ARIADNE

Pathway Studio® Explore 1.1, Affymetrix Edition

Training Manual

Version 2.0

Preface

This manual is for users of Pathway Studio® Explore, Affymetrix Edition Version 1.1.

Instructions for the installation of Pathway Studio Explore are not included in this manual. Please see the Pathway Studio Explore Installation Guide for information on the installation of Pathway Studio Explore and the ResNet Explore Database. The Installation Guide can be found on Ariadne's Technical Support site: http://www.ariadnegenomics.com/support/pathway-studio-explore/training-material/.

How to Use this Manual

This manual is designed to walk you through some major workflows in Pathway Studio Explore. Each section has an introduction to the tools in Pathway Studio examples of how they can be used, followed by a hands-on work example. Each example builds on information from the previous exercises, and sometimes utilizes files generated from previous examples.

Two example data files accompany this training manual:

- GDS2126.gepr and GDS2126.txt – This files contains the information needed to import a microarray experiment
- SCLC genes – this is an MS Excel spreadsheet containing genes differentially expressed between normal lung and small cell lung tumor samples.

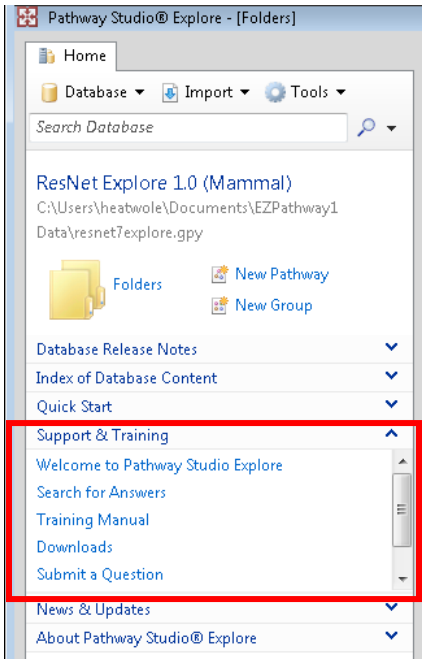
You will need to download these files to be able to reproduce the hands-on exercises. You can find these files in the same location in the Support section of Ariadne's website as this Training Manual.

Other Training and Support Resources

The Ariadne Technical Support site has additional resources available to enable Pathway Studio Explore users found at:
http://www.ariadnegenomics.com/support/pathway-studio-explore

Access to Technical Support

Technical Support for Ariadne's products can be easily accessed from Pathway Studio:



Links within the Information Pane in Pathway Studio take you directly to the support website. Registration and log-in to this website is required.

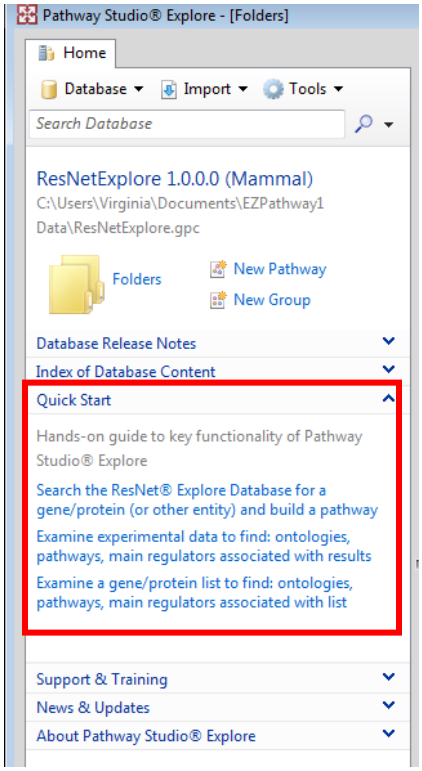
In addition, you can access the support site directly at:

http://www.ariadnegenomics.com/support/pathway-studio-explore

A link is provided here to submit questions directly to Technical Support.

In addition this Training Manual, you can access the Quick Start Guide through the Information Pane. This Quick Start Guide will walk you through the steps of some common analysis workflows for Pathway Studio.

Quick Start Guides



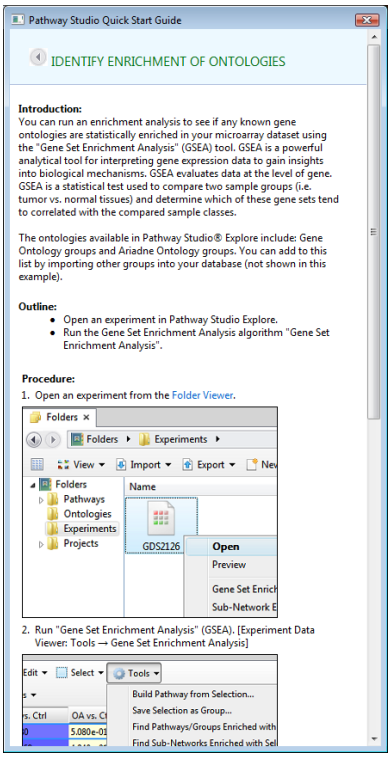


Table of Contents

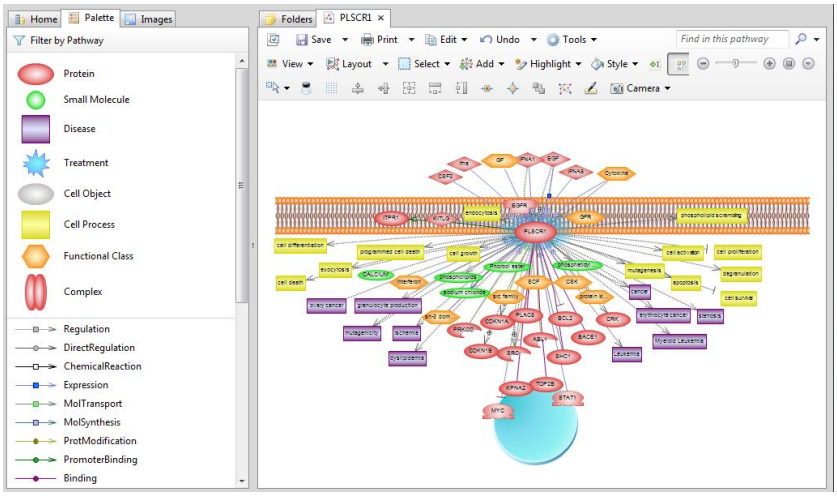
Section 1: Introduction to Pathway Studio Explore and ResNet Explore Database

Congratulations on your decision to utilize Pathway Studio Explore as an important tool to support your biological research. Pathway Studio Explore helps you to interpret experimental data in the context of pathways, gene regulation networks and protein interaction maps; interpret microarray and proteomics data, classify and prioritize proteins, draw pathway diagrams, and automatically update your pathways with newly published facts using MedScan Technology.

What can Pathway Studio Explore do for you?

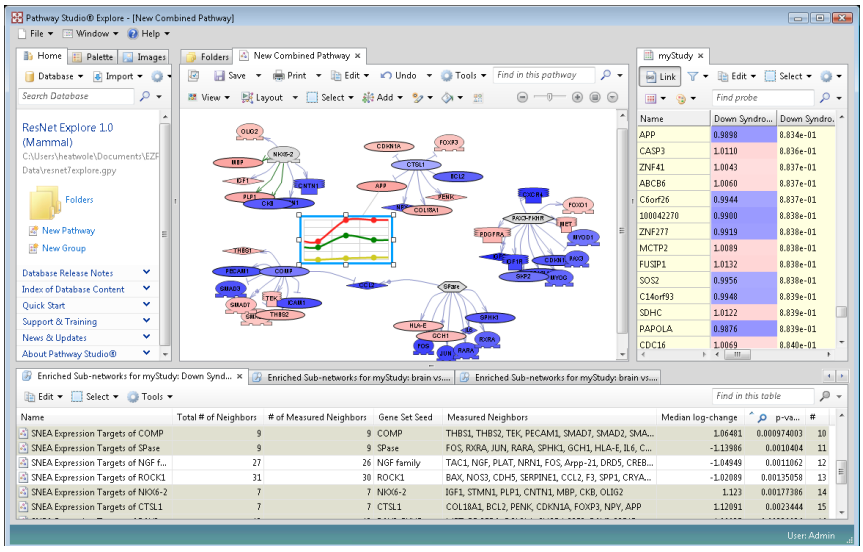
- **Identify relationships among proteins, small molecules, cell processes and diseases**

  What is known to interact with my protein? What processes are associated with my protein?



- **Build and reconstruct pathways from your microarray and other high-throughput data**

  Analysis Tools to find enriched networks in experimental data



MedScan Reader 3.0

MedScan technology is a Natural Language Processing Technology used to extract relationship information from biomedical literature. Ariadne utilizes MedScan technology to build the ResNet Explore Database, the mammalian (human, mouse and rat) database, of relationship information summarized from all abstracts in PubMed as well as information contained in 61 free full-text journals. ResNet Curator was applied to the extracted data to condense information and remove some technical false positives.

Here is an example of how MedScan works. MedScan reads sentences and identifies entities (proteins, complexes, small molecules, diseases etc.) in sentences, here depicted in red.

Sentence in scanned literature: **"Axin binds beta-catenin and inhibits GSK-3beta."**

- Identify Proteins in Dictionary (in red): **[Entities]**

"Axin binds beta-catenin and inhibits GSK-3beta."

Next MedScan utilizes pattern rules to identify described relationships between the sentences

- Identify Interaction Type (in blue): **[Relationships]**

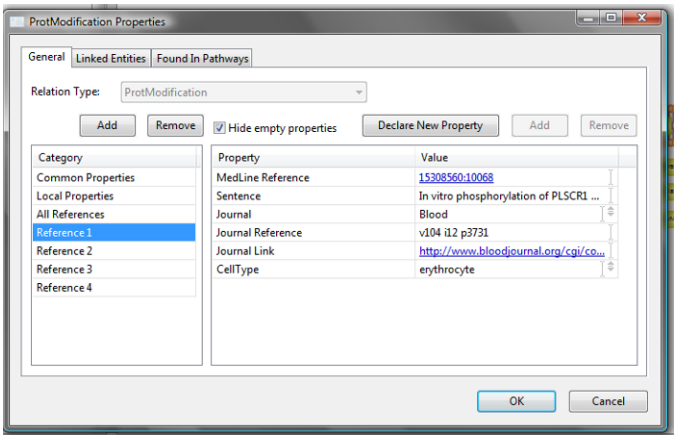
"Axin **binds** beta-catenin and **inhibits** GSK-3beta."

The extracted facts are the relationships, which are added to the ResNet database:

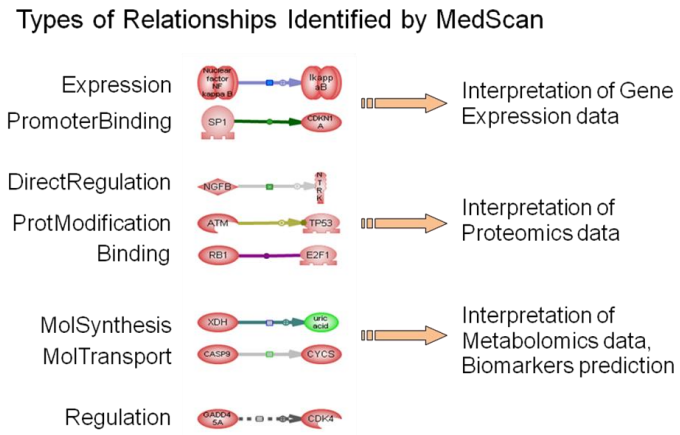
Extracted Facts:

Axin - beta-catenin relation: **Binding**
Axin -> GSK-3beta relation: **Regulation,** effect: **Negative**

The sentences containing identified relationships are available for examination within the Pathway Studio interface as well as the literature reference.



The MedScan dictionary contains lists of entity names. (There are eight types of relationships between entities identified by MedScan. To see a definition of entity types and relationships types, see Appendix A. All relations have directionality except Binding.)

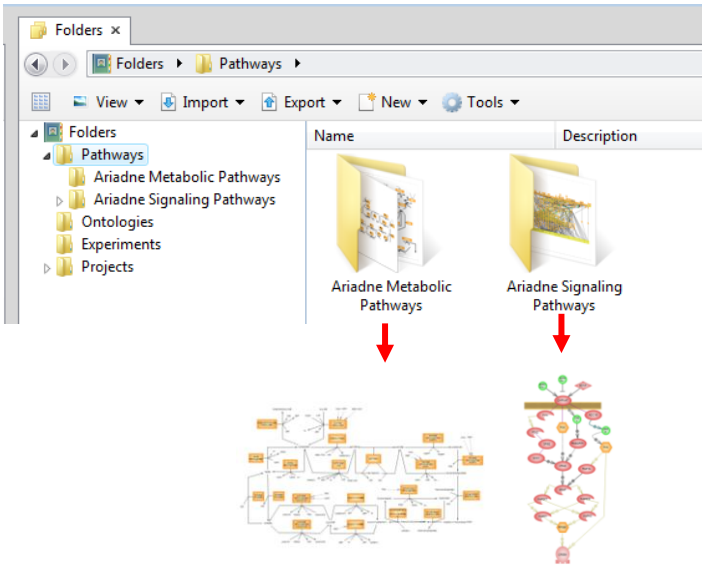


ResNet Explore Database

The ResNet Explore database includes almost 700,000 unique relationships derived from over 19 million PubMed abstracts as well as 61 full-text journals. In addition to information extracted by MedScan, ResNet Explore includes MeSH terms for diseases based on the Medical Subject Headings (MeSH) from the National Library of Medicine (http://www.nlm.nih.gov/mesh/meshhome.html), GO terms from The Gene Ontology Consortium (http://www.geneontology.org/), Ariadne Ontology terms, and a collection of Ariadne curated reference pathways (227 receptor signaling pathways, 21 cellular process pathways and 39 metabolic pathways). Most small molecules in ResNet have identifiers from either PubChem (http://pubchem.ncbi.nlm.nih.gov/) or from the American Chemical Society (http://www.cas.org/).

Curated Pathway Collection

The ResNet Explore Database includes a collection of reference pathways including a large number of receptor signaling pathways, cellular process pathways and metabolism pathways. These can be found in the Pathways folder. Ariadne scientists have built these pathways based on general knowledge in order to provide you with useful building blocks to extend with your own specific expertise.



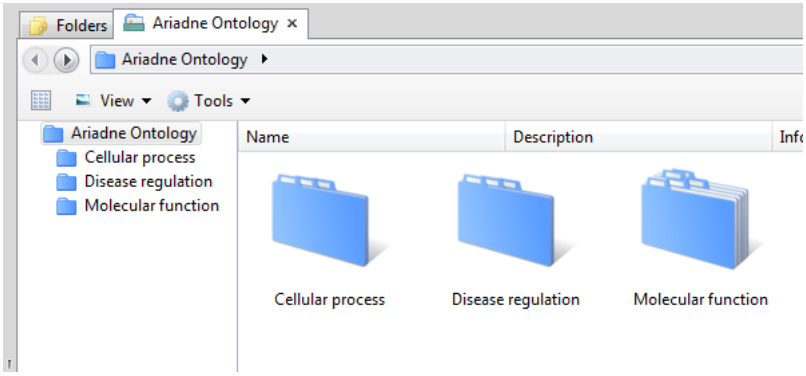
Receptor pathways curated into 227 signaling pathways

21 Cellular Process pathways

39 Metabolic Pathways

Ariadne Gene Ontology/Gene Ontology

In addition to the well-known “GO” vocabulary from the Gene Ontology Consortium, Ariadne has prepared a robust ontology that has been optimized to provide the best results from analysis with Pathway Studio. Ariadne’s Gene Ontology is designed to sort genes into the appropriate category, rather than to assign multiple categories to a gene. The ontology is relatively flat, containing only two groups, cellular process and molecular function. Additionally, the Ariadne Gene Ontology has been designed to minimize redundancy in the classification, and consequently avoids much of the redundancy found in analysis results produced using the Gene Ontology Consortium’s vocabulary.

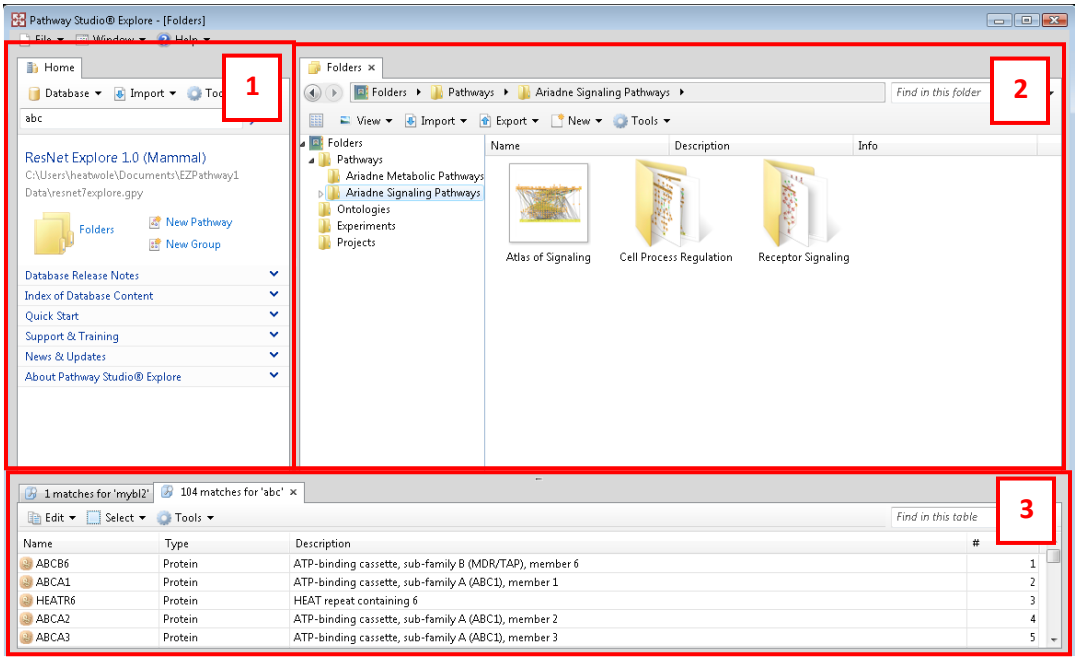


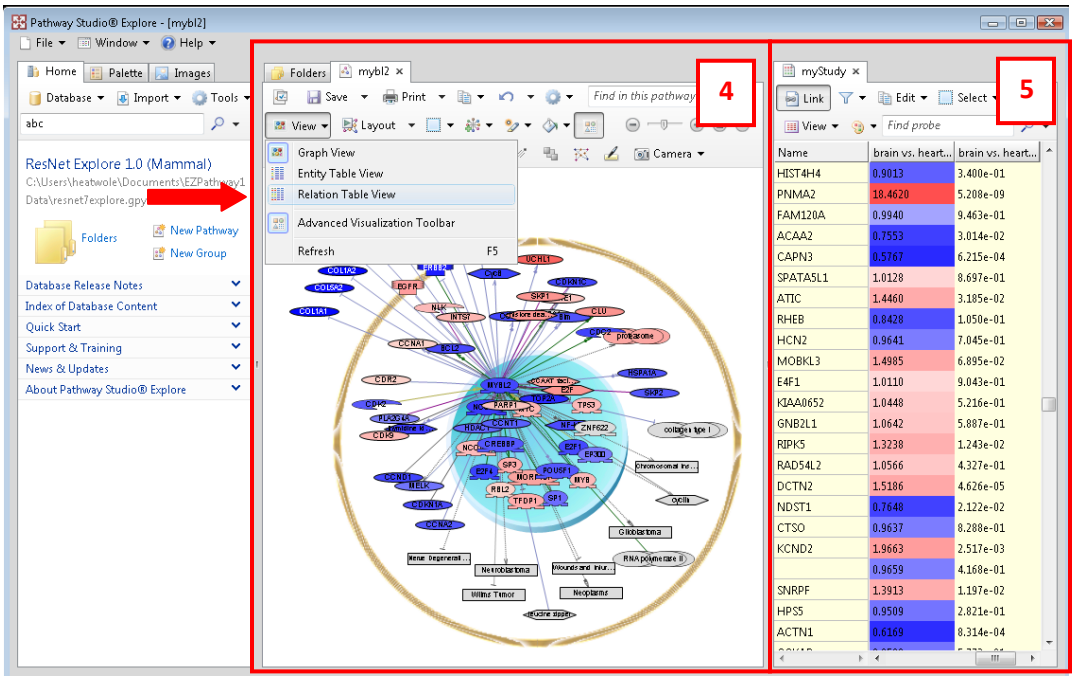
Organizes almost 9000 genes into 505 groups with three-tier biological hierarchy (created by Ariadne scientists)

This more succinct ontology can be used with Gene Set Enrichment Analysis and Fisher’s Exact test to obtain meaningful results.

Section 2: Introduction to the Pathway Studio interface

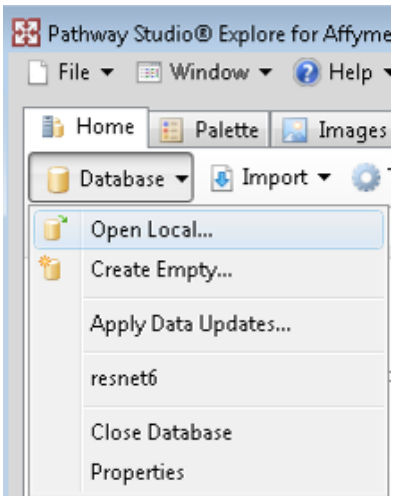
The major panes in the interface include: Information Pane (1), Folders Views (2), List Pane (3), Graph (and Relationship and Entity) View (4), Experiment Pane (5) (see following two figures).





Opening a Database

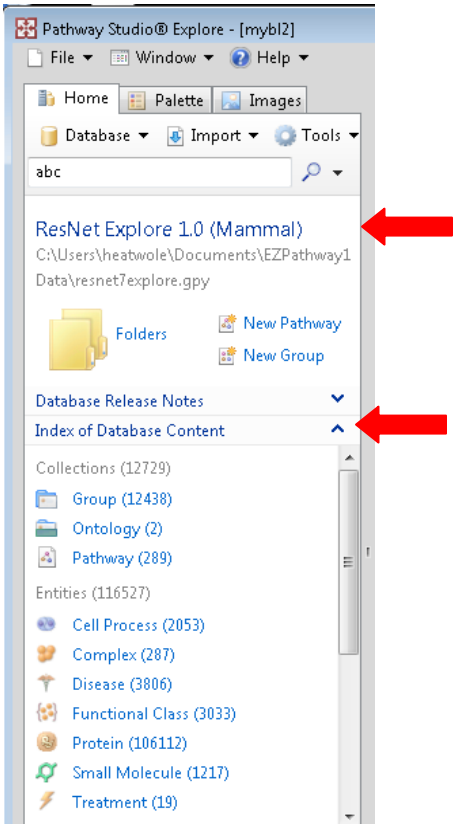
To before working with Pathway Studio, you must first select to open the Explore Database (if it has not opened by default). The database hosted locally on your computer's hard drive.



To open a local database go to the Information Pane and select: Database > Open local.

Information Pane

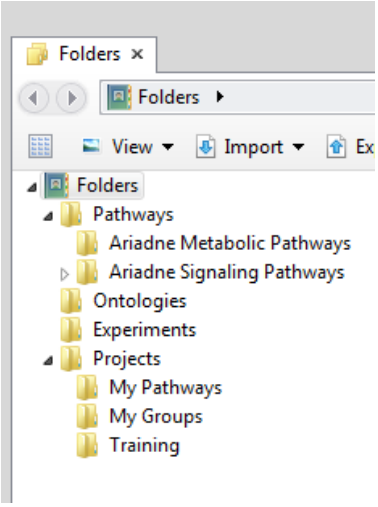
The Information Pane contains subheadings that provide useful links and information.



The header in the Information Pane indicates the database that is currently active in the Pathway Studio application.

Opening the "Index of the Database Content" tab reveals a summary of the information contained in the current database, such as totals for entity and relationship types, numbers of groups, pathways and experiments as well as ontologies.

Folders View



The Folders View allows you to browse through the complete folder tree to find specific files. Pathways and Ontologies are provided in their respective folders. In the Experiments Projects folder you can create folders and subfolders as needed, and you now have the flexibility to save related items (group, pathway and experiment) in the same folder. This allows you easily organize your work.

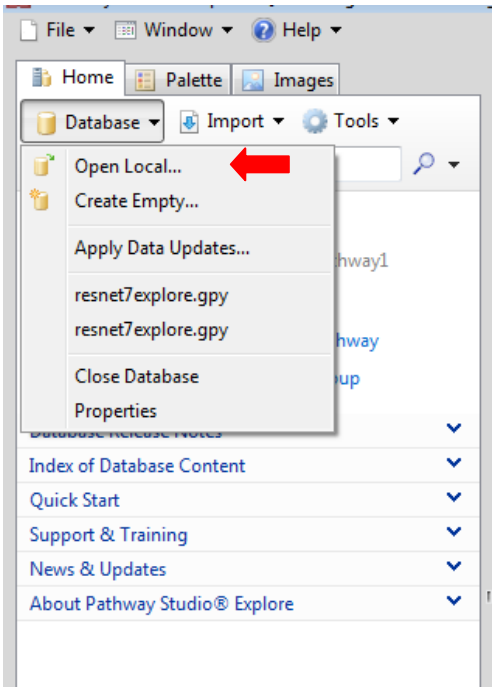
Note: When working with this database you have complete freedom to manipulate the folders. This includes the ability to (accidently) delete reference pathways and ontology groups. It is advisable to keep all your work in the Experiments and Projects folders.

● Exercise One: Open the local ResNet Explore Database

Begin Exercise:

Objective: To learn how open the Explore Database.

Let's begin using Pathway Studio by opening the local ResNet Explore Database. (To do this, the database must first be installed on your local computer.) Note: the first time you use Pathway Studio Explore, the Explore Database should automatically open.



1. In the Information Pane, go to Database > Open Local.
2. Select resnet7explore (gyp) to open the ResNet Explore database.
3. In the top of the Information Pane see "ResNet Explore 1.0 (Mammal)"
4. Select "Index of Database Content" to see a summary of the content in the local database: types and numbers of collections, experiments, entities and relationships.

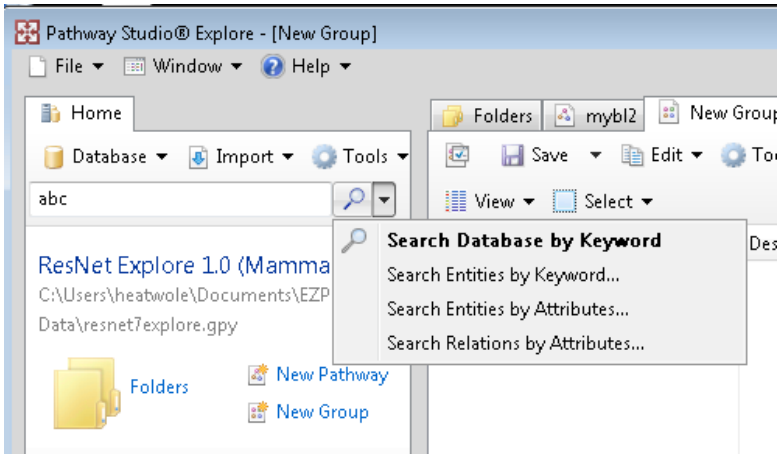
End Exercise: Open the local ResNet Explore Database

Section 3: Building Pathways

Pathway Studio Explore provides the tools to build pathways (networks) from the entities and relationships found in the ResNet Explore database. There are many ways to start to build pathways and only some examples will be shown here. You can start by searching the database to find an entity of interest and then query for relationships to that entity. Alternatively you can import a list of entities and query the relationships they have with each other and what other relationships in the database connect to them.

Search ResNet for an Entity

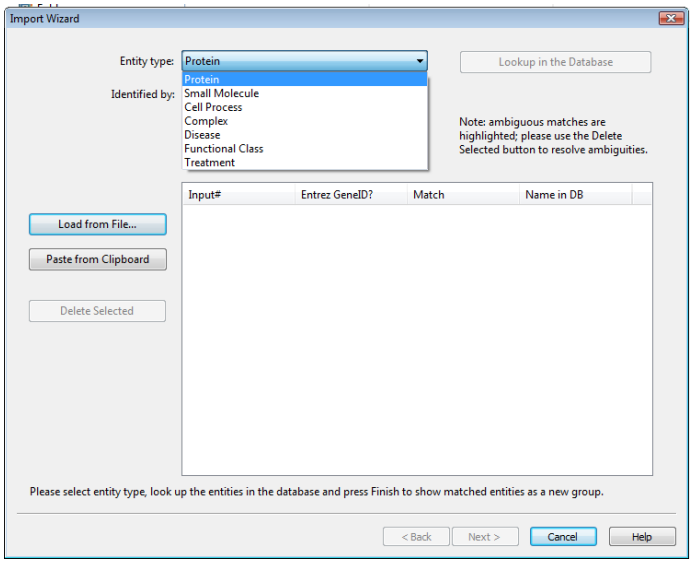
The Quick Search Box in the Information Pane allows for a keyword search of the entire database. Simply type the keyword in the box and select the magnifying glass to the right of the box. To define more specific searches, utilize the options in the drop-down menu to the right of the magnifying glass. Note: you can search for relationships as well as entities.



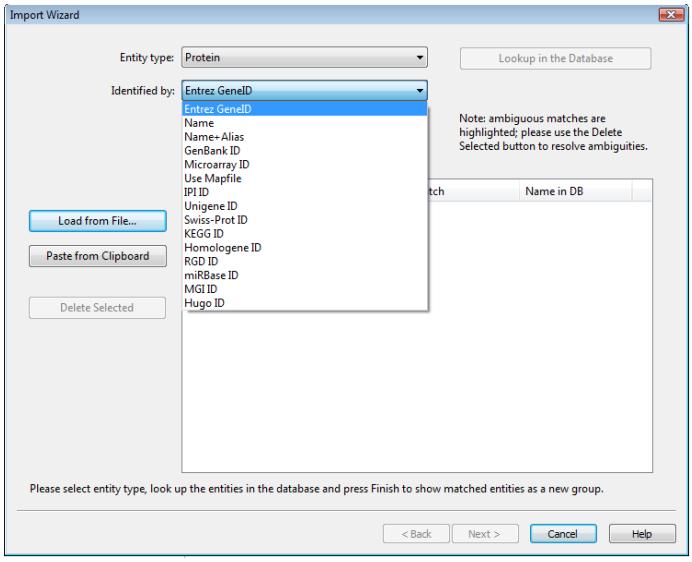
The results of the search will appear in the bottom list pane.

Import a list of Entities

You can import a list of entities by selecting the Import > Gene List. The Import Wizard appears

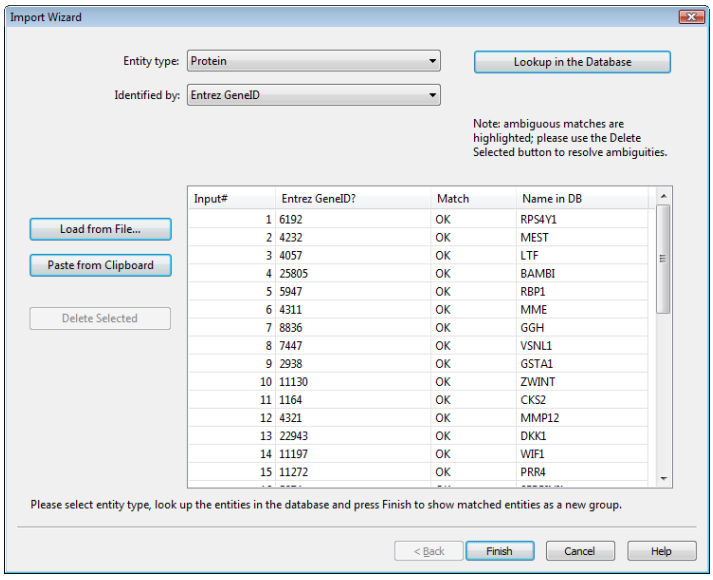


Note: Select this option for importing any entity list of IDs including: proteins/genes, small molecules, cell objects, cell processes, complexes, diseases, functional classes and treatments.



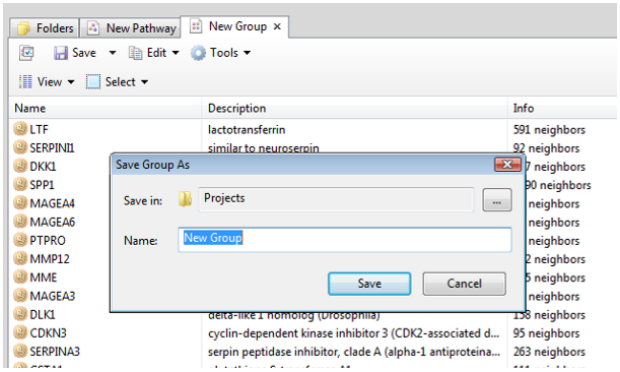
Once the type of entity to import is selected, a second menu is available to identify the specific ID types.

You can simply copy a list of IDs from an Excel spreadsheet or txt file to your clipboard, and then select Paste from Clipboard. The list will appear in the window. Select Lookup in the Database to find the entities.



Note: if the imported ID list produces some ambiguous mapping, you have the opportunity to manually select the desired mapping. Use "Delete Selected" to remove any unwanted mapping.

Choose to save your imported list as a group.



Once the entities that are the starting point in pathway building have been either identified by searching ResNet Explore or imported and saved as a Group, you are ready to start to build a pathway.

● Exercise Two: Search ResNet Explore for Entities and Relationships and Import a Protein List

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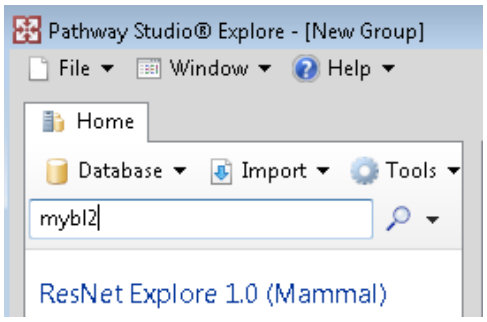
Begin Exercise:

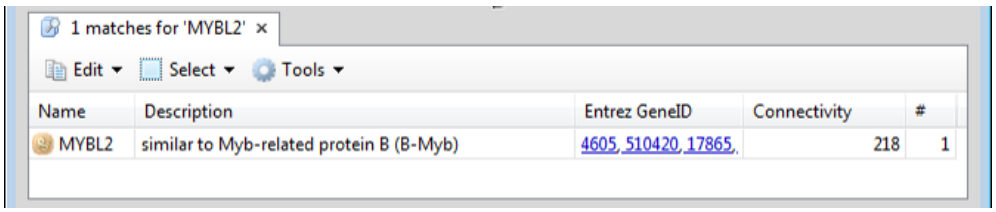
Objective: To learn how to find entities and relationships contained in the ResNet Explore database either by searching the database or by importing a list of entities.

Let's begin by searching the ResNet Explore database for a specific protein:

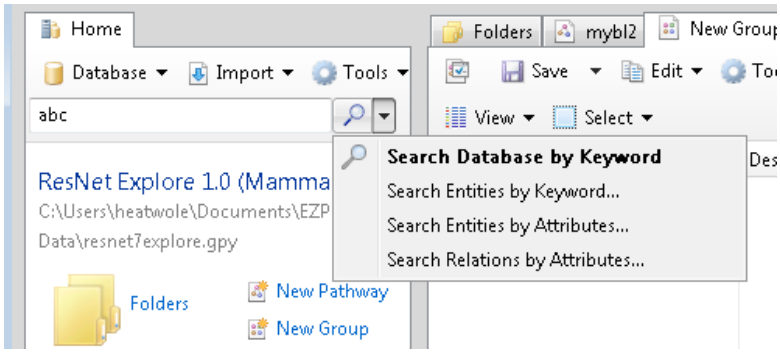
1. Select the Search Database by Keyword option in the Information Pane and type in the protein name: MYBL2



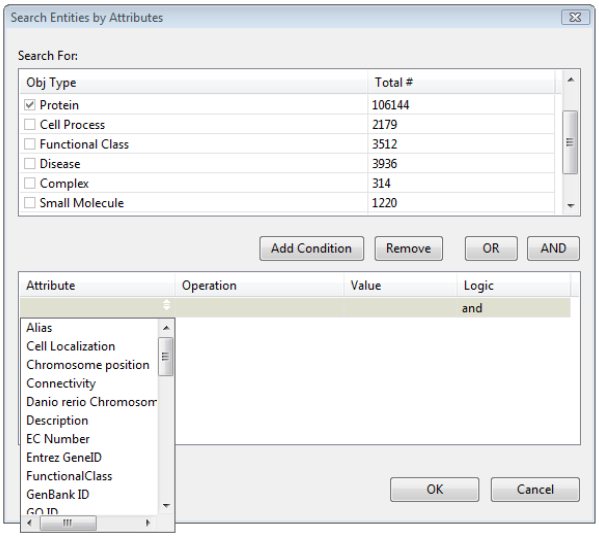
The results of your search are found in the List Pane at the bottom of the screen.

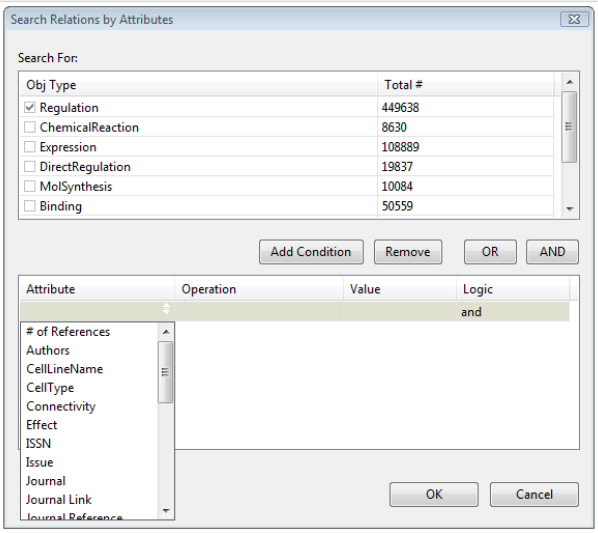


2. Examine the more specific search options found in the drop down menu just to the right of the Keyword search box:



a. Search Entities by Keyword – can specify specific entity types for your search
b. Search Entities by Attribute – use this option to search specific annotation fields for a selected entity type (left figure below)
c. Search Relations by Attribute – use this option to search specific annotation fields for a selected relationship type (right figure below)

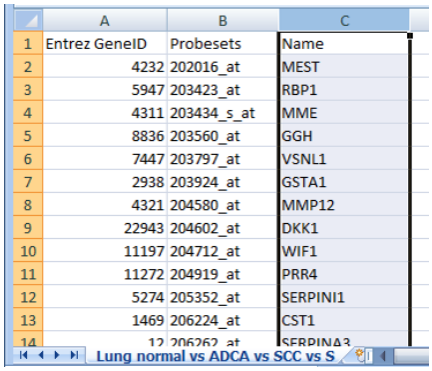




Leave the tab in the List Pane containing the search results for MYBL2 open for now.

Now let's import of list of entity IDs. Use the MS Excel file provided with this training manual.

3. Open the MS Excel file that accompanies this manual (SCLC genes) and copy all the column contents with the header "name" to the clipboard.



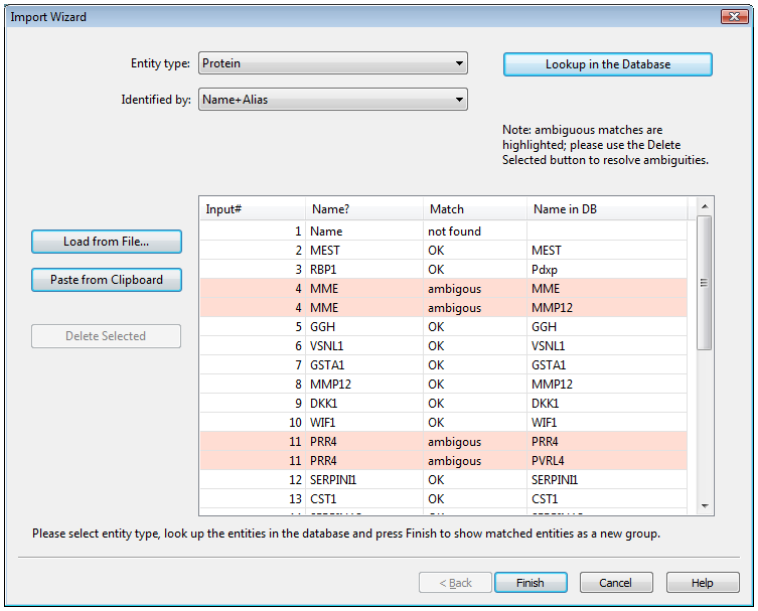
	A	B	C
1	Entrez GeneID	Probesets	Name
2	4232	202016_at	MEST
3	5947	203423_at	RBP1
4	4311	203434_s_at	MME
5	8836	203560_at	GGH
6	7447	203797_at	VSNL1
7	2938	203924_at	GSTA1
8	4321	204580_at	MMP12
9	22943	204602_at	DKK1
10	11197	204712_at	WIF1
11	11272	204919_at	PRR4
12	5274	205352_at	SERPINI1
13	1469	206224_at	CST1
14	12	206262_at	SERPINA3

Lung normal vs ADCA vs SCC vs S

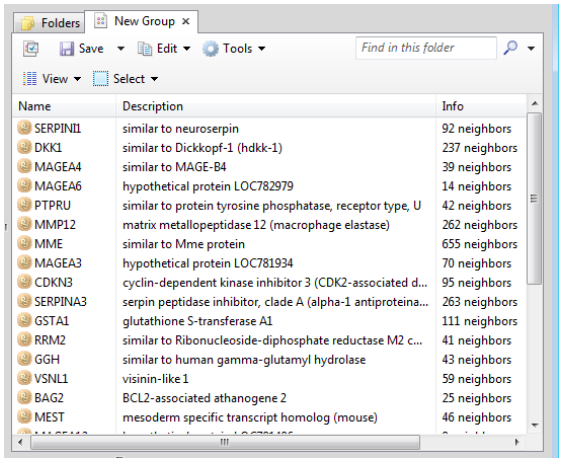
4. Go to Import > Gene List. The Import Wizard is displayed.

Note: Use Import > Gene List to import any list of entities: proteins (genes), small molecules, cell objects, cell processes, complexes, diseases, functional classes and treatments. The "Identified by" ID list will reflect the selected entity type.
Note: Recall that in Pathway Studio the gene and the product of the gene (protein) are merged into one entity.

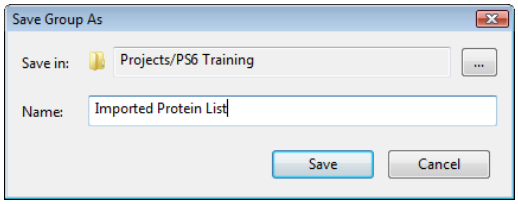
5. Select “Paste from Clipboard” to copy the list of protein names into the Wizard.
6. For “Entity type” select: Protein. For “Identified by” select: “Name+alias”.
7. Select “Lookup in the Database” to identify the imported entities in the ResNet database.
8. “Not found” in the Match column indicates the entity was not found in (note that “Name” from the column header in the MS Excel sheet is indicated as “not found”).



9. Items with ambiguous mapping are highlighted (Note: lower case and capital letters are recognized differently).
10. Remove undesired mapping by highlighting the rows and click “Delete Selected” button to remove.
11. When all undesired mapping is removed, select “Finish” to import the list. The imported list will appear in a New Group window.



12. Choose Save to save the group (provide a destination folder and name for the group – create a new folder if desired).



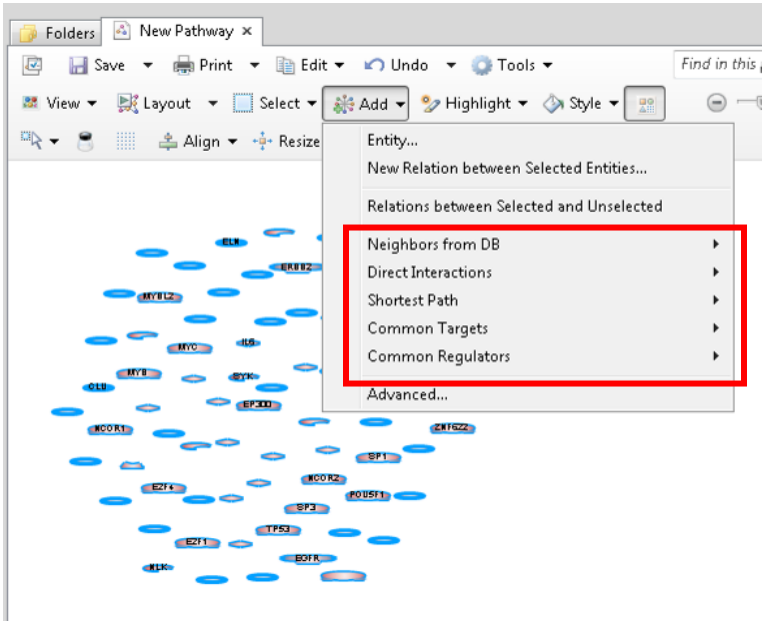
End Exercise: Search ResNet for Entities and Relationships and Import a Protein List

Build Pathway Tools in Pathway Studio

Pathway Studio provides both quick short cut menus for pathway building as well as an advanced menu that allows you to define more specific network options.

Build Pathway Tool – Quick Menus

With the entities in a new Pathway View, select the Add tool to see the short cut menu for building pathways.



Each of these short cut menu options has easy-to-interpret submenus. Select the appropriate option for the desired pathway.

Note: If an option is grayed out, it is not an appropriate option for the number of entities selected.

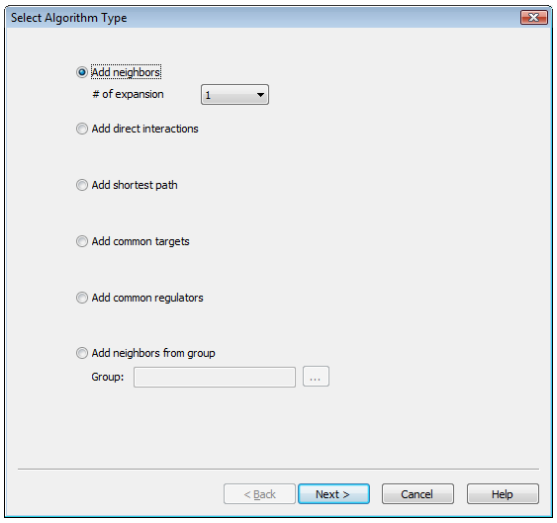
The Relationship types and Entity types included in the Shortcut menu options:

		Type(s) of Relationships	Type(s) of Entities
Neighbors from DB	Expression Targets	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Expression Regulators	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Physical Interactions	Direct Regulation, Binding	Proteins, Complexes, Functional Classes
	Protein Modification Targets	Prot Modification	Proteins, Complexes, Functional Classes
	Protein Modification Enzymes	Prot Modification	Proteins, Complexes, Functional Classes
	All	All	All
Direct Interactions	Physical Interactions	Direct Regulation, Binding	Proteins, Complexes, Functional Classes
	Expression Regulation	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Protein Modification	Prot Modification	Proteins, Complexes, Functional Classes
Shortest Path	Physical Interactions	Direct Regulation, Binding	Proteins, Complexes, Functional Classes
	Expression Regulation	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Protein Modification	Prot Modification	Proteins, Complexes, Functional Classes
Common Targets	Expression Targets	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Physical Interactions	Direct Regulation, Binding	Proteins, Complexes, Functional Classes
	Protein Modification Targets	Prot Modification	Proteins, Complexes, Functional Classes
	All	All	All
Common Regulators	Expression Regulators	Expression, PromoterBinding	Proteins, Complexes, Functional Classes
	Physical Interactions	Direct Regulation, Binding	Proteins, Complexes, Functional Classes
	Protein Modification Enzymes	Prot Modification	Proteins, Complexes, Functional Classes

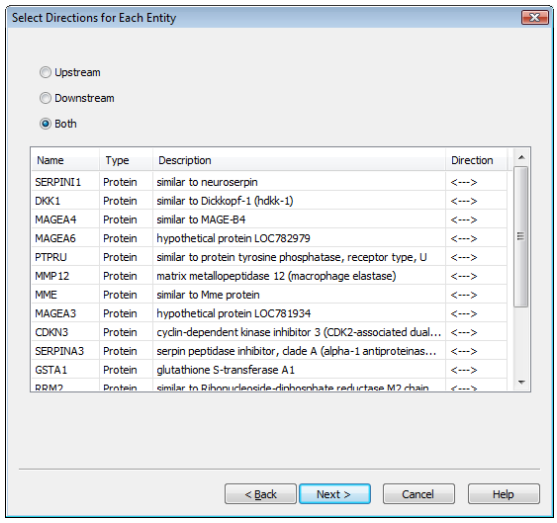
Build Pathway Tool – Advanced Menu Desktop

The Advanced Menu option (Add > Advanced) opens a wizard that provides you to options for more specifically defining the pathway building filter options. After selecting the entities to including in your build pathway algorithm, the four wizard windows allow you to specify: a.) algorithm type for pathway building, b.) select the directionality of the resultant relationships, c.) the entities to be included in the pathway and d.) the relationships to be included in the new pathway.

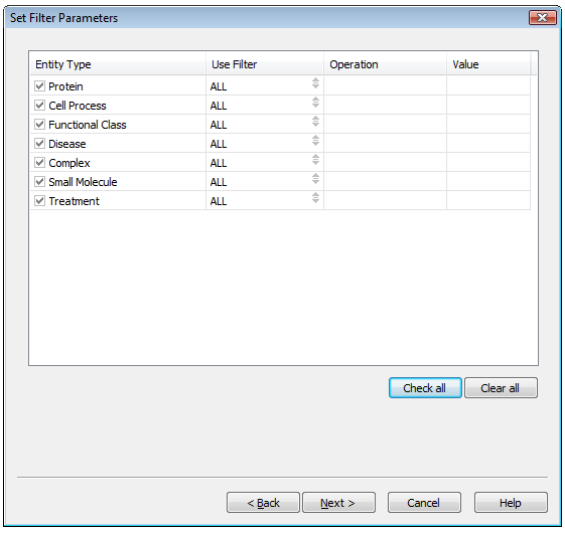
a.



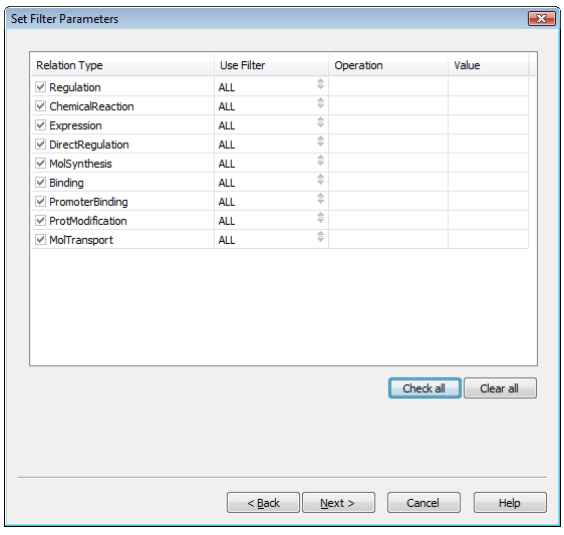
b.



c.

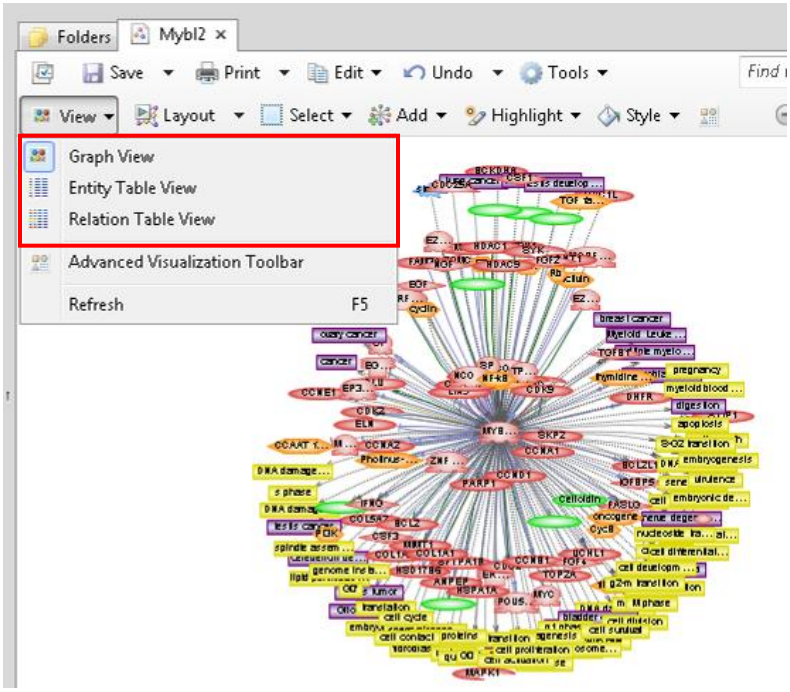


d.



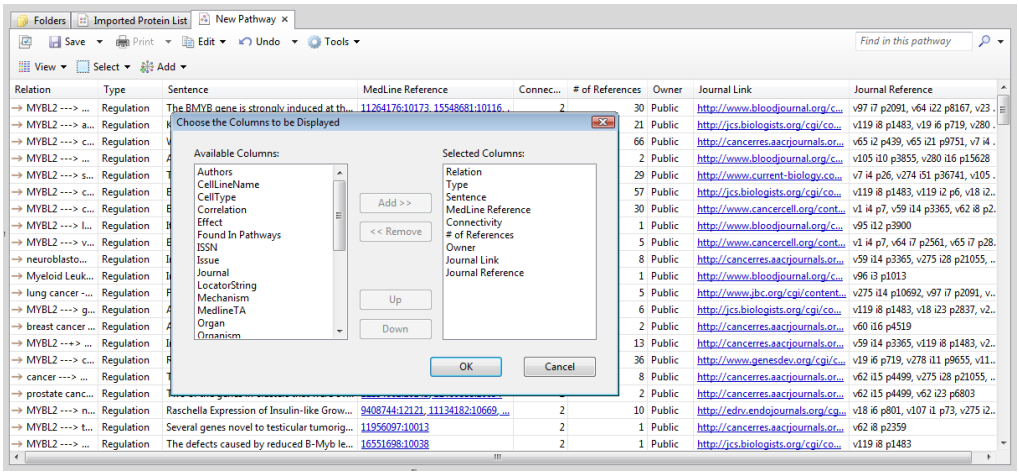
Graph View/ Entity Table view / Relation Table view

There are three options for viewing resultant pathways: Graph View, Entity Table View and Relation Table View.



Customizing tables, filtering by significance

The Entity Table view and Relation Table view provide customizable tables of summary information of the pathway. Any information deleted from either of these tables is also deleted from the Graph View. Customize the columns of information by selecting Tools > Customize Columns.

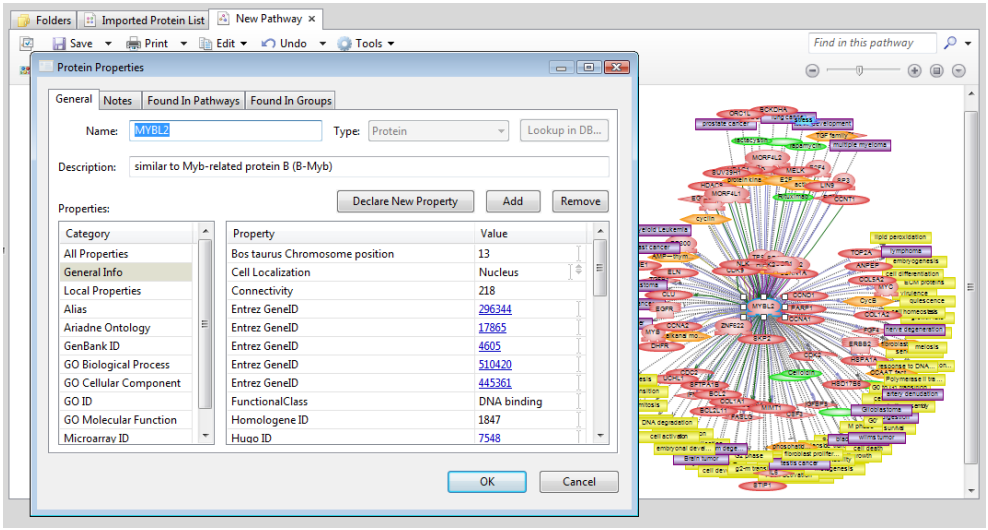


Note: In the Relationship View table you can see the number of references in the database that support an individual relationship. High numbers of references can be used as a measure of confidence for a relationship. Lower numbers of references can indicate newly identified relationships or potential false positives from MedScan. The reference sentence is available for you to examine in order for you to determine the accuracy of the interpretation by MedScan. Undesired (false positive) relationships can be manually deleted from the database. See **Appendix B**: Deleting Entities and Relations from a Local Database.

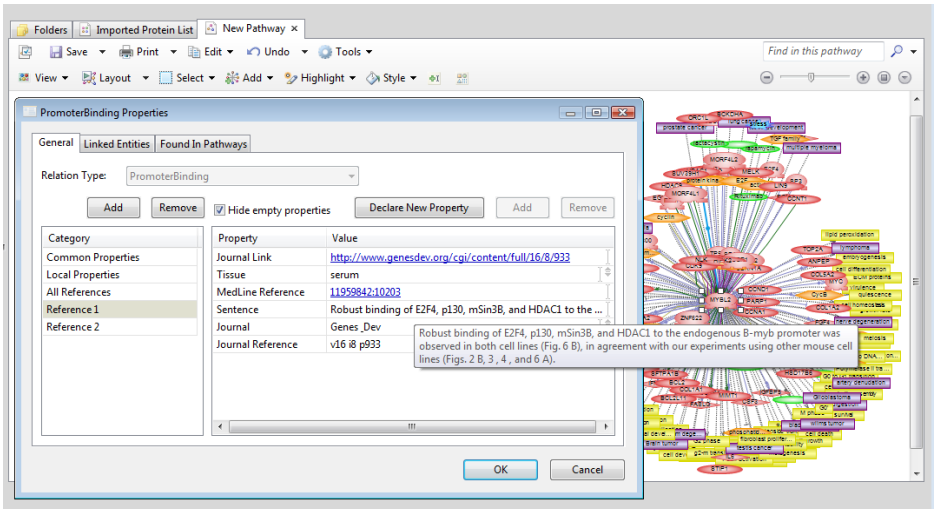
Viewing Details about Entities/Relationships

Detailed information about entities and relationships displayed in the Graph View can be seen by a.) mouse over the object or b.) double click to open the properties dialog.

Here is an example of attribute information for a selected entity, viewed in the Properties dialog:



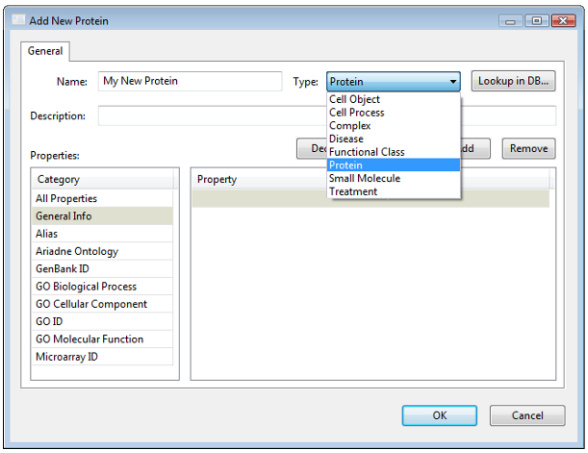
The properties dialog for the relationship provides reference information that supports the relationship. You can view the sentences identified by MedScan that support the relationship.



Creating new Entities and Relationships

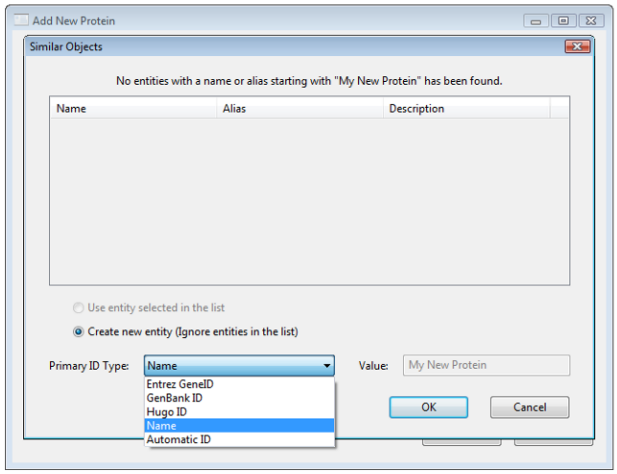
You can create a new entity or a new relationship between entities by using the Add > Entity or Add > New Relation between selected entities options in the New Pathway window.

To add a new Entity, provide appropriate information in the Add New Protein dialog:



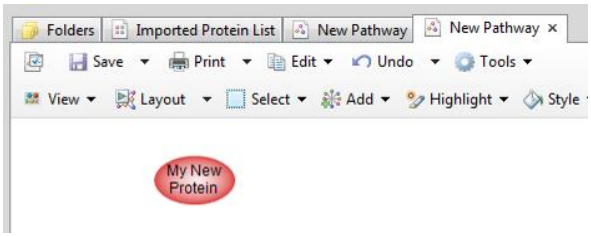
Note: When you attempt to add a new entity to ResNet, Pathway Studio will first check to see if an entity with the given name already exists in the database. It is advisable not to create a new entity with the same name or identifier as an existing entity.

Use the Add New Protein dialog for all entities you choose to add to ResNet, not just protein entities.



If your identifier is unique, complete the dialog by providing the type of primary identifier (in the example shown "name") and select OK. This will create this new entity in ResNet.

Newly created entity:



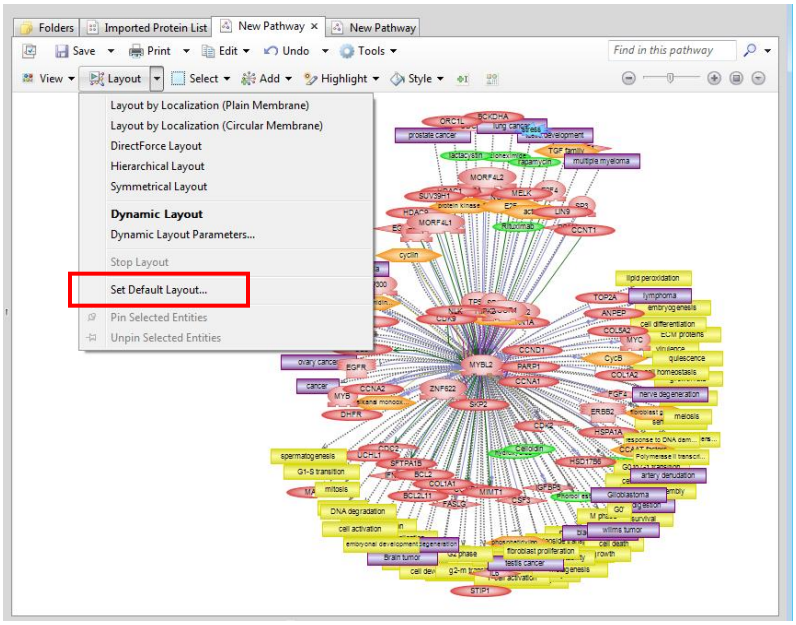
You can permanently delete an entity or relationship from ResNet by selecting it in a Graph View, then when the mouse is in the white space of the graph, right-click and select "Delete Selected Entities/Relations from the Database." Note: if you do not find this option available in the menu it can be enabled by going to the Information Pane, selecting Tools > Program Options > Menu > Enable Advanced Menu for Pathways > Yes.

Customization of Pathway Layouts

There are multiple layout options for displaying a pathway. (Please see the User's Manual for a description of how each layout is calculated.)

The Layout by Localization options utilizes Gene Ontology cellular localization assignments when placing the entity with respect to cellular objects. Be aware that entities can have more than one legitimate localization assignment within Gene Ontology (an example would be a protein that shuttles between the nucleus and cytoplasm). In this case the layout displays only one of the localization assignments. You can change the assigned localization of an entity by selecting the entity, right-click, choose "Localization in Pathway" and choose the desired cellular localization.

You can change the default layout view by choosing Layout > Set Default Layout.


Folders
Imported Protein List
New Pathway
New Pathway
Save
Print
Edit
Undo
Tools
Find in this pathway
View
Layout
Select
Add
Highlight
Style
Layout by Localization (Plain Membrane)
Layout by Localization (Circular Membrane)
DirectForce Layout
Hierarchical Layout
Symmetrical Layout
Dynamic Layout
Dynamic Layout Parameters...
Stop Layout
Set Default Layout...
Pin Selected Entities
Unpin Selected Entities

● Exercise Three: Building Pathways

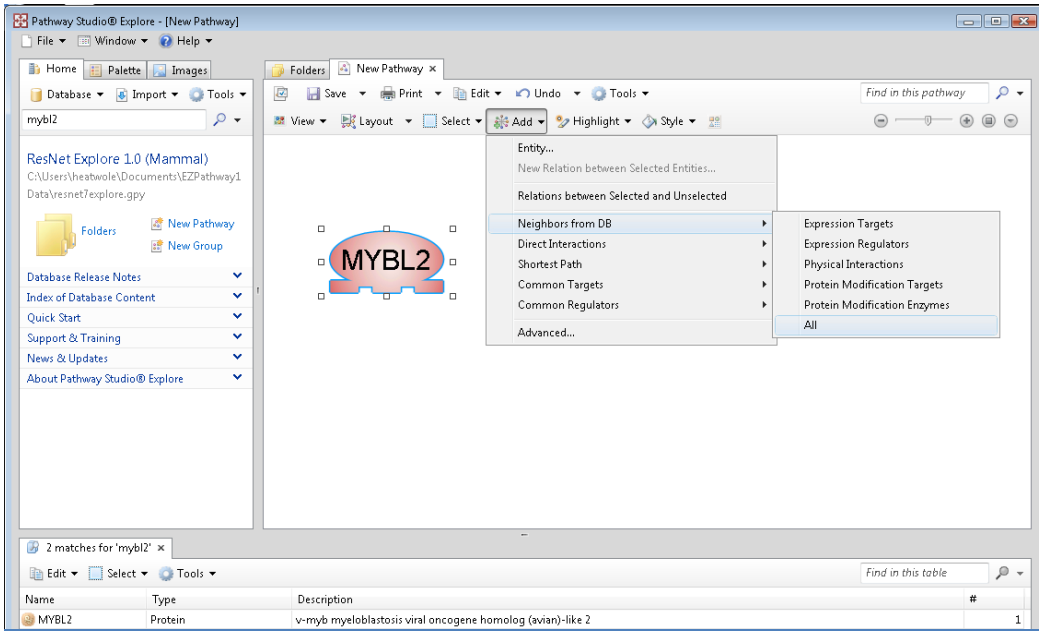
Begin Exercise:

Objective: To build pathways using both the Quick Menu and the Advanced Menu option and to become familiar with basic functionality used for viewing and modifying pathways.

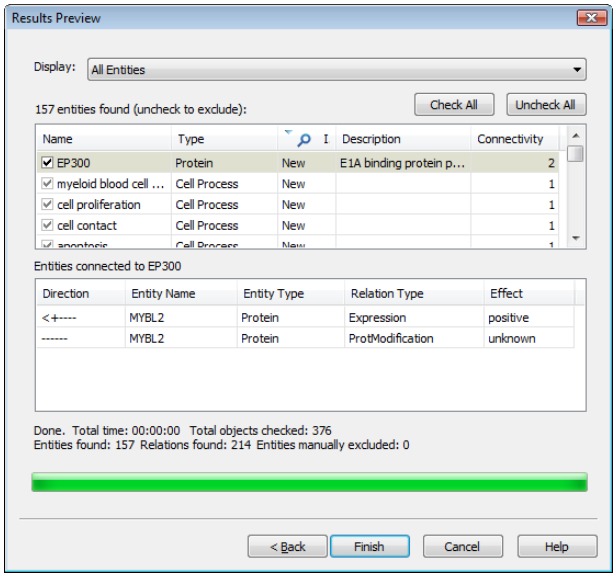
Build Pathway Tool – Quick Menu

Let's build a simple pathway displaying all connections in the ResNet Explore database to our protein MYBL2.

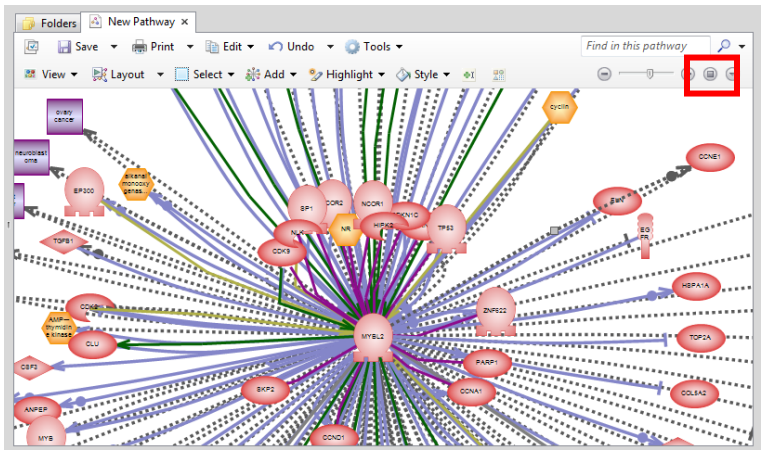
1. Select "New Pathway" from the Information Pane. A new pathway view will open to the right.
2. Click and drag the MYBL2 protein icon from the List Pane below and drag it to the new pathway window.
3. With the MYBL2 protein entity selected (highlighted in blue) go to the Add menu and select > Neighbors from DB > All. This will identify all entities connected to MYBL2 in the database.



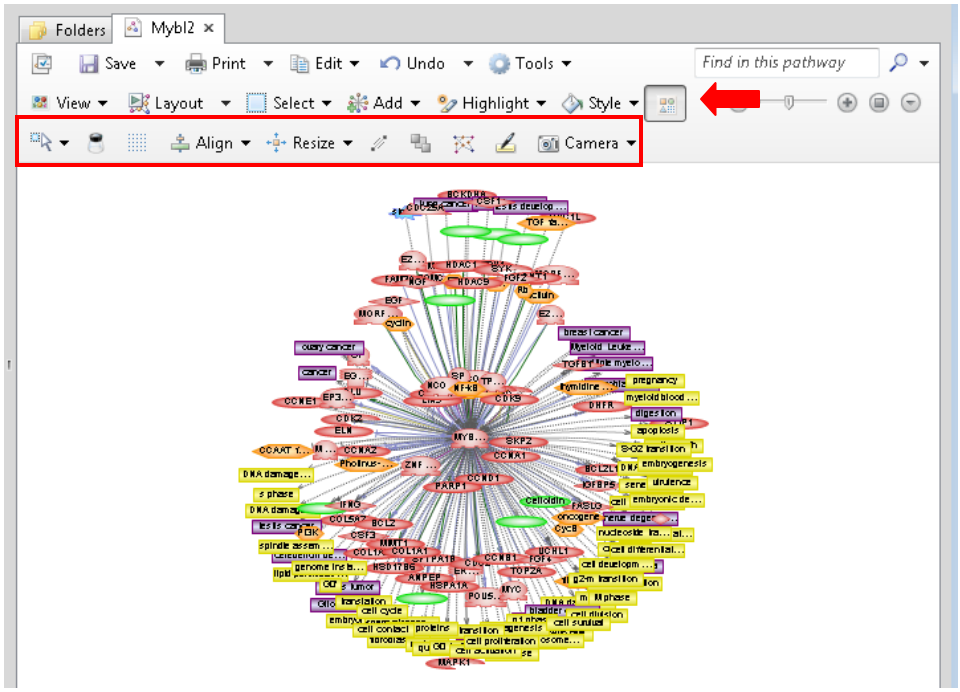
4. The Results Preview window displays a summary of information about the resultant pathway. You can manually review the results here and manually delete any undesired entities or relationships at this time.



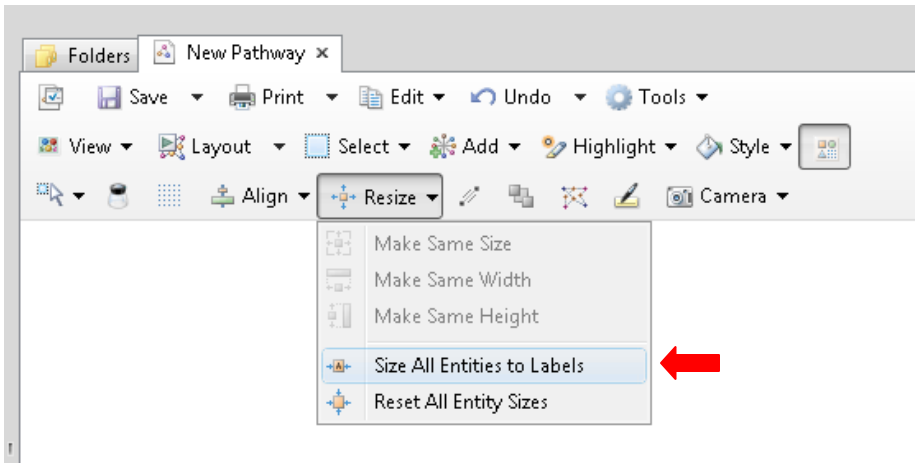
5. To visualize the resultant pathway, select “Finish”.
6. Select the “Fit All Entities to Window” button (found in upper right) to view the entire pathway in the window.



7. The Advanced Visualization Tool bar provides many functions to optimize your pathway view. Select the icon to display the bottom tool bar row.



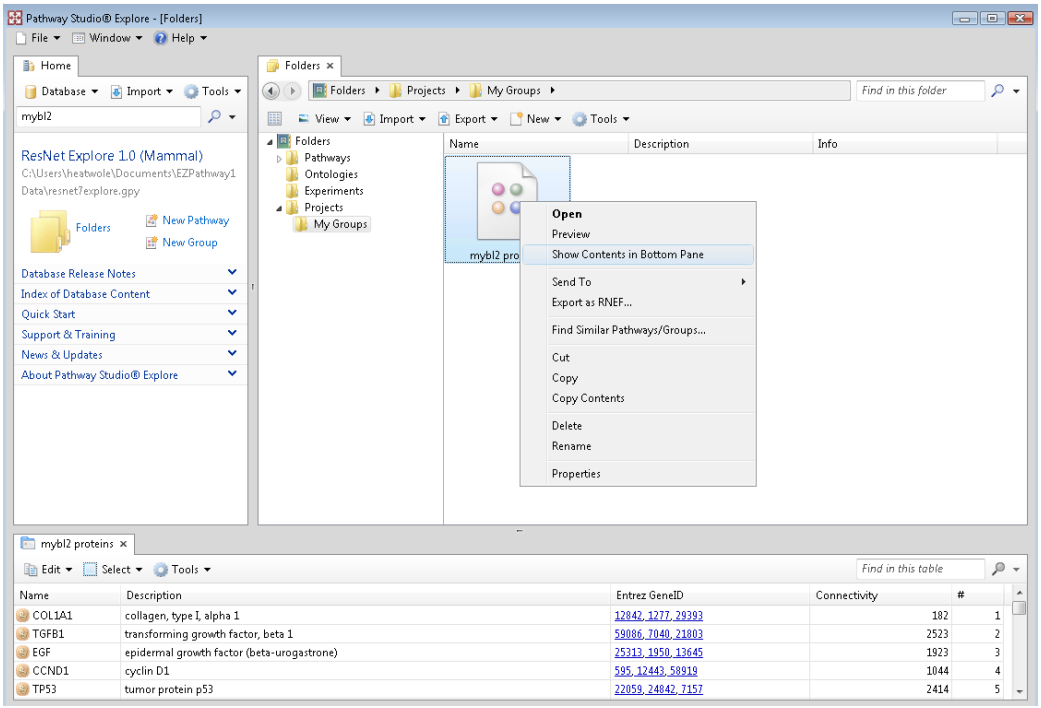
8. Select: Resize > Size All Entities to Labels to better visualize the names of the entities.



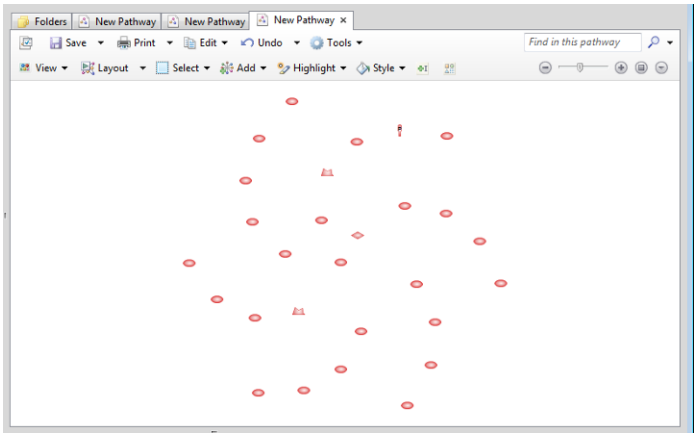
Build Pathway Tool – Advanced Menu

Now let's use a different Build Pathway algorithm to build a pathway from the list of imported proteins.

1. Right-click the group icon from the saved list of imported proteins. Select "Show contents in Bottom Pane." This will open the group list in the List Pane.



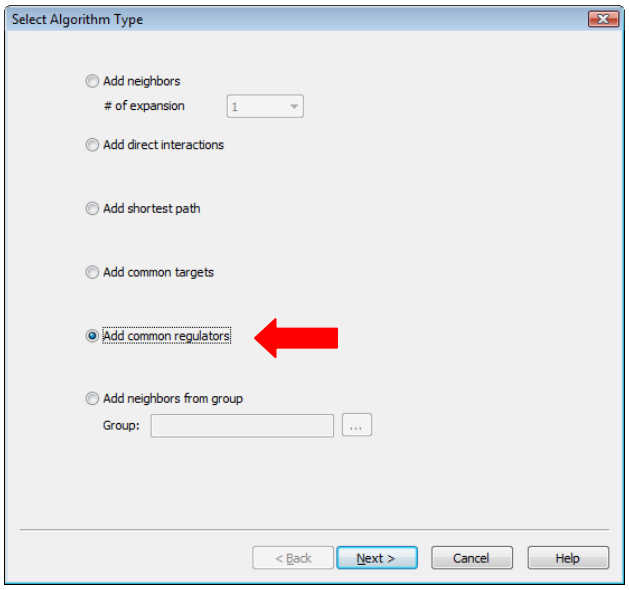
2. Select “New Pathway” the Information Pane and select all the entities in the List Pane. Click and drag the entire list to the new pathway window. Select “Fit all Entities to Window” in the upper right to visualize all the entity icons.

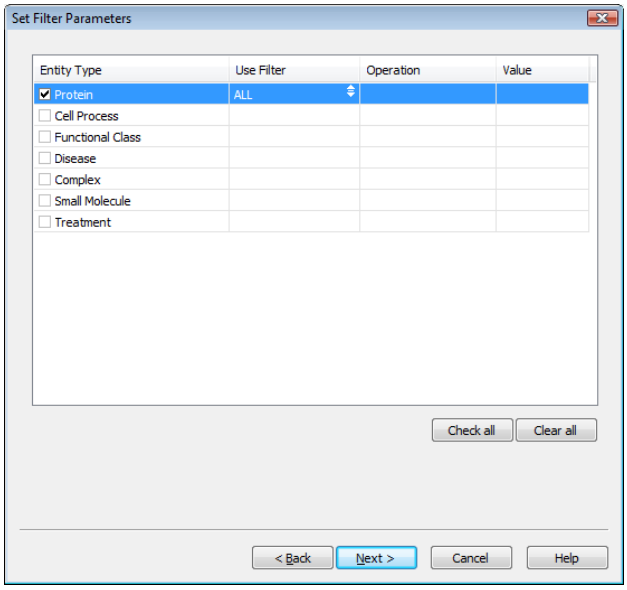


3. Choose Select > All to select all entities. Then choose Advanced from the Add menu. The Advanced Build Pathway wizard will appear.

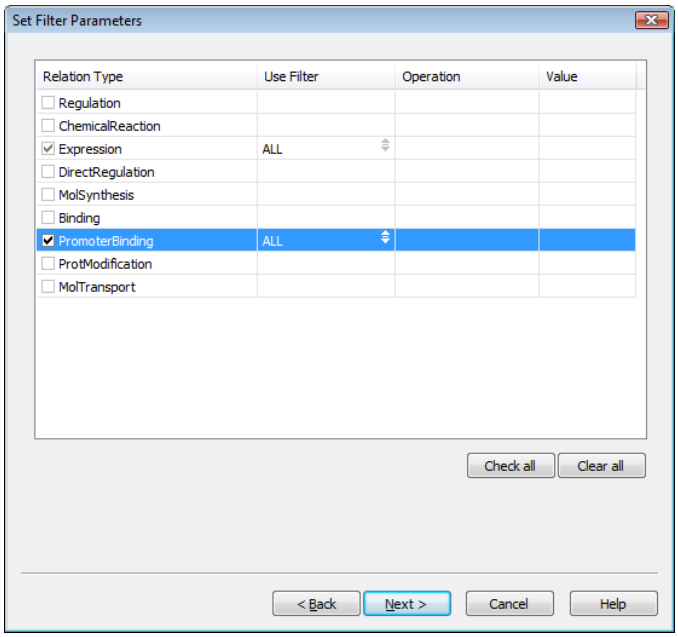
Let’s find common transcriptional regulators shared by at least two members of our group.

4. In the Select Algorithm window, select “Add common regulators”. This will identify upstream regulators that shared by two or more members of the group. Recall that some relations have directionality, which allows us to define direction of influence.
5. Next select the type of entity or entities for the regulators. In this example, select proteins.

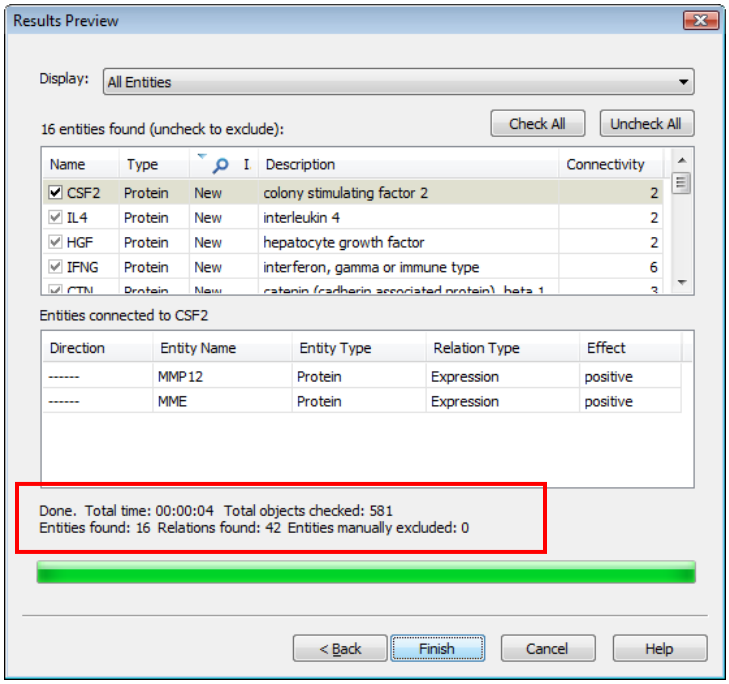




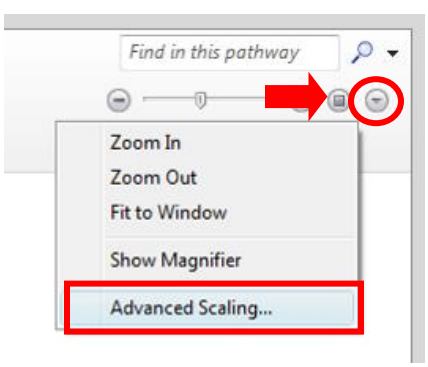
6. Finally, select the types of relation the transcriptional regulators will have with the targets. In this example, we will select Expression and PromoterBinding. Although regulation could also be utilized, the category of Regulation contains by far the largest number of relationships and is less specific, so we will leave it out for now.

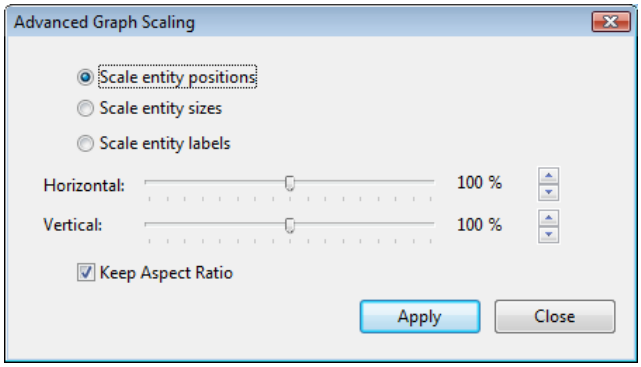


7. When the algorithm is finished, select Finish to view the pathway. Note: at the bottom of the Results Preview window there is a summary of the resultant pathway. In the example below, 16 shared potential transcriptional regulators were identified and 42 relations between those regulators and the starting group of entities will be added to the pathway view.

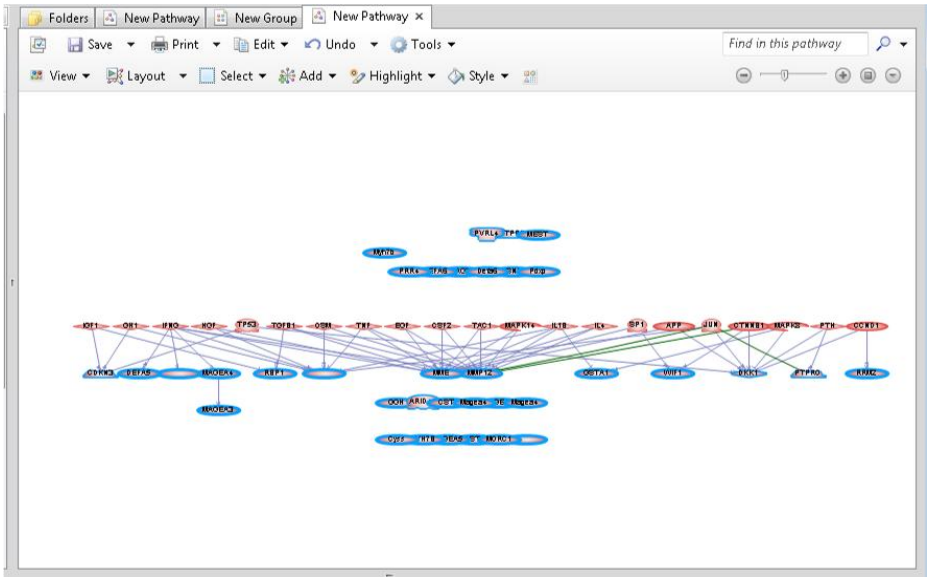


8. Use the “Fit All Entities to Window” button to maximize the pathway view.
9. Select “Resize > Fit All Entities to Labels” in the Advanced Visualization tool bar.
10. As this pathway depicts upstream transcriptional regulators, select “Hierarchical Layout” from the layout menu. This will display the transcriptional regulators above the targets they regulate.
11. If needed, adjust the x and y-axis scale of the image by selecting Advanced Scaling from the menu in the far upper right. (Uncheck “Keep Aspect Ratio” before changing horizontal and vertical proportions.)





12. The new pathway contains the original group list and new transcriptional regulators. Let’s select the members of the original group. From the List Pane, choose Select > All, then Select > Mirror Selection to active pathway. The original members will be selected (blue line around selected entities). Alternatively you can Select > All, Edit > copy the list, then in the pathway view Select > Entities on Clipboard.

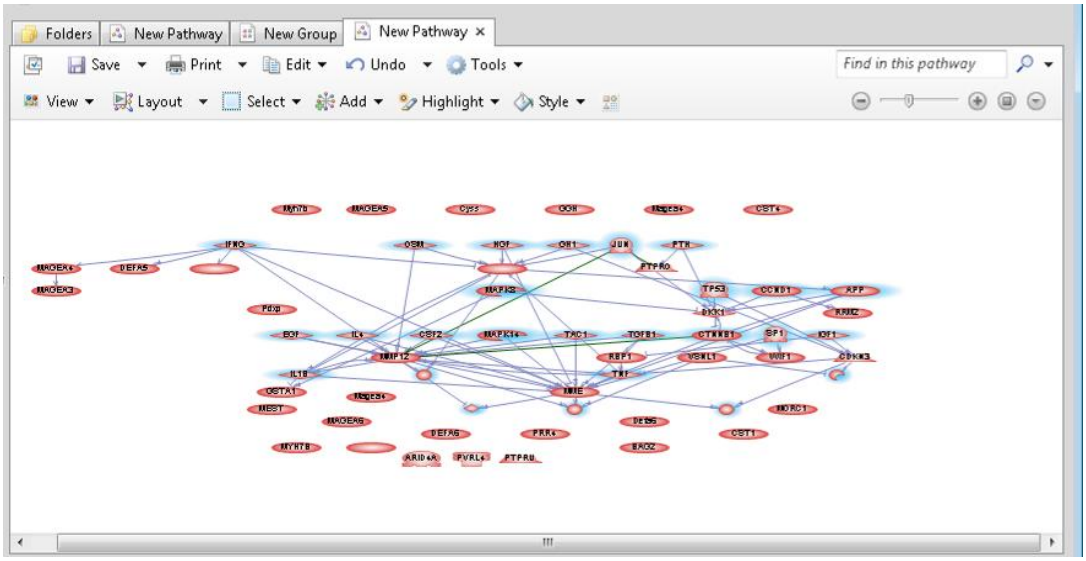


Now let's find shared transcriptional targets for our original list of proteins.

13. With the original group members selected, go to the Add menu and select Advanced.
14. Select algorithm type > Add common targets. This will find targets shared by two or more from our selected list.
15. Select Entity type Proteins and Relation type Expression and PromoterBinding.
16. Select Finish to view the pathway. Adjust the visualization by first selecting Layout > Hierarchical Layout, fit the pathway to the window.
17. Select the members of the original group by Select > All and then Select > Mirror Selection to Active Pathway

Let's add highlighting (a color halo) to the upstream regulators and the downstream targets.

18. With the original list selected, go to Select > Invert Selection. Now the added upstream regulators and downstream targets are selected. (Note: relations will also be selected which is ok as highlight won't apply to relationships.)
19. Choose Highlight > blue to highlight the upstream regulators and downstream targets. None of the members of your original list will be highlighted.
20. Save the pathway with the name "Upstream Regulators and Downstream Targets."



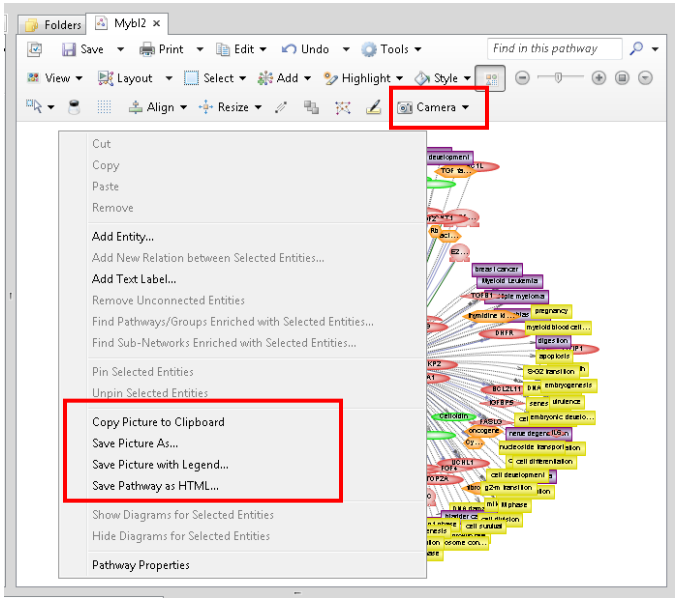
End Exercise: Building Pathways

Saving Pathway Images

Right-click on the pathway to access the four options for saving an image:

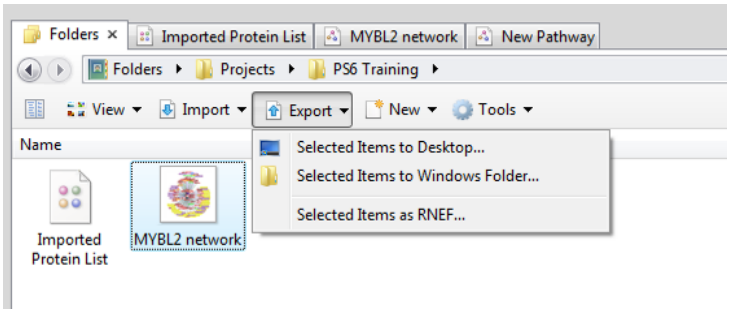
a.) Copy Picture to clipboard (available to paste into many programs),
b.) Save Picture As (saves the pathway image as a *.gif – default or *.jpeg, *.png, *.tif, and *.bmp),
c.) Save Picture with Legend (save an image of a pathway with a legend included. There are also options to scale the image size –height/width and a choice of image resolutions from screen resolution up to 1200 dpi).
d.) Save Pathway as HTML (export and save the pathway to an HTML file which enables the visualization of a pathway outside of Pathway Studio such as Internet Explorer. The HTML figure has hyperlinks for entities.)

Options for saving a pathway image are also found in the Advanced Visualization Tool bar menu when selecting the camera icon.

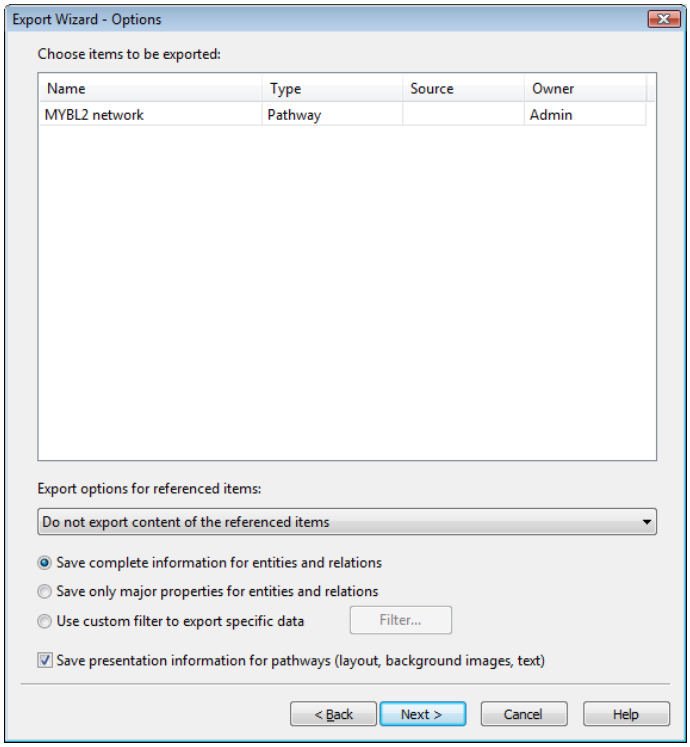


Exporting Pathways

From the folder view select the pathway file for export and select Export. A menu box appears with options for destinations to export the file.



The third option, “Selected Items as RNEF” opens an export wizard that allows for more flexibility in selecting data about the pathway for export.



Section 4: Importing Experimental Data

Experimental data will be directly imported into Pathway Studio from Partek® after an initial analysis of the differentially expressed genes has been performed. See instructions provided by Partek for explanations of the data generated and exported into Pathway Studio Explore.

For the purpose of this Training Manual, two files of microarray data, GDS2126.gepr and GDS2126.txt have been provided. The following exercise demonstrates how to import this file so that the experiment data is available for use in the remaining training exercises. This experiment contains gene expression data from synovial tissues from patients with rheumatoid arthritis and osteoarthritis and well as normal controls.

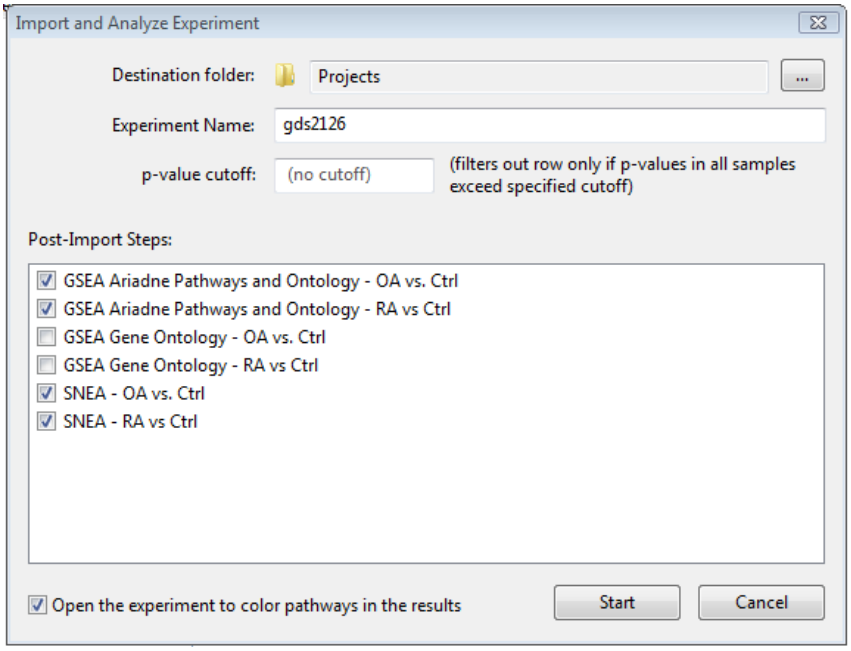
● Exercise Four: Import data

Note: This example is not your standard workflow for moving experimental data into Pathway Studio Explore. This example is solely to generate an experiment data file for further training examples to be used here. When you use Pathway Studio Explore for your research needs, your data will be imported directly from Partek.

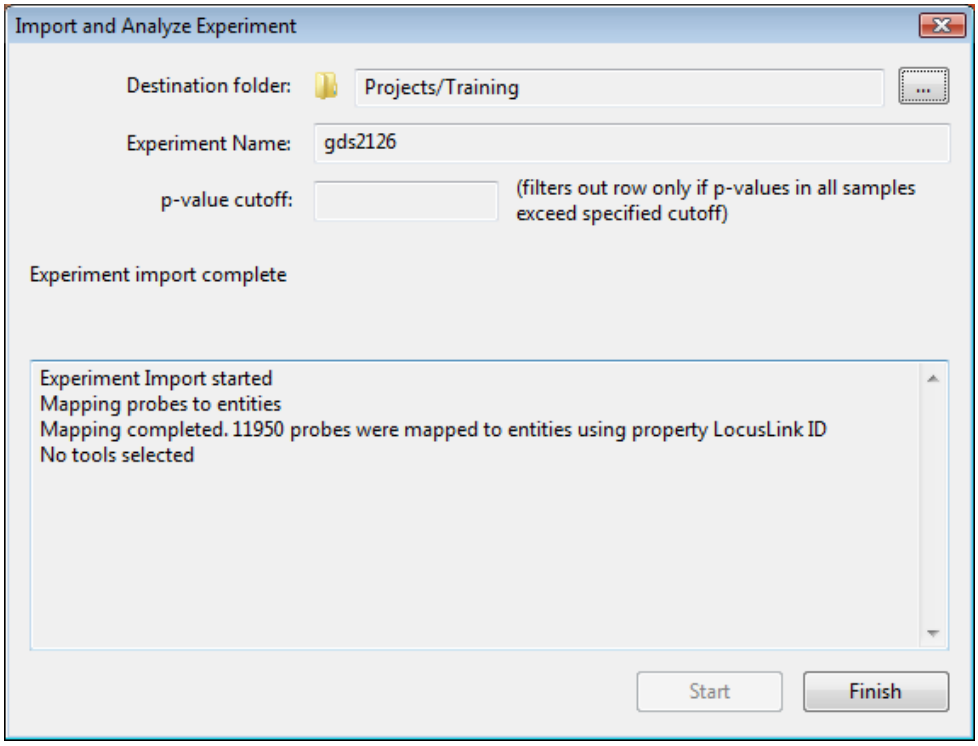
Begin Exercise: Import data (non standard workflow)

Objective: import a file of microarray data into Pathway Studio Explore.

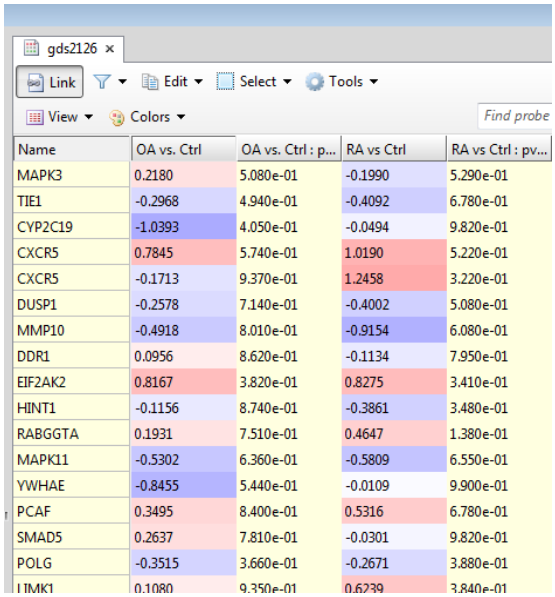
1. Place the files, GDS2126.gepr and GDS2126.txt on your computer desktop.
2. With Pathway Studio Explore open, double click on the GDS2126.gepr file. The following dialog will appear:



3. Select the desired destination folder where the experiment will be imported.
4. Some experimental analysis options have been selected by default (Gene Set Enrichment Analysis and Sub-Network Enrichment Analysis). For the purpose of this training session, uncheck all the analysis. We will introduce and explain these tools below prior to running these analyses. When the import has completed, select “Finish”



The Experiment Table will open automatically.



gds2126 ×

Link | Edit | Select | Tools | View | Colors | Find probe

Name	OA vs. Ctrl	OA vs. Ctrl : p...	RA vs Ctrl	RA vs Ctrl : pv...
MAPK3	0.2180	5.080e-01	-0.1990	5.290e-01
TIE1	-0.2968	4.940e-01	-0.4092	6.780e-01
CYP2C19	-1.0393	4.050e-01	-0.0494	9.820e-01
CXCR5	0.7845	5.740e-01	1.0190	5.220e-01
CXCR5	-0.1713	9.370e-01	1.2458	3.220e-01
DUSP1	-0.2578	7.140e-01	-0.4002	5.080e-01
MMP10	-0.4918	8.010e-01	-0.9154	6.080e-01
DDR1	0.0956	8.620e-01	-0.1134	7.950e-01
EIF2AK2	0.8167	3.820e-01	0.8275	3.410e-01
HINT1	-0.1156	8.740e-01	-0.3861	3.480e-01
RABGGTA	0.1931	7.510e-01	0.4647	1.380e-01
MAPK11	-0.5302	6.360e-01	-0.5809	6.550e-01
YWHAE	-0.8455	5.440e-01	-0.0109	9.900e-01
PCAF	0.3495	8.400e-01	0.5316	6.780e-01
SMAD5	0.2637	7.810e-01	-0.0301	9.820e-01
POLG	-0.3515	3.660e-01	-0.2671	3.880e-01
LIMK1	0.1080	9.350e-01	0.6239	3.840e-01

End Exercise: Import data

Section 5: Experimental data analysis tools

The experimental data analysis tools in Pathway Studio provide two different enrichment analysis algorithms, the Fisher's Exact Test and Gene Set Enrichment Analysis. Identification of enrichment of defined gene sets (pathways and groups) as well as user-defined sub-network identified in the ResNet database are both possible.

gene sets algorithms	**Known Gene Sets** (ontologies, curated pathways)	**Sub-Networks** (user defined from ResNet)
Fisher's Exact Test (experimental values not utilized)	**Find Pathways/Groups Enriched with Selected Entities**	**Find Sub-Networks Enriched with Selected Entities**
Gene Set Enrichment Analysis (GSEA) (experimental values are considered in the analysis)	**Gene Set Enrichment Analysis**	**Sub-Network Enrichment Analysis**

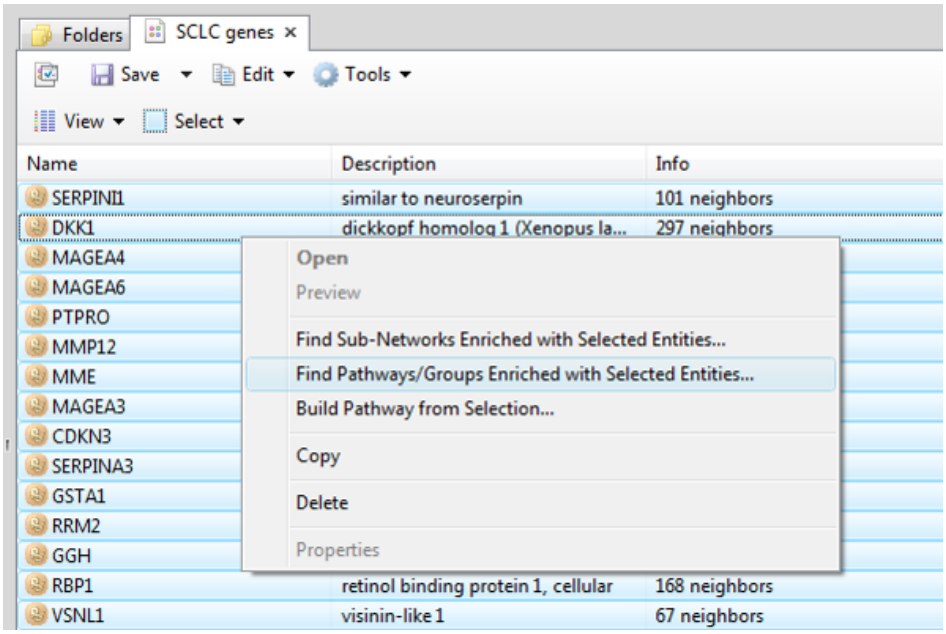
Names of the tools as they appear in Pathway Studio

Fisher's Exact Test

Fisher's Exact test is a statistical test used to determine if there are nonrandom associations between two categorical variables. You can use the Fisher's Exact test to see if there are gene groups (such as ontology groups) or pathways that are statistically enriched in your list of genes. In addition you can identify if sub-networks are enriched.
For more information about the Fisher's Exact test see: http://mathworld.wolfram.com/FishersExactTest.html

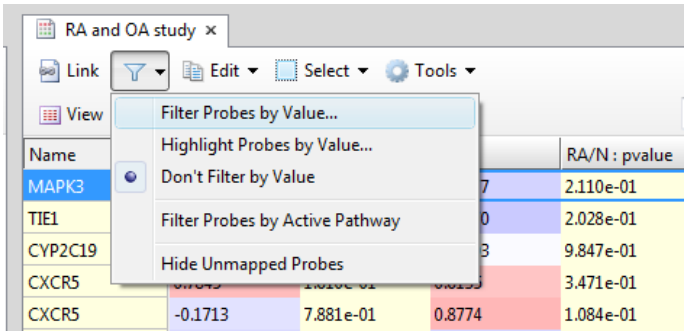
In gene expression analysis, the Fisher's Exact test is typically run on the list of genes that have been determined (ex. by fold change / p-value) to be statistically significantly differently expressed between experimental conditions. **The experimentally derived values are not utilized in the Fisher's Exact test when calculating enrichment in the gene list.**

You can launch the Fisher's Exact test from the Group view by selecting the proteins in the group, then right-click and select "Find Pathways/Groups Enriched with Selected Entities".

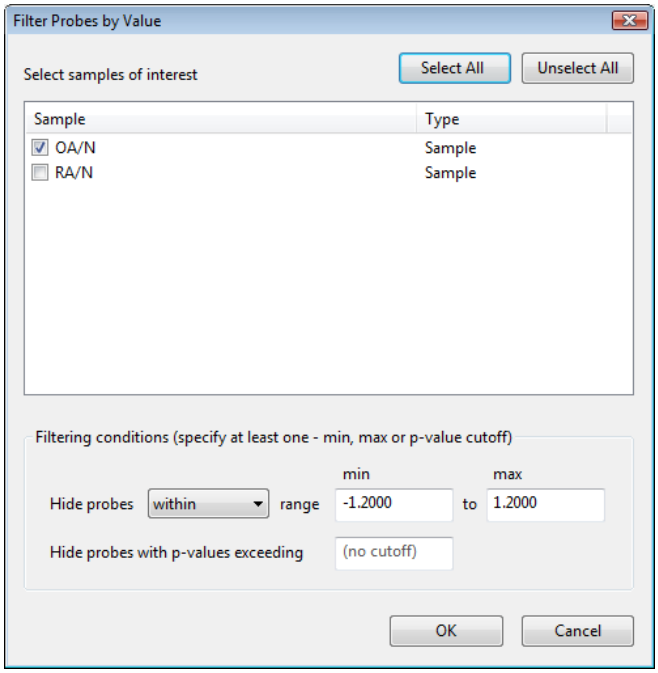


You can also launch the Fisher's Exact Test from the Experiment Pane. First filter the view of your results table by fold change / p-value to identify the list of statistically significant genes.

Filter a table by selecting the filter icon and choosing: "Filter Probes by Value"



You can define two filters, in this example fold change range and p-value range.



Note: Applying this filter does not remove the rows that are filtered out. The rows containing values that do not meet the filter criteria are displayed in gray.

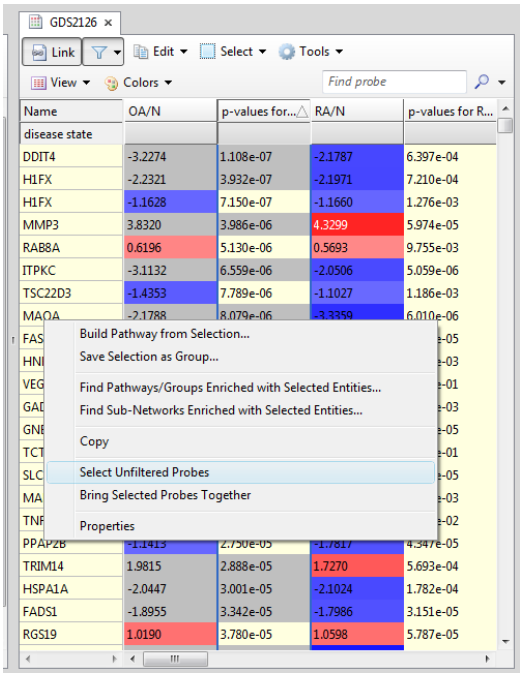
To run an analysis on only the probes that did meet the filter criteria, you must first choose "Select Unfiltered Probes."

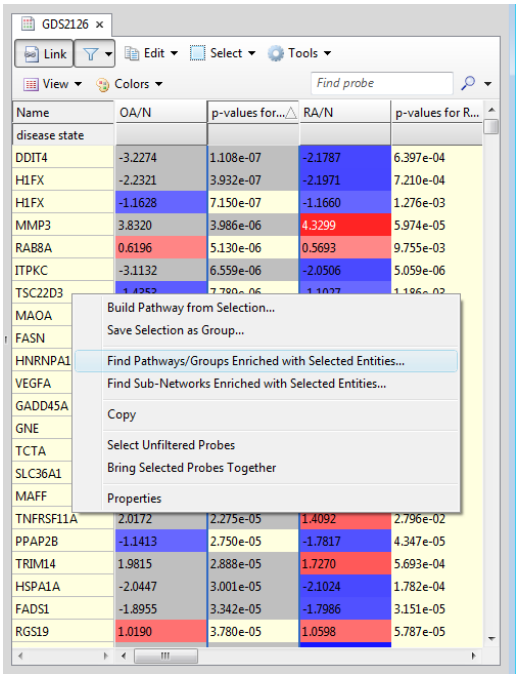
You can apply this filter to one or more than one dataset. In addition, you can choose Highlight Probes by value to highlight the probes in the list that meet the filter criteria.

Name	OA/N	OA/N : pvalue
CD19	0.1164	7.711e-01
CCR7	0.1429	7.293e-01
THPO	-0.5120	6.201e-01
GSTT2	0.6160	2.957e-01
RABEPK	-0.1451	4.270e-01
IRAK1	-0.3257	1.490e-01
APBB1	-0.1878	7.976e-02
NR3C1	0.1583	6.077e-01
ABO	-0.9554	4.373e-02
BIRC3	-2.0447	3.001e-05
GPR33	0.9192	7.073e-03
CCR9	0.8968	2.277e-03
ISG15	0.5487	6.431e-02
EPHA1	0.4140	1.652e-01

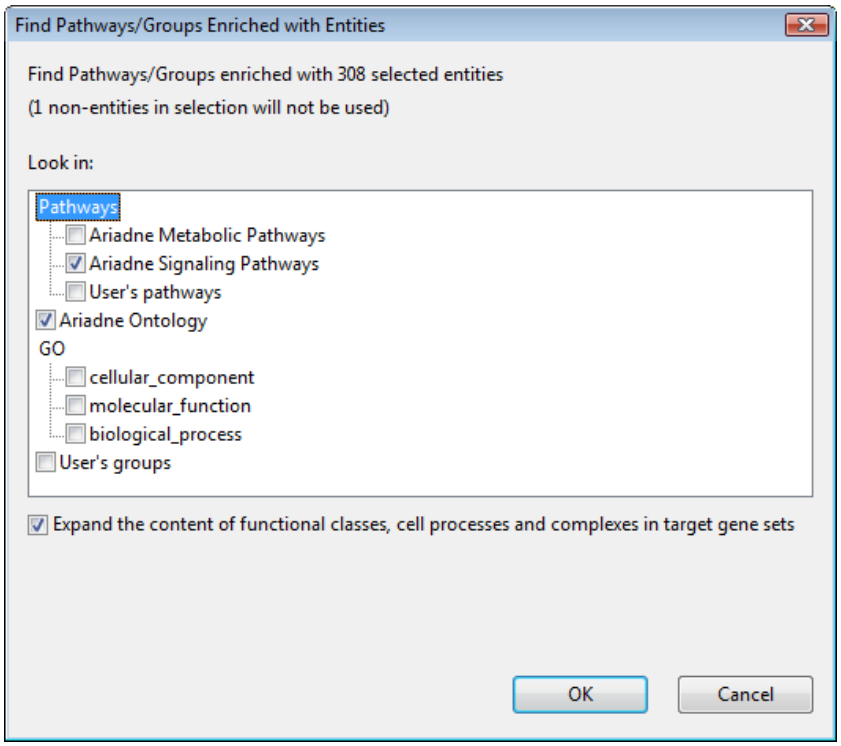
In addition, you can determine this list of genes in an analysis outside of Pathway Studio, and import the resultant list into Pathway Studio for Fisher's Exact test analysis.

To run the Fisher's Exact test from the Experiment Pane, select the genes to be included in the analysis by choosing "Select Unfiltered Probes", then right-click on the gene name column and select "Find Pathways/Groups Enriched with Selected Entities."



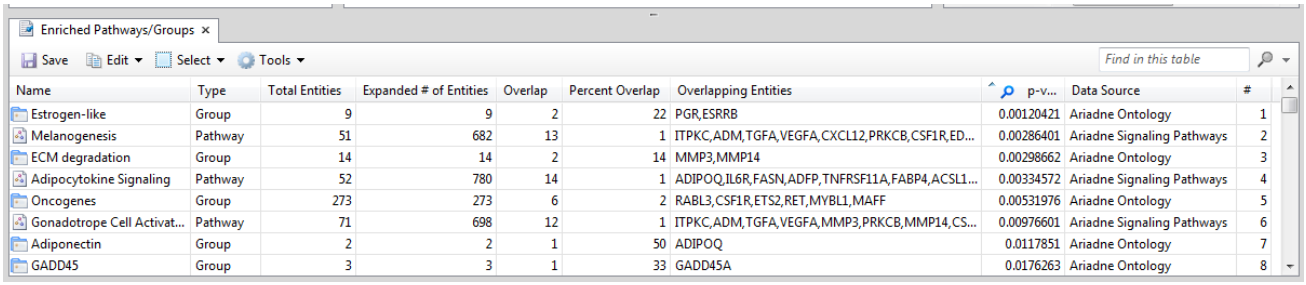


The Find Pathways/Groups Enriched with Entities dialog opens. Here you can select by check box the groups and pathways to include in the analysis. In Pathway Studio you have available the classic Gene Ontology as well as Ariadne Ontology and the Ariadne reference pathway collection to use in the analysis. In addition you can include any pathways and groups you have created in the analysis.



Note: Some types of entities, such as functional classes in the Cellular Process pathways contain proteins that have been mapped to them. To include these proteins in the analysis make sure the check box labeled, “Expand the content of functional classes and complexes in target gene sets” is selected.

The results of the enrichment analysis are displayed in a table in the List Pane, ranked by p-value.



Name	Type	Total Entities	Expanded # of Entities	Overlap	Percent Overlap	Overlapping Entities	p-v...	Data Source	#
Estrogen-like	Group	9	9	2	22	PGR,ESRRB	0.00120421	Ariadne Ontology	1
Melanogenesis	Pathway	51	682	13	1	ITPKC,ADM,TGFA,VEGFA,CXCL12,PRKCB,CSF1R,ED...	0.00286401	Ariadne Signaling Pathways	2
ECM degradation	Group	14	14	2	14	MMP3,MMP14	0.00298662	Ariadne Ontology	3
Adipocytokine Signaling	Pathway	52	780	14	1	ADIPOQ,IL6R,FASN,ADFP,TNFRSF11A,FABP4,ACSL1...	0.00334572	Ariadne Signaling Pathways	4
Oncogenes	Group	273	273	6	2	RABL3,CSF1R,ETS2,RET,MYBL1,MAFF	0.00531976	Ariadne Ontology	5
Gonadotrope Cell Activat...	Pathway	71	698	12	1	ITPKC,ADM,TGFA,VEGFA,MMP3,PRKCB,MMP14,CS...	0.00976601	Ariadne Signaling Pathways	6
Adiponectin	Group	2	2	1	50	ADIPOQ	0.0117851	Ariadne Ontology	7
GADD45	Group	3	3	1	33	GADD45A	0.0176263	Ariadne Ontology	8

The “# of Entities” indicates the number of entities shown in the group or pathway. The “Expanded # of Entities” includes the total number of entities shown on the pathway and contained in complexes and functional classes. The “Overlap” is the number of proteins shared in common between your input group and the resultant group or pathways. The Percent Overlap expresses the overlap of the input list with the entities in the identified object in percent value. The overlapping entities are listed in the Overlapping Entities column. Data Source indicates the source of the group or pathway.

You can save the results of the analysis by exporting to MS Excel: Select > All, Tools > Send Data to Excel. In addition, you can save the results in Pathway Studio by selecting “Save” from the tool bar just above the table.

● Exercise Five: Experimental Data Analysis – Fisher's Exact Test for Enriched Groups and Pathways

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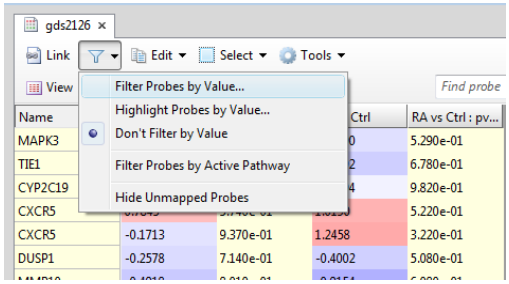
Begin Exercise:

Objective: To run a Fisher's Exact test analysis on a filtered list from gene expression data to identify ontology groups and pathways enriched in the significantly differentially expressed gene set.

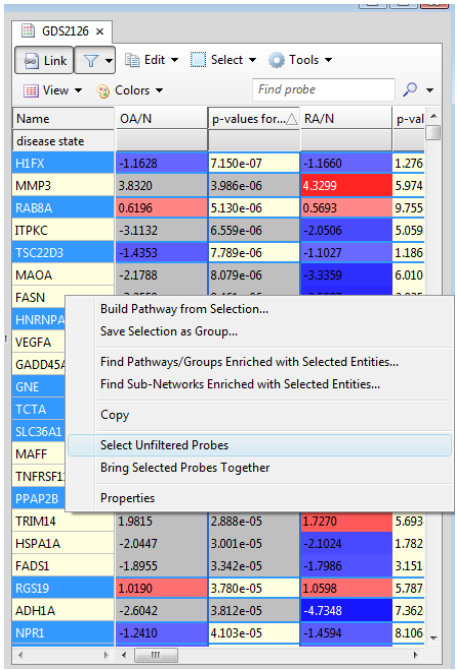
Let's use the osteoarthritis and rheumatoid arthritis synovial tissue data set to run the Fisher's Exact test.

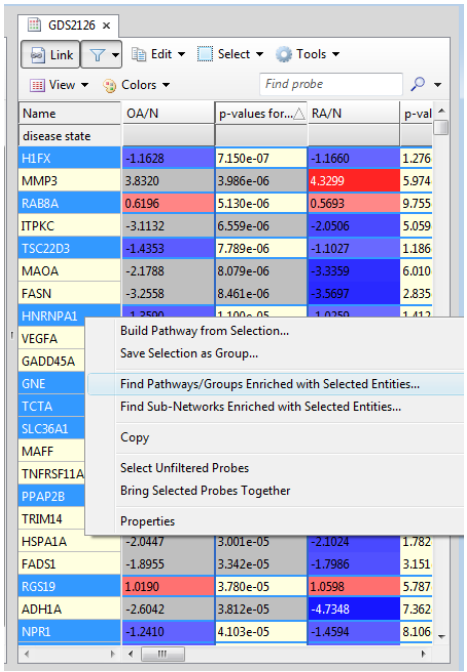
Fisher's Exact Test

1. Filter the table by selecting the filter icon and selecting "Filter Probe by Value".

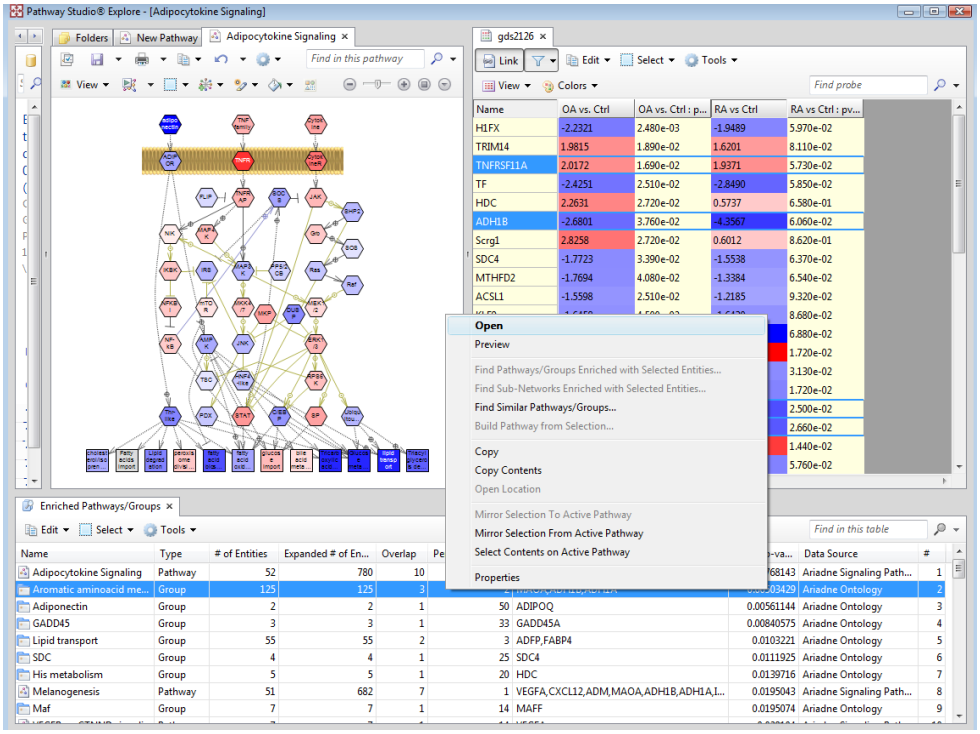


2. Set the filter for the Osteoarthritis data set (check that box) to include only genes with a log change range greater than 1.5 (i.e. set the range to be outside of -1.5 and 1.5), and set the p-value cut off at 0.05.
3. Select "Set Filter." All genes that don't meet the filter criteria and displayed in gray.
4. Right click and choose "Select Unfiltered Probes." Then right click and select "Find pathways/groups Enriched with Selected Entities". This runs the Fisher's Exact test.

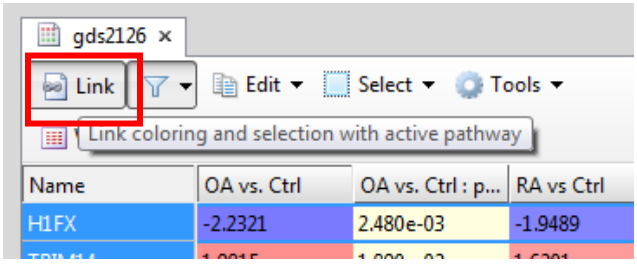




5. Select “Ariadne Signaling Pathways” and “Ariadne Ontology” for the analysis and then select “OK” to run the analysis. (Make sure the check box for “Expand the content of functional classes and complexes in target gene sets” is selected.
6. See the results in the List Pane.
7. Find the top pathway in the list and right-click and select “Open” to see it in a pathway view.

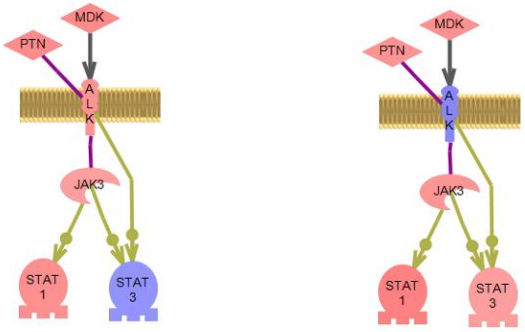


8. If the proteins in the pathway are not colored by the experimental values (here red and blue), then select the “Link” button above the Experiment Pane. This will apply the colorized values of the experiment to the nodes on the pathway.

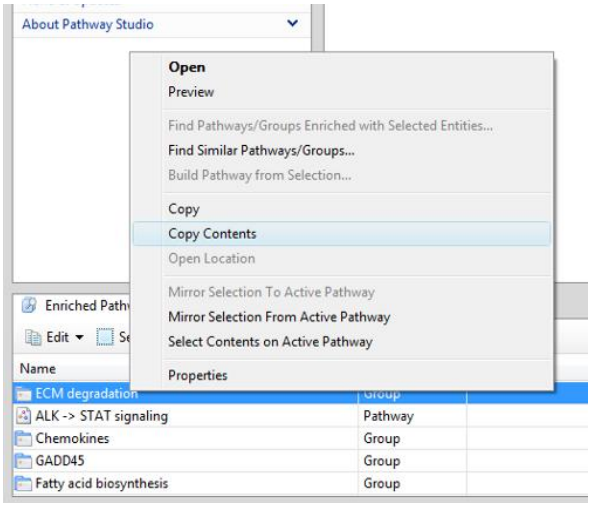


9. With the “Link” function active (the pathway nodes colored by the experiment values) select the second dataset, rheumatoid arthritis, by clicking that column header. Do you see any change in the expression levels of any of the proteins in the pathway?

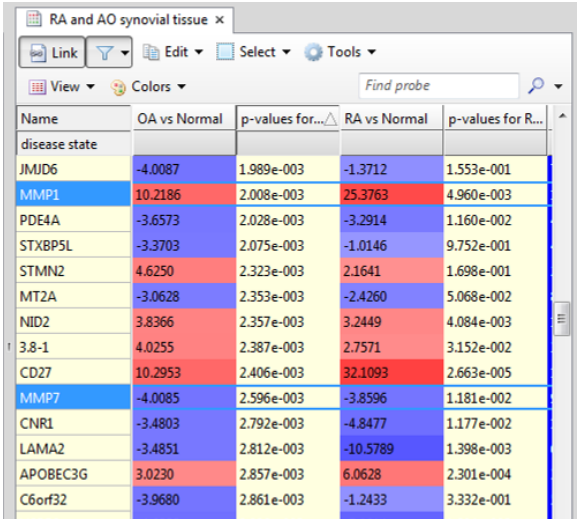
Osteoarthritis Rheumatoid arthritis



10. Find the members of an ontology group that overlap with your gene list, right click on the ontology group and select “Copy Contents.” This will copy the proteins in the ontology to the clipboard. Note: if the ontology contains a child ontology group, the contents of that lower level will not be copied.



11. In the Experiment Pane choose: Select > Entities on Clipboard. The proteins of the ontology group will be highlighted in the Experiment table.



Name	OA vs Normal	p-values for...	RA vs Normal	p-values for R...
disease state				
JMJD6	-4.0087	1.989e-003	-1.3712	1.553e-001
MMP1	10.2186	2.008e-003	25.3763	4.960e-003
PDE4A	-3.6573	2.028e-003	-3.2914	1.160e-002
STXBP5L	-3.3703	2.075e-003	-1.0146	9.752e-001
STMN2	4.6250	2.323e-003	2.1641	1.698e-001
MT2A	-3.0628	2.353e-003	-2.4260	5.068e-002
NID2	3.8366	2.357e-003	3.2449	4.084e-003
3.8-1	4.0255	2.387e-003	2.7571	3.152e-002
CD27	10.2953	2.406e-003	32.1093	2.663e-005
MMP7	-4.0085	2.596e-003	-3.8596	1.181e-002
CNR1	-3.4803	2.792e-003	-4.8477	1.177e-002
LAMA2	-3.4851	2.812e-003	-10.5789	1.398e-003
APOBEC3G	3.0230	2.857e-003	6.0628	2.301e-004
C6orf32	-3.9680	2.861e-003	-1.2433	3.332e-001

Right-click and select “Bring Selected Probes Together” to see all overlapping probes groups together in the experiment list.

12. Results of the analysis can be saved by exporting to MS Excel: Select > All, Tools > Send Data to Excel.
13. Leave the experiment tab open for use in the following exercise.

End Exercise: Experimental Data Analysis – Fisher's Exact Test

Gene Set Enrichment Analysis

Gene Set Enrichment Analysis (GSEA) is similar to the Fisher's Exact test in that it identifies statistical enrichment in experimental data of known groups (such as ontologies) and curated pathways. Gene Set Enrichment analysis differs from Fisher's Exact test in that the **rank** (based on the absolute value of the ratios of the experimental data values) of the genes in the experimental dataset is taken into consideration when identifying enrichment and the entire dataset can be used (no statistical threshold needs to be initially defined).

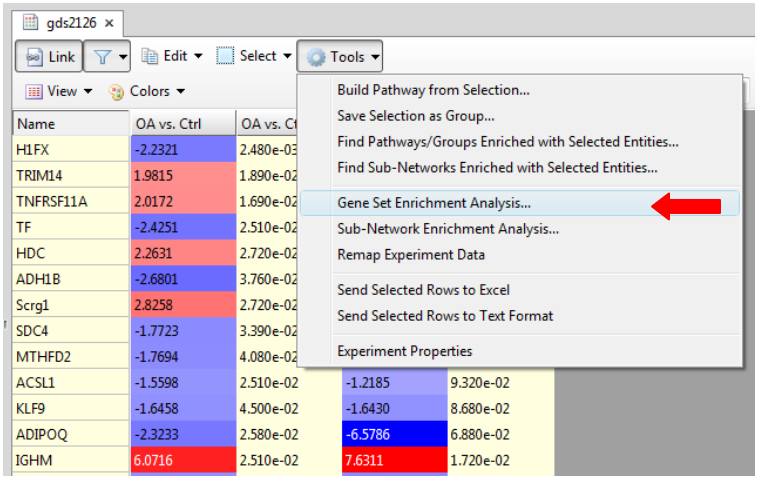
Potential Advantages of Gene Set Enrichment over Fisher's Exact Test:

- Threshold relevance – In Fisher's Exact test only the subset of genes determined by a relevance threshold is considered. This list can be variable depending on the user defined chosen threshold (ex. fold change or p-value).
- In Fisher's Exact Test the rank position of the gene in the experimental results is not considered (it is either on the list or its not). In GSEA rank is considered.
- GSEA is able to identify when many members of a pathway are changed even if none are changed above the threshold used in the Fisher's Exact test (correlation).

It is unnecessary and not recommended to filter your data set by fold change/p-value prior to running the Gene Set Enrichment Analysis; however you can filter to remove genes with high p-values if desired.

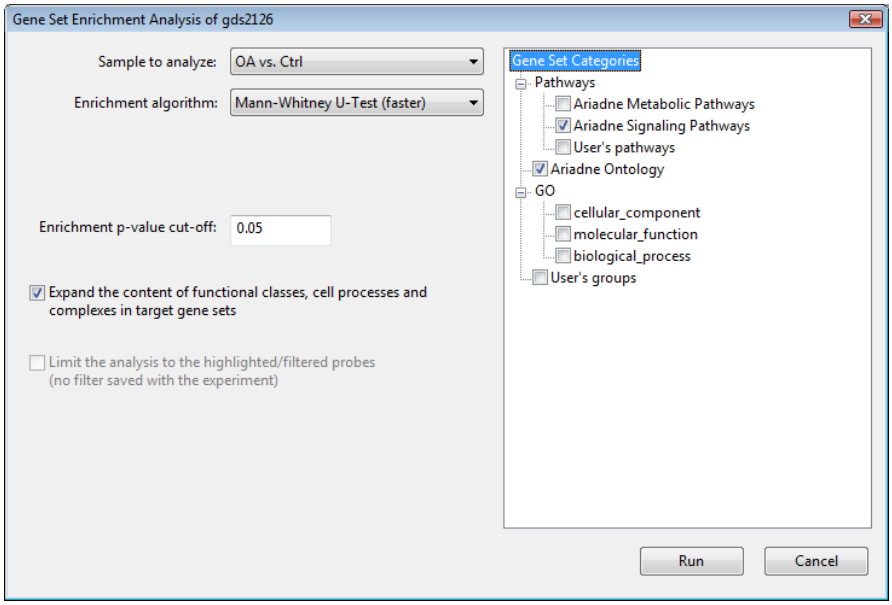
Note: The Gene Set Enrichment Analysis algorithm was developed at the Broad Institute, (http://www.broad.mit.edu/gsea/). The Broad Institute has curated a large number of gene sets that can be used in this analysis. Gene sets downloaded from the Broad Institute can then be imported into Pathway Studio by selecting Advanced in the Import menu in the Information Pane. Select Gene Sets/Gene Sets in Broad Institute Format to download these gene sets.

Launch GSEA from the Tools menu in the Experiment Pane.

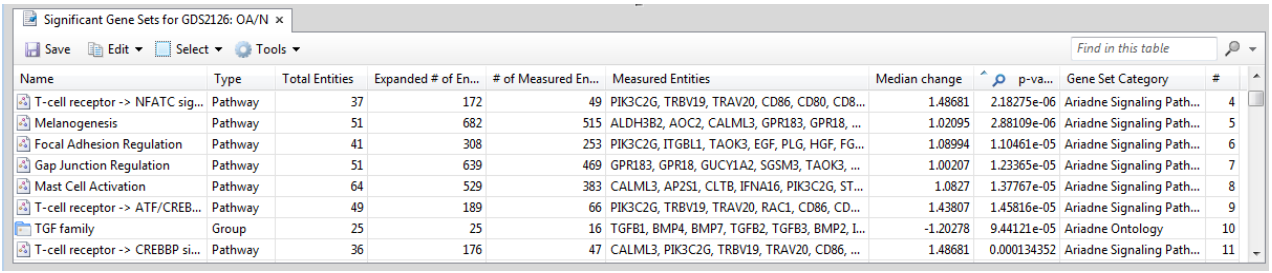


Select the dataset for the analysis, the gene sets (ontologies/pathways,) and the preferred algorithm. You will get similar results from both the Mann-Whitley U-Test or the Kolmogorov-Smirnov algorithms however the

Mann-Whitley U-Test will run much faster. As with the Fisher's Exact test, check the box "Expand the contents of functional classes and complexes in target gene sets, to include those genes in your analysis.



Select "Run" to compute the results and view the results in the bottom List Pane. Enriched gene sets are ranked by p-value.



Name	Type	Total Entities	Expanded # of En...	# of Measured En...	Measured Entities	Median change	p-va...	Gene Set Category	#
T-cell receptor -> NFATC sig...	Pathway	37	172	49	PIK3C2G, TRBV19, TRAV20, CD86, CD80, CD8...	1.48681	2.18275e-06	Ariadne Signaling Path...	4
Melanogenesis	Pathway	51	682	515	ALDH3B2, AOC2, CALML3, GPR183, GPR18, ...	1.02095	2.88109e-06	Ariadne Signaling Path...	5
Focal Adhesion Regulation	Pathway	41	308	253	PIK3C2G, ITGBL1, TAOK3, EGF, PLG, HGF, FG...	1.08994	1.10461e-05	Ariadne Signaling Path...	6
Gap Junction Regulation	Pathway	51	639	469	GPR183, GPR18, GUCY1A2, SGSM3, TAOK3, ...	1.00207	1.23365e-05	Ariadne Signaling Path...	7
Mast Cell Activation	Pathway	64	529	383	CALML3, AP2S1, CLTB, IFNA16, PIK3C2G, ST...	1.0827	1.37767e-05	Ariadne Signaling Path...	8
T-cell receptor -> ATF/CREB...	Pathway	49	189	66	PIK3C2G, TRBV19, TRAV20, RAC1, CD86, CD...	1.43807	1.45816e-05	Ariadne Signaling Path...	9
TGF family	Group	25	25	16	TGFB1, BMP4, BMP7, TGFB2, TGFB3, BMP2, I...	-1.20278	9.44121e-05	Ariadne Ontology	10
T-cell receptor -> CREBBP si...	Pathway	36	176	47	CALML3, PIK3C2G, TRBV19, TRAV20, CD86, ...	1.48681	0.000134352	Ariadne Signaling Path...	11

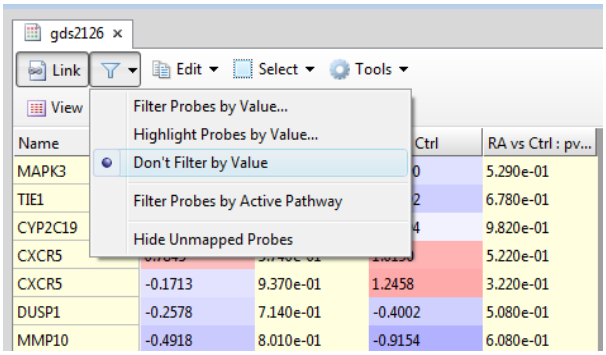
- **Exercise Six: Experimental Data Analysis – Gene Set Enrichment Analysis**

Begin Exercise:

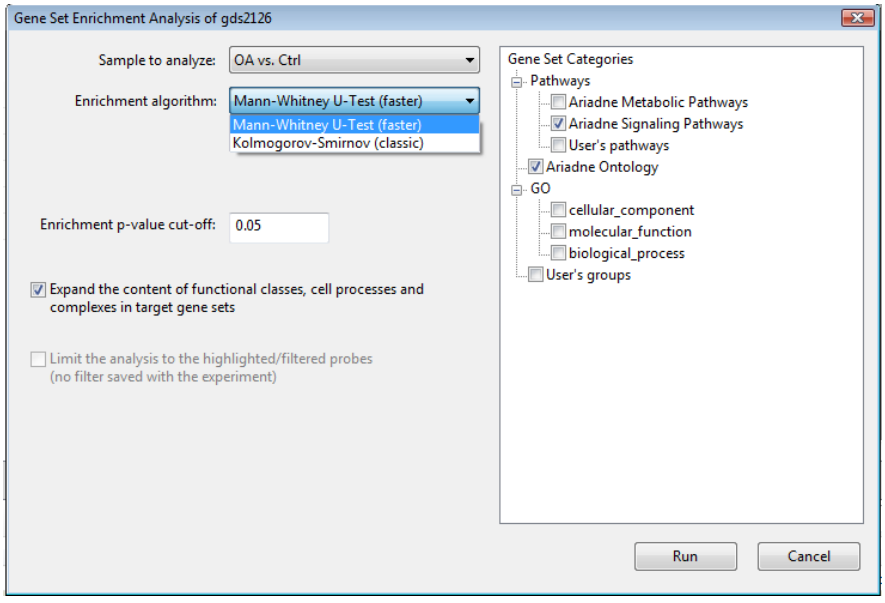
Objective: To run the Gene Set Enrichment Analysis (GSEA) on a gene expression data set to find ontology groups and pathways that are enriched in the dataset and to compare the results of GSEA to the results obtained by the Fisher's Exact test.

Let's use the osteoarthritis and rheumatoid arthritis synovial tissue data set to run the Gene Set Enrichment Analysis and find Ariadne Pathways and Ariadne Ontology groups enriched in the experimental results.

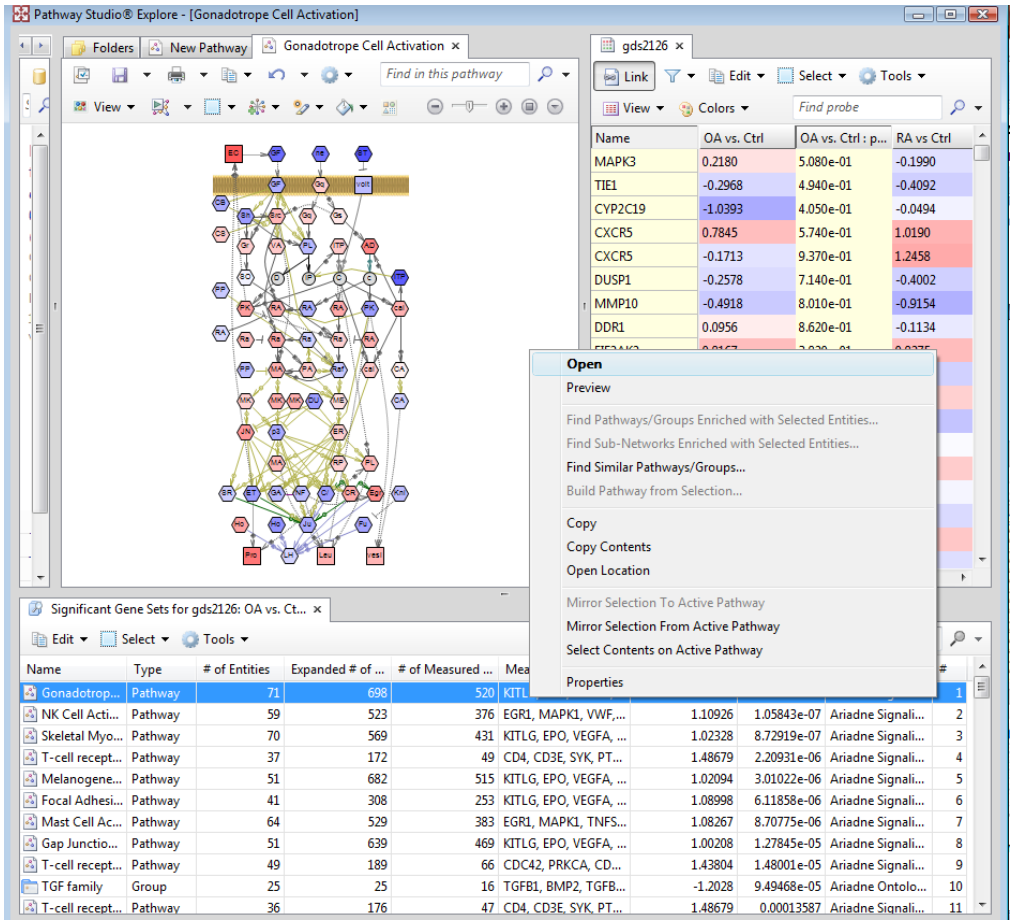
1. If a filter has been applied to the experimental dataset, remove the filter before continuing.



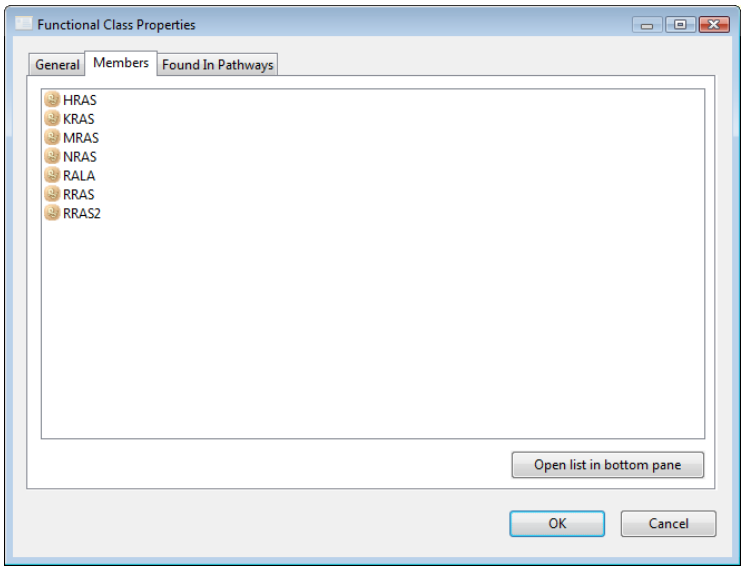
2. Select Tools > Gene Set Enrichment Analysis.



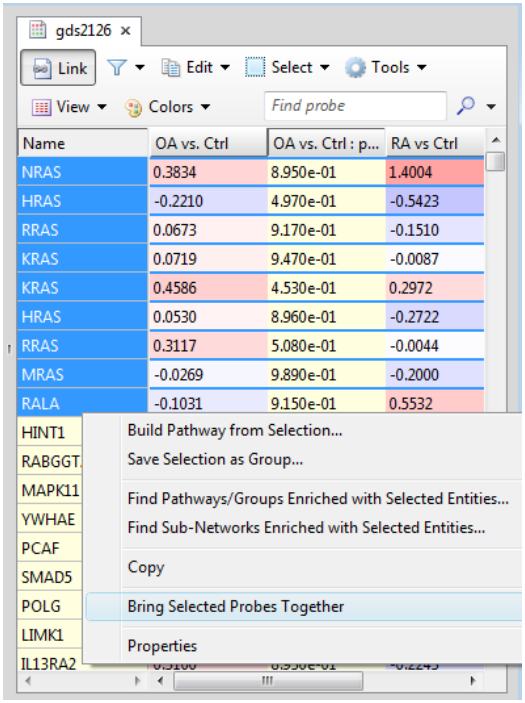
3. Select: Sample to analysis: (osteoarthritis vs control), Gene Set Categories: Ariadne Signaling Pathways and Ariadne Ontology, Enrichment algorithm, Mann-Whitney U-Test, p-value cut-off: 0.05, check "Expand the contents of functional classes and complexes in target gene sets." When finished, select "Run" to compute the results. The results appear in the List Pane, sorted by p-value.
4. Right-click on the top pathway and select open to view the pathway. See that this pathway contains many functional classes.



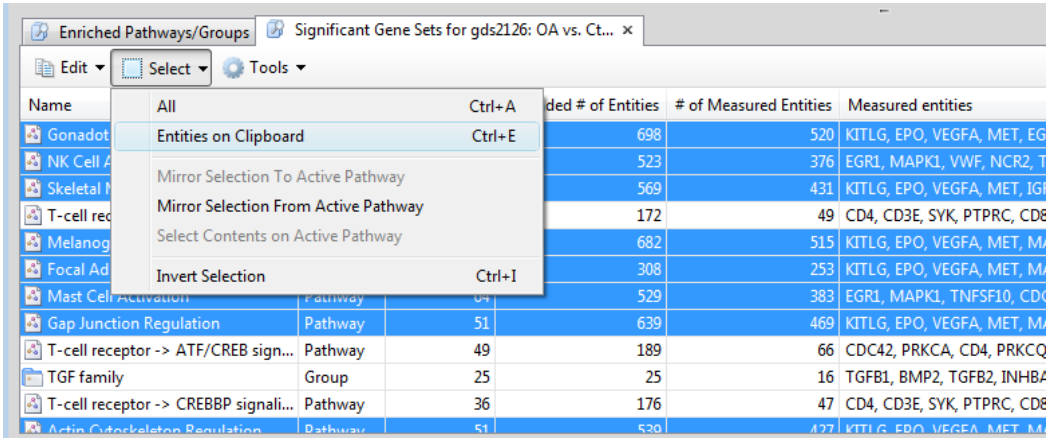
5. Select a functional class and right-click to open the properties view. Select the members tab to see the members of the functional class. Select “Open list in bottom pane.”



6. With the Functional Class open in the list pane choose Select > All, Edit > Copy. This copies the list of proteins to the clipboard.
7. In the Experiment Pane, choose Select > Entities on Clipboard. Next, right-click and choose "Bring Selected Probes Together." This will group all the selected proteins in the table together. If no proteins are selected, there is no overlap between the selected functional class and your dataset.



8. To find the overlap of pathways and groups between the results of the Fisher's Exact test and the Gene Set Enrichment Analysis for a dataset, first choose the tab containing the Fisher's Exact test result (labeled "Enriched Pathways/Groups"). This will allow you to examine what pathways/groups in your results that are unique to one of the algorithms and which are common to both.
9. Choose Select > All, Edit > Copy.
10. Next, choose the GSEA results tab "Significant Gene Sets". Choose Select > Entities on Clipboard. The groups and pathways that are in both analysis results will be selected.



11. Choose Tools > Send Data to Excel to save the selected groups and pathways to an Excel spreadsheet.

End Exercise: Experimental Data Analysis – Gene Set Enrichment Analysis

Sub-Network Enrichment Analysis

For every entity in the ResNet database, the Sub-Network Enrichment Analysis (SNEA) algorithm uses the relationships in the ResNet database to build “sub-networks” based on user specified criterion. It then uses these sub-networks and the Fisher’s Exact test or GSEA algorithm to identify the networks that are significantly enriched.

The user-defined sub-networks consist of a single “regulator” gene and its targets. The significance of the target expression levels in every built network is evaluated. The result is the identification of individual “regulators” which most likely affect the differentially expressed genes, thus providing the one plausible explanation for the observed expression changes in the experiment.

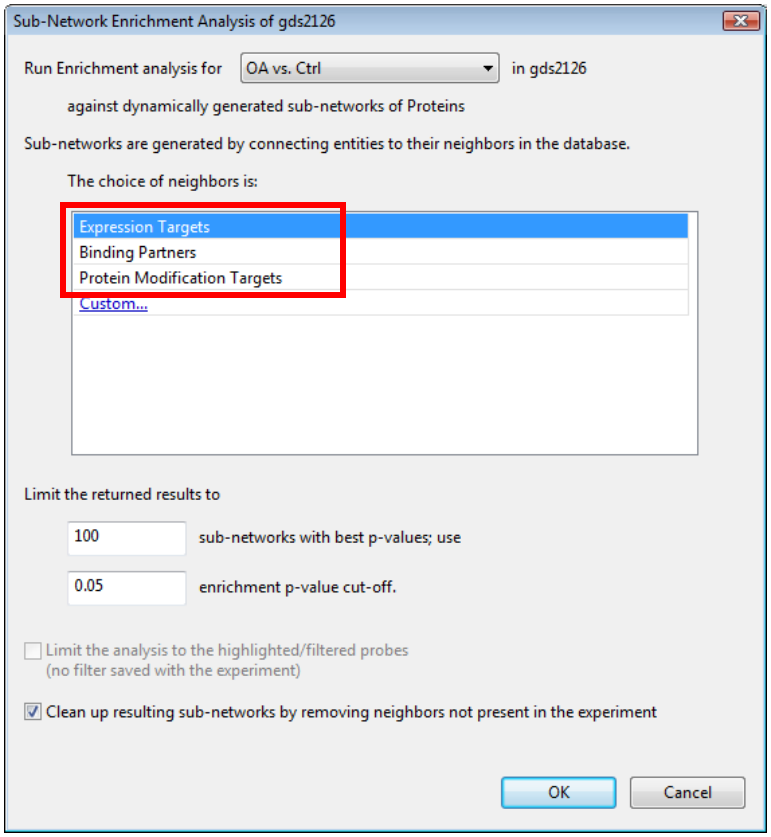
Defining the Sub-Networks

Setting user-defined criteria for building the sub-networks involves first defining the “regulator” (also referred to as the “seed”) and then the “neighbors” (the “targets” defined by selecting specific relationship types).

For example, defining a protein seed and promoter binding relationships will give these types of networks:

The network consists of the target proteins with which the seed protein has a promoter binding relationship.

The Sub-Network Enrichment Analysis menu provides some short-cut menu options (see table below) or you can use the “custom” options for more flexibility in sub-network definitions.



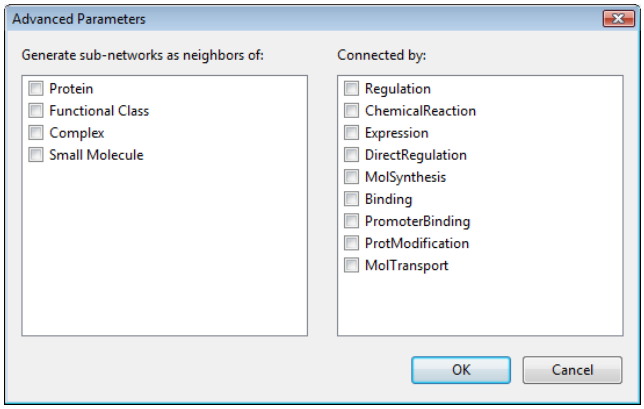
See definitions for these short-cut menu options in table below.

Note: Ariadne recommends that you check the box "Clean up resulting sub-networks by removing neighbors not present in the experiment." This way, only measured entities for which experimental data exists (for example proteins/genes in a microarray experiment) are included in the network. (A small molecule or a disease would not have experimental data on a gene expression microarray and would have no expression data associated with it.)

Sub-network preset conditions for short-cut menu:

Sub-network Type	Seed	Neighbors	Relationship	Direction
Expression Targets	Gene	Gene	Promoter Binding, Expression	Outbound from seed
Protein Binding Partners	Protein	Protein	Binding	No direction
Protein Modification Targets	Protein	Protein / Complex	Protein Modification	Outbound from seed

Use the custom menu to select specific seeds and relationships.



Note: all relationships are outbound from seed to regulators except when relation is binding, which has no directionality.

The results of the SNEA are viewed in a table sorted by p-value. Each network is named by the regulator of the network.

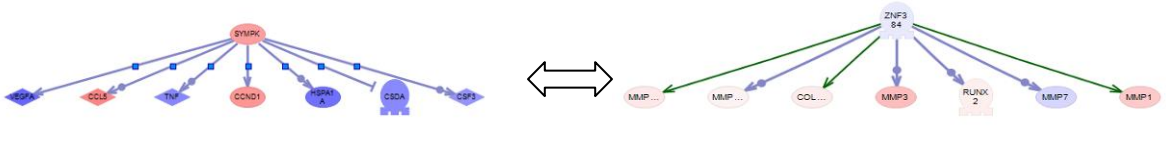
Enriched Sub-networks for gds2126: OA vs. Ct... ×

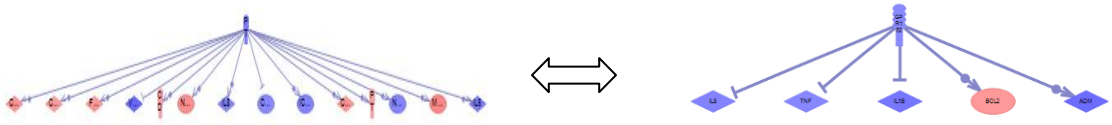
Edit ▾ Select ▾ Tools ▾ Find in this table

Name	Total # of Neighbors	# of Measured Neighbors	Gene Set Seed	Measured Neighbors	Median change	p-value	#
Expression Targets of HSD11B1	7	7	HSD11B1	TIMP1, MMP9, TIMP2, MMP7, MMP1, I...	1.50483	0.00135834	1
Expression Targets of LCN2	7	7	LCN2	PPARG, HMOX1, CDH1, IRS1, ATF5, VEG...	-2.17543	0.00149916	2
Expression Targets of IL18BP	7	7	IL18BP	CCL5, IL1B, TNF, MMP9, IFNG, VCAM1, ...	-1.42662	0.00223295	3
Expression Targets of DCN	23	21	DCN	RHOA, IL1B, IL6, MMP14, MMP1, NCAN...	-1.58195	0.00245946	4
Expression Targets of SPARC	20	20	SPARC	MMP3, MMP1, FN1, MMP2, CDK2, TNF...	1.31887	0.00252243	5
Expression Targets of EIF2S1	11	9	EIF2S1	DDIT3, MYC, PHLDA1, ATF3, NFKBIA, C...	-2.34404	0.00269447	6
Expression Targets of CCNC	5	5	CCNC	VCAM1, TYMS, CDC2, MYC, CCNH	2.25355	0.00287694	7
Expression Targets of exosome	6	6	exosome	MMP1, IFNG, TNF, SERPINE1, IL6, PRF1	-1.69525	0.00295389	8
Expression Targets of chemokine	62	57	chemokine	AKT1, CSF2, SELE, TNFSF11, CXCL12, CX...	1.28138	0.00311814	9
Expression Targets of CCL3	25	24	CCL3	ITGAM, CCL21, IL10, MMP9, IL2, FLT3L...	1.31741	0.00332672	10
Expression Targets of SREBF1	105	88	SREBF1	PCSK6, IL8, VEGFA, PDX1, ESR1, PPAT, F...	-1.28129	0.00412663	11
Expression Targets of CD8A	120	105	CD8A	IL8RA, GZMA, ICAM1, PRF1, KLRK1, CD...	1.31695	0.00496164	12

When networks are the same size, those with greater differential gene expression will have better p-values. When differential expression is equal, larger networks will have better p-values than smaller networks.

Better p-value





Both enrichment algorithms, Fisher's Exact Test and Gene Set Enrichment Analysis, can be used to identify enriched Sub-Networks.

	Known Gene Sets (ontologies, curated pathways)	**Sub-Networks** (user defined from ResNet)
Fisher's Exact Test (experimental values not utilized)	**Find Pathways/Groups Enriched with Selected Entities**	**Find Sub-Networks Enriched with Selected Entities**
Gene Set Enrichment Analysis (GSEA) (experimental values are considered in the analysis)	**Gene Set Enrichment Analysis**	**Sub-Network Enrichment Analysis**

Names of the tools as they appear in Pathway Studio

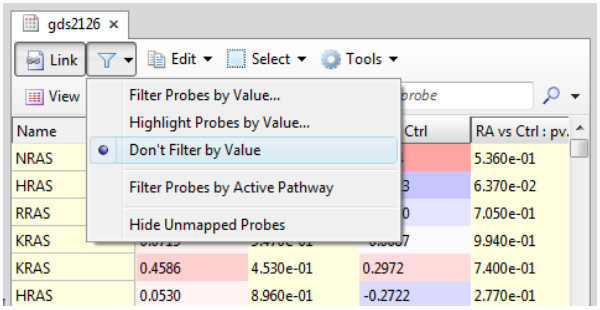
● Exercise Seven: Experimental Data Analysis – Sub-Network Enrichment Analysis (with GSEA)

Begin Exercise:

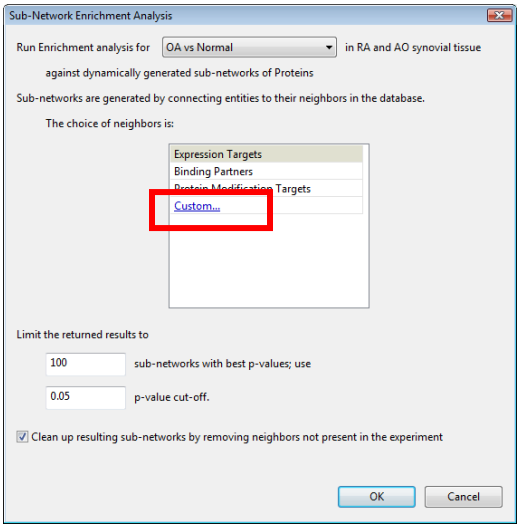
Objective: To run the Sub-Network Enrichment Analysis on a gene expression dataset to identify dynamically defined transcriptional regulatory networks enriched in the dataset and to visualize multiple networks in one view to allow for identification of overlap between networks.

Let's use the osteoarthritis and rheumatoid arthritis synovial tissue data set to run the Sub-Network Enrichment Analysis and find transcriptional regulatory networks enriched in the experimental dataset.

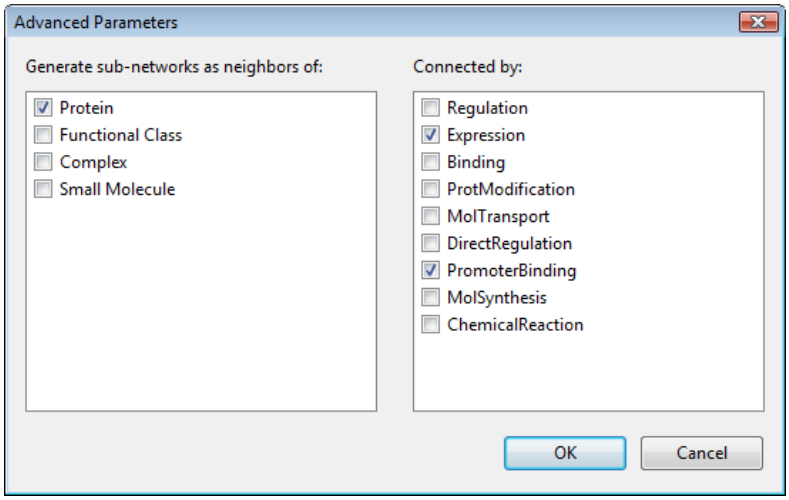
1. If a filter has been applied to the experimental dataset, remove the filter before continuing.



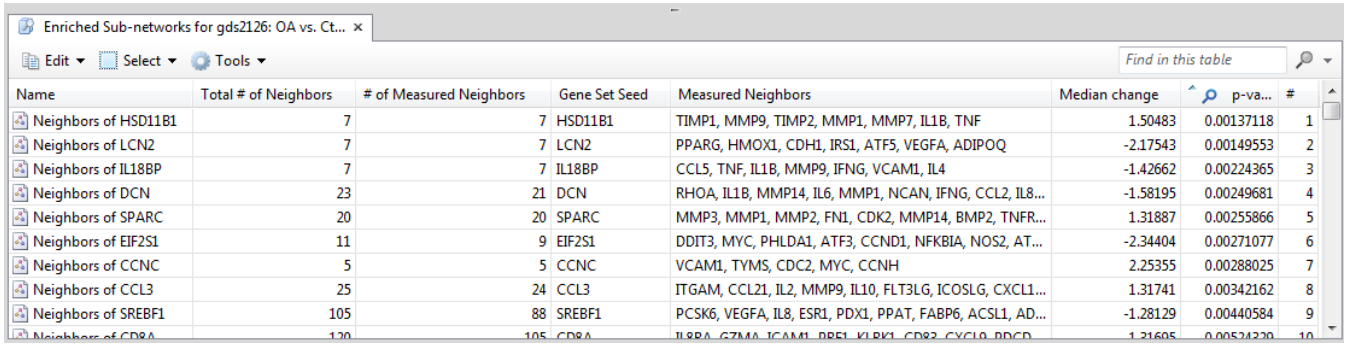
2. Select Tools > Sub-Network Enrichment Analysis
3. In the Run Enrichment analysis menu, select the osteoarthritis study.
4. In the choice of neighbors menu select "Custom"



5. Here we will define transcriptionally regulated networks: Select Protein for the (transcriptional) regulator "seed" and select Expression and PromoterBinding to define the transcriptionally regulated "target" network. Note: We will not utilize Regulation in this example, as this is the largest relation category in ResNet.

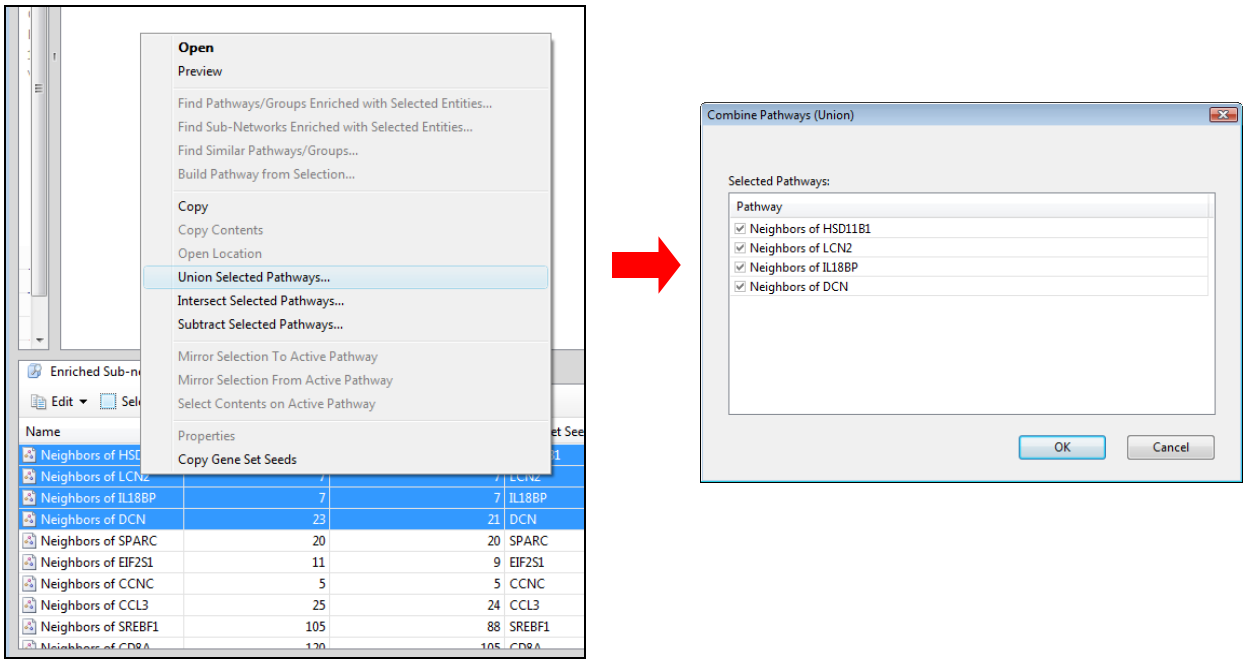


6. Select OK
7. In the Sub-Network Analysis tool ensure that the box “Clean up resulting sub-networks by removing neighbors not present in the experiment”, then select OK to run the analysis.
8. View the results in the List Pane. Each network is named by the “seed” (the regulator).

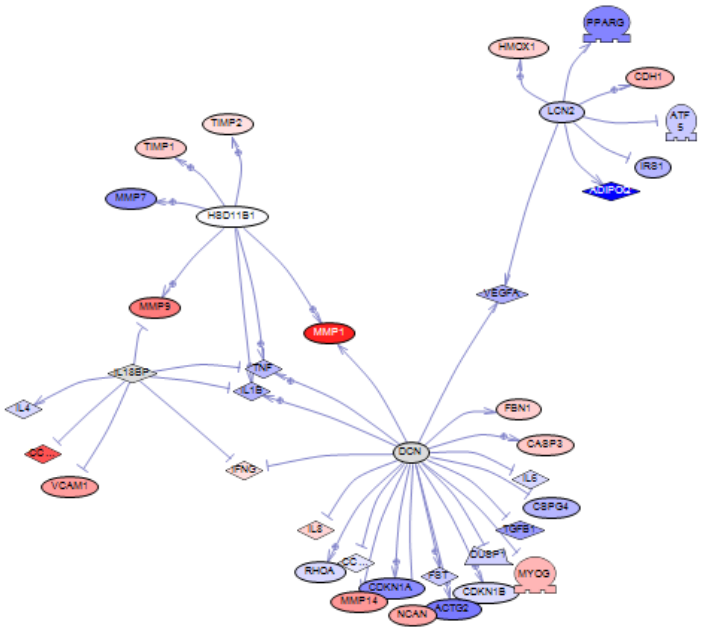


Name	Total # of Neighbors	# of Measured Neighbors	Gene Set Seed	Measured Neighbors	Median change	p-va...	#
Neighbors of HSD11B1	7	7	HSD11B1	TIMP1, MMP9, TIMP2, MMP1, MMP7, IL1B, TNF	1.50483	0.00137118	1
Neighbors of LCN2	7	7	LCN2	PPARG, HMOX1, CDH1, IRS1, ATF5, VEGFA, ADIPOQ	-2.17543	0.00149553	2
Neighbors of IL18BP	7	7	IL18BP	CCL5, TNF, IL1B, MMP9, IFNG, VCAM1, IL4	-1.42662	0.00224365	3
Neighbors of DCN	23	21	DCN	RHOA, IL1B, MMP14, IL6, MMP1, NCAN, IFNG, CCL2, IL8...	-1.58195	0.00249681	4
Neighbors of SPARC	20	20	SPARC	MMP3, MMP1, MMP2, FN1, CDK2, MMP14, BMP2, TNFR...	1.31887	0.00255866	5
Neighbors of EIF2S1	11	9	EIF2S1	DDIT3, MYC, PHLDA1, ATF3, CCND1, NFKBIA, NOS2, AT...	-2.34404	0.00271077	6
Neighbors of CCNC	5	5	CCNC	VCAM1, TYMS, CDC2, MYC, CCNH	2.25355	0.00288025	7
Neighbors of CCL3	25	24	CCL3	ITGAM, CCL21, IL2, MMP9, IL10, FLT3LG, ICOSLG, CXCL1...	1.31741	0.00342162	8
Neighbors of SREBF1	105	88	SREBF1	PCSK6, VEGFA, IL8, ESR1, PDX1, PPAT, FABP6, ACSL1, AD...	-1.28129	0.00440584	9

9. Let’s view the top four networks in one pathway. Select the top four networks, right-click and choose “Union Selected Pathways.” In the “Combine Pathway (Union)” window, select OK.



The resultant network should look something like this (using the dynamic layout). Note: although your network may look similar, the dynamic layout may generate a slightly different layout than the one see here.

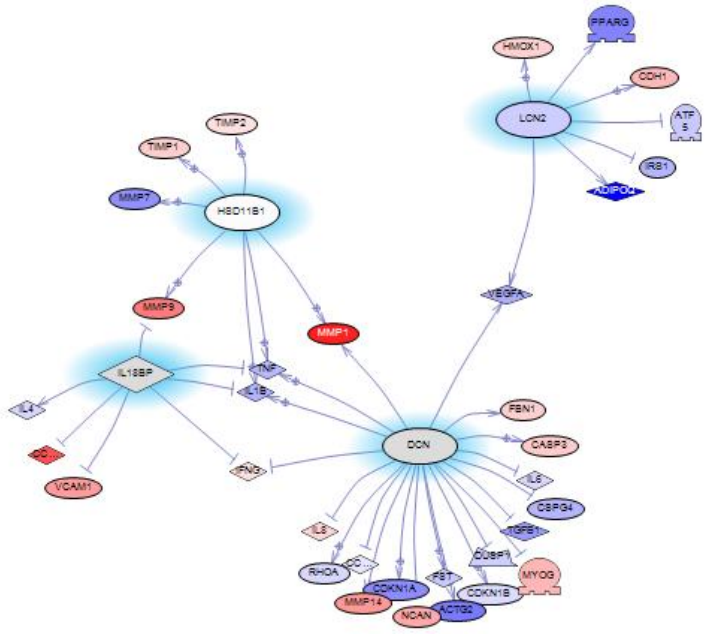


Now let's modify this network to better view the entity names and emphasize the four regulators in this network by applying highlighting.

10. Select "Fit all entities to window" to maximize the size of the network in the window.
11. Open the Advanced Visualization tool bar and select "Size All Entities to Labels" in the Resize menu.

12. Next, using the control key, select the four regulators, BP1, CCR7, IV and CD69. Note that selected entities have a blue halo. One of the selected entities will also have small white boxes around it. Click on the corner white box and expand the size of this entity.

13. With the other three entities (regulators) still selected, go to the Advanced Visualization tool bar and select “Make Same Size” from the Resize menu. This will expand all the regulators to the same larger size.
14. While the four regulators remain selected, go to the highlight menu and select the blue highlight. This will put a blue halo around the regulators. Your network should look similar to this:



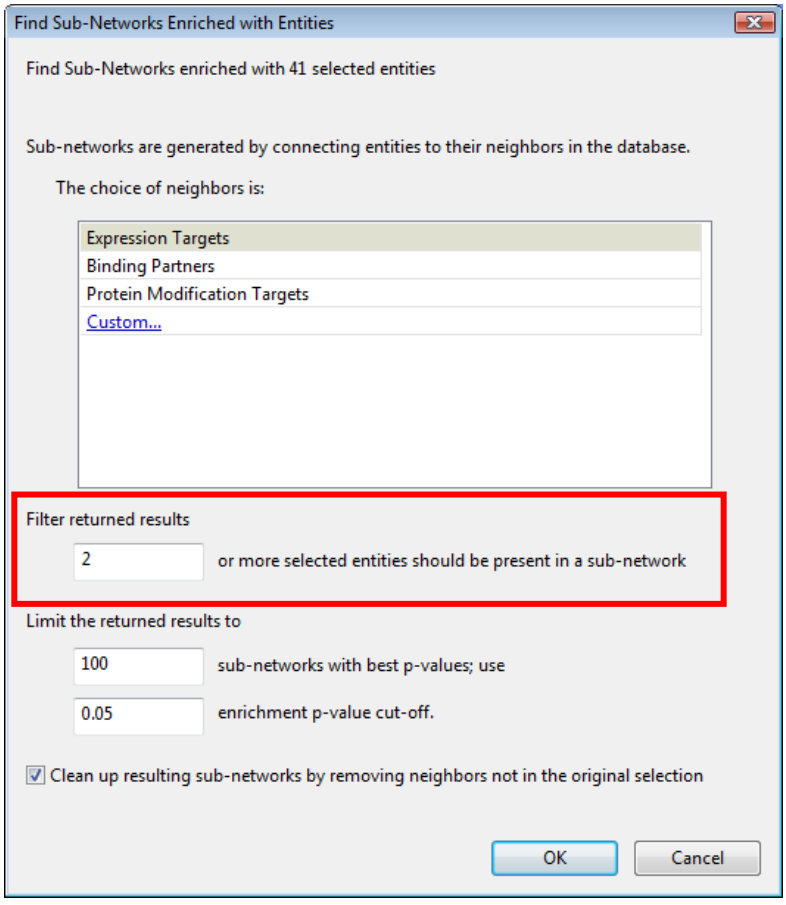
Note that two of the four regulators is gray. This is because these regulator were not measured in the gene expression experiment. The Sub-Network Enrichment Analysis is able to utilize all the relationship information in ResNet to identify potentially important regulators for a gene expression experiment, even if that specific regulator was not included on the microarray.

End Exercise: Experimental Data Analysis – Sub-Network Enrichment Analysis (with GSEA)

This Training Manual gave examples of three of the four enrichment analysis options highlighted below: Fisher's Exact Test of known gene sets, Gene Set Enrichment Analysis of known gene sets and Sub-Network Enrichment Analysis (GSEA) of Sub-Networks.

gene sets algorithms	**Known Gene Sets** (ontologies, curated pathways)	**Sub-Networks** (user defined from ResNet)
Fisher's Exact Test (experimental values not utilized)	**Find Pathways/Groups Enriched with Selected Entities**	**Find Sub-Networks Enriched with Selected Entities**
Gene Set Enrichment Analysis (GSEA) (experimental values are considered in the analysis)	**Gene Set Enrichment Analysis**	**Sub-Network Enrichment Analysis**

In addition, you can run the Fisher's Exact Test on any list of proteins to find enriched sub-networks. Keep in mind that if you start with a short protein list, most of your enriched sub-networks will also be small. The Find Sub-Networks Enriched with Entities dialog allows you to set a threshold for the size of the sub-network to be returned.



Appendix A: Definitions

Entity definitions:

Protein: The principal source of proteins and their annotation in ResNet is Entrez Gene. The level of detalization of proteins in ResNet Mammal is the Gene. This means that if proteins are encoded by the same gene, they will have the same identifier.

Small molecule: Symbolizes metabolites, drug and other chemicals of low molecular weight (< 1 KDa). It also can be used to represent non-biological polymers of larger molecular weight.

Cell object: Symbolizes cellular organelles and other sub-cellular components. A majority of cell process entities coincide with the part of the Gene Ontology cellular component classification that excludes protein complexes.

Cell process: Symbolizes biological processes. A majority of Cell process entities coincide with Gene Ontology biological processes classification.

Disease: Symbolizes diseases and other health conditions and processes.

Treatment: Symbolizes non-chemical treatments and environmental conditions such as cold shock, draught etc.

Complex: This is a container entity that symbolizes several polypeptides that form the complex via physical interaction. The complex is usually well-characterized in the literature, performs well-defined function and is referred in the literature by a specific name. A majority of complex entities coincide with part of Gene Ontology cellular classification that describes protein complexes.

Functional class: This is a container entity that symbolizes functional classes of proteins. Majority of functional class entities coincide with Gene Ontology molecular function classifications.

Relationship definitions:

MolTransport: Indicates that the regulator changes the localization of the target. Describes events of molecular translocation, export, import or release

Regulation: Indicates that the regulator changes the activity of the target. The mechanism of the regulation is either unknown or has not been specified in the sentence describing the relation.

Chemical Reaction: Symbolizes either enzyme catalyzed or spontaneous chemical reaction, i.e. transformation of one set of Small Molecules into another. Usually must have at least one substrate show with the link incoming to control and one product shown with the link outgoing from control. Enzyme is shown as undirected link between Functional Class or Protein entity and control

Binding: Physical interaction between molecules

ProtModification: Indicates that the regulator molecule changes the protein modification of the target molecule. Usually indicates the direct interaction, i.e. the regulator catalyses the chemical modification reaction.

DirectRegulation: Indicates that the regulator influences the target activity by physically interacting with it.
Expression: Indicates that the regulator changes the protein level of the target, by means of regulating its gene expression or protein stability.

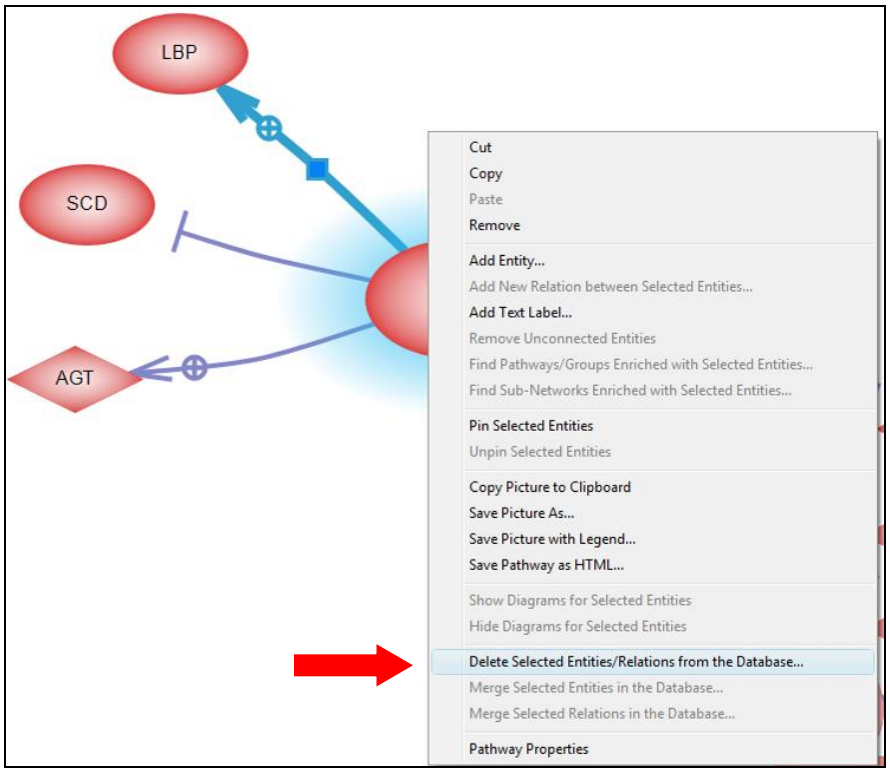
PromoterBinding: Indicates that the regulator binds the promoter of the target.

MolSynthesis: Indicates that the regulator changes the concentration of the target. Usually Small Molecule is a target in MolSynthesis

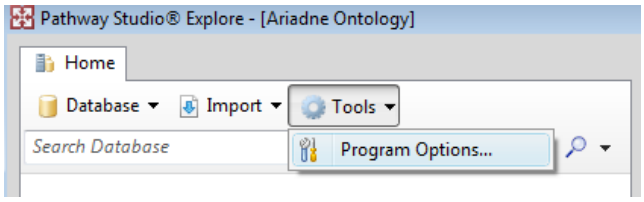
Appendix B: Deleting Entities and Relations from a Local Database (Pathway Studio Explore and ResNet Explore)

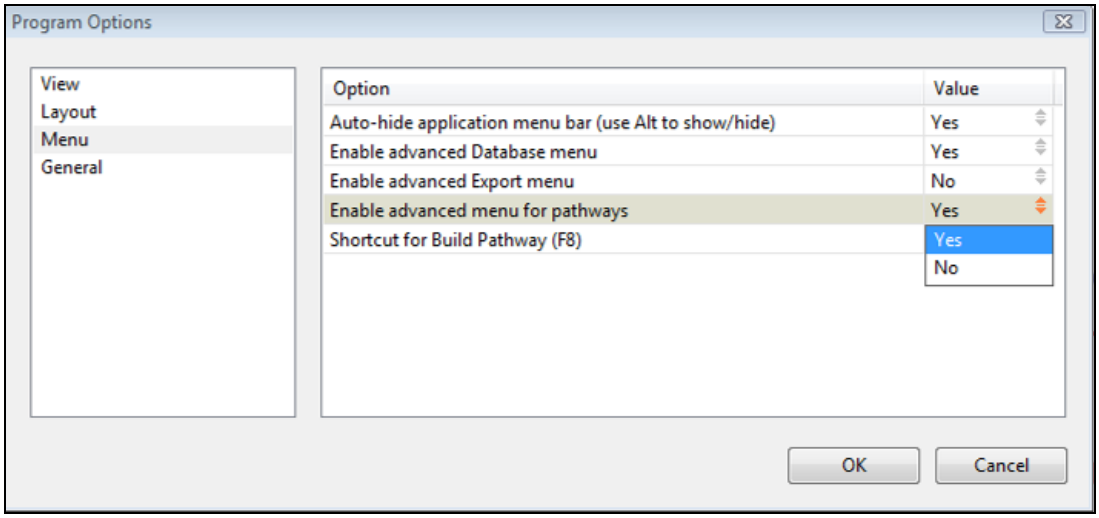
When you delete a relation or entity from a pathway view, it is only removed from that specific pathway but still remains in the ResNet database.

To permanently delete a relation or entity from the ResNet database, select the relation or entity, move the mouse cursor to an empty area of the pathway, right-click and select “Delete Selected Entities/Relations from Database.”



Note: If “Delete Selected Entities/Relations from the Database” does not appear as a menu option, in the Information Pane go to Tools > Program Options. Next, select “Menu” then change “Enable Advanced Menu for Pathways” from “No” to “Yes.”





If you have any questions about Pathway Studio Explore, please contact us at:

Ariadne Support Team

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