# Bioinformatics

## Proteins II. - Pattern, Profile, & Structure Database Searching

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# Proteins I.-III. - Syllabus

- Proteins I.
  - Phylogenetic Trees
  - Multiple Sequence Alignments
- Proteins II.
  - Searching for Homologous Sequences
  - Working with Protein Structure Information
- Proteins III.
  - Comparing Protein Structures
  - Building Structural Models

# Last Week

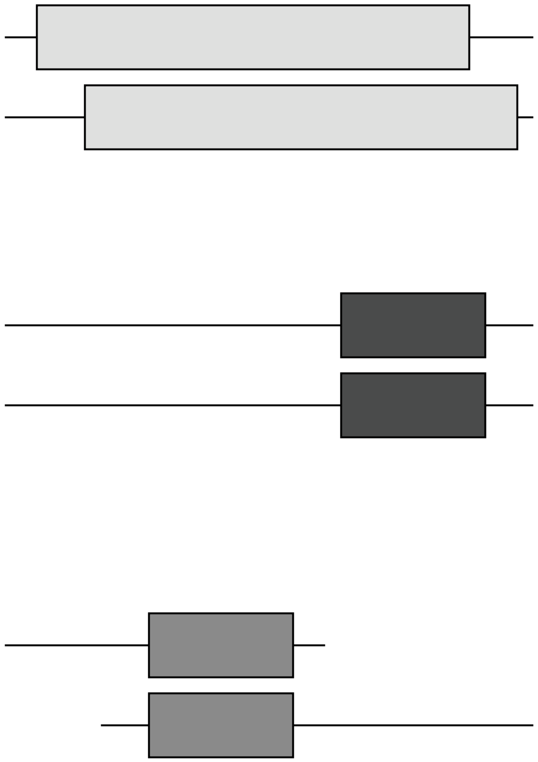
- Phylogenetic Trees
  - Trees show the relationship between sequences
  - Approaches: Maximum Parsimony, Distance, Maximum Likelihood
  - Distance scores should be considered secondary to tree shape
- Multiple Sequence Alignments
  - Approaches
    - Global: Dynamic Programming, Progressive, Iterated
    - Local: Profiles, Block-Based, Motif-Based
  - Manual manipulation of alignments with applications like Jalview is acceptable and recommended (esp. for pattern identification)
  - Scoring is usually the Sum of Pair-wise alignment scores

# Proteins II. - Syllabus

- Searching for Homologous Sequences
  - Pattern Searches
    - Patscan
  - Profile Searches
    - PSI-BLAST/HMMER2
- Working with Protein Structure Information
  - Coordinate Files, Databases, Classification
  - Structure Viewers

# Protein Modules

- Proteins are derived from a limited number of basic building blocks (Domains)
- Evolution has shuffled these modules giving rise to a diverse repertoire of protein sequences
- As a result, proteins can share a global or local relationship



(Higgins )

# Protein Families

- Protein Family - a group of proteins that share a common function and/or structure, that are potentially derived from a common ancestor (set of homologous proteins)

- Characterizing a Family - Compare the sequence and structure patterns of the family members to reveal shared characteristics that potentially describe common biological properties

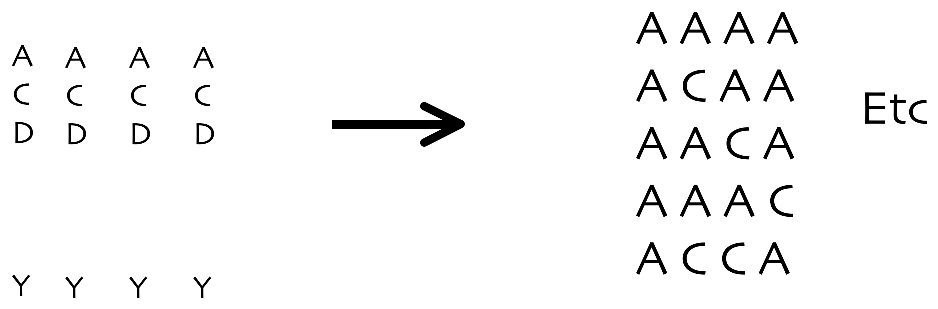
- Motif/Domain - sequence and/or structure patterns common to protein family members

# Patterns & Profiles

- Techniques for searching sequence databases to uncover common domains/motifs of biological significance that categorize a protein into a family
- Pattern - a deterministic syntax that describes multiple combinations of possible residues within a protein string
- Profile - probabilistic generalizations that assign to every segment position, a probability that each of the 20 aa will occur

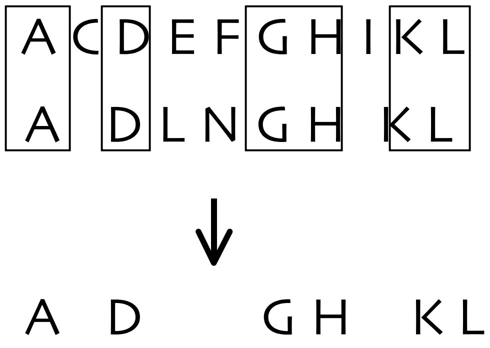
# Pattern/Profile Discovery Algorithms

- Pattern Driven Methods
  - Enumerate all possible patterns in solution space and try matching them to a set of sequences



# Pattern/Profile Discovery Algorithms

- Sequence Driven Methods
  - Build up a pattern by pair-wise comparisons of input sequences, storing positions in common, removing positions that are different



A C D E F G H I K L

A D L N G H K L

↓

A D G H K L

# Sequence Patterns

- Specification - a single residue K, set of residues (KPR), exclusion {KPR}, wildcards X, varying lengths x(3,6) -> variable gap lengths
- General Syntax
  - C-x(2,4)-C-x(3)-[LIVMFYWC]-x(8)-H-x(3,5)-H
- Patscan Syntax (http://web.wi.mit.edu/bio/pub/patscan.html)
  - C 2…4 C 3…3 any(LIVMFYWC) 8…8 H 3…5 H
- Patscan command
  - %scan_for_matches -p pattern_file < /db0/Data/nr > output_file

# Sequence Pattern Concerns

- Pattern descriptors must allow for approximate matching by defining an acceptable distance between a pattern and a potential hit
  - Weigh the sensitivity and specificity of a pattern
- What is the likelihood that a pattern would randomly occur?

# Sequence Profiles

- Consensus - mathematical probability that an aa will be located at a given position
- Probabilistic pattern constructed from a MSA
- Opportunity to assign penalties for insertions and deletions (not well suited for variable gap lengths)
- PSSM - Position Specific Scoring Matrix
  - Columns of weights for every aa corresponding to each column of a MSA

# PSSM Example

| C<br>O<br>N | A | C | D | E | F | G | H | I | K | L | M | N | P | Q | R | S | T | V | W | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 8 | -2 | 5 | 4 | 5 | 5 | -4 | 24 | 0 | 15 | 13 | 1 | 1 | 1 | -7 | 2 | 22 | 21 | -18 | -6 |
| T | 13 | -5 | 24 | 18 | -18 | 19 | 7 | 1 | 7 | -7 | -4 | 14 | 11 | 10 | -1 | 9 | 29 | 3 | -28 | -14 |
| L | 5 | -5 | 3 | 4 | 13 | 4 | 2 | 8 | -4 | 14 | 12 | 8 | -5 | 0 | -10 | 0 | 10 | 10 | -1 | 5 |
| S | 17 | 17 | 13 | 10 | -12 | 29 | -5 | -5 | 6 | -14 | -9 | 12 | 10 | 0 | -2 | 34 | 19 | 1 | -8 | -15 |

# PSSM Properties

- Score-based sequence representations for searching databases

- Scores Calculation
  - Log odds score representing: Distribution of aa in an MSA column, substitution matrices
- Goal
  - Limit the diversity in each column to improve reliability
- Problems
  - Differing length gaps between conserved positions (unlike patterns)

# PSSM Weighting

- Differentially weight sequences to reduce redundancy from non-representative sampling
  - Similar sequences get low weights, diverged sequences get higher weights
  - Maximum discrimination: weights that best discriminate between positives and background
  - Use root to sequence distance to calculate weight
  - Average distance of a sequence to all other sequences

# Building Profiles: Hidden Markov Models

- Statistical model that considers all the possible combinations of matches, mismatches, and gaps to generate a consensus (Higgins, 2000)
- Sequence ordering and alignments are not necessary at the onset (but in many cases alignments are recommended)
- Ideally use at least 20 sequences in the training set to build a model
- Calibration prevents over-fitting training set (i.e. Ala scan)
- Generate a model (profile), then search a database with it
- Limitations
  - Not all potential domains are represented in each family database, therefore, should develop own models/profiles

# Searching Family Databases

- BLAST searches provide a great deal of information, but it is difficult to select out the important sequences (listed by score, not family)
- Family searches can give an immediate indication of a protein’s classification/function
- Identify domains and family members
  - Use a sequence to search a database and characterize a pattern and identify its protein family
  - Use a specific pattern to identify homologous sequences (family members)

# Family Database Resources

- Curated Databases*
  - Proteins are placed into families with which they share a specific sequence pattern
- Clustering Databases*
  - Sequence similarity-based without the prior knowledge of a specific patterns
- Derived Databases*
  - Pool other databases into one central resource

*(Higgins )

# Curated Family Databases

- Pfam (http://pfam.wustl.edu/hmmsearch.shtml/) **
  - Uses manually constructed seed alignments and HMM PSSMs to automatically extract domains
  - db of protein families and corresponding profile-HMMs
  - Searches report e-value and bits score
- Prosite (http://www.expasy.ch/tools/scanprosite/)
  - Hit or Miss -> no stats
- PRINTS (http://www.bioinf.man.ac.uk/fingerPRINTScan/)
  - Find fingerprints in your sequence

# Clustering Family Databases

- Search a database against itself and cluster similar sequences into families
- ProDom (http://prodes.toulouse.inra.fr/prodom/doc/prodom.html)
  - Searchable against MSAs and consensus sequences
- Protomap (http://www.protomap.cs.huji.ac.il/)
  - Swiss-Prot based and provides a tree-like view of clustering

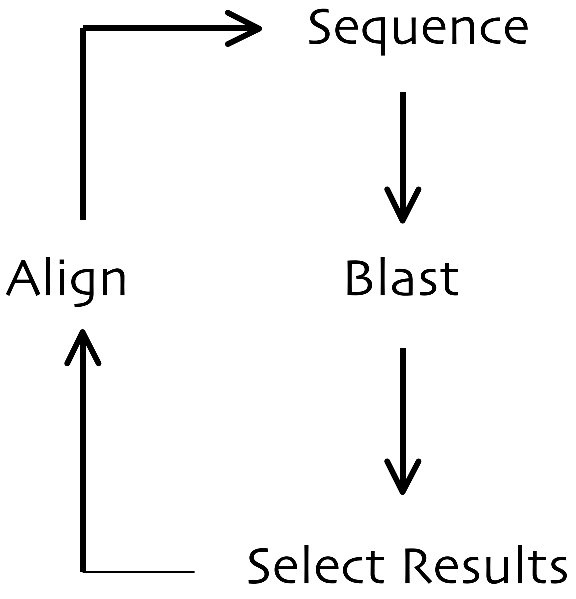
# Derived Family Databases

- Databases that utilize protein family groupings provided by other resources
- Blocks - Search and Make (http://blocks.fhcrc.org/blocks/)
  - Uses Protomap system for finding blocks that are indicative of a protein family (GIBBS/MOTIF)
- Proclass (http://pir.georgetown.edu/gfserver/proclass.html)
  - Combines families from several resources using a neural network-based system (relationships)
  - Not an alignment-based system
- MEME (Locally available through GCG on fladda)

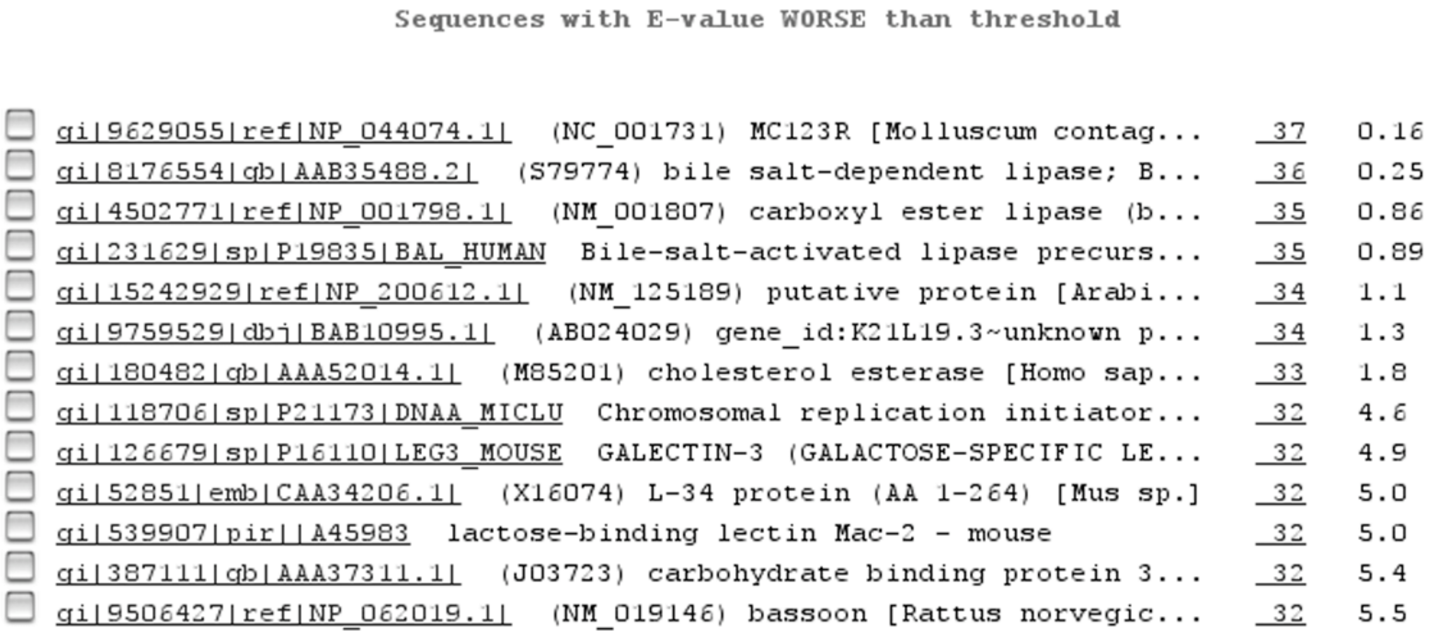
  (http://meme.sdsc.edu/meme/website/intro.html)

# HMM Implementations Profile Building & Searching

- PSI-BLAST http://www.ncbi.nlm.nih.gov/BLAST/
  - Start with a sequence, BLAST it, align select results to query sequence, estimate a profile with the MSA, search DB with the profile
  - Iterate until process stabilizes
  - Focus on domains, not entire sequences
  - Greatly improves sensitivity



# PSI-BLAST Sample Output



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                    Sequences with E-value WORSE than threshold

□ gi|9629055|ref|NP_044074.1|  (NC_001731) MC123R [Molluscum contag...   37   0.16
□ gi|8176554|gb|AAB35488.2|  (S79774) bile salt-dependent lipase; B...   36   0.25
□ gi|4502771|ref|NP_001798.1|  (NM_001807) carboxyl ester lipase (b...   35   0.86
□ gi|231629|sp|P19835|BAL_HUMAN  Bile-salt-activated lipase precurs...   35   0.89
□ gi|15242929|ref|NP_200612.1|  (NM_125189) putative protein [Arabi...   34   1.1
□ gi|9759529|dbj|BAB10995.1|  (AB024029) gene_id:K21L19.3~unknown p...   34   1.3
□ gi|180482|gb|AAA52014.1|  (M85201) cholesterol esterase [Homo sap...   33   1.8
□ gi|118706|sp|P21173|DNAA_MICLU  Chromosomal replication initiator...   32   4.6
□ gi|126679|sp|P16110|LEG3_MOUSE  GALECTIN-3 (GALACTOSE-SPECIFIC LE...   32   4.9
□ gi|52851|emb|CAA34206.1|  (X16074) L-34 protein (AA 1-264) [Mus sp.]   32   5.0
□ gi|539907|pir||A45983  lactose-binding lectin Mac-2 - mouse            32   5.0
□ gi|387111|gb|AAA37311.1|  (J03723) carbohydrate binding protein 3...   32   5.4
□ gi|9506427|ref|NP_062019.1|  (NM_019146) bassoon [Rattus norvegic...   32   5.5
```

# HMM Implementations
# Profile Building & Searching

- HMMER2 (http://hmmer.wustl.edu/)
  - Determine which sequences to include/exclude
  - Perform alignment, select domain, excise ends, manually refine MSA (pre-aligned  sequences better)
  - Build profile
    - %hmmbuild [-options] <hmmfile output> <alignment file>
  - Calibrate profile (re-calc. Parameters by making a random db)
    - %hmmcalibrate [-options] <hmmfile>
  - Search database
    - %hmmsearch [-options] <hmmfile> <database file>

# HMMER2 Output

- Hmmsearch returns e-values and bits scores
- Repeat process with selected results
  - Unfortunately need to extract sequences from the results and manually perform MSA before beginning next round of iteration

# Validating HMMER2 Profiles

- Including false positives into the building process will reduce the accuracy of the profile (increase the noise)
- Validate by
  - Blast results against each other
  - Demonstrate that you can get from one sequence to another

# Patterns vs. Profiles

- Patterns
  - Easy to understand (human-readable)
  - Account for different length gaps
- Profiles
  - Sensitivity, better signal to noise ratio
  - Teachable

# Protein Structures

- Protein Structure Classification
- Coordinate Files
- Structure Coordinate Databases
- Structure Family Databases
- Structure Visualization

# Protein Structure Classification

- Proteins can adopt only a limited number of possible 3D conformations
  - Combinations of □ helices, □ sheets, loops, and coils
- Completely different sequences can fold into similar shapes
- Protein Structure Classes
  - Class □: bundles of □ helices
  - Class □: antiparallel □ sheets (sandwiches and barrels)
  - Class □ / □: parallel □ sheets with intervening □ helices
  - Class □ + □: segregated □ helices and antiparallel □ sheets
  - Multidomain
  - Membrane/Cell surface proteins

(Higgins )

# Coordinate Files

- Coordinate Data: location of a molecule's atoms in space (XYZ triple)
- XYZ triple is labeled with an atom, residue, chain, and molecule
  - Modified aa are labeled with X, H's not usually listed
- Data Representation
  - Chemistry Rules Approach: connect the dots utilizing a standard rules base to specify bond distances (not consistent among applications)
  - Explicit Bonding Approach: explicit bonding information is specified in the file (very consistent)

# Coordinate File Formats

- MMDB http://www.ncbi.nlm.nih.gov/Structure/
  - ASN.1 standard data description language
- PDB
  - Column oriented, “flexible format”
  - Sequence - Explicit SEQRES and Implicit ATOM lines
- mmCIF
  - Chemical Interchange Format - relational db format
  - http://web.wi.mit.edu/proteins/education/1F3J.cif

# Structure Coordinate Databases

- RCSB (Research Collaboratory for Structural Bioinformatics) http://www.rcsb.org/
  - Formally know as the Protein Data Bank at Brookhaven National Laboratories
  - Structure Explorer PDB search engine
    - Text and PDB ID (4 letter code) searching
- MMDB (Molecular Modeling Database @NCBI)
  - Compilation of structures represented in multiple formats
  - Provides structure summaries
  - BLAST sequences to search for available structures

# Structure Families

- Divide structures into the limited number of possible structure families
  - Homologous proteins can be identified by examining their respective structures for conserved fold patterns
  - Representative members can be used for modeling sequences of unknown structure

# Structure Family Databases

- SCOP: Structural Classification Of Proteins
  - based on a definition of structural similarities. Hierarchical levels to reflect evolutionary and structural relationships
- CATH: Classification by Class, Architecture, Topology, and Homology
  - classified first into hierarchical levels like SCOP
- FSSP: Fold classification based on Structure-structure alignment of proteins
  - based on structural alignment of all pair-wise combinations of proteins in PDB by DALI (used to id common folds and place into groups)
- MMDB
  - Aligns 3D structures based on similar arrangements of secondary structural elements (VAST)
- SARF
  - categorized on the basis of structural similarity, categories are similar to other dbs

# Resource Links

- Protein data bank (PDB)
  - http://www.rcsb.org/pdb
- Molecular Modeling Database
  - http://www.ncbi.nlm.nih.gov/Structure/
- Structural Classification of Proteins SCOP
  - http://scop.mrc-lmb.cam.ac.uk/scop
- CATH
  - http://www.biochem.ucl.ac.uk/bsm

# Next Week

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- Proteins II.
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  - Working with Protein Structure Information
- Proteins III.
  - Comparing Protein Structures
  - Building Structural Models

# Visualizing Structural Information

- Hand edit files
- Different representations of molecule
  - wire, backbone, space-filling, ribbon
- NMR ensembles
  - Models showing dynamic variation of molecules in solution
- VIEWERS
  - RasMol and Chime is the Netscape plug-in
    - http://www.umass.edu/microbio/rasmol/index2.html
  - Cn3D MMDB viewer (See in 3D) with explicit bonding
    - http://www.ncbi.nlm.nih.gov/Structure
  - SwissPDB Viewer
    - http://www.expasy.ch/spdbv/mainpage.html

# References

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