Gene Function Prediction

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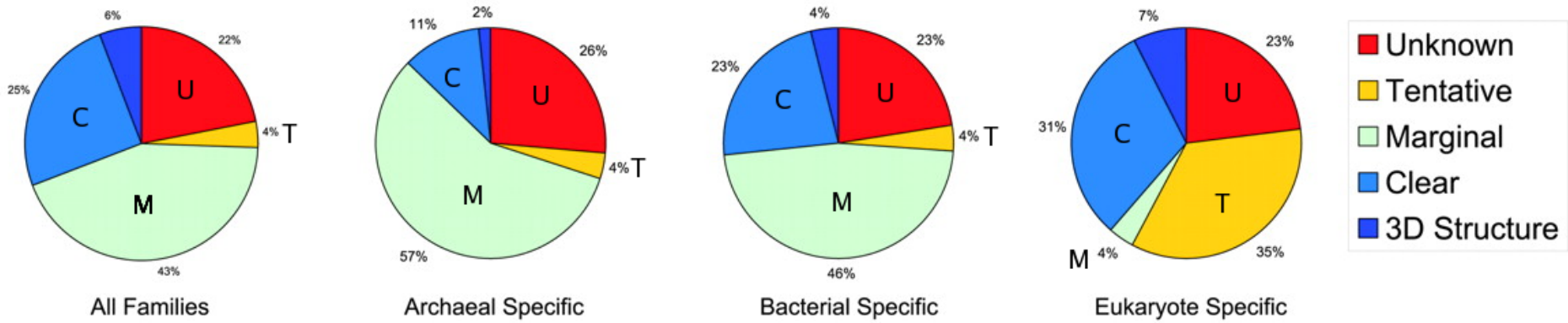
Data, Data, Data

- $\geq$ 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.

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.
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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

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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*.

Jump to FLNs

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  - RCA: inferred from reviewed computational analysis

Potential Advantages of GO

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

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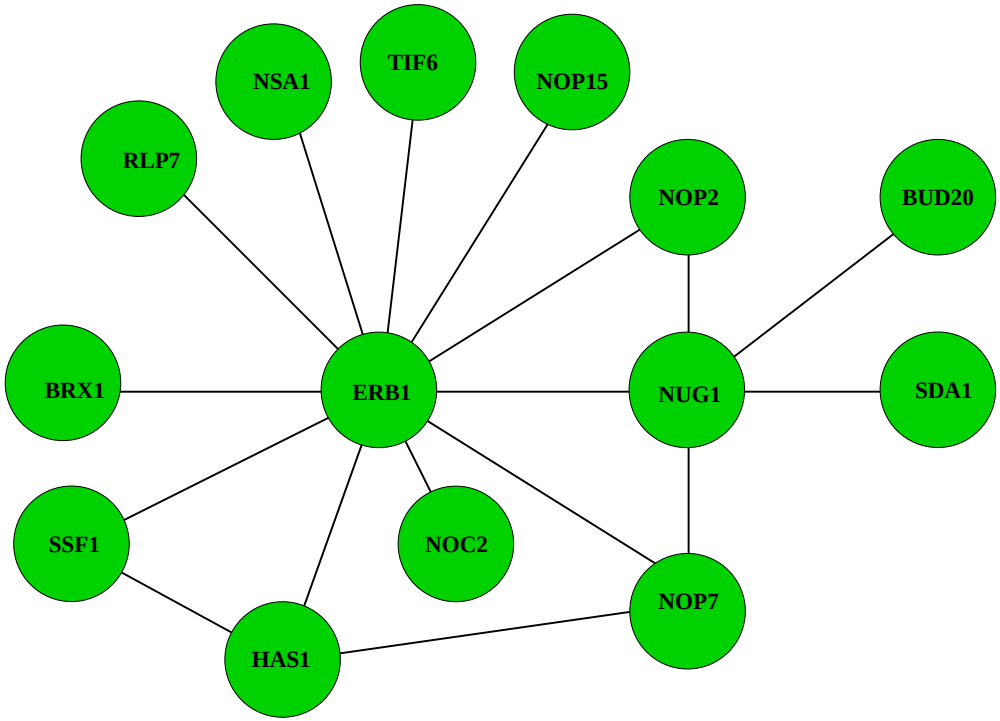
- "Cross-products" of different ontologies: combine different (independent) ontologies to derive richer vocabularies.
- "For example, by combining the developmental terms in the GO process ontology with a second ontology that describes Drosophila anatomical structures, we could create an ontology of fly development."
- "We could create an ontology of biosynthetic pathways by combining the biosynthesis terms in the GO process ontology with a chemical ontology."

Functional Linkage Networks



- 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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  - Co-expression from DNA microarray data.
  - Protein products interact.
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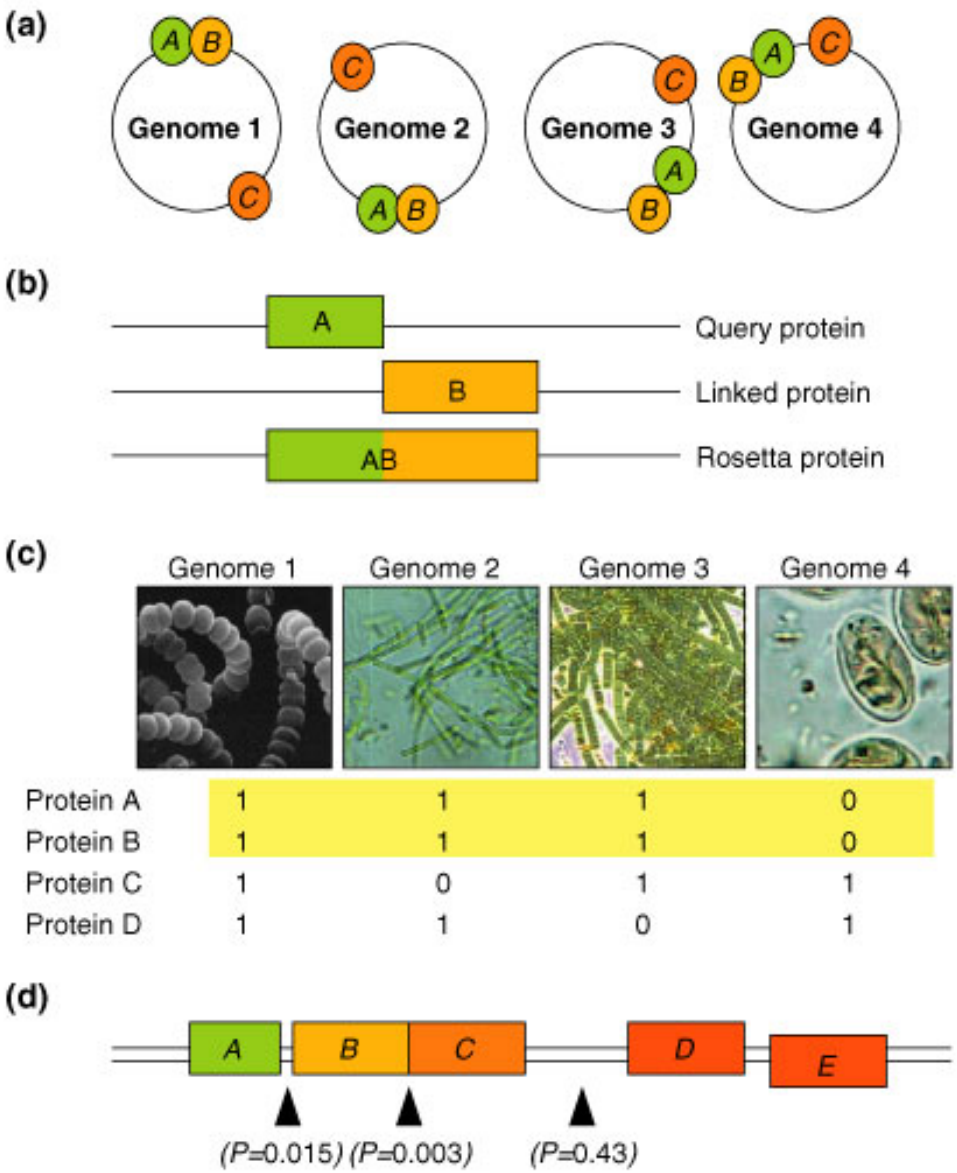
- Cross-organism
  - Information on co-evolution encoded in genomic context.

▸ Onward to Challenges

Cross-Organism Functional Associations



Previous Research on Functional Links

- Databases: BIND, DIP, GRID, IDSERVE, PROLINKS, PREDICTOME, REACTOME, STRING, ...
- Techniques for predicting functional associations, e.g., protein-protein interactions (Jansen et al., Science, 302, 2003; Zhang et al., BMC Bioinformatics, 5, 2005).
- Techniques for integrating diverse pieces of evidence into a single integrated FLN (Lee et al., Science, 306, 2005).

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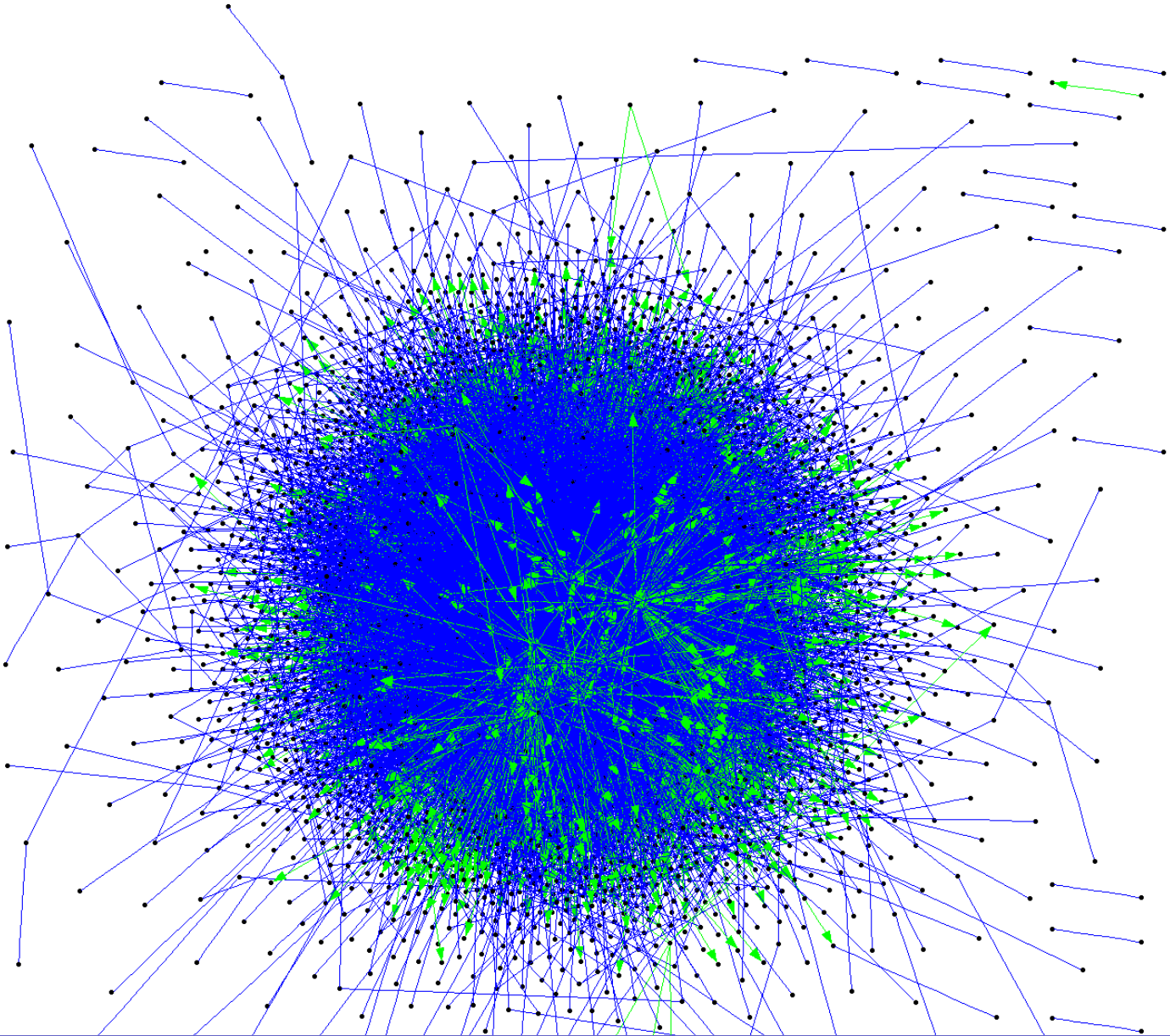
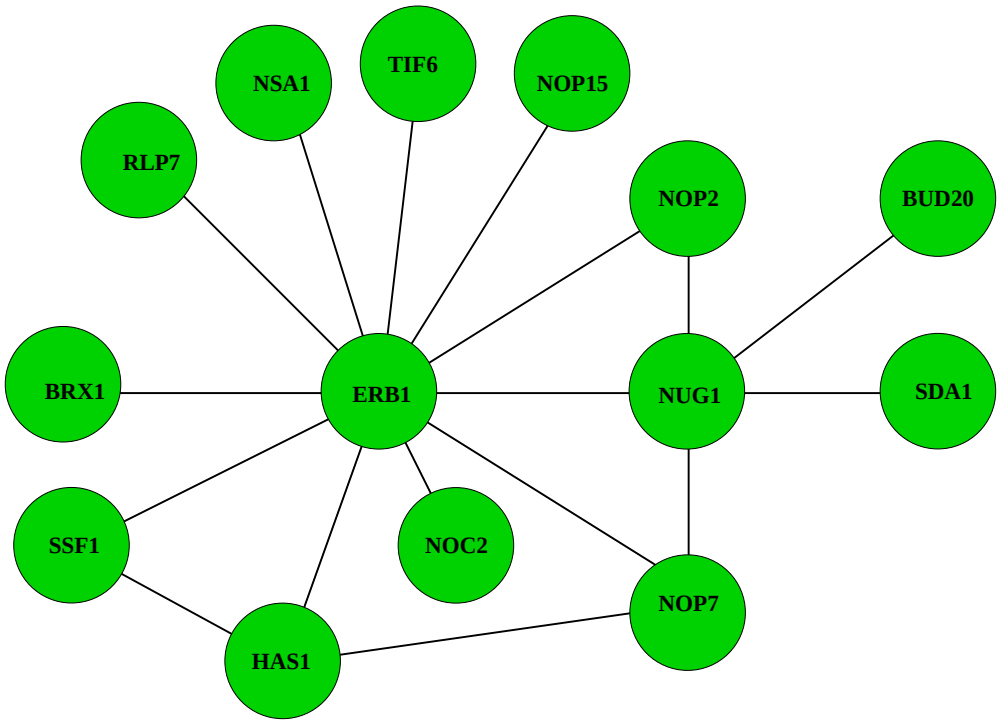
- Techniques for integrating diverse pieces of evidence into a single integrated FLN (Lee et al., Science, 306, 2005).
- How do we systematically use FLNs to make robust and quantified predictions of function?

Example of an FLN in Saccharomyces cerevisiae



Why is Functional Annotation Difficult?



- Functional associations are not perfect indicators of shared function.

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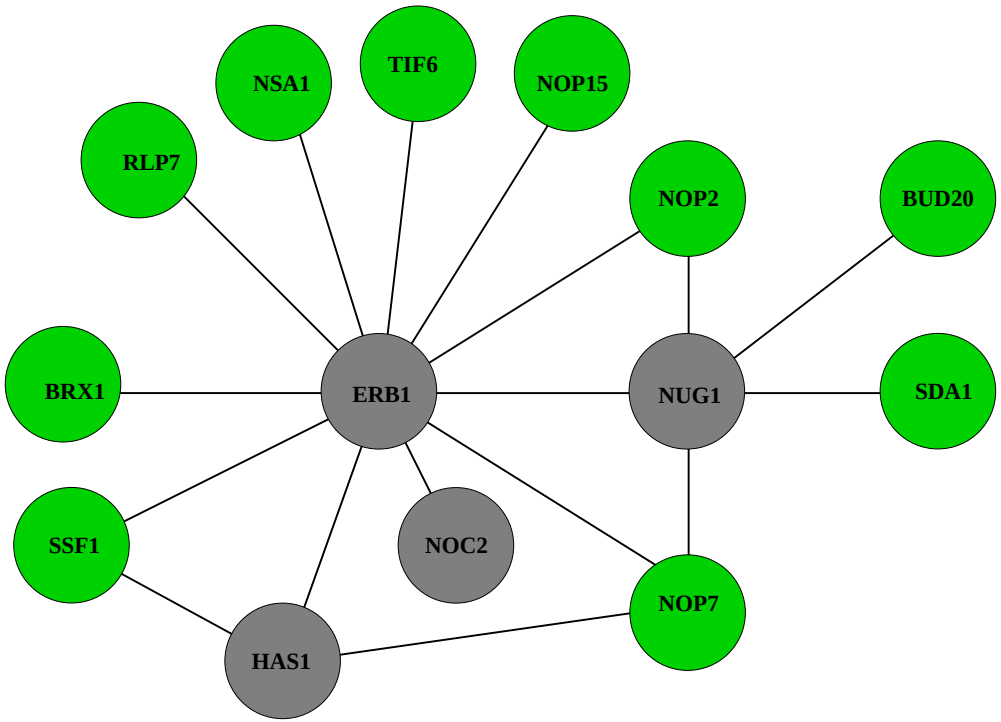

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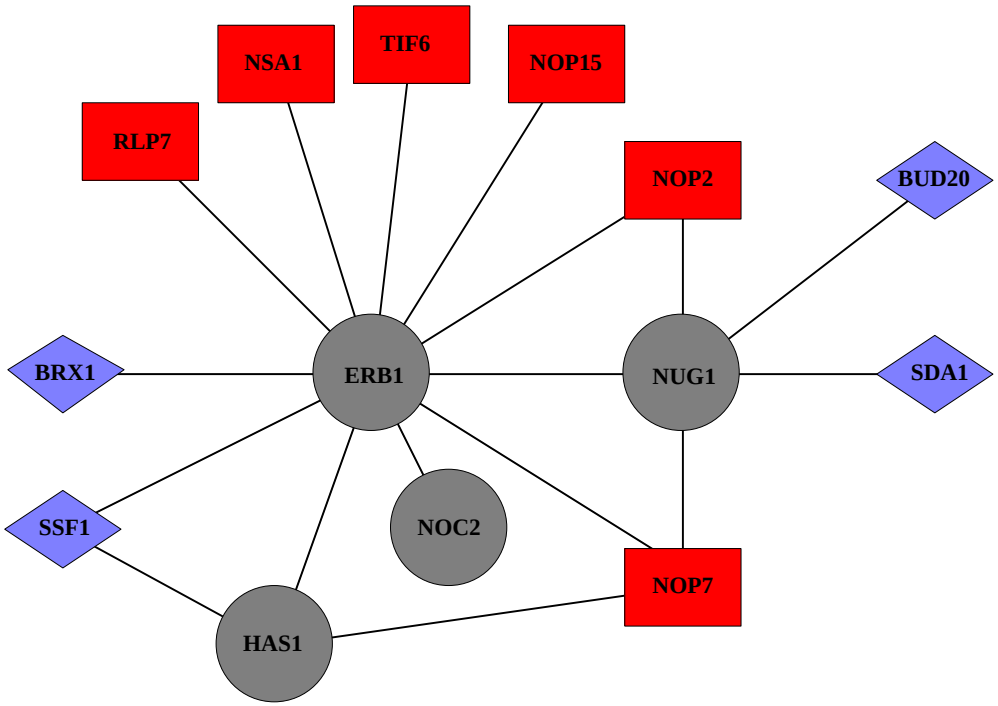

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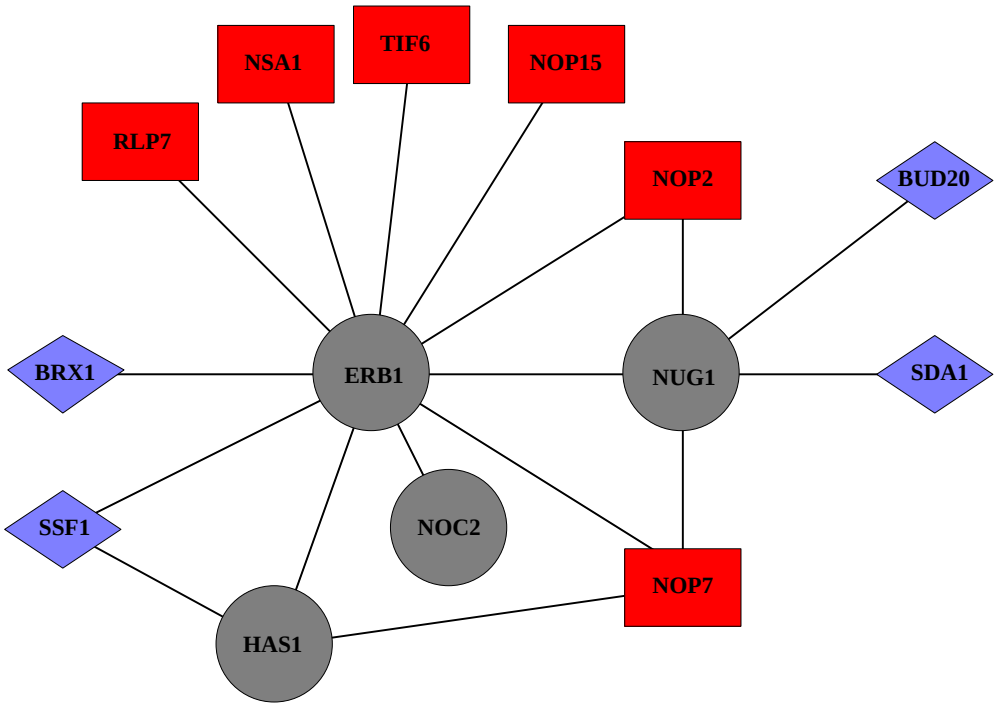
- Neighbourhood structure is ambiguous.

The GAIN System



Gene Annotation Using Integrated Networks (GAIN):

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

(Karaoz, Murali, Letovsky, Zheng, Ding, Cantor and Kasif, "Whole genome annotation using evidence integration in functional linkage networks," *PNAS*, 2004, 101, 2888–2893.)

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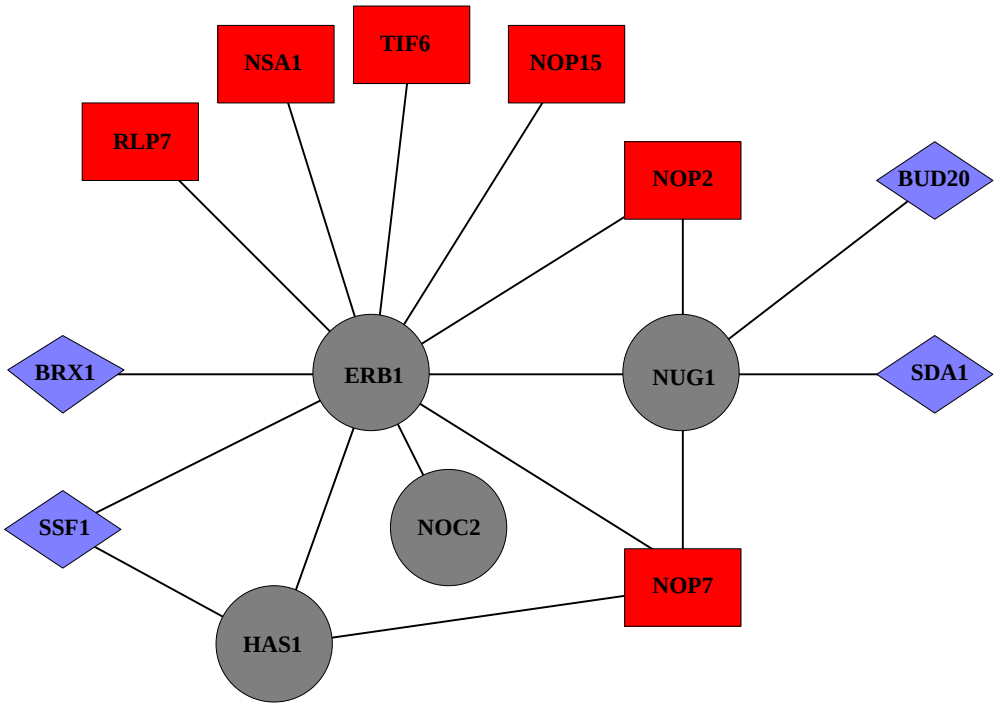

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Overview of the GAIN Pipeline

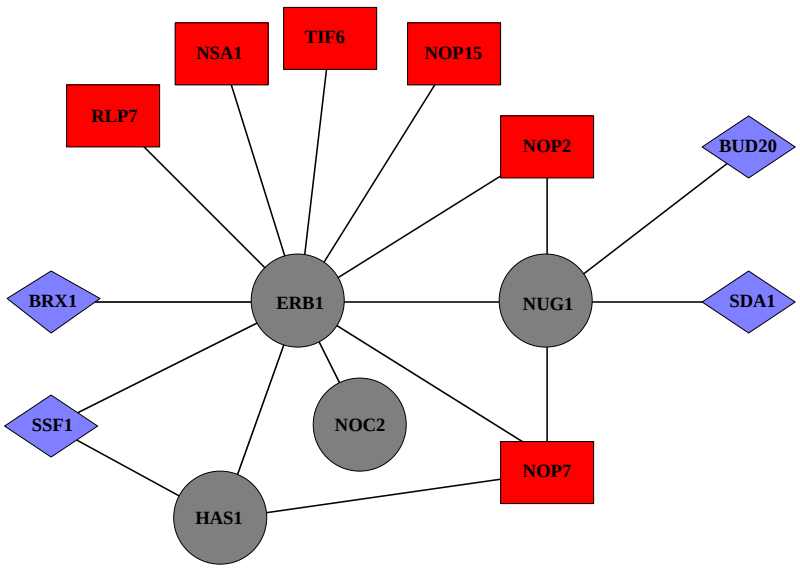
- Inputs: Functional genomic data sets, GO functional annotations.
- Outputs: For each function in GO, a set of genes predicted to have that function.

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 $\mathrm{not}f$ 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

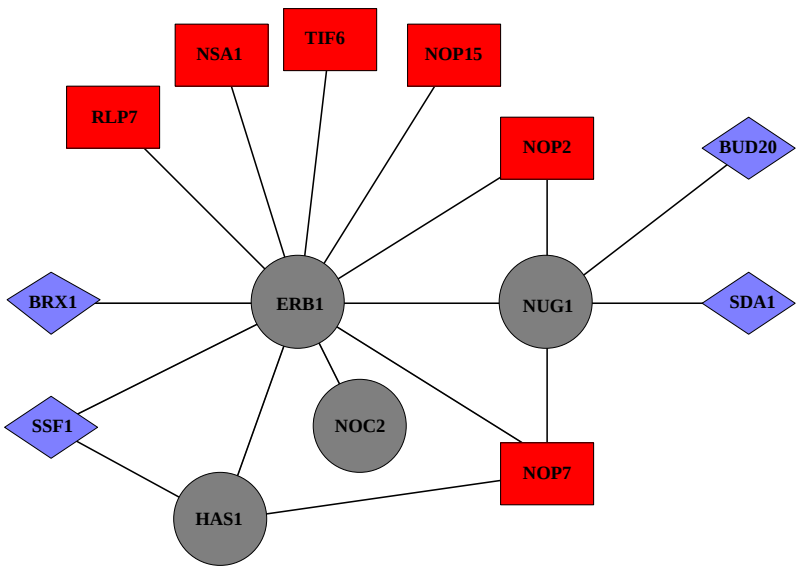
- *Labelled FLN* G_f for a function $f \equiv$ the FLN G with states (labels) attached to nodes.



- 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}.

Hopfield Networks

- Functional linkage graph $\longrightarrow$ discrete Hopfield network.
  - Gene $\equiv$ node.
  - Interaction $\equiv$ 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.
  - $s_i = -1$: gene i is annotated with another function f'.
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Skip Node States

Assigning Node States

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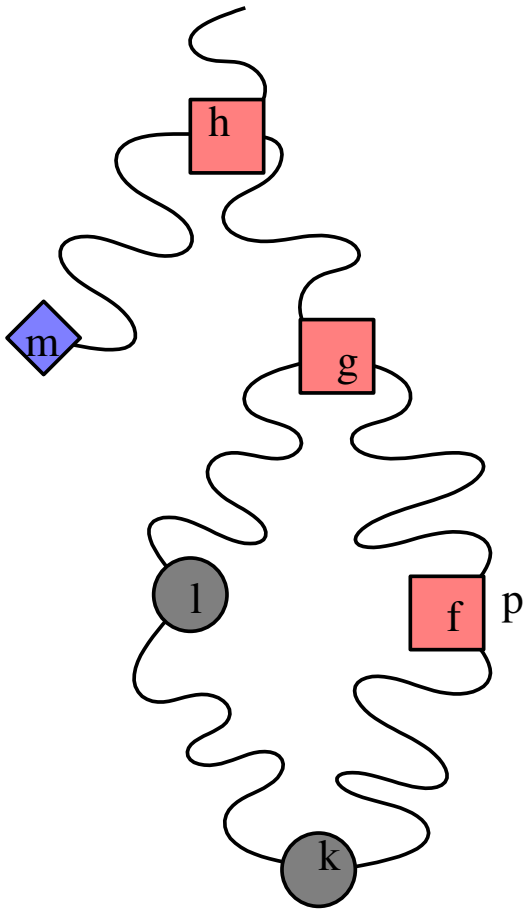

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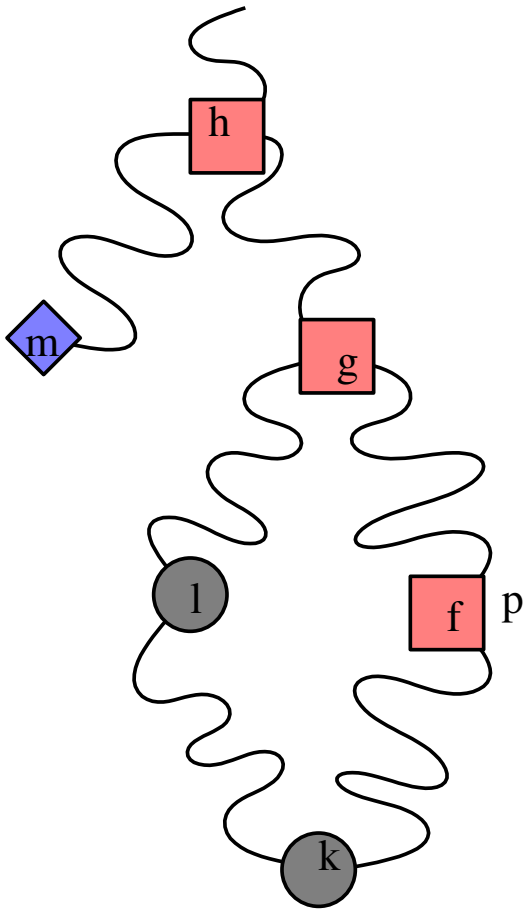

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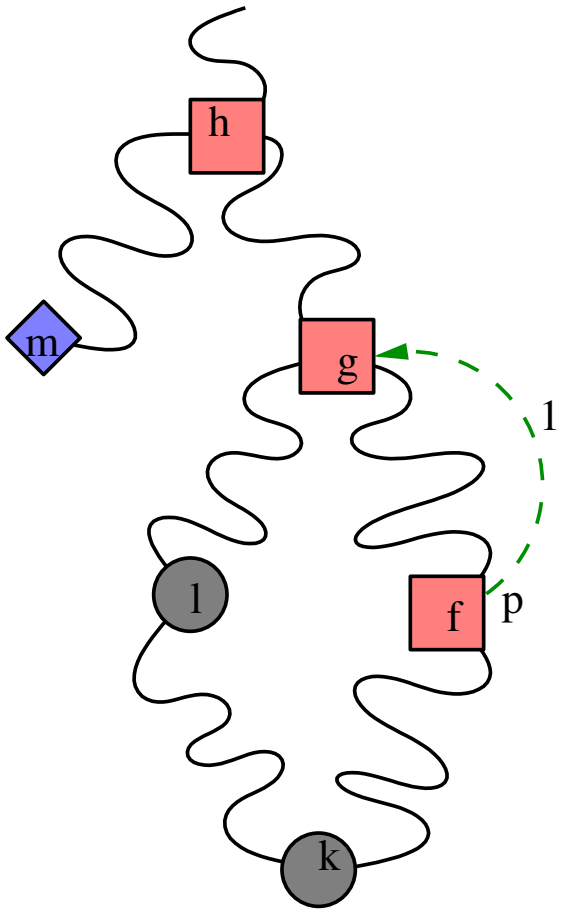

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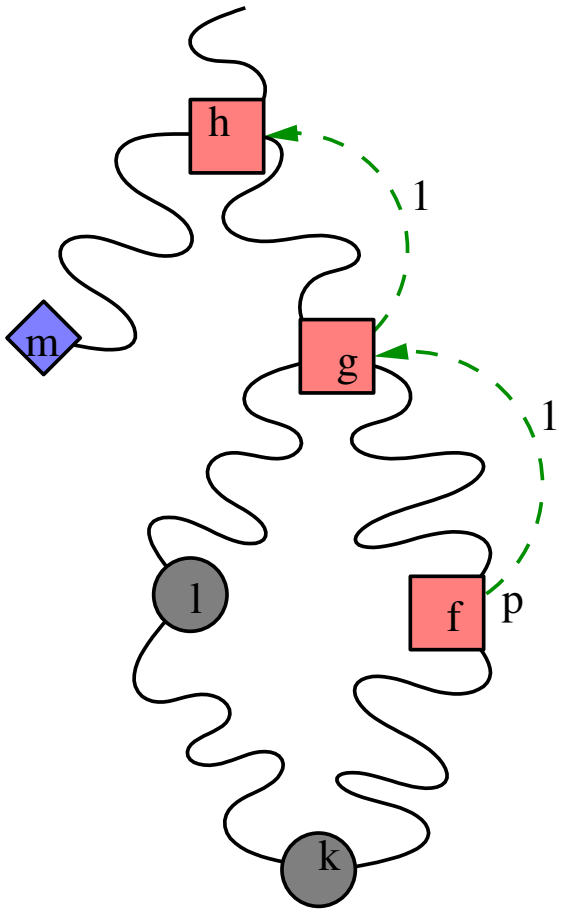

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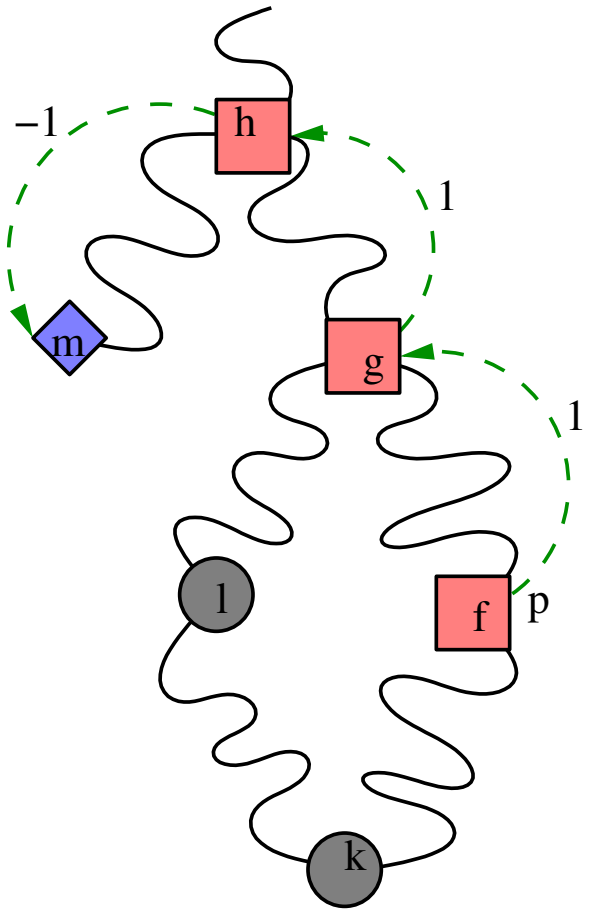

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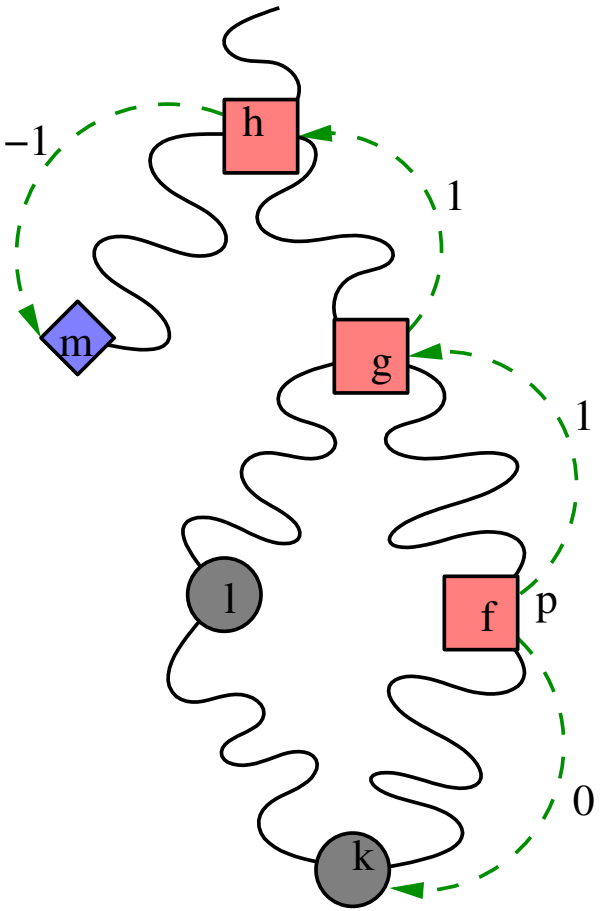

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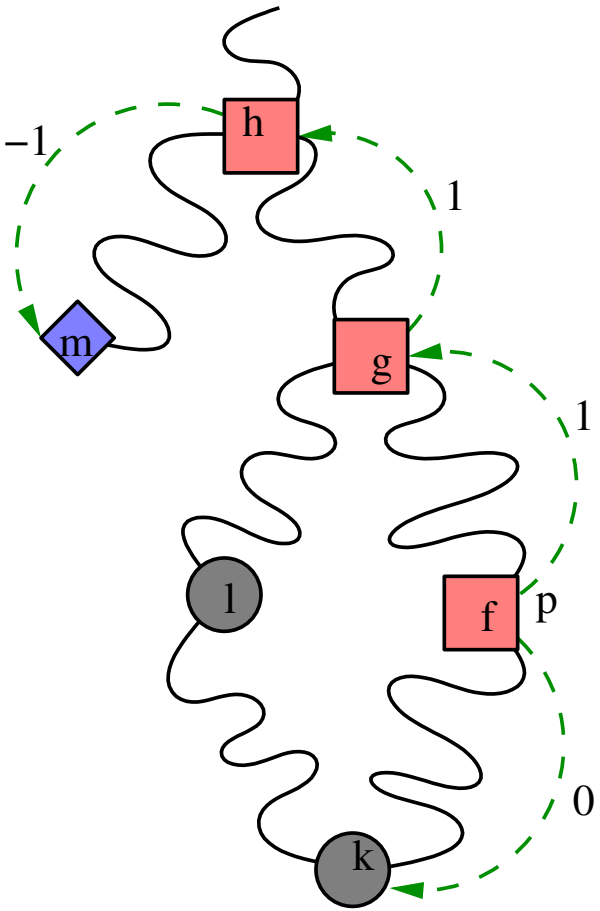

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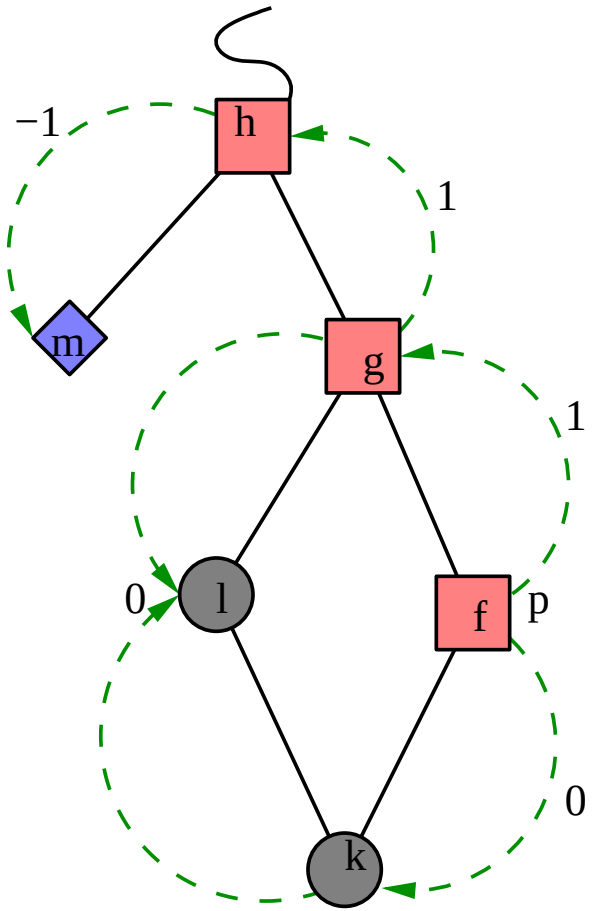

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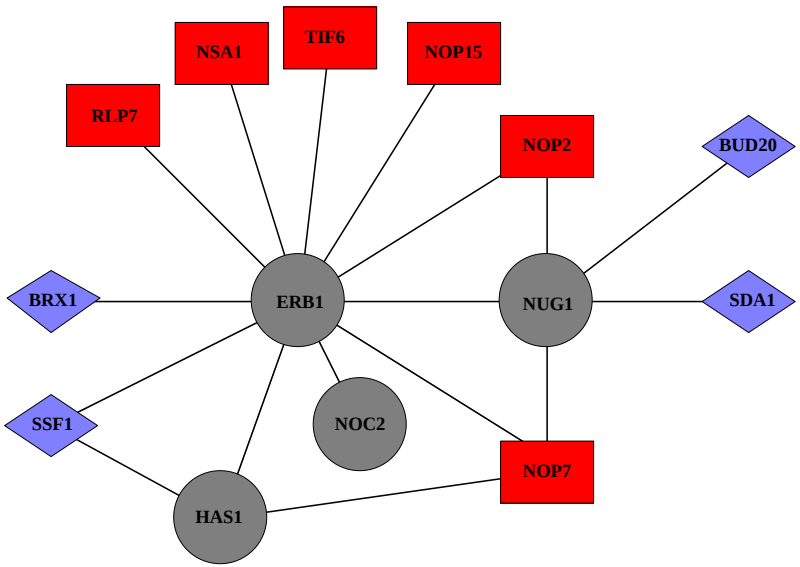
  - l: -1 or 0? Correct state is 0.

Goal: Maximally-Consistent Assignments



- An edge is *consistent* if it is incident on nodes with the same state.
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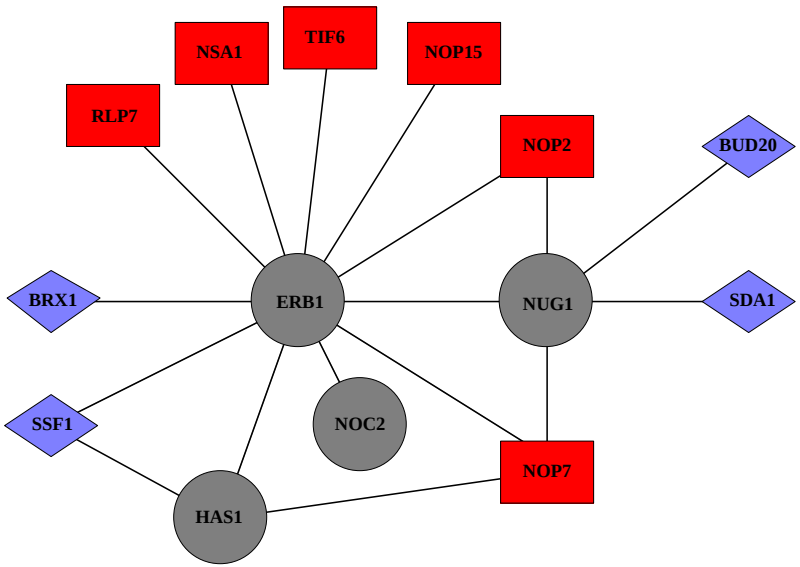

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- *Maximally-consistent assignment*: number of consistent edges is maximised.

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

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- Vasquez et al., *Nature Biotech. 2003* use a simulated annealing-based approach.
- Our approach is based on the idea of *local updates*: each node looks at its neighbours and decides what its state should be.
- Both approaches are well-known and well-studied.

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.
- Vasquez et al., *Nature Biotech. 2003* use a simulated annealing-based approach.
- Our approach is based on the idea of *local updates*: each node looks at its neighbours and decides what its state should be.
- Both approaches are well-known and well-studied.
- Can use minimum cuts and integer programming (Nabieva et al., Proc. ISMB 2005; Murali, Wu, and Kasif, *Nature Biotech.*, 2006).

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$$s_i = \mathrm{sgn}\left(\sum_{j \in N_i} w_{ij} s_j \right),$$

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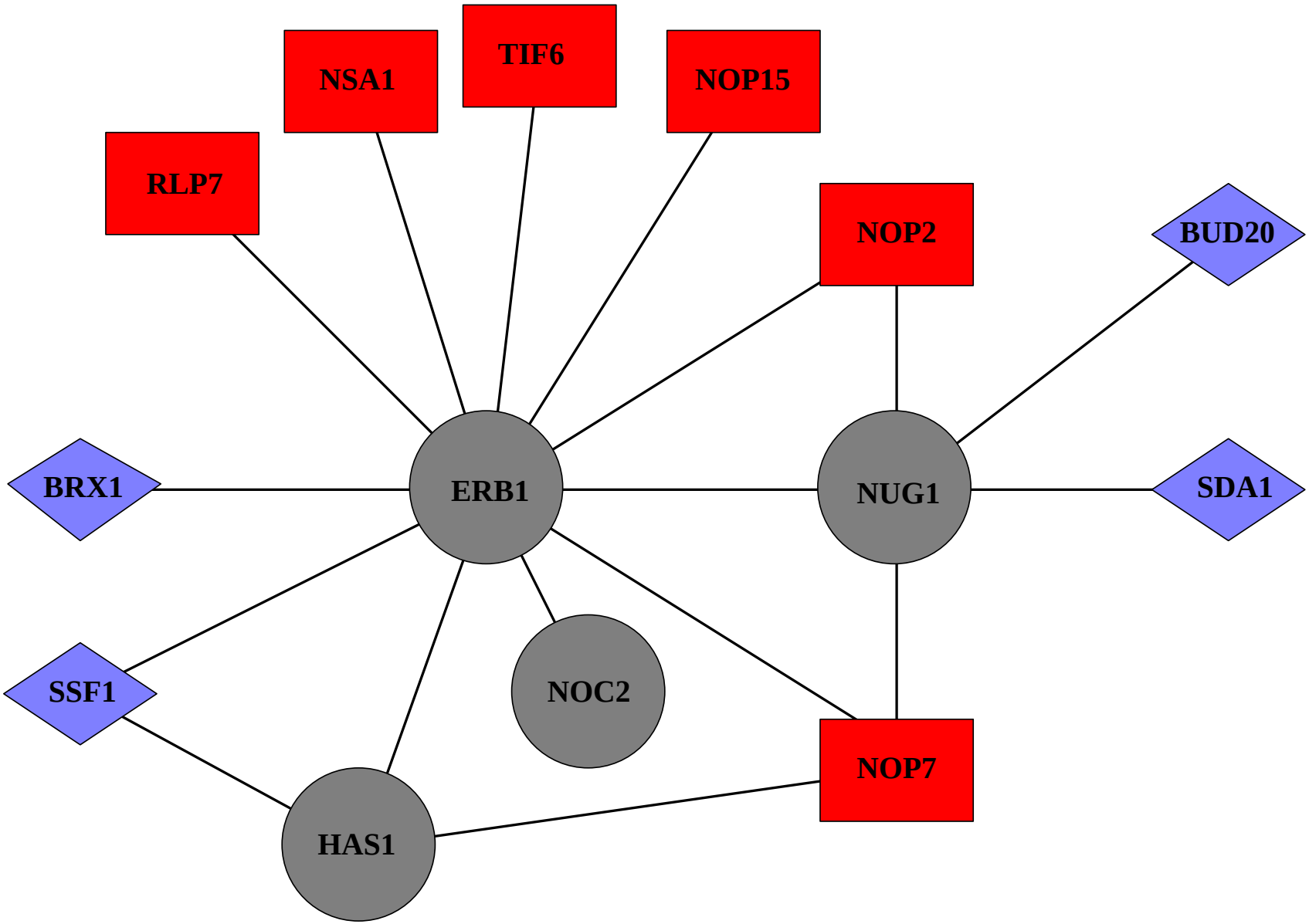
  - Serial update: go through each node in sequence.
- Stopping criterion: converge when no node's state changes.

Example of Local Updates



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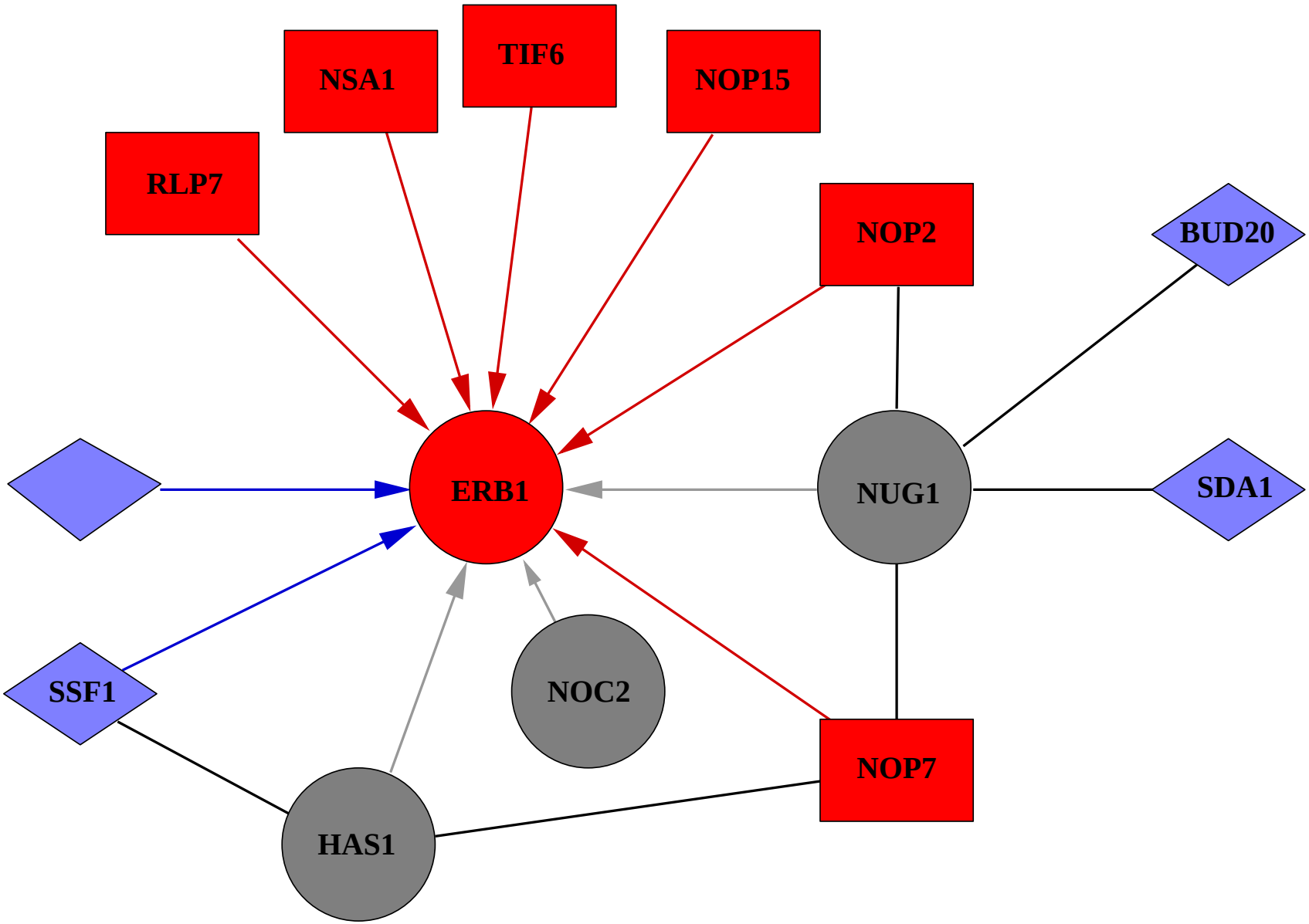

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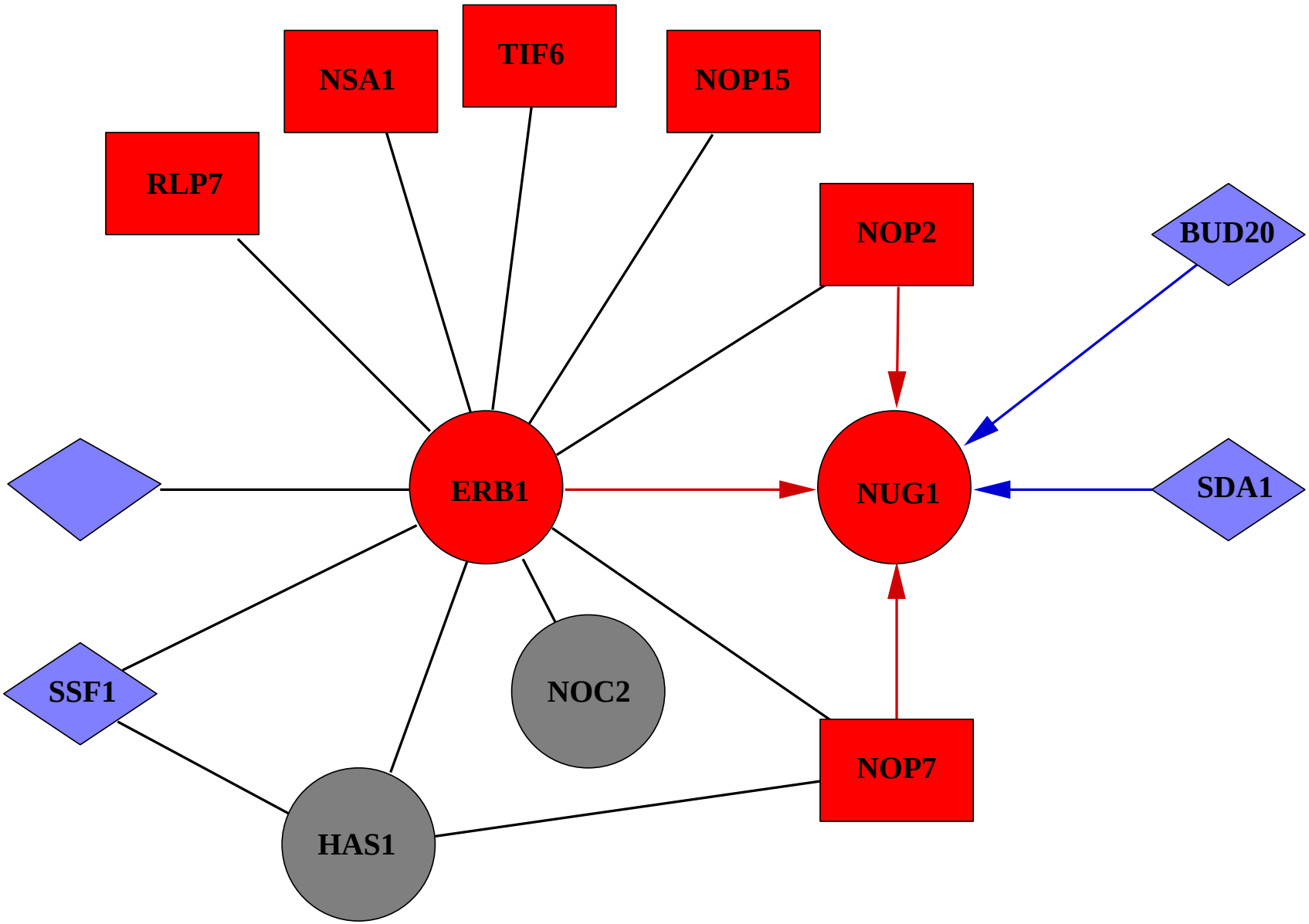

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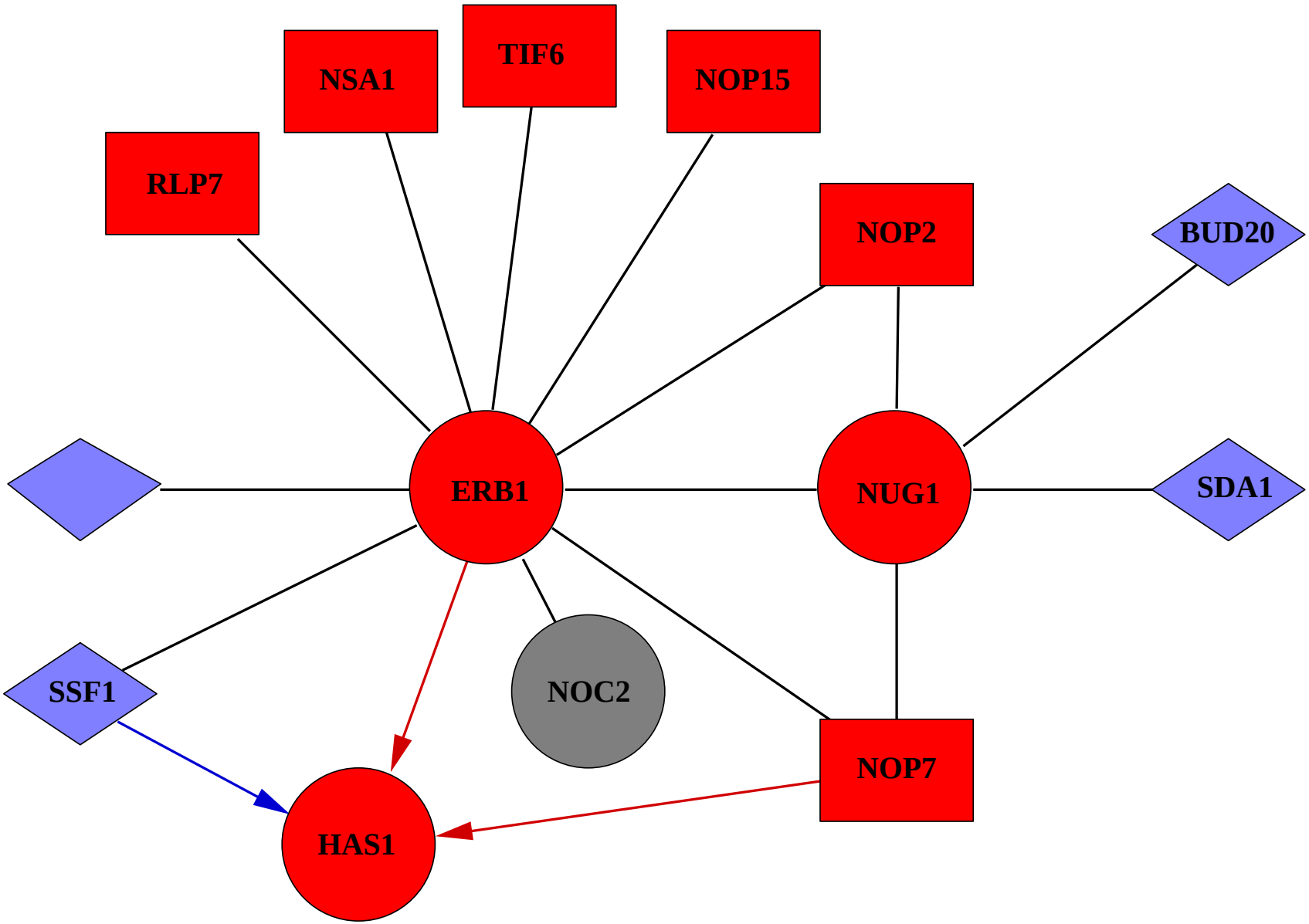

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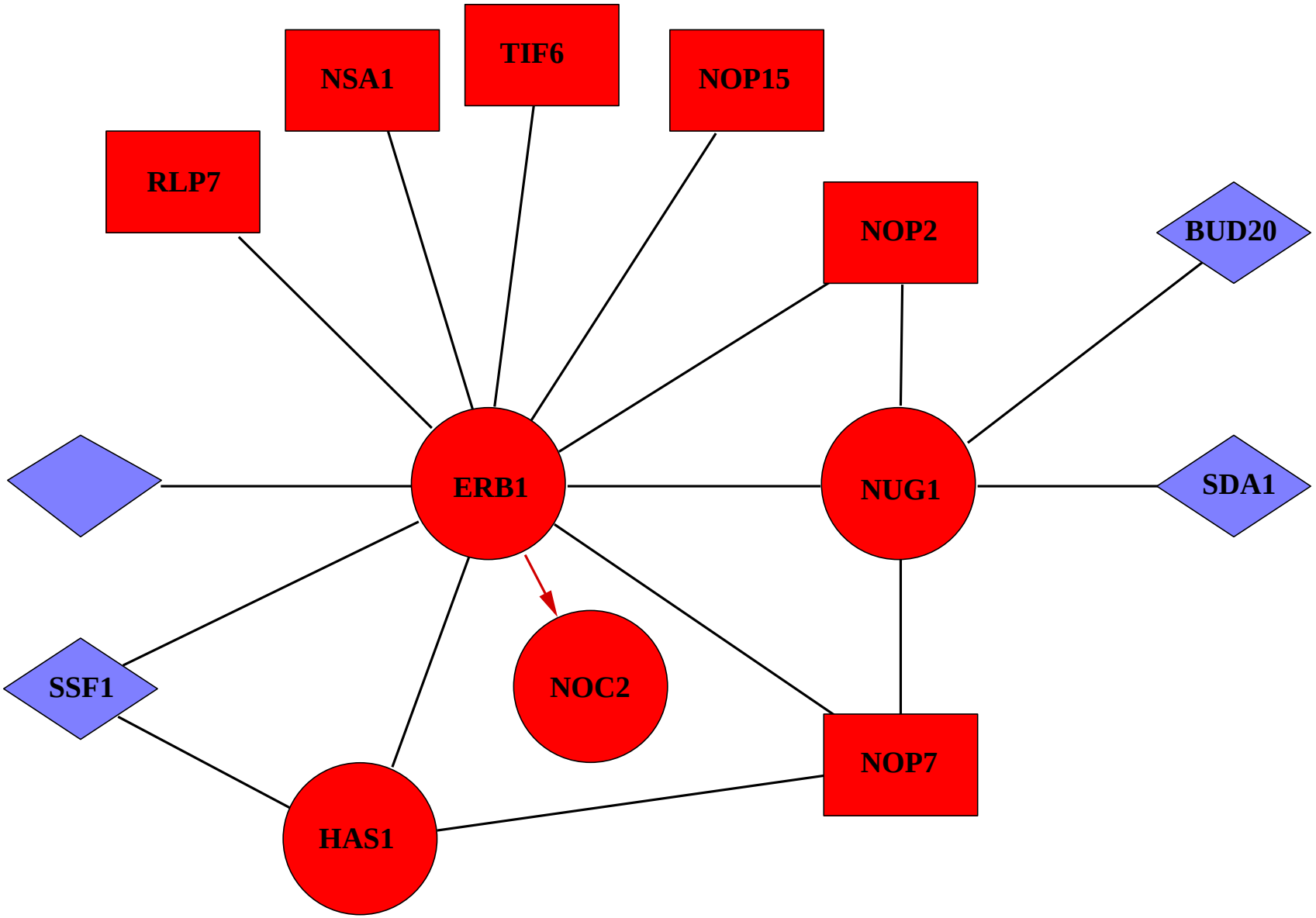

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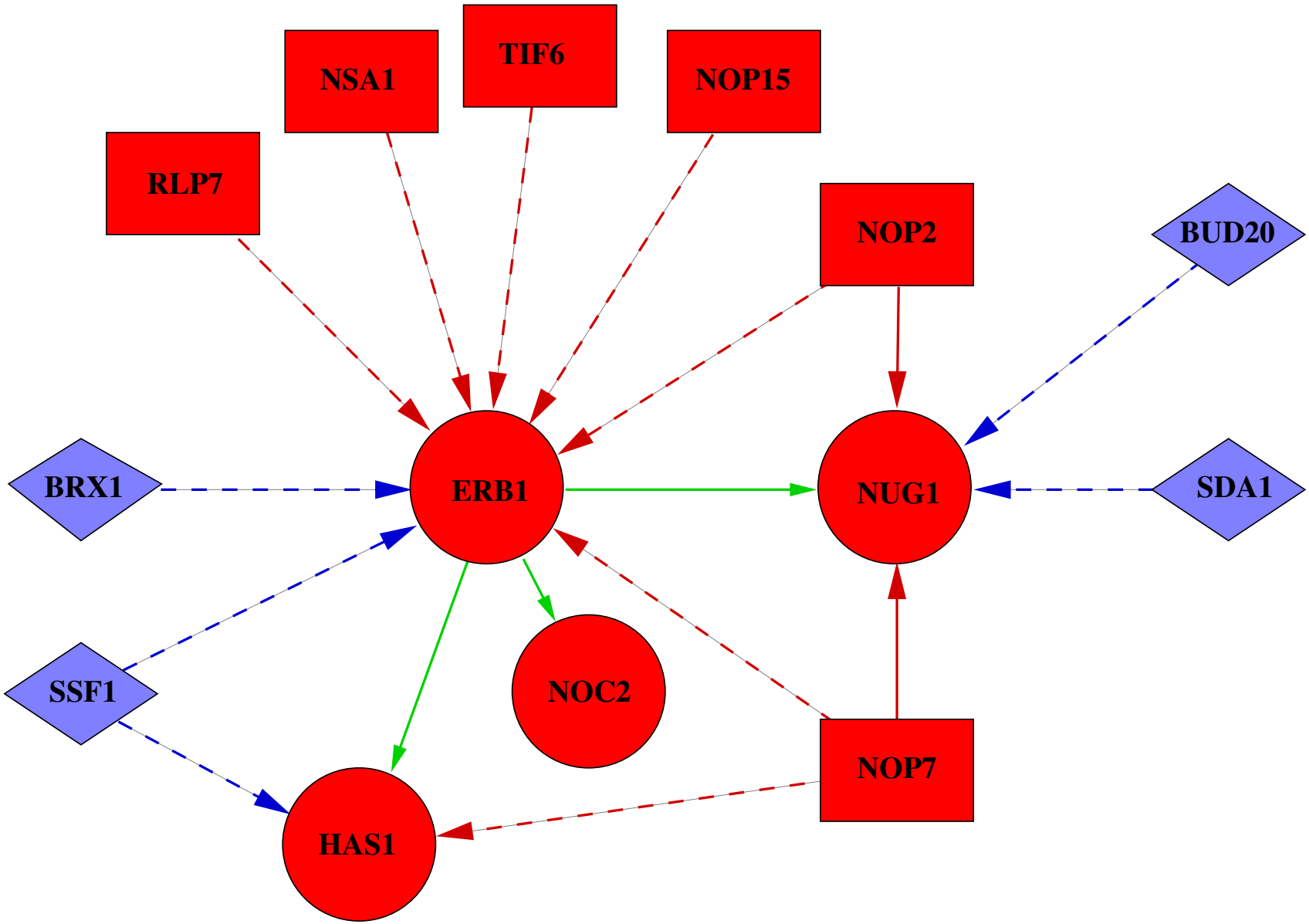

Data Sets

- Interactions: General Repository of Interaction Datasets (GRID).
- Microarray: *Functional discovery via a compendium of expression profiles*. Hughes TR et al. *Cell*. 2000 102: 109–26.
- 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.

ORF_A	ORF_B	EXPERIMENTAL_SYSTEM	SOURCE	PUBMED_ID
YER006W	YPL211W	Affinity Precipitation	Bassler et al.	;11583615;
YDL140C	YBR154C	Two Hybrid	BIND	;2496296;9207794;10393904;

- 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.

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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: $s_i : 1 \rightarrow 0 \rightarrow -1$

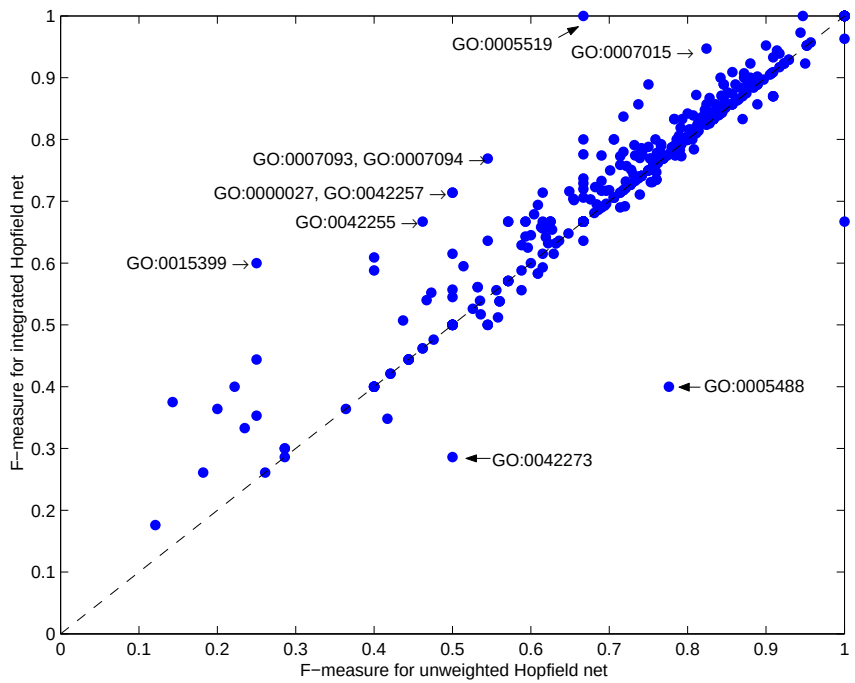
- Precision $=$ TP/(TP $+$ FP)
- Sensitivity $=$ Recall $=$ TP/(TP $+$ FN)
- F-measure $=$ Harmonic mean of precision and recall.

Results for Both Variants

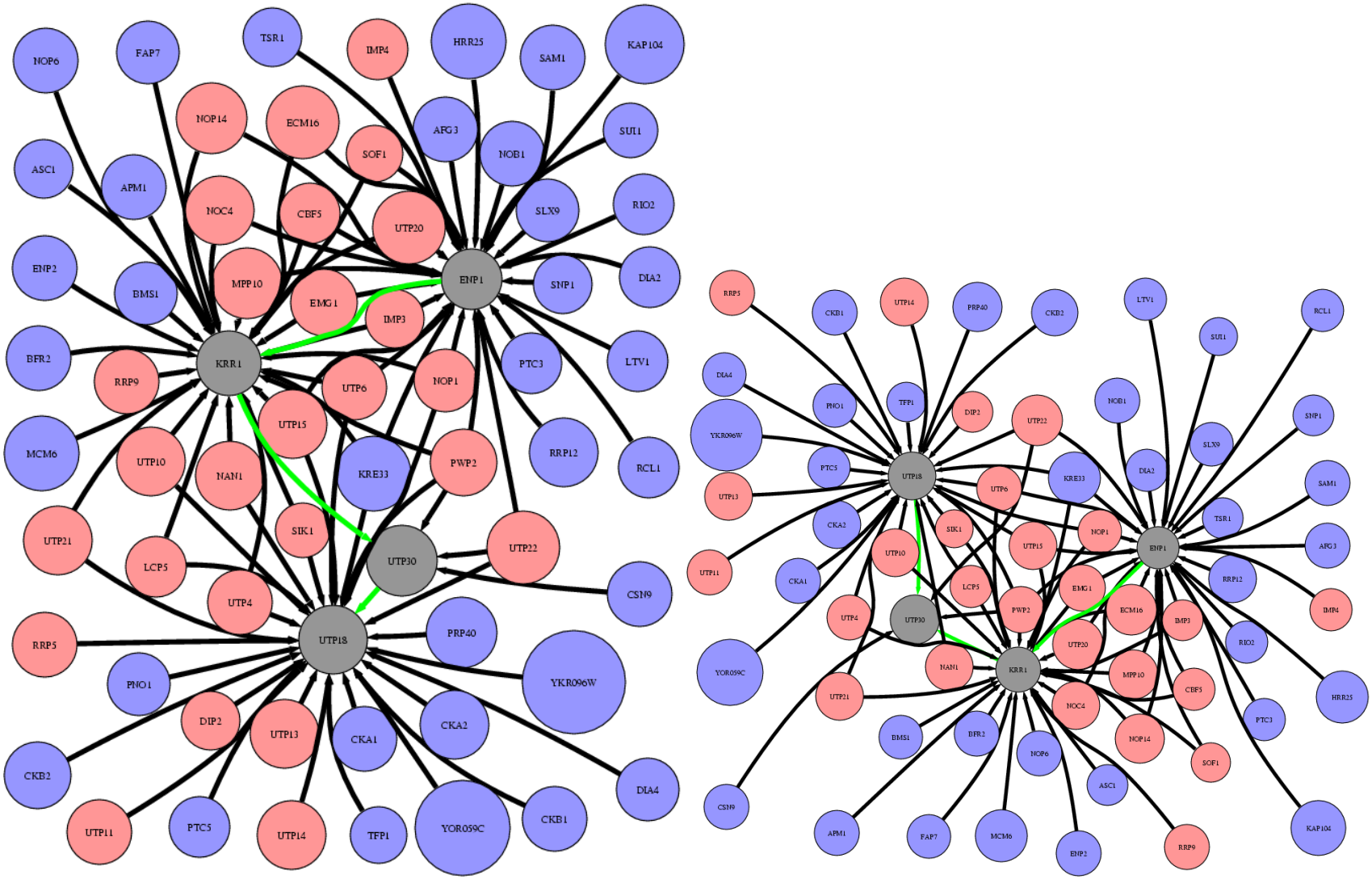
1. Overall comparison of cross-validation.
2. Specific examples of genes that perform better on cross-validation (see paper).
3. Novel functional annotations.
4. Propagation diagrams.

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



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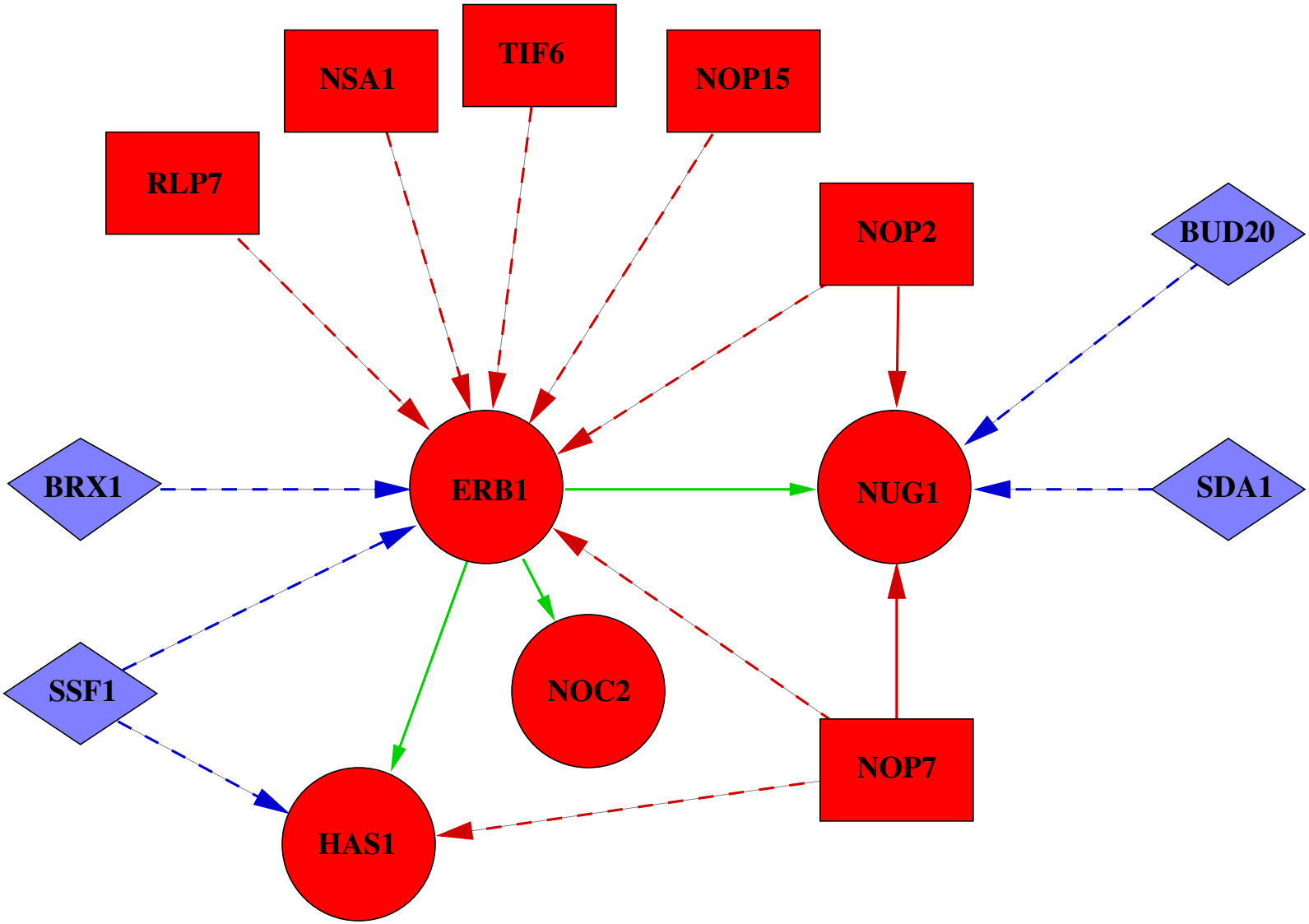
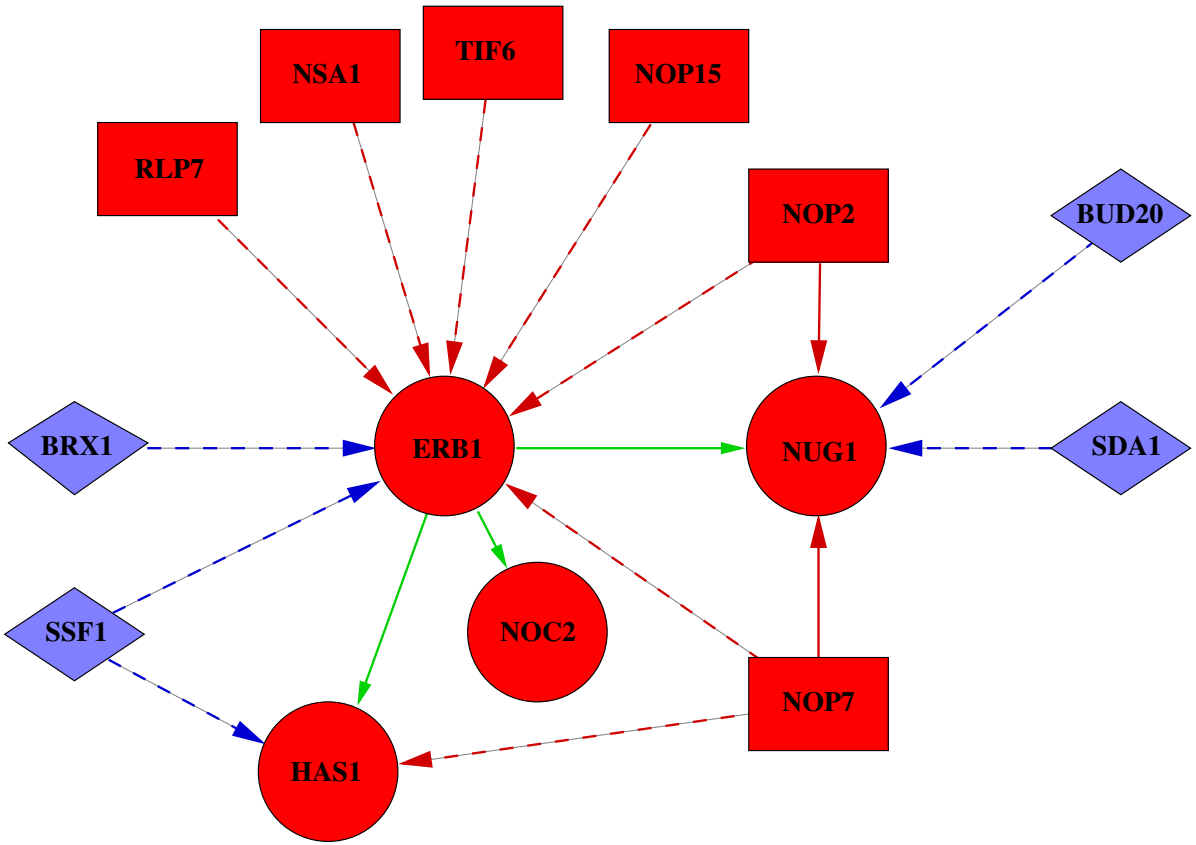


Novel Functional Annotations



- 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 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.