CS 5854: Gene Function Prediction

T. M. Murali

January 31, February 7, 12, 2013
Data, Data, Data

- ≥ 10,000+ genomes sequenced, mainly microbial and 100s of eukaryotic.
- Computational identification of genes.
- Systematic gene knockouts.
- Gene expression data, proteomic data, metabolic data.
- Molecular interaction networks, metabolic pathways.
“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.)
Gene Functions in Arabidopsis thaliana

- All functions
- Specific functions

Fraction of genes

EXP
AUTH
IC
ISS
IEA
Solution: Automated Gene Function Prediction

- 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.

BUT

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. We need techniques for gene function prediction that go beyond sequence similarity.

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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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  - 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.
Features of GO: Hierarchy

- A team of experts defines GO terms.
- GO terms are described at multiple levels of detail.
- Explicit parent-child relationships between terms, forming a directed acyclic graph (DAG).
Features of GO: Evidence Codes

- Annotations typically done by individual genome databases.
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- Evidence code attached to annotation:
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  - IPI: inferred from physical interaction (2-hybrid)
  - IGI: inferred from genetic interaction (suppressor, synthetic lethal)
  - IEP: inferred from expression pattern (microarray)
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  - RCA: inferred from reviewed computational analysis
Revisit Gene Functions in Arabidopsis thaliana

Graph showing the fraction of genes with all functions and specific functions for different methods: EXP, AUTH, IC, ISS, IEA. The graph indicates a trend with an increase in the fraction of genes across methods.
Potential Advantages of GO

- The vocabulary is controlled ⇒ 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 gene function prediction algorithms).
  - isa complete.
  - Automated Ontology engineering (Alterovitz et al., Nat. Biotech., 2010).
- Freely available to the community.
Moving Beyond GO

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- “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.”
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
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- Organism specific
- Cross-organism
Constructing FLNs

- **Organism specific**
  - Co-expression from DNA microarray data.
  - Protein products interact.
  - Enzymes that catalyse different reactions in the same metabolic pathway.
  - Genes co-regulated by the same transcription factor.
  - Double mutants are lethal (synthetic lethality).

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

(a) 
```
Genome 1    Genome 2    Genome 3    Genome 4
A  B  C  A  B  C  B  A  C
```

(b) 
```
Query protein
A
B
AB
Linked protein
Rosetta protein
```

(c) 
```
Genome 1    Genome 2    Genome 3    Genome 4
Protein A  1  1  1  0
Protein B  1  1  1  0
Protein C  1  0  1  1
Protein D  1  1  0  1
```

(d) 
```
A
B
C
(D=0.015) (P=0.003) (P=0.43)
E
```
Research on Functional Links

- Databases: BIND, DIP, GRID, IDSERVE, PROLINKS, PREDICTOME, REACTOME, STRING, ....
- Techniques for integrating diverse pieces of evidence into a single integrated FLN (Lee et al., *Science*, 306, 2005; papers by Troyanskaya’s group; Mostafavi et al., *Genome Biology*, 2008).
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▶ How do we systematically use FLNs to make robust and quantified predictions of function?
Example of an FLN in Saccharomyces cerevisiae
Functional associations are not perfect indicators of shared function.
Why is Gene Function Prediction Difficult?

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- 20–30% of genes of unknown function have only such genes as neighbours.
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- Neighbourhood structure is ambiguous.
The GAIN System

Gene Annotation Using Integrated Networks (GAIN):

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Gene Annotation Using Integrated Networks (GAIN):
- 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.
- FLN $\rightarrow$ discrete Hopfield network.
  - Gene $\equiv$ node.
  - Interaction $\equiv$ edge.

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

An edge between nodes $u$ and $v$ has a weight $w_{uv}$. 
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 $f$?
- $f$: 
- $g$: 
- $h$: 
- $m$: 
- $k$: 
- $l$: 

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

$$E = \sum_{(u,v) \text{ is an edge}} -w_{uv}s_us_v$$

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

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

- Activation rule is

\[ s_u = \text{sgn} \left( \sum_{v \in N_u} w_{uv} s_v \right) , \]

where \( N_v \) = neighbours of node \( u \).
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- Stopping criterion: converge when no node’s state changes.
Why does the Local Update Algorithm Converge?

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- Every time a node $x$’s state changes, $E$ decreases.
- Let $E^o$ and $E^n$ be the old and values of energy, respectively.
- Let $s^o_x$ and $s^n_x$ be the old and new states of $x$, respectively.
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\]

- What are the maximum and minimum values of $E$?
Example of Local Updates

RLP7 - NSA1 - TIF6 - NOP15

BRX1 - HAS1 - NOC2 - NOP2 - NOP7

BUD20 - SDA1
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RLP7 → NSA1 → TIF6 → NOP15 → ERB1

NOP7 → SSF1 → NOC2 → NOP2 → NUG1

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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>
<thead>
<tr>
<th>ORF_A</th>
<th>ORF_B</th>
<th>EXPERIMENTAL_SYSTEM</th>
<th>SOURCE</th>
<th>PUBMED_ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>YER006W</td>
<td>YPL211W</td>
<td>Affinity Precipitation</td>
<td>Bassler et al.;11583615;</td>
<td></td>
</tr>
<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: $w_{uv} = 1$.
- Integrated: $w_{uv}$ is the absolute value of correlation coefficient of the expression profiles of gene $u$ and gene $v$ in the “Compendium” data set.
Leave One-Out Cross Validation

For each function $f$,

1. for each gene $u$ annotated with $f$, set initial value of $s_u = 0$ and compute state assigned to $u$ by the Hopfield network.
2. Perform a similar operation for each gene not annotated with $f$. 

Measurement of performance:

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

Precision = $\frac{TP}{TP + FP}$

Sensitivity = Recall = $\frac{TP}{TP + FN}$

F-measure = Harmonic mean of precision and recall.
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- Sensitivity = Recall = \( \frac{TP}{TP + FN} \)
- F-measure = Harmonic mean of precision and recall.
**k-fold cross validation**

1. Partition union of positive and negative examples into \(k\) groups, uniformly at random.

2. For each group, use algorithm to predict the state of each positive/negative example in that group using all other examples.

3. Sort all positive and negative examples in decreasing order of prediction confidence.

4. For each threshold on prediction confidence, compute the number of true positives (\(tp\)), false positives (\(fp\)), true negatives (\(tn\)), and false negatives (\(fn\)).

5. For each threshold on prediction confidence, compute precision (\(tp/(tp + fp)\)), recall (\(tp/(tp + fn)\)), and false positive rate (\(fp/(fp + tn)\)).

6. As prediction confidence varies, plot precision against recall.
Results for Both Variants

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

- RLP7
- NSA1
- TIF6
- NOP15
- BRX1
- SSF1
- HAS1
- ERB1
- NOP2
- BUD20
- SDA1
- NOC2
- NOP7

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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 Functional Annotations

- **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.
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.
Algorithms: Local and Local+

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<th>Local+</th>
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<td>$s_u = \frac{\sum_{v \in N_u} w_{uv} s_v}{\sum_{v \in N_u} w_{uv}}$</td>
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</tbody>
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- $N_u$ is the set of neighbours of gene $u$.
- Local+ does not use negative examples, i.e., $s_v$ is initially 0 for negative examples.
Algorithm: FunctionalFlow

(Nabieva et al., ISMB 2005.)

- No negative examples.
- Each node sends flow to or receives flow from each neighbour.
- $s(v)$ is the total inflow into node over multiple phases.
- Number of phases is input to the algorithm (half the diameter of the network suggested.)
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g_0(u, v) = 0
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\[
s_0(u) = \begin{cases} 
\infty & \text{if } u \text{ is a positive example} \\
0 & \text{otherwise}
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\[
s_t(u) = s_{t-1}(u) + \sum_{v \in N_u} \left( g_t(v, u) - g_t(u, v) \right)
\]
Algorithm: SinkSource

▶ Compute voltage at each unknown example by minimising \( \sum (u, v) w_{uv} (s_u - s_v)^2 \)

▶ Solve linear system of equations:
  \[ s_v = \sum u w_{uv} s_u \sum u w_{uv} \]
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Matrix Formulation of SinkSource

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\[ \left( \sum_u W_{uv} \right) S_v = \sum_u W_{uv} S_u \]

Define \( y_u = s_u \) only for positive and negative examples and split RHS,

\[ \left( \sum_u W_{uv} \right) S_v = \sum_u W_{uv} S_u + \sum_u W_{uv} y_u \]
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Define \( W = [w_{uv}] \), \( D = [\sum_u w_{uv}] \), \( L = D - W \), \( s = [s_u] \) and \( y = [\sum_u w_{uv} y_u] \).

\[ Ds =Ws + y \]

\[ (D - W)s = Ls = y \]

\[ s = L^{-1}y \]
Algorithm: SinkSource+

Compute voltage at each unknown example by minimising
\[ \sum (u, v) w_{uv} (s_u - s_v)^2 + \lambda \sum v s_v^2 \]

Solve linear system of equations:
\[ s_v = \sum u w_{uv} s_u + \lambda + \sum u w_{uv} \]

Matrix form is
\[ s = (\lambda I + L)^{-1} y. \]

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Algorithm: SinkSource+

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Solve linear system of equations:
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