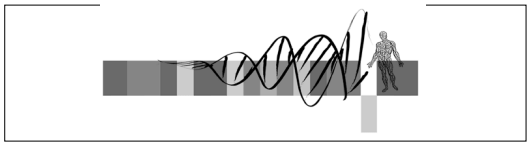

Getting To Know Your Protein

Comparative Protein Analysis:
Part II. Protein Domain Identification & Classification

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Comparative Protein Analysis

- **Part I. :**
  - **Phylogenetic Trees** and **Multiple Sequence Alignments** are important tools to understand global relationships between sequences.
  - Tree Building Tools with Different Algorithms
    - http://bioweb.pasteur.fr/seqanal/phylogeny/intro-uk.html
    - http://evolution.genetics.washington.edu/phylip/software.xref.html
  - Tree Reliability
    - Bootstrapping 1. Randomly re-sample MSA columns to produce a random alignment (equal length as original MSA), 2. Build tree based on random alignment, 3. Predicted branches are significant if they occur in ~ >70% of the trees from multiple, randomized alignments.
    - Use a several tree building algorithms to determine whether they produce similar trees as the original.

Comparative Protein Analysis

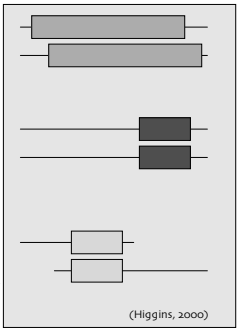
- **Part II. :**
  - How do you identify sequence relationships that are restricted to localized regions?
  - Can you apply phylogenetic trees and MSAs to only sub-regions of sequences?
  - How do you apply what you know about a group of sequences to finding additional, related sequences?
  - What can the relationship between your sequences and previously discovered ones tell you about their function?
- Assigning sequences to **Protein Families**

Syllabus

- **Protein Families**
  - Identifying Protein Domains
  - Family Databases & Searches
- Searching for Family Members
  - Pattern Searches
    - Patscan
  - Profile Searches
    - PSI-BLAST/HMMER2

Proteins As Modules

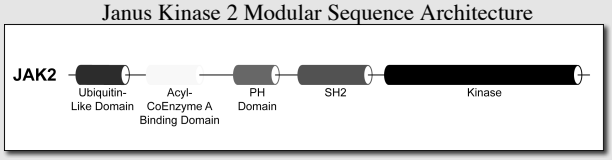
- Proteins are derived from a limited number of basic building blocks (**Domains**)
- Evolution has shuffled these modules giving rise to a diverse repertoire of protein sequences
- As a result, proteins can share a global or local relationship



Protein Domains

SH2 Motif

Janus Kinase 2 Modular Sequence Architecture

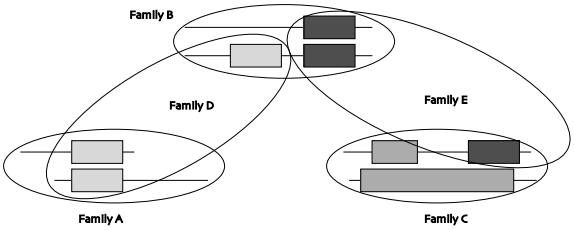


Motifs describe the domain

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 (trait/feature/characteristic)

Protein Families

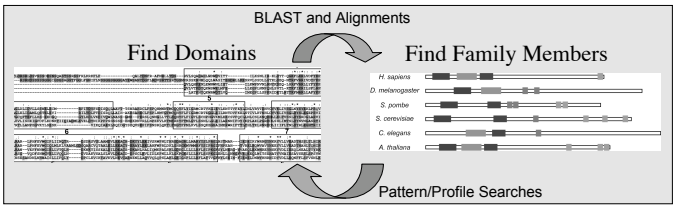


Separate Families Can Be Interrelated

Proteins Can Belong To Multiple Families

Creating Protein Families

- Use domains to identify family members
  - Use a sequence to search a database and characterize a pattern/profile
  - Use a specific pattern/profile to identify homologous sequences (family members)



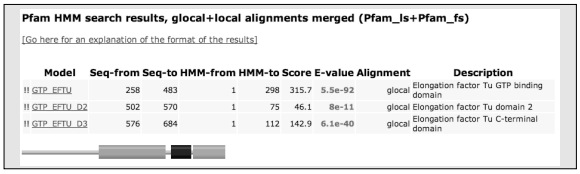
Family Database Resources

- **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 specific patterns
- **Derived** Databases*
  - Pool other databases into one central resource
- **Search** and **Browse**
  - **InterPro** http://www.ebi.ac.uk/interpro/

*(Higgins, 2000)

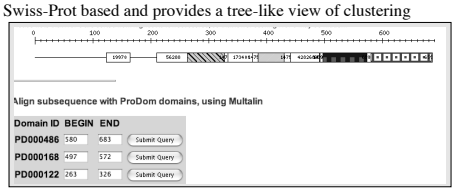
Curated Family Databases

- **Pfam** (http://pfam.wustl.edu) **
  - Uses manually constructed seed alignments and PSSM to automatically extract domains
  - db of protein families and corresponding profile-HMMs of prototypic domains
  - 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/)



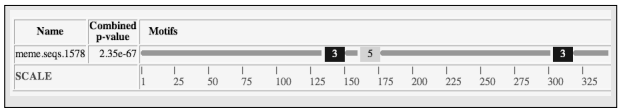
Clustering Family Databases

- Search a database against itself and cluster similar sequences into families
- **ProDom** (http://prodes.toulouse.inra.fr/prodom/current/html/home.php)
  - Searchable against MSAs and consensus sequences
- **Protomap** (http://protomap.cornell.edu/)
  - 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)
- **MEME** (http://meme.sdsc.edu/meme/website/intro.html)



Syllabus

- Protein Families
  - Identifying Protein Domains
  - Family Databases & Searches
- **Searching for Family Members**
  - Pattern Searches
    - Patscan
  - Profile Searches
    - PSI-BLAST/HMMER2

Searching Databases By Family

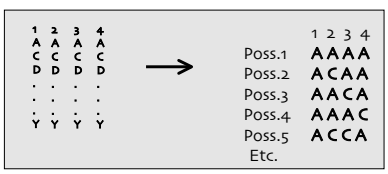
- BLAST searches provide a great deal of information, but it is difficult to select out the important sequences (listed by score, not family)
- Family searches can give an immediate indication of a protein's classification/function
- Use Family Database search tools to identify domains and family members

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

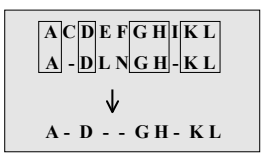
Pattern Discovery Algorithms

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



Pattern 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



Pattern Building

- Find patterns like “pos1 xx pos2 xxxx pos3”
  - Definition of a non-contiguous motif

```
1. C Y D - - C A F T L R Q S A V M H K H A R E H
2. C A T Y - C R T A I D T V K N S L K H H S A H
3. C W D G G C G I S V E R M D T V H K H D T V H
4. C Y C - - C S D H M K K D A V E R M H K K D H
5. C N M F - C M P I F R Q N S L A R E H E R M H
6. C L N N T C T A F W R Q K K D D T V H N S L H

   C xxxx C xxxx [LIVMFW] xxxxxxxx H xxxxx H
```

Define/Search A Motif http://us.expasy.org/tools/scanprosite/

Pattern Properties

- **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://jura.wi.mit.edu/bio/education/bioinfo/homework/hw8/patscan.txt
  - C 2…4 C 3…3 any(LIVMFYWC) 8…8 H 3…5 H
- **Pattern Database Searching**
  - %scan_for_matches -p pattern_file < nr > output_file

Sequence Pattern Concerns

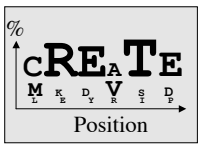
- Pattern descriptors must allow for approximate matching by defining an acceptable distance between a pattern and a potential hit
  - Weigh the sensitivity and specificity of a pattern
- What is the likelihood that a pattern would randomly occur?

Sequence Profiles

- **Consensus** - mathematical probability that a particular aa will be located at a given position
- **Probabilistic** pattern constructed from a MSA
- Opportunity to assign penalties for insertions and deletions, but not well suited for variable gap lengths
- **PSSM** - (Position Specific Scoring Matrix)
  - Represents the sequence profile in tabular form
  - Columns of weights for every aa corresponding to each column of a MSA

Profile Discovery/Analysis

- Perform global MSA on group of sequences
- Move highly conserved regions to smaller MSAs
- Generate scoring table with log odds scores
  - Each column is independent
  - Average Method: profile matrix values are weighted by the proportion of each amino acid in each column of MSA
  - Evolutionary Method: calculate the evolutionary distance (Dayhoff model) required to generate the observed amino acid distribution



PSSM Example

```
1. I T I S
2. T D L S
3. V T M G
4. I T I G
5. V G F S
6. I E L T
7. T T T S
8. I T L S
```

(i.e. Distribution of aa in an MSA column)

← Target sequences

Resulting Consensus: I T L S

PSSM

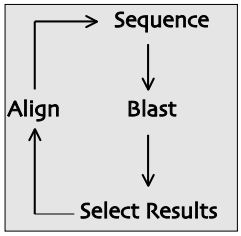
POS	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
1	8	-2	5	4	5	5	-4	24	0	15	13	1	1	1	-7	2	22	21	-18	-6
2	13	-5	24	18	-18	19	7	1	7	-7	-4	14	11	10	-1	9	29	3	-28	-14
3	5	-5	3	4	13	4	2	8	-4	14	12	8	-5	0	-10	0	10	10	-1	5
4	17	17	13	10	-12	29	-5	-5	6	-14	-9	12	10	0	-2	34	19	1	-8	-15

PSSM Properties

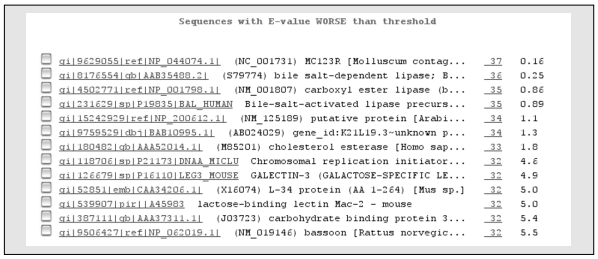
- Score-based sequence representations for searching databases
- Goal
  - Limit the diversity in each column to improve reliability
- Problems
  - Differing length gaps between conserved positions (unlike patterns)

PSI-BLAST Implementation

- **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 - constructs PSSM
  - Iterate until process stabilizes
  - Focus on domains, not entire sequences
  - Greatly improves sensitivity (but may affect specificity)



PSI-BLAST Sample Output


Sequences with E-value WORSE than threshold

gi|9629055|ref|NP_044074.1|	(NC_001731) MC123R [Molluscum contag...	37	0.16
gi|8176554|db|AAB35408.2|	(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|231625|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|db|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 Building

- **Hidden Markov Models** are Statistical methods that consider 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/PSSM), then search a database with it

HMM Implementation

- **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> > out

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

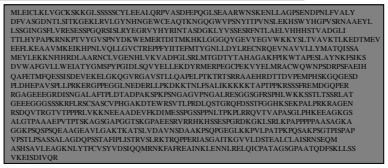
```
HMMER 2.2g (August 2001)
Copyright (C) 1992-2001 HHMI/Washington University School of Medicine
Freely distributed under the GNU General Public License (GPL)
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
HMM file:                 pfam_hsd.hmm [Hydrolase]
Sequence database:        /cluster/db0/Data/nr
per-sequence score cutoff:  [none]
per-domain score cutoff:    [none]
per-sequence Eval cutoff:   <= 10
per-domain Eval cutoff:     [none]
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

Query HMM:  Hydrolase
Accession:  PF00702
Description: haloacid dehalogenase-like hydrolase
  [HMM has been calibrated; E-values are empirical estimates]

Scores for complete sequences (score includes all domains):
Sequence                  Description              Score  E-value  N
--------                  -----------              -----  -------  ---
gi|16131263|ref|NP_417844.1|  phosphoglycolat  168.4  2.9e-45  1
gi|24114648|ref|NP_709158.1|  phosphoglycolat  167.8  4.2e-45  1
gi|15803888|ref|NP_289924.1|  phosphoglycolat  167.8  4.2e-45  1
gi|26249979|ref|NP_756019.1|  Phosphoglycolat  166.4  1.1e-44  1
```

Patterns vs. Profiles

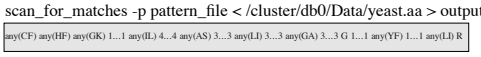
- **Patterns**
  - Easy to understand (human-readable)
  - Account for different length gaps
- **Profiles**
  - Sensitivity, better signal to noise ratio
  - Teachable

Domain ID & Searching

- Family/Domain Search
  - http://pfam.wustl.edu



- Pattern Search
  - scan_for_matches (Patscan)
    - scan_for_matches -p pattern_file < /cluster/db0/Data/yeast.aa > output_file



- Profile Search
  - HMMER2
    - hmmbuild [-options] <hmmfile output> <alignment file>

Exercises

- Use PFAM to identify domains within your sequence
- Scan your sequences with ProSite to find a pattern to represent the domain
- Use the ProSite pattern to search the non-redundant db
- Use PSI-BLAST to build a sequence profile and search the non-redundant db

References

- 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.