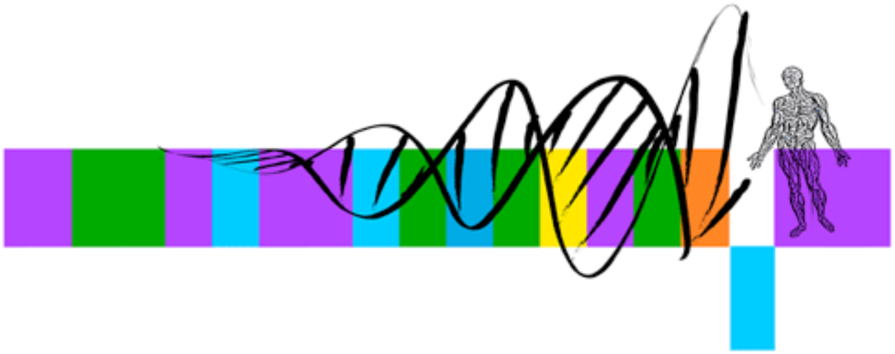


Getting To Know Your Protein

Comparative Protein Analysis:

Part II. Protein Domain Identification & Classification

Robert Latek, PhD
Sr. Bioinformatics Scientist
Whitehead Institute for Biomedical Research

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

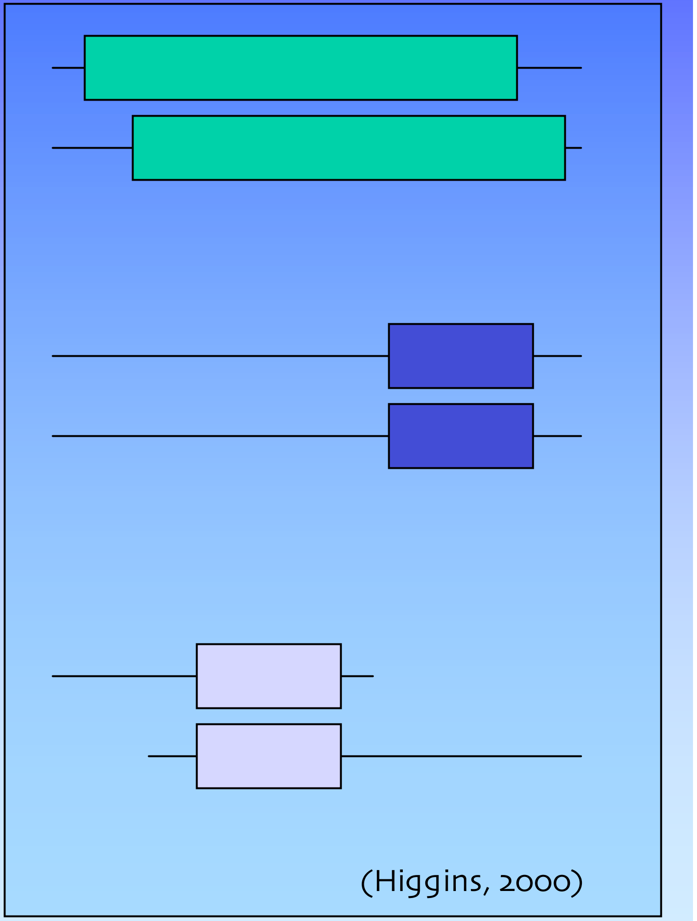
- **Part II. :**
  - How do you identify sequence relationships that are restricted to localized regions?
  - Can you apply phylogenetic trees and MSAs to only sub-regions of sequences?
  - How do you apply what you know about a group of sequences to finding additional, related sequences?
  - What can 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

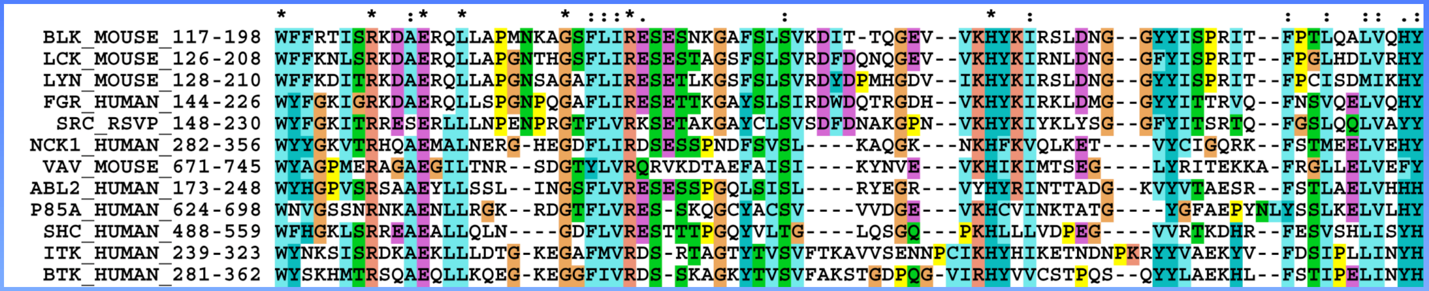
- Proteins are derived from a limited number of basic building blocks (**Domains**)
- Evolution has shuffled these modules giving rise to a diverse repertoire of protein sequences
- As a result, proteins can share a global or local relationship



(Higgins, 2000)

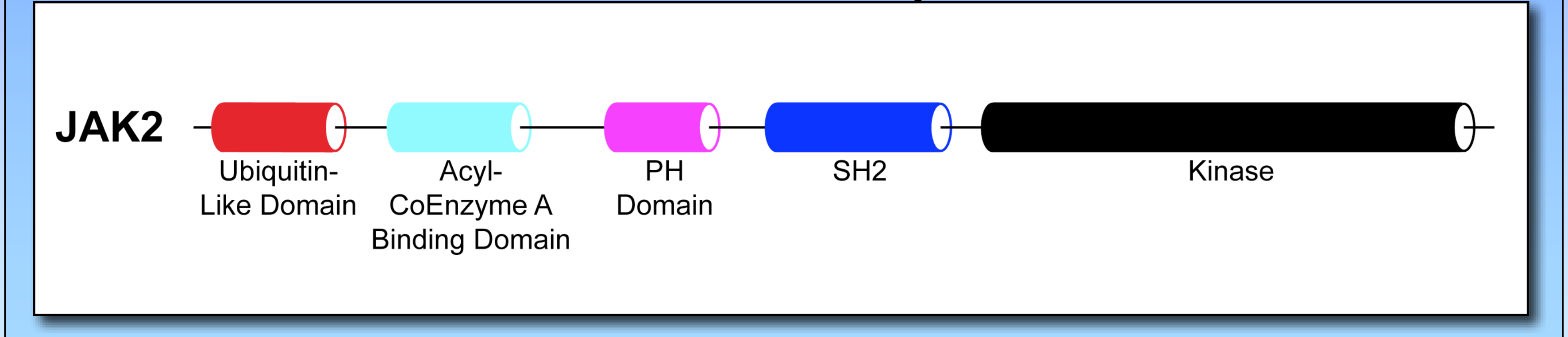
Protein Domains

SH2 Motif



```
                   *      *  :*  *       * :::*.          :               *  :                   :  :  :: .:
 BLK_MOUSE_117-198 WFFRTISRKDAERQLLAPMNKAGSFLIRESESNKGAFSLSVKDIT-TQGEV--VKHYKIRSLDNG--GYYISPRIT--FPTLQALVQHY
 LCK_MOUSE_126-208 WFFKNLSRKDAERQLLAPGNTHGSFLIRESESTAGSFSLSVRDFDQNQGEV--VKHYKIRNLDNG--GFYISPRIT--FPGLHDLVRHY
 LYN_MOUSE_128-210 WFFKDITRKDAERQLLAPGNSAGAFLIRESETLKGSFSLSVRDYDPMHGDV--IKHYKIRSLDNG--GYYISPRIT--FPCISDMIKHY
 FGR_HUMAN_144-226 WYFGKIGRKDAERQLLSPGNPQGAFLIRESETTKGAYSLSIRDWDQTRGDH--VKHYKIRKLDMG--GYYITTRVQ--FNSVQELVQHY
  SRC_RSVP_148-230 WYFGKITRRESERLLLNPENPRGTFLVRKSETAKGAYCLSVSDFDNAKGPN--VKHYKIYKLYSG--GFYITSRTQ--FGSLQQLVAYY
NCK1_HUMAN_282-356 WYYGKVTRHQAEMALNERG-HEGDFLIRDSESSPNDFSVSL----KAQGK---NKHFKVQLKET----VYCIGQRK--FSTMEELVEHY
 VAV_MOUSE_671-745 WYAGPMERAGAEGILTNR--SDGTYLVRQRVKDTAEFAISI----KYNVE---VKHIKIMTSEG----LYRITEKKA-FRGLLELVEFY
ABL2_HUMAN_173-248 WYHGPVSRSAAEYLLSSL--INGSFLVRESESSPGQLSISL----RYEGR---VYHYRINTTADG--KVYVTAESR--FSTLAELVHHH
P85A_HUMAN_624-698 WNVGSSNRNKAENLLRGK--RDGTFLVRES-SKQGCYACSV----VVDGE---VKHCVINKTATG----YGFAEPYNLYSSLKELVLHY
 SHC_HUMAN_488-559 WFHGKLSRREAEALLQLN----GDFLVRESTTTPGQYVLTG----LQSGQ---PKHLLLVDPEG----VVRTKDHR--FESVSHLISYH
 ITK_HUMAN_239-323 WYNKSISRDKAEKLLLDTG-KEGAFMVRDS-RTAGTYTVSVFTKAVVSENNPCIKHYHIKETNDNPKRYYVAEKYV--FDSIPLLINYH
 BTK_HUMAN_281-362 WYSKHMTRSQAEQLLKQEG-KEGGFIVRDS-SKAGKYTVSVFAKSTGDPQG-VIRHYVVCSTPQS--QYYLAEKHL--FSTIPELINYH
```

Janus Kinase 2 Modular Sequence Architecture

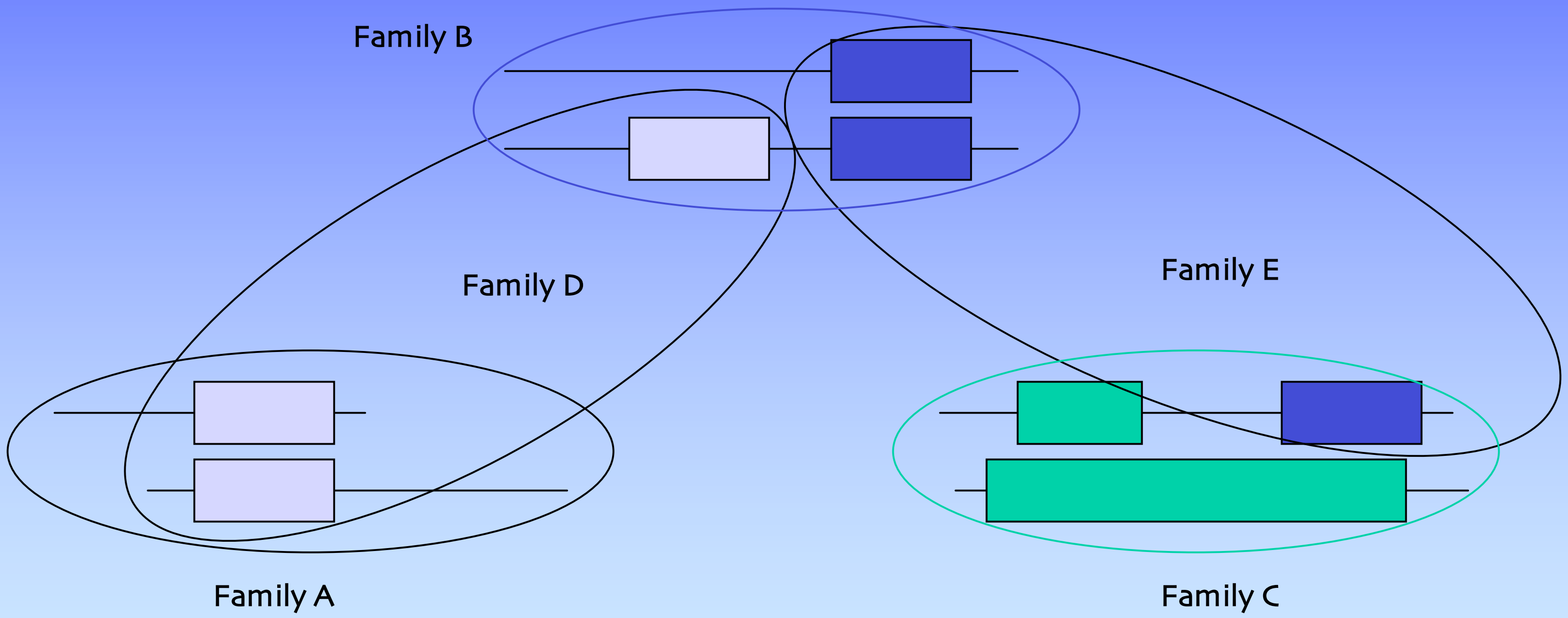


Motifs describe the domain

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

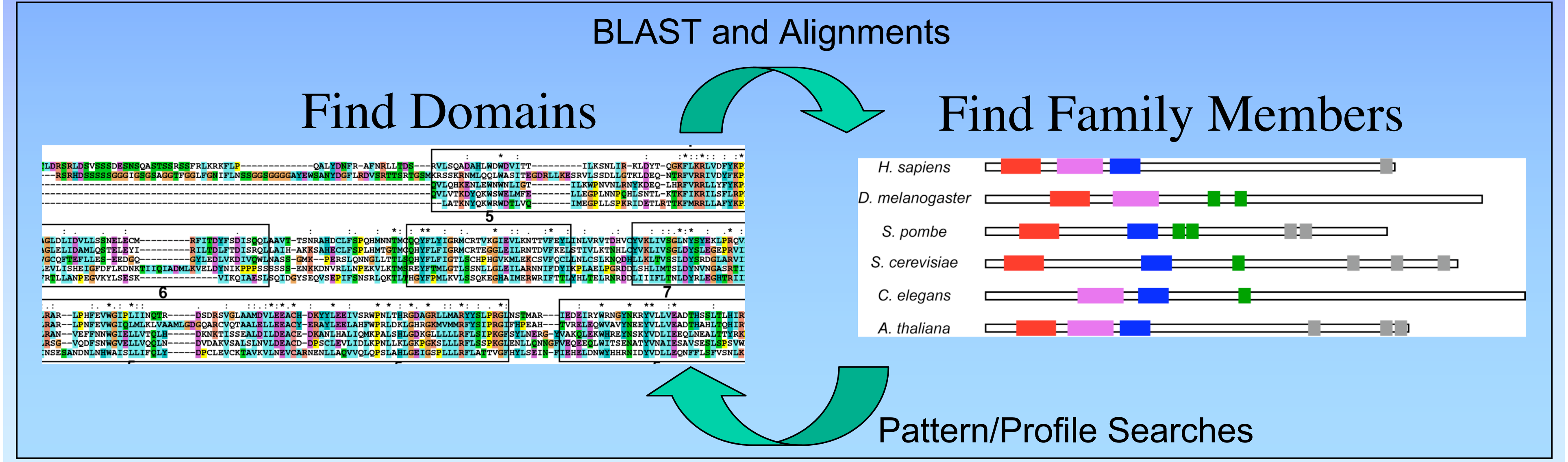


Separate Families Can Be Interrelated

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)



BLAST and Alignments

Find Domains

Find Family Members

Pattern/Profile Searches

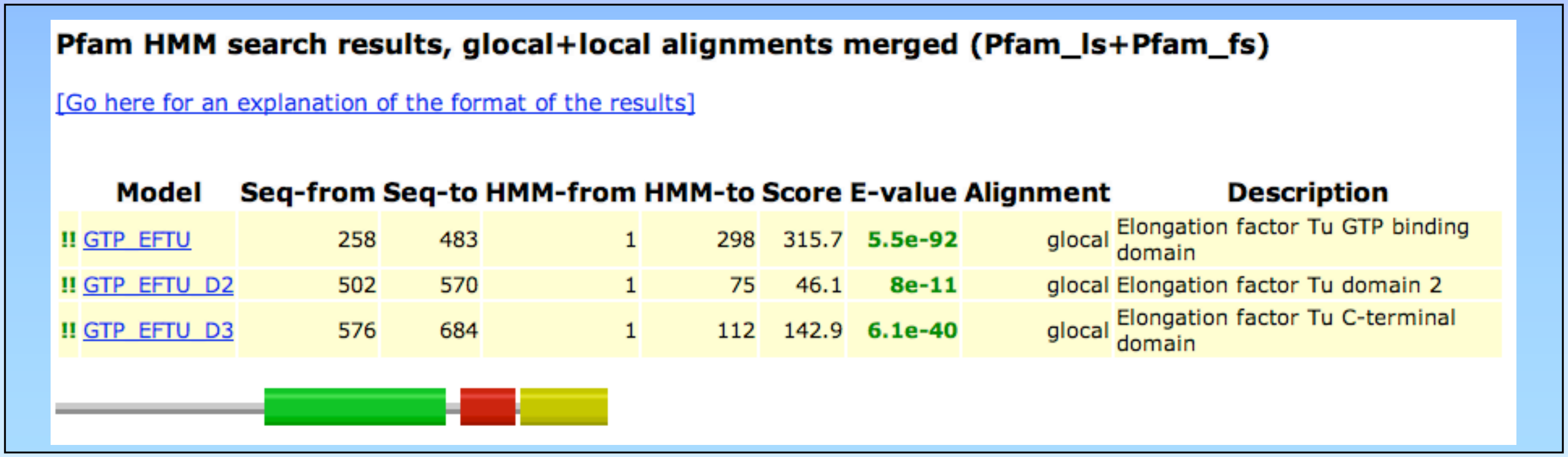
Family Database Resources

- **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/)



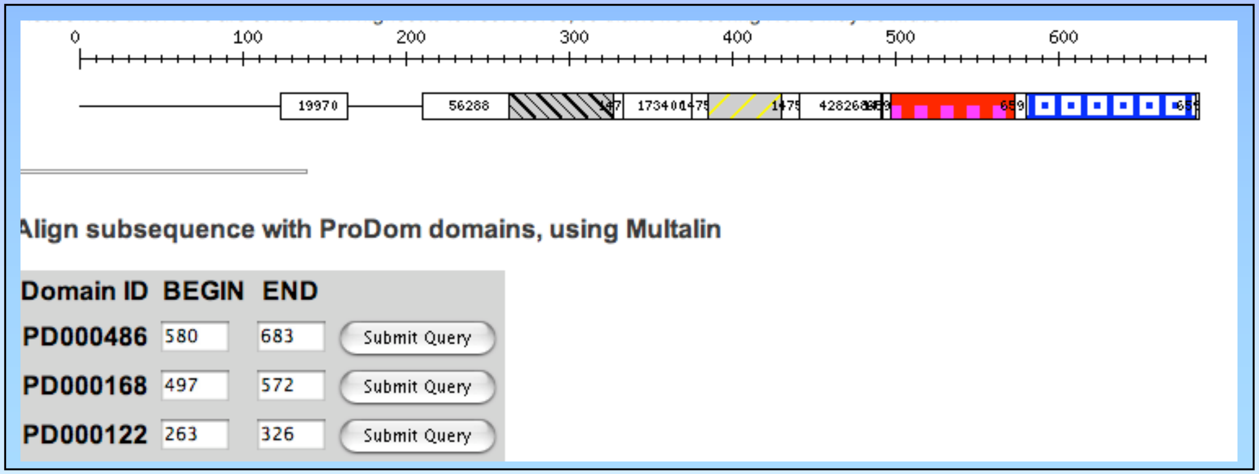
Pfam HMM search results, glocal+local alignments merged (Pfam_ls+Pfam_fs)

[Go here for an explanation of the format of the results]

	Model	Seq-from	Seq-to	HMM-from	HMM-to	Score	E-value	Alignment	Description
!!	GTP_EFTU	258	483	1	298	315.7	5.5e-92	glocal	Elongation factor Tu GTP binding domain
!!	GTP_EFTU_D2	502	570	1	75	46.1	8e-11	glocal	Elongation factor Tu domain 2
!!	GTP_EFTU_D3	576	684	1	112	142.9	6.1e-40	glocal	Elongation factor Tu C-terminal domain

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

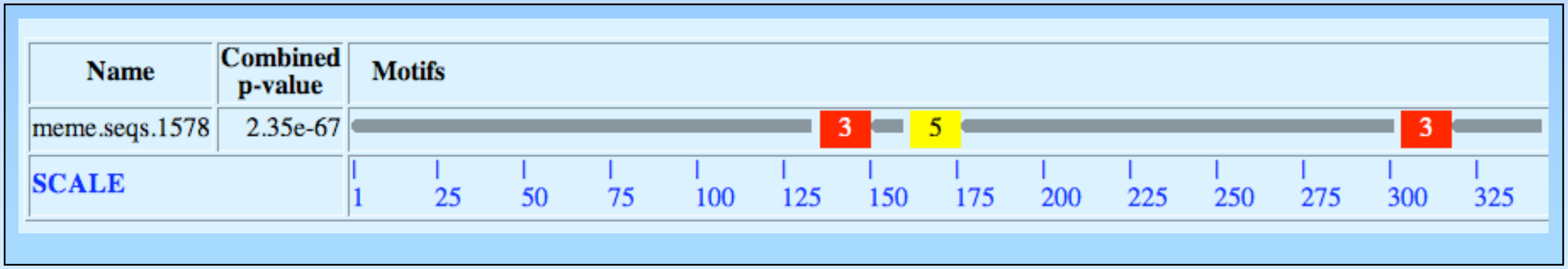


Align subsequence with ProDom domains, using Multalin

Domain ID	BEGIN	END	
PD000486	580	683	Submit Query
PD000168	497	572	Submit Query
PD000122	263	326	Submit Query

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)



Name	Combined p-value	Motifs
meme.seqs.1578	2.35e-67	3 5 3
SCALE		1 25 50 75 100 125 150 175 200 225 250 275 300 325

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

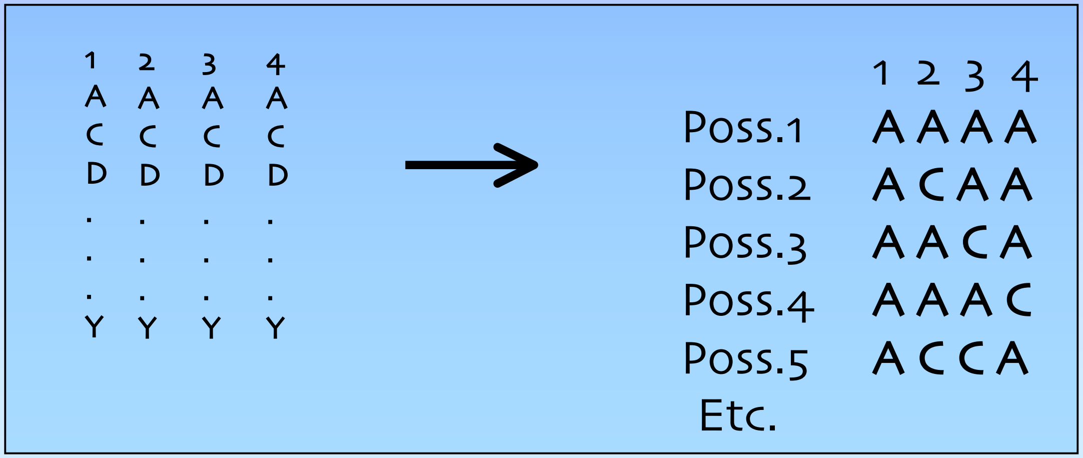
- Use Family Database search tools to identify domains and family members

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



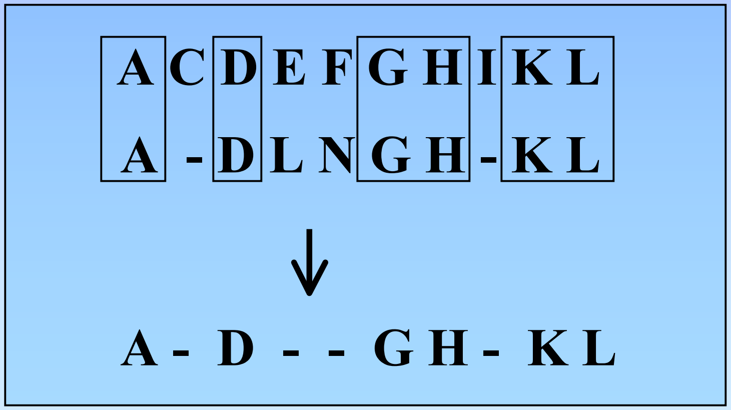
1	2	3	4
A	A	A	A
C	C	C	C
D	D	D	D
.	.	.	.
.	.	.	.
.	.	.	.
Y	Y	Y	Y

→

	1	2	3	4
Poss.1	A	A	A	A
Poss.2	A	C	A	A
Poss.3	A	A	C	A
Poss.4	A	A	A	C
Poss.5	A	C	C	A
Etc.				

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



A C D E F G H I K L

A - D L N G H - K L

↓

A - D - - G H - K L

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

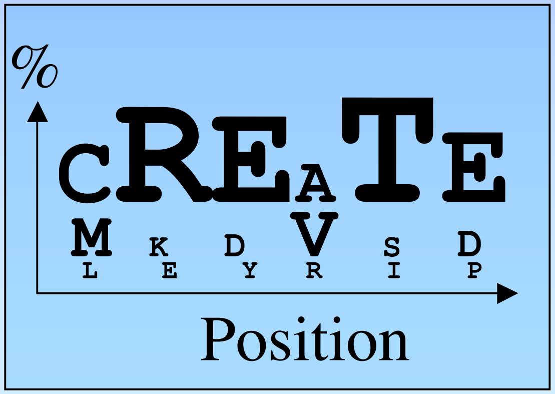
- Pattern descriptors must allow for approximate matching by defining an acceptable distance between a pattern and a potential hit
  - Weigh the sensitivity and specificity of a pattern
- What is the likelihood that a pattern would randomly occur?

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

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

```
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
```

← Target sequences

Resulting Consensus: I T L S

PSSM

P O S	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

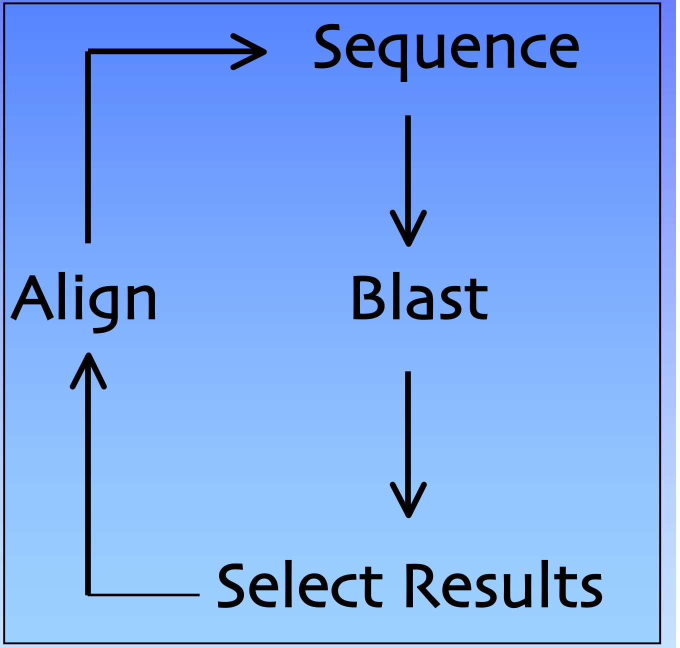
- Score-based sequence representations for searching databases
- Goal
  - Limit the diversity in each column to improve reliability
- Problems
  - Differing length gaps between conserved positions (unlike patterns)

PSI-BLAST Implementation

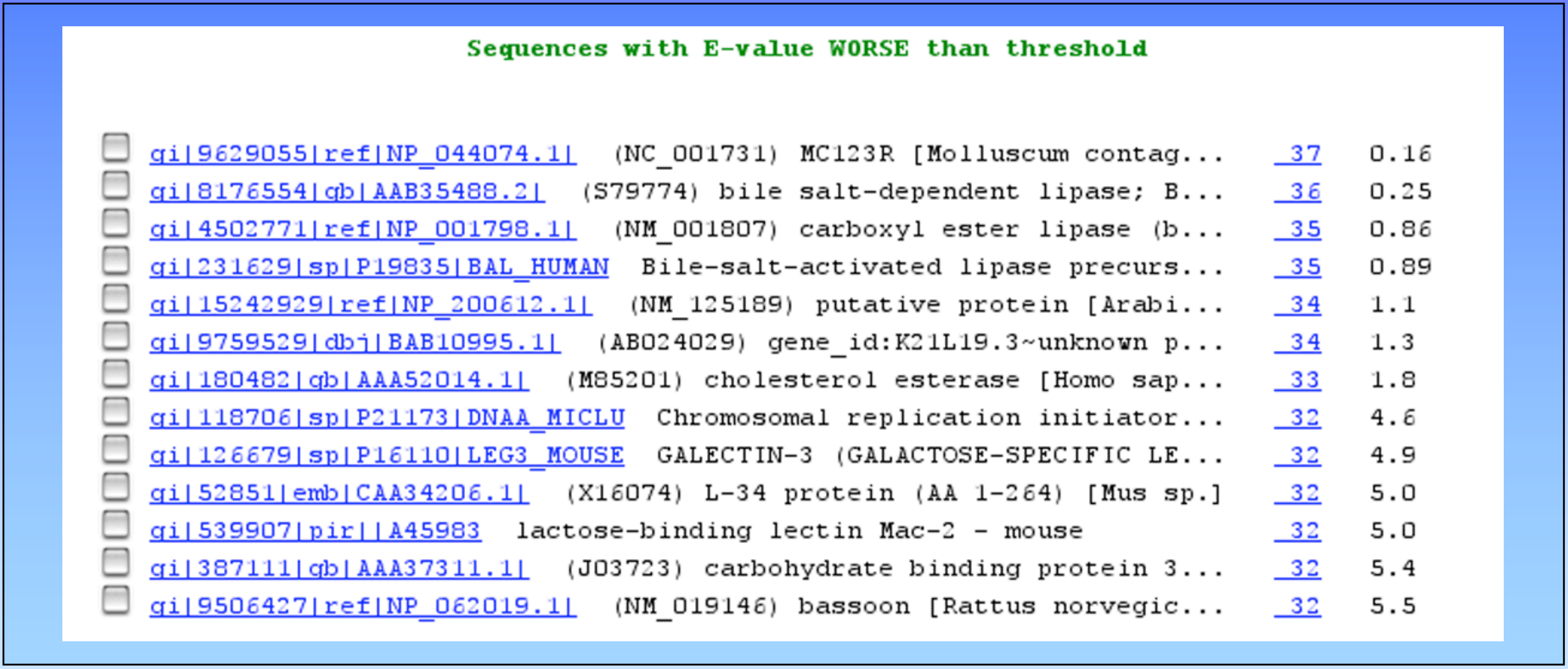
- **PSI-BLAST**

  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|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 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

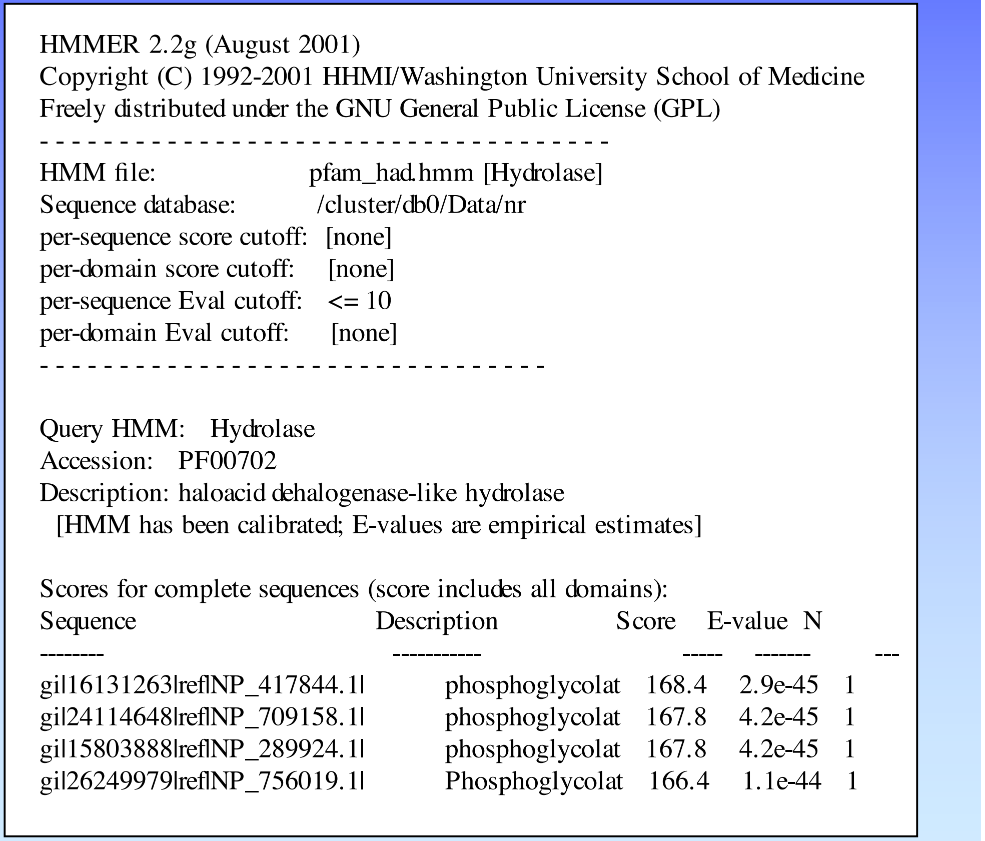
    -
      ```
      %hmmbuild [-options] <hmmfile output> <alignment file>
      ```
  - Calibrate profile (re-calc. Parameters by making a random db)

    -
      ```
      %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_had.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

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

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

```
any(CF) any(HF) any(GK) 1…1 any(IL) 4…4 any(AS) 3…3 any(LI) 3…3 any(GA) 3…3 G 1…1 any(YF) 1…1 any(LI) R
```

- 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 Ouellete. Wiley Interscience, 2001.
- Bioinformatics: Sequence, structure, and databanks. Des Higgins and Willie Taylor. Oxford University Press, 2000.