Spring 2025

BIOS 477/877

Bioinformatics and Molecular Evolution

Lecture 10

TODAY'S TOPICS

- ➢ Assignment 2 Review
- ➢ Amino Acid Substitution Matrix
  - Information Theory
- ➢ Statistical Significance of Alignment Scores
- ➢ Assignment 5

Log Odds Matrix

- PAM matrix

  $S(i,j) = 10\log_{10}\{M(i,j)/f(i)\}$

  $M(i,j)$: Mutation probability from AA_j to AA_i
  $f(i)$: Frequency of AA_i (number of AA_i / total number of residues)
  Probability to find AA_i by chance

- BLOSUM matrix

  $S(i,j) = 2\log_2(q_{ij}/e_{ij})$

  q_{ij}: Observed frequency of AA_i, AA_j pairs
  e_{ij}: Expected frequencies of AA_i, AA_j pairs

- General form

  $S(i,j) = 1/\lambda \log_2(q_{ij}/p_ip_j)$ [in bit unit]
  $S(i,j) = 1/\lambda \log_e(q_{ij}/p_ip_j)$ [in nat unit]

Relative Entropy (H)

	AA_1	AA_2	
AA_1	S_{11}	S_{21}	
AA_2	S_{12}	S_{22}	

➢ **Expected Score (E)**

$E = \Sigma_i\Sigma_j\, p_ip_jS(i,j)$ [p_i , p_j: expected freq. of AA_i, AA_j]

➢ **Relative Entropy (H)**

This is "ENTROPY" in Information Theory; Completely unrelated to "entropy" in thermodynamics!!

$H = \Sigma_i\Sigma_j\, q_{ij}\, \lambda S(i,j)$ [q_{ij} is observed freq. of AA_i, AA_j pair]

- the average information per residue pair
- summarizes the behavior of the scoring matrix
- the ability of the matrix to discriminate related from unrelated (nonrandom matching from random matching)
  - → $H = 0$ when target distribution equals to background distribution
    [If $q_{ij}=p_ip_j$, $S(i,j) = 1/\lambda\log_e(q_{ij}/p_ip_j) = 1/\lambda\log_e(1) = 0$]
  - → H increases when the two distributions become more distinguishable

→ can be used to compare scoring matrices

Introduction to Information Theory

➢ **Information:**
a decrease in uncertainty (unpredictability, a degree of surprise)

- If you are asking questions to somebody...
  - → if you can guess every answer correctly
  - → there is no surprise
  - → you cannot gain any new information
  - → but if you have no idea what answer you get
  - → every answer is a surprise
  - → you gain a lot of information

Information Theory Primer by Tom Schneider (also on Canvas): http://users.fred.net/tds/lab/papers/primer/

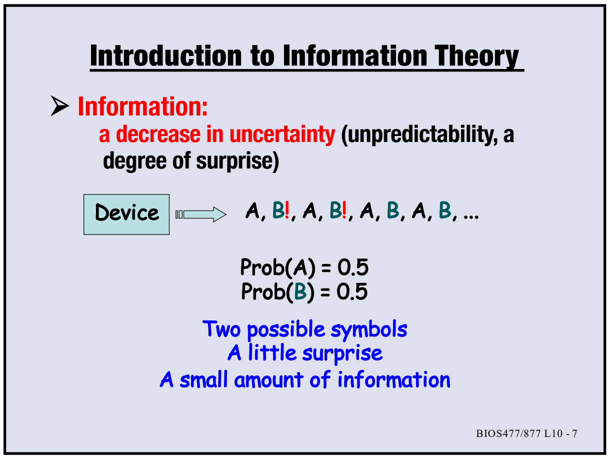
Introduction to Information Theory

➢ **Information:**
a decrease in uncertainty (unpredictability, a degree of surprise)

Device ⇨ A, A, A, A, A, A, A, A, ...

Prob(A) = 1
Only one possible symbol

No surprise
No information



Introduction to Information Theory

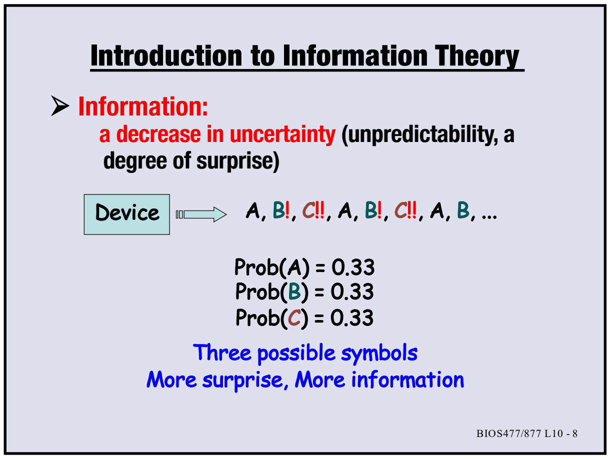
- **Information:**
  a decrease in uncertainty (unpredictability, a degree of surprise)

Device ⇨ A, B!, A, B!, A, B, A, B, ...

Prob(A) = 0.5
Prob(B) = 0.5

Two possible symbols
A little surprise
A small amount of information

7



Introduction to Information Theory

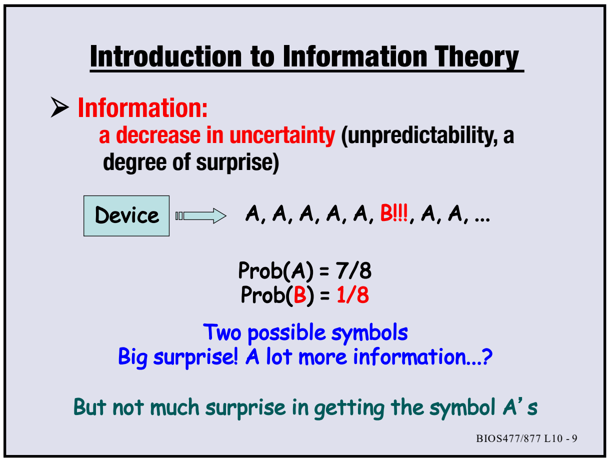
- **Information:**
  a decrease in uncertainty (unpredictability, a degree of surprise)

Device ⇨ A, B!, C!!, A, B!, C!!, A, B, ...

Prob(A) = 0.33
Prob(B) = 0.33
Prob(C) = 0.33

Three possible symbols
More surprise, More information

8



Introduction to Information Theory

- **Information:**
  a decrease in uncertainty (unpredictability, a degree of surprise)

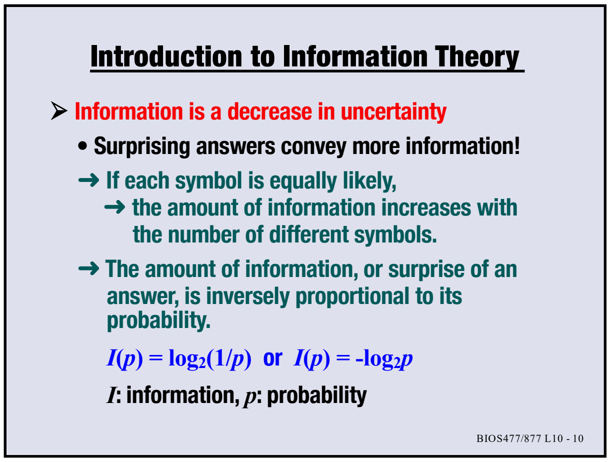
Device ⇨ A, A, A, A, A, B!!!, A, A, ...

Prob(A) = 7/8
Prob(B) = 1/8

Two possible symbols
Big surprise! A lot more information...?

But not much surprise in getting the symbol A's

9



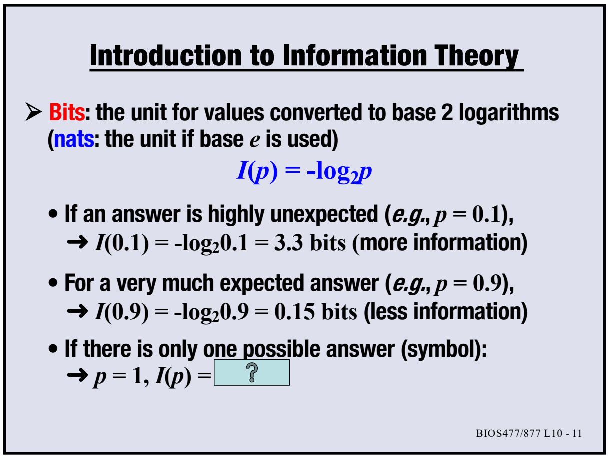
Introduction to Information Theory

- **Information is a decrease in uncertainty**
  - Surprising answers convey more information!
  - ➜ If each symbol is equally likely,
    - ➜ the amount of information increases with the number of different symbols.
  - ➜ The amount of information, or surprise of an answer, is inversely proportional to its probability.

    $I(p) = \log_2(1/p)$ or $I(p) = -\log_2 p$

    I: information, p: probability

10



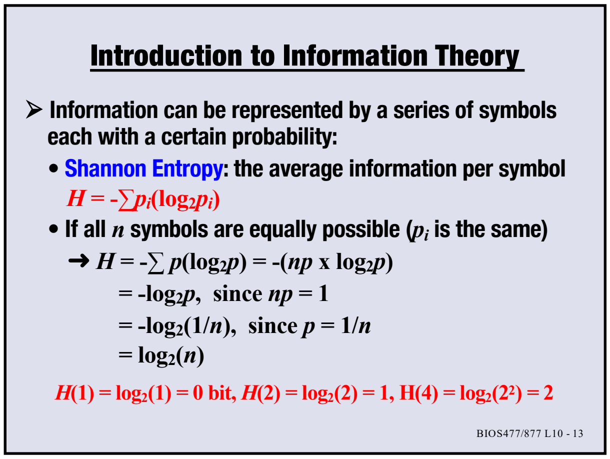
Introduction to Information Theory

- **Bits**: the unit for values converted to base 2 logarithms (**nats**: the unit if base e is used)

  $I(p) = -\log_2 p$

  - If an answer is highly unexpected (*e.g.*, $p = 0.1$),
    ➜ $I(0.1) = -\log_2 0.1 = 3.3$ bits (more information)
  - For a very much expected answer (*e.g.*, $p = 0.9$),
    ➜ $I(0.9) = -\log_2 0.9 = 0.15$ bits (less information)
  - If there is only one possible answer (symbol):
    ➜ $p = 1$, $I(p) = \boxed{?}$

11



Introduction to Information Theory

- Information can be represented by a series of symbols each with a certain probability:
  - **Shannon Entropy**: the average information per symbol
    $H = -\sum p_i(\log_2 p_i)$
  - If all n symbols are equally possible (p_i is the same)

$$\begin{aligned} \rightarrow H &= -\sum p(\log_2 p) = -(np \times \log_2 p) \\ &= -\log_2 p, \text{ since } np = 1 \\ &= -\log_2(1/n), \text{ since } p = 1/n \\ &= \log_2(n) \end{aligned}$$

$H(1) = \log_2(1) = 0$ bit, $H(2) = \log_2(2) = 1$, $\mathrm{H}(4) = \log_2(2^2) = 2$

13

Introduction to Information Theory

- Information can be represented by a series of symbols each with a certain probability:
  - **Shannon Entropy**: the average information per symbol
    $H = -\sum p_i(\log_2 p_i)$
  - For a random DNA sequence: ATGC (p = 0.25 for all)
    H = -(0.25 x 4) x $\log_2(0.25)$ or $\log_2(4)$ = 2 bits
  - For a AT-rich DNA sequence: $p_A = p_T = 0.45$ and $p_G = p_C = 0.05$
    H = {-0.45 x ($\log_2 0.45$)} + {-0.45 x ($\log_2 0.45$)} +
    {-0.05 x ($\log_2 0.05$)} + {-0.05 x ($\log_2 0.05$)}
    = {-0.45 x (-1.15)} x 2 + {-0.05 x (-4.32)} x 2 = 1.47 bits

Relative Entropy (H)

- Expected Score (E)
  $E = \Sigma_i\Sigma_j\, p_i p_j S(i,j)$ [p_i , p_j: expected freq. of AA_i, AA_j]
- Relative Entropy (H)
  $H = \Sigma_i\Sigma_j\, q_{ij}\, \lambda S(i,j)$ [q_{ij} is observed freq. of AA_i, AA_j pair]
  Since $S(i,j) = 1/\lambda \log_2(q_{ij}/p_ip_j)$ or $1/\lambda \log_e(q_{ij}/p_ip_j)$
  $H = \Sigma_i\Sigma_j\, q_{ij} \log_2(q_{ij}/p_ip_j)$ or $\Sigma_i\Sigma_j\, q_{ij} \log_e(q_{ij}/p_ip_j)$
  $= \Sigma_i\Sigma_j\{q_{ij}\log_2(q_{ij}) - q_{ij}\log_2\{p_ip_j)\}$

Connection to
$H = -\Sigma p_i(\log_2 p_i)$

Note: Both Expected Score and Relative Entropy have their units in bit or nat.

Comparing Scoring Matrices

- Relative Entropy (H) of a scoring matrix
  $H = \Sigma_i\Sigma_j q_{ij}\lambda S(i,j)$ [q_{ij} is observed freq. of AA_i, AA_j pair]
  - the average information per residue pair for a scoring matrix
  - summarizes the behavior of the scoring matrix
  - the ability of the matrix to discriminate related from unrelated (nonrandom matching from random matching)
    → H=0 when target distribution equals to background distribution [If $q_{ij}=p_ip_j$, $S(i,j) = 1/\lambda log_e(q_{ij}/p_ip_j) = 1/\lambda log_e(1) = 0$]
    → H increases when the two distributions become more distinguishable

→ can be used to compare scoring matrices

Comparing Scoring Matrices

- Relative Entropy (H)
  H(PAM1) = 4.17 bits
  H(PAM50) = 2.00
  H(PAM120) = 0.98
  H(PAM160) = 0.70
  H(PAM250) = 0.36
  from Altschul (1991)

H decreases with increasing PAM;
H increases with increasing BLOSUM

Higher BLOSUM is generated including sequences that are more similar to one another
↓
More amino acid pairs are used

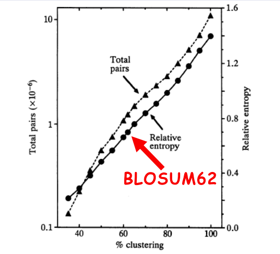


FIG. 1. Relationship between percentage clustering and total amino acid pair counts plotted on a logarithmic scale and relative entropy.

from Henikoff and Henikoff (1992)

Comparing Scoring Matrices

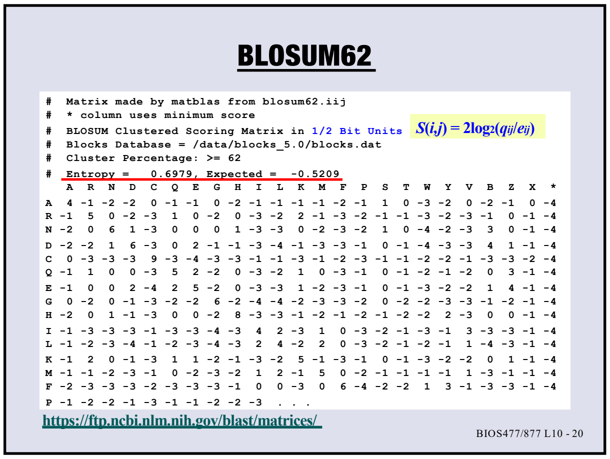
- Relative Entropy (H) of a scoring matrix
  $H = \Sigma_i\Sigma_j q_{ij}\lambda S(i,j)$
  - The average information per residue pair for a scoring matrix
  - Decreases with increasing PAM: H(PAM1) > H(PAM120)
    → All PAMn is extrapolated from PAM1
    → Higher PAM is less specific, contains less information, thus has a lower H
  - Increases with increasing BLOSUM: H(BLOSUM45) < H(BLOSUM80)
    → Higher BLOSUM is generated using more data (fewer information is eliminated), thus has a higher H

[*e.g.*, BLOSUM100 is generated using the threshold 100%; only identical sequences are down-weighted for calculation]

BLOSUM and PAM matrices

(default in BLAST)

BLOSUM80	BLOSUM62	BLOSUM45
Less divergent	↔	More divergent
PAM120	PAM160	PAM250
H = 0.98 bits	0.7 bits	0.36 bits



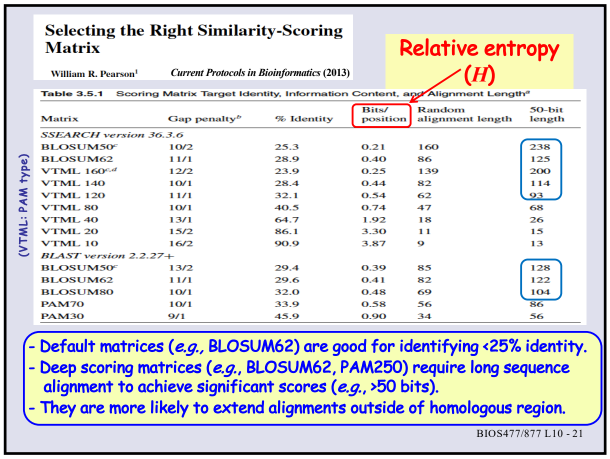
BLOSUM62

```
#  Matrix made by matblas from blosum62.iij
#  * column uses minimum score
#  BLOSUM Clustered Scoring Matrix in 1/2 Bit Units
#  Blocks Database = /data/blocks_5.0/blocks.dat
#  Cluster Percentage: >= 62
#  Entropy =   0.6979, Expected =  -0.5209
   A  R  N  D  C  Q  E  G  H  I  L  K  M  F  P  S  T  W  Y  V  B  Z  X  *
A  4 -1 -2 -2  0 -1 -1  0 -2 -1 -1 -1 -1 -2 -1  1  0 -3 -2  0 -2 -1  0 -4
R -1  5  0 -2 -3  1  0 -2  0 -3 -2  2 -1 -3 -2 -1 -1 -3 -2 -3 -1  0 -1 -4
N -2  0  6  1 -3  0  0  0  1 -3 -3  0 -2 -3 -2  1  0 -4 -2 -3  3  0 -1 -4
D -2 -2  1  6 -3  0  2 -1 -1 -3 -4 -1 -3 -3 -1  0 -1 -4 -3 -3  4  1 -1 -4
C  0 -3 -3 -3  9 -3 -4 -3 -3 -1 -1 -3 -1 -2 -3 -1 -1 -2 -2 -1 -3 -3 -2 -4
Q -1  1  0  0 -3  5  2 -2  0 -3 -2  1  0 -3 -1  0 -1 -2 -1 -2  0  3 -1 -4
E -1  0  0  2 -4  2  5 -2  0 -3 -3  1 -2 -3 -1  0 -1 -3 -2 -2  1  4 -1 -4
G  0 -2  0 -1 -3 -2 -2  6 -2 -4 -4 -2 -3 -3 -2  0 -2 -2 -3 -3 -1 -2 -1 -4
H -2  0  1 -1 -3  0  0 -2  8 -3 -3 -1 -2 -1 -2 -1 -2 -2  2 -3  0  0 -1 -4
I -1 -3 -3 -3 -1 -3 -3 -4 -3  4  2 -3  1  0 -3 -2 -1 -3 -1  3 -3 -3 -1 -4
L -1 -2 -3 -4 -1 -2 -3 -4 -3  2  4 -2  2  0 -3 -2 -1 -2 -1  1 -4 -3 -1 -4
K -1  2  0 -1 -3  1  1 -2 -1 -3 -2  5 -1 -3 -1  0 -1 -3 -2 -2  0  1 -1 -4
M -1 -1 -2 -3 -1  0 -2 -3 -2  1  2 -1  5  0 -2 -1 -1 -1 -1  1 -3 -1 -1 -4
F -2 -3 -3 -3 -2 -3 -3 -3 -1  0  0 -3  0  6 -4 -2 -2  1  3 -1 -3 -3 -1 -4
P -1 -2 -2 -1 -3 -1 -1 -2 -2 -3  . . .
```

$S(i,j) = 2\log_2(q_{ij}/e_{ij})$

https://ftp.ncbi.nlm.nih.gov/blast/matrices/

20



Selecting the Right Similarity-Scoring Matrix

William R. Pearson[1] *Current Protocols in Bioinformatics* (2013)

Relative entropy (H)

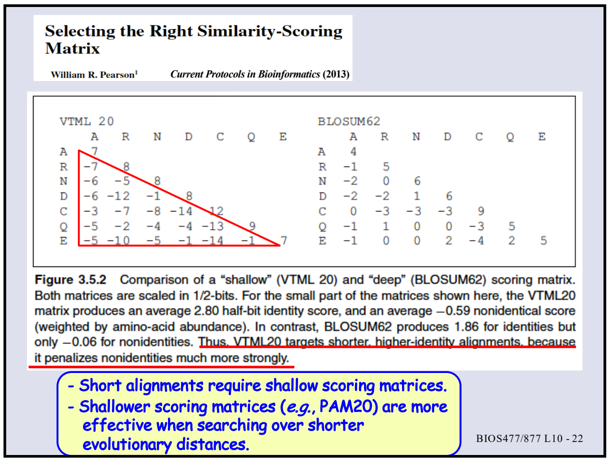
Table 3.5.1 Scoring Matrix Target Identity, Information Content, and Alignment Length[a]

(VTML: PAM type)

Matrix	Gap penalty[b]	% Identity	Bits/ position	Random alignment length	50-bit length
SSEARCH version 36.3.6					
BLOSUM50[c]	10/2	25.3	0.21	160	238
BLOSUM62	11/1	28.9	0.40	86	125
VTML 160[c,d]	12/2	23.9	0.25	139	200
VTML 140	10/1	28.4	0.44	82	114
VTML 120	11/1	32.1	0.54	62	93
VTML 80	10/1	40.5	0.74	47	68
VTML 40	13/1	64.7	1.92	18	26
VTML 20	15/2	86.1	3.30	11	15
VTML 10	16/2	90.9	3.87	9	13
BLAST version 2.2.27+					
BLOSUM50[c]	13/2	29.4	0.39	85	128
BLOSUM62	11/1	29.6	0.41	82	122
BLOSUM80	10/1	32.0	0.48	69	104
PAM70	10/1	33.9	0.58	56	86
PAM30	9/1	45.9	0.90	34	56

- Default matrices (*e.g.*, BLOSUM62) are good for identifying <25% identity.
- Deep scoring matrices (*e.g.*, BLOSUM62, PAM250) require long sequence alignment to achieve significant scores (*e.g.*, >50 bits).
- They are more likely to extend alignments outside of homologous region.

21



Selecting the Right Similarity-Scoring Matrix

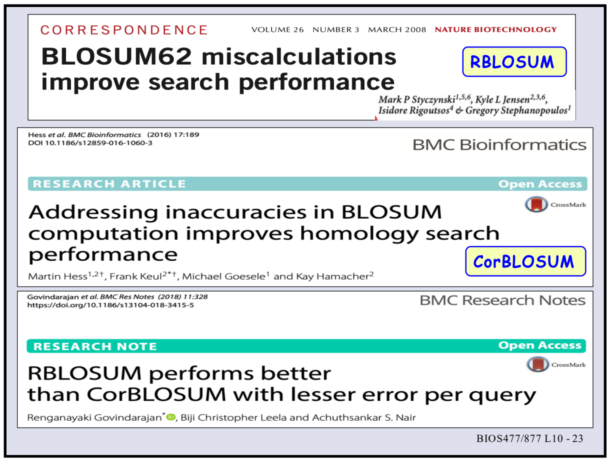
William R. Pearson[1] *Current Protocols in Bioinformatics* (2013)

```
VTML 20                              BLOSUM62
    A   R   N   D   C   Q   E            A   R   N   D   C   Q   E
A   7                                A   4
R  -7   8                            R  -1   5
N  -6  -5   8                        N  -2   0   6
D  -6 -12  -1   8                    D  -2  -2   1   6
C  -3  -7  -8 -14  12                C   0  -3  -3  -3   9
Q  -5  -2  -4  -4 -13   9            Q  -1   1   0   0  -3   5
E  -5 -10  -5  -1 -14  -1   7        E  -1   0   0   2  -4   2   5
```

Figure 3.5.2 Comparison of a “shallow” (VTML 20) and “deep” (BLOSUM62) scoring matrix. Both matrices are scaled in 1/2-bits. For the small part of the matrices shown here, the VTML20 matrix produces an average 2.80 half-bit identity score, and an average –0.59 nonidentical score (weighted by amino-acid abundance). In contrast, BLOSUM62 produces 1.86 for identities but only –0.06 for nonidentities. Thus, VTML20 targets shorter, higher-identity alignments, because it penalizes nonidentities much more strongly.

- Short alignments require shallow scoring matrices.
- Shallower scoring matrices (*e.g.*, PAM20) are more effective when searching over shorter evolutionary distances.

22



CORRESPONDENCE VOLUME 26 NUMBER 3 MARCH 2008 NATURE BIOTECHNOLOGY

BLOSUM62 miscalculations improve search performance

RBLOSUM

Mark P Styczynski[1,5,6], Kyle L Jensen[2,3,6], Isidore Rigoutsos[4] & Gregory Stephanopoulos[1]

Hess *et al. BMC Bioinformatics* (2016) 17:189
DOI 10.1186/s12859-016-1060-3

BMC Bioinformatics

RESEARCH ARTICLE Open Access

Addressing inaccuracies in BLOSUM computation improves homology search performance

CorBLOSUM

Martin Hess[1,2†], Frank Keul[2*†], Michael Goesele[1] and Kay Hamacher[2]

Govindarajan *et al. BMC Res Notes* (2018) 11:328
https://doi.org/10.1186/s13104-018-3415-5

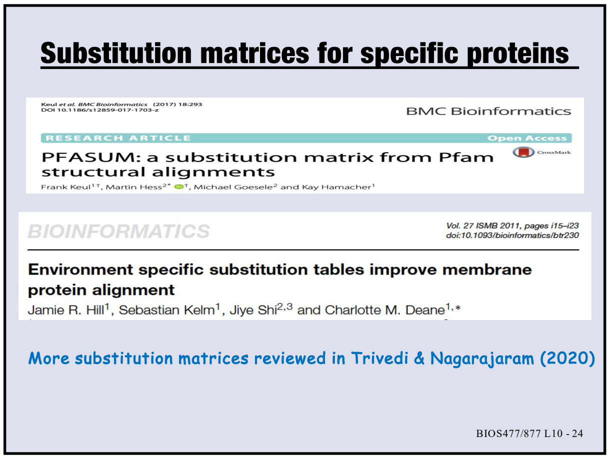
BMC Research Notes

RESEARCH NOTE Open Access

RBLOSUM performs better than CorBLOSUM with lesser error per query

Renganayaki Govindarajan*, Biji Christopher Leela and Achuthsankar S. Nair

23



Substitution matrices for specific proteins

Keul *et al. BMC Bioinformatics* (2017) 18:293
DOI 10.1186/s12859-017-1703-z

BMC Bioinformatics

RESEARCH ARTICLE Open Access

PFASUM: a substitution matrix from Pfam structural alignments

Frank Keul[1†], Martin Hess[2*†], Michael Goesele[2] and Kay Hamacher[1]

BIOINFORMATICS

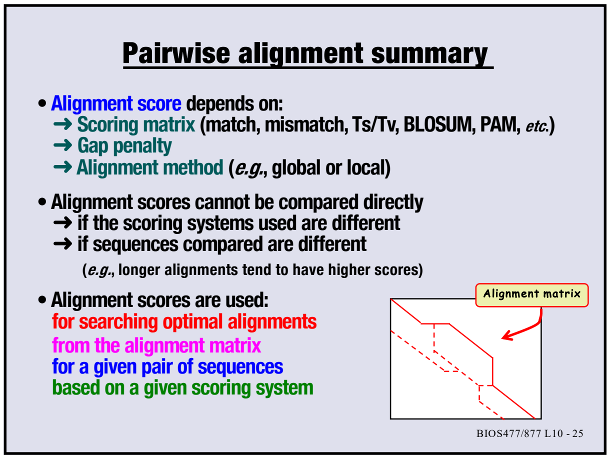
Vol. 27 ISMB 2011, pages i15–i23
doi:10.1093/bioinformatics/btr230

Environment specific substitution tables improve membrane protein alignment

Jamie R. Hill[1], Sebastian Kelm[1], Jiye Shi[2,3] and Charlotte M. Deane[1,*]

More substitution matrices reviewed in Trivedi & Nagarajaram (2020)

24



Pairwise alignment summary

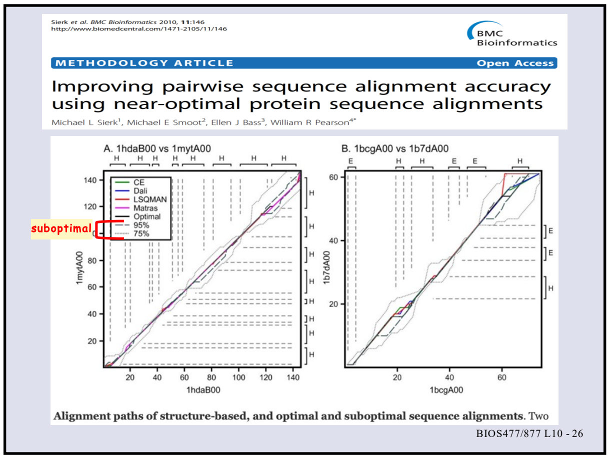
- **Alignment score** depends on:
  - ➔ **Scoring matrix** (match, mismatch, Ts/Tv, BLOSUM, PAM, *etc.*)
  - ➔ **Gap penalty**
  - ➔ **Alignment method** (*e.g.*, global or local)
- Alignment scores cannot be compared directly
  - ➔ if the scoring systems used are different
  - ➔ if sequences compared are different

    (*e.g.*, longer alignments tend to have higher scores)
- Alignment scores are used:

  for searching optimal alignments
  from the alignment matrix
  for a given pair of sequences
  based on a given scoring system

Alignment matrix

25



Sierk *et al. BMC Bioinformatics* 2010, **11**:146
http://www.biomedcentral.com/1471-2105/11/146

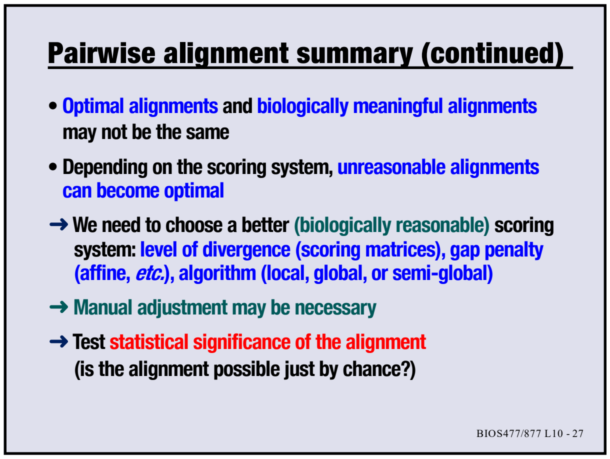
BMC Bioinformatics

METHODOLOGY ARTICLE **Open Access**

Improving pairwise sequence alignment accuracy using near-optimal protein sequence alignments

Michael L Sierk[1], Michael E Smoot[2], Ellen J Bass[3], William R Pearson[4*]

Alignment paths of structure-based, and optimal and suboptimal sequence alignments. Two



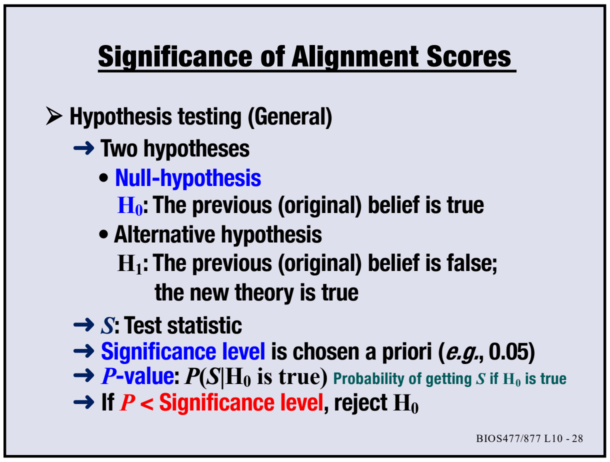
Pairwise alignment summary (continued)

- **Optimal alignments** and **biologically meaningful alignments** may not be the same
- Depending on the scoring system, **unreasonable alignments can become optimal**

→ We need to choose a better (biologically reasonable) scoring system: level of divergence (scoring matrices), gap penalty (affine, *etc.*), algorithm (local, global, or semi-global)

→ Manual adjustment may be necessary

→ Test statistical significance of the alignment (is the alignment possible just by chance?)



Significance of Alignment Scores

➢ Hypothesis testing (General)

→ Two hypotheses

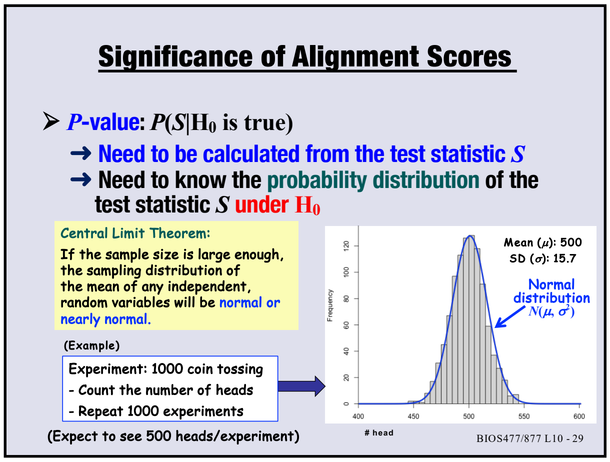
- Null-hypothesis
  H_0: The previous (original) belief is true
- Alternative hypothesis
  H_1: The previous (original) belief is false; the new theory is true

→ S: Test statistic

→ Significance level is chosen a priori (*e.g.*, 0.05)

→ P-value: $P(S|H_0 \text{ is true})$ Probability of getting S if H_0 is true

→ If P < Significance level, reject H_0



Significance of Alignment Scores

➢ P-value: $P(S|H_0 \text{ is true})$

→ Need to be calculated from the test statistic S

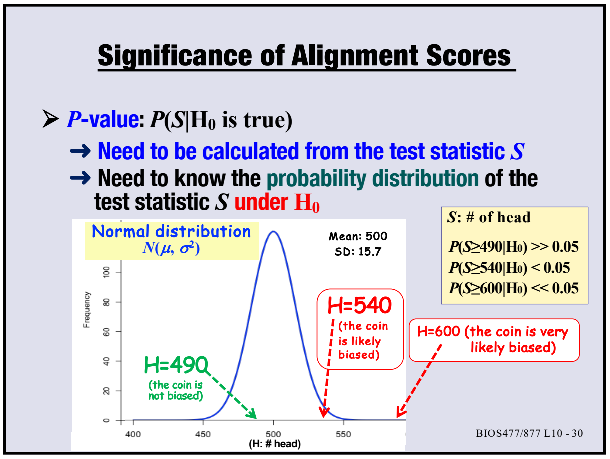
→ Need to know the probability distribution of the test statistic S under H_0

Central Limit Theorem:
If the sample size is large enough, the sampling distribution of the mean of any independent, random variables will be normal or nearly normal.

(Example)

Experiment: 1000 coin tossing
- Count the number of heads
- Repeat 1000 experiments

(Expect to see 500 heads/experiment)



Significance of Alignment Scores

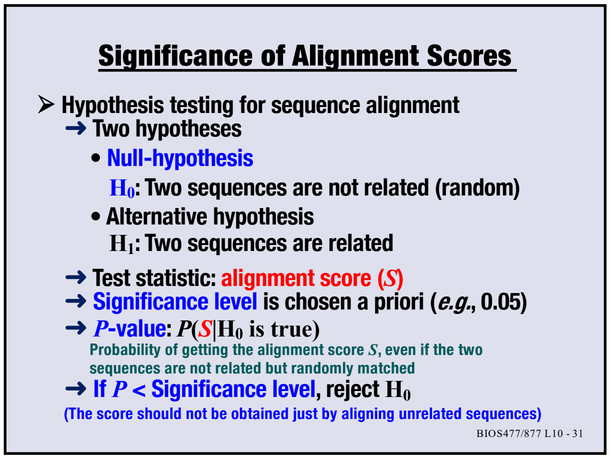
➢ P-value: $P(S|H_0 \text{ is true})$

→ Need to be calculated from the test statistic S

→ Need to know the probability distribution of the test statistic S under H_0

S: # of head

$P(S \geq 490|H_0) >> 0.05$
$P(S \geq 540|H_0) < 0.05$
$P(S \geq 600|H_0) << 0.05$



Significance of Alignment Scores

➢ Hypothesis testing for sequence alignment

→ Two hypotheses

- Null-hypothesis
  H_0: Two sequences are not related (random)
- Alternative hypothesis
  H_1: Two sequences are related

→ Test statistic: alignment score (S)

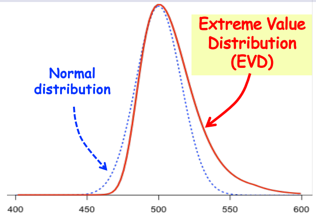
→ Significance level is chosen a priori (*e.g.*, 0.05)

→ P-value: $P(S|H_0 \text{ is true})$
Probability of getting the alignment score S, even if the two sequences are not related but randomly matched

→ If P < Significance level, reject H_0
(The score should not be obtained just by aligning unrelated sequences)

Significance of Alignment Scores

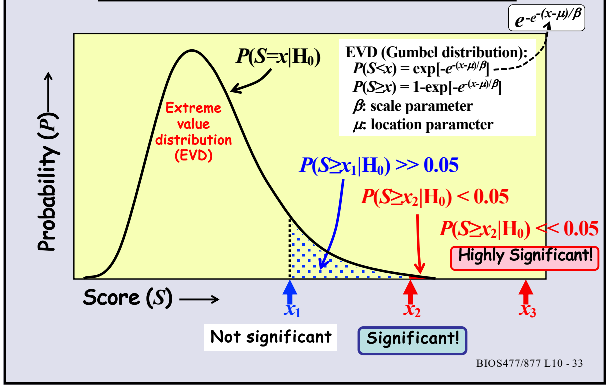
- **P-value**: $P(S|H_0$ is true)
  - ➜ Need to be calculated from the test statistic S
  - ➜ Need to know the probability distribution of the test statistic S under H_0



Distribution of alignment scores follow Extreme Value Distribution (Gumbel distribution)

The probability distribution of highest values in an experiment (*e.g.*, optimal alignment scores)

Significance of Alignment Scores



EVD (Gumbel distribution):
$P(S<x) = \exp[-e^{-(x-\mu)/\beta}]$
$P(S\geq x) = 1-\exp[-e^{-(x-\mu)/\beta}]$
β: scale parameter
μ: location parameter

Significance of Alignment Scores

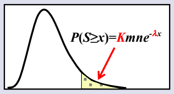
- $P(S\geq x|H_0)$: Probability of getting the alignment score $S \geq x$

Karlin-Altschul equation (Karlin and Altschul 1990)

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

EVD (Gumbel distribution):
$P(S\geq x) = 1-\exp[-e^{-(x-\mu)/\beta}]$
$\lambda = 1/\beta$, $\mu = (\ln Kmn)/\lambda$

K and λ: calculated from the empirical distribution of S based on a given scoring matrix and amino acid composition
m and n: lengths of sequences aligned



➜ Solved for ungapped local alignments
➜ Can be applied for gapped local alignments

- E-value = $P(S\geq x|H_0)$ x N, where N is the number of sequences in the dataset
  - ➜ Expected number of sequences in the dataset to have a score $\geq x$

E-value ≠ P-value

Significance of Alignment Scores

- How to calculate K and λ (in LALIGN and PRSS)
  - ➜ estimated from an empirical probability distribution.

1) The second sequence is shuffled many times. (simulates random sequences)

2) Smith-Waterman local alignment score is calculated from each alignment: $P(S=x|H_0)$

3) The distribution is fitted to an extreme value distribution to obtain estimates of K and λ

4) P-value is estimated based on the K and λ, and the original alignment score x: $P(S\geq x) \approx Kmne^{-\lambda x}$

Simulation of Alignment Scores

- RECA_ECOLI (P0A7G6; 353 amino acids)
- RAD51_YEAST (P25454; 400 amino acids)

Smith-Waterman local alignment score = 293
(BLOSUM50, gap opening: -10, gap extension: -1)

```
RECA_ECOLI          3 IDENK-QKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLDIA     51
                      |.|.| .|.|..|...:...|...:...:   |..::..::|||.:||..
RAD51_YEAST       124 ISEAKADKLLNEAARLVPMGFVTAADFHM---RRSELICLTTGSKNLDTL    170

RECA_ECOLI         52 LGAGGLPMGRIVEIYGPESSGKTTL------TLQV-IAAAQREGKTCAFI     94
                      || ||:..|.|.|::|...:||:.|      |.|: :.....||| |.:|
RAD51_YEAST       171 LG-GGVETGSITELFGEFRTGKSQLCHTLAVTCQIPLDIGGGEGK-CLYI    218

RECA_ECOLI         95 DAEHALDPI----YARKLGVDID----NLLCSQPDTGEQALEICDALAR-    135
                      |.|..:.|:    .|:::|:|.|    |:..::....::.|.:.||.|:
RAD51_YEAST       219 DTEGTFRPVRLVSIAQRFGLDPDDALNNVAYARAYNADHQLRLLDAAAQM    268

RECA_ECOLI        136 --SGAVDVIVVDSVAALTPKAEI--EGEIGDSHMGLAARMMSQAMRKLAG    181
                        .....:||||||.||. :.:.  .||:...:|.||..|  :|:::||
RAD51_YEAST       269 MSESRFSLIVVDSVMALY-RTDFSGRGELSARQMHLAKFM--RALQRLA-    314

RECA_ECOLI        182 NLKQSNTLLIFINQIRMKI--GVMFG-NPETTTGGNALKFYASVRLDIRR    228
                        .|....::..||:..::  |:.|. :|:...|||.:....::.||
RAD51_YEAST       315 --DQFGVAVVVTNQVVAQVDGGMAFNPDPKKPIGGNIMAHSSTTRL----    358

RECA_ECOLI        229 IGAVKEGENVVGSETRVKVVKNKIAAPFKQAEFQILYGEGI    269
                        ..|:|:   |.:...||| :....|..:..|.| |.:|:
RAD51_YEAST       359 --GFKKGK---GCQRLCKVV-DSPCLPEAECVFAI-YEDGV    392
```

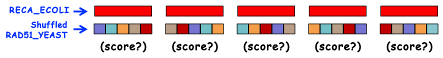
https://www.ebi.ac.uk/jdispatcher/psa/emboss_water

Simulation of Alignment Scores

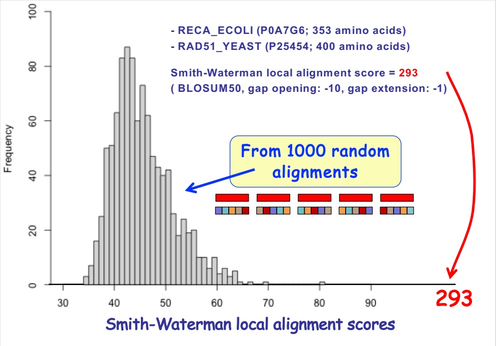
- RECA_ECOLI (P0A7G6; 353 amino acids)
- RAD51_YEAST (P25454; 400 amino acids)

Smith-Waterman local alignment score = 293
(BLOSUM50, gap opening: -10, gap extension: -1)

Shuffle RAD51_YEAST 1000 times
(generate 1000 random sequences)
⬇
Align with RECA_ECOLI
(generate 1000 random local alignments)



Simulation of Alignment Scores

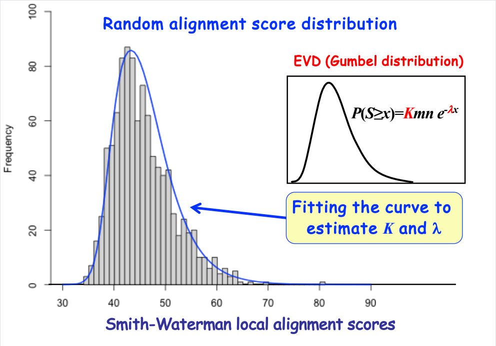


- RECA_ECOLI (P0A7G6; 353 amino acids)
- RAD51_YEAST (P25454; 400 amino acids)

Smith-Waterman local alignment score = 293
(BLOSUM50, gap opening: -10, gap extension: -1)

From 1000 random alignments

Simulation of Alignment Scores



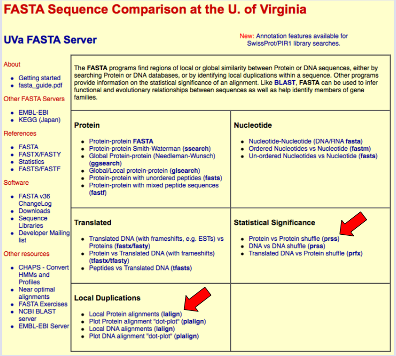
Random alignment score distribution

EVD (Gumbel distribution)

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

Fitting the curve to estimate K and λ

FASTA Web server by William Pearson



FASTA Sequence Comparison at the U. of Virginia

UVa FASTA Server

New: Annotation features available for SwissProt/PIR1 library searches.

About

- Getting started
- fasta_guide.pdf

Other FASTA Servers

- EMBL-EBI
- KEGG (Japan)

References

- FASTA
- FASTX/FASTY
- Statistics
- FASTS/FASTF

Software

- FASTA v36 ChangeLog
- Downloads
- Sequence Libraries
- Developer Mailing list

Other resources

- CHAPS - Convert HMMs and Profiles
- Near optimal alignments
- FASTA Exercises
- NCBI BLAST server
- EMBL-EBI Server

The **FASTA** programs find regions of local or global similarity between Protein or DNA sequences, either by searching Protein or DNA databases, or by identifying local duplications within a sequence. Other programs provide information on the statistical significance of an alignment. Like **BLAST**, **FASTA** can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

Protein

- Protein-protein **FASTA**
- Protein-protein Smith-Waterman (**ssearch**)
- Global Protein-protein (Needleman-Wunsch) (**ggsearch**)
- Global/Local protein-protein (**glsearch**)
- Protein-protein with unordered peptides (**fasts**)
- Protein-protein with mixed peptide sequences (**fastf**)

Nucleotide

- Nucleotide-Nucleotide (DNA/RNA **fasta**)
- Ordered Nucleotides vs Nucleotide (**fastm**)
- Un-ordered Nucleotides vs Nucleotide (**fasts**)

Translated

- Translated DNA (with frameshifts, e.g. ESTs) vs Proteins (**fastx/fasty**)
- Protein vs Translated DNA (with frameshifts) (**tfastx/tfasty**)
- Peptides vs Translated DNA (**tfasts**)

Statistical Significance

- Protein vs Protein shuffle (**prss**)
- DNA vs DNA shuffle (**prss**)
- Translated DNA vs Protein shuffle (**prfx**)

Local Duplications

- Local Protein alignments (**lalign**)
- Plot Protein alignment "dot-plot" (**plalign**)
- Local DNA alignments (**lalign**)
- Plot DNA alignment "dot-plot" (**plalign**)

https://fasta.bioch.virginia.edu/fasta_www2/fasta_list2.shtml

https://fasta.bioch.virginia.edu/wrpearson/

Original FASTA package was released on 1988 (earlier than BLAST)

The origin of FASTA format