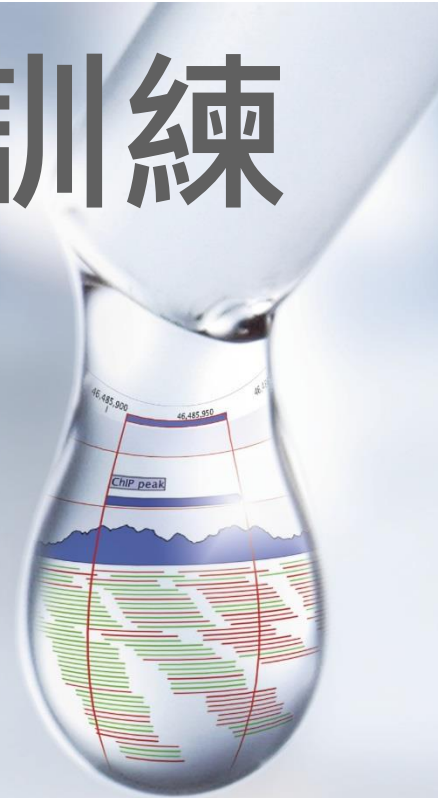# IPA進階模組教育訓練

多實驗組別等IPA分析方式及結果意義闡釋

Clair Tsai 蔡宜庭

創源生技 業務主任

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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.
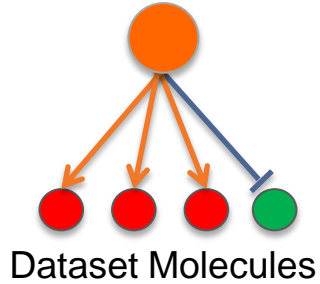
- Significant p-value ≤ 0.05

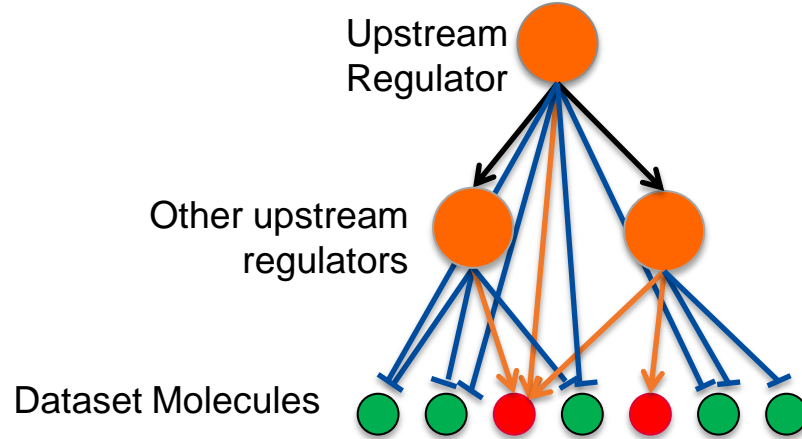Note: Benjamini-Hochberg correction for multiple testing can be implemented in some cases

Reference Set

Dataset

Pathway
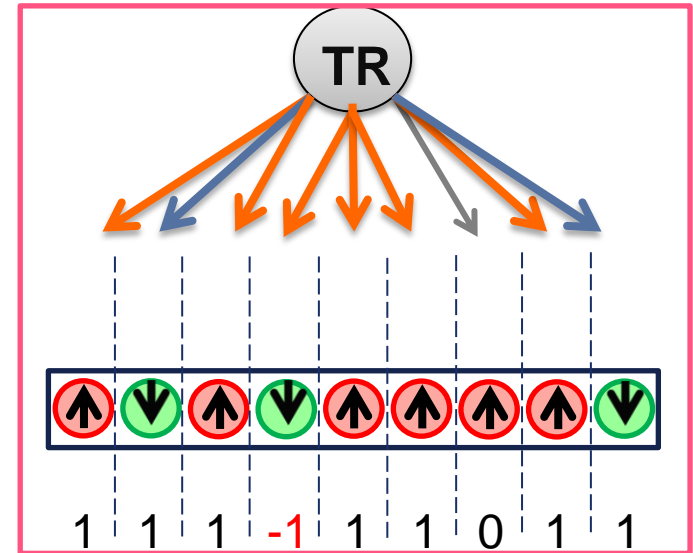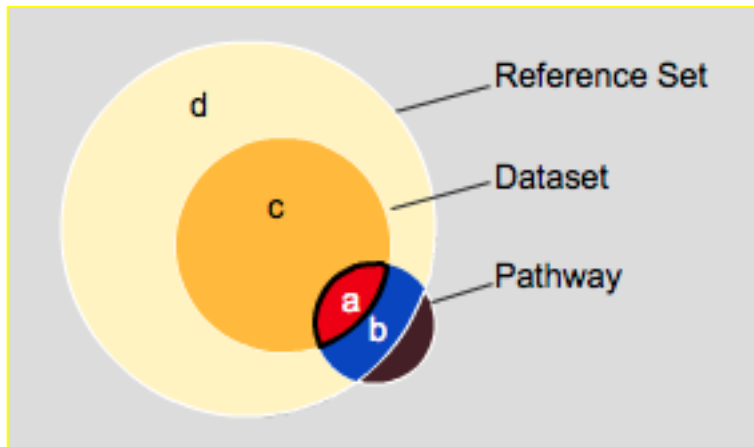
z-score

- Predicts Activation or Inhibition

- Correlation between what is known (IPA Knowledge Base) and your expression data

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Upstream Analysis

Dataset Molecules

Mechanistic Network of Upstream Regulators

Upstream Regulator

Other upstream regulators

Dataset Molecules

Function Analysis

Dataset Molecules

Diseases / functions
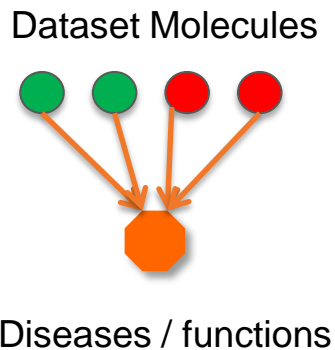
Regulator Effect Network

Any

Diseases / functions

Interaction Network

Dataset Molecules

Summary: 將顯示前五名各頁籤之分析結果

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

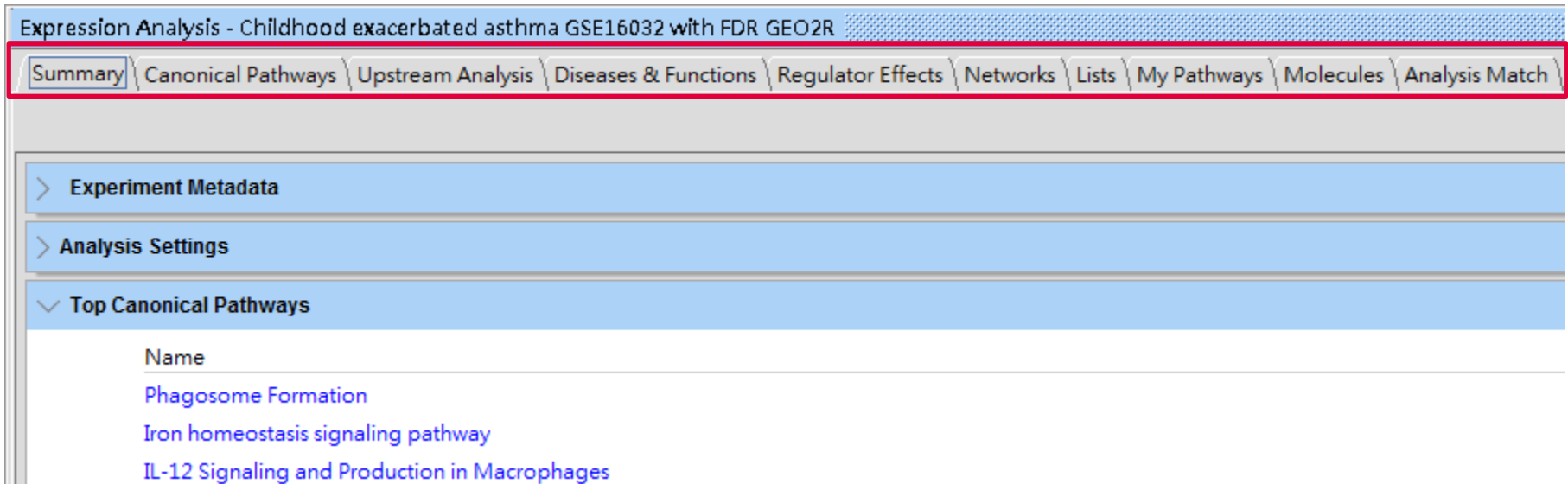**Upstream Analysis: 列出與資料中變動分子有關的Upstream molecules，以及根據研究文獻預測它們是否是被啟動或是被抑制。**

Disease&Function : 了解實驗結果在各分析疾病調控上之結果

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。

Regulator effect:將上下游之調控路徑整合

Analysis Match:比對外部實驗組資訊與實驗之調控相似相異性

Expression Analysis - Childhood exacerbated asthma GSE16032 with FDR GEO2R

Summary \ Canonical Pathways \ Upstream Analysis \ Diseases & Functions \ Regulator Effects \ Networks \ Lists \ My Pathways \ Molecules \ Analysis Match \

Experiment Metadata

Analysis Settings

Top Canonical Pathways

Name

Phagosome Formation

Iron homeostasis signaling pathway

IL-12 Signaling and Production in Macrophages

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

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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)**

Click to show bar chart

Summary: 將顯示前五名各頁籤之分析結果

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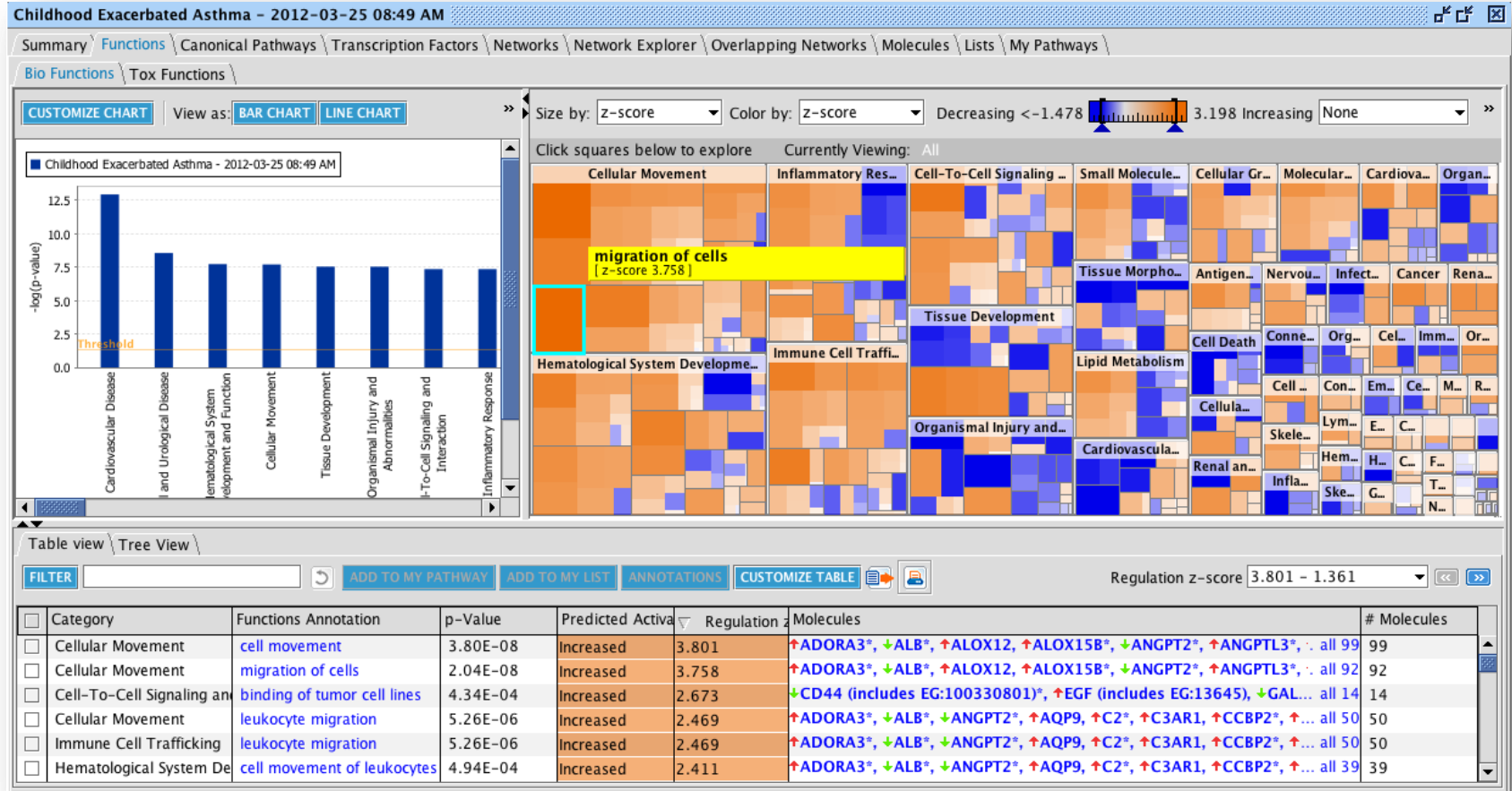Expression Analysis - Childhood exacerbated asthma GSE16032 with FDR GEO2R

Summary | Canonical Pathways | Upstream Analysis | Diseases & Functions | Regulator Effects | Networks | Lists | My Pathways | Molecules | Analysis Match

> Experiment Metadata
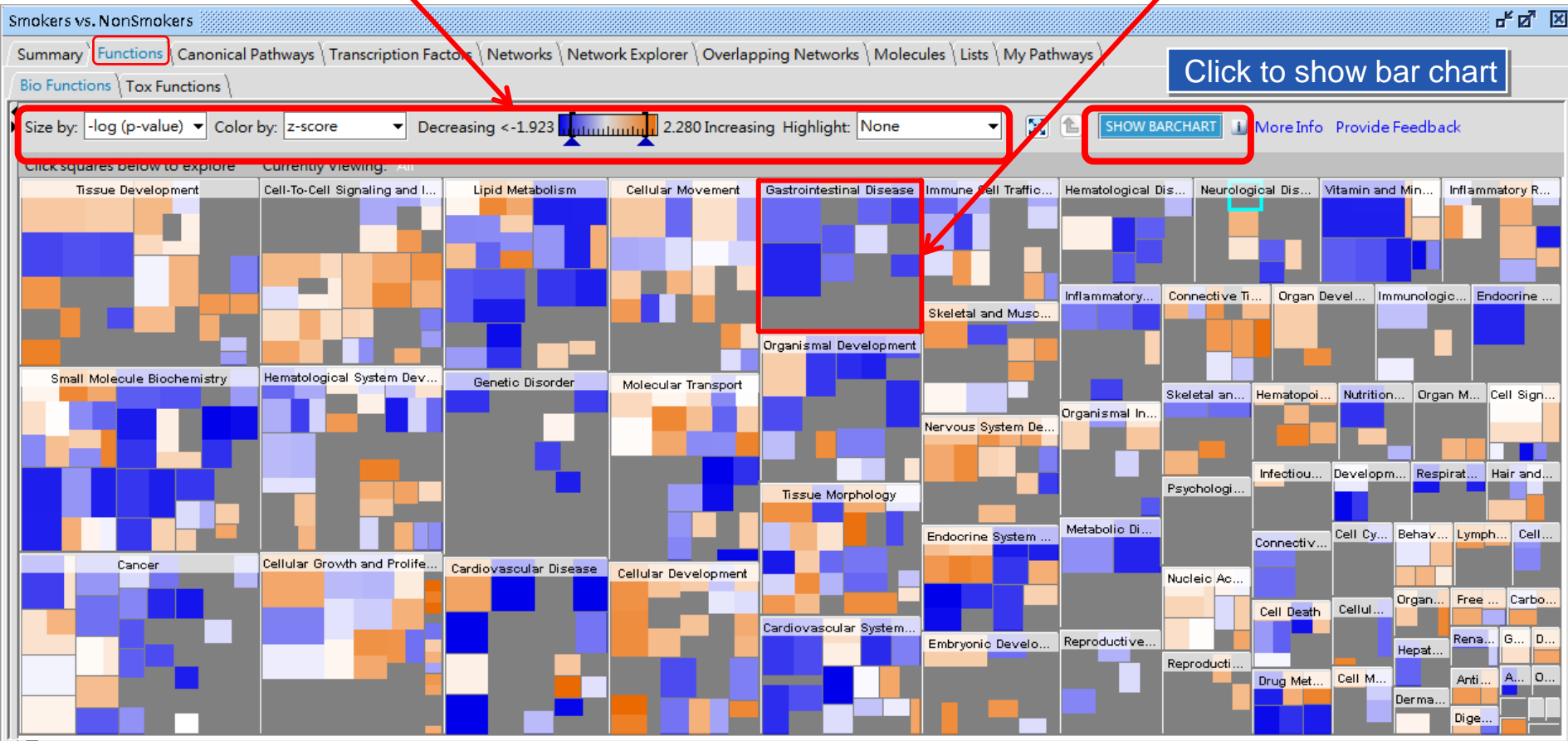
> Analysis Settings

∨ Top Canonical Pathways

Name

Phagosome Formation

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IL-12 Signaling and Production in Macrophages

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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

Summary: 將顯示前五名各頁籤之分析結果

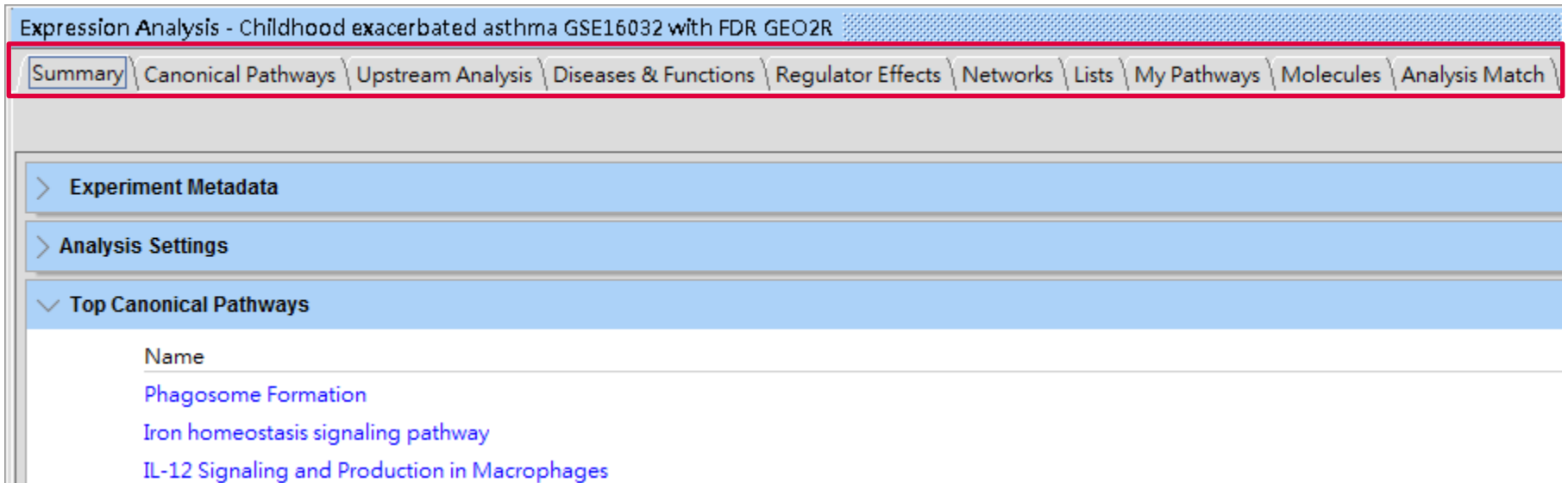Canonical Pathways：列出受實驗影響的Signaling Pathway與Metabolic Pathway

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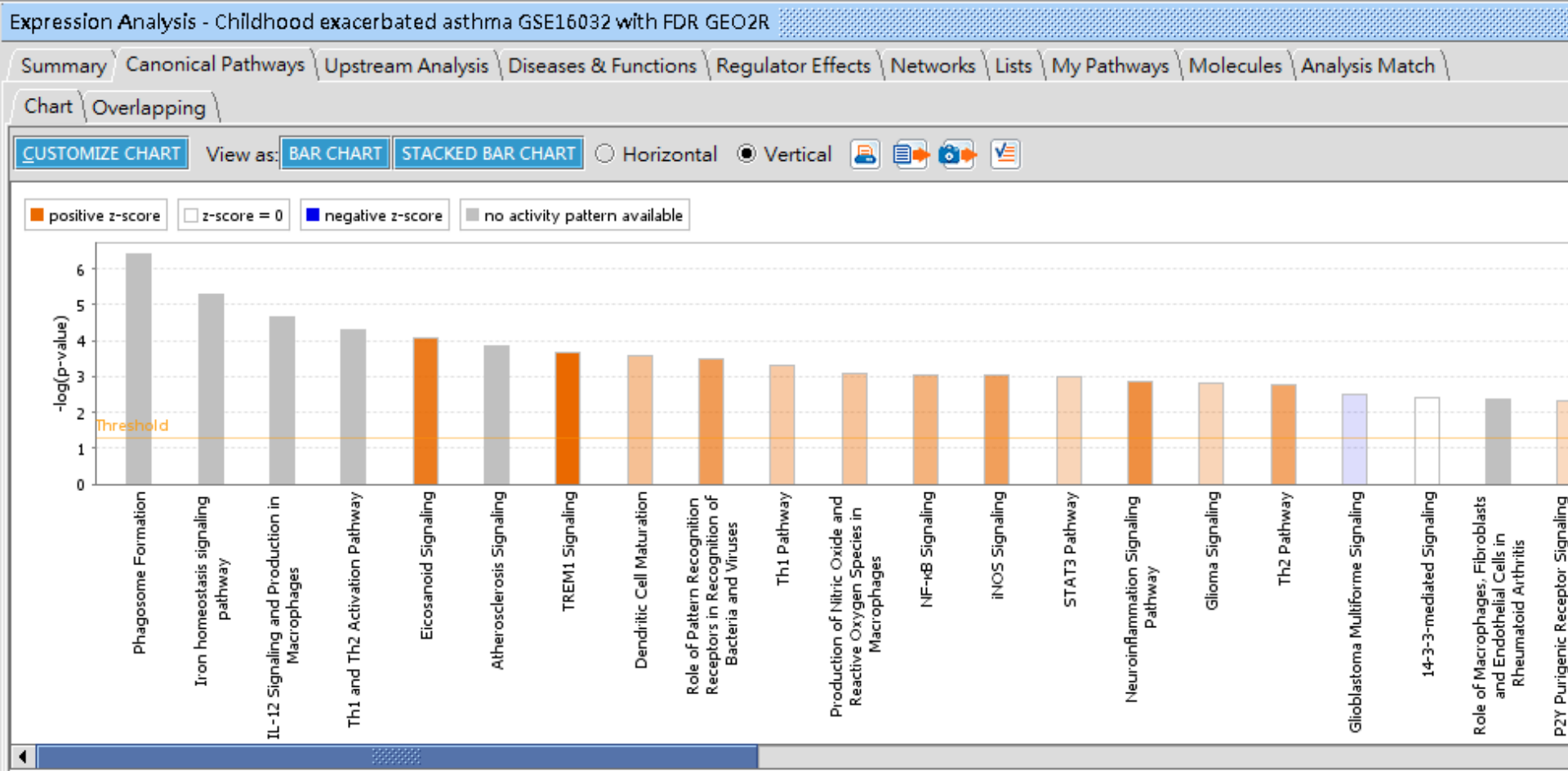| Summary | Canonical Pathways | Upstream Analysis | Diseases & Functions | Regulator Effects | Networks | Lists | My Pathways | Molecules | Analysis Match |

> **Experiment Metadata**

> **Analysis Settings**

∨ **Top Canonical Pathways**

Name

Phagosome Formation

Iron homeostasis signaling pathway

IL-12 Signaling and Production in Macrophages

Sample to Insight

Statistical measure of correlation between the transcription regulator (TR) and resulting gene expression
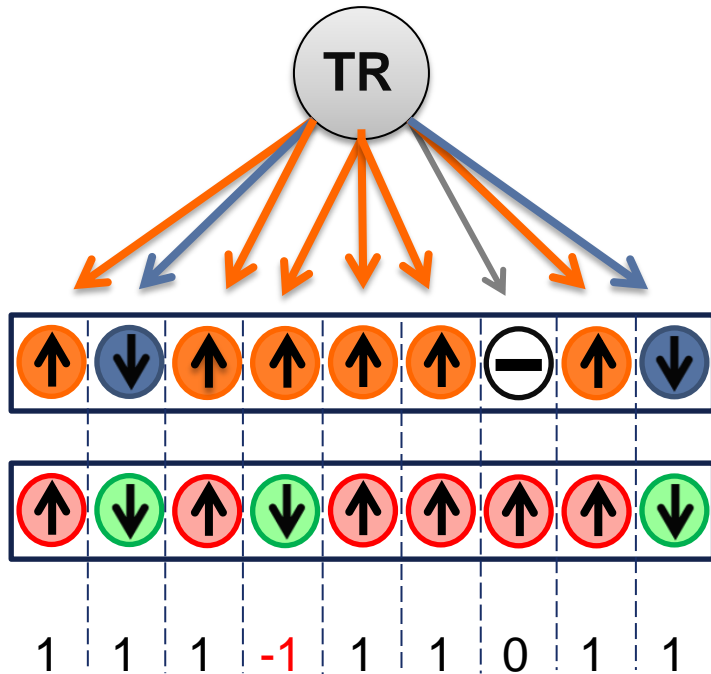
N = 8 genes

TR effect on downstream genes
(Literature)

Differential gene expression
(Uploaded Data)

| 1 | 1 | 1 | -1 | 1 | 1 | 0 | 1 | 1 |

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

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

Summary: 將顯示前五名各頁籤之分析結果

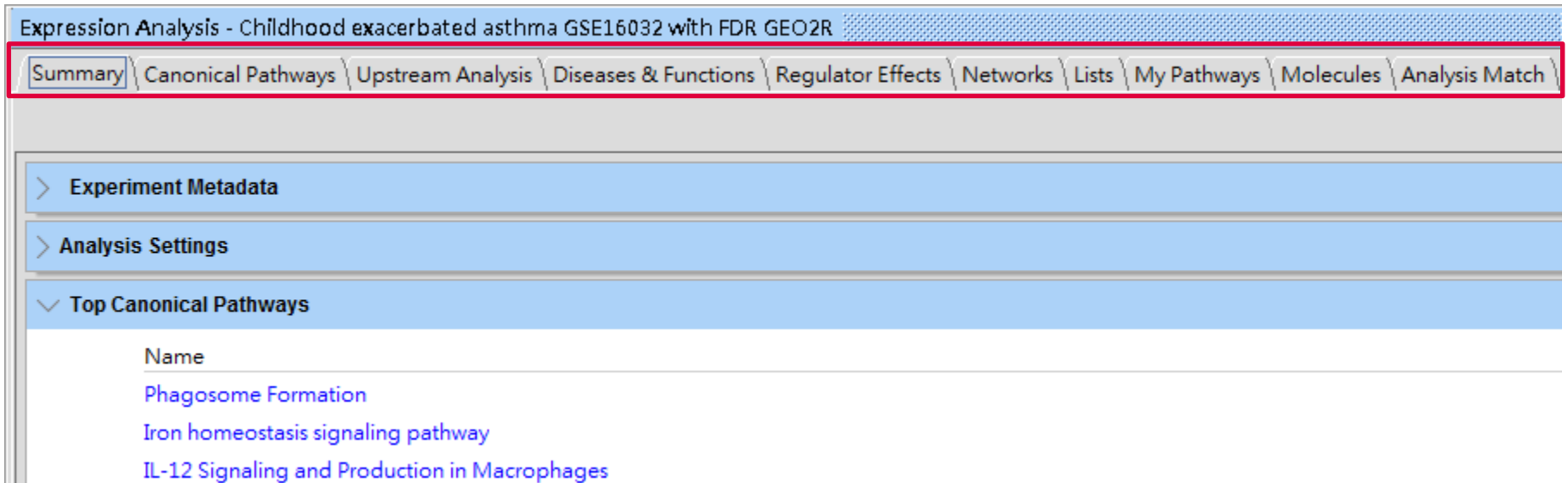Canonical Pathways：列出受實驗影響的Signaling Pathway與Metabolic Pathway

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

Upstream Regulators

Simplest Regulator Effects result

Molecules in the dataset

Algorithm

First iteration

Disease or Function

Disease or Function

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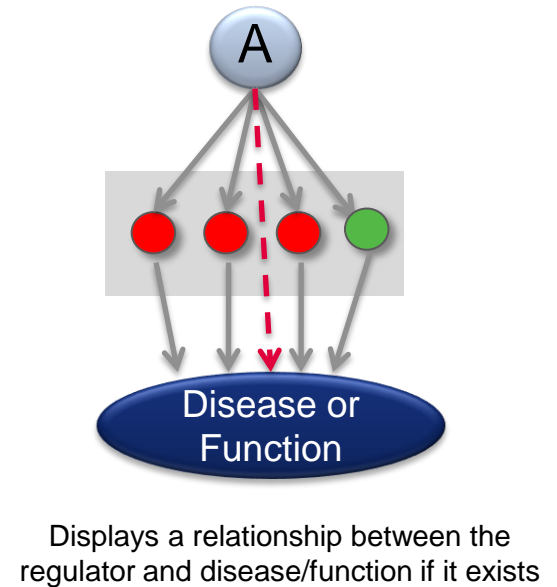
Downstream Effects Analysis

Causally consistent networks score higher
The algorithm runs iteratively to merge additional regulators with diseases and functions

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Upstream Regulator Analysis

Regulator Effects

Algorithm **Next iteration**

Disease or Function "A"

Downstream Effects Analysis

Additional iterations

Displays relationship between the regulators as well as regulators and functions if they exist

Disease or Function "A"

Disease or Function "B"
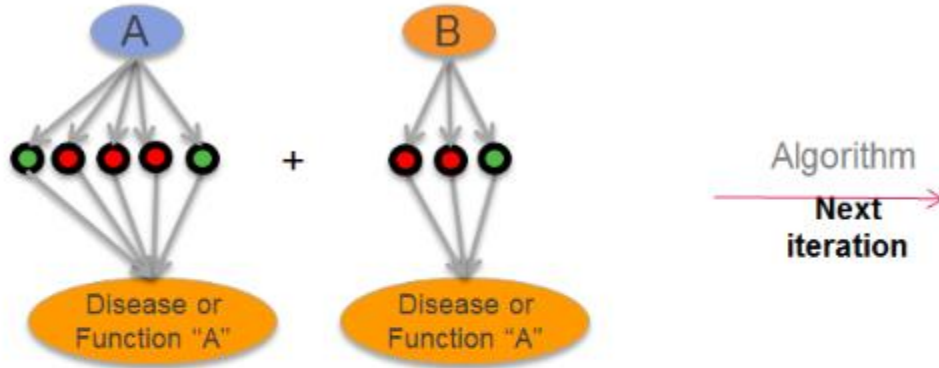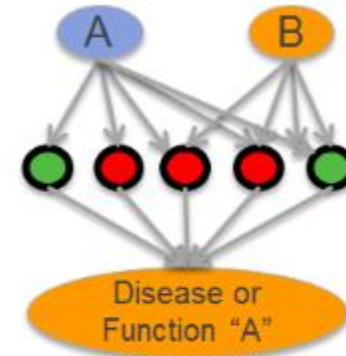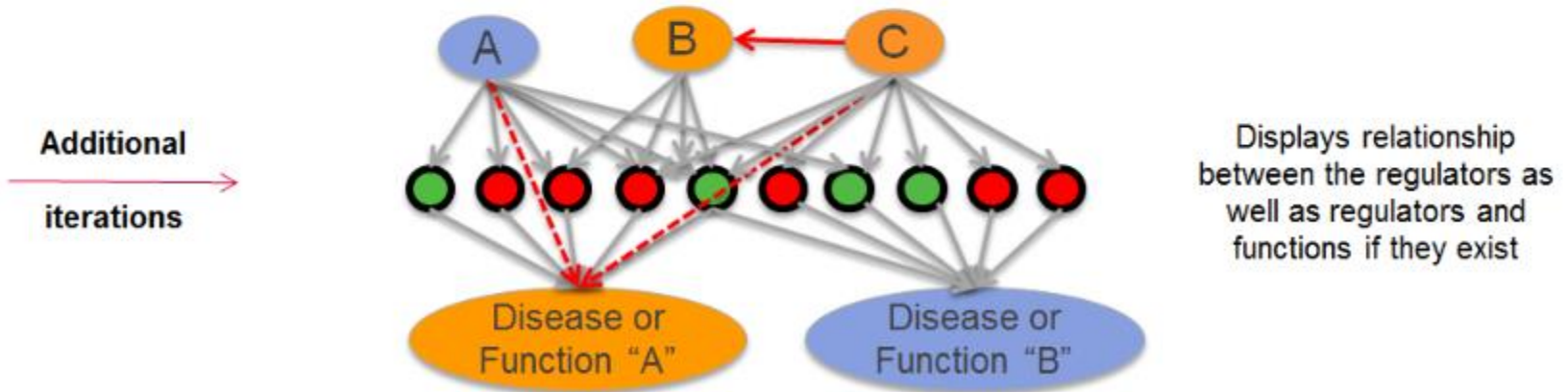
$$\text{Consistency Score} = \frac{Pc \cdot Wc + Pi \cdot Wi + Pn \cdot Wn}{(S)^{Ws}}$$

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Summary: 將顯示前五名各頁籤之分析結果

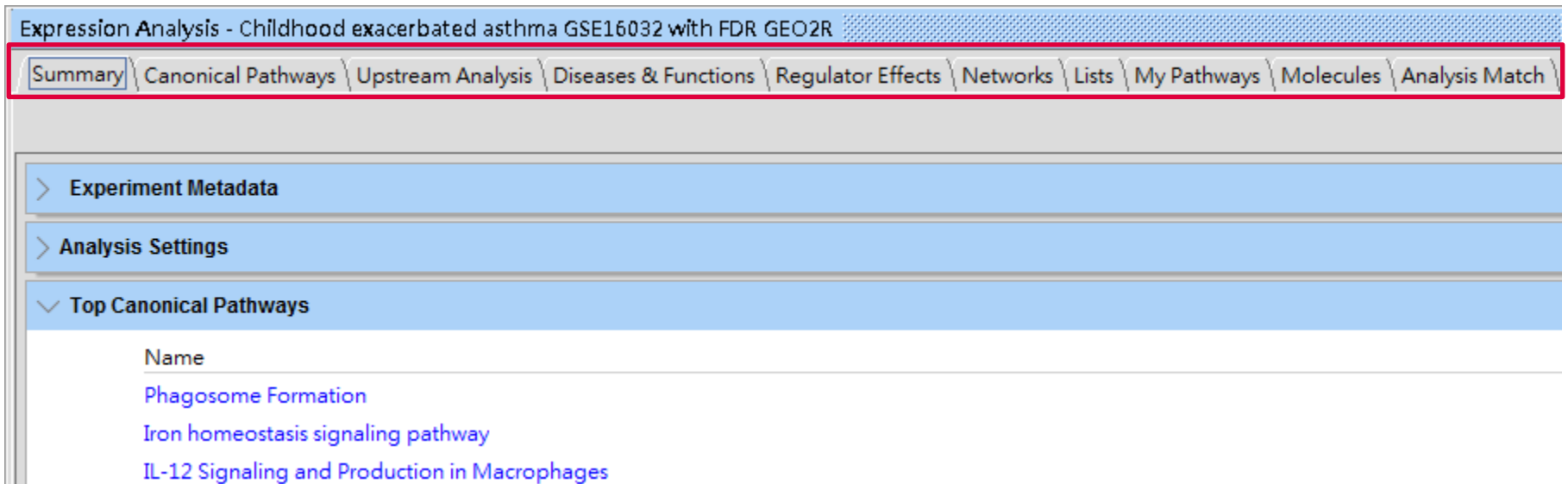Canonical Pathways：列出受實驗影響的Signaling Pathway與Metabolic Pathway

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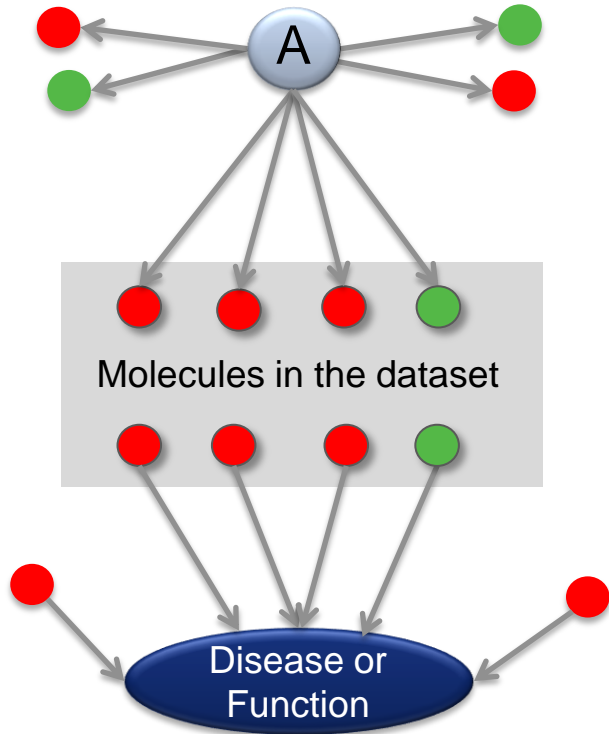> Experiment Metadata

> Analysis Settings

∨ Top Canonical Pathways

Name

Phagosome Formation

Iron homeostasis signaling pathway

IL-12 Signaling and Production in Macrophages

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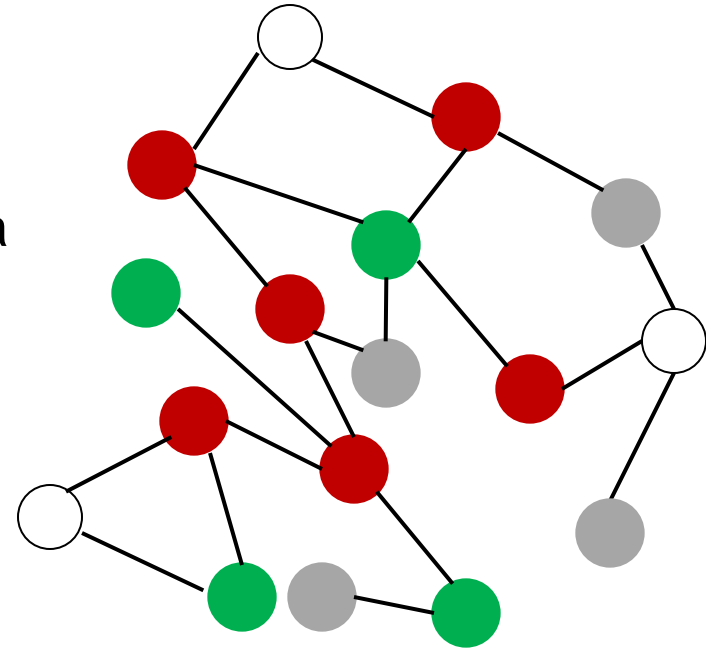
Molecules per Network	Networks per Analysis
35 ▼	25 ▼
35	10
70	25
140	50

Live Demo

A. 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)

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)

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?

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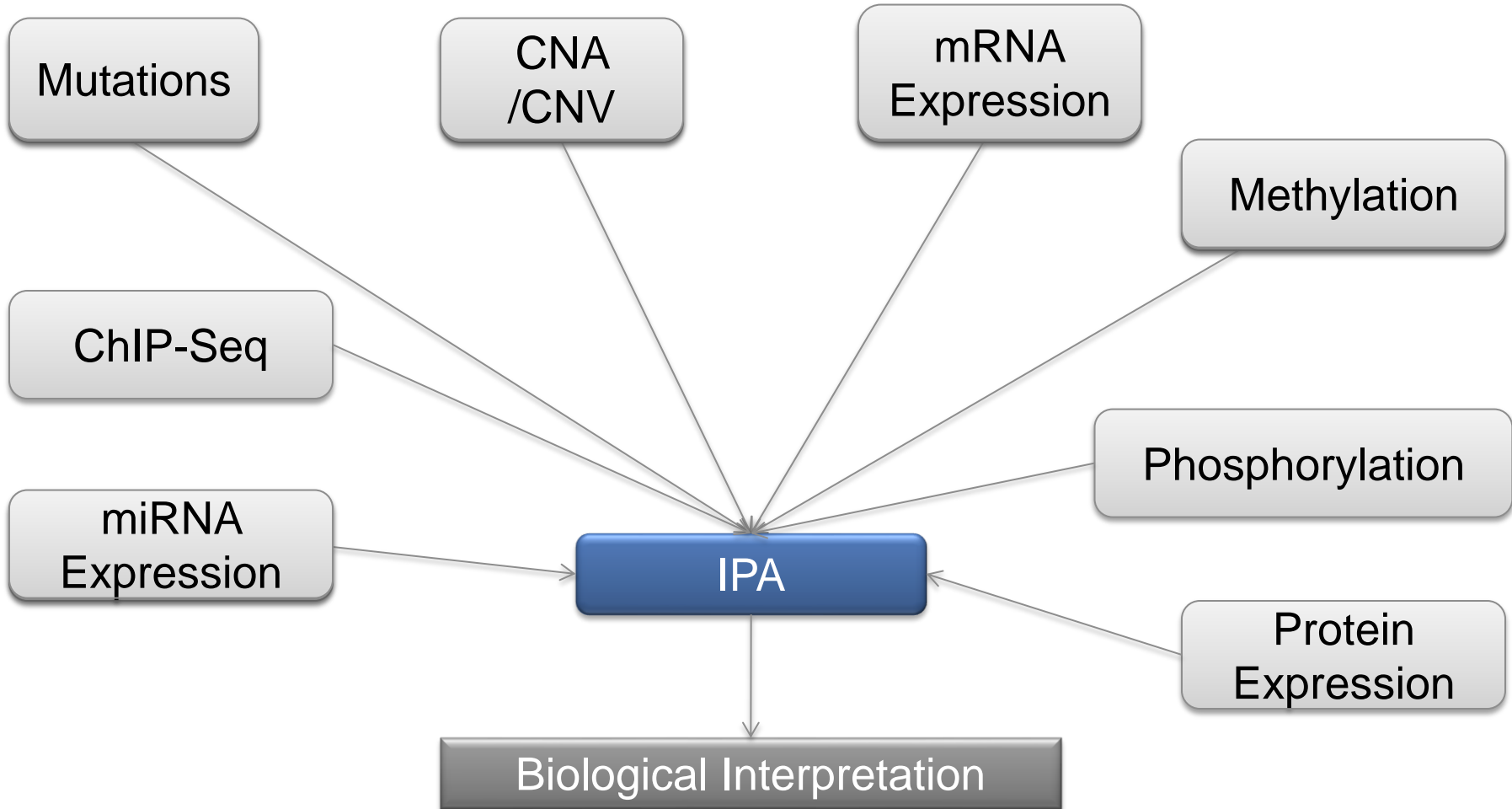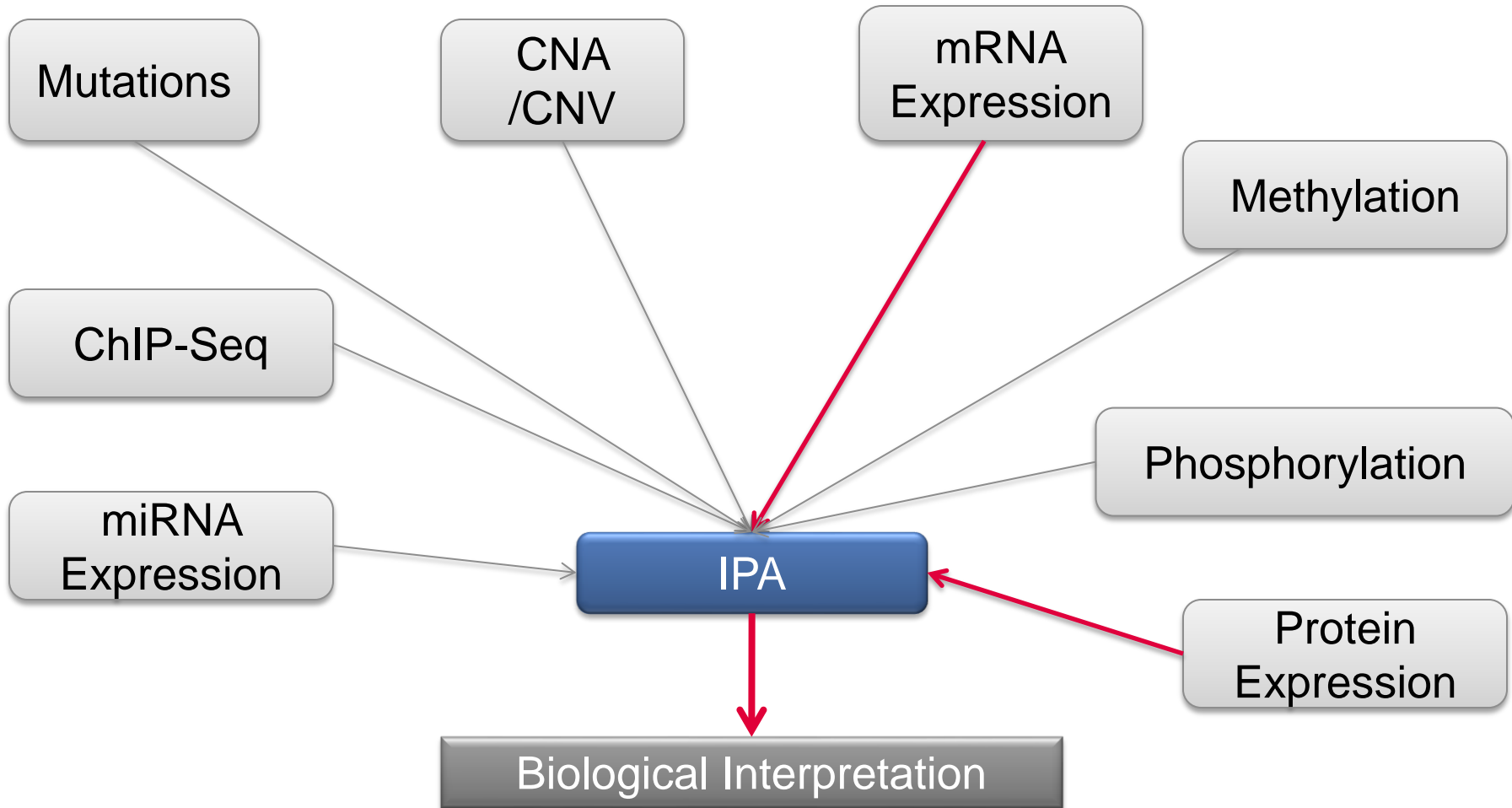
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Review your workflow – What are your goals?
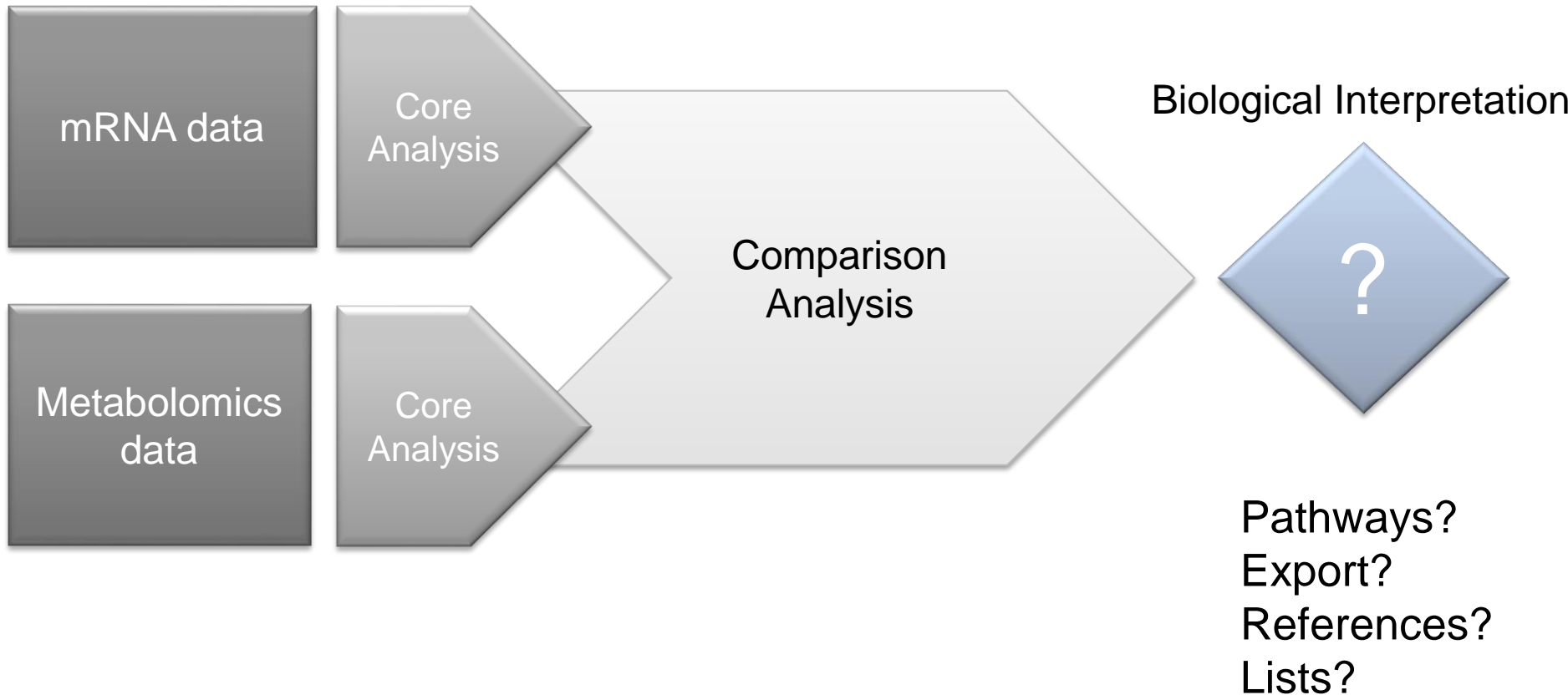
mRNA data

Core Analysis

Metabolomics data

Core Analysis

Comparison Analysis

Biological Interpretation

?

Pathways?
Export?
References?
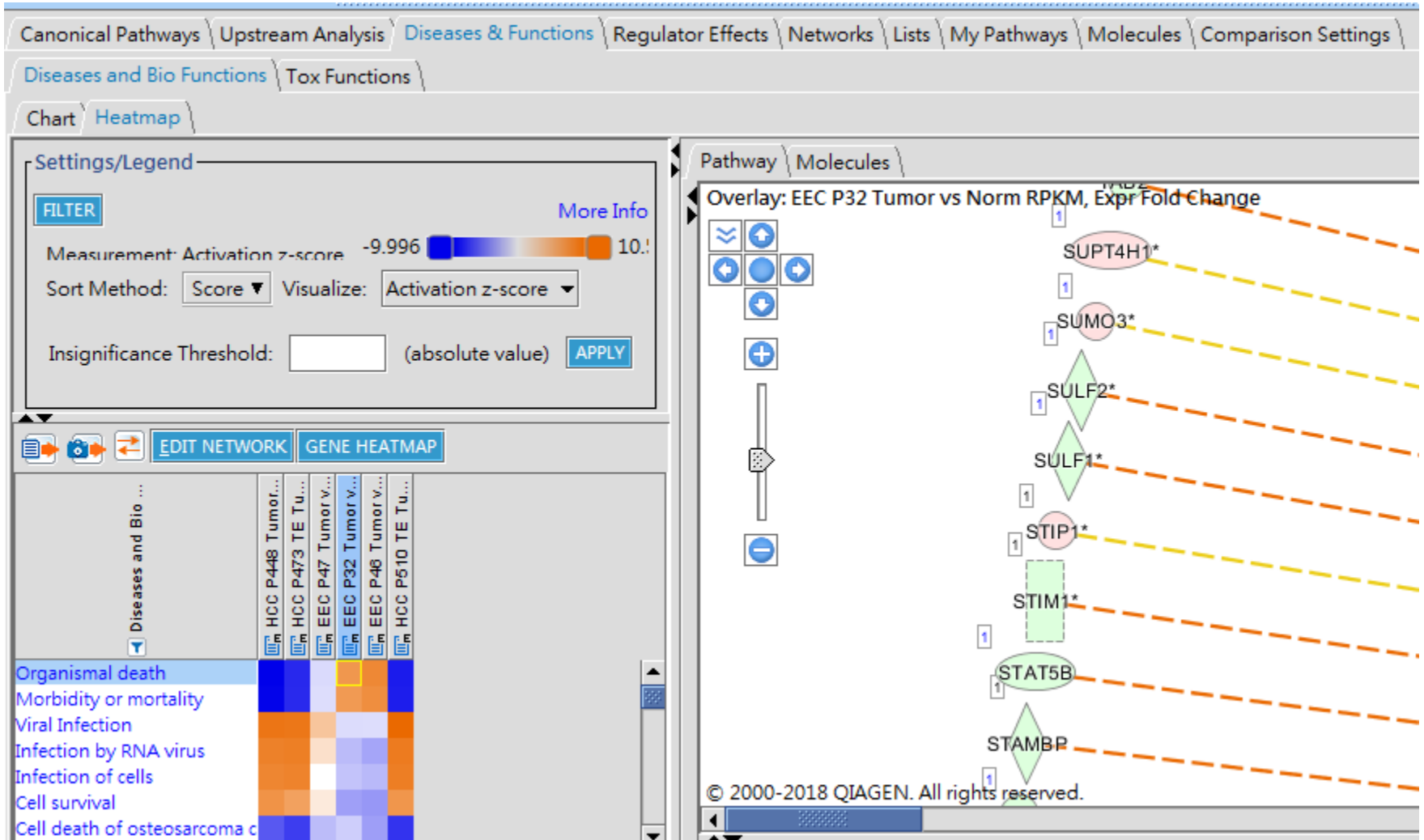Lists?

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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]

Metabolomics result

Transcriptomic result

IPA analysis (biomarker)

urine

liver adipose muscle

db/db diabetic mouse model

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

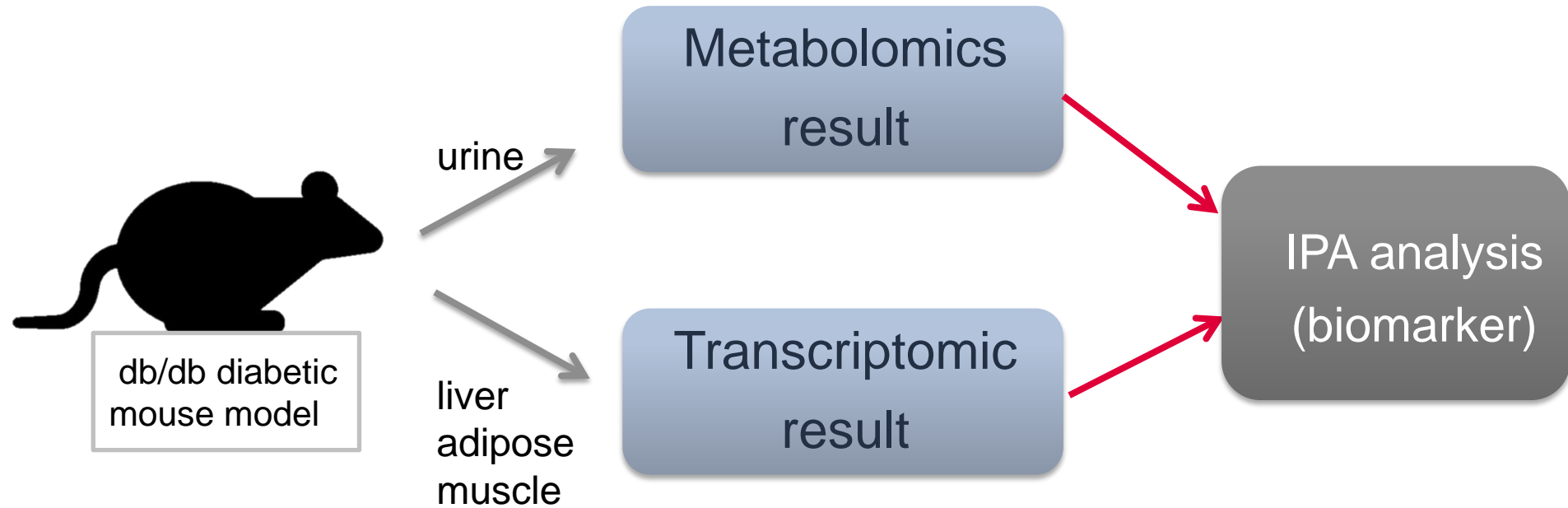| ID	Symbol	Fold Change	p-value
C00671	(3S)-3-methyl-2-oxopentanoate	-1.344	0.00E+00
C01089	(R)-3-hydroxybutyric acid	-1.311	0.00E+00
C05984	2-hydroxybutyric acid	-1.344	0.00E+00
C00322	2-oxoadipic acid	2	
C00026	2-oxoglutaric acid	2.031	0.00E+00
C01468	4-cresol	1.64	0.00E+00
C00642	4-hydroxyphenylacetic 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 Change	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
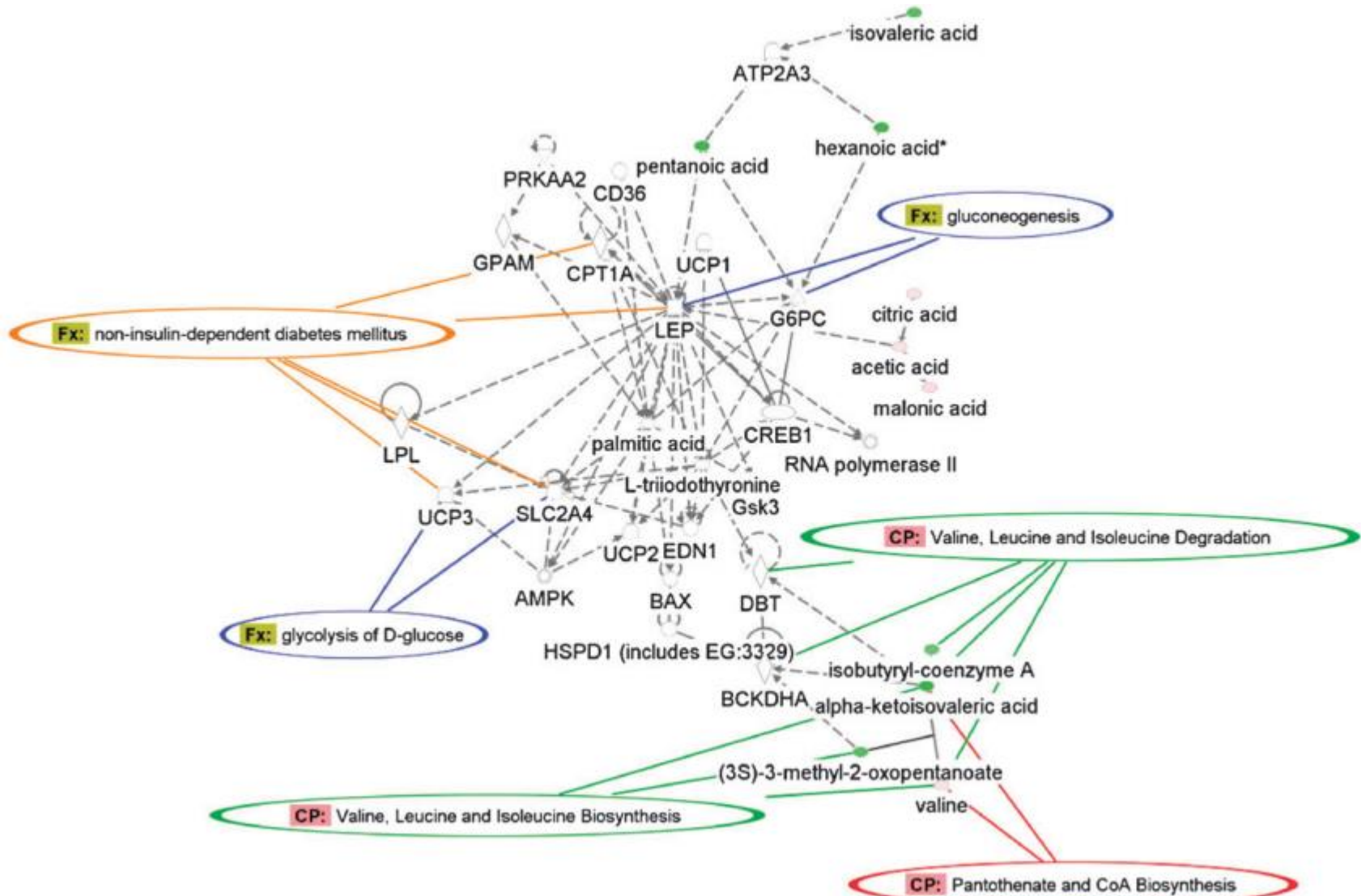
Network describing lipid metabolism, small molecule biochemistry and transport
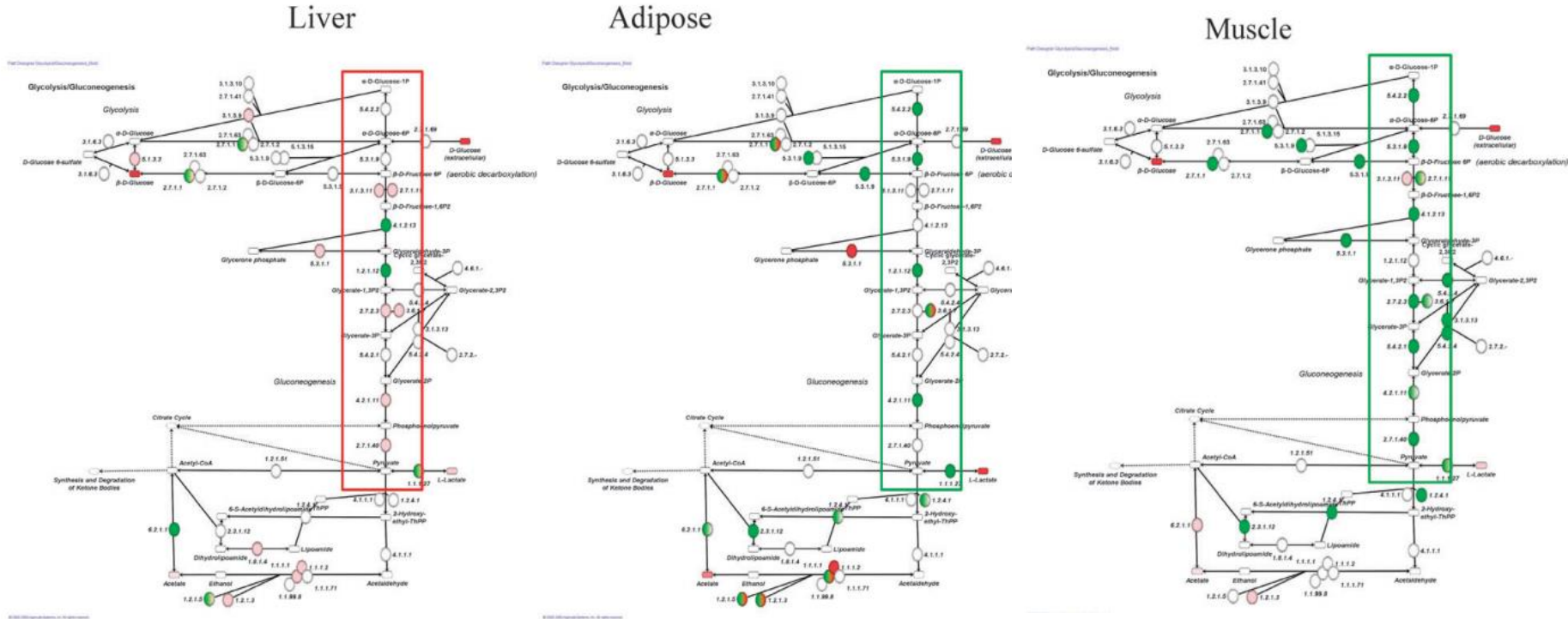
Gluconeogenesis pathway mapping metabolomics and transcriptomics results

Result:

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

- metabolomics highlighted at least <u>24 distinct pathways</u> 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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Upstream Analysis

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

p-value of over... 1.68E-11 - 7.92E-04 (p1 of 9) | More Info

Upstream Regulator	Log Ratio	Molecule Type	Predicted Activation State	Activation z-score	p-value of overlap	Target molecules in dataset	Mechanistic Network
rosiglitazone		chemical drug	Activated	3.746	1.68E-11	↑ACAA2, ↑ACADVL, ...all 59	240 (15)
PPARG	↑0.706	ligand-dependent nuclear receptor	Activated	4.793	2.22E-09	↑ACAA2, ↑ACOX1, ↑...all 53	185 (18)
TNF	↑5.150	cytokine	Inhibited	-3.414	2.62E-09	↓ABCC2, ↓ABR, ↑A...all 147	244 (18)
9-cis-retinoic acid		chemical drug		1.201			
				4.599			
				3.145			

Functions

CUSTOMIZE CHART | View as: » | Size by: -log (p-value) | Color by: z-score | None

Click squares below to explore | Currently Viewing: All > Lipid Metabolism

concentration | synthesis | oxidation | transport | homeostasis

oxidation of lipid
[-log (p-value) 5.272 : z-score 2.104]

uptake | quantity | storage | cleav...

accumulation

Rosiglitazone

-log(p-value): 10.0, 7.5, 5.0, 2.5, 0.0
Threshold
Lipid Metabolism

Mechanistic Networks

rosiglitazone
TNF
ADIPOQ
STAT3*
NFKB1
CEBPB

Canonical Pathways

LPS/IL-1 Mediated Inhibition of RXR Function
Overlay: Rosiglitazone_10uM

FXR ligand | Retinoic acid
HL*
RXRA

Rosiglitazone | Ratio
-log(p-value): 4.5, 4.0, 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0.0
Threshold
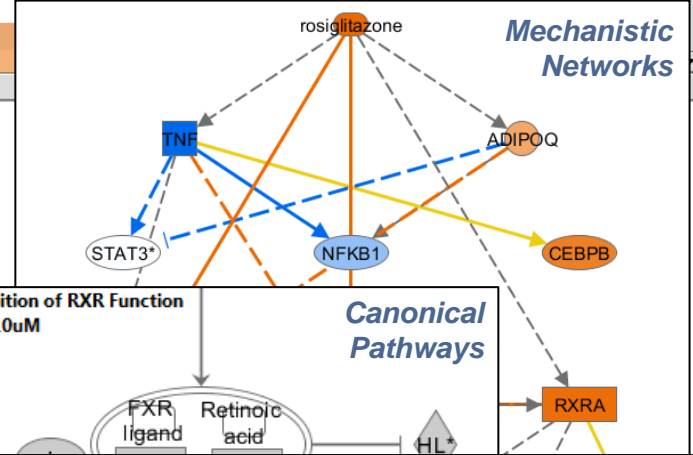LPS/IL-1 Mediated Inhibition of RXR Function | Retinol Biosynthesis | Triacylglycerol Biosynthesis | LXR/RXR Activation

Networks

PEMT*
HDL-chole
PLTP*
LIPG*
H2AFV
VLDL-cholesterol
HDL
tria
FKBP9*
PELI2
GK
MAFF*
PDK4
AGTRAP*
PDCD10*
ERK
EPHA7
CCM2*

Regulator Effects

TLR7
1.230E-3
0.346

CXCL10	IRF7	ICAM1	CCL2	MYD88	IL1B	CCL3L1/CCL3L3	CCL5	CXCL2	CCR7
2.960E-5	2.530E-6	1.600E-4	5.190E-6	2.530E-4	1.370E-2	3.360E-4	2.400E-3	6.990E-5	3.080E-3
1.810	2.240	0.462	1.240	0.430	0.359	0.684	0.854	0.949	0.227

recruitment of leukocytes

Sample to Insight

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Live Demo

快速篩選microRNA的潛在標的

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**

Use ▼ to filter a column. Add data or more columns using 'Add column(s) ⊞ '.

Rows: 1 - 131

| 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 Regula...
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				7994131		↑4.995		

miRNA Data → miRNA Target Filter → Molecule Type → Pathways (Cancer/Growth) → mRNA → ↑↓/↓↑ → ?

| 88 data points | 13,690 targets | 1,090 targets | 333 targets | 39 targets | 32 targets |

Use Pathway tools to build hypothesis for microRNA to mRNA target association

- **Goal**: Utilize newly discovered microRNAs to better understand biology around potential mRNA targets/disease

- **Challenge**: New and rapidly evolving field with different measurement techniques and prediction algorithms leading to variability in data

- **Need**: Identify mRNA targets to microRNAs using biological and experimental information, correlate microRNA and mRNA target expression, specify easy to use confidence levels of interaction predictions, annotate mRNA targets with biological context, pathways, species, etc., all within a single workflow

- **Outcome**: Reduce time of identification of relevant mRNA targets from months to minutes
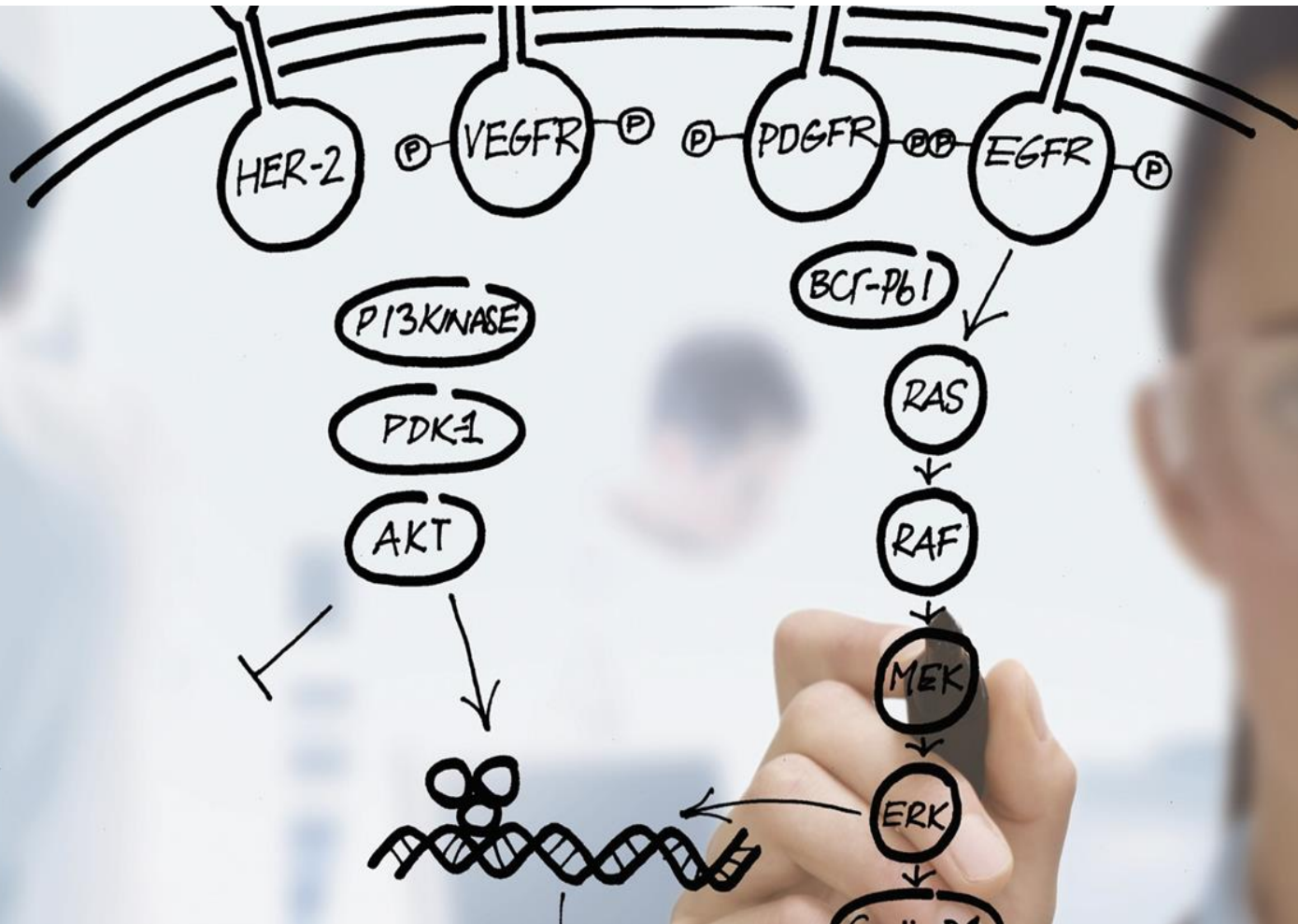
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