Gene Function Prediction

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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

GO FLNs GAIN Results Other Algorithms

Keith Haring, *Untited*, 1986

- Molecular biology: what are the parts of the cell? what functions does each part perform?

Urs Wehrli, *Tidying Up Art*, 2003

→

Molecular biology: what are the parts of the cell? what functions does each part perform?
Molecular biology: what are the parts of the cell? what functions does each part perform?

Systems biology: how do the parts make up the whole? how do genes and their products collectively carry out complex cellular functions?

We need to understand how genes, proteins, and other molecules interact with other in different cell states, different tissues, and under different external conditions.
Sea Urchin (Strongylocentrotus purpuratus)

- Very important in developmental biology.
- Many principles of embryo development were discovered in the sea urchin.
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A Cell
A Cell is a Modular Network that Computes Gene Function Prediction
A Cell is a Modular

Introduction

GO

FLNs

GAIN

Results

Other Algorithms

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A Cell is a Modular Network

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Gene Function Prediction
A Cell is a Modular Network

C Module A functions:

Vegetal plate expression in early development:

Synergism with modules B and G enhancing endoderm expression in later development:

Repression in ectoderm (modules E and F) and skeletogenic mesenchyme (module DC):

Modules E, F and DC with LiCl treatment:
A Cell is a Modular Network that Computes

**B**

if (F = 1 or E = 1 or CD = 1) and (Z = 1)

\[ \alpha = 1 \]

else \[ \alpha = 0 \]

if (P = 1 and CG₁ = 1)

\[ \beta = 2 \]

else \[ \beta = 0 \]

if (CG₂ = 1 and CG₃ = 1 and CG₄ = 1)

\[ \gamma = 2 \]

else \[ \gamma = 1 \]

\[ \delta(t) = B(t) + G(t) \]

\[ \epsilon(t) = \beta \cdot \delta(t) \]

if (\( \epsilon(t) = 0 \))

\[ \xi(t) = \text{Otx}(t) \]

else \[ \xi(t) = \epsilon(t) \]

if (\( \alpha = 1 \))

\[ \eta(t) = 0 \]

else \[ \eta(t) = \xi(t) \]

\[ \Theta(t) = \gamma \cdot \eta(t) \]

Repression functions of modules F, E, and DC mediated by Z site

Both P and CG₁ needed for synergistic link with module B

Final step up of system output

Positive input from modules B and G

Synergistic amplification of module B output by CG₁-P subsystem

Switch determining whether Otx site in module A, or upstream modules (i.e., mainly module B), will control level of activity

Repression function inoperative in endoderm but blocks activity elsewhere

Final output communicated to BTA
Network is Complex
Network is Complex
Network is Complex but Very Poorly Understood
“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

- **EXP**
- **AUTH**
- **IC**
- **ISS**
- **IEA**

Plot showing the fraction of genes with all functions and specific functions.
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.

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 functional annotation 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

- Collaborative effort to define a controlled vocabulary to describe gene and gene product attributes in any organism.
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Introduction  GO  FLNs  GAIN  Results  Other Algorithms

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    that is part of some larger object, which may be an anatomical
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- 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 define GO terms.
- GO functions, processes, and components are described at multiple levels of detail.
- Explicit parent-child relationships between terms.
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- Annotations typically done by individual genome databases.
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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

![Bar chart and line graph showing the fraction of genes with different gene functions.]

- **EXP**
  - All functions: 0.15
  - Specific functions: 0.05

- **AUTH**
  - All functions: 0.10
  - Specific functions: 0.02

- **IC**
  - All functions: 0.05
  - Specific functions: 0.01

- **ISS**
  - All functions: 0.10
  - Specific functions: 0.03

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**Legend:**
- Black: All functions
- Gray: Specific functions

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Gene Function Prediction
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 functional annotation algorithms).
  - isa complete.
  - Automated Ontology engineering (Alterovitz et al., Nat. Biotech., 2010).
- 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
  - 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
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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).

- **Cross-organism**
Constructing FLNs

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

(a) Genome 1

(b) Query protein
Linked protein
Rosetta protein

(c)

(d)

Protein A
Protein B
Protein C
Protein D

(P=0.015) (P=0.003) (P=0.43)
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).
**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).
- **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?

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- 20–30% of genes of unknown function have only such genes as neighbours.
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- 20–30% of genes of unknown function have only such genes as neighbours.
- Neighbourhood structure is ambiguous.
Gene Annotation Using Integrated Networks (GAIN):

- Propagate evidence systematically across the entire FLN.
- Integrate information from different sources to improve robustness.

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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.
- 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 $w_{uv}$. 

> Skip Node States
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 $f$:

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

$$E = -\frac{1}{2} \sum_u \sum_v w_{uv} s_u s_v$$

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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Both approaches are well-known and well-studied.

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.
Example of Local Updates

- RLP7
- NSA1
- TIF6
- NOP15
- BRX1
- SSF1
- HAS1
- ERB1
- NOP2
- NUG1
- NOP7
- BUD20
- SDA1
Example of Local Updates
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Example of Local Updates
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.

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<th>ORF_B</th>
<th>EXPERIMENTAL_SYSTEM</th>
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<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.
**Evaluation**

- 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 $f$. 
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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 $= \text{Recall} = \frac{TP}{TP + FN}$
- 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).
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.
▷ 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+ 

<table>
<thead>
<tr>
<th>Local</th>
<th>Local+</th>
</tr>
</thead>
<tbody>
<tr>
<td>$s_u = \sum_{v \in N_u} w_{uv} s_v / \sum_{v \in N_u} w_{uv}$</td>
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</tr>
</tbody>
</table>

- $N_u$ 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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\[ g_0(u, v) = 0 \]

\[ s_0(u) = \begin{cases} 
\infty & \text{if } u \text{ is a positive example} \\
0 & \text{otherwise} 
\end{cases} \]
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$$g_t(u, v) = \begin{cases} 0 & \text{if } s_{t-1}(u) < s_{t-1}(v) \\ \min \left( w_{uv}, s_{t-1}(u) \frac{w_{uv}}{\sum_{y \in N_u} w_{uy}} \right) & \text{otherwise} \end{cases}$$
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  \end{cases} \\
  s_t(u) &= s_{t-1}(u) + \sum_{v \in N_u} (g_t(v, u) - g_t(u, v))
\end{align*}
\]
Algorithm: SinkSource

- **Intuition:** Consider the network to be an electrical network.
  - connect positive examples to a source of 1V.
  - connect negative examples to ground (0V).
  - Treat each edge weight as a conductance.
Algorithm: SinkSource

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- Solve linear system of equations:
  \[
  f(v) = \frac{\sum_u w_{uv} f(u)}{\sum_u w_{uv}}
  \]

T. M. Murali
CS 3824
Gene Function Prediction
Matrix Formulation of SinkSource

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Define \( y(u) = f(u) \) only for positive and negative examples and split RHS,

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Define \( W = [w_{uv}] \), \( D = [\sum_u w_{uv}] \), \( L = D - W \), \( f = [f(u)] \) and \( y = [\sum_u w_{uv} y(u)] \).

\[ Df = Wf + y \]

\[ (D - W)f = Lf = y \]

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

- No negative examples.

- Minimise

\[ \sum_{(u,v)} w_{uv} (f(u) - f(v))^2 \]

- by solving linear system of equations

\[ f(v) = \frac{\sum_u w_{uv} f(u)}{\sum_u w_{uv}} \]
Algorithm: SinkSource+

- No negative examples.
- Add an artificial node $t$ with $s_t$ set to 0.
- Connect $t$ to each node $u$ with $s_u \neq 1$ with an edge of weight $\lambda$.
- Minimise

$$\sum_{(u,v)} w_{uv} (f(u) - f(v))^2$$

- by solving linear system of equations

$$f(v) = \frac{\sum_u w_{uv} f(u)}{\sum_u w_{uv}}$$
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- Matrix form is $f = (\lambda I + L)^{-1} y$. 