Sequence comparison: Significance of similarity scores

Genome 559: Introduction to Statistical and Computational Genomics

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The null hypothesis

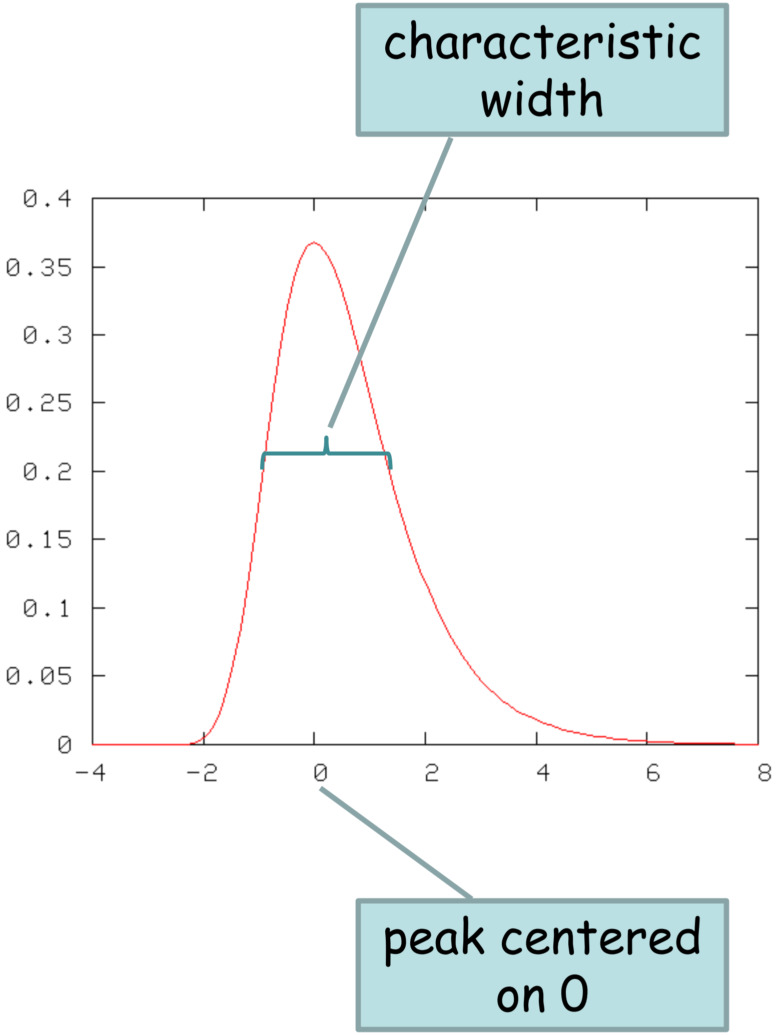
- We are interested in characterizing the distribution of scores from pairwise sequence alignments.
- We measure how surprising a given score is, assuming that the two sequences are not related.
- This assumption is called the null hypothesis.
- The purpose of most statistical tests is to determine whether the observed result(s) provide a reason to reject the null hypothesis.

Sequence similarity score distribution

Frequency
?
Sequence comparison score

- Search a randomly generated database of sequences using a given query sequence.
- What will be the form of the resulting distribution of pairwise sequence comparison scores?

Unscaled EVD equation



characteristic width

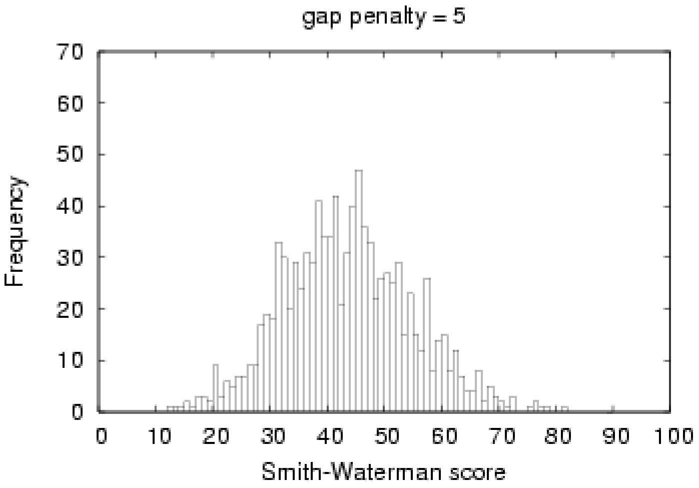
peak centered on 0

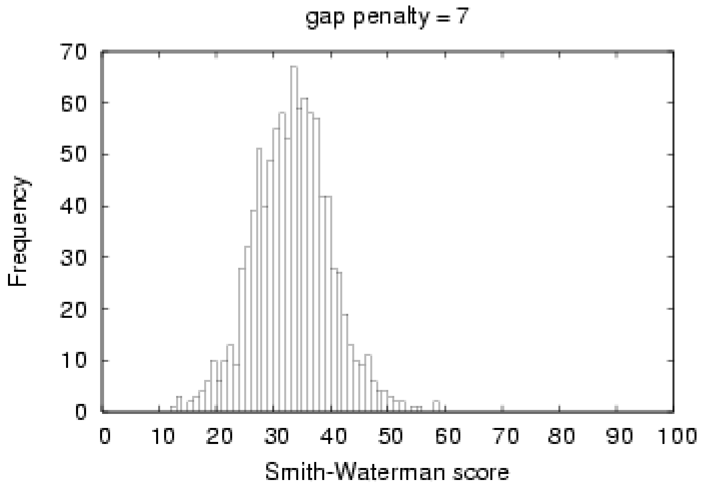
$$P(S \geq x) = 1 - e^{(-e^{-x})}$$

S is data score, x is test score

(FYI this is 1 minus the cumulative density function or CDF)

Scaling the EVD





- An EVD derived from, e.g., the Smith-Waterman algorithm with BLOSUM62 matrix and a given gap penalty has a characteristic mode μ and scale parameter λ.

$$P(S \geq x) = 1 - e^{(-e^{-x})}$$ scaled: $$P(S \geq x) = 1 - e^{(-e^{-\lambda(x-\mu)})}$$

λ and μ depend on the <u>size</u> of the query, the <u>size</u> of the target database, the <u>substitution matrix</u> and the <u>gap penalties</u>.

Similar to scaling the standard normal

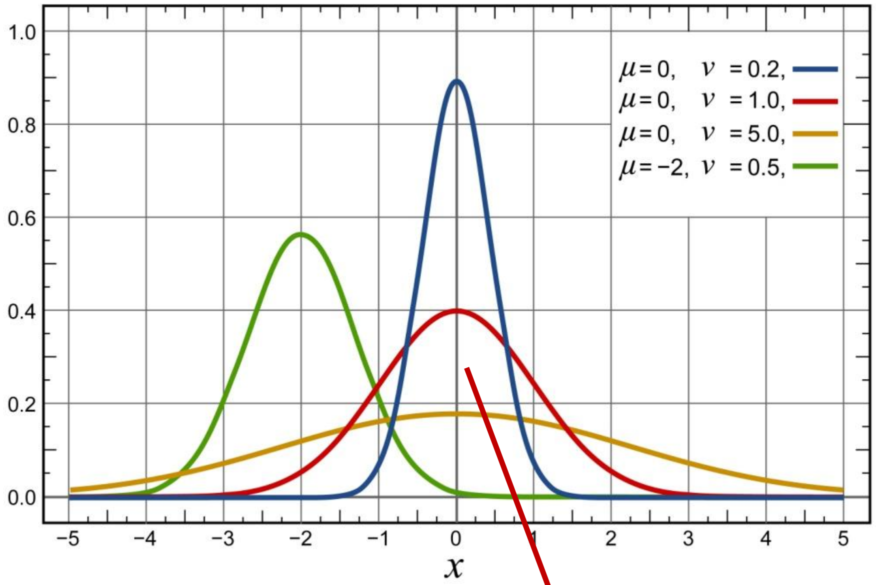
$$PDF_{snormal} = Ce^{-x^2/2}$$

where $C = 1/\sqrt{2\pi}$

$$PDF_{gnormal} = Ce^{-(x-\mu)^2/2v}$$

where $C = 1/\sqrt{2\pi v}$

v is variance, μ is mean



standard normal

(μ moves peak and v adjusts width)

An example

You run BLAST and get a score of 45. You then run BLAST on a shuffled version of the database, and fit an EVD to the resulting empirical distribution. The parameters of the EVD are $\mu = 25$ and $\lambda = 0.693$. What is the p-value associated with score 45?

$$P(S \geq 45) = 1 - e^{(-e^{-0.693(45-25)})}$$

$$= 1 - e^{(-e^{-13.86})}$$

$$= 1 - e^{-9.565 \times 10^{-7}}$$

$$= 1 - 0.999999043$$

$$= 9.565 \times 10^{-7}$$

BLAST has precomputed values of μ and λ for all common matrices and gap penalties (and the run scales λ for the size of the query and database)

What p-value is significant?

- The most common thresholds are 0.01 and 0.05.
- A threshold of 0.05 means you are 95% sure that the result is significant.
- Is 95% enough? It depends upon the *cost* associated with making a mistake.
- Examples of costs:
  - Doing extensive wet lab validation (expensive)
  - Making clinical treatment decisions (very expensive)
  - Misleading the scientific community (very expensive)
  - Doing further simple computational tests (cheap)
  - Telling your grandmother (very cheap)

Multiple testing

- Say that you perform a statistical test with a 0.05 threshold, but you repeat the test on twenty different observations (e.g. 20 different blast runs)

- Assume that all of the observations are explainable by the null hypothesis.

- What is the chance that at least one of the observations will receive a p-value of 0.05 or less?

$$1 - 0.95^{20} = 0.6415$$

Bonferroni correction

- Assume that individual tests are *independent.*
- Divide the desired p-value threshold by the number of tests performed.

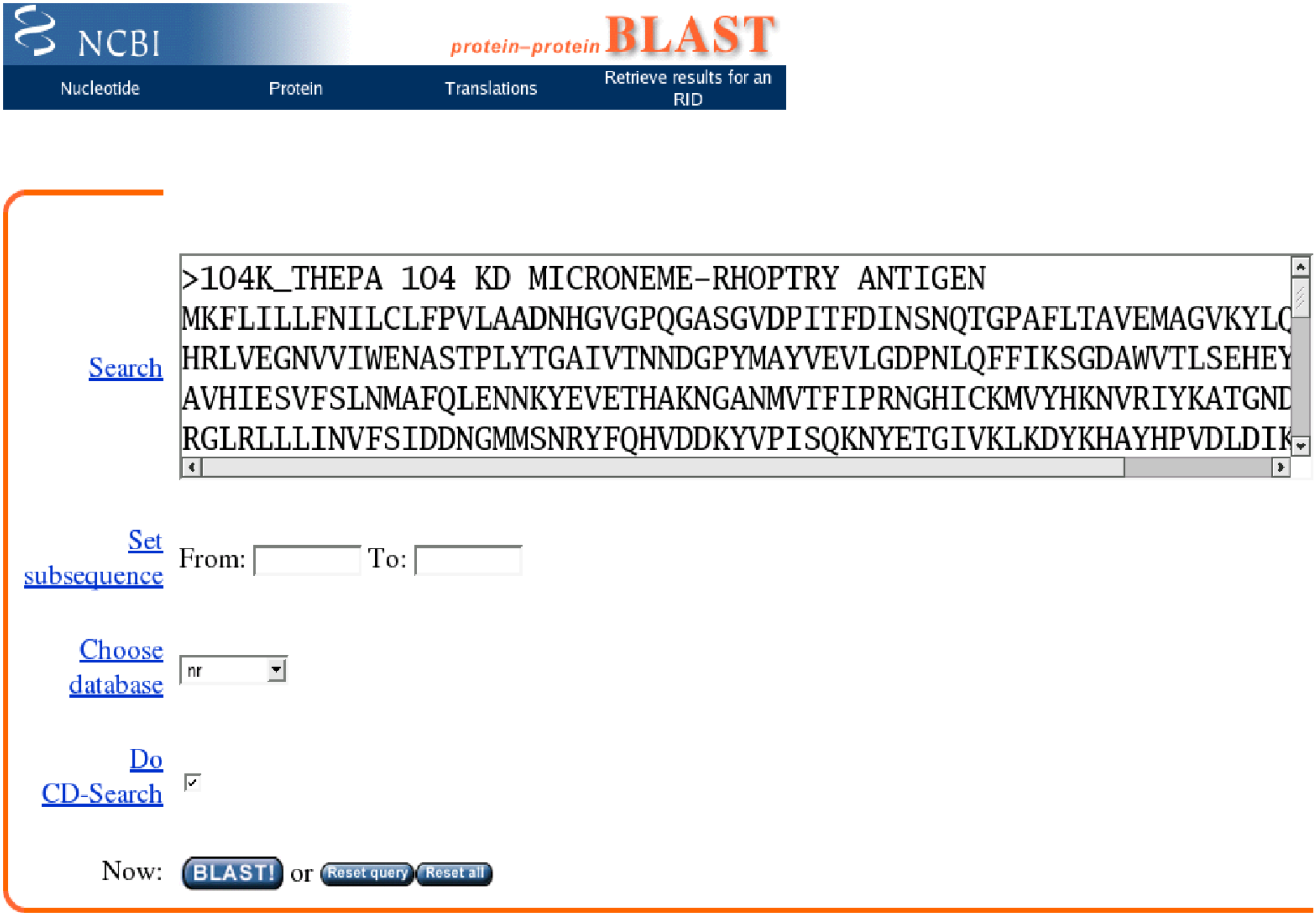
Database searching

- Say that you search the non-redundant protein database at NCBI, containing roughly one million sequences (i.e. you are doing 10^6 pairwise tests). What p-value threshold should you use?
- Say that you want to use a conservative p-value of 0.001.
- Recall that you would observe such a p-value by chance approximately every 1000 times in a random database.

E-values

- A p-value is the probability of making a mistake.
- An E-value is the expected number of times that the given score would appear in a random database of the given size.
- One simple way to compute the E-value is to multiply the p-value times the size of the database.
- Thus, for a p-value of 0.001 and a database of 1,000,000 sequences, the corresponding E-value is 0.001   1,000,000 = 1,000.

(BLAST actually calculates E-values in a different way, but they mean about the same thing)



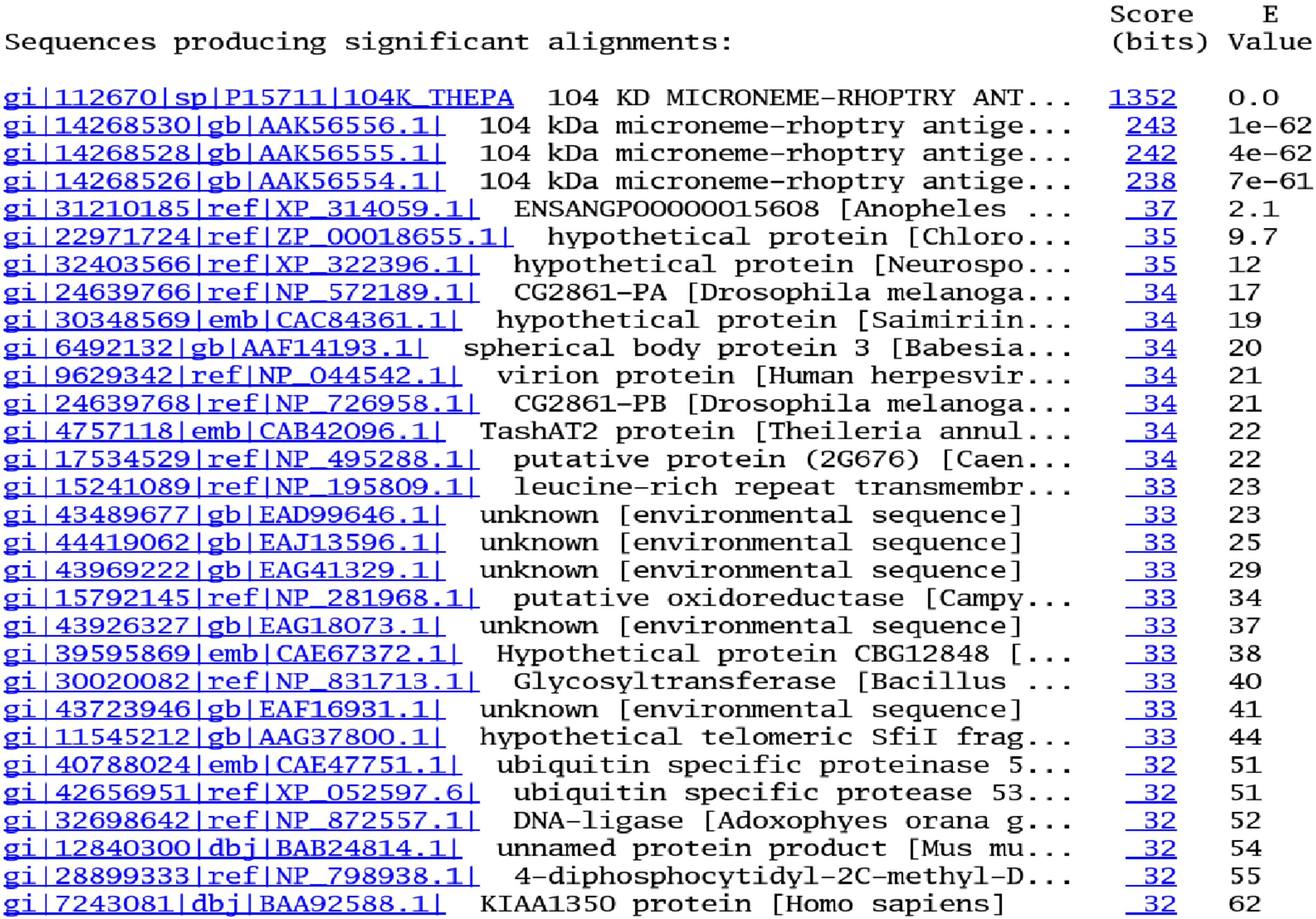
Search

```
>104K_THEPA 104 KD MICRONEME-RHOPTRY ANTIGEN
MKFLILLFNILCLFPVLAADNHGVGPQGASGVDPITFDINSNQTGPAFLTAVEMAGVKYLQ
HRLVEGNVVIWENASTPLYTGAIVTNNDGPYMAYVEVLGDPNLQFFIKSGDAWVTLSEHEY
AVHIESVFSLNMAFQLENNKYEVETHAKNGANMVTFIPRNGHICKMVYHKNVRIYKATGND
RGLRLLLINVFSIDDNGMMSNRYFQHVDDKYVPISQKNYETGIVKLKDYKHAYHPVDLDIK
```

Set subsequence From: To:

Choose database nr

Do CD-Search ☑



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

gi|112670|sp|P15711|104K_THEPA  104 KD MICRONEME-RHOPTRY ANT...  1352   0.0
gi|14268530|gb|AAK56556.1|  104 kDa microneme-rhoptry antige...   243   1e-62
gi|14268528|gb|AAK56555.1|  104 kDa microneme-rhoptry antige...   242   4e-62
gi|14268526|gb|AAK56554.1|  104 kDa microneme-rhoptry antige...   238   7e-61
gi|31210185|ref|XP_314059.1|  ENSANGP00000015608 [Anopheles ...    37   2.1
gi|22971724|ref|ZP_00018655.1|  hypothetical protein [Chloro...    35   9.7
gi|32403566|ref|XP_322396.1|  hypothetical protein [Neurospo...    35   12
gi|24639766|ref|NP_572189.1|  CG2861-PA [Drosophila melanoga...    34   17
gi|30348569|emb|CAC84361.1|  hypothetical protein [Saimiriin...    34   19
gi|6492132|gb|AAF14193.1|  spherical body protein 3 [Babesia...    34   20
gi|9629342|ref|NP_044542.1|  virion protein [Human herpesvir...    34   21
gi|24639768|ref|NP_726958.1|  CG2861-PB [Drosophila melanoga...    34   21
gi|4757118|emb|CAB42096.1|  TashAT2 protein [Theileria annul...    34   22
gi|17534529|ref|NP_495288.1|  putative protein (2G676) [Caen...    34   22
gi|15241089|ref|NP_195809.1|  leucine-rich repeat transmembr...    33   23
gi|43489677|gb|EAD99646.1|  unknown [environmental sequence]       33   23
gi|44419062|gb|EAJ13596.1|  unknown [environmental sequence]       33   25
gi|43969222|gb|EAG41329.1|  unknown [environmental sequence]       33   29
gi|15792145|ref|NP_281968.1|  putative oxidoreductase [Campy...    33   34
gi|43926327|gb|EAG18073.1|  unknown [environmental sequence]       33   37
gi|39595869|emb|CAE67372.1|  Hypothetical protein CBG12848 [...    33   38
gi|30020082|ref|NP_831713.1|  Glycosyltransferase [Bacillus ...    33   40
gi|43723946|gb|EAF16931.1|  unknown [environmental sequence]       33   41
gi|11545212|gb|AAG37800.1|  hypothetical telomeric SfiI frag...    33   44
gi|40788024|emb|CAE47751.1|  ubiquitin specific proteinase 5...    32   51
gi|42656951|ref|XP_052597.6|  ubiquitin specific protease 53...    32   51
gi|32698642|ref|NP_872557.1|  DNA-ligase [Adoxophyes orana g...    32   52
gi|12840300|dbj|BAB24814.1|  unnamed protein product [Mus mu...    32   54
gi|28899333|ref|NP_798938.1|  4-diphosphocytidyl-2C-methyl-D...    32   55
gi|7243081|dbj|BAA92588.1|  KIAA1350 protein [Homo sapiens]        32   62
```

Summary

- A distribution plots the frequencies of types of observation.
- The area under the distribution curve is 1.
- Most statistical tests compare observed data to the expected result according to a null hypothesis.
- Sequence similarity scores follow an extreme value distribution, which is characterized by a long tail.
- The p-value associated with a score is the area under the curve to the right of that score.
- Selecting a significance threshold requires evaluating the cost of making a mistake.
- Bonferroni correction: Divide the desired p-value threshold by the number of statistical tests performed.
- The E-value is the expected number of times that a given score would appear in a randomized database.