2003

Searching Sequence Databases

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Searching Sequence Databases

Database Searching

- How can we find a particular short sequence in a database of sequences (or one HUGE sequence)?
- Problem is identical to local sequence alignment, but on a much larger scale.
- We must also have some idea of the *significance* of a database hit.
  - Databases always return some kind of hit, how much attention should be paid to the result?

BLAST

- BLAST – Basic Local Alignment Search Tool
- An approximation of the Needleman & Wunsch algorithm
- Sacrifices some search sensitivity for speed

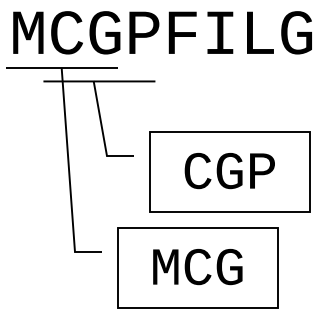
Scoring Matrices

- DNA
  - Identity
  - Transition/Transversion
- Proteins
  - PAM
  - BLOSUM

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

The BLAST algorithm

- Break the search sequence into *words*
  - $W = 3$ for proteins, $W = 12$ for DNA




```
MCG, CGP, GPF, PFI, FIL,
ILG, LGT, GTY, TYC
```

- Include in the search all words that score above a certain value (T) for any search word

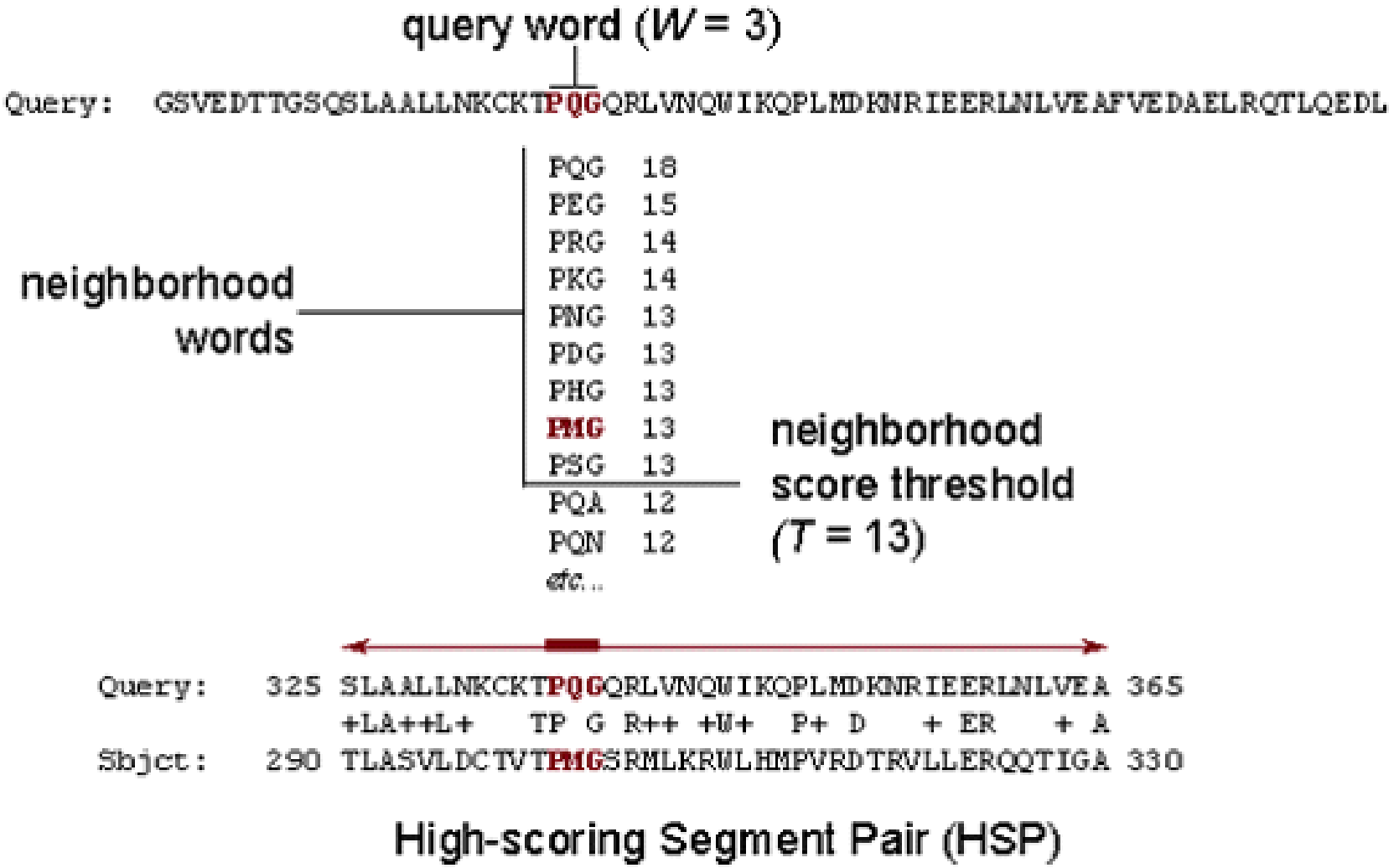
MCG	CGP	
MCT	MGP	…
MCN	CTP	
…	…	

This list can be computed in linear time

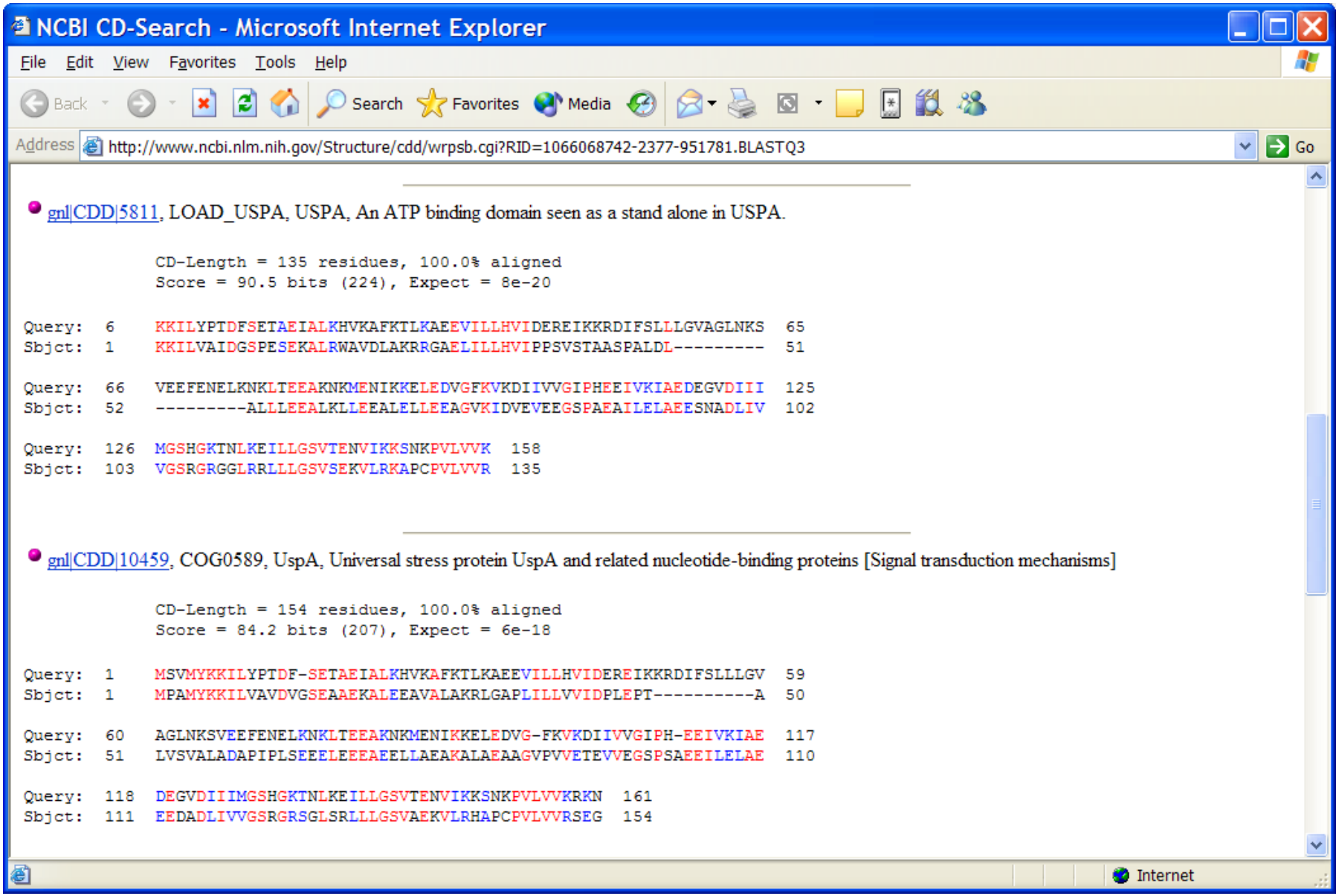
The Blast Algorithm (2)

- Search for the words in the database
  - Word locations can be precomputed and indexed
  - Searching for a short string in a long string
    - Regular expression matching: FSA
- HSP (High Scoring Pair) = A match between a query word and the database
- Find a “hit”: Two non-overlapping HSP’s on a diagonal within distance A
- Extend the hit until the score falls below a threshold value, X

The BLAST Search Algorithm



Results from a BLAST search



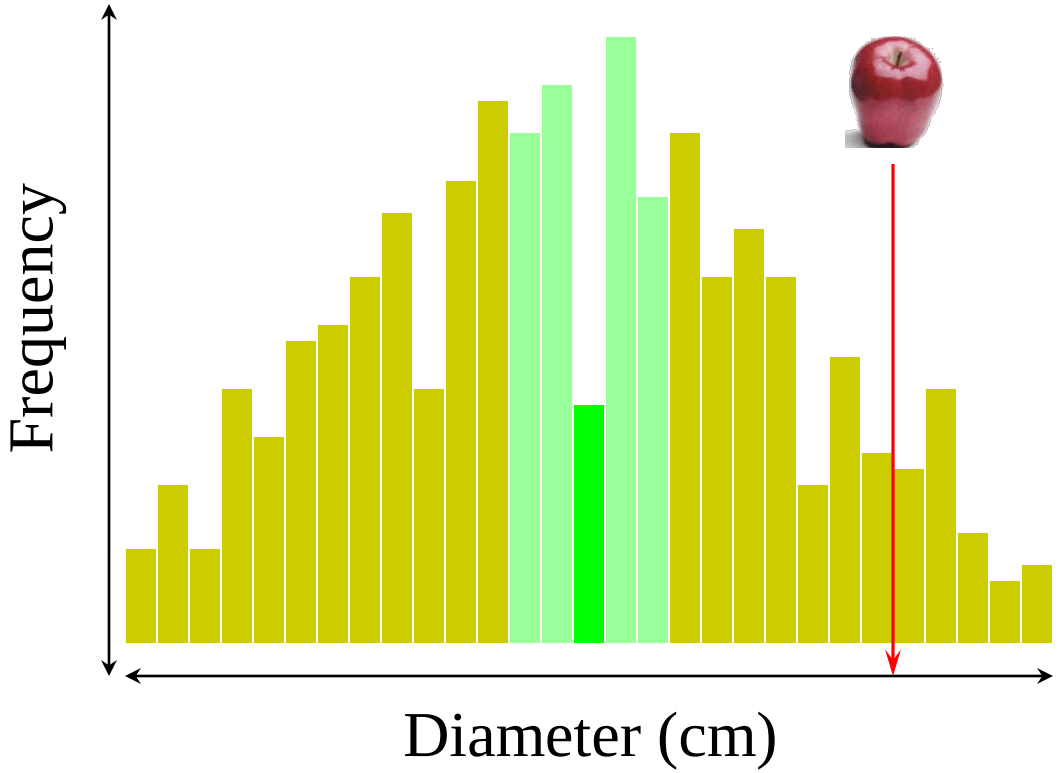
Search Significance Scores

- A search will *always* return some hits.

- How can we determine how “unusual” a particular alignment score is?
  - ORF’s
    - Assumptions

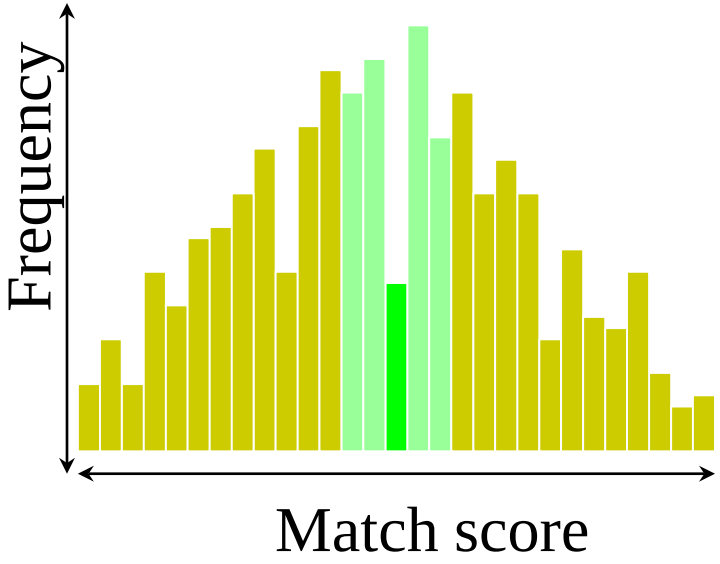
Assessing significance requires a *distribution*

- I have an apple of diameter 5”. Is that unusual?



Is a match significant?

- Match scores for aligning my sequence with ***random sequences***.
- Depends on:
  - Scoring system
  - Database
  - Sequence to search for
    - Length
    - Composition



- How do we determine the ***random sequences?***

Generating “random” sequences

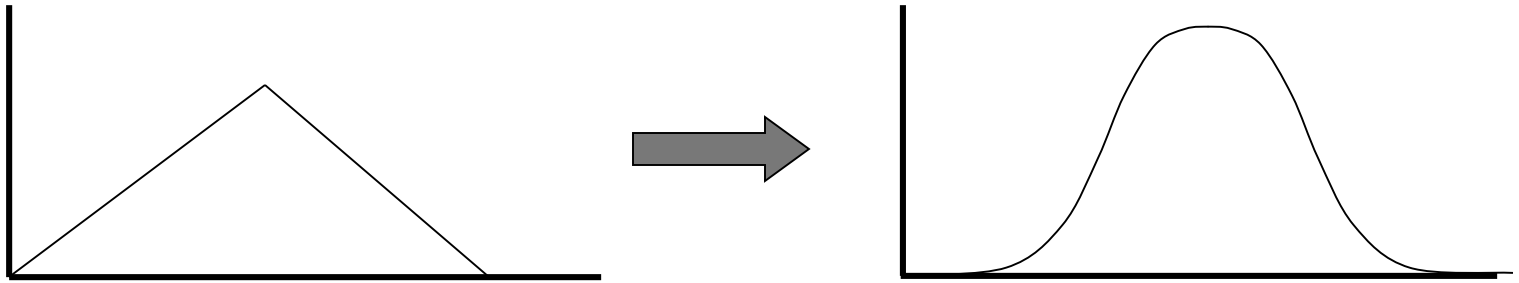
- Random uniform model:

$$P(G) = P(A) = P(C) = P(T) = 0.25$$

  - Doesn’t reflect nature
- Use sequences from a database
  - Might have genuine homology
    - We want unrelated sequences
- Random shuffling of sequences
  - Preserves composition
  - Removes true homology

What distribution do we expect to see?

- The mean of *n* random (i.i.d.) events tends towards a Gaussian distribution.
  - Example: Throw *n* dice and compute the mean.
  - Distribution of means:



n = 2

n = 1000

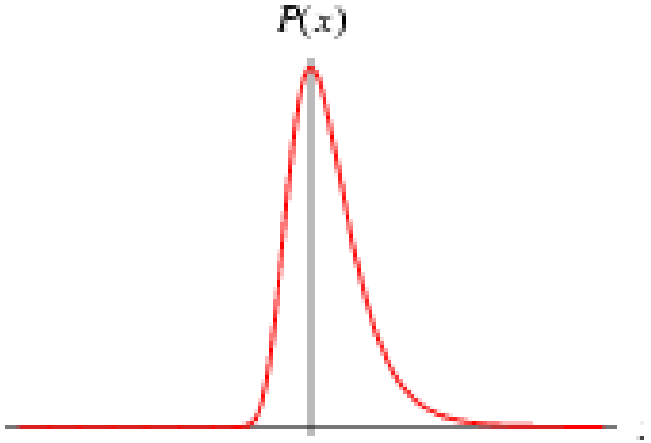
The extreme value distribution

- This means that if we get the match scores for our sequence with *n* other sequences, the mean would follow a Gaussian distribution.

- The **maximum** of *n* (i.i.d.) random events tends towards the **extreme value distribution** as *n* grows large.

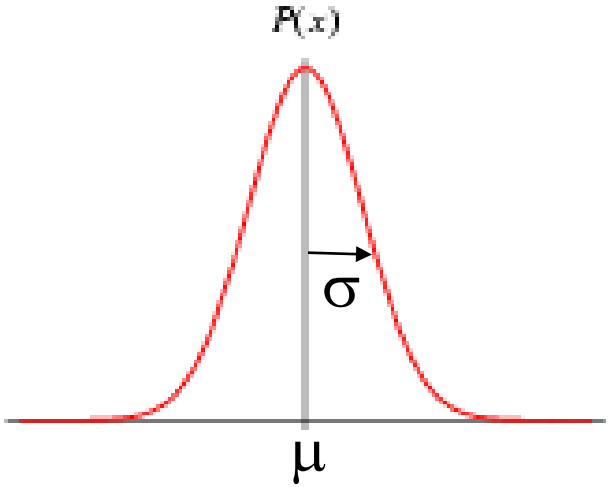
Comparing distributions

Extreme Value:



$$f(x) = \frac{1}{\beta} e^{-\frac{x-\mu}{\beta}} e^{-e^{-\frac{x-\mu}{\beta}}}$$

Gaussian:



$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2 / 2\sigma^2}$$

Determining P-values

- If we can estimate β and μ, then we can determine, for a given match score *x*, the probability that a random match with score *x* or greater would have occurred in the database.
- For sequence matches, a scoring system and database can be parameterized by two parameters, *K* and λ, related to β and μ.
  - It would be nice if we could compare hit significance without regard to the database and scoring system used!

Bit Scores

- The expected number of hits with score ≥ *S* is:

$$E = Kmn\, e^{-\lambda s}$$

  - Where *m* and *n* are the sequence lengths
- Normalize the raw score using:

$$S' = \frac{\lambda S - \ln K}{\ln 2}$$

- Obtains a "bit score" S', with a *standard set of units.*
- The new E-value is: $E = mn\, 2^{-S'}$

P values and E values

- Blast reports *E*-values
- $E = 5$, $E = 10$ versus $P = 0.993$ and $P = 0.99995$
- When $E < 0.01$ *P*-values and *E*-values are nearly identical

BLAST parameters

- Lowering the neighborhood word threshold (T) allows more distantly related sequences to be found, at the expense of increased noise in the results set.
- Raising the segment extension cutoff (X) returns longer extensions for each hit.
- Changing the minimum *E*-value changes the threshold for reporting a hit.