

Construct, Analyze and Compare Biological Networks with InSyBio BioNets

November 2019

Insybio Suite v2.6



InSyBio
Intelligent Systems Biology

User Manual

www.insybio.com

InSyBio BioNets

BioNets is a biological networks analysis tool for the

- Preprocessing and analysis of gene expression data
- Biomarker discovery through differential expression analysis and/or network comparison
- Preprocessing, meta-analysis and visualization of biological networks

The execution of complex biological processes requires the precise interaction and regulation of thousands of molecules. Systematic approaches to study large numbers of proteins, metabolites, and their modifications have revealed complex molecular networks. Network representation of intracellular biological networks typically considers molecular components within a cell as nodes and their direct or indirect interactions as edges. Network representation enables integration of data from many different studies into a single framework.

Biological networks are significantly different from random networks and often exhibit ubiquitous properties in terms of their structure and organization. The analysis of these networks provides novel insights in understanding basic cellular mechanisms and the mechanisms of disease pathologies.

There exist different categories of biological networks. With InSyBio BioNets users can handle undirected biological networks. The main undirected biological networks are gene co-expression and protein-protein interaction networks. InSyBio BioNets can also analyze directed biological networks but the edges' direction will be ignored.

In gene co-expression networks, each node is a gene and each edge indicates a correlation between the expression profiles of the two-node genes it connects. Gene co-expression networks can be used to find genes with similar functional properties and to uncover biomarkers through the comparison of networks derived from different states (e.g. control vs disease states). Gene co-expression networks can be

constructed by using gene expression experimental techniques, such as microarray or RNA-seq experiments.

Protein-protein Interaction (PPI) networks consist of nodes which represent proteins and edges which represent the physical or functional interactions between the nodes-proteins. PPI networks can be constructed using experimental (e.g. Yeast2hybrid technique [1], Tandem Affinity Purification [2] and so on) or computational techniques. The edges of the PPI networks can have weights representing the strength or the confidence score of the interaction. PPI networks can be used to functionally characterize proteins, predict protein complexes and protein biomarkers when comparing PPI networks from different cellular states to locate significantly altered regions of the network.

BioNets provides a set of tools for the construction, preprocessing, meta-analysis and visualization of biological networks. Moreover, additional tools for parsing gene expression files, creating gene co-expression files and uncovering differential expression biomarkers have been added to InSyBio BioNets to enable the construction and analysis of gene co-expression networks. Additional tools are also offered to ease InSyBio BioNets users. Specifically, the users can perform time consuming analysis steps and analyze large biological networks and large gene expression files using our user friendly job scheduling mechanism. Regarding the uncovered biomarkers, users have access to informative biomarker reports which include links to other publicly available databases for these biomarkers, links to our PPI interaction repository (InSyBio Interact) and information about the prior knowledge on associating these biomarkers to diseases.

With InSyBio BioNets you can:

1. Parse .soft files
2. Parse gene expression data files
3. Construct gene co-expression networks from gene expression data
4. Uncover biomarkers using differential expression analysis
5. Uncover biomarkers using network comparison analysis
6. Merge differential expression biomarkers with network comparison biomarkers

7. Access informative biomarker reports on the extracted biomarkers
8. Analyze biological networks to control their quality, find significant nodes and edges, conduct shortest path analysis and so on.
9. Visualize biological networks
10. Predict clusters (e.g. protein complexes) from a biological network (e.g. Protein-Protein Interaction graph)
11. Combine networks
12. Analyze large biological networks and large gene expression files using our user friendly job scheduling mechanism

BioNets Jobs Dashboard

The analysis of biological networks and of gene expression data includes some time intensive steps. To deal with this complexity, InSyBio BioNets offers a simple and user friendly Jobs Dashboard. Users can start as many jobs as they need and monitor the progress of their jobs using BioNets Jobs Dashboard. Metadata concerning the execution of the analysis task (start date, duration, job type, and so on) are presented in the Dashboard. When a job is completed, the results can be accessed through the View Results link.

Status	Job ID	Job Type	Input File(s)	Submission Date	Start Execution Date	Completion Date	Actions
Completed	35	Merging network and differential expression biomarkers	dsfile1570631741_6842.txt (diffexpr_21.txt) Biomarkers from the comparison of a)Co-expression Network from healthy control and b)Co-expression Network from Parkinson's disease (netcompbiomarkers_23.txt)	11/19/19 11:24 AM	11/19/19 11:24 AM	11/19/19 11:24 AM	View Results
Completed	34	Merging network and differential expression biomarkers	dsfile1570631741_6842.txt (diffexpr_21.txt) Biomarkers from the comparison of a)Co-expression Network from healthy control and b)Co-expression Network from Parkinson's disease (netcompbiomarkers_23.txt)	11/19/19 11:20 AM	11/19/19 11:22 AM	11/19/19 11:22 AM	View Results
Error	33	Merging network and differential expression biomarkers	Differential expression file created from MQ_significant_1_VS_MQ_significant_0 (diff_express_file_MQ_significant_1_VS_MQ_significant_0.tsv) Biomarkers from the comparison of a)Co-expression Network from healthy control and b)Co-expression Network from Parkinson's disease (netcompbiomarkers_23.txt)	11/19/19 11:16 AM	11/19/19 11:16 AM	11/19/19 11:16 AM	View Details
Completed	32	Analyse biological networks	gene network (gene_net.tsv)	11/15/19 3:03 PM	11/15/19 3:03 PM	11/15/19 3:03 PM	View Results
Completed	31	Predict Network Complexes from Biological Networks	Co-expression Network from Parkinson's disease (coexpnet_9.txt)	10/15/19 12:57 PM	10/15/19 12:57 PM	10/15/19 12:57 PM	View Results
Error	30	Predict Network Complexes from Biological Networks	Co-expression Network from Parkinson's disease (coexpnet_9.txt)	10/15/19 12:51 PM	10/15/19 12:51 PM	10/15/19 12:51 PM	View Details
Error	29	Predict Network Complexes from Biological Networks	Co-expression Network from healthy control (coexpnet_8.txt)	10/15/19 12:50 PM	10/15/19 12:50 PM	10/15/19 12:50 PM	View Details
Error	28	Predict Network Complexes from Biological Networks	create_net_1 (dsfile1570793154_9932.tsv)	10/15/19 12:26 PM	10/15/19 12:26 PM	10/15/19 12:26 PM	View Details
Error	27	Predict Network Complexes from Biological Networks	Co-expression Network from Parkinson's disease (coexpnet_9.txt)	10/15/19 12:26 PM	10/15/19 12:26 PM	10/15/19 12:26 PM	View Details

Gene Expression Data Parsing, Preprocessing and Analysis

BioNets offers a set of tools for handling, preprocessing and analyzing gene expression data in order to construct gene co-expression networks, and predict biomarkers.

SOFT Files Parsing

InSyBio BioNets supports the universally accepted format for gene expression data named SOFT. Simple Omnibus Format in Text (SOFT) is the format supported by Gene Expression Omnibus database [13] (<http://www.ncbi.nlm.nih.gov/geo/>) and it is the prevalent format for gene expression experiments. It is a simple line-based, plain text format, meaning that SOFT files may be readily generated from common spreadsheet and database applications. A single SOFT file can hold both data tables and accompanying descriptive information for multiple, concatenated Platforms, Samples, and/or Series records.

InSyBio BioNets soft parsing includes the following preprocessing steps:

- logarithmic normalization (if the expression values are not normalized),
- missing values estimation with the knn-impute method,
- filtering using minimum average expression values and minimum expression values variance filters.

The different experimental states (conditions) defined in the SOFT file will be automatically recognized and a gene expression tab delimited file will be constructed for each state.

Users are enabled either to use default parameter values or to tune the following parameters:

- Minimum number of experiments in every single experimental state (default value 3)

- Minimum average expression value (default value 0.0)
- Minimum allowed variance of gene expression values (default value 0.0)

Do you have a GDS or a GSE file:

Title:

Filename:

Advanced Options

Minimum number of experiments:

Minimum average expression value:

Minimum allowed variance of gene expression values:

When a SOFT file is parsed, the users can further analyze the extracted tab delimited gene expression files. They can use them either to create a co-expression network or to extract biomarkers using differential expression analysis.

Job Status Job ID Submission Date Execution Time Input Data and Parameters

COMPLETED 7 Oct 8, 2019 1:34:56 PM 00 hours, 00 minutes, 49 seconds

Molecules Quantification File	State	Download	Next Action
softparse_7_healthy control.txt	healthy control	<input type="button" value="Download"/>	--Select Action--
softparse_7_neurodegenerative disease control.txt	neurodegenerative disease control	<input type="button" value="Download"/>	--Select Action--
softparse_7_Parkinson's disease.txt	Parkinson's disease	<input type="button" value="Download"/>	--Select Action--

First Previous 1 Next Last Show 10 entries Showing 1 to 3 of 3 entries

Upload gene expression files

Users can generate gene expression files using their own methods and directly upload them to InSyBio DataStore. The uploaded files should be tab delimited files

with the first column reporting the gene's symbol or name and the other columns being the expression values in different experiments, conditions and/or samples.

Gene co-expression network creation

Gene expression files, either uploaded directly by the users or generated through soft files parsing, can be used to generate weighted gene co-expression networks. Experienced users can tune the parameters of the algorithms used for this step and select the most suitable algorithm for them. Three options are offered:

- **Pearson Correlation [14]:** This method adds an edge to a network if the Pearson correlation of the nodes adjacent to the edge exceeds a threshold.
- **Mutual information [15]:** This method adds an edge to a network if the mutual information among the expression profiles of the two nodes of the edge exceeds a threshold.
- **Spearman Correlation [23]:** This method adds an edge to a network if the Spearman correlation of the nodes adjacent to the edge exceeds a threshold.

The thresholds for adding edges are dynamically generated to alleviate problems occurring by using the same threshold for all nodes. In particular, for a single node Pearson correlations or Mutual Information or Spearman correlations between this node and all other nodes are calculated. Assuming that the Pearson correlation/Mutual Information/Spearman correlation values between a single node and all other nodes follow a normal distribution, then the threshold for adding edges is selected to be in a predefined confidence interval (90%, 95% or 99%). The confidence interval is predefined at 99% but the users can change this value in order to get denser or sparser networks. In order to force nodes to have a minimum number of edges users can also specify a minimum value for the threshold of adding an edge in the network. Experienced users can further filter nodes from the network by altering the minimum expression variance threshold and the minimum average of the logarithmized expression values threshold.

InSyBio Suite - Gene Co-expression Network Creation

Title:

Filename:

[Select file from Data Store](#) [Go to Data Store to Upload File](#)

Advanced Options

Method:

Method's minimum threshold:

Interval of trust:

Filtering parameter minimum variance:

Filtering parameter minimum average logarithmized expression:

[Submit Job](#)

View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of the analysis you can select the “View Results” at the Actions column and view the created network.

InSyBio Suite - Gene Co-expression Network Creation Results

Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters
COMPLETED	26	Oct 15, 2019 12:02:11 PM	03 hours, 34 minutes, 37 seconds	View Results

Network Title: Co-expression Network from Parkinson's disease

File: coexpnet_26.txt

[Download](#)

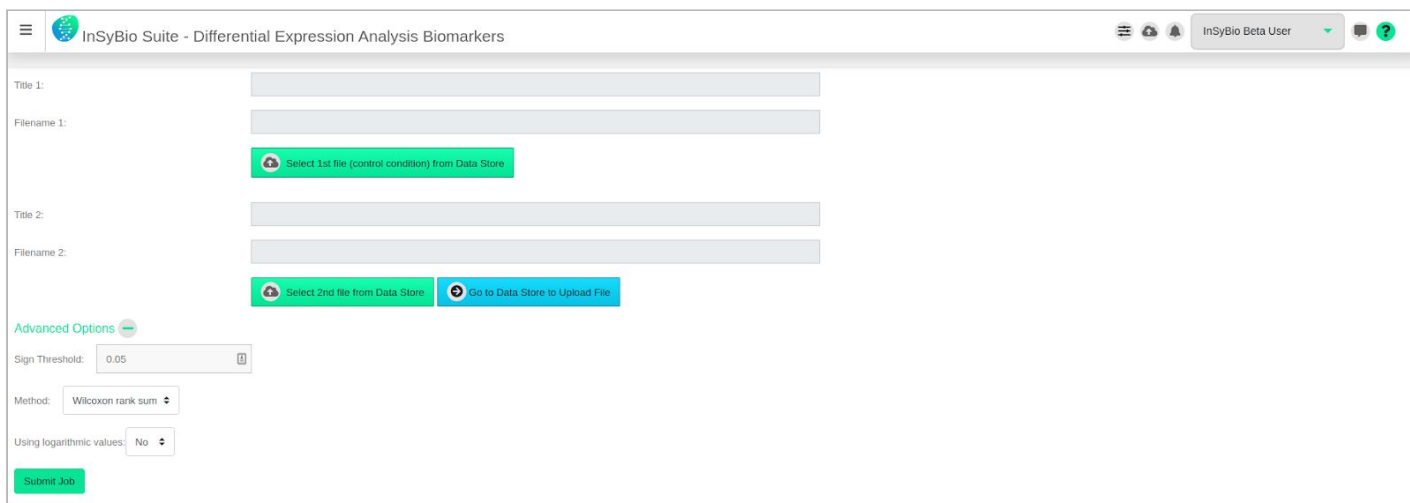
Next Action: --Select Action--

Showing 1 to 1 of 1 entries

Differential Expression Analysis Biomarkers

Another action the users can perform if they have two gene expression files is the differential expression analysis to uncover biomarkers. Experienced users can select among T-Test [16] and Wilcoxon Rank Sum [17] statistical methods, stating their

p-value thresholds. Default method and threshold proposed by InSyBio are Wilcoxon Rank Sum with p-value threshold equal to 0.05. Bonferoni corrections [18] are by default applied in the computation of a p-value to reduce the number of false positive predictions. Also the users can choose to use logarithmic values.



The screenshot shows the InSyBio Suite interface for Differential Expression Analysis Biomarkers. It features a header with the InSyBio logo and the text "InSyBio Suite - Differential Expression Analysis Biomarkers". The main area contains input fields for "Title 1:" and "Filename 1:", followed by a green button "Select 1st file (control condition) from Data Store". Below these are input fields for "Title 2:" and "Filename 2:", followed by two buttons: "Select 2nd file from Data Store" and "Go to Data Store to Upload File". An "Advanced Options" section is expanded, showing a "Sign Threshold" input field with the value "0.05", a "Method" dropdown menu set to "Wilcoxon rank sum", and a "Using logarithmic values" dropdown menu set to "No". A green "Submit Job" button is located at the bottom left of the form.

View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of the analysis you can select the “View Results” at the Actions column and view the differential expression analysis biomarkers.

The results are presented in a table with Gene, P-value, Average expression in control samples, Average expression in examined phenotype/condition samples, Fold-change or log-fold change in logarithmic expression values (Phenotype/Control), Database, Related Uniprot ID, Link to External Databases columns. Clicking a Gene Expression field the user can view diseases associated with that gene. Clicking a Related Uniprot ID field the user can view the related protein in our InSyBio Interact tool. Clicking a Link to External Databases the user can view the gene in external databases.

The users are enabled to download detailed reports about their extracted biomarkers.

InSyBio Suite - Differential Expression Analysis Biomarkers Results

Job Status: **COMPLETED** Job ID: 17 Submission Date: Oct 9, 2019 2:24:00 PM Execution Time: 00 hours, 00 minutes, 04 seconds

Merge it with Network Comparison Biomarkers [Export Results](#)

Gene Expression	P-value	Average expression in control samples	Average expression in examined phenotype/condition samples	Fold-change or log-fold change in logarithmic expression values (Phenotype/Control)	Database	Related Uniprot ID	Link to External Databases
ST13	4.2176647935e-05	0.5509855393915455	0.53489031930978	0.9787883076213952	Gene Symbols	P50502, Q3KMR6, Q0I156, Q9P114, H7C311, F6VDH7, F8WA07	Genecards OMIM
ITCH	5.20270174719e-05	0.465061060698	0.40151230014936014	1.035374363807483	Gene Symbols	Q96J02	Genecards OMIM
SKP1	0.27458591157e-05	0.5906480927294543	0.5785392819772199	0.9794991113976546	Gene Symbols	P63208, F8W8N3, E5RJRS, E5RGM3, E7ERH2, E5RGM4, E5RK33, E5RHM3	Genecards OMIM
ATP8A1	0.000325618020956	0.4380780004832727	0.40601145370454014	0.9286279001816106	Gene Symbols	Q9Y200, Q4G1C1, H0YAF4, H0YAJ4, H0YAA1, H0Y8I6	Genecards OMIM
UCHL1	0.000411036153619	0.441796919744	0.4762137208538999	1.0779018584598616	Gene Symbols	P89936, A6NLJ7, D6R974, D6RE83, D6RF53, D6R956, V9HW74, D6RJ09	Genecards OMIM
FOFT1	0.000430457720909	0.5920600765785454	0.5023757091605203	0.9036429311802443	Gene Symbols	P37260, Q6IAX1, E9PNJ2, E9PNM1, E9PJG4, E9PQ00, E9PS69, E9PSH1	Genecards OMIM
GPR68	0.000724626682887	0.35344894982290903	0.400668274592590003	1.1336368268363985	Gene Symbols	Q15743	Genecards OMIM
PDE6D	0.000774499269602	0.42039439936181017	0.40075438792591994	0.9333013856760496	Gene	Q43924, B8ZZK5, Q6I024, C9I252	Genecards

Biomarker Discovery

Using InSyBio BioNets, users can uncover biomarkers by applying:

- Differential Expression Analysis on gene expression files
- Biological Networks Comparison
- Differential Expression Biomarkers and Network Based Biomarkers merging

InSyBio BioNets automatically detects the type of symbols used for the network's nodes. Most known symbol types are supported by InSyBio to generate advanced reports including:

- Gene Symbols,
- Uniprot ids,
- Gene ids,
- EMBL ids,
- Refseq ids,
- RefseqNT ids,
- Kegg ids,
- Reactome ids,
- and many more (this list is continuously updated)

When the symbol's type is detected, then the biomarkers report provides information about the significance of the biomarker, links to InSyBio Interact Tool about the proteins being related with this biomarker, links to Genecards [19] and OMIM [20] and information about prior knowledge associating this biomarker with diseases with information mined from DisGeNet database [21].

Differential expression biomarkers are measured with a single p-value and network based biomarkers with a confidence score. Users have the option to combine the two experiments by predicting combined biomarkers and there BioNets uses a combined confidence score. The combined biomarkers have significantly different expression profiles on the two examined conditions while their role in the network is significantly altered.

Differential Expression Analysis Biomarkers

Differential expression analysis predicts differentially expressed biomarkers. [See above.](#)

Network Comparison Biomarkers

It is widely accepted lately that differential expression biomarkers are large in numbers, contain a large number of false positives and mainly depict the outcome of disease mechanism and not its cause. For this reason, the current trend in biomarker discovery is to detect biomarkers by comparing biological networks. Biological networks are slightly altered in different biological conditions and changes on them are associated with the causes of disease mechanisms with high probability.

When having two biological networks of different conditions, users can use them to predict network biomarkers with an InSyBio's novel methodology. In particular, a certain network metric is selected and InSyBio BioNets attempts to detect network's nodes with significantly altered values for this network metric. Thus, our approach finds nodes whose role in the network has significantly changed among the different conditions. Experienced users can select a specific network metric among the following ones:

- Degree Centrality [9]
- Clustering Coefficient [8]
- Pagerank method [6, 7]

Pagerank method is the default one. This method triggers random walkers starting from each node. Significant nodes are collecting more information from the diffused quantities of the random walkers over time. Experienced users can also select the confidence interval for tuning the threshold of assigning a node as biomarker. Higher confidence interval values lead to the extraction of more compact sets of biomarkers.

View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of the analysis you can select the “View Results” at the Actions column and view the network comparison biomarkers.

Gene Expression	Confidence Score	Centrality metric in control network	Centrality metric in examined phenotype/condition network	Difference in centrality metric between examined phenotype and control networks	Database	Related Uniprot ID	Link to External Databases
HBA2	1.0	0.02940816326530612	0.1	0.003401360544217687	Gene Symbols	P69905, G3VIN2, Q7Z6G4, U6A493, D1MGQ2, Q9GT46, E9LUX2, U3PXP0, Q86YQ1, Q86YQ5, U6A3P2	Genecards OMIM
B2M	1.0	0.003401360544217687	0.1	0.01020408163265306	Gene Symbols	P61769, Q16446, F5H6I0, K7NSM3, H0YLF3, K7NSM4, J3KNU0	Genecards OMIM
HBB	0.9877450980392157	0.013605442176870748	0.1	0.030612244897959183	Gene Symbols	P68871, Q7Z2K5, Q4TWB7, Q0Z944, Q6VF05, Q14484, Q9UBV6, Q9GZL9, F8W6P5, B2M0Y1, Q8IUL9, Q4TZM4, A9YUX2, Q631Z8, Q5GM01, Q9H115, J7LKS8, Q14477, Q95412, E9M263, Q52MT0, Q9HAR8, Q4JLR8, Q9BWV6, B5ANL9, B2M157, Q9UKS4, Q3Y918... This entity is associated with more UniProt IDs.	Genecards OMIM
ACTR2	0.6642156862745099	0.02940816326530612	0.2	0.003401360544217687	Gene Symbols	P61160, F5H6T1, Q8IY98	Genecards OMIM
YWHAZ	0.6642156862745099	0.027210884353741496	0.1	0.10979591836734694	Gene Symbols	P63104, B8AZ56, B7Z2E6, D0PN11, E5RGE1, E7EVZ2, E7ESK7, E7EX29, H0YB80, E9PD24, E5RIR4	Genecards OMIM
RPL3	0.6642156862745099	0.03401360544217687	0.1	0.003401360544217687	Gene Symbols	P39023, G5E9G0, Q49A39, B4DN06, B5MCW2, H7C422, H7C3M2, F8WCRI1, Q960L0, Q9NY85, Q9BT63	Genecards OMIM
RPL4	0.6519607843137255	0.006802721088435374	0.2	0.006802721088435374	Gene Symbols	P36578, H3BM89, H3BU31, H3BT97	Genecards OMIM

The results are presented in a table with Gene, Confidence Score, Centrality metric in control network, Centrality metric in examined phenotype/condition network, Difference in centrality metric between examined phenotype and control networks,

Database, Related Uniprot ID, Link to External Databases columns. Clicking a Gene Expression field the user can view diseases associated with that gene. Clicking a Related Uniprot ID field the user can view the related protein in our InSyBio Interact tool. Clicking a Link to External Databases the user can view the gene in external databases.

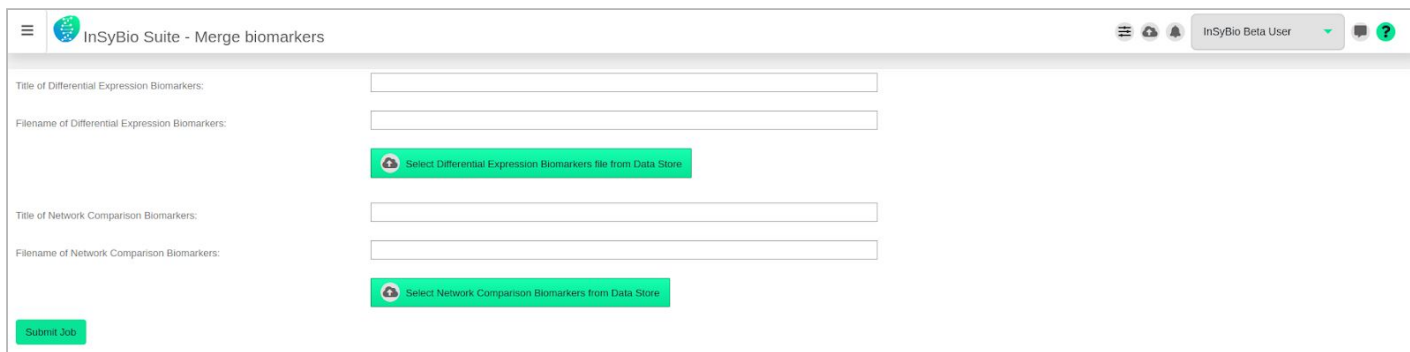
The users are allowed to download detailed reports about their extracted biomarkers.

The screenshot displays the InSyBio Suite interface. A modal window titled "HBA2 (GENESYMBOLS)" is open, showing a table of "Related Diseases". The table has columns for Disease ID, Disease Name, Gene Symbol, Official Gene Symbol, Uniprot ID, Score, and Association Type. The table lists 10 entries, with the first 5 visible. The background shows the InSyBio Suite navigation menu and a list of biomarkers.

Disease ID	Disease Name	Gene Symbol	Official Gene Symbol	Uniprot ID	Score	Association Type
unls:C0902312	alpha-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.367556	Biomarker
unls:C0902312	alpha-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.367556	GeneticVariation
unls:C0902312	alpha-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.367556	AlteredExpression
unls:C0905283	beta-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.348328	Biomarker
unls:C0905283	beta-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.348328	AlteredExpression
unls:C0905283	beta-Thalassemia	HBA2	hemoglobin, alpha 2	-	0.348328	GeneticVariation
unls:C0709299	Heinz Body Anemias	HBA2	hemoglobin, alpha 2	-	0.3	Biomarker
unls:C0263454	Chloracne	HBA2	hemoglobin, alpha 2	-	0.3	Biomarker
unls:C1415477	HEMOGLOBIN--ALPHA LOCUS 1	HBA2	hemoglobin, alpha 2	-	0.1	Biomarker
unls:C1415481	HEMOGLOBIN--BETA LOCUS	HBA2	hemoglobin, alpha 2	-	0.1	Biomarker

Merge Biomarkers

Users can select to merge the biomarker results of differential expression analysis and network comparison analysis. In fact the real biomarkers should have different expression profiles in the examined biological conditions and their role in the biological networks should be significantly altered in order to reassure that they are the real cause of biological variation among the condition and not a result of this variation.



The screenshot displays the 'Merge biomarkers' interface in the InSyBio Suite. It features two main sections for inputting biomarker data. The first section, 'Differential Expression Biomarkers', includes text input fields for 'Title' and 'Filename', and a green button labeled 'Select Differential Expression Biomarkers file from Data Store'. The second section, 'Network Comparison Biomarkers', also includes text input fields for 'Title' and 'Filename', and a green button labeled 'Select Network Comparison Biomarkers from Data Store'. A 'Submit Job' button is located at the bottom left. The top navigation bar contains the InSyBio logo, the page title 'InSyBio Suite - Merge biomarkers', and the user profile 'InSyBio Beta User'.

View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of the analysis you can select the “View Results” at the Actions column and view the merged biomarkers.

The results are presented at a table with Gene, Combined confidence score, Differential expression analysis confidence score, Average expression in control samples, Average expression in examined phenotype/condition samples, Fold-change or log-fold change in logarithmic expression values (Phenotype/Control), Network comparison confidence score, Centrality metric in control network, Centrality metric in examined phenotype/condition network, Difference in centrality metric between examined phenotype and control networks, Database, Related Uniprot ID, Link to External Databases columns. Clicking a Gene Expression field the user can view diseases associated with that gene. Clicking a Related Uniprot ID field the user can view the related protein in our InSyBio Interact tool. Clicking a Link to External Databases the user can view the gene in external databases.

The users are allowed to download detailed reports about their extracted biomarkers.

InSyBio Suite - Merge Biomarkers Results

Job Status: **COMPLETED** Job ID: 34 Submission Date: Nov 19, 2019 11:20:02 AM Execution Time: 00 hours, 00 minutes, 01 seconds

Export Results

Gene Expression	Combined confidence score	Differential expression analysis confidence score	Average expression in control samples	Average expression in examined phenotype/condition samples	Fold-change or log-fold change in logarithmic expression values (Phenotype/Control)	Network comparison confidence score	Centrality metric in control network	Centrality metric in examined phenotype/condition network	Difference in centrality metric between examined phenotype and control networks	Database	Related Uniprot ID	Link to External Databases
SEH1L	0.5650777554480388	0.999957823952065	0.5509855393915455	0.53489031930978	0.9707883076213952	1.0	0.02848816326530612	0.1	0.003401360544217687	Gene Symbols	Q06EE3, K7EP25, K7EN15, K7ELV2, K7EP88	GeneCards OH1H

First Previous 1 Next Last Show 25 entries Showing 1 to 1 of 1 entries

Biological Networks Preprocessing and Analysis

Biological Network Analysis

When users create or upload a biological network, they can access a menu of six analytical options described below. In order to analyze a biological network the users should:

- Select a biological network file from the ones in InSyBio DataStore or upload a new biological network file using InSyBio DataStore.
- If they are experienced, they can tune the following parameters:
 - Method for selecting significant nodes (Pagerank (default) [6, 7], Clustering Coefficient [8], degree centrality [9])
 - Confidence interval for locating significant nodes
 - Method for selecting significant edges (Edge weight (default), Inbetweenness centrality [10])
 - Confidence interval for locating significant edges

The screenshot displays the InSyBio Suite interface for Biological Network Analysis. At the top, the title bar reads "InSyBio Suite - Biological Network Analysis" and the user is identified as "InSyBio Beta User". The main form includes input fields for "Title:" and "Filename:". Below these fields are two buttons: "Select file from Data Store" (with a folder icon) and "Go to Data Store to Upload File" (with a plus icon). An "Advanced Options" section is expanded, showing four configuration items: "Method (Most Significant Nodes)" set to "Pagerank", "Interval of Trust (Most Significant Nodes)" set to "95%", "Method (Most Significant Edges)" set to "Edge weight", and "Interval of Trust (Most Significant Edges)" set to "95%". A green "Submit Job" button is located at the bottom left of the form.

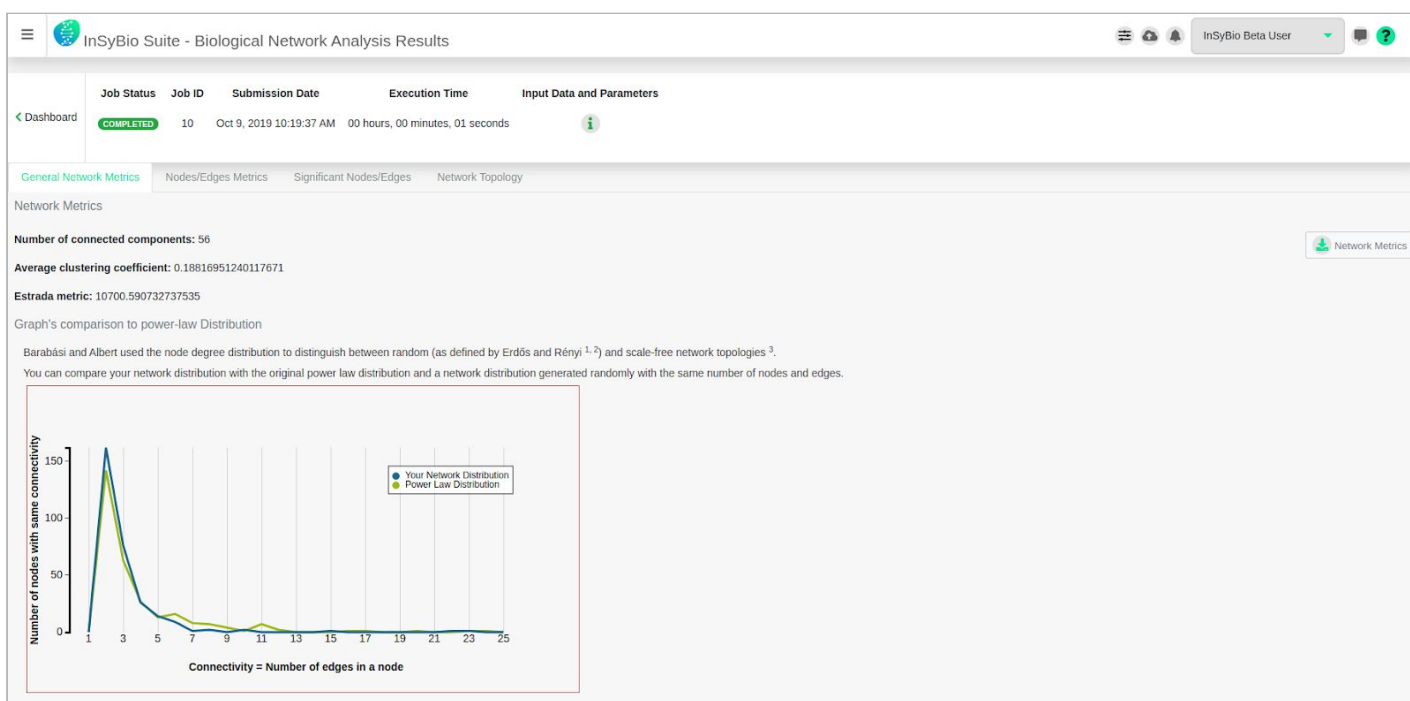
View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of

the analysis you can select the “View Results” at the Actions column and view the Network Analysis details.

General Network Analysis

In the General Network Analysis tab users can view the most significant network metrics (clustering coefficient [8], Estrada index [11] and so on) and compare the degree distribution of their network with the a random network’s power law distribution. Information in this tab is not available for networks with more than 225000 edges.



Node/Edges Metrics

In the Node/Edges Metrics tab users can find the metrics for all nodes (degree centrality, clustering coefficient and pagerank centrality) and edges (edge weight and in betweenness centrality) of your network.

InSyBio Suite - Biological Network Analysis Results
InSyBio Beta User

Job Status COMPLETED | **Job ID** 10 | **Submission Date** Oct 9, 2019 10:19:37 AM | **Execution Time** 00 hours, 00 minutes, 01 seconds | **Input Data and Parameters**

General Network Metrics | **Nodes/Edges Metrics** | Significant Nodes/Edges | Network Topology

Node Metrics

Browse among metrics

Node	Degree Centrality	Clustering Coefficient	Pagerank Centrality
KDM4C	0.02840816326530612	0.08333333333333333	0.00545874705967949
PWP1	0.013605442176870748	0.42857142857142855	0.0038529591022577053
GPR137B	0.017006802721088433	0.125	0.004691050656523418
TMEM19	0.013605442176870748	0.15555555555555556	0.0033742694808678014
CDK12	0.02840816326530612	0.14285714285714285	0.005425151458877989
TBC1D2B	0.006802721088435374	0.25	0.002110636154453804
TDP1	0.03401360544217687	0.03169811320754717	0.007636343728023451
RBH4B	0.027210884353741496	0.16666666666666666	0.005939693364823362
QR5L1	0.003401360544217687	0	0.0011416364047241953
TBC1D31	0.03401360544217687	0.036317567567567564	0.006436571356618464
BLM	0.023809523809523808	0.08333333333333333	0.004783777627885641

Showing 1 to 25 of 295 entries

Edge Metrics

Browse among metrics

Node 1	Node 2	Edge weight	In Betweenness Centrality
KDM4C	PWP1	0.5055	0.0026675012216347393
KDM4C	GPR137B	0.5134	0.0005116041574014593
KDM4C	TMEM19	0.6113	0.00019601660763288367
KDM4C	CDK12	0.7999	0.00014604711941273687
KDM4C	TBC1D2B	0.6214	0.0026211614589338557
KDM4C	TDP1	0.6328	0.00722526944595433
PWP1	CXXC1	0.5245	0.0026980283638879284
PWP1	CDK12	0.601	0.0026670619821778595
PWP1	TBC1D2B	0.5427	7.606690495407202e-05
GPR137B	FN3KRP	0.702	0.0039277085928139405
GPR137B	GFDD1	0.509	0.0026980283638879284

Showing 1 to 25 of 403 entries

Uncovering Significant Nodes/Edges

In the Uncovering Significant Nodes/Edges tab users can access two tables including the uncovered significant nodes and edges. For each node and edge, the respective metrics and the p-values of their significance are shown. Significant edges are not available for networks with more than 225000 edges.

InSyBio Suite - Biological Network Analysis Results
InSyBio Beta User

Job Status **Job ID** **Submission Date** **Execution Time** **Input Data and Parameters**

COMPLETED 10 Oct 9, 2019 10:19:37 AM 00 hours, 00 minutes, 01 seconds

General Network Metrics Nodes/Edges Metrics **Significant Nodes/Edges** Network Topology

Most Significant Nodes

Browse among metrics Most Significant Nodes Metrics

Node	Degree Centrality	Clustering Coefficient	Pagerank Centrality	P-value
SEH1L	0.07482993197278912	0.041346585671423346	0.014742792981727363	6.06964794635e-10
POM121	0.0782312925170668	0.026769564693114676	0.014549429134455425	1.15139913003e-09
HILPDA	0.06462585834813686	0.035512256442489	0.013432126379302443	3.79481067388e-08
PRKDC	0.05442176870748299	0.0321955003878976	0.010893210706800677	0.000165974598913
KANSL2	0.05102040816326531	0.053111587982832616	0.009596211634244486	0.000445425156985
SLC4A1	0.03401360544217687	0.07459207459207459	0.008522390798449988	0.00299817965494
MKRN1	0.03401360544217687	0.08970099667774087	0.008207088012754453	0.0049519472131
MCTS1	0.03741496598639456	0.04932472108044627	0.007951337083452987	0.00729827227717
TDP1	0.03401360544217687	0.03169811320754717	0.007636343728023451	0.0114953400335
GGT2	0.03741496598639456	0.03853853853853854	0.007562216980743118	0.0127447636834
WDR73	0.03401360544217687	0.10301507537668442	0.00730826935813476	0.0181453725339

Showing 1 to 11 of 11 entries

Most Significant Edges

Browse among metrics Most Significant Edges Metrics

Node 1	Node 2	Edge weight	In Betweenness Centrality	P-value
HBB	HBA2	0.9784	4.6120142972443216e-05	1.38524115427e-10
BNIP3L	BPGM	0.8167	0.00012106537530266344	5.17926623801e-05
SEH1L	KANSL2	0.804	0.0004971733110785342	0.000116358373571
KDM4C	CDK12	0.7909	0.000146604711941273687	0.000257577375122
ZZZ3	NDST2	0.7631	6.918021445866483e-05	0.00121575837359
WDR73	HILPDA	0.7518	0.002059482546087248	0.00216901109883
ARPC5L	PTPRCAP	0.7417	2.3060071486221608e-05	0.00354858075572
KANSL2	MCTS1	0.7411	0.0008711041831069584	0.00365117176753
SEH1L	MCTS1	0.7329	0.002359168433191263	0.0053453593906
HILPDA	KRT2	0.7298	0.0022675516509829098	0.00614908572273
RP55	RP516	0.7265	4.6120142972443216e-05	0.00712061261435
NDST2	TCERG1	0.7264	2.3060071486221608e-05	0.00715205700881
AIFM1	RBM42	0.7218	9.224028594488643e-05	0.00873744485776
SELENBP1	RPIA	0.7211	0.00038433452477036015	0.00900394266668
LOC100506123	CLCN6	0.7154	0.0026749682924017064	0.0114519986631
PCYOX1L	PRKDC	0.7145	0.0010167160047489436	0.0118872229838
POM121	PRKDC	0.705	0.001513665754412901	0.0174270867824
GPR137B	FN3KRP	0.702	0.0039277085928139405	0.0195821180706
MCTS1	APH1A	0.6986	0.0004227679772473951	0.0222940233474

Showing 1 to 19 of 19 entries

Biological Network Visualization

The Biological Network Visualization tab offers an interactive visual representation of the biological network. When networks have more than 10,000 edges, a haircut filter is applied before the visualization of the network. If the haircut filter cannot reduce the number of edges below 10,000 edges then no network visualization is provided. Networks' visualization is based on the Cytoscape plugin [12] and it provides an interactive graphical interface. Users can retrieve information about clicked nodes and edges, export the image in different formats (a PNG, SVG, PDF, XGMML, GraphML or SIF document), decrease opacity on mouseover and view the network using different visualization layouts (force-directed, circle or radial).

☰

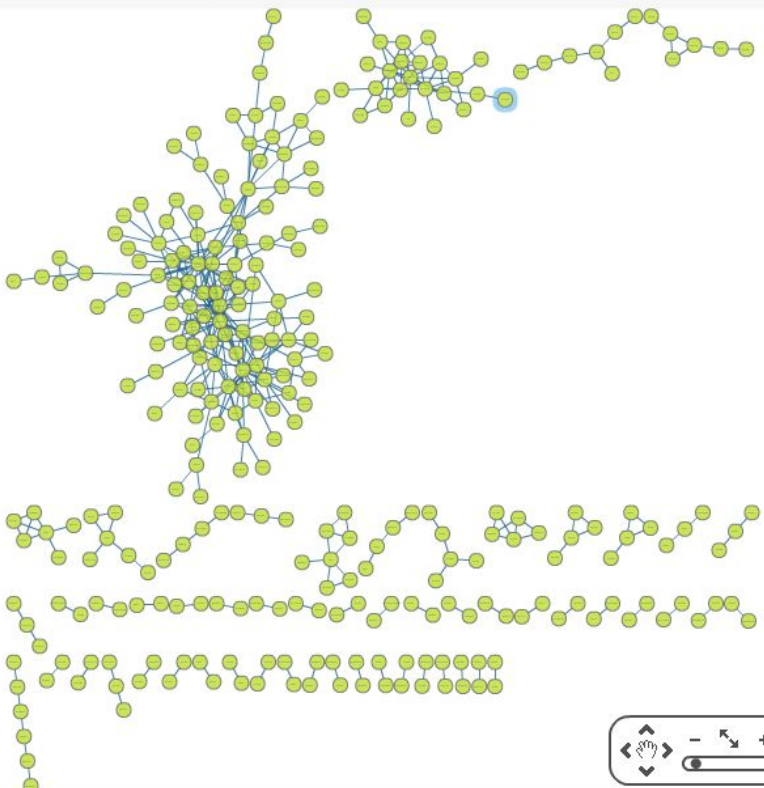
InSyBio Suite - Biological Network Analysis Results

	Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters
← Dashboard	COMPLETED	10	Oct 9, 2019 10:19:37 AM	00 hours, 00 minutes, 01 seconds	i

General Network Metrics
Nodes/Edges Metrics
Significant Nodes/Edges
Network Topology

Network's Graph Visualization

The visualization capability of network's graph is highly depended in the size of the network and the size of the memory of your workstation computer. In some cases, especially in large networks, the visualization will not be possible.



↕ ↔ ↔ ↕

- +

Network Information ?

Node: TRAK2

Decrease opacity on mouseover
(Might become slow for large networks).

Layout Force Directed

Export Network Layout ?

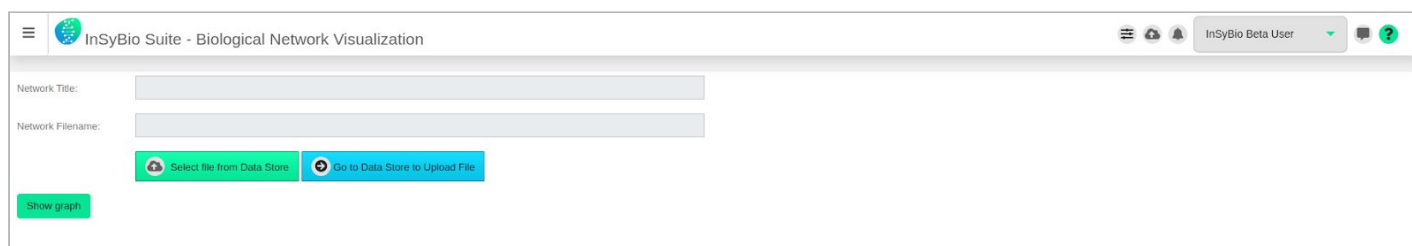
PNG image
▼

Save Visualized Network

Biological Network Visualization

The Biological Network Visualization function offers an interactive visual representation of the biological network. When networks have more than 10,000 edges, a haircut filter is applied before the visualization of the network. If the haircut filter cannot reduce the number of edges below 10,000 edges then no network

visualization is provided. Networks' visualization is based on the Cytoscape plugin [12] and it provides an interactive graphical interface. Users can retrieve information about clicked nodes and edges, export the image in different formats (a PNG, SVG, PDF, XGMML, GraphML or SIF document), decrease opacity on mouseover and view the network using different visualization layouts (force-directed, circle or radial).

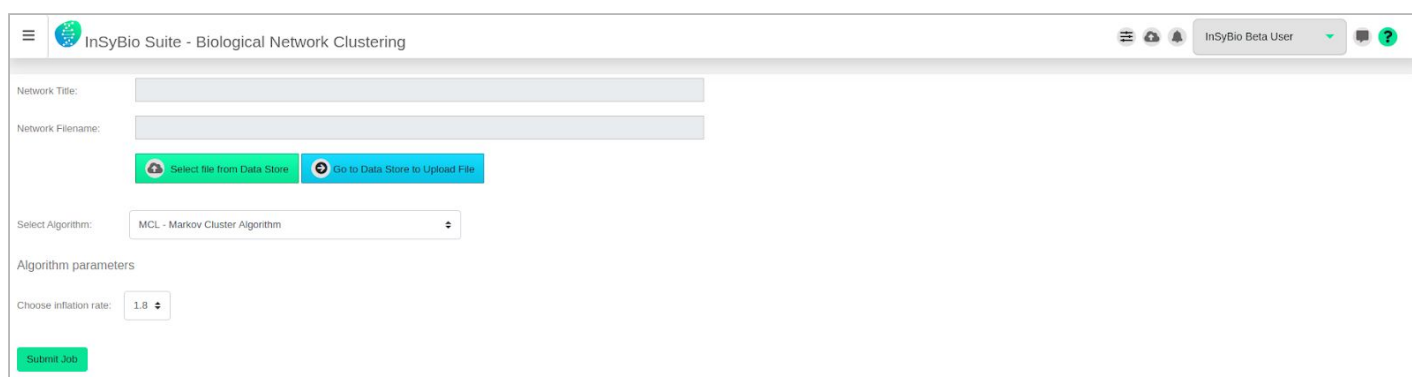


The screenshot shows the 'InSyBio Suite - Biological Network Visualization' interface. It features a header with the InSyBio logo and the text 'InSyBio Suite - Biological Network Visualization'. Below the header, there are two input fields: 'Network Title:' and 'Network Filename:'. Underneath these fields are two buttons: 'Select file from Data Store' (with a red upload icon) and 'Go to Data Store to Upload File' (with a blue download icon). At the bottom left, there is a green 'Show graph' button. The top right corner shows the user 'InSyBio Beta User' and a help icon.

Biological Network Clustering

With this tool, you can analyze your Biological Network to extract complexes of similar nodes (i.e protein complexes). Weighted and unweighted Biological Networks can be handled. Three options are supplied for the prediction of Biological Network complexes: Markov Clustering (MCL) [3], Restricted Neighborhood Search Clustering (RNSC) [4] and Clustering with Overlapping Neighborhood Expansion (ClusterONE) [22]. You can name and enter your network via a file or by a saved snapshot in your Data Store and specify the algorithm parameters:

MCL algorithm



The screenshot shows the 'InSyBio Suite - Biological Network Clustering' interface. It features a header with the InSyBio logo and the text 'InSyBio Suite - Biological Network Clustering'. Below the header, there are two input fields: 'Network Title:' and 'Network Filename:'. Underneath these fields are two buttons: 'Select file from Data Store' (with a red upload icon) and 'Go to Data Store to Upload File' (with a blue download icon). Below these buttons is a dropdown menu for 'Select Algorithm:' with 'MCL - Markov Cluster Algorithm' selected. Underneath is a section for 'Algorithm parameters' with a 'Choose inflation rate:' label and a dropdown menu showing '1.8'. At the bottom left, there is a green 'Submit Job' button. The top right corner shows the user 'InSyBio Beta User' and a help icon.

- Inflation Rate parameter (default value 1.8)

The output is a list of the clusters created from the algorithm. For each cluster, the node IDs are listed.

RNSC algorithm

The screenshot shows the InSyBio Suite - Biological Network Clustering interface. It includes a header with the InSyBio logo and the text 'InSyBio Suite - Biological Network Clustering'. On the right, there is a user profile 'InSyBio Beta User' and a help icon. The main area contains input fields for 'Network Title' and 'Network Filename'. Below these are two buttons: 'Select file from Data Store' and 'Go to Data Store to Upload File'. A dropdown menu for 'Select Algorithm:' is set to 'RNSC - Restricted Neighborhood Search Clustering Algorithm'. Under 'Algorithm parameters', there are input fields for: 'Max Cluster Number' (100), 'Tabu Length' (1), 'Tabu List Tolerance' (1), 'Naive Stopping Tolerance' (5), 'Scaled Stopping Tolerance' (5), and 'Number of Experiments' (1). A green 'Submit Job' button is located at the bottom left.

- Maximum number of clusters (default value 100),
- Tabu length (default value 1),
- Tabu list tolerance (default value 1),
- Naive stopping tolerance (default value 5),
- Scaled stopping tolerance (default value 5), and
- Number of experiments (default value 1)

Each type of graph has a fairly similar response to the changing tabu length, and RNSC clusters each quite well (and quite quickly) with a tabu length of around $n/100$, where n the number of nodes in the graph.

King et al [4] showed that a diversification frequency of $n/50$ and length diversification = $n/5$ (n the number of nodes in the graph) yield fairly good results in terms of both score and time, but no set of parameters is clearly the best for all classes

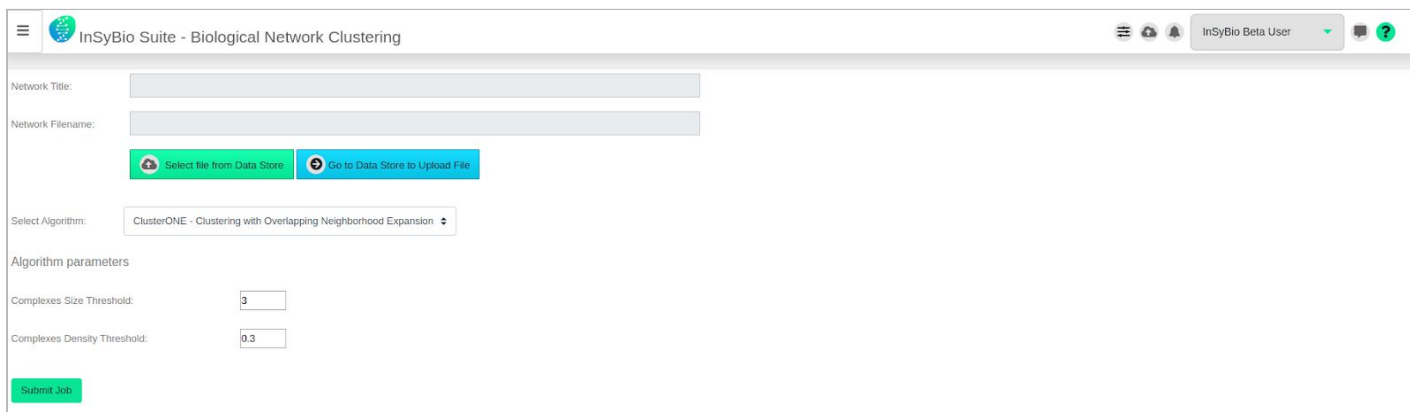
of graphs. For more information on Parameter Training and Statistical Results please consult [5].

The RNSC has the capability to efficiently searching many minima in the search space. In this section we will consider a standard tabu list, i.e. one with tabu list tolerance equal to 1, since it was shown in [5] that extending the tabu tolerance did not offer an advantage for the problem.

For the naive and scaled stopping tolerance, there is always a choice between speed and quality. For all graph types, the final scaled cost decreases as the stopping tolerance is increased and the standard deviation of the cost decreases similarly.

The output is a list of the clusters created from the algorithm. For each cluster, the node IDs are listed.

ClusterONE algorithm



The screenshot displays the InSyBio Suite - Biological Network Clustering interface. It includes the following elements:

- Network Title:
- Network Filename:
- Buttons: [Select file from Data Store](#) and [Go to Data Store to Upload File](#)
- Select Algorithm: ClusterONE - Clustering with Overlapping Neighborhood Expansion
- Algorithm parameters:
 - Complexes Size Threshold:
 - Complexes Density Threshold:
- Submit Job:

- Complexes size threshold: (default value 3)
- Complexes density threshold: (default value 0.3)

The output is a list of the clusters created from the algorithm. For each cluster, the node IDs are listed.

View Results

After starting an analysis job you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of

the analysis you can select the “View Results” at the Actions column and view the Network Clusters.

Job Status	Job ID	Submission Date	Execution Time	Input Data and Parameters
COMPLETED	31	Oct 15, 2019 12:57:44 PM	00 hours, 00 minutes, 01 seconds	

Cluster ID	Nodes	Action
cluster_1	B2M, HBB, HBA2	View Complex
cluster_2	SLC4A1, SELENBP1	View Complex
cluster_3	HBD, AHSP	View Complex
cluster_4	ACTR2, YWHAZ	View Complex

The results are presented in your screen as a list or you can download them as a TAB delimited tsv file.

Clusters Visualization

For each computed cluster the participating nodes are presented and it can be visualized. Clusters' visualization is based on the Cytoscape plugin [12] and it provides an interactive graphical interface. Users can retrieve information about clicked nodes and edges, export the image in different formats (a PNG, SVG, PDF, XGMML, GraphML or SIF document), decrease opacity on mouseover and view the network using different visualization layouts (force-directed, circle or radial).

The screenshot displays the InSyBio Suite interface. On the left, a sidebar lists job clusters: cluster_1 (B2M, HBB, HBA2), cluster_2 (SLC4A1, SELENBP1), cluster_3 (HBD, AHSP), and cluster_4 (ACTR2, YWHAZ). The main area is a 'Visualize Complex' window showing a network diagram with three green nodes labeled B2M, HBA2, and HBB. Below the diagram are navigation controls (pan, zoom, reset) and an 'Export Network Layout' section with a 'PNG image' dropdown and a 'Save Visualized Network' button. A 'Selected Element' panel is empty on the right. The top right shows the user 'InSyBio Beta User' and a 'Close' button.

Combine Networks

You can select from Data Store two files, representing Biological Networks, to combine with same or different node ids types. If they have the same node type they are merged as is, if they have different node type they are changed to GeneSymbols ids using the Insybio Interact knowledgebase. You can perform normalization to the edge scores, according to your specifications and also use different weights for the combination of networks.

InSyBio Suite - Combine Networks

Combined Network Title:

Network Title 1:

Network Filename 1:

Network Title 2:

Network Filename 2:

Show More Options

Weight 1:

Weight 2:

Normalize:

- Upper Limit:
- Lower Limit:

View Results

In order to access your network you can go to “BioNets Jobs Dashboard”, where you can view the status of your current and previous BioNets jobs. After the completion of the graph you can select the “View Results” at the Actions column and view the combined network results.

InSyBio Suite - Combine Networks Results

Job Status: **COMPLETED** Job ID: 5 Submission Date: Oct 4, 2019 1:25:19 PM Execution Time: 00 hours, 00 minutes, 01 seconds

Network File

Selected Element
Node : KRT17
Connected Nodes :
• KRT6A

Network Layout: Force Directed

Export Network Layout
PNG image Save Visualized Network

References

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About Us

InSyBio Ltd is a bioinformatics pioneer company (www.insybio.com) in personalized healthcare, that focuses on developing computational frameworks and tools for the analysis of complex life-science and biological data in order to develop predictive integrated biomarkers (biomarkers of various categories) with increased prognostic and diagnostic aspects for the personalized Healthcare Industry.

InSyBio Suite consists of tools for providing integrated biological information from various sources, while at the same time it is empowered with robust, user-friendly and installation-free bioinformatics tools based on intelligent algorithms and methods.

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