Heuristic Methods for Sequence Database Searching

BMI/CS 576

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Heuristic alignment motivation

- $O(mn)$ too slow for large databases with high query traffic
- heuristic methods do fast approximation to dynamic programming
  - FASTA [Pearson & Lipman, 1988]
  - BLAST [Altschul *et al*., 1990; Altschul et al., *Nucleic Acids Research* 1997]

Heuristic alignment motivation

- consider the task of searching UnitProtKB/Swiss-Prot against a query sequence:
  - say our query sequence is 362 amino-acids long
  - most recent release of DB contains 188,719,038 amino acids
  - finding local alignments via dynamic programming would entail $O(10^{11})$ matrix operations

- many servers handle thousands of such queries a day (NCBI > 500,000)

Heuristic alignment

- heuristic algorithm: a problem-solving method which isn’t guaranteed to find the optimal solution, but which is efficient and finds good solutions

- key heuristics in BLAST
  - look for seeds of high scoring alignments
  - use dynamic programming selectively

- key tradeoff made: sensitivity vs. speed

$$\text{sensitivity} = \frac{\#\ \text{significant matches detected}}{\#\ \text{significant matches in DB}}$$

Overview of BLAST (Basic Alignment Search Tool)

- given: query sequence *q*, word length *w*, word score threshold *T*, segment score threshold *S*
  - compile a list of “words” (of length *w*) that score at least *T* when compared to words from *q*
  - scan database for matches to words in list
  - extend all matches to seek high-scoring alignments
- return: alignments scoring at least *S*

Determining query words

Given:

query sequence: QLNFSAGW

word length *w* = 2 (default for protein usually *w* = 3)

word score threshold *T* = 9

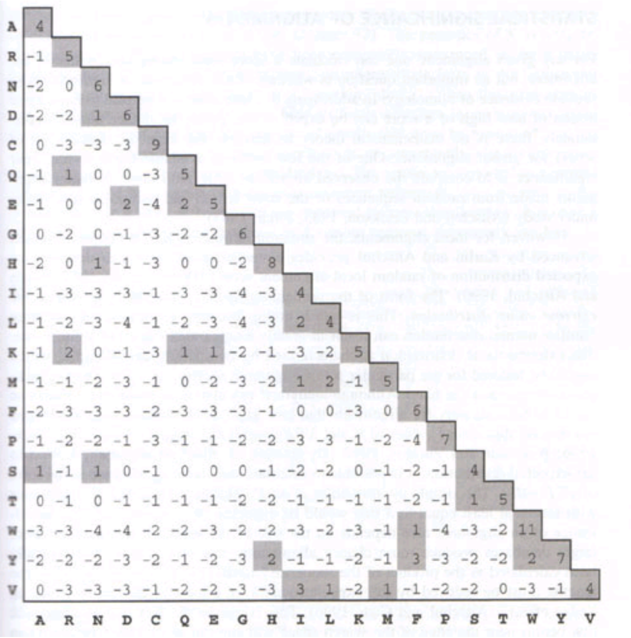
Step 1: determine all words of length *w* in query sequence

QL LN NF FS SA AG GW

Determining query words

Step 2: determine all words that score at least *T* when compared to a word in the query sequence

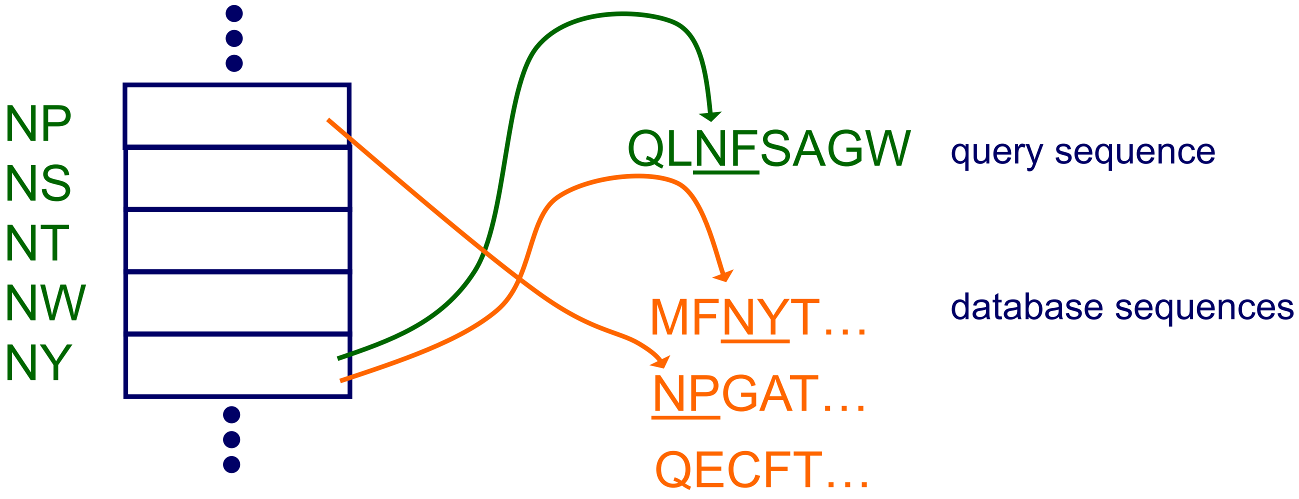
words from sequence	query words w/ T≥9
QL	QL=9
LN	LN=10
NF	NF=12, NY=9
…	
SA	none
…	



	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

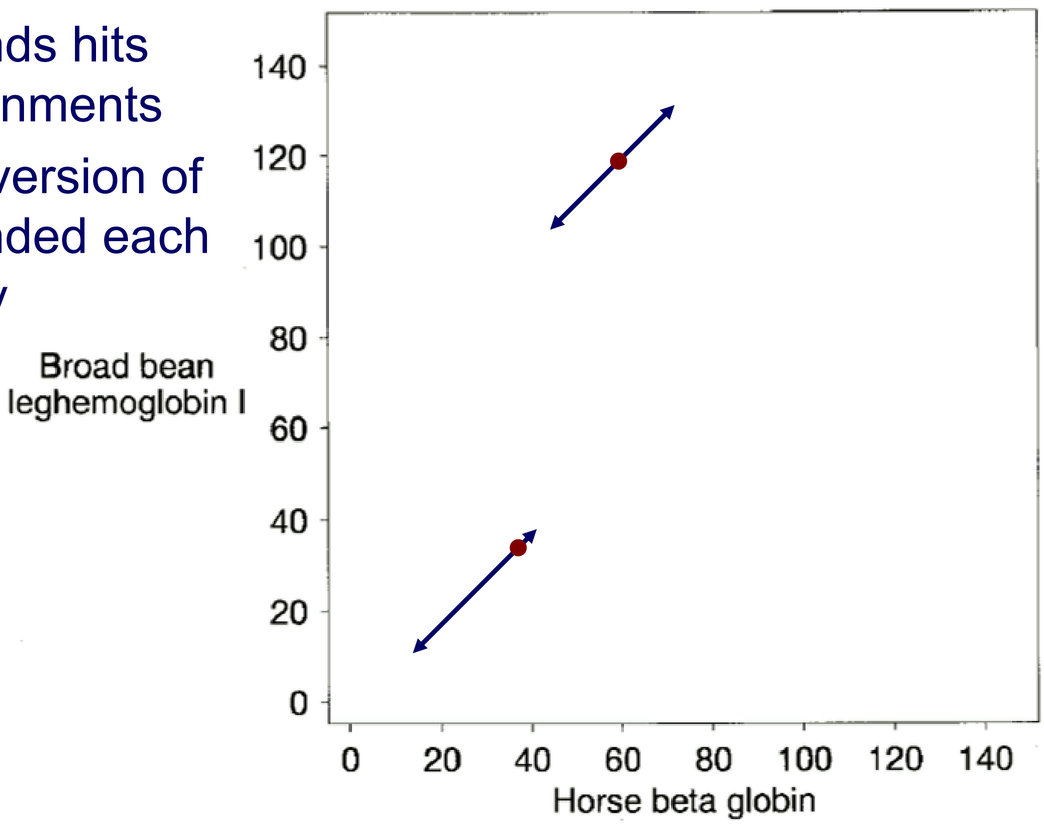
Scanning the database

- search database for all occurrences of query words
- approach:
  - – index database sequences into table of words (pre-compute this)
  - – index query words into table (at query time)



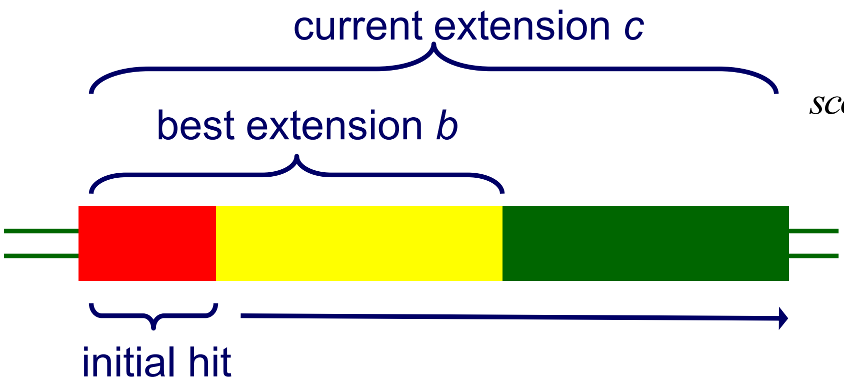
Extending hits

- BLAST extends hits into local alignments
- The original version of BLAST extended each hit separately



Extending hits in original BLAST

- extend hits in both directions (without allowing gaps)
- terminate extension in one direction when score falls certain distance below best score for shorter extensions



$score(c) \geq score(b) - \varepsilon$?

- return segment pairs scoring at least *S*

Sensitivity vs. running time

- the main parameter controlling the sensitivity vs. running-time trade-off is *T* (threshold for what becomes a query word)
  - small *T*: greater sensitivity, more hits to expand
  - large *T*: lower sensitivity, fewer hits to expand

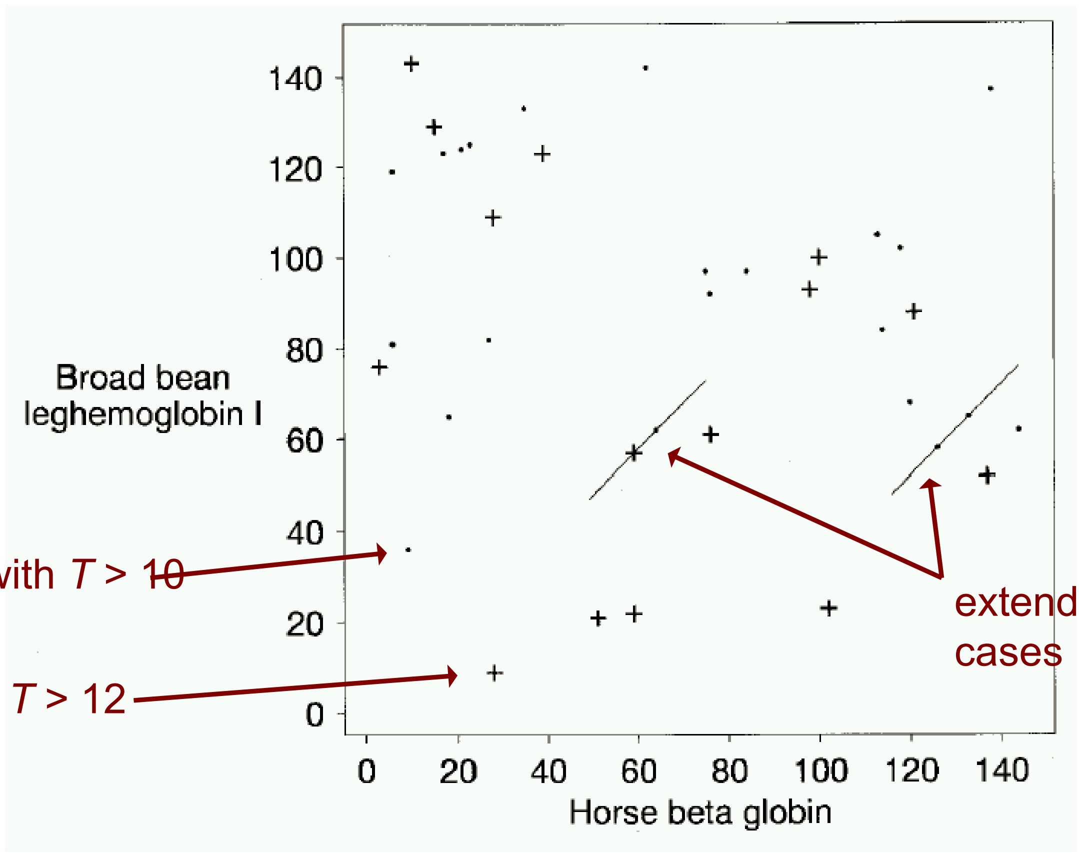
The two-hit method

- extension step typically accounts for 90% of BLAST’s execution time

- key idea: do extension only when there are two hits on the same diagonal within distance A of each other

- to maintain sensitivity, lower *T* parameter
  - more single hits found
  - but only small fraction have associated 2nd hit

The two-hit method



❻•❼ hits with *T* > 10

'+' hits with *T* > 12

extend these cases

Figure from: Altschul et al. *Nucleic Acids Research* 25, 1997

Gapped BLAST

- trigger gapped alignment if two-hit extension has a sufficiently high score
- find length-11 segment with highest score; use central pair in this segment as seed
- run DP process both forward & backward from seed
- prune cells when local alignment score falls a certain distance below best score yet

Gapped BLAST

filled cells show alignment pairings considered

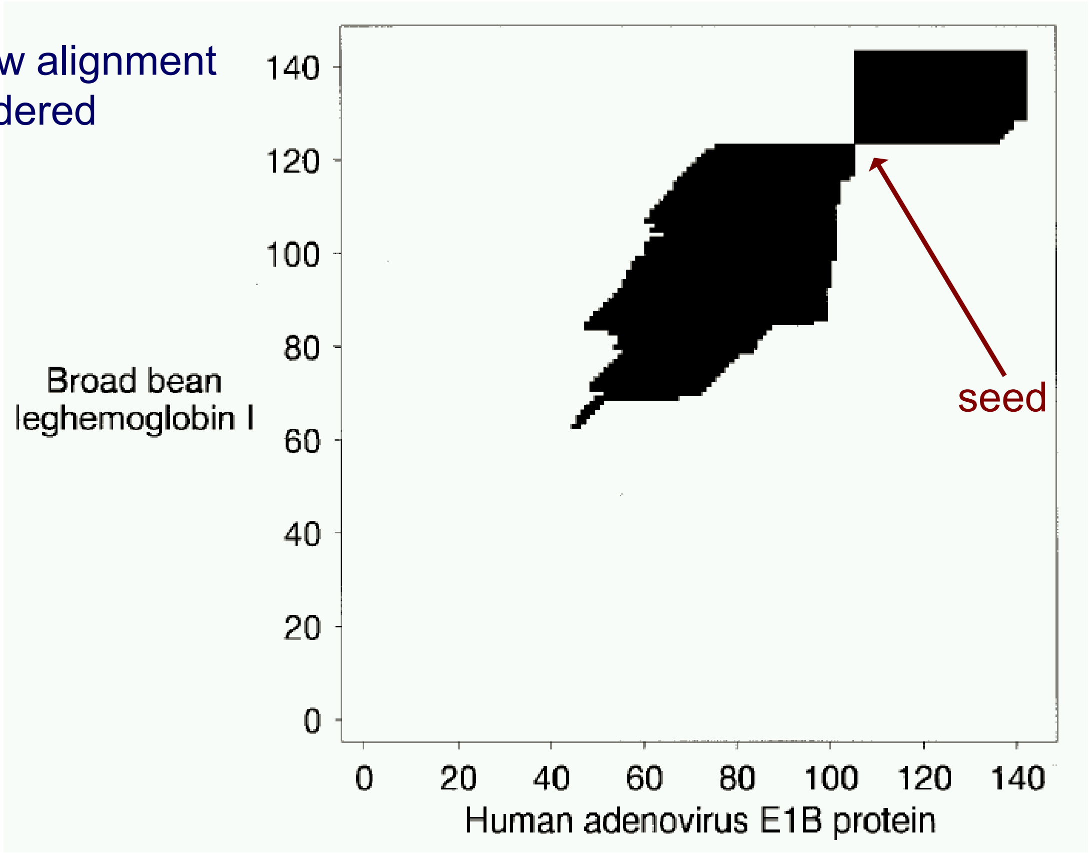


Figure from: Altschul et al. *Nucleic Acids Research* 25, 1997

PSI (*Position Specific Iterated*) BLAST

- basic idea
  - use results from BLAST query to construct a *profile matrix*
  - search database with profile instead of query sequence
  - iterate

A profile matrix

sequence positions

amino acids

	1	2	3	4	5	6	7	8
A			-2.4					
R			1.2					
D			0.5					
N			-0.2					
C			-3.1					

•••

⋮

PSI BLAST: searching with a profile

- aligning profile matrix to a simple sequence
  - like aligning two sequences
  - except score for aligning a character with a matrix position is given by the matrix itself – not a substitution matrix

sequence C N A R •••

profile

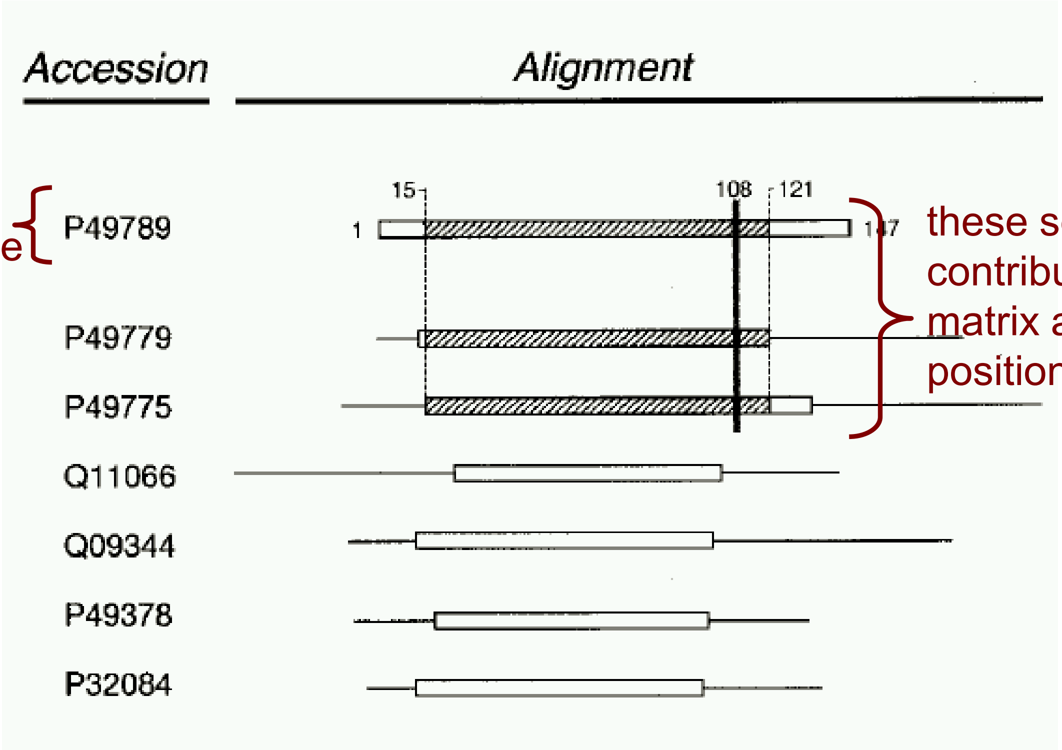
	C	N	A	R				
A			■					
R				■				
D								
N		■						
C	■							

•••

⋮

PSI BLAST:
constructing the profile matrix

query sequence

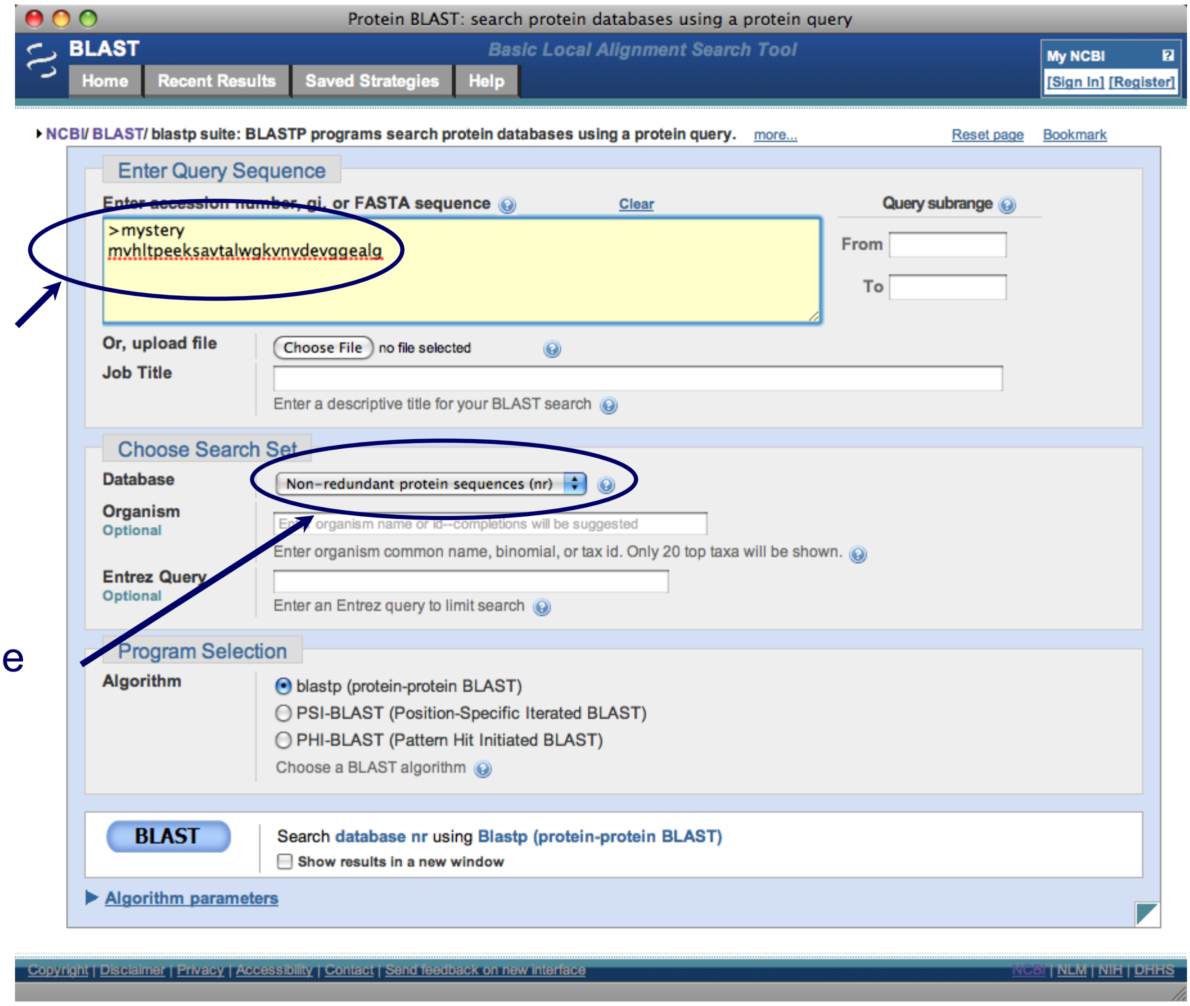


these sequences contribute to the matrix at position 108

Figure from: Altschul et al. Nucleic Acids Research 25, 1997

query

database



Protein BLAST: search protein databases using a protein query

NCBI/ BLAST/ blastp suite: BLASTP programs search protein databases using a protein query. more...

Enter Query Sequence

Enter accession number, gi, or FASTA sequence

```
>mystery
mvhltpeeksavtalwgkvnvdevggealg
```

Query subrange

From

To

Or, upload file: no file selected

Job Title

Enter a descriptive title for your BLAST search

Choose Search Set

Database: Non-redundant protein sequences (nr)

Organism Optional: Enter organism name or id--completions will be suggested

Enter organism common name, binomial, or tax id. Only 20 top taxa will be shown.

Entrez Query Optional

Enter an Entrez query to limit search

Program Selection

Algorithm

- ● blastp (protein-protein BLAST)
- ○ PSI-BLAST (Position-Specific Iterated BLAST)
- ○ PHI-BLAST (Pattern Hit Initiated BLAST)

Choose a BLAST algorithm

Search database nr using Blastp (protein-protein BLAST)

□ Show results in a new window

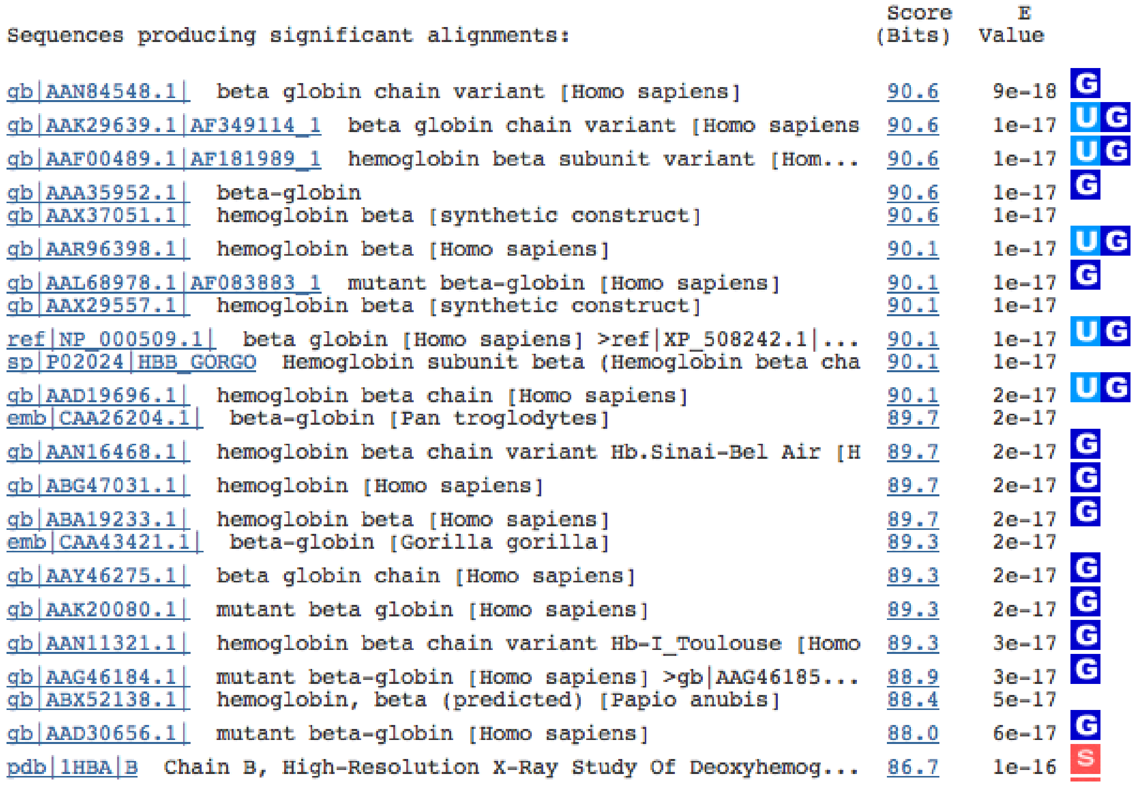
Algorithm parameters

www.ncbi.nlm.nih.gov/BLAST/

BLAST programs

Program	Query	Database
BLASTP	Protein	Protein
BLASTN	DNA	DNA
BLASTX	Translated DNA	Protein
TBLASTN	Protein	Translated DNA
TBLASTX	Translated DNA	Translated DNA

BLAST results



```
                                                                 Score    E
Sequences producing significant alignments:                      (Bits)  Value

gb|AAN84548.1|  beta globin chain variant [Homo sapiens]          90.6    9e-18  G
gb|AAK29639.1|AF349114_1  beta globin chain variant [Homo sapiens  90.6    1e-17  UG
gb|AAF00489.1|AF181989_1  hemoglobin beta subunit variant [Hom...  90.6    1e-17  UG
gb|AAA35952.1|  beta-globin                                       90.6    1e-17  G
gb|AAX37051.1|  hemoglobin beta [synthetic construct]             90.6    1e-17
gb|AAR96398.1|  hemoglobin beta [Homo sapiens]                    90.1    1e-17  UG
gb|AAL68978.1|AF083883_1  mutant beta-globin [Homo sapiens]       90.1    1e-17  G
gb|AAX29557.1|  hemoglobin beta [synthetic construct]             90.1    1e-17
ref|NP_000509.1|  beta globin [Homo sapiens] >ref|XP_508242.1|...  90.1    1e-17  UG
sp|P02024|HBB_GORGO  Hemoglobin subunit beta (Hemoglobin beta cha  90.1    1e-17
gb|AAD19696.1|  hemoglobin beta chain [Homo sapiens]              90.1    2e-17  UG
emb|CAA26204.1|  beta-globin [Pan troglodytes]                    89.7    2e-17
gb|AAN16468.1|  hemoglobin beta chain variant Hb.Sinai-Bel Air [H  89.7    2e-17  G
gb|ABG47031.1|  hemoglobin [Homo sapiens]                         89.7    2e-17  G
gb|ABA19233.1|  hemoglobin beta [Homo sapiens]                    89.7    2e-17  G
emb|CAA43421.1|  beta-globin [Gorilla gorilla]                    89.3    2e-17
gb|AAY46275.1|  beta globin chain [Homo sapiens]                  89.3    2e-17  G
gb|AAK20080.1|  mutant beta globin [Homo sapiens]                 89.3    2e-17  G
gb|AAN11321.1|  hemoglobin beta chain variant Hb-I_Toulouse [Homo  89.3    3e-17  G
gb|AAG46184.1|  mutant beta-globin [Homo sapiens] >gb|AAG46185...  88.9    3e-17  G
gb|ABX52138.1|  hemoglobin, beta (predicted) [Papio anubis]       88.4    5e-17
gb|AAD30656.1|  mutant beta-globin [Homo sapiens]                 88.0    6e-17  G
pdb|1HBA|B  Chain B, High-Resolution X-Ray Study Of Deoxyhemog...  86.7    1e-16  S
```

BLAST comments

- it’s heuristic: may miss some good matches
- it’s fast: empirically, 10 to 50 times faster than Smith-Waterman
- PSI-BLAST can detect more distant relationships among protein sequences, but the process of generalizing the query can also lead it astray
- large impact:
  - NCBI’s BLAST server handles more than 500,000 queries a day
  - most used bioinformatics program in the world