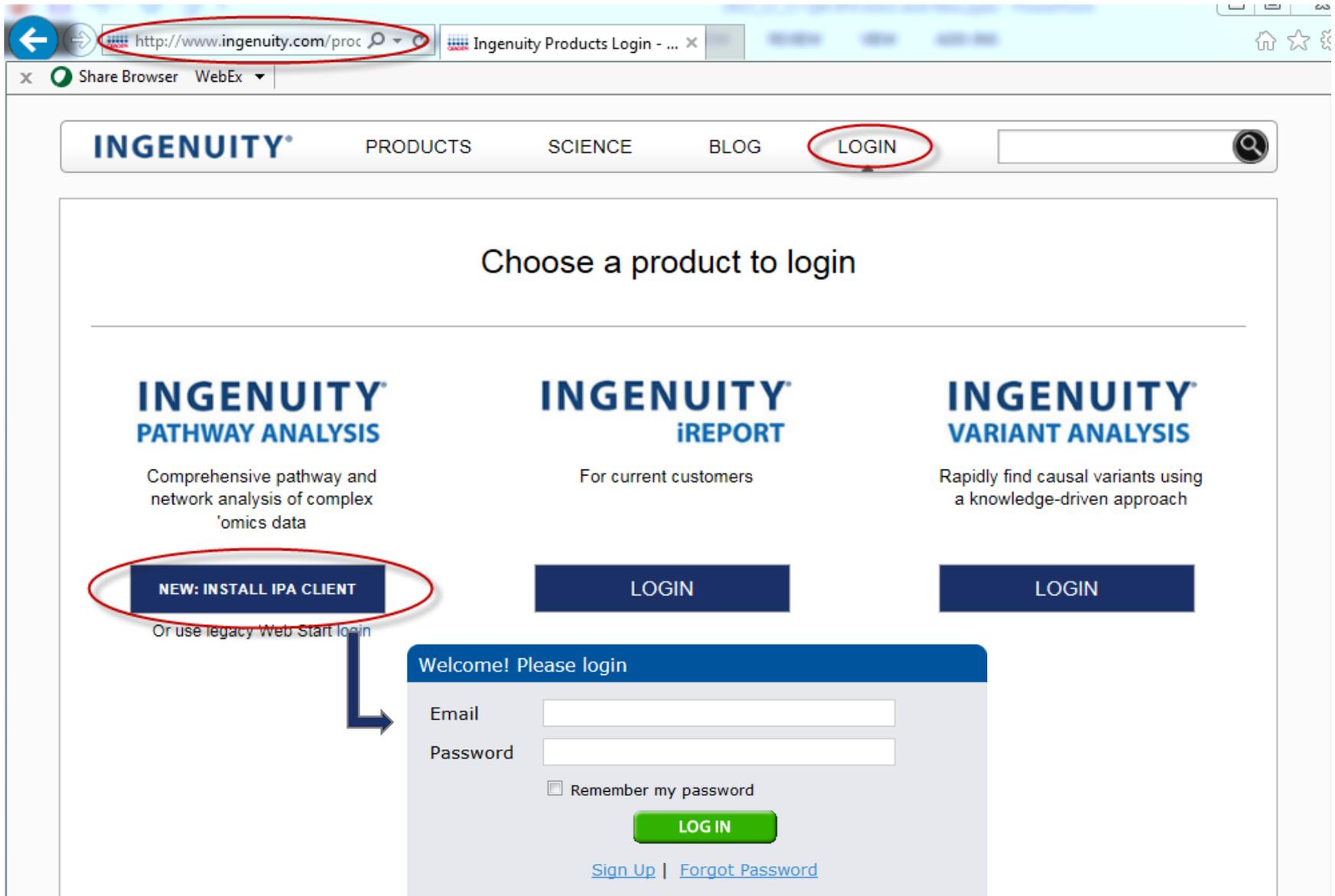


IPA Training:  
Maximizing the Biological Interpretation of Gene, Transcript &  
Protein Expression Data with IPA

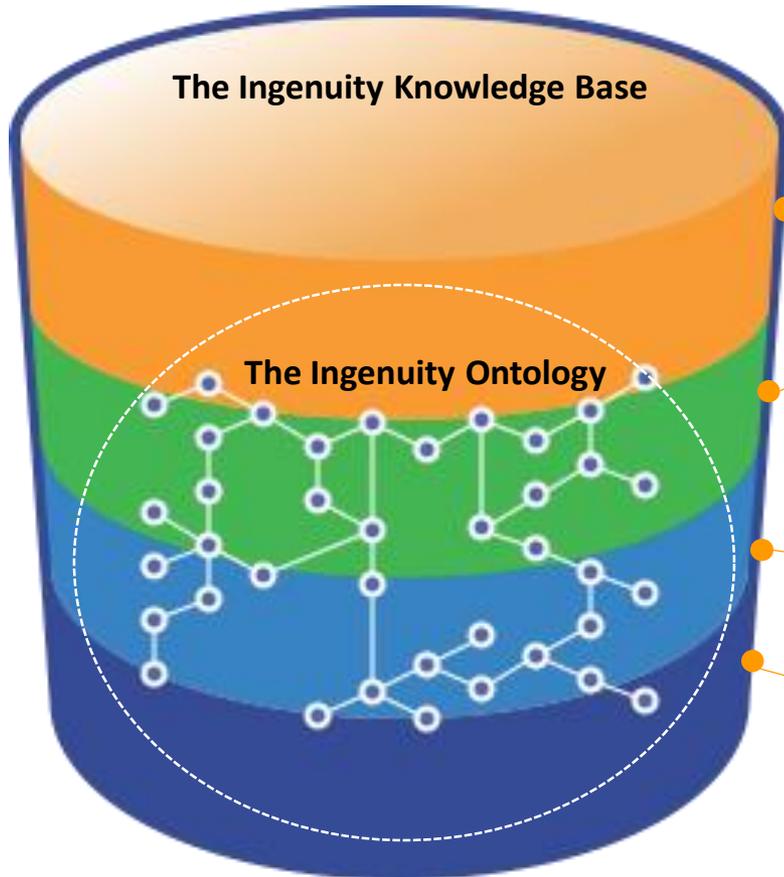


The screenshot shows a web browser window with the URL <http://www.ingenuity.com/proc> in the address bar. The page header includes the Ingenuity logo and navigation links for PRODUCTS, SCIENCE, BLOG, and LOGIN. The main content area is titled "Choose a product to login" and features three product cards: Ingenuity Pathway Analysis, Ingenuity iReport, and Ingenuity Variant Analysis. The Pathway Analysis card has a "NEW: INSTALL IPA CLIENT" button circled in red, with a sub-link "Or use legacy Web Start login" below it. A blue arrow points from this button to a login form. The iReport and Variant Analysis cards each have a "LOGIN" button. The login form, titled "Welcome! Please login", contains fields for Email and Password, a "Remember my password" checkbox, and a green "LOG IN" button. At the bottom of the form are links for "Sign Up" and "Forgot Password".

## IPA

- Deep pathway understanding of a single gene/protein
  - Drug/therapeutic target discovery
  
- Biological understanding of large data sets, including
  - Differential gene expression, array and RNAseq (transcriptomics)
    - **Isoform annotation (New)**
  - Differential protein expression (proteomics)
  - **Genes with loss/gain-of-function variants (New)**
  - Metabolomics
  - miRNA expression
  - Gene List
    - Chip-seq
    - siRNA screening
    - Sequence Variants (see also Ingenuity Variant Analysis)
  - Methylation
  - Protein phosphorylation

## Ingenuity Knowledge Base



The Ingenuity Knowledge Base

The Ingenuity Ontology

### Ingenuity Findings

**Ingenuity® Expert Findings** – Manually curated Findings that are reviewed, from the full-text, rich with contextual details, and are derived from top journals.

**Ingenuity® ExpertAssist Findings** – Automated text Findings that are reviewed, from abstracts, timely, and cover a broad range of publications.

### Ingenuity Modeled Knowledge

**Ingenuity® Expert Knowledge** – Content we model such as pathways, toxicity lists, etc.

**Ingenuity® Supported Third Party Information** – Content areas include Protein-Protein, miRNA, biomarker, clinical trial information, and others



- File > New > Core Analysis
  - Or File > Data Set Upload
  
- Upload Data (gene expression, protein expression, metabolomics, etc.)
  
- Set Core Analysis Settings
  
- Run Analysis
  
- Interpret Results

## Data Upload

Typical value-types that are uploaded to IPA

Identifier List

	A
1	ID
2	NM_130786
3	NR_015380
4	NM_138932
5	NM_014576
6	NM_138933
7	NM_000014
8	NR_026971
9	NM_144670
10	NM_001080438
11	NM_017436
12	NM_016161
13	NM_015665

+differential expression

	A	B
		Log2Ratio
1	130786	0.14
2	015380	-0.99
3	138932	-0.02
4	014576	-0.02
5	138933	0.02
6	000014	-4.79
7	026971	-0.67
8	144670	-5.96
9	001080438	-1.97
10	017436	-1.09
11	016161	2.02
12	015665	-0.27

+significance stat

	B	C
	Log2Ratio	p-value
1	0.14	8.68E-01
2	-0.99	2.24E-01
3	-0.02	9.83E-01
4	-0.02	9.85E-01
5	0.02	9.79E-01
6	-4.79	1.02E-01
7	-0.67	6.17E-01
8	-5.96	1.30E-01
9	-1.97	3.47E-01
10	-1.09	5.02E-01
11	2.02	5.97E-02
12	-0.27	5.68E-01

+RPKM

(maximum RPKM between experimental condition and control recommended for RNAseq)

	B	C	D
	Log2Ratio	p-value	Intensity/ RPKM/FPKM
1	0.14	8.68E-01	2931.69
2	-0.99	2.24E-01	1649.26
3	-0.02	9.83E-01	1.67
4	-0.02	9.85E-01	1.77
5	0.02	9.79E-01	1.83
6	-4.79	1.02E-01	239.75
7	-0.67	6.17E-01	213.79
8	-5.96	1.30E-01	610.64
9	-1.97	3.47E-01	3.91
10	-1.09	5.02E-01	6186.83
11	2.02	5.97E-02	149.85
12	-0.27	5.68E-01	13330.34

## Format for multi-observation upload

- Multiple experimental differential expressions can be grouped into a single spreadsheet and upload
  - Nice-to-have if you are comparing a series of expression values such as a time-course
  - Be sure and name your observations at the time of upload in IPA

		Observation 1			Observation 2		
	A	B	C	D	E	F	G
1	ID	12 Hour Log2Ratio	12 Hour p-value	12 Hour Intensity/	24 Hour Log2Ratio	24 Hour p-value	24 Hour Intensity/
2	NM_130786	0.14	8.68E-01	2931.69	-0.83	4.65E-01	4791.17
3	NR_015380	-0.99	2.24E-01	1649.26	0.72	5.32E-01	198.72
4	NM_138932	-0.02	9.83E-01	1.67	1.58	8.31E-03	7879.80
5	NM_014576	-0.02	9.85E-01	1.77	-0.77	1.26E-02	46757.06
6	NM_138933	0.02	9.79E-01	1.83	0.90	2.03E-02	26426.36
7	NM_000014	-4.79	1.02E-01	239.75	-0.01	9.82E-01	2117.73
8	NR_026971	-0.67	6.17E-01	213.79	0.12	8.64E-01	14076.24
9	NM_144670	-5.96	1.30E-01	610.64	-1.62	1.46E-01	31.85
10	NM_001080438	-1.97	3.47E-01	3.91	0.12	8.25E-01	10491.96
11	NM_017436	-1.09	5.02E-01	6186.83	2.02	4.44E-01	14788.50
12	NM_016161	2.02	5.97E-02	149.85	-0.57	1.09E-01	273101.00
13	NM_015665	-0.27	5.68E-01	13330.34	0.36	4.87E-01	11876.00
14	NM_023928	-1.12	1.03E-02	22828.15	-0.17	7.18E-01	3339.36

## Verify the differential expression calculation

- Recommend  $\text{Log}_2(\text{ratio})$  differential expression

$$\text{Log}_2\left(\frac{\textit{Experimental Condition Exp.}}{\textit{Control Exp.}}\right)$$

- Ratio differential expression

$$\left(\frac{\textit{Experimental Condition Exp.}}{\textit{Control Exp.}}\right)$$

- Fold Change

- If increased differential expression

$$\left(\frac{\textit{Experimental Condition Exp.}}{\textit{Control Exp.}}\right)$$

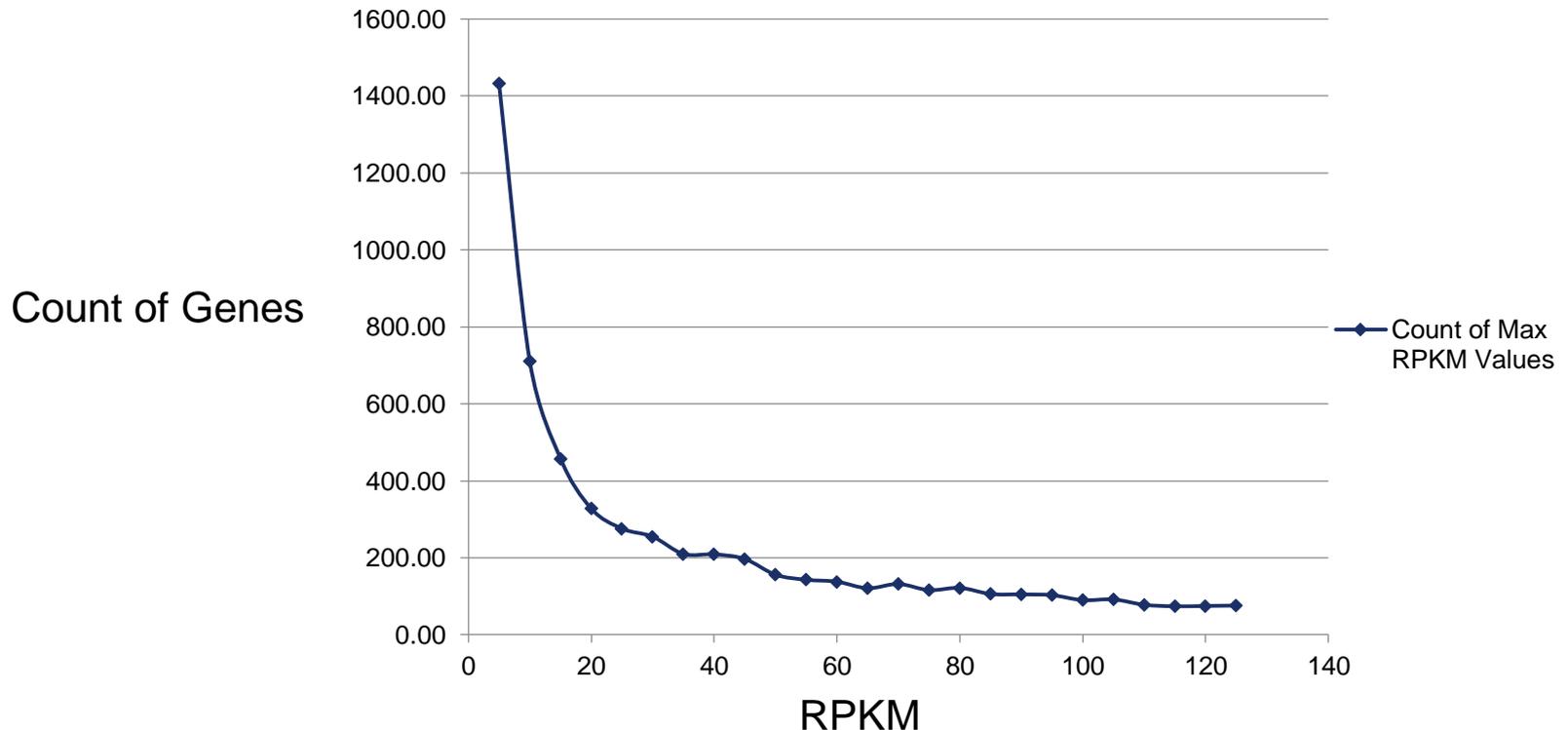
- If decreased differential expression

$$-1 \left(\frac{\textit{Control Exp.}}{\textit{Experimental Condition Exp.}}\right)$$

Fold change will never have values between 1 and -1

## Filtering on absolute expression

- RNAseq measurements often result in many significant differential fold changes at low absolute transcript expression levels
- Including the maximum RPKM value of your experimental condition and control allows for later filtering on absolute expression value in addition to fold change and p-value



## Best practices

- Calculate metrics outside of IPA (e.g. fold-change, p-value)
- Create an Excel spreadsheet or tab delimited file
  - Only 1 header row allowed
  - One column must have identifiers, preferably the left-most column
  - Can have up to 20 observations
  - IPA will only look at the top worksheet in an Excel workbook
- Group related observations into a single spreadsheet if possible
  - Time course, drug concentration, cell lines, etc.
- Specify array platform (chip) if possible
  - It is OK to use “Not specified/applicable”
- Pre-filter data at the lowest threshold that you have confidence in
  - For example, probe measurement p-value of .05 or other criteria
  - Use the Recalculate button to refresh the screen

- Examples of data set types
  - Differential gene expression, array and RNAseq (transcriptomics)
    - **Isoform annotation (New)**
  - Differential protein expression (proteomics)
  - **Genes with loss/gain-of-function variants (New)**
  - Metabolomics
  - miRNA expression
  - Gene List
    - Chip-seq
    - siRNA screening
    - Sequence Variants (see also Ingenuity Variant Analysis)
  - Methylation
  - Protein phosphorylation

## Why don't all of the molecules map?

- The gene ID might not correspond to a known gene product. For example, most ESTs are not found in the knowledge base (exception: ESTs that have a corresponding Entrez Gene identifier are found in the knowledge base).
- A gene/protein ID might correspond to several loci or more than one gene. Such identifiers are left unmapped in the application due to the ambiguity of the identity.
- Identifiers for species other than human, mouse or rat must map to human, mouse or rat orthologues in order to map in IPA.
- SNPs must map to a single gene. SNPs that fall greater than 2 KB upstream or 0.5 KB downstream of a gene coding region will not be mapped in IPA during data upload, since they cannot be unambiguously mapped to a single gene.
- There may be insufficient findings in the literature regarding some molecules.

If you are using a standard vendor platform supported by IPA, then that platform should be selected as your reference set.

If you do not know the platform or the data were taken from different platforms, select a reference set that best estimates the entire population you evaluated.

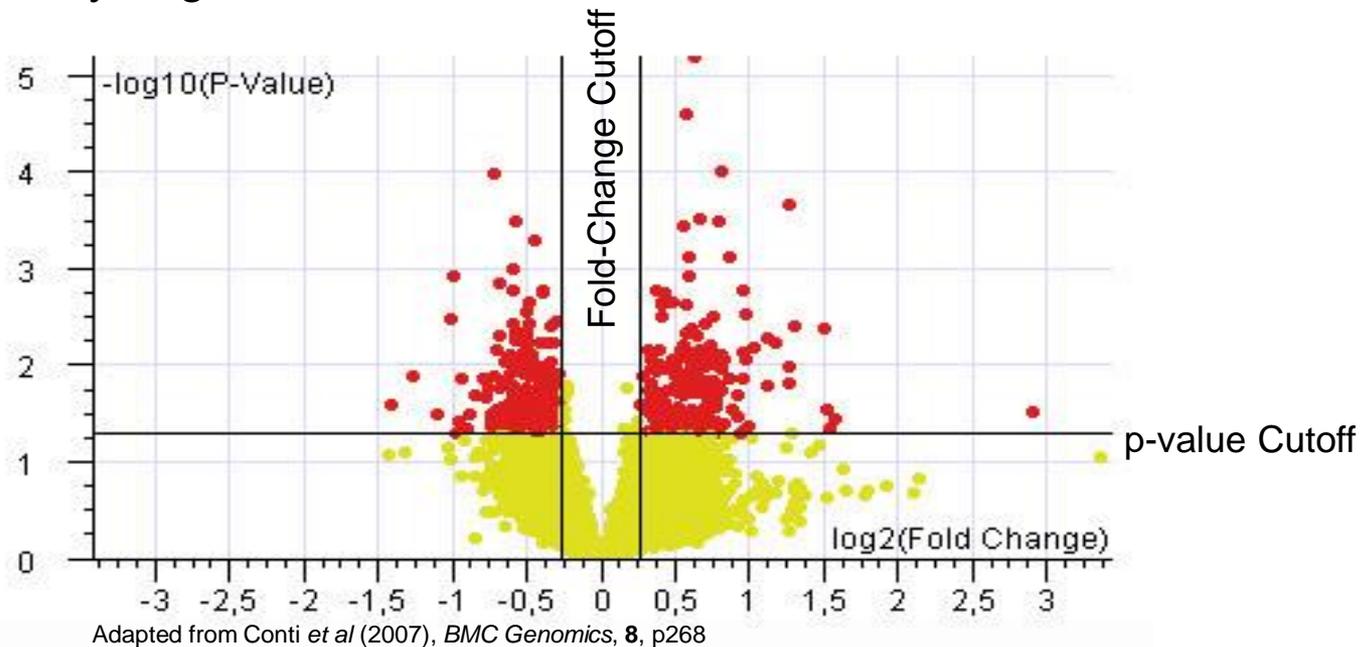
- For gene expression data, select the “Ingenuity Knowledge Base (genes only)”
- For metabolomics, select the “Ingenuity Knowledge Base (endogenous chemicals only)”
- You have the option to having your uploaded data set used as the reference set (User Data Set)



## Core Analysis Set-up

'Ideal' set size for IPA core analysis from gene expression data is typically 200-3000

- Small sets will not have many directional effect z-scores (downstream functions, upstream regulators)
- Very large data sets will tend to have more 'noise'



Create Core Analysis - [analysis : GSE26129\_MCF-7\_A2780\_IPA.xls]

**General Settings**

- Networks Interaction & Causa...
- Data Sources
- Confidence Experimentally Ob...
- Species All
- Tissues & Cell Lines
- Mutation All

Population of genes to consider for p-value calculations:

Reference Set: Whole Human Genome Microarray 4x44K v2

Relationships to consider:  
Affects networks and upstream regulator analysis

Direct and Indirect Relationships  
 Direct Relationships

Optional Analyses:  
 My Project

Analysis Filter Summary  
Consider only relationships where confidence = Experimentally Observed

ADVANCED SAVE

Set data cutoff filters

Set Cutoffs

Expression Value Type	Cutoff	Range	Focus On
Log Ratio		-8.8771 to 8.2299	Both Up/Downregulated
Fold Change	3.0	-470.206 to 300.24	Both Up/Downregulated
p-value	0.01	0.0 to 0.9999	

RECALCULATE

19495 analysis-ready molecules across observations

Click here to apply filter cutoffs and see number that are network and function eligible

Network and function eligible molecules should be 100-2000 for best results, but other values can work

Preview Dataset GSE26129\_MCF-7\_A2780\_IPA.xls Observation: MCF-7TxD10 vs. MCF-7cc (19495)

Analysis-Ready (19495) Mapped IDs (31984) Unmapped IDs (41000)

View other observations if a multi-observation data set

Log	Symbol	Entrez Gene Name	Location	Type(s)	Drug(s)
↓ -1.326	A1BG*	alpha-1-B glycop	Extracellular Space	other	
↓ -0.407	A1BG-AS1	A1BG antisense R	unknown	other	
↑ 0.715	A1CF*	ADP-DEF1	Nucleus	enzyme	

RUN ANALYSIS CANCEL

# Creating an IPA Core Analysis- Network Generation

Option to exclude endogenous chemicals from networks

Option to turn off molecular networks for a faster analysis

Fine-tune format of networks

Turn On Causal Network (Advanced Analytics)

ADVANCED SAVE AS DEFAULTS

Set Cutoffs

Expression Value Type	Cutoff	Range	Focus On
Log Ratio		-8.8771 to 8.2299	Both Up/Downregulated
Fold Change	3.0	-470.206 to 300.24	Both Up/Downregulated
p-value	0.01	0.0 to 0.9999	

RECALCULATE 2026 analysis-ready molecules across observations

Preview Dataset GSE26129\_MCF-7\_A2780\_IPA.xls Observation: MCF-7TxD10 vs. MCF-7cc (840)

Analysis-Ready (840) \ Mapped IDs (31984) \ Unmapped IDs (9016) \ All IDs (41000)

ADD TO MY PATHWAY ADD TO MY LIST CREATE DATASET CUSTOMIZE TABLE

	Log Ratio	Fold Change	p-value	ID	Notes	Symbol	Entrez Gene Name	Location	Type(s)	Drug(s)
<input type="checkbox"/>	↑3.668	↑12.712	1.69E-04	A_24_P235429	D	ABCA1*	ATP-binding cas	Plasma Membra	transporter	probucol
<input type="checkbox"/>	↑1.697	↑3.242	7.76E-04	A_24_P67096	D	ABCA5*	ATP-binding cas	Plasma Membra	transporter	
<input type="checkbox"/>	↑1.380	↑20.815	1.10E-04	A_22_P83533		ABCA1	ATP-binding cas	Plasma Membra	transporter	defevidec XR

RUN ANALYSIS CANCEL

Create Core Analysis - [analysis : Time course. Treated vs untreated]

**General Settings**

Network Generation O...

Data Sources All

Confidence Experiment...

Species All

Tissues & Cell Lines All

Mutation All

**ADVANCED** **SAVE AS DEFAULTS**

Population of Relationships: **Relationships**

Relationships: **Relationships**

Affect: **Relationships**

Direct Relationships

Indirect Relationships

Optional Analyses:

- My Project
  - My Pathways
  - My Lists
- Ingenuity CWS
  - My Pathways
  - My Lists
- Alcon
  - My Pathways
  - My Lists

**Analysis Filter Summary**

Consider only relationships where confidence = **Experimentally Observed**

**Set Cutoffs**

Expression Value Type	Cutoff	Range	Focus On
Fold Change	2	-17.2747 to 46.8718	Both Up/Downregulated
p-value	.05	0.0 to 0.9994	

**RECALCULATE** 363 analysis-ready molecules across observations

**Preview Dataset** Time course. Treated vs untreated Observation: 120 hours (304)

Analysis-Ready (304) | Mapped IDs (461) | Unmapped IDs (20) | All IDs (481)

**ADD TO MY PATHWAY** **ADD TO MY LIST** **CREATE DATASET** **CUSTOMIZE TABLE**

	Fold Change	p-value	ID	Notes	Symbol	Entrez Gene Name	Location	Type(s)	Drug(s)
<input type="checkbox"/>	+2.974	2.00E-06	209459_s_at		ABAT	4-aminobutyrate a	Cytoplasm	enzyme	valproic acid, vig...
<input type="checkbox"/>	+14.652	1.59E-04	207692_s_at	D	ACAN*	aggrecan	Extracellular Space	other	
<input type="checkbox"/>	+41.570	0.00E00	205132_at		ACTC1	actin, alpha, cardia	Cytoplasm	enzyme	
<input type="checkbox"/>	+19.161	0.00E00	226814_at	D	ADAMTS9*	ADAM metallopep	Extracellular Space	peptidase	
<input type="checkbox"/>	+4.054	0.00E00	213974_at		ADAMTSL3	ADAMTS-like 3	unknown	other	
<input type="checkbox"/>	+2.267	3.00E-06	205771_s_at		AKAP7	A kinase (PRKA) an	Plasma Membrane	other	
<input type="checkbox"/>	+4.429	1.00E-06	215783_s_at		ALPL	alkaline phosphata	Plasma Membrane	phosphatase	

Rows: 1 - 50

**RUN ANALYSIS** **CANCEL**

Several filters available. Set criteria to filter out findings of less interest.

Create Core Analysis - [analysis : Time course. Treated vs untreated]

**General Settings**

Network Generation O...

Data Sources All

Confidence Experiment...

Species All

Tissues & Cell Lines All

Mutation All

**ADVANCED**

SAVE AS DEFAULTS

**Set Cutoffs**

Expression Value Type Cutoff

Fold Change

2

p-value

.05

**Preview Dataset Time course. Trea**

Analysis-Ready (304) Mapped IDs

ADD TO MY PATHWAY

ADD TO MY LIST

<input type="checkbox"/>	Fold Change	p-value
<input type="checkbox"/>	↑2.974	2.00E-06
<input type="checkbox"/>	↑14.652	1.59E-04
<input type="checkbox"/>	↑41.570	0.00E00
<input type="checkbox"/>	↑19.161	0.00E00
<input type="checkbox"/>	↑4.054	0.00E00
<input type="checkbox"/>	↑2.267	3.00E-06
<input type="checkbox"/>	↑4.429	1.00E-06

**Population of genes to consider for p-value calculations:**

Reference Set: Human Genome U133 Plus 2.0 Array

**Relationships to consider:**

Affects networks and transcription factor analysis

Direct and Indirect Relationships

Direct Relationships

Optional Analyses:

**Analysis Filter Summary**

Consider only relationships where confidence = Experimentally Observed

Make sure molecule coloring is set for a metric such as fold change, log ratio, etc.

**Advanced Settings**

Select expression value for node coloring: Fold Change

This expression value type will be used to calculate the directionality of functions and will be displayed in color on pathways and networks.

**Duplicate Resolution**

When IDs map to the same gene, protein, or other molecule:

Apply cutoffs before consolidating IDs: Yes (recommended)

Resolve duplicates using Exp Value: Fold Change

Consolidate IDs using the expression value: maximum

Confirm how you would like to resolve duplicates

**Observation to Include**

Include	Observation Name	Analysis Ready
<input type="checkbox"/>	#1 2 hours	9
<input checked="" type="checkbox"/>	#2 24 hours	169
<input checked="" type="checkbox"/>	#3 120 hours	304

Deselect any observations that you would like to exclude from the analysis

OK CANCEL

ANALYSIS CANCEL

Set criteria to filter out findings of less interest.

- Species
- Tissue

Filter stringency

- A “Stringent” setting requires that each of a pair of molecules and the relationship that connects them meet the filter criteria
- A “Relaxed” filter requires that the gene or protein expression of the molecules connected by a relationship meet the filter criteria

Unspecified refers to findings or molecules where cell/tissue/organ is not specified or classified

protein-protein interactions [1]

+ Binding of **MATRILYSIN [MMP7]** protein and human **TIMP2** protein occurs in a cell-free system.

### Pre-filter Advantages

- Focuses IPA analysis on networks, biological functions, and canonical pathways on molecules and relationships closely related to the experiment.

### Pre filter Disadvantages

- Loss of information
- Loss of relationships that may be applicable to your species or tissue but were described in a different species or tissue.

Create Core Analysis - [analysis : Prostate Disease.txt]

### Filters and General Settings

**General Settings** ?

**Species** All ?

**Tissues & Cell Lines** D11-145... ?

**Data Sources** All ?

**SAVE AS DEFAULT FILTERS**

3 of 3 Observations selected for Analysis **EDIT** Click Edit to select observations to be analyzed.

### Expression Value Parameters

- Liver
- Lung
- Mammary Gland
- Ovary
- Pancreas
- Placenta
- Prostate Gland
- Retina
- Salivary Gland
- Skeletal Muscle
- Small Intestine
- Spleen

Stringent filter (filter molecules and relationships) ?

Relaxed filter (filter molecules) ?

## Large Scale Data Analysis

## IPA Core Analysis

- Pathway Analysis
  - Predicts pathways that are changing based on gene expression
  - New tools to predict directional effects on the pathway (MAP overlay tool)
- Upstream Regulator Analysis
  - Predicts what regulators caused changes in gene expression
  - Predicts directional state of regulator
  - Creates de novo pathways based on upstream regulators (Mechanistic Networks)
- Diseases and Functions Analysis
  - Predicts effected biology (cellular processes, biological functions) based on gene expression and predicts directional change on that effect
    - “Increase in cell cycle”
    - “Decrease in apoptosis”
- Regulator Effects
  - Models pathway interactions from predicted upstream regulators, through differentially expressed genes, to biological processes
- Networks
  - Predicts non-directional gene interaction map

**CLEAR**

You can predict the upstream and downstream effects of activation or inhibition on other molecules. Begin by applying expression values from a dataset or analysis, or interactively in silico.

**Predict effect of dataset or in silico changes**

**PREDICTION ON**  Display prediction legend

Predict effects:  
Upstream and Downstream

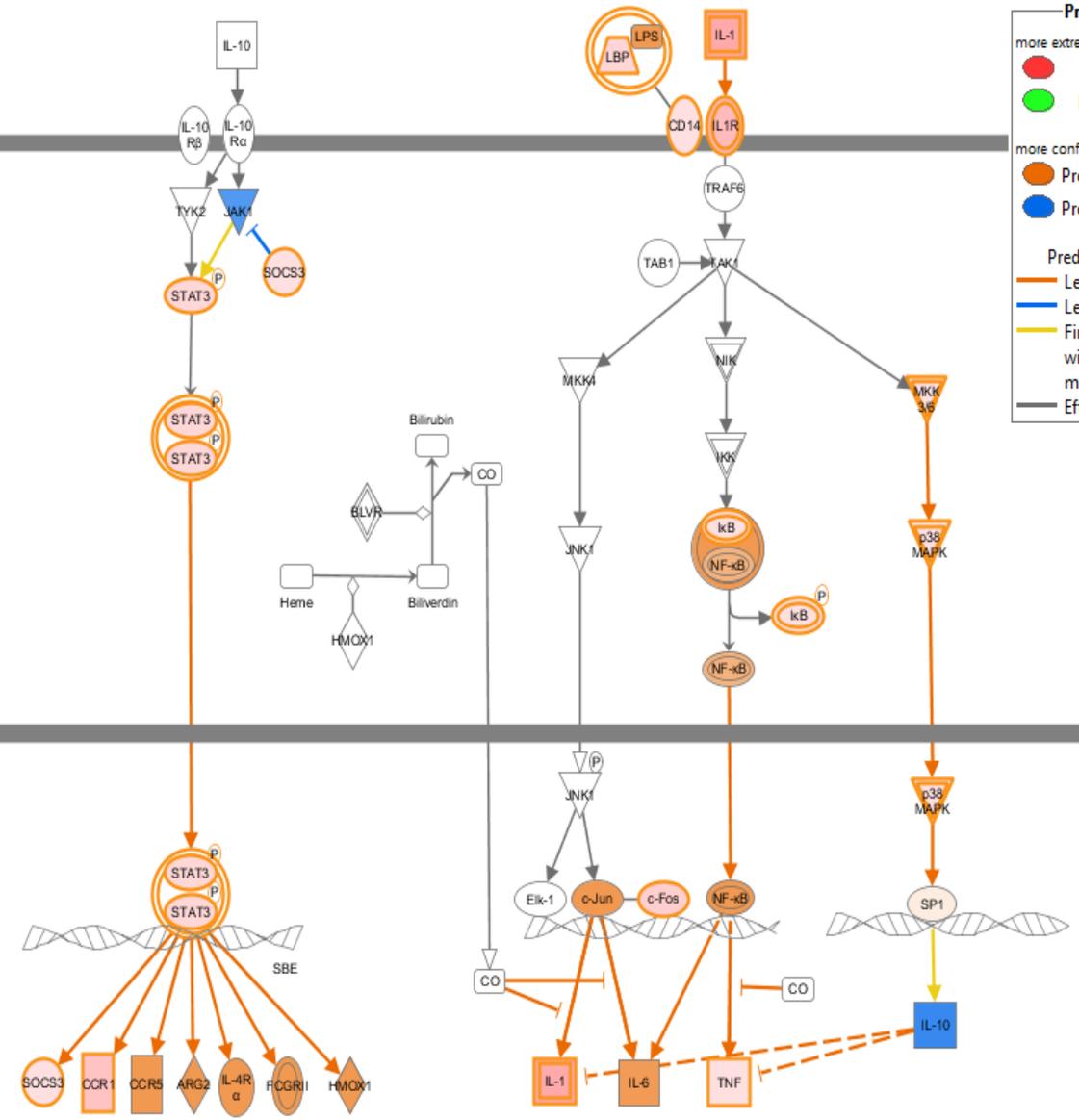
**Activate or inhibit molecules interactively in silico**

Select the value to apply and then click the molecules you wish to apply them to.

Use expression values from a Dataset or Analysis  
Current Analysis/Dataset/List: Day 10  
[Change Analysis/Dataset/List](#)

**IL-10 Signaling**

Navigation icons: Home, Back, Forward, Refresh, Search, etc.



**Prediction Legend**

more extreme | less

● Upregulated (red) | ● Downregulated (green)

more confidence | less

● Predicted activation (orange) | ● Predicted inhibition (blue)

**Predicted Relationships**

— Leads to activation (orange line)

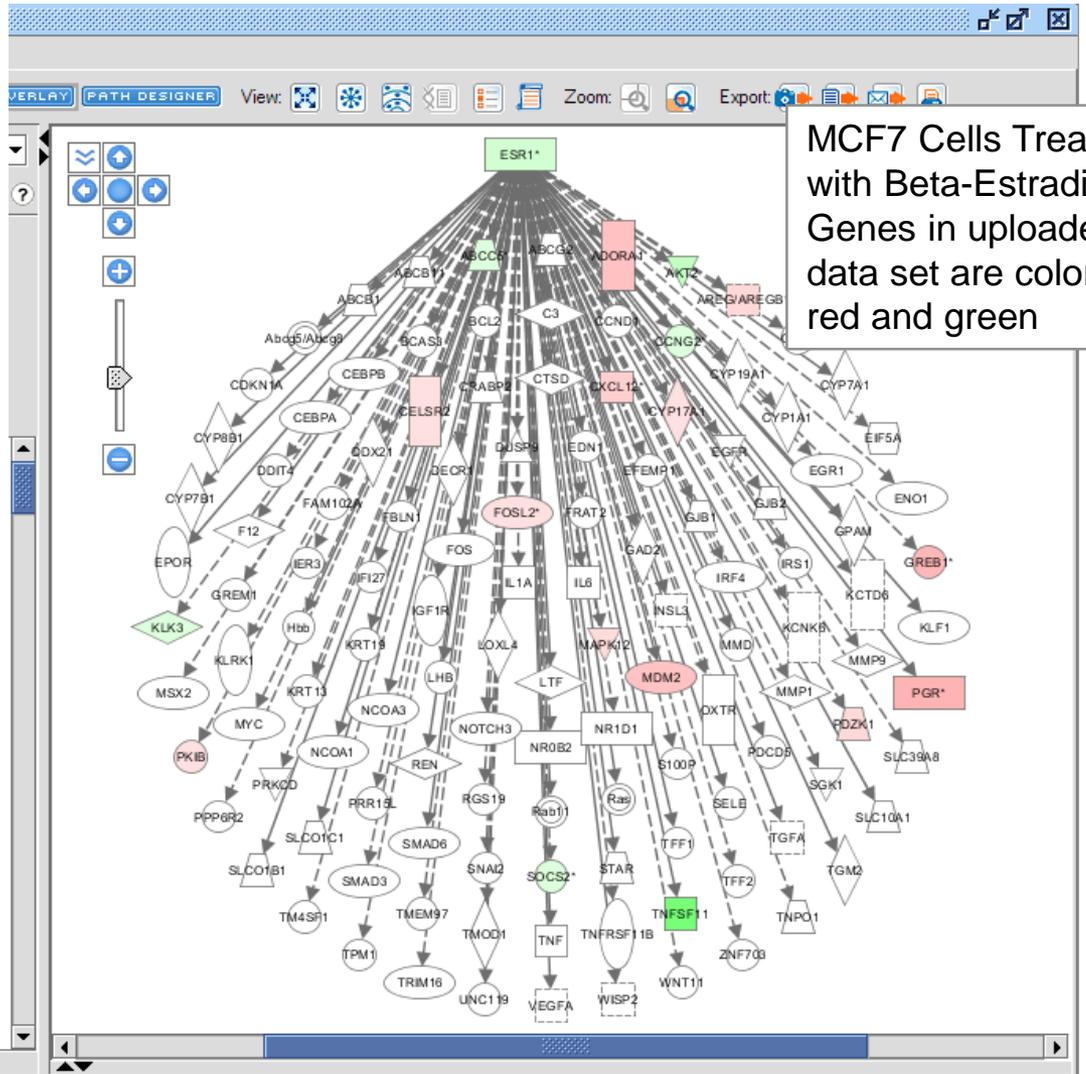
— Leads to inhibition (blue line)

— Findings inconsistent with state of downstream molecule (yellow line)

— Effect not predicted (grey line)

## IPA Upstream Regulator Analysis

- Use published experimental molecular interactions to identify upstream regulators
- Identify upstream regulators by determining gene enrichment in downstream genes
- Predict the activity state of regulators by correlating literature reported effects with observed gene expression

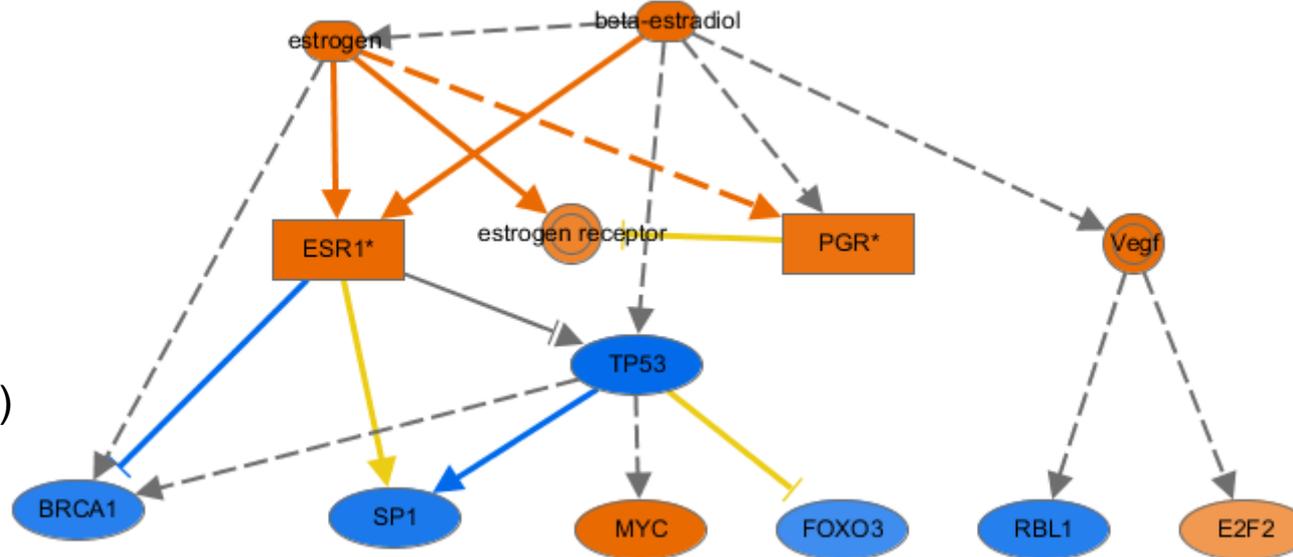


MCF7 Cells Treated with Beta-Estradiol. Genes in uploaded data set are colored red and green

## IPA Mechanistic Networks

Upstream Regulator	Fold Change	Molecule Type	Predicted Activation	Activation z-sc...	p-value of overlap	Target molecules in...	Mechanistic Net...
<input type="checkbox"/> beta-estradiol		chemical - endogen	Activated	6.097	1.24E-26	↓ABCA1, ↓... all 122	186 (13)
<input type="checkbox"/> Mek		group	Activated	3.683	7.37E-07	↑ABCE1, ↑... all 16	
<input type="checkbox"/> estrogen		chemical drug	Activated	3.661	2.00E-04	↓ABCA1, ↑... all 19	129 (13)
<input type="checkbox"/> ESR1	↓-1.708	ligand-dependent nu	Activated	3.504	2.84E-13	↓ABCC5, ↑... all 37	183 (13)
<input type="checkbox"/> IL3		cytokine	Activated	3.190	1.74E-02	↑ADA, ↑AR... all 16	

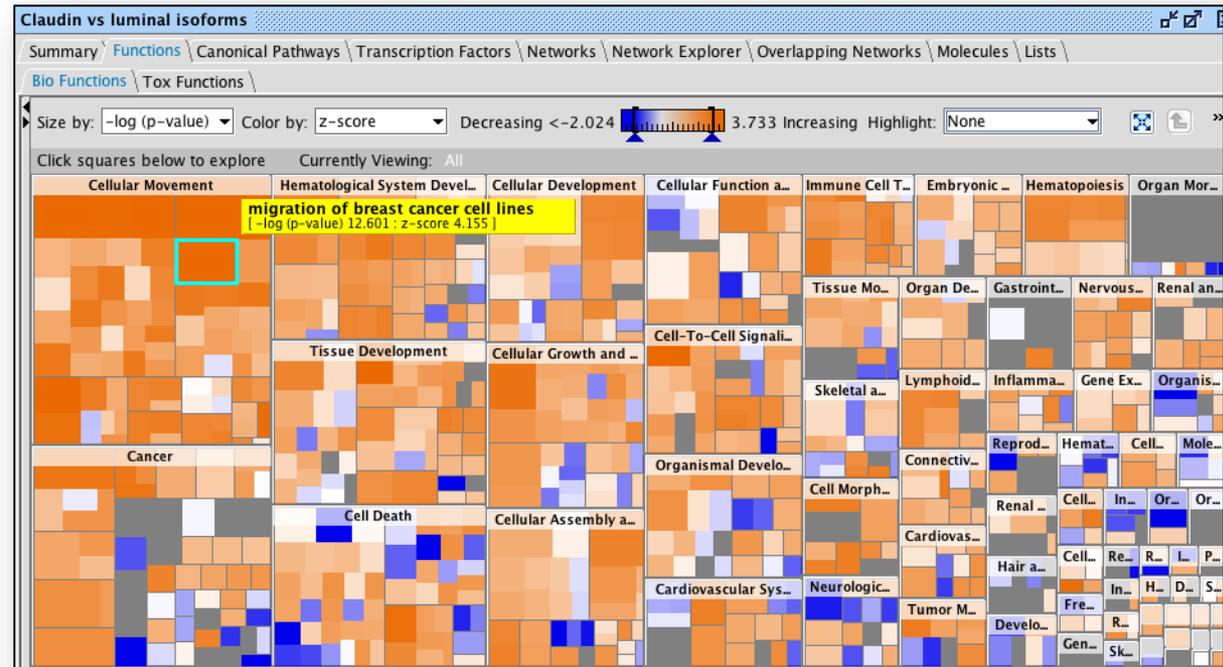
- Identify potential upstream regulator signal transduction
- Using shared downstream gene effects and gene-gene interactions, pathways (mechanistic networks) are created.



## Downstream Effects Analysis

Identify key biological processes influenced by differentially expressed genes

Understand whether cellular processes are being driven up or down by correlating observed expression with reported experimental gene effects

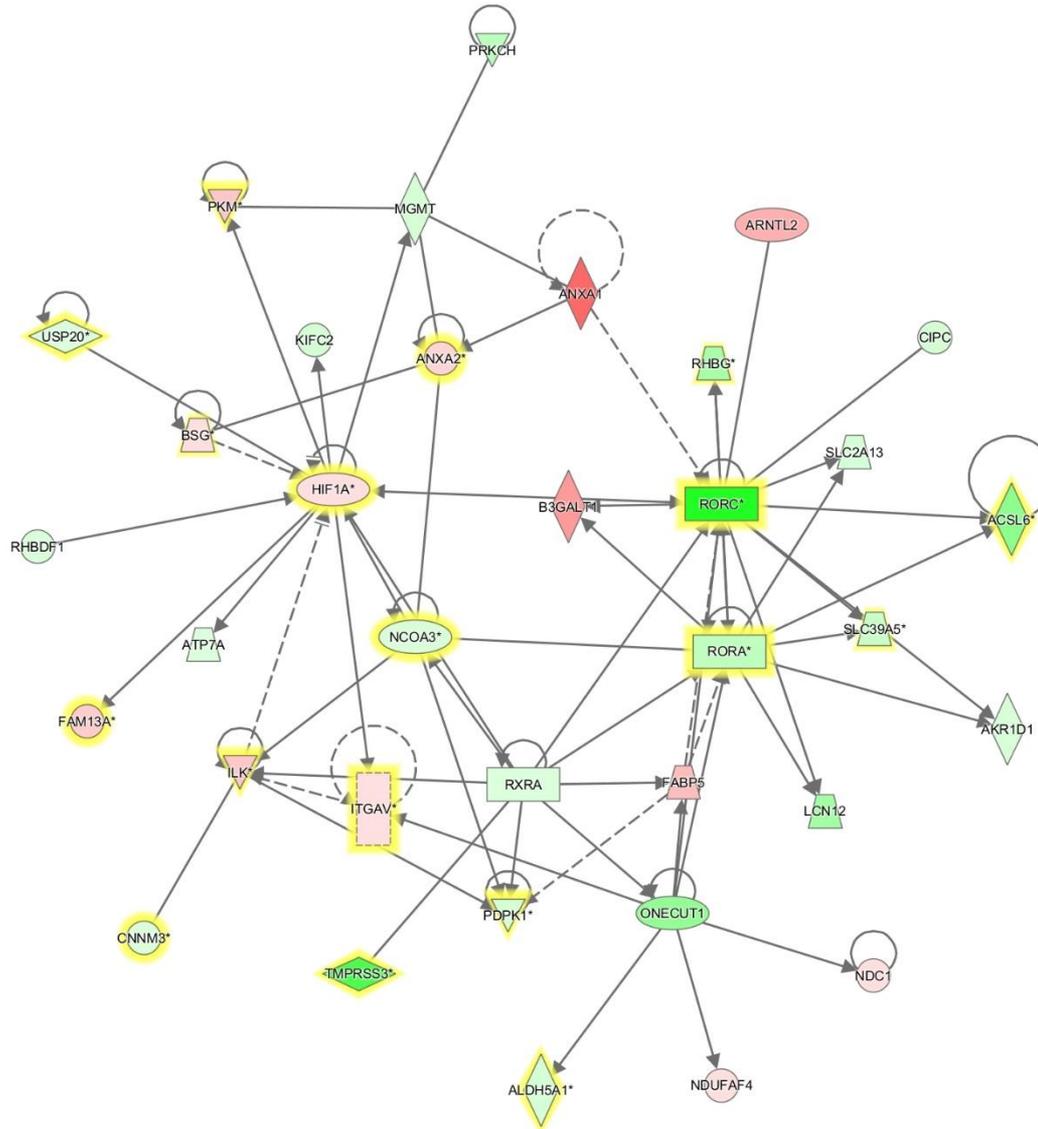


- Each box represents a biological process or disease
- The size of the box represents gene enrichment
- The color of the box indicates the predicted increase or decrease

## Regulator Effects

- Hypothesis for how a phenotype, function or disease is regulated in the dataset by activated or inhibited upstream regulators
- Explain impact of upstream molecules on downstream biology
- Explain potential mechanism for a phenotype or drug
- Define drug targets
- 





# Analyzing and Interpreting Results

- IPA will subdivide your data into slices based on molecule connectivity (networks), cellular functions, and involvement in canonical pathways
- Spend time surveying the information. Not everything is of scientific interest, look for slices of your data that address your scientific question, are consistent with known biological processes, are consistent with pathology, etc.
- Typically the goal will be to find a set of genes/molecules that can be looked at in greater detail by building a custom pathway
- If you are comparing observations, run comparison analysis.

IPA calculates two distinct statistics as part of a core analysis

■ P-value:

- Calculated using a Right-Tailed Fisher's Exact Test
- Reflects the likelihood that the association or overlap between a set of significant molecules from your experiment and a given process/pathway/transcription neighborhood is due to random chance. The smaller the p-value the less likely that the association is random.
- The p-value does not consider the directional effect of one molecule on another, or the direction of change of molecules in the dataset.

■ Z-score:

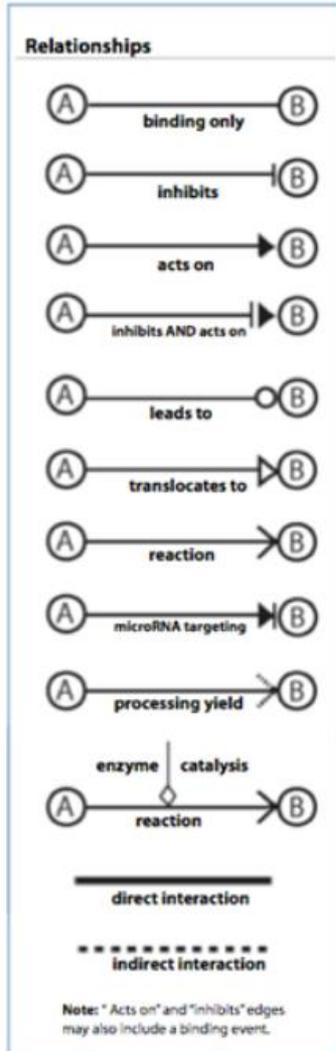
- Applied in some analysis types and provides predictions about upstream or downstream processes.
- Takes into account the directional effect of one molecule on another molecule or on a process, and the direction of change of molecules in the dataset.

# Analyzing Results

## Canonical Pathway Analysis

- What known biological pathways appear most significantly affected by the genes in my data set?
- What genes within a pathway are changing in expression and what effect might that change have on the pathway?

- Bar-chart represents significance of gene enrichment for any given pathway
  - Significance is most important metric
  
- Ignore bumpy yellow line: ratio/percent coverage of a pathway subject to pathway size bias
  
- Bar-chart color indicates predicted directionality
  - When considering pathway directionality, focus on  $2 < z\text{-score} < -2$
  - Just because a pathway does not have a good z-score does not make it uninteresting
  
- To open pathway, look for open pathway button on far right after bar-chart selection



### Relationship Labels

A	Activation
B	Binding
C	Causes/Leads to
CC	Chemical-Chemical interaction
CP	Chemical-Protein interaction
E	Expression (includes metabolism/ synthesis)
EC	Enzyme Catalysis
I	Inhibition
L	Proteolysis (includes degradation for Chemicals)
LO	Localization
M	Biochemical Modification
mIT	microRNA Targeting
MB	Group/complex Membership
nTRR	Non-Targeting RNA-RNA Interaction
P	Phosphorylation/Dephosphorylation
PD	Protein-DNA binding
PP	Protein-Protein binding
PR	Protein-RNA binding
PY	Processing Yields
RB	Regulation of Binding
RE	Reaction
RR	RNA-RNA Binding
T	Transcription
TR	Translocation
UB	Ubiquitination

### Network Shapes

	Cytokine
	Growth Factor
	Chemical /Drug/ Toxicant
	Enzyme
	G-protein Coupled Receptor
	Ion Channel
	Kinase
	Ligand-dependent Nuclear Receptor
	Peptidase
	Phosphatase
	Transcription Regulator
	Translation Regulator
	Transmembrane Receptor
	Transporter
	Complex / Group
	microRNA
	Mature microRNA
	Other

## Interpretation Tips

- Look for pathway biological themes
  - Use Overlapping Pathway tab to filter and view pathways with shared genes
  - Often lesser scoring pathways of a theme are simply subsets of genes found in a better scoring pathway
  
- Scan CP names for pathways of particular interest
  - Statistical significance does not equal biological significance and visa-versa
  - Pathways may have many second messengers which can be regulated post-transcriptionally
  
- View pathways by clicking the bar-chart and the OPEN PATHWAY button on right
  
- Use MAP tool (OVERLAY tool) to help interpretation
  
- Overlay other analyses as applicable
  
- Toggle overlay options

- Scroll-wheel on mouse controls zoom, or use toolbar zoom buttons
- Left-click selects (turns blue)
- Left-click-drag on nodes moves the node
- Right-click hold-and-drag moves your view
- Right-click brings up menu for controlling
  - tool tip (mouse-over node pop-up)
  - copy/paste
  - Highlighting (colored outline)
  - selection
- Node shapes indicate a protein's primary function, see Help>Legend
- Relationship lines indicate the type of relationship and the mouse-over letter the type of relationship, see Help>Legend

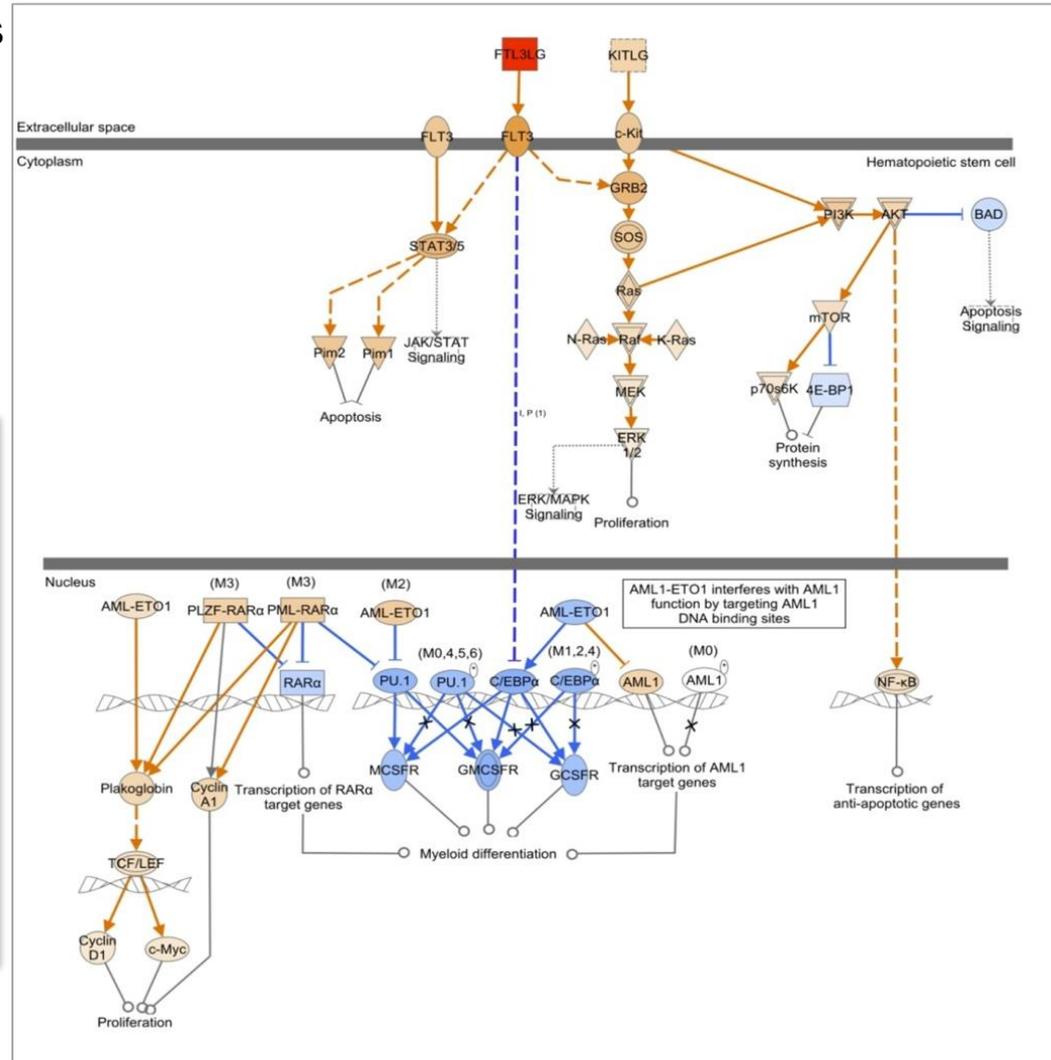
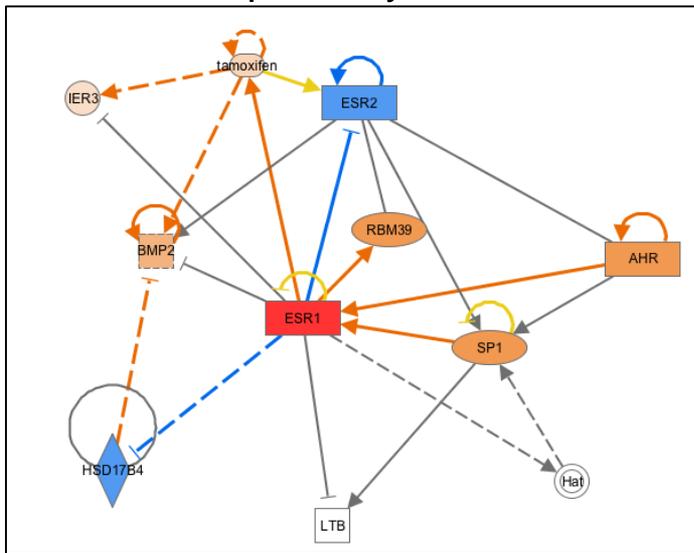


Navigation Control

- Double-clicking a node brings up the node summary
  - You can navigate to the Gene/Chem View page by clicking the protein name at the top of the summary window pane.
- Double-clicking a relationship line brings up the relationship summary
  - You can to the literature evidence findings by clicking the “[View relationships between:...](#)” link at the top of the summary window pane.
- Groups
  - Groups are represented by a double outline applicable to any molecule shape. These represent cases where findings use a general gene name to describe a gene class or group of isoforms
  - Complexes of different proteins are also given a double outline
  - **View members by left-click selecting, then right-click>Show Membership**

## OVERLAY button -> MAP (Molecule Activity Predictor)

- Use observed expression changes to suggest functional effects on neighboring molecules
- Manually set activation states to observe predicted effects on canonical pathways



OVERLAY button -> Analyses, Data sets, and Lists

- Select other analyses from projects
- Useful for comparisons

# Analyzing Results

## Upstream Regulators

- What transcription factors likely led to observed gene expression changes?
- What *de novo* pathways can be created based on predicted upstream regulator interactions?

Identify important signaling molecules for a more complete regulatory picture

Hr12FC

Summary | Functions | Canonical Pathways | **Upstream Analysis** | Networks | Molecules | Lists | My Pathways

Upstream Regulators | Causal Networks

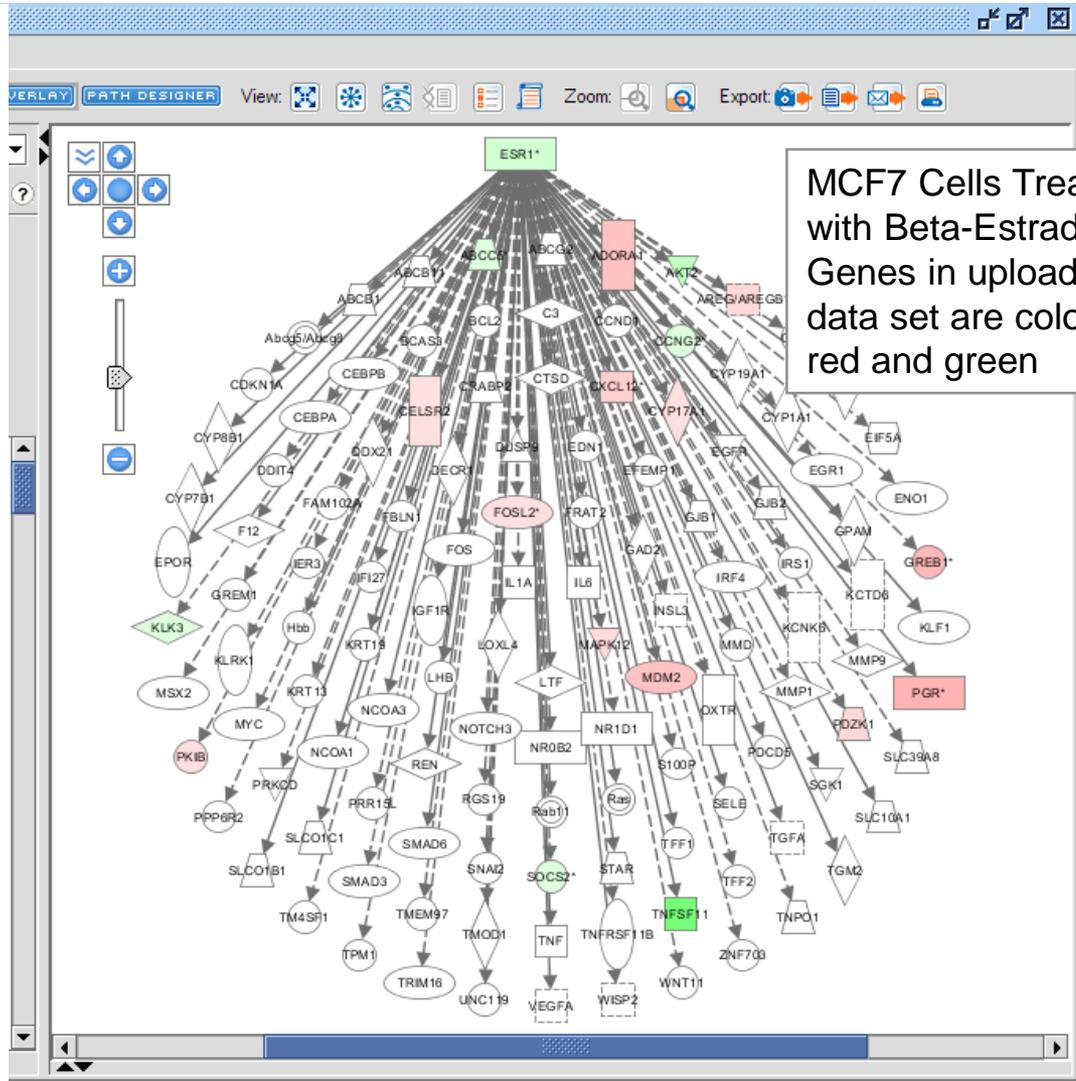
ADD TO MY PATHWAY | ADD TO MY LIST | CUSTOMIZE TABLE | DISPLAY AS NETWORK | MECHANISTIC NETWORKS

<input type="checkbox"/>	Upstream Regulator	Fold Change	Molecule Type	Predicted Activatio...	Activation z-score	p-value of over...	Target molecules in ...	Mechanistic Net...
<input type="checkbox"/>	beta-estradiol		chemical - endogen	Activated	6.097	1.24E-26	↓ABCA1, ↓... all 122	186 (17)
<input type="checkbox"/>	raloxifene		chemical drug		-0.751	1.83E-14	↑AREG/AREGB...all 28	125 (15)
<input type="checkbox"/>	ESR1	↓-1.708	ligand-dependent nu	Activated	3.504	2.84E-13	↓ABCC5, ↑... all 37	186 (20)
<input type="checkbox"/>	trichostatin A		chemical drug		-0.620	1.02E-11	↓ABCA1, ↑... all 45	189 (20)
<input type="checkbox"/>	valproic acid		chemical drug		-1.593	5.88E-11	↓ABCG1, ↓... all 33	193 (18)
<input type="checkbox"/>	fulvestrant		chemical drug	Inhibited	-2.826	2.72E-10	↑ADRB1, ↑... all 27	181 (22)
<input type="checkbox"/>	TGFB1		growth factor		-0.325	4.02E-10	↓ABCA1, ↑... all 86	187 (20)
<input type="checkbox"/>	RAF1		kinase		-0.321	6.96E-10	↑AREG/AREGB...all 25	145 (19)
<input type="checkbox"/>	ESR2		ligand-dependent nu		0.095	9.98E-09	↑ADORA1, ↑... all 18	184 (19)
<input type="checkbox"/>	MYC	↑1.855	transcription regulat	Activated	2.599	1.02E-08	↑ABCE1, ↓... all 52	157 (15)
<input type="checkbox"/>	CCND1	↑1.371	other		0.777	1.28E-08	↑BCL2, ↑B... all 24	154 (18)
<input type="checkbox"/>	ERBB2	↓-1.822	kinase		0.591	1.68E-08	↑AREG/AREGB...all 43	144 (20)
<input type="checkbox"/>	TNF		cytokine		-0.134	2.00E-08	↓ABCA1, ↓... all 77	227 (22)
<input type="checkbox"/>	dexamethasone		chemical drug		-0.930	2.64E-08	↑ABHD2, ↓... all 79	203 (18)
<input type="checkbox"/>	Salmonella enterica s		chemical toxicant		1.149	5.11E-08	↑AREG/AREGB...all 20	
<input type="checkbox"/>	ZNF217	↓-1.315	transcription regulat		0.555	7.38E-08	↓ADM, ↓A... all 13	
<input type="checkbox"/>	PGR	↑5.528	ligand-dependent nu		1.879	8.41E-08	↑AREG/AREGB...all 18	168 (20)
<input type="checkbox"/>	tretinoin		chemical - endogen	Inhibited	-2.611	1.02E-07	↓ABCA1, ↑... all 70	159 (20)
<input type="checkbox"/>	methylselenic acid		chemical reagent		0.152	1.16E-07	↓ACSL3, ↑... all 24	180 (12)

Selected/Total upstream regulators : 0/709

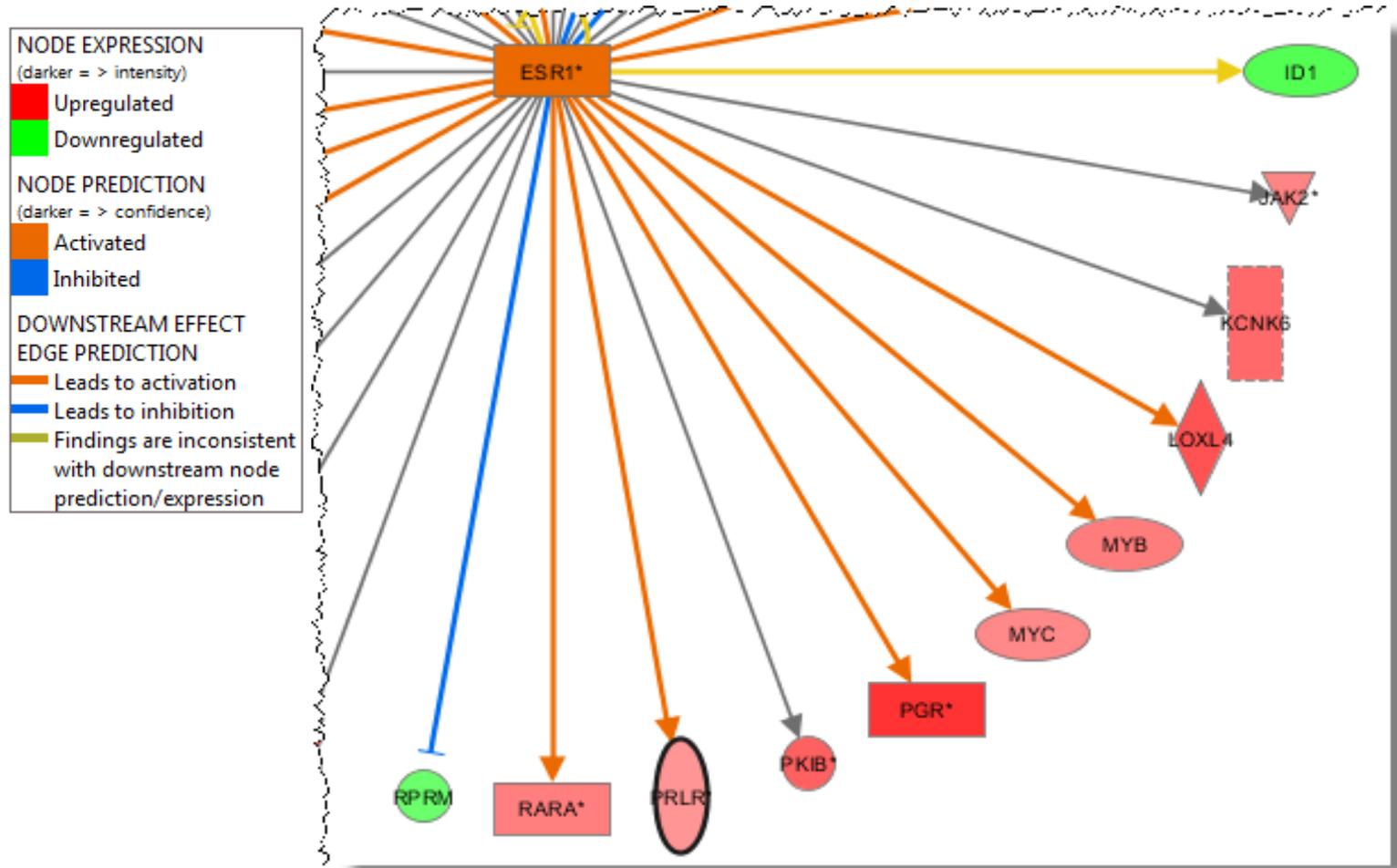
- Quickly filter by molecule type
- Filter by biological context
- Generate regulators-targets Network to identify key relationships

- Use experimentally observed relationships (not predicted binding) between regulators and dataset genes to predict upstream transcriptional regulators.
- Calculate z-score to predict activation or inhibition of regulators based on relationships with dataset genes and direction of change of dataset genes.



# IPA Upstream Regulator Analysis

Directional Effects: Molecule Activity Predictor  
Examine Expression Relationship Consistency



# IPA Upstream Regulator Analysis

Summary | Functions | Canonical Pathways | Upstream Analysis | Networks | Molecules | Lists | My Pathways

Upstream Regulators | Causal Networks

ADD TO MY PATHWAY | ADD TO MY LIST | CUSTOMIZE TABLE | DISPLAY AS NETWORK | MECHANISTIC NETWORKS

<input type="checkbox"/>	Upstream Regulator	Fold Change	Molecule Type	Predicted Activation...	Activation z-score	p-value of over...	Target molecules in ...	Mechanistic Net...
<input type="checkbox"/>	beta-estradiol		chemical - endogen	Activated	6.097	1.24E-26	↓ABCA1, ↓... ..all 122	186 (17)
<input type="checkbox"/>	raloxifene		chemical drug		-0.751	1.83E-14	↑AREG/AREGB...all 28	125 (15)
<input type="checkbox"/>	ESR1	↓-1.708	ligand-dependent nu	Activated	3.504	2.84E-13	↓ABCC5, ↑... ..all 37	186 (20)
<input type="checkbox"/>	trichostatin A		chemical drug		-0.620	1.02E-11	↓ABCA1, ↑... ..all 45	189 (20)
<input type="checkbox"/>	valproic acid		chemical drug		-1.593	5.88E-11	↓ABCG1, ↓... ..all 33	193 (18)
<input type="checkbox"/>	fulvestrant		chemical drug	Inhibited	-2.826	2.72E-10	↑ADRB1, ↑... ..all 27	181 (22)
<input type="checkbox"/>	TGFB1		growth factor		-0.325	4.02E-10	↓ABCA1, ↑... ..all 86	187 (20)
<input type="checkbox"/>	RAF1		kinase		-0.321	6.96E-10	↑AREG/AREGB...all 25	145 (19)
<input type="checkbox"/>	ESR2		ligand-dependent nu		0.095	9.98E-09		
<input type="checkbox"/>	MYC	↑1.855	transcription regulat	Activated	2.599	1.02E-08		
<input type="checkbox"/>	CCND1	↑1.371	other		0.777	1.28E-08		
<input type="checkbox"/>	ERBB2	↓-1.822	kinase		0.591	1.68E-08		
<input type="checkbox"/>	TNF		cytokine		-0.134	2.00E-08		
<input type="checkbox"/>	dexamethasone		chemical drug		-0.930	2.64E-08		
<input type="checkbox"/>	Salmonella enterica s		chemical toxicant		1.149	5.11E-08		
<input type="checkbox"/>	ZNF217	↓-1.315	transcription regulat		0.555	7.38E-08		
<input type="checkbox"/>	PGR	↑5.528	ligand-dependent nu		1.879	8.41E-08		
<input type="checkbox"/>	tretinoin		chemical - endogen	Inhibited	-2.611	1.02E-07		
<input type="checkbox"/>	methylselenic acid		chemical reagent		0.152	1.16E-07		

Selected/Total upstream regulators : 0/709

Molecule Types

- Unfiltered
- Transcription Factors
- miRNA
- Drugs and Chemicals
- Select from list below

Select all

- biologic drug
- chemical - endogenous mammalian
- chemical - endogenous non-mammalian
- chemical - kinase inhibitor
- chemical - other
- chemical - protease inhibitor
- chemical drug

Apply Cancel

- Quickly filter by molecule type
- Filter by biological context
- Generate regulators-targets Network to identify key relationships

- Entities with positive z-scores are known to elicit the same gene expression changes as seen in your data
  - Entities you might want to knock-down to inhibit effects of experiment
- Entities with negative z-scores are known to elicit the opposite gene expression when active
  - Entities you could add to an experiment to counter effects of experiment
  
- Contradictions between z-score direction prediction and measured gene expression could be the result of
  - A discrepancy between protein activity and expression level
  - Lag time between change in gene expression and effect of that expression
  
- A regulator with significant z-score but poor p-value could represent a situation where only a few downstream genes in your experimental condition correlate in expression, but many other genes may be expressed in other conditions (or is junk).
- A regulator with insignificant z-score and significant p-value could represent a situation where the genes in your data are downstream of the regulator, but their expression pattern is unique to your experimental condition (or is junk).

## IPA Upstream Regulator Analysis

Summary | Functions | Canonical Pathways | **Upstream Analysis** | Networks | Molecules | Lists | My Pathways

Upstream Regulators | Causal Networks

ADD TO MY PATHWAY | ADD TO MY LIST | CUSTOMIZE TABLE | DISPLAY AS NETWORK | MECHANISTIC NETWORKS

<input type="checkbox"/>	Upstream Regulator	Fold Change	Molecule Type	Predicted Activation	Activation z-sc...	p-value of overlap	Target molecules in ...	Mechanistic Net...
<input type="checkbox"/>	beta-estradiol		chemical - endogeno	Activated	6.097	1.24E-26	↓ABCA1, ↓... all 122	186 (17)
<input type="checkbox"/>	Mek		group	Activated	3.683	7.37E-07	↑ABCE1, ↑... all 16	116 (13)
<input type="checkbox"/>	estrogen		chemical drug	Activated	3.661	2.00E-04	↓ABCA1, ↑... all 19	160 (18)
<input type="checkbox"/>	ESR1	↓-1.708	ligand-dependent nu	Activated	3.504	2.84E-13	↓ABCC5, ↑... all 37	186 (20)
<input type="checkbox"/>	IL3		cytokine	Activated	3.190	1.74E-02	↑ADA, ↑AR... all 16	
<input type="checkbox"/>	MYC	↑1.855	transcription regulat	Activated	2.500	1.02E-08	↑ABCE1, ↓... all 52	157 (15)
<input type="checkbox"/>	LEP						↓ESR1 ...all 13	
<input type="checkbox"/>	Cg						↑A... all 21	215 (19)
<input type="checkbox"/>	Vegf						↑C... all 27	141 (19)
<input type="checkbox"/>	PI3K (complex)						A1, ↑... all 14	
<input type="checkbox"/>	FSH		complex	Activated	2.291	8.10E-07	↓ADM, ↑A... all 29	202 (22)
<input type="checkbox"/>	F2		peptidase	Activated	2.287	1.50E-03	↑B4GALT1, ↑... all 16	163 (16)
<input type="checkbox"/>	NFkB (complex)		complex	Activated	2.258	2.02E-02	↓ABCG1, ↑... all 24	
<input type="checkbox"/>	Immunoglobulin		complex	Activated	2.236	7.32E-02	↓ADM, ↑B... all 10	
<input type="checkbox"/>	lithium chloride		chemical drug	Activated	2.213	4.22E-04	↑BCL2, ↑CDC6 ...all 9	147 (17)
<input type="checkbox"/>	ERK		group	Activated	2.200	1.05E-02	↑AREG/AREGB...all 12	
<input type="checkbox"/>	MYB	↑2.039	transcription regulat	Activated	2.199	6.99E-02	↑BCL2, ↓CD... all 5	
<input type="checkbox"/>	NFKBIA	↓-1.204	transcription regulat	Activated	2.183	7.24E-02	↑ATP11A, ↑... all 17	
<input type="checkbox"/>	CSF1	↓-1.195	cytokine	Activated	2.154	4.52E-01	↑BCL2, ↑EGR3 ...all 5	

Selected/Total upstream regulators : 0/709

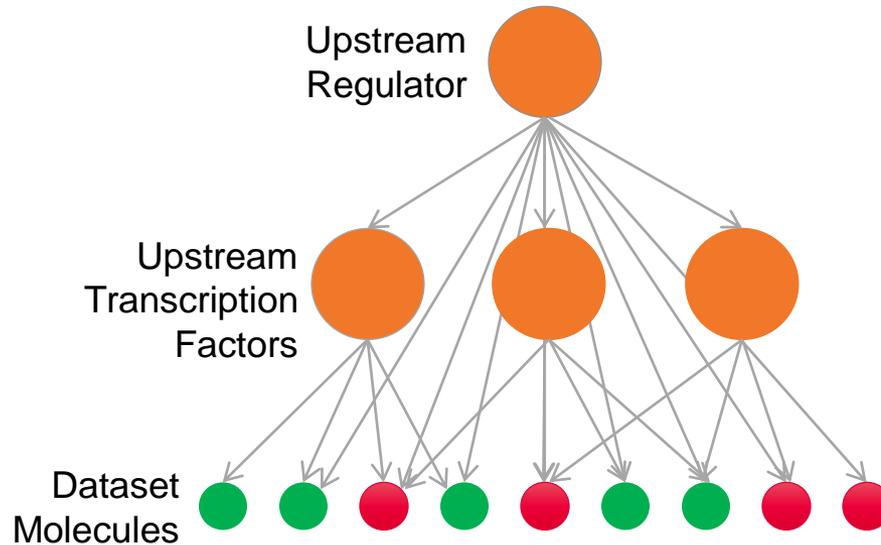
How might these upstream regulators interact?

- Quickly filter by molecule type
- Filter by biological context
- Generate regulators-targets Network to identify key relationships

## IPA Mechanistic Networks

Goal: To discover plausible sets of connected upstream regulators that can work together to elicit the gene expression changes observed in a dataset

How: Take IPA Upstream Regulator results and computationally seek pairs of regulators predicted to affect the expression of a similar set of genes. Repeat to build a network:

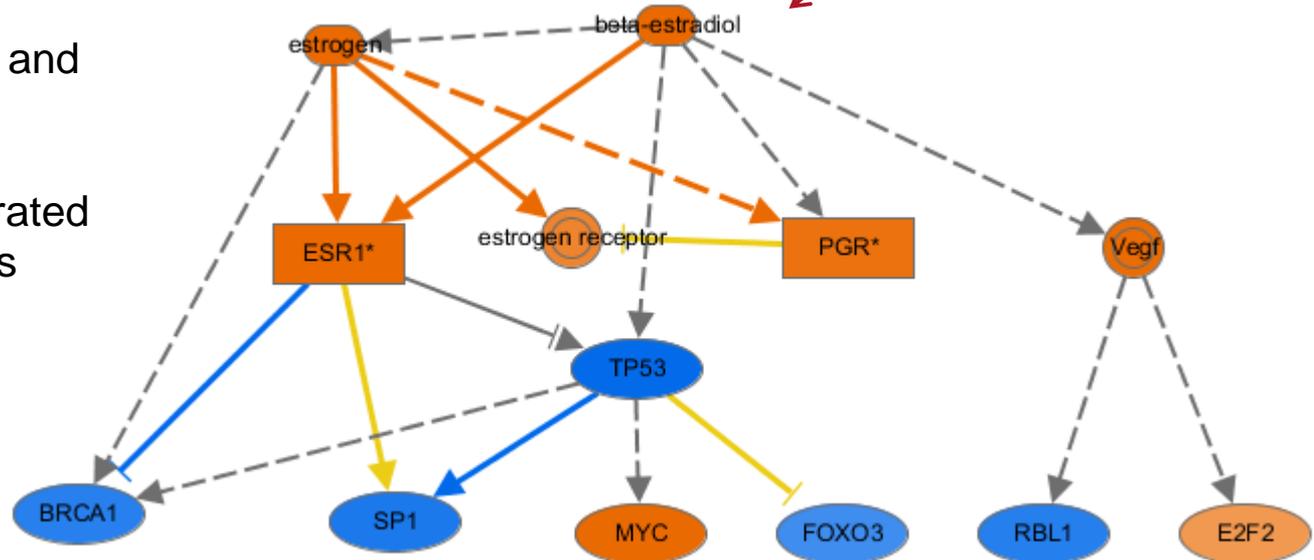


How might the upstream molecule drive the observed expression changes?

Upstream Regulator	Fold Change	Molecule Type	Predicted Activation	Activation z-score	p-value of overlap	Target molecules in	Mechanistic Net...
<input type="checkbox"/> beta-estradiol		chemical - endogen...	Activated	6.097	1.24E-26	↓ABCA1, ↓... all 122	186 (13)
<input type="checkbox"/> Mek		group	Activated	3.683	7.37E-07	↑ABCE1, ↑... all 16	
<input type="checkbox"/> estrogen		chemical drug	Activated	3.661	2.00E-04	↓ABCA1, ↑... all 19	129 (13)
<input type="checkbox"/> ESR1	↓-1.708	ligand-dependent nu...	Activated	3.504	2.84E-13	↓ABCC5, ↑... all 37	183 (13)
<input type="checkbox"/> IL3		cytokine	Activated	3.190	1.74E-02	↑ADA, ↑AR... all 16	

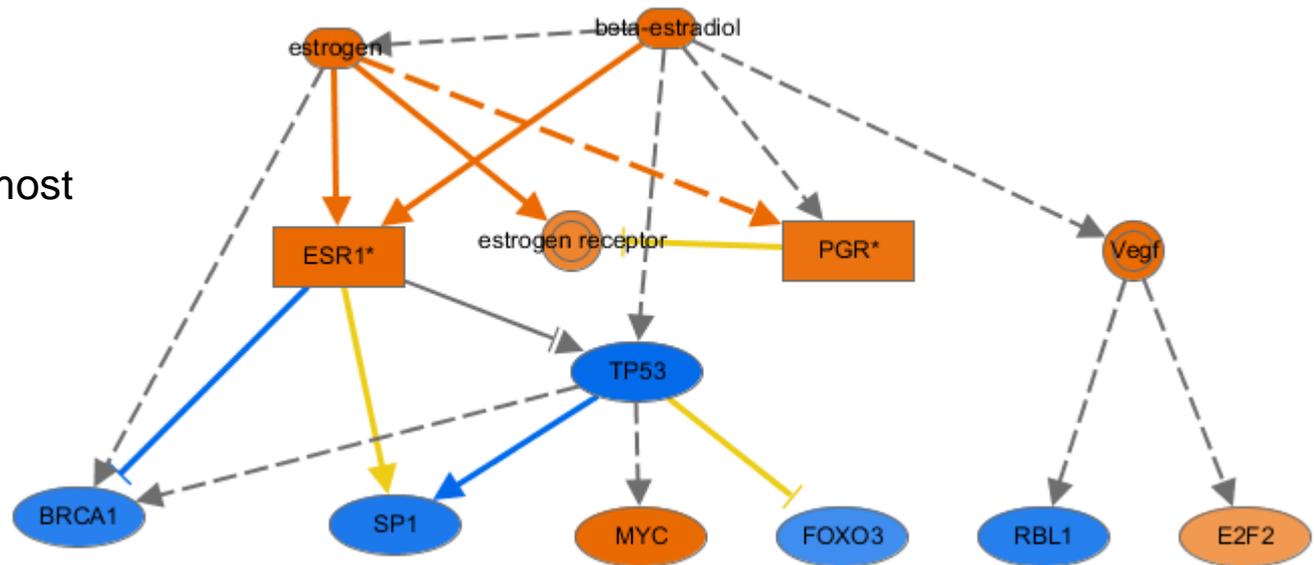
Hypothesis generation and visualization

Each hypothesis generated indicates the molecules predicted to be in the signaling cascade



Summary   Functions   Canonical Pathways   Upstream Analysis   Networks   Molecules   Lists   My Pathways								
Upstream Regulators   Causal Networks								
<input type="button" value="ADD TO MY PATHWAY"/> <input type="button" value="ADD TO MY LIST"/> <input type="button" value="CUSTOMIZE TABLE"/> <input type="button" value="DISPLAY AS NETWORK"/> <input type="button" value="MECHANISTIC NETWORKS"/>								
<input type="checkbox"/>	Upstream Regulator	Fold Change	Molecule Type	Predicted Activation	Activation z-sc...	p-value of overlap	Target molecules in ...	Mechanistic Net...
<input type="checkbox"/>	beta-estradiol		chemical - endogen	Activated	6.097	1.24E-26	↓ABCA1, ↓... ...all 122	186 (13)
<input type="checkbox"/>	Mek		group	Activated	3.683	7.37E-07	↑ABCE1, ↑... ...all 16	
<input type="checkbox"/>	estrogen		chemical drug	Activated	3.661	2.00E-04	↓ABCA1, ↑... ...all 19	129 (13)
<input type="checkbox"/>	ESR1	↓-1.708	ligand-dependent nu	Activated	3.504	2.84E-13	↓ABCC5, ↑... ...all 37	183 (13)
<input type="checkbox"/>	IL3		cytokine	Activated	3.190	1.74E-02	↑ADA, ↑AR... ...all 16	

Recommend increasing stringency of criteria in most cases



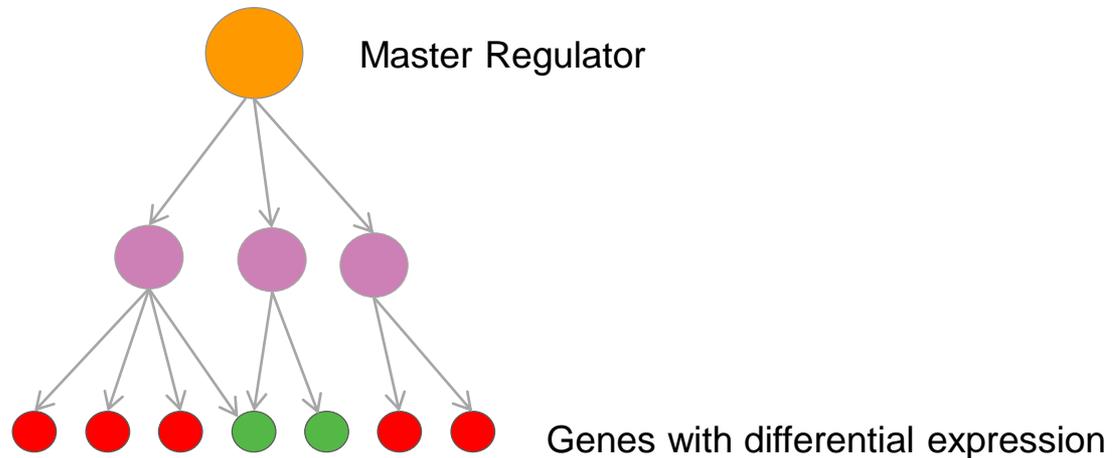
# Advanced Analytics

## Causal Networks

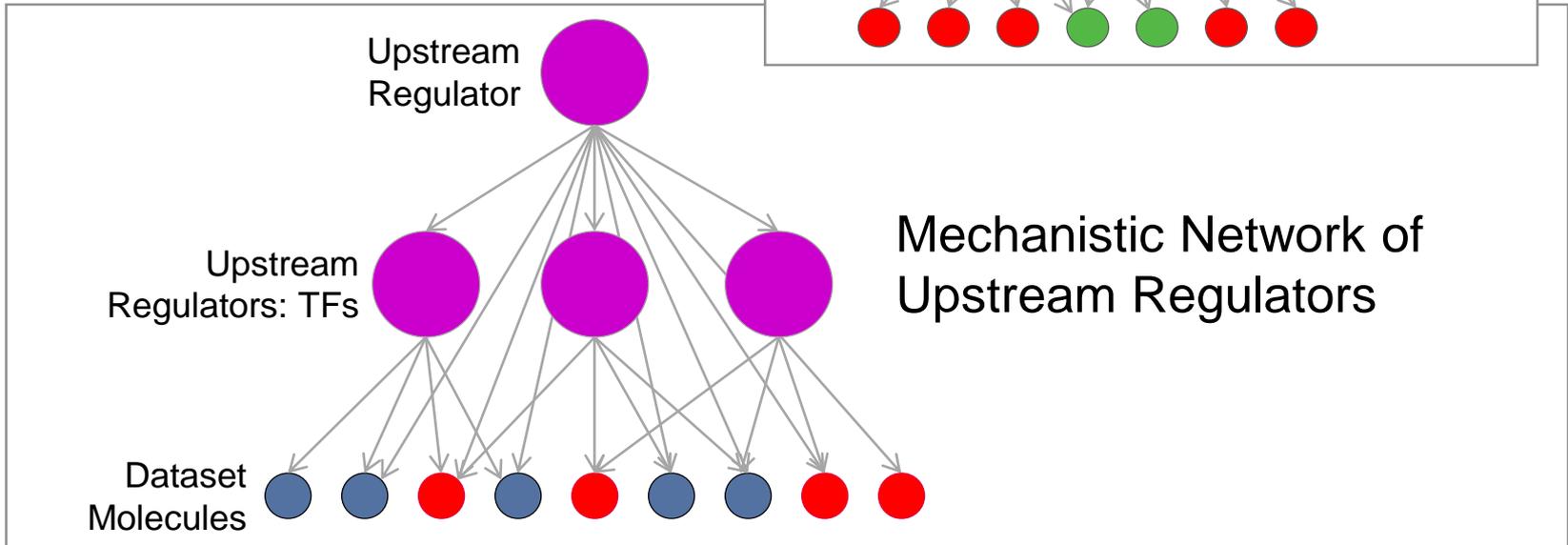
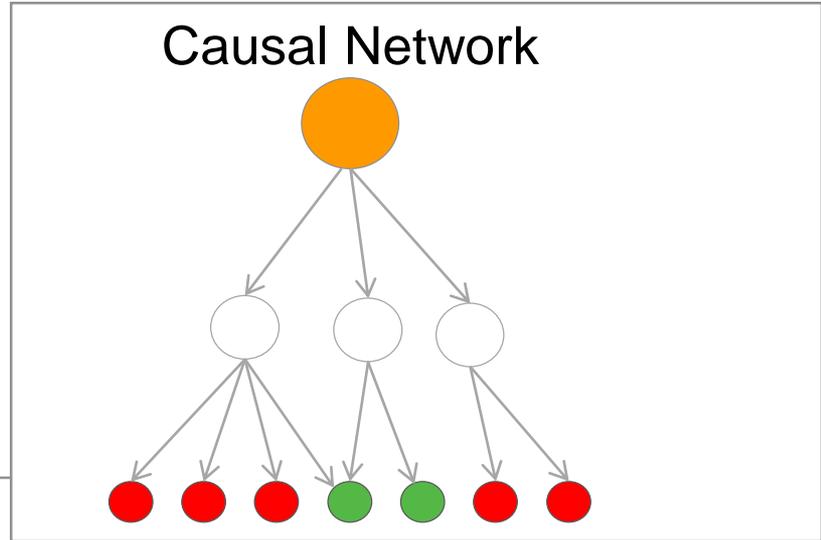
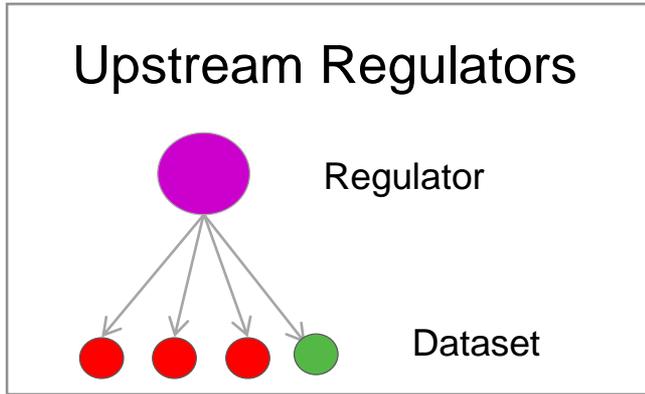
## Advanced Analytics

- Alternate method of predicting upstream regulators based on causal relationships and allowing multiple interaction steps to gene expression changes
- Identify potential novel master-regulators of your gene expression by creating pathways of literature-based relationships
- Expands predictions to include indirect upstream regulators not in mechanistic networks

## Causal Networks



### Advanced Analytics: Causal Network Analysis



Create Core Analysis - [analysis : E2 of MCF7 P05.xls]

**General Settings** ?

**Networks** Interaction & Causa... ?

**Node Types** ?

**Data Sources** All ?

**Confidence** Experimentally Ob... ?

**Species** All ?

**Tissues & Cell Lines** All ?

**Mutation** All ?

**Generate the following Networks** (increases analysis time)

**Interaction networks**

Include endogenous chemicals    Molecules per network    Networks per analysis

*Genes are always included*       

**Causal networks**

Score master regulators for relationships to diseases, functions, genes, or chemicals (max 50)

Score using causal paths only

[ADD...](#)  
[REMOVE](#)

[ADVANCED](#)   [SAVE AS DEFAULTS](#)

**Set Cutoffs**

Expression Value Type	Cutoff	Range	Focus On	
Exp Fold Change	<input type="text"/>	-22.7434 to 25.1208	<input type="text" value="Both Up/Downregulated"/>	<a href="#">RECALCULATE</a> <b>9574</b> analysis-ready molecules across observations
Exp p-value	<input type="text"/>	0.0 to 0.05		

Preview Dataset E2 of MCF7 P05.xls    Observation:

[Analysis-Ready \(4532\)](#)   [Mapped IDs \(13871\)](#)   [Unmapped IDs \(1496\)](#)   [All IDs \(15367\)](#)

# Advanced Analytics: Causal Network Analysis

Summary \ Functions \ Canonical Pathways \ Upstream Analysis \ Networks \ Molecules \ Lists \ My Pathways \

Upstream Regulators \ Causal Networks

ADD TO MY PATHWAY ADD TO MY LIST

p-value of over... 2.02E-21 - 2.42E-11 (p1 of 10)

Master Regulator	Fold Cha...	Molecule Type	Participati...	Depth	Predicted ...	Activation z-score	p-value...	Net...	Target molec...	Causal network	Target...	In...	D...
SULT1E1		enzyme	beta-estr... all 32		Inhibited	-4.951	2.02E-21	1.00E-04	↑ABCA1, ↑AB... all 101	102 (3)	2	.. an... all 8	
beta-estradiol		chemical - endogenous ...	beta-estr... all 11		Activated	5.528	2.52E-21	1.00E-04	↑ABCA1, ↑ABCC5... all 99	99 (1)	1	8-bro... all 8	8stres
trans-hydroxytamoxifen		chemical drug	trans-hy... all 1			0.928	4.24E-20	1.00E-04	↓ABCC5, ↑ARE... all 29	29 (1)	1		
fulvestrant		chemical drug	Akt, AKT1... all 212			-1.555	2.12E-16	1.00E-04	↓ABCA1, ↑ADA... all 93	93 (21)	21		
bisindolylmaleimide I		chemical - kinase inhibitor	ATF2, b... all 442		Inhibited	-2.502	3.98E-16	2.00E-04	↑ABCE1, ↓AB... all 108	108 (44)	44		
SORBS3		other	AKT1, a... all 493			1.362	1.69E-15	1.00E-04	↑ABCE1, ↑AB... all 138	138 (49)	47	EGF	all 1
raloxifene		chemical drug	Akt, AKT1... all 152			0.000	5.66E-15	1.00E-04	↓ABCA1, ↑ADA... all 82	82 (15)	15		
NR1B		ligand-dependent nucle...	acetami... all 233			-0.913	6.47E-15	6.00E-04	↓ABCA1, ↑AB... all 120	120 (23)	20	.. 8-... all 10	andr.
1,4-bis[2-(3,5-dichlorophenyl)acetyl]piperazine		chemical toxicant	.. Ahr-a... all 183			-0.368	6.78E-15	7.00E-04	↓ABCA1, ↑AB... all 118	118 (18)	18		
CSF1	↓-1.195	cytokine	Akt, AKT1... all 372			1.616	1.18E-14	5.00E-04	↑ABCE1, ↑AB... all 124	124 (37)	37	C3, ... all 12	forsk
BAG1	↑1.110	other	AR, ↑BAG1... all 92			1.890	1.31E-14	1.00E-04	↑ABHD2, ↑ADA... all 63	63 (9)	9	IL2, ↑... all 2	
RAC3		enzyme	Akt, AKT1... all 413		Activated	2.514	1.85E-14	3.00E-04	↓ABCA1, ↑AB... all 124	124 (41)	39		
UBE2L3	↓-1.071	enzyme	AR, EGFR... all 82			1.722	1.95E-14	1.00E-04	↑ABHD2, ↑ARE... all 57	57 (8)	7		FSH
MMP11		peptidase	AGT, Akt... all 63		Activated	2.214	2.46E-14	2.00E-04	↓ABCA1, ↑AB... all 160	160 (63)	61	↓FURIN... all 3	prog.
Ap1		complex	Akt, AKT1... all 493			0.709	3.00E-14	3.00E-04	↓ABCA1, ↑AB... all 161	161 (49)	49	beta... all 38	AR, ↑
NCOA4		transcription regulator	AHR, AR... all 52			1.980	3.04E-14	1.00E-04	↑ADA, ↑ADM, ↑... all 50	50 (5)	4		
BAD		other	Akt, AKT1... all 453		Inhibited	-2.227	3.57E-14	1.00E-04	↓ABCA1, ↑AB... all 147	147 (45)	44	8-(4-... all 32	Calc...
dihydrotestosterone		chemical - endogenous ...	Akt, AR... all 262		Activated	2.636	4.20E-14	5.00E-04	↓ABCA1, ↑AB... all 121	121 (26)	26	8-bro... all 6	finast
androstenedione		chemical - endogenous ...	Akt, AKT1... all 333			1.281	5.48E-14	7.00E-04	↓ABCA1, ↑AB... all 103	103 (33)	31	8-bro... all 6	ESR
CMA1		peptidase	APP, C... all 523			-0.077	6.06E-14	1.50E-03	↓ABCA1, ↑AB... all 167	167 (52)	49		
SMARCE1	↓-1.177	transcription regulator	Akt, AKT1... all 293			1.400	6.65E-14	5.00E-04	↓ABCA1, ↑AB... all 100	100 (29)	29		
FKBP4	↑2.700	enzyme	AKT1, a... all 363			-1.754	9.04E-14	1.70E-03	↑ABCE1, ↑AB... all 130	130 (36)	35	Ca2+... all 1	
CSF2		cytokine	Akt, AKT1... all 342		Activated	2.307	9.28E-14	2.00E-03	↓ABCA1, ↑AB... all 137	137 (34)	34	CCL21... all 9	beta-
Cxcl11		cytokine	Akt, ↑C... all 163		Activated	3.305	1.13E-13	4.00E-04	↓ABCA1, ↑ABCE1... all 77	77 (16)	13	MAP... all 2	CSF2
raloxifene		chemical drug	raloxifene... all 1			-0.784	1.19E-13	1.00E-04	↑AREG/AREGB, ↑... all 26	26 (1)	1		
FURIN	↓-1.256	peptidase	ADAM12... all 473			1.698	1.76E-13	6.00E-04	↓ABCA1, ↑AB... all 153	153 (47)	44	MAPK3... all 2	PPP2
SMAD2	↓-1.153	transcription regulator	androgen... all 53			0.811	1.83E-13	9.00E-04	↓ABCA1, ↑AB... all 152	152 (53)	50	ACV... all 20	beta...
Hdac		group	Akt, AKT1... all 523		Inhibited	-2.280	1.83E-13	6.00E-04	↑ABCE1, ↑AB... all 130	130 (52)	51	7S NGF... all 4	romi.
VEGFA		growth factor	ADAM17... all 1113		Activated	2.154	2.22E-13	6.00E-04	↓ABCC5, ↑AB... all 194	194 (111)	94	12-h... all 50	ACV...
TCF7L2	↓-1.576	transcription regulator	Akt, AKT1... all 333			-1.106	2.42E-13	8.00E-04	↓ABCA1, ↑ABCE1... all 99	99 (33)	32	LN2... all 1	triam
cardiolipin		chemical - endogenous ...	↓ABL1, ... all 363		Activated	2.108	2.75E-13	1.00E-04	↑ABCE1, ↓ABCG1... all 90	90 (36)	30		

Selected/Total rows : 0 / 968

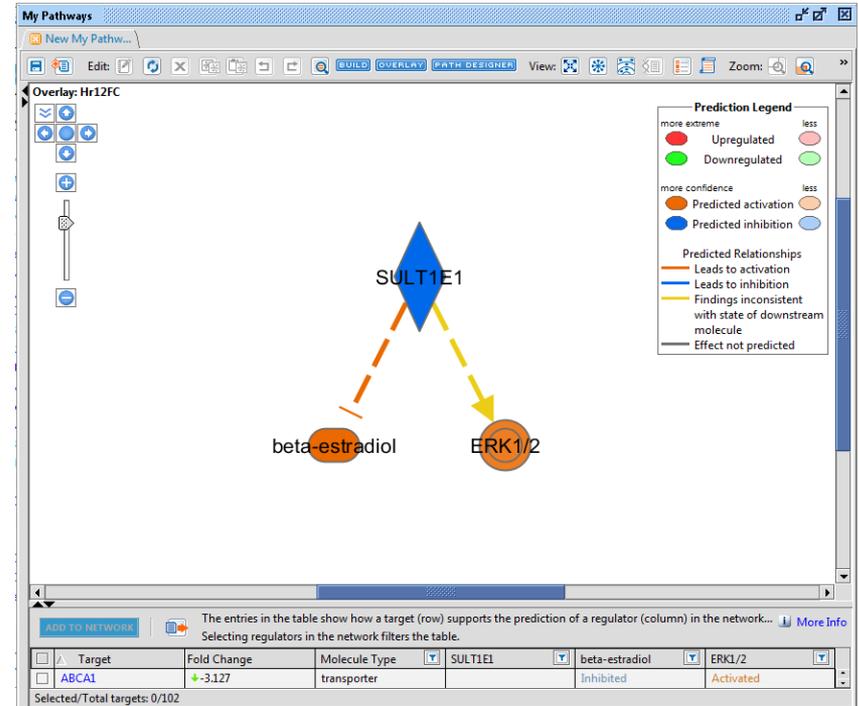
Beta-estradiol of MCF7 cells at 12 hr.  
SULT1E1 is top master regulator, but does not appear in upstream regulator table



# SULT1E1 Causal Network

## Advanced Analytics: Causal Network Analysis

- SULT1E1 is an enzyme that converts estrone and estradiol to an inactive form
- Causal network predicts the absence, inhibition, or saturation of this enzyme in this experiment where estradiol was added exogenously
- SULT1E1 does not have downstream gene expression relationships and, thus, does not appear in the Upstream Regulator table or Mechanistic Networks
- Hypothesis: increasing SULT1E1 activity can have an anti-estrogen effect



## Advanced Analytics: Causal Network Analysis

Only considers edges of unambiguous direction of regulation to downstream genes

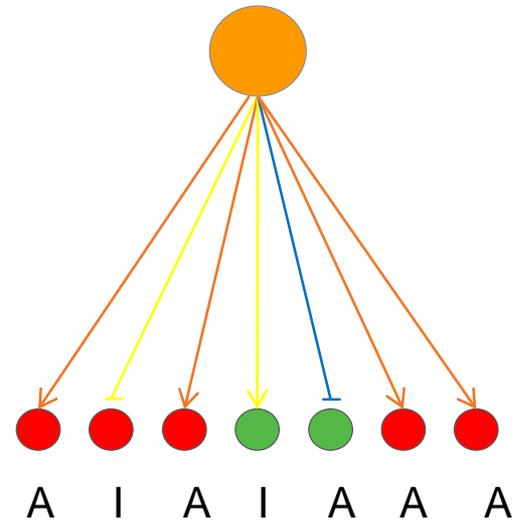
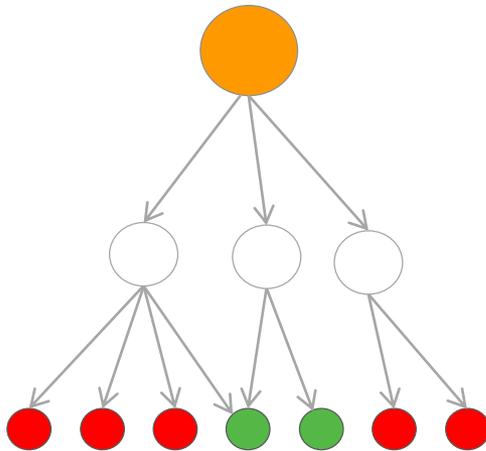
Edges that cannot be assigned a direction of regulation, including all types of binding edges are excluded

- Included relationship types
  - activation (A)
  - inhibition (I)
  - expression (E)
  - transcription (T)
  - group/complex
  - membership edges (MB, considered activating)
  - phosphorylation (P)

Up to 3 interactions edges from root are considered

- Expression/Transcription must be last edge type

## Advanced Analytics: Causal Network Analysis



2 inhibitory edges  
5 activating edges

## Advanced Analytics: Causal Network Analysis

Two p-values are calculated

- Fishers Exact Test of whether there is a greater than expected proportion of downstream data set genes than expected by chance
- Network bias corrected p-value is a measure of how often a more significant result was seen in 10K iterations of selecting random data sets of genes with similar relationship number.

z-score

- Activation z-score is calculated and represents the bias in gene regulation that predicts whether the upstream regulator exists in an activated or inactivated state

$$z = \frac{N^+ - N^-}{\sqrt{N}},$$

z-score represents the number of standard deviations from the mean of a normal distribution of activity edges.

# Analyzing Results

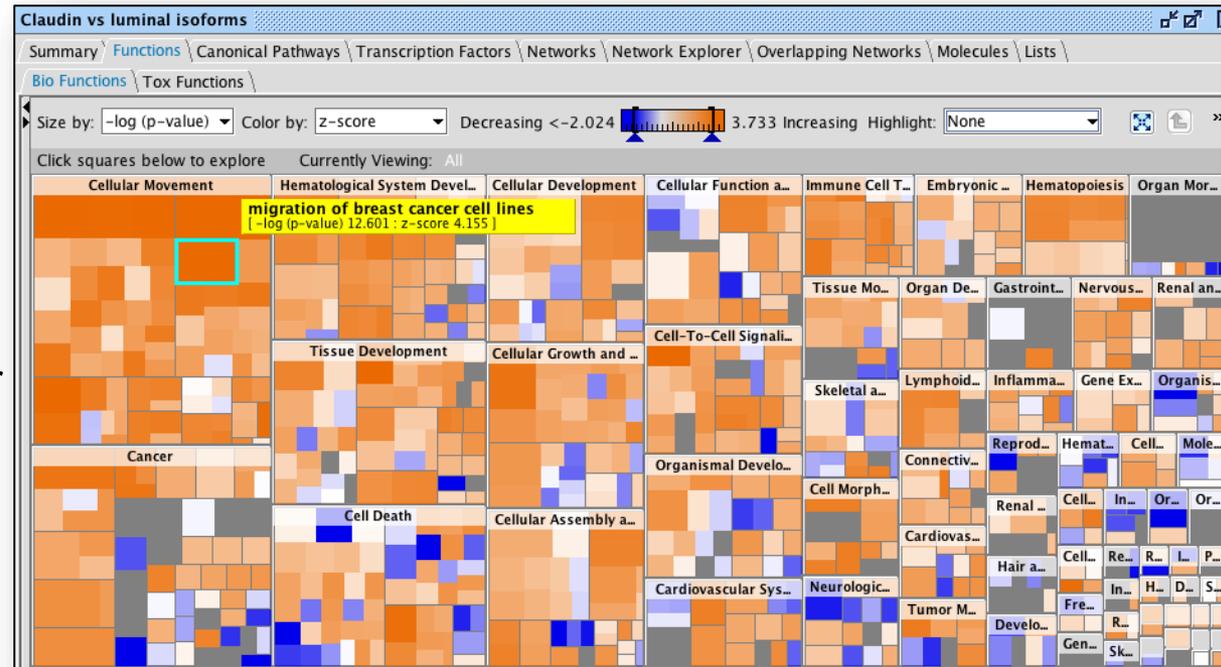
Diseases & Functions  
(Downstream Effects)

- How are cellular processes are predicted to be changing based on my gene expression data?
- What genes are driving these directional changes?

## Downstream Effects Analysis

Identify key biological processes influenced by differentially expressed genes

Understand whether cellular processes are being driven up or down by correlating observed expression with reported experimental gene effects



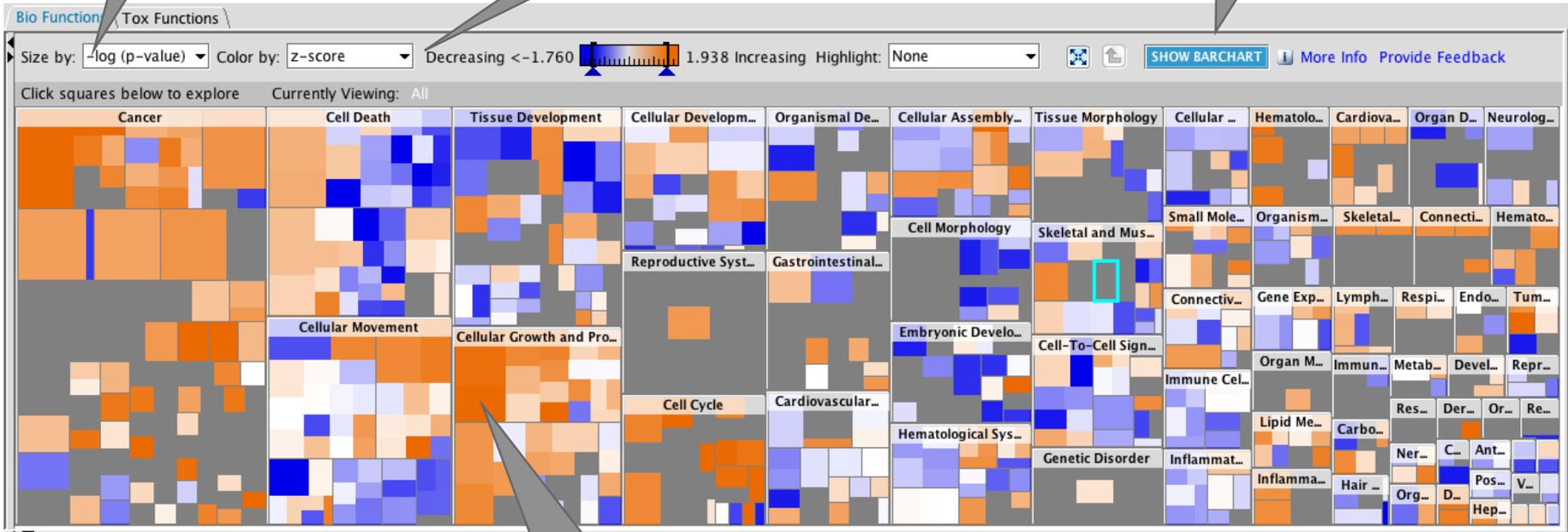
- Each box represents a biological process or disease
- The size of the box represents gene enrichment
- The color of the box indicates the predicted increase or decrease

# Downstream Effect on Bio Function

Size of the Square

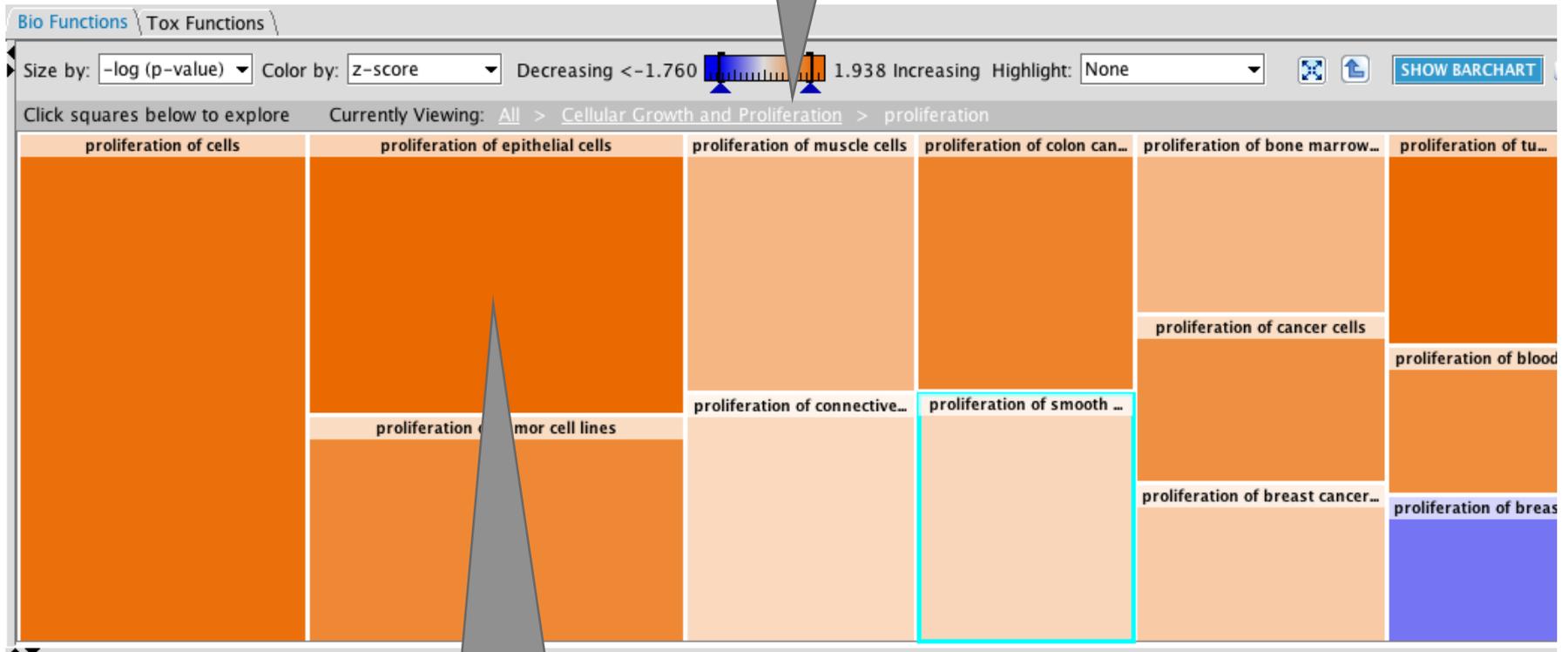
Color by and Scale

Toggle to the Bar Chart



Click on a Square to Drill Down within that function

Ontology Levels



Click to See the Specific Genes and Findings

Functional Category and Statistical Result

Access Findings

Downstream Effects Analysis Evidence for Effects

proliferation of epithelial cells(z-score 1.991). Overlap p-value 8.80E-10

ADD TO MY PATHWAY ADD TO MY LIST CUSTOMIZE TABLE CREATE DATASET More Info

ID	Genes in dataset	Prediction (based on expression)	Fold Change	Findings
H62162	HPN	Decreased	↑3.016	Decreases (1)
AA464600	MYC	Increased	↑2.761	Increases (8)
AA030029	PRKCA	Increased	↑2.175	Increases (3)
H24650	LAMC1	Increased	↑1.917	Increases (1)
N71159	MTA1	Increased	↑1.873	Increases (1)
R19956	VEGFA	Increased	↑1.807	Increases (2)
N41			↑1.787	Affects (1)
H3		Decreased		Decreases (1)
AA459263	BCL2A1	Increased		Increases (1)
AA488645	NAB1	Decreased	↑1.576	Decreases (1)
N74882	DLX5	Increased	↑1.572	Increases (1)
H65052	F2	Increased	↑1.564	Increases (2)
W48713	EGFR	Increased	↑1.515	Increases (10)
H84048	RBL1	Increased	↓-1.559	Decreases (5)
AA456439	SMAD4	Increased	↓-1.565	Decreases (4)
N67039	CDK6	Increased	↓-1.570	Decreases (1)
AA487589	METAP2	Decreased	↓-1.570	Increases (1)
AA489752	CCNG2	Increased	↓-1.578	Decreases (1)
AA489617	TCF7L2	Decreased	↓-1.721	Affects (1)

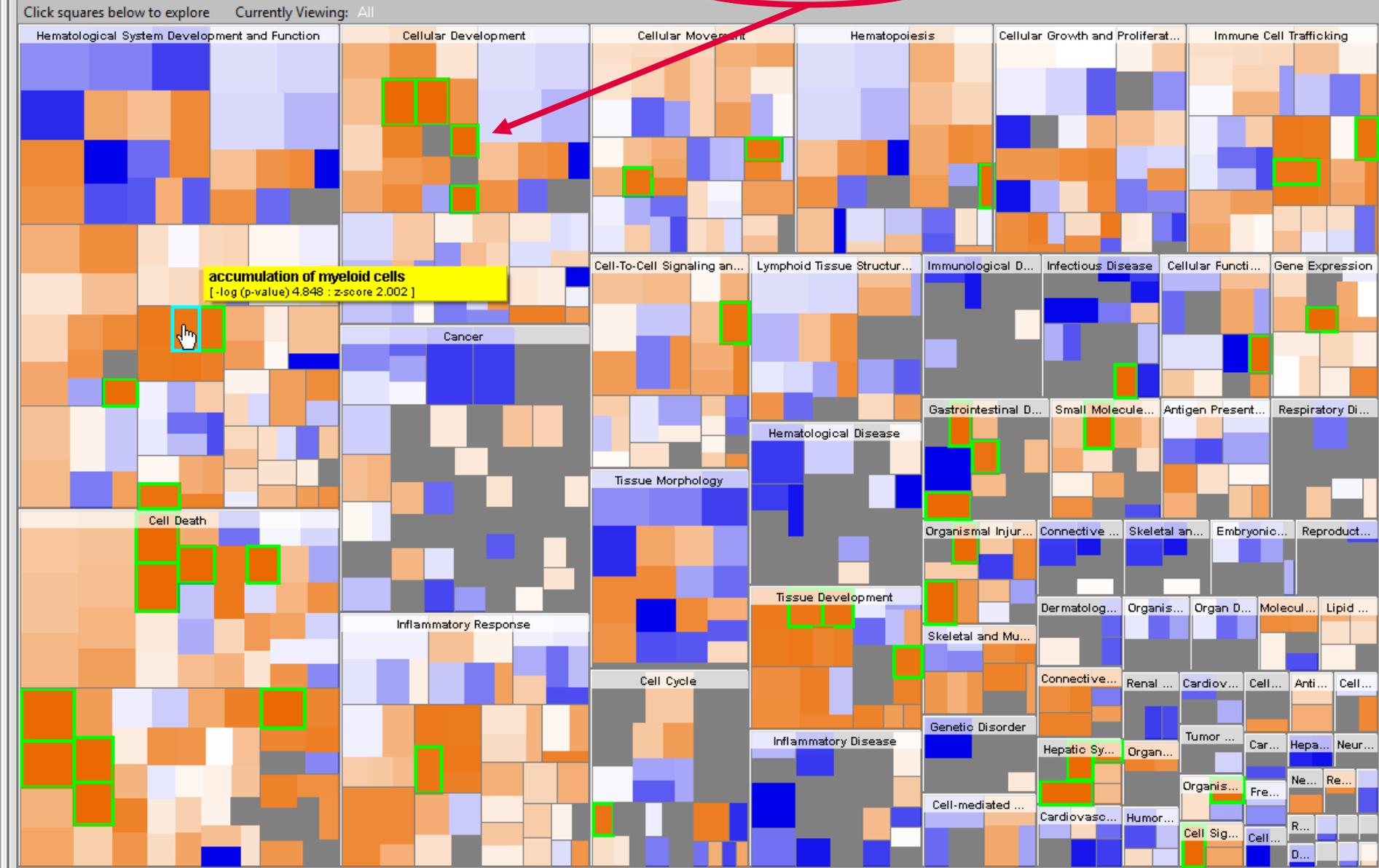
Selected/Total rows : 0/42

Prediction Logic

VEGFA: Known to increase proliferation of epithelial cells and is upregulated in the dataset therefore predicted to increase the function

Expression Value in Your Dataset

Size by:  Color by:  Decreasing <-1.779 2.280 Increasing Highlight:



Goal is understand biology and identify smaller subsets of genes that are of interest

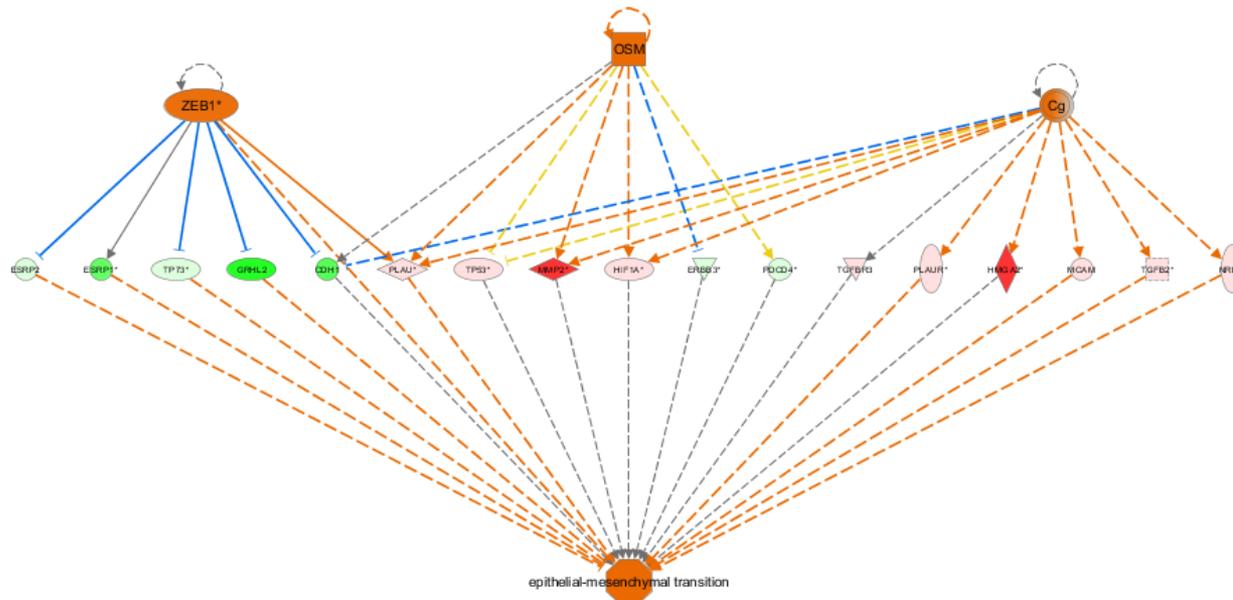
Genes related to a particular function can be :

- sent to a pathway for building and/or overlay analysis
- saved as a new Data Set and sent to Core Analysis for additional categorization and segmentation

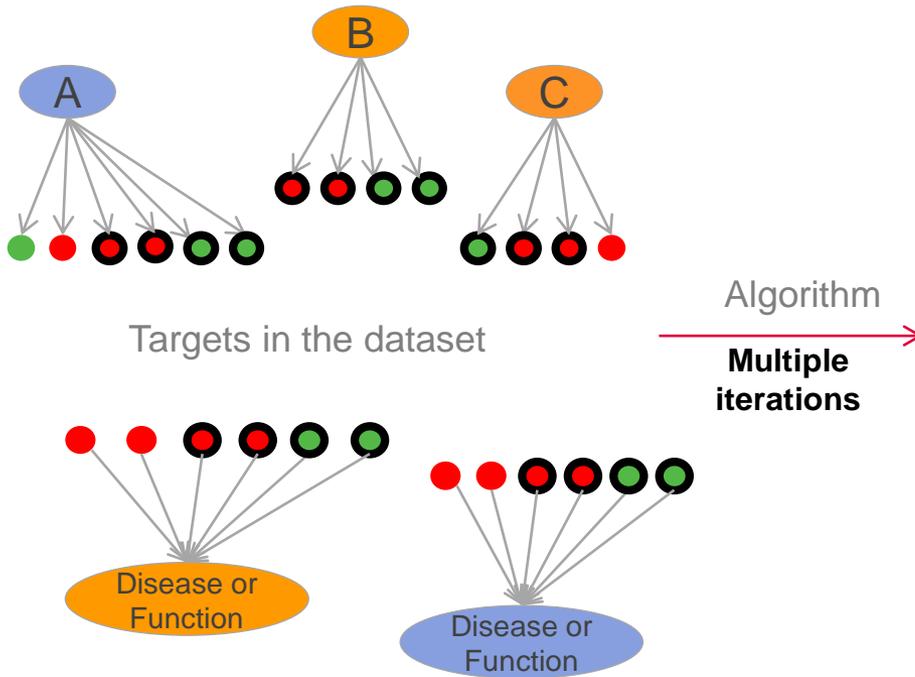
# Analyzing Results

## Regulator Effects

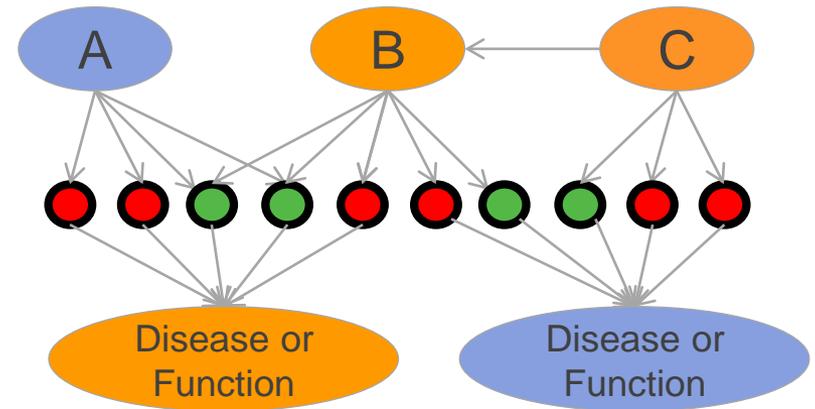
- Hypothesis for how a phenotype, function or disease is regulated in the dataset by activated or inhibited upstream regulators
- Explain impact of upstream molecules on downstream biology
- Explain potential mechanism for a phenotype or drug
- Define drug targets
- Discover novel (or confirm known) regulator → disease/phenotype/function relationships



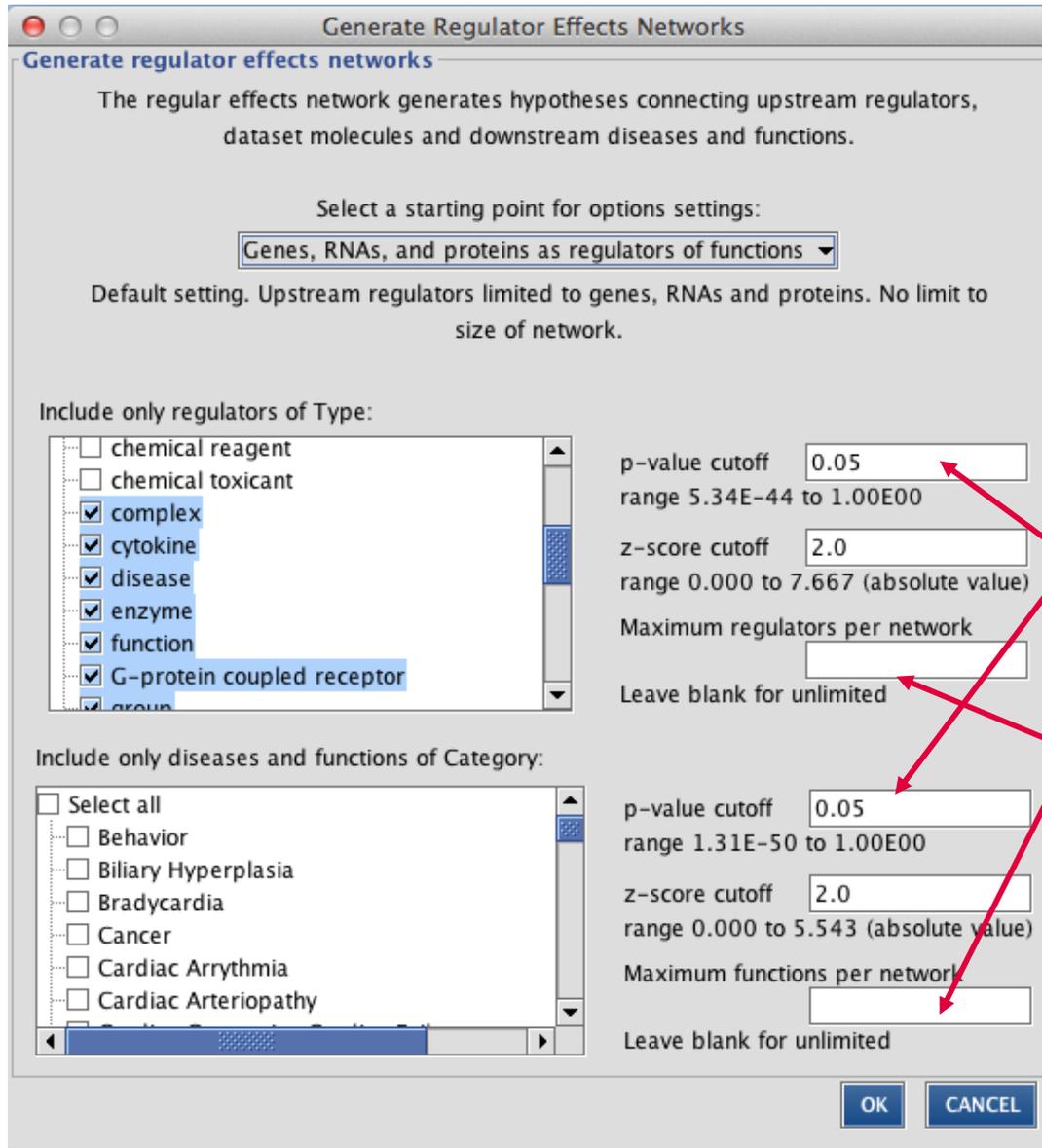
## Upstream Regulator Analysis



## Functional Network Analysis



## Downstream Effects Analysis



Default:

Genes, RNAs,  
proteins as  
regulators

vs.

Any type of  
disease or  
function

Recommend  
decreasing p-value  
cutoffs to 1.0E-3 or  
lower in most cases.

Recommend setting  
network size to 1  
regulator and 1 function  
in addition to viewing  
with no value



# Analyzing Results

## Networks

- To show as many interactions between user-specified molecules in a given dataset and how they might work together at the molecular level
- Highly-interconnected networks are likely to represent significant biological function

- Networks are assembled based on gene/molecule connectivity with other gene/molecules.
  - Assumption: the more connected a gene/molecule, the more influence it has and the more “important” it is.
  
- Networks are assembled using decreasingly connected molecules from your data set.
  
- Genes/molecules from the Knowledge Base may be added to the network to fill or join areas lacking connectivity.
  
- A maximum of 35, 70, or 140 genes/molecules can comprise a network based on parameter settings.
  
- Networks are annotated with high-level functional categories.

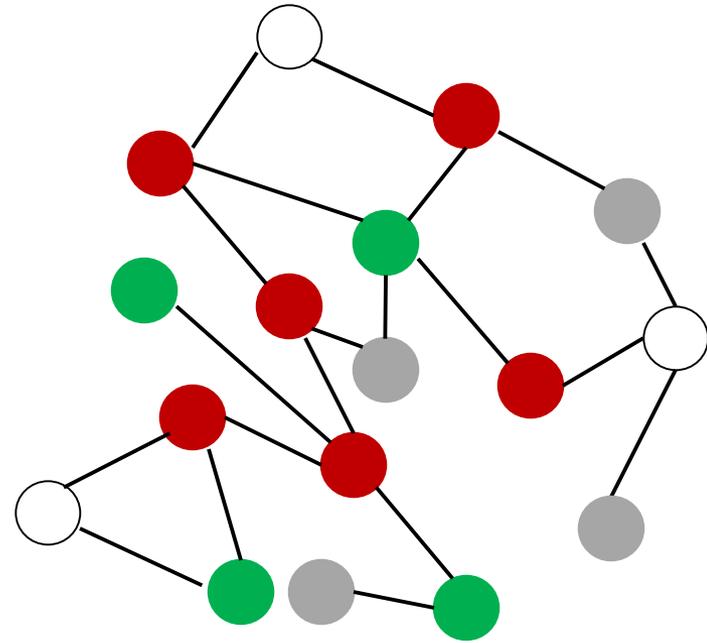
Focus molecules are “seeds”

Focus molecules with the most interactions to other focus molecules are then connected together to form a network

Non-focus molecules from the dataset are then added

Molecules from the Ingenuity’s KB are added

Resulting Networks are scored and then sorted based on the score



Keep in mind..

- Networks may contain smaller networks of connectivity related to specific functions. It might make sense to subset a network. (What does this mean? Just focus on subportions of the network?)
- Larger cellular networks may span IPA assembled networks. Merging networks may allow you to visualize these larger networks.
- Networks should be treated as “starter pathways” that you then modify based on your biological understanding of the system and the questions that you want to answer. Use the pathway building (‘Build’ button) and Overlay tools to expand on your initial results.

# Getting Help

**support@ingenuity.com**

**support-ingenuity@qiagen.com**

+1 650 381-5111  
6am-5pm Pacific Time (M-F)

**QIAGEN Redwood City/Silicon Valley**

1700 Seaport Blvd., 3rd Floor

Redwood City

CA 94063, USA



**INGENUITY**  
PATHWAY ANALYSIS

**INGENUITY**  
iREPORT

**INGENUITY**  
VARIANT ANALYSIS

For Help and Technical Support contact our Customer Support team by email to [support@ingenuity.com](mailto:support@ingenuity.com), or by phone to +1 650-381-5111

For getting started tutorials and training videos see the 'Tutorials' link on the help menu within IPA

To see case studies, application notes, and white papers visit [www.ingenuity.com/library](http://www.ingenuity.com/library)

To view our future scientific seminars, and to watch the series archive visit [www.ingenuity.com/science/scientific-seminar-series.html](http://www.ingenuity.com/science/scientific-seminar-series.html)

To see how IPA has been used and cited in over 9000 publications visit [www.ingenuity.com/science/search-pub.html](http://www.ingenuity.com/science/search-pub.html)

### IPA search and explore series videos:

- The Ingenuity Knowledge Base for IPA <http://youtu.be/4IFxsfMkpQg>
- Searching and accessing the Knowledge Base <http://youtu.be/iU9ihqzfeEY>
- Building a pathway: Filtering and growing <http://youtu.be/8rYEs8F0Cws>
- Building a pathway: Exploring the path of interaction <http://youtu.be/--TRmuMVP9E>
- Overlay contextual information  
<http://www.youtube.com/watch?v=rSp8X6Y6Wlc>
- Editing a pathway for publication <http://youtu.be/yEJjqlUM4So>

## IPA data analysis series videos:

- Data analysis : Part 1 (Data upload) <http://youtu.be/XrdMN9eGWjg>
- Data analysis : Part 2 (Results interpretation) [http://youtu.be/PfF\\_Ru73-1o](http://youtu.be/PfF_Ru73-1o)
- Comparison analyses <http://youtu.be/JCanWpyfvQE>
- Analysis results <http://youtu.be/rrppi9OGPUY>
- Statistical calculation <http://www.youtube.com/watch?v=0oxCQ9dOQIE>
- Canonical pathways <http://youtu.be/6iZdD9Ojll0>
- Network Analysis <http://youtu.be/eReZrNE2bWY>
- Downstream effects analysis <http://youtu.be/CYMrhwuvVKs>
- Upstream regulator analysis <http://www.youtube.com/watch?v=X2bStYNJXm4>
- Human isoforms <http://youtu.be/Po07vk3pOVE>
- Molecular toxicology [http://youtu.be/m1nYDFdY\\_Zg](http://youtu.be/m1nYDFdY_Zg)
- Biomarker filter and comparison analysis <http://youtu.be/XQFUy0s6wCU>
- MicroRNA target filter <http://www.youtube.com/embed/06xoKQL9-KA>

<http://ingenuity.force.com/ipa/articles/Tutorial/Tutorials>

- Search for genes tutorial
- Analysis results tutorial
- Upload and analyze example eata tutorial
- Upload and analyze your own expression data tutorial
- Visualize connections among genes tutorial
- Learn about specialized features
- Human isoforms view tutorial
- Transcription factor analysis tutorial
- Downstream effects analysis tutorial

# Comparing Core Analyses

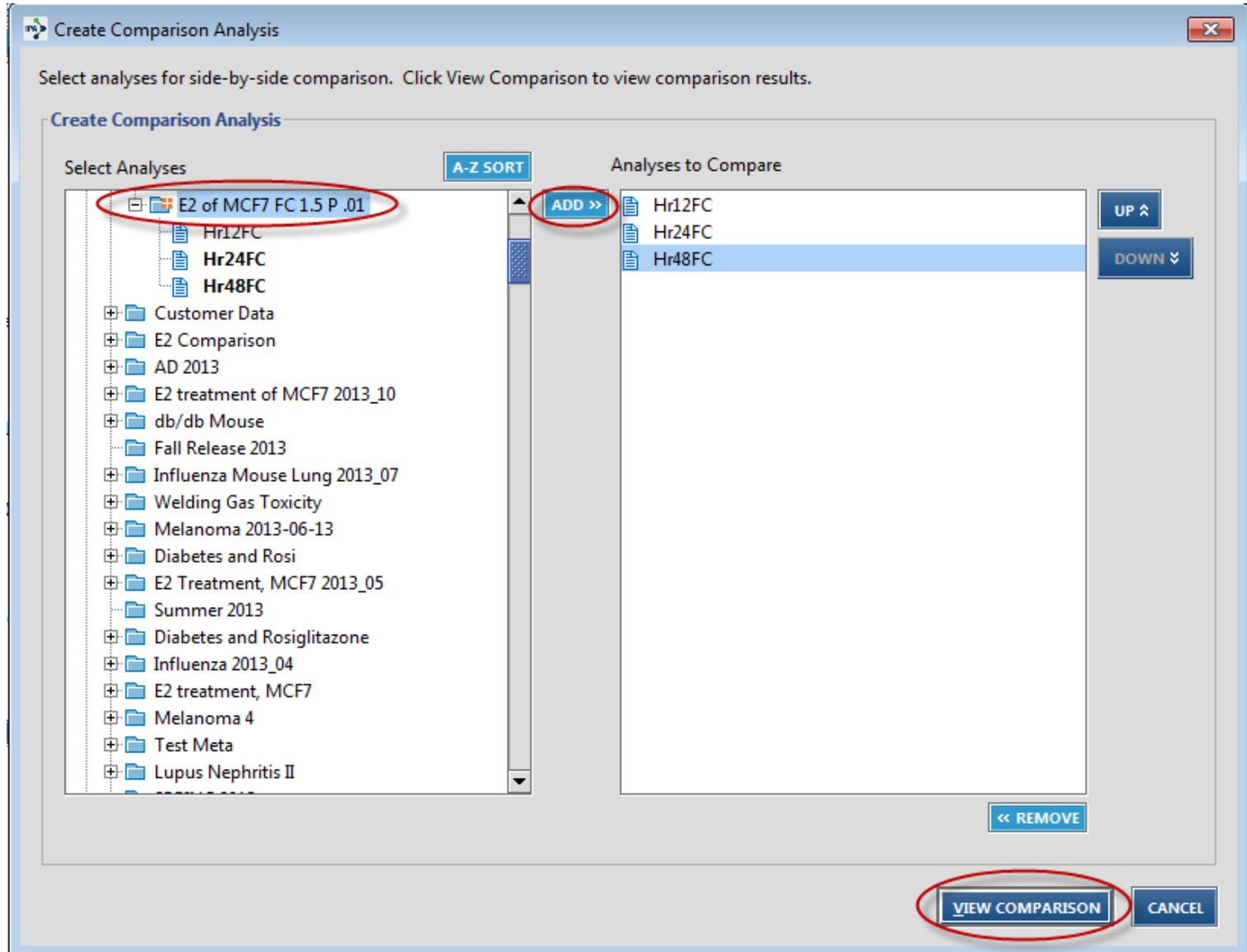
- Multiple Comparison
  - Time course
  - Does response
  
- Multiple Platforms and Data Integration
  - Systems biology
  - Combining SNP, CNA, mRNA, microRNA, proteomics, etc.
  
- Analysis Comparisons work best with Canonical Pathways, Upstream Regulators, and Disease and Functions
  
- Regulator Effects and Mechanistic Networks are similarly difficult to compare because these networks are created in the context of the single analysis.
  - To compare these networks across analyses, open, view the network, and then use the OVERLAY -> “Data Sets, Analyses, and Lists” to overlay colored representation of gene changes.

The screenshot shows the IPA software interface. The 'File' menu is open, displaying various options. A mouse cursor is pointing at the option 'Core, Tox or Metabolomics Comparison Analysis...', which is highlighted in blue. The menu items and their keyboard shortcuts are as follows:

Menu Item	Keyboard Shortcut
New	Ctrl-N
Open	Ctrl+Shift-T
Save	Ctrl-S
Save As...	
Upload Dataset...	Ctrl-U
Batch Upload Datasets...	
Search Datasets and Analyses...	
Refresh Project Manager	F5
View References	
Export Data...	Ctrl-E
Export Image...	Ctrl+Shift-E
Send To	
Share	
Properties	
Preferences	
Print...	Ctrl-P
Close IPA	Ctrl-Q
Core Analysis...	Ctrl-N
Tox Analysis...	Ctrl+Shift-T
Biomarker Filter...	Ctrl+Shift-B
Metabolomics Analysis...	Ctrl+Shift-A
<b>Core, Tox or Metabolomics Comparison Analysis...</b>	<b>Ctrl+Shift-C</b>
Biomarker Comparison Analysis...	Ctrl+Shift-K
miRNA Target Filter...	Ctrl+Shift-I
My Pathway	Ctrl+Shift-N
Path Designer	Ctrl+Shift-D
Advanced Search	Ctrl+Shift-S
Project...	Ctrl+Shift-P
Compare...	Ctrl-R
Filtered Dataset...	Ctrl-D
Import Pathway	

In the background, a search bar is visible with the text 'SEARCH' and 'Advanced Search'. Below the search bar, a list of project folders is shown, including 'Influenza mouse Lung 2013\_07', 'Welding Gas Toxicity', 'Melanoma 2013-06-13', 'Diabetes and Rosi', 'E2 Treatment, MCF7 2013\_05', 'Summer 2013', 'Diabetes and Rosiglitazone', and 'Influenza 2013\_04'.

# Selecting Analyses to Compare



Create Comparison Analysis

Select analyses for side-by-side comparison. Click View Comparison to view comparison results.

Create Comparison Analysis

Select Analyses A-Z SORT Analyses to Compare

**E2 of MCF7 FC 1.5 P .01**

- Hr12FC
- Hr24FC**
- Hr48FC

Customer Data

E2 Comparison

AD 2013

E2 treatment of MCF7 2013\_10

db/db Mouse

Fall Release 2013

Influenza Mouse Lung 2013\_07

Welding Gas Toxicity

Melanoma 2013-06-13

Diabetes and Rosi

E2 Treatment, MCF7 2013\_05

Summer 2013

Diabetes and Rosiglitazone

Influenza 2013\_04

E2 treatment, MCF7

Melanoma 4

Test Meta

Lupus Nephritis II

Hr12FC

Hr24FC

**Hr48FC**

ADD >>

UP ^

DOWN v

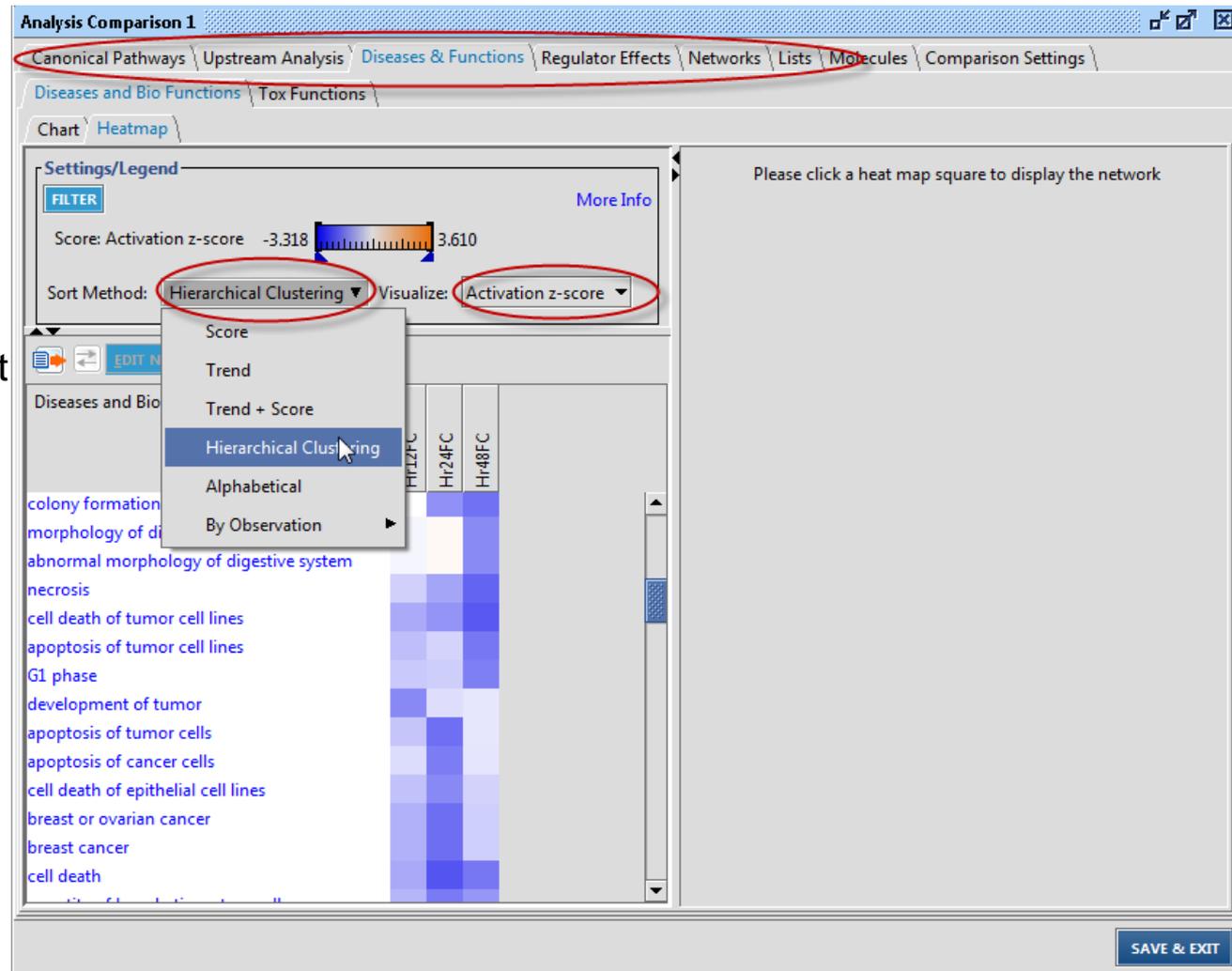
<< REMOVE

VIEW COMPARISON CANCEL

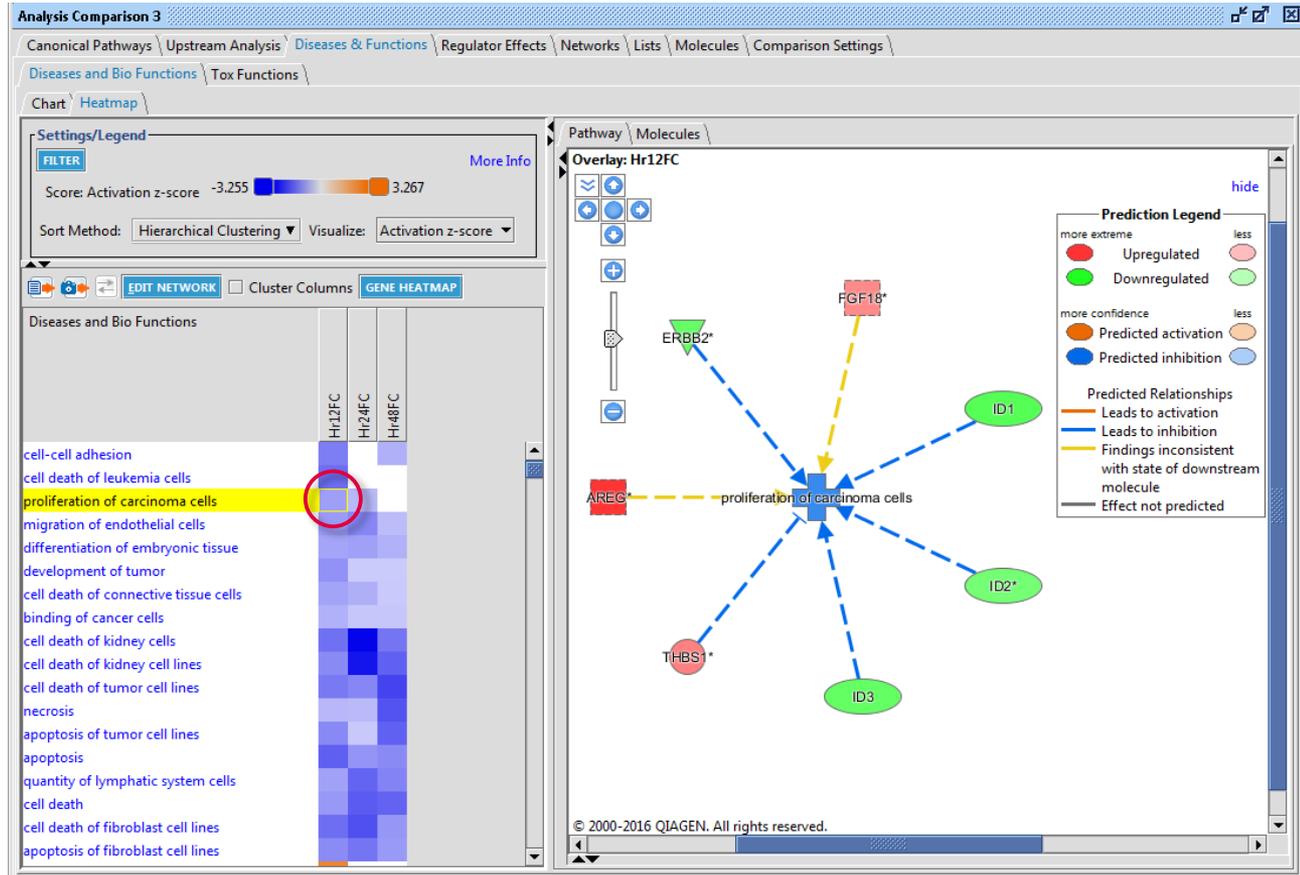
Tabs at top navigate to the analysis-type of interest

Heatmap can be generated using different calculation methods

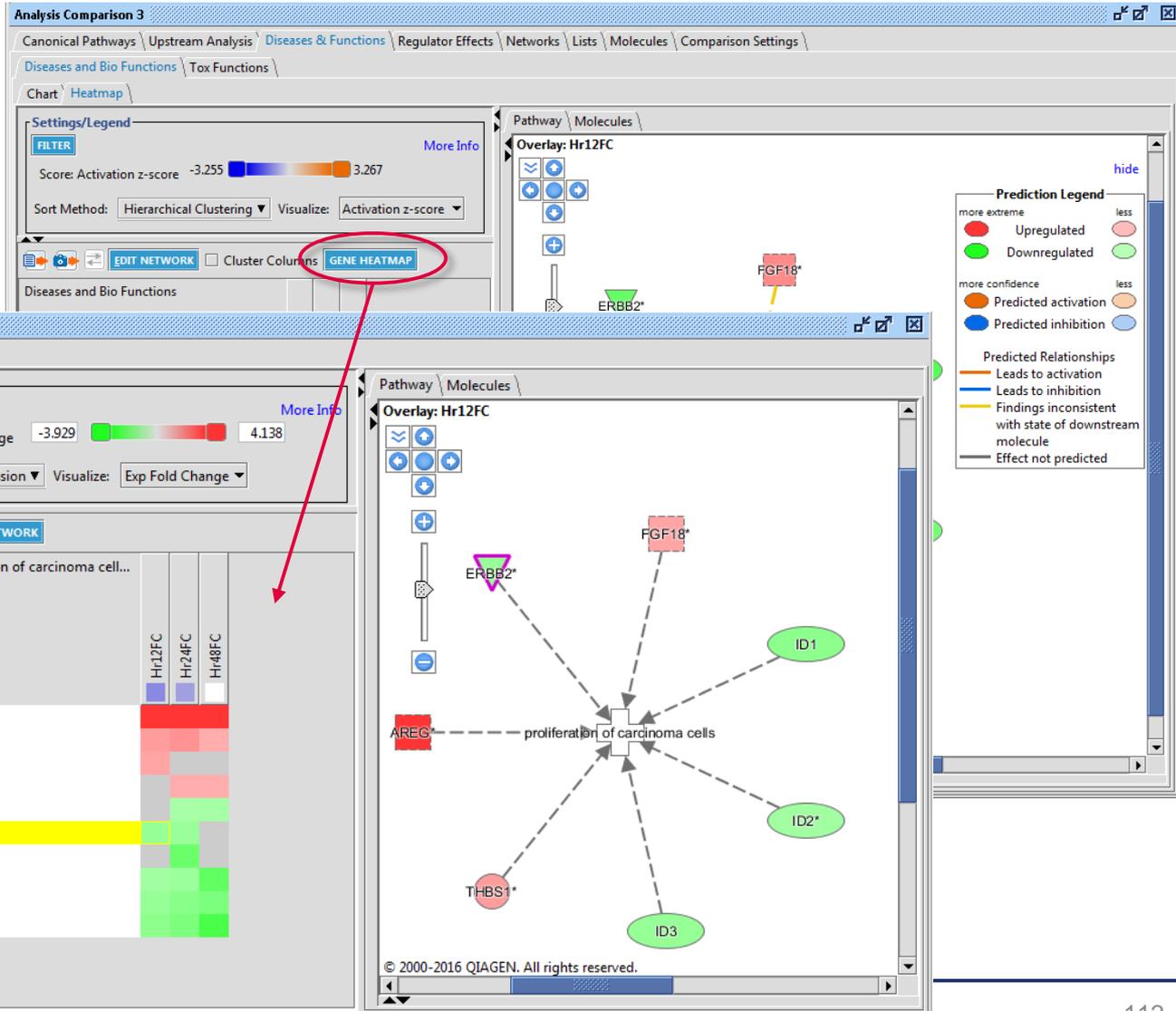
Heatmap can be based on different metrics depending on analysis-type.



Selecting heatmap element displays pathway or network with data-values overlay and MAP coloring (if applicable)



Clicking GENE HEATMAP button displays gene expression values across observations



# Micro-RNA Target Filter

Filter miRNA differential expression data set (if corresponding mRNA differential expression data, filter as well)

- File -> New -> Filtered Data Set

Start microRNA Target Filter

- File -> New -> miRNA Target Filter
- Open miRNA filtered data set

Using funnel in column headers, filter mapping based on information type/confidence

Add annotation columns, if desired, by clicking the plus sign in column header and filter as desired

If corresponding mRNA, click “ADD/REPLACE MRNA DATA SET” to filter mRNA mappings to genes in the mRNA expression data set

- Click “EXPRESSION PAIRING” to pair the expression between the miRNA and mRNA
- Click the funnel in the column header of the expression pairing column to filter for the miRNA-mRNA pairing desired

Click to summary tab to view a summary of miRNA-mRNA mappings

For further analysis, select one or more miRNAs from the summary tab and add the miRNA and targets to a new pathway and perform overlays for interpretation of functions, pathways, drug targets, etc.

**microRNA Target Filter**

68 microRNA families have targeting information available.  
Filtered to [51 microRNAs](#) targeting 32 mRNAs.

ADD/REPLACE MRNA DATASET   EXPRESSION PAIRING

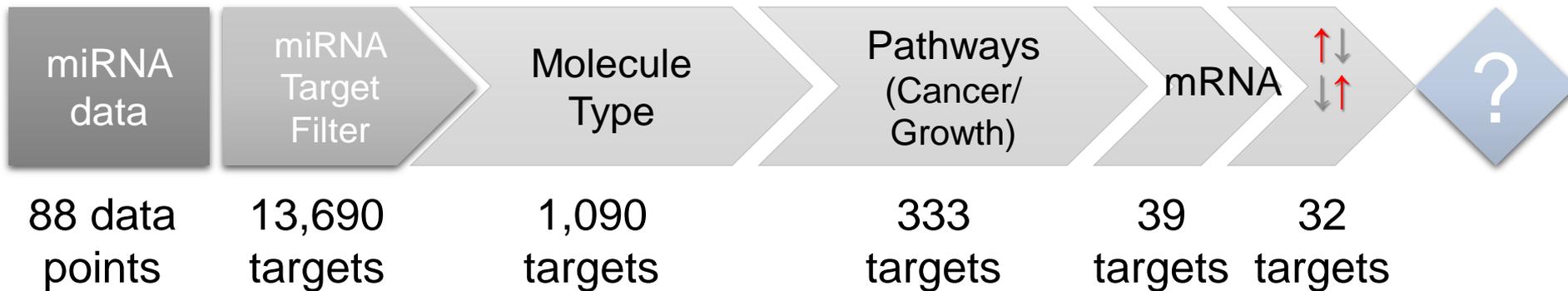
Details | Summary

ADD TO MY PATHWAY   ADD TO MY LIST

Rows: 1 - 131

Use [v] to filter a column. Add data or more columns using 'Add column(s) [+]'.

microRNA dataset: melanoma_microRNA_data				Relationship			mRNA dataset: mRNA Metastasis vs Normal - 2FC,0.05PV				
ID	Symbol	metastatic melanoma (Fold C...	Source	Confidence	Expression Pairing	ID	Symbol	Fold Change	Molecular Type	Pathway	
hsa-let-7c	let-7	↓-3.120	TargetScan Human	High (predicted)	↓↑	8072015	ADRBK2	↑3.394	kinase	Colorectal Cancer Met...	
hsa-let-7c	let-7	↓-3.120	TargetScan Human	Moderate (predicted)	↓↑	8067167	AURKA	↑2.136	kinase	Molecular Mechanisms...	
hsa-let-7c	let-7	↓-3.120	TargetScan Human	High (predicted)	↓↑	8105121	GHR	↑2.052	transmembrane receptor	Growth Hormone Signa...	
hsa-let-7c	let-7	↓-3.120	TargetScan Human	Moderate (predicted)	↓↑	7994131	PRKCB	↑4.995	kinase	Breast Cancer Regulat...	
hsa-miR-206	mir-1	↑1.880	TargetScan Human	Moderate (predicted)	↑↓	7956301	LRP1	↓-3.463	transmembrane receptor	Colorectal Cancer Met...	
hsa-miR-206	mir-1	↑1.880	TargetScan Human	High (predicted)	↑↓	8008201	NGFR	↓-2.917	transmembrane receptor	PTEN Signaling	
hsa-miR-122	mir-122	↑1.970	TargetScan Human	High (predicted)	↑↓	7963670	MAP3K12	↓-3.119	kinase	Germ Cell-Sertoli Cell J...	
hsa-miR-122	mir-122	↑1.970	TargetScan Human	Moderate (predicted)	↑↓	8157524	TLR4	↓-6.290	transmembrane receptor	Colorectal Cancer Met...	
hsa-miR-125a-5p	mir-125	↓-1.450	TargetScan Human	Moderate (predicted)	↓↑	7985213	CHRNA5	↑2.965	transmembrane receptor	AMPK Signaling	
hsa-miR-125a-5p	mir-125	↓-1.450	TargetScan Human	Moderate (predicted)	↓↑	7994131	PRKCB	↑4.995	kinase	Breast Cancer Regulat...	



Use Pathway tools to build hypothesis for microRNA – target association to melanoma metastasis.



Ingenuity®  
Knowledge Base

IPA has high-quality microRNA-related findings (including both experimentally validated and predicted interactions)

- **TarBase**: experimentally validated microRNA-mRNA interactions
- **Target Scan**: predicted microRNA-mRNA interactions (low-confidence interactions were excluded)
- **miRecords**: experimentally validated human, rat, and mouse microRNA-mRNA interactions
- **Literature Findings**: microRNA-related findings manually curated from published literature by scientific experts and structured into the Ingenuity® Knowledge Base

Single source for microRNA content plus related biology enables biologically relevant target prioritization in minutes vs. weeks

Extensive human, mouse, and rat coverage

- For Searching, IPA Supports:
  - miRBase Identifiers
  - Entrez Gene Symbols and Entrez Gene IDs
  - Other synonyms used in the literature
  
- For Data Upload, IPA Supports:
  - miRBase Identifiers for mature miRNAs
  - miRBase Accession Numbers (format MIMAT#####) are preferred. These are stable identifiers.
  - miRBase Name Identifiers (format: mmu-miR-###) are allowed. Since some miRNA arrays provide annotations only with the name, we have provided mappings for them. These change over time so use MIMAT instead if available.
  - Precursor identifiers are NOT supported
  - Entrez Gene IDs (not Entrez Gene Symbols)
  - HUGO gene symbols (human only)

## Mapping microRNA IDs in IPA during Data Upload

- A given ID can only map to a single node in IPA
- miRNA identifiers each map to either a group node or a locus-specific node:
  - miRNA identifiers that correspond to mature miRNAs that do NOT appear in a group (ie, they arise from only one known precursor, and that precursor has no more than one known Entrez Gene ID/locus) are mapped to a locus-specific node.
  - miRNA identifiers corresponding to mature miRNAs that ARE in a group map to that group.
  - No miRNA maps to more than one group node in IPA.

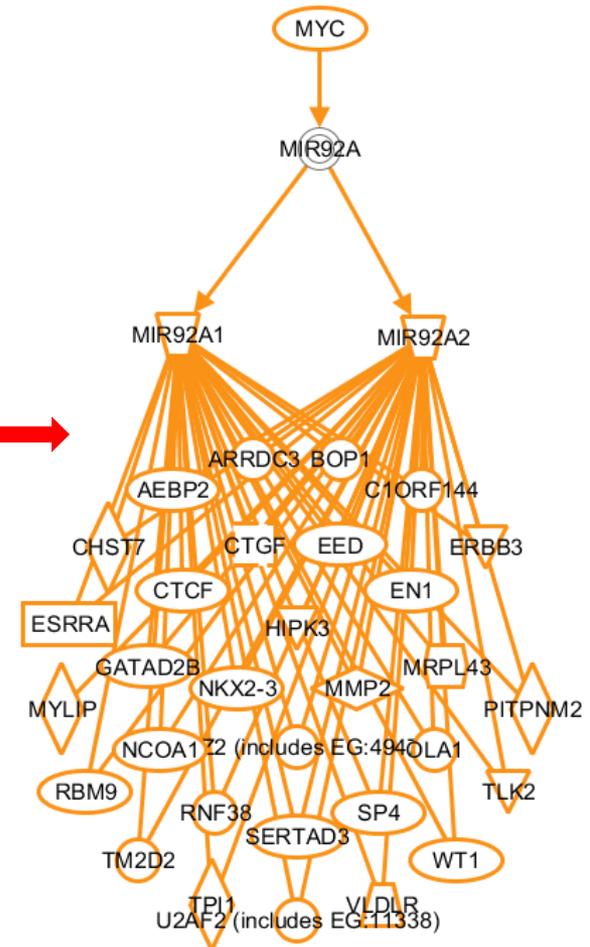
- Mature miRNAs may arise from multiple precursors:
  - A given mature form may arise from multiple distinct miRNA precursors.
  - A given precursor may arise from multiple distinct loci.
  
- Groups are created in the knowledge base to represent mature miRNA's that may arise from multiple precursors or multiple loci.
  - When authors refer to a particular mature miRNA form that may arise from multiple distinct precursors and/or multiple genetic loci, the finding is mapped to a group concept that contains all potential “parent” precursors.
  
- Groups might have different network connections compared to the individual members of the group.
  - Findings might be mapped to the individual members or to the group, depending on information provided by the author.
  - ‘Grow’ functionality does not ‘look inside’ the groups.
  - Additional steps will ensure that all members of group will be considered when applying ‘Grow’



Expanding groups prior to Growing will provide information on known molecular interactions for all members of the group.

MIR92A

- Hide Tool Tips
- Show Members/Membership
- Edit Custom Molecule
- Full Screen View
- Zoom Selected
- Magnifying Lens
- Delete
- Cut
- Copy
- Paste
- Print
- Send By E-Mail
- Select All
- Select Highlighted
- Select Nearest Neighbor(s)
- Highlight Selected
- Unhighlight Selected
- Reset Highlight
- Invert Selected
- Invert Highlight
- View References





## Biomarker Filter

IPA-Biomarker™ analysis filters/refines candidate lists based on biological criteria such as association to a disease, normal presence in a fluid, or normal expression in a tissue/cell type/cell line and/or clinical usage.

- Species
- Tissues and Cell Lines
- Biofluids
- Diseases
- Clinical Biomarkers

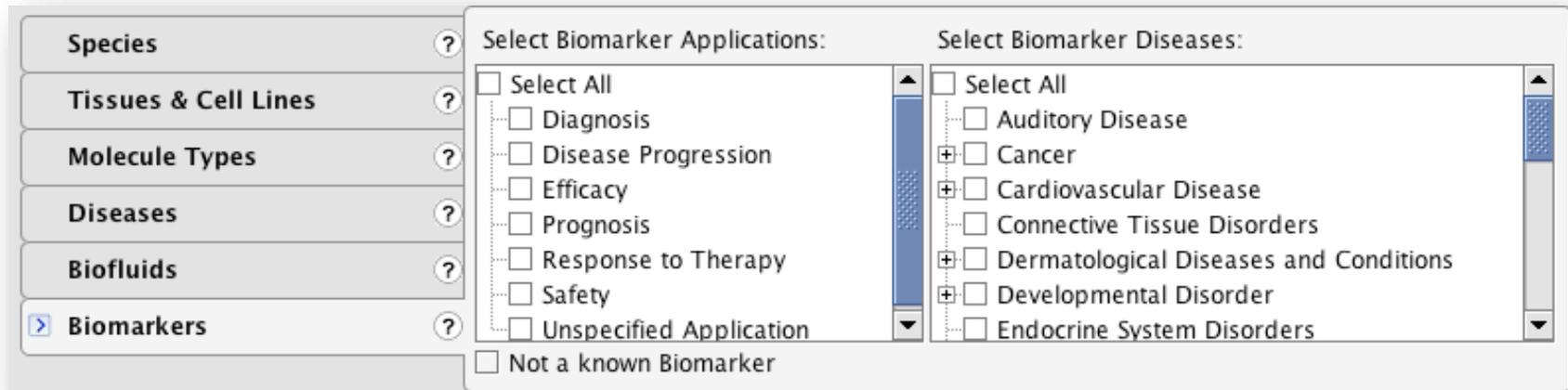
The output is a refined list of candidates

- It does not calculate functions, Canonical Pathways, or networks

Different observations or datasets can be compared using the Comparison Biomarker Analysis

- Calculates unique and common molecules

The Biomarker Filter capability rapidly priorities biomarker candidates based on biological characteristics and clinical usage.



The screenshot shows a software interface for filtering biomarkers. On the left is a sidebar with the following categories, each with a question mark icon:

- Species
- Tissues & Cell Lines
- Molecule Types
- Diseases
- Biofluids
- Biomarkers** (highlighted with a blue arrow icon)

The main area is divided into two panels:

- Select Biomarker Applications:**
  - Select All
  - Diagnosis
  - Disease Progression
  - Efficacy
  - Prognosis
  - Response to Therapy
  - Safety
  - Unspecified Application
  - Not a known Biomarker
- Select Biomarker Diseases:**
  - Select All
  - Auditory Disease
  - Cancer
  - Cardiovascular Disease
  - Connective Tissue Disorders
  - Dermatological Diseases and Conditions
  - Developmental Disorder
  - Endocrine System Disorders

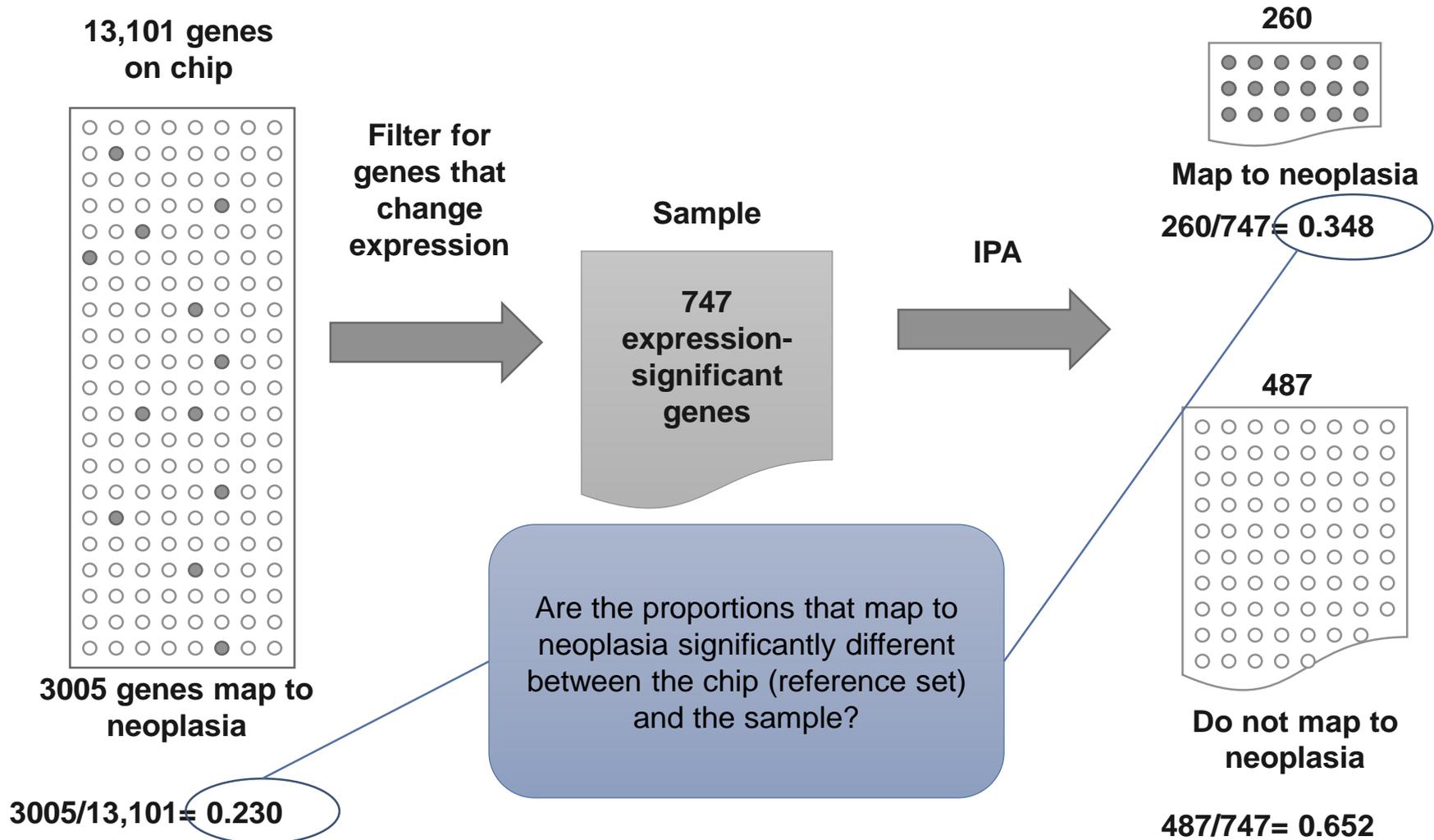
## Clinical Usage (Biomarkers):

Identify biomarkers by their specific application, including markers for Disease Diagnosis and Prognosis, Disease Progression, markers of Drug Efficacy and Safety, and Patient Response to Therapy

# Statistics in IPA

- Is the proportion of genes in my sample mapping to a gene set (those that are significant) similar to the proportion of all measureable genes (reference set) that map in the gene set?
  - If the proportions are similar, there is no biological effect

# Mapping Colorectal Cancer Expression Data to the Function “Neoplasia”



A 2x2 contingency table is created based on the total population, the sample, and how many genes map to the function/pathway. This table is used to calculate the Fisher's Exact Test

	Neoplasia	Not Neoplasia	
In Sample	$k$	$n - k$	$n$
Not in Sample	$m - k$	$N + k - n - m$	$N - n$
	$m$	$N - m$	$N$

$m$ = Total that map to function/pathway

$N$ = Total

$k$ = Number that map to function/pathway in sample

$n$ = Total sample

Numbers based on the colorectal cancer data mapping to neoplasia

	Neoplasia	Not Neoplasia	
In Sample	260	487	747
Not in Sample	2745	9609	12354
	3005	10096	13101

3005 = Total that map to neoplasia on chip

13101 = Total on chip

260 = Number that map to neoplasia in sample

747 = Total sample

Fisher's Exact Test p-value = 2.13 E-14



# What Can We Say About Our Colorectal Cancer Data Set And Neoplasia?

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- We can conclude that the proportion, or over representation, of genes mapping to neoplasia is not likely the result of sampling (and is likely an effect of the disease)

If you are using a standard vendor platform supported by IPA, then that platform should be selected as your reference set.

If you do not know the platform or the data were taken from different platforms, select a reference set that best estimates the entire population you evaluated.

- For gene expression data, select the “Ingenuity Knowledge Base (genes only)”
  - This setting uses all function- and pathway-eligible genes in the knowledge base.
- For metabolomics, select the “Ingenuity Knowledge Base (endogenous chemicals only)”
- You have the option to having your uploaded data set used as the reference set (User Data Set)

Low density arrays are problematic because the genes that are being measured are usually not randomly chosen to start with, but are typically selected based on a priori function or pathway knowledge

Let's assume a inflammatory cytokine array

- If you select the Ingenuity Knowledge Base as your reference, your p-values for inflammation functions and pathways will be artificially low (significant) because the array was heavily biased for these genes.
- If you upload every gene on the array, and select the “User Data Set” reference option, your p-values are statistically accurate, but inflammatory functions and pathways may not appear significant because the likelihood of having a random sample with similar proportions to inflammation processes is extremely high.

Benjamini-Hochberg method of multiple testing correction

Based on the Fisher's exact test p-value

Calculates false discovery Rate

- Threshold indicates the fraction of false positives among significant functions



5% (1/20) may be a false positive

“What is the significance of function X in relation to my dataset?”

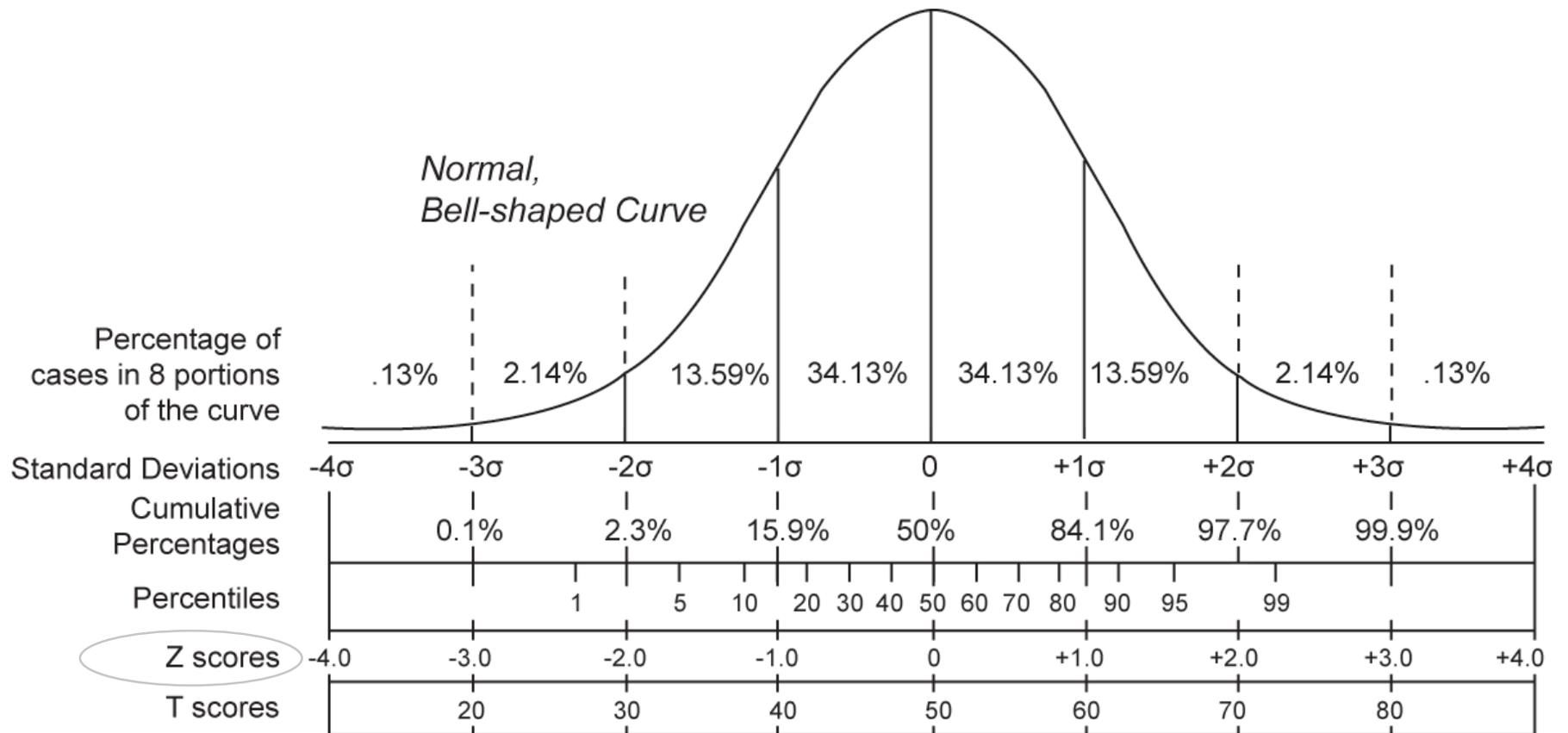
- Use Fisher’s Exact test result

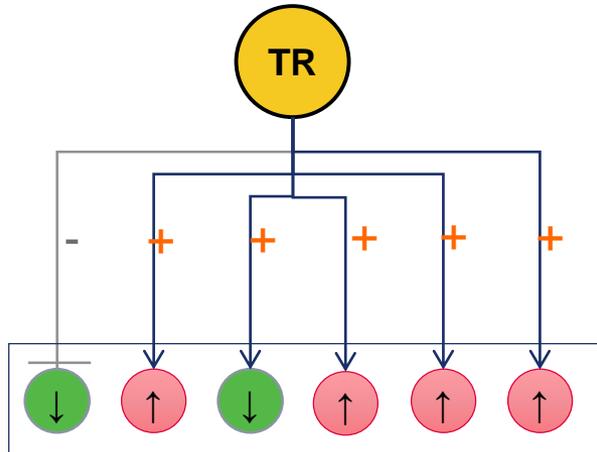
“What are all significant functions within this dataset?”

- Use Benjamini-Hochberg multiple testing correction

A set of genes chosen at random should be about equally likely to have an increasing or decreasing effect, thus, about 50% each direction, or a  $z=0$ .

A z-score represents the non-randomness of directionality within a gene set





Every TR is analyzed

Literature-based effect TR has on downstream genes

Differential Gene Expression (Uploaded Data)

1    1    -1    1    1    1

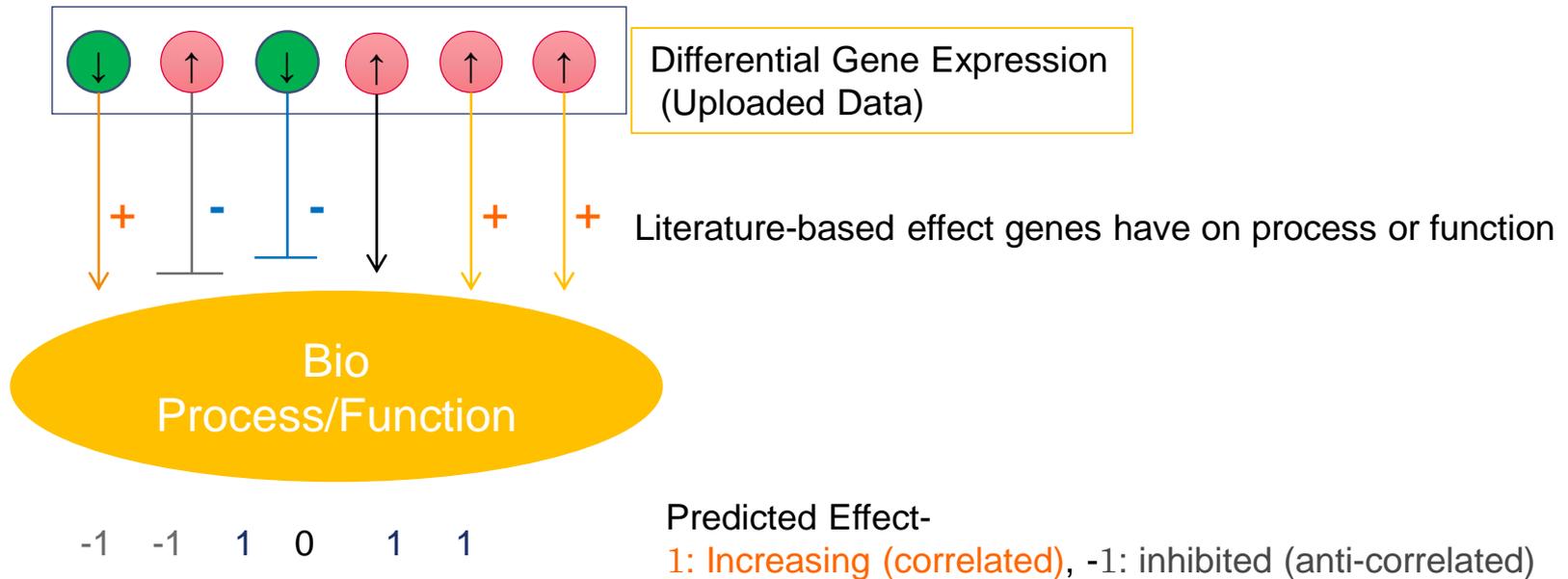
Predicted activation state of TR:

1: activated (correlated), -1: inhibited (anti-correlated)

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}} = \frac{4}{\sqrt{6}} = 2.04$$

- z-score is statistical measure of correlation between relationship direction and gene expression.
- z-score > 2 or < -2 is considered significant

Actual z-score *can* weighted by relationship, relationship bias, data bias



$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}} = \frac{1}{\sqrt{5}} = .447$$

- “z-score” is statistical measure of correlation between relationship direction and gene expression.
- z-score > 2 or < -2 is considered significant

Actual z-score is weighted by relationship, relationship bias, data bias