MetScape 3.1 User Manual

An App for Cytoscape

National Center for Integrative Biomedical Informatics

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Contents

Please note that due to continuous software upgrades, the images in this handout may not exactly mimic what you see on the screen.

OVERVIEW

About data sources

MetScape is an app for Cytoscape, the bioinformatics network visualization tool. The app can be used to visualize and interpret metabolomics and gene expression data in the context of human metabolic networks.

MetScape uses a metabolite database developed by extracting and integrating information from the following sources:

1. Edinburgh Human Metabolic Network (EHMN) —

http://www.ehmn.bioinformatics.ed.ac.uk/

2. KEGG COMPOUND Database —
http://www.genome.jp/kegg/compound/

MetScape allows users to load a list of metabolites with experimentally determined concentrations, a list of genes with experimentally determined expression values, and a list of concepts or pathways, and display them in the context of relevant metabolic networks.

Workflow overview

With MetScape, you can:

- Trace the connections between metabolites and genes.
- Integrate multidimensional data
- Visualize compound, reaction, enzyme and gene networks and display compound structures, as well as information for reactions, enzymes, genes, and pathways.
- Visually animate changes in compound concentrations over time and across experimental conditions.

Pathway-Based Workflow

The basic steps in the **pathway** workflow include:

1. Enter data. You can type or paste a list of compounds and/or genes, load a file containing experimental data, or start from a biological pathway.
2. Select compound and reaction attributes. Choose which attributes to display in a table as you work with your visual network graph.
3. Explore the visual network and table of attributes:
    - Expand and collapse a network.
    - Create a subnetwork
    - Visualize your data in a wide variety of network layouts provided by Cytoscape.
    - Use color, size, and other effects to visually reflect a set of attribute values.
4. Save your session and reopen it later.

Correlation-Based Workflow

The basic steps in the **correlation** workflow include:

1. Enter data. You can load a file containing experimental data.
2. Select the desired Edge Mapping and Significance Range. Use the slider to designate the desired significance range.
3. Explore the visual network and table attributes.
4. Save your session and reopen it later.

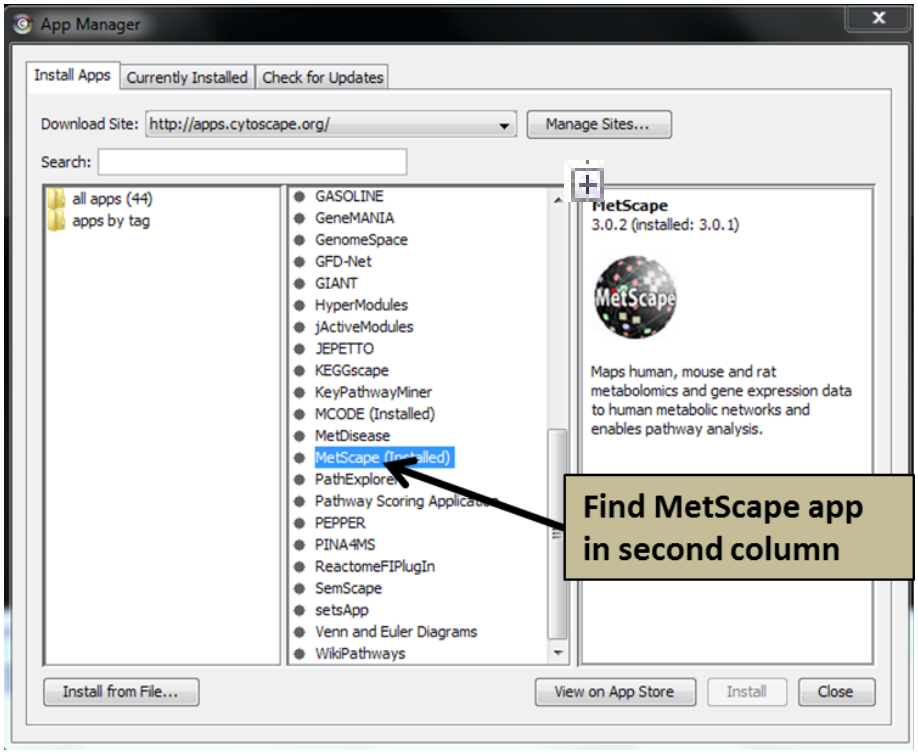
Installing Cytoscape and the MetScape app on your local computer

1. Install Cytoscape on your computer. For more information, go to the Cytoscape website at http://cytoscape.org/.

2. After Cytoscape is installed, start the application.

3. To install the MetScape app, select **Apps -> App Manager** from the Cytoscape menu.

4. Under the **Install Apps** tab, scroll down until you find MetScape in the second column.



5. Click on the **MetScape** app.

6. Click **Install.**

7. When installation is complete, click **Close.**

A MetScape option is added to the Cytoscape **Apps** menu.

Registration: MetScape is a free program. We ask you to register because it helps us to keep track of the number of downloads. Your information will be stored in a secure database and we will not share it with anyone. We may send you infrequent e-mails about future MetScape releases.

Note: This app requires Cytoscape 3.0+ to run correctly.

PART 1: PATHWAY-BASED NETWORK

Entering Data

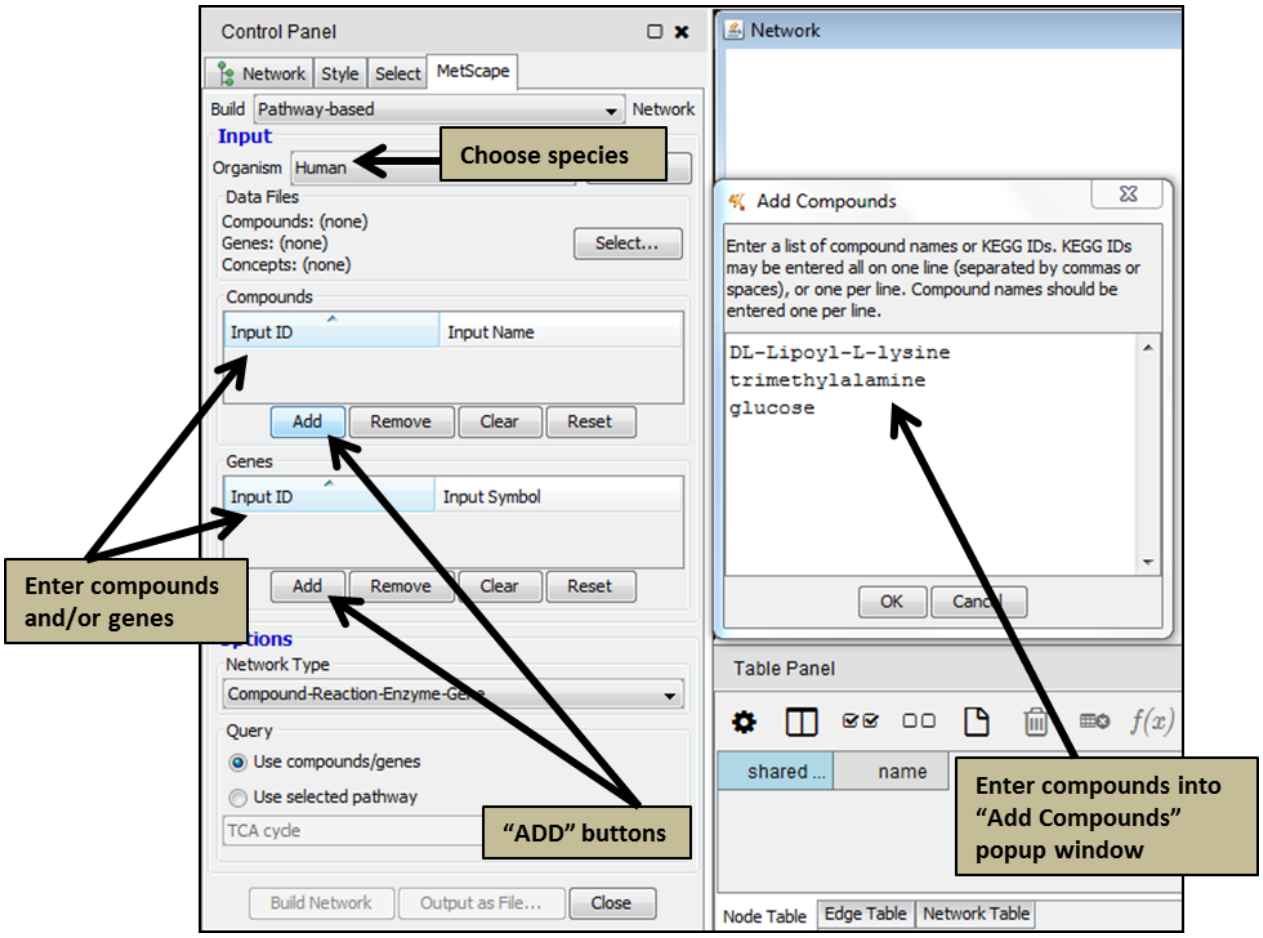
1. To begin a Cytoscape session with the MetScape app, first start Cytoscape.

2. Choose one of the following methods to get started.

Option 1: Enter a list of compounds

1. Select **Apps -> MetScape-> Build Network -> Pathway-based** from the Cytoscape menu.

2. A MetScape tab now appears on the left of the Cytoscape screen.

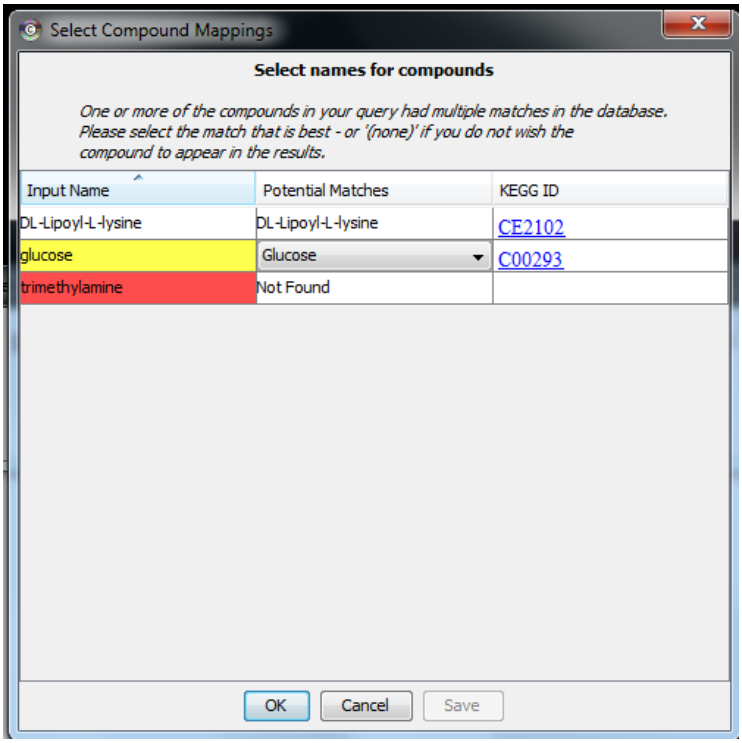


3. Select a species. Choices are: human, rat, mouse

4. Manually enter (or copy and paste) compound ID(s) or name(s) and/or Entrez gene ID(s) or symbol(s). Click **Add** and enter the appropriate ID type in the popup box. Lists of IDs should be separated by spaces; lists of compound names should be entered one per line.

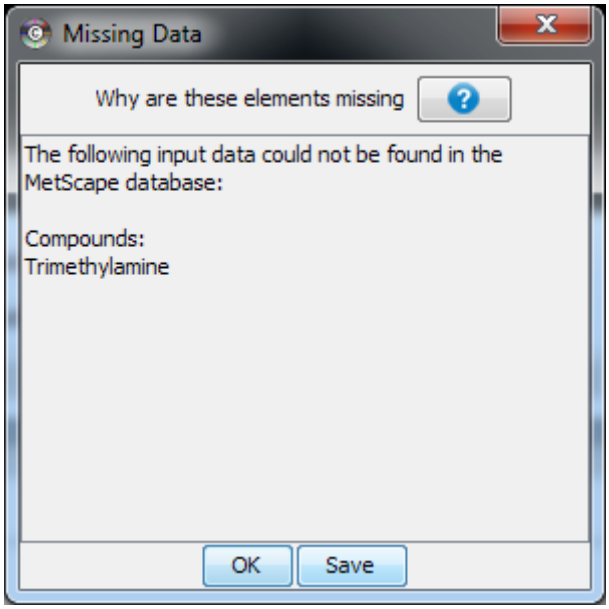
5. Click **OK**

6. If you enter a compound name, it will map to its KEGG ID a popup window will appear. If there is more than one potential match, use the dropdown arrow to choose the best match. If the compound is not found in the system, it will say "Not Found." The mapping selection will be saved so that your selection will appear as the default option in the future.



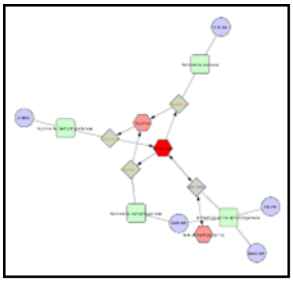
7. Click **OK**

8. After the experimental data has been loaded, if any of the genes/compounds that you submitted were not mapped to the database objects, a **Missing Data** window will appear. After viewing the **Missing Data** information, click **SAVE** to save the data for later viewing or click **OK** to close the window without making it available for future viewing. (See the Missing Data Window section below, under Option 2: Load an experiment file, for more information about missing data).

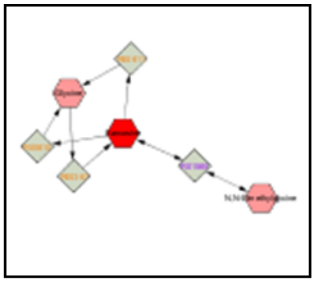


9. Select a **Network Type** by selecting one of the following from the dropdown menu:

   - **Compound-Reaction-Enzyme-Gene** (1)
   - **Compound-Reaction** (2)
   - **Compound-Gene** (3)
   - **Compound** (4)



1



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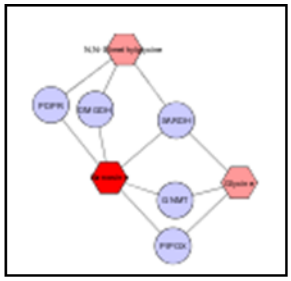

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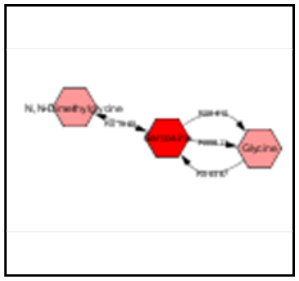

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Note: When selecting Compound as the Network Type, a dropdown menu appears under Query, providing the option of choosing between compounds or genes. When any other Network Type is selected, the only option is to use compounds/genes unless using a selected pathway.

10. Click **Build Network** to query the database and create the network.

Option 2: Load an experiment file

Use to load experimental data to visualize and explore compound networks over time or in varying experimental conditions. The input can be an Excel, comma, or tab delimited file. MetScape allows users to load three types of files – compound file, gene file, and concept file. Each type is optional, e.g. you can load only compounds, only genes, only concepts, or any combination of the above.

1. Select **Apps -> MetScape-> Build Network -> Pathway-based** from the Cytoscape menu.

2. A MetScape tab now appears on the left of the Cytoscape screen.

3. Select a species. Choices are: human, rat, mouse

4. Load experimental data by clicking the **Select…** button on the MetScape tab.

Compound File

The compound data file must meet the following requirements:

- The first row must be a heading row, in which:
    - The columns in the first row are column headings to label the data.
- All other rows contain experimental data, in which:
    - The first column contains KEGG Compound IDs or names.
    - The remaining columns contain experimental data. Multiple experimental values are permissible in the same spreadsheet.

Below is a portion of an example compound file with Compound IDs, significance values, and fold change values:

	A	B	C
1		Fold Change	P-value
2	C00002	0.51	0.006174265
3	C00004	1.14	0.646801757
4	C00008	0.99	0.970303452
5	C00020	2.12	0.068052875
6	C00025	0.91	0.115376267
7	C00037	0.99	0.904658012
8	C00041	0.95	0.574835153
9	C00042	1.09	0.809172269
10	C00044	0.56	0.100724407
11	C00047	1.36	0.189989302
12	C00049	0.92	0.755789133
13	C00064	0.48	0.186707182
14	C00065	1.22	0.805151078
15	C00073	0.80	0.356405722

Gene File

The gene file must meet the following requirements:

- The first row must be a heading row that includes column headings to label the data.
- The first column contains Entrez Gene IDs or Official Gene Symbols.
- The remaining columns contain experimental data. Multiple experimental values are permissible in the same spreadsheet.

Below is a portion of an example gene file with Gene IDs, significance values, and fold change values:

	A	B	C
1	ENTREZ_GENE_ID	Pvalue	Log_fold_change
2	1	0.002335904	-0.02
3	2	2.07E-08	-0.06
4	2	2.03E-08	0.04
5	9	0.003919472	0.04
6	10	0.00026716	-0.08
7	12	0.000237925	0.05
8	13	0.002489364	-0.05
9	14	0.506396205	0.00
10	15	8.25E-06	-0.04
11	16	0.022222108	-0.01
12	18	1.47E-08	-0.07
13	18	5.24E-06	-0.09
14	18	2.12E-05	-0.07
15	19	0.820594937	0.00
16	19	1.76E-08	0.06
17	19	9.46E-09	0.04
18	19	0.660631201	0.00
19	20	1.16E-07	-0.05

Concept File

The concept file can be generated by a gene set enrichment analysis tool such as LRpath or GSEA from gene expression data.

Note: Gene set enrichment testing is an approach used to test for predefined biologically-relevant gene sets that contain more significant genes from an experimental dataset than expected by chance.

- *GSEA (Subramanian at al., Proc. Natl. Acad. Sci. USA, 2005, 102:15545-15550).*
- *LRpath (Sartor et al., Bioinformatics, 2009, 25(2):211-7).*

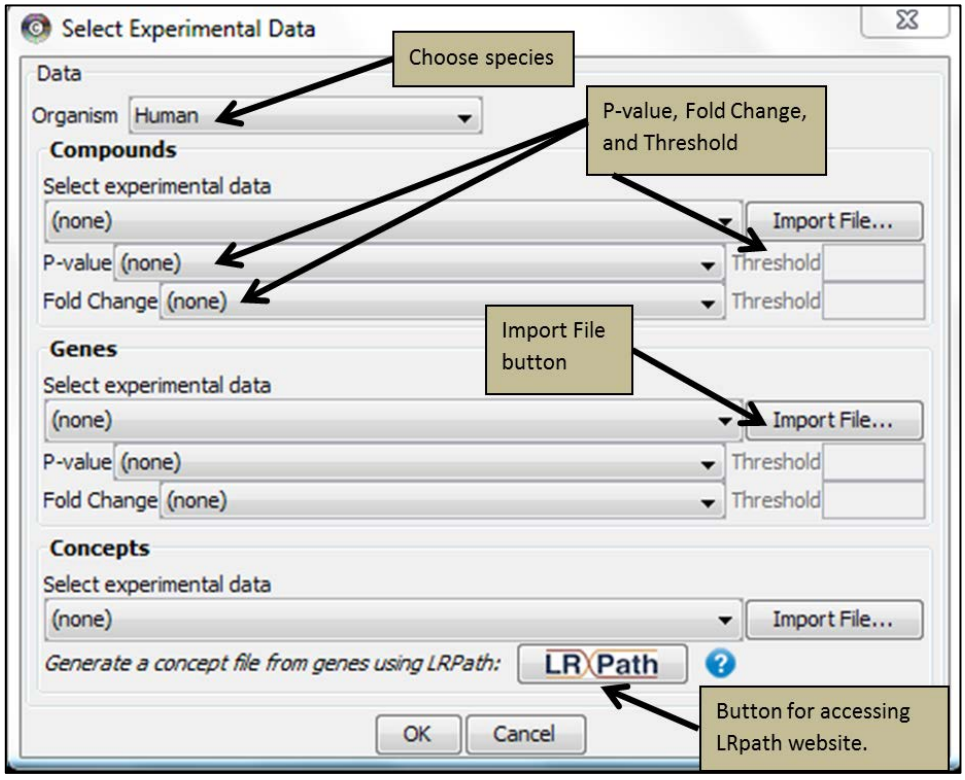
Below is a portion of an example concept file:

	A	B	C	D	E	F	G	H	I
1	Concept.name	ConceptType	n.genes	coeff	odds.ratio	p.value	FDR	Direction	sig.genes
2	Citrate cycle (TCA	KEGG Pathway	32	0.44858373	16.2447876	1.23E-08	1.67E-06	up	24368, 24399, 24401, 25179, 25721,
3	Fatty acid metab	KEGG Pathway	28	0.40269739	12.2142885	1.10E-06	7.48E-05	up	24158, 25363, 25618, 25757, 140547
4	Alanine and aspa	KEGG Pathway	18	0.47651019	19.323541	1.93E-06	8.75E-05	up	24379, 24401, 25721, 81670, 81829,
5	Reductive carbox	KEGG Pathway	11	0.55839769	32.1439027	6.68E-06	2.27E-04	up	24368, 24399, 24401, 25721, 79250,
6	Oxidative phospl	KEGG Pathway	44	0.27758123	5.61284966	3.64E-05	9.89E-04	up	116550, 291103, 295923, 301011, 31
7	Urea cycle and m	KEGG Pathway	25	0.32301096	7.44383786	2.00E-04	0.00441518	up	24368, 24379, 24399, 24401, 24600,
8	PPAR signaling p	KEGG Pathway	32	0.28585824	5.90912002	2.33E-04	0.00441518	up	24158, 24450, 25045, 25757, 29171,
9	Carbon fixation	KEGG Pathway	11	0.44333373	15.7233268	3.59E-04	0.00542234	up	24401, 25721, 81670, 81829, 114508
10	Arginine and pro	KEGG Pathway	20	0.33385131	7.96259603	5.14E-04	0.00699086	up	24368, 24379, 24399, 24401, 24600,
11	Butanoate metab	KEGG Pathway	30	0.26301141	5.12695967	0.00101503	0.01254945	up	24379, 24399, 24401, 24450, 25721,
12	Valine, leucine a	KEGG Pathway	24	0.27898384	5.66198894	0.00167291	0.01625109	up	24158, 24450, 29711, 140547, 17046
13	Glutathione met	KEGG Pathway	24	0.27372392	5.4799003	0.00205204	0.01860519	up	24379, 24399, 24401, 24422, 25721,
14	Propanoate meta	KEGG Pathway	16	0.32105291	7.35380592	0.00265316	0.02122524	up	24158, 140547, 170465, 171155, 298
15	Nitrogen metabo	KEGG Pathway	15	0.30373897	6.60362465	0.00596513	0.04056287	up	24379, 24399, 24401, 25721, 29242,
16	C5-Branched diba	KEGG Pathway	19	0.26819205	5.29471153	0.00686636	0.04226683	up	24158, 24379, 24399, 24401, 25721,
17	Pyruvate metabo	KEGG Pathway	19	0.26689922	5.25234187	0.00714807	0.04226683	up	81829, 298942, 306198, 307858, 361
18	Phenylalanine m	KEGG Pathway	21	0.25034932	4.73898475	0.00822755	0.0447139	up	24368, 24401, 25721, 81683
19	Antigen processi	KEGG Pathway	21	0.2496329	4.71793226	0.00841626	0.0447139	up	24223, 24812, 25217, 25599

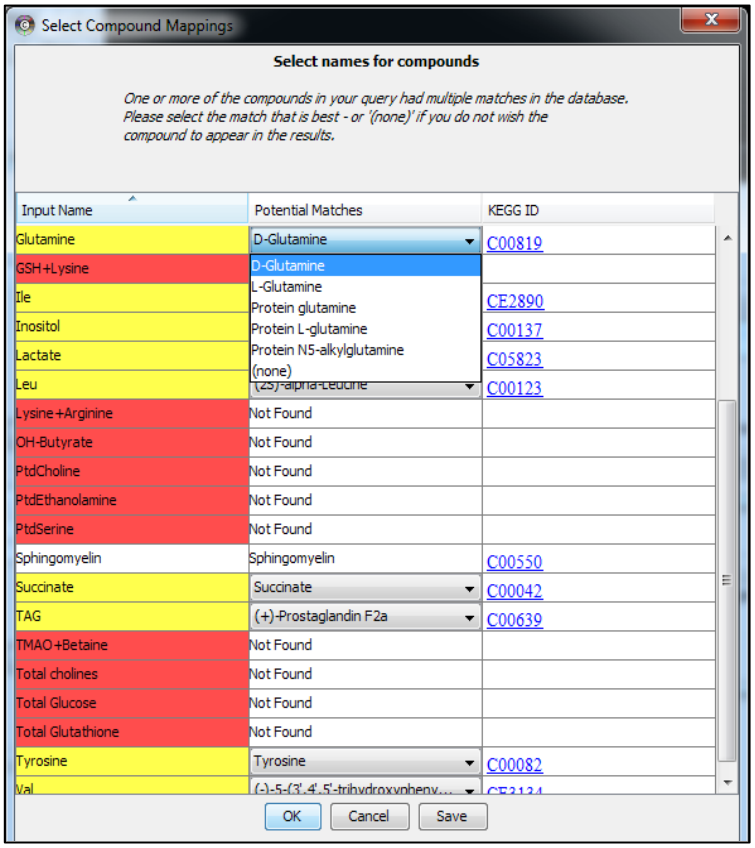
To generate a concept file, you can click the LRpath button from MetScape that will take you to the LRpath website.

Note: LRpath performs gene set enrichment testing, an approach used to test for predefined biologically-relevant gene sets that contain more significant genes from an experimental dataset than expected by chance (Sartor et al., 2009). To run LRpath, you need a Gene Expression file with fold change (or log fold change) values and p-values. The Gene Expression file needs to contain all gene records, not just those for significant genes; LRpath will determine the significant genes from the input.

Example of selecting experimental data, including importing files and designating fold change, p-values, and thresholds:

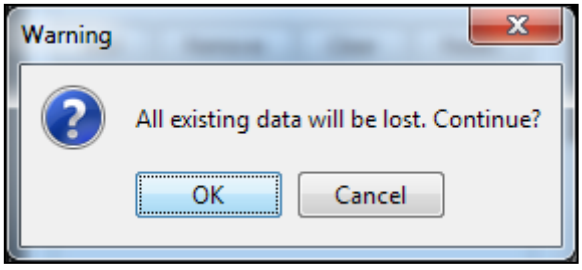


Below is an example of the ID mapping window from a loaded compound experiment file. The mapping selection will be saved so that your selection will appear as the default option in the future.



Note: If you select **Save** on the **Select Compound Mappings** window, you will get a .csv file containing mapping information for all compounds that MetScape successfully mapped.

Note: Multiple networks can be built within the same user session. After a network is built, pull up the **Select Experimental Data** window by clicking on the **Select…** button and change the data files. Then build the new network. Both networks will remain accessible. If you change species, however, all existing data will be lost. The below warning window will pop up in this situation.



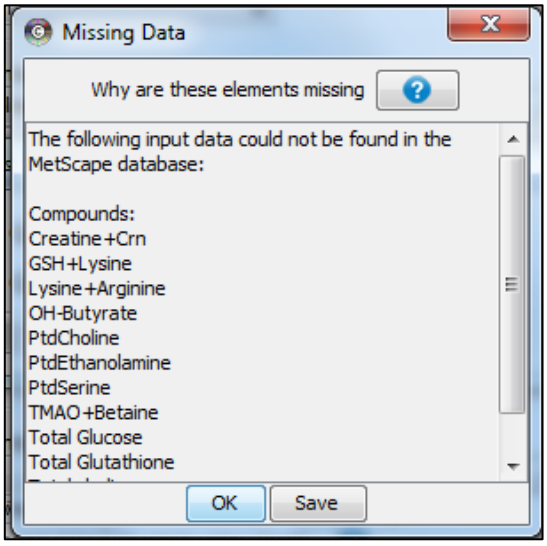
Missing Data Window

After the experimental data has been loaded, if any of the genes/compounds that you submitted were not mapped to the database objects, a **Missing Data** window will appear.

Genes, compounds, and concepts may appear on this missing elements list because:

- Genes and compounds that you supply may not be in the database. If they are not found in the database then they are reported as missing.
- If your input genes are not human (Rat, for example), then they are mapped to human genes using homologs from NCBI's HomoloGene. If this mapping fails, then those genes are reported as missing.
- MetScape will display only the genes that encode metabolic enzymes. If an input gene does not encode metabolic enzymes, it will appear on the missing elements list.
- A concept (pathway) will appear on the missing list if all of its significant genes are missing. The list of significant genes for a concept comes from the input file or from LRpath.

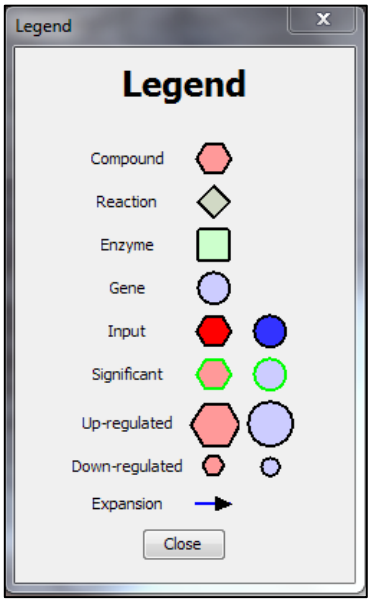
After viewing the **Missing Data** information, click **SAVE** to save the data for later viewing or click **OK** to close the window without making it available for future viewing.



Pathway-Based Visualization

MetScape includes a legend explaining its various shapes and colors. The legend will be specific to the current network type (pathway or correlation).

Access the MetScape **Legend** from **Apps -> MetScape -> Show Legend**.



Additional information about the network is expressed through visualization:

- A compound is red in the original network and subsequent subnetworks if it was in the original data loaded into MetScape.
- A green border surrounding a node represents a significant gene/compound.
- Node size represents the direction of the change. Larger nodes represent an increase and smaller nodes represent a decrease in gene/metabolite. The actual amount of the change is not represented visually.
- When a node is expanded, the edges between the original node and the expanded nodes become blue.

Note: In a pathway-based network, selected edges are highlighted yellow instead of red.

MetScape Tab

This tab has options for choosing Network Types, the data you entered, and more.

From this tab you can:

- Select between building a **pathway-based network** or a **correlation-based network**
- Select the **type of network** that you want to build

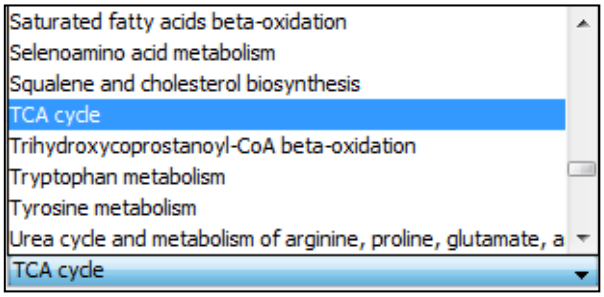
  Select **Network Type** by selecting one of the following from the dropdown menu:

  - **Compound-Reaction-Enzyme-Gene**
  - **Compound-Reaction**
  - **Compound-Gene**
  - **Compound**

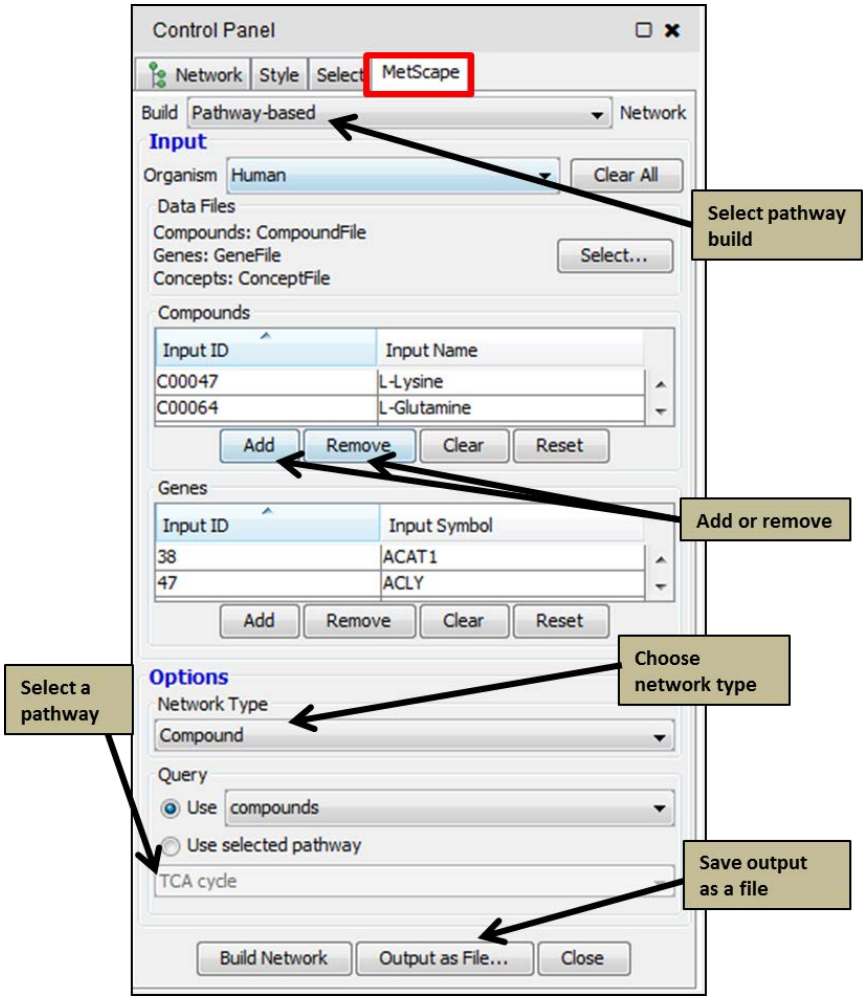
Note: When selecting Compound as the Network Type, a dropdown menu appears under Query, providing the option of choosing between compounds or genes. When any other

Network Type is selected, the only option is to use compounds/genes unless using a selected pathway.

- **Add** or **Remove** data.
- Select a **pathway**
    - Use to view all the compounds and reactions associated with a metabolic pathway. Select a specific pathway from the drop-down list.



- Save the **Output as a File**.
- **Build Network** graph based on the data.



Note: When the organism is human, the Genes section will show Input ID and Input Symbol. However, when the organism is not human (ex. rat), the Genes section will show Input ID, Input Symbol, and Human Symbol.

Rules Used to Build Different Network Types

- **Compound-Reaction-Enzyme-Gene (C-R-E-G)**, **Compound-Reaction (C-R), and Compound–Gene (C-G) networks**

The C-R-E-G, C-R, and C-G networks are all built from the same underlying data. That data is derived, in each case, by finding compounds that participate in reactions that are catalyzed by enzymes that are encoded by genes. If only genes are input, then all the enzymes, reactions, and compounds that match those genes are used. If only compounds are input, then all the reactions, enzymes, and genes that match those compounds are used. If both genes and compounds are input, then only those C-R-E-G couplings that match both a compound from the compound input and a gene from the gene input are used.

If a concept file is provided, genes from that file will be used as input instead of the genes from a provided gene file.

If only a gene file is provided, all genes from that file are used as input. In this case, we recommend that you load a smaller set of genes (e.g. most significant differentially expressed genes).

- **Compound (C) networks built from compound input**

If a list of compounds is provided, the resulting network will include the query compounds (shown in red) plus any compounds that participate in the same reactions as query compounds. The edges will be drawn between "seed" compounds and their neighboring compounds

- **Compound (C) networks built from gene input**

If a list of genes is provided, the resulting network will include the compounds that are related to the query genes via the reactions in which they participate, and the enzymes that catalyze these reactions. The edges will be drawn between all compounds.

- **Pathway networks**

Pathway-specific C-R-E-G, C-R, and C-G networks are built from a set of genes, enzymes, reactions and compounds defined in the EHMN database. Pathway-specific Compound networks are built similarly to the Compound networks built from a set of input genes.

Table Panel Display

Attributes of compounds, reactions, pathways, and concepts that you have selected in the network appear in the Cytoscape Table Panel.

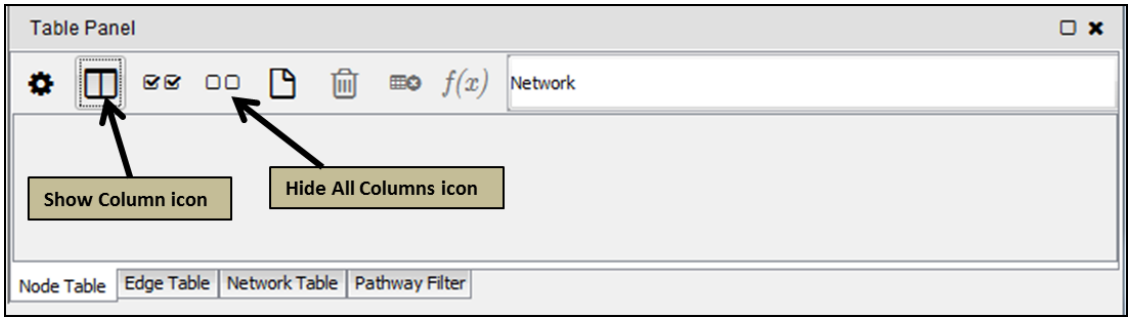
Choosing attributes provided through MetScape

To choose which attributes to view:

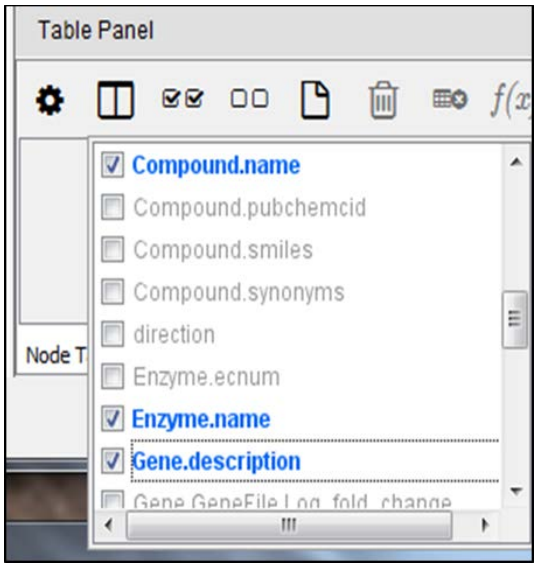
1. To choose attributes for compounds, click the **Node Table** tab in the Table Panel. You can choose to view **node**, **edge**, or **network** attributes by clicking on the respective tabs at the bottom of the Table Panel. Pathway data can be viewed under the **Pathway Filter tab** and concept data can be viewed under the **Concept Filter** tab in the Table Panel.

2. Click the **Hide All Columns** icon in the Table Panel toolbar**.** This clears the Table Panel of all columns.

3. Click the **Show Column** icon in the Table Panel toolbar. A list of attributes with check boxes will appear.



4. Select the attributes you want displayed in the Table Panel. Attributes are listed with item type before the attribute (ex. Compound.name; Enzyme.name). The available attributes will depend on the type of network created.



5. When you are done, click anywhere outside of the list and the table will be populated with the appropriate data.

Note: In Cytoscape 3.0, clicking on the two horizontal squares with check marks will select all attributes.

Below is a table of available attributes for each node type:

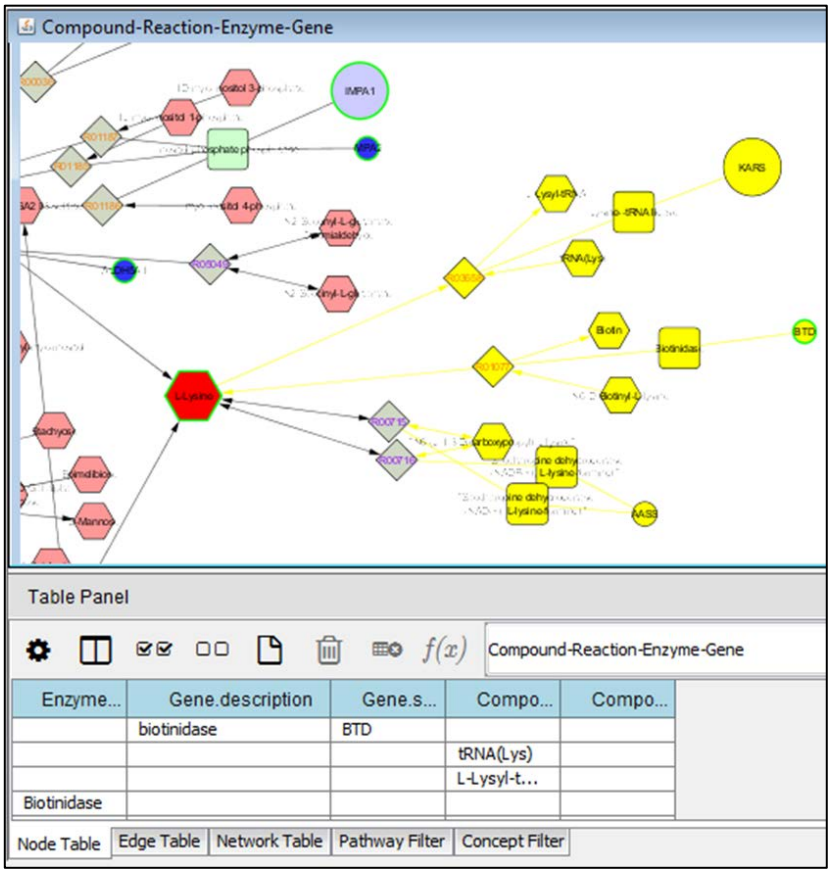
A	B
Attribute	**Description**
ID	Unique identifier, required by Cytoscape
Compound.approximateMW	Compound Molecular weight (integer, data source - EHMN database)
Compound.casnum	Chemical Abtracts Service compound identifier
Compound.cid	KEGG compound unique identifier
Compound.*file_name*	Rows with data specific to your experiments
Compound.formula	Molecular formula
Compound.mw	Molecular weight
Compound.name	Compound name
Compound.pubchemcid	PubChem compound identifier
Compound.smiles	SMILES string for compound
Compound.synonyms	Other names for compound
Enzyme.ecnum	Enzyme Comission number
Enzyme.name	Enzyme name
Gene.*file_name*	Relative gene data values
Gene.*column_name*	Gene data p-value
Gene.description	Gene description
Gene.human.geneid	Human gene identifier
Gene.locations	Subcellular location
Gene.symbol	Official gene symbol
Gene...geneid	Ortholog gene ID if the input organism is not human
Reaction.equation	Equation for a reaction
Reaction.locations	Reaction subcellular location (EHMN database)
Reaction.pathway	Pathway involving reaction
Reaction.reversible	T= reaction is reversible; F=reaction is non reversible
Reaction.rid	Reaction identifier
Type	Node type (gene, enzyme, reaction or compound)
canonicalName	Node name that is shown on node label

*Please note that the reactions shown in the Reaction.equation attribute display all compounds, while the networks built from the list of input genes/compounds show only "main" compounds (see Ma et al., Mol. Syst. Biol., 2007, 3:135 for details). Pathway-specific networks show all compounds.

Below is a table of available attributes for each edge:

A	B
Attribute	**Description**
Enzyme.names	Enzyme name
Enzyme.ecnums	Enzyme Comission number
Reaction.equation	Equation for reaction
Reaction.locations	Reaction subcellular location (EHMN database)
Reaction.pathway	Pathway involving reaction
Reaction.reversible	T= reaction is reversible; F=reaction is non reversible
Reaction.rid	Unique identifier for reaction
canonicalName	Common name
direction	Direct or undirected
interaction	Type of interaction

6. Select nodes and/or edges in the graph to view their attributes in the Table Panel. If nothing is selected, attributes for all nodes and edges are shown.

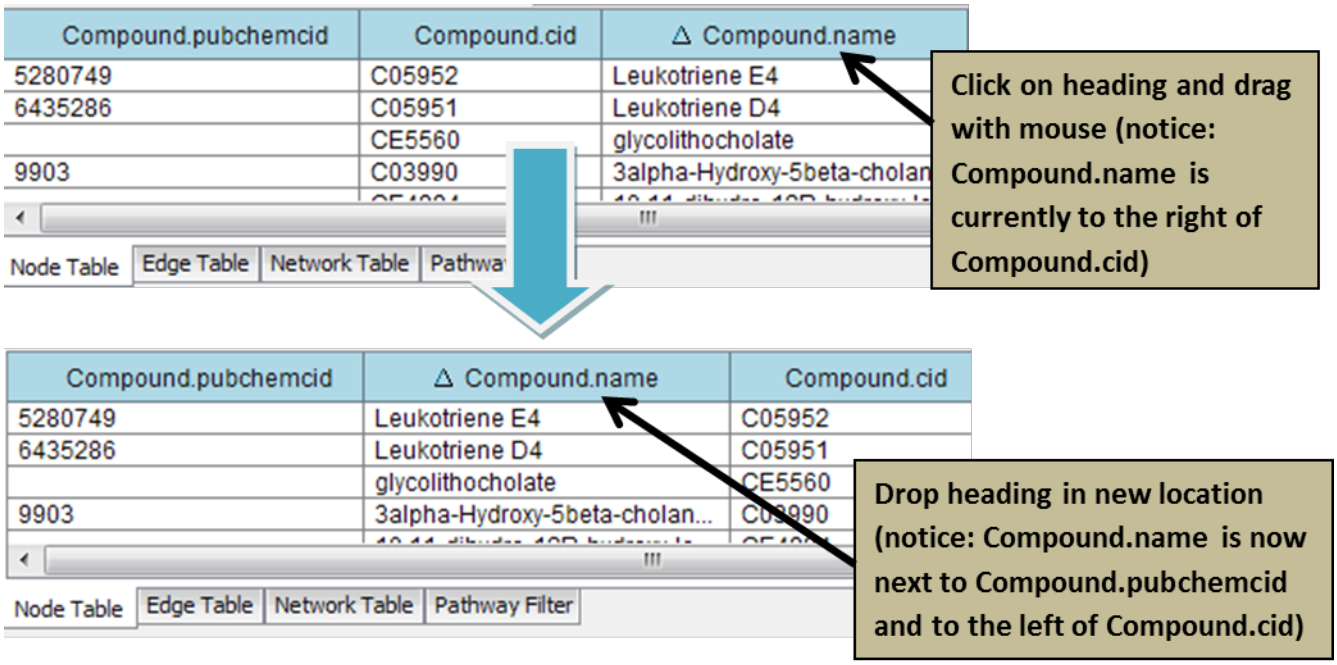


Notes:

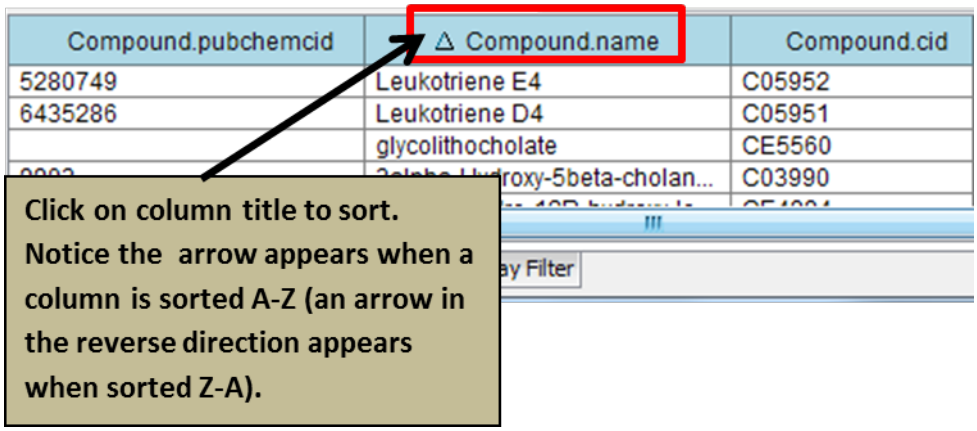
- Some attributes (such as Formula, Mass, and Smile) apply only to compound nodes, while others (such as Enzyme, Pathway, and Reversibility) apply only to reaction nodes.

Rearranging attributes in the table panel

To reorder attributes in the Table Panel, click on an attribute column heading and drag it to a new location:

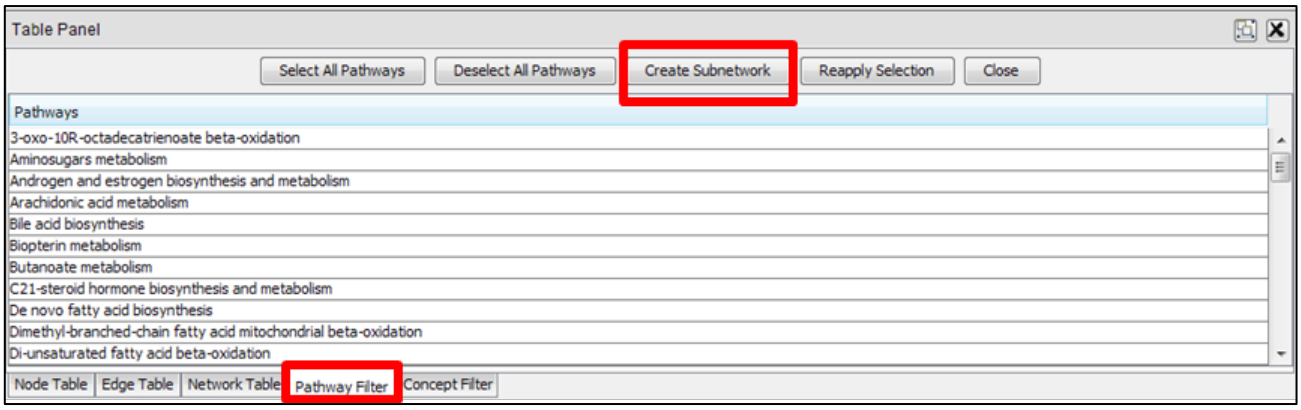


To sort by an attribute, click the attribute column heading; to sort in the reverse direction, click again.



Pathway filter in the table panel

MetScape provides two ways to access pathway information. The **Pathway Filter** tab lists all pathways represented in the network. Selecting one or more pathways will highlight in the network all nodes in the pathway(s). Pathways are also displayed as attributes in the Node Table tab.



A subgraph can be created for a given pathway. The subgraph consists of a subset of nodes from an active network that belong to the selected pathway. To create a subgraph:

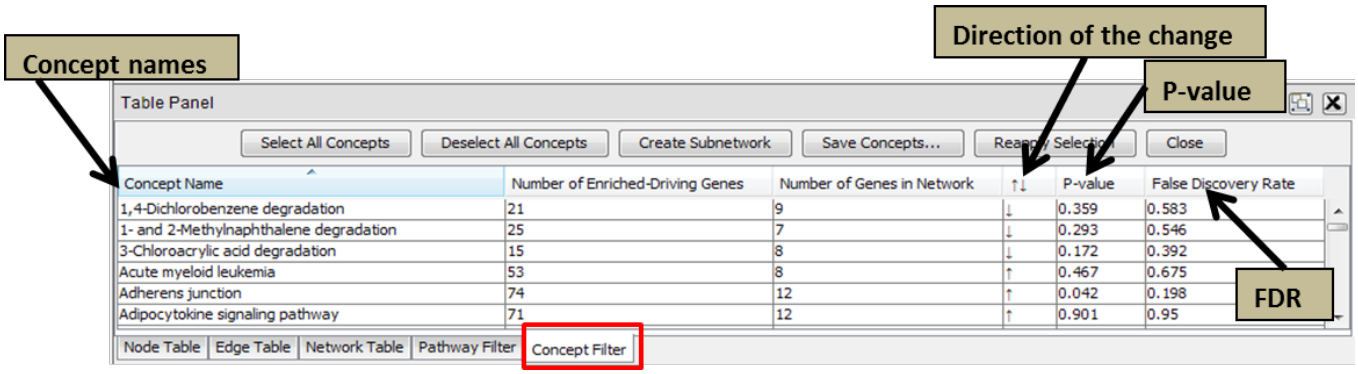
1. Select a pathway in the **Pathway Filter** tab.

2. Click the **Create Subnetwork** button at the top of the Table Panel (make sure you are on the **Pathway Filter** tab).

3. A new graph appears in the graph window. This is the subnetwork for the selected pathway.

Concept filter in the table panel

The content of the created or loaded concept file is put into the **Concept Filter** tab.



1. Concept Name - official name of the concept.

2. Number of Enriched-Driving Genes – the number of enriched-driving genes in the concept.

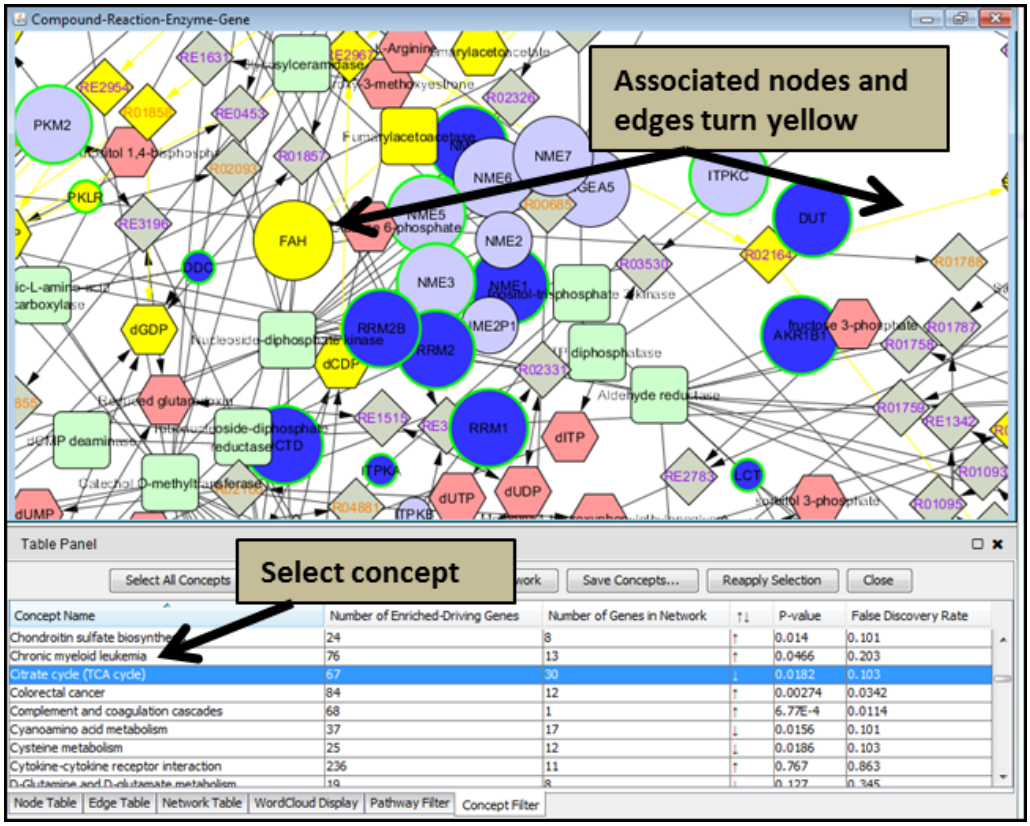
3. Number of Genes in Network - the number of input genes that belong to the concept.

4. Direction – direction of the change.

5. P-value - the p-value for enrichment/depletion.

6. False Discovery Rate - the FDR, estimated using the method of Benjamini and Hochberg (When results are sorted by p-value, a FDR < 0.05 is interpreted to mean that approximately 5% of the concepts with lesser or equal p-value are false positives).

To map a concept on the graph, click on the concept name in the Table Panel. The concept will then be highlighted in the graph (associated nodes and edges turn yellow.).

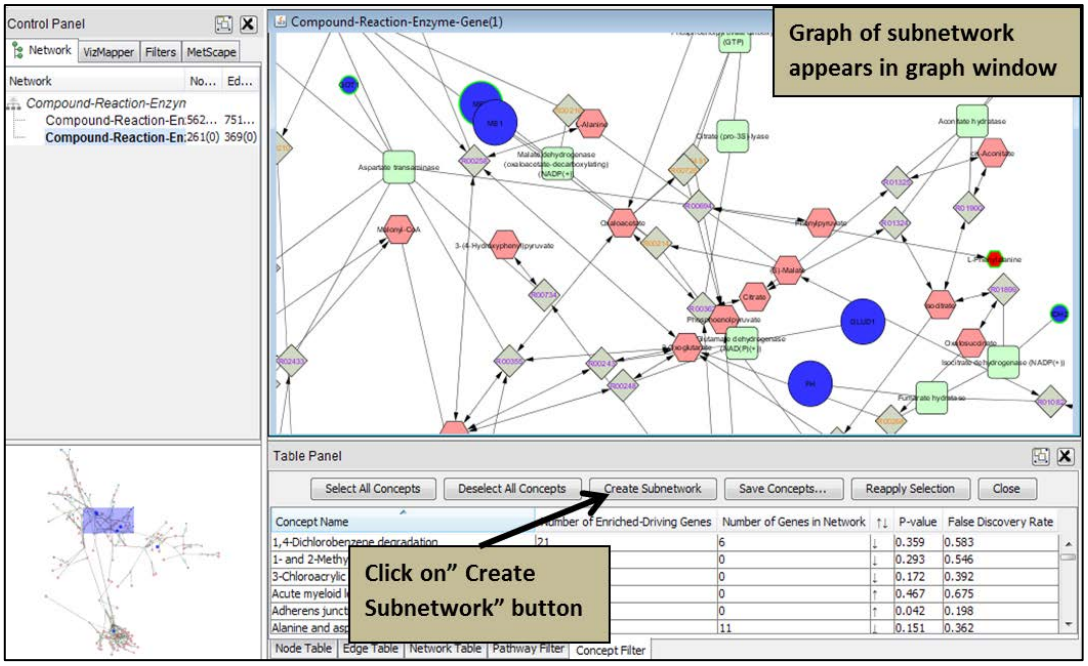


A subgraph can be created for a given concept. The subgraph consists of genes considered to be significant to the concept (those genes that drive the concept), not all genes in the concept. To create a subgraph:

1. Select a concept in the **Concept Filter** tab.

2. Click the **Create Subnetwork** button at the top of the Table Panel (make sure you are on the **Concept Filter** tab).

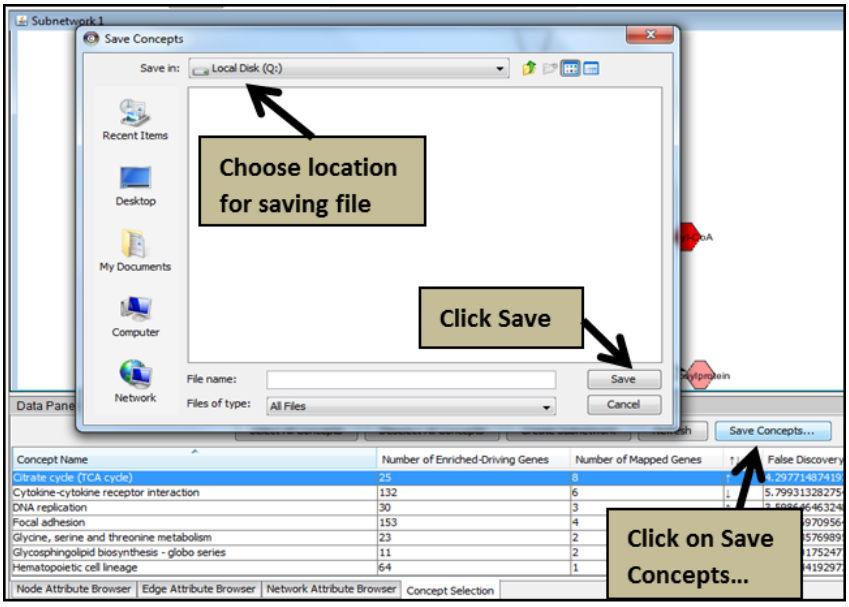
3. A new graph appears in the graph window. This is the subnetwork for the selected concept.



The **Reapply Selection** button will re-select the last selected concept in the graph. If you select a concept and then select off of it, clicking the **Reapply Selection** button will re-select that concept.

The concept information can be saved as a file. To save this information:

1. Under **Concept Filter** tab, click the **Save Concepts…** button.
2. A new window pops up, asking where to save the file. Choose the desired location.
3. Click **Save**. The file saves as a CSV file that can be opened with most text editing or spreadsheet programs. The file format is identical to the input concept file.

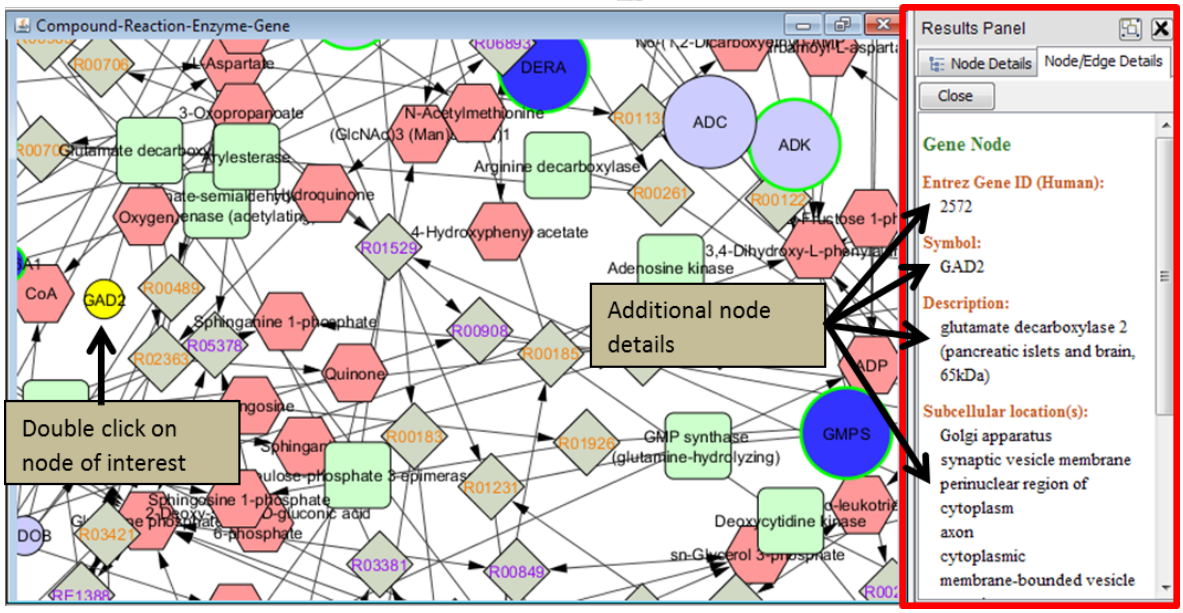


MetScape Results Panel (Additional Node Information)

Additional information can be obtained for each node by double clicking on it.

To get additional information about any compound, gene, reaction, or enzyme:

1. Double click on the node of interest.
2. On the right side of the screen, the **MetScape Results Panel** will show up.
3. Use the scroll bar or click on the undock icon ☐ to see all the data.



Animation of Data

Create an animation of the data to see how it changes over time and across treatments.

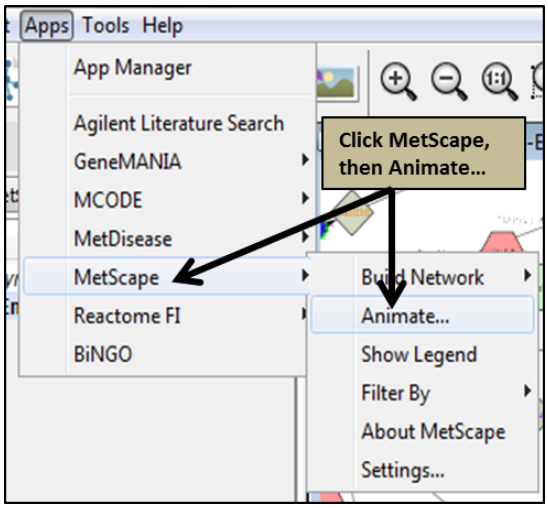
Building an Animation

1. Create a subnetwork (for example, create a subnetwork for the TCA Cycle. See the **Concept Filter** section in the Table Panel section for how to do this).

Note: It is not necessary to create a subnetwork before doing data animation; the animation can be done on any network. For example, the Compounds file can have additional data columns (see image below) that are loaded when initially importing the Compounds file.

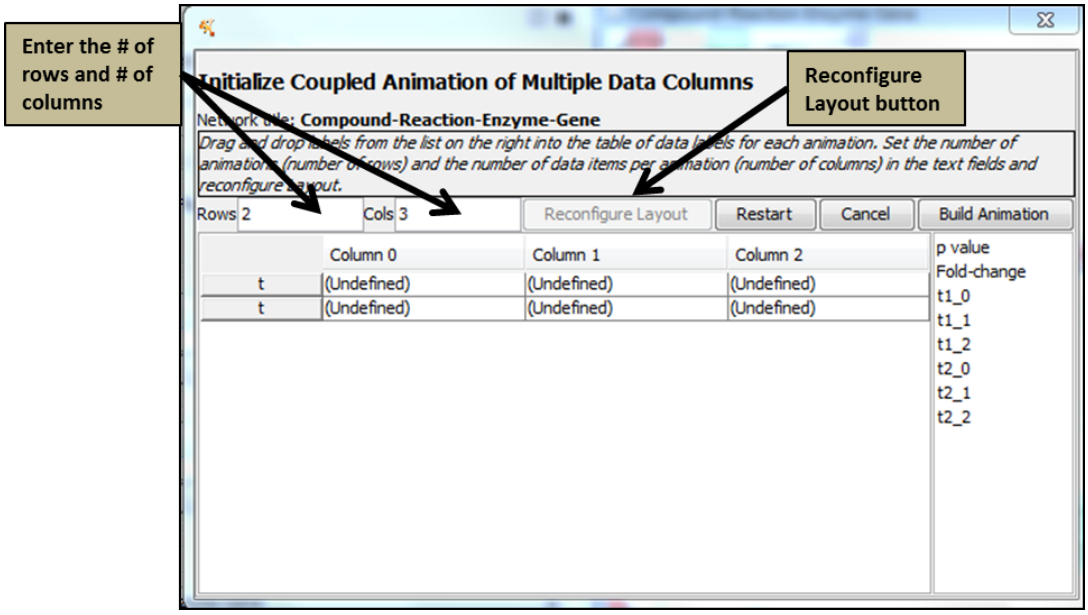
A	B	C	D	E	F	G	H	I
CID	**p value**	**Fold-change**	**t1_0**	**t1_1**	**t1_2**	**t2_0**	**t2_1**	**t2_2**
C00219	0.040982	1.49	0.477255	0.811411	0.907349	0.677623	0.349178	0.686385
C00503	0.008525	1.53	0.902665	0.698903	0.309585	0.33507	0.761891	0.042653
C00187	0.030047	1.85	0.489314	0.029276	0.314592	0.705353	0.084872	0.340032
C02721	0.024311	2.81	0.86923	0.125058	0.843886	0.667656	0.008175	0.131301
C00047	0.017356	1.03	0.312654	0.651588	0.386625	0.413499	0.650567	0.926665
C16868	0.004029	2.01	0.19994	0.379436	0.374019	0.064547	0.325439	0.605136
C05122	0.000312	1.75	0.002804	0.477482	0.486432	0.683021	0.562158	0.268456
C00064	0.000021	-1.2	0.113069	0.635121	0.403885	0.769097	0.885804	0.783052
C05629	0.000252	-1.38	0.775584	0.198474	0.490499	0.713089	0.756346	0.96571
C00079	0.036583	-1.15	0.139549	0.464563	0.037484	0.041068	0.592104	0.816224
C00398	0.016353	-1.07	0.115421	0.541825	0.8363	0.829824	0.001483	0.302741
C00294	0.000014	-1.4	0.125432	0.275928	0.741933	0.513364	0.90882	0.575744

2. From the **Apps** menu, choose **MetScape -> Animate…**

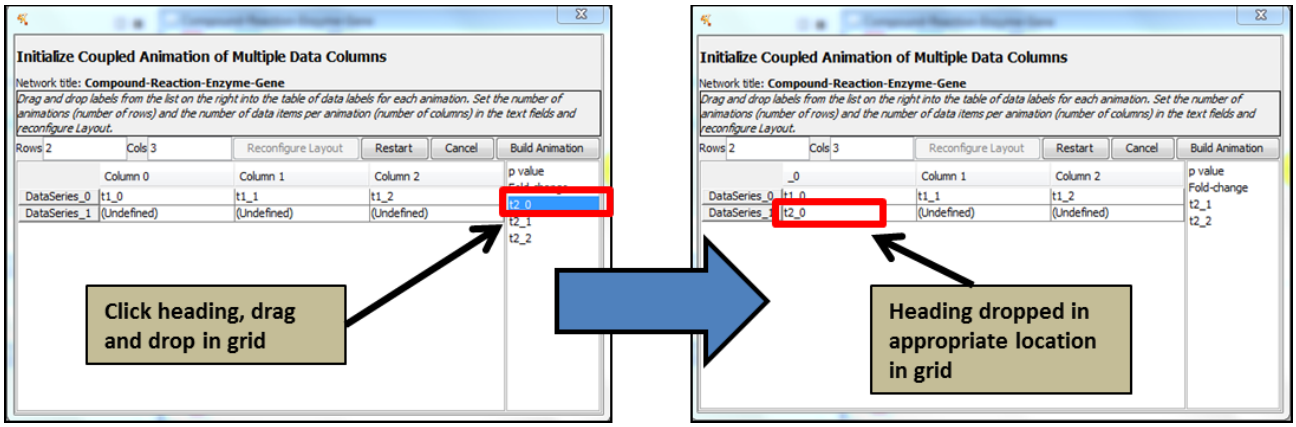


3. A new window pops up, titled **Initialize Coupled Animation of Multiple Data Columns.**

4. Column headings from your data are listed on the right side of the new window. Determine how many animations (number of rows) and data items per animation (number of columns) you will need. Enter this information into the Rows and Cols boxes. If you change these numbers from the default, click on **Reconfigure Layout.**



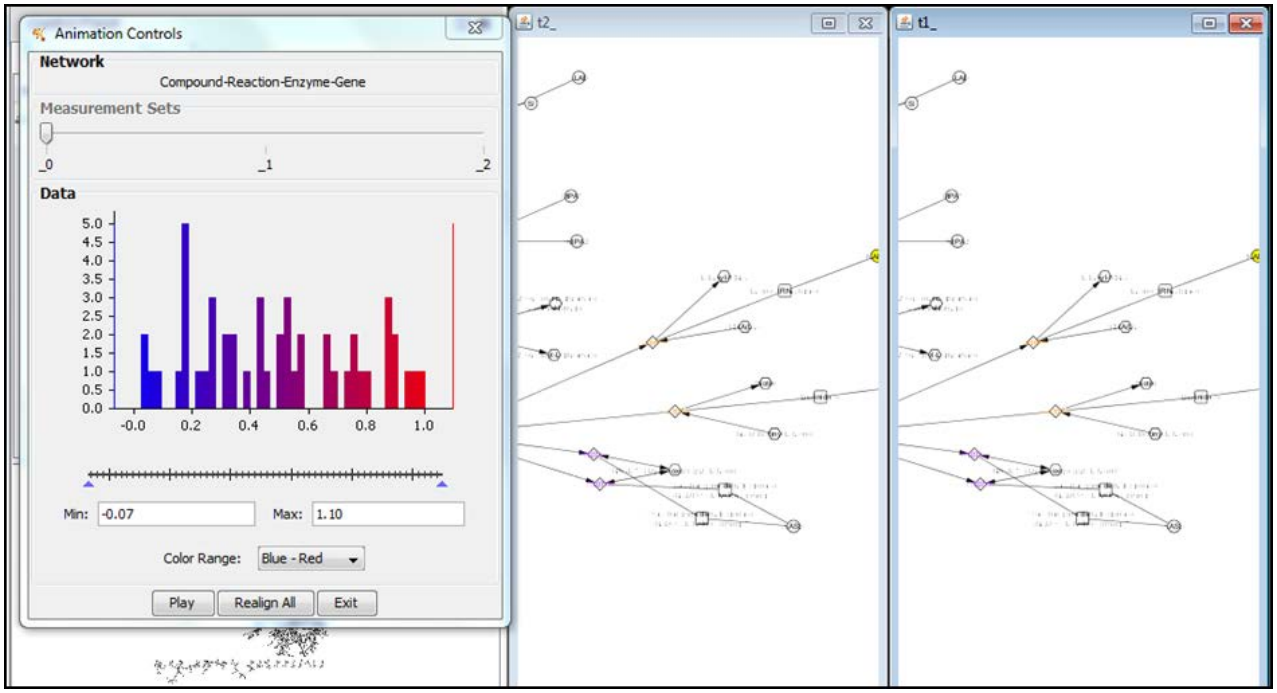
5. Click and drag the column names into the grid to build the animation.



6. After grid is completely filled in, click on **Build Animation.**

Note: If you want to undo what you filled out in the grid, click on **Restart** and the grid will be cleared.

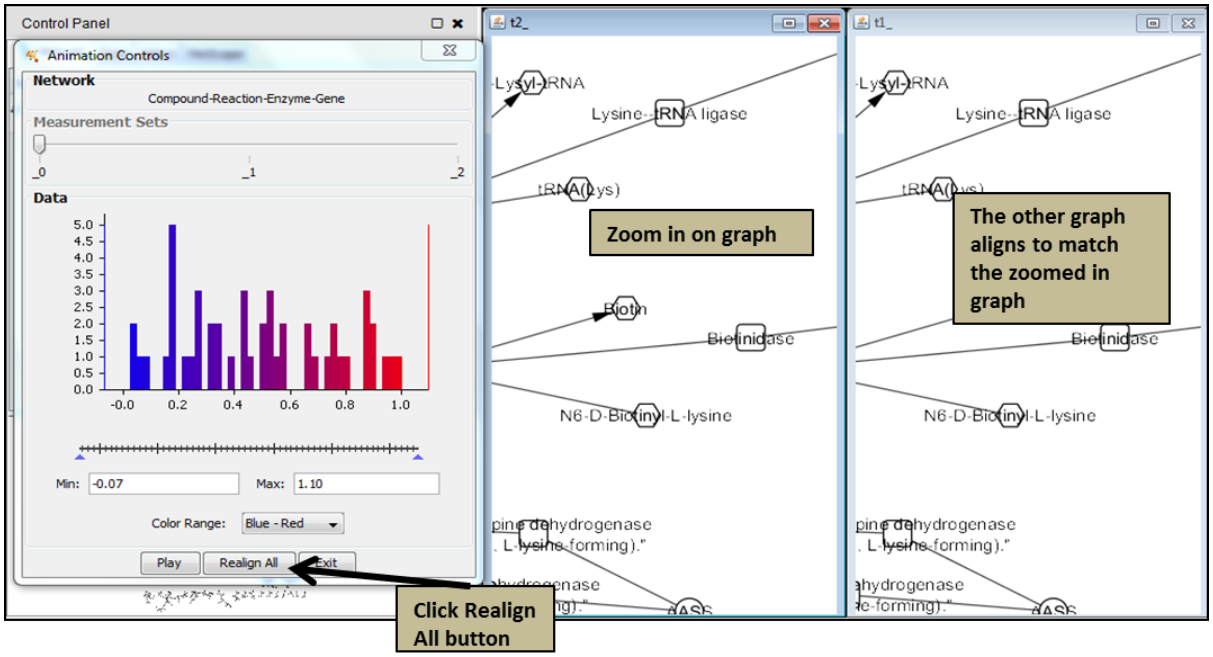
7. Two new windows appear in the graph panel, each representing a different treatment. In addition, an **Animation Controls** window appears.



Manipulating the animation: zooming

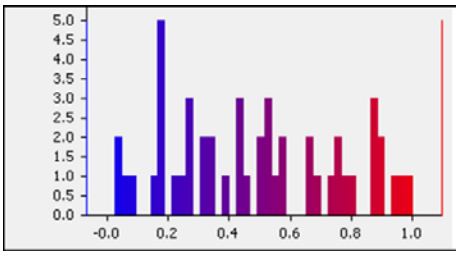
1. **Zoom** in on one graph to the appropriate view size (zoom the same way you do with any Cytoscape graph).

2. On the **Animation Controls** window, click on **Realign All.** This brings all the treatment graphs to the same view.



Manipulating the animation: bar chart

1. The bar chart in the **Animation Controls** window shows the range of the data and its frequency. The range of measurements are on the x-axis, while the frequency with which they occur in the experimental data set are on the y-axis.



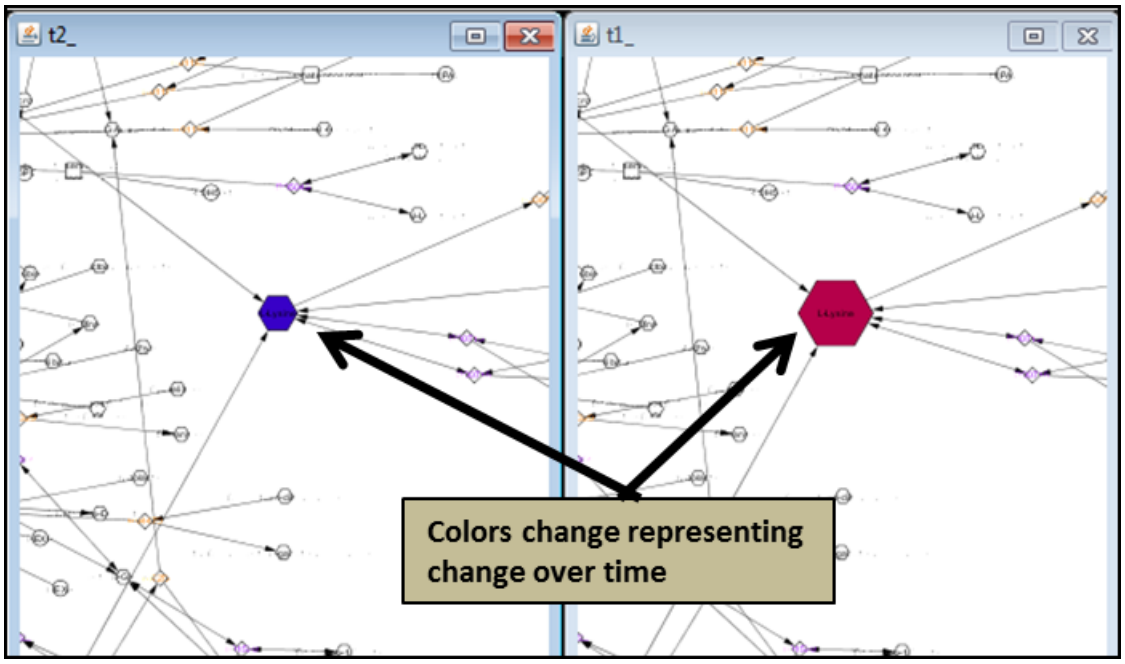
2. Click on a bar in the bar chart and a vertical line (slider) will show up on the bar chart. The slider can be moved by clicking on the bar chart and dragging the line. When moved, the values shown in black are also shown in black in the graph and the set of the color scale is reset.

3. The selected portion of the graph determines what the animation will show. This allows outliers to be removed if desired.

4. The color range can be changed by clicking the dropdown arrow next to **Blue-Red.**

Playing the animation

To play the animation, click **Play** on the **Animation Controls** window**.** Colors and node sizes change within the three treatments, showing changes over time. To stop the animation, click **Stop** on the **Animation Controls** window**.**



Manipulating Network

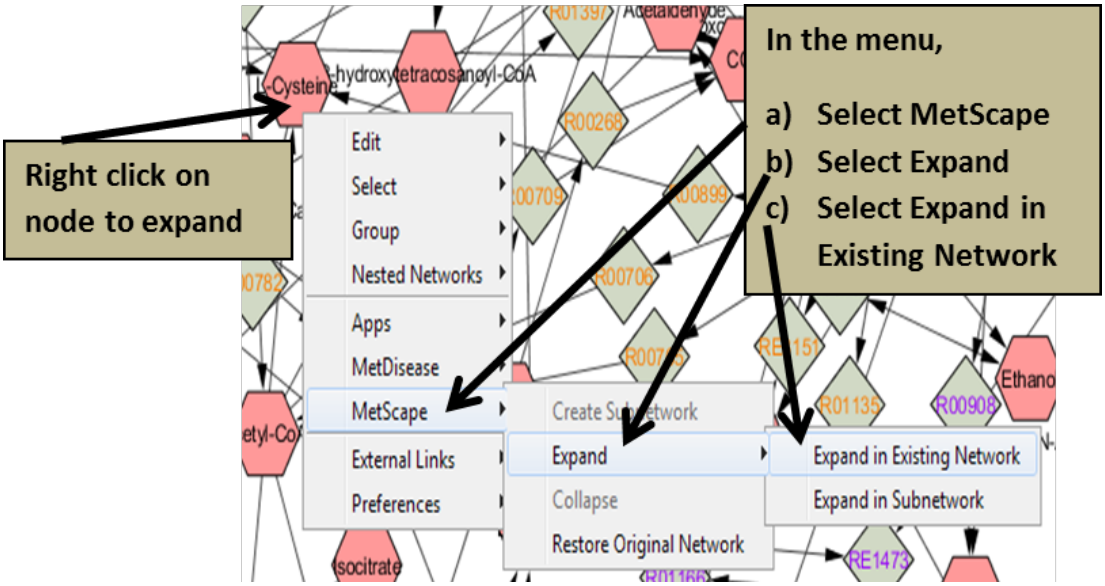
Expanding a network

From a compound node, you can expand the current network to include additional reactions and related compounds, genes, and enzymes.

Option 1: Expanding a network in the current network window (Example using Compound-Reaction Network)

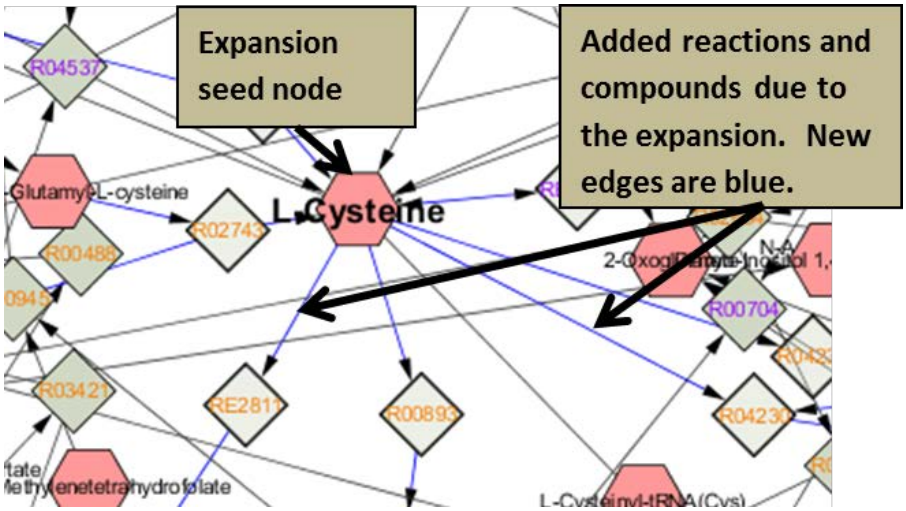
1. Right click on the compound node you want to expand (this node is known as the expansion seed node). A menu of options will pop up.

2. Go to **MetScape -> Expand -> Expand in Existing Network**



3. Additional compounds and reactions are added to the network. As a result, the network is often redrawn.

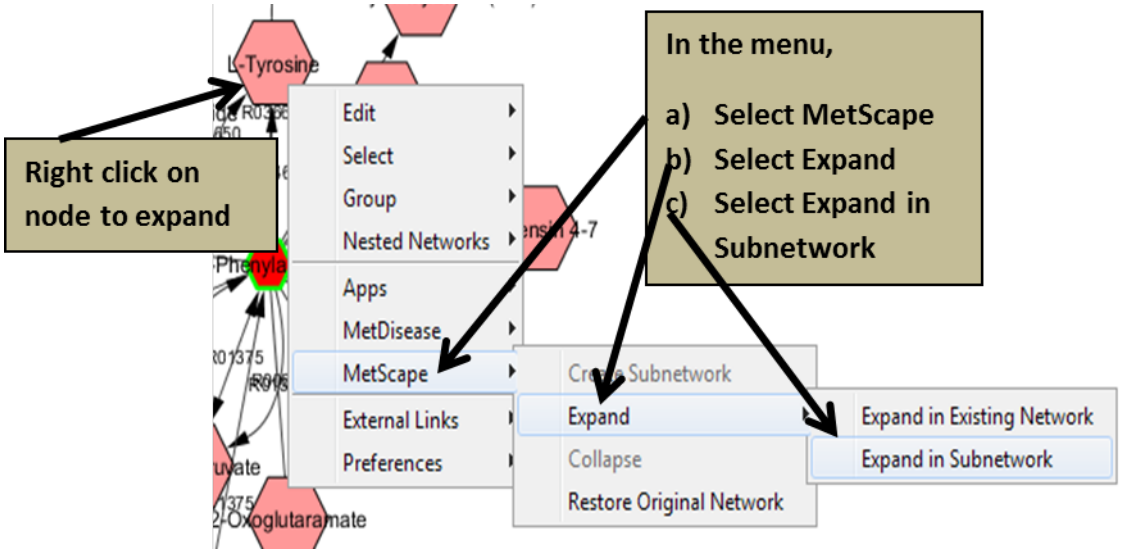
4. The edges between the original node and the expanded nodes are now blue.



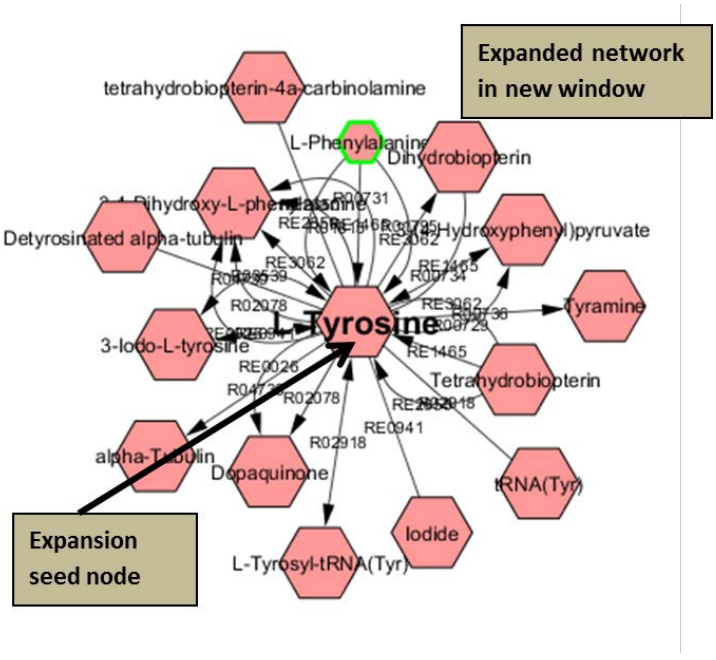
Option 2: Expanding a network in a new window (Example using Compound Network from Selected Genes)

1. Right click on the compound node you want to expand (this node is known as the expansion seed node). A menu of options will pop up.

2. Select **MetScape -> Expand -> Expand in Subnetwork**



3. A new network is created in a new window. This network includes only the expansion seed compound and its related compounds, reactions, enzymes, and genes (depending on the Network Type). The original color designations are used when the graph is created in a new window.



Collapsing a network

To collapse a network that is expanded in a current network window:

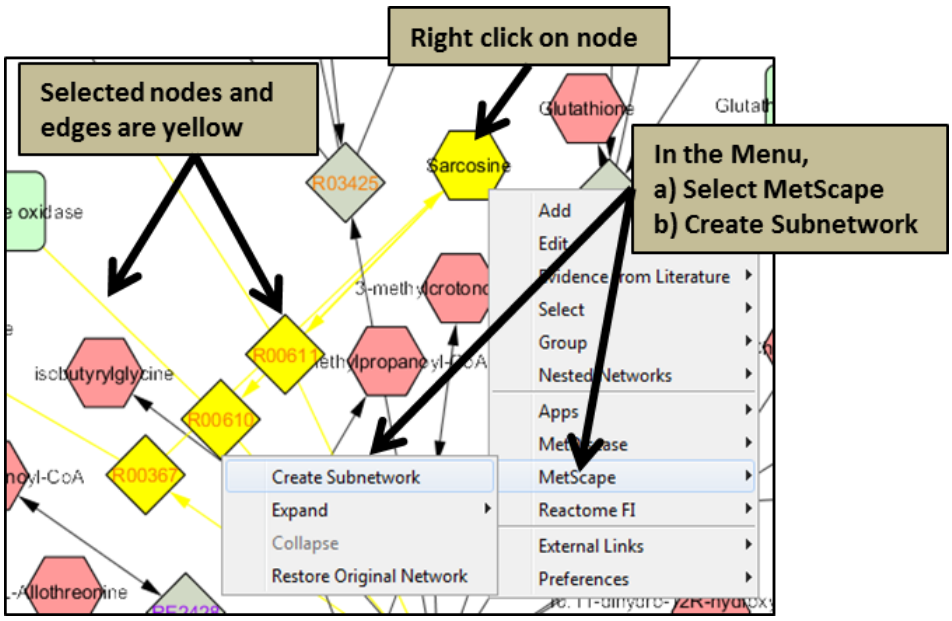
1. Right click on the expansion seed compound node.
2. Select **MetScape -> Collapse** and then:
    - To collapse only the branch expanded from that expansion seed compound, select **Collapse.**
    - To collapse all expanded branches, select **Restore Original Network.**

Creating a subnetwork

A subnetwork of a current network can be created and will appear in a separate window. A subnetwork will include all highlighted nodes and edges (selected nodes and edges should be yellow).

For example:

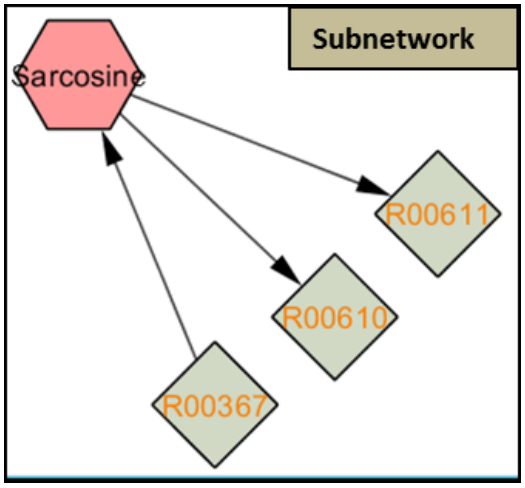
1. Select a compound in the built network. For this example, I will select Sarcosine.
2. Go to the **Select** menu in Cytoscape.
3. Choose **Nodes -> First Neighbors of Selected Nodes -> Undirected.** Now all the first neighbors of Sarcosine should be yellow.
4. Go to the **Select** menu in Cytoscape.
5. Choose **Edges -> Select Adjacent Edges.** Now all the adjacent edges should be yellow.
6. Right click on Sarcosine. A menu of options will pop up.



7. Choose **MetScape -> Create Subnetwork.**

Note: When choosing options from the menu, be careful not to move the cursor outside the menu panels. Doing so will remove the highlighting of the nodes and edges, resulting in an empty subnetwork.

8. A new subnetwork is created in the graph window.



Destroying a network

To destroy a network no longer needed:

1. Make sure you really do want to destroy the network.
    - Cytoscape will ask you to confirm the deletion.
    - Destroying the network is irreversible.
    - **Note:** If you want to destroy a network view without destroying the network itself, use **Destroy View** instead of **Destroy Network.**
2. On the **Network** tab in the Cytoscape Control Panel, right click on the network you want to destroy and select **Destroy Network.**

Note: If you destroy a network that has subnetworks, the subnetworks are **NOT** destroyed. They are promoted up one network level.

PART 2: CORRELATION-BASED NETWORK

Entering Data

File Formats

Two correlation file formats are accepted (column-based is recommended):

1. Column-based
    - The first row must contain column headings of the user's choosing
    - The first two columns contain metabolite names
    - The next column(s) contains values, such as p-values.

Below is a portion of an example column-based correlation file:

	A	B	C	D	E
1	metab1	metab2	pcor	pval	qval
2	Alanine	Alanine	1	0	0
3	Alanine	Sarcosine	0.110571	0.101242	0.287801
4	Alanine	Glycine	0.255278	0.000101	0.001017
5	Alanine	Alpha-am	0.091832	0.1743	0.428134
6	Alanine	Valine	0.040047	0.554916	0.74299
7	Alanine	Leucine	0.006732	0.921006	0.960585
8	Alanine	Isoleucine	0.071432	0.291444	0.548247
9	Alanine	Threonine	0.100668	0.136092	0.358658
10	Alanine	Serine	-0.13329	0.047578	0.164894
11	Alanine	Proline	0.468989	5.19E-15	6.56E-13
12	Alanine	Asparagin	-0.05741	0.396934	0.616099
13	Alanine	Aspartic a	0.059375	0.380926	0.609963
14	Alanine	Methionir	0.102612	0.128614	0.346165
15	Alanine	4-Hydroxy	-0.11061	0.101123	0.287801

2. Matrix format
    - The first row and column contain metabolite names
    - The rest of the rows/columns contain correlation values

Below is a portion of an example matrix format correlation file:

	A	B	C	D	E	F	G	H	I
1		Alanine	Sarcosine	Glycine	Alpha-ami	Valine	Leucine	Isoleucine	Threonine
2	Alanine	1	0.215426	0.171592	0.167991	0.450516	0.287503	0.352786	0.213065
3	Sarcosine	0.215426	1	0.060618	0.158684	0.21722	0.106856	0.175509	0.074988
4	Glycine	0.171592	0.060618	1	0.130999	-0.08656	-0.12006	-0.09012	0.164396
5	Alpha-amin	0.167991	0.158684	0.130999	1	0.417381	0.322295	0.266963	0.064821
6	Valine	0.450516	0.21722	-0.08656	0.417381	1	0.773163	0.775301	0.131847
7	Leucine	0.287503	0.106856	-0.12006	0.322295	0.773163	1	0.884062	0.165156
8	Isoleucine	0.352786	0.175509	-0.09012	0.266963	0.775301	0.884062	1	0.258682
9	Threonine	0.213065	0.074988	0.164396	0.064821	0.131847	0.165156	0.258682	1
10	Serine	0.070274	-0.03839	0.441288	0.104243	0.046118	0.023038	0.09493	0.553733
11	Proline	0.593237	0.129416	0.071866	0.02707	0.517814	0.420597	0.452117	0.306851
12	Asparagine	0.110042	0.039533	0.282938	0.156343	-0.01262	0.106846	0.066391	0.602028

Building a Network

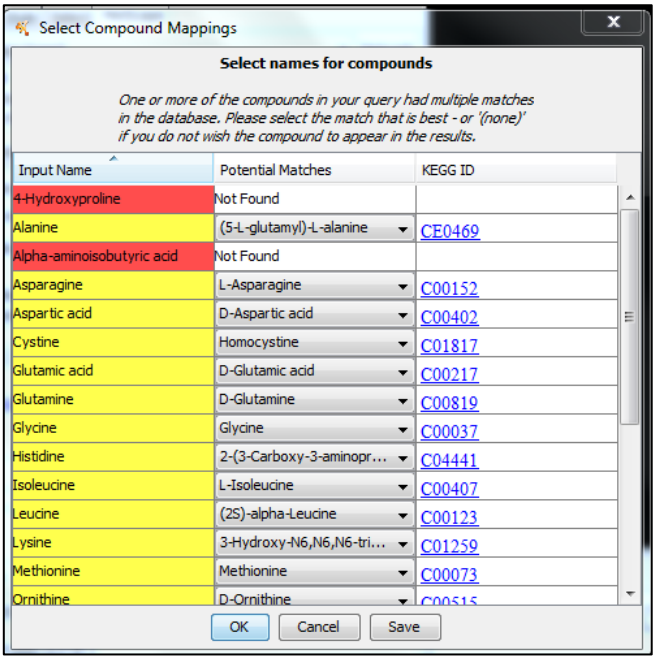
1. To begin a Cytoscape session with the MetScape app, first start Cytoscape.

2. Select **Apps -> MetScape-> Build Network -> Correlation-based** from the Cytoscape menu.

3. A MetScape tab now appears on the left of the Cytoscape screen.

4. Use the **Select** button to upload the appropriate file.

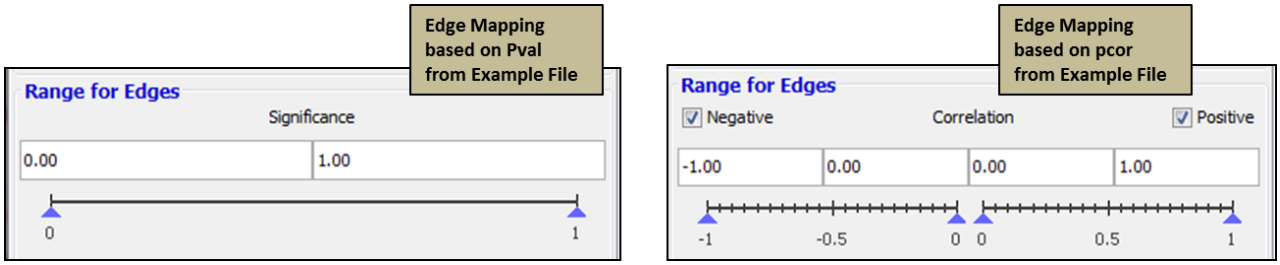
5. Compound names will map to their KEGG id; a popup window will appear. If there is more than one potential match, use the dropdown arrow to choose the best match. If a compound is not found in the system, it will say "Not Found." The mapping selection will be saved so that your selection will appear as the default option in the future.



5. Click **OK.**

6. Under Edge Mapping, use the dropdown menu next to **Base Edges on** and select the appropriate column from your data file.

Note: The **Range for Edges** slider changes to match the data type chosen. In the example files used in this manual, the slider is 0 to 1 for pval but -1 to 1 for pcor.

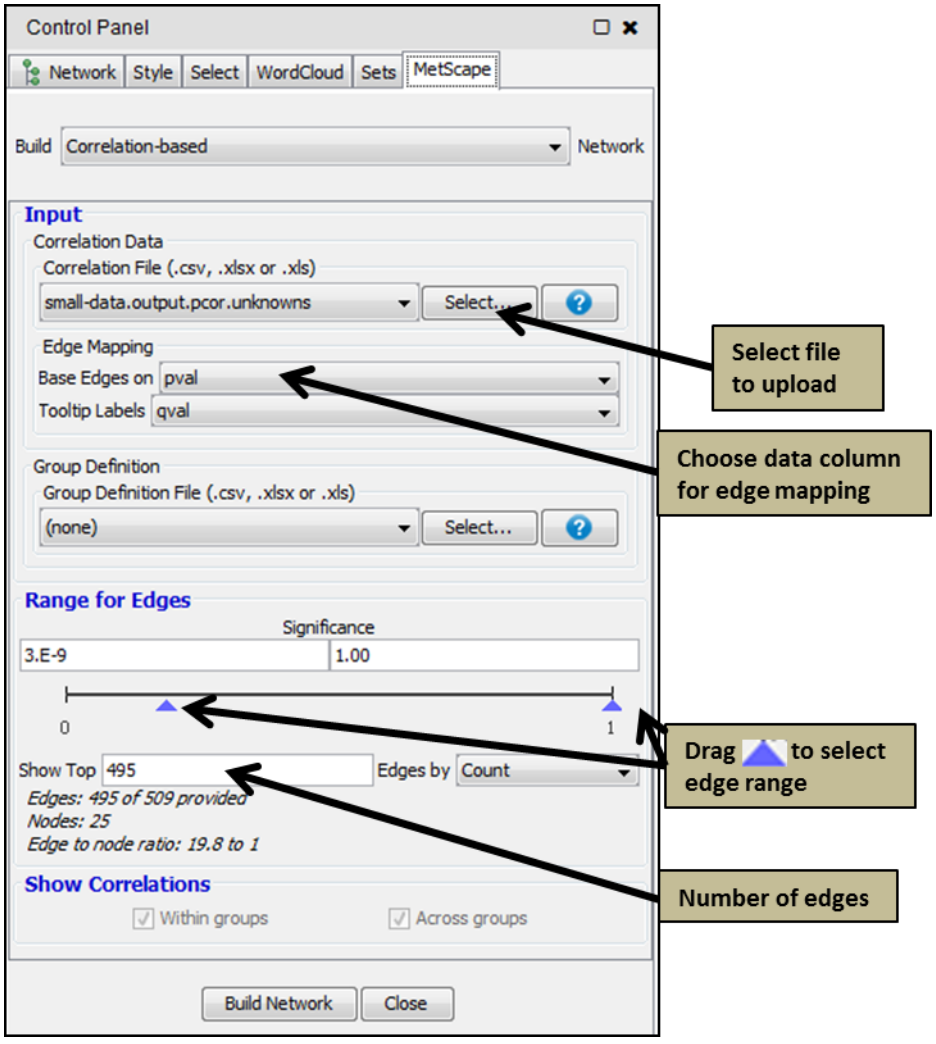


7. Under Edge Mapping, use the dropdown menu next to **Tooltip Labels** to select additional values that will be viewable when mousing over an edge in the built network.

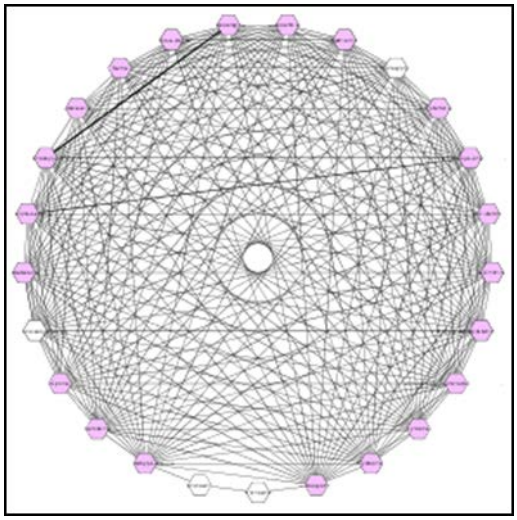
8. **Optional:** Load **group definition** file. For more information, go to the Group Definition section of this document.

9. Under Range for Edges, drag the arrows left and right OR enter numbers in the text boxes to select the desired **significance range**. A log scale is used to allow for very small p-values.

10. The number of edges that will appear in the network built with the current parameters will appear below the slider.



11. Click **Build Network**.



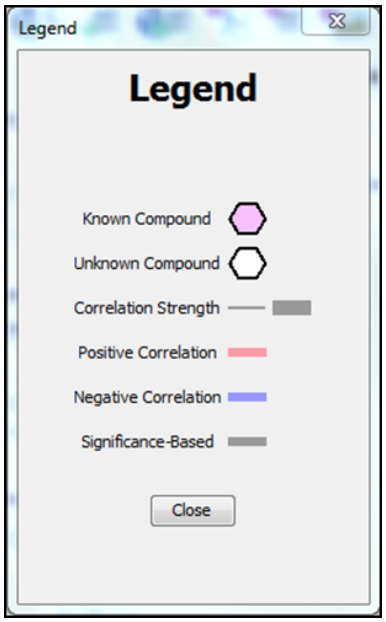
Group Definition

MetScape provides the option of creating groups using a **group definition file**. A group definition file is a simple 2-column file with metabolite names in the first column and group names in the second column. Group names can be anything that you choose. A group definition file can be loaded using the **Select…** button under Group Definition on the MetScape tab. If a group definition file is used, a **Group Filter tab** will appear in the Table Panel after the network is drawn. Clicking on a group name in the Group Filter tab will select all nodes in the network that are part of that group.

Correlation Visualization

MetScape provides a legend explaining its various shapes and colors. The legend will be specific to the current network type (pathway or correlation).

While on a correlation network, access the MetScape **Legend** from **Apps -> MetScape -> Show Legend**.



Additional information about the network is expressed through visualization:

- Edges
  - If the dataset only has values 0 to 1, all edges will be black.
  - If a dataset has positive and negative correlation values:
    - A pink edge represents a positive correlation.
    - A blue edge represents a negative correlation.
  - The thicker the edge, the stronger the correlation.

Note: In a correlation-based network, selected edges are highlighted yellow instead of red.

- Nodes:
  - Purple nodes represent mapped compounds.
  - White nodes represent compounds that did not map to a known compound in the MetScape database.

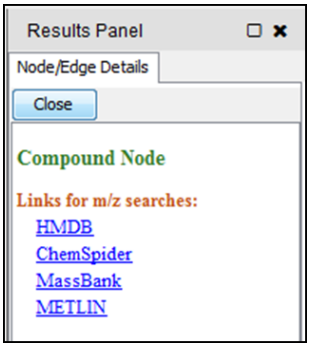
Additional Information

Node and edge attributes are displayed in the Table Panel. See the Table Panel Display section above for details on this panel. A Pathway Filter tab is also available in the Table Panel. See the Pathway Filter in the Table Panel section above for details about this panel.

When a correlation-based pathway is built, its name in the Network tab reflects significance information for that network. This naming convention is to help distinguish between networks when creating multiple networks in the same Cytoscape session.

Correlation Network Results Panel

Details about nodes can be viewed by double clicking on a node of interest. To learn more about the details window for **known compounds**, see the MetScape Results Panel section above. Double clicking on an **unknown compound** will bring up a Results Panel that contains links to HMDB, ChemSpider, MassBank, and METLIN.



Each of these links will allow you to search based on **mass-to-charge values**, helping to identify the unknown compound.

In addition, a **node attribute file** can be imported into Cytoscape with one column for nodes and a second column for mass-to-charge. The mass-to-charge column needs to have one of the following as the heading: m/z, M/Z, m\z, M\Z, mz, or MZ. If the mass-to-charge values are entered for unknown compounds, clicking on the HMDB link in the Results Panel will go to the HMDB results for that mass-to-charge value. Please note that the other three links will still go to their search pages.

SAVING AND REOPENING A SESSION

Saving a session

To save a Cytoscape session containing one or more MetScape app networks:

1. Select **File -> Save** (or **Save As…**) from the Cytoscape menu.
2. Browse to a location for saving the file.
3. Name the file.
4. Click **Save.**

Reopening a session

To reopen a saved session containing MetScape app data:

1. Select **File -> Open** from the Cytoscape menu.
2. Navigate to the saved file location.
3. Select the file.
4. Click **Open.**
5. After Cytoscape reports that the session file was successfully loaded, click **Close.**

SUPPLEMENT: METDISEASE APP

MetDisease is designed to annotate metabolites with Medical Subject Headings (MeSH) disease terms. The underlying data comes from the Metab2MeSH data set (Sartor et al., Bioinformatics. 2012 May 15;28(10):1408-10). To use MetDisease, you need a network of compounds with KEGG or PubChem Ids or compound name. If using MetScape 2.3.2 or higher, you can use MetDisease on the MetScape networks.

How to use MetDisease

Once you have built a MetScape network:

1. Select **Apps -> MetDisease -> Find MeSH Terms...**

2. Choose the appropriate identifier type and attribute. Click **OK**.

3. A hierarchical tree of MeSH Disease terms should be displayed in the Table Panel.

4. Selecting a tree node will select respective metabolites in the network. The numbers shown after the descriptors represent the number of matching metabolites in the active network.

Note: Descriptors that have no nodes in the active network can be collapsed and hidden.

5. To view relevant PubMed citations, right click on a selected compound node and select **MetDisease -> PubMed Citations**.

For more information about MetDisease, go to http://metdisease.ncibi.org/. The MetDisease user manual can be downloaded at http://metdisease.ncibi.org/pdf/MetDisease-User-Manual.pdf.