Basic Local Alignment Search Tool

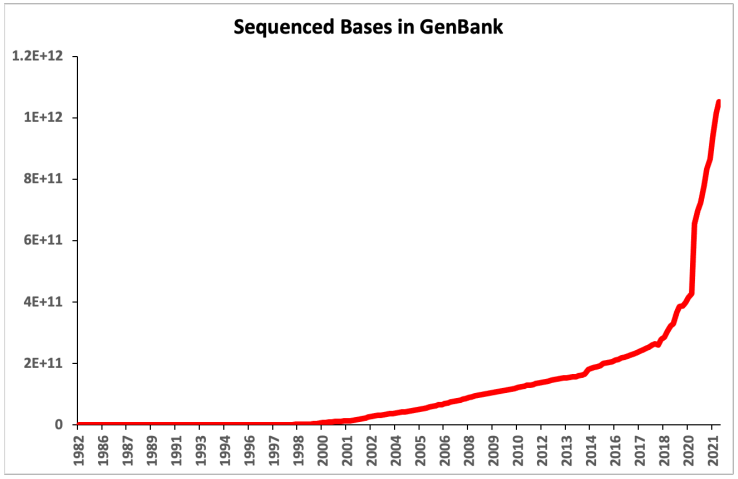
A blast from the past…

AGATCAC
CGACAG

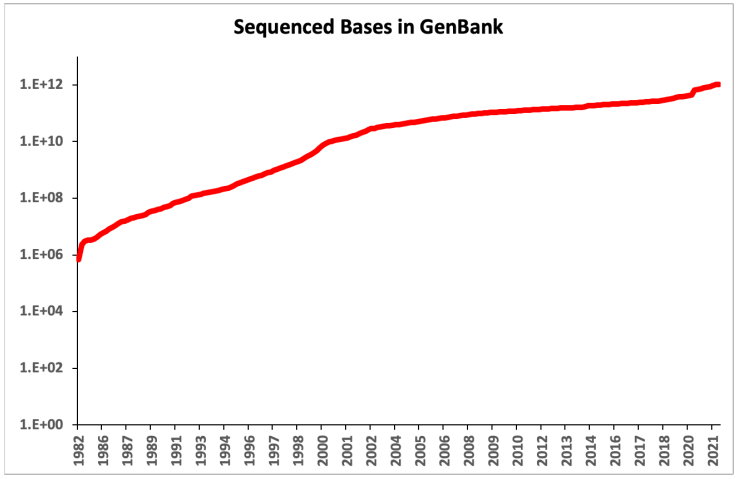
		C	G	A	C	A	G
	0	0	0	0	0	0	0
A	0	0	0	5	0	5	0
G	0	0	5	0	1	0	10
A	0	0	0	10	4	6	4
T	0	0	0	4	6	0	2
C	0	5	0	0	9	3	0
A	0	0	0	5	3	14	8
C	0	5	0	0	10	8	10

GATCA
|| ||
GA-CA

Why BLAST?



Why BLAST?



How Does BLAST Work?

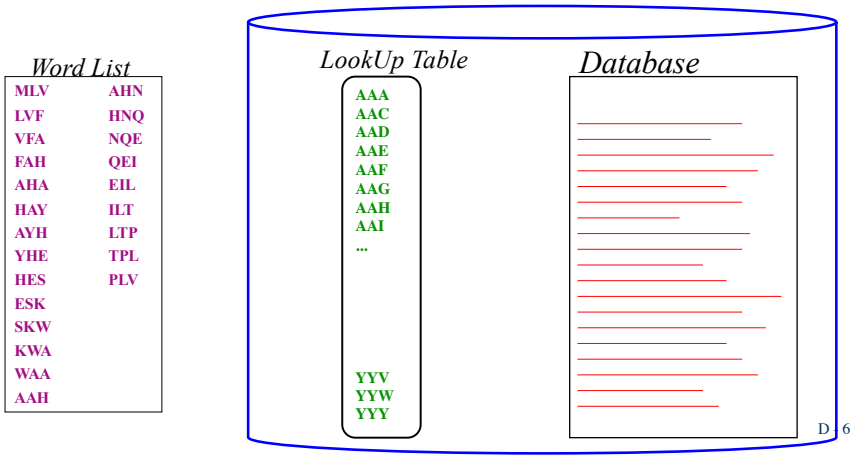
Query sequence

MLVFAHAYHESKWAAHNQEILTPLV

BLAST Example

Query sequence

MLVFAHAYHESKWAAHNQEILTPLV



BLAST Example

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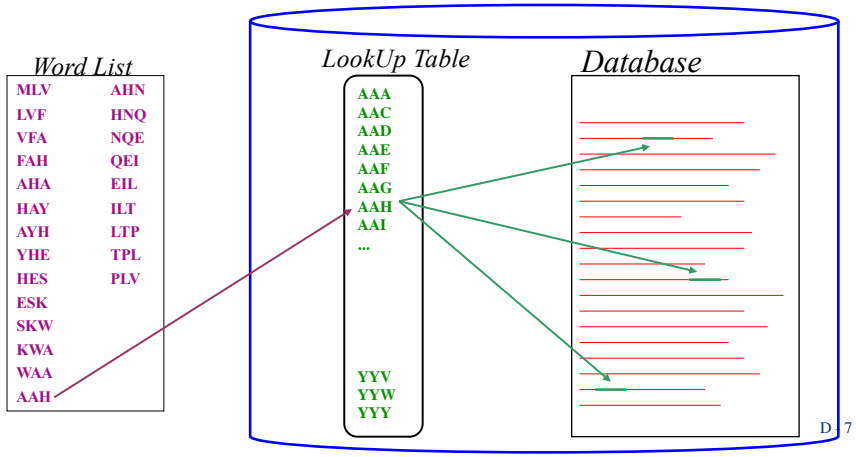
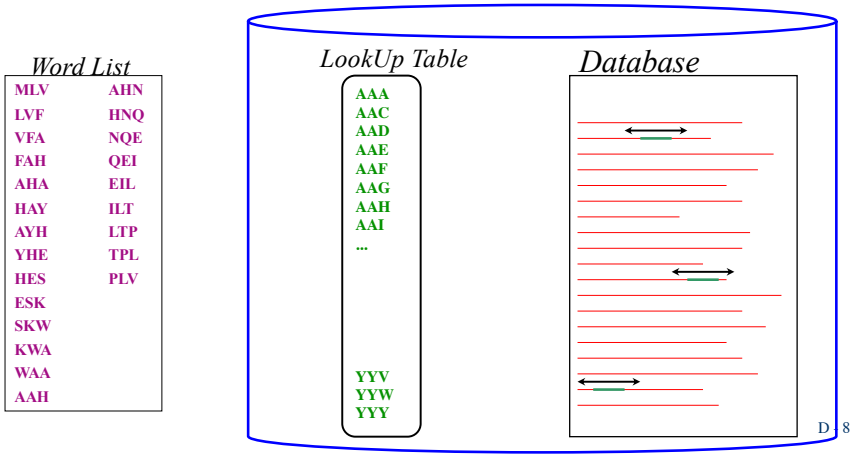


BLAST Example

Query sequence

MLVFAHAYHESKWAAHNQEILTPLV

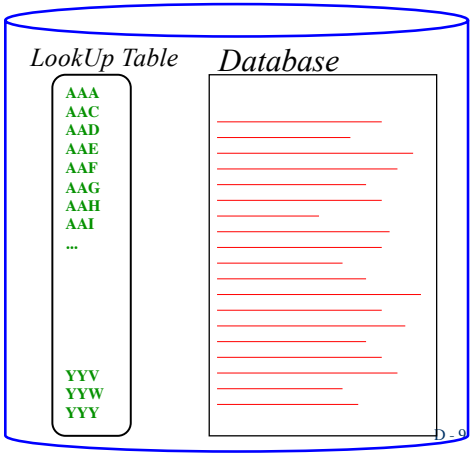


BLAST In a Nutshell

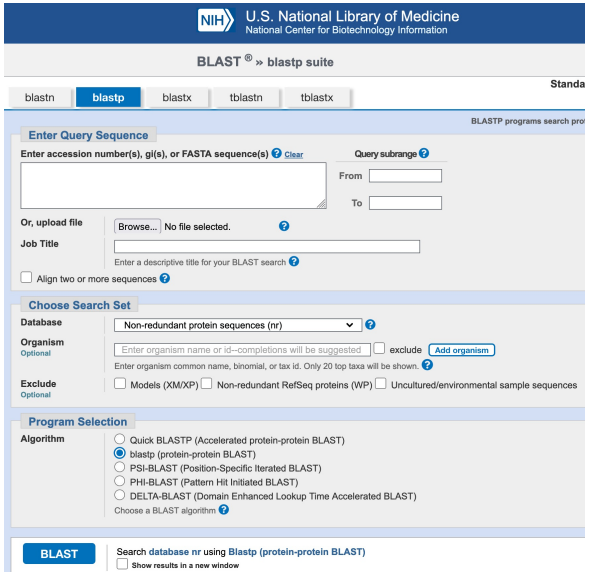
Query sequence

MLVFAHAYHESKWAAHNQEILTPLV

- Create "word list" from query sequence
- Locate *words* in database via "lookup table"
- Determine similarity of query sequence to each word-match sequence in database



BLAST Program



BLAST Output

universal stress protein [Pyrococcus horikoshii]

Sequence ID: WP_048053230.1 **Length:** 167 **Number of Matches:** 1

Range 1: 1 to 152 GenPept Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Method	Identities	Positives	Gaps
96.7 bits(239)	2e-22	Compositional matrix adjust.	62/157(39%)	97/157(61%)	8/157(5%)

```
Query  4    MYKKILYPTDFSETAEIALKHVKAFKTLKAEEVILLHVIDEREIKKRDIFSLLGVAGLNK  63
            M++K+L+PTDFSE A  A++  +    ++  EVILLHVIDE  +++     + G +
Sbjct  1    MFRKVLFPTDFSEGAYRAVEVFEKRNKMEVGEVILLHVIDEGTLEE----LMDGYSFFYD  56

Query  64   SVEEFENELKNKLTEEAKNKMENIKKELEDVGFKVKD---IIVVGIPHEEIVKIAEDEGV  120
            + E    ++K KL EEA  K++   +E++   F+ K+   II  GIP +EIVK+AE+E V
Sbjct  57   NAEIELKDIKEKLKEEASRKLQEKAEEVKR-AFRAKNVRTIIRFGIPWDEIVKVAEEENV  115

Query  121  DIIIMGSHGKTNLKEILLGSVTENVIKKSNKPVLVVK  157
              +II+ S GK +L    LGS    V++K+ KPVL++K
Sbjct  116  SLIILPSRGKLSLSHEFLGSTVMRVLRKTKKPVLIIK  152
```


BLAST Output

Search Parameters	
Program	blastn
Word size	28
Expect value	0.05
Hitlist size	100
Match/Mismatch scores	1,-2
Gapcosts	3,1
Low Complexity Filter	Yes
Filter string	L;m;
Genetic Code	1

Database	
Posted date	Feb 14, 2022 11:51 AM
Number of letters	673,799,021,858
Number of sequences	79,695,929
Entrez query	None

Karlin-Altschul statistics		
Lambda	1.33271	1.32
K	0.620991	0.57
H	1.12409	1

Results Statistics	
Length adjustment	32
Effective length of query	1429
Effective length of database	671248752130
Effective search space	959214466793770
Effective search space used	959214466793770

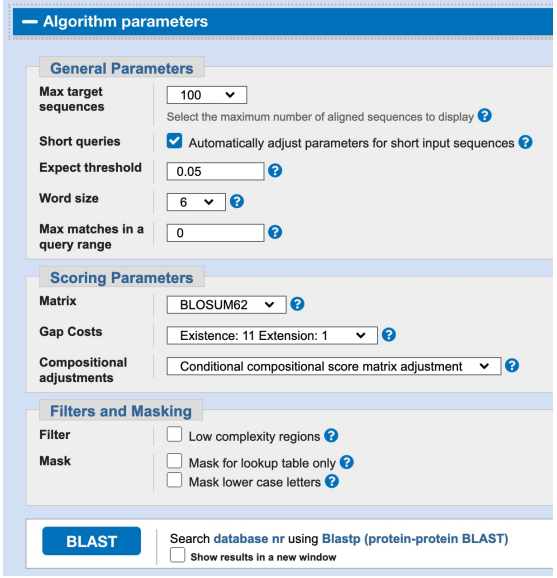
Search Parameters	
Program	blastp
Word size	6
Expect value	0.05
Hitlist size	100
Gapcosts	11,1
Matrix	BLOSUM62
Filter string	F
Genetic Code	1
Window Size	40
Threshold	21
Composition-based stats	2

Database	
Posted date	Feb 12, 2022 2:31 AM
Number of letters	175,661,398,663
Number of sequences	460,231,190
Entrez query	None

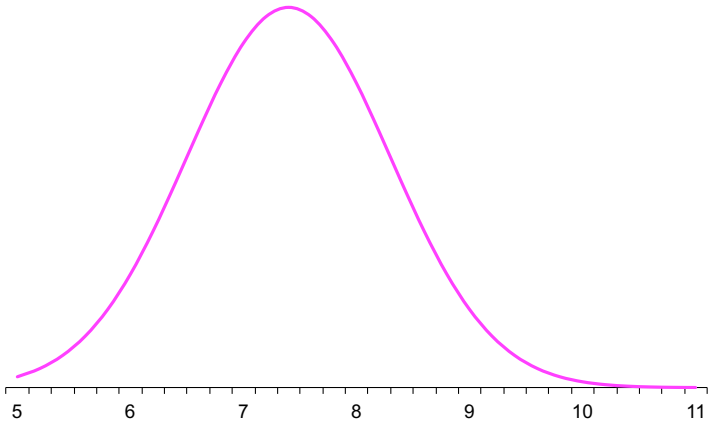
Karlin-Altschul statistics		
Lambda	0.316534	0.267
K	0.135598	0.041
H	0.365158	0.14
Alpha	0.7916	1.9
Alpha_v	4.96466	42.6028
Sigma		43.6362

Results Statistics

BLAST Options

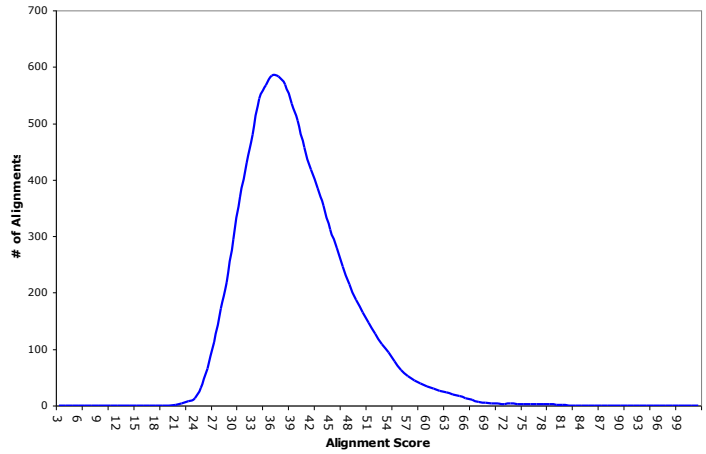


Normal Distributions



The widths of zebra stripes are normally distributed, with a mean of 7.3 centimeters and a standard deviation of 0.9 centimeters.

Extreme Value Distributions



Scores of optimal local alignments correspond to extreme value distributions.

Statistical Significance

Suppose we align two sequences, a query sequence and a target sequence, and we determine that their optimal local alignment score is $S = 60$.

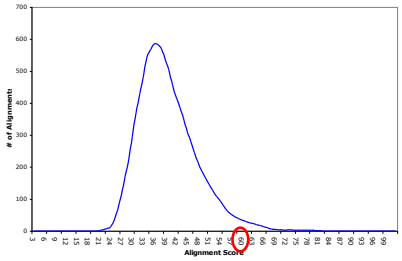
Are the sequences similar? In other words, is a score of $S = 60$ significant? How likely is it that we would observe an alignment score of $S = 60$ by chance?

The *p*-value of an optimal local alignment score, S, is the likelihood that two random sequences* would have an optimal local alignment score greater than or equal to S.

* of the same lengths and compositions as the query and target sequences

p-values for pairs of sequences

What is the probability that the optimal local alignment score for two random sequences will be at least 60?



Solution 1: Count up all of the alignment scores greater than or equal to 60 and divide by the total number of alignment scores, i.e., 10,000.

Solution 2: Plug $x = 60$ into the the following expression, where $\mu = 34.2$ and $\beta = 6.1$

$$1.0 - e^{-e^{-\frac{x-\mu}{\beta}}}$$

p-values for databases

When searching a large database with many target sequences, our previous definition of the *p*-value is problematic because we can expect some small *p*-values by chance. For example, if we align a query sequence to 6,000,000 target sequences in a database, we can expect 60,000 scores with a *p*-value less than 0.01.

When we BLAST a query sequence against a database of many target sequences, the *p*-value of one of the alignment scores, S, indicates the likelihood that we would see a score of at least S when BLASTing the query sequence against a comparable random database.

E-values

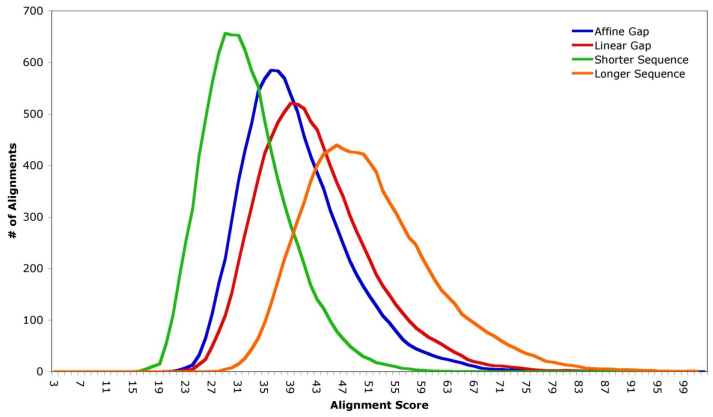
Instead of *p*-values, BLAST reports E-values. If the alignment score of a query sequence and some target sequence in the database is S, the E-value is the expected number of alignments with score S or higher in a random database.

Score	Expect	Method	Identities	Positives	Gaps
40.0 bits(92)	1.4	Compositional matrix adjust.	27/88(31%)	42/88(47%)	3/88(3%)

```
Query  9    MSKGAPWAKGRRGIAVLSRRLAGETSSVAQSTPSYSD---KNLTQPSLNNINAYDSIILS  65
            MSK  P   G RGI + +  +A     + Q+  + S     +LT P+   +  +  ++S
Sbjct  127  MSKAEPVESGERGIIINTASIAAFEGQIGQAAYAASKGAVHSLTLPAARELARHGIRVMS  186

Query  66   MDQSNIGTPLTSGGSDDVQEQGAAGVKF  93
            +     GTP+ SG  DDVQ+  AA   F
Sbjct  187  IAPGLFGTPMLSGLPDDVQDSLAANTPF  214
```


E-values depend on sequences and scoring



Runtime of BLAST?

If *n* is the length of the query sequence and *m* is the length of the target database...

What is the runtime of computing a pairwise alignment?

What is the runtime of BLAST?

How can we improve this runtime?

Linear Alignment