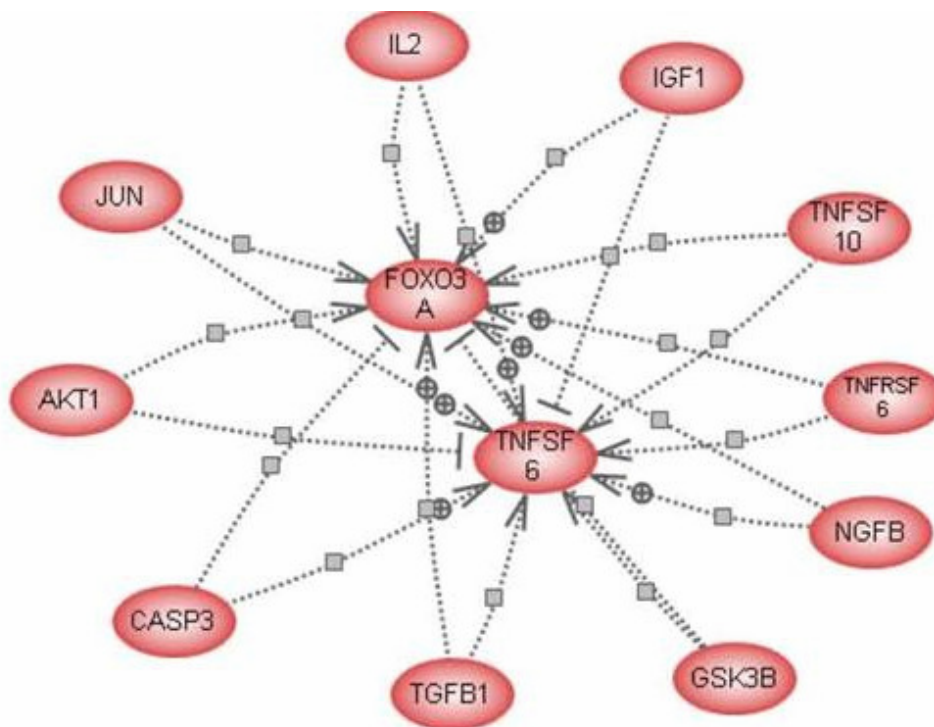


## Find Common Regulator for Selected Entities



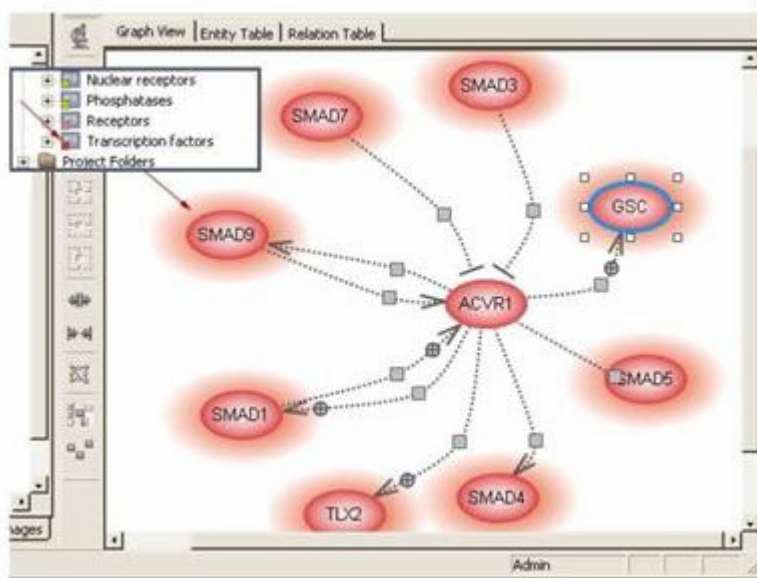
Filters used:

Pathway Entities: Human proteins

Pathway Relations: Regulation

# Find Pathways and Find Group Options

- ❖ Find all pathways or all groups your object of interest (protein, small molecule, cell process, relation, etc.) is a member.



- ❖ Use Highlight operation in the Database Pane to all entities that belong to both your pathway and the group you are interested in.

## Menu for Network Manipulation and Information

- ❖ Graph view, entity table, relation table
- ❖ Statistics: total number of each type of entities and relations (in the left Pane)
- ❖ Graph view manipulations: changing the graph view manually, zooming, rotating, etc.
- ❖ Create new entity (protein, small molecule, ...), define its properties
- ❖ Create new link between selected nodes, select the type and all required attributes of the new relation.
- ❖ Build pathway for selected nodes

## MedScan Search

(Human only)

❖ MedScan Reader

[Search PubMed](#)

[Search BioMed Central](#)

[Search HighWire Press](#)

[Search Google Scholar \(beta\)](#)

[Search Google](#)

Query and Import

atherosclerosis

❖ MedScan Update a Pathway

❖ ID mapping service

## Pathway Studio: Exercise 1

### Building a Network From an Imported List of Genes

- Copy the list of ten genes in a Notepad file

#### 7 genes

MMP8  
MMP9  
CD163  
CTSS  
VCL  
HSPB7  
UCP2

- Go to Tools → Import protein list → Paste the 10 genes  
→ Click on Name → Click on Lookup → Click on Import  
→ Close the initial window.

## Pathway Studio: Exercise 1 (Contd.)

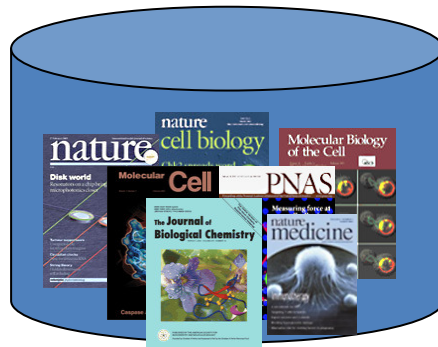
- Go to the New Group Table. Read it.
- Select all lines → use the command “Build pathway for selection”
- Select in the new window “Find only direct interactions”
- Click Start in the new window. Close the window after the job is finished. Analyze and manipulate the graph.
- Come back to the New Group Table, and repeat building a pathway with the option “ Find all shortest paths between the selected nodes”.
- Analyze and manipulate. Read statistics in the left screen field.
- Repeat constructing pathways by expanding to all first neighbors, then with common regulators, and finally, with common targets.
- Use Edit → Combine → Union to combine some of the pathways

# The Ingenuity Platform

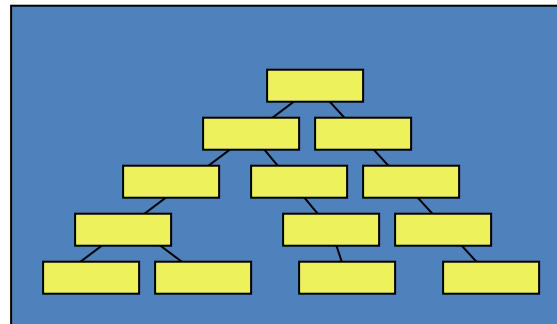
*Pathways  
Knowledge Base*

*Ontology and Knowledge  
Infrastructure*

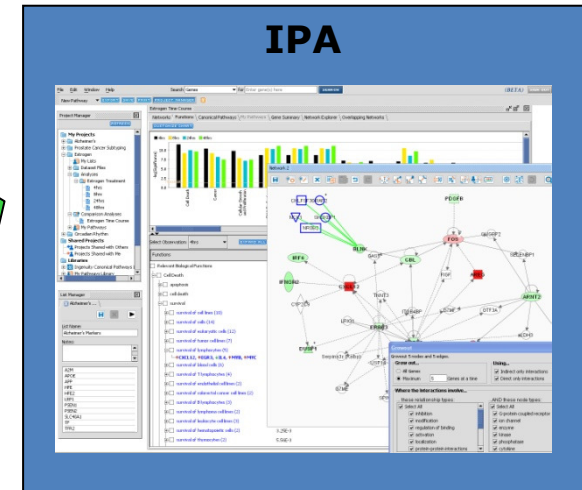
*Solutions*



- **Biological findings manually extracted from full text**
- **Scalable best-in-class Content Acquisition tools and processes**



- **Ingenuity Ontology of biological objects and processes in 12 major branches**
- **Knowledge Infrastructure tools and processes for structuring biological knowledge**



**IPA Integration Module**  
**Knowledge Base Query Tools: Ask**  
**Knowledge Base Databases**  
**Enterprise Search Capabilities**

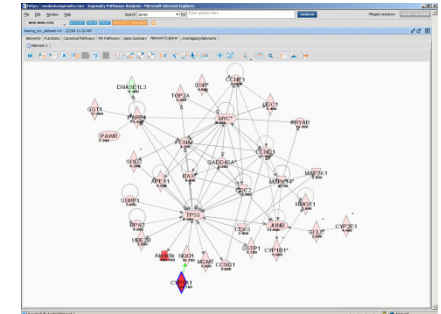
# Example Applications in Research and Development

- **Disease Sub-typing:** Coupling pathways analysis with gene expression to characterize disease subtypes
- **Biomarker Research:** Coupling pathways analysis with gene expression to elucidate biomarkers
- **Disease Models:** Using networks and pathways to build molecular models
- **Mechanism of Action:** Network-assisted transcription profiling to identify mechanisms of action

# Ingenuity Pathways Analysis: Three Approaches

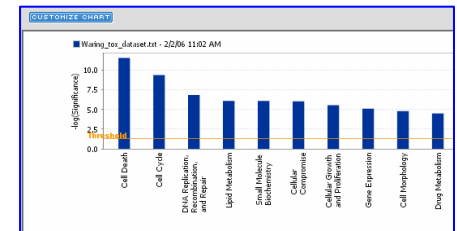
What regulatory relationships exist between the genes, proteins in my dataset?

➔ **Networks**



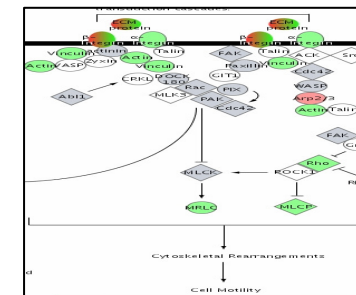
Which biological and disease processes are most relevant to my genes of interest?

➔ **Functional Analysis**



Which well-characterized cell signaling and metabolic pathways are most relevant to my experimental data?

➔ **Metabolic and Signaling Pathways**



# Major IPA Functions

**Analyze your experimental data to understand mechanism and function**

- Gain context and understanding from microarray, proteomics, and other experimental data
- Analyze changes in biological states across time and dosages

**Build your own pathways**

- Create customized pathways for your targets, biomarkers, processes, and diseases of interest
- Integrate your proprietary biological and chemical relationships into IPA pathways
- Analyze your customized pathways to derive biological function
- Compute "shortest paths" between genes or proteins

# Major IPA Functions - 2

## Use IPA as a tool for searching literature findings

- Search based on genes, proteins, diseases, processes, functions, protein family, tissue, sub-cellular location, drugs, and more
- Expand search results to elucidate pathways and functions

## Personalize and share your IPA experience

- Share your analyses and personalized pathways with colleagues
- Create, store, and manage customized lists of genes and proteins
- Generate high-resolution images for publication and presentations
- Create a bibliography of pathway information

**Very large database of expert curated metabolic and signaling pathways – Ingenuity Pathway Knowledge Database (IPKB)**

**23,900 mammalian genes (10,300 human, 5,200 rat and 8,400 mouse)**

**80 metabolic and 67 signaling curated pathways**

# Getting Started

## Menu

### Start a New Analysis

- Analyze a data set
- Compare two or more results

### Create a Pathway

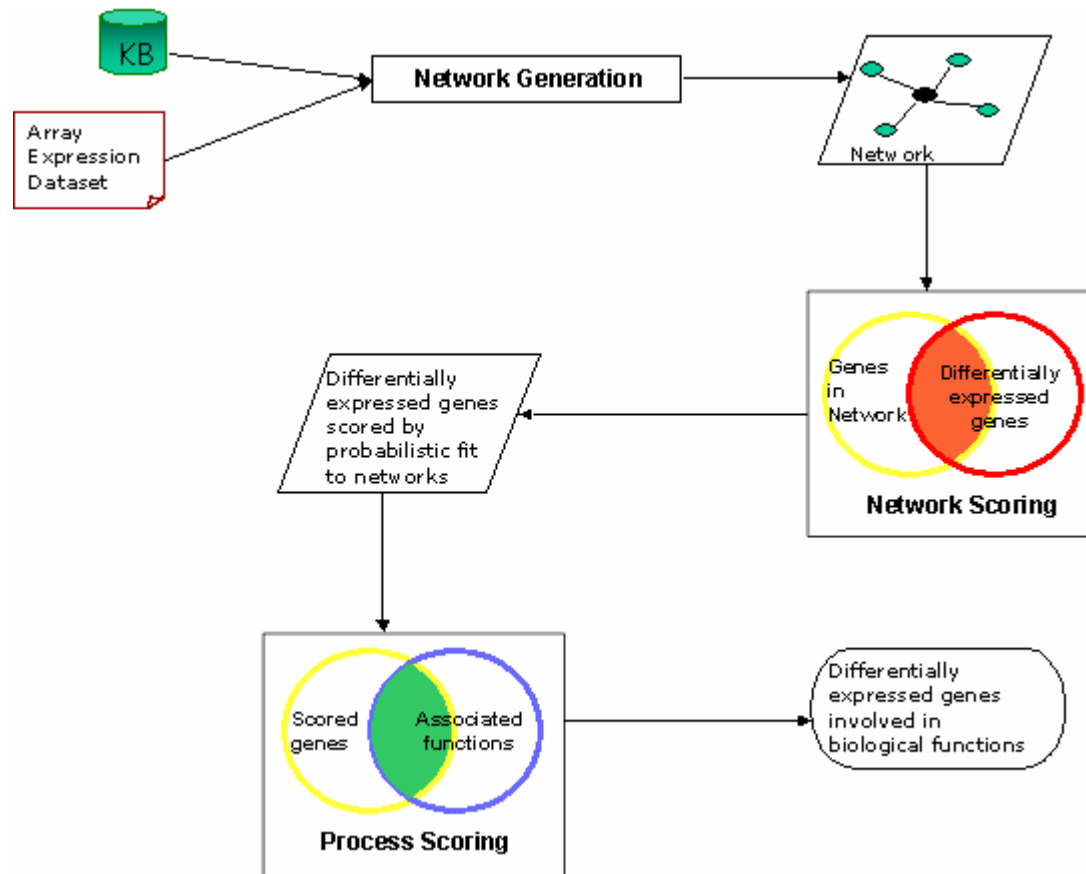
**Search** for genes associated with  
functions and diseases  
drugs  
protein families  
subcellular location

### Help

# Help Resources

- Click here to download a [PDF file](#) of this Help manual
- View [training](#) videos
- Download [templates](#) to format and upload your data
- Find out [how](#) Ingenuity Pathways Analysis works
- Get answers to [Frequently Asked Questions](#)
- Read about example [use cases](#) in our Application Notes
- Identify the various node shapes displayed with the [Legend](#)
- Verify that your computer meets the [technical requirements](#)
- See the [troubleshooting](#) page
- Click here for the [PDF Library](#)

# The Flow of Information in Ingenuity Pathways Analysis



# Ingenuity's Global Molecular Network

**Input:** dataset file containing differentially expressed genes or selected list of genes

Annotations; Network Focus Genes

**Creates** both: a global network and local networks relevant to experiment

- Starts with specifying an expression value (a fold change or p-value)
- Builds Level 1 network with interactions between Focus Genes
- Extend the network to 35 genes with specific interactions with other genes
- Ranks these local networks by a score that takes into account the number of Focus Genes and the size of the networks.

**Provides** tools for network manipulation: adding nodes and relations, building neighborhoods, merging networks

Grow, Neighborhood Explorer, Merge Network, Node View

(drug target overlay, cellular process and disease overlay, canonical pathway overlay, subcellular localization)

# Single Dataset Analysis

## Step 1: Sign In

- 1) Go to <https://analysis.ingenuity.com>
- 2) Use your email address and password
- 3) You will be brought to the Welcome screen

## Step 2: Format Your Dataset

- 1) Use the template from Template Downloads (for expression data)  
Enter a Gene/Protein ID list in the first column, then the columns for Expression Value. Absent and Override are entered for the first observation.
- 3) Repeat for the second observation, and so on

## Step 3: Create a Project Folder to Store Results

- 1) Click on the **Start a New Analysis** link on the Welcome screen.
- 2) Select **Analyze** a dataset and click Next

## Step 4: Upload File

- 3) Click the **Upload** button to upload a new dataset.
- 4) Click on **New Project** to create a destination file for your dataset
- 5) **Name** and **Save** your project. With **Next** you upload your file: Browse → Next → Select Format A or B → Name your file → Next

## Step 5: Create Analysis

- 1) Enter a name for the analysis in the **Analysis Name** field. The uploaded dataset is listed under **Dataset Files** and is selected for the analysis **Next**.
- 2) Run **My Pathways Analysis** - the uploaded dataset is scored against all currently defined custom pathways (View in My Pathways).
- 3) **Build Networks Using** Direct & Indirect Relationships or only one of them
- 4) Specify **Expression Value Cutoff** - determines which genes from the dataset file are selected as **Focus Genes** (e.g.  $p < 0.05$ ). Click **Recalculate**.

## Step 6: Review Dataset File Mapping in the IPKB

- 1) Lists generated: **Mapped Genes, Unmapped Genes, All Genes IDs, Network Focus Genes, and Functions/Pathways Analysis Genes.**
- 2) Options to review the mappings of the genes in each list:
  - A) Before running the analysis: scroll to the bottom of the Create Analysis screen; click on the specific list tabs.
  - B) After running an analysis: right-click on the completed analysis in the Project Manager window, then select Settings.

The Dataset File Mapping section is at the bottom of the Analysis Settings window.

## Step 7: Run Ingenuity Pathways Analysis

- 1) Click on the Run Analysis button (specify a cut-off first if applicable)
- 2) The Project Manager window will appear, and a clock icon will appear next to the name of the running analysis.

## Step 8: Access the Results

- 1) Open the Project Manager by selecting Window and then Project Manager.
- 2) Locate the folder created in Step 3, press the + button to expand the folder.
- 3) Scroll down to the sub-folder entitled Analysis and expand it.
- 4) Once the analysis has completed, it will appear tagged with an icon , and the name of the analysis will appear in bold.

Up to 15 min to complete, depending on the size of the dataset.

E-mail notification or **Refresh** Button

- 5) Double-click on the analysis name to open the Analysis Summary page  
Analyze your results, either by selecting a network for further study, or by viewing the **Functions** or **Canonical Pathways** results.

## Step 9: Select Networks for Further Study

The results of the analysis are presented in the Analysis Summary by a List of Networks view, where networks are displayed according to their score.

### Step 9A: Select networks for further study

**A. Score-centric approach**

**B. Attribute-centric approach** (gene family, location,...)

**C. Gene-centric approach** (use the **Filter** box and Select the Network identifier)

### Step 9B: View Functions

- 1) Click on the Functions tab in the Analysis Summary. A bar graph displayed in the top panel shows the significance of a list of functional categories for the dataset. The bottom panel lists all the functional categories, along with the significance values and associated number of genes.

- 2) To view the more specific functions grouped together under the functional categories, select Expand Functions. This will display all specific functions and associated genes.
- 3) To view the genes associated with a specific function, click on the + sign next to the given function. This will display all associated genes.
- 4) To drill down to the individual findings used to establish these functional annotations, click on the name of the specific function highlighted in blue (e.g. metabolism of amino acids).
- 5) Click on **Expand All** link or the + sign located next to each finding to view the literature references.

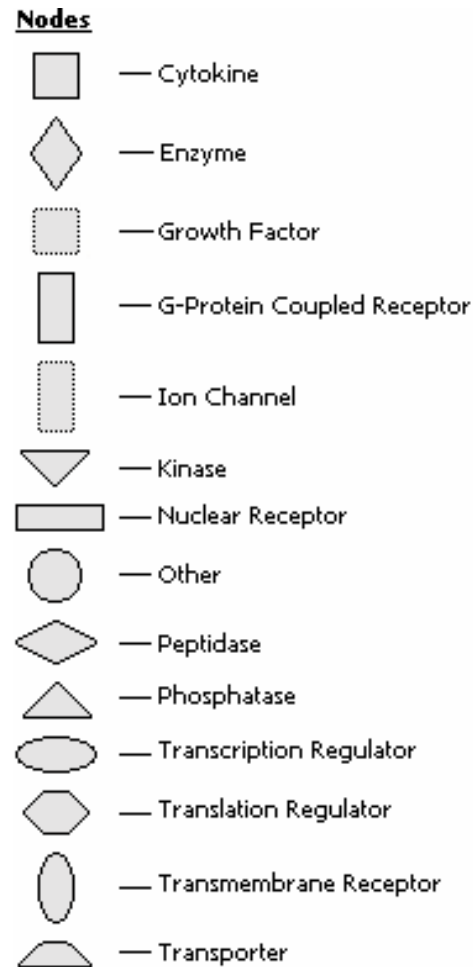
## **Step 10. Access Network Explorer**

- 1) From **Analysis Summary**, click on the Network tab to view the list of networks.
- 2) Access the network of interest by a) clicking on the **Network ID** column or b) clicking on the box the left of it. Then click on **View Networks** to open the network diagram.

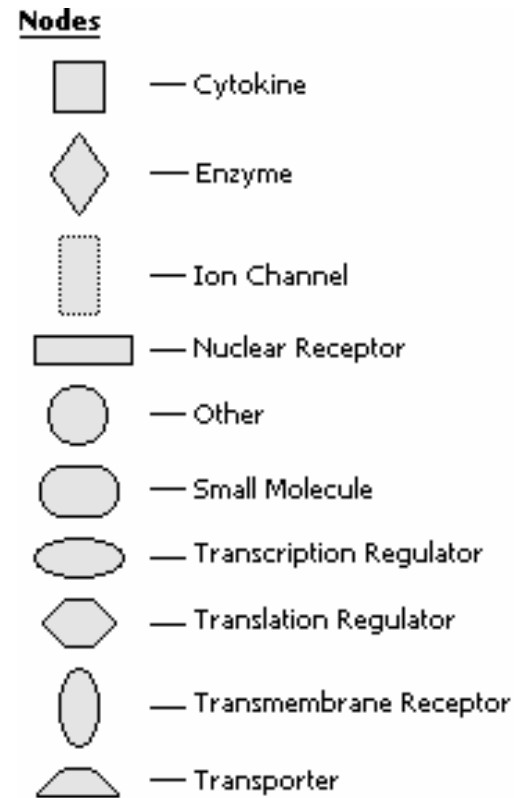
- 3) Use the **Zoom Fit** button, located in the **Network Explorer Toolbar** at the top of the window, to center the network.
- 4) Use the **Zoom in** and **Zoom out** buttons to adjust the size of the display. Alternatively, hold down and drag the cursor to select a portion of the diagram you are interested in viewing more closely, then click the **Zoom selected area** button .
- 5) Select **Legend** from the **Help menu** to view the descriptions of the colors and symbols used in **Network Explorer**. Node color preferences can be changed in the **Preferences** page.
- 6) Use the **Subcellular** layout toggle button to sort the network nodes (genes) based on their subcellular locations.
- 7) Use the **Highlight Drug Targets** button to highlight genes in the network that are known drug targets. At the same time, a separate **Drug Targets** window opens up with a list of the gene name, drug name, and disease indication for each highlighted node (drug target). Close the **Highlight Drug Targets** window to toggle back to the default network view.

# Network Explorer Node Shapes

## Network Explorer Node Shapes



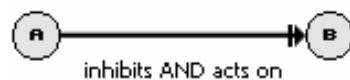
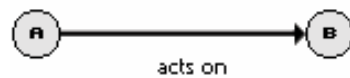
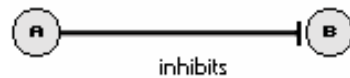
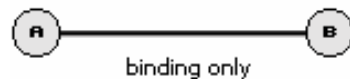
## Canonical Pathways Node Shapes



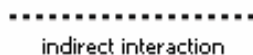
# Network Explorer & Canonical Pathways Edges

## Network Explorer Edge Types

### Edges

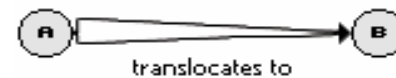
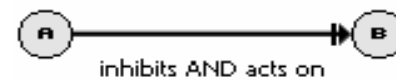
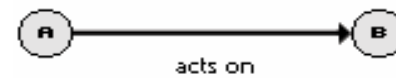
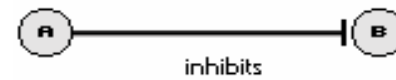


**Note:** "Acts on" and "Inhibits" edges may also include a binding event.

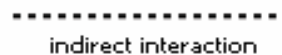
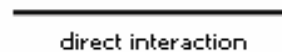


## Canonical Pathways Edge Types

### Edges



**Note:** "Acts on" and "Inhibits" edges may also include a binding event.



## Edge Labels

- A** Activation / Deactivation
- RB** Regulation of Binding
- PR** Protein-MRNA binding
- PP** Protein-Protein binding
- PD** Protein-DNA binding
- B** Binding (only appears prior to IPA 3.0)
- E** Expression
- I** Inhibition
- L** ProteoLysis
- M** Biochemical Modification
- O** Other (only appears prior to IPA 3.0)
- P** Phosphorylation / Desphosphorylation
- T** Transcription
- LO** Localization

## **Step 10B: View Canonical Pathways**

**Global Canonical Pathways** provides a summary of the most relevant metabolic and signaling pathways for the entire dataset. To access Canonical Pathways from the Analysis Summary:

- 1) Click on the Canonical Pathways tab. The window that appears will display a bar graph, with all the relevant pathways based on significance.
- 2) To view the associated genes, click on the bar representing your pathway of interest. This will display list of all the genes involved in the bottom panel of the screen.
- 3) To access the pathway diagram, click on the View Pathway located above the gene list. This opens a new window with a diagram of the pathway. All nodes corresponding to genes that were part of the dataset file are colored. For a key of the various symbols and colors in a pathway diagram, click on the Legend link.

## **Step 11: View the Node Summary**

- 1) Go to the Network Explorer diagram for the top-scoring network.
- 2) Double-click on a node.

The Node View enables drawing rapid conclusions about the gene's function.

## **Step 12: Access Gene View**

(Explores literature about molecular interactions)

1. Go to **Network Explorer** diagram and select a network.
2. Double click on a node. This brings up **Node Summary View** in a new window.
- 3) Click on the node name at the top of the **Node Summary View**. This opens a new window with the full **Gene View** for the gene.
- 4) Click on the link in the **Categorized Literature Findings** section to view a list of all the findings associated with this gene that can be found in the IPKB.
- 5) Link to individual findings by clicking on the number next to the functional category of interest.
- 6) Click on the **Expand All** link or to the + sign to view the literature.

## **Step 13: View the Edge Summary**

(A quick summary of the biological interactions used to construct an edge)

- 1) Go to the Network Explorer diagram for the top-scoring network.
- 2) Move your mouse over an edge. Double-click on the edge.

The Edge Summary View will appear in a separate window.

## **Step 14: Access Edge View**

Provides more detailed information about the edge (molecular interaction) connecting two nodes (genes)

- 1) Go to the **Network Explorer** diagram for the top-scoring network.
- 2) Double-click on an edge. This brings up the **Relationship Summary View** in a new window.
- 3) Click on the link at the top of the **Edge Summary View** that displays the names of the two genes separated by a forward slash (/).
- 4) Click on the **Expand All link** or the + sign located next to each finding to view the original literature reference and a link to the PubMed abstract for each finding supporting the edge.

## **Step 15: Access Functional Analysis for a Network**

To view Functional Analysis from the Network List view:

- 1) Select the Networks tab to view the list of networks. In the Top Functions column, the top three functions are displayed.

To view Functional Analysis from Network Explorer:

- 1) Click on the Functional Analysis button in the toolbar at the top of the screen.

- 2) Use the + sign to expand the functions to view the specific functions associated with them, including the underlying genes associated with the specific functional categories.
- 3) To view the underlying biological evidence from the literature (findings) that were used to generate these gene-function associations, click on the name of a specific functional category. This will display all relevant findings.
- 4) To view the PubMed abstracts for these findings, select **Expand all**, then click on the PubMed ID.

## **Step 16: View Canonical Pathways for a Network**

Canonical Pathways enables you to discover which genes in a network participate in known pathways established in the scientific literature.

- 1) Go to **Network Explorer** to view a network.
- 2) Click on the **Overlay Canonical Pathways** button in the **Network Explorer Toolbar**. This opens a window listing the pathways associated with genes from the network.
- 3) Select the pathway you wish to view. Nodes associated with this pathway are tethered to the pathway label. Single click on the pathway label to highlight the nodes associated with this pathway.
- 4) Double click the pathway label to view the canonical pathway.

- 4) Double click the pathway label to view the canonical pathway.
- 5) Click on one of the gene names within the text box. This causes a **Node Summary** of that gene to appear on the left.

### **Step 17: Access Neighborhood Explorer**

In this step, you can view in one diagram the genes that directly interact with a particular gene of interest and the molecular relationships that connect them to this gene.

To access **Neighborhood Explorer** from **Network Explorer**:

- 1) Double-click on a node. This brings up the **Node Summary** in a new window.

To access **Neighborhood Explorer** from **Node View**:

- 1) Go to **Node View** for a gene by any one of the following ways:
  - a) Click on the gene name within the application where it appears as a link
  - b) from the **Node Summary View**; c) from **Network Explorer**
- 2) Click on the Neighborhood Explorer link next to the gene name within **Node View**. This brings the network extended with the neighborhood.
- 3) Click on the gene of interest. This will highlight the gene and all of its associated edges. You can then select any other node within this diagram and navigate to the neighborhoods centered around them.

# Exercise: Search for Genes Related to Atherosclerosis

Go to GETTING STARTED → Search

Type in the following genes symbols:

MMP8 MMP9 CD163 CTSS VCL HSPB7 UCP2

Leave “All identifiers” in “Identifier Type”

Enter “Atherosclerosis” in “Functions and Diseases”

Check “cytoplasm”, “extracellular space”, “nucleus”, and “plasma membrane”  
in “Subcellular Locations”

Check “Functions and Diseases” in “Display results as” → Click Search

## Exercise – Part 2

In the “Search” window select the box next to  
“Matching Functions and Diseases”

Select “Add to List”, “New List”, then name it and save it

Select “Add to Pathway” and New Pathway

View the pathway with “Auto-Layout”

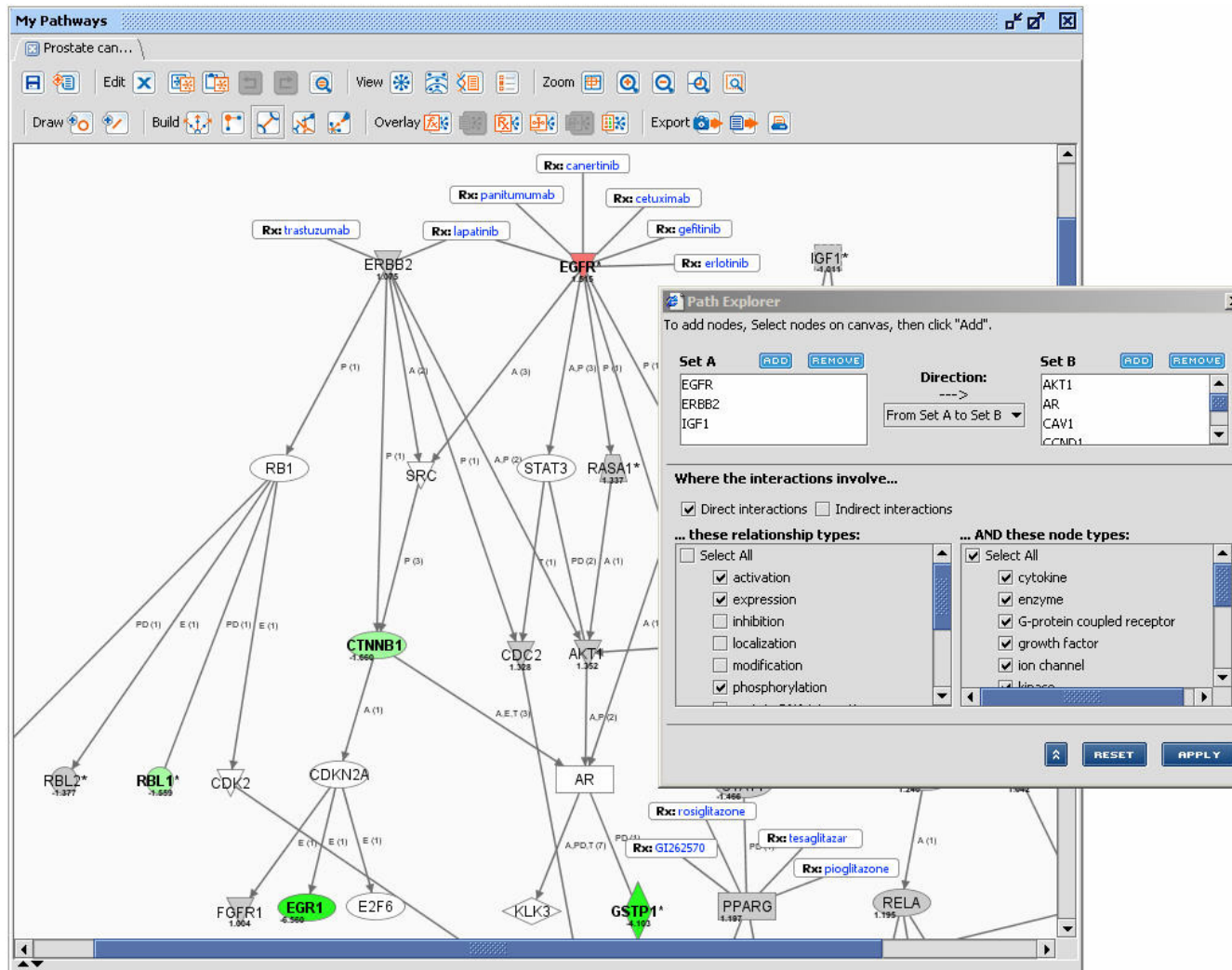
Use “Overlay Drug” with beta-estradiol

Export the built pathway as a JPEG file

# Path Explorer:

## Find Shortest Paths Between Genes of Interest

“What molecular paths exist between cancer drug tamoxifen and targets and genes that affect lung cancer.”



# How to find the relevant biology of a list of chemicals/chemical library?

Visualize relationships between chemicals and gene products and understand their impact on normal cellular processes and diseases

**ChemView: beta-estradiol** (Neighborhood Explorer)

Review the categorized literature findings and database information for this node.

**beta-estradiol**

**Synonyms:** 1,3,5(10)-estratriene-3,17beta-diol; 17-beta-E2; 17-beta-estradiol; 17-beta-oestradiol; E2; estradiol; estradiol hemihydrate; oestradiol

**Systematic Name:** estra-1, 3, 5(10)-triene-3, 17b-diol

**IUPAC Name:**

**CAS Registry Number:** 50-28-2

**SMILES:** C[C@]12CC[C@@H]3[C@@H]([C@@H]1CC[C@@H]2O)CCC4=C3C=CC(=C4)O

**InChI:** InChI=1/C18H24O2/c1-18-9-8-14-13-5-3-12(19)/10-11(13)2-4-15(14)16(18)6-7-17(18)20/h3,5,10,14-17,19-20h,2,4,6-9H2,1H3/t14-,15-,16+,17+,18+/m1/s1

**Chemical Formula:** C<sub>18</sub>H<sub>24</sub>O<sub>2</sub>

**PubChem Link:** [beta-estradiol](#)

**Group/Family of Compound**

**Family Members:** estrogen

**Drug Information**

**Brand Names:** Alora, Climara, Estrace, Estraderm, Estrasorb, Estring, Fempatch, Vivelle

**Approval Status:** Approved

**Therapeutic Indications:** atrophic\_vaginitis, breast\_carcinoma, female\_hypogonadism, hypogestrogenism, kraurosis\_vulvae, menopause, osteoporosis, ovarian\_failure, prostatic\_carcinoma

**7309 Categorized Literature Findings** (Hide details)

**Biological Relevance** | **Pharmacological Relevance** | **Additional Findings**

**Biological Relevance**

**Chemical-Cell or Tissue Interaction**

**Positive (23)** beta islet cells, alpha islet cells, bone cell lines, fibroblast cell lines, [parovus.tissue.cell.lines](#), underspecified cellular component, intestinal cell lines

**Negative (2)** fibroblast cell lines

**Chemical-Nucleic Acid Interaction**

**Positive (9)** [SLC9A3R1](#)

**Chemical-Protein Interaction**

**Positive (328)** ESR1, ESR2, Estrogen Receptor, SHBG, AR, NCOA3, ERBB2, Ste, CCND1, KCNNB1, NCOA2, protein fragment, KCNNA1, APF, CYP3A4, FLP RECOMBINASE, FSH, Fsh, GST A1-1, GST A1-1, NR3B1, protein

**Negative (19)** ESR1, protein fragment, ESR2, NCOA3, KCNNA1, Pgrmc1

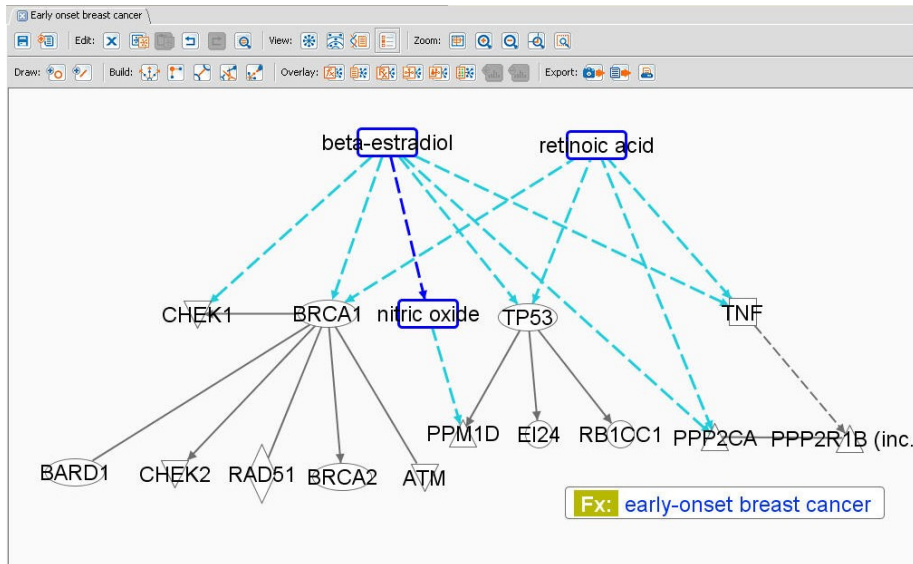
**Regulation**

**quantity regulated by (97)** INHBA, Fsh, estradiol benzoate, testosterone propionate, formestane, GAL, GH1, Hcg (chorionic gonadotropin complex), Immg, hypophysectomy, CYP19A1, LEP, AFP, ESR1, ESR2, FSHB, GnRH1, IGF1, LHCSR, LH, cetrorelix, estrone, goserelin, tetraclerodibenzodioxin, CGA, COMT, D-tyrothionine, OAZ, ECH1, EDN3 (includes EG1908), EM 800, FSHR, GLP1R, GnRH, IRS2, LHB, NOS3, Na-Glu, PGR, PRL, RJ486, SRD5A1, TAC1, THRA, THRB, epicatechin, epicatechin gallate, finazoles, fulvestrant, gamma radiation, ovariectomy, pimgadine, removal, tamoxifen, toproline

**binding regulated by (53)** heavy metal, AKT, resveratrol, ESR1, H7, PTPN1, clonidine, dopamine, epinephrine, norepinephrine, phorbol myristate acetate, propranolol, quipirole, urea, 3',5'-ADP, 3-phosphoserine, 4-vinyl-1-cyclohexene dioxide, L-triiodothyronine, NCOA4 (includes EG8031), SNGC, SRC, TGFB1, TNF, cadmium chloride, estriol, mercuric chloride, methylselenic acid, metribolone, phosphotyrosine, pregnenolone, progesterone, sodium arsenite, vanadate, zinc chloride

**metabolism regulated by (40)** 3-methylcholanthrene, dexamethasone, phenobarbital, CYP1A1, tetrachlorodibenzodioxin, clofibrate, PGR, isoniazid

**oxidation regulated by (20)** HSD17B2, 17-alpha-ethinylestradiol, 17alpha-hydroxypregnenolone, 17beta-dihydroequilin, 2-hydroxyestradiol, 20-alpha-hydroxyprogesterone, 3-alpha,17-beta-androstane-diol, 3-beta,17-beta-androstane-diol, 5-beta-androstane 3 alpha, 17-beta-diol, CYP3A4, alpha-estradiol, androst-5-ene-3beta,17beta-diol, apigenin, coumestrol, estriol, medroxyprogesterone acetate, morphine, prednisolone, pregnenolone, testosterone



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