MINING MICROARRAY DATA FOR BIOLOGICALLY IMPORTANT GENE SETS

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ABSTRACT

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Microarray technology enables researchers to measure the expression levels of thousands of genes simultaneously to understand relationships between genes, extract pathways, and in general understand a diverse amount of biological processes such as diseases and cell cycles. While microarrays provide the great opportunity of revealing information about biological processes, it is a challenging task to mine the huge amount of information contained in the microarray datasets. Generally, since an accurate model for the data is missing, first a clustering algorithm is applied and then the resulting clusters are examined manually to find genes that are related with the biological process under inspection. We need automated methods for this analysis which can be used to eliminate unrelated genes from data and mine for biologically important genes. Here, we introduce a general methodology which makes use of traditional clustering algorithms and involves integration of the two main sources of biological information, Gene Ontology and interaction networks, with microarray data for eliminating unrelated information and find a clustering result containing only genes related with a given biological process. We applied our methodology successfully on a number of different cases and on different organisms. We assessed the results with Gene Set

Enrichment Analysis method and showed that our final clusters are highly enriched. We also analyzed the results manually and found that most of the genes that are in the final clusters are actually related with the biological process under inspection.

Keywords: microarray analysis, Gene Ontology, interaction networks, clustering

ÖZ

BİYOLOJİK ÖNEM TAŞIYAN GEN LİSTELERİNİN BULUNMASI İÇİN MİKRODİZİ VERİ MADENCİLİĞİ

Korkmaz, Gülberal Kırçiçeği Yoksul
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Mikrodizi teknolojisi araştırmacıların aynı anda birçok gen ifade seviyesini ölçerek genler arasındaki ilişkileri anlamalarını, yolakları bulmalarını ve genel olarak hastalıklar, hücre döngüsü gibi birçok biyolojik olayı anlayabilmelerini sağlamaktadır. Aynı anda büyük sayılarda deneyin yapılmasına olanak sağlamakla birlikte, bu büyük miktarda mikrodizi verisini araştırmak ilgi çekici bir konudur. Veri hakkında önceden fazla bilginin olmaması nedeni ile genellikle önce veriye bir bölümleme algoritması uygulandıktan sonra bulunan bölümler konu ile ilgili önemli genleri bulmak amacı ile araştırmacılarca incelenir. Bu incelemeyi kolaylaştırmak için gereksiz genleri eleyerek biyolojik olarak önemli genleri bulacak otomatik metotlar gereklidir. Bu tezde mevcut bölümleme algoritmalarını kullanan, Gen Ontolojisi ve etkileşim ağları olmak üzere iki ana biyolojik bilgi kaynağını birleştirerek mikrodizi verisindeki gereksiz bilgileri eleyen ve çıktı olarak sadece deneyle ilgili genleri içeren bir bölümleme veren genel bir metodoloji sunulmaktadır. Sunulan metodoloji birçok farklı veri üzerinde denenmiş ve umut verici sonuçlar elde edilmiştir. Sonuçlar Gen Seti Zenginleştirme Metodu (GSEA) ile karşılaştırılmış ve metodoloji ile bulunan bölümlerin yüksek zenginleştirme skorlarına sahip olduğu görülmüştür. Sonuçlar üzerinde yapılan detaylı incelemelerde

de bölümleme sonucunda bulunan genlerin büyük çoğunluğunun deney konusu olan biyolojik süreç ile ilişkili olduğu tespit edilmiştir.

Anahtar Kelimeler: bölümleme, mikrodizi, gen ontolojisi, etkileşim ağları

To my dearest daughters Ece and Eda

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CHAPTER 1

INTRODUCTION

1.1 Motivation

There are thousands of genes in the genome of an organism. At a given instant of time, only a small percentage of them are expressed. Thanks to the microarray technology, the expression levels of thousands of genes can be measured simultaneously. Microarray technology enables researchers to study the expressions of entire genomes under different conditions. Microarray experiments are used to understand relationships among genes, extract pathways, and in general to understand a diverse amount of biological processes. While microarrays provide great opportunity of revealing information about biological processes, it is a challenging task to mine the the massive quantity of microarray datasets to identify important aspects of biological processes. The methods to analyze microarray data should address the curse of dimensionality problem raised by the tens of thousands of genes and small sample sizes, i.e. small sizes of experimental conditions or time points compared to the number of measurements.

1.2 Problem Definition

A widely used technique in mining microarray data is to apply clustering since an accurate model for the data is missing. There are dozens of clustering algorithms available in the literature, each of which is better under certain conditions. Generally, clustering algorithms all result with different number of clusters and cluster contents differ greatly as can be seen from the results presented in Chapter 5. To solve these stability problems, ensembles of clusters approach may be considered. However, even

when the *perfect* clustering that fits to the data is achieved, the interpretation of the clustering still remains as a problem to solve. For the interpretation of results, biologists apply enrichment methods to select important clusters and then manually analyze the genes in the selected clusters. In the analysis, they make use of the information contained in databases such as Gene Ontology, pathway databases and biological networks. Among tens of thousands of genes, at most hundreds of them are related with the biological process under inspection. We need automated methods to mine for that hundreds of biologically important genes.

Another problem to be solved in mining microarray data is the combination of heterogeneous datasets. There are experiments made at different times and conditions about the same process, with different number of samples and genes involved. We need methodologies to find the genes that play a common role among these experiments about the same biological process performed at different times and conditions.

The problem definition is to find biologically important genes in an experiment or locate the common characteristics between heterogeneous datasets without facing the curse of dimensionality problem. We should make use of the previous knowledge about genes contained in the biological information resources. Gene Ontology is an important resource containing information about genes and it is widely used. Gene interaction network is yet another important source of information that enriches the knowledge about genes and gene lists. Furthermore, the number of known gene interactions is constantly growing thanks to the recent developments in research. Therefore, it is valuable to combine these two important sources of information in the analysis.

1.3 Contributions

In this thesis, we provide a general methodology which involves integration of Gene Ontology, interaction networks and microarray data to eliminate unrelated information from microarray data and find a clustering result containing only genes which are related with the biological process under inspection. The methodology is also applicable to the problem of combination of heterogeneous datasets.

The contributions of this thesis are as follows:

- We describe and assess ***Interaction Based Homogeneity***, a measure to evaluate the relationship of a gene list with respect to an interaction network. To the best of our knowledge, this is the first study to use interaction networks in the calculation of homogeneity of gene lists.

- We propose a ***gene weight measure*** calculated from *Interaction Based Homogeneity* values of the clusters that a gene belongs to and use it to clean up clusters.

- We propose a novel and robust methodology called ***Cluster-Eliminate-Combine (CEC)*** integrating Gene Ontology and interaction networks for mining microarray data.

- We show that *instead of using whole interaction networks, taking their subset using Gene Ontology terms* dramatically improves the performance of the analysis.

- We show that the *CEC* methodology is applicable for different cases such as combining multiple clustering algorithms for the analysis of the same microarray data and for combining heterogeneous microarray experiments to find their common characteristics.

1.4 Organization of the Thesis

A brief introduction is already given in this chapter. Background and literature information about microarray data analysis, clustering ensembles, gene ontology, interaction networks, enrichment methods and data integration is presented in Chapter 2. Interaction Based Homogeneity is presented in Chapter 3. Cluster-Eliminate-Combine methodology is outlined in Chapter 4 along with the results proving the necessity of each component of the methodology. Chapter 5 contains detailed results and discussions of applying the CEC methodology to a diverse set of datasets and cases. The thesis ends with Chapter 6 which contains conclusions and future work.

CHAPTER 2

BACKGROUND INFORMATION

One of the biggest challenges in bioinformatics research is to infer networks which represents relations among genes. Usually, high throughput experiments such as microarray experiments are applied to gather information for network construction. Generally, a two-step approach has been taken in order to interpret the results of the microarray experiments to infer relationships. First, a clustering algorithm is applied onto the data. The clustering results are then interpreted to extract relationships [1, 2, 3]. In the first section of this chapter, clustering algorithms for microarray data is summarized. In the second section, a survey on clustering combination methods is given. The third section describes methods for the interpretation of clustering results. The next section summarizes partition similarity metrics used in our study. The last section contains a survey on methods integrating different biological information sources.

2.1 Clustering Microarray Data

For clustering microarray data, general clustering methods such as k-means and hierarchical clustering [4, 5, 6, 7] are widely used. In addition to general algorithms, there are algorithms designed specifically for the analysis of microarray data. Fu and Medico proposed the Fuzzy Clustering by Local Approximation of Membership (FLAME) method which is based on a fuzzy clustering algorithm which makes use of neighborhood information. The implementation of the algorithm can be found in Gene Expression Data Analysis Studio (GEDAS) [2]. Huttenhower et al. proposed Nearest Neighbor Networks (NNN) clustering algorithm which uses neighborhood information to construct an interaction graph and searches for mutual cliques in this

graph to find clusters [8].

A popular and recent method is biclustering which clusters simultaneously genes and samples or experimental conditions [9]. A survey on biclustering algorithms is provided by Tanay et al. [10]. Prelic´ et al. provides a comparison of biclustering methods for gene expression data [11].

A specific type of microarray experiments is called time-series microarray experiments at which the expression of genes are measured at different time points. There are algorithms designed specifically for time series microarray data. Schliep et al. proposes a mixture model with Hidden Markov Models for analyzing time series data [12]. They apply partially supervised learning of mixtures through a modification of Expectation Maximization algorithm. Ernst et al. proposed Short Time Series Expression Miner method which first finds model profiles from the data and then uses them to find clusters [3]. The method is implemented in Short Time Series Expression Miner (STEM) tool. Sacchi et al. proposed Temporal Abstraction method for clustering short time series data [13]. The method is a generalization of the template-based clustering. The implementation of the algorithm can be found in TimeClust application [14]. Bin and Russo [15] filter genes and apply dimensionality reduction to the data before clustering.

In this work, we use five different clustering methods: FLAME, GQL, NNN, STEM and TAC. The details of these methods are given in the following subsections.

2.1.1 FLAME: Fuzzy Clustering by Local Approximation of Membership

Fu and Medico proposed the FLAME method which is a fuzzy clustering algorithm based on neighborhood information for the analysis of DNA microarray data. The implementation of the algorithm can be found in Gene Expression Data Analysis Studio (GEDAS) [2]. The method has three steps. In the first step, the object density of each gene (object) is calculated by using the distance between its k nearest neighbors. By using these densities, Cluster Supporting Objects and outliers are defined. In the next step, each object is assigned to a fuzzy membership vector in an iterative process which makes use of the object densities and the Cluster Supporting

Objects. In the final step, clusters are constructed by using the fuzzy membership vectors. In the following three subsections, the details of each step is given.

2.1.1.1 Extraction of Local Structure Information and CSO Identification

In this step, the similarities between each pair of objects are calculated and the k-nearest neighbors are identified for each object. The density of each gene is calculated as one over the average distance of the gene to its k nearest neighbors. Cluster Supporting Objects are identified as the genes with higher densities than all of its neighbors. Similarly, outliers are then identified as genes with lower densities than its neighbors. In addition, a density threshold is also applied to locate the outliers. The density threshold is calculated by using the mean and variance of the densities.

2.1.1.2 Local Approximation of Fuzzy Membership

In this step, each gene x is associated with a fuzzy membership vector $p(x)$ such that

$p(x) = (p_1(x), p_2(x), ..., p_M(x))$ where,

$p_i(x)$ denotes the membership of gene x to cluster i,

$0 \leq p_i(x) \leq 1; \sum_{i=1}^{M} p_i(x) = 1$, and

M is the number of clusters defined as the number of Cluster Supporting Objects plus one (for outliers).

For the calculation of the membership vector, the weights defining how much each neighbor contributes to the approximation is calculated by

$$w_{xy} = \frac{s(x,y)}{\sum_{z \in KNN(x)} s(x,z)},$$

where $s(x,y)$ is the similarity between x and y.

The membership vector is then calculated in an iterative process of local approximation:

$p^{t+1}(x) = \sum_{y \in KNN(x)} w_{xy} p^t(y)$,

$p_i^0(x) = 1, p_j^0(x) = 0, j \neq i, 1 \leq j \leq M$ for Cluster Supporting Objects representing cluster i,

$p_M^0(x) = 1, p_j^0(x) = 0, 1 \leq j < M$ for outliers and,

$p_i^0(x) = \frac{1}{M}$, for every other gene.

2.1.1.3 Cluster Construction

In this step, membership vectors are used to construct clusters. One object can be assigned to multiple clusters if it has a high membership score for more than one cluster. Also, some objects are not assigned to any clusters if they don't have a high membership score for any of the clusters. These objects are also labeled as outliers.

2.1.2 GQL-Cluster: Graphical Query Language

Schliep et al. proposed a mixture model for analyzing time series data with Hidden Markov Models [12]. They apply partially supervised learning of mixtures through a modification of Expectation Maximization algorithm. The method has four main parts. The first part is a class of Hidden Markov models. They applied a linear chain HMM topology with the addition of possibility of transition from the last to the first state for cyclic behavior. In the models, states do not have a specific semantic. The second part is for selection of an initial collection of models. The authors proposed three methods for choosing a starting point for mixture estimation. The first method is expert selection by using a graphical tool. The second method is to use randomized models. The third method is to learn initial models. The third part is for estimating a finite mixture. The last part is to infer groups from the mixture.

2.1.3 NNN: Nearest Neighbor Networks

Huttenhower et al. proposed Nearest Neighbor Networks (NNN) clustering algorithm which makes use of small cliques of mutual nearest neighbors in an interaction network to find clusters [8]. The input of NNN is a set of m genes, a similarity measure $s(x, y)$, and a neighborhood size k. For each gene g_i, $N(g_i)$ which represents the set of k nearest

neighbors of g_i according to the similarity matrix s is calculated. An undirected graph is constructed such that the vertices are the genes and there is an edge between gene g_i and gene g_j if g_j is in $N(g_i)$ and g_i is in $N(g_j)$, i.e. the two genes are mutual nearest neighbors. Then, all cliques of size g are identified, overlapping cliques are merged to produce preliminary networks representing potential clusters. Subsequently, clusters which has cut vertices are divided into two clusters and the cut vertices are included in both of the clusters. Here, cut vertices represents genes connecting clusters which shares no other interactions.

2.1.4 STEM: Short Time Series Expression Miner

Ernst et al. proposed a method designed specifically for short time series microarray data [3]. The method is implemented in Short Time Series Expression Miner (STEM) tool. The method has three main steps. In the first step, the model profiles are selected. In the next step, genes are assigned to a model profiles and the significant profiles are located. And in the final step, the significant profiles are grouped to find final clusters. In the following subsections, details of these steps are given.

2.1.4.1 Selecting Model Profiles

In this step, first, model profiles are generated by using a user-defined parameter c which controls the amount of change a gene can exhibit between successive time points. For n time points, a profile is a vector of size $n-1$, and each entry in the vector has a value between $-c$ and c. As an example, if c=2, a gene can go up either one or two units (1 and 2), stay the same (0), or go down one or two units (-1 and -2). For n time points, $(2c+1)^{(n-1)}$ profiles are generated. If P represents the set of profiles, the set R of model profiles is constructed such that the minimum distance between any two profiles in R is maximized. The algorithm starts with $R = p_1$ where $p_1 = -1, -1, ..., -1$. Then, in each iteration the profile r which satisfies:

$$r = \max_{p \in P \setminus R} \min_{q \in R} d(p, q)$$

is added to R. This process is repeated m times where m is the user-defined number of model profiles.

2.1.4.2 Identifying Significant Model Profiles

In this step, each gene g is assigned to a model profile with the smallest distance. After this assignment, the significant model profiles which deviates significantly from the null hypothesis are identified by using a permutation based test. Each gene has $n!$ permutations. Each possible permutation is assigned to its closest model profile. Let s_i^j be the number of genes assigned to model profile i in permutation j. The expected number of genes for model profile i is calculated by

$$E_i = \frac{\sum_j s_i^j}{n!}$$

If the number of genes assigned to model profile i is greater than the expected value, it is selected as a significant model profile.

2.1.4.3 Grouping Significant Profiles

The last step is to determine and group similar significant profiles. For this reason, a graph $G = (V, E)$ is constructed in which the nodes V are the significant model profiles and there is an edge between two nodes if the distance between two profiles are smaller than a threshold. Cliques in this graph represents the profiles that should be grouped. In order to locate cliques, a greedy algorithm which grows a cluster C_i around each significant model profile p_i is applied. The algorithm starts with $C_i = p_i$. Then, at each step, a profile p_j which is connected to every node in C_i is selected and added to C_i. After obtaining clusters for each significant profile, the largest cluster is selected and removed from the graph. The process is repeated until every profile is assigned to a cluster.

2.1.5 TAC: Temporal Abstraction Clustering

Sacchi et al. proposed Temporal Abstraction method for clustering short time series data [13]. Temporal Abstraction Clustering is a generalization of the template-based clustering. The implementation of the algorithm can be found in TimeClust application [14]. The algorithm has two steps. In the first step, qualitative representation of the time series is inferred from the data. In the next step, the qualitative represen-

tation is used to cluster data. In the following subsections, details of these steps are given.

2.1.5.1 Temporal Abstraction Detection and Time Series Representation

Time series are represented with a qualitative label consisting of trend temporal abstractions which are inferred from the expression profiles by applying piecewise linear approximations. There are three types of trends: increasing, decreasing and steady. For each expression profile, a set of dominant points which are time points at which is the start of the significant change of the trend starts are determined. Given two time points t_i and t_j, the arc length S_{ij} is defined as the sum of the lengths of all the segments joining pairs of consecutive points between t_i and t_j. The chord length C_{ij} is the length of the segment joining t_i and t_j. Then, the point t_{j-1} is a dominant point if $\frac{\sqrt{S_{ij}^2 - C_{ij}^2}}{2} > T$, where T is a predefined threshold, in other words if the slope change that occur between t_i and t_j is higher than a threshold. After finding dominant points, each interval between two dominant points is labeled with a trend according to the slope between them. Then, a three level representation of the qualitative pattern is created. The first level, named L_1 is the immediate output of the temporal abstraction detection phase. The next level, L_2 is created by combining consecutive interval labels of the same type into same label. The last level L_3 is obtained by removing all elements of type steady from the temporal abstractions.

2.1.5.2 Temporal Abstraction Clustering

The clustering step starts with the first gene and build initial sets of clusters for each level of representation. Then, for each gene, the temporal abstraction pattern of the gene and each cluster is compared. If the temporal abstraction pattern of the gene is matched with a cluster, the gene is assigned to the cluster. If there is no match, a new cluster is created and the gene is assigned to the newly created cluster.

2.2 Combination of Multiple Clustering Algorithms

There are a dozen of different clustering algorithms, each of which is suitable for a different model or cluster shape. Since in most of the cases, the shape of the cluster is not known in advance, combination of clusterings is widely applied to obtain a stable and robust clustering solution [16, 2, 17, 18]. This approach is also called clustering ensembles or consensus clustering. The approach is summarized in Figure 2.1. The partitions, i.e. different clustering solutions, can be obtained in several ways such as applying the same algorithm with different parameters, using different distance metrics or applying different clustering algorithms to the data. Topchy et al. showed that with the increasing number of partitions, the clustering ensembles method approaches to a true clustering solution [19, 17].

Fred and Jain [20, 21] introduced the idea of evidence accumulation which creates a new similarity matrix from the initial partitions by a voting mechanism and then perform hierarchical clustering based on the new similarity matrix. The evidence accumulation clustering method makes no assumptions on the number of clusters in each partition. Assuming that patterns belonging to natural clusters are more likely to co-exist in the same cluster than in different partitions, they propose a new measure based on voting mechanism to combine partitions. They create a new nxn similarity matrix C called *co-association matrix* from N partitions of n patterns as:

$$C(i, j) = \frac{n_{ij}}{N},$$

where n_{ij} is the number of co-occurrences of pattern pair (i, j) in N partitions.

Clustering ensembles method is also applied to different bioinformatics problems. Hu and Yoo applied the ensembles method to gene expression data analysis [22]. They first create a distance matrix based on clustering solutions, then apply a graph based clustering algorithm to obtain a consensus clustering. Yu et al. applied a graph based consensus clustering algorithm for class discovery from microarray data [23]. Chakrabarti and Panchenko applied clustering ensembles method to the problem of finding determining sites for functional specification or diversification in protein families by combining three best performing methods [24]. Glaab et al. created a web based

tool which provides ensemble and consensus methods for microarray analysis [25].

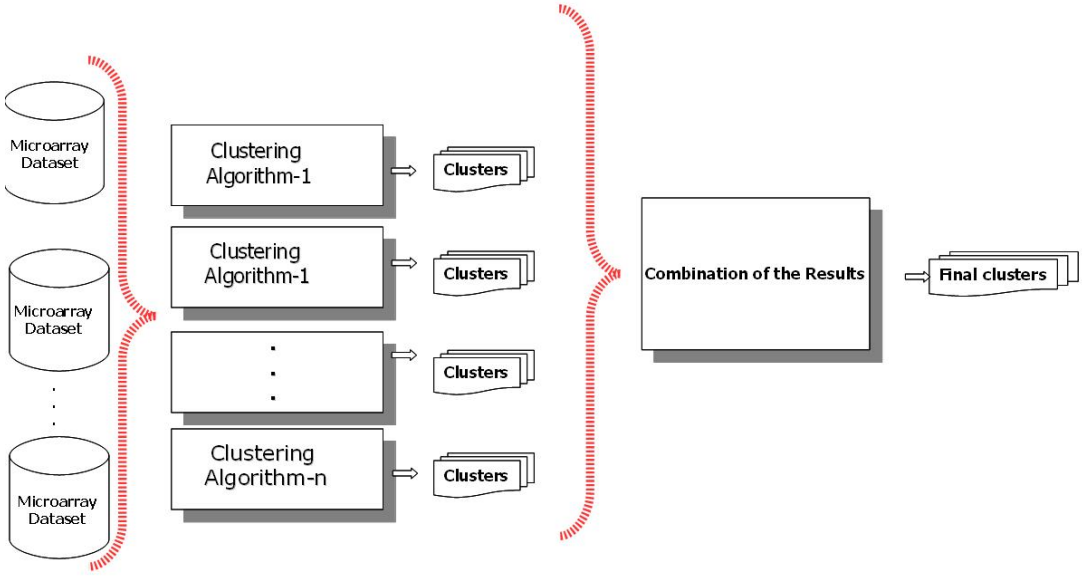


Figure 2.1: Outline of the clustering ensembles method.

2.3 Interpretation of Clustering Results

Interpretation of clustering results is an important step in microarray data analysis. While interpreting clustering results, methods annotate each cluster with GO terms [26], protein structural information [27], MeSH categories [28], protein-protein interactions, pathways [29], functional categories [30] and enrichment according to predefined gene lists [31] along with some statistical significance measures. The method of annotating each cluster is applicable when the assignments are to be analyzed and interpreted by experts. However, in some cases interpretation by experts can be very difficult and time consuming, especially when there are dozens of clusters which contains thousands of genes and each of the clusters have several annotations. When we need to decide which clusters are more important and contains biologically relevant genes, we need some quantitative measures of the quality of gene lists. Such measures can also be used in other applications such as evaluation and comparison of different clustering algorithms.

Most of the popular measures are based on Gene Ontology (GO) [32]. Wang et al. defines a GO-based measure which gives weights to different relationships among GO terms and calculate semantic values of GO terms by taking ancestor terms into con-

sideration [33]. The semantic values of terms are then used to calculate the similarity between GO terms. Datta and Datta propose two performance measures called Biological Homogeneity Index and Biological Stability Index for cluster evaluation in terms of the algorithm's capability to produce biologically meaningful clusters using a reference set of functional classes [34]. Resnik's similarity is defined to calculate the semantic similarity based on the information content. It is applied to GO terms first by Lord et al. [35]. Resnik's similarity is widely used in GO-based evaluation [36, 37, 38, 39, 40]. GOSemSim package [41] which is available through Bioconductor [42], contains implementations of various GO-based measures including Resnik's similarity.

Interaction network is another important source of information. There is a growing number of known interactions with the contribution of recent work [43, 44, 45]. Pattin and Moore provide a good review on the importance of interaction networks in genetic research and state that knowledge on interaction networks complements the knowledge on genome and the use of these two sources of information together can provide an in-depth understanding of biological phenomena such as diseases [46]. As stated by Marco and Marin, Gene Ontology and interaction networks are mostly correlated; however, there are cases in which these two show significant differences [47].

2.4 Comparison of Partition Similarity

At different stages of our work, we needed comparison of partitions. For this purpose, we applied three different metrics which are defined in [48] as:

When comparing two partitions M and N:

- Rand statistic represents the average number of agreements between clusters M and N and defined as

$$Rand = \frac{(a+d)}{(a+b+c+d)}.$$

- Jaccard coefficient represents the average number of elements contained in the intersection of clusters and defined as

$$Jaccard = \frac{(a)}{(a+b+c)}$$

- Folkes and Mallows index represents agreements between clusters M and N. The index is successful in discriminating unrelated clusters from related ones and defined as

$$FolkesAndMallows = \sqrt{\frac{a}{(a+b)} * \frac{a}{(a+c)}},$$

where

- a is the number of pairs of data points which are in the same cluster of M and in the same cluster of N,
- b is the number of pairs of data points which are in the same cluster of M but in different clusters of N,
- c is the number of pairs of data points which are in different clusters of M but in the same cluster of N, and,
- d is the number of pairs of data points which are in different clusters of M an in different clusters of N.

For each of the three measures, higher values represent more similar clusterings.

2.5 Integration of Biological Information Resources

Recently, there are methods that integrate biological information resources such as Gene Ontology and interaction networks into microarray data analysis. Yeh et al. integrates microarray data, disease genes and interaction networks to locate drug targets [49]. Weights are assigned to interactions in network by using the microarray data, model the problem of finding drug targets as a maximum flow problem and use disease genes to solve the maximum flow problem to locate drug targets. Zhao et al. [50] combine interactions and microarray data to locate drug targets. They assign weights to genes by using the distance of a gene to known disease genes in the interaction network and gene expression values. Both of the methods are specific to finding drug targets and do not make use of information about genes contained in Gene Ontology.

Lee et al. [51] incorporate microarray and interaction data to construct a subnetwork of abnormally expressed genes in postmortem brain samples of schizophrenia, bipolar disorder, and major depression patients. After constructing the subnetwork, they analyzed abnormally expressed genes by using topological features of the subnetwork and with several enrichment tools. The study does not provide an automated methodology to incorporate microarrays and interaction networks, however, it clearly shows the significance of incorporating microarrays and interaction networks.

Smoot et al. [52] incorporate Gene Ontology and interaction networks to visualize the subnetworks that are enriched by the input GO terms. Although it provides users to visually analyze the subnetwork to locate candidate genes, they do not make use of microarray data in their study.

From these examples, we can clearly say that the integration of biological information sources enables us to reveal previously unknown biological infomation. We need methods such as the Cluster-Eliminate-Combine that we propose in our study to automate this integration and analysis.

CHAPTER 3

INTERACTION BASED HOMOGENEITY

The number of known interactions is growing significantly thanks to the current research. In order to incorporate the interaction information source in the analysis of microarrays, we should apply measures based on interaction networks. In this chapter, we describe Interaction Based Homogeneity (IBH) which is a measure to evaluate the relation of the gene lists with respect to a known interaction network.

3.1 Homogeneity

Homogeneity is widely used to evaluate similarity of gene lists, especially in the evaluation of clustering results [53, 54, 55, 1, 34]. Assume that we have a gene list $L = \{g_1, g_2, ..., g_n\}$ of size n. By using the similarity measure $S(g_i, g_j)$ of two genes g_i and g_j, the homogeneity of list L is defined as follows:

$$Homogeneity(L) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} S(g_i, g_j)}{n^2}.$$

.

Here, homogeneity is defined as the average of the similarities between each element in the list. Homogeneity ranges from 0 to 1; 0 meaning that genes in list are not similar and 1 meaning that all genes in list are similar.

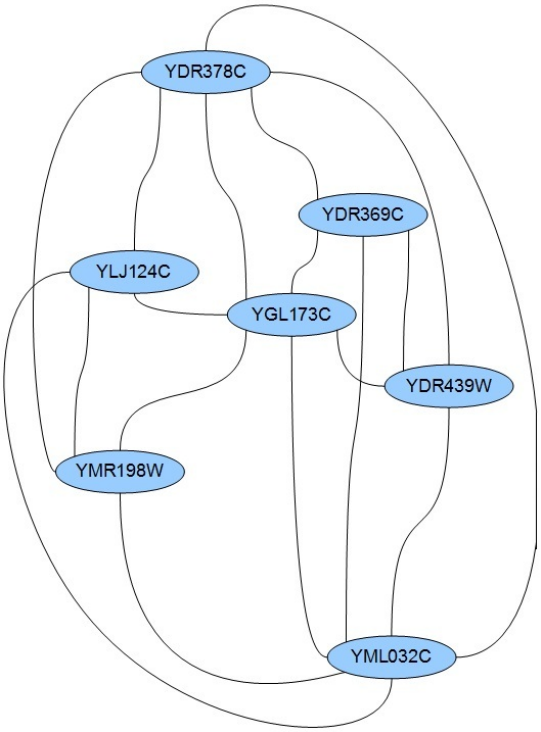


Figure 3.1: The graph representation of a very small sub-network of yeast.

3.2 Interaction Network

An interaction network is a representation of the cell as a biological model. There are two types of interactions in an interaction network. The first one is the protein-protein interactions, which represent the dynamics of cell function. The second type of interactions is genetic interactions that represent the relationship between regulatory modules of the cell. The interaction network can be modeled as a graph in which proteins or genes are represented as nodes and the relationship between them is represented as edges. As an example, the graph representation of a very small sub-network of yeast is given in Figure 3.1.

There are several repositories containing interactions such as IntAct [56], STRING [57], MIPS [58], MINT [59] and BioGRID [45]. Among them, Biological General Repository for Interaction Datasets (BioGRID) is a unified database of interactions which contains both protein-protein and genetic interactions for various organisms.

Table 3.1: Adjacency matrix of the graph in Figure 3.1.

	YDR378C	YDR369C	YDR439W	YLJ124C	YGL173C	YML032C	YMR198W
YDR378C	0	1	1	1	1	1	1
YDR369C	1	0	1	0	1	1	0
YDR439W	1	1	0	0	1	1	0
YLJ124C	1	0	0	0	1	1	1
YGL173C	1	1	1	1	0	1	1
YML032C	1	1	1	1	1	0	1
YMR198W	1	0	0	1	1	1	0

3.3 Interaction Based Homogeneity

Given a gene list L of n genes and a network E, we first form an adjacency matrix A whose rows and columns are genes in L where $A_{ij} = 1$ if genes i and j have an interaction in the E and $A_{ij} = 0$ otherwise. Interaction Based Homogeneity for a gene list $L = \{g_1, g_2, ..., g_n\}$ with respect to a network E is then calculated as follows:

$$IBH_E(L) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} A_{ij}}{n^2}.$$

.

As an example, the adjacency matrix for the sub-network in Figure 3.1 is given in Table 3.1. IBH for the gene list

$$L_{example} = \{YDR369C, YDR378C, YDR439W, YJL124C, YGL173C, YML032C, YMR198W\}$$

is

$$IBH(L_{example}) = 0.694.$$

3.3.1 Relationship with Other Network-Based Measures

There are a numerous number of measures based on interaction network. The degree of a gene g on an interaction network E of n nodes that has the adjacency matrix A is defined as:

$$D_E(g) = \sum_{i=1}^{n} A_{gi}.$$

The interaction based homogeneity is equal to the normalized average degree of genes in a gene list L on the interaction network E:

$$IBH_E(L) = \frac{\sum_{i=1}^{n} D_E(L(i))}{n^2}.$$

There is also a popular measure called clustering coefficient that is defined as the normalized number of edges (interactions) between the neighbors of a node. In other words, the clustering coefficient of a gene g given an interaction network represents the number of interactions between genes interacting with g. The clustering coefficient of a gene g given an interaction network E of n nodes that has the adjacency matrix A is defined as:

$$\frac{C_E(g) = \sum_{i=1}^{n} \sum_{j=1}^{n} A_{gi} A_{gj} A_{ij}}{k_g * (k_g - 1),}$$

where k_g is the number of genes interacting with gene g and calculated as:

$$k_g = \sum_{i=1}^{n} A_{gi}.$$

Both of the IBH and clustering coefficient measure the connectedness of the interaction network. When the interaction network is fully connected, the interaction based homogeneity and clustering coefficient are equal.

3.4 Comparison

To evaluate the performance of Interaction Based Homogeneity, we compared it with four different enrichment methods. First two methods are GO-based homogeneities calculated by two different popular similarity measures: Resnik's similarity and Wang's similarity. The third one is DAVID (Database for Annotation, Visualization and Integrated Discovery) Gene Functional Classification method which uses an integrated biological knowledge base to extract biological meaning from gene lists [60, 61]. The last method is a KEGG-based domainSignatures enrichment method proposed by Hahne et al. [62].

For GO-based enrichment, we downloaded a recent snapshot of GO database [32]. We eliminated 'IEA' annotations which means that the annotation is inferred from electronic annotation. The number of non-IEA annotations for each of the selected

Table 3.2: Number of non-IEA annotations for the selected organisms.

Organism	Number of Annotations
Yeast	47.882
Fruit Fly	59.515
Human	90.011

Table 3.3: Number of interactions in interaction databases for the selected organism.

Organism	Interaction Database	Number of Interactions
Yeast	BioGRID	186.589
Yeast	IntAct	98.475
Fruit Fly	BioGRID	47.761
Fruit Fly	DroID	16.858
Fruit Fly	IntAct	28.992
Human	BioGRID	40.584
Human	IntAct	68.415

organism is given in Table 3.2. We used GOSemSim [41] package in R [63] for GO-based Resnik's and Wang's similarity based homogeneity between two genes in the lists.

To calculate Interaction Based Homogeneity, we combined several interaction databases: Biological General Repository for Interaction Datasets (BioGRID) database [45], droID [64, 65], i2d [66, 67] and IntAct [56]. Extracting new interactions is still an ongoing study and the amount of known interactions varies from one organism to another one. The amount of known interactions may affect the performance of Interaction Based Homogeneity. To see the effects of the knowledge level of interactions on Interaction Based Homogeneity, we selected three different organisms: fruit fly, human and yeast. The number of interactions in each interaction database for the selected organisms are given in Table 3.3.

In order to show the accuracy of Interaction Based Homogeneity, we tailored the evaluation strategy of Ruths et al. [68] that makes use of predefined gene sets and adds randomly selected genes to measure the performance of their similarity measure. Similarly, we compared the performance of the measures by adding randomly selected genes that are not in the original list. We expected a linear decrease in the measure with the increase of the percentage of the randomness.

In the last section of the comparison, we ranked a microarray data with Interaction Based Homogeneity and apply Gene Set Enrichment Analysis to the ranked list.

3.4.1 Gene Ontology (GO)

Gene Ontology is a database of hierarchical annotations of genes, gene products and sequences and it is organized as three non-overlapping ontologies. Molecular Function (MF) ontology describes activities at the molecular level while Biological Process (BP) ontology describes biological goals. The last ontology is the Cellular Component (CC) ontology describing locations of genes and gene products. There are evidence codes for each of the annotation that describes the method by which the annotation is extracted.

3.4.2 Homogeneity Based on GO Based Resnik's Similarity

Resnik defined semantic similarity between terms by using the concept of information content [69]; less frequent terms are accepted as more informative GO terms. For each GO term t, the frequency of the term $frequency(t)$ can be calculated as follows:

$$frequency(t) = annotations(t) + \sum_{c \in children(t)} frequency(c),$$

where $annotations(t)$ is the number of gene products annotated by t and $children(t)$ is the set of child terms of t.

The information content is the probability $p(t)$ of the term t which is calculated as follows:

$$p(t) = \frac{frequency(t)}{frequency(root)}.$$

Resnik's semantic similarity between two terms t_1 and t_2 is then defined as given below.

$$S_{Resnik}(t_1, t_2) = max_{t \in A(t_1,t_2)}(-\log p(t)),$$

where $A(t_1, t_2)$ is the set of common ancestors of t_1 and t_2.

The similarity between two genes g_1 and g_2 is defined as the maximum Resnik's similarity between terms annotated by g_1 and g_2:

$$S_{maxResnik}(g_1, g_2) = max_{S_{Resnik(t_1,t_2)}}, \quad t_1 \in T(g_1), t_2 \in T(g_2),$$

where $T(g_i)$ is the set of terms annotated by g_i.

Finally, GO Based Resnik's Homogeneity (GBRH) for a gene list $L = \{g_1, g_2, ..., g_n\}$ of size n is defined as follows:

$$GBRH(L) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} S_{maxResnik}(g_i, g_j)}{n^2}.$$

3.4.3 Homogeneity Based on GO Based Wang's Similarity

Wang et al. defined the semantic similarity between GO terms [33]. A GO term A can be represented as a directed acyclic graph $G_A = \{A, T_A, E_A\}$ where $T_A = A \bigcup P(A)$, $P(A)$ is the set of ancestors of A in G_A, and E_A are the edges connecting the terms in G_A. There are two types of edges: "is a" edge and "part of" edge. For any term $t \in T_A$, $S_A(t)$ is defined as follows:

$$S_A(A) = 1$$

$$\mathrm{S}_A(t) = max\{w_e * S_A(t') | t' \in C(t) if (t \neq A)\},$$

where $C(t)$ is the set of children of term t, w_e is the semantic contribution factor for edge e, $0 < w_e < 1$, which depends on the type of the edge. The semantic value of GO term A, $SV(A)$ is defined as follows:

$$SV(A) = \sum_{t \in T_A} S_A(t).$$

and the semantic similarity between GO terms A and B, $S_{GO}(A, B)$ is defined as follows:

$$S_{Wang}(A,B) = \frac{\sum_{t \in T_A \bigcup T_B} S_A(t) + S_B(t)}{SV(A) + SV(B)}.$$

The similarity between two genes g_1 and g_2 is defined as the maximum Wang's similarity between terms annotated by g_1 and g_2.

$$S_{maxWang}(g_1,g_2) = max_{S_{Wang}(t_1,t_2)}, t_1 \in T(g_1), t_2 \in T(g_2),$$

where $T(g_i)$ is the set of terms annotated by g_i.

Finally, GO Based Wang Homogeneity (GBWH) for a gene list $L = \{g_1, g_2, ..., g_n\}$ of size n is defined as follows:

$$GBWH(L) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} S_{maxWang}(g_i, g_j)}{n^2}.$$

3.4.4 David Gene Functional Classification

David Functional Classification Tool uses 14 functional annotation sources to create similarity matrix between genes. Genes are clustered using this matrix by a heuristic fuzzy partition algorithm to group genes into functionally related clusters. For the comparison, we take the highest *Enrichment Score* of the found clusters.

3.4.5 KEGG Based DomainSignatures Method

Hahne et al. [62] proposed a method to assign lists of genes to previously described functional gene collections or pathways by comparing InterPro domain signatures of the candidate gene lists with domain signatures of gene sets derived from KEGG pathways. For the comparison, we take the maximum similarity in the pathway similarity matrix.

3.4.6 Comparison on Lists of Highly Interacting Genes

We created a 25 gene lists for each of the three organisms so that the lists contain highly interacting genes.

We first calculated the degree of each gene in the interaction network. Then, the first 25 genes with the highest degrees were selected as prototype genes. The lists were created from the prototype genes by adding genes interacting with prototype gene to the list.

The results of the comparison of Interaction Based Homogeneity on lists of highly interacting genes with GO Based Resnik's Homogeneity, GO Based Wang's Homogeneity, David Gene Functional Classification and domainSignatures are given in Figure 3.2, Figure 3.3, Figure 3.4 and Figure 3.5 respectively.

In the comparisons with David Gene Functional Classification, over 25 gene lists, 23 of them have 0 enrichment score in yeast, only two of the lists have non zero enrichment score. For fruit fly 3 gene lists and for human 9 gene lists have non zero enrichment scores.

Similar results are found in the comparison of domainSignatures, all of the gene lists have zero similarity scores in the case of yeast and fruit fly, for this reason only results for human are given in the figure. In the human gene lists, 3 lists have zero enrichment scores.

We can observe from the results that Interaction Based Homogeneity describes the gene lists better than GO-based measures especially when the number of known interactions is high. With the increased level of randomness, there is only a slight decrease in GO-based measures which indicates that genes in the lists are not evaluated as similar and that totally random lists also has non-zero homogeneity values.Similar results can be observed in the David and domainSignature comparisons, and in these cases we can not find any enrichment for most of the highly interacting gene lists. There is an unexpected increase in David's enrichment score for yeast gene lists with 40% to 60% randomness. And for fruit fly, it gives zero enrichment for all of the gene lists that have more than 40% randomness. In domainSignature method, an unexpected

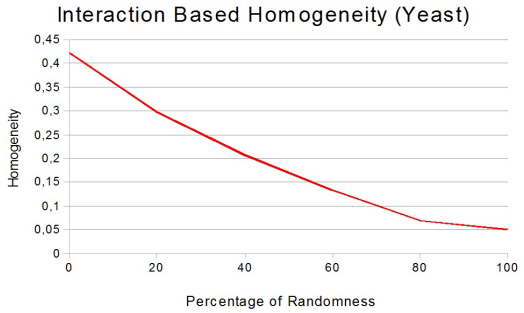
increase in similarity is observed for gene lists with 20% randomness. On the other hand, Interaction Based Homogeneity starts with higher values of homogeneity and homogeneity goes to nearly zero with the increased randomness.

3.4.7 Comparison on Lists That Are Similar According to Gene Ontology

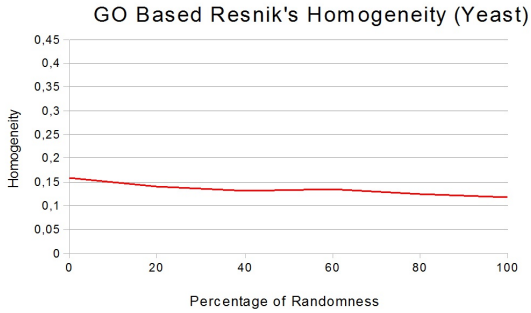
We created gene lists in which GO-based measures would perform well. We created 25 gene lists for each of the organism by following the strategy of Ruth et al. [68]. We first chose 25 prototype genes for each organism which have highest number of GO annotations. The lists were then created by adding genes that shared more than 7 GO terms with the lists of prototype genes. Finally, we have gene lists which had different number of total GO terms but all of the genes in the list shared at least 7 GO terms with the prototype gene. The results of the evaluation of Interaction Based Homogeneity on lists containing similar genes according to Gene Ontology with GO Based Resnik's Homogeneity, GO Based Wang's Homogeneity, David Gene Functional Classification and domainSignatures are given in Figure 3.6, Figure 3.7, Figure 3.8 and Figure 3.9 respectively.

In the comparison of David Gene Functional Classification enrichment scores with IBH on lists similar according to GO, we see that 4 lists have zero enrichment scores for yeast and 5 for human. In this case, for yeast there are no gene lists that have zero enrichment scores. The domainSignatures method yields zero similarity score for 8 gene lists.

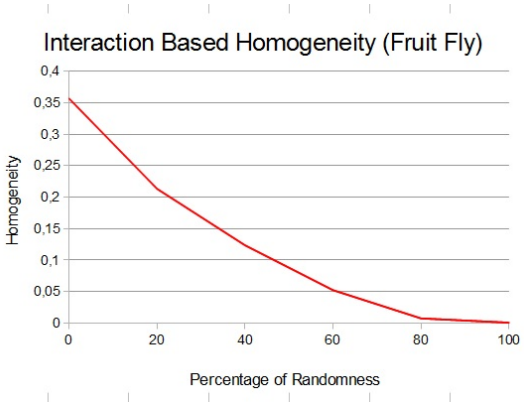
As expected, GO Based Homogeneity measures performs better in this case than in the case of highly interacting genes. Interaction Based Homogeneity performs well in this case, too. More interestingly, Interaction Based Homogeneity performs better than GO Based Resnik's and Wang's Homogeneities in gene lists of yeast, an organism for which lots of interactions are known. This surprising result indicates that the performance of Interaction Based Homogeneity will increase in parallel with the number of known interactions. David Gene Functional Classification gives better results for gene lists similar according to GO, however it cannot discriminate gene lists with 80% and 100% randomness in yeast and fruit fly. In domainSignature method, an unexpected increase in similarity is observed for gene lists with 20% randomness.



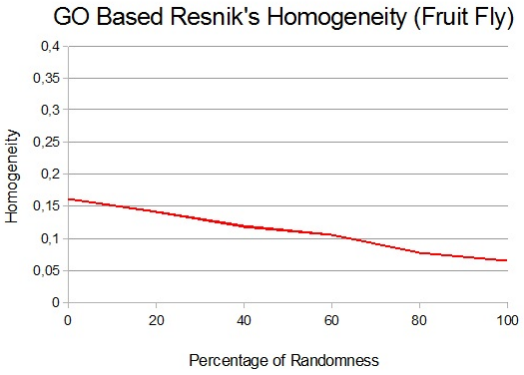
(a) Interaction Based Homogeneity on highly interacting yeast genes.



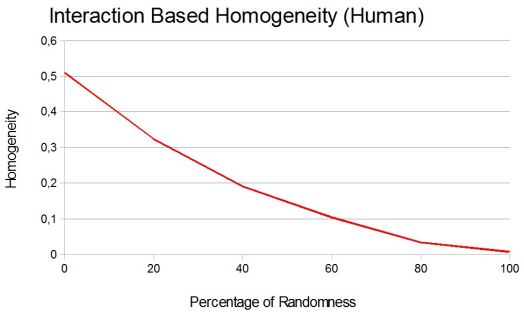
(b) GO Based Resnik's Homogeneity on highly interacting yeast genes.



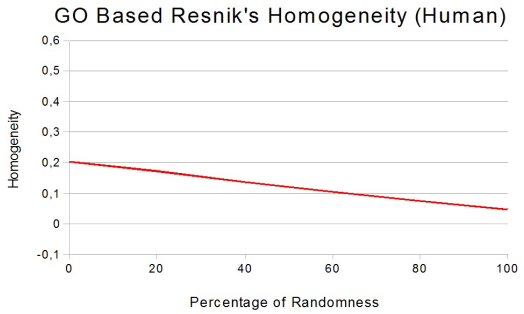
(c) Interaction Based Homogeneity on highly interacting fly genes.



(d) GO Based Resnik's Homogeneity on highly interacting fly genes.

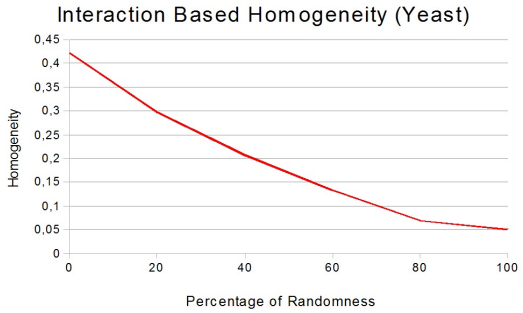


(e) Interaction Based Homogeneity on highly interacting human genes.

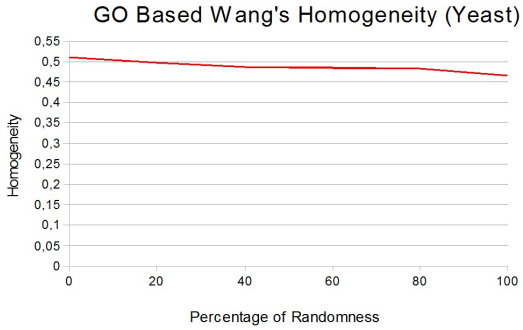


(f) GO Based Resnik's Homogeneity on highly interacting human genes.

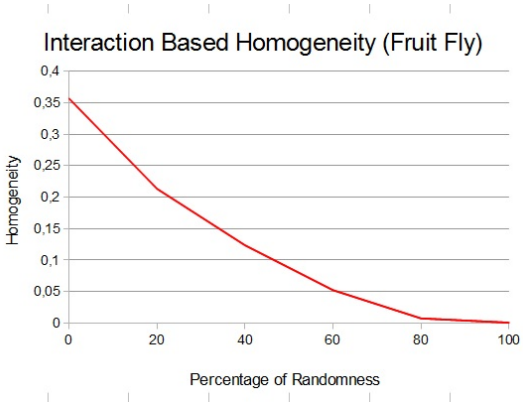
Figure 3.2: Comparison of GO based Resnik's Homogeneity and Interaction Based Homogeneity on gene lists of highly interacting genes for 3 different organisms.



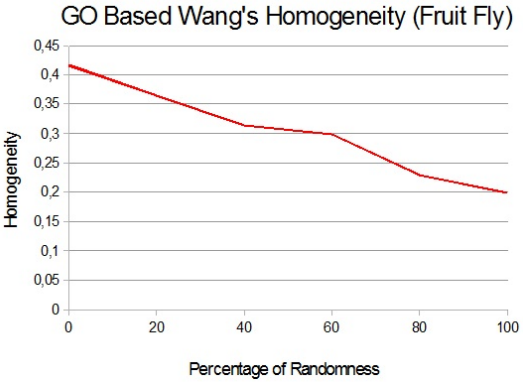
(a) Interaction Based Homogeneity on highly interacting yeast genes.



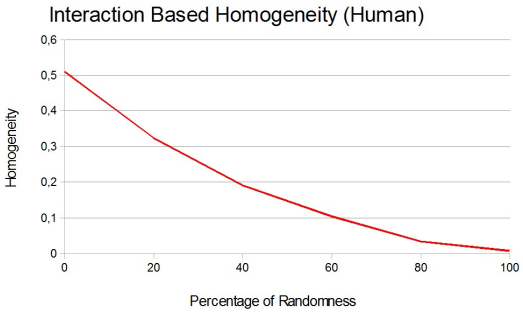
(b) GO Based Wang's Homogeneity on highly interacting yeast genes.



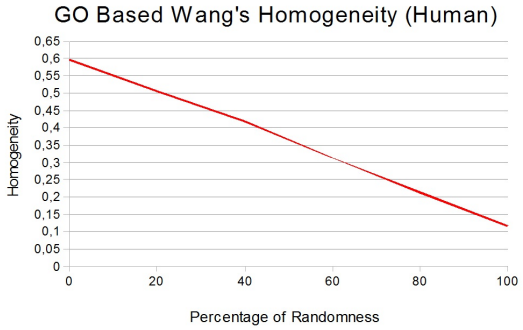
(c) Interaction Based Homogeneity on highly interacting fly genes.



(d) GO Based Wang's Homogeneity on highly interacting fly genes.

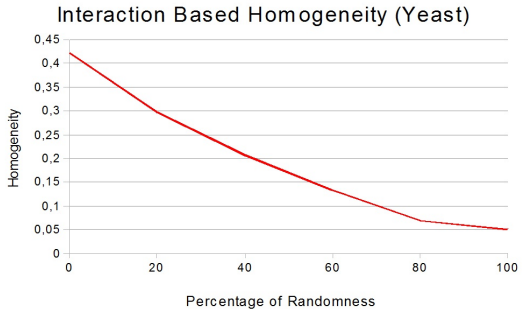


(e) Interaction Based Homogeneity on highly interacting human genes.

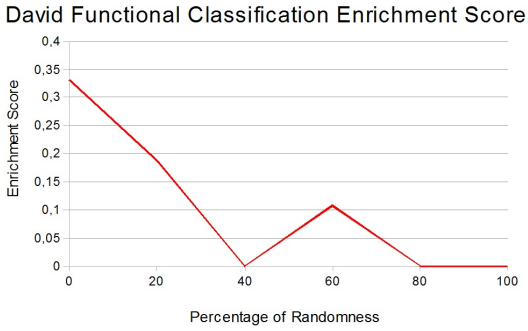


(f) GO Based Wang's Homogeneity on highly interacting human genes.

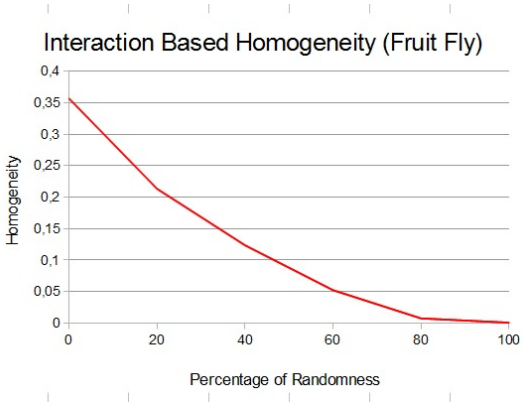
Figure 3.3: Comparison of GO based Wang's Homogeneity and Interaction Based Homogeneity on gene lists of highly interacting genes for 3 different organisms.



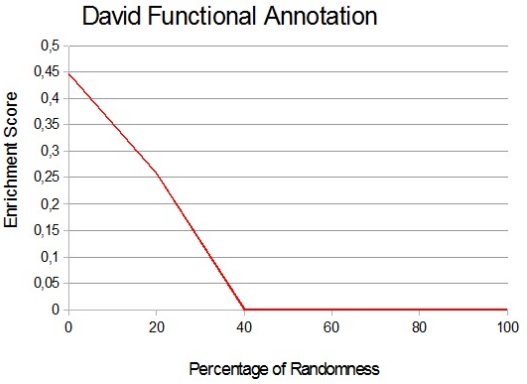
(a) Interaction Based Homogeneity on highly interacting yeast genes.



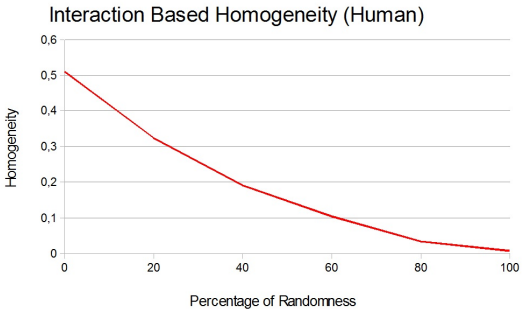
(b) DAVID Functional Classification Enrichment Score on highly interacting yeast genes.



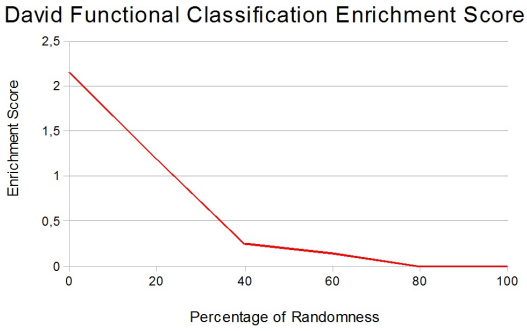
(c) Interaction Based Homogeneity on highly interacting fly genes.



(d) DAVID Functional Classification Enrichment Score highly interacting fly genes.

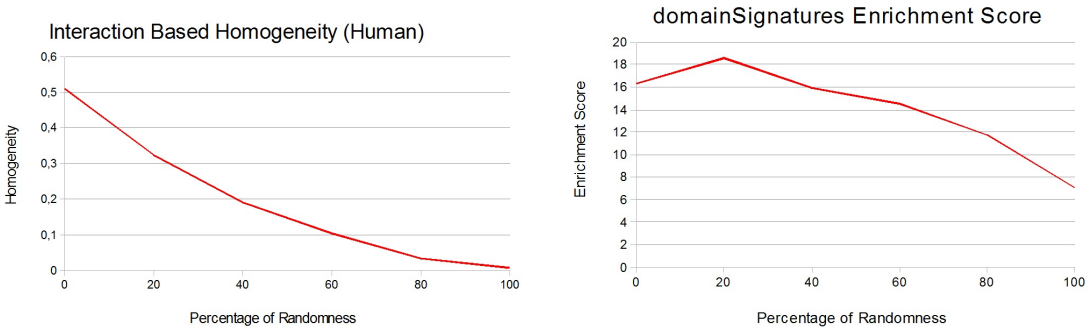


(e) Interaction Based Homogeneity on highly interacting human genes.



(f) DAVID Functional Classification Enrichment Score on highly interacting human genes.

Figure 3.4: Comparison of DAVID Functional Classification Enrichment Score and Interaction Based Homogeneity on gene lists of highly interacting genes for 3 different organisms.



(a) Interaction Based Homogeneity on highly interacting human genes.

(b) domainSignatures on highly interacting human genes.

Figure 3.5: Comparison of domainSignatures and Interaction Based Homogeneity on gene lists of highly interacting human genes.

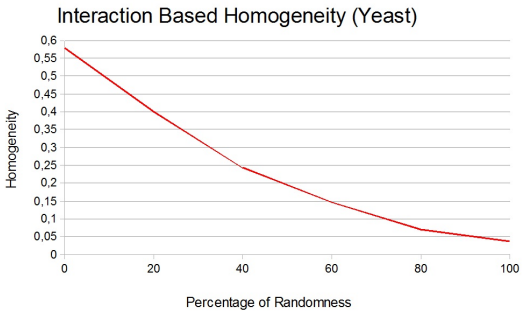
3.4.8 Gene Set Enrichment Analysis of p53 Dataset Ranked by Interaction Based Homogeneity

We take the a gene expression microarray data from the original GSEA paper measuring the p53 status in cancer cell lines (p53 dataset) and created a ranked list of genes based on the Interaction Based Homogeneity. For creating a ranked list of genes, we first created different partitions of the p53 dataset by applying different clustering algorithms to the dataset. When clustering, we applied algorithms implemented in GEDAS [70] to the microarray data with different parameters. Then, we calculated IBH for each of the resulting clusters. The weight w_g of a gene g is then calculated as the sum of the Interaction Based Homogeneities of the clusters that gene g belongs to:

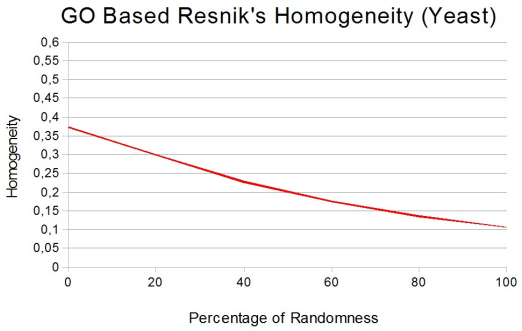
$$w_g = \sum_{i=1}^{k} IBH(C_i),$$

where $C_1 ... C_k$ are the clusters that gene g belongs to.

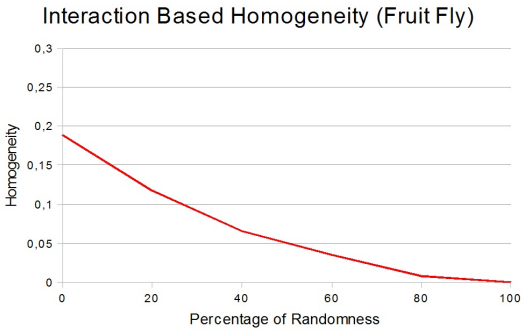
We applied Gene Set Enrichment Analysis to the ranked list based on p53 dataset to identify functional gene sets (C_2). Several gene sets related with p53 function such as Biocarta p53 pathway, KEGG p53 pathway are enriched. Some of the enrichment results are given in Figure 3.10.



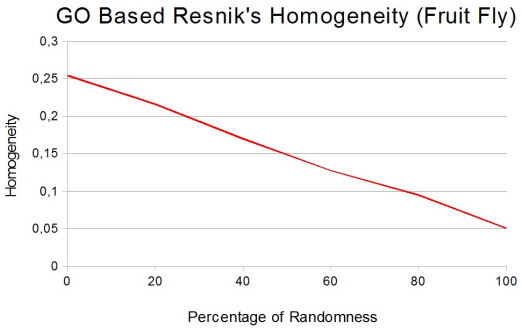
(a) Interaction Based Homogeneity on yeast genes similar according to Gene Ontology.



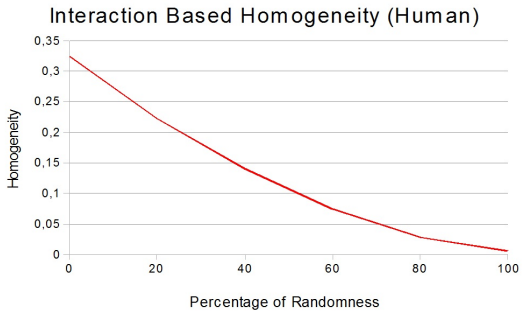
(b) GO Based Resnik's Homogeneity on yeast genes similar according to Gene Ontology.



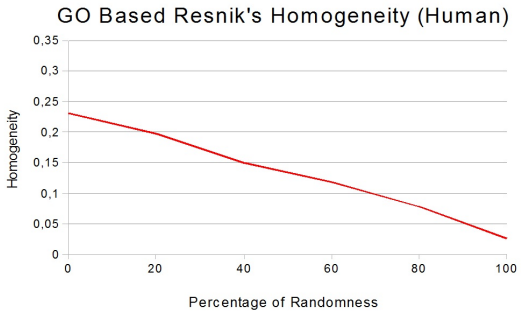
(c) Interaction Based Homogeneity on fly genes similar according to Gene Ontology.



(d) GO Based Resnik's Homogeneity on fly genes similar according to Gene Ontology.

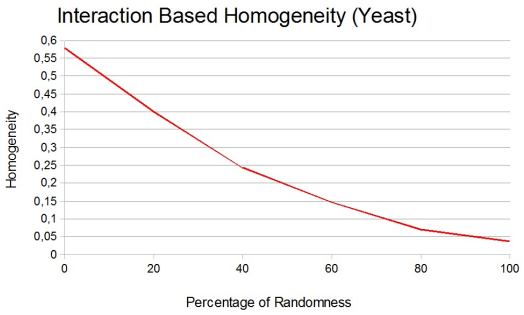


(e) Interaction Based Homogeneity on human genes similar according to Gene Ontology.

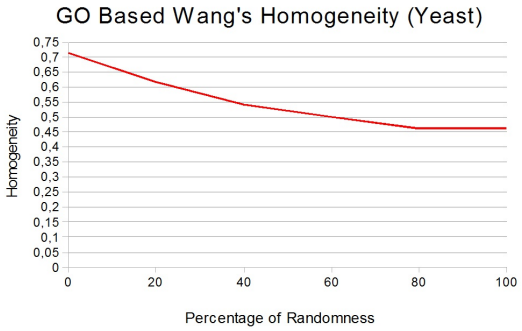


(f) GO Based Resnik's Homogeneity on human genes similar according to Gene Ontology.

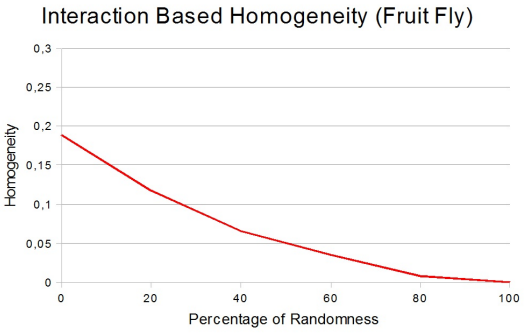
Figure 3.6: Comparison of GO based Resnik's Homogeneity and Interaction Based Homogeneity on gene lists similar according to Gene Ontology for 3 different organisms.



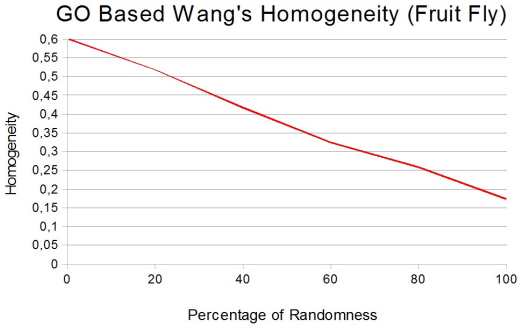
(a) Interaction Based Homogeneity on yeast genes similar according to Gene Ontology.



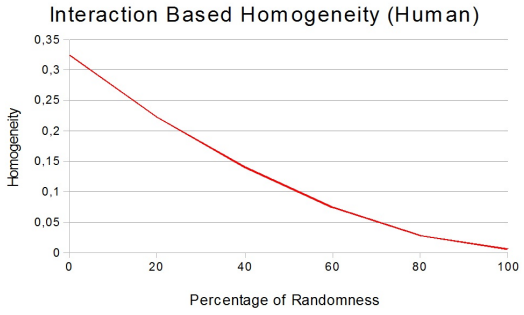
(b) GO Based Wang's Homogeneity on yeast genes similar according to Gene Ontology.



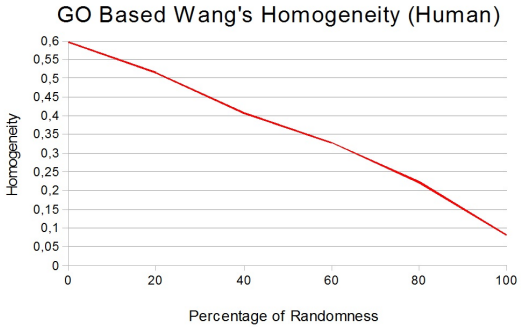
(c) Interaction Based Homogeneity on fly genes similar according to Gene Ontology.



(d) GO Based Wang's Homogeneity on fly genes similar according to Gene Ontology.

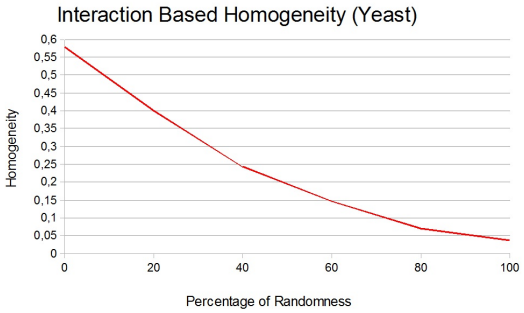


(e) Interaction Based Homogeneity on human genes similar according to Gene Ontology.

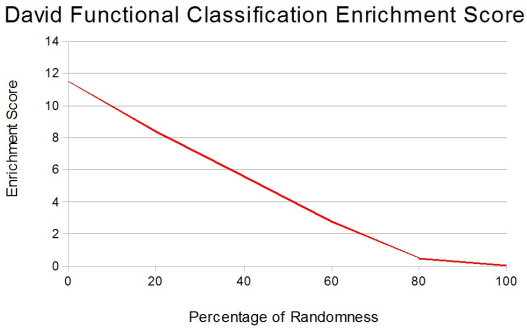


(f) GO Based Wang's Homogeneity on human genes similar according to Gene Ontology.

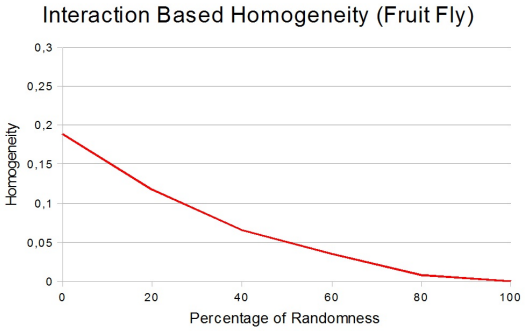
Figure 3.7: Comparison of GO based Wang's Homogeneity and Interaction Based Homogeneity on gene lists similar according to Gene Ontology for 3 different organisms.



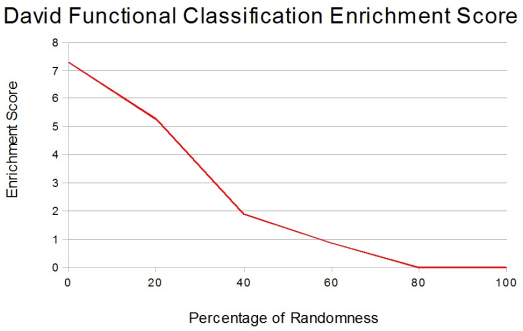
(a) Interaction Based Homogeneity on yeast genes similar according to Gene Ontology.



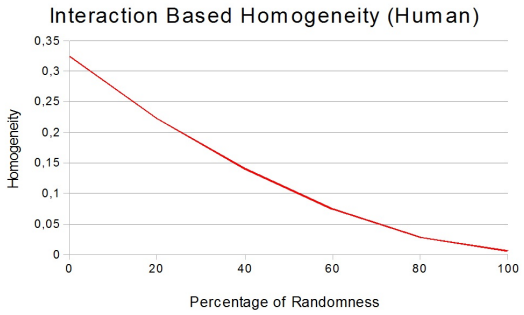
(b) DAVID Functional Classification Enrichment Score on yeast genes similar according to Gene Ontology.



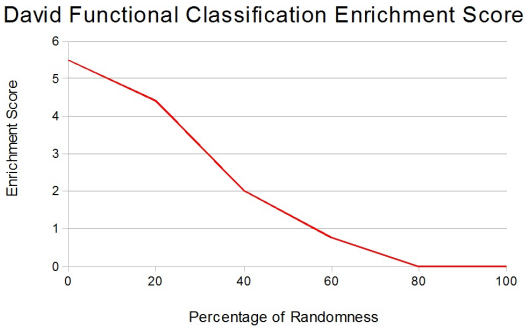
(c) Interaction Based Homogeneity on fly genes similar according to Gene Ontology.



(d) DAVID Functional Classification Enrichment Score on fly genes similar according to Gene Ontology.

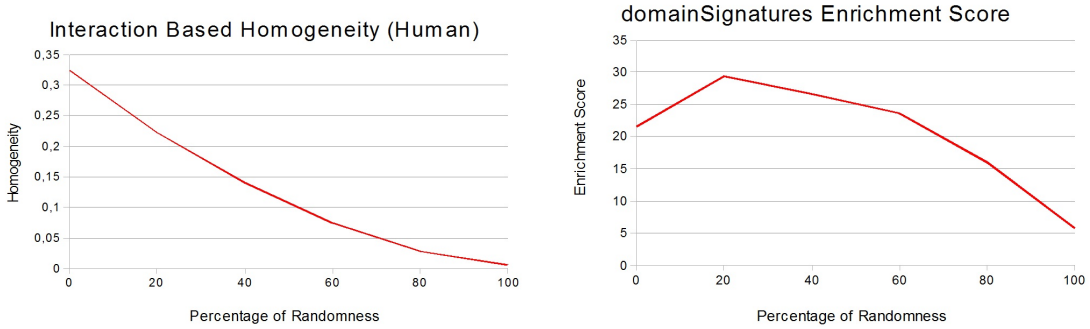


(e) Interaction Based Homogeneity on human genes similar according to Gene Ontology.



(f) DAVID Functional Classification Enrichment Score on human genes similar according to Gene Ontology.

Figure 3.8: Comparison of DAVID Functional Classification Enrichment Score and Interaction Based Homogeneity on gene lists similar according to Gene Ontology for 3 different organisms.



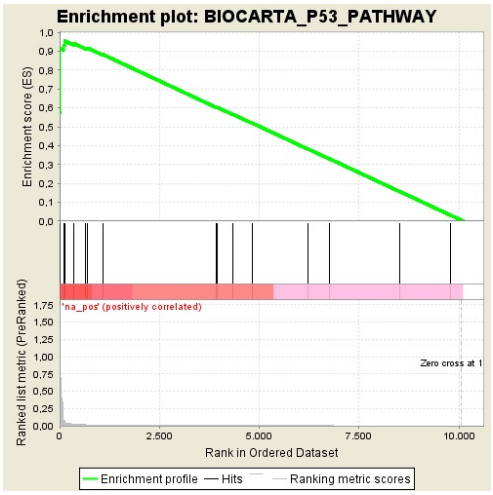
(a) Interaction Based Homogeneity on human genes similar according to Gene Ontology.

(b) domainSignatures on human genes similar according to Gene Ontology.

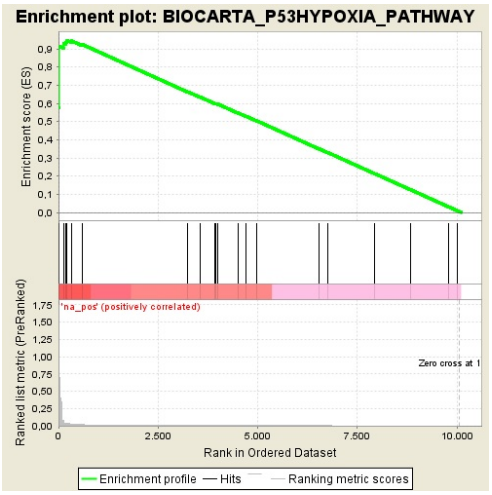
Figure 3.9: Comparison of domainSignatures and Interaction Based Homogeneity on gene lists similar according to Gene Ontology for human genes.

3.5 Results and Discussion on IBH

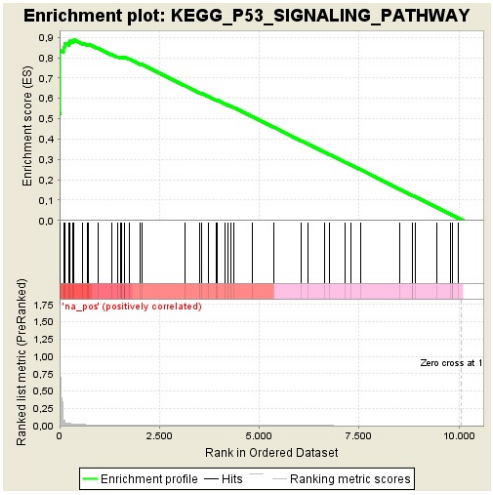
We compared IBH with four different enrichment methods in two different cases: two GO based measure which employs popular Resnik's distance and Wang's distance, David annotation method and a KEGG-based enrichment method using domain signatures. We proved the effectiveness of the Interaction Based Homogeneity measure in two different cases in which the measure's ability to distinguish related lists from random lists and to measure the randomness is presented. In the first case, we compared results for 75 gene lists of highly interacting genes. The results show that Interaction Based Homogeneity is especially more useful in detecting highly interacting gene lists than GO-based measure. In the second case, 75 gene lists which are similar according to GO is used. Interaction Based Homogeneity also performs well in the comparison of lists that are similar according to Gene Ontology. We also create an R package called ibh implementing Interaction Based Homogeneity in different cases. It is accepted to Bioconductor [42] and freely available. Details of the package can be found in Appendix A.



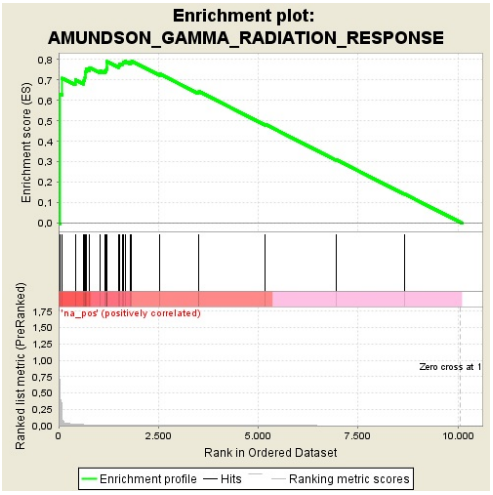
(a) Enrichment plot for biocarta_p53_pathway.



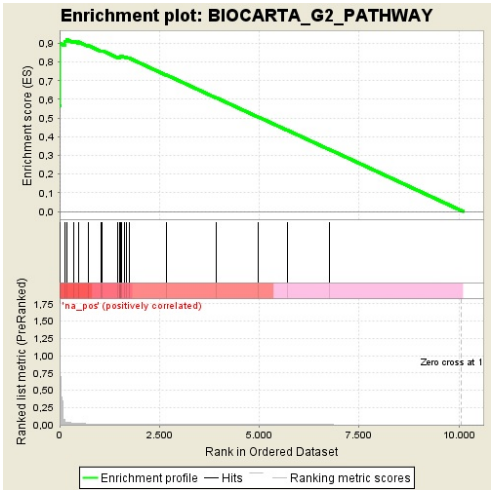
(b) Enrichment plot for biocarta_p53hypoxia_pathway.



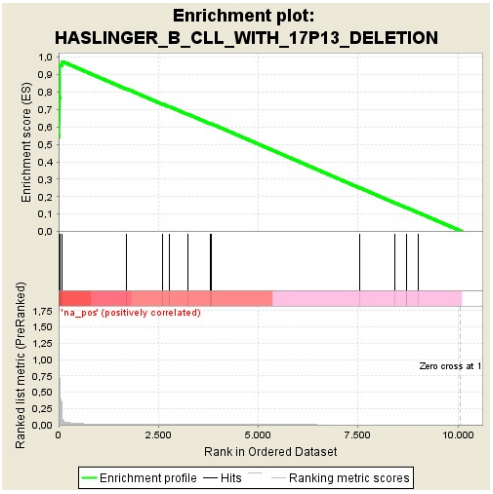
(c) Enrichment plot for kegg_p53_signalling_pathway.



(d) Enrichment plot for amundson_gamma_radiation_response.



(e) Enrichment plot for biocarta_g2_pathway.



(f) Enrichment plot for haslinger_b_cll_with_17p13_deletion.

Figure 3.10: Results of GSEA analysis of p53 dataset which is ranked by Interaction Based Homogeneity.

CHAPTER 4

CLUSTER-ELIMINATE-COMBINE METHOD

In this chapter, we describe Cluster-Eliminate-Combine (CEC) method that take as input an interaction network, a list of GO terms representing the functional class of the genes that are of the interest and a set of partitions of microarray data and give as output a clustering result containing only genes that are related with the biological process under inspection. CEC method is mainly based on clustering ensembles method described in Section 2.2. Several improvements are made to the clustering ensembles method as indicated below.

1. Instead of using every cluster in combination, a subset of clusters are selected according to the relevance with the biological process of the experiment. The relevance is measured by Interaction Based Homogeneity described in Chapter 3. Gene Ontology terms related with the experiment are provided to the method as input to describe the biological process under inspection.

2. The selected clusters are cleaned up by applying a gene weight measure which is calculated for each gene by using clusters and Interaction Based homogeneity.

3. The clustering ensembles method is finally applied to the selected and cleaned clusters.

The outline and the algorithm of the proposed method is given in Figure 4.1 and Algorithm 1 respectively. First, a subnetwork of the interaction network is constructed by using the GO terms provided as input. The constructed interaction subnetwork represents the relations that are more likely to be related according to the functional

class that are of the current interest. Second, Interaction Based Homogeneity of each cluster is calculated by using this subnetwork. Third, for each gene, a gene weight is calculated. The gene weight represents the importance of the gene according to the experiments and the interaction subnetwork. As the next step, the genes that have high weights are selected and the others are eliminated from the clusters. As the final step, the remaining clusters are combined to achieve the final relationship information.

Algorithm 1 Outline of the CEC algorithm.

Require: A set of partitions P of microarray data M resulting from N clustering algorithms, a threshold T for cluster elimination, interaction network I, a set of Gene Ontology terms O

$C \leftarrow createSetOfClusters(P)$ {Create the set of clusters}

$S \leftarrow createInteractionSubnetwork(I, O)$ {Create interaction subnetwork}

for $i = 1 \rightarrow length(C)$ **do**

calculate $IBH_S(C_i)$

end for{Calculate IBH for each cluster}

$G \leftarrow SetOfGenes(C)$

for all *gene* $g \in G$ **do**

$weight(g) \leftarrow \sum_i IBH_S(C_i), g \in C_i$

end for{Calculate gene weights}

for $i = 1 \rightarrow length(C)$ **do**

for all *gene* $g \in C_i$ **do**

if $weight(g) \leq T$ **then**

remove g from $C[i]$

end if

end for

end for{Clean clusters}

for $i = 1 \rightarrow length(G)$ **do**

for $j = 1 \rightarrow length(G)$ **do**

$n(i, j) \leftarrow 0$

for $c = 1 \rightarrow N$ **do**

if $G[i] \in P_c$ $G[j] \in P_c$ **then**

$n(i, j) \leftarrow n(i, j) + 1$

end if

end for{Create co-association matrix from C}

$C(i, j) \leftarrow \frac{n(i,j)}{N}$

end for

end for

return $Cluster(C)$

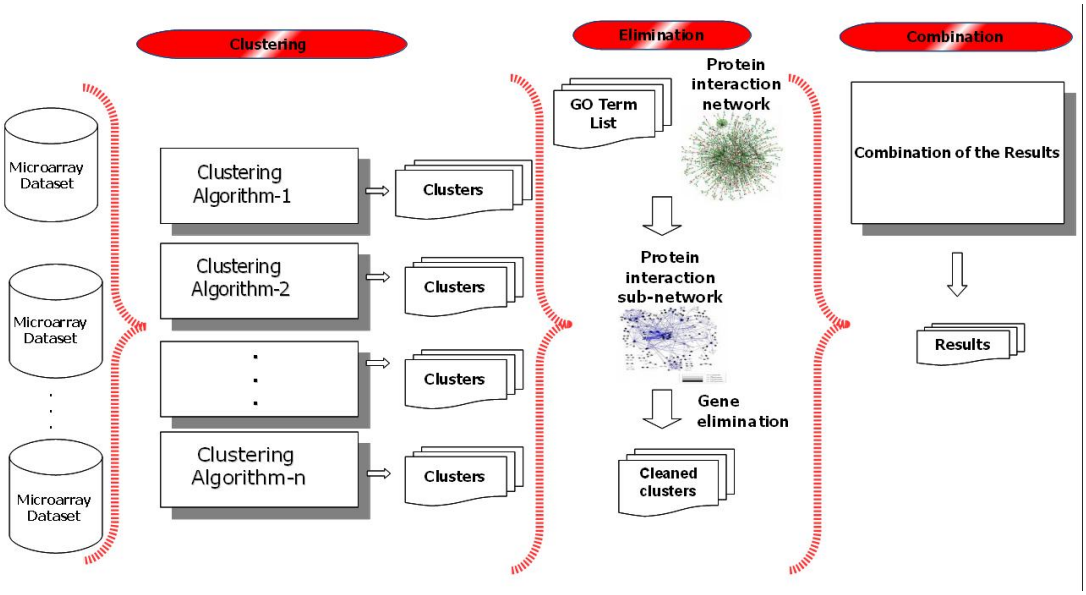


Figure 4.1: Outline of the CEC method.

4.1 Details of CEC Method

In the following sections, each step of the methodology are described in detail.

4.1.1 Interaction Subnetwork

An interaction network can be represented as a graph whose edges are genes. There is an edge in the graph between nodes X and Y only if there is an interaction between gene X and Y. Given an interaction network N and a set of GO terms G, an interaction subnetwork S is created by eliminated edges *(X, Y)* such that both X and Y are not annotated by GO terms contained in G.

4.1.2 Interaction Based Homogeneity

Interaction Based Homogeneity is described in detail in Chapter 3. Given a gene list of n genes, we first form an adjacency matrix A whose rows and columns are genes in the list where $A_{ij} = 1$ if genes i and j have an interaction in the network and $A_{ij} = 0$ otherwise. The Interaction Based Homogeneity for a gene list $L = \{g_1, g_2, ..., g_n\}$ of size n is then calculated as

$$IBH(L) = \frac{\sum_{i=1}^{n} \sum_{j=1}^{n} A_{ij}}{n^2}.$$

4.1.3 Calculating Gene Weights

The weight $W(g)$ of a gene g is defined as the sum of weights of clusters that g belongs to: $W(g) = \sum_{i=1}^{n} \sum_{g \in C_i} IBH(C_i)$

4.1.4 Cleaning the Clusters

Before clustering combination, the clusters are cleaned by removing unnecessary genes from the clusters. Genes that have weight smaller than a given threshold T are assumed to be unrelated. That is, for each cluster C_i, a new cluster C_i' is constructed

such that $C_i' = \{g | g \in C_i \text{ and } W(g) > T\}$.

4.1.5 Cluster Combination

The result of each method is used as a partition and the evidence accumulation method is applied, the co-association value is calculated as the new similarity matrix. Evidence accumulation method and co-association value are described in Section 2.2. Then, a model based algorithm is used to cluster the genes with the new similarity matrix to obtain the combined clusters. For the model based clustering, we used *mclust* package [71].

4.2 Dataset

Gasch et al. [72] use DNA microarrays to measure changes in transcript levels over time for almost every yeast gene and the dataset contains 5457 genes. The GDS36 dataset that is provided in their study is a yeast heat shock dataset in which measurements are taken at 5, 15, 30 and 90 minutes on a heat shock from 29°C to 33°C. There are 4 samples in the dataset. The log ratio values of the measurements are used. We used the whole dataset as is in all of the analyses without performing a preprocessing step for selecting differentially expressed genes.

4.3 Results of Cluster-Eliminate-Combine on GDS36 Dataset

For analyzing GDS36 dataset, in the clustering step, the dataset is clustered by five algorithms to obtain different partitions. The algorithms used are CAGED, GEDAS, NNN, STEM and TAC. The algorithms are described in detail in Section 2.1. Number of clusters found in each partition is given in Table D. As can be seen from the table, the number of clusters found varies from 1 to 50 and each partition contains different numbers of genes ranging from 5 to 5457.

Then, we run the elimination step of the algorithm. Genes annotated with GO term *Response to Heat (GO:0009408)* is selected as the gene list. There are 192 genes annotated by this GO term with experimental evidence codes. The subnetwork of

Table 4.1: Number of clusters found in each partition for GDS36 dataset.

Algorithm	Number of Clusters	Total Number of Genes in Clusters
CAGED	7	5457
FLAME	1	11
NNN	1	5
STEM	50	1461
TAC	3	5457

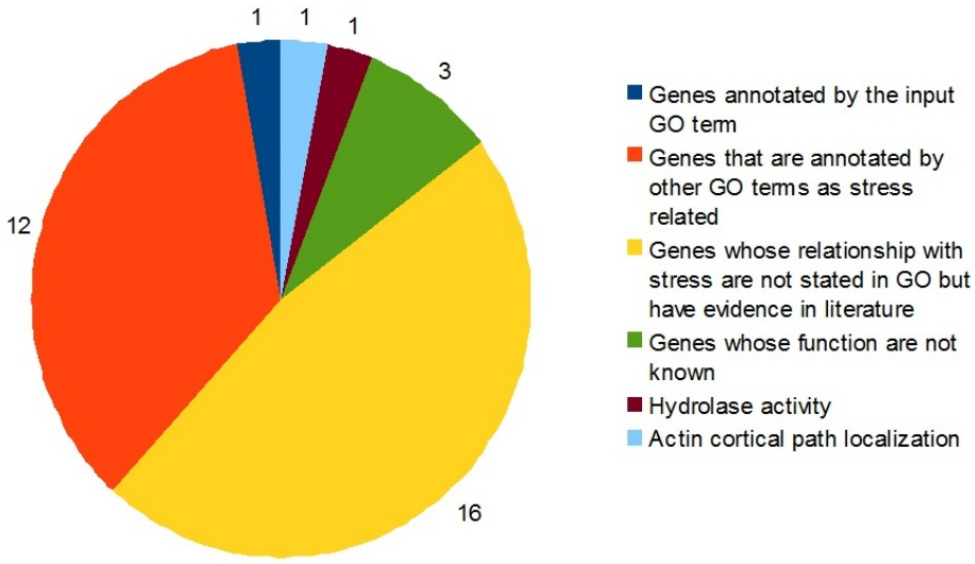


Figure 4.2: CEC on yeast heat shock GDS36 dataset.

BioGRID interactions related with the 192 genes in the selected GO term is prepared. First, the IBH for each cluster is calculated. Then, from IBH values of each cluster, gene weights are calculated. The unrelated genes are eliminated from the clusters in partitions by using threshold being equal to 0.01.

The distance matrix among genes that passed the elimination step is calculated by using the evidence accumulation. Then, the distance matrix is used by a model based clustering algorithm to obtain the final clustering. The results after combination step can be found in Figure 4.2 and detailed analysis of the results are given in Table D.2 in Appendix D.

We can see from the results that the analysis suggests 1 cluster that contains 36 genes. We analyzed the results on gene by gene basis. First, GO annotations for each gene is

searched to locate annotations related with heat shock. After GO search, a literature survey is performed for genes that do not have related annotations in GO.

The results can be summarized as follows:

- *Genes that are annotated by the input GO term:* Only 1 gene (*HSP104*) is annotated by the input GO term.
- *Genes that are annotated by other GO terms as stress related:* 12 genes (SSE2, SSA4, HSP42, TSL1, DDR2, TPS3, HOR7, GAD1, CTT1, GRE3, BDH1, SPI1) are annotated by other GO terms as stress related.
- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* 16 genes (PRB1 [73], PHR1 [74], TMA17 [75, 76], TPK1 [73], CIT1 [73], PGM2 [73], HXK1 [73], CWP1 [77], RTN2 [78], UGP1 [79], PNC1 [80], DCS2 [81], GLK1 [73], TFS1 [82], SOL4 [83], GTO3 [80]) are found to be related with stress in literature.
- *The unrelated genes and genes whose functions are not known yet:* These genes grouped for further analysis:
  - *Genes whose function are not known:* 3 genes have unknown functions (YNL195C, FMP16 and YCL042W).
  - *Other genes:* AMS1 gene is related with hydrolase activity and YSC84 gene works in actin cortical path localization.

Several interesting and promising results can be seen:

1. Many stress response genes that are not annotated by *Heat Shock GO term*, and therefore not given to the method as input, are extracted from the dataset. Examples of those genes are HSP42, TSL1, DDR2, TPS3 and HOR7.
2. Response to oxidative stress can be seen as well as response to stress genes. Lee et al. [84] states that oxidation and heat shock have common physiological effect on cells, so it is meaningful that oxidative response genes such as CIT1 and BDH1 are included in the final clustering by CEC method.

3. When cell response to heat, the amount of hydrolysis also increases since it generates the energy needed to refold the proteins and to the synthesis of biological polymers. A more detailed description about the process can be found in [85]. Genes related with hydrolase activity such as PNC1, DC2, SOL4 and AMS1 have been found in the final clustering.

We compared the input partitions and the clustering found by CEC method by 3 different similarity measures which are described in Section 2.4. The results for Rand statistics, Jaccard coefficient and Folkes and Malkows index are given in Table 4.3, Table 4.3 and Table 4.3, respectively. To make results more clear, we included tables containing average statistics among input partitions for Rand statistics, Jaccard coefficient and Folkes and Malkows index in Table 4.3, Table 4.3 and Table 4.3, respectively. We can see from the analysis that the CEC method finds a partition that is the most similar to all of the partitions.

Table 4.2: Rand Statistics Between Partitions on GDS36 Dataset.

	CAGED	GEDAS	NNN	STEM	TAC	**CEC**
CAGED	1,00	0,97	0,97	0,54	0,37	0,99
GEDAS	0,97	1,00	0,99	0,52	0,36	0,98
NNN	0,97	0,99	1,00	0,52	0,36	0,98
STEM	0,54	0,52	0,52	1,00	0,49	0,53
TAC	0,37	0,36	0,36	0,49	1,00	0,36
CEC	0,99	0,98	0,98	0,53	0,36	1,00

Table 4.3: Average Rand Statistics Between Input Partitions and Between Input Partitions and CEC on GDS36 Dataset.

Partition Name	Average
CAGED	0,71
GEDAS	0,71
NNN	0,71
STEM	0,50
TAC	0,40
CEC	0,77

4.4 Contributions of Each Component of the Method

In order to demonstrate the contributions of each of the component of the method, we will show and compare several different versions of the method as follows:

Table 4.4: Jaccard Coefficient Between Partitions on GDS36 Dataset.

	CAGED	GEDAS	NNN	STEM	TAC	**CEC**
CAGED	1,00	0,97	0,97	0,53	0,36	0,99
GEDAS	0,97	1,00	0,99	0,52	0,36	0,98
NNN	0,97	0,99	1,00	0,52	0,36	0,98
STEM	0,53	0,52	0,52	1,00	0,27	0,53
TAC	0,36	0,36	0,36	0,27	1,00	0,36
CEC	0,99	0,98	0,98	0,53	0,36	1,00

Table 4.5: Average Jaccard Coefficient Between Input Partitions and Between Input Partitions and CEC on GDS36 Dataset.

Partition Name	Average
CAGED	0,71
GEDAS	0,71
NNN	0,71
STEM	0,46
TAC	0,34
CEC	0,77

- A first improvement to the clustering ensembles method is the IBH based elimination of unrelated clusters before clustering combination. We will show the results of using the interaction network, without using Gene Ontology to create interaction subnetwork and without the gene cleaning step. The details and results are given in Section 4.4.1.

- The second improvement is the addition of Gene Ontology terms and the idea of taking the interaction subnetworks. We will show the results of using the interaction network and Gene Ontology to create an interaction subnetwork and using it to eliminate unrelated clusters, without cluster cleaning. The details and results are given in Section 4.4.2.

- In order to demonstrate the significance of using a real interaction network, we will apply CEC with a fully connected network as input. The details and results are given in Section 4.4.3.

Table 4.6: Folkes and Mallows Index Between Partitions on GDS36 Dataset.

	CAGED	GEDAS	NNN	STEM	TAC	CEC
CAGED	1,00	0,99	0,99	0,73	0,59	0,99
GEDAS	0,99	1,00	1,00	0,72	0,6	0,99
NNN	0,99	1,00	1,00	0,72	0,6	0,99
STEM	0,73	0,72	0,72	1,00	0,43	0,73
TAC	0,59	0,6	0,6	0,43	1,00	0,6
CEC	0,99	0,99	0,99	0,73	0,6	1,00

Table 4.7: Average Folkes and Mallows Index Between Input Partitions and Between Input Partitions and CEC on GDS36 Dataset.

Partition Name	Average
CAGED	0,82
GEDAS	0,83
NNN	0,83
STEM	0,65
TAC	0,56
CEC	0,86

4.4.1 Elimination of Unrelated Clusters Before Clustering Combination

The idea behind this component of the CEC method is to incorporate the knowledge contained in interaction networks to clustering combination. We calculate the fitness of a cluster to the interaction network by Interaction Based Homogeneity. In this step, we calculate IBH for each cluster and eliminate clusters that has small IBH values. The resulting clusters are then combined by using the evidence accumulation method. The outline and the algorithm of this step is given in Figure 4.3 and Algorithm 2, respectively.

Algorithm 2 Algorithm for elimination of unrelated clusters before clustering combination.

Require: A set of partitions P of microarray data M resulting from N clustering algorithms, a threshold T for cluster elimination, interaction network I

{Create the set of clusters}

$C \leftarrow createSetOfClusters(P)$

{Calculate Interaction Based Homogeneity for each Cluster}

for $i = 1 \rightarrow length(C)$ **do**

  if $IBH_I(C_i) < T$ **then**

    remove C_i from C

  end if

end for

$G \leftarrow SetOfGenes(C)$

for $i = 1 \rightarrow length(G)$ **do**

  for $j = 1 \rightarrow length(G)$ **do**

    $n(i, j) \leftarrow 0$

    for $c = 1 \rightarrow N$ **do**

      if $G[i] \in P_c$ $G[j] \in P_c$ **then**

        $n(i, j) \leftarrow n(i, j) + 1$

      end if

    end for{Create co-association matrix from C}

    $C(i, j) \leftarrow \frac{n(i,j)}{N}$

  end for

end for

$R \leftarrow Cluster(C)$

return R

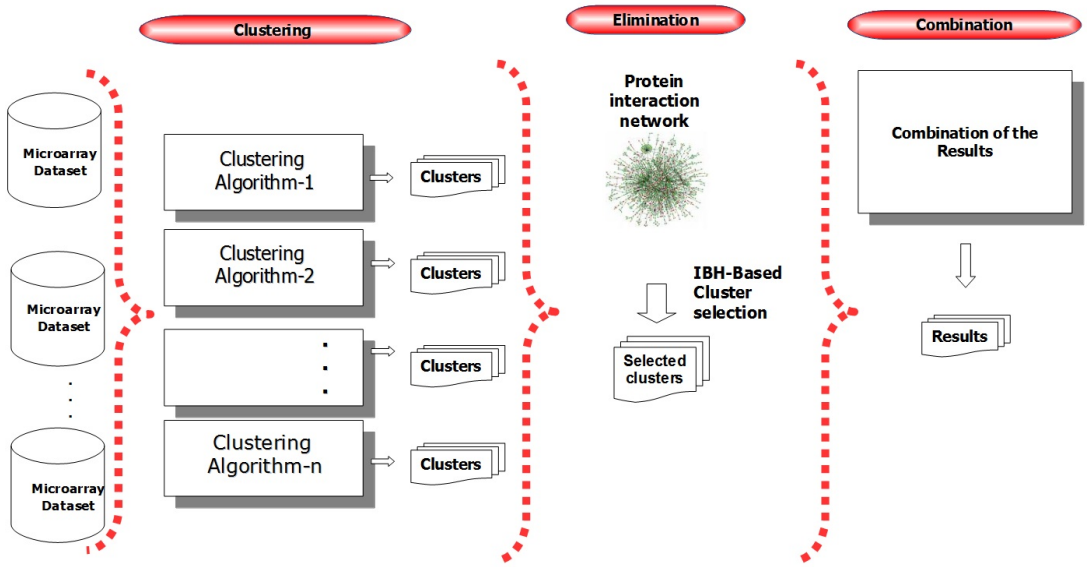


Figure 4.3: Elimination of unrelated clusters before clustering combination.

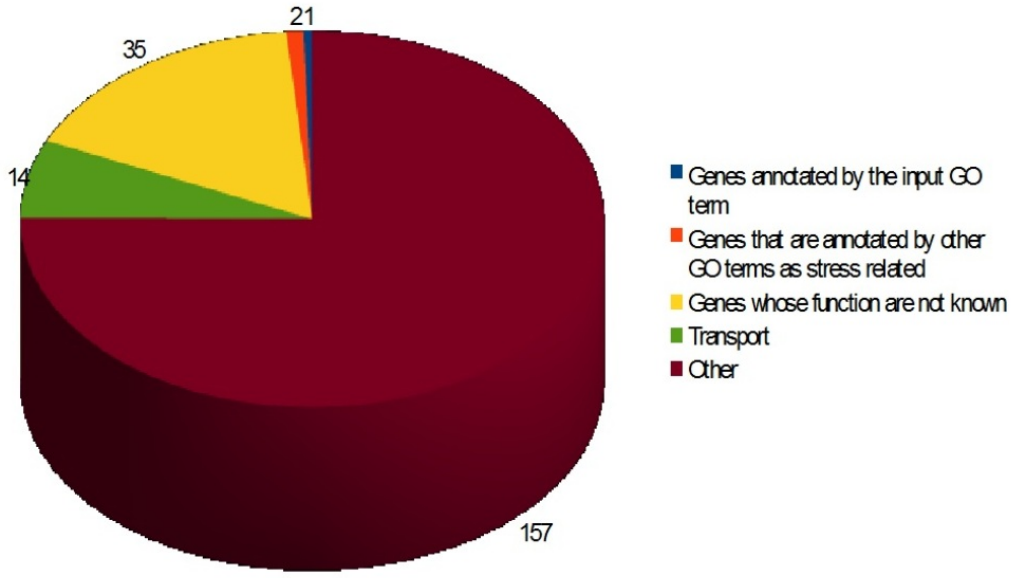


Figure 4.4: Elimination of unrelated clusters before clustering combination on yeast heat shock GDS36 dataset.

4.4.1.1 Results of Elimination of Unrelated Clusters Before Clustering Combination

The results of analyzing GDS36 data is given in Figure 4.4.

We analyzed the results on a gene by gene basis. First, GO annotations for each gene is searched to locate annotations related with heat shock. After that a literature survey is performed for genes who do not have related annotations in GO. The results can be summarized as:

- *Genes that are annotated by the input GO term:* LCB5 gene is annotated by the input GO term.
- *Genes that are annotated by other GO terms as stress related:* 2 genes (ZEO1, ATC1) are annotated by other GO terms as stress related.
- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* No genes are found to be related with stress in literature.
- *The unrelated genes and genes whose functions are not known yet:* These genes grouped for further analysis:

- *Other genes that may be related with stress:* 14 genes are transport related: HXT5, AVT6, GOT1, SAM1, PPM2, SAM2, YMC2, YJR129C,YNL022C, YGR283C, AIR1, PHO84, ARE1, TRM44, TRM5, FRE1, YBR141C, FRE7 and HXT3. It is known that the expression levels of transport-related genes decreases under stress [73]. The role of these genes should be further investigated to make sure of their relevance with stress conditions, especially the heat shock. Other genes in this group are PTH2 and YHR113W which are responsible from hydrolase activity.
- *Genes whose function are not known:* 35 genes have unknown functions.
- *Other genes:* 157 genes have no evidence for relevance with heat shock. When we examine genes in this category, we see that they are mostly transcription, processing and binding related genes.

Since there are lots of known interactions on transcription and binding related genes, the results are dominated by them. This situation may be the result of the fact that some biological phenomena may involve more interactions than another. In order to prevent these interactions to dominate the results, we should include some functional information about the experiment.

4.4.2 Create Interaction Subnetworks Using Gene Ontology

The problem in the method described in previous section is the lack of functional information related with the experiment. The Gene Ontology is an excellent resource from which the functional classes of genes can be obtained. The idea introduced in this section is to take as input Gene Ontology terms related with the experiment and incorporate knowledge contained in these Gene Ontology terms into the analysis. The Gene Ontology terms are used to create an interaction subnetwork from the whole network of the organism. The subnetwork contains the interactions of genes related with the functional classes. As an example, when analyzing sporulation dataset, Gene Ontology terms related with sporulation are given as input, the interaction subnetwork are than created by selecting interactions among genes annotated by the input Gene Ontology term. Then, we calculate IBH for each cluster by using the subnetwork and eliminate clusters that has small IBH values. The resulting clusters are than combined

by using the evidence accumulation method. The outline and the algorithm of this step is given in Figure 4.5 and Algorithm 3, respectively.

Algorithm 3 Algorithm for creating interaction subnetworks using Gene Ontology.

Require: A set of partitions P of microarray data M resulting from N clustering algorithms, a threshold T for cluster elimination, interaction network I, a set of Gene Ontology terms O

{Create the set of clusters}

$C \leftarrow createSetOfClusters(P)$

{Create interaction subnetwork}

$S \leftarrow createInteractionSubnetwork(I, O)$

{Calculate Interaction Based Homogeneity for each Cluster}

for $i = 1 \rightarrow length(C)$ **do**

  if $IBH_S(C_i) < T$ **then**

    remove C_i from C

  end if

end for

$G \leftarrow SetOfGenes(C)$

{Create co-association matrix from C}

for $i = 1 \rightarrow length(G)$ **do**

  for $j = 1 \rightarrow length(G)$ **do**

    $n(i, j) \leftarrow 0$

    for $c = 1 \rightarrow N$ **do**

      if $G[i] \in P_c$ $G[j] \in P_c$ **then**

        $n(i, j) \leftarrow n(i, j) + 1$

      end if

    end for

    $C(i, j) \leftarrow \frac{n(i,j)}{N}$

  end for

end for

$R \leftarrow Cluster(C)$

return R

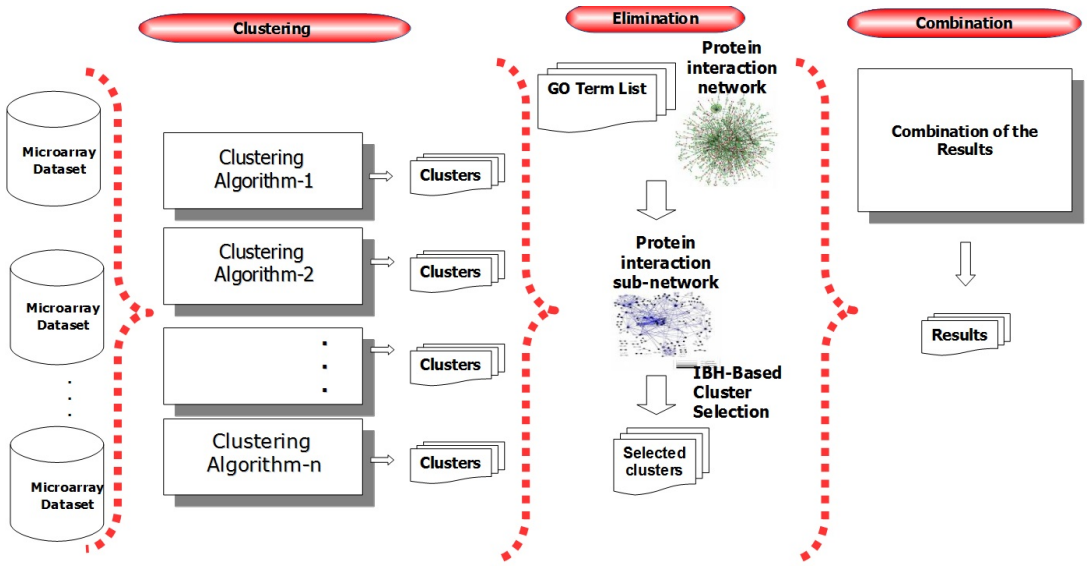


Figure 4.5: Outline of creating interaction subnetworks using Gene Ontology.

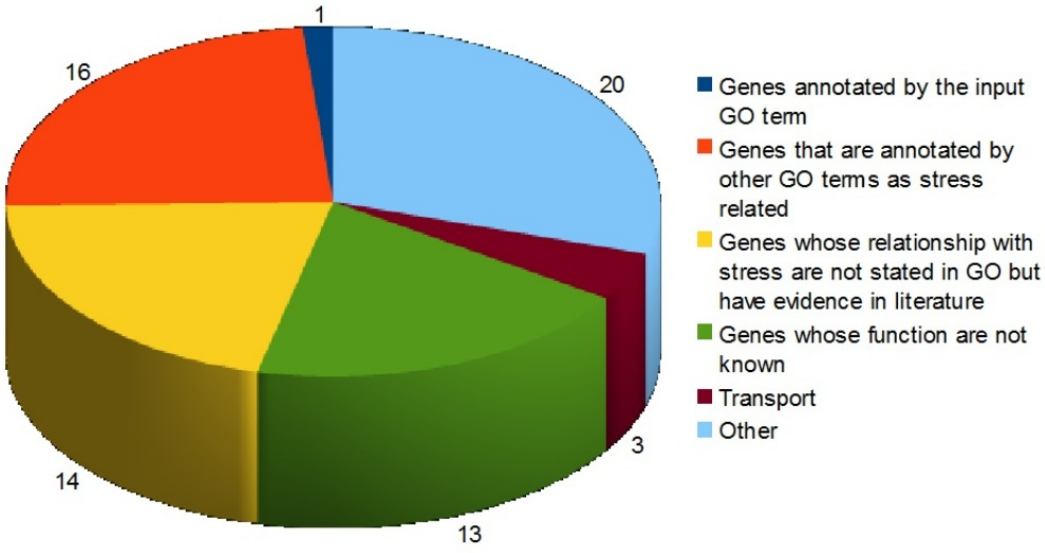


Figure 4.6: Results of creating interaction subnetworks using Gene Ontology on yeast heat shock GDS36 dataset.

4.4.2.1 Results of Creating Interaction Subnetworks Using Gene Ontology

The results of analyzing GDS1711 data are given in Figure 4.6.

We analyzed the results on a gene by gene basis. First, GO annotations for each gene is searched to locate annotations related with heat shock. After that a literature survey is performed for genes who do not have related annotations in GO. The results can be summarized as follows:

- *Genes that are annotated by the input GO term:* 1 gene (HSP12) is annotated by the input GO term.
- *Genes that are annotated by other GO terms as stress related:* 16 genes (BDH1, DDR2, GAD1, GRE3, HOR7, HSP104, HSP42, NCE103, SPI1, SSA4, SSE2, TPS1, TPS3, TSL1, YHN1, YOL053C) are annotated by other GO terms as stress related.
- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* 14 genes (CIT1, CWP1, DCS2, GLK1, GTO3, HXK1, PGM2, PHR1, PRB1, RTN2, SOL4, TMA17, TPK1, UGP1) are found to be related

with stress in literature.

- *The unrelated genes and genes whose functions are not known yet:* These genes grouped for further analysis:
  - *Other genes that may be related with stress:* 3 genes are *transport related genes:* (FTH1, GYP5, THI17). It is known that the expression levels of transport-related genes decreases under stress [73]. The role of these genes should be further investigated to make sure of their relevance with stress conditions, especially the heat shock.
  - *Genes whose function are not known:* 13 genes have unknown functions.
  - *Other genes:* 20 genes have no evidence for relevance with heat shock.

We can see that the results are significantly improved with the inclusion of the functional class information contained in the provided Gene Ontology terms. However, we have lots of unrelated genes in the result. The unrelated genes come from clusters with high IBH values. Although some of the genes in such clusters are important, we should find a way to eliminate the unrelated genes. Another problem is that with this method, some related genes contained in clusters with low IBH values are eliminated. The last problem to be solved before finalizing the method is that the method eliminates clusters and some important genes are lost since the method tends to eliminate larger clusters. When small cluster thresholds are used, the method outputs large clusters having more than 1000 genes in a cluster since the clustering algorithms used to create partitions generate such clusters. The solution found to this problem is that instead of eliminating clusters, we clean them. For cleaning clusters, first, an IBH based weight is calculated for each gene. Than, genes that have small weights are removed from clusters. The resulting clusters are than combined by using the evidence accumulation method.

4.4.3 Contribution of Using Interaction Networks

From the results presented in previous sections, we can see the contribution of Gene Ontology terms. The other important part of the algorithm is the interaction network. To show the contribution of the interaction network, we give as input a fully

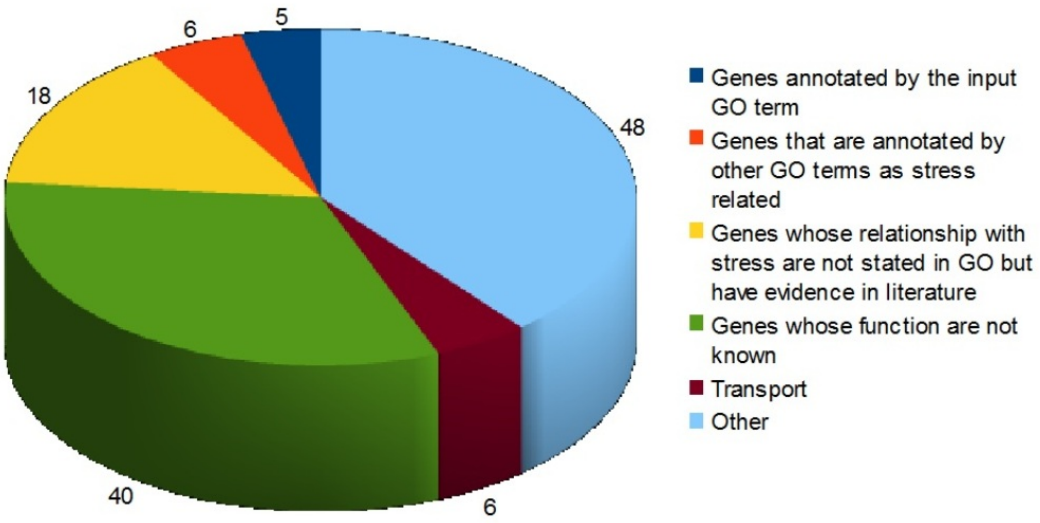


Figure 4.7: CEC on yeast heat shock GDS36 dataset–with a fully connected interaction network.

connected graph as an interaction network. The result without the contribution of a real interaction network is given in Figure 4.7.

The results can be summarized as:

- *Genes that are annotated by the input GO term:* 5 genes (YAP1, HSP12, WSC4, WSC3, LCB5) are annotated by the input GO term.
- *Genes that are annotated by other GO terms as stress related:* 6 genes (RIM15, SIN3, MHR1, TIF4632, YOL053C, HSP30) are annotated by other GO terms as stress related.
- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* 18 genes (RRN5, MMS22, FAB1, TOM1, DRS2, TFC3, YTA7, YME1, RML2,IRA1, SWI4, HSP12, KAP104, YHL037C, YFL052W, ICS3, OCA5, TFP1) are found to be related with stress in literature.
- *The unrelated genes and genes whose functions are not known yet:* These genes grouped for further analysis:
  - *Other genes that may be related with stress:* 6 genes are *transport related*

genes: LRO1, YHR009C, DAN1, QDR2, COX5A, FRE7. It is known that the expression levels of transport-related genes decreases under stress [73]. The role of these genes should be further investigated to make sure of their relevance with stress conditions, especially the heat shock.

- *Genes whose function are not known:* 40 genes have unknown functions.
- *Other genes:* 48 genes have no evidence for relevance with heat shock.

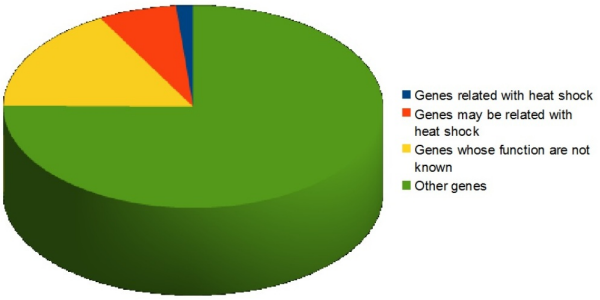
We can see from the results that although the number of genes that are annotated by the input GO term is increased from 1 to 5, the number of unrelated genes is also increased significantly, proving the necessity of using a real interaction network.

4.5 Summary

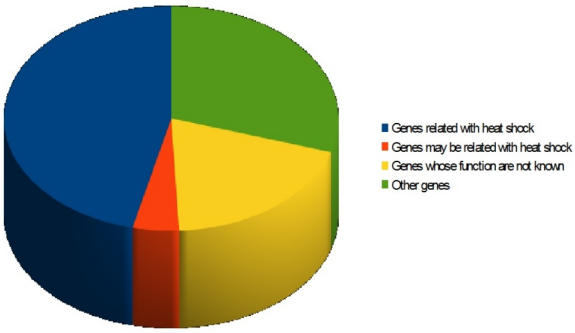
In this chapter, we described the Cluster-Eliminate-Combine method and demonstrate the significance of its components. A summary of the results can be found in Figure 4.8:

- The results of applying whole interaction network to select clusters with high IBH are given in Figure 4.8-(a). We can see that the results are dominated with transcription and binding related genes since there are a lot of known interactions for them and we do not use any functional class information.
- The results of using the functional class information contained in Gene Ontology by creating a subnetwork of the interaction network by a GO term which represents the biological process related with the microarray dataset are shown in Figure 4.8-(b). We can see from the results that the number of related genes are increased, however there are lots of unrelated genes in the result.
- In the final CEC method, a subnetwork of the interaction network is taken by a GO term which represents the biological process related with the microarray dataset, in this case *heat shock*. Then clusters are cleaned by gene weight. After cleaning the resulting clusters are combined. The results are shown in Figure 4.8-(a). The results show the step-by-step contributions made by each of the components of CEC method.

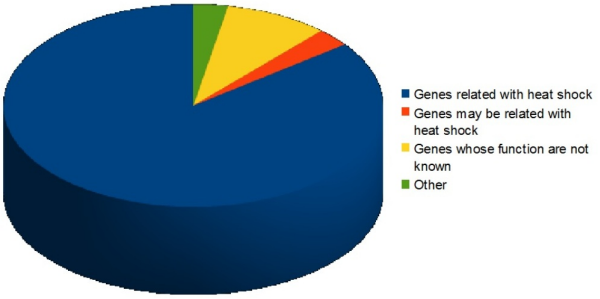
Finally, in order to show the significance of the interaction network, CEC is applied to the same dataset with a fully connected interaction network. The results are shown in Figure 4.9. This results proves the significance of using a real interaction network.



(a) Summarized Results of the Step-2 of CEC on Yeast heat shock GDS36 dataset

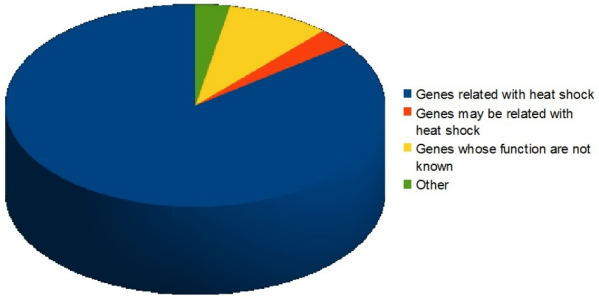


(b) Summarized Results of the Step-3 of CEC on Yeast heat shock GDS36 dataset

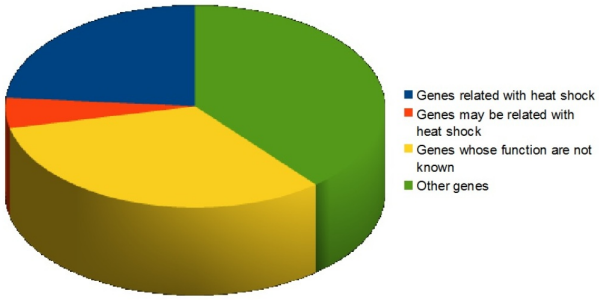


(c) Summarized Results of CEC on Yeast heat shock GDS36 dataset-Final Method

Figure 4.8: Contribution of the components of CEC method on GDS36 heat shock dataset.



(a) Summarized Results of CEC on Yeast heat shock GDS36 dataset-With real interaction network



(b) Summarized Results of CEC on Yeast heat shock GDS36 dataset with a fully connected interaction network.

Figure 4.9: Significance of using a real interaction network.

CHAPTER 5

RESULTS AND DISCUSSIONS

In this chapter, we show the results of applying the *CEC* method in different cases. Interactions are taken from BioGRID interaction repository [45]. While taking genes annotated by related Gene Ontology terms, only experimental Evidence Codes (*EXP: Inferred from Experiment , IDA: Inferred from Direct Assay, IPI: Inferred from Physical Interaction , IMP: Inferred from Mutant Phenotype, IGI: Inferred from Genetic Interaction , IEP: Inferred from Expression Pattern*) are taken into consideration. For the GO searches in the first two subsections, AMIGO tool is used [86]. In Section 5.1, two time-series datasets are analyzed by CEC. As mentioned in previous section, CEC can be applied to the problem of combining heterogeneous datasets. In Section 5.2, an example application of CEC to the combination of 7 different heat shock datasets is presented. Finally, in Section 5.3, CEC is analyzed with GSEA [87] on two example datasets.

5.1 Different Algorithms on Time-Series Microarray Data

5.1.1 Description

In time series experiments, the expression levels of genes are measured at different time points. There are algorithms designed specifically to cluster time series gene expression data. In this section, we applied 5 different clustering algorithms to a time series dataset and apply CEC method to the clustering results. The experiment is outlined in Figure 5.1. We selected two different time series datasets which are described in the first subsection for the analysis. The results of analyzing the datasets

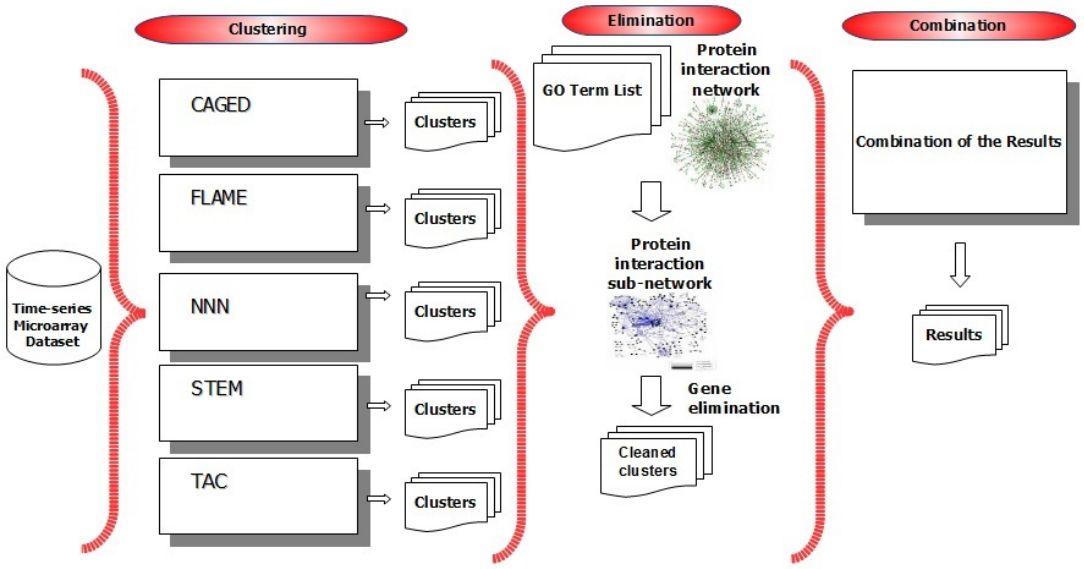


Figure 5.1: Outline of the application of CEC method to time-series microarray data.

by CEC is presented in the following sections.

5.1.2 Datasets

Yeast is a model organism in which most of the experiments are carried on very simply and quickly. For this reason, yeast genes are more annotated and its interactions are better known compared to many other organisms. We selected two different yeast time series datasets.

1. **Yeast sporulation dataset:** Chu et al. [88] measured the changes of the expression levels of yeast genes during sporulation. There are 7 time points in this datasets. They identified 485 differentially expressed genes and we use them as input.

2. **GDS1711:** Matsumoto et al. [89] measured the genomic response at the level of mRNA expression to the deletion of SSA1/2 in comparison with the mild heat-shocked wild-type using cDNA microarray. The GDS1711 microarray data prepared in this study is a heat shock dataset in which measurements are taken at 30 and 60 minutes on heat shock treatment at 43°C for wild types and ssa1 ssa2 double deletion mutant. There are 12 samples. The log ratio values of

the measurements are used. The dataset contains 6020 genes. We used the whole dataset in all of the analyses without performing a preprocessing step for selecting differentially expressed genes.

5.1.3 Results on Yeast Sporulation Dataset

For analyzing Yeast sporulation dataset, in the clustering step, the dataset is clustered by five algorithms to obtain different partitions. Number of clusters found in each partition is given in Table F. As can be seen from the table, the number of clusters found varies from 5 to 18.

Table 5.1: Number of clusters found in each partition for yeast sporulation dataset.

Algorithm	Number of Clusters
CAGED	6
FLAME	13
GQL	5
NNN	18
STEM	8
TAC	7

We apply subsequently the elimination step of the algorithm. Genes annotated with GO term *Sporulation (GO:0043934)* is selected as the gene list. The subnetwork of BioGRID interactions related with the genes in the selected GO term is prepared. First, the IBH for each cluster is calculated. Then, from IBH values of each cluster, gene weights are calculated. The unrelated genes are eliminated from the clusters in partitions by using threshold as 0.01.

The distance matrix between genes that passed the elimination step is calculated by using the evidence accumulation. Then, the distance matrix is used by a model based clustering algorithm to obtain the final clustering. The results after combination step can be found in Figure 5.2 and detailed analysis of the results are given in Table F.2 in Appendix F.

We can see from the results that the analysis suggests 7 clusters. When we examine the clusters, we can see that Cluster-1 contains mostly genes related with meiosis, Cluster-6 contains stress response genes, Cluster-7 contains transport related genes.

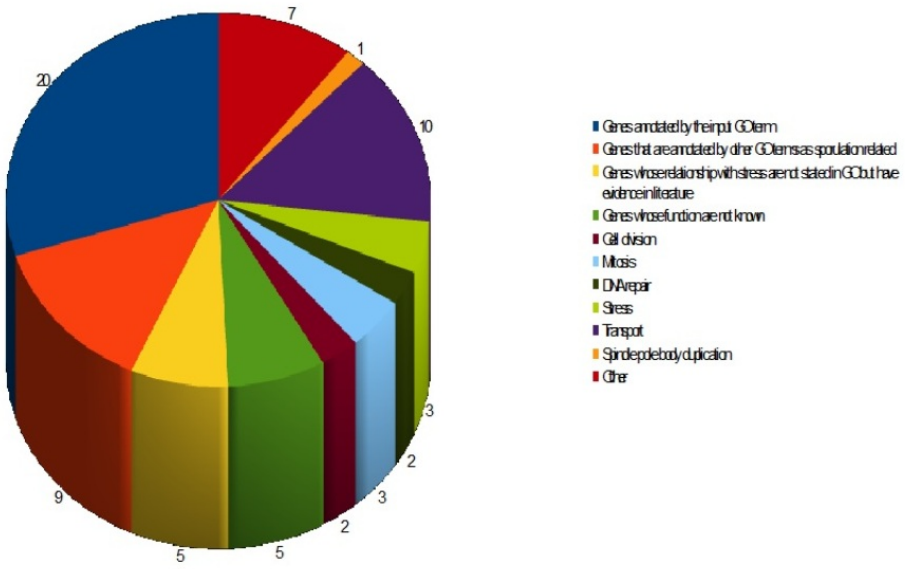


Figure 5.2: CEC on yeast sporulation dataset.

We then analyzed the results on a gene by gene basis. First, GO annotations for each gene is searched to locate annotations related with heat shock. After GO search, a literature survey is performed for genes that do not have related annotations in GO.

The results can be summarized as follows:

- *Genes that are annotated by the input GO term:* 20 genes (YDR523C, YLR213C, YLR227C, YOR313C, YPL130W, YHR184W, YLR307W, YOL091W, YOR298W, YLR343W, YNL128W, YNL204C, YOL132W, YDR273W, YDR522C, YGL170C, YHR185C, YNL098C, YHR139C, YJL074C) are annotated by the input GO term.

- *Genes that are annotated by other GO terms as sporulation related:* 9 genes (YBR268W, YDL154W, YOR033C, YER106W, YKL042W, YHR124W, YGR059W, YJL038C, YAR007C) are annotated by other GO terms as sporulation related.

- *Genes whose relationship with sporulation are not stated in GO bu have evidence in literature:* YDR065W [90], YGR229C [91], YFR032C [92] YGL138CBriza2002, YMR125W [93], genes are found to be related with sporulation.

- The unrelated genes and genes whose functions are not known yet: These genes

Table 5.2: Number of clusters found in each partition for GD1711 dataset.

Algorithm	Number of Clusters	Total Number of Genes in Clusters
CAGED	16	5959
FLAME	63	6020
NNN	4	36
STEM	50	4305
TAC	13	5949

grouped for further analysis:

- Cell division related genes: YDL008, YDR218C,
- Mitosis related genes: YIL139C, YEL061C, YAL040C,
- DNA repair genes: YDR263C, YDR317W,
- Stress related genes: YAL062W, YDR256C, YOR375C,
- Transport related genes: YFL011W, YLR209C, YMR272C, YGL179C, YJR152W, YML008C, YNL142W, YAL067C, YGR224W, YPL208W,
- Spindle pole body duplication: YPL124W,
- Genes whose function are not known: YER182W, YDL186W, YPR027C, YGL015C, YOL015W,
- Other genes: YDL103C, YEL016C, YIL159W, YFR023W, YHR015W, YKR016W, YOR051C.

5.1.4 Results on Yeast Heat Shock GDS1711 Dataset

For analyzing GDS1711 dataset, in the clustering step, the dataset is clustered by five algorithms to obtain different partitions. Number of clusters found in each partition is given in Table 5.2. As can be seen from the table, the number of clusters found varies from 4 to 63 and each partition contains different numbers of genes ranging from 36 to 6020.

Then, we run the elimination step of the algorithm. As in the GDS36 heat shock dataset, genes annotated with GO term *Response to Heat (GO:0009408)* is selected as the gene list. There are 192 genes annotated by this the GO term with experimental evidence codes. The subnetwork of BioGRID interactions related with the 192 genes

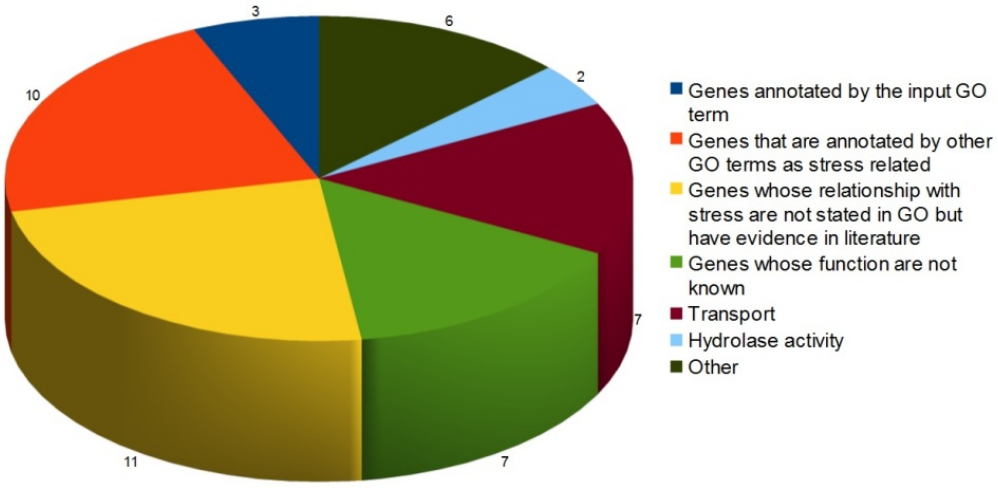


Figure 5.3: CEC on yeast heat shock GDS1711 dataset.

in the selected GO term is prepared. First, the IBH for each cluster is calculated. Then, from IBH values of each cluster, gene weights are calculated. The unrelated genes are eliminated from the clusters in partitions by using threshold being equal to 0.01.

The distance matrix between genes that passed the elimination step is calculated by using the evidence accumulation. Then, the distance matrix is used by a model based clustering algorithm to obtain the final clustering. We analyzed the results on a gene by gene basis. First, GO annotations for each gene is searched to locate annotations related with heat shock. After that a literature survey is performed for genes who do not have related annotations in GO. The results are summarized in Figure 5.3 and detailed analysis of the results are given in Table H.1 in Appendix H. We can see from the results that the analysis suggests 6 clusters and 55 genes.

The results can be summarized as:

- *Genes that are annotated by the input GO term:* 3 genes (*SGT2, NUP84, ASM4*) are annotated by the input GO term.

- *Genes that are annotated by other GO terms as stress related:* 10 genes (TDH3,

MRK1, HSC82, MSN4, SSE1, STI1, ACT1, LRE1, YOR356W, MET22) are annotated by other GO terms as stress related.

- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* 11 genes (YOR356W [94], PRE7 [94], YGR207C [95], AIM2 [94], NSE5 [94], TOK1 [96], TPM1 [94], VPS53 [94], NGL1 [97], BPT1 [98], ESC2 [94]) are found to be related with stress in literature.

- *The unrelated genes and genes whose functions are not known yet:* These genes grouped for further analysis:

  - *Other genes that may be related with heat shock:* Among these genes, most of them are *transport related genes:* PFA5, AVT2, YGL114W, YPL236C, ATF2, DSL1 and EST1. It is known that the expression levels of transport-related genes decreases under stress [73]. The role of these genes should be further investigated to make sure of their relevance with stress conditions, especially the heat shock.

  - *Genes whose function are not known:* 7 genes (YOR352W, YFR032C, YIR043C, AIM38, AIM43, DLT1, GTT3) have unknown functions.

  - *Other genes:* There are 8 genes that have no evidence for relevance with heat shock. PTH2 and YHR113W which are responsible from hydrolase activity, YBL036C for pyridoxal phosphate binding, YNR048W for phospholipid-translocating ATPase activity, UBA for protein ubiquitination, EDC3 for cytoplasmic mRNA processing body assembly, AEP1 for regulation of translation and GTB1 polysaccharide biosynthetic process.

5.2 Combining Different Microarray Experiments

5.2.1 Description

In heat shock response of the cell, several groups of genes are expressed in order to adapt the cell to the new environment. Mainly, carbohydrate metabolism, fatty acid metabolism, respiration, oxidative stress defense autophagy and vacuolar function, protein folding and degradation, cytoskeletal reorganization and signaling genes are

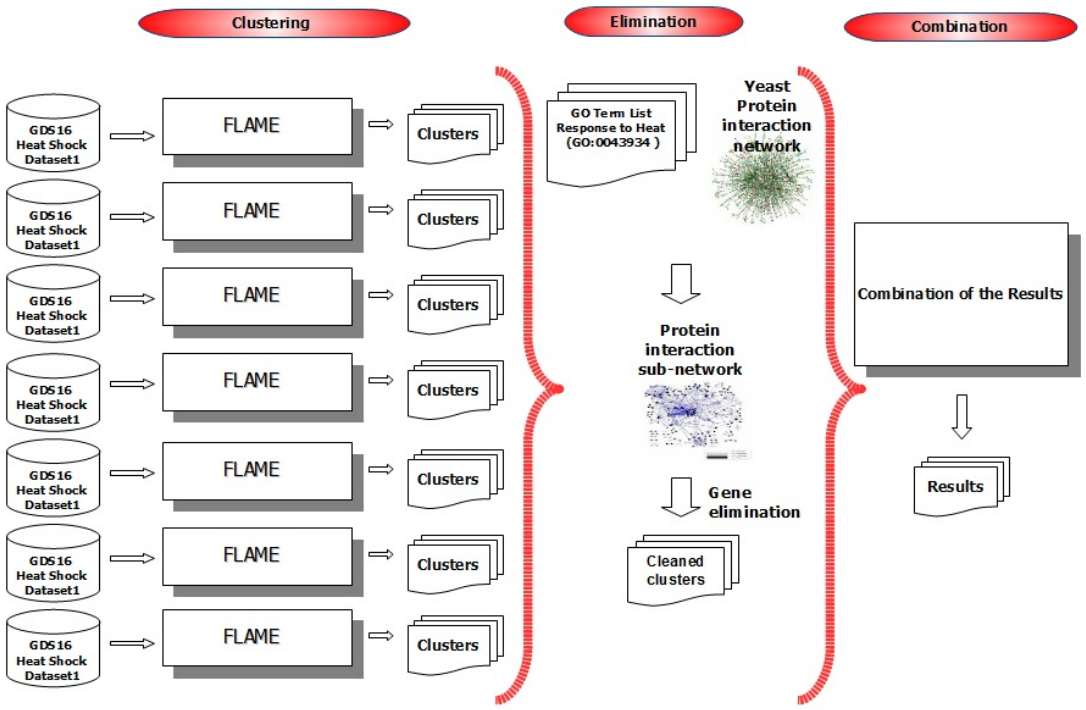


Figure 5.4: Outline of the application of CEC method to combination of heterogeneous datasets, 7 different heat shock microarray datasets in this example.

expressed. We take seven different time series datasets, each representing a heat shock time series experiment. Our aim is to find the common characteristics that can be observed on these 7 experiments in the context of heat shock. For clustering, FLAME algorithm is used [2]. The outline of the combination is shown in Figure 5.4. The selected datasets are described in the next section and the results are then given.

5.2.2 Datasets

Gasch et al. measured genomic expression patterns in the yeast Saccharomyces cerevisiae responding to diverse environmental transitions over time for almost every yeast gene [72]. We selected heat shock experiments from the experiment data they provide, namely GDS15, GDS16, GDS34, GDS35, GDS36 and GDS112. In addition to these datasets, we included a gene deletion experiment on SSA1/2 gene heat shock response by Matsumoto et al. [89]. All datasets can be downloaded from Gene Expression Omnibus (GEO).

- **GDS15:** In this dataset, measurements are taken 20 min after temperature shift from 17°C, 21°C, 25°C, 29°C or 33°C to 37°C [72]. There are 6 samples and 9345 genes/gene candidates in the dataset.

- **GDS16:** In this dataset, measurements are taken at several time points up to 80 minutes on heat shock from 25°C to 37°C [72]. There are 8 samples and 9388 genes/gene candidates in the dataset.

- **GDS34:** In this dataset, measurements are taken at 15, 30, 45, 60 and 90 minutes on heat shock from 37°C to 25°C [72]. There are 5 samples and 7467 genes/gene candidates in the dataset.

- **GDS35:** In this dataset, measurements are taken on mild heat shocks from 29°C to 33°C at variable osmolarity, with or without addition of 1 M sorbitol. There are 6 samples and 7424 genes/gene candidates in the dataset. The samples are collected at 5, 15 and 30 minutes [72].

- **GDS36:** In this dataset, measurements are taken at 5, 15, 30 and 90 minutes on a heat shock from 29°C to 33°C [72]. There are 4 samples and 7578 genes/gene candidates in the dataset.

- **GDS112:** In this dataset, measurements are taken at 0, 5, 15, 30 and 60 minutes on a heat shock from 30°C to 37°C [72]. There are 5 samples and 8582 genes/gene candidates in the dataset.

- **GDS1711:** In this dataset,measurements are taken at 30 and 60 minutes on heat shock treatment at 43°C for wild types and ssa1 ssa2 double deletion mutant. There are 12 samples [89] and 6974 genes/gene candidates.

5.2.3 Results

In the clustering step, the 7 different heat shock datasets are clustered by FLAME algorithm to obtain different partitions.

Then, we run the elimination step of the algorithm. Genes annotated with GO term *Response to Heat (GO:0009408)* is selected as the gene list. There are 192 genes annotated by this the GO term with experimental evidence codes. The subnetwork of

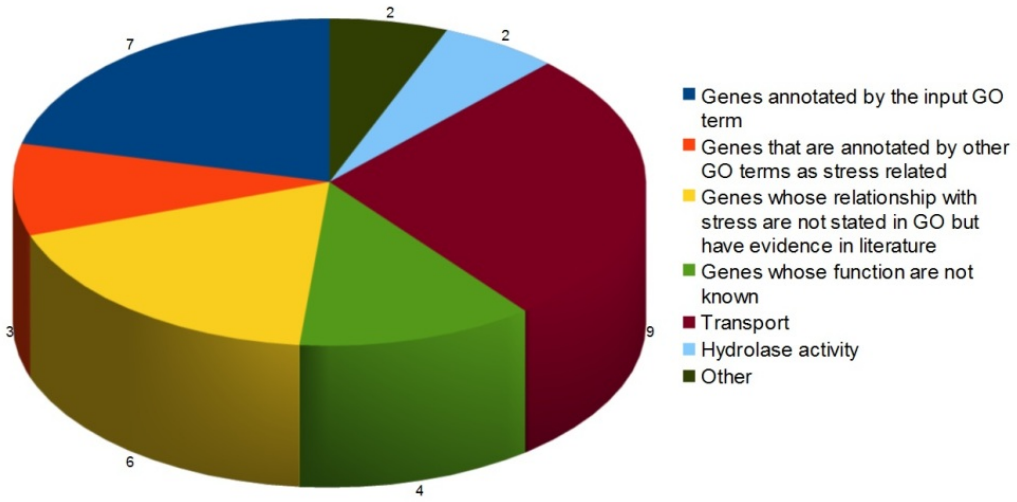


Figure 5.5: CEC on application of CEC method to combination of heterogeneous datasets, 7 different heat shock microarray datasets in this example.

BioGRID interactions related with the 192 genes in the selected GO term is prepared. First, the IBH for each cluster is calculated. Then, from IBH values of each cluster, gene weights are calculated. The unrelated genes are eliminated from the clusters in partitions by using threshold being equal to 0.01.

The distance matrix between genes that passed the elimination step is calculated by using the evidence accumulation. Then, the distance matrix is used by a model based clustering algorithm to obtain the final clustering. The results after combination step can be found in Figure 5.5.

We examined the results on gene basis and the results are given in Table I.1. Most of the genes resulting from the CEC analysis are related to heat shock experiments. In addition to genes that are annotated with the input GO term *Heat shock response*, genes that have functions related with heat shock response are found. Some of the genes are not annotated in GO with heat shock or stress related terms. However, when we look at the literature, we found some genes are actually related with heat shock response.

To summarize,

- *Genes that are annotated by the input GO term:* Genes STE20, ASM4, HSP104, NUP100, NUP84, SGT2 and MDJ1 are annotated by the GO term Response to heat are given as output of CEC analysis.

- *Genes that are annotated by other GO terms as stress related:* DAN1, LRE1 and PSR2 are annotated as stress related genes in GO.

- *Genes whose relationship with stress are not stated in GO bu have evidence in literature:* ARP4 [99], DOG2 [100], AIM45 [95], BPT1 [98], PFK26 [73] and TOK1 [96] genes are found to be related with stress response.

- *The unrelated genes and genes whose functions are not known yet:* Among these genes, most of them are transport related genes: APL1, ATF2, GGA1, SSH4, THI72, VTI1, YCK3, YPL236C and YGL14W. It is known that the expression levels of transport-related genes decreases under stress [73]. The role of these genes should be further investigated to make sure of their relevance with stress conditions, especially the heat shock. Other genes in this group are HOS3 and SGA1 which are responsible from hydrolase activity, CFT2 for mRNA cleavage and MOT2 for protein polyubiquitination. The last subgroup of genes are those whose function are not yet known: YHL005C, YJR141W, YLR177W and YLR345W.

5.3 Evaluation of CEC With the Gene Set Enrichment Analysis Method

5.3.1 Description

In order to assess the success of the method, we checked the GSEA enrichment scores of the analysis results. To make the comparisons more accurate, we selected two datasets from the original GSEA study. We first clustered the datasets with different algorithms such as FLAME, k-means and fuzzy c-means with different parameters and then applied the CEC method. The dataset is described in the first subsection. The results of analyzing the datasets by CEC is presented in the following sections.

5.3.2 Datasets

In order to make accurate comparisons, we selected two datasets from the original GSEA study.

1. *Male vs. Female Lymphoblastoid Cells.* Subramanian et al. measured expression profiles from lymphoblastoid cell lines derived from 15 males and 17 females to identify gene sets correlated with the distinctions [31]. They achieved enrichment of male related genes in male>female comparison.

2. *p53 Status in Cancer Cell Lines* Olivier et al. measured gene expressions from the NCI-60 collection of cancer cell lines [101]. Subramanian et al. used 50 of them in which mutation status was reported. The p53 gene was normal in 17 of them and mutated in the other 33.

5.3.3 Results on Male vs. Female Lymphoblastoid Cells Dataset

We selected male sex differentiation GO term as input to CEC method. We used each cluster found by CEC as a gene list as the input to Gene Set Enrichment analysis method.

We performed 2 runs with different cluster cleaning thresholds. The enrichment plots of enriched clusters with t=0.01 are given Figure 5.6.

We can see from the GSEA analysis results that each of the two clusters resulted from the CEC analysis of *Male vs. Female Lymphoblastoid Cells Dataset* are enriched, one positively correlated with male phenotypes and one negatively correlated with female phenotypes.

In order to show the affect of the gene cleaning threshold, the enrichment plots of the analysis with t=0.001 is given in 5.7.

When threshold being equal to 0.001 is used, 9 clusters are found by CEC analysis. We can see from the results that each of the 9 clusters are enriched by GSEA method and 4 clusters are positively correlated with male phenotypes and 5 clusters are negatively correlated with female phenotypes.

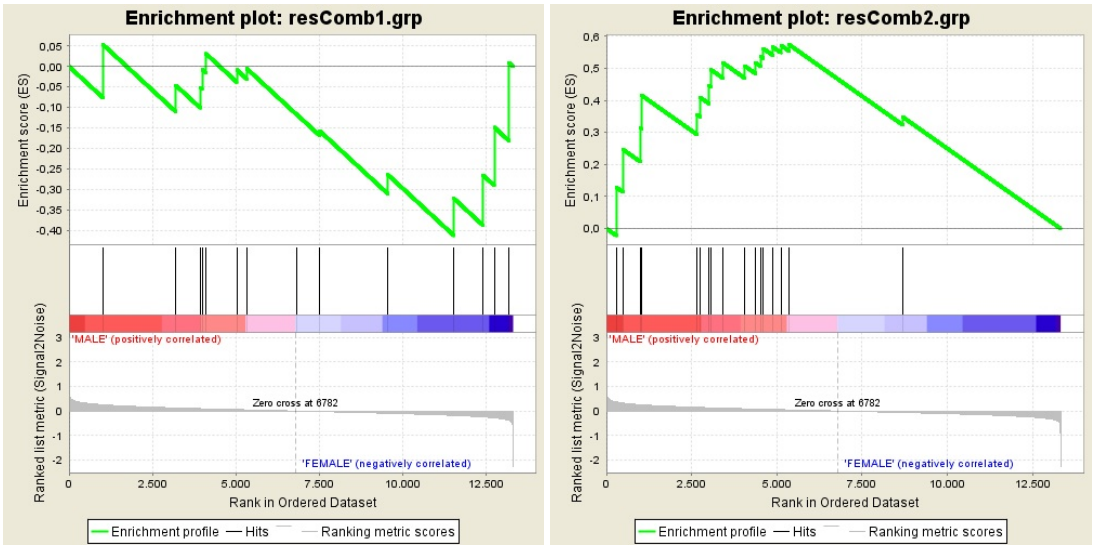


Figure 5.6: GSEA enrichment plot of enriched clusters found in CEC analysis of Male vs. Female Lymphoblastoid Cells Dataset with t=0.01.

5.3.4 Results on p53 Status in Cancer Cell Lines Dataset

We selected *p53 Binding* GO term as input to CEC method. The enrichment plots of enriched clusters are given in Figure 5.8.

We can see from the results of analysis of clusters found by CEC method by GSEA that all of the clusters are enriched. 2 clusters are positively correlated with normal phenotypes and 3 clusters are negatively correlated with phenotypes having p53 mutation.

5.3.5 Stability and Complexity Analysis

The only variable that can affect the stability of the method is the threshold value used in cleaning step. In order to see the effect of it on the method, we make slight changes on the threshold value and compared the resulting partitions by Rand Statistics, Jaccard Coefficient and Folkes and Mallow Index. As can be seen from the results in Table 5.3, Table 5.4 and Table 5.5, slight changes in the threshold do not cause dramatic changes in the results, showing the stability of the method on threshold values.

The algorithm of CEC method is $O(n^2)$, where n is the number of genes in the mi-

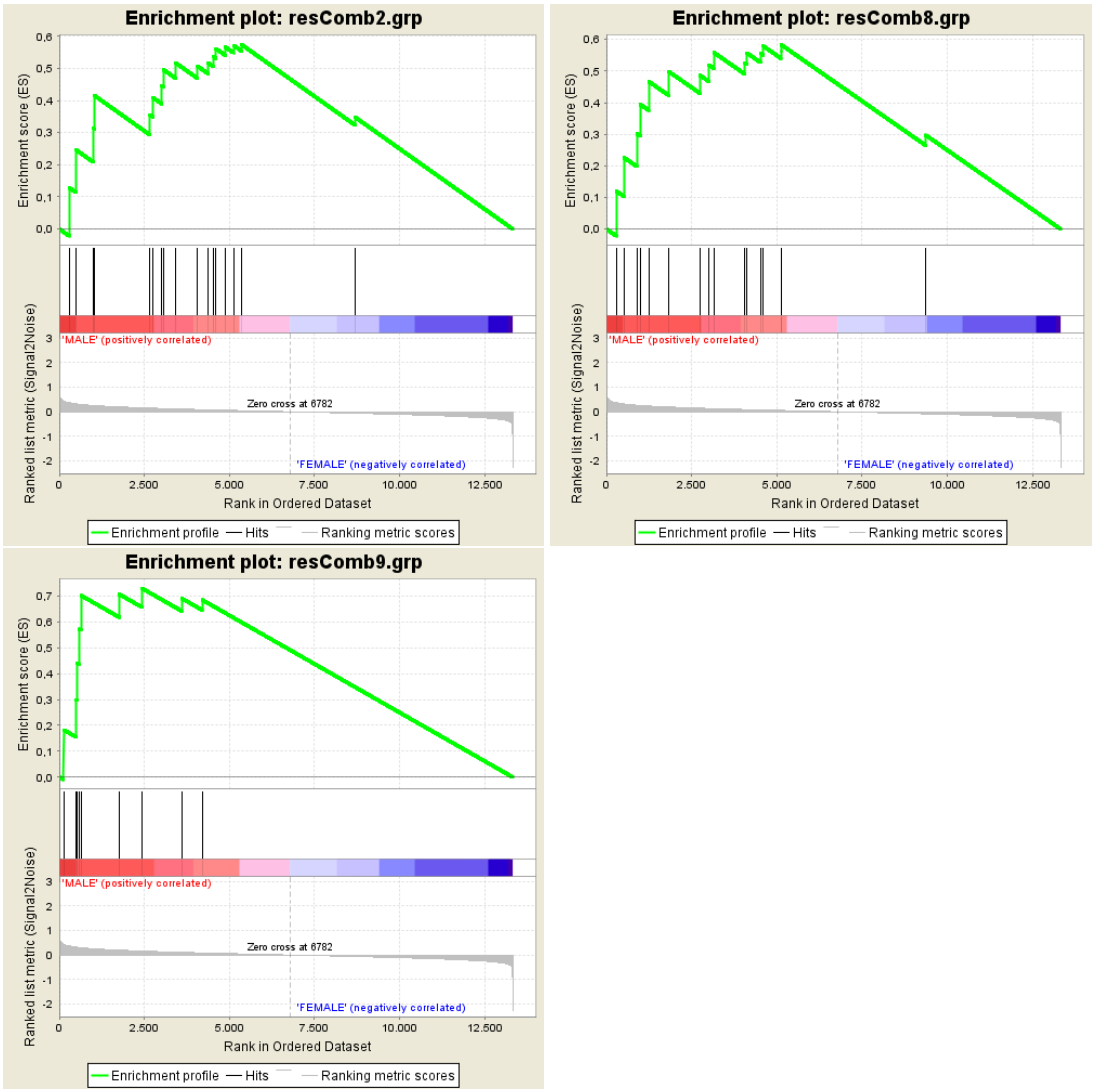


Figure 5.7: GSEA enrichment plot of enriched clusters found in CEC analysis of Male vs. Female Lymphoblastoid Cells Dataset with t=0.001.

Table 5.3: Rand Statistics among partitions with different threshold t values.

	t=0.003	t=0.004	t=0.005	t=0.006	t=0.007
t=0.003	1,00	0,95	0,95	0,81	0,79
t=0.004	0,95	1,00	1,00	0,86	0,84
t=0.005	0,95	1,00	1,00	0,86	0,84
t=0.006	0,81	0,86	0,86	1,00	0,98
t=0.007	0,79	0,84	0,84	0,98	1,00

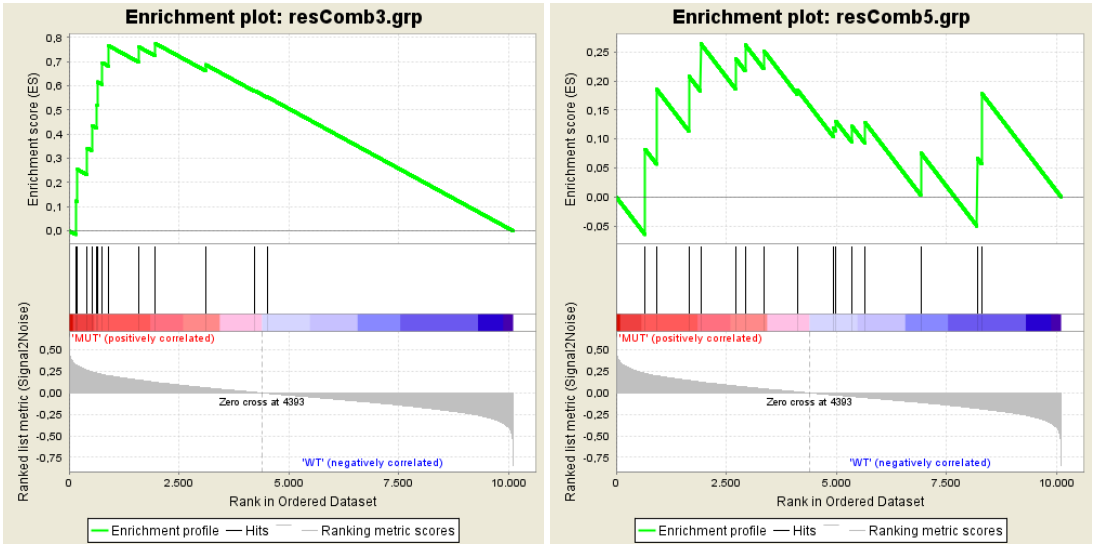


Figure 5.8: GSEA enrichment plot of enriched clusters found in CEC analysis of p53 Status in Cancer Cell Lines Dataset with t=0.01.

Table 5.4: Jaccard Coefficient among partitions with different threshold t values.

	t=0.003	t=0.004	t=0.005	t=0.006	t=0.007
t=0.003	1,00	0,93	0,93	0,78	0,76
t=0.004	0,93	1,00	1,00	0,84	0,82
t=0.005	0,93	1,00	1,00	0,84	0,82
t=0.006	0,78	0,84	0,84	1,00	0,98
t=0.007	0,76	0,82	0,82	0,98	1,00

Table 5.5: Folkes and Mallows Coefficient among partitions with different threshold t values.

	t=0.003	t=0.004	t=0.005	t=0.006	t=0.007
t=0.003	1,00	0,96	0,96	0,88	0,87
t=0.004	0,96	1,00	1,00	0,91	0,90
t=0.005	0,96	1,00	1,00	0,91	0,90
t=0.006	0,88	0,91	0,91	1,00	0,99
t=0.007	0,87	0,90	0,90	0,99	1,00

croarray dataset.

5.3.6 Results on Yeast Sporulation Dataset with STRING Interaction Database

In order to see the effect of using another interaction database, we used STRING interaction database [57] to the yeast sporulation database. For analyzing Yeast sporulation dataset, in the clustering step, the dataset is clustered by five algorithms to obtain different partitions. Number of clusters found in each partition is given in Table F in Appendix G. As can be seen from the table, the number of clusters found varies from 5 to 18.

Then, we run the elimination step of the algorithm. Genes annotated with GO term *Sporulation (GO:0043934)* is selected as the gene list. The subnetwork of STRING interactions related with the genes in the selected GO term is prepared. First, the IBH for each cluster is calculated. Then, from IBH values of each cluster, gene weights are calculated. The unrelated genes are eliminated from the clusters in partitions by using threshold being equal to 0.01.

The distance matrix between genes that passed the elimination step is calculated by using the evidence accumulation. Then, the distance matrix is used by a model based clustering algorithm to obtain the final clustering. The results after combination step can be found in Figure 5.9 and detailed analysis of the results are given in Table G.1 in Appendix G.

We analyzed the results on a gene by gene basis. First, GO annotations for each gene is searched to locate annotations related with heat shock. After GO search, a literature survey is performed for genes that do not have related annotations in GO.

The results can be summarized as follows:

- *Genes that are annotated by the input GO term:* 18 genes (YLR227C, YOR313C, YPL130W, YDR523C, YLR213C, YHR184W, YOR298W, YLR307W, YOL091W, YHR185C, YDR273W, YDR522C, YLR343W, YNL128W, YNL204C, YOL132W, YDR273W, YGL170C) are annotated by the input GO term.

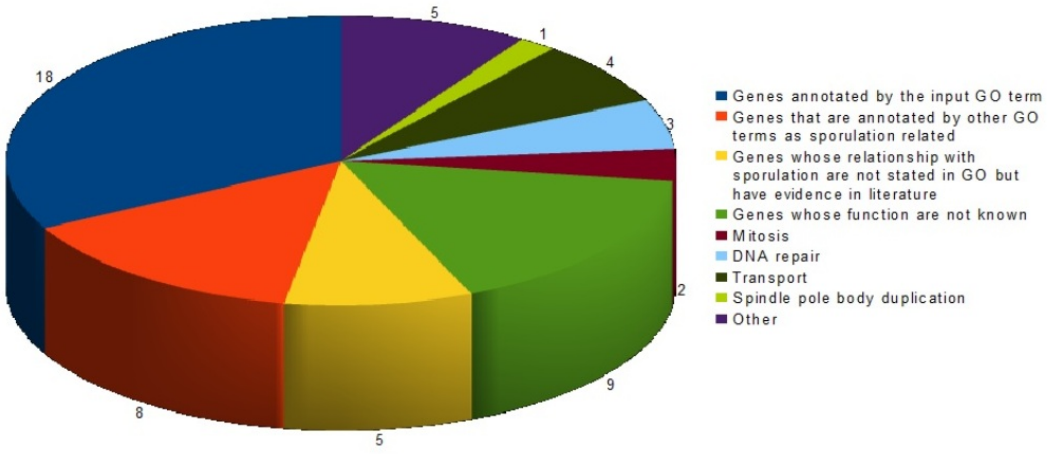


Figure 5.9: CEC on yeast sporulation dataset with STRING interaction database.

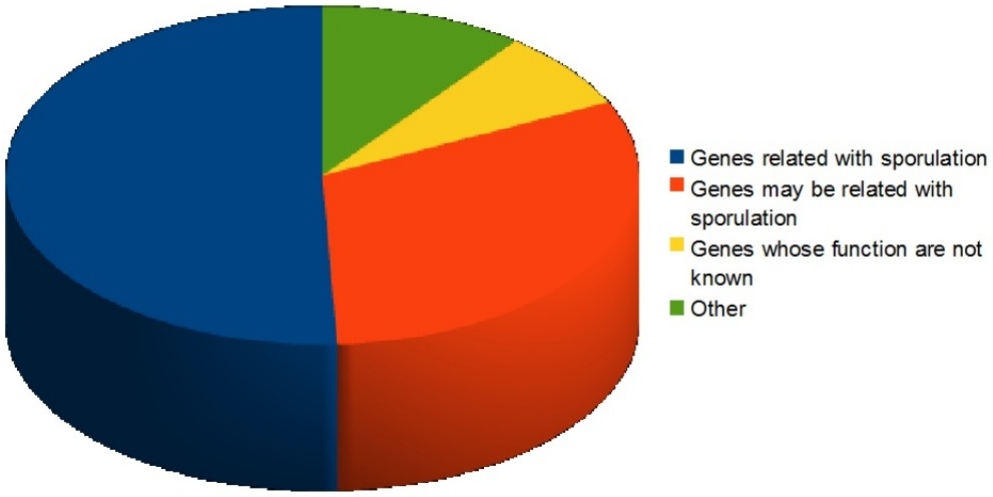
- *Genes that are annotated by other GO terms as sporulation related:* 8 genes (YDR218C, YHR124W, YDL154W, YOR33C, YER106W, YKL042W, YGR059W, YJL038C) are annotated by other GO terms as sporulation related.

- *Genes whose relationship with sporulation are not stated in GO bu have evidence in literature:* 6 genes (YDR065W, YGR229C,M YFR032C, YMR125W, YGL138C) are found to be related with sporulation.

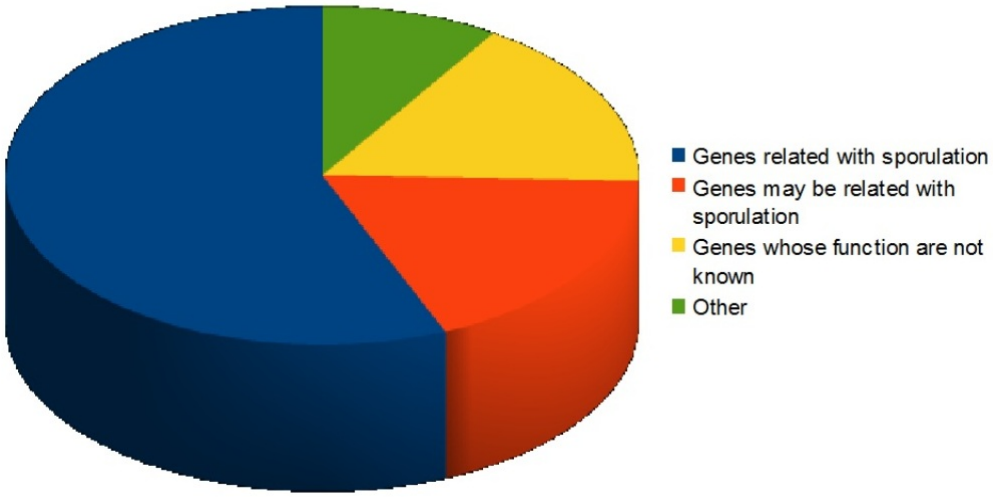
- The unrelated genes and genes whose functions are not known yet: These genes grouped for further analysis:

  - Mitosis related genes: YIL139C, YEL061C,
  - DNA repair genes: YDR263C, YDR317W, YIL159W,
  - Transport related genes: YFL011W, YLR209C, YMR272C, YHR015W,
  - Spindle pole body duplication: YPL124W,
  - Genes whose function are not known: YGL015C, YCRX06W, YDR355C, YGR228W, YCRX07W, YPR027C, YOL015W, YER182W, YDL186W.
  - Other genes: YBR268W, YDL008W, YDL103C, YEL016C, YFR023W.

The summarized results of the application of CEC method on yeast sporulation datasets with two different interaction databases, BioGRID and STRING are given in 5.10. As can be seen from the results, the results of CEC method does not change significantly with the change of interaction database used.



(a) Analysis with BioGRID Interaction Database.



(b) Analysis with String Interaction Database.

Figure 5.10: Summarized results of the analysis of sporulation dataset with two different interaction databases.

CHAPTER 6

CONCLUSION

Microarrays are used to understand relationships between genes, extract pathways, and in general to understand biological processes. With microarray technology, the expression levels of the entire genome can be measured simultaneously. While microarrays provide a great opportunity, mining microarray data is a challenging task. Methods to analyze microarray data should address the curse of dimensionality problem raised by the tens of thousands of genes versus small sample sizes. In order to solve the curse of dimensionality problem and find genes related with the biological process under inspection, methods should incorporate biological sources and use them in the analysis.

In this study, we describe *Cluster-Eliminate-Combine* method which involves integration of two important biological resources, Gene Ontology (GO) [32] and interaction networks in the analysis microarray data for eliminating unrelated genes from microarray data and allows us to find a clustering result containing only genes which are related with the biological process under inspection.

CEC method depends heavily on Interaction Based Homogeneity (IBH) that we describe in Chapter 3. Interaction Based Homogeneity measure is the first study to use interaction networks in the calculation of homogeneity, to the best of our knowledge. In order to demonstrate the effectiveness of IBH, we compared it with different similar popular measures. We applied two GO-based homogeneity measures, one using *Resnik's similarity* [69, 35] and the other using *Wang's similarity* [33]. We applied a KEGG-based enrichment score which is based on *domainSignatures* method [62]. Finally, we compared IBH with the popular *DAVID* tool's enrichment scores with

IBH [60, 61]. We performed our experiments on two cases. In the first case, lists of highly interacting genes were used and in the second case lists that are similar according to Gene Ontology were used. We showed that IBH is very successful in distinguishing related lists from random lists and measuring randomness in both cases.

Gene Set Enrichment Analysis (GSEA) [31] is a popular method in the analysis of microarray data. We applied IBH to create a ranked list from a microarray data, *p53 dataset*, and used GSEA method to locate enriched gene lists according to the ranked list. Gene lists related with the biological process under inspection were enriched by GSEA method, which proves the quality of the ranked list created by IBH.

We implemented an R [63] package called ***ibh*** containing several methods to calculate IBH. The package is accepted to be included in *Bioconductor* which provides tools for the analysis and comprehension of high-throughput genomic data and has an active user community. *ibh* package is freely available through *Bioconductor* [42].

After demonstrating the effectiveness of Interaction Based Homogeneity, we presented details of *CEC* method in Chapter 4. In CEC method, first, several partitions from microarray data are made by applying different clustering algorithms to the data or using different parameters on the same algorithm. An interaction subnetwork is created by using the whole interaction network of the genome and the Gene Ontology terms provided by the user presenting the biological process under inspection. Each cluster contained in the partitions are evaluated by IBH and a gene weight is calculated for each gene in the microarray data by using IBH values of the clusters that the gene belongs to. The clusters are then cleaned up by gene weights and a threshold. Finally, the cleaned clusters are combined by the cluster ensembles method using evidence accumulation strategy [20, 21].

We selected an example dataset, GDS36 yeast heat shock dataset, and showed the significance of each component of the method individually on this dataset. We presented results without contributon of Gene Ontology terms, without providing a real interaction network and without the cluster cleaning. The addition of each component have significant effects on the results as shown in Chapter 4.

Detailed results of CEC are given in Chapter 5. We presented results on another

yeast heat shock dataset, GDS1711 and on a popular yeast sporulation dataset. We analyzed two microarray datasets contained in the original GSEA study, *gender and p53 datasets*, by CEC method and applied GSEA on the resulting clusters. We showed that the resulting clusters are also enriched by the GSEA method.

Another challenge in mining microarray data is to combine heterogeneous datasets which contains different number of genes and different samples and measurement conditions. CEC method can also address combination of heterogeneous datasets. We provided an example application on the combination of seven heat shock datasets, showing that CEC method reveals the genes that play a common role among seven experiments.

The results given in Chapter 5 proves that application of CEC method reveals previously unknown biological information. As an example, in heat shock experiment analysis the resulting clustering reveals the heat shock relevance of genes that plays role in oxidative stress genes, hydrolase genes and general stress genes, although the input GO term provided to the algorithm does not contain information about them. The results of the analyses of CEC method are also supported by recent research as can be seen from the manual analysis of CEC results.

In all of the above experiments we used BIOGRID [45] interaction database as the information resource of the interaction network. To show that the method is not very dependent on the BIOGRID database, we used another popular interaction database, STRING [57], which contains known and predicted interactions. The results of CEC by using STRING database are similar to the previous results.

Several improvements can be made to the CEC method. The current work on interaction networks has a focus on weighted interaction networks [102, 103, 104]. Certain interaction networks such as STRING [57] provides scores for interactions. CEC method can be extended to make use of such weighted interaction networks and interaction scores. When the weighted interaction networks become more publicly available, Interaction Based Homogeneity measure and gene weight calculation may be adapted to use weighted interaction networks.

Interaction Based Homogeneity gives good results on protein complexes since is based

on pairwise interactions, but it does not perform well on signaling cascades. Another improvement on the method can be on increasing the performance of Interaction Based Homogeneity measure on signaling cascades. Interaction Based Homogeneity can be changed to take transitive interactions into consideration to solve this problem and increase its performance on signaling cascades.

Since the clustering part of the CEC method does not involve any special steps, the method can be generalized to analyze other *omics* data such as proteomics or metabolomics. The data can be clustered with different algorithms or parameters, and then the elements in the clusters can be eliminated and combined in the same way as analyzing microarray data.

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Appendix A

ibh SOFTWARE PACKAGE

ibh software package contains methods to calculate Interaction Based Homogeneity (IBH), a measure to evaluate the relationship of gene lists with respect to an interaction network. *ibh* package is developed in R and it is freely available through Bioconductor. In *ibh* package, researchers may make use of predefined interaction networks as well as their own interaction networks. In addition to using gene lists as input, methods in the package enable the user to directly employ the clustering results as input for the evaluation with IBH.

ibh software package is implemented in R and it is submitted to Bioconductor. *ibh* package contains easy to use functions. We also created an experimental data package called *simpIntLists*. *simpIntLists* package contains interaction networks which are generated from the latest version of BioGRID interactions. *simpIntLists* is used by functions of *ibh* package in which predefined interactions are used. *simpIntLists* contains interactions for seven organisms: Arabidopsis thaliana, Caenerhabditis elegans, Drosophila melanogaster, Homo sapiens, Mus musculus, Saccharomyces cerevisae, and Schizosaccharomyces pombe. Unique identifiers, official names or Entrez identifiers can be used as identifiers for each organism. When using the predefined interactions, the user should provide the name of the organism and type of the identifier.

ibh and *simpIntLists* packages require R programming language and they can be installed by entering the following commands from R command line.

```
> source("http://Bioconductor.org/biocLite.R")
> biocLite("ibh")
> biocLite("simpIntLists")
```

In the following sections, we give examples on how the methods in *ibh* package can be applied.

A.0.7 Loading the Package

In order to employ the methods implemented in *ibh* package, the following command should be entered in R command line:

```
> library(ibh)
```

This command will also load *simpIntLists* package that contains experimental data.

A.0.8 Interaction Based Homogeneity to Evaluate Gene Lists

Interaction Based Homogeneity can be calculated for multiple gene lists by providing an organism name and identifier type as shown in the example below.

```
> listofGeneList <- list(list("YJR151C", "YBL032W", "YAL040C",
+     "YBL072C", "YCL050C", "YCR009C"), list("YDR063W", "YDR074W",
+     "YDR080W", "YDR247W", "YGR183C", "YHL033C"), list("YOL068C",
+     "YOL015W", "YOL009C", "YOL004W", "YOR065W"))
> ibhForMultipleGeneListsPreDefined(listofGeneList,
+     organism = "yeast", idType = "UniqueId")

[1] 0.4722222 0.2222222 0.0400000
```

In this example, we evaluated the IBH for three lists that contain yeast genes. Gene names are unique identifiers and the predefined BioGRID interactions are used in evaluation. The IBH for gene list {*"YJR151C", "YBL032W", "YAL040C", "YBL072C", "YCL050C", "YCR009C"*} is 0.4722222, for gene list {*"YDR063W", "YDR074W", "YDR080W", "YDR247W", "YGR183C", "YHL033C"*} is 0.2222222 and for gene list {*"YOL068C", "YOL015W", "YOL009C", "YOL004W", "YOR065W"*} is 0.0400000. The first gene list is more homogeneous according to the interaction network than the other two gene lists.

A.0.9 Interaction Based Homogeneity to Evaluate Clustering Results

ibh package contains methods that can be used in clustering evaluation based on Interaction Based Homogeneity for each cluster. As an example, we show the evaluation of results of clustering on a sample data set, *yeastCC* which can also be downloaded from Bioconductor. *yeastCC* package contains an expression set of yeast cell cycle microarray experiment dataset[105]. We use the *kmeans* method implemented in *stats* package to cluster the data.

```
> require(yeastCC)
> require(stats)
> library(ibh)
> data(yeastCC)
> subset <- exprs(yeastCC)[1:50, ]
> d <- dist(subset, method = "euclidean")
> k <- kmeans(d, 3)
```

With the commands above, we clustered the data into 3 clusters. We can now evaluate the result of clustering as follows:

```
> ibhClusterEvalPredefined(k$cluster,
+     rownames(subset), organism = "yeast", idType = "UniqueId")
```

```
[1] 0.00925926 0.02378121 0.11111111
```

.

The result shown above contains Interaction Based Homogeneity for each of the three cluster based on predefined interactions. The results show that the third cluster is more homogeneous than the other clusters with respect to the predefined yeast interaction network.

A.0.10 Creating and Using Proprietary Interaction Lists

Users can also provide proprietary interaction lists for any type of organism and identifier. As an example, consider an organism with genes *A, B,C and D* and the interaction network as given in Figure 3. User can provide interactions in the network by filling a list:

```
> intList <- list(list(name="A", interactors=as.vector(c("B", "C"))),
  list(name="C", interactors=as.vector(c("A","D"))))
```

Interaction Based Homogeneity can then be calculated with respect to interaction network defined by *intList* as:

```
> listOfGeneList <- list(list("A","C"),list("C", "D"))
> ibhForMultipleGeneLists(intList, listOfGeneList)

[1] 0.50 0.25
```

Appendix B

DETAILED RESULTS ON ELIMINATION OF UNRELATED CLUSTERS BEFORE CLUSTERING COMBINATION

Table B.1: Analysis of the GDS36-heat shock time course dataset with elimination of unrelated clusters before clustering combination

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	HXT5	fructose transmembrane transporter activity glucose transmembrane transporter activity mannose transmembrane transporter activity	no	no
2	STU2	microtubule binding	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	FPR4	ubiquitin-protein ligase activity mitosis	no	no
4	MAE1	malic enzyme activity	no	no
5	YDL129W	Putative protein of unknown function	no	no
5	IZH1	metal ion binding	no	no
1	AVT6	transporter activity	no	no
1	PIR1	structural constituent of cell wall	no	no
1	UGA2	succinate-semialdehyde dehydrogenase	no	no
1	CSI1	cullin deneddylation	no	no
2	SPR28	structural molecule activity	no	no
2	YMR082C	unknown	no	no
2	SRM1	signal transducer activity	no	no
2	GPI17	GPI-anchor transamidase activity	no	no
2	GOT1	Golgi to endosome transport	no	no
3	ZEO1	response to stress	no	yes

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	DAS2	unknown	no	no
3	HIF1	histone binding	no	no
3	NET1	rDNA binding	no	no
3	POL5	rRNA transcription	no	no
3	POL5	rRNA transcription	no	no
4	YJR071W	unknown	no	no
4	NIP7	rRNA processing	no	no
4	KRE33	ribosomal small subunit biogenesis	no	no
4	URA7	CTP synthase activity	no	no
4	NUG1	rRNA processing	no	no
4	RRB1	ribosome biogenesis	no	no
4	RRP5	poly(U) RNA binding	no	no
4	SAM1	methionine adenosyltransferase activity	no	no
4	RRP5	poly(U) RNA binding	no	no
4	PPM2	tRNA methyltransferase activity	no	no
4	SDA1	ribosomal large subunit biogenesis	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
4	ILV1	L-threonine ammonia-lyase activity	no	no
4	SQT1	ribosomal large subunit assembly	no	no
4	IZH2	metal ion binding	no	no
4	ESF1	rRNA processing	no	no
4	LHP1	RNA binding	no	no
4	PPT1	protein serine/threonine phosphatase activity	no	no
4	PHO5	acid phosphatase activity	no	no
4	UTP10	snoRNA binding	no	no
4	MTR4	poly(A) RNA binding	no	no
4	MTR4	poly(A) RNA binding	no	no
4	SVS1	response to chemical stimulus	no	no
4	RPC40	contributes to DNA-directed RNA polymerase activity	no	no
4	ELP2	regulation of transcription from RNA polymerase II promoter	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
4	YDL050C	unknown	no	no
4	UTP20	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
4	TYW1	wybutosine biosynthetic process	no	no
4	NOP58	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
4	YBR238C	aerobic respiration	no	no
4	PHO3	acid phosphatase activity	no	no
4	RPA135	contributes to DNA-directed RNA polymerase activity	no	no
4	RNR1	nucleotide binding	no	no
4	UTP10	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
4	IMD4	IMP dehydrogenase activity	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
4	SNU13	RNA binding	no	no
4	YMR304CA	unknown	no	no
4	PRS3	contributes to ribose phosphate diphosphokinase activity	no	no
4	SAM2	methionine adenosyltransferase activity	no	no
5	YGR160W	unknown	no	no
5	ARX1	ribosomal large subunit biogenesis	no	no
5	RRP12	ribosome biogenesis	no	no
5	RPF1	rRNA primary transcript binding	no	no
5	ROK1	rRNA processing	no	no
5	UTP23	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	TRM1	tRNA methylation	no	no
5	YNL060C	unknown	no	no
5	PWP1	rRNA processing	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	NOC2	ribosome assembly	no	no
5	DBP3	rRNA processing	no	no
5	RPF2	rRNA binding	no	no
5	DBP9	rRNA processing	no	no
5	NOP4	rRNA processing	no	no
5	RRP1	rRNA processing	no	no
5	RRP8	rRNA processing	no	no
5	RLP7	rRNA binding	no	no
5	UTP11	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	EFG1	G1 phase of mitotic cell cycle	no	no
5	YCL053C	unknown	no	no
5	RLP24	unknown	no	no
5	YOR287C	unknown	no	no
5	PWP2	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	RRS1	ribosomal large subunit biogenesis	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	IPI3	rRNA processing	no	no
5	DBP7	rRNA processing	no	no
5	TRM2	tRNA modification	no	no
5	FAF1	maturation of SSU-rRNA from tricistronic rRNA transcript	no	no
5	YDL062W	unknown	no	no
5	PNO1	protein complex assembly	no	no
5	UTP5	positive regulation of transcription from RNA polymerase I promoter	no	no
5	DBP8	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	YCR056W	unknown	no	no
5	SSF1	rRNA binding	no	no
5	TRF5	histone mRNA catabolic process	no	no
5	YBR266C	endocytosis	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	YDR491C	unknown	no	no
5	PUF6	ribosomal large subunit biogenesis	no	no
5	CGR1	rRNA processing	no	no
5	YCLX02C	unknown	no	no
5	PUF6	ribosomal large subunit biogenesis	no	no
5	RPC34	tRNA transcription from RNA polymerase III promoter	no	no
5	ERG3	ergosterol biosynthetic process	no	no
5	YBL028C	unknown	no	no
5	RRP17	rRNA processing	no	no
5	YDR413C	unknown	no	no
5	RAS1	GTPase activity	no	no
5	YMC2	transmembrane transport	no	no
5	NSA2	ribosomal large subunit biogenesis	no	no
5	YLR003C	unknown	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	MPP10	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	YDR274C	unknown	no	no
5	IMP4	rRNA processing	no	no
5	MRT4	rRNA processing	no	no
5	NOC3	rRNA processing	no	no
5	NOP12	RNA binding	no	no
5	RRP15	maturation of 5.8S rRNA from tricistronic rRNA transcript	no	no
5	MPP10	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	RIO1	protein kinase activity	no	no
5	TMA16	unknown	no	no
5	YPL044C	unknown	no	no
5	NOP2	rRNA processing	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	YJR129C	S-adenosylmethionine-dependent methyltransferase activity	no	no
5	NSA1	ribosomal large subunit biogenesis	no	no
5	RRP6	histone mRNA catabolic process	no	no
5	YNL022C	tRNA (cytosine-5-)-methyltransferase activity	no	no
5	RPP1	rRNA processing	no	no
5	YNL114C	unknown	no	no
5	YLR400W	unknown	no	no
5	BUD21	endonucleolytic cleavage to generate mature 5'-end of SSU-rRNA	no	no
5	GCD14	tRNA methylation	no	no
5	BUD27	formation of translation preinitiation complex	no	no
5	TRM13	tRNA methylation	no	no
5	UTP30	ribosomal small subunit biogenesis	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	DUS3	tRNA modification	no	no
5	BUD22	ribosomal small subunit biogenesis	no	no
5	UTP6	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	GCD10	tRNA methylation	no	no
5	DIP2	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	YGR283C	S-adenosylmethionine-dependent methyltransferase activity	no	no
5	UTP18	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	LCP5	rRNA processing	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	UTP4	positive regulation of transcription from RNA polymerase I promoter	no	no
5	ATC1	response to stress	no	yes
5	NRP1	unknown	no	no
5	UTP13	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	UTP8	tRNA binding	no	no
5	AIR1	contributes to polynucleotide adenylyltransferase activity	no	no
5	YIL127C	unknown	no	no
5	FCF2	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	YPR142C	unknown	no	no
5	NOG1	rRNA processing	no	no
5	NSR1	rRNA processing	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	RSA4	ribosomal large subunit assembly	no	no
5	REI1	ribosomal large subunit biogenesis	no	no
5	NAF1	RNA binding	no	no
5	UTP21	rRNA processing	no	no
5	PWP2	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	RCL1	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	TRM11	RNA binding	no	no
5	ALB1	ribosomal large subunit biogenesis	no	no
5	NOG2	ribosomal large subunit export from nucleus	no	no
5	YOR146W	unknown	no	no
5	IMP3	rRNA processing	no	no
5	FAL1	ATP-dependent RNA helicase activity	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	FYV7	maturation of SSU-rRNA from tricistronic rRNA transcript	no	no
5	BFR2	rRNA processing	no	no
5	YIL091C	rRNA binding	no	no
5	PHO84	inorganic phosphate transmembrane transporter activity	no	no
5	YML018C	unknown	no	no
5	ARE1	ergosterol O-acyltransferase activity	no	no
5	TRM44	tRNA (uracil) methyltransferase activity	no	no
5	DBP6	rRNA processing	no	no
5	YIH1	regulation of cellular amino acid metabolic process	no	no
5	TRM5	tRNA (guanine) methyltransferase activity	no	no
5	SFL1	gene silencing (	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	AQR1	amino acid export	no	no
5	FRE1	iron ion transport	no	no
5	RTT10	endocytic recycling	no	no
5	YBR141C	S-adenosylmethionine-dependent methyltransferase activity	no	no
5	HMT1	mRNA export from nucleus	no	no
5	YGR079W	unknown	no	no
5	UTP15	snoRNA binding	no	no
5	NOC4	endonucleolytic cleavage in 5'-ETS of tricistronic rRNA transcript	no	no
5	UBP10	ubiquitin-specific protease activity	no	no
5	YPR136C	unknown	no	no
5	YPL068C	unknown	no	no
5	GIT1	transmembrane transport	no	no
5	YIL091C	ribosomal small subunit biogenesis	no	no

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	YDL151C	ascospore formation	no	no
5	FOB1	rDNA condensation	no	no
5	SWC7	chromatin remodeling	no	no
5	NIP1	translation initiation factor activity	no	no
5	YBL054W	regulation of transcription from RNA polymerase II promoter	no	no
5	YOR342C	unknown	no	no
5	DPH1	peptidyl-diphthamide biosynthetic process from peptidyl-histidine	no	no
5	HNM1	choline transport	no	no
6	RRN11	transcription initiation from RNA polymerase I promoter	no	no
6	YER130C	sequence-specific DNA binding	no	no
6	YDL063C	ribosomal large subunit biogenesis	no	no
6	YDR539W	cinnamic acid catabolic process	no	no
6	LCB5	response to heat	yes	yes

Continued on next page

Table B.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
6	YPR157W	unknown	no	no
6	TFP1	cellular protein metabolic process	no	no
6	YER0191C	unknown	no	no
6	CHA1	threonine catabolic process	no	no
6	FRE7	iron ion transport	no	no
6	GAL10	galactose catabolic process via UDP-galactose	no	no
7	HXT3	transmembrane transport	no	no
7	ECM34	fungal-type cell wall organization	no	no
7	YJL215C	unknown	no	no
7	SPG1	unknown	no	no
7	MDE1	methylthioribulose 1-phosphate dehydratase activity	no	no
7	NMT1	replicative cell aging	no	no
7	THI4	ferrous iron binding	no	no

Appendix C

DETAILED RESULTS ON CREATING INTERACTION SUBNETWORKS USING GENE ONTOLOGY

Table C.1: Analysis of the GDS36 time course dataset with creating interaction subnetworks using Gene Ontology.

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	AIM17	unknown	no	no
1	ALD3	aldehyde dehydrogenase (NAD) activity	no	no
1	ALD4	aldehyde dehydrogenase	no	no
1	AMS1	cellular carbohydrate metabolic process	no	no
1	BDH1	oxidation-reduction process	no	yes
1	CIT1	cellular carbohydrate metabolic process	no	yes [73]

Continued on next page

Table C.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	CWP1	ascospore-type prospore membrane assembly	no	yes [77]
1	CY3 0.1X	unknown	no	no
1	DCS2	hydrolase activity	no	yes [81]
1	DDR2	response to stress	no	yes
1	DNL4	replicative cell aging	no	no
1	DSE4	glucan endo-1,3-beta-D-glucosidase activity	no	no
1	ESC1	chromatin silencing at telomere	no	no
1	FAA2	long-chain fatty acid-CoA ligase activity	no	no
1	FMP16	unknown	no	no
1	FMP33	unknown	no	no
1	FTH1	high-affinity iron ion transport	no	no
1	GAD1	cellular response to oxidative stress	no	yes
1	GAL11	negative regulation of transcription from RNA polymerase II promoter	no	no

Continued on next page

Table C.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	GLK1	carbohydrate metabolic process	no	yes [73]
1	GPH1	glycogen catabolic process	no	no
1	GRE3	cellular response to oxidative stress, response to stress	no	yes
1	GSY2	glycogen biosynthetic process	no	no
1	GTO3	glutathione metabolic process	no	yes [80]
1	GYP5	vesicle-mediated transport	no	no
1	HOR7	response to stress	no	yes
1	HSP104	response to stress	no	yes
1	HSP12	cellular response to heat	yes	yes
1	HSP42	Small heat shock protein (sHSP) with chaperone activity, response to stress	no	yes
1	HXK1	carbohydrate metabolic process	no	yes [73]

Continued on next page

Table C.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	NCE103	cellular response to oxidative stress	no	yes
1	NIP100	microtubule binding	no	no
1	NORF 108	unknown	no	no
1	NORF 13	unknown	no	no
1	PGM2	carbohydrate metabolic process	no	yes [73]
1	PHR1	DNA repair, response to DNA damage stimulus	no	yes [74]
1	PNS1	unknown	no	no
1	PRB1	hydrolase activity	no	yes [73]
1	PYC1	pyruvate carboxylase activity	no	no
1	RTN2	Protein of unknown function	no	yes [78]
1	SDS24	cytokinetic cell separation	no	no
1	SOL4	carbohydrate metabolic process, hydrolase activity	no	yes [83, 76]
1	SPI1	response to acid	no	yes

Continued on next page

Table C.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	SSA4	Heat shock protein that is highly induced upon stress, response to stress	no	yes
1	SSE2	Member of the heat shock protein 70 (HSP70) family, response to stress	no	yes
1	TFS1	regulation of proteolysis	no	no
1	THI7	thiamine transmembrane transporter activity	no	no
1	TMA17	Protein of unknown function that associates with ribosomes	no	yes [75, 76]
1	TPK1	Ras protein signal transduction	no	yes [73]
1	TPS1	response to stress	no	yes
1	TPS3	response to stress	no	yes
1	TSL1	response to stress	no	yes
1	UGP1	glycogen biosynthetic process	no	yes [79]

Continued on next page

Table C.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	URK1	pyrimidine-containing compound salvage	no	no
1	YAR002AC	unknown	no	no
1	YCLX05C	unknown	no	no
1	YER067W	energy reserve metabolic process	no	no
1	YGP1	cell wall assembly	no	no
1	YHB1	response to stress	no	yes
1	YHR079BC	double-strand break repair	no	no
1	YJL217W	unknown	no	no
1	YKL202W	unknown	no	no
1	YLR111W	unknown	no	no
1	YNL195C	Putative protein of unknown function	no	no
1	YOL053C	response to stress	no	yes
1	YPL185W	unknown	no	no
1	YSC84	actin filament binding	no	no

Appendix D

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO GDS36 YEAST HEAT SHOCK DATASET

Table D.1: Number of clusters found in each partition for GDS36Dataset.

Algorithm	Number of Clusters	Total Number of Genes in Clusters
CAGED	7	5457
FLAME	1	11
NNN	1	5
STEM	50	1461
TAC	3	5457

Table D.2: Analysis of the GDS36-heat shock from 29°C to 33°C time course dataset with CEC method.

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	HSP104	Heat shock protein that refolds denatured, aggregated proteins, trehalose metabolism in response to heat stress,	yes	yes
Continued on next page				

Table D.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		response to stress		
1	SSE2	Member of the heat shock protein 70 (HSP70) family, response to stress	no	yes
1	SSA4	Heat shock protein that is highly induced upon stress, response to stress	no	yes
1	HSP42	Small heat shock protein (sHSP) with chaperone activity, response to stress	no	yes
1	TSL1	response to stress	no	yes
1	DDR2	response to stress	no	yes
1	TPS3	response to stress	no	yes
1	HOR7	response to stress	no	yes
1	GAD1	cellular response to oxidative stress	no	yes
1	CTT1	oxidation-reduction process, response to oxidative stress,	no	yes

Continued on next page

Table D.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		cellular response to water deprivation, response to stress		
1	GRE3	cellular response to oxidative stress, response to stress	no	yes
1	BDH1	oxidation-reduction process	no	yes
1	PRB1	sporulation resulting in formation of a cellular spore, hydrolase activity, cellular response to starvation,	no	yes [73]
1	PHR1	DNA repair, response to DNA damage stimulus	no	yes [74]
1	TMA17	Protein of unknown function that associates with ribosomes	no	yes [75, 76]
1	TPK1	Ras protein signal transduction	no	yes [73]
1	CIT1	cellular carbohydrate metabolic process	no	yes [73]

Continued on next page

Table D.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	PGM2	carbohydrate metabolic process	no	yes [73]
1	HXK1	carbohydrate metabolic process	no	yes [73]
1	SPI1	response to acid	no	yes
1	CWP1	ascospore-type prospore membrane assembly	no	yes [77]
1	RTN2	Protein of unknown function	no	yes [78]
1	UGP1	glycogen biosynthetic process	no	yes [79]
1	PNC1	hydrolase activity	no	yes [80]
1	DCS2	hydrolase activity	no	yes [81]
1	GLK1	carbohydrate metabolic process	no	yes [73]
1	TFS1	negative regulation of peptidase activity	no	yes [82]
1	SOL4	carbohydrate metabolic process, hydrolase activity	no	yes [83, 76]
1	GTO3	glutathione metabolic process	no	yes [80]

Continued on next page

Table D.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	YSC84	actin cortical patch localization	no	no
1	YNL195C	Putative protein of unknown function	no	no
1	FMP16	Putative protein of unknown function	no	no
1	YCL042W	Putative protein of unknown function	no	no
1	AMS1	cellular carbohydrate metabolic process, hydrolase activity, acting on glycosyl bonds	no	no

Appendix E

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO GDS36 YEAST HEAT SHOCK DATASET WITH A FULLY CONNECTED INTERACTION NETWORK

Table E.1: Analysis of the GDS36-heat shock time course dataset with CEC method and a fully connected interaction network.

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	NORF 78	unkown	no	no
1	NORF 17	unkown	no	no
1	RRN5	chromatin organization	no	yes [106]
1	RIM15	response to stress	no	yes
1	GCV2	glycine dehydrogenase (decarboxylating) activity	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	NCA3	mitochondrion organization	no	no
1	DLD3	lactate metabolic process	no	no
1	MMS22	double-strand break repair	no	yes [107]
1	YBR286W50	unknown	no	no
1	FAB1	endosome membrane	no	yes [107]
1	YML003W	unknown	no	no
1	MCM4	DNA replication origin binding	no	no
1	YPR127W	unknown	no	no
1	MEC1	protein kinase activity	no	no
1	YDR186C	unknown	no	no
1	RPH1	histone demethylation	no	no
1	TOM1	ubiquitin-protein ligase activity	no	yes [107]
1	GZF3	sequence-specific DNA binding	no	no
1	DRS2	phospholipid-translocating ATPase activity	no	yes [107]

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	GDH2	glutamate dehydrogenase (NAD+) activity	no	no
1	GCV1	glycine catabolic process	no	no
1	286W750	unknown	no	no
1	CIT2	citrate metabolic process	no	no
1	TOR2	protein binding	no	no
1	YNR040W	unknown	no	no
1	CHO2	phosphatidylcholine biosynthetic process	no	no
1	AIM23	unknown	no	no
1	286W700	unknown	no	no
1	TFC3	contributes to DNA binding, bending	no	yes [107]
1	YTA7	chromatin binding	no	yes [107]
1	YME1	ATP-dependent peptidase activity	no	yes [108]
1	SIN3	positive regulation of transcription from RNA polymerase II promoter in response to heat stress	no	yes

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	COG4	CVT pathway	no	no
1	YOR022C	phospholipase activity	no	no
1	RML2	structural constituent of ribosome	no	yes [107]
1	ISM1	isoleucine-tRNA ligase activity	no	yes [107]
1	GPB1	signal transducer activity	no	no
1	TCB3	lipid binding	no	no
1	TGL4	phospholipid metabolic process	no	no
1	LRO1	phospholipid:diacylglycerol acyltransferase activity	no	no
1	YAP1	response to heat	yes	yes
1	MHR1	cellular response to oxidative stress	no	yes
1	YAL004W	unknown	no	no
1	BNA3	kynurenic acid biosynthetic process	no	no
1	YAL004W	unknown	no	no
1	BNA3	kynurenic acid biosynthetic process	no	no
1	CHA4	cellular amino acid catabolic process	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	YHR009C	retrograde transport, endosome to Golgi	no	no
1	IRA1	Ras GTPase activator activity	no	yes [107]
1	YHR009C	retrograde transport, endosome to Golgi	no	no
1	SWI4	DNA binding	no	yes [107]
1	HSP12	cellular response to heat	yes	yes
1	PYC1	pyruvate carboxylase activity	no	no
2	MET1	sulfate assimilation	no	no
2	YOR356W	unknown	no	no
2	YEL020C	unknown	no	no
2	KAP104	nuclear localization sequence binding	no	yes [107]
2	TPD3	actin filament organization	no	no
2	YKL136W	unknown	no	no
2	MDV1	ubiquitin binding	no	no
2	BEM3	phosphatidylinositol-3-phosphate binding	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
2	KHA1	potassium:hydrogen antiporter activity	no	no
2	UBP5	protein deubiquitination	no	no
3	NORF 3	unknown	no	no
3	COX4	zinc ion binding	no	no
3	DAN1	sterol transport	no	no
3	YER135C	unknown	no	no
3	YHL037C	DNA binding	no	yes [109]
3	YFL052W	unknown	no	yes [109]
3	YER181C	unknown	no	no
3	YLR365W	unknown	no	no
3	YDL186W	unknown	no	no
3	WSC4	response to heat	yes	yes
3	YHL041W	unknown	no	no
3	YKL031W	unknown	no	no
3	YGL188C	unknown	no	no
3	YGL109W	unknown	no	no
3	YGR066C	unknown	no	no
3	FDH2	formate catabolic process	no	no
3	MMS4	DNA repair	no	no
3	YOR055W	unknown	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	TIF4632	stress granule assembly	no	yes
3	YGR053C	unknown	no	no
3	YLR296W	unknown	no	no
3	YGL118C	unknown	no	no
3	SPG4	unknown	no	no
3	YJR157W	unknown	no	no
3	SPO19	meiosis	no	no
3	YDR220C	unknown	no	no
3	QDR2	drug transmembrane transporter activity	no	no
3	YPT53	endocytosis	no	no
4	YOL053C	response to stress	no	yes
4	CY3 0.1X	unknown	no	no
5	HXT2	glucose transport	no	no
5	ECM22	sequence-specific DNA binding	no	no
5	HSP30	response to stress	no	yes
5	HIS4	histidinol dehydrogenase activity	no	no
5	YOR013W	mitotic recombination	no	no
5	MIP6	RNA binding	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	COX5A	mitochondrial electron transport, cytochrome c to oxygen	no	no
5	GSM1	DNA binding	no	no
5	YDR537C	unknown	no	no
5	YIG1	glycerol biosynthetic process	no	no
5	YML090W	unknown	no	no
5	PAC1	microtubule plus-end binding	no	no
5	ICS3	unknown	no	yes [108]
5	YER188W	unknown	no	no
5	ATP20	structural molecule activity	no	no
5	CTH1	mRNA binding	no	no
5	PAU11	unknown	no	no
5	WSC3	response to heat	yes	yes
6	RRN11	TBP-class protein binding	no	no
6	OCA5	unknown	no	yes [107]
6	YER130C	unknown	no	no
6	YDL063C	ribosomal large subunit biogenesis	no	no

Continued on next page

Table E.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
6	YDR539W	cinnamic acid catabolic process	no	no
6	YPR157W	unknown	no	no
6	TFP1	intron homing	no	yes [107]
6	YER0191C	unknown	no	no
6	FRE7	iron ion transport	no	no
6	GAL10	galactose catabolic process via UDP-galactose	no	no
6	LCB5	response to heat	yes	yes
6	CHA1	L-serine catabolic process	no	no

Appendix F

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO YEAST SPORULATION DATASET

Table F.1: Number of clusters found in each partition for yeast sporulation dataset.

Algorithm	Number of Clusters
CAGED	6
FLAME	13
GQL	5
NNN	18
STEM	8
TAC	7

Table F.2: Analysis of the Sporulation time course dataset with CEC method.

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
1	YBR268W	sporulation resulting in formation of a cellular spore	no	yes

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
1	YDL103C	UDP-N-acetylglucosamine biosynthetic process	no	no
1	YDL008W	mitosis cell division	no	no
1	YDL154W	reciprocal meiotic recombination	no	yes
1	YDR065W	vacuolar acidification	no	yes [90]
1	YDR523C	meiosis ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
1	YEL016C	nucleoside-triphosphate diphosphatase activity	no	no
1	YFL011W	transmembrane transport	no	no
1	YLR213C	ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
2	YER182W	Putative protein of unknown function(FMP10)	no	no

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
1	YGR229C	regulation of mitotic cell cycle cellular bud neck cell wall biogenesis	no	yes [91]
1	YLR227C	meiosis spindle pole body sporulation resulting in formation of a cellular spore	yes	yes
1	YOR033C	meiotic DNA double-strand break processing DNA repair	no	yes
1	YOR313C	meiosis protein whose expression is induced during sporulation sporulation resulting in formation of a cellular spore ascospore formation	yes	yes
1	YPL130W	meiosis-specific prospore protein, meiosis,	yes	yes

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
		sporulation resulting in formation of a cellular spore		
2	YER106W	meiotic chromosome segregation, meiosis, meiotic sister chromatid cohesion involved in meiosis I	no	yes
2	YFR032C	(RRT5)Putative protein of unknown function, regulation of transcription, DNA-dependent	no	yes [92]
2	YHR184W	meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore	yes	yes
2	YIL139C	mitosis	no	no
2	YIL159W	DNA repair	no	no

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
2	YKL042W	spindle pole body duplication in nuclear envelope	no	yes
2	YLR209C	transferase activity (PNP1)	no	no
2	YPL124W	spindle pole body duplication in nuclear envelope	no	no
2	YLR307W	ascospore wall assembly, chitosan layer of spore wall, sporulation resulting in formation of a cellular spore	yes	yes
2	YMR272C	fatty acid biosynthetic process electron transport chain	no	no
2	YOL091W	ascospore wall assembly, spindle pole body ,	yes	yes

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
		sporulation resulting in formation of a cellular spore		
2	YOR298W	ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
3	YDR218C	cellular bud neck, fungal-type cell wall organization, cell morphogenesis, cell division	no	no
3	YDR263C	response to DNA damage stimulus DNA repair	no	no
3	YDR317W	response to DNA damage stimulus DNA repair	no	no
3	YGL138C	Putative protein of unknown function	no	yes [110]
3	YHR124W	meiosis	no	yes
3	YLR343W	ascospore wall assembly	yes	yes
3	YNL128W	ascospore wall assembly	yes	yes

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
3	YMR125W	(STO1) mRNA capping, nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	no	yes [93]
3	YNL204C	sporulation resulting in formation of a cellular spore	yes	yes
3	YOL132W	ascospore wall assembly	yes	yes
4	YDL186W	Putative protein of unknown function	no	no
4	YDR273W	Meiosis-specific component of the spindle pole body, meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore, cell division	yes	yes

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
4	YPR027C	Putative protein of unknown function	no	no
4	YDR522C	meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore, ascospore formation	yes	yes
4	YGL170C	Component of the meiotic outer plaque of the spindle pole body, ascospore formation, sporulation resulting in formation of a cellular spore, spindle pole body	yes	yes
4	YHR185C	ascospore wall assembly, sporulation resulting in formation of a cellular spore, spindle pole body	yes	yes
5	YFR023W	(PES4) RNA binding	no	no

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
5	YEL061C	mitosis, spindle pole body separation	no	no
5	YGL015C	Putative protein of unknown function	no	no
5	YHR015W	(MIP6)mRNA export from nucleus	no	no
5	YJL038C	sporulation resulting in formation of a cellular spore	no	yes
5	YGR059W	ascospore wall assembly, sporulation resulting in formation of a cellular spore, ascospore wall, cellular bud neck	no	yes
5	YOL015W	Putative protein of unknown function	no	no
6	YAL040C	G1/S transition of mitotic cell cycle	no	no
6	YAL062W	oxidation-reduction process	no	no

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
6	YAR007C	reciprocal meiotic recombination, DNA repair, DNA replication	no	yes
6	YDR256C	oxidation-reduction process, age-dependent response to reactive oxygen species	no	no
6	YGL179C	transferase activity	no	no
6	YJR152W	(DAL5) dipeptide transporter activity, allantoate transmembrane transporter activity	no	no
6	YKR016W	cristae formation	no	no
6	YML008C	transferase activity	no	no
6	YNL098C	ascospore formation, signal transduction	yes	yes
6	YNL142W	ammonium transmembrane transport	no	no
6	YGL179C	regulation of translation	no	no

Continued on next page

Table F.2 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
6	YOR375C	oxidation-reduction process	no	no
7	YAL067C	transmembrane transport	no	no
7	YGR224W	transmembrane transport	no	no
7	YHR139C	meiosis, sporulation resulting in formation of a cellular spore, ascospore wall assembly	yes	yes
7	YJL074C	ascospore formation, mitosis	yes	yes
7	YPL208W	methyltransferase activity	no	no

Appendix G

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO YEAST SPORULATION DATASET WITH STRING DATABASE

Table G.1: Analysis of the Sporulation dataset with CEC method and STRING interaction database

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as playing role in sporulation
1	YDR263C	DNA repair	no	no
1	YDR317W	DNA repair	no	no
1	YDR218C	sexual sporulation resulting in formation of a cellular spore	no	yes
1	YGL015C	unknown	no	no
1	YHR124W	meiosis	no	yes
2	YBR268W	mitochondrial translation	no	no

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
2	YDL008W	ubiquitin-protein ligase activity	no	no
2	YDL103C	UDP-N-acetylglucosamine biosynthetic process	no	no
2	YDL154W	reciprocal meiotic recombination	no	yes
2	YFL011W	hexose transport	no	no
2	YLR227C	sporulation resulting in formation of a cellular spore	yes	yes
2	YOR313C	ascospore formation	yes	yes
2	YPL130W	meiosis	yes	yes
2	YCRX06W	unknown	no	no
2	YDR065W	vacuolar acidification	no	yes [90]
1	YLR213C	ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
1	YOR033C	meiotic DNA double-strand break processing DNA repair	no	yes

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	YEL016C	nucleoside-triphosphate diphosphatase activity	no	no
2	YDR523C	meiosis ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
2	YGR229C	regulation of mitotic cell cycle cellular bud neck cell wall biogenesis	no	yes [91]
3	YER106W	meiotic chromosome segregation, meiosis, meiotic sister chromatid cohesion involved in meiosis I	no	yes
3	YHR184W	meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore	yes	yes
2	YIL159W	DNA repair	no	no

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	YDR355C	unknown	no	no
3	YER182W	Putative protein of unknown function(FMP10)	no	no
3	YOR298W	ascospore wall assembly sporulation resulting in formation of a cellular spore	yes	yes
3	YFR032C	(RRT5)Putative protein of unknown function, regulation of transcription, DNA-dependent	no	yes [92]
3	YGR228W	unknown	no	no
3	YIL139C	mitosis	no	no
3	YKL042W	spindle pole body duplication in nuclear envelope	no	yes
3	YLR209C	transferase activity (PNP1)	no	no
3	YPL124W	spindle pole body duplication in nuclear envelope	no	no

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	YLR307W	ascospore wall assembly, chitosan layer of spore wall, sporulation resulting in formation of a cellular spore	yes	yes
3	YMR272C	fatty acid biosynthetic process electron transport chain	no	no
3	YOL091W	ascospore wall assembly, spindle pole body , sporulation resulting in formation of a cellular spore	yes	yes
4	YCRX07W	unknown	no	no
4	YDL186W	Putative protein of unknown function	no	no
4	YPR027C	Putative protein of unknown function	no	no

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
4	YHR185C	ascospore wall assembly, sporulation resulting in formation of a cellular spore, spindle pole body	yes	yes
4	YDR273W	Meiosis-specific component of the spindle pole body, meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore, cell division	yes	yes
4	YDR273W	Meiosis-specific component of the spindle pole body, meiosis, ascospore wall assembly,	yes	yes

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		sporulation resulting in formation of a cellular spore, cell division		
5	YFR023W	(PES4) RNA binding	no	no
5	YOL015W	Putative protein of unknown function	no	no
5	YEL061C	mitosis, spindle pole body separation	no	no
5	YDR522C	meiosis, ascospore wall assembly, sporulation resulting in formation of a cellular spore, ascospore formation	yes	yes
5	YGL170C	Component of the meiotic outer plaque of the spindle pole body, ascospore formation, sporulation resulting in formation of a cellular spore,	yes	yes

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		spindle pole body		
5	YHR015W	(MIP6)mRNA export from nucleus	no	no
5	YJL038C	sporulation resulting in formation of a cellular spore	no	yes
5	YGR059W	ascospore wall assembly, sporulation resulting in formation of a cellular spore, ascospore wall, cellular bud neck	no	yes
6	YLR343W	ascospore wall assembly	yes	yes
6	YMR125W	(STO1) mRNA capping, nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	no	yes [93]
6	YNL128W	ascospore wall assembly	yes	yes

Continued on next page

Table G.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
6	YNL204C	sporulation resulting in formation of a cellular spore	yes	yes
6	YOL132W	ascospore wall assembly	yes	yes
6	YGL138C	Putative protein of unknown function	no	yes [110]

Appendix H

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO GDS1711 YEAST HEAT SHOCK DATABASE

Table H.1: Analysis of the GDS1711 time course dataset with CEC method

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	SIP1	regulation of protein complex assembly signal transduction	no	yes [94]
1	YBL036C	pyridoxal phosphate binding	no	no
1	YNR048W	phospholipid-translocating ATPase activity	no	no
1	YOR352W	Putative protein of unknown function	no	no

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	TDH3	oxidation-reduction process, oxidoreductase activity, apoptosis	no	yes
1	YGR207C	electron transport chain	no	yes [95]
1	PRE7	hydrolase activity, proteasomal ubiquitin-independent protein catabolic process	no	yes [94]
1	PTH2	hydrolase activity, negative regulation of proteasomal ubiquitin-dependent protein catabolic process	no	no
1	YFR032C	Putative protein of unknown function Pregulation of transcription,	no	no
1	YPR196W	sequence-specific DNA binding transcription factor activity,	no	no

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		regulation of transcription, DNA-dependent		
2	PUS9	pseudouridine synthesis	no	no
2	ADD66	ER-associated protein catabolic process, proteasome assembly	no	no
2	DMA2	ubiquitin-protein ligase activity, mitosis	no	no
2	MRK1	response to stress	no	yes
2	ADE12	purine nucleotide biosynthetic process, DNA replication origin binding	no	no
2	AIM2	hydrolase activity	no	yes
2	HCH1	Heat shock protein regulator that binds to Hsp90p, response to stress	no	yes
2	HSC82	Cytoplasmic chaperone of the Hsp90 family, response to stress,	no	yes

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		telomere maintenance		
2	SGT2	response to heat	yes	yes
2	SSE1	response to stress	no	yes
2	MSN4	heat acclimation, response to stress, response to freezing, cellular response to oxidative stress	no	yes
2	PYC1	gluconeogenesis, NADPH regeneration, biotin carboxylase activity	no	no
2	STI1	Hsp90 cochaperone, response to stress, Hsp70 protein binding, Hsp90 protein binding	no	yes
3	ACT1	cellular response to oxidative stress	no	yes
3	NSE5	response to DNA damage stimulus, DNA repair	no	yes [94]

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
3	NUP84	mRNA export from nucleus in response to heat stress	yes	yes
3	TOK1	regulation of ion transmembrane transport, potassium ion transmembrane transport	no	yes [96]
3	YGL114W	transmembrane transport	no	no
3	YPL236C	transferase activity	no	no
3	YIR043C	unknown function, membrane	no	no
4	GYL1	regulation of exocytosis	no	no
4	LRE1	Protein involved in control of cell wall structure and stress response fungal-type cell wall organization	no	yes
4	TPM1	actin polymerization or depolymerization, exocytosis	no	yes

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
4	EPL1	response to DNA damage stimulus, DNA repair	no	no
4	UBA1	protein ubiquitination	no	no
4	VPS53	Golgi to vacuole transport	no	yes [94]
4	YOR356W	oxidoreductase activity, oxidation-reduction process, transport	no	yes
4	NGL1	hydrolase activity	no	yes [97]
4	PFA5	transferase activity	no	no
4	AVT2	amino acid transport, transporter activity	no	no
4	YHR113W	hydrolase activity	no	no
4	ASM4	transmembrane transport, mRNA export from nucleus in response to heat stress	yes	yes
4	EDC3	cytoplasmic mRNA processing body assembly	no	no

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
5	AEP1	regulation of translation	no	no
5	AIM38	Putative protein of unknown function	no	no
5	ATF2	transferase activity, response to toxin	no	no
5	MET22	response to stress, hydrolase activity	no	yes
6	AIM43	Protein of unknown function	no	no
6	BPT1	transmembrane transport, cadmium ion transmembrane transporter activity, bilirubin transmembrane transporter activity	no	yes [98]
6	DLT1	Protein of unknown function	no	no
6	DSL1	protein transport	no	no
6	ESC2	intra-S DNA damage checkpoint,	no	yes [94]

Continued on next page

Table H.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		double-strand break repair via homologous recombination		
6	EST1	transferase activity, telomere maintenance via telomerase, G-quadruplex DNA formation	no	no
6	GTB1	polysaccharide biosynthetic process	no	no
6	GTT3	Protein of unknown function may be involved in glutathione metabolism	no	no

Appendix I

DETAILED RESULTS OF APPLYING CLUSTER-ELIMINATE-COMBINE METHOD TO COMBINATION OF HETEROGENEOUS DATASETS PROBLEM

Table I.1: Result of applying CEC method to 7 different heat shock time-series datasets.

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	ARP4	DNA repair response to DNA damage stimulus	no	yes [99]
1	DAN1	response to stress	no	yes
1	DOG2	hydrolase activity	no	yes [100]
1	HOS3	hydrolase activity	no	no
1	LRE1	Protein involved in control of cell wall structure and stress response,	no	yes

Continued on next page

Table I.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
		fungal-type cell wall organization		
1	PSR2	response to stress hydrolase activity	no	yes
1	SGA1	hydrolase activity	no	yes
1	STE20	cellular response to heat	yes	yes
1	ASM4	mRNA export from nucleus in response to heat stress	yes	yes
1	HSP104	cellular heat acclimation, response to stress, unfolded protein binding, chaperone binding	yes	yes
1	NUP100	mRNA export from nucleus in response to heat stress	yes	yes
1	NUP84	mRNA export from nucleus in response to heat stress	yes	yes
1	SGT2	response to heat	yes	yes

Continued on next page

Table I.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	AIM45	flavin adenine dinucleotide binding, transport	no	yes [95]
1	APL1	protein transport	no	no
1	ATF2	transferase activity, response to toxin	no	no
1	BPT1	ATP binding, transmembrane transport	no	yes [98]
1	CFT2	mRNA polyadenylation, mRNA cleavage	no	no
1	GGA1	intracellular protein transport	no	no
1	MDJ1	protein folding, response to heat response to stress	yes	yes
1	MOT2	protein polyubiquitination	no	no
1	PFK26	ATP binding, transferase activity	no	yes [73]
1	SSH4	protein transport	no	no

Continued on next page

Table I.1 – continued from previous page

Cluster	Gene	Description	Contained in the input of selected GO terms	Known as responsive to stress
1	THI72	transmembrane transport	no	no
1	TOK1	regulation of ion transmembrane transport, potassium ion transmembrane transport	no	yes [96]
1	ULA1	ATP binding	no	no
1	VTI1	protein transport	no	no
1	YCK3	ATP binding, transferase activity	no	no
1	YPL236C	ATP binding, transferase activity	no	no
1	YGL114W	transmembrane transport	no	no
1	YHL005C	not found in GO database	no	no
1	YJR141W	Essential protein of unknown function	no	no
1	YLR177W	Putative protein of unknown function	no	no
1	YLR345W	not found in GO database	no	no

VITA

PERSONAL

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EDUCATION

Doctorate of Philosophy (2006- 2012)	Middle East Technical University , Computer Engineering Dep. CGPA : 3.57
Master of Science (1999-2002)	Middle East Technical University , Computer Engineering Dep. CGPA : 3.71
Bachelor of Science (1994-1999)	Middle East Technical University, Computer Engineering Dep. CGPA : 3.64 High Honor Student

SKILLS

Specialties	Project Management	Expert, >5 years experience
	Application Security	Expert, >5 years experience
	Bioinformatics	Expert, >5 years experience
	Image processing	Expert, >5 years experience
	Machine Learning	Expert, >5 years experience
	Application Development	Expert, >10 years experience
Foreign Languages	English	KPDS 2011, Grade 97.5
Programming Languages	Java	Expert, >10 years experience
	C	Expert, >10 years experience
	C++	Expert, >10 years experience
	Delphi	Expert, >10 years experience
	COBOL	Expert, >10 years experience
	PHP	Good, >5 years experience
	ASP	Good, >5 years experience
	R	Good, >3 years experience
Operating Systems	OS/400	Expert, >10 years experience
	Windows	Expert, >10 years experience, administrator of 5 windows servers at CBT
	Linux	Expert, >5 years experience, administrator of 4 servers at CBT

	DB/400	Expert, >10 years experience
DBMS	DB2	Good, >5 years experience
	Derby	Expert, >5 years experience
	UML	Expert, >5 years experience
	Spring	Expert, >5 years experience
	Hibernate	Expert, >5 years experience
	Web services	Expert, >5 years experience
	XML	Expert, >5 years experience
Technologies	WSS4J	Expert, >5 years experience
	Spring Web Services	Expert, >3 years experience
	Junit	Expert, >5 years experience
	Drools	Expert, >3 years experience
	Struts	Good, >3 years experience
	E-signature	Good, >3 years experience
	JSP	Good, >3 years experience
Application	Tomcat	Expert, >5 years experience
Servers	Websphere Application Server	Good, >3 years experience
Project	Compuware Changepoint	Expert, >5 years experience
Management	IBM Rational products	Expert, >5 years experience
Tools	MsProject	Expert, >5 years experience

EMPLOYMENT

1. Central Bank of Turkey (CBT, TCMB)

(July 1999 – Now)

I am working in CBT as the project manager of the *Banking Group* which is responsible for developing banking applications for the branches of CBT. Some projects that I worked on are summarized in the following sections.

1.1. Public Electronic Payment System (PEPS-KEÖS (Kamu Elektronik Ödeme Sistemi))

Public Electronic Payment System is one of the biggest e-government projects in Turkey. It is designed to safely and efficiently perform government expenditures such as provision of social benefits, salaries, pensions, travel and miscellaneous expenses incurred by government employees on behalf of the government. It is a successful implementation of the Treasury Single Account. By the use of PEPS, Turkish cash management was developed from simple to active cash management and the Turkey's note on cash management was raised from 1.2 to 3.6, which made the Turkish applications better than most developed countries.

PEPS is a joint project of CBT, Treasury and Ministry of Finance. I was responsible from the technical coordination between the organizations and I worked on the analysis, design and coding of the project. I also worked in the regulatory phases of the project by participating to the working group that wrote the protocol which was signed between three organizations, prepared the changes in the related law and wrote the legal regulations of the system.

1.2. Treasury Internet Banking System

The system enables Treasury to perform real-time inquiries about the balance and movements of its accounts. The infrastructure that accepts e-signed payment orders and performs the appropriate payments automatically is developed and in the testing phase now. The project was developed by Java, Spring Web Services, Spring(Struts, Hibernate, JSP) and COBOL. I worked both in the technical and regulatory phases of the project. As well as

actively developing the project, I contributed to the protocol that was signed between CBT and Treasury.

1.3. Telephone Banking System

The Telephone Banking System serves the accountants of CBT since 2001. It was developed by Delphi, COBOL and Java. The system makes use of special hardware called voice boards to make use of telephone lines automatically.

1.4. Internet Banking System

The system is a web service based application developed for banks to perform real-time inquiries about the balance and movements of their accounts.

1.5. TIC-ESTS(EMKT) Branch Interface

Turkish Interbank Clearing–Electronic Security Transfer and Settlement System (*TIC-ESTS*) works in an integrated manner with the TIC-RTGS (EFT) to electronically transfer and settle Turkish government securities with "delivery *versus* payment" (DVP) principle. I worked on the CBT Branch interface of TIC-ESTS to enable CBT branches send, receive and process messages of TIC-ESTS. The application was developed in Cobol and run in AS/400.

1.6. Message Transfer System

The system provides modules for applications to define, send and receive messages between branches. By the message transfer system, applications can send their messages to other AS/400 systems by just writing to a file and calling a module. The modules of the system encapsulates all of the APPC programming and error handling mechanisms. The system was developed in Cobol.

1.7. Application Security Group

The group worked on the possible attacks to applications and methods for preventing them. We created reports that can be used as an internal reference for all of the software developers in CBT for secure application development.

1.8. Cheque Clearing System

I first worked on the group that developed the cheque clearing system interface of CBT in Cobol and Delphi. Then, for the new version of Cheque Clearing System, instead of

using Biztalk client internally, we created a Java based a web service client to communicate with Microsoft Biztalk.

1.9. Central Bank of Northern Cyprus (KKTCMB) Cheque Clearing System

Worked on the KKTCMB interface of the Cheque Clearing System which was developed by Java and COBOL. I detected critical security defects of the general Cheque Clearing System of the country and reported them, which postponed the production date of the system. The report is then used as a checklist and after all of the found defects are cleared out second examination was requested. After our second examination, the cheque clearing system was taken into production.

1.10. CBT Software Development Methodology Group

I worked in the methodology group whose aim is to determine and standardize an application development methodology for CBT. We examined different methodologies and decided to tailor Extreme Programming to our organization. The methodology is now actively used by software development groups.

1.11. Microcomputer Environment Standardization Project

I worked in the group that aims to standardize the microcomputer environment. We reported the problems in the environment that are mostly caused by the use of different programming languages and configurations. The findings of the group is used as a basis for a standardized microcomputer environment.

1.12. Informatics Crime Law Draft

Worked in the group that created the critics of CBT for Informatic Crimes Law Draft.

1.13. Year 2000 project

I worked on resolving the year 2000 problem of various applications of CBT.

1.14. YTL project

I worked on the TL-YTL conversion and the removal of 6 zeros from the TL projects in order to adapt the branch applications to the changes needed by the conversions.

1.15. Accounting Archive System

The system generates reports from past accounting reports that are taken to the archive. The reports are usually requested by the Government Accounting Bureau. In order to create the archive, we processed and organized cartridges from all of the 21 branches of CBT for the past 10 years. The system is developed by using Jasper Reports and Java.

1.16. CBT Branches Consolidation Project

As a part of the consolidation project that aims to centralize the branch applications that were previously developed in a distributed manner, the applications used by CBT branches' banking services are re-developed by using agile methodologies and Spring infrastructure. I worked as the project manager of the development of the following systems:

1.16.1. Money Transfer System between Branches

The branches can send and receive money orders by this system.

1.16.2. Treasury Exportation Funds Payment System

The exporters and exportation funds that they receive from treasury are followed by this system. The system is integrated by money order system, RTGS (EFT) and the accounting system.

1.16.3. Treasury R&D Funds Payment System

The firms that receive R&D funds from fairs are followed and their payments are made by this system. The system is integrated by money order system, RTGS (EFT) and the accounting system.

1.16.4. Accounting Offices Payments System

Accounting offices sends payment orders to our branches and the orders are processed by this system and sent to RTGS (EFT).

1.16.5. Branch Expense System

It is a basic module that are used by other branch applications. If an expense should be taken from an accounting process, it is taken automatically by using predefined rules by expense system. The system uses Drools to process the rules on the fly.

1.16.6. Electronic Banking System

The telephone banking system and its maintenance applications (for creating passwords, adding/removing accounts to system, etc.) are redeveloped in Java.

2. The Scientific and Technological Research Council of Turkey (TUBITAK)

(November 1997 – June 1999)

I worked as a part-time technical researcher at TUBITAK on Form Archiving Prototype System (FORMAR) and Constitutional Court Automation System. The FORMAR project is selected as the best graduation project in 1999 in the research and development area in computer engineering department.

3. PhD Thesis

In my PhD, I worked on data integration and bioinformatics. I started with the short time series microarray data analysis, and then generalized the method to combine microarray data, interaction networks and Gene Ontology. We created the ***ibh*** package, which is accepted to Bioconductor, which computes the fitness of a given gene list to an interaction network. The ibh package is available from http://www.bioconductor.org/packages/release/bioc/html/ibh.html.

4. Master's Thesis

In the master's thesis, I worked on creating an OCR for the Turkish language. I used image processing techniques for preprocessing such as skew correction, noise removal and segmentation; and applied neural network to create a classifier for Turkish characters. We combined it with a Turkish post-processor and achieved very successful results that are far better than commercially available tools at that time.

PUBLICATIONS

1. Kırçiçeği KORKMAZ, Rengül ÇETİN-ATALAY, Volkan ATALAY, ***Results of the combination of multiple partitions for the analysis of short time series microarray data***, European Conference on Computational Biology (ECCB), 2008 (Poster presentation)

2. Kırçiçeği KORKMAZ, Rengül ÇETİN-ATALAY, Volkan ATALAY, ***Benefits of Incorporating Biological Knowledge in Clustering Short Time Series Data*** , International Symposium on Health Informatics and Bioinformatics (HIBIT), 2008 (Poster presentation)

3. Sait Ulas KORKMAZ, G. Kircicegi Y.AKINCI, Volkan ATALAY, ***A Character Recognizer For Turkish Language***, ICDAR 2003 : 1238-1241.

4. G.Kircicegi AKINCI, Sait Ulas KORKMAZ, Fatos Yarman VURAL, ***Karmasik Renkli Dokumanlarda Sayfa Cozumlemesi,*** Sinyal Isleme veUygulamalari Kurultayi, pp 114-120 Ankara 1999.