

Visualizing Pathway-Level Co-expression Results in Cytoscape

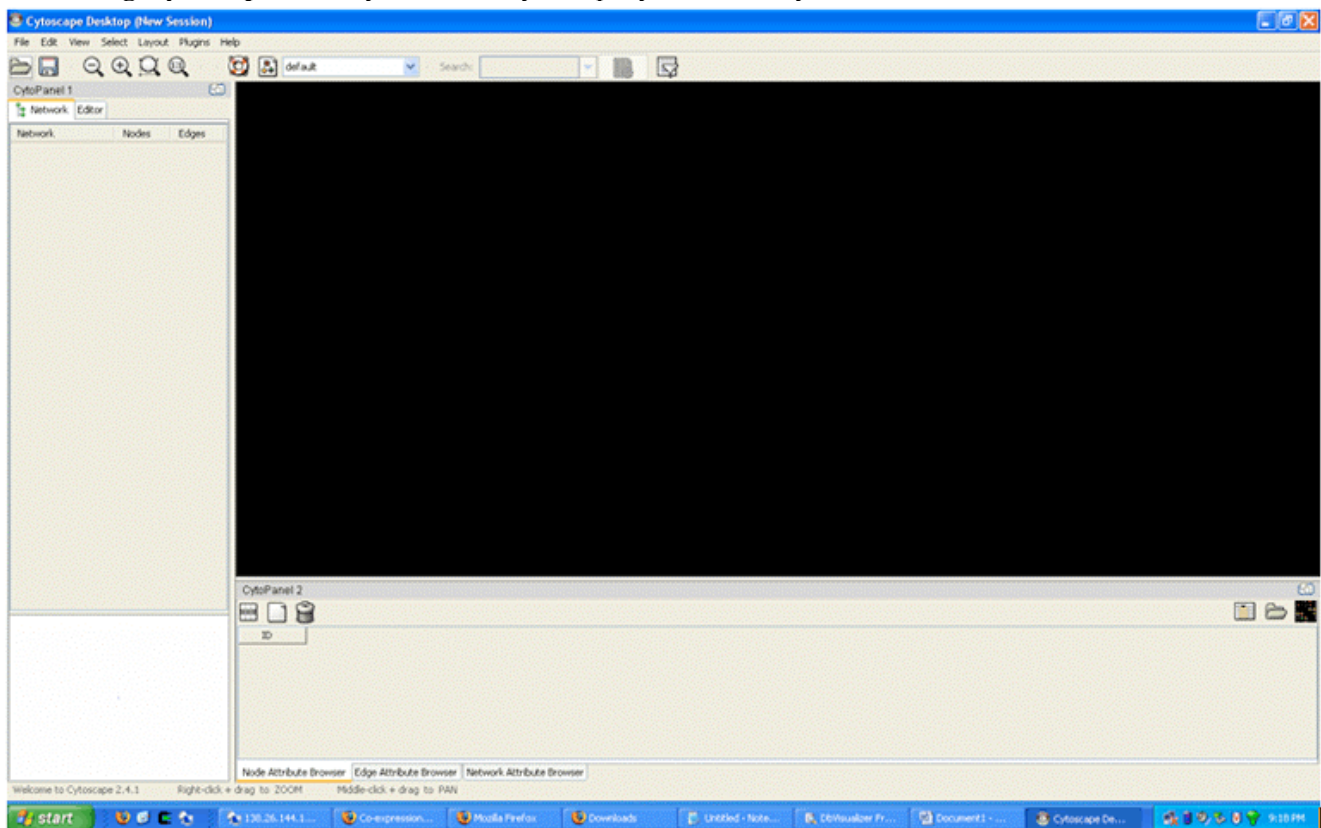
Introduction. Cytoscape is an open source, freely-available network visualization tool. Detailed instructions on how to obtain and operate Cytoscape are available at the Cytoscape Web site: <http://www.cytoscape.org>.

This tutorial is designed to help you get started using Cytoscape to view data files packaged with Arabidopsis Co-expression tool results.

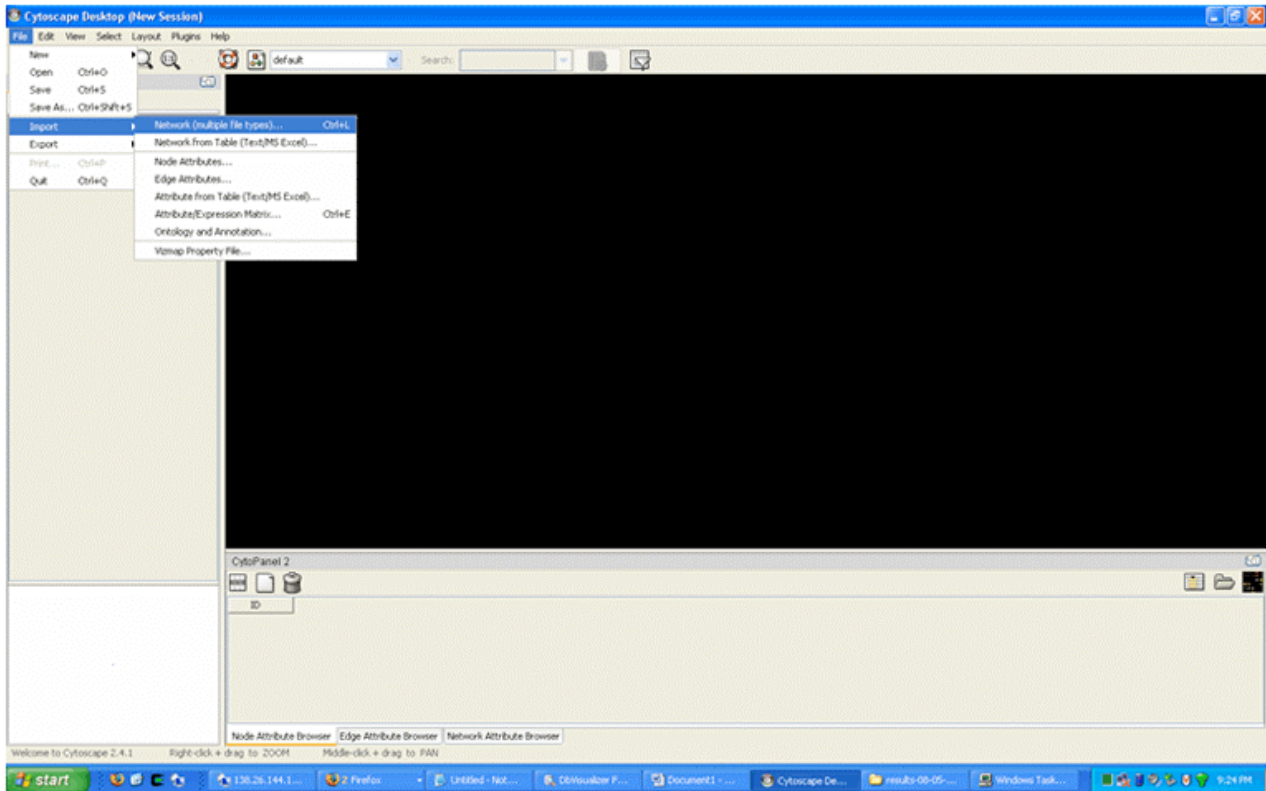
Get the files. After you run the Co-expression tool, you'll receive a set of files designed to be loaded into Cytoscape. These are:

- **Coexp.sif** – this is the network file that reports connections between the query (“bait”) genes you entered at Step 1 of running the tool
- **Node attribute (“.noa”) files** – These contain mappings between nodes (genes or probe sets) and their attributes, such as gene name synonyms
- **Edge attribute (“.eda”) files** – These contains mappings between edges (connections between genes) and other data, such as correlation coefficients between gene expression values.

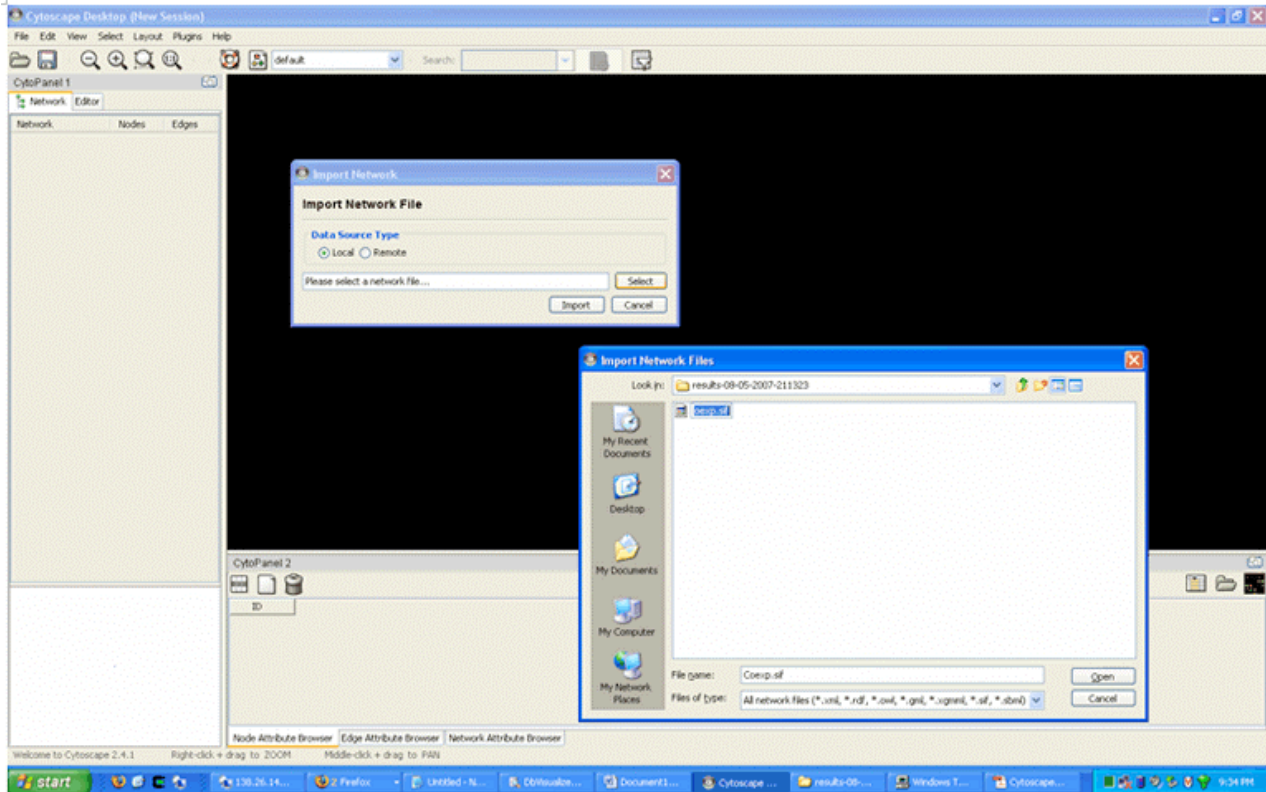
Launching Cytoscape. When you launch Cytoscape, you will likely see a window that looks like this:



Use the **File-> Import->Network (Multiple File types)...** command to open and load the network file Coexp.sif. Choose



In the 'Import Network' panel, select the Data Source Type as 'Local'. Use the "Select" key to browse the coexpression results directory and select the file ending with '.SIF'. Click 'Import' to load the data.



Successful SIF file import shows simple interaction statistics, like 'Description', 'Status' and 'Progress'. Click 'Close' on the 'Loading Network' panel.

Loading attribute files (*.noa and *.eda)

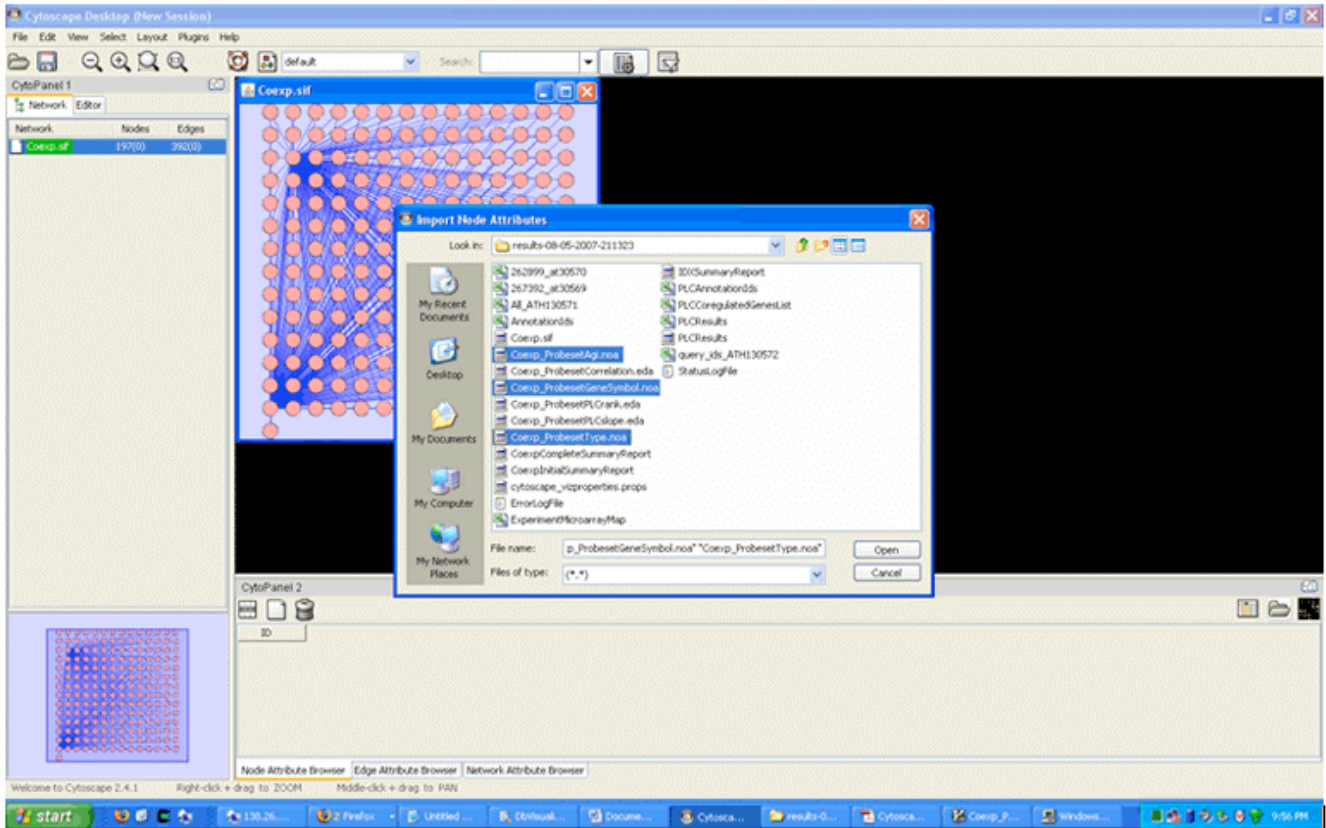
After successfully importing the network data (interactions), node and edge attribute files need to be imported and applied on the recently created network chart.

Coexpression tool's PLC analysis engine outputs three node attribute files:

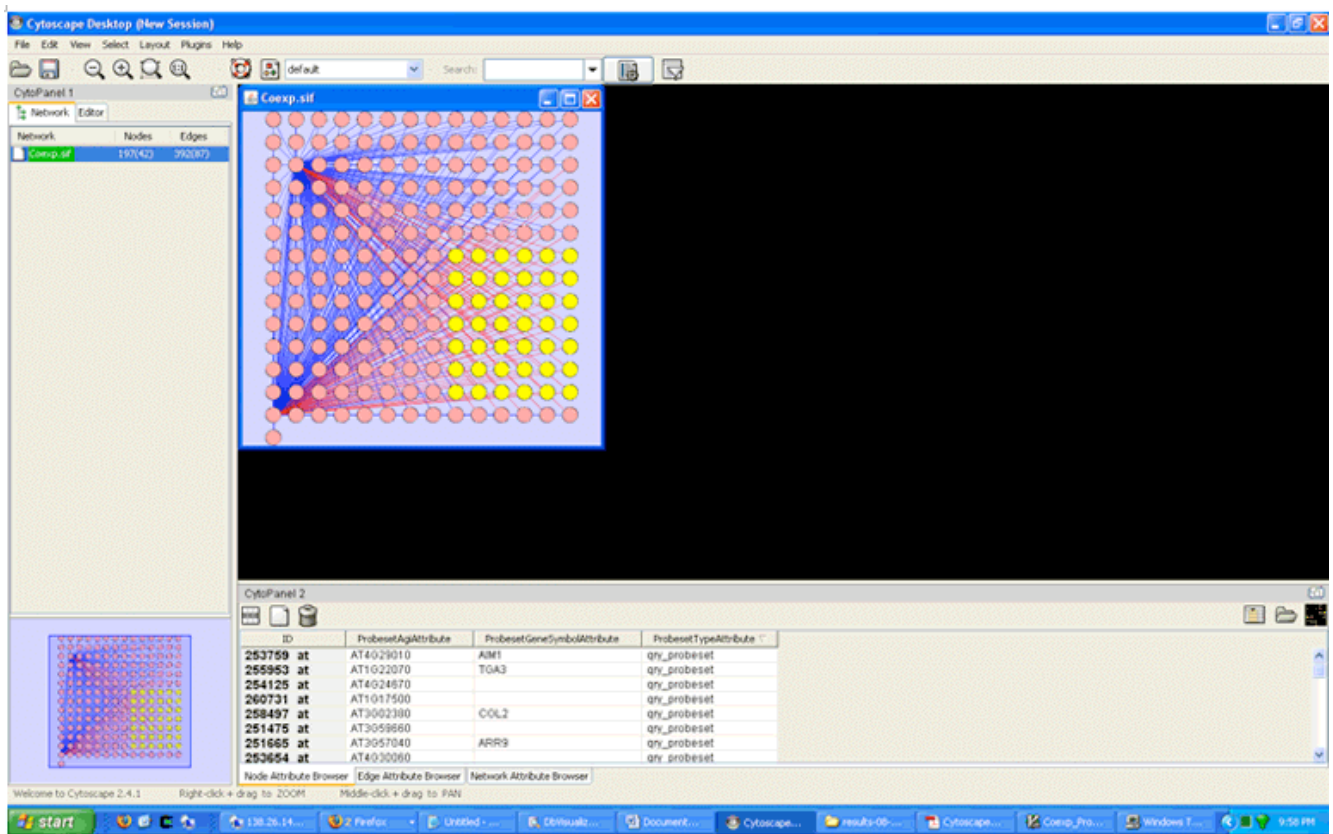
File	Description
Coexp_ProbesetType.noa	Categories probesets as 'bait_probeset' and 'qry_probeset'. 'bait' probes are the probes of interest that were either directly entered in the Step1 of the tool or those probes that got mapped to the AGI Ids entered in Step1.
Coexp_ProbesetAgi.noa	Maps probes to AGI Ids.
Coexp_ProbesetGeneSymbol.noa	Maps probes to Gene Symbols (through their AGI ids)

To import node attributes file all at once, select the **Node Attribute Browser** tab-panel found on the bottom of the cytoscape tool window.

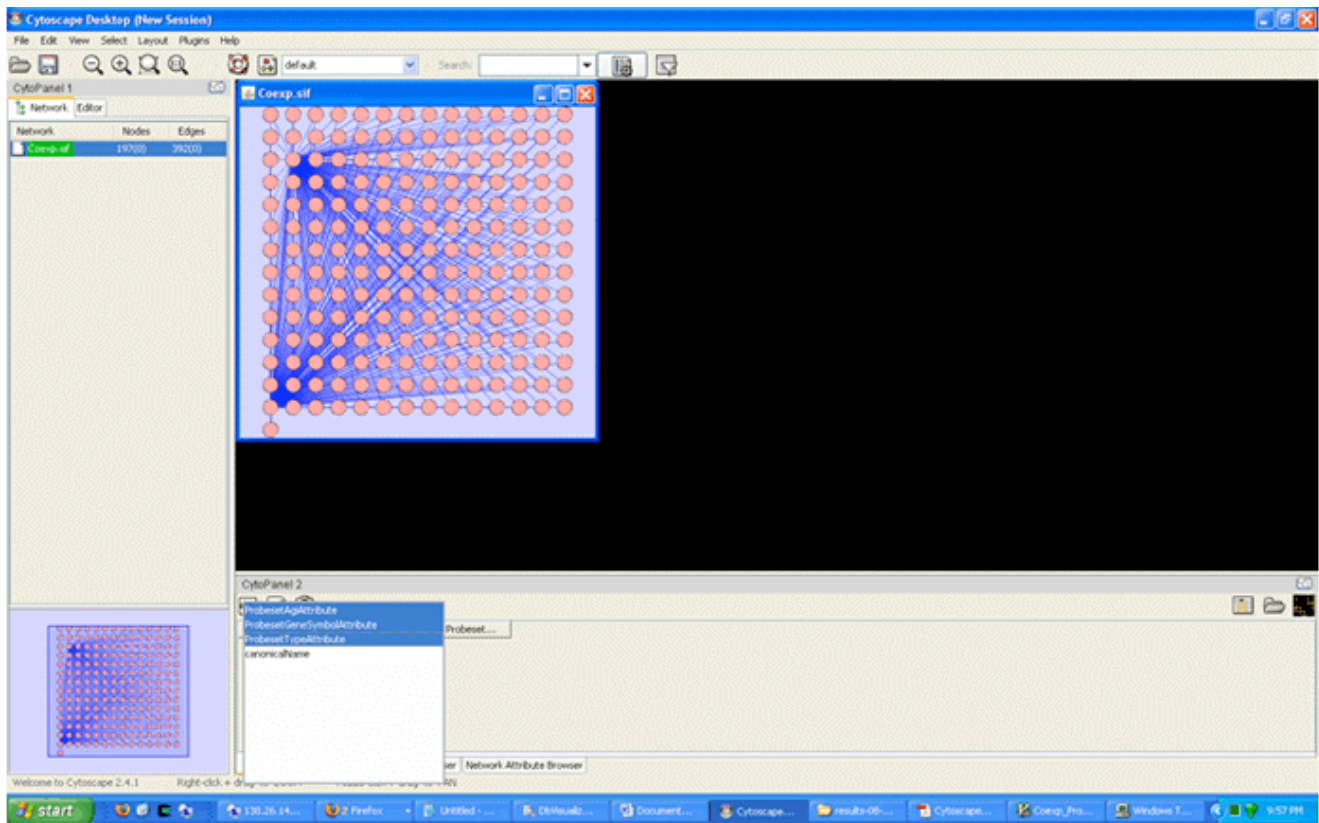
Click the **Folder-open** icon found on the right-side of the **CytoPanel 2**, and use the **select** option to browse and select node attribute files – these end with the suffix .noa. An alternative way of importing node attribute files is by selecting **File -> Import -> node attributes**. Note that you can select multiple files at a time by control- or shift-clicking the file names.



Click on the 'Select attributes' icon to select the attribute columns from the imported node attribute files and modify node appearance.



To check if the node attribute data applies to the network, select a set of nodes from the network chart and observe the selected nodes attribute data reflecting on the bottom node attribute panel.



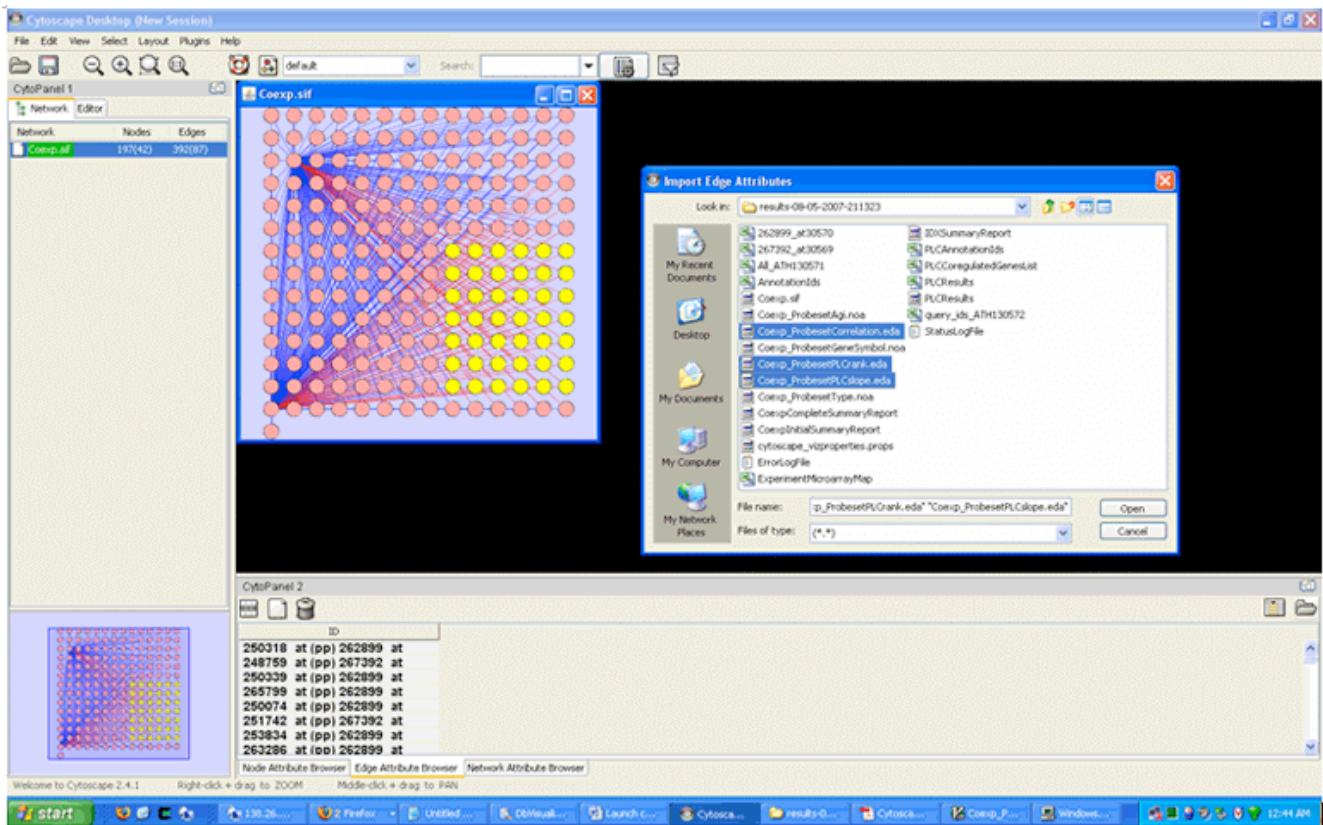
Loading Edge Attributes

The coexpression tool's PLC analysis outputs three edge attribute files:

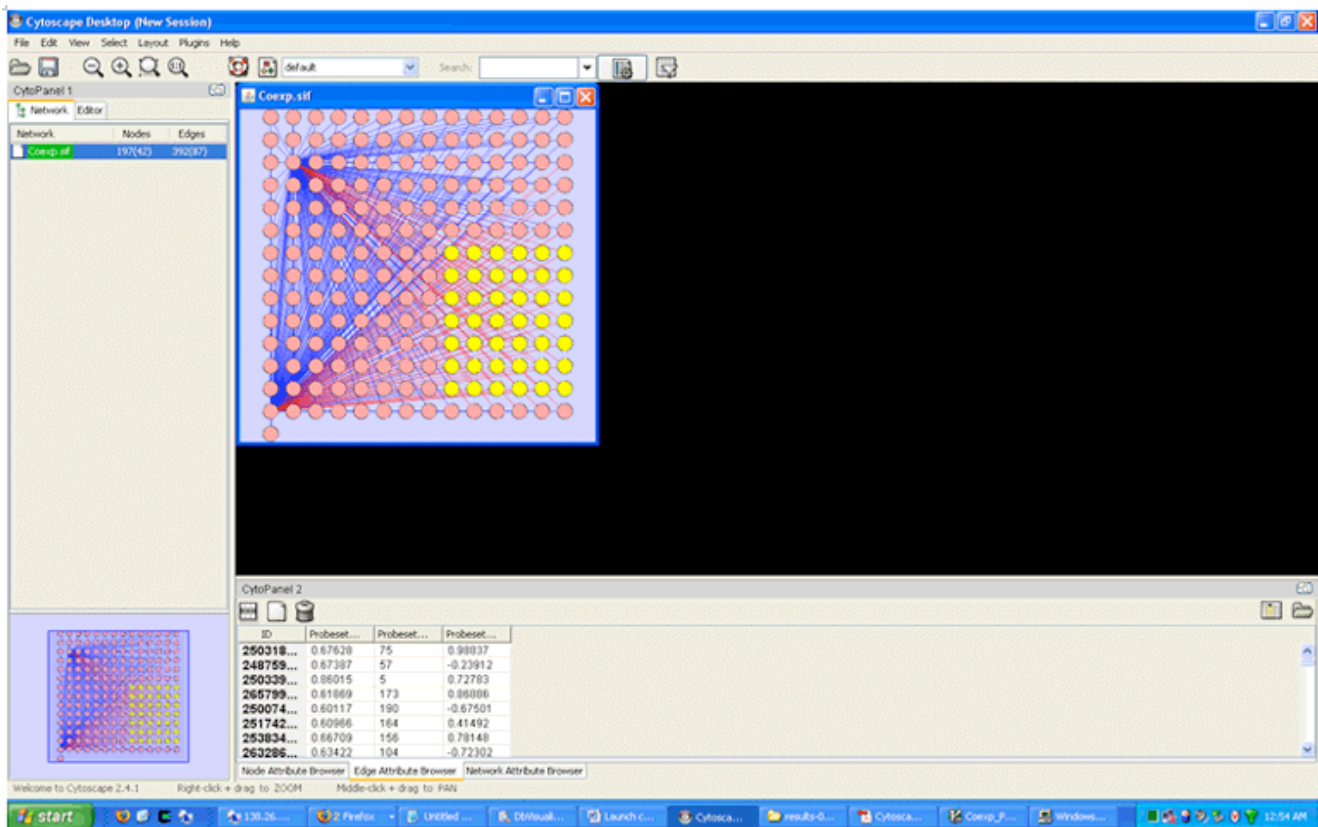
File	Description
Coexp_ProbesetCorrelation.eda	Records the correlation relationship between connected nodes, which are probesets.
Coexp_ProbesetPLCrank.eda	Records PLC rank between connected nodes.
Coexp_ProbesetPLCslope.eda	Records relationship between nodes based of their regression slope. Slope is a metric of correlation. A higher Correlation with a positive slope means positive correlation.

To import node attribute files all at once, select the **Edge Attribute Browser** tab-panel found on the bottom of the Cytoscape tool window.

Click the **Folder-open** icon found on the right-side of the **CytoPanel 2**, and use the **select** option to browse and select edge attribute files. These files end with the suffix `.eda`. An alternate way of importing edge attribute files is by selecting **File -> Import -> node attributes**.

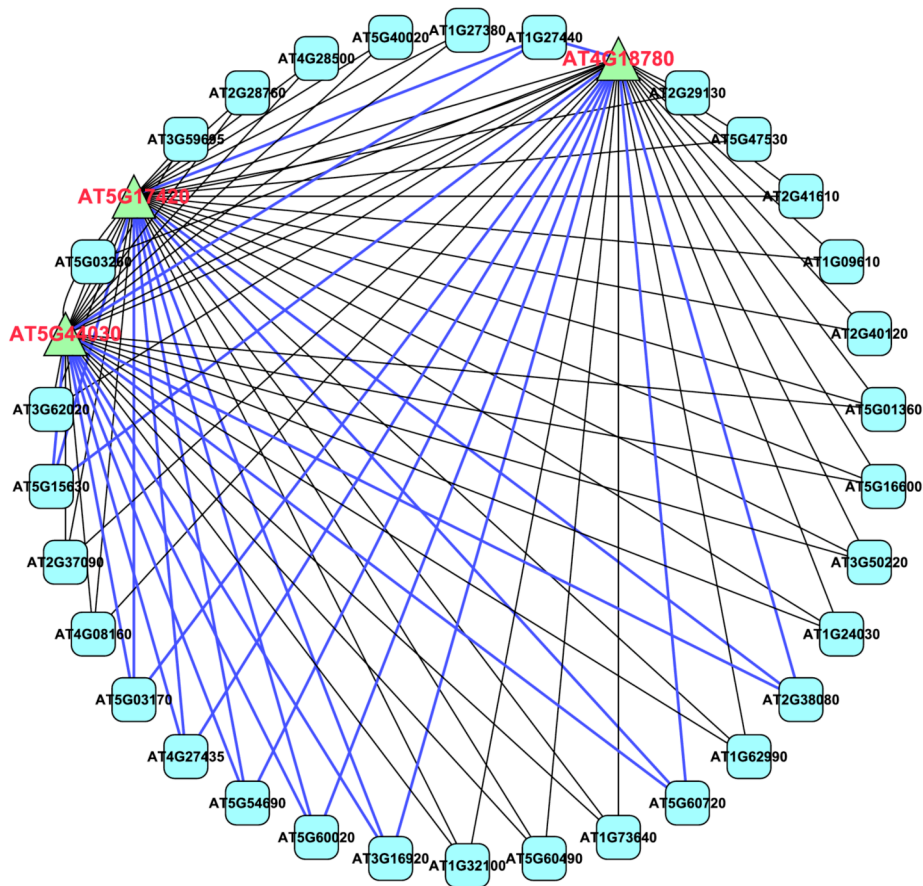


To test if the node and edge attributes have been imported correctly, select few random nodes from either the network chart OR from the **CytoPanel2 -> Node attribute browser** and examine the node attributes and edge attributes for those selected nodes.



Applying new styles to the network

Cytoscape offers capability to customize visual appearance of your network. The Coexpression tool provides a visual properties file (cytoscape_vizproperties.props) which specifies styles for nodes and edges. To apply these styles to your network, load the properties file 'cytoscape_vizproperties.props' can be applied to your network by selecting **File -> Import -> Vizmap Property File**. Once you do this, you should see an image that looks something like the following:



In this image, the query (“bait”) genes from the PLC analysis are labeled in red. Genes that are co-expressed with all three of the query genes are connected to them via blue lines.

For tips on how to further modify the appearance of the network, see the Cytoscape manual, available at the Cytoscape Web site: www.cytoscape.org.