IPA robustness analysis *v1.2 (2020-07-07)*

June 15/17, 2020
July 1/3, 2020

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

Stephane Plaisance (stephane.plaisance@vib.be)

Himes et al 2014
GSE52778

Series GSE52778 | Query DataSets for GSE52778

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	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. 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
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. *PLoS One* 2014;9(6):e99625. PMID: 24926665

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

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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 Wagner[4], Ruoxi Hu[4], Qiyu Wang[4], Barbara Klanderman[2], Reid M. Whitaker[1], Qingling Duan[1], Jessica Lasky-Su[1], Christina Nikolos[5], William Jester[5], Martin Johnson[5], Reynold A. Panettieri Jr.[5], Kelan G. Tantisira[1], 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, 5 Pulmonary, 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 μM for 18 hours). Based on a Benjamini-Hochberg corrected p-value <0.05, we identified 316 differentially expressed genes, including both well known (*DUSP1*, *KLF15*, *PER1*, *TSC22D3*) 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 IL1β, and small interfering RNA-mediated knockdown of *CRISPLD2* further increased IL1β-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

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

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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 (NFκB) [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

Filter

	ENSGID	baseMean	log2FoldChange	lfcSE	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

IPA®

Filter

	ENSGID	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
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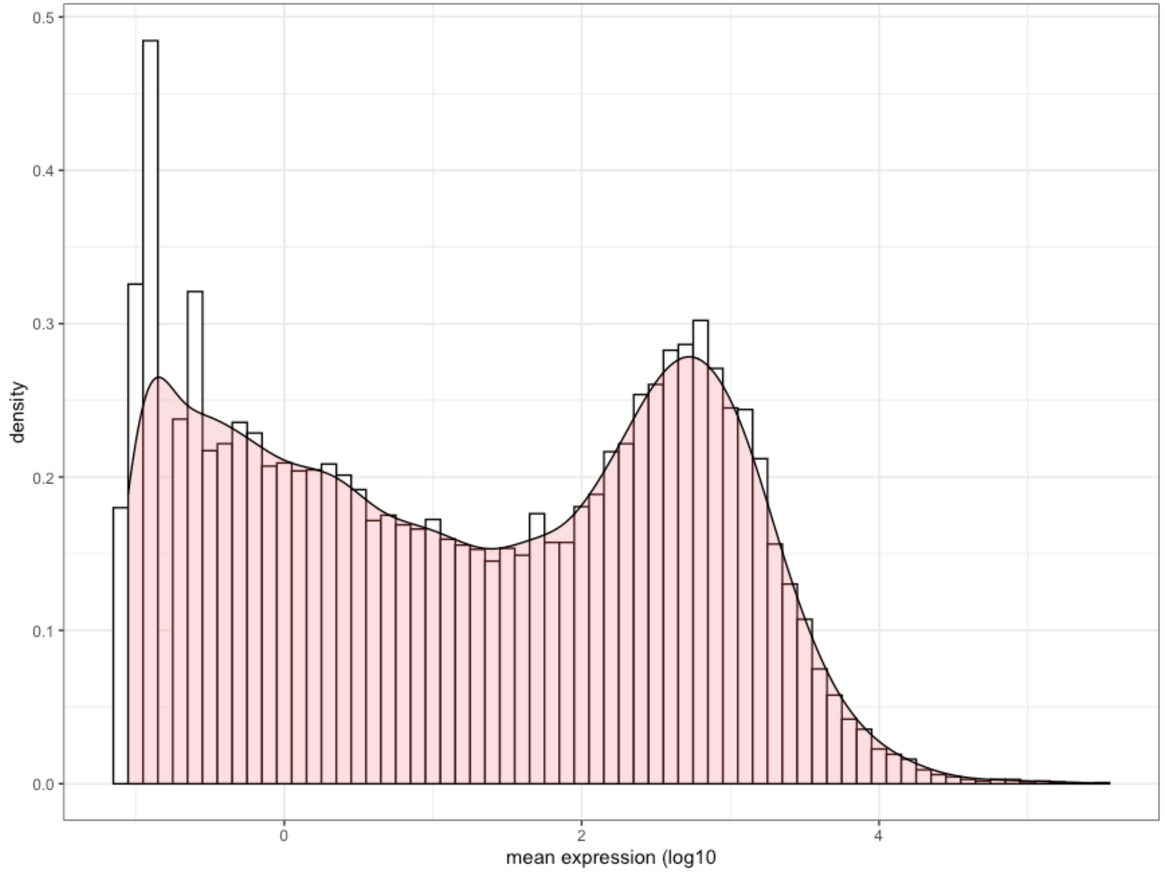
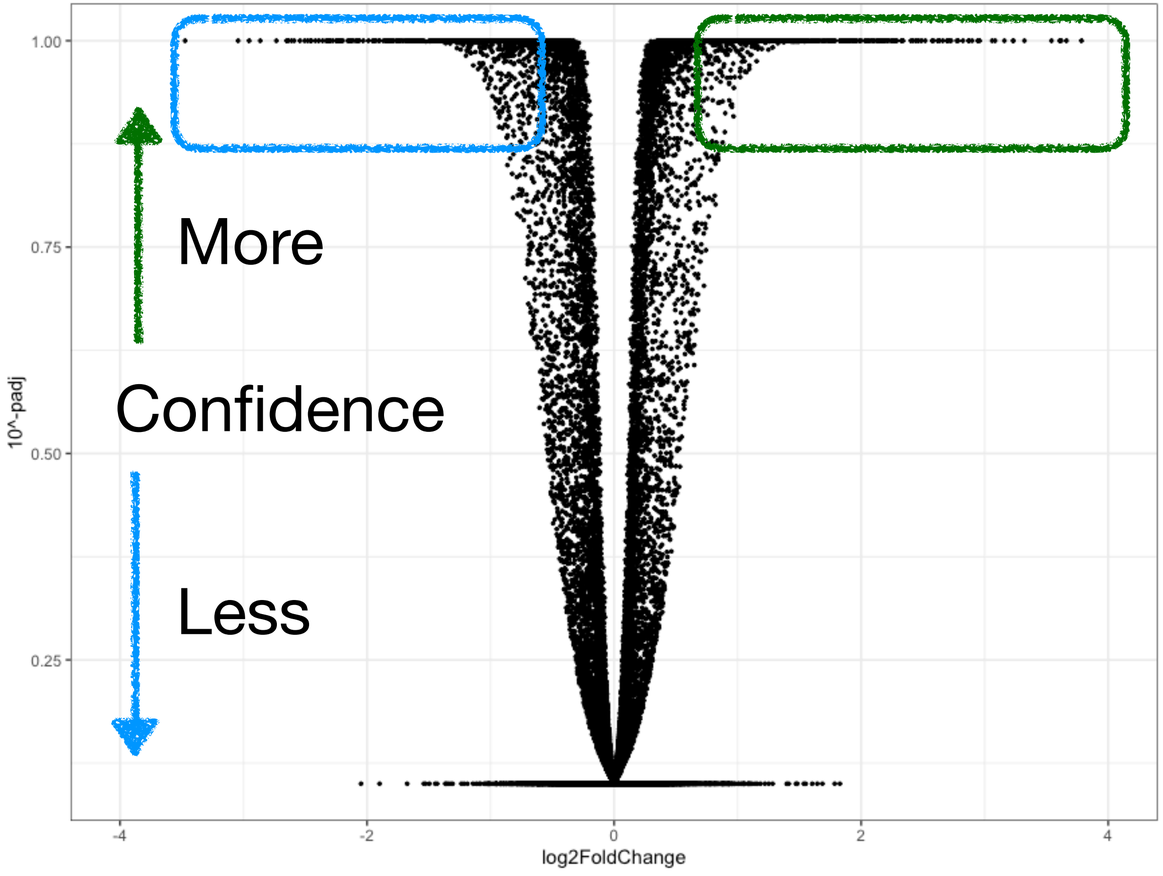
quick exploration of our dataset

Distribution of expression levels
'across all samples'



Low ← expression level → High

Differential expression
Dex / Cont



← Down Up →

IPA robustness analyses

- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- data acquired with a low coverage technology
- Importance of the background

DE cutoff effect

log2FoldChange	-3.4729 to 5.0905
padj	0.0 to 0.9988

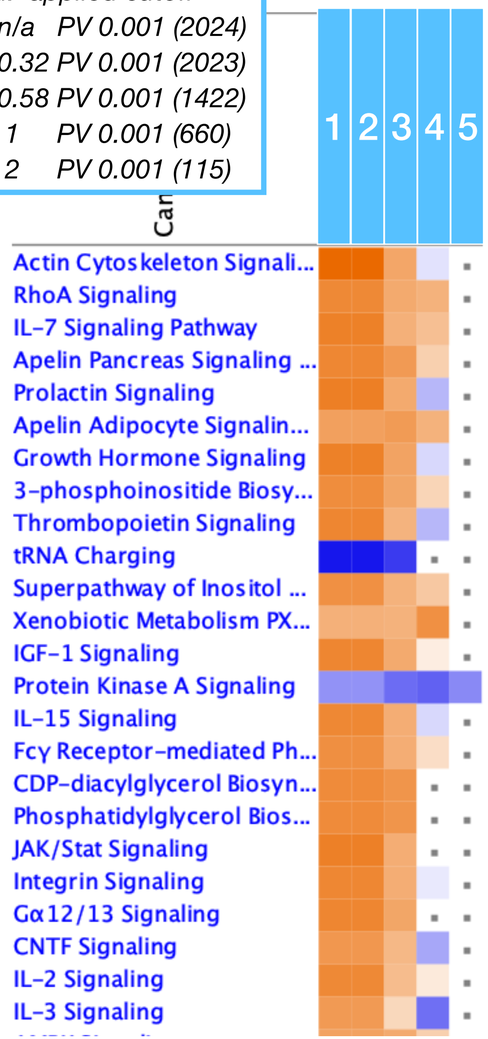
1	log2FoldChange		Down		Up	RECALCULATE	2024 analysis-ready molecules across observations (909 Down and 1115 Up)
	padj	0.001				
2	log2FoldChange	-0.322	Down	0.322	Up	2023 analysis-ready molecules across observations (908 Down and 1115 Up)
	padj	0.001				
3	log2FoldChange	-0.585	Down	0.585	Up	1422 analysis-ready molecules across observations (646 Down and 776 Up)
	padj	0.001				
4	log2FoldChange	-1.0	Down	1.0	Up	660 analysis-ready molecules across observations (295 Down and 365 Up)
	padj	0.001				
5	log2FoldChange	-2	Down	2	Up	RECALCULATE	115 analysis-ready molecules across observations (44 Down and 71 Up)
	padj	0.001				

Compare

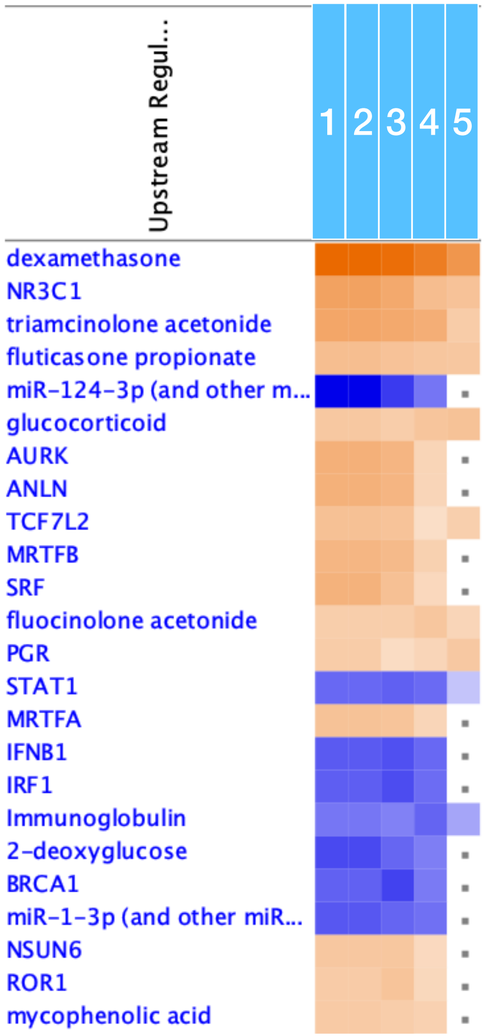
all features are quite robust to cutoff BUT <u>excess cutoff is detrimental</u>

Canonical Pathways

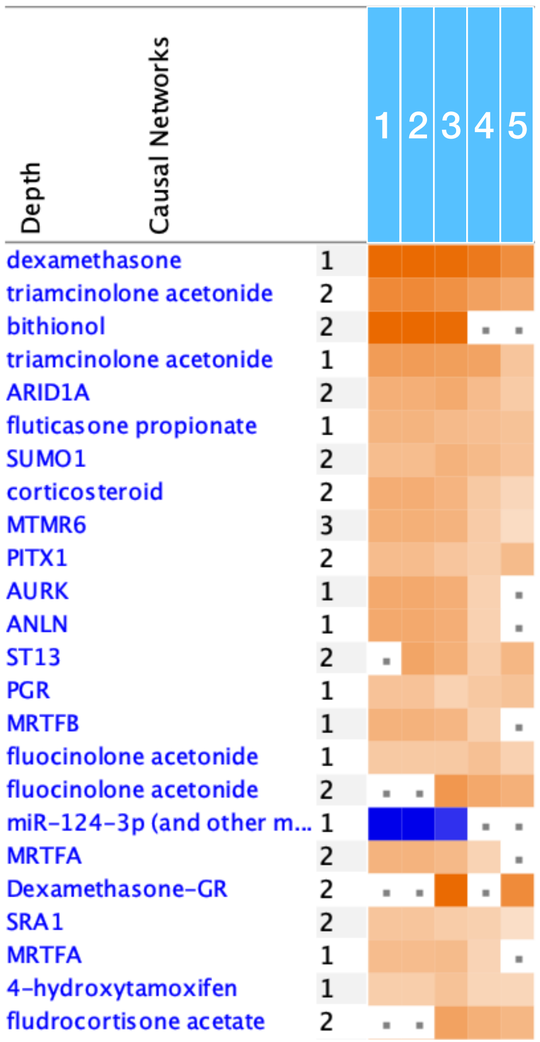
legend: 'applied cutoff'
***1**: LR n/a PV 0.001 (2024)*
***2**: LR 0.32 PV 0.001 (2023)*
***3**: LR 0.58 PV 0.001 (1422)*
***4**: LR 1 PV 0.001 (660)*
***5**: LR 2 PV 0.001 (115)*



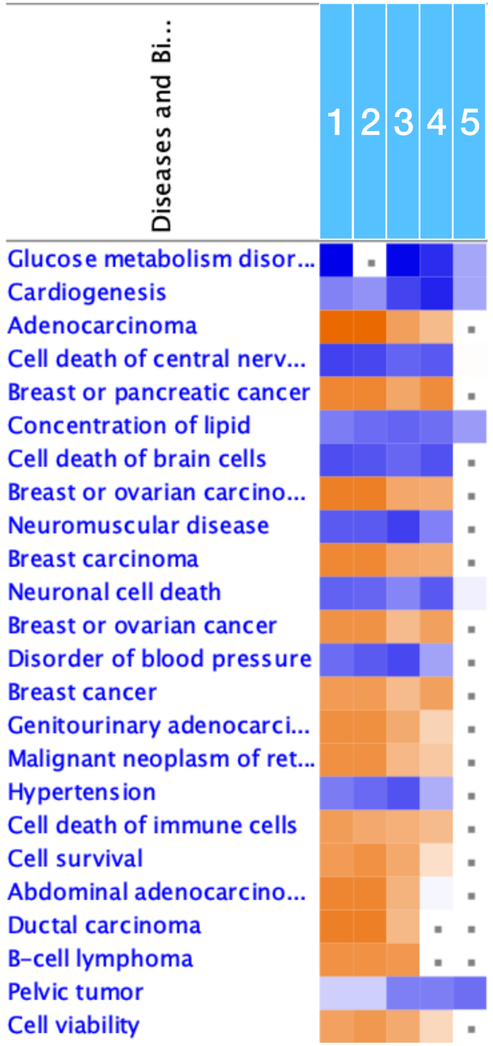
Upstream regulators



Causal Networks



Diseases & Functions



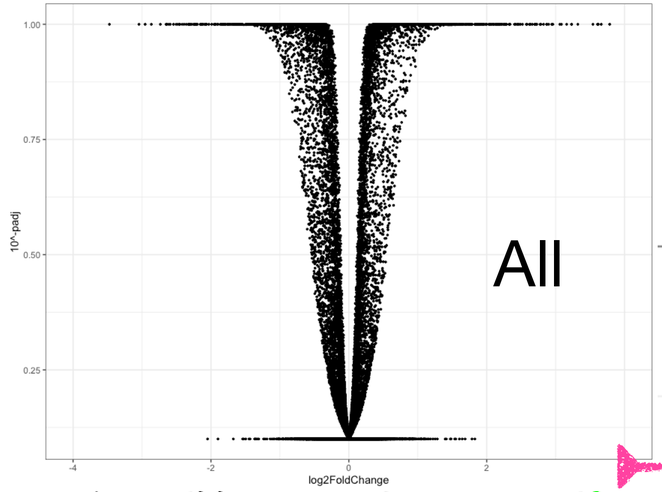
'.' Not significant at 0.05 ^

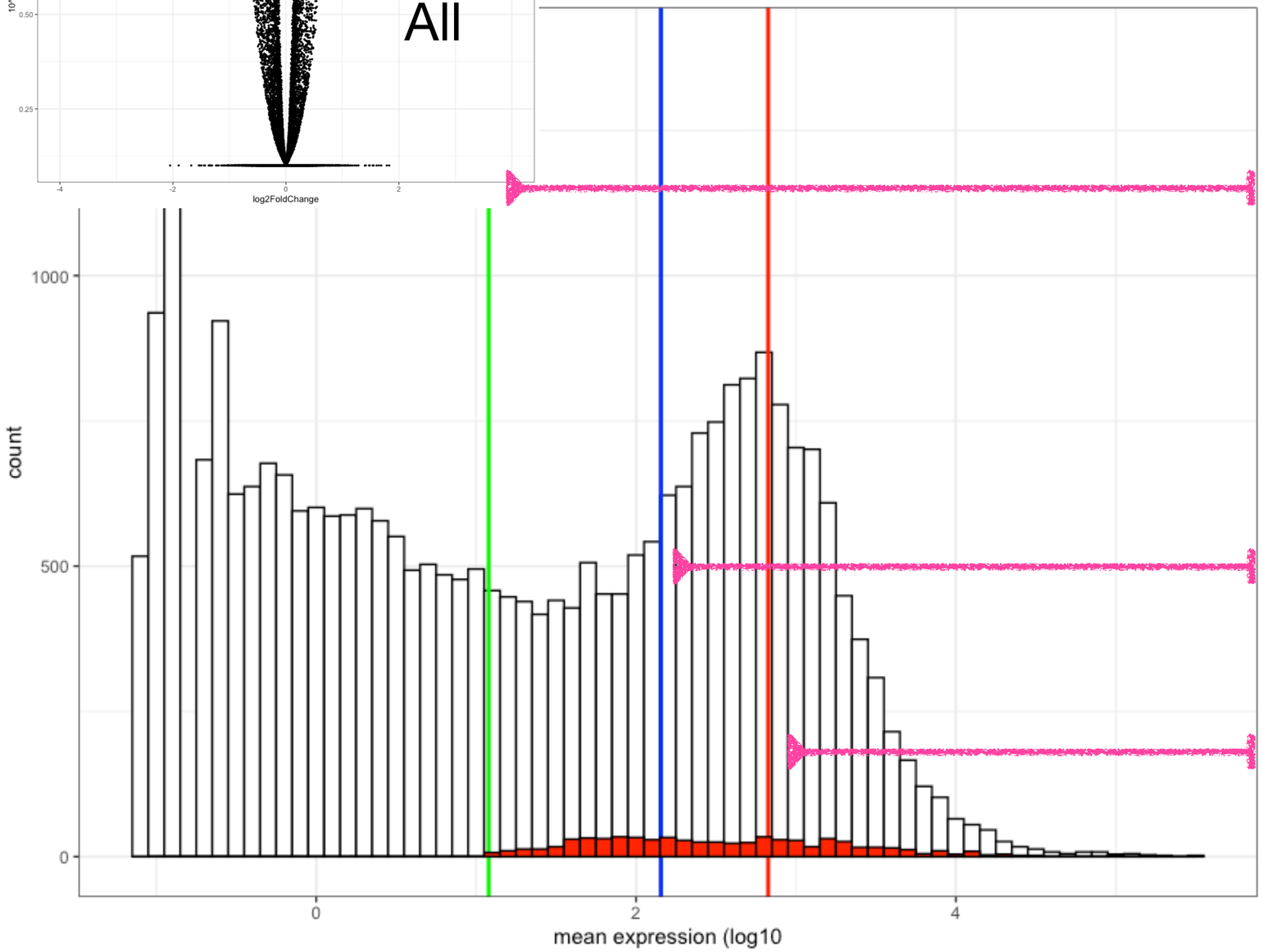
IPA robustness analyses

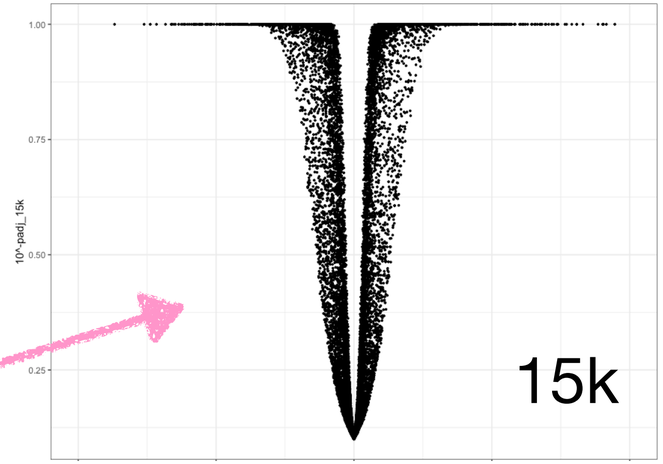
- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- data acquired with a low coverage technology
- Importance of the background

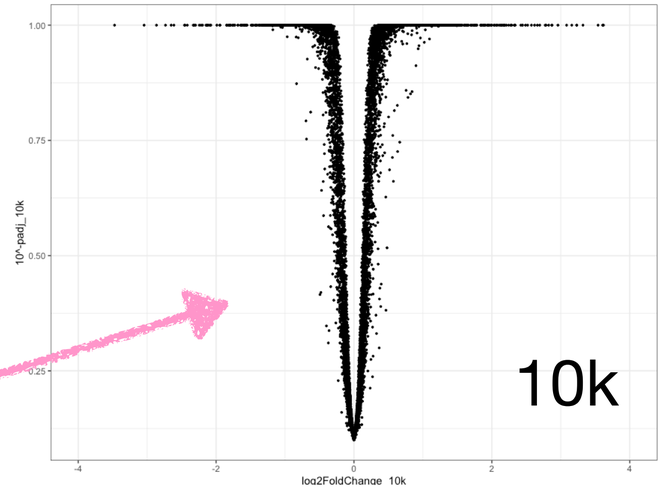
Platform sensitivity effect

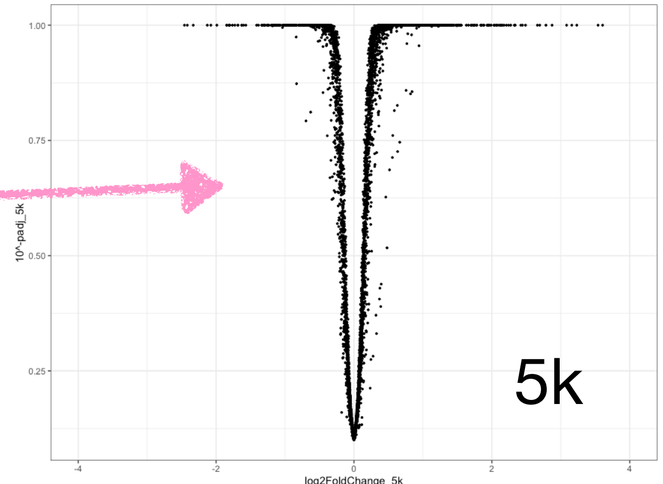
only keeping the top-N expressed genes



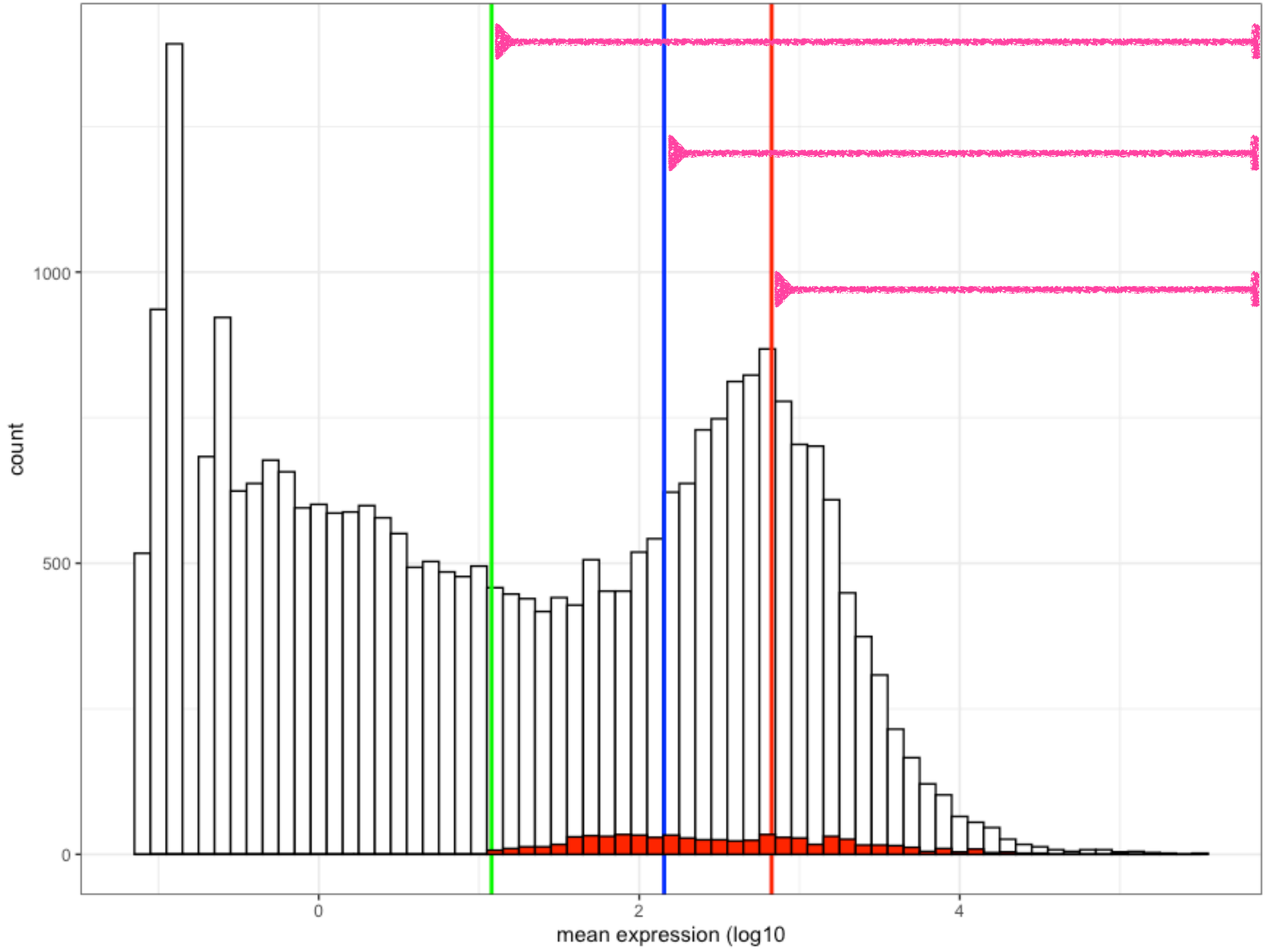








robustness to sensibility of detection



15k genes

10k genes

5k genes

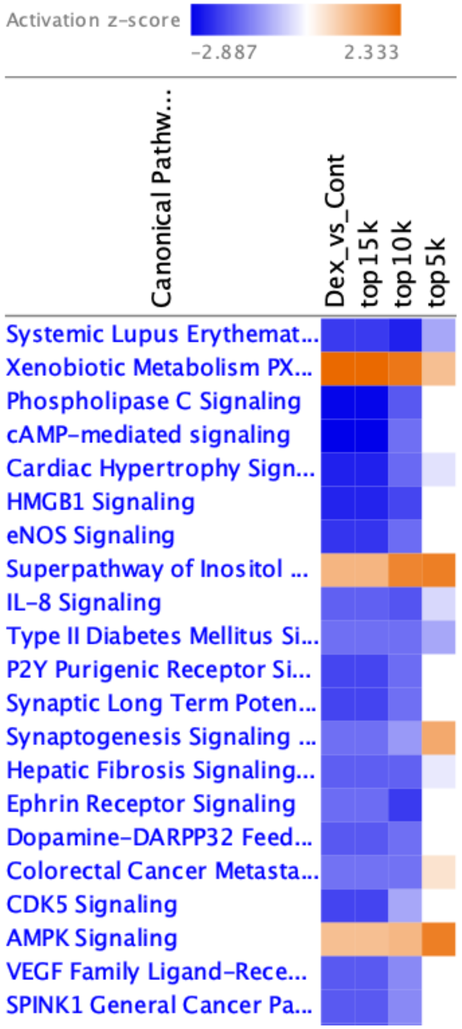
IPA

|logR| >1 & adjp < 0.001

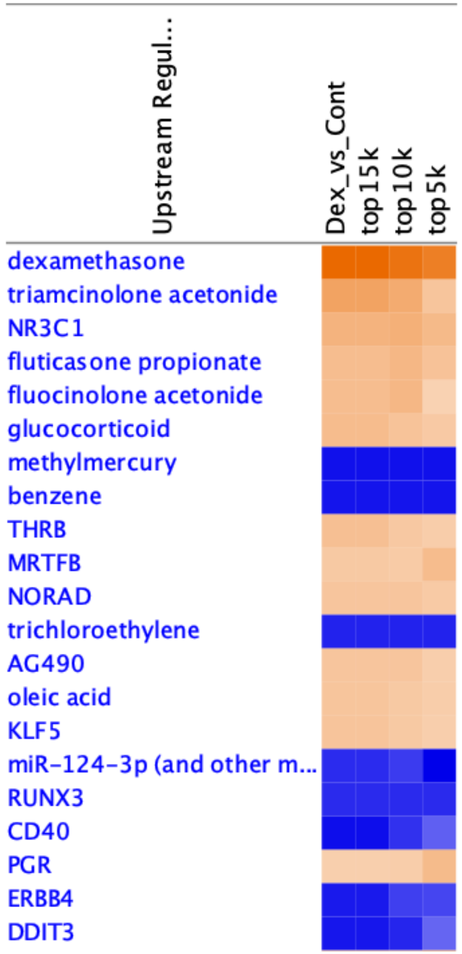
Compare

large data loss leads to less sensitivity due to loss of lower-expression DE genes

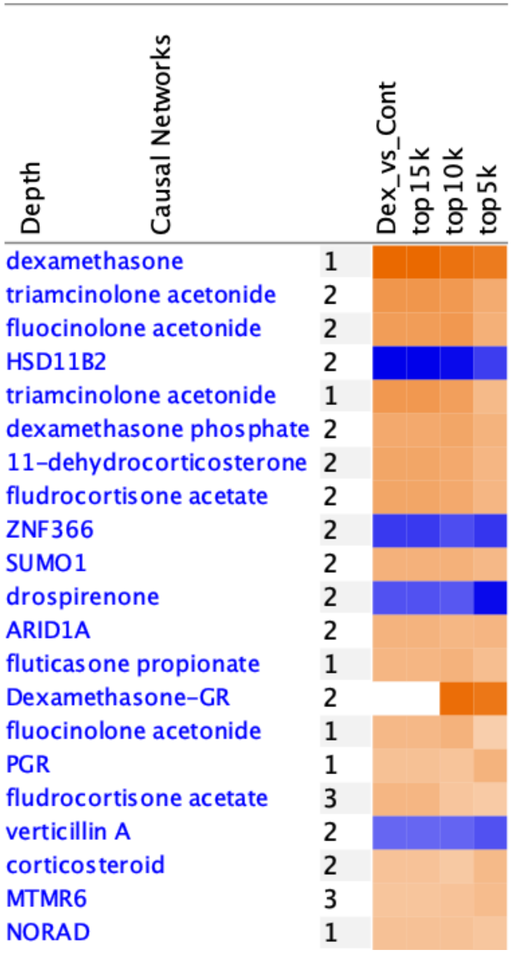
Canonical Pathways



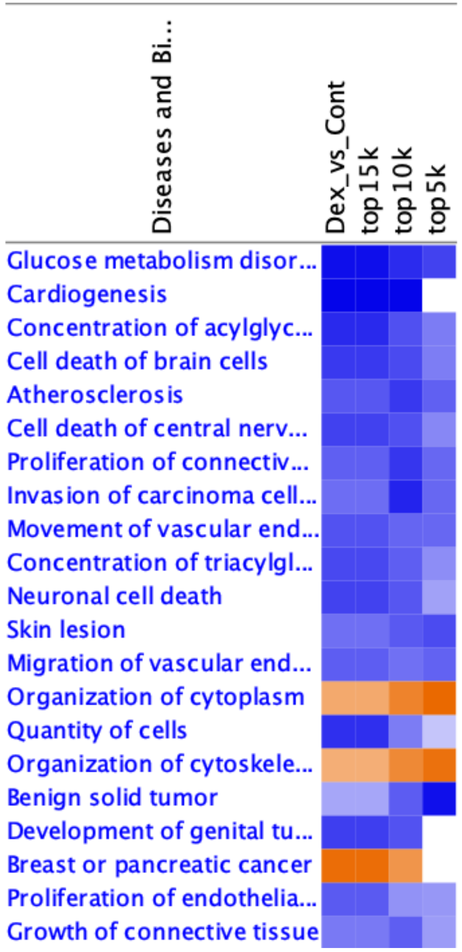
Upstream regulators



Causal Networks



Diseases & Functions



IPA robustness analyses

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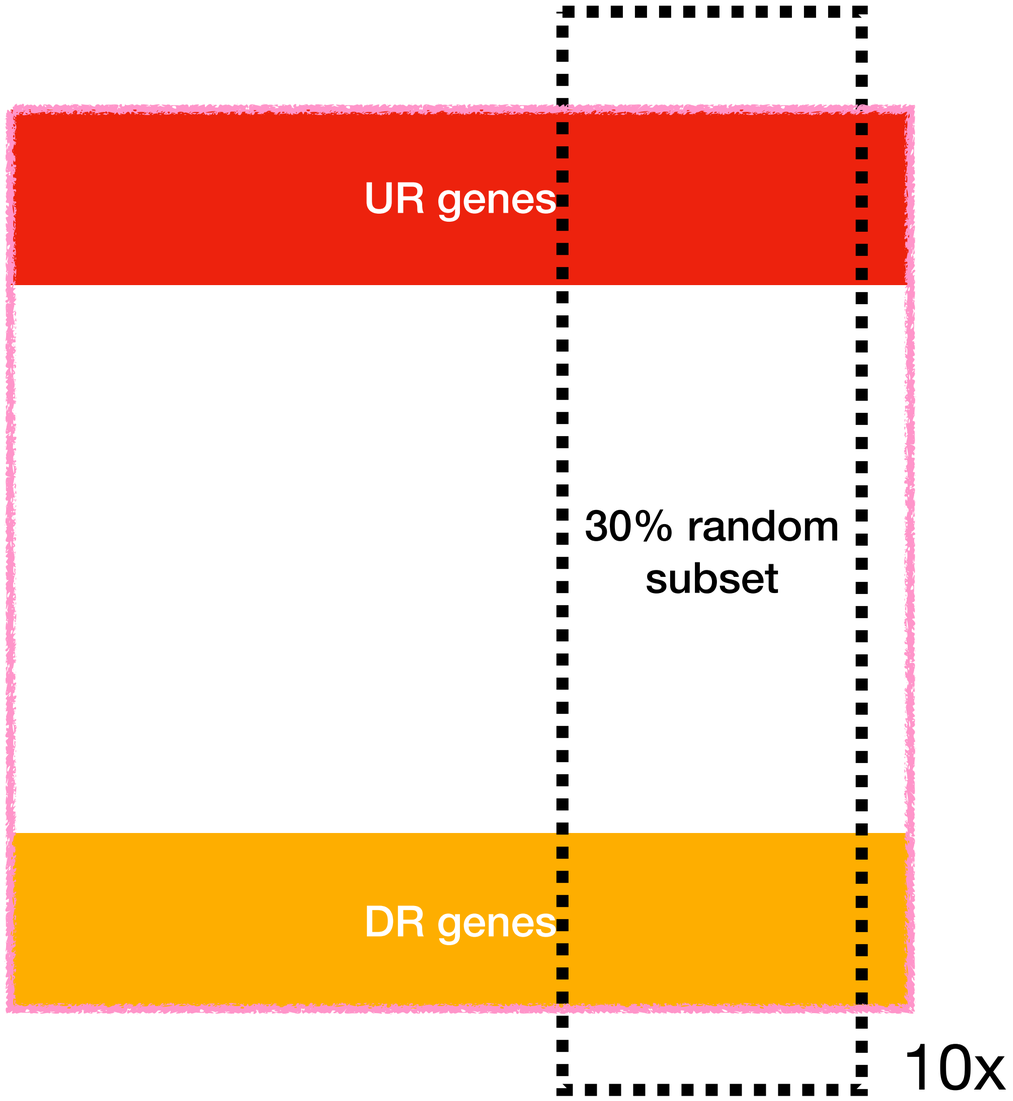
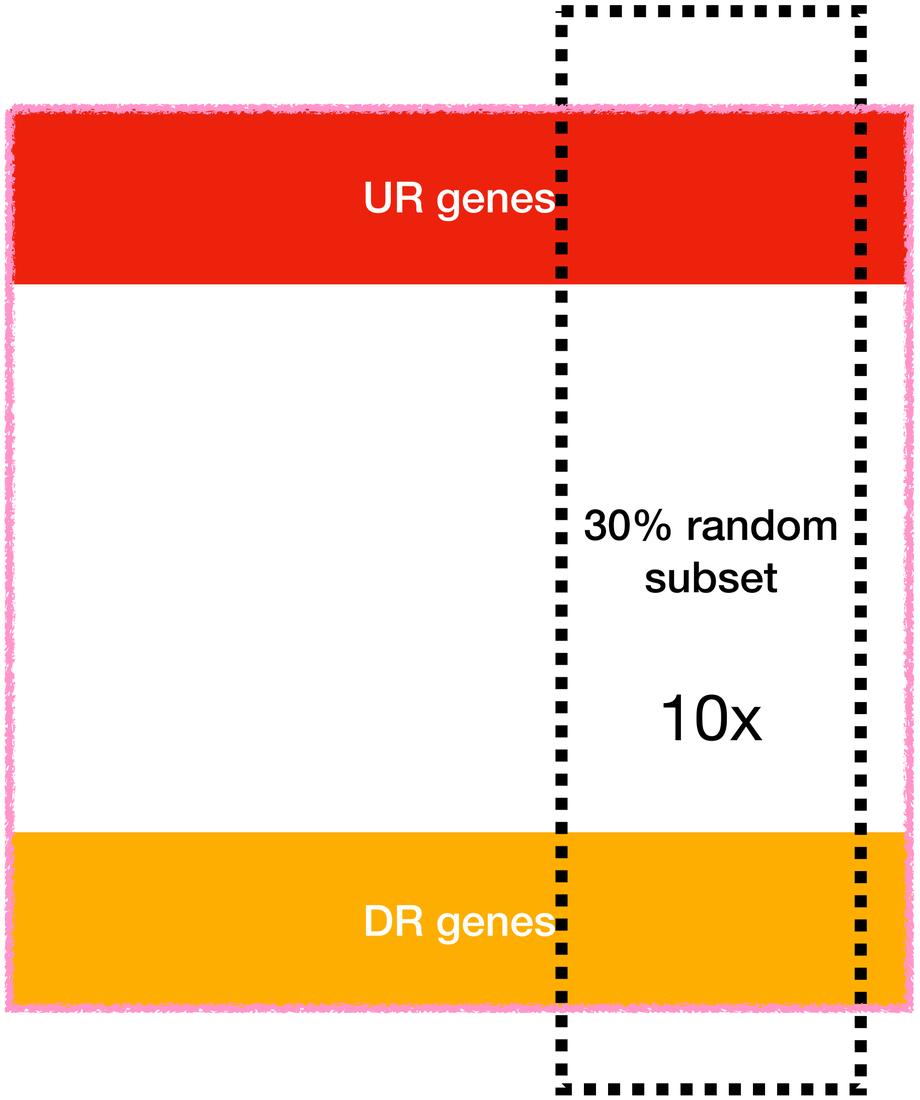
- Importance of the background

Sampling effect



IPA®

Robustness to random sampling

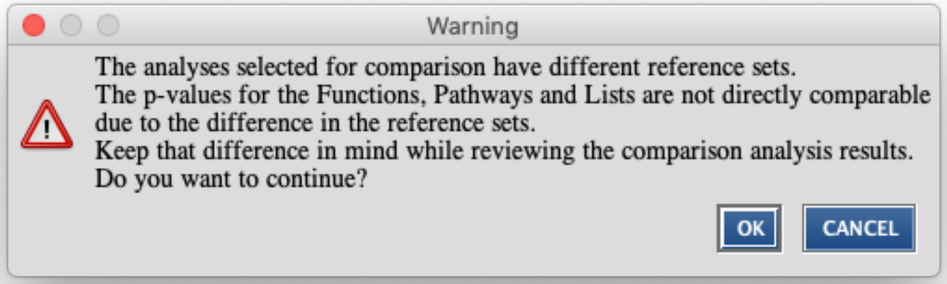


10x core analysis

KB as background

|logR| >1 & adjp < 0.001

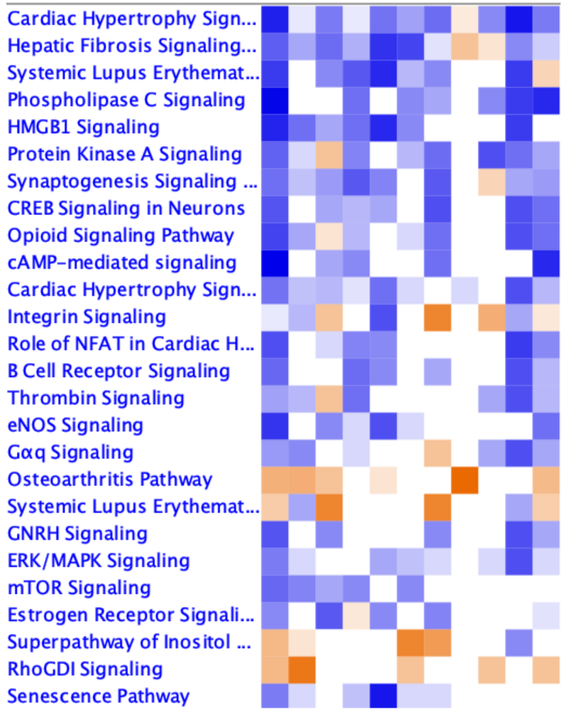
Compare



Platforms that only 'see' a fraction of the truth are less robust although globally IPA resists well to random sampling

Canonical Pathways

+1234567890



Upstream regulators

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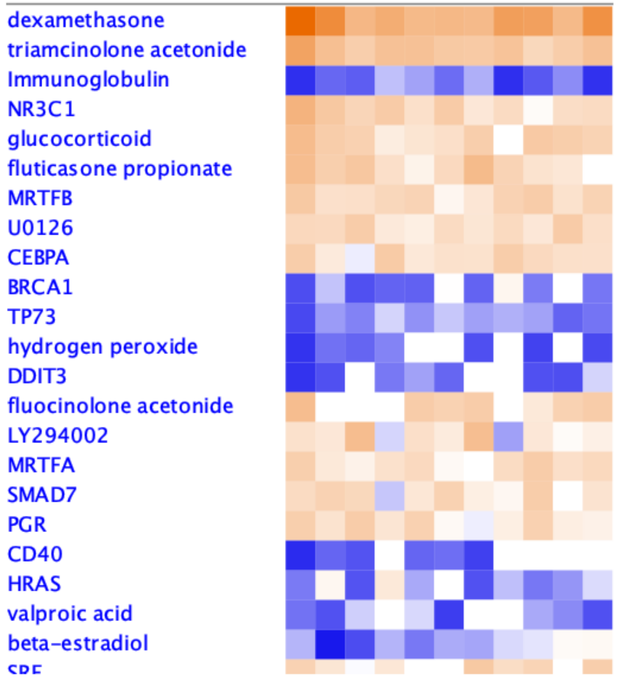

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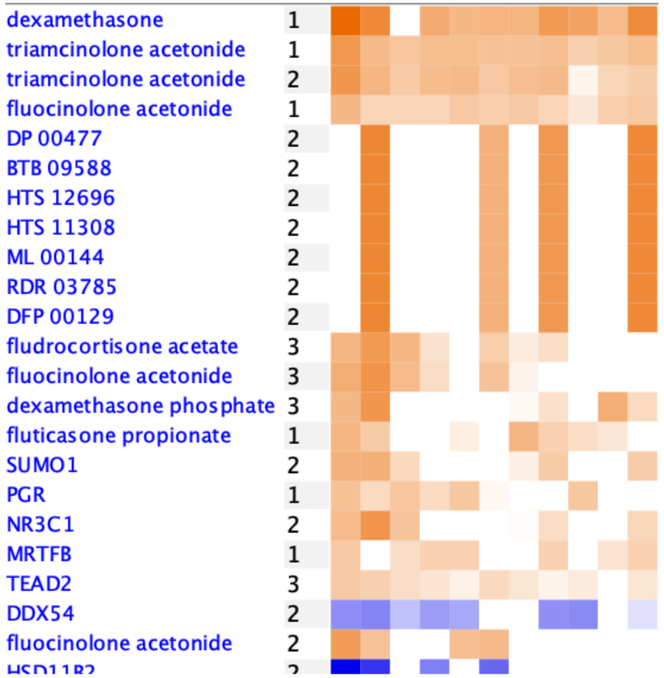
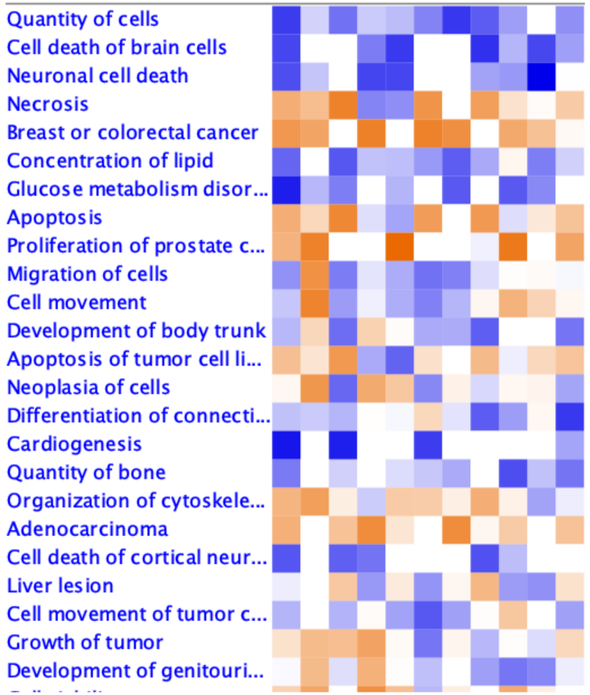


Diseases & Functions

+1234567890



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Platform sensitivity matters !

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