

# QIAGEN<sup>®</sup> IPA<sup>®</sup>

For the analysis and interpretation of 'omics data

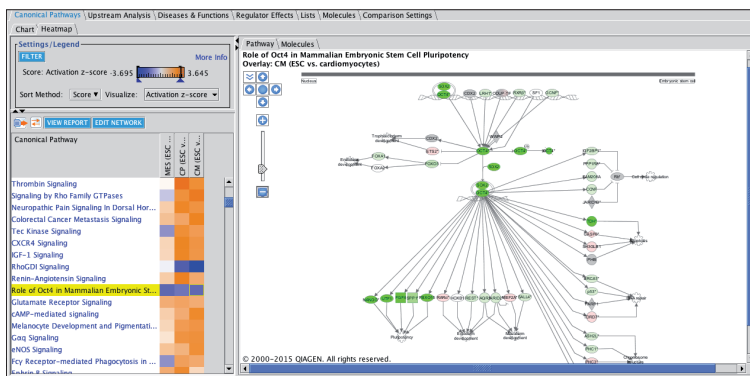
QIAGEN Ingenuity<sup>®</sup> Pathway Analysis (QIAGEN IPA) is a web-based software application for the analysis, integration and interpretation of data derived from 'omics experiments, such as RNA-seq, microarrays including miRNA and SNP, metabolomics, proteomics and small-scale experiments that generate gene and chemical lists. Powerful analysis and search tools uncover the significance of data and identify new targets or candidate biomarkers within the context of biological systems.

QIAGEN IPA goes beyond pathway analysis by:

- Identifying key regulators and activity to explain expression patterns
- Predicting downstream effects on biological and disease processes
- Providing targeted data on genes, proteins, chemicals and drugs
- Building interactive models of experimental systems

## Insightful data analysis and interpretation

Data analysis and interpretation with QIAGEN IPA builds on the comprehensive, manually curated content of the QIAGEN Knowledge Base. Powerful algorithms identify regulators, relationships, mechanisms, functions and pathways relevant to changes observed in an analyzed dataset. Analytics go beyond pathway analysis to help you understand experimental results within the context of biological systems (Tables 1 and 2) and interactive tools allow detailed exploration of results, including comparisons across multiple analyses (Figure 1), discovery of novel biological connections and generation of testable hypotheses.



**Figure 1. Interactive tools to explore and compare datasets.** Trends and similarities across analyses can be quickly compared using heatmaps and interactive pathway graphics within the context of canonical pathways, analysis of downstream effects and examination of potential upstream regulators.

**Table 1. Applications supported by IPA**

- Target identification and validation
- Biomarker discovery
- Drug mechanism of action
- Drug mechanism of toxicity
- Disease mechanisms

**Table 2. Experimental approaches supported by IPA**

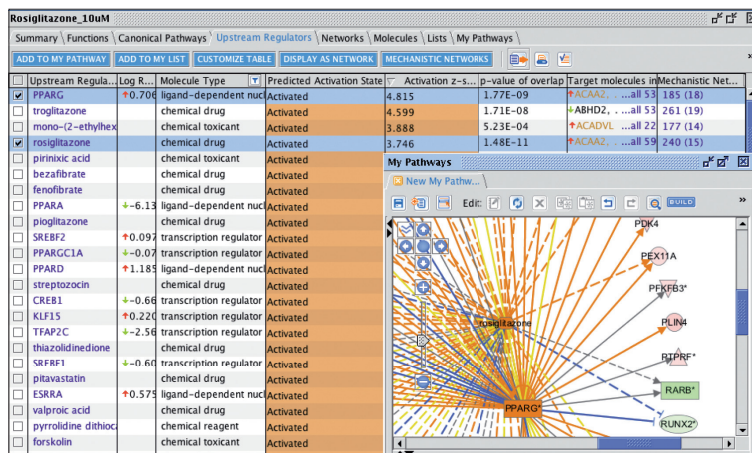
- RNA-seq
- Microarray
- miRNA
- mRNA
- qPCR
- Proteomics
- Metabolomics

## Unlock insights and develop novel hypotheses

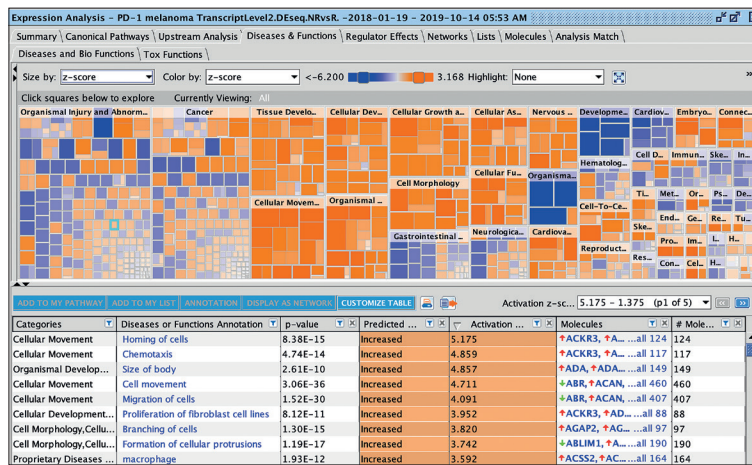
The Core Analysis in IPA quickly identifies relationships, mechanisms, functions and pathways relevant to a dataset. Upstream Regulator Analysis surfaces molecules, including miRNA and transcription factors, which may be causing observed gene expression changes (Figure 2) while Downstream Effects Analysis predicts downstream biological processes that are increased or decreased based on the analyzed data (Figure 3).

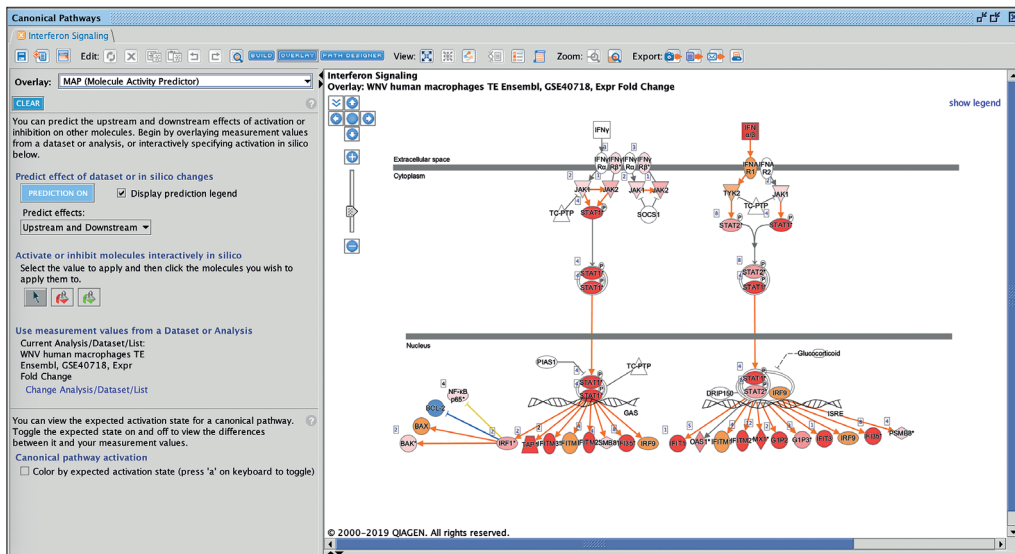
Integrating results about potential regulators and effects, the Regulator Effects tool highlights connections to create hypotheses about upstream triggers responsible for downstream phenotypic or functional outcomes. To further explore potential hypotheses, Molecule Activity Predictor (MAP) enables the user to interrogate subnetworks and canonical pathways by selecting a molecule of interest, indicating up- or downregulation, and simulating directional consequences on downstream molecules and the inferred activity upstream in the examined network or pathway (Figure 4).

**Figure 2. Interactive analysis of plausible upstream regulators and networks.** Insightful analyses predict upstream molecules, including miRNA and transcription factors, which may be causing observed gene expression changes.



**Figure 3. Detailed examination of downstream effects.** Detailed heatmaps highlight significant downstream biological processes that are increased or decreased based on gene expression results.





**Figure 4. Simulation of perturbations in subnetworks and canonical pathways.** Molecule Activity Predictor (MAP) interactively interrogates sub-networks and canonical pathways to simulate the downstream consequences of up- or downregulating a molecule and to predict the inferred activity upstream.

Advanced Analytics goes beyond immediate connections

Building on the Core Analysis, Causal Network Analysis, which is a component of QIAGEN IPA Advanced Analytics, uncovers multi-level causal relationships relevant to experimental data by expanding upstream analysis to include regulators that are not directly connected to targets in the analyzed dataset. Another Advanced Analytics component, BioProfiler, quickly surfaces molecules that are causally relevant to a disease or phenotype of interest, helping to identify potential therapeutic or toxicity targets, as well as associated known drugs and biomarkers.

Automatically compare your analysis with thousands of other analyses

QIAGEN IPA is also available with Analysis Match to automatically discover other QIAGEN IPA Core Analyses with similar (or opposite) biological results as com-

pared to yours. These matches can help confirm your interpretation of the results or provide unexpected insights into underlying shared biological mechanisms among supposedly unrelated datasets. It scans the analyses you have created in your Project Manager, as well as thousands of other human and mouse expression analyses curated from public sources, seeking shared patterns of Canonical Pathways, Upstream Regulators, Causal Networks and Diseases and Functions (Figure 5).

The analyses included in Analysis Match were created in QIAGEN IPA from more than 50,000 highly curated and quality-controlled human and mouse disease and oncology datasets re-processed from SRA, GEO, Array Express, LINCS and TCGA. These datasets were generated by QIAGEN OmicSoft and are the “comparisons” found in DiseaseLand and OncoLand representing various contrasts between disease versus normal, treatment versus non-treatment and much more (Figure 6).

Analysis Name	Project	case.disease...	cas...	co...	UR (...)	CN (...)	DE (...)	...
Estradiol(E2)treatedMCF7 12hr FC1.5 P<.05 GSE11352	Example Analyses				97.67		97.89	48.89
Estradiol (E2) treated MCF7 at 12, 24, 48 hrs (GSE11352)1, 48hr Fold Change	Example Analyses				69.06	46.06	64.55	44.92
test2- breast cancer [breast] estradiol	OncoGEO	breast cancer	breast	Treatme...	58.18	30.15	54.01	35.58
test1- breast cancer [breast] beta-estradiol (E2)	OncoGEO	breast cancer	breast	PreTreat...	49.61	37.61	54.01	35.31
test1- asthma [airway smooth muscle] FBS	HumanDisease	asthma	airway sm...	TreatTime...	42.97	42.64	50.00	33.90
test3- breast cancer [breast] estrogen	OncoGEO	breast cancer	breast	TreatTime...	65.63	28.43	40.82	33.72
Estradiol (E2) treated MCF7 at 12, 24, 48 hrs (GSE11352)1, 24hr Fold Change	Example Analyses				75.45	49.24		31.17
test2- breast cancer [breast] beta-estradiol (E2)	OncoGEO	breast cancer	breast	PreTreat...	42.97	20.10	61.24	31.08
test3- breast cancer [breast] estradiol	OncoGEO	breast cancer	breast	TreatTime...	54.07	22.47	45.64	30.55
test7- colorectal adenocarcinoma [colon] DMSO	OncoGEO	colorectal adenocar...	colon	TreatTime...	39.22	28.43	54.01	30.41
test3- NA [colon] surfactant coated silver nanoparticles	HumanDisease	NA	colon	Dosage =...	51.14	24.62	45.64	30.35
test1- breast adenocarcinoma [breast] liquiritigenin	OncoGEO	breast adenocarcinoma	breast	TreatTime...	35.08	24.62	54.01	28.43
test1- Simpson-Golabi-Behmel syndrome (SCBS) [adipose tissue] culture medium	HumanDisease	Simpson-Golabi-Beh...	adipose ti...	TreatTime...	46.41	24.62	40.82	27.96
test10- breast adenocarcinoma [breast] genistein	OncoGEO	breast adenocarcinoma	breast	TreatTime...	37.21	22.47	50.00	27.42
test2- breast cancer [breast] estrogen	OncoGEO	breast cancer	breast	TreatTime...	60.76	48.20		27.24
test1- breast adenocarcinoma [breast] beta-estradiol (E2)	OncoGEO	breast adenocarcinoma	breast	TreatTime...	41.14	20.10	45.64	26.72
test28- normal control [peripheral blood] anti-CD28 antibody;anti-CD3 antibody	HumanDisease	normal control	peripheral...	CellType:T...	37.21	20.10	40.82	24.53
test1- endometrial carcinoma (UCEC) [uterus] estradiol	OncoGEO	endometrial carcinom...	uterus	TreatTime...	55.47	42.64		24.53
test10- atherosclerosis;hyperlipidemia [liver] NA	MouseDisease	atherosclerosis;hyperl...	liver	AnimatStra...	27.74	20.10	50.00	24.46
test3- normal control [kidney] post-dialysis uremic patient plasma;probenecid	HumanDisease	normal control	kidney	Tissue:Sm...	24.81	30.15	40.82	23.95
test1- hepatoblastoma [liver] NA	OncoGEO	hepatoblastoma	liver	Classificati...	55.47	40.20		23.92
Mouse adipocyte Insulin stimulation, class I sites PMC3690479, 10min	Example Analyses				24.81	24.62	45.64	23.77
test1- ulcerative colitis (UC) [large intestine] NA	MouseDisease	ulcerative colitis (UC)	large intes...	Genotype...	42.97	24.62	27.39	23.74
test1- type 1 diabetes mellitus [terminal ileum] NA	MouseDisease	type 1 diabetes mellit...	terminal il...	SubjectTre...	55.47	38.92		23.60
test10- endometrial carcinoma (UCEC) [uterus] estradiol	OncoGEO	endometrial carcinom...	uterus	TreatTime...	52.62	41.44		23.52
test12- endometrial carcinoma (UCEC) [uterus] estradiol	OncoGEO	endometrial carcinom...	uterus	TreatTime...	52.62	41.44		23.52
test1- brain medulloblastoma [brain] Transfection,Alk shRNA	OncoGEO	brain medulloblastoma	brain	Transfecti...	27.74	20.10	45.64	23.37
test6- non-small cell lung carcinoma [lung] bexarotene (LGD1069);gemcitabine	OncoGEO	non-small cell lung ca...	lung	TreatTime...	31.38	20.10	40.82	23.08
test3- breast carcinoma [lymphoid tissue] MRK003	OncoGEO	breast carcinoma	lymphoid t...	TreatTime...	-49.92	-42.64		-23.14
test1- heart failure [ventricular myocardium] NA	HumanDisease	heart failure	ventricular...	DiseaseSta...	-32.82	-20.10	-40.82	-23.44
test6- normal control [tracheal epithelium] NA	HumanDisease	normal control	tracheal e...	Tissue:Sm...	-32.82	-20.10	-40.82	-23.44
test5- breast cancer [breast] hormone starvation	OncoGEO	breast cancer	breast	TreatTime...	-54.07	-40.20		-23.57
test9- lung adenocarcinoma (LUAD) [lung] erlotinib	OncoGEO	lung adenocarcinoma...	lung	TreatTime...	-27.74	-26.59	-40.82	-23.79

Figure 5. Analysis Match automatically displays analyses that are similar or dissimilar to your analysis. Analyses can be filtered by percentage similarity or by metadata keywords.

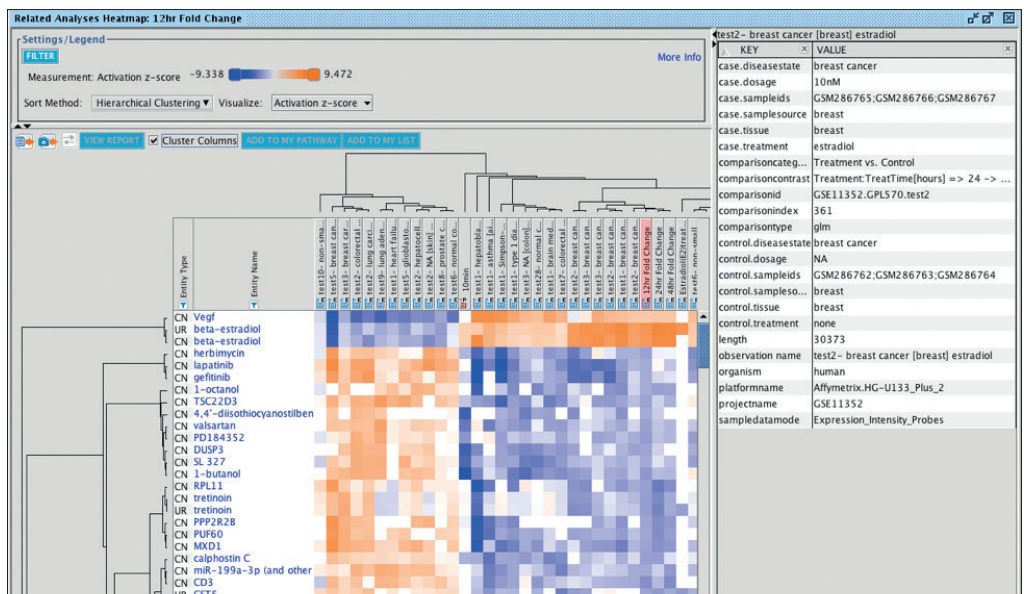


Figure 6. Selected analyses displayed as a heat map. This view enables you to drill down to explore the underlying drivers of the similarity or dissimilarity to your analysis.

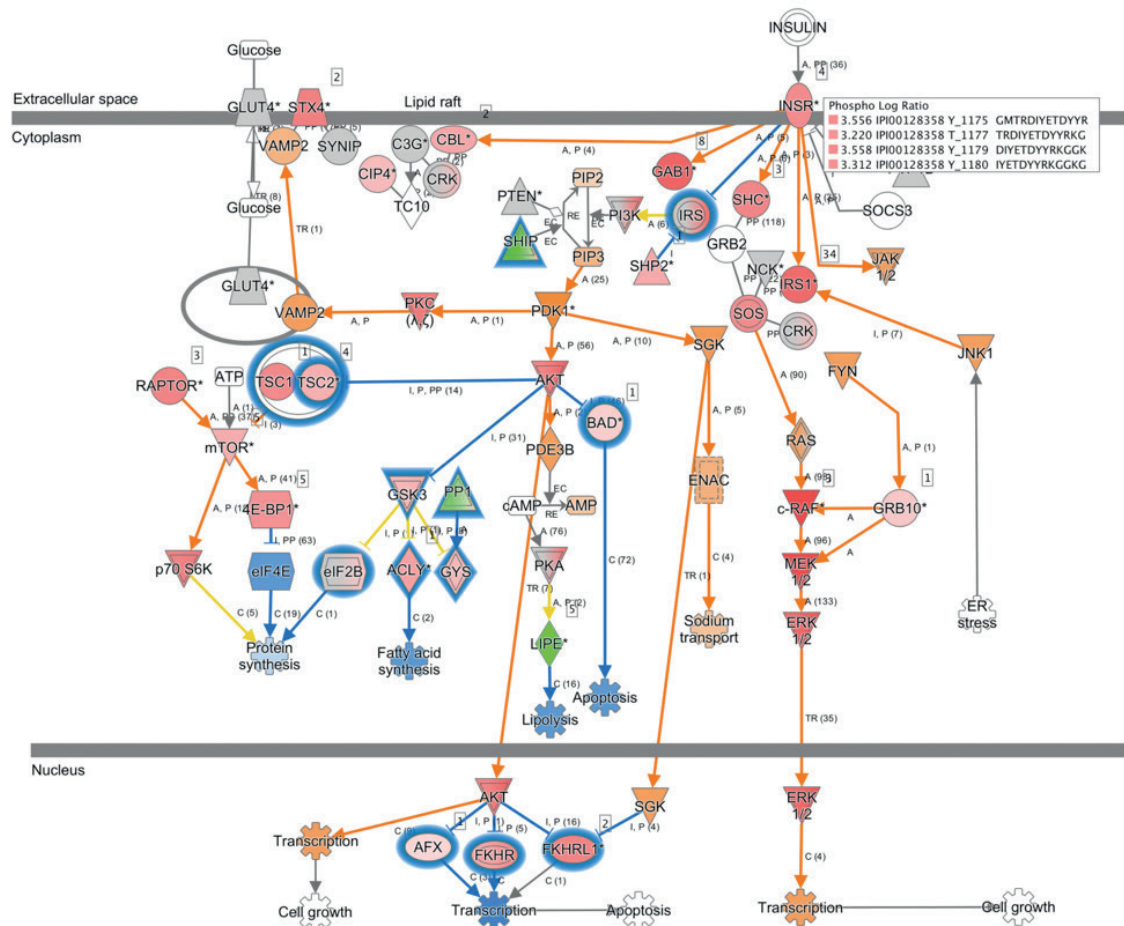


## Cause and effect for phosphoproteomics and metabolomics

QIAGEN IPA helps you understand the cause and effect of phosphorylation changes and endogenous metabolite concentration changes. You can predict which upstream regulators are responsible and whether those regulators are activated or inhibited, as well as visualize effects on downstream biological processes, diseases and established biological pathways (Figure 7).

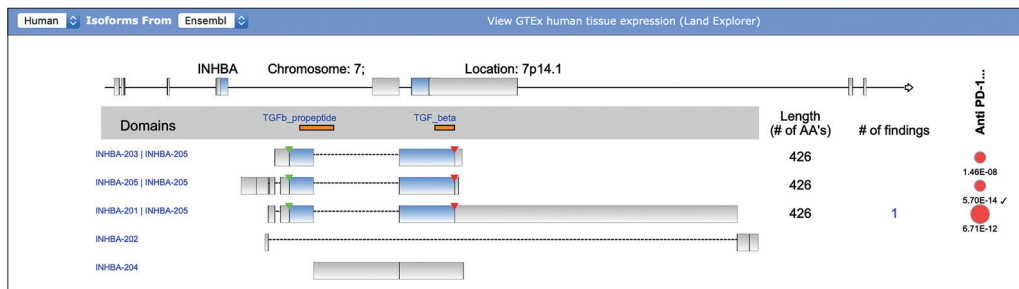
## Powerful tools for deep analysis of RNA-seq and miRNA data

Every feature of QIAGEN IPA is aimed at maximizing the impact of the information in an analysis so that the interpretation of a dataset is comprehensive. For example, the Isoform View displays expression data associated with each isoform from uploaded RNA-seq data in an intuitive graphical overview. Significantly regulated isoforms are listed in this view along with impacted functional protein domains and links to supporting publications (Figure 8).



**Figure 7. Canonical pathway with phosphorylation overlay.** QIAGEN IPA leverages the known effects of phosphorylation to predict activity of phosphorylated proteins in your phosphoprotein dataset. For example, proteins with blue halos are proteins with increased phosphorylation which inhibits their activity.

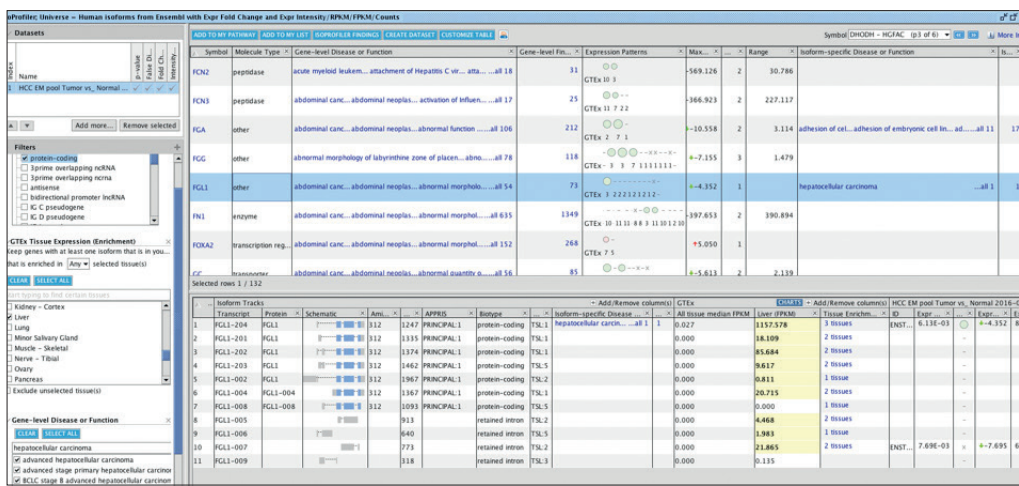
**Figure 8. Intuitive visualization of RNA-seq data.** Significantly regulated isoforms in experimental data are intuitively displayed to facilitate exploration of their impact on functional protein domains with links to isoform-specific publications.

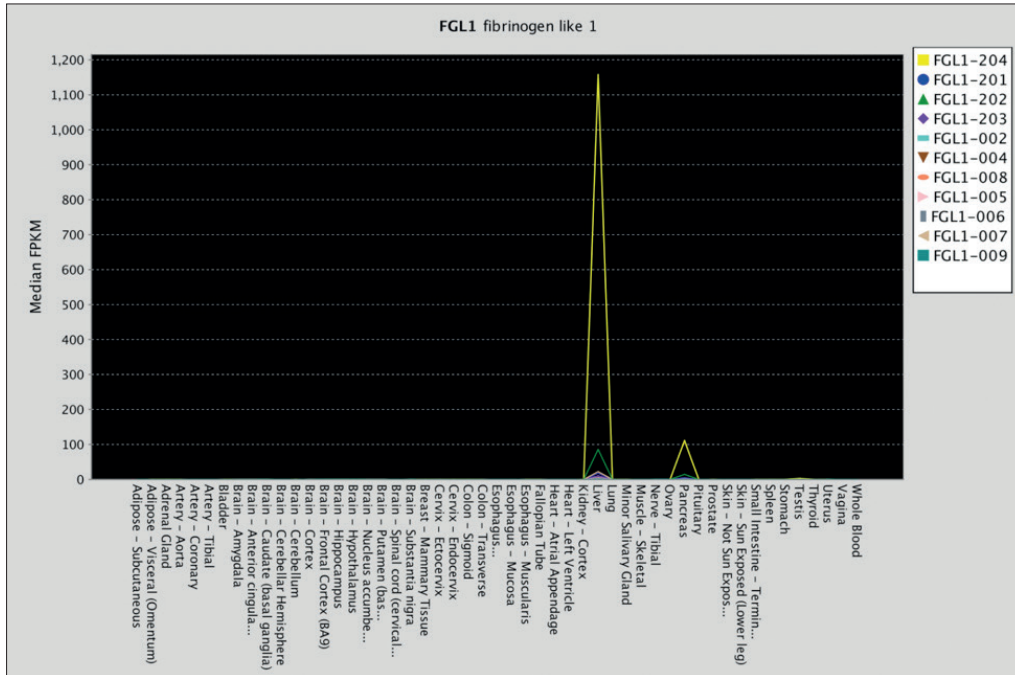


IsoProfilr helps you determine which isoforms from your RNA-seq datasets have interesting biological properties relevant to your research project. For example, use this function to identify genes where isoforms are both upregulated and down regulated in the same dataset, which may have important functional consequences. Find cases of isoform switching – when the most highly expressed (highest FPKM) isoform for a gene differs between the experiment and the control, or explore all the cases where a gene expresses multiple protein-coding isoforms or is known to impact a disease or function. Explore the tissue-specific expression patterns of the transcripts in your dataset with fully integrated GTEx data (Figures 9–10).

Another powerful capability, the MicroRNA Target Filter, combines interactive filtering and comprehensive content to identify and prioritize miRNA-mRNA target pairings and provide insight into the biological effects of miRNA (Figure 11). Additionally, Upstream Regulator Analysis predicts miRNAs that may be regulating genes in an experimental dataset.

**Figure 9. IsoProfilr window enabling filtering and prioritization of splice variants in your RNA-seq data.** IsoProfilr helps identify and prioritize isoforms having interesting biological properties relevant to your research. Find genes with RNA transcripts having unusual pattern(s) of expression such as isoform switching or with known disease associations or functions.





**Figure 10. Graph of tissue expression across human tissues for all known splice variants for a gene.** Use the fully reprocessed and integrated human GTEx expression data to identify transcripts with known tissue-specific expression.

**microRNA Target Filter**

50 microRNAs have targeting information available. Filtered to 50 microRNAs targeting 1339 mRNAs.

ADD/REPLACE MRNA DATASET   EXPRESSION PAIRING

Details | Summary

ADD TO MY PATHWAY   ADD TO MY LIST   CREATE DATASET

Rows: 1 - 1000 (p1 of 5)

Use [v] to filter a column. Add data or more columns using 'Add column(s)'. Add column(s)

microRNA dataset: MicroRNA...	Add column(s)	Relationship	Add column(s)	mRNA dataset: OVCa wil		
ID	Symbol	Fold Ch...	Source	Confidence	Expression Pairi...	Symbol
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TarBase, TargetScan Hu	Experimentally Observed	↕	HOXD10
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	IL6R
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	ITGA1
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	KCNQ3
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	KIAA0319
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	KIAA0753
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	KLF11
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	MAN1A2
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	MAP4K4
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	MDM2
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	NCOR2
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	Ingenuity Expert Findings	Experimentally Observed	↕	NF1
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	NFAT5
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	NFIX
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	Moderate (predicted)	↕	NFX1
<input type="checkbox"/> hsa-mir-10b	miR-10a-5p (and other	+2.940	TargetScan Human	High (predicted)	↕	NKTR

Selected/Total rows : 0/4511

VIEW FILTER SUMMARY   SAVE   CANCEL

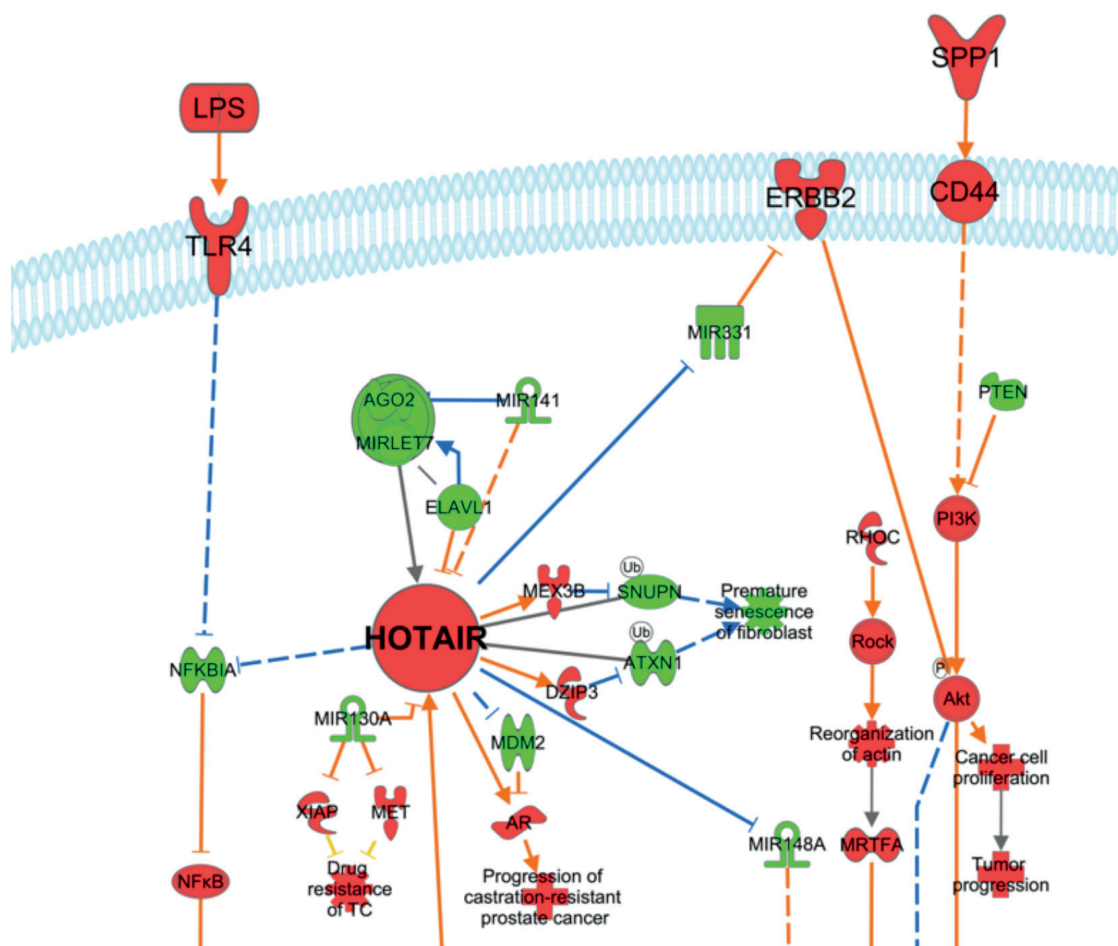
**Figure 11. Intuitive filtering tools to confidently identify mRNA targets.** The miRNA Target Filter in QIAGEN IPA provides insights into the biological effects of miRNA, based on experimentally validated interactions from TarBase and miRecords, predicted miRNA-mRNA interactions from TargetScan, and miRNA-related findings from peer-reviewed literature.

Build custom pathways and gene or chemical list libraries

Create custom pathways with My Pathways and gene or chemical list libraries from a range of input data: Gene lists from QIAGEN IPA search results, existing QIAGEN IPA networks or canonical pathways, uploaded lists of targets or biomarkers, or imported pathways using XGML, BioPax, SBML or GPML. Integrated tools guide the identification of upstream regulators or downstream targets of genes, enable layering of biological information or experimental data, and facilitate interrogation of hundreds of indexed ubnetworks and canonical pathways to simulate

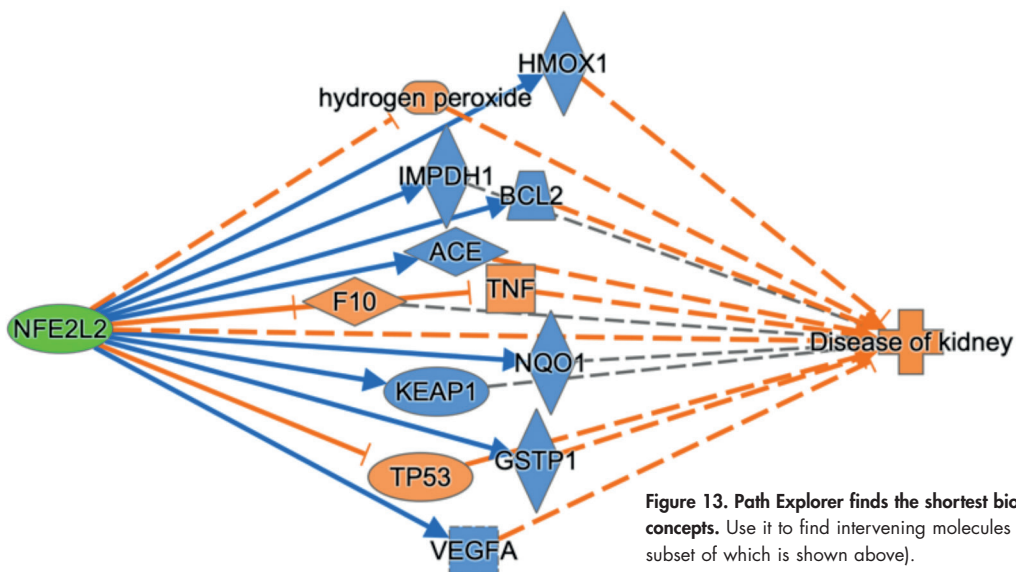
effects and mechanisms of altered activity of target molecules (Figure 12). Highly interactive, these features afford intuitive exploration of connections between targets in a dataset to generate testable hypotheses and construct event-specific pathways such as:

- miRNA-mRNA target networks
- Transcriptional networks
- Phosphorylation cascades
- Protein-protein or protein-promoter interaction networks
- Chemical/drug effects on proteins



**Figure 12. Powerful pathway editing tools to create interactive biological models.** Transform your networks and pathways in QIAGEN IPA into publication-quality pathway graphics rich with color, customized text and fonts, biological icons, organelles and custom backgrounds. Expand and explore pathways using the high-quality content stored in QIAGEN IPA.





**Figure 13. Path Explorer finds the shortest biological distance between two concepts.** Use it to find intervening molecules between two concepts (a subset of which is shown above).

### Explore pathways and interactions of interest

Path Explorer is an interactive tool that uncovers relevant relationships among genes of interest. By exploring these connections, the shortest paths between molecules associated with a disease or toxicity phenotype can be quickly identified, including access to supporting literature. Gene, Chemical & Pathway Search quickly generates and compares targeted lists of genes, druggable proteins, biomarkers and chemicals (Figure 13).

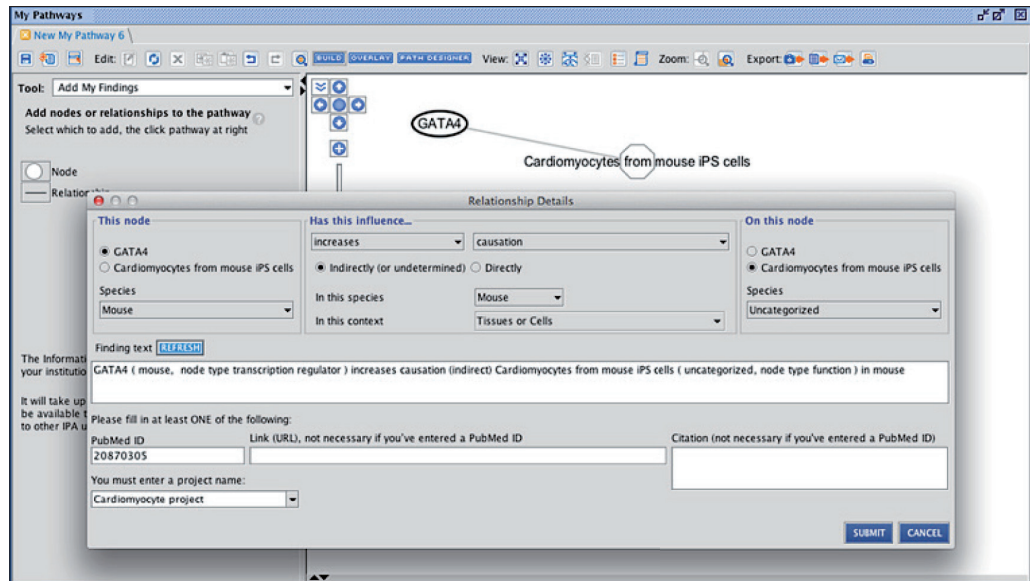
### Leverage internal knowledge for a better understanding

QIAGEN IPA can incorporate your own or your institution's internal data curation efforts for a disease or therapeutic area of interest. With the My Findings module, proprietary molecule-to-molecule relationships and molecule-to-disease or molecule-to-function relationships are uploaded to a secure, customer dedicated repository, making the content accessible throughout QIAGEN IPA. Any hypothetical or empirically demonstrated relationships can be imported or drawn and annotated on a new or existing pathway and then used in subsequent analyses to increase confidence in predicted upstream regulators, interaction or causal networks and downstream effects (Figure 14).

### Seamless sharing and communication of results

QIAGEN IPA functions as a central platform for the analysis of biological data, generation of testable hypotheses and construction and visualization of molecular models of experimental systems. The communication and collaboration tools of QIAGEN IPA enable collaborative work on models and creation of interactive reports to share with colleagues. Collaborators with a license for QIAGEN IPA can be invited to share datasets and analyses or a customized Collaboration Workspace can serve as a shared results repository within or across institutions or consortia. QIAGEN IPA creates detailed summaries of analysis results that highlight the broader biological and therapeutic relevance of a particular pathway, gene or molecule list (including uploaded proprietary lists). The detailed tabular data and dynamic features of these reports enable fast decision making and hypothesis generation. Finally, Path Designer transforms networks and pathways into publication-quality colored graphics with species-specific nomenclature, biological icons and organelles, and with customized text, fonts and backgrounds. Path Designer pathways are intuitive, fully interactive graphics supported by the comprehensive content of QIAGEN IPA.

**Figure 14. Quickly customize relationship content for analyses.** My Findings incorporates your own or your institution's internal knowledge for a disease or therapeutic area to strengthen analyses and insights most relevant to your research question.



## Understanding biological connections in a variety of applications

### Biomarker discovery

Prioritize molecular biomarker candidates based on key biological properties and elucidate mechanisms linking markers to a disease or phenotype of interest.

### NGS/RNA-seq data analysis

Streamline data analysis with rapid visualization and biological interpretation of expressed isoforms.

### Metabolomics

Leverage critical biological context in QIAGEN IPA to overcome the challenges of analyzing metabolomics data and infer impacted cell function from metabolite lists.

### Proteomics and phosphoproteomics

Uncover mechanistic links in complex proteomics data, identify potentially implicated regulators and predict impacted downstream processes or diseases.

### miRNA research

Predict miRNAs regulating gene expression patterns and find mRNA targets based on content from miRBase, TargetScan and the QIAGEN Knowledge Base.

### Toxicogenomics

Generate focused toxicity and safety assessments of candidate compounds and gain insight into pharmacological response and mechanism of action and toxicity.

## Powered by content of the QIAGEN Knowledge Base

The QIAGEN Knowledge Base is a data repository like no other. It organizes biological interactions and functional annotations created from millions of individually modeled relationships between proteins, genes, complexes, cells, tissues, drugs and diseases. These modeled relationships, or Findings, are manually reviewed for accuracy and include rich contextual details and links to

original publications (Figure 14). The QIAGEN Knowledge Base enables access to relevant and substantiated knowledge from primary literature, as well as public and third-party databases (Tables 3–4), for the comprehensive interpretation of experimental results within the context of larger biological systems.

**Table 3. Additional content sources in QIAGEN IPA**

Entrez Gene	GTEx Isoform-level Tissue Expression	Orphanet
RefSeq	HumanCyc metabolic pathway information	Hazardous Substance Database (HSDB)
Conserved Domain Database (CDD)	BIND, DIP, MIPS, BioGRID, IntAct, Cognition protein-protein interactions	TargetScan
OMIM	clinicaltrials.gov	miRBase
ClinVar	Drugs@FDA	miRecords
GWAS Database	European Medicines Agency	OncoTree
Gene Ontology (GO)	Pharmaceuticals and Medical Devices Agency	TarBase
Human Metabolome Database (HMDB)	Mosby's Drug Consult	COSMIC
Human Phenotype Ontology (HPO)	Goodman & Gilman's Pharmacological Basis of Therapeutics	Chemical Carcinogenesis Research Information System Database (CCRIS)
GNF Tissue Expression Body Atlas	DrugBank	The Mouse Genome Database (MGD) from The Jackson Laboratory (JAX)
NCI-60 Cell Line Expression Atlas	Obesity Gene Map Database	

**Table 4. Identifiers supported in QIAGEN IPA**

Affymetrix®	Gene Symbol – human (Hugo/HGNC)	PubChem CID
Affymetrix SNP ID	GenPept	Refseq
Agilent®	GI Number	UCSC (hg18)
CAS Registry Number	Human Metabolome Database (HMDB)	UCSC (hg19)
CodeLink	Illumina®	Unigene
dbSNP	Ingenuity	Uniprot/Swiss-Prot Accession
Ensembl	International Protein Index	<b>Species-specific Identifiers supported in IPA</b>
Entrez Gene	KEGG	Human
GenBank	Applied Biosystems®	Mouse
Gene Symbol – mouse (Entrez Gene)	miRBase (mature)	Rat
Gene Symbol – rat (Entrez Gene)	miRBase (stemloop)	Additional species via ortholog mapping

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