# Previous recordings, slides, etc.

# QIAGEN

### Popular recordings from recent trainings (2022)

RNA-seq analysis and interpretation: https://qiagen.showpad.com/share/FFpWhr5b1aVbQbcQD0bs5

miRNA analysis using Ingenuity Pathway Analysis: https://qiagen.showpad.com/share/aDzTaHl4GBasbDcuyJWQp

Single-cell RNA-seq analysis and interpretation: https://qiagen.showpad.com/share/u5Sv8rbZzED2aLe2bc4s4

Public single cell data analysis and interpretation: https://qiagen.showpad.com/share/wMpUrxwnrJk4Zc6iP9jzm

Single-cell ATAC-seq analysis: https://giagen.showpad.com/share/weaxKgKrGxtnW4Udz5Toa

SARS-CoV-2 detection in wastewater w/ CLC Genomics Workbench: https://qiagen.showpad.com/share/PZmv2KNXNT1yDTITUNmca

Long reads sequencing analysis: https://qiagen.showpad.com/share/EXDUJ3RKHsjXNxQpCiVnA

#### Software trials

CLC Genomics Workbench trial: https://qiagen.showpad.com/share/I6MqdhlybpllyvuYW3AKy

Human Somatic Mutation Database trial: https://qiagen.showpad.com/share/ELhnINyfamHr1Q1mmQnuv Ingenuity Pathway Analysis resources

IPA beginner training recording: https://qiagen.showpad.com/share/t2cGMdF3NjVucBA0b9tIT

IPA deeper dive training recording: https://qiagen.showpad.com/share/wCQrTeE2ftSQkgFTooHSu

IPA step-by-step guide w/ data format (slide 9) and upload (slide 28) instructions: https://qiagen.showpad.com/share/VjizSLJVBw1qfVnyoVJ8B

#### Resources in this presentation

Formatting transcriptomics data before<br/>uploading to IPAEnrichment in IPAData upload step by stepIPA Analysis TabsHow to access Land Explorer dataIPA HelpLand Explorer navigationColors and z-scores in IPA

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## **QIAGEN IPA Boston University Workshop**

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Technical Support: <u>ts-bioinformatics@qiagen.com</u>



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# Agenda

### **Topics for today's workshop**

### Interpreting your 'omics data using IPA Core Analysis

QIAGEN

- Setting up a core analysis
- Canonical pathways
- Comparison analysis

### **Creating networks from scratch**

- Connecting genes in a network
- In silico testing
- Overlay drugs and biomarkers

### Exploring publicly available data

- Activity plot, pattern search, Analysis Match
- Land Explorer

### Summary



# Interpret your data with causal analytics and find key pathways





# Compare multiple experiments and build hypotheses without data

Treated Untreated





### **Compare observations**





### **Build networks from scratch**

# IPA is powered by the QIAGEN Knowledge Base



Over 12.2 million research findings accessible by you in seconds Over two decades of expert literature curation Manuscripts, clinical trials, and 'omics data obtained from public and commercial sources (TCGA, GTEx, DrugBank, HumanCyc, OMIM, etc.) Weekly and quarterly updates with the latest The high-quality, manually curated data disease, genetics, cancer and drug findings allows for causality prediction

# IPA is used and cited widely across many scientific disciplines



=	Google Scholar	"Ingenuity pathway analysis"
•	Articles	About 51,900 results (0.09 sec)
	Any time Since 2023 Since 2022 Since 2019 Custom range	Causal analysis approaches in <b>ingenuity pathway analysis</b> <u>A Krämer</u> , J Green, J Pollard Jr, <u>S Tugendreich</u> - Bioinformatics, 2014 - academic.oup.com In this article, we describe causal analysis approaches that have been implemented in <b>Ingenuity Pathway Analysis</b> (IPA) with particular focus on the details of the underlying algorithms, ☆ Save 50 Cite Cited by 3740 Related articles All 8 versions
	Sort by relevance Sort by date	[нтмL] Biological pathway analysis by ArrayUnlock and <b>ingenuity pathway</b> analysis Á Jiménez-Marín, M Collado-Romero, M Ramirez-Boo, - BMC proceedings, 2009 - Springer
	Any type Review articles	Once a list of differentially expressed genes has been identified from a microarray experiment, a subsequent post-analysis task is required in order to find the main biological processes ☆ Save ワワ Cite Cited by 155 Related articles All 21 versions
	<ul> <li>include patents</li> <li>✓ include citations</li> </ul>	[нтмь] <b>Ingenuity pathway analysis</b> of gene expression profiles in distal nerve stump following nerve injury: insights into wallerian degeneration
	Create alert	J Yu, X Gu, S Yi - Frontiers in cellular neuroscience, 2016 - frontiersin.org Ingenuity pathway analysis indicated that inflammation and immune response, cytokine signaling, cellular growth and movement, as well as tissue development and function were ☆ Save ワワ Cite Cited by 72 Related articles All 9 versions ≫
		Potential predictive plasma biomarkers for cervical cancer by 2D-DIGE proteomics and <b>Ingenuity Pathway Analysis</b> X Guo, Y Hao, M Kamilijiang, A Hasimu, J Yuan, G Wu Tumor Biology, 2015 - Springer Bioinformatic analysis within the framework of <b>Ingenuity Pathway Analysis</b> (IPA @ ) showed that 10 plasma proteins as candidate biomarker were screened, mainly including lipid ☆ Save 奶 Cite Cited by 85 Related articles All 8 versions

## Find public data with similar or dissimilar activation patterns for key pathways, regulators, diseases and functions with Activity Plot

0(p



# Identify patterns seen in your data and match with public data sets





# Compare your entire core analysis with public data sets

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Expression Analysis - Disease vs Normal	xpression Analysis - Disease vs Normal — 🗇 🗙 🎽													
Summary Graphical Summary Pathways Up	mmary Graphical Summary Pathways Upstream Analysis Diseases & Functions Regulator Effects Networks Lists Analysis Match Molecules													
Evaluate Metadata View As Heatmap View	ew Comparison	Customize Table	<b>*</b>								z-sc 76.88	- 67.75 (1/514)	✓ ≪ » ()	4 ∗★
Analysis Name T	Project	τ × <sub>case.diseas</sub> τ × <sub>c</sub>	ase.target T × 🧃	ase tissue 🛛 🕇	× case.treatm T ×	compariso T ×	comparison T ×	compariso T × weblink	× CP (z-score)	T × UR (z-score) T	× CN (z-score) T	K DE (z-score) T	× <sub>\(\tag{z-score}\)</sub> × ×	
14- Alzheimer's disease (AD) [cerebral cortex] NA 972	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Genotype:Age[month	GSE104775.GPL21103 https://www.ncbi.	nlm 79.77	76.81	59.16	91.77	76.88	5
3- Alzheimer's disease (AD) [cerebral cortex] NA 989	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Disease vs. Normal	Age[months]:Disease	GSE104775.GPL21103 https://www.ncbi.	nlm 79.77	77.46	64.81	82.72	76.19	*
16- Alzheimer's disease (AD) [cerebral cortex] NA 974	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Genotype:Age[month	GSE104775.GPL21103 https://www.ncbi.	nlm 85.28	77.46	57.45	83.77	75.99	
22- Alzheimer's disease (AD) [cerebral cortex] NA 981	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Genotype:Age[month	GSE104775.GPL21103 https://www.ncbi.	nlm 85.28	74.83	54.77	87.86	75.69	6
39- Alzheimer's disease (AD) [cerebral cortex] NA 999	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Age[months]:Genoty	GSE104775.GPL21103 https://www.ncbi.	nlm 85.28	77.46	59.16	80.57	75.62	° •
12- Alzheimer's disease (AD) [cerebral cortex] NA 970	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Age[months]:Genoty	GSE104775.GPL21103 https://www.ncbi.	nlm 85.28	75.50	54.77	86.86	75.60	
1- amyotrophic lateral sclerosis (ALS) [spinal cord] NA 672 N	MouseDisease	amyotrophic lateral	5	pinal cord	NA	Disease vs. Normal	DiseaseState:Age[w	GSE43879.GPL11002.I http://www.ncbi.n	m 79.77	74.16	60.00	87.86	75.45	
8- Alzheimer's disease (AD) [cerebral cortex] NA 5229	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Other Comparisons	Tissue:SubjectTreat	GSE134151.GPL21103 https://www.ncbi.	nlm 79.77	77.46	58.31	85.84	75.35	7
2- normal control [spinal cord] NA 1225	MouseDisease	normal control	5	pinal cord	NA	Other Comparisons	AgeCategory => old	GSE106803.GPL13112 https://www.ncbi.	nlm 79.77	76.16	55.68	88.85	75.12	*
3- Alzheimer's disease (AD) [cerebral cortex] NA 12038	MouseDisease	Alzheimer's disease	c	erebral cortex	NA	Disease vs. Normal	DiseaseState:Age[m	E-MTAB-2121.A-GEOE http://www.ebi.ac	uk/ 79.77	76.81	58.31	84.81	74.93	
3- Alzheimer's disease (AD) [hippocampus] NA 9063	MouseDisease	Alzheimer's disease	h	ippocampus	NA	Disease vs. Normal	DiseaseState:Subjec	GSE93678.GPL17021.I https://www.ncbi.	nlm 79.77	72.11	61.64	85.84	74.84	
3- amyotrophic lateral sclerosis (ALS) [spinal cord] NA 184 M	MouseDisease	amyotrophic lateral	s	pinal cord	NA	Other Comparisons	AgeCategory:Genoty	GSE113924.GPL19057 https://www.ncbi.	nlm 73.85	80.62	60.00	84.81	74.82	× ×
6- Alzheimer's disease (AD) [hippocampus] NA 952	MouseDisease	Alzheimer's disease	h	ippocampus	NA	Disease vs. Normal	SubjectTreatment:Di	GSE104424.GPL13112 https://www.ncbi.	nlm 79.77	72.80	60.00	85.84	74.60	*

#### Expression Analysis - Disease vs Normal

												- H
Summary Graphical Summary Pathways	Upstream Analysis	Diseases & Functions Regulator Effects	Networks Lists	Analysis Match	Molecules							
Evaluate Metadata View As Heatmap	View Comparison	Customize Table							z-sc74.4	862.45 (1/514)	- « » <b>)</b> 0	4 *
Analysis Name	Project	T × case.diseas T × case.target T >	< case.tissue T	< case.treatm T	× compariso T × comparison 1	× compariso • × weblink • ×	CP (z-score) T ×	UR (z-score) 🔻 🗙	CN (z-score) T ×	DE (z-score) T ×	∠ z-score ▼ ×	
9- neurodegenerative disease [spinal cord] NA 5116	MouseDisease	neurodegenerative d	spinal cord	NA	Other Comparisons Gender:Genotype	= GSE133047.GPL21103 https://www.ncbi.nlm	-79.77	73.48	-53.85	-90.81	-74.48	
1- Alzheimer's disease (AD) [cerebral cortex] NA 5207	MouseDisease	Alzheimer's disease	cerebral cortex	NA	Treatment vs. Control Tissue:Genotype:	ub GSE134151.GPL21103 https://www.ncbi.nlm	-73.85	-72.11	-65.57	-83.77	-73.83	5
5- NA [liver] NA 22191	MouseDisease	NA	liver	NA	Treatment vs. Control Age[months]:Subj	ect GSE84495.GPL11533.t https://www.ncbi.nlm	-85.28	-76.16	-53.85	-77.23	-73.13	*
8- neurodegenerative disease [spinal cord] NA 5115	MouseDisease	neurodegenerative d	spinal cord	NA	Other Comparisons Gender:Genotype	= GSE133047.GPL21103 https://www.ncbi.nlm	-79.77	-75.50	-50.00	-84.81	-72.52	
1- colorectal cancer [colonrectum] NA 5714	OncoHuman	colorectal cancer	colonrectum	NA	Disease1 vs. Diseas DiseaseSubtype :	> GSE116305.GPL4133.t https://www.ncbi.nlm	-85.28	-70.00	-55.68	-73.75	-71.18	
1- glioma [brain] NA 8144	OncoHuman	glioma	brain	NA	Other Comparisons GeneticSubtype =	> GSE24072.GPL96.test https://www.ncbi.nlm	-79.77	72.11	-50.99	-81.65	-71.13	6
4- normal control [liver] NA 19591	MouseDisease	normal control	liver	NA	Treatment1 vs. Treat SubjectTreatmen	= GSE51432.GPL1261.te https://www.ncbi.nlm	-85.28	76.16	-52.92	-70.09	-71.11	*
2- adrenoleukodystrophy (ALD) [skin] culture medium 84	40 HumanDisease	adrenoleukodystroph	skin	culture medium	CellType1 vs. CellTy ExperimentGroup	= GSE85839.GPL16791.I https://www.ncbi.nlm	-85.28	72.11	-48.99	-77.23	-70.90	
54- glioblastoma (GBM) [brain] NA 2634	TCGA	glioblastoma (GBM)	brain	NA	Other Comparisons DNASeq_Somatio	M TCGA_GBM.GPLna.DE	-90.45	-72.80	-50.00	-70.09	-70.84	
2- skin melanoma (SKCM) [skin] NA 7	OncoMouse	skin melanoma (SKC	skin	NA	Other Comparisons CellType:Genotyp	e[h GSE101690.GPL17021 https://www.ncbi.nlm	-79.77	71.41	-52.92	-78.36	-70.62	-
24- normal control [liver] NA 12486	MouseDisease	normal control	liver	NA	Treatment vs. Control SubjectTreatmen	:G GSE10493.GPL1261.te http://www.ncbi.nlm	-79.77	-70.00	-56.57	-76.09	-70.61	
1227- normal control [skeletal muscle] NA 14810	MouseDisease	normal control	skeletal muscle	NA	Treatment1 vs. Treat Tissue:Experimen	Gr GSE120661.GPL10787 https://www.ncbi.nlm	-79.77	72.80	-46.90	-82.72	-70.55	*
5- Alzheimer's disease (AD) [thalamus] NA 5226	MouseDisease	Alzheimer's disease	thalamus	NA	Treatment vs. Control Tissue:Genotype:	ub GSE134151.GPL21103 https://www.ncbi.nlm	-67.42	-70.00	-62.45	-81.65	-70.38	
73- crohn's disease (CD) (peripheral blood) NA 9776	HumanDisease	crohn's disease (CD)	peripheral blood	NA	Disease1 vs. Diseas CellType:Disease	.oc., E-MTAB-331.A-AFFY-1 http://www.ebi.ac.uk/.	-79.77	72,80	-50.00	-78.36	-70.23	

# Explore genes in Land Explorer





# Explore public data comparisons in Land Explorer and IPA





# Agenda

### **Topics for today's workshop**

### Interpreting your 'omics data using IPA Core Analysis

QIAGEN

- Setting up a core analysis
- Canonical pathways
- Comparison analysis

### **Creating networks from scratch**

- Connecting genes in a network
- In silico testing
- Overlay drugs and biomarkers

### Exploring publicly available data

- Activity plot, pattern search, Analysis Match
- Land Explorer

### Summary



# Previous recordings, slides, etc.

# QIAGEN

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Public single cell data analysis and interpretation: https://qiagen.showpad.com/share/wMpUrxwnrJk4Zc6iP9jzm

Single-cell ATAC-seq analysis: https://qiagen.showpad.com/share/weaxKgKrGxtnW4Udz5Toa

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#### Software trials

CLC Genomics Workbench trial: https://qiagen.showpad.com/share/I6MqdhlybpllyvuYW3AKy

Human Somatic Mutation Database trial: https://qiagen.showpad.com/share/ELhnINyfamHr1Q1mmQnuv Ingenuity Pathway Analysis resources

IPA beginner training recording: https://qiagen.showpad.com/share/t2cGMdF3NjVucBA0b9tIT

IPA deeper dive training recording: https://qiagen.showpad.com/share/wCQrTeE2ftSQkgFTooHSu

IPA step-by-step guide w/ data format (slide 9) and upload (slide 28) instructions: <u>https://qiagen.showpad.com/share/VjizSLJVBw1qfVnyoVJ8B</u>

Resources in this presentation

Formatting transcriptomics data before uploading to IPA

Data upload step by step

How to access Land Explorer data

Land Explorer navigation

Colors and z-scores in IPA

# IPA investigates biology of anti-PD1 immunotherapy resistance in Metastatic Melanoma



#### Volume 26, Issue 14

15 July 2020





### Tumor CD155 Expression Is Associated with Resistance to Anti-PD1 Immunotherapy in Metastatic Melanoma



Ailin Lepletier<sup>1</sup>, Jason Madore<sup>1</sup>, Jake S. O'Donnell<sup>1,2,3</sup>, Rebecca L. Johnston<sup>4</sup>, Xian-Yang Li<sup>1</sup>, Elizabeth McDonald<sup>2</sup>, Elizabeth Ahern<sup>1,3,5</sup>, Anna Kuchel<sup>1,3,5</sup>, Melissa Eastgate<sup>3,5</sup>, Sally-Ann Pearson<sup>1</sup>, Domenico Mallardo<sup>6</sup>, Paolo A. Ascierto<sup>6</sup>, Daniela Massi<sup>7</sup>, Barbara Merelli<sup>8</sup>, Mario Mandala<sup>8</sup>, James S. Wilmott<sup>9</sup>, Alexander M. Menzies<sup>9</sup>, Charles Leduc<sup>10</sup>, John Stagg<sup>1,11</sup>, Bertrand Routy<sup>11</sup>, Georgina V. Long<sup>9</sup>, Richard A. Scolyer<sup>9,12,13</sup>, Tobias Bald<sup>14</sup>, Nicola Waddell<sup>4</sup>, William C. Dougall<sup>1</sup>, Michele W.L. Teng<sup>2,3</sup>, and Mark J. Smyth<sup>1,3</sup>

- Metastatic melanoma treated with immune checkpoint blockade therapy (n=155) and BRAF/MEK-directed targeted therapy (n=50)
- RNA sequencing performed on samples and clinical RECIST response and progression-free survival data were collected
- High pretreatment levels of CD155 correlated with high parenchymal PD1+CD8+/CD8+ T-cell ratios and poor response to anti-PD1 therapy

## Formatting 'omics data before uploading to IPA

Observation 1



		Observatio		Observation 2				
	А	В	С	D	E			
1	geneid	UCvsNormal.Log2FoldChange	UCvsNormal.pval	52wksVedolizumabvsBaseline.Log2FoldChange	52wksVedolizumabvsBaseline.pval			
2	DDX11L1	 -0.1067	0.2878	0.1183	0.1624			
3	WASH7P	-0.1883	0.0097	0.3063	0.0006			
4	FAM138F	-0.0761	0.4699	0.2466	0.0191			
5	OR4F5	0.1474	0.5311	0.1713	0.2913			
6	LOC729737	0.4789	0.0017	0.029	0.8331			
7	LOC100133331	0.4789	0.0017	0.029	0.8331			
8	LOC100132062	 0.4789	0.0017	0.029	0.8331			
9	OR4F29	 0.2495	0.2389	0.2181	0.1887			
10	JA429831	 0.1215	0.3338	0.2556	0.0004			

# Analyte identifier REQUIRED to explore enrichment

### RNA examples: Gene symbols, array identifiers from Affymetrix, Ensembl, etc.

Protein examples: UniProt, GenPept, Gene symbols, Ensembl. etc.

Metabolite examples: KEGG, CAS registry number, etc. \*add multiple columns of ids to ensure best mapping

### Change values needed to calculate activity predictions

Change value examples: fold changes, ratios, etc.

Significance values: P-values \*optional but recommended to enable filtering for significance

#### Accepted file formats:

- ✓ .txt (tab-delimited text files)
- ✓ .xls, .xlsx, .csv (Excel tables
- ✓ .diff (Cuffdiff output

Multiple comparisons or observations may be uploaded in one file

## **IPA** important colors





### **Activity Prediction**



## Z-scores predict activity



# Compares the EXPECTED gene expression pattern for activation in the knowledgebase to the ACTUAL gene expression measurements

#### Z-scores are used throughout IPA to predict activation or inhibition of:

- Canonical pathways
- Upstream regulators

- Master regulators
- Diseases and functions



#### Simplified z-score formula:

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$$

### **Z-score calculation summary**



#### Compares the EXPECTED gene expression pattern for activation in the knowledgebase to the ACTUAL data set gene expression measurements



# NO! a NEGATIVE Z-score is calculated indicating 4 predicted inhibition

#### Z-score= -2.236

Symbol	Measurement	+ Expected	Matching?
	/ Expr Log Ratio	×	
CREB3L3	+-1.536	🕈 Up	×
IHH	+-1.173	🕈 Up	×
PBX1	+-1.037	🕈 Up	×
РКМ	<b>1.082</b>	🕈 Up	$\checkmark$
CREB5	<b>†</b> 1.148	🕈 Up	<b>√</b> .
CREB3L2	<b>†</b> 1.179	🕈 Up	$\checkmark$
CCN4	<b>†</b> 1.204	🕈 Up	✓
IL10RA	<b>†</b> 1.246	🕈 Up	✓
FCGR2C	<b>†</b> 1.256	🕈 Up	$\checkmark$
INPP5D	<b>†</b> 1,297	🕈 Up	$\checkmark$
CD86	<b>†</b> 1.016	+ Down	×
IL1RAP	<b>†</b> 1.046	+ Down	×
HLA-DMB	<b>†</b> 1.106	🕂 Down	×
IL18RAP	<b>†</b> 1.124	+ Down	×
TLR4	<b>†</b> 1.213	+ Down	×
HLA-DQA2	<b>†</b> 1.225	+ Down	×
HLA-DQA1	<b>†</b> 1.225	+ Down	×

YES! a POSITIVE Z-score is calculated indicating predicted activation

#### Z-score= 2.427

Symbol	Measurement	+ 🛆 Expected	Matching?
	Expr Log Ratio	×	4.
NOS2	<b>†</b> 3,434	+ Down	X
IL1A	<b>†</b> 2,425	+ Down	×
HLA-DQA2	<b>†</b> 1,225	🕇 Up	<b>v</b>
TGFB1	<b>†</b> 1.095	🕈 Up	× /
HLA-DQA1	<b>†</b> 1.225	🕈 Up	*
JAK3	<b>†</b> 1.383	🕈 Up	×
FCER1G	<b>†</b> 1.313	🕈 Up	<b>√</b>
FPR1	<b>†</b> 2.117	🕈 Up	v te
PPARG	+-1.376	🕈 Up	×
IL10RA	<b>†</b> 1.246	🕈 Up	× /
HLA-DQB2	<b>†</b> 1.674	🕈 Up	¥ 5
ACADM	+-1.378	↑ Up	×
TLR4	<b>†</b> 1.213	🕈 Up	× /
IGH*	<b>†</b> 1.334	🕈 Up	v 14
PPARGC1A	+-2.495	🕈 Up	×
IL1RN	<b>†</b> 3.188	↑ Up	×,
HLA-DRB1	<b>†</b> 1.728	↑ Up	✓

## Pathway enrichment: P-value of overlap



- Reference set is typically all genes or proteins that can be reliably detected by the 'omics technology used
- Most perturbed is a subset of measured genes/proteins that were significantly different between experimental groups
  - Fold change cut offs and p-value cut offs used
- IPA calls these "Analysis-ready molecules"
- Molecules associated with a biological category are a set of genes/proteins that the IPA knowledgebase has annotated to be important for a biological pathway, function, or disease.

## Testing for Enrichment using the Fisher's Exact Test





## P-Value from the Right-tailed Fisher's Exact Test



# p-value: the probability of observing a result as extreme or more extreme, if the null hypothesis is true



H<sub>o</sub> = Overlap of molecules for a particular biological category is due to chance

 $\alpha$  (significance level) = 0.05



 $p-value = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}$ 

**P-value = 2.08 x 10<sup>-12</sup>** 

We can reject the null hypothesis

# Agenda

**Refresher on QIAGEN Ingenuity Pathway Analysis** 

### Interpreting your 'omics data using IPA

- Setting up a comparison analysis
- Upstream regulators
- Causal networks

### Exploring publicly available data

- Land Explorer
- Activity plot, pattern search, Analysis Match

Summary



# Activity Plot, Pattern Search, and Analysis Match





#### Activity Plot: compare single entities

- Discover analyses with similar downstream activities of a particular node or entity
- Input: one gene, canonical pathway, or other "molecule" within IPA
- How: uses all downstream targets of the entity as a signature for comparison



#### Pattern Search: compare networks

- Determine if your pattern of activity is seen among a massive collection of expression data
- Input: any network or pathway with multiple genes and a pattern
- How: uses the signature you provide to query our OmicSoft database

Nrf2 in Kidney							
> Dataset Files							
🗸 🗁 Analyses							
VIE Nrf2 Transcriptomics	r	× comp., T × weblink T ×	OP (2 * ×	UR (z Y ×	CN (z * ×	DE (2 * ×	💎 🖬 🗖 🗙
		GSE5364 GPL96 https://www.nc	73.85	38.73	22.36	66.14	50.27
Drug vs DMSO [RN/	A]	GSE119933/GPL https://www.nc	67.42	46.90	33,17	50.00	49.37
KO ve WT (RNA)		GSE118339/GPL https://www.nc	73405	37.71	26.45	\$5,90	48.43
			60.30	36.06	38,73	50.00	46.27
4- kidney disease (kidney glomeruli) NA 12973	MouseDisease	GSE117987.GPL https://www.nc	60.30	44.72	24.49	50.00	44.83
93- nonalcoholic fatty liver disease (NAFLD) [liver] N	MouseDisease	GSE115766/GPL https://www.nc	67.42	26,40	26,45	55.90	44.55
NRI2-Proteome-modified 2 - 2022-04-18 02:34 PM, I	Example Analyses		60.30	34.64	33.17	50,00	44.53
161- normal control Jung NA 16452	MouseDisease	GSE132040/GPL https://www.nc	67.A2	28.87	20.00	61.24	44.38
1- normal control [liver] NA 20804	MouseDisease	GSE83940.GPL1 https://www.nc	60.30	41.45	24,49	50.00	44.05
163- normal control Jung NA 16434	MouseDisease	GSE132040/GFL https://www.nc	60.30	30.51	16.90	6124	42.74
165- normal control (itxer) gettinib 2295	RefDisease	GSESTEDS.GPUT https://www.nc	60.50	40.00	20.00	50.00	42.53
9- diet induced obesity (cluodenum) NA 7407	MouseDisease	GSE69305.GPL1 Http://www.mcb	79.77	53.85	33.17		41.70
7- normal control (liver) NA 4273	MouseDisease	GSE33295.GPL6 https://www.nc	79.77	46.90	34.64		40.33
944- normal control (liver) propranolol 6001	RatDisease	GSE119933/GPL https://www.nc	67.42	54.77	36,73		40.23
2- cellar disease (duodenan) NA 25220	HumanDisease	GSE77625.GPU1 https://www.nc	60.50	46,90	20,00	33.54	40.19
1- diet induced obesity (liver) NA 4283	MouseDisease	GSE33575.CPL1 Http://www.ncbu	-73.85	-51.95	-36.06		-40.47
16- crohn's disease (CD) (ileal mucosa) NA 13918	HumanDisease	GSE16879.GPL5 http://www.ncb	-60.30	-31.62	-20.00	-50.00	-40.48
1- alcoholic fatty liver (liver) NA 5669	MouseDisease	GSE52644/GPL1. http://www.ncb	-79.77	-52.92	-31.62		-41.08
1002- normal control (liver) NA 4139	RefDisease	GSES7815.GPU1 https://www.nc	-67,42	-54.77	-45.59		-41.45
927- normal control (liver) NA 3318	RatDisease	GSE57815.CPL1 https://www.nc	-73.85	-49.14	-44.72		-41.93
4- emphysema (Jung) NA 8597	MouseDisease	GSE8790 GPL12 http://www.ncb	-73.85	-50.99	-45.83		-42.67
2- disease control (kidney) NA 11770	HumanDisease	GSE124203/GPL https://www.nc	-60.30	-30.51	-24.49	-55.90	42.00
63- normal control [lave] NA 15602	MouseDisease	GSE151754/GPL https://www.nc	-67.42	-54.77	-50,00		-45.05
9- crohris disease (CD) (iteal mucosa) NA 25527	HumanDisease	GSE75214.CPL6 https://www.nc	-60.30	-31.62	-26.45	-55.90	-43.57
69- disease control [peyer's patches] NA 9305	MouseDisease	GSE94517.GPL1 https://www.nc	-60.30	-41.23	-26.45	-50.00	-44.50
67- disease control (peyer's patches) NA 9304	MouseDisease	GSE94517/GPL1 https://www.nc	-60.30	-37.42	-24/49	-55.90	-44.53
542- NA (neostriatum) NA 8860	MouseDisease	GSESSKAD.GPU1 https://www.nc	-661.90	-55.17	-50,00	-55.90	-44,84
47- toronlasmosis flund NA 13191	MouseDisease	GSE119855.GPL https://www.nc.	-60.30	-46.90	-36.05	-50.00	-48.32

#### Analysis Match: compare entire studies

- Validate your biological model, find potential disease targets for drug repositioning, etc.
- Input: any IPA core analysis
- How: uses canonical pathways, upstream regulators, causal networks, and disease enrichment as signatures for comparison

### Signatures used to match against other analyses





Upstream regulators



Disease enrichment



# Agenda

### **Topics for today's workshop**

### Interpreting your 'omics data using IPA Core Analysis

QIAGEN

- Setting up a core analysis
- Canonical pathways
- Comparison analysis

### **Creating networks from scratch**

- Connecting genes in a network
- In silico testing
- Overlay drugs and biomarkers

### Exploring publicly available data

- Activity plot, pattern search, Analysis Match
- Land Explorer

### Summary







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#### **Feedback and contact information**

Post-training survey: https://www.surveymonkey.com/r/QLYLYK8 Software questions and support: Sales and licensing questions: Additional feedback and training: ts-bioinformatics@qiagen.com bioinformaticssales@qiagen.com devendra.mistry@qiagen.com



# Data formatting

# Formatting transcriptomics data before uploading to IPA



	IDs (required)			• Ratio, fo	old change, etc. (recommended)
	Ţ		•	Signi	ficance (optional)
	А	В	С	D	E
1	geneid	UCvsNormal.Log2FoldChange	UCvsNormal.pval	52wksVedolizumabvsBaseline.Log2FoldChange	52wksVedolizumabvsBaseline.pval
2	DDX11L1	-0.1067	0.2878	0.1183	0.1624
3	WASH7P	-0.1883	0.0097	0.3063	0.0006
4	FAM138F	-0.0761	0.4699	0.2466	0.0191
5	OR4F5	0.1474	0.5311	0.1713	0.2913
6	LOC729737	0.4789	0.0017	0.029	0.8331
7	LOC100133331	0.4789	0.0017	0.029	0.8331
8	LOC100132062	0.4789	0.0017	0.029	0.8331
9	OR4F29	0.2495	0.2389	0.2181	0.1887
10	JA429831	0.1215	0.3338	0.2556	0.0004
		Observatior	า 1	Observatio	in 2

### Common identifier types

- Arrays from Affymetrix, Illumina, etc.
- Gene symbols (Entrez or HUGO)
- Ensembl, RefSeq, UCSC, etc.

### Accepted file formats

- .txt (tab-delimited text files)
- .xls, .xlsx, .csv (Excel tables)
- .diff (Cuffdiff output)

IDs are the only required column

**Change measurements** are needed for IPA to make activity predictions

# Formatting proteomics data before uploading to IPA





Observation 1

Phosphorylation changes (ratio, fold change, etc.) and sites are supported, but these columns must be assigned

## Formatting proteomics data before uploading to IPA



IDs	(require	ed)				• Ratio, fold change, etc. (recommended)
	ļ			•		<ul> <li>Significance (optional)</li> </ul>
	Α	B	С	D	E	Common protein IDs
1 10	)	Symbol	Phospho Fold Change	Phospho p-value	Phospho Site	Common protein 123
2 IF	9100137139	1700003H04Rik	-1.271	0.221	_M(ox)ET(ph)LGEK_	
3 IF	PI00224491	2900026A02Rik	-1.244	0.25	_RQS(ph)LYENQA_	<ul> <li>Ensembl</li> </ul>
4 IF	PI00224491	2900026A02Rik	-1.404	0.156	_SEECS(ph)PQWLK_	
5 IF	PI00652957	4930594M22Rik	-5.729	5.47E-09	_MFKSS(ph)PR_	<ul> <li>Gene symbols (Entrez or HUGO)</li> </ul>
6 IF	PI00137111	4933402E13Rik	2.196	0.000423	_AWALNDS(ph)ANT(ph)SPNAWFVER_	
7 IF	9100137111	4933402E13Rik	2.196	0.000423	_AWALNDS(ph)ANT(ph)SPNAWFVER_	Or Brack and Or Brack
8 IF	9100137111	4933402E13Rik	2.196	0.000423	AWALNDS(ph)ANT(ph)SPNAWFVER	<ul> <li>GenPept and GenBank</li> </ul>
9 IF	PI00654190	4933431E20Rik	-1.184	0.304	VGGLS(ph)PR	
10 IF	PI00654176	4933439C10Rik	-1.097	0.431	SPHLSGS(ph)LPR	<ul> <li>International Protein Index</li> </ul>
11 IF	PI00225598	A430057M04Rik	1.079	0.299	ALPT(ph)EPR	
12 IF	PI00227449	A730008H23Rik	-1.448	0.133	GM(ox)TLQWLIS(ph)PVK	<ul> <li>UniProt and SwissProt</li> </ul>
13 IF	PI00311509	AAAS	-1.085	0.37	ITHIPLYFVNAQFPRFS(ph)PVLGR	onin rot and owissi rot
14 IF	PI00458612	AAK1	1.07	0.311	VGSLT(ph)PPSS(ph)PKTQR	
15 IF	PI00458612	AAK1	1.07	0.311	VGSLT(ph)PPSS(ph)PKTQR	
16 IF	PI00458612	AAK1	1.057	0.332	AGQTQPNPGILPIQPALT(ph)PR	
			I.			UniProt ID conversion tool:

**Observation 1** 

<u>https://www.uniprot.org/mapping/</u>

Phosphorylation changes (ratio, fold change, etc.) and sites are supported, but these columns must be assigned

# Formatting metabolomics data before uploading to IPA



	Multiple	ID colun	nns		Ratio, fold change, etc. (recommended)							
		1			(optional)	Ţ	Significance (optional)					
					¬ <b>‡</b>			•				
1	A	В	С	D	E	F	G	Н				
1	Pubchem	Kegg	HMDB	CAS	Metabolites	Fold change	P_value	P_value_adjust				
2					(2 or 3)-decenoate (10:1n7 or n8)	1.212936133	4.44028E-05	0.000585189				
3	6443013	C14762	HMDB0004667	29623-28-7	13-HODE + 9-HODE	0.584109411	0.003698077	0.016919182				
4	10111	C02294	HMDB01522	471-29-4	1-methylguanidine	1.219937764	0.015399637	0.049446834				
5	5462190	C15606	HMDB0012134	746507-19-7	2,3-dihydroxy-5-methylthio-4-pentenoate (DMTPA)*	1.566518315	0.002802172	0.013670263				
6	80283	C02356	HMDB00452	1492-24-6	2-aminobutyrate	0.633800292	0.011016709	0.038805594				
7	10796774		HMDB00317	488-15-3	2-hydroxy-3-methylvalerate	0.997343835	0.006172648	0.024774766				
8	11427		HMDB37115	120-91-2	2-hydroxy-4-(methylthio)butanoic acid	1.294720456	0.000305912	0.002622524				

**Observation 1** 

### Common metabolite IDs

- CAS registry number
- Human Metabolome Database
- KEGG
- PubChem CID

Metabolite ID conversion tools:

- <u>https://biodbnet-abcc.ncifcrf.gov/db/db2db.php</u>
- <u>https://cts.fiehnlab.ucdavis.edu/batch</u>
- http://csbg.cnb.csic.es/mbrole2/conversion.php

## Formatting 'omics data before uploading to IPA

Observation 1



	Observation 1			DH I	Observation 2	
	А		В	С	D	E
1	geneid		UCvsNormal.Log2FoldChange	UCvsNormal.pval	52wksVedolizumabvsBaseline.Log2FoldChange	52wksVedolizumabvsBaseline.pval
2	DDX11L1		-0.1067	0.2878	0.1183	0.1624
3	WASH7P		-0.1883	0.0097	0.3063	0.0006
4	FAM138F		-0.0761	0.4699	0.2466	0.0191
5	OR4F5		0.1474	0.5311	0.1713	0.2913
6	LOC729737		0.4789	0.0017	0.029	0.8331
7	LOC100133331		0.4789	0.0017	0.029	0.8331
8	LOC100132062		0.4789	0.0017	0.029	0.8331
9	OR4F29		0.2495	0.2389	0.2181	0.1887
10	JA429831		0.1215	0.3338	0.2556	0.0004

# Analyte identifier REQUIRED to explore enrichment

### RNA examples: Gene symbols, array identifiers from Affymetrix, Ensembl, etc.

Protein examples: UniProt, GenPept, Gene symbols, Ensembl. etc.

Metabolite examples: KEGG, CAS registry number, etc. \*add multiple columns of ids to ensure best mapping

### Change values needed to calculate activity predictions

Change value examples: fold changes, ratios, etc.

Significance values: P-values \*optional but recommended to enable filtering for significance

#### Accepted file formats:

- ✓ .txt (tab-delimited text files)
- ✓ .xls, .xlsx, .csv (Excel tables
- ✓ .diff (Cuffdiff output

Multiple comparisons or observations may be uploaded in one file



# IPA colors and z-scores



### Pathway or gene activity predicted by IPA

Inhibited

Activated

### Actual measurement of gene expression in your dataset

Downregulated Upregulated
#### What do z-scores mean in IPA?



Actual dataset<br/>measurementVS.What IPA expects if pathway<br/>is activated

🛆 Symbol	Measurement	+	Expected
	Expr Log Ratio	х	
CCL2	+-2.030		🕇 Up
CD44	<b>↓</b> -1.634		🕇 Up
CD274	+-2.218		🕇 Up
COL1A1	<b>↓</b> -2.040		🕇 Up
COL1A2	<b>↓</b> -1.920		🕈 Up

Pathway inhibited

Z score

A Symbol	Measurement +	Expected
	Expr Log Ratio ×	:
CCL2	+-2.030	🕇 Up
CD44	<b>†</b> 1.634	🕇 Up
CD274	<mark>↓</mark> -2.218	🕇 Up
COL1A1	<b>†</b> 2.040	🕇 Up
COL1A2	↓-1.920	🕇 Up

No clear signal for prediction Z score = 0

Symbol	Measurement	+	Expected
	Expr Log Ratio	×	
CCL2	<b>†</b> 2.030		🕇 Up
CD44	<b>†</b> 1.634		🕇 Up
CD274	<b>†</b> 2.218		🕇 Up
COL1A1	<b>†</b> 2.040		🕇 Up
COL1A2	<b>†</b> 1.920		🕇 Up

Pathway activated

+ Z score

How well do the actual measurements match the expected measurements?

### Examples of z-scores

	ÌÒÒ
	CEN
GIA	GEIN

Symbol	Measurement	+	A Expected
	Expr Log Ratio	×	
NR5A2	+-1.002		🕹 Down
ABCB11	+-1.056		🕂 Down
CYP2B6	+-3.063		+ Down
PPARGC1A	+-2.495		🕂 Down
ACOX1	+-1.727		+ Down
SLCO1B3	<b>†</b> 3.223		+ Down
TLR4	<b>†</b> 1,213		🕈 Up
LY96	<b>†</b> 1.189		🕈 Up
IL1R1	<b>†</b> 1.634		🕈 Up
IL1RAP	<b>†</b> 1.046		🕈 Up
IL1B	<b>†</b> 3.890		🕈 Up
LIPC	<b>↓</b> -1.375		🕈 Up

**Z-score = 2.4** 

**10/12 measurements match expected** 

Mostly matching

Signal predominantly points to predicted activation

Symbol	Measurement	+	Expected
	A Expr Log Ratio	×	
CREB3L3	<b>↓</b> -1.536		🕈 Up
ІНН	<b>↓</b> -1.173		🕈 Up
PBX1	+-1.037		🕈 Up
CD86	<b>†</b> 1.016		+ Down
IL1RAP	<b>†</b> 1.046		+ Down
РКМ	<b>†</b> 1.082		🕈 Up
HLA-DMB	<b>†</b> 1.106		+ Down
IL18RAP	<b>†</b> 1.124		+ Down
CREB5	<b>†</b> 1.148		🕈 Up
CREB3L2	<b>†</b> 1.179		🕈 Up
CCN4	<b>†</b> 1.204		🕈 Up
TLR4	<b>†</b> 1.213		+ Down

#### Z-score = -2.236

4/12 measurements match expected

Mostly anti-matching

Signal predominantly points to predicted inhibition

## Z-scores predict activity



# Compares the EXPECTED gene expression pattern for activation in the knowledgebase to the ACTUAL gene expression measurements

#### Z-scores are used throughout IPA to predict activation or inhibition of:

- Canonical pathways
- Upstream regulators

- Master regulators
- Diseases and functions



#### Simplified z-score formula:

$$z = \frac{x}{\sigma_x} = \frac{\sum_i x_i}{\sqrt{N}} = \frac{N_+ - N_-}{\sqrt{N}}$$

#### **Z-score calculation summary**



#### Compares the EXPECTED gene expression pattern for activation in the knowledgebase to the ACTUAL data set gene expression measurements



# NO! a NEGATIVE Z-score is calculated indicating 4 predicted inhibition

#### Z-score= -2.236

Symbol	Measurement	+ Expected	Matching?
	/ Expr Log Ratio	×	
CREB3L3	+-1.536	🕈 Up	×
IHH	+-1.173	🕈 Up	×
PBX1	+-1.037	🕈 Up	×
РКМ	<b>1.082</b>	🕈 Up	$\checkmark$
CREB5	<b>†</b> 1.148	🕈 Up	<b>√</b> .
CREB3L2	<b>†</b> 1.179	🕈 Up	$\checkmark$
CCN4	<b>†</b> 1.204	🕈 Up	✓
IL10RA	<b>†</b> 1.246	🕈 Up	✓
FCGR2C	<b>†</b> 1.256	🕈 Up	$\checkmark$
INPP5D	<b>†</b> 1,297	🕈 Up	$\checkmark$
CD86	<b>†</b> 1.016	+ Down	×
IL1RAP	<b>†</b> 1.046	+ Down	×
HLA-DMB	<b>†</b> 1.106	🕂 Down	×
IL18RAP	<b>†</b> 1.124	+ Down	×
TLR4	<b>†</b> 1.213	+ Down	×
HLA-DQA2	<b>†</b> 1.225	+ Down	×
HLA-DQA1	<b>†</b> 1.225	+ Down	×

YES! a POSITIVE Z-score is calculated indicating predicted activation

#### Z-score= 2.427

Symbol	Measurement	+ 🛆 Expected	Matching?
	Expr Log Ratio	×	4.
NOS2	<b>†</b> 3,434	+ Down	X
IL1A	<b>†</b> 2,425	+ Down	×
HLA-DQA2	<b>†</b> 1,225	🕇 Up	<b>v</b>
TGFB1	<b>†</b> 1.095	🕈 Up	× /
HLA-DQA1	<b>†</b> 1.225	🕈 Up	*
JAK3	<b>†</b> 1.383	🕈 Up	×
FCER1G	<b>†</b> 1.313	🕈 Up	<b>√</b>
FPR1	<b>†</b> 2.117	🕈 Up	v te
PPARG	+-1.376	🕈 Up	×
IL10RA	<b>†</b> 1.246	🕈 Up	× /
HLA-DQB2	<b>†</b> 1.674	🕈 Up	¥ 52
ACADM	+-1.378	↑ Up	×
TLR4	<b>†</b> 1.213	🕈 Up	× /
IGH*	<b>†</b> 1.334	🕈 Up	v 10
PPARGC1A	+-2.495	🕈 Up	×
IL1RN	<b>†</b> 3.188	↑ Up	×,
HLA-DRB1	<b>†</b> 1.728	↑ Up	✓



# **Enrichment in IPA**

## Pathway enrichment: P-value of overlap



- Reference set is typically all genes or proteins that can be reliably detected by the 'omics technology used
- Most perturbed is a subset of measured genes/proteins that were significantly different between experimental groups
  - Fold change cut offs and p-value cut offs used
- IPA calls these "Analysis-ready molecules"
- Molecules associated with a biological category are a set of genes/proteins that the IPA knowledgebase has annotated to be important for a biological pathway, function, or disease.



## Testing for Enrichment using the Fisher's Exact Test





## P-Value from the Right-tailed Fisher's Exact Test



p-value: the probability of observing a result as extreme or more extreme, if the null hypothesis is true



 $H_o$  = Overlap of molecules for a particular biological category is due to chance

 $\alpha$  (significance level) = 0.05



 $p-value = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!a!b!c!d!}$ 

**P-value = 2.08 x 10<sup>-12</sup>** 

We can reject the null hypothesis



# Land explorer

## How to access Land Explorer data

#### 1) Click on Genes and Chemicals





# IPA Gene View report



IPA Gene View: BHI HE40	(Mammalian) > Interaction Network			Contact Support Help Documentatio	n						
Boview the estegarized literature fit	ndings and database information for this ps	odo		Contact Support Theip Documentatio							
Summary Human Mouse Rat	nungs and database information for this ne	uue.									
	Member Of: BHLH, BHLHB										
Entrez Gene Name: basic helix-loop-helix family member e40											
	Synonym(s): Basic helix loop helix, Basic he Stra14	elix-loop-helix domain containing, class b2, basic helix-loo	op-helix family, member e40, basic helix-loop-helix	family member e40, BHLHB2, C130042M06Rik,	Clast5, CR8, DEC1, HLHB2, SHARP-2, STF						
NCBI CDD Domains (Superf	amilies / Multi- basic Helix Loop Helix (bHLH) Domains):	domain superfamily, Hairy Orange, helix loop helix doma	ain								
Protein Functions / Functi	onal Domains: DNA binding, double-stranded transcription factor activity, seq transcription regulator	DNA binding, helix 3-helix 4 domain, helix-loop-helix don quence-specific DNA binding, RNA polymerase II transcription	nain, nucleic acid binding, protein binding, protein o ption regulatory region sequence-specific binding tr	domain specific binding, protein heterodimerization ranscriptional repressor activity, sequence-specific	on, protein homodimerization, RNA polymeras ic DNA binding, transcription factor binding,						
Subcel	Iular Location: chromatin, nuclear bodies, nuc	cleoplasm, Nucleus									
Cano	nical Pathway: Circadian Rhythm Signaling; IE	D1 Signaling Pathway; IL-10 Signaling; Pathogen Induced	d Cytokine Storm Signaling Pathway; Senescence	Pathway							
	(miRNAs wissed AAUAUAU), 1534.5 (j) (and other miRNAs w miR-148b.5 (and other miRNA miR-1993.9 (and other miRNA 1971 (and other miRNAs wisse miR-218-1.5 (miRNAs wisse 3098-th land other miRNAs wise 3098-th land other miRNAs wise	mR-1955 miRNAs wissed UAGGCCG), miR-194-59 issed GGGAGO, mR-1955 miRNAs wissed CCUG As wissed AGUUCUG), miR-1915 p. miRNAs wissed AGUGAGGC), miR-211-59 (miRNAs wissed UAAAGGC), miR-2159 (miRNAs wissed CAAGGA d UGQUUCC), miR-2152 (miRNAs wissed AGGAUGG), issed UCUCC), mR-252 (miRNAs wissed AGGAUGG), issed UCUCC), mR-252 (miRNAs wissed AGGAUGG), issed UCUCC), mR-252 (miRNAs wissed AGGAUGG),	(and other miRNAs wiseed CUCACUG, miR.105 GUC), miR.1393 (miRNAs wiseed GAGACG), s wiseed ACALUCA), miR.1873 (miRNAs wiseed ACALUCA), miR.1943-39 (miRNAs wiseed ACGU GGAGAU), miR.1943-39 (miRNAs wiseed ACGU C), miR.21-59 (miRNAs wiseed GUUCCU), miR.28 (miR.23)-59 (miR.23)-59 (miR.23)-50	-5g (mRNAs wiseed CACCUCC), mR1430a-3g mR14143; Gind other mRNAs wiseed AACAC d CGUGUCU), mR-1956 (mRNAs wiseed AACAC GCC), mR-1956 (mRNAs wiseed AGAAAG), GCC), mR-153g (mRNAs wiseed CUGUCAU), n mR-253g (and other mRNAs wiseed UCAAGUA), m	(and other miRNAs wiseed AGUCAA), min UG), miR-145-3p (miRNAs wiseed CGCAA), min CUGA), miR-1966 (miRNAs wiseed GGACGA), min miR-1963 (miRNAs wiseed GGACGA), min miR-21-5p (miRNAs wiseed AGCUCA), min miR-21-5p (miRNAs wiseed GGUGCCA), min min the ministration of the						
OmicSoft Land Explorer: Sample-	lev experimental data										
Data Type / Data Source RNA-seq expression:	Solid tissue (GTEx), Solid tissue (HPA).	Cell Lines Cancer cell lines (CCLE)	Oncology Consortia TCGA, TARGET, BeatAML, ICGC, CGCI, CCLE+GTEx+TCGA, ENCODE RNA-	Oncology Studies General oncology, Hematology, Pediatrics, Mouse studies	Disease Studies Human disease, Mouse disease, Rat dis <sup>,</sup>						
	Solid tissue (GTEx)	Cancer cell lines (CCLE), GSK cell lines, NCI cell lines, Pfizer cell lines	TARGET, expO, METABRIC, CCLE+GTEx	General oncology, Hematology, Metastasis, Pediatrics, Mouse studies	Human disease, Mouse disease, Rat dis						
Microarray expression:			TCGA, ENCODE RNA-associated gene	General oncology, Hematology, Metastasis,	Human disease. Mouse disease. Rat dis						
Microarray expression: Differential regulation:	Solid tissue (GTEx)	Treated cells (LINCS)	KNOCKOOWN	Pediatrics, Mouse studies							
Microarray expression: Differential regulation: Alteration frequency:	Solid tissue (GTEx)	Treated cells (LINCS) Cancer cell lines (CCLE), NCI cell lines	TCGA, TRACERX, BeatAML, ICGC, TARGET, METABRIC	General oncology, Hematology, Metastasis, Pediatrics							
Microarray expression: Differential regulation: Alteration frequency: Survival by expression:	Solid tissue (GTEx)	Treated cells (LINCS) Cancer cell lines (CCLE), NCI cell lines	KNOCKGOWN TCGA, TRACERX, BeatAML, ICGC, TARGET, METABRIC TCGA, BeatAML, TARGET, CGCI	General oncology, Hematology, Metastasis, Pediatrics General oncology, Clinical outcomes, Hematology							
Microarray expression: Differential regulation: Alteration frequency: Survival by expression: Single Cell differential regulation:	Solid tissue (GTEx) Human Cell Landscape (HCL), Tabula Sapiens	Treated cells (LINCS) Cancer cell lines (CCLE), NCI cell lines	KIDOCKOWI TCGA, TRACERX, BeatAML, ICGC, TARGET, METABRIC TCGA, BeatAML, TARGET, CGCI	General oncology, Hematology, Metastasis, Pediatrics General oncology, Hematology, Metastasis, Pediatrics General oncology, Clinical outcomes, Hematology Human Disease (UMI), Human Disease (nor UMI), Human Disease (UMI), Mouse Disease (non-UMI), Mouse Disease (UMI), Mouse Disease (non-UMI), Mouse Disease UMI (author annotations)	Human Disease (UMI), Human Disease ( UMI), Human Disease UMI (author annotations), Mouse Disease (UMI), Mou Disease (UMI), Mouse Disease UMI (author annotations)						
Microarray expression: Differential regulation: Alteration frequency: Survival by expression: Single Cell differential regulation:	Solid tissue (GTEx) Human Cell Landscape (HCL), Tabula Sapiens	Treated cells (LINCS) Cancer cell lines (CCLE), NCI cell lines	KIDOCKOWI TCGA, TRACERX, BeatAML, ICGC, TARGET, METABRIC TCGA, BeatAML, TARGET, CGCI	General oncology, Hematology, Metastasis, Pediatrics General oncology, Hematology, Metastasis, Pediatrics General oncology, Clinical outcomes, Hematology Human Disease (UMI), Human Disease (nor UMI), Human Disease (UMI), Mouse Disease (nor-UMI), Mouse Disease (UMI) (author annotations)	Human Disease (UMI), Human Disease ( UMI), Human Disease UMI (author annotations), Mouse Disease (UMI), Mou Disease (UMI), Mouse Disease UMI (author annotations)						



# Data upload step by step

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	Genes and Chemicals	Diseases and Functions	Pathways and Lists	Datasets and Ana	lyses		. 0	QIAGEN Land Explorer
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S Select Identifier Type:	Assign at	dditional columns as ID t	o improve mapping co	ign the identifier type( verage if desired	5).			
4 Array platform used fo	r experiments: Not spe	cified/applicable	Select relevant :	array platform as a refe	rence set for data an:	alveie	It is not already	1. To upload a dataset file, <u>click here</u> .
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1	geneid	UCvsNormal.Log2F	0.2070	52wksVedolizumab	52wksVedolizumab.			
3	WASH7P	-0.1883	9.700000000000000000	0.30630000000000000	5,99999999999999999999			<ol><li>Select the dataset file (.txt, .xls, .xlsx, .csv, or .diff) from your computer and click the Open https://doi.org/10.1016/j.csv.</li></ol>
4	FAM138F	-7.61000000000000	0.469899999999999998	0.2466000000000000000	1.9099999999999999999			buaon.
5	OR4F5	0.1474	0.5311000000000002	0.17130000000000000	0.2913			3. Select Flexible format for the file format from the dropdown menu if it is not already selected.
6	LOC729737	0.478899999999999999	9 1.6999999999999999	2.90000000000000	0.83309999999999999	15		A Assign on array platform used for the dataset if applicable. Assigning the appropriate array
7	LOC100133331	0.478899999999999999	9 1.6999999999999999	2.900000000000000	0.83309999999999999	15		4. Assign an array platform used for the dataset, if applicable. Assigning the appropriate array platform improves the accuracy of the statistics by assigning the platform as the reference set
8	LOC100132062	0.4788999999999999999	9 1.69999999999999999	2.9000000000000000	0.8330999999999999999	15		or "universe" of all possible measured molecules for that dataset. Leave as "Not
9	UK4F29	0.2495	0.2389	0.21809999999999999999	4.0000000000000000000000000000000000000			specified/applicable_for RINA-seq, metabolomics, or phosphoproteomics datasets.
11	JB137814	-0.67400000000000	1.6381E-6	0.13919999999999999999	0.3422			5. If your dataset is fairly simple (without many extraneous columns that you intend to ignore)
12	M37726	-1.055099999999999999	5.75999999999999999	0.336799999999999999	2.3400000000000000000			click the Infer Observations button. IPA will try to automatically assign the columns for you. If this is successful, you may ont to assign additional ID columns (see step 5) or just skin to step
13	LINC00115	-0.1666	2.10000000000000	0.14630000000000001	0.06			<ol> <li>If Infer Observations is unsuccessful, just click the button again to toggle it off.</li> </ol>
14	LOC643837	0.1024999999999999999	9 0.30209999999999998	8.50999999999999999	8.8000000000000005			6 Apping at least one column as an identifier column ("ID") from the drandourn many than
15	FAM41C SAMD11	-5.5100000000000000000000000000000000000	9 0.2554000000000000002	-1 7500000000000	7.92999999999999999999	0		select its identifier type(s) in the secondary dropdown menu. IPA supports many identifiers and
17	NOC2L	0.3407999999999999999	9 1.2575E-6	-5.580000000000000000	0.4404000000000000000	1		symbols and will attempt to guess the type of identifier in your dataset file if the identifiers are in
18	KLHL17	0.1497	8.200000000000000	-7.109999999999999	0.256799999999999999	7		appropriate identifier type. If more than one type of identifier exists in your dataset, select all
19	PLEKHN1	0.14630000000000000	1 8.80000000000000	-5.33E-2	0.3573000000000000	1		types that are appropriate, but refrain from selecting all identifier types at once as it can lead to
20	Clorf170	-0.164899999999999	8.50000000000000	0.1165000000000001	7.0499999999999993			mis-mapping.
21	HES4	0.1274000000000000	1 5.39999999999999999	-2.63E-2	0.693100000000000	5		Bound have (B lag Blow is to a standor to Blow) = 0.7 State Tryle and Analy Street = B Analy St
22	ISG15	0.332000000000000000	2 4.5167000000000000	-0.22700000000000	4.59999999999999999999			3 Conservation and March 2014 and 2014
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12	M37726		-1.05509999999999999	5.7599999999999999	0.336799999999999999	2.3400000000000001			
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17	NOC2L		0.3407999999999999999	1.2575E-6	-5.5800000000000	0.44040000000000001			
18	KLHL17		0.1497	8.200000000000000	-7.109999999999999	0.256799999999999997	37		
19	PLEKHN1		0.146300000000000001	8.800000000000000	-5.33E-2	0.35730000000000001	21		
20	C1orf170		-0.164899999999999	8.500000000000000	0.1165000000000000	7.04999999999999993	3aa		
21	HES4		0.127400000000000001	5.3999999999999999	-2.63E-2	0.6931000000000005	55		
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Provide Feedback | Support Tim Hou Close IPA Genes and Chemicals Diseases and Functions Pathways and Lists Datasets and Analyses **QIAGEN Land Explorer** .... Advanced Search Create New... Enter gene names/symbols/IDs or chemical/drug names here Search Dataset Upload - GSE73661-UC VDZ with pval.xlsx – 🗆 × **Q** 1. Select File Format: Flexible Format 2. Contains Column Header: Yes No 3. Select Identifier Type: Please assign at least one column below as "ID", and assign the identifier type(s). Assign additional columns as ID to improve mapping coverage if desired. 4. Array platform used for experiments: Not specified/applicable Select relevant array platform as a reference set for data analysis. 5. Use the dropdown menus to specify the column names that contain identifiers and observations. For observations, select the appropriate measurement value type. Raw Data (21563) Dataset Summary (20250) Metadata 0 Edit Observation Names Infer Observations ID Observation 1 Observation 1 Observation 2 Observation 2 ID/Observation Name Expr Log Ratio Expr p-value Expr Log Ratio Expr p-value Measurement/Annotation 2 types selec... UCvsNormal.Log2F... UCvsNormal.pval geneid 52wksVedolizumab... 52wksVedolizumab.. -0.1067 0.1183 9. Assign all the measurements as "Observation 1" (or 2 DDX11L1 0.2878 0.1623999999999999999 WASH7P -0.1883 9.700000000000003 0.3063000000000002 5.999999 the name chosen by Infer Observations) if they FAM138F -7.6100000000000... 0.46989999999999998 0.2466000000000001 1.9099999 0.1474 OR4F5 0.53110000000000 0.1713000000000001 0.291 represent different value types for one "comparison." LOC729737 0.47889999999999999 1.699999999999999999 2 9000000000000000 6 LOC100133331 2.9000000000000000 LOC100132062 8 2 90000000000000000 10. If your dataset contains multiple comparisons 0.2495 9 OR4F29 0.2389 0.21809999999999999 0.1887000 0.1215 10 JA429831 0.333799999999999 0.255599999999999999 4.0000 (observations), then you will need to assign each 11 JB137814 -0.6740000000000... 1.6381E-6 0.13919999999999999 0.3422 batch of additional columns to Observation 2, 12 M37726 1.0550999999999999 5,75999999999999999. . 0.33679999999999999 2.3400000 13 LINC00115 -0.1666 2,1000000000000001 0.14630000000000001 0.06 **Observation 3, etc.** 14 LOC643837 0.1024999999999999 0.302099999999 8.5099999999999999... 8.800000 FAM41C 0.20979999999999999 0.2554000000000000 0.1237 15 7,929999999999999999 16 SAMD11 -5.51999999999999... 0.4088 -1.7500000000000... 0.78839999999 17 NOC2L 0.3407999999999999 1.2575E-6 -5.5800000000000... 0.440400000000000 0.1497 18 KLHL17 8.2000000000000007 -7.1099999999999999. 0.25679999 19 PLEKHN1 0.1463000000000001 8.8000000000000000... -5.33E-2 0.35730000000000000 20 Clorf170 -0.164899999999999... 8.500000000000006. 0.1165000000000001 7.0499999999999993 21 HES4 0.1274000000000001 5.39999 -2.63E-2 0.6931000000000000 22 ISG15 0.332000000000002 4.516700000000003. -0.2270000000000... 4.599999 23 AGRN 0.8164000000000001 4.414299999999999... - -0.3106999999999... 1E-3

New user training: Large dataset analysis and knowledge base queries using QIAGEN IPA

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File Edit View Window	Help					Provide Feedback   Support Tim Hou Close IPA
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							To lat	abel each observation, select an existing name from the pull-down lists,			
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	5	OR4F5	0.1474	0.5311000000000002	0.171300000000000001	0.2913	4.	bservation 4			
	6	LOC729737	0.47889999999999999999	1.699999999999999999	2.900000000000000	0.833099999999					
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	9	OR4F29	0.2495	0 2389	0.21809999999999999999	0.8550999999999					
	10	JA429831	0.1215	0.33379999999999999999	0.255599999999999999999	4.0000000000000000000000000000000000000					
	11	JB137814	-0.67400000000000	1.6381E-6	0.1391999999999999999	0.3422					
	12	M37726	-1.055099999999999999	5.759999999999999999	0.3367999999999999999	2.34000000000		OK Cancel			
	13	LINC00115	-0.1666	2.10000000000001	0.146300000000000001	0.06					
	14	LOC643837	0.10249999999999999999	0.302099999999999998	8.50999999999999999	8.8000000000000000000000000000000000000	0005				
	15	FAM41C	0.20979999999999999999	0.25540000000000002	0.1237	7.92999999999999	9995				
	16	SAMD11	-5.5199999999999999	0.4088	-1.75000000000000	0.7883999999999	999999				
	17	NUC2L	0.340799999999999999999	9 2000000000000000	-7.100000000000000	0.44040000000	00001	,			
	19	PLEKHN1	0.14630000000000000000	8.800000000000000000	-5.33E-2	0.357300000000	00001				
	20	Clorf170	-0.164899999999999	8.50000000000000000	0.11650000000000000000	7.04999999999999	9993				
	21	HES4	0.12740000000000000000	5.399999999999999999	-2.63E-2	0.69310000000	00005				
	22	ISG15	0.33200000000000002	4.516700000000003	-0.22700000000000	4.59999999999999	9999				
	23	AGRN	0.81640000000000000000	4.41429999999999999	-0.310699999999999	1E-3					
	24	Clorf159	-8.0600000000000	0.1613999999999999999	9.28999999999999999	0.13400000000	00001				
	25	JA715134	-0.2087	0.1124	0.3785	2.50000000000	0001				
	26	TTLL10	-8.649999999999999	0.2422	0.1138	0.133500000000	00001				

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1	geneid	UCvsNormal.Log2F	UCvsNormal.pval	52wksVedolizumab	52wksVedolizumab			
2	DDX11L1	-0.1067	0.2878	0.1183	0.162399999999999999			
3	WASH7P	-0.1883	9.700000000000003	0.30630000000000002	5.999999999999999995			
4	FAM138F	-7.6100000000000	0.469899999999999998	0.24660000000000000	1.90999999999999999			
5	OR4F5	0.1474	0.53110000000000002	0.17130000000000000	0.2913			
6	LOC729737	10 4700000000000000000000000000000000000						
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7	LOC100133331	0.4788999999999999999999	1.6999999999999999999 1.6999999999999	2.900000000000000 2.900000000000000	0.833099999999999999 0.833099999999999999			
7 8	LOC100133331 LOC100132062	0.478899999999999999999999999999999999999	1.6999999999999999999 1.6999999999999	2.900000000000000 2.90000000000000 2.90000000000	0.8330999999999999999 0.833099999999999999 0.8330999999999999999 0.833099999999999999999			
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7 8 9 10 11 12 13	LOC100133331 LOC100132062 OR4F29 JA429831 JB137814 M37726 LINC00115 LOC640977	0.478899999999999999999 0.478899999999999999 0.2495 0.1215 -0.67400000000000 -1.055099999999999999 -0.1666 0.10340000000000000000000000000000000000	1.69999999999999999 1.699999999999999 1.69999999999999 0.2389 0.333799999999999999 1.6381E-6 5.7599999999999999 2.10000000000001	2.90000000000000 2.9000000000000 2.90000000000	0.833099999999999999 0.8330999999999999 0.8330999999999999 0.188700000000000000 1.887000000000000000 0.3422 2.340000000000000000000  0.06			
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7 8 9 10 11 12 13 14 15 16 17	LOC100133331 LOC100132062 OR4F29 JA429831 JB137814 M37726 LINC00115 LOC643837 FAM41C SAMD11 NOC21	0.478899999999999999999 0.4788999999999999999 0.478899999999999999 0.478899999999999999 0.1215 -0.674000000000000 -1.0550999999999999 -0.1666 0.10249999999999999 -5.519999999999999 -5.51999999999999	1.6999999999999999 1.699999999999999 1.699999999999999 0.2389 0.3337999999999999999 0.333799999999999999 0.332799999999999999 0.33209999999999999 0.325400000000002 0.4088 1.2554.6	2.90000000000000 2.9000000000000 2.90000000000	0.333099999999999999 0.8330999999999999 0.8330999999999999 0.1887000000000000 1.00000000000000 0.3422 2.3400000000000001 0.06 8.8000000000000005 7.929999999999999 0.7883999999999999 0.78839999999999999			
7 8 9 10 11 12 13 14 15 16 17 18	LOC100133331 LOC100132062 OR4F29 JA429831 JB137814 M37726 LINC00115 LOC643837 FAM41C SAMD11 NOC2L KI H17	0.47839999999999999999 0.47889999999999999 0.2495 0.1215 -0.67400000000000 -1.05509999999999 0.102499999999999 0.1024999999999999 0.2037999999999999 0.3407999999999999	1.6999999999999999 1.699999999999999 1.69999999999	2.90000000000000 2.9000000000000 2.90000000000	0.333099999999999999 0.8330999999999999 0.8330999999999999 0.1887000000000000 1.40000000000000 0.3422 2.340000000000000 0.06 8.80000000000000 0.06 8.80000000000000 0.78399999999999999 0.4404000000000001 0.256700000000001			
7 8 9 10 11 12 13 14 15 16 17 18 19	LOC100133331 LOC100132062 OR4F29 JA429831 JB137814 M37726 LINC00115 LOC643837 FAM41C SAMD11 NOC2L KLHL17 DI EKHM1	0.4783999999999999999999 0.47889999999999999 0.4788999999999999 0.2495 0.1215 -0.67400000000000 -1.055099999999999 -0.1666 0.1024999999999999 0.102499999999999 0.102499999999999  0.3407999999999999 0.146300000000000000000	1.6999999999999999 1.69999999999999 1.69999999999	2.90000000000000 2.9000000000000 2.90000000000	0.3330999999999999999 0.83309999999999999 0.1887000000000000 1.400000000000000 2.34000000000000 0.06 8.80000000000000 0.06 8.80000000000000 0.06 0.7929999999999999999 0.44040000000000 0.25679999999999999 0.357300000000000 0.357300000000000 0.35730000000000000 0.35730000000000000000000000000000000000			

#### 13. Click the <u>Save</u> button to proceed.

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Provide Feedback | Support Tim Hou Close IPA Genes and Chemicals Diseases and Functions Pathways and Lists Datasets and Analyses QIAGEN Land Explorer .... Advanced Search Create New... Enter gene names/symbols/IDs or chemical/drug names here Search Dataset Upload - GSE73661-UC VDZ with pval.xlsx – 🗗 🗙 ~ 0 1. Select File Format: Flexible Format 2. Contains Column Header: Yes No 3. Select Identifier Type: Please assign at least one column below as "ID", and assign the identifier type(s). Assign additional columns as ID to improve mapping coverage if desired. 4. Array platform used for experiments: Not specified/applicable Select relevant array platform as a reference set for data analysis. 5. Use the dropdown menus to specify the column names that contain identifiers and observations. For observations, select the appropriate measurement value type. Raw Data (21563) Dataset Summary (20250) Metadata 0 Edit Observation Names Infer Observations ID UCvsNormal UCvsNormal 52wksVedoli... 52wksVedoli... ID/Observation Name Expr Log Ratio Expr p-value Expr Log Ratio Expr p-value Measurement/Annotation 2 types selec... UCvsNormal.Log2Fol... UCvsNormal.pval 52wksVedolizumabvs... 52wksVedolizumabvs. geneid 14. Click the <u>OK</u> button to proceed. DDX11L1 2 -0.1067 0.2878 0.1183 0.1623999999999999999 WASH7P -0.1883 3 9700000000000003 X FAM138F 7.6100000000000... 0.46989999999999999 0.24660000000000001 1.90999999999999 Your dataset does not contain any metadata. 0.1474 5 OR4F5 0.5311000000000 0.1713000000000001 0.2913 Metadata is useful when you search for datasets or subsequent analyses. LOC729737 0.47889999999999999 1.69999999999999999. 2.900000000000001... 0.83309999999999 6 Please click on the Metadata tab to add keywords that describe the data. LOC100133331 0.478899999999999999 1.69999999999999999. 2.90000000000000001.. . 0.8330999999999 0.47889999999999999 1.6999999999999999... 8 LOC100132062 2,900000000000001... 0,8330999999999 Click OK to continue to save the dataset without metadata 9 OR4F29 0.2495 0.2389 0.21809999999999999 0.18870000000db OK Cancel 10 0.1215 JA429831 0.333799999999999999 0.25559999999999999 4.0000 11 -0.67400000000000... 1.6381E-6 JB137814 0.1391999999999999 0.3422 12 M37726 -1.0550999999999999 5.7599999999999999... 0.33679999999999999 2.3400000 00000001. 13 LINC00115 -0.1666 2.1000000000000001. 0.1463000000000001 0.06 14 0.1024999999999999 0.30209999999999 LOC643837 8.5099999999999995... 8.800000 00000005 15 FAM41C 0.20979999999999999 0.2554000000000000 0.1237 7.92999999999999999 16 SAMD11 -5.519999999999999... 0.4088 17 NOC2L 0.3407999999999999 1.2575E-6 -5.58000000000000... 0.4404000000000000 18 KLHL17 0.1497 -7.109999999999999... 0.256799999999999999 8,20000000000000007. 19 PLEKHN1 0.1463000000000001 8.800000000000000. -5.33E-2 0.3573000000000000 20 C1orf170 -0.1648999999999999... 8.5000000000000006. 0.1165000000000001 7.0499999999999993. 21 HES4 -2.63E-2 0.1274000000000001 5.39999999999999999... 0.6931000000000005 22 ISG15 0.3320000000000002 4.516700000000003... -0.22700000000000... 4.59999999999999999. 23 AGRN 0.8164000000000001 4.41429999999999999... -0.310699999999999... 1E-3 24 -8.0600000000000... 0.161399999999999999 9.289999999999996... 0.1340000000000000 C1orf159 25 JA715134 -0.2087 0.1124 0.3785 2.5000000000000001. 0.1138 26 TTLL10 -8.649999999999999... 0.2422 0.133500000000000001

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24	C1orf159	-8.060000000000000	. 0.1613999999999999999	9.289999999999999999	. 0.134000000000000000							
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Cancer

4.48F-24 - 1.89F-88

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# **IPA Analysis Tabs**

# Summary Tab

Expression Analysis - UCvsNormal		- 0
Summary Graphical Summary Pathways Upstream Analysis Diseases & Functions Regulator Effects Networks Lists Analysis Match Molecu	ules	
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> Experiment Metadata		
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Name	p-value Overlar	зр
Granulocyte Adhesion and Diapedesis	· 2.71E-31 32.8 %	62/189
Pathogen Induced Cytokine Storm Signaling Pathway	• 3.35E-28 22.4 %	83/371
Agranulocyte Adhesion and Diapedesis	· 7.93E-26 28.1 %	59/210
L - 10 Signaling	• 9.12E-21 29.2 %	45/154
Hepatic Fibrosis / Hepatic Stellate Cell Activation	• 1.67E-19 25.3 %	49/194
✓ Top Upstream Regulators		
✓ Upstream Regulators		
Name	p-value Predicted Act	ctivation
lipopolysaccharide	1.17E-129 Activated	
TNF	• 7.30E-113 Activated	
dexamethasone		
IFNG	• 4.27E-90 Activated	
ILIB	• 6.80E-90 Activated	
✓ Causal Network		
Name	p-value Predicted Act	tivation
APOC1	9,19E-127 Activated	
lipopolysaccharide		
SC-58125		
infliximab	- 2.78E-108 Inhibited	
TAT	2.9IE-107 Activated	
imes Top Diseases and Bio Functions		
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Name	p-value range # Molect	cules
Inflammatory Response	+ 2.42E-24 - 1.97E-103 640	)
Organismal Injury and Abnormalities	+ 3.04E-24 - 1.97E-103 1301	1
Immunological Disease	+ 2.49E-24 - 5.20E-91 682	2
Inflammatory Disease	+ 1.65E-24 - 5.20E-91 580	1

#### Top 5 for all analysis modules and a quick high-level look at your data



# **Graphical Summary Tab**





Graphical display of the top biological themes and features within your data with added AI inferences (dotted lines)

## Pathways Tab





Metabolic and cell signaling pathways that are enriched in your data with activity prediction

# Upstream Analysis Tab



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One	dexaptethasone	A 9 995	chemical drug	Inhibited	-3.118		4.26E-109	◆ABCB1, ◆ACADM, ◆ACADS, ◆all 397 735 (15)	Filter	CONS
a dell'Canal	IF IG	<b>↑</b> 0.896	Linklinkt	Activated	10,480		4.27E-90	TA2M, +ABCB1, +ABCD3, TAD.,all 298 627 (12)		
additional	STAT2	\$0.541	Hignlight row	Activated	9.421		2755 93	↑ A2M, ♥ ADCD11, ♥ ADCD3,♥ ADminial 255 500 (15)		
	TGER1	1 095	and click to	Activated	7.417		5.75E-62 5.50E-78	+ARCG2 +ACAA2 +ACKR2 +A all 326 715 (10)		
		+ 0.090		Activated	6082		2 33E 77	+ACOX1 +ACS11 +ACS14 +A all 247 636 (16)		
upstream of	Immunoglobulin	• -0.050	display at	Activated	1515		1895.76	+ACKR2 +ACSI1 +ADAM19 + all 219 735 (18)		
apolioanioi	II.6	<b>†</b> 1969	diopidy de	Activated	8004		1055 71		Activ	/ity
regulatory	beta-estradiol	1905	network map	Activated	3.368		3.05E-71	2011 P8 (119)	prodio	tion
notworko	tetradecanoviphorbol acetate		chemical grug	Activated	9,577		1.54E-65	+ABCB1, ↑ACSL4, ↑ADAM9, ↑Aall 227 750 (19)	predic	lion
networks	IL13	+-0.146	cvtokine	Activated	3,622		147E-62	↓ACOX1, ↑ACSL1, ↑ADAMTS4, ▶all 136 583 (14)		
	tretinoin		chemical drug	Activated	6.148		1.20E-59	↑A2M, ↑ABCA12, ↓ACKR2, ↓Aall 268 731 (19)		
	SB203580		chemical drug	Inhibited	-8.915		1.05E-56	♦ACOX1, ↑ADAMT54, ↑ADAMTall 133 573 (14)		
	PD98059		chemical - kinase inhibitor	Inhibited	-7.587		4.57E-53	↑AGT, ↑ANGPT2, ↓ANPEP, ↑Aall 140 640 (19)		
Measurement	STAT1	1.375	transcription regulator	Activated	5.905		5.45E-53	A2M, AGT, ANGPT2, APOall 119 579 (13)		
Measurement	IL10	↑0.342	cytokine		-1.493		6.06E-53	↓ABCB1, ↑ADM, ↑BCL2A1, ↑CCall 125 595 (14)	Genes	, that
from your	meet augurndin E2		chemical - endogenous mammalian	Activated	2.723		1.27E-52	↑ADAMTS1, ↑ADAMTS4, ↑ANall 114 633 (16)	thic rog	ulator
	NFkB (complex)		complex	Activated	9.687		7.11E-52	↑A2M, ↓ABCB1, ↓ABCD3, ↑ADall 146 598 (12)	unsieg	ulator
dataset for	OSM	<b>1.647</b>	cytokine	Activated	8.233		1.46E-50	↑A2M, ↓ACKR2, ↑ADAMTS1, ↑all 134 499 (14)	targe	ote I
this regulator	IL1		group	Activated	7.204		1.51E-50	↓ABCG2, ↑ADAMTS1, ↑ADAMTall 106 673 (14)	targe	210
ins regulator	CSF2	<b>†</b> 0.605	cytokine	Activated	7.860		2.54E-50	ALOX5, ANXA1, ATM, BCLall 130 619 (14)	prese	nt in 🔰
	IL2	+-0.189	cytokine	Activated	6.149		3.31E-50	ACVR2A, *ADAM19, *ANXA1,all 158 591 (15)	vour file	torod
	Interferon alpha		group	Activated	8.165		9.76E-50	ADA2, ADAM19, ADAMTS1,all 135 490 (14)	your III	relea
	progesterone		chemical - endogenous mammalian		1,499		2.30E-49	♦ABCB1, ♦ABCG2, ♦ACAT1, ♦Aall 147 673 (23)	eteb	sot
	IL1A	<b>↑</b> 2,425	cytokine	Activated	8.386		6.59E-49	ADAMTS1, ADAMTS4, BIRC3,all 89 509 (10)	uala	
	CD40LG	<b>↑</b> 0.184	cytokine	Activated	6.426		7.03E-49	↑ANXA6, ↑BCL2A1, ↑BCL6, ↑BIall 111 601 (15)		
Degulator	RELA	<b>†</b> 0.323	transcription regulator	Activated	7.160		3.09E-48	↑A2M, +ABCB1, +ABCG2, ↑AGT,all 121 585 (12)		
Regulator	poly rl:rC-RNA		biologic drug	Activated	9.733		2.83E-47	ANXA1, TAPOL1, TAPOL2, TAall 146 474 (12)		
predicted by	AGT	1.115	growth factor	Activated	7.558		4.06E-45	TADAM12, TAD. 194 ADHall 168 670 (18)		
predicted by	EGF	+-0.136	growth factor	Activated	7.198		6.25E-45	ADUGZ, TAUSLA, TADAMIST, Taban 144 611 (16)		
IPA using	IL1/A	T0.612	cytokine	Activated	5.991		/.15E-45	HAREG, FOLLZAI, TCS, TCCLTT,		
	Nord	T1.916	cytokine	Activated	7,039		2.10E-44	ACATT, FADAMIST, TAKEG, Tdll 109 554 (15)	P-valu	ie of
patterns seen	Vegi Liotas		group	Inhibited	7.228		0.10E-40 4 E4E 40	LARCEL LARCELL LARCE2 + 301126 515 (15)	cignific	anco
in your data	fluticasone propionato		chemical drug	minuted	-7220		4.346-45		Signine	ance
in your uata	II 10RA	<b>†</b> 1246	transmembrane recentor	Inhibited	-1.701		3.63E_42			
(no	resignimod	• 1:240	chemical drug	Activated	5321		1565-41			
	STAT6	+-0.034	transcription regulator	Activated	2.151		1.85E-40	+ACOX1. +ACSL1. +ADAM19.+all 110 627 (15)		
measurement)		. 00031			2.03.					

Regulators that may be contributing to the signal observed in your data. Some have been measured in your dataset and some have been predicted by IPA

#### \*\* ALL COLUMNS ARE FILTERABLE\*\*

#### **Diseases & Functions Tab**





Diseases and functions that may be key to the biology in your experimental data based on patterns of expression observed
### **Regulator Effects Tab**





Ties dataset molecules and regulators to a predicted phenotypic outcome

#### **Networks** Tab

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TX

....all 3

...all 3

Network of Expression Analysis - UCvsNormal highly Summary Graphical Summary Pathways Upstream Analysis Diseases & Functions Regulator Effects Networks Lists Analysis Match Molecules connected Networks Overlapping Networks molecules. View Networks Add To My Pathway Add To My List Merge Networks Annotation Customize Table 📇 🌐 🗘 🗌 Expand click to open network map 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. Total selected molecules: 0 T × Score ▼ × Focus Molecules ▼ × **Top Diseases and Functions** ID Molecules in Network 35 47 ↓AKAP1, ↑ARFGAP3, ↑ARHGAP25, ↑BACE2, ↑BASP1, ↑BIRC3, ↓C1orf210, ↓CKB, ↓CLYBL, ↓DYNC2H1 34 Connective Tissue Disorders, Developmental Disorder, Hereditary Diso.....all 3 .all 35 45 33 ↓ABCB1, antioxidant, ↑CAV1, ↓CDHR5, ↑CHAC1, ↓CHRNA1, ↓CNTN4, ↑DERL3, ↓EPB41L3, ↑FAM20A Connective Tissue Development and Function, Skeletal and Muscular S.....all 3 ↓ACOX1, ↓ADAMTSL1, ↓AIFM3, ↑ASS1, ↑CCDC88A, ↑CD38, ↑CEP128, ↑DMD, ↓EPB41L4B, ↓FHIP1A ...all 35 40 31 Cancer, Endocrine System Disorders, Organismal Injury and Abnormalit.....all 3 ↓ BDH1, ↓ BRINP3, cldn, ↑ CLDN1, ↑ CLDN2, ↓ CLDN8, Collagen type V, ↑ CRELD2, ↑ CSGALNACT2, ↑ DRAM1 ...all 35 38 30 Cardiovascular System Development and Function, Cell Morphology, C.,. ...all 3 ↑ADAM19, ↑APLNR, ↑AQP9, Caspase 3/7, ↓CFTR, ↑DYSF, ↑ELOVL5, ↑FADS2, ↑FAIM2, ↑FPR2 ...all 35 33 28 Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry ate O-acyltransferase, 75 N. ...all 35 30 26 1-acylglycerol-3-phosphate O-acyltransferase, 1-acylglycerophosphocholine O-acyltransferase, 2-acylglycerol-3-p Developmental Disorder, Endocrine System Disorders, Molecular Trans.....all 3 Molecule in ABCA13, ADA2, AHNAK2, Ap2, Ap2 alpha, ATPase, BST2, CTSK, FKBP11, GBP1 ...all 35 30 26 **bold** is in your ↑BCL6, ↑BHLHE40, ↑BTK, CALC, ↑CCR7, ↑CD40, ↑CD69, ↑CD86, Csf2ra-Csf2rb, ↑CSF2RB ...all 35 30 26 Cellular Function and Maintenance, Humoral Immune Response Protei.....all 3 26s Proteasome, Cdc2, Cyclin A, +DAPP1, +ELL2, +FOSB, FSH, +GEM, +HBEGF, +HSD11B2 Cellular Development, Cellular Growth and Proliferation, Digestive ...all 35 30 26 dataset: 10 →AFICYL2, ↑ALOX5, Alp, ↓ANK3, BMP, CaMKII, ↑CCDC3, collagen type i (famil) CR1L ...all 35 28 25 Connective Tissue Development and Function, Hematological System ... unbolded +A1CF, Alpha catenin, +ANPEP, +BAG3, +BGN, +CCN2, +CHST15, +CNTN3 +COL1A1, +COL1A2 ...all 35 28 25 Dermatological Diseases and Conditions, Organismal Injury and Abnor.....all Alpha Actinin, Cadherin, calpain, +CAPN9, +CDH11, +CDH3, +CLCA1, +CCN2, cyclooxygenase, +DMBT1 ...all 35 26 24 Cancer, Cell-To-Cell Signaling and Interaction Organismal Injury and A.....all 3 12 have been 13 ↑ADAM12, ↑ADAMTS1, ↑ADAMTS4, c-Src, ↑CCN1, collagen, complement receptor, Complement receptor 4, ↑CR1, ↑CR2 ...all 35 26 Cell-To-Cell Signaling and Interaction, Cellular Movement, Hematologi.....all 3 24 added to 14 + ACKR2, + ACKR4, Adaptor protein 1, C-C chemokine receptor, C-X-C chemokine receptor, + CCL11, + CCL13, + CCL23, + CCL4L1/CCL4L2, chemo....all 35 26 24 Cell-To-Cell Signaling and Interaction, Cellular Movement, Immune Cel.....all 3 15 +A2M, +ANGPT2, +BTG2, +CD55, +COL4A1, Colla en type I (complex), Collagen(s), EGFR ligand, **†F2R,** Fibrinogen ...all 35 26 24 Cardiovascular Disease, Organismal Injury and Abnormalities, Post-Tra.....all 3 maximize +ACAA2, +ACAT1, Apolipoprotein, C1q, +C3 +CATSPERB, +CD80, +CEBPB, +CFH, +CLU 16 ...all 35 26 24 Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry ....all 3 connectivity 17 14-3-3, **† AIM2, † ANKRD22, † CCL20**, 103, **† CLEC7A, † CLMP, † CXCL11, † CXCL9, † CYTIP** ...all 35 26 Dermatological Diseases and Conditions, Inflammatory Disease, Inflam.....all 3 24 18 ↓ASB13, ↑C1R, ↑C15, ↑C2, C4 aspase-3, **↑CFB**, ENaC, **↑GBP1P1**, growth factor ...all 35 24 23 Cancer, Organismal Injury and Abnormalities, Reproductive System Dis.....all 3 19 ↑ACP3, ADORA2, ↑CD22 ↑CD37, CD8, ↑CHST11, ↑CRISPLD2, ↑EGR1, Fc gamma receptor, ↑FCRL5 ...all 35 24 23 Cell-To-Cell Signaling and Interaction, Hematological System Develop... ...all 3 20 Abl1/2. + ACSM3. AMY2B, + AQP11, + AQP7, + AQP8, AQUAPORIN, C2-C4b, C3-Cfb, C4BF ...all 35 23 22 Carbohydrate Metabolism, Increased Levels of Potassium, Molecular Tr....all 3 20s protea ne, acad, 🕇 ACADM, 🔸 ACADS, 🕹 ACADSB, Acox, acyl-CoA dehydrogenase, 🕈 ANGPTL4, 🕈 ART3, 🕹 B4GALNT2 21 ...all 35 23 22 Energy Production, Lipid Metabolism, Small Molecule Biochemistry Based on a p-22 Atrial Natriuretic Peptide, BETA TUBULIN, + CPA6, CYP, CYP19, CYP19A, Cyp1a/2a/3a/4a/2c, + CYP27A1, Cyp2b ...all 35 23 22 Lipid Metabolism, Small Molecule Biochemistry, Vitamin and Mineral ... ...all 3 value 23 ABCG2, +ADGRA3, +ADGRE3, +ADGRF1, +ADGRF5, +ADGRL4, +ATP23, atvpical protein kinase C. +CALCRL, +CROT ...all 35 23 22 Cell Signaling, Connective Tissue Development and Function, Nucleic A.....all 3 24 ♦ ACVR1C, ♦ ACVR2A, alcohol group acceptor phosphotransferase, APC/APC2, ♦ CDC14A, ♦ CDKN2B, ♦ CNKSR3, cytochrome-c oxidase, ♦ DHFR, ...all 35 23 22 Cancer, Neurological Disease, Organismal Injury and Abnormalities calculation 25 ↑ACSL1, AMPK, ↑ATM, ATP synthase, BCR (complex), ↑CASP1, ↑CASP4, caspase, Cyclin B, ↑DDIT4 ...all 35 23 Cell Death and Survival, Dermatological Diseases and Conditions, Org... ...all 3 22 how likely molecules exist as part Selected 0 / 25 of a network

Disease or function predicted to be an outcome

Networks constructed from your dataset with level of connectivity prioritized

than chance alone

### **Analysis Match Tab**



	Expression An	alysis - UCvsNormal													– 🗆 🗙		
	Summary	Graphical Summary	Pathways	Upstream Analy	sis Diseases & Functions	Regulator Effec	ts Networks	Lists Ana	alysis Match	Molecules							Average z-
previously run	Evaluate Metadata View As Heatmap View Comparison Customize Table													۲ ا		score from all	
analyses with	Analysis Name		т	Project 🛛 🛪 🗡	case.d T × case.t T	× <sub>case.ti</sub> <b>v</b> ×	case.t T ×	comp T ×	сотр т ×	сотр т × у	weblink T ×	CP (zT × UR	(z T × CN (z-	T × DE (z )	r × 🗸 z 🕇 ×		foaturos
matching or	SSE73661-UC	VDZ dataset 2022 - 2022	-09-08 09:05 k	Kristin Practice							1	79.47 92.2	88.32	91.61	87.90		leatures
antimatabing	Causal network exercise GSE73661-UC VDZ dataset Kristin Practice 79.47 92.20 88.32 91.61 87.9											87.90					
antimatering	GSE73661-IBD	Vedolizumab-w-metadat	ta - 2022-12·E	Example Analyses	ulcerative colit	colonic mucosa						72.55 89.4	4 78.74	90.63	82.84		
results	3- ulcerative co	litis (UC) [colonic mucosa	a] NA 24615 H	HumanDisease	ulcerative colit	colonic mucosa	NA	Other Compari	. DiseaseStage	GSE48958.GPL6 h	ttp://www.ncb	72.55 81	5 69.28	85.57	77.21	(	
	2- ulcerative co	litis (UC) [colonic mucosa	a] NA 26850 H	HumanDisease	ulcerative colit	colonic mucosa	NA	Disease vs. No	DiseaseState:T	GSE65114.GPL1 h	ttp://www.ncb	58.82 83.6	6,82	85.57	76.47		
	1- crohn's dise	ase (CD) [colonic mucosa]	] NA 3988 H	HumanDisease	crohn's diseas	colonic mucosa	NA	Disease1 vs. D	. DiseaseState	GSE123141.GPL h	ttps://www.nc	72.55 83.0	71.41	73.19	75.06		Downstream
Double click to open core analysis	4- crohn's dise	ase (CD) [colonic mucosa]	] NA 28859 H	HumanDisease	crohn's diseas	colonic mucosa	NA	Disease vs. No	Tissue:Experim	GSE75214.GPL6 h	ttps://www.nc	54.89 86.0	67.82	80.18	74.73		effects (i.e.
	th crohn's dise	ase (CD) [colonic mucosa]	] NA 26115 H	HumanDisease	crohn's diseas	colonic mucosa	NA	Disease vs. No	ExperimentGro	GSE59071.GPL6.h	ttp://www.ncb	54.89 86.0	67.82	80.18	74.73		discosos and
	20- obesity [va:	tus lateralis muscle] NA	5260 H	HumanDisease	obesity	vastus laterali	NA	reatment1 vs	SubjectTreatm	GSE129843.GPL h	ttps://www.nc	54,89 81.2	4 3,48	79.06	4.67		diseases and
	TU- ANCA-asso	clated vasculitis (kidney	giomerulij IN F	HumanDisease	ANCA-associat	kidney glomer	NA	Disease I vs. D	DiseaseState	GSE104948.GPL h	ittps://www.nc	72.55 81.8	65 05.20 IS CO.05	70.06	74.15		functions
analysis	5- rapidly prog	essive giomeruionephriti	IS (RPGN) [KI F	HumanDisease	rapidly progres	kidney glomer	NA I	Disease I vs. D	Tissue Complin	GSE4/183.GPL1 h	ttp://www.ncb	2.55 01.0	6 69 56	79.0	74.18		
	11- ulcerative	olitis (UC) [signoid colonj	cal NA 2864: F	HumanDisease	ulcerative colit	colonic mucosa	NA	Disease vs. No.	DiceaseState	GSE73661 GPL6 H		58.82 84.9	65 57	76.76	74.00		
Link to Land Explorer to view volcano plot of comparison	47- crobo's dis	ease (CD) [rectum] NA 11	332 1	HumanDisease	crohn's diseas	rectum	NA	A Other Compari	Tissue/Samplin	GSE100833 GPL https://www.nc	54.89 84.8	5 72.11	73.19	73.76			
	14- ulcerative	olitis (UC) (nouch mucos	al NA 26872 H	2 HumanDisease	ulcerative colit	nouch mucosa	NA C	Other Comparia	Tissue:Clinical	GSE65270 GPL6 h	http://www.ncb 76.09	76.09 84.8	5 63.25	7171	73.72		CN <sup>.</sup> Causal
	17- obesity (va	tus lateralis musclel NA	5256 H	HumanDisease	obesity	vastus laterali	NA	reatment1 vs	. SubjectTreatm	GSE129843.GPL h	ttps://www.nc	72.55 81.2	4 67.82	73.1	73.0		Notworko
			252			1555900	NO.			GSE100833.GPL h	ttps://www.nc	72.55 81.2	4 64.81	75.59	73.55		INELWOIKS
	1- chronic idio	athic urticaria [skin] NA	25905 H	HumanDisease	chronic idiopat	skin	NA	Disease vs. No	SamplePatholo	GSE57178.GPL6.h	ttps://www.nc	72.55 82.4	6 58.31	80.18	73.37		
	3- inflammator	/ bowel disease (IBD) [col	lonic mucosa H	HumanDisease	inflammatory b	colonic mucosa	NA	Disease vs. No	DiseaseState	GSE4183.GPL57 h	http://www.ncb	54.89 84.2	6 70.00	73.19	73.09		
	2- ulcerative co	litis (UC) [colonic mucosa	a] NA 28644 H	HumanDisease	ulcerative colit	colonic mucosa	NA	reatment vs	TreatmentStat	GSE73661.GPL6 h	ttps://www.nc	-64.89 -81.	85 -68.56	-76.76	73.02		
	11- obesity [va	tus lateralis muscle] NA	5250 H	HumanDisease	obesity	vastus laterali	NA	reatment1 vs	. SubjectTreatm	GSE129843.GPL h	ttps://www.nc	-56.20 -78	10 -76.81	-81.28	-7.10		UR: Unstream
	565- normal co	ntrol (nucleus accumbens	s] NA 830 🛛 H	Human Tissues (GT	normal control	nucleus accum.	NA	Other Compari	. Gender:Tissue	GTEx.GPL16791. h	https://gtexpor	-68.82 -83	67 -65.57	-76.76	-73.7		Degulatora
	16- thyroid eye	disease [lacrimal gland]	NA 26057 H	HumanDisease	thyroid eye dis	lacrimal gland	NA	Disease1 vs. D	Tissue:Disease	GSE58331.GPL5 h	https://www.nc	-60.70 -83	07 -69.28	-84.52	-74.39		Regulators
	5- asthma [nas	al epithelium] NA 10078	H	HumanDisease	asthma	nasal epitheliu.	NA	Freatment1 vs	. DiseaseState:S	GSE97668.GPL1.h	ttps://www.nc	-68.82 -74	83 -67.08	-87.63	-74.59		
	41- crohn's dis	ease (CD) [colonic mucos	a] NA 16850 H	HumanDisease	crohn's diseas	colonic mucosa	NA	Responder vs	DiseaseState:S	GSE16879.GPL5 h	ttp://www.ncb	-60.70 -82	46 -71.41	-86.60	-75.29		
	8- ulcerative co	litis (UC) [colonic mucosa	a] NA 28650 H	HumanDisease	ulcerative colit	colonic mucosa	NA	Responder vs	Response:Trea	GSE73661.GPL6.h	https://www.nc	-68.82 -81	24 -72.80	-83.45	-76.58		CP <sup>.</sup>
	GSE73661-UC	VDZ dataset 2022 - 2022	-09-08 09:05 k	Kristin Practice								-68.82 -85	44 -76.81	-81.28	-78.09		Capaniaal
	1- ulcerative co	litis (UC) [colonic mucosa	a] NA 24767 H	HumanDisease	ulcerative colit	colonic mucosa	NA	Freatment vs	SubjectTreatm	GSE50594.GPL6 h	https://www.nc	-68.82 -87.	18 -74.16	-82.38	-78.14		Canonical
	Causal network	exercise GSE73661-UC	VDZ dataset k	Kristin Practice								-68.82 -85	44 -77.46	-81.28	-78.25		Pathways
	GSE73661-IBD	Vedolizumab-w-metadat	ta - 2022-12·E	Example Analyses	ulcerative colit	colonic mucosa						-82.72 -86	60 -76.16	-92.58	-84.51		

Matches your core analysis against public datasets in IPA as well as previously run core analyses and returns similar and dissimilar datasets based on 4 core analysis features: CP, UR, CN, DE

\*\*All columns are filterable\*\*

Positive z-score: Pink: matching

Negative z-score: Blue: antimatching



# IPA Help

## **IPA Help Resources**



