Sequence Alignment: A General Overview

COMP 571
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Life through Evolution

- All living organisms are related to each other through evolution
- This means: any pair of organisms, no matter how different, have a common ancestor sometime in the past, from which they evolved
- Evolution involves
  - inheritance: passing of characteristics from parent to offspring
  - variation: differentiation between parent and offspring
  - selection: favoring some organisms over others

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Sequence Variations Due to Mutations

- Mutations and selection over millions of years can result in considerable divergence between present-day sequences derived from the same ancestral sequence.
- The base pair composition of the sequences can change due to point mutation (substitutions), and the sequence lengths can vary due to insertions/deletions

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DNA Sequence Evolution

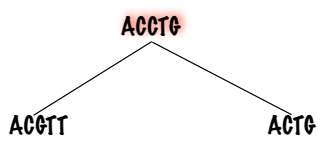
4-1

DNA Sequence Evolution

ACCTG

4-2

DNA Sequence Evolution



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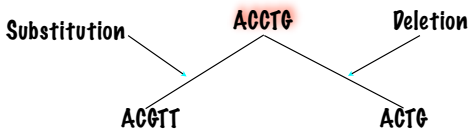
DNA Sequence Evolution



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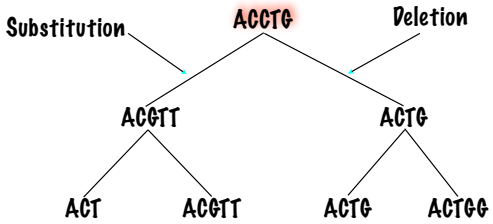
DNA Sequence Evolution



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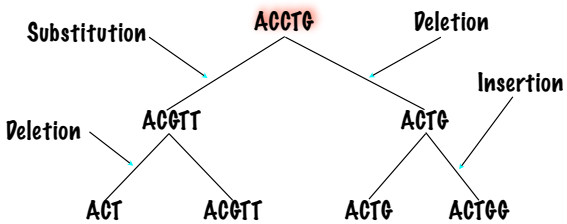
DNA Sequence Evolution



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Principles of Sequence Alignment

1. Alignment is the task of locating "equivalent" regions of two or more sequences to maximize their similarity

Mismatches

T H A T S E Q U E N C E
T H I S S E Q U E N C E

T H I S I S A - S E Q U E N C E
T H - - - - A T S E Q U E N C E

gap (indels: insertions/deletions)

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Principles of Sequence Alignment

2. Alignment can reveal homology between sequences
   1. Similarity is descriptive term that tells about the degree of match between the two sequences
   2. Homology tells that the two sequences evolved from a common ancestral sequence
   3. Sequence similarity does not not always imply a common function
   4. Conserved function does not always imply similarity at the sequence level
   5. Convergent evolution: sequences are highly similar, but are not homologous

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Principles of Sequence Alignment

3. It is easier to detect homology when comparing protein sequences than when comparing nucleic acid sequences
   1. The probability of a "match by chance" is much higher in DNA sequences than in protein sequences.
   2. The genetic code is redundant: identical amino acids can be encoded by different codons.
   3. The complex 3D structure of a protein, and hence its function, is determined by the amino acid sequence. Hence, conserving function leads to fewer changes in the amino acids than in the nucleotide sequence.

Scoring Alignments

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- Given two sequences, the number of possible alignments is exponential
- Finding the "correct" alignment involves defining a scoring scheme and finding an alignment with optimal score

Scoring Alignments: The Main Principles

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- Alignments of related sequences should give good scores compared with alignments of randomly chosen sequences
- The correct alignment of two related sequences should ideally be the one that gives the best score
- (In practice, the correct alignment does not necessarily have the best score, since no "perfect" scoring scheme has been devised)

Percent Identity as a Measure for Quantifying Sequence Similarity

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- Identity is the number of identical bases or amino acids matched between two aligned sequences
- Percent identity is obtained by dividing this number by the total length of the aligned sequences and multiplying by 100
- Sequence similarity based on identity is usually visualized using the dot-plot representation

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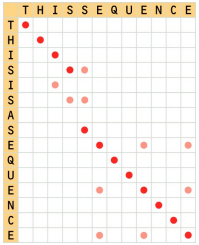
Dot-plot



Red dots represent identities that are due to true matching of identical residue-pairs and pink dots represent identities that are due to noise (matching of random identical residue-pairs)

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Dot-plot

- Dot-plots suffer from background noise
- To overcome this problem it is necessary to apply a filter
- The most-commonly used filtering method uses a sliding window and requires that the comparison achieves some minimum identity score summed over that window before being considered

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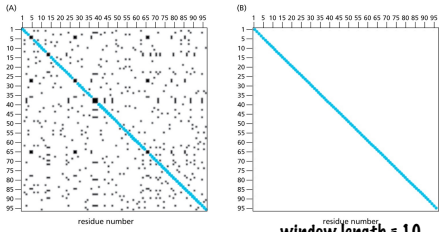
Dot-plot

Dot-plot of an SH2 sequence compared to itself



window length = 1

window length = 10
min. ident. score = 3

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Scoring Alignments

- Genuine matches do not have to be identical
  - Mutations that replace one amino acid with another having similar physiochemical properties are more likely to have been accepted during evolution
  - So, pairs of amino acids with similar properties will often represent genuine matches rather than matches occurring randomly
- To take this into account, percent identity is replaced by percent similarity

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Scoring Alignments

- There is a minimum percent identity that can be accepted as significant
- It has been found that, in general, sequence pairs with identity at or greater than 30% over their whole length are pairs of structurally similar proteins
- In the region between 20% and 30% identity, referred to as the twilight zone, evolutionary relatedness may exist but cannot be reliably assumed in the absence of other evidence

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Substitution Matrices and Gaps

- Scoring schemes have to represent two salient features of an alignment
  1. They must reflect the degree of similarity of each pair of residues (the likelihood that both are derived from the same residue in the presumed common ancestral sequence). This is achieved through the use of an appropriate substitution matrix
  2. They must asses the validity of inserted gaps

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Substitution Matrices

- Mostly apply to amino acids
- The score of a protein sequence alignment is assigned to each aligned pair of amino acids by reference to a substitution matrix, which defines values for all possible pairs of residues (a 20x20 matrix)
- Several such matrices have been devised, and which one to use depends on the "evolutionary distance" of the sequences being aligned

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Substitution Matrices

- Commonly used substitution matrices include PAM (Point Accepted Mutation) and BLOSUM (Blocks of Amino Acid Substitution Matrix)
- PAM substitution matrices were derived based on substitution frequencies in sets of closely related protein sequences
- BLOSUM substitution matrices were derived based on mutation data in highly conserved local regions of sequences

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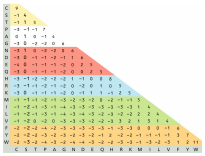
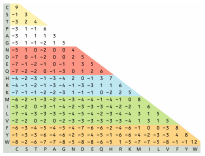
Substitution Matrices



BLOSUM62



PAM120

Substitution Matrices

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- There are other matrices: JTT (Jones et al., 1992), VT (Muller and Vingron, 2000), STR (uses information about protein structure), SLIM and PHAT (for membrane proteins), ...
- It is very hard to determine which matrix to use
- When aligning distantly related sequences, PAM250 and BLOSUM-50 are preferable, whereas when aligning closely related sequences PAM120 and BLOSUM-80 may be better

Handling Gaps

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- Homologous sequences are often of different lengths as the result of insertions and deletions (indels) that have occurred in the sequences as they diverged from the ancestral sequences
- Their alignment is generally dealt with by inserting gaps in the sequences to achieve as correct a match as possible
- Gaps must be introduced judiciously
- To place limits on the introduction of gaps, alignment programs use a gap penalty: each time a gap is introduced, the penalty is subtracted from the score

Handling Gaps

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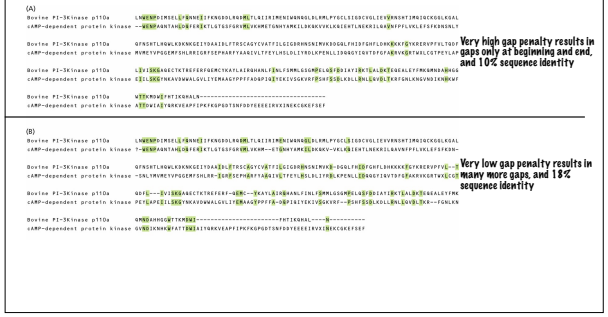
- Structural analysis has shown that fewer indels occur in sequences of structural importance, and that insertions tend to be several residues long rather than just a single residue long
- This informs what gap penalty model to define
- Different gap penalty models may result in different alignments

Handling Gaps



Very high gap penalty results in gaps only at beginning and end, and 10% sequence identity

Very low gap penalty results in many more gaps, and 18% sequence identity

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Types of Alignment

- Global alignment: Aligning the whole sequences
  - Appropriate when aligning two very closely related sequencs
- Local alignment: Aligning certain regions in the sequences
  - Appropriate for aligning multi-domain protein sequences
- It is important to use the "appropriate" type

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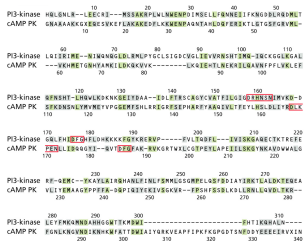
Local vs. Global Alignment

Local

Global



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Types of Alignment

- Pairwise alignment: Aligning a pair of sequences
  - Computationally "easy"
- Multiple alignment: Aligning more than two sequences
  - Computationally "hard"
  - Advantageous when sequences of low similarity are being aligned

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Pairwise vs. Multiple Alignment

```
(A) p110α          TFILGIGDRHNSNIMVKDDG-QLFHIDFGHFLDHKKKKFGYKRERVPFVLT--QDFLIVI 142
    cAMP-kinase    QIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWXLCGTPEYLAPE 179

(B) p110β          SYVLGIG----------DRHSDNINVKKTGQLFHIDFGHILGNFKSKFGIKRERVPFILT 136
    p110δ          TYVLGIG----------DRHSDNIMIRESGQLFHIDFGHFLGNFKTKFGINRERVPFILT 136
    p110α          TFILGIG----------DRHNSNIMVKDDGQLFHIDFGHFLDHKKKKFGYKRERVPFVLT 135
    p110γ          TFVLGIG----------DRHNDNIMITETGNLFHIDFGHILGNYKSFLGINKERVPFVLT 135
    p110_dicti     TYVLGIG----------DRHNDNLMVTKGGRLFHIDFGHFLGNYKKKFGFKRERAPFVFT 135
    cAMP-kinase    QIVLTFEYLHSLDLIYRDLKPENLLIDQQGYIQVTDFGFAKRVKGRTWXLCG--TPEYLA 177
```

The pairwise alignment does not align the important active-site residues
The multiple alignment does align the important active-site residues

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Searching Databases

- Searching databases has become an integral part of molecular biology
- Searching sequence databases typically entails finding sequences in the database that are "similar" to a query sequence
- Such a search amounts to aligning the query sequence to sequences in the database and returning ones with "good" alignment score
- Given the sizes of available biological databases, heuristics are employed instead of the exact, yet expensive, alignment algorithms

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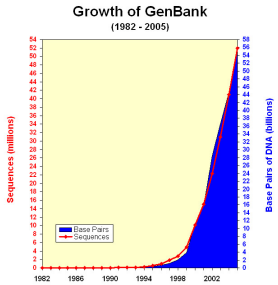
Growth of GenBank



Source: http://www.ncbi.nlm.nih.gov/Genbank/genbankstats.html

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Database Search

- Database search needs to be both sensitive, in order to detect distantly related homologs and avoid false-negative searches, and also specific, in order to reject unrelated sequences with high similarity (false positives)
- The two most-commonly used heuristics for sequence database search are FASTA and BLAST

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- In the next set of slides we will cover
  - the theory behind scoring schemes
  - algorithmic techniques for computing optimal alignments
  - significance of alignments