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Gene expression data analysis[#]

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ABSTRACT – Microarrays are one of the latest breakthroughs in experimental molecular biology, which allow monitoring of gene expression for tens of thousands of genes in parallel and are already producing huge amounts of valuable data. Analysis and handling of such data is becoming one of the major bottlenecks in the utilization of the technology. The raw microarray data are images, which have to be transformed into gene expression matrices, tables where rows represent genes, columns represent various samples such as tissues or experimental conditions, and numbers in each cell characterize the expression level of the particular gene in the particular sample. These matrices have to be analyzed further if any knowledge about the underlying biological processes is to be extracted. In this paper we concentrate on discussing bioinformatics methods used for such analysis. We briefly discuss supervised and unsupervised data analysis and its applications, such as predicting gene function classes and cancer classification as well as some possible future directions.
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microarray / bioinformatics

1. Introduction

With several eukaryotic genomes completed and the draft human genome published, we are now entering the postgenomic age. The main focus in genomic research is switching from sequencing to using the genome sequences in order to understand how genomes are functioning. Some questions we would like to ask are:
– what are the functional roles of different genes and in what cellular processes they participate;
– how genes are regulated, how genes and gene products interact, what are these interaction networks;
– how gene expression levels differ in various cell types and states, how gene expression is changed by various diseases or compound treatments.

Knowing the gene transcript abundance in various tissues, developmental stages and under various conditions is important for attacking these questions. Although mRNA is not the ultimate product of a gene, transcription is the first step in gene regulation, and information about the transcript levels is needed for understanding gene regulatory networks. Moreover, the measurement of mRNA levels currently is considerably cheaper and can be done in a more high-throughput way than direct measurements of the protein levels. The correlation between the mRNA and protein abundance in the cell may not be straightforward; nevertheless the absence of mRNA in a cell is likely to imply a not-very-high level of the respective protein and thus at least qualitative estimates about the proteome can be based on the transcriptome information. The mRNA and protein level correlation studies are under way (see [1]).

The ability to monitor the gene expression on the transcript level has become possible due to the advent of DNA microarray technologies (see [2]). A microarray is a glass slide, onto which single-stranded DNA molecules are attached at fixed locations (spots). There may be tens of thousands of spots on an array, each related to a single gene. Microarrays exploit the preferential binding of complementary single-stranded nucleic acid sequences. There are several variations of microarray technologies, each used in a specific way.

One of the most popular experimental platforms is used for comparing mRNA abundance in two different samples (or a sample and control). RNA from the sample and control cells are extracted and labeled with two different fluorescent labels: e.g., a red dye for the RNA from the sample population and a green dye for that from the control population. Both extracts are washed over the

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microarray. Gene sequences from the extracts hybridize to their complementary sequences in the spots.

To measure the relative abundance of the hybridized RNA the array is excited by a laser. If the RNA from the sample population is in abundance, the spot will be red, if the RNA from the control population is in abundance, it will be green. If sample and control bind equally, the spot will be yellow, while if neither bind, it will not fluoresce and appear black. Thus, from the fluorescence intensities and colors for each spot, the relative expression levels of the genes in the sample and control populations can be estimated.

By measuring transcription levels of genes in an organism under various conditions, at different developmental stages and in different tissues, we can build up 'gene expression profiles' which characterize the dynamic functioning of each gene in the genome. We can imagine the expression data represented in a matrix with rows representing genes, columns representing samples (e.g., various tissues, developmental stages and treatments), and each cell containing a number characterizing the expression level of the particular gene in the particular sample. We will call such a table a 'gene expression matrix'. Building up a database of such matrices will help us to understand gene regulation, metabolic and signaling pathways, the genetic mechanisms of disease, and the response to drug treatments. For instance, if overexpression of certain genes is correlated with a certain cancer, we can explore which other conditions affect the expression of these genes and which other genes have similar expression profiles. We can also investigate which compounds (potential drugs) lower the expression level of these genes.

2. From raw data to gene expression matrix

Like many experimental technologies, microarrays measure the target quantity (i.e. relative or absolute mRNA abundance) indirectly by measuring another physical quantity, the intensity of the fluorescence of the spots on the array for each fluorescent dye, i.e. for each optical wavelength (so-called channel). Therefore the raw data produced by microarrays are in fact monochrome images (*figure 1*). Transforming these images into the gene expression matrix is a nontrivial process: the spots corresponding to genes on the microarray should be identified, their boundaries determined, the fluorescence intensity from each spot measured and compared to the background intensity and to these intensities for other channels. The software for this initial image processing is often provided with the image scanner, since it will depend on particular properties of the hardware. Often laborious manual adjustment of the grid for spots is used. We will not discuss the raw data processing in detail in this paper; some survey of image analysis software can be found on http://cmpteam4.unil.ch/biocomputing/array/software/MicroArray_Software.html.

In any physical experiment it is important to know not only the value of the measurement, but also the standard error or some other identifier of reliability for each data point. For most microarray technology platforms only the ratio of the background-subtracted signals of the given sample and the control is meaningful. If the spot intensity is low, the ratio of these numbers may be high, but the measurement may not be reliable. The spot quality can be assessed not only by the absolute intensity in each channel, but also by many other factors, such as uniformity of the individual pixel intensities, or the shape of the spot. Unfortunately currently there is no standard way of assessing the spot measurement reliability. If experiments have been done in replicates, they can be used to assess the standard errors in addition to the single measurement quality assessments. Little has been published yet on how to use the reliability of gene expression measurements by combining the information about the spot image in each channel and the replicate images.

Another difficulty in creating a gene expression matrix comes from the necessity to identify each spot with the respective gene. This is not always possible, since spots are typically based on EST sequences, and linking the EST to the respective gene may be nontrivial. Typically it is done through EST clusterings. Additionally, the same gene

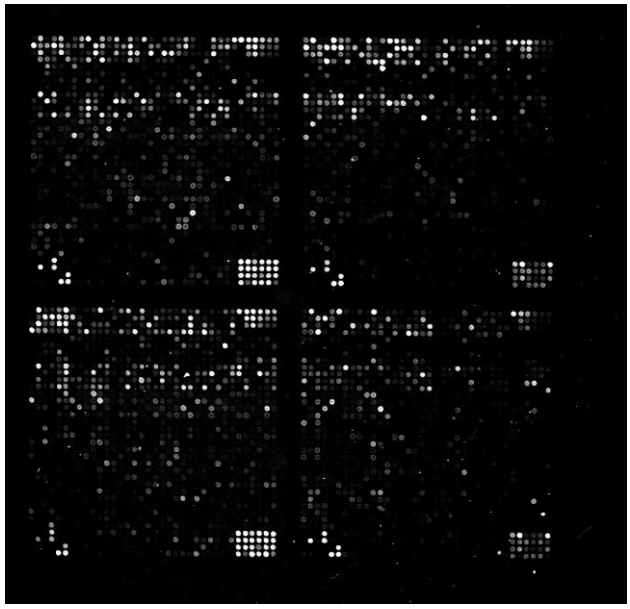


Figure 1. A sample image from scanning a hybridized rat microarray containing over 5 000 genes. Each spot features a pool of identical single-stranded DNA molecules representing a single gene. The brightness of the spot is proportional to the amount of fluorescent mRNA hybridized to the DNA of the spot. Automated image analysis software should identify these fluorescence spots, determine their boundaries, and the fluorescence intensity from each spot should be measured and compared to the background fluorescence. Moreover, the image should be compared to a similar image obtained from the control measurements and ratio of background subtracted intensities calculated. In this way images are transformed into the gene expression matrix, which can be analyzed further by numerical methods.
The image was kindly provided by Tom Freeman (Sanger Centre, Cambridge, UK).

may be represented by several spots on the array, either by exactly the same, or a different sequence. What expression level to attribute to the gene, if measurements from these different spots differ?

Microarray-based gene expression measurements are still far from giving estimates of mRNA counts per cell in the sample. The measurements are relative by nature: essentially we can either compare the expression level of the same gene in different samples, or different genes in the same sample. Moreover, appropriate normalization should be applied to enable any data comparisons. It is typically assumed that abundance ratios of 1.5 to 2 are indicative of a change in gene expression, but such estimates are very crude, since the reliability of ratios depends on the absolute intensity values, as well as varying from spot to spot due to the dependence of hybridization efficiency on the particular sequence and cross-hybridization between homologous sequences (for instance see [3]). This should be kept in mind while analyzing the gene expression matrix. The value of microarray-based gene expression measurements would be considerably higher, if reliability and limitations of particular microarray platforms for particular kinds of measurements, as well as cross-platform comparison and normalization, were studied and published.

After we have processed the raw image data into the gene expression matrix, the next task is to analyze this matrix and to try to extract from it some knowledge about the underlying biological processes.

3. Gene expression matrix analysis

There are two straightforward ways in which the gene expression matrix can be studied: 1) comparing expression profiles of genes by comparing rows in the expression matrix; 2) comparing expression profiles of samples by comparing columns in the matrix.

Additionally both methods can be combined (provided that the data normalization allows it). When comparing rows or columns, we can look either for similarities or differences. If we find that two rows are similar, we can hypothesize that the respective two genes are coregulated and possibly functionally related. By comparing samples, we can find which genes are differentially expressed and, for instance, study effects of various compounds.

Before we can perform any comparisons, we need a way to measure the similarity (or distance) between the objects we are comparing. We can regard these objects (rows or columns in the matrix) as points in n-dimensional space or as n-dimensional vectors, where n is the number of samples for gene comparison, or number of genes for sample comparison. The natural, so-called Euclidean distance (for definition see [4]) between these points in the n-dimensional space may be the most obvious, but not necessarily the best choice. Intuitively appealing is to use the correlation coefficient calculated by treating the two n-dimensional vectors as a series of random variables. In fact this distance is related to the angle between the two n-dimensional vectors. Euclidean and correlation distance measures are related, if we normalize the length of the n-dimensional vectors to 1. This allows us to use correlation distance even in the cases when Euclidean properties are important. Some other distance measures, including rank correlation coefficient and mutual information-based measure are proposed in D'haesleer et al. [5]. Currently, to the best of our knowledge, there is no theory on how to choose the best distance measure. Possibly one 'right' distance measure in the expression profile space does not exist, and the choice should depend on the questions that we are asking. Standard sets of known coregulated genes in various organisms and gene regulatory network modeling can potentially help in finding theoretically substantiated similarity measures.

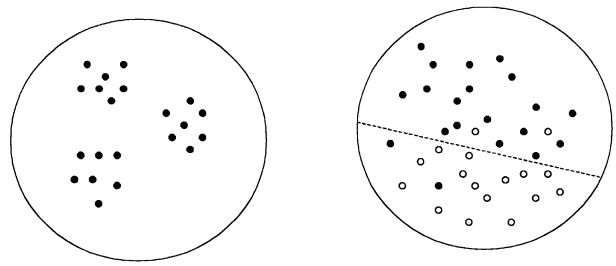


Figure 2. Supervised and unsupervised data analysis. In the unsupervised case (left) we are given data points in n-dimensional space ($n = 2$ in the example) and we are trying to find ways of how to group together points with similar features. For instance, there are three natural clusters in the example each consisting of datapoints close to each other in a sense of Euclidean distance. A clustering algorithm should identify these clusters. In the supervised case (right), the objects are labeled (e.g., we have dark and hollow points in the example), and the task is to find a set of classification rules allowing us to discriminate between these points as precisely as possible. For instance, the dotted line in the drawing discriminates most of the points correctly, allowing us to predict their 'labels', dark or hollow, by their position above or below the dotted line.

After having chosen the similarity measure in the expression profile space we can study the expression matrix either in a supervised or unsupervised manner. The supervised approach assumes that for some (or all) profiles we have additional information, such as functional classes for the genes, or diseased/normal states attributed to the samples. We can view this additional information as labels attached to the rows or columns. Having this information, a typical task is to build a classifier able to predict the labels from the expression profile. A typical example of unsupervised data analysis is expression profile clustering to find groups of coregulated genes or related samples. For conceptual illustration of unsupervised and supervised analysis see *figure 2*. First we discuss the clustering approach.

3.1. Unsupervised analysis

The goal of clustering is to group together objects (genes or samples) with similar properties. This can be viewed also as the reduction of the dimensionality of the system. Clustering is not a new technique, many algorithms have been developed for it and many of these

algorithms have been applied to analyze expression data. The hierarchical [3, 6] and *K*-means clustering algorithms [7, 8], as well as self-organizing maps [9] have all been used for clustering expression profiles. Even a simple clustering algorithm based on binning (i.e. discretizing the expression profile space and clustering together the profiles that map into the same bin) has been shown to be useful for clustering genes and subsequent discovering of transcription factor binding sites [10]. More recently new algorithms have been developed specifically for gene expression profile clustering, for instance [11] based on finding approximate cliques in graphs.

Hierarchical clustering works by iteratively joining the two closest clusters starting from singleton clusters [6] or iteratively partitioning clusters starting with the complete set [12]. After each joining of two clusters, the distances between all the other clusters and a new joined cluster are recalculated. The complete linkage, average linkage, and single linkage methods use maximum, average, and minimum distances between the members of two clusters respectively. Note that to obtain a particular partitioning into clusters, the threshold distance should be chosen by independent means (typically by the user himself).

The *K*-means clustering algorithm typically uses the Euclidean properties of the vector space. The desired number of clusters *K* has to be chosen a priori. After the initial partitioning of the vector space into *K* parts, the algorithm calculates the center points in each subspace and adjusts the partition so that each vector is assigned to the cluster the center of which is the closest. This is repeated iteratively until either the partitioning stabilizes or the given number of iterations is exceeded. The approaches for the initial selection of the first set of *K* cluster centers can vary.

Clustering of expression profiles has been used for grouping genes as well as samples. The clustering of genes for finding coregulated and functionally related groups is particularly interesting in the cases when we have complete sets of organisms' genes. In a frequently quoted paper DeRisi et al. [13] used a DNA array containing a complete set of yeast genes to study the diauxic shift time course. They selected small groups of genes with similar expression profiles and showed that these genes are functionally related and contain relevant transcription factor binding sites upstream to their open reading frames. More systematic studies of this data set for regulatory elements were done in [10] and [14].

Later, more expression studies of yeast under various conditions were carried out, including sporulation [15], cell cycle [16] and yeast gene regulatory machinery [17]. Clustering has been applied to the obtained gene expression matrices, and groups of functionally related and coregulated genes have been revealed. Tavazoie et al. [8] clustered expression profiles of the 3 000 most variable yeast genes during the cell cycle (15 time points, data from Cho et al. [18]) into 30 clusters by the *K*-means algorithm. They found that for half of these clusters, strong sequence patterns are present in the gene upstream sequences. Note that expression profiles of cell-cycle-dependent genes are periodic and Fourier analysis has been used to discover these genes [16].

Eisen et al. [6] have developed a hierarchical clustering-based algorithm and visualization software package, which is currently one of the most frequently used tools for expression profile clustering and data visualization. They applied their software to gene expression matrices obtained by combining 80 different yeast samples (experimental conditions) studied in various hybridization experiments in Stanford University (including the ones mentioned above).

Gene expression profile clustering does not necessarily require the full genome. For instance Iyer et al. [19] studied 8 600 genes in human fibroblast and obtained 10 distinct gene clusters each associated with genes with particular functional roles, such as signal transduction, coagulation, hemostasis, inflammation, etc.

A simple method for finding sets of interesting genes is comparing expression profiles of two or more samples for differentially expressed genes. For instance, Lee et al. [20] have used this method to find genes that are differentially expressed in skeletal muscle of adult (5-month) and old (30-month) mice. Of over 6 347 mice genes surveyed by a microarray, 58 displayed a greater than two-fold increase, whereas 55 displayed a greater than two-fold decrease in expression in the skeletal muscles of the old mice. Of the genes that increased the expression, 16% were mediators or stress response genes and 9% were involved in neuronal growth. Of genes that decreased in expression, 13% were participating in energy metabolism. In the same study gene expression profiles from 30-month-old mice with restricted calorie intake (76% of that of control population) were compared to 30-month-old control population, and it was shown that the expression profile of restricted calorie intake mice was closer to that of younger mice.

Hierarchical clustering of Eisen et al. [6] has also been used for sample clustering. An interesting application of this approach is the clustering of tumors to find new possible tumor subclasses. In a recent paper by Alizadeh et al. [21], diffuse large B-cell lymphoma (DLBCL) has been studied using 96 samples of normal and malignant lymphocytes. Applying hierarchical clustering algorithm of [7] to these samples they showed that there is a diversity in gene expression among the tumors of DLBCL patients. They identified two molecularly distinct forms of DLBCL, which had gene expression patterns indicative of different stages of B-cell differentiation. Interestingly, these two groups correlated well with the patient survival rates, thus confirming the clusters are meaningful.

The sample clustering has been combined with gene clustering to identify which genes are the most important for the sample clustering [12, 21]. Alon et al. [12] have applied a partitioning-based clustering algorithm to study 6 500 genes of 40 tumor and 22 normal colon tissues for clustering both genes and samples. They call this method two-way clustering.

3.2. Supervised analysis

One of the goals of supervised expression data analysis is to construct classifiers, such as linear discriminants, decision trees or support vector machines (SVM), which assign a predefined class to a given expression profile. For

instance, if a classifier can be constructed based on gene expression profiles that is able to distinguish between two different but morphologically closely related tumor tissues, such a classifier can be used for diagnostics. Moreover, if such a classifier is based on a set of relatively simple rules, it can help to understand the mechanisms involved in each tumor. Typically, such classifiers are trained on a subset of data with a priori given classification and tested on another subset with known classification. After assessing the quality of the prediction they can be applied to data the classification of which is unknown.

Brown et al. [22] have applied various supervised learning algorithms to six functional classes of yeast genes using gene expression matrices from 79 samples [6]. Genes from some of the classes such as ribosomal proteins and histones are expected to be coexpressed. For these classes a good classification accuracy were achieved. Some other functional classes such as protein kinases are not expected to have distinct gene expression profiles. It was shown that the SVM provides the best prediction accuracy for the functional classes that are expected to be coregulated.

Golub et al. [23] applied neighborhood analysis to construct class predictors for samples, concretely for leukemias. They were looking for genes the expression of which is best correlated with two known classes of leukemias, acute myeloid leukemia and acute lymphoblastic leukemia. They constructed a classifier based on 50 genes (from 6 817) using 38 samples and applied it to a collection of 34 new samples. The classifier correctly predicted 29 of these 34 samples.

Note that when classifying samples, we are confronted with a problem that there are many more attributes (genes) than objects (samples) that we are trying to classify. This makes it always possible to find a perfect discriminator if we are not careful in restricting the complexity of the permitted classifiers. To avoid this problem we must be looking for very simple classifiers compromising between the simplicity and the classification accuracy. Ben-Dor et al. [24] applied a new clustering algorithm for classification of colon and ovarian cancer data sets. They used unsupervised clustering to find a hierarchical structure in the expression profile space, and supervised learning to find the best threshold to correlate the clustering structure with the known cancer classes.

Whether we use supervised or unsupervised expression profile analysis, they are only the first steps in expression data analysis. It is a long way from finding gene clusters to finding functional roles of the respective genes, and moreover, understanding underlying biological processes. A natural step downstream from expression profile clustering is the usage of putative promoter sequences of similarly expressed genes for finding regulatory sequence elements in genomes. This is easier for yeast, since typically yeast promoters are relatively close to open reading frames (ORFs).

It seems reasonable to hypothesize that genes with similar expression profiles, i.e. genes that are coexpressed, may share something common in their regulatory mechanisms, i.e. may be coregulated. Therefore by clustering together genes with similar expression profiles one can find groups of potentially coregulated genes and search for putative regulatory signals.

A systematic application of this approach has been carried out in [25].

4. Conclusions

Expression data analysis methods are currently only in their infancy. Even the rather obvious approaches, such as cluster analysis and finding differentially expressed genes, have been used only rather crudely. For instance, the appropriateness of similarity measures has not been systematically explored and they are used on an ad-hoc basis. The information characterizing the measurement quality of different data points is typically not used. Advances in this area are hindered by the lack of systematic research on ways of assessing the measurement quality and comparing data from various technology platforms. These shortcomings can be overcome only if the journals encourage publications exploring the gene expression measurement technologies themselves, rather than always concentrating on the biological subject. In the long run the advancement of biological knowledge will be accelerated by technology centric studies, with biology becoming more of a quantitative science.

Gene expression data analysis methods will develop similarly as sequence analysis methods have developed over the past decades. The amounts of gene expression data will continue growing and the data will become more systematic. Currently, the gene expression profiling is similar to gene sequencing before the era of genome sequencing: the measurements are carried out to attack particular questions or sometimes just to demonstrate the concept.

With the technology becoming more reliable, with introduction of standard controls in the experiments and developing generally accepted data normalization and quality control methods, it will become possible to systematically profile genes in various organisms, tissues, developmental stages and conditions. Various chemical compounds will be profiled for their possible toxicity and other effects on organisms, and various signatures will be associated with various toxicity mechanisms or cellular processes. This approach will resemble systematic genome sequencing. Algorithms for reliable searching of similar expression profiles, or analyzing sets of related profiles to discover common signatures will be needed, in the same way as searching and pattern discovery algorithms are needed to explore sequences.

However, there is a major difference between gene sequence and expression data. Even if eventually we are able to overcome various technological limitations, and even if we are able to measure gene expression in terms of absolute units such as mRNA counts, the gene expression profiles are meaningful only in the context of the experimental conditions in which they have been measured. This requires detailed and systematic annotation of samples and experimental conditions. For this to become a reality, agreed ontologies and controlled vocabularies for tissues, cell types, and treatments, as well as for array designs, image analyses and hybridization protocols have to be

developed. Systematic building up of gene expression matrices for various organisms would be facilitated by establishing a public repository for gene expression data [26].

Like genome sequencing, the systematic gene expression profiling is not the end in itself. It is a long way from having detailed gene expression profiles to real understanding of underlying cellular processes. Bioinformatics methods and tools will be needed to cope with the huge amounts of data, but they will not bring any deep understanding by themselves. On the other hand, the traditional 'gene by gene' methods will not be sufficient to understand gene regulatory networks consisting of thousands or tens of thousands of genes. One of the most challenging downstream goals of gene expression profiling and data analysis is the reverse engineering and modeling of gene regulatory networks (see for instance [27–29]). With biology becoming a more quantitative science, modeling approaches will become more and more usual.

References

[1] Celis J.E., Kruhøffer M., Gromova I., Frederiksen C., Østergaard M., Thykjaer T., Gromov P., Yu Y., Pálsdóttir H., Ørntoft T.F., Gene expression profiling: Monitoring transcription and translation products using DNA microarrays and proteomics, FEBS Lett. 480 (2000) 2–16.

[2] The Chipping Forecast, Nat. Genet. 21 (1999) suppl.

[3] Claverie J.M., Computational methods for the identification of differential and coordinated gene expression, Hum. Mol. Genet. 8 (1999) 1821–1832.

[4] Legendre P., Legendre L., Numerical, Ecology. Developments in Environmental Modelling, Elsevier, Amsterdam, 1998.

[5] D'haesleer P., Wen X., Fuhrman S., Somogyi R., Information Processing in Cells and Tissues, Plenum Press, New York, 1998.

[6] Eisen M., Spellman P.T., Botstein D., Brown P.O., Cluster analysis and display of genome-wide expression patterns, Proc. Natl. Acad. Sci. USA 95 (1998) 14863–14867.

[7] Hartigan J.A., Clustering Algorithms, John Wiley & Sons, New York, 1975.

[8] Tavazoie S., Hughes D., Campbell M.J., Cho R.J., Church G.M., Systematic determination of genetic network architecture, Nat. Genet. 22 (1999) 281–285.

[9] Tamayo P., Slonim D., Mesirov J., Zhu Q., Kitareewan S., Dmitrovsky E., Lander E., Golub T., Interpreting patterns of gene expression with self-organizing maps: Methods and application to hematopoietic differentiation, Proc. Natl. Acad. Sci. USA 96 (1999) 2907–2912.

[10] Brazma A., Jonassen I., Vilo J., Ukkonen E., Predicting gene regulation elements in silico on a genomic scale, Genome Res. 8 (1998) 1202–1215.

[11] Ben-Dor A., Yakhini Z., Proceedings of the Third Annual International Conference on Computational Molecular Biology, ACM Press, Lyon, 1999, pp. 33–42.

[12] Alon U., Barkai N., Notterman D.A., Gish K., Ybarra S., Mack D., Levine A.J., Broad patterns of gene expression revealed by clustering analysis of tumor and normal colon tissues probed by oligonucleotide arrays, Proc. Natl. Acad. Sci. USA 96 (1999) 6745–6750.

[13] DeRisi J.L., Iyer V.R., Brown P.O., Exploring the metabolic and genetic control of gene expression on a genomic scale, Science 278 (1997) 680–686.

[14] van Helden J., André B., Collado-Vides J., Extracting regulatory sites from the upstream region of yeast genes by computational analysis of oligonucleotide frequencies, J. Mol. Biol. 281 (1998) 827–842.

[15] Chu S., DeRisi J.L., Eisen M., Mulholland J., Botstein D., Brown P.O., Herskowitz I., The transcription program of sporulation in budding yeast, Science 282 (1998) 699–705.

[16] Spellman P.T., Sherlock G., Zhang M., Iyer V.R., Anders K., Eisen M., Brown P.O., Botstein D., Futcher B., Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization, Mol. Biol. Cell 9 (1998) 3273.

[17] Holstege F., Jennings E., Wyrick J., Lee T., Hengartner C., Green M., Golub T., Lander E., Young R., Dissecting the regulatory circuitry of a eukaryotic genome, Cell 95 (1998) 717–728.

[18] Cho R.J., Campbell M.J., Winzeler E.A., Steinmetz L., Conway A., Wodicka L., Wolfsberg T.G., Gabrielian A.E., Landsman D., Lockhart D.J., Davis R.W., A genome wide transcriptional analysis of gene expression of the mitotic cell cycle, Mol. Cell 2 (1998) 65–73.

[19] Iyer V.R., Eisen M.B., Ross D.T., Schuler G., Moore T., Lee J.C.F., Trent J.M., Staudt L.M. Jr, J.H., Boguski M.S., Lashkari D., Shalon D., Botstein D., Brown P.O., The transcriptional program in the response of human fibroblasts to serum, Science 283 (1999) 83–87.

[20] Lee C., Klopp R.G., Weindruch R., Prolla T.A., Gene expression profile of aging and its retardation by caloric restriction, Science 285 (1999) 1390–1393.

[21] Alizadeh A.A., Eisen M.B., Davis R.E., Ma C., Lossos I.S., Rosenwald A., Boldrick J.C., Sabet H., Tran T., Yu X., Powell J.I., Yang L., Marti G.E., Moore T., Hudson J. Jr, Lu L., Lewis D.B., Tibshirani R., Sherlock G., Chan W.C., Greiner T.C., Weisenburger D.D., Armitage J.O., Warnke R., Levy R., Wilson W., Grever M.R., Byrd J.C., Botstein D., Brown P.O., Staudt L.M., Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling, Nature 403 (2000) 503–511.

[22] Brown M.P.S., Grundy W.N., Lin D., Cristianini N., Sugnet C.W., Furey T.S., Ares M.J., Haussler D., Knowledge-based analysis of microarray gene expression data by using support vector machines, Proc. Natl. Acad. Sci. 97 (2000) 262–267.

[23] Golub T.R., Slonim D.K., Tamayo P., Huard C., Gaasenbeek M., Mesirov J.P., Coller H., Loh M.L., Downing J.R., Caligiuri M.A., Bloomfield C.D., Lander E.S., Molecular classification of cancer: Class discovery and class prediction by gene expression monitoring, Science 286 (1999) 531–537.

[24] Ben-Dor A., Bruhn L., Friedman N., Nachman I., Schummer M., Yakhini Z., Tissue classification with gene expression profiles, in: Shamir R., Miyano S., Istrail S., Pevzner P., Waterman M. (Eds.), The Fourth Annual International Conference on Computational Molecular Biology RECOMB-2000, ACM Press, Tokyo, 2000.

[25] Vilo J., Brazma A., Jonassen I., Robinson A., Ukkonen E., Proceedings of Eighth International Conference on Intelligent Systems for Molecular Biology, AAAI Press, La Jolla, CA, 2000, pp. 384–394.

[26] Brazma A., Robinson A., Cameron G., Ashburner M., One stop shop for microarray data, Nature 403 (2000) 699–700.

[27] Akutsu T., Miyano S., Kuhara S., The Pacific Symposium on Biocomputing '99 (PSB'99), vol. 3, World Scientific, Hawaii, 1999, pp. 17–28.

[28] Liang S., Fuhrman S., Somogyi R., The Pacific Symposium on Biocomputing, vol. 3, World Scientific, Hawaii, 1998, pp. 18–29.

[29] Thieffry D., Colet M., Thomas R., Formalization of regulatory networks: a logical method and its automation, Math. Model. Sci. Comput. 55 (1993) 144–151.