IPA進階模組教育訓練

利用整合Omicsoft自有實驗數據與重要公開資料庫提昇數據分析能力



Clair Tsai　蔡宜庭

創源生技 業務主任

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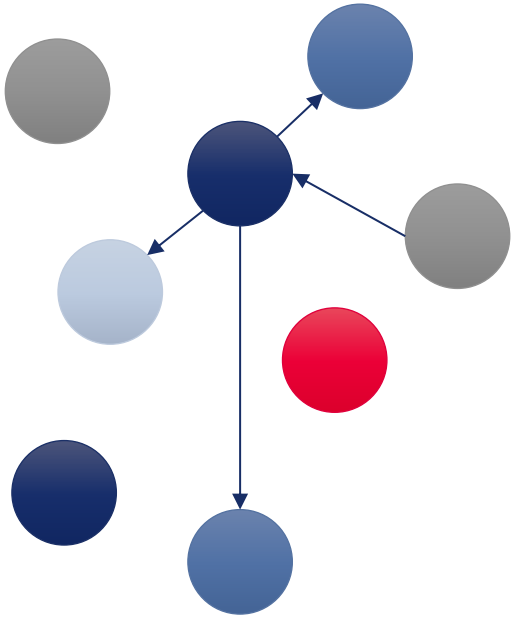
Agenda

- **Uncover causality — the next level of pathway analysis**
- **Ingenuity Pathway Analysis Advanced Analytics**
  — Analysis functions in IPA AA module
- **Case study**
  1. **Analysis match**
     將您的分析結果與IPA收錄上千筆分析資訊做比較
  2. **Phosphoproteomics Analysis**
     闡釋磷酸化蛋白分析意義結果
  3. **Isoprofiler**
     快速篩選實驗中目標興趣之Isoform

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Omics and Multi-Omics

- **Gene expression:**
  - qPCR analysis
  - Microarray
  - RNA-Seq (NGS)
  - microRNA
  - mRNA
- **Proteomics:**
  - PhosphoProteomics[New]
- **Metabolomics**

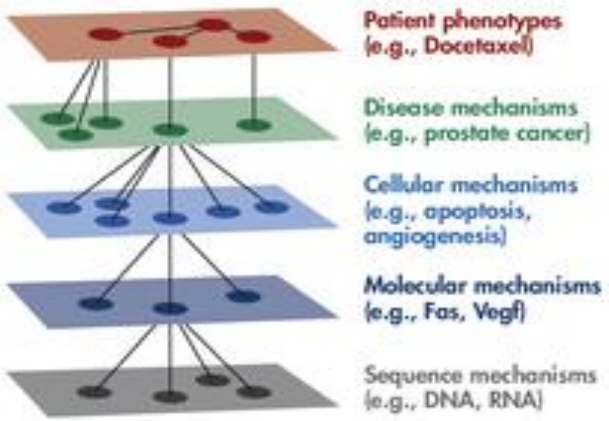


Omics

- **Build Pathway**
  - Grow Pathway
  - Path Explorer
  - Connect Molecules
  - Overlay and Predict Activity
- **Pathway Analysis**
  - Up Stream Regulator
  - Down Stream Effect
  - Canonical Pathway Analysis
  - Network Analysis

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Omics and Multi-Omics

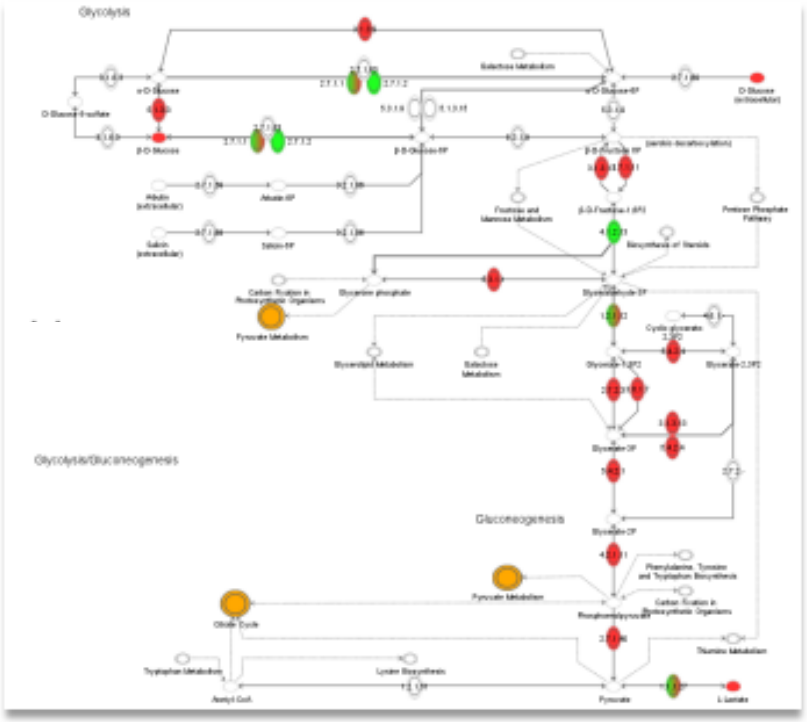


Multi-Omics

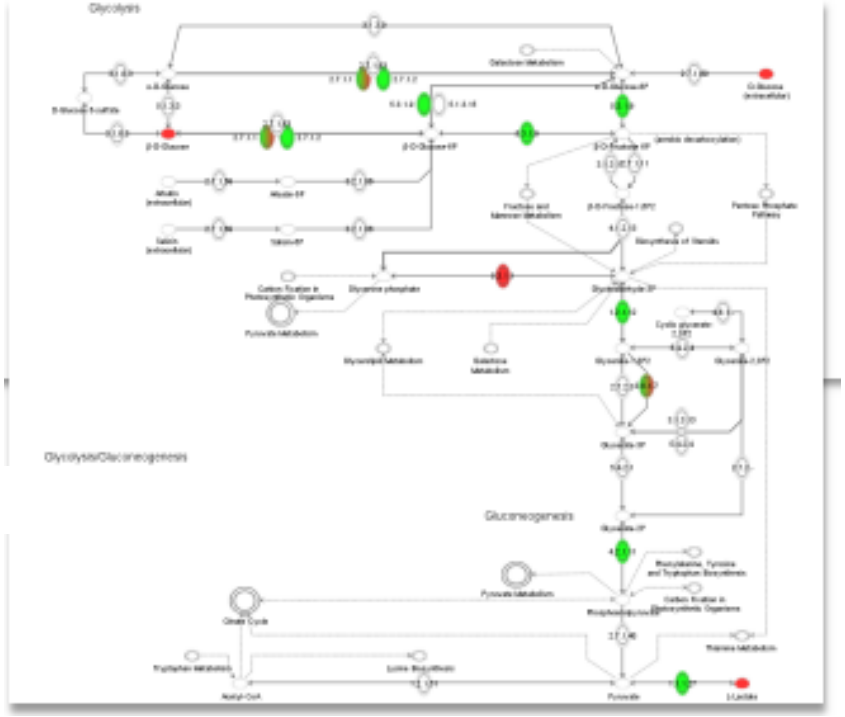
- **Single Experiment**
  - Time Course
  - Dose Response
  - Before/After Treatment
- **Multi Experiments**
  - System biology
  - Combining SNP, CNA, mRNA, microRNA, proteomics, metabolomics, PTM, etc
- **Host-Microbiota Interactions**
  - Metagemonics

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Bringing together multiple types of genomic data



Genomics Level



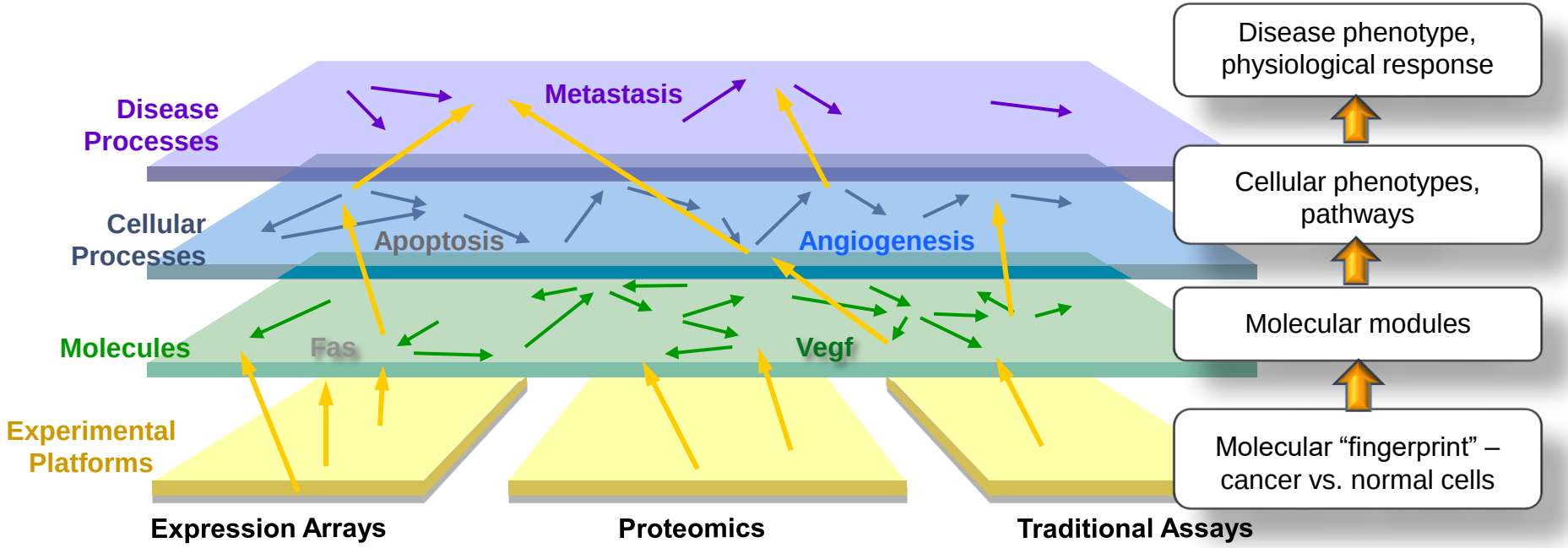
Proteomics Level

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The challenge

Bottom to top experiment model

- **Therapeutic Insight from Experimental Data**
  - Integrate – Interpret – Gain

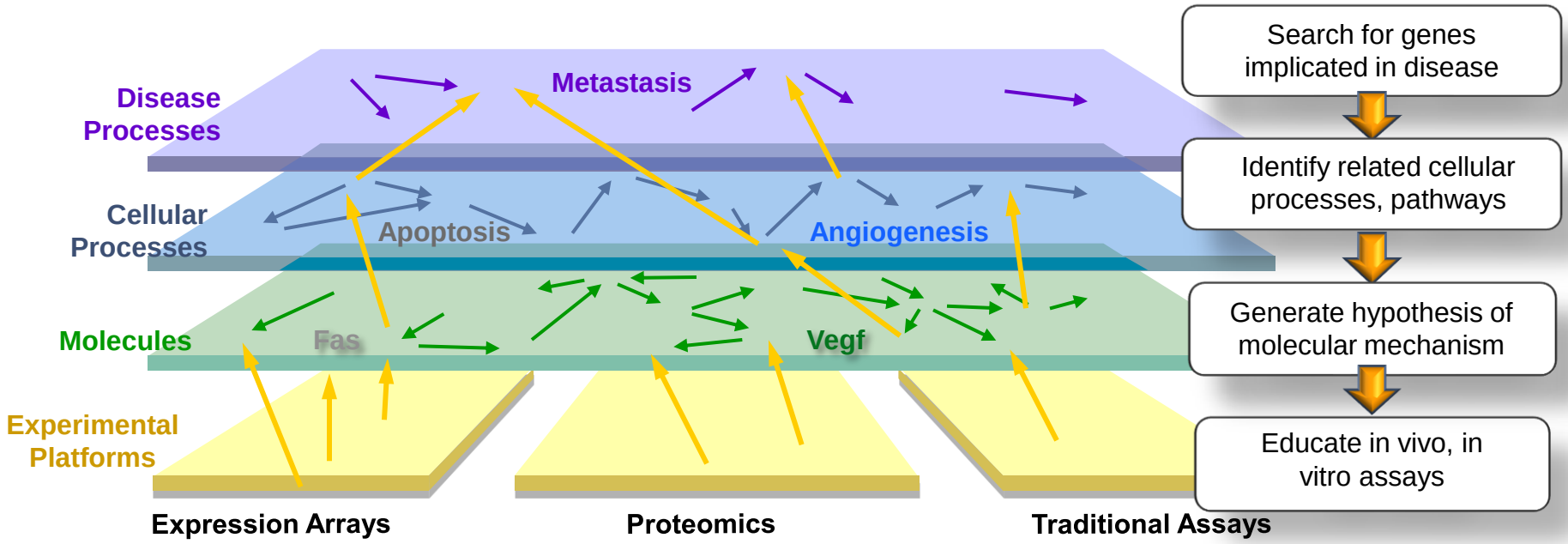


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The challenge

Top-to-bottom experiment model

- Rapid understanding and interpretation of experimental systems

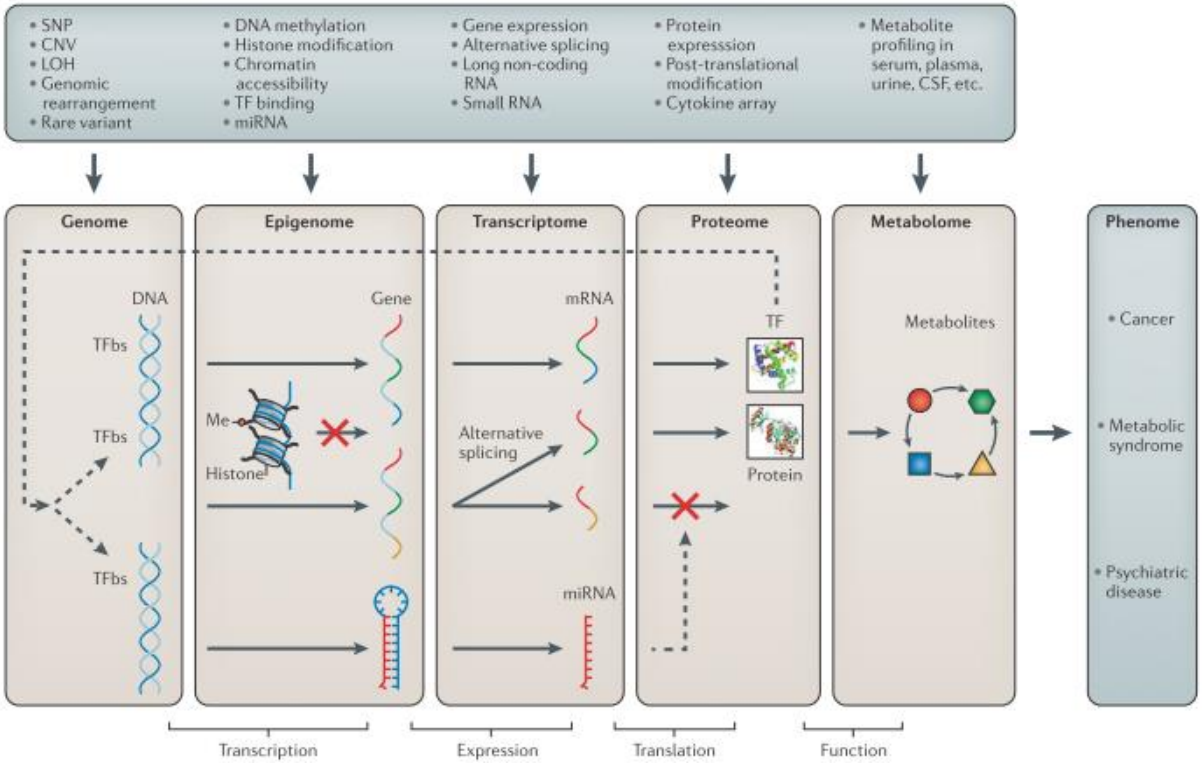


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IPA Advanced Analytics — get the most out of your data

The innovative features of IPA Advanced Analytics:

- Generate novel hypotheses for mechanisms of action or drug targets
- Prioritize predicted regulatory networks by connection to disease or phenotype of interest
- Uncover causal relationships relevant to your experimental data



Methods of integrating data to uncover genotype–phenotype interactions, *Nature Reviews Genetics* (2015)

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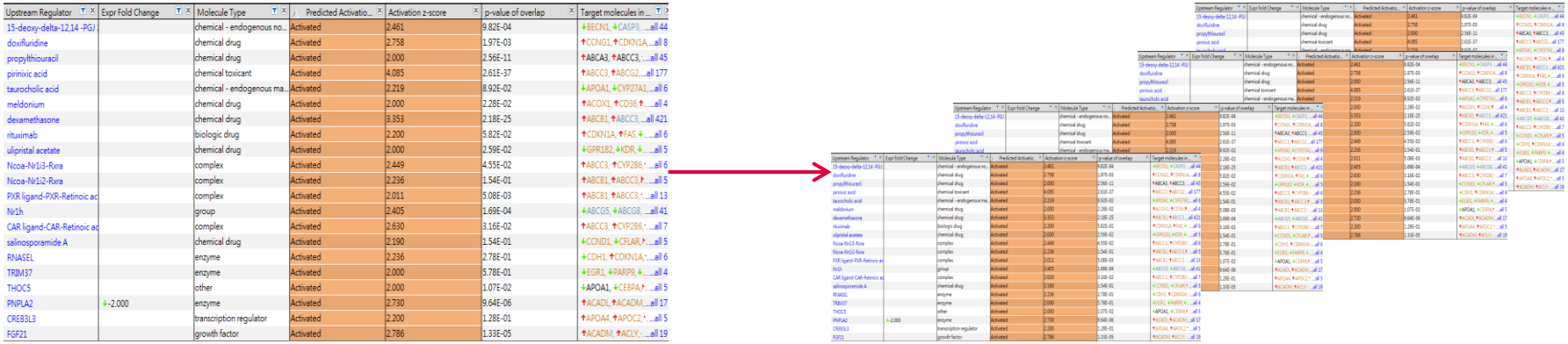
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Analysis Match

Analysis Match enables you to automatically match your analysis against:

- All your own analysis
- Analyses of 49000+ expression datasets processed and curated from public sources by Omicsoft (QIAGEN)

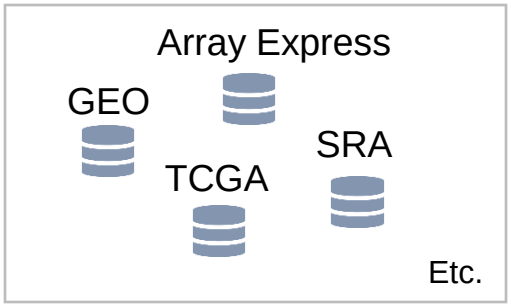
- Build confidence in your analysis results
- Discover unexpected insights into mechanisms shared between studies
- Gain insight from ‘anti-matches’

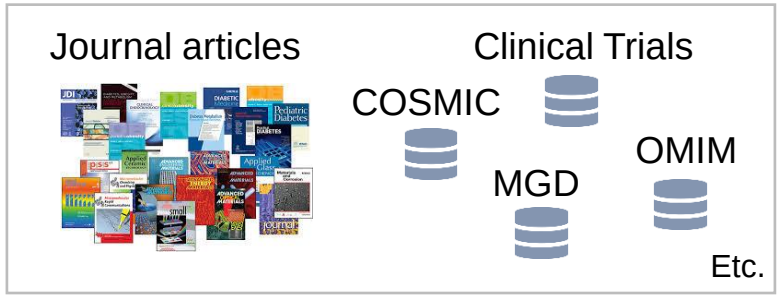


Upstream Regulator	Expr Fold Change	Molecule Type	Predicted Activatio...	Activation z-score	p-value of overlap	Target molecules in ...
15-deoxy-delta-12,14 -PGJ...		chemical - endogenous no...	Activated	2.461	9.82E-04	↓BECN1, ↓CASP3, ...all 44
doxifluridine		chemical drug	Activated	2.758	1.97E-03	↑CCNG1, ↑CDKN1A, ...all 8
propylthiouracil		chemical drug	Activated	2.000	2.56E-11	↑ABCA3, ↑ABCC3, ...all 45
pirinixic acid		chemical toxicant	Activated	4.085	2.61E-37	↑ABCC3, ↑ABCG2, ...all 177
taurocholic acid		chemical - endogenous ma...	Activated	2.219	8.92E-02	↓APOA1, ↓CYP27A1, ...all 6
meldonium		chemical drug	Activated	2.000	2.28E-02	↑ACOX1, ↑CD36, ↑...all 4
dexamethasone		chemical drug	Activated	3.353	2.18E-25	↑ABCB1, ↑ABCC3, ...all 421
rituximab		biologic drug	Activated	2.200	5.82E-02	↑CDKN1A, ↑FAS, ↓...all 6
ulipristal acetate		chemical drug	Activated	2.000	2.59E-02	↓GPR182, ↓KDR, ↓...all 5
Ncoa-Nr1i3-Rxra		complex	Activated	2.449	4.55E-02	↑ABCC3, ↑CYP2B6, ↑...all 6
Ncoa-Nr1i2-Rxra		complex	Activated	2.236	1.54E-01	↑ABCB1, ↑ABCC3, ↑...all 5
PXR ligand-PXR-Retinoic ac...		complex	Activated	2.011	5.08E-03	↑ABCB1, ↑ABCC3, ↑...all 13
Nr1h		group	Activated	2.405	1.69E-04	↓ABCG5, ↓ABCG8, ...all 41
CAR ligand-CAR-Retinoic ac...		complex	Activated	2.630	3.16E-02	↑ABCC3, ↑CYP2B6, ↑...all 7
salinosporamide A		chemical drug	Activated	2.190	1.54E-01	↓CCND1, ↓CFLAR, ↑...all 5
RNASEL		enzyme	Activated	2.236	2.78E-01	↓CDH1, ↑CDKN1A, ↑...all 6
TRIM37		enzyme	Activated	2.000	5.78E-01	↓EGR1, ↓PARP9, ↓...all 4
THOC5		other	Activated	2.000	1.07E-02	↓APOA1, ↓CEBPA, ↑...all 5
PNPLA2	↓-2.000	enzyme	Activated	2.730	9.64E-06	↑ACADL, ↑ACADM, ...all 17
CREB3L3		transcription regulator	Activated	2.200	1.28E-01	↑APOA4, ↑APOC2, ↑...all 5
FGF21		growth factor	Activated	2.786	1.33E-05	↑ACADM, ↑ACLY, ...all 19

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Unprecedented discovery, together





Curation, Processing, & QA

Curation & QA

Datasets integrated into OmicSoft Lands

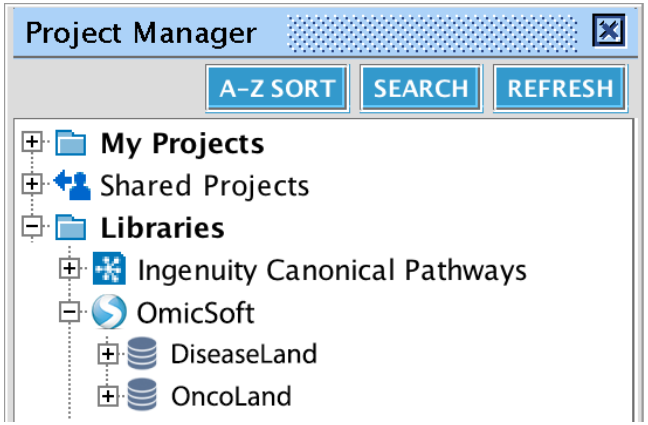
Curated Findings

OmicSoft Array Suite

OncoLand
DiseaseLand

49,000+ Expression comparison datasets

INGENUITY PATHWAY ANALYSIS





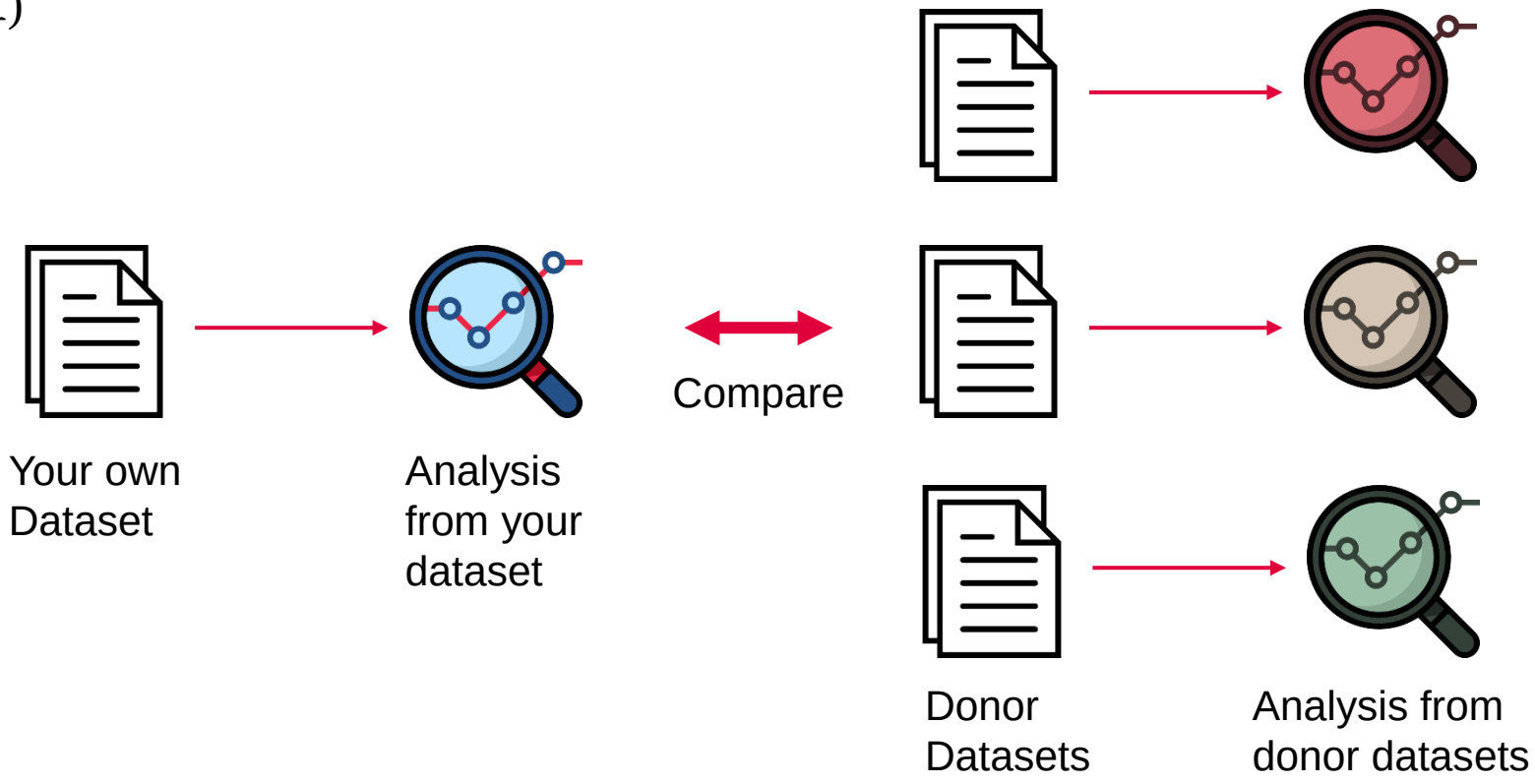
- Biological analyses of each dataset
- Compare your analysis to all OmicSoft analyses

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Analysis Match

截至今日，本次更新增加了19,000組分析完成的數據至Analysis Match分析功能中，您可以在Analysis Match分析模組當中，比較您的實驗資料與外部資料庫如TCGA、LINCS等，在不同的癌症類型，疾病類型與實驗組中分析其相似及相異關係度。

並於七月中旬，會自**LINCS（NIH Library of Integrated Network-Based Cellular Signatures）**再添加大約**28,000**個分析組，總數將超過**49,000**個(相較於上季僅有8100+個分析組)

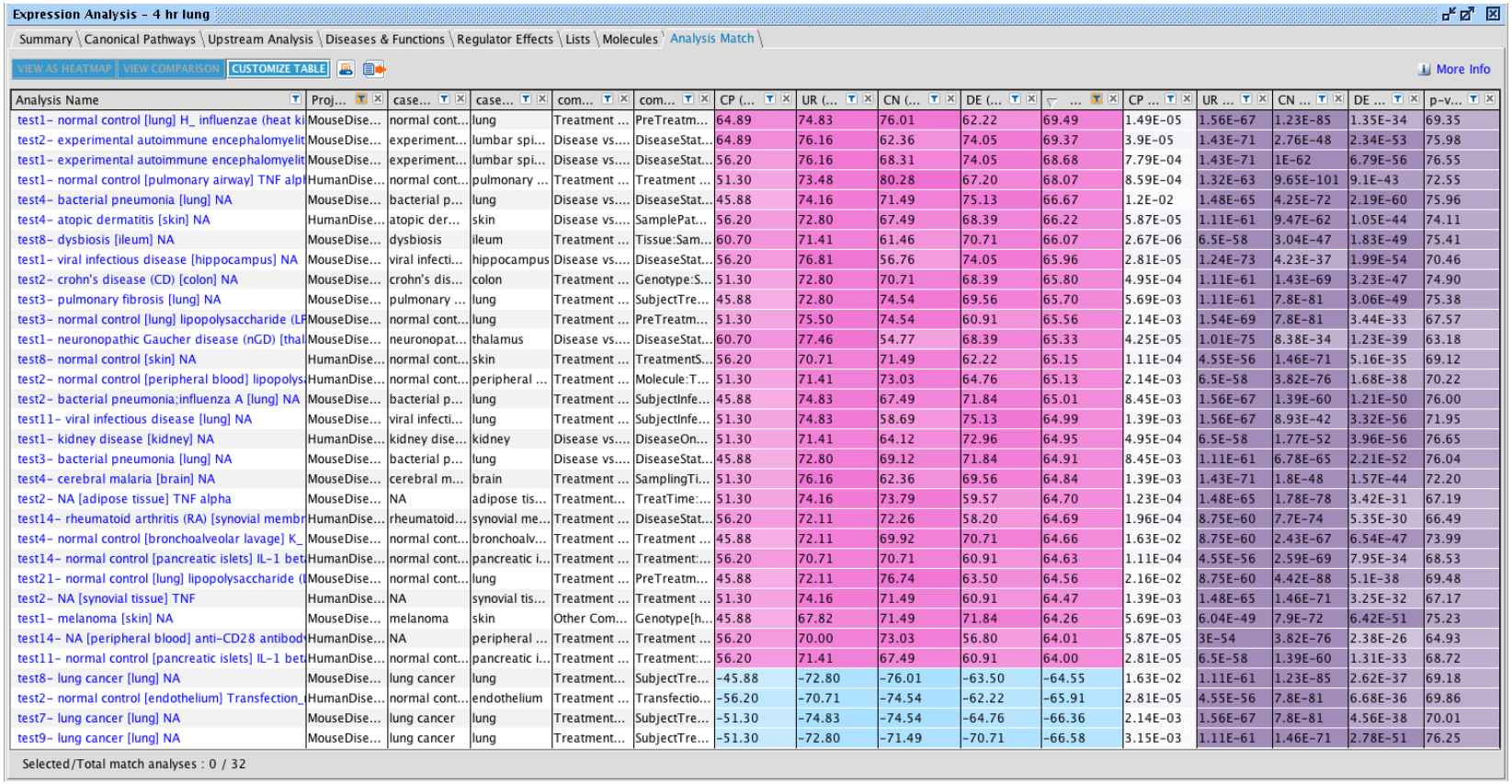


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Analysis Name	Proj...	case...	case...	com...	com...	CP (...	UR (...	CN (...	DE (...	...	CP ...	UR ...	CN ...	DE ...	p-v...
test1- normal control [lung] H_ influenzae (heat ki	MouseDise...	normal cont...	lung	Treatment ...	PreTreatm...	64.89	74.83	76.01	62.22	69.49	1.49E-05	1.56E-67	1.23E-85	1.35E-34	69.35
test2- experimental autoimmune encephalomyelit	MouseDise...	experiment...	lumbar spi...	Disease vs....	DiseaseStat...	64.89	76.16	62.36	74.05	69.37	3.9E-05	1.43E-71	2.76E-48	2.34E-53	75.98
test1- experimental autoimmune encephalomyelit	MouseDise...	experiment...	lumbar spi...	Disease vs....	DiseaseStat...	56.20	76.16	68.31	74.05	68.68	7.79E-04	1.43E-71	1E-62	6.79E-56	76.55
test1- normal control [pulmonary airway] TNF alpl	HumanDise...	normal cont...	pulmonary ...	Treatment ...	Treatment ...	51.30	73.48	80.28	67.20	68.07	8.59E-04	1.32E-63	9.65E-101	9.1E-43	72.55
test4- bacterial pneumonia [lung] NA	MouseDise...	bacterial p...	lung	Disease vs....	DiseaseStat...	45.88	74.16	71.49	75.13	66.67	1.2E-02	1.48E-65	4.25E-72	2.19E-60	75.96
test4- atopic dermatitis [skin] NA	HumanDise...	atopic der...	skin	Disease vs....	SamplePat...	56.20	72.80	67.49	68.39	66.22	5.87E-05	1.11E-61	9.47E-62	1.05E-44	74.11
test8- dysbiosis [ileum] NA	MouseDise...	dysbiosis	ileum	Treatment ...	Tissue:Sam...	60.70	71.41	61.46	70.71	66.07	2.67E-06	6.5E-58	3.04E-47	1.83E-49	75.41
test1- viral infectious disease [hippocampus] NA	MouseDise...	viral infecti...	hippocampus	Disease vs....	DiseaseStat...	56.20	76.81	56.76	74.05	65.96	2.81E-05	1.24E-73	4.23E-37	1.99E-54	70.46
test2- crohn's disease (CD) [colon] NA	MouseDise...	crohn's dis...	colon	Treatment ...	Genotype:S...	51.30	72.80	70.71	68.39	65.80	4.95E-04	1.11E-61	1.43E-69	3.23E-47	74.90
test3- pulmonary fibrosis [lung] NA	MouseDise...	pulmonary ...	lung	Treatment ...	SubjectTre...	45.88	72.80	74.54	69.56	65.70	5.69E-03	1.11E-61	7.8E-81	3.06E-49	75.38
test3- normal control [lung] lipopolysaccharide (LF	MouseDise...	normal cont...	lung	Treatment ...	PreTreatm...	51.30	75.50	74.54	60.91	65.56	2.14E-03	1.54E-69	7.8E-81	3.44E-33	67.57
test1- neuronopathic Gaucher disease (nGD) [thal	MouseDise...	neuronopat...	thalamus	Disease vs....	DiseaseStat...	60.70	77.46	54.77	68.39	65.33	4.25E-05	1.01E-75	8.38E-34	1.23E-39	63.18
test8- normal control [skin] NA	HumanDise...	normal cont...	skin	Treatment ...	TreatmentS...	56.20	70.71	71.49	62.22	65.15	1.11E-04	4.55E-56	1.46E-71	5.16E-35	69.12
test2- normal control [peripheral blood] lipopolys	HumanDise...	normal cont...	peripheral ...	Treatment ...	Molecule:T...	51.30	71.41	73.03	64.76	65.13	2.14E-03	6.5E-58	3.82E-76	1.68E-38	70.22
test2- bacterial pneumonia;influenza A [lung] NA	MouseDise...	bacterial p...	lung	Treatment ...	SubjectInfe...	45.88	74.83	67.49	71.84	65.01	8.45E-03	1.56E-67	1.39E-60	1.21E-50	76.00
test11- viral infectious disease [lung] NA	MouseDise...	viral infecti...	lung	Treatment ...	SubjectInfe...	51.30	74.83	58.69	75.13	64.99	1.39E-03	1.56E-67	8.93E-42	3.32E-56	71.95
test1- kidney disease [kidney] NA	HumanDise...	kidney dise...	kidney	Disease vs....	DiseaseOn...	51.30	71.41	64.12	72.96	64.95	4.95E-04	6.5E-58	1.77E-52	3.96E-56	76.65
test3- bacterial pneumonia [lung] NA	MouseDise...	bacterial p...	lung	Disease vs....	DiseaseStat...	45.88	72.80	69.12	71.84	64.91	8.45E-03	1.11E-61	6.78E-65	2.21E-52	76.04
test4- cerebral malaria [brain] NA	MouseDise...	cerebral m...	brain	Treatment ...	SamplingTi...	51.30	76.16	62.36	69.56	64.84	1.39E-03	1.43E-71	1.8E-48	1.57E-44	72.20
test2- NA [adipose tissue] TNF alpha	MouseDise...	NA	adipose tis...	Treatment...	TreatTime:...	51.30	74.16	73.79	59.57	64.70	1.23E-04	1.48E-65	1.78E-78	3.42E-31	67.19
test14- rheumatoid arthritis (RA) [synovial membr	HumanDise...	rheumatoid...	synovial me...	Treatment ...	DiseaseStat...	56.20	72.11	72.26	58.20	64.69	1.96E-04	8.75E-60	7.7E-74	5.35E-30	66.49
test4- normal control [bronchoalveolar lavage] K_	MouseDise...	normal cont...	bronchoalv...	Treatment ...	Treatment ...	45.88	72.11	69.92	70.71	64.66	1.63E-02	8.75E-60	2.43E-67	6.54E-47	73.99
test14- normal control [pancreatic islets] IL-1 bet	HumanDise...	normal cont...	pancreatic i...	Treatment ...	Treatment:...	56.20	70.71	70.71	60.91	64.63	1.11E-04	4.55E-56	2.59E-69	7.95E-34	68.53
test21- normal control [lung] lipopolysaccharide (I	MouseDise...	normal cont...	lung	Treatment ...	PreTreatm...	45.88	72.11	76.74	63.50	64.56	2.16E-02	8.75E-60	4.42E-88	5.1E-38	69.48
test2- NA [synovial tissue] TNF	HumanDise...	NA	synovial tis...	Treatment ...	Treatment ...	51.30	74.16	71.49	60.91	64.47	1.39E-03	1.48E-65	1.46E-71	3.25E-32	67.17
test1- melanoma [skin] NA	MouseDise...	melanoma	skin	Other Com...	Genotype[h...	45.88	67.82	71.49	71.84	64.26	5.69E-03	6.04E-49	7.9E-72	6.42E-51	75.23
test14- NA [peripheral blood] anti-CD28 antibod	HumanDise...	NA	peripheral ...	Treatment ...	Treatment ...	56.20	70.00	73.03	56.80	64.01	5.87E-05	3E-54	3.82E-76	2.38E-26	64.93
test11- normal control [pancreatic islets] IL-1 bet	HumanDise...	normal cont...	pancreatic i...	Treatment ...	Treatment:...	56.20	71.41	67.49	60.91	64.00	2.81E-05	6.5E-58	1.39E-60	1.31E-33	68.72
test8- lung cancer [lung] NA	MouseDise...	lung cancer	lung	Treatment...	SubjectTre...	-45.88	-72.80	-76.01	-63.50	-64.55	1.63E-02	1.11E-61	1.23E-85	2.62E-37	69.18
test2- normal control [endothelium] Transfection_	HumanDise...	normal cont...	endothelium	Treatment ...	Transfectio...	-56.20	-70.71	-74.54	-62.22	-65.91	2.81E-05	4.55E-56	7.8E-81	6.68E-36	69.86
test7- lung cancer [lung] NA	MouseDise...	lung cancer	lung	Treatment...	SubjectTre...	-51.30	-74.83	-74.54	-64.76	-66.36	2.14E-03	1.56E-67	7.8E-81	4.56E-38	70.01
test9- lung cancer [lung] NA	MouseDise...	lung cancer	lung	Treatment...	SubjectTre...	-51.30	-72.80	-71.49	-70.71	-66.58	3.15E-03	1.11E-61	1.46E-71	2.78E-51	76.25

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Analysis Match with over 49,200+ Omicsoft datasets

DiseaseLand	OncoLand
HumanDisease (7075) •437 diseases •225 tissues •62 expression platforms •>750 RNA-seq datasets	OncoGeo (1781) •113 cancers •60 tissues •32 expression platforms •>200 RNA-seq datasets
MouseDisease (6443) •261 diseases •177 tissues •50 expression platforms •>1000 RNA-seq datasets	TCGA (4789) •33 cancers •27 tissues •385 different mutational status / clinical signs
RatDisease (441) •23 diseases •33 tissues	Pediatrics (315) •38 cancers •14 tissues
LINCS (28,234) •23 cell lines •374 chemical treatments or gene overexpression • 226 different targets (or groups of target genes)	Metastatic Cancer (81) •27 cancers •18 tissues
Hematology (128) •22 cancers •7 tissues	

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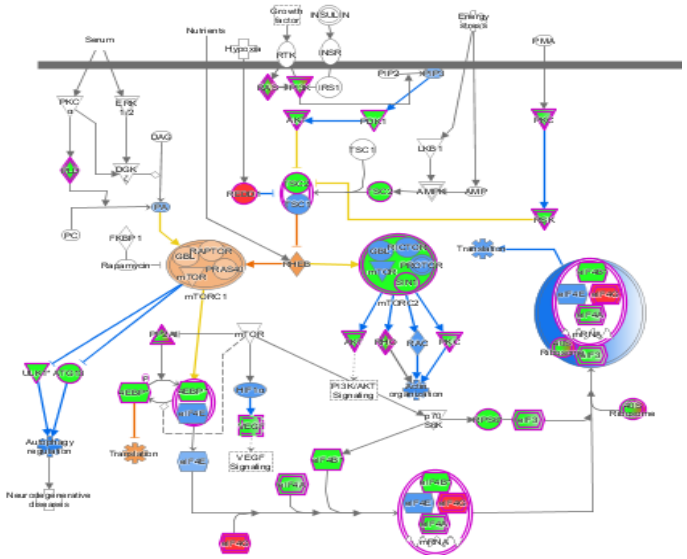
Types of comparisons of analyses in the lands

Comparison Type	Example
Treatment vs. Control	Anti-TNF therapy vs none
Disease vs. Normal	Non-lesional psoriasis vs normal
Treatment 1 vs. Treatment 2	MLL5 vs EGFP
Disease 1 vs. Disease 2	Irritable bowel syndrome(IBS) vs inflammatory bowel syndrome(IBD)
CellType1 vs. CellType 2	Oligodendrocyte vs astrocyte
Responder vs. non-Responder	Partial response vs progressive disease
Tissue 1 vs. Tissue 2	Brown adipose tissue vs White adipose tissue
Other Comparison	Disease stage → In remission vs onset

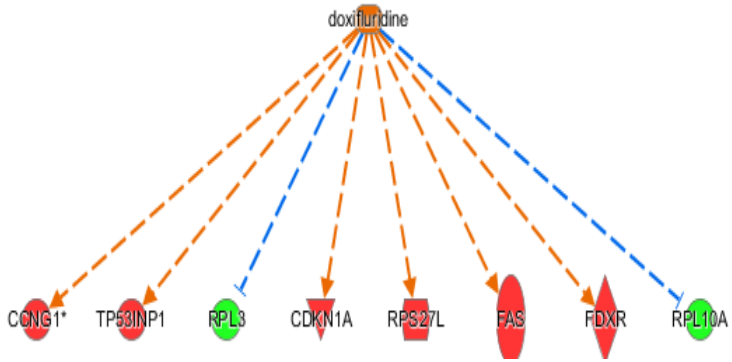
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Analysis Match ‘Entities’

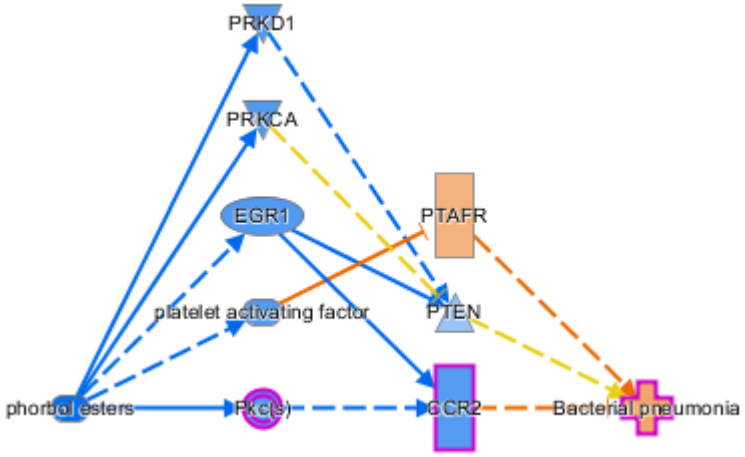
Canonical pathways



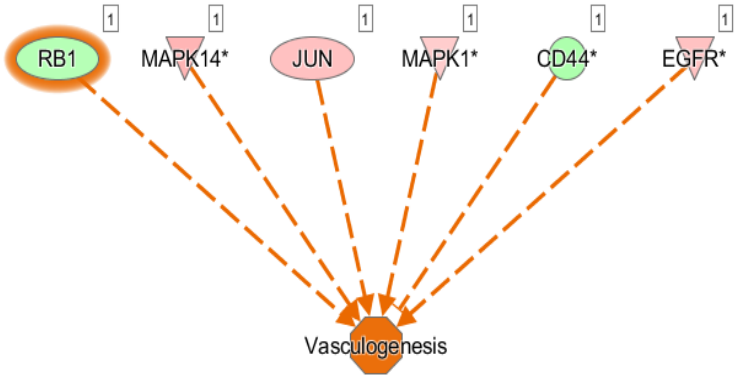
Upstream regulators



Causal networks (‘master’ regulator)



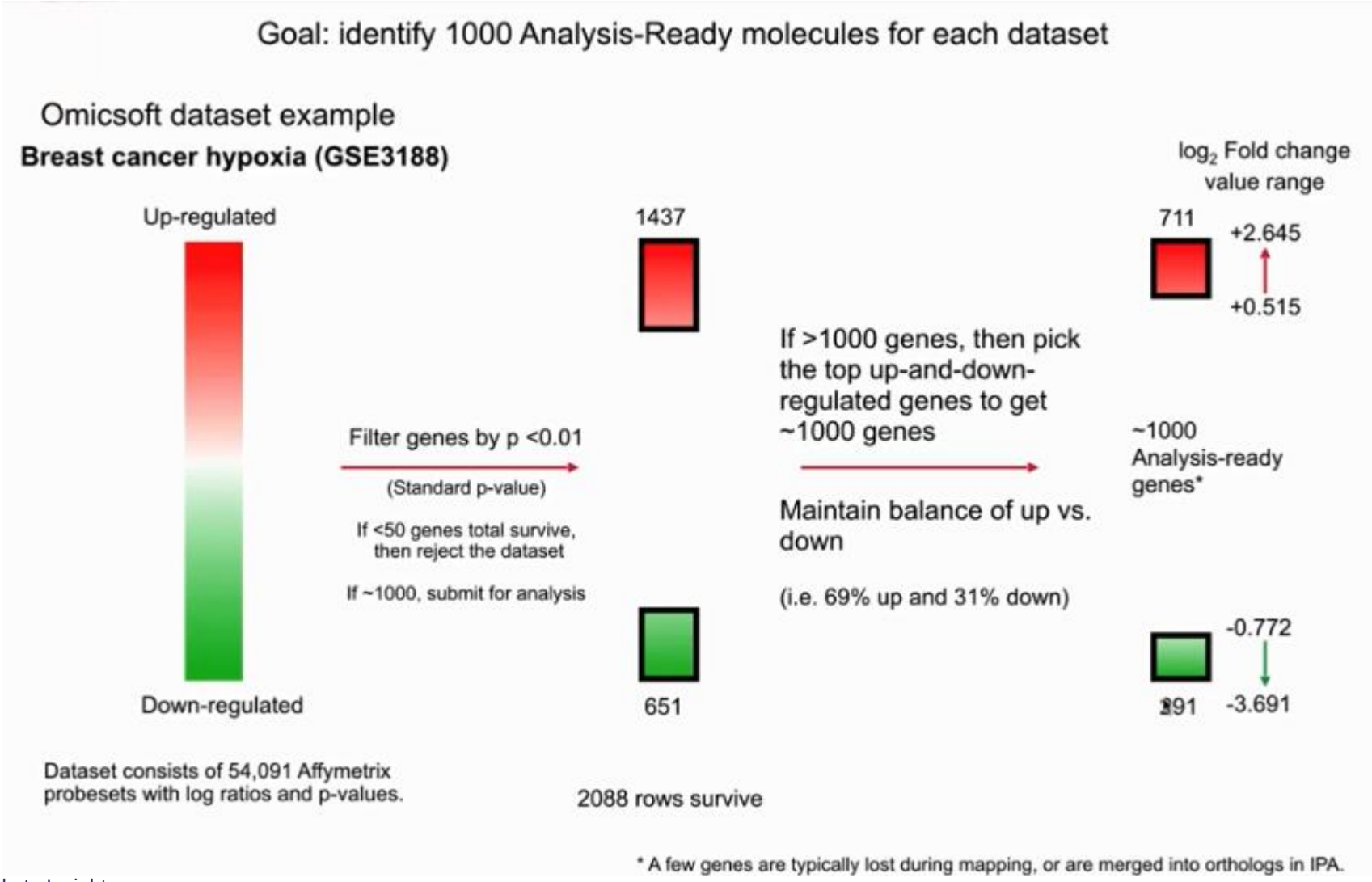
Disease and functions



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Strategy to create the Omicsoft dataset for analyses

Goal: identify 1000 Analysis-Ready molecules for each dataset



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Case study

Metastatic melanoma (PMID 20442294)

- Arises from melanocytes in the epidermis
- Primarily caused by UV-induced DNA damage
- Occurs in skin, eyes and mucosal organs (e.g., intestines, lungs, mouth)
- Often metastasizes to liver, lungs, bones, brain
- Median 5-year survival 15–20%



Photo credit: **Skincancer.org**

- Metastatic melanoma cell line ratioed to normal human epidermal melanocytes skin cell line
- Differential gene expression (Affymetrix microarray)

Source: Philippidou, D. et al. (2010) Signatures of miRNAs and selected miRNA target genes in human melanoma Cancer Research. 70 (10): 4163-73.

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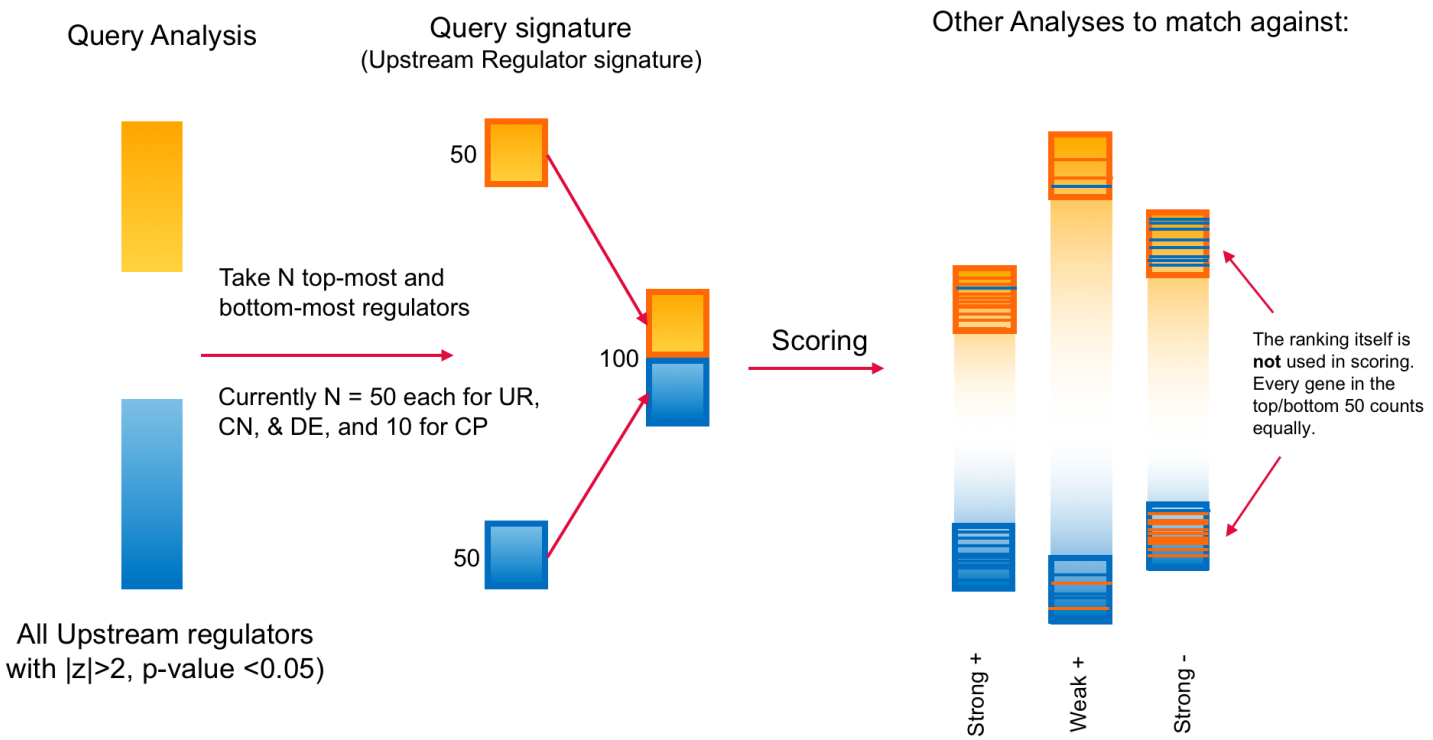
Live Demo

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How signatures are created and compared

Canonical Pathways (up to 20 pathways)
Upstream Regulators (up to 100 regulators)
Causal Networks (up to 100 master regulators)
Diseases & Functions (up to 100 diseases or functions)

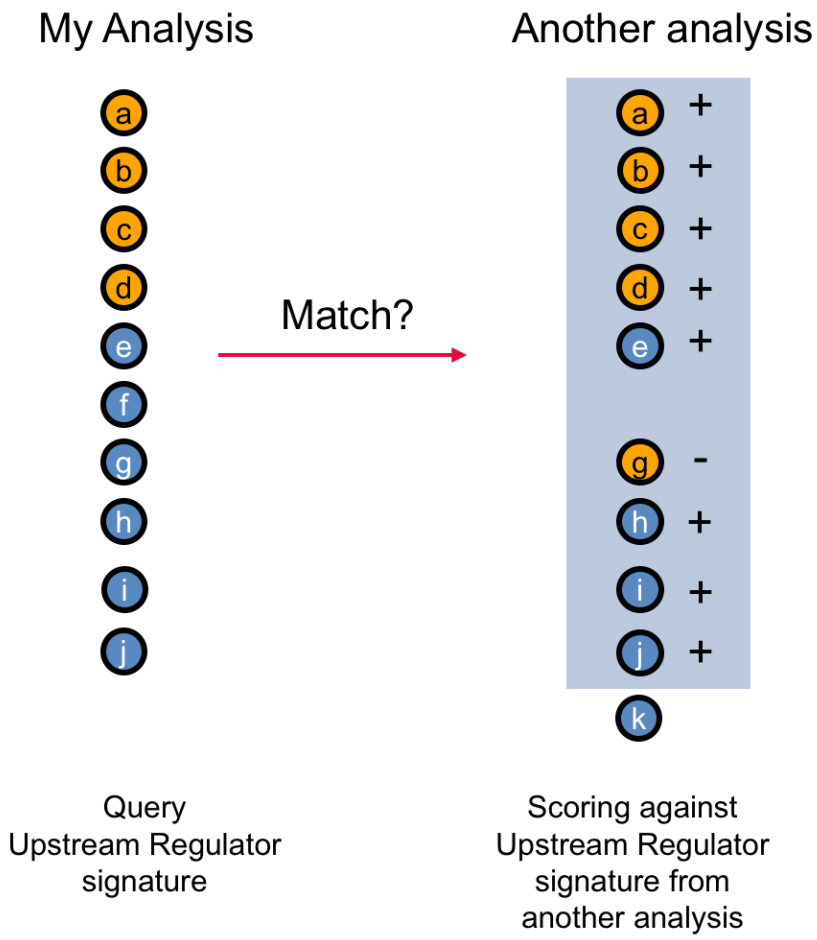
Example



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Analysis Match – explanation of z-score

How signatures are created and compared



$$Z = \frac{\text{matches - mismatches}}{\text{Square root of all matches}}$$

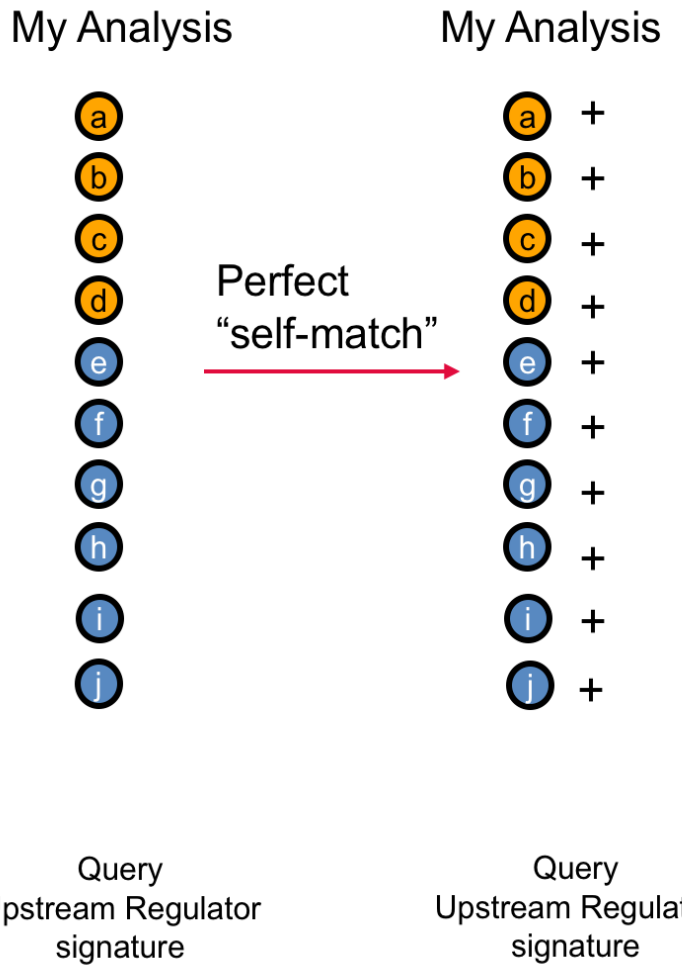
- z-score is a measure of the match between two patterns
- Assumes the pattern is created from two sets of entities where the sign of the matching entities is random

$= (8-1)/\sqrt{9} = 2.33$ (raw z-score)

Yes, it matches (because z>2)

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Steps for normalizing the z-score for a match percentage



First, find out the **max** possible z-score for a perfect match for this particular analysis:
= (10-0)/√10 = 3.16 (It’s really just equal to √N)

Then divide the **actual** z-score for the matching analysis by the calculated **max**.

In this example:
Normalized z-score for the matching analysis is 2.33/3.16 = 74%

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How are Overall scores calculated in Analysis match

For z-score

- Simply take average of percentages for all four entities

CP (z-score)	UR (z-score)	CN (z-score)	DE (z-score)	z-score overall score
50.00	60.91	53.85	40.49	51.31

Average of the four percentages

For p-value

- P-value percentage for entities are not reported
- Take the –log 10 of each p-value (if –log 10 of p-value is >50, then just set it to 50)
- Calculate % vs. the maximum possible p-value (self vs. self match)
- Take average of percentages for all four entities

CP (p-value)	UR (p-value)	CN (p-value)	DE (p-value)	p-value overall score
2.21E-03	1.86E-43	1.11E-35	1.97E-10	45.02

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Live Demo

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Analysis Match allow you to:

1. Obtain and evaluate hypotheses across an extensive collection of public data
2. Build confidence in your analysis result
3. Discover unexpected insights into the biology shared among studies
4. Find “anti-matches” to identify important differences.
5. Develop greater insight into the biological mechanisms driving your experimental results.

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Agenda

- Uncover causality — the next level of pathway analysis
- **Ingenuity Pathway Analysis Advanced Analytics**
  — Analysis functions in IPA AA module
- Case study
  1. Isoprofiler
     快速篩選實驗中目標興趣之Isoform
  2. Phosphoproteomics Analysis
     闡釋磷酸化蛋白分析意義結果
  3. Analysis match
     將您的分析結果與IPA收錄上千筆分析資訊做比較

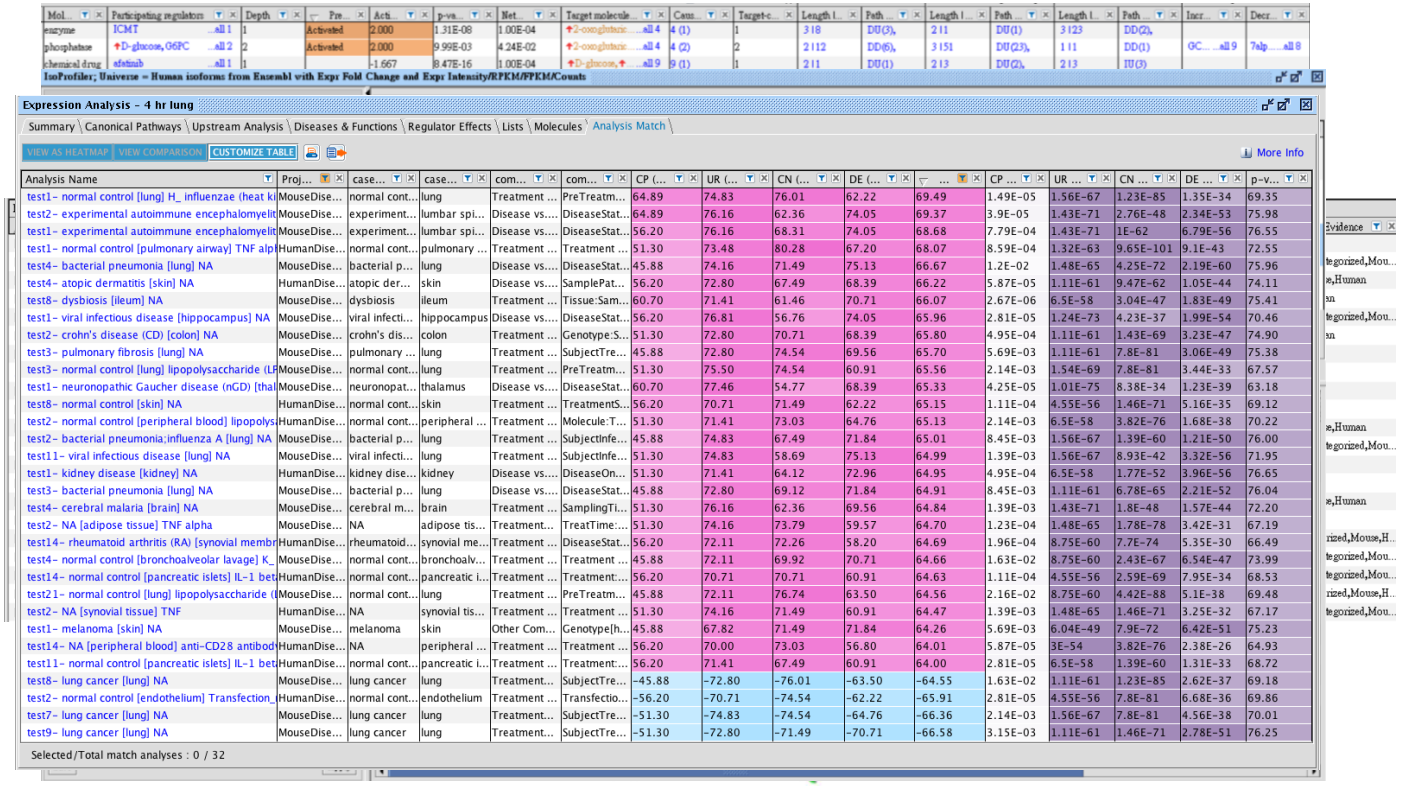
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Advanced Analytics

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.

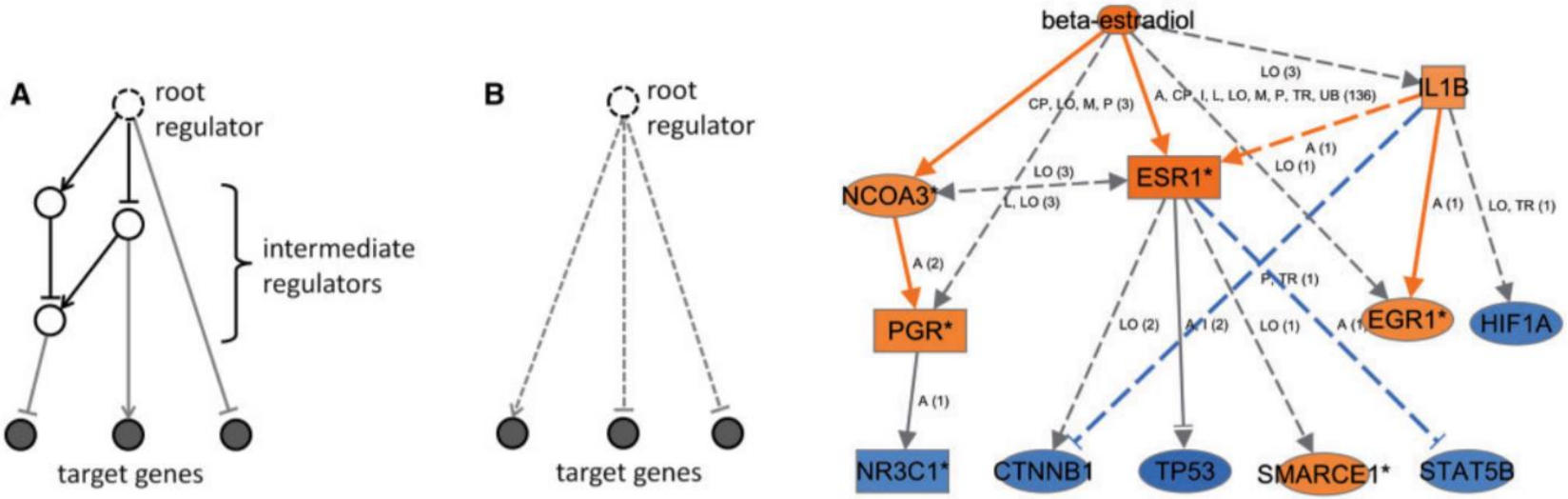
- Causal Network Analysis
- BioProfiler
- Relationship Export
- IsoProfiler
- Phosphoproteomics Analysis
- Analysis Match



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Causal Network

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.



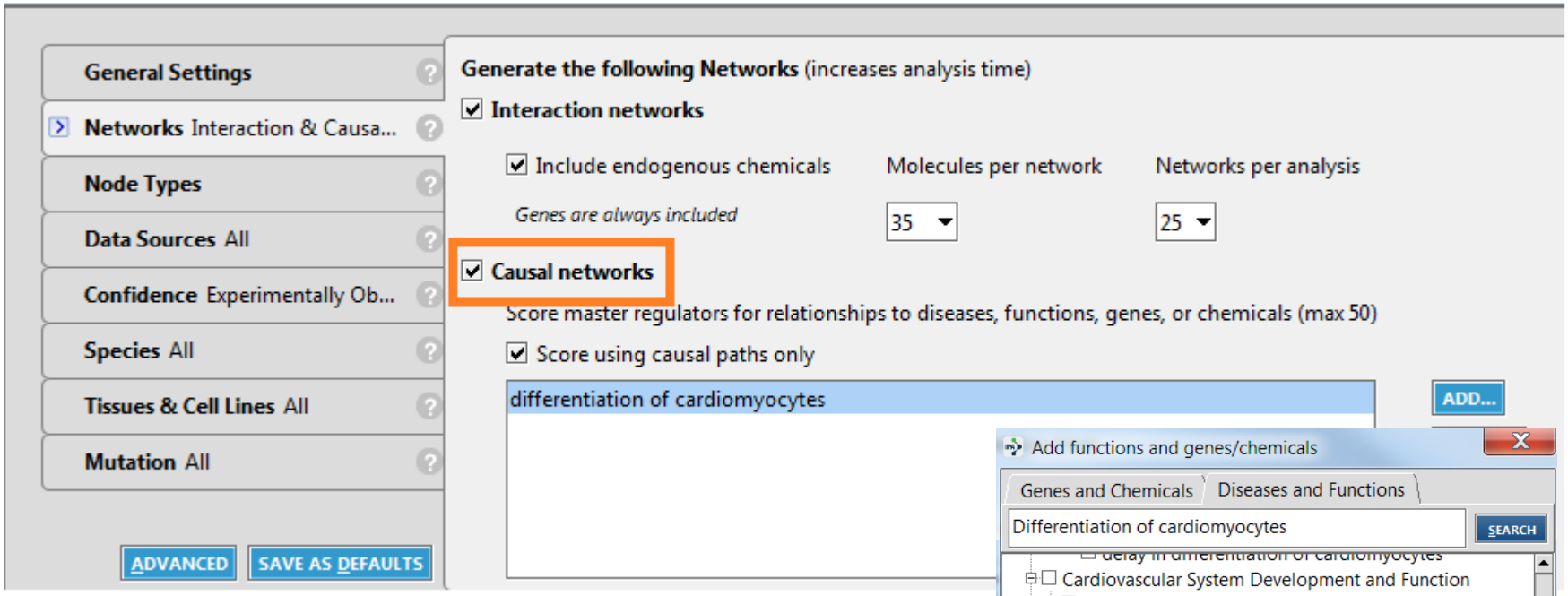
Causal analysis approaches in Ingenuity Pathway Analysis. 2014 *Bioinformatics*

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Causal Network

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.



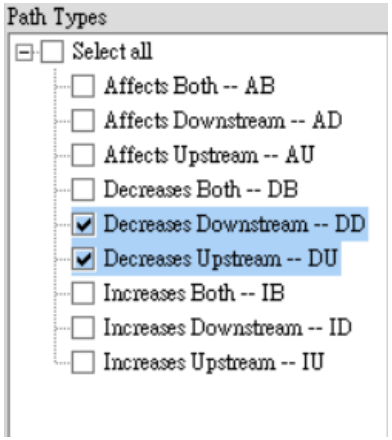
Click on Add to begin searching for specific function, disease or genes and chemicals that you may wish to score against your causal networks.

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Causal Network

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

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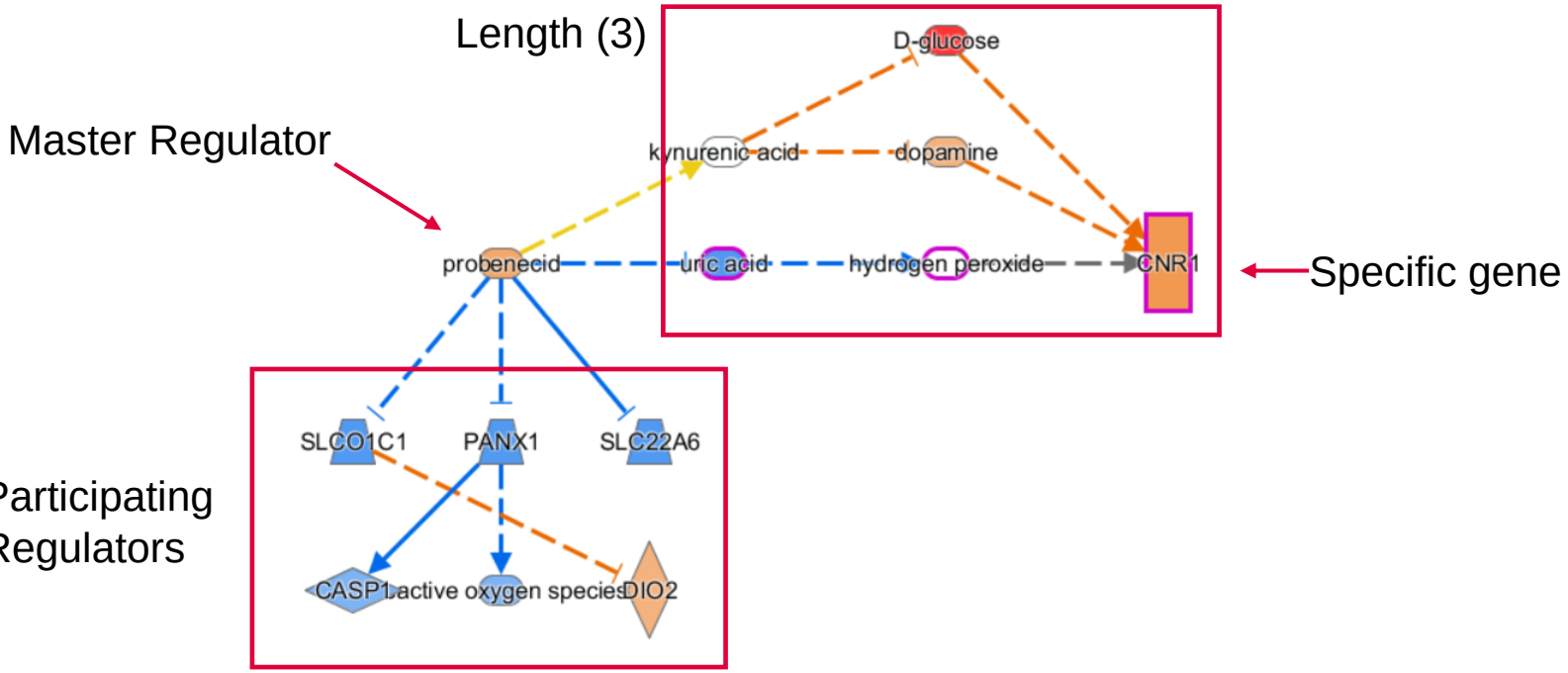
Causal Network

Result

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

Master Regulator and Networks

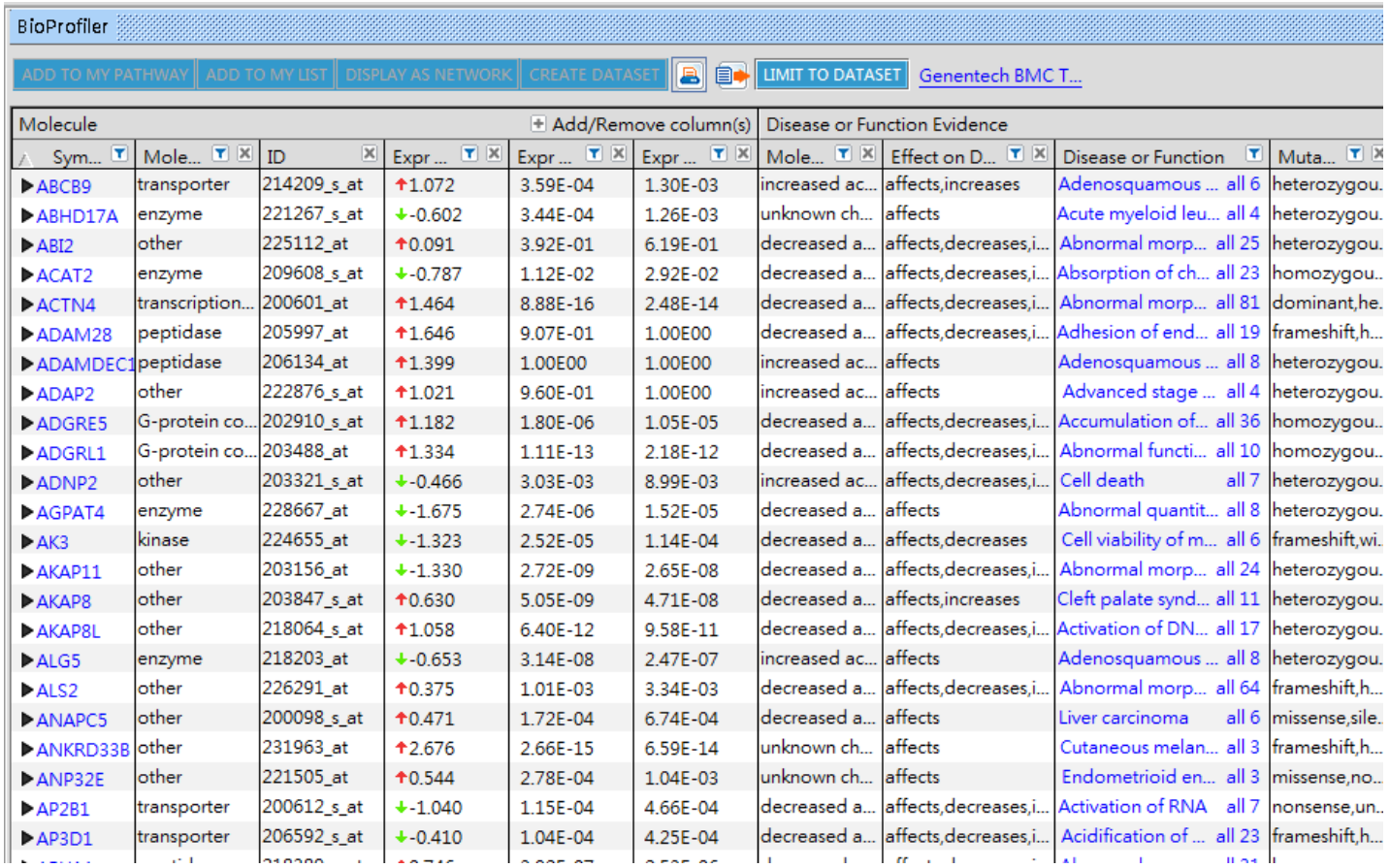
Master ...	Expr...	Mol...	Participating regulators	Depth	Pre...	Acti...	p-va...	CNR1 Length l...	CNR1 Path ...	Diabetes mellitus [diabetes] Length l...	Diabetes mellitus [diabetes] Path ...	Insulin Length l...	Insulin Path ...	Relationships Between M... Incr...	Relationships Between M... Decr...
ICMT		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



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BioProfiler

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.


BioProfiler

ADD TO MY PATHWAY | ADD TO MY LIST | DISPLAY AS NETWORK | CREATE DATASET | LIMIT TO DATASET | Genentech BMC T...

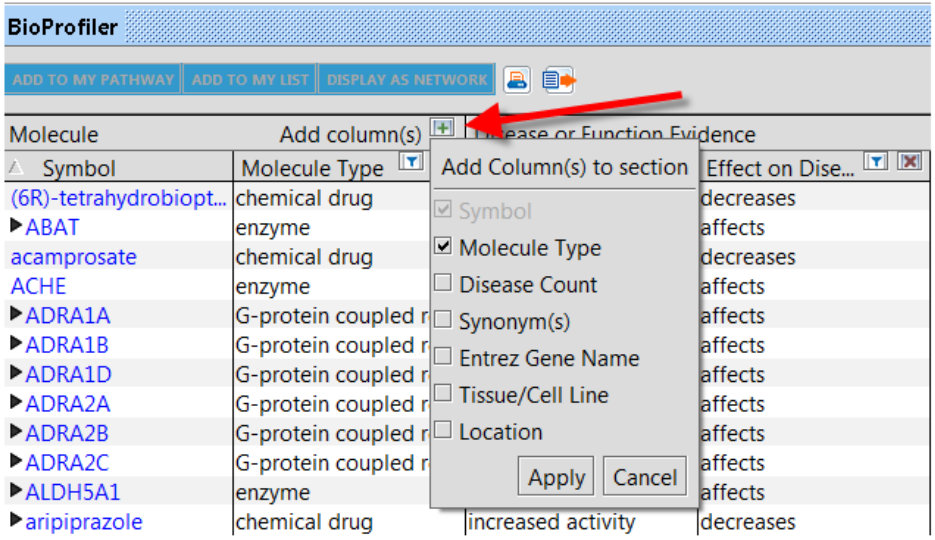
Molecule | Add/Remove column(s) | Disease or Function Evidence

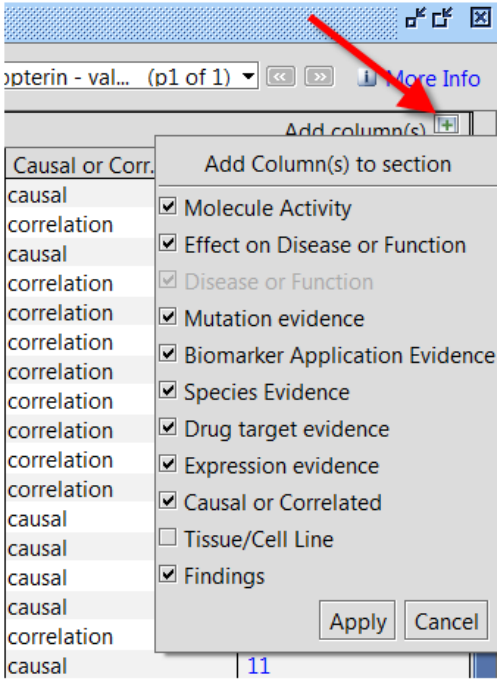
Sym...	Mole...	ID	Expr ...	Expr ...	Expr ...	Mole...	Effect on D...	Disease or Function	Muta...
ABCB9	transporter	214209_s_at	↑1.072	3.59E-04	1.30E-03	increased ac...	affects,increases	Adenosquamous ... all 6	heterozygou.
ABHD17A	enzyme	221267_s_at	↓-0.602	3.44E-04	1.26E-03	unknown ch...	affects	Acute 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...

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When will I use BioProfiler?

- 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?





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Example

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

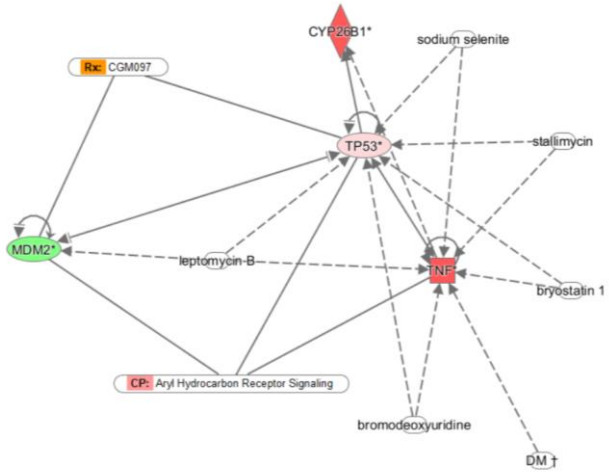
- What kind of **Transporter** express in **Liver**?
  - ABCD2, HBZ

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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.

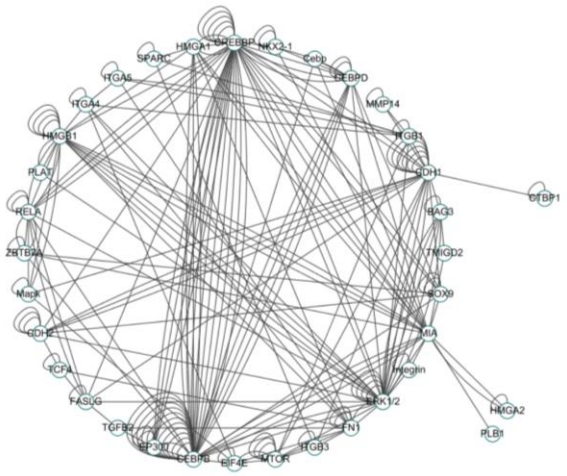
The export format contains relationships modeled as triples: Node A --> Relationship -->Node B



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

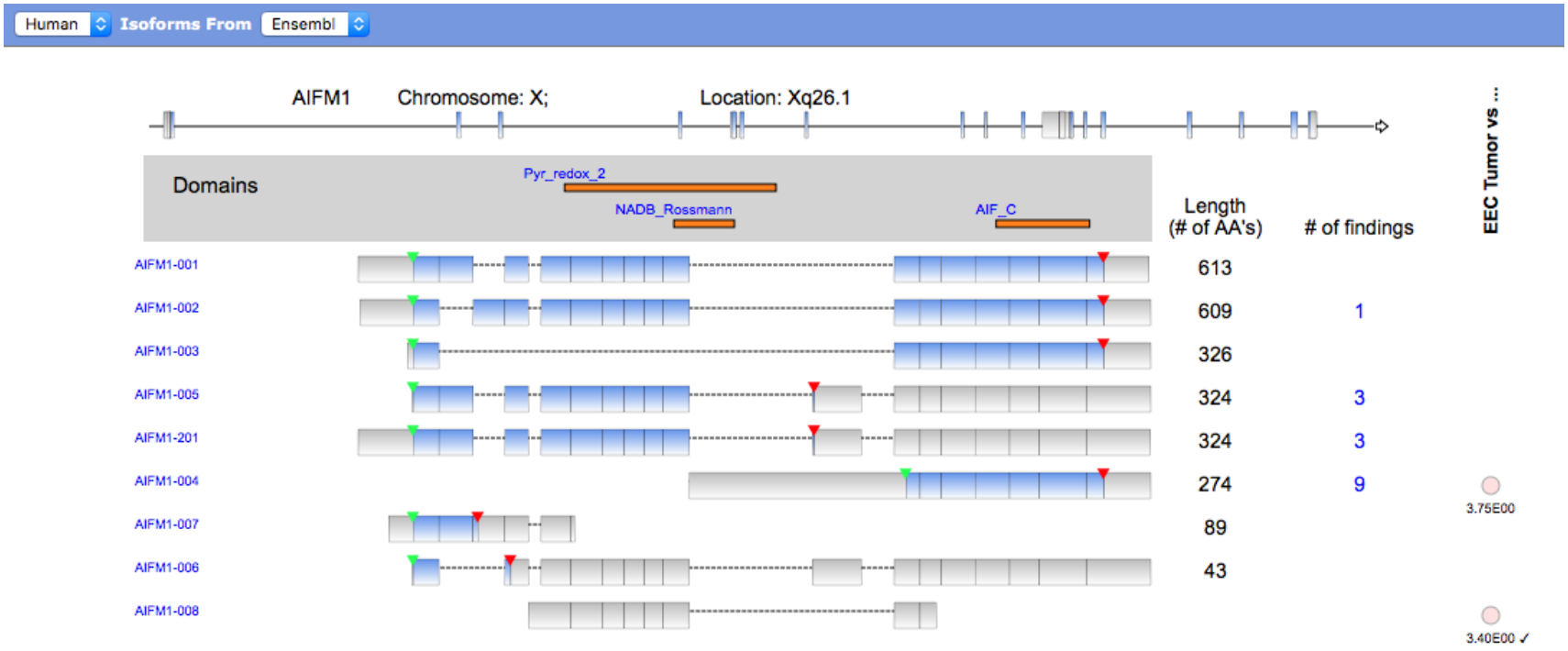
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IsoProfiler

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

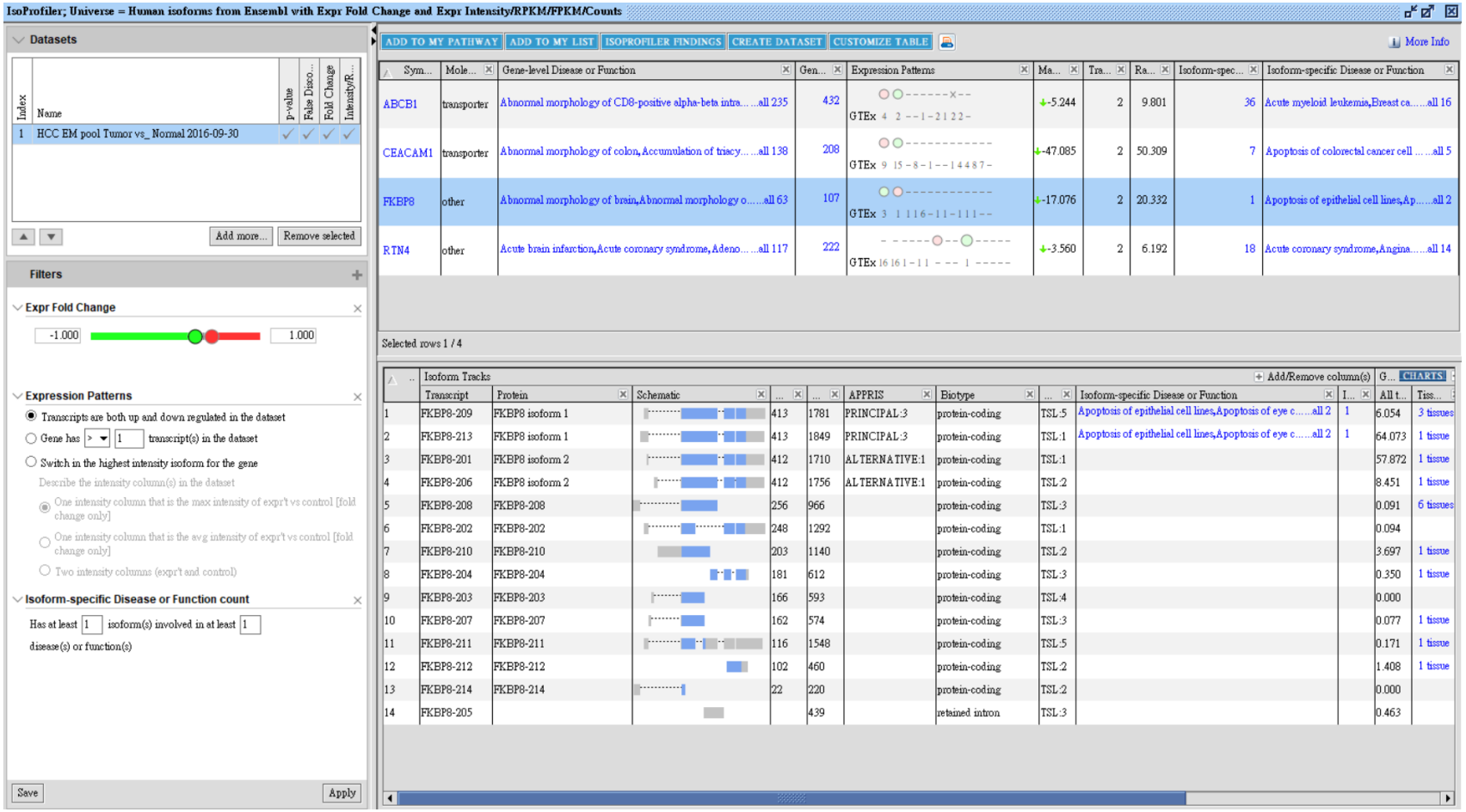
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IsoProfiler

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

Dataset Chooser

Genes and their isoforms from the dataset



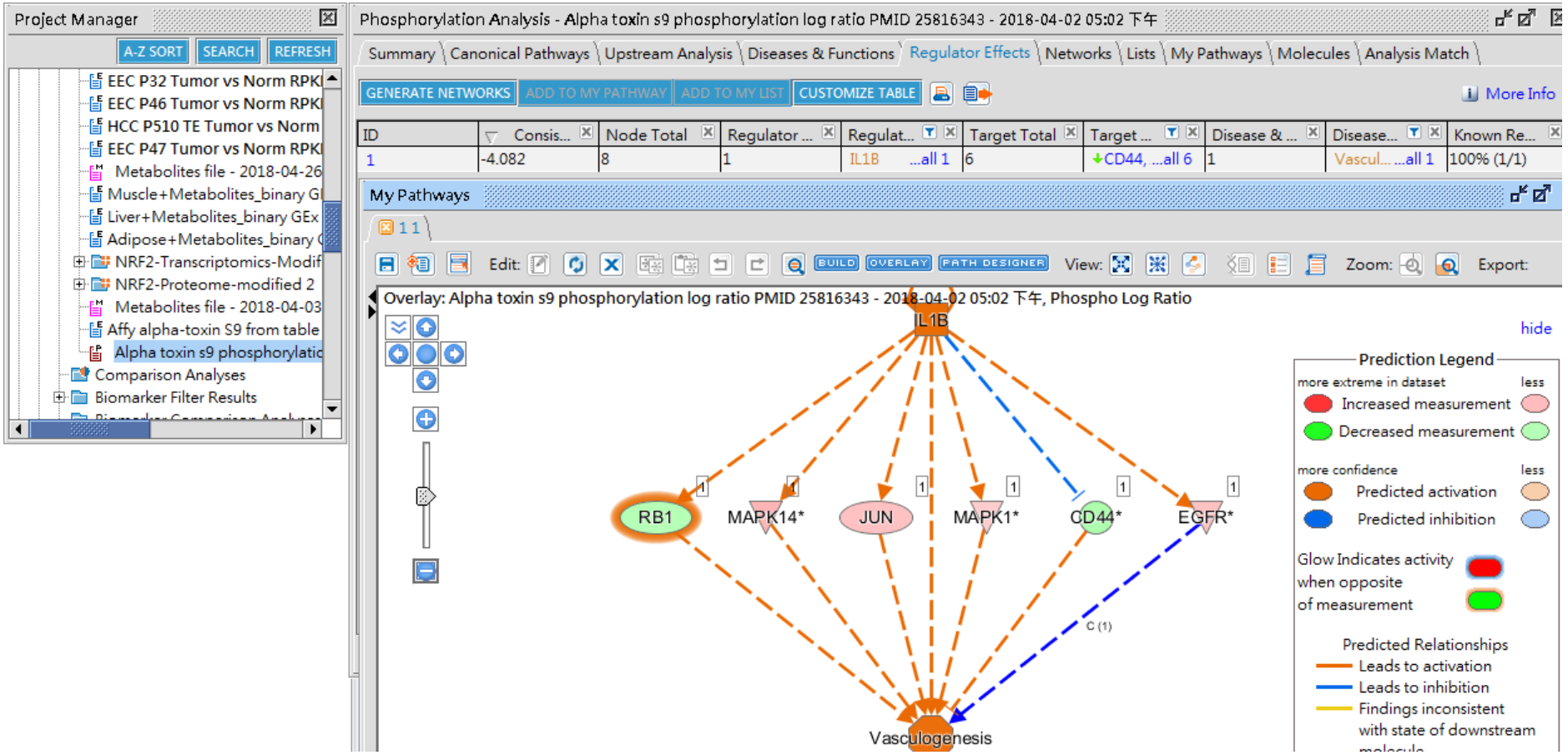
Isoform filters

Isoform details on the selected gene

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PhosphoProteomics Analysis

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.

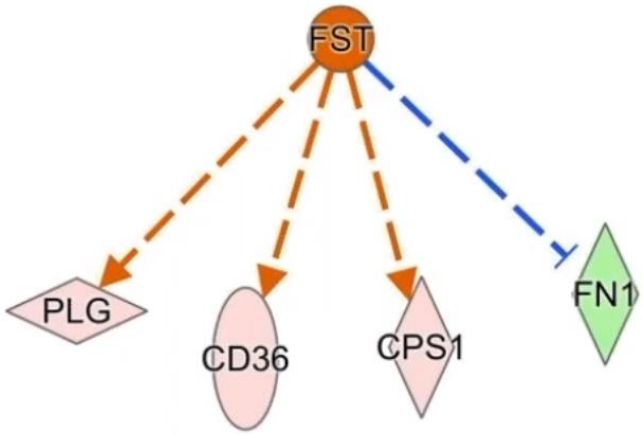


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PhosphoProteomics Analysis

Gene expression data:

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

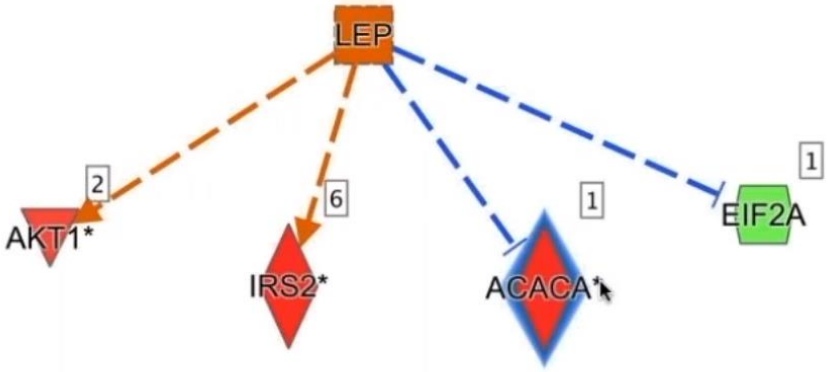


PLG, CD36, CPS1 are *up-regulated* by *activated* PST

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

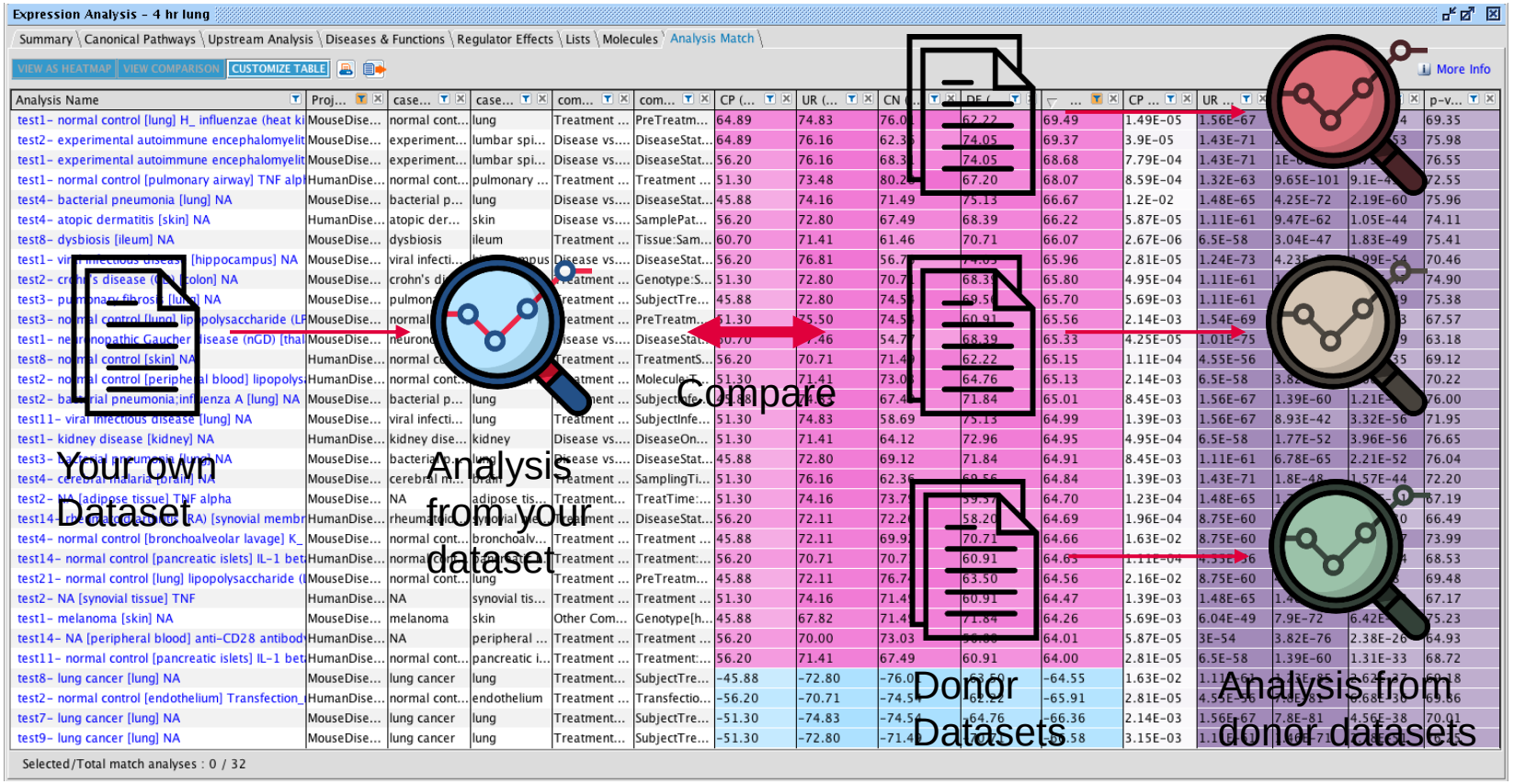
ACACA has *increased* phosphorylation from *activated* LEP

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

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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.



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Agenda

- Case study

1. Isoprofiler

   Identifying key isoforms in your RNA-seq

   Example datasets: EEC & HCC

   快速篩選實驗中目標興趣之Isoform

2. Phosphoproteomics Analysis

   Interpreting the Results of Phosphoproteomics Analysis

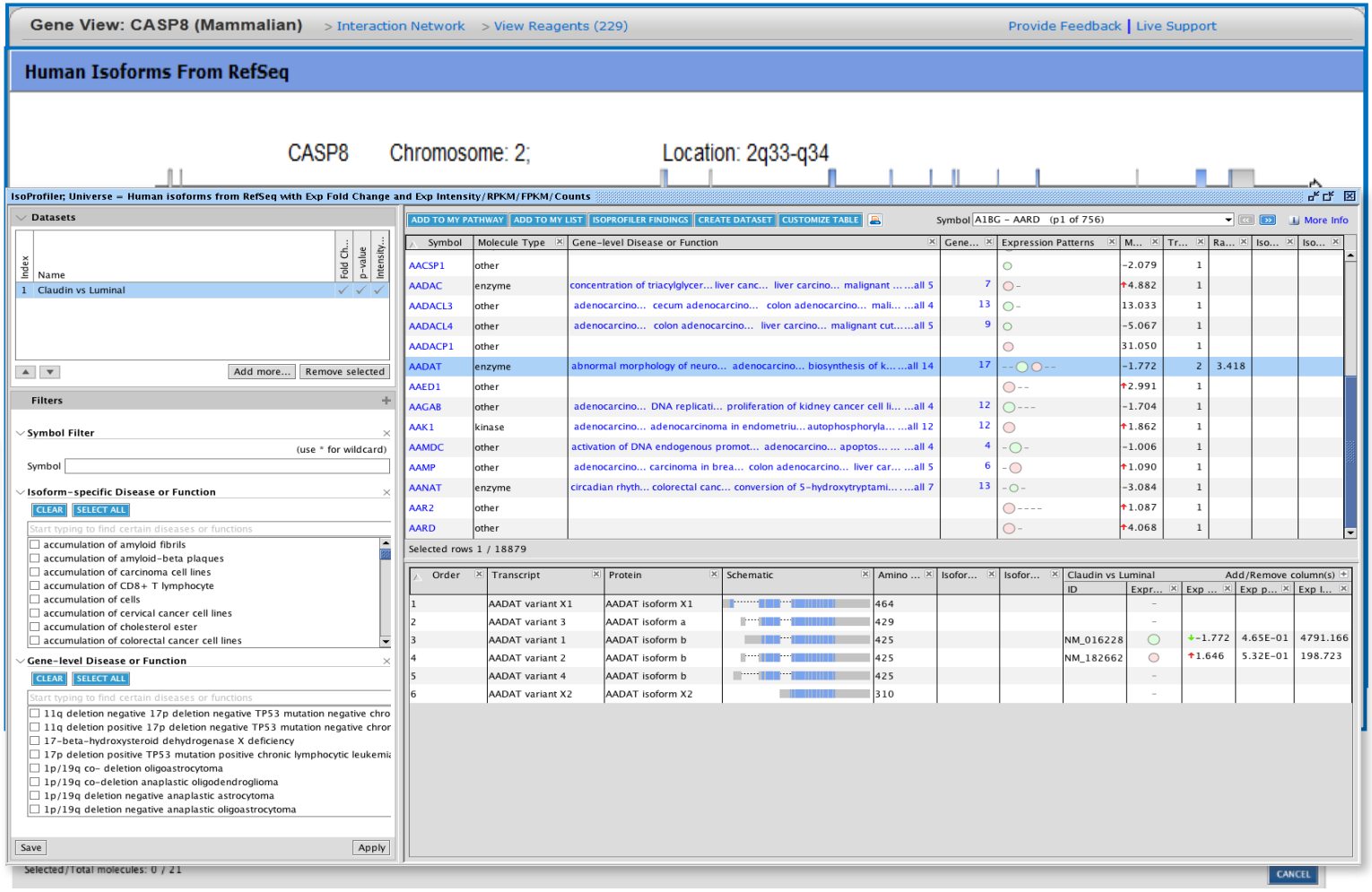
   Example datasets: Alpha toxin treatment in S9 cell

   闡釋磷酸化蛋白分析意義結果

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Tools for the analysis of RNA-seq Data in IPA

- Gene View Summaries
- Human & Mouse Isoform Views
- IsoProfiler
- IsoProfiler Findings



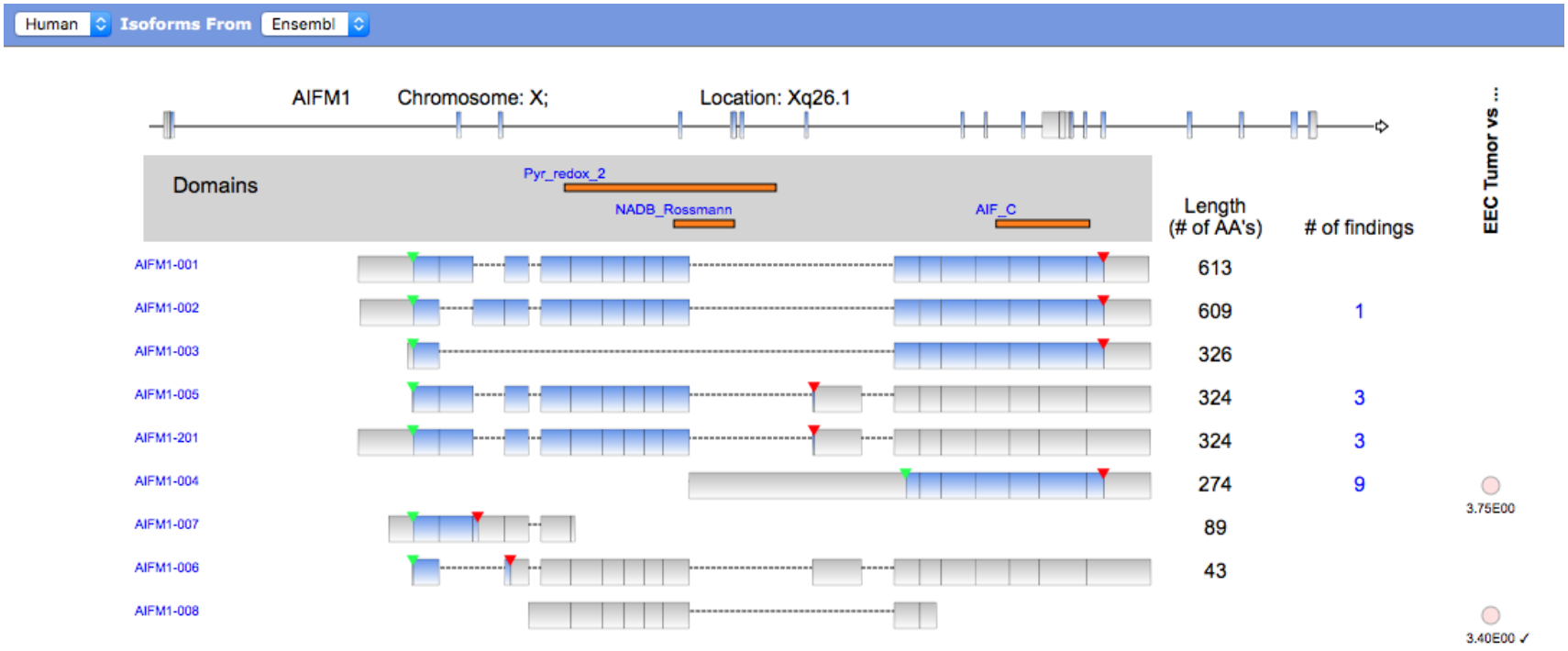
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IsoProfiler

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**.

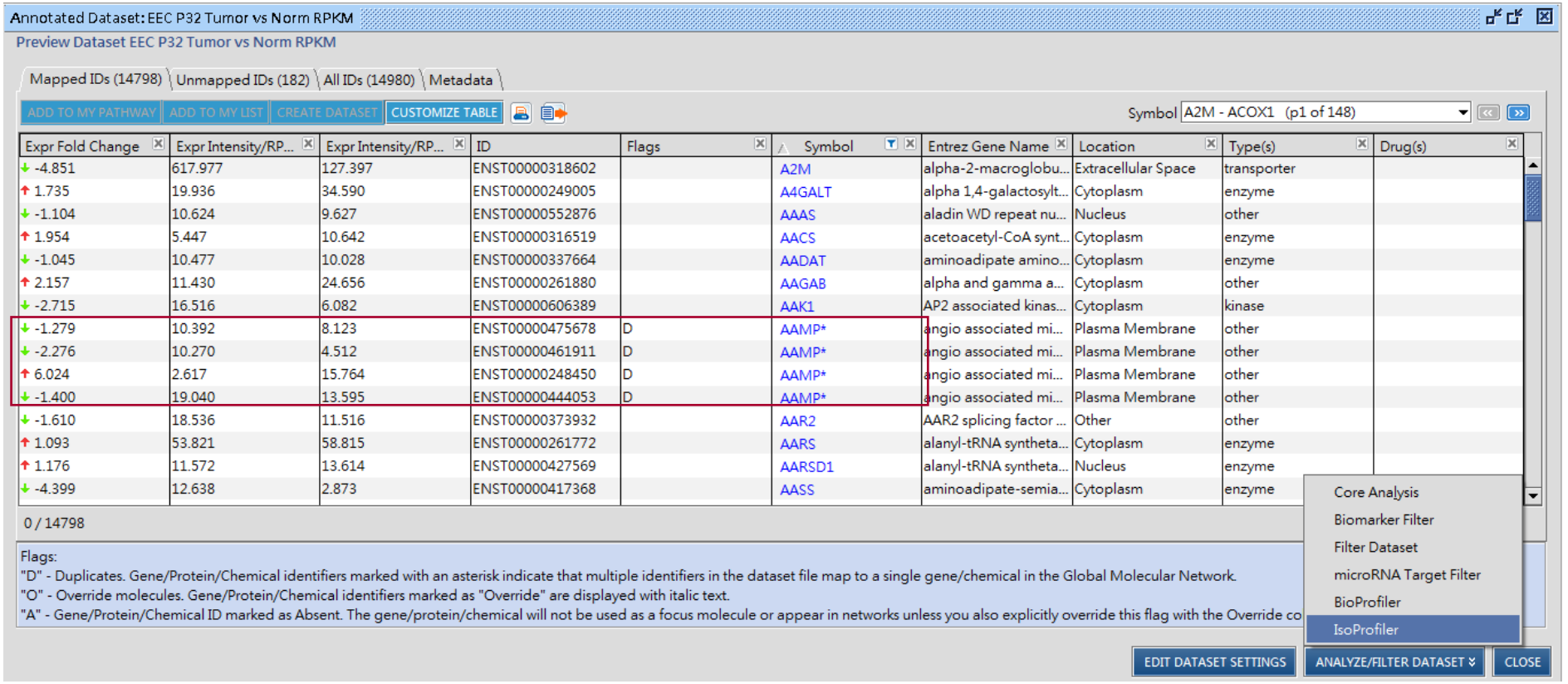


Ensembl ID with isoform viewer in IPA

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Dataset Analysis with Isoprofiler

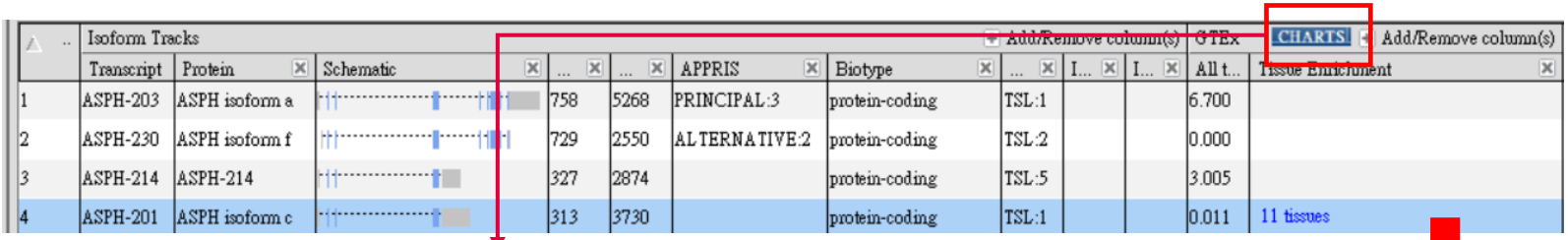
Without Isoprofiler Analysis, different treanscript ID with same gene name can only analysis with the one which has the max expression value.



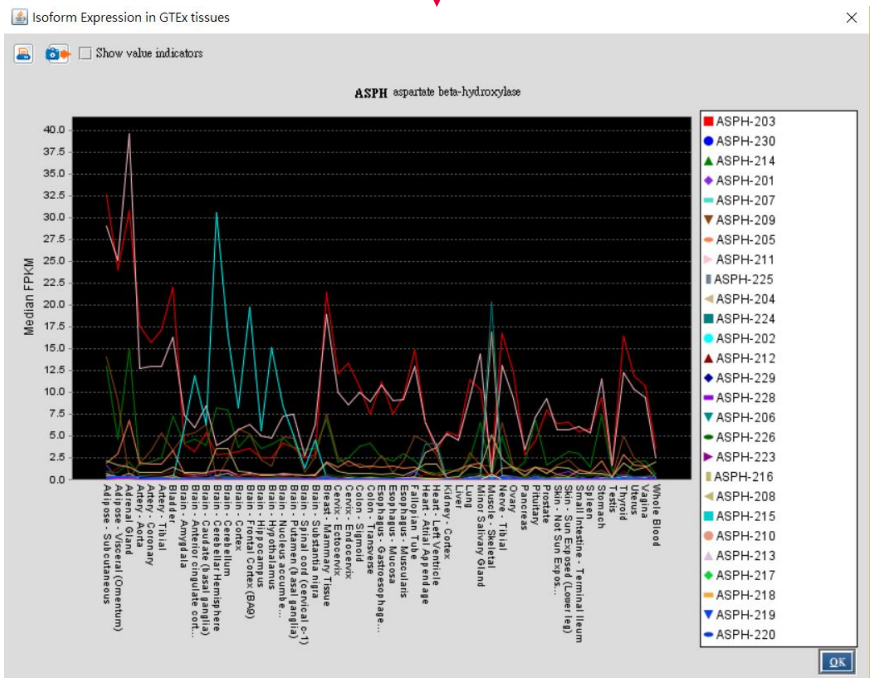
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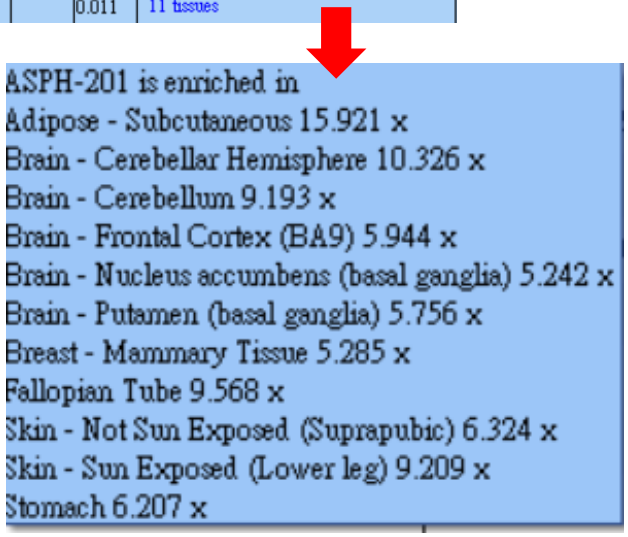
IsoProfiler with **GTEx Tissue Expression**

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.



	Isoform Tracks										GTEx	CHARTS	Add/Remove column(s)
	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	



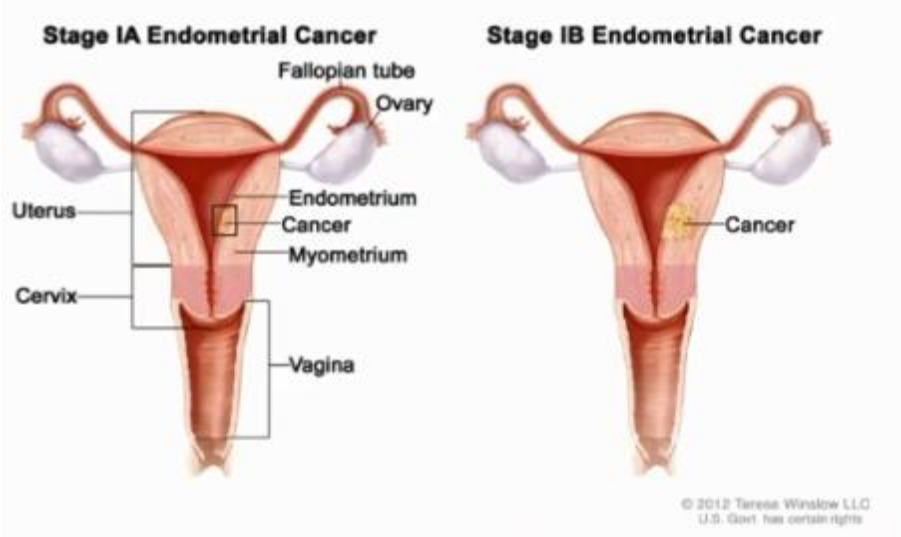


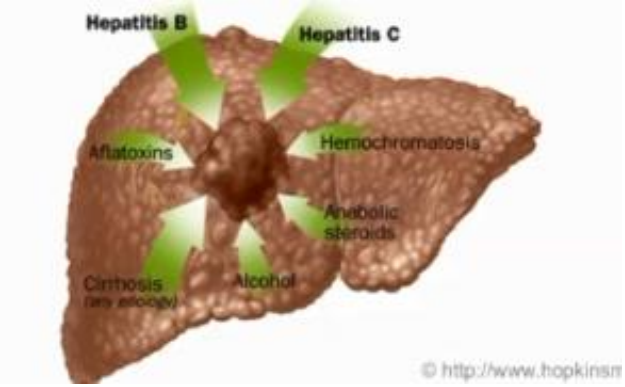
The GTEx consortium has performed over 9,800 paired-end RNA-Seq experiments across 51 human tissues

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Two datasets:

- Endometrioid Endometrial Adenocarcinoma (EEC)
  - Most common gynecological cancer in Europe & North America
  - 75% of diagnosed women have disease confined to the uterus (early, stage I)
- Hepatocellular carcinoma (HCC)
  - One of the most common types of cancer
  - 3rd most common cause of cancer-related deaths worldwide



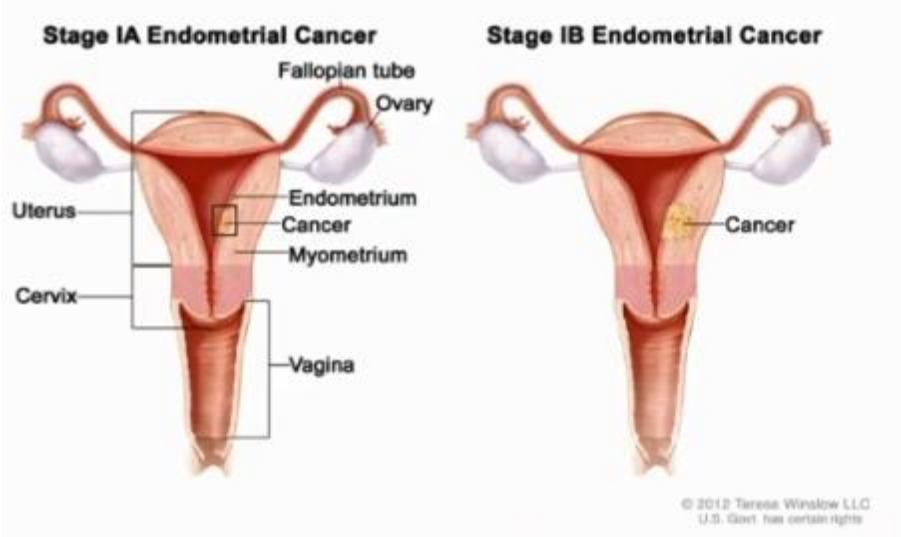


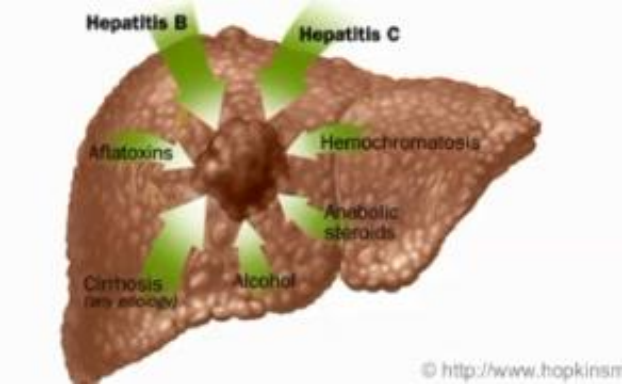
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Two datasets:

- RNA-seq datasets
  - Three EEC patients (SRP0456)

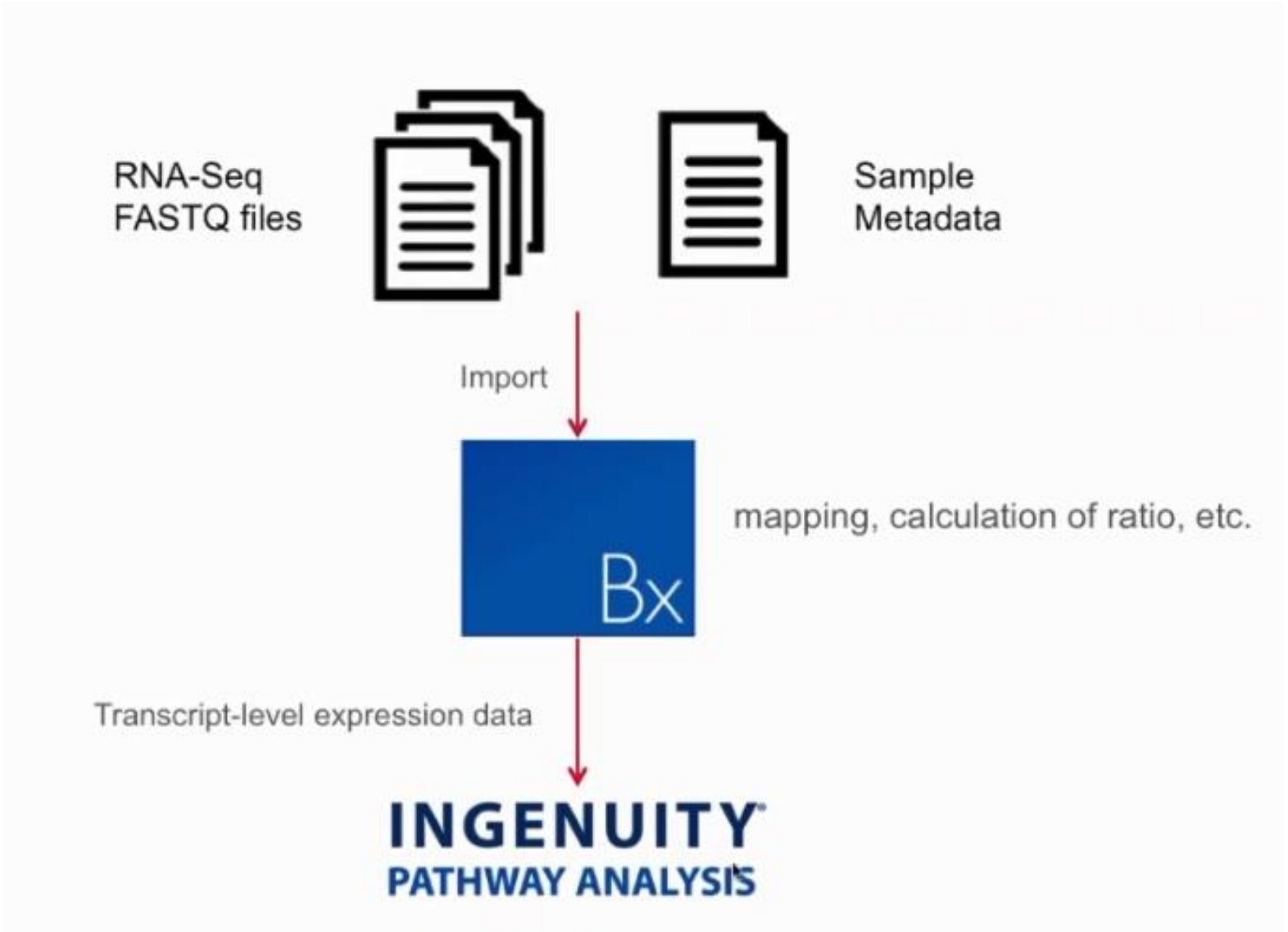
    --- Two stage 1A, one stage 1B
  - Three HCC patients (GSE33294)
  - Tumorous vs. adjacent normal tissue





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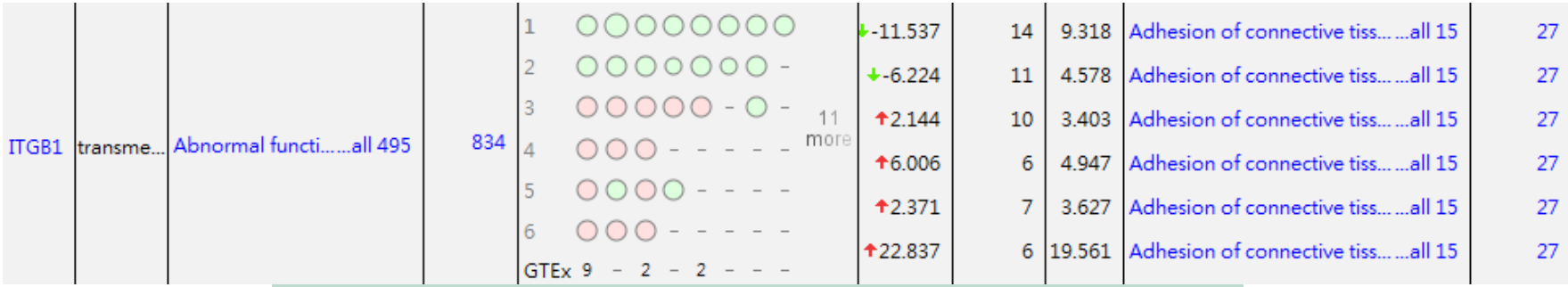
RNA-seq Solution



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Questions that can be solved by Isoprofiler

- Focus on important attributes of the isoforms
  - Which isoforms are protein-coding and associated with disease or function?
- Discover genes with isoforms having unusual expression patterns
  - Can we identify cases if isoform switching?
- Explore the isoform-specific disease/function associations
  - How can we find the most relevant associations?



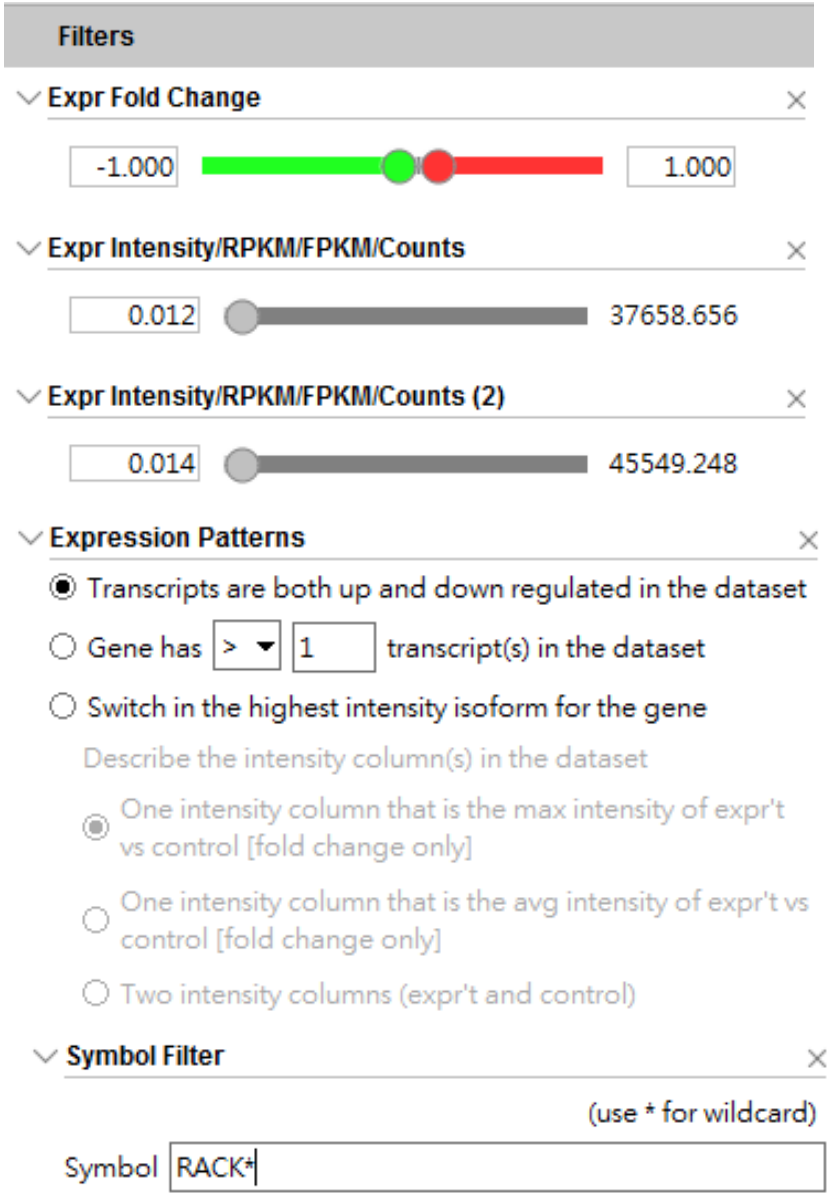
All Questions can be solved by the right setting filter

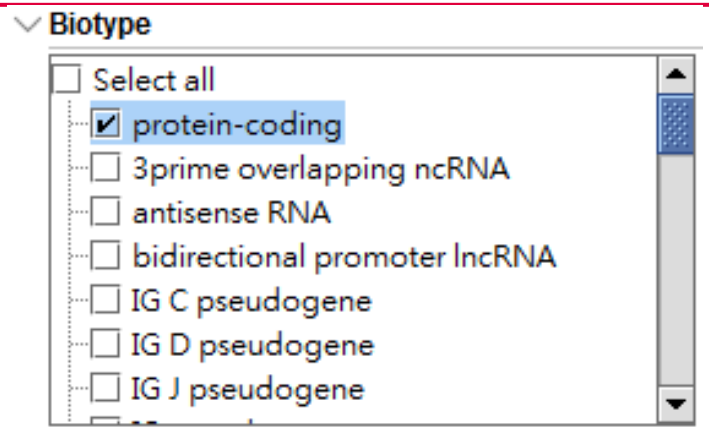
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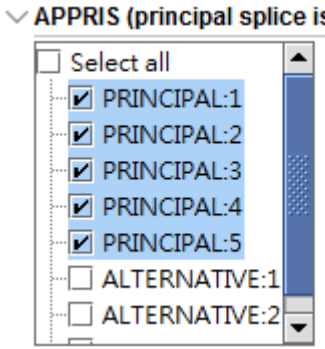
Live Demo

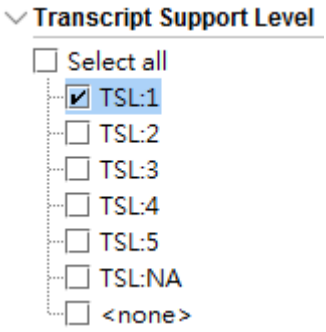
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Isoprofiler filter panel







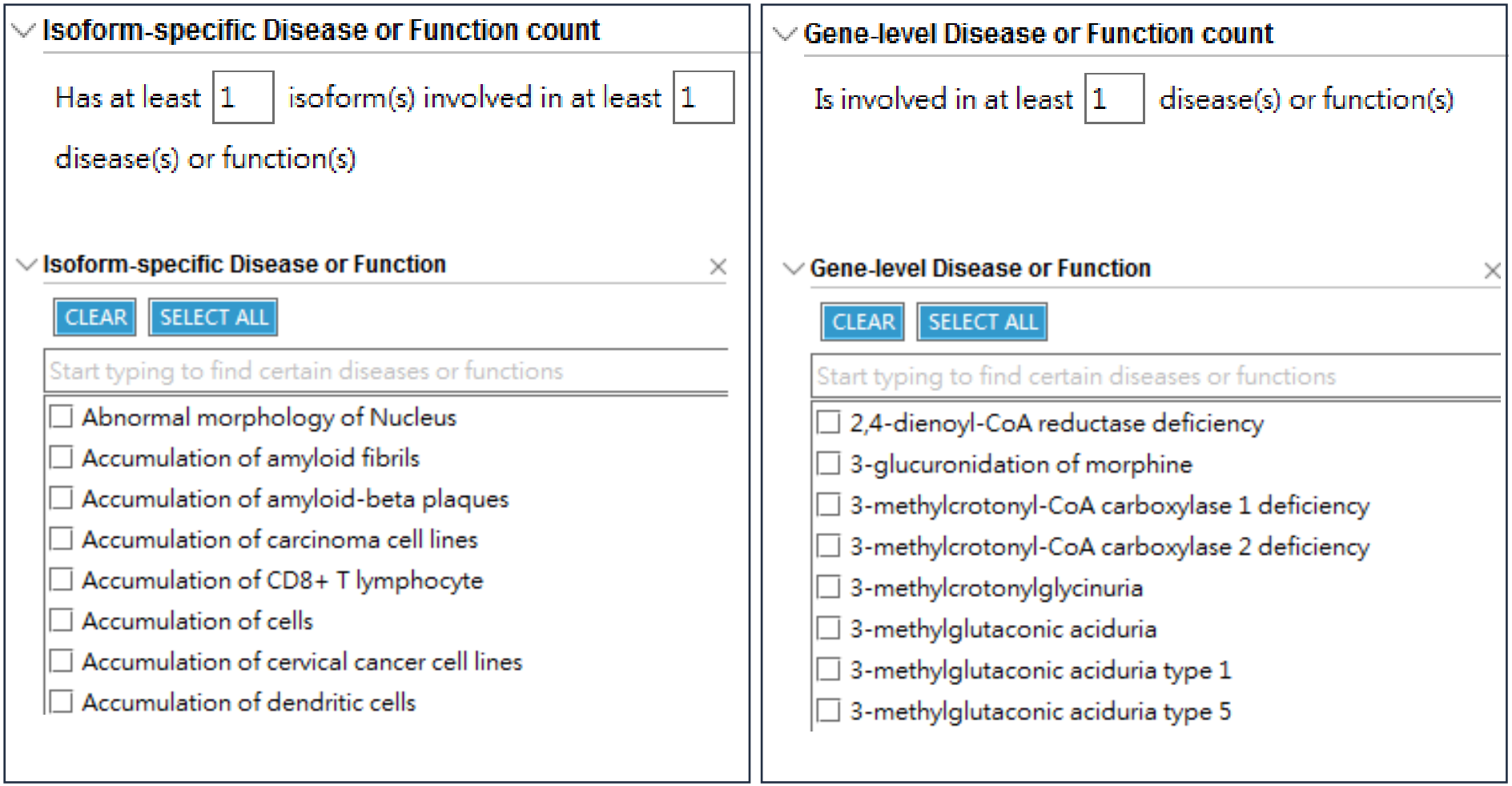


Column name	Applicable to identifier source
Nucleotides	Ensembl only
APPRIS	All three
Biotype	Ensembl only
TSL	Ensembl only
Transcript Type	RefSeq datasets only

Protein coding or non protein-coding. The value is automatically inferred from the identifier prefix: NM, XM = protein-coding, whereas NR, XR = non protein-coding.

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Isoprofiler filter panel



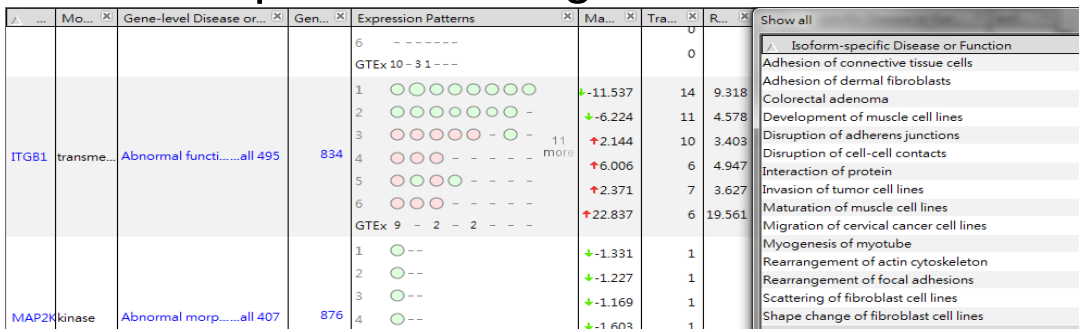
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Live Demo

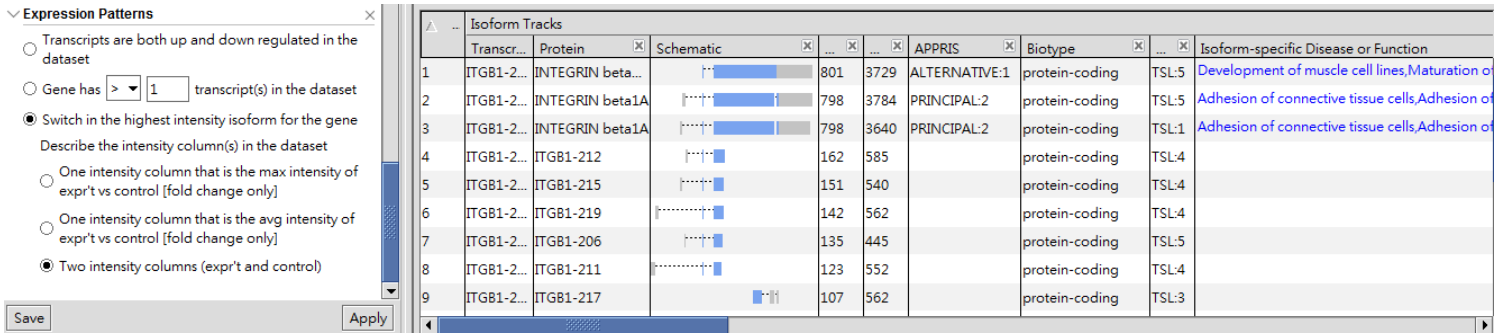
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Summary

- Focus on important attributes of the isoforms
  - Which isoforms are protein-coding and associated with disease or function?



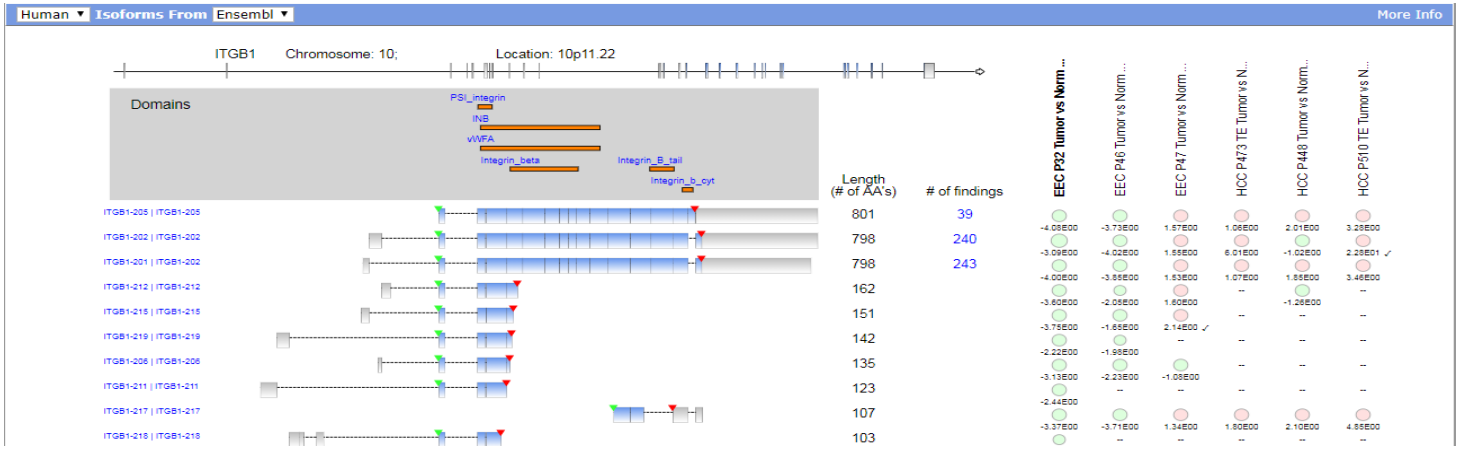
- Discover genes with isoforms having unusual expression patterns
  - Can we identify cases if isoform switching?
- Explore the isoform-specific disease/function associations
  - How can we find the most relevant associations?



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Summary

- Used the Gene View Page
  - To learn more about the gene and its isoforms



- Used the IsoProfiler Findings
  - To explore the details about isoform-specific disease or function findings

Molecule						Add/Remove column(s)	Disease or Function Evidence					
...	T...	P...	...	...	S...	...	Effect on Dis...	Dise...	...	Bi...	S...	D...
▼ITGB1	ITGB all 3	INTE all 2	protein i...	transme...	Human	increase...	affects,decreases,inc...	A... all 15	wild type	not appl...	Mouse,...	not appl...
	ITGB all 2	INTE all 1	protein i...		Human	increase...	increases	Dis... all 1	wild type	not appl...	Mouse	not appl...
	ITGB all 2	INTE all 1	protein i...		Human	increase...	increases	Sh... all 1	wild type	not appl...	Mouse	not appl...
	ITGB all 2	INTE all 1	protein i...		Human	increase...	increases	Dis... all 1	wild type	not appl...	Mouse	not appl...
	ITGB all 2	INTE all 1	protein i...		Human	increase...	increases	Re... all 1	wild type	not appl...	Mouse	not appl...

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Agenda

- Case study

  1. Isoprofiler

     Identifying key isoforms in your RNA-seq

     Example datasets: EEC & HCC

     快速篩選實驗中目標興趣之Isoform

  2. Phosphoproteomics Analysis

     Interpreting the Results of Phosphoproteomics Analysis

     Example datasets: Alpha toxin treatment in S9 cell

     闡釋磷酸化蛋白分析意義結果

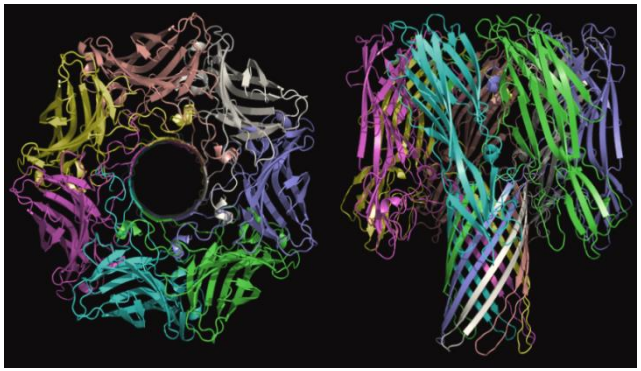
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Case study

Alpha-toxin, alpha-hemolysin (Hla)

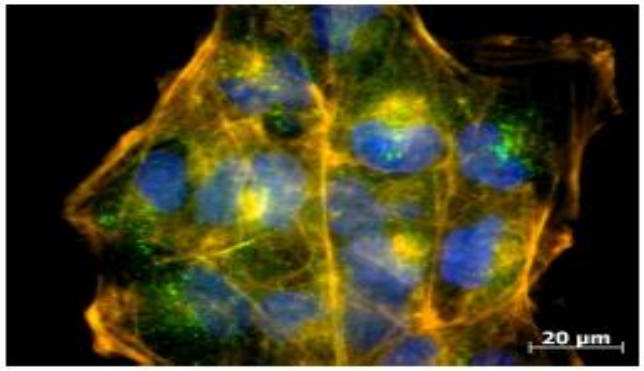
- The major pore-forming cytotoxin released by most *Staphylococcus aureus* strains
- The key factor in the pathogenesis of S. aureus diseases, including pneumonia





Human bronchial epithelial cell lines

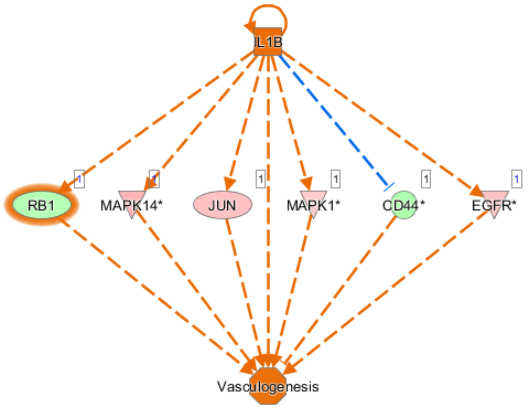
- Alpha toxin treatment in S9 cell
- Quantitative Mass spectrometry
- Phosphoproteomics analysis to highlight critical pathways affected by Hla-treatment.



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Questions that can be search in phosphoproteomics

- **Signaling and metabolic canonical pathways are enriched in the data?**
- **Predicted upstream regulator are significant?**
- **Disease and biological functions are over-represented?**
- **Molecular Networks (potential molecular interactions) pinpoint important molecules in the system**
- **Molecules and biological process are predicted to be activated or inhibited?**



Upstream R...	Phospho L...	Molecule T...	Predicted ...	Activation z-sc...	p-value of ove...	Target mol...	Mechanisti...
MAP2K4		kinase	Activated	2.181	4.52E-03	↑JUN, ↑M... ...all 5	
IFNG		cytokine	Activated	2.229	1.40E-03	↓ABL2, ↓... ...all 14	32 (8)
IL1B		cytokine	Activated	2.319	2.48E-03	↓CD44, ↑.....all 12	29 (9)
sirolimus		chemical drug	Activated	2.347	2.68E-02	↓AKT1S1, ↑.....all 8	
sodium arsenite		chemical drug	Activated	2.000	6.06E-03	↑EGFR, ↑J... ...all 4	28 (7)
MTOR		kinase	Inhibited	-2.156	1.54E-01	↓AKT1S1, ↓.....all 5	
ROCK1		kinase	Inhibited	-2.000	4.81E-02	↓DSTN, ↓... ...all 4	
GH1		growth factor	Inhibited	-2.462	5.70E-11	↓AHNAK, ↓....all 26	
CDK2		kinase	Inhibited	-2.284	1.45E-02	↓HIST1H... ...all 10	
CSNK2A1		kinase	Inhibited	-2.268	8.58E-07	↓ATP2B1, ↓....all 28	
propranolol		chemical drug	Inhibited	-2.000	1.73E-03	↑BAD, ↓C... ...all 4	
phorbol esters		chemical - other	Inhibited	-2.219	2.90E-03	↓EIF3A, ↓... ...all 5	
dihydrotestosteron		chemical - endoge...	Inhibited	-2.101	2.54E-04	↑ACACA, ↑.....all 8	37 (7)

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Upstream regulator Analysis in Phosphoproteomics

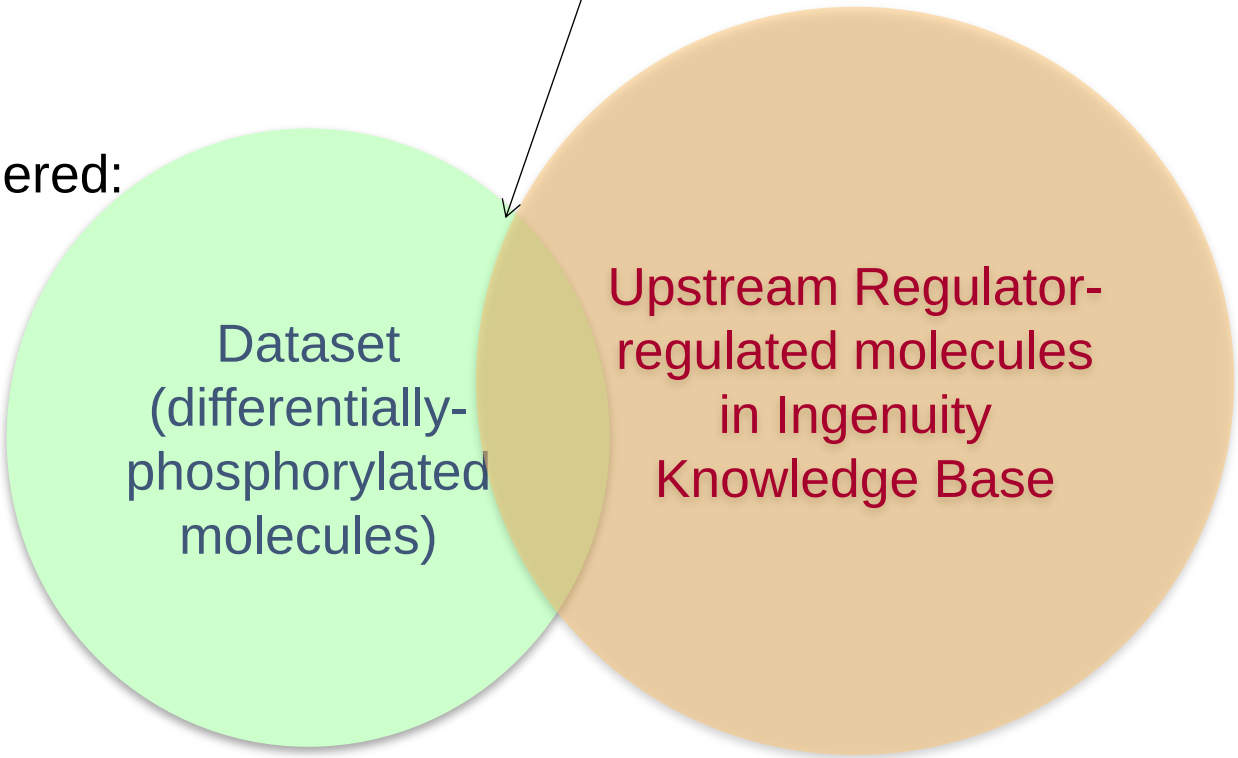
Upstream Regulator → Any molecule that
Has a downstream effect.

- Transcription factor
- miRNAs
- Drugs
- Compounds, etc.

Target edge type considered:

- Phosphorylation

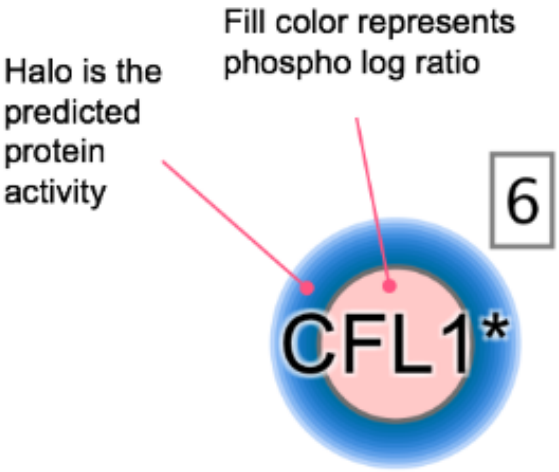
Evaluate the perturbed genes
Un the dataset that are known
Targets of a particular regulator.



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PhosphoProteomics Analysis

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



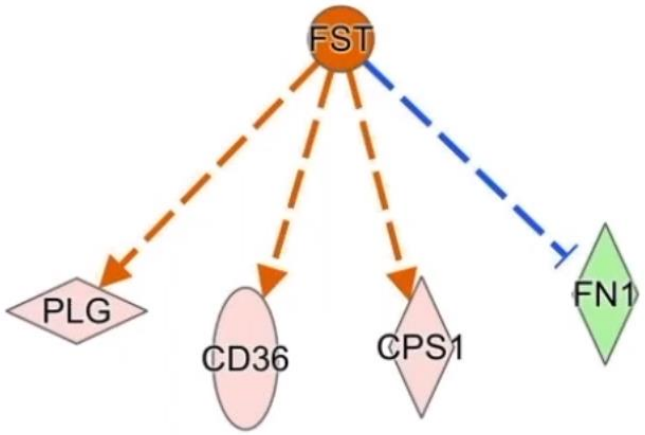
GSK3B has *decreased* phosphorylation in dataset but prediction of *increased* protein activity

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.

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Gene expression data:

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

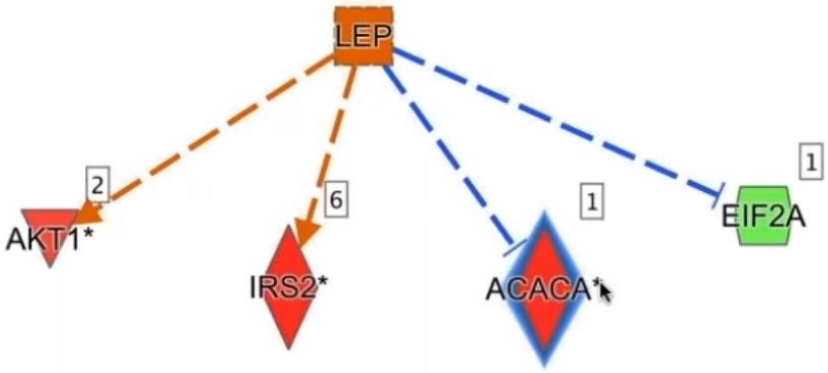


PLG, CD36, CPS1 are *up-regulated* by *activated* PST

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

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Live Demo

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Questions that address today

Canonical Pathways

- Signaling metabolic canonical pathways enriched in the data.

Upstream Analysis

- Predicted upstream regulators and their interactions.

Disease and Functions

- Over-represented diseases and biological functions.

Regulator Effects

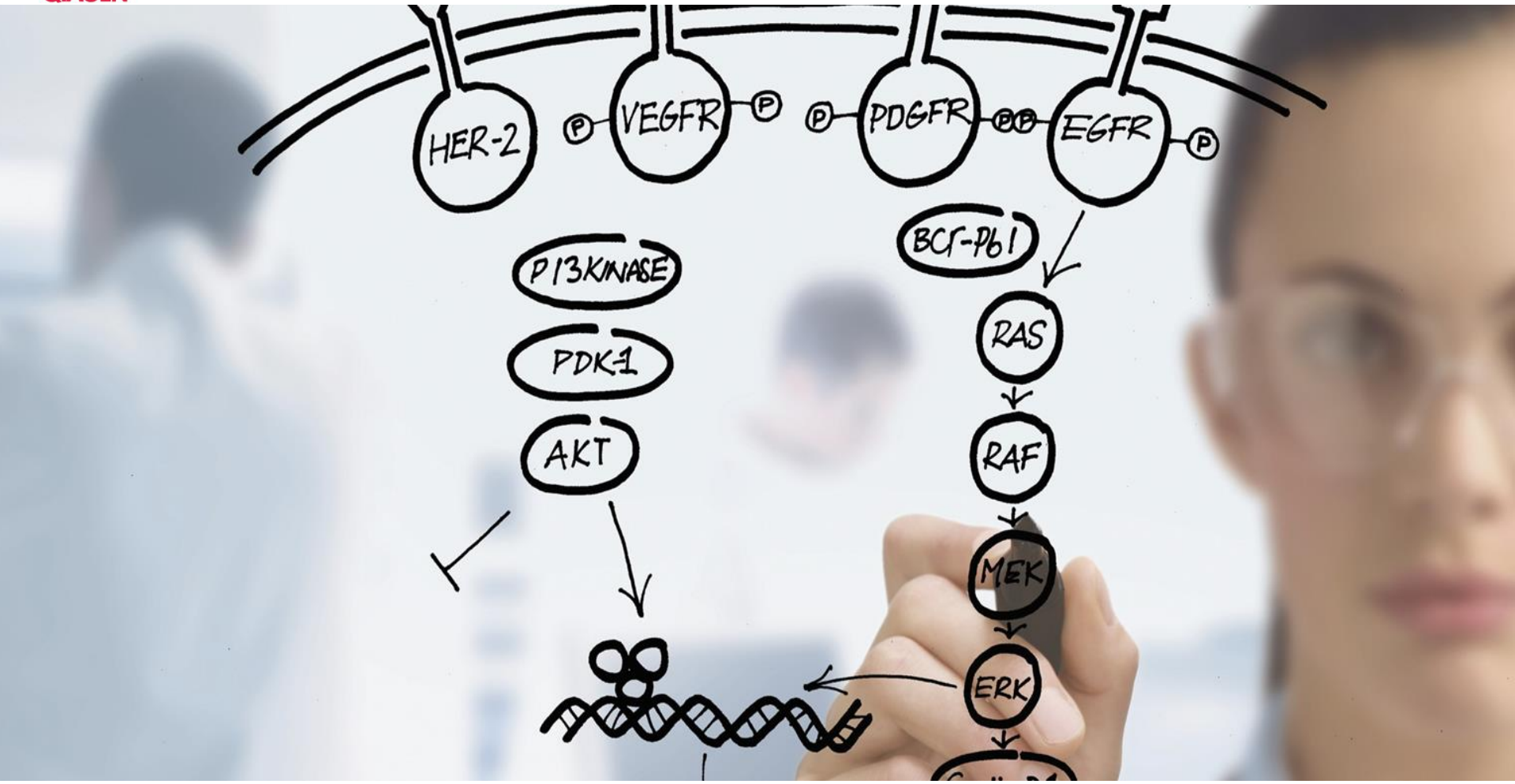
- Links between upstream regulators, dataset molecules, and diseases and functions.

Networks

- Molecular Networks that pinpoint important molecules.

Predictions of activation or inhibition for these molecules, diseases, and functions.

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Office: +886-2-2795-1777#3012
Fax: +886-2-2793-8009 EXT 1022
My E-mail: clairtsai@gga.asia
MSC Support: **msc-support@gga.asia**

Sample to Insight

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