An Effectiveness Evaluation of Information Technology of Gene Expression Profiles Processing for Gene Networks Reconstruction

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Received: 03 March 2018; Accepted: 24 May 2018; Published: 08 July 2018

Abstract—The paper presents the research results concerning an effectiveness evaluation of information technology of gene expression profiles processing for purpose of gene regulatory networks reconstruction. The information technology is presented as a structural block-chart of step-by-step stages of the studied data processing. The DNA microchips of patients, who were investigated on different types of cancer, were used as experimental data. The optimal parameters of data processing algorithm at appropriate stage of this process implementation by quantity criteria of data processing quality were determined during simulation. Validation of the reconstructed gene networks was performed with the use of ROC-analysis by comparison of character of genes interconnection in both the basic network and networks reconstructed based on the obtained biclusters.

Index Terms—Gene expression profiles, Filtering, Reducing, Clustering, Biclustering, Gene network reconstruction, Gene network validation.

I. INTRODUCTION

Reconstruction and simulation of gene regulatory networks (GRN) based on gene expression profiles is one of the current problems of modern bioinformatics. The implementation of this process corresponds to better understanding of genes interactions character and influence of these interactions to functional possibilities of biological objects. The gene expression profile is a vector of gene expressions, which are determined on different conditions of experiment performing. Two technologies of gene expressions array formation are actual nowadays: DNA microchip technology [1,2] and mRNA sequencing method [3,4]. Each of them has own advantages and disadvantages. However, in any case, the final result is a matrix of genes expressions. The rows of this matrix are genes and the columns present the conditions of experiment performing. Peculiarities of the experimental data are high dimension of feature space and existence of complex noise component, which arises in the case of DNA microchip technology implementation at the stage of experiment performing and initial data formation. The complexity of GRN reconstruction is determined by the following:

- the experimental data do not allow us usually to define the network structure and character of genes interaction;
- the large number of genes, which determine the structure and amount of the network, complicates the process of the obtained results interpretation.

Solving this problem is possible by developing modern methods for processing gene expression profiles obtained by DNA microchip experiments or mRNA molecules sequencing method to reduce non-informative genes and further grouping of remaining genes using modern clustering and biclustering technologies. The final step of this process is the reconstruction and validation of gene networks and following simulation in order to evaluate their adequacy.

The first works concerning gene regulatory networks reconstruction were published at the end of the last century. The papers [5,6] presents the technologies of GRN reconstruction based on linear modeling methods and mutually correlation of gene expression profiles appropriately. The methods of GRN reconstruction and simulation based on Bayes networks and differential equation were analysed in [7,8]. However, it should be noted that the presented methods are based on the use of a small number of genes. The methods of forming an experimental data in order to reconstruct GRN were not consider in these papers. The comparison analysis of different methods of GRN reconstruction with allocation their advantages and disadvantages are presented in reviews [9-12]. However, the methods of optimizing the parameters of the proposed models and algorithms were not considered in these works.
Modern technologies for obtaining gene expression data tends to cover the maximum number of system variables [13]. For example, the technology of DNA microchip or mRNA molecules sequencing method allow measuring the expression of tens of thousands of genes concurrently. In this case, each of the studied object is characterized by a numerical vector of genes expression in the length of tens of thousands of units. GRN reconstruction based on a complete set of genes is problematic because this process requires a large amount of computer resources and the interpretation of the model is a very complicated problem. Biclustering technology is actual to group the genes and samples nowadays [14-16]. However, it should be noted that the application of this technology does not solve the problem of studied data grouping. The use of biclustering algorithms allows us to obtain clusters of mutually correlated genes and samples, but there is a problem of the choice of biclusters quantity and the level of detail of this process. The results of comparison analysis of biclustering algorithms effectiveness with the use of the tested data and gene expression profiles are presented in [17]. The hybrid model of gene expression profiles cluster-bicluster analysis was proposed as the result of research. Data clustering within the framework of this model was carried out with the use of objective clustering inductive technology, the main conception of which is described in [18]. The paper [19] presents the results of research concerning practical implementation the objective clustering inductive technology based on SOTA (Self Organizing Tree Algorithm) and DBSCAN (Density Based Spatial Clustering of Application with Noise) clustering algorithms. The gene expression profiles were used as the test data during simulation process. The results of research concerning development of technology for reconstruction and validation of gene networks based on the obtained biclusters are presented in [20]. However, it should be noted that in spite of achievements in this subject area there are some unsolved problems. So, nowadays there are no effective technologies for both gene expression profiles preprocessing and following grouping genes and samples for purpose of gene regulatory network reconstruction. This fact indicates the relevance of the presented research topic.

The aim of the research is an effectiveness evaluation of the information technology of gene expression profiles processing for gene regulatory networks reconstruction with the use of gene expression profiles of patients, who were investigated on different types of cancer.

The paper is organized as follows. Section 2 presents the structural block-chart of the information technology of gene expression profiles processing and step-by-step procedure of its implementation. Section 3 is devoted to practical implementation of the technology to process the DNA microchip data of patients, who were investigated on different type of cancer. The results of the reconstructed models of gene regulatory networks validation are presented in section 4. This section contains also the discussion of the obtained results. The conclusions are presented in section 5.

II. INFORMATION TECHNOLOGY OF GENE EXPRESSION PROFILES PROCESSING

The architecture of the information technology of gene expression profiles processing for gene regulatory networks reconstruction and validation is presented in Fig. 1.

![Diagram](image)

Fig.1. Information technology of gene expression profiles processing

The practical implementation of this technology involves the following stages:

**Stage I. Formation of genes expressions array**

1. Transformation of the matrix of DNA microchips light intensities to the expressions of the corresponding genes. Implementation of this stage involves four steps: background correction, normalization, PM correction and summarization. Each of these steps can be implemented with the use of different methods. Choice of the optimal combination of the methods within the framework of the
proposed technology was performed based on minimum value of Shannon entropy calculated with the use of James-Stein shrinkage estimator [21].

**Stage II. Gene expression profiles wavelet filtering**

2. Determination of the wavelet filter optimal parameters (type of wavelet, wavelet decomposition level and thresholding coefficient value).
3. Step-by-step gene expression profiles wavelet filtering in accordance with the technology presented in [22].

**Stage III. Gene expression profiles wavelet reducing**

4. Calculation of variance, average absolute value and Shannon entropy for the studied gene expression profiles. Statistical analysis of the obtained vectors. Setup of range of the appropriate parameters change. It was supposed that if values of variance and average absolute value of gene expression profile is less and Shannon entropy is more of the appropriate boundary values, this gene is removed from data as non-informative. Determination of the input parameters boundary values was performed with the use of fuzzy logic inference technology [23-26].
5. Setup of fuzzy logic inference system. Formation of the basic term-set for input variables (variance, average, Shannon entropy), and output parameter, which determines the level of informativity of gene expression profiles \( QL \) (Quality). Formation of fuzzy rules, which are agreed between both the input variables and output parameter.
6. Determination of the boundary value of the output parameter \( QL_{lim} \), which allows us the gene expression profiles to divide into informative and non-informative. Determination of the step of the input variables changing within a given range.
7. Determination of the input parameters boundary values according to technology presented in [27]. Gene expression profiles reducing. Formation of a new array of gene expression profiles.

**Stage IV. Gene expression profiles clustering**

8. Formation of two equal power gene expression profiles subsets (contain the same quantity of pairwise similar profiles).
9. Determination of optimal parameters of clustering algorithms SOTA and DBSCAN using inductive technology of objective clustering [18,19].
10. Gene expression profiles clustering with the use of DBSCAN clustering algorithm. Allocated genes, which are identified as noise. Formation of a new data array.
11. Gene expression profiles clustering with the use of SOTA clustering algorithm. Formation of two clusters of gene expression profiles for the following step of bicluster analysis.

**Stage V. Gene expression profiles biclustering**

12. Determination of “ensemble” biclustering algorithm optimal parameters within the framework of the technology presented in [17].

**Stage VI. Gene regulatory networks reconstruction with the use of correlation inference algorithm**

14. Determination of thresholding coefficient optimal parameters for the basic gene network (reconstructed based on the set of gene expression profiles obtained at step 10 of this procedure) and for gene networks reconstructed on the basis of the obtained biclusters according to the technology presented in [20].

**Stage VII. Validation of the reconstructed models of gene networks**

16. ROC-analysis of the obtained models of gene networks according to technology presented in [20]. Calculation of the validation relative criteria for obtained models of gene networks.
17. Analysis of the obtained results.

III. PRACTICAL IMPLEMENTATION OF THE INFORMATION TECHNOLOGY OF GENE EXPRESSION PROFILES PROCESSING

Three types of DNA microchips of patients, who were investigated on different types of cancer, were used during simulation process. The first data contained 64 DNA microchips investigated on colorectal cancer [28]. 36 patients from them were healthy and other 32 patients had this type of disease. The second data included 26 DNA microchips investigated on prostate cancer [29]. 13 patients from them were healthy and other 9 ones were ill. The third data included 88 DNA microchips investigated on lung cancer [30]. 44 patients from them were recognized as healthy. Fig. 2 shows the results of the first stage of the information technology implementation. This figure contains the charts of Shannon entropy values distribution versus the used method and step of the DNA microchip data processing.

![Fig. 2. Charts of the average of Shannon entropy values distribution versus the step and method of the DNA microchip data processing](image)

Analysis of the obtained results allows us to conclude that optimal in terms of Shannon entropy criterion is the following combination of the methods: “rma”
background correction method, “quantile” normalization method and “mas” methods of PM correction and summarization. The average of Shannon entropy in this case is the minimal that indicates the maximum informativity of the obtained gene expression profiles to compare with the use of other combination of the methods. The obtained data are presented as a matrix, where rows are the studied samples or condition of the experiment performing and columns are the studied genes. The sizes of the studied data are the following:

- patients’ array investigated on colorectal cancer: (64×54675);
- patients’ array investigated on prostate cancer: (26×22277);
- patients’ array investigated on lung cancer: (88×54675).

According to the proposed technology, the next stage of the data processing is the obtained gene expression profiles wavelet filtering. Determination of optimal parameters of wavelet filter was performed by the method, which was described in detail in [22]. Biorthogonal wavelet bior1.5 was used during simulation process. The optimal level of wavelet decomposition was determined based on maximum value of Shannon entropy for allocated noise component. Determination of thresholding coefficient optimal value was carried out on the basis of minimum value of Shannon entropy for filtered data. The results of the simulation are presented in Fig. 3.

![Fig.3. Results of the simulation to determine the wavelet filter optimal parameters: a) chart of Shannon entropy of allocated noise component versus the wavelet decomposition level; b) chart of Shannon entropy of filtered data versus the thresholding coefficient](image)

The analysis of the obtained results allows us to conclude that in all cases the fourth wavelet decomposition level is the optimal one therefore the value of Shannon entropy in these cases for the allocated noise component achieved the maximum values. The optimal thresholding coefficient values are 1.4 for patients’ gene expression profiles investigated on colorectal cancer and lung cancer. In the case of the DNA microarray of patients, who were investigated on prostate cancer the thresholding coefficient optimal value was taken 1.6. In these cases Shannon entropy criterion for the filtered data was minimal. This fact indicates the maximum informativity of the studied gene expression profiles.

Gene expression profiles reducing involves removing genes whose expression profiles have variance and average absolute value less and Shannon entropy more than corresponding boundary values. The technology of gene expression profiles reducing based on fuzzy logic inference system with the use of statistical criteria and Shannon entropy has been proposed in [27]. Variance, average absolute value and Shannon entropy of gene expression profiles were used as input variables. The quality of gene expression profiles was used as output parameter. The range of the output parameter change was divided into five equal sections (very low, low, median, high and very high). Statistical characteristics of input variables for the studied gene expression profiles are presented in tables 1-3. Formalization of the input variables to linguistic estimates for the studied data within the framework of the proposed fuzzy logic inference model are presented in tables 4-6. The Gaussian and triangular membership functions were used for the input and output variables respectively. The genes, which were indicated as very high by quality parameter (≥0.8) for patients’ data, who were studied on colorectal cancer and lung cancer and as high quality (≥0.6) for patients’ data investigated on prostate cancer, were allocated for the following investigation.

<table>
<thead>
<tr>
<th>Table 1. Variation of gene expression profiles variance</th>
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<tbody>
<tr>
<td><strong>Type of cancer</strong></td>
</tr>
<tr>
<td>Colorectal</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Lung</td>
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<table>
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<tr>
<th>Table 2. Variation of gene expression profiles average absolute values</th>
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</thead>
<tbody>
<tr>
<td><strong>Type of cancer</strong></td>
</tr>
<tr>
<td>Colorectal</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Lung</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Table 3. Variation of gene expression profiles Shannon entropy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of cancer</strong></td>
</tr>
<tr>
<td>Colorectal</td>
</tr>
<tr>
<td>Prostate</td>
</tr>
<tr>
<td>Lung</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4. Formalization of input parameters to linguistic estimates for patients’ data investigated on colorectal cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Input parameters</strong></td>
</tr>
<tr>
<td>Variance (Vr)</td>
</tr>
<tr>
<td>Average absolute value (Abs)</td>
</tr>
<tr>
<td>Shannon entropy (Entr)</td>
</tr>
</tbody>
</table>
As the result of the simulation the initial data were transformed as following:

- patients’ gene expression profiles investigated on colorectal cancer: (64 × 54675) → (64 × 1758);
- patients’ gene expression profiles investigated on prostate cancer: (26 × 22277) → (26 × 1990);
- patients’ gene expression profiles investigated on lung cancer: (88 × 54675) → (88 × 2126).

The next stage of the proposed technology implementation involves cluster-bicluster analysis in order to allocate groups of genes and samples for following gene networks reconstruction. Structural block-chart of cluster-bicluster technology based on DBSCAN and SOTA clustering algorithms and biclustering method “ensemble” is presented in Fig. 5 [17-19]. Practical implementation of this technology involves the following steps:

1. Division of the initial gene expression profiles dataset into two equal power subsets, which contain the same quantity of pairwise similar objects;
2. Determination of the optimal parameters for DBSCAN and SOTA clustering algorithms operation according to the technology presented in [19].
4. Clustering of the obtained gene expression profiles with the use of SOTA clustering algorithm. Formation of two subsets of gene expression profiles for the following bicluster analysis.
5. Determination of the optimal parameters for “ensemble” biclustering algorithm according to the technology presented in [17].

![Problem statement, aim formation](image)

![Data clustering with the use of DBSCAN algorithm](image)

![Data clustering with the use of SOTA algorithm](image)

Fig. 6 shows the simulation results to determine the optimal parameters of DBSCAN clustering algorithm operation within the framework of the objective clustering inductive technology. Minimal quantity of points (MinPts) inside epsilon-neighborhood (EPS) was changed from 3 to 4. Increase of MinPts value promoted to unsatisfactory results because many little clusters and many genes, which were identified as noise, appeared in these cases. The analysis of the obtained results allows us...
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to determine the following parameters of DBSCAN clustering algorithm operation: gene expression profiles of patients, who were investigated on colorectal cancer: 1) EPS = 0.22, MinPts = 3; 2) EPS = 0.27, MinPts = 4. In the first case 92 genes and in the second 343 ones were identified as noise. The second case was selected. The number of genes was change from 1758 to 1666;

- gene expression profiles of patients, who were investigated on prostate cancer: 1) EPS = 0.19, MinPts = 3; 2) EPS = 0.26, MinPts = 4. In the first case 285 genes and in the second 428 ones were identified as noise. The first case was selected. The number of genes was change from 1990 to 1705;

- gene expression profiles of patients, who were investigated on lung cancer: 1) EPS = 0.2, MinPts = 3; 2) EPS = 0.42, MinPts = 3; 3) EPS = 0.2, MinPts = 4; 4) EPS = 0.4, MinPts = 4. In the first case 523 genes, in the second 331, in the third 570 and in the fourth 470 genes were identified as noise. The second case was selected. The number of genes was change from 2126 to 1795.

The results of the DBSCAN clustering algorithm operation is shown in Fig. 7.

Fig. 6. Charts of complex balance criterion versus the EPS and MinPts values for patients’ gene expression profiles, who were investigated on: a,b) colorectal cancer; c,d) prostate cancer; e,f) lung cancer

The results of simulation to determine the scell optimal parameter of SOTA clustering algorithm. The value of this parameter was changed within the range from 0.001 to 0.2 with step 0.005. The general Harrington desirability index (complex balance criterion) was calculated at each step of data clustering according to the technology presented in [19]. The following values of scell parameter were determined during simulation process:

- gene expression profiles of patients, who were investigated on colorectal cancer: scell = 0.051;
- gene expression profiles of patients, who were investigated on prostate cancer: scell = 0.051;
- gene expression profiles of patients, who were investigated on lung cancer: scell = 0.26.

In all cases the studied gene expression profiles were divided into two clusters. The results of the algorithm operation is presented in Fig. 9.

Bicluster analysis was performed according to the technology presented in [17] with the use of "ensemble" biclustering algorithm. Firstly, the optimal parameters of “ensemble” biclustering algorithm were determined. Then, the biclustering of the studied gene expression profiles was carried out with the use of the optimal parameters of the biclustering algorithm operation. Five the largest biclusters were selected from each subset of the studied gene expression profiles. The results of biclustering for all types of gene expression profiles are presented in tables 7-9.
Table 7. Results of biclustering of patients’ gene expression profiles investigated on colorectal cancer

<table>
<thead>
<tr>
<th>Objects</th>
<th>911 of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biclust.</td>
<td>BC1 BC2 BC4 BC5 BC8</td>
</tr>
<tr>
<td>Genes</td>
<td>108 97 18 24 243</td>
</tr>
<tr>
<td>Samples</td>
<td>29 26 25 23 32</td>
</tr>
</tbody>
</table>

Table 8. Results of biclustering of patients’ gene expression profiles investigated on prostate cancer

<table>
<thead>
<tr>
<th>Objects</th>
<th>823 of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biclust.</td>
<td>BC1 BC2 BC4 BC5 BC6</td>
</tr>
<tr>
<td>Genes</td>
<td>79 84 97 72 126</td>
</tr>
<tr>
<td>Samples</td>
<td>8 5 4 7 4</td>
</tr>
</tbody>
</table>

Table 9. Results of biclustering of patients’ gene expression profiles investigated on lung cancer

<table>
<thead>
<tr>
<th>Objects</th>
<th>950 of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biclust.</td>
<td>BC1 BC3 BC4 BC5 BC6</td>
</tr>
<tr>
<td>Genes</td>
<td>54 29 19 27 16</td>
</tr>
<tr>
<td>Samples</td>
<td>29 28 28 14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Objects</th>
<th>845 of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biclust.</td>
<td>BC1 BC2 BC5 BC6 BC7</td>
</tr>
<tr>
<td>Genes</td>
<td>18 41 17 21 18</td>
</tr>
<tr>
<td>Samples</td>
<td>26 30 52 29 26</td>
</tr>
</tbody>
</table>

IV. GENE REGULATORY NETWORKS RECONSTRUCTION AND VALIDATION

Gene regulatory networks reconstruction and their validation were carried out with the use of technology, which was described in [20]. Correlation inference algorithm of gene regulatory network reconstruction was used during the simulation process. Firstly, the thresholding coefficient optimal value of the used algorithm was determined. At this stage it were investigated both the basic gene networks, which were reconstructed based on the data obtained as the result of DBSCAN clustering algorithm operation and gene networks, which were reconstructed based on the obtained biclusters. Gene network validation involves the comparison analysis of interconnection character between genes in both the basic networks and networks reconstructed on the basis of obtained biclusters. The technology of ROC-analysis was used at this stage. A structural block chart of gene networks validation technology is presented in Fig. 10. Practical implementation of this technology involves the following steps:

1. Data preprocessing: filtering, reducing, clustering and biclustering of gene expression profiles.
2. Reconstruction of basic gene network based on the data from the obtained clusters after BDSCAN clustering algorithm operation.
3. Reconstruction of gene networks based on the data from the relevant biclusters.
4. Determination of the quality parameters for the classification of interconnection character between genes in the appropriate networks (TP(true positive), TN(true negative), FP(false positive), FN(false negative)).
5. Calculation of relative quality parameters for the model quality estimation Sc(sensitivity), Sp(specificity), FPR(false positive ratio).
6. Calculation of relative validation criterion: \( RVC = \frac{Sc}{FPR} \). Analysis of the obtained results.

![Fig. 10. Structural block chart of technology of gene regulatory networks validation](image_url)

Fig. 11 shows the results of validation of the reconstructed gene networks models.
The analysis of the obtained results allows us to conclude that the reconstructed gene network have high level of adequacy since the value of relative validation criterion in all cases is essentially more than 1 (statistically insignificant). The gene networks reconstructed on the basis of data of the biclusters for which the value of the relative validation criterion is greatest have the particular interest for further modeling. These networks are the most adequate to the basic gene network reconstructed based on full dataset of studied genes and samples. Further modeling of gene regulatory networks allows us to consider the particularities of influence of the expression of individual genes to the expression values of other genes of network for purpose of better understanding character of interconnection between genes for different type of diseases and for different state of the investigated biology object.

V. CONCLUSION

The research concerning practical implementation of the information technology of gene expression profiles processing for purpose of gene regulatory networks reconstruction and validation has been presented in the paper. The DNA microchips of patients, who were investigated on colorectal cancer, prostate cancer and lung cancer, have been used during simulation process. The first step involved determination of optimal combination methods to obtain the array of genes expression profiles. Shannon entropy calculated with the use of James-Stein shrinkage estimator has been used as criterion during implementation of this stage. The following combination of methods has been determined as the result of simulation: “rma” background correction method, “quantile” normalization method and “mas” methods of PM correction and summarization. The second stage involved wavelet filtering of the studied gene expression profiles. Biorthogonal wavelet bior1.5 have been used during simulation process. The optimal level of wavelet decomposition was determined based on maximum value of Shannon entropy for allocated noise component. Determination of thresholding coefficient optimal value was carried out on the basis of minimum value of Shannon entropy for filtered data. The results of the simulation have shown that in all cases the fourth level of wavelet decomposition was an optimal. The thresholding coefficients values to process the detail coefficients were the following: 1.4 for patients’ gene expression profiles, who were investigated on colorectal cancer, and 1.6 for patients’ gene expression profiles, who were investigated on prostate cancer and lung cancer. Implementation of gene expression profiles reducing technology has been performed with the use of statistical criteria and Shannon entropy. It was supposed that if values of variance and average of absolute value of gene expression profile is less and Shannon entropy is more of boundary values of the appropriate parameters, this gene is removed from data as non-informative. Determination of the input parameters boundary values has been performed with the use of fuzzy logic inference technology. The quantity of genes for the following investigation has been essentially decreased during simulation process.

Implementation of the cluster-bicluster technology involved three steps. Firstly, the genes which were identified as noise have been removed by DBSCAN clustering algorithm operation. Then, the gene expression profiles have been divided into two groups with the use of SOTA clustering algorithm. Finally, the biclusters have been allocated by the use of “ensemble” biclustering algorithm. Determination of optimal parameters of the clustering algorithms has been carried out within the framework of objective clustering inductive technology. The optimal parameters of biclustering algorithm have been performed based on minimum value of internal biclustering quality criterion.

The correlation inference algorithm has been used for gene networks reconstruction. Topology of the reconstructed networks is determined by thresholding coefficient value. Determination of optimal value of the thresholding coefficient has been performed on the basis of maximum value of the complex topological parameter. The finally stage of the proposed technology implementation is validation of the reconstructed networks. ROC-analysis has been used to implement of this stage. The charts of relative validation criteria distribution for reconstructed gene networks have been constructed. The analysis of the obtained results has shown high effectiveness of the proposed technology because the value of relative validation criterion for all gene networks was essentially more than 1. This fact indicates the high level of adequate of networks reconstructed based on biclusters to network reconstructed on the basis of full set of genes.

REFERENCES


Authors’ Profiles

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