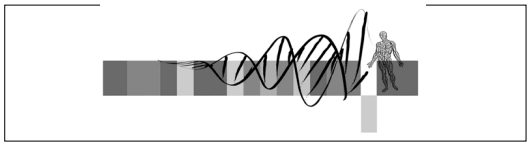

Getting To Know Your Protein

Comparative Protein Analysis:
Part I. Phylogenetic Trees and Multiple Sequence Alignments

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Meeting Your Protein *In Silico*

- Define and characterize your favorite sequence
  - Identify homologous sequences
  - Predict function
  - Examine potential mutations
  - Study in 3D
  - Make manuscript figures :-)

Comparative Protein Analysis

Definition

Use information regarding a group of sequences to determine the function of an undefined sequence.

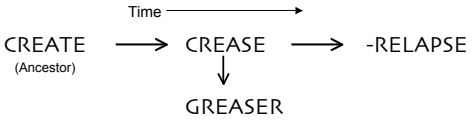
Extract novel information about a protein, or a series of proteins, through comparisons with other, related sequences.

Application

What are they?
What are their functions?
Why are they important?

Comparative Protein Analysis

- Identify proteins within an organism that are related to each other and across different species
- Generate an evolutionary history of related genes
- Locate insertions, deletions, and substitutions that have occurred during evolution

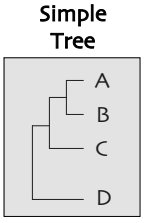


Syllabus

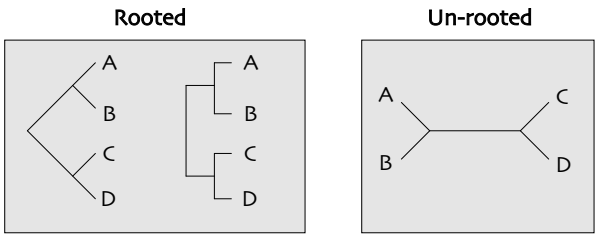
- **Phylogenetic Trees**
- Multiple Sequence Alignments
- From Trees and MSAs to Manuscript Figures
- Exercises

Phylogenetic Trees

- A graph representing the evolutionary history of a sequence
- Relationship of one sequence to other sequences
- Dissect the order of appearance of insertions, deletions, and mutations
- Predict function, observe epidemiology, analyzing changes in viral strains
- Tree of Life http://tolweb.org/tree/phylogeny.html



Tree Shapes

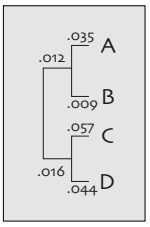


Branches intersect at Nodes
Leaves are the topmost branches

Tree Characteristics

- **Tree Properties**
  - **Clade**: all the descendants of a common ancestor represented by a node
  - **Distance**: number of changes that have taken place along a branch
- **Tree Types**
  - **Cladogram**: shows the branching order of nodes
  - **Phylogram**: shows branching order and distances

Phylogram



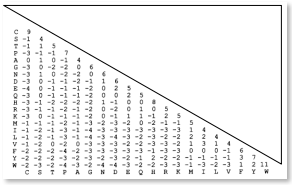
Tree Building Algorithms

- Maximum Parsimony
- Distance Methods
  - UPGMA
  - Neighbor Joining
- Maximum Likelihood

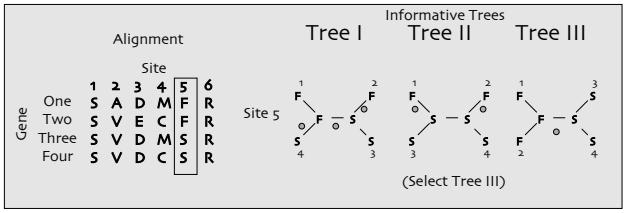
Amino Acid Substitutions

- Substitute one aa with another based on:
  - Chemical property
  - Size
  - Evolution (matrices)

BLOSUM 62
(Blocks Amino Acid Substitution Matrices)
(62% Identical Sequences)



Maximum Parsimony



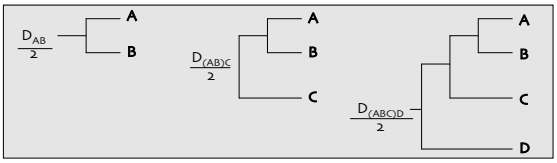
- Find the tree that changes one sequence into all of the others by the least number of steps [Focus solely on end product sequences, ignore evolutionary history]
- Only informative sites are analyzed (no gaps or conserved positions)
- Can be misleading when rates of change vary in different tree branches

Distance Methods

- **Distance** is expressed as the fraction of sites that differ between two sequences in an alignment
- Sequences with the smallest number of changes (shortest distance) are "related taxa"

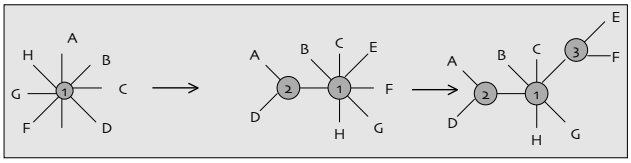
Distance Methods - UPGMA

- **UPGMA** (Unweighted Pair-Group Method with Arithmetic mean)
  - Sequentially find pair of taxa with smallest distance between them, and define branching as midpoint of two
  - Assumes the tree is additive and that rate of change is constant in all of the branches



Distance Methods - NJ

- **Neighbor-Joining** (NJ): useful when there are different rates of evolution within a tree
  - Each possible pair-wise alignment is examined. Calculate distance from each sequence to every other sequence
  - Choose the pair with the lowest distance value and join them to produce the minimal length tree
  - Update distance matrix where joined node is substituted for two original taxa and then repeat process



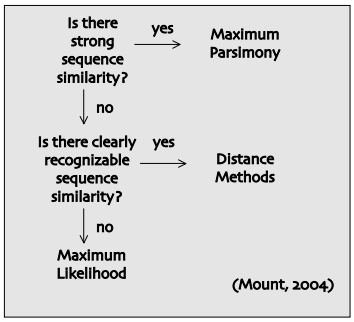
Maximum Likelihood

- Best accounts for variation in sequences
- Establish a **probabilistic model** with multiple solutions and determine which is most likely
- All possible trees are considered, therefore, only suitable for small number of sequences
  - Maximizes probability of finding optimal tree

Tree Reliability

- Probability that the members of a clade are always members of that clade
- Sample by **Bootstrapping**
  - Random sites of an alignment are randomly sampled so as to create a dataset the same size as the original. The same analysis as applied to the original data set is performed on the bootstrap dataset
  - Construct a consensus bootstrap tree and compare to the original tree

Which Method to Use?



Syllabus

- Phylogenetic Trees
- **Multiple Sequence Alignments**
- From Trees and MSAs to Manuscript Figures
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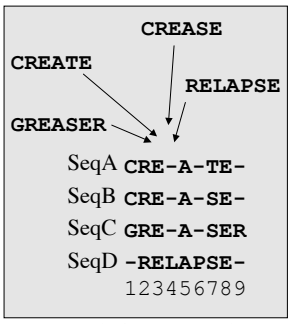
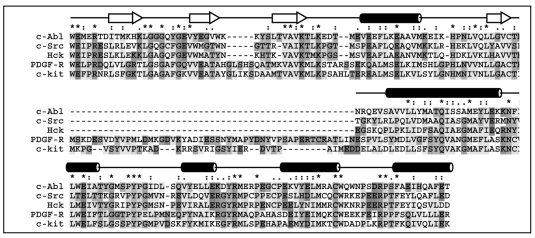
Multiple Sequence Alignments

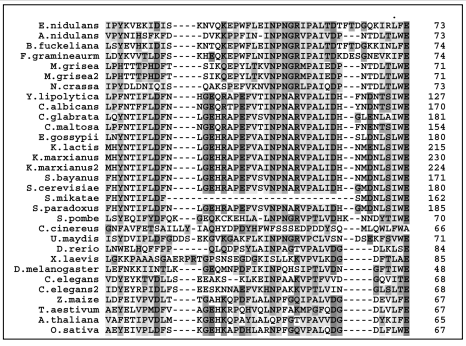
- Place residues in columns that are derived from a common ancestral residue
- MSA can reveal sequence patterns
  - Demonstration of homology between >2 sequences
  - Identification of functionally important sites
  - Protein function prediction
  - Structure prediction
  - Search for weak but significant similarities in databases
  - Design PCR primers for related gene identification
  - Genome sequencing: contig assembly



Multiple Sequence Alignment



Multiple Sequence Alignment



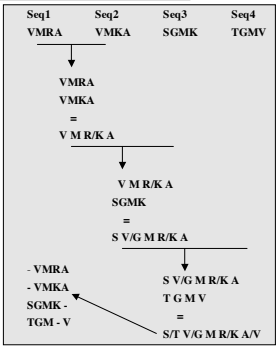
Approaches

- **Optimal Global Alignments** -Dynamic programming
  - Build matrices with every possible combination and search for optimal solution
  - Align 10 sequences of 100 aa length
  - Optimal in the mathematical sense

  = 100^{10} possibilities
- **Global Progressive Alignments** - Match most common sequences together
- **Global Iterative Alignments** - Multiple re-building attempts to find best alignment
- **Local alignments**
  - Profiles, Blocks, Patterns

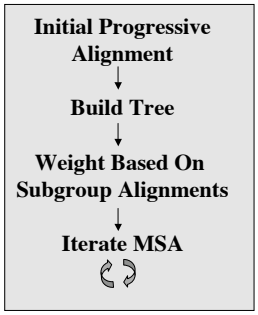
Global Progressive Alignment

- A heuristic approach that utilizes phylogenetic information to assist in routing the alignment (clustalw/clustalx)
- Feng & Doolittle1987, Higgins and Sharp 1988
- Most alike sequences are aligned together in order of their similarity (tree-based), a consensus is determined and then aligned to next most similar sequence



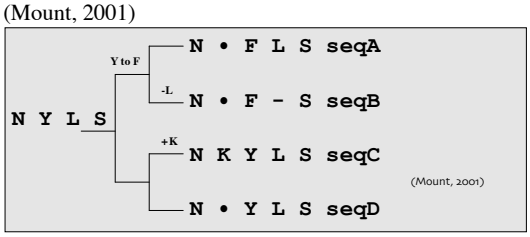
Iterative Multiple Alignment

- "Repeatedly re-align subgroups of sequences into a global alignment to improve alignment score" (Mount, 2004)
- Start with a progressive alignment and tree
- Recalculate pair-wise scores during progressive alignment, use new scores to rebuild the tree, which is used to improve alignments



MSA and Tree Relationship

- "The optimal alignment of several sequences can be thought of as minimizing the number of mutational steps in an evolutionary tree for which the sequences are the leaves" (Mount, 2001)



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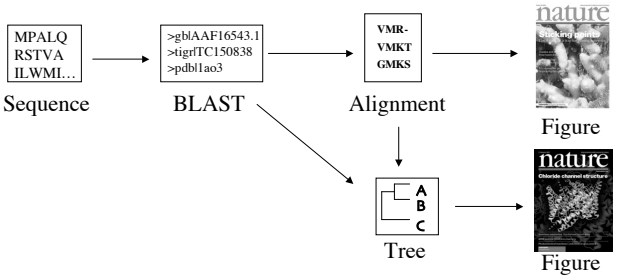
Manuscript Figures 101



1. Find Related Sequences

- **BLAST**
  - www.ncbi.nih.gov/BLAST

```
MLEICLKLVGCKSKKGLSSSSSCYLEEALQRPVASDFEPQGLSEAARWNSKENLLAGPSENDPNLFVALY
DFVASGDNTLSITKGEKLRVLGYNHNGEWCEAQTKNGQGWVPSNYITPVNSLEKHSWYHGPVSRNAAEYL
LSSGINGSFLVRESESSPGQRSISLRYEGRVYHYRINTASDGKLYVSSESRFNTLAELVHHHSTVADGLI
TTLHYPAPKRNKPTVYGVSPNYDKWEMERTDITMKHKLGGGQYGEVYEGVWKKYSLTVAVKTLKEDTMEV
EEFLKEAAVMKEIKHPNLVQLLGVCTREPPFYIITEFMTYGNLLDYLRECNRQEVNAVVLLYMATQISSA
MEYLEKKNFIHRDLAARNCLVGENHLVKVADFGLSRLMTGDTYTAHAGAKFPIKWTAPESLAYNKFSIKS
DVWAFGVLLWEIATYGMSPYPGIDLSQVYELLEKDYRMERPEGCPEKVYELMRACWQWNPSDRPSFAEIH
QAFETMFQESSISDEVEKELGKQGVRGAVSTLLQAPELPTKTRTSRRAAEHRDTTDVPEMPHSKGQGESD
PLDHEPAVSPLLPRKERGPPEGGLNEDERLLPKDKKTNLFSALIKKKKKTAPTPPKRSSSFREMDGQPER
RGAGEEEGRDISNGALAFTPLDTADPAKSPKPSNGAGVPNGALRESGGSGFRSPHLWKKSSTLTSSRLAT
GEEEGGGSSSKRFLRSCSASCVPHGAKDTEWRSVTLPRDLQSTGRQFDSSTFGGHKSEKPALPRKRAGEN
RSDQVTRGTVTPPPRLVKKNEEAADEVFKDIMESSPGSSPPNLTPKPLRRQVTVAPASGLPHKEEAGKGS
ALGTPAAAEPVTPTSKAGSGAPGGTSKGPAEESRVRRHKHSSESPGRDKGKLSRLKPAPPPPPAASAGKA
GGKPSQSPSQEAAGEAVLGAKTKATSLVDAVNSDAAKPSQPGEGLKKPVLPATPKPQSAKPSGTPISPAP
VPSTLPSASSALAGDQPSSTAFIPLISTRVSLRKTRQPPERIASGAITKGVVLDSTEALCLAISRNSEQM
ASHSAVLEAGKNLYTFCVSYVDSIQQMRNKFAFREAINKLENNLRELQICPATAGSGPAATQDFSKLLSS
VKEISDIVQR
```


2. Compile & Align Sequences

- **Compile** Sequences into FASTA format

```
>Human
MPALGYKFSTW…
>Mouse
MDGSTDYGILQINS…
>Rat
MKKP..
>Murine_Leukemia_Virus
MTSR….
```

- **Align**
  - PC: www-igbmc.u-strasbg.fr/BioInfo/ClustalX/Top.html
  - OS X: www.embl.de/~chenna/clustal/darwin/
  - Web: pir.georgetown.edu/pirwww/search/multaln.html
  - Jalview: http://www.jalview.org/Web_Installers/install.htm

3. Build Tree

- **Create** tree
  - Clustalx Neighbor Joining method
- **Draw** tree
  - TreeView:
    - taxonomy.zoology.gla.ac.uk/rod/treeview.html
  - Web:
    - iubio.bio.indiana.edu/treeapp/treeprint-form.html

4. Create Figures

- MSAs are often multipage
  - **Convert** to PDF with **Acrobat Distiller** or open with **Ghostview** (http://www.cs.wisc.edu/~ghost/ or http://www.kiffe.com/macghostview.html)
  - Extract pages individually and save as separate PDF/PS files
  - **Open** images in favorite illustration program
  - **Export** annotated alignments/trees to Powerpoint
- **Publish** paper, give award-winning presentation!

Exercise I

- **BLAST your sequence**
  - NCBI BLAST
  - Collate and edit sequences in a text editor
- **Perform multiple sequence alignment**
  - Clustalx
- **Build Phylogenetic Tree**
  - Clustalx and TreeView
- **Manage Postscript Files**
  - Adobe Acrobat Distiller/Ghostview
- **Create Figure**
  - Illustrator - > Powerpoint

References

- Bioinformatics: Sequence and genome Analysis. David W. Mount. CSHL Press, 2001 and 2004.
- Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins. Andreas D. Baxevanis and B.F. Francis Ouellette. Wiley Interscience, 2001.
- Bioinformatics: Sequence, structure, and databanks. Des Higgins and Willie Taylor. Oxford University Press, 2000.