H3ABioNet

Pan African Bioinformatics Network for H3Africa

Introduction to Bioinformatics online course: IBT

Sequence alignment theory and applications

Session 3: BLAST algorithm

Learning Objectives

- Understand the principles of the BLAST algorithm
- Understand the different BLAST programs, parameters and their applications
- Be able to adjust the sensitivity and specificity of BLAST searches by adjusting parameters
- Understand and evaluate BLAST results

Learning Outcomes

- Select the correct BLAST algorithm and database for the appropriate biological question being asked, i.e. select between blastp, blastn, blastx etc. based on the question being addressed
- Understand the meaning of the various output metrics/results
- Comment on results based on the various output metrics

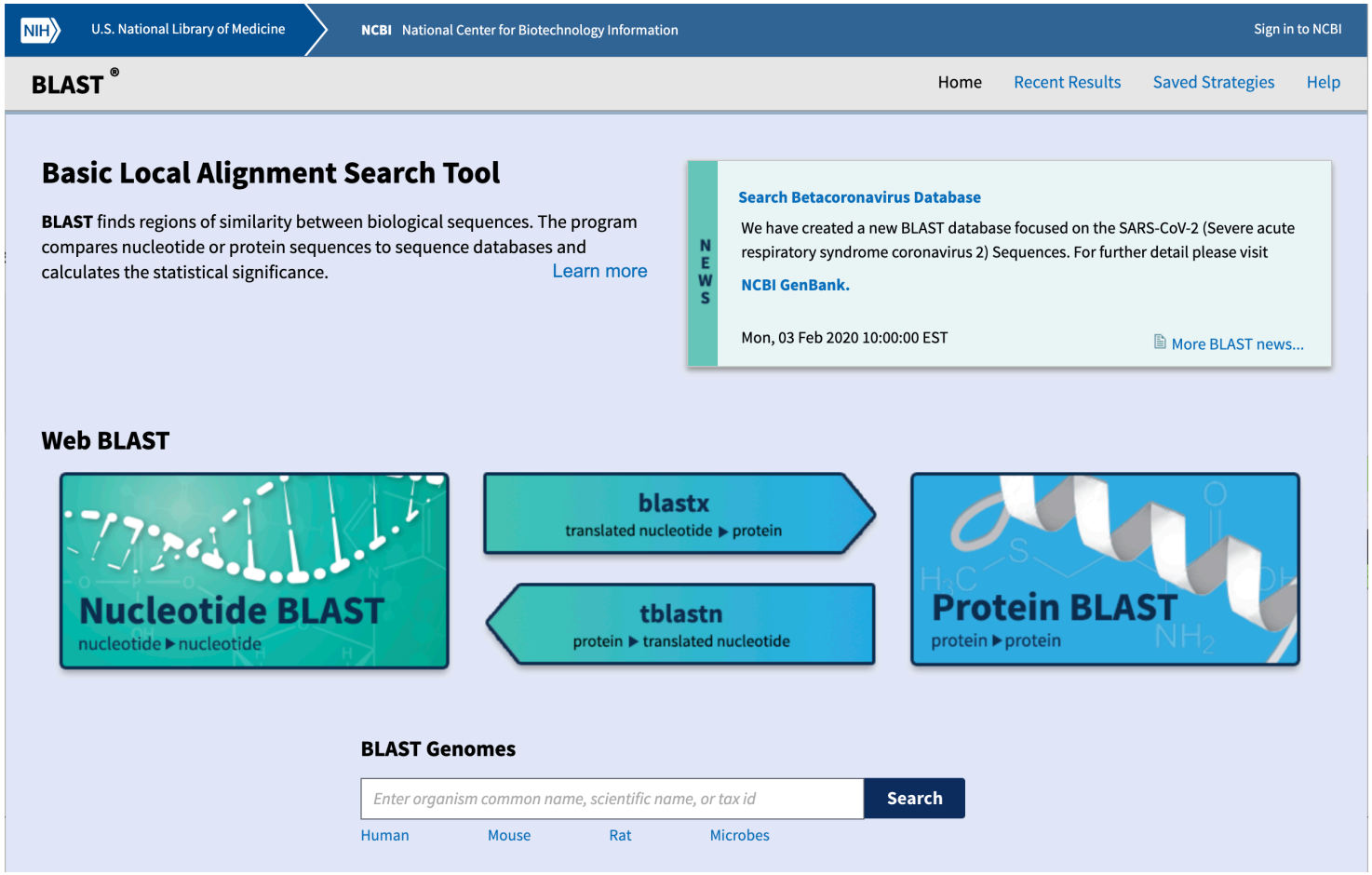
Why do a BLAST search?

- You can get important clues about the function of an as yet uncharacterised sequence.
- Identify homologous species for unknown species.
- Locate domains in protein sequence.
- Establish phylogeny.
- Mapping DNA from unknown location.

Principles of BLAST

- Basic Local Alignment Search Tool (Altschul et al., 1990) finds regions of local similarity between sequences.
- Compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches.
- Heuristic method for local alignment.
- Based on the assumption that good alignments contain short lengths of exact matches.

https://blast.ncbi.nlm.nih.gov/Blast.cgi



Specialized Searches

Specialized searches

SmartBLAST

Find proteins highly similar to your query

Primer-BLAST

Design primers specific to your PCR template

Global Align

Compare two sequences across their entire span (Needleman-Wunsch)

CD-search

Find conserved domains in your sequence

IgBLAST

Search immunoglobulins and T cell receptor sequences

VecScreen

Search sequences for vector contamination

CDART

Find sequences with similar conserved domain architecture

Multiple Alignment

Align sequences using domain and protein constraints

MOLE-BLAST

Establish taxonomy for uncultured or environmental sequences

3 Parts of BLAST

1. Set-up

2. Find local alignments between the query sequence and a sequence in a database.

3. Produce p-values and a rank ordering of the local alignments according to the p-values.

BLAST - Part 1

1. Set-up
   - Read in query, search parameters and database
   - Check for low complexity or other repeats (optional)

3 Parts of BLAST

1. Set-up

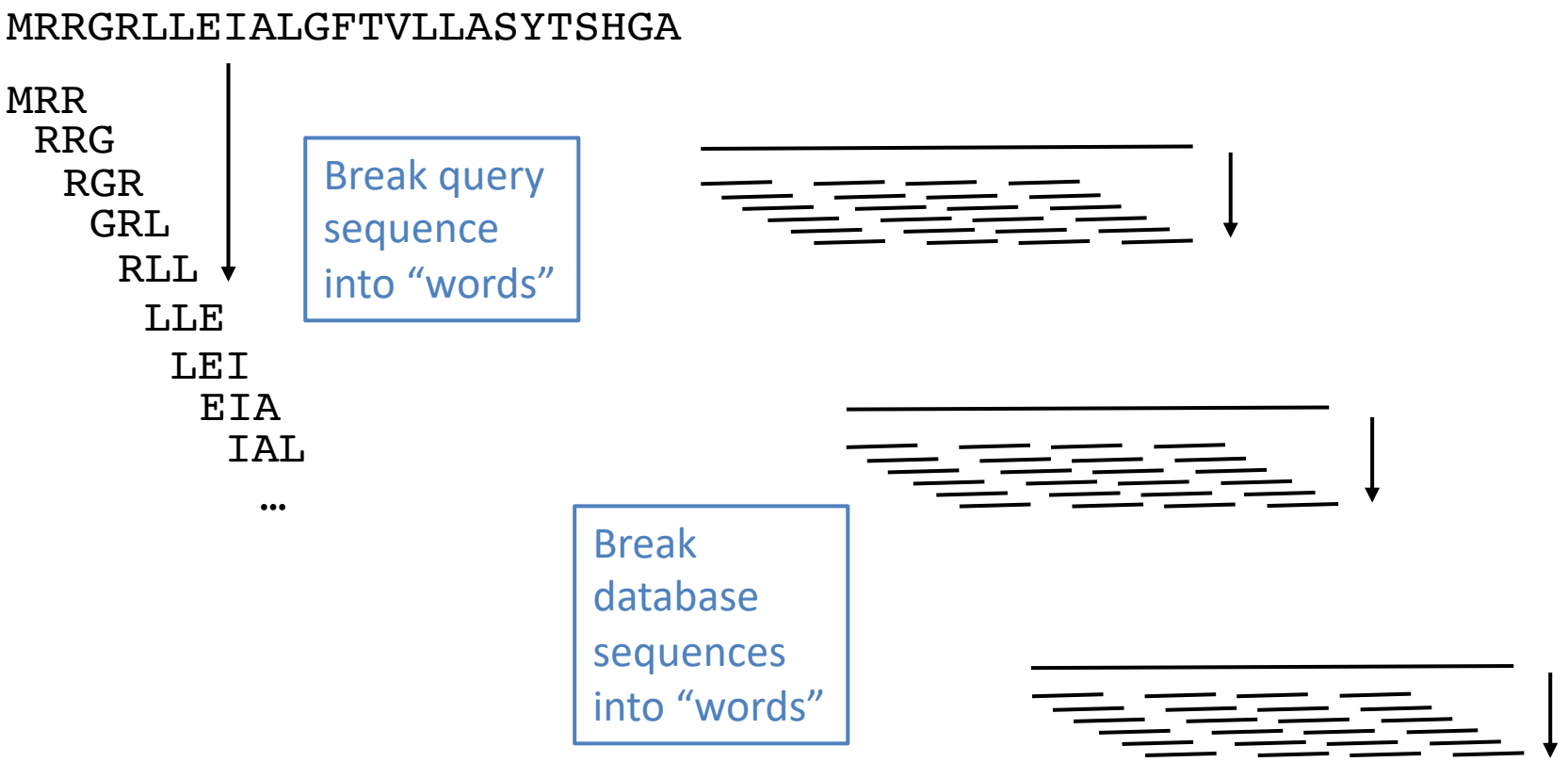
2. Find local alignments between the query sequence and a sequence in a database.

3. Produce p-values and a rank ordering of the local alignments according to the p-values.

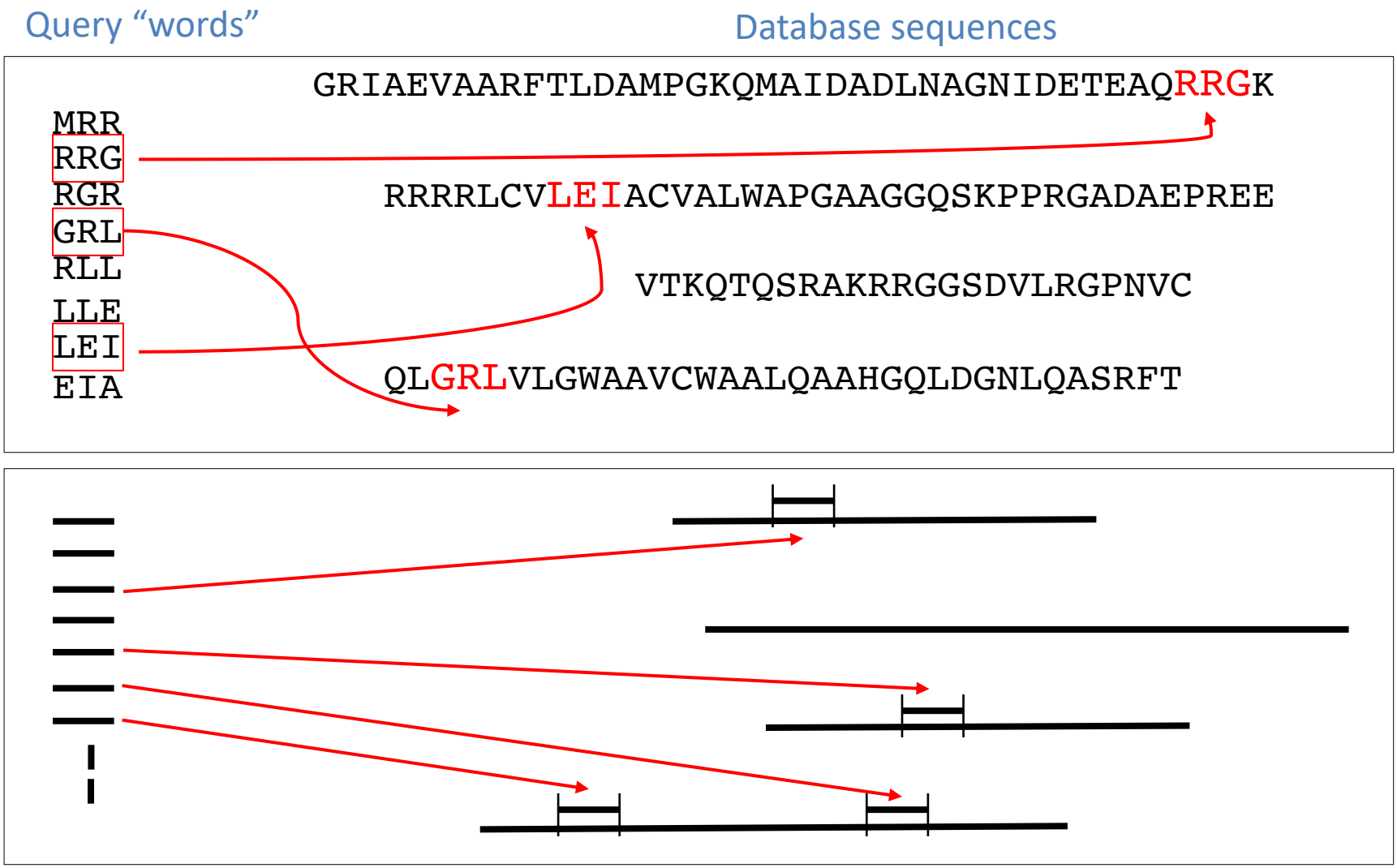
BLAST - Part 2

2. Find local alignments
   - Break query sequence into “words”
     - Typically, 3 for protein sequence and 11 for nucleotide sequence
   - Matches between the “words” in the “query” and “words” in the database sequences are found.
   - These can be exact matches (for nt-nt search) or matches that satisfy some positive-valued threshold score (for prot-prot search) as determined using a scoring matrix.

Sequences to “words”



Find location of matching “words” in the database sequences



BLAST - Part 2

2. Find local alignments, continued (1)
   - Each “word” match is scored
     - The score is computed by assigning a value to each aligned pair of letters and then summing these values over the length of the alignment.
     - For protein sequence alignments, scores for every possible amino acid letter pair are given in a “substitution matrix” where likely substitutions have positive values and unlikely substitutions have negative values. Default matrix used by BLASTP is BLOSUM62.
     - For nucleotide alignments, BLAST uses a reward of +2 for aligned pairs of identical letters and a penalty of −3 for each non-identical aligned pair.

BLAST - Part 2

BLOSUM62 matrix

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

MRRGRLLEIAAI

MRR	GRL	LEI	AAI
M=5	G=6	L=4	A=4
R=5	R=5	E=5	A=4
R=5	L=4	I=4	I=4
Total= 15	15	13	12

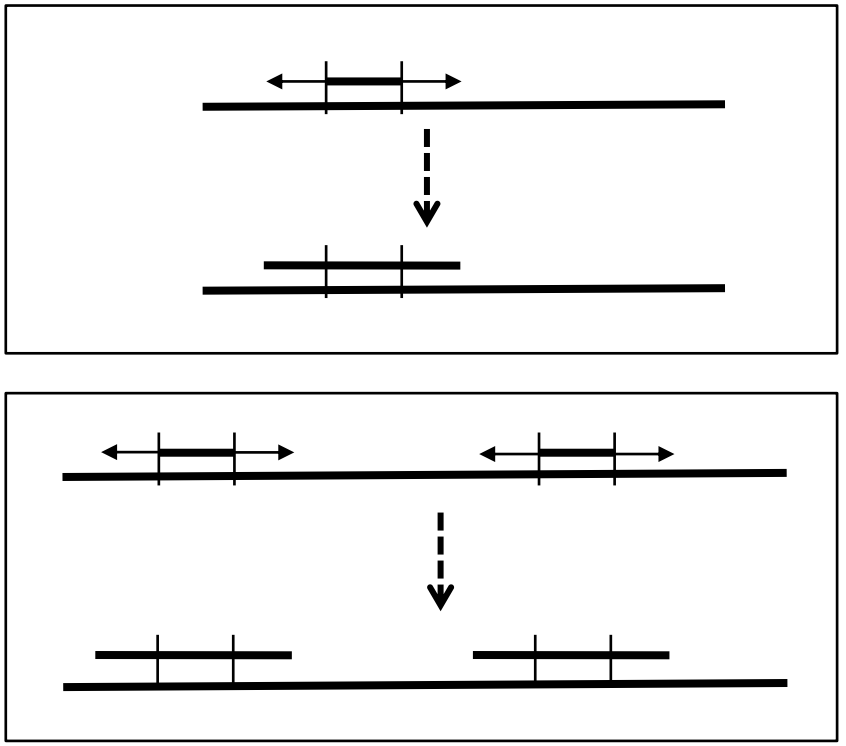
- Words that do not score above a given threshold (T) are removed from the list.
  - E.g. When T is 13, AAI is removed.
- Matches with a score above a given threshold are used to "seed" an ungapped alignment.

BLAST - Part 2

Extend Hits

- The “words” are extended in both directions into gap-free extensions for as long as the cumulative alignment score increases or stays the same.
- Gap-free extensions with alignment score above a given threshold are used to seed gapped extensions

- Gapped extensions (HSPs) scoring above an empirically determined cut-off score, S, are retained.



BLAST - Part 2

- HSP is the fundamental unit of BLAST algorithm output.
- An HSP consists of two sequence fragments of arbitrary but equal length whose alignment is locally maximal and for which the alignment score meets or exceeds a cut-off score, S.

```
Query  1  MRRGRLLEIALGFTVLLASYTSHGA  25
          M RGRL+ +A+G    +L+ +T  G
Sbjct  1  MCRGRLVRLAVGLVAVLSLWTEPGG  25
```

High-Scoring Segment Pair (HSP)

- 2 sequences
- Scoring system
- Cut-off score

3 Parts of BLAST

1. Set-up

2. Find local alignments between the query sequence and a sequence in a database.

3. Produce p-values and a rank ordering of the local alignments according to the p-values.

BLAST - Part 3

3. Determine the statistical significance of each HSP score (Raw score -> P-value -> E-value).
   - What is the probability (P-value) of the HSP alignment occurring by chance?
   - Are the two sequences descended from the same common ancestor?
   - P-value is the probability of finding exactly a HSPs with score >=*S*

BLAST - Part 3

Problems:

- Longer sequences are more likely to find higher scoring pairs.
- Longer databases are more likely to result in higher scoring pairs.

Solution:

- Convert Probability values (P-values) to Expectation values (E-values)

BLAST – Part 3

- The “Expect Value” (E-value) is the number of times that an alignment as good or better than that found by BLAST would be expected to occur by chance, given the size of the database searched.

E = Length of Database X Length of Sequence X Probability

- E-value is dependent on the length of the sequence and the length of the database

As the database grows the E-value will change.

- E-value < 1e-179 is written as 0.0

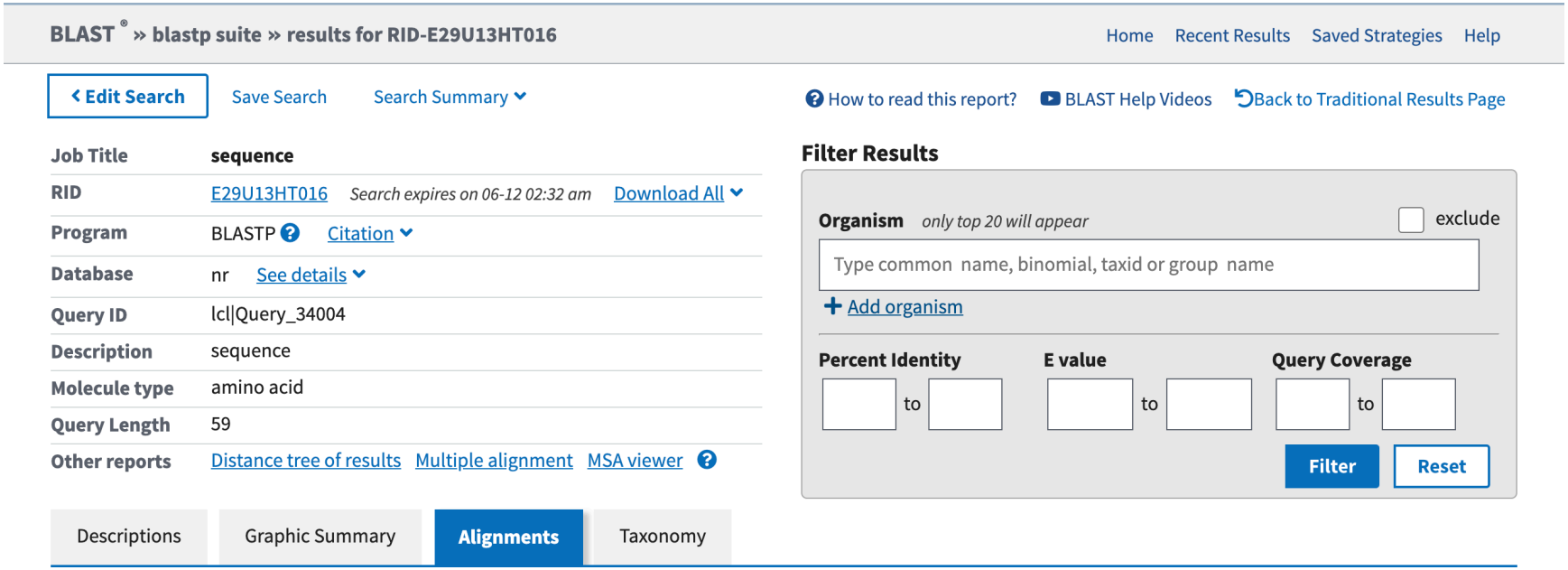
Bit Score

- Bit score (S') is a **normalised** raw score that is independent of the search space.
  - Search space (N) = Query length (n) x Sum of length of sequences in the database (m)
- It measures sequence similarity independent of query sequence length and database size and is normalized based on the raw pairwise alignment score.
  - It is linearly related to the raw score.
  - The higher the bit score, the more significant the match is.
- An alignment may have different E-values if searched against different databases but will have the same bit score.

Take a moment to reflect

1. How are the scores and E-value related? Will a high score give a high or low E-value?
2. Would a longer sequence give a higher or lower E-value?
3. How would an increase in database size affect the E-value?
4. What effect will adjusting the gap penalty have on the alignment?

BLAST Output (1)



BLAST Output (2)

Descriptions | **Graphic Summary** | Alignments | Taxonomy

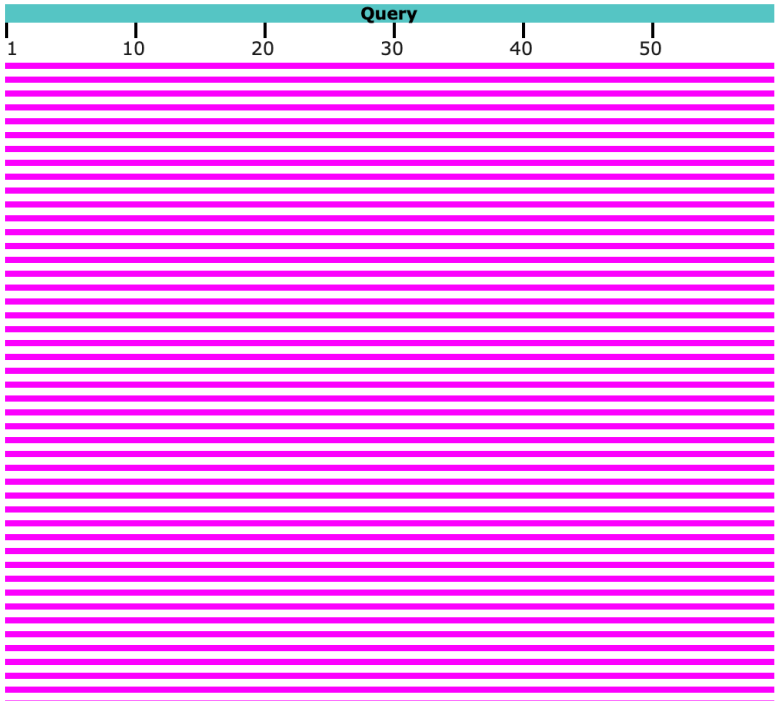
hover to see the title *click to show alignments* *Show Conserved Domains*

Alignment Scores: < 40 | 40 - 50 | 50 - 80 | 80 - 200 | >= 200

100 sequences selected

No putative conserved domains have been detected

Distribution of the top 100 Blast Hits on 100 subject sequences



BLAST Output (3)

Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments Download Manage Columns Show 100

select all *100 sequences selected* GenPept Graphics Distance tree of results Multiple alignment

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
fibrillin 1 (Marfan syndrome), isoform CRA_a [Homo sapiens]	126	126	100%	6e-32	100.00%	EAW77353.1
fibrillin 1 [Homo sapiens]	126	126	100%	6e-32	100.00%	BAD16739.1
fibrillin [Homo sapiens]	126	126	100%	6e-32	100.00%	AAB02036.1
fibrillin-1 preproprotein [Homo sapiens]	126	126	100%	6e-32	100.00%	NP_000129.3
epididymis secretory sperm binding protein [Homo sapiens]	126	126	100%	6e-32	100.00%	ADO22212.1
fibrillin 1 [Homo sapiens]	125	125	100%	8e-32	100.00%	BAD16738.1
fibrillin [Homo sapiens]	124	124	100%	1e-31	100.00%	CAA45118.1
unnamed protein product [Homo sapiens]	124	124	100%	2e-31	100.00%	BAG65498.1
fibrillin 1 [Homo sapiens]	114	114	100%	2e-30	100.00%	BAD16737.1
FBN1 isoform 5 [Pan troglodytes]	124	124	100%	2e-31	98.31%	PNI74996.1
fibrillin-1 [Pan troglodytes]	124	124	100%	2e-31	98.31%	XP_001149266.4
fibrillin-1 [Carlito syrichta]	123	123	100%	7e-31	98.31%	XP_008068093.1
FBN1 isoform 3 [Pan troglodytes]	118	118	100%	1e-30	98.31%	PNI74995.1
fibrillin 1 [Grampus griseus]	116	116	100%	7e-32	96.61%	QEQ26544.1
PREDICTED: LOW QUALITY PROTEIN: fibrillin-1 [Rousettus aegyptiacus]	122	122	100%	7e-31	96.61%	XP_016018715.1
fibrillin-1 isoform X2 [Delphinapterus leucas]	122	122	100%	9e-31	96.61%	XP_022442869.1
fibrillin-1 isoform X2 [Equus caballus]	122	122	100%	9e-31	96.61%	XP_023473665.1

BLAST Output

Descriptions | Graphic Summary | **Alignments** | Taxonomy

Alignment view: Pairwise | Restore defaults | Download

100 sequences selected

Download GenPept Graphics ▼ Next ▲ Previous ◀◀Descriptions

fibrillin 1 (Marfan syndrome), isoform CRA_a [Homo sapiens]

Sequence ID: EAW77353.1 **Length:** 2869 **Number of Matches:** 1

Range 1: 1 to 59 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
126 bits(316)	6e-32	Composition-based stats.	59/59(100%)	59/59(100%)	0/59(0%)

```
Query  1   MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC  59
           MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC
Sbjct  1   MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC  59
```

Related Information
Gene - associated gene details

Download GenPept Graphics ▼ Next ▲ Previous ◀◀Descriptions

fibrillin 1 [Homo sapiens]

Sequence ID: BAD16739.1 **Length:** 2871 **Number of Matches:** 1

Range 1: 1 to 59 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
126 bits(316)	6e-32	Composition-based stats.	59/59(100%)	59/59(100%)	0/59(0%)

```
Query  1   MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC  59
           MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC
Sbjct  1   MRRGRLLEIALGFTVLLASYTSHGADANLEAGNVKETRASRAKRRGGGGHDALKGPNVC  59
```

Related Information
Gene - associated gene details

BLAST Output (nucleotide)

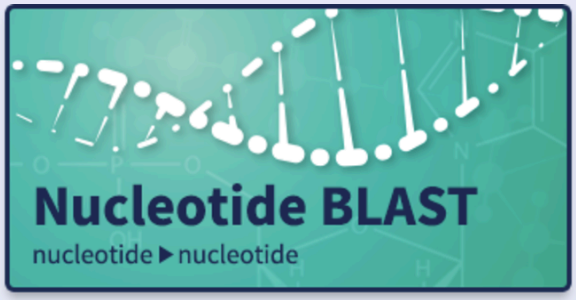
Score	Expect	Identities	Gaps	Strand
366 bits(198)	1e-94	274/311(88%)	4/311(1%)	Plus/Minus

```
Query  48468796  CGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGGCAGGCAGATCACGAGGT  48468855
                 ||||||  | ||||||||||||||||||||||| |||||||||||||||  |||||| ||||
Sbjct  11303     CGGTGGTTTACGCCTGTAATCCCAGCACTTTGAGAGGCCGAGGCAGGCGGATCACAAGGT  11244

Query  48468856  CAGGAGATCGAGACCATCCTGGCTAACACAGTGAAACCTCATCTCTACTAAAAATACAAA  48468915
                 ||||||||  |||||  ||||||||||||||||||||||    ||||||||||||||||||
Sbjct  11243     CAGGAGATTGAGACTGTCCTGGCTAACACAGTGAAACCCTGTCTCTACTAAAAATACAAA  11184

Query  48468916  AAATTAGCCAGGCGTGGTGGTGGGTGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAG  48468975
                 ||||||||   |||  ||||||    ||||||||||||||||||||||||||||||||||
Sbjct  11183     AAATTAGCTGGGCATGGTGGCACATGCCTGTAGTCCCAGCTACTCGGGAGGCTGAGGCAG  11124

Query  48468976  GAGAATGGCATGAACCTGGGAGGCGGAGCTTGCAGTGAGCCAAGATGGTACCACTGTACT  48469035
                 |||||||||||||||||||||| ||||||||||||||||  ||||  |  ||||||  |||
Sbjct  11123     GAGAATGGCATGAACCTGGGAGACGGAGCTTGCAGTGAGCAGAGATTGCGCCACTGCACT  11064

Query  48469036  CCAGCCTGGGCGACAGAGCGAGACTCCGTCTCaaaaaa-aaa--aaaatatatatatata  48469092
                 ||||||||||| |||||||||||||||| |||||||||| |||  |||| | | ||| ||
Sbjct  11063     CCAGCCTGGGTGACAGAGCGAGACTCCATCTCAAAAAACAAAGCAAAACAAA-ATAAATT  11005

Query  48469093  taaaatataat  48469103
                 || |||| |||
Sbjct  11004     TACAATAAAAT  10994
```

Low Complexity Region

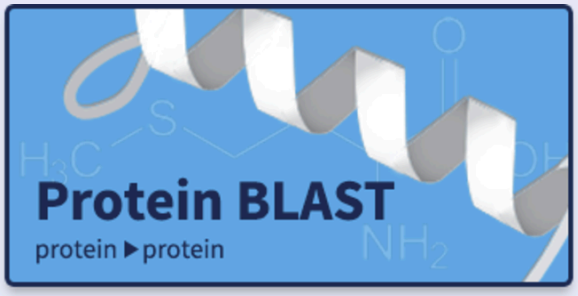
- "Low complexity region" is a region of sequence composed of few kinds of elements (low compositional complexity).
- They might give high scores that confuses the program to find the actual significant sequences in the database.
- Marked with X (protein sequences) or N (nucleotide sequences) and ignored in the BLAST program.
- SEG (proteins) or DUST (nucleotides)

BLAST Algorithms



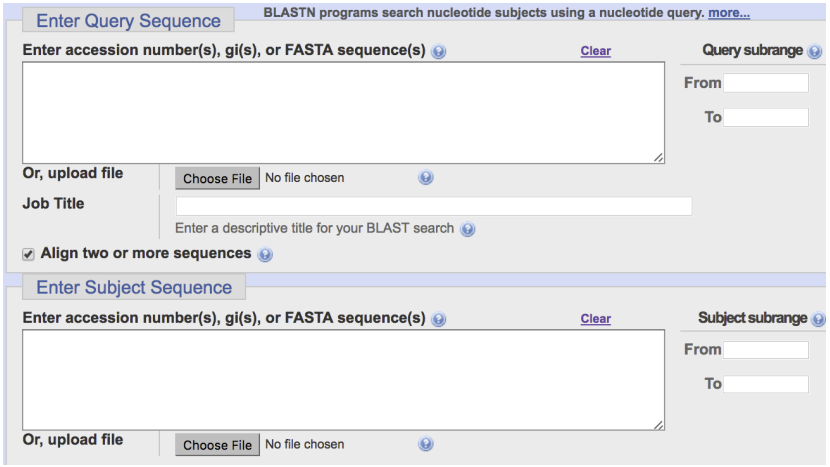
blastx
translated nucleotide ▶ protein

tblastn
protein ▶ translated nucleotide



- (•) Highly similar sequences (megablast)
- ( ) More dissimilar sequences (discontiguous megablast)
- ( ) Somewhat similar sequences (blastn)

- ( ) Quick BLASTP (Accelerated protein-protein BLAST)
- (•) blastp (protein-protein BLAST)
- ( ) PSI-BLAST (Position-Specific Iterated BLAST)
- ( ) PHI-BLAST (Pattern Hit Initiated BLAST)
- ( ) DELTA-BLAST (Domain Enhanced Lookup Time Accelerated BLAST)



Can align two or more sequences in all BLAST algorithms

PSI-BLAST

- Used to find distant relatives of a protein.
- Does an iterative search.
  - First search a regular BlastP
  - Generates a multiple alignment of the HSPs above an E-value threshold and calculates a profile or a Position Specific Scoring Matrix (PSSM)
    - PSSM captures the conservation pattern in the alignment and records it as a scoring matrix
  - In the next iteration this profile is used instead of the original substitution matrix to detect sequences that match the conservation pattern specified by the PSSM
  - After every iteration new sequences above the threshold are added to the the PSSM
- In this way, PSI-BLAST allows detection of distant sequence similarities.

Summary

- The algorithm underlying a BLAST search is complex.
- By understanding it you can adjust the sensitivity and specificity of the search.
- Higher the Bit score the lower the E-value
  - E-value depends on the size of the database and the length of the query.
  - A “significant” E-value of a result will depend on your sequence and goal of the search.
- How to choose amongst the different “flavours” of BLAST for proteins and nucleotides

Useful Resources

- BLAST QuickStart
- BLAST Handbook
- PSI-BLAST
- NCBI – Expect Values, part 1 – video
- NCBI – Expect Values, part 2 – video