetical protein [Nostoc s...	57	7e-08
gi 15668761 ref NP_247560.1 conserved hypothetical protein...	57	7e-08
gi 23048165 ref ZP_00075893.1 COG0526: Thiol-disulfide iso...	56	1e-07
gi 21228351 ref NP_634273.1 hypothetical protein [Methanos...	55	4e-07
gi 29345530 ref NP_809033.1 conserved hypothetical protein...	53	1e-06
gi 20092738 ref NP_618813.1 conserved hypothetical protein...	53	1e-06
gi 11499829 ref NP_071073.1 conserved hypothetical protein...	53	1e-06
gi 22299430 ref NP_682677.1 ORF_ID:ts11887~hypothetical pr...	52	2e-06
gi 29346208 ref NP_809711.1 conserved hypothetical protein...	52	3e-06
gi 23001521 ref ZP_00045426.1 COG0526: Thiol-disulfide iso...	46	2e-04
gi 23015000 ref ZP_00054791.1 COG0526: Thiol-disulfide iso...	45	3e-04
gi 22960655 ref ZP_00008294.1 COG0526: Thiol-disulfide iso...	45	3e-04
gi 24372130 ref NP_716172.1 redox-active disulfide protein...	42	0.002
gi 39936619 ref NP_948895.1 Thiol-disulfide isomerase and ...	39	0.016
gi 1169967 sp P42035 THIO METTM Probable Thioredoxin (Gluta...	36	0.17
gi 15678829 ref NP_275946.1 thioredoxin [Methanothermobact...	34	0.46
gi 23060730 ref ZP_00085617.1 COG0438: Glycosyltransferase...	34	0.77
gi 23121123 ref ZP_00103523.1 COG0526: Thiol-disulfide iso...	32	1.9
gi 34860800 ref XP_215715.2 similar to Alcohol dehydrogena...	32	3.5
gi 19684184 gb AAH26035.1 C4orf9 protein [Homo sapiens]	32	3.5
gi 17933966 ref NP_530756.1 glutamine amidotransferase [Ag...	32	3.8

Click here to begin

start 5 Netscape BioInfo3.1 10:27 AM

BLAST Output

[gi|2621990|gb|AAB85393.1](#) conserved protein [Methanothermobacter thermautotrophicus str. Delta H]
Length = 77

Score = 124 bits (310), Expect = 5e-28
Identities = 77/77 (100%), Positives = 77/77 (100%)

Query: 1 MMKIQIYGTGCANCQMLEKNAREAVKELGIDAEFEKIKEMDQILEAGLTALPGLAVDGEL 60
MMKIQIYGTGCANCQMLEKNAREAVKELGIDAEFEKIKEMDQILEAGLTALPGLAVDGEL
Sbjct: 1 MMKIQIYGTGCANCQMLEKNAREAVKELGIDAEFEKIKEMDQILEAGLTALPGLAVDGEL 60

Query: 61 KIMGRVASKEEIKKILS 77
KIMGRVASKEEIKKILS
Sbjct: 61 KIMGRVASKEEIKKILS 77

>[gi|23111526|ref|ZP_00097156.1](#) COG0526: Thiol-disulfide isomerase and thioredoxins
[Desulfitobacterium hafniense]
Length = 76

Score = 71.2 bits (173), Expect = 4e-12
Identities = 40/76 (52%), Positives = 57/76 (75%)

Query: 2 MKIQIYGTGCANCQMLEKNAREAVKELGIDAEFEKIKEMDQILEAGLTALPGLAVDGELK 61
M I+I GTGCANC+ LE NA+EA+KELG+DA EK++++ I+ G+ P L V+ ++K
Sbjct: 1 MVIKILGTGCANCKLEANAKEAIKELGLDAVVEKVEDLQAIMAYGVMKTPALVVNEQVK 60

Query: 62 IMGRVASKEEIKKILS 77
+MG+V S EEIKK L+
Sbjct: 61 VMGKVLSAEEIKKYLN 76

BLAST - Rules of Thumb

- **Don't trust a BLAST alignment with an Expect score > 0.01**
- **Expect and Score are related, but Expect contains more information. Note that %Identities is more useful than the bit Score**
- **If uncertain about a hit, perform a PSI-BLAST search**

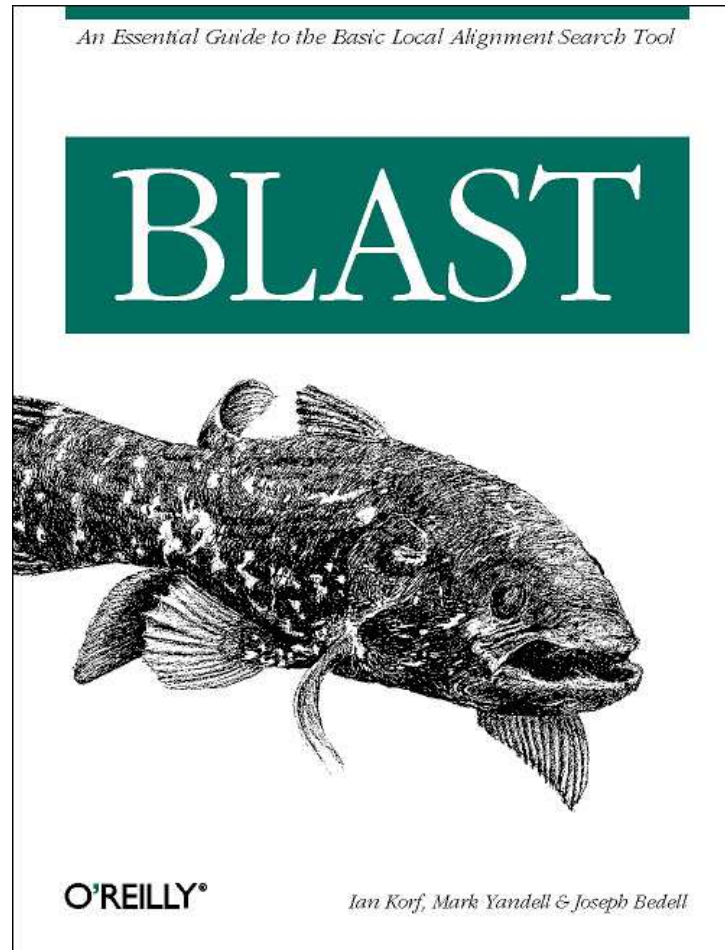
Different Flavours of BLAST

- **BLASTP** - protein query against protein DB
- **BLASTN** - DNA query against GenBank (DNA)
- **BLASTX** - 6 frame trans. DNA query against proteinDB
- **TBLASTN** - protein query against 6 frame GB transl.
- **TBLASTX** - 6 frame DNA query to 6 frame GB transl.
- **PSI-BLAST** - protein 'profile' query against protein DB

References

- https://bioinf.comav.upv.es/courses/biotech3/theory/sequence_alignment.html
- <https://www.ebi.ac.uk/Tools/psa/>
- <https://www.ebi.ac.uk/Tools/msa/clusterw2/>

O'Reilly Book



Home Assignment

- 1. Discuss the importance of sequence alignment.**
- 2. Differentiate between global and local sequence alignment with examples.**
- 3. Describe the BLAST algorithm for database searching.**

Last data of submission 20.04.2020

Thank you.

Email: sprakashsingh@mgcub.ac.in