2018 IPA 系統生物學分析軟體暨資料庫

進階操作課程

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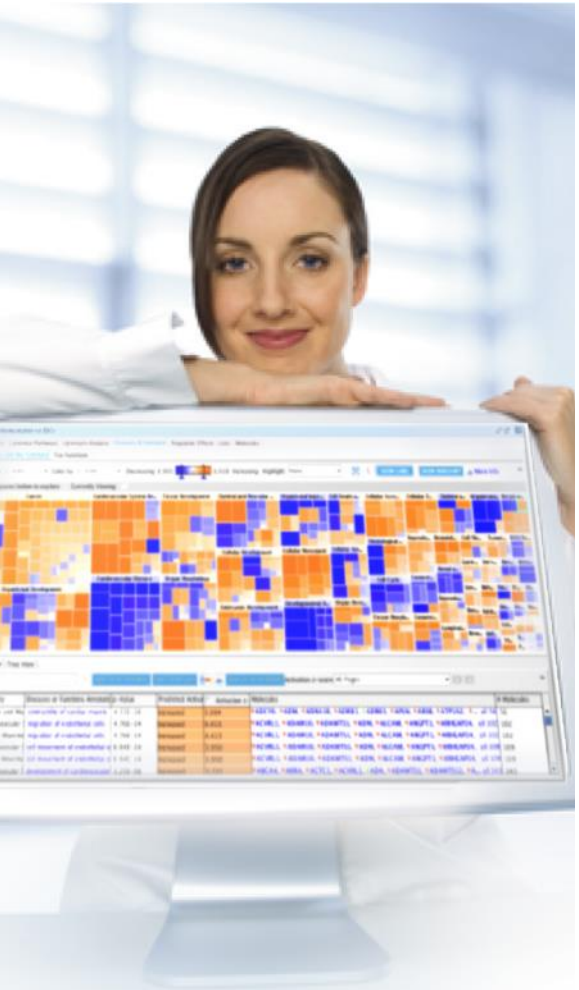
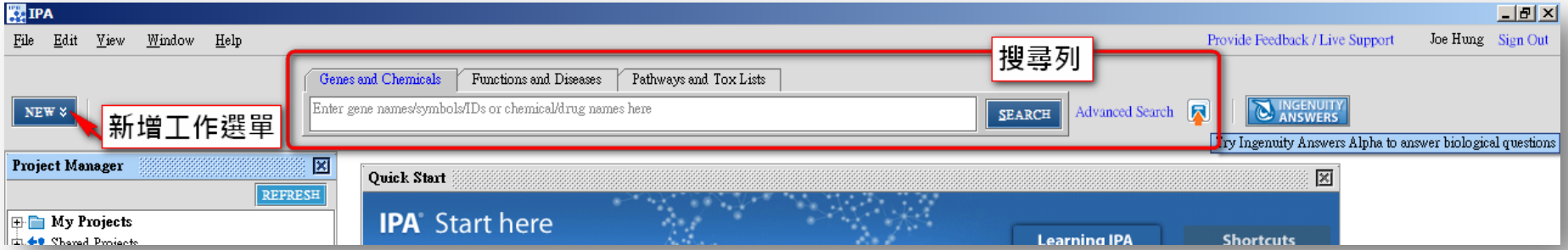


Review for Introductory Training course

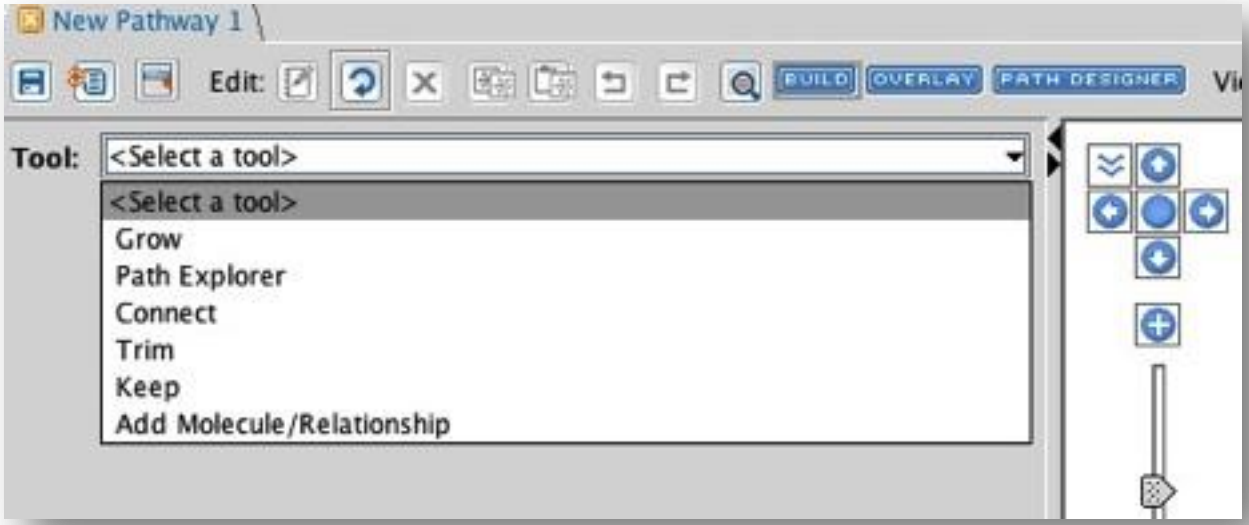
- IPA介紹
- 利用IPA進行搜尋
- 使用IPA進行分子模型建構並繪製訊息傳遞路徑

Searching

- Searching Basics
- Gene/chemical search and results
- Function/Disease search and results
- Drug target search and results
- Advanced search: Limiting results to a molecule type, family or subcellular location



Build Tools 的功能

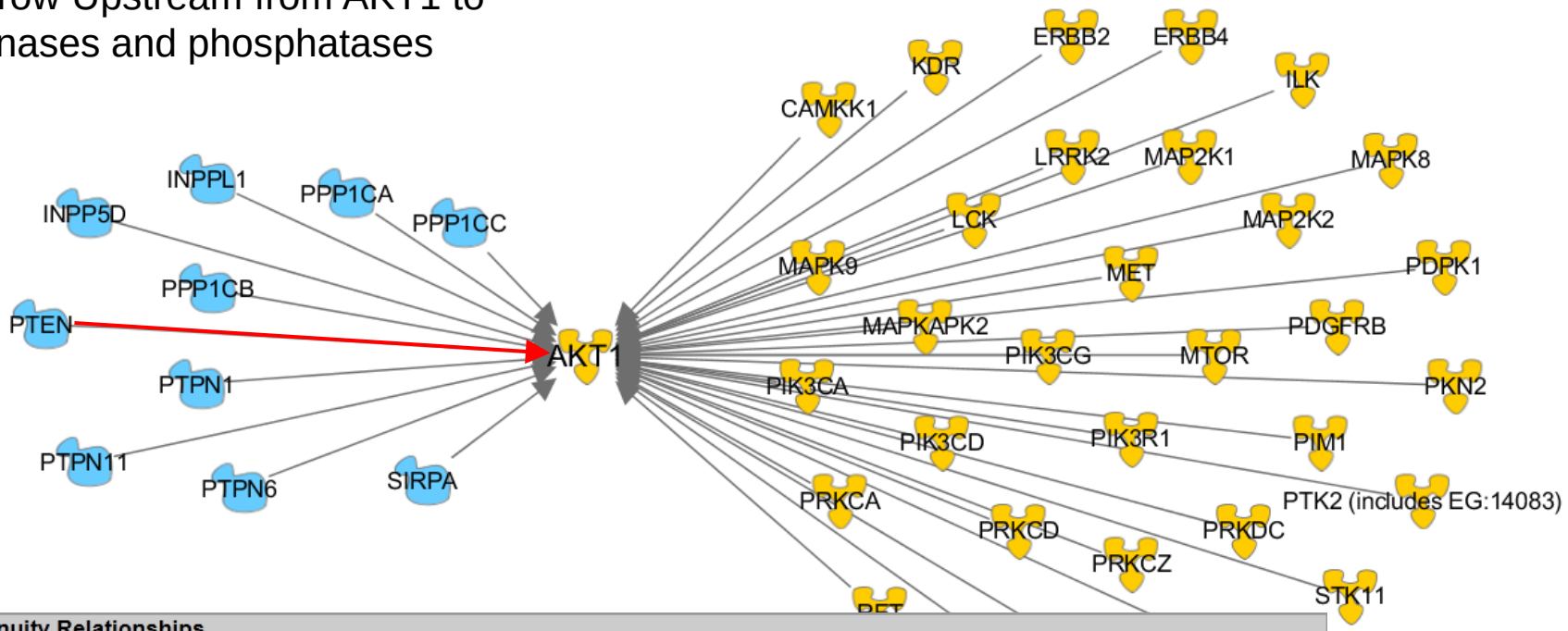


Build Tools包含下列數個建構pathway圖型的工具:

- Grow: 依照使用者的篩選以及參數設定，找出與Pathway圖型目標分子下有關係的其他分子
- Path Explorer: 此工具可以找出兩群分子的最短關係途徑
- Connect: 依照使用者的條件設定，迅速將Pathway圖型內的各分子關係找出並連結
- Trim: 依照使用者的條件設定，移除Pathway圖型的分子
- Keep: 依照使用者的條件設定，保留符合條件的Pathway圖型內的分子
- Add Molecule/Relationship: 讓使用者加入自行訂定名稱以及相關註解的資訊到Pathway圖型裡面，但此資訊只限定在使用者自己的帳號內可使用

Build and Grow Networks of Molecules

Grow Upstream from AKT1 to kinases and phosphatases



Ingenuity Relationships

phosphorylation [9]

In Pc 3 cells, **PTEN protein decreases** (in a dose-dependent manner) **phosphorylation of human PKB [AKT1] protein to** phosphorylated (S473) **human PKB [AKT1] protein**.

10716737 Persad S, Attwell S, Gray V, Delcommenne M, Troussard A, Sanghera J, Dedhar S. Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells. Proc Natl Acad Sci U S A 2000 Mar 28;97(7):3207-12.

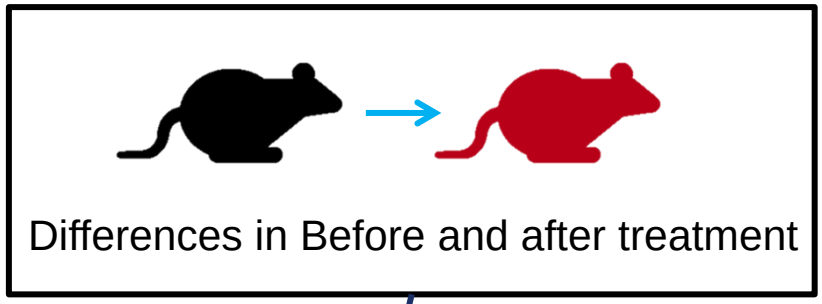
Source: Ingenuity Expert Findings

In U87MG cells, **human PTEN protein decreases phosphorylation of human PKB/AKT [AKT1] protein**.

10554022 Maier D, Jones G, Li X, Schonthal AH, Gratzl O, Van Meir EG, Merlo A. The PTEN lipid phosphatase domain is not required to inhibit invasion of glioma cells. Cancer Res 1999 Nov 1;59(21):5479-82.

Source: Ingenuity Expert Findings

Why are we using IPA?



Differences in Before and after treatment

Your dataset

pathway

Relationship With other Observation

Disease

function

What are the relationship between each molecules?

What do they relate to each other?

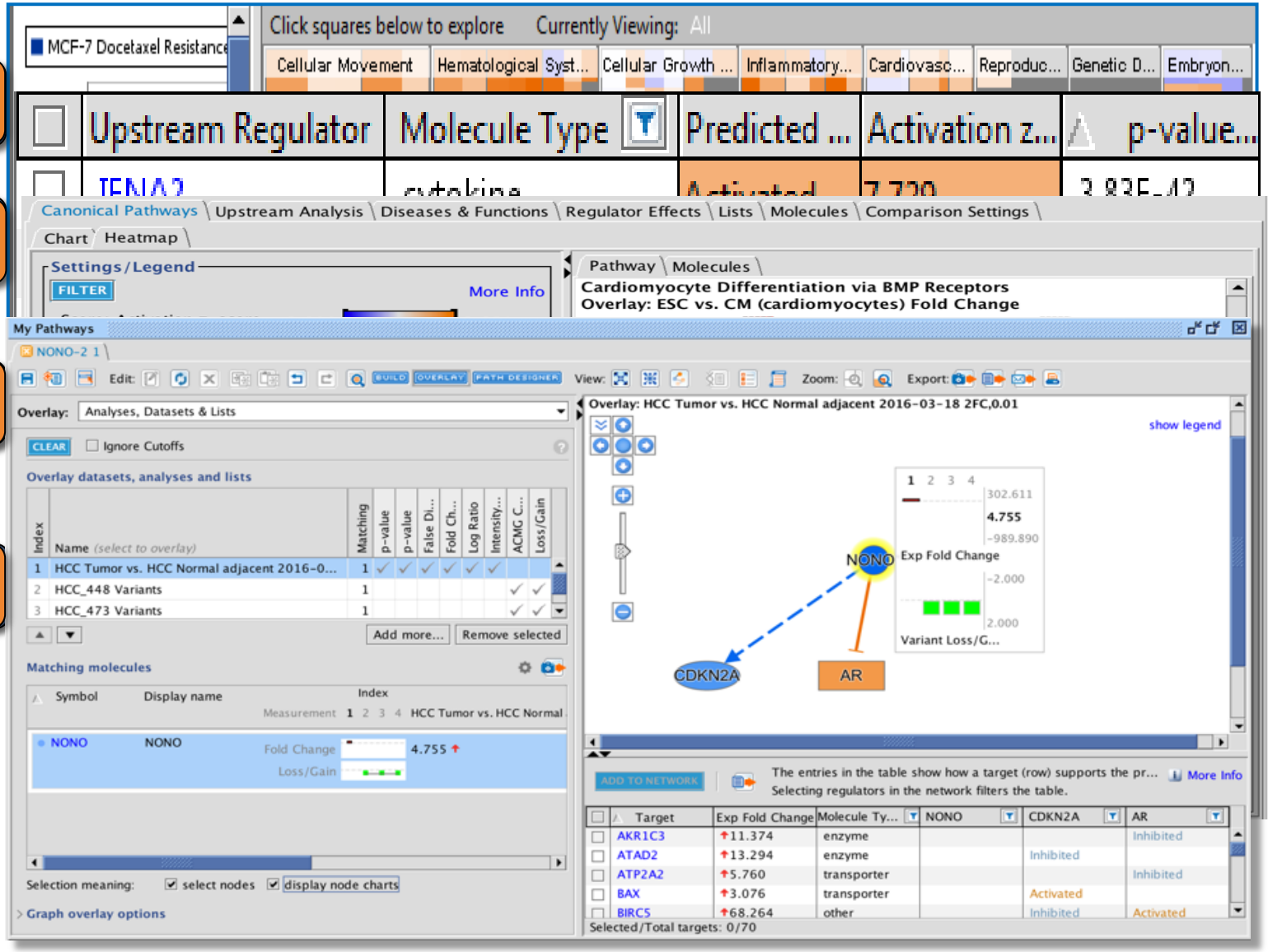
Unique Tools for Biological Analysis and Interpretation

Diseases & Bio Functions

Upstream Regulator Analysis

Comparison Analysis

Multi-omics Overlay

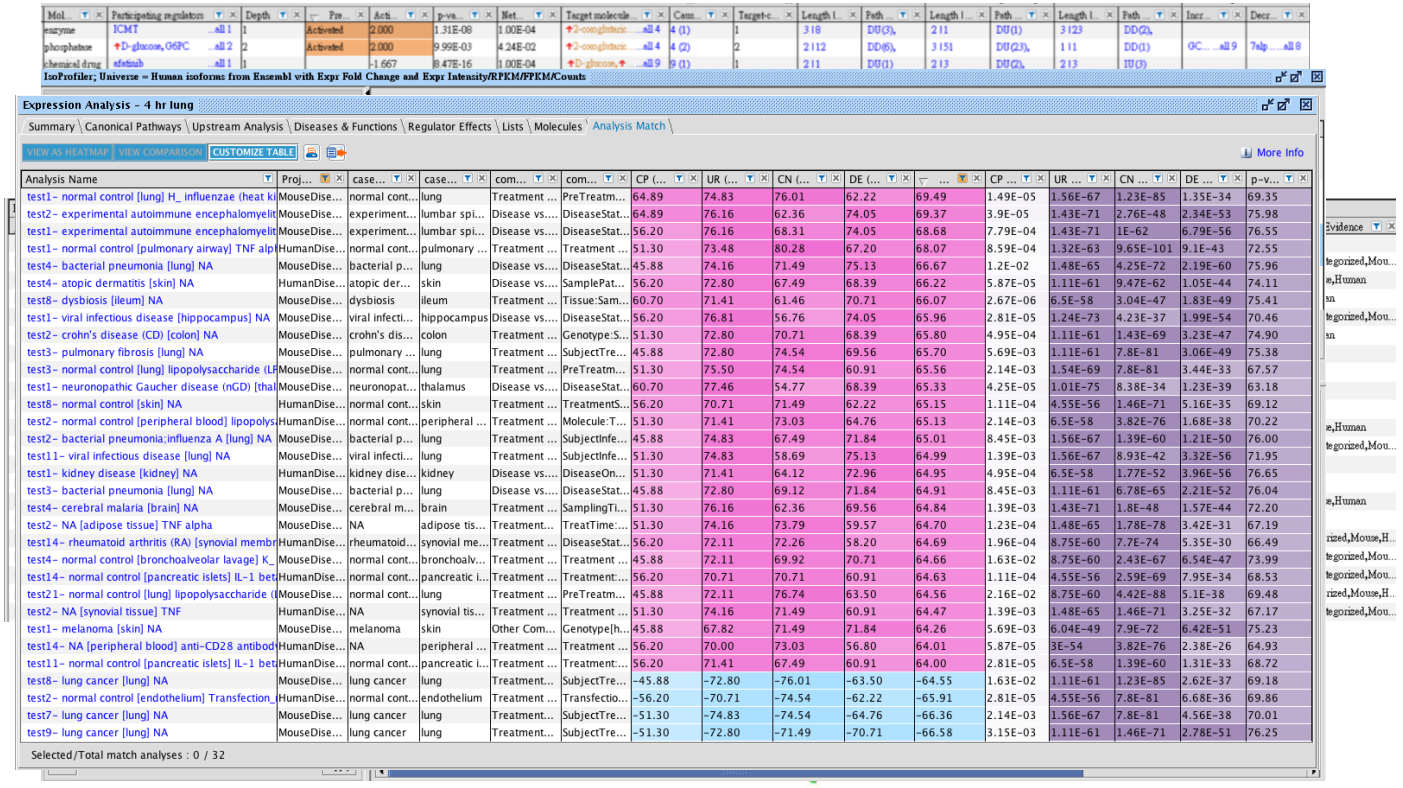


Advanced Analytics

Uncover causality — the next level of pathway analysis

This set of capabilities includes Causal Network Analysis, BioProfiler, IsoProfiler, Relationship Export, and Phosphoproteomics Analysis, all designed to help you understand causal connections between diseases, genes, and networks of upstream regulators.

- Causal Network Analysis
- BioProfiler
- Relationship Export
- IsoProfiler
- Phosphoproteomics Analysis
- Analysis Match



Basic Module and Advanced module on IPA

Basic Module

- Canonical Pathway
- Isoform View
- Disease View
- Gene and Chem View
- Path Designer
- Interactive Disease and Functions Nodes
- Biomarker filter
- Molecule Activity Predictor (MAP)
- Mechanistic Network
- Upstream regulator Analysis
- Downstream Effects Analysis
- Regulator Effects
- Network Analysis
- Comparison Analysis
- MicroRNA Target Filter
- Tox Lists and Tox Functions

Advanced Analytics (AA)

- Causal Network Analysis
- BioProfiler
- Relationship Export
- IsoProfiler
- PhosphoProteomics Analysis
- Analysis Match (Pay extra)

https://www.qiagenbioinformatics.com/products/features/

https://www.qiagenbioinformatics.com/files/flyers/IPA_Advanced_Analytics_WEB.pdf

A. Data Upload and How to Run a Core Analysis

1. **Upload experiment data**
2. **Data process with IPA Core Analysis**

上傳實驗資料並使用IPA分析功能

B. Functional Interpretation in IPA

- **Hands-on Exercises**

1. **Introduction for Analysis Tools**
2. **Interpret the data output information**

IPA分析結果介紹

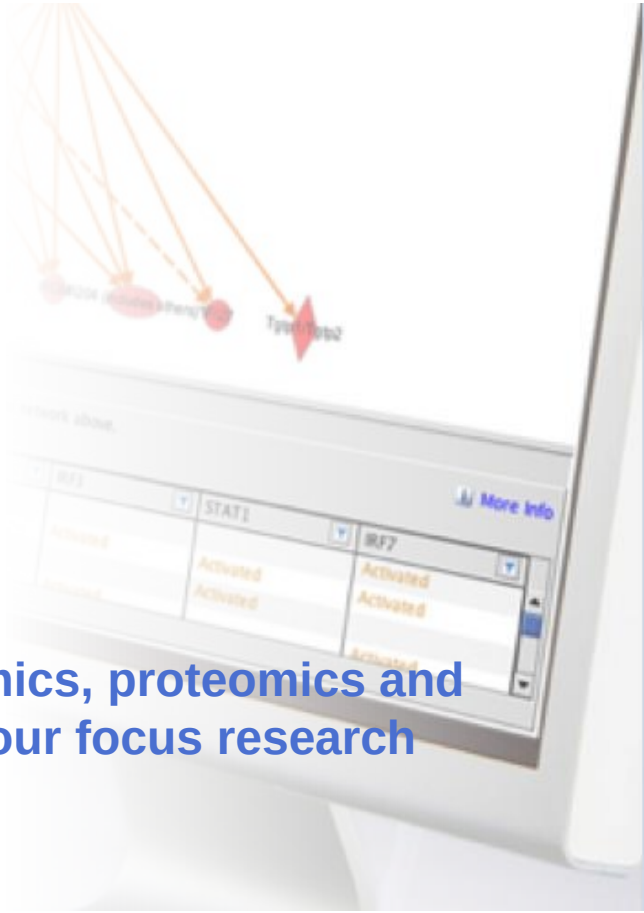
C. Multi-Omics Analysis using IPA

Integrate and compare genomics, transcriptomics, proteomics and metabolomics data to see the big picture on your focus research

比較分析結果的差異

D. Case study

E. Q & A



Ingenuity Pathways Analysis的分析的結果回傳

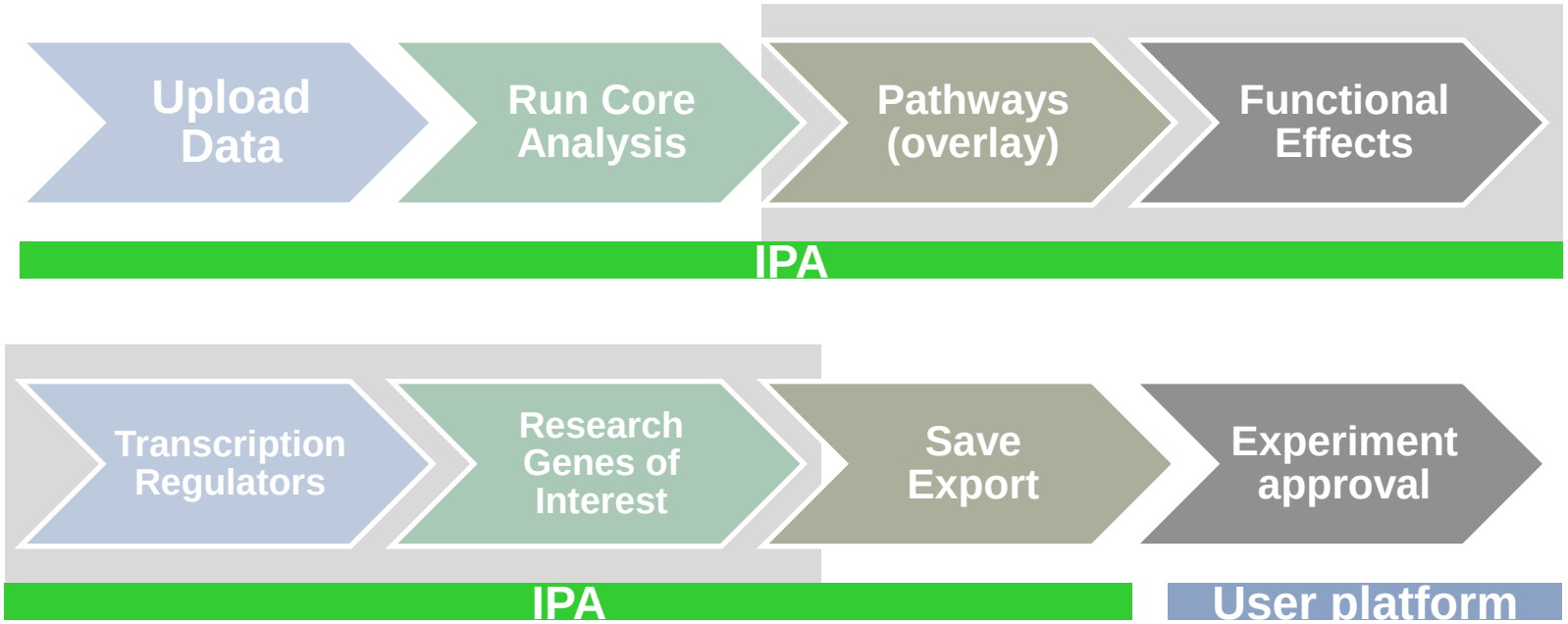
- 與實驗資料相關的生物功能或是疾病分析
- 所影響的Signaling Pathway與Metabolic Pathway以及裡面的組成分子
- 受影響的Transcription regulator的種類以及相關基因與蛋白
- 實驗資料中的分子關係如何形成的網路

分析功能種類:

IPA-Core Analysis 分析mRNA, miRNA或是protein的實驗資料

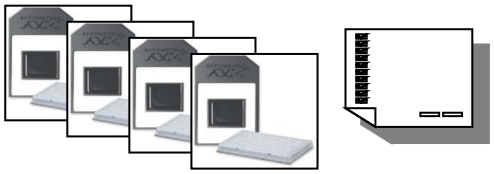
IPA-Tox Analysis: 分析後得到毒性學相關結果

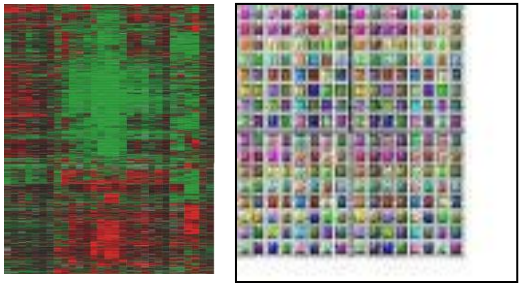
IPA-Metabolomics Analysis: 主要用於分析代謝體(Metabolomics)實驗相關資料



General Analysis Workflow in IPA

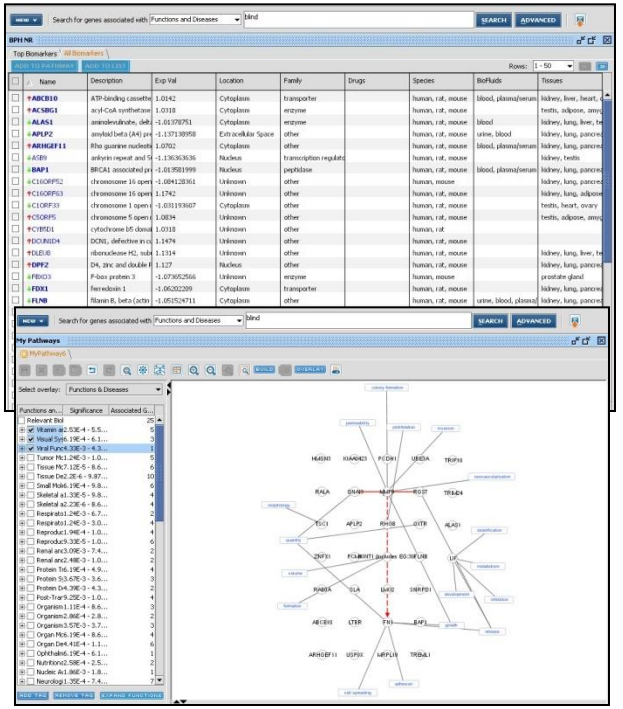
Workflow for Dataset Analysis





Genomic, exon, miRNA, SNP, protein arrays; Any molecule lists; Other proteomic & metabolomic assays

IPA



Identify functions, diseases, and canonical pathways associated with your data

Observation:

- An experimental condition such as a time point, disease subtype, or compound concentration

Expression Value:

- Numerical value indicating level of expression, significance, or other assay result for a specific identifier (gene, RNA, protein, or chemical)

Reference Set:

- The set of molecules used as the universe of molecules when calculating the **statistical relevance of biological functions and pathways with respect to a dataset file**. The set of molecules are the user's dataset or molecules in Ingenuity's Knowledge Base (genes, endogenous chemicals, or both).

Focus Molecule:

- Molecules that are from uploaded list, pass filters are applied, and are available for generating networks

準備IPA分析用的Dataset

必須有一欄放入ID

Replicates

Average

Other observations (Comparison)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	CBCR-T In	Clone ID	Unigene C	Accession	Gene Sym	NAME	BPH-205	BPH-202	BPH-203	BPH-201	BPH-204	BPH Ave F	PCA-402	PCA-403	PCA-404	PCA-410	PCA-408	PCA-401	PCA-409
2	1-10-10-18	770890	Hs.109851	AA434409		ESTs	0.938	0.993	0.944	1.201	0.995	1.019	0.912	0.861	0.869	0.706	0.781	0.716	0.821
3	1-10-10-2	753420	Hs.240112	AA406422	KIAA0276	KIAA0276	0.942	1.083	1.312	1.115	1.285	1.113	0.724	0.868	1.021	0.742	0.591	0.491	0.531
4	1-10-10-3	366154	Hs.222909	AA062813	DKFZP434	DKFZP434	0.941	0.913	0.968	0.941	0.908	-1.06298	1.458	0.991	1.052	0.903	1.087	1.07	0.907
5	1-10-10-5	51746	Hs.79348	H23046	RGS7	regulator	1.054	0.9	0.853	0.826	0.8	-1.10102		1.113	1.082	0.803	1.031	1.003	0.942
6	1-10-10-6	781704	Hs.77558	AA431611	TRIP7	thyroid ho	0.996	1.38	1.605	1.172	1.115	1.28825	0.692	0.944	1.022	0.91	0.91	0.769	0.747
7	1-10-10-8	282051	Hs.71741	N53616		ESTs, High	1.016	1.051	1.062	0.992	1.027	1.03025	1.03	1.043	1.054	1.043	1.237	1.117	1.105
8	1-10-1-11	366966	Hs.27865	AA026562		ESTs	0.965	2.153	2.193	1.665	1.789	1.744	0.47	0.833	0.291	0.644	0.669	0.683	0.775
9	1-10-11-1	280752	Hs.79362	N50554	RBL2	retinoblas	0.993	1.229	1.39	1.146	1.107	1.1895	0.776	0.89	1.125	0.938	0.964	0.736	0.752
10	1-10-11-10	123646	Hs.117331	R02728		ESTs	1.007	0.904	0.895	0.818	0.892	-1.10375	1.033	0.889	1.07	0.919	1.081	1.143	1.016
11	1-10-11-13	200307	Hs.68647	R96804		ESTs, Wea	1.031	1.085	1.396	1.268	1.091	1.195	0.773	1.025	0.998	0.958	0.987	0.982	1.086
12	1-10-11-17	325138	Hs.82035	W49785		ESTs	0.868	0.995	1.124	1.211	1.219	1.0495	0.626	0.823		0.724	0.798	0.611	0.661
13	1-10-11-19	502287	Hs.83992	AA156781		ESTs	0.918	1.246	1.253	1.419	1.51	1.209	1.402	0.931	1.26	1.896	1.277	1.004	0.834
14	1-10-1-17	809473	Hs.29759	AA443119		Homo sap	0.929	0.993	1.796	1.359	2.58	1.26925	0.571	0.743	1.471	0.626	0.464	0.514	0.628
15	1-10-1-20	137890	Hs.92202	R68581		ESTs	0.931	1.218	1.226	0.969	1.313	1.086		0.415	0.689	0.744	0.728	0.946	0.897
16	1-10-12-12	213118	Hs.37978	H69576		ESTs	0.893	0.796	0.973	0.796	0.951	-1.15674	1.089	0.995	1.19	1.111	1.153	1.164	0.978
17	1-10-12-20	198607	Hs.58617	R94947	ROCK2	Rho-assoc	0.963	1.137	1.236	1.038	1.483	1.0935	0.65	0.866	1.154	0.68	0.76	0.778	0.758
18	1-10-14-2	755752	Hs.6151	AA496327		Human mI	0.944	1.141	1.221	1.11	1.077	1.104	0.963	0.987	1.053	1.053	0.973	0.942	0.873
19	1-10-14-20	427980	Hs.150390	AA001835	ZNF262	zinc finger	0.951	1.043	0.91	1.004	0.86	-1.02354	0.807	1.116	0.922	1.032	1.054	0.932	1.083
20	1-10-14-6	49260	Hs.12840	H16573		Homo sap	0.975	0.861	0.907	0.9	0.92	-1.098	1.124		1.067	0.938	1.171	1.122	1.113
21	1-10-15-14	810741	Hs.7719	AA457725	GABARAP	GABA(A) r	1.016	1.096	1.281	1.318	1.17	1.17775	1.14	1.291	1.178	1.156	0.986	0.908	1.128
22	1-10-15-17	265592	Hs.29826	N21407		ESTs	1.11	1.261	1.391	1.482	1.084	1.311	0.842	1.073	1.068	1.507	0.847	0.755	0.989
23	1-10-15-20	428737	Hs.103280	AA004648		ESTs	1.089	0.879	0.884	0.819	1.069	-1.08962	1.191	1.179	1.092	0.979	1.147	1.086	1.114
24	1-10-15-21	50182	Hs.89591	H17882	KAL1	Kallmann	0.958	1.564	1.308	1.224	0.79	1.2635	0.662	1.162	0.652	0.456	0.749	0.73	0.558
25	1-10-15-5	484535	Hs.198241	AA036974	AOC3	amine oxi	1.145	1.175	1.307	1.286	1.172	1.22825	0.731	0.929	1.248	0.873	1.041	0.673	0.999

- **重複性實驗的數值平均、p-vlaue或fold-change等統計計算，要先在IPA分析之前完成。**

- **將實驗資料用 Excel 表格檔案儲存，檔案裡面只能有一個Sheet存在。**
  - Excel Sheet當中必須要有一欄是列出**分子的ID** (如Gene Symbol, Refseq number, Uniprot number, HMDB等常用命名皆支援)
  - 每個Excel Sheet 最多可以放入 **20個 observations** (即20個實驗變因的資料欄的意思)
  - 每個Observation可以有3個不同的表現值種類 (ex. p-Value，fold-change等)
  - **表格欄位最上方只能有一個Head row (首行)**
  - 資料上傳到IPA後，可以在cut-off 值欄位進行設定，讓使用者決定門檻來決定**表現顯著有差異**的生物分子。意味著原始實驗資料中有些分子的數值不夠顯著，可以用cut-off值作為門檻排除於分析運算中。那些通過cut-off值的分子們在IPA中稱之為Analysis-Ready Molecules。

分析用的Dataset的範例格式

這表格為標準IPA分析用資料表格範例，裡面的數值類型是 Log Ratio
這組實驗資料裡面有三個Observation :

- Observation 1 : Smokers vs. NonSmokers
- Observation 2 : Early COPD vs. NonSmokers
- Observation 3 : COPD vs. NonSmokers

	A	B	C	D
1	ID_REF	*Smokers vs. NonSmokers*	*Early COPD vs. NonSmokers*	*COPD vs. NonSmokers*
2	1007_s_at	-0.006955963	-0.028339307	0.06209247
3	1053_at	-0.047503628	-0.001610169	0.060261582
4	117_at	-0.110988314	0.193030977	-0.079160692
5	121_at	0.050275771	-0.010810624	0.078980219
6	1255_g_at	0.01098737	-0.151880946	0.271391848
7	1294_at	-0.05090447	0.020144002	0.045157579
8	1316_at	0.041293255	0.040339731	0.101614517
9	1320_at	-0.086868115	-0.050757375	-0.746738716
10	1405_i_at	0.374965097	-0.01805262	0.474615195
11	1431_at	-0.084408555	0.014600862	0.010719682
12	1438_at	-0.043223369	0.04906669	0.072519797
13	1487_at	0.016570535	0.017204065	0.140111634
14	1494_f_at	0.074827039	-0.171039972	-0.053830243
15	1552256_a_at	0.146508027	0.156529919	0.264935712

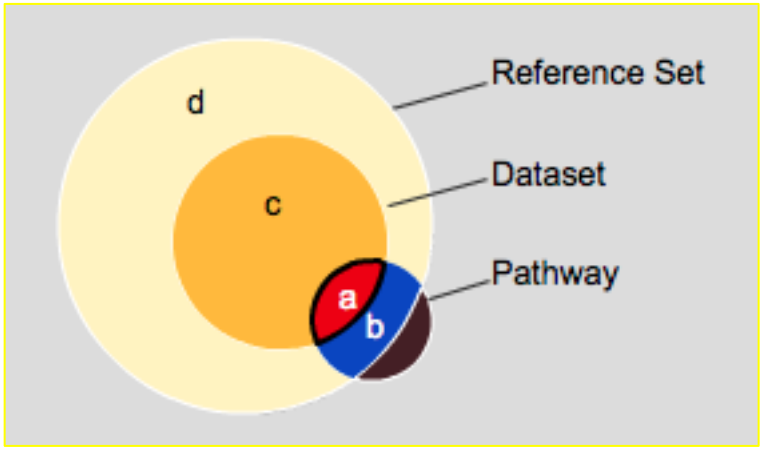
*不同observation的重複實驗數值已經在先前經過平均才放入此表格

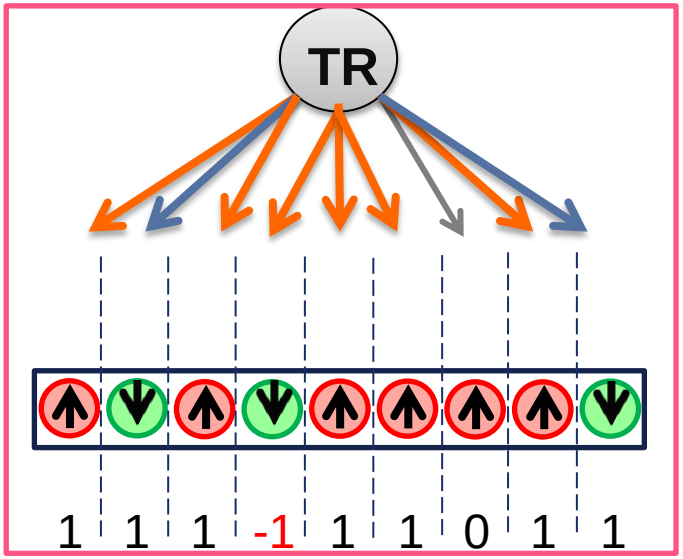
Live Demo

p-value of overlap

- Null hypothesis: No overlap between molecules from dataset and disease/function/upstream regulator/pathway.
- Calculate using the right-tailed Fisher's Exact Test.
- Significant p-value ≤ 0.05

Note: Benjamini-Hochberg correction for multiple testing can be implemented in some cases





z-score

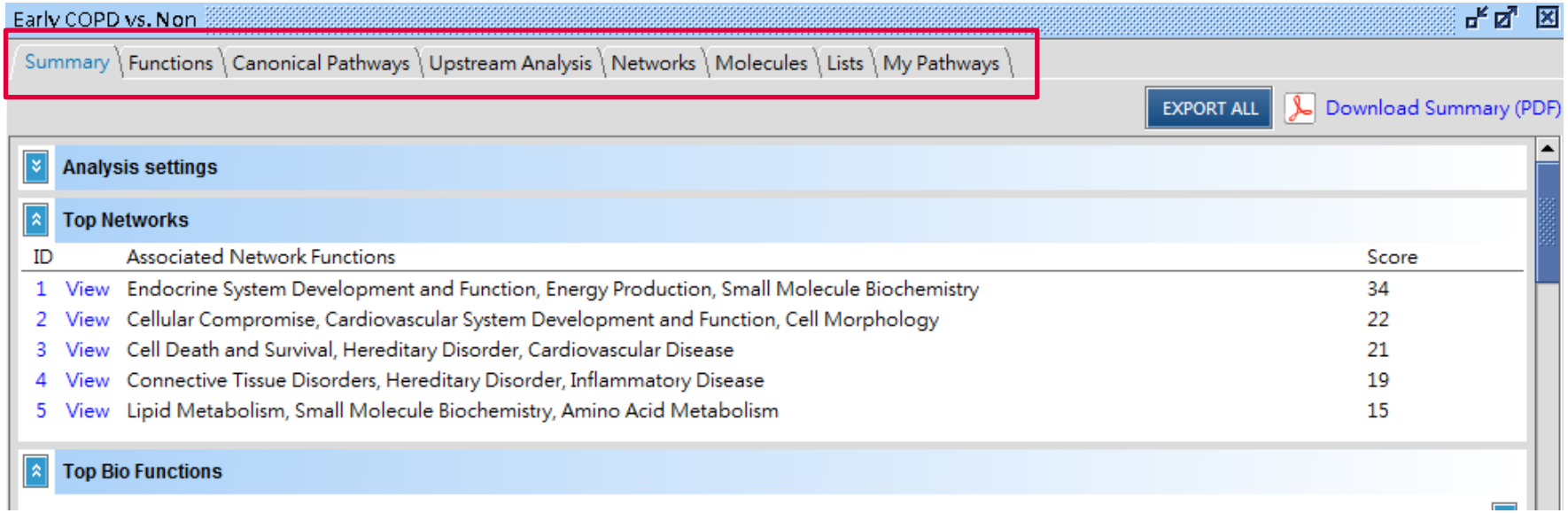
- Predicts Activation or Inhibition
- Correlation between what is known (IPA Knowledge Base) and your expression data

Functions analysis: 呈現因為分子變化而受影響的生物功能、疾病與毒性學結果

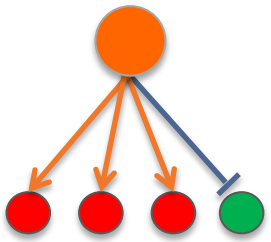
Canonical Pathways : 列出受實驗影響的Signaling Pathway與Metabolic Pathway

Upstream Analysis: 列出與資料中變動分子有關的Upstream molecules，以及根據研究文獻預測它們是否是被啟動或是被抑制。

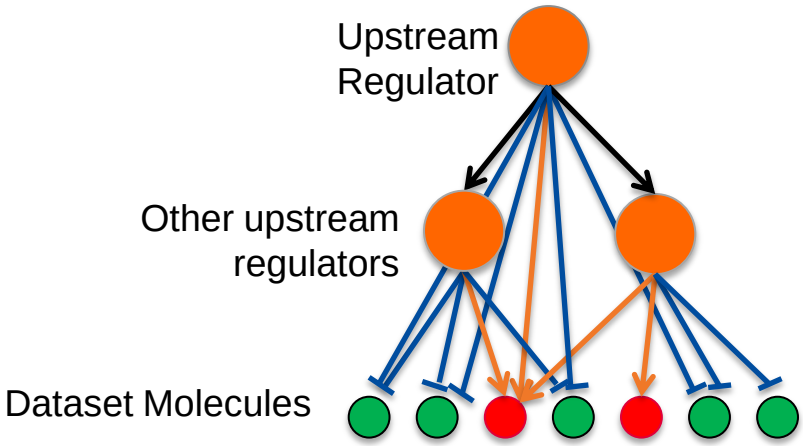
Networks : 呈現實驗資料中的分子間的網路關係 。並且可以利用Build Tool與Overlay Tool進行延伸與知識的拓展，以上各分析結果都是用來解釋實驗觀察到的現象的重要依據。



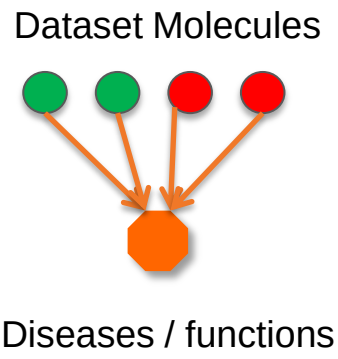
Upstream Analysis



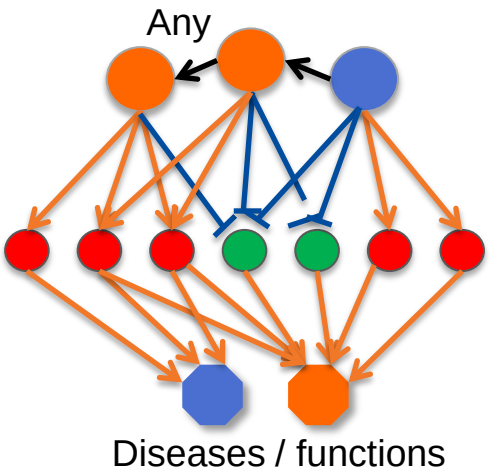
Mechanistic Network of Upstream Regulators



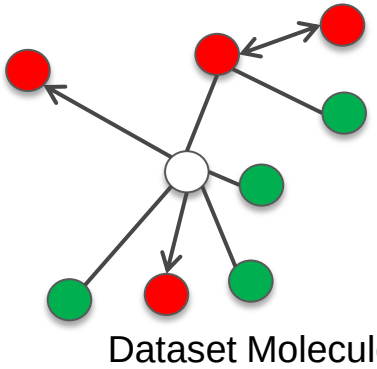
Function Analysis



Regulator Effect Network



Interaction Network



Functions analysis: 呈現因為分子變化而受影響的生物功能、疾病與毒性學結果

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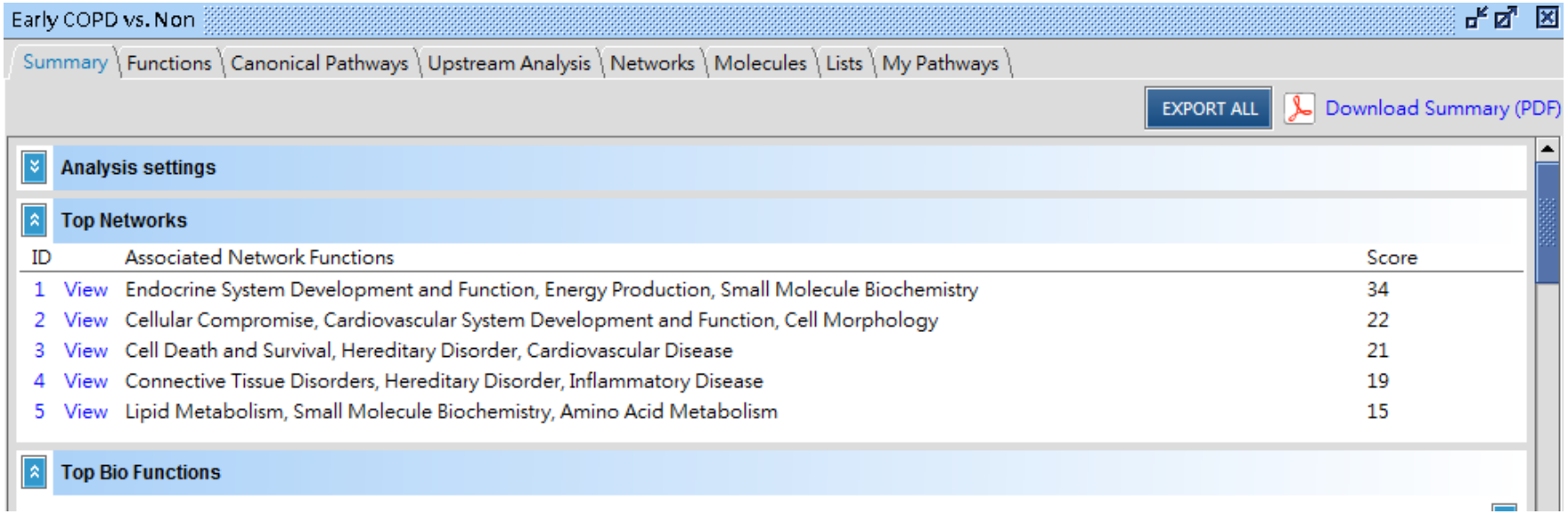
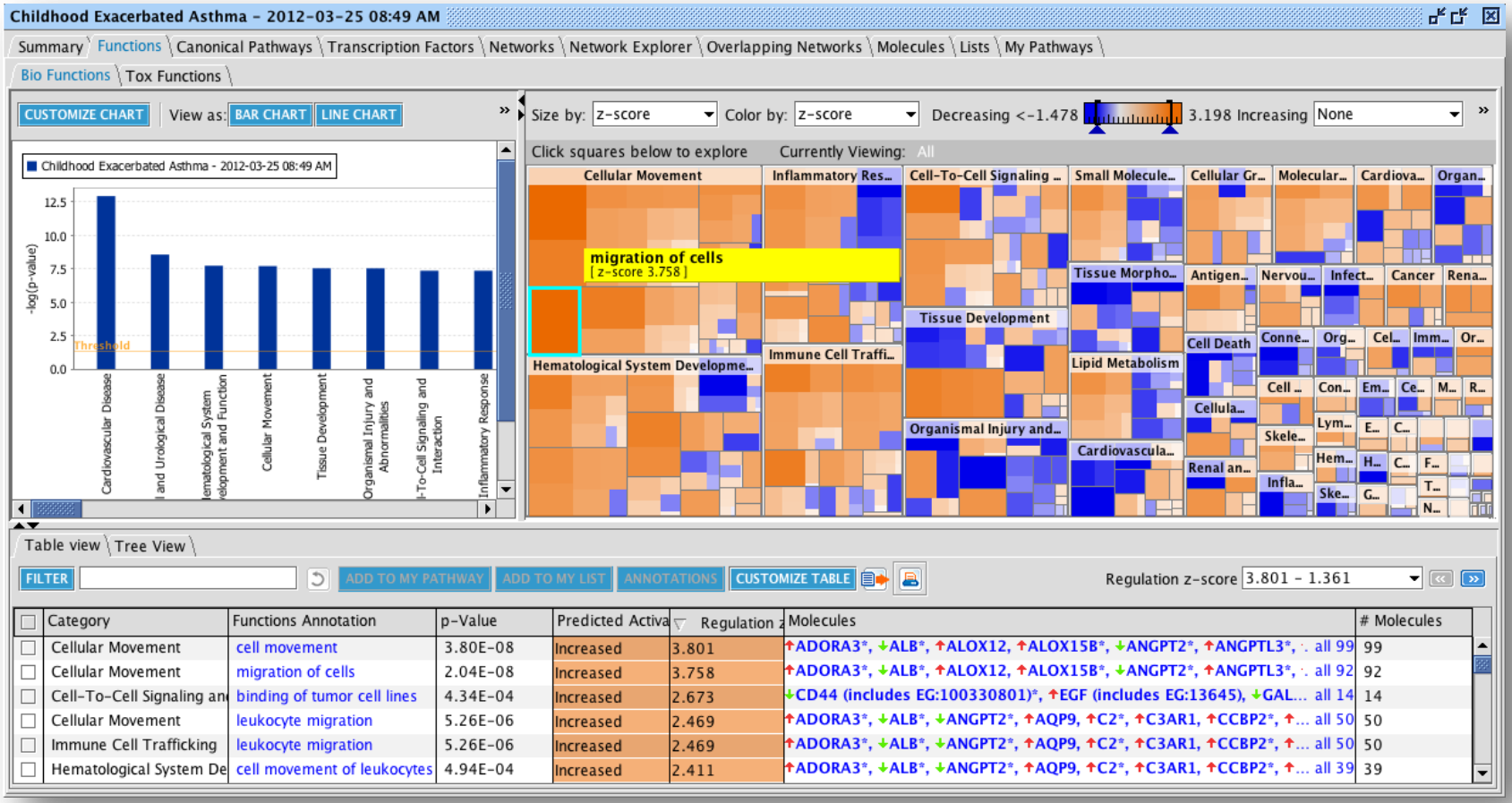


Interpret Downstream Biological Functions

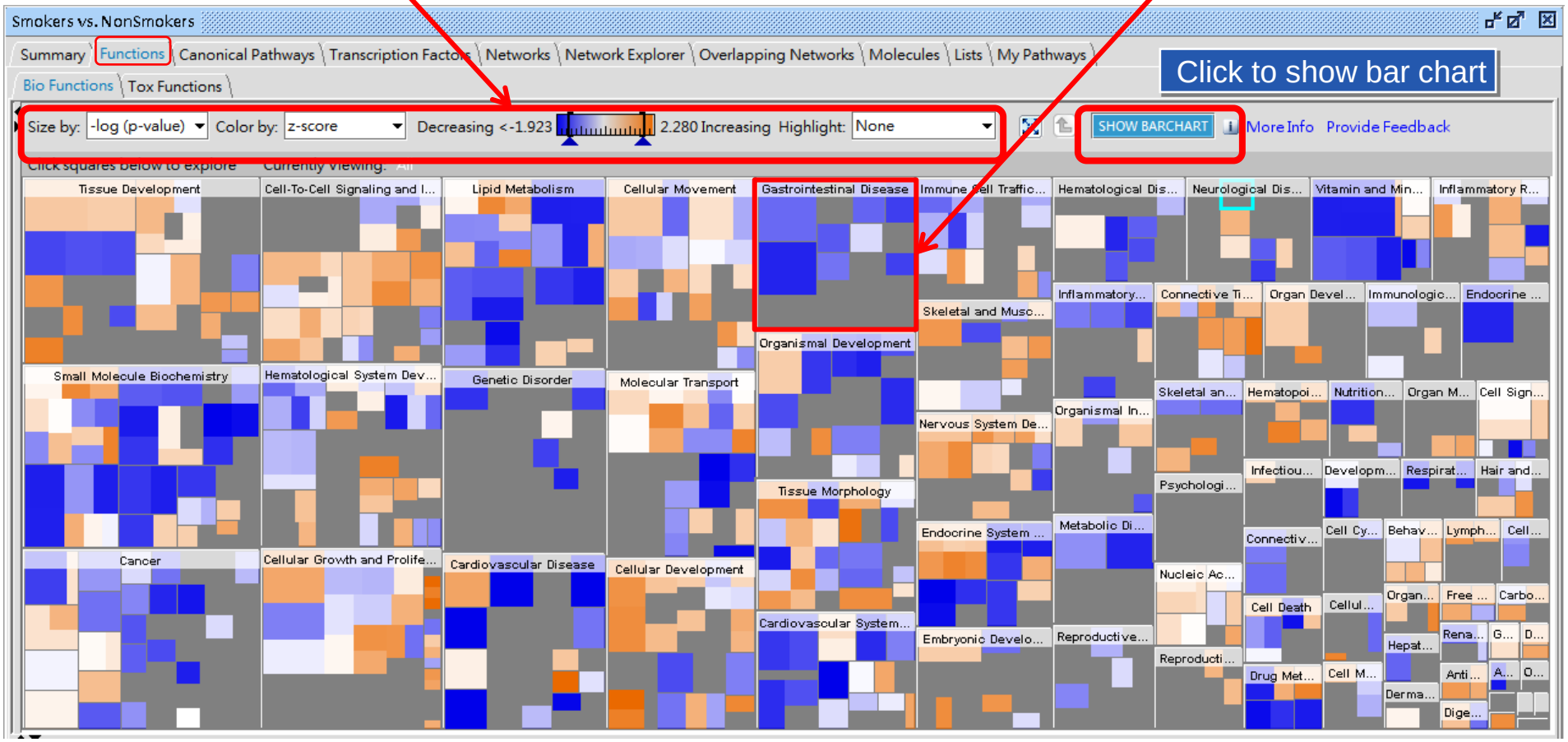


Identify over-represented biological functions and predict how those functions are increased or decreased in the experiment

Downstream Effects Analysis介紹

方塊代表受實驗影響的生物功能與疾病，顏色可以用[Color by]指定是**z-score, -log (p-value), 或是 # of genes上色。如果是用z-score上色的話，藍色區塊是預測被減低的功能，橘色則是此功能會增加。是根據實驗資料做出的演算。**

每個矩形可以經由點擊進入下一層分區: **Mid-level functional category (level 2)** 與 **Specific functions (level 3)**



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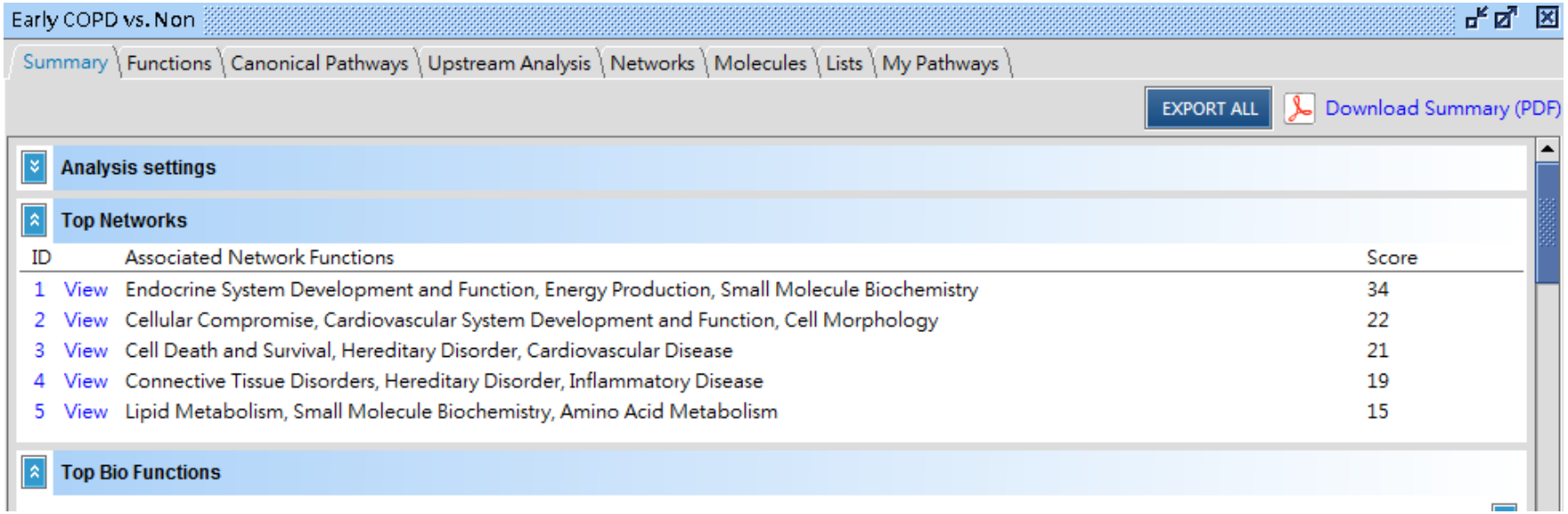
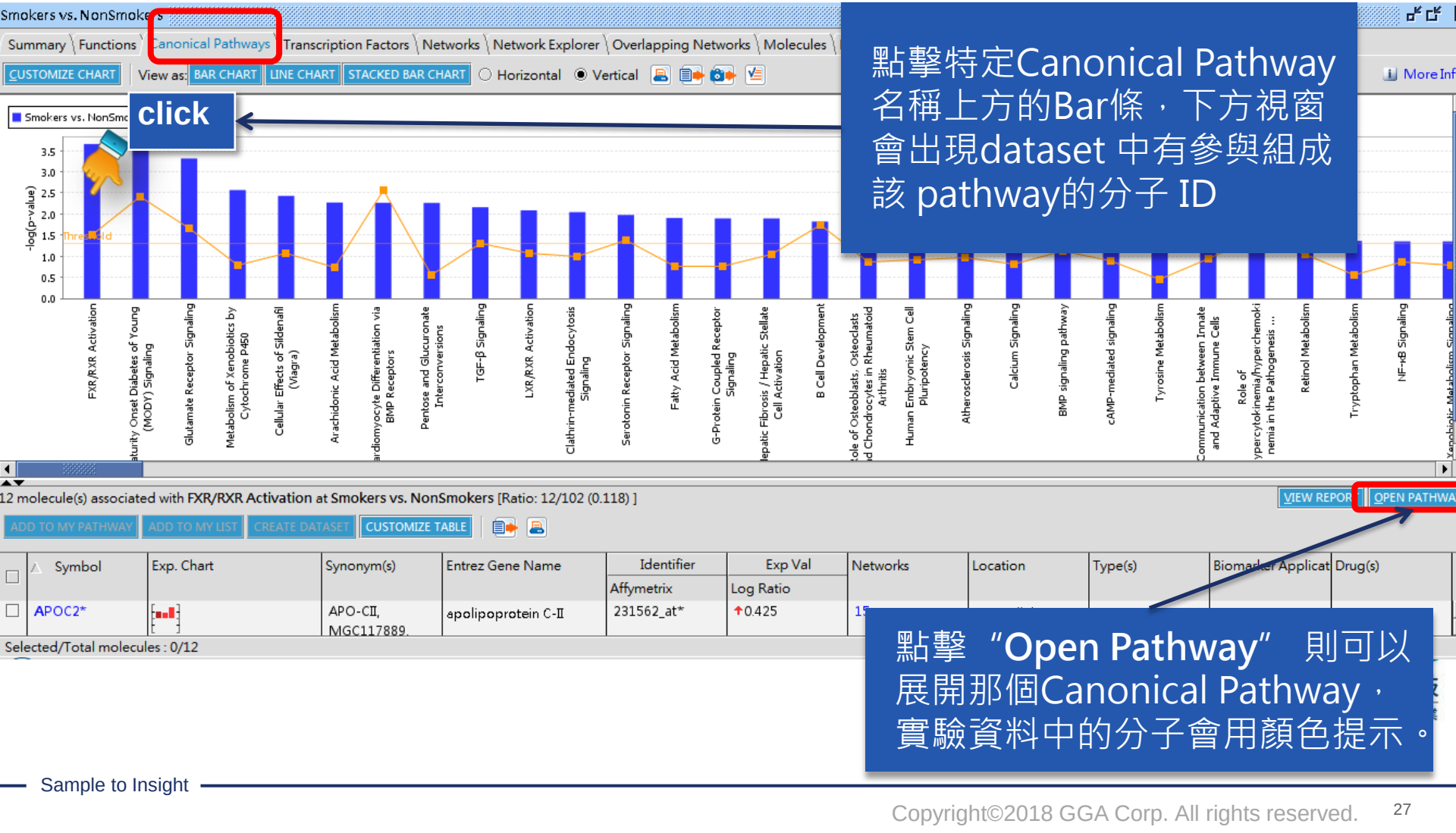


Canonical Pathways結果標籤:
受影響的Signaling Pathway與Metabolic Pathway 依照顯著性用條狀圖排列



點擊特定Canonical Pathway名稱上方的Bar條，下方視窗會出現dataset 中有參與組成該 pathway的分子 ID

點擊 “Open Pathway” 則可以展開那個Canonical Pathway，實驗資料中的分子會用顏色提示。

IPA 分析結果

Functions analysis: 呈現因為分子變化而受影響的生物功能、疾病與毒性學結果

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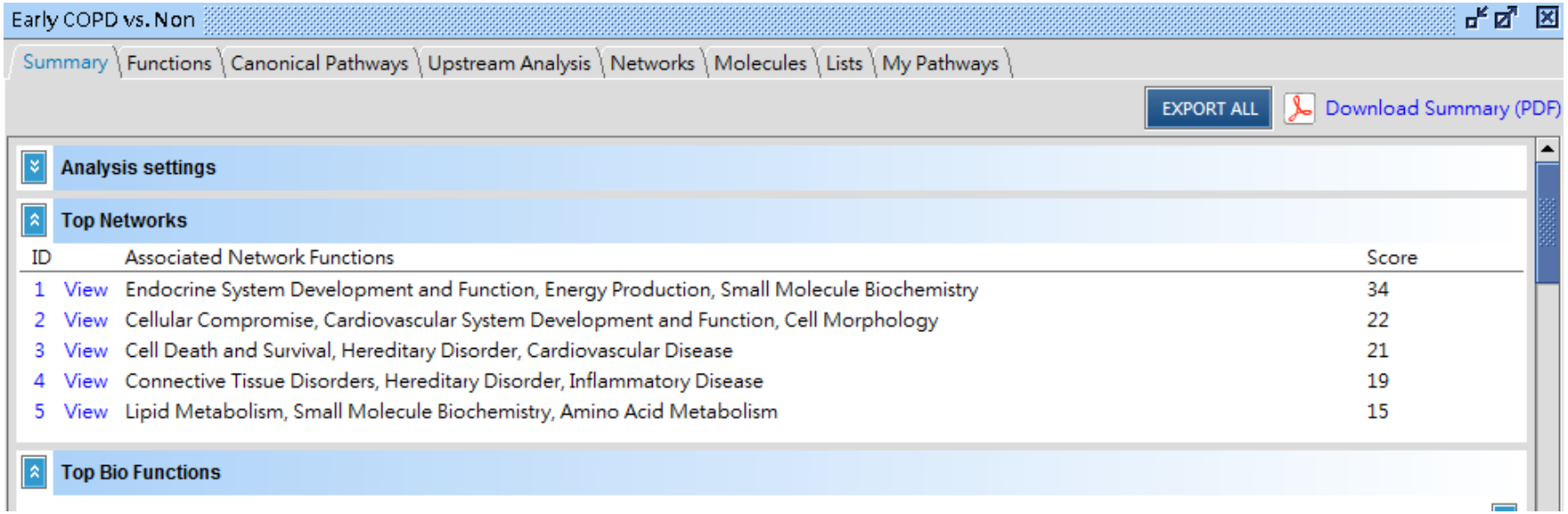
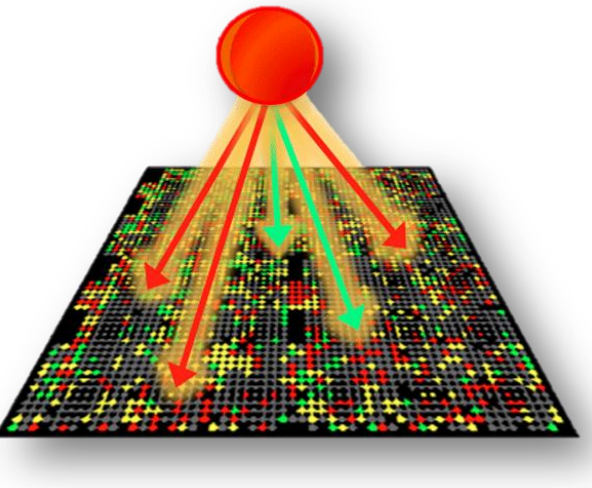


Upstream Regulator Analysis: *How does it work?*



Use experimentally observed relationships (vs. Predicted event) between Upstream Regulators and genes to predict potential regulator and activation

Predict activation or inhibition of regulator to explain the changes in gene expression in your dataset

Calculates two complementary statistical measures:

- Activation z-score
- Overlap p-value

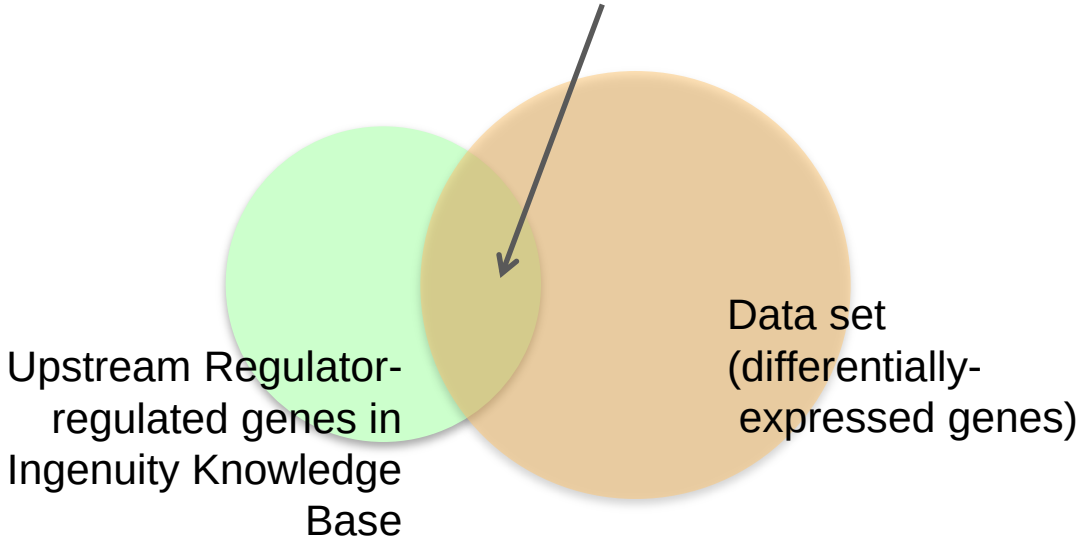
Upstream Regulator Analysis: *How does it work?*

Can we predict the activation state (activated/inhibited) of a potential regulator from expression data?

Approach: Two complementary statistical measures:
Activation z-score and Overlap p-value

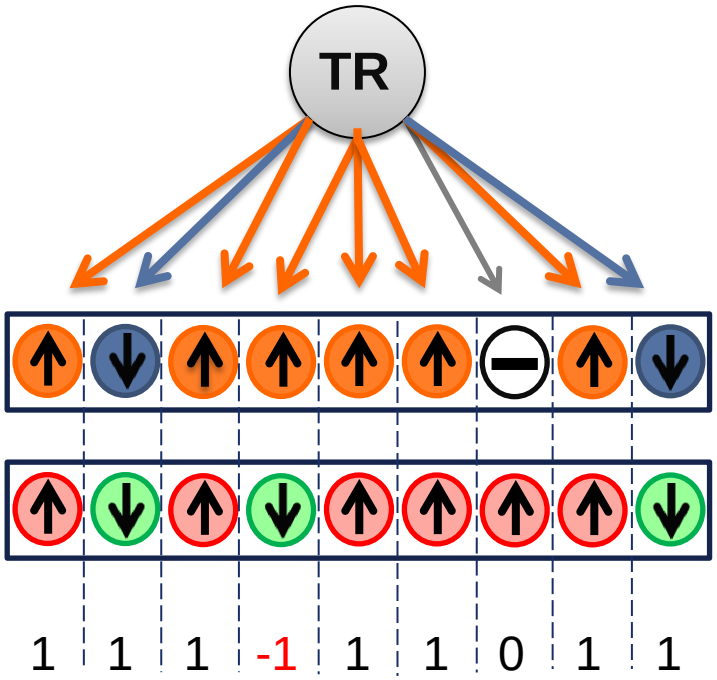
TR → target edge types considered:
- Expression
- Transcription
- Protein-DNA binding

Evaluate the perturbed genes in the dataset that are known targets of a particular regulator



Upstream Analysis Activation z–score

Statistical measure of correlation between the transcription regulator (TR) and resulting gene expression



N = 8 genes

TR effect on downstream genes
(Literature)

Differential gene expression
(Uploaded Data)

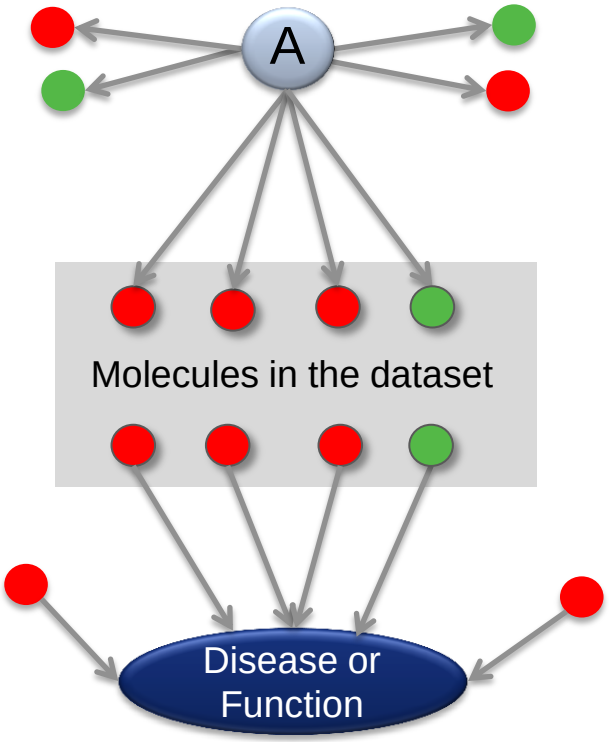
z-score > 2 or < -2 is considered significant

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

Concept of “Regulator Effects” - Spring 2014

Hypotheses for how activated or inhibited upstream regulators cause downstream effects on biology

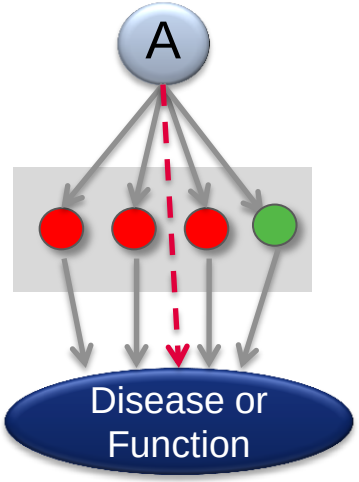
Upstream Regulators



Algorithm

First iteration

Simplest Regulator Effects result



Displays a relationship between the regulator and disease/function if it exists

Downstream Effects Analysis

Causally consistent networks score higher

The algorithm runs iteratively to merge additional regulators with diseases and functions

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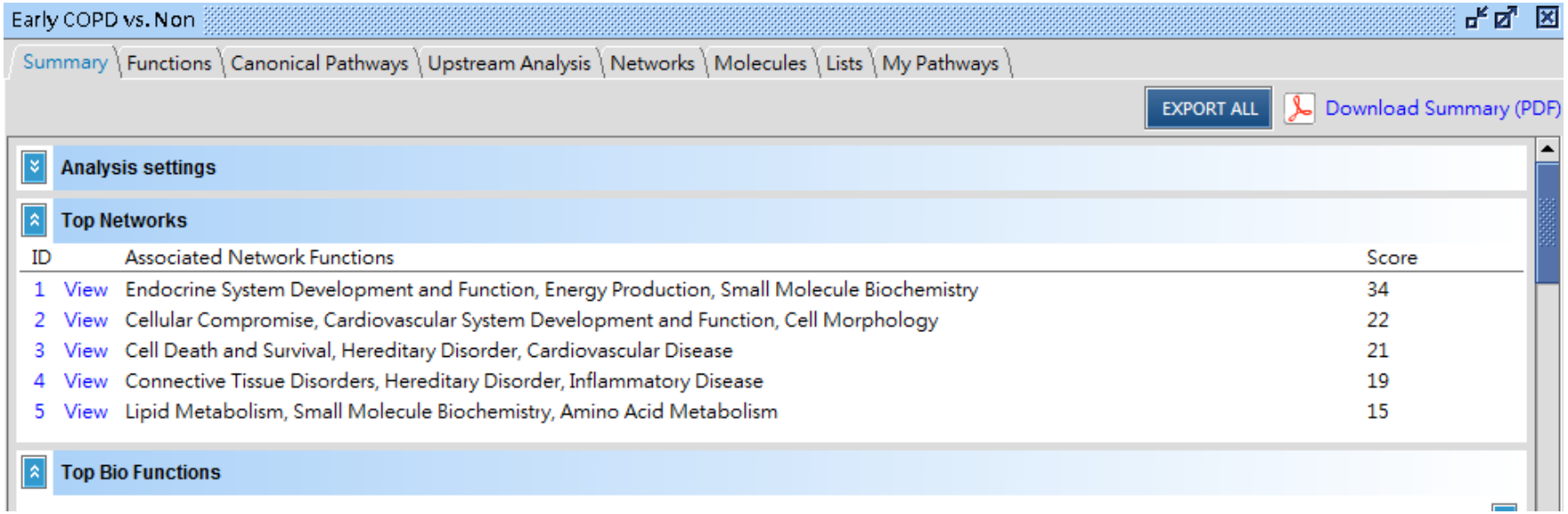
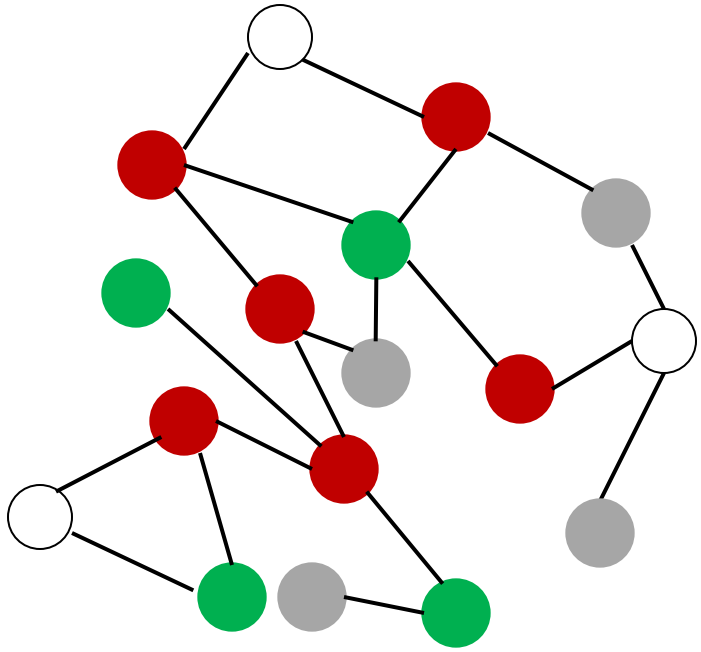


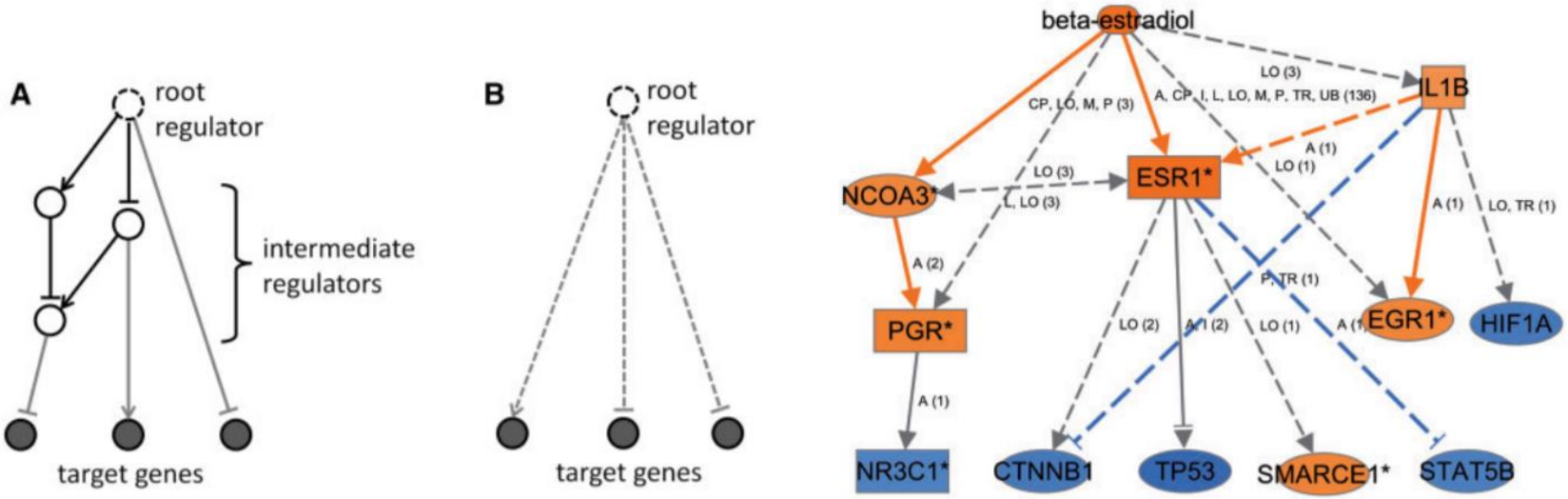
1. Focus molecules are “seeds”
2. Focus molecules with the most interactions to other focus molecules are then connected together to form a network
3. Non-focus molecules from the dataset are then added
4. Molecules from the Ingenuity’s Knowledge Base are added
5. Resulting Networks are scored and then sorted based on the score



Molecules per Network	Networks per Analysis
35	25
35	10
70	25
140	50

Live Demo

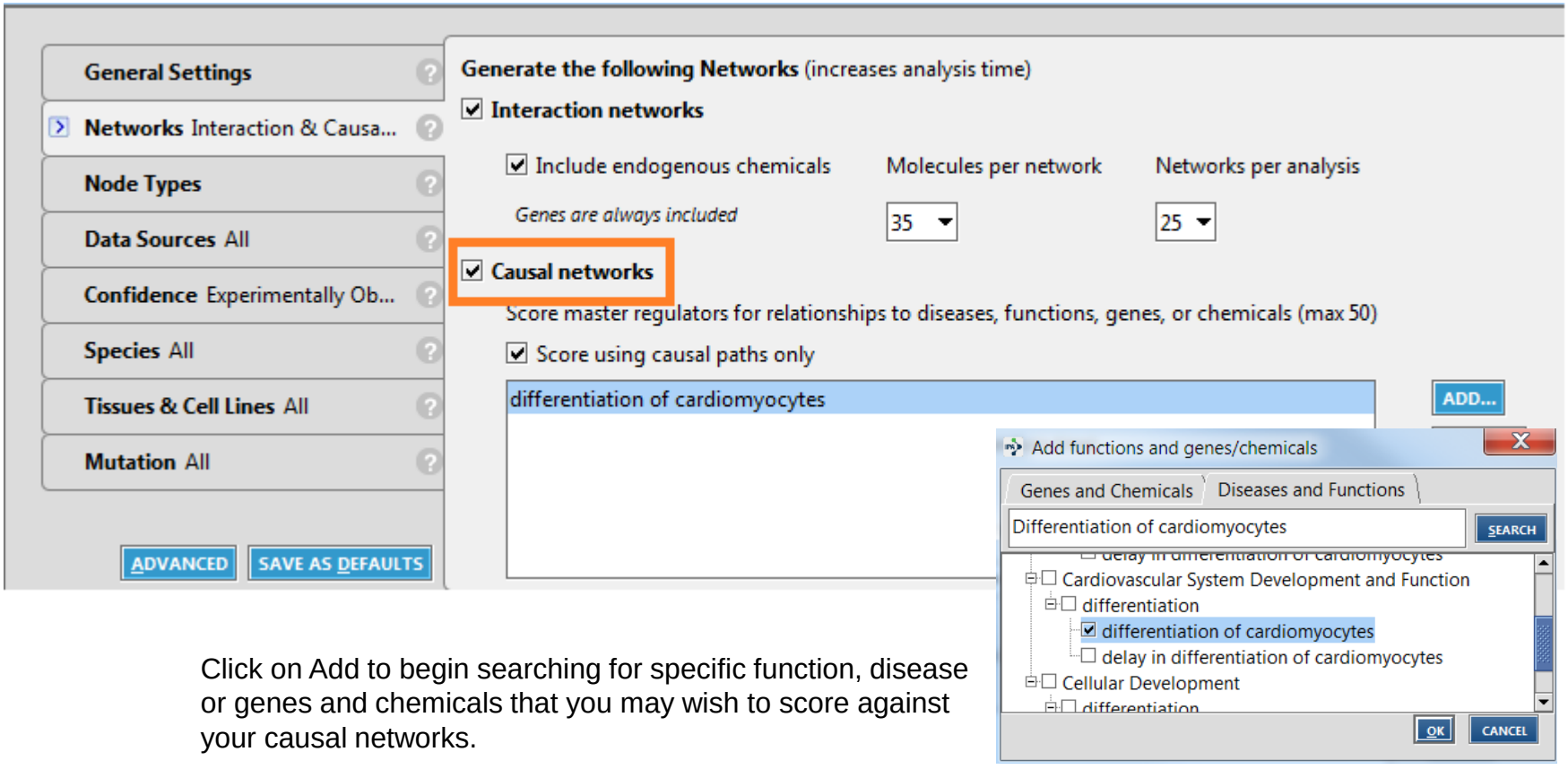
The enhanced Causal Network Analysis (CNA) provides a comprehensive approach to identifying upstream molecules that control the expression of the genes in your datasets. You can now, in a single click, visualize the diseases and functions you are scoring against, understand the effect of the master regulator on that disease or function, and drill-down to the evidence supporting those relationships. In addition, increase the predictive power by allowing intervening molecules or functions, up to three steps or 'hops', to connect a hypothesis to the scoring criteria.



Causal analysis approaches in Ingenuity Pathway Analysis. 2014 *Bioinformatics*

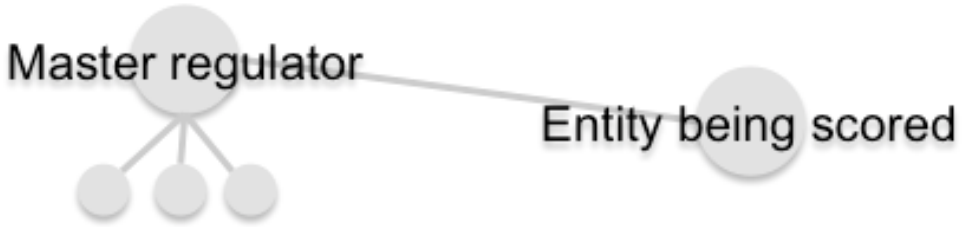
How to Create Causal networks?

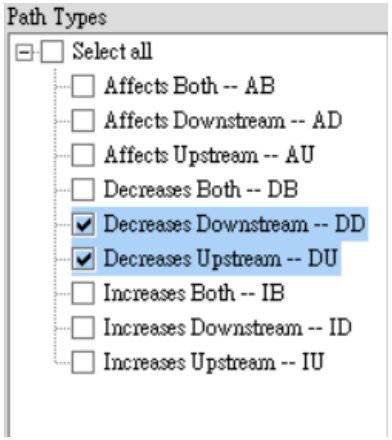
The option to build causal networks is available in IPA on the Create Analysis page. Select the check box for Causal network under General Settings > Networks to include Casual Analysis in your analysis results.



Click on Add to begin searching for specific function, disease or genes and chemicals that you may wish to score against your causal networks.

Mechanism of scoring path types





The effect of the upstream node on the downstream node:

- **Increases:** The upstream node *increases* the activity of the downstream node

- **Deceases:** The upstream *decreases* the activity of the downstream node

- ***Affects:** The upstream node neither increases nor decreases the activity of the downstream node

The position of the master regulator node relative to the entity being scored:

- **Upstream:** The master regulator is upstream (acts on) of the entity being scored

- **Downstream:** The master regulator is downstream (is acted upon) by the entity being scored

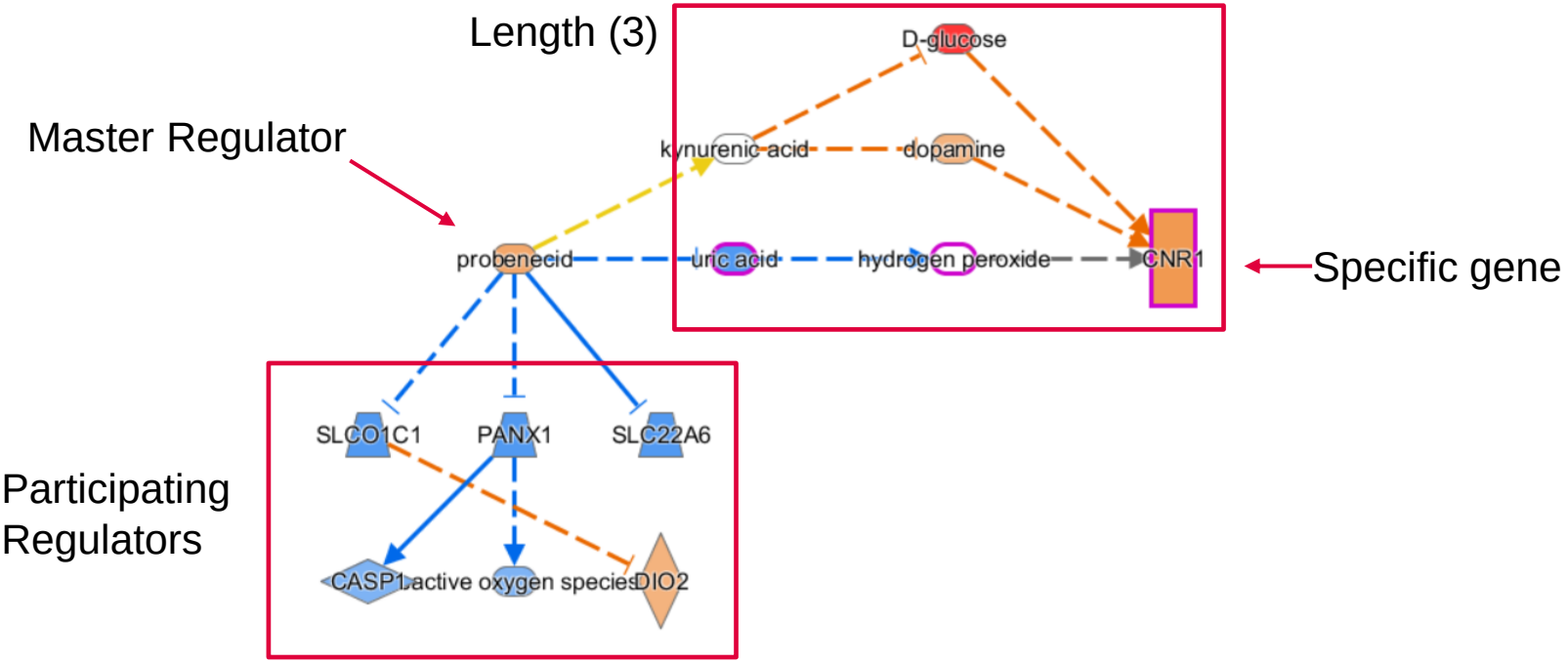
- ***Both:** The master regulator is neither upstream nor downstream of the entity being scored

Result

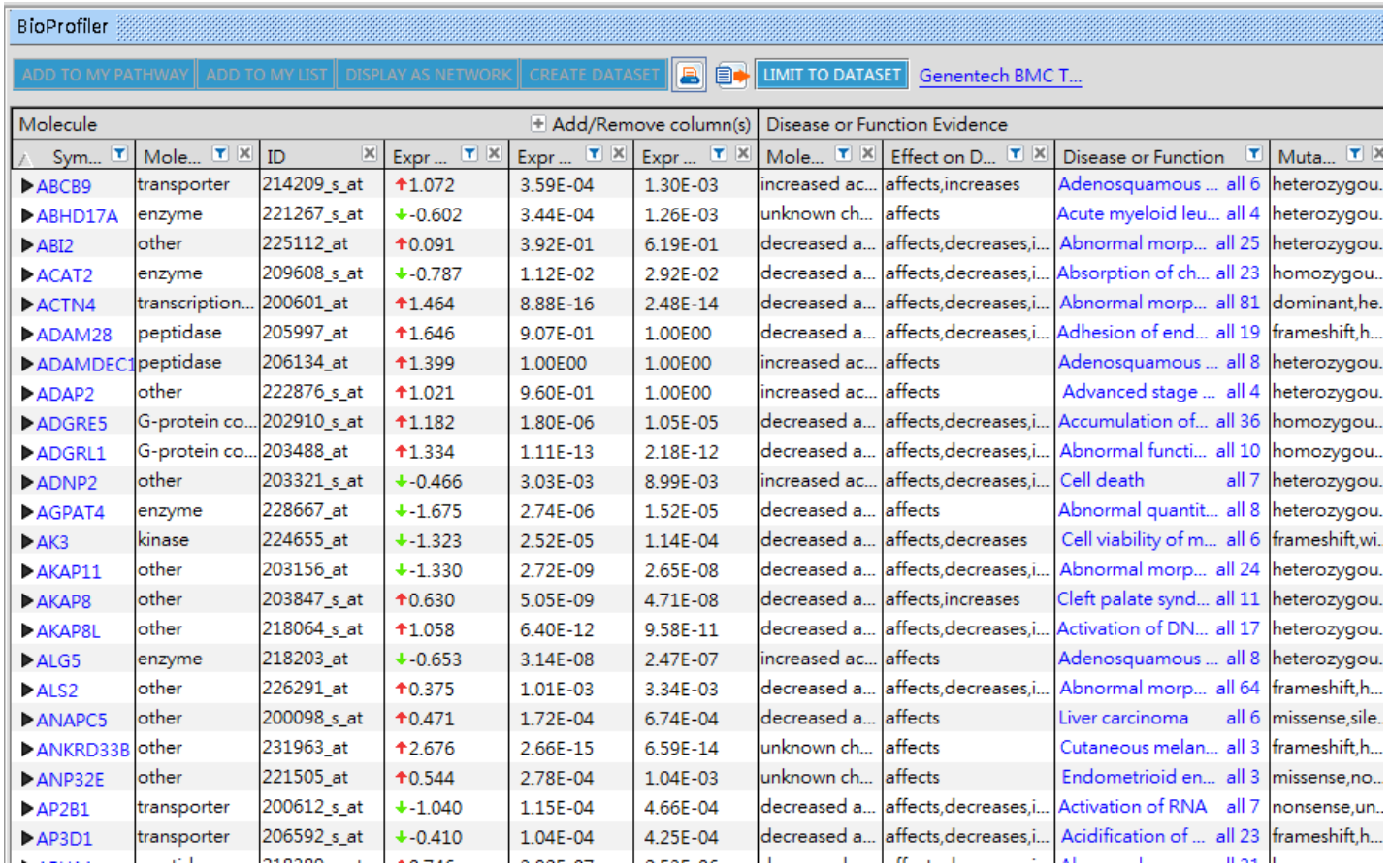
Specific function, disease or genes and chemicals that you wish to score against your causal networks.

Master Regulator and Networks

Master ...	Expr...	Mol...	Participating regulators	Depth	Pre...	Acti...	p-va...	CNR1 Length l...	CNR1 Path ...	Diabetes mellitus [diabetes] Length l...	Diabetes mellitus [diabetes] Path ...	Insulin Length l...	Insulin Path ...	Relationships Between M... Incr...	Relationships Between M... Decr...
ICMT		enzyme	ICMT ...all 1	1	Activated	2.000	1.31E-08	3 | 8	DU(3),	2 | 1	DU(1)	3 | 23	DD(2),		
G6PC		phosphatase	↑D-glucose, G6PC ...all 2	2	Activated	2.000	9.99E-03	2 | 12	DD(6),	3 | 51	DU(23),	1 | 1	DD(1)	GC... ...all 9	7alp.....all 8
afatinib		chemical drug	afatinib ...all 1	1		-1.667	8.47E-16	2 | 1	DU(1)	2 | 3	DU(2),	2 | 3	IU(3)		
UCP2		transporter	UCP2 ...all 1	1		-1.633	6.82E-13	2 | 7	DD(4),	1 | 1	IU(1)	1 | 1	DD(1)	sirol.....all 1	PLI... ...all 5
propylthiourac		chemical drug	propylthiouracil ...all 1	1		-1.134	8.83E-13	2 | 1	IU(1)	2 | 3	DU(3)	2 | 1	IU(1)		
sirolimus		chemical drug	sirolimus ...all 1	1		-1.265	3.23E-09	2 | 2	DU(2)	1 | 1	IU(1)	1 | 1	IU(1)		
methimazole		chemical drug	methimazole ...all 1	1		-1.342	2.62E-08	2 | 2	DU(1),	2 | 1	DU(1)	2 | 2	DU(1),		
probenecid		chemical drug	CASP1, DIO2, PANX1, ...all 7	3		0.816	1.23E-06	3 | 5	DU(5)	3 | 77	DU(36),	3 | 7	DU(2),		
HTT		transcription ...	HTT ...all 1	1		-0.447	2.05E-06	1 | 1	IU(1)	1 | 1	DU(1)	2 | 7	DD(2),		sirol.....all 2

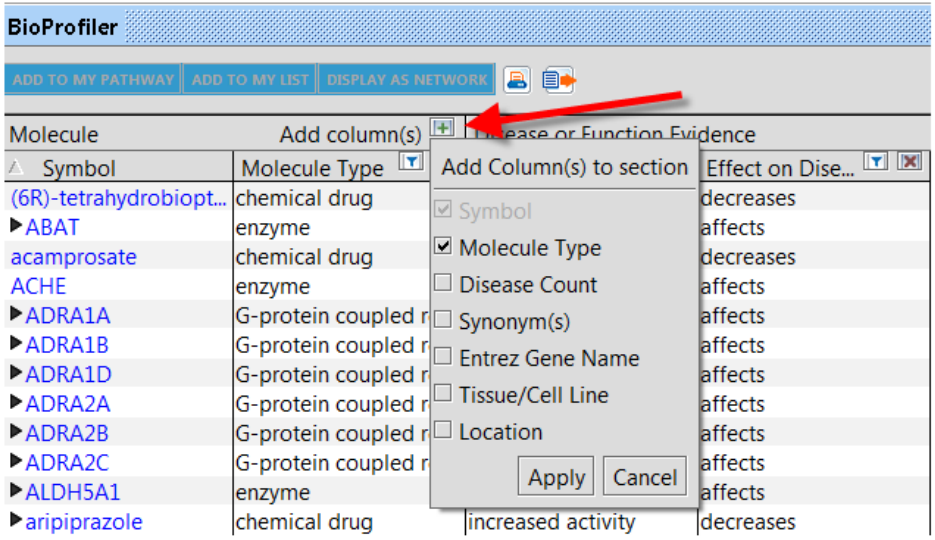


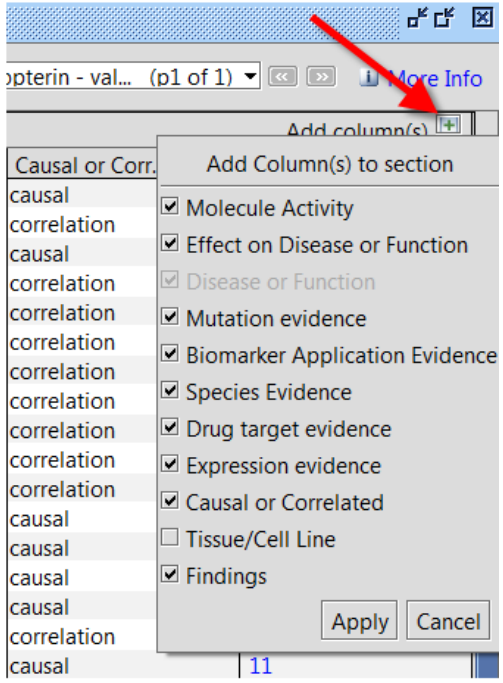
BioProfiler allows you to make novel discoveries by providing you the ability to filter the fine-grained relationships between molecules (genes, RNAs, proteins, and chemicals) and diseases or functions.



Sym...	Mole...	ID	Expr ...	Expr ...	Expr ...	Mole...	Effect on D...	Disease or Function	Muta...
ABCB9	transporter	214209_s_at	↑1.072	3.59E-04	1.30E-03	increased ac...	affects,increases	Adenosquamous ... all 6	heterozygou.
ABHD17A	enzyme	221267_s_at	↓-0.602	3.44E-04	1.26E-03	unknown ch...	affects	Acute myeloid leu... all 4	heterozygou.
ABI2	other	225112_at	↑0.091	3.92E-01	6.19E-01	decreased a...	affects,decreases,i...	Abnormal morp... all 25	heterozygou.
ACAT2	enzyme	209608_s_at	↓-0.787	1.12E-02	2.92E-02	decreased a...	affects,decreases,i...	Absorption of ch... all 23	homozygou..
ACTN4	transcription...	200601_at	↑1.464	8.88E-16	2.48E-14	decreased a...	affects,decreases,i...	Abnormal morp... all 81	dominant,he.
ADAM28	peptidase	205997_at	↑1.646	9.07E-01	1.00E00	decreased a...	affects,decreases,i...	Adhesion of end... all 19	frameshift,h...
ADAMDEC1	peptidase	206134_at	↑1.399	1.00E00	1.00E00	increased ac...	affects	Adenosquamous ... all 8	heterozygou.
ADAP2	other	222876_s_at	↑1.021	9.60E-01	1.00E00	increased ac...	affects	Advanced stage ... all 4	heterozygou.
ADGRE5	G-protein co...	202910_s_at	↑1.182	1.80E-06	1.05E-05	decreased a...	affects,decreases,i...	Accumulation of... all 36	homozygou..
ADGRL1	G-protein co...	203488_at	↑1.334	1.11E-13	2.18E-12	decreased a...	affects,decreases,i...	Abnormal functi... all 10	homozygou..
ADNP2	other	203321_s_at	↓-0.466	3.03E-03	8.99E-03	increased ac...	affects,decreases,i...	Cell death all 7	heterozygou.
AGPAT4	enzyme	228667_at	↓-1.675	2.74E-06	1.52E-05	decreased a...	affects	Abnormal quantit... all 8	heterozygou.
AK3	kinase	224655_at	↓-1.323	2.52E-05	1.14E-04	decreased a...	affects,decreases	Cell viability of m... all 6	frameshift,wi.
AKAP11	other	203156_at	↓-1.330	2.72E-09	2.65E-08	decreased a...	affects,decreases,i...	Abnormal morp... all 24	heterozygou.
AKAP8	other	203847_s_at	↑0.630	5.05E-09	4.71E-08	decreased a...	affects,increases	Cleft palate synd... all 11	heterozygou.
AKAP8L	other	218064_s_at	↑1.058	6.40E-12	9.58E-11	decreased a...	affects,decreases,i...	Activation of DN... all 17	heterozygou.
ALG5	enzyme	218203_at	↓-0.653	3.14E-08	2.47E-07	increased ac...	affects	Adenosquamous ... all 8	heterozygou.
ALS2	other	226291_at	↑0.375	1.01E-03	3.34E-03	decreased a...	affects,decreases,i...	Abnormal morp... all 64	frameshift,h...
ANAPC5	other	200098_s_at	↑0.471	1.72E-04	6.74E-04	decreased a...	affects	Liver carcinoma all 6	missense,sile.
ANKRD33B	other	231963_at	↑2.676	2.66E-15	6.59E-14	unknown ch...	affects	Cutaneous melan... all 3	frameshift,h...
ANP32E	other	221505_at	↑0.544	2.78E-04	1.04E-03	unknown ch...	affects	Endometrioid en... all 3	missense,no..
AP2B1	transporter	200612_s_at	↓-1.040	1.15E-04	4.66E-04	decreased a...	affects,decreases,i...	Activation of RNA all 7	nonsense,un..
AP3D1	transporter	206592_s_at	↓-0.410	1.04E-04	4.25E-04	decreased a...	affects,decreases,i...	Acidification of ... all 23	frameshift,h...

- Targets of toxicity: Which genes when [decreased] in activity [increase][liver cholestasis]? What types of [genetic] evidence support this?
- Target discovery: What [heterozygous knockouts] in [mouse] can [decrease] [asthma]?
- Which drugs or which targets have been in late stage clinical trials or approved to decrease [diabetes]?
- Biomarker research: Which genes are potential [diagnosis OR prognosis] biomarkers of [breast cancer] and are [upregulated] in breast cancer?





ABCD2

- Type: Transporter
- Location: Cytoplasm
- Tissue: Adipose, Liver
- Disease: Abnormal Conduction by Nerves
- Effect: Decrease

HBZ

- Type: Transporter
- Location: Cytoplasm
- Tissue: Liver, Lung
- Disease: Abnormal Conduction by Nerves
- Effect: Increase

ARTN

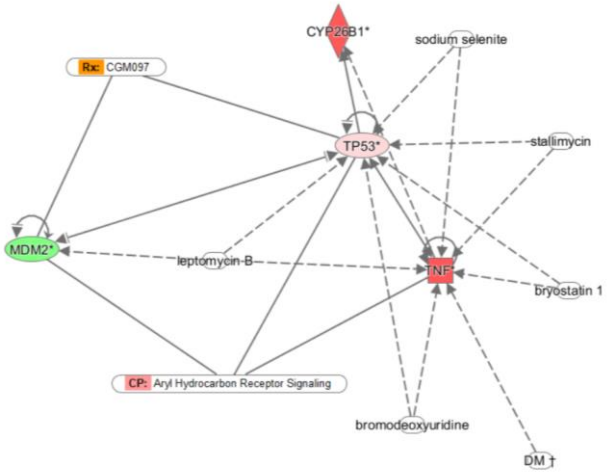
- Type: Growth Factor
- Location: Extracellular Space
- Tissue: Adrenal
- Disease: Abnormal Conduction by Nerves
- Effect: Decrease

- What kind of **Transporter** function in **Liver**?
  - ABCD2, HBZ
- Which genes **decrease** the expression of **Abnormal conduction by nerves**?
  - ABCD2, ARTN

Relationship Export

The new Relationship Export capability in IPA enables you to export the structural information contained within IPA networks or pathways for visualization in other tools such as Cytoscape.

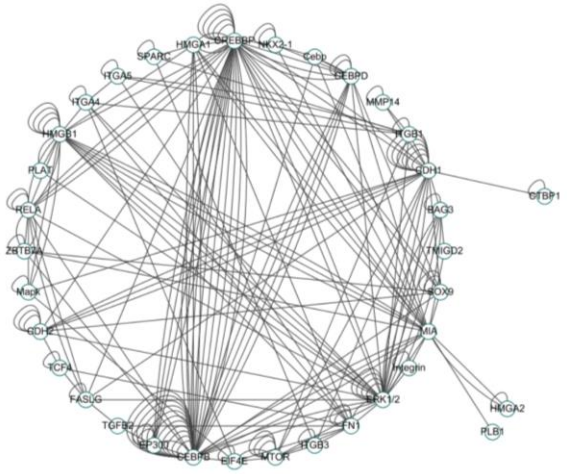
The export format contains relationships modeled as triples: Node A --> Relationship -->Node B



IPA Network

	A	B	C
1	From Molecule(s)	Relationship Type	To Molecule(s)
2	BAG3	activation	ERK1/2
3	BAG3	phosphorylation	ERK1/2
4	BAG3	protein-protein interaction	BAG3
5	BAG3	protein-protein interaction	ERK1/2
6	BAG3	regulation of binding	ERK1/2
7	CDH1	activation	ERK1/2
8	CDH1	activation	Mapk
9	CDH1	expression	CDH1
10	CDH1	expression	CDH2
11	CDH1	expression	ERK1/2
12	CDH1	expression	ITGB3
13	CDH1	expression	MMP14
14	CDH1	expression	MTOR
15	CDH1	expression	SOX9
16	CDH1	phosphorylation	ERK1/2
17	CDH1	protein-protein interaction	BAG3
18	CDH1	protein-protein interaction	CDH1
19	CDH1	protein-protein interaction	CDH2
20	CDH1	protein-protein interaction	ITGB1
21	CDH1	regulation of binding	CDH1

Relationship Export

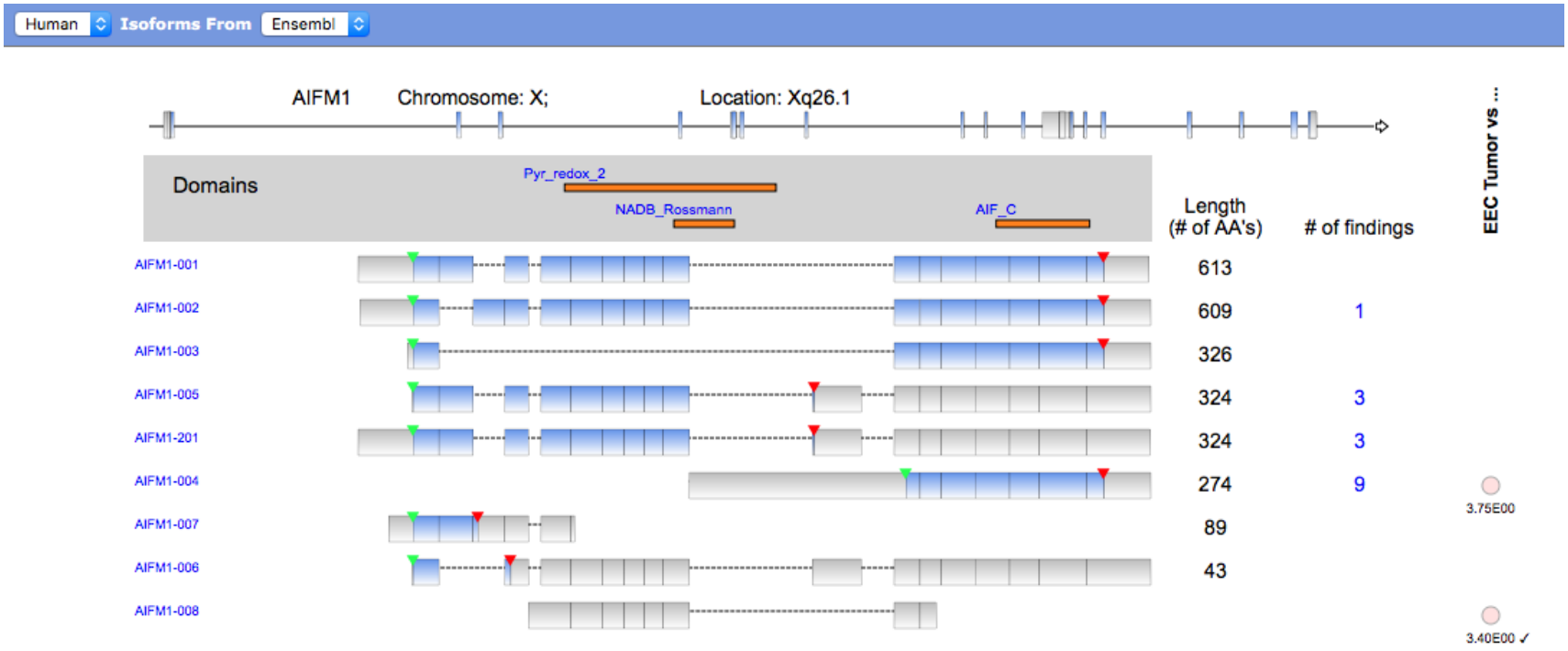


Cytoscape

IsoProfiler displays and enables filtering on transcripts & isoforms in your RNA-seq dataset(s)

Your data must be mapped using **RefSeq**, **Ensembl**, or **UCSC** identifiers. You cannot use gene names or gene-level IDs to map your dataset for IsoProfiler, you must use **transcript** IDs.

Furthermore, for IsoProfiler your dataset must consist of a single source for your dataset-- i.e. only Ensembl, or only RefSeq. **You cannot mix sources**.

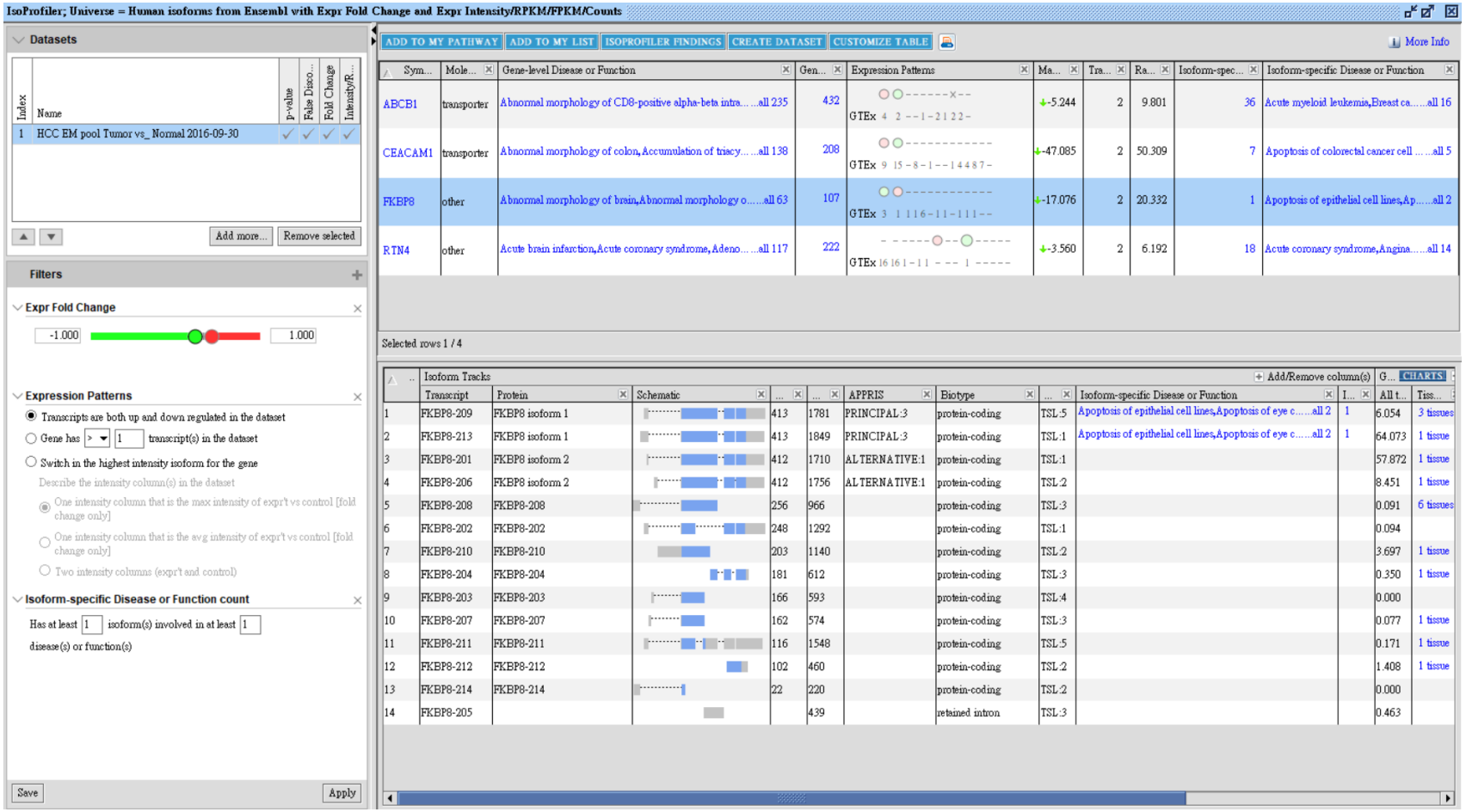


Ensembl ID with isoform viewer in IPA

Once IsoProfiler launches, it will display results such as in this example

Dataset Chooser

Genes and their isoforms from the dataset

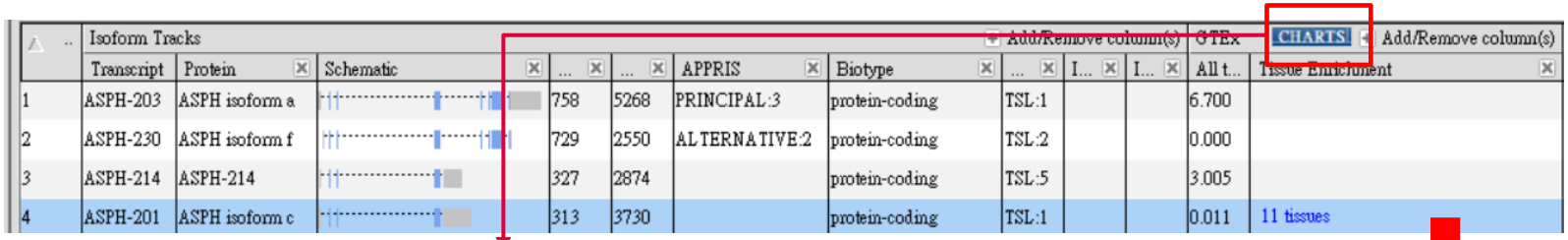


Isoform filters

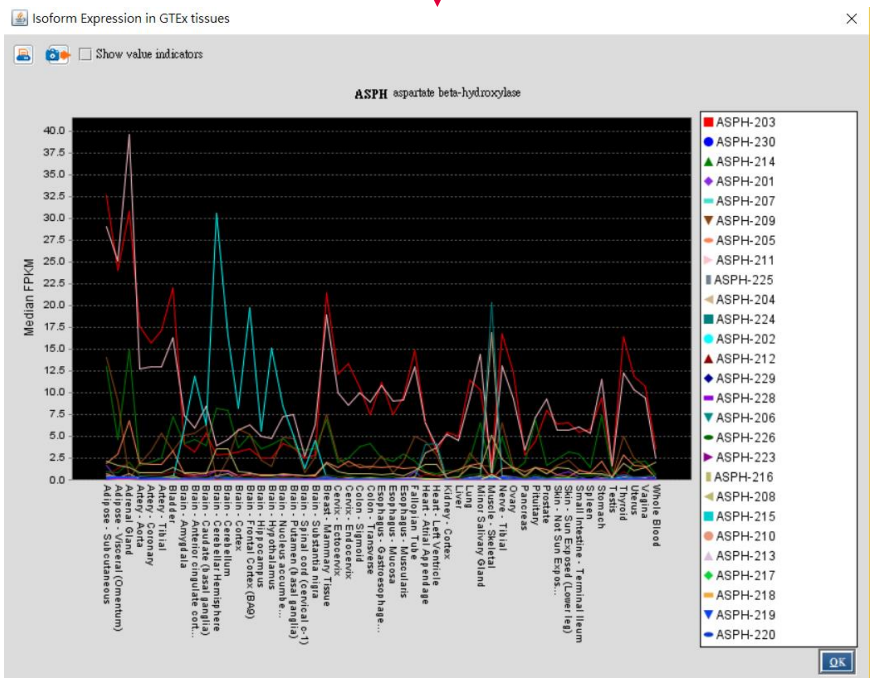
Isoform details on the selected gene

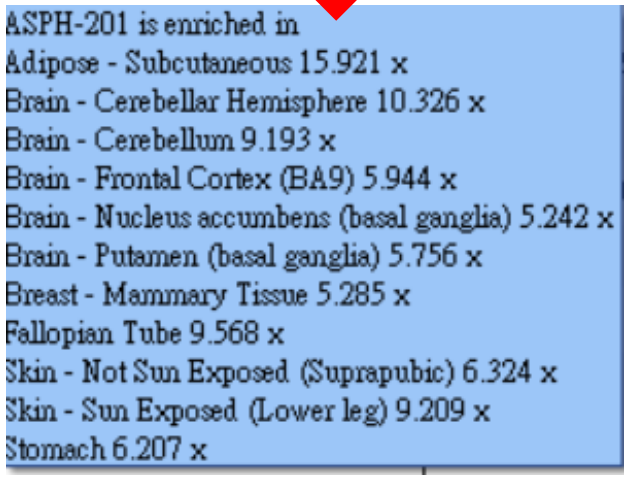
IsoProfiler with **GTEx Tissue Expression**

If your dataset is based on human expression data, additional functionality appears in IsoProfiler to help you explore tissue expression information from the **GTEx consortium**, which profiled **51 tissues** from multiple human tissue donors by RNA-seq.



	Transcript	Protein	Schematic	...	...	APPRIS	Biotype	...	I...	I...	All t...	Tissue Enrichment
1	ASPH-203	ASPH isoform a		758	5268	PRINCIPAL:3	protein-coding	TSL:1			6.700	
2	ASPH-230	ASPH isoform f		729	2550	ALTERNATIVE:2	protein-coding	TSL:2			0.000	
3	ASPH-214	ASPH-214		327	2874		protein-coding	TSL:5			3.005	
4	ASPH-201	ASPH isoform c		313	3730		protein-coding	TSL:1			0.011	11 tissues

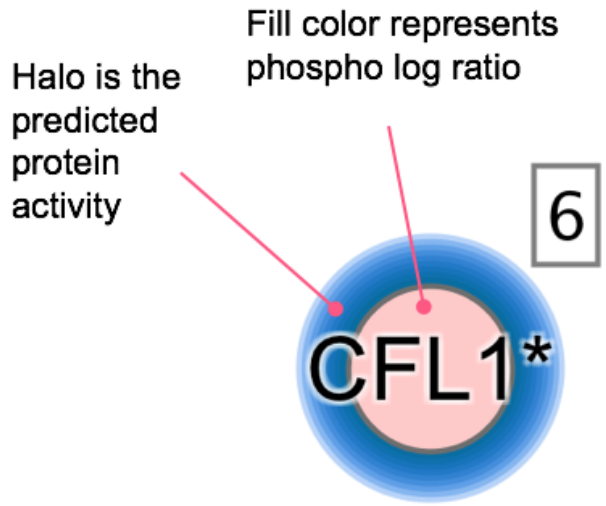




The GTEx consortium has performed over 9,800 paired-end RNA-Seq experiments across 51 human tissues

Find out how to understand your **Phosphoproteomics** Analysis and about the multiple ways of relating the molecules in your dataset to the body of information in the Ingenuity Knowledge Base.

Halos indicate the protein's activity when it is *opposite* of the direction of phosphorylation

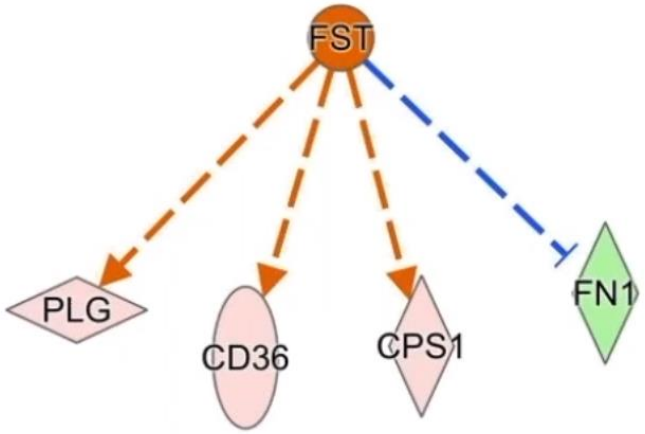


CFL1 has *increased* phosphorylation in dataset but prediction of *decreased* protein activity

GSK3B has *decreased* phosphorylation in dataset but prediction of *increased* protein activity

Gene expression data:

FST causes the up-or-down-regulation of target genes

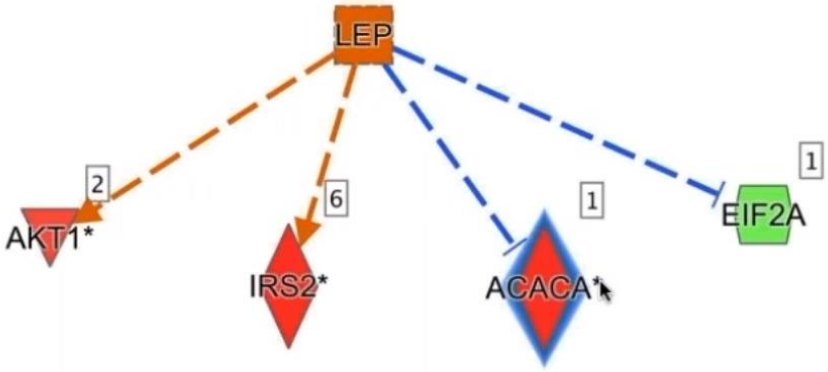


PLG, CD36, CPS1 are *up-regulated* by *activated* PST

FN1 is *down-regulated* by *activated* PST

Phosphorylation data:

LEP causes the up-or-down phosphorylation of target proteins



AKT1, IRS2 are *activated* by *increased* phosphorylation from *activated* LEP

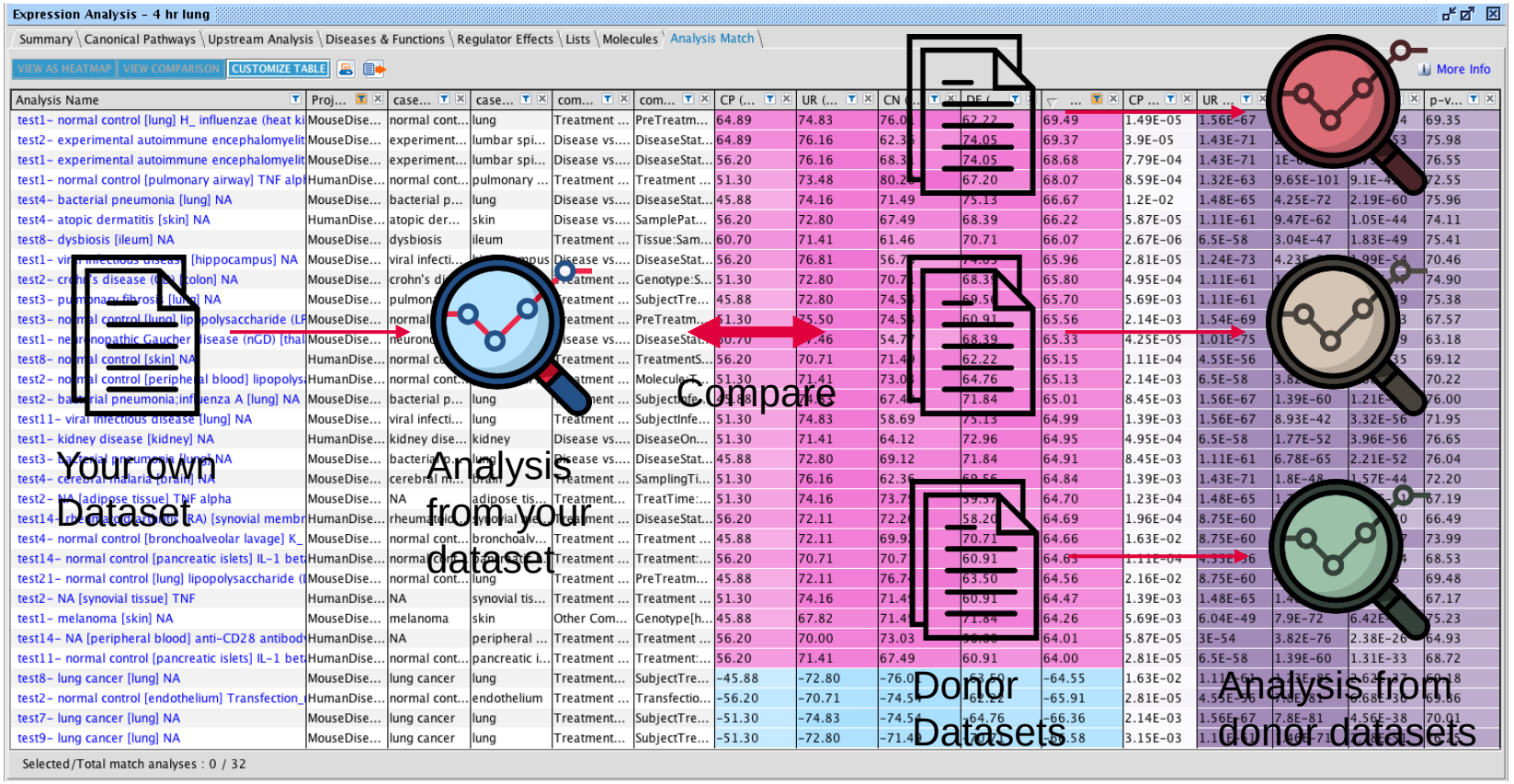
EIF2A's activity is *inhibited* by *decreased* phosphorylation from *activated* LEP

ACACA has *increased* phosphorylation from *activated* LEP

The blue halo indicates that phosphorylation cause *inhibition* of ACACA's activity

Analysis Match

Automatically discover other IPA Core Analyses with **similar (or opposite) biological results** as compared to yours, to help confirm your interpretation of the results or to provide unexpected insights into underlying shared biological mechanisms.



A. Data Upload and How to Run a Core Analysis
上傳實驗資料並使用IPA分析功能

B. Functional Interpretation in IPA
IPA分析結果介紹
Hands-on Exercises

C. Multi-Omics Analysis using IPA

Integrate and compare genomics, transcriptomics, proteomics and metabolomics data to see the big picture on your focus research

比較分析結果的差異

D. Q&A

Single Experiment

- Time Course
- Dose Response

Multi Experiment

- System biology
- Combining SNP, CNA, mRNA, microRNA, proteomics, etc

Set Analysis

- Exploring Common Molecules across one or more experiment (s)

Bringing together multiple types of genomic data

Research AIM:

- To attain a systems biology understanding of your research by bringing multiple types of genomic data together (SNP, CNA, mRNA, microRNA, proteomics, etc.).

Challenge:

- Data types measured different molecular status in experiment
- Too much data, some data types may have extra ‘noise’(i.e. arrays)
- Venn Diagram-type comparison excludes ‘A affects B’ information

Solution:

- Identify phenotypes, disease associations, and pathways that are common themes for multiple data types using Comparison Analysis
- Interactive pathways overlay multiple data types and find genes up or down-stream that change in the various data types.
- Pathway tools find regulatory connections between molecules of interest and the various data types
- microRNA Target Filter can link microRNAs and targets from miRNA and target data sets

How do you integrate multiple data types now?

Single Experiment

- Time Course
- Dose Response

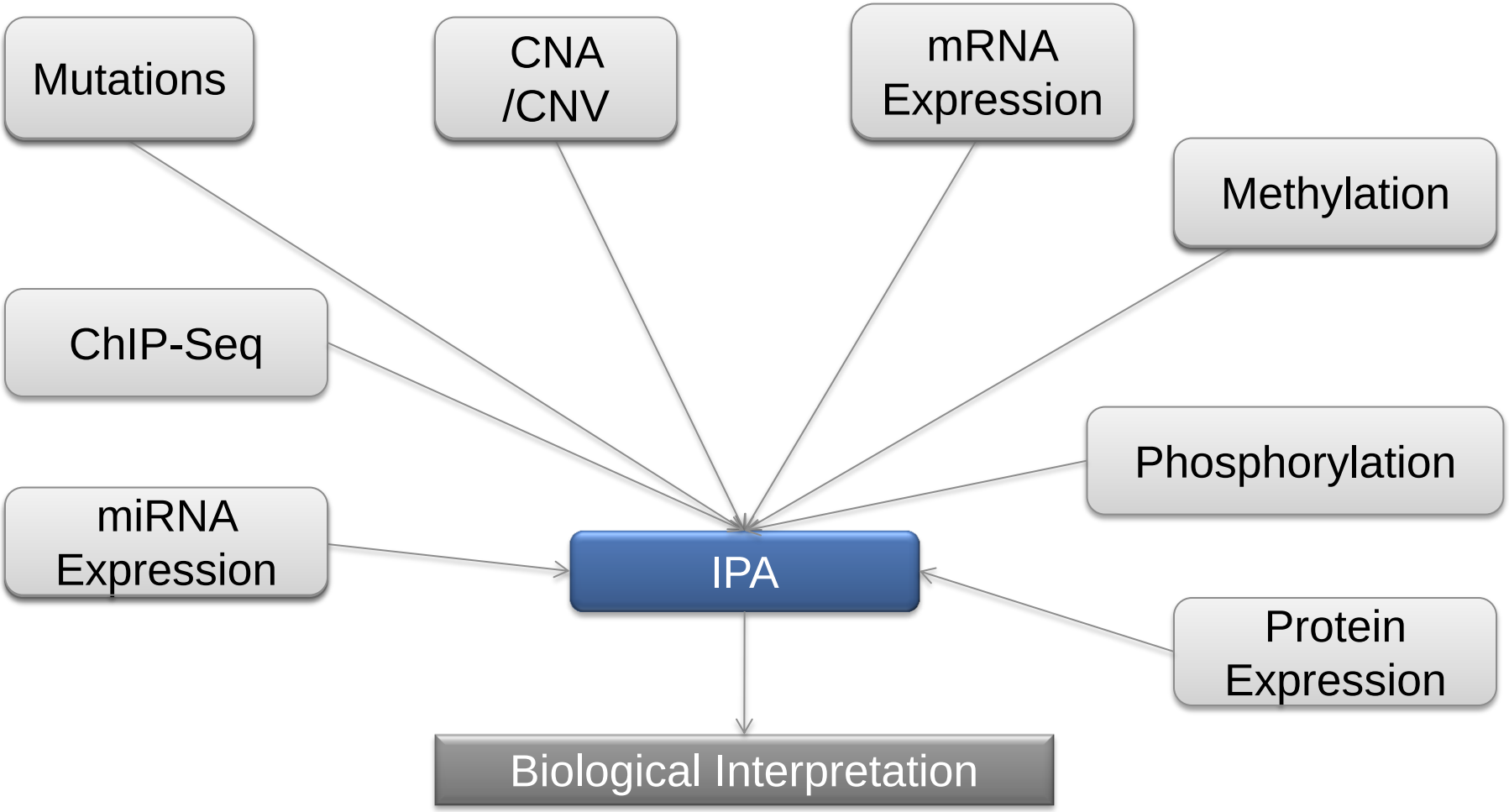
Multi Experiment

- System biology
- Combining SNP, CNA, mRNA, microRNA, proteomics, etc.

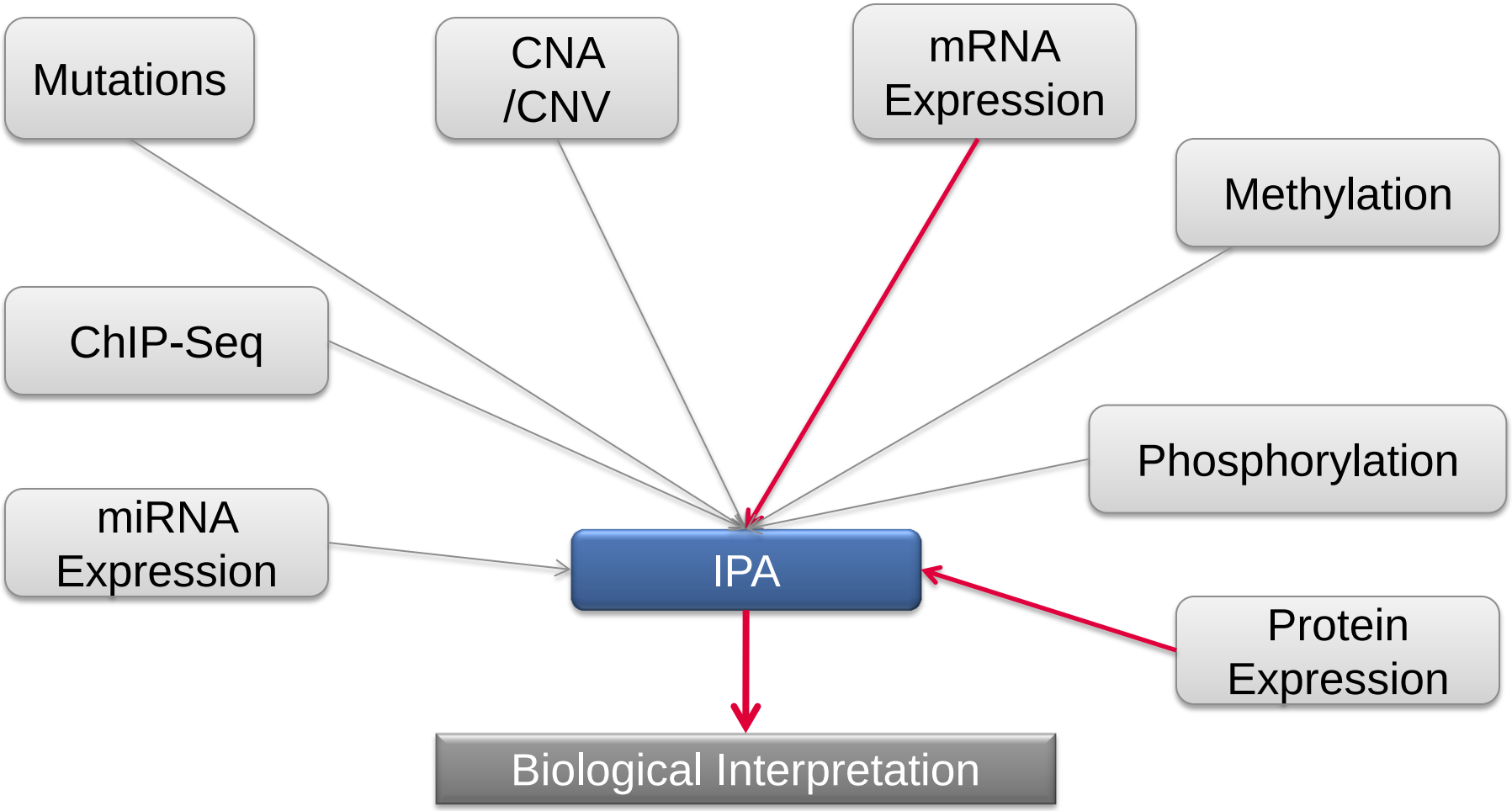
Set Analysis

- Exploring Common Molecules across one or more experiment (s)

IPA: A Point of Data Integration

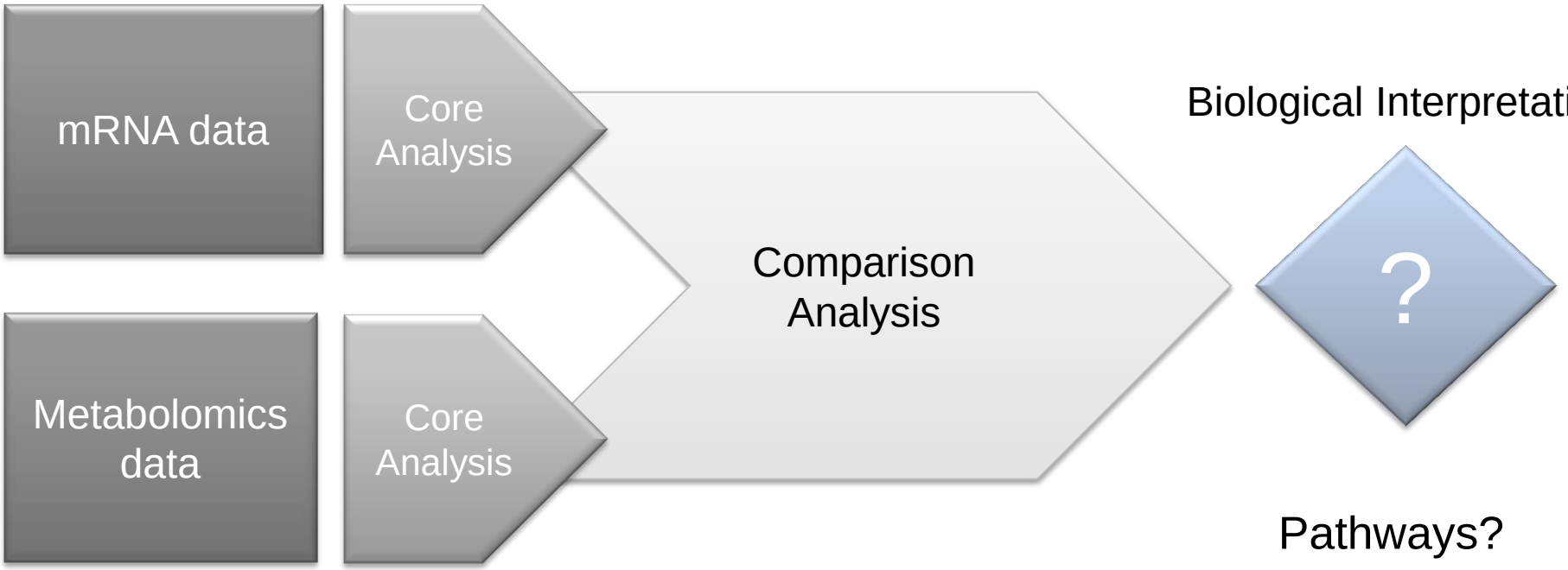


IPA: A Point of Data Integration



What do you want out of this comparison?

Review your workflow – What are your goals?



Single Experiment

- Time Course
- Dose Response

Multi Experiment

- System biology
- Combining SNP, CNA, mRNA, microRNA, proteomics, etc

Set Analysis

- Exploring Common Molecules across one or more experiment (s)

IPA Analysis of Metabolomics Data Including Cross-Platform Integration with Transcriptomics Data from a Diabetic Mouse Model

Integration of metabolomics and transcriptomics data to aid biomarker discovery in type 2 diabetes

Susan C. Connor,†*[a] Michael K. Hansen,‡[b] Adam Corner,[c] Randall F. Smith[d] and Terence E. Ryan‡[b]

Received 15th July 2009, Accepted 4th December 2009
First published as an Advance Article on the web 23rd March 2010
DOI: 10.1039/b914182k

db/db diabetic mouse model

urine

liver
adipose
muscle

Metabolomics result

Transcriptomic result

IPA analysis (biomarker)

Goal:

- providing novel biomarkers of T2D and drug efficacy

Challenge:

- difficult to determine the precise pathophysiology in individual T2D patients

Solution:

- Integrate metabolomics of study data from other platforms, such as transcriptomics, thus linking known metabolites and genes to relevant biochemical pathways.

Outcome:

- These biomarkers may be useful by identifying patient populations that share common disease and may respond better to a particular class of anti-diabetic drugs.

Input dataset

- Observation 1 : urinary NMR-based metabolomics data
- Observation 2 : liver transcriptomic data
- Observation 3 : adipose transcriptomic data
- Observation 4 : muscle transcriptomic data

metabolomics data

ID	Symbol	Fold Chang	p-value
C00671	(3S)-3-methyl-2-oxopentanoate	-1.344	0.00E+00
C01089	(R)-3-hydroxybutyric acid	-1.311	0.00E+00
C05984	2-hydroxybutyric acid	-1.344	0.00E+00
C00322	2-oxoadipic acid	2	
C00026	2-oxoglutaric acid	2.031	0.00E+00
C01468	4-cresol	1.64	0.00E+00
C00642	4-hydroxyphenylacetic acid	1.002	0.00E+00
C00033	acetic acid	1.226	1.83E-02
C02571	acetyl-L-carnitine	-1.011	0.00E+00
C00212	adenosine	-1.066	0.00E+00
C01551	allantoin	1.081	0.00E+00
C00233	alpha-ketoisocaproic acid	-1.371	0.00E+00
C00141	alpha-ketoisovaleric acid	-1.559	0.00E+00
C00417	cis-aconitic acid	1.208	0.00E+00
C00158	citric acid	1.262	0.00E+00
C00327	citrulline	2	
C00300	creatine	-1.33	0.00E+00
C00791	creatinine	-1.281	6.00E-06
C00879	D-galactaric acid	2.896	0.00E+00
C00124	D-galactose	3.183	0.00E+00
C00031	D-glucose	10.945	0.00E+00

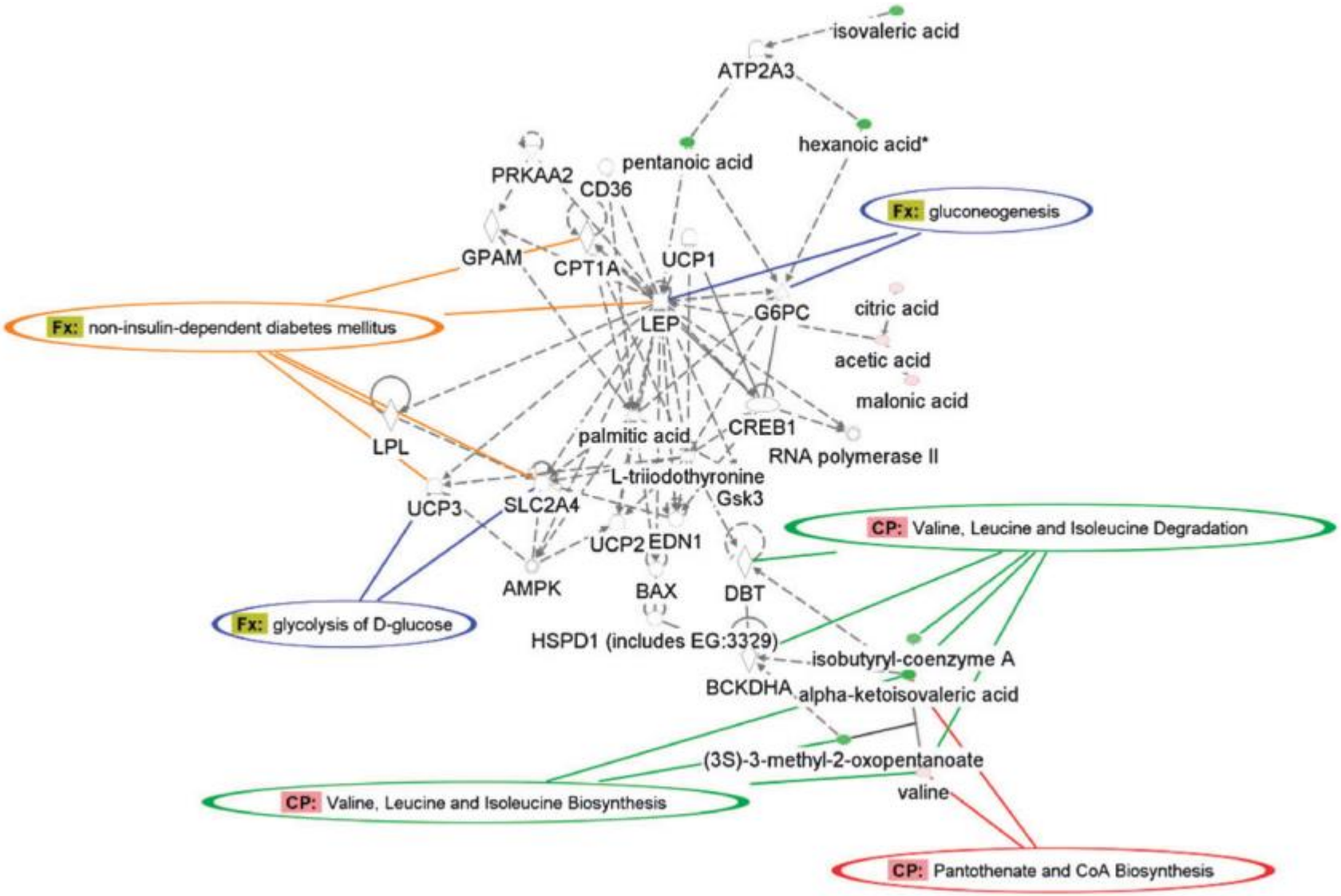
+

transcriptomic data

ID	Fold Change	p-value
C00671	-1.344	0.00E+00
C01089	-1.311	0.00E+00
1418083_at	-2	3.83E-02
1433936_at	-2	2.86E-03
1434484_at	2	1.86E-02
1453238_s_at	2	8.93E-05
1435137_s_at	2	3.06E-06
1427932_s_at	2	9.77E-08
1430989_a_at	-2	3.71E-02
1448038_at	-2	3.91E-02
1424365_at	-2	2.54E-07
C05984	-1.344	0.00E+00
C00322	2	0.00E+00
C00026	2.031	0.00E+00
1429115_at	-2	4.59E-02
1435522_a_at	-2	1.86E-08
1428083_at	-2	1.06E-09
1455207_at	-2	2.75E-04
1420269_at	2	5.55E-05
1423357_at	-2	2.68E-03
1453207_at	-2	4.41E-03

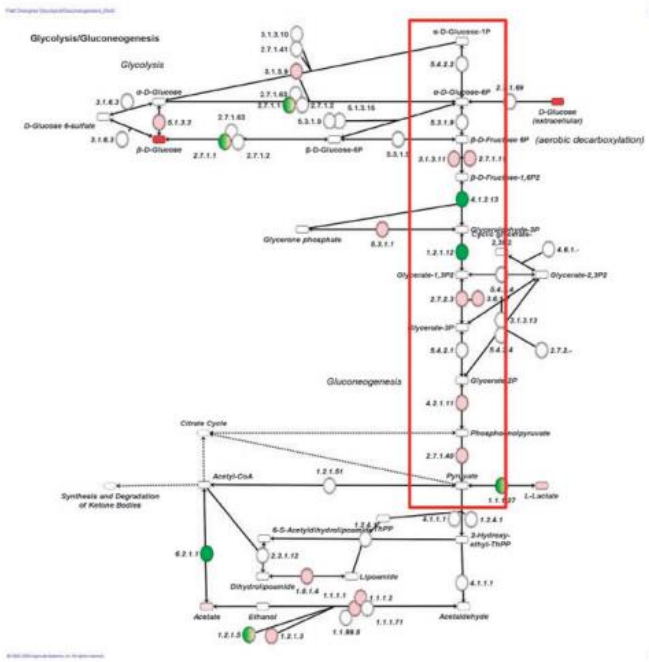
ID	Fold Chang	p-value
C05270	-1.49	0.00E+00
C00888	-1.49	0.00E+00
C00026	2.031	0.00E+00
C00233	-1.371	0.00E+00
C00141	-1.559	0.00E+00
C01089	-1.311	0.00E+00
C11457	-1.838	0.00E+00
C02642	-1.072	0.00E+00
C02571	-1.011	0.00E+00
C00212	-1.066	0.00E+00
C00041	1.119	0.00E+00
C01551	1.081	0.00E+00
C05984	-1.344	0.00E+00
C01585	-1.49	0.00E+00
C00803	-1.49	0.00E+00
C00671	-1.344	0.00E+00
C00417	1.208	0.00E+00
C00158	1.262	0.00E+00
C00300	-1.33	0.00E+00
C00543	-1.12	0.00E+00
C00346	1.248	0.00E+00

ID	Fold Change	p-value
C00671	-1.344	0.00E+00
C01089	-1.311	0.00E+00
1452730_at	2	2.60E-03
1436187_at	-2	2.94E-12
1424722_at	-2	3.67E-02
1456546_at	-2	1.13E-02
1455692_x_	-2	1.11E-02
1448038_at	-2	4.14E-04
1451588_at	-2	6.20E-27
1424365_at	2	1.04E-04
1436339_at	2	2.61E-12
C05984	-1.344	0.00E+00
C00322	2	
C00026	2.031	0.00E+00
1435524_at	-2	7.42E-06
1452170_at	2	6.82E-08
1424968_at	-2	1.94E-06
1433530_at	-2	1.46E-03
1428500_at	-2	4.00E-05
1439962_at	-2	5.34E-05
1435522_a_	2	2.54E-03

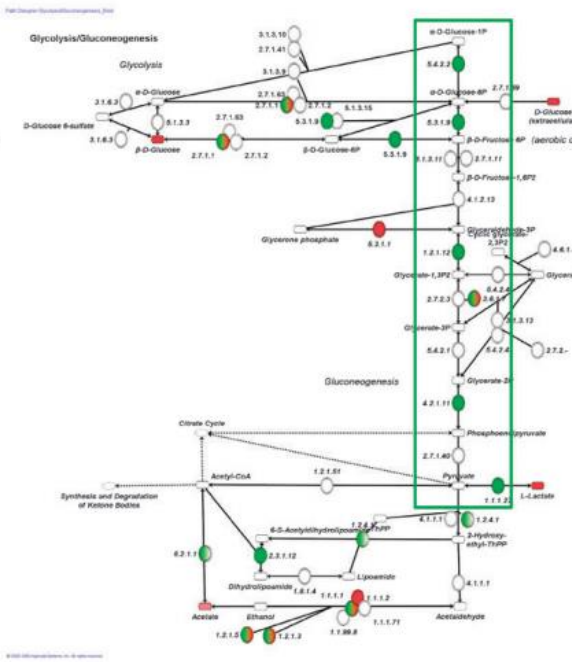


Network describing lipid metabolism, small molecule biochemistry and transport

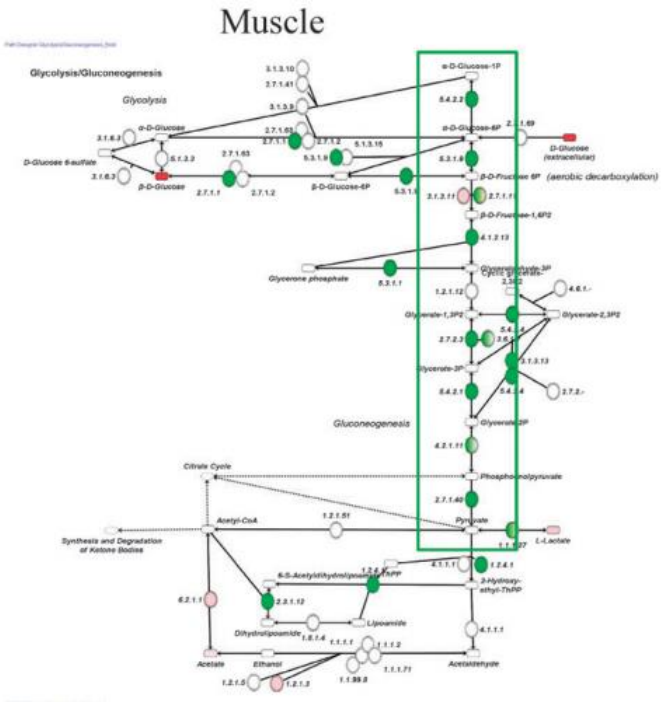
Liver



Adipose



Muscle



Gluconeogenesis pathway mapping metabolomics and transcriptomics results

Result:

- Several biomarkers (e.g., BCAAs, nicotinamide metabolites, pantothenic acid) have not previously been suggested as possible biomarkers for diabetes.

- metabolomics highlighted at least 24 distinct pathways that distinguish diabetic and control mice. The pathways most affected were amino acid, amino group metabolism and the urea cycle. Also affected were fatty acid biosynthesis, degradation and transport, DNA and protein synthesis changes in urinary protein, MUP and NAG, energy metabolism, and steroid hormone synthesis.

Live Demo

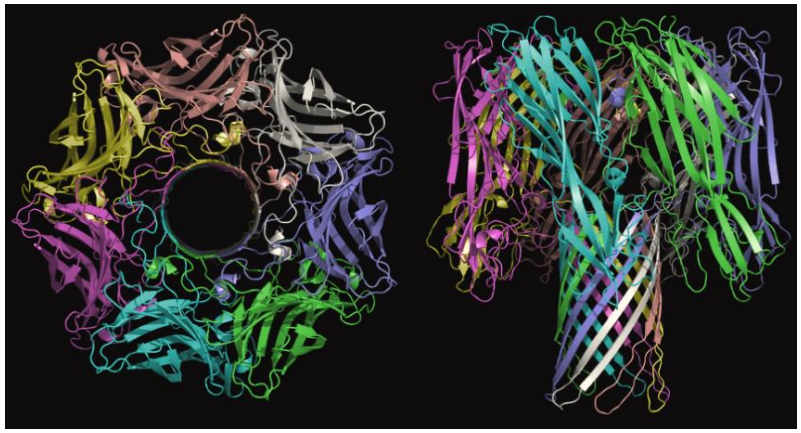
RESEARCH ARTICLE

A Multi-Omics Approach Identifies Key Hubs Associated with Cell Type-Specific Responses of Airway Epithelial Cells to Staphylococcal Alpha-Toxin

Erik Richter[1], Manuela Harms[1], Katharina Ventz[1], Philipp Gierok[2], Ravi Kumar Chilukoti[3], Jan-Peter Hildebrandt[4], Jörg Mostertz[1], Falko Hochgräfe[1]*

Staphylococcus aureus

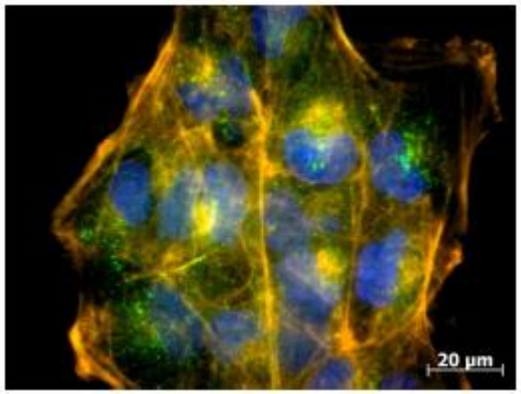
secret



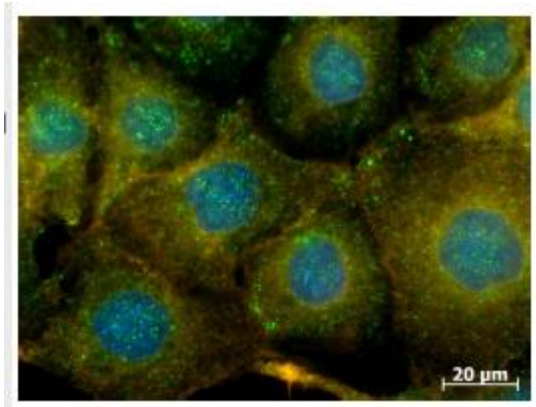
Alpha-toxin, or alpha-hemolysin (Hla)

ADAM10

Hla-resistant S9 cells



Hla-susceptible 16HBE14o-



Phosphoproteomics

S9 Alpha toxin treatment

Transcriptomics

Goal:

- Better understanding of Hla-induced cellular programs

Challenge:

- Diverse cellular reactions of Hla-treatment, difficult to defined cell-specific responses

Solution:

- Integrate phosphoproteomics and transcriptomics data, thus linking known metabolites and genes to revealed a substantial impact on phosphorylation-dependent signaling and the interaction between gene expression

Outcome:

- Revealed a substantial in both cell models and highlights alterations in signaling pathways associated with contacts

Input dataset

- Observation 1 : S9 alpha-toxin treatment phosphoproteomics data
- Observation 2 : S9 alpha-toxin treatment transcriptomic data

Phosphoproteomics data + transcriptomic data

First Hit ID	PhospAlpha
Q9UNL2	5.0485912
P49207	5.0406868
O95747	3.5411981
Q14573	3.5139138
Q9NZQ3	3.2174572
P04792	3.2136851
Q13085	3.1679433
Q9Y580	3.1565325
P04792	3.155894
P46779	3.0451337
P57678	3.0223869
Q8NC51	3.0177892
P17858	2.9980667
A6ND36	2.7129297
Q96N67	2.6753043
O94874	2.6740569
P29966	2.6586675
Q9NS69	2.6523421
Q8ND04	2.5458685
Q14573	2.4490019
Q8NDI1	2.444508

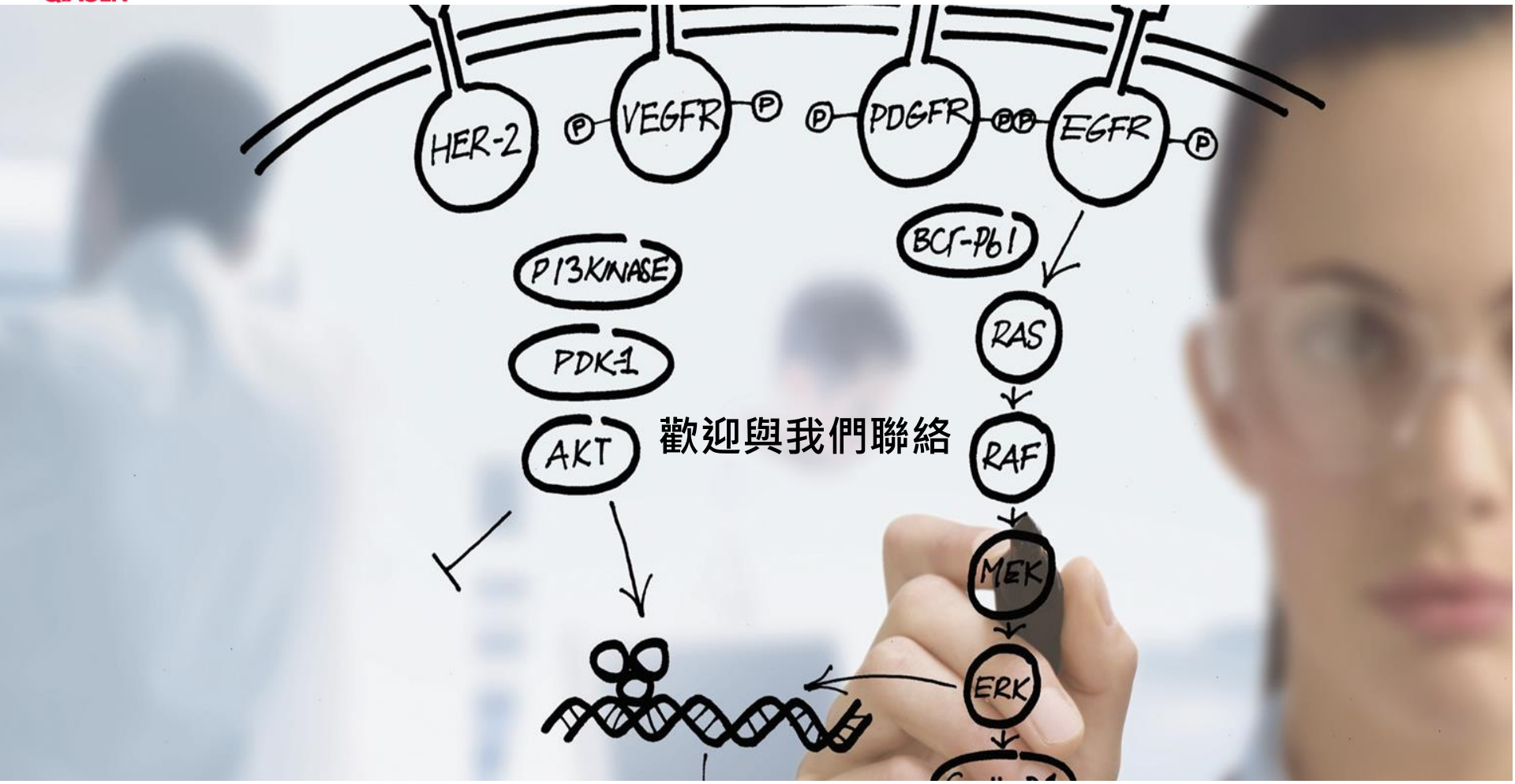
Affy ID	Gene Name	Exp Fold Change [S9 vs. HLA
8055952	NR4A2	7.99979
7975779	FOS	6.99416
8131803	IL6	4.07129
8115831	DUSP1	3.85729
8108370	EGR1	3.82366
8095680	IL8	3.33594
7938390	ADM	3.31279
8069676	ADAMTS1	3.18191
8163002	KLF4	2.84788
8029693	FOSB	2.75585
7909610	ATF3	2.73689
8083594	PTX3	2.68894
8148304	TRIB1	2.68555
7955589	NR4A1	2.66317
8129677	SGK1	2.61712
8148317	MYC	2.52871
7965335	DUSP6	2.44648
8156848	NR4A3	2.33387
7926677		2.3149
8030128	PPP1R15A	2.19422
8100994	CXCL2	2.1804

Result:

- Several biomarkers (e.g., BCAAs, nicotinamide metabolites, pantothenic acid) have not previously been suggested as possible biomarkers for diabetes.

- metabolomics highlighted at least 24 distinct pathways that distinguish diabetic and control mice. The pathways most affected were amino acid, amino group metabolism and the urea cycle. Also affected were fatty acid biosynthesis, degradation and transport, DNA and protein synthesis changes in urinary protein, MUP and NAG, energy metabolism, and steroid hormone synthesis.

Live Demo



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Sample to Insight