Genome Analyis (Integrative Bioinformatics & Genomics) 2008

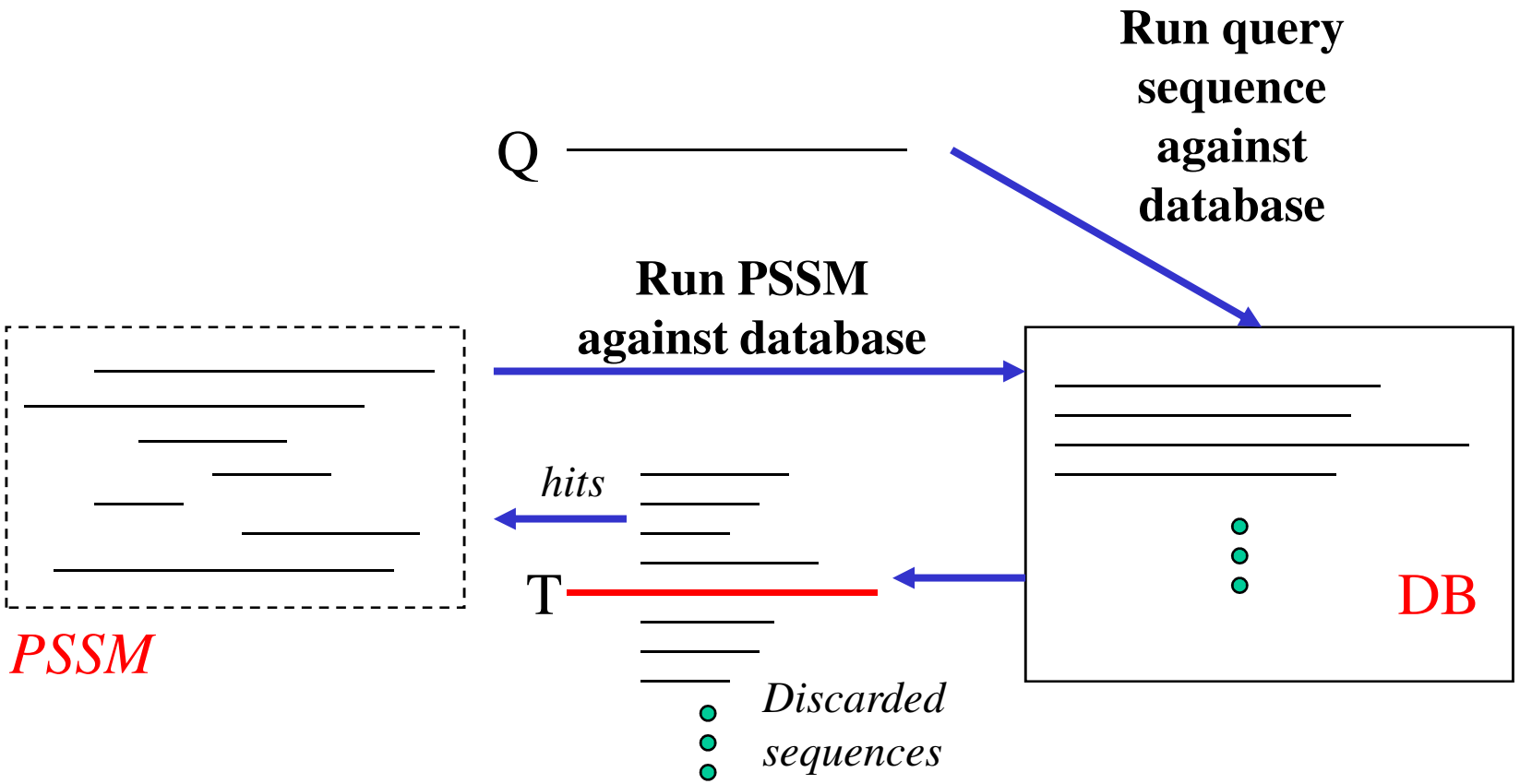
Lecture 7

Iterative homology searching using PSI-BLAST, scoring statistics and performance evaluation

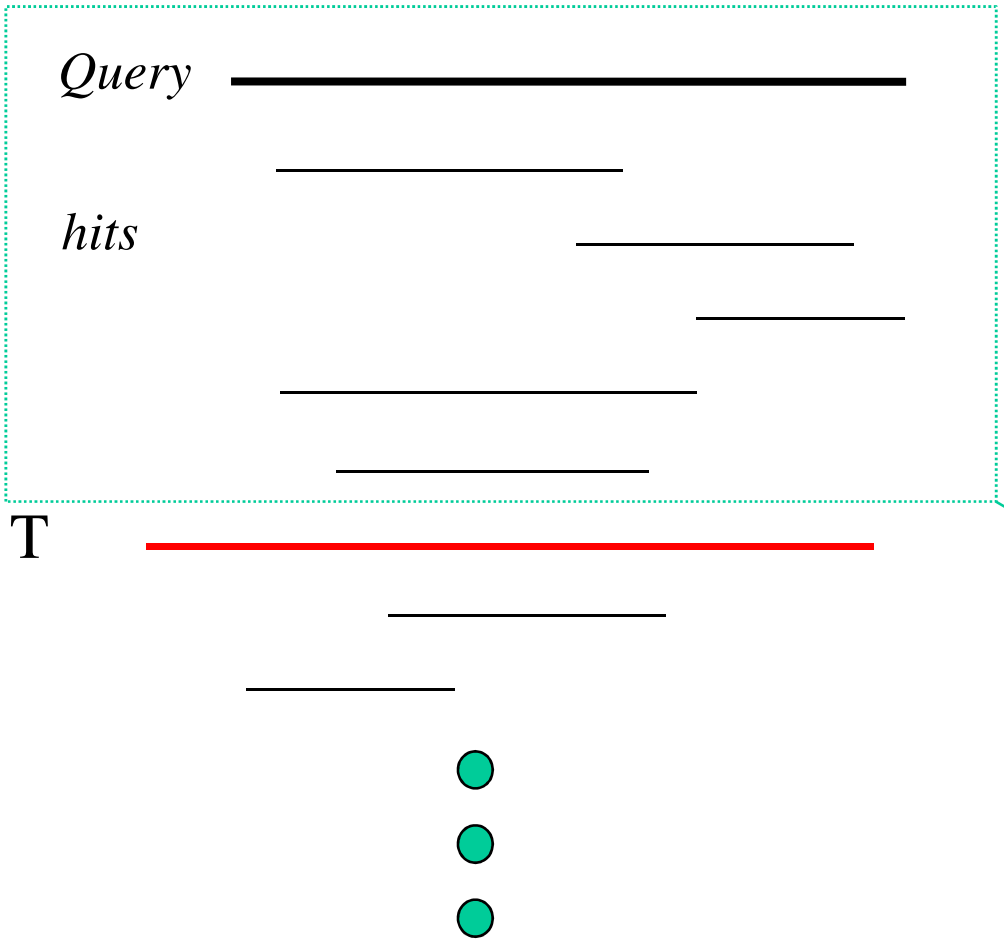
PSI (*Position Specific Iterated*) BLAST

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

PSI-BLAST iteration



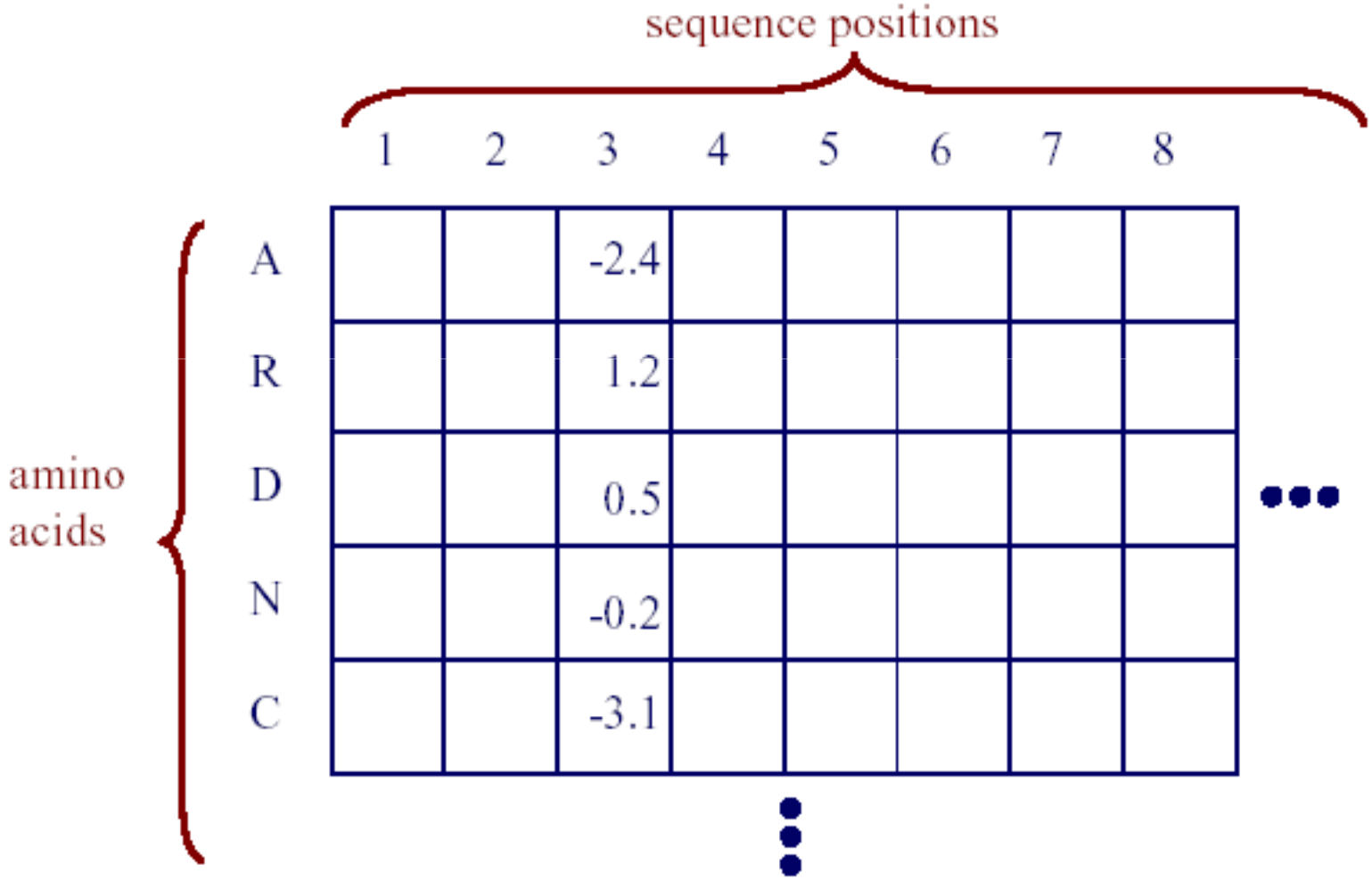
PSI-BLAST iteration



During iteration, new hits can come in and hits can drop out of the hit-list (also the query sequence..)

At each iteration a new profile is made of the master-slave alignment

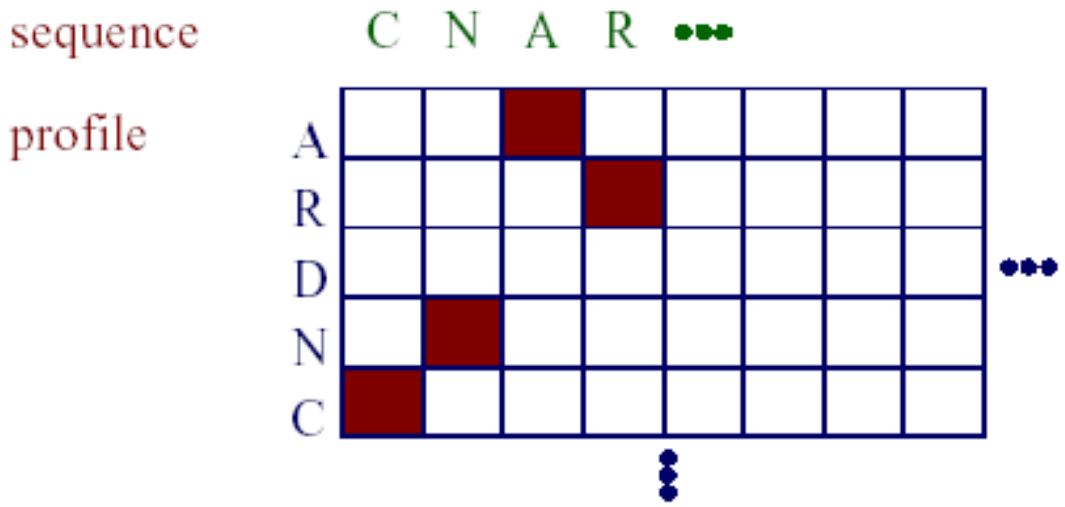
A Profile Matrix (Position Specific Scoring Matrix – PSSM)



This is the same as a profile without position-specific gap penalties

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



BLAST PSSM calculation using frequency normalisation and log conversion (A) and scoring a sequence against a PSSM (B)

(A)

```
   12345
S1 GCTCC
S2 AATCG
S3 TACGC
S4 GTGTT
S5 GTAAA
S6 CGTCC
```

	1	2	3	4	5	Overall
A	.17	.33	.17	.17	.17	6/30 = .20
C	.17	.17	.17	.50	.50	9/30 = .30
G	.50	.17	.17	.17	.17	7/30 = .23
T	.17	.33	.50	.17	.17	8/30 = .27

Normalise by dividing by overall frequencies

	1	2	3	4	5	Overall
A	.85	1.65	.85	.85	.85	6/30 = .20
C	.57	.57	.57	1.67	1.67	9/30 = .30
G	2.17	.74	.74	.74	.74	7/30 = .23
T	.63	1.22	1.85	.63	.63	8/30 = .27

Convert to log to base of 2

profile

	1	2	3	4	5
A	-0.23	0.72	-0.23	-0.23	-0.23
C	-0.81	-0.81	-0.81	0.74	0.74
G	1.11	-0.43	-0.43	-0.43	-0.43
T	-0.66	0.29	0.89	-0.66	-0.66

(B)

Match **GATCA** to PSSM

Find nucleotides at corresponding positions

	1	2	3	4	5
A	-0.23	0.72	-0.23	-0.23	-0.23
C	-0.81	-0.81	-0.81	0.74	0.74
G	1.11	-0.43	-0.43	-0.43	-0.43
T	-0.66	0.29	0.89	-0.66	-0.66

Sum corresponding log odds matrix scores

Score = 1.11 + 0.72 + 0.89 + 0.74 - 0.23 = **3.23**

PSI BLAST:
Constructing the Profile Matrix

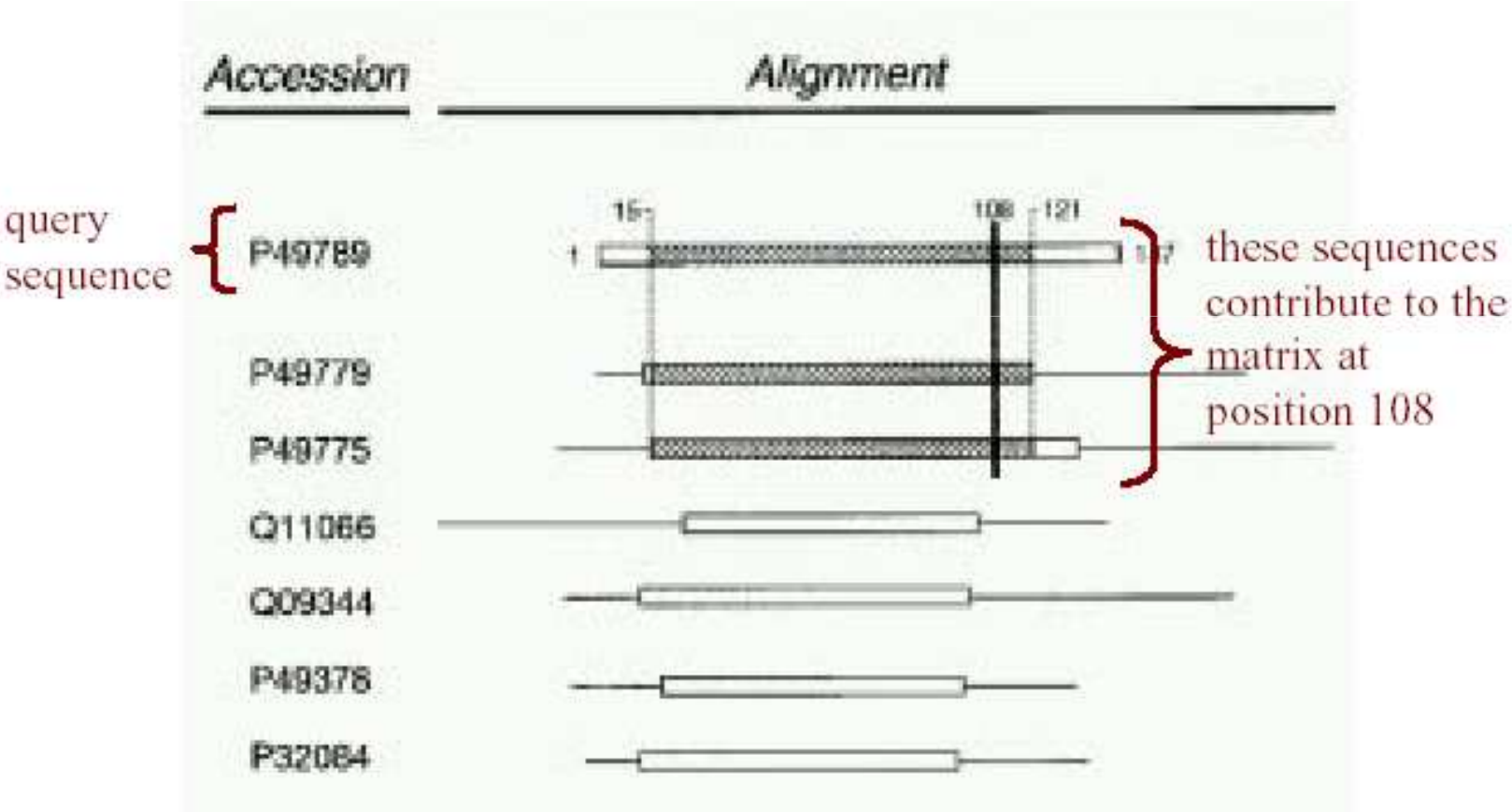


Figure from: Altschul et al. Nucleic Acids Research 25, 1997

PSI BLAST: Determining Profile Elements

- the value for a given element of the profile matrix is given by:

$$matrix(i,j) = \log\left(\frac{\Pr(a_i \mid \text{col} = j)}{\Pr(a_i)}\right)$$

- where the probability of seeing amino acid a_i in column j is estimated as:

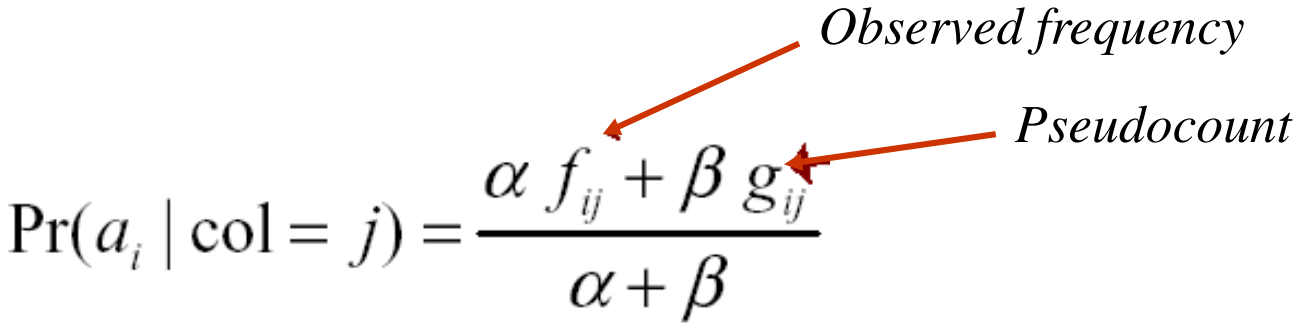
Observed frequency

Pseudocount

$$\Pr(a_i \mid \text{col} = j) = \frac{\alpha f_{ij} + \beta g_{ij}}{\alpha + \beta}$$

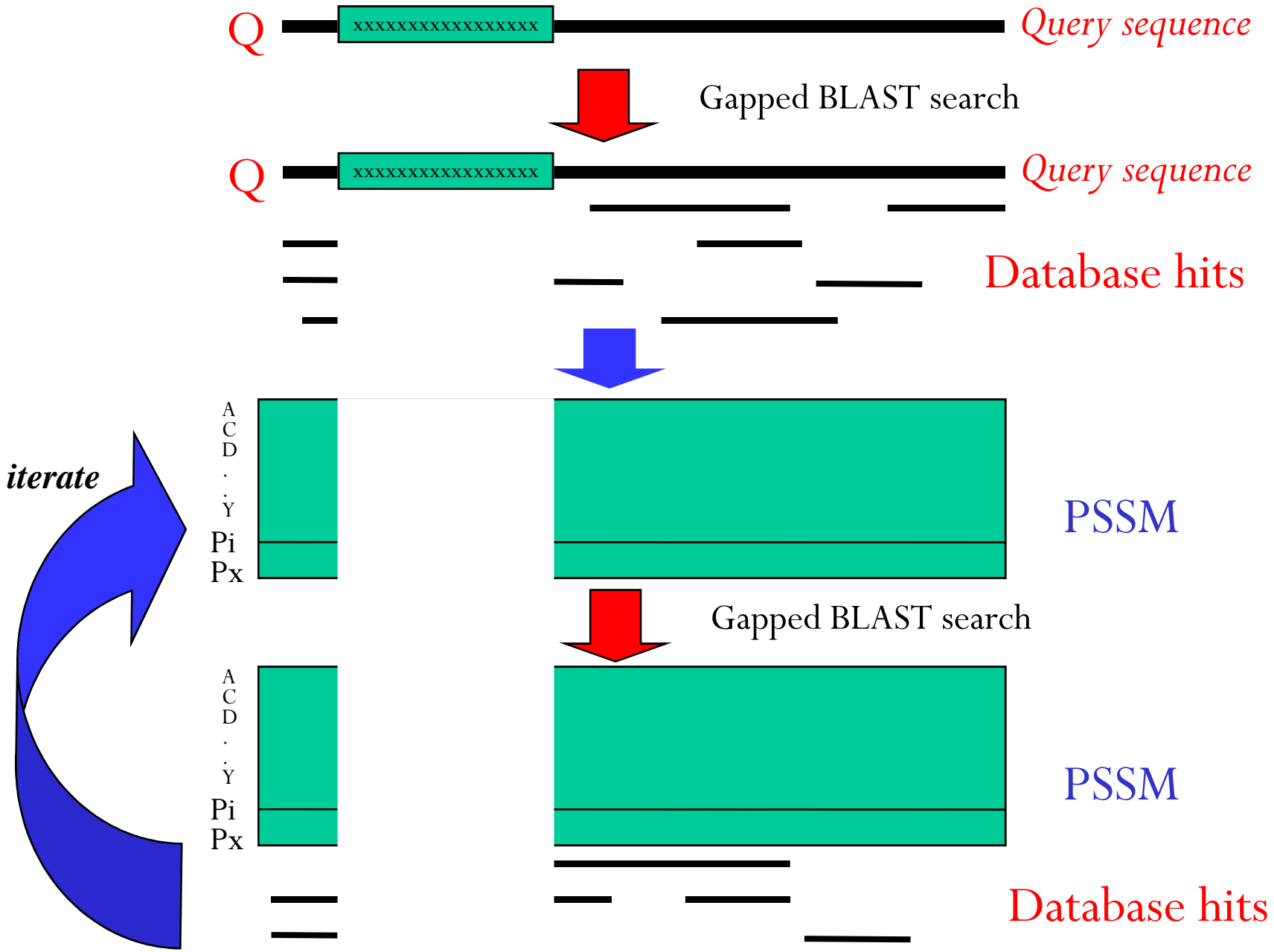
e.g. α = number of sequences in profile, β=1

PSI BLAST: Determining Profile Elements



- f_{ij} denote the frequencies in a given profile position (alignment column) while g_{ij} are the frequencies observed in a database (e.g., the complete alignment, sequence family, or complete sequence database)
- α and β are scaling factors to weigh the f and g contributions: if α equals the number of sequences in profile, and β=1 (preceding slide), then the influence of the observed frequencies in the database becomes less with an increased number of sequences in the alignment (from which the profile is taken). The rationale for this is that when there is only one sequence in the alignment, one cannot be sure about the observed frequencies (all the time a single amino acid), such that a greater emphasis on the database frequency is a good thing. With greater numbers of sequences in the alignment, the database influence can become less and less.

PSI-BLAST iteration



PSI-BLAST steps in words

- Query sequences are first scanned for the presence of so-called ***low-complexity regions*** (Wooton and Federhen, 1996 – next slide), *i.e.* regions with a biased composition likely to lead to spurious hits, are excluded from alignment.
- The program then initially operates on a single query sequence by performing a gapped BLAST search
- Then, the program takes significant local alignments (hits) found, constructs a multiple alignment (master-slave alignment) and abstracts a position-specific scoring matrix (PSSM) from this alignment.
- Rescan the database in a subsequent round, using the PSSM, to find more homologous sequences. Iteration continues until user decides to stop or search has converged

Low-complexity sequences

- For example: AAAAA… or AYLAYLAYL… or AYLLYAALY…
- Low-complexity (sub)sequences have a biased composition and contain less information than high-complexity sequences
- Because of the low information content, they often lead to spurious hits without a biological basis (for example, you can't tell whether a poly-A sequence is more similar to a globin, an immunoglobulin or a kinase sequence).

The innovation and power of BLAST is the statistical scoring system

- (PSI-)BLAST converts raw alignment scores based on the
  - (query-database) sequence lengths
  - the size of the data base
  - the (amino acid or nucleotide) composition of the database
- It also checks to what extend a hit score is higher than randomly expected
  - BLAST has a clever and fast way for doing this
- This makes the scores really comparable, so that the hit list can be ordered based on their statistical scores (bit-scores and E-values)

Statistical scoring using scrambled sequences (Z-scores)

- Biological sequence AVTCAAG can be randomly scrambled into VCAGATA (keeping the residue composition intact)
- For assessing the statistical significance of a given pairwise alignment (query against database sequence) the Z-score can be used:

$$Z = (x - \text{mean}) / \text{standard\_deviation},$$

  where x is the (raw) alignment score between the query and database sequence, and the mean and standard-deviation are calculated over a large number (100-1000) of pairwise alignments of scrambled sequence pairs
- Z-scores are calculated independently, each time only the query and a database sequence are needed
- Z-score calculation becomes computationally prohibitive with large sequence databases

Statistics and thresholds

- Simple idea: accept only hits above a certain threshold value *T*
- The likelihood of random sequences to yield a score >*T* increases linearly with the logarithm of the 'search space' *n***m* (query sequence length *n* and total database sequence length *m*)
- This gives the following formula for accepting hits:

$$\mathbf{S > T + \log(m*n)/\lambda},$$

where λ is depending upon the scoring scheme (substitution matrix, gap penalties)

Alignment Bit Score

$$B = (\lambda S - \ln K) / \ln 2$$

- S is the raw alignment score
- The bit score ('bits') B has a standard set of units
- The bit score B is calculated from the number of gaps and substitutions associated with each aligned sequence. The higher the score, the more significant the alignment
- λ and K and are the statistical parameters of the scoring system (BLOSUM62 in Blast).
- See Altschul and Gish, 1996, for a collection of values for λ and K over a set of widely used scoring matrices.
- Because bit scores are normalized with respect to the scoring system, they can be used to compare alignment scores from different searches based on different scoring schemes (e.g., using different amino acid exchange matrices)

Normalised sequence similarity

The **p-value** is defined as the probability of seeing at least one unrelated score S greater than or equal to a given score x in a database search over n sequences.

This probability follows the Poisson distribution (Waterman and Vingron, 1994):

$$P(x, n) = 1 - e^{-n \cdot P(S \geq x)},$$

where n is the number of sequences in the database

Depending on x and n (fixed)

Normalised sequence similarity

Statistical significance

The **E-value** is defined as the expected number of non-homologous sequences with score greater than or equal to a score x in a database of n sequences:

$$E(x, n) = n \cdot P(S \geq x)$$

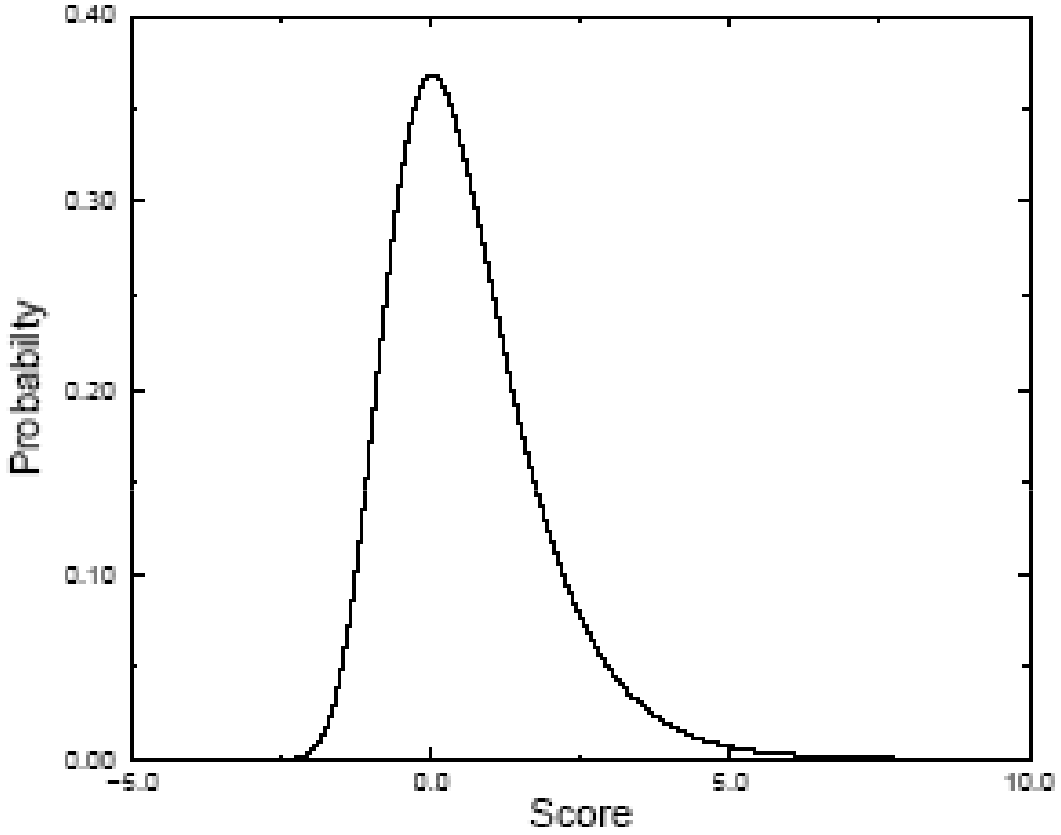
For example, if E-value = 0.01, then the expected number of random hits with score $S \geq x$ is 0.01, which means that this E-value is expected by chance only once in 100 independent searches over a randomised database.

if the E-value of a hit is 5, then five fortuitous hits with $S \geq x$ are expected within a single randomised database search, which renders the hit not significant.

A model for database searching score probabilities

- Scores resulting from searching with a query sequence against a database follow the Extreme Value Distribution (EDV) (Gumbel, 1955).
- Using the EDV, the raw alignment scores are converted to a statistical score (E value) that keeps track of the database amino acid composition and the scoring scheme (a.a. exchange matrix)

Extreme Value Distribution (EVD)

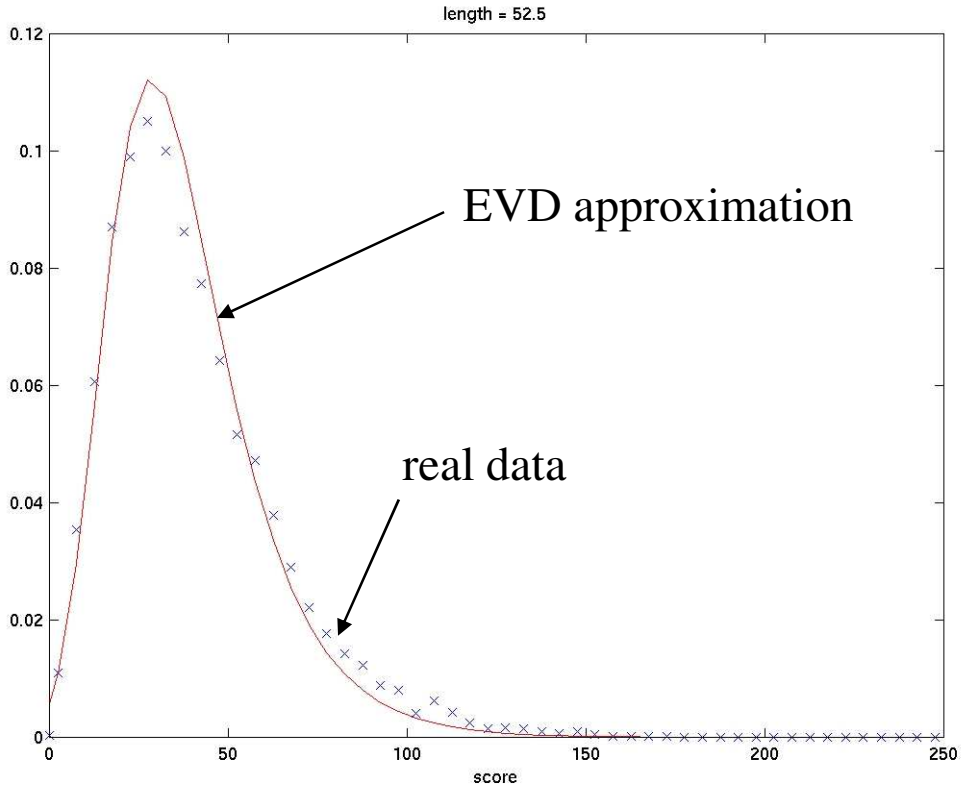


$$y = 1 - \exp(-e^{-\lambda(x-\mu)})$$

An exponent in an exponent..

Probability density function for the extreme value distribution resulting from parameter values $\mu = 0$ and $\lambda = 1$, [$y = 1 - \exp(-e^{-x})$], where μ is the characteristic value and λ is the decay constant.

Extreme Value Distribution (EVD)



You know that an optimal alignment of two sequences is selected out of many suboptimal alignments, and that a database search is also about selecting the best alignment(s). This bodes well with the EVD which has a right tail that falls off more slowly than the left tail. Compared to using the normal distribution, when using the EVD an alignment has to score further beyond the expected mean value to become a significant hit.

Extreme Value Distribution

The probability of a score S to be larger than a given value x can be approximated following the EVD as:

E-value: $P(S \geq x) = 1 - \exp(-e^{-\lambda(x-\mu)})$,

where $\mu = (\ln Kmn)/\lambda$, and K a constant that can be estimated from the background amino acid distribution and scoring matrix (see Altschul and Gish, 1996, for a collection of values for λ and K over a set of widely used scoring matrices).

Extreme Value Distribution

Using the equation for μ (preceding slide), the probability for the raw alignment score S becomes

$$P(S \geq x) = 1 - \exp(-Kmne^{-\lambda x}).$$

In practice, the probability $P(S \geq x)$ is estimated using the approximation $1 - \exp(-e^{-x}) \approx e^{-x}$, which is valid for large values of x. This leads to a simplification of the equation for $P(S \geq x)$:

$$P(S \geq x) \approx e^{-\lambda(x-\mu)} = Kmne^{-\lambda x}.$$

The lower the probability (E value) for a given threshold value x, the more significant the score S.

Normalised sequence similarity

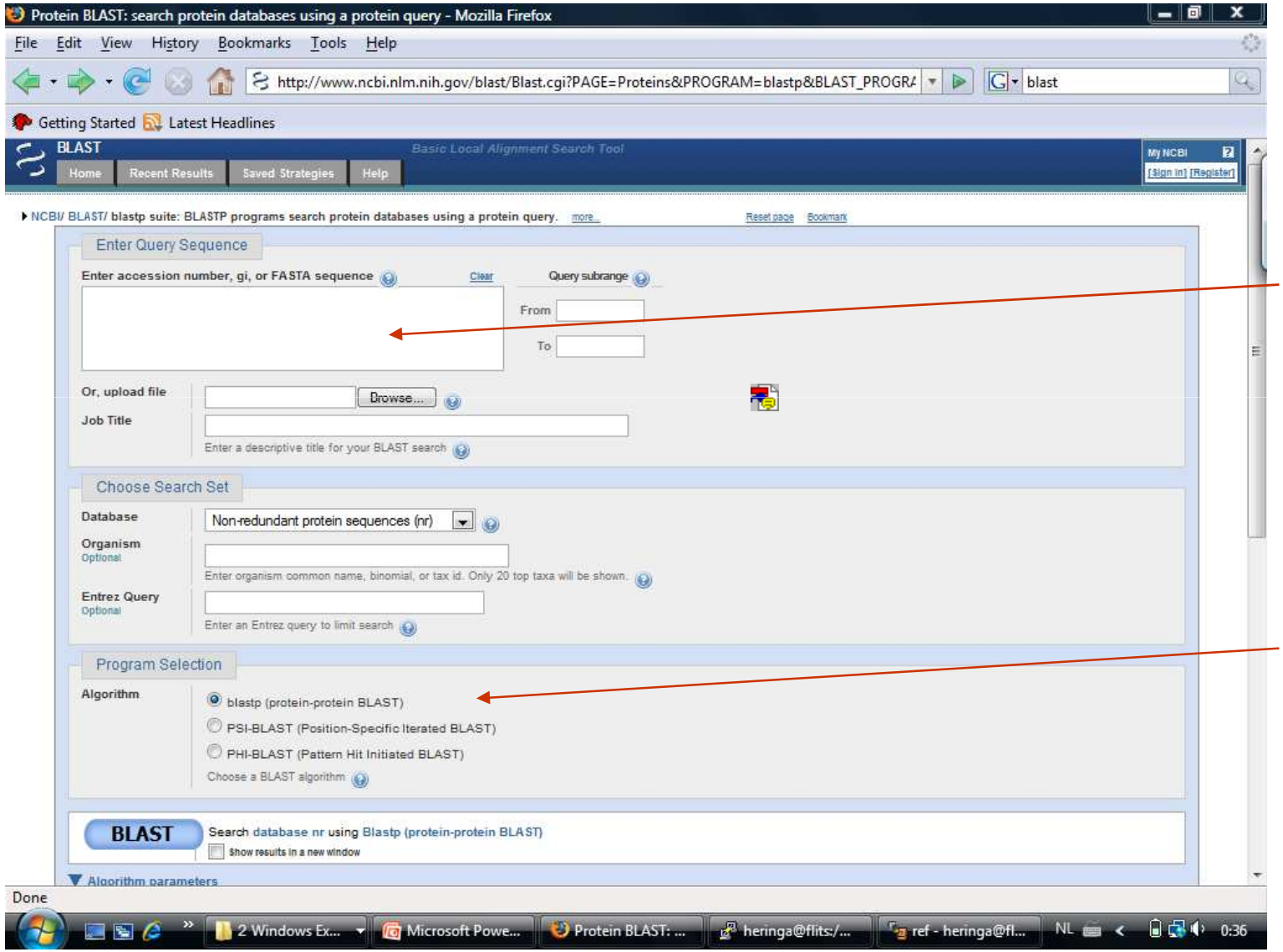
Statistical significance

- Database searching is commonly performed using an E-value threshold in between 0.1 and 0.001.
- Low E-value thresholds decrease the number of false positives in a database search, but increase the number of false negatives, thereby lowering the sensitivity of the search.
- Each time the database grows, the BLAST team has to recalculate the $\mu = (\ln Kmn)/\lambda$ and λ values (one time per DB update)

Words of Encouragement

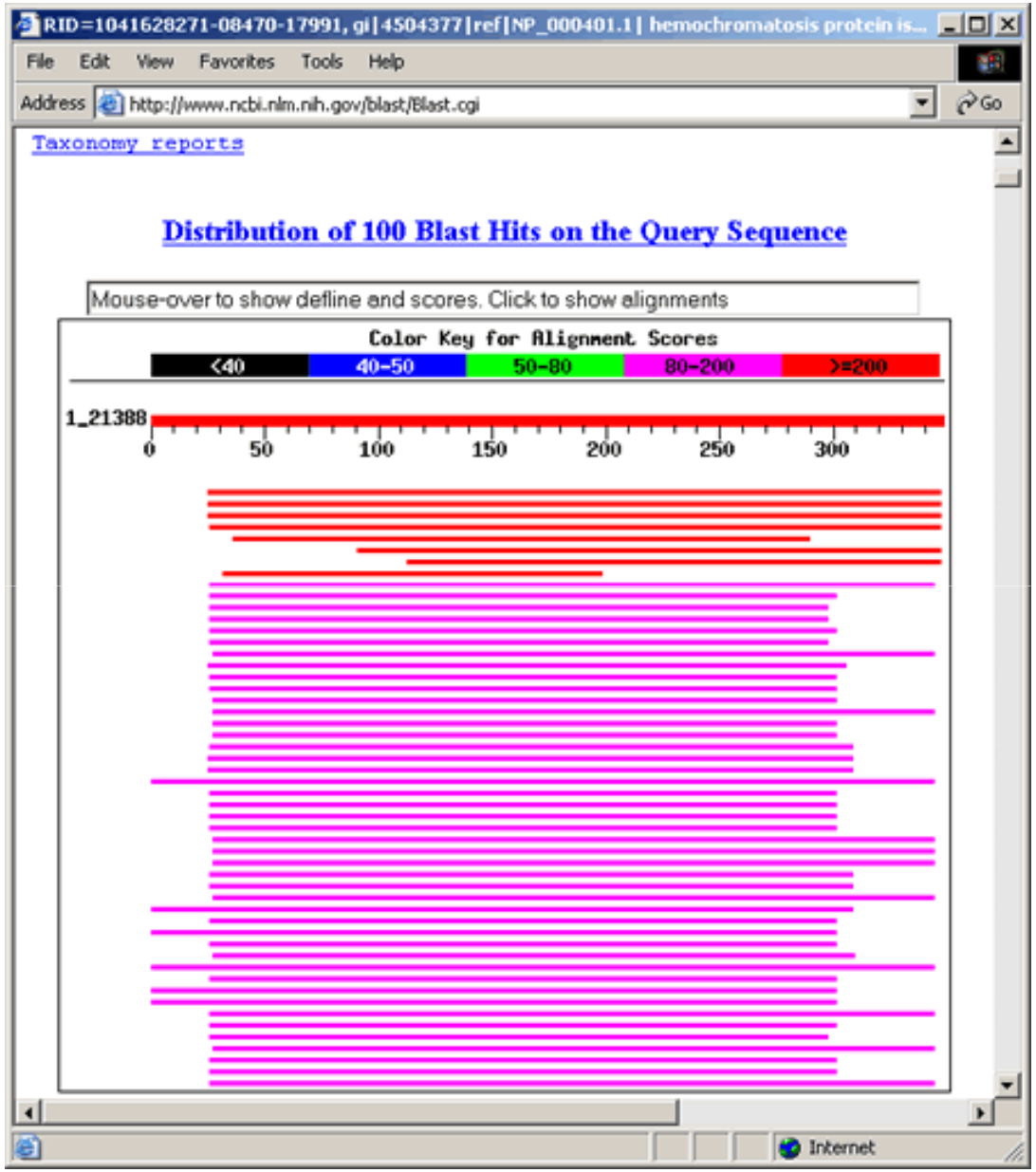
- *"There are three kinds of lies: lies, damned lies, and statistics"* – Benjamin Disraeli
- *"Statistics in the hands of an engineer are like a lamppost to a drunk – they're used more for support than illumination"*
- *"Then there is the man who drowned crossing a stream with an average depth of six inches."* – W.I.E. Gates

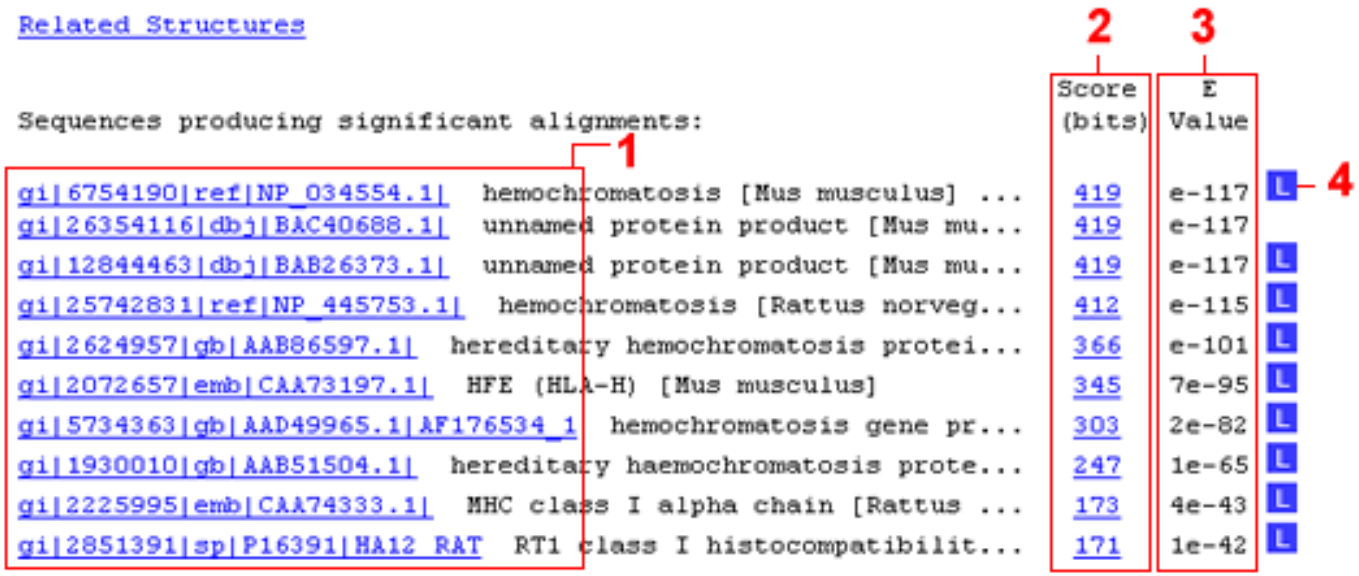
PSI-BLAST entry page



Paste your query sequence

Choose the BLAST program you want


RID=1041628271-08470-17991, gi|4504377|ref|NP_000401.1| hemochromatosis protein is...
File Edit View Favorites Tools Help
Address http://www.ncbi.nlm.nih.gov/blast/Blast.cgi
Go
Taxonomy reports
Distribution of 100 Blast Hits on the Query Sequence
Mouse-over to show defline and scores. Click to show alignments
Color Key for Alignment Scores
<40
40-50
50-80
80-200
>=200
1_21388
0
50
100
150
200
250
300
Internet

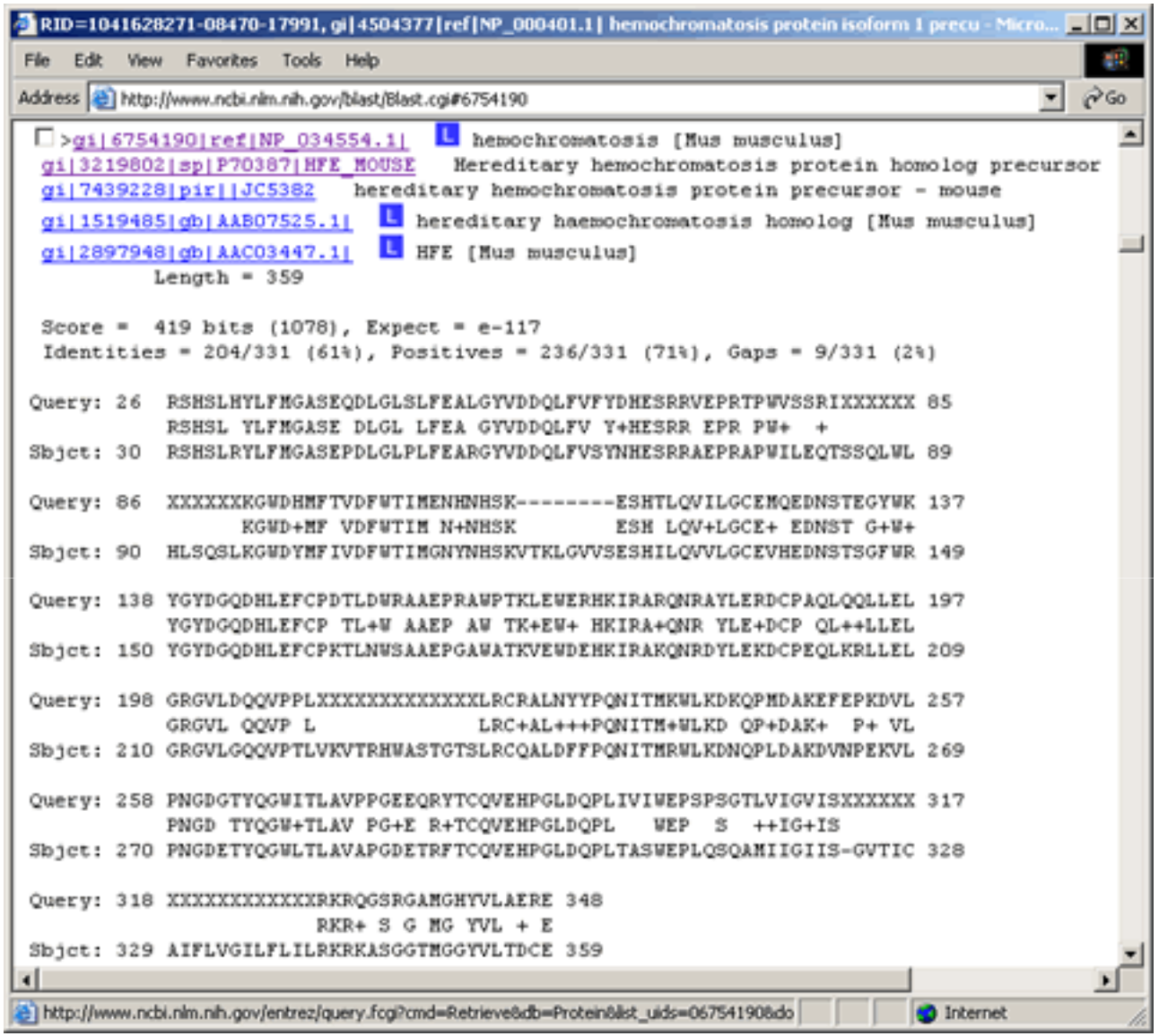


1 - This portion of each description links to the sequence record for a particular hit.

2 - Score or bit score is a value calculated from the number of gaps and substitutions associated with each aligned sequence. The higher the score, the more significant the alignment. Each score links to the corresponding pairwise alignment between query sequence and hit sequence (also referred to as subject or target sequence).

3 - E Value (Expect Value) describes the likelihood that a sequence with a similar score will occur in the database by chance. The smaller the E Value, the more significant the alignment. For example, the first alignment has a very low E value of e^{-117} meaning that a sequence with a similar score is very unlikely to occur simply by chance.

4 - These links provide the user with direct access from BLAST results to related entries in other databases. 'L' links to LocusLink records and 'S' links to structure records in NCBI's Molecular Modeling DataBase.



'X' residues denote low-complexity sequence fragments that are ignored during the search

Protein Database	Description
nr	All non-redundant GenBank CDS translations+PDB+SwissProt+PIR+PRF
month	All new or revised GenBank CDS translation+PDB+SwissProt+PIR released in the last 30 days.
swissprot	The last major release of the SWISS-PROT protein sequence database (no updates). These are uploaded to our system when they are received from EMBL.
patents	Protein sequences derived from the Patent division of GenBank.
yeast	Yeast (Saccharomyces cerevisiae) protein sequences. This database is not to be confused with a listing of all Yeast protein sequences. It is a database of the protein translations of the Yeast complete genome.
E. coli	E. coli (Escherichia coli) genomic CDS translations.
pdb	Sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank.
kabat [kabatpro]	Kabat's database of sequences of immunological interest. For more information http://immuno.bme.nwu.edu/
alu	Translations of select Alu repeats from REPBASE, suitable for masking Alu repeats from query sequences. It is available at ftp://ncbi.nlm.nih.gov/pub/jmc/alu. See "Alu alert" by Claverie and Makalowski, Nature vol. 371, page 752 (1994).

Nucleotide Database	Description
nr	All non-redundant GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS, or HTGS sequences).
month	All new or revised GenBank+EMBL+DDBJ+PDB sequences released in the last 30 days.
dbest	Non-redundant database of GenBank+EMBL+DDBJ EST Divisions.
dbsts	Non-redundant database of GenBank+EMBL+DDBJ STS Divisions.
mouse ests	The non-redundant Database of GenBank+EMBL+DDBJ EST Divisions limited to the organism mouse.
human ests	The Non-redundant Database of GenBank+EMBL+DDBJ EST Divisions limited to the organism human.
other ests	The non-redundant database of GenBank+EMBL+DDBJ EST Divisions all organisms except mouse and human.
yeast	Yeast (Saccharomyces cerevisiae) genomic nucleotide sequences. Not a collection of all Yeast nucelotides sequences, but the sequence fragments from the Yeast complete genome.
E. coli	E. coli (Escherichia coli) genomic nucleotide sequences.
pdb	Sequences derived from the 3-dimensional structure of proteins.
kabat [kabatnuc]	Kabat's database of sequences of immunological interest. For more information http://immuno.bme.nwu.edu/
patents	Nucleotide sequences derived from the Patent division of GenBank.
vector	Vector subset of GenBank(R), NCBI, (ftp://ncbi.nlm.nih.gov/pub/blast/db/ directory).
mito	Database of mitochondrial sequences (Rel. 1.0, July 1995).
alu	Select Alu repeats from REPBASE, suitable for masking Alu repeats from query sequences. It is available at ftp://ncbi.nlm.nih.gov/pub/jmc/alu. See "Alu alert" by Claverie and Makalowski, Nature vol. 371, page 752 (1994).
epd	Eukaryotic Promotor Database ISREC in Epalinges s/Lausanne (Switzerland).
gss	Genome Survey Sequence, includes single-pass genomic data, exon-trapped sequences, and Alu PCR sequences.
htgs	High Throughput Genomic Sequences.

Making things even faster- indexing the complete database (or genome sequence)

- SSAHA – Sequence Search and Alignment by Hashing Algorithms (Ning et al., 2001)
- BLAT – BLAST-like Alignment Tool (Kent, 2002)
- PatternHunter (Ma et al., 2002)
- BLASTZ – alignment of genomic sequences (Schwartz et al., 2003)

BLAT – BLAST-Like Alignment Tool

- Analyzing vertebrate genomes requires rapid mRNA/DNA and cross-species protein alignments. **BLAT** (the BLAST-like alignment tool) was developed by Jim Kent from UCSC. It is more accurate and 500 times faster than popular existing tools such as BLAST for mRNA/DNA alignments and 50 times faster for protein alignments at sensitivity settings typically used when comparing vertebrate sequences (e.g. BLAST).
- BLAT's speed stems from an index of all nonoverlapping k-mers in the genome. This index fits inside the RAM of inexpensive computers, and need only be computed once for each genome assembly. BLAT has several major stages. It uses the index to find regions in the genome likely to be homologous to the query sequence. It performs an alignment between homologous regions. It stitches together these aligned regions (often exons) into larger alignments (typically genes). Finally, BLAT revisits small internal exons possibly missed at the first stage and adjusts large gap boundaries that have canonical splice sites where feasible.
- **From Wikipedia, the free encyclopedia**

BLAT – BLAST-Like Alignment Tool

- Blat produces two major classes of alignments:
- at the DNA level between two sequences that are of 95% or greater identity, but which may include large inserts
- at the protein or translated DNA level between sequences that are of 80% or greater identity and may also include large inserts.
- The output of BLAT is flexible. By default it is a simple tab-delimited file which describes the alignment, but which does not include the sequence of the alignment itself.

Indexing (hashing) the database

- BLAT - The Blast-Like Alignment Tool
- For large-scale genome comparison

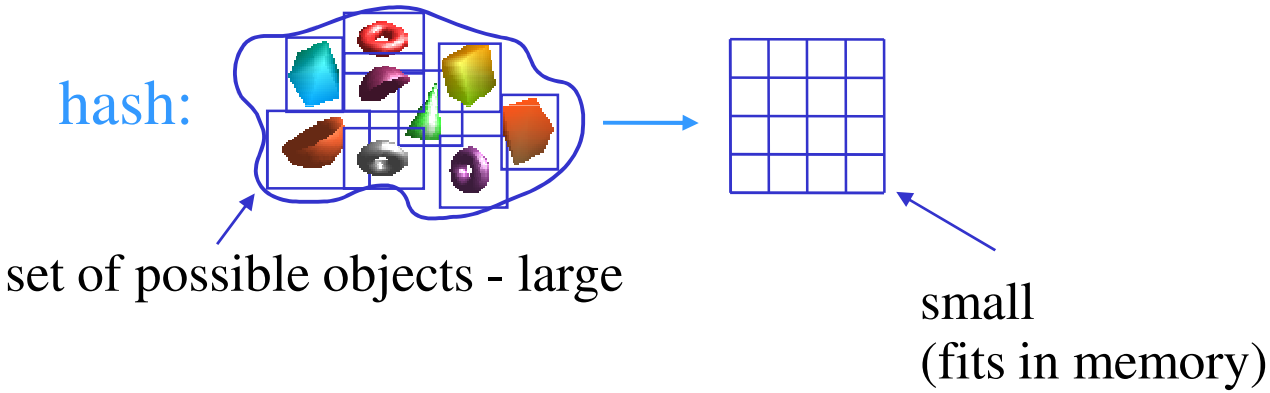
– query can be as large as a complete genome

Preprocessing phase:

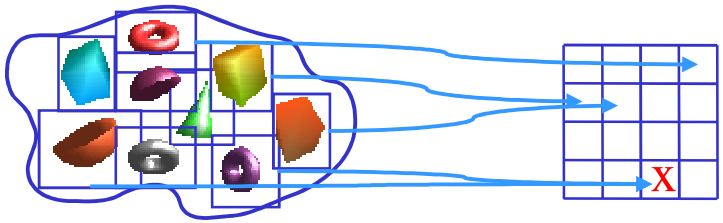
- BLAST: indexes only the query sequence
- BLAT: indexes the complete database

Hashing – associative arrays (recap)

- Indexing with the object, the
- Hash function:



- Objects should be “well spread”

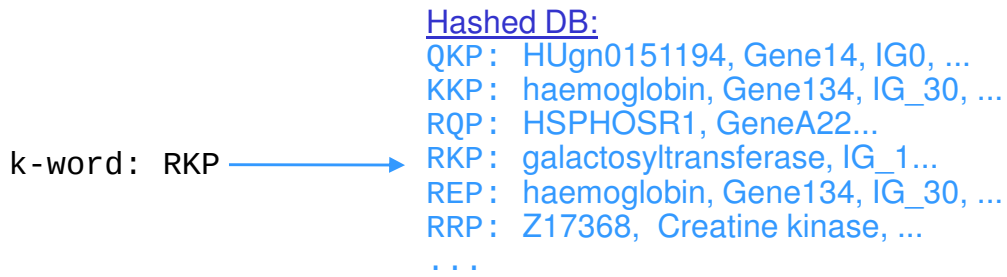


Hashing – widely used implementation

- *T9 Predictive Text* in mobile phones
  - "hello" in Multitap:
    4, 4, 3, 3, 5, 5, 5,
    (*pause*) 5, 5, 5, 6, 6, 6
  - "hello" in T9:
    4, 3, 5, 5, 6
  - Collisions: 4, 6:
    "*in*", "*go*"

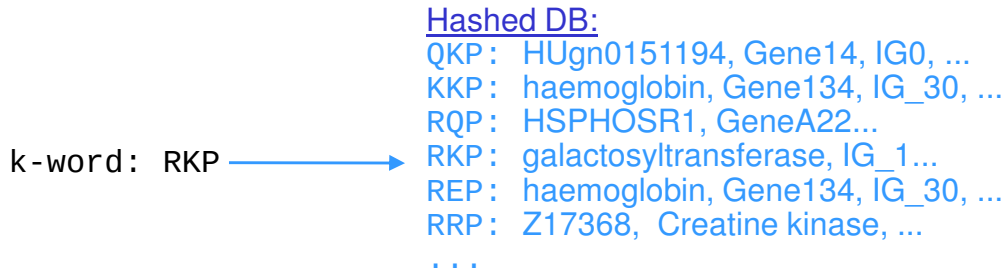
BLAT step 1- indexing the database: Find ”exact” matches with hashing

- Preprocess the database
  - Hash the database with k-words
  - For each k-word store in which sequences it appears



BLAT step 1- indexing the database: Find "exact" matches with hashing

- The database is preprocessed only once! (independent from the query)
- In a constant time we can get the sequences with a certain *k-word*



BLAT – step2: scanning the DB

- Hit criteria
- In a constant time we can get the
- Sequences with a certain *k-word*
- Relaxing hit definition -> improve sensitivity

  - allow imperfect hits

  - costly, huge hash grows a few times!

➔ shorten k (would lead to FP), but expect two hits (see BLAST two-hit method)

BLAT, Step 3 – Identifying homologous regions

- Exclude common k-words
- For all k-words from query
  – find out the position in db

- For results (qpos, dbpos):
  – split into buckets (64kbp)
  – sort on the diagonal (diag=qposdbpos)

BLAT, Step 3 – Identifying homologous regions (Continued)

- from diagonally close hits (gap limit) create "pre-clusters"
  - sort each "pre-cluster" on dbpos
  - create clusters from close hits
  - run **Local Alignment** for each cluster

Seeds – improving sensitivity

- More general form of k-word is **a seed**
- The seed

CT.GT.AT.

gives “hits” with both sequences

...CTCGTTATA...

...CTAGTAATG...

HMM-based homology searching

- Most widely used HMM-based profile searching tools currently are SAM-T2K (Karplus *et al.*, 2000) and HMMER2 (Eddy, 1998)
- Formal probabilistic basis and consistent theory behind gap and insertion scores
- HMMs good for profile searches, not as good for alignment
- HMMs are slow

The Framework

Databank searching can be split into three phases:

1. Matching phase

   The query sequence is compared by (partial) alignment with the databank sequence. Most programs pre-select potential hits at this level. The comparison is usually heuristic and fast (see previous lecture).

2. Scoring phase

   If the database sequence passed the matching phase, the query-hit sequence pair is re-aligned and scored, mostly using a pairwise scoring matrix. (see previous lecture)

3. Selection phase

   Based on a statistical criterion, sequences with a score above a user defined score cutoff are returned as hits. The hitlist is the result of the databank searching and each hit is a potential homologue.

Some Tricky Problems

Repeats

Multi-domain proteins

Low-complexity regions

Reduncancy

Very short queries

Very distant sequences

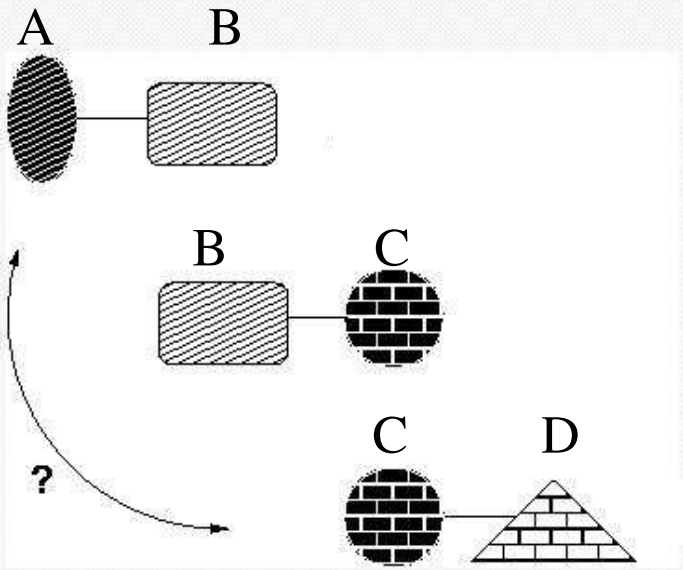
Un-annotated sequences ('conserved hypothetical' proteins)

Profile wander

Multi-domain Proteins

It can be disadvantageous to search with multi-domain proteins. If a multi-domain protein contains domains AB, and B is a common domain, many other (multi-domain) proteins containing this domain will be matched. The interesting domain could be A, but the majority of reported hit matches B.

In iterative sequence searching, the multi-domain proteins BC that were detected in the first round will produce hits to other proteins containing CD. The search may drift from AB to CD.



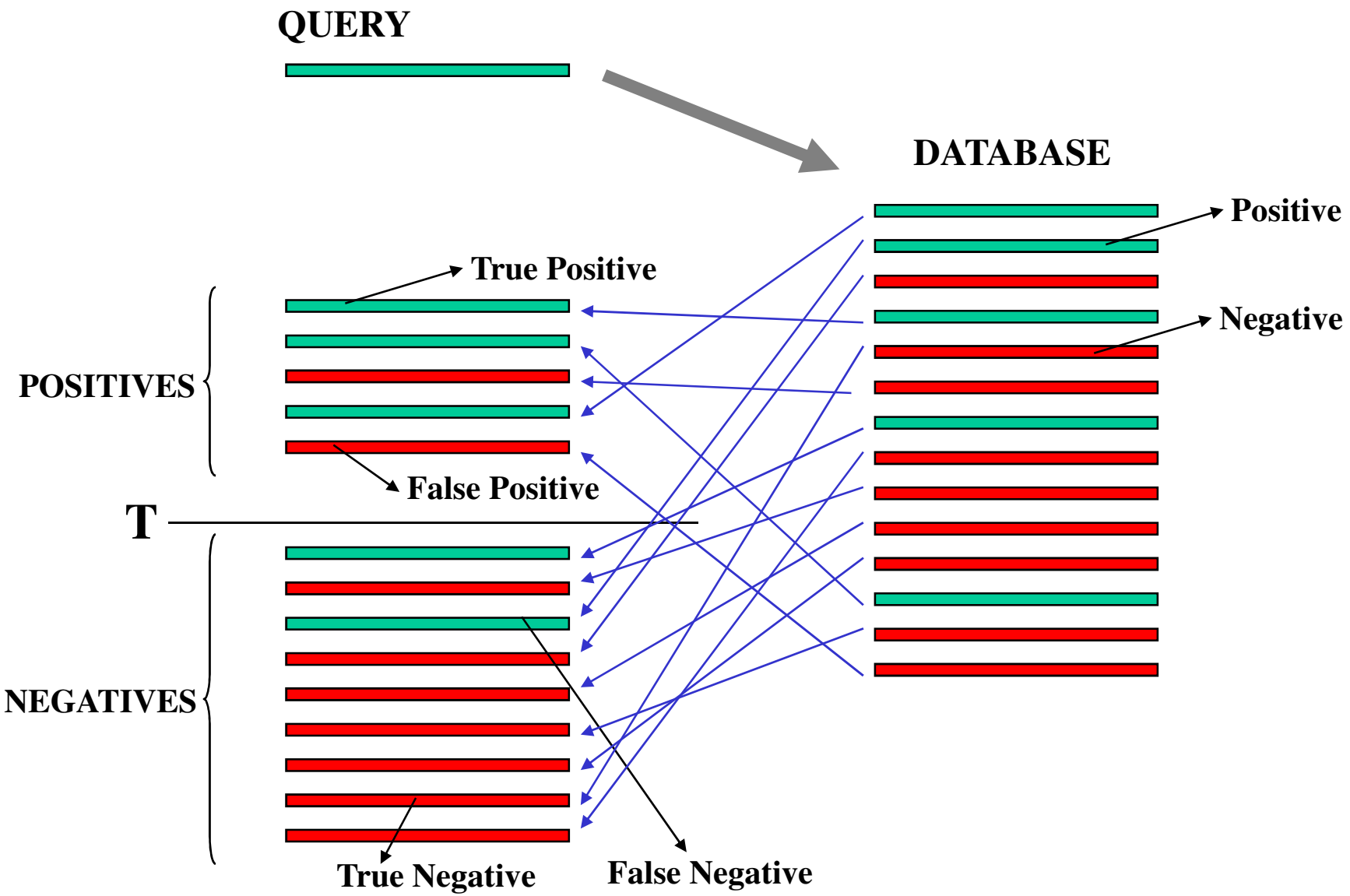
Multi-domain Proteins (cont.)

- A common conserved protein domain such as the tyrosine kinase domain can make weak but relevant matches to other domain types appear very low in the hit list, so that they are missed (e.g. only appearing after 5000 kinase hits)
- Sequences containing low-complexity regions, such as coiled coils and transmembrane regions, can cause an explosion of the search rather than convergence because of the absence of any strong sequence signals.
- Conversely, some searches may lead to premature convergence; this occurs when the PSSM is too strict only allowing matches to very similar proteins, i.e., sequences with the same domain organization as the query are detected but no homologues with different domain combinations. In this case the power of iteration is not used fully.

How to assess homology search methods

- We need an annotated database, so we know which sequences belong to what homologous families
- Examples of databases of homologous families are PFAM, Homstrad or Astral
- The idea is to take a protein sequence from a given homologous family, then run the search method, and then assess how well the method has carried out the search
- This should be repeated for many query sequences and then the overall performance can be measured

Sequence searching



So what have we got

		Observed: P	Observed: N
Predicted	P	TP	FP
Predicted	N	FN	TN

Sensitivity and Specificity – medical world

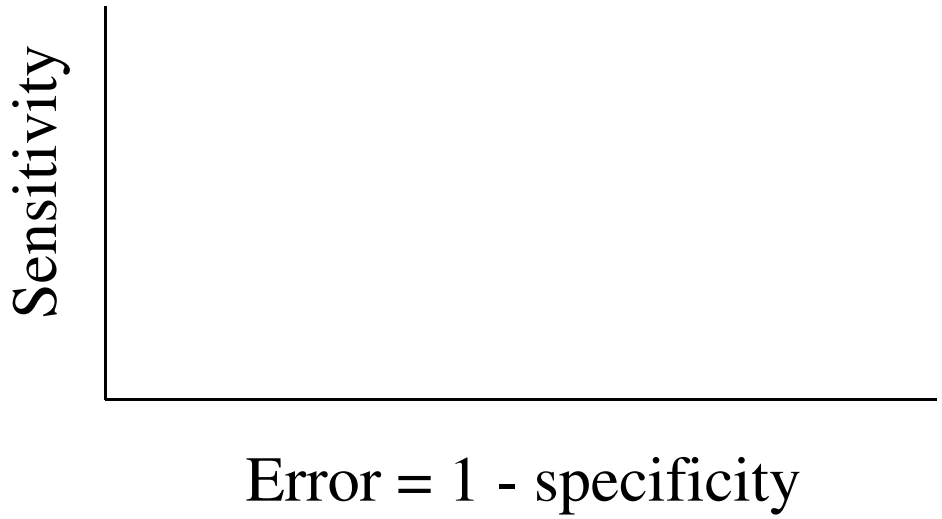
Test	+	-		
+	9990 True Positive (TP)	990 False Positive (FP)	All with Positive Test TP+FP	Positive Predictive Value= TP/(TP+FP) 9990/(9990+990) =91%
-	10 False Negative (FN)	989,010 True Negative (TN)	All with Negative Test FN+TN	Negative Predictive Value= TN/(FN+TN) 989,010/(10+989,010) =99.999%
	All with Disease 10,000	All without Disease 999,000	Everyone= TP+FP+FN+TN	
	Sensitivity= TP/(TP+FN) 9990/(9990+10)	Specificity= TN/(FP+TN) 989,010/(989,010+990)	Pre-Test Probability= (TP+FN)/(TP+FP+FN+TN) (in this case = prevalence) 10,000/1,000,000 = 1%	

Coverage and specificity

- Easy to remember:
  - Coverage = TP/all positives
  - Specificity = TN / all negatives

Receiver Operator Curve (ROC)

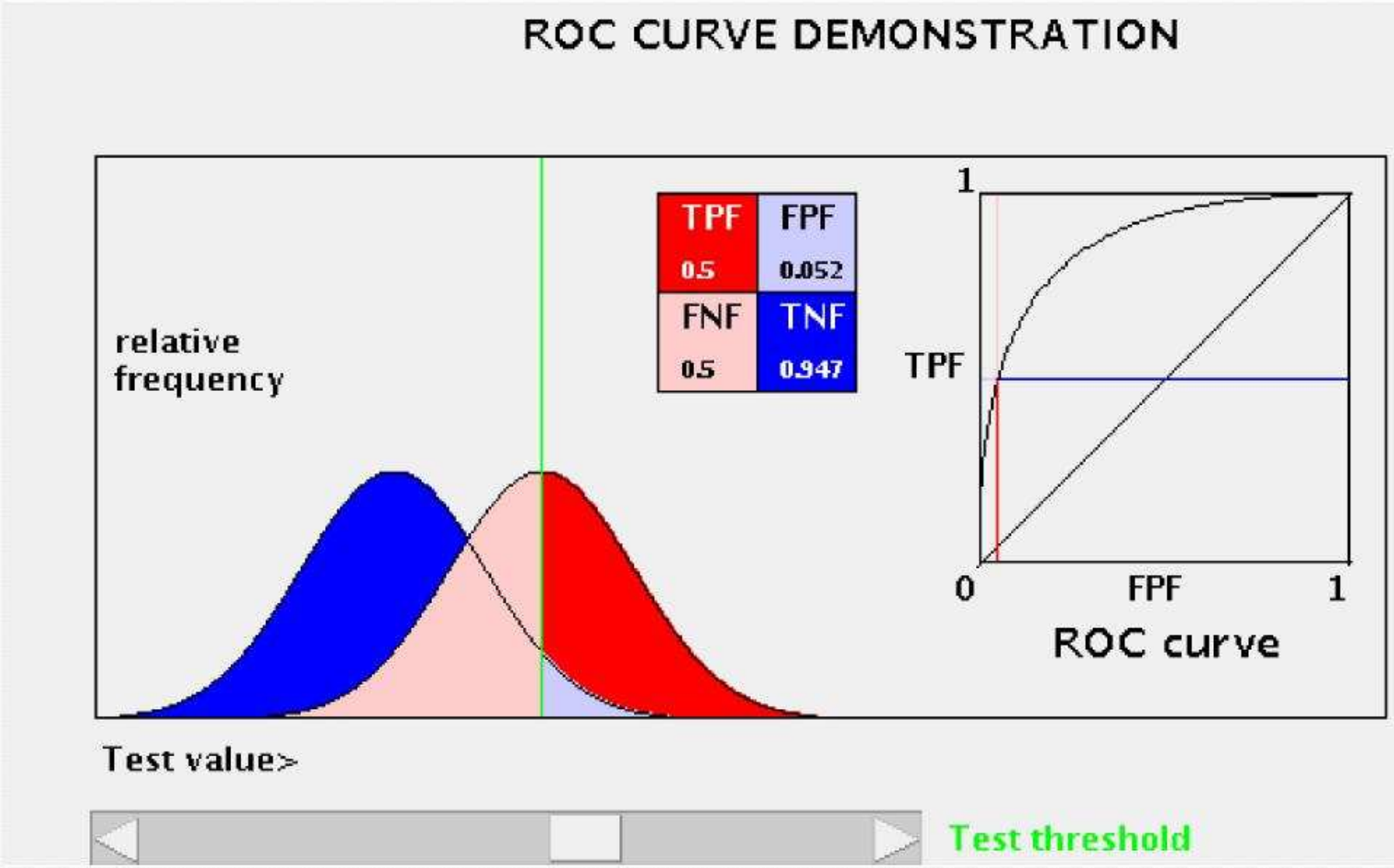
- Plot Sensitivity (TP/(TP+FN)) against 1-specificity (1-TN/(FP+TN)), where the latter is called **error**



Sensitivity is also called **Coverage**

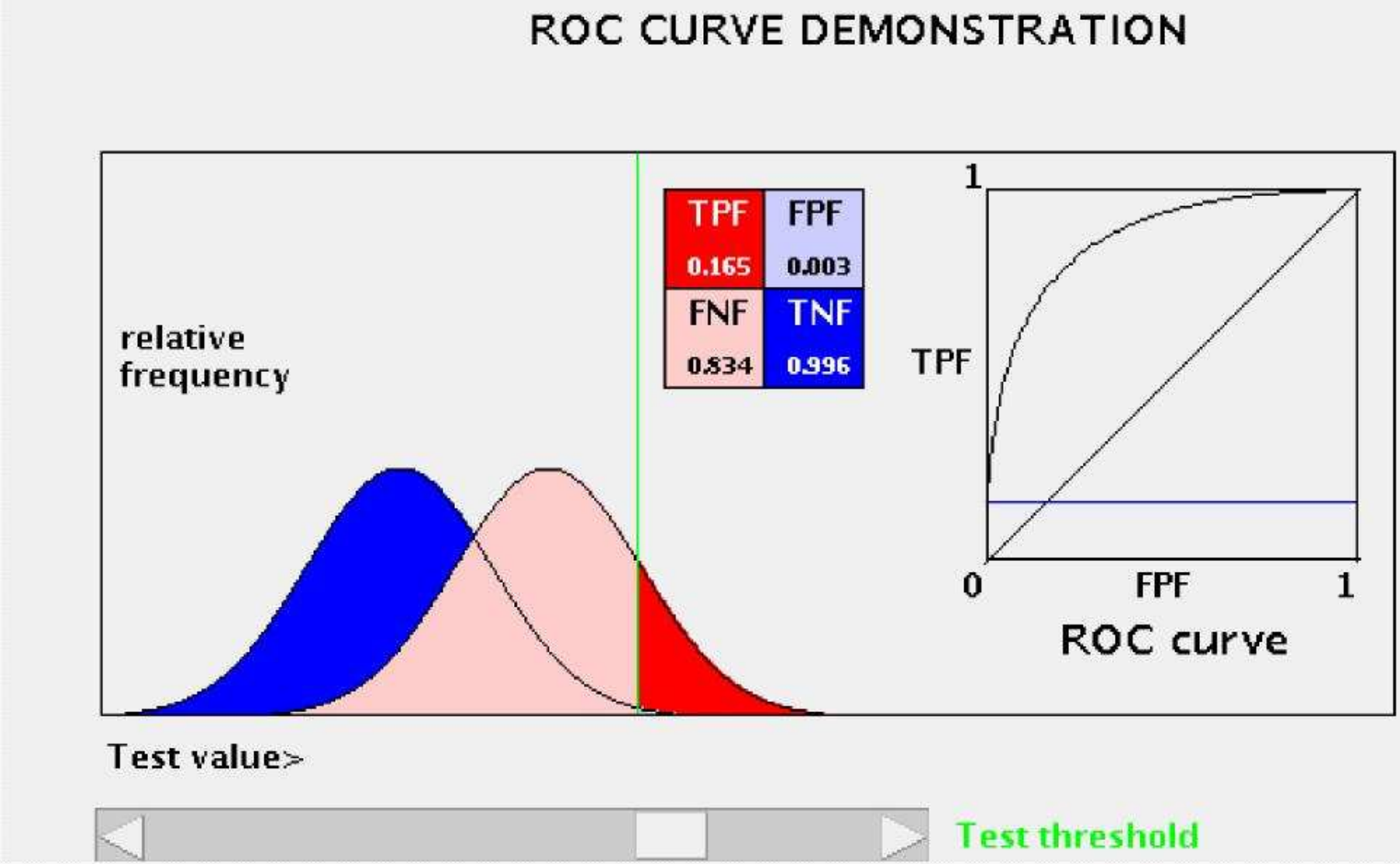
Going down the prediction list: correct prediction - one step up; false prediction - one step to the right

Sequence Searching



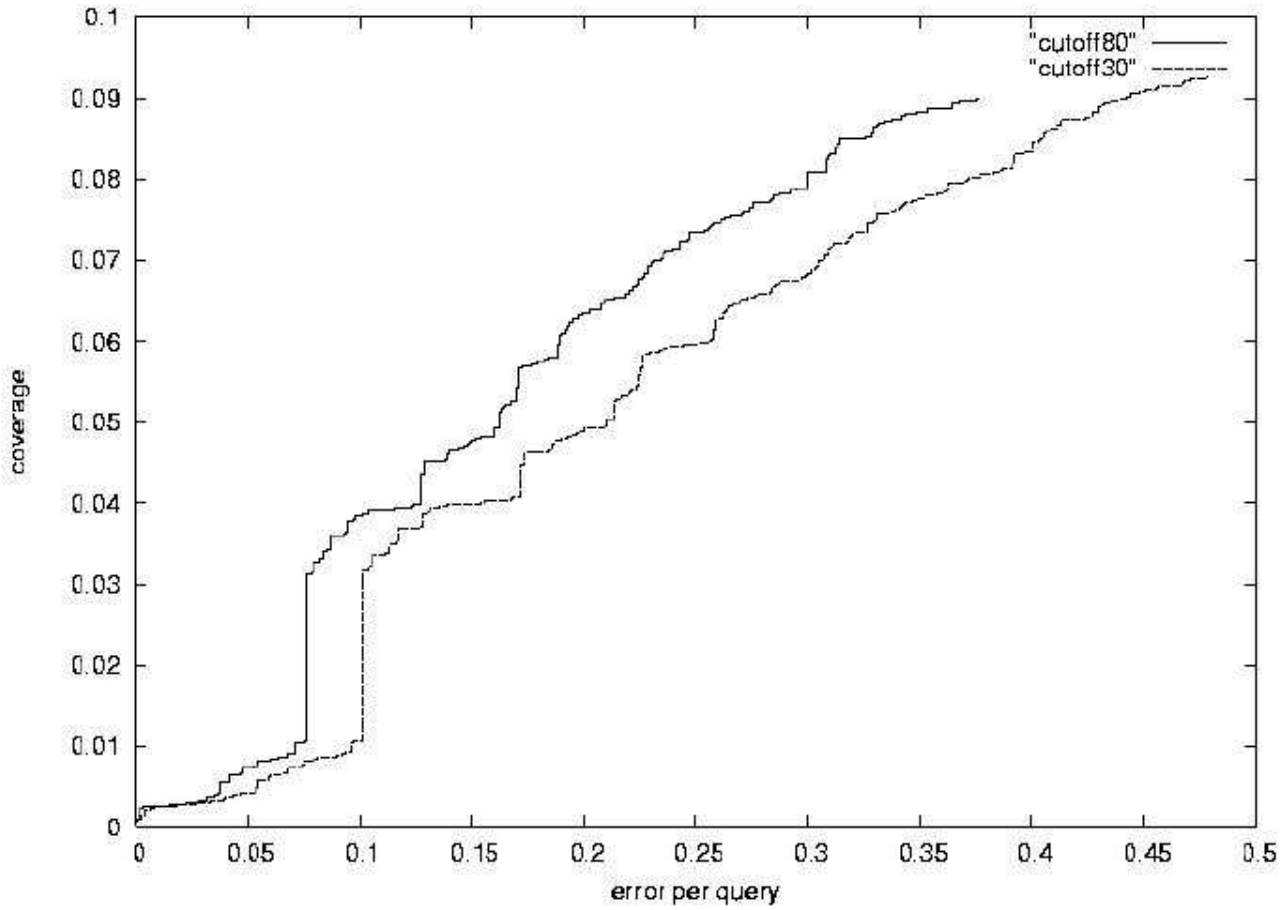
http://www.anaesthetist.com/mnm/stats/roc/

Sequence Searching



Benchmark (see also ROC curve in previous lecture)

A benchmark is a performance test of a method on a representative set of examples, for which the sequence relationships are known. Therefore, each hit can be judged as true/false. The performance of the search program is reflected in the benchmark curve.



Methods for Sequence Searching

Heuristic Alignment

Matching of short identical words and match extension
(Blast, Fasta)

Dynamic Programming

Parallelised searching on distributed database
(Bioccelerator)

Suffix Tree

Identity matching of substrings
(Mummer)

Pattern Matching

Matching of conserved motifs using HMMs or regular expressions
(HMMer, PROSITE)

Iterative databank searching

Homologously related proteins are classified into families. If the homology can only be derived from similarities in structure or function, they are classified into super-families. The ideal sequence search program would find all family members and all super-family members.

In reality many homologous sequences are missed, because the sequence similarity has been lowered by evolution beyond recognition.

The idea of iterative sequence searching is to use the hit list of an initial search as query for a consecutive search. This can be repeated (iterated) until no more sequences are added to the hit-list.

How do we search a databank with a list of query sequences?

Sequence identity scoring zones

- >25-30%: **homology** zone
- 15-25%: twilight zone
- <15%: midnight zone (Rost, 1999)

Is midnight zone properly definable?