Whole-Genome Annotation using Functional Linkage Networks

T. M. Murali

February 21, 2006
Data, Data, Data

- ≥ 150 genomes sequenced, 100 microbial and 50 eukaryotic.
- Computational identification of genes.
- Systematic gene knockouts.
- Gene expression data, proteomic data, metabolic data.
- Molecular interaction networks, metabolic pathways.
Introduction

Advent of Systems Biology
Introduction

**Roadblock: What do the Genes do?**

“During the last few years, we have seen enormous strides in our abilities to sequence genomes, . . . With more than 150 complete genome sequences now available and many laboratories rushing into microarray analysis, proteomic initiatives, and even systems biology, it seems an appropriate time to consider not just the opportunities those sequences present, but also their shortcomings. **By far the most serious problem is the quality and degree of completeness of the annotation of those genomes.**” (Identifying Protein Function—A Call for Community Action. Roberts RJ (2004), PLoS Biol 2(3): e42.)
Solution: Automated Functional Annotation

- Develop computational techniques that automatically integrate diverse source of data to predict function.
- Provide measures of confidence and statistical significance for each prediction.
- Present the predictions in a user-friendly manner to a biologist for designing experiments to validate prediction.
How do you Predict Function?

- Genes with similar sequences in different organisms are likely to have the same function.
- Use algorithms for computing sequence and structural similarity.
- Transfer the known function of a well-studied gene to a gene with a similar sequence that has no known functions.
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- 25% of the genes have no known sequence or structural similarity to any gene in any other organism (60% in *Plasmodium falciparum*).
- An additional 50% have poor annotations.
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We need techniques for functional annotation that go beyond sequence similarity.
What is Gene Function?

- Not an easy question to answer!
- A gene’s function has many aspects.
- Different aspects are interesting to different biologists.
- There are many ways to describe a gene’s function.
- Different groups of biologists have derived different vocabularies.
- A number of different functional catalogues exist: MultiFun (for *E. coli*), MIPS FunCat, structure-based (e.g., PFam/ProSite domains, SCOP), COG, EC, Uniprot
The Gene Ontology

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- Three Gene Ontology (GO) categories:
  - a molecular function: an activity, such as catalytic or binding activity, carried out by the gene product at the molecular level;
  - is used in a biological process: a series of events accomplished by one or more ordered assemblies of molecular functions; and
  - might be associated with a cellular component: a component of a cell that is part of some larger object, which may be an anatomical structure or a gene product group.
  - For example, the gene product cytochrome c can be described by
    - the molecular function term oxidoreductase activity,
    - the biological process terms oxidative phosphorylation and induction of cell death, and
    - the cellular component terms mitochondrial matrix and mitochondrial inner membrane.
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Features of GO

- GO is hierarchical: functions, processes, and components, described at multiple levels of detail.
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- A team of experts define GO terms.
- Annotations typically done by individual genome databases.

Evidence code attached to annotation:

- IDA - inferred from direct assay (enzyme assay, cell fractionation)
- IPI - inferred from physical interaction (2-hybrid)
- IGI - inferred from genetic interaction (suppressor, synthetic lethal)
- IEP - inferred from expression pattern (microarray)
- IMP - inferred from mutant phenotype
- ISS - inferred from sequence or structure similarity
- TAS - traceable author statement
- NAS - non-traceable author statement
- RCA: reviewed computational analysis.
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Potential Advantages of GO

- The vocabulary is controlled $\Rightarrow$ common vocabulary for all biologists.
- Designed to apply across species.
- Computed mappings from other functional catalogues to GO.
- The GO terms are constantly updated (actually a headache for functional annotation algorithms).
- Freely available to the community.
Moving Beyond GO

- GO does not describe many aspects of a gene’s function:

  - which cells or tissues it is expressed in, which developmental stages it is expressed in, or its involvement in disease.
  - Other ontologies are being developed to meet these needs.
  - Open Biomedical Ontologies: http://obo.sourceforge.net/
  - “Cross-products” of different ontologies: combine different (independent) ontologies to derive richer vocabularies.
  - “For example, by combining the developmental terms in the GO process ontology with a second ontology that describes Drosophila anatomical structures, we could create an ontology of fly development.”
  - “We could create an ontology of biosynthetic pathways by combining the biosynthesis terms in the GO process ontology with a chemical ontology.”
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A functional linkage network (FLN) is a graph where each node corresponds to a gene and each edge connects two genes that may share a similar function.

An edge may not indicate which function the connected genes share.
Constructing FLNs

- Organism specific
  - Co-expression from DNA microarray data.
  - Protein products interact.
  - Genes co-regulated by the same transcription factor.
  - Double mutants are lethal (synthetic lethality).
  - Knockout mutants have the same metabolic profiles.

- Cross-organism
  - Information on co-evolution encoded in genomic context.
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Cross-Organism Functional Associations

(a) Diagram showing functional associations across different genomes:
- Genome 1: A, B, C
- Genome 2: C, A, B
- Genome 3: A, B, C
- Genome 4: B, A

(b) Schematic of protein interactions:
- Query protein: A
- Linked protein: B
- Rosetta protein: AB

(c) Images of proteins in different genomes:
- Genome 1: Protein A = 1, Protein B = 1, Protein C = 0, Protein D = 1
- Genome 2: Protein A = 1, Protein B = 1, Protein C = 0, Protein D = 1
- Genome 3: Protein A = 1, Protein B = 1, Protein C = 1, Protein D = 1
- Genome 4: Protein A = 0, Protein B = 0, Protein C = 1, Protein D = 1

(d) Diagram showing expression levels:
- Protein A: (P=0.015)
- Protein B: (P=0.003)
- Protein C: (P=0.43)
Previous Research on Functional Links

- Databases: BIND, DIP, GRID, IDSERVE, PROLINKS, PREDICTOME, REACTOME, STRING, . . . .


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- Databases: BIND, DIP, GRID, IDSERVE, PROLINKS, PREDICTOME, REACTOME, STRING, . . . .
- How do we systematically use FLNs to make robust and quantified predictions of function?
Example of an FLN in Saccharomyces cerevisiae
Why is Functional Annotation Difficult?

- Functional associations are not perfect indicators of shared function.

- 20–30% of genes of unknown function have only such genes as neighbours.

- Neighbourhood structure is ambiguous.
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**Gene Annotation Using Integrated Networks (GAIN):**

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- Propagate evidence systematically across the entire FLN.
- Integrate information from different sources to improve robustness: protein-protein interactions and gene expression data.

Overview of the GAIN Pipeline

- Inputs: Functional genomic data sets, GO functional annotations.
- Outputs: For each function in GO, a set of genes predicted to have that function.

1. Construct FLN $G$ from functional genomic data sets.
2. For each function $f$ in GO
   2.1 Construct a labelled FLN $G_f$ for $f$.
   2.2 Propagate the label $f$ or $\neg f$ across $G_f$.
   2.3 Output set of genes that have been assigned the function $f$.

- Can predict multiple functions for a gene.
Labelled FLNs

- **Labelled FLN** $G_f$ for a function $f \equiv$ the FLN $G$ with states (labels) attached to nodes.

- Each node $i$ has an associated state $s_i$:
  - $s_i = 1$: gene $i$ is annotated with $f$.
  - $s_i = -1$: gene $i$ is annotated with another function $f'$.
  - $s_i = 0$: otherwise.

- An edge between nodes $i$ and $j$ has a weight $w_{ij}$. 

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Hopfield Networks

- Functional linkage graph → discrete Hopfield network.
  - Gene ≡ node.
  - Interaction ≡ edge.

- Build a separate Hopfield network for each function.
- Given a function $f$, each node $i$ has an associated state $s_i$:
  - $s_i = 1$: gene $i$ is annotated with $f$.
  - $s_i = 0$: gene $i$ is hypothetical.
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- An edge between nodes $i$ and $j$ has a weight $w_{ij}$. 
Assigning Node States

- Assigning node states correctly is not a trivial manner.
- We must respect/exploit GO’s hierarchical structure.
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What is state of gene $p$ with respect to function

- $f$: 
- $g$: 
- $h$: 
- $m$: 
- $k$: 
- $l$: 

Correct state is 0.
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- $l$: -1 or 0? Correct state is 0.
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Goal: Maximally-Consistent Assignments

- An edge is consistent if it is incident on nodes with the same state.
- Maximally-consistent assignment: number of consistent edges is maximised.
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- An edge is *consistent* if it is incident on nodes with the same state.
- *Maximally-consistent assignment*: number of consistent edges is maximised.

Computational goal: Assign state of $-1$ or $+1$ to nodes with initial state 0 to achieve maximal consistency by minimising

$$E = -\frac{1}{2} \sum_i \sum_j w_{ij} s_i s_j$$

Predict nodes in state 1 as being annotated with the function.
Minimising $E$

- Finding state assignments to all nodes with initial $s_i = 0$ to minimise $E$ is NP-complete if some edge weights are negative.
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- Vasquez et al., *Nature Biotech.* 2003 use a simulated annealing-based approach.
Minimising $E$

- Finding state assignments to all nodes with initial $s_i = 0$ to minimise $E$ is NP-complete if some edge weights are negative.
- Our approach is based on the idea of *local updates*: each node looks at its neighbours and decides what its state should be.
Minimising $E$

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- Both approaches are well-known and well-studied.
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Both approaches are well-known and well-studied.

Can use minimum cuts and integer programming (Nabieva et al., Proc. ISMB 2005).
Local Update Rule

- Activation rule is

\[ s_i = \text{sgn} \left( \sum_{j \in N_i} w_{ij} s_j \right), \]

where \( N_i \) = neighbours of node \( i \).
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- Applying this rule:
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  - Parallel update: each node updates itself in parallel with the other nodes.
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  - Serial update: go through each node in sequence.
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- Applying this rule:
  - Parallel update: each node updates itself in parallel with the other nodes.
  - Serial update: go through each node in sequence.

- Stopping criterion: converge when no node’s state changes.
Example of Local Updates
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GAIN

ERB1

TIF6

NOP15

RLP7

NSA1

NOP2

NUG1

NOP7

BUD20

SSF1

NOC2

HAS1

SDA1

NOP7

NOC2

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Example of Local Updates

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Example of Local Updates

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Data Sets

- Interactions: General Repository of Interaction Datasets (GRID).
- Functional Annotations: Gene Ontology, three categories are biological process, molecular function, and cellular component.
Cleaning Up PPI Network

- GRID data set has 4711 genes and 13607 interactions.
- GRID data set has information on publications.

<table>
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<th>ORF_A</th>
<th>ORF_B</th>
<th>EXPERIMENTAL_SYSTEM</th>
<th>SOURCE</th>
<th>PUBMED_ID</th>
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<td>YER006W</td>
<td>YPL211W</td>
<td>Affinity Precipitation</td>
<td>Bassler et al.</td>
<td>;11583615;</td>
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<tr>
<td>YDL140C</td>
<td>YBR154C</td>
<td>Two Hybrid</td>
<td>BIND</td>
<td>;2496296;9207794;10393904;</td>
</tr>
</tbody>
</table>

- We only consider interactions reported by at least two different experiments to obtain 997 interactions between 1004 genes.
Data Integration

- Unweighted: $w_{ij} = 1$.
- Integrated: $w_{ij}$ is the absolute value of correlation coefficient of the expression profiles of gene $i$ and gene $j$ in the “Compendium” data set.
Evaluation

Leave one-out cross validation: For each function $f$,
1. for each gene $i$ annotated with $f$, set initial value of $s_i = 0$ and compute state assigned to $i$ by the Hopfield network.
2. Perform a similar operation for each gene not annotated with $f$. 

- True positive: $s_i: 1 \rightarrow 0 \rightarrow 1$
- False positive: $s_i: -1 \rightarrow 0 \rightarrow 1$
- True negative: $s_i: -1 \rightarrow 0 \rightarrow -1$
- False negative: $s_i: 1 \rightarrow 0 \rightarrow -1$

Precision = $TP/(TP + FP)$
Sensitivity = Recall = $TP/(TP + FN)$
F-measure = Harmonic mean of precision and recall.
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Measurement:

- True positive: $s_i : 1 \rightarrow 0 \rightarrow 1$
- False positive: $s_i : -1 \rightarrow 0 \rightarrow 1$
- True negative: $s_i : -1 \rightarrow 0 \rightarrow -1$
- False negative: $s_i : 1 \rightarrow 0 \rightarrow -1$

- Precision $= TP/(TP + FP)$
- Sensitivity $= \text{Recall} = TP/(TP + FN)$
- F-measure $= \text{Harmonic mean of precision and recall.}$
Results for Both Variants

1. Overall comparison of cross-validation.
2. Specific examples of genes that perform better on cross-validation (see paper).
Results from GAIN

Overall Cross-Validation Results

- Restricted to 828 functions for which F-score > 0.
- Unweighted network: Precision = 94%, Recall = 64%.
- Integrated network: Among 440 functions for which we make at least one novel prediction,
  - 168 function had better F-measures, 227 the same, and 45 smaller F-measures in the integrated network.
Results from GAIN

Propagation Diagrams

Whole-Genome Functional Annotation
Novel Functional Annotations

- ERB1, HAS1, and NUG1: validated to have the function “rRNA processing.”
- NOC2: validated to have the function “ribosome assembly and ribosome-nuclear export.”
Novel Functional Annotations

▶ NHP10
  ▶ biological process *chromatin modeling* and cellular component *chromatin remodeling complex*.
  ▶ HMG1 proteins are involved in chromatin structure.

▶ UFO1
  ▶ cellular component *nuclear ubiquitin ligase complex*
  ▶ molecular function *ubiquitin-protein ligase activity* and biological processes *ubiquitin-dependent protein catabolism*.

▶ PKC1
  ▶ cellular component *1,3 beta-glucan synthase complex*.
  ▶ known: cellular component *intracellular* and biological processes *cell wall organization and biogenesis*.
More Novel FunctionalAnnotations

- YKL067W
  - biological process *signal transduction* and cellular component *spindle pole body*.
  - molecular function *nucleoside-diphosphate kinase (NDK) activity*; NDK interferes with the mating pheromone signal transduction in *S. pombe*.

- YCR099C and YBL059W
  - biological process *ER to Golgi transport* and cellular component *COPII vesicle coat*.
  - Vesicles with COPII coats are found associated with ER membranes at steady state.
Results from GAIN

Overall Correctness of Predictions

- 207 predictions for functions with F-score > 75%.
- 15 predictions are correct.
- 11 predictions at distance 1 from true function.
- 49 predictions at distance 2 from true function.
- Remaining predictions not validated.
- Validated functions include nucleolus, chromatin remodeling complex, snoRNA binding, RNA binding, vesicle-mediated transport.
Features of the GAIN System

- Systematic algorithm for propagating evidence in an FLN.
- Clean separation between construction of functional links and prediction of function.
- For each function, predictions are maximally consistent.
- Each prediction associated with measures of confidence.
- Propagation diagrams provide intuitive visualisation of evidence flow.
- VIRGO webserver for invoking GAIN and querying and browsing its predictions.
Future Research

- Cross-microbe functional annotation with Allan Dickerman, Eric Nordberg, and Brett Tyler.
- Cross-species functional annotation with Allan Dickerman and Brett Tyler. Collect gene expression profiles after oxidative stress for *S. cerevisiae*, *A. thaliana*, *Phytophthora sojae*, and *P. falciparum*.
- Algorithmic and technical improvements to the GAIN prediction system with Simon Kasif, Madhav Marathe, Henning Mortveit, and Anil Vullikanti.