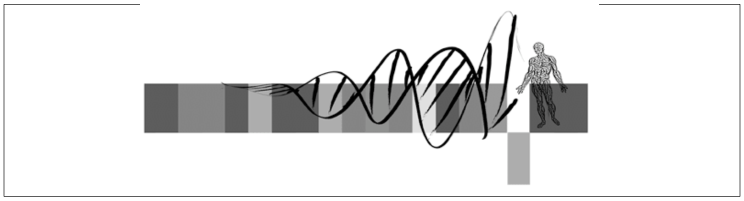

# Bioinformatics for Biologists

## Comparative Protein Analysis:

## Part II. Sequence Pattern and Profile Database Searching

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# Knowledge Exploration

- **Phylogenetic Trees** and **Multiple Sequence Alignments** are important tools to understand relationships between known 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 newly discovered tell you about their function?

- Discovering sequence **Families**

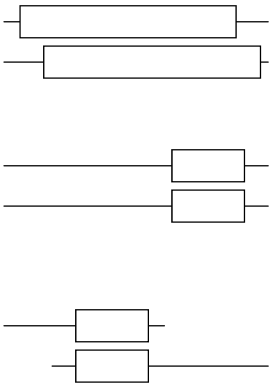
# Syllabus

(Finding Family Members)

- **Protein Families**
  - Protein Domains
  - Family Databases & Searches
- Searching for Homologous Sequences Using Patterns/Profiles
  - Pattern Searches
    - Patscan
  - Profile Searches
    - PSI-BLAST/HMMER2

# Proteins As Modules

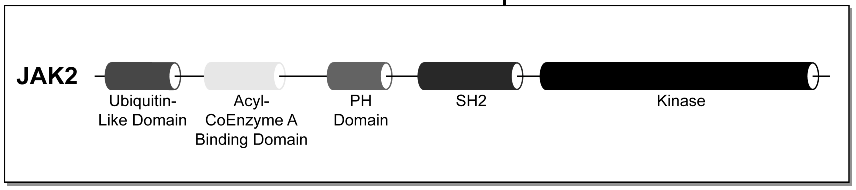
- Proteins are derived from a limited number of basic building blocks (**Domains**)
- Evolution has shuffled these modules giving rise to a diverse repertoire of protein sequences
- As a result, proteins can share a global or local relationship



(Higgins, 2000)

# Protein Domains

Janus Kinase 2 Modular Sequence Architecture



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
```

Motifs describe the domain

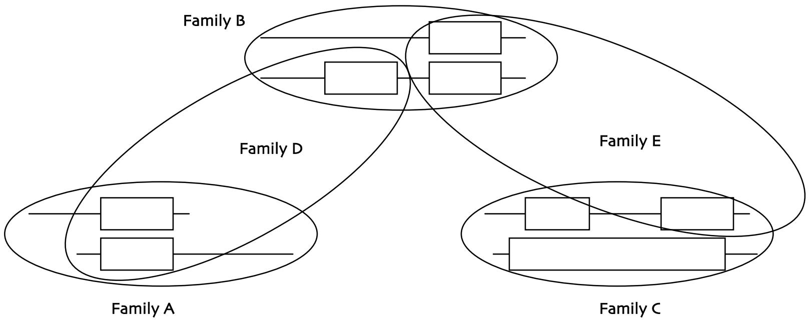
# Protein Families

- **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

# Protein Families



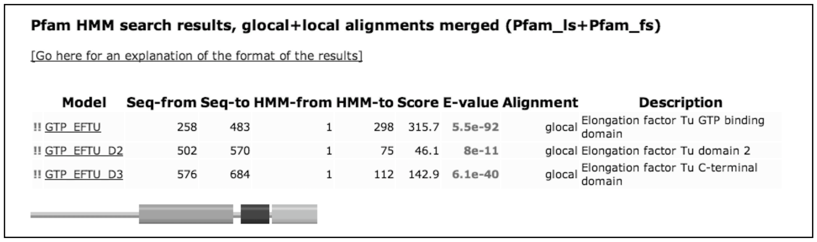
# Family Database Resources

- **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
- **Search** and **Browse**

*(Higgins, 2000)

# Curated Family Databases

- **Pfam** (http://pfam.wustl.edu/hmmsearch.shtml/) **
  - Uses manually constructed seed alignments and PSSM 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/)



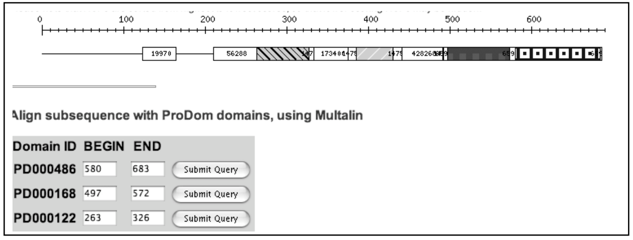
**Pfam HMM search results, glocal+local alignments merged (Pfam_ls+Pfam_fs)**

[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/doc/prodom.html)
  - Searchable against MSAs and consensus sequences
- **Protomap** (http://protomap.cornell.edu/)
  - Swiss-Prot based and provides a tree-like view of clustering

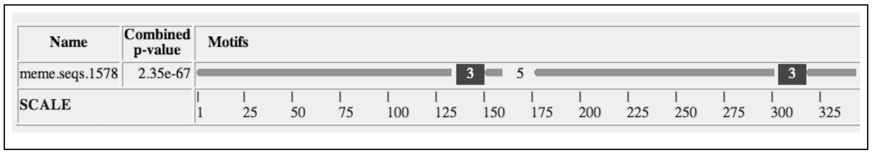


**Align subsequence with ProDom domains, using Multalin**

| 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 |

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

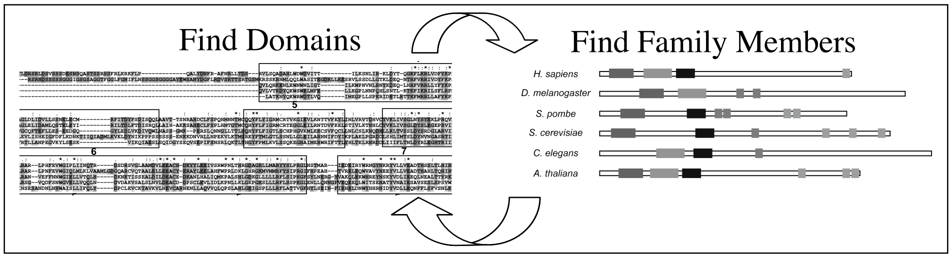
# Syllabus

(Finding Family Members)

- Protein Families
  - Protein Domains
  - Family Databases & Searches
- **Searching for Homologous Sequences** (Finding Family Members)
  - Pattern Searches
    - Patscan
  - Profile Searches
    - PSI-BLAST/HMMER2

# 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)


Find Domains

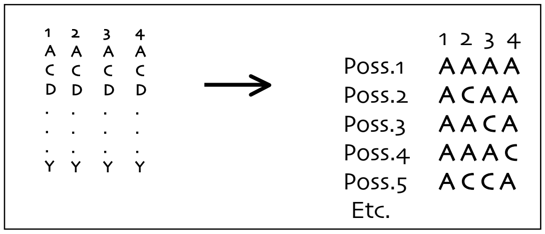
Find Family Members

# 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

# Discovery Algorithms

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



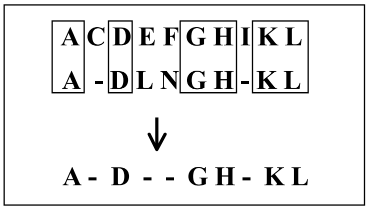
```
1 2 3 4
A A A A
C C C C
D D D D
. . . .
. . . .
. . . .
Y Y Y Y
```

→

```
        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.
```

# 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
```

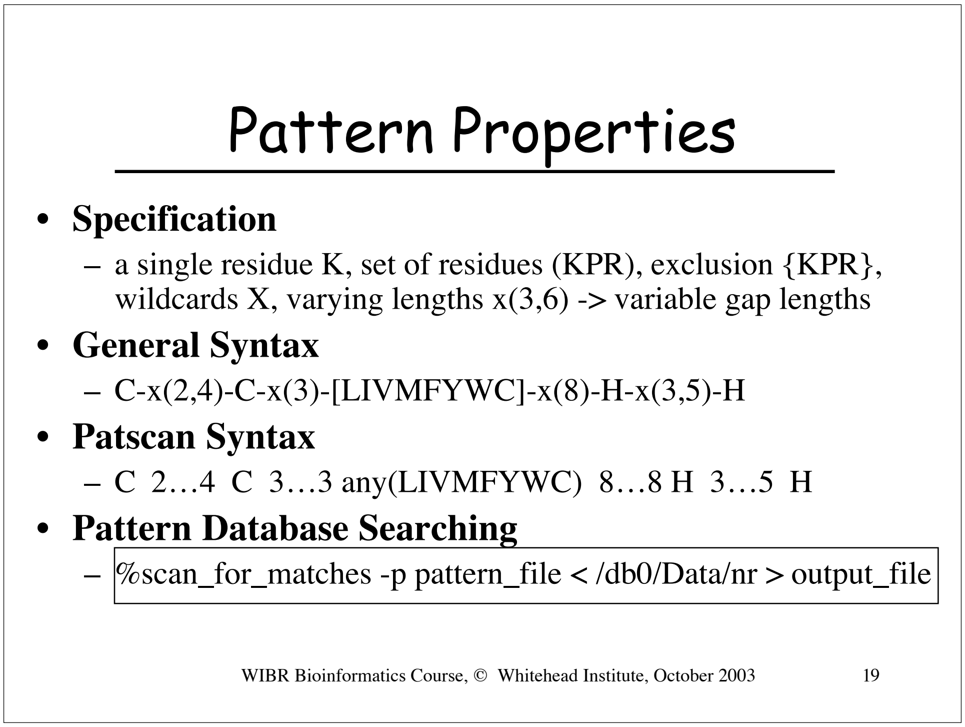
# Pattern Building

- Find patterns like “aa1 xx aa2 xxxx aa3”
  - 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
```

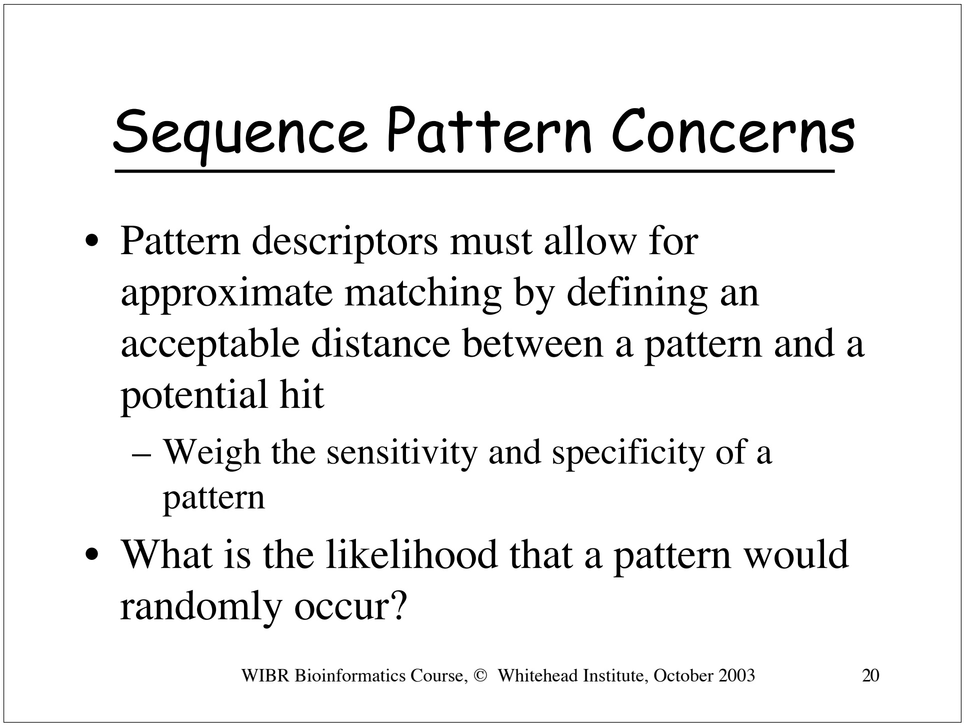
**C xxxx C xxxx [LIVMFW] xxxxxxxx H xxxxx H**

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**
  - C  2…4  C  3…3 any(LIVMFYWC)  8…8 H  3…5  H
- **Pattern Database Searching**
  - 
    ```
    %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, 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

# 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
```

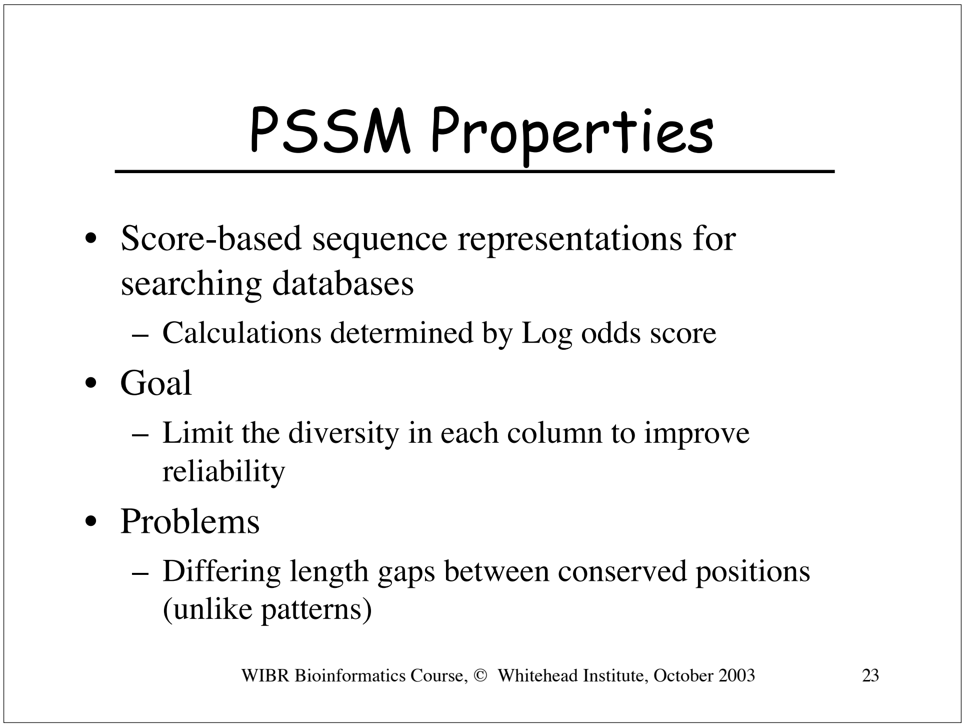
( i.e. Distribution of aa in an MSA column)

← Target sequences

*Resulting Consensus:* I T L S

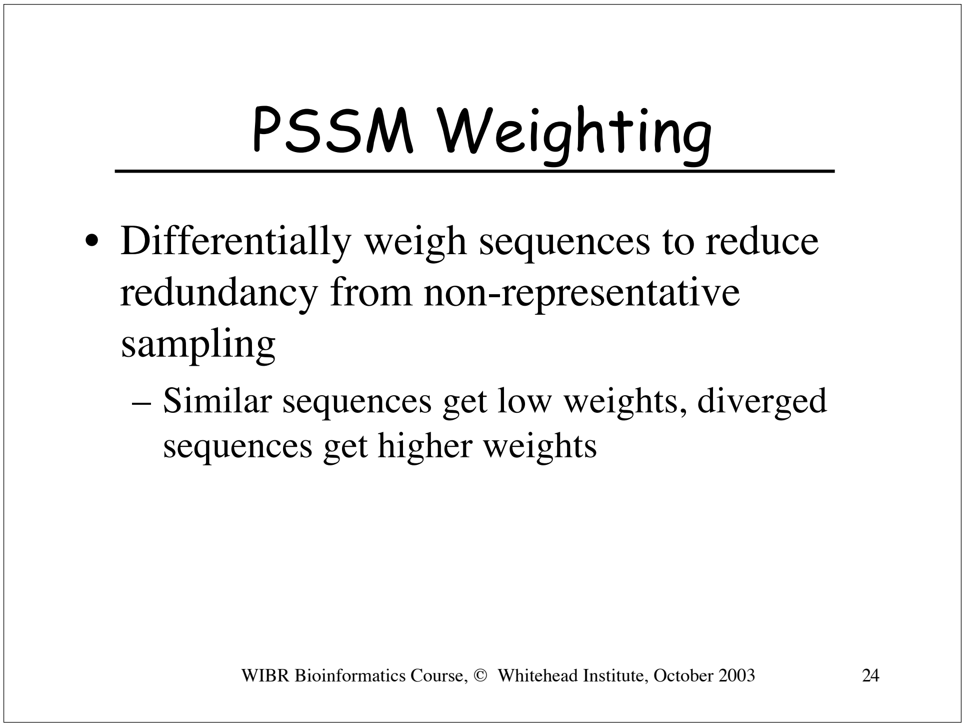
PSSM

| P<br>O<br>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
  - Calculations determined by Log odds score
- Goal
  - Limit the diversity in each column to improve reliability
- Problems
  - Differing length gaps between conserved positions (unlike patterns)



## PSSM Weighting

- Differentially weigh sequences to reduce redundancy from non-representative sampling
  - Similar sequences get low weights, diverged sequences get higher weights

# PSI-BLAST Implementation

- **PSI-BLAST**

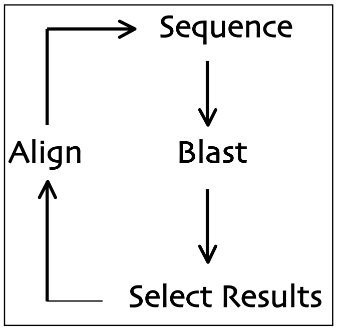
  http://www.ncbi.nlm.nih.gov/BLAST/

  – 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

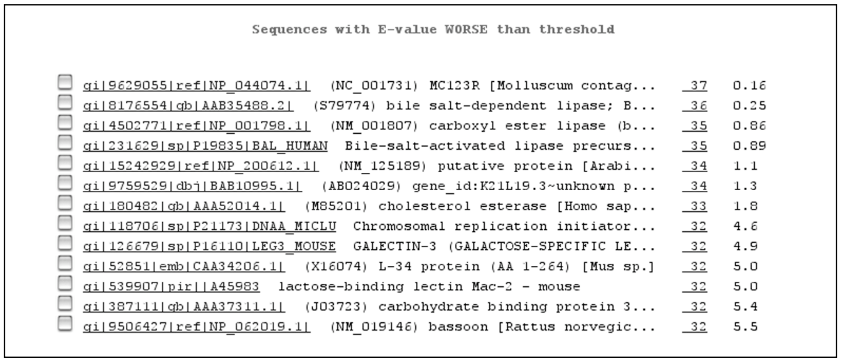
  – Iterate until process stabilizes

  – Focus on domains, not entire sequences

  – Greatly improves sensitivity



# 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 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/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

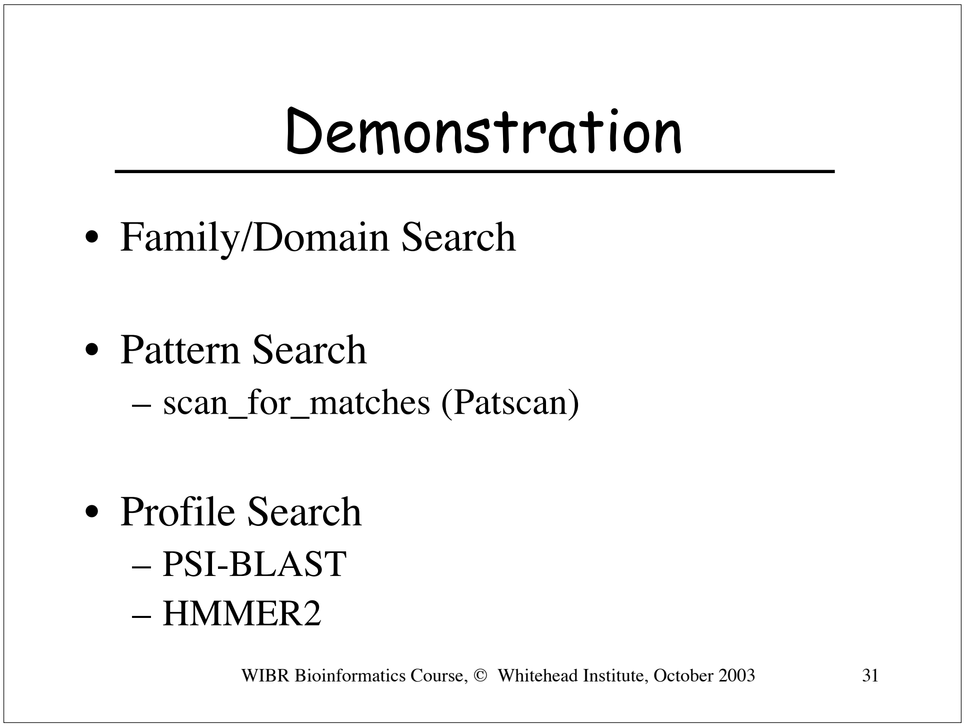
```
HMMER 2.2g (August 2001)
Copyright (C) 1992-2001 HHMI/Washington University School of Medicine
Freely distributed under the GNU General Public License (GPL)
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
HMM file:                pfam_had.hmm [Hydrolase]
Sequence database:         /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
```

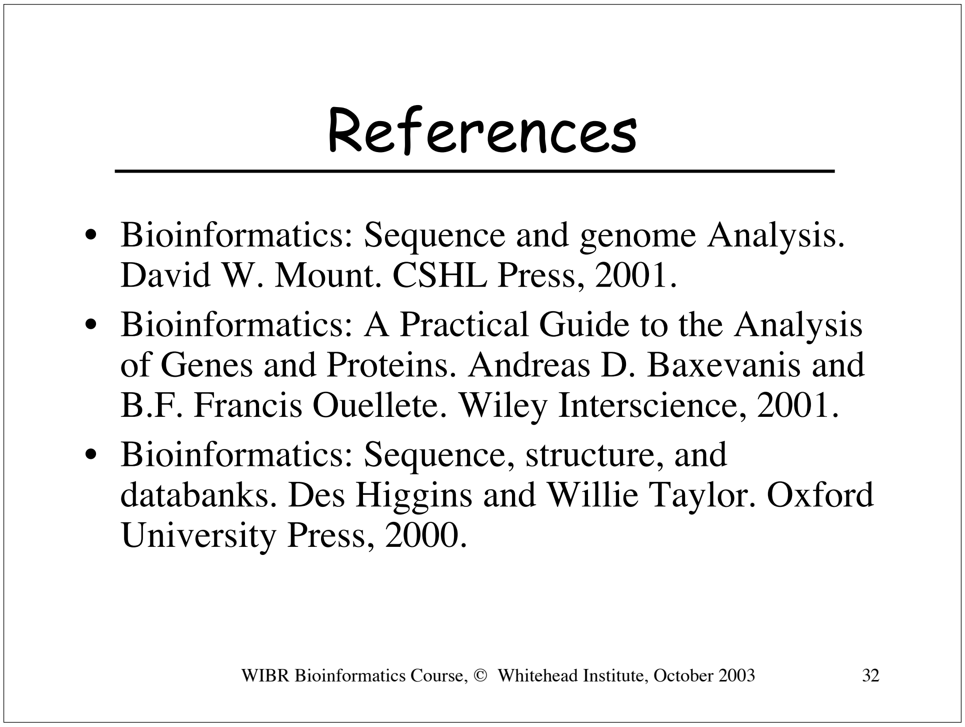
# Patterns vs. Profiles

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



# Demonstration

- Family/Domain Search
- Pattern Search
  - scan_for_matches (Patscan)
- Profile Search
  - PSI-BLAST
  - HMMER2



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