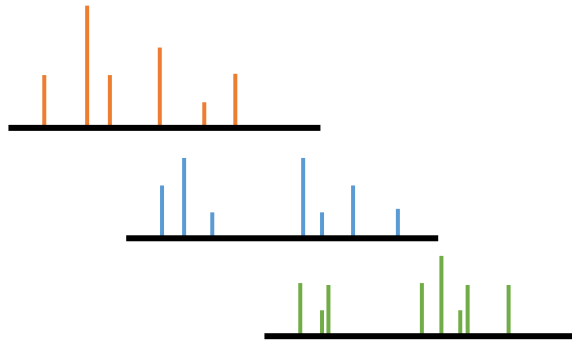
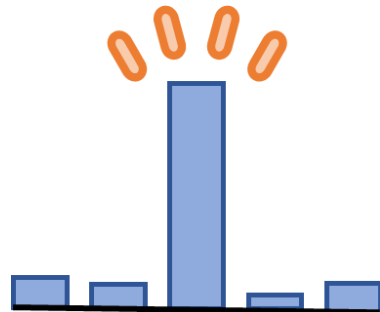


プロテオミクスデータの三次解析および生物学的解釈

フィルジェン株式会社 バイオインフォマティクス部
(biosupport@filgen.jp)



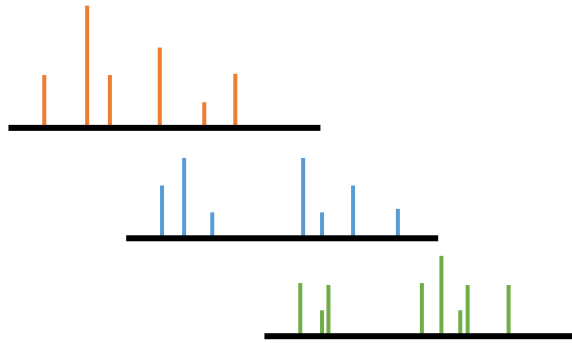
	Sample 1	Sample 2	Sample 3	...
Gene A	0	0	10	
Gene B	5	5	6	
Gene C	5	1	7	
...				



一次解析：スペクトルの取得

二次解析：発現定量

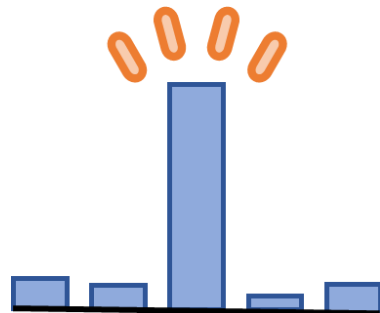
三次解析：データの可視化、統計解析



一次解析：スペクトルの取得

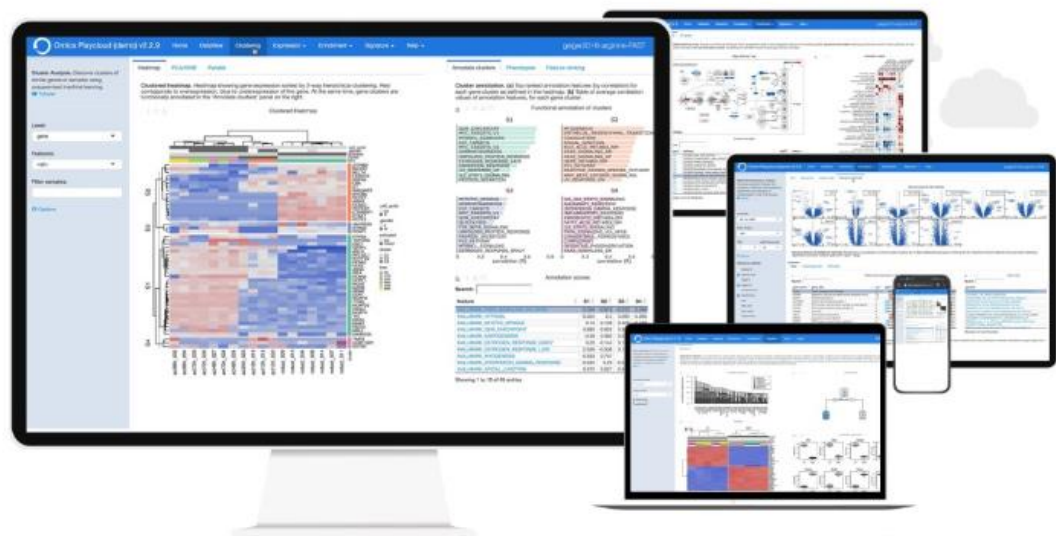
	Sample 1	Sample 2	Sample 3	...
Gene A	0	0	10	
Gene B	5	5	6	
Gene C	5	1	7	
...				

二次解析：発現定量



解析者の技量に依拠するところが大きい

三次解析：データの可視化、統計解析



Omics Playground

- 定量済みデータをアップロードするだけで、データの解釈に必要な100以上のプロットを生成可能
- 生物学で用いられる様々な統計手法をカバー（Deseq2, EdgeR, limmaなど）
- プロテオミクスデータの外、トランスクリプトミクスデータ、マイクロアレイデータもアップロード可能
- ユーザーどうしで解析結果の共有が可能

データさえあれば、誰でも重要な遺伝子を特定できる

	Sample A	Sample B	Sample C
Gene 1	5.4	4.1	0.1
Gene 2	0.1	0.3	8.9
Gene3	6.7	5.2	4.4

① **expression.csv** :
サンプルごとの発現定量データ

	Sex	Country	Dominant hand
Sample A	male	Japan	right
Sample B	male	USA	left
Sample C	female	Japan	right

② **sample.csv** :
各サンプルの属性を記述するデータ

	Left vs Right	male vs female
Sample A	right	male
Sample B	left	male
Sample C	right	female

③ **contrast.csv** :
比較解析の内容を記述するデータ

Bojkova, Denisa, et al. "**Proteomics of SARS-CoV-2-infected host cells reveals therapy targets.**"
Nature 583.7816 (2020): 469-472.

Caco-2細胞をSARS-CoV2に感染させ、経時的なプロテオームの変化を追跡した。

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BigOmics Analytics
bojkova2020-sarscov2

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Menu

- Load
- Load dataset
- New dataset
- DataView
- Clustering
- Expression
- GeneSets
- Compare
- SystemsBio

Data View

[Gene overview](#)
[Sample QC](#)
[Counts table](#)
[Sample information](#)
[Contrasts](#)

This Gene overview panel displays data for a selected gene. The 'gene info' box provides more information about the gene and hyperlinks to external databases. The upper plots show the expression level, average expression ranking, and distribution of expression among the samples. The remaining plots, display the most correlated genes and expression in the GTEX tissue database.

Gene info

symbol: COPA
name: COPI coat complex subunit alpha
map_location: 1q23.2

summary: In eukaryotic cells, protein transport between the endoplasmic reticulum and Golgi compartments is mediated in part by non-clathrin-coated vesicular coat proteins (COPs). Seven coat proteins have been identified, and they represent subunits of a complex known as coatomer. The subunits are designated alpha-COP, beta-COP, beta-prime-COP, gamma-COP, delta-COP, epsilon-COP, and zeta-COP. The alpha-COP, encoded by COPA, shares high sequence similarity with RET1P, the alpha subunit of the coatomer complex in yeast. Also, the N-terminal 25 amino acids of alpha-COP encode the bioactive peptide, xenin, which stimulates exocrine pancreatic secretion and may act as a gastrointestinal hormone. Alternative splicing results in multiple splice forms encoding distinct isoforms. [provided by RefSeq, Jul 2008].

OMIM: 601924, 616414
KEGG: NA
GO: pancreatic juice secretion (GO:0030157)

Gene expression

Average rank

t-SNE clustering

Top correlated genes

Tissue expression (GTEx)

Settings

Gene: COPA

Filter samples:

Group by: group

Options

7

Clustering

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Menu

- Load
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- Features
- Expression
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Cluster features

Gene Geneset

Visually explore and compare expression signatures on UMAP plots. Feature-level clustering is based on pairwise co-expression between genes (or genesets). This is in contrast to sample-level clustering which clusters samples by similarity of their expression profile. Feature-level clustering allows one to detect gene modules, explore gene neighbourhoods, and identify potential drivers. By coloring the UMAP with the foldchange, one can visually compare the global effect between different conditions.

Geneset UMAP

Geneset signatures

Control

Virus

Settings

Show:

phenotype

comparisons

treatment

Reference:

<average>

Filter genesets:

<all>

Show full table

Geneset table

Search:

DB	geneset	sd.X	Control	Virus
C2	ZHAN_MULTIPLE_MYELOMA_PR_UP	0.041	0.041	-0.041
STAUDT	MYELOMA_PR_SUBGROUP_UP	0.041	0.041	-0.041
DRUG	Phytoestrogens_CTD_00007437	0.037	0.037	-0.037
C2	WHITFIELD_CELL_CYCLE_LITERATURE	0.034	0.034	-0.034
C2	ODONNELL_TARGETS_OF_MYC_AND_TFRC_DN	0.033	0.033	-0.033
DISEASE	breast adenocarcinoma DOID-3458 human GSE61304 sample 1071 (up)	0.032	0.032	-0.032
C2	REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	0.031	0.031	-0.031
PATHWAY	E2F mediated regulation of DNA replication_Homo sapiens_R-HSA-113510	0.031	0.031	-0.031

Showing 1 to 13 of 299 entries

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Expression

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Differential expression

Overview | Top genes | Volcano by comparison | Volcano by method

Menu

- Load
- DataView
- Clustering
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 - Differential expression**
 - Correlation analysis
 - Find biomarkers
- GeneSets
- Compare
- SystemsBio

Volcano plot

Bland-Altman (MA) plot

Differential expression

Gene in comparison

Table | Foldchange (all) | FDR table

Differential expression analysis

symbol	gene_title	chr	logFC	stars	meta.q	AveExpr0	AveExpr1	trend.limma
LYPD3	LY6/PLAUR domain containing 3	19q13.31	1.020	★★★	0.00002991	2.235	3.256	0.00002991
HKDC1	hexokinase domain containing 1	10q22.1	1.018	★★★	0.06904	9.120	10.14	0.06904
INSL4	insulin like 4	9p24.1	-1.009	★★★	0.003866	3.100	2.091	0.002478
DCD	dermcidin	12q13.2	0.9475	★★★	0.01820	3.310	4.258	0.01820

Showing 22 to 32 of 1,379 entries

Gene sets with gene

- COMPARTMENTS: Integrin_alpha6-beta4_complex
- DRUG: pioglitazone_PC3_UP
- TISSUE: SKIN_UP
- DRUG: (-)-isoprenaline_PC3_UP
- DRUG: Lapatinib DB01259 human GSE38376 sample 2584 (up)
- DRUG: TELENZEPINE_UP
- GOBP: cell-matrix adhesion (GO_0007160)

Showing 1 to 9 of 158 entries

Settings

Contrast: Virus24h_vs_Control24h

Gene family: <all>

FDR: 0.2 | logFC: 0

Options

Expression Analysis

Expression

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Differential expression

Overview Top genes Volcano by comparison Volcano by method

Expression of top differentially expressed genes

Table Foldchange (all) FDR table

Differential expression analysis

Search:

symbol	gene_title	chr	logFC	stars	meta.q	AveExpr0	AveExpr1	trend.limma
KIF23	tubing protein 23	2q33	-1.143	***	0.0001720	3.404	4.607	0.0003230
AFP	alpha fetoprotein	4q13.3	-1.103	***	0.00002119	9.043	7.940	0.00009563
TTR	transthyretin	18q12.1	-1.082	***	0.000003237	8.181	7.099	0.000002159
TMBIM6	transmembrane BAX inhibitor motif containing 6	12q13.12	-1.050	***	2.297e-12	6.009	4.959	1.205e-12
RALYL	RALY RNA binding protein like	8q21.2	-1.021	***	0.02227	3.451	2.430	0.02227
LYPD3	LY6/PLAUR domain containing 3	19q13.31	1.020	***	0.00002991	2.235	3.256	0.00002991
HKDC1	hexokinase domain containing 1	10q22.1	1.018	***	0.06904	9.120	10.14	0.06904
INSL4	insulin like 4	9p24.1	-1.009	***	0.003866	3.100	2.091	0.002478
DCD	dermcidin	12q13.2	0.9475	***	0.01820	3.310	4.258	0.01820

Showing 22 to 31 of 1,379 entries

Gene sets with gene

Search:

geneset	rhc
COMPARTMENTS: Integrin_alpha6-beta4_complex	
DRUG: pioglitazone_PC3_UP	
TISSUE: SKIN_UP	
DRUG: (-)-isoprenaline_PC3_UP	
DRUG: Lapatinib DB01259 human GSE38376 sample 2584 (up)	
DRUG: TELENZEPINE_UP	
GOBP: cell-matrix adhesion (GO_0007160)	

Showing 1 to 8 of 158 entries

Settings

Contrast: Virus24h_vs_Control24h

Gene family: <all>

FDR: 0.2 logFC: 0

Options

Expression Analysis

Expression

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Biomarker Selection

Feature selection | Feature-set ranking

Variable importance

Biomarker expression

Decision tree

Heatmap

Settings: Predicted target: group, Filter samples: [empty], Feature set: <all>, Compute

Menu: Load, DataView, Clustering, Expression, Differential expression, Correlation analysis, Find biomarkers, GeneSets, Compare, SystemsBio


Biomarker Board

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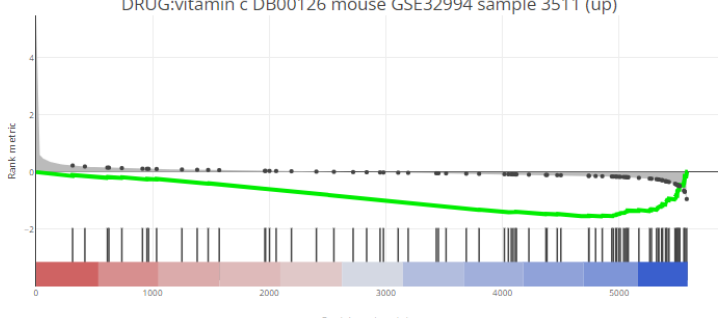

 Geneset Enrichment

Geneset enrichment

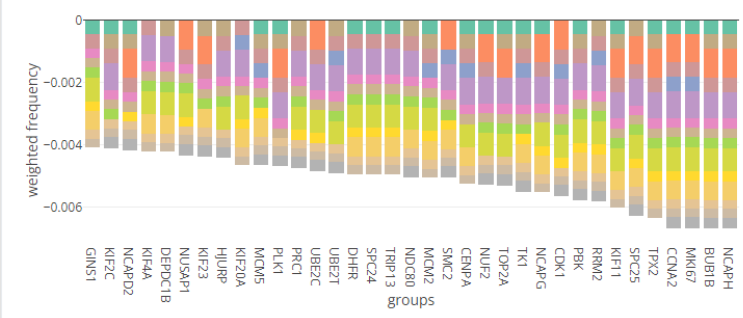
[Enrichment](#)
[Geneset expression](#)
[Enrichment by comparison](#)
[Volcano by comparison](#)
[Volcano by method](#)

Top enriched gene sets

DRUG:vitamin c DB00126 mouse GSE32994 sample 3511 (up)



Frequency in top gene sets



Enrichment analysis

Search:

geneset	logFC	meta.q	size	stars	AveExpr0	AveExpr1
DRUG:vitamin c DB00126 mouse GSE32994 sample 3511 (up)	-0.03360	0.01170	80	★★★	5.527	5.494
GENE:Pdk1 knockout mouse GSE42187 sample 2009 (down)	-0.03430	0.01170	70	★★★	5.867	5.832
GENE:Notch1 KO mouse GSE57655 sample 1698 (up)	-0.03780	0.01170	41	★★★	5.661	5.623
GENE:NOTCH3 KD human GSE27424 sample 1410 (up)	-0.03900	0.01170	83	★★★	5.385	5.346
GENE:ErbB2 druginhibition mouse GSE4066 sample 1854 (up)	-0.03920	0.01170	83	★★★	6.376	6.337
GENE:MELK druginhibition human GSE50227 sample 1956 (down)	-0.04160	0.01170	49	★★★	5.694	5.652
DRUG:estradiol DB00783 human GSE46924 sample 2487 (up)	-0.04180	0.01170	133	★★★	5.650	5.608
LIGAND:estradiol human MCF-7 cells GSE46924 lizand163 (uo)	-0.04180	0.01170	133	★★★	5.650	5.608

Showing 1 to 8 of 904 entries

Genes in gene set

Search:

symbol	fc	p	q
CEBPA	-0.9417	0.000	0.000
HPGD	-0.6855	0.000	0.000
AURKA	-0.6796	0.000	0.000
TK1	-0.6468	0.000	0.000
TPX2	-0.4879	0.000	0.000
DHFR	-0.4818	0.000	0.000
ISYNA1	-0.4676	0.002400	0.01950
CHTF18	-0.4522	0.07030	0.2065
CCNA2	-0.4519	0.000	0.000
BUB1B	-0.4280	0.0002000	0.002500

Showing 1 to 10 of 80 entries

Settings

Contrast: Virus24h_vs_Control2 4h

Gene set collection: <all>

FDR: 0.2

logFC threshold: 0

Options

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Geneset enrichment

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[Geneset expression](#)
[Enrichment by comparison](#)
[Volcano by comparison](#)
[Volcano by method](#)

Volcano plot

Enrichment barplot

Expression geneplot

Enrichment vs. expression

[Table](#)
[Enrichment \(all\)](#)
[FDR table](#)

Enrichment analysis

Search:

geneset	logFC	meta.q	size	stars	AveExpr0	AveExpr1
C2:KEGG_ECM_RECEPTOR_INTERACTION	0.04450	0.1986	31	***	5.186	5.2
DRUG:morphine 5288826 mouse GSE7762 sample 3487 (up)	-0.02340	0.1988	102	***	7.433	7.4
AGING:Mouse_kidney_25 weeks vs 100 weeks_GSE41018_aging399 (up)	-0.02860	0.1995	167	***	7.178	7.1
COMPARTMENTS:pre-autophagosomal_structure	0.07100	0.1995	15	***	5.195	5.2
PATHWAY:FGF signaling pathway_Homo sapiens_98ed0df6-6192-11e5-8ac5-06603...	-0.03810	0.1995	27	***	6.364	6.3
DRUG:Alkbh1_Embryonic_stem_cells_Knockout_GSE71943_up	-0.02480	0.1997	331	***	7.523	7.5
DRUG:TRICHLOROETHYLENE 6575 mouse GSE24278 sample 3309 (up)	-0.01680	0.1998	68	***	6.330	6.3

Showing 897 to 904 of 904 entries

Genes in gene set

Search:

symbol	fc	p	q
SQSTM1	0.4714	0.000	0.000
RB1CC1	0.3093	0.3123	0.5866
TRAPPC8	0.2784	0.001600	0.01430
WIPI1	0.2248	0.03340	0.1439
ATG16L1	-0.1804	0.07060	0.2403
RAB1B	0.1529	0.005900	0.03960
WIPI2	0.1367	0.1982	0.4578
BECN1	0.1319	0.5852	0.8005
STBD1	0.1288	0.1475	0.3824
VMP1	0.03810	0.8942	0.9571

Showing 1 to 10 of 15 entries

Settings

Contrast: Virus24h_vs_Control24h

Gene set collection: <all>

FDR: 0.2

logFC threshold: 0

Options

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- Signature Analysis

Test signatures

Volcano plots Enrichment Overlap/similarity Markers

This panel shows your custom list of genes on top of the GSEA enrichment plots for each comparison. Enter your list of genes in the right box.

Enrichment plots

Virus24h_vs_Control24h

Virus2h_vs_Control2h

Virus6h_vs_Control6h

Virus10h_vs_Control10h

Enrichment table

Enrichment by contrasts

contrast	NES	q
Virus24h_vs_Control24h	-2.219	0.007400
Virus2h_vs_Control2h	-1.720	0.007500
Virus6h_vs_Control6h	1.500	0.05160
Virus10h_vs_Control10h	-0.9898	0.4680

Showing 1 to 4 of 4 entries

Genes in signature

Please select a contrast

Settings

Signature type: <custom>

Genes:

- MCM5 PCNA TYMS FEN1
- MCM2 MCM4 RRM1 UNG
- GINS2 MCM6 CDCA7 DTL
- PRM1 UHRF1 MLF1IP HELLS
- RFC2 RPA2 NASP RADS JAP1
- GMNN WDR76 SLBP CCNE2
- UBR7 POLD3 MSH2 ATAD2
- RAD51 RRM2 CDC45 CDC6
- EXO1 TIPIN DSCC1 BLM
- CASP8A2 USP1 CLSPN
- POLA1 CHAF18 BRIP1 E2F8
- HMGB2 CDK1 NUSAP1
- UBE2C BIRC5 TPX2 TOP2A
- NDC80 CKS2 NUF2 CKS1B
- NK167 TMOPO CENPF TACC3
- FAM64A SMC4 CCNB2

[apoptosis] [cell_cycle]

[immune_chkpt]

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Pathway Analysis

WikiPathways Reactome GO graph

WikiPathway

+ RESET

Activation matrix

Enrichment table

Search:

pathway.id	pathway	logFC	meta.q
WP150	PATHWAY:DNA Replication_Mus musculus_WP150	-0.08668	0.1282
WP466	PATHWAY:DNA Replication_Homo sapiens_WP466	-0.08526	0.1096
WP78	PATHWAY:TCA Cycle_Homo sapiens_WP78	0.08249	0.2559
WP244	PATHWAY:Alpha 6 Beta 4 signaling pathway_Homo sapiens_WP244	0.08212	0.2867
WP434	PATHWAY:TCA Cycle_Mus musculus_WP434	0.07830	0.2743
WP1601	PATHWAY:Fluoropyrimidine Activity_Homo sapiens_WP1601	-0.07674	0.4484
WP2446	PATHWAY:Retinoblastoma (RB) in Cancer_Homo sapiens_WP2446	-0.07258	0.02397
WP2363	PATHWAY:Gastric Cancer Network 2_Homo sapiens_WP2363	-0.06874	0.4001
WP1982	PATHWAY:SRFRP signaling_Homo sapiens_WP1982	-0.04858	0.1896

Showing 1 to 34 of 62 entries

Settings >>

Contrast:

Virus24h_vs_Control2
4h

Options

Pathway Analysis

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Pathway Analysis

WikiPathways | **Reactome** | GO graph

Reactome pathway

RESET

Enrichment table

Search:

reactome.id	pathway
1 R-HSA-975634	PATHWAY:Retinoid metabolism and transport_Homo sapiens_R-HSA-975634
1 R-HSA-3000157	PATHWAY:Laminin interactions_Homo sapiens_R-HSA-3000157
1 R-HSA-191273	PATHWAY:Cholesterol biosynthesis_Homo sapiens_R-HSA-191273
1 R-HSA-162588	PATHWAY:Budding and maturation of HIV virion_Homo sapiens_R-HSA-162588
1 R-HSA-174417	PATHWAY:Telomere C-strand (Lagging Strand) Synthesis_Homo sapiens_R-HSA-174417
1 R-HSA-167238	PATHWAY:Pausing and recovery of Tat-mediated HIV elongation_Homo sapiens_R-HSA-167238
1 R-HSA-167243	PATHWAY:Tat-mediated HIV elongation arrest and recovery_Homo sapiens_R-HSA-167243
1 R-HSA-167242	PATHWAY:Abortive elongation of HIV-1 transcript in the absence of Tat_Homo sapiens_R-HSA-167242

Showing 1 to 8 of 198 entries

Activation matrix

	Virus24h_vs_Control24h	Virus6h_vs_Control6h	Virus10h_vs_Control10h	Virus2h_vs_Control2h
formation of hiv elongation complex in the absence of hiv ta...				
myd88 dependent cascade initiated on endosome				
mma capping				
regulation of tp53 activity through phosphorylation				
tp53 regulates transcription of dna repair genes				
abortive elongation of hiv-1 transcript in the absence of ta...				
amyloid fiber formation				
dual incision in tc-ner				
toll like receptor 10 (tlr10) cascade				
telomere c-strand (lagging strand) synthesis				
retinoid metabolism and transport				
transcriptional regulation of pluripotent stem cells				
pausing and recovery of tat-mediated hiv elongation				
rna pol ii ctd phosphorylation and interaction with ce				
cholesterol biosynthesis				
tat-mediated hiv elongation arrest and recovery				
traf6 mediated induction of nfkb and map kinases upon tlr7/8...				
gap-filling dna repair synthesis and ligation in tc-ner				
toll like receptor 7/8 (tlr7/8) cascade				
myd88 cascade initiated on plasma membrane				
activation of the pre-replicative complex				
mhc class ii antigen presentation				
myd88mal cascade initiated on plasma membrane				
toll like receptor tlrtlr2 cascade				
transcription-coupled nucleotide excision repair (tc-ner)				
rna pol ii ctd phosphorylation and interaction with ce				
traf6 mediated induction of proinflammatory cytokines				
toll like receptor tlr1lr2 cascade				
toll like receptor 4 (tlr4) cascade				
signaling by fgfr2 in disease				
glycosphingolipid metabolism				
toll like receptor 5 (tlr5) cascade				
microRNA (mirna) biogenesis				
tat-mediated elongation of the hiv-1 transcript				
formation of rna pol ii elongation complex				
formation of hiv-1 elongation complex containing hiv-1 tat				
generic transcription pathway				
glycerophospholipid biosynthesis				
non-integrin membrane-ecm interactions				
g alpha (12/13) signalling events				
hexose transport				
citric acid cycle (tca cycle)				
cell surface interactions at the vascular wall				
smooth muscle contraction				
platelet degranulation				
response to elevated platelet cytosolic ca2+				
laminin interactions				
extracellular matrix organization				
integrin cell surface interactions				
glycolysis				

Settings

Contrast: Virus24h_vs_Control24h

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Pathway Analysis

Compare

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Menu << Compare signatures >>

Load > Pairwise scatter Signature clustering

DataView

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Compare signatures

Compare datasets

Similar experiments

SystemsBio >

Compare Signatures

Scatterplot pairs

Venn diagram

A = Virus10h_vs_Control10h C = Virus2h_vs_Control2h
B = Virus24h_vs_Control24h

Leading-edge table

name	intersection	fc.A	fc.B	fc.C
SOX11	ABC	0.718	0.259	0.598

Showing 1 to 1 of 1 entries

Settings >>

Contrasts:

- Virus10h_vs_Control10h
- Virus24h_vs_Control24h
- Virus2h_vs_Control2h

Options

https://pro.playground.bigomics.ch/?email=ikenaga@filgen.jp#tab-2343-1

Compare

Omics Playground v3 | pro.playground.bigomics.ch/?email=ikenaga@filgen.jp | bojkova2020-sarscov2 | Invite! Discuss! Help | ikenaga@filgen.jp

Similar experiments

FC correlation | FC Heatmap | Meta-network

Compare different experiments by correlating their fold-change signatures. Highly correlated logFC signatures suggest similar experiments.

FC scatter plots

Similarity scores

Search:

dataset/contrast	score	rho
[GSE89505] treatment_dose:032mgkg_vs_other	0.132	-0.302
[GSE89627] genotype_variation:sema3dnul1_vs_other	0.105	-0.272
[GSE89122] source:kidneycollectingduct_vs_Normalkidneycollecti	0.0868	0.268
[GSE85133] radiated:U_vs_N	0.0796	-0.247
[GSE85135] radiated:U_vs_N	0.0796	-0.247
[GSE115232] cell_line:C2C12_vs_4T1	0.0794	-0.249
[GSE118973] ent:16hfasting_vs_16hfasting+60mincage_vs_16hf	0.0788	0.271

Showing 1 to 9 of 594 entries

FC-FC scatterplot

$r = -0.0093$

LRRCC1

Virus10h_vs_Control10h (logFC)

[GSE89505] treatment_dose:032mgkg_vs_other (logFC)

Settings

Contrast: Virus10h_vs_Control10h

Signature DB: sigdb-archs4.h5

Advanced options

Similar Experiments | <https://pro.playground.bigomics.ch/?email=ikenaga@filgen.jp#>

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Menu

- Load
- DataView
- Clustering
- Expression
- GeneSets
- Compare
- SystemsBio
- Drug connectivity

Drug Connectivity

Drug enrichment

Drug connectivity

Mechanism of action

Activation matrix

Enrichment table

Search:

drug	NES	pval	padj	MOA	target
phorbol-12-myristate-13-acetate	2.6143	0.0014	0.0366		
cucurbitacin-i	2.4089	0.0014	0.0366		
ingenol	2.2764	0.0014	0.0366		
tricitiribine	2.2439	0.0014	0.0366	AKT inhibitor	AKT1 AKT2 AKT3
vinblastine	2.2144	0.0014	0.0366	microtubule inhibitor tubulin polymerization inhibitor	JUN TUBA1A TUBB TUBD1 TUBE1 TU...
TNF- α	2.2098	0.0014	0.0366		
phorbol-myristate-acetate	2.2075	0.0014	0.0366		
spiramide	2.1803	0.0014	0.0366		
digitoxin	2.1489	0.0014	0.0366	ATPase inhibitor	ATP1A1
AG-879	2.1458	0.0014	0.0366		
podophyllotoxin	2.134	0.0014	0.0366	microtubule inhibitor tubulin polymerization inhibitor	IGF1R TOP2A TUBA4A TUBB

Showing 1 to 9 of 621 entries

Settings

Contrast: Virus24h_vs_Control2 4h

Analysis type: activity/L1000

only annotated drugs

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