



IPA training

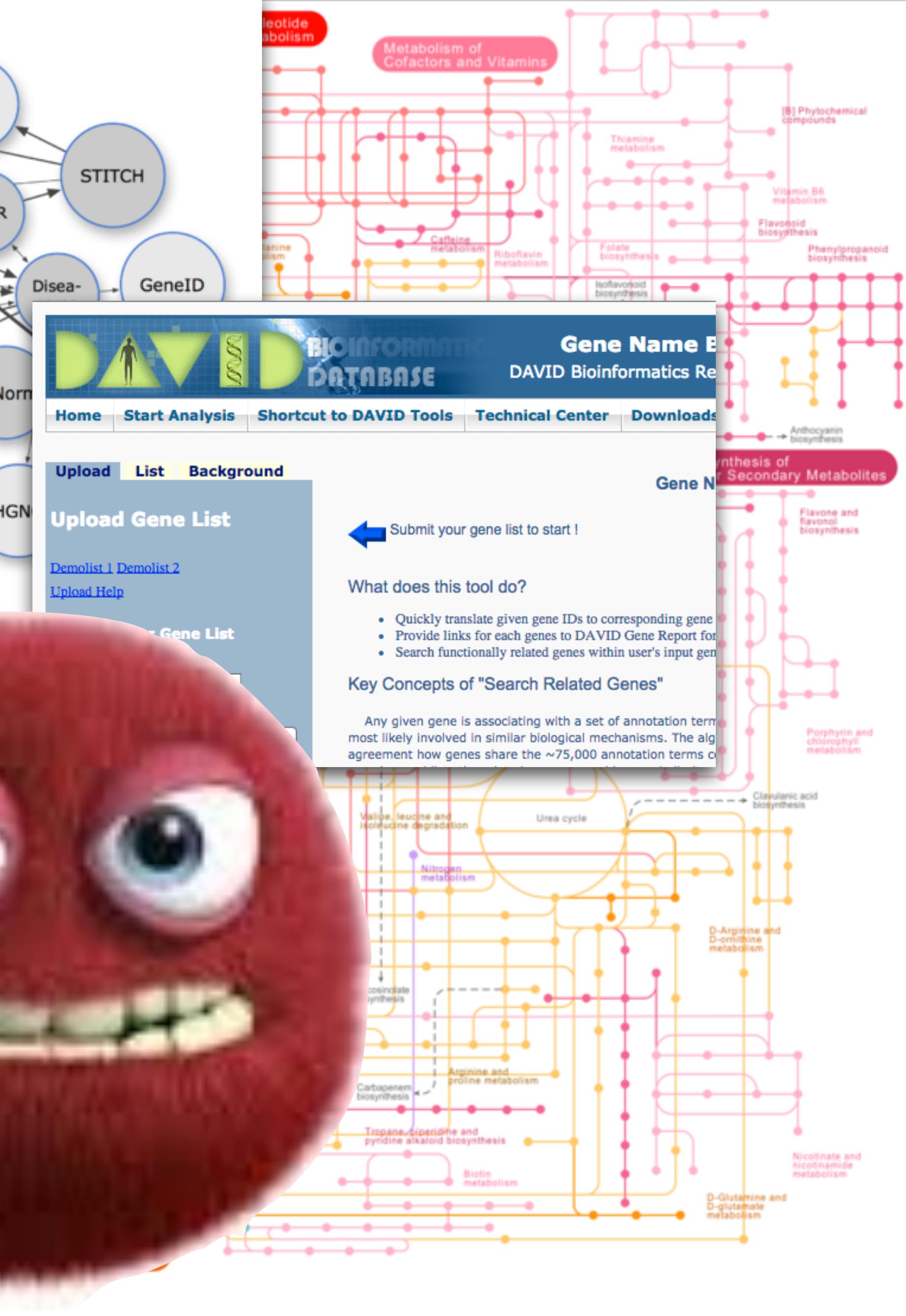
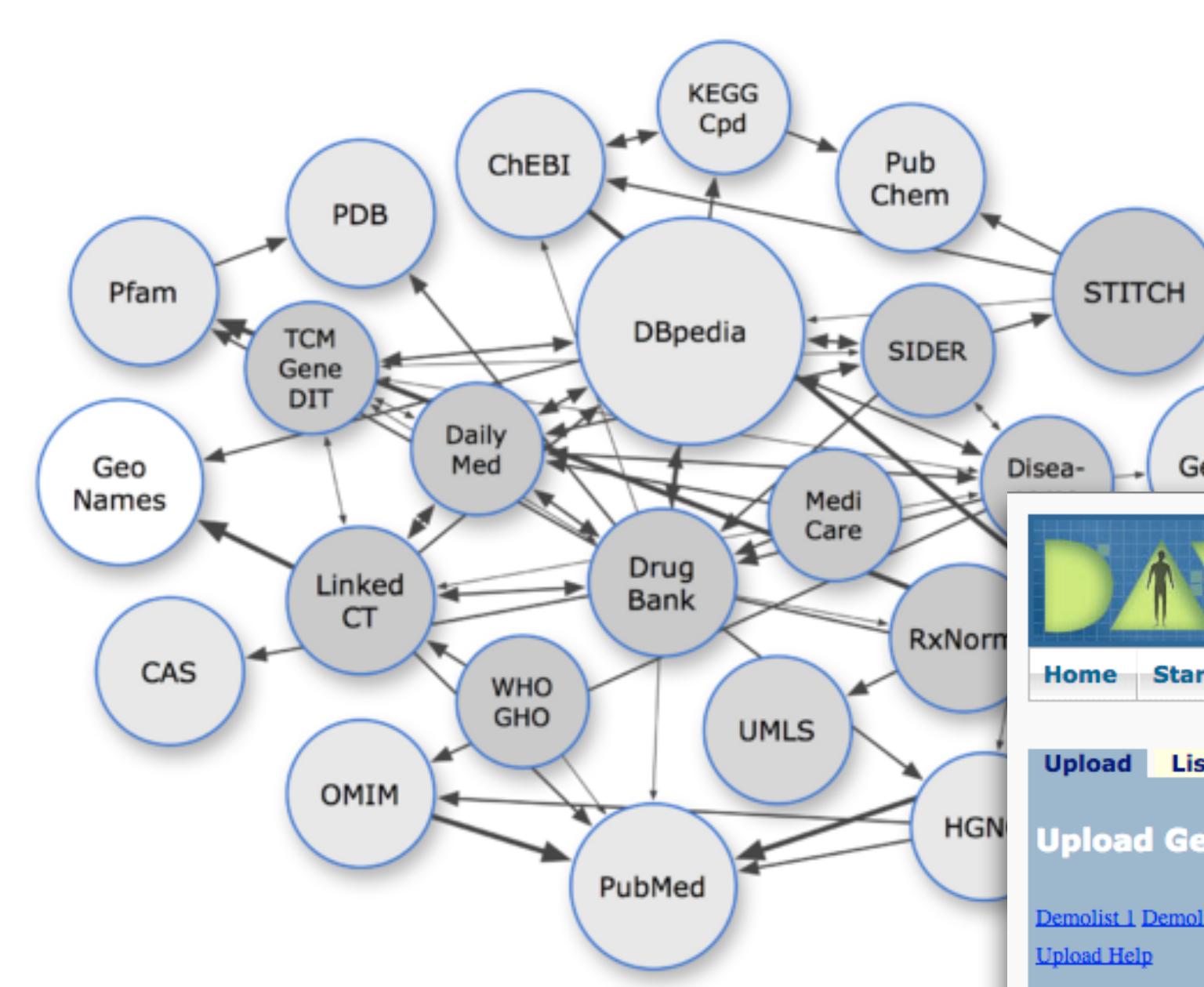
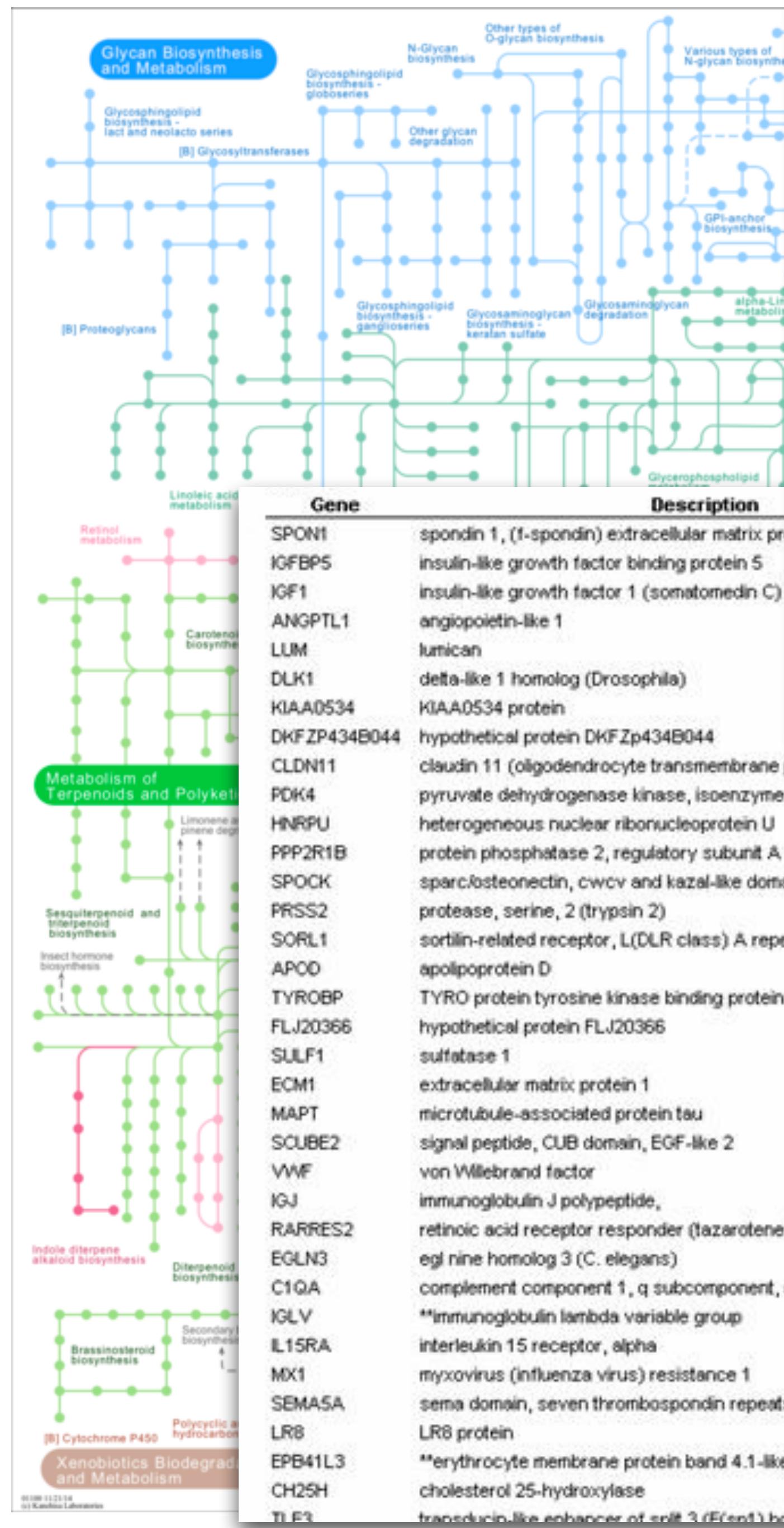
June 15/17, 2020

July 1/3, 2020

*For feedback or questions, please email **bits@vib.be***

Stephane Plaisance (stephane.plaisance@vib.be)





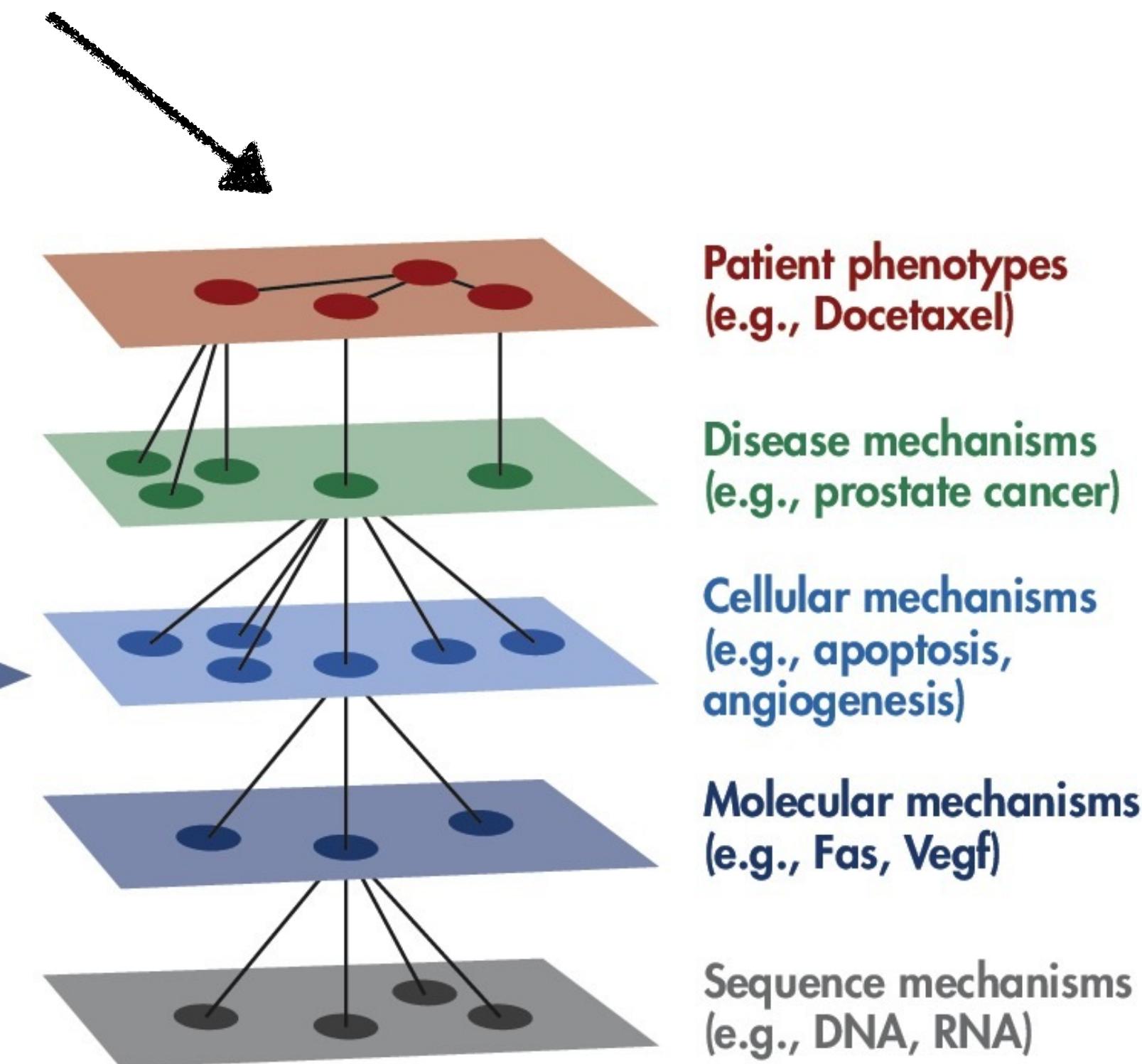
IPA knowledge is **curated** and **connected**

Ingenuity® Knowledge Base:



Content acquisition

different levels are linked (relational DB)



Ingenuity ontology

controlled vocabulary

Expert Findings ... manually curated ... from the full-text of articles ... extraction protocol ... Findings from ~300 top journals ... , including tables and figures ...

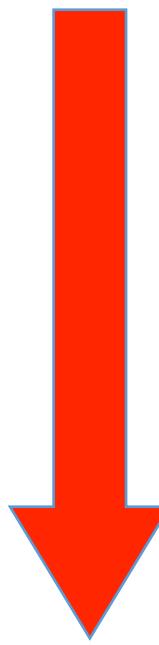
Supported Third Party Information is manually reviewed content from selected sources and databases. This includes findings and annotations from major **NCBI databases** (**EntrezGene, RefSeq, OMIM disease associations**), targets and pharmacological relevance of FDA approved and **clinical trial drugs, clinical biomarkers, Gene Ontology annotations**, a **normal gene expression body atlas for over 30 tissues and the NCI-60 panel of cancer cell lines, microRNA-mRNA target databases** and **GWAS databases** :



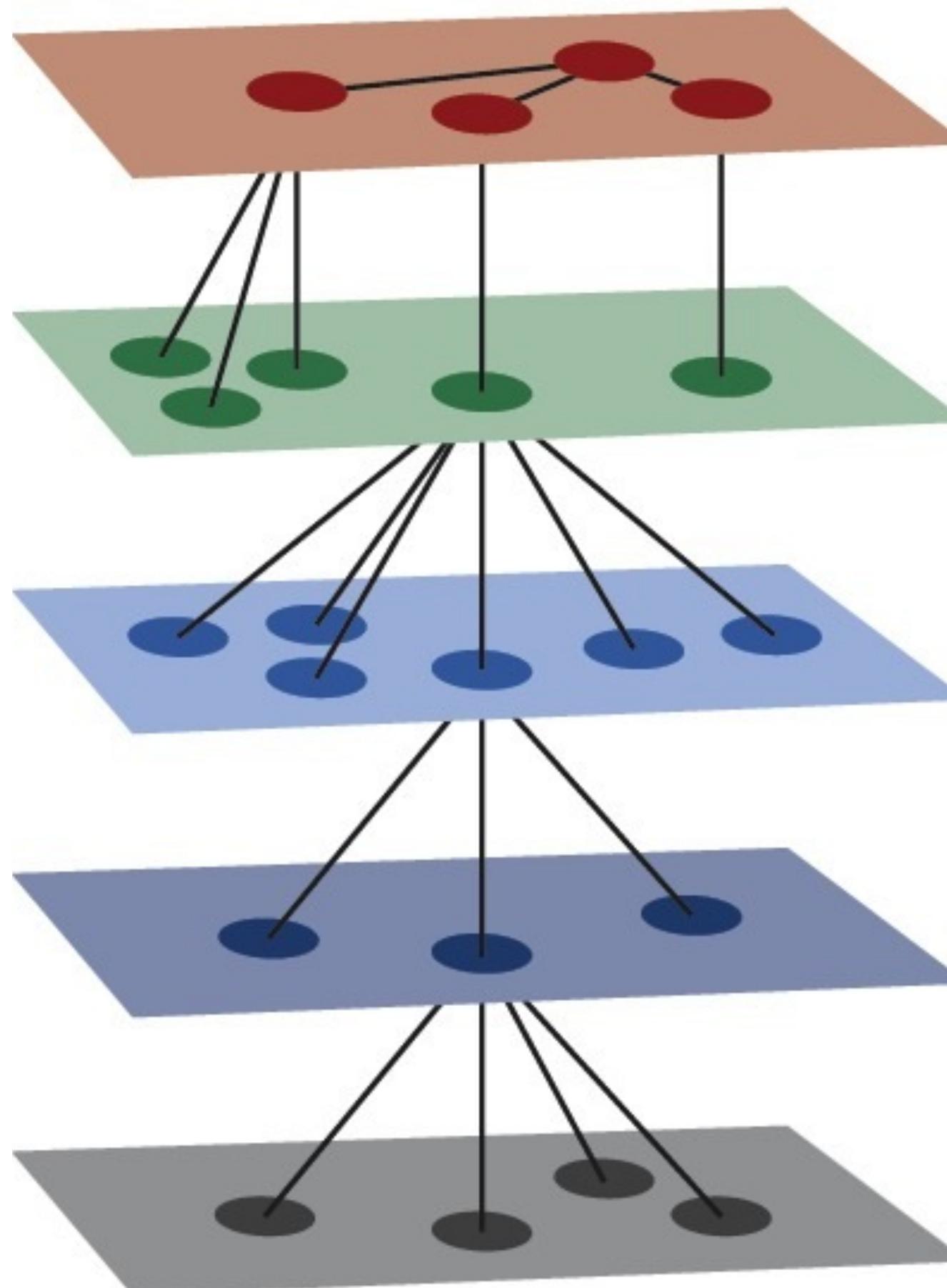
Entrez Gene
RefSeq
OMIM
ClinVar
COSMIC
GWAS Database
Gene Ontology
Human Metabolome Database (HMDB)
GNF Tissue Expression Body Atlas
NCI-60 Cell Line Expression Atlas
BIND, DIP, MINT, MIPS, BIOGRID, INTACT, COGNIA protein-protein interactions (updated)
TarBase
TargetScan
miRecords
Clinicaltrials.gov
Drugs@FDA.gov
Mosby's Drug Consult
Goodman & Gilman's 'Pharmacological Basis of Therapeutics'
DrugBank
Hazardous Substance Database (HSDB)
Chemical Carcinogenesis Research Information System database (CCRIS)

How can I use IPA ?

top-DOWN



KNOWLEDGE



DATA

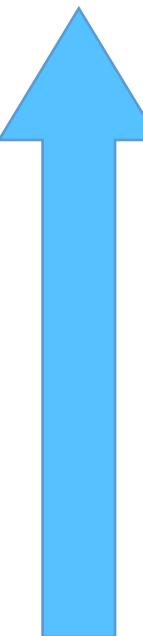
Patient phenotypes
(e.g., Docetaxel)

Disease mechanisms
(e.g., prostate cancer)

Cellular mechanisms
(e.g., apoptosis,
angiogenesis)

Molecular mechanisms
(e.g., Fas, Vegf)

Sequence mechanisms
(e.g., DNA, RNA)



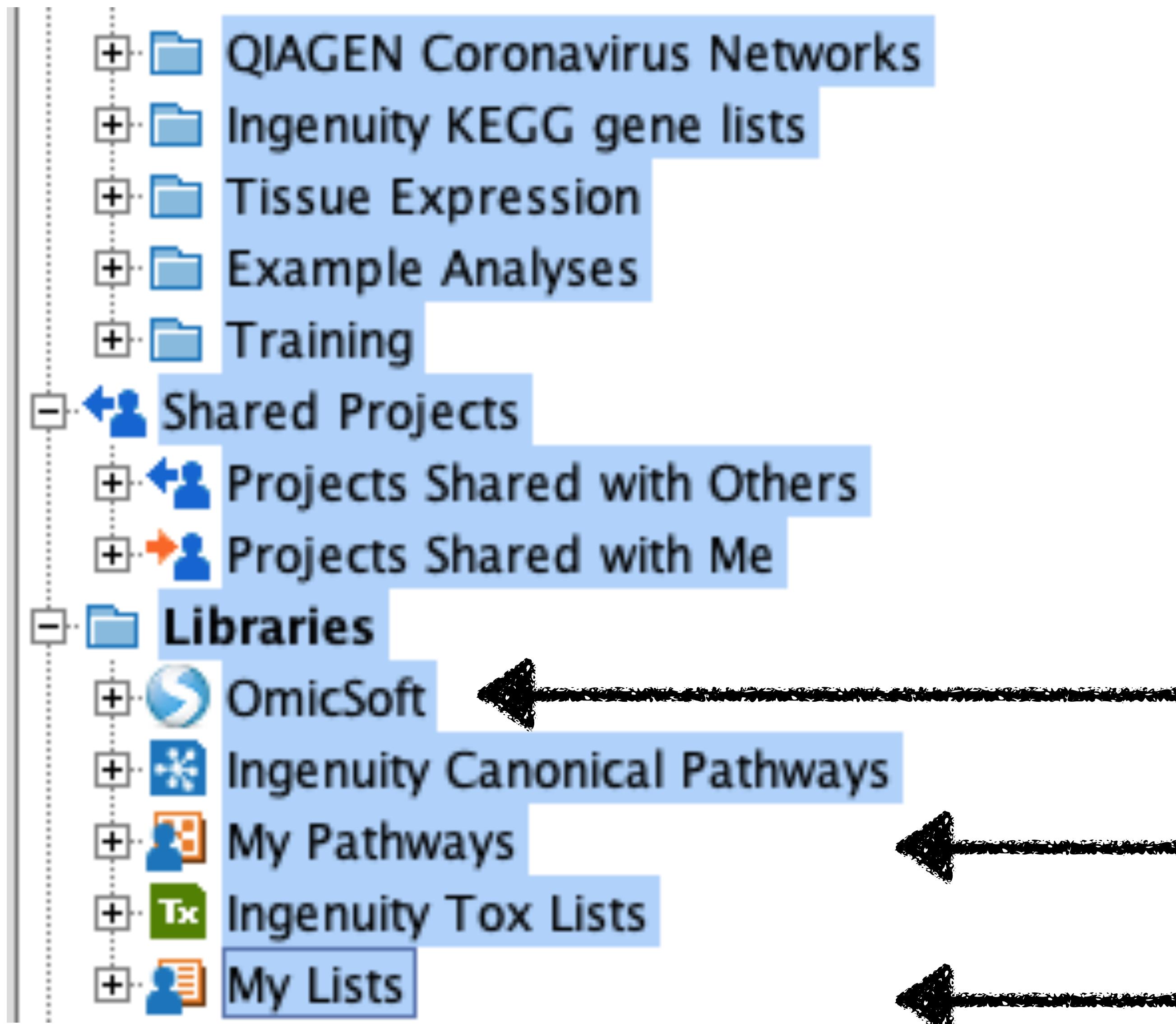
bottom-UP
GSE52778



DATA

Valid Expression Value Type	Expected Values
Ratio	(0, +INF)
Fold Change	(-INF, -1) (1, +INF)
Log Ratio	(-INF, +INF)
P-Value	(0, 1)
False Discovery Rate, q-value	(0, 100)
Intensity	(0, +INF)
RPKM/FPKM	(0, +INF)
Other (normalized around zero)	(-INF, +INF)

Data added to each account



> 57'000 Omicsoft datasets !

All YOUR PW's and Lists

IPA is all about Gene Lists

IPA gene list enrichment

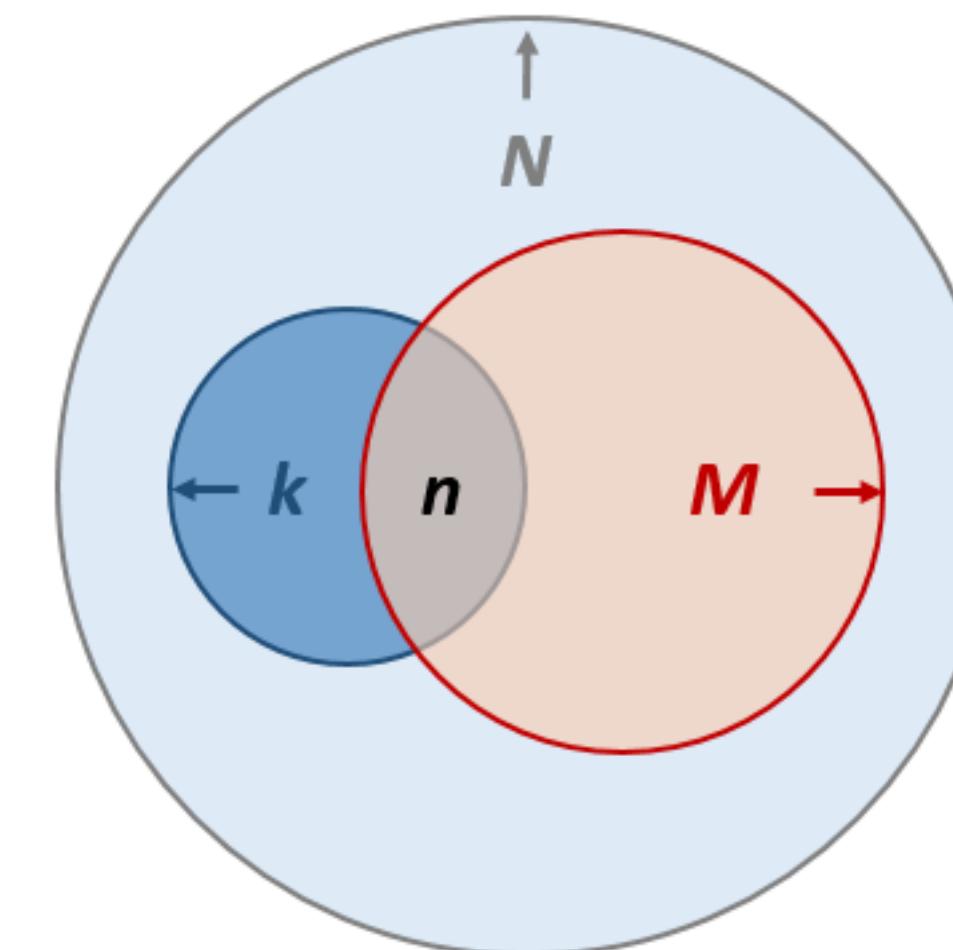
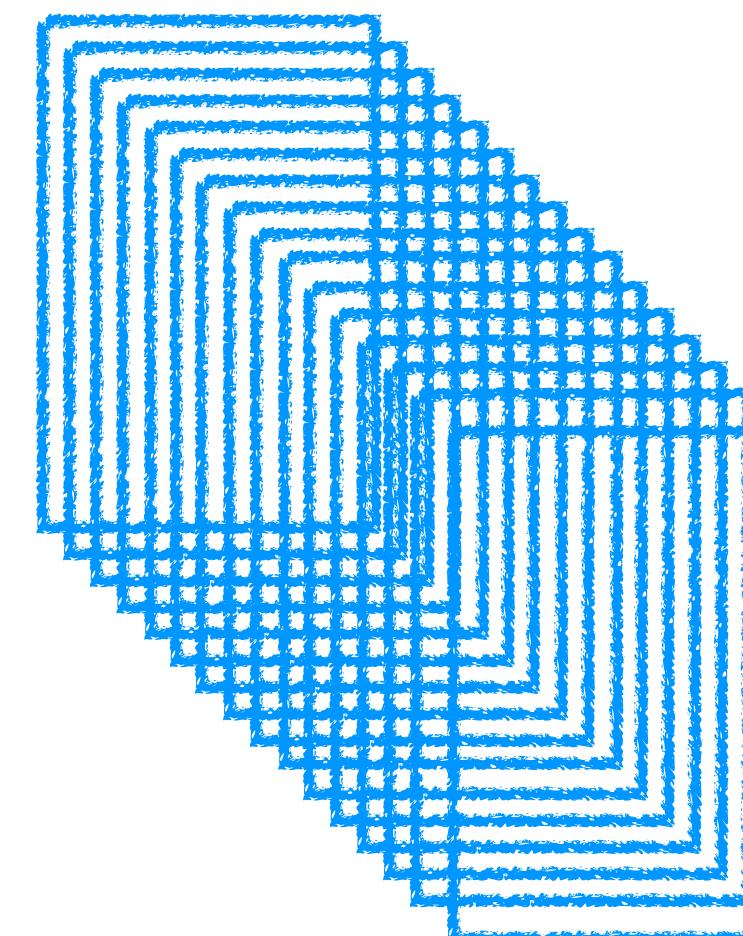


Dataset

UR genes
DR genes

User list

X



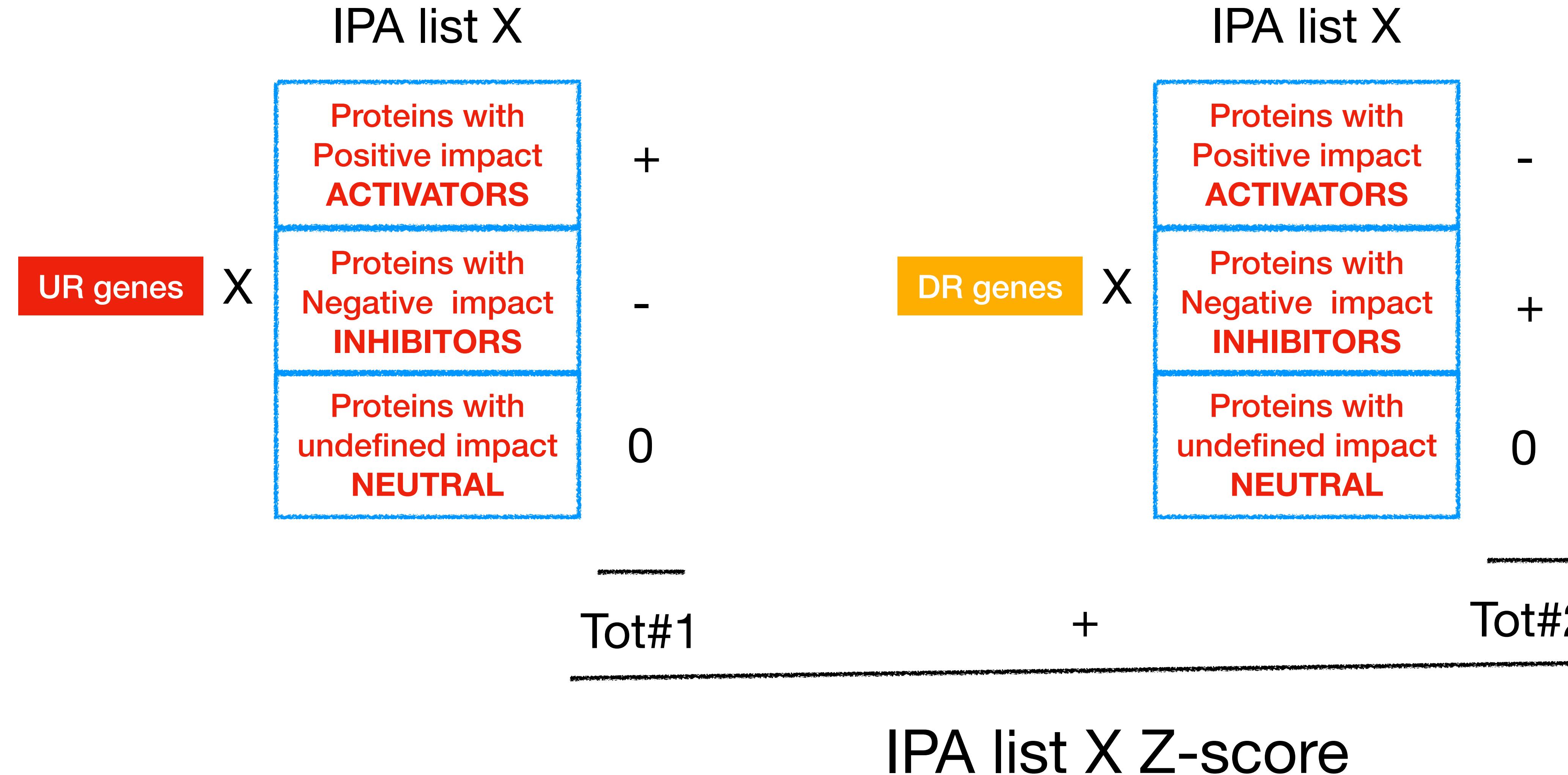
$$p = \sum_{i=n}^{\min(M, K)} \binom{i}{n} \binom{N-k}{M-i}.$$

Title	Enrichment pValue
1	1x10^-10
2	1x10^-9
3	1x10^-8
...	...
N	1

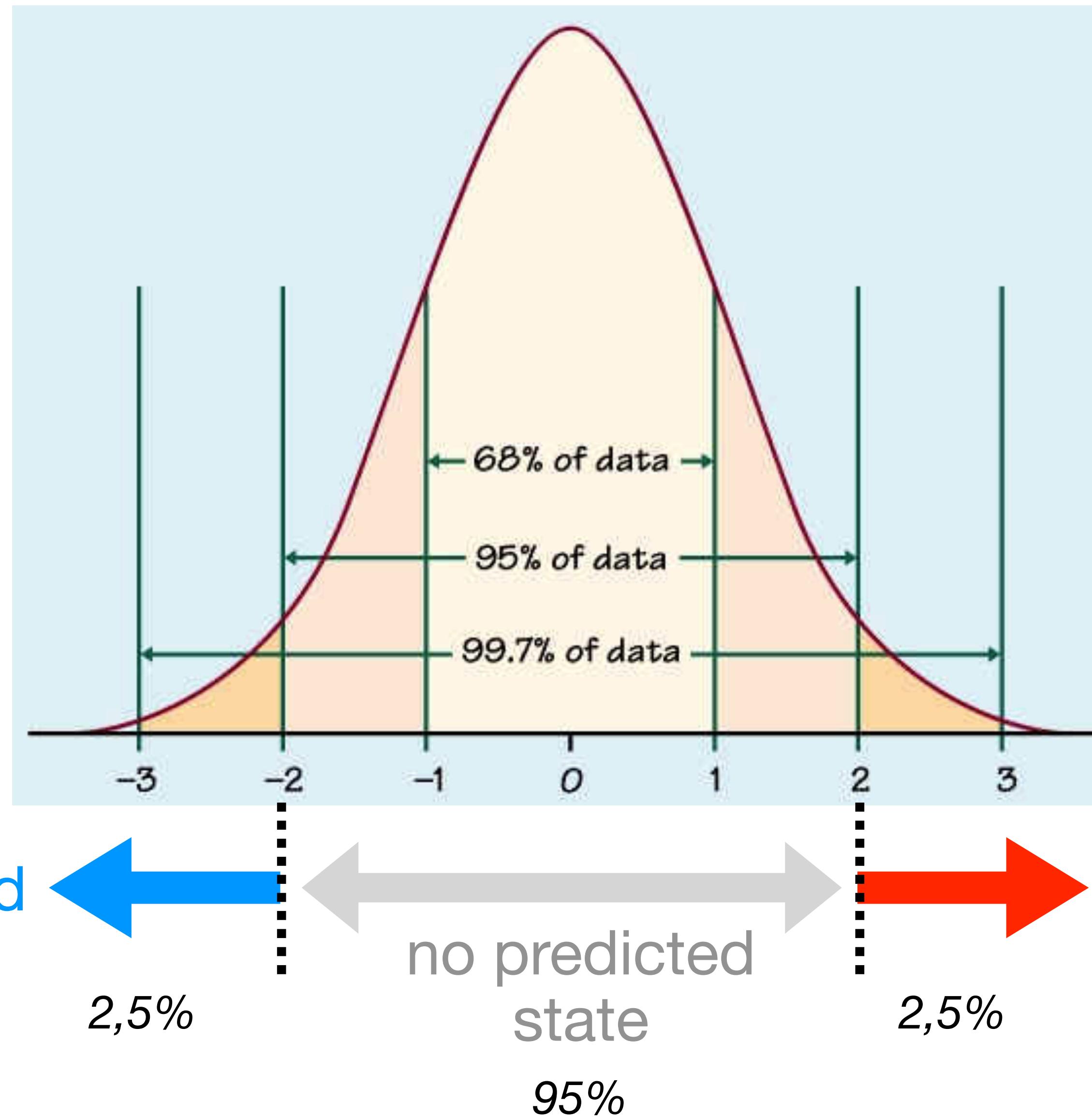
There are N total number of genes in our study pool (this is also known as the “background” gene list, defaults to all genes in the IPA KB). A given pathway of interest consists of k gene members. Our input gene list consists of M genes, among which n are found to fall into the same given pathway.

<http://tv.qiagenbioinformatics.com/video/19605716/understanding-the-p-value-of>

IPA Z-scores and effect predictions

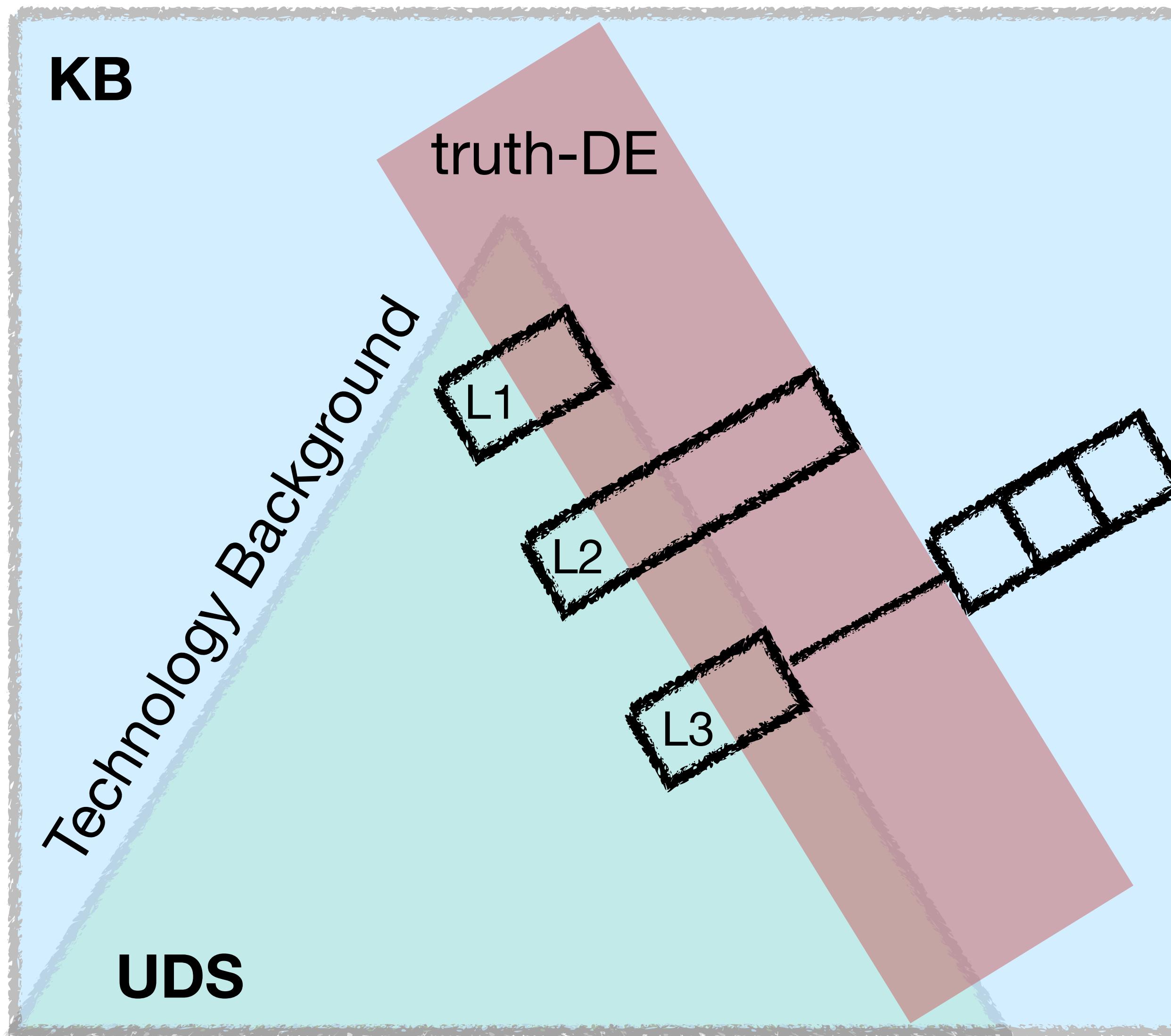


IPA Z-scores for all tested lists



Background effect

Genome Background



DE%

	UDS	KB	Effect
L1	50%	50%	"="
L2	50%	75%	<i>underestimate</i>
L3	50%	20%	<i>overestimate</i>

Top Down

NEW

Genes and Chemicals

Diseases and Functions

Pathways and Tox Lists

Core Analysis...

Comparison Analysis...

Biomarker Filter...

Biomarker Comparison Analysis...

microRNA Target Filter...

BioProfiler

IsoProfiler

My Pathway

Path Designer

Filter Dataset

Upload Dataset...

Advanced Search

Project...

Compare

Import Pathway



Enter gene names/symbols/IDs or chemical/drug names here

SEARCH

Advanced Search



Hide Tool Tips

Show Members/Membership

Edit Custom Molecule

Full Screen View

Zoom Selected

Magnifying Lens

Delete

Cut

Copy

Paste

Print

Send By E-Mail

Select All

Select Opaque Area

Select Highlighted

Select Nearest Neighbor(s)

Highlight Selected

Unhighlight Selected

Reset Highlight

Invert Selected

Invert Highlight

View References

BUILD

Grow

Path Explorer

Connect

Trim

Keep

Add Molecule/Relationship

OVERLAY

Analyses, Datasets & Lists

MAP (Molecule Activity Predictor)

Drug

Disease & Function

My List

Canonical Pathway

My Pathway

Ingenuity Tox List

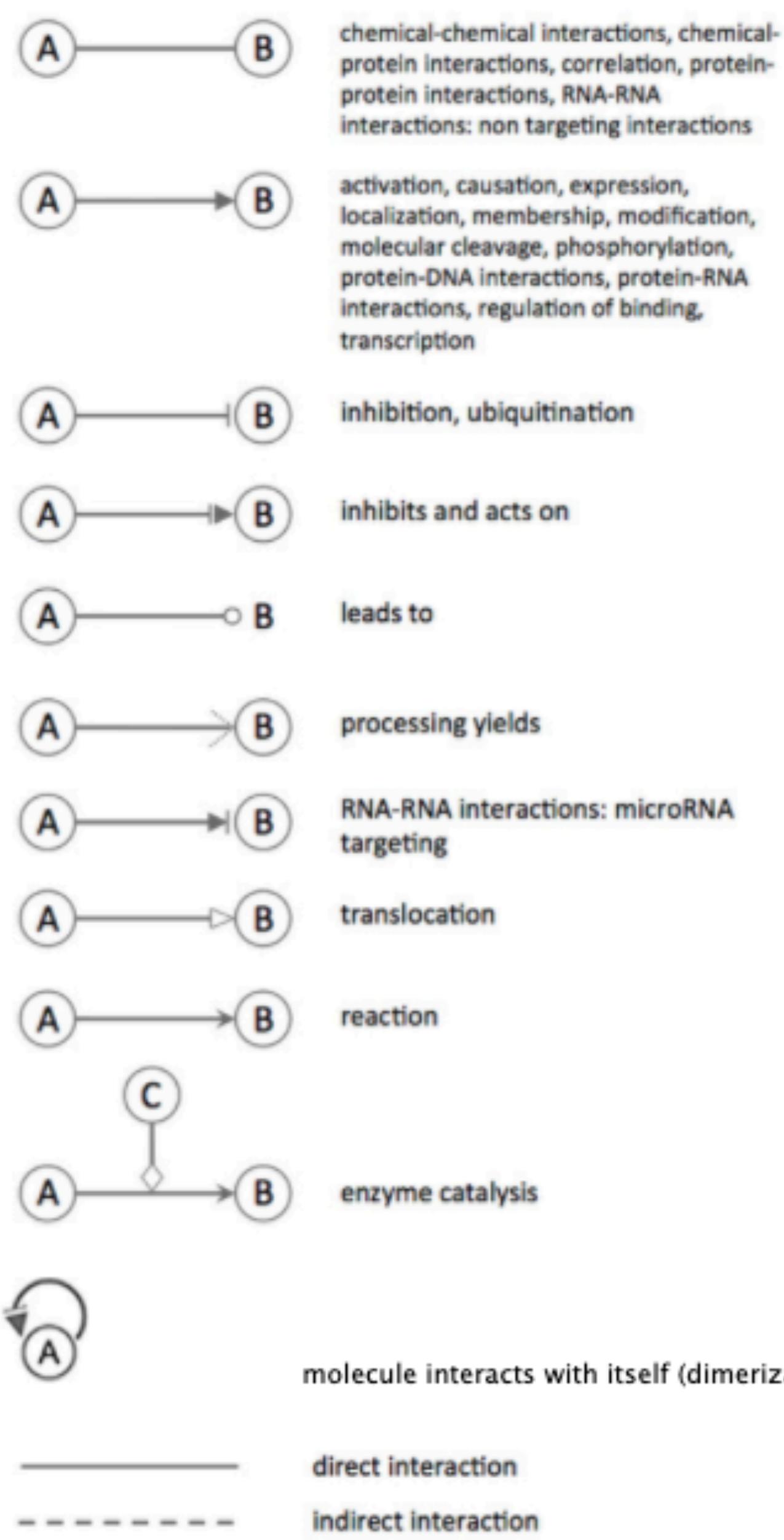
Biomarkers

Highlight or Select

shapes = nodes

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

Relations = edges



Annotations = weight

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

Bottom UP

Status	Public on Jan 01, 2014
Title	Human Airway Smooth Muscle Transcriptome Changes in Response to Asthma Medications
Organism	Homo sapiens
Experiment type	Expression profiling by high throughput sequencing
Summary	<p>Rationale: Asthma is a chronic inflammatory airway disease. The most common medications used for its treatment are β2-agonists and glucocorticosteroids, and one of the primary tissues that these drugs target in the treatment of asthma is the airway smooth muscle. We used RNA-Seq to characterize the human airway smooth muscle (HASM) transcriptome at baseline and under three asthma treatment conditions.</p> <p>Methods: The Illumina TruSeq assay was used to prepare 75bp paired-end libraries for HASM cells from four white male donors under four treatment conditions: 1) no treatment; 2) treatment with a β2-agonist (i.e. Albuterol, 1μM for 18h); 3) treatment with a glucocorticosteroid (i.e. Dexamethasone (Dex), 1μM for 18h); 4) simultaneous treatment with a β2-agonist and glucocorticoid, and the libraries were sequenced with an Illumina Hi-Seq 2000 instrument. The Tuxedo Suite Tools were used to align reads to the hg19 reference genome, assemble transcripts, and perform differential expression analysis using the protocol described in https://github.com/blancahimes/taffeta</p>
Overall design	mRNA profiles obtained via RNA-Seq for four primary human airway smooth muscle cell lines that were treated with dexamethasone, albuterol, dexamethasone+albuterol or were left untreated.
Contributor(s)	Himes B, Lu Q
Citation(s)	Himes BE, Jiang X, Wagner P, Hu R et al. RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates cytokine function in airway smooth muscle cells. <i>PLoS One</i> 2014;9(6):e99625. PMID: 24926665

[https://www.ncbi.nlm.nih.gov/gds/?term=GSE52778\[Accession\]](https://www.ncbi.nlm.nih.gov/gds/?term=GSE52778[Accession])

OPEN ACCESS Freely available online

PLOS ONE



RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells

Blanca E. Himes^{1,2,3*}, Xiaofeng Jiang^{4*}, Peter Wagner⁴, Ruoxi Hu⁴, Qiyu Wang⁴, Barbara Klanderman², Reid M. Whitaker¹, Qingling Duan¹, Jessica Lasky-Su¹, Christina Nikolos⁵, William Jester⁵, Martin Johnson⁵, Reynold A. Panettieri Jr.⁵, Kelan G. Tantisira¹, Scott T. Weiss^{1,2}, Quan Lu^{4*}

¹ Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, ² Partners HealthCare Personalized Medicine, Boston, Massachusetts, United States of America, ³ Children's Hospital Informatics Program, Boston, Massachusetts, United States of America, ⁴ Program in Molecular and Integrative Physiological Sciences, Departments of Environmental Health, and Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts, United States of America, ⁵Pulmonary, Allergy and Critical Care Division, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

Abstract

Asthma is a chronic inflammatory respiratory disease that affects over 300 million people worldwide. Glucocorticoids are a mainstay therapy for asthma because they exert anti-inflammatory effects in multiple lung tissues, including the airway smooth muscle (ASM). However, the mechanism by which glucocorticoids suppress inflammation in ASM remains poorly understood. Using RNA-Seq, a high-throughput sequencing method, we characterized transcriptomic changes in four primary human ASM cell lines that were treated with dexamethasone—a potent synthetic glucocorticoid (1 μ M for 18 hours). Based on a Benjamin-Hochberg corrected p-value <0.05, we identified 316 differentially expressed genes, including both well known (*DUSP1*, *KLF15*, *PER1*, *TSC2D3*) and less investigated (*C7*, *CCDC69*, *CRISPLD2*) glucocorticoid-responsive genes. *CRISPLD2*, which encodes a secreted protein previously implicated in lung development and endotoxin regulation, was found to have SNPs that were moderately associated with inhaled corticosteroid resistance and bronchodilator response among asthma patients in two previously conducted genome-wide association studies. Quantitative RT-PCR and Western blotting showed that dexamethasone treatment significantly increased *CRISPLD2* mRNA and protein expression in ASM cells. *CRISPLD2* expression was also induced by the inflammatory cytokine IL1 β , and small interfering RNA-mediated knockdown of *CRISPLD2* further increased IL1 β -induced expression of *IL6* and *IL8*. Our findings offer a comprehensive view of the effect of a glucocorticoid on the ASM transcriptome and identify *CRISPLD2* as an asthma pharmacogenetics candidate gene that regulates anti-inflammatory effects of glucocorticoids in the ASM.

Citation: Himes BE, Jiang X, Wagner P, Hu R, Wang Q, et al. (2014) RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells. *PLoS ONE* 9(6): e99625. doi:10.1371/journal.pone.0099625

Editor: Jan Peter Tuckermann, University of Ulm, Germany

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Funding: Funding was provided by National Institutes of Health (NIH) U01 HL65899, an NIH Pharmacogenomics Research Network (PGRN) – RIKEN Center for Genomic Medicine (CGM) Global Alliance, R01 HL097796, R01 HL114769, and P30 ES013508. BEH was funded by NIH K99 HL105663. XI was supported by NIH training grant T32 HL007118. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors declare that no competing interests exist.

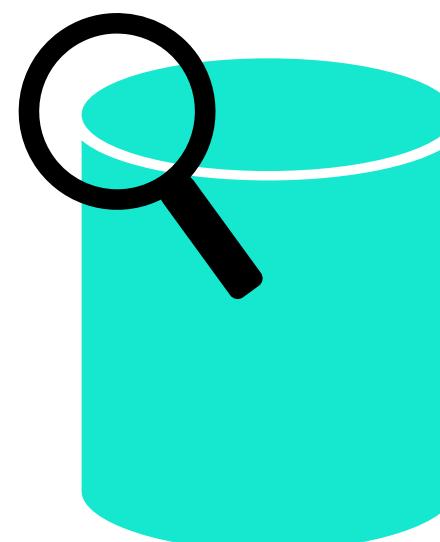
* Email: blanca.himes@channing.harvard.edu (BH); qiu@hsph.harvard.edu (QJ)

These authors contributed equally to this work.

Introduction

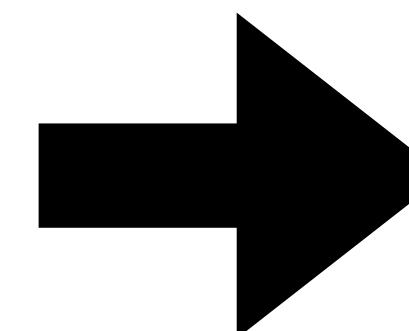
Asthma, a chronic inflammatory respiratory disease that affects over 25 million Americans and 300 million people world-wide, is characterized by variable airflow limitation and airway hyperresponsiveness [1,2]. Glucocorticoids (GCs) are common medications used to treat various inflammatory diseases, including asthma [3]. Inhaled corticosteroids, GC medications that act directly in the lung, are among the most common asthma controller medications and treatment of asthma patients with them leads to improved clinical outcomes, including decreased asthma symptoms and exacerbations [4]. At a cellular level, GCs act by binding to GC receptors (GRs), causing them to translocate to cell nuclei where they modulate transcription of various genes in a tissue-dependent fashion [5]. The anti-inflammatory action of GCs is partly a result of 1) GC-GR complexes stimulating anti-inflammatory genes by directly binding to DNA at glucocorticoid receptor enhancer elements, and of 2) GC-GR complexes inhibiting proinflammatory transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) [6]. In addition to directly reducing inflammation, GCs have been shown to affect other asthma-related phenotypes, including bronchodilation [7], airway hyperresponsiveness [8], and airway smooth muscle (ASM) contractility [9].

Many cells and tissues are involved in asthma and are targeted by GCs, including inflammatory [10,11], airway epithelium [12], and ASM [13]. Of these, the ASM is involved in altered airway contractility [14], a major asthma-specific trait that is assessed clinically and for research studies by measures such as bronchodilator response [15] and airway hyperresponsiveness [16].



Raw mapping counts

DESeq2 pipeline



DEX

Cont

	ENSGID	baseMean	log2FoldChange	IfcSE	stat	pvalue	padj
1	ENSG00000165995	514.28409	3.3216624	0.13073664	25.407280	2.095444e-142	3.170407e-138
2	ENSG00000152583	985.55928	4.3408121	0.17608576	24.651693	3.529480e-134	2.670051e-130
3	ENSG00000120129	3325.40270	2.8731495	0.11677344	24.604478	1.131211e-133	5.705074e-130
4	ENSG00000101347	13616.93476	3.6065585	0.15175505	23.765657	7.569343e-125	2.863104e-121
5	ENSG00000189221	2294.73000	3.2319825	0.13961723	23.148881	1.491976e-118	4.514719e-115
6	ENSG00000211445	12162.48685	3.5406811	0.15734396	22.502809	3.895921e-112	9.824214e-109
7	ENSG00000162614	5410.96706	1.9889528	0.09230661	21.547241	5.619692e-103	1.214656e-99
8	ENSG00000157214	2925.97934	1.9403761	0.09059393	21.418390	9.004693e-102	1.703013e-98
9	ENSG00000154734	29962.15322	2.2726505	0.11453294	19.842767	1.272588e-87	2.139362e-84
10	ENSG00000179094	763.98622	3.0843241	0.15766714	19.562250	3.244382e-85	4.908750e-82
11	ENSG00000125148	1882.40635	2.0790623	0.10639385	19.541188	4.902803e-85	6.743582e-82
12	ENSG00000163884	545.77230	4.0811465	0.21032420	19.404075	7.129014e-84	8.988498e-81
13	ENSG00000134243	5497.56910	2.1329937	0.11058860	19.287645	6.821066e-83	7.938671e-80
14	ENSG00000139132	1205.02704	2.1776906	0.11309502	19.255406	1.271748e-82	1.374396e-79
15	ENSG00000162493	1082.40622	1.8435294	0.09583897	19.235696	1.860277e-82	1.876399e-79
16	ENSG00000178695	2674.93415	-2.4593228	0.13041743	-18.857317	2.558795e-79	2.419660e-76
17	ENSG00000162692	502.78754	-3.4729419	0.18631326	-18.640336	1.512866e-77	1.346451e-74
18	ENSG00000146250	315.44553	-2.6514559	0.14556181	-18.215326	3.900843e-74	3.278875e-71
19	ENSG00000148848	1348.87125	-1.8136130	0.10181953	-17.812034	5.699996e-71	4.538997e-68
20	ENSG00000198624	2022.68664	2.7847634	0.15667199	17.774481	1.114232e-70	8.429166e-68
21	ENSG00000124766	1319.42200	-2.3315246	0.13494507	-17.277582	6.939659e-67	4.999859e-64

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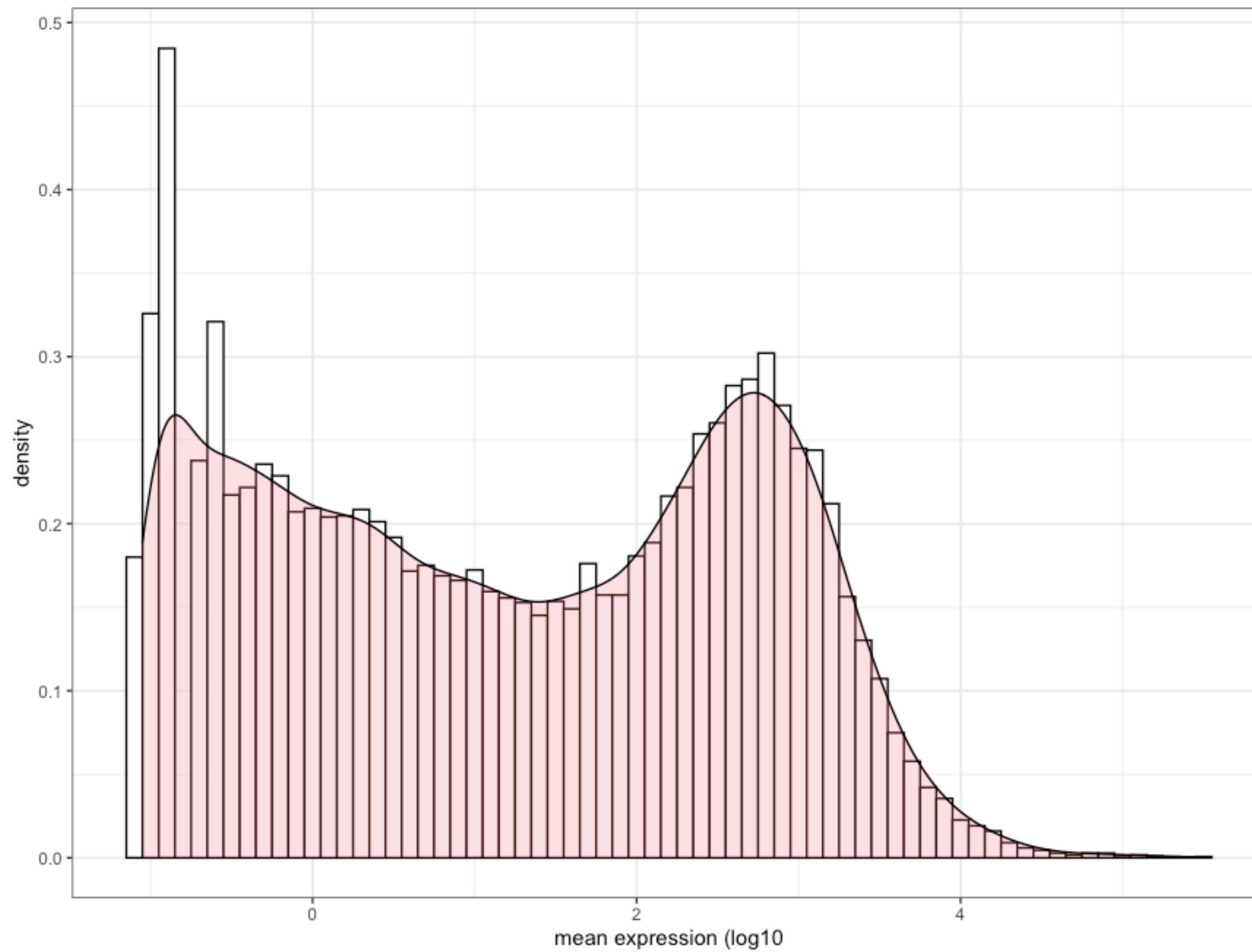


Filter

	ENSGID	baseMean	log2FoldChange	IfcSE	stat	pvalue	padj
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2	ENSG00000152583	985.55928	4.3408121	0.17608576	24.651693	3.529480e-134	2.670051e-130
3	ENSG00000120129	3325.40270	2.8731495	0.11677344	24.604478	1.131211e-133	5.705074e-130
4	ENSG00000101347	13616.93476	3.6065585	0.15175505	23.765657	7.569343e-125	2.863104e-121
5	ENSG00000189221	2294.73000	3.2319825	0.13961723	23.148881	1.491976e-118	4.514719e-115
6	ENSG00000211445	12162.48685	3.5406811	0.15734396	22.502809	3.895921e-112	9.824214e-109
7	ENSG00000162614	5410.96706	1.9889528	0.09230661	21.547241	5.619692e-103	1.214656e-99
8	ENSG00000157214	2925.97934	1.9403761	0.09059393	21.418390	9.004693e-102	1.703013e-98
9	ENSG00000154734	29962.15322	2.2726505	0.11453294	19.842767	1.272588e-87	2.139362e-84
10	ENSG00000179094	763.98622	3.0843241	0.15766714	19.562250	3.244382e-85	4.908750e-82
11	ENSG00000125148	1882.40635	2.0790623	0.10639385	19.541188	4.902803e-85	6.743582e-82
12	ENSG00000163884	545.77230	4.0811465	0.21032420	19.404075	7.129014e-84	8.988498e-81
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17	ENSG00000162692	502.78754	-3.4729419	0.18631326	-18.640336	1.512866e-77	1.346451e-74
18	ENSG00000146250	315.44553	-2.6514559	0.14556181	-18.215326	3.900843e-74	3.278875e-71
19	ENSG00000148848	1348.87125	-1.8136130	0.10181953	-17.812034	5.699996e-71	4.538997e-68
20	ENSG00000198624	2022.68664	2.7847634	0.15667199	17.774481	1.114232e-70	8.429166e-68
21	ENSG00000124766	1319.42200	-2.3315246	0.13494507	-17.277582	6.939659e-67	4.999859e-64

quick exploration of our dataset

Distribution of expression levels 'across all samples'



Low ← expression level → High

Differential expression Dex / Cont



← Down → Up

Advanced Analytics

Expression MATCH

Given your wet-lab expression profile

- Find other expression data with similar DE genes
- Discover what topic they cover
- Select interesting data and look for shared functions

Results

- Table of related profiles
- Heatmap showing shared functions
- Comparison analysis gateway

+ ***OmicSoft LandExplorer*** license open until end of this year

[follow the introductory webinar HERE](#)

BioProfiler

Given a list of candidate genes

- Filter based on multiple queries
- Identify subset relevant for your needs
- Use the resulting list in IPA

Results

- Focussed list of candidates

IPA

<https://www.bits.vib.be/software-overview/ingenuity-pathways-analysis>

bits.vib.be/software-overview/ingenuity-pathways-analysis

Extensions WA Home 365 SMRT ONT R710 NC VIB BITS Covid-19 perso myPages Git Biocomputing LOCALHOST utils

VIB Bioinformatics Core

Search

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Ingenuity Pathways Analysis

 With Ingenuity Pathways Analysis you can summarize a variety of biological information around your gene of interest. Create visually appealing networks of your pathway or your gene lists and start discovering biology! Which biological processes does your gene list represent? IPA can tell you immediately, based on their curated information source extracted from the literature.
We have several free IPA licenses for use by VIB scientists.

Get it!

How to access IPA? (VIB scientists only)

IPA news

Read the latest release notes ([here](#)).
Read the archived release notes ([here](#)).

Online Tutorials

Recorded IPA webinars and tutorial videos (in particular the 3-part series covering most of IPA)

Software Support

Overview

- > CLC Main Workbench
- > Tibco Spotfire
- > CLC Genomics Workbench
- > ELN
- > Genevestigator
- > GraphPad Prism
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