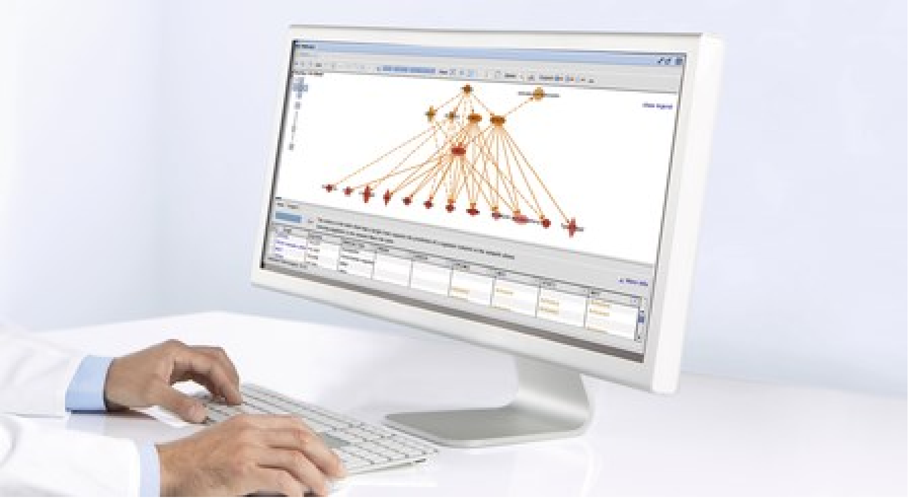


Ingenuity Pathway Analysis (IPA) : Maximizing the Biological Interpretation of Gene, Transcript & Protein Expression Data with IPA

Jennifer Poitras, Ph.D.
Field Applications Scientist
Qiagen Advanced Genomics
Jennifer.Poitras@qiagen.com

IPA provides efficient insight

With IPA you can:

- Analyze and visually integrate multi-omics datasets
  - Identify pathway enrichment AND directionality
  - Predict upstream molecules that may be driving expression changes
    - Identify druggable targets
  - Link differentially expressed genes to your phenotype

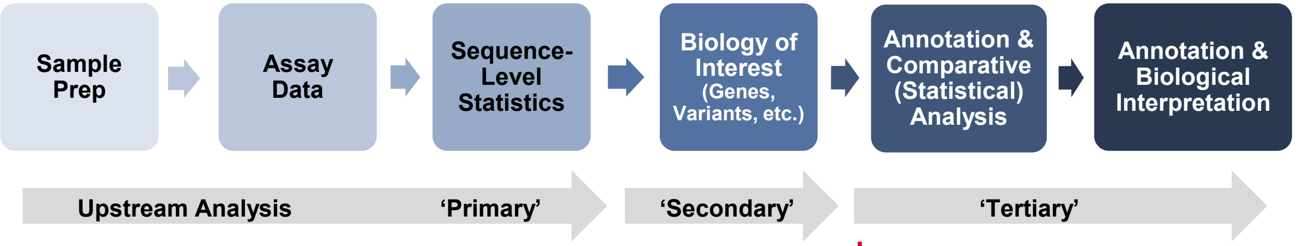
- Mining the Knowledgebase: One stop shop
  - Look up all curated knowledge about gene/disease of interest
  - Explore and create pathways
  - Bioprofiler: identify actionable targets

Overview

- Introduction to IPA
- Large Dataset Analysis
  - Core Analysis
  - Comparison Analysis
- Newer IPA Functionalities
  - Overlay variant data
  - Phosphoproteomic analysis
  - miRNA Target Filter
  - Isoprofiler
  - Bioprofiler
  - Search and Explore
- Questions/Answer

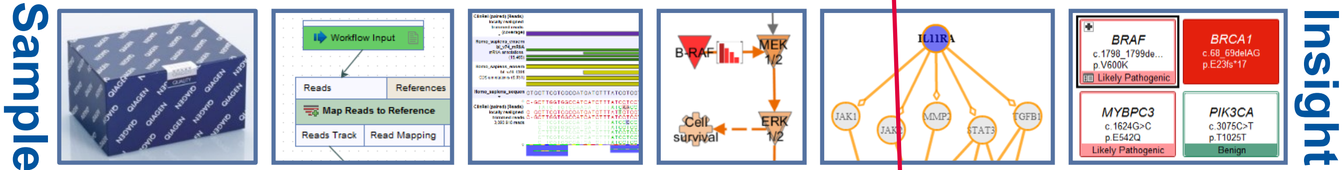
When do you use IPA?

QIAGEN Sample to Insight



Sample Prep → **Assay Data** → **Sequence-Level Statistics** → **Biology of Interest (Genes, Variants, etc.)** → **Annotation & Comparative (Statistical) Analysis** → **Annotation & Biological Interpretation**

Upstream Analysis **'Primary'** → **'Secondary'** → **'Tertiary'**



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CLC bio A QIAGEN Company

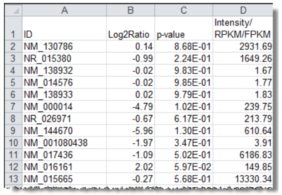
Gx Bx

INGENUITY VARIANT ANALYSIS

QIAGEN Clinical Insight

BIOBASE BIOLOGICAL DATABASES

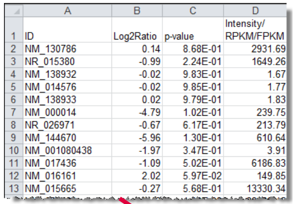
INGENUITY PATHWAY ANALYSIS



	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 is Ingenuity Pathway Analysis?

What can IPA do?



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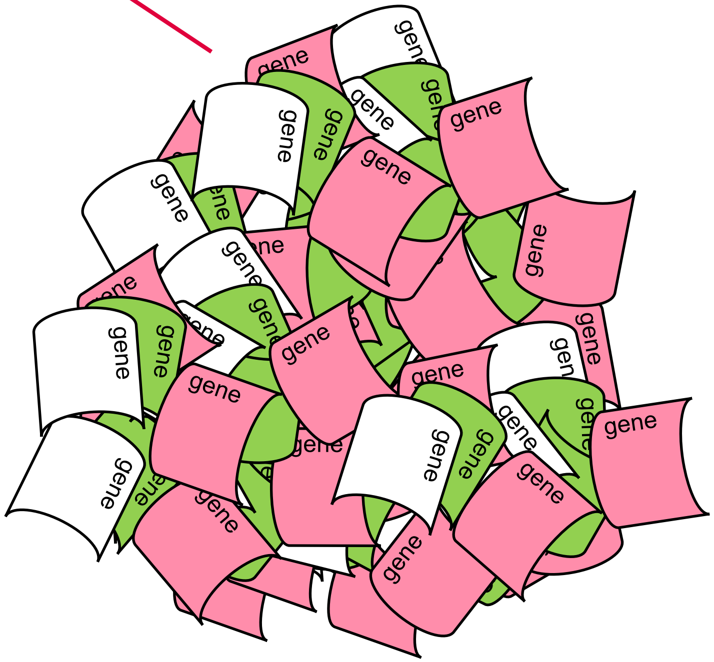
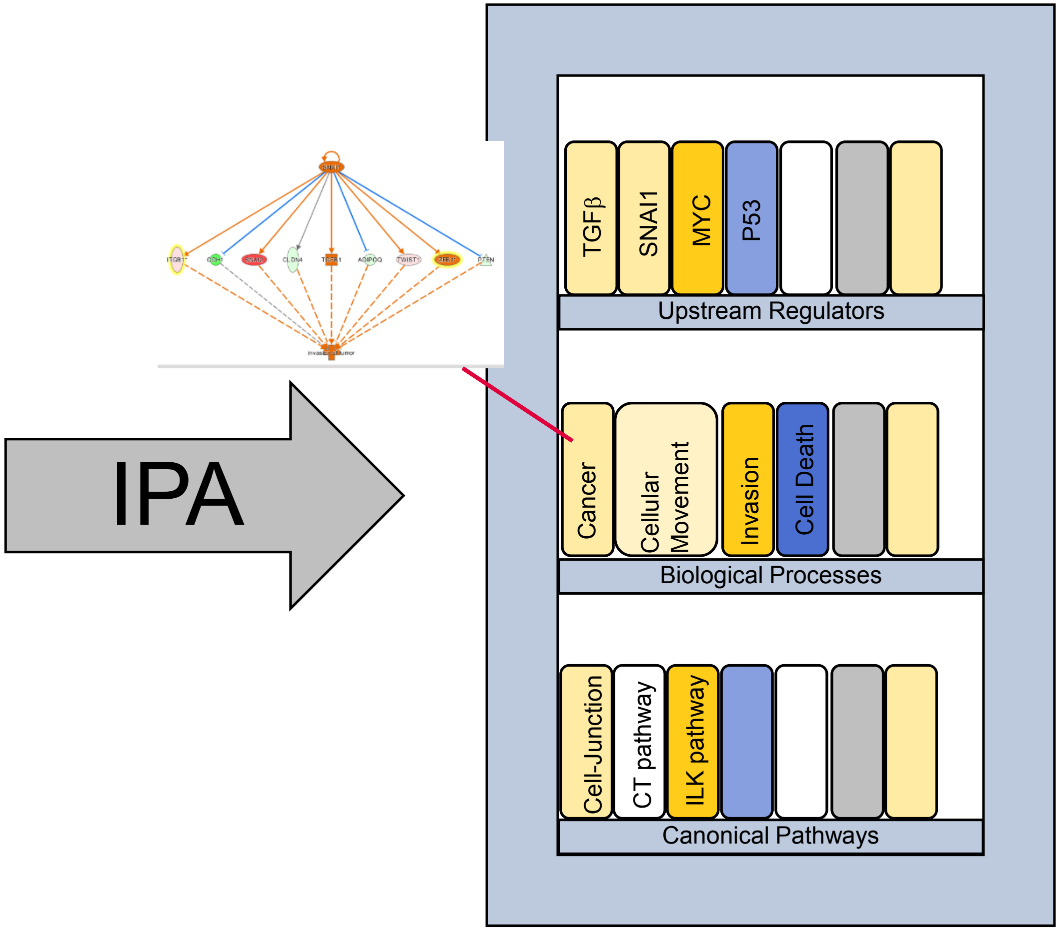


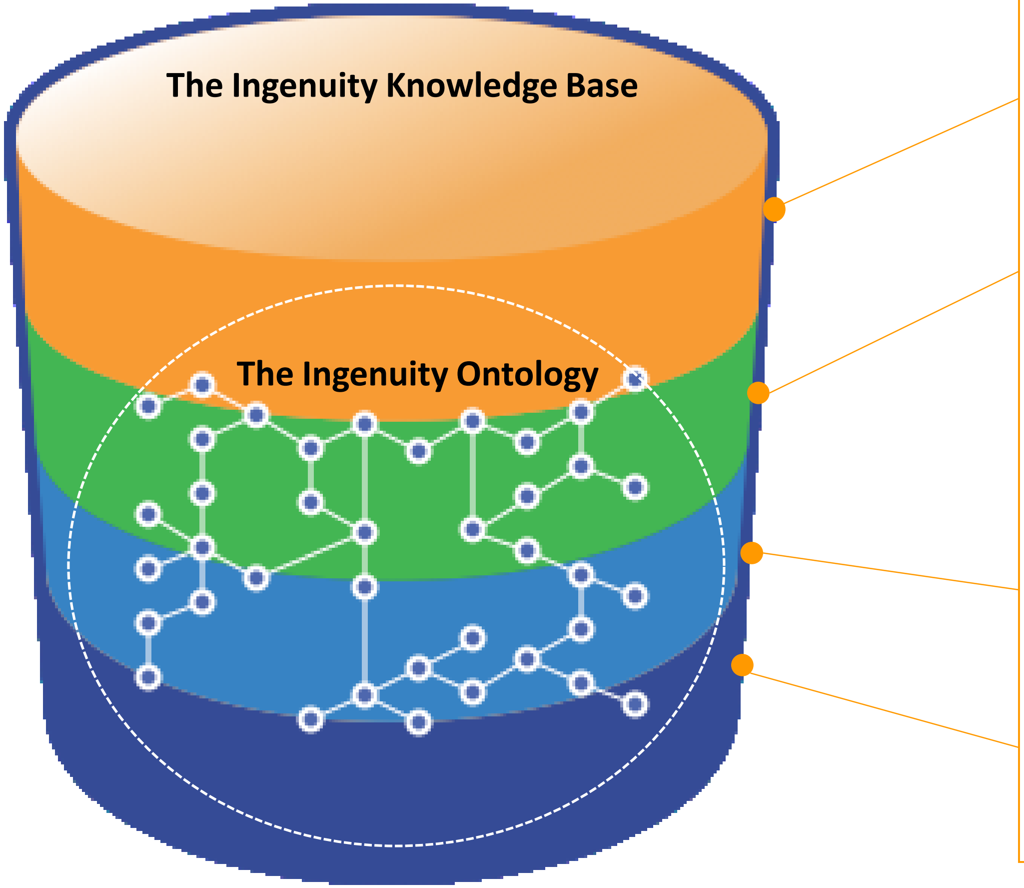
Large RNA seq dataset in form of a huge pile of papers



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

Ingenuity Content

Ingenuity Knowledge Base



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 Supported

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

How can IPA help you?

IPA

- Deep pathway understanding of a single gene/protein
  - Drug/therapeutic target discovery

- Biological understanding of large data sets
  - Differential gene expression, array and RNAseq (transcriptomics)
  - Differential protein expression (proteomics)
  - Metabolomics
  - miRNA expression
  - Gene List
    - Chip-seq
    - siRNA screening
    - Sequence Variants (see also Ingenuity Variant Analysis)
  - Methylation
  - Protein phosphorylation

How can IPA help you?

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  - Gene List
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    - siRNA screening
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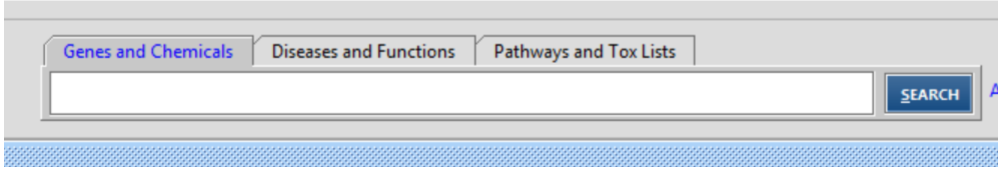
  - Protein phosphorylation

- **Deep pathway understanding of a single gene/protein**
  - What other molecules does it interact with?
  - What processes is it associated with?
  - What compounds affect its activity?



How can IPA help you?

- **Biological understanding of large data sets**
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  - Differential protein expression (proteomics)
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- **Deep pathway understanding of a single gene/protein**
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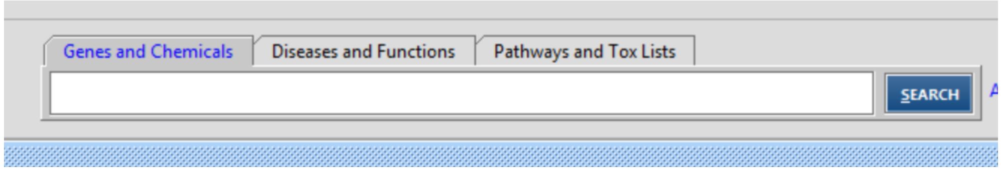
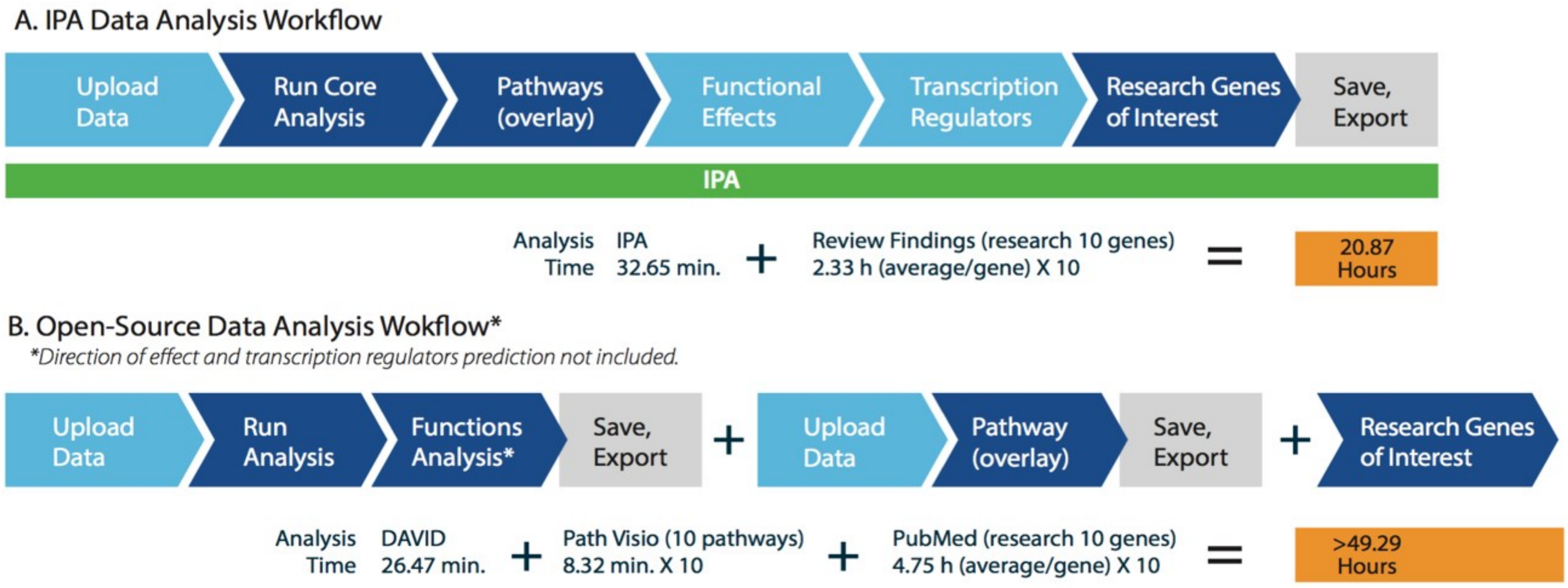


- **Investigate a disease or process of interest**
  - Which genes/proteins/metabolites are good biomarker candidates?
  - What are promising treatment targets?

Return on Investment (ROI)

Significant time savings vs open source tools


A. IPA Data Analysis Workflow

Analysis Time IPA 32.65 min. + Review Findings (research 10 genes) 2.33 h (average/gene) X 10 = 20.87 Hours

B. Open-Source Data Analysis Wokflow*

*Direction of effect and transcription regulators prediction not included.

Analysis Time DAVID 26.47 min. + Path Visio (10 pathways) 8.32 min. X 10 + PubMed (research 10 genes) 4.75 h (average/gene) X 10 = >49.29 Hours

- Open source tools **do not** generally have directional content
- Many have woefully stale content: DAVID had no content updates for several **years**

IPA has directional content that is updated every week

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- Questions/Answer

IPA Core Analysis

Canonical Pathways

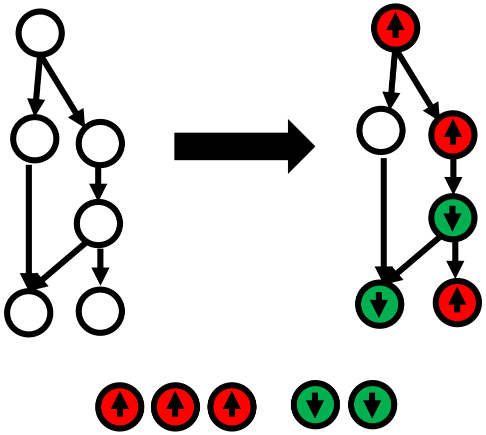
☐ Predicts activation/inhibition of predefined pathways based on gene expression

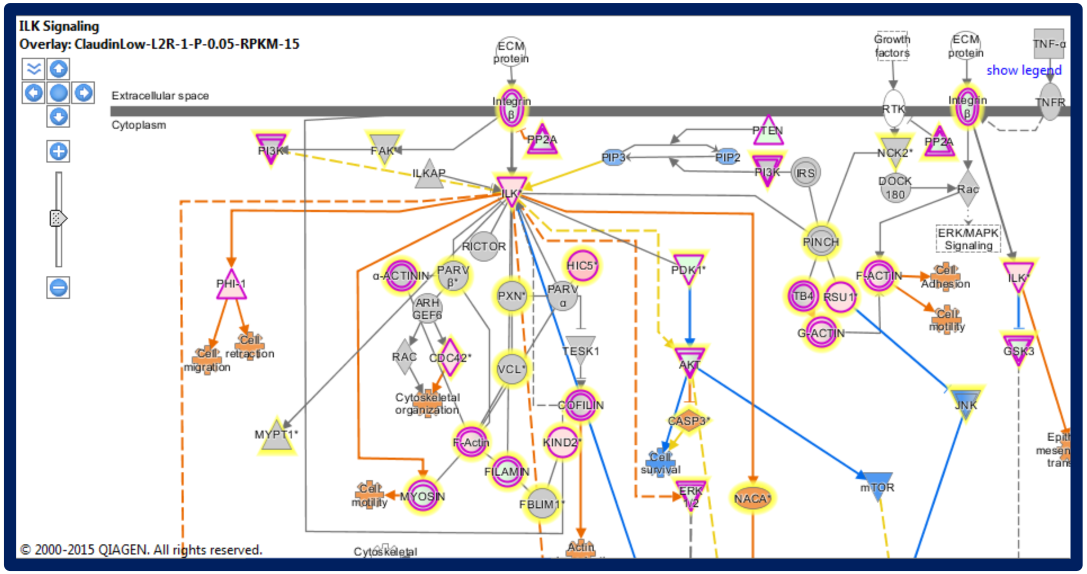
Upstream Analysis

Diseases and Functions

Regulator Effect

Networks





ILK Signaling
Overlay: ClaudinLow-L2R-1-P-0.05-RPKM-15

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IPA Core Analysis

Canonical Pathways

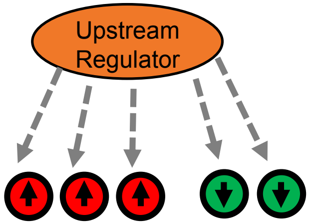
Upstream Analysis

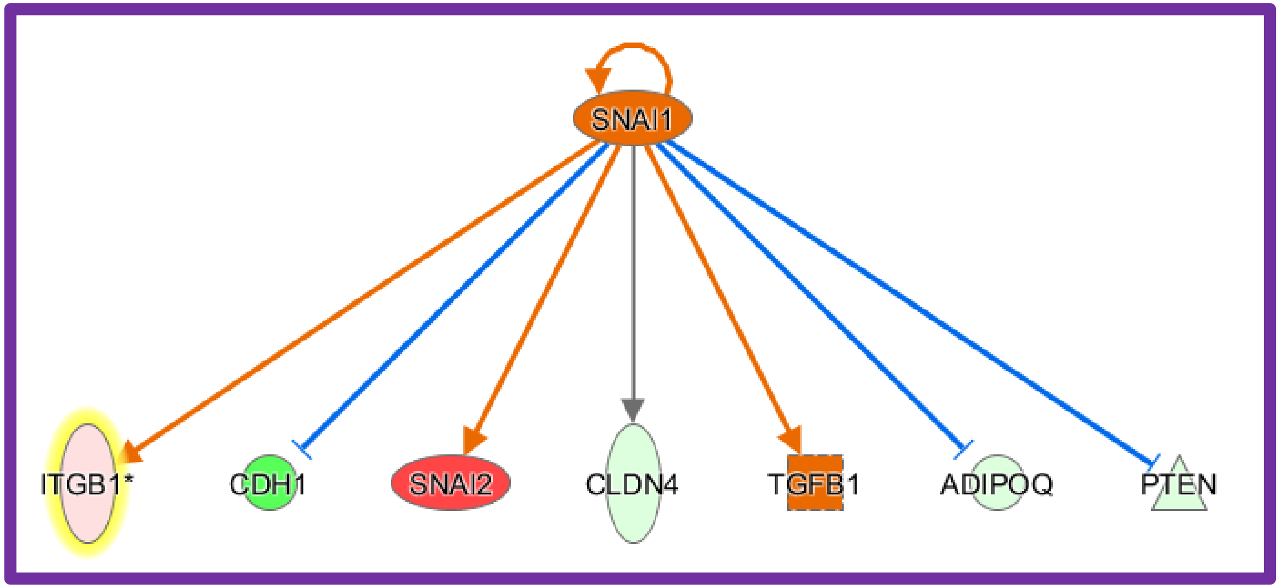
- ☐ Identifies upstream regulators eliciting expression changes
- ☐ Predicts activation state

Diseases and Functions

Regulator Effect

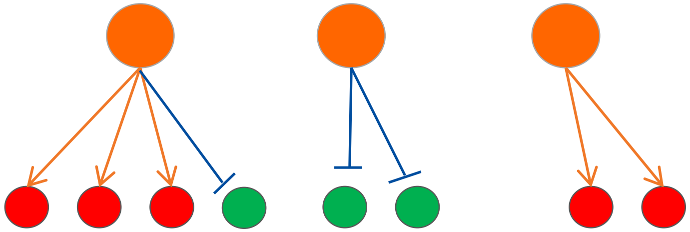
Networks



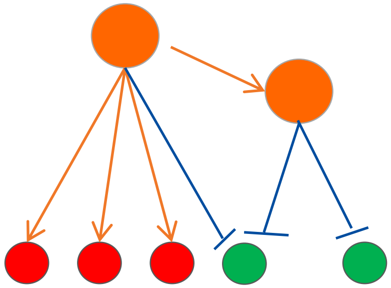


Upstream Regulators, Mechanistic Networks and Causal Networks

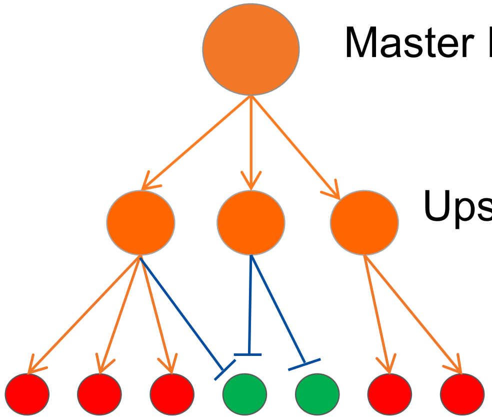
Upstream Regulators



Mechanistic Networks



Advanced Analytics: Causal Network Analysis



Master Regulators

Upstream Regulators

Your Dataset Genes/Molecules

IPA Core Analysis

Canonical Pathways

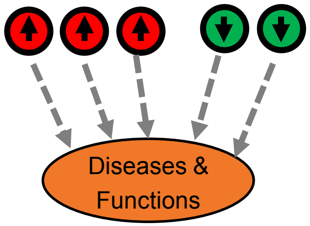
Upstream Analysis

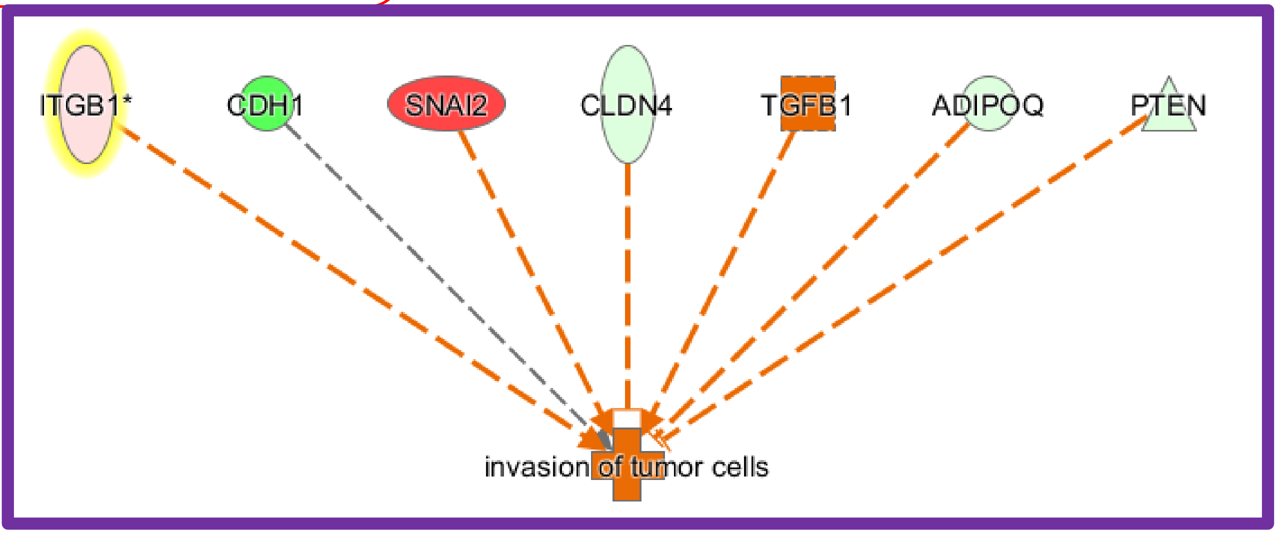
Diseases and Functions

☐ Predicts biological effects based on gene expression

Regulator Effect

Networks





IPA Core Analysis

Canonical Pathways

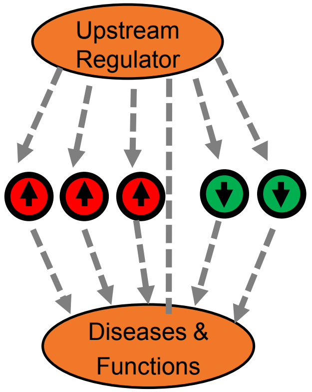
Upstream Analysis

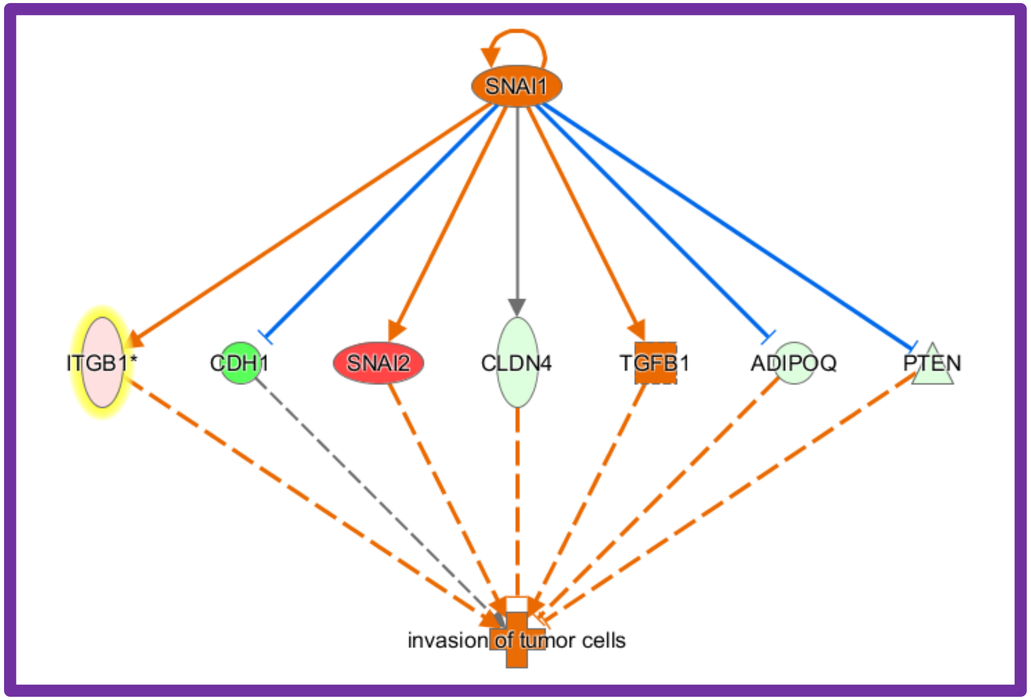
Diseases and Functions

Regulator Effect

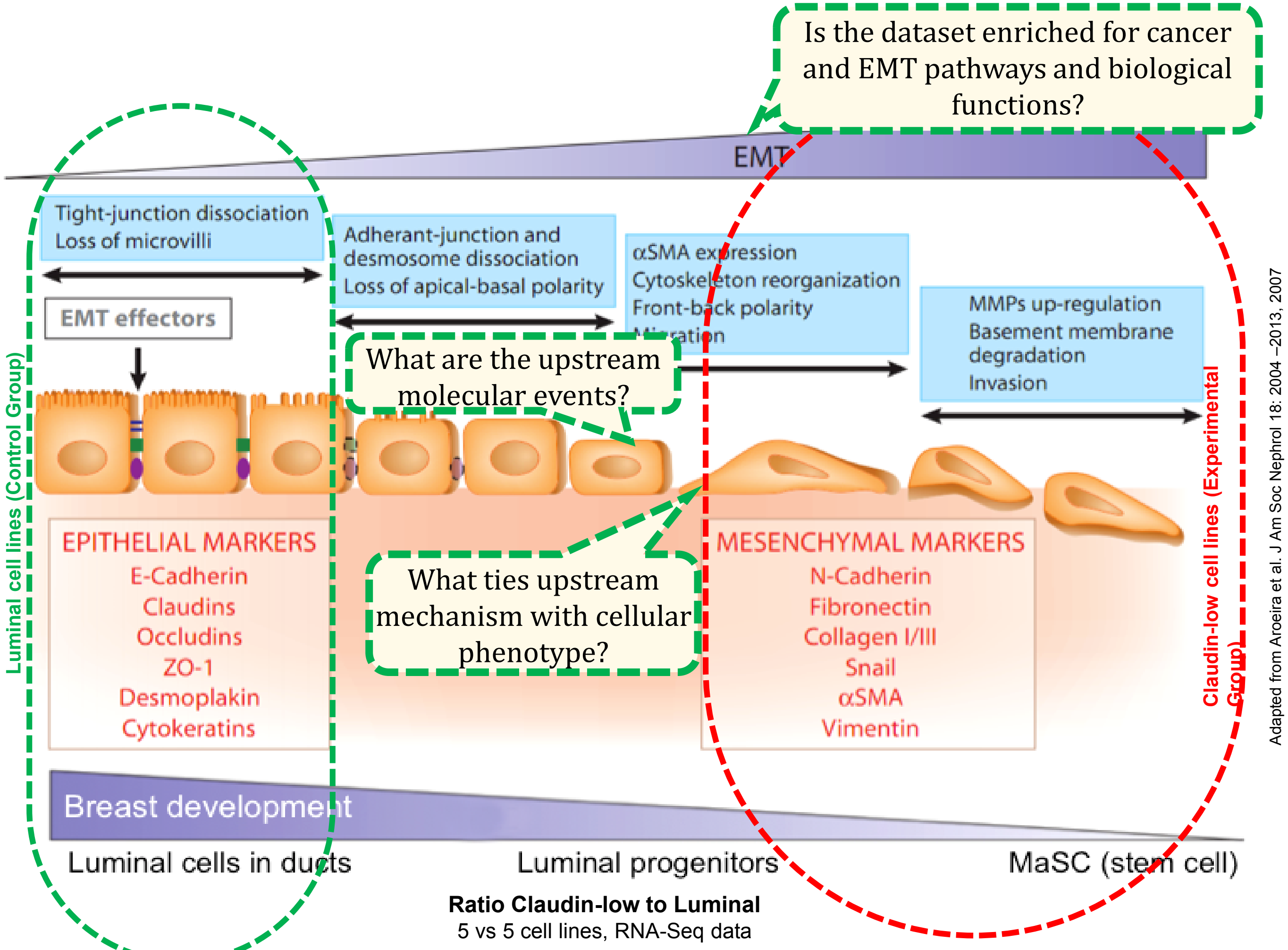
- ☐ Models pathway interactions
- ☐ How do dysregulated genes mediate a potential path from upstream regulator to function?

Networks





Core Analysis: Claudin low vs Luminal Breast Cancer



Is the dataset enriched for cancer and EMT pathways and biological functions?

What are the upstream molecular events?

What ties upstream mechanism with cellular phenotype?

Ratio Claudin-low to Luminal
5 vs 5 cell lines, RNA-Seq data

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

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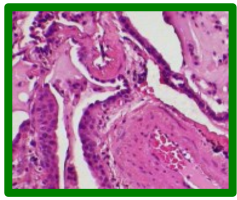
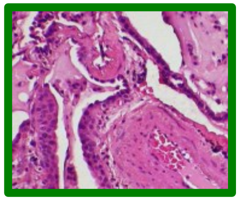
Comparison Analysis: BPH, PCA, MET

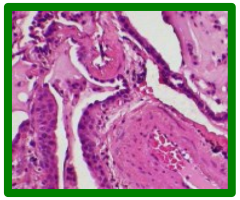
BPH



PCA

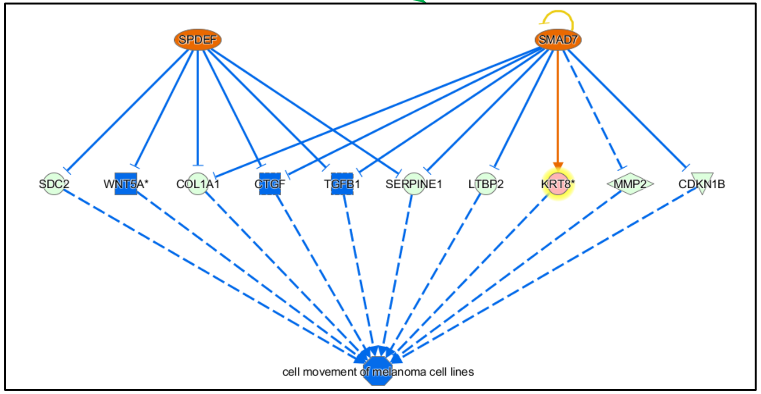


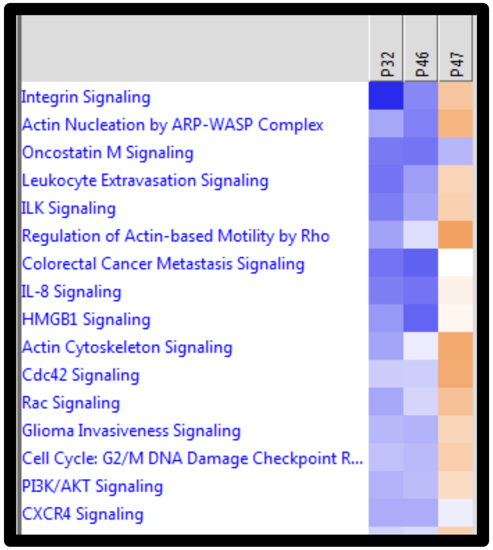
MET



Can we form new hypothesis/conclusions?

What are the upstream molecular events?



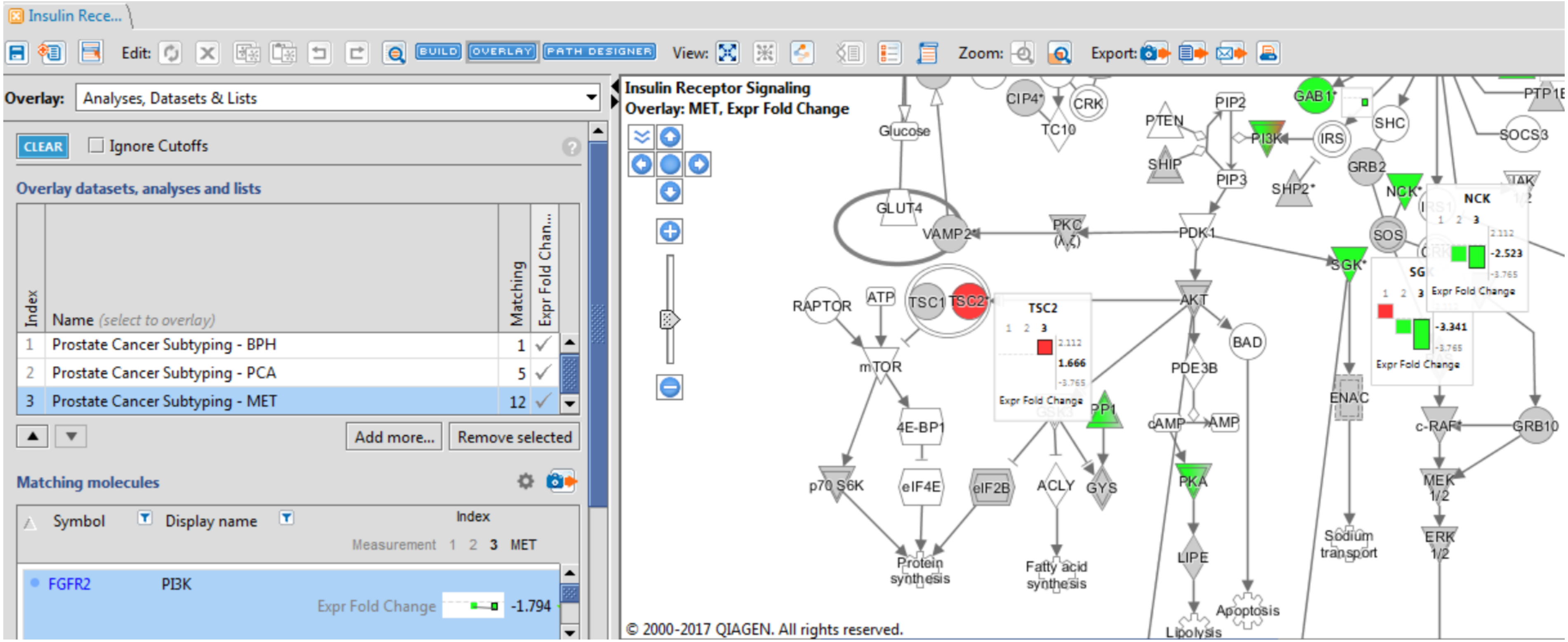


P32 P46 P47

Integrin Signaling
Actin Nucleation by ARP-WASP Complex
Oncostatin M Signaling
Leukocyte Extravasation Signaling
ILK Signaling
Regulation of Actin-based Motility by Rho
Colorectal Cancer Metastasis Signaling
IL-8 Signaling
HMGB1 Signaling
Actin Cytoskeleton Signaling
Cdc42 Signaling
Rac Signaling
Glioma Invasiveness Signaling
Cell Cycle: G2/M DNA Damage Checkpoint R...
PI3K/AKT Signaling
CXCR4 Signaling

How are these events different among the samples?

Overlay expression values for multiple experiments/disease conditions



Overlay: Analyses, Datasets & Lists

☐ Ignore Cutoffs

Overlay datasets, analyses and lists

Index	Name *(select to overlay)*	Matching	Expr Fold Chan...
1	Prostate Cancer Subtyping - BPH	1	✓
2	Prostate Cancer Subtyping - PCA	5	✓
3	Prostate Cancer Subtyping - MET	12	✓

Matching molecules

Symbol	Display name	Index Measurement 1 2 3 MET
FGFR2	PI3K	Expr Fold Change -1.794

Insulin Receptor Signaling
Overlay: MET, Expr Fold Change

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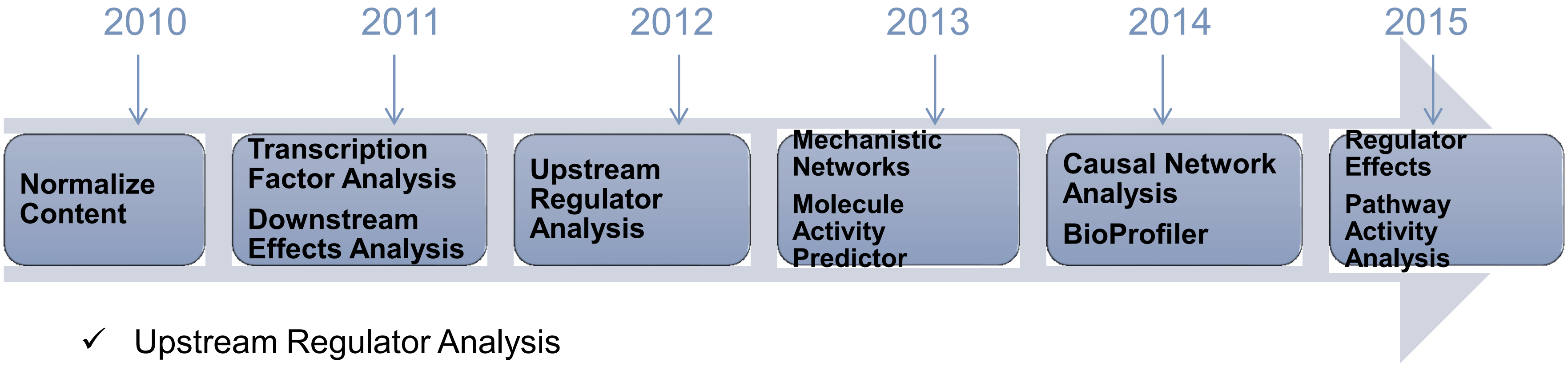
  - ☐ Search and Explore
- Questions/Answer

IPA: Focused on Hypothesis Generation



2010

Normalize Content

2011

Transcription Factor Analysis

Downstream Effects Analysis

2012

Upstream Regulator Analysis

2013

Mechanistic Networks

Molecule Activity Predictor

2014

Causal Network Analysis

BioProfiler

2015

Regulator Effects

Pathway Activity Analysis

- ✓ Upstream Regulator Analysis
  - Predict upstream molecules which may be causing the observed gene expression changes
- ✓ Downstream Effects Analysis
  - Identify whether significant downstream biological processes are increased or decreased based on gene expression results
- ✓ Causal Network Analysis*
  - Uncover hidden connections in upstream regulators to generate plausible causal networks which explain observed expression changes
- ✓ Regulator Effects
  - Integrate Upstream Regulator results with Downstream Effects results predict what may occur upstream to cause phenotypic or functional outcomes downstream.

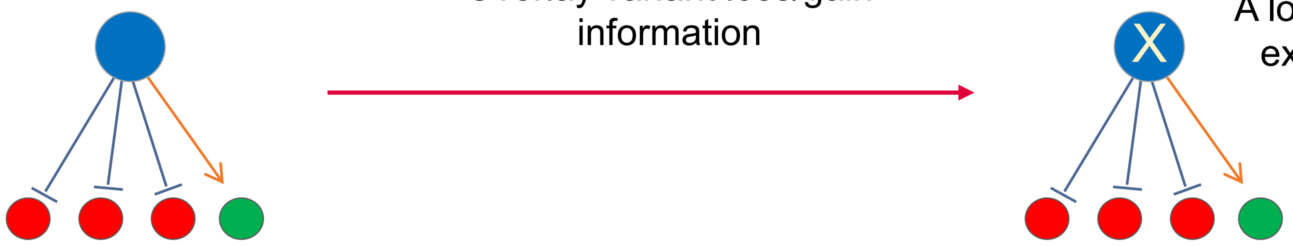
From Casual Analytics to Multi-omics

5 years of progress: IPA highlights 2012-2016

Capabilities	2012	2013	2014	2015	2016
Causal Content and Analysis	• Upstream Regulator Analysis • Downstream Effects Analysis • Mechanistic Networks • Molecule Activity Predictor	• BioProfiler • Causal Network Analysis • Diseases and functions on networks	• Regulator Effects • Diseases & Functions on Canonical Pathways • Grow to Diseases and Functions • Pathway Activity Analysis		
Enterprise Support		• Batch dataset upload (with metadata) • Dataset & analysis search • My Findings spreadsheet	• My Findings graphical entry interface • Relationship Export	• IPA client Installer	
Comparison Analysis		• Comparison analysis heatmaps trends, clusters	• Filtering of heatmaps	• Gene-level heatmaps	
RNA-seq Support	• Isoform view for human	• Overlay data in isoform view	• Ensembl support for Isoforms • IPA Plugin for CLC	• Isoform view for mouse • IsoProfiler (v1) • CuffDiff import	• IsoProfiler (v2) • Isoform names / heat maps on pathways • Automated processing of data to IPA from CLC
Multi-omics Support				• Variant loss/gain & ACMG import • Core Analysis Variant loss/gain • Set color range on pathways	• New bar charts on pathways • Overlay multiple analyses / datasets of different types
Phospho-proteomics					• Phospho data import • Initial Phospho analysis (CP, DEA) with flipped nodes • Overlay phospho-sites on pathways

Synergism between expression and variant data

Variants can provide a mechanism to explain the observed biology



Upstream Regulator
(predicted to be inhibited
based on expression)

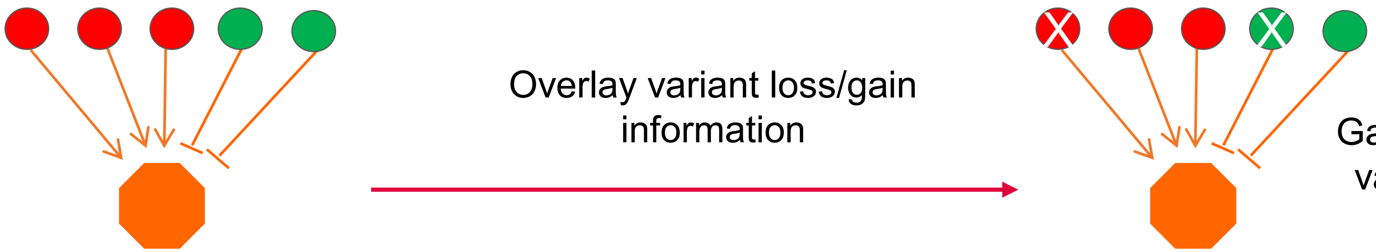
Dataset Molecules

Overlay variant loss/gain
information

Upstream Regulator

A loss-of-function variant
explains/validates the
prediction

Dataset Molecules



Dataset Molecules

Diseases / functions

Overlay variant loss/gain
information

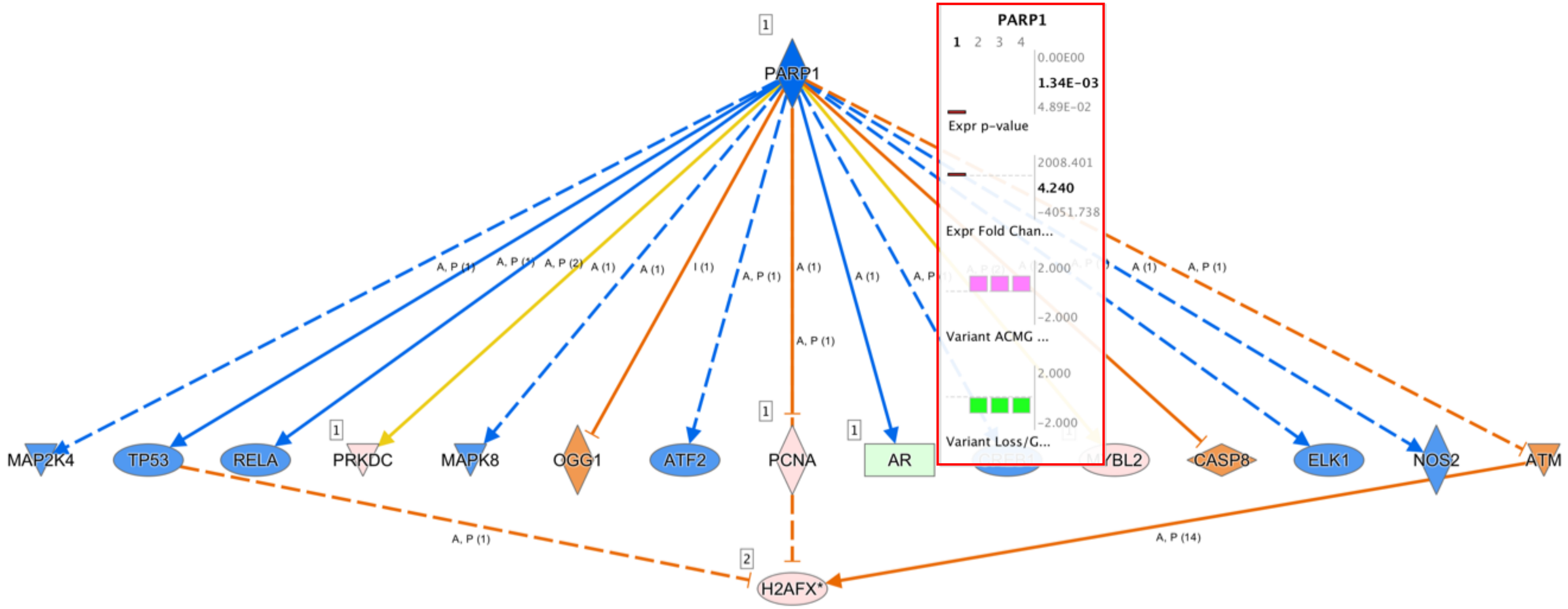
Dataset Molecules

Gain or loss of function
variants contribute to
known phenotype

Diseases / functions

Elucidating mechanism of expression changes and phenotypes

Integrate expression results with gene variant information

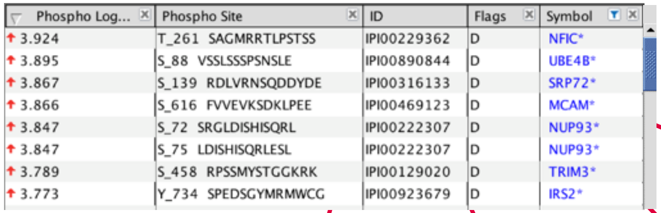


Example: HCC RNA-seq dataset and individual patient variant datasets overlaid on causal network.

- → PARP1 is *upregulated* in the three patients but is likely *inactivated* by mutations.
- → The inactivation is correspondingly predicted from expression data as shown above
- → Some disease findings for PARp1 are only found in HGMD and Ingenuity Expert Mutation content

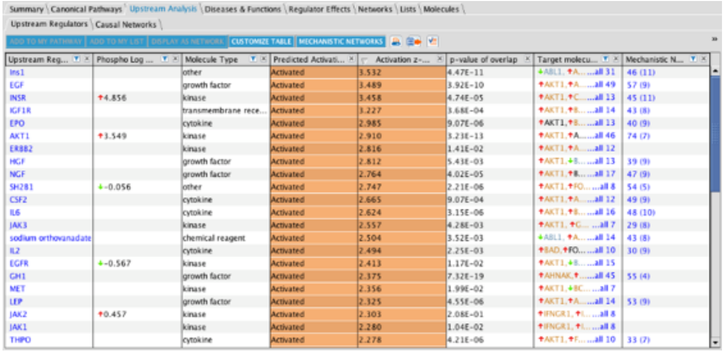
Phosphorylation Analysis in IPA

Phosphoproteomics dataset



Phospho Log...	Phospho Site	ID	Flags	Symbol
↑3.924	T_261 SAGMRRTLPSTSS	IPI00229362	D	NFIC*
↑3.895	S_88 VSSLSSSPSNSLE	IPI00890844	D	UBE4B*
↑3.867	S_139 RDLVRNSQDDYDE	IPI00316133	D	SRP72*
↑3.866	S_616 FVVEVKSDKLPEE	IPI00469123	D	MCAM*
↑3.847	S_72 SRGLDISHISQRL	IPI00222307	D	NUP93*
↑3.847	S_75 LDISHISQRLESL	IPI00222307	D	NUP93*
↑3.789	S_458 RPSSMYSTGGKRK	IPI00129020	D	TRIM3*
↑3.773	Y_734 SPEDSGYMRMWCG	IPI00923679	D	IRS2*

Upstream phospho regulators and causal phospho networks (March 2017)



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

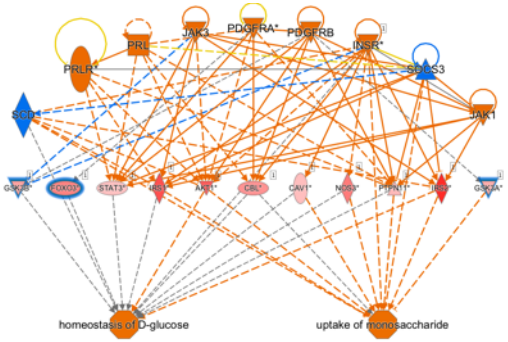
Upstream Regulators | Causal Networks

Upstream Reg...	Phospho Log ...	Molecule Type	Predicted Activati...	Activation z-...	p-value of overlap	Target molecu...	Mechanistic N...
Ins1		other	Activated	3.532	4.47E-11	↓ABL1, ↑A... ...all 31	46 (11)
EGF		growth factor	Activated	3.489	3.92E-10	↑AKT1, ↑A... ...all 49	57 (9)
INSR	↑4.856	kinase	Activated	3.458	4.74E-05	↑AKT1, ↑C... ...all 13	45 (11)
IGF1R		transmembrane rece...	Activated	3.227	3.68E-04	↑AKT1, ↑B... ...all 14	43 (8)
EPO		cytokine	Activated	2.985	9.07E-06	↑AKT1, ↑B... ...all 13	40 (9)
AKT1	↑3.549	kinase	Activated	2.910	3.23E-13	↑AKT1, ↑A... ...all 46	74 (7)
ERBB2		kinase	Activated	2.816	1.41E-02	↑AKT1, ↑A... ...all 12	
HGF		growth factor	Activated	2.812	5.43E-03	↑AKT1, ↓B... ...all 13	39 (9)
NGF		growth factor	Activated	2.764	4.02E-05	↑AKT1, ↑B... ...all 17	47 (9)
SH2B1	↓-0.056	other	Activated	2.747	2.21E-06	↑AKT1, ↑FO... ...all 8	54 (5)
CSF2		cytokine	Activated	2.665	9.07E-04	↑AKT1, ↑A... ...all 12	49 (9)
IL6		cytokine	Activated	2.624	3.15E-06	↑AKT1, ↑B... ...all 16	48 (10)
JAK3		kinase	Activated	2.557	4.28E-03	↑AKT1, ↑C... ...all 7	29 (8)
sodium orthovanadate		chemical reagent	Activated	2.504	3.52E-03	↓ABL1, ↑A... ...all 14	43 (8)
IL2		cytokine	Activated	2.494	2.25E-03	↑BAD, ↑FO... ...all 10	30 (9)
EGFR	↓-0.567	kinase	Activated	2.413	1.17E-02	↑AKT1, ↓B... ...all 15	
GH1		growth factor	Activated	2.375	7.32E-19	↑AHNAK, ↑... ...all 45	55 (4)
MET		kinase	Activated	2.356	1.99E-02	↑AKT1, ↓BC... ...all 7	
LEP		growth factor	Activated	2.325	4.55E-06	↑AKT1, ↑A... ...all 14	53 (9)
JAK2	↑0.457	kinase	Activated	2.303	2.08E-01	↑IFNGR1, ↑I... ...all 8	
JAK1		kinase	Activated	2.280	1.04E-02	↑IFNGR1, ↑I... ...all 8	
THPO		cytokine	Activated	2.278	4.21E-06	↑AKT1, ↑F... ...all 10	33 (7)

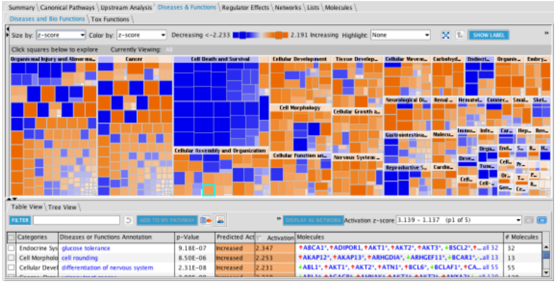
Canonical Pathways

Phospho Regulator Effects

(March 2017)



Downstream Effects



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

Diseases and Bio Functions | Tox Functions

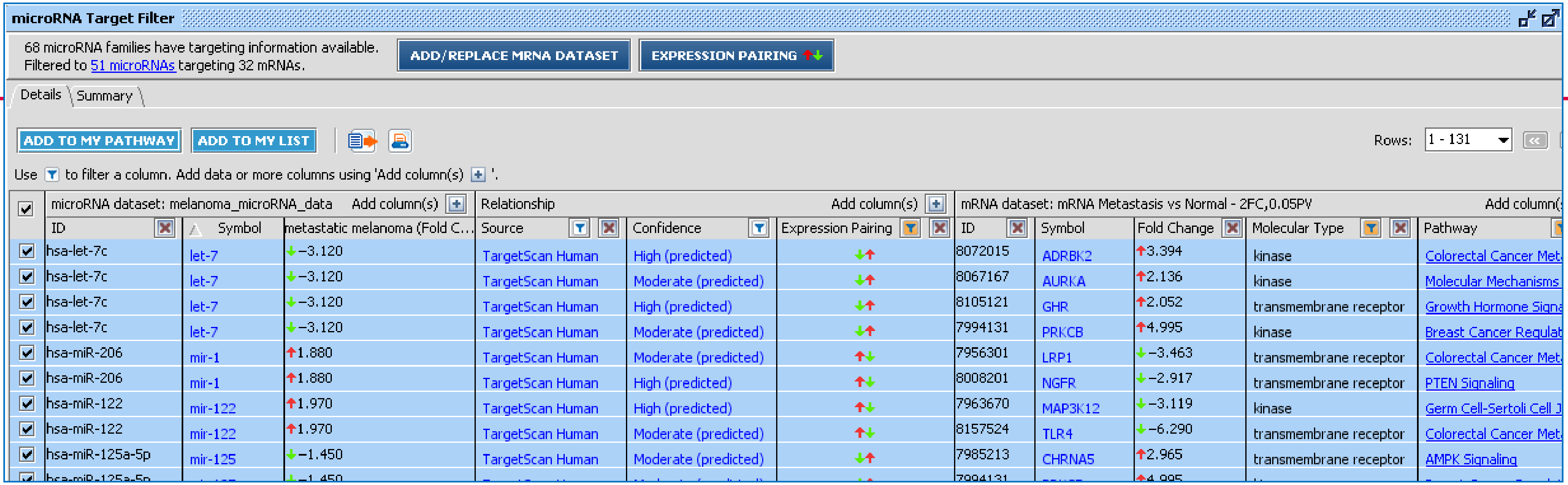
Table View | Tree View

Activation z-score 3.139 - 1.137 (p1 of 5)

☐	Categories	Diseases or Functions Annotation	p-Value	Predicted Act	Activation	Molecules	# Molecules
☐	Endocrine Sys	glucose tolerance	9.18E-07	Increased	2.347	↑ABCA1*, ↑ADIPOR1, ↑AKT1*, ↑AKT2*, ↑AKT3*, ↓BSCL2*, ↑... all 32	32
☐	Cell Morpholo	cell rounding	8.50E-06	Increased	2.253	↑AKAP12*, ↑AKAP13*, ↑ARHGDIA*, ↓ARHGEF11*, ↓BCAR1*, ...all 13	13
☐	Cellular Devel	differentiation of nervous system	2.31E-08	Increased	2.231	↓ABL1*, ↑AKT1*, ↑AKT2*, ↑ATN1*, ↑BCL6*, ↓BCLAF1*, ↑CA... all 55	55

Overview

- Introduction to IPA
- Large Dataset Analysis
  - Core Analysis
  - Comparison Analysis
- Newer IPA Functionalities
  - Overlay variant data
  - **Phosphoproteomic analysis**
  - miRNA Target Filter
  - Isoprofiler
  - Bioprofiler
  - Search and Explore
- Questions/Answer



68 microRNA families have targeting information available.
Filtered to 51 microRNAs targeting 32 mRNAs.

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

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

miRNA data

miRNA Target Filter

Molecule Type

Pathways (Cancer/ Growth)

mRNA

↑↓
↓↑

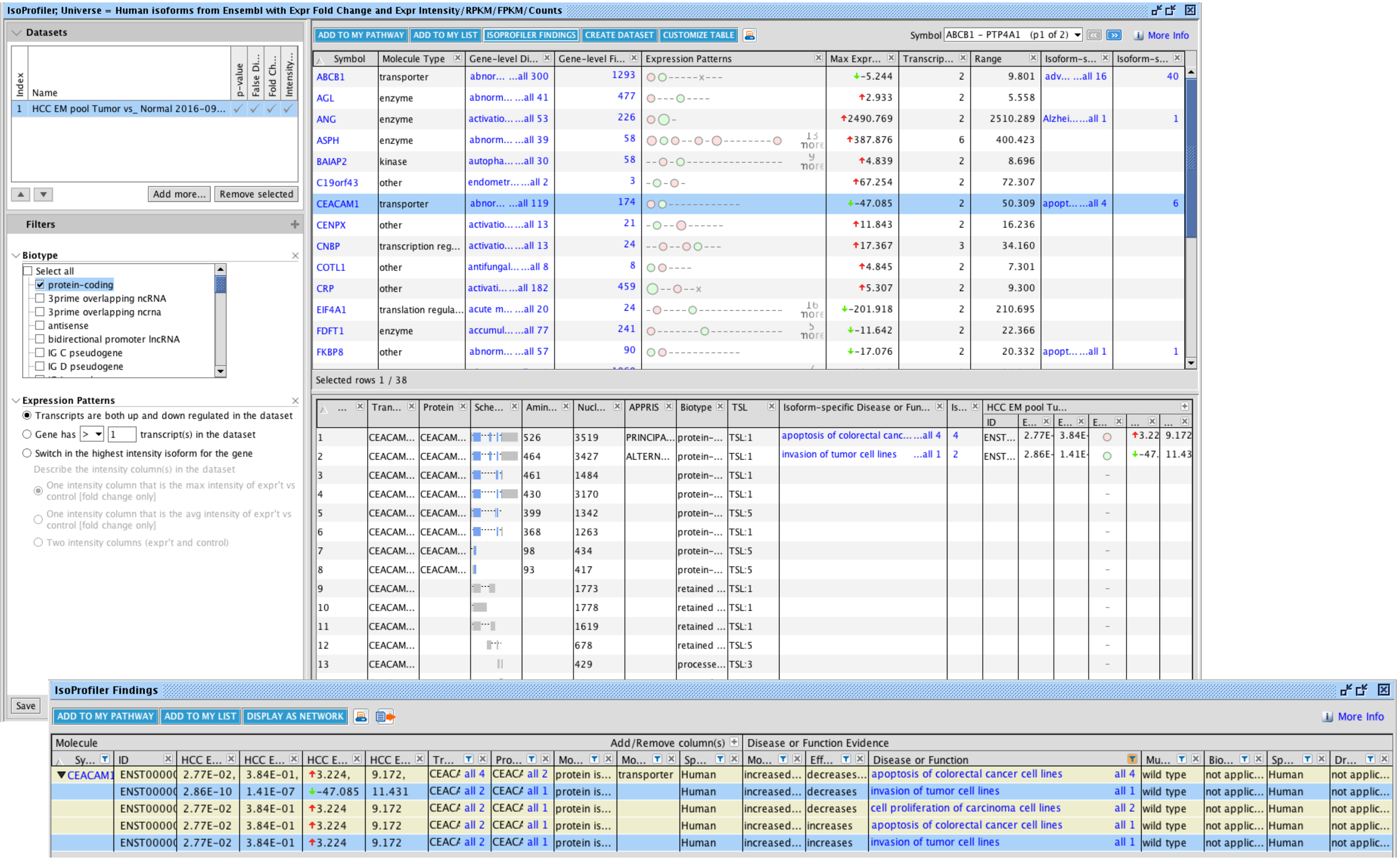
?

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

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

IsoProfiler helps you discover biologically relevant transcripts

CEACAM1 has oppositely regulated transcripts in HCC, leading to same outcome



IsoProfiler; Universe = Human isoforms from Ensembl with Expr Fold Change and Expr Intensity/RPKM/FPKM/Counts

Datasets

Index	Name	p-value	False Di...	Fold Ch...	Intensity...
1	HCC EM pool Tumor vs_ Normal 2016-09...	✓	✓	✓	✓

Filters

Biotype

- ☐ Select all
  - ☑ protein-coding
  - ☐ 3prime overlapping ncRNA
  - ☐ 3prime overlapping ncrna
  - ☐ antisense
  - ☐ bidirectional promoter lncRNA
  - ☐ IG C pseudogene
  - ☐ IG D pseudogene

Expression Patterns

- ● Transcripts are both up and down regulated in the dataset
- ○ Gene has > 1 transcript(s) in the dataset
- ○ Switch in the highest intensity isoform for the gene

Describe the intensity column(s) in the dataset

- ● One intensity column that is the max intensity of expr't vs control [fold change only]
- ○ One intensity column that is the avg intensity of expr't vs control [fold change only]
- ○ Two intensity columns (expr't and control)

Symbol ABCB1 – PTP4A1 (p1 of 2)

Symbol	Molecule Type	Gene-level Di...	Gene-level Fi...	Expression Patterns	Max Expr...	Transcrip...	Range	Isoform-s...	Isoform-s...
ABCB1	transporter	abnor... ...all 300	1293	○○-----x---	↓-5.244	2	9.801	adv... ...all 16	40
AGL	enzyme	abnorm... ...all 41	477	○---○----	↑2.933	2	5.558		
ANG	enzyme	activatio... ...all 53	226	○○-	↑2490.769	2	2510.289	Alzhei... ...all 1	1
ASPH	enzyme	abnorm... ...all 39	58	○○○--○-○--------○ 13 more	↑387.876	6	400.423		
BAIAP2	kinase	autopha... ...all 30	58	--○-○--------------- 9 more	↑4.839	2	8.696		
C19orf43	other	endometr... ...all 2	3	-○-○-	↑67.254	2	72.307		
CEACAM1	transporter	abnor... ...all 119	174	○○------------	↓-47.085	2	50.309	apopt... ...all 4	6
CENPX	other	activatio... ...all 13	21	-○--○------	↑11.843	2	16.236		
CNBP	transcription reg...	activatio... ...all 13	24	--○--○○---	↑17.367	3	34.160		
COTL1	other	antifungal... ...all 8	8	○○----	↑4.845	2	7.301		
CRP	other	activati... ...all 182	459	○--○--x	↑5.307	2	9.300		
EIF4A1	translation regula...	acute m... ...all 20	24	-○----○-------------- 16 more	↓-201.918	2	210.695		
FDFT1	enzyme	accumul... ...all 77	241	○-------○------------ 5 more	↓-11.642	2	22.366		
FKBP8	other	abnorm... ...all 57	90	○○------------	↓-17.076	2	20.332	apopt... ...all 1	1

Selected rows 1 / 38

...	Tran...	Protein	Sche...	Amin...	Nucl...	APPRIS	Biotype	TSL	Isoform-specific Disease or Fun...	Is...	HCC EM pool Tu... ID	E...	E...	E...	...	...
1	CEACAM...	CEACAM...		526	3519	PRINCIPA...	protein-...	TSL:1	apoptosis of colorectal canc... ...all 4	4	ENST...	2.77E-	3.84E-	○	↑3.22	9.172
2	CEACAM...	CEACAM...		464	3427	ALTERN...	protein-...	TSL:1	invasion of tumor cell lines ...all 1	2	ENST...	2.86E-	1.41E-	○	↓-47.	11.43
3	CEACAM...	CEACAM...		461	1484		protein-...	TSL:1						-		
4	CEACAM...	CEACAM...		430	3170		protein-...	TSL:1						-		
5	CEACAM...	CEACAM...		399	1342		protein-...	TSL:5						-		
6	CEACAM...	CEACAM...		368	1263		protein-...	TSL:1						-		
7	CEACAM...	CEACAM...		98	434		protein-...	TSL:5						-		
8	CEACAM...	CEACAM...		93	417		protein-...	TSL:5						-		
9	CEACAM...				1773		retained ...	TSL:1						-		
10	CEACAM...				1778		retained ...	TSL:1						-		
11	CEACAM...				1619		retained ...	TSL:1						-		
12	CEACAM...				678		retained ...	TSL:5						-		
13	CEACAM...				429		processe...	TSL:3						-		

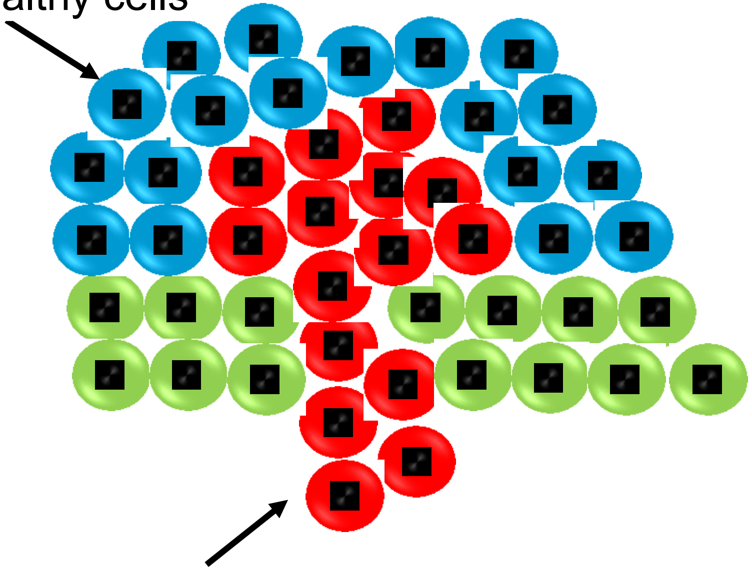
IsoProfiler Findings

Molecule											Disease or Function Evidence						
Sy...	ID	HCC E...	HCC E...	HCC E...	HCC E...	Tr...	Pro...	Mo...	Mo...	Sp...	Mo...	Eff...	Disease or Function		Mu...	Bio...	Sp...	Dr...
CEACAM1	ENST00000	2.77E-02,	3.84E-01,	↑3.224,	9.172,	CEACA all 4	CEACA all 2	protein is...	transporter	Human	increased...	decreases...	apoptosis of colorectal cancer cell lines	all 4	wild type	not applic...	Human	not applic...
	ENST00000	2.86E-10	1.41E-07	↓-47.085	11.431	CEACA all 2	CEACA all 1	protein is...		Human	increased...	decreases	invasion of tumor cell lines	all 1	wild type	not applic...	Human	not applic...
	ENST00000	2.77E-02	3.84E-01	↑3.224	9.172	CEACA all 2	CEACA all 1	protein is...		Human	increased...	decreases	cell proliferation of carcinoma cell lines	all 2	wild type	not applic...	Human	not applic...
	ENST00000	2.77E-02	3.84E-01	↑3.224	9.172	CEACA all 2	CEACA all 1	protein is...		Human	increased...	increases	apoptosis of colorectal cancer cell lines	all 1	wild type	not applic...	Human	not applic...
	ENST00000	2.77E-02	3.84E-01	↑3.224	9.172	CEACA all 2	CEACA all 1	protein is...		Human	increased...	increases	invasion of tumor cell lines	all 1	wild type	not applic...	Human	not applic...

Search and Explore

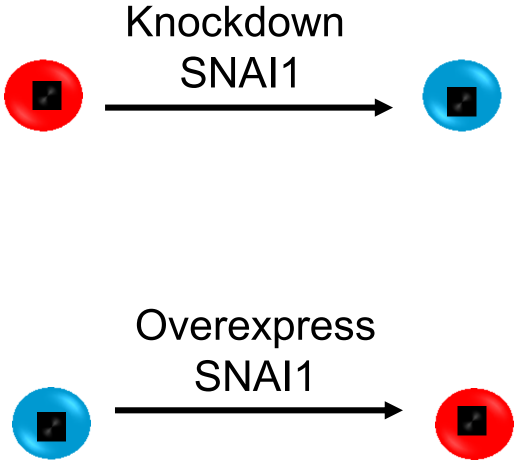
(Data not required)

Case Study: SNAI1 knockdown making cells less invasive



Blue = healthy cells

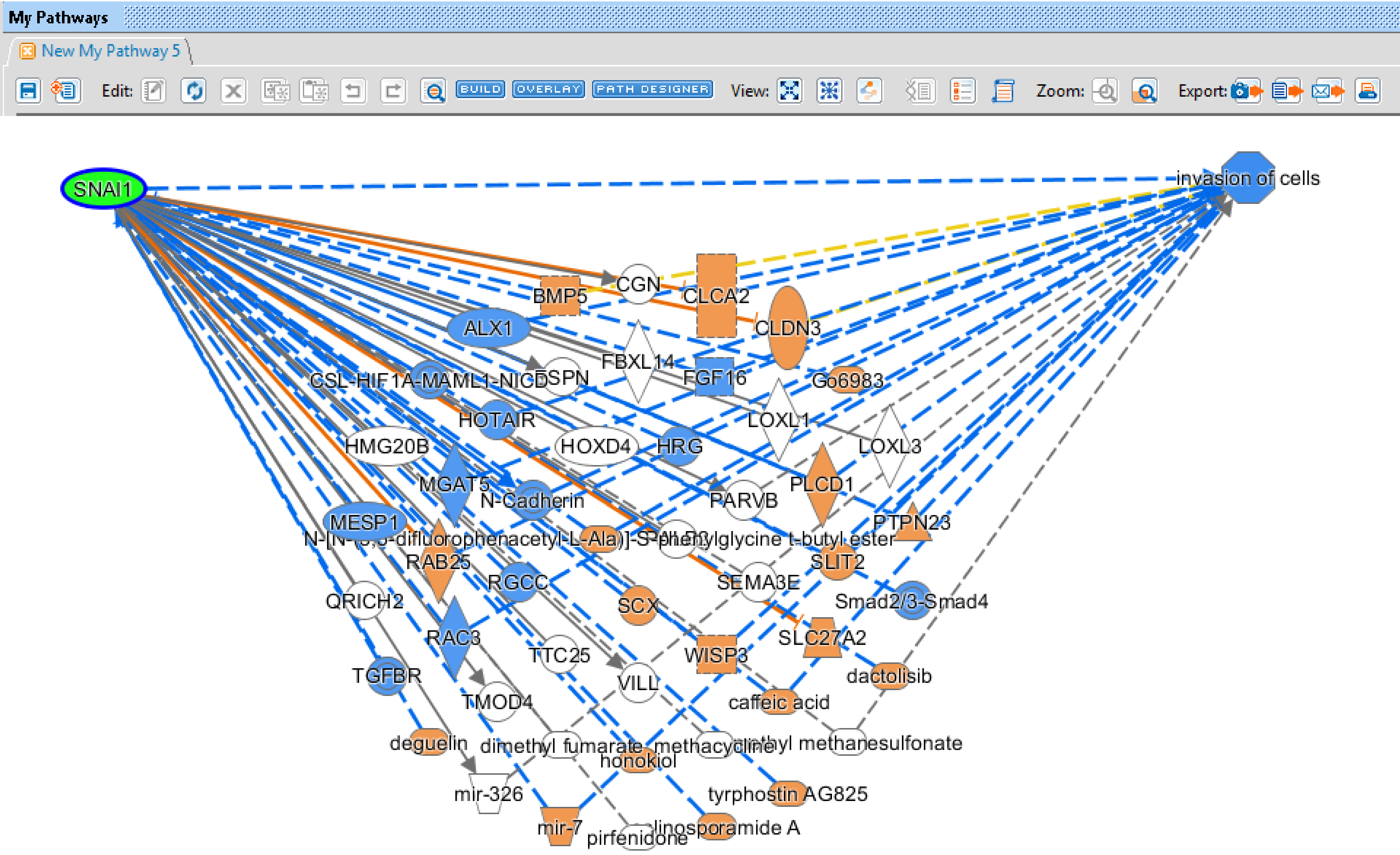
Red = invasive cells



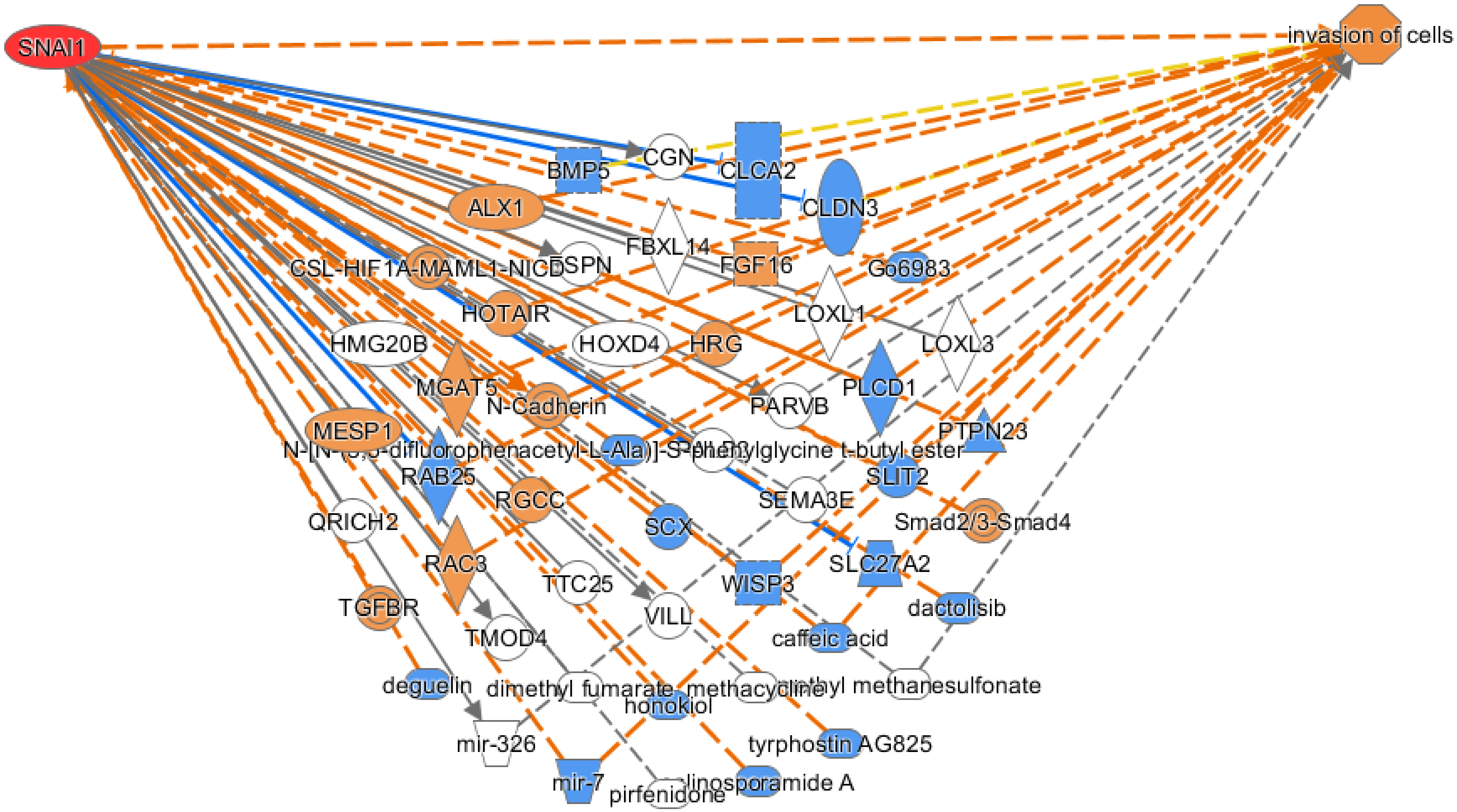
Knockdown SNAI1

Overexpress SNAI1

Case Study: SNAI1 knockdown making cells less invasive

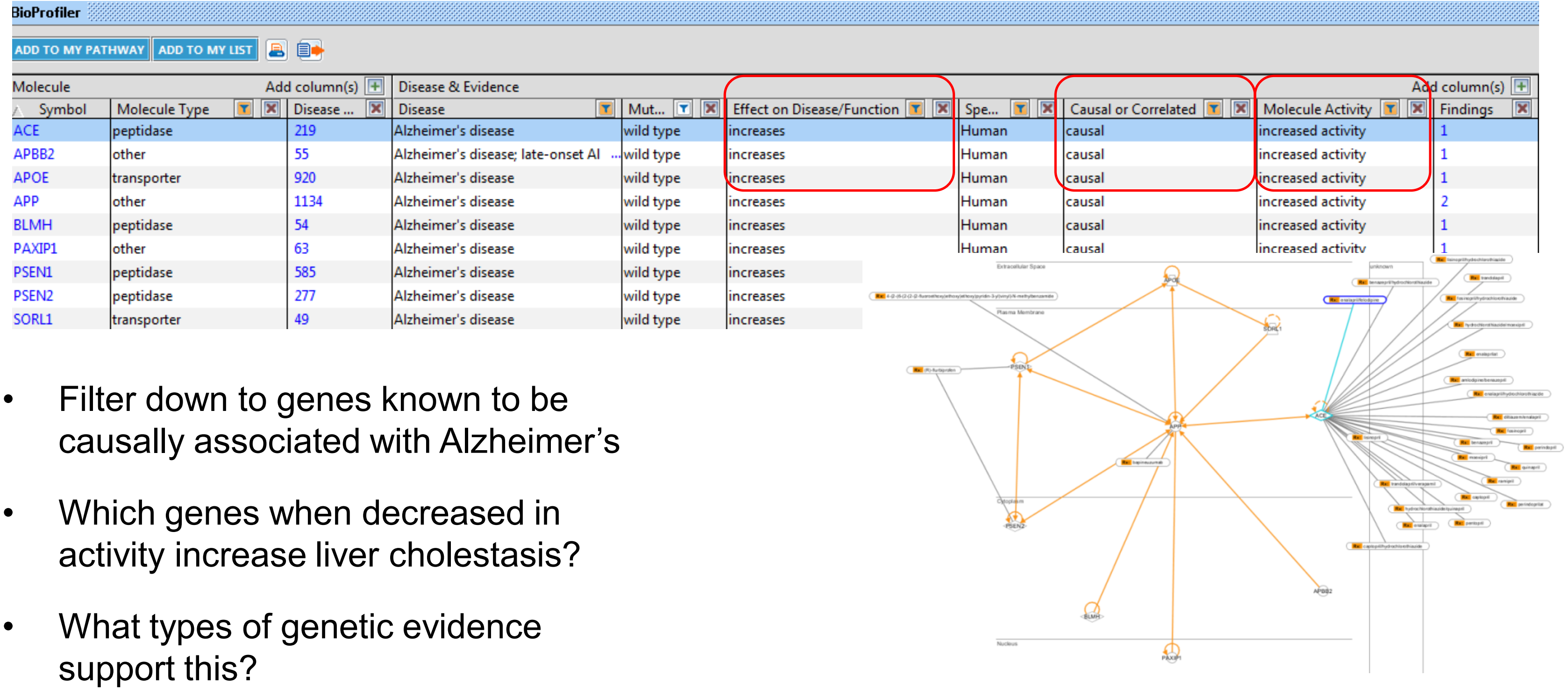


Case Study: SNAI1 knockdown making cells less invasive



BioProfiler (new)
Quickly profile a disease, phenotype, or function

Identify genes known to be causally relevant as potential targets or identify targets of toxicity, associated known drugs, biomarkers and pathways



Symbol	Molecule Type	Disease ...	Disease	Mut...	Effect on Disease/Function	Spe...	Causal or Correlated	Molecule Activity	Findings
ACE	peptidase	219	Alzheimer's disease	wild type	increases	Human	causal	increased activity	1
APBB2	other	55	Alzheimer's disease; late-onset Al ...	wild type	increases	Human	causal	increased activity	1
APOE	transporter	920	Alzheimer's disease	wild type	increases	Human	causal	increased activity	1
APP	other	1134	Alzheimer's disease	wild type	increases	Human	causal	increased activity	2
BLMH	peptidase	54	Alzheimer's disease	wild type	increases	Human	causal	increased activity	1
PAXIP1	other	63	Alzheimer's disease	wild type	increases	Human	causal	increased activity	1
PSEN1	peptidase	585	Alzheimer's disease	wild type	increases				
PSEN2	peptidase	277	Alzheimer's disease	wild type	increases				
SORL1	transporter	49	Alzheimer's disease	wild type	increases				

- Filter down to genes known to be causally associated with Alzheimer's
- Which genes when decreased in activity increase liver cholestasis?
- What types of genetic evidence support this?

Demo Overview

- Introduction to IPA
- Large Dataset Analysis
  - Data upload
  - Core Analysis
  - Comparison Analysis
- Other IPA Functionalities
  - Search and Explore
  - miRNA Target Filter
  - Isoprofiler
  - **Bioprofiler**
- Questions/Answer

IPA provides efficient insight

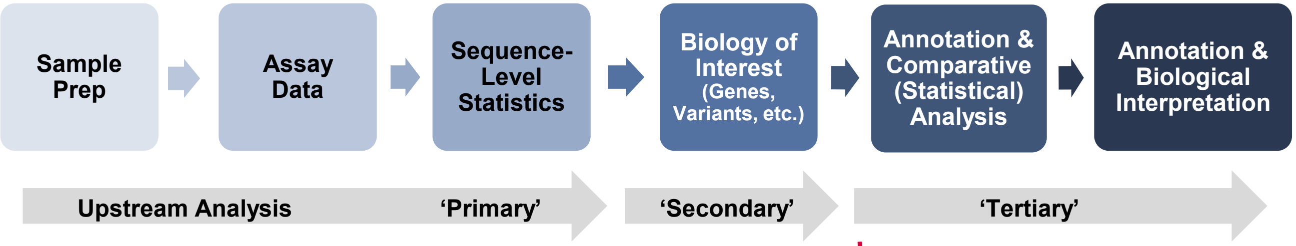
With IPA you can:

- Analyze and visually integrate multi-omics datasets
  - ☐ Identify pathway enrichment AND directionality
  - ☐ Predict upstream molecules that may be driving expression changes
    - Identify druggable targets
  - ☐ Link differentially expressed genes to your phenotype

- Mining the Knowledgebase: One stop shop
  - ☐ Look up all curated knowledge about drug/gene of interest
  - ☐ Explore and create pathways
  - ☐ Bioprofiler: identify actionable targets

When do you use IPA?

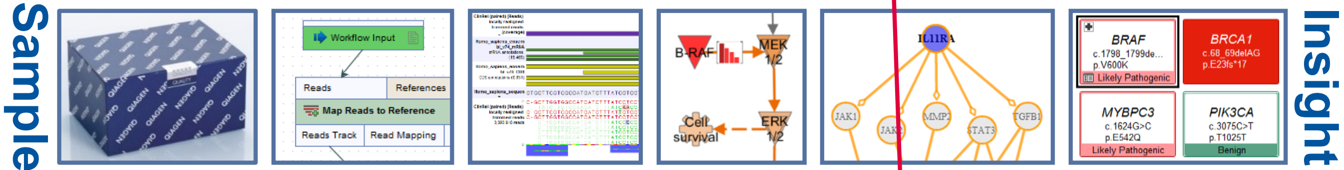
QIAGEN Sample to Insight



Sample Prep → **Assay Data** → **Sequence-Level Statistics** → **Biology of Interest (Genes, Variants, etc.)** → **Annotation & Comparative (Statistical) Analysis** → **Annotation & Biological Interpretation**

Upstream Analysis **'Primary'** → **'Secondary'** → **'Tertiary'**

Sample



Insight

QIAGEN

CLC bio A QIAGEN Company

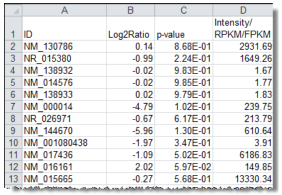
Gx Bx

INGENUITY VARIANT ANALYSIS

QIAGEN Clinical Insight

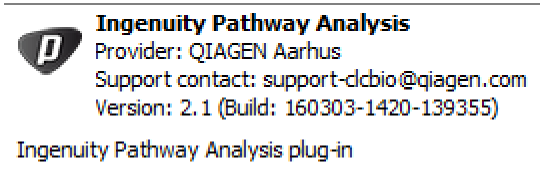
BIOBASE BIOLOGICAL DATABASES

INGENUITY PATHWAY ANALYSIS



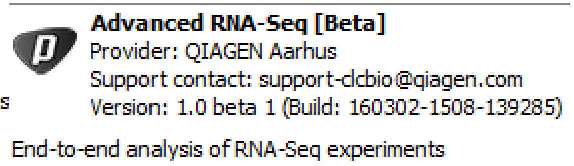
	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
4	NM_138932	-0.02	9.83E-01	1.67
5	NM_014576	-0.02	9.85E-01	1.77
6	NM_138933	0.02	9.79E-01	1.83
7	NM_000014	-4.79	1.02E-01	239.75
8	NR_026971	-0.67	6.17E-01	213.79
9	NM_144670	-5.96	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

The Ingenuity Pathway Analysis plugin – in BxWB



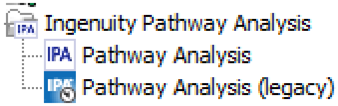
Ingenuity Pathway Analysis
Provider: QIAGEN Aarhus
Support contact: support-clcbio@qiagen.com
Version: 2.1 (Build: 160303-1420-139355)

Ingenuity Pathway Analysis plug-in

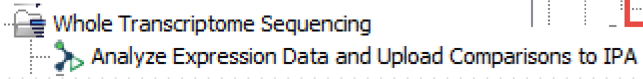


Advanced RNA-Seq [Beta]
Provider: QIAGEN Aarhus
Support contact: support-clcbio@qiagen.com
Version: 1.0 beta 1 (Build: 160302-1508-139285)

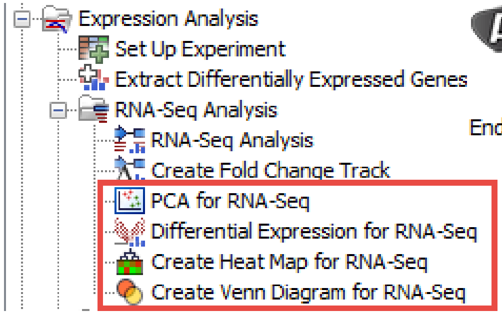
End-to-end analysis of RNA-Seq experiments



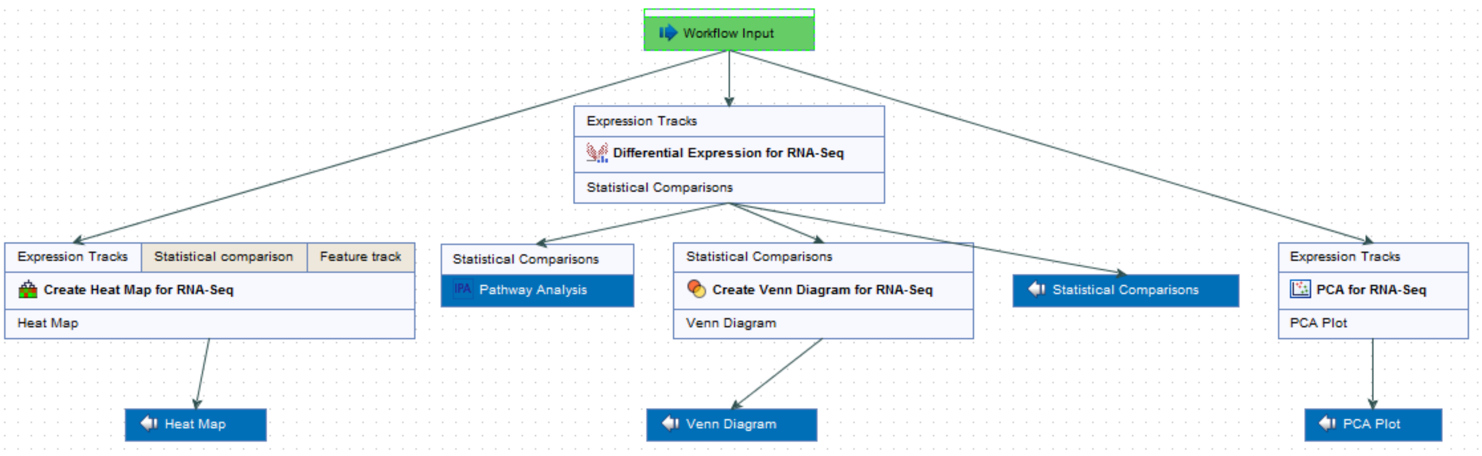
- Ingenuity Pathway Analysis
  - Pathway Analysis
  - Pathway Analysis (legacy)

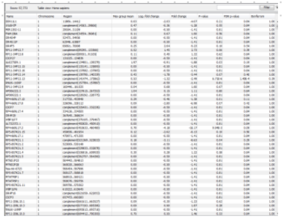


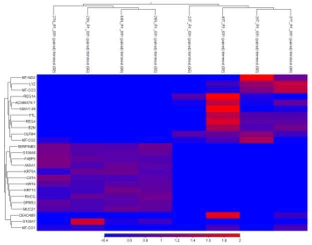
- Whole Transcriptome Sequencing
  - Analyze Expression Data and Upload Comparisons to IPA

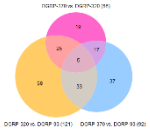


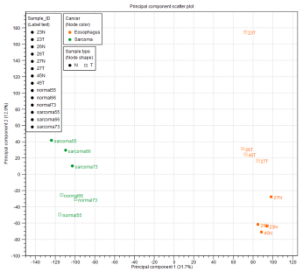
- Expression Analysis
  - Set Up Experiment
  - Extract Differentially Expressed Genes
  - RNA-Seq Analysis
    - RNA-Seq Analysis
    - Create Fold Change Track
    - PCA for RNA-Seq
    - Differential Expression for RNA-Seq
    - Create Heat Map for RNA-Seq
    - Create Venn Diagram for RNA-Seq



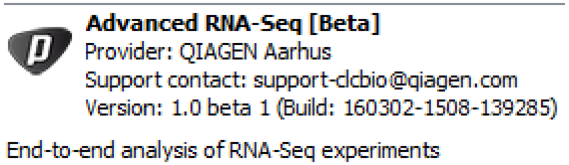








Working in BxWB to IPA



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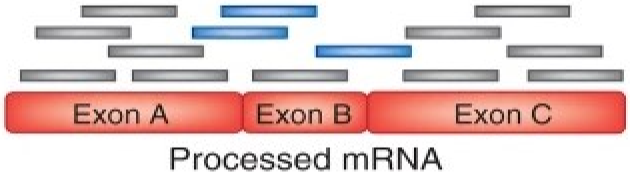
Support contact: support-clcbio@qiagen.com
Version: 1.0 beta 1 (Build: 160302-1508-139285)

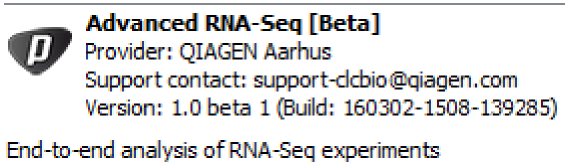
End-to-end analysis of RNA-Seq experiments

Map reads to reference



Working in BxWB to IPA

Map reads to reference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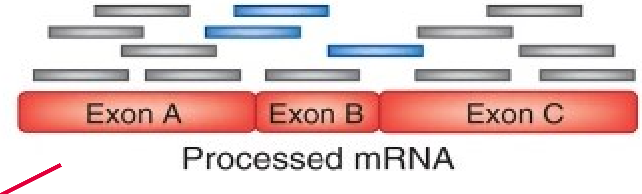
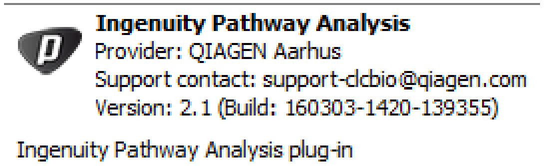
End-to-end analysis of RNA-Seq experiments



Processed mRNA


Ingenuity Pathway Analysis
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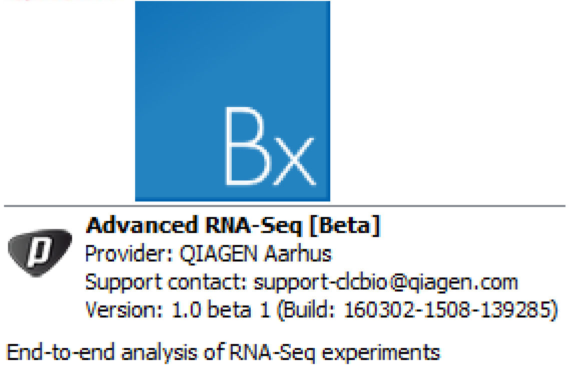
Support contact: support-clcbio@qiagen.com
Version: 2.1 (Build: 160303-1420-139355)

Ingenuity Pathway Analysis plug-in

Working in BxWB to IPA



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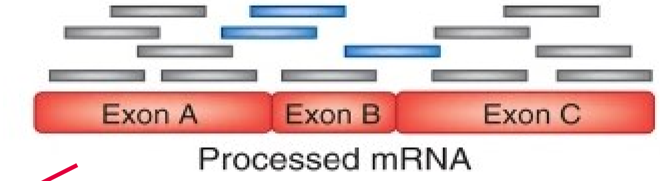

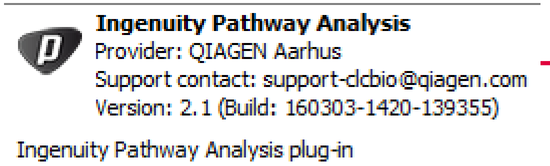

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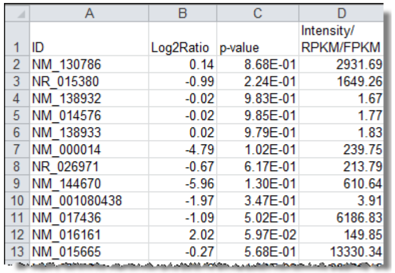

	A	B	C	D
1	ID	Log2Ratio	p-value	Intensity/ RPKM/FPKM
2	NM_130786	0.14	8.68E-01	2931.69
3	NR_015380	-0.99	2.24E-01	1649.26
4	NM_138932	-0.02	9.83E-01	1.67
5	NM_014576	-0.02	9.85E-01	1.77
6	NM_138933	0.02	9.79E-01	1.83
7	NM_000014	-4.79	1.02E-01	239.75
8	NR_026971	-0.67	6.17E-01	213.79
9	NM_144670	-5.96	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

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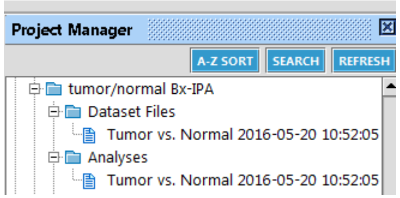
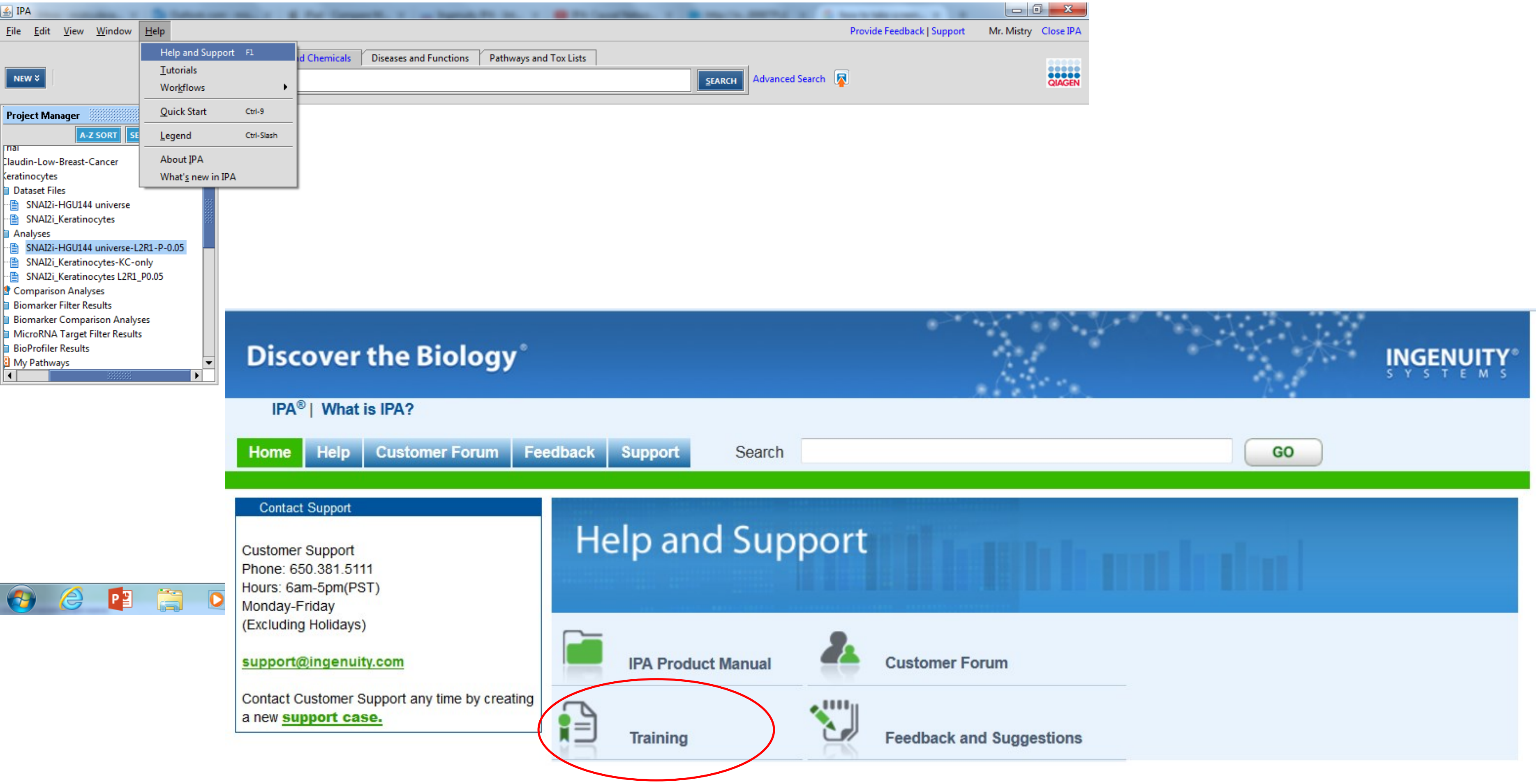


- tumor/normal Bx-IPA
  - Dataset Files
    - Tumor vs. Normal 2016-05-20 10:52:05
  - Analyses
    - Tumor vs. Normal 2016-05-20 10:52:05

Resources: Training Manuals and Videos



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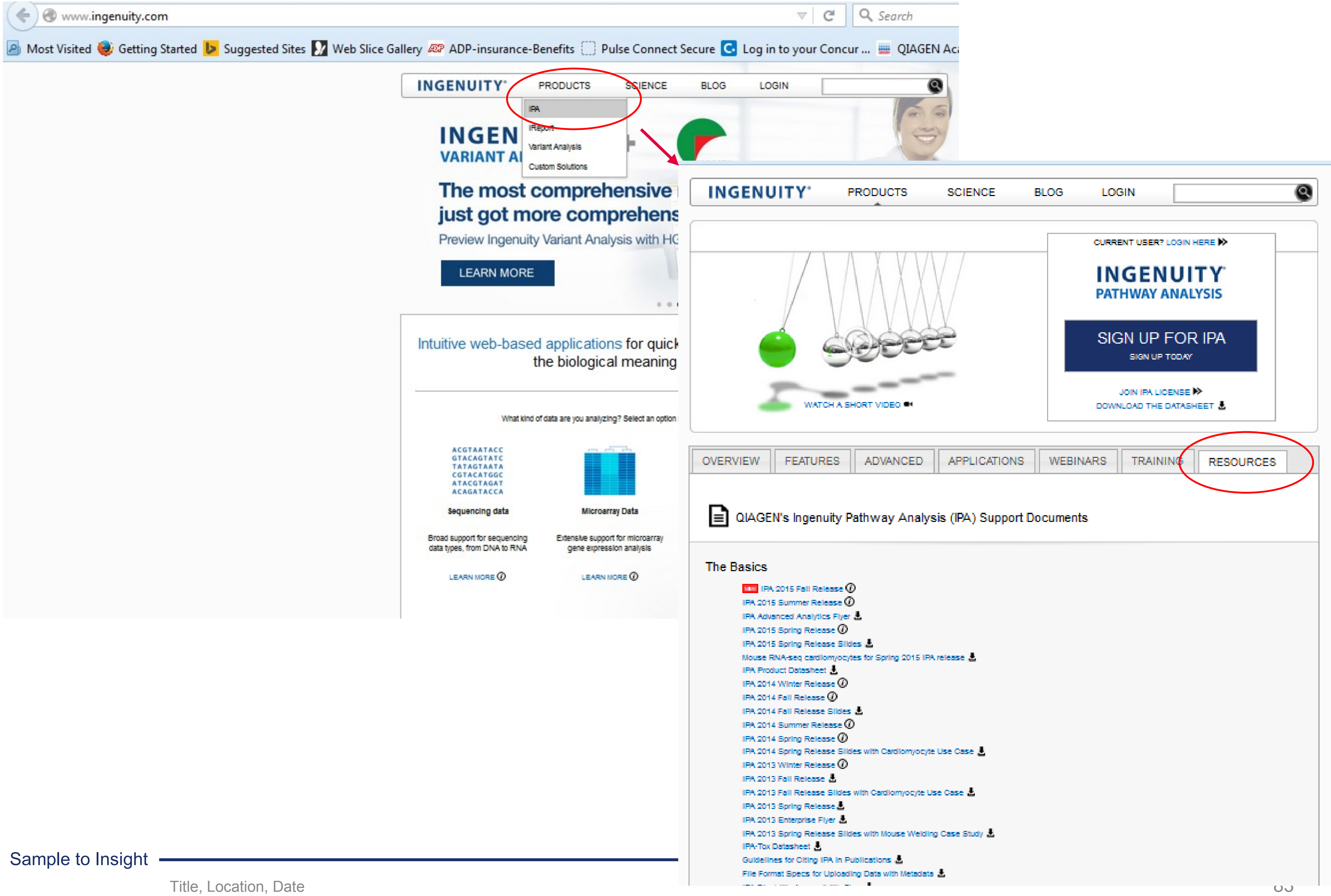
Resources: White Papers



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Intuitive web-based applications for quick
the biological meaning

What kind of data are you analyzing? Select an option

Sequencing data

Broad support for sequencing data types, from DNA to RNA

Microarray Data

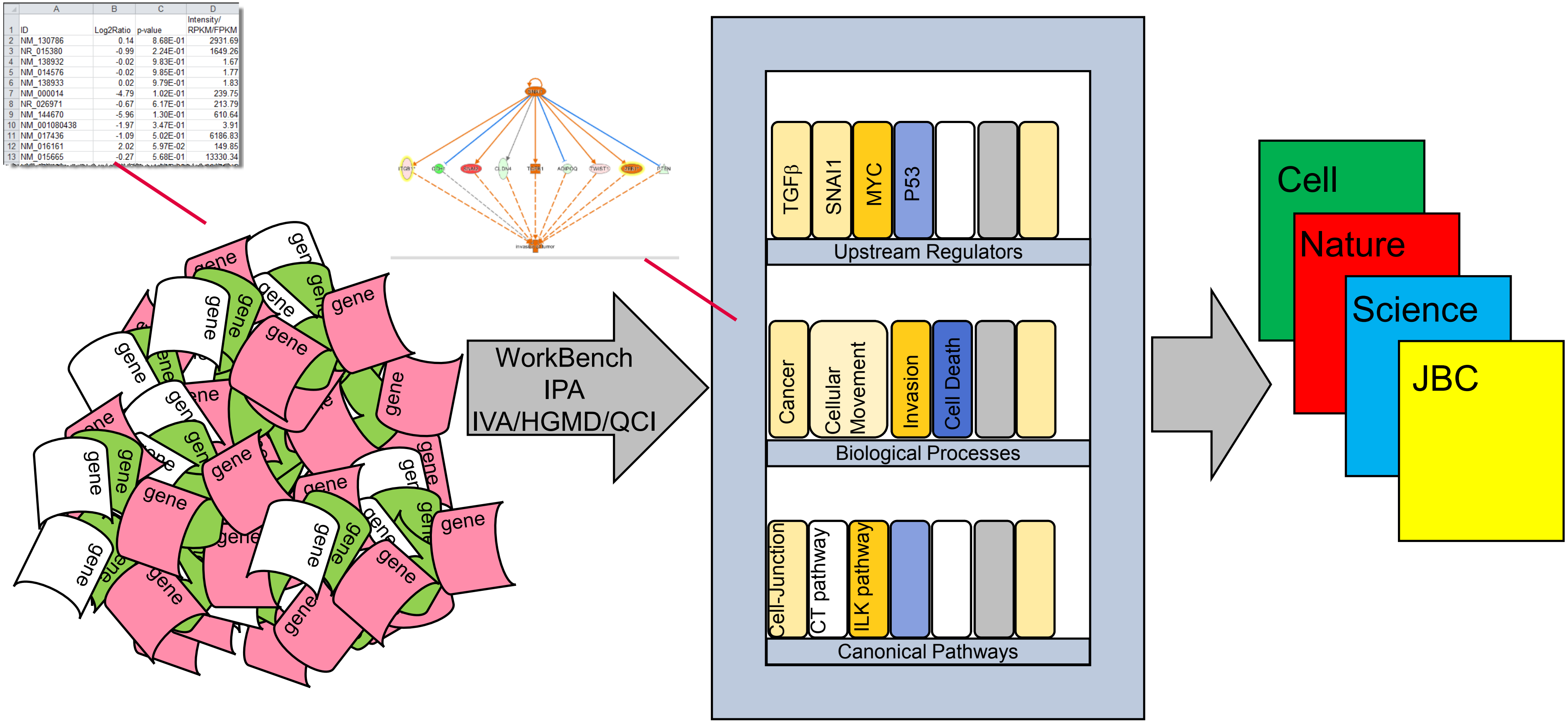
Extensive support for microarray gene expression analysis

QIAGEN's Ingenuity Pathway Analysis (IPA) Support Documents

The Basics

- NEW IPA 2015 Fall Release
- IPA 2015 Summer Release
- IPA Advanced Analytics Flyer
- IPA 2015 Spring Release
- IPA 2015 Spring Release Slides
- Mouse RNA-seq cardiomyocytes for Spring 2015 IPA release
- IPA Product Datasheet
- IPA 2014 Winter Release
- IPA 2014 Fall Release
- IPA 2014 Fall Release Slides
- IPA 2014 Summer Release
- IPA 2014 Spring Release
- IPA 2014 Spring Release Slides with Cardiomyocyte Use Case
- IPA 2013 Winter Release
- IPA 2013 Fall Release
- IPA 2013 Fall Release Slides with Cardiomyocyte Use Case
- IPA 2013 Spring Release
- IPA 2013 Enterprise Flyer
- IPA 2013 Spring Release Slides with Mouse Welding Case Study
- IPA-Tox Datasheet
- Guidelines for Citing IPA in Publications
- File Format Specs for Uploading Data with Metadata

What can Qiagen bioinformatics products do for you?



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WorkBench
IPA
IVA/HGMD/QCI

TGFβ
SNAI1
MYC
P53
Upstream Regulators

Cancer
Cellular Movement
Invasion
Cell Death
Biological Processes

Cell-Junction
CT pathway
ILK pathway
Canonical Pathways

Cell
Nature
Science
JBC

Questions?

Thank You!!

Jennifer Poitras, Ph.D.
Field Applications Scientist
Jennifer.Poitras@qiagen.com