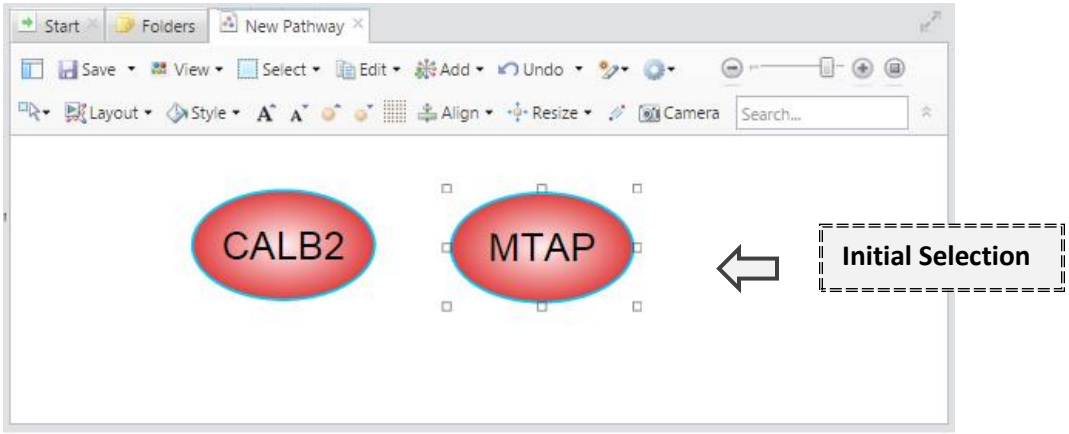
USING PATHWAY STUDIO® TO ANSWER BIOLOGICAL QUESTIONS BY PATHWAY BUILDING

This tutorial describes how to use the powerful build pathway wizard in Pathway Studio to find answers to biological questions. The first section demonstrates the build pathway wizard through step by step visual instructions. The table that follows provides quick summaries of dozens of biological questions and the settings used in the wizard to answer them. Note that the critical settings that are summarized in following table are highlighted in the images by a dotted line surround text box like this:

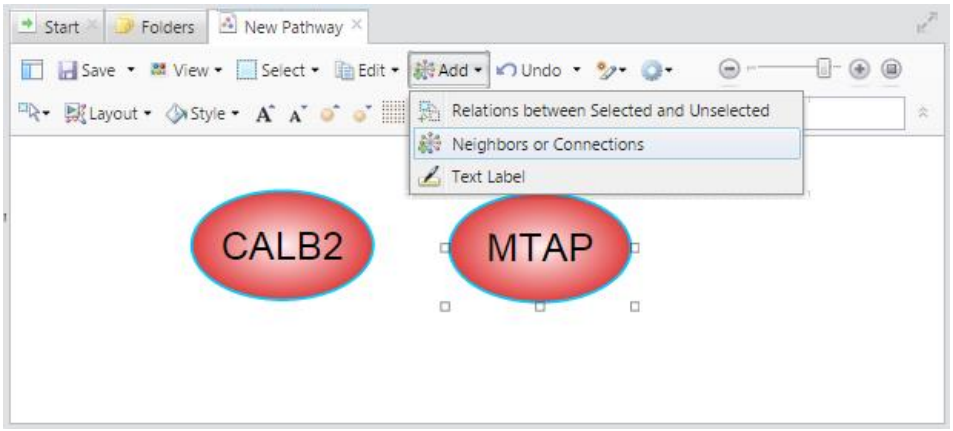
Indicates Wizard Selection

INTRODUCTION TO THE BUILD PATHWAY WIZARD

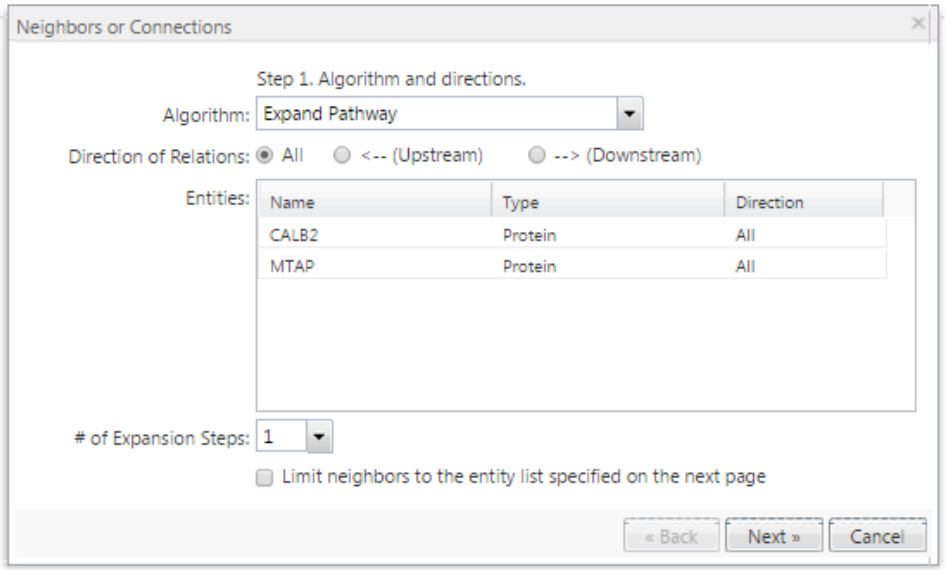
To start building a pathway, open a new pathway view and add the entity(s) of interest. Next, select these entities. (Selected entities will have a blue highlight.)



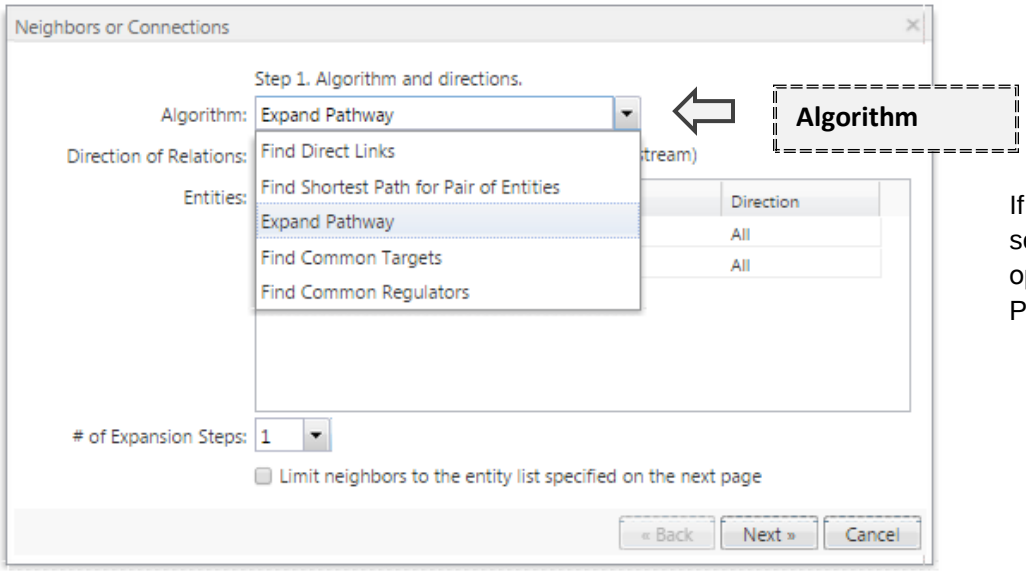
With the entities selected, go to the Add menu and select “Neighbors and Connections.”



The build pathway wizard window will open.



Select the desired algorithm from the drop down menu.

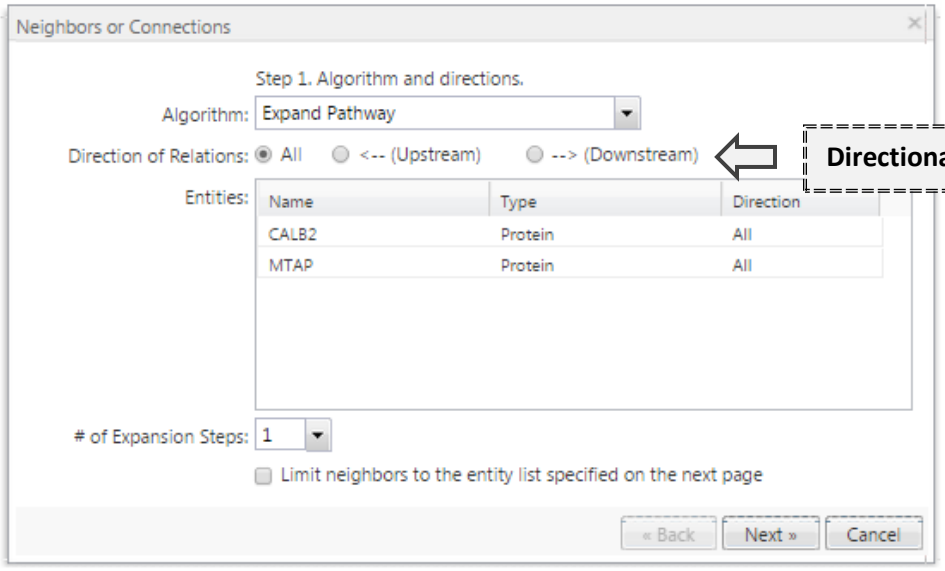


If only one entity is initially selected then the only option available is “Expand Pathway.”

The table below provides a brief description of each build pathway algorithm. Each algorithm allows you to answer different types of biological questions.

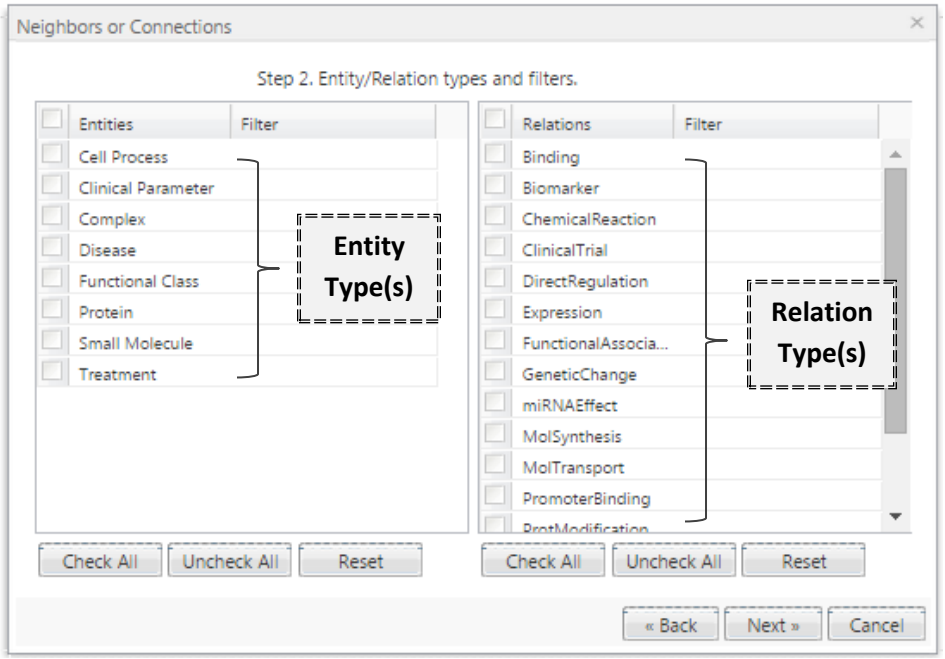
Algorithm	Definition
Find Direct Links	find relationships between two or more selected entities
Find Shortest Path for a Pair of Entities	find relationships between two selected entities, adding entities as needed to form the relationships
Expand Pathway	find entities in the database directly connected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

Next, select the desired directionality of the relation(s).



Two relation types: "binding" and "functional association" do not have directionality assigned.

Now select the entity type(s) and relation type(s) desired.

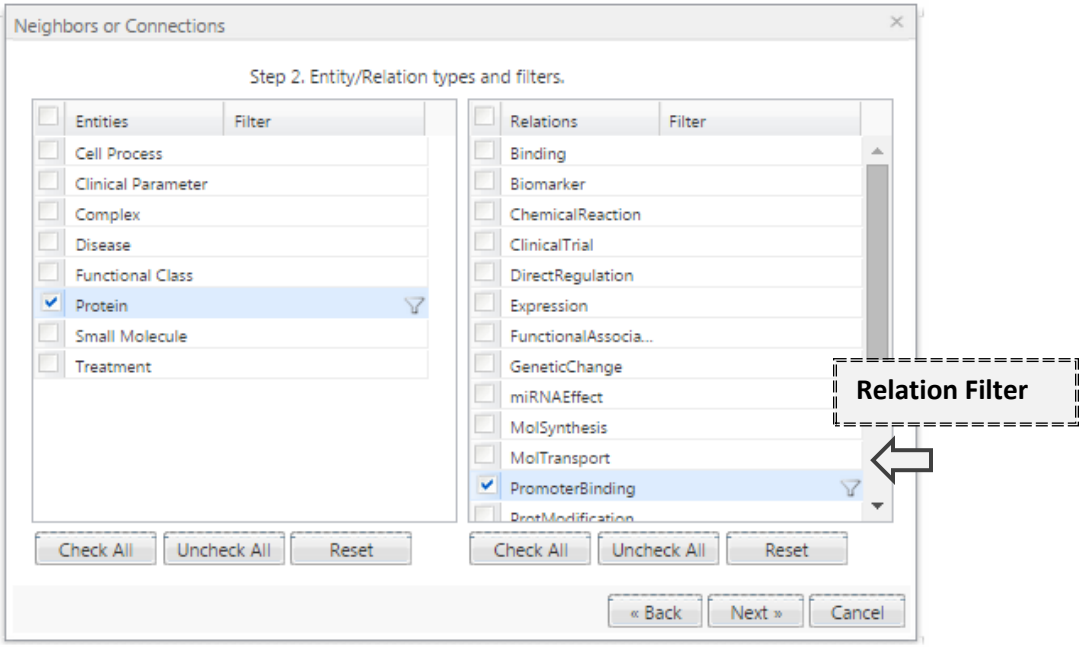


Please see the "**Quick Reference Guide to the Data Model**" found on the support page: http://help.elsevier.com/app/answers/detail/a_id/2683/p/9049 for detailed definitions of the entity and relation terms.

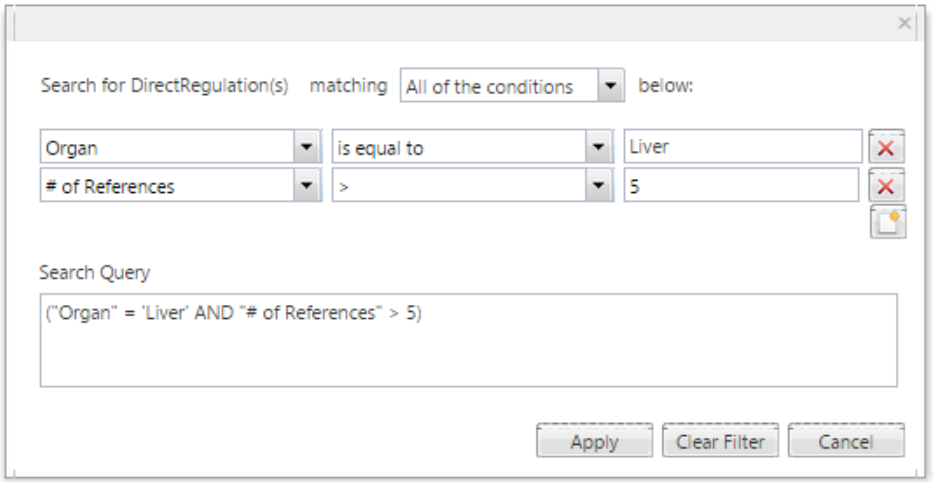
Points to consider:

- It is reasonable to combined "protein" and "functional class" and "complex" when defining queries that include proteins.
- It is best practice to select the most specific relation types first when building a pathway. If in doing so, the resultant pathway is small, you can consider adding addition relations to the pathway to expand the results.
- Regulation is the least specific all relation types. Use more specific relations first, when possible, and include regulation only if the more specific relations did not identify desired results.

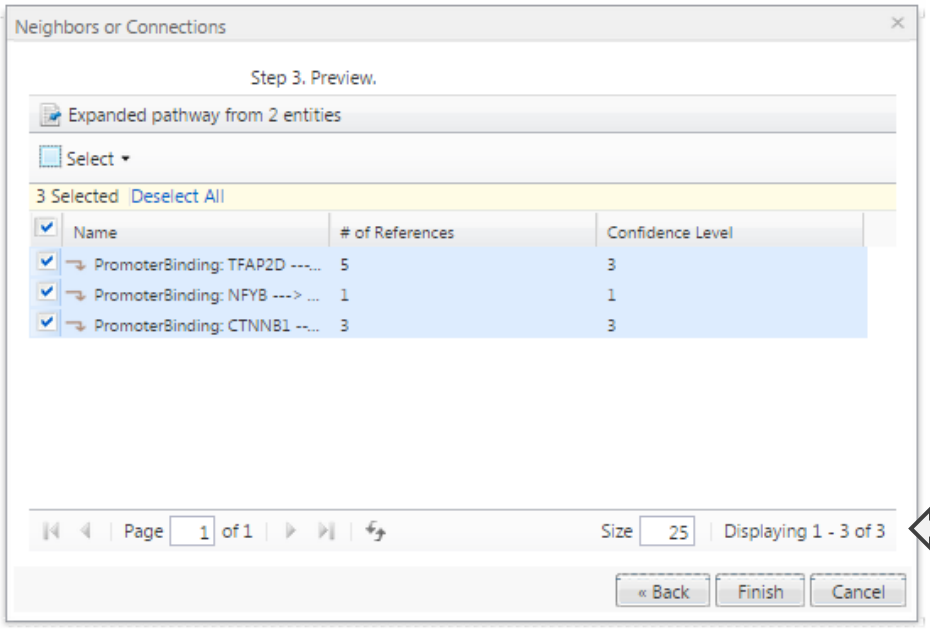
When an entity or relation is selected, a filter icon appears to the right of it.



The filter dialog allows for the definition of more specific queries based on filtering by entity or relation attribute information contained in the database. This is a powerful tool for answering more specific biological questions.

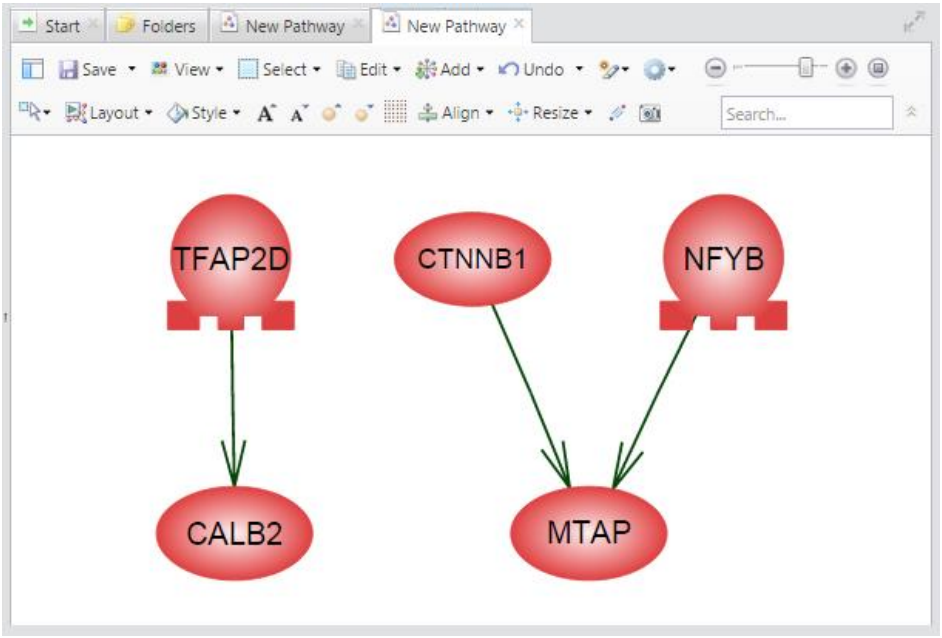


When all the selections have been made in the wizard, a final results summary screen appears. Select “Finish” to view the resulting pathway.



Please note the resultant number of relations that will be displayed (3 in this example). If this number is in the thousands, consider using the back button and applying more specific filters to reduce the size of the network.

The results of the query are displayed in the pathway view.



EXAMPLES OF BIOLOGICAL QUESTIONS AND THE BUILD PATHWAY WIZARD SETTINGS USED TO FIND RESULTS

This section provides many commonly used workflows for building pathways in Pathway Studio. Each example defines the biological question, provides the wizard setting used to build the desired pathway to answer the question and additional considerations to better clarify or refine results.

	Question	Wizard Selections	Considerations
Gene/Protein Expression			
1	What proteins (transcription factors) bind to the promoter of a gene(s)?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** promoterbinding	Finds transcription factors for genes (directly binding to promoters)
2	What predicted miRNAs may regulate expression of a gene(s)?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** miRNAEffect Build the pathway, switch to the Relation Table view, add "Source" to the table and sort on that column. Relations with Source annotation of public datasets "miRanda" "TargetScan" "PicTar" "TarBase" etc are predicted miRNA targets.	Finds predicted miRNA targets (from public prediction datasets - identified in "source" annotation field of the relation)
3	What known miRNAs regulate expression of a gene(s)?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** miRNAEffect Build the pathway, switch to the Relation Table view, add "Sentence" to the table and sort on that column. Any relation with a supporting sentence is a literature confirmed relation.	Finds literature confirmed miRNA targets. (Differentiate from predicted miRNAEffect relations by reference annotation)
4	What proteins are involved in the expression of a gene(s), either directly or indirectly?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** promoterbinding + expression	Finds both direct expression regulators (promoterbinding) and proteins with possibly an indirect effect on expression (expression)
5	Does a group of genes share common expression regulators?	**initial selection:** proteins **algorithm:** find common regulators ***connectivity minimum:*** 2 or higher **entity type:** protein **relation type:** promoterbinding + expression	Finds common expression regulators that impact multiple targets in a select group If promoterbinding + expression gives too many results, try examining only promoterbinding

	Question	Wizard Selections	Considerations
6	Does a group of proteins regulate expression of similar gene(s)?	**initial selection:** proteins **algorithm***:* find common targets ***connectivity minimum:*** 2 or higher **entity type:** protein **relation type(s):** promoterbinding + expression	Finds targets that share a common regulator from a select group If promoterbinding + expression gives too many results, try examining only promoterbinding
Physical Interaction with Proteins			
7	What proteins bind to a protein?	**initial selection:** protein **algorithm:** binding **directionality:** (all) **entity type:** protein **relation type:** binding	Identifies protein binding partners (no additional regulatory event known) Binding relations have no directionality (DirectRegulation is regulation through a direct physical interaction and can also be considered here.)
8	What small molecules bind to a protein?	**initial selection:** protein **algorithm:** binding ***directionality:*** (all) **entity type:** small molecules **relation type:** binding	Identifies small molecules that bind to a protein (no additional regulatory event known) (Drugs/non-naturally occurring small molecules included in ChemEffect data) (DirectRegulation is regulation through a direct physical interaction and can also be considered here.)
9	What proteins regulate a protein through a direct physical interaction?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** directregulation	Finds proteins that regulate the activity of a target protein through a direct physical interaction Can also consider "protmodification" relations
10	What small molecules regulate a protein through direct physical interactions?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type***:* small molecule **relation type:** directregulation	Finds small molecules that regulate the activity of a protein through a direct physical interaction (Drugs/non-naturally occurring small molecules included in ChemEffect data)
Protein Modification(s)			
11	What protein(s) acetylate/deacetylate a protein?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** protmodification **applied relation filter:** mechanism "is equal to" acetylation or deacetylation	Identifies proteins involved in acetylation/deacetylation of target protein(s).
12	What protein(s) cleave a protein?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** protmodification *applied relation filter:* mechanism "is equal to" cleavage	Identifies proteins involved in the proteolytic cleavage of target protein(s).

	Question	Wizard Selections	Considerations
13	What proteins(s) methylate/demethylate a protein?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** protmodification *applied relation filter:* mechanism "is equal to" methylation/demethylation	Identifies proteins involved in the methylation/demethylation of target protein(s).
14	What protein(s) phosphorylate/dephosphorylate a protein?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type***:* protein **relation type:** protmodification *applied relation filter:* mechanism "is equal to" phosphorylation/ dephosphorylation	Identifies protein(s) involved in the phosphorylation/ dephosphorylation of target protein(s).
15	What protein(s) ubiquitinate a protein?	**initial selection:** protein **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** protmodification *applied relation filter:* mechanism "is equal to" ubiquitination	Identifies proteins involved in the ubiquitination of target protein(s).
Relations localized in a tissue/organ/cell type/cell line			
16	What relations are known to be found in a specific organ?	In Build Pathway Wizard, for each relation type, select relation filter: organ "is equal to" and select desired organ from controlled vocabulary. Alternatively: After pathway is built, in the Relation Table View, add column for "organ."	Identifies relations with annotation of organ type.
17	What relations are known to be found in a specific tissue?	In Build Pathway Wizard, for each relation type, select relation filter: tissue "is equal to" and select desired tissue from controlled vocabulary. Alternatively: After pathway is built, in the Relation Table View, add column for "tissue."	Identifies relations with annotation of tissue type.
18	What relations are known to be found in a specific cell type?	In Build Pathway Wizard, for each relation type, select relation filter: celltype "is equal to" and select desired cell type from controlled vocabulary. Alternatively: After pathway is built, in the Relation Table View, add column for "celltype."	Identifies relations with annotation of Cell type.

	Question	Wizard Selections	Considerations
19	What relations are known to be found in a specific cell line name?	In Build Pathway Wizard, for each relation type, select relation filter: celllinename "is equal to" and select desired cell line from controlled vocabulary. Alternatively: After pathway is built, in the Relation Table View, add column for "celllinename."	Identifies relations with annotation of cell line name.
Finding Connections between entities not directly connected			
20	Can two proteins not directly connected, be connected through protein / small molecule intermediates?	**initial selection:** pair of proteins **algorithm:** find shortest path for a pair of entities **directionality:** all **entity type(s):** proteins + small molecules **relation type(s):** binding, chemical reaction, directregulation, expression, miRNAEffect, molsynthesis, moltransport, promoterbinding (physical interaction relations)	Identifies shortest path of molecular connection (physical interactions) between two proteins in the database through proteins/small molecule intermediates through selected relations type. (Can be extended to non-physical interactions by selecting additional relation types) Note: Regulation is the least specific relation type and should be excluded unless more specific relations do not produce results.
21	Can two proteins not directly connected, be connected through association to diseases/cell processes?	**initial selection:** pair of proteins **algorithm:** find shortest path for a pair of entities **directionality:** all **entity type(s):** cell process + disease **relation type:** regulation	Identifies if unconnected proteins share connections to similar diseases or cellular processes. (Note: this not identifying connections through physical interactions) For DiseaseFx® data include quantitivechange, statechange, geneticchange
Protein /Small Molecule Transport			
22	What protein mediates the translocation of a protein or small molecule?	**initial selection:** protein or small molecule **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** moltransport	Identifies proteins involved in the translocation of a protein or small molecule target.
23	What small molecule mediates the translocation of a protein	**initial selection:** protein **algorithm***:* expand pathway *directionality:* upstream *entity type:* small molecule *relation type:* moltransport	Identifies small molecules involved in the translocation of a protein target.

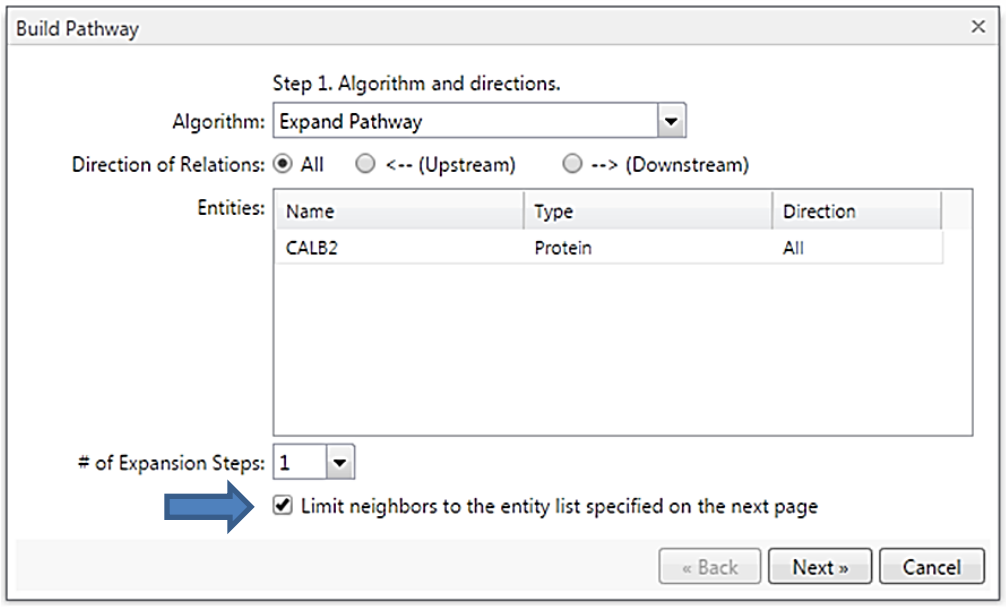
	Question	Wizard Selections	Considerations
Proteins/Small molecules involved in chemical interactions			
24	What enzymes are involved in a chemical reaction with a small molecule?	**initial selection:** small molecule **algorithm:** expand pathway **directionality:** all **entity type(s):** proteins, functional classes **relation type:** chemical reaction	Identifies functional classes and proteins that catalyze chemical reactions of small molecules. Most metabolism enzymes in the metabolism pathways are represented by functional classes.
Protein/Small Molecule associations and changes in Diseases and Cell Processes			
25	What proteins are known to be associated with a disease or cellular process?	**initial selection:** disease or cell process **algorithm:** expand pathway **directionality:** upstream **entity type:** protein **relation type:** regulation	Identifies proteins known to be associated with a specific disease or cellular process. (More specific data relating proteins to diseases is available in DiseaseFx data including statechange, genetic change and quantitativechange.)
26	What small molecules are associated with a disease or cellular process?	**initial selection:** disease or cell process **algorithm:** expand pathway **directionality:** upstream **entity type:** small molecules **relation type:** regulation	Identifies small molecules that are associated with diseases or cellular processes. Small molecule association with diseases and cell processes through regulation relations are found in the ChemEffect® Database. In addition, more information about small molecules associated with diseases can be found in the DiseaseFx database through quantitivechange and biomarker relations.
27	What proteins are known to change in expression, activity or abundance in a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality***:* downstream **entity type:** protein **relation type:** quantitativechange **applied relation filter:** filtering field name: quantitative type; sub-categories: expression, abundance, activity	Identifies proteins that are changed in activity abundance or expression in a disease. Quantitativechange relations are found only in DiseaseFx data
28	What small molecules are known to change in abundance in a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** small molecules **relation type:** quantitativechange **applied relation filter:** filtering field name: quantitative type; sub-category: abundance	Identifies small molecules that are changed in abundance in a disease. Quantitativechange relations are found in DiseaseFx data

	Question	Wizard Selections	Considerations
29	What proteins with genetic mutations are associated with a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** protein **relation type:** genetic change **applied relation filter:** filtering field name: *change type;* subcategories: gene deletion, mutation, gene amplification, epigenic, methylation	Identifies proteins with genetic changes (gene deletions, amplifications, mutations, epigenic changes, or methylation) associated with a disease. Geneticchange relations are found in DiseaseFx data.
30	What proteins or small molecules are diagnostic for a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** protein **relation type:** biomarker **applied relation filter:** filtering field name: biomarker type; sub-category: diagnostic	Identifies proteins/small molecules know to be diagnostic for a disease. Biomarker relations are found in DiseaseFx data.
31	What proteins or small molecules are prognostic for a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** protein **relation type:** biomarker **applied relation filter:** filtering field name: biomarker type; sub-category: prognostic	Identifies proteins/small molecules known to be prognostic for a disease. Biomarker relations are found in DiseaseFx data.
32	What protein phosphorylation/dephosphorylation events are associated with a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** protein **relation type:** statechange **applied relation filter:** filtering field name: *change type*; sub-category: phosphorylation/dephosphorylation	Identifies post translational protein phosphorylation/dephosphorylation events associated with a disease. Statechange relations are found in DiseaseFx data.
33	What protein/gene splice variants are associated with a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** downstream **entity type:** protein **relation type:** statechange **applied relation filter:** filtering field name: *change type*; sub-category: alternate splicing	Identifies alternate gene splicing events/splice variants associated with a disease. Statechange relations are found only in DiseaseFx data.

	Question	Wizard Selections	Considerations
Small Molecule concentrations			
34	What proteins regulate the synthesis or catabolism of a small molecule?	**initial selection:** small molecule **algorithm:** expand pathway **directionality:** upstream **entity type(s):** protein **relation type(s):** molsynthesis	Identifies proteins that regulat the concentrations of small molecules through metabolic events
Clinical Trials			
35	What small molecules/drugs have been tested in clinical trials for a disease?	**initial selection:** small molecules **algorithm:** expand pathway **directionality:** downstream **entity type(s):** disease + cell process **relation type:** clinicaltrials	Identifies small molecules/drugs that have been involved in clinical trials. Drugs are included in ChemEffect Data. Clinicaltrials relations are included in DiseaseFx data. Monoclonal antibodies are represented as small molecules on the ChemEffect database.
Functional Associations between Diseases and Cell Processes			
36	What cellular processes are associated with a disease?	**initial selection:** disease **algorithm:** expand pathway **directionality:** (all) **entity type:** cellular process **relation type:** functional class	Identifies associations between cellular processes and diseases (no directionality in the relations). Functionalassociation relations are found in DiseaseFx data.
Additional Pathway Building Workflows (below)			

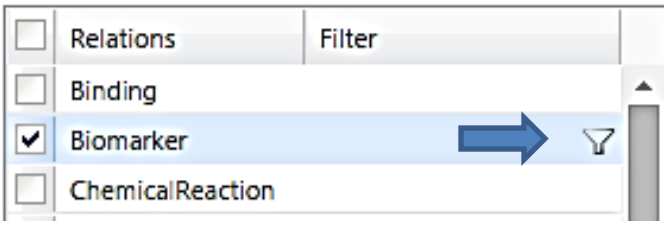
37 Build pathway filtered by group.

In step 1 of the build pathway wizard check the box “Limit neighbors to the entity list specified on the next page” and chose Next. In the next dialog select the desired group (the group must first be saved in your workspace before it can be selected).

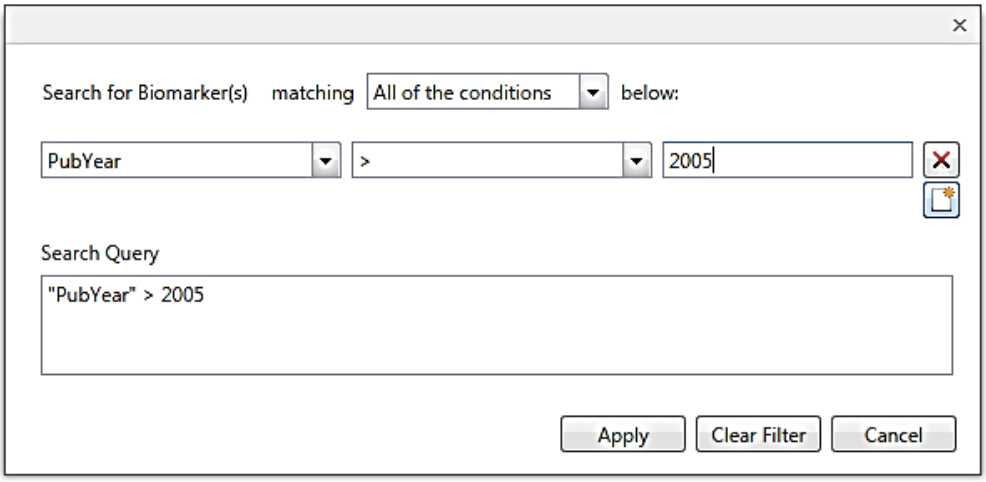


38 Build Pathways with relations filtered by publication year

In step 2 of the build pathway wizard, select the filter icon for the desired relation



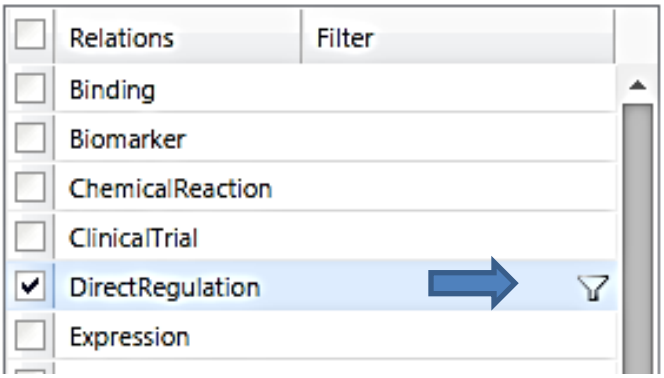
Next define the filter for PubYear to filter for the desired date range. See example below:



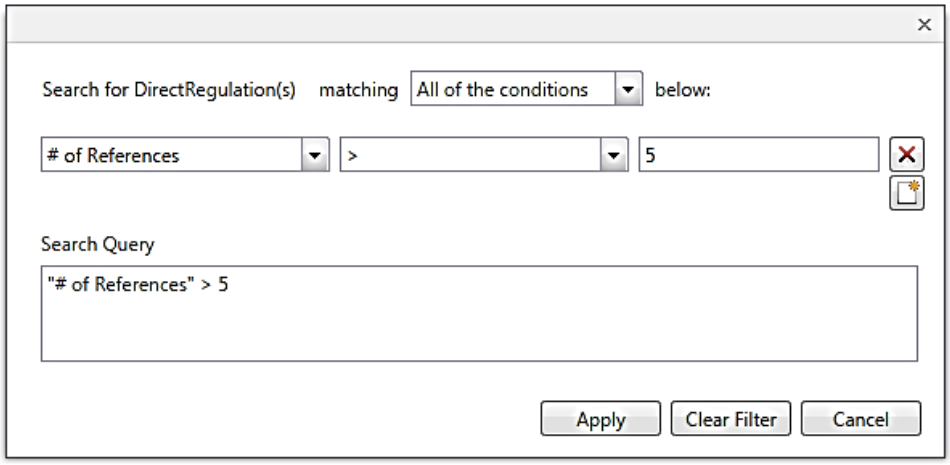
This powerful advanced filter dialog can be used to query on any relation attribute in the database.

39 Build Pathways with relations filtered by reference count

In step 2 of the build pathway wizard, select the filter icon for the desired relation



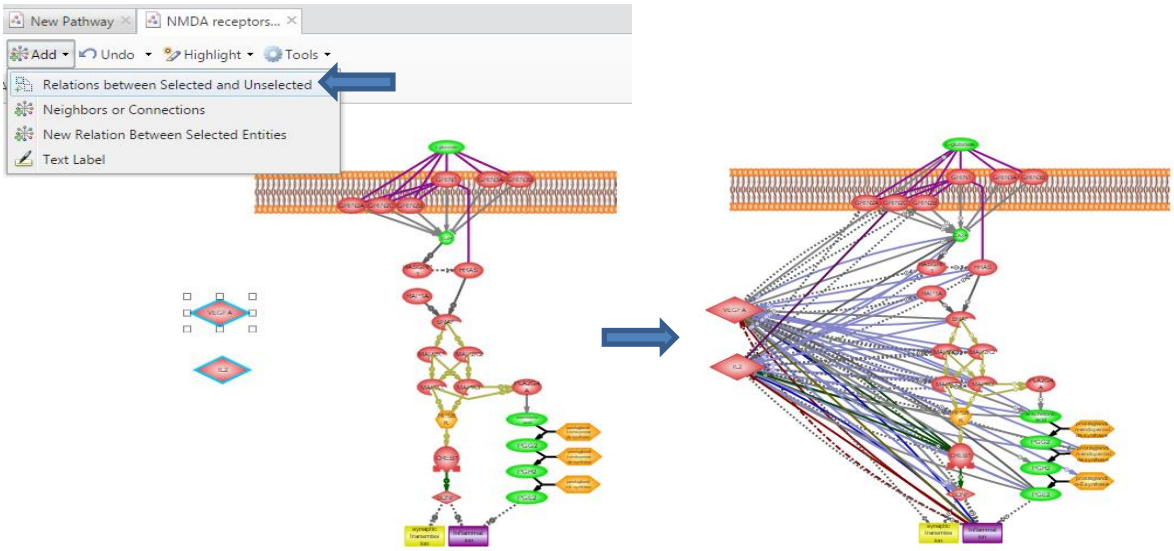
Next define the desired range of # of references. See example below:



This powerful advanced filter dialog can be used to query on any relation attribute in the database.

40 Add a protein/small molecule etc. to an existing pathway

Add new entities to existing pathway, select. Go to Add>Relations between Selected and Unselected.



If you have any questions about Pathway Studio please contact Elsevier Customer Care:

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