Identification of essential and functionally modulated genes through the microarray assay

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Identification of essential genes is one of the ultimate goals of drug designs. Here we introduce an in silico method to select essential genes through the microarray assay. We construct a graph of genes, called the gene transcription network, based on the Pearson correlation coefficient of the microarray expression level. Links are connected between genes following the order of the pair-wise correlation coefficients. We find that there exist two meaningful fractions of links connected, $p_m$ and $p_s$, where the number of clusters becomes maximum and the connectivity distribution follows a power law, respectively. Interestingly, one of clusters at $p_m$ contains a high density of essential genes having almost the same functionality. Thus the deletion of all genes belonging to that cluster can lead to lethal inviable mutant efficiently. Such an essential cluster can be identified in a self-organized way. Once we measure the connectivity of each gene at $p_s$. Then using the property that the essential genes are likely to have more connectivity, we can identify the essential cluster by finding the one having the largest mean connectivity per gene at $p_m$. 
Thousands of genes and their products in a given living organism is believed to function in a concerted way that creates the mystery of life [1]. Such cooperative functions between genes can be visualized through a graph where nodes denote genes and links do activating or repressing effects on transcription [2, 3]. Traditional methods in molecular biology are very limited to analyze such large-scale interactions among thousands of genes, so that a global picture of gene functions is hard to obtain. The recent advent of the microarray assay has enough attraction to researcher, allowing them to decipher gene interactions in a more efficient way [4]. While the data through the microarray assay are not sufficiently accumulated yet and they are also susceptible to errors in detecting the expression level, the microarray assay is an potential candidate for a fundamental approach to understand large-scale gene complexes and can be used in many applications such as drug design and toxicological research.

Since the microarray technology is having a significant impact on genomics study, many methods for pattern interpretation have been developed, including the K-means clustering [5], the self-organizing map [6], the hierarchical method [7], the relevance network method [8], etc. All such methods, however, contain tunable thresholds, so that the results obtained through those methods could be misled by the thresholds artificially chosen. While those methods are useful for clustering genes, they cannot give any information needed to identify essential genes. Here the essential genes mean target genes for drug designs, because the deletion of them leads to inviable mutant to the organism.

In this paper, we propose a novel in silico method to identify essential genes from microarray database. Our method is inspired by the combination of the gene clustering and the close relationship between the lethality or essentiality of genes and the connectivity in a network. Once genes are clustered by using a graph theory and the cluster containing a high density of essential genes is identified by using the relationship between the lethality and the connectivity of the graph [9]. The main difference from the previous work [9] lies in that while the previous method mainly deals with genes individually, our method does rather with clusters of genes modulated by their functionality, which turns out to be much more efficient in selecting essential genes. In our method, we do not use any threshold artificially. Thus the essential genes can be identified in a self-organized way. Moreover we find that the genes belonging to the same cluster are modulated in their functions. Since the essential genes we select belong to the same cluster, we can select the essential genes from almost the same functional module. Finally we
propose the functions of unknown genes in the yeast protein database classification as the major function of the known genes belonging to each cluster.

**Basic Concepts**

The method is inspired from the two concepts: (i) the percolation clustering modulated by their functions and (ii) the relationship between the essentiality and the inhomogeneous connectivity distribution in biochemical networks. First the percolation concept \[ p_c \] is well known to physicists and has been applied to many systems including the composite system of metals and insulators exhibiting the transition as the metal concentration \( p \) changes. When \( p \) is small enough, there are many small size clusters of metal and no giant cluster spanning from one end to the other, leading the system to be in an insulator phase. As \( p \) increases, the number of clusters increases. There exists a critical value \( p_c \) called the percolation threshold above which small-size clusters are connected together and a giant cluster forms, spanning the entire system. Then the system turns into a metal state.

Next recently there are many studies for complex systems in terms of graph. In graph theory, a graph is composed of nodes and links. Degree of a certain node is the number of links connected to that node. The emergence of a power law in the degree distribution,

\[
P(k) \sim k^{-\gamma},
\]

in complex networks has recently attracted many attentions \[11, 12\]. The network following such a power-law degree distribution is called scale-free network. The scale-free networks are ubiquitous in nature such as social, biological, information systems, etc. For example, for the protein interaction network where nodes represent proteins and links do their interactions, the degree distribution follows a power law \[13, 14\]. Such a behavior implies there exist a few hub proteins having a large number of connections compared with other proteins. Recently it was shown that such hub proteins are more likely to be essential \[9\]. For the yeast protein interaction network, the probability of the proteins with the first 0.7% ranks is as high as 62%. Thus it was proposed that the selection of essential proteins can be made by finding highly connected proteins.

**Microarray data**

We apply those concepts to the microarray data downloaded from Ref. \[15\] containing 287 single gene deletion \( S. \text{cerevisiae} \) mutant strains. The deletion data elucidate generic relationships among perturbed transcriptomes \[16\].
The data contain two large, internally consistent, global mRNA expression subsets of the yeast *S. cerevisiae*. One of them provides steady state mRNA expression data in wild-type *S. cerevisiae* sampled 63 separate times (the ‘control’ set), and the other provides individual measurements on the genomic expression program of 287 single gene deletion mutant *S. cerevisiae* strains grown under identical cell culture conditions as wide-type yeast cells (the ‘perturbation’ set). Each of the microarray data is the ratio between the expression levels of wild-type and perturbed one. Thus the data can be written in terms of a $N \times M$ matrix denoted as $C$, where $N = 6316$ and $M = 287$, representing the total number of genes and different-deletion experiments, respectively. Each element $c_{i,j}$ of the matrix $C$ means the logarithmic value (base 10) of the ratio of the expression levels for the $i$-th gene under the $j$-th perturbation condition [$17$].

**Percolation clustering**

To obtain the correlations among the transcription genes, we compare each pair of the expression levels from different genes. For each pair, we first select the list of genes of which the expression levels are known in both transcriptomes. Next the Pearson correlation coefficient $\rho_{i,j}$ between $i$ and $j$ genes is calculated, defined as

$$\rho_{i,j} \equiv \frac{\langle c_{i,k}c_{j,k} \rangle - \langle c_{i,k} \rangle \langle c_{j,k} \rangle}{\sqrt{(\langle c_{i,k}^2 \rangle - \langle c_{i,k} \rangle^2)(\langle c_{j,k}^2 \rangle - \langle c_{j,k} \rangle^2)}}, \tag{2}$$

where $\langle \cdots \rangle$ means average over $k$, different-deletion experiments. As shown in Fig.1, the distribution of the correlations $\{\rho_{i,j}\}$ is of a bell shape, ranged between -1 and 1. Based on the Pearson’s coefficients, we generate a network by connecting genes whose the Pearson’s coefficient is larger than a parameter $\rho$. That is, the link between nodes $i$ and $j$ is connected if $\rho_{i,j} > \rho$. The parameter $\rho$ will be determined later in a self-organized way. Each link is assumed to have a unit weight. Let $p$ mean the fraction of connected links among $N(N-1)/2$ possible pairs. Then $p$ depends on $\rho$. When $p$ is close to zero ($\rho$ is close to 1), the number of links is small, and most nodes remain as isolated nodes or form small-size clusters. As $p$ increases ($\rho$ decreases), the size of each cluster grows or the number of clusters $N(p)$ including at least two genes increases. At a certain value of $p$, denoted as $p_m$, the number of clusters becomes maximum shown in Fig.2, which is different from the percolation threshold $p_c$. $p_m$ is estimated to be $p_m \approx 0.0002$. Beyond $p_m$, the number of clusters decreases, however, the mean size of each cluster increases.

**Scale-free network**
As $p$ increases, the mean size of each cluster increases. While the giant cluster forms at $p_c$, the degree distribution of the giant cluster does not follow a power law. The critical state where the degree distribution follows a power law can be reached at a higher fraction $p_s \approx 0.0063$ in Fig.3. Note that the degree distribution needs an exponential cutoff in the tail part, which is a generic behavior due to finite number of genes. The degree exponent is estimated to be $\gamma \approx 0.9$, which is close to the values obtained by others in different systems [18, 19], but smaller than typical values occurring in many other systems in the range of $2 < \gamma \leq 3$. For $p > p_s$, the connectivity distribution does not follow the power law.

To understand the biological implication of the scale-free network, we investigate the relationship between the degree of a certain gene and its essentiality. In Fig.4, we plot the fraction of the essential genes with degree larger than $k_{\text{min}}$. Up to $k_{\text{min}} \approx 250$, the genes with a larger number of connectivity is more likely to be essential, but for $k_{\text{min}} > 250$, this tendency does not hold any more. Even for the case of $k_{\text{min}} < 250$, the fraction of the essentiality is not larger than 40%, less than the rate of 62% in the protein interaction network. Thus as a whole, the way of identifying essential genes from the information of the connectivity of the gene transcription network alone is not good enough.

**Method**

To improve the success rate of identifying essential genes through the microarray assay, here we introduce a new method as follows. First links are connected between a pair of genes $\{i, j\}$ one by one in descending order of $\rho_{i,j}$. Whenever a link is connected, we measure the number of clusters $N(p)$ including at least two genes as a function of $p$. Second we identify $p_m$ where the number of clusters becomes maximum. Third we find the critical fraction $p_s$ where the connectivity distribution follows a power law and measure the connectivity of each gene $k_i(p_s)$. Finally keeping the information of the degree of each gene $k_i$ at $p_s$, we return to the gene transcription network at $p_m$. For each cluster $J$, we calculate the average connectivity per node, that is,

$$\langle k^J \rangle = \frac{\sum_{i \in J} k_i^J(p_s)}{N^J(p_m)},$$

where $N^J(p_m)$ is the number of genes belonging to a cluster $J$. Based on the fact that genes with a larger number of connectivity are more likely to be essential, we think that the cluster with the largest value of $\langle k^J \rangle$ is the most likely to contain essential genes.
Essential cluster
To confirm this idea, we directly measure the essentiality $E_J$, that is the fraction of known essential genes among the genes belonging to a given cluster $J$. Indeed, as shown in Fig. 5, the two quantities, $\langle k_J \rangle$ and $E_J$, behave in the same manner. Thus we can select the cluster containing the largest fraction of essential genes by finding the cluster with the largest $\langle k_J \rangle$. We find that for the yeast data, the third largest cluster with 64 genes turns out to have the largest value of $\langle k_J \rangle$, containing 47 essential genes, 17 nonessential genes, and 1 unidentified genes (Fig. 6). Thus the certainty of selecting essential genes is remarkable improved as high as 73% or even higher when the unidentified gene is excluded, much larger than the one obtained only through the connectivity distribution in the gene transcription network.

Functional clustering
It is known that many biochemical networks are composed of modular structure according to their functional role. For the yeast, it is known that there are 43 categories by their functions [15]. We classify genes into 43 categories for each cluster at $p_m$. Fig. 7 shows the ratio of genes belonging to each functional category for the first five largest clusters. Fig. 8 also shows the functional module structure in the gene transcription network. From those figures, one can find that there exist major functions for each cluster, implying that the genes belonging to the same cluster are likely to have the same function. For example, the majority of the genes in the largest cluster belong to the functional class of amino-acid metabolism. Those of the second, third and fourth largest cluster are of small molecule transport, RNA processing/modification, and protein synthesis, respectively. The reason of such functional clustering in the gene transcription network lies in that the genes having the same function are likely to respond to external perturbation in the same manner, making the Pearson correlation coefficients between them large. Our result is consistent with the recent discovery of revealing modular organization in the yeast transcription network [20] and in the metabolic networks [21]. Next by using the fact of the gene clustering by their functional module, we assign function candidate of unknown functional annotation as the major one of the genes belonging to the same cluster, which are listed in Table 1.

Conclusion and discussion
By using the facts that (i) the genes with the same function are highly correlated in the expression level of the microarray and (ii) the essential genes are likely to have a larger number of connectivity in the large-scale gene tran-
scription network, we have proposed an *in silico* method to identify a cluster containing a high density of essential genes. Since the selected genes are from the same cluster, they are likely to be of the same function. These essential and functionally moduled genes will be useful for drug designs. Note that since our method does not include any tuning parameter, it has no ambiguity to identify the essential cluster in contrast to previous other methods used in gene clustering, where some ambiguity is included. Finally our work is similar in idea to a recent one that the microarray-driven gene expression can be studied much efficiently in parallel to the functional analysis of many gene products [22].

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Figure Legends

Fig. 1: The distribution of the correlation functions

The distribution of the Pearson correlation coefficients $\rho_{i,j}$ for the yeast *S. cerevisiae*.

Fig. 2: The number of clusters

Plot of the number of clusters as a function of the fraction $p$ of connected links.

Fig. 3: The connectivity distribution of the gene transcription network

Plot of the connectivity distribution of the gene transcription network at various fractions of link connections, $p = 0.0003$ (□), $p = 0.0016$ (○), $p = 0.0063 \approx p_s$ (●), and $p = 0.0032$ (▽). At $p_s$, the degree distribution follows a power law with an exponential cutoff. Dotted line having a slope -0.9 is drawn for the eye.

Fig. 4: The fraction of the essentiality

Plot of the fraction of the essentiality of nodes having degree larger than $k_{\text{min}}$ as a function of $k_{\text{min}}$

Fig. 5: The identification of the essential cluster

The comparison between $\langle k^J \rangle$ (●) and $E^J$ (□) for each cluster indexed by cluster size at $p_m$.

Fig. 6: The gene transcription network colored by their essentiality

The gene transcription network at $p_m$ of the yeast *S. cerevisiae*. The green, white and yellow nodes represent essential, nonessential, and unidentified genes, respectively.

Fig. 7: The functional genes ratio for each cluster
The genes ratio belonging to each functional category for the first five largest clusters.

**Fig. 8: The gene transcription network colored by their functions.**

The gene transcription network at $p_m$ of the yeast *S. cerevisiae*. The genes with the functions, amino-acid metabolism, small molecule transport, RNA processing/modification, protein synthesis are distinguished by the different colors, red, blue, green, and brown, respectively. The white and the yellow represent other functions and unknown function, respectively.
Table Legend

Table 1: Function candidates for unknown genes

Assigned functions for unknown genes by following the major function of the genes of each cluster at $p_m$. 
Figure 1: Rho et al.
Figure 2: Rho et al.
Figure 3: Rho et al.
Figure 4: Rho et al.
Figure 5: Rho et al.
Figure 6: Rho et al.
Figure 7: Rho et al.
Figure 8: Rho et al.
### Table 1: Rho et al.

| Amino-acid metabolism or Other metabolism - 52 genes |
|-----------------------------------------------------|
| YAL014C | YBR046C | YBR047W | YBR147W | YBR261C | YCL028W | YCL044C |
| YCR051W | YDL054C | YDR425W | YDR426C | YER152C | YER175C | YFL010C |
| YFL028C | YGL117W | YGL224C | YHR029C | YHR122W | YHR162W | YIL041W |
| YIL056W | YIL164C | YIL165C | YJL072C | YJL200C | YJL213W | YJR111C |
| YJR130C | YJR154W | YLR152C | YLR193C | YLR267W | YLR290C | YLR539C |
| YML113W | YMR097C | YMR321C | YNL129W | YNL276C | YNL311C | YNR069C |
| YOL118C | YOR042W | YOR044W | YOR203W | YPL135W | YPL251W | YPL264C |
| YPR059C | YPR114W | YKL033W-A |

| Small molecule transport - 48 genes |
|------------------------------------|
| YAL065C | YBR301W | YCR104W | YDR542W | YEL049W | YER188W | YFL020C |
| YGL261C | YGR150C | YGR169C | YGR294W | YHL046C | YHR049W | YIL176C |
| YIR041W | YJL218W | YJL223C | YKL005C | YKL224C | YLL025W | YLL056C |
| YLL064C | YLR037C | YLR091W | YLR269C | YLR461W | YMR020W | YMR107W |
| YLM252C | YMR253C | YNL285W | YNL310C | YNR014W | YNR076W | YOL161C |
| YOR134W | YOR205C | YOR286W | YOR389W | YOR394W | YPL107W | YPL277C |
| YPL282C | YPR053C | YAL068C | YHR049C-A | YMR316C-B | YMR325W |

| RNA Processing/modification - 31 genes |
|---------------------------------------|
| YBL028C | YCL059C | YDL063C | YDL148C | YDR101C | YDR152W | YDR165W |
| YDR324C | YDR361C | YDR496C | YER126C | YGR128C | YGR145W | YHR052W |
| YHR085W | YHR196W | YHR197W | YKR060W | YKR081C | YLR068W | YLR129W |
| YML093W | YNL002C | YNL182C | YNL207W | YNR053C | YOR004W | YOL077C |
| YOR145C | YPR112W | YPL146C |

| Protein Synthesis - 5 genes |
|----------------------------|
| YGL102C | YJL188C | YLR062C | YPL142C | YPR044C |

| Carbohydrate metabolism or Cell stress - 17 genes |
|--------------------------------------------------|
| YBR053C | YDL110C | YDL204W | YDR032C | YER067W | YIL136W | YIL70C |
| YJR161W | YLR149C | YLR270W | YML128C | YMR110C | YNL115C | YNL200C |
| YOL082W | YPL123C | YMR169C |

| Energy generation - 7 genes |
|-----------------------------|
| YGL069C | YKL169C | YKL195W | YMR158W | YPR099C | YPR100W | YKL053C-A |

| Chromatin/chromosome structure - 3 genes |
|------------------------------------------|
| YBL113C | YFL068W | YML133C |