Soft Computing in Bioinformatics

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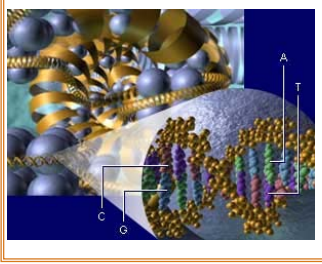
With a lot of help from our friends at MU, Univ of Utah, Univ. West FL and Indian Statistical Institute



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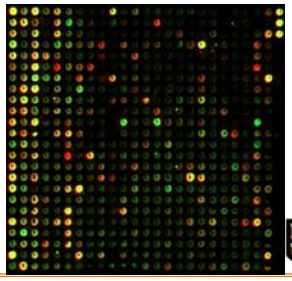

Outline

I. Background

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   ii. Structure
2. Microarrays (expression, hypermethylation)
3. Taxonomies: Gene Ontology and MeSH.

II. Gene Product Similarity Measures

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4. BLAST
5. GO-based measures
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Outline (Continued)

III. Visualization and Clustering

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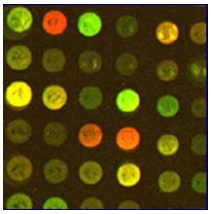
2. Visual Assessment of cluster Tendency
3. FCM and NERFCM
4. Bi-clustering (AKA co-clustering, two-way clustering)

IV. Knowledge Discovery

1. Functional annotation of gene products
2. Functional Clustering of proteins in families
3. Summarization of a set of gene products
4. Hot applications:
   i. Methylation microarrays
   ii. Learning biochemical networks from microarray data

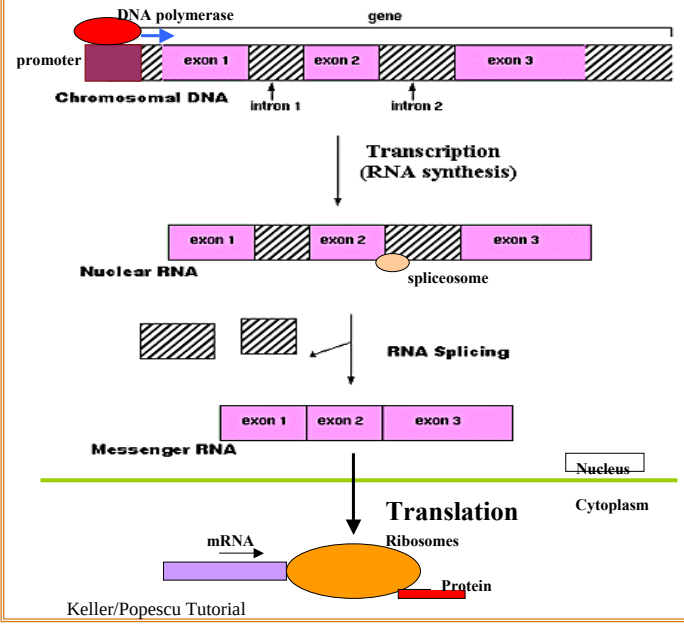
I. Background

Introduction



- **Principal features of gene products are**
  - **the sequence and expression values following a microarray experiment**
- **Sequence comparisons**
  - **DNA, Amino Acids, Motifs, Secondary Structure**
- **For many gene products, additional functional information comes from**
  - **the set of Gene Ontology (GO) annotations and**
  - **the set of journal abstracts related to the gene (MeSH annotations)**
- **For these genes, it is reasonable to include similarity measures based on these terms**

I.1. Gene Product Sequences



- DNA=sequence of nucleotides {A,C,**T**,G,(N-any)}
- RNA= sequence of nucleotides {A,C,**U**,G,(N-any)}
- Protein=sequence of 20 amino acids

Protein structure:

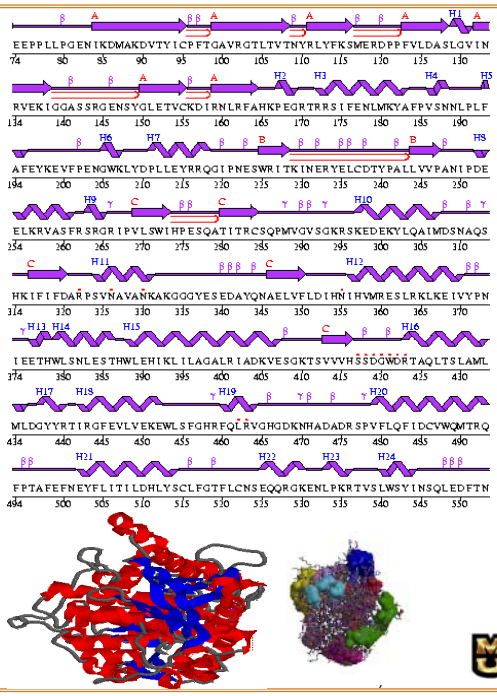
-primary (AA sequence)

-secondary (coils, β-sheets, turns)

-tertiary structure

-quaternary structure

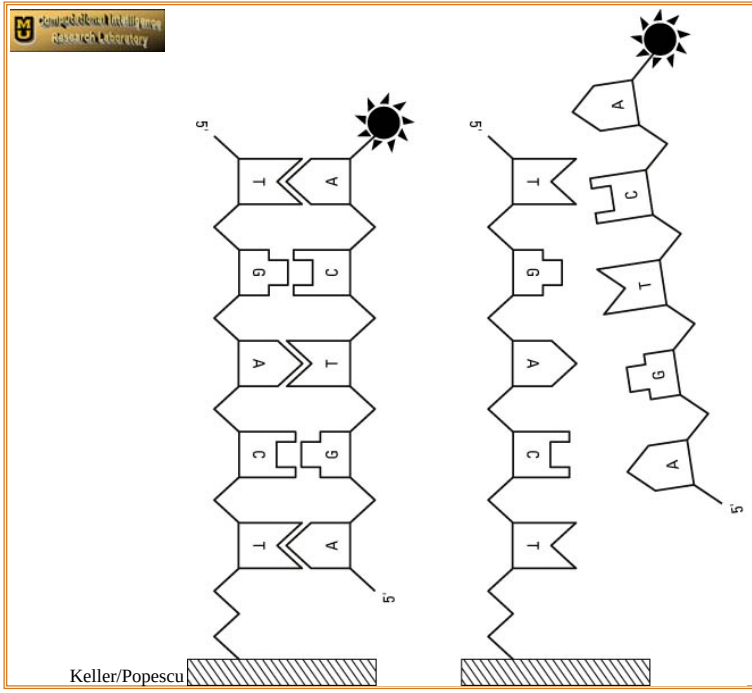
Example: MTMR2 (Myotubularin-Related Protein-2)

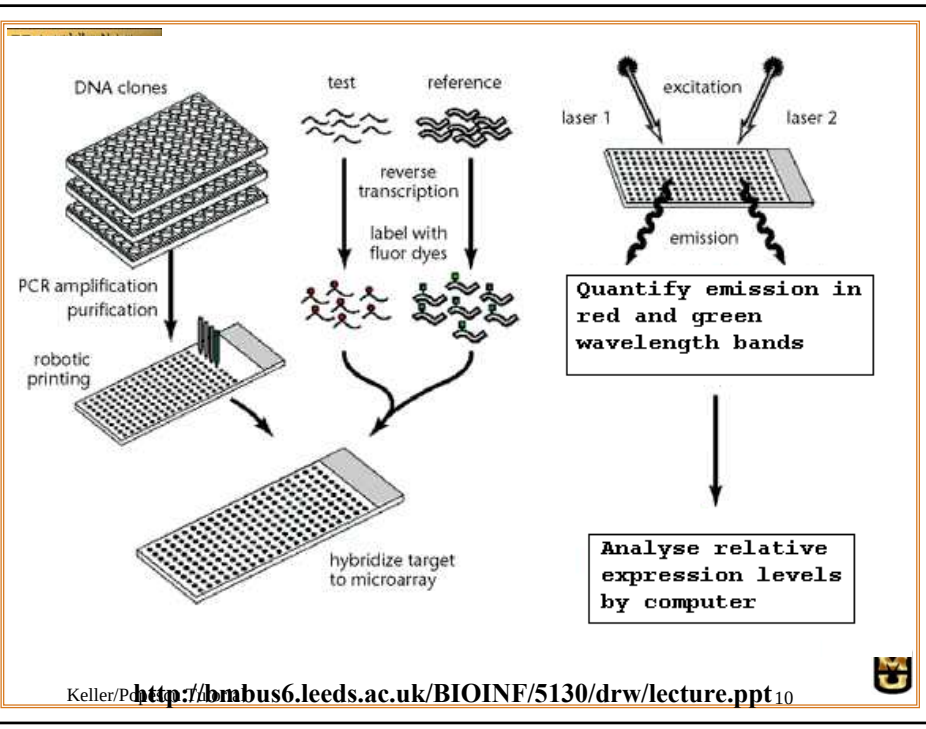


Keller/Popescu Tutorial

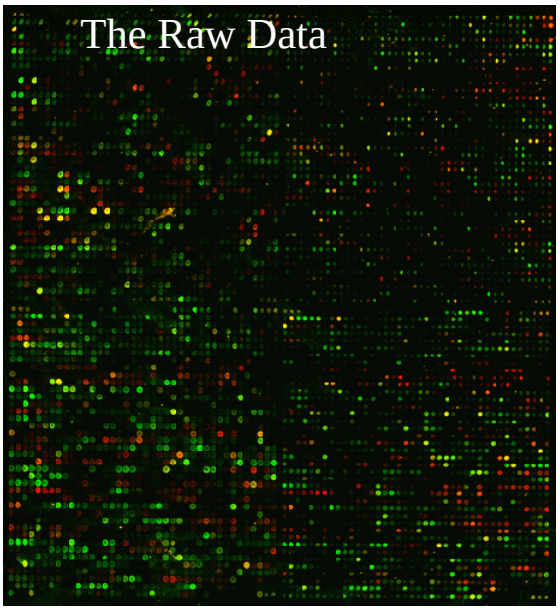
I.2. How Do Microarrays Work?

- **Conceptual description:**
  - **Set of targets are immobilized in predetermined positions on a substrate**
  - **Solution containing tagged molecules capable of binding to the targets is placed over the immobilized targets**
  - **Binding between targets and tagged molecules occurs**
  - **Tags allow you to visualize which targets have been bound (and thereby tell you something about the molecules that were present in your solution or about the location of the targets)**

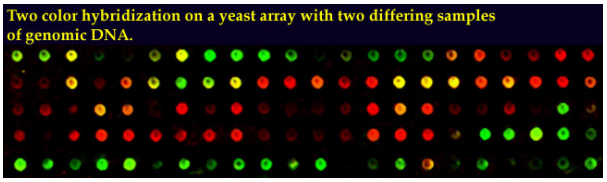

5'
T
A
G
C
A
T
C
G
T
A
5'
A
C
T
G
A
C
T
G
A
5'


DNA clones
test
reference
excitation
laser 1
laser 2
reverse transcription
label with fluor dyes
emission
PCR amplification
purification
robotic printing
Quantify emission in red and green wavelength bands
hybridize target to microarray
Analyse relative expression levels by computer

The Raw Data



The Raw Data



- **Measuring mRNA expression levels of many genes in a single experiment**
- **Conceptually: one spot per gene, 10s of thousands spots per array**
- **Relative mRNA levels between two samples are being measured**
- **Red (from cy5) sample 1 > sample 2**
- **Green (from cy3) sample 2 > sample 1**
- **Yellow (cy3 + cy5) sample 1 = sample 2**
- **Black nothing in either**

Taken from http://cmgm.stanford.edu/pbrown/scanner.html

and http://bmbus6.leeds.ac.uk/BIOINF/5130/drw/lecture.ppt

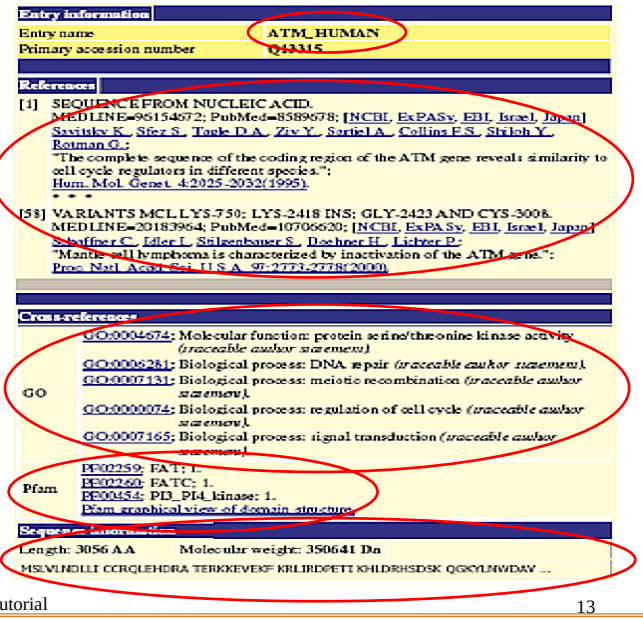
Bioinformatics Databases (Swiss-Prot, http://www.ncbi.nlm.nih.gov, etc)

Literature

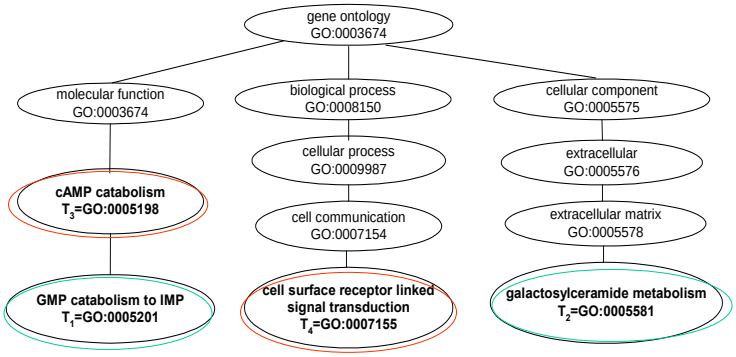
GO terms

Domains

Sequence

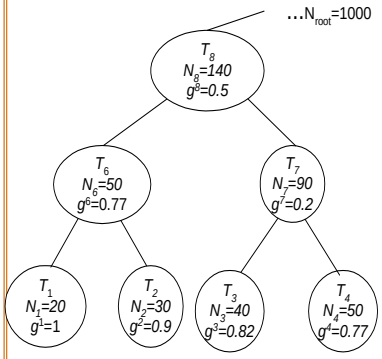


I.3. Taxonomies: Gene Ontology



- **Gene ontology (GO) = a controlled terminology**
- **DAG with "is-a" and "part-of" relationships**
- **http://www.geneontology.org**
- **COL21A1 : $G_1=\{T_1,T_2\}$ COL27A1 $G_2=\{T_3,T_4\}$.**

Similarity in an Ontology (DAG)



$s(T_1, T_3) = IC(T_8) = 0.5$

- **Problem: s(T1, T3)=?**
- **Many approaches: path-based, depth-based, density-based, information content…**
- **We use information content:**
  - **Count the occurrence N_i of each term and all children in a corpus (Swiss-Prot)**
  - **Compute term probability**

$$p(T_k) = \left(\frac{N_k}{N_{root}} \right)$$

  - **Information content of a term is**

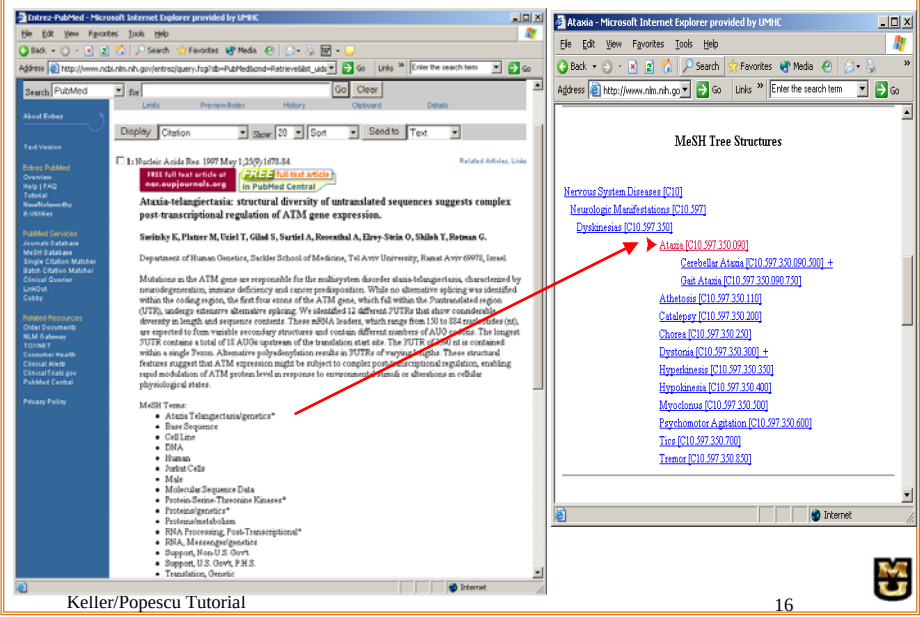
$$g^k = IC(T_k) = -\ln(p(T_k)) / \max_{T_j \in GO} \{-\ln(p(T_j))\}$$

  - **Similarity between two terms is (Resnik):**

$$s(T_i, T_j) = IC[nearest_ancestor(T_i, T_j)]$$

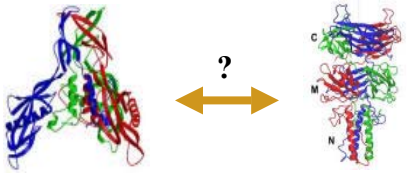
1. Resnik P., J. of Art. Int. Res. (JAIR), 11, pp. 95-130, 1999.

Medical Subject Heading (MeSH)



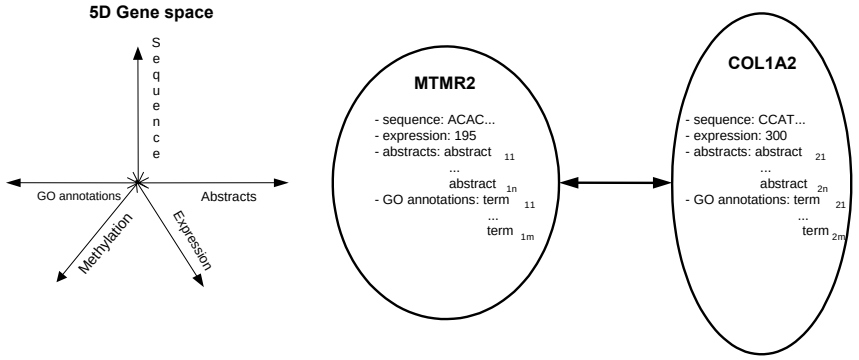
II. Gene Product Similarity Measures

II.1. How Can We Compare Two Gene Products?



World	Strings	Strings	3D Space	Gene Ontology	Domains	Medline Articles
Representation	Primary Structure (Nucleotide, Amino acids)	Secondary Structure (Coils, Sheets…)	Tertiary structure (network of atoms)	Set of GO terms	Set of Domains	Set of articles
Similarity	Blast, Fasta, Smith-Waterman	Blast, Fasta, Smith-Waterman	Euclidean, etc.	Jaccard, fuzzy measure, etc.	Fuzzy cosine, Fuzzy Jaccard...	Fuzzy cosine, Fuzzy Jaccard...

Features for Gene Product Similarity – Our Goal



MTMR2: myotubularin related protein 2

COL1A2: collagen alpha chain Type 1 Protein 2

Sequence Comparison: Definitions

- **Sequence alignment:**
  - **A one-to-one matching of two sequences so that each character in a pair of sequences is associated with a single character of the other sequence or with a null character (gap)**

```
-AGGCTATCACCTGACCTCCAGGCCGA--TGCCC---
 || ||||||| |||| |  || |||  |||||
TAG-CTATCAC--GACCGC--GGTCGATTTGCCCGAC
```

- **Types of alignment:**
  - **Pair-wise vs. multiple**
  - **Global vs. local**
  - **Gapped vs ungapped**
- **Homologous proteins: share a common ancestor**
  - **Orthologous: differ because they are found in different species**
  - **Paralogous: differ due to a gene duplication event**

Scoring the Sequence Similarity

- **Scoring matrices: each symbol pair is assigned a numerical value based on their biochemical properties**
  - **DNA scoring matrices**
  - **Protein scoring matrices: PAM, BLOSUM**
- **Gap penalties**
  - **allowing gaps can lead to high similarity values for non-homologous sequences**
  - **Penalizing gaps reduce the number of gaps**
  - **the cost of a gap is: C=a+gap_length*b**
- **Sequencer actually produces memberships in bases**
  - **Potential use of fuzzy dynamic programming**

DNA Scoring Matrices

Sequence 1

Sequence 2

```
actaccagttcatttgatacttctcaaa
     | |     |        ||
  taccattaccgtgttaactgaaaggacttaaagact
```

	A	G	C	T
A	**1**	0	0	0
G	0	**1**	0	0
C	0	0	**1**	0
T	0	0	0	**1**

Match = 1
Mismatch = 0
Score = 5

- **Other choices, e.g., Match = 5, Mismatch = –4: Score = -51**

Protein Scoring Matrices

- **Scoring matrices reflect:**
  - **# of mutations to convert one to another**
  - **chemical similarity**
  - **observed mutation frequencies**
  - **the probability of occurrence of each amino acid**
- **Widely used scoring matrices:**
  - **PAM** [Dayhoff 1978]

    PAM[1-250]: average change of all amino acid positions
  - **BLOSUM** [Henikoff 1992],

    BLOSUM[50-85]: identity between sequences used to build matrix
- **Tips on choosing a scoring matrix**:
  - ✓ **For database search the commonly used is BLOSUM62**
  - ✓ **For closely related proteins use low PAM or high BLOSUM**

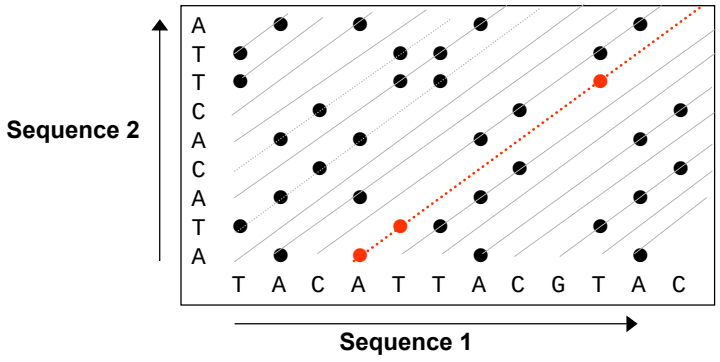
BLOSUM50

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	5	-2	-1	-2	-1	-1	-1	0	-2	-1	-2	-1	-1	-3	-1	1	0	-3	-2	0
R	-2	7	-1	-2	-4	1	0	-3	0	-4	-3	3	-2	-3	-3	-1	-1	-3	-1	-3
N	-1	-1	7	2	-2	0	0	0	1	-3	-4	0	-2	-4	-2	1	0	-4	-2	-3
D	-2	-2	2	8	-4	0	2	-1	-1	-4	-4	-1	-4	-5	-1	0	-1	-5	-3	-4
C	-1	-4	-2	-4	13	-3	-3	-3	-3	-2	-2	-3	-2	-2	-4	-1	-1	-5	-3	-1
Q	-1	1	0	0	-3	7	2	-2	1	-3	-2	2	0	-4	-1	0	-1	-1	-1	-3
E	-1	0	0	2	-3	2	6	-3	0	-4	-3	1	-2	-3	-1	-1	-1	-3	-2	-3
G	0	-3	0	-1	-3	-2	-3	8	-2	-4	-4	-2	-3	-4	-2	0	-2	-3	-3	-4
H	-2	0	1	-1	-3	1	0	-2	10	-4	-3	0	-1	-1	-2	-1	-2	-3	2	-4
I	-1	-4	-3	-4	-2	-3	-4	-4	-4	5	2	-3	2	0	-3	-3	-1	-3	-1	4
L	-2	-3	-4	-4	-2	-2	-3	-4	-3	2	5	-3	3	1	-4	-3	-1	-2	-1	1
K	-1	3	0	-1	-3	2	1	-2	0	-3	-3	6	-2	-4	-1	0	-1	-3	-2	-3
M	-1	-2	-2	-4	-2	0	-2	-3	-1	2	3	-2	7	0	-3	-2	-1	-1	0	1
F	-3	-3	-4	-5	-2	-4	-3	-4	-1	0	1	-4	0	8	-4	-3	-2	1	4	-1
P	-1	-3	-2	-1	-4	-1	-1	-2	-2	-3	-4	-1	-3	-4	10	-1	-1	-4	-3	-3
S	1	-1	1	0	-1	0	-1	0	-1	-3	-3	0	-2	-3	-1	5	2	-4	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	2	5	-3	-2	0
W	-3	-3	-4	-5	-5	-1	-3	-3	-3	-3	-2	-3	-1	1	-4	-4	-3	15	2	-3
Y	-2	-1	-2	-3	-3	-1	-2	-3	2	-1	-1	-2	0	4	-3	-2	-2	2	8	-1
V	0	-3	-3	-4	-1	-3	-3	-4	-4	4	1	-3	1	-1	-3	-2	0	-3	-1	5

Algorithms for Gene Product Sequence Similarity (Alignment)

- **Visualization: Dot Plot**
- **Dynamic programming (slow):**
  - **Smith-Waterman (local alignment) [Smith 1981]**
  - **Needleman-Wunsch (global alignment) [Needleman 1970]**
- **Heuristic (fast):**
  - **Fasta [Pearson 1990]**
  - **BLAST [Altschul 1990, 1997]**

II.2. Dot Plot Similarity Visualization



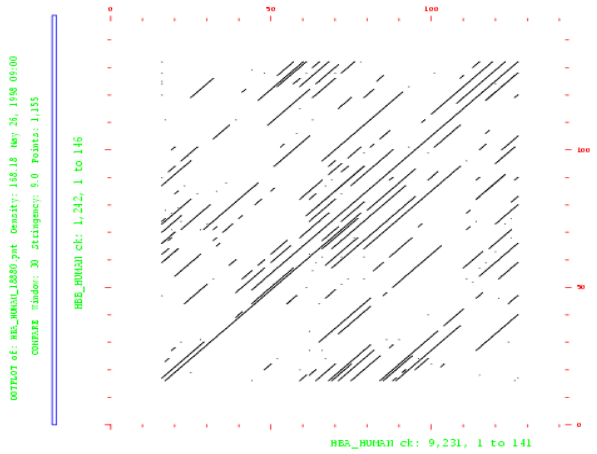
One possible alignment:

```
T A C A T T A C G T A C
    |   |       |
    A T A C A C T T A
```

Window=3, Threshold=2

Dot Plot Example

- **Hemoglobin α chain (X) vs. Hemoglobin β chain (Y) {window =30, stringency (threshold) =9}**



II.3. Smith-Waterman

- **Recurrence equation:**

$$F(i,j) = \max \{ 0,\ F(i-1, j-1) + s(x_i, y_j),\ F(i-1,j) - d,\ F(i, j-1) - d \}$$

- **Example: Align HEAGAWGHEE and PAWHEAE**

Use BLOSUM 50 for substitution matrix and d = 8 for gap penalty

		H	E	A	G	A	W	G	H	E	E
	0	0	0	0	0	0	0	0	0	0	0
P	0	0	0	0	**0**	0	0	0	0	0	0
A	0	0	0	5	0	**5**	0	0	0	0	0
W	0	0	0	0	2	0	**20**	12	0	0	0
H	0	10	2	0	0	0	12	**18**	**22**	14	6
E	0	2	16	8	0	0	4	10	18	**28**	20
A	0	0	8	21	13	5	0	4	10	20	27
E	0	0	0	13	18	12	4	0	4	16	26

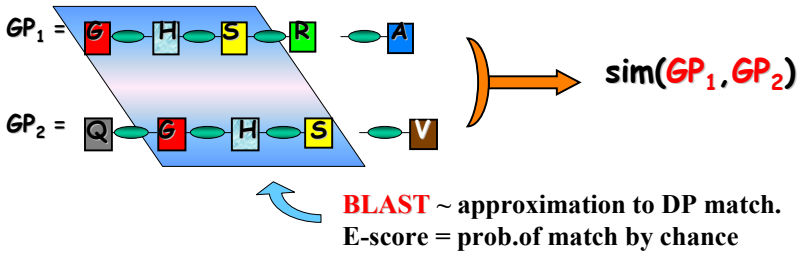
AWGHE

AW-HE

II.4. BLAST

- **B**asic **L**ocal **A**lignment **T**ool [Altschul et al. 1990, 1997]
- **Designed for searches in large sequence databases**
- **BLAST: is a heuristic that works by finding word-matches between the query and database sequences**
- **BLAST:**
  - **Searches for high-scoring local alignments between two sequences**
  - **Tests for significance of the scores found via P-values.**
- **Mathematical basis: random walk [Ewens et al. 2001, Korf et al. 2003]**

Similarity Between Pairs of Sequences



BLAST ~ approximation to DP match.
E-score = prob.of match by chance

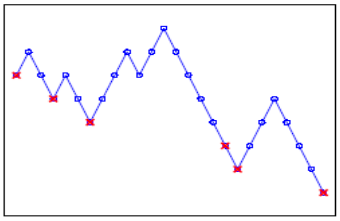
Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402.

Example of a Random Walk

DNA sequence alignment:

A G A C T G T A G A C A G C T A A T T A T G C A A
A C G C C C T A G C C A C G A G C G T A T C G C G

Score function $S(i,k)$, example: $S(i,k) = 2\delta_{ik} - 1 = \begin{cases} +1 & \text{if } i = k \\ -1 & \text{if } i \neq k \end{cases}$



Ladder points: Points in the walk lower than any previously reached point.

- Statistic of interest Y_i: Maximum height of the ith excursion after leaving the ith ladder point and before arriving at the $(i+1)$th ladder point.
- Statistic of interest $Y_{max} = \max\{Y_1, \ldots, Y_M\}$

Steps of BLAST

- **1. Filter out low-complexity regions**
  - **eliminate statistically significant but biologically uninteresting regions of the query sequence**
- **2. Create query words of length 3 (for proteins) or 11 (for DNA) from query sequence using a sliding window**

```
MEFPGLGSLGTSEPLPQFVDPALVSS
MEF
 EFP
  FPG
```

- **3. Using a scoring matrix (BLOSUM62 for proteins or +5/-4 for DNA) score all possible words of length w=3 (proteins) or w=11(DNA) against each query word**

Steps of BLAST (cont.)

- **4. Select a word threshold (T=14) and keep only the words with score>T (about 50 for each query word)**
  - **The total number of high scoring words is about 50*sequence_length**
- **5. Scan each database sequence for a match to high scoring-words. Use each match as a seed for an un-gapped alignment**
- **6. Extend each match to the left and right as long as the score increases. This extended matches are called HSP (high-scoring segment pair)**

Steps of BLAST (cont.)

- **7. Determine the statistical significance of each extended match (expect E and p-value) with score > cutoff score S**
  - **The expected number of extended matches with score >S expected by chance (E) is:**

$$E = kmne^{-\lambda S}$$

m=number of letters in the query
n=number of letters in the database
λ=normalization constant dependent on the scoring matrix
k=~0.1, accounts for possible correlation between matches

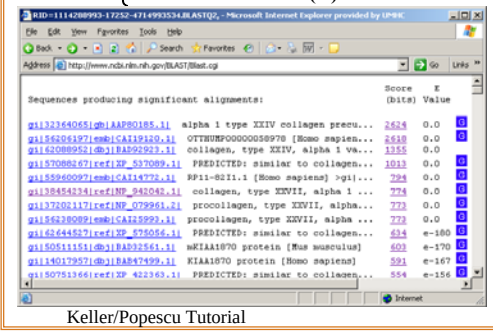
  - **The probability of such an alignment (p-value) is:**

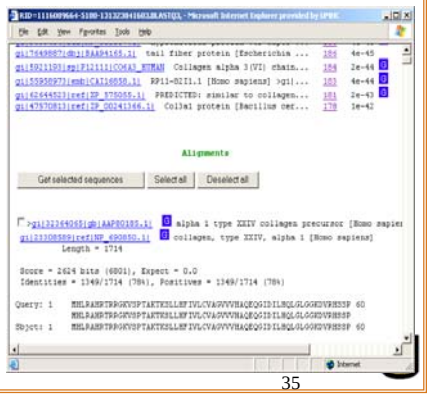
$$p\text{-}value = 1 - e^{-E}$$

Practical Considerations

- **Where to blast?**
  - **NCBI: www.ncbi.nlm.nih.gov/BLAST/**
  - **blastp for proteins and blastn for DNA**
- **How do we compute the similarity?**
  - **Use the expect E, rather than the score (S or λS)**

$$sim(s_1, s_2) = \begin{cases} 0 & \ln(E) > 0 \\ -\ln(E)/100 & -100 < \ln(E) < 0 \\ 1 & \ln(E) < -100 \end{cases}$$





References for Sequence Matching

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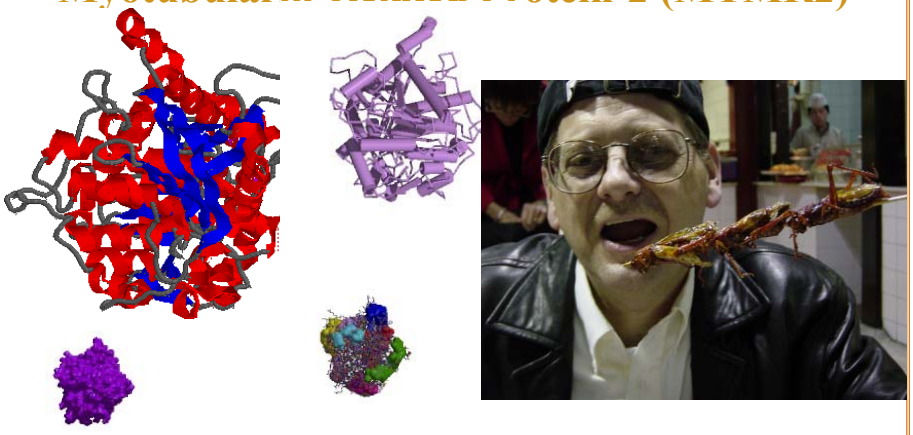
Myotubularin-Related Protein-2 (MTMR2)

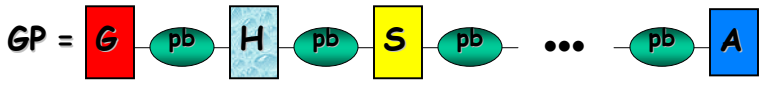


Different "looks" at the structure through a study of sequence info

II.5 Gene Ontology (GO)-based Similarity Measures

- **Gene Ontology is a Directed Acyclic Graph**
- **Three Subgraphs:**
  - **Molecular Function**
  - **Biological Process**
  - **Cellular Component**
- **Can use Information Theory to compute importance of a term and association between terms**
- **Similar Gene Products should have similar annotations**

Gene Products as Sequences of 20 AA symbols



Linear polypeptide chains of (20) Amino Acids

Gene Products as Sets of Terms from GO Nodes

GP =
- **T(4674) = protein serine/threonine kinase activity**
- **T(4672) = protein kinase activity**
- **T(4722) = protein serine/threonine phosphatase activity**
- **T(5077) = fibroblast growth factor receptor activity**

Term-Based Similarity

Given two gene products, G_1 and G_2, we can consider them as being represented by collections of terms

$$G_1 = \{T_{11},...,T_{1i},...,T_{1n}\} \qquad G_2 = \{T_{21},...,T_{2j},...,T_{2m}\}$$

The goal is to define a "natural" similarity: $s(G_1, G_2)$

There are two main approaches

- **similarities between pair-wise elements of the two sets are defined and aggregated using a given fusion operator**
- **the similarity degree can be defined globally for the two entire sets.**
  - **In a sense, here the "aggregation" is performed before the similarity is computed.**

Gene Product Similarities

Set-based Measures

Jaccard similarity: $s_J(G_1,G_2)=\frac{|G_1\cap G_2|}{|G_1\cup G_2|}$

Set Cosine similarity: $s_C(G_1,G_2)=\frac{|G_1\cap G_2|}{\sqrt{|G_1||G_2|}}$

Dice similarity: $s_D(G_1,G_2)=\frac{2|G_1\cap G_2|}{|G_1|+|G_2|}$

Vector Space-based Cosine similarity: $s_V(G_1,G_2)=\frac{v_1\bullet v_2}{|v_1||v_2|}$

v_1 v_2 are augmented vectors in an augmented space

$$G=G_1\cup G_2$$

Problems With Existing Approaches

- "Bag of word" approaches do not account for the information content of the terms
  - Example 1: $Diet_1$={apple, bread}; $Diet_2$={pork, bread}. Jaccard: 0.33 (close?)
  - Example 2: $Diet_1$={apple}; $Diet_2$={orange}. Jaccard: 0 (far?)
- Existent pair-wise approaches are inconsistent:
  - Average: $Diet_1$={apple, oranges}; $s(Diet_1, Diet_1)<1$
  - Maximum: $Diet_1$={apple, bread}, $Diet_2$={fish, bread}; $s(Diet_1, Diet_2)=1$
- No approach accounts for uncertainty
  - $Diet_1$={pork(seldom), fish (often), bread (all the time)}, $Diet_2$={pork(often), fish (seldom), bread (seldom)};

Building GO Similarities

- Similarity is computed pair-wise: $s_{ij}(T_{1i}, T_{2j})$
  - We Don't compute similarity directly, but
    - Coefficients of Association
    - Uses Information Theoretic approach
- In papers under review (with UWF and ISI)
  - We fuse with normalized LOS Operators
- Here, we look at the sets themselves

Our New Similarities

- Based on the concept of Fuzzy Measures
- Idea:
  - Terms describing gene products can be given natural "weights" if they come from taxonomies, like the GO
  - Weights may be based on "information theory" or "depth in tree"
  - Weights might be assigned by experts
  - Fuzzy measures allow the measure of the "whole" to be more (or less) than the "sum of its parts"

Novel Similarities

1. **Fuzzy measure similarity (FMS)[1]**
   - Considers the context of a term in a set
2. **Augmented fuzzy measure similarity (AFMS)[1]**
   - Addresses the case when there are no common terms
3. **Choquet integral similarity[1]**
   - Considers the uncertainty of the objects (annotations)
4. **Linear order statistics similarity (LOS)[2]**
   - A generalization of the pair-wise maximum and average
5. **PFAM domain similarity[3]**
   - Uses the distance between two HMM instead of a tree

1. M. Popescu, J.M. Keller, J.A. Mitchell, "Fuzzy Measures on the Gene Ontology for Gene Product Similarity", *IEEE Trans. Computational Biology and Bioinformatics*, accepted for publication 2005.
2. J.M. Keller, J.C. Bezdek, M. Popescu, N. Pal, J.A. Mitchell, J. Huband, "Gene Ontology-based Knowledge Discovery using GO Similarity Measures based on Linear Order Statistics", *International Journal Uncertainty, Fuzziness and Knowledge-Based Systems*, under review, 2005.
3. M. Popescu, J. M. Keller, J.A. Mitchell, "Gene Ontology Automatic Annotation Using a Domain Based Gene Product Similarity Measure", *14th IEEE International Conference on Fuzzy Systems*, Reno, Nevada, May 21-25, 2005, pp. 108-111.

II.5.i Fuzzy Measure Similarity

- **Sources of information in a set G (sensors, features, algorithms, etc.)**
  - Here, $G = \{T_1, \ldots, T_n\}$, the set of terms describing G
- **Worth of sources comes from a Fuzzy Measure:**
  $g: 2^G \rightarrow [0,1]$ such that
  - $g(\phi) = 0$ and $g(G) = 1$
  - $g(A) \leq g(B)$ if $A \subseteq B$
  - If $\{A_i\}$ is an increasing sequence of subsets of G, then

$$\lim_{i \to \infty} g(A_i) = g\left(\bigcup_{i=1}^{\infty} A_i\right)$$

Fuzzy Measures

- For a fuzzy measure *g*, let $g^i = g(\{T_i\})$

  The mapping $T_i \rightarrow g^i$ is called a fuzzy density function

- The fuzzy density value, g^i , is interpreted as the (possibly subjective) importance of the single information source T_i in determining the similarity of two genes

- General fuzzy measures are broad, but often the densities can be extracted from the problem domain or supplied by experts

- Need fuzzy measures that can be "built" from densities

Fuzzy Measures

- A fuzzy measure g is called a lamda measure (g_λ-fuzzy measure) if additionally:

  For all $A, B \subseteq X$ with $A \cap B = \phi$,

$$g(A \cup B) = g(A) + g(B) + \lambda \cdot g(A) \cdot g(B) \text{ for some } \lambda > -1$$

- For any lamda fuzzy measure λ can be uniquely determined for a finite set G by solving

$$1 + \lambda = \prod_{i=1}^{n} \left(1 + \lambda g^i\right)$$

- where $G = \{T_1, \ldots, T_n\}$ and $g^i = g(\{T_i\})$ interpreted as the (possibly subjective) importance of the single information source T_i in determining the evaluation of a hypothesis

Construction of Fuzzy Densities

- Collect all the terms (T_i) for all Gene Products in Database
- Compute Information Theoretic Content
  - Use a Corpus (like Swiss-Prot)
  - Certainly other ways to get Term importance

$$p(T_k) = \left(\frac{\text{count}(T_k + \text{children of } T_k \text{ in CORPUS})}{\text{count}(\text{all GO terms in CORPUS})} \right)$$

$$g^k = ic(T_k) = -\log(p(T_k)) / \max_{T_j \in GO} \{-\log(p(T_j))\}$$

Example GenBank ID AAN03650 (COL24A1 gene)

G = { T_1 = 5201 ("extracellular matrix structural component"),
T_2 = 7155 ("cell adhesion"), T_3 = 5581("collagen") }

$$\{g^k\} = \{0.58,\ 0.44,\ 0.65\}$$

$$1 + \lambda = (1 + 0.58\lambda)(1 + 0.44\lambda)(1 + 0.65\lambda) \Rightarrow \lambda = -0.86$$

$$g(\{T_1\}) = g^1 = 0.58,\ g(\{T_2\}) = g^2 = 0.44,\ g(\{T_3\}) = g^3 = 0.65,$$
$$g(\{T_1, T_2\}) = g^1 + g^2 + \lambda g^1 g^2 = 0.8.$$

$$g(\{T_1, T_3\}) = 0.9,\ g(\{T_3, T_2\}) = 0.84,\ g(G) = 1$$

Fuzzy Measure Similarity

New fuzzy measure similarity between two sets G_1 and G_2 of terms is defined as:

$$s_{FMS}(G_1, G_2) = \frac{g_1(G_1 \cap G_2) + g_2(G_1 \cap G_2)}{2}$$

where g_1 is a fuzzy measure defined on G_1 and g_2 is a fuzzy measure defined on G_2

Example: Two Genes From the Same Family

G_1: GenBank ID AAH35609 (MTMR4 gene)
G_2: GenBank ID AAH12399 (MTMR8 gene)

G_1={T_1=4721(protein phosphatase activity), T_2=6470(protein amino acid dephosphorylation), T_3=8270(zinc ion binding)},

G_2={T_1=4721(protein phosphatase activity), T_2=6470(protein amino acid dephosphorylation), T4=16787(hydrolase activity)}.

Densities: $\{g^{1i}\} = \{0.52, 0.57, 0.54\}$; $\{g^{2j}\} = \{0.52, 0.57, 0.33\}$

Here, the set of common terms that supports the similarity of G_1 and G_2 is {T_1,T_2}

Intra Family Example (continued)

$$s_D = \frac{4}{6} \approx 0.67, \qquad s_J = \frac{2}{4} = 0.5.$$

$$v_1 = (0.52 \quad 0.57 \quad 0.54 \quad 0.0), v_2 = (0.52 \quad 0.57 \quad 0.0 \quad 0.33) \Rightarrow$$

$$s_V = \frac{(0.52 \quad 0.57 \quad 0.54 \quad 0.0) \bullet (0.52 \quad 0.57 \quad 0.0 \quad 0.33)}{0.94 * 0.84} \approx 0.75.$$

Lambda measure for G_1 has λ=-0.84 $\quad g_1(\{T_1, T_2\}) = 0.84.$

Lambda measure for G_2 has λ=-0.72 $\quad g_2(\{T_1, T_2\}) = 0.88.$

$$s_{FMS}(G_1, G_2) = \frac{g_1(\{T_1, T_2\}) + g_2(\{T_1, T_2\})}{2} = \frac{0.84 + 0.88}{2} = 0.86$$

II.5.ii Augmented Sets

What happens if $G_1 \cap G_2 = \varnothing$?

Suppose that G_1 and G_2 are as before (terms from a taxonomy):

$$G_1 = \{T_{11}, ..., T_{1i}, ..., T_{1n}\} \qquad G_2 = \{T_{21}, ..., T_{2j}, ..., T_{2m}\}$$

Augment each set as: $G_1' = G_1 \cup \{T_{1i,2j}\}$ $\quad G_2' = G_2 \cup \{T_{1i,2j}\}$

$\{T_{1i,2j}\}$ is the set of nearest common ancestors (NCA) of every pair (T_{1i}, T_{2j})

Then $[G_1 \cap G_2]' = [G_1' \cap G_2'] = [G_1 \cap G_2] \cup \{T_{1i,2j}\}$ and calculate FMS on it

Construction of Augmented Densities

- Note: Root node (GO) has
  - Probability 1 and info content 0
- For each pair of terms (T_i, T_j) in the set of distinct terms
  - Find the Nearest Common Ancestor node NCA

$$T_{ij} = T(g_{ij}) = T(NCA\ (g_i\ ,\ g_j))$$

- And set the "augmented density" to

$$g^k = ic(T_{ij}) = -\log_2(p(T_{ij}))$$

or

$$g^k = ic(T_{ij}) = 1 - p(T_{ij})$$

Our Second Approach

- What if pairs of terms have both similarities and "importance" towards determining total gene similarity?
- For example, same or similar annotation terms to generate pair similarity and use "reliability of annotation" to create importance (fuzzy measure)
- Useful (we conjecture) for comparing based on abstracts
  - Keywords build pairwise similarities
  - Impact factors (or source of terms) give importance

II.5.iii Choquet Fuzzy Integral

Suppose that G_1 and G_2 are as before (terms from a taxonomy):

$$G_1 = \{T_{11},...,T_{1i},...,T_{1n}\} \qquad G_2 = \{T_{21},...,T_{2j},...,T_{2m}\}$$

Let $X = G_1 \times G_2$ and $s : X \to [0,1]$

To simplify the notation, we reorder the term pairs and label them by a single subscript so that $X = \{T_1, T_2, \cdots, T_{nm}\}$

$T_k = (T_{1i}, T_{2j})$ for some pair (i,j)

Then we compute $s(T_k) = s_{ij}(T_{1i}, T_{2j})$

Choquet Fuzzy Integral

Let g be a fuzzy measure on (finite set) X

Then the Choquet fuzzy integral of s with respect to g is given by

$$C(s) = \sum_{i=1}^{nm} \left[s(T_{(i)}) - s(T_{(i+1)}) \right] \cdot g(S_i)$$

where the function values are reordered so that

$$s(T_{(1)}) \geq s(T_{(2)}) \geq \cdots \geq s(T_{(nm)}) \quad s(T_{(nm+1)}) = 0$$

and

$$S_i = \{T_{(1)}, \cdots, T_{(i)}\}$$

Choquet Fuzzy Integral

Define $w_i = g(S_i) - g(S_{i-1})$ $\quad g(S_0) \equiv 0$

Then the Choquet fuzzy integral can be rewritten as

$$C(s) = \sum_{i=1}^{nm} w_i \cdot s(T_{(i)})$$

Looks linear, but isn't - Depends on the sort

II5.iv Linear Order Statistics (LOS) Similarity

- Two gene products, G_1 and G_2, represented by collections of GO terms, journal abstracts or PFAM domains:

$$G_1 = \{T_{11},...,T_{1i},...,T_{1n}\} \qquad G_2 = \{T_{21},...,T_{2j},...,T_{2m}\}$$

- The LOS similarity between G_1 and G_2 is:

$$s_{LOS}(G_1,G_2) = \sum_{i=1}^{nm} w_i s(T_{(i)}) \qquad s(T_{(1)}) \geq s(T_{(2)}) \geq \cdots \geq s(T_{(nm)})$$

where

- $s(T_{(i)})$= ordered pair-wise similarities
- $T_{(i)}$ = a pair of objects (GO terms, journal abstracts or PFAM domains), (T_{1j},T_{2k})
- $w = (w_1,\ldots,w_{nm})$ is a weight vector

- OBS: maximum=(1,0,…,0), average=(1/nm,…,1/nm);

Annotation Reliability

Numeric values chosen for the reliability of the GO annotation

Traceable author statement	Inferred from sequence similarity	Inferred from electronic annotation	Non-traceable author statement	Not docu-mented	Not recorded
TAS	ISS	IEA	NAS	ND	NR
1	0.8	0.6	0.4	0.1	0.1

Example1: Annotation Reliability Example

Earlier Intra family example:

G_1 = AAH35609 (MTMR4 gene)
G_2 = AAH12399 (MTMR8 gene)

G_1={T_1=4721(TAS), T_2=6470(IEA), T_3=8270(NR)}

G_2={T_1=4721(ISS), T_2=6470(NAS), T4=16787(NR)}

$$s(T_{1i}, T_{2j}) = \begin{vmatrix} 0.52 & 0.33 & 0 \\ 0.1 & 0.1 & 0 \\ 0 & 0 & 0.57 \end{vmatrix}$$

Annotation Reliability Example

How to Generate Densities?

Reliabilities: $\{c^{1i}\} = \{1, 0.6, 0.1\}$ $\{c^{2i}\} = \{0.8, 0.4, 0.1\}$

Densities:
$$c^{ij} = c(T_{1i}, T_{2j}) = \begin{vmatrix} 0.8 & 0.4 & 0.1 \\ 0.6 & 0.4 & 0.1 \\ 0.1 & 0.1 & 0.1 \end{vmatrix}$$

$$c^{ij} = \min(c(T_{1i}), c(T_{2j}))$$

Annotation Reliability Example

Sorted Similarities and Associated Densities

$\{s(T_{(i)})\} = \{0.58,\ 0.52,\ 0.33,\ 0.1,\ 0.1, 0, 0, 0, 0\}$

$\{c^{(i)}\} = \{0.1, 0.8, 0.4, 0.4, 0.6, 0.1, 0.1, 0.1, 0.1\}$

Use Decomposable Measure

$$g(\{c_{(1)}, c_{(2)}\}) = \min(1, g(\{c_{(1)}\}) + g(\{c_{(2)}\}))$$

$$s_{Choquet} = [0.1(0.58 - 0.52) + 0.9(0.52 - 0.33) + 1(0.33 - 0.1) + 1(0.1 - 0.1) + 1(0.1 - 0)$$
$$= 0.5$$

Example 2: LOS Using GO Annotations

- Same 2 gene products (myotubularin family):
  - G_1= AAH35609 (MTMR4 gene) ={T_{11}=4721, T_{12}=6470, T_{13}=8270}
  - G_2= AAH12399 (MTMR8 gene) ={T_{21}=4721, T_{22}=6470, T_{23}=16787}

- Other similarities: FMS=0.86, Blast=0.85, Average=0.28, Maximum=1, Jaccard=0.5

- LOS similarity:

1. Compute pair-wise similarities and order them:

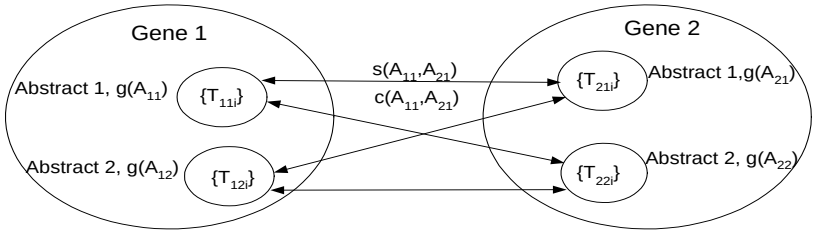
$$\{s(T_{(i)})\} = \{0.58, 0.52, 0.33, 0.1, 0.1, 0, 0, 0, 0\}$$

2. Choose the weight vector: w=(0.4 0.4 0.2 0 0 0 0 0 0)

3. Compute the LOS similarity:

$$s_{LOS} = w_1 s(T_{(1)}) + w_2 s(T_{(2)}) + w_3 s(T_{(3)}) = 0.4 * 0.58 + 0.52 * 0.4 + 0.33 * 0.2 = 0.5$$

Example 3: Gene Similarity Based on Abstract Matching



- Use Choquet Fuzzy Integral to fuse!
- What can we discover?:
  - Genes that co-occur in experiments (as reported in journals) are <u>believed</u> by the authors to be connected (even if they are not homologues)

Abstract Term Example

MeSH Terms for L32132:	Tree ID	Density
Amino Acid Sequence	G06.184.603.060	0.2
Animal	check tag	0
Base Sequence	G06.184.603.080	0.2
Carrier Proteins/analysis	D12.776.157	0.18
Carrier Proteins/chemistry	D12.776.157	0.18
Carrier Proteins/genetics*	D12.776.157	0.18
Cattle	B02.649.077.380.271	0.22
Cloning, Molecular	E05.393.220	0.18
DNA, Complementary/analysis	D13.444.308.497.220	0.22
Human	check tag	0
Liver/metabolism	A03.620	0.16
Male	check tag	0
Molecular Sequence Data	L01.453.245.667	0.2
RNA, Messenger/analysis	D13.444.735.544	0.2
Rabbits	B02.649.521.700	0.2
Rats	B02.649.865.635.560	0.22
Rats, Sprague-Dawley	B02.649.865.635.560.670	0.23
Sequence Homology, Amino Acid	G06.184.842.200	0.2
Support, Non-U.S. Gov't	check tag	0
Support, U.S. Gov't, P.H.S.	check tag	0

Matching by Abstract

- **s(ATM, STK11)=? Expert: Should be "Medium" Similar**
- **Algorithm:**
  - **Retrieve PubMed abstracts for ATM, STK11**
  - **Calculate all the pair-wise distances based on the MeSH indexing**
  - **Keep the 4 best-matching pairs**
  - **Find the impact factor for each journal: $g(A_i)$, i=1…8**

ATM	**12917635-Oncogene (6.737)**	**12970738-Oncogene (6.737)**	**14500819-Nucleic Acids Res. (6.373)**	**14499692-Science (23.329)**
STK11	**12183403 – Cancer Res (8.30)**	**12234250 – Biochem J (4.326)**	**12805220 - EMBO J. (12.459)**	**11853558-Biochem J (4.326)**

Abstract Similarity Example

Calculate the confidence of the pair (use IF, here)
$g^{ij}=g(A_1, A_2) = IF(A_1)*IF(A_2)$ and normalize to max

$$\{g^{ij}\}=\begin{vmatrix} 0.19 & 0.10 & 0.29 & 0.10 \\ 0.19 & 0.10 & 0.29 & 0.10 \\ 0.18 & 0.09 & 0.27 & 0.09 \\ 0.67 & 0.35 & 1.00 & 0.35 \end{vmatrix}$$

Abstract Similarity Example

Abstract Pairwise Similarity by FMS

$$s(A_k)_{FMS}=\begin{vmatrix} 0.44 & 0.0 & 0.00 & 0.00 \\ 0.07 & 0.29 & 0.1 & 0.11 \\ 0.00 & 0.13 & 0.26 & 0.32 \\ 0.00 & 0.20 & 0.16 & 0.24 \end{vmatrix}$$

Weighted Average:
$s_a(ATM, STK11)=0.37$

Choquet Integral
$s_{Choquet}(ATM, STK11)=0.53$

II.5.v: Domain-Based Similarity

- Two gene products described by sets of PFAM (http://pfam.wustl.edu) domains $G_1=\{M_{11}, \ldots M_{1N}\}$, $G_2=\{M_{21}, \ldots M_{2K}\}$ where M_{ij} is the number of PFAM domains λ_j contained in gene product i
- Define a PFAM domain pair-wise similarity using [1]:

$$D_s(\lambda_1,\lambda_2)=\frac{D(\lambda_1,\lambda_2)+D(\lambda_2,\lambda_1)}{2}$$

$$D(\lambda_1,\lambda_2)=\frac{1}{T}[\log P(O_1 \mid \lambda_1)-\log P(O_1 \mid \lambda_2)]$$

where O_1 is a sequence of length T generated with λ_1

- Assumption:
  - neglect the order of the domains
  - To account for the domain order we use dynamic programming together with the above HMM distance

(1) Rabiner L, Juang BH, Fundamentals of Speech Recognition, Prentice Hall, New Jersey, 1993.

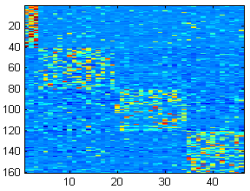
PFAM Similarity Between ATM & STKB

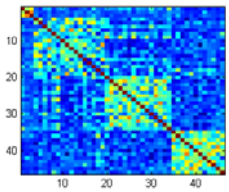
- G_1=ATM_HUMAN={M11=1(PI3_PI4_KINASE)}
  G_2=STKB_HUMAN={M21=1(PKINASE)}
- Smith-Waterman=0.04, Jaccard=0
- The HMM similarity between the 2 domains= 0.17 ➔ the gene similarity is $s(G_1, G_2)$=0.17
- OBS: in the general case we:
  - Use $M_{1i}*M_{2j}*s(\lambda_i, \lambda_j)$ for measures such as FMS, Jaccard, etc
  - Integrate $M_{1i}*M_{2j}$ w.r.t. the HMM similarity $s(\lambda_i, \lambda_j)$ for the Choquet similairty

III. Visualization and Clustering

Two Types of Data

- **Relational:**
  - **Obtained by computing the similarities between a set of objects**
  - **Examples: patient-patient in microarray experiments, gene-gene in family classification.**
  - **Algorithms: hierarchical, VAT, FCM, NERFCM**
- **Object data**
  - **Examples: Patient-genes in microarray experiments, gene-domains, gene-GO terms**
  - **C-means algorithms (hard, fuzzy, possibilistic) do not usually work due to the high dimensionality of the data (8000-30000 dimensions).**
  - **Algorithms: bi-clustering (co-clustering)**





Our Experimental Design

- Extract "families" of Gene Products
  - Sequence ID
- Get Sequence data
  - Compute sequence-based similarities
- Get GO annotations
  - Construct similarities from sets of annotating terms
  - We'll use set-based methods (like fuzzy measures)
- Visual Comparisons
- Clustering and Knowledge Discovery

Construction of $GPD194_{12.10.03}$

- 194 human gene products clustered into three protein families using the Markov clustering algorithm (Enright 2002)
- From ENSEMBL Genome Browser: www.ensembl.org

Characteristics of the $GPD194_{12.10.03}$ data set

Ensembl ID	N_i = Number of Human Gene Products	F_i = Protein Family	No. of genes
ENSF00000 000339	21	myotubularin	7
ENSF00000 000073	87	receptor precursor	7
ENSF00000 000042	86	collagen alpha chain	13

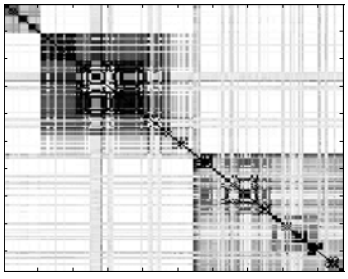
Sequence Comparison

- The 194 DNA sequences are submitted to
  - the Smith-Waterman routine and
  - the BLAST procedure to obtain
- Sets of pairwise numerical similarities
  - $\{s_{ij} : s_{ij} \in [0, 1] ; 1 \le i, j \le 194\}$ and $\{b_{ij} : b_{ij} \in [0, 1] ; 1 \le i, j \le 194\}$

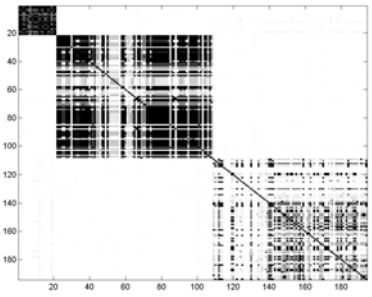
$$s_{ij} = \frac{\text{alignment_length}(\text{gene_product}_i, \text{gene_product}_j)}{\min\{\text{length}(\text{gene_product}_i), \text{length}(\text{gene_product}_j)\}}$$

$$b_{ij} = \begin{cases} 0 & \text{if } -\log(\text{E-score}) < 0 \\ 1 & \text{if } -\log(\text{E-score}) > 100 \\ -\log(\text{E-score})/100 & \text{else} \end{cases}$$

Sequence (Dis)similarity Images



Smith-Waterman



Blast

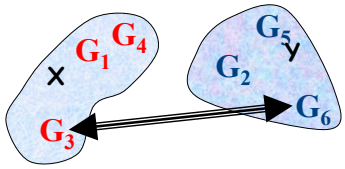
Pretty Binary!

III.1 Hierarchical Clustering ➲ U_{crisp}

Most used clustering in microarray studies

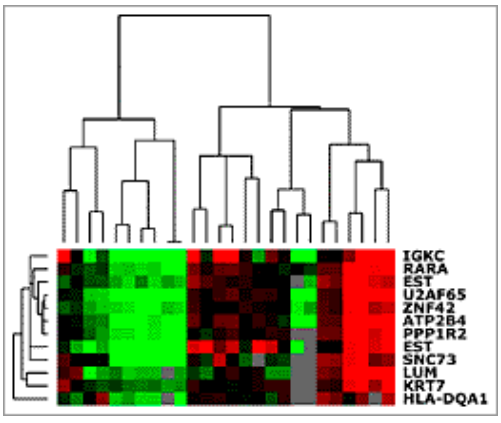
Different linkage types: complete (max), single (min), average

Input D_{nxn}	$D_{ij} \geq 0$ ⧗ $D_{ii} = 0$ ⧗ $D_{ij} = R_{ji}$
Pick	A metric δ on *pairs of sets*
From : c = n To : c = 1	Merge most similar clusters c ➲ c-1 ⧗ D_n ➲ D_{n-1}



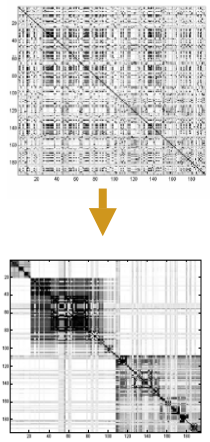
$$d_{CompleteLinkage}(X,Y) = \max_{\substack{x\in X \\ y\in Y}} \{d(x,y)\} = \max_{\substack{1\le i,j\le n \\ i\neq j}} \{d_{ij}\}$$

Example of Hierarchical Clustering



III.2 Visual Assessment of cluster Tendency (VAT)

- **Based on Minimum Spanning Tree (Prim's algorithm)**
- **Input: Dissimilarity matrix**



VAT Ordering and Display Algorithm

Step 1 Set $K = \{1,2,\ldots,n\}$; $I = J = \emptyset$; $P[0] = (0,\ldots,0)$.

Step 2 Select $(i,j) \in \arg\max_{p \in K, q \in K} \{R_{pq}\}$.

Set $P(1) = i$; $I = \{i\}$; and $J = K - \{i\}$.

Step 3 For $r = 2,\ldots, n$:

Select $(i,j) \in \arg\min_{p \in I, q \in J} \{R_{pq}\}$.

Set $P(r) = j$; Replace $I \leftarrow I \cup \{j\}$ and $J \leftarrow J - \{j\}$.

Next r.

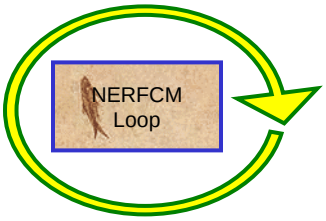
Step 4 Obtain the ordered dissimilarity matrix $\tilde{R}$ using the ordering array P as: $\tilde{R}_{ij} = R_{P(i)P(j)}$, for $1 \le i,j \le n$.

Step 5 Display the reordered matrix $\tilde{R}$ as the ODI $\tilde{I}$ using the conventions given above.

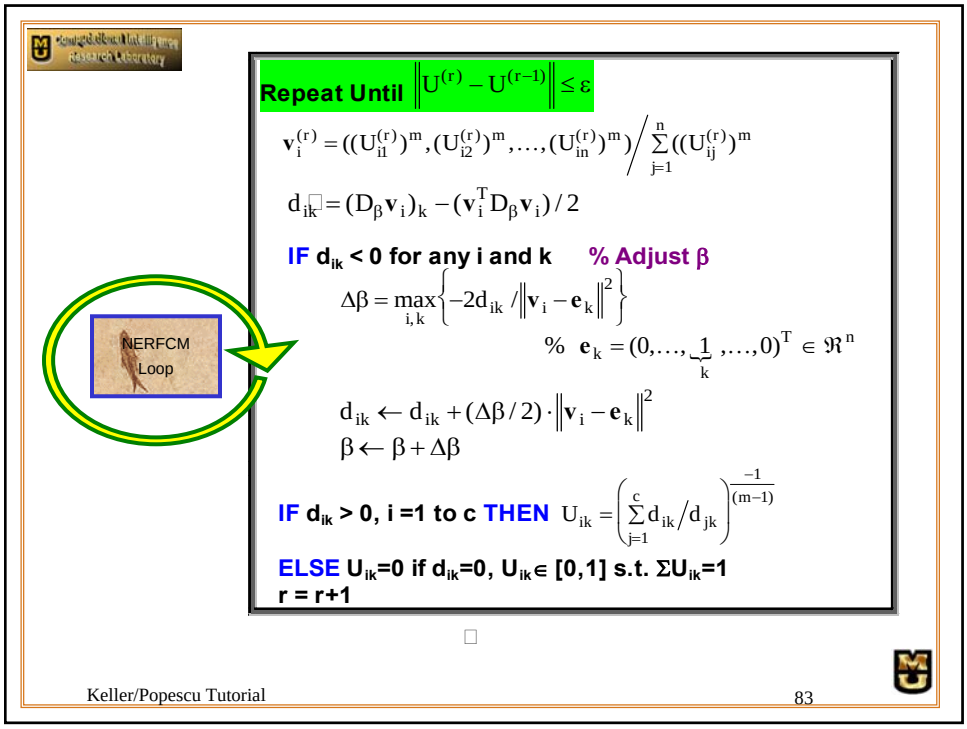
III.3 Non-Euclidean Relational Fuzzy C-means (NERFCM)

input: Dissimilarity matrix,D; output: Fuzzy memberships, U_{fuzzy}

Input	$D_{ij} \ge 0$	$D_{ij} = D_{ji}$	$D_{ii} = 0$	$D^{\circ} \alpha([1]_n - I_n)$
Pick	$2 \le c < n$	$m > 1$	$\varepsilon > 0$	
Initialize	$r = 0$	$\beta = 0$	$D_\beta = D + \beta[1]_n$	$U^{(0)} \in M_{fcn}$



Outputs	$U^* \in M_{fcn}$	$\{v_1,\ldots,v_c\}$



III.4 Co-Clustering

- **AKA simultaneous clustering, two-way clustering, biclustering**
- **Applied mainly in two fields: text (web) mining and bioinformatics (microarrays)**
- **Text mining: each column represents a key word, each row represent a document**
- **Microarray: each column represents a patient and each row represent a gene**
- **Idea: cluster patient (documents) and genes (key words) *simultaneously***

Why Co-Clustering?

	p_1	p_2	p_3	p_4	p_5	p_6
g_1	1	1	1	0	1	0
g_2	0	1	1	0	1	1
g_3	0	0	1	1	1	0
g_4	1	0	1	1	0	0
g_5	1	1	0	0	1	1
g_6	1	1	0	0	1	1
g_7	0	1	0	0	1	0

- **Problem reduces to finding dense submatrices**
- **Exact solution is impractical**
- **Only patients $\{p_1,p_2,p_3\}$ have the genes $\{g_1, g_2\}$ expressed**
- **Microarray significance: find subgroups of given cancer (leukemia) patients that respond different to different treatments, that is, $\{p_1, p_2, p_3\}$ respond to drug A while $\{p_4, p_5, p_6\}$ not.**

- **Web significance: documents $\{p_1, p_2\}$ can be summarized by words $\{g_1,g_2,g_3\}$; If $\{g_1,g_2,g_3\}$ can be in turn summarized by {G} (using an ontology)=> G can link to $\{p_1, p_2\}$**

Application Algorithms

- **Web mining:**
  - **Marker propagation, ping-pong: Oyanagi 2001**
  - **Fuzzy co-clustering, FCCM: Oh 2001**
  - **Fuzzy co-clustering, FSKWIC, Frigui 2002**
  - **Fuzzy co-clustering, CoDoK, Kummamuru 2003**
- **Bioinformatics:**
  - **Residue minimization biclustering: Cheng & Church 2001**
  - **Spectral graph approach: Cho & Dhillon 2001**
  - **Coupled two way clustering (CTWC): Getz 2000**

III.5 Other Clustering Algorithms Used in Bioinformatics

- **Markov Clustering**
  - **Used to cluster Swiss-Prot gene products (~150000) in families**
  - **Used Blast similarity (E-score)**
  - **Results: Ensembl browser (www.ensembl.org)**
- **Minimum spanning trees (MST)**
  - **Used for gene expression data**
- **Super paramagnetic clustering (SPC)**
  - **Used in CTWC (Getz 2000)**
  - **Uses paramagnetic spin propagation to define a local similarity measure**

Clustering References

- **Hierarchical**
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  - S. Raychaudhuri, PD Sutphin, JT Chang, RB Altman, "Basic microarray analysis: grouping and feature reduction", Trends in Biotechnology, Vol. 19, No5, May 2001.
  - Mei-Ling Ting Lee, Analysis of microarray gene expression data, Kluwer AP, Boston, MA, 2004.
- **VAT**
  - Bezdek, J.C.; Hathaway, R.J.;VAT: a tool for visual assessment of (cluster) tendency, Neural Networks, 2002. Proceedings, IJCNN '02, Volume 3, May, 2002, pp. 2225-2230.
- **NERFCM**
  - R. J. Hathaway and J. C. Bezdek, "NERF C-Means: Non-Euclidean relational fuzzy clustering", Pattern Recognition, vol. 27, No. 3, pp. 429-437, 1994.
- **FCM**
  - Claverie J.-M., Human Molecular Genetics, No. 8, pp. 1821-1183, 1999.
- **Bi-clustering (Co-clustering)**
  - Y. Cheng, G. M. Church, Biclustering of Expression Data, Proceedings of the Eighth International Conference on ISMB, 2000, Pages: 93 - 103
  - G Getz, E Levine and E Domany, Coupled two-way clustering analysis of gene microarray data. Proc Natl Acad Sci U S A 2000, 97:12079-12084
  - H. Cho, I. S. Dhillon, Y. Guan, and S. Sra, Minimum Sum-Squared Residue Co-clustering of Gene Expression Data, Proc. of the 4tf SIAM International Conference on Data Mining, pages 114-125, April 2004
  - Kummamuru, K., Dhawale, A.K., Krishnapuram, R.: Fuzzy co-clustering of documents and keywords. In: Proc. of FUZZIEEE, St. Louis, USA (2003)
  - Oh, C.H., Honda, K., Ichihashi, H.: Fuzzy clustering for categorical multivariate data. In:Proc. of IFSA/NAFIPS, Vancouver (2001) 2154-2159
  - Oyanagi, S., Kubota, K., Nakase, A.: Application of matrix clustering to web log analysis and access prediction. In: Proceedings of WEBKDD, San Francisco (2001)
  - Frigui, H., Nasraoui, O.: Simultaneous categorization of text documents and identification of cluster-dependent keywords. In: Proceedings of FUZZIEEE, Honolulu, (2002) 158-163
  - Raghuram Krishnapuram, Introduction to Knowledge Management and Text Mining, Tutorial FUZZIEEE 2003, St Louis, MO.
- **Other**
  - MCL: Enright A.J., Van Dongen S., Ouzounis C.A., *Nucleic Acids Res.*, vol. 30, no. 7, 2002.
  - MST: Ying Xu , Victor Olman, Dong Xu, Clustering gene expression data using a graph-theoretic approach: an application of minimum spanning trees Bioinformatics, Vol. 18 no. 4 2002, Pages 536-545

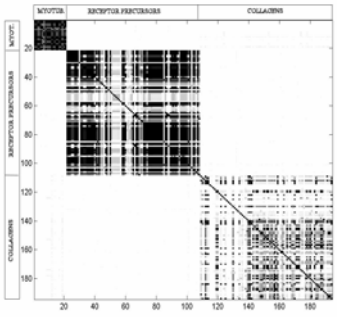
IV. Knowledge Discovery

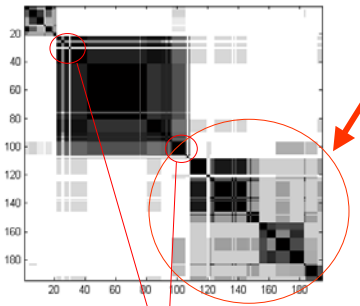
Knowledge Discovery in Bioinformatics

- **1. Clustering (and cluster validity) of gene products in families;**
- **2. Automatic annotation (GO, Domains, etc) of gene products (verification of the existent ones)**
- **3. Functional summarization of gene products (what are the main functions of a set of genes?)**
- **4. Other bioinformatics applications**
  - **a. Phylogenetic trees**
  - **b. Secondary structure prediction**
  - **c. Learning biochemical networks from microarray data**

IV.1 Clustering of Gene Products in Families

- **BLAST and FMS similarity matrix among the 194 gene products**
- **Cluster the similarity matrix using FCM[1]**
- **Collagen superfamily substructure was later confirmed by biologists[2]**





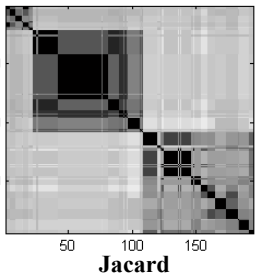
COL1A2,
COL21A1,
COL24A1,
COL27A1,
COL2A1,
COL3A1,
COL4A1,
COL4A2,
COL4A3,
COL4A6,
COL5A3,
COL9A1,
COL9A2

BLAST FMS

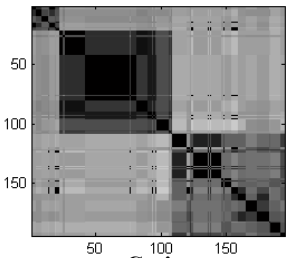
Annotation errors!

1. Claverie J.-M., Human Molecular Genetics, No. 8, pp. 1821-1183, 1999.
2. Myllyharju J, Kivirikko K.I., Trends in Genetics 2004; 20(1), pp. 33-43.

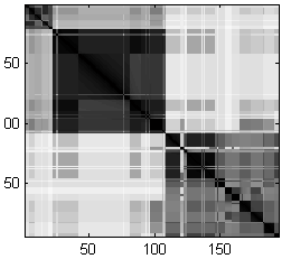
Visual Inspection: Augmented Sets



Jacard

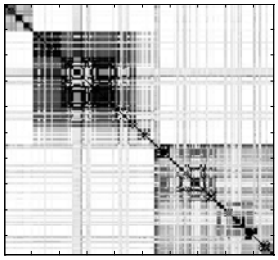


Cosine



FMS

- **Raises all similarities**
- **Somewhat stronger within family values**



Smith-Waterman

Anything More Than Just a "Pretty Face"?

Pearson's coefficient between similarities and BLAST and "Ideal"

GO similarity	FMS	AFMS	Jaccard	Average	Maximum
Person's Coefficient (vs. BLAST)	0.52	0.54	0.44	0.44	0.47
Person's Coefficient (vs. Ideal)	0.9	0.86	0.72	0.82	0.84

Pearson's coefficient for the measures using the information reliability

Similarity Measure/ Comparison target	Reliability Weighted Jaccard	Choquet
Pearson coefficient (BLAST)	0.41	0.49
Pearson coefficient (Ideal case 1-0 similarity)	0.65	0.85

Simple Clustering Example

Number of mismatches between three gene families from MCL (Ensembl) and respective similarity type using complete linkage in Hierarchical Clustering

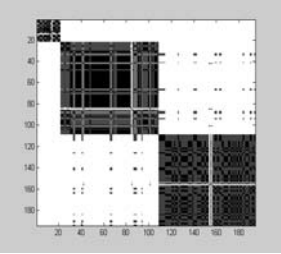
	Jaccard	Cosine	FMS	Blast
Nonaugmented	105	105	35	85
Augmented	11	27	0	---

Simple Clustering Example

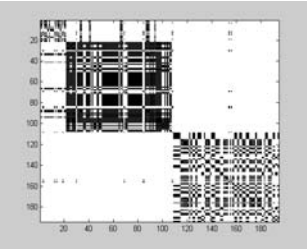
Number of mismatches between three gene families from MCL and respective similarity type using single linkage in Hierarchical Clustering

	Jaccard	Cosine	FMS	Blast
Nonaugmented	0	0	84	105
Augmented	0	106	0	---

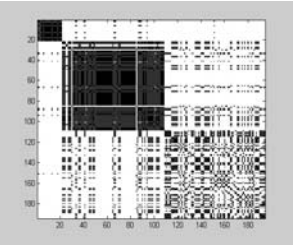
Can Look at Sub-Taxonomies, mf,cc,bp



mf-molecular function



cc-cellular component



bp-biological process

Actually we have used the MF branch for functional summarization

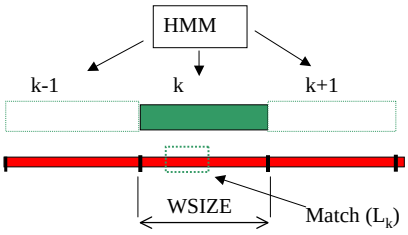
IV.2 Automatic Functional Annotation of Gene Products

- **GO similarity measures work for known genes**
  - **Where annotation terms are known**
- **What is wrong with BLAST?**
  - **The match might not be related to the function**
  - **Score accounts for the largest match => tends to be binary**
- **Represent gene products using DOMAINS**
  - **A DOMAIN is a structurally compact, independently folding unit that forms a stable 3D structure and shows a certain level of conservation**
- **We use the hidden Markov model (HMM) of a domain as found in the PFAM database http://pfam.wustl.edu/**

GO Functional Annotation (cont.)

- **Problem statement: find the functions an unknown gene product P_{unk} using a set of N genes $\{P_k\}_{k=1,N}$ with known functions**
- **Approach:**
  - **Use Gene Ontology annotations**
  - **Use HMM representation of protein domains (PFAM) to compute the similarity**
  - **Use fuzzy K-nearest neighbor to find k-most similar gene products to the unknown one**
  - **Score the annotation algorithm using a receiver-operator characteristic (ROC) curve.**

How Do We Extract Domain Features?



$$M_i = \frac{1}{WSIZE} \sum_{k=1}^{[L/WSIZE]+1} L_k$$

- **Use each HMM as a feature extractor (as implemented in hmmer-http://hmmer.wustl.edu/)**
- **Use a sliding window: WSIZE=length(HMM)**
- **For each window k, record the length of the match L_k that has a log-likelihood > THRESHOLD**
- **M_i=the amount of match ($\in$R) of domain i in the sequence**
- **A unknown sequence P: $P=\{M_1, \ldots M_N\}$**

Computation of the Similarity

- $P_1=\{M_{11}, \ldots M_{1N}\}$, $P_2=\{M_{21}, \ldots M_{2M}\}$
- Could use set-based similarity measures if we consider only M_{ij}>THRESHOLD.
- If we use vector representation ($M_{ij} \geq 0$, hence N=M=ND), the domain similarity $s_{DOM}(P_1,P_2)$ is:

$$s_{DOM}(P_1, P_2) = \frac{\sum_{i=1}^{ND} \min(M_{1i}, M_{2i})}{\sum_{i=1}^{ND} \max(M_{1i}, M_{2i})}$$

Example of Domain Similarity

- Two collagen genes, COL1A2 (collagen 1 alpha 2) and COL21A1 (collagen 21 alpha 1), contain ND=3 PFAM domains, namely:
  - COLLAGEN("Collagen triple helix repeat")
  - COLFI("Fibrillar collagen C-terminal domain")
  - VWA("von Willebrand factor type A domain").
- The domain representation is P_1 = COL1A2 = (18,1,0) and P_2 = COL21A1 = (6,0,1), the above similarity is:

  s(P1, P2) = (min(18,6)+min(1,0)+min(0,1))/
  (max(18,6)+max(1,0)+max(0,1))
  = 0.3
- This low value is "good" since they are not in the same family

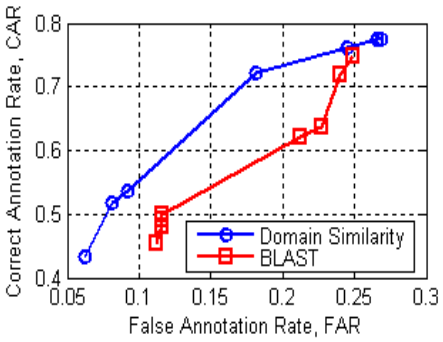
Automatic GO Annotation Algorithm

- Want to annotate an unknown gene product P_{Unk}.
- Algorithm:
  - Compute the ND features of the unknown sequence P_{Unk}
  - Compute the similarities between P_{Unk} and all N annotated gene products $\{s_{Unk,k} = s_{DOM}(P_{Unk}, P_k)\}_{k=1,N}$
  - Pick K most similar gene products $\{P_k\}_{k=1,K}$
  - Use the similarities as fuzzy memberships in fuzzy K-NN

$$w(i) = \sum_{k=1}^{K} \frac{s_{Unk,k}\,\delta_{ik}}{K}$$

  - Annotate P_{Unk} with terms i for which w(i)>THRESHOLD

Results



$$CAR = \frac{|\{t \mid t \in T_{True} \cap T_{Computed}\}|}{|T_{True}|}$$

$$FAR = \frac{|\{t \mid t \in T_{Computed} - T_{True}\}|}{|T_{Computed}|}$$

- Use the previous data set (194 known gene products, containing 13 domains)
- Use a leave-one-out scheme
- Compute CAR, FAR for THRESHOLD=0.1…0.9
- OBS: we can reach much lower FAR than BLAST for the same CAR

IV.3 Functional Summarization Using the GO

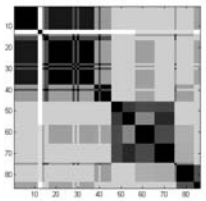
- **Given a group of N gene products, find M <N Gene Ontology terms that describes them (microarray experiments)**
- **Algorithm**:

1. **Compute the similarity matrix between the N gene products**
2. **Cluster the gene products in M clusters (M could be determined using a cluster validity measure)**
3. **Represent each cluster using i∈[1,M] the most frequent term found in cluster i.**

Functional Summarization Using the GO: Example

COL1A2, COL21A1, COL24A1, COL27A1, COL2A1, COL3A1, COL4A1, COL4A2, COL4A3, COL4A6, COL5A3, COL9A1, COL9A2

Cluster	1	2	3
FMS	5581/1 (collagen)	5587/1 (collagen type 4)	5594/1 (collagen type 9)
BLAST	16740/1 (transferase activity)	5201/0.95 (extracellular matrix structural constituent)	5201/1 (extracellular matrix structural constituent)



FMS-based Clusters Produce More Specific Summaries

IV.4 Hot Applications

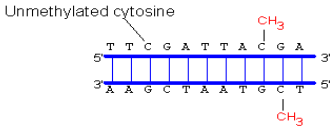
- **i. Methylation Microarrays**
- **ii. Learning Biochemical Networks From Microarrays**

IV.4.i Epigenetic Alterations in Cancer Hot Off the Press (For Us)

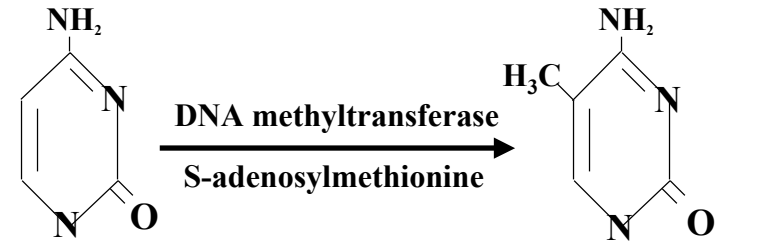
"A study of heritable changes that modulate chromatin organization and gene expression without changes in DNA sequences"

Differential Gene Methylation Microarray Data

- **Gene expression = whether the gene was transcribed or not**
- **Methylation = method of controlling gene expression**
- **Mechanism: an enzyme (methyltransferase) "tags" cytosine with a methyl group**
- **Outcome: If the promoter region (rich in CG) of a gene is heavily methylated, the gene is not expressed**
- **Reason: Not every gene should be expressed in every cell of our bodies (don't want our brain cells to make hemoglobin, the protein required to carry oxygen around in our blood)**



CpG Methylation

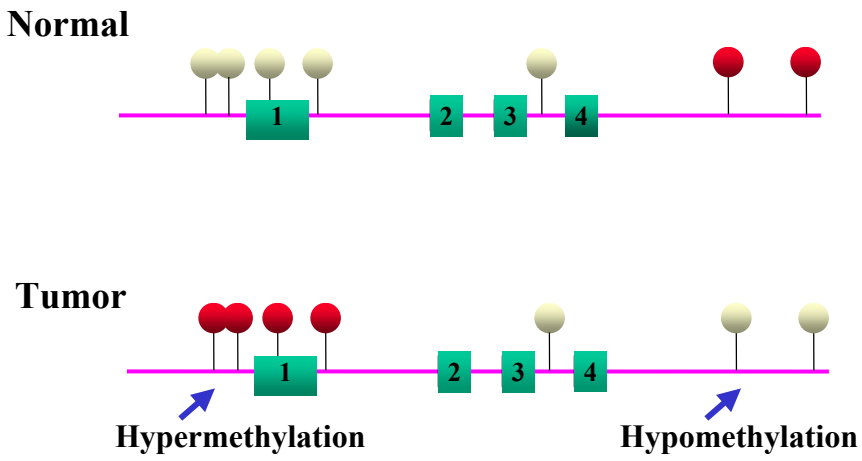


Cytosine **5-methylcytosine**

Some Facts Related to CpG Islands

- ~28,529 CpG islands per haploid genome in humans. (Based on Celera Sequencing data)
- 40-50% of all genes are associated with CpG islands.
- Other CpG islands are located in regions containing no genes.
  - Most CpG islands are <u>un</u>methylated in normal cells
  - <u>Exception</u>
    - Genes on the inactive X chromosome
    - Imprinted genes
    - Repeated sequences or transposable elements

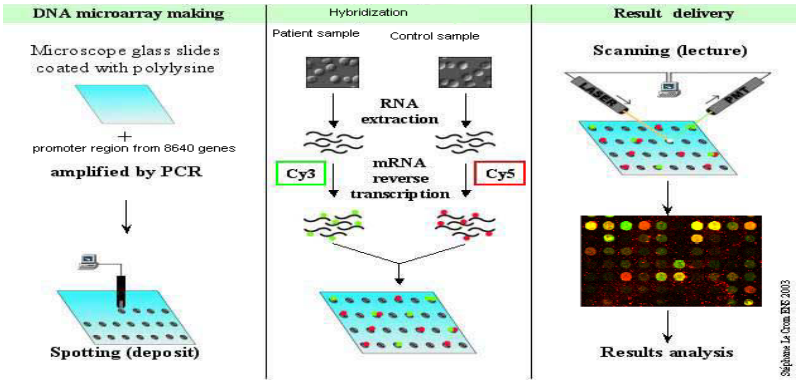
Aberrant DNA Methylation in Cancer



Hypothesis

- **CpG island hypermethylation is heritable in tumor cells**
- **Multiple methylated loci are progressively accumulated during tumorigenesis**
- **As a result, tumor cells can generate unique epigenetic signatures that are associated with specific cancer subtypes**

Methylation DNA Microarray

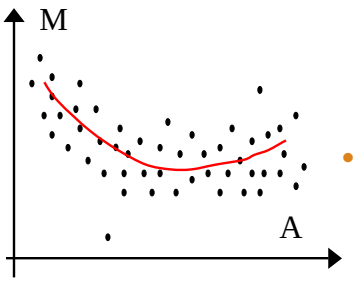


- **Sample (patient) and control genes (normal) are marked with different fluorescent dyes (Cy5-red, Cy3-green)**
- **Use a scanner to obtain for each spot 3 values: R, G, B**
- **Advantage vs. Chip microarray: can select your own genes**
- **Disadvantage: noisier data due to quality control problems**

Lymphoma Experiment

- 4 groups of patients:
  - Hyperplasia, normal (HP)-3 patients
  - 3 types of lymphoma
    - chronic lymphocytic leukemia (CLL) –16 patients
    - Follicular lymphoma (FL) – 15 patients
    - Mantle cell lymphoma (MCL)-12 patients
- The number of genes investigated is 8640.
- Goals:
  - Improve the accuracy of lymphoma classification
  - Find differentially methylated genes
- Questions:
  - What are the genes that are uniquely methylated in each group?
  - What are the genes that are uniquely unmethylated in each group?
  - Can we cluster the patients such that we match the conventional pathologically determined lymphoma diagnoses?

Normalization of Methylation DNA Microarray

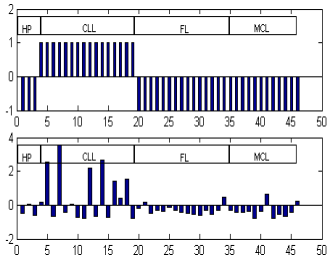


- Many normalization procedures
- Use a goal-driven approach to select best normalization: select the normalization that produces 4 clusters of patients that match best the pathologically determined lymphoma diagnoses
- Intensity-dependent normalization
  - M = log R – log G
  - A = ½[log R + log G]
  - Fit a curve (LOWESS, loess)[1]: L(A)
  - Normalize: M-L(A)

1.Y.H. Yang *et al,* Nucl. Acid. Res. 30 (2002)

Lymphoma Patient Clustering

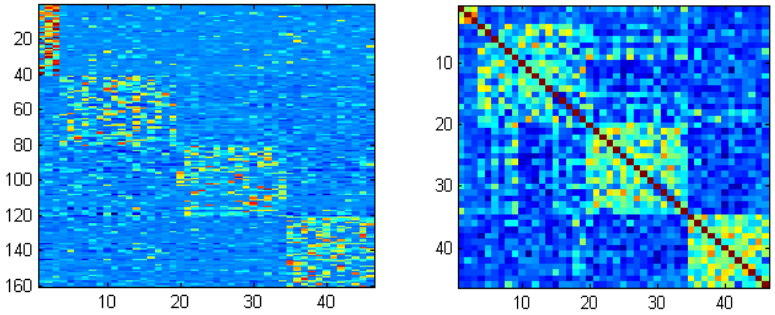
	p_1	p_2	p_3	p_4	p_5	p_6
g_1	5	6	1	0	1	0
g_2	8	7	1	0	1	1
g_3	0	0	5	7	1	0
g_4	1	0	6	9	0	0
g_5	1	1	0	0	6	8
g_6	1	1	0	0	4	6
g_7	0	1	0	0	1	0



- Select gene uniquely hypermethylated in one group
- Use a modified "idealized expression pattern" algorithm (Golub, 1999) *: compute the correlation between a gene profile and the "idealized" profile
- Use again a goal-driven approach
- Select 40 genes in each group=>each patient has 160 features
- Compute cross-correlation between patients

*Golub, TR, et al.,Science, vol 286, 531:537, 1999

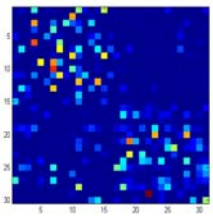
Lymphoma Patient Clustering-Results

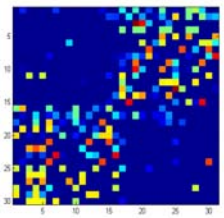


- Clustering was performed using FCM (Claverie 1999)
- The clustering of the patients based on the selected 160 genes was able to match perfectly the pathologically determined lymphoma classes.
- Initial evaluation indicates that the identified genes are indeed involved in essential cellular processes including apoptosis, and proliferation

Simultaneous Gene Selection From Methylation and Expression Microarrays

- **Data set: 31 expression microarray and 31 methylation microarray from two types of lymphoma: CLL and FL**
- **Question: select genes that are not expressed but methylated for each type of lymphoma**

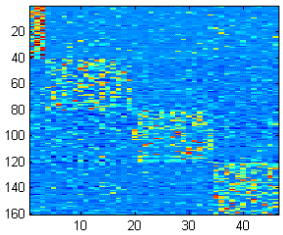




Results: Genes exclusively methylated and not expressed in FL : PSMB4, LRP1B, TSPY1/2, EIF4EBP1, MYOD1, MNAT1

IV.4.ii Learning Signaling Pathways From Microarray Data

- **For each lymphoma, find the unique gene hypermethylation pattern of signaling pathways such as apoptosis and cell proliferation**



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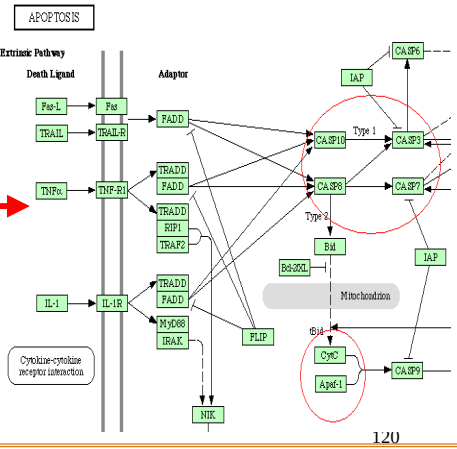

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Conclusions

- **Introduced Soft computing methods to determine gene product similarity from taxonomy terms**
  - **Use fuzzy measures on (augmented) term intersection set**
  - **Have fuzzy integrals to fuse confidence and "worth" (very general)**
  - **Investigating other combination schemes**
- **Results can (should) be combined with sequence information, e.g., domains and motifs, and expression values for robust similarity**
- **Next steps**
  - **Apply to new database of hand curated (RefSeq) proteins (~9000 proteins/~6000 Annotated)**
  - **Clustering and classification on microarray data Expression and Hyper/Hypo Methylation**
- **Knowledge Discovery**
  - **Do the clusters found exhibit linguistic similarity?**
  - **Unknown gene product maps into cluster by sequence: share the linguistic properties?**

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