Basic Local Alignment Search Tool

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History of BLAST

- the program was designed by *Stephen W. Altschul*, *Warren Gish*, *Webb Miller*, *Eugene W. Myers* and *David J. Lipman*
- it was published in 1990 in the Journal of Molecular Biology *(J. Mol. Biol.)*
- it is one of the most common and best programs to compare new sequences *(amino acid or DNA)* with existing sequences in a database

global Alignments

- global Alignments completely align two given sequences
- a general technique to determine this type of Alignment is the *Needleman-Wunsch algorithm*
- an example with *GACTA* and *ACT*:

G	A	C	T	A
-	A	C	T	-

local Alignments

- a local Alignment is the best possible Alignment of two *substrings* of two given Sequences
- a general technique to determine this type of Alignment is the *Smith-Waterman algorithm*
- an example with *PQRAXABCSTVQ* and *XYAXBACSLL*:

A	X	A	B	-	C	S
A	X	-	B	A	C	S

- **BLAST** uses local Alignments

Score functions

- scoring functions usually asign numerical values to specific operations which are executed to align the given sequences
- in our case we have to asign values to *match*, *mismatch* and *Indel(Insertion or Deletion)*
- asigned scores (example): *match* = *+1*, *mismatch* = *-1* and *Indel* = *-2*

Score functions

G A C T A
\- A C T -

- the score for the above example would be:
  (-2) + 1 + 1 + 1 + (-2) = -1

important

BLAST does not allow Indels!
For nucleotide sequences **BLAST** uses the following scores
match = +5 mismatch = -4 Indel = NOT ALLOWED

Abstract

- the scoring according to amino acid sequences is more complicated than the scoring for nucleotide sequences
- $\longrightarrow$ development and usage of PAM-matrices
  (PAM = Point Accepted Mutation)
- PAM is a measure for the evolutionary distance between two amino acid sequences
- the developed matrices reflect the physiochemical properties of specific groups of amino acids

A matrix for DNA-sequences

	A	T	C	G
A	5	-4	-4	-4
T	-4	5	-4	-4
C	-4	-4	5	-4
G	-4	-4	-4	5

Remark

BLAST uses this score matrix for nucleotide sequences.

The PAM 120 matrix

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

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$$M_{ij} = \begin{cases} \frac{m_j * A_{ij}}{\sum_{(i)} A_{ij}}, & \text{if } i \neq j \\ 1 - m_j, & \text{otherwise} \end{cases}$$

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Methods

- maximal segment pair *(MSP)*
- local maximal segment pair *(LMSP)*
- approximation of MSP scores
- implementation

MSP

Definition

A MSP is the highest scoring pair of identical length segments chosen from two sequences.

- variable boundaries $\longrightarrow$ any length
- MSP provides a measure of local similarity
- allows the estimation of the statistical significance of the calculated scores under an appropriate *random sequence model*
  $\longrightarrow$ tractability to mathematical analysis

LMSP

Definition

A segment pair is locally maximal if its score cannot be improved either by extending or by shortening both segments.

Approximation of MSP scores

Definition

Let S be the threshold of the estimated MSP scores.

Definition

Let a word pair be a segment pair of fixed length w.

Definition

Let T be the threshold of the estimated word pair scores.

Approximation of MSP scores

- a scientist is only interested in those sequence entries with MSP scores over some cutoff score S
  1. sequences with high similarity
     $\longrightarrow$ biologically significant
  2. sequences with borderline scores
     $\longrightarrow$ helpful in distinguishing biologically interesting relationships
- **BLAST** searches the database for segments that contain a word pair with a score of at least T
  $\longrightarrow$ seeking for a word pair of fixed length w instead of searching the whole query minimizes time

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Implementation

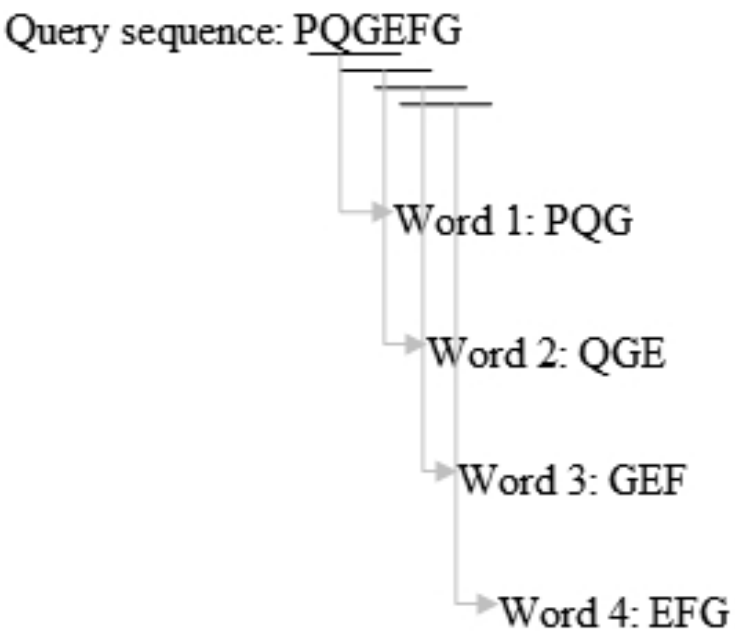
Algorithm

Three algorithmic steps have to be implemented:

1. compiling a list of high-scoring words from the query sequence
2. scanning the database for hits
3. extension of the hits

1. Creating the list of words

- example sequence: *PQGEFG*
- value for $w = 3$



1. Creating the list of words

- all the generated w-words from the query will be compared with all possible w-words and the individual scores will be calculated
- only those words with a score above or equal the threshold T are stored
- example: assume the first word of the previous slide $\longrightarrow$ PQG
- a comparison with PEG and PQA leeds to the scores 15 and 12
- if T is 13 PEG will be stored and PQA will be abandoned

1. Creating the list of words

Problem

DNA sequences are highly non-random, with locally biased base composition (e.g. A+T-rich regions), and repeated sequence elements and this has important consequences for the design of a DNA database search tool.
$\longrightarrow$ a database search will produce a copious output of matches with little interest

Solution: words generated by repetitive or unbalanced regions are removed from the query word list

2. Scanning the database

- the database is scanned for w-words that pair with w-words of the generated list of the query $\rightarrow$ BLAST-hits
- example for the query sequence: *PQGEFG*
- example for the database sequence: *PEGVVG*
- scanning process:

Query-sequence	P	Q	G	E	F	G
Database-sequence	**P**	**E**	**G**	V	V	G

2. Scanning the database

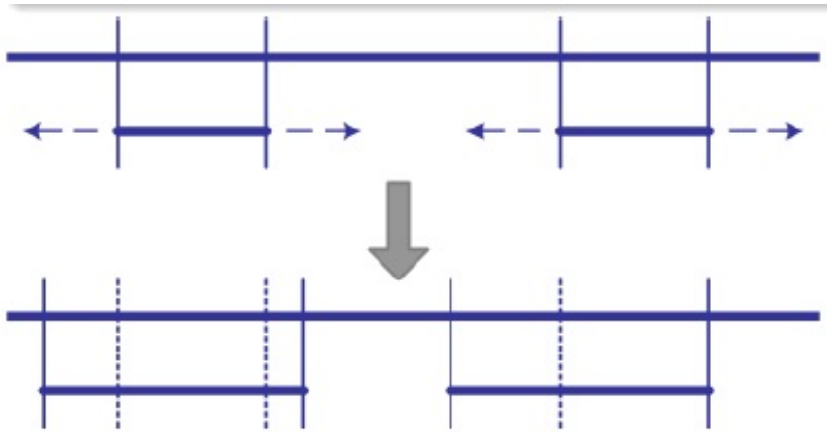
Problem

The scan of the database arouses a classic algorithmic problem, i.e. search a long sequence for all occurrences of certain short sequences.

There exist two main approaches for solving this problem:

1.
   - map each word to an integer between 1 an 20^w
   - a word can be uses as an index for an array
   - each database word leads directly to the corresponding hits
2.
   - usage of a deterministic finite automaton or finite state machine
     $\rightarrow$ utilization in BLAST because of efficiency

3. Extension of the found hits



- the hits from the database are extended in both directions
- the extensions terminate when a segment pair whose score falls a certain distance below the best score found for shorter extensions is found

Statistical significance

BLAST is able to return a value for the statistical significance of a found and extended hit in the database.

- when two sequences are compared the probability of finding a segment pair with a score greater or equal S is:

$$1 - e^{-(Kmne^{-\lambda S})}$$

- the probability of finding c or more distict segment pairs with a score greater or equal S is:

$$1 - e^{-(Kmne^{-\lambda S})} \sum_{i=0}^{c-1} \frac{y^i}{i!}$$

Complexity

Complexity

The expected-time computational complexity of the BLAST-algorithm is approximately:

$$\mathcal{O}(aW + bN + \frac{cNW}{20^w})$$

where:

W = the number of words generated

N = the number of residues in the database

a,b,c = constants

- W-term accounts for compiling the word list
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Performance of BLAST with "random sequences"

- the main question with regard to the performance of the algorithm is the selection of values for w, T and S
- therefore *Altschul et al.* generated one million pairs of "random protein sequences" of length 250 and subsequently searched the MSP for each using the PAM-120 matrix
- according to their investigations the most balancing values for w and T are:

	DNA-sequences	Protein-sequences
w	12	4
T	17	17

Performance of BLAST with "random sequences"

Results for the comparison of sequences with different w- and T-values

w	T	Implied % of MSPs missed by BLAST when S equals						
		45	50	55	60	65	70	75
3	14	20	16	12	10	8	6	5
4	16	18	14	11	8	6	5	4
	17	28	23	19	16	13	11	9
5	18	20	15	12	9	7	5	4

According to these results one may assume that the best value for T would be either 14 or 16.

Performance of BLAST with "random sequences"

- the number of words in the query-word list increases exponentially with decreasing T
- the values 14 or 16 for T lead to a crucial higher amount of words in the query-word list than a value of 17

Remark

If the amount of words in the query-word list increases it subsequently increases the needed CPU time exponentially.

$\rightarrow$ $T = 17$ balances the needed CPU time and the amount of probably missed MSPs

Performance of BLAST with homologous sequences

- comparison of proteins with other members of their respective superfamilies
  $\longrightarrow$ computing the true MSP scores
  $\longrightarrow$ computing the BLAST approximation with $w = 4$ and various settings of T
  $\Rightarrow$ comparison of the results with the ones from the previous random model
- two tests with BLAST:
  1. searching the globins with woolly monkey myoglobin
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Performance of BLAST with homologous sequences

Performance on real data

In general, the distribution of mutations along sequences has been shown to be more clustered than predicted by a Poisson process, and thus the BLAST approximation should, on average, **perform better on real sequences than predicted by the random model**.

Performance of BLAST with two long DNA sequences

- comparison of a 73.360bp section of the human genome containing the β-like globin gene cluster with a corresponding 44.595bp section of the rabbit genome with $w = 12$
- the pair exhibits three main classes of locally similar regions

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