

IPA :

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

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- Introduction to IPA
- Search and Explore
 - Growing a network out of a molecule
 - Bioprofiler (Advanced Analytics)

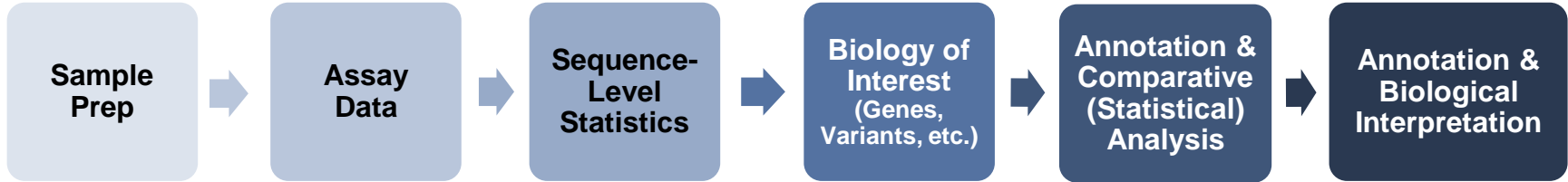
- Large Dataset Analysis
 - Uploading your dataset(s) and starting a core analysis
 - Core Analysis
 - Canonical Pathways
 - Upstream Regulators
 - Causal Network (Advanced Analytics)
 - Diseases and Functions
 - Regulator Effect
 - Networks
 - Comparison Analysis

- Questions/Answer

Introduction

When do you use IPA?

QIAGEN Sample to Insight



Sample

Insight



**INGENUITY®
VARIANT ANALYSIS**

**INGENUITY®
CLINICAL**

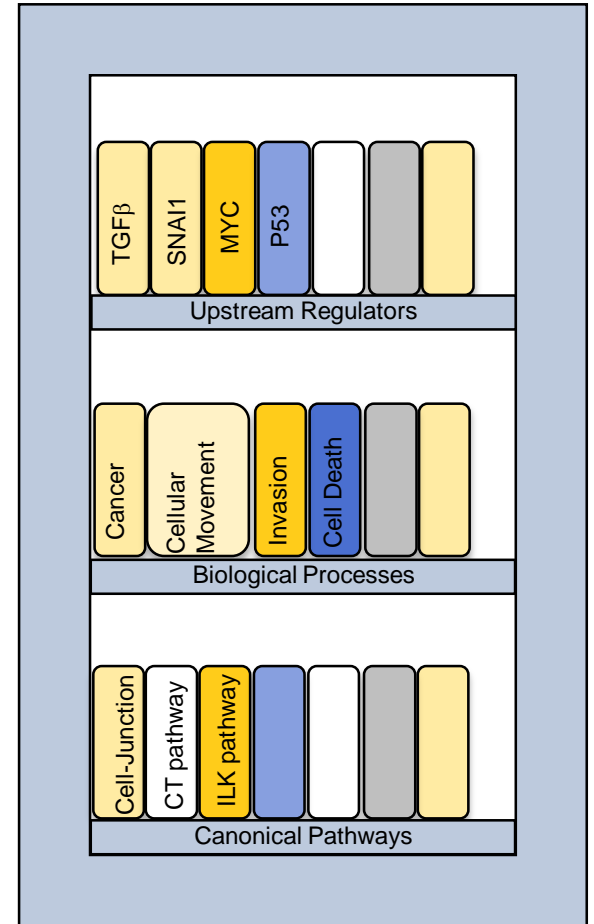
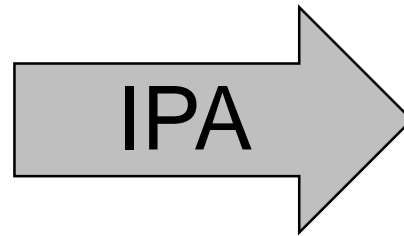
**INGENUITY®
PATHWAY ANALYSIS**

	A	B	C	D
1 ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM	
2 NM_130786	0.14	8.68E-01	2931.69	
3 NR_015380	-0.99	2.24E-01	1649.26	
4 NM_138932	-0.02	9.83E-01	1.67	
5 NM_014576	-0.02	9.85E-01	1.77	
6 NM_138933	0.02	9.79E-01	1.83	
7 NM_000014	-4.79	1.02E-01	239.75	
8 NR_026971	-0.67	6.17E-01	213.79	
9 NM_144670	-5.96	1.30E-01	610.64	
10 NM_001080438	-1.97	3.47E-01	3.91	
11 NM_017436	-1.09	5.02E-01	6186.83	
12 NM_016161	2.02	5.97E-02	149.85	
13 NM_015665	-0.27	5.68E-01	13330.34	

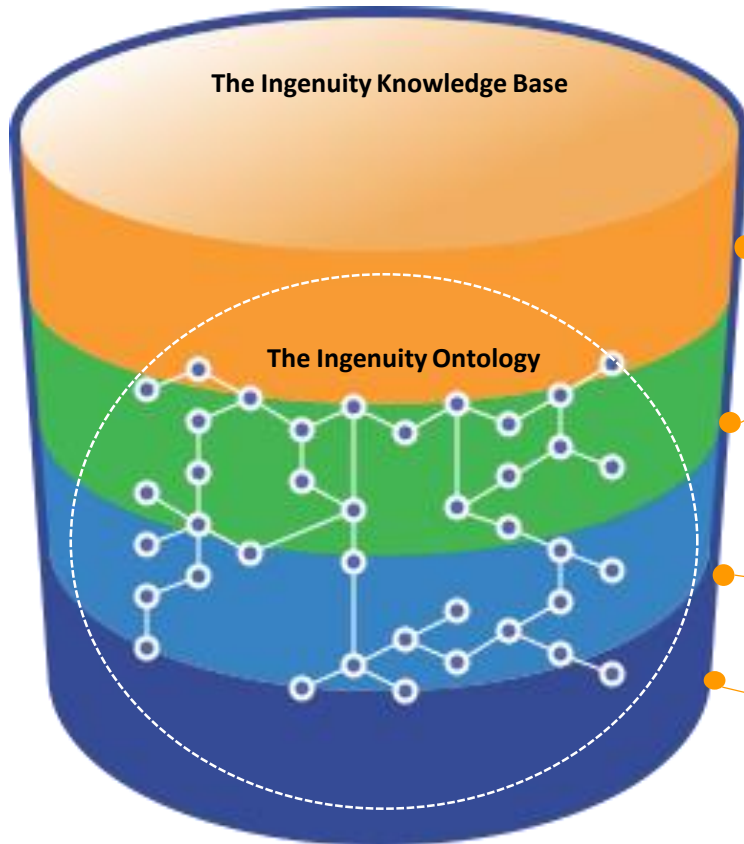
What can IPA do?



Large RNA seq dataset in form of a huge pile of papers



Systematic analysis by IPA in form of organized binders on a bookshelf



Ingenuity Content

Ingenuity Findings

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

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

Ingenuity Modeled Knowledge

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

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

Species: human, mouse and rat

Data from other species can be mapped to human, mouse and rat orthologues

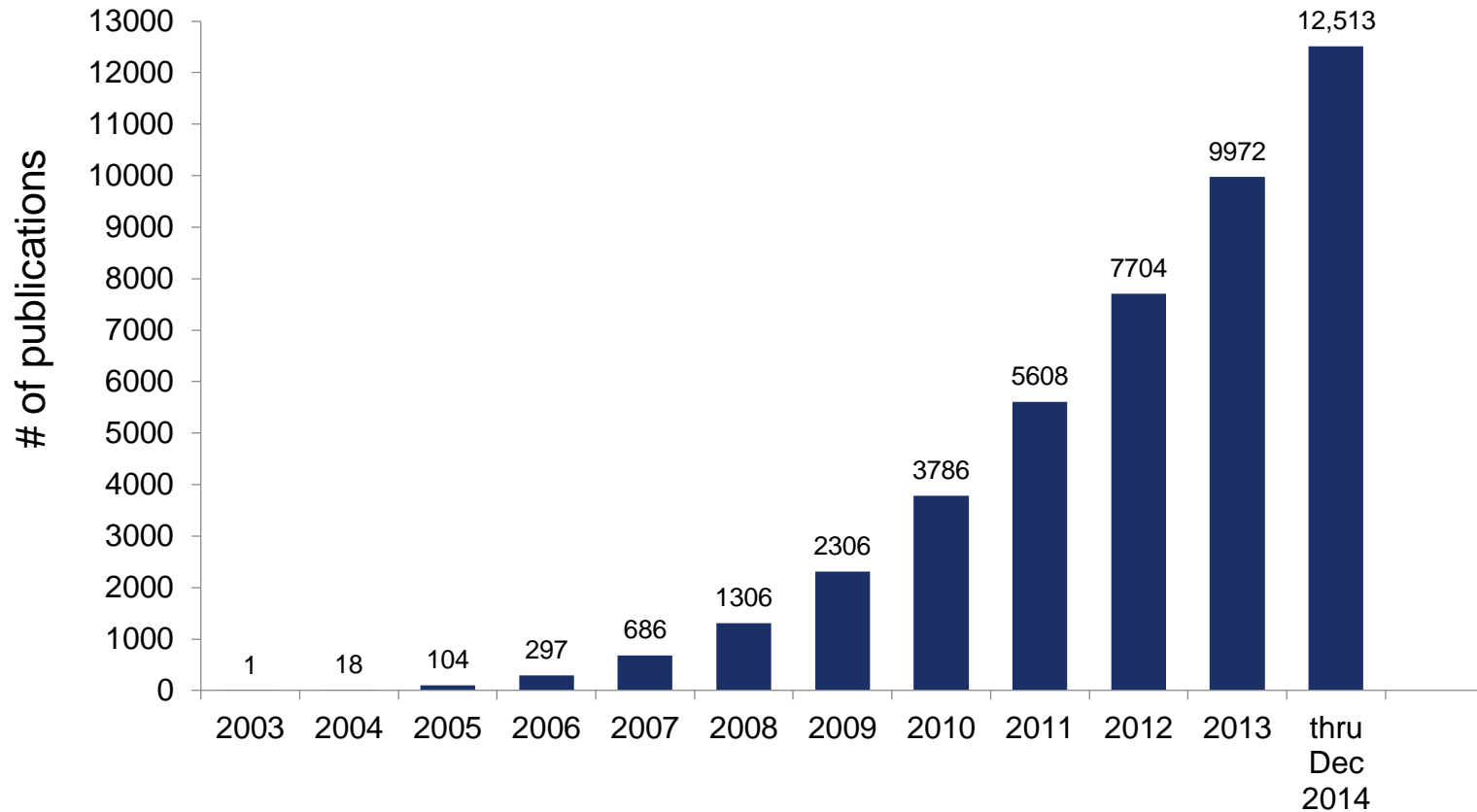
Species Supported

- **Human, Mouse, Rat in full content**
- **IPA uses HomoloGene to map other identifiers to human/mouse/rat orthologs** (though supporting content for the additional species will be specific to human, mouse, and rat)
 - Arabidopsis thaliana
 - Bos taurus (bovine)
 - Caenorhabditis elegans
 - Gallus gallus (chicken)
 - Pan troglodytes (chimpanzee)
 - Danio rerio (zebrafish)
 - Canis lupus familiaris (canine)
 - Drosophila melanogaster
 - Macaca mulatta (Rhesus Monkey)
 - Saccharomyces cerevisiae
 - Schizosaccharomyces pombe



Peer-reviewed publications citing QIAGEN's Ingenuity products

12,513 publications and growing!

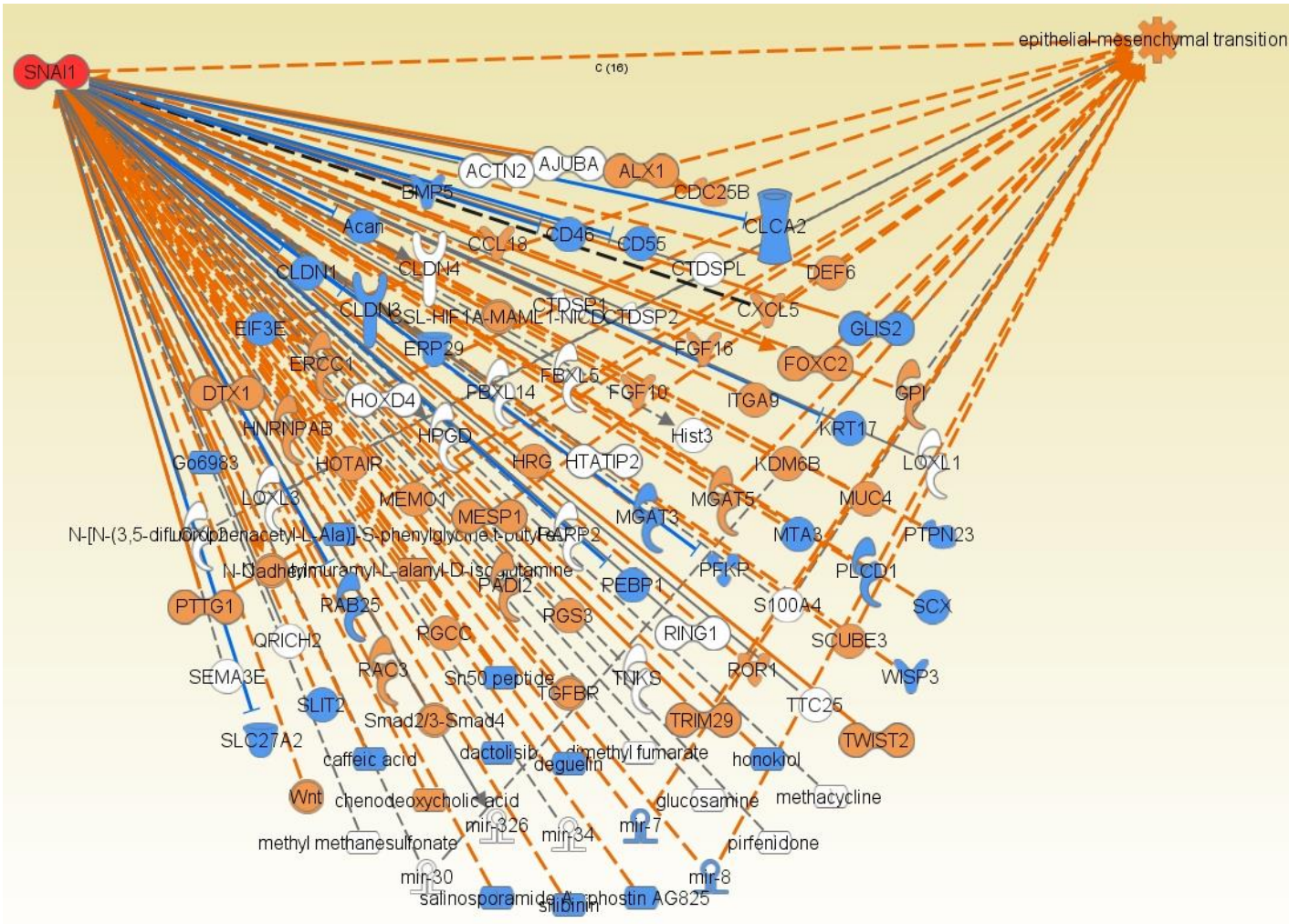


Two different types of analyses by IPA

- Deep pathway understanding of a single gene/protein
- Biological understanding of large data sets

Deep pathway understanding of a single gene/protein

- Drug/therapeutic target discovery



Biological understanding of large data sets

- Differential gene expression, array and RNA-seq (transcriptomics)
- Differential protein expression (proteomics)
- Metabolomics
- miRNA expression
- Gene List
 - Chip-seq
 - siRNA screening
- Methylation
- Protein phosphorylation



Transcriptomics

IPA can help with almost any transcriptomics-related question or application



Biomarker Discovery

Identifies the most promising and relevant biomarker candidates within experimental datasets



microRNA Research

Combines filtering tools and microRNA-mRNA content to provide insight into the biological effects of microRNAs



Toxicogenomics

Delivers a focused toxicity and safety assessment of candidate compounds, and provides a more complete understanding of pharmacological response, drug mechanism of action, and mechanism of toxicity



Metabolomics

Overcomes the metabolomics data analysis challenge by providing the critical context necessary to gain biological insight into cell physiology and metabolism from metabolite data



Drug Repositioning

Expression profiling of approved drugs and comparison to profiles of diseased tissue can lead to discovery of new uses for these already approved entities



Proteomics

Perform a comprehensive analysis of your proteomics for a deep understanding of proteins and related biological processes

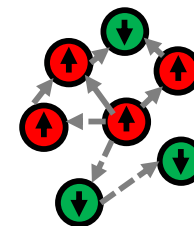
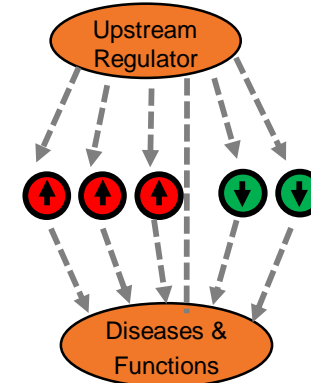
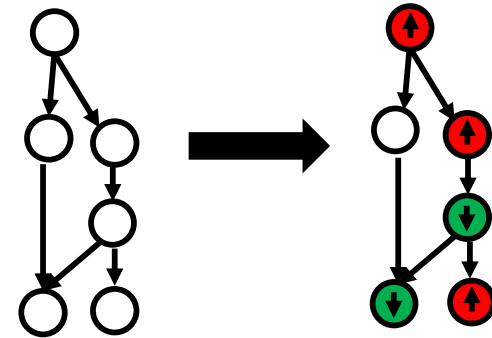


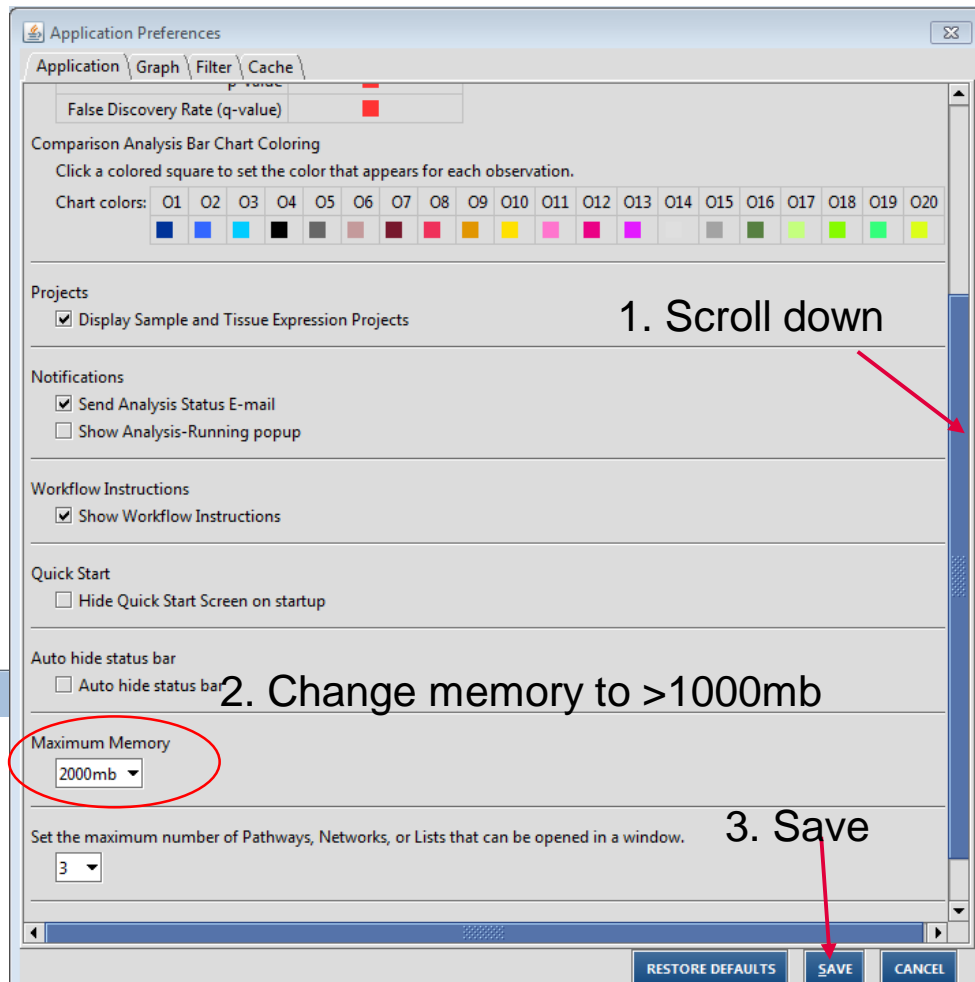
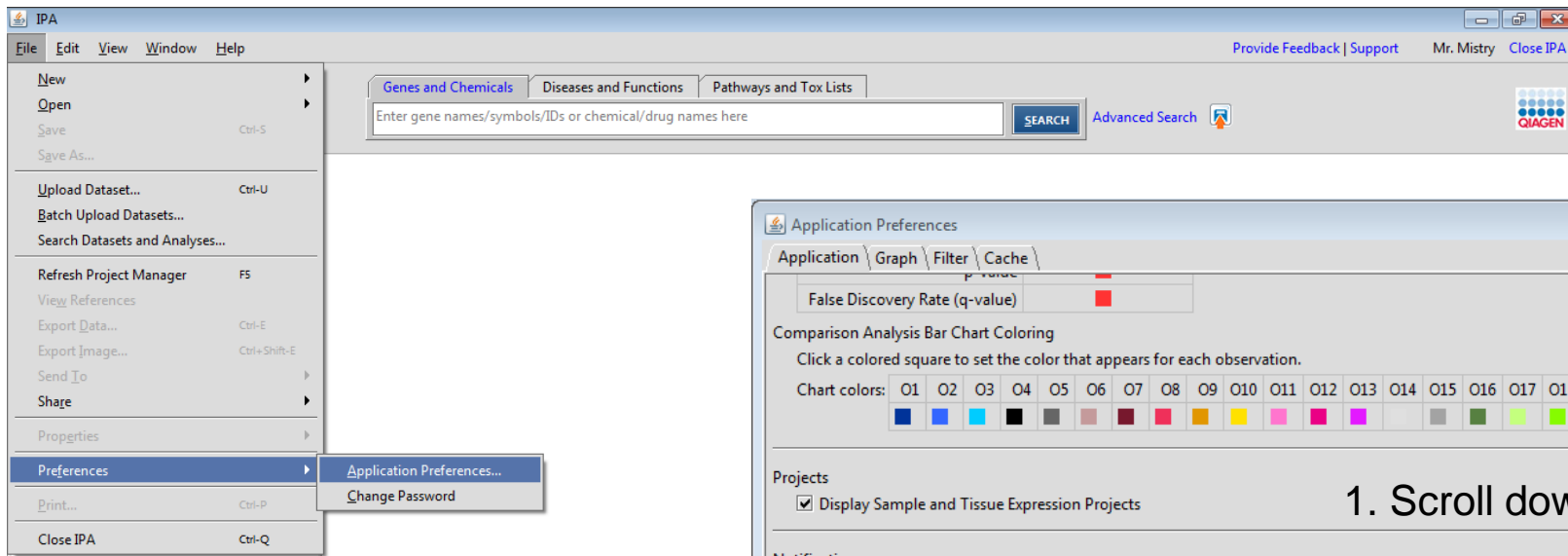
Target Discovery

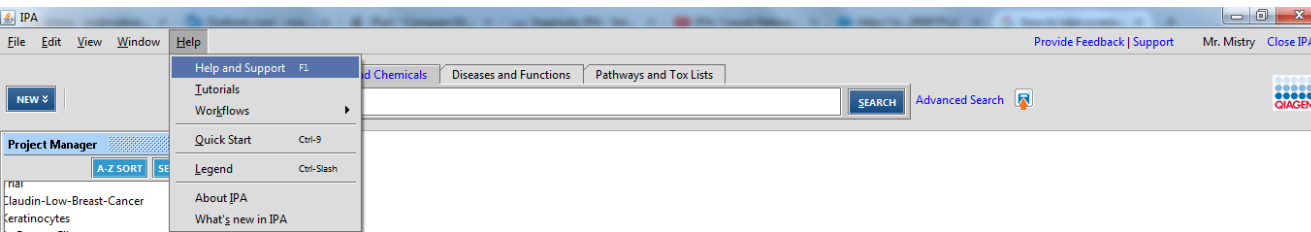
Genes that are shown to be activated in a pathological condition may serve as promising targets for therapeutic development efforts

IPA Core Analysis

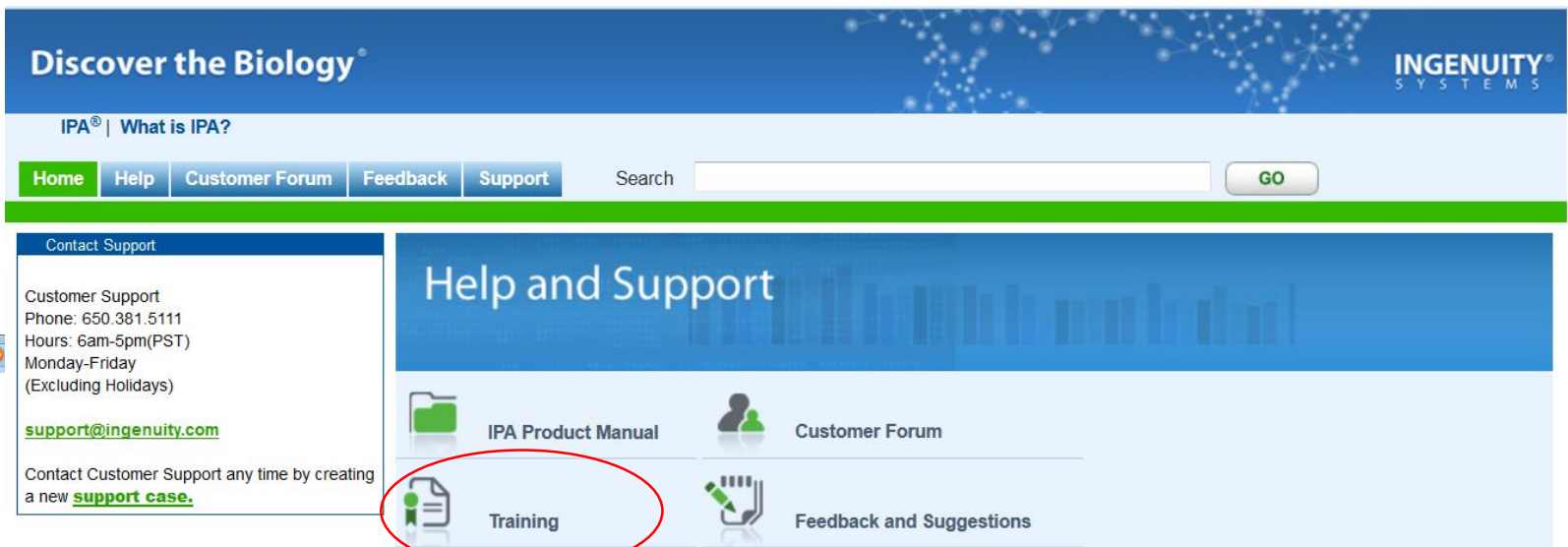
- **Canonical Pathway Analysis**
 - Predicts pathways that are changing based on gene expression
 - New tools to predict directional effects on the pathway (MAP overlay tool)
- **Upstream Regulator Analysis**
 - Predicts what regulators caused changes in gene expression
 - Predicts directional state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







The screenshot shows the IPA software interface. The top menu bar includes File, Edit, View, Window, and Help. The Help menu is open, showing options: Help and Support (F1), Tutorials, Workflows, Quick Start (Ctrl-9), Legend (Ctrl-Slash), About IPA, and What's new in IPA. The Project Manager sidebar on the left lists various datasets and analyses, including 'SNAI2i-HGU144 universe' and 'SNAI2i_Keratinocytes'.



The screenshot shows the Ingenuity Systems website. The main header reads 'Discover the Biology' and 'INGENUITY SYSTEMS'. Below the header, there is a navigation bar with 'Home', 'Help', 'Customer Forum', 'Feedback', and 'Support' buttons. A search bar is also present. The 'Help and Support' section is highlighted, featuring four icons: 'IPA Product Manual', 'Customer Forum', 'Training' (circled in red), and 'Feedback and Suggestions'. The 'Training' icon is a document with a green checkmark.

Don't worry too much about notes or if you fall behind during the point and click training. We have manuals/videos for everything.

Uploading your dataset

Suggested Format for uploading RNA-seq data

Required Recommended Max RPKM recommended for RNA seq

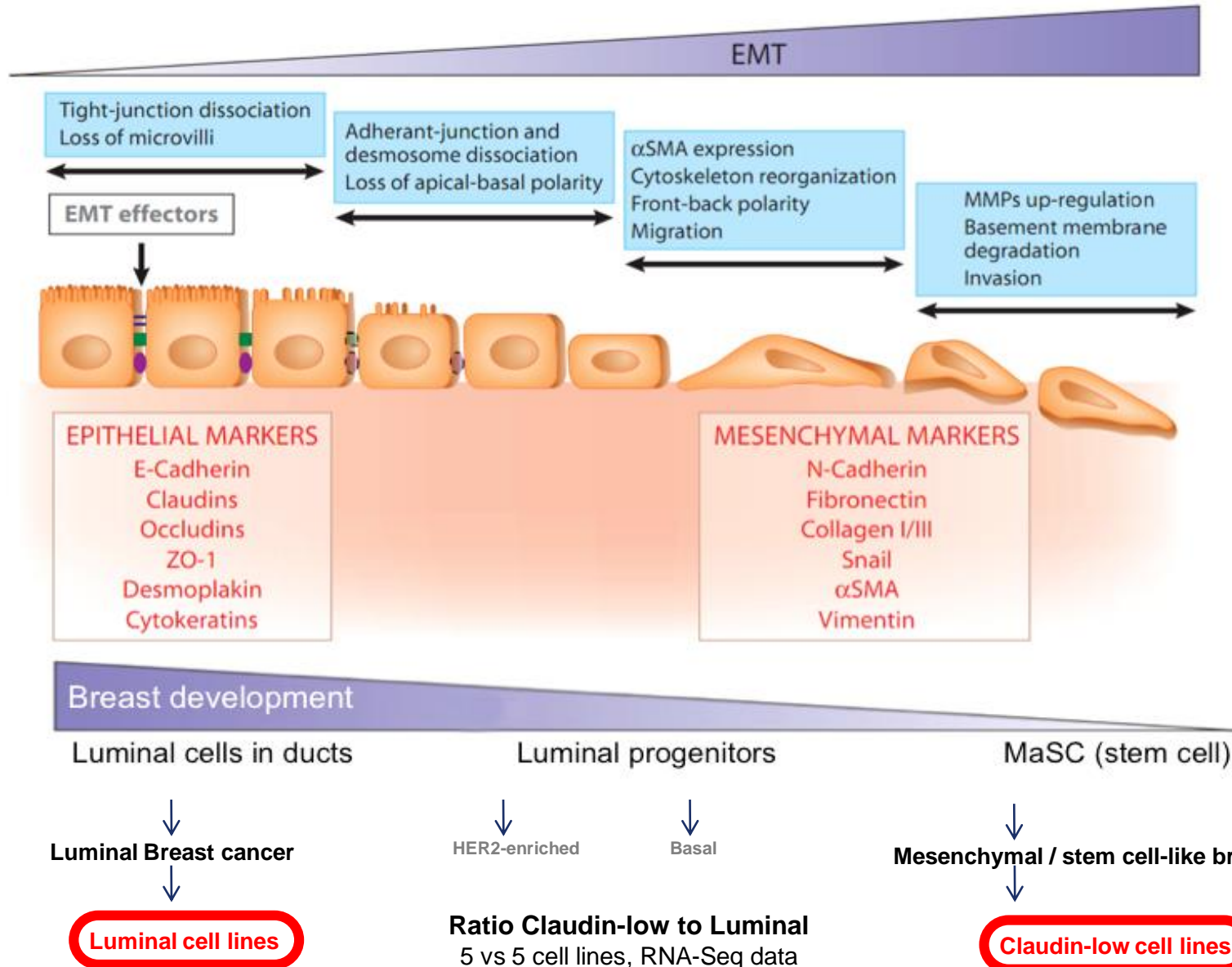
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12	NM_016161	2.02	5.97E-02	149.85
13	NM_015665	-0.27	5.68E-01	13330.34

	A	B	C	D	E
		Exp	Ctl		
P53		300	100	=Max(B2:C2)	
SNAI1		1000	100	1000	
KRT1		10	1	10	

Case Study

RNA Seq: Claudin Low vs Luminal Breast cancer cell lines

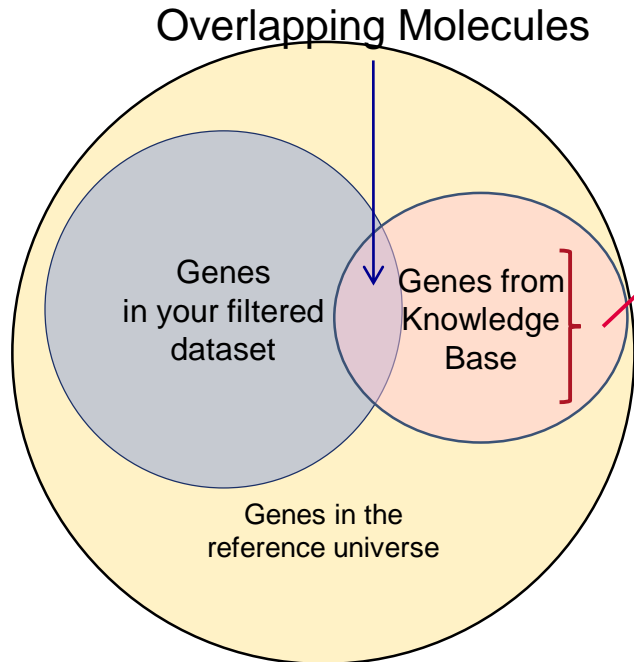
Epithelial to Mesenchymal Transition



Adapted from Aroeira et al. J Am Soc Nephrol 18: 2004 –2013, 2007

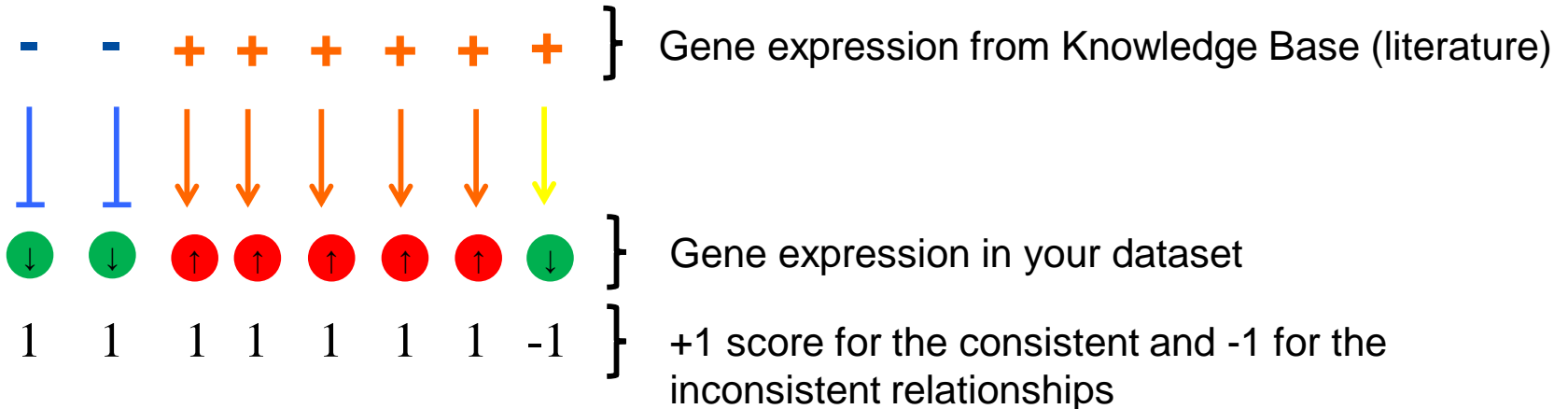
- Verify the biology
 - Can IPA identify cancer and EMT related pathways and biological functions in this dataset?
 - What are some of the relevant pathways?
 - What are some of the relevant biological functions?
- Identification of transcriptional regulators
 - What are the transcriptional regulators that are causing the gene expression changes in this dataset?
 - Are they activated or inhibited?
- Hypothesis generation
 - Are the predicted upstream regulators increasing or decreasing downstream biological functions?

P value and Z Score



- Genes from previous literature that belong to
 - A canonical pathway OR
 - Downstream of an upstream regulator OR
 - Upstream of a disease or function

- Different from the “Expression P-value” uploaded with your dataset
- Calculated using **Fisher’s exact test**
- The statistical test looks for an unexpectedly large overlap given the number of molecules in each category
- p-values should be insignificant (<0.05) for random datasets
- Gene expression direction is **not** taken into account for this calculation



$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}} = (7-1)/\sqrt{8} = 2.12 \text{ (= predicted activation)}$$

- z-score is a statistical measure of the match between expected relationship direction and observed gene expression
- z-score > 2 or < -2 is considered significant
- Note that the actual z-score is weighted by the underlying findings, the relationship bias, and dataset bias

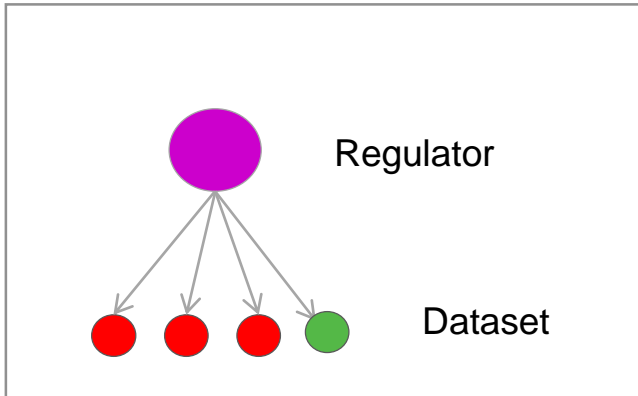


Upstream Regulators vs. Causal Networks

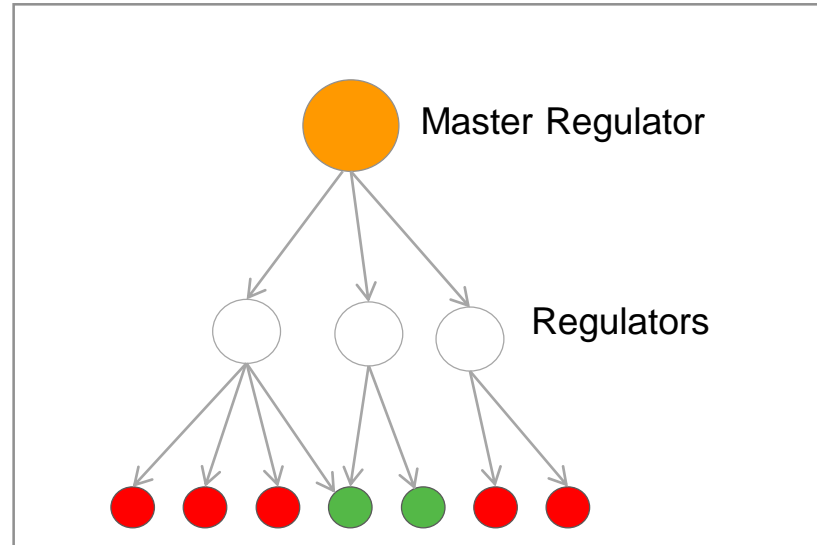
Leveraging the network to create more upstream regulators

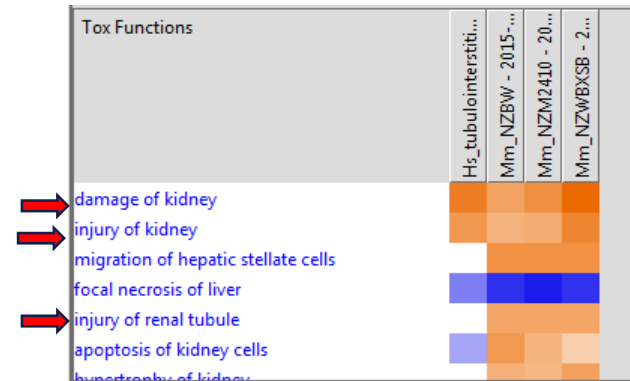
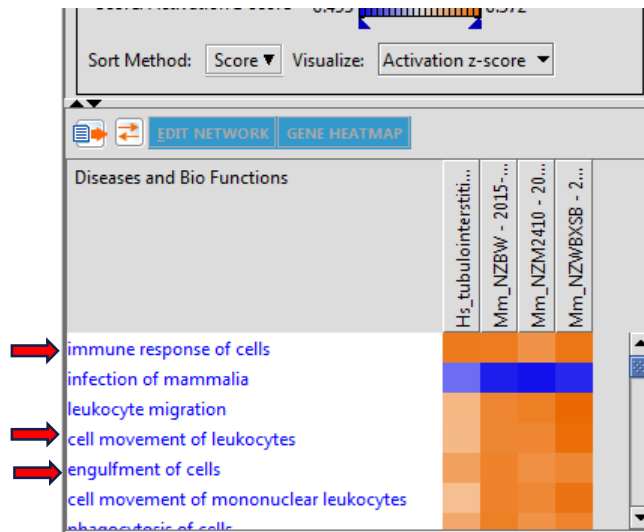
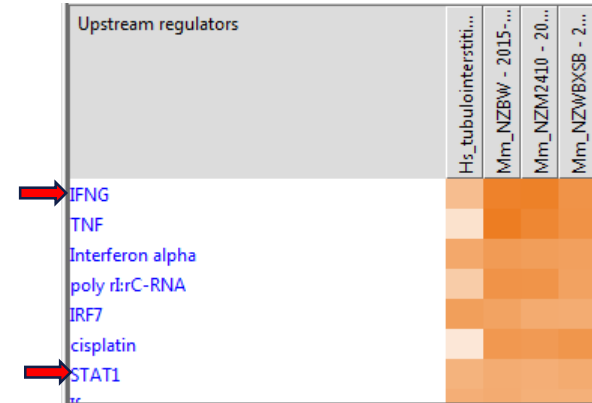
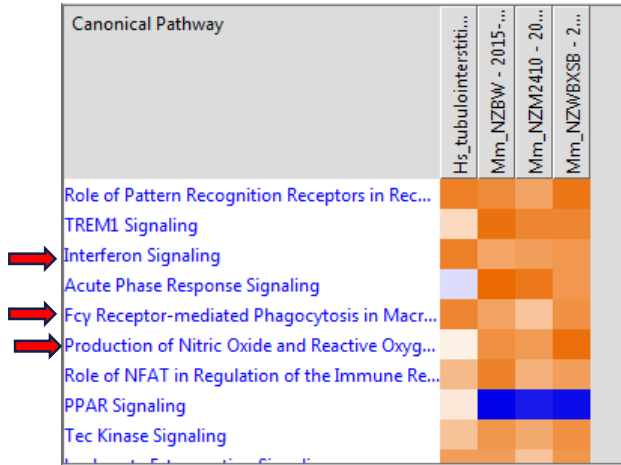
Advanced Analytics: Causal Network Analysis

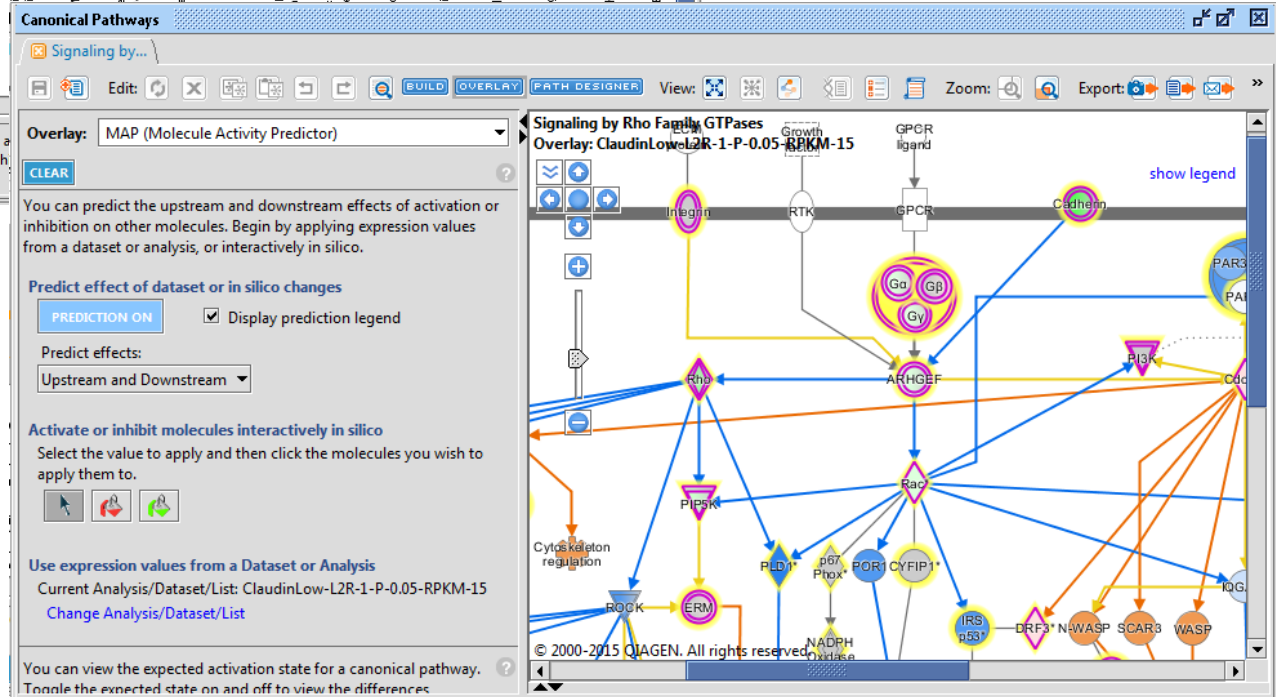
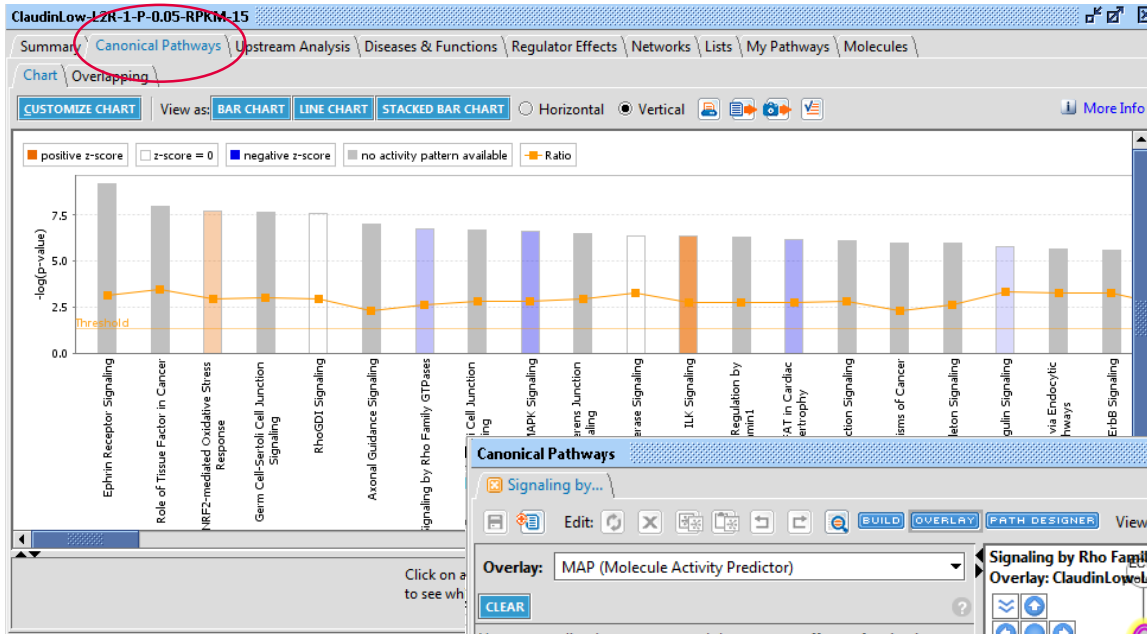
Upstream Regulators



Causal Network





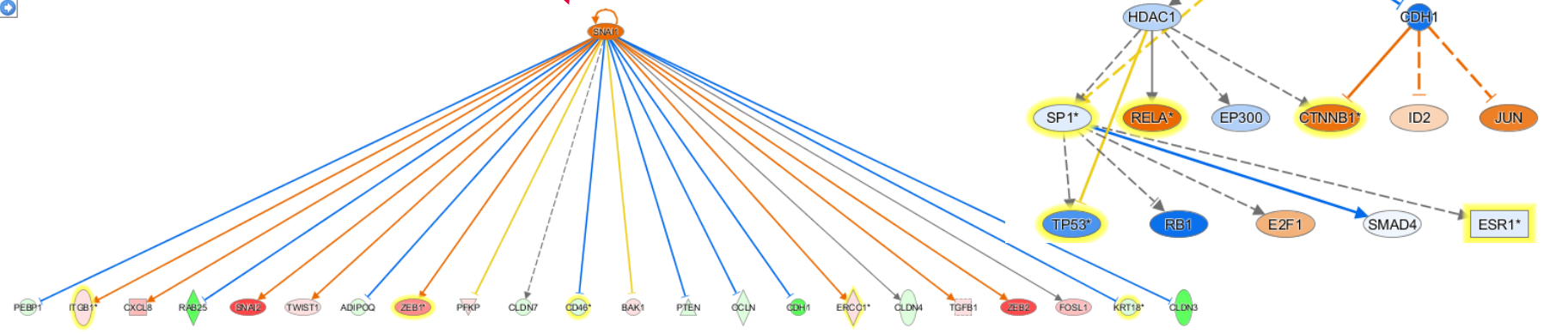


Upstream Regulators Causal Networks

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

Upstream Reg...	Log Ratio	Molecule...	Predicted Acti...	Activation z-s...	p-value o...	Target m...	Mechanis...
Sos		group			1.15E-06	↑ABCC1, ↓... all 58	679 (10)
IDH2	↓-7.249	enzyme			1.40E-06	↓ADIPOQ, ↓... all 10	
miR-155-5p (miRNAs)		mature microRNA	Inhibited	-2.464	1.48E-06	↑ABHD16A, ↓... all 47	
FOS	↓-70.043	transcription regula...		0.713	1.62E-06	↑ABCC1, ↓... all 123	1000 (18)
ID3	↑6.045	transcription regula...		0.611	2.29E-06	↓ADGRG1, ↓... all 52	
SYVN1	↓-2.290	transporter	Activated	3.507	7.51E-06	↑ABCC4, ↑... all 41	612 (10)
miR-1-3p (and other)		mature microRNA		0.248	1.04E-05	↓ABHD11, ↑... all 55	
MAPK1	↑1.387	kinase		1.885	1.04E-05	↑ADAM12, ↓... all 81	991 (18)
MTOR	↑1.161	kinase		0.509	1.25E-05	↑ACADL, ↓... all 66	948 (17)
PML	↑4.199	transcription regula...		0.178	1.37E-05	↑ACADL, ↓... all 37	957 (19)
miR-27a-3p (and other)		mature microRNA		-0.262	1.72E-05	↑BBC3, ↓C... all 17	
SDCBP	↑5.305	enzyme		1.149	1.97E-05	↓CDH1, ↑CT... all 9	389 (11)
AGT	↑1.637	growth factor	Activated	2.660	2.82E-05	↑ACAT2, ↓... all 99	1163 (19)
mir-8		microRNA	Inhibited	-2.104	3.24E-05	↑ABL2, ↓AKT1... all 19	
SNAI1	↑1.041	transcription regula...	Activated	3.473	3.56E-05	↓ADIPOQ, ↑... all 22	807 (14)
Vegf		group	Activated	4.387	3.59E-05	↓ADAM15, ↓... all 94	692 (15)
MGEA5	↓-1.305	enzyme		0.000	3.62E-05	↓ABLIM1, ↑... PKM-15	
TP63	↑23.730	transcription regula...		0.879	4.24E-05	↑AHR, ↓ARAF	
ZEB1	↑370.218	transcription regula...	Activated	2.003	5.08E-05	↓CCNG2	

Selected/Total molecules : 1 / 747



ClaudinLow-L2R-1-P-0.05-RPKM-15-endo

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

Diseases and Bio Functions | Tox Functions

Size by: $-\log(p\text{-value})$ Color by: z-score Decreasing <-1.430 3.671 Increasing Highlight: Top 5 z-score [SHOW LABEL](#)

Click squares below to explore Currently Viewing: All

Organismal Injury and Abnormalit... Cancer Cellular Movement Cellular Devel... Tissue Deve... Cellular Growth... Reproductive... Embryon... Cell Cycle

Hepati... Gene E... Tumor ... Hemato...

Organismal De...

Cellular Assemb... Cell Death a...

Devel... Hair... Neur... Org... Conn...

Gastrointestinal Di...

Cardiovascula...

Hema... En... Inf... Sk... Im...

Organ...

Ner... Rep... Ce... Ca... Di...

Renal ... Imm... Tis... Re... O...

Hem... Ly...

Cell Morphology Cellular Fun... Cell-To-Cell Sig...

Table View | Tree View

[FILTER](#) [DISPLAY AS NETWORK](#) p-Value 7.34E-50 - 6.73E-16

Downstream Effects Analysis: Evidence for Effects

epithelial-mesenchymal transition predicted to be increased (z-score 3.548). Overlap p-value 3.31E-07

37 of 68 genes have expression direction consistent with increased in epithelial-mesenchymal transition.

[ADD TO MY PATHWAY](#) [ADD TO MY LIST](#) [CUSTOMIZE TABLE](#) [CREATE DATASET](#) Prediction (bas... Increased - Decreased (p1 of 2) [More Info](#)

ID	Genes in dataset	Prediction (based on expressi...	Log Ratio	Findings
<input type="checkbox"/> NM_015888	HOOK1	Increased	-1518.575	Decreases (2)
<input type="checkbox"/> NM_005401	PTPN14	Increased	3.117	Increases (1)
<input type="checkbox"/> NM_198381	ELF5	Increased	-8.484	Decreases (3)
<input type="checkbox"/> NM_001098210	CTNNB1	Increased	2.888	Increases (10)
<input type="checkbox"/> NM_001135599	TGFB2	Increased	24.919	Increases (3)
<input type="checkbox"/> NM_001664	RHOA	Increased	2.137	Increases (2)
<input type="checkbox"/> NM_001014795	ILK	Increased	5.874	Increases (3)
<input type="checkbox"/> NM_020824	ARHGAP21	Increased	2.004	Increases (1)
<input type="checkbox"/> NM_006500	MCAM	Increased	24.908	Increases (2)
<input type="checkbox"/> NM_002613	PDPK1	Increased	-3.432	Decreases (4)
<input type="checkbox"/> NM_001080123	PRNP	Increased	15.895	Increases (2)
<input type="checkbox"/> NM_003068	SNAI2	Increased	590.706	Increases (9)
<input type="checkbox"/> NM_001145103	SMAD3	Increased	1.621	Increases (11)
<input type="checkbox"/> NM_000660	TGFB1	Increased	8.019	Increases (153)
<input type="checkbox"/> NM_001024628	NRP1	Increased	13.102	Increases (2)
<input type="checkbox"/> NM_023106	FGFR1	Increased	16.259	Increases (2)

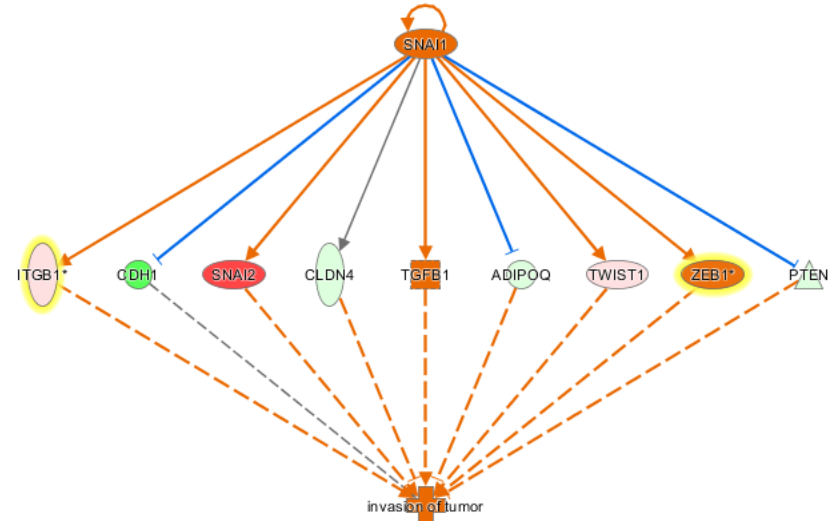
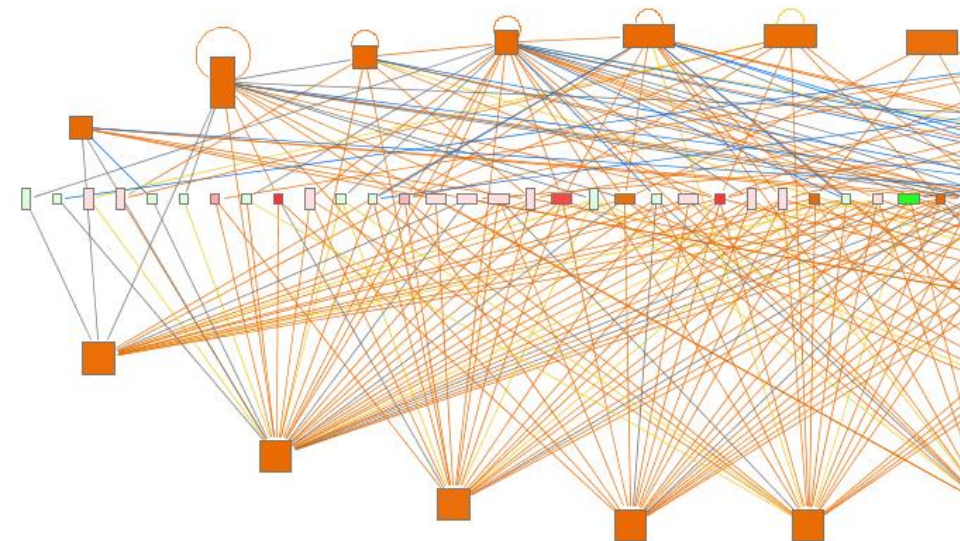
Selected/Total rows: 0/68

ClaudinLow-L2R-1-P-0.05-RPKM-15-endo

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

GENERATE NETWORKS | ADD TO MY PATHWAY | ADD TO MY LIST | CUSTOMIZE TABLE | More Info

ID	Const...	Node Total	Regulator...	Regul...	Target To...	Targe...	Disease &...	Disea...	Known Re...
1	2.333	11	1	↑SNAI1 ...all 19	↓ADIPOQ ...all 9	1	invasion ...	0% (0/1)	
2	1.890	9	1	↑SNAI1 ...all 17	↓ADIPOQ ...all 7	1	invasion ...	0% (0/1)	
3	1.789	7	1	miR-155-... all 15	↑EGFR, ↑... all 5	1	invasion ...	0% (0/1)	
4	0.522	35	1	↑F2 ...all 133	↑ALDH... all 33	1	tumorige...	100% (1/1)	
5	0.514	36	1	↑F2 ...all 134	↑ALDH... all 34	1	female g...	100% (1/1)	
6	0.000	25	1	Vegf ...all 123	↓CDH1, ↓... all 23	1	endocrin...	100% (1/1)	
7	0.000	9	1	↓WISP2 ...all 17	↓CDH1, ↓... all 7	1	endocrin...	0% (0/1)	
8	-3.020	60	1	↓TNF ...all 158	↑AR, ↑... all 58	1	endocrin...	0% (0/1)	
9	-3.441	21	1	estrogen ... all 19	↑AXL, ↓... all 19	1	endocrin...	0% (0/1)	
10	-3.667	11	1	↓IL2 ...all 19	↓CD44, ↓... all 9	1	invasion ...	0% (0/1)	
11	-4.373	43	1	Cg ...all 141	↓ACPP, ↓... all 41	1	hepatobil...	0% (0/1)	
12	-4.427	42	1	Cg ...all 140	↓ACPP, ↓... all 40	1	liver cancer	0% (0/1)	
13	-4.690	90	1	↑MYC ...all 188	↑ABCE1, ...all 88	1	female g...	100% (1/1)	



ClaudinLow-L2R-1-P-0.05-RPKM-15-endo

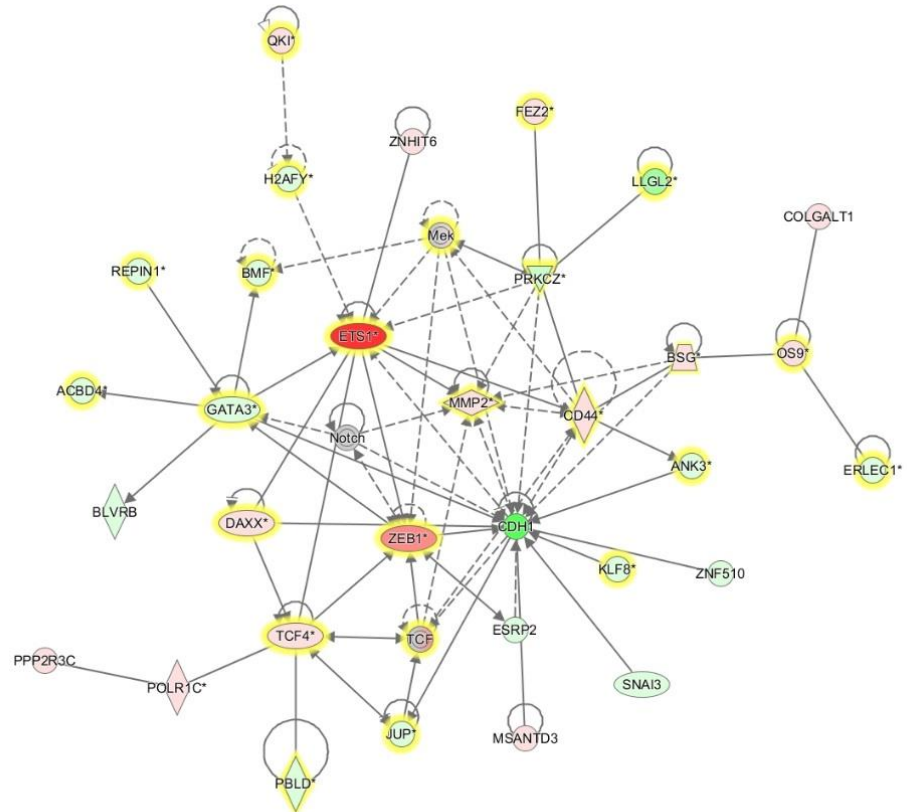
Summary | Canonical Pathways | Upstream Analysis | Diseases & Functions | Regulator Effects | **Networks** | Lists | My Pathways | Molecules

Networks | Overlapping Networks

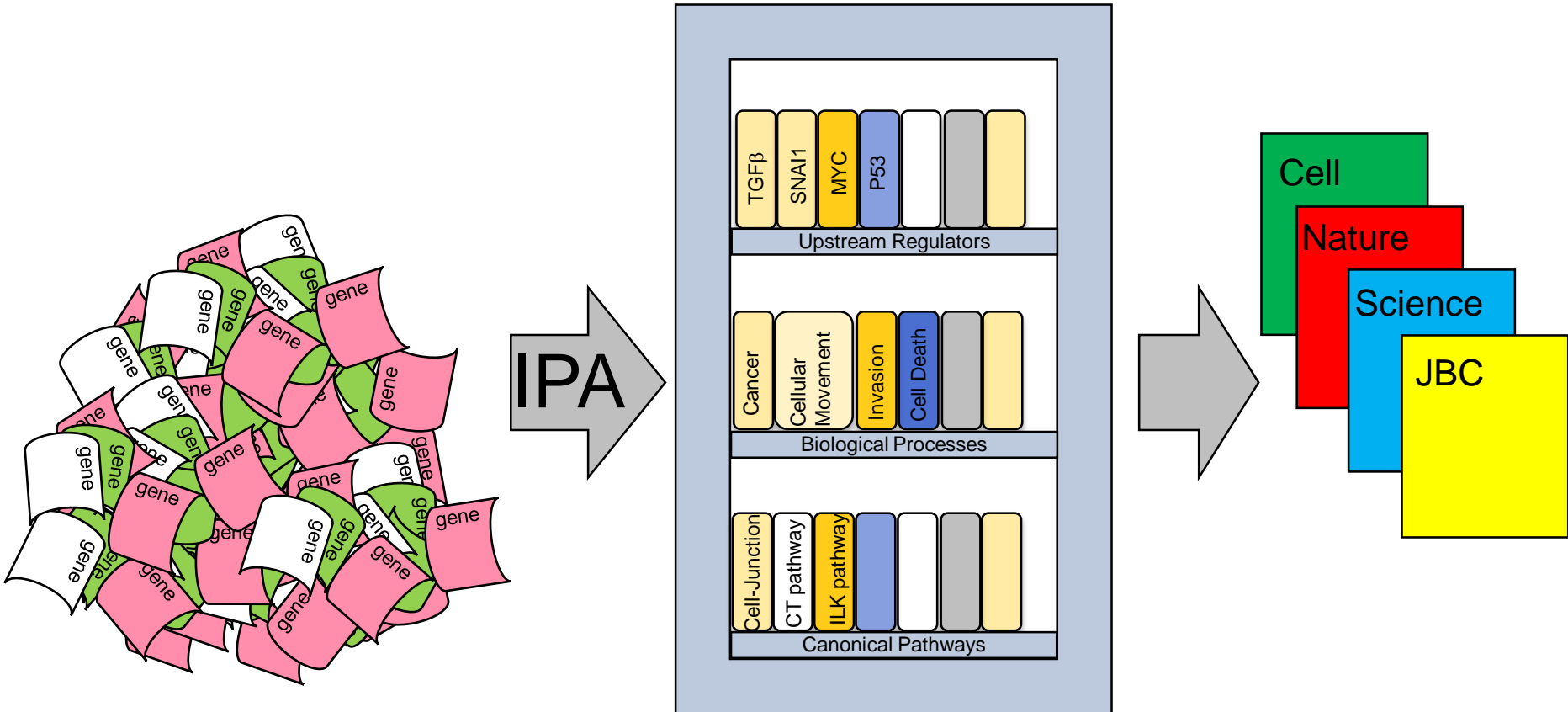
FILTER [] VIEW NETWORKS ADD TO MY PATHWAY ADD TO MY LIST MERGE NETWORKS FUNCTIONS ANNOTATIONS

The analysis is composed of 25 networks. To view a network, select the appropriate network(s) and click View Networks. To merge selected networks, click Merge Networks.

ID	Molecules in Network	Score	Focus Molecule	Top Diseases and Functions
1	↓ANXA9, ↓BFSP1*, ↑BIN1*, ↓CD164*, ↓CNFN, ↓CTPS2*, ↑CUTA*, ↓EVPL, ↓FAM110A*, ↓GRB2*, ↓HELZ, ↓KIF13A*, ↓KIF26A, ↑KIRREL, ↓LONRF2, ↓MSI2*, ↓NCKAP5*, ↓NUTM2X/NUTM2G*, ↓OTUD5*, ↓PEX7, ↓PEX13, ↓PPL, ↑RIN3, ↑SH2D5*, ↑SH3D19*, ↑SH3GL1, ↓SH3YL1*, ↓SLC23A1*, ↓SLC24A1, ↓SNX4, ↑SNX7*, ↓SOS2, ↑TJAP1*, ↓TULP1, ↓ZNF609	34	35	Connective Tissue Disorders, Developmental Disorder, Hereditary Disorder
2	↓ARRB1*, ↓BPHL*, ↑CCDC86, ↓CMBL, ↑CNBP*, ↓DDX21, ↑DKC1*, ↓DNAH1, ↓DNAH3, ↑FBL, ↑GNL3*, ↓GPBAR1*, ↑IFRD2, ↓IGF2BP2*, ↓MINA*, ↑MYBBP1A*, ↑NAA15, ↑NAA50, ↑NHP2L1*, ↑NOC3L, ↑NOP56*, ↑PABPC4*, ↑PPRC1, ↑RPP9, ↓RSL1D1, ↑RTCA*, ↑SAFB2, ↑SF3B5, ↓SRPK3, ↑STXBP5*, ↑SUN3*, ↑TCOF1*, ↓THUMPDL1, ↓WWC2, ↑ZNHIT6			Network 2 : Claudin Low-L2R-2-P-0.01 : Claudin Low : Claudin Low-L2R-2-P-0.01
3	↑C2orf44*, ↓CA8, ↓CAB39L*, ↑CCDC186, ↑CEP85L*, ↓COBLL1, ↓COL4A3BP*, ↓CSNK1G1, ↑CSNK1G2, ↓DCAF7, ↑DOCK7, ↑FANCB*, ↓FANCF, ↑HBEGF, ↓KIAA1324, ↑KIAA1324, ↓LLGL2*, ↓LRCH4, ↓MARK2*, ↑MARK3*, ↓MARK4, ↓MKRN1*, ↑MYO10, ↑NRD1*, ↓PLEKHA5*, ↓POM121/POM121C*, ↓PPM1B*, ↑SEC13*, ↑SEH1L*, ↓SH3BP5L, ↓SOGA1, ↑SPDL1, ↓STRA13, ↓STRADA*, ↓TMC4*			
4	↓ABCA3, ↓ANO2, ↓BRWD1*, ↑C18orf25*, ↓C1QTNF6*, ↑CRIM1, ↓DCDC2, ↑ENY2, ↓ESR1*, ↓FAM102A*, ↓FAM186A, ↓KCNK6, ↓MACROD1, ↓METTL7A, ↓MTLS*, ↑NDC1, ↑PHF5A, ↓PSD4, Rab11, ↓RAB11A, ↓RAB11FIP3*, ↓RAB11FIP4, ↓RERG, ↓RTN2*, ↓SEMA4A, ↑SLC35B4, ↓SLC44A2*, ↓SLC4A5*, ↑TC2N*, ↓TMPRSS3*, ↓TTC9, ↓VPS13D*, ↓ZNF107*, ↓ZNF141, ↓ZNF703			
5	↑ABCF2*, ↓ABLIM1*, ↓AKTIP*, ↓BRICD5, ↓C10orf82, ↓CALCOCO2, ↓CCNB1IP1*, ↓COMTD1, ↑FAM107A*, ↑GADD45GIP1, ↓HOOK1, ↓HOOK2*, IKK (complex), ↑KATNAL1*, ↑KHDRBS3, ↓KLC4*, ↓KRT15, ↓MPPED2*, ↑NOR1, ↑PAOR5*, ↓RPAP2, ↓RPS6KA6, ↓SAP30RP, ↓SEMP2*			



What can IPA do?



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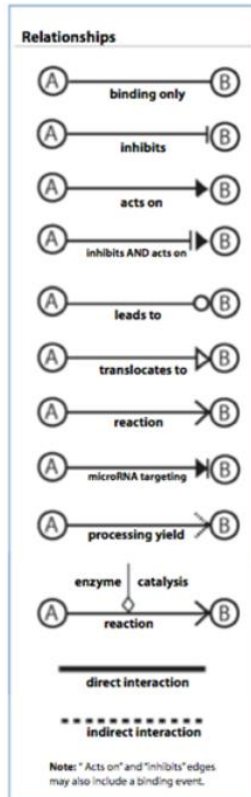
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Relationship Labels

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

Network Shapes

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

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