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Protein Alignment Scoring - PAM and BLOSUM

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Sequence Alignments Revisited

- Scoring nucleotide sequence alignments was easier
  - Match score
  - Possibly different scores for transitions and transversions
- For amino acids, there are many more possible substitutions
- How do we score which substitutions are highly penalized and which are moderately penalized?
  - Physical and chemical characteristics
  - Empirical methods

Scoring Mismatches

- Physical and chemical characteristics
  - V $\rightarrow$ I – Both small, both hydrophobic, conservative substitution, small penalty
  - V $\rightarrow$ K – Small $\rightarrow$ large, hydrophobic $\rightarrow$ charged, large penalty
  - *Requires some expert knowledge and judgement*
- Empirical methods
  - How often does the substitution V $\rightarrow$ I occur in proteins that are known to be related?
    - Scoring matrices: PAM and BLOSUM

PAM matrices

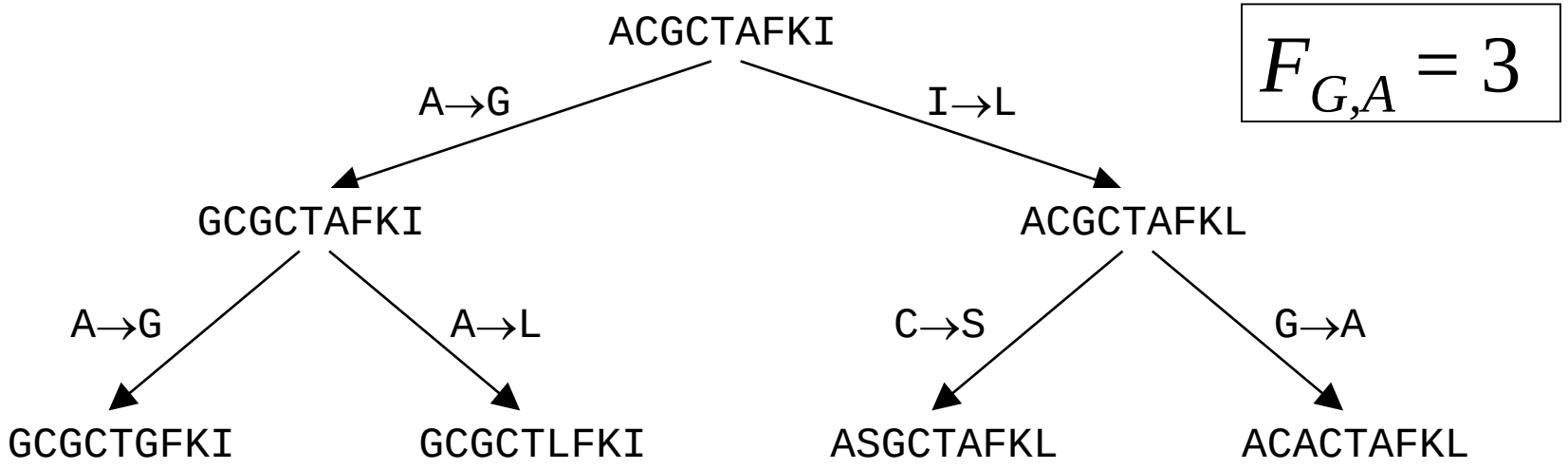
- PAM = “Point Accepted Mutation” interested only in mutations that have been “accepted” by natural selection
- Starts with a multiple sequence alignment of very similar (>85% identity) proteins. Assumed to be homologous
- Compute the *relative mutability*, m_i, of each amino acid
  - e.g. m_A = how many times was alanine substituted with anything else?

Relative mutability

- ACGCTAFKI
  GCGCTAFKI
  ACGCTAFKL
  GCGCTGFKI
  GCGCTLFKI
  ASGCTAFKL
  ACACTAFKL
- Across *all pairs* of sequences, there are 28 $A \rightarrow X$ substitutions
- There are 10 ALA residues, so $m_A = 2.8$

Pam Matrices, cont'd

- Construct a phylogenetic tree for the sequences in the alignment



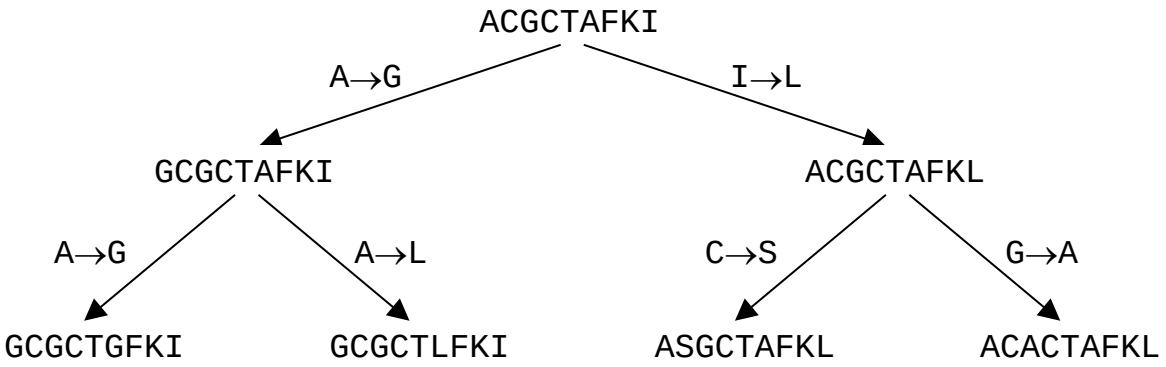
$F_{G,A} = 3$

- Calculate substitution frequences $F_{X,X}$
- Substitutions may have occurred either way, so A $\rightarrow$ G also counts as G $\rightarrow$ A.

Mutation Probabilities

- $M_{i,j}$ represents the probability of J $\rightarrow$ I substitution.

$$M_{ij} = \frac{m_j F_{ij}}{\sum_i F_{ij}}$$



- $M_{G,A} = \frac{2.7 \times 3}{4} = 2.025$

The PAM matrix

- The entries, $R_{i,j}$ are the $M_{i,j}$ values divided by the frequency of occurrence, f_i, of residue *i*.
- f_G = 10 GLY / 63 residues = 0.1587
- $R_{G,A} = \log(2.025/0.1587) = \log(12.760) = 1.106$
- The log is taken so that we can add, rather than multiply entries to get compound probabilities.
- *Log-odds* matrix
- Diagonal entries are $1 - m_j$

Interpretation of PAM matrices

- PAM-1 – one substitution per 100 residues (a PAM unit of time)
- Multiply them together to get PAM-100, etc.
- "Suppose I start with a given polypeptide sequence *M* at time *t*, and observe the evolutionary changes in the sequence until 1% of all amino acid residues have undergone substitutions at time *t*+*n*. Let the new sequence at time *t*+*n* be called *M′*. What is the probability that a residue of type *j* in *M* will be replaced by *i* in *M′*?"

PAM matrix considerations

- If $M_{i,j}$ is very small, we may not have a large enough sample to estimate the real probability. When we multiply the PAM matrices many times, the error is magnified.
- PAM-1 – similar sequences, PAM-1000 very dissimilar sequences

BLOSUM matrix

- Starts by clustering proteins by similarity
- Avoids problems with small probabilities by using averages over clusters
- Numbering works opposite
  - BLOSUM-62 is appropriate for sequences of about 62% identity, while BLOSUM-80 is appropriate for **more** similar sequences.