Statistics for matching runs

- Statistics of matching runs:

$$E(l) \cong mnp^{l}$$

- Length versus score?
  - Consider all mismatches receive a negative score of -∞ and $a_i b_j$ match receives a positive score of $s_{i,j}$.
- What is the expected number of matching runs with a score x or higher?

$$E(S >= x) \propto mnp^{x}$$

  - Using this theory of matching runs, Karlin and Altschul developed a theory for statistics of local alignments without gaps (extended this theory to allow for mismatches).

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Statistics of local alignments without gaps

- A scoring matrix which satisfy the following constraint:
  - The expected score of a single match obtained by a scoring matrix should be negative.

$$E(s_{i,j}) = \sum_{i,j} p_i p_j s_{i,j} < 0$$

  - Otherwise?
    - Arbitrarily long random sequences will get higher scores just because they are long, not because there's a significant match.
- If this requirement is met then the expected number of alignments with score x or higher is given by:

$$E(S \geq x) = Kmne^{-\lambda x}$$

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Statistics of local alignments without gaps

$$E(S \geq x) = Kmne^{-\lambda x}$$

- $K < 1$ is a proportionality constant that corrects the mn "space factor" for the fact that there are not really mn independent places that could have produced score $S \geq x$.
- K has little effect on the statistical significance of a similarity score
- λ is closely related to the scoring matrix used and it takes into account that the scoring matrices do not contain actual probabilities of co-occurence, but instead a scaled version of those values. To understand how λ is computed, we have to revisit the construction of scoring matrices.

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Scoring Matrices

- In 1970s there were few protein sequences available. Dayhoff used a limited set of families of protein sequences multiply aligned to infer mutation likelihoods.

```
PGNPFATPLEILPEWYLYPVFQILRVLPNKLLGIACQGAIPLGLMMVPFIE
PANPFATPLEILPEWYFYPVFQILRTVPNKLLGVLAMAAVPVGLLTVPFIE
PANPMSTPAHIVPEWYFLPVYAILRSIPNKLGGVAAIGLVFVSLLALPFIN
PANPLVTPPHIKPEWYFLFAYAILRSIPNKLGGVLALLFSILMLLLVPFLH
PANPLSTPAHIKPEWYFLFAYAILRSIPNKLGGVLALLFSILVLIFIPMLQ
PANPLSTPPHIKPEWYFLFAYAILRSIPNKLGGVLALLLSILILIFIPMLQ
IANPMNTPTHIKPEWYFLFAYSILRAIPNKLGGVIGLVMSILIL..YIMIF
ESDPMMSPVHIVPEWYFLFAYAILRAIPNKVLGVVSLFASILVL..VVFVL
IVDTLKTSDKILPEWFFLYLFGFLKAIPDKFMGLFLMVILLFSL..FLFIL
```

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Scoring Matrices

```
PGNPFATPLEILPEWYLYPVFQILRVLPNKLLGIACQGAIPLGLMMVPFIE
PANPFATPLEILPEWYFYPVFQILRTVPNKLLGVLAMAAVPVGLLTVPFIE
PANPMSTPAHIVPEWYFLPVYAILRSIPNKLGGVAAIGLVFVSLLALPFIN
PANPLVTPPHIKPEWYFLFAYAILRSIPNKLGGVLALLFSILMLLLVPFLH
PANPLSTPAHIKPEWYFLFAYAILRSIPNKLGGVLALLLSILVLIFIPMLQ
PANPLSTPPHIKPEWYFLFAYAILRSIPNKLGGVLALLLSILILIFIPMLQ
IANPMNTPTHIKPEWYFLFAYSILRAIPNKLGGVIGLVMSILIL..YIMIF
ESDPMMSPVHIVPEWYFLFAYAILRAIPNKVLGVVSLFASILVL..VVFVL
IVDTLKTSDKILPEWFFLYLFGFLKAIPDKFMGLFLMVILLFSL..FLFIL
```

- Dayhoff represented the similarity of amino acids as a log odds ratio:

$$s_{ij} = \log(q_{ij} / p_i p_j)$$

where q_{ij} is the observed frequency of co-occurrence, and p_i, p_j are the individual frequencies.

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Example

- If M occurs in the sequences with 0.01 frequency and L occurs with 0.1 frequency. By random pairing, you expect 0.001 amino acid pairs to be M-L. If the observed frequency of M-L is actually 0.003, score of matching M-L will be
  - $\log_2(3)$=1.585 bits or $\log_e(3) = \ln(3)$ = 1.1 nats
- Since, scoring matrices are usually provided as integer matrices, these values are scaled by a constant factor. λ is approximately the inverse of the original scaling factor.

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How to compute λ

- Recall that:

$$\lambda s_{ij} = \log(q_{ij} / p_i p_j)$$

$$\Rightarrow q_{ij} = p_i p_j e^{\lambda s_{ij}}$$

and: $\sum_{i=1}^{n}\sum_{j=1}^{i} q_{ij} = 1$ Sum of observed frequencies is 1.

$\Rightarrow \sum_{i=1}^{n}\sum_{j=1}^{i} p_i p_j e^{\lambda s_{ij}} = 1$ Given the frequencies of individual amino acids and the scores in the matrix, λ can be estimated.

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Extreme value distribution

- Consider an experiment that obtains the maximum value of locally aligning a random string with query string (without gaps). Repeat with another random string and so on. Plot the distribution of these maximum values.
- The resulting distribution is an extreme value distribution, called a *Gumbel distribution*.

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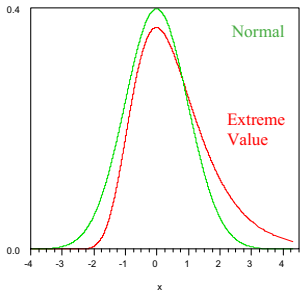
Normal vs. Extreme Value Distribution



Normal distribution:

$y = (1/\sqrt{2\pi})e^{-x^2/2}$

Extreme value distribution:

$y = e^{-x - e^{-x}}$

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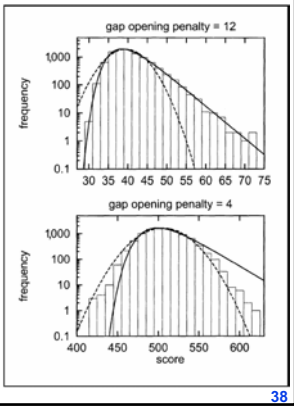
Local alignments with gaps

- The EVD distribution is not always observed. Theory of local alignments with gaps is not well studied as in without gaps. Mostly empirical results. For example, BLAST allows only a certain range of gap penalties.



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BLAST statistics

- Pre-computed λ and K values for different scoring matrices and gap penalties are used for faster computation.
- Raw score is converted to bit score:

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

- E-value is computed using

$$E = sss \cdot 2^{-S_{bit}}$$

$$sss = (m - L)(n - N \cdot L)$$

- *m* is query size, *n* is database size and *L* is the typical length of maximal scoring alignment.

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FASTA Statistics

- FASTA tries to estimate the probability distribution of alignments for every query.
- For any query sequence, a large collection of scores is gathered during the search of the database.
- They estimate the parameters of the EVD distribution based on the histogram of scores.
- Advantages:
  - reliable statistics for different parameters
    - different databases, different gap penalties, different scoring matrices, queries with different amino acid compositions.

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Statistical significance another example

- Suppose, we have a huge graph with weighted edges and I want to find strongly connected clusters of nodes.
- Suppose, an algorithm for this task is given.
- The algorithms gives you the best hundred clusters in this graph.
- How do you define best?
- Cluster size?
- Total weight of edges?

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Statistical significance

- How different is a found cluster of size N from a random cluster of the same size?
- This measure will enable comparison of clusters of different sizes.

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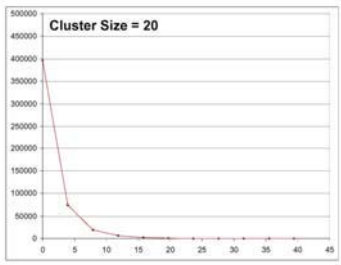
Statistical significance of a cluster

- Use maximum spanning tree weight of a cluster as a quantitative representation of that cluster.
- And see what values random clusters get. (sample many random clusters)



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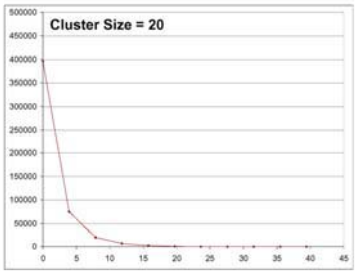
Statistical significance of a cluster



Looks like an exponential decay. We may fit an exponential distribution on this histogram.

$$y = \lambda e^{-\lambda x}$$

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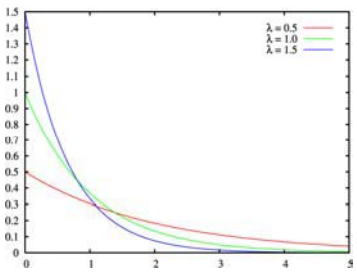
Fitting an exponential



$$y = \lambda e^{-\lambda x}$$

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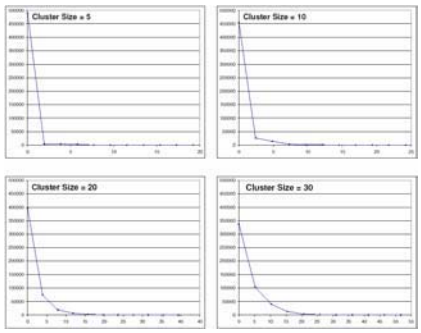
Statistical significance of a cluster



After we fit an exponential distribution, we compute the probability that another random cluster gets a higher score than the score of found cluster.

$$P(x \geq w) = e^{-\lambda_k w}$$

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Examples

- $\lambda_5 = 1.7$ for clusters of size 5 and $\lambda_{20} = 0.36$ for clusters of size 20.
- Suppose you have found a cluster of size 5 with weights of its edges sum up to 15 and you have found a cluster of size 20 with weight 45 which one would you prefer?

$$P(x \geq 15) = e^{-\lambda_5 15} = 8.42 \times 10^{-12}$$

$$P(x \geq 45) = e^{-\lambda_{20} 45} = 9.21 \times 10^{-8}$$

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