Introduction to Bioinformatics

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Topics

- Introduction
- Biological Sequence Alignment and Database Search
- Analysis of gene expression data

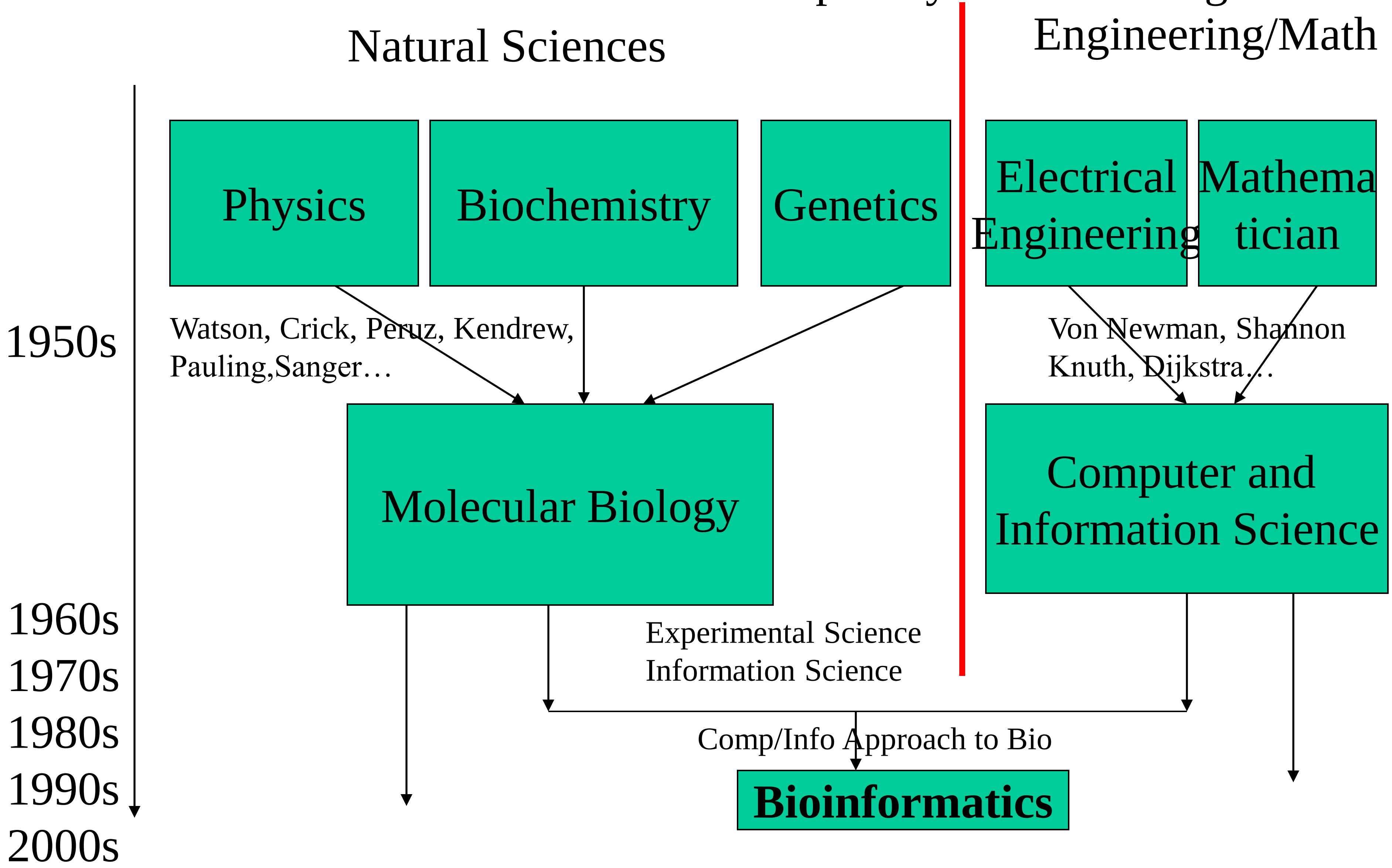
What’s Bioinformatics?

An interdisciplinary science of developing and applying computational techniques to address problems in molecular biology

- Develop bioinformatics algorithms and tools
- Apply bioinformatics tools to address biological problems

History of Bioinformatics

How does a new interdisciplinary science emerge?



Natural Sciences

Engineering/Math

Physics

Biochemistry

Genetics

Electrical Engineering

Mathematician

1950s

Watson, Crick, Peruz, Kendrew,
Pauling,Sanger…

Von Newman, Shannon
Knuth, Dijkstra…

Molecular Biology

Computer and Information Science

1960s

1970s

1980s

1990s

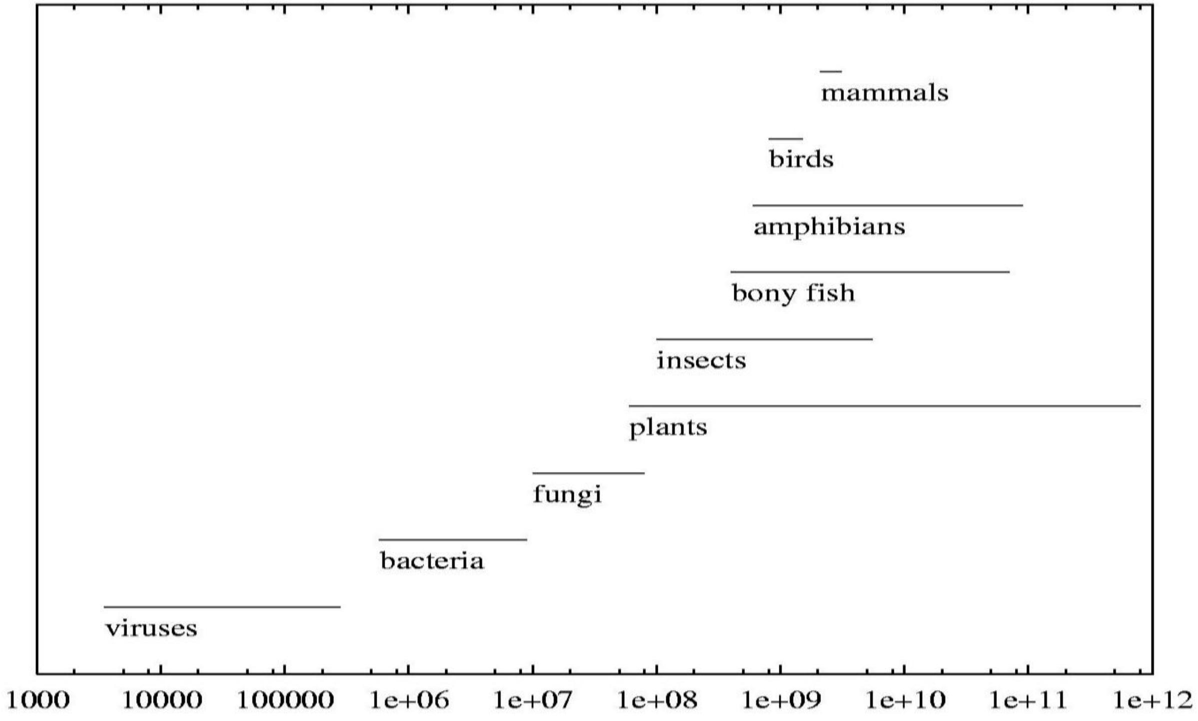
2000s

Experimental Science
Information Science

Comp/Info Approach to Bio

Bioinformatics

Genome Sequencing

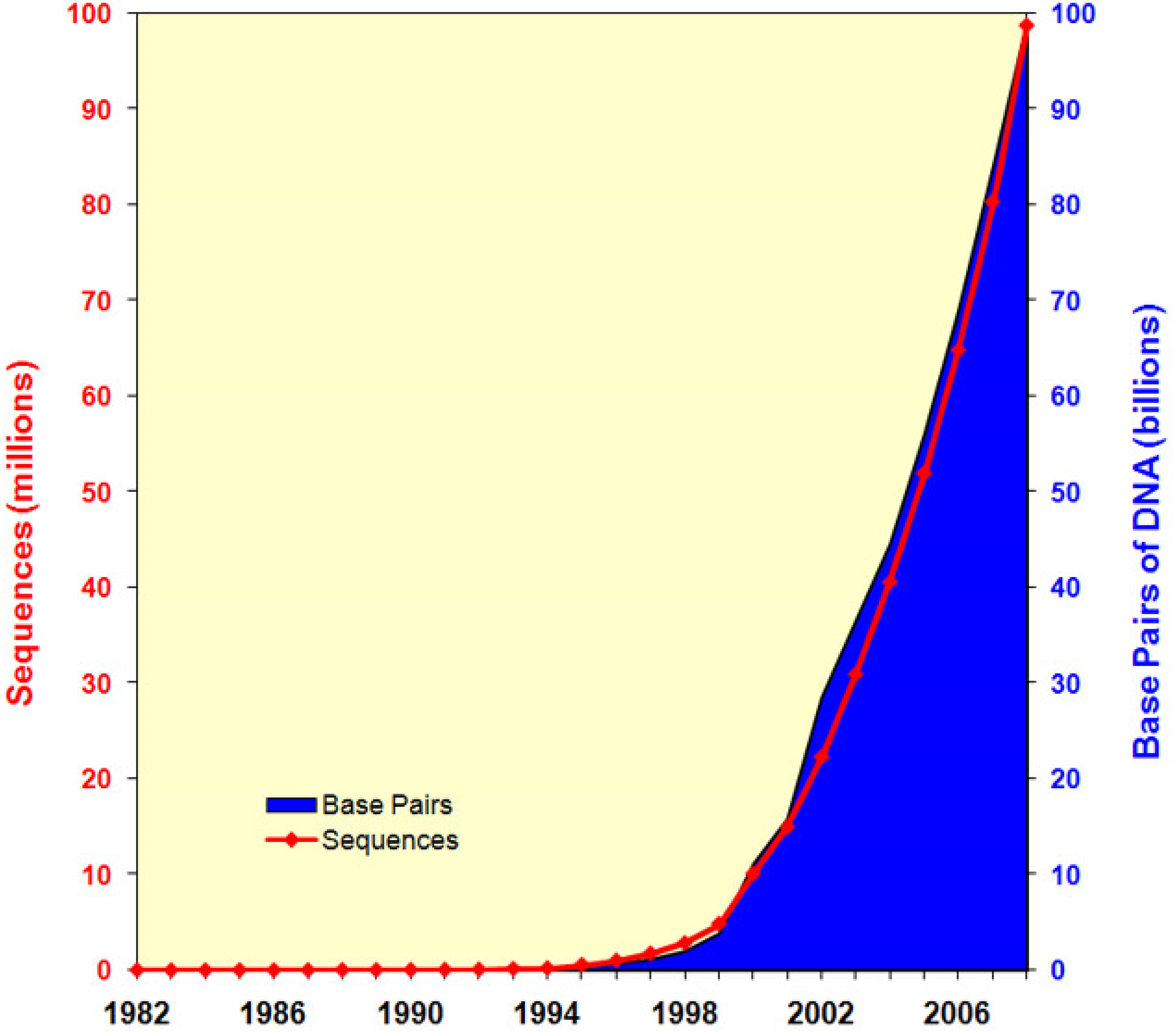


High-Throughput Sequencing

- Transcriptome (EST, RNA-Seq, Chip-Seq)
- Proteomics (Mass Spectrometry)
- Metabolomics

Growth of GenBank

(1982 - 2008)



What can we do with these huge amount of data?

WHY ARE YOU DIGGING A HOLE?
I'M LOOKING FOR BURIED TREASURE!
WHAT HAVE YOU FOUND?
A FEW DIRTY ROCKS, A WEIRD ROOT, AND SOME DISGUSTING GRUBS.
ON YOUR FIRST TRY??
THERE'S TREASURE EVERYWHERE!
WATTERSON

Find buried treasure - Doug Brutlag, 1999.

Typical Bioinformatics Problems

- What family does this gene / protein belong to?
- Are there other known homologous proteins?
- What is the function and structure of this protein?
- What biological pathway does this protein participate in?
- Is a mutation on a gene / protein related to a phenotype or disease?
- Is a gene differentially expressed in a biological condition?

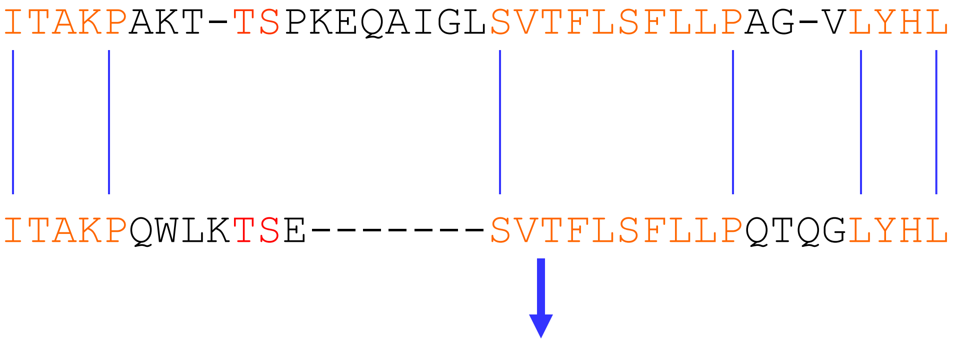
Fundamental Problems: Sequence Comparison

- Why do we compare sequences?
- What’s similarity between two sequences?
- How to compare sequences?
- Is similarity significant?

Importance of Similarity Comparison

- Identify evolutionary relationship between genes and proteins
- Similar genes/proteins have similar function
- Similar proteins have similar structures

Global Pairwise Sequence Alignment



```
ITAKPAKT-TSPKEQAIGLSVTFLSFLLPAG-VLYHL

ITAKPQWLKTSE-------SVTFLSFLLPQTQGLYHL
```

Alignment (similarity) score

Three Main Issues

1. Definition of alignment score
2. Algorithms of finding the optimal alignment
3. Evaluation of significance of alignment score

A simple scoring scheme

- Score of character pair: S(match)=1, S(not_match) = -1, S(gap-char) = -1
- Score of an alignment = $\sum_{1}^{n} S_i$

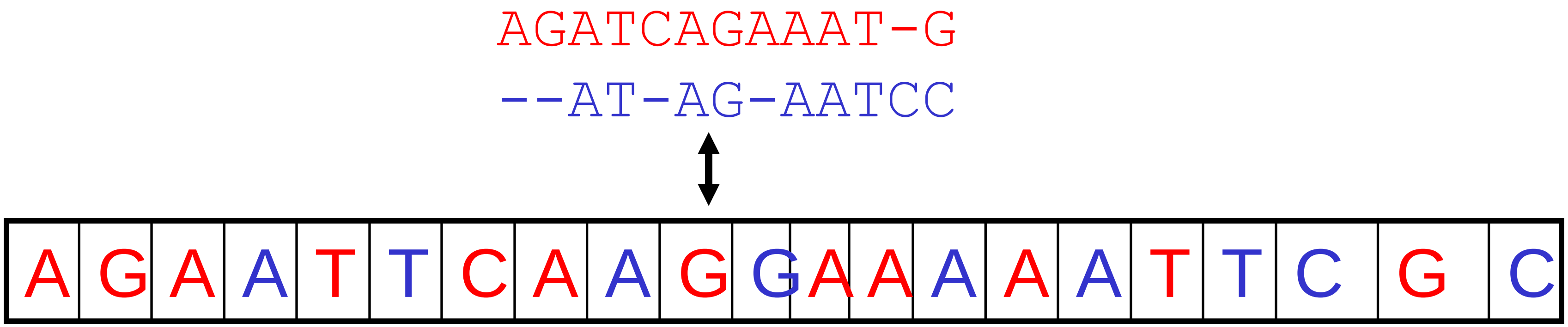
```
ITAKPAKTPTSPKEQAIGLSVTFLSFLLPAGWVLYHL
ITAKPQWLKSTE-------SVTFLSFLLPQTQGLYHL
```

5 – 7 – 7 +10 -4 + 4= 1

Optimization

- How can we find the best alignment to maximize alignment score?
- How many possible alignments exist for two sequences with length m and n?

Total Number of Possible Alignments



```
AGATCAGAAAT-G
--AT-AG-AATCC
```

A	G	A	A	T	T	C	A	A	G	G	A	A	A	A	A	T	T	C	G	C

m + n

Total Number of Alignments

Select m positions out of *m+n* possible positions:

$$\binom{m+n}{m} = \frac{(m+n)!}{m!n!}$$

Exponential!

If m = 300, n = 300, total = 10^{37}

Divide and Conquer

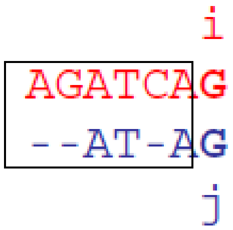
Goal: align prefix P[1..i] and prefix Q[1..j]

```
                  i
Seq P: AGATCAGAAATGG
Seq Q: ATAGAATCC
              j
```

Three possibilities assuming we know the optimal alignment of smaller prefixes:

Case 1

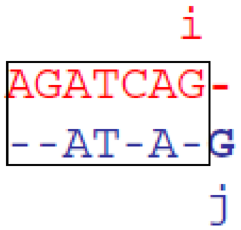
Use alignment of P[1..i-1] and Q[1..j-1], pair P[i] and Q[j]



```
      i
AGATCAG
--AT-AG
      j
```

Case 2

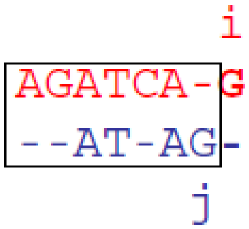
Use alignment of P[1..i] And Q[1..j-1], pair Q[j] with gap



```
      i
AGATCAG-
--AT-A-G
       j
```

Case 3

Use alignment of P[1..i-1] and Q[1..j], pair P[i] with gap.



```
       i
AGATCA-G
--AT-AG-
      j
```

Needleman and Wunsch Algorithm

- Given sequences P and Q, we use a matrix M to record the optimal alignment scores of all prefixes of P and Q. M[i,j] is the best alignment score for the prefixes P[1..i] and Q[1..j].
- **M[i,j] =**

max [

M[i-1,j-1] + S(P[i],Q[j]),

M[i,j-1] + S(-, Q[j])

M[i-1,j] + S(P[i], -)

]

Dynamic Programming

Dynamic Programming Algorithm

Three-Step Algorithm:

- Initialization
- Matrix fill (scoring)
- Trace back (alignment)

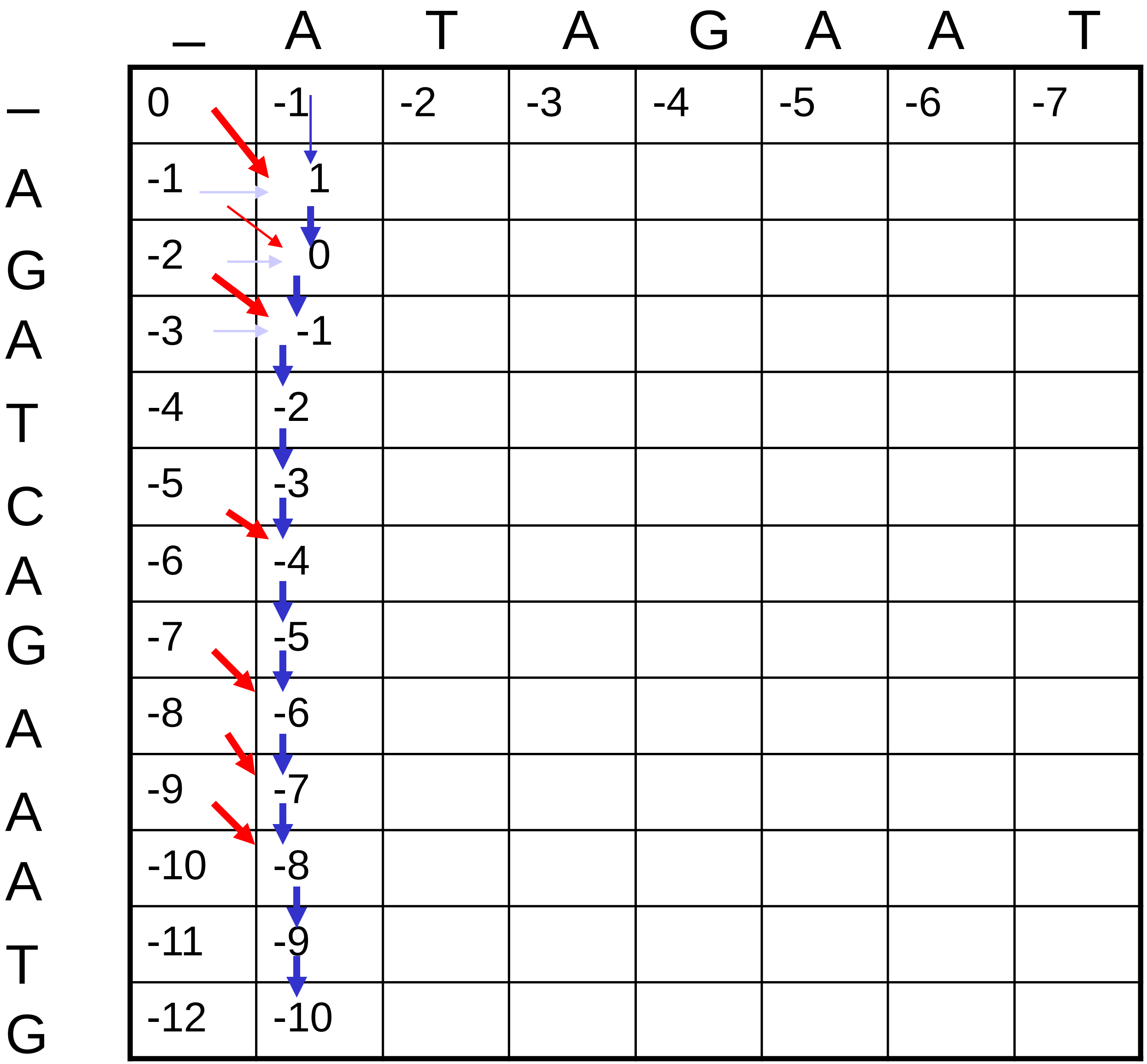
1. Initialization of Matrix M

j

i

	_	A	T	A	G	A	A	T
–	0	-1	-2	-3	-4	-5	-6	-7
A	-1							
G	-2							
A	-3							
T	-4							
C	-5							
A	-6							
G	-7							
A	-8							
A	-9							
A	-10							
T	-11							
G	-12							

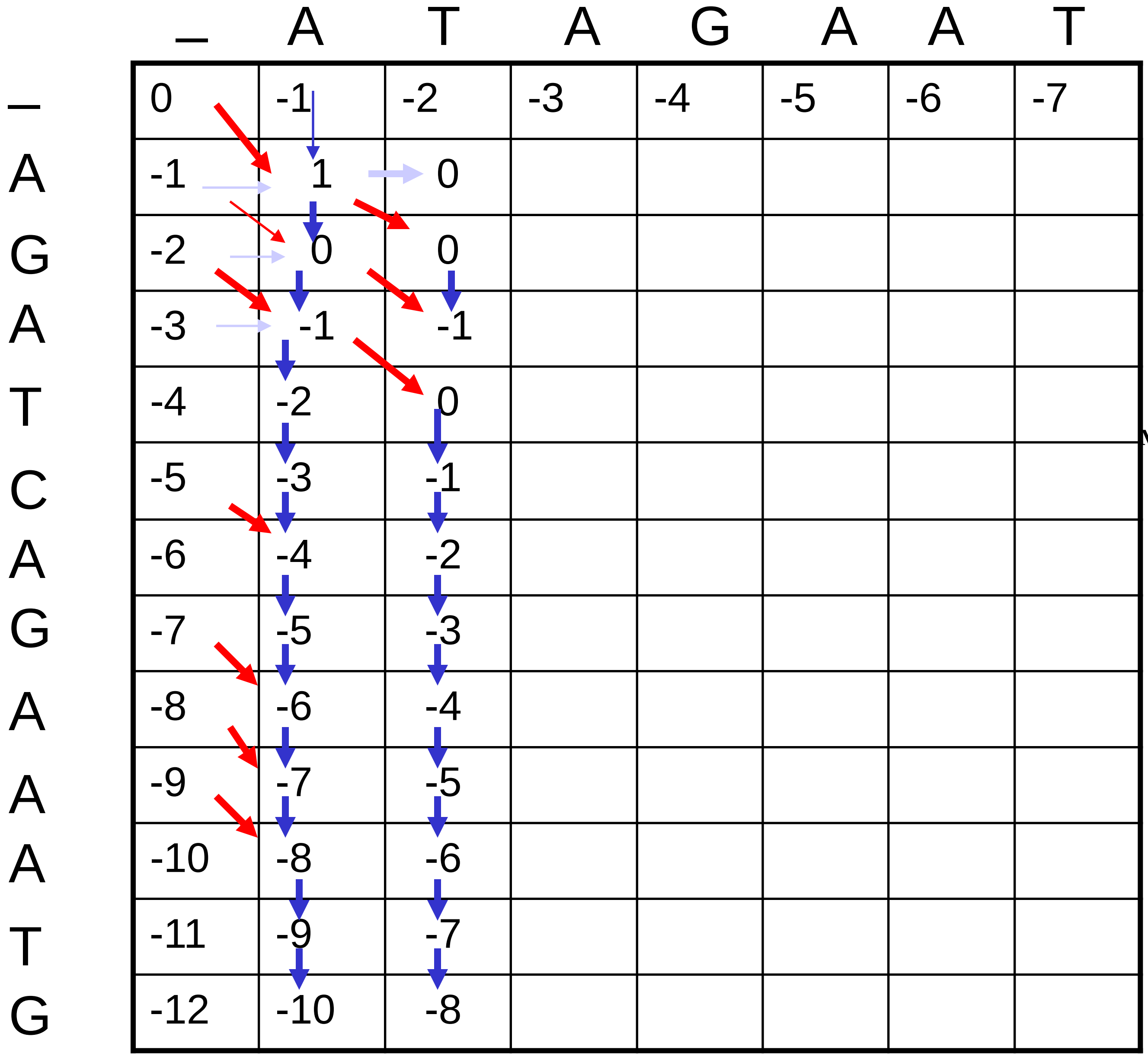
2. Fill Matrix



	_	A	T	A	G	A	A	T
_	0	-1	-2	-3	-4	-5	-6	-7
A	-1	1						
G	-2	0						
A	-3	-1						
T	-4	-2						
C	-5	-3						
A	-6	-4						
G	-7	-5						
A	-8	-6						
A	-9	-7						
A	-10	-8						
T	-11	-9						
G	-12	-10						

M[i,j] =
max [
M[i-1,j-1] + S(P[i],Q[j]),
M[i,j-1] + S(-, Q[j])
M[i-1,j] + S(P[i], -)
]

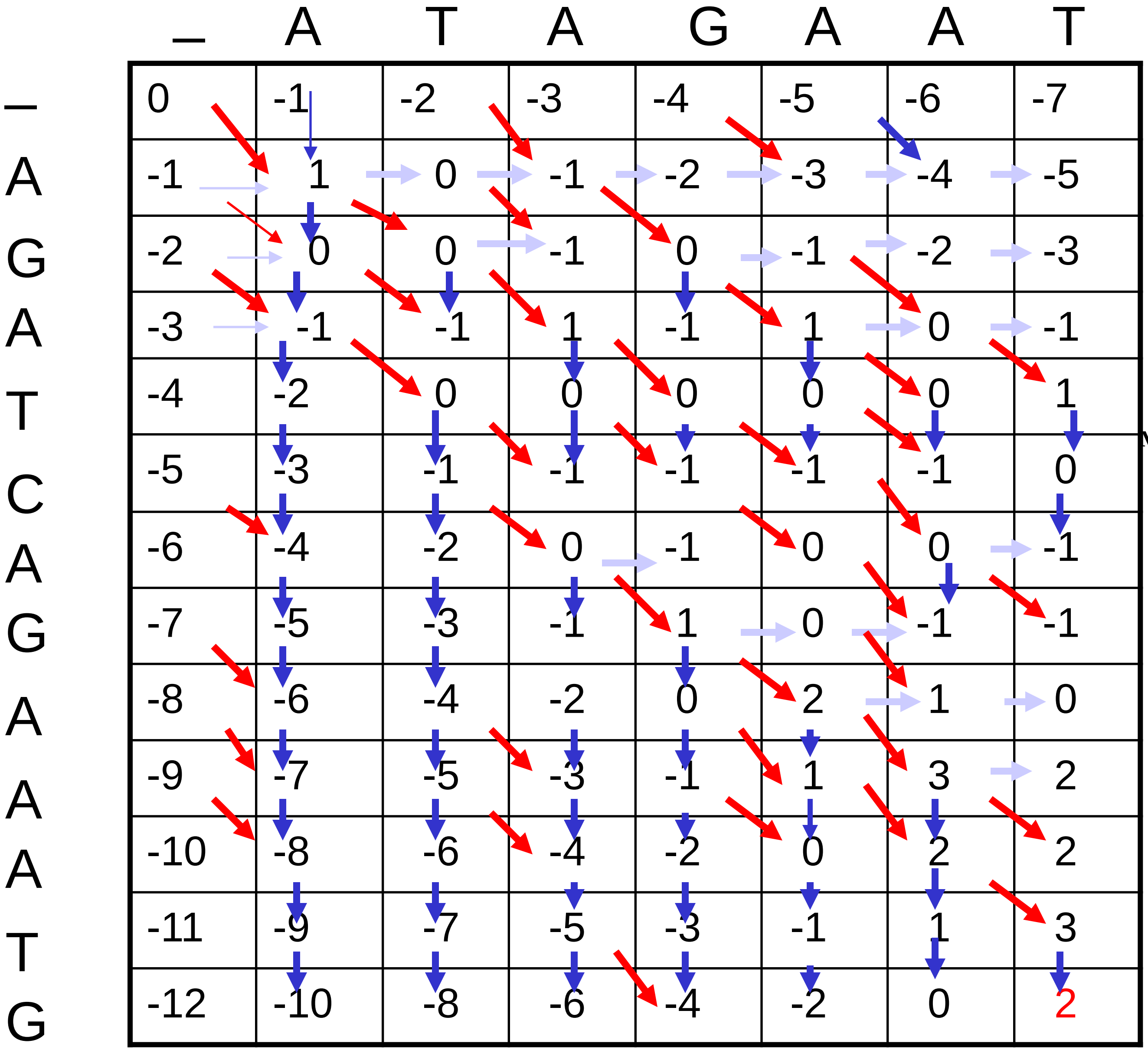
2. Fill Matrix



	_	A	T	A	G	A	A	T
_	0	-1	-2	-3	-4	-5	-6	-7
A	-1	1	0					
G	-2	0	0					
A	-3	-1	-1					
T	-4	-2	0					
C	-5	-3	-1					
A	-6	-4	-2					
G	-7	-5	-3					
A	-8	-6	-4					
A	-9	-7	-5					
A	-10	-8	-6					
T	-11	-9	-7					
G	-12	-10	-8					

M[i,j] =
max [
M[i-1,j-1] + S(P[i],Q[j]),
M[i,j-1] + S(-, Q[j])
M[i-1,j] + S(P[i], -)
]

2. Fill Matrix

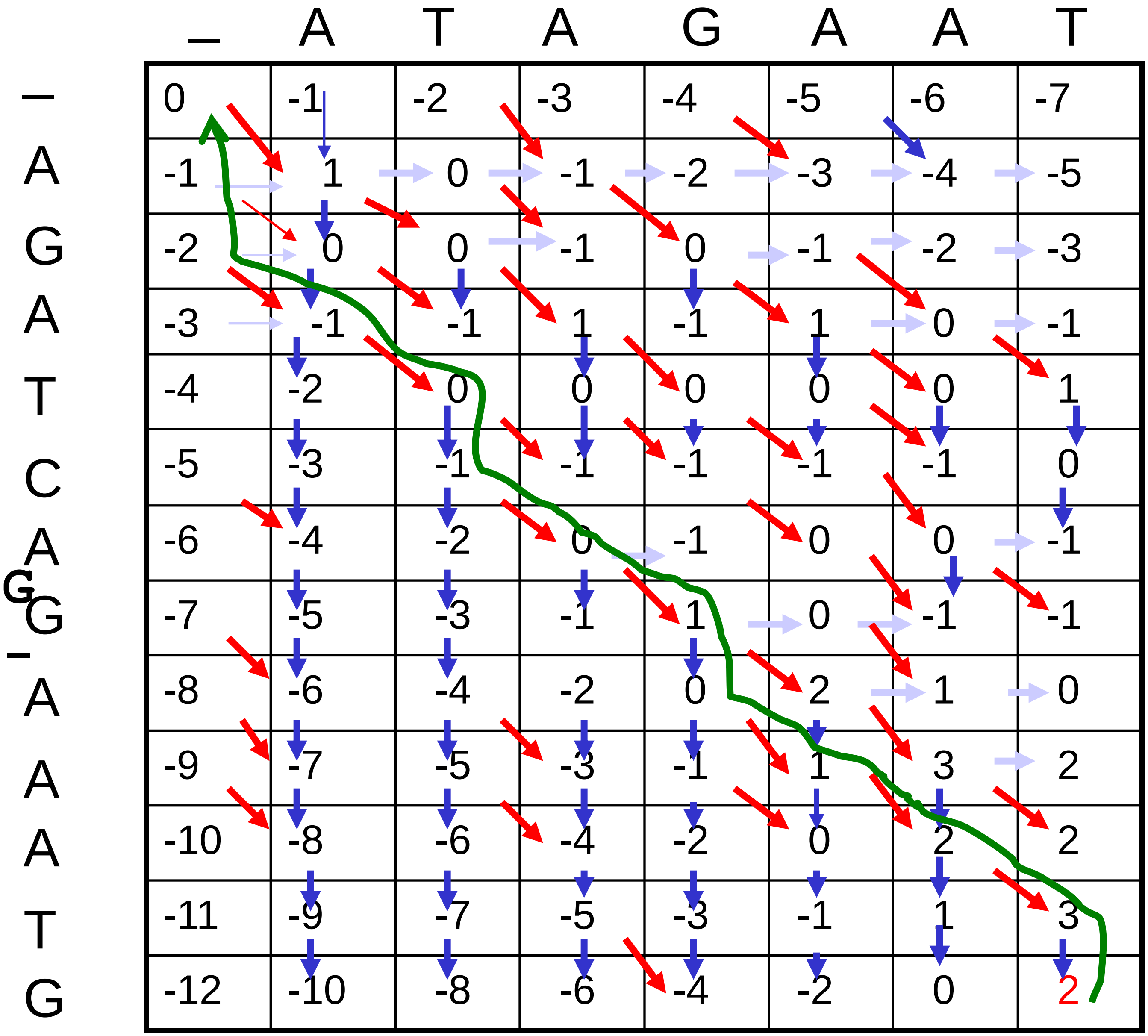


	_	A	T	A	G	A	A	T
_	0	-1	-2	-3	-4	-5	-6	-7
A	-1	1	0	-1	-2	-3	-4	-5
G	-2	0	0	-1	0	-1	-2	-3
A	-3	-1	-1	1	-1	1	0	-1
T	-4	-2	0	0	0	0	0	1
C	-5	-3	-1	-1	-1	-1	-1	0
A	-6	-4	-2	0	-1	0	0	-1
G	-7	-5	-3	-1	1	0	-1	-1
A	-8	-6	-4	-2	0	2	1	0
A	-9	-7	-5	-3	-1	1	3	2
A	-10	-8	-6	-4	-2	0	2	2
T	-11	-9	-7	-5	-3	-1	1	3
G	-12	-10	-8	-6	-4	-2	0	2

M[i,j] =
max [
M[i-1,j-1] + S(P[i],Q[j]),
M[i,j-1] + S(-, Q[j])
M[i-1,j] + S(P[i], -)
]

3. Trace Back

```
AGATCAGAAATG
--AT-AG-AAT-
```



	_	A	T	A	G	A	A	T
_	0	-1	-2	-3	-4	-5	-6	-7
A	-1	1	0	-1	-2	-3	-4	-5
G	-2	0	0	-1	0	-1	-2	-3
A	-3	-1	-1	1	-1	1	0	-1
T	-4	-2	0	0	0	0	0	1
C	-5	-3	-1	-1	-1	-1	-1	0
A	-6	-4	-2	0	-1	0	0	-1
G	-7	-5	-3	-1	1	0	-1	-1
A	-8	-6	-4	-2	0	2	1	0
A	-9	-7	-5	-3	-1	1	3	2
A	-10	-8	-6	-4	-2	0	2	2
T	-11	-9	-7	-5	-3	-1	1	3
G	-12	-10	-8	-6	-4	-2	0	2

Local vs. Global Alignment

- Global Alignment

```
--T—-CC-C-AGT—-TATGT-CAGGGGACACG—A-GCATGCAGA-GAC
  |  || |  ||  | | | |||    || | | |  | ||||   |
AATTGCCGCC-GTCGT-T-TTCAG----CA-GTTATG—T-CAGAT--C
```

- Local Alignment—better alignment to find conserved segment

Transcription binding site

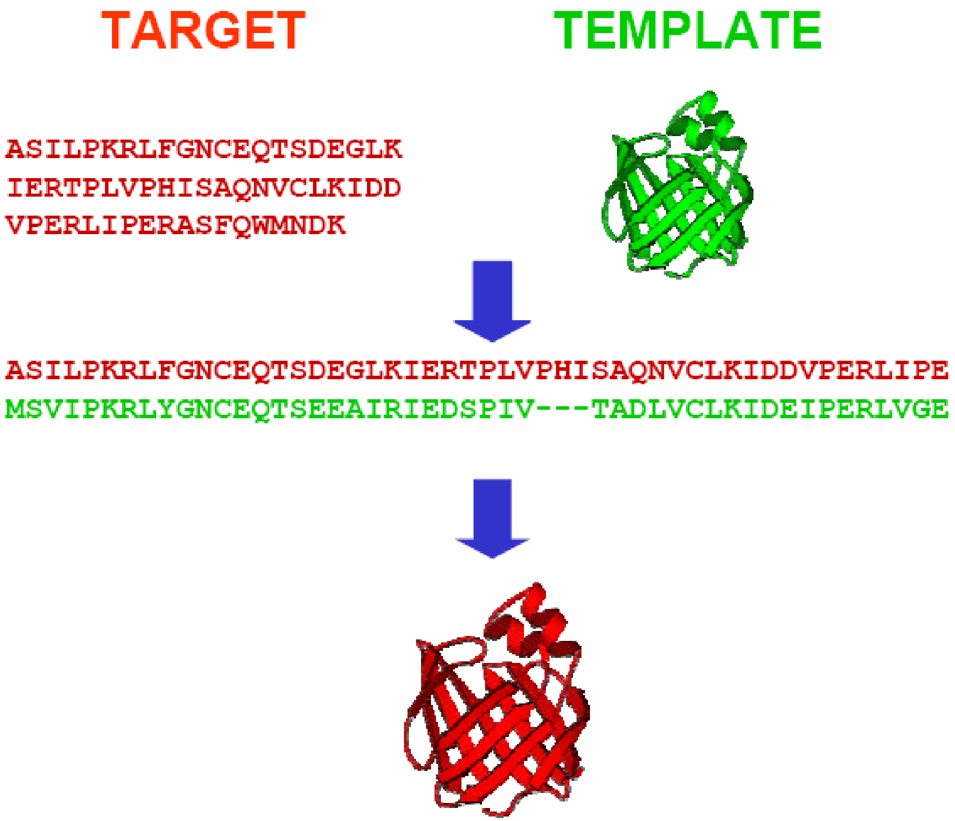
```
                  tccCAGTTATGTCAGggacacgagcatgcagagac
                     ||||||||||||
aattgccgccgtcgttttcagCAGTTATGTCAGatc
```

Smith-Waterman Algorithm

Same dynamic program algorithm as global alignment except for three differences.

1. All negative scores is converted to 0
2. Alignment can start from anywhere in the matrix
3. Alignment can end at anywhere in the matrix

Application Example (Alignment – Structure)



```
ASILPKRLFGNCEQTSDEGLK
IERTPLVPHISAQNVCLKIDD
VPERLIPERASFQWMNDK
```

```
ASILPKRLFGNCEQTSDEGLKIERTPLVPHISAQNVCLKIDDVPERLIPE
MSVIPKRLYGNCEQTSEEAIRIEDSPIV---TADLVCLKIDEIPERLVGE
```

Source: A. Fisher, 2005

Global and Local Alignment Tools

- NEEDLE (global alignment)

  http://bioweb.pasteur.fr/seqanal/interfaces/needle.html

- WATER (local alignment)

  http://bioweb.pasteur.fr/seqanal/interfaces/water.html

Scoring Matrix

- How to accurately measure the similarity between amino acids (or nucleotides) is one key issue of sequence alignment.
- For nucleotides, a simple identical / not identical scheme is mostly ok.
- Due to various properties of amino acids, it is hard and also critical to measure the similarity between amino acids.

Evolutionary Substitution Approach

- During evolution, the substitution of similar (or dissimilar) amino acids is more (or less) likely to be selected within protein families than random substitutions (M. Dayhoff)
- The frequency / probability one residue substitutes another one is an indicator of their similarity.

PAM Scoring Matrices

(M. Dayhoff)

- Select a number of protein families.
- Align sequences in each family and count the frequency of amino acid substitution of each column. The frequency is used to compute the empirical substitution probability of which residue i substitutes residue j (P_{ij}).
- Similarity score is ratio of observed substitution probability over the random substitution probability. **$S(i,j) = \log(P_{ij} / (P_i * P_j))$**. P_i is the observed probability of residue i and P_j is the observed probability of residue j
- PAM: Point Accepted Mutation

A Simplified Example

```
ACGTCGAGT
ACCACGTGT
CACACTACT
ACCGCATGA
ACCCTATCT
TCCGTAACA
ACCATAAGT
AGCATAAGT
ACTATAAGT
ACGATAAGT
```

Chars	Prob.
A	6 / 10
C	1 / 10
G	2 / 10
T	1 / 10

Substitution Frequency Table

	A	C	G	T
A	30	6	12	6
C	6	0	2	1
G	12	2	1	2
T	6	1	2	0

Total number of substitutions: 90

	A	C	G	T
A	.33	.07	.14	.07
C	.07	0	.02	.01
G	.14	.02	.01	.02
T	.07	.01	.02	0

P(A<->C) = 0.07+0.07=0.14

A Simplified Example

```
ACGTCGAGT
ACCACGTGT
CACACTACT
ACCGCATGA
ACCCTATCT
TCCGTAACA
ACCATAAGT
AGCATAAGT
ACTATAAGT
ACGATAAGT
```

Chars	Prob.
A	6 / 10
C	1 / 10
G	2 / 10
T	1 / 10

Substitution Frequency Table

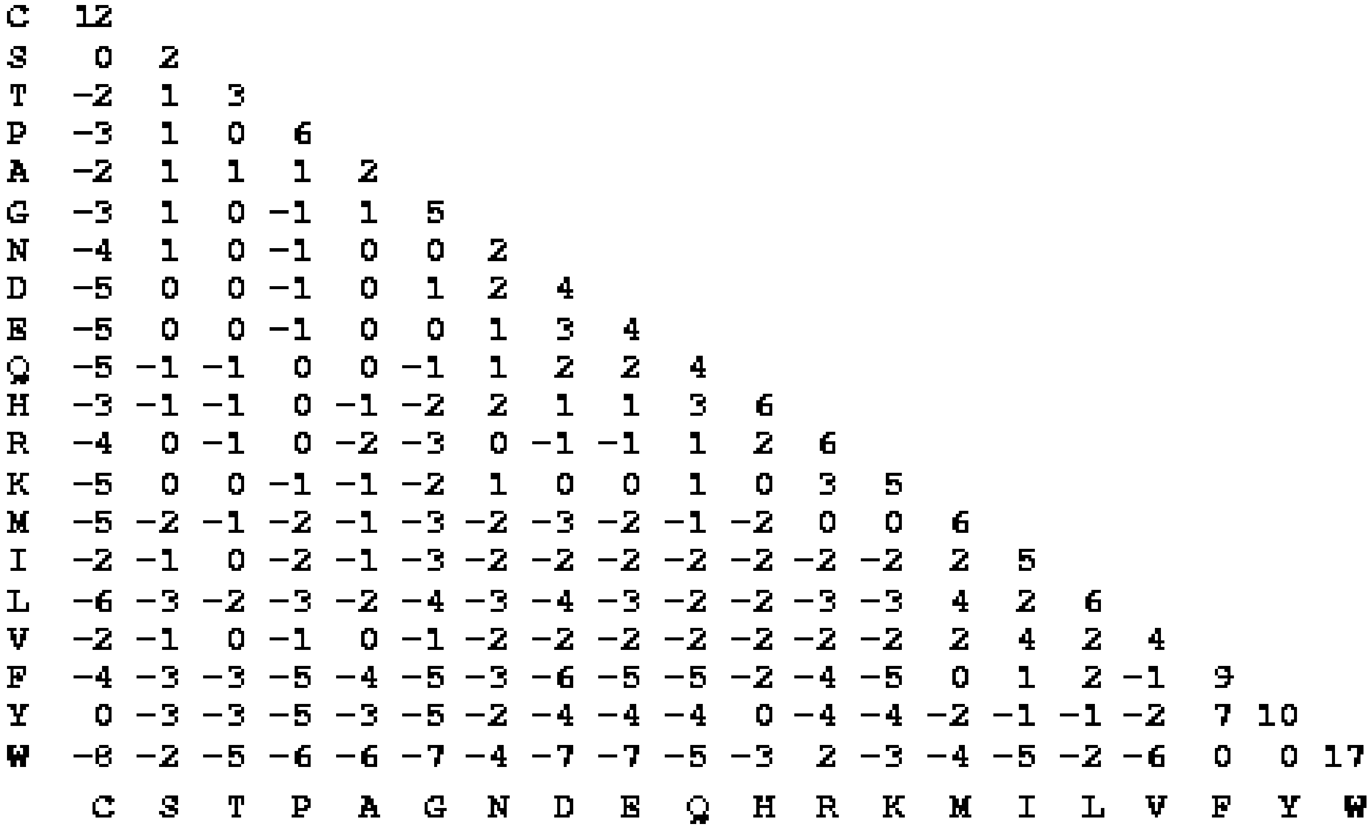
	A	C	G	T
A	30	6	12	6
C	6	0	2	1
G	12	2	1	2
T	6	1	2	0

Total number of substitutions: 90

	A	C	G	T
A	.33	.07	.14	.07
C	.07	0	.02	.01
G	.14	.02	.01	.02
T	.07	.01	.02	0

P(A<->C) = 0.07+0.07=0.14

S(A,C) = log(0.14/(0.6*0.1)) = 0.36



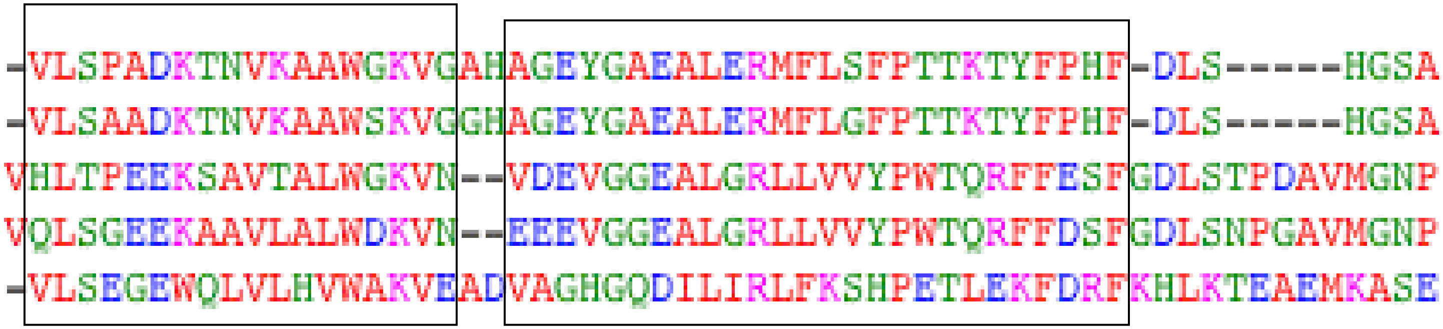
C	12																			
S	0	2																		
T	−2	1	3																	
P	−3	1	0	6																
A	−2	1	1	1	2															
G	−3	1	0	−1	1	5														
N	−4	1	0	−1	0	0	2													
D	−5	0	0	−1	0	1	2	4												
E	−5	0	0	−1	0	0	1	3	4											
Q	−5	−1	−1	0	0	−1	1	2	2	4										
H	−3	−1	−1	0	−1	−2	2	1	1	3	6									
R	−4	0	−1	0	−2	−3	0	−1	−1	1	2	6								
K	−5	0	0	−1	−1	−2	1	0	0	1	0	3	5							
M	−5	−2	−1	−2	−1	−3	−2	−3	−2	−1	−2	0	0	6						
I	−2	−1	0	−2	−1	−3	−2	−2	−2	−2	−2	−2	−2	2	5					
L	−6	−3	−2	−3	−2	−4	−3	−4	−3	−2	−2	−3	−3	4	2	6				
V	−2	−1	0	−1	0	−1	−2	−2	−2	−2	−2	−2	−2	2	4	2	4			
F	−4	−3	−3	−5	−4	−5	−3	−6	−5	−5	−2	−4	−5	0	1	2	−1	9		
Y	0	−3	−3	−5	−3	−5	−2	−4	−4	−4	0	−4	−4	−2	−1	−1	−2	7	10	
W	−8	−2	−5	−6	−6	−7	−4	−7	−7	−5	−3	2	−3	−4	−5	−2	−6	0	0	17
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

PAM250 Matrix (log odds multiplied by 10)

BLOSUM Matrices

(Henikoff and Henikoff)

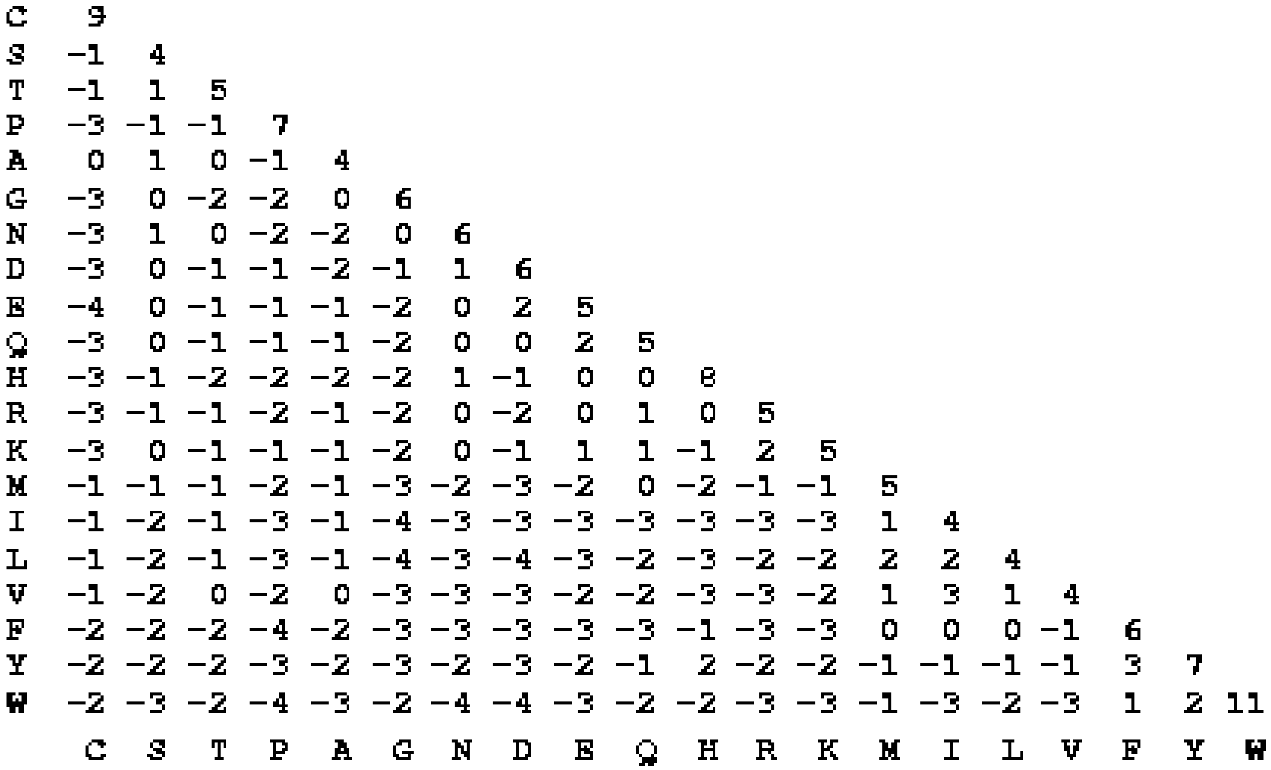
- PAM matrices don’t work well for aligning evolutionarily divergent sequences.
- BLOSUM: BLOcks SUbstitution Matrix
- PAM based on observed mutations throughout global alignment. BLOSUM based on highly conserved local regions /blocks without gaps.
- BLOSUMn is a matrix calculated from proteins share at most n% identity. BLOSUM62 is the most widely used matrix (BLAST, PSI-BLAST, CLUSTALW)



```
-VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS-----HGSA
-VLSAADKTNVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHF-DLS-----HGSA
VHLTPEEKSAVTALWGKVN--VDEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGNP
VQLSGEEKAAVLALWDKVN--EEEVGGEALGRLLVVYPWTQRFFDSFGDLSNPGAVMGNP
-VLSEGEWQLVLHVWAKVEADVAGHGQDILIRLFKSHPETLEKFDRFKHLKTEAEMKASE
```

Block 1

Block2



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

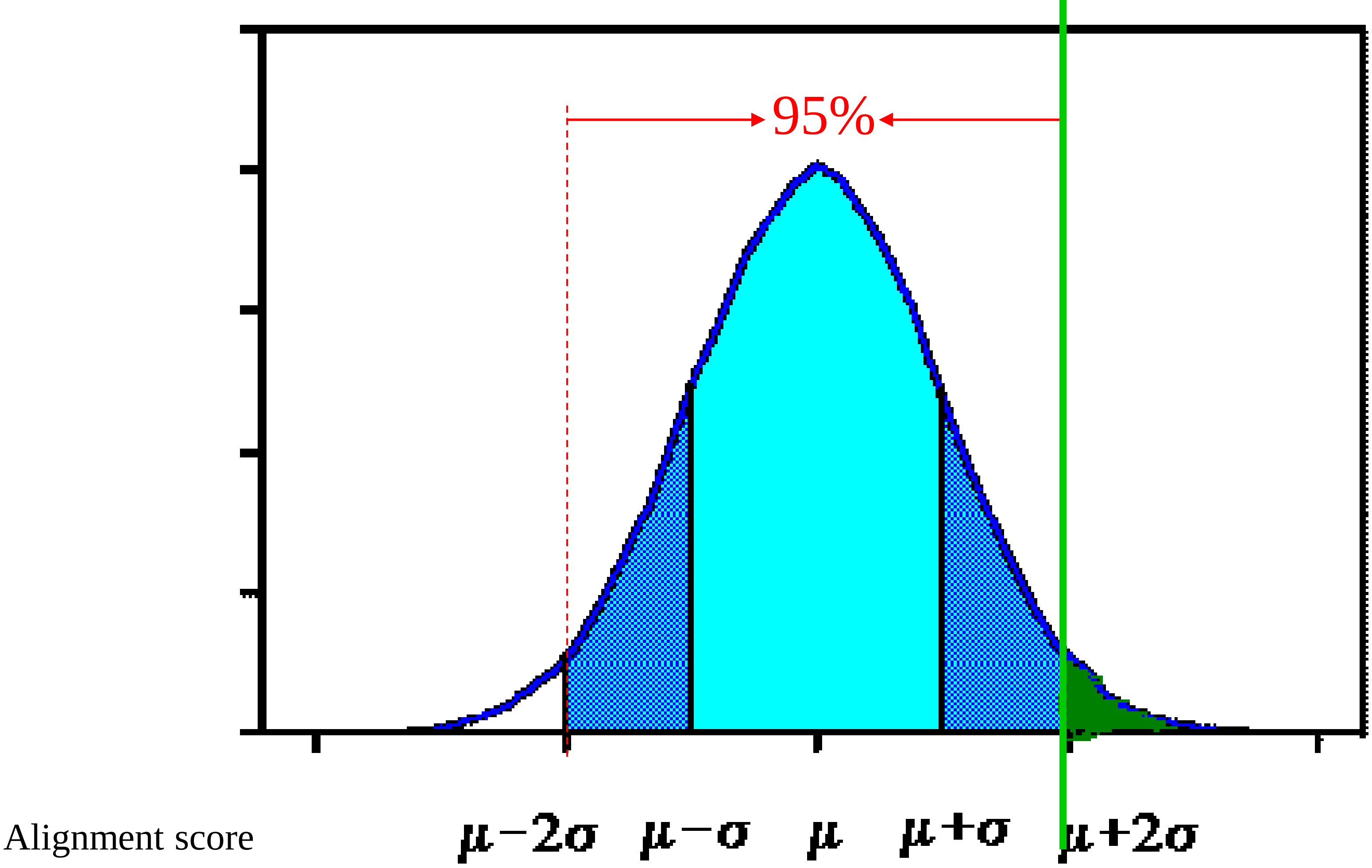
BLOSUM62 Matrix

Significance of Sequence Alignment

- Why do we need significant test?
- Mathematical view: unusual versus “by chance”
- Biological view: evolutionary related or not?

Randomization Approach

- Randomization is a fundamental idea due to Fisher.
- Randomly permute chars within sequence P and Q to generate new sequences (P’ and Q’). Align new sequences and record alignment scores.
- Assuming these scores obey normal distribution, compute mean (u) and standard derivation (σ) of alignment scores



Normal distribution of alignment scores of two sequences

- If S = u+2 σ, the probability of observing the alignment score equal to or more extreme than this by chance is 2.5%, e.g., P(S>=u+2 σ) = 2.5%.

Thus we are 97.5% confident that the alignment score is significant (not by chance).

- For any score x, we can compute P(S >= x), which is called p-value.

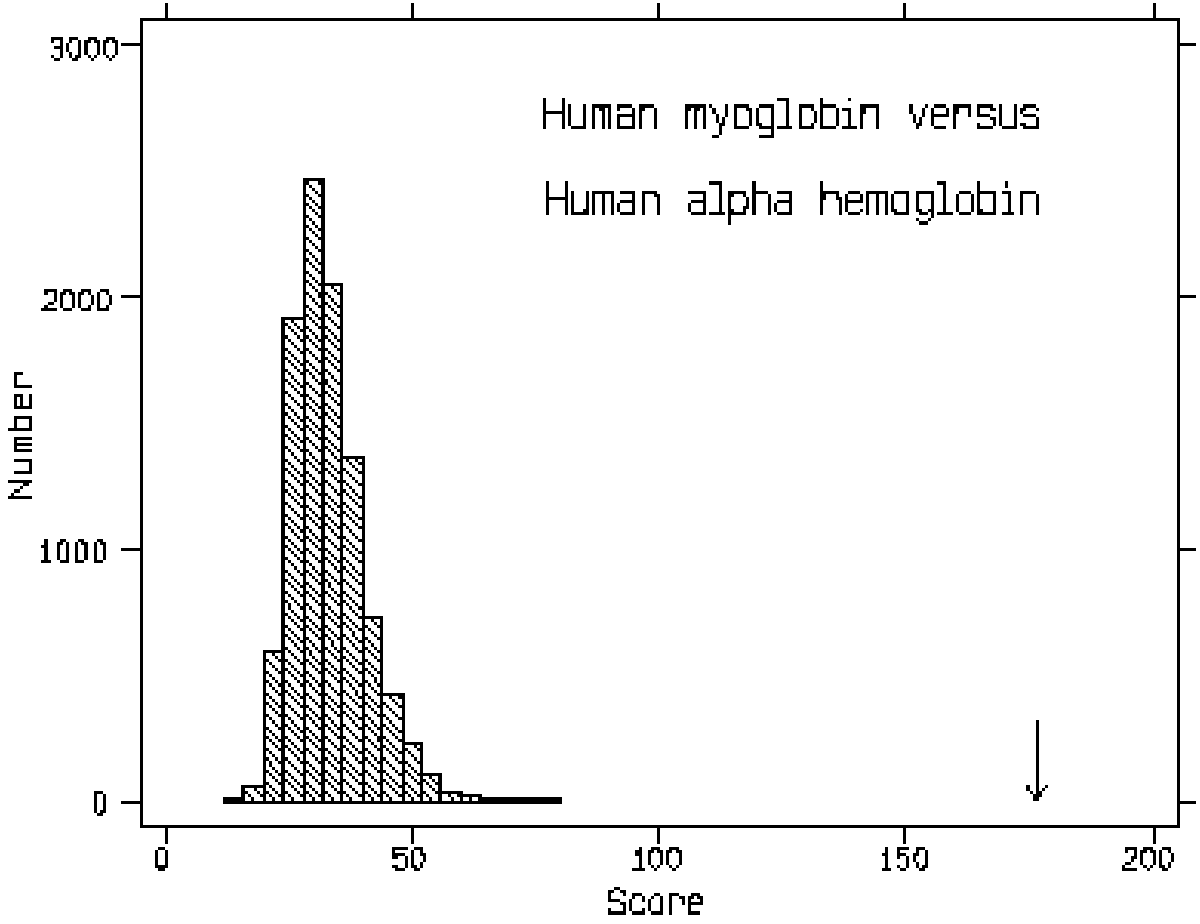


Figure: Histogram of alignment scores

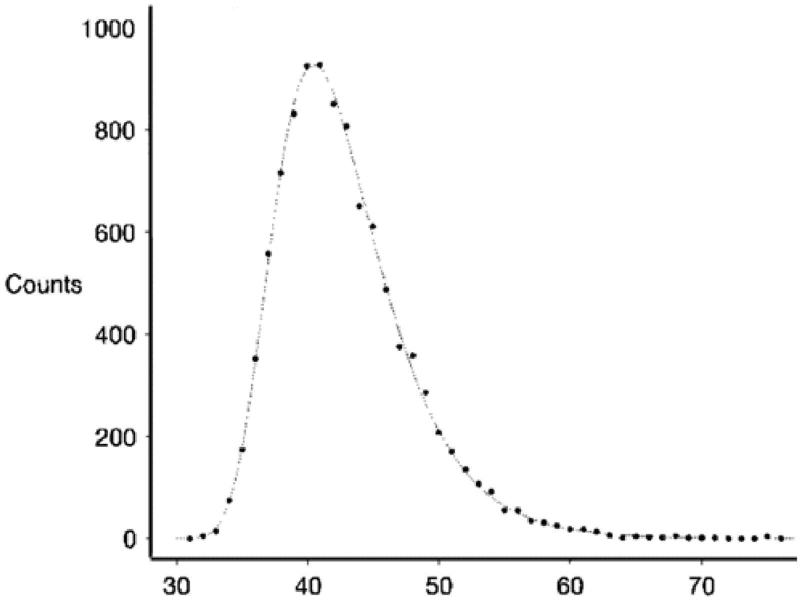
Model-Based Approach

(Karlin and Altschul)

http://www.people.virginia.edu/~wrp/cshl02/Altschul/Altschul-3.html

- Extreme Value Distribution

$$P(S \geq x) = 1 - \exp(-Kmn\, e^{-\lambda x})$$



K and lamda are statistical parameters depending on substitution matrix. For BLOSUM62, lamda=0.252, K=0.35

P-Value

- $P(S \geq x)$ is called **p-value**. It is the probability that random sequences has alignment score equal to or bigger than x.
- Smaller -> more significant.

Problems of Using Dynamic Programming to Search Large Sequence Database

- Search homologs in DNA and protein database is often the first step of a bioinformatics study.
- DP is too slow for large sequence database search such as Genbank and UniProt. Each DP search can take hours.
- Most DP search time is wasted on unrelated sequences or dissimilar regions.
- Developing fast, practical sequence comparison methods for database search is important.

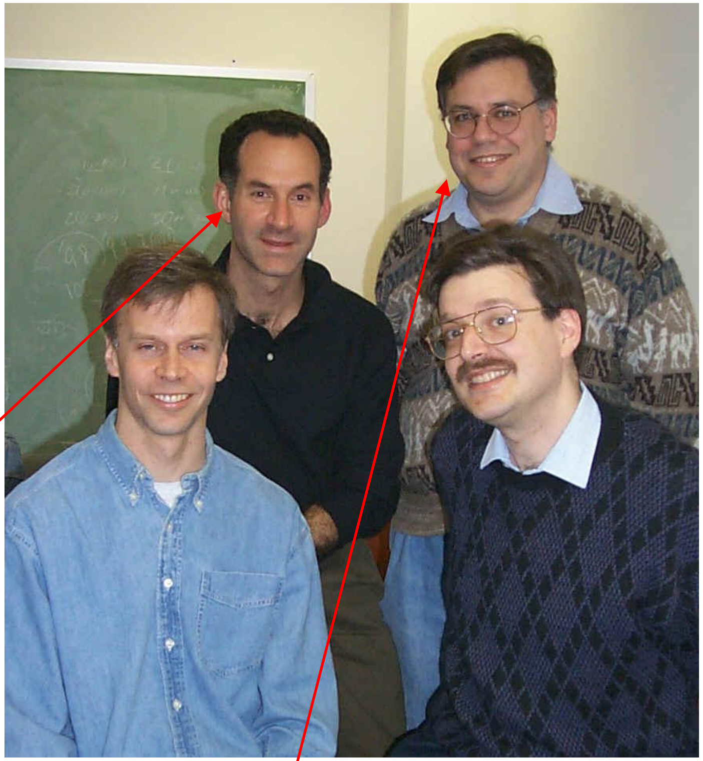
Fast Sequence Search Methods

- All successful, rapid sequence comparison methods are based on a simple fact: similar sequences /regions **share some common words**.
- First such method is FASTP (Pearson & Lipman, 1985)
- Most widely used methods are BLAST (Altschul et al., 1990) and PSI-BLAST (Altschul et al., 1997).

Basic Local Alignment Search Tool

(**S. Altschul, W. Gish, W. Miller, E. Meyer and D. Lipman**)

1. Compile a list of words for a query
2. Scan sequences in database for word hits
3. Extending hits



David Lipman

Stephen Altschul

Compile Word List

- Words: w-mer with length w.
- Protein 4-mer and DNA 12-mer

Query:

DSRSKGEPRDSGTLQSQEAKAVKKTSLFE

Words: DSRS, SRSK, RSKG, KGEP….

Example of extension

Query: DSRSKGEPRDSGTLQSQEAKAVKKTSLFE

Words: DSRS, **SRSK**, RSKG, KGEP….

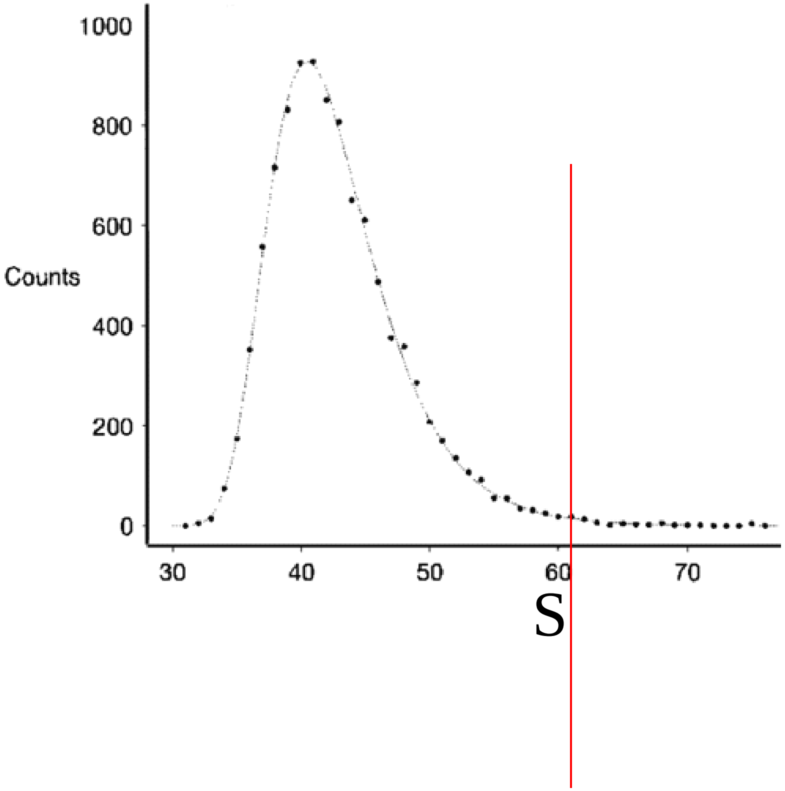
Database Sequence: PE**SRSK**GEPRDSGKKQMDSOKPD

← →

Maximum Segment Pair: **ESRSKGEPRDSG**

P-Value and E-Value

- P-value
- E-value = database size * p-value
- Common threshold: 0.01

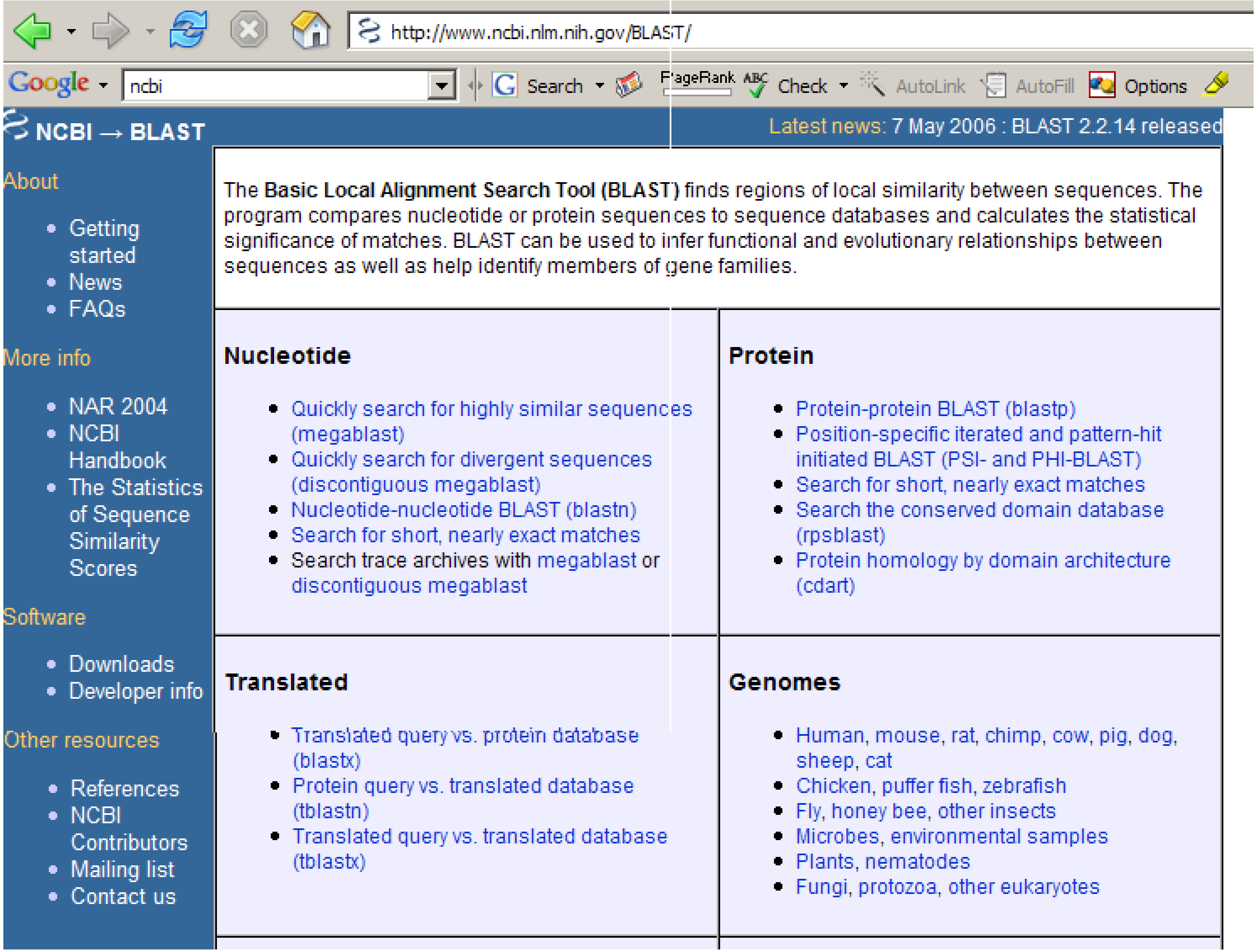


P-value = Prob(score >=S)

Usage of BLAST

- Versions: BLASTP, BLASTN, BLASTX (translated)
- Sequence Databases: NR, PDB, SwissProt, Gene databases of organisms, or your own databases
- Expectation value
- Low complexity
- Similarity matrix (PAM or BLOSUM)
- Output format

NCBI Online Blast



NCBI → BLAST

Latest news: 7 May 2006 : BLAST 2.2.14 released

About

- Getting started
- News
- FAQs

More info

- NAR 2004
- NCBI Handbook
- The Statistics of Sequence Similarity Scores

Software

- Downloads
- Developer info

Other resources

- References
- NCBI Contributors
- Mailing list
- Contact us

The **Basic Local Alignment Search Tool (BLAST)** finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Nucleotide

- Quickly search for highly similar sequences (megablast)
- Quickly search for divergent sequences (discontiguous megablast)
- Nucleotide-nucleotide BLAST (blastn)
- Search for short, nearly exact matches
- Search trace archives with megablast or discontiguous megablast

Protein

- Protein-protein BLAST (blastp)
- Position-specific iterated and pattern-hit initiated BLAST (PSI- and PHI-BLAST)
- Search for short, nearly exact matches
- Search the conserved domain database (rpsblast)
- Protein homology by domain architecture (cdart)

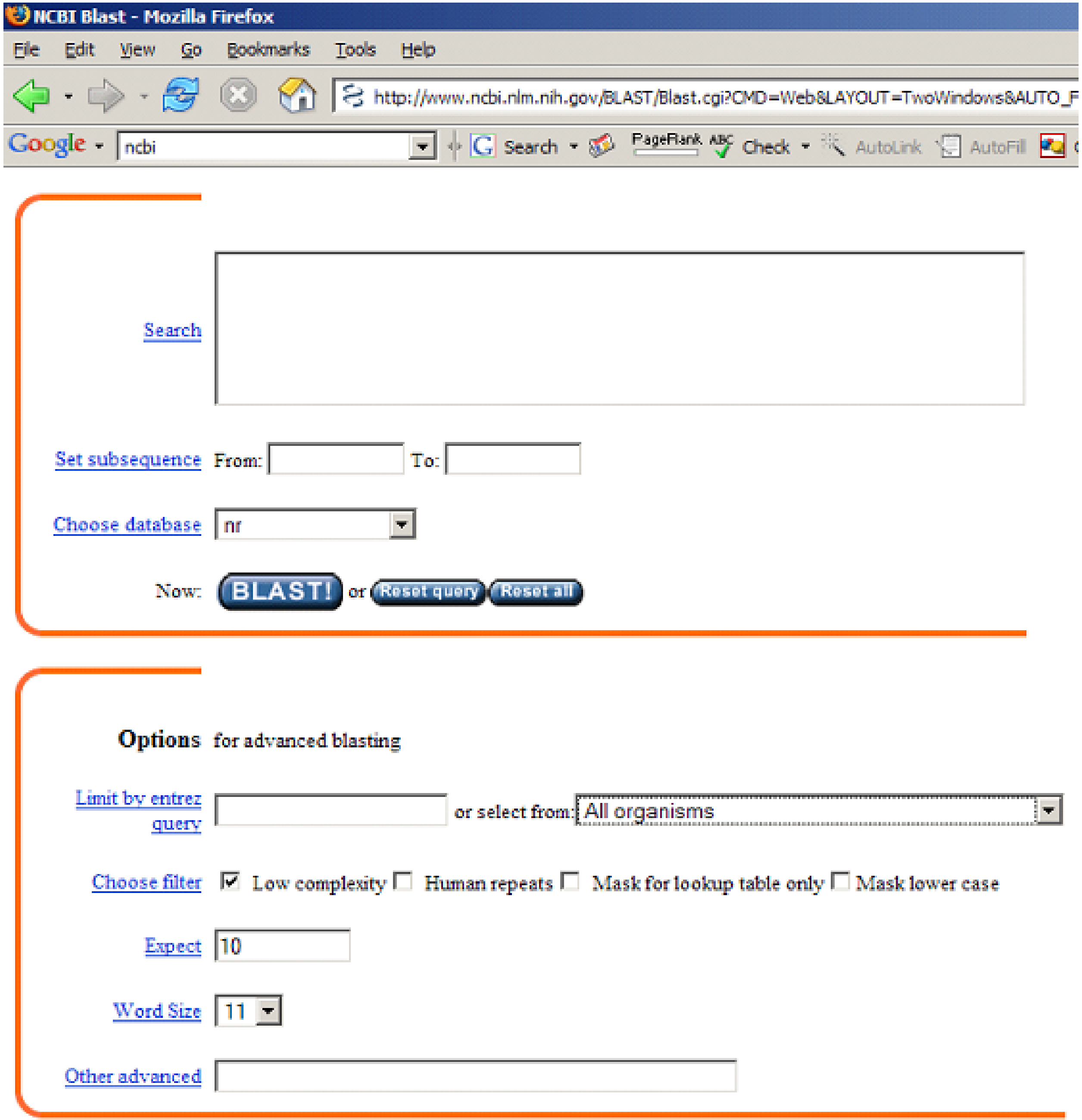
Translated

- Translated query vs. protein database (blastx)
- Protein query vs. translated database (tblastn)
- Translated query vs. translated database (tblastx)

Genomes

- Human, mouse, rat, chimp, cow, pig, dog, sheep, cat
- Chicken, puffer fish, zebrafish
- Fly, honey bee, other insects
- Microbes, environmental samples
- Plants, nematodes
- Fungi, protozoa, other eukaryotes

DNA Blast



NCBI Blast - Mozilla Firefox
File Edit View Go Bookmarks Tools Help
http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi?CMD=Web&LAYOUT=TwoWindows&AUTO_F
Google ncbi Search PageRank Check AutoLink AutoFill

Search

Set subsequence From: To:

Choose database: nr

Now: BLAST! or Reset query Reset all

Options for advanced blasting

Limit by entrez query: or select from: All organisms

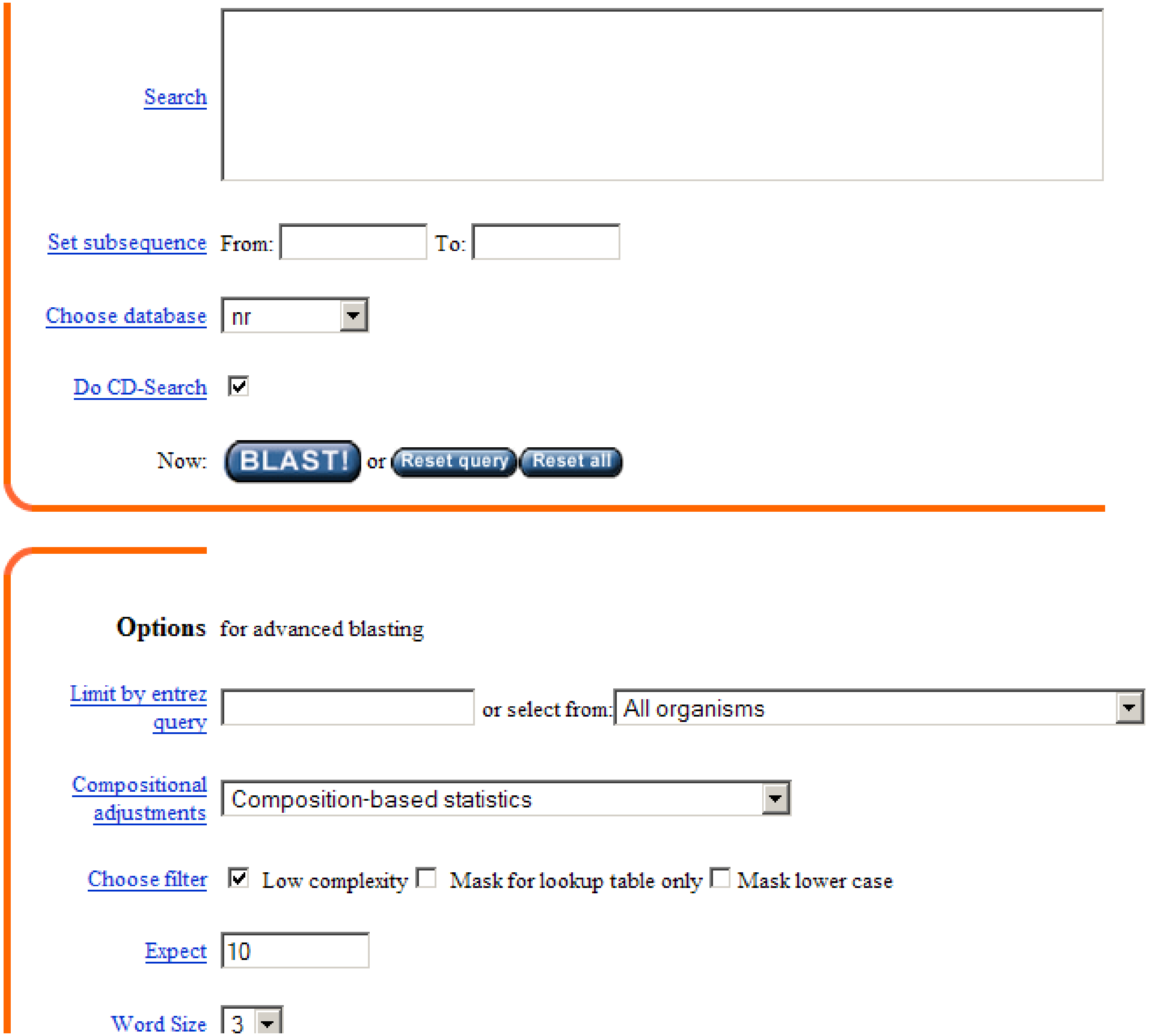
Choose filter: ☑ Low complexity □ Human repeats □ Mask for lookup table only □ Mask lower case

Expect: 10

Word Size: 11

Other advanced:

Protein Blast



Search

Set subsequence From: To:

Choose database nr

Do CD-Search ☑

Now:

Options for advanced blasting

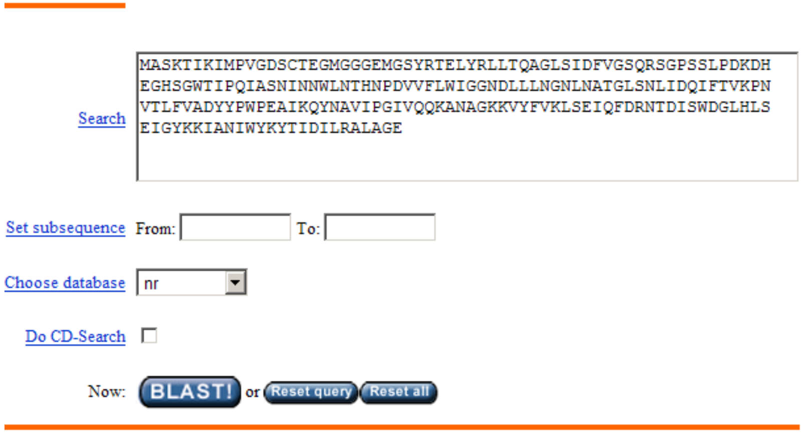
Limit by entrez query or select from: All organisms

Compositional adjustments Composition-based statistics

Choose filter ☑ Low complexity □ Mask for lookup table only □ Mask lower case

Expect 10

Word Size 3



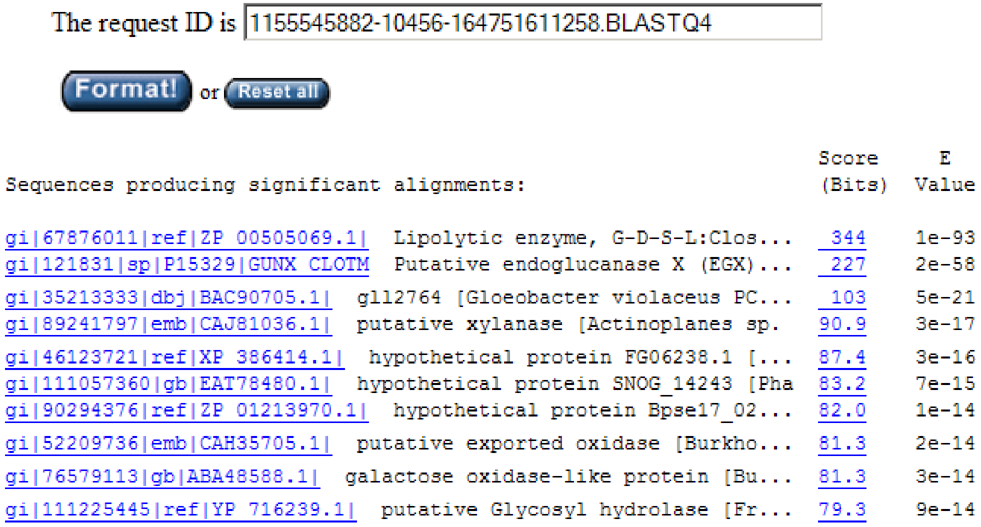
Search
```
MASKTIKIMPVGDSCTEGMGGGEMGSYRTELYRLLTQAGLSIDFVGSQRSGPSSLPDKDH
EGHSGWTIPQIASNINNWLNTHNPDVVFLWIGGNDLLLNGNLNATGLSNLIDQIFTVKPN
VTLFVADYYPWPEAIKQYNAVIPGIVQQKANAGKKVYFVKLSEIQFDRNTDISWDGLHLS
EIGYKKIANIWYKYTIDILRALAGE
```

Set subsequence From: To:

Choose database nr

Do CD-Search □

Now: BLAST! or Reset query Reset all



The request ID is 1155545882-10456-164751611258.BLASTQ4

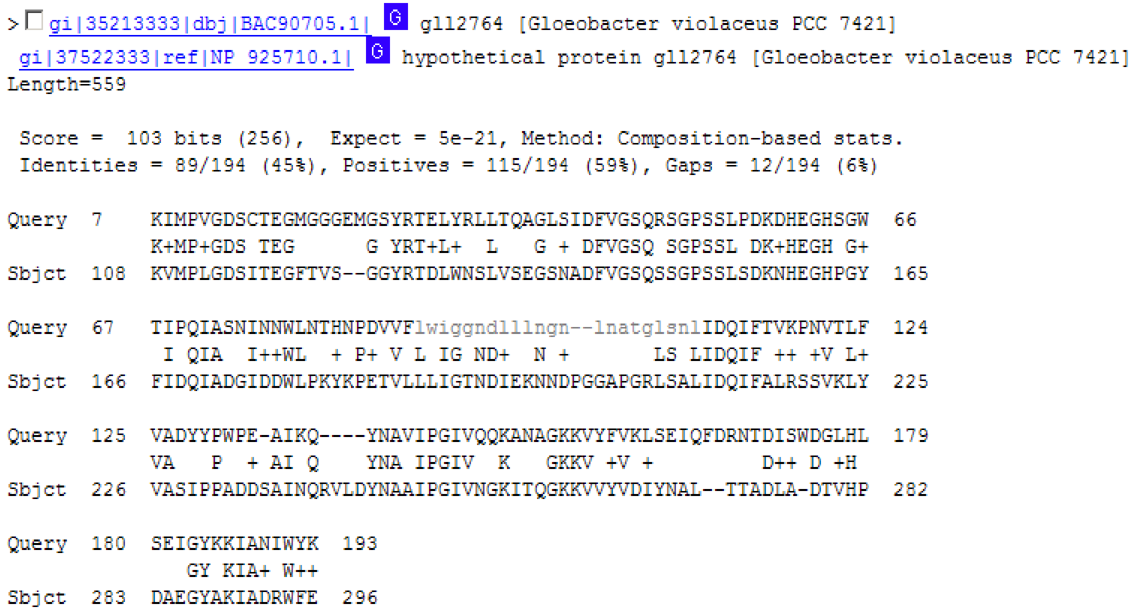
Format! or Reset all

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

gi|67876011|ref|ZP_00505069.1|  Lipolytic enzyme, G-D-S-L:Clos...  344    1e-93
gi|121831|sp|P15329|GUNX_CLOTM  Putative endoglucanase X (EGX)...  227    2e-58
gi|35213333|dbj|BAC90705.1|  gll2764 [Gloeobacter violaceus PC...  103    5e-21
gi|89241797|emb|CAJ81036.1|  putative xylanase [Actinoplanes sp.  90.9    3e-17
gi|46123721|ref|XP_386414.1|  hypothetical protein FG06238.1 [...  87.4    3e-16
gi|111057360|gb|EAT78480.1|  hypothetical protein SNOG_14243 [Pha  83.2    7e-15
gi|90294376|ref|ZP_01213970.1|  hypothetical protein Bpse17_02...  82.0    1e-14
gi|52209736|emb|CAH35705.1|  putative exported oxidase [Burkho...  81.3    2e-14
gi|76579113|gb|ABA48588.1|  galactose oxidase-like protein [Bu...  81.3    3e-14
gi|111225445|ref|YP_716239.1|  putative Glycosyl hydrolase [Fr...  79.3    9e-14
```

Matched sequences ranked by score and evalue

Output Format



```
>□ gi|35213333|dbj|BAC90705.1| G gll2764 [Gloeobacter violaceus PCC 7421]
 gi|37522333|ref|NP_925710.1| G hypothetical protein gll2764 [Gloeobacter violaceus PCC 7421]
Length=559

 Score =  103 bits (256),  Expect = 5e-21, Method: Composition-based stats.
 Identities = 89/194 (45%), Positives = 115/194 (59%), Gaps = 12/194 (6%)

Query  7    KIMPVGDSCTEGMGGGEMGSYRTELYRLLTQAGLSIDFVGSQRSGPSSLPDKDHEGHSGW  66
            K+MP+GDS TEG      G YRT+L+  L   G + DFVGSQ SGPSSL DK+HEGH G+
Sbjct  108  KVMPLGDSITEGFTVS--GGYRTDLWNSLVSEGSNADFVGSQSSGPSSLSDKNHEGHPGY  165

Query  67   TIPQIASNINNWLNTHNPDVVFlwiggndlllngn--lnatglsnlIDQIFTVKPNVTLF  124
             I QIA  I++WL  + P+ V L IG ND+  N +       LS LIDQIF ++ +V L+
Sbjct  166  FIDQIADGIDDWLPKYKPETVLLLIGTNDIEKNNDPGGAPGRLSALIDQIFALRSSVKLY  225

Query  125  VADYYPWPE-AIKQ----YNAVIPGIVQQKANAGKKVYFVKLSEIQFDRNTDISWDGLHL  179
            VA   P  + AI Q    YNA IPGIV  K   GKKV +V +         D++ D +H
Sbjct  226  VASIPPADDSAINQRVLDYNAAIPGIVNGKITQGKKVVYVDIYNAL--TTADLA-DTVHP  282

Query  180  SEIGYKKIANIWYK  193
               GY KIA+ W++
Sbjct  283  DAEGYAKIADRWFE  296
```

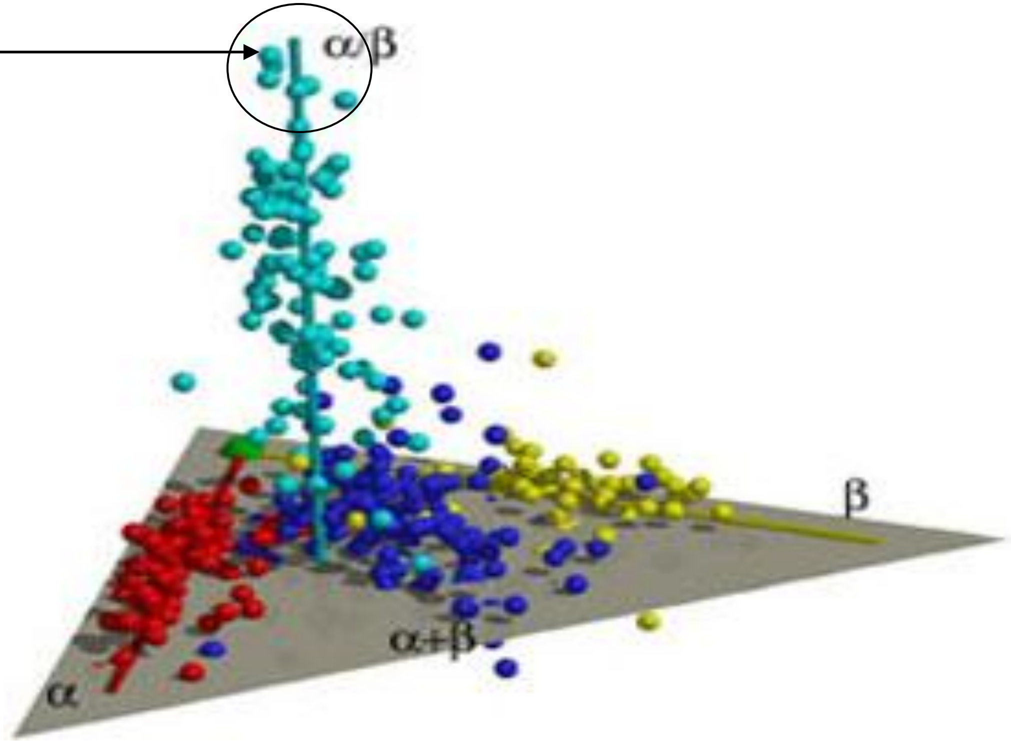
Significant local alignments

Database Search Using Sequence Profiles

- Multiple related sequences in protein family and super family (profile)
- More data, more robust, more sensitive
- Consider a group of related sequences (profile) is a **POWERFUL** idea

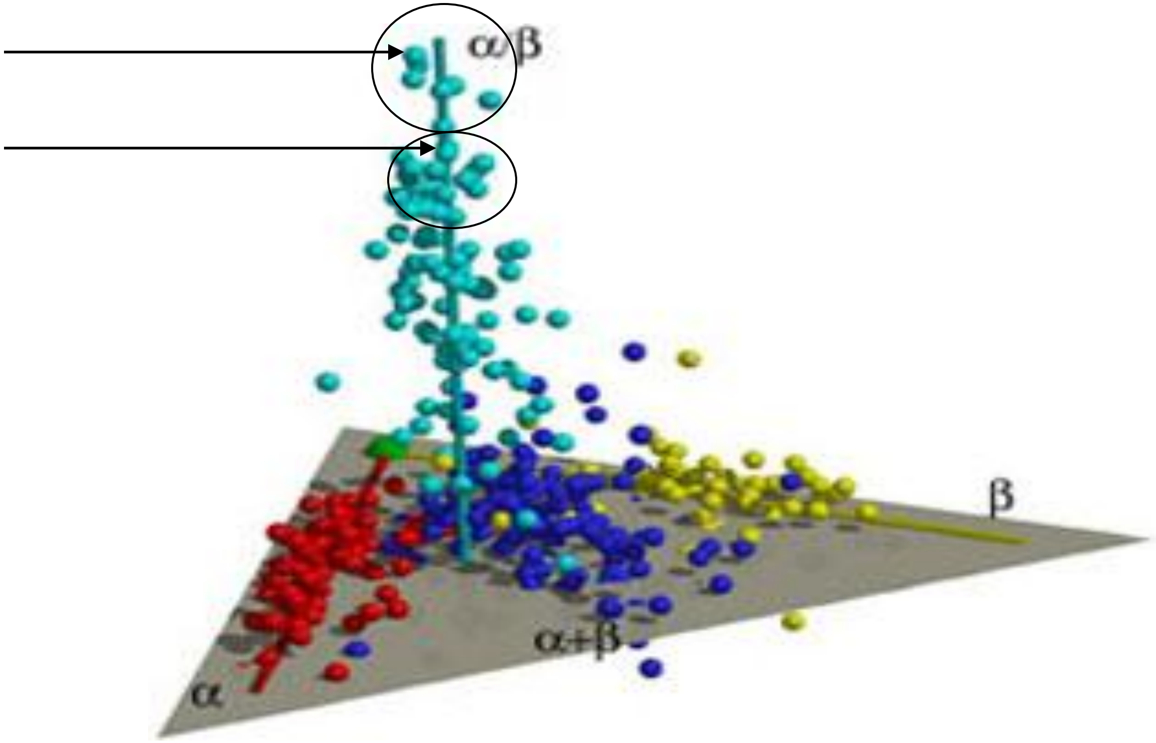
Why does a family of sequences help?

Step 1



Protein Universe

Why does a family of sequences help?

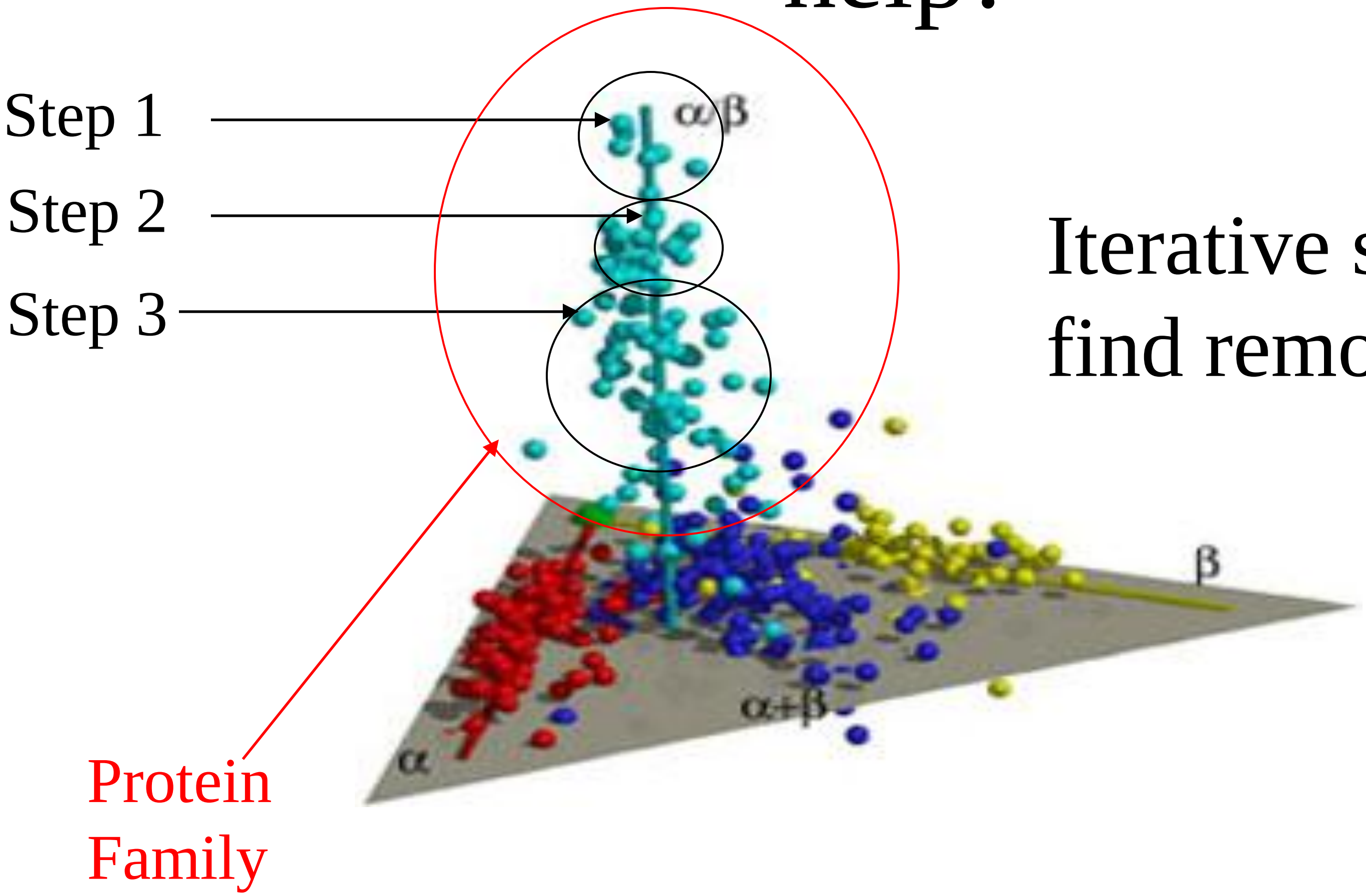


Step 1

Step 2

Protein Universe

Why does a family of sequences help?



Step 1

Step 2

Step 3

Protein Family

Iterative search helps find remote homologs.

Protein Universe

PSI-BLAST Algorithm

- Use BLAST to search database. Use significantly matched sequences to construct a profile / PSSM
- Repeat

  Use PSSM to search database

  Use significant matched sequences to construct a PSSM
- Until no new sequence is found or reach the maximum number of iterations.

Use PSI-BLAST Software

- Download: http://130.14.29.110/BLAST/download.shtml
- Command:

```
blastpgp –i seq_file  –j iteration –h include_evalue_threshold –e report_evalue_threshold –d database –o output_file
```

-i: input sequence file in FASTA format

-j: number of iterations

-d: sequence database

-h: cut-off e-value of including a sequence into PSSM (profile)

-e: cut-off e-value of reporting a sequence

-o: output file