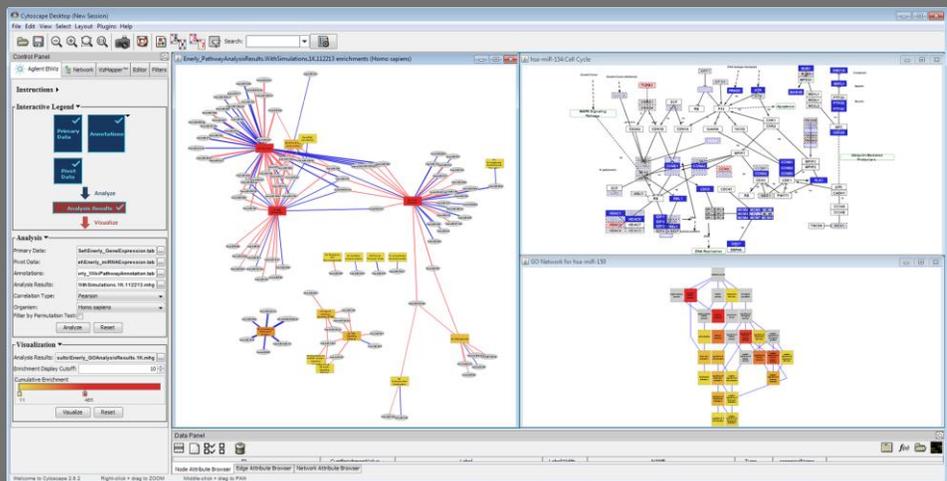


ENViz: A Cytoscape App for Integrated Statistical Analysis and Visualization of Sample-Matched Data with Multiple Data Types

User Tutorial

ENViz is software for extracting biological insights from multiple types of measurements via integrated statistical analysis of these measurements and available systematic annotations, such as ontology or pathway annotations. Visualization of the analyses is provided by an app of the Cytoscape network biology software platform.



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In memory

Allan Kuchinsky, a colleague, a mentor, a fellow scientist, a dedicated friend.

Allan was the living spirit behind ENViz. He passed away not long before we finished working on this project, leaving us all in shock and agony. Allan identified the potential for weaving a joint data analysis approach into Cytoscape and led us all through executing and completing this task. Even though Allan was constantly fighting cancer and its complications, he led our team with great enthusiasm to cross countless obstacles to make ENViz a reality. Allan also worked with great excitement with collaborators to apply ENViz to scientific studies and to learn how we could improve, provide more information, and add the correct touch of extra functionality. Allan's work and enthusiasm brought the software to its current state, ready for release. We continue to learn from Allan--his work is still here for us to follow and trust. Above all, Allan was a dedicated friend. We will always remember Allan's human warmth and his appreciation to the team's work.

We dedicate ENViz to Allan's memory, as a scientist and as a human being. Always with a smile and always excited to discuss new science and how software can make it better.



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- A. Appendix - Overview of ENViz Enrichment Analysis**

1. Welcome to ENViz

ENViz (**E**nrichment Analysis and **V**isualization) performs joint enrichment analysis of two types of sample matched datasets and available systematic annotations. Examples of such data sets may be gene expression, miRNA or other non-coding RNA expression, or proteomics measurements collected in the same set of samples, together with pathway, gene ontology (GO) or any custom annotation of the data. Enrichment analysis is based on minimum hypergeometric statistics [2, 3]. Results of enrichment analysis are visualized as an interactive Cytoscape network, and could be visually overlaid on biological pathways or GO hierarchy.

1.1. Introduction to ENViz

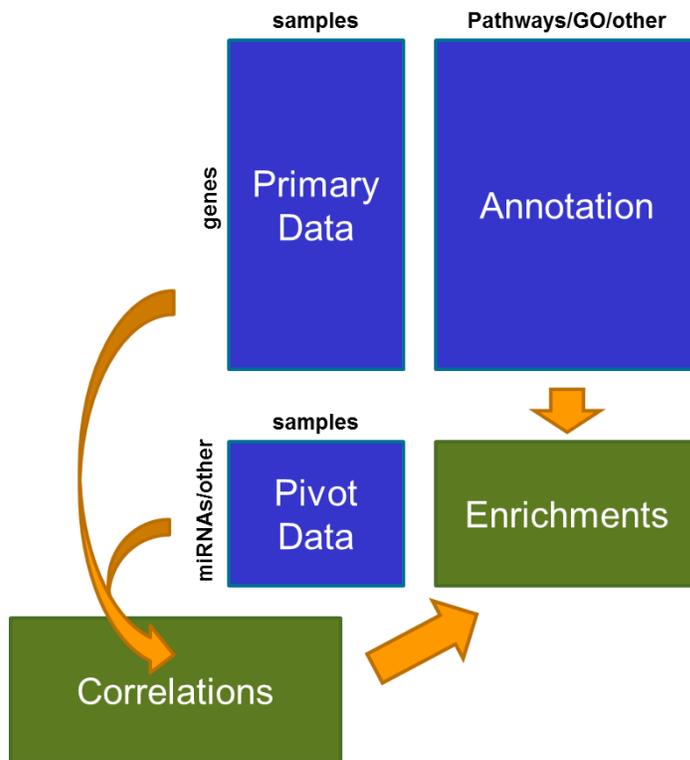
Over recent years, modern biology has undergone an information revolution, which is evident in a shift of thinking and practice. While typical biological studies are focused on specific pathways, like the p53 signaling pathway, the emergence of novel high-throughput technologies now enables the quantification of biological features in a genome-wide scale. The rapid development of technology, in particular, enabled measurements of mRNA expression levels, miRNA expression levels, DNA methylation states, DNA copy number, etc. Various methodologies have been developed, in recent years, to handle integrated analysis of functional genomics data, mainly by studying the transcriptional programs and global organization of biological processes. Still, only a few tools support routine joint analysis of sample cohort with multiple genomic measurement results. No such tools provide the visualization strength of Cytoscape. In ENViz we aimed to provide a tool that enables joint analysis and harnesses Cytoscape to support and provide a visualization interface.

ENViz approach to integrated data analysis uses the power of enrichment statistics and knowledge of genomic annotation databases to assign relevant function annotations to profiled elements and to gain better understating of the relationship between different molecular levels in the cells.

The basic idea is to first rank the informative elements according to a particular aspect of the non-informative dataset and then search for enrichment of interesting annotations in the ranked list of elements. The enrichment results are therefore directly assigned to the un-annotated profiled elements.

Even though development of ENViz was motivated by available modern biological measurements, joint analysis of two sample matched datasets and systematic annotations may be applied to other measurement that fit in the setup described below.

ENViz follows an enrichment analysis approach, driven by three input matrices.



ENViz input consists of primary data matrix (e.g. a set of genes with expression measured across a set of samples), pivot data matrix (e.g. a set of miRNAs with expression measured in the same set of samples), and primary data annotation matrix (e.g. pathway or GO annotation of genes).

Note that in the figure above, the boxes representing the various input matrices are drawn to their relative dimensions. Consider the case where:

N = number of biological entities, such as genes, in the *Primary Data Matrix*

M = number of biological entities, such as miRNAs, in the *Pivot Data Matrix*

A = number of annotation entries, such as pathways or GO terms, in the *Annotation Matrix*,



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S = number of samples.

Then

- $N * S$ = dimensions of *Primary Data Matrix*
- $M * S$ = dimensions of *Pivot Data Matrix*
- $N * A$ = dimensions of *Annotation Matrix*
- $M * A$ = dimensions of *Enrichments Matrix*
- $N * M$ = dimensions of *Correlations Matrix*

ENViz analyses a *primary* data set with respect to a *pivot* data set and *primary data annotation* in the following way. For each *pivot* entry,

- we compute the correlation of *pivot* data to each element of *primary* data across all samples
- we rank elements of the *primary* data based on this correlation (or anti-correlation), and
- compute the statistical enrichment of *annotation* elements (gene sets) in the top of this ranked list based on an mHG (minimum hypergeometric [2,3]) statistics.

Details of mHG statistics are explained in [2, 3, 5]. Briefly, for each annotation element, we do the following. We consider top k ranking (by correlation) primary data elements (genes), and compute enrichment of this annotation element in the top k genes based on the hypergeometric statistics. We repeat this process for all threshold values, k , and choose k that optimizes the hypergeometric significance. A bound on the corresponding p -value is calculated to correct for multiple testing [2, 3] and an mHG score $\leq -\log_{10}(\text{mHG } p\text{-value})$ is reported (large values of the mHG score represent significant enrichments). The correction is valid for every individual pivot element, but all enrichment results are not corrected for the number of pivot elements and the number of annotation elements. For guidance on this next level of correction please see Section 2.6 below.

Significant results are represented in Cytoscape as an *enrichment network* - a bipartite graph with nodes corresponding to *pivot* and *annotation* elements, and edges corresponding to *pivot-annotation* entry pairs with enrichment scores better than the user defined threshold. In addition,

- for analysis of enrichment in biological pathways, correlations of primary data and corresponding pivot data are visually overlaid on biological pathways for each significant pivot-annotation pair, using the WikiPathways [4] resource.
- enrichments of GO categories are overlaid on top of the Gene Ontology DAG. Edges of the enrichment network, representing significant associations, may point to functionally relevant mechanisms.

Development

ENViz is a research prototype tool developed by the Computational Biology and Informatics project at Agilent Laboratories, the central research organization of Agilent Technologies.

License

ENViz is protected under the license agreement terms in Section 5.



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ENViz also includes a number of other open source resources, which are detailed in Section 6.

References

- [1] Cline, MS, *et al.*, (2007) "*Integration of biological networks and gene expression data using Cytoscape*". Nat Protoc 2: 10. 2366-2382
- [2] Eden, E, Lipson, D, Yogev, S, and Yakhini, Z. (2007), "*Discovering Motifs in Ranked Lists of DNA sequences*", PLoS Computational Biology, 3(3):e39.
- [3] Eden, E, Navon, R, *et al.* (2009) "*GOrilla: A Tool For Discovery And Visualization of Enriched GO Terms in Ranked Gene Lists*", BMC Bioinformatics, 10:48
- [4] Kelder, T, *et al* (2011) "*WikiPathways: building research communities on biological pathways*" NAR. doi: 10.1093/nar/gkr1074
- [5] Enerly, E, *et al* (2011), "*miRNA-mRNA Integrated Analysis Reveals Roles for miRNAs in Primary Breast Tumors*", PLoS One. Feb 22;6(2):e16915

1.2. Installation

For installation details, see the [Cy2 User Readme](#) or the [Cy3 User Readme](#) documents.

1.3. Input files

ENViz requires two input files - the Primary data file and Pivot data file:

Primary data file: tab delimited text file with one row for each primary data element (e.g. gene), and one column for each sample. First column should have primary data element identifier. If primary data contains gene based measurements, first column should contain a gene identifier, which should be an EntrezID or a GeneSymbol. The first row should have sample identifiers. Other entries in this file have measurements for each primary data element in each sample. Missing values should be denoted by '#'. In the sample dataset primary data file is *Enerly_GeneExpression.tab*.

Pivot data file: tab delimited file with one row for each pivot element, and one column for each sample. The first column should have pivot identifiers. The first row should have sample identifiers. Samples should be ordered in the same way in primary and pivot data files and use the same identifiers, i.e. the first row in primary data file and the first row in the pivot data file should be the same. Other entries in this file have measurements for each pivot element in each sample. Missing values should be denoted by '#'. In the sample dataset pivot data file is *Enerly_miRNAExpression.tab*.

Note that ENViz is not performing any pre-processing or filtering of the input data files. You should apply the normalization/filtering processes best suited for your data. Primary and pivot data should not have any duplicate entries.

Annotation file: optional tab delimited file containing systematic annotations of primary data elements that will be used for enrichment analysis. First column should have primary data element identifiers. Primary data elements in the annotation file and in the primary data file should be ordered in the same way and use the same identifiers, i.e. the first columns in primary data file and in annotation file should be the same. First row should have annotation categories. Other entries of this file are 0s and 1s. 1 in row i column j indicates that primary element from row i belongs to annotation category in column j . If an annotation file is not provided, ENViz can generate one for GO (Gene Ontology) and for pathway annotations (using WikiPathways). EntrezIDs or GeneSymbols are used to assign genes to pathway or GO categories.

In the sample dataset, the GO annotation file is *Enerly_GO_Annotation.tab.gz*, and the pathway annotation file is *Enerly_WikiPathway_Annotation.tab.gz* (you may notice the different *.gz* extension that specifies the files are compressed because the annotation files can be quite large).

1.4 Example dataset



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For this tutorial, we use the dataset published in [5]. Data formatted for ENViz can be downloaded from <http://bioinfo.cs.technion.ac.il/people/zohar/ENViz/data.html>

This dataset consists of 100 breast tumor samples with various characteristics. Primary data is gene expression profiles of 13,639 genes for 100 samples from microarray experiments. Pivot data is microarray based miRNA profiles of 489 microRNAs measured for the same 100 samples. The sample dataset includes pathway annotation files generated using WikiPathways and GO annotation file.

Primary data file: *Enerly_GeneExpression.tab*, a tab delimited file containing gene expression data for 13,639 genes and 100 samples. Gene expression data is taken from gProcessedSignal Agilent Feature Extraction files. The expression levels were log2 transformed and quantile normalized [5].

Pivot data file: *Enerly_miRNAExpression.tab*, a tab delimited file containing data for 489 miRNAs and 100 samples. miRNA expression data is log2 transformed and normalized to the 75th percentile [5].

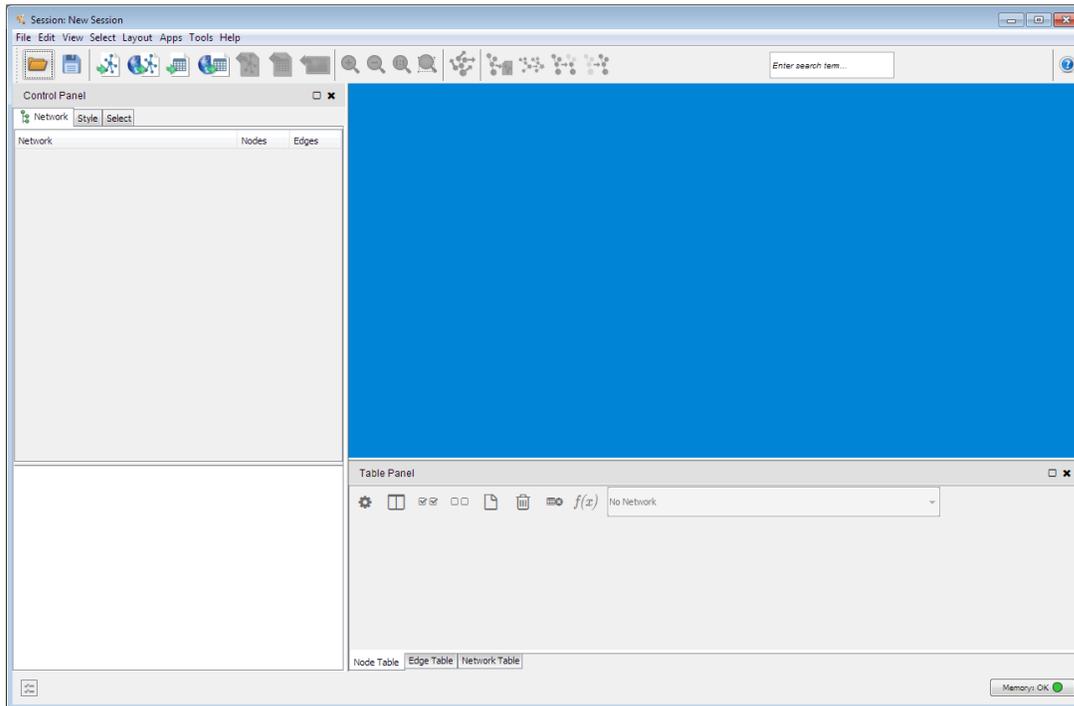
Annotation files: GO annotation file *Enerly_GO_Annotation.tab*, and WikiPathway annotation file *Enerly_WikiPathway_Annotation.tab*. The GO annotation file contains gene annotations for 11,177 GO terms. GO annotation is based on GO database release downloaded on 08/22/2013. The pathway annotation file contains gene annotations for 204 pathways.

In all examples and descriptions below, we will refer to primary data as gene expression data from the sample dataset, pivot data as miRNA expression data from pivot dataset, and annotation data as either GO annotation or pathway annotation from the sample dataset.

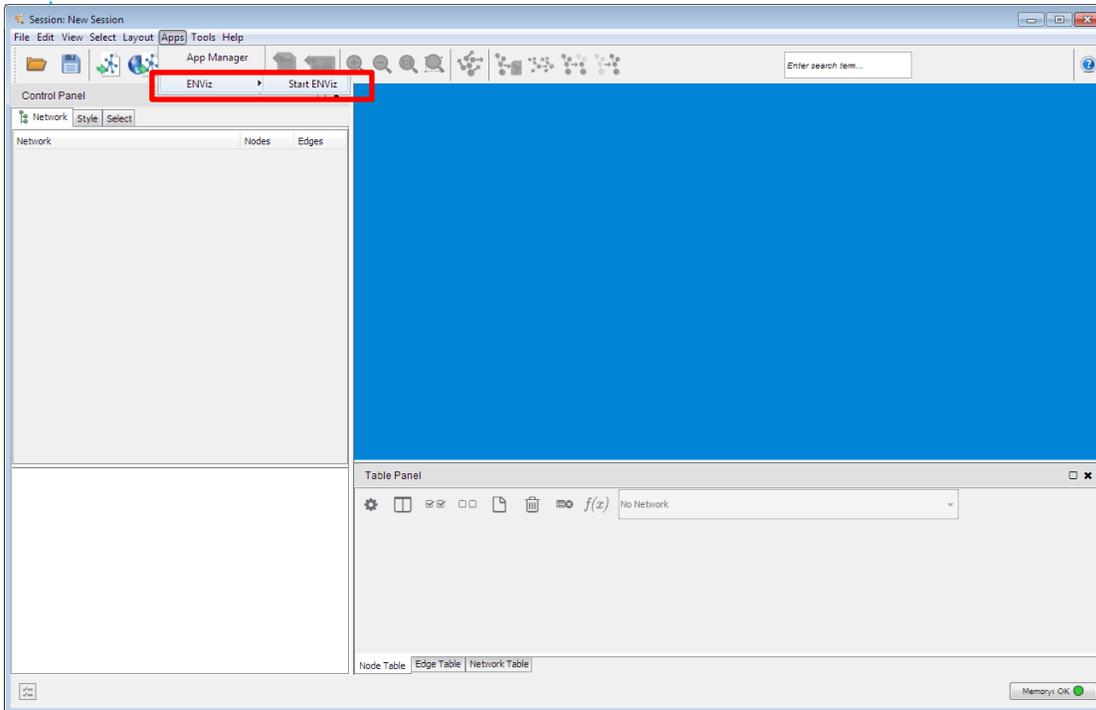
2. ENViz Analysis

2.1 Starting ENViz

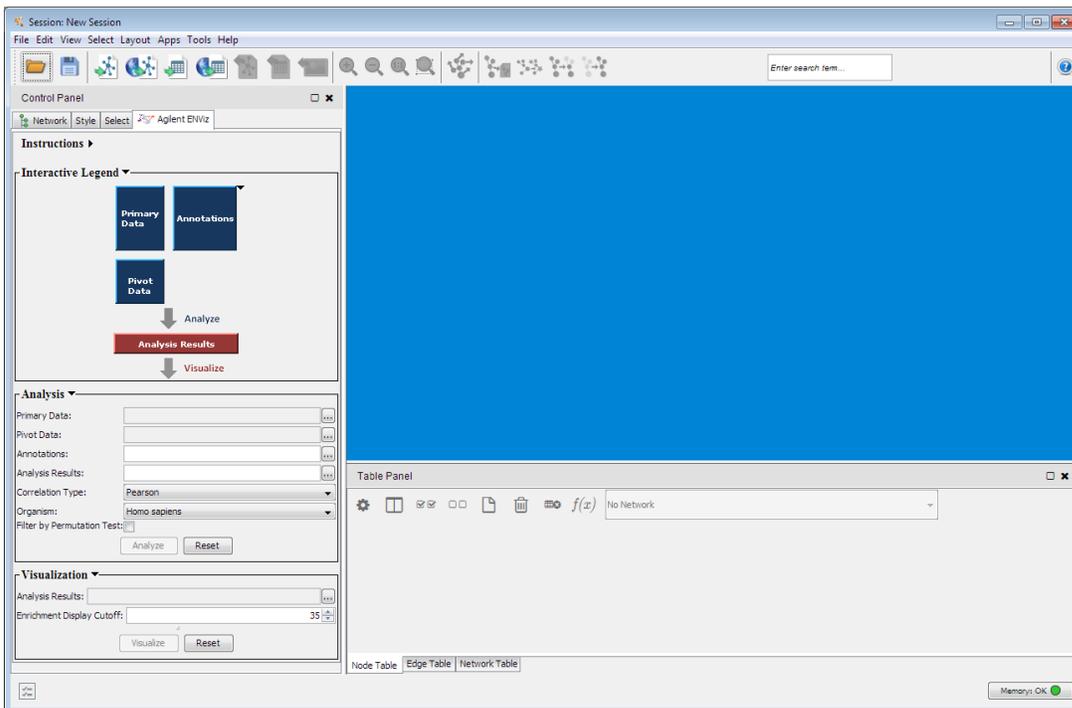
Launch Cytoscape. You should see a window that looks something like:



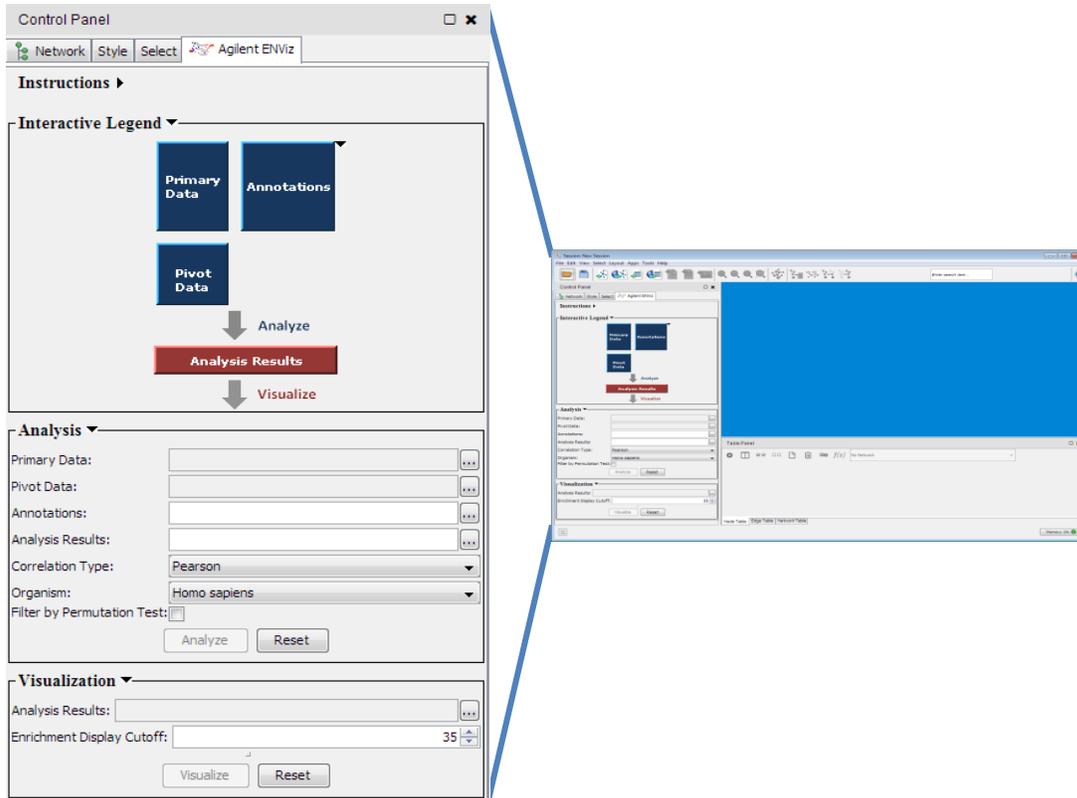
Start ENViz by selecting the **Apps --> ENViz--> Start ENViz** menu item



When ENViz starts, it opens a new *Agilent ENViz* tab and places a control panel in the left subpanel of the Cytoscape window. This includes controls for inputting the primary data, pivot, and annotations; buttons for running analysis and visualization; and controls for setting thresholds that control the size of the networks to visualize:



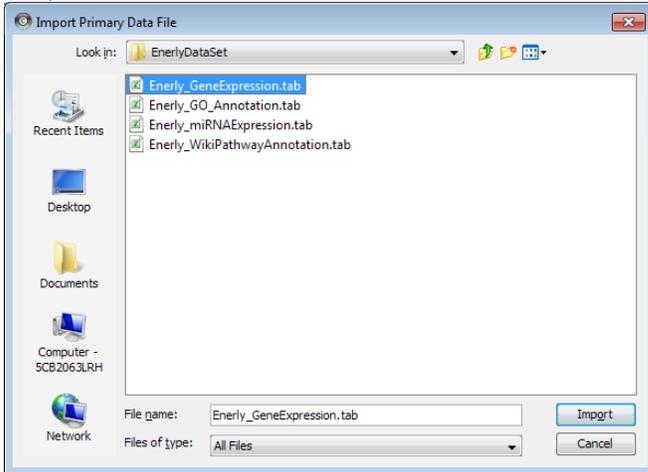
The Control Panel contains Instructions, an Interactive Legend, Analysis and Visualization controls. Interactive legend provides a graphical overview of the workflow. Under the Interactive Legend subpanel, you can click on the labeled boxes and be prompted for the appropriate files. You can also drag and drop a file reference onto a labeled box.



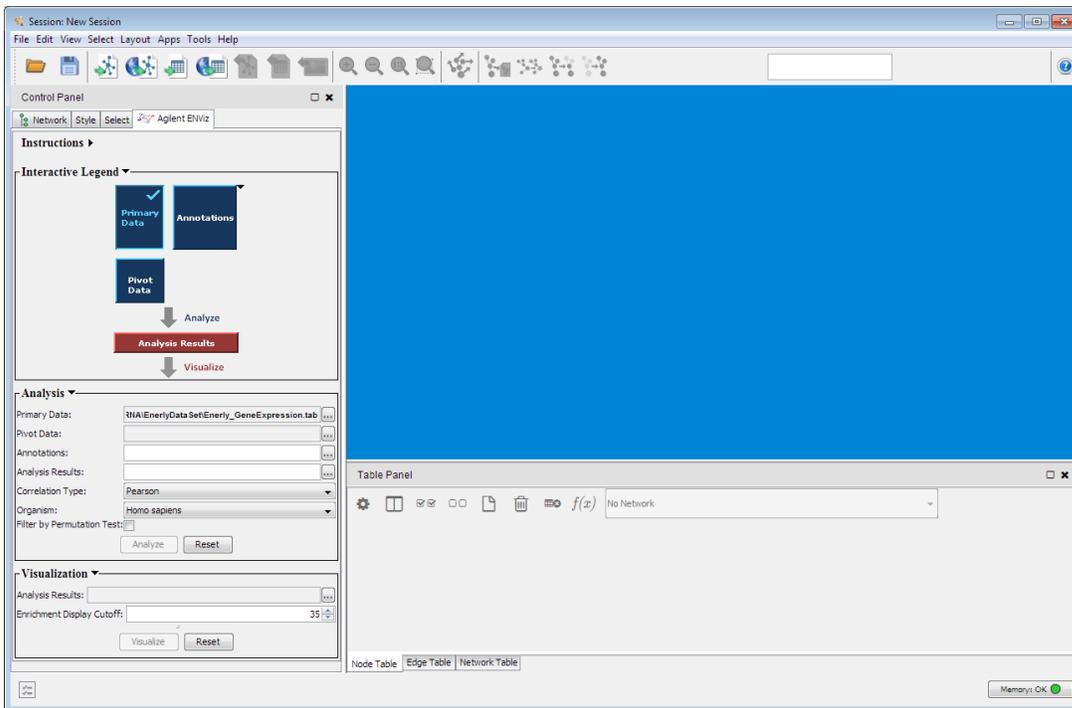
Separate sub-panels can be collapsed or expanded by clicking on their handles. This is based upon *collapsible subpanels* (Bader Lab, University of Toronto).

2.2 Setting up input and results files

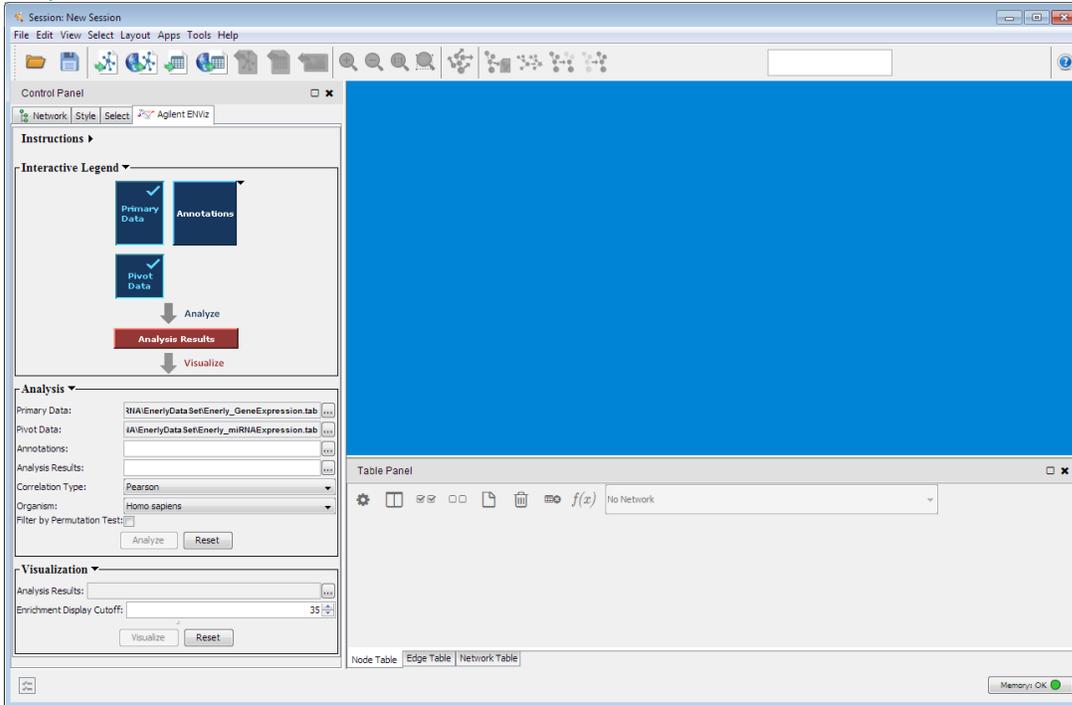
To load primary data, click on the box labeled *Primary Data* on the interactive legend. Navigate to the folder with your input data and select your primary data file. For the sample dataset, select *Enerly_GeneExpression.tab* and click *Import* (or simply double-click on *Enerly_GeneExpression.tab*):



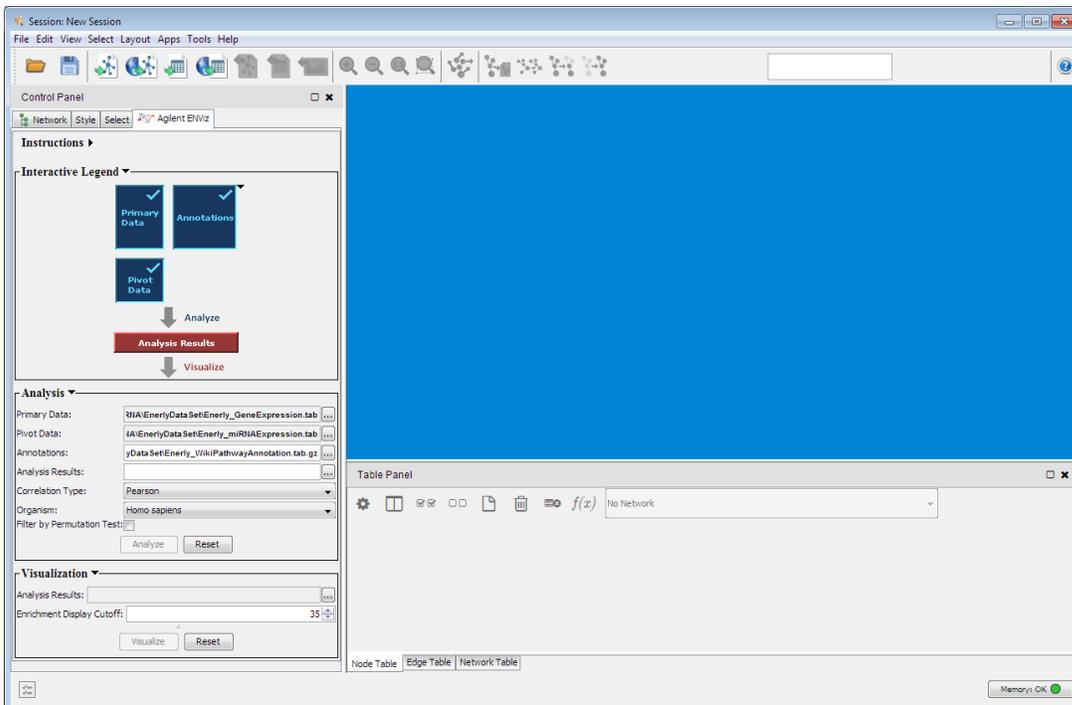
Notice the checkmark that now appears on the *Primary Data* box. These checkboxes inform you that the given box has been filled in. Also notice that the *Primary Data* *textbox* in the *Analysis* subpanel is filled in with your choice:



Similar to *Primary Data*, to load *pivot data* click on the box labeled *Pivot Data*, and navigate to the pivot data file. For the sample dataset, double-click on *Enerly_miRNAExpression.tab*:



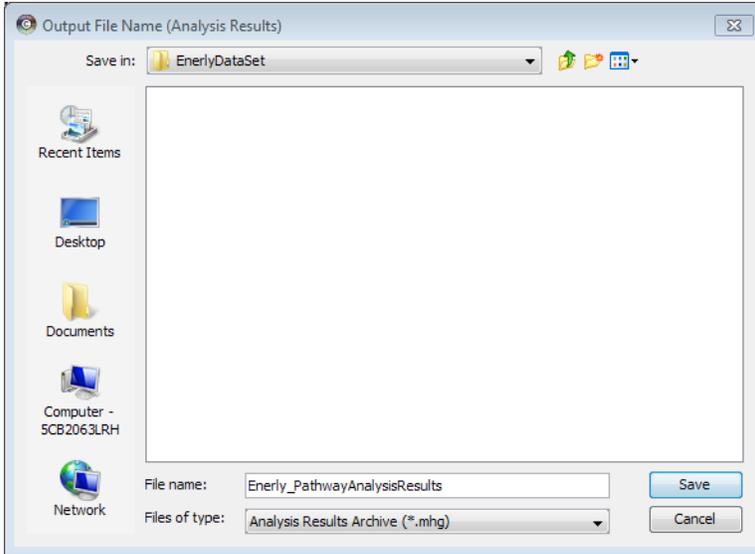
Click on the *Annotations* box, and navigate to the annotation file *Energy_WikiPathway_Annotation.tab.gz*, and click *New or Import*.



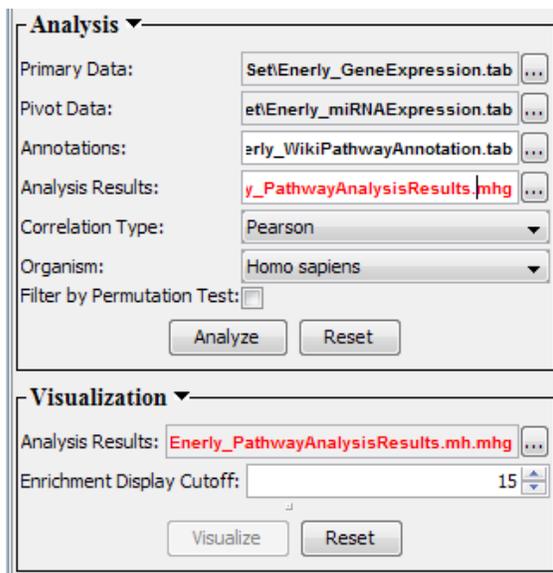
You've now set up the input for a pathway-oriented analysis.

To run the analysis, specify an *Analysis Results* file for the results of analysis. This is an archive file that will contain the correlations, enrichments, and thresholds that are produced by the analysis. It also serves as input to *Visualization*.

Click on the box labeled *Analysis Results*. You will be prompted for a file name:



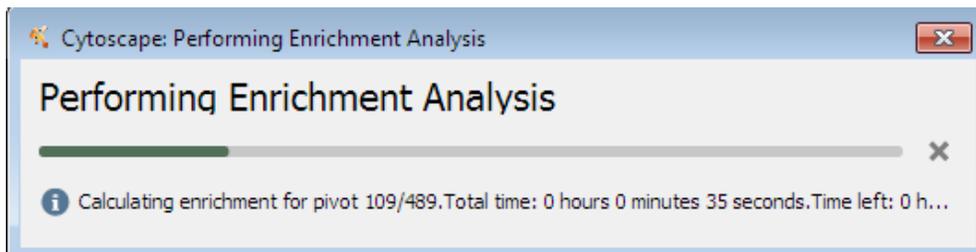
Enter a new file name of your choice. You can do this by choosing an existing file and modifying the name in the *File name*: field, or by just typing a new name. You don't need to enter the .mhg extension - it will be added automatically. After entering a name, click on *Save*. The output file name will be shown in red text on the control panel when the file doesn't currently exist. It will also be shown, in red, in the *Analysis Results* input box for the *Visualization* sub-panel. You can also directly enter or edit the pathname in the *Analysis Results* input box, however the pathname must be a full pathname to the file.



The analysis results file is a zipped archive containing all the analysis data files needed for visualizing your enrichment results as an enrichment network.

2.3 Running analysis

Once the input and results files are set up, the *Analyze* button becomes enabled. Select *Correlation Type* (Pearson or Spearman), *Organism* (*Homo Sapiens* for sample dataset), *Filter by Permutation Test* (unchecked for this example) and click the *Analyze* button to run the analysis. You will see a task monitor dialog that displays the status of the analysis as it executes, along with the total and remaining time estimates:



When the analysis is completed, the analysis results file name becomes black (meaning the results file now exists), and you can now visualize the results as a Cytoscape network as described in Section 3.

To run an analysis using GO annotations, perform the same operations above, but select "Enerly_GO_Annotation.tab.gz" as the annotation file, and change the name of the analysis results archive and click *Analyze*. Note that since GO annotation has more than 11K terms, enrichment analysis takes much longer than for pathways.

For your own data, if you don't have an annotation file ready, see Section 2.5 on how to generate annotation files using ENViz.

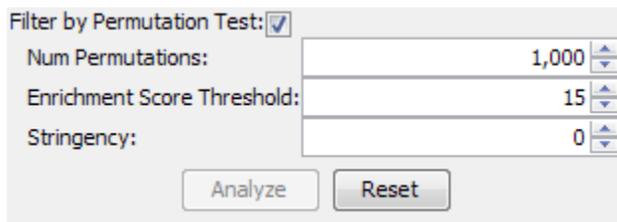
2.4 Filtering analysis results by permutation test

When the option *Filter by Permutation Test* is selected, the following filtering of analysis results based on randomized shuffled data is performed in addition to enrichment analysis.

To run this analysis, you specify

- *Num Permutations* - number of permutations to run (default is 1000),
- *Enrichment Score Threshold* - default is 15. For GO analysis, 15 is the recommended threshold for the sample dataset, for pathway analysis the recommended threshold is 6. See Section *Good analysis practices* for guidelines on how to set this threshold for other datasets.
- *Stringency* - the number of random scores above observed mHG score that still allow passing the filter (default is 0).

For each permutation, samples in the pivot data are randomly shuffled. This means that the columns of the matrix are permuted. For each pivot with at least one enrichment score above *Enrichment Score Threshold*, the correlations between unchanged primary data and randomized pivot data, and the corresponding enrichment scores S_{rand} are calculated. If for a given pivot-annotation element pair with enrichment score S , we observe $S_{rand} \geq S$ more than *Stringency* times across all permutations, this pivot-annotation element pair is considered not significant and its enrichment score is set to 0.



Filter by Permutation Test:

Num Permutations:

Enrichment Score Threshold:

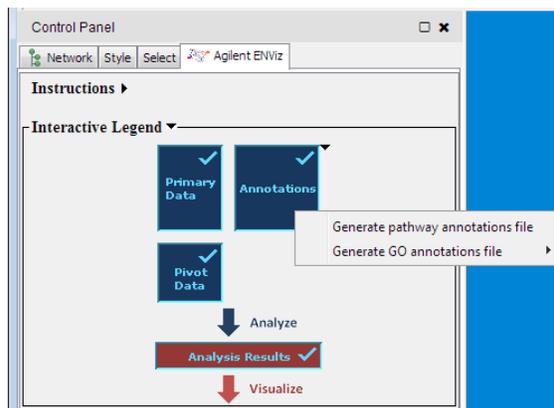
Stringency:

Analyze Reset

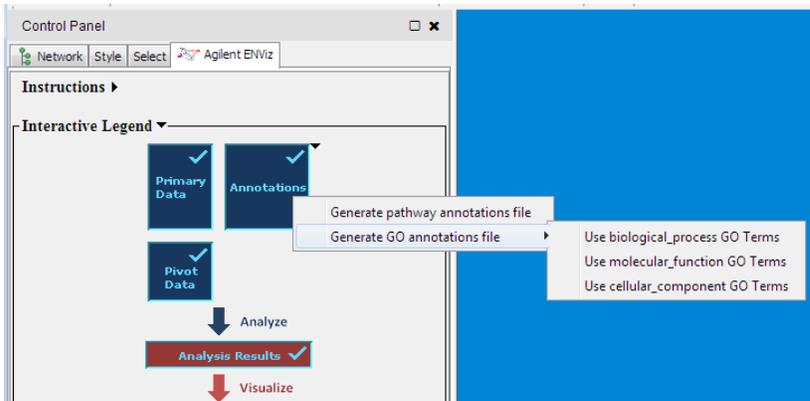
For pivot-annotation pairs that survive this permutation test filtering, the original mHG score is reported as the enrichment score.

2.5 Generating annotation files

To generate a pathway-based annotation file (based on WikiPathways), provide an annotation file name in the *Annotations* box. Remember that you can do this by clicking on the *Annotations* box in the interactive legend or by clicking the '...' button next to the *Annotations* field and changing an existing file name, or by directly typing the full pathname of file in the *Annotations* field. After selecting a name, right-click on the *Annotations* box in the Interactive Legend (or alternatively click on the triangle next to the top of the *Annotations* box). Select 'Generate pathway annotations file'. This will generate a tab delimited file with one row for each gene and one column for each pathway will be generated. Entry in row i column j of annotation file will be 1 if gene i belongs to pathway j , 0 otherwise. If the annotation file name has the extension '.gz', the file will be automatically compressed (zipped).



To generate a GO annotations file, right-click on the *Annotations* box (or click the little triangle next to the annotations box) and select 'Generate GO annotations file' and then select one of the options 'Use biological_process GO terms', 'Use molecular_function GO terms', 'or 'Use cellular_component GO terms'.



When GO annotation is generated for the first time, 'gene_info.gz', 'gene2go.gz' and 'gene_ontology_edit.obo' are downloaded from <ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/> website and copied to your ENViz app's data directory (see the [Cy2 User Readme](#) or the [Cy3 User Readme](#) documents for details). These files are used to generate GO annotation matrices and visualize the GO hierarchy. These files are updated monthly.

2.6 Good analysis practices

1. Make sure that samples are ordered in the same way in primary and pivot data files. ENViz checks that sample names in the first rows in primary and pivot data are the same and will generate an error otherwise.
2. Since ENViz does not do any pre-processing of the input data, it is very important that input data does not have any duplicate elements. Having duplicate primary data elements will compromise mHG statistical model, and having duplicate pivot entries will generate duplicate enrichment network nodes and edges. When primary data is gene based and has GeneSymbol as gene identifier, check also that different GeneSymbols do not represent the same gene.
3. In case of GO annotation, remove primary data elements that do not belong to any annotation categories. This will improve both the speed of the analysis and the accuracy of the statistics.
4. To reduce false positive results it is preferred that the *Enrichment Score Threshold* T be set to correct for multiple testing. For example, according to Bonferroni multiple testing correction, $T = -\log_{10}(p_0/(M*A))$, where M , A are the dimensions of *Enrichments Matrix* and p_0 is the desired significance level.
5. Running analysis with *filtering by permutation test option* reduces the level of false positive results. Recommended Enrichment Score Threshold for filtering by permutation test is the same T as above.



3. Visualizing Analysis Results

To visualize the results of a completed analysis, select your *Enrichment Display Cutoff* (10 in this example) and press the *Visualize* button.

The screenshot shows two panels in the ENVIz software interface. The top panel, titled "Analysis", contains several input fields: "Primary Data" (Set1Enerly_GeneExpression.tab), "Pivot Data" (etfEnerly_miRNAExpression.tab), "Annotations" (erly_WikiPathwayAnnotation.tab), "Analysis Results" (y_PathwayAnalysisResults.mhg), "Correlation Type" (Pearson), and "Organism" (Homo sapiens). There is also a checkbox for "Filter by Permutation Test" and "Analyze" and "Reset" buttons. The bottom panel, titled "Visualization", has "Analysis Results" (Set1Enerly_PathwayAnalysisResults.mhg) and "Enrichment Display Cutoff" (10) with a spin button. It also includes "Visualize" and "Reset" buttons.

3.1 Enrichment Networks

As you may recall, ENVIz represents significant analysis results as an *enrichment network* - a bipartite graph with nodes corresponding to *pivot* and *annotation* elements, and edges corresponding to *pivot-annotation* pairs with annotation enrichment scores better than the user-defined threshold (cutoff) value.

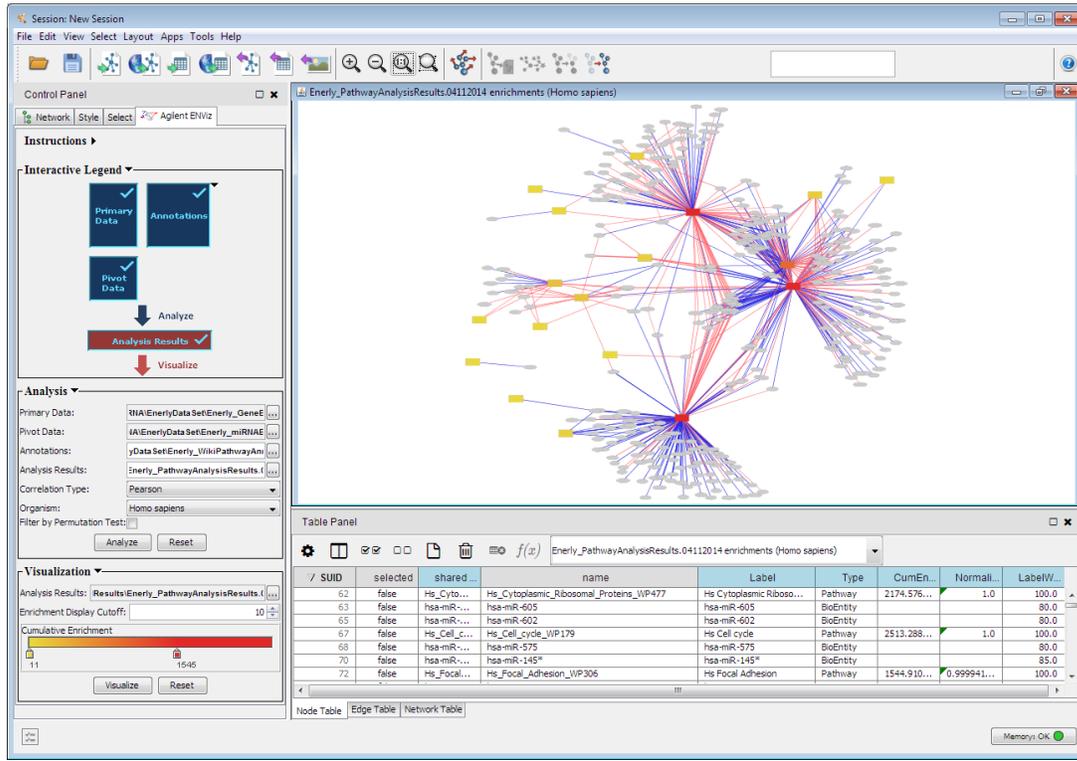
Once your enrichment network is visible in Cytoscape, you can **pan** and **zoom** the network using standard Cytoscape commands and menu items (see the Cytoscape User Manual).

3.2 Pathway enrichment visualization

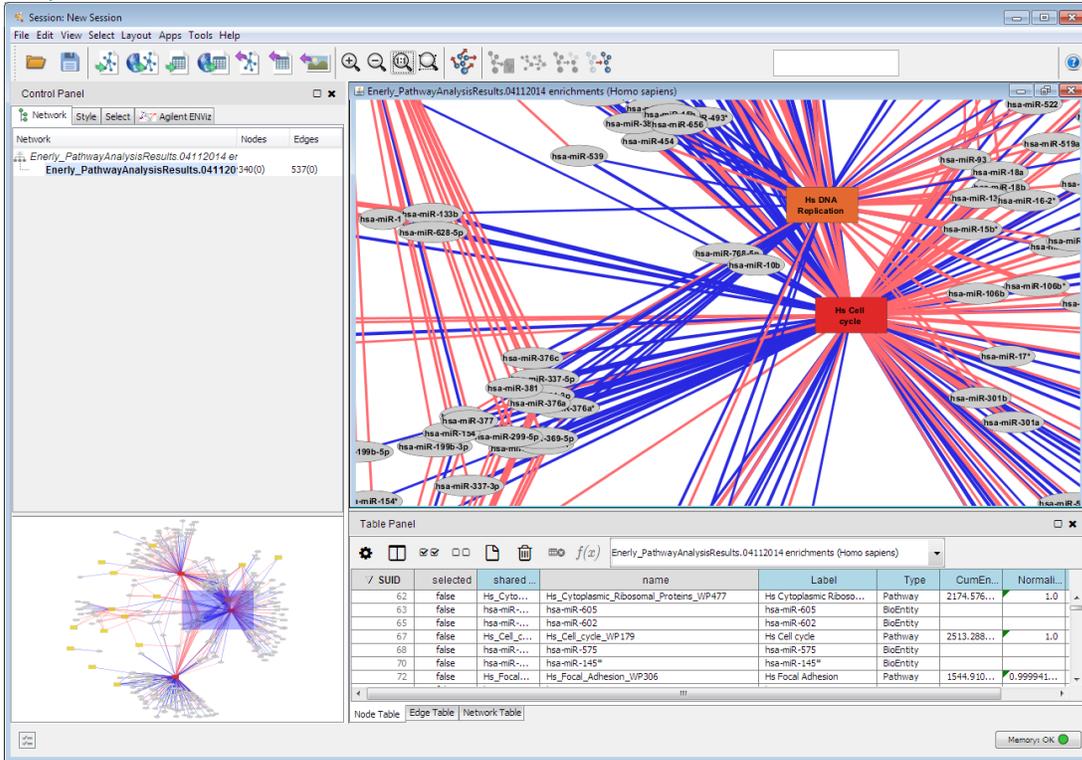
In the figure below,

- **Nodes** are pathways (color coded yellow → red by cumulative enrichment score) and miRNAs (color-coded **grey**). The cumulative enrichment score for each pathway node is calculated as the sum of enrichment scores for all edges connected to this pathway node.
- **Edges** between pathway and miRNA nodes are color-coded by direction of gene-pivot correlation. **Red** corresponds to the enrichment among genes positively correlated to the pivot miRNA, and **blue** corresponds to the enrichment among genes negatively correlated to the pivot miRNA. Thickness of the edge is proportional to the enrichment score.

The network below is generated for the enrichment score cutoff of 10 and contains 340 nodes (miRNAs and pathways) and 537 edges:



To see specific areas of the network in more detail, use the Cytoscape pan and zoom operations. By clicking on the Control Panel Network tab, you can also pan around the network and see the zoomed-in network in the context of the whole network on the bottom left:

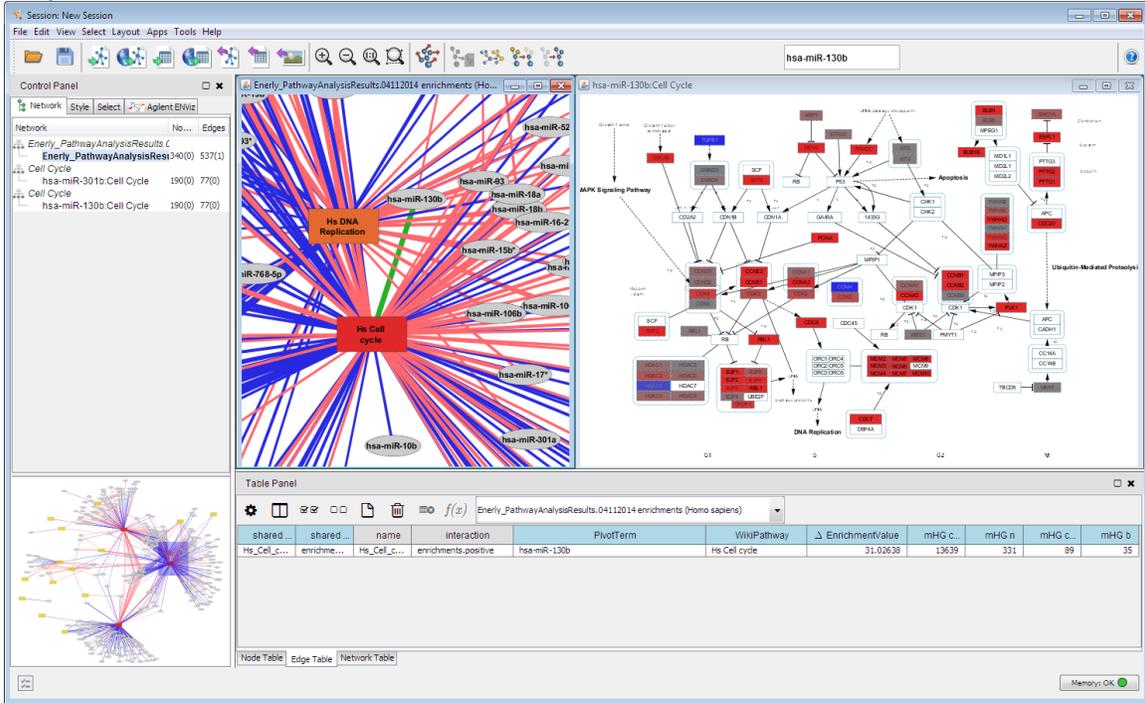


Visually overlaying enrichment information on pathways

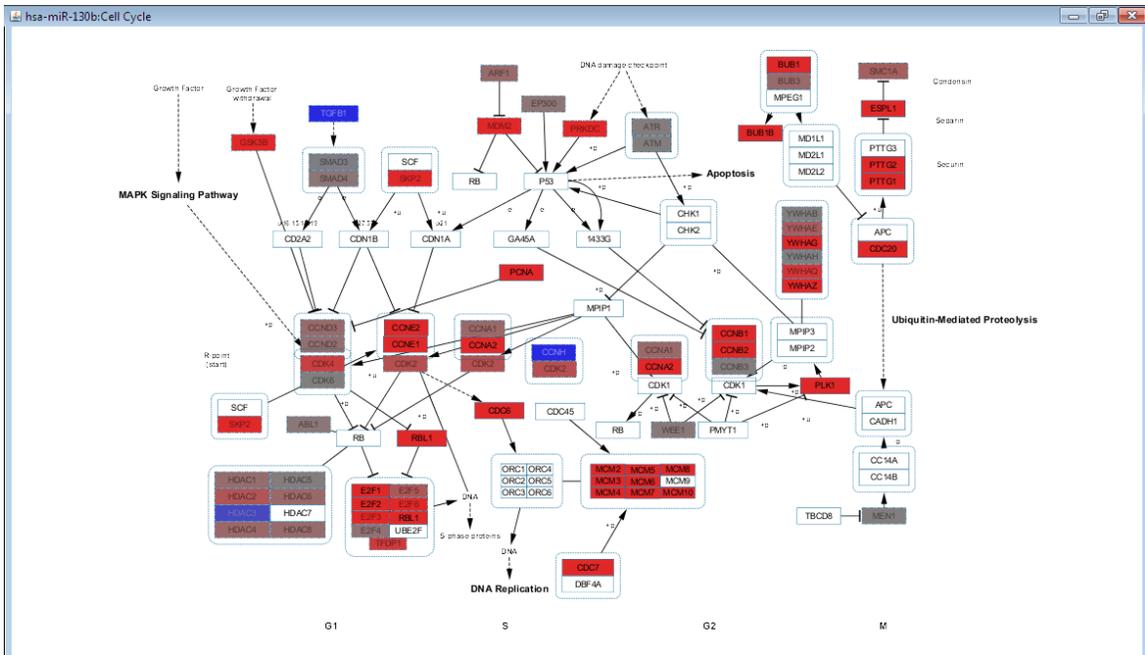
For analysis of enrichment in biological pathways, correlations of primary data and corresponding pivot data are visually overlaid on biological pathways for each significant pivot-annotation pair, using WikiPathways [4]. You can overlay enrichment information on pathways by double-clicking on edges, pathways, and pivot nodes in the enrichment network. Besides selecting the node or edge you clicked on (and displaying it in green), one of the following pathway visual overlays takes place:

Displaying a pathway colored by one connecting edge

Double-clicking on an edge in the enrichment network displays the WikiPathway corresponding to the pathway node connected to the edge. In this example, the selected pathway is *Cell cycle*. Genes in the selected pathway are colored by correlation to the pivot node at the other end of the selected edge (*hsa-miR-130b*). In the right part of the figure below, all gene nodes in the *Cell cycle* pathway that map to primary data elements are color-coded (blue -> red) for the correlation score between the primary data element (gene) and the pivot data element *hsa-miR-130b*. Solid borders and high opacity show genes above correlation threshold that were included in the gene set used for enrichment analysis. Genes colored red have positive correlations to *hsa-miR-130b*, genes colored blue have negative correlations to *hsa-miR-130b*.



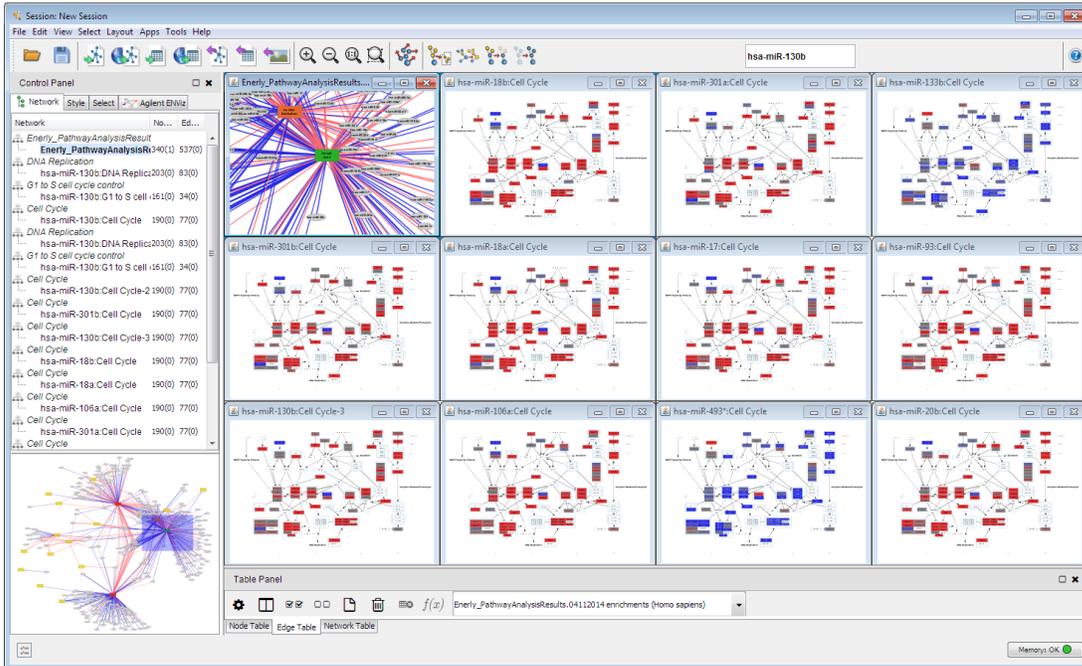
Here is a slightly larger view of the *Cell cycle* pathway with genes color-coded by correlations to *hsa-miR-130b*:



Displaying a pathway colored by several connecting edges

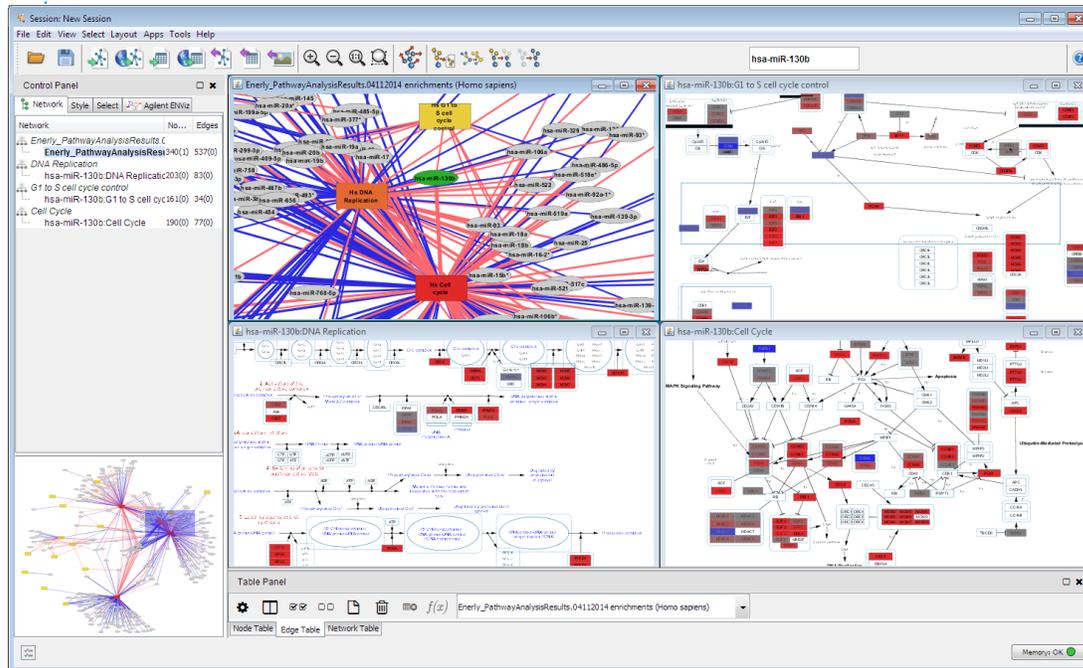
Double-clicking on a pathway node displays the pathway multiple times (up to a configurable limit) with each pathway instance colored by the data associated with a different "coloring" edge connected to this pathway. The coloring edge is used to color the pathway through the use of the correlation to the pivot node connected to

that *coloring* edge. If the number of edges connected to the pathway node is bigger than the limit, pathway instances are generated for the edges with the highest enrichment scores. Network views are tiled in a *small multiples* view that accentuates contrasts between correlations for different pivot data. In the Figure below, 11 different views of cell cycle-2 pathway are shown; genes in each view are colored by the correlation to the corresponding miRNA. The title of each corresponding panel has the name of the pathway and the name of the miRNA.



Displaying multiple pathways colored by one pivot

Double-clicking on a pivot node displays each of the pathways connected to that pivot, up to a configurable limit. Each pathway is colored by its correlation with the selected pivot node:



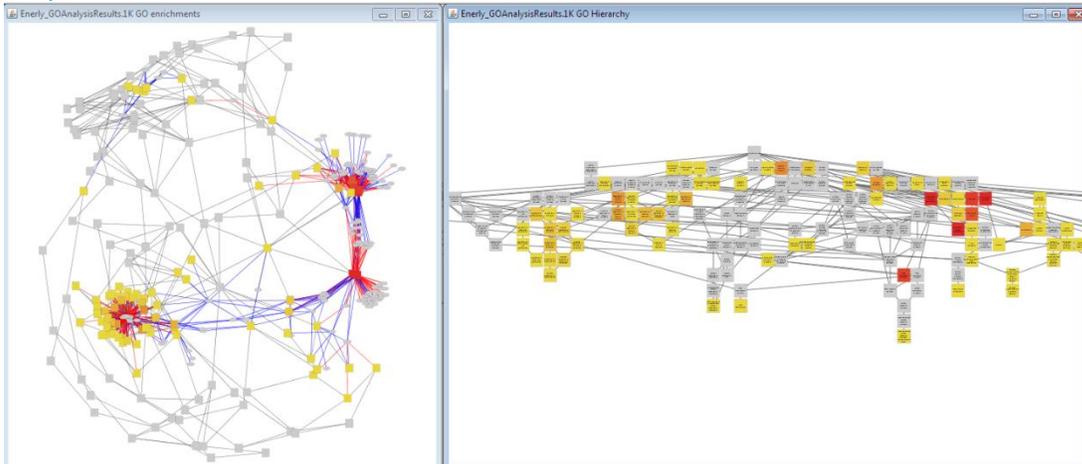
3.3 Gene Ontology enrichment and visualization

Enrichments of GO categories are overlaid on top of the Gene Ontology DAG in addition to the bi-partite graph representing the enrichment network.

In the Figure below, the **left** panel shows the pi-partite GO enrichment network with:

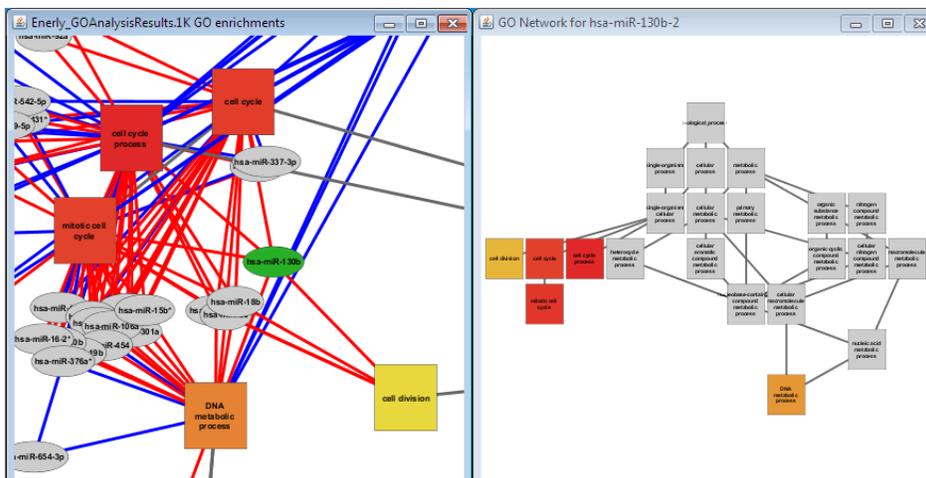
- **GO term nodes**, colored on a yellow to red scale, according to the GO term cumulative enrichment value. Cumulative enrichment score for each GO term is the sum of enrichment scores for all edges connected to the GO term node.
- **Nodes for included parents** of these GO terms - even if they don't have edges connecting them to a pivot. Unconnected parents are colored gray.
- **Pivot nodes** (miRNAs) colored gray.
- **Edges** between GO and miRNA nodes corresponding to pivot-annotation pairs with annotation enrichment scores better than the *Enrichment Display Cutoff* value. These edges are color-coded by direction of gene-pivot correlation. **Red** corresponds to the enrichment among genes positively correlated to the pivot miRNA, and **blue** corresponds to the enrichment among genes negatively correlated to the pivot miRNA. Thickness of the edge is proportional to the enrichment score.
- **Edges** between GO terms represent parent-child relationship between GO terms.

The **right** panel shows a GO summary network (part of GO DAG) for GO terms from the left enrichment network [3]. Each GO node is color-coded by the cumulative enrichment score for its set of pivot miRNA nodes. Parent terms are added to complete the GO hierarchy. Parent GO terms that do not have individual miRNA-GO term edges above the enrichment score cutoff are colored gray:



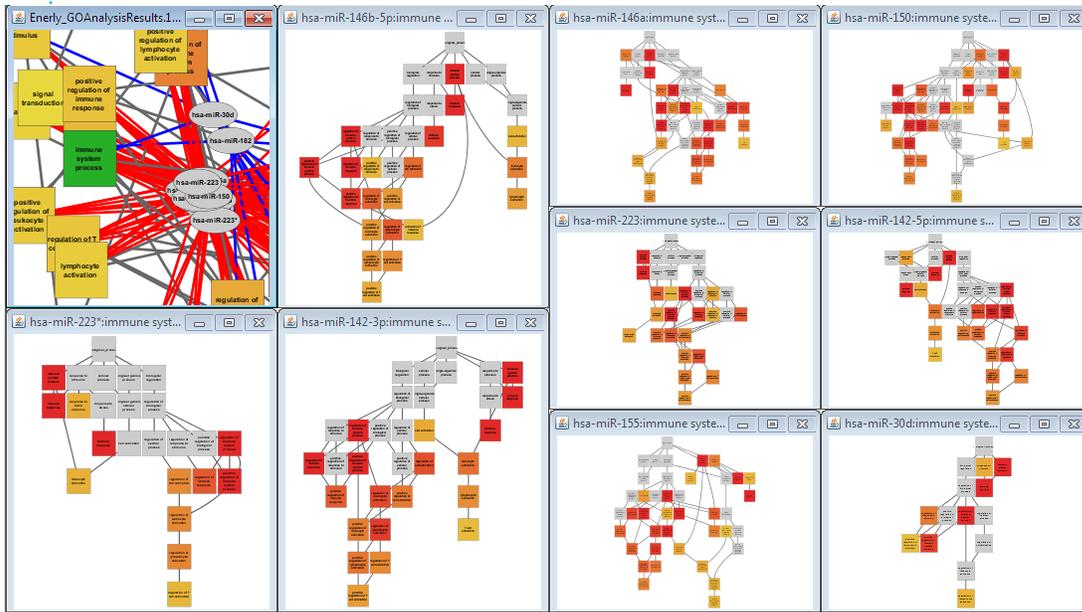
Displaying GO Summary network colored by one pivot

Double-clicking on a pivot node (gray) in the enrichment network (**left**) shows the GO summary network (**right** panel) colored based on the enrichment scores for the selected pivot. This GO summary network includes only GO terms enriched in the genes correlated (anti-correlated) to the selected miRNA pivot and their parents:



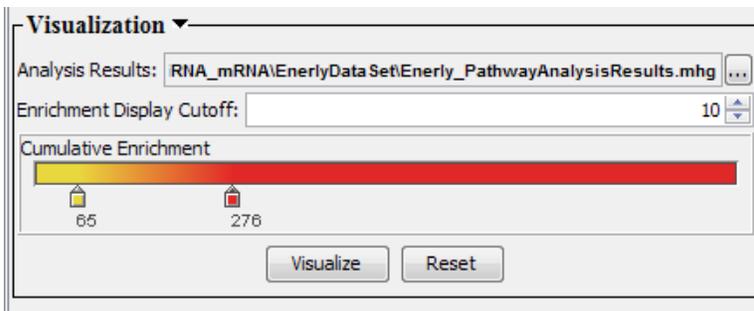
Displaying multiple GO Summary networks colored by multiple pivots

Double-clicking on a GO node displays multiple, different GO Summary networks, one per connected pivot, up to a configurable limit. Each network is populated by the GO terms that have significant enrichment values for the corresponding pivot datum and their parents:



3.4 Color controls

The coloring of annotation nodes in each enrichment network can be adjusted by the color slider:



When analysis results are visualized for the first time, the coloring scheme is defined in the following way. Nodes with cumulative enrichment scores in the bottom 10% are colored yellow. Nodes with cumulative enrichment in the top 10% are colored red, colors for all other nodes are scaled linearly. These color assignments can be manually adjusted using the left and right slider thumbs to get the best visual representation of the network.

Cumulative enrichment values are used to color pathway enrichment networks, GO cumulative enrichment networks and cumulative GO graphs. Enrichment score values are used to color selected pivot GO graphs.

3.5 Viewing and saving enrichment statistics

To see enrichment scores corresponding to individual edges, right-click on an edge and select *Enrichment Statistics*. A window will pop-up showing the enrichment score together with the details of mHG statistics. In the example below, enrichment of



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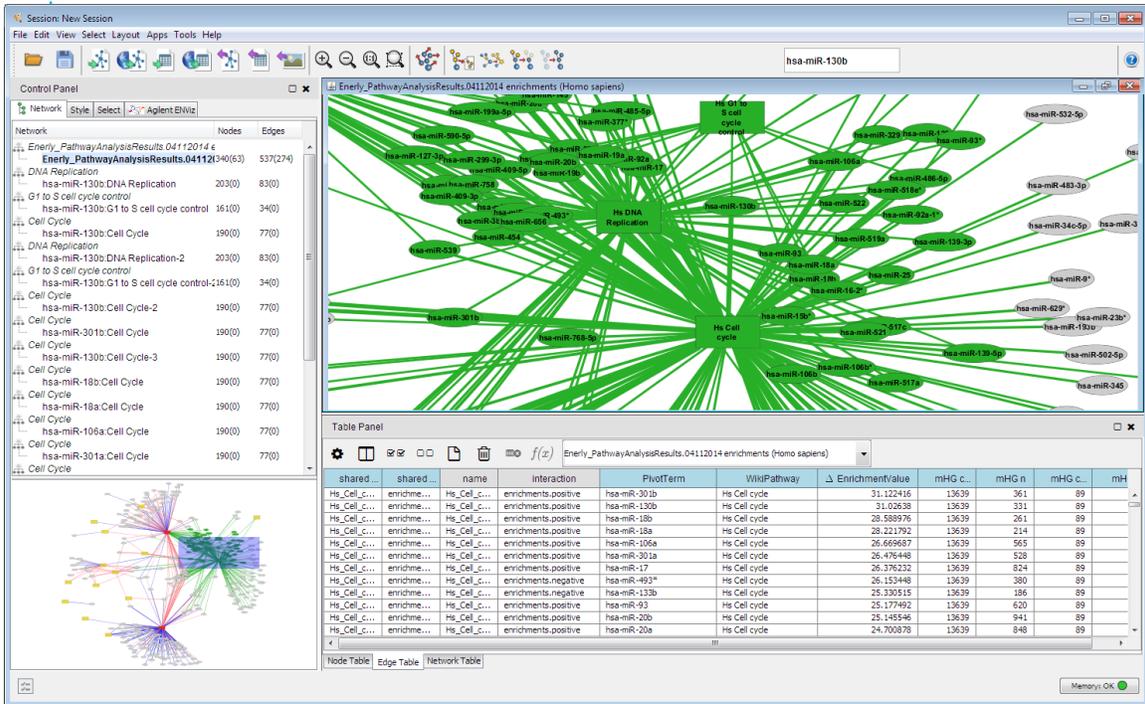
pathway *Cell cycle* for genes positively correlated with *hsa-miR-130b* has mHG score of 31. In more detail, out of a total of 13,639 genes in the dataset, 89 belong to *Cell cycle* pathway. Of the top 331 genes most correlated to *hsa-miR-130b*, 35 belong to *Cell Cycle* pathway.

Statistic	Value
Enrichment value	31
N (total primary genes)	13639
n (genes above correlation cutoff)	331
B (Genes in 'Hs Cell cycle')	89
b (Genes above cutoff in 'Hs Cell cycle')	35

The enrichment statistics are stored as attributes associated with edges. So, to see the enrichment statistics for many edges, you can use Cytoscape's Table Panel to view and export the edge attributes of an enrichment network. The attribute names of the enrichment statistics are prefaced with 'mHG' and the capital names are prefaced with 'cap'. For example, the 'B' statistic is represented by the attribute 'nHG capB'.

Here's the steps to view and export an enrichment network's statistics:

1. Select edges of interest in your enrichment network by dragging the mouse over them or using other Cytoscape operations for selecting edges (e.g., Select->Edges).
2. Go to the Edge Table and click on the *Select All Attributes* button (the button with checkmarks).
3. To export enrichment statistics for all edges in the network, use **File->Export->Table** from the Cytoscape menu.



For each visualized enrichment network, False Discovery rate (FDR) rate is calculated and reported in the Network Table. FDR is calculated as the following ratio:

$$\text{expected number of significant edges} / \text{observed number of significant edges}$$

Where *expected number of significant edges* is:

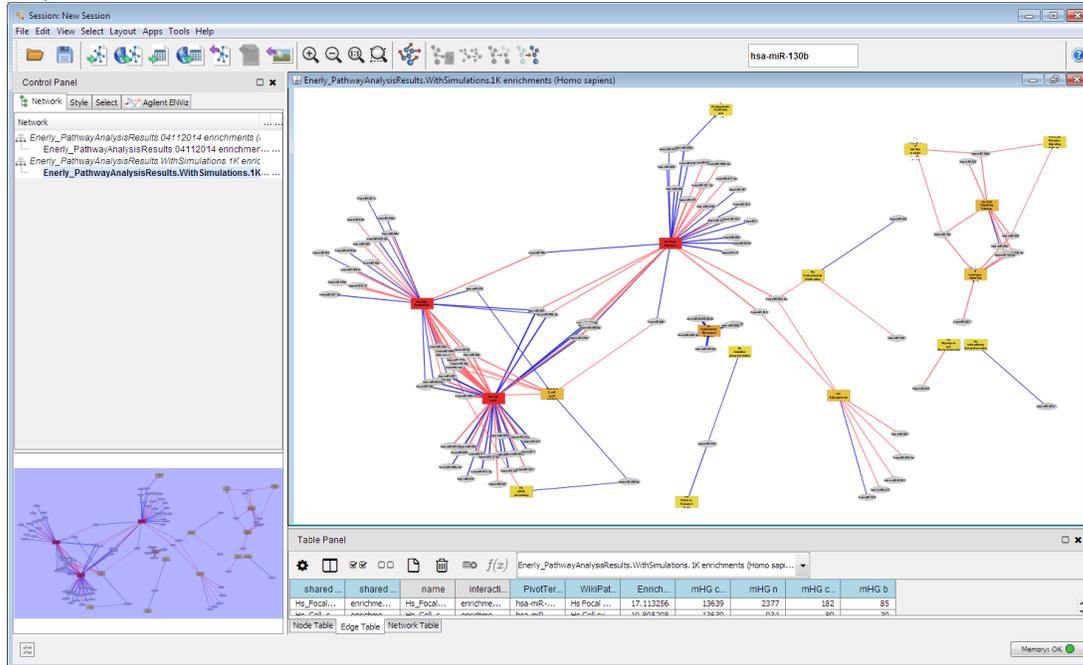
$$\text{num-annotation-nodes (A) x num-pivots (M) x } 10^{(-\text{Enrichment Display Cutoff})}$$

And *observed number of significant edges* is the number of edges in the enrichment network with enrichment scores above Enrichment Display Cutoff.

3.6 Visualization of saved results

Any analysis results archive file generated by ENViz can be visualized as an enrichment network independent of running enrichment analysis.

To visualize a previously generated analysis results file, select it using the Visualization subpanel and then click *Visualize*. The figure below shows the visualization of our sample pathway analysis results when filtering by permutation test (sample file *Enerly_PathwayAnalysisResults.WithSimulations.1K.mhg*):



This network is much smaller than pathway enrichment network generated without the filtering by permutation test. This network has 114 nodes and 142 edges at the display cutoff of 10, compare to 340 nodes and 537 edges in the network obtained without the permutation test filter.

3.7 Visualization of generic enrichment data

When generic annotation data is used for enrichment analysis, ENViz only displays the enrichment network as a bi-partite graph with pivot and annotation nodes. As before, edges connect pivot and annotation nodes corresponding to pivot-annotation pairs with annotation enrichment scores better than the *Enrichment Display Cutoff* value. These edges are color-coded by direction of gene-pivot correlation. Red corresponds to the enrichment among genes positively correlated to the pivot, and blue corresponds to the enrichment among genes negatively correlated to the pivot. Thickness of the edge is proportional to the enrichment score.



4. ENViz Operational Notes

For details on recommended computer configuration, ENViz configuration, and migrating data to new versions of ENViz, see the [Cy2 User Readme](#) or the [Cy3 User Readme](#) documents.

4.1 Destroying and recreating network views

ENViz fully supports destroying and creating Cytoscape views of ENViz-generated CyNetworks. Because ENViz can quickly generate many Cytoscape views, you may wish to destroy some network views and then bring them back at a later time. To remove views:

1. Click on Cytoscape's Control Panel Network tab
2. Select the network views to remove
3. Bring up the context menu on any of the selected networks (e.g., right-click on Windows) and perform *Destroy View*.

To recreate views:

1. Select some or all of the networks for which you destroyed views.
2. Bring up the context menu on any of these selected networks and perform *Create View*.

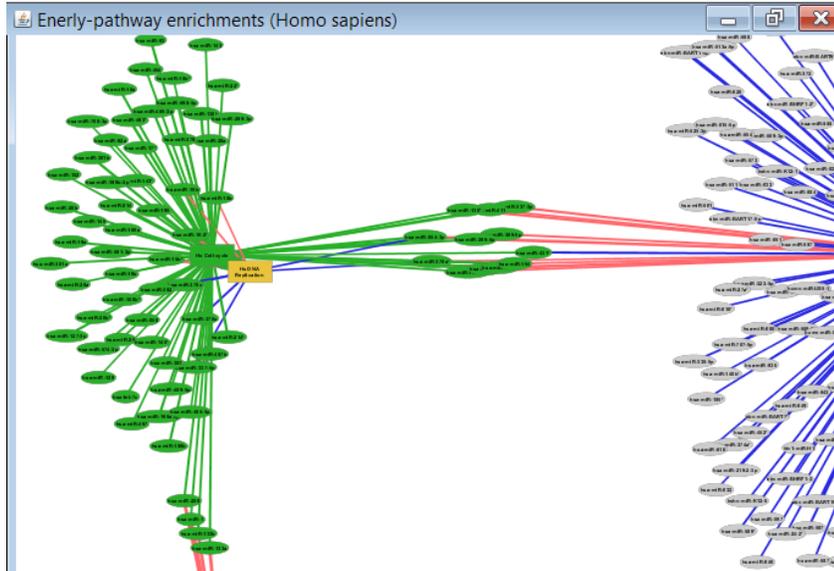
The networks for which you recreated views should behave normally and support all ENViz operations.

4.2 Creating sub-networks from ENViz enrichment networks

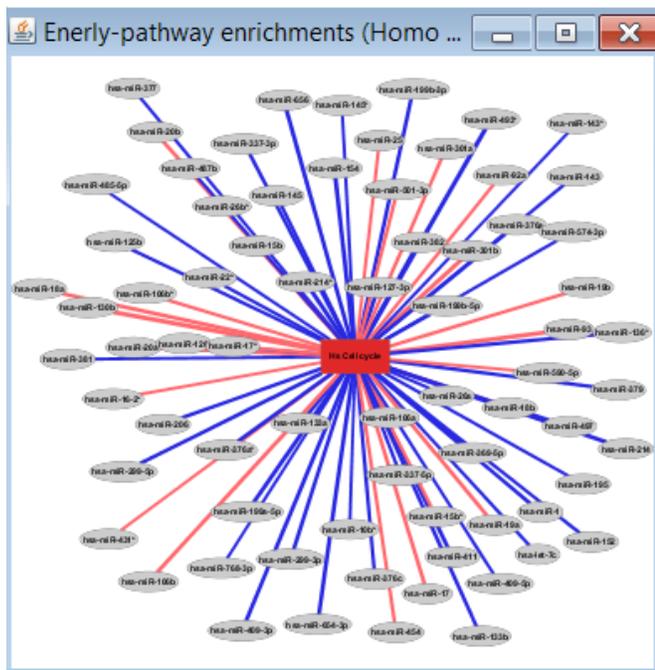
For many cases, you can use Cytoscape to create sub-networks of ENViz networks and have these sub-networks behave like the original enrichment network in that the various ENViz operations will work on edges, pivots, and annotations in the sub-network.

Consider the example of pulling out one pathway from an enrichment network. We will pull out the *Hs Cell cycle* pathway and place it in a separate CyNetwork:

1. Visualize the Enerly pathway data (see Visualizing Analysis Results, above)
2. Select the *Hs Cell cycle* pathway node and perform *Select->Edges->Select Adjacent Edges*
3. Now select all the nodes connected to your selected edges by choosing *Select->Nodes->Nodes Connected by Selected Edges*. Your enrichment network will look similar to:



4. Now create a new sub-network by performing *File->New->Network->From Selected Nodes, Selected Edges*. You should now see a new sub-network like:



This new sub-network should behave just like the original enrichment network for ENViz operations.

ENViz uses a heuristic to determine the originating enrichment network so that it can associate the appropriate ENViz state with the sub-network. This heuristic can fail when there isn't a clear originating network as in creating a network from ten different enrichment networks. When there is more than one possible originating enrichment network, ENViz currently arbitrarily chooses one (a future release of ENViz will allow you to choose the originating network). When an originating enrichment network is not found, the sub-network will not behave as an ENViz network.



4.3 Saving and restoring sessions

Cytoscape networks created by ENViz can be saved and restored¹ in Cytoscape sessions, however the functionality of these networks is limited to displaying the networks as they appeared when saved and to having the correct attributes associated with the nodes and edges in each of these networks.

All ENViz-specific operations, like double-clicking on nodes and edges are *not* supported.

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¹ Note that you may see some error messages when restoring your session about the CytoscapeSessionReader not being able to read files (NullPointerException). You can ignore these messages-the networks referenced seem to be restored correctly.



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6. Acknowledgments

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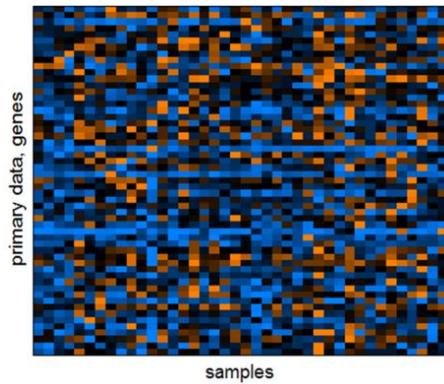
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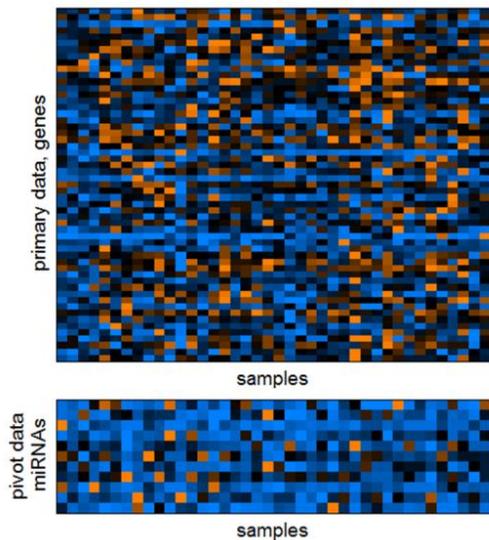
A. Appendix

Overview of ENViz Enrichment Analysis

Consider a primary data matrix with N rows and S columns. In the sample data set this matrix has gene expression data.

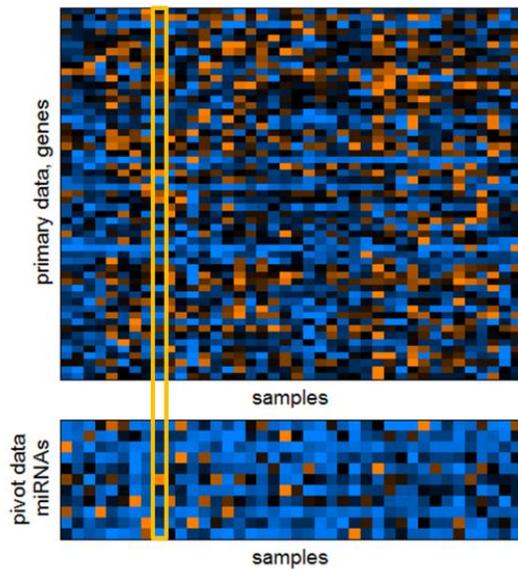


Next, consider pivot data matrix with M rows and S columns. In the sample data set this matrix has miRNA expression data:

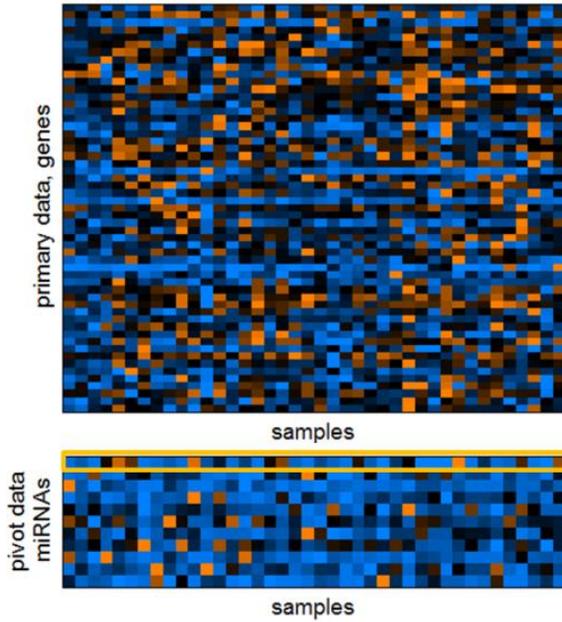




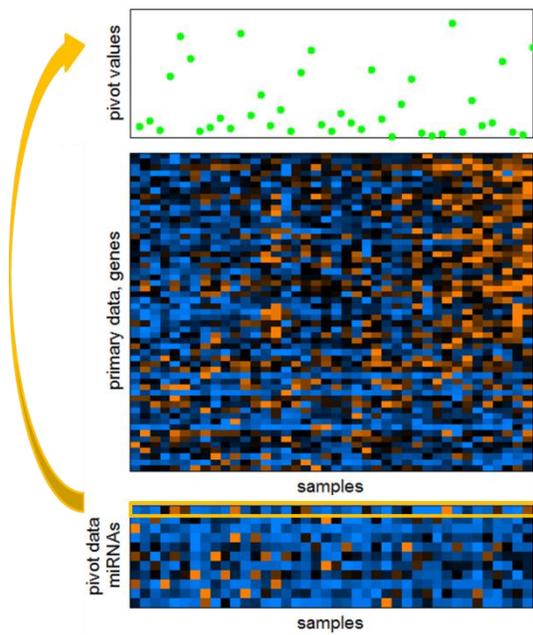
Samples in the primary and pivot data matrices should be ordered in the same way, i.e., sample s in primary data is the same as sample s in pivot data:



Now consider the first row in the pivot matrix (first pivot):



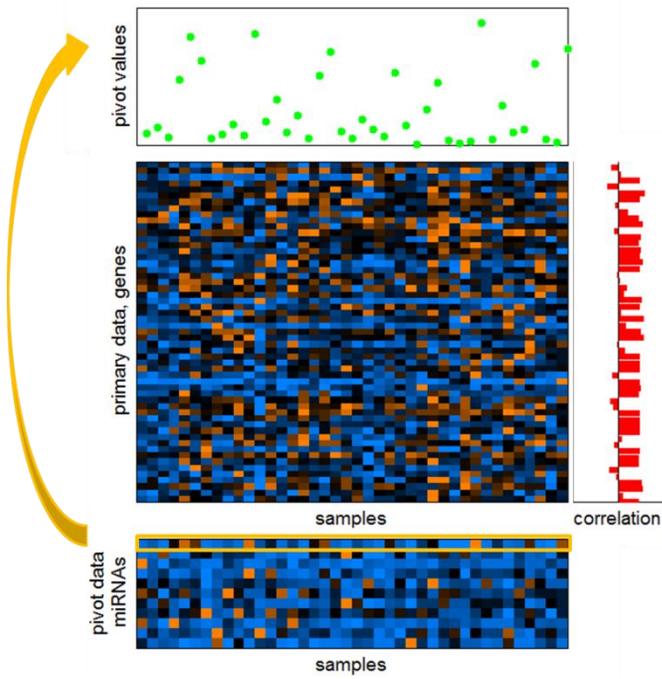
In the Figure below, values for the first pivot are plotted in the top panel:



A correlation value is calculated for every row/gene in the primary data with respect to the first pivot. Correlation could be Pearson or Spearman. Correlation values to the first pivot are plotted on the right hand side of the pivot data matrix heatmap:



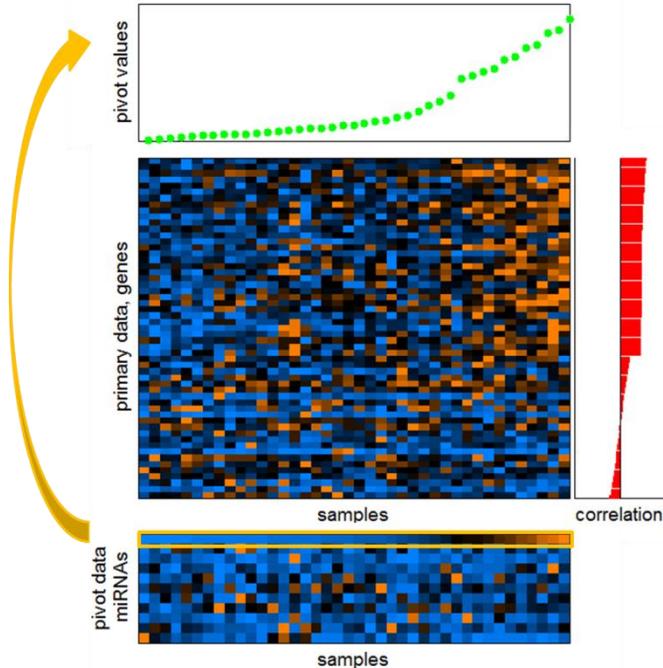
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Next genes/rows are ranked according to the correlation with pivot. In the Figure below the samples are reordered by monotonically increasing pivot values to produce more clear visualization of the analysis:



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We then compute the statistical enrichment of primary *data annotation* elements (gene sets) in the top of this ranked list based on mHG (minimum hypergeometric) statistics.

Details of mHG statistics are explained in [2, 3, 5] and briefly in section 1.1 above. Note that whereas standard statistical enrichment tools set arbitrary thresholds (such as top 100 genes, top 10% of the genes or genes with at least 2-fold change), the mHG model seeks enrichment in the top of the ranked list without predefining the top of the list

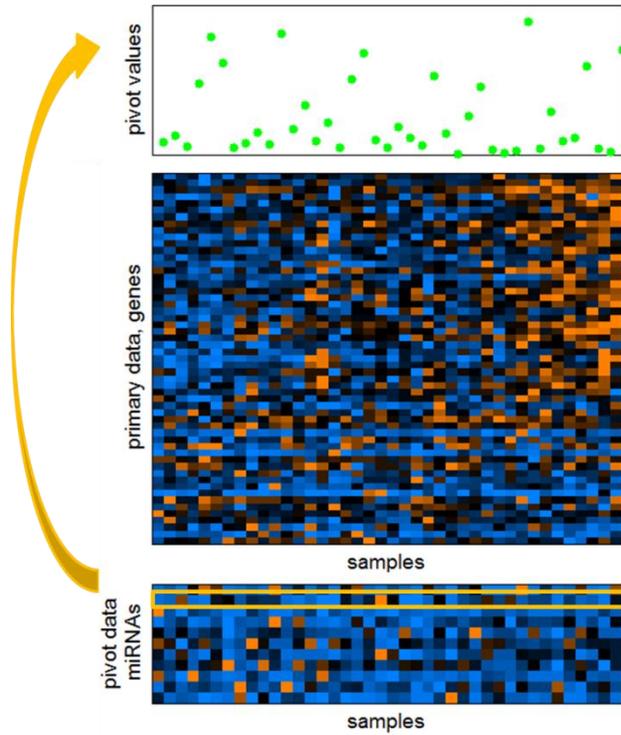
The process results in an enrichment score calculated for each pivot datum and each annotation element (e.g. pathway or GO term). The enrichment score is $-\log_{10}(\text{mHG } p\text{-value})$. Note that mHG p -values are corrected for multiple testing done in mHG calculation, but not for number of pivots testes or number of annotation terms in the annotations matrix.

This enrichment analysis process is also done for anti-correlation, where the primary data are arranged according to anti-correlation with the pivot datum being analysed.

We then repeat the same process using the second row as the point of focus:



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At the end of the analysis, we generate two enrichment matrices of size $M \times A$, one based on positive correlations, and one based negative correlations. These matrices are used for visualization of analysis results described in Section 3.