

Bioinformatics — Lecture 3

Sequence alignments

(EG Chps. 6, 10; MM Chps. 4–6)

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Today

Sequence similarity

Alignment

Are sequences related?

Sequence distances

Global alignment algorithms

Multiple sequence alignment

Local alignment

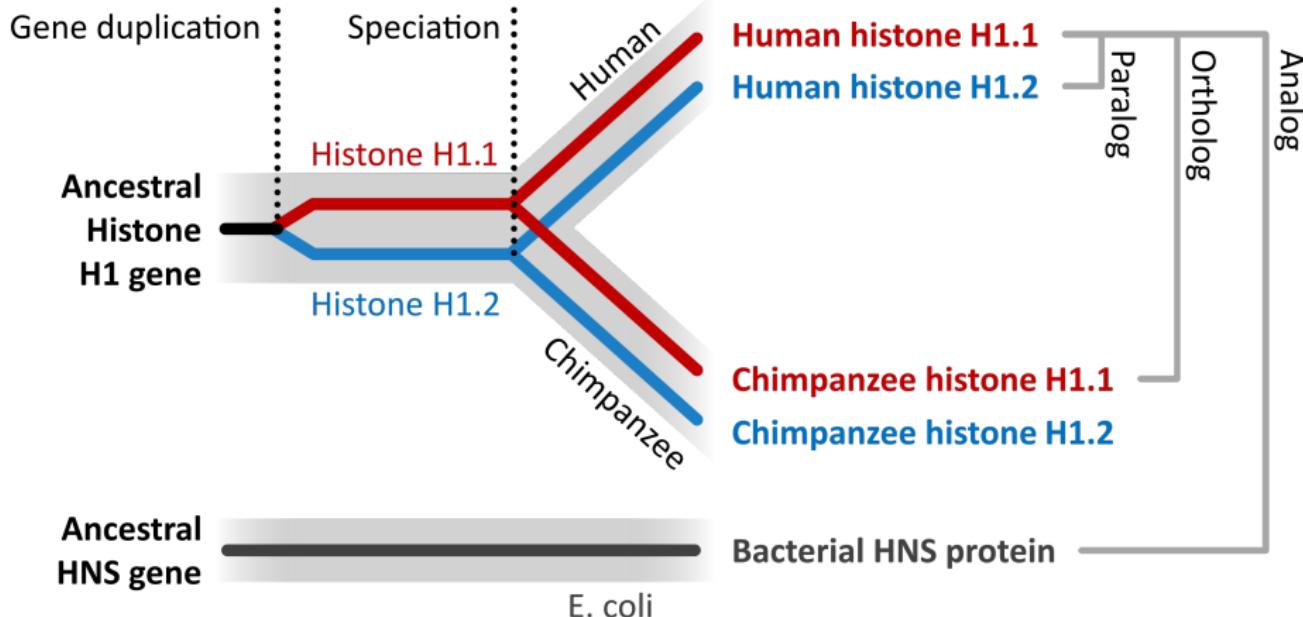
BLAST

Additional reading

A computer science perspective: chapter 7 in
[CHL] M. Crochemore, C. Hancart, T. Lecroq. Algorithms on
Strings, Cambridge, 2007, Cambridge University Press.

Multiple alignments: chapter 6 in
[DEKM] R. Durbin, S. Eddy, A. Krogh, G. Mitchison. Biological
Sequence Analysis: Probabilistic Models of Proteins and Nucleic
Acids, Cambridge, 1998, Cambridge University Press.

Gene homology (MM Ch. 3.4)



https://en.wikipedia.org/wiki/File:Ortholog_paralog_analog_examples.svg, by Thomas Shafee, CC BY 4.0

Gene homology

Orthologues: genes from different species that are descended from a common ancestral sequence, often carry out the same function

Paralogues: genes derived from a gene duplication event, can be in same or different species, and can have same or different functions

Identify genes in one species, given gene sequences in another species: *sequence alignment*

Alignment (simple) example

Original sequence: *CGGTATGCCA*

Descendant 1 sequence: *CGGGTATCCAA*

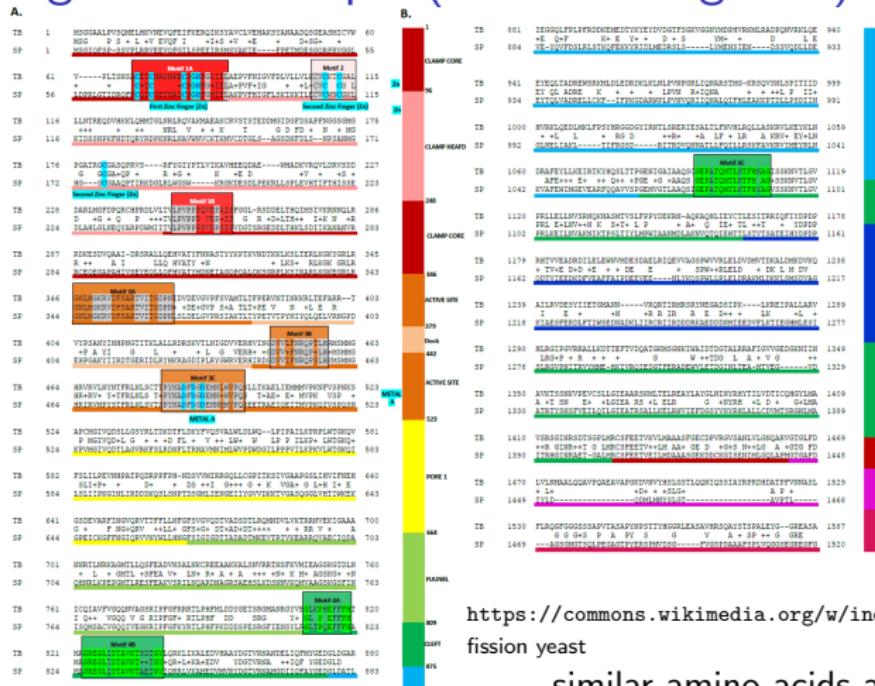
Descendant 2 sequence: *CCCTAGGTCCCCA*

Alignment:

C	G	G	G	T	A	-	-	T	C	C	A	A
C	C	C	-	T	A	G	G	T	C	C	C	A

https://es.wikipedia.org/wiki/Trypanosoma_brucei#/media/Archivo:Tb_brucei.jpg, by Torsten Ochsenreiter, public domain

Alignment example (also MM Fig. 3.4)



(sub-Saharan African parasite)



<https://commons.wikimedia.org/w/index.php?curid=8627875>, public domain
fission yeast

similar amino acids are marked, also . : |

Fig. 3 in L. Papageorgiou, V. Megalooikonomou, D. Vlachakis, 2017. Genetic and structural study of DNA-directed RNA polymerase II of *Trypanosoma brucei*, towards the designing of novel antiparasitic agents. PeerJ 5:e3061 doi:10.7717/peerj.3061. CC-BY 4.0

Alignment example (also MM Fig. 3.12)



	Bt-Chy	Ha-Chy	Br-Try	He-Try	Mc-Try	Dp-ChyL	Dp-ChyS	Pc-Try	Aa-Try	Al-Try	Dp-Try	Up-Try	Pc-GP	Pv-ChyBII	Mj-Chy	Ea-Bu	Dp-ChyL	Fo-Cap
Pos:	111/116	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
Pos:	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136
Pos:	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132
Pos:	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132
Pos:	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132
Pos:	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132
Pos:	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132
Pos:	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132

	Bt-Chy	Ha-Chy	Br-Try	He-Try	Mc-Try	Dp-ChyL	Dp-ChyS	Pc-Try	Aa-Try	Al-Try	Dp-Try	Up-Try	Pc-GP	Pv-ChyBII	Mj-Chy	Ea-Bu	Dp-ChyL	Fo-Cap
Pos:	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136	589/136
Pos:	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132	630/132
Pos:	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132	631/132
Pos:	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132	632/132
Pos:	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132	633/132
Pos:	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132	634/132
Pos:	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132	635/132

" Multiple sequence alignment of the trypsin-like protease from *M. carcinus* (Mc-TryL), 12 mature brachyurins and 2 mammalian trypsins and 2 mammalian chymotrypsins as the reference. Grey shadows show sequence motif at the beginning of the mature protein, or conserved cysteines. The catalytic triad is denoted by black boxes, while substrate binding residues and residues that confer the peptide-bond specificity are shown by white boxes."

(trypsin: enzyme in small intestine starting digestion of protein molecules)

Fig. 2 in J.M Viader-Salvadó, J.A. Aguilar Briseño, J.A. Gallegos-López, J.A. Fuentes-Garibay, C.A. Alvarez-González, M. Guerrero-Olazarán, 2020. Identification and *in silico* structural and functional analysis of a trypsin-like protease from shrimp *Macrobrachium carcinus*. PeerJ 8:e9030 doi:10.7717/peerj.9030. CC-BY 4.0

Sequence alignment (also MM Fig. 3.13)

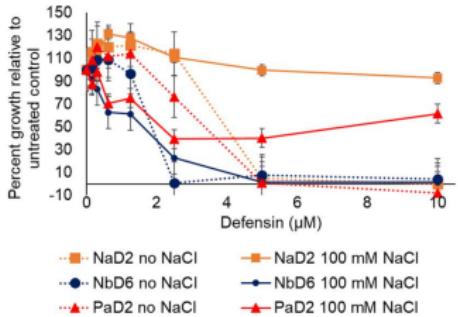
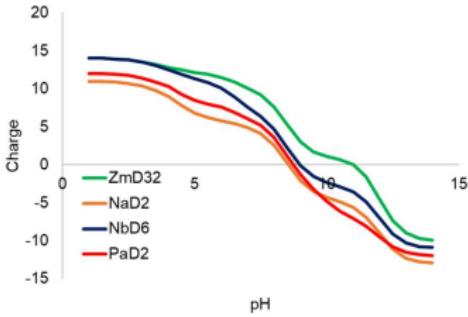


National Museum of Anthropology
(Mexico)

A

		Ident	Sim	Charge
PaD2	RTCE S QSH K F G T L S D TNC G NV C H S E G F P G G K C R G L R R C F C T K N C	70.2	87.2	+6.1
NbD6	RTCE S QSH R F K G L C F S R S N C A S V C H T E G F N G G H C R G F R R R C F C T R H C	78.7	87.2	+7.6
NaD2	RTCE S QSH R F K G P C A R D S N C A T C V L T E G F S G G D C R G F R R R C F C T R P C	78.7	85.1	+4.9
ZmD32	RT C Q S Q H R F R G P C L R R S N C A N V C R T E G F P G G R C R G F R R R C F C T T H C C	100	100	+10.1

: *; *: * ; ** .. ** : *** .. *** ; ***: ***** * *

B**C**

different defensin (against microbes) proteins share similar sequences
behave differently with varying salinity

Fig. 8 in K. Kerenga Bomai et. al., 2019. Salt-Tolerant Antifungal and Antibacterial Activities of the Corn Defensin ZmD32. Front. Microbiol. 10 doi:10.3389/fmicb.2019.00795. CC BY.

Comparing sequences (frequency EG Ch. 6.1)

	nucleotide				Total
sequence 1	#A ₁	#C ₁	#G ₁	#T ₁	L ₁
sequence 2	#A ₂	#C ₂	#G ₂	#T ₂	L ₂
Total	#A	#C	#G	#T	L

H_0 : both sequences are drawn from populations with identical nucleotide frequencies

$$p_{1N} = \#N_1/L_1, p_{N2} = \#N_2/L_2, p_N = \#N/L$$

$$2L \left(\frac{L_1}{L} \sum p_{1N} \log p_{1N} + \frac{L_2}{L} \sum p_{2N} \log p_{2N} - \sum p_N \log p_N \right) \sim \chi^2(3)$$

Comparing sequences (EG Ch. 6.3)

Ungapped alignment assumed (uniformly generated in R, 4 runs needed for $\text{LCS} \geq 3$)

```
T G T G T T T G C C C T A G A C A T A G G G T A G A T
C A T G T C G C T C A A C A T A G T G A A C A C T C
```

H_0 : two sequences in ungapped alignment are random with respect to each other

Exact-matching test statistic: Y_{\max} —length of longest common subsequence (here 3)

$$Y_{\max} = y_{\max} \text{ has } p\text{-value } \cong 1 - \exp(-(1-p)Np^{y_{\max}})$$

(EG Eq. 5.15, Lec 2 slide 30), where

$$p = P(A)^2 + P(G)^2 + P(C)^2 + P(T)^2 \text{ (nucleotide frequencies)}$$

Well-matching test statistic: $Y_{\max}^{(k)}$ —length of longest subsequence with up to k mismatches

p-value approximate (see EG Eqs. 6.5. 6.6, 3.51)

Distance on X (CHL Ch. 7.1)

a function, $d : X \times X \rightarrow \mathbb{R}$ satisfying

positivity: $\forall_{u,v \in X} d(u, v) \geq 0$

separation: $\forall_{u,v \in X} d(u, v) = 0$ iff $u = v$

symmetry: $\forall_{u,v \in X} d(u, v) = d(v, u)$

triangle inequality: $\forall_{u,v,w \in X} d(u, v) \leq d(u, w) + d(v, w)$

Similarity score $s(u, v) = (-1) \cdot d(u, v)$

Distances on sequences (CHL Ch. 7.1)

Prefix distance:

$$d_{pref}(u, v) = |u| + |v| - 2|\text{longest common prefix}(u, v)|$$

Suffix distance:

$$d_{pref}(u, v) = |u| + |v| - 2|\text{longest common suffix}(u, v)|$$

Factor distance:

$$d_{pref}(u, v) = |u| + |v| - 2|\text{longest common subsequence}(u, v)|$$

Hamming distance: for two strings of same length, number of positions on which they differ

Levenshtein (edit) distance (CHL Ch. 7.1)

$Lev(x, y) = \min(\text{cost of transformation } u \text{ to } v)$

permitted operations (single character edits):

substitution: replace a letter by another letter

deletion: remove a letter

insertion: insert a letter at a given position

TACA → TATATA

operation	result	alignment	cost
T→T	TACA	(T,T)	0
A→A	TACA	(A,A)	0
insert T	TATCA	(-,T)	1
insert A	TATACA	(-,A)	1
C→T	TATATA	(C,T)	1
A→A	TATATA	(A,A)	0

see also Crochemore, Hancart, Lecroq : Figs. 7.1, 7.2

$$Lev(TACA, TATATA) = 3 \cdot \text{cost} \left(\begin{array}{ccccccc} T & A & - & - & C & A \\ T & A & T & A & T & A \end{array} \right) = 3$$

DEFINE CUSTOM COSTS FOR EACH OPERATION AND LETTER CHANGE
MATCH SCORE, MISMATCH PENALTY

The approach (EG Ch. 6.4, MM Ch. 5)

Define scores for matches and penalties for mismatches
(biology: substitution matrices, gap cost function)

Align two sequences by finding transformation steps minimizing
 $\text{Lev}(\text{Seq1}, \text{Seq2})$ under these scores
(dynamic programming: Needleman–Wunsch algorithm)

Gap cost function: $\delta(l)$, e.g. linear $\delta(l) = -ld$ (gap of length l)

Needleman–Wunsch (EG Ch. 6.4.2, MM Ch. 5.3–4)

Input: two sequences $\mathbf{x} = X_1 X_2 \dots X_m$ and $\mathbf{y} = Y_1 Y_2 \dots Y_n$

denote: $\mathbf{x}_{1i} = X_1 X_2 \dots X_i$

define: $B(i, j)$ score of the best alignment between \mathbf{x}_{1i} and \mathbf{y}_{1j}

initial conditions: $B(i, 0) = -id$, $B(0, j) = -jd$, $B(0, 0) = 0$

recursion $w(X, Y)$ score/penalty of match/mismatch:

$$B(i, j) = \max \begin{cases} B(i - 1, j - 1) + w(X_i, Y_j) & \\ B(i - 1, j) - d & \text{gap in sequence } \mathbf{y} \\ B(i, j - 1) - d & \text{gap in sequence } \mathbf{x} \end{cases}$$

Complexity: $O(mn)$

Example

$$w(x, x) = 1, w(x, y) = -1 \quad x \neq y, d = 1$$

$\mathbf{x} = GCATGCG, \mathbf{y} = GATTACA$

Alignments with a max score of 0

GCA-TGCG

| | | |

G-ATTACA

GCAT-GCG

| | | |

G-ATTACA

GCATG-CG

| | | |

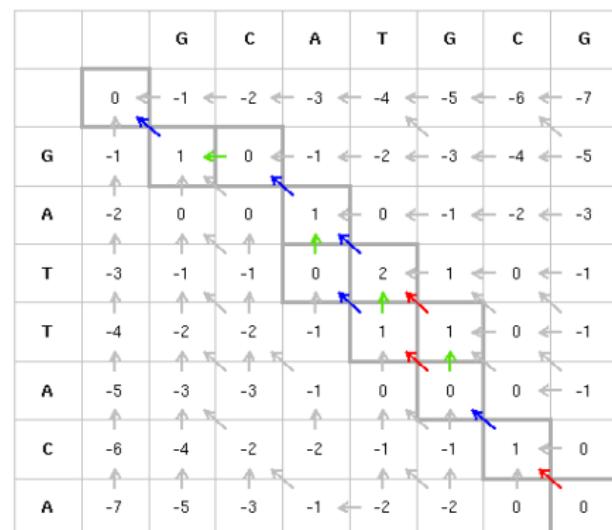
G-ATTACA

Needleman-Wunsch

match = 1

mismatch = -1

gap = -1



SeqAlignR by Leonard Persson Norblad 732A51_BioinformaticsHT2024_global_alignment_by_SeqAlignR.example.R

see also Ewens, Grant: Fig. 6.1 (there $d = 2$)

Fitting one sequence into another (EG Ch. 6.4.3)

Input: two sequences $\mathbf{x} = X_1 X_2 \dots X_m$ and $\mathbf{y} = Y_1 Y_2 \dots Y_n$
but $n \gg m$ (\mathbf{x} is to be aligned to a subsequence of \mathbf{y})

find :
$$\max\{B(\mathbf{x}, \mathbf{y}_{k,j}) : 1 \leq k \leq j \leq n\}$$

define $F(i, j) = \max\{B(\mathbf{x}_{1,i}, \mathbf{y}_{k,j}) : 1 \leq k \leq j\}$

initial conditions: $F(i, 0) = -id$, $F(0, j) = 0$

recursion:

$$F(i, j) = \max \begin{cases} F(i-1, j-1) + w(X_i, Y_j) \\ F(i-1, j) - d & \text{gap in sequence } \mathbf{y} \\ F(i, j-1) - d & \text{gap in sequence } \mathbf{x} \end{cases}$$

Variations

Other gap models (EG Ch. 6.4.5)

gap open penalty: $\delta(l) = -d - (l - 1)e$

d : gap open, e : gap extension

Ends-free alignment (MM Ch. 5.5)

$B(i, 0) = B(0, j) = 0$

(sequences of significantly different lengths)

R packages (BioConductor)

- ▶ **SeqAlignR**, by Leonard Persson Norblad, on [CRAN](#)
- ▶ msa
- ▶ DECIPHER
- ▶ DIAlignR

BONUS POINT(S): extend **SeqAlignR** for the $n \gg m$ case, with the Smith–Waterman algorithm, and other possibilities

FIRST DISCUSS YOUR IDEAS AND POSSIBLE POINTS

$w(x, y)$ (MM Ch. 4.3)

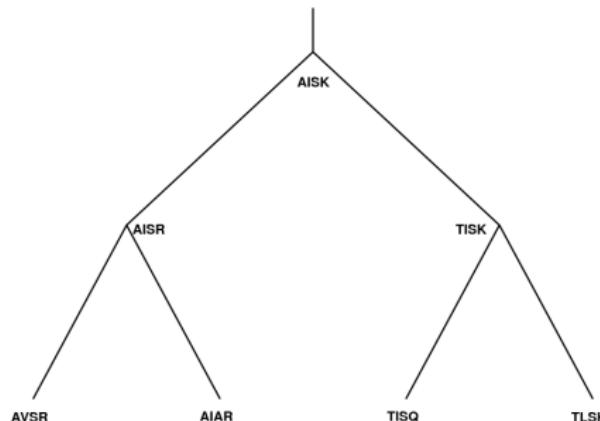
“Physical and chemical properties of replacement residues (amino acids) should be nearly the same as of the original one.”

Radically different ones can compromise the protein’s function.

Idea: Make a table of similarities of amino acids

Not possible: from sequence data see what replacements occurred.

Nucleotide level (gene): synonymous changes “should” be neutral



	A	I	K	L	Q	R	S	T	V
A	0	0	0	0	0	0	1	1	0
I	0	0	0	1	0	0	0	0	1
K	0	0	0	0	1	1	0	0	0
L	0	1	0	0	0	0	0	0	0
Q	0	0	1	0	0	0	0	0	0
R	0	0	1	0	0	0	0	0	0
S	1	0	0	0	0	0	0	0	0
T	1	0	0	0	0	0	0	0	0
V	0	1	0	0	0	0	0	0	0

PAM matrix (EG Ch. 6.5.3, MM Ch. 4.4–4.10)

Accepted Point Mutations: collect closely related protein sequence and record matrix (**A**) of frequencies of point substitutions

Margaret Dayhoff (1978) 1572 substitutions recorded
(MM Fig. 4–4)

Jones, Taylor, Thornton (1992) 59190 substitutions recorded
(similar, MM Fig. 4–5)

PAM Matrix, Dayhoff (1978)

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	30	109	154	33	93	266	579	21	66	95	57	29	20	345	772	590	0	20	365	
Arg		17	0	10	120	0	10	103	30	17	477	17	7	67	137	20	27	3	20	
Asn			532	0	50	94	156	226	36	37	322	0	7	27	432	169	3	36	13	
Asp				0	76	831	162	43	13	0	85	0	0	10	98	57	0	0	17	
Cys					0	0	10	10	17	0	0	0	0	10	117	10	0	30	33	
Gln						422	30	243	8	75	147	20	0	93	47	37	0	0	27	
Glu							112	23	35	15	104	7	0	40	86	31	0	10	37	
Gly								10	0	17	60	7	17	49	450	50	0	0	97	
His									3	40	23	0	20	50	26	14	3	40	30	
Ile										253	43	57	90	7	20	129	0	13	661	
Leu											39	207	167	43	32	52	13	23	303	
Lys												90	0	43	168	200	0	10	17	
Met													17	4	20	28	0	0	77	
Phe														7	40	10	10	260	10	
Pro															269	73	0	0	50	
Ser																696	17	22	43	
Thr																	0	23	186	
Trp																		6	0	
Tyr																			17	
Val																				

<https://github.com/brouwern/dayoff> GNU GPL
 Margaret Dayhoff recorded 1572 substitutions

Fig 80 in M.O. Dayhoff, R.M. Schwartz RM, B.C. Orcutt BC 1978. A model of Evolutionary Change in Proteins.

Atlas of protein sequence and structure (vol. 5, suppl 3ed.). Washington, DC.: National Biomedical Research Foundation. p.345-358.

D.T. Jones, W.R. Taylor, J.M Thornton JM 1992. The rapid generation of mutation data matrices from protein sequences.

Comp. Appl. Biosci. 8:275-282, recorded 59190 substitutions (their Tab. I; MM Fig. 4.5)

PAM matrix (EG Ch. 6.5.3, MM Ch. 4.4–4.10)

Relative mutability: probability that one amino acid will mutate into another in a short period of time

$$m(j) = \sum_{i \neq j} \mathbf{A}_{i,j} / n(i), \quad n(i) \text{ number of observed amino acids } i$$

	A	I	K	S	T
Amino acid	A	I	K	S	T
Changes	0	0	1	1	2
Freq	2	4	5	3	2
Rel. mutability	0	0	$\frac{1}{5}$	$\frac{1}{3}$	1

λ solves (99% sequence identity after mutation)

$$0.99 = 1 - \lambda \sum_j \frac{n(j)}{N} m(j) \quad N : \text{total number of observed amino acids}$$

$M_n = M_1^n$, M_n : PAM $_n$ matrix, PAM250 is often used

$$w_n(i, j) = 10 \log_{10} \left(\frac{M_n(i, j)}{n(j)/N} \right)$$

BLOSUM matrix (EG 6.5.2, MM Ch. 4.11)

BLOcks SUbstitution Matrix,

align multiple short, related sequences

Count the number of (ordered) pairs of positions where one amino acid changes into another, $\tilde{\mathbf{A}}$ matrix

$$\mathbf{Q}_{i,j} = \tilde{\mathbf{A}} / \sum_{i,j} \tilde{\mathbf{A}}_{i,j}$$

$$\tilde{\mathbf{R}}_{i,j} = \mathbf{Q}_{i,j} / ((n(i)/N)(n(j)/N))$$

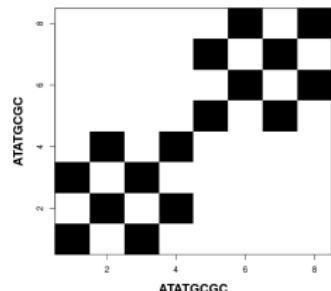
A	I	S	K	T	I	S	K
A	R	S	K	T	V	S	K
A	R	S	K	T	V	S	Q
A	R	S	K	T	V	S	Q
A	R	S	K	T	V	S	K
A	R	S	K	T	V	S	K
A	R	S	K	T	I	S	K
A	R	S	K	T	I	S	K
A	R	T	K	K	I	S	K

$$w(i,j) = 2 \log_2 \tilde{\mathbf{R}}_{i,j}$$

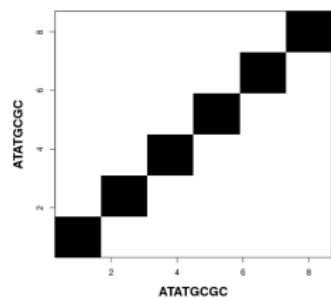
A	56	I	12	7	16
K		86	12	7	7
Q		12	2		
R		7		42	
S				98	7
T			7	7	42
V		16			12

Dot plots (MM Ch. 5.2)

`seqinr::dotPlot()`

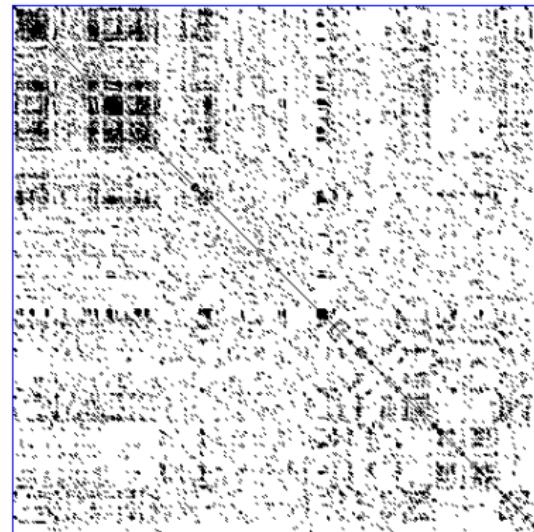


`sliding window:1 nmatch=wsize=1`



`sliding window:3 nmatch=wsize=3`

Human zincfinger TF versus itself dotplot.



<https://commons.wikimedia.org/w/index.php?curid=949276>,
by Opabinia regalis, CC BY-SA 3.0

Multiple sequences (EG Ch. 6.6, MM Ch. 6.3)

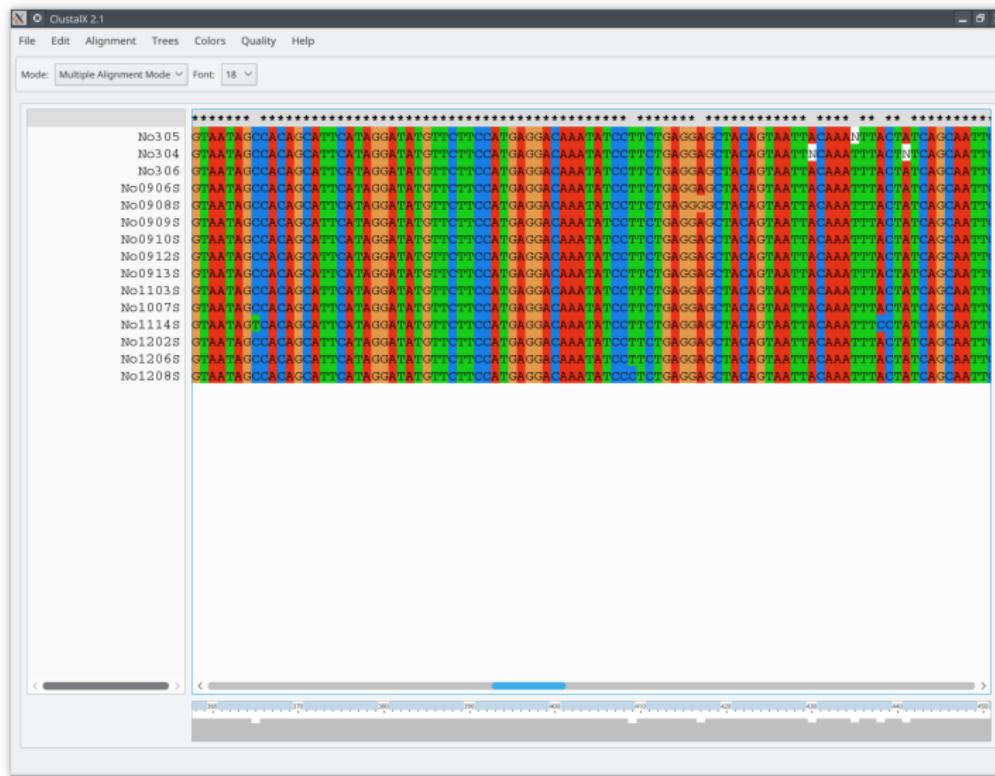
Needleman–Wunsch algorithm can be directly generalized but impractical for large number of sequences (more than 20 in 2005)

Heuristics developed e.g. CLUSTAL W
(<http://www.clustal.org/>);
(<https://www.ebi.ac.uk/Tools/msa/clustalo/>)

1. Create all pairwise alignments and calculate scores
2. Create a guide tree (neighbour-joining) based on the scores
3. Align sets of sequences going from tips to root See also Ch. 6 in DEKM

ClustalX (woodmouse.fasta from phangorn)

<http://www.clustal.org/>



Variations: Smith–Waterman algorithm (EG Ch. 6.4.4, MM Ch. 5.6)

Local alignment: given two sequences, which sub–sequences have the highest–scoring alignment

Smith–Waterman: linear gap penalty

find : $\max\{B(\mathbf{x}_{h,i}, \mathbf{y}_{k,j}) : 1 \leq h \leq i \leq m, 1 \leq k \leq j \leq n\}$

define : $L(i, j) = \max\{0, B(\mathbf{x}_{h,i}, \mathbf{y}_{k,j}) : 1 \leq h \leq i, 1 \leq k \leq j\}$

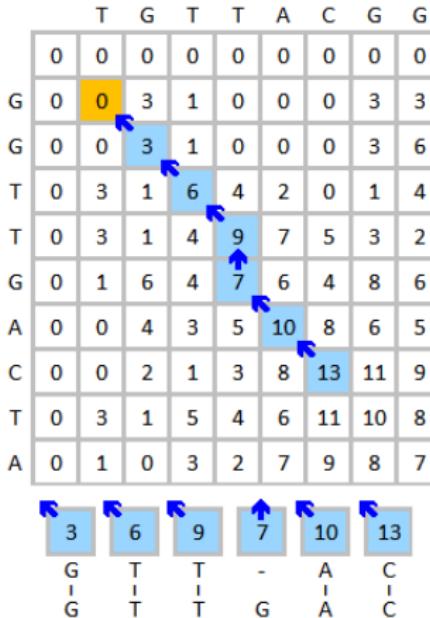
idea: $L(i, j)$ negative then remove first part of alignment

initial conditions: $L(i, 0), L(0, j) = 0$

recursion:

$$L(i, j) = \max \begin{cases} 0 \\ L(i - 1, j - 1) + w(X_i, Y_j) & \text{gap in sequence } \mathbf{y} \\ L(i - 1, j) - d & \text{gap in sequence } \mathbf{x} \\ L(i, j - 1) - d & \text{gap in sequence } \mathbf{x} \end{cases}$$

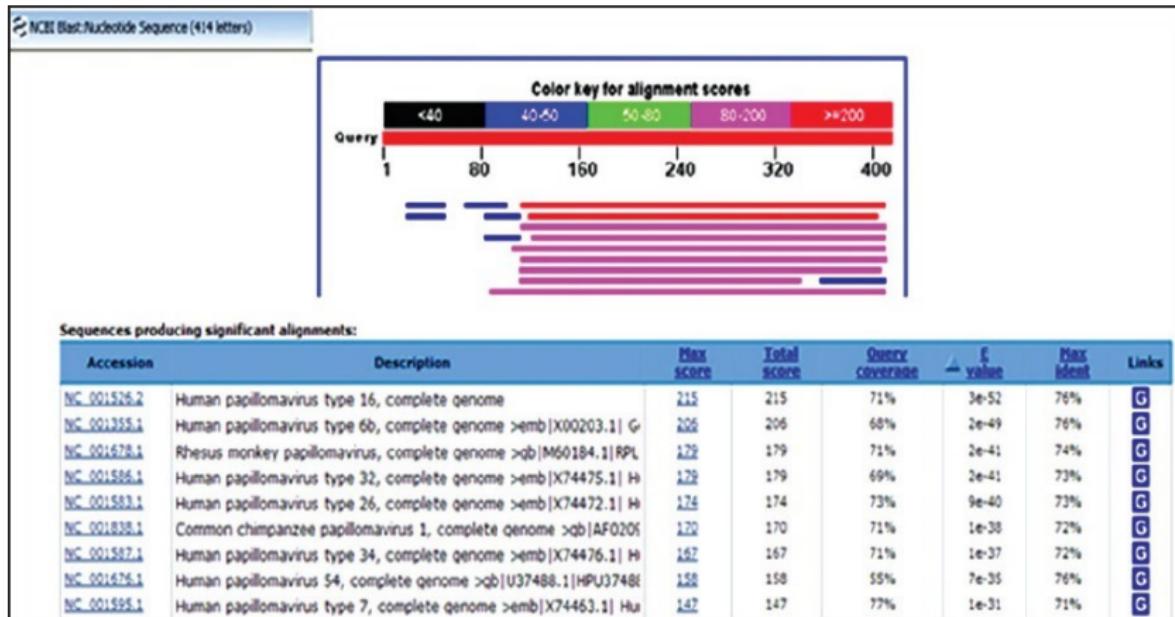
Example



<https://commons.wikimedia.org/w/index.php?curid=54407062>, by Yz cs5160, CC BY-SA 4.0

$d = 2$, match:+3, mismatch:-3

BLASTing (MM Ch. 6.2)



Linda Bruslind, General Microbiology, 2019, CC BY-NC 4.0,

see also NCBI user's manual: <https://www.ncbi.nlm.nih.gov/books/NBK279690/>

BLAST phases (MM Fig. 6.2, [https://en.wikipedia.org/wiki/BLAST_\(biotechnology\)](https://en.wikipedia.org/wiki/BLAST_(biotechnology)))

high-scoring word: (short subsequence of given length(s))

Phase 1: Remove regions of low complexity (i.e., those with few different elements)

Phase 2: Create a list of k (longer than word threshold) letter *high scoring* words derived from query sequence.

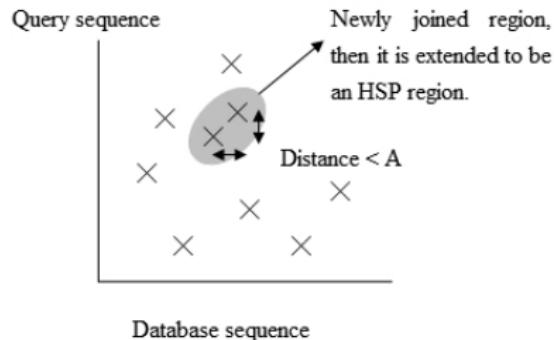
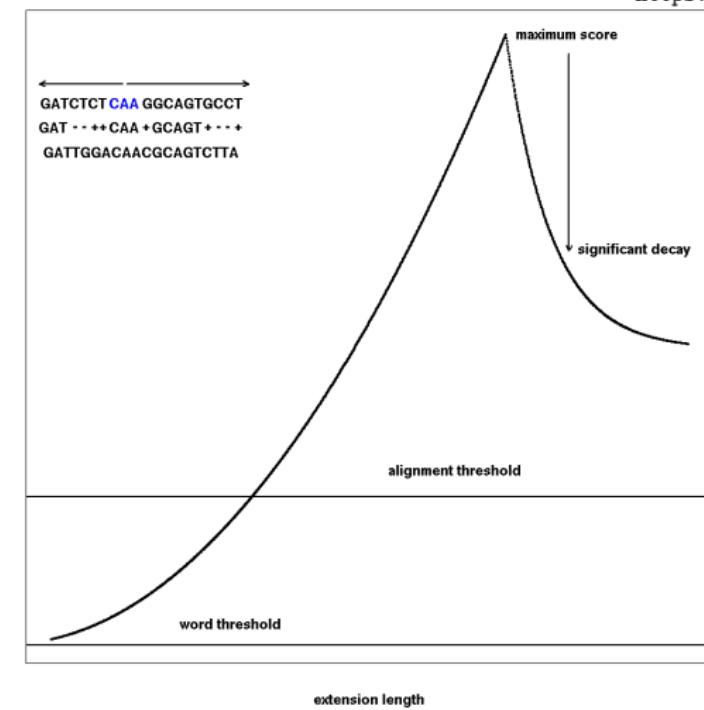
Phase 3: Using a chosen substitution (scoring) matrix (BLOSUM62) create a list of *high scoring pairs* (**HSP**; query/database; longer than word threshold) by searching through the sequence database (against which query is *blasted*).

Phase 4: Extend each HSP, both ways, until a significant decay in its score is reached.

Phase 5: Reduce back each HSP until the maximum score for the pair is attained.

BLAST phases (MM Figs. 6.3, 6.4)

<https://commons.wikimedia.org/w/index.php?curid=33587927>



Database sequence

by DISP, public domain

E-value (EG Ch. 10.3.4, 10.4, MM p. 104)

Multiple testing issue: multiple hits between query and database sequence, where to cut off?

H_0 : The two sequences are unrelated
(each pair of aligned amino acids is independent)

Score $\mathbf{S}(j, k)$ (assumed substitution matrix) assigned to each position where amino acids j, k are observed

accumulated score(i): sum of scores from positions $1, \dots, i$
(random walk)

EG Ch. 10.2.5–10.3.3 p-value derivations

E-value

E : number of hits of score at least s expected by chance
(given the query and database sizes)

$$E = 2N'_1 N'_2 K e^{-\lambda s}$$

N'_1, N'_2 : sizes of query, database sequences corrected for edge effects

K, λ : estimated model parameters (from **S**)

s : similarity score

D : database size

$$\text{Expect} = \frac{(1 - e^E)D}{N_2} \quad \text{p-value} = 1 - e^{-\text{Expect}}$$

BLAST's applications (MM Tab. 6.1)

IDEA: Function based on sequence similarity to other sequences

- ▶ Identify genes in newly sequenced genomes.
- ▶ Identify non-coding DNA sequences.
- ▶ Identify distantly related proteins (similar substructures).
- ▶ Predict protein structures.
- ▶ Predict protein functions
(similar sequences—similar structures).

BLAST printout (EG Ch. 10.5.1)

Range 1: 94 to 309 GenPept Graphics					▼ Next Match	▲ Previous Match
Score	Expect	Method	Identities	Positives	Gaps	
129 bits(323)	1e-30	Compositional matrix adjust.	79/221(36%)	123/221(55%)	13/221(5%)	
Query 93	VIFSLVTPQVSTYINNYVGQLIREVSDETVKAVQIAVNQGVVTGRNPRQIARDFRSSIGL					152
Sbjct 94	V F V + ++ +LIRE + E +A+A+G+ G N P R A R F R S I G L					153
Query 153	TTRQEMTVQRRLRASLETGDVGYVNSLTTVTDS-----AKNAVSAGKLSQAKIDQIVEQT					206
Sbjct 154	T RQ++ VQR R+ LE+G ++ + D A+ A+ L++A++D++VE+					211
Query 207	TERQQQLAVQRYRSLLESGSSEALSR--QLRDRRFDRTVARAARTGEPLTRAQVDRMVERY					265
Sbjct 212	R L R Y I K Q R T E I A R T E S L R A V S V G Q D Q A I R Q G Q V T G S I S N E L L K P - W L Y R K D G R T R D V H I RY++ R+E I A R T E + L R A V G D+ RQ + G ++ E L+R W+ + D R R H S D R Y L R Y R S E V I A R T E A L R A V H A G N D E M Y R Q A V E S G H V A Q E Q I Q R T W V T A R D E V R H S H S					271
Query 266	ST-GETNGWIPMNRPFPSTPLGPLMFP RD PNGSAANVINCRC		305			
Sbjct 272	+ G+T G ++ + G L +P DP A+ + CRC					
	ALGGQTRG--LDEVWEAAGGVLRYPGDPEAPASETVQCRC		309			

<https://cpt.tamu.edu/bich464/doc/C2.html>, Center for Phage Technology, CC BY-SA 4.0

BioPerl for graphically presenting BLAST's output

https://bioperl.org/howtos/BioGraphics_HOWTO.html

Questions?