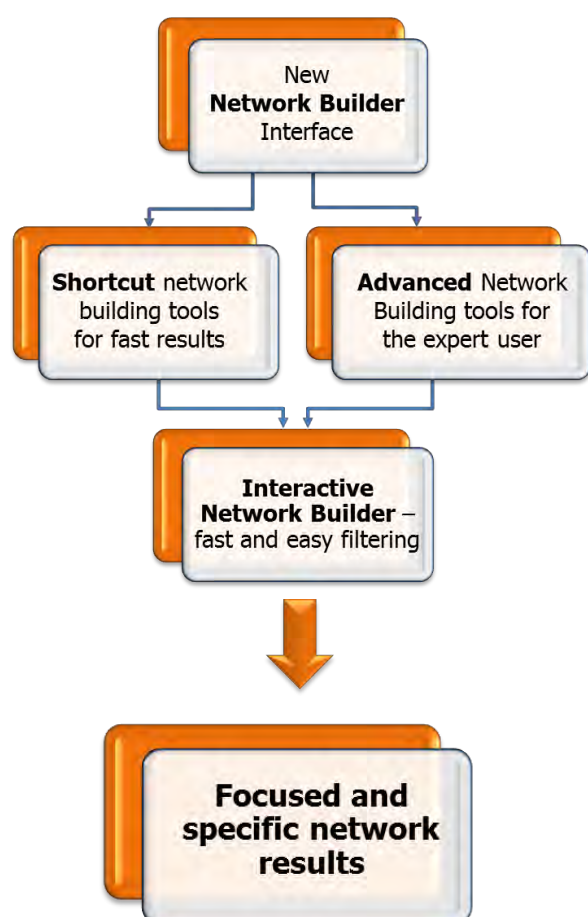


## Pathway Building in Pathway Studio Web 11.1

### Introduction to Pathway Building Tools in Pathway Studio Web 11.1

The newly designed Pathway Studio Web 11.1 makes pathway building faster, easier and more intuitive. The interface provides a simple path to build and filter pathways, providing powerful easy-to-use filtering tools and short cuts, designed to satisfy the novice user while still maintaining advanced filter options for the expert user.



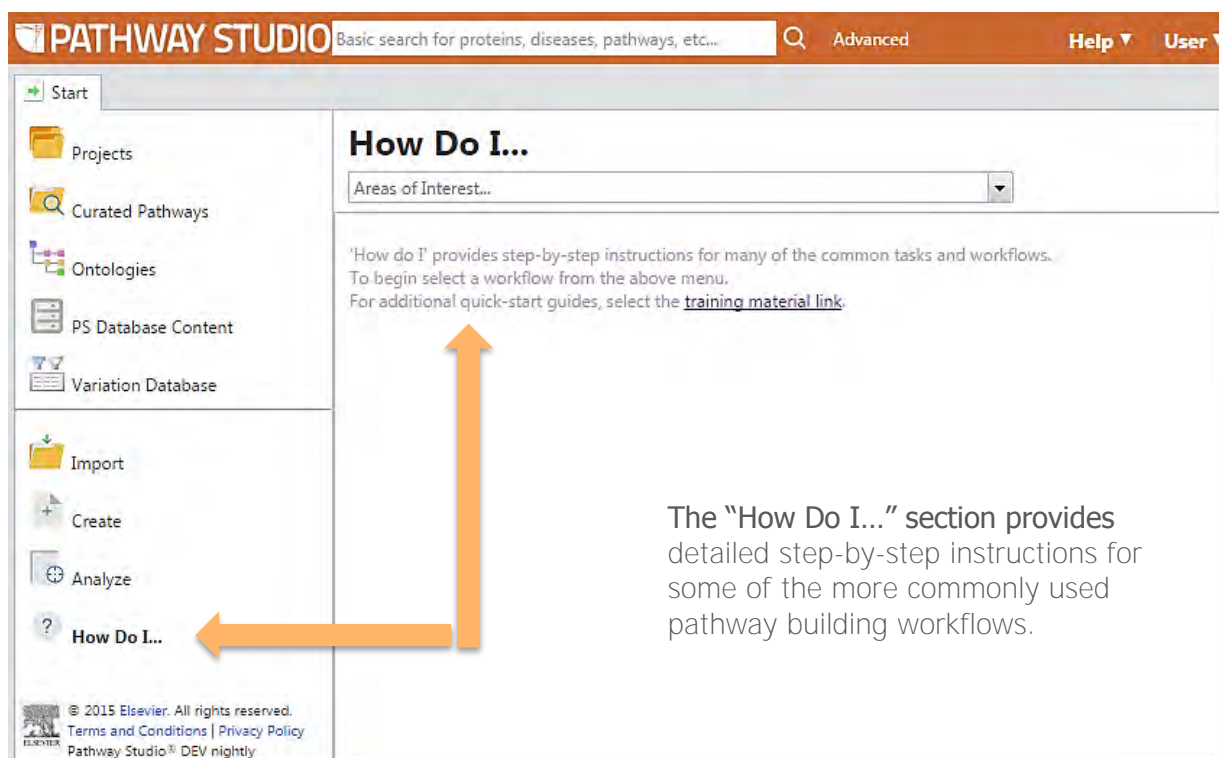
Start your network building activities from the new **Network Builder** interface.

**Shortcut** menu options allow for quick visualization of network results from preset menus.

**Advanced** network building tools allow the expert user to apply specific entity and relation filters for more focused and specific results.

The **Interactive Network Builder** is a simple-to-use interface for filtering larger networks to produce smaller, more manageable and focused results.

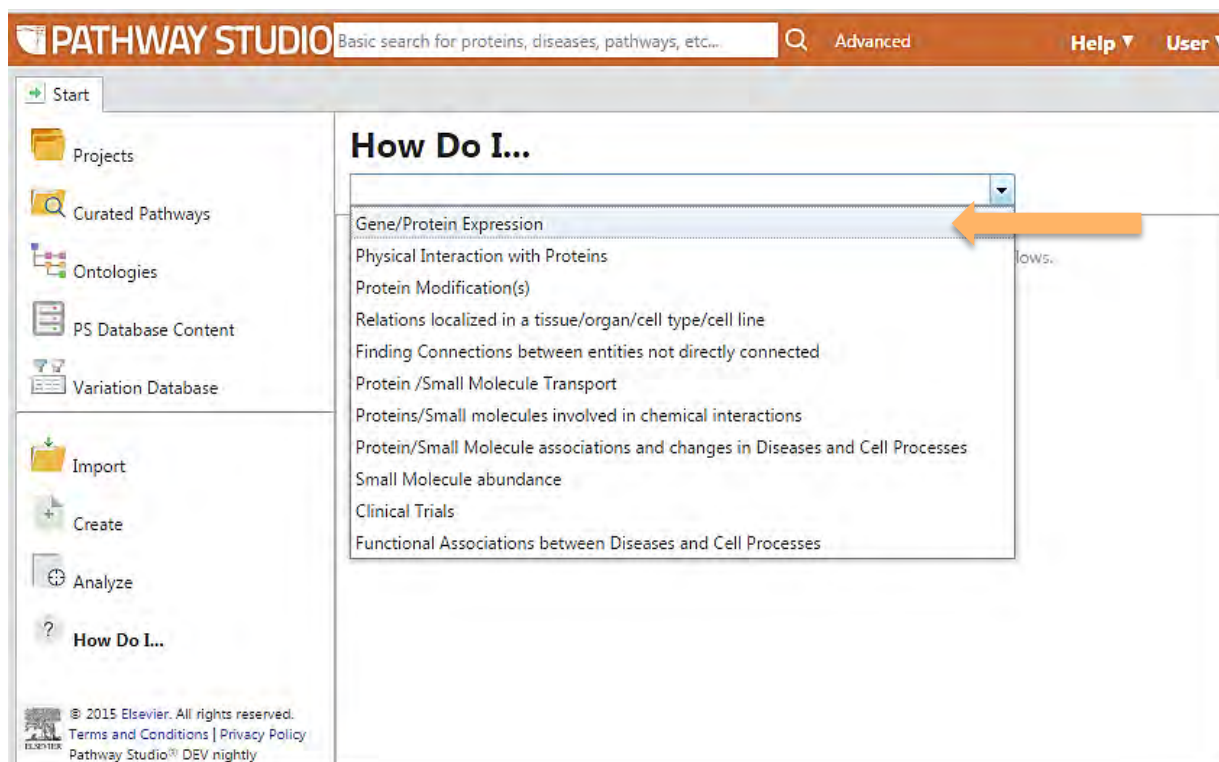
In addition, Pathway Studio Web 11.1 also includes detailed step-by-step instructions for advanced pathway building for many of the most commonly used workflows. The “**How Do I...**” section organizes information based on biological and molecular categories. Access the “How Do I...” section from the lower left pane.



The screenshot shows the Pathway Studio Web 11.1 interface. The top navigation bar includes the logo, a search bar, and links for 'Advanced', 'Help', and 'User'. The left sidebar contains a 'Start' menu with options: Projects, Curated Pathways, Ontologies, PS Database Content, Variation Database, Import, Create, Analyze, and 'How Do I...'. The main content area is titled 'How Do I...' and features a dropdown menu labeled 'Areas of Interest...'. Below the dropdown, text explains that 'How do I' provides step-by-step instructions for common tasks and workflows, and directs users to select a workflow from the menu or use a 'training material link' for quick-start guides. An orange arrow points from the 'How Do I...' menu item in the sidebar to the 'How Do I...' section header, and another orange arrow points from the 'How Do I...' section header to the 'Areas of Interest...' dropdown menu.

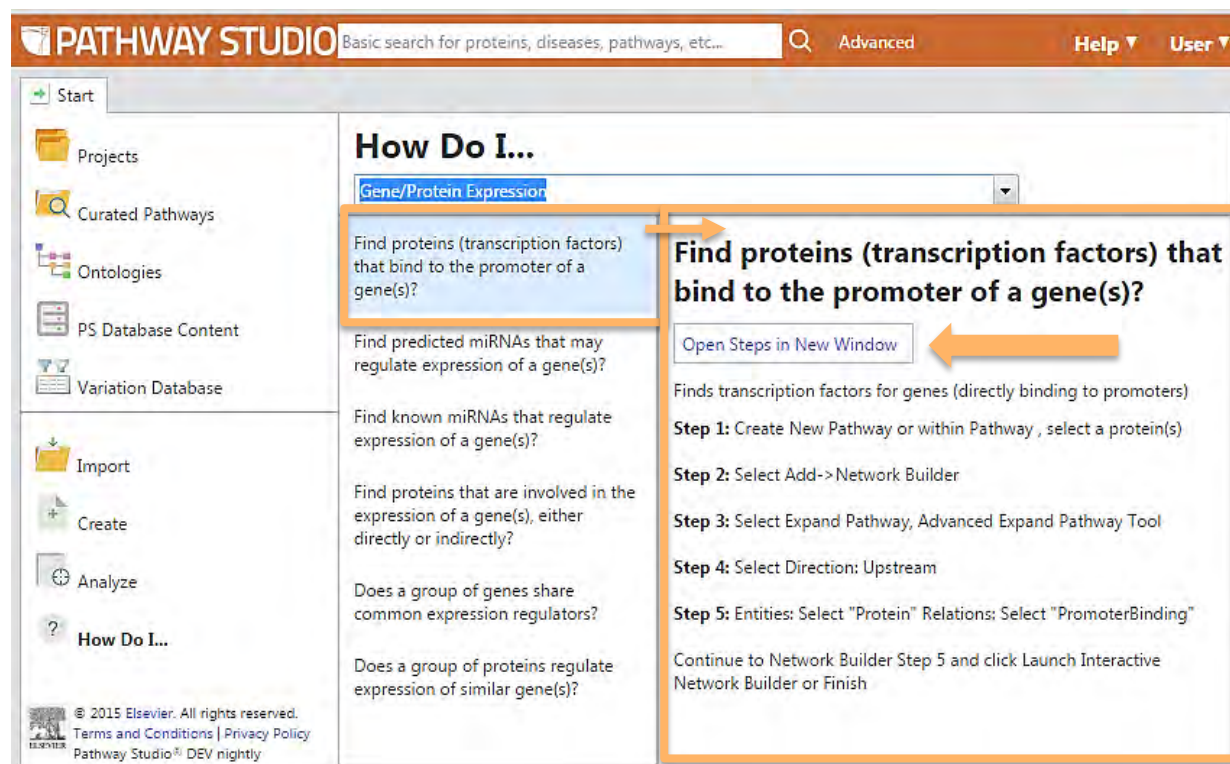
The “How Do I...” section provides detailed step-by-step instructions for some of the more commonly used pathway building workflows.

Next, select an area of interest from the drop-down menu.

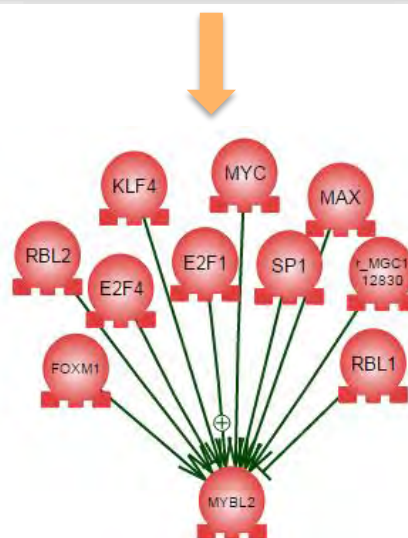


The screenshot shows the Pathway Studio Web 11.1 interface with the 'How Do I...' section. The dropdown menu is open, displaying a list of areas of interest: Gene/Protein Expression, Physical Interaction with Proteins, Protein Modification(s), Relations localized in a tissue/organ/cell type/cell line, Finding Connections between entities not directly connected, Protein /Small Molecule Transport, Proteins/Small molecules involved in chemical interactions, Protein/Small Molecule associations and changes in Diseases and Cell Processes, Small Molecule abundance, Clinical Trials, and Functional Associations between Diseases and Cell Processes. An orange arrow points to the 'Gene/Protein Expression' option in the dropdown menu.

Finally, select a specific workflow from the options provided. Once selected, the detailed instructions for building this pathway (using the Advanced Pathway Tool provided in the Network Builder) are provided on screen. For ease-of-use you can select to view the step-by-step instructions in a new window. See more description about the Advanced Pathway Tool later in this document.



The detailed workflows provided allow you to quickly and easily find answers to your biological questions.

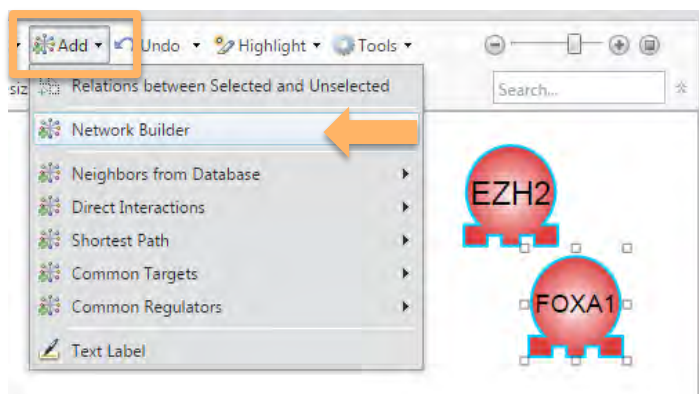


**Note:** Not all workflows are possible for all database types. For example, subscribers of the Mammal only database cannot examine networks that include relations between small molecules and diseases or cell processes as this data is only included in the ChemEffect<sup>®</sup> dataset. Subscribers to the Plant database could not, for example, see associations with Clinical Trials, as this is associated only with mammalian data and included in the DiseaseFx<sup>®</sup> dataset.

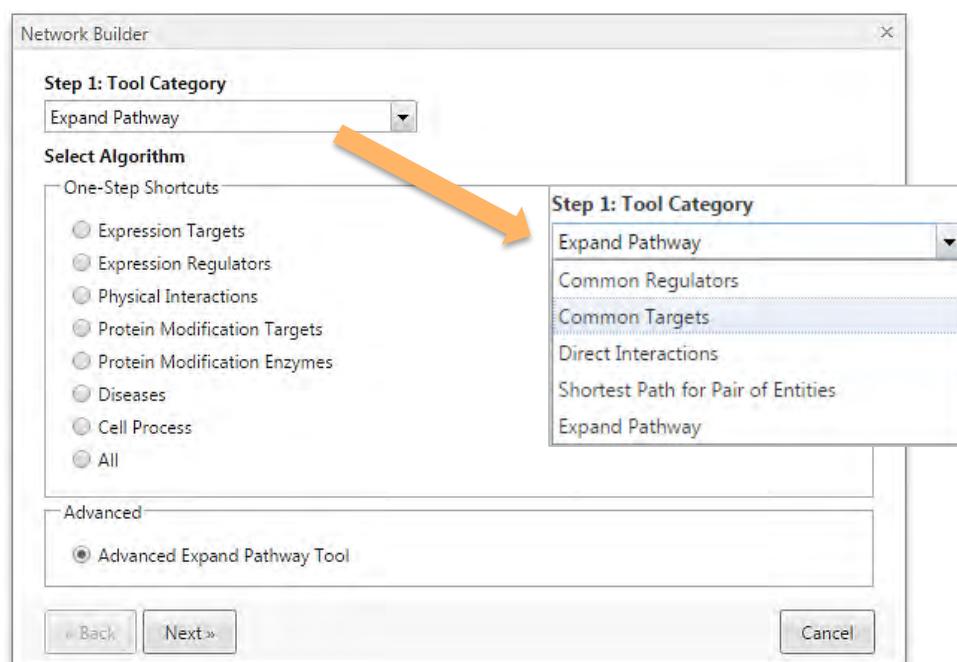
Now, let's take a closer step-by-step look at both basic and advanced network building workflows.

## Shortcut (Basic) Workflows for Network Building

The both the shortcut (basic) menus and the advanced pathway building tools can be accessed from the **Network Builder** dialog. With a pathway view open, select the desired entity/entities to start. Go to the Add menu and select "Network Builder."



In the Network Builder dialog, first select the type of network you want to build from the Tool Category drop-down menu in Step 1.

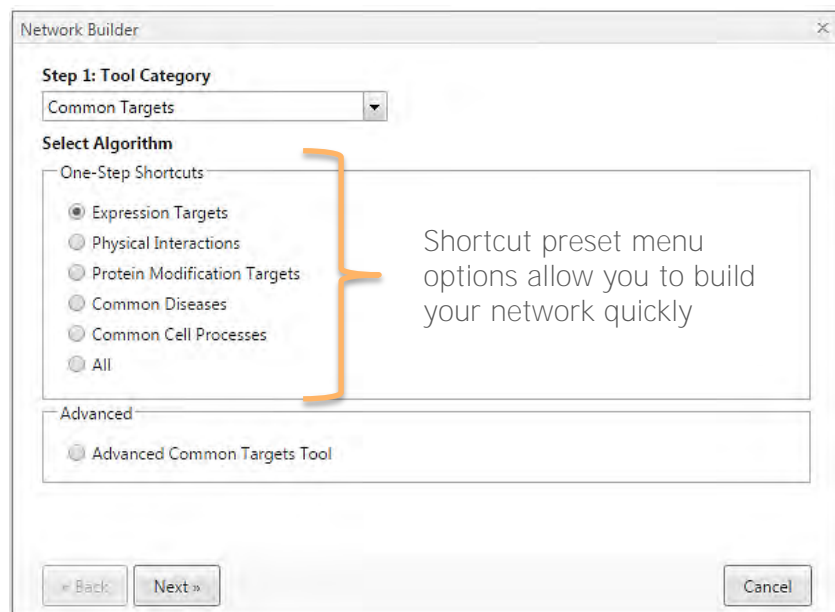


In Step 1, the tool definitions are as follows:

- **Direct Interactions:** find relationships between two or more selected entities
- **Shortest Path for a Pair of Entities:** find relationships between two selected entities, adding entities as needed to form the relationships
- **Expand Pathway:** find entities in the database directly connected to the entity /entities selected
- **Common Targets:** find one or more downstream targets that are regulated by at least two or more of the selected entities
- **Common Regulators:** find one or more upstream regulators that regulate two or more of the selected entities

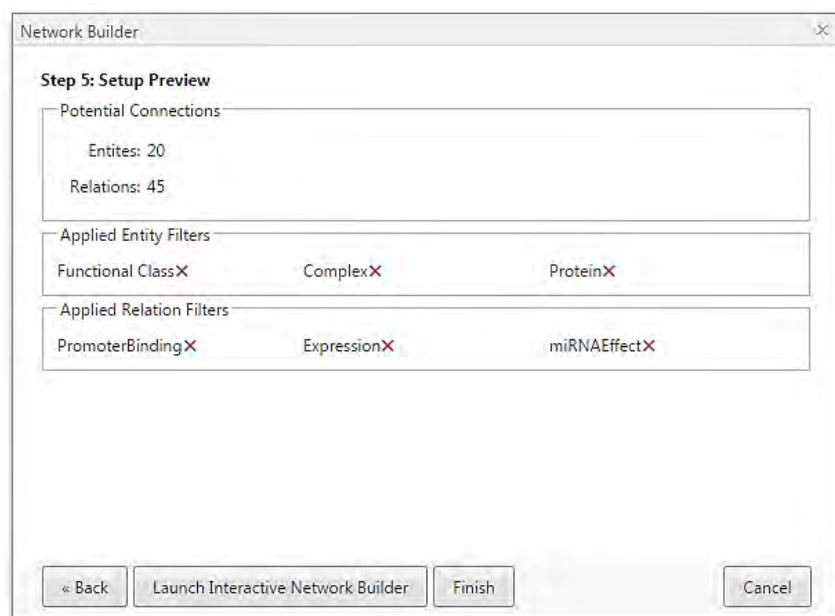


Once the desired build pathway algorithm is selected in Step 1, the One-Step Shortcuts menu directly below lists options that are available for that algorithm. Chose the desired shortcut and then select "Next."



**Appendix One** lists definitions of each shortcut option, including entity and relation selection, directionality and minimum limits (if applicable).

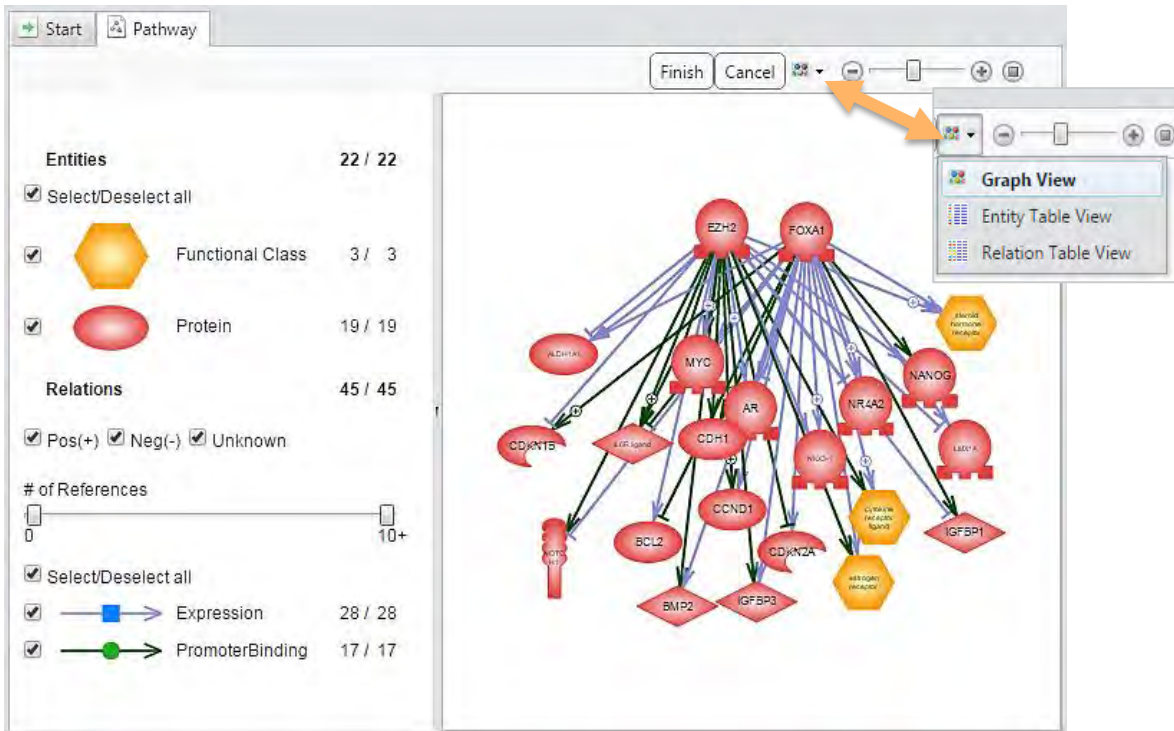
Next the Step 5 Setup Preview dialog appears. This displays the applied entity and relation filters, each followed by a red "x." If you want to further refine your network at this point by removing an entity or relation category, select the "x" to remove that category. [Note: once removed, the only way to add the entity or relation type back is to start your network building workflow from the beginning.]



At this point you can select "Finish" to see the resultant network.

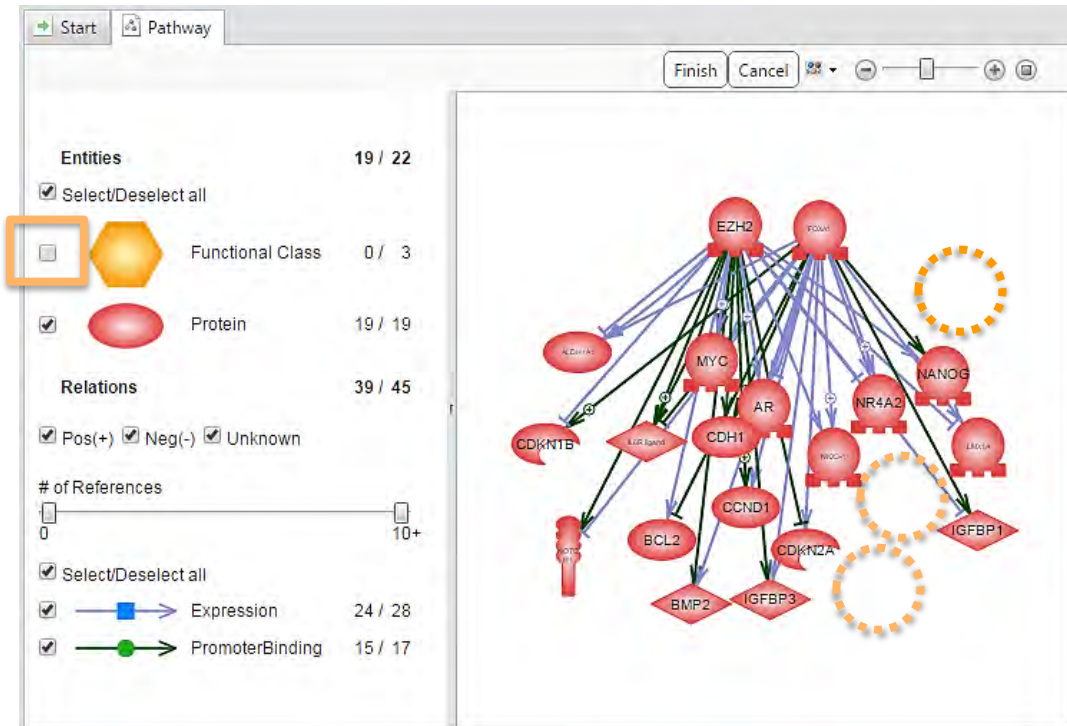
Alternatively, you can select "Launch Interactive Network Builder." This will allow you to see statistics of your network for entities and relations. It will allow you to filter your network by entity type, relation type, relation effect (positive, negative, unknown) and by the reference count for the relations.

The **Interactive Network Builder** is a powerful easy-to-use dialog to facilitate reducing larger networks to smaller more precise results.

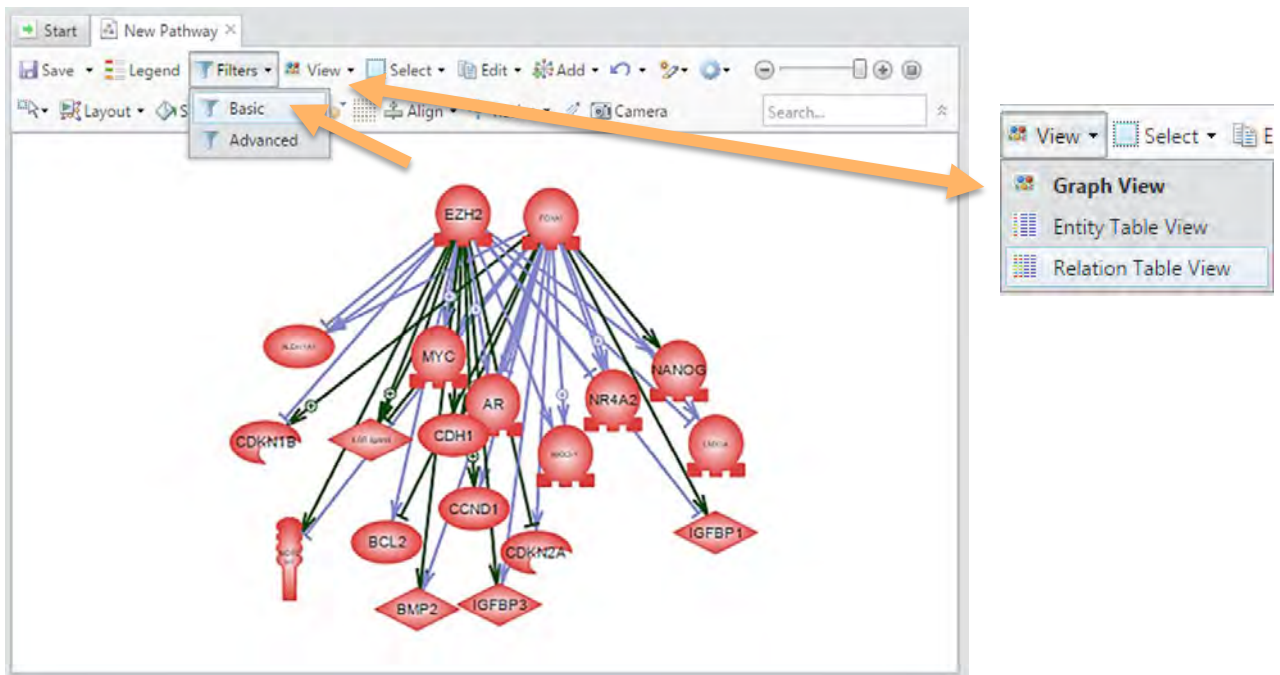


Note: If the network is larger than 200 entities, the graph view will not display on the right. Instead the Entity Table View will display. The checkbox filters on the left are still available.

In the example below, the Functional Class entity type was deselected (uncheck box). This removes the functional class entities from the resultant network.



After using the Interactive Network Builder to apply the desired filters, select the "Finish" button to see the resultant network. If your network is still large and you would like to apply additional filters, you can access the Interactive Network Builder again by selecting Filters > Basic from the graph view.

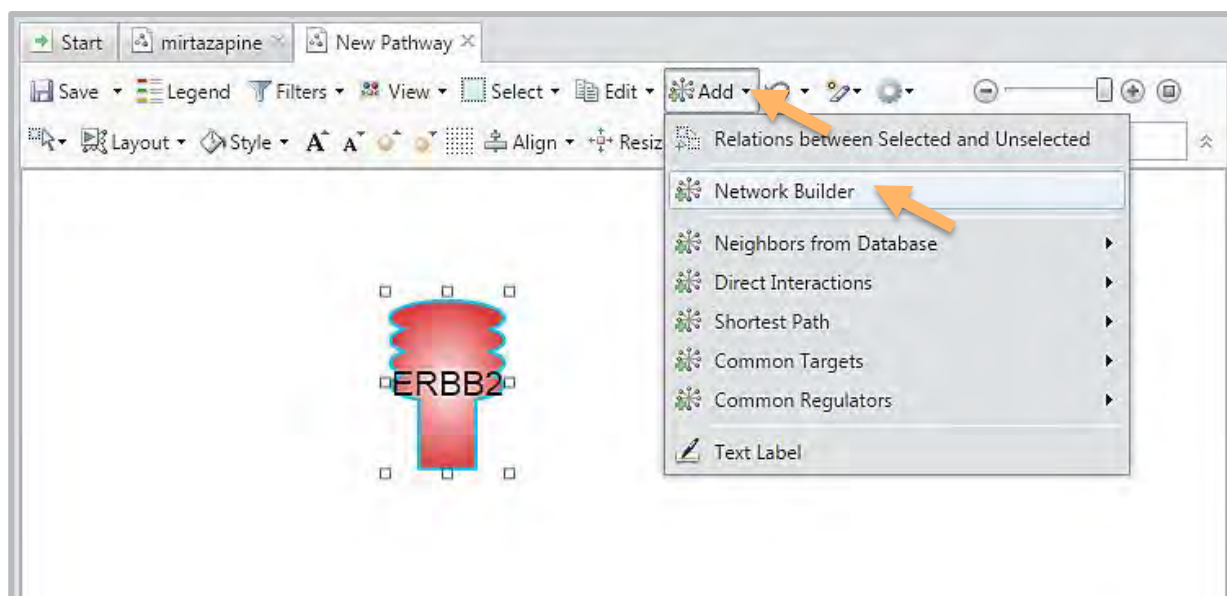


Network results can always be viewed in tabular form by selecting either Entity Table View or Relation Table View from the View menu.

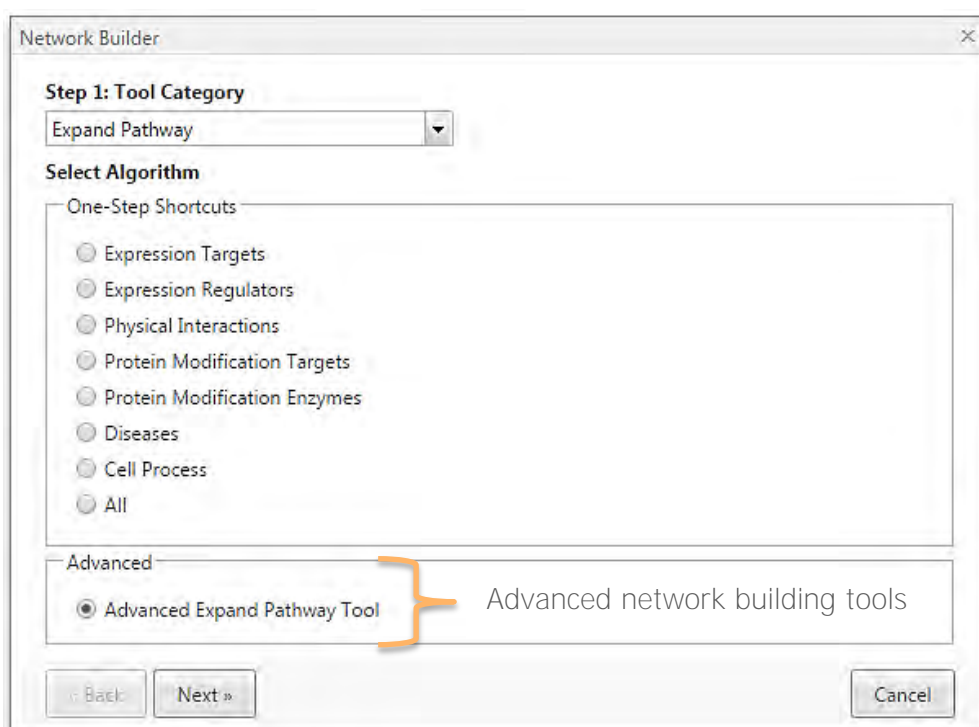
## Advanced Network Building Tools for Expert Users: Building Focused Networks

Pathway Studio Web 11.1 retains advanced pathway building tools that allow expert users to define very specific networks through advanced filtering options, utilizing relation subtype categories and other specific entity and relation properties fields. Filtering steps can be applied during the network building process or after the initial network has been completed.

Similar to the shortcut (basic) workflows, advanced workflows are accessed through the Network Builder in the Add menu.



In Step 1 of the Network Builder dialog, select the desired network type from the drop-down menu. Then select "Advanced Pathway Tool" in the Advanced box. Finally, chose "Next."

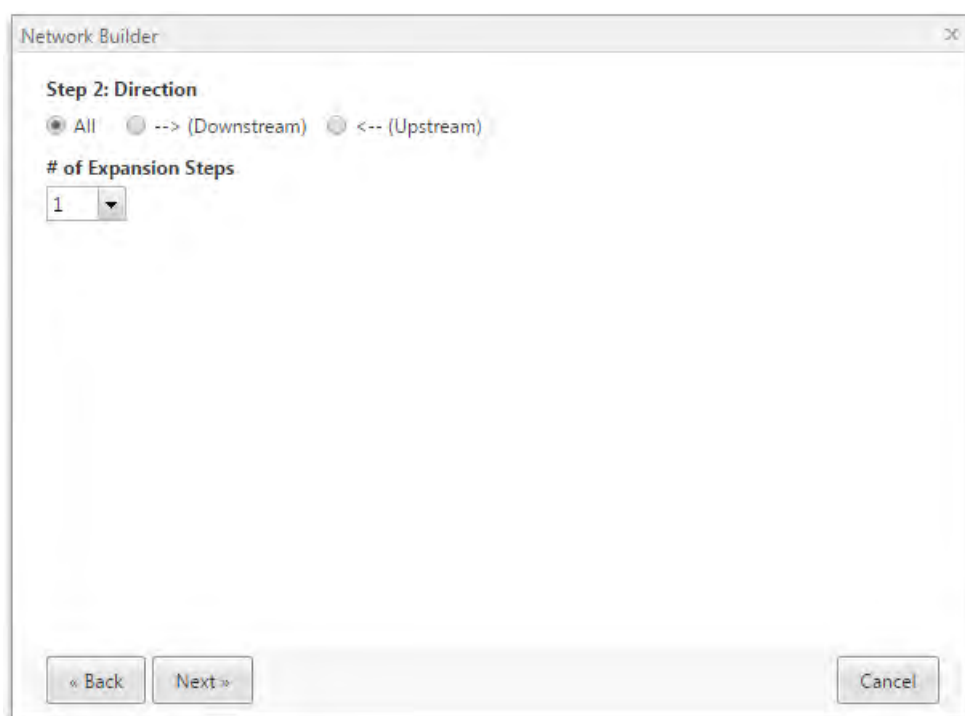




In Step 1, the tool definitions are as follows:

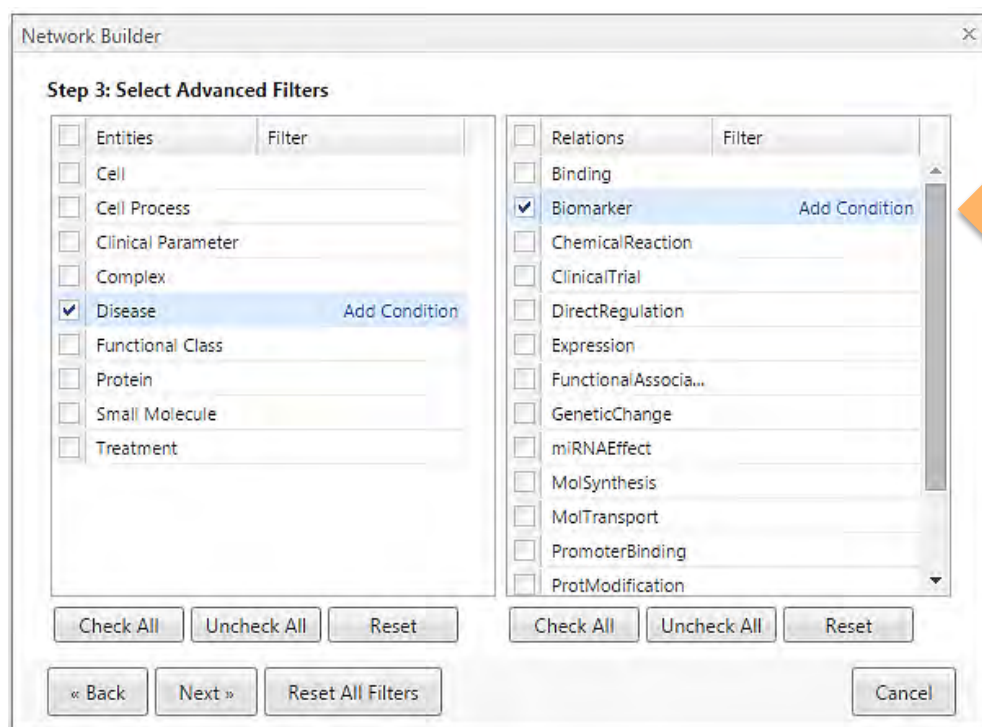
- **Direct Interactions:** find relationships between two or more selected entities
- **Shortest Path for a Pair of Entities:** find relationships between two selected entities, adding entities as needed to form the relationships
- **Expand Pathway:** find entities in the database directly connected to the entity /entities selected
- **Common Targets:** find one or more downstream targets that are regulated by at least two or more of the selected entities
- **Common Regulators:** find one or more upstream regulators that regulate two or more of the selected entities

In Step 2, select the desired directionality of the relations. For some algorithms, such as Common Targets, or Common Regulators, the directionality is already defined. If in doubt of the directionality of a specific relation type, select "All" at this step. For most applications keep the # of Expansion Steps at 1. Select "Next."



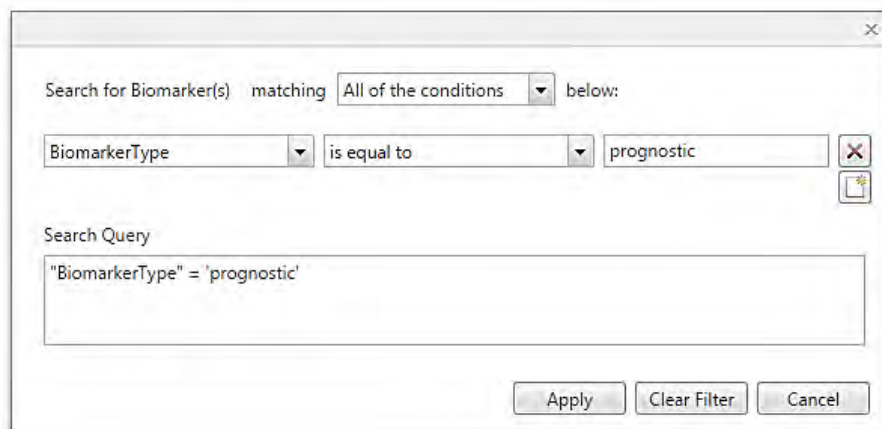
Next, select the entity type(s) and relation type(s) to include in the network. See **Appendix Two** for definitions of each entity and relation type. The entity and relation types displayed in this view will be dependent upon your individual Pathway Studio subscription.

When an entity or relation is selected, the "Add Conditions" options becomes available which allows for filtering based on specific properties of that entity or relation.

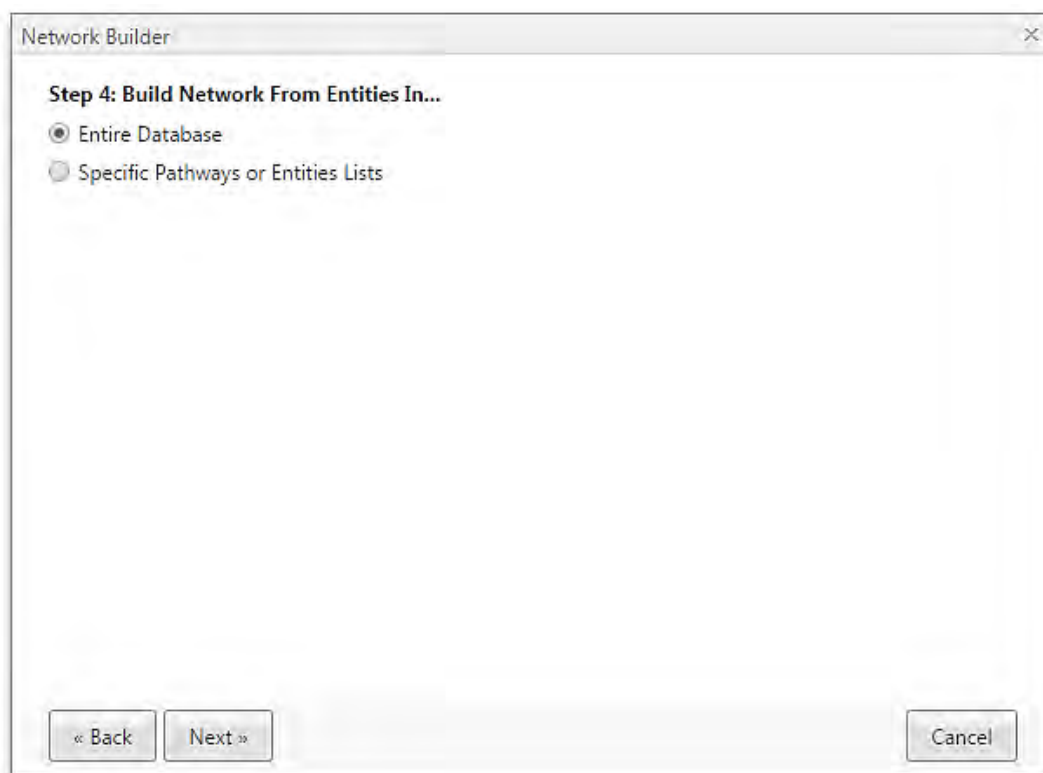


To apply advanced filtering on properties data for an **entity or relation**, click on **“Add Conditions.”** The advanced filtering dialog will appear. Select the properties field and the desired filtering parameter(s). In the example below, the applied filter will identify only Biomarker relations that have a property **“Biomarker Type” of “prognostic.”**

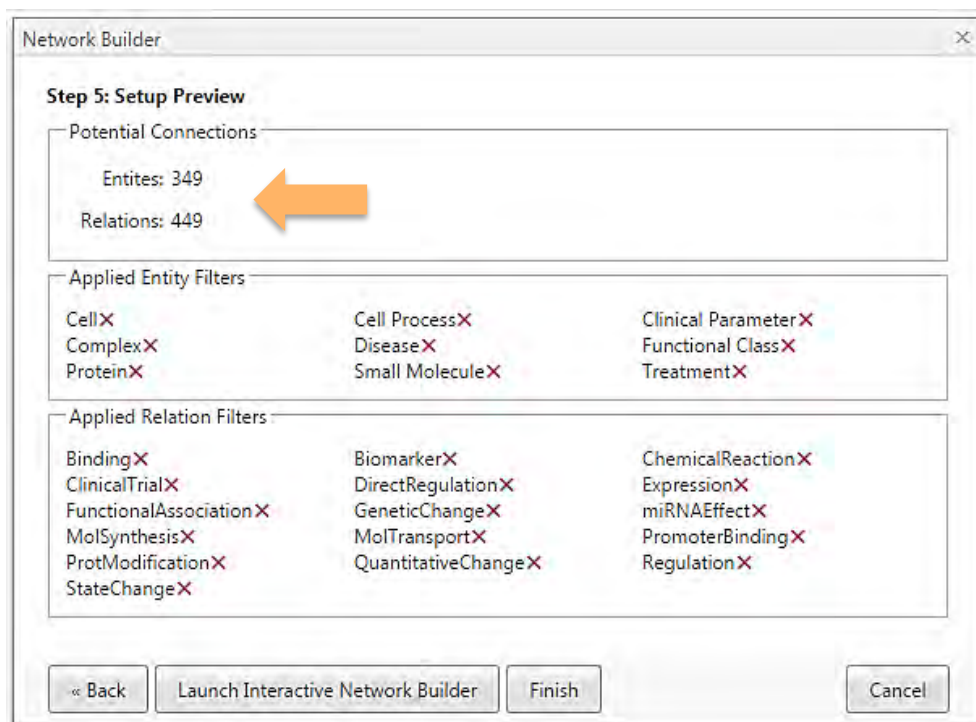
Note: Filtering on specific properties can be applied during network building, or later, after a network has been completed.



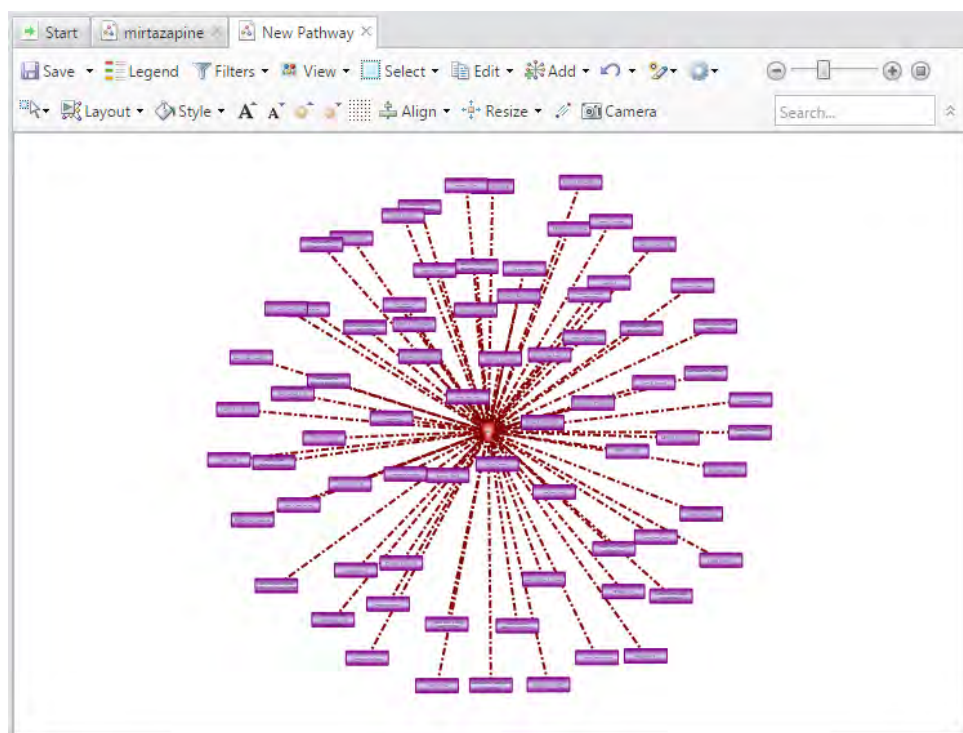
Next, select the source of entity and relation data for network building, either from the entire database (more common), or from a designated saved pathway/group. **Then select, “Next.”**



The Setup Preview step provides an additional opportunity to reduce the size of your network by removing an entity or relation type(s). Total number of entities and relations are summarized and the types included are listed. A specific entity or relation type can be removed by selecting the red "x." . [Note: once removed, the only way to add the entity or relation type back is to start your network building workflow from the beginning.]

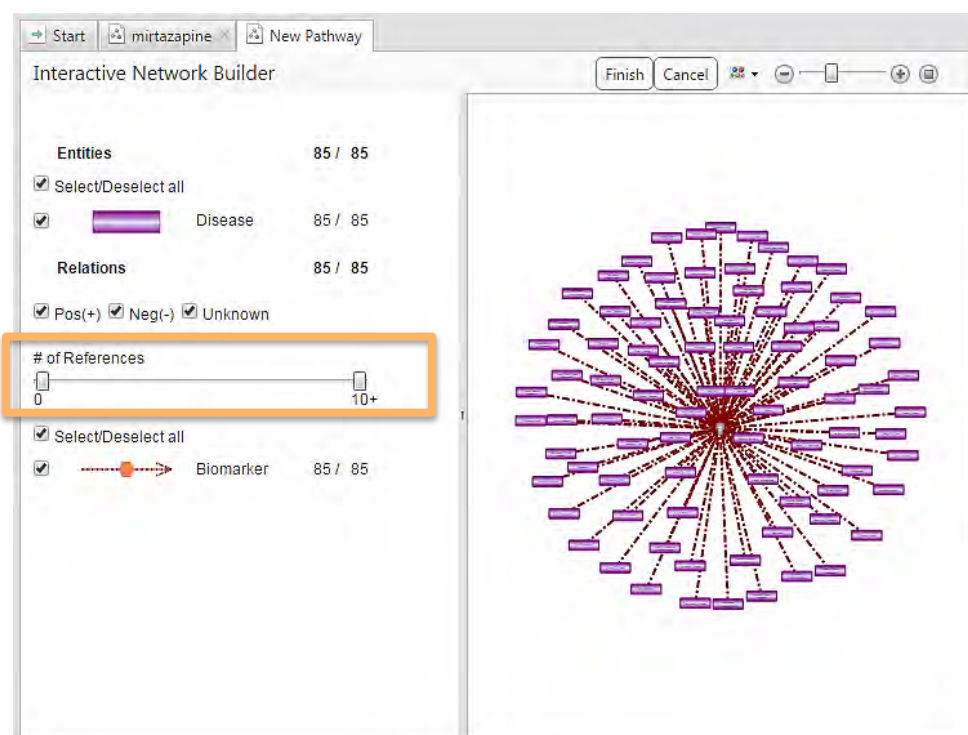


Select "Finish" to see your resulting network.



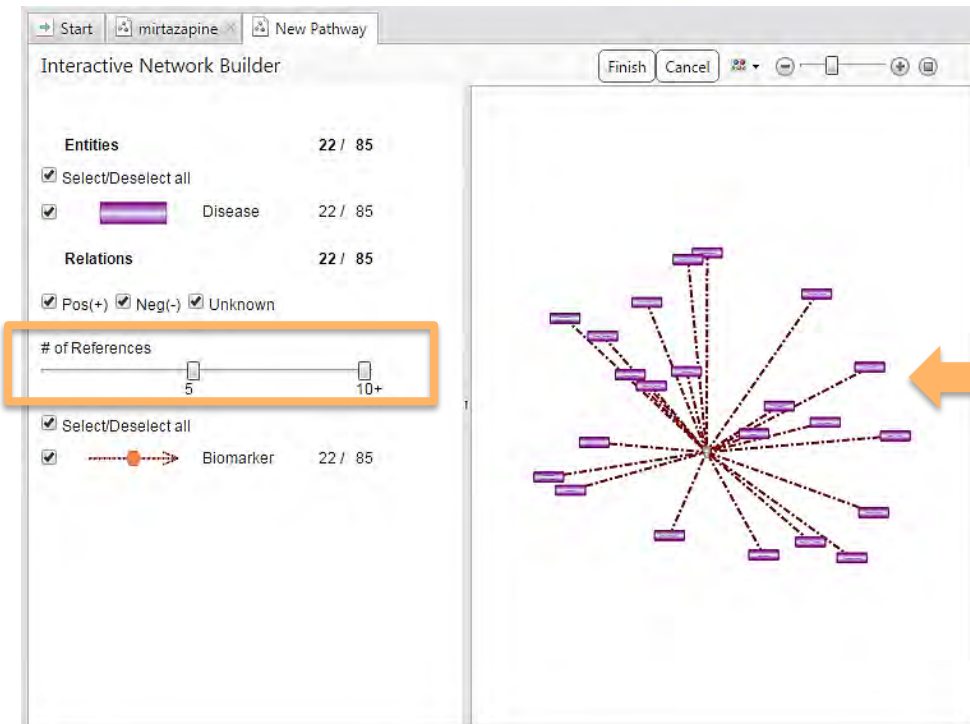
Alternatively, in Step 5 Setup Preview, you can select "Launch Interactive Network Builder." This will allow you to see statistics of your network for entities and relations. It will allow you to filter your network by entity type, relation type, relation effect (positive, negative, unknown) and by the reference count for the relations. The **Interactive Network Builder** is a powerful easy-to-use dialog to facilitate reducing larger networks to smaller more precise results.

In the Interactive Network Builder example shown in the following two figures a relation reference count filter of  $\geq 5$  references is applied to a network



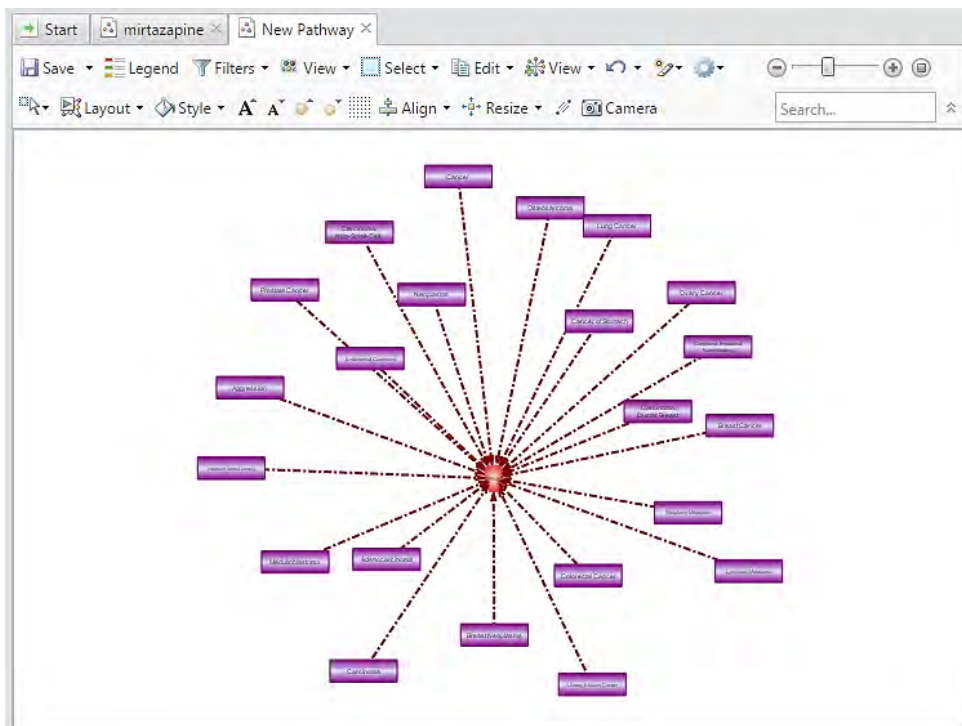


By moving the scroll bar for # of References to five, only relations with a reference of five or above remain.



The confidence level of the network is increased by including only relations with higher numbers of literature references.

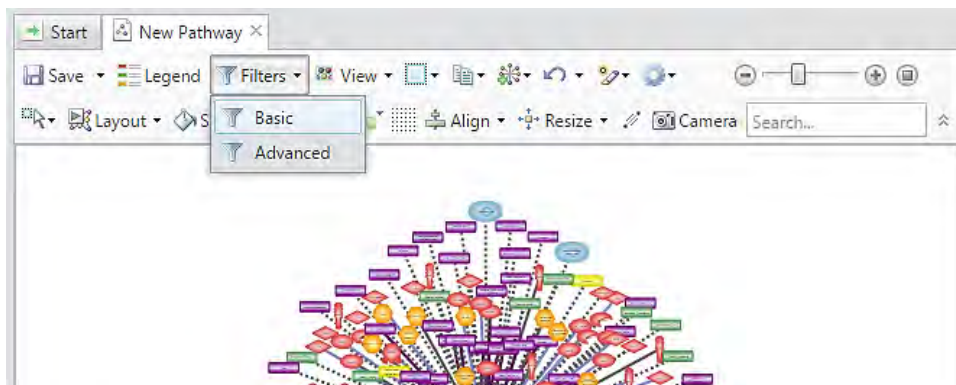
Select "Finish" to view the resultant network in the graph view.



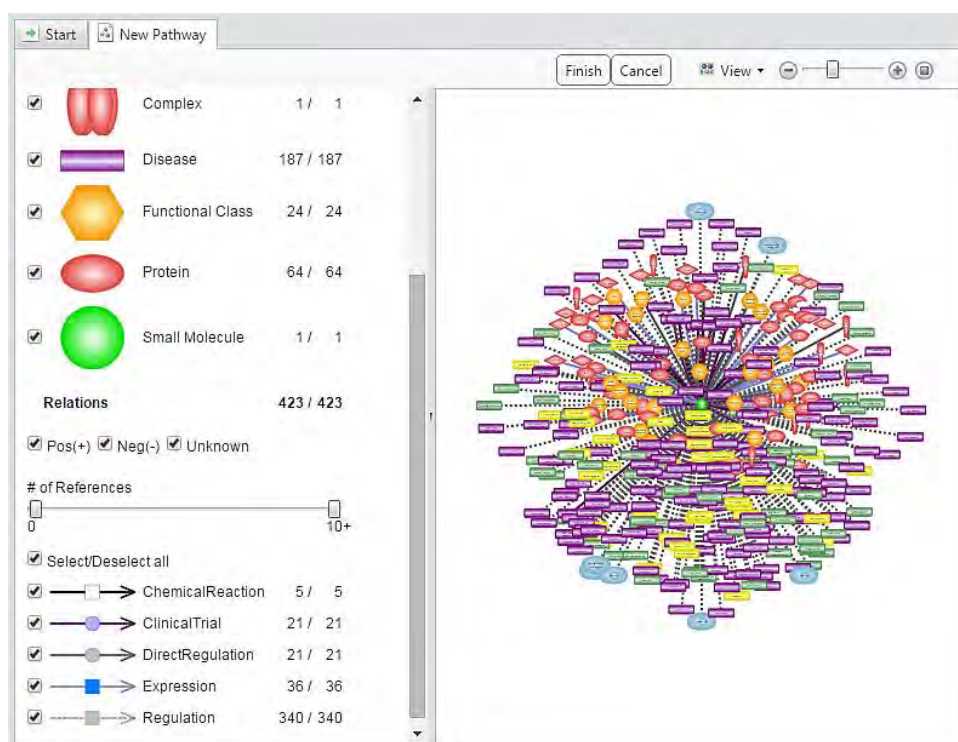
Once a network is completed, additional subtractive filters can be applied to reduce the network to more specific results. Alternatively, a subset of the network can be selected for further analysis.

### Subtractive Filter - Basic

To apply a subtractive filter to a network, in the graph view select Filter > Basic.



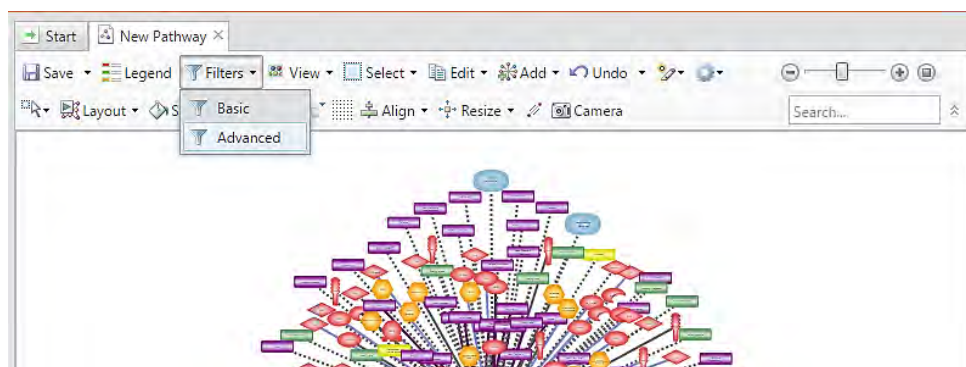
This will open the Interactive Network Builder. From here you can select to remove any entity or relation type, remove relations based on effect (positive, negative, or unknown), or filter relations based on the number of literature references. Simply uncheck the box to remove a specific category, or move the scroll bar to set the threshold for references for all relation.



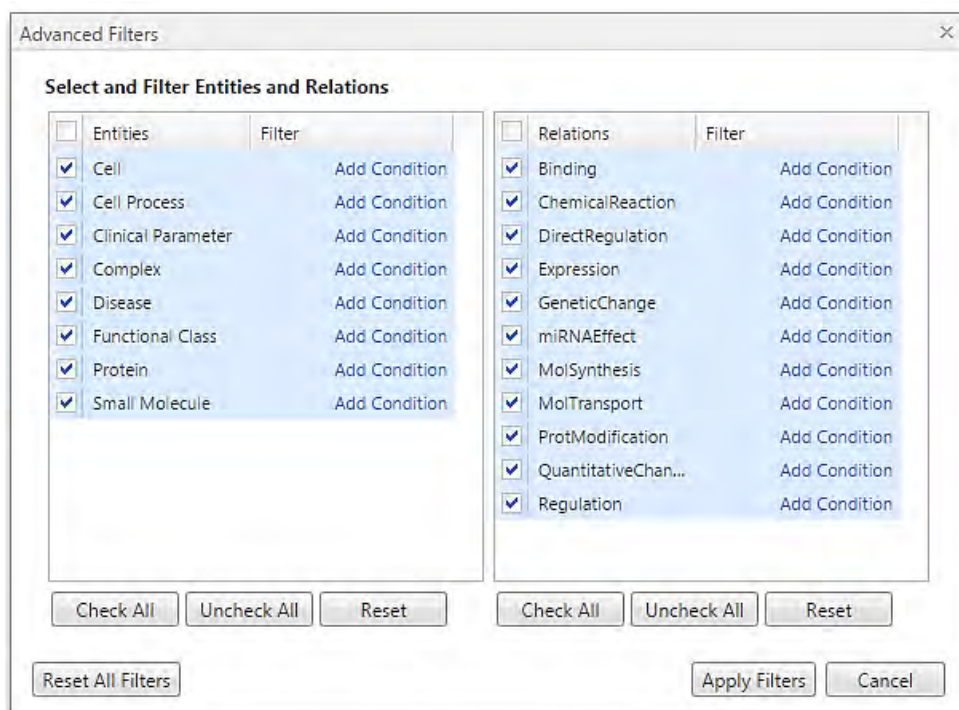
When done, select the "Finish" button to see the resultant network.

### Subtractive Filter - Advanced

To apply subtractive filters based on properties data, in the graph view select Filter > Advanced.



This will open the list of entities and relations included in the network. Select "Add Condition" to open the properties filter.

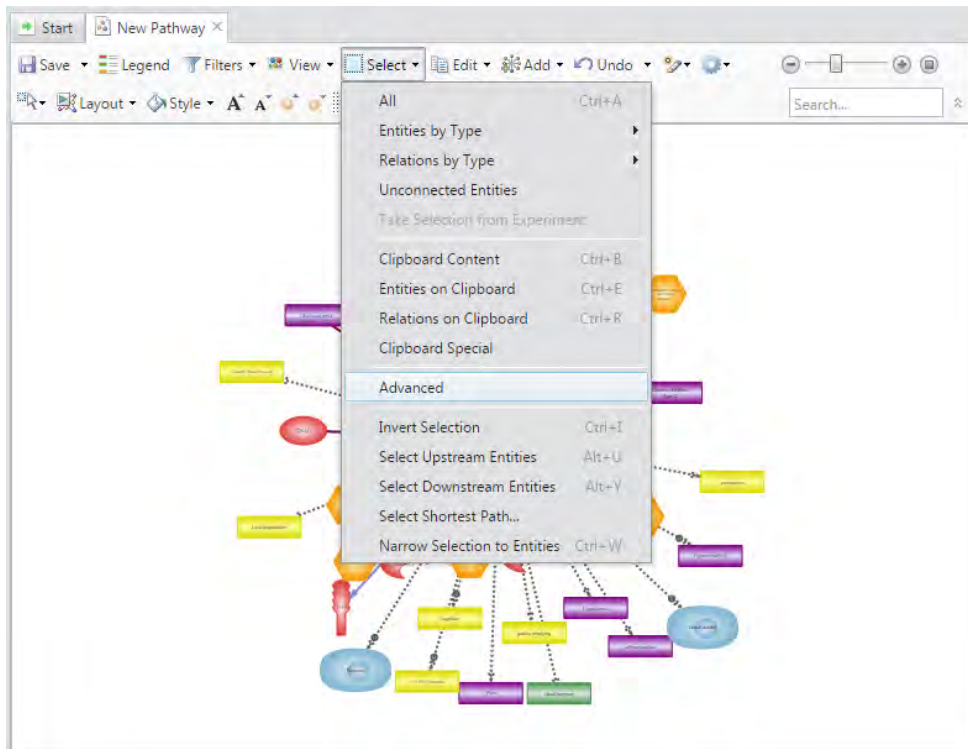


In this example, the properties filters identifies Regulation relations with a positive effect. By applying this filter, all Regulation relations that are NOT positive (in other words, negative or unknown Regulation relations) will be removed from the network.

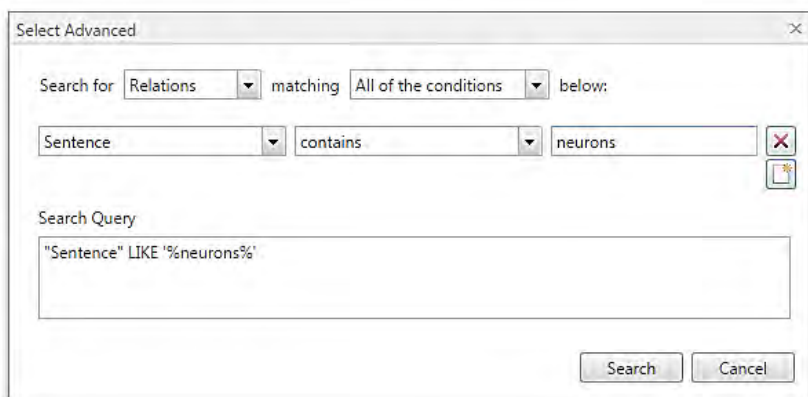


Selection Filters

To apply a positive selection to a network to go the Select menu and chose "Advanced." The advanced properties filter will appear.

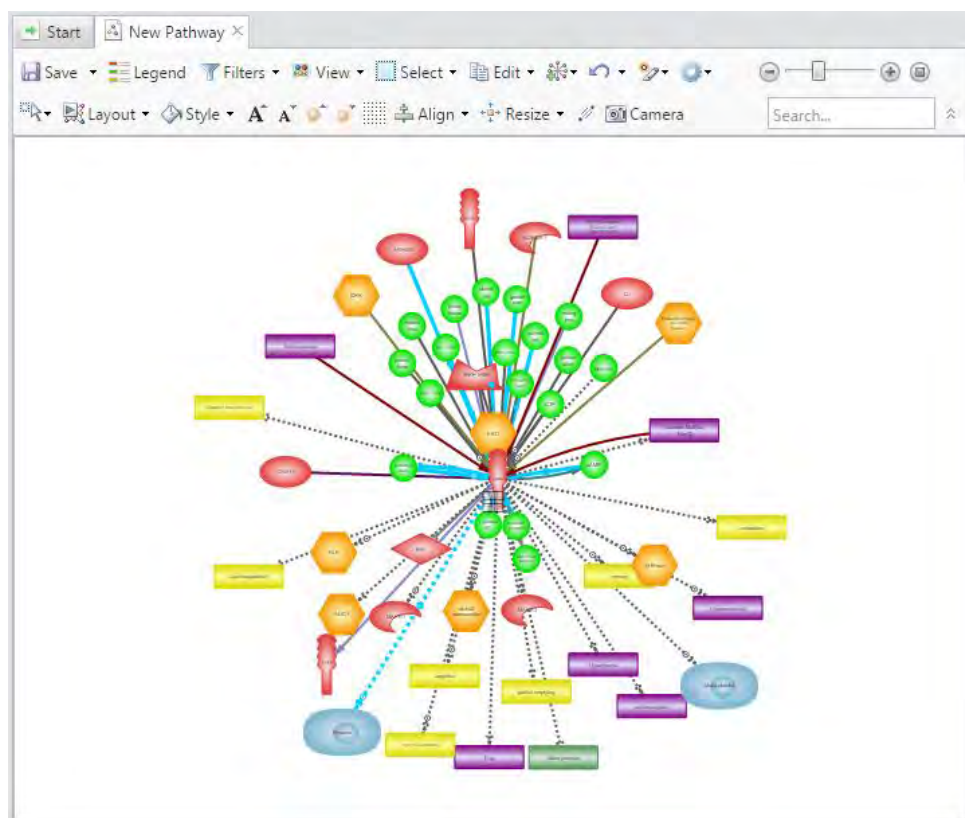


Use the Select Advanced menu to identify desired properties data. In the instance shown below, this search will identify relations with supportive sentences that contain the word "neuron." Select "Search."



The relations that have supportive sentences in their references that contain the key word are highlighted in blue.





Once the selection has been made, you can copy the highlighted relations into a new graph view for further analysis.

If you have any questions about Pathway Studio Web 11.1 contact [Customer Care](#).

**Appendix One: Pathway building shortcut definitions.**

| Category                   | Sub-Category                 | Relationship(s)  | Entity(ies)  | Direction  | Minimum Limits |
|----------------------------|------------------------------|--|--|------------|----------------|
| <b>Neighbors from DB</b>   | Expression Targets           | Expression, PromoterBinding, miRNAEffect   | Proteins, Complexes, Functional Classes                  | Downstream | NA             |
|                            | Expression Regulators        | Expression, PromoterBinding, miRNAEffect   | Proteins, Complexes, Functional Classes, small molecules | Upstream   | NA             |
|                            | Physical Interactions        | DirectRegulation, Binding  | Proteins, Complexes, Functional Classes, small molecules | All        | NA             |
|                            | Protein Modification Targets | ProtModification   | Proteins, Complexes, Functional Classes                  | Downstream | NA             |
|                            | Protein Modification Enzymes | ProtModification   | Proteins, Complexes, Functional Classes                  | Upstream   | NA             |
|                            | Diseases                     | Biomarker, ClinicalTrial, GeneticChange, QuantitiveChange, Regulation, StateChange   | Diseases   | All        | NA             |
|                            | Cell Processes               | ClinicalTrials, Regulation   | Cell Processes   | Downstream | NA             |
|                            | All                          | All  | All  | All        | NA             |
| <b>Direct Interactions</b> | Physical Interactions        | DirectRegulation, Binding  | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | Expression Regulation        | Expression, PromoterBinding, miRNAEffect   | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | Protein Modification         | ProtModification   | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | All                          | All  | All  | All        | NA             |
| <b>Shortest Path</b>       | Physical Interactions        | DirectRegulation, Binding  | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | Expression Regulation        | Expression, PromoterBinding, miRNAEffect   | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | Protein Modification         | ProtModification   | Proteins, Complexes, Functional Classes                  | All        | NA             |
|                            | All                          | DirectRegulation, Binding, Expression, PromoterBinding, miRNAEffect, ProtModification, Chemical Reaction, MolSynthesis, MolTransport | Proteins, Complexes, Functional Classes, small molecule  | All        | NA             |
| <b>Common Targets</b>      | Expression Targets           | Expression, PromoterBinding, miRNAEffect   | Proteins, Complexes, Functional Classes                  | Downstream | 2              |
|                            | Physical Interactions        | DirectRegulation, Binding  | Proteins, Complexes, Functional Classes                  | Downstream | 2              |
|                            | Protein Modification Targets | ProtModification   | Proteins, Complexes, Functional Classes                  | Downstream | 2              |

|                          |                              |   |   |            |   |
|--------------------------|------------------------------|---|---|------------|---|
|                          | Common Diseases              | Regulation, Clinical Trial  | Diseases                                | Downstream | 2 |
|                          | Common Cell Processes        | Regulation, Clinical Trial  | Cell Processes                          | Downstream | 2 |
|                          | All                          | All   | All                                     | Downstream | 2 |
| <b>Common Regulators</b> | Expression Regulators        | Expression, PromoterBinding, miRNAEffect                              | Proteins, Complexes, Functional Classes | Upstream   | 2 |
|                          | Physical Interactions        | DirectRegulation, Binding   | Proteins, Complexes, Functional Classes | Upstream   | 2 |
|                          | Protein Modification Enzymes | ProtModification  | Proteins, Complexes, Functional Classes | Upstream   | 2 |
|                          | Common Diseases              | Biomarker, GeneticChange, QuantitativeChange, Regulation, StateChange | Diseases                                | Upstream   | 2 |
|                          | All                          | All   | All                                     | Upstream   | 2 |

## Appendix Two: Entity and Relation Definitions.

### **ENTITIES:**

**Cell** – mammal cell types, including immunology subtypes  
(mammal+ChemEffect+DiseaseFx+CellEffect only)

**Cell Process** \* – biological processes, most coincide with Gene Ontology.

**Clinical Parameter** – measured parameters of the human body used in clinical practice (mammal and mammal+CE+DFx only).

**Complex** \* – several polypeptides that form a complex via physical interactions.

**Disease** – Mammal: health conditions and disease terms from MeSH; plant: Plant diseases.

**Functional Class** \* – most functional classes coincide with Gene Ontology.

**Protein** – defined by Entrez Gene - represents both genes and the gene products, including proteins and miRNAs.

**Small Molecule** – Mammal: naturally occurring metabolites and small molecules found in cells; ChemEffect® adds drugs (including some biologically active polypeptides that work as drugs such as monoclonal antibodies) and nonnaturally occurring small molecules to the mammal database. Plant: naturally occurring metabolites and small molecules and other plant related chemicals (ex. herbicides or research related chemicals).

**Treatment** – non-chemical treatments and environmental conditions, such as cold shock.

*\*Container Entities – these are valid entities but also can have proteins mapped to them. You can see the proteins for the container entities in the "child concepts" in the property records for the specific entity.*

### **RELATIONS:**

(Mammal and Plant):

**Binding** - direct physical interaction between two molecules.

**ChemicalReaction** - enzyme catalyzed reaction involving small molecules.

**DirectRegulation** - influences target activity by direct physical interaction (excluding promoter binding interactions).

**Expression** - regulator changes protein abundance by affecting levels of transcript or protein stability. miRNAEffect the inhibitory effect of a miRNA on its mRNA target.

**Regulation** - changes the activity of the target by an unknown mechanism (may be direct or indirect). This is a less specific relation type than others provided.

**MolSynthesis** - regulator changes the concentrations of the target (usually a small molecule target).

**MolTransport** - regulator changes the localization of the target (molecular translocation, export, import etc.).

**PromoterBinding** - regulator binds to the promoter of a gene.

**ProtModification** - regulator changes the modification of the target molecule, usually by a direct interaction.

Filtering Field Name: Mechanism; Sub-Categories: acetylation, cleavage, deacetylation, demethylation, dephosphorylation, direct interaction, methylation, phosphorylation, posttranscriptional inhibition, proteolysis, ubiquitination.



(Mammal + ChemEffect+DiseaseFx + CellExpress – in addition to relations listed above):

**CellExpression** - proteins expressed in a particular cell type

Filtering Field Name: Mechanism; Sub-category: surface (or null)

**Quantitative Change** - Changes in abundance/activity/expression of a gene/protein/small molecule in a disease state (between disease-protein/complex/functional class/small molecules).

Filtering Field name: Quantitative Type; Sub-Categories: Expression, Abundance, Activity, Secretion

**Genetic Change** - Genetic changes in a gene in a disease state such as gene deletions, amplifications, mutations or epigenetic changes (between diseaseprotein/complex/functional class).

Filtering Field Name: Change Type; Sub-Categories: Gene Deletion, Mutation, Gene Amplification, Epigenic methylation

**Biomarkers** - Identification of proteins/complexes/functional classes/metabolites that are prognostic or diagnostic biomarkers for a disease (between diseaseprotein/complex/functional class/naturally occurring small molecules).

Filtering Field Name: Biomarker Type; Sub-Categories: Diagnostic, Prognostic

**State Change** - Changes in a protein's posttranslational modification status or alternative splicing events associated with a disease (between diseaseprotein/ complex/functional class).

Filtering Field Name: Change Type; Sub-Categories: Alternate Splicing, Phosphorylation

**Functional Association** - Different types of functional associations between a disease and a cellular process or another disease (between Disease – Cell Process) (no sub-types).

**Clinical Trials** - Disease/cell process relation representing clinical trials conducted for a drug against a disease (from ClinicalTrials.gov) (between Disease/Cell Process – Small Molecule) (no sub-types).