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Densely-connected Biclustering for Mining Interaction Networks with Expression Data

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Outline

- Introduction
- Related work
- Densely-connected biclustering
  [Moser et al, SDM 2009]
- Discovery of functional modules
  [Colak et al, PLoS ONE 2010]
- Inference of cancer subnetwork markers
  [Dao et al, Bioinformatics 2010]
- Conclusion

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- Biological networks: molecules and their interactions and attributes
- Nodes: proteins, protein/protein complexes, genes, mRNAs, . . .
- Attributes: properties of molecules such as expression values
- Edges: interactions / reactions between corresponding molecules

Introduction

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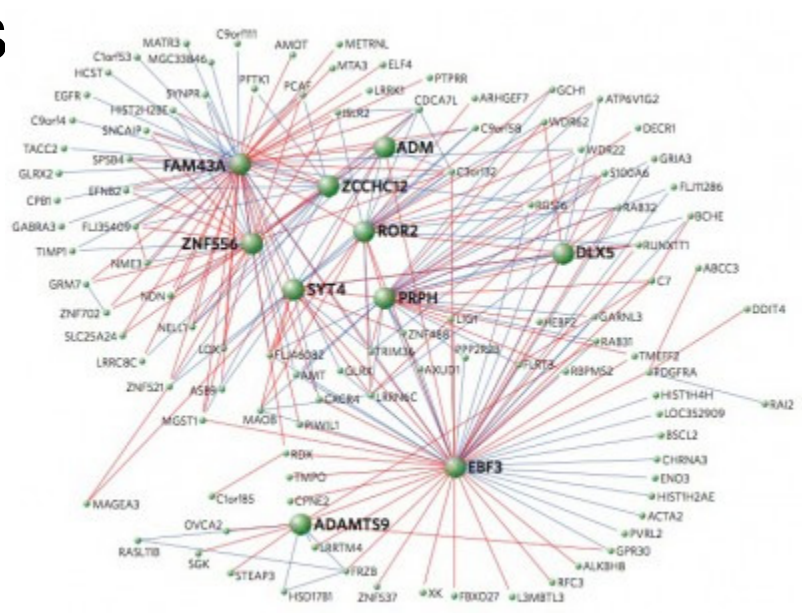
- Protein-protein interaction (PPI) networks
  interactions of protein molecules, which are important for many biological functions
- Gene regulatory networks / Transcriptional regulatory networks
  promotion / repression interactions between genes, transcription factors (TFs)



Introduction

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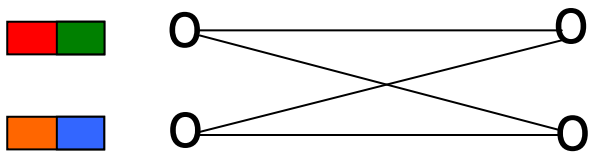
- High-throughput data to infer networks
- Attributes such as gene expression (microarray, SAGE)
- Interactions such as regulatory protein-gene interactions (ChIP)
- Interactions are often condition-specific

  if some genes are regulated by the same transcription factors, they are expressed at similar levels under the same conditions



Introduction

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Advanced biclustering methods

- Order preserving submatrices [Ben-Dor et al 2002]
  - considers relative expression values
  - subset of genes whose expression values induce a linear order across a subset of conditions
- Significant biclusters (Samba) [Tanay et al 2002]
  - subset of genes that jointly respond across a subset of conditions
  - gene responds if its expression level changes significantly

Related Work

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Finding dense components

- MCL (Markov Clustering) [Enright et al., 2002] simulates flow in a network by computing successive powers of the adjacency matrix
- Quasi-cliques [Pei et al 2005] subnetworks of nodes that all have more than a specified percentage of all possible out-edges within the subnetwork

Related Work

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Motivation for integrated methods

- High-throughput data of either type is noisy
  e.g. high-throughput PPI data can contain up to 50% false positives
- Single data types provide only partial information on the underlying biological system
  e.g. interaction network is static picture of the interactome and cannot yield insights into the dynamic behavior of cellular systems

Related Work

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Integrated methods

- Co-clustering [Hanisch et al. 2002]
  distance function combining expression and network distance, apply standard (distance-based) clustering algorithm

  → does not guarantee connectivity, partitions the data
- Matisse [Ulitsky & Shamir 2007]
  test the hypothesis of a group of genes being co-expressed, find connected subnetworks which pass the test on a high significance level

→ does not guarantee density, clusters are partitioning

Related Work

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- Given a network (graph) with d-dimensional (node) attributes.
- Want to find subnetworks that
  - are dense,
  - connected,
  - and have similar attribute values in a subspace.

→ can overlap

Densely-connected Biclustering

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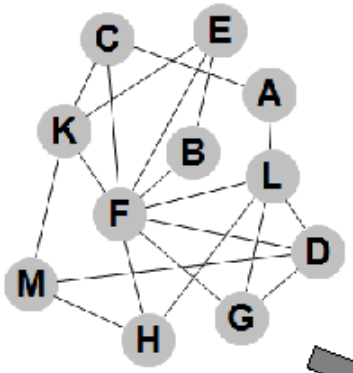
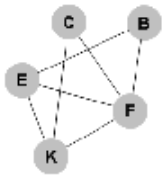
- *Densely-connected bicluster (DCB)*:
  subgraph G' satisfying three conditions:
  (1) subspace *homogeneity*, i.e. attribute values are within a range of at most ω in at least δ dimensions,
  (2) *density*, i.e. has at least α of all possible edges, and
  (3) *connectedness*, i.e. each pair of nodes has a connecting path in G'.
- Task
  Find all *maximal* DCBs.

Densely-connected Biclustering

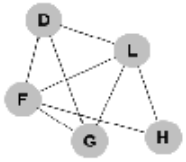


density = 7/10



	Con-1	Con-5	Con-6
B	1	0	2
C	1	0	2
E	1	0	2
F	1	0	2
K	1	0	2

DCB



	Con-3	Con-4	Con-7
D	-1	-1	0
F	-1	-1	0
G	-1	-1	0
H	-1	-1	0
L	-1	-1	0

density = 8/10

	Con-1	Con-2	Con-3	Con-4	Con-5	Con-6	Con-7
A	2	-2	1	0	2	1	2
B	1	1	0	-2	0	2	1
C	1	0	0	1	0	2	1
D	0	0	-1	-1	2	1	0
E	1	1	2	0	0	2	2
F	1	1	-1	-1	0	2	0
G	0	2	-1	-1	2	0	0
H	1	1	-1	-1	1	1	0
K	1	0	2	0	0	2	1
L	2	2	-1	-1	-1	-1	0
M	2	0	1	1	1	-2	-1

$\alpha = 0.7$
$\delta = 3$
$\omega = 0.0$

Densely-connected Biclustering

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- DCB problem is NP-hard
  its decision version can be reduced from the Max-Clique problem.
- Search space of all potential DCBs (subgraphs) is a lattice:

- a subnetwork is a *child* of another one if it can be obtained by adding exactly one node and the corresponding edges to the parent network
- *bottom* of lattice: the complete network
  top of lattice: pairs of nodes

Densely-connected Biclustering

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- A constraint is *loose anti-monotone* if for each network G of size n that satisfies the constraint, there is *at least one* induced subnetwork G' of G of size n - 1 satisfying the constraint.
- For α >= 0.5, the DCB constraints are loose anti-monotone.
- level-wise (breadth-first) search of the lattice structure in a top-down manner

→ construct only connected subgraphs

→ prune all candidates that do not satisfy the constraints of density and homogeniety

Densely-connected Biclustering

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- Pre-processing

remove edges between nodes that cannot belong to same DCB

- Seed generation

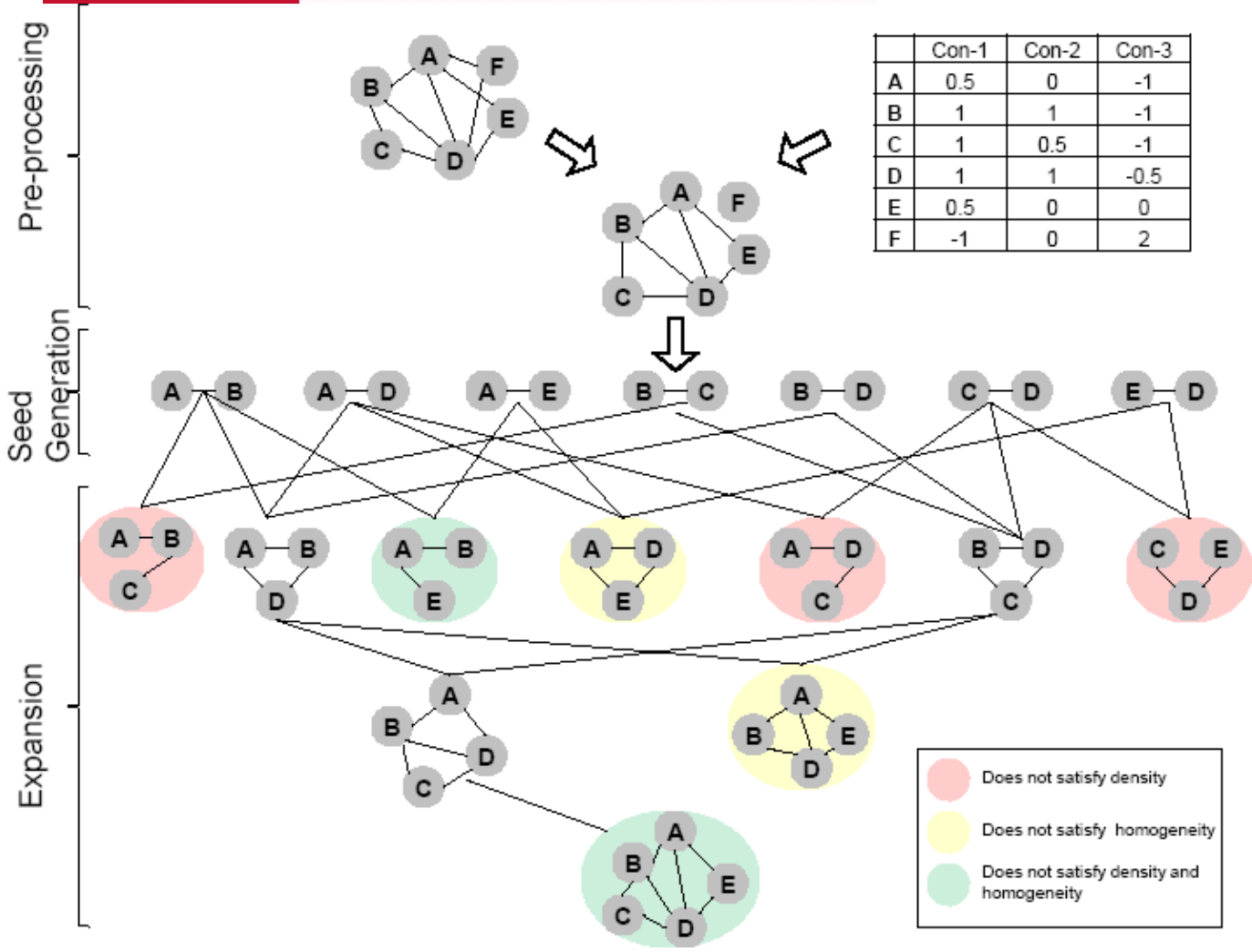
create DCBs of size 2

- Expansion

level-wise search of the lattice structure

→ Algorithm is efficient
as long as the network is not too dense

Densely-connected Biclustering



$\alpha = 0.8$
$\delta = 2$
$\omega = 0.5$

Densely-connected Biclustering

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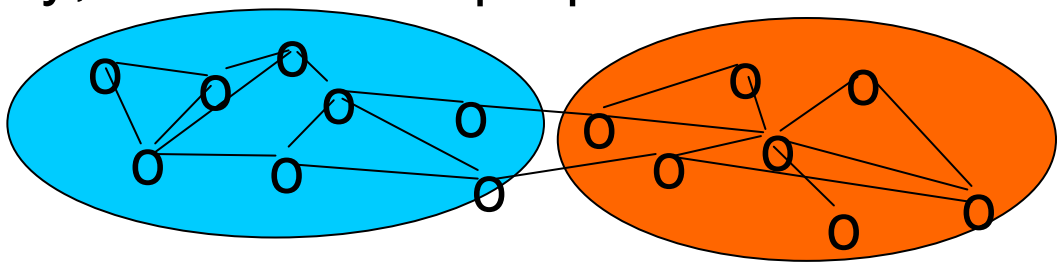
- Modularity is fundamental design principle in natural and man-made systems
- Modules are formed according to common physical, regulatory, or functional properties



- Many interactions within modules,
  few interactions between different modules

→ Easy debugging and replacement of modules

Discovery of Functional Modules

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- Functional modules in PPI network
  static molecular complex (e.g. ribosome)
  or a dynamic signaling pathway
  e.g. MAP kinase cascade
- Functional modules in gene regulatory networks
  regulatory modules, in which every gene is controlled by the same TFs under the same environmental conditions
- Here: discovery of modules in PPI networks
  using DCB

Discovery of Functional Modules

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- Yeast dataset
  PPI network from BioGRID database
  gene expression data from yeast compendium dataset
- Human dataset
  PPI network from BioGRID database
  human tissue expression dataset
- GO-based evaluation
  enrichment: percentage of modules that are enriched with at least one GO term
  coverage: percentage of GO-terms that are enriched in at least one module
  F-value: harmonic mean of enrichment and coverage

Discovery of Functional Modules

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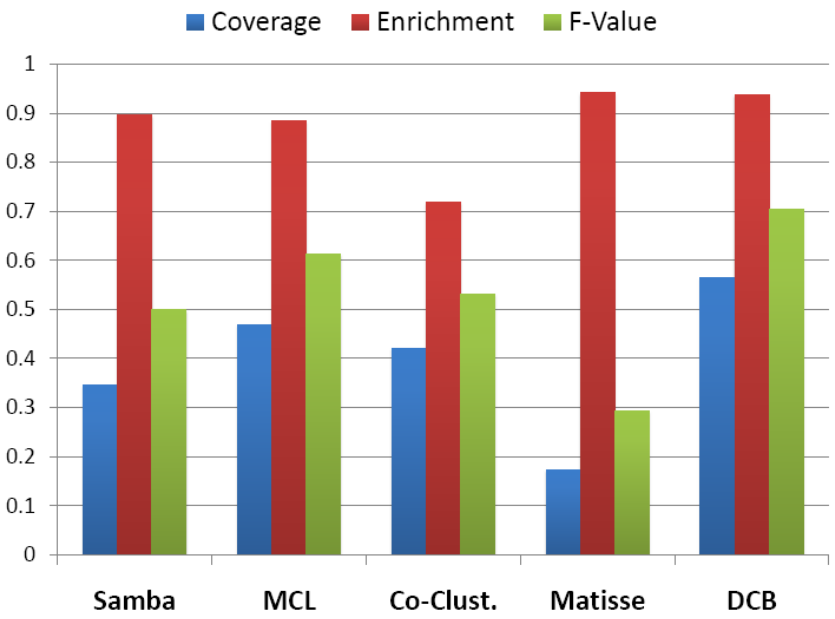
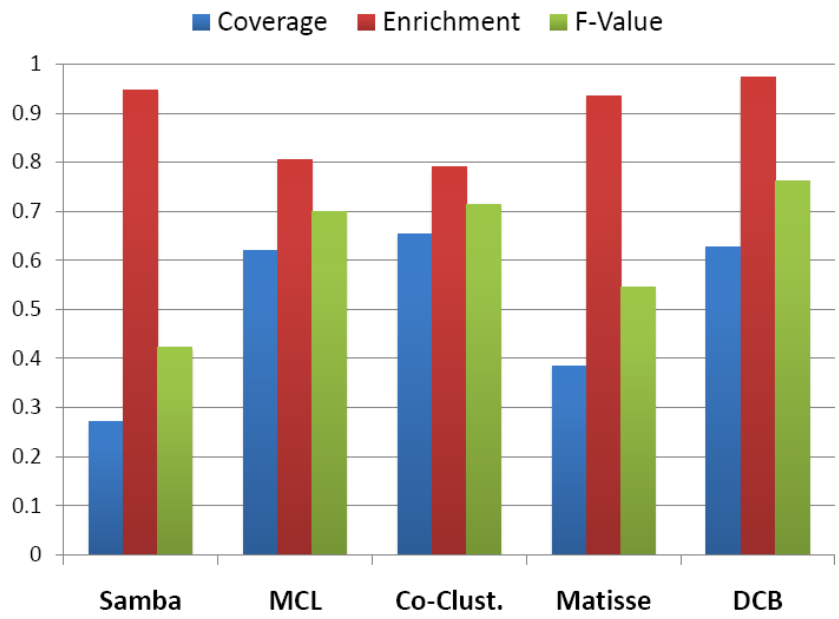
Yeast



Human



→ DCB consistently outperforms the comparison partners

Discovery of Functional Modules

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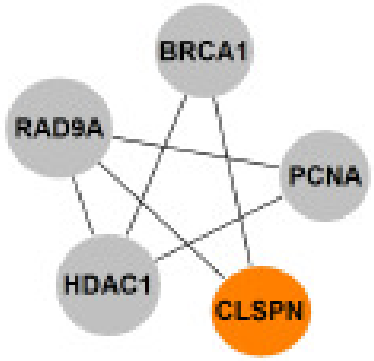
Annotation Prediction

- Using annotations of neighboring genes in an enriched module → based on the guilt-by association principle
- Examine genes that do not have detailed GO term annotations but have some related work in the literature



Annotations predicted for Claspin:

DNA replication checkpoint
regulation of DNA replication initiation
regulation of DNA repair
DNA damage checkpoint

Discovery of Functional Modules

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Biomarker Discovery

•**Single gene markers:** Each gene is ranked according to their ability to distinguish samples of different classes

• **Multigenic markers:** Each subset G of genes is ranked based on the aggregate ability of all genes in G to distinguish samples of different classes

Inference of Cancer Subnetwork Markers

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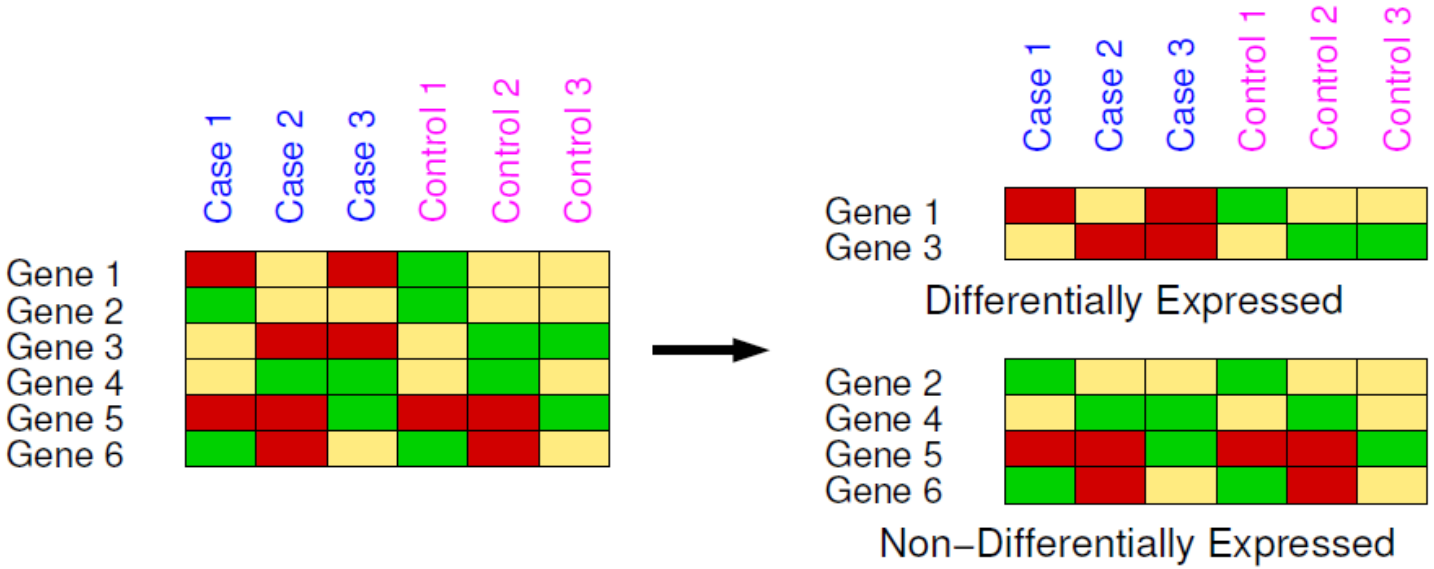
Single gene markers



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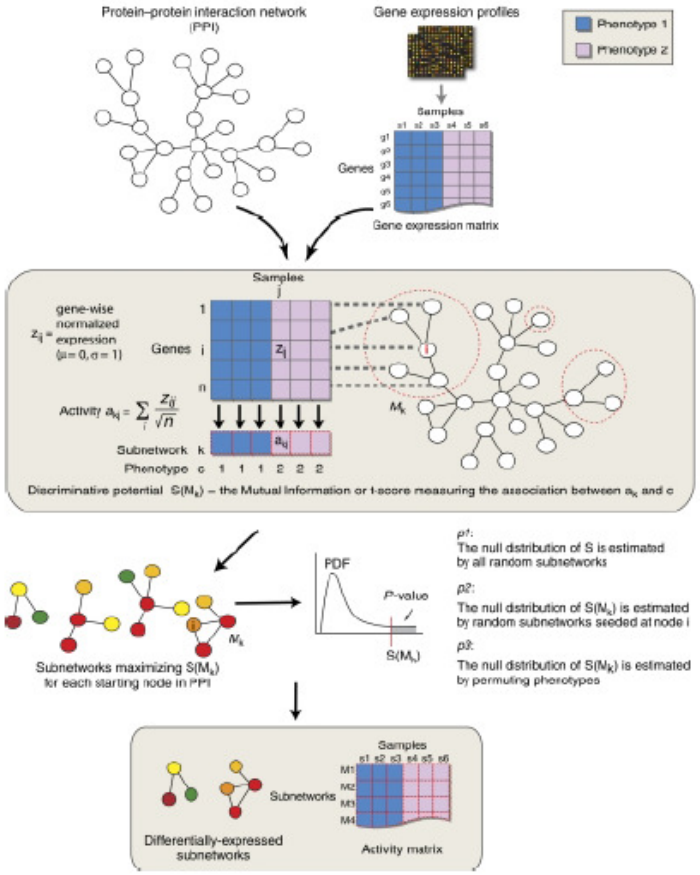
Multigenic markers

[Chuang et al., Mol.Sys.Biol. (2007)]:

•Predicting progression of breast cancer

•Markers are connected subgraphs with aggregate expression profiles that correlate the most with the labels of the samples

•Greedy heuristics for searching for optimal subnetwork markers



Inference of Cancer Subnetwork Markers

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Multigenic markers

[Chowdhury et al., PSB 2010]:
•Predicting colon cancer subtypes

•Each marker is a small connected subnetwork *G* such that genes in *G* **cover** all disease samples (gene *g* **covers** sample *s* if *g* is differentially expressed in *s*)

•Greedy heuristics for searching for the smallest subnetwork markers

Inference of Cancer Subnetwork Markers

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Motivations

[Goh et al., PNAS (2007)]:

•The protein products of genes related to the same disease tend to interact with one another.

•Genes related to a disease have coherent functions with respect to the Gene Ontology hierarchy.

Inference of Cancer Subnetwork Markers

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Our Approach

Our subnetwork markers:

•include genes that have higher interaction among them than expected (**densely connected subnetworks**), and

•contain differentially expressed genes in a fraction of all the samples from cancer tissues (**partially differential expression**).

Reduce problem to Densely-Connected Biclustering

1: gene is differentially expressed in sample

0: gene is not differentially expressed in sample

Inference of Cancer Subnetwork Markers

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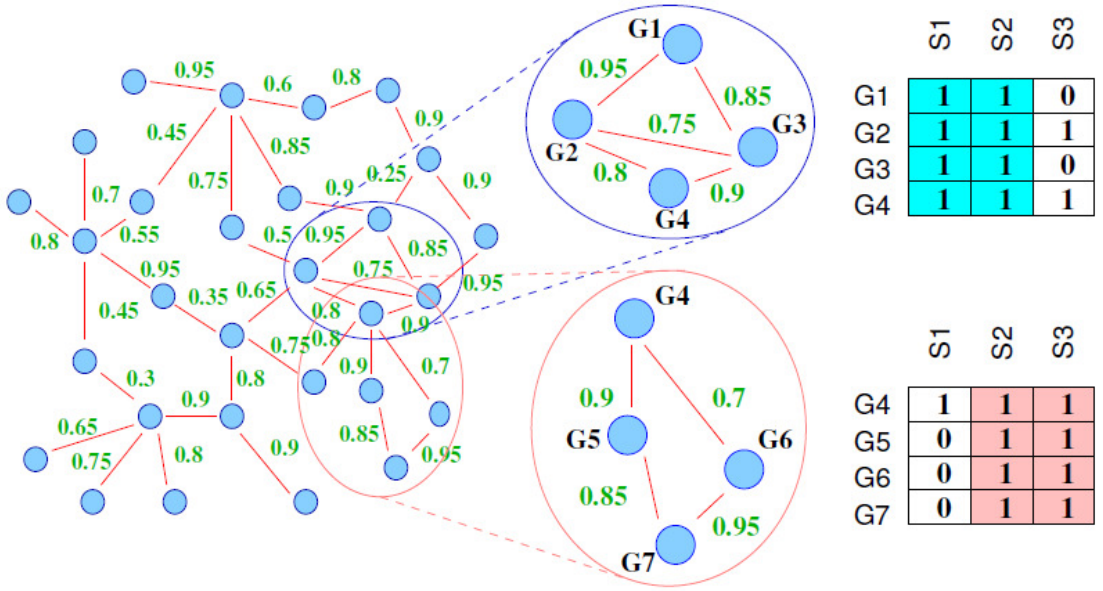
Partially Differential Expressed



Compute all densely connected subnetworks whose genes are differentially expressed in a subset of patients of size at least k (here: $k = 2$).

Inference of Cancer Subnetwork Markers

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Classifier Construction

1.Rank density-connected biclusters according to density significance

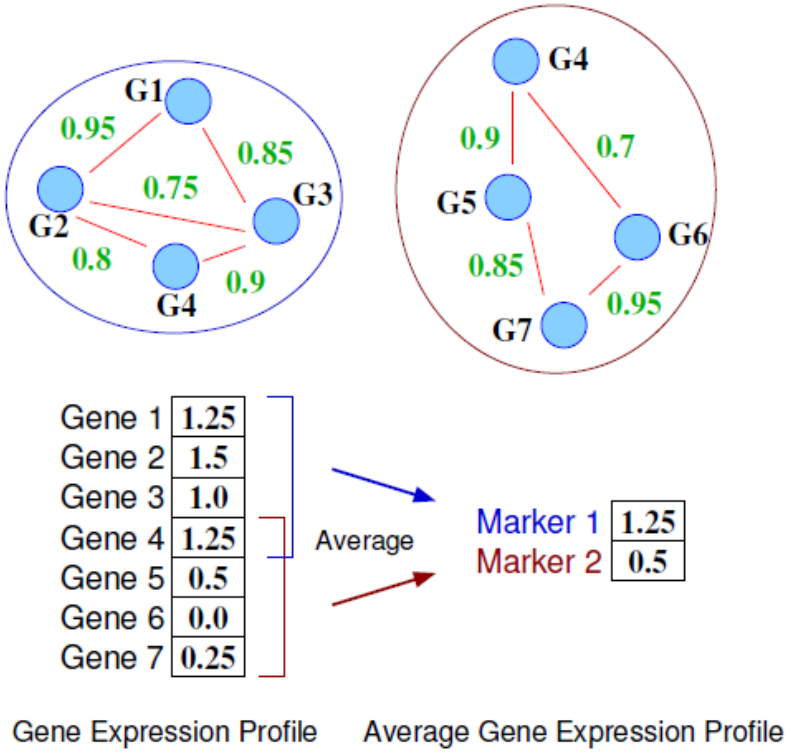
2.Keep only highly-ranked subnetworks with little overlap

3.Feature generation

dimension of feature space = number of markers

feature values = average expression of genes

4. SVM classification



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Confidence-scored PPI network

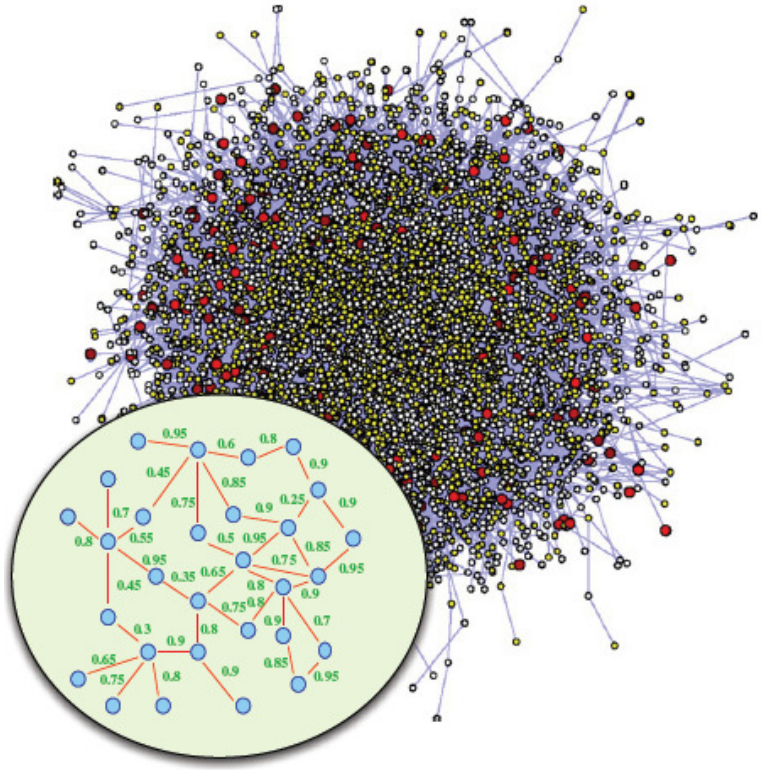
[STRING, von Mering et al., NAR 2009]:

•Edges reflect physical protein-protein interactions

•Confidence scores reflect the probability that the interaction is associated with a cellular phenomenon (and not an experimental artifact)

•Scoring system based on KEGG pathways

→ DCB can be extended to edge-weighted networks (wDCB)



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Gene Expression Datasets

Three colon cancer datasets:

- GSE8671, 32 patients / tissue pairs
- GSE10950, 24 patients / tissue pairs
- GSE6988, 123 samples across several cancer subtypes

Inference of Cancer Subnetwork Markers

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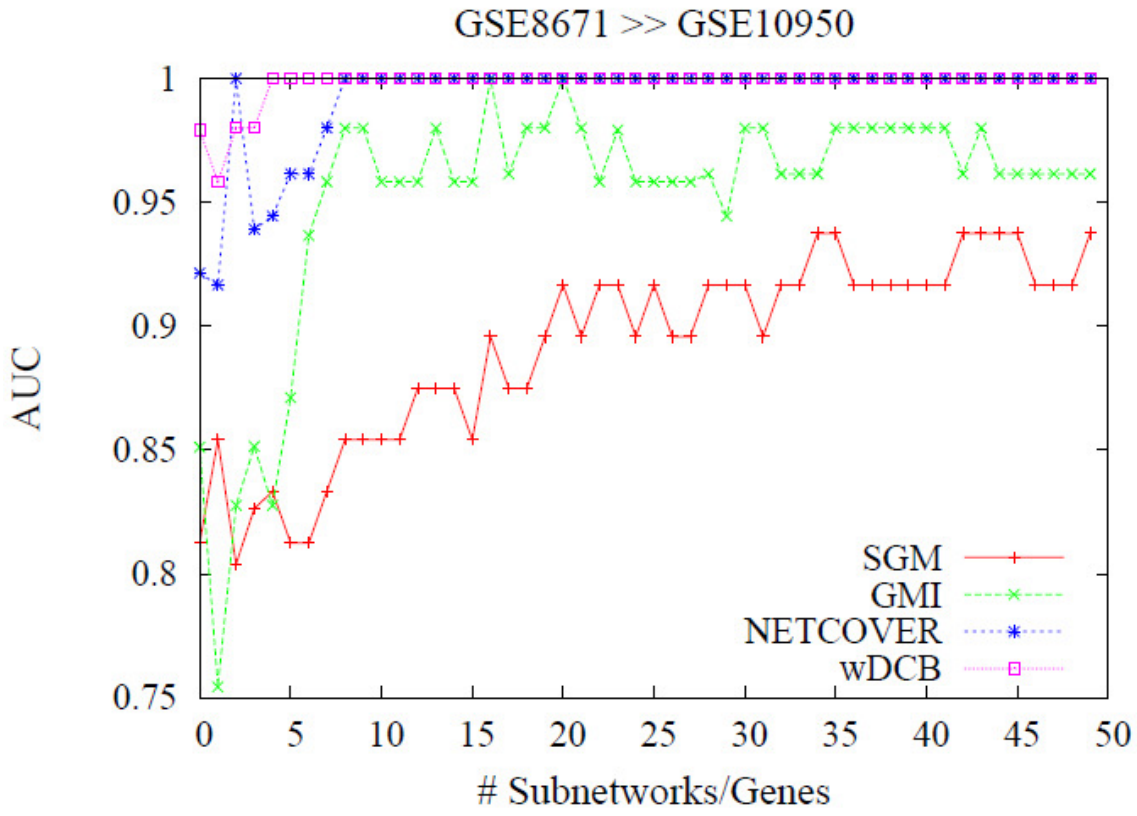
Classification Performance



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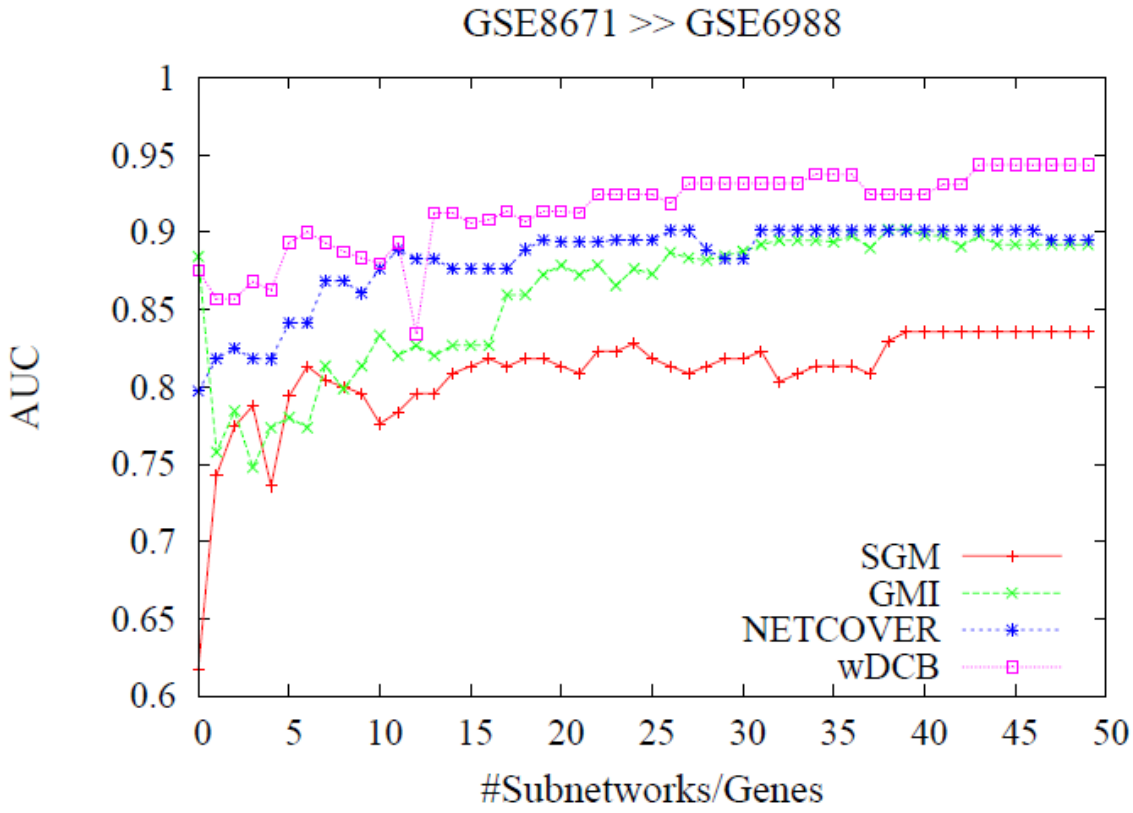
Classification Performance



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Subnetwork Marker Statistics

			Avg AUC				Avg AUC	
	#	ER-50	6988	10950	#	ER-50	6988	8671
GMI	806	0.38	0.86	0.95	755	0.34	0.84	0.99
NC	923	0.12	0.87	0.99	N/A	N/A	0.86	N/A
wDCB	282	0.76	0.91	1.00	216	0.74	0.91	1.00
	8671 Subnetworks				**10950 Subnetworks**			

GMI = Greedy Mutual Information (Chuang et al.)
NC = NetCover (Chowdhury et al.)
wDCB = weighted Density Constrained Biclustering
# = total number of subnetworks computed
ER-50 = enrichment rate of the top-50 markers

Inference of Cancer Subnetwork Markers

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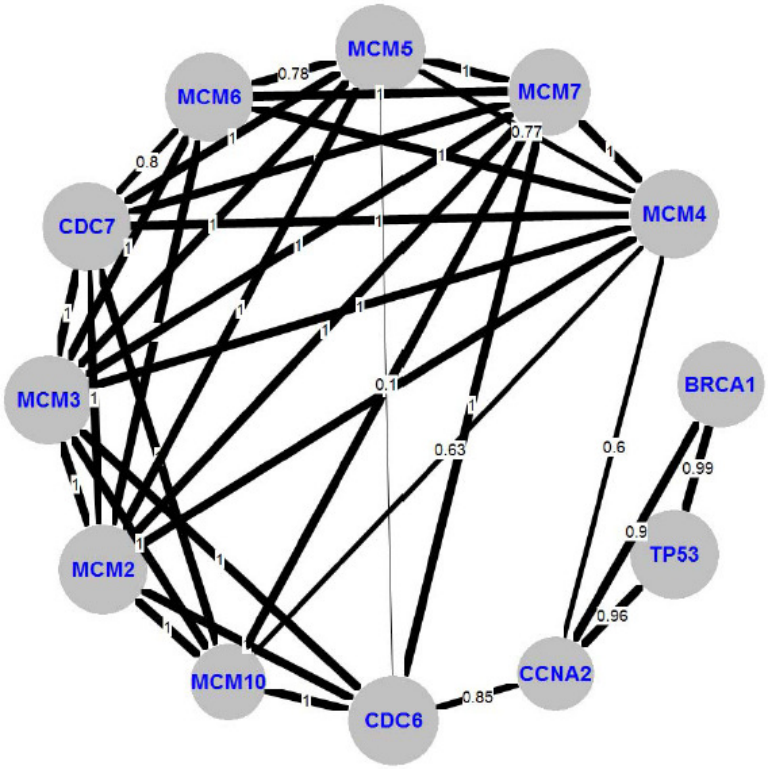
Top Marker from GSE8671

- DNA replication initiation
- DNA metabolic process
- TP53, BRCA1: tumor
- suppressor genes
- Minichromosome maintenance complex (MCM complex)
- Protein kinase CDC7 phosphorylates MCM2



Inference of Cancer Subnetwork Markers

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Future Work

•Compare subnetwork signatures of different cancers or subtypes of a particular cancer

•Extend the interaction network, e.g.with ncRNA and ncRNA-protein interactions

•Redesign novel methods to work with real-valued continuous phenotype variables

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References

Ben-Dor et al (2002): “Discovering local structure in gene expression data: the order-preserving submatrix problem”, Proc. RECOMB 2002.

Colak, R. et al (2010): “Inferring the active modulome: an approach based onDensely Connected Biclustering”, PLoS ONE 5(10): e13348.

Dao, P. et al (2010): “Inferring cancer subnetwork markers using density-constrained biclustering”, Bioinformatics 26(18).

Enright, A. J. et al. (2002): “An efficient algorithm for large-scale detection of protein families”, Nucleic Acids Research 30, 1575-1584.

Hanisch, D. et al. (2002): “Co-clustering of biological networks and gene expression Data”, Bioinformatics, 18, (Suppl. 1): 145-154.

Moser et al (2009): “Mining Cohesive Patterns from Graphs with Feature Vectors”, Proc. SDM 2009.

Pei, J. et al. (2005): “Mining Cross-Graph Quasi-Cliques in Gene Expression and Protein Interaction Data”, Proc. 21st IEEE Int. Conf. on Data Engineering.

Tanay A., Sharan R., Shamir R. (2002): “Discovering statistically significant biclusters in gene expression data”, Bioinformatics, 18:Suppl1, S136-S144.

Ulitsky, I. and Shamir, R. (2007): “Identification of functional modules using network topology and high-throughput data”, BMC Systems Biology, 1:8