Journal of Bioinformatics and Computational Biology

COMBINING MICROARRAYS AND BIOLOGICAL KNOWLEDGE FOR ESTIMATING GENE NETWORKS VIA BAYESIAN NETWORKS

SEIYA IMOTO[1], TOMOYUKI HIGUCHI[2], TAKAO GOTO[1],
KOUSUKE TASHIRO[3], SATORU KUHARA[3], SATORU MIYANO[1]

[1] *Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108-8639, Japan*
[2] *The Institute of Statistical Mathematics, 4-6-7, Minami-Azabu, Minato-ku, Tokyo, 106-8569, Japan*
[3] *Graduate School of Genetic Resources Technology, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka, 812-8581, Japan*
imoto@ims.u-tokyo.ac.jp
higuchi@ism.ac.jp
takao@ims.u-tokyo.ac.jp
ktashiro@grt.kyushu-u.ac.jp
kuhara@grt.kyushu-u.ac.jp
miyano@ims.u-tokyo.ac.jp

Received 19 August 2003
Revised 21 November 2003
Accepted 7 December 2003

We propose a statistical method for estimating a gene network based on Bayesian networks from microarray gene expression data together with biological knowledge including protein-protein interactions, protein-DNA interactions, binding site information, existing literature and so on. Microarray data do not contain enough information for constructing gene networks accurately in many cases. Our method adds biological knowledge to the estimation method of gene networks under a Bayesian statistical framework, and also controls the trade-off between microarray information and biological knowledge automatically. We conduct Monte Carlo simulations to show the effectiveness of the proposed method. We analyze *Saccharomyces cerevisiae* gene expression data as an application.

Keywords: Gene network; Bayesian network; biological knowledge; microarray data.

1. Introduction

In recent years, a large amount of gene expression data has been collected. Estimating a gene network that shows regulatory relationships between genes has become one of the central topics in the field of bioinformatics. Several methodologies have been proposed for constructing a gene network based on gene expression data, such as Boolean networks,[1,2,16,37,49] differential equation models[7,11,12,37] and Bayesian networks.[14,15,19,20,22,24,25,42] The main drawback for the gene network construction from microarray data is that while the gene network contains a large number of genes, the information contained in gene expression data is limited by the number of microarrays, their quality, the experimental design, noise, and measurement errors. Therefore, estimated gene networks contain some incorrect gene regulations, which cannot be evaluated from a biological viewpoint. In particular, it is difficult to determine the direction of gene regulation using gene expression data only. Hence, the use of biological knowledge, including protein-protein and protein-DNA interactions,[3,5,18,23,27] sequences of the binding site of the genes controlled by transcription regulators,[36,45,55] literature and so on, are considered to be a key for microarray data analysis. The use of biological knowledge has previously received considerable attention for extracting more information from microarray data.[4,6,20,38,41,43,46,47,48]

In this paper, we provide a general framework for combining microarray data and biological knowledge aimed at estimating a gene network by using a Bayesian network model. If the gene regulation mechanisms are completely known, we can model the gene network easily. However, many parts of the true gene network are still unknown and need to be estimated from data. Hence, it is necessary to construct a suitable criterion for evaluating estimated gene networks in order to obtain an optimal network. While criteria, such as BDe[8] and MDL,[14,51] proposed previously for evaluating a Bayesian network model, only measure the closeness between a model and microarray data, we derive a criterion for selecting networks based on microarray data and biological knowledge. The proposed criterion consists of two components: One shows the fitness of the model to the microarray data, while the other reflects biological knowledge, which is modeled under a probabilistic framework. Our proposed method automatically tunes the balance between the biological knowledge and microarray data based on our criterion and estimates a gene network from the combined data.

In Section 2.1, we describe our statistical model for constructing gene networks and introduce a criterion for evaluating networks in Section 2.2. A statistical framework for representing biological knowledge is described in Section 2.3. In Section 2.4, we illustrate how to model various types of biological knowledge in practice. Monte Carlo simulations, in Section 3.1, are conducted to show the effectiveness of the proposed method. We apply our method to *Saccharomyces cerevisiae* gene expression data in Section 3.2.

2. Method for Estimating Gene Networks

2.1. *Bayesian network and nonparametric heteroscedastic regression model*

Bayesian networks[28] are a type of graphical models for capturing complex relationships among a large amount of random variables by the directed acyclic graph encoding the Markov assumption. In the context of Bayesian networks, a gene corresponds to a random variable shown as a node, while gene regulations are shown by directed edges. Thus gene interactions are modeled by the conditional distribution of each gene. We use Bayesian network and nonparametric heteroscedastic regression models[25] for constructing gene networks from microarray data.

Suppose that we have n sets of microarrays $\{\boldsymbol{x}_1, ..., \boldsymbol{x}_n\}$ of p genes, where $\boldsymbol{x}_i = (x_{i1}, ..., x_{ip})^T$ is a p dimensional gene expression vector obtained by ith microarray. Here, x_{ij} is an expression value of jth gene, denoted by gene$_j$, measured by ith microarray after required normalizations and transformation.[44] For cDNA microarray data, x_{ij} is given by $\log_2(R_{ij}/G_{ij})$, where R_{ij} and G_{ij} are normalized intensities of Cy5 and Cy3 for gene$_j$ measured by ith microarray. The interaction between gene$_j$ and its parents is modeled by the nonparametric additive regression model[21] with heterogeneous error variances

$$x_{ij} = m_{j1}(p_{i1}^{(j)}) + \cdots + m_{jq_j}(p_{iq_j}^{(j)}) + \varepsilon_{ij},$$

where $p_{ik}^{(j)}$ is the expression value of kth parent of gene$_j$ measured by ith microarray and ε_{ij} depends independently and normally on mean 0 and variance σ_{ij}^2. Here, $m_{jk}(\cdot)$ is a smooth function constructed by B-splines[10,13,26] of the form

$$m_{jk}(p_{ik}^{(j)}) = \sum_{l=1}^{M_{jk}} \gamma_{lk}^{(j)} b_{lk}^{(j)}(p_{ik}^{(j)}),$$

where $\{b_{1k}^{(j)}(\cdot), ..., b_{M_{jk},k}^{(j)}(\cdot)\}$ is a prescribed set of B-splines and $\gamma_{mk}^{(j)}$ are parameters. Below we use 20 B-splines of degree 3, that is $M_{jk} = 20$. Hence, a Bayesian network and nonparametric heteroscedastic regression model can be represented as

$$f(\boldsymbol{x}_i|\boldsymbol{\theta}_G) = \prod_{j=1}^{p} f_j(x_{ij}|\boldsymbol{p}_{ij}, \boldsymbol{\theta}_j)$$

for $i = 1, ..., n$, where $\boldsymbol{\theta}_G$ is a parameter vector and $f_j(x_{ij}|\boldsymbol{p}_{ij}, \boldsymbol{\theta}_j)$ is a density of Gaussian distribution with mean $m_{j1}(p_{i1}^{(j)}) + \cdots + m_{jq_j}(p_{iq_j}^{(j)})$ and variance σ_{ij}^2. If gene$_j$ has no parent genes, we use μ_j and σ_j^2 instead of $m_{j1}(p_{i1}^{(j)}) + \cdots + m_{jq_j}(p_{iq_j}^{(j)})$ and σ_{ij}^2, respectively.

This model has several advantages. Unlike Boolean networks and discrete Bayesian networks,[14,15,19,20,22,42] no discretization of gene expression data, which leads to information loss, is required. Second, even nonlinear relationships between genes are automatically extracted based on gene expression data.

2.2. *Criterion for evaluating networks*

Some gene networks are partially known, but many mechanisms of gene regulations are still unknown. Therefore we need to estimate unknown structures of the gene network from the data. Hence, the construction of a suitable criterion for measuring the closeness between an estimated gene network and the true one is an essential problem for statistical gene network modeling. Following the result of Imoto *et al.*[25], a criterion for evaluating an estimated gene network can be derived from Bayes approach. At first, we briefly introduce the derivation of their criterion. We then explain how to extend their criterion to combine microarray data and biological knowledge.

When we construct a gene network G by using a Bayesian network model, the posterior probability of the network is obtained as the product of prior probability of the network $\pi(G)$ and the marginal likelihood divided by the normalizing constant. After dropping the normalizing constant, the posterior probability of the network is proportional to

$$\pi(G)\int\prod_{i=1}^{n}f(\boldsymbol{x}_i|\boldsymbol{\theta}_G)\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda})d\boldsymbol{\theta}_G,$$

where $\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda})$ is a prior distribution on the parameter vector $\boldsymbol{\theta}_G$ with hyperparameter vector $\boldsymbol{\lambda}$ satisfying $\log\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda}) = O(n)$. The essential problem for constructing a criterion based on the posterior probability of the network is how to compute the marginal likelihood given by a high dimensional integral. Imoto *et al.*[25] used the Laplace approximation for integrals[9,35,53] and derived a criterion, named BNRC$_{hetero}$ (Bayesian network and Nonparametric heteroscedastic Regression Criterion), of the form

$$\mathrm{BNRC}_{hetero}(G) = -2\log\pi(G) + \log\left|\frac{n}{2\pi}J_\lambda(\hat{\boldsymbol{\theta}}_G)\right| - 2nl_\lambda(\hat{\boldsymbol{\theta}}_G|\boldsymbol{X}),$$

where

$$l_\lambda(\boldsymbol{\theta}_G|\boldsymbol{X}) = \frac{1}{n}\sum_{i=1}^{n}\log f(\boldsymbol{x}_i|\boldsymbol{\theta}_G) + \frac{1}{n}\log\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda}),$$

$$J_\lambda(\boldsymbol{\theta}_G) = -\frac{\partial^2\{l_\lambda(\boldsymbol{\theta}_G|\boldsymbol{X})\}}{\partial\boldsymbol{\theta}_G\partial\boldsymbol{\theta}_G^T}$$

and $\hat{\boldsymbol{\theta}}_G$ is the mode of $l_\lambda(\boldsymbol{\theta}_G|\boldsymbol{X})$.

Suppose that the prior distribution $\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda})$ is factorized as

$$\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda}) = \prod_{j,k}\pi_{jk}(\boldsymbol{\gamma}_{jk}|\lambda_{jk}),$$

where $\boldsymbol{\gamma}_{jk} = (\gamma_{1k}^{(j)},...,\gamma_{M_{jk},k}^{(j)})^T$ is a parameter vector and λ_{jk} is a hyperparameter. We use a singular M_{jk} variate normal distribution as the prior distribution on $\boldsymbol{\gamma}_{jk}$,

$$\pi_{jk}(\boldsymbol{\gamma}_{jk}|\lambda_{jk}) = \left(\frac{2\pi}{n\lambda_{jk}}\right)^{-(M_{jk}-2)/2}|K_{jk}|_+^{1/2}\exp\left(-\frac{n\lambda_{jk}}{2}\boldsymbol{\gamma}_{jk}^TK_{jk}\boldsymbol{\gamma}_{jk}\right),$$

where K_{jk} is an $M_{jk} \times M_{jk}$ symmetric positive semidefinite matrix satisfying $\boldsymbol{\gamma}_{jk}^T K_{jk} \boldsymbol{\gamma}_{jk} = \sum_{\alpha=3}^{M_{jk}} (\gamma_{\alpha k}^{(j)} - 2\gamma_{\alpha-1,k}^{(j)} + \gamma_{\alpha-2,k}^{(j)})^2$. This prior is related to the smoothness of the fitted B-splines by tuning the hyperparameter λ_{jk}. If we choose λ_{jk} large, the fitted B-spline reduces to a linear function. On the other hand, when we use a small λ_{jk}, the B-spline overfits the data. Therefore the choice of the value of the hyperparameters is an essential problem for computing the criterion. We optimize them by minimizing the proposed criterion, BNRC_{hetero}.

By using the prior distrubution $\pi(\boldsymbol{\theta}_G|\boldsymbol{\lambda})$ defined above, we then have the decomposition

$$\mathrm{BNRC}_{hetero} = -2\log\pi(G) + \sum_{j=1}^{p} \mathrm{BNRC}_{hetero}^{(j)}.$$

Here $\mathrm{BNRC}_{hetero}^{(j)}$ is a score for gene_j and given by

$$\begin{aligned}\mathrm{BNRC}_{hetero}^{(j)} = &-(\sum_{k=1}^{q_j} M_{jk} + 1)\log(\frac{2\pi}{n}) - \sum_{i=1}^{n}\log w_{ij} + n\log(2\pi\hat{\sigma}_j^2) + n \\ &+ \sum_{k=1}^{q_j}\{\log|\Lambda_{jk}| - M_{jk}\log(n\hat{\sigma}_j^2)\} - \log(2\hat{\sigma}_j^2) - \log|K_{jk}|_+ \\ &+ \sum_{k=1}^{q_j}\{(M_{jk}-2)\log\left(\frac{2\pi\hat{\sigma}_j^2}{n\beta_{jk}}\right) + \frac{n\beta_{jk}}{\hat{\sigma}_j^2}\hat{\boldsymbol{\gamma}}_{jk}^T K_{jk}\hat{\boldsymbol{\gamma}}_{jk}\},\end{aligned}$$

where $w_{ij},\ i = 1, ..., n$ are weights of the heterogeneous error variance $\sigma_{ij}^2 = w_{ij}^{-1}\sigma_j^2$ and $\Lambda_j = B_{jk}^T W_j B_{jk} + n\beta_{jk}K_{jk}$ with $B_{jk} = (\boldsymbol{b}_{jk}(p_{1k}^{(j)}), ..., \boldsymbol{b}_{jk}(p_{nk}^{(j)}))^T$, $\boldsymbol{b}_{jk}(p_{ik}^{(j)}) = (b_{1k}^{(j)}(p_{ik}^{(j)}), ..., b_{M_{jk},k}^{(j)}(p_{ik}^{(j)}))^T$, $W_j = \mathrm{diag}(w_{1j}, ..., w_{nj})$ and $\beta_{jk} = \sigma_j^2\lambda_{jk}$. The details of the parameter estimation are described in Imoto *et al.*[25].

2.3. *Adding biological knowledge*

The criterion $\mathrm{BNRC}_{hetero}(G)$, introduced in the previous section, contains two quantities: the prior probability $\pi(G)$ of the network, and the marginal likelihood of the data. The marginal likelihood shows the fitness of the model to the microarray data. The biological knowledge can then be added into the prior probability of the network $\pi(G)$.

Let U_{ij} be the interaction energy of the edge from gene_i to gene_j and let U_{ij} be categorized into I values, $H_1, ..., H_I$, based on biological knowledge. For example, if we know *a priori* that gene_i regulates gene_j, we set $U_{ij} = H_1$. However, if we do not know whether gene_k regulates gene_l or not, we set $U_{kl} = H_2$. We treat the prior information of each edge independently. Note that $0 < H_1 < H_2$, it is more natural to choose the network with a large number of H_1 edges rather than H_2 edges in the sense of prior information. Our setting, $H_1 < H_2$, gives a higher prior probability to the graph with a lot of H_1 edges than to the graph with a lot of H_2 edges.

The total energy of the network G can then be defined as

$$E(G) = \sum_{\{i,j\} \in G} U_{ij},$$

where the sum is taken over the existing edges in the network G. Under the Bayesian network framework, the total energy can be decomposed into the sum of the local energies

$$E(G) = \sum_{j=1}^{p} \sum_{i \in L_j} U_{ij} = \sum_{j=1}^{p} E_j, \tag{1}$$

where L_j is an index set of parents of gene$_j$ and $E_j = \sum_{i \in L_j} U_{ij}$ is a local energy defined by gene$_j$ and its parents. Fig. 2.3 shows an example of a gene network and its energy.

The probability of a network G, $\pi(G)$, is modeled by the Gibbs distribution[17]

$$\pi(G) = Z^{-1} \exp\{-\zeta E(G)\}, \tag{2}$$

where ζ (> 0) is a hyperparameter and Z is a normalizing constant called the partition function

$$Z = \sum_{G \in \mathcal{G}} \exp\{-\zeta E(G)\}.$$

Here $\mathcal{G}$ is the set of possible networks. By replacing $\zeta H_1, ..., \zeta H_I$ with $\zeta_1, ..., \zeta_I$, respectively, the normalizing constant Z is a function of $\zeta_1, ..., \zeta_I$. We call ζ_j an

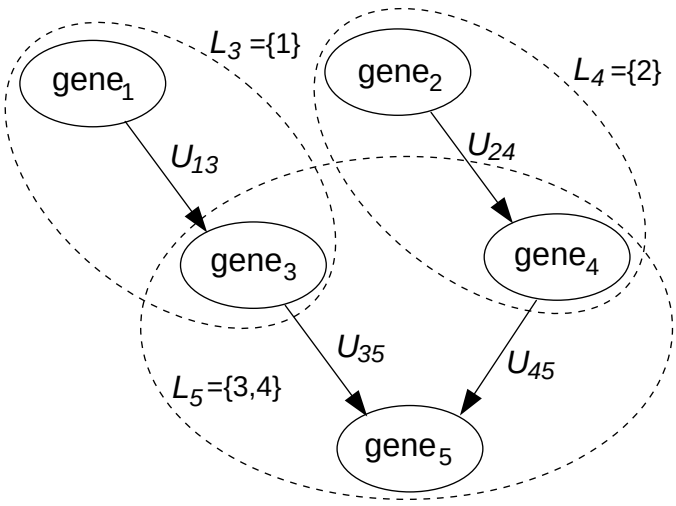


Fig. 1. A gene network and its energy. The index sets L_3, L_4 and L_5 are illustrated and L_1 and L_2 are defined by empty sets. The local energies are $E_3 = U_{13}$, $E_4 = U_{24}$ and $E_5 = U_{35} + U_{45}$. The total energy of this network is $E = E_3 + E_4 + E_5 = U_{13} + U_{24} + U_{35} + U_{45}$.

inverse normalized temperature. By substituting (1) into (2), we have

$$\pi(G) = Z^{-1} \prod_{j=1}^{p} \exp\{-\zeta E_j\} = Z^{-1} \prod_{j=1}^{p} \prod_{i \in L_j} \exp(-\zeta_{\alpha(i,j)}),$$

with $\alpha(i,j) = k$ for $U_{ij} = H_k$. Hence, by adding biological knowledge into the prior probability of the network, BNRC_{hetero} can be rewritten as

$$\mathrm{BNRC}_{hetero}(G, \zeta_1, ..., \zeta_I) = 2 \log Z + \sum_{j=1}^{p} \{2 \sum_{i \in L_j} \zeta_{\alpha(i,j)} + \mathrm{BNRC}^{(j)}_{hetero}\}. \quad (3)$$

We can choose an optimal network under the given $\zeta_1, ..., \zeta_I$. Also the optimal values of $\zeta_1, ..., \zeta_I$ are obtained as the minimizer of (3). Therefore, we can represent an algorithm for estimating a gene network from microarray data and biological knowledge as follows:

Step1: Set the values $\zeta_1, ..., \zeta_I$.

Step2: Estimate a gene network by minimizing $\mathrm{BNRC}_{hetero}(G)$ under the given $\zeta_1, ..., \zeta_I$.

Step3: Repeat Step1 and Step2 against the candidate values of $\zeta_1, ..., \zeta_I$.

Step4: An optimal gene network is obtained from the candidate networks obtained in Step3.

In Step2, we use the greedy hill-climbing algorithm for learning networks as follows:

Step1: Start from the empty graph.

Step2: For each gene, either add, remove, or reverse an edge, if it leads to a reduction in the criterion.

Step3: Repeat Step 2 until the value of the criterion reaches a minimum.

The details of the greedy hill-climbing algorithm and the computation of the criterion are shown in Imoto *et al.*[25]. Note that the proposed prior probability of the network can be used for other types of Bayesian network models, such as discrete Bayesian networks and dynamic Bayesian networks.[32,33,39,41,50]

The computation of normalizing constant, Z, is intractable even for moderately sized gene networks. To avoid this problem, we compute upper and lower bounds of the partial function and use them to choose the optimal value of inverse normalized temperature. An upper bound is obtained by directed graphs that are allowed to contain cyclic graphs. The number of graphs that has $b_1, b_2, ..., b_I$ edges

of $\zeta_1, \zeta_2, ..., \zeta_I$ out of $a_1, a_2, ..., a_I$ edges, respectively, is obtained by

$$S(b_1, ..., b_I) = \prod_{i=1}^{I} \frac{a_i}{b_i!(a_i - b_i)!}.$$

The upper bound of Z is then

$$\sum_{b_1,...,b_I} S(b_1, ..., b_I) \exp(-\sum_{i=1}^{I} b_i \zeta_i).$$

Thus the true value of the partition function is not greater than the upper bound. A lower bound is computed by multi-level directed graphs with following assumptions: (A1) There is one top gene and (A2) Genes at the same level have a common direct parent gene. We also consider joined graphs of some multi-level directed graphs satisfying (A1) and (A2). Since the number of possible graphs is much larger than those included in the computation, the true value of the partition function should be greater than the lower bound. Since the optimization of the network structure for fixed $\zeta_1, ..., \zeta_I$ does not depend on the value of the partition function, our method works well in practice. Of course, when the number of genes is small, we can perform an exhaustive search and compute the partition function completely. However, we think that the development of an effective algorithm to enumerate all possible networks or approximate the partition function is an important problem.

As a related work, Segal *et al.*[47] proposed an interesting method for combining protein-protein interaction data with microarray gene expression data. They modeled protein-protein interaction data based on Markov networks[34] and considered the joint probability of microarray data and protein-protein data for estimating molecular pathways. Although our model is different from their model, their model contains a hyperparameter, denoted by α, that plays quite similar role of ζ_1 and ζ_2. Similar to our criterion, their joint probability contains the normalizing constant, which is a function of the hyperparameter, α. While we optimize the hyperparameters by our criterion, they did not compute the normalizing constant and chose the value of α heuristically.

2.4. *Prior design for various biological knowledge*

In this subsection, we show some examples of biological knowledge and how to include them into the prior probability in practice. We consider using two values ζ_1 and ζ_2 satisfying $0 < \zeta_1 < \zeta_2$ for representing biological knowledge. Basically, we allocate ζ_1 to a known relationship and ζ_2 otherwise. The prior information can be summarized as a $p \times p$ matrix U whose (i, j) element, u_{ij}, corresponds to ζ_1 or ζ_2. Note that not all prior knowledge can be easily interpreted as binary values.

Protein-protein interactions

The number of known protein-protein interactions is rapidly increasing and stored in some public databases such as GRID[18] and BIND.[3,5] Protein-protein interactions show at least two proteins that form a complex. Therefore, representing protein-protein interactions by a directed graph is not suitable. However, they can be included in our method. If we know gene$_i$ and gene$_j$ create a protein-protein interaction, we set $u_{ij} = u_{ji} = \zeta_1$. In such a case, we will decide whether we make a virtual node corresponding to a protein complex theoretically.[40]

Protein-DNA interactions

Protein-DNA interactions show gene regulation by transcription factors and can be modeled more easily than protein-protein interactions. When gene$_i$ is a transcription regulator and controls gene$_j$, we set $u_{ij} = \zeta_1$ and $u_{ji} = \zeta_2$.

Sequences

Genes that are controlled by a transcription regulator might have a consensus motif in their promoter DNA sequences. If gene$_{j_1}$,...,gene$_{j_n}$ have a consensus motif and are controlled by gene$_i$, we set $u_{ij_1} = \cdots = u_{ij_n} = \zeta_1$ and $u_{j_1 i} = \cdots = u_{j_n i} = \zeta_2$. Previously, we used the information of consensus motifs to evaluate estimated gene networks from a biological viewpoint. This information, however, can be introduced directly into our method. One straightforward way is the use of known regulatory motifs kept in public databases such as SCPD[45] and YTF.[55] As for an advanced method, Tamada *et al.*[52] proposed a method for simultaneously estimating a gene network and detecting regulatory motifs based on our method, and succeeded in estimating an accurate gene network and detecting a true regulatory motif.

Gene networks and pathways

The information of gene networks can be introduced directly into our method by transforming the prescribed network structures into the matrix U. We can then estimate a gene network based on U and microarray data. Our method can also use gene networks estimated by other techniques such as boolean networks, differential equation models, and so on. Also, some databases, such as KEGG,[30,31] contain several known gene networks and pathways. This information can be used similarly.

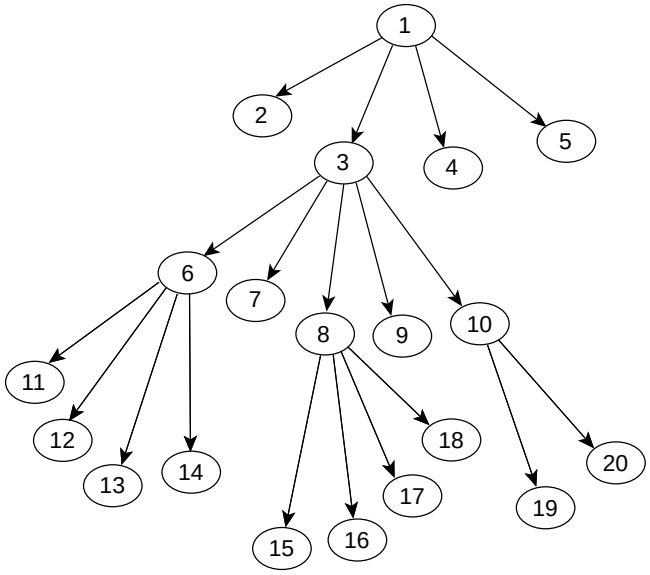
Literature

Some research has been performed to extract information from a huge amount of literature.[29] Literature contain various kinds of information including biological knowledge described above. So we can model literature information in the same way.

3. Computational Experiments

3.1. *Monte Carlo simulations*

Before analyzing real gene expression data, we perform Monte Carlo simulations to examine the properties of the proposed method. We assume an artificial network with 20 nodes shown in Fig. 2 (a). The functional relationships between nodes are



(a)

$$g_1 = \varepsilon_1, \quad g_2 = .7g_1 + \varepsilon_2 \quad g_5 = .7g_1 + \varepsilon_5, \quad g_{10} = 1/\{1 + \exp(-4g_3)\} + \varepsilon_{10}$$

$$g_3 = \begin{cases} -1 + \varepsilon_3 \ (g_1 \le -.5) \\ g_1 + \varepsilon_3 \ (|g_1| < .5) \\ 1 + \varepsilon_3 \ (g_1 \ge .5) \end{cases} \qquad g_6 = \begin{cases} .8g_3 + \varepsilon_6 \ (g_3 \le -1) \\ (g_3 + 1)^{1.5} + \varepsilon_6 \ (-1 < |g_3| < 0) \\ 1 + \varepsilon_6 \ (g_3 \ge 1) \end{cases}$$

$$g_4 = \begin{cases} .4g_1 + 1 + \varepsilon_4 \ (|g_1| \le .3) \\ (g_1 + 1)^2 + \varepsilon_4 \ (|g_1| < .3) \end{cases} \quad g_8 = \begin{cases} .2g_3 - 1 + \varepsilon_8 \ (g_3 \le .2) \\ 1.4g_3 + \varepsilon_8 \ (g_3 > .2) \end{cases}$$

$$g_{11} = .7g_6 + \varepsilon_{11}, \ g_{14} = .7g_6 + \varepsilon_{14}, \ g_{15} = 1/\{1 + \exp(-4g_8)\} + \varepsilon_{15}$$

$$g_9 = \begin{cases} .4g_3 + 1 + \varepsilon_9 \ (|g_3| \le .3) \\ (g_3 + 1)^{1.2} + \varepsilon_9 \ (|g_3| < .3) \end{cases} \quad g_{13} = \begin{cases} .4g_6 + 1 + \varepsilon_{13} \ (|g_6| \le .3) \\ (g_6 + 1)^2 + \varepsilon_{13} \ (|g_6| < .3) \end{cases}$$

$$g_{12} = \begin{cases} -1 + \varepsilon_{12} \ (g_6 < -.5) \\ g_6 + \varepsilon_{12} \ (|g_6| \le .5) \\ 1 + \varepsilon_{12} \ (|g_6| > .5) \end{cases} \quad \begin{aligned} g_{16} &= .8g_8 + \varepsilon_{16} \\ g_{19} &= 1/\{1 + \exp(-4g_{10})\} + \varepsilon_{19} \\ g_{20} &= 1.1g_{10} + \varepsilon_{20} \end{aligned}$$

$$g_{17} = \begin{cases} .2g_8 - 1 + \varepsilon_{17} \ (g_8 \le .2) \\ 1.4g_8 + \varepsilon_{17} \ (g_8 > .2) \end{cases} \quad g_{18} = \begin{cases} .4g_8 + 1 \ (|g_8| > .3 \\ (g_8 + 1)^{1.2} \ (g_8 \le .3) \end{cases}$$

(b)

Fig. 2. Artificial gene network and functional structures between nodes.

listed in Fig. 2 (b). The data ware generated from the artificial network of Fig. 2 (a) with the functional structures between nodes shown in Fig. 2 (b). Then the observations of the child variable are generated after transforming the observations of the parent variables to mean 0 and variance 1. A network was rebuilt from simulated data consisting of 50 or 100 observations, which corresponds to 50 or 100 microarrays. Since, recently more microarray data have become available and it is often the case that we can use more than 100 microarrays. While at the starting point of the analysis, we have over 6000 genes for yeast, after some pre-treatments of the data or using some prior knowledge, the number of target genes is typically less than 50 or so. We consider such a case in this simulation. As for the biological knowledge, we tried the following situations: **(Case 1)** we know some gene regulations (100%, 75%, 50% or 25% out of 19 edges shown in Fig. 2 (a)) and **(Case 2)** we know some gene regulations, but some (1, 2, or 3) incorrect edges are kept in the database. We set $\{0.5,\ 1.0\}$ and $\{\zeta_1,\ 2.5,\ 5.0,\ 7.5,\ 10.0\}$ as the candidate values of ζ_1 and ζ_2, respectively.

Fig. 4 shows two estimated networks: One is estimated by 100 observations (microarrays) alone. We use $\zeta_1 = \zeta_2 = 0.5$, i.e. we did not use any prior knowledge (we denote this network by N_0 for convenience). The other is estimated by 100 observations and prior information of 75% gene regulations, i.e. we know 14 correct relations out of the all 19 correct edges (we denote this network by N_1). Edges appearing in both networks are colored green, while edges appearing in N_0 or N_1 only are colored blue and red, respectively. By adding prior knowledge, it is clear that we succeeded in reducing the number of false positives. We also find additional four correct relationships. Fig. 3 shows the behavior of BNRC_{hetero} when $\zeta_1 = 0.5$.

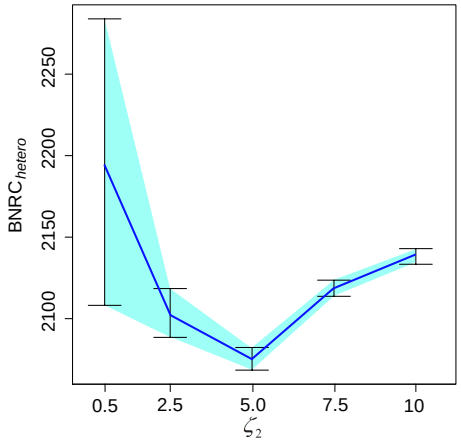


Fig. 3. The behavior of BNRC_{hetero} when $\zeta_1 = 0.5$. We can find out the optimal inverse normalized temperature ζ_2 is 5.0.

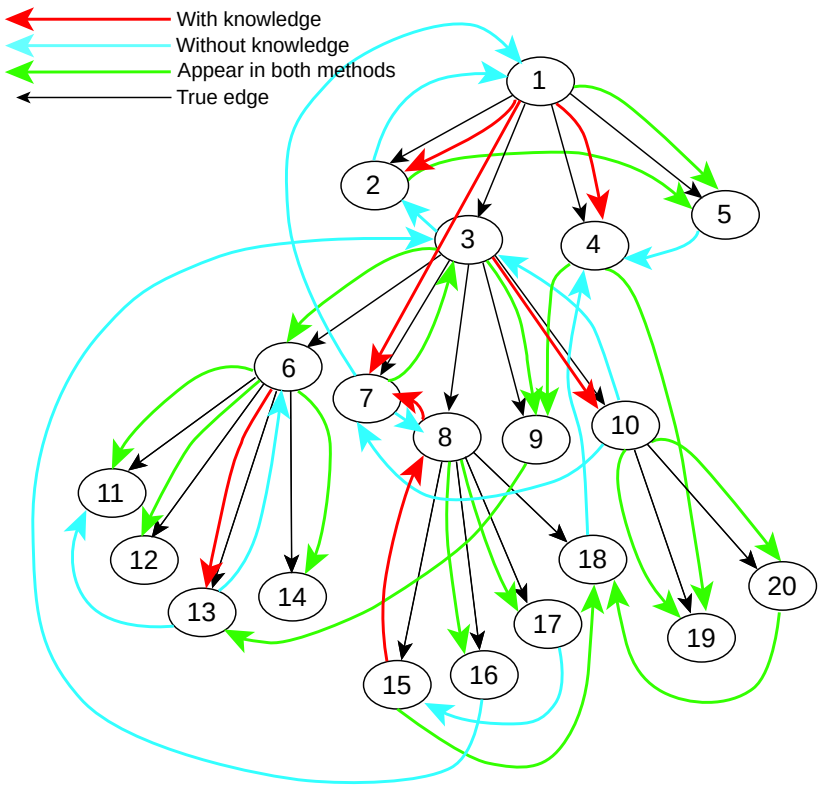


Fig. 4. An example of resulting networks based on 100 samples. We used $\zeta_1 = 0.5$ and $\zeta_2 = 5.0$ that are selected by our criterion (see Fig. 3).

We find that the optimal value of ζ_2 is 5.0. From the Monte Carlo simulations, we observed that ζ_2 can be selected by using middle values (depicted by a blue line) of upper and lower bounds or upper bounds in practice. For the selection of ζ_1, we use the middle value of the upper and lower bounds of the score of our criterion.

Fig. 5 shows the boxplots of the average squared errors (ASEs) that are defined by

$$\mathrm{ASE} = \sum_{i=1}^{100} \sum_{j=1}^{20} (x_{ij}^* - \hat{x}_{ij})^2,$$

where x_{ij}^* is the true value of x_{ij}, that is x_{i2}^* is given by $0.7x_{i1}$, and $\hat{x}_{ij}$ is the estimate of x_{ij} based on the estimated network. Since we repeated the Monte Carlo simulation 1000 times, each boxplot is obtained from 1000 ASEs. Smaller ASE means a more accurate estimated network. From Fig. 5, it is clear that by adding prior information we succeeded in reducing the ASE. The distributions of the number of true positives and false positives of the estimate networks are shown in Fig. 6. While

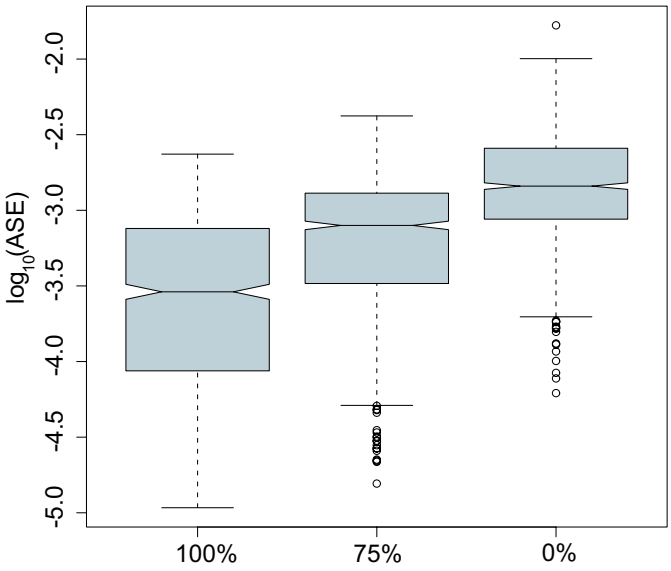


Fig. 5. Boxplots of the average squared errors.

the estimated networks without prior information contain many correct edges, we observe that the proposed method could reduce the number of false positives even if we added only a part of true relations.

The results of the Monte Carlo simulations are summarized as follows:

In **(Case 1)**, we obtained networks more accurately as long as we add correct knowledge. We observed that the number of false positives decreased drastically. We presume the reason is the nature of directed acyclic graphs. Since a Bayesian network model is a directed acyclic graph, one incorrect estimate may affect the relations in its neighborhood. However, by adding some correct knowledge, we can restrict the search space of the Bayesian network model learning effectively.

In **(Case 2)**, the results depend on the type of incorrect knowledge.
(i) If we use misdirected relations, e.g. $\text{gene}_8 \rightarrow \text{gene}_3$, as prior knowledge, serious problems occur. Since microarray data to some degree support the misdirected relations, they tend to receive a better criterion score.
(ii) If we add indirect relations such as $\text{gene}_1 \rightarrow \text{gene}_8$, we observed that our method controlled the balance between this prior information and microarray data and could decide whether the prior relation is true.
(iii) If irrelevant relations such as $\text{gene}_{20} \rightarrow \text{gene}_5$ are added as prior information, our method could reject these prior information, because, the microarray data do not support these relations.

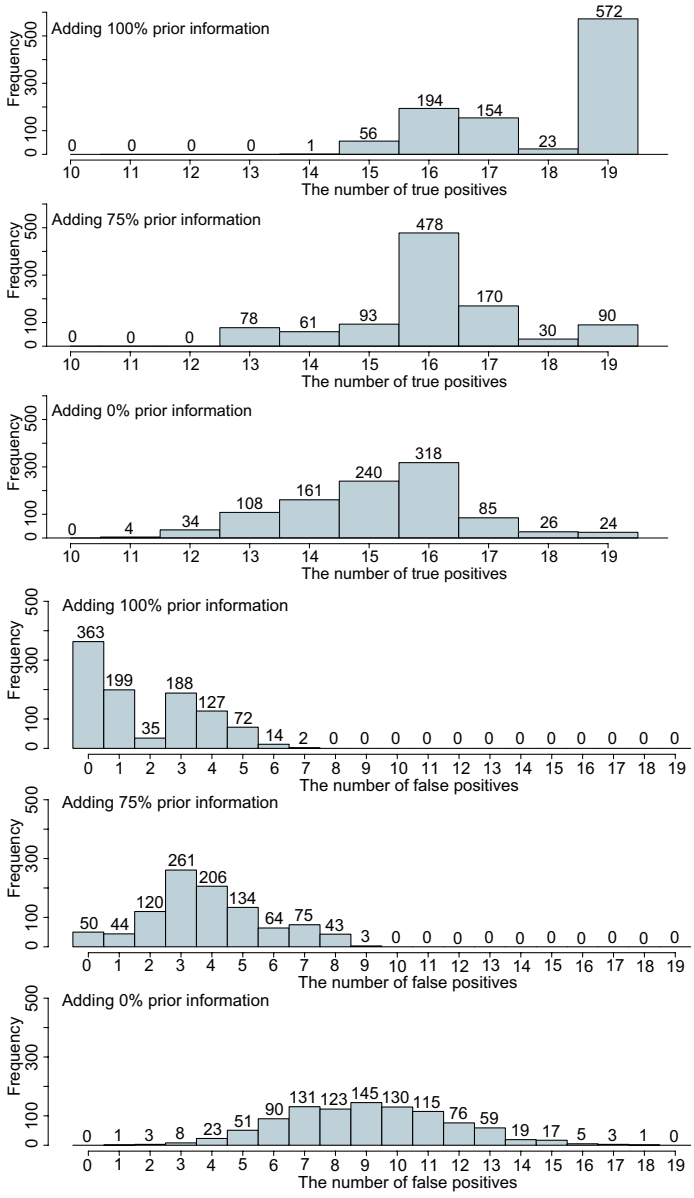


Fig. 6. Distribution of the number of true positives and false positives of the estimated networks. Upper three figures show the number of true positives when we use 100%, 75% and 0% prior information, respectively. Lower three figures show the number of false positives with respect to 100%, 75% and 0%. Note that since 19 edges in the true network, the maximum number of true positives is 19.

3.2. *Example using experimental data*

In this section, we demonstrate our method by analyzing *Saccharomyces cerevisiae* gene expression data obtained by disrupting 100 genes, which are almost all transcription factors. We used the BY4741 (*MATa*, *HIS3D1*, *LEU2D0*, *MET15D0*, *URA3D0*) as the wild type strain and purchased gene disruptions from Research Genetics, Inc. We focus on five genes, *MCM1*, *SWI5*, *ACE2*, *SNF2* and *STE12* (see Table 1) and extract genes that are regulated by these 5 genes from the Yeast Proteome Database.[54] Thus, we construct a prior network shown in Fig. 7, based on the database information. We include the prior network in our Bayesian network estimation method. That is, the purpose of this analysis is to estimate the gene network containing above 36 genes from microarray data together with the prior network. For constructing a Bayesian network with prior knowledge, the simplest way is to fix the prior edges and learn the other parts of the network based on the observed data. However, we observed that the score of our criterion, BNRC_{hetero}, of the estimated network learned with fixed prior edges cannot decrease compared with the optimal one. Fig. 8 shows the estimated gene network using microarray data only. There are many non-prior edges and many of them are probably false positives. In addition, we find three misdirected relations: "*SWI5* → *MCM1*", "*HO* → *ACE2*" and "*STE6* → *STE12*". By adding the prior network, we obtain the gene network shown in Fig. 10. As for the inverse normalized temperatures ζ_1 and ζ_2, we set $\zeta_1 = 0.5$ and choose the optimal value of ζ_2. We also estimated a gene network based on $\zeta_1 = 1$ and found the results described below to be essentially unchanged.

Fig. 9 shows the behavior of BNRC_{hetero} with respect to ζ_2. We find that the optimal value of ζ_2 is 2.5. Fig. 10 shows the resulting network based on microarray data and the biological knowledge represented by the prior network in Fig. 7. We show the edges that correspond to the prior knowledge in black. The edges between genes that are regulated by the same transcription factor in the prior network are shown in blue. The red edges do not correspond to the prior knowledge. In particular, we find that the relationships around *MCM1* improve drastically. The network

Table 1. Five transcription factors and their regulating genes.

MCM1 :	transcription factor of the MADS box family
	MET14, *CDC6*, *MET2*, *CDC5*, *MET6*, *SIC1*, *STE6*, *CLN2*, *PCL2*, *STE2*, *ACE2*, *MET16*,*MET3*, *MET4*, *CAR1*, *SWI5*, *PCL9*, *CLB1*, *MET17*, *EGT2*, *ARG5,6*, *PMA1*, *RME1*, *CLB2*
SWI5 :	transcription factor
	CDC6, *SIC1*, *CLN2*, *PCL2*, *PCL9*, *EGT2*, *RME1*, *CTS1*, *HO*
ACE2 :	metallothionein expression activator
	CLN2, *EGT2*, *HO*, *CTS1*, *RME1*
SNF2 :	component of SWI/SNF global transcription activator complex
	CTS1, *HO*
STE12 :	transcriptional activator
	STE6, *FAR1*, *KAR3*, *SST2*, *FUS1*, *STE2*, *BAR1*, *AGA1*, *AFR1*, *CIK1*

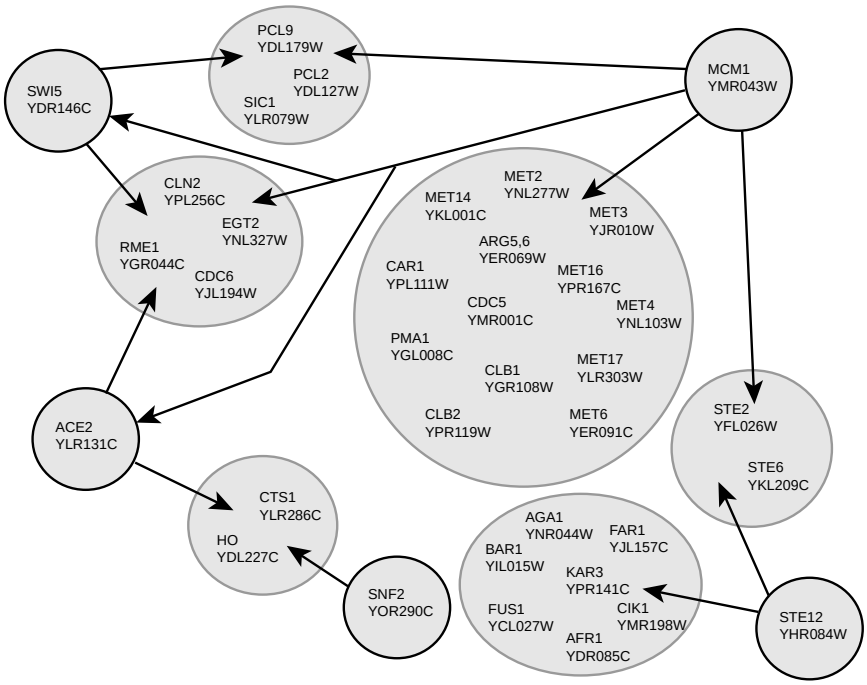


Fig. 7. Prior knowledge network. The genes that are in each shadowed circle are regulated by the parent genes.

based on microarray only (Fig. 8) indicates that only *SIC1* and *ACE2* are regulated by *MCM1*. Note that the underlined genes correspond to the prior network information. After adding the prior knowledge and optimizing the inverse normalized temperatures, we find that 10 genes out of 24 genes that are listed as co-regulated genes of *MCM1* in Table 1 are extracted. Also, the relationships around *STE12* become clearer. Before adding prior knowledge, the estimated network in Fig. 8 suggests *FUS1*, *AFR1*, *KAR3*, *BAR1*, *MET4*, *MET16* and *MCM1* are regulated by *STE12*, while *STE12* is controlled by *HO*, *STE6* and *MET3*. On the other hand, the network in Fig. 10 shows that *STE12* regulates *FUS1*, *AFR1*, *KAR3*, *CIK1*, *STE2*, *STE6*, *HO* and *MCM1*. Note that the three misdirected relations described above are corrected in Fig. 10. The difference between the inverse normalized temperatures $\zeta_1 = 0.5$ and $\zeta_2 = 2.5$ is small, because the score of the criterion is added as $2\zeta_1$ or $2\zeta_2$, when we add an edge that is listed or not listed in the prior network, respectively. Therefore, microarray data contain this information and we succeeded in extracting this information with the slight help of the prior network.

We optimized the inverse normalized temperature ζ_2 based on the proposed criterion. From the network based on the optimal inverse normalized temperatures, we can find the diffrence between microarray data and biological knowledge. By

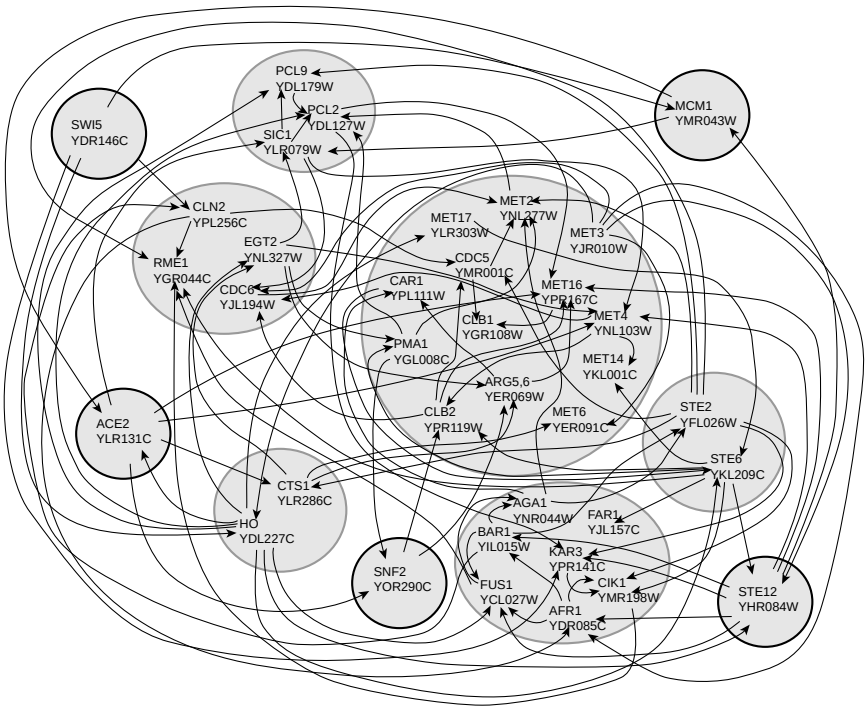


Fig. 8. Resulting network based on microarray only.

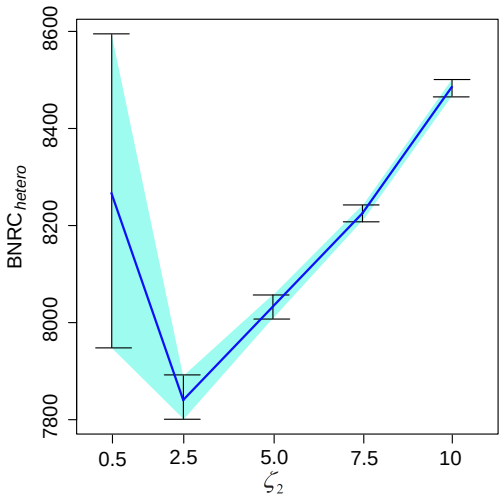


Fig. 9. Optimization of ζ_2. We can find out that the optimal value of ζ_2 is 2.5.

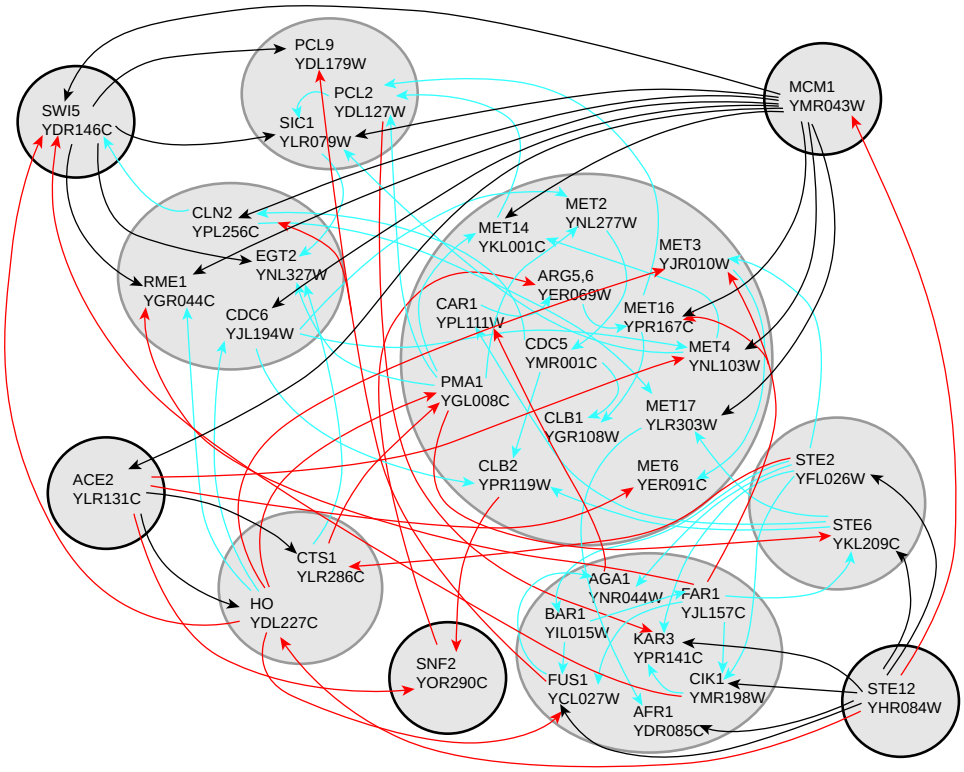


Fig. 10. Resulting network based on microarray data and biological knowledge. The inverse normalized temperatures are selected by our criterion ($\zeta_1 = 0.5$, $\zeta_2 = 2.5$).

comparing Fig. 8 with Fig. 10, we find that the microarray data reflect the relationship between seven genes (*CLN2*, *RME1*, *CDC6*, *EGT2*, *PCL2*, *PCL9* and *SIC1*) and two transcription factors (*MCM1* and *SWI5*). On the other hand, we find that there are somewhat large differences between microarray data and the prior network for the relationship between *MCM1* and the thirteen genes that are in the biggest circle.

4. Discussion

In this paper we proposed a general framework for combining microarray data and biological knowledge aimed at estimating a gene network. An advantage of our method is the balance between microarray information and biological knowledge is optimized by the proposed criterion. By adding biological knowledge into our Bayesian network estimation method, we succeeded in extracting more information from microarray data and estimating the gene network more accurately. We believe that the combination of microarray data and biological knowledge gives a new

perspective for understanding the systems of living creatures.

We consider the following problems as our future works: (1) In this paper we focused on how to use biological knowledge together with microarray data. However, of course, the development of more effective models and criteria for estimating a gene network is an important problem. (2) Our Bayesian network model does not treat the experimental conditions. However, real biological processes are often condition specific. Therefore, considering experimental conditions is an important problem. (3) Recently, joint learning methods aimed at extracting more effective information from multi-types of genomic data have recieved considerable attention. We plan to extend our method so that it can handle such data effectively. (4) When we use some databases that have various confidence information as the biological knowledge, we may need to use more hyperparameters for specifying $\pi(G)$. (5) From biological knowledge, we deterministically decided the category to which edges belong, e.g. $u_{11} = \zeta_1$, $u_{12} = \zeta_2$, and so on. However, biological knowledge contains some errors. In fact, u_{ij} can be interpreted as a random variable, and a statistical model can be constructed for u_{ij}. In that sense, our method can be extended as a Bayesian network estimation method with a self-repairing database mechanism. We would like to investigate these problems in a future paper.

Acknowledgments

The authors would like to thank four referees for constructive and helpful comments that improved the quality of the paper considerably.

References

1. T. Akutsu, S. Miyano and S. Kuhara. Identification of genetic networks from a small number of gene expression patterns under the Boolean network model. *Pacific Symposium on Biocomputing*, **4**, 17-28, 1999.
2. T. Akutsu, S. Miyano and S. Kuhara. Inferring qualitative relations in genetic networks and metabolic pathways. *Bioinformatics*, **16**, 727-734, 2000
3. G.D. Bader, I. Donaldson, C. Wolting, B.F.F. Ouellette, T. Pawson and C.W.V. Hogue. BIND-The biomolecular interaction network database. *Nucleic Acids Research*, **29**, 242-245, 2001.
4. H. Bannai, S. Inenaga, A. Shinohara, M. Takeda and S. Miyano. A string pattern regression algorithm and its application to pattern discovery in long introns. *Genome Informatics*, **13**, 3-11, 2002.
5. BIND `http://www.blueprint.org/`
6. H.J. Bussemaker, H. Li and E.D. Siggia. Regulatory element detection using correlation with expression. *Nature Genetics*, **27**, 167-171, 2001.
7. T. Chen, H. He and G. Church. Modeling gene expression with differential equations. *Pacific Symposium on Biocomputing*, **4**, 29-40, 1999.
8. G. Cooper and E. Herskovits. A Bayesian method for the induction of probabilistic networks from data. *Machine Learning*. **9**, 309-347, 1992.
9. A.C. Davison. Approximate predictive likelihood. *Biometrika*, **73**, 323-332, 1986.
10. C. de Boor. *A Practical Guide to Splines*. Springer, Berlin. 1978.

11. M.J.L. de Hoon, S. Imoto and S. Miyano. Inferring gene regulatory networks from time-ordered gene expression data using differential equations. *Proc. 5th International Conference on Discovery Science*, Lecture Note in Artificial Intelligence, **2534**, Springer-Verlag, 267-274, 2002.
12. M.J.L. de Hoon, S. Imoto, K. Kobayashi, N. Ogasawara and S. Miyano. Inferring gene regulatory networks from time-ordered gene expression data of *Bacillus subtilis* using differential equations. *Pacific Symposium on Biocomputing*, **8**, 17-28, 2003.
13. P.H.C. Eilers and B. Marx. Flexible smoothing with B-splines and penalties (with discussion). *Statistical Science*, **11**, 89-121, 1996.
14. N. Friedman and M. Goldszmidt. *Learning Bayesian networks with local structure*. in M.I. Jordan ed., Kluwer Academic Publishers, 421-459, 1998.
15. N. Friedman, M. Linial, I. Nachman and D. Pe'er. Using Bayesian network to analyze expression data. *J. Comp. Biol.*, **7**, 601-620, 2000.
16. I. Gat-Viks and R. Shamir. Chain functions and scoring functions in genetic networks. *Bioinformatics*, **19** (ISMB2003), i108-i117, 2003.
17. S. Geman and D. Geman. Stochastic relaxation, Gibbs distribution and the Bayesian restorations. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, **6**, 721-741, 1984.
18. GRID `http://biodata.mshri.on.ca/grid/servlet/Index`
19. A.J. Hartemink, D.K. Gifford, T.S. Jaakkola and R.A. Young. Using graphical models and genomic expression data to statistically validate models of genetic regulatory networks. *Pacific Symposium on Biocomputing*, **6**, 422-433, 2001.
20. A.J. Hartemink, D.K. Gifford, T.S. Jaakkola and R.A. Young. Combining location and expression data for principled discovery of genetic regulatory network models. *Pacific Symposium on Biocomputing*, **7**, 437-449, 2002.
21. T. Hastie and R. Tibshirani. *Generalized Additive Models*. Chapman & Hall, 1990.
22. D. Heckerman. *A Tutorial on Learning with Bayesian Networks*. in M.I. Jordan ed., Kluwer Academic Publishers, 301-354, 1998.
23. T. Ideker, O. Ozier, B. Schwikowski and A.F. Siegel. Discovering regulatory and signalling circuits in molecular interaction networks. *Bioinformatics*, **18** (ISMB 2002), S233-S240, 2002.
24. S. Imoto, T. Goto and S. Miyano. Estimation of genetic networks and functional structures between genes by using Bayesian networks and nonparametric regression. *Pacific Symposium on Biocomputing*, **7**, 175-186, 2002.
25. S. Imoto, S. Kim, T. Goto, S. Aburatani, K. Tashiro, S. Kuhara and S. Miyano. Bayesian network and nonparametric heteroscedastic regression for nonlinear modeling of genetic network. *Journal of Bioinformatics and Computational Biology*, **1**(2), 231-252. (Preliminary version has appeared in *Proc. 1st IEEE Computer Society Bioinformatics Conference*, 219-227, 2002).
26. S. Imoto and S. Konishi. Selection of smoothing parameters in B-spline nonparametric regression models using information criteria. *Ann. Inst. Statist. Math.* in press.
27. T. Ito, T. Chiba, R. Ozawa, M. Yoshida, M. Hattori and Y. Sakaki. A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc. Natl. Acad. Sci. USA*, **97**, 4569-4574, 2001.
28. F.V. Jensen. *An Introduction to Bayesian Networks*. University College London Press, 1996.
29. T.-K. Jenssen, A. Lægreid. J. Komorowski and E. Hovig. A literature network of human genes for high-throughput analysis of gene expression. *Nature Genetics*, **28**, 21-28.
30. M. Kanehisa, S. Goto, S. Kawashima and A. Nakaya. The KEGG databases at

GenomeNet. *Nucleic Acids Res.*, **30**, 42-46, 2002.
31. KEGG `http://www.genome.ad.jp/kegg/kegg2.html`
32. S. Kim, S. Imoto and S. Miyano. Dynamic Bayesian network and nonparametric regression for nonlinear modeling of gene networks from time series gene expression data. *Proc. 1st International Workshop on Computational Methods in Systems Biology*, Lecture Note in Computer Science, **2602**, Springer-Verlag, 104-113, 2003.
33. S. Kim, S. Imoto and S. Miyano. Inferring gene networks from time series microarray data using dynamic Bayesian networks. *Briefings in Bioinformatics*, **4**(3), 228-235.
34. R. Kindeman and J. Snell. *Markov Random Fields and Their Applications*. American Mathematical Society, Providence, RI, 1980.
35. S. Konishi, T. Ando and S. Imoto. Bayesian information criteria and smoothing parameter selection in radial basis function networks. *Biometrika*, **91**, in press.
36. T.I. Lee, N.J. Rinaldi, F. Robert, D.T. Odom, Z. Bar-Joseph, G.K. Gerber, N.M. Hannett, C.T. Harbison, C.M. Thompson, I. Simon, J. Zeitlinger, E.G. Jennings, H.L. Murray, D.B. Gordon, B.Ren, J.J. Wyrick, J-B. Tagne, T.L. Volkert, E. Fraenkel, D.K. Gifford and R.A. Young. Transcriptional regulatory networks in *Saccharomyces cerevisiae*. *Science*, **298**, 799-804, 2002.
37. Y. Maki, D. Tominaga, M. Okamoto, S. Watanabe and Y. Eguchi. Development of a system for the inference of large scale genetic networks. *Pacific Symposium on Biocomputing*, **6**, 446-458, 2001.
38. D.R. Masys. Linking microarray data to the literature. *Nature Genetics*, **28**, 9-10, 2001.
39. K. Murphy and S. Mian. Modelling gene expression data using dynamic Bayesian networks. *Technical report, Computer Science Division, University of California, Berkeley, CA*. 1999.
40. N. Nariai, S. Kim, S. Imoto and S. Miyano. Using protein-protein interactions for refining gene networks estimated from microarray data by Bayesian networks. *Pacific Symposium on Biocomputing*, **9**, in press.
41. I.M. Ong, J.D. Glasner and D. Page. Modelling regulatory pathways in *E. coli* from time series expression profiles. *Bioinformatics*, **18** (ISMB2002), S241-S248, 2002.
42. D. Pe'er, A. Regev, G. Elidan and N. Friedman. Inferring subnetworks from perturbed expression profiles. *Bioinformatics*, **17** (ISMB 2001), S215-S224, 2001.
43. Y. Pilpel, P. Sudarsanam and G.M. Church. Identifying regulatory networks by combinatorial analysis of promoter elements. *Nature Genetics*, **29**, 153-9, 2001.
44. J. Quackenbush. Microarray data normalization and transformation. *Nature Genetics*, **32**, 496-501, 2002.
45. SCPD `http://cgsigma.cshl.org/jian/`
46. E. Segal, Y. Barash, I. Simon, N. Friedman and D. Koller. From promoter sequence to expression: a probabilistic framework. *Proc. 6th Annual International Conference on Research in Computational Molecular Biology* (RECOMB2002), 263-272, 2002.
47. E. Segal, H. Wang and D. Koller. Discovering molecular pathways from protein interaction and gene expression data. *Bioinformatics*, **19** (ISMB2003), i264-i272, 2003.
48. E. Segal, R. Yelensky and D. Koller. Genome-wide discovery of transcriptional modules from DNA sequence and gene expression. *Bioinformatics*, **19** (ISMB2003), i273-i282, 2003.
49. I. Shmulevich, E.R. Dougherty, S. Kim and W. Zhang. Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks. *Bioinformatics*, **18**, 261-274, 2002.
50. V.A. Smith, E.D. Jarvis and A.J. Hartemink. Evaluating functional network inference using simulations of complex biological systems. *Bioinformatics*, **18** (ISMB2002),

S216-S224, 2002.
51. J. Suzuki. A construction of Bayesian networks from databases on an MDL principle. *Proc. 9th Conference on Uncertainty in Artificial Intelligence*. 266-273, 1993.
52. Y. Tamada, S. Kim, H. Bannai, S. Imoto K. Tashiro, S. Kuhara and S. Miyano. Estimating gene networks from gene expression data by combining Bayesian network model with promoter element detection. *Bioinformatics*, **19** (ECCB2003), ii227-ii236.
53. L. Tinerey and J.B. Kadane. Accurate approximations for posterior moments and marginal densities. *J. Amer. Statist. Assoc.*, **81**, 82-86, 1986.
54. YPD `http://www.incyte.com/sequence/proteome/databases/YPD.shtml`
55. YTF `http://biochemie.web.med.uni-muenchen.de/YTFD/`