Introduction to Bioinformatics

Pairwise Sequence Alignment

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Outline

- Introduction to sequence alignment
- pair wise sequence alignment
  - The Dot Matrix
  - Scoring Matrices
  - Gap Penalties
  - Dynamic Programming

Introduction to sequence alignment

- In molecular biology, a common question is to ask whether or not two sequences are related.
- The most common way to tell whether or not they are related is to compare them to one another to see if they are similar.
- Question:
  - Are two sequences related?
- Compare the two sequences,
  - see if they are similar

Sequence Alignment

- Sequence Alignment
  - the identification of residue-residue correspondences.
- It is the basic tool of bioinformatics.
- Example:
  - **pear** and **tear**
- Similar words, different meanings

Biological Sequences

- Similar biological sequences tend to be related
- Information:
  - Functional
  - Structural
  - Evolutionary
- Common mistake:
  - **sequence similarity is not homology!**
- Homologous sequences:
  - derived from a common ancestor

Relation of sequences

- **Homologs**:
  - similar sequences in 2 different organisms derived from a common ancestor sequence.
- **Orthologs**:
  - Similar sequences in 2 different organisms that have arisen due to a speciation event. Functionality Retained.
- **Paralogs**:
  - Similar sequences within a single organism that have arisen due to a gene duplication event.
- **Xenologs**:
  - similar sequences that have arisen out of horizontal transfer events (symbiosis, viruses, etc)

Relation of sequences

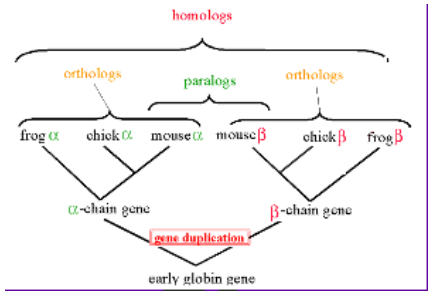

Image Source:
http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/Orthology.html

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Use Protein Sequences for Similarity Searches

- DNA sequences tend to be less informative than protein sequences
- DNA bases vs. 20 amino acids - less chance similarity
- Similarity of AAs can be scored
  - # of mutations, chemical similarity, PAM matrix
- Protein databanks are much smaller than DNA databanks
  - less random matches.
- Similarity is determined by pairwise alignment of different sequences

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Pairwise Alignment

- The alignment of two sequences (DNA or protein) is a relatively straightforward computational problem.
- There are lots of possible alignments.
- Two sequences can always be aligned.
- Sequence alignments have to be scored.
- Often there is more than one solution with the same score.

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Sequence Alignment

The concept

- An alignment is a mutual arrangement of two sequences.
  - Pairwise sequence alignment
- It exhibits where the two sequences are similar, and where they differ.
- An optimal alignment is one that exhibits the most correspondences, and the least differences.
- Sequences that are similar probably have the same function

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Sequence Alignment

Terms of sequence comparison

- Sequence identity
  - exactly the same Amino Acid or Nucleotide in the same position
- Sequence similarity
  - substitutions with similar chemical properties
- Sequence homology
  - general term that indicates evolutionary relatedness among sequences
  - sequences are homologous if they are derived from a common ancestral sequence

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Sequence Alignment

Things to consider:

- to find the best alignment one needs to examine all possible alignments
- to reflect the quality of the possible alignments one needs to score them
- there can be different alignments with the same highest score
- variations in the scoring scheme may change the ranking of alignments

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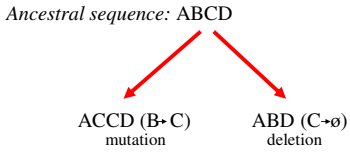
Sequence Alignment

Evolution:



ACCD
AB—D

or

ACCD
A—BD

Pairwise Alignment

true alignment

Sequence Alignment

A protein sequence alignment

```
MSTGAVLIY--TSILIKECHAMPAGNE-----
---GGILLFHRTHELIKESHAMANDEGGSNNS
   *  *     *  **** ***
```

A DNA sequence alignment

```
attcgttggcaaatcgcccctatccggccttaa
att---tggcggatcg-cctctacgggcc----
***    ****  **** **     ******
```


Hamming or edit distance

- Simplest method in determining sequence similarity is to determine the edit distance between two sequences
- If we take the example of pear and tear, how similar are these two words?
- An alignment of these two is as follows:

```
P E A R
  | | |
T E A R
```


Hamming Distance

- Minimum number of letters by which two words differ
- Calculated by summing number of mismatches
- Hamming Distance between PEAR and TEAR is 1

Gapped Alignments

- With biological sequences, it is often necessary to align two sequences that are of
  - different lengths,
  - that have regions that have been inserted or deleted over time.
- Thus, the notion of gaps needs to be introduced.
  - gaps denoted by '-'
- Consider the words alignment and ligament.
  - One alignment of these two words is as follows:

```
A L I G N M E N T
  | | |   | | | |
- L I G A M E N T
```


Possible Residue Alignments

- An alignment can produce one of the following:
  - a match between two characters
  - a mismatch between two characters
    - also called a substitution or mutation
  - a gap in the first sequence
    - which can be thought of as the deletion of a character in the first sequence
  - a gap in the second sequence
    - which can be thought of as the insertion of a character in the first sequence

Alignments

- Consider the following two nucleic acid sequences:
  - ACGGACT and ATCGGATCT.
- The followings are two valid alignments:

```
A - C - G G - A C T
|   |   |       | |
A T C G G A T _ C T
```

```
A T C G G A T C T
|   | | |       | |
A - C G G - A C T
```

- Which alignment is the better alignment?

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Alignment Scoring Scheme

- One way to judge this is to assign
  - a + score for each match,
  - a - score for each mismatch,
  - a - score for each insertion/deletion (indels).
- Possible scoring scheme:
  match: +2 mismatch: -1 indel: –2
  - Alignment 1:
    - 5 * 2 – 1(1) – 4(2) = 10 – 1 – 8 = 1
  - Alignment 2:
    - 6 * 2 – 1(1) – 2 (2) = 12 – 1 – 4 = 7
- Using the above scoring scheme, the 2nd alignment is a better alignment,
  - since it produces a higher alignment score.

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Alignment Methods

- Visual
- Brute Force
- Dynamic Programming
- Word-Based (k tuple)

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Visual Alignments (Dot Plots)

- One of basic techniques for determining the alignment between two sequences is by using a visual alignment known as dot plots.
- Matrix
  - Rows:
    - Characters in one sequence
  - Columns:
    - Characters in second sequence
- Filling
  - Loop through each row;
    - if character in row-column match, fill in the cell
  - Continue until all cells have been examined

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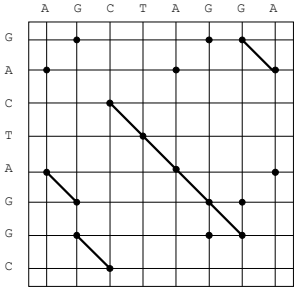
The Dot Matrix

- established in 1970 by A.J. Gibbs and G.A.McIntyre
- method for comparing two amino acid or nucleotide sequences



- each sequence builds one axis of the grid
- one puts a dot, at the intersection of same letters appearing in both sequences
- scan the graph for a series of dots
  - reveals similarity
  - or a string of same characters
- longer sequences can also be compared on a single page, by using smaller dots

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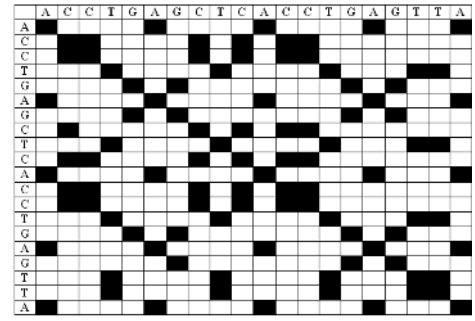
Example Dot Plot



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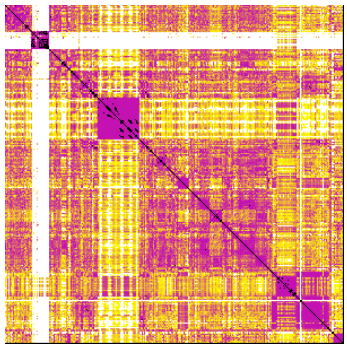
An entire software module of a telecommunications switch; about two million lines of C



- Darker areas indicate regions with a lot of matches
  - a high degree of similarity
- Lighter areas indicate regions with few matches
  - a low degree of similarity
- Dark areas along the main diagonal indicate sub-modules.
- Dark areas off the main diagonal indicate a degree of similarity between sub-modules.
- The largest dark squares are formed by redundancies in initializations of signal-tables and finite-state machines.

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Information within Dot Plots

- Dot plots are useful as a first-level filter for determining an alignment between two sequences.
  - It reveals the presence of insertions or deletions
- Comparing a single sequence to itself can reveal the presence of a repeat of a subsequence
  - Inverted repeats = reverse complement
    - Used to determine folding of RNA molecules
- Self comparison can reveal several features:
  - similarity between chromosomes
  - tandem genes
  - repeated domains in a protein sequence
  - regions of low sequence complexity (same characters are often repeated)

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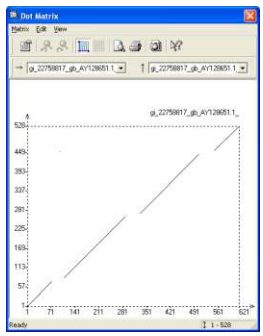
Insertions/Deletions



- Regions containing insertions/deletions can be readily determined.
- One potential application is to determine the number of coding regions (exons) contained within a processed mRNA.

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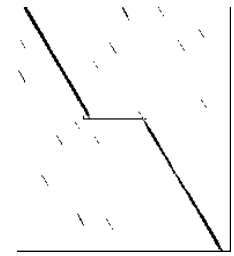
Insertions/Deletions

Two similar, but not identical, sequences

An indel (insertion or deletion):



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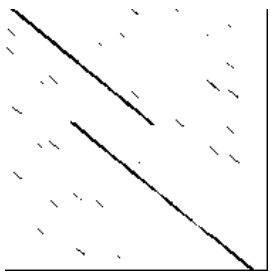
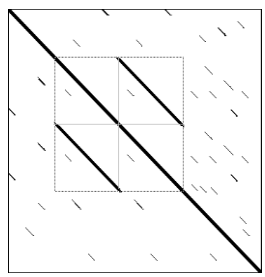
Duplication

A tandem duplication:



Self-dotplot of a tandem duplication:



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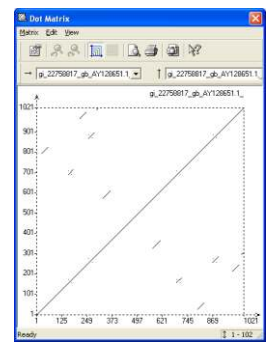
Repeats/Inverted Repeats



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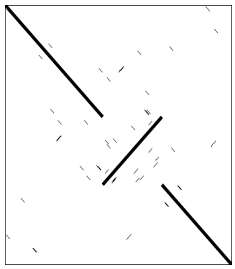
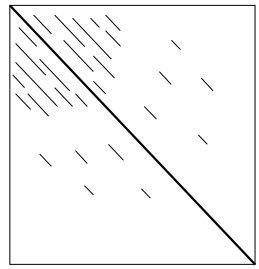
Repeats/Inverted Repeats

An inversion:

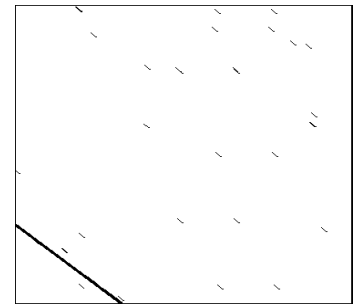


Self dot plot with repeats:



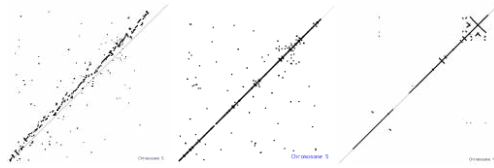
The Dot Matrix

Joining sequences:



Comparing Genome Assemblies

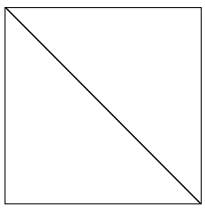
- Dot plots can also be used in order to compare two different assemblies of the same sequence.
- Below are three dotplots of various chromosomes.



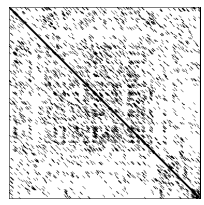
- The 1st shows two separate assemblies of human chromosome 5 compared against each other.
- The 2nd shows one assembly of chromosome 5 compared against itself, indicating the presence of repetitive regions.
- The 3rd shows chromosome Y compared against itself, indicating the presence of inverted repeats.

Noise in Dot Plots

The very stringent, self-dotplot:



The non-stringent self-dotplot:

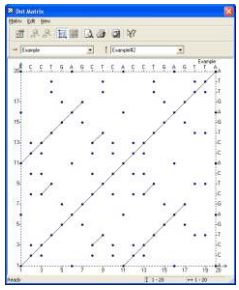


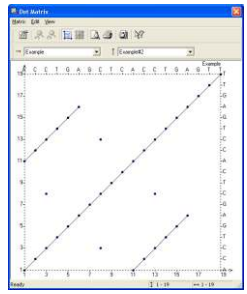
- Stringency is the quality or state of being stringent.
- stringent: marked by rigor, strictness, or severity especially with regard to rule or standard
- Stringency is a situation in which a law, test, etc. is extremely severe or limiting and must be obeyed

Noise in Dot Plots

- Nucleic Acids (DNA, RNA)
  - 1 out of 4 bases matches at random
- To filter out random matches,
  - sliding windows are used
  - Percentage of bases matching in the window is set as threshold
    - A dot is printed only if a minimal number of matches occur
- Rule of thumb:
  - larger windows for DNAs (only 4 bases, more random matches)
  - typical window size is 15 and stringency of 10

Reduction of Dot Plot Noise





Self alignment of ACCTGAGCTCACCTGAGTTA

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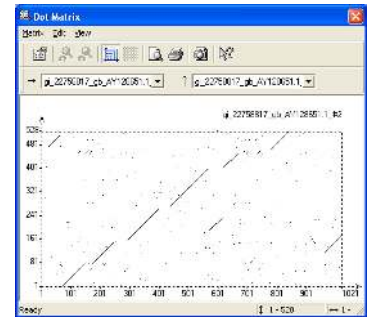
Available Dot Plot Programs

- Vector NTI software package (under AlignX)



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Available Dot Plot Programs

- Vector NTI software package (under AlignX)

GCG software package:

- Compare http://www.hku.hk/bruhk/gcgdoc/compare.html
- DotPlot+ http://www.hku.hk/bruhk/gcgdoc/dotplot.html
- http://www.isrec.isb-sib.ch/java/dotlet/Dotlet.html
- http://bioweb.pasteur.fr/cgi-bin/seqanal/dottup.pl
- Dotter (http://www.cgr.ki.se/cgr/groups/sonnhammer/Dotter.html)

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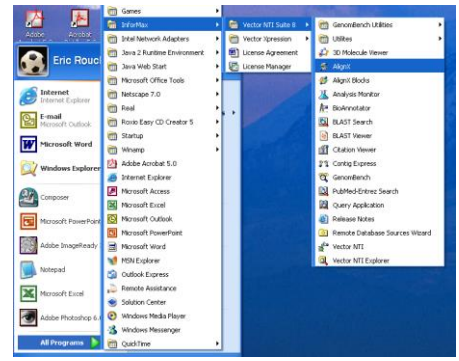
Available Dot Plot Programs



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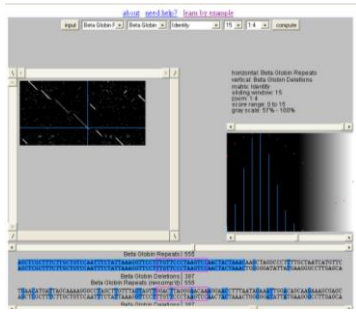
Available Dot Plot Programs

Dotlet (Java Applet) http://www.isrec.isb-sib.ch/java/dotlet/Dotlet.html



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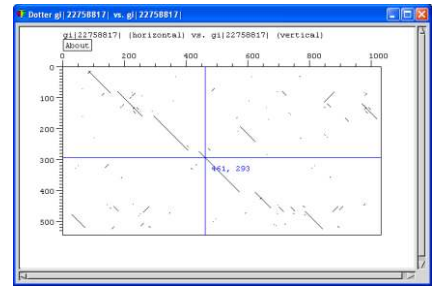
Available Dot Plot Programs

Dotter (http://www.cgr.ki.se/cgr/groups/sonnhammer/Dotter.html)



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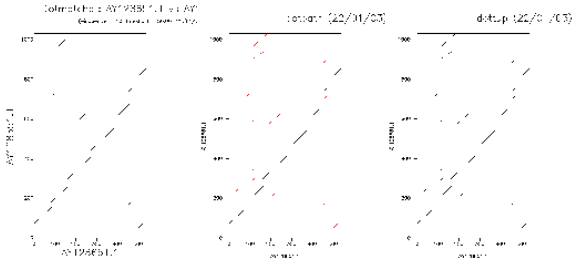
Available Dot Plot Programs

EMBOSS DotMatcher, DotPath,DotUp



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The Dot Plot (Dot Matrix)

- When to use the Dot Plot method?
  - unless the sequences are known to be very much alike
- limits of the Dot Matrix
  - doesn't readily resolve similarity that is interrupted by insertion or deletions
  - Difficult to find the best possible alignment (optimal alignment)
  - most computer programs don't show an actual alignment

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Dot Plot References

Gibbs, A. J. & McIntyre, G. A. (1970).
The diagram method for comparing sequences. its use with amino acid and nucleotide sequences.
Eur. J. Biochem. **16**, 1-11.

Staden, R. (1982).
An interactive graphics program for comparing and aligning nucleic-acid and amino-acid sequences.
Nucl. Acid. Res. **10** (9), 2951-2961.

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Next step

- We must define quantitative measures of sequence similarity and difference!
  - Hamming distance:
    - # of positions with mismatching characters

      AGTC
      CGTA Hamming distance = 2
  - Levenshtein (or edit) distance:
    - # of operations required to change one string into the other (deletion, insertion, substitution)

      AG-TCC
      CGCTCA Levenshtein distance = 3

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Scoring

- +1 for a match -1 for a mismatch?
- should gaps be allowed?
  - if yes how should they be scored?
- what is the best algorithm for finding the optimal alignment of two sequences?
- is the produced alignment significant?

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Determining Optimal Alignment

- Two sequences: X and Y
  - |X| = m; |Y| = n
  - Allowing gaps, |X| = |Y| = m+n
- Brute Force
- Dynamic Programming

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Brute Force

- Determine all possible subsequences for X and Y
  - 2^{m+n} subsequences for X, 2^{m+n} for Y!
- Alignment comparisons
  - $2^{m+n} * 2^{m+n} = 2^{(2(m+n))} = 4^{m+n}$ comparisons
- Quickly becomes impractical

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Dynamic Programming

- Used in Computer Science
- Solve optimization problems by dividing the problem into independent subproblems
- Sequence alignment has optimal substructure property
  - Subproblem: alignment of prefixes of two sequences
  - Each subproblem is computed once and stored in a matrix

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Dynamic Programming

- Optimal score:
  - built upon optimal alignment computed to that point
- Aligns two sequences beginning at ends, attempting to align all possible pairs of characters

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Dynamic Programming

- Scoring scheme for matches, mismatches, gaps
- Highest set of scores defines optimal alignment between sequences
- Match score: DNA – exact match; Amino Acids – mutation probabilities
- Guaranteed to provide optimal alignment given:
  - Two sequences
  - Scoring scheme

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Steps in Dynamic Programming

- Initialization
- Matrix Fill (scoring)
- Traceback (alignment)

DP Example:
Sequence #1: GAATTCAGTTA; M = 11
Sequence #2: GGATCGA; N = 7

- $s(a_i b_j) = +5$ if $a_i = b_j$ (match score)
- $s(a_i b_j) = -3$ if $a_i \neq b_j$ (mismatch score)
- $w = -4$ (gap penalty)

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View of the DP Matrix

- M+1 rows, N+1 columns

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

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Global Alignment (Needleman-Wunsch)

- Attempts to align all residues of two sequences
- ***INITIALIZATION***: First row and first column set
- $S_{i,0} = w * i$
- $S_{0,j} = w * j$

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Initialized Matrix(Needleman-Wunsch)

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	-4	-8	-12	-16	-20	-24	-28	-32	-36	-40	-44
G	-4											
G	-8											
A	-12											
T	-16											
C	-20											
G	-24											
A	-28											

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Matrix Fill (Global Alignment)

$S_{i,j}$ = MAXIMUM[

$S_{i-1, j-1} + s(a_i, b_j)$ (match/mismatch in the diagonal),

$S_{i,j-1} + w$ (gap in sequence #1),

$S_{i-1,j} + w$ (gap in sequence #2)

]

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Matrix Fill (Global Alignment)

- $S_{1,1} = MAX[S_{0,0} + 5, S_{1,0} - 4, S_{0,1} - 4] = MAX[5, -8, -8]$

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	-4	-8	-12	-16	-20	-24	-28	-32	-36	-40	-44
G	-4	5										
G	-8											
A	-12											
T	-16											
C	-20											
G	-24											
A	-28											

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Matrix Fill (Global Alignment)

- $S_{1,2} = MAX[S_{0,1} - 3, S_{1,1} - 4, S_{0,2} - 4] = MAX[-4 - 3, 5 - 4, -8 - 4] = MAX[-7, 1, -12] = 1$

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	-4	-8	-12	-16	-20	-24	-28	-32	-36	-40	-44
G	-4	5	1									
G	-8											
A	-12											
T	-16											
C	-20											
G	-24											
A	-28											

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Matrix Fill (Global Alignment)

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	-4	-8	-12	-16	-20	-24	-28	-32	-36	-40	-44
G	-4	5	1	-3	-7	-11	-15	-19	-23	-27	-31	-35
G	-8											
A	-12											
T	-16											
C	-20											
G	-24											
A	-28											

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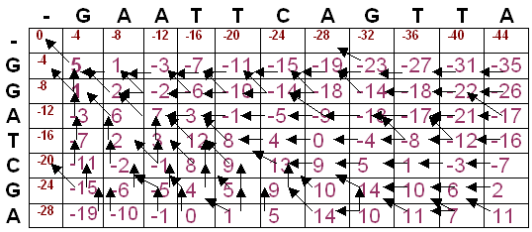
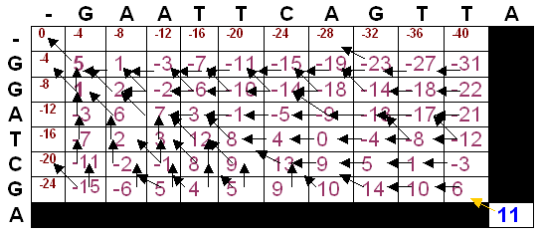
Filled Matrix (Global Alignment)



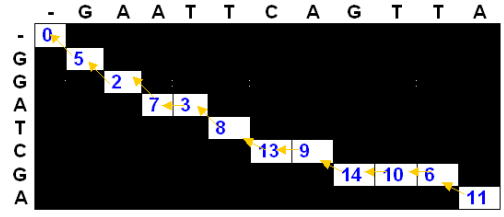
Trace Back (Global Alignment)

- maximum global alignment score = 11 (value in the lower right hand cell).
-
- Traceback begins in position $S_{M,N}$; i.e. the position where both sequences are globally aligned.
-
- At each cell, we look to see where we move next according to the pointers.

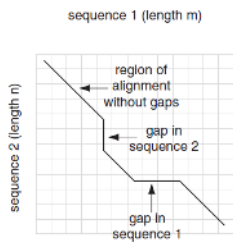
Trace Back (Global Alignment)



Global Trace Back



G A A T T C A G T T A
| | | | | | |
G G A – T C – G – – A



Checking Alignment Score

G A A T T C A G T T A
| | | | | | |
G G A – T C – G – – A

\+ – + – + + – + – – +
5 3 5 4 5 5 4 5 4 4 5

5 – 3 + 5 – 4 + 5 + 5 – 4 + 5 – 4 – 4 + 5 = 11 ✓

Local Alignment

- Smith-Waterman: obtain highest scoring local match between two sequences
- Requires 2 modifications:
  - Negative scores for mismatches
  - When a value in the score matrix becomes negative, reset it to zero (begin of new alignment)

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Local Alignment Initialization

- Values in row 0 and column 0 set to 0.

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	0	0	0	0	0	0	0	0	0	0	0
G	0											
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

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Matrix Fill (Local Alignment)

$S_{i,j}$ = MAXIMUM [

$S_{i-1, j-1} + s(a_i, b_j)$ (match/mismatch in the diagonal),

$S_{i,j-1} + w$ (gap in sequence #1),

$S_{i-1,j} + w$ (gap in sequence #2),

0]

69

Matrix Fill (Local Alignment)

$S_{1,1} = MAX[S_{0,0} + 5, S_{1,0} - 4, S_{0,1} - 4, 0] = MAX[5, -4, -4, 0] = 5$

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5										
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

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Matrix Fill (Local Alignment)

$S_{1,2} = MAX[S_{0,1} - 3, S_{1,1} - 4, S_{0,2} - 4, 0] = MAX[0 - 3, 5 - 4, 0 - 4, 0] = MAX[-3, 1, -4, 0] = 1$

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	1									
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

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Matrix Fill (Local Alignment)

$S_{1,3} = MAX[S_{0,2} - 3, S_{1,2} - 4, S_{0,3} - 4, 0] = MAX[0 - 3, 1 - 4, 0 - 4, 0] = MAX[-3, -3, -4, 0] = 0$

	-	G	A	A	T	T	C	A	G	T	T	A
-	0	0	0	0	0	0	0	0	0	0	0	0
G	0	5	1	0								
G	0											
A	0											
T	0											
C	0											
G	0											
A	0											

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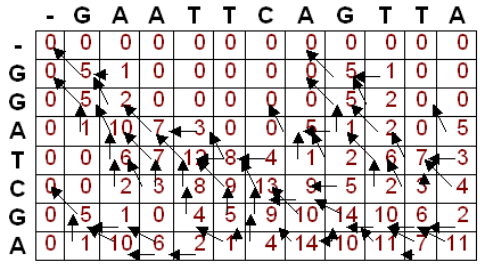
Filled Matrix (Local Alignment)



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Trace Back (Local Alignment)

- maximum local alignment score for the two sequences is 14
- found by locating the highest values in the score matrix
- 14 is found in two separate cells, indicating multiple alignments producing the maximal alignment score

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Trace Back (Local Alignment)

- Traceback begins in the position with the highest value.
- At each cell, we look to see where we move next according to the pointers
- When a cell is reached where there is not a pointer to a previous cell, we have reached the beginning of the alignment

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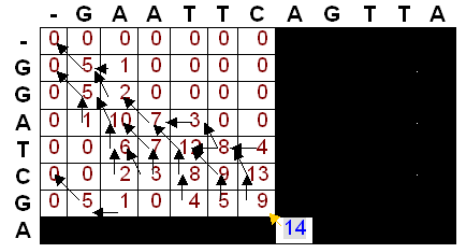
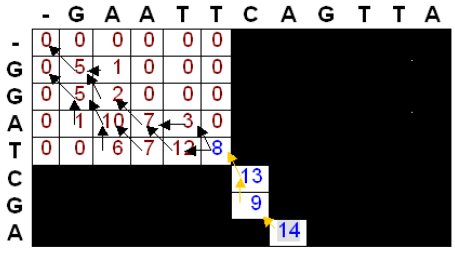
Trace Back (Local Alignment)



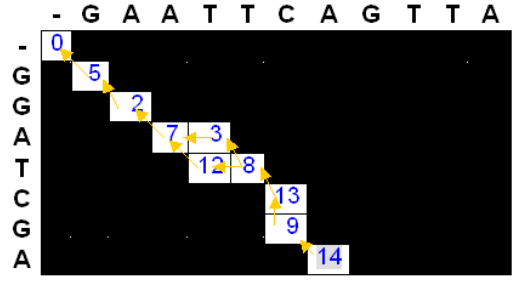
76

Trace Back (Local Alignment)



77

Trace Back (Local Alignment)



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Maximum Local Alignment

```
G A A T T C - A     G A A T T C - A
|   | |   |   |     |   |   | |   |
G G A T - C G A     G G A - T C G A

+ - + + - + - +     + - + - + + - +
5 3 5 5 4 5 4 5     5 3 5 4 5 5 4 5
```


Overlap Alignment

Consider the following problem:

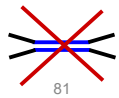
- Find the most significant overlap between two sequences?
- Possible overlap relations:

a.

b.

Difference from local alignment:

Here we require alignment between the endpoints of the two sequences.



Overlap Alignment

Initialization: $S_{i,0} = 0$, $S_{0,j} = 0$

Recurrence: as in global alignment

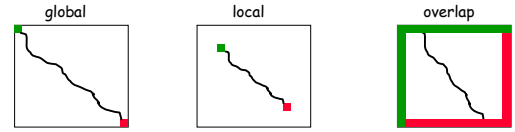
$S_{i,j}$ = **MAXIMUM** [

$S_{i-1, j-1} + s(a_i, b_j)$ (match/mismatch in the diagonal),

$S_{i,j-1} + w$ (gap in sequence #1),

$S_{i-1,j} + w$ (gap in sequence #2)]

Score: maximum value at the bottom line and rightmost line



Overlap Alignment - example

PAWHEAE
HEAGAWGHEE

Scoring scheme :
Match: +4
Mismatch: -1
Gap penalty: -5

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0										
A	0										
W	0										
H	0										
E	0										
A	0										
E	0										

Overlap Alignment

PAWHEAE
HEAGAWGHEE

Scoring scheme :
Match: +4
Mismatch: -1
Gap penalty: -5

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
A	0	-1									
W	0	-1									
H	0	4									
E	0	-1									
A	0	-1									
E	0	-1									

Overlap Alignment

PAWHEAE
HEAGAWGHEE

Scoring scheme:
Match: +4
Mismatch: -1
Gap penalty: -5

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
A	0	-1	-2	3	-2	3	-2	-2	-2	-2	-2
W	0	-1	-2	-2	2	-2	7	2	-3	-3	-1
H	0	4	-1	-3	-3	1	2	6	6	1	-2
E	0	-1	8	3	-2	-3	0	1	5	10	5
A	0	-1	3	12	7	2	-2	-1	0	5	9
E	0	-1	3	7	11	6	1	-3	-2	4	9

Overlap Alignment

The best overlap is:

P A W H E A E - - - - - -
- - - H E A G A W G H E E

Pay attention!
A different scoring scheme could yield a different result, such as:

Scoring scheme :
Match: +4
Mismatch: -1
Gap penalty: -2

- - - P A W- H E A E
H E A G A W G H E E -

Sequence Alignment Variants

- Global alignment (The Needelman-Wunsch Algorithm)
  - Initialization: $S_{i,0} = i*w$, $S_{0,j} = j*w$
  - Score: $S_{i,j} = MAX [S_{i-1, j-1} + s(a_i, b_j), S_{i,j-1} + w, S_{i-1,j} + w]$
- Local alignment (The Smith-Waterman Algorithm)
  - Initialization: $S_{i,0} = 0$, $S_{0,j} = 0$
  - Score: $S_{i,j} = MAX [S_{i-1, j-1} + s(a_i, b_j), S_{i,j-1} + w, S_{i-1,j} + w, 0]$
- Overlap alignment
  - Initialization: $S_{i,0} = 0$, $S_{0,j} = 0$
  - Score: $S_{i,j} = MAX [S_{i-1, j-1} + s(a_i, b_j), S_{i,j-1} + w, S_{i-1,j} + w]$

Scoring Matrices

- match/mismatch score
  - Not bad for similar sequences
  - Does not show distantly related sequences
- Likelihood matrix
  - Scores residues dependent upon likelihood substitution is found in nature
  - More applicable for amino acid sequences

Parameters of Sequence Alignment

Scoring Systems:

- Each symbol pairing is assigned a numerical value, based on a symbol comparison table.

Gap Penalties:

- Opening: The cost to introduce a gap
- Extension: The cost to elongate a gap

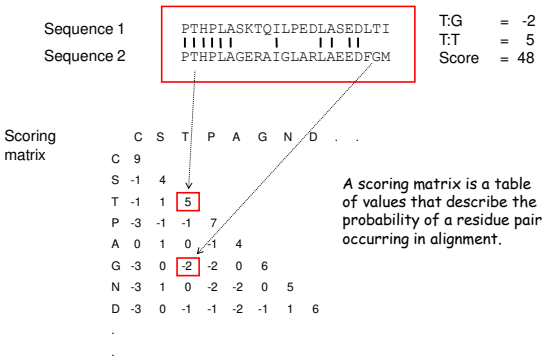
DNA Scoring Systems -very simple

Sequence 1 actaccagttcatttgatacttctcaaa
Sequence 2 taccattaccgtgttaactgaaaggacttaaagact

	A	G	C	T
A	1	0	0	0
G	0	1	0	0
C	0	0	1	0
T	0	0	0	1

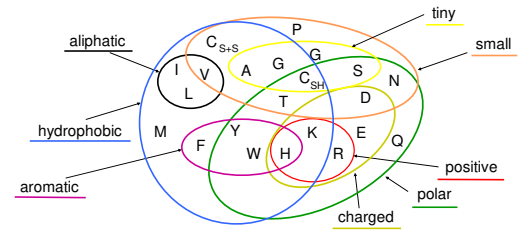
Match: 1
Mismatch: 0
Score = 5

Protein Scoring Systems



Protein Scoring Systems

- Amino acids have different biochemical and physical properties that influence their relative replaceability in evolution.

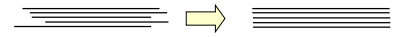


Protein Scoring Systems

- Scoring matrices reflect:
  - # of mutations to convert one to another
  - chemical similarity
  - observed mutation frequencies
- Log odds matrices:
  - the values are logarithms of probability ratios of the probability of an aligned pair to the probability of a random alignment.
- Widely used scoring matrices:
  - PAM
  - BLOSUM

PAM matrices (Percent Accepted Mutations)

- Derived from global alignments of protein families . Family members share at least 85% identity (Dayhoff *et al.*, 1978).

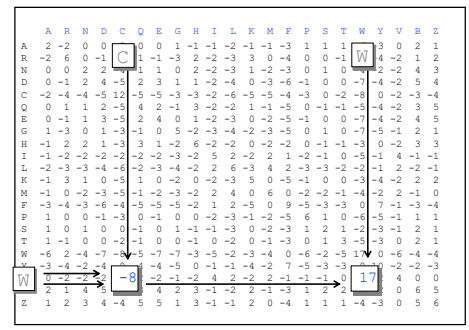


- Construction of phylogenetic tree and ancestral sequences of each protein family
- Computation of number of replacements for each pair of amino acids

PAM matrices

- The numbers of replacements were used to compute a so-called PAM-1 matrix.
- The PAM-1 matrix reflects an average change of 1% of all amino acid positions.
- PAM matrices for larger evolutionary distances can be extrapolated from the PAM-1 matrix by multiplication.
- PAM250 = 250 mutations per 100 residues.
- Greater numbers mean bigger evolutionary distance

PAM 250



A value of 0 indicates the frequency of alignment is random

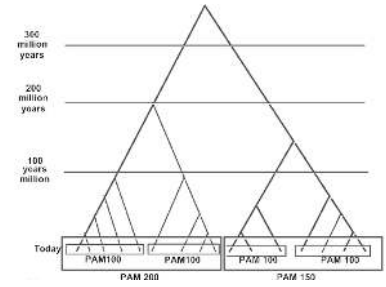
log(freq(observed)/freq(expected))

Amino Acid Frequency

freq(expected) = $f(AA_i)*f(AA_j)$

	1978	1991
L	0.085	0.091
A	0.087	0.077
G	0.089	0.074
S	0.070	0.069
V	0.065	0.066
E	0.050	0.062
T	0.058	0.059
K	0.081	0.059
I	0.037	0.053
D	0.047	0.052
R	0.041	0.051
P	0.051	0.051
N	0.040	0.043
Q	0.038	0.041
F	0.040	0.040
Y	0.030	0.032
M	0.015	0.024
H	0.034	0.023
C	0.033	0.020
W	0.010	0.014

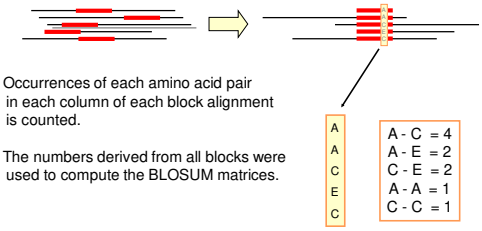
Use Different PAM's for Different Evolutionary Distances



(Adapted from D Brutlag, Stanford)

BLOSUM (Blocks Substitution Matrix)

- Derived from alignments of domains of distantly related proteins (Henikoff & Henikoff,1992).



- Occurrences of each amino acid pair in each column of each block alignment is counted.
- The numbers derived from all blocks were used to compute the BLOSUM matrices.

A
A
C
E
C

A - C = 4
A - E = 2
C - E = 2
A - A = 1
C - C = 1

BLOSUM (Blocks Substitution Matrix)

- Sequences within blocks are clustered according to their level of identity.
- Clusters are counted as a single sequence.
- Different BLOSUM matrices differ in the percentage of sequence identity used in clustering.
- The number in the matrix name (e.g. 62 in BLOSUM62) refers to the percentage of sequence identity used to build the matrix.
- Greater numbers mean smaller evolutionary distance.

TIPS on choosing a scoring matrix

- Generally, BLOSUM matrices perform better than PAM matrices for local similarity searches (Henikoff & Henikoff, 1993).
- When comparing closely related proteins one should use lower PAM or higher BLOSUM matrices,
- For distantly related proteins higher PAM or lower BLOSUM matrices.
- For database searching the commonly used matrix is BLOSUM62.

Nucleic Acid Scoring Scheme

- Transition mutation (more common)
  - purine ⟷ purine A ⟷ G
  - pyrimidine ⟷ pyrimidine T ⟷ C
- Transversion mutation
  - purine ⟷ pyrimidine A, G ⟷ T, C

	A	G	T	C
A	20	10	5	5
G	10	20	5	5
T	5	5	20	10
C	5	5	10	20

Amino acid exchange matrices

Amino acids are **not** equal:

1. Some are easily substituted because they have similar:
   - physico-chemical properties
   - structure
2. Some mutations between amino acids occur more often due to similar codons

The two above observations give us ways to define *substitution matrices*

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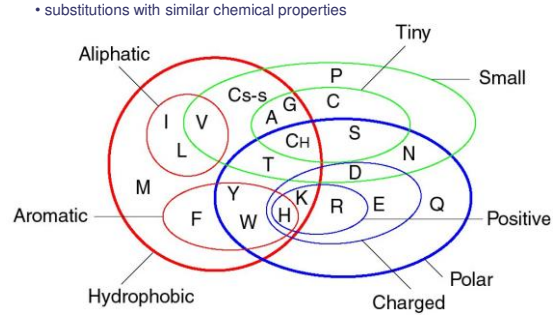
Properties of Amino Acids

Sequence similarity

- substitutions with similar chemical properties



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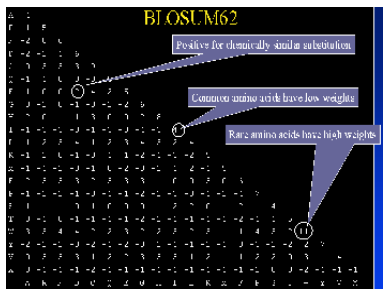
Scoring Matrices

- table of values that describe the probability of a residue pair occurring in an alignment
- the values are logarithms of ratios of two probabilities
  1. probability of random occurrence of an amino acid (diagonal)
  2. probability of meaningful occurrence of a pair of residues



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Scoring Matrices

Widely used matrices

- **PAM** (Percent Accepted Mutation) **/ MDM** (Mutation Data Matrix) **/ Dayhoff**
  - Derived from *global* alignments of *closely* related sequences.
  - Matrices for greater evolutionary distances are extrapolated from those for lesser ones.
  - The number with the matrix (PAM40, PAM100) refers to the evolutionary distance; greater numbers are greater distances.
  - PAM-1 corresponds to about 1 million years of evolution
  - for distant (global) alignments, Blosum50, Gonnet, or (still) PAM250
- **BLOSUM** (Blocks Substitution Matrix)
  - Derived from *local, ungapped alignments of distantly related sequences*
  - *All matrices are directly calculated; no extrapolations are used*
  - *The number after the matrix (BLOSUM62) refers to the minimum percent identity of the blocks used to construct the matrix; greater numbers are lesser distances.*
  - *The BLOSUM series of matrices generally perform better than PAM matrices for local similarity searches.*
  - For local alignment, Blosum 62 is often superior
- ***Structure-based matrices***
- ***Specialized Matrices***

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Scoring Matrices

The relationship between BLOSUM and PAM substitution matrices

- BLOSUM matrices with higher numbers and PAM matrices with low numbers are designed for comparisons of closely related sequences.
- BLOSUM matrices with low numbers and PAM matrices with high numbers are designed for comparisons of distantly related proteins.

BLOSUM 80	BLOSUM 62	BLOSUM 45
PAM 1	PAM 120	PAM 250
Less divergent	← →	*More divergent*

http://www.ncbi.nlm.nih.gov/Education/BLASTinfo/Scoring2.html

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Percent Accepted Mutation (PAM or Dayhoff) Matrices

- Studied by Margaret Dayhoff
- Amino acid substitutions
  - Alignment of common protein sequences
  - 1572 amino acid substitutions
  - 71 groups of protein, 85% similar
- "Accepted" mutations – do not negatively affect a protein's fitness
- Similar sequences organized into phylogenetic trees
- Number of amino acid changes counted
- Relative mutabilities evaluated
- 20 x 20 amino acid substitution matrix calculated

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Percent Accepted Mutation (PAM or Dayhoff) Matrices

- PAM 1: 1 accepted mutation event per 100 amino acids; PAM 250: 250 mutation events per 100 …
- PAM 1 matrix can be multiplied by itself N times to give transition matrices for sequences that have undergone N mutations
- PAM 250: 20% similar; PAM 120: 40%; PAM 80: 50%; PAM 60: 60%

PAM1 matrix

normalized probabilities multiplied by 10000

	Ala A	Arg R	Asn N	Asp D	Cys C	Gln Q	Glu E	Gly G	His H	Ile I	Leu L	Lys K	Met M	Phe F	Pro P	Ser S	Thr T	Trp W	Tyr Y	Val V
A	9867	2	9	10	3	8	17	21	2	6	4	2	6	2	22	35	32	0	2	18
R	1	9913	1	0	1	10	0	0	10	3	1	19	4	1	4	6	1	8	0	1
N	4	1	9822	36	0	4	6	6	21	3	1	13	0	1	2	20	9	1	4	1
D	6	0	42	9859	0	6	53	6	4	1	0	3	0	0	1	5	3	0	0	1
C	1	1	0	0	9973	0	0	0	1	1	0	0	0	0	1	5	1	0	3	2
Q	3	9	4	5	0	9876	27	1	23	1	3	6	4	0	6	2	2	0	0	1
E	10	0	7	56	0	35	9865	4	2	3	1	4	1	0	3	4	2	0	1	2
G	21	1	12	11	1	3	7	9935	1	0	1	2	1	1	3	21	3	0	0	5
H	1	8	18	3	1	20	1	0	9912	0	1	1	0	2	3	1	1	1	4	1
I	2	2	3	1	2	1	2	0	0	9872	9	2	12	7	0	1	7	0	1	33
L	3	1	3	0	0	6	1	1	4	22	9947	2	45	13	3	1	3	4	2	15
K	2	37	25	6	0	12	7	2	2	4	1	9926	20	0	3	8	11	0	1	1
M	1	1	0	0	0	2	0	0	0	5	8	4	9874	1	0	1	2	0	0	4
F	1	1	1	0	0	0	0	1	2	8	6	0	4	9946	0	2	1	3	28	0
P	13	5	2	1	1	8	3	2	5	1	2	2	1	1	9926	12	4	0	0	2
S	28	11	34	7	11	4	6	16	2	2	1	7	4	3	17	9840	38	5	2	2
T	22	2	13	4	1	3	2	2	1	11	2	8	6	1	5	32	9871	0	2	9
W	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	9976	1	0
Y	1	0	3	0	3	0	1	0	4	1	1	0	0	21	0	1	1	2	9945	1
V	13	2	1	1	3	2	2	3	3	57	11	1	17	1	3	2	10	0	2	9901

Log Odds Matrices

- PAM matrices converted to log-odds matrix
  - Calculate odds ratio for each substitution
    - Taking scores in previous matrix
    - Divide by frequency of amino acid
  - Convert ratio to log10 and multiply by 10
  - Take average of log odds ratio for converting A to B and converting B to A
  - Result: Symmetric matrix
  - EXAMPLE: Mount pp. 80-81

PAM250 Log odds matrix

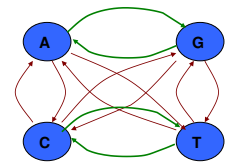
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	12																				C
S	0	2																			S
T	-2	1	3																		T
P	-3	1	0	6																	P
A	-2	1	1	1	2																A
G	-3	1	0	-1	1	5															G
N	-4	1	0	-1	0	0	2														N
D	-5	0	0	-1	0	1	2	4													D
E	-5	0	0	-1	0	0	1	3	4												E
Q	-5	-1	-1	0	0	-1	1	2	2	4											Q
H	-3	-1	-1	0	-1	-2	2	1	1	3	6										H
R	-4	0	-1	0	-2	-3	0	-1	-1	1	2	6									R
K	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5								K
M	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6							M
I	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	5						I
L	-6	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	6					L
V	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4				V
F	-4	-3	-3	-5	-4	-5	-4	-6	-5	-5	-2	-4	-5	0	1	2	-1	9			F
Y	0	-3	-2	-5	-3	-5	-2	-4	-4	-4	0	-4	-4	-2	-1	-1	-2	7	10		Y
W	-8	-2	-5	-6	-6	-7	-4	-7	-7	-5	-3	2	-3	-4	-5	-2	-6	0	0	17	W
	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	

Blocks Amino Acid Substitution Matrices (BLOSUM)

- Larger set of sequences considered
- Sequences organized into signature blocks
- Consensus sequence formed
  - 60% identical: BLOSUM 60
  - 80% identical: BLOSUM 80

DNA Mutations

In addition to using a match/mismatch scoring scheme for DNA sequences, nucleotide mutation matrices can be constructed as well. These matrices are based upon two different models of nucleotide evolution: the first, the Jukes-Cantor model, assumes there are uniform mutation rates among nucleotides, while the second, the Kimura model, assumes that there are two separate mutation rates: one for transitions (where the structure of purine/pyrimidine stays the same), and one for transversions. Generally, the rate of transitions is thought to be higher than the rate of transversions.



PURINES: A, G
PYRIMIDINES C, T

Transitions: A↔G; C↔T

Transversions: A↔C, A↔T, C↔G, G↔T

Nucleic Acid Scoring Matrices

- Two mutation models:
  - Jukes-Cantor Model of evolution: α = common rate of base substitution
  - Kimura Model of Evolution: α = rate of transitions; β = rate of transversions
    - Transitions
    - Transversions

$$R = \begin{array}{c} \\ A \\ C \\ G \\ U \end{array} \begin{array}{c} \begin{array}{cccc} A & C & G & U \end{array} \\ \begin{pmatrix} * & 0.25\alpha & 0.25\alpha & 0.25\alpha \\ 0.25\alpha & * & 0.25\alpha & 0.25\alpha \\ 0.25\alpha & 0.25\alpha & * & 0.25\alpha \\ 0.25\alpha & 0.25\alpha & 0.25\alpha & * \end{pmatrix} \end{array}$$

$$R = \begin{array}{c} \\ A \\ C \\ G \\ U \end{array} \begin{array}{c} \begin{array}{cccc} A & C & G & U \end{array} \\ \begin{pmatrix} * & 0.25\beta & 0.25\alpha & 0.25\beta \\ 0.25\beta & * & 0.25\beta & 0.25\alpha \\ 0.25\alpha & 0.25\beta & * & 0.25\beta \\ 0.25\beta & 0.25\alpha & 0.25\beta & * \end{pmatrix} \end{array}$$

Nucleotide substitution matrices with the equivalent distance of 1 PAM

A. Model of uniform mutation rates among nucleotides.

	A	G	T	C
A	0.99			
G	0.00333	0.99		
T	0.00333	0.00333	0.99	
C	0.00333	0.00333	0.00333	0.99

B. Model of 3-fold higher transitions than transversions.

	A	G	T	C
A	0.99			
G	0.006	0.99		
T	0.002	0.002	0.99	
C	0.002	0.002	0.006	0.99

Nucleotide substitution matrices with the equivalent distance of 1 PAM

A. Model of uniform mutation rates among nucleotides.

	A	G	T	C
A	2			
G	-6	2		
T	-6	-6	2	
C	-6	-6	-6	2

B. Model of 3-fold higher transitions than transversions.

	A	G	T	C
A	2			
G	-5	2		
T	-7	-7	2	
C	-7	-7	-5	2

Linear vs. Affine Gaps

- The scoring matrices used to this point assume a linear gap penalty where each gap is given the same penalty score.
- However, over evolutionary time, it is more likely that a contiguous block of residues has become inserted/deleted in a certain region (for example, it is more likely to have 1 gap of length k than k gaps of length 1).
- Therefore, a better scoring scheme to use is an initial higher penalty for opening a gap, and a smaller penalty for extending the gap.

Linear vs. Affine Gaps

- Gaps have been modeled as linear
- More likely contiguous block of residues inserted or deleted
  - 1 gap of length k rather than k gaps of length 1
- Scoring scheme should penalize new gaps more

Affine Gap Penalty

- $w_x = g + r(x\text{-}1)$
- w_x : total gap penalty; g: gap open penalty; r: gap extend penalty; x: gap length
-
- gap penalty chosen relative to score matrix
  - Gaps not excluded
  - Gaps not over included
  - Typical Values: g = -12; r = -4

Affine Gap Penalty and Dynamic Programming

$$M_{i,j} = \max \{ D_{i-1,j-1} + \text{subst}(A_i, B_j) , M_{i-1,j-1} + \text{subst}(A_i, B_j) , I_{i-1,j-1} + \text{subst}(A_i, B_j) \}$$

$$D_{i,j} = \max \{ D_{i,j-1} - \text{extend}, \quad M_{i,j-1} - \text{open} \}$$

$$I_{i,j} = \max \{ M_{i-1,j} - \text{open}, \quad I_{i-1,j} - \text{extend} \}$$

where M is the match matrix, D is delete matrix, and I is insert matrix

Drawbacks to DP Approaches

- Dynamic programming approaches are guaranteed to give the optimal alignment between two sequences given a scoring scheme.
- However, the two main drawbacks to DP approaches is that they are compute and memory intensive, in the cases discussed to this point taking at least $O(n^2)$ space, between $O(n^2)$ and $O(n^3)$ time.
- Linear space algorithms have been used in order to deal with one drawback to dynamic programming. The basic idea is to concentrate only on those areas of the matrix more likely to contain the maximum alignment. The most well-known of these linear space algorithms is the Myers-Miller algorithm. Compute intensive

Alternative DP approaches

- Linear space algorithms Myers-Miller
- Bounded Dynamic Programming

- Ewan Birney's Dynamite Package
  - Automatic generation of DP code

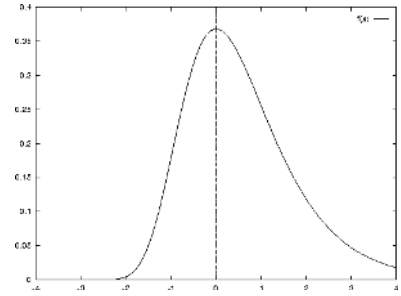
Assessing Significance of Alignment

- When two sequences of length m and n are not obviously similar but show an alignment, it becomes necessary to assess the significance of the alignment. The alignment of scores of random sequences has been shown to follow a Gumbel extreme value distribution.

Significance of Alignment

- Determine probability of alignment occurring at random
  - Sequence 1: length m
  - Sequence 2: length n
- Random sequences:
  - When two sequences of length m and n are not obviously similar but show an alignment, it becomes necessary to assess the significance of the alignment.
  - The alignment of scores of random sequences has been shown to follow a Gumbel extreme value distribution.

Gumbel Extreme Value Distribution



- http://roso.epfl.ch/mbi/papers/discretechoice/node11.html
- http://mathworld.wolfram.com/GumbelDistribution.html
- http://en.wikipedia.org/wiki/Generalized_extreme_value_distribution

Probability of Alignment Score

- Using a Gumbel extreme value distribution, the expected number of alignments with a score at least S (E-value) is:

$$E = Kmn\, e^{-\lambda S}$$

  - m,n: Lengths of sequences
  - *K* ,λ: statistical parameters dependent upon scoring system and background residue frequencies

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- Recall that the log-odds scoring schemes examined to this point normally use a $S = 10*\log_{10}x$ scoring system.
- We can normalize the raw scores obtained using these non-gapped scoring systems to obtain the amount of bits of information contained in a score (or the amount of nats of information contained within a score).

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Converting to Bit Scores

A raw score can be normalized to a bit score using the formula:

$$S' = \frac{\lambda S - \ln K}{\ln 2}$$

- The E-value corresponding to a given bit score can then be calculated as:

$$E = mn\, 2^{-S'}$$

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- Converting to nats is similar. However, we just substitute e for 2 in the above equations. Converting scores to either bits or nats gives a standardized unit by which the scores can be compared.

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P-Value

- P values can be calculated as the probability of obtaining a given score at random. P-values can be estimated as:

$$P = 1 - e^{-E}$$

which is approximately e^{-E}

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A quick determination of significance

- If a scoring matrix has been scaled to bit scores, then it can quickly be determined whether or not an alignment is significant.
- For a typical amino acid scoring matrix, K = 0.1 and lambda depends on the values of the scoring matrix.
- If a PAM or BLOSUM matrix is used, then lambda is precomputed.
- For instance, if the log odds matrix is in units of bits, then lambda = $\log_e 2$, and the significance cutoff can be calculates as $\log_2(mn)$.

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Significance of Ungapped Alignments

- PAM matrices are $10 * \log_{10}x$
- Converting to $\log_2 x$ gives bits of information
- Converting to $\log_e x$ gives nats of information

Quick Calculation:

- If bit scoring system is used, significance cutoff is:

$$\log_2(mn)$$

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Example

- Suppose we have two sequences, each approximately 250 amino acids long that are aligned using a Smith-Waterman approach.
- Significance cutoff is:

  – $\log_2(250 * 250) = 16$ bits

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Example

- Using PAM250, the following alignment is found:

- F W L E V E G N S M T A P T G
- F W L D V Q G D S M T A P A G

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Example

- Using PAM250, the score is calculated:

- F W L E V E G N S M T A P T G
- F W L D V Q G D S M T A P A G

- S = 9 + 17 + 6 + 3 + 4 + 2 + 5 + 2 + 2 + 6 + 3 + 2 + 6 + 1 + 5 = 73

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PAM250 matrix

	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	12																				C
S	0	2																			S
T	−2	1	3																		T
P	−3	1	0	6																	P
A	−2	1	1	1	2																A
G	−3	1	0	−1	1	5															G
N	−4	1	0	−1	0	0	2														N
D	−5	0	0	−1	0	1	2	4													D
E	−5	0	0	−1	0	0	1	3	4												E
Q	−5	−1	−1	0	0	−1	1	2	2	4											Q
H	−3	−1	−1	0	−1	−2	2	1	1	3	6										H
R	−4	0	−1	0	−2	−3	0	−1	−1	1	2	6									R
K	−5	0	0	−1	−1	−2	1	0	0	1	0	3	5								K
M	−5	−2	−1	−2	−1	−3	−2	−3	−2	−1	−2	0	0	6							M
I	−2	−1	0	−2	−1	−3	−2	−2	−2	−2	−2	−2	−2	2	5						I
L	−6	−3	−2	−3	−2	−4	−3	−4	−3	−2	−2	−3	−3	4	2	6					L
V	−2	−1	0	−1	0	−1	−2	−2	−2	−2	−2	−2	−2	2	4	2	4				V
F	−4	−3	−3	−5	−4	−5	−4	−6	−5	−5	−4	−5	−5	0	1	2	−1	9			F
Y	0	−3	−3	−5	−3	−5	−2	−4	−4	−4	0	−4	−4	−2	−1	−1	−2	7	10		Y
W	−8	−2	−5	−6	−6	−7	−4	−7	−7	−5	−3	2	−3	−4	−5	−2	−6	0	0	17	W
		C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	

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Significance Example

- S is in $10 * \log_{10}x$, so this should be converted to a bit score:

- $S = 10 \log_{10}x$
- $S/10 = \log_{10}x$
- $S/10 = \log_{10}x * (\log_2 10/\log_2 10)$
- $S/10 * \log_2 10 = \log_{10}x / \log_2 10$
- $S/10 * \log_2 10 = \log_2 x$
- $1/3\ S \sim \log_2 x$

- $S' \sim 1/3S$

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Significance Example

- S' = 1/3S = 1/3 * 73 = 24.333 bits
- The significance cutoff is:
  log2(mn) = log2(250 * 250) = 16 bits
- Since the alignment score is above the significance cutoff, this is a significant local alignment.

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Estimation of E and P

- When a PAM250 scoring matrix is being used, K is estimated to be 0.09, while lambda is estimated to be 0.229.
- For PAM250, K = 0.09; λ = 0.229
- We can convert the score to a bit score as follows:
- $S' = \lambda S - \ln Kmn$
- S' = 0.229 * 73 – ln 0.09 * 250 * 250
- S' = 16.72 – 8.63 = 8.09 bits
- $P(S' >= x) = 1 - e^{(-e^{-x})}$
- $P(S' >= 8.09) = 1 - e^{(-e^{-8.09})} = 3.1 * 10^{-4}$
- Therefore, we see that the probability of observing an alignment with a bit score greater than 8.09 is about 3 in 1000.

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Significance of Gapped Alignments

- Gapped alignments make use of the same statistics as ungapped alignments in determining the statistical significance.
- However, in gapped alignments, the values for λ and K cannot be easily estimated.
- Empirical estimations and gap scores have been determined by looking at the alignments of randomized sequences.

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Bayesian Statistics

- Bayesian statistics are built upon conditional probabilities,
  - which are used to derive the joint probability of two events or conditions.
- P(B|A) is the probability of B given condition A is true.
- P(B) is the probability of condition B occurring, regardless of conditions A.
- P(A, B): Joint probability of A and B occurring simultaneously

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Bayesian Statistics

- Suppose that A can have two states, A1 and A2, and B can have two states, B1 and B2.
- Suppose that P(B1) = 0.3 is known.
- Therefore, P(B2) = 1 – 0.3 = 0.7.
- These probabilities are known as ***marginal probabilities***.
- Now we would like to determine the probability of A1 and B1 occurring together, which is denoted as: P(A1, B1) and is called the joint probability

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Joint Probabilities

- Note that in this case the marginal probabilities A1 and A2 are missing. Thus, there is not enough information at this point to calculate the marginal probability.
- However, if more information about the joint occurrence of A1 and B1 are given, then the joint probabilities may be derived using Bayes Rule:
  - P(A1,B1) = P(B1)P(A1|B1)
  - P(A1,B1) = P(A1)P(B1|A1)

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Bayesian Example

- Suppose that we are given P(A1|B1) = 0.8.
- Then, since there are only two different possible states for A,
- P(A2|B1) = 1 – 0.8 = 0.2.
- If we are also given P(A2|B2) = 0.7,
- then P(A1|B2) = 0.3.
- Using Bayes Rule, the joint probability of having states A1 and B1 occurring at the same time is
- P(B1)P(A1|B1) = 0.3 * 0.8 = 0.24 and
- P(A2,B2) = P(B2)P(A2|B2) = 0.7 * 0.7 = 0.49.
- The other joint probabilities can be calculated from these as well.

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Posterior Probabilities

- Calculation of joint probabilities results in posterior probabilities
  - Not known initially
  - Calculated using
    - Prior probabilities
    - Initial information

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Applications of Bayesian Statistics

- Evolutionary distance between two sequences
- Sequence Alignment
- Significance of Alignments
- Gibbs Sampling

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Pairwise Sequence Alignment Programs

- needle
  - Global Needleman/Wunsch alignment
- water
  - Local Smith/Waterman alignment
- Blast 2 Sequences
  - NCBI
  - word based sequence alignment
- LALIGN
  - FASTA package
  - Mult. Local alignments

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Various Sequence Alignments

Wise2 -- Genomic to protein

Sim4 -- Aligns expressed DNA to genomic sequence

spidey -- aligns mRNAs to genomic sequence

est2genome -- aligns ESTs to genomic sequence

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