BIOS 477/877
Bioinformatics and Molecular Evolution

Instructor: Etsuko Moriyama
(School of Biological Sciences)

Spring 2026 | Lecture 8

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Today's topics

- Alignment strategy: a good practice
- Gap Penalty
- Scoring (Substitution) Matrix
- Graduate Only Assignment
- Assignment 4

2

Alignment Strategy

- Protein alignment is easier than DNA alignment
  - → DNA has only 4 nucleotide types (they can match just by chance more easily)
  - → Protein sequences evolve more slowly than DNA sequences (genetic code is redundant; nonsynonymous substitutions are less frequent than synonymous substitutions)
- If DNA sequences are from coding regions:
  - → Translate them, and align at the protein level first
  - → This ensures gaps inserted between codons (prevents insertion of frame-shifting gaps)
- Do not blindly rely on the default parameter set
  - → Try various scoring matrices, gap penalties, etc.

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DNA alignment at protein level

1. Translate DNA sequences to amino acid sequences
2. Align them at the protein level
3. Reverse translate the protein alignment to DNA alignment

[Some useful tools]

- TranslatorX
- RevTrans 2.0
- PAL2NAL
- tranalign

(included in EMBOSS servers; see the course/Link page.)

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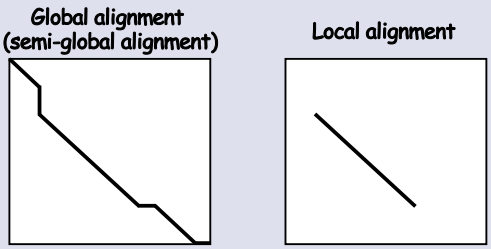
Global vs. local alignments



$s(a_i,b_j) = 2$ where $a_i=b_j$
$s(a_i,b_j) = -1$ where $a_i \neq b_j$
$w = -2$
(w=0 for free end gap)

Match/Mismatch scores
Gap penalty

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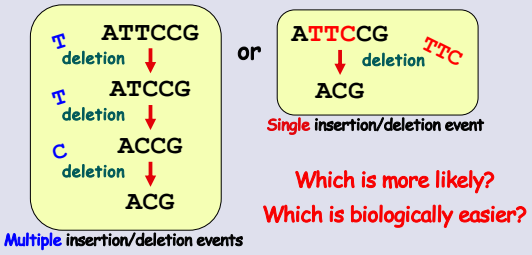
Insertions and deletions: mechanisms

- A gap of length $k \neq k$ gaps of length 1



Which is more likely?
Which is biologically easier?

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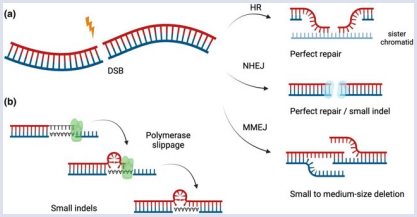
Insertions and deletions: mechanisms
(continued)

- Indel mutations are often strongly deleterious
- Indel events are rare (less common than point mutations)
- Multi-residue indels are not uncommon (e.g., hotspot, repetitive DNA)



DSB: double-stranded breaks
HR: homologous repair
NHEJ: non-homologous end joining
MMEJ: microhomology-mediated end joining

Redeling et al. (2024)

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Insertions and deletions: mechanisms
(continued)

- Indel mutations are often strongly deleterious
- Indel events are rare
  (less common than point mutations)
- Multi-residue indels are not uncommon
- Fewest number of unlikely events
  → most likely evolutionary hypothesis
  → Maximum parsimony

AATCTATA → AA-G-ATA (2 indels)
AATCTATA → AA--GATA (1 indel (more likely than 2 indel events))

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Indel evolution and gap penalty

- Fewer, but longer, indel event is more likely than too many small indels

ATTCCG → deletion (TTC) → ACG

- Indels of multiple residues
- Single insertion/deletion event

During alignment:
Extension of existing gaps should cost less than creation (or opening) of a new gap

Two types of gap penalties are required

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Gap penalty functions

- Linear (length-proportional) gap penalty:
  $w(x) = gx$
  g: gap penalty
  x: length of a gap
- Affine gap penalty:
  $w(x) = \begin{cases} g_o + g_e(x-1) & \text{when } x > 0 \\ 0 & \text{when } x = 0 \end{cases}$
  g_o: gap opening penalty
  g_e: gap extension penalty [usually $g_o > g_e$]
  x: length of a gap

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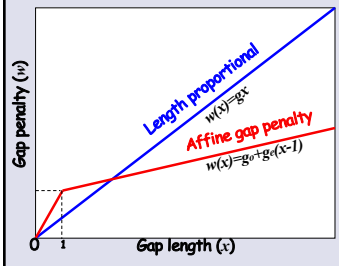
Gap penalty functions



Affine gap penalty:
Extension of existing gaps costs less than creation/opening of new gaps

(g_o: gap opening penalty, g_e: gap extension penalty)

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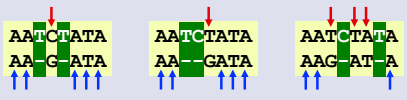
Simple alignments

- Varied length & gaps considered



Score= 3 3 1

Alignment Score =
(match score) x (the number of matched pairs) +
(mismatch score) x (the number of mismatched pairs) +
(gap penalty) x (the number of gaps)

Use match score = 1, mismatch score = 0, gap penalty = -1
using linear gap penalty

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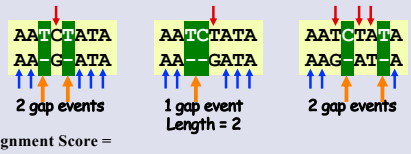
Simple alignments (with affine gap penalty)

- Varied length & gaps considered



Alignment Score =
(match score) x (the number of matched pairs) +
(mismatch score) x (the number of mismatched pairs) +
∑[(for each gap event) → {(gap opening penalty) +
(gap extension penalty) x (gap length - 1)}]

Match score = 1, mismatch score = 0, & using affine gap penalty:
gap opening penalty = -2, gap extension penalty = -1

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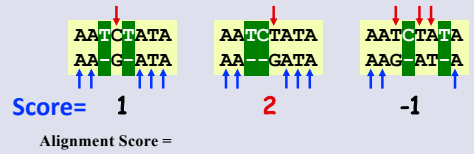
Simple alignments (with affine gap penalty)

- Varied length & gaps considered



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∑[(for each gap event) → {(gap opening penalty) +
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Match score = 1, mismatch score = 0, & using affine gap penalty:
gap opening penalty = -2, gap extension penalty = -1

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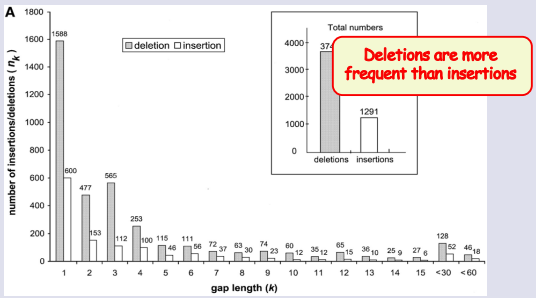
Empirical indel distribution: DNA

Based on the comparisons of >1700 processed pseudogenes against their functional homologues in the human genome



Deletions are more frequent than insertions

Zhang and Gerstein (2003)

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Empirical indel distribution: DNA

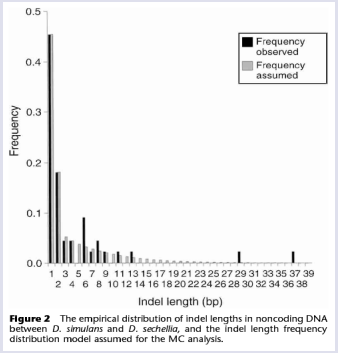

Figure 2 The empirical distribution of indel lengths in noncoding DNA between *D. simulans* and *D. sechellia*, and the indel length frequency distribution model assumed for the MC analysis.

Based on the comparisons of 23 noncoding region sequences between *Drosophila simulans* and *D. sechellia*

Keightley and Johnson (2004)

This distribution was later used in MCALIGN2 (Wang *et al.* 2006)

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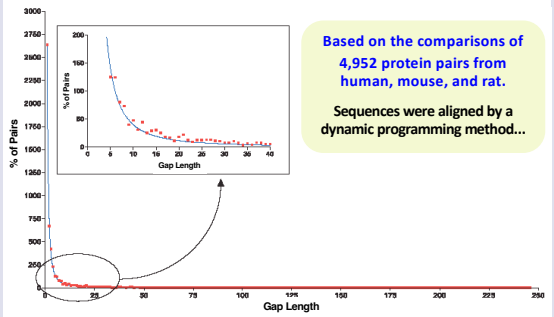
Empirical indel distribution: protein



Based on the comparisons of 4,952 protein pairs from human, mouse, and rat.

Sequences were aligned by a dynamic programming method...

Chang and Benner (2004)

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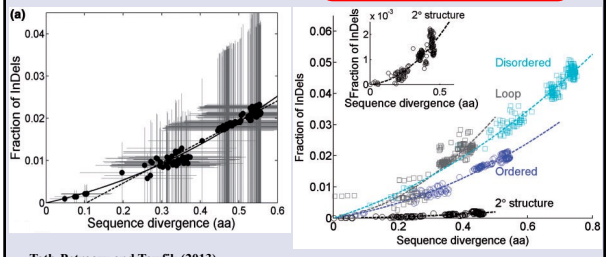
Empirical indel distribution: protein

Based on the comparisons of 1,310 orthologous families from 22 fungal species.

Loop regions have more indels compared to the regions with secondary structures



Toth-Petroczy and Tawfik (2013)

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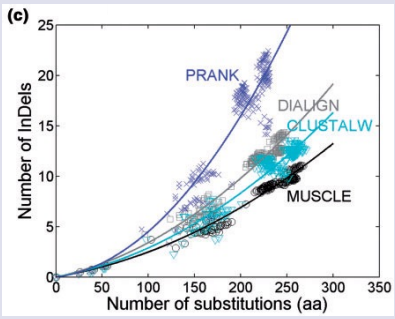
Empirical indel distribution: protein



Indel rates/lengths are affected by different alignment methods!

Toth-Petroczy and Tawfik (2013)

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Gap penalty function (more realistic)

- Empirical indel size distributions (both for DNA and proteins) can be described by a power law:

  $f_k = Ck^{-b}$ [k: indel size, b: the power parameter]

  → Corresponding gap penalty function

  $w = a + b\ln(k)$

  a: gap opening penalty

  b: gap extension penalty

  Affine gap penalty: $w = a + bk$

  - Gap extension penalty is proportional to the logarithm of gap length k (logarithmic gap penalty system)
  - increases more slowly with gap length than in the affine gap penalty system (easier long gaps)

e.g., Cartwright (2006 and 2009); Loewenthal *et al.* 2021; Wygoda *et al.* (2024)
Review: Redelings *et al.* (2024)

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Scoring (substitution) matrix: DNA identity matrix

i \ j	A	T	C	G
A	1	0	0	0
T	0	1	0	0
C	0	0	1	0
G	0	0	0	1

Match/mismatch scores can be expressed in a matrix format

Match score = 1
Mismatch score = 0

$s(i, j)$: the similarity score between nucleotides i and j

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Scoring (substitution) matrix: Transition/transversion matrix

	A	T	C	G
A	1	?	?	?
T	?	1	?	?
C	?	?	1	?
G	?	?	?	1

Match score = 1
Mismatch score:
{ Transition = ?
{ Transversion = ?

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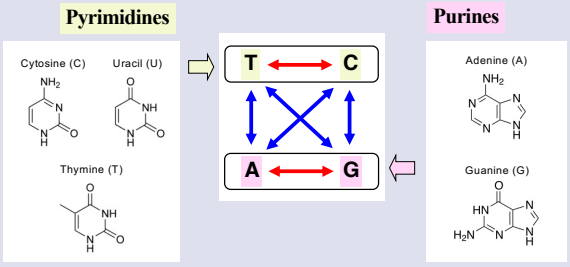
Types of DNA substitutions



Transition substitutions: between pyrimidines, between purines
Transversion substitutions: between pyrimidines and purines

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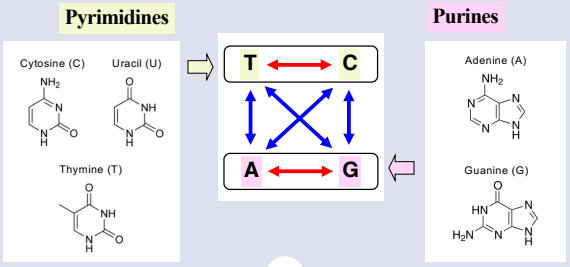
Types of DNA substitutions



↔ Transition ? ↔ Transversion

Which substitutions happen more often?

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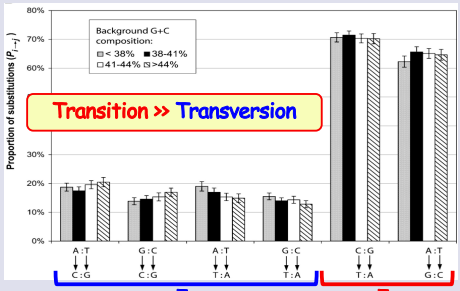
Types of DNA substitutions

Based on the comparisons of >1700 processed pseudogenes against their functional homologues in the human genome



Zhang and Gerstein (2003)

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Scoring (substitution) matrix: Transition/transversion matrix

	A	T	C	G
A	1	-5	-5	-1
T	-5	1	-1	-5
C	-5	-1	1	-5
G	-1	-5	-5	1

Match score = 1

Mismatch score:

- Transition = -1 (more allowed → smaller penalty)
- Transversion = -5 (fewer allowed → larger penalty)

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Amino acid substitution matrices

- Identity matrix
- Genetic code matrix
- Matrices based on AA properties
- Matrices based on empirical data
  - Dayhoff matrices (PAM120 etc.)
  - BLOSUM matrices (BLOSUM62 etc.)
  - Gonnet matrices (Gonnet 250 etc.)
  - JTT matrices
  - and more …

AAindex (566 indices + ~150 matrices)

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Protein identity matrix

	C	S	T	P	A	G	⋯
C	1	0	0	0	0	0	
S	0	1	0	0	0	0	
T	0	0	1	0	0	0	
P	0	0	0	1	0	0	
A	0	0	0	0	1	0	
G	0	0	0	0	0	1	
⋮							

Match score = 1
Mismatch score = 0

(20 x 20)

	C	S	T	P	A	G	⋯
C	6	-1	-1	-1	-1	-1	
S	-1	6	-1	-1	-1	-1	
T	-1	-1	6	-1	-1	-1	
P	-1	-1	-1	6	-1	-1	
A	-1	-1	-1	-1	6	-1	
G	-1	-1	-1	-1	-1	6	
⋮							

Match score = +6
Mismatch score = -1

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Genetic code matrix

(A simpler version)

	A	B	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	Z
A	3.0	2.0	1.0	2.0	2.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	2.0	2.0	2.0	1.0	1.0	2.0
B		3.0	1.0	3.0	2.0	1.0	2.0	2.0	2.0	2.0	1.0	1.0	3.0	1.0	2.0	1.0	2.0	2.0	2.0	0.0	2.0	2.0
C			3.0	1.0	0.0	2.0	2.0	1.0	1.0	0.0	1.0	1.0	0.0	1.0	0.0	2.0	1.0	1.0	1.0	2.0	0.0	0.0
D				3.0	2.0	1.0	2.0	2.0	1.0	1.0	1.0	0.0	2.0	1.0	1.0	1.0	1.0	1.0	2.0	0.0	2.0	2.0
E					3.0	0.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	1.0	2.0	1.0	1.0	3.0
F						3.0	1.0	1.0	2.0	0.0	2.0	1.0	1.0	1.0	0.0	1.0	2.0	1.0	2.0	1.0	2.0	0.0
G							3.0	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.0	2.0	2.0	1.0	2.0	2.0	2.0
H								3.0	1.0	1.0	2.0	0.0	2.0	2.0	2.0	2.0	1.0	1.0	1.0	0.0	2.0	2.0
I									3.0	2.0	2.0	2.0	2.0	1.0	1.0	1.0	2.0	2.0	2.0	0.0	1.0	1.0
K										3.0	1.0	2.0	2.0	1.0	2.0	2.0	2.0	1.0	2.0	1.0	1.0	2.0
L											3.0	2.0	1.0	2.0	2.0	2.0	2.0	1.0	2.0	1.0	1.0	2.0
M												3.0	1.0	1.0	1.0	1.0	2.0	2.0	1.0	0.0	1.0	0.0
N													3.0	1.0	1.0	1.0	1.0	1.0	2.0	0.0	2.0	2.0
P														3.0	2.0	2.0	2.0	2.0	2.0	1.0	1.0	2.0
Q															3.0	2.0	1.0	1.0	1.0	1.0	1.0	3.0
R																3.0	2.0	2.0	1.0	2.0	1.0	2.0
S																	3.0	2.0	1.0	1.0	1.0	1.0
T																		3.0	1.0	1.0	1.0	1.0
V																			3.0	1.0	1.0	2.0
W																				3.0	1.0	1.0
Y																					3.0	1.0
Z																						3.0

3.0: Identical (no change required)
2.0: One nucleotide change required
1.0: Two nucleotide changes required
0.0: Three nucleotide changes required

(B: D/Asp or N/Asn, Z: E/Glu or Q/Gln)

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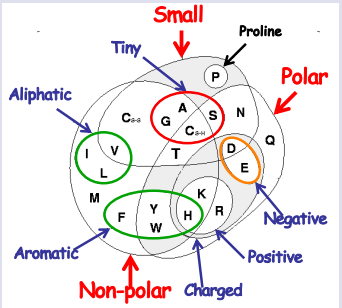
Amino acid physico-chemical properties



Physicochemical properties of amino acids:

- Major factors in protein folding protein functions
- → Substitutions between similar amino acids are more common
- → In functionally or structurally important regions, substitutions are limited between similar amino acids

Livingstone and Barton (1993)

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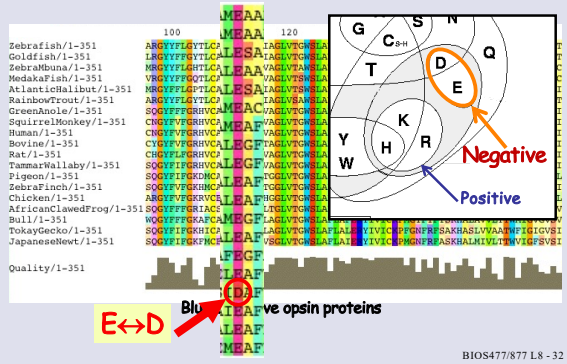
Amino acid substitutions



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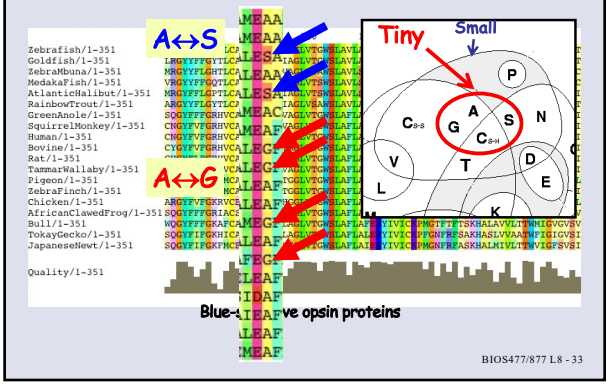
Amino acid substitutions



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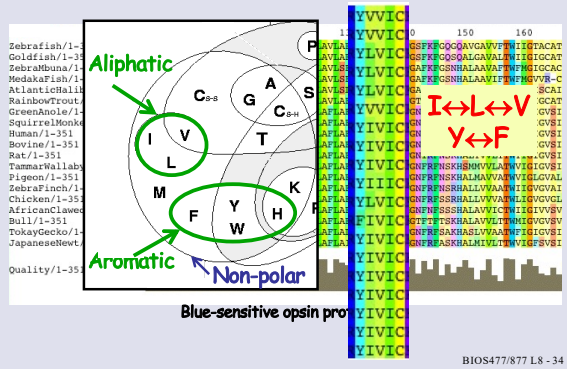
Amino acid substitutions



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Amino acid substitution matrices

- Matrices based on various amino acid properties (hydrophobicity, charge, electronegativity, size, etc.)
  - Biologically meaningful matrix can be obtained by combining all of these matrices (including even genetic code matrix). But this is not easy!
- Matrices based on empirical data
  - Alignments show the results of experiments done by the Nature
  - Capture the relative substitutability of amino acid pairs in the context of evolution
  - The model of protein evolution

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Amino acid substitution matrices

- Identity matrix
- Genetic code matrix
- Matrices based on AA properties
- Matrices based on empirical data
  - Dayhoff matrices (PAM120 etc.)
  - BLOSUM matrices (BLOSUM62 etc.)
  - Gonnet matrices (Gonnet 250 etc.)
  - JTT matrices
  - and more …

AAindex (566 indices + ~150 matrices)

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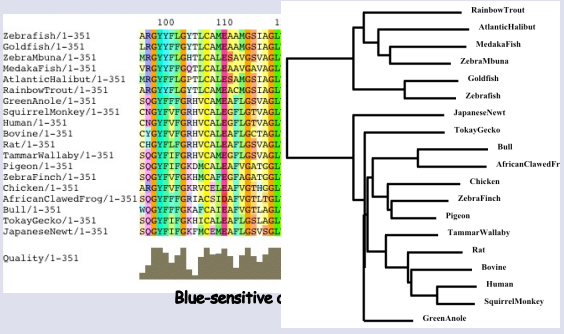
How to count amino acid substitutions



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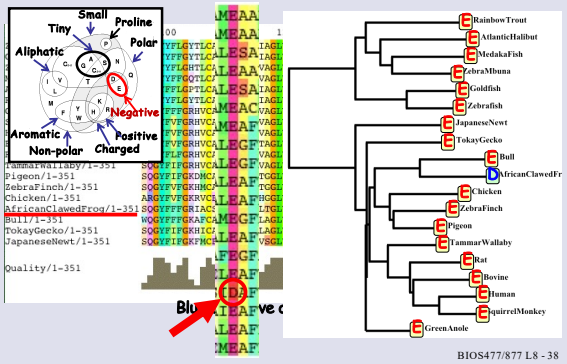
How to count amino acid substitutions



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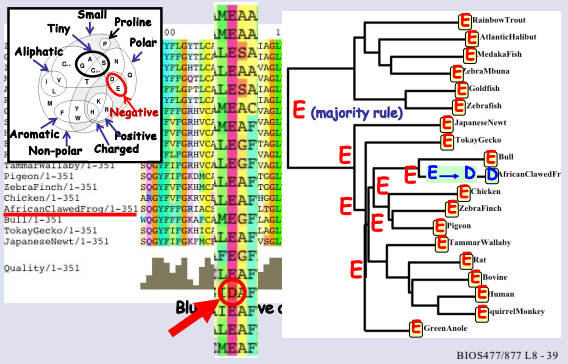
How to count amino acid substitutions



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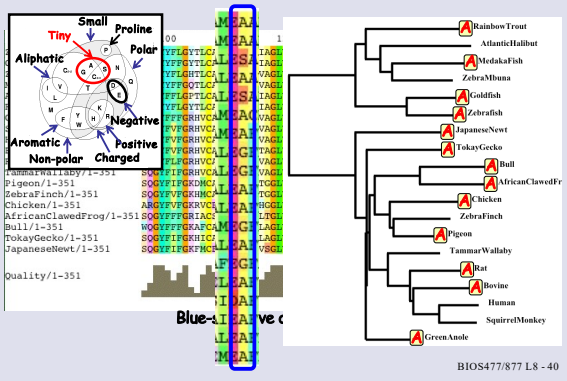
How to count amino acid substitutions



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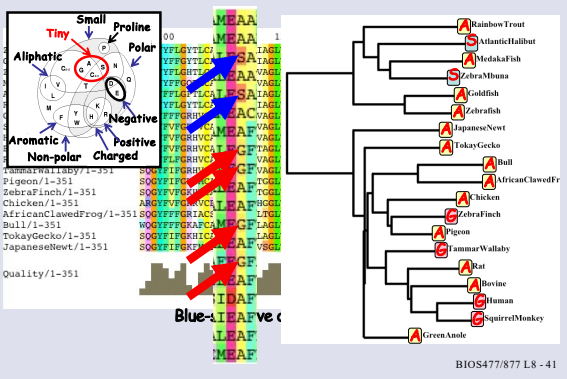
How to count amino acid substitutions



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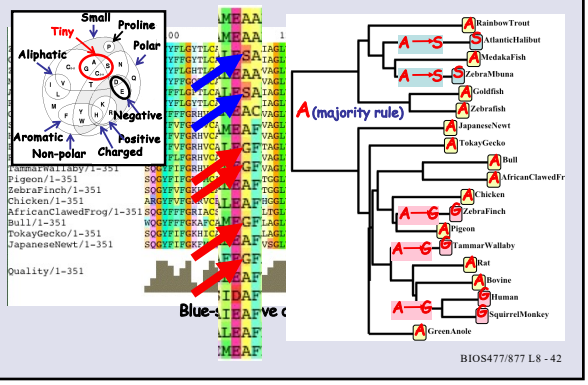
How to count amino acid substitutions



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Amino acid substitution matrices based on empirical data

- **PAM matrices**
  Dayhoff, Schwartz, and Orcutt (1978)
- **BLOSUM matrices**
  Henikoff and Henikoff (1992)
  - Also see Eddy (2004)

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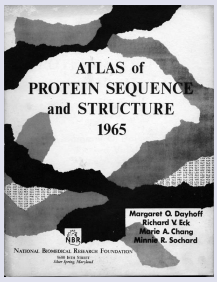
Margaret O. Dayhoff
(1925-1983)

Founder of
the field of Bioinformatics
The first woman in the field



Collection of all known protein sequences
1st *Atlas* contained 65 proteins
Developed into PIR (Protein Information Resource), a brain-child of Dayhoff

Dayhoff developed a single letter code for the amino acids

Read the Smithsonian website; also in Strasser (2010)

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PAM matrices (Dayhoff et al. 1978)

- **Accepted point mutations** (point accepted mutations, percent accepted mutations)
  - → **accepted by selection**: no (or very weak) deleterious effect, maintaining the function
- Based on 1,572 changes in 71 groups of **closely related proteins** (34 protein families)
  - at least 85% identical
  - no ambiguity in alignments, no gap
  - most likely observed substitutions do not affect protein functions (accepted by selection, close to neutral)
  - successive (multiple) substitutions at one site are minimal (no hidden substitution)

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Graduate Only Assignment

- **Report 1: March 8**
  - → Short proposal (2-3 pages)
- **Report 2: May 3**
  - → Final report (5-7 pages)
- Comparative analysis of bioinformatics methods
  - e.g., Multiple alignment methods:
    Clustal W vs. MUSCLE vs. MAFFT
    - → **Compare three methods**
    - → Choose appropriate input datasets (3 or more)
    - → Read the Guidelines!
- **Undergraduate students can do these assignments for bonus points (both reports need to be submitted)**

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