Swiss Institute of Bioinformatics

Enrichment analysis

tania.wyss@sib.swiss

gustavo.ruizbuendia@sib.swiss

ELIXIR SWITZERLAND

Schedule

- **9:00 - 9:30**
- Introduction
- **9:30 – 10:30**
- Over-representation analysis
- Exercise
- **10:30ish** break
- **10:50 - 12:30**
- Method of gene set enrichment analysis
- Exercise
- **12:30ish - 13:30** lunch break
- **13:30 - 15:30**
- Visualization of enrichment results
- Exercise
- **15:30ish - 15:50** break
- **15:50 - 16:50**
- Ontologies and sources of gene sets
- Exercise
- **16:50 - 17:00** Feedback and end of day

The Translational Data Science group

- Part of the **SIB Swiss Institute of Bioinformatics**
- Located at the AGORA Cancer Research Center in **Lausanne**
- Provides **the statistics, bioinformatics and computational expertise** to molecular biology and applied research labs.
- Participates in fundamental and translational research by providing expertise in **data analysis** of single-cell and bulk multi-omics, spatial transcriptomics, flow cytometry, etc

For core facility service inquiry: nadine.fournier@sib.swiss
https://agora-cancer.ch/scientific-platforms/translational-data-science-facility/
https://www.sib.swiss/raphael-gottardo-group

Tell us about yourself !

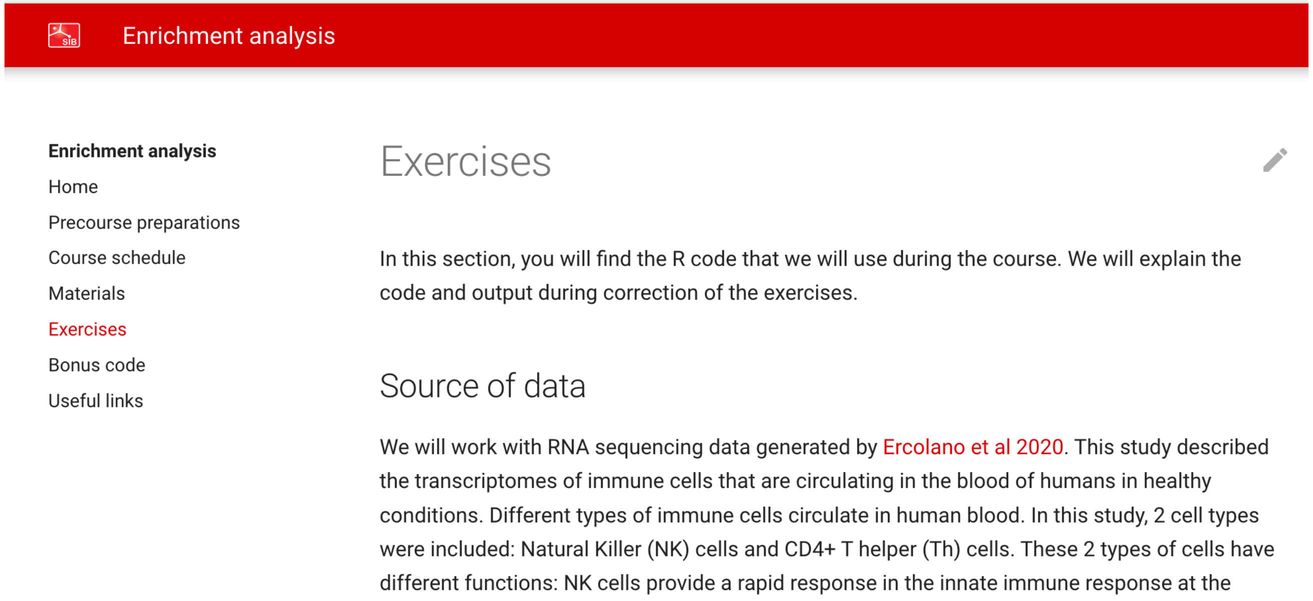
- Write your name and some keywords about yourself and/or your research into the Google doc, to share about yourself.
- vevox poll

Photo by National Cancer Institute, Unsplash

Photo by Scott Graham, Unsplash

Course material

- https://sib-swiss.github.io/enrichment-analysis-training/



Enrichment analysis

Exercises

In this section, you will find the R code that we will use during the course. We will explain the code and output during correction of the exercises.

Source of data

We will work with RNA sequencing data generated by Ercolano et al 2020. This study described the transcriptomes of immune cells that are circulating in the blood of humans in healthy conditions. Different types of immune cells circulate in human blood. In this study, 2 cell types were included: Natural Killer (NK) cells and CD4+ T helper (Th) cells. These 2 types of cells have different functions: NK cells provide a rapid response in the innate immune response at the

- Feedback: survey at the end of the day about your opinion on this course (link sent by course organizer).

Credits: 0.25 ECTS

- Please provide answers and R code for an additional exercise (eg 1 Word with answers and figures and 1 script file, or 1 file generated using Rmarkdown)

https://sib-swiss.github.io/enrichment-analysis-training/exercises/#extra-exercise-for-ects-credits

- Sign up for credit by adding your name to the google Doc file (email sent by course organizer)
- Send answers to tania.wyss@sib.swiss within 1 week

Questions and Exercises

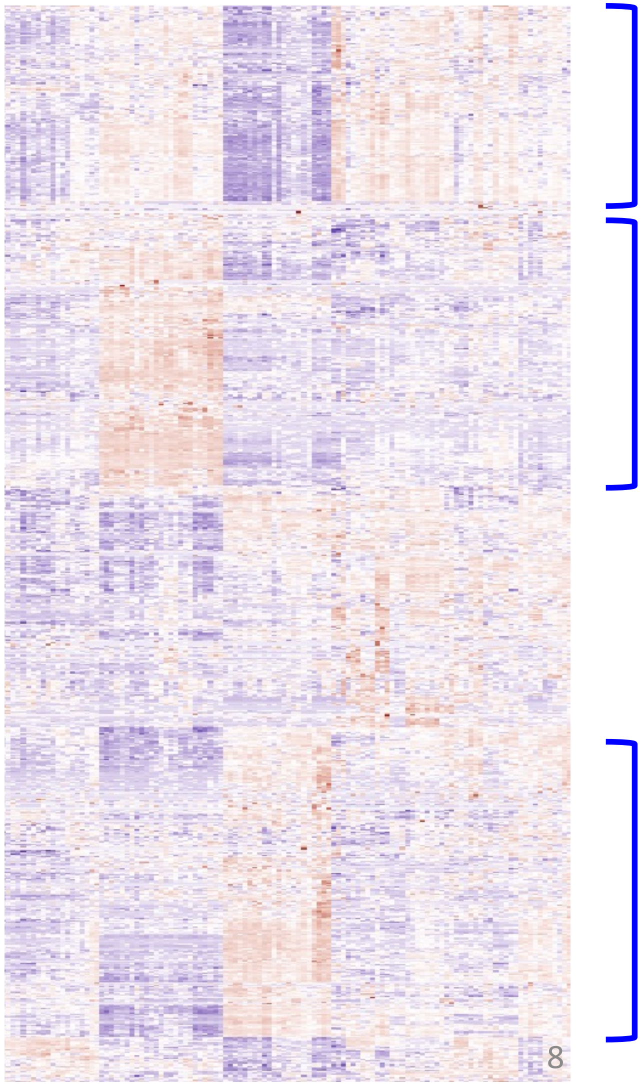
- Feel free to interrupt with questions by asking them directly or raising your (virtual) hand.
- Use the Q&A in Google Doc (or Zoom chat), we will provide answers
- Add a ✅ when you are done with the current exercise

- Exercises in R:
  - We will try to debug as much as possible
  - We are happy if you share your results or alternative code!

Why do we perform enrichment analysis?

- Gene expression analysis yields hundreds to thousands of significant genes
  - We need to summarize the information provided by so many genes
  - Understand their biological relationships
  - Understand the genes' function (**functional analysis**)
  - Identify overarching biological processes or molecular pathways taking place in your system

Some genes have similar expression pattern across samples

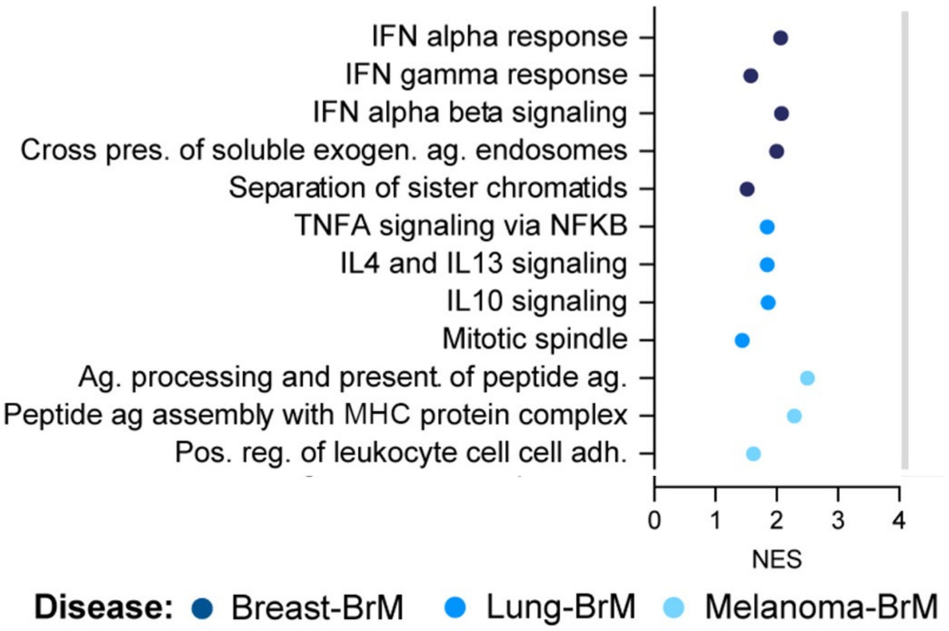


IVY GAP: https://glioblastoma.alleninstitute.org/

Enrichment analysis in the literature – non-exhaustive examples

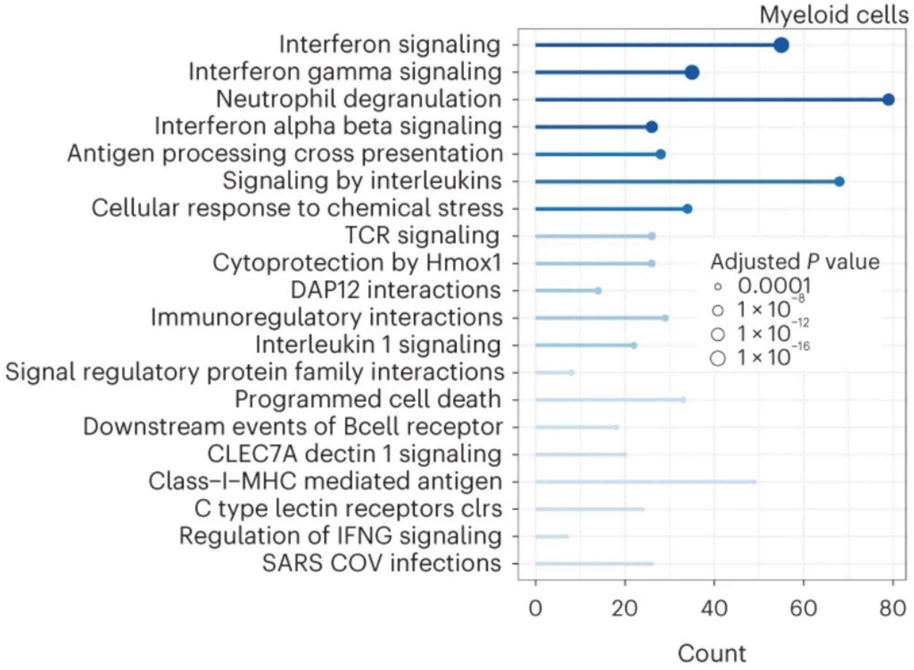
Often presented in *omics* studies

Different molecular alterations in vasculature of brain metastasis from different origins, compared to normal brain vasculature



Bulk RNAseq (GSEA)
https://doi.org/10.1016/j.ccell.2023.12.018

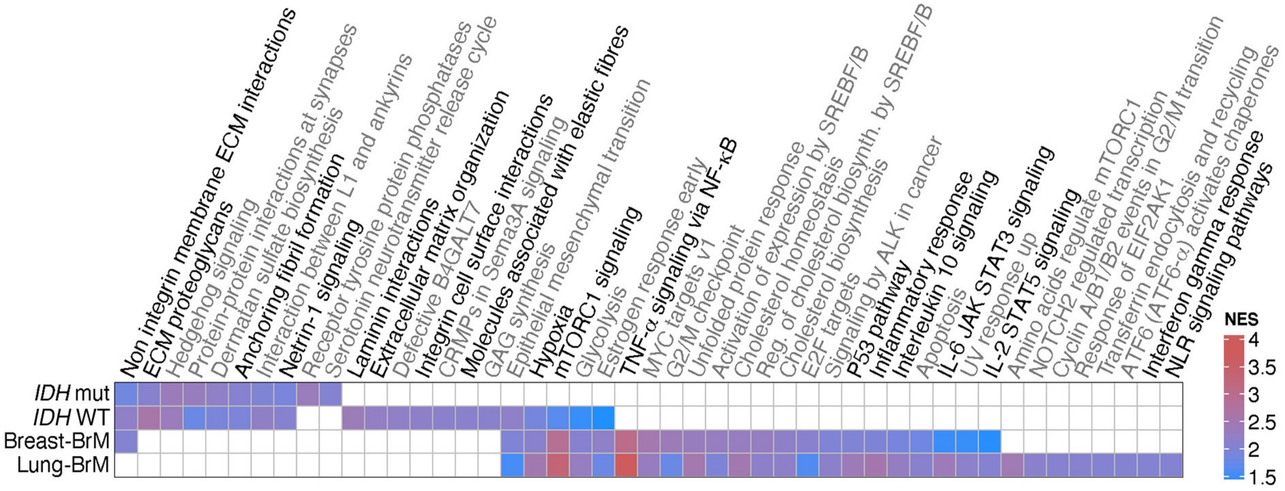
Impact of a treatment on myeloid cells, pathways that could contribute to tumor growth limitation



Single-cell RNAseq (ORA)
https://doi.org/10.1038/s43018-023-00668-y

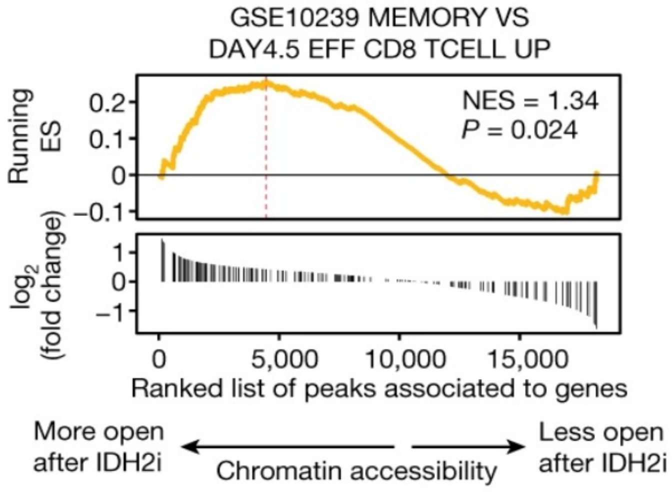
Enrichment analysis in the literature – non-exhaustive examples

Neutrophils (immune cells) express different pathways depending on the brain tumor genotype (mut/WT) or origin (primary vs metastatic tumor)



Bulk RNAseq (GSEA)

https://doi.org/10.1016/j.cell.2023.08.043



Increased memory phenotype in immune cells exposed to a component.

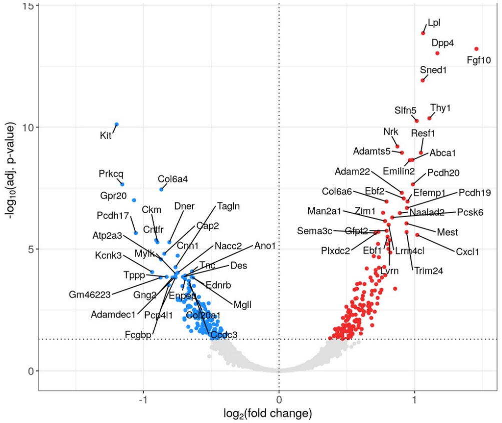
Bulk ATACseq (GSEA)

https://doi.org/10.1038/s41586-023-06546-y

Enrichment analysis – input data

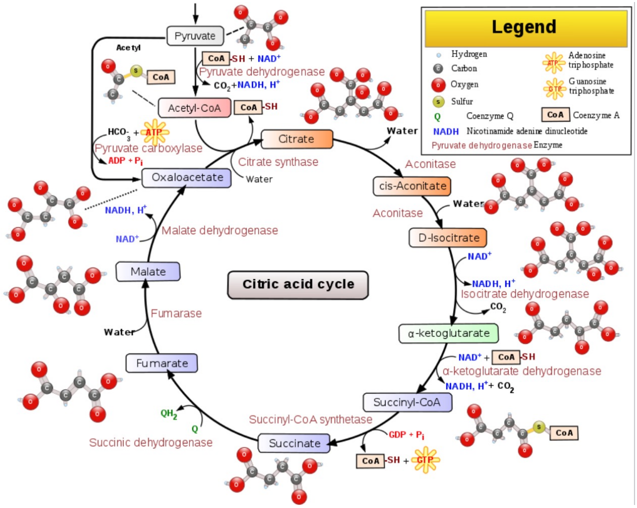
List of genes/proteins that are:

- Differentially expressed between 2 conditions
- Similar expression pattern across samples
- …
- Either available as a list of gene symbols/IDs or with a score associated to each gene: *e.g.* T statistic or fold change



Database of gene/protein functional annotation

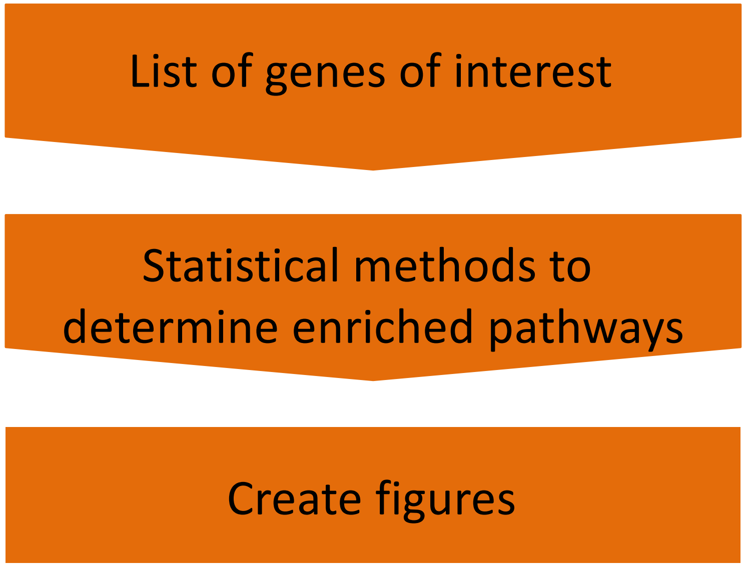
- Genes need to be grouped into gene sets/pathways/functional annotations.
- Consortia of researchers usually create these gene groupings/annotations



https://en.wikipedia.org/wiki/Citric_acid_cycle

Enrichment analysis - three major steps

- Obtain a gene/protein list from omics data
- Apply statistical methods to identify pathways enriched in the gene list relative to what is expected by chance
- Visualize and interpret the results



List of genes of interest

Statistical methods to determine enriched pathways

Create figures

Enrichment analysis in non-model organisms

- Need functional annotation of genes: genes need to be grouped into pathways/functions.
- If not available, convert your genes into the orthologs of a closely related species that has such a database.
- Will require effort to find a gene functional annotation database. All statistical analyses are otherwise the same.

See Useful links:

https://sib-swiss.github.io/enrichment-analysis-training/links/#tools-for-species-other-than-human-or-mouse

Approaches used in enrichment analysis

Test your gene list for enrichment of:

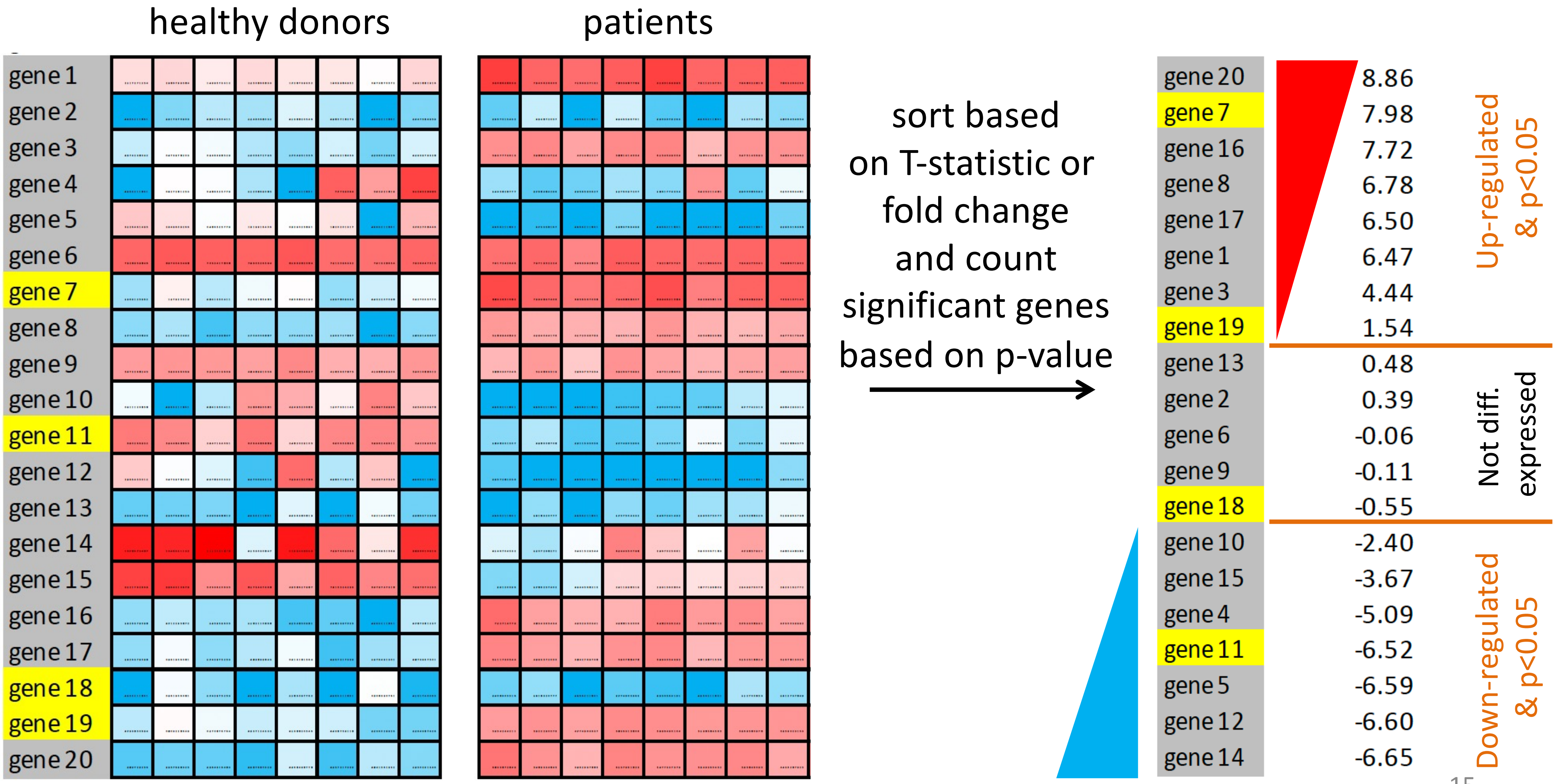
- Genes associated with a particular function or pathway (targeted)
- Genes annotated into a large collection of gene sets (exploratory)

Statistical methods available (covered today):

- over-representation analysis (ORA)
- gene set enrichment analysis (GSEA)

Over-representation analysis (ORA)

Are the DE genes overlapping with the genes contained within the yellow set?



sort based on T-statistic or fold change and count significant genes based on p-value →

gene 20	8.86	Up-regulated & p<0.05
gene 7	7.98	
gene 16	7.72	
gene 8	6.78	
gene 17	6.50	
gene 1	6.47	
gene 3	4.44	
gene 19	1.54	
gene 13	0.48	Not diff. expressed
gene 2	0.39	
gene 6	-0.06	
gene 9	-0.11	
gene 18	-0.55	
gene 10	-2.40	Down-regulated & p<0.05
gene 15	-3.67	
gene 4	-5.09	
gene 11	-6.52	
gene 5	-6.59	
gene 12	-6.60	
gene 14	-6.65	

Fisher’s exact test

2 x 2 count table	**Up-regulated**	**Not up-regulated**	**Total**
Yellow	2	2	4
Not yellow	6	9	15
Total	8	11	19

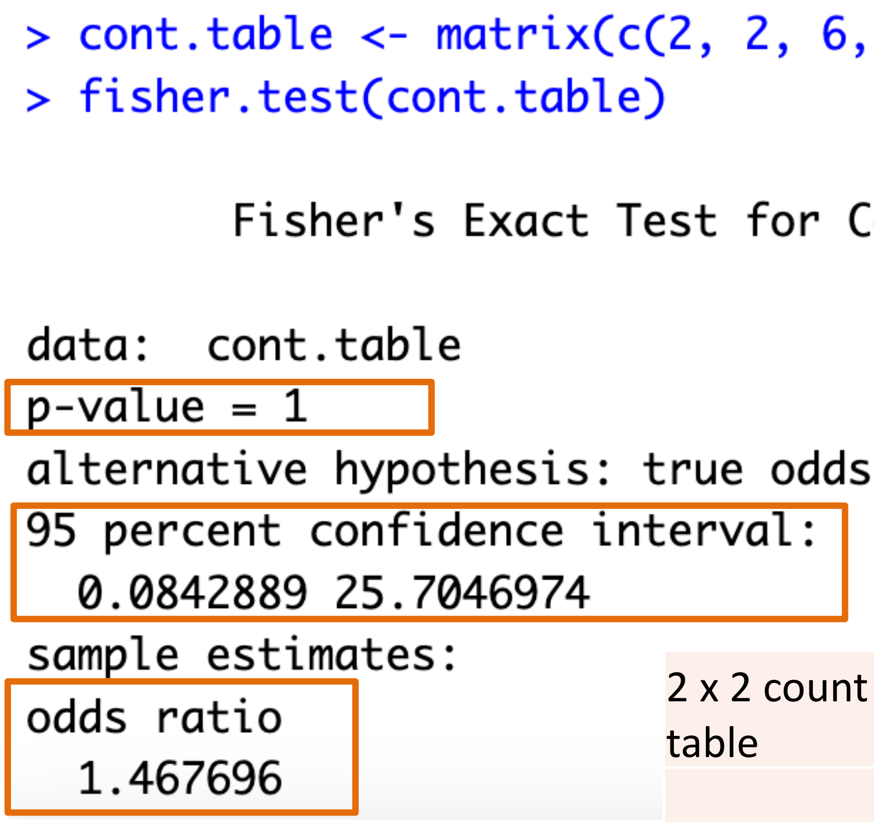
contingency table

H_0: The proportion of yellow genes up-regulated is the same as the proportion of yellow genes that are not up-regulated.

H_1: The proportion of yellow genes up-regulated is not the same as the proportion of yellow genes that are not up-regulated.

http://mengnote.blogspot.com/2012/12/calculate-correct-hypergeometric-p.html

Fisher’s exact test in R



```
> cont.table <- matrix(c(2, 2, 6, 9), ncol=2, byrow = T)
> fisher.test(cont.table)

	Fisher's Exact Test for Count Data

data:  cont.table
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.0842889 25.7046974
sample estimates:
odds ratio
  1.467696
```

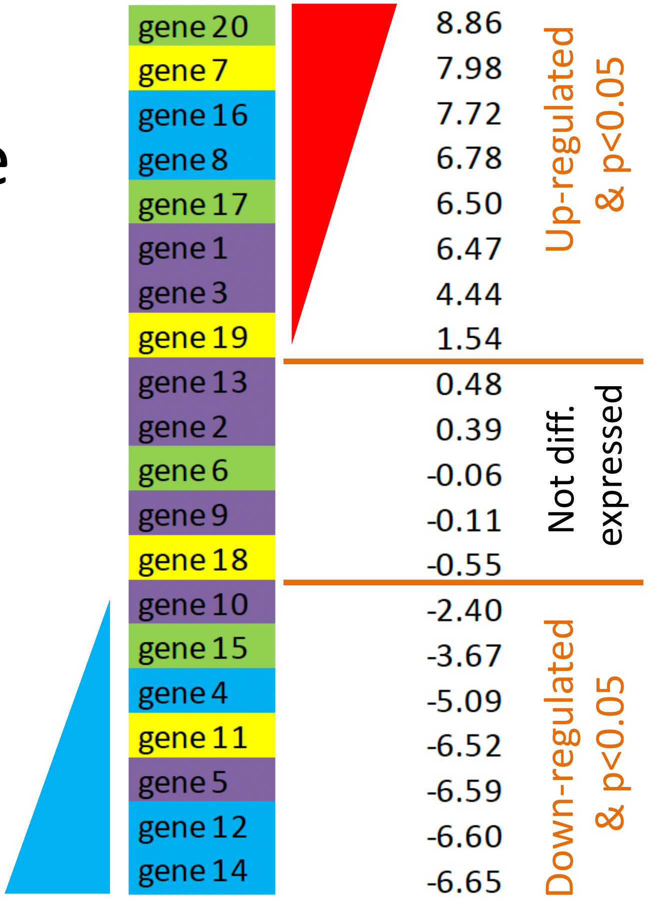
2 x 2 count table	**Up-regulated**	**Not up-regulated**	**Total**
Yellow	2	2	4
Not yellow	6	9	15
Total	8	11	19
	2/8 = 0.25	2/12 = 0.167	

Which gene sets are differentially expressed?

🟩	gene 20	8.86
🟨	gene 7	7.98
🟦	gene 16	7.72
🟦	gene 8	6.78
🟩	gene 17	6.50
🟪	gene 1	6.47
🟪	gene 3	4.44
🟨	gene 19	1.54
🟪	gene 13	0.48
🟪	gene 2	0.39
🟩	gene 6	-0.06
🟪	gene 9	-0.11
🟨	gene 18	-0.55
🟪	gene 10	-6.27
🟩	gene 15	-6.30
🟦	gene 4	-6.50
🟨	gene 11	-6.52
🟪	gene 5	-6.59
🟦	gene 12	-6.60
🟦	gene 14	-6.65

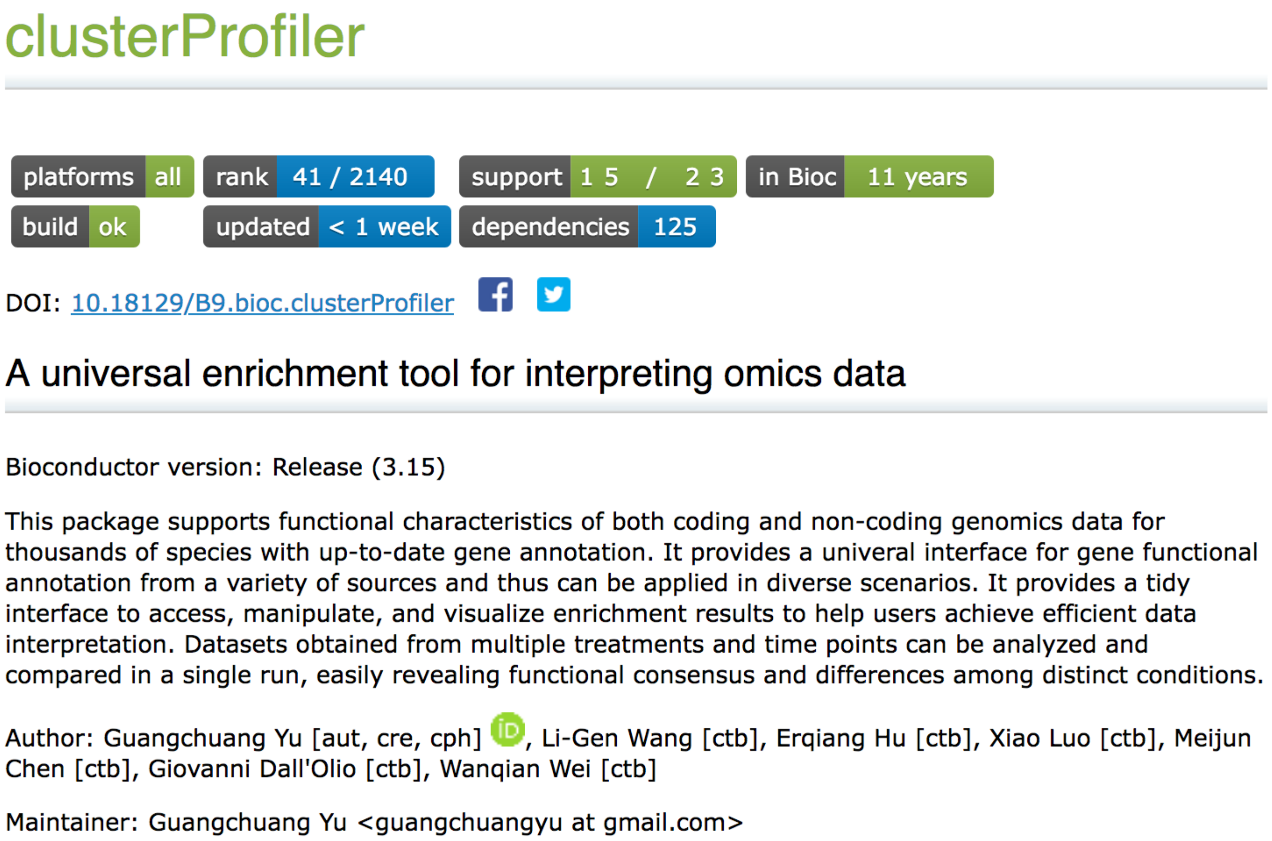
Run individual Fisher's exact tests for each gene set, yellow, blue, purple, green

⇒Multiple tests need p-value adjustment.



🟩	gene 20	8.86	Up-regulated & $p<0.05$
🟨	gene 7	7.98	
🟦	gene 16	7.72	
🟦	gene 8	6.78	
🟩	gene 17	6.50	
🟪	gene 1	6.47	
🟪	gene 3	4.44	
🟨	gene 19	1.54	
🟪	gene 13	0.48	Not diff. expressed
🟪	gene 2	0.39	
🟩	gene 6	-0.06	
🟪	gene 9	-0.11	
🟨	gene 18	-0.55	
🟪	gene 10	-2.40	Down-regulated & $p<0.05$
🟩	gene 15	-3.67	
🟦	gene 4	-5.09	
🟨	gene 11	-6.52	
🟪	gene 5	-6.59	
🟦	gene 12	-6.60	
🟦	gene 14	-6.65	

Enrichment analysis using R: one possibility among others



clusterProfiler

platforms all | rank 41 / 2140 | support 1 5 / 2 3 | in Bioc 11 years

build ok | updated < 1 week | dependencies 125

DOI: [10.18129/B9.bioc.clusterProfiler]()

A universal enrichment tool for interpreting omics data

Bioconductor version: Release (3.15)

This package supports functional characteristics of both coding and non-coding genomics data for thousands of species with up-to-date gene annotation. It provides a univeral interface for gene functional annotation from a variety of sources and thus can be applied in diverse scenarios. It provides a tidy interface to access, manipulate, and visualize enrichment results to help users achieve efficient data interpretation. Datasets obtained from multiple treatments and time points can be analyzed and compared in a single run, easily revealing functional consensus and differences among distinct conditions.

Author: Guangchuang Yu [aut, cre, cph], Li-Gen Wang [ctb], Erqiang Hu [ctb], Xiao Luo [ctb], Meijun Chen [ctb], Giovanni Dall'Olio [ctb], Wanqian Wei [ctb]

Maintainer: Guangchuang Yu <guangchuangyu at gmail.com>

Built-in functions for enrichment analysis

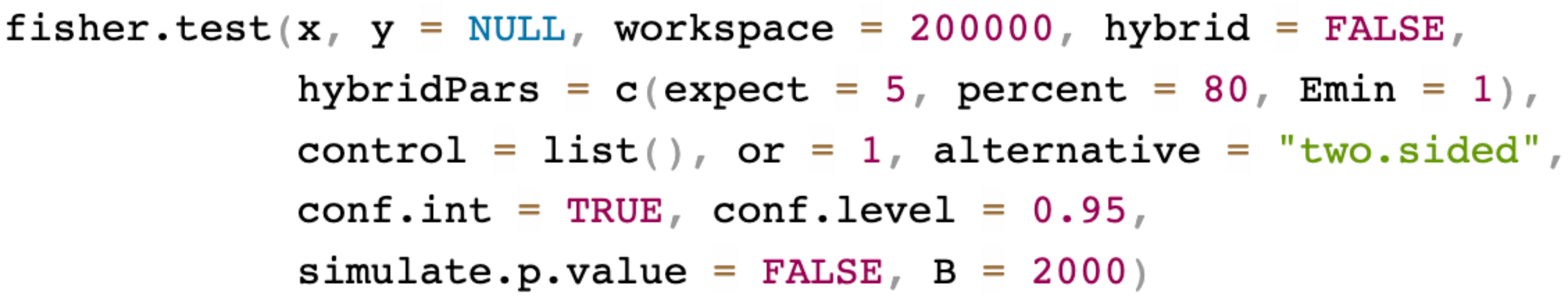
Built-in gene sets for human, mouse, yeast, etc

Built-in GO and KEGG (see later)

- ***https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html***
- ***G Yu***, LG Wang, Y Han, QY He. clusterProfiler: an R package for comparing biological themes among gene clusters. ***OMICS: A Journal of Integrative Biology*** 2012, 16(5):284-287. doi:[10.1089/omi.2011.0118](http://dx.doi.org/10.1089/omi.2011.0118)
- Full vignette: http://yulab-smu.top/clusterProfiler-book/

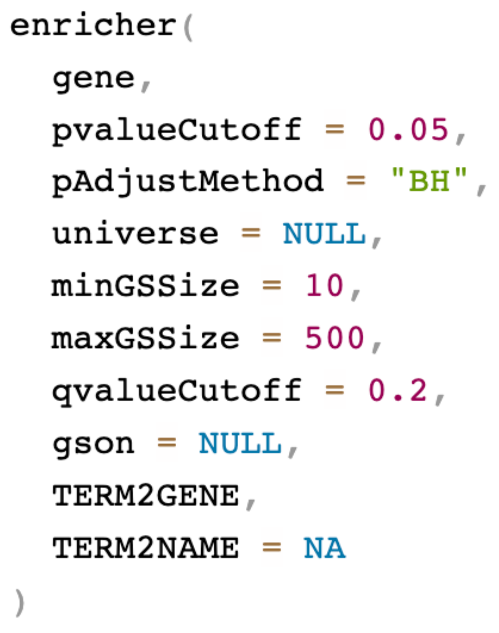
Functions for Fisher test and for ORA with R and clusterProfiler

Fisher exact test (package stats)



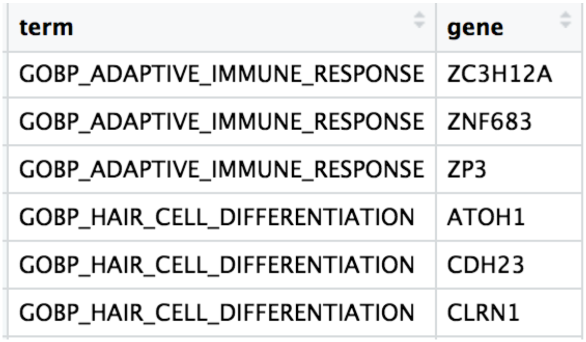
```
fisher.test(x, y = NULL, workspace = 200000, hybrid = FALSE,
            hybridPars = c(expect = 5, percent = 80, Emin = 1),
            control = list(), or = 1, alternative = "two.sided",
            conf.int = TRUE, conf.level = 0.95,
            simulate.p.value = FALSE, B = 2000)
```

enricher(): implementation of hypergeometric test (one-sided Fisher test) for user defined gene list and gene set collections (package clusterProfiler)



```
enricher(
  gene,
  pvalueCutoff = 0.05,
  pAdjustMethod = "BH",
  universe = NULL,
  minGSSize = 10,
  maxGSSize = 500,
  qvalueCutoff = 0.2,
  gson = NULL,
  TERM2GENE,
  TERM2NAME = NA
)
```

TERM2GENE:
A 2-column
data frame

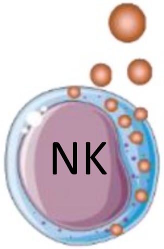


term	gene
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZC3H12A
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZNF683
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZP3
GOBP_HAIR_CELL_DIFFERENTIATION	ATOH1
GOBP_HAIR_CELL_DIFFERENTIATION	CDH23
GOBP_HAIR_CELL_DIFFERENTIATION	CLRN1

Eg genes that are markers of cell clusters of single-cell RNA seq

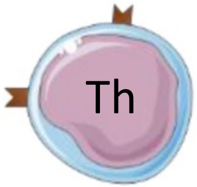
vevox

Innate immunity



Recap and exercise 1

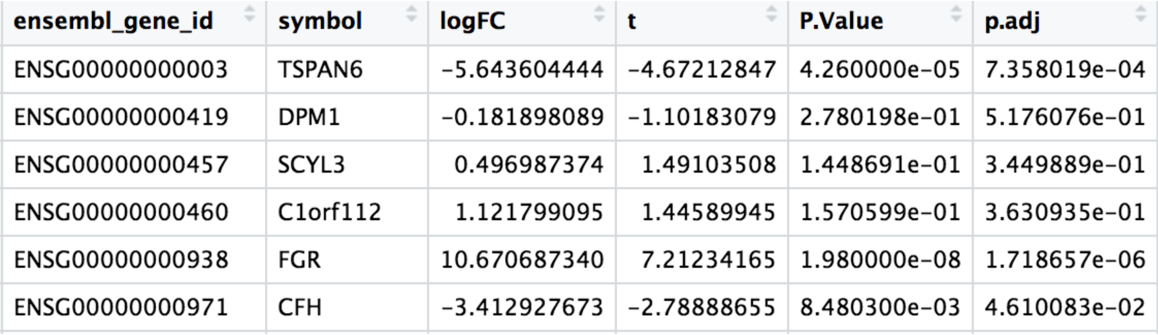
Adaptive immunity



https://www.mdpi.com/1420-3049/24/24/4530/htm

- Once we have identified differentially expressed (DE) genes, we can use an over-representation analysis to determine whether or not the genes of a gene set of interest are over-represented among the DE genes or not.

- Exercise 1:
- Results table of differential gene expression analysis between 2 human immune cell types, natural killer (NK) cells and CD4 T helper cells (Th):



ensembl_gene_id	symbol	logFC	t	P.Value	p.adj
ENSG00000000003	TSPAN6	-5.643604444	-4.67212847	4.260000e-05	7.358019e-04
ENSG00000000419	DPM1	-0.181898089	-1.10183079	2.780198e-01	5.176076e-01
ENSG00000000457	SCYL3	0.496987374	1.49103508	1.448691e-01	3.449889e-01
ENSG00000000460	C1orf112	1.121799095	1.44589945	1.570599e-01	3.630935e-01
ENSG00000000938	FGR	10.670687340	7.21234165	1.980000e-08	1.718657e-06
ENSG00000000971	CFH	-3.412927673	-2.78888655	8.480300e-03	4.610083e-02

Positive logFC = higher in NK
Negative logFC = lower in NK

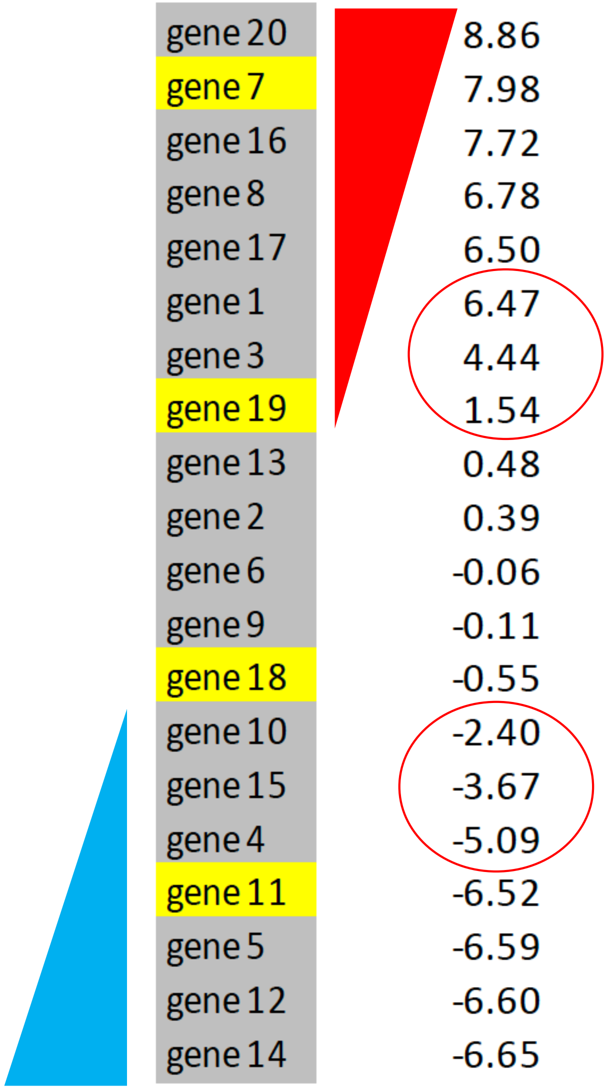
- Run a **Fisher’s exact test** to determine whether genes involved in the **adaptive immune response** are over-represented among the genes up-regulated in Th cells.

RNA sequencing data from:
https://jlb.onlinelibrary.wiley.com/doi/full/10.1002/JLB.5MA0120-209R?af=R
https://ashpublications.org/bloodadvances/article/3/22/3674/428873/CD56-as-a-marker-of-an-ILC1-like-population-with

Fisher’s exact test is threshold-based

2 x 2 count table	**Up-regulated**	**Not up-regulated**	**Total**
Yellow	2	2	4
Not yellow	6	9	15
Total	8	11	19

Contingency table with count of genes, does not take into account the magnitude of the change of each gene.



gene20	8.86
gene7	7.98
gene16	7.72
gene8	6.78
gene17	6.50
gene1	6.47
gene3	4.44
gene19	1.54
gene13	0.48
gene2	0.39
gene6	-0.06
gene9	-0.11
gene18	-0.55
gene10	-2.40
gene15	-3.67
gene4	-5.09
gene11	-6.52
gene5	-6.59
gene12	-6.60
gene14	-6.65

http://mengnote.blogspot.com/2012/12/calculate-correct-hypergeometric-p.html

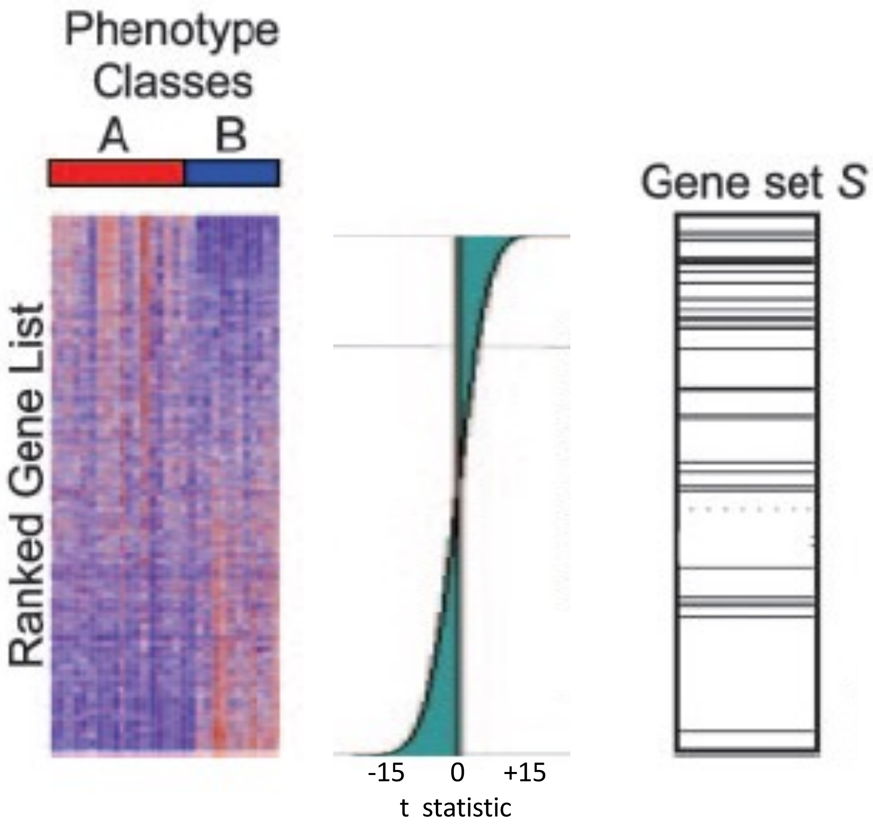
Gene set enrichment analysis (GSEA)

- **Threshold-free**: the whole list of genes detected in the omics data is used.
- GSEA is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (MSigDB)
- Rank all genes based on score (eg t-statistic) and calculate an enrichment score (ES) that reflects the degree to which the members of a gene set are overrepresented at the top or bottom of the ranked genes.

Subramanian et al PNAS 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles

Method of GSEA

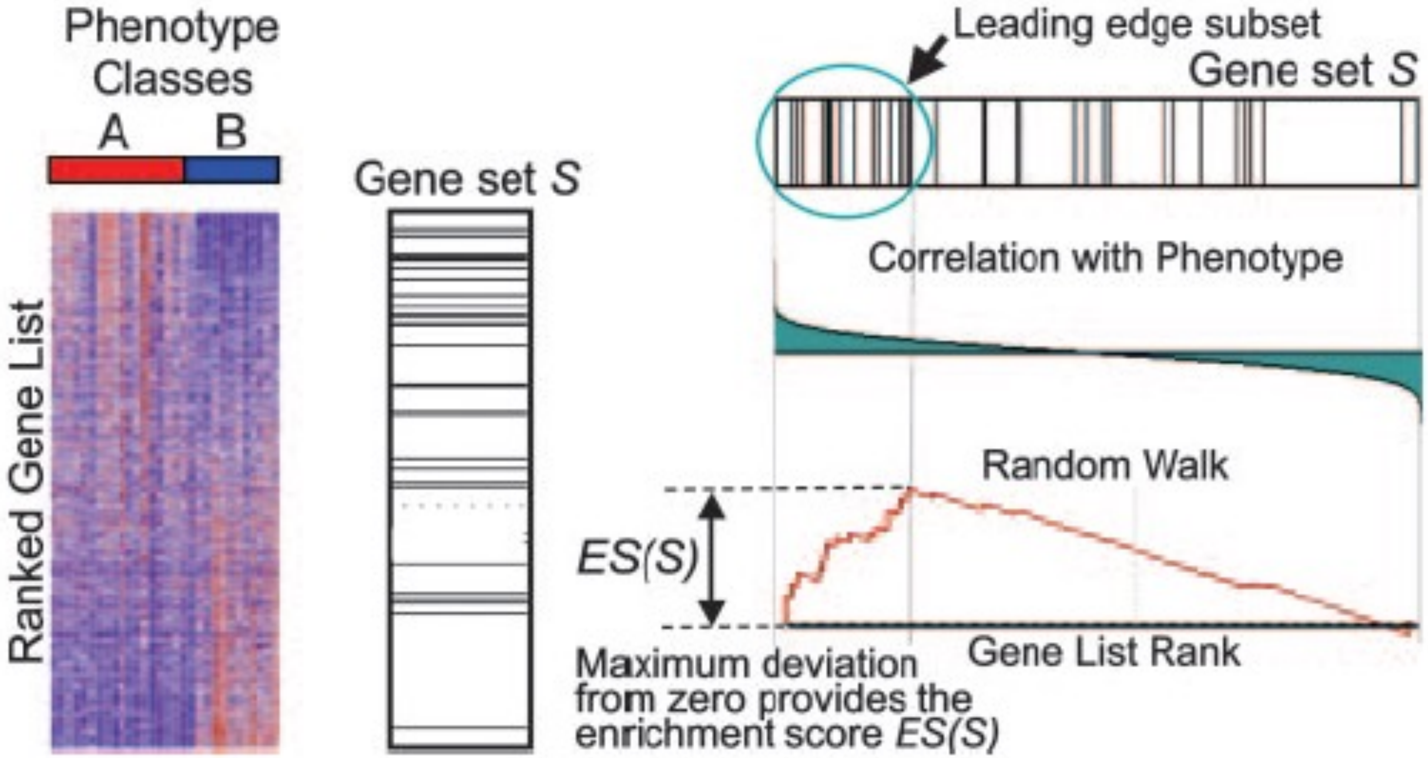
Goal: determine whether the members of a gene set **S** are randomly distributed throughout a ranked gene list or if they are located at the top or bottom of the ranked gene lists



1. Sort the genes based on the t statistic (=weight)

Subramanian et al PNAS 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles

Method of GSEA

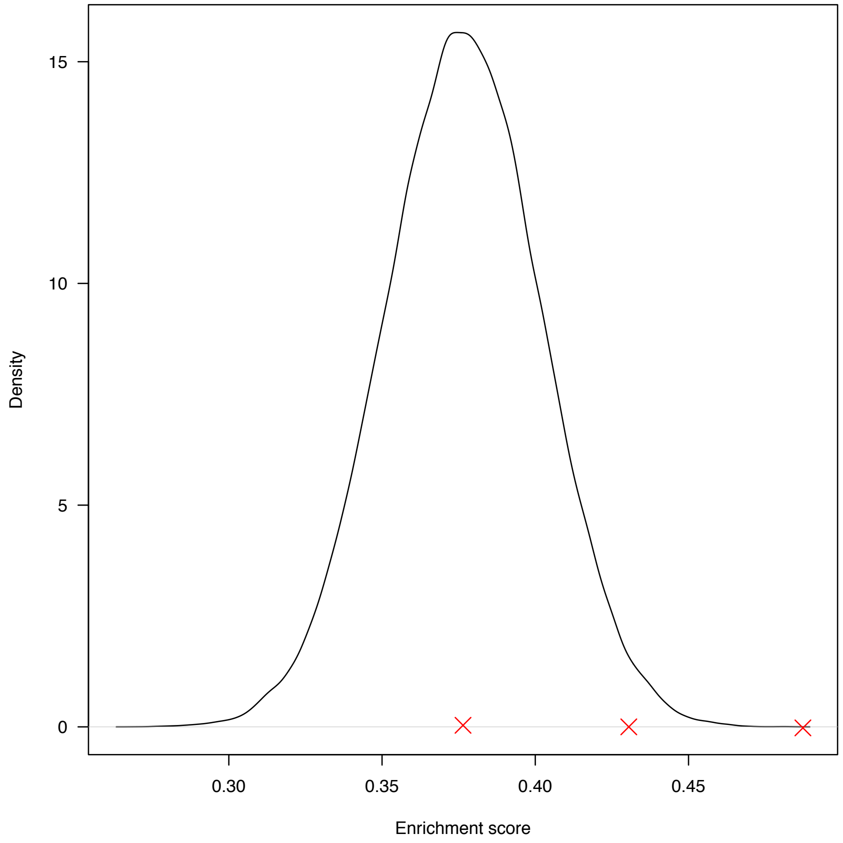


1. Sort the genes based on the t statistic (=weight)
2. Calculate enrichment score ES using weight. The ES for a set is the maximum value reached (pos. or neg.)

For ES equation, see Appendix in Subramanian et al 2005: https://www.pnas.org/doi/epdf/10.1073/pnas.0506580102

Method of GSEA

1. Sort the genes based on the t statistic (=weight)
2. Calculate enrichment score ES using weight. The ES for a set is the maximum value reached (pos. or neg.)
3. Perform permutations of samples and/or genes to recalculate random ES scores
4. Calculate Normalized ES (NES) and estimate p-value of each gene set based on randomized ES scores
5. Adjust p-value



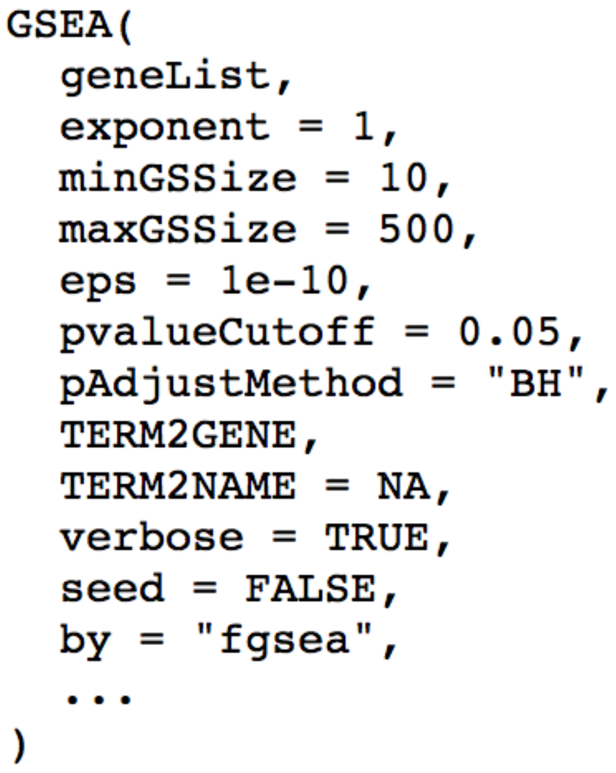
NES: 1	NES: 1.16	NES: 1.32
p: 0.5	p: 0.05	p: 0.001

$$\text{NES} = \frac{\text{actual ES}}{\mathit{mean}(\text{ESs against all permutations of the dataset})}$$

Do not forget p-value adjustment if more than 1 gene set is tested!

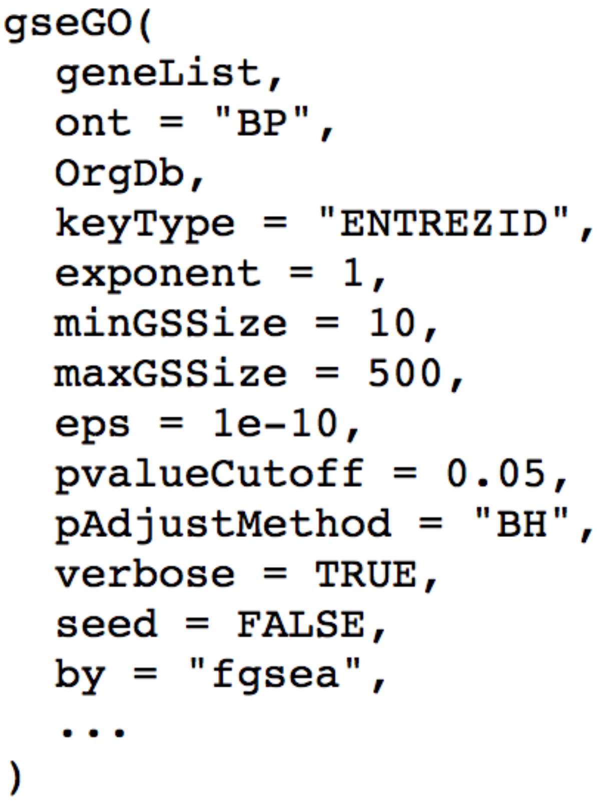
Functions for GSEA with clusterProfiler

GSEA(): GSEA of user-defined gene sets using all ranked genes



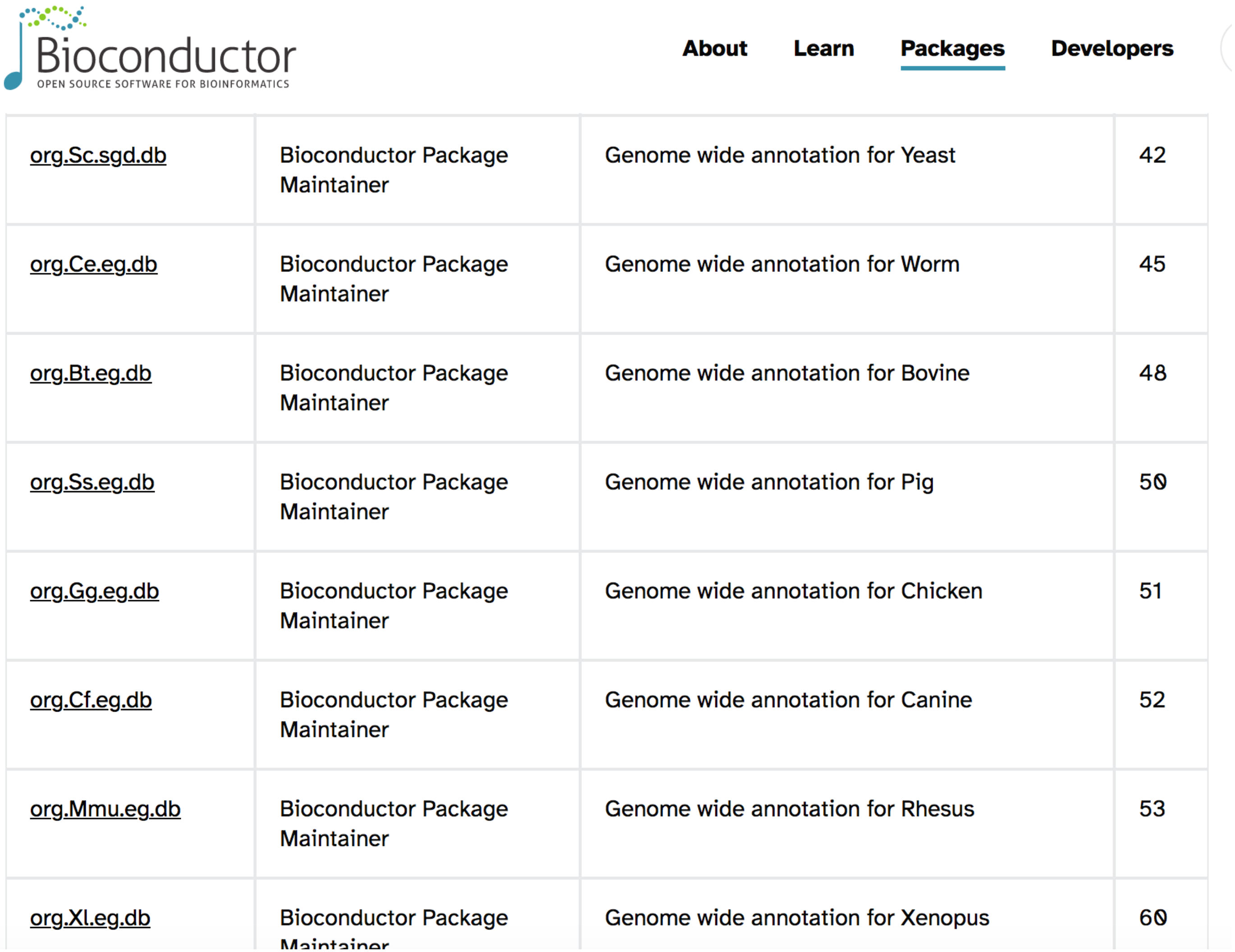
```
GSEA(
  geneList,
  exponent = 1,
  minGSSize = 10,
  maxGSSize = 500,
  eps = 1e-10,
  pvalueCutoff = 0.05,
  pAdjustMethod = "BH",
  TERM2GENE,
  TERM2NAME = NA,
  verbose = TRUE,
  seed = FALSE,
  by = "fgsea",
  ...
)
```

gseGO(): GSEA of GO gene sets using all ranked genes



```
gseGO(
  geneList,
  ont = "BP",
  OrgDb,
  keyType = "ENTREZID",
  exponent = 1,
  minGSSize = 10,
  maxGSSize = 500,
  eps = 1e-10,
  pvalueCutoff = 0.05,
  pAdjustMethod = "BH",
  verbose = TRUE,
  seed = FALSE,
  by = "fgsea",
  ...
)
```

Bioconductor orgDb packages



org.Sc.sgd.db	Bioconductor Package Maintainer	Genome wide annotation for Yeast	42
org.Ce.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Worm	45
org.Bt.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Bovine	48
org.Ss.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Pig	50
org.Gg.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Chicken	51
org.Cf.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Canine	52
org.Mmu.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Rhesus	53
org.Xl.eg.db	Bioconductor Package Maintainer	Genome wide annotation for Xenopus	60

https://bioconductor.org/packages/3.18/BiocViews.html#___OrgDb

vevox

Recap and exercise 2

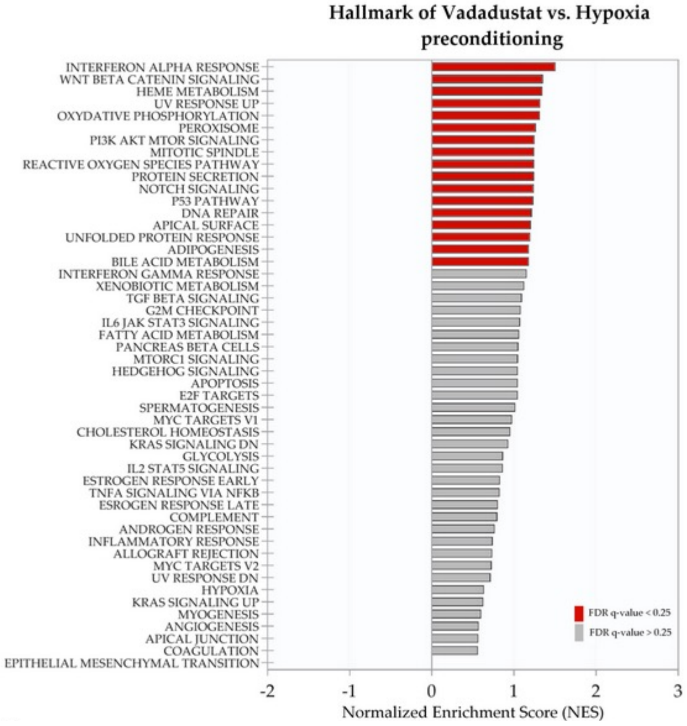
- Fisher test is a threshold-based method, while GSEA is a threshold-free enrichment method. Both can be used for single or multiple gene sets.

- Exercise 2: use functions of clusterProfiler and data provided in Ex. 1
  - Run a GSEA for the Gene Ontology gene sets (more details on this collection later)
  - Explore the results: how many gene sets are significant? Are the gene sets up-regulated or down-regulated in NK cells?

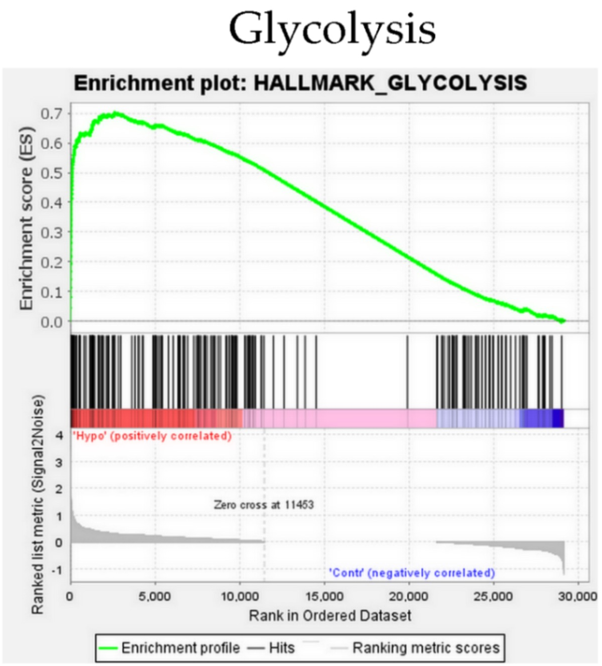
Visualization of enrichment results

There are many options, here some common ones:

Barplot of NES for many pathways:



GSEA plot for a single pathway:



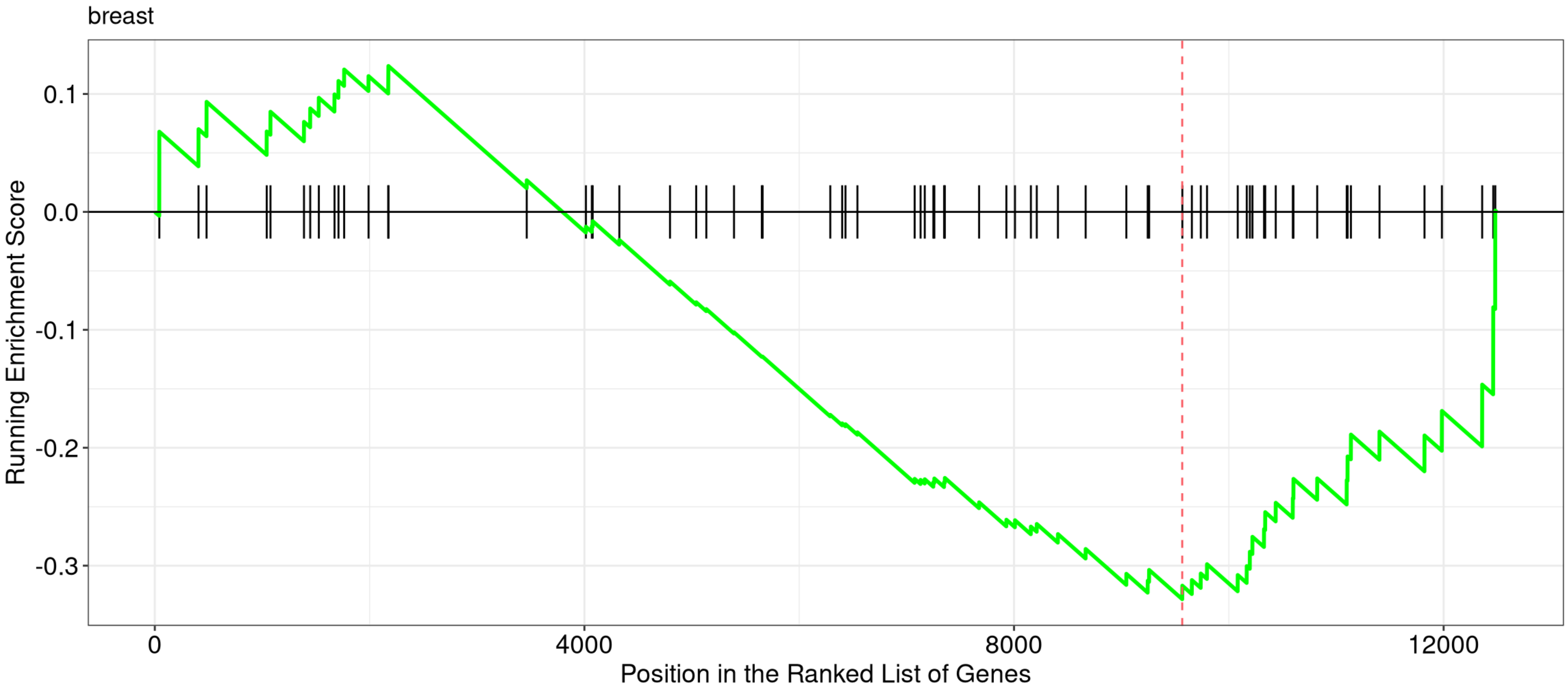
Zielniok et al 2021
Int. J. Mol. Sci. **2021**, *22*(15), 8160; https://doi.org/10.3390/ijms22158160

Via enrichplot: package for visualization using clusterProfiler objects
https://www.bioconductor.org/packages/release/bioc/html/enrichplot.html

Visualizations available in clusterProfiler

GSEA plot (or barcode plot; for gseaResult objects)

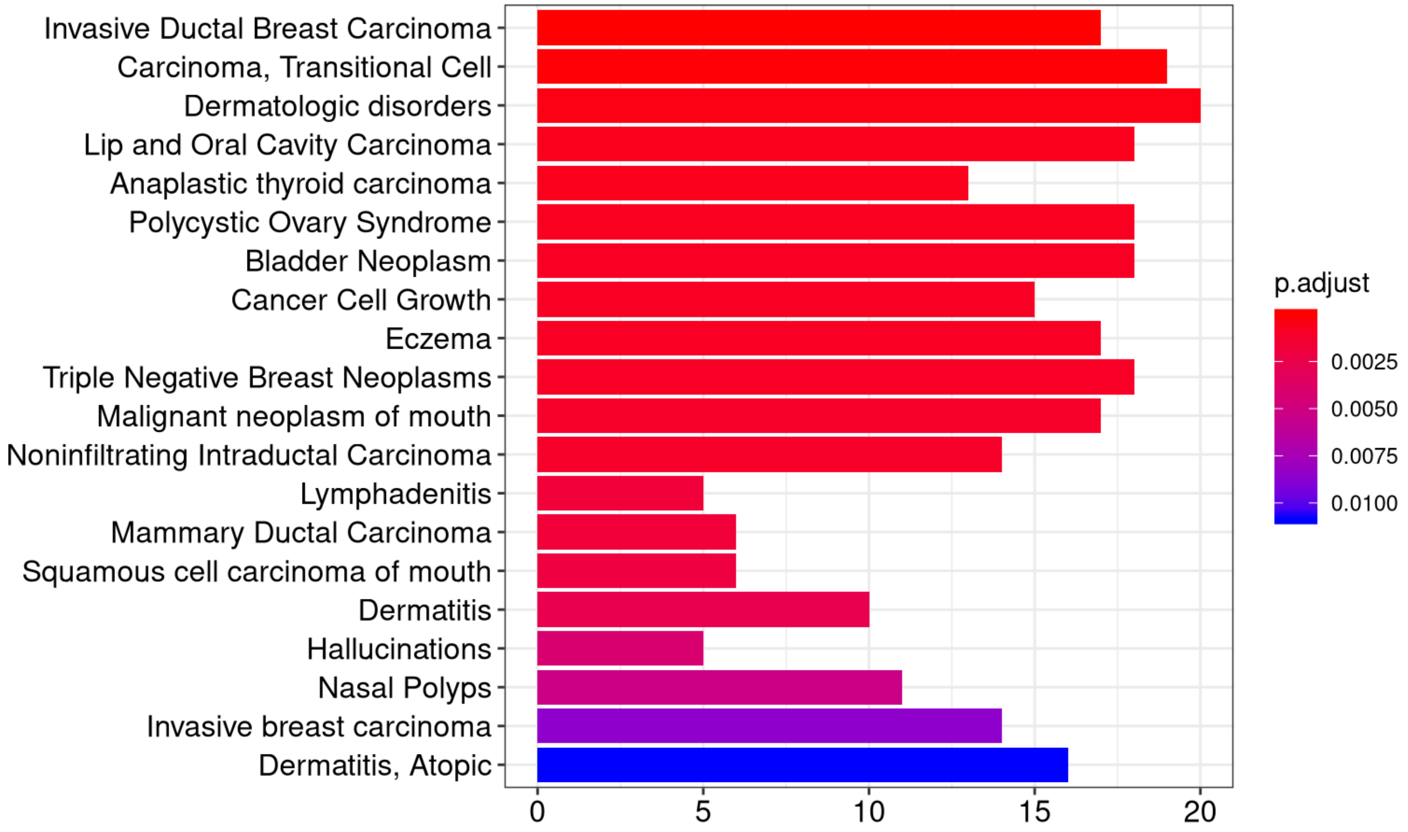
```
> gseaplot(h_NK_vs_Th, geneSetID = “breast", title=" breast")
```



Visualizations available in clusterProfiler

barplot (from graphics package but works on enrichResult objects)

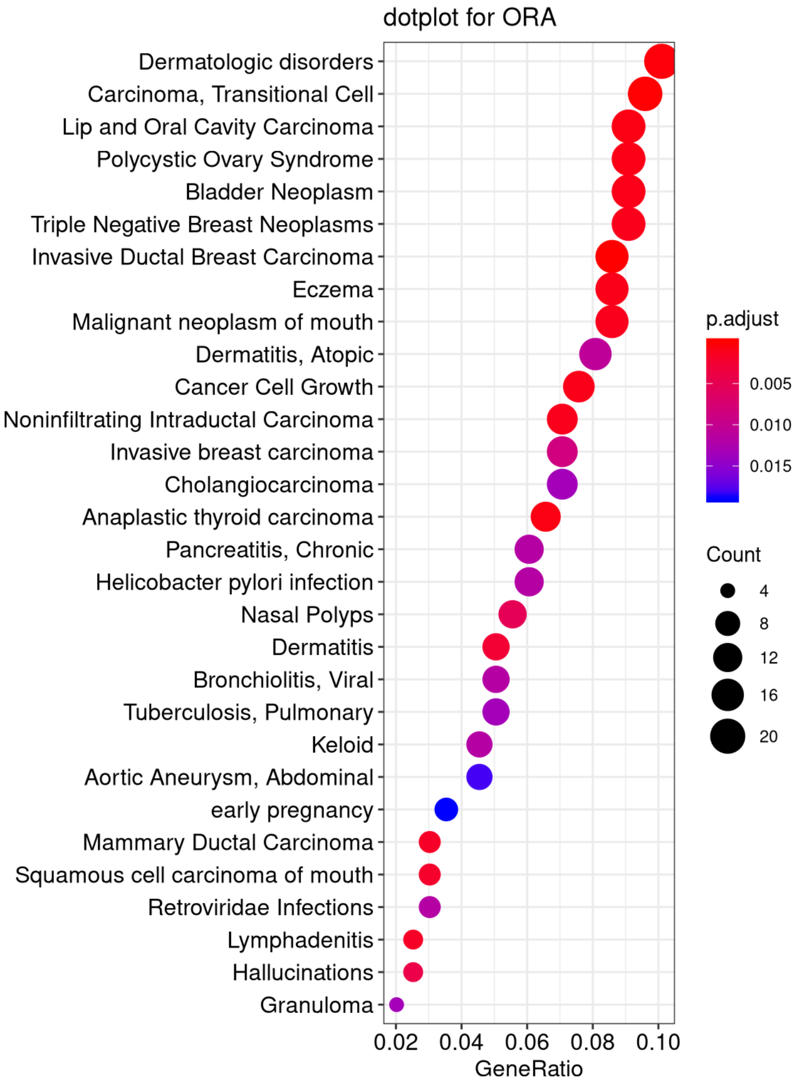
```
> ego <- enrichGO(de, OrgDb=org.Hs.eg.db, ont="BP", keyType = "SYMBOL")
> barplot(ego, showCategory=20)
```

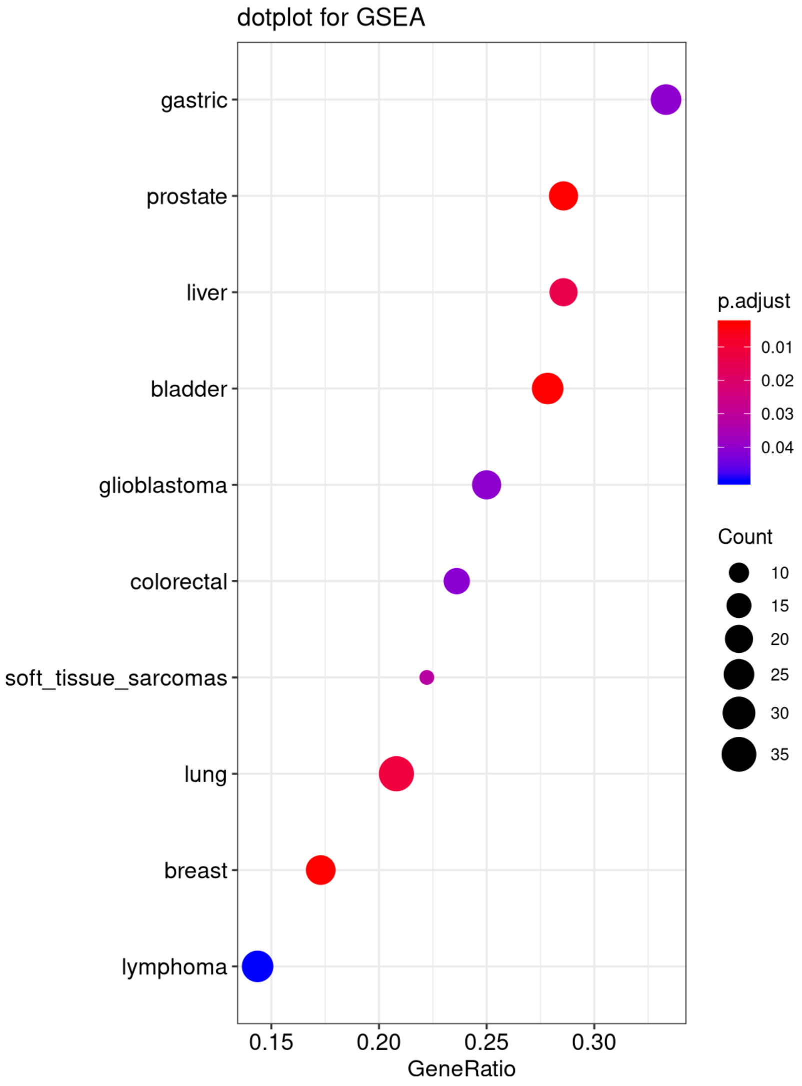


Visualizations available in clusterProfiler

dotplot

```
> ego <- enrichGO(de)
> dotplot(ego, showCategory=20)
```

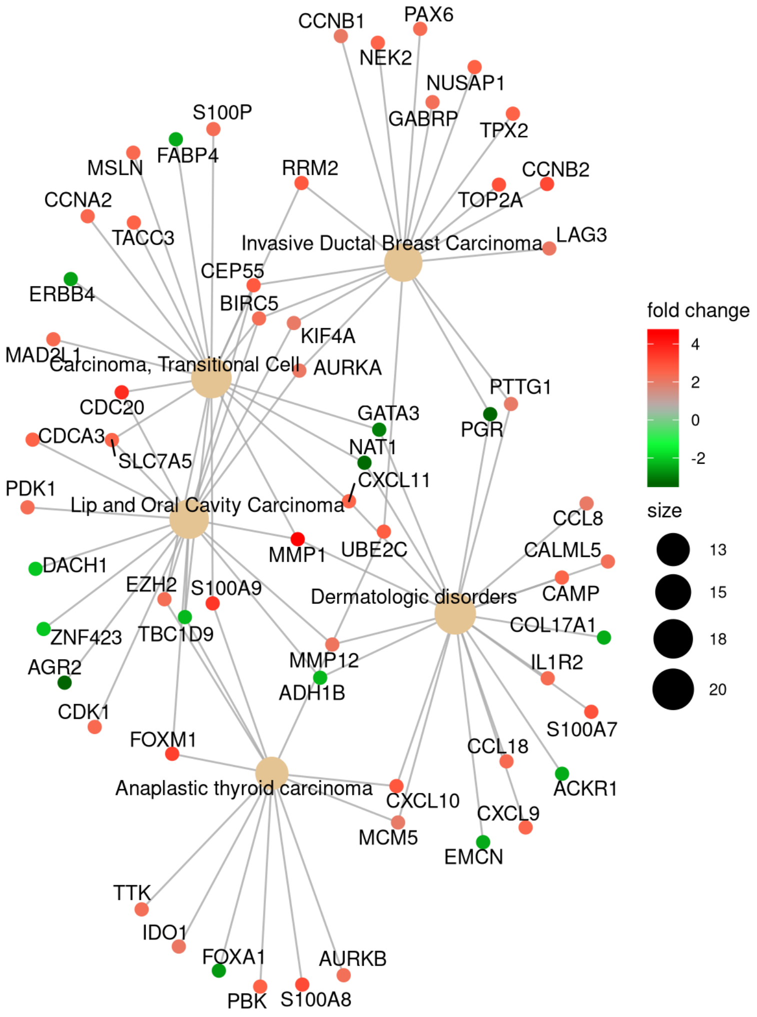

dotplot for ORA


dotplot for GSEA

Visualizations available in clusterProfiler

```
> cnetplot(ego, categorySize="pvalue", foldChange=geneList)
```

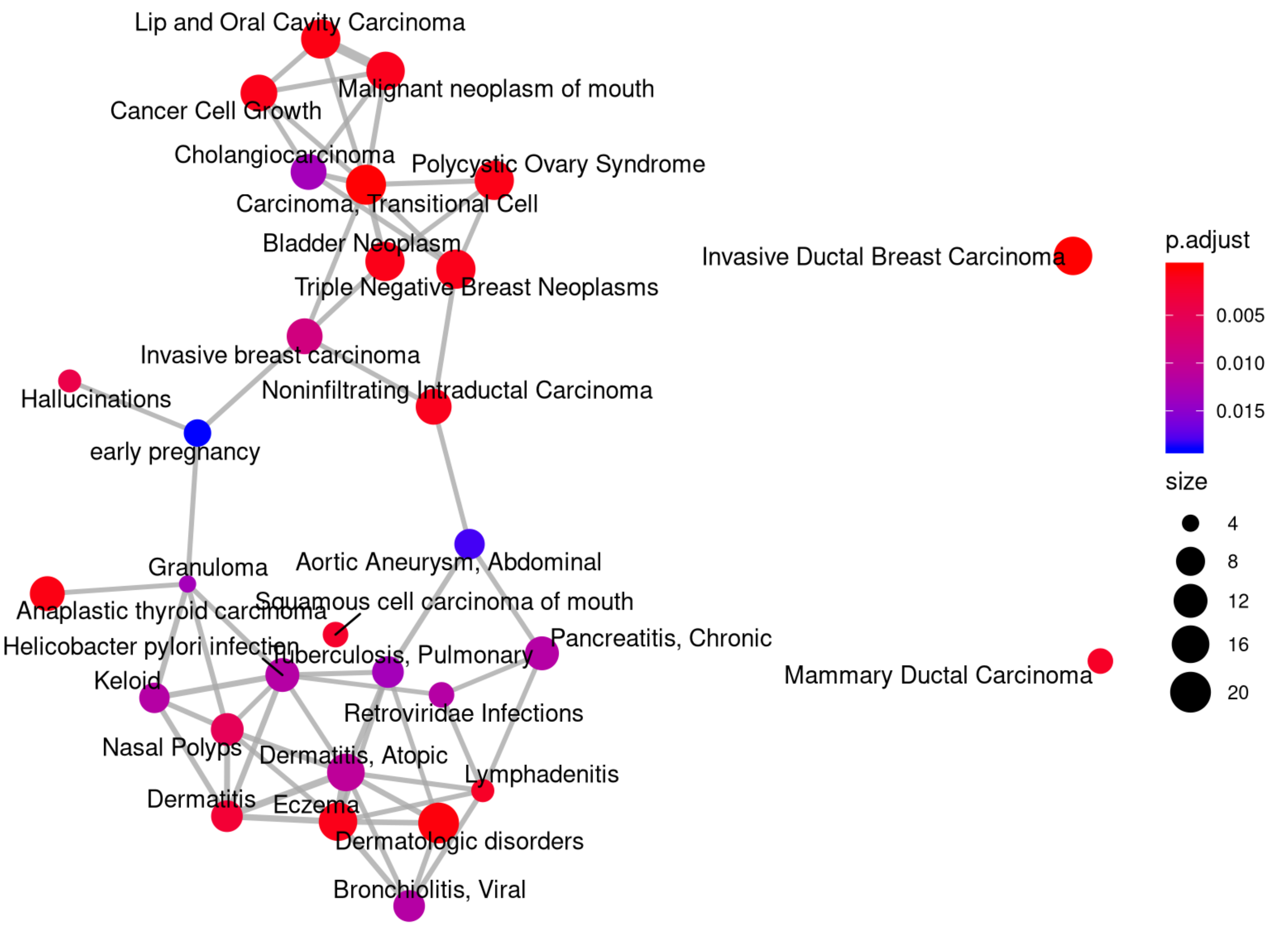
- Gene-concept network



Visualizations available in clusterProfiler

- Enrichment map

```
> ego <- enrichGO(de)
> emapplot(ego)
```

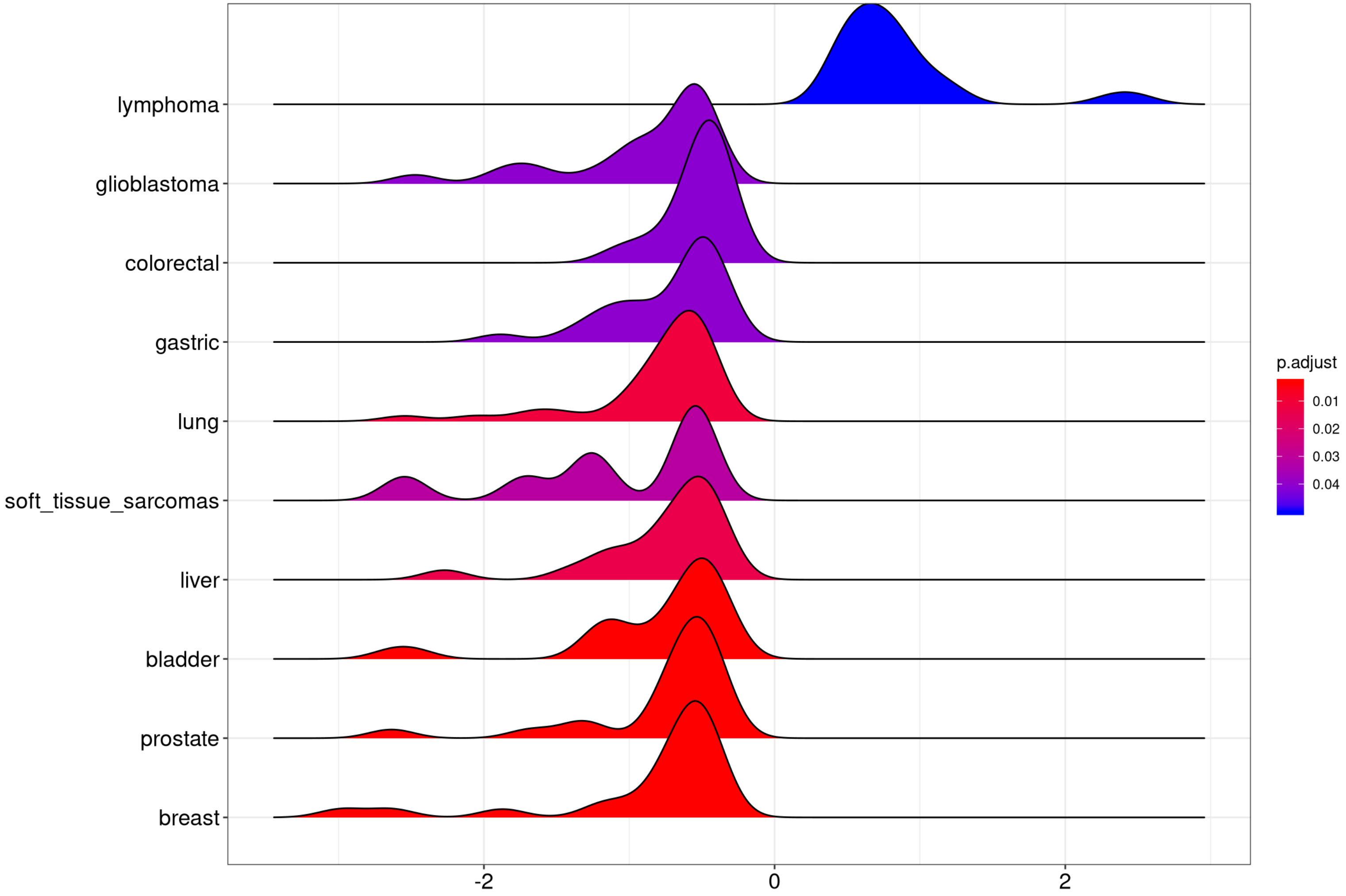


Lip and Oral Cavity Carcinoma
Malignant neoplasm of mouth
Cancer Cell Growth
Cholangiocarcinoma
Polycystic Ovary Syndrome
Carcinoma, Transitional Cell
Bladder Neoplasm
Triple Negative Breast Neoplasms
Invasive Ductal Breast Carcinoma
Invasive breast carcinoma
Hallucinations
Noninfiltrating Intraductal Carcinoma
early pregnancy
Granuloma
Aortic Aneurysm, Abdominal
Anaplastic thyroid carcinoma
Squamous cell carcinoma of mouth
Helicobacter pylori infection
Tuberculosis, Pulmonary
Pancreatitis, Chronic
Mammary Ductal Carcinoma
Keloid
Retroviridae Infections
Nasal Polyps
Dermatitis, Atopic
Lymphadenitis
Dermatitis
Eczema
Dermatologic disorders
Bronchiolitis, Viral
p.adjust
0.005
0.010
0.015
size
4
8
12
16
20

Visualizations available in clusterProfiler

- Ridgeplot

```
> ego <- gseGO(de)
> ridgeplot(ego)
```



vevox

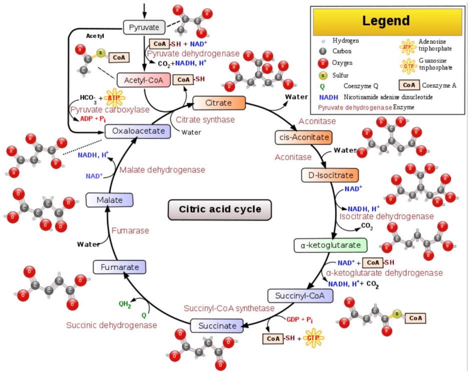
Recap and Exercise 3

Several visualization methods can be used to represent the results, either for single gene sets (barcode plot) or for several gene sets (barplots, etc).

Exercise 3: Create figures for the enrichment results:

- barplot of -log$_{10}$(p-value) of the p-values of the top 10 GO gene sets, or of positive and negative NES values
- Enrichment maps, gene-concept networks, ridge plots, etc

What is a gene set?



https://en.wikipedia.org/wiki/Citric_acid_cycle

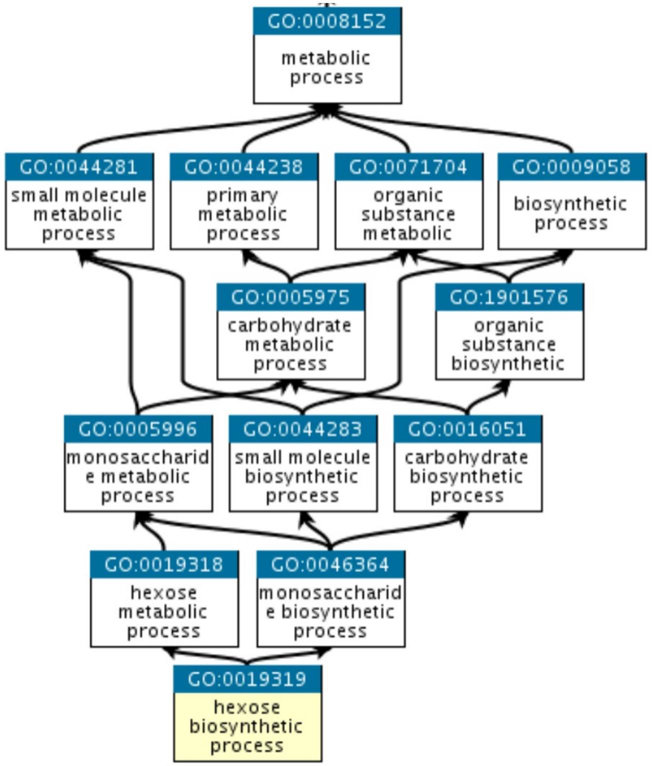
- Genes working together in a pathway (e.g. energy release through Krebs cycle)
- Genes located in the same compartment in a cell (e.g. all proteins located in the cell nucleus)
- Proteins that are all regulated by a same transcription factor
- Custom gene list that comes from a publication and that are down-regulated in a mutant
- List of SNPs associated with a disease
- … etc!
- Several gene sets are grouped into Knowledge bases

Gene ontology

- http://geneontology.org/

Collaborative effort to address the need for consistent descriptions of gene products across databases

- GO Consortium: develop a comprehensive, computational model of biological systems, ranging from the molecular to the organism level, across the multiplicity of species in the tree of life
- GO terms = GO categorizations
- GO term: each with a name (DNA repair) and a unique accession number (GO:0005125)


GO:0008152
metabolic process
GO:0044281
small molecule metabolic process
GO:0044238
primary metabolic process
GO:0071704
organic substance metabolic
GO:0009058
biosynthetic process
GO:0005975
carbohydrate metabolic process
GO:1901576
organic substance biosynthetic
GO:0005996
monosaccharide metabolic process
GO:0044283
small molecule biosynthetic process
GO:0016051
carbohydrate biosynthetic process
GO:0019318
hexose metabolic process
GO:0046364
monosaccharide biosynthetic process
GO:0019319
hexose biosynthetic process

The Gene Ontology (GO) knowledgebase is the world's largest source of information on the functions of genes.

Not covered today: GOSemSim (bioconductor), Revigo (http://revigo.irb.hr/)

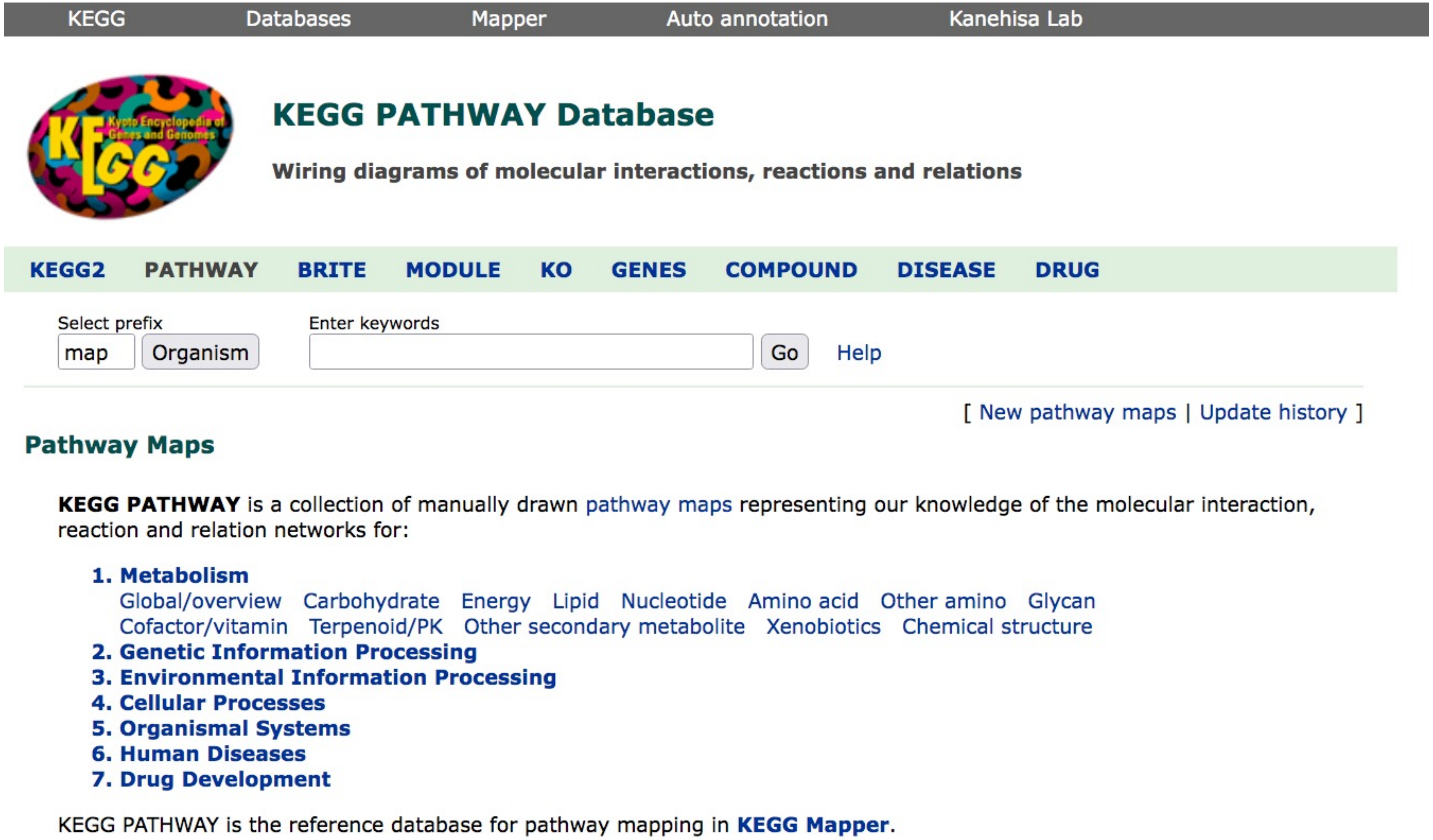
Gene ontology

GO ontologies: GO terms organized in 3 independent controlled vocabularies

- **Molecular function**: represents the biochemical activity of the gene product, such activities could include "ligand", "GTPase”, and "transporter".
- **Cellular component**: refers to the location in the cell of the gene product. Cellular components could include "nucleus”, "lysosome", and "plasma membrane”.
- **Biological process**: refers to the biological role involving the gene or gene product, and could include "transcription", "signal transduction", and "apoptosis". A biological process generally involves a chemical or physical change of the starting material or input.

KEGG

https://www.genome.jp/kegg/pathway.html



KEGG PATHWAY Database

Wiring diagrams of molecular interactions, reactions and relations

[New pathway maps | Update history]

Pathway Maps

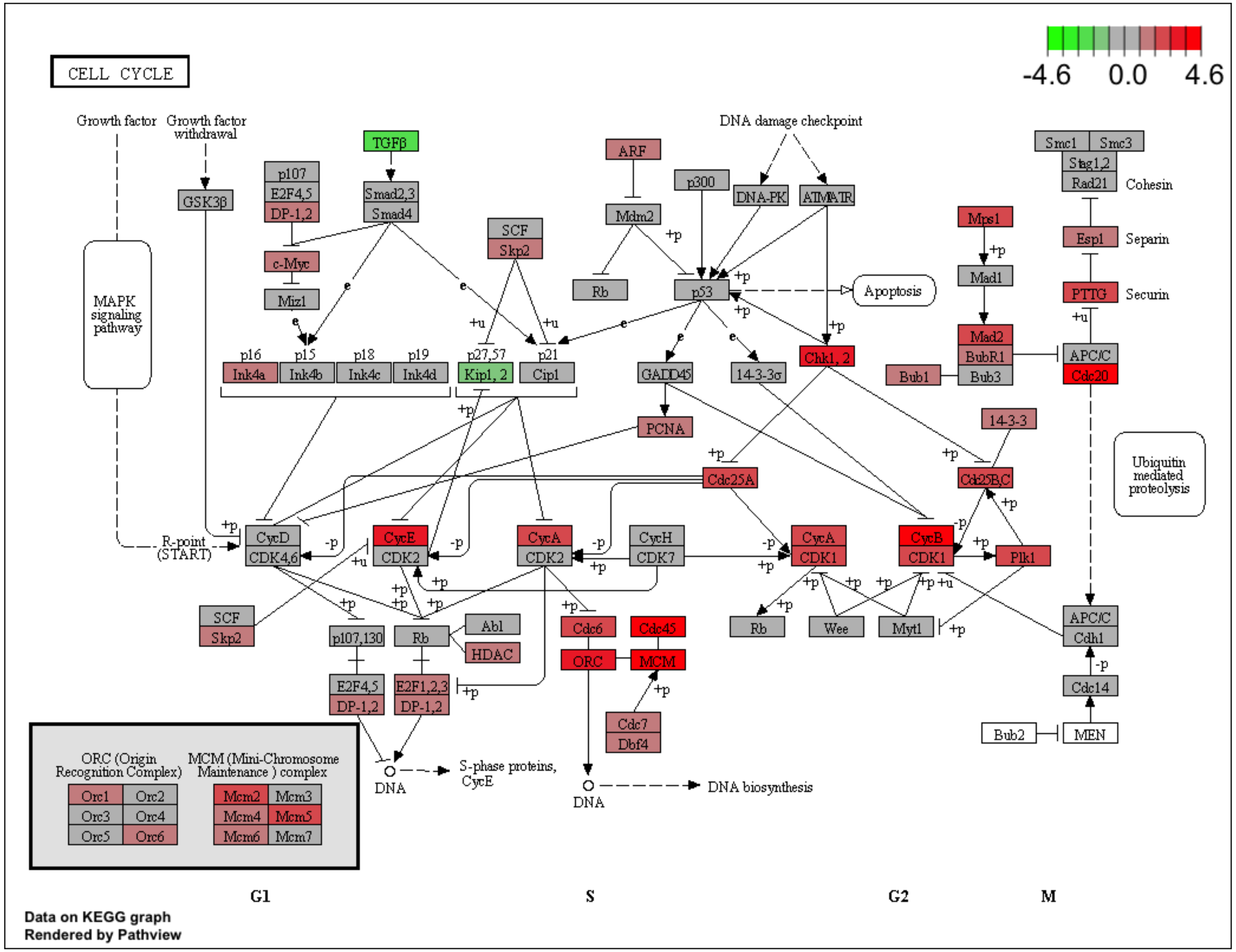
KEGG PATHWAY is a collection of manually drawn pathway maps representing our knowledge of the molecular interaction, reaction and relation networks for:

1. **Metabolism**
   Global/overview Carbohydrate Energy Lipid Nucleotide Amino acid Other amino Glycan
   Cofactor/vitamin Terpenoid/PK Other secondary metabolite Xenobiotics Chemical structure
2. **Genetic Information Processing**
3. **Environmental Information Processing**
4. **Cellular Processes**
5. **Organismal Systems**
6. **Human Diseases**
7. **Drug Development**

KEGG PATHWAY is the reference database for pathway mapping in **KEGG Mapper**.

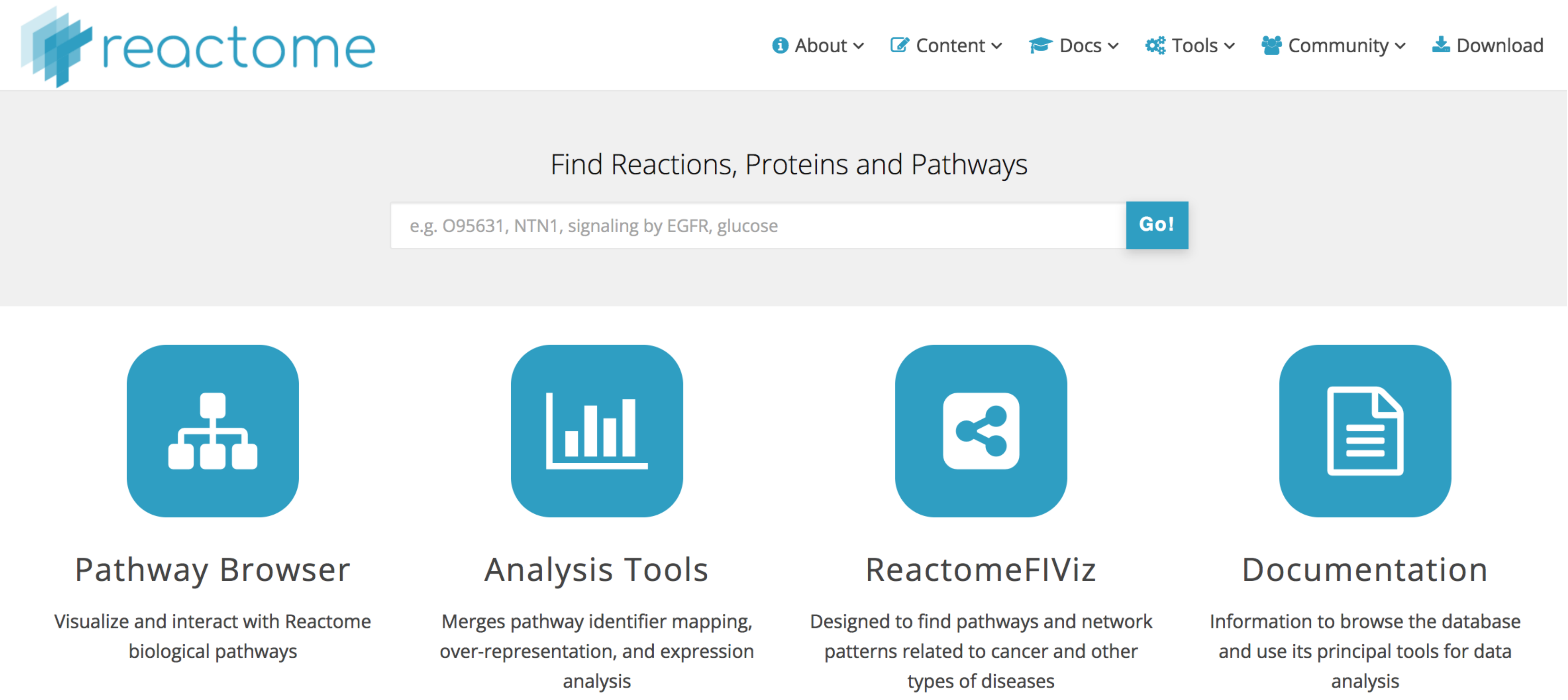
Visualization for KEGG pathways pathview package

```
> pathview(gene.data = geneList, pathway.id = "hsa04110", species = "hsa",
           limit = list(gene=max(abs(geneList)), cpd=1))
```



Reactome

https://reactome.org/



Find Reactions, Proteins and Pathways

e.g. O95631, NTN1, signaling by EGFR, glucose

Go!

Pathway Browser

Visualize and interact with Reactome biological pathways

Analysis Tools

Merges pathway identifier mapping, over-representation, and expression analysis

ReactomeFIViz

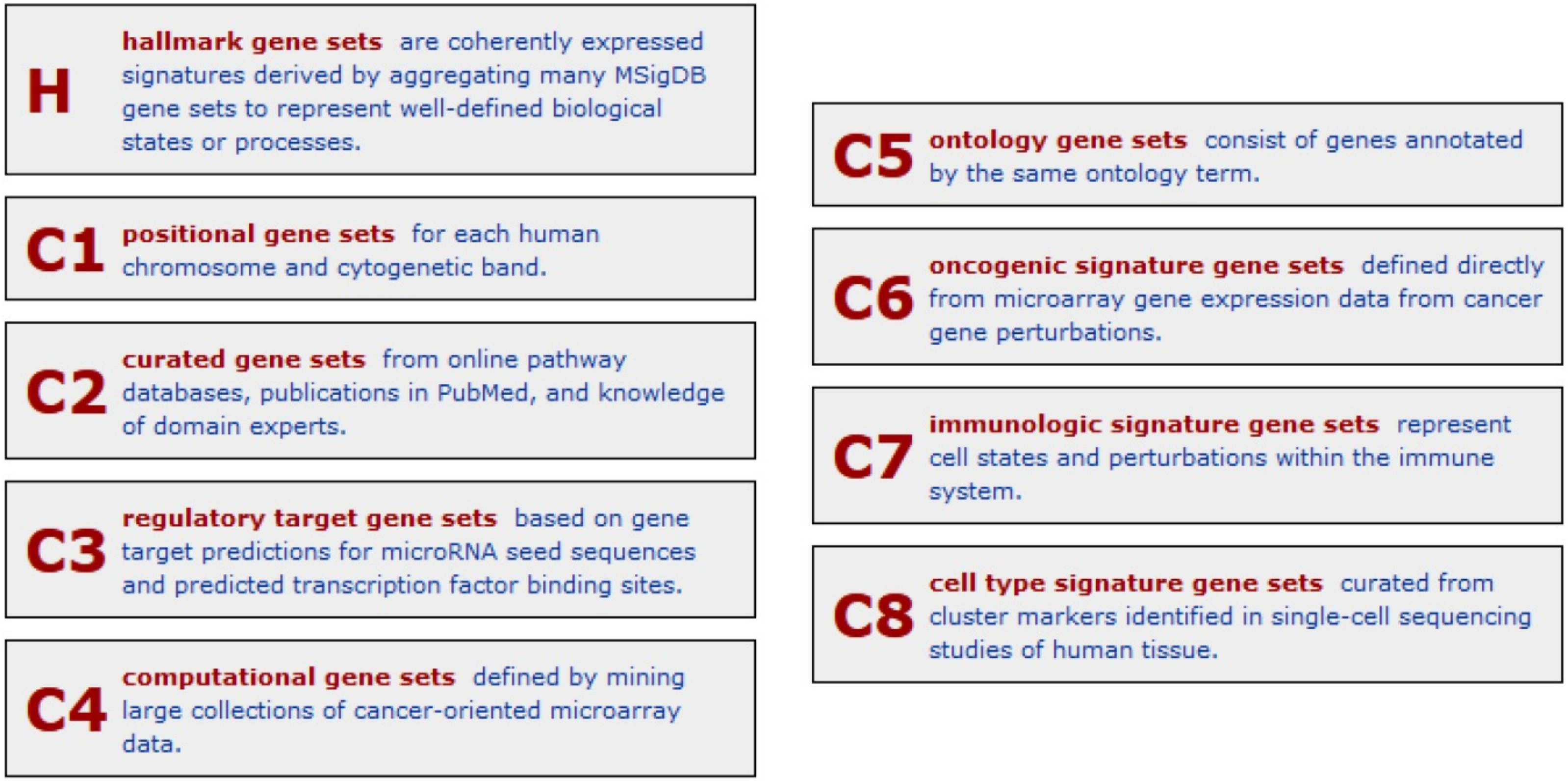
Designed to find pathways and network patterns related to cancer and other types of diseases

Documentation

Information to browse the database and use its principal tools for data analysis

MSigDB

https://www.gsea-msigdb.org/gsea/msigdb/index.jsp



H **hallmark gene sets** are coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes.

C1 **positional gene sets** for each human chromosome and cytogenetic band.

C2 **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.

C3 **regulatory target gene sets** based on gene target predictions for microRNA seed sequences and predicted transcription factor binding sites.

C4 **computational gene sets** defined by mining large collections of cancer-oriented microarray data.

C5 **ontology gene sets** consist of genes annotated by the same ontology term.

C6 **oncogenic signature gene sets** defined directly from microarray gene expression data from cancer gene perturbations.

C7 **immunologic signature gene sets** represent cell states and perturbations within the immune system.

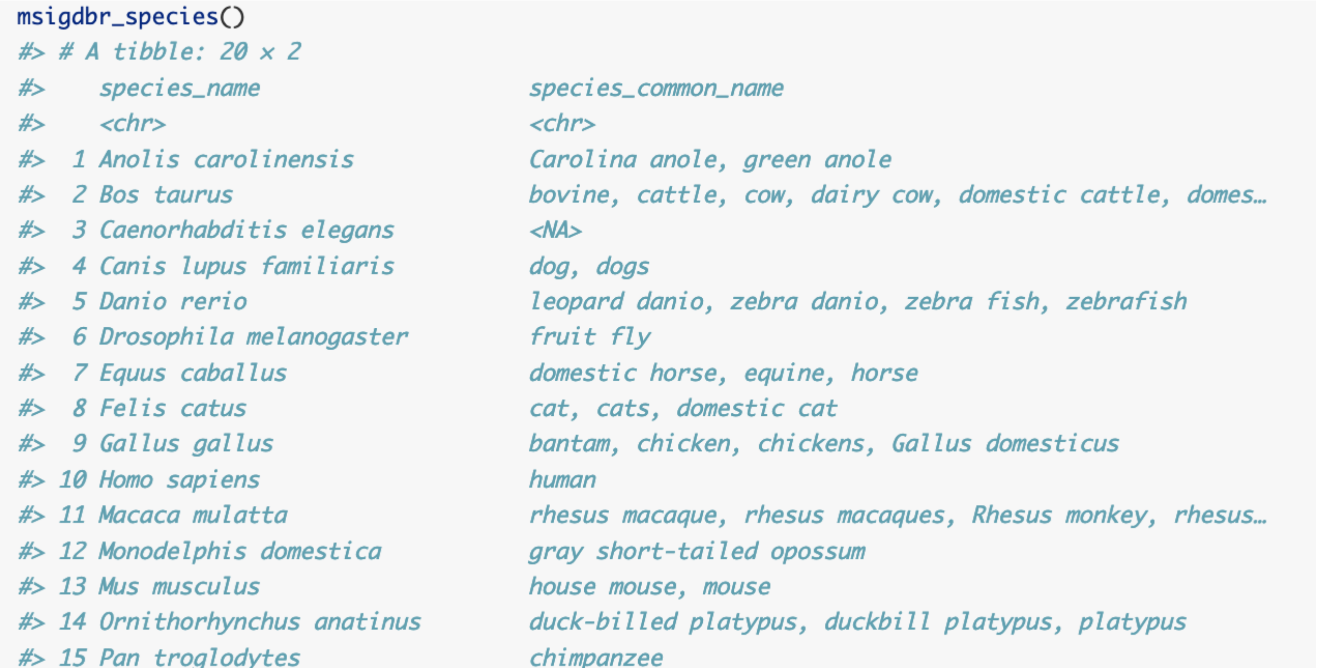
C8 **cell type signature gene sets** curated from cluster markers identified in single-cell sequencing studies of human tissue.

Download gmt files with version number:
https://www.gsea-msigdb.org/gsea/downloads.jsp

The Hallmark collection:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707969/

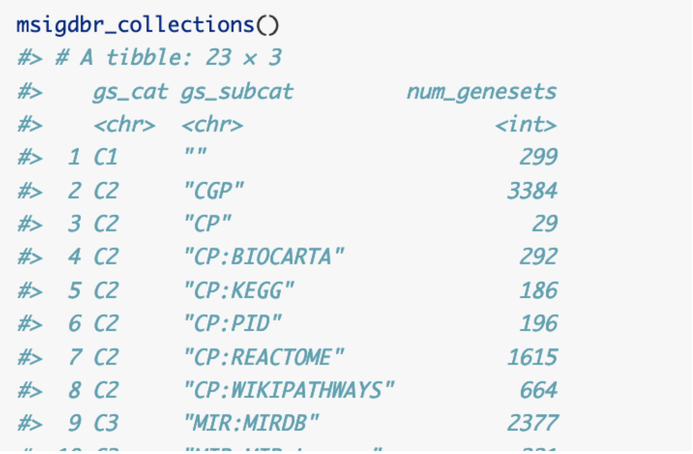
msigdbr package

Homologues for other species



```
msigdbr_species()
#> # A tibble: 20 × 2
#>    species_name                    species_common_name
#>    <chr>                           <chr>
#>  1 Anolis carolinensis             Carolina anole, green anole
#>  2 Bos taurus                      bovine, cattle, cow, dairy cow, domestic cattle, domes…
#>  3 Caenorhabditis elegans          <NA>
#>  4 Canis lupus familiaris          dog, dogs
#>  5 Danio rerio                     leopard danio, zebra danio, zebra fish, zebrafish
#>  6 Drosophila melanogaster         fruit fly
#>  7 Equus caballus                  domestic horse, equine, horse
#>  8 Felis catus                     cat, cats, domestic cat
#>  9 Gallus gallus                   bantam, chicken, chickens, Gallus domesticus
#> 10 Homo sapiens                    human
#> 11 Macaca mulatta                  rhesus macaque, rhesus macaques, Rhesus monkey, rhesus…
#> 12 Monodelphis domestica           gray short-tailed opossum
#> 13 Mus musculus                    house mouse, mouse
#> 14 Ornithorhynchus anatinus        duck-billed platypus, duckbill platypus, platypus
#> 15 Pan troglodytes                 chimpanzee
```

Helper function to view available collections

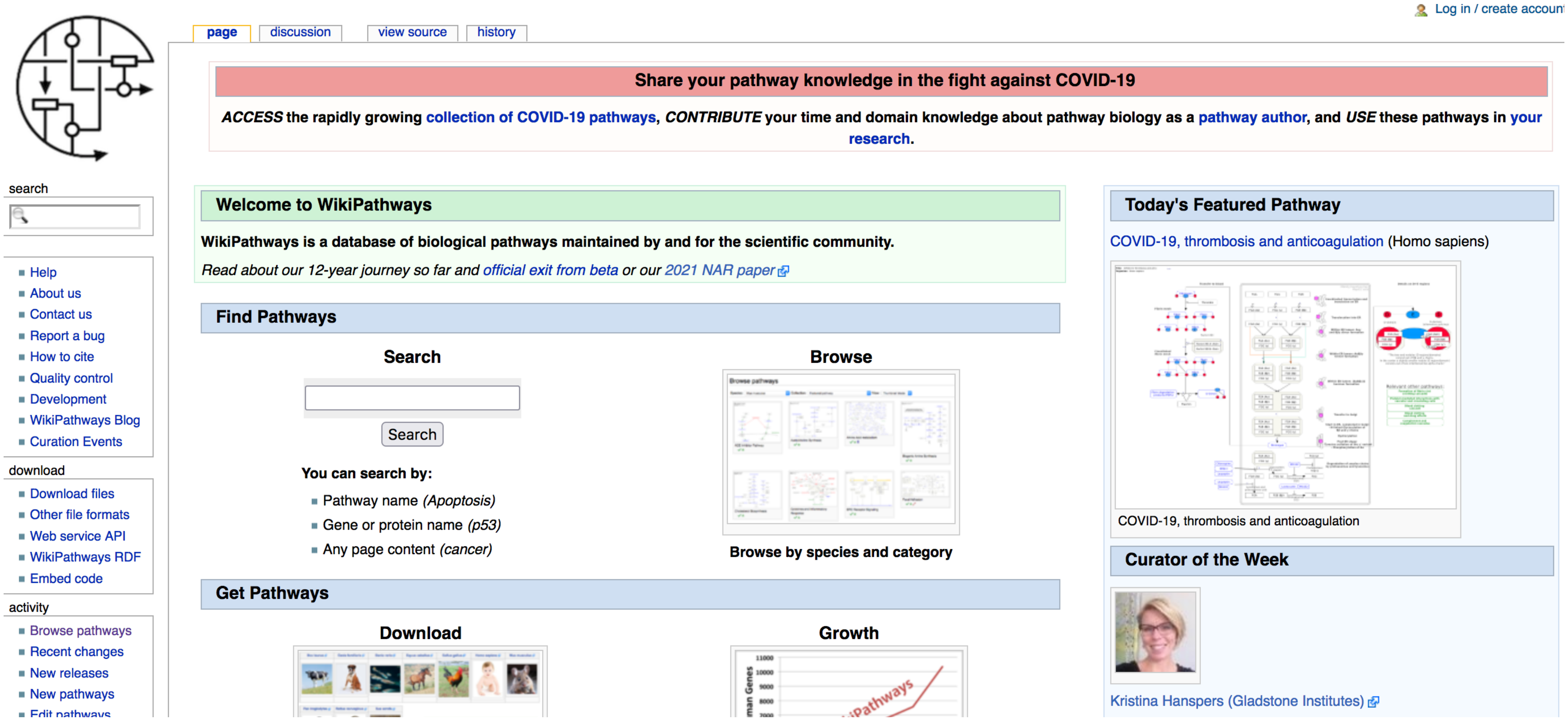


```
msigdbr_collections()
#> # A tibble: 23 × 3
#>    gs_cat gs_subcat         num_genesets
#>    <chr>  <chr>                    <int>
#>  1 C1     ""                         299
#>  2 C2     "CGP"                     3384
#>  3 C2     "CP"                        29
#>  4 C2     "CP:BIOCARTA"              292
#>  5 C2     "CP:KEGG"                  186
#>  6 C2     "CP:PID"                   196
#>  7 C2     "CP:REACTOME"             1615
#>  8 C2     "CP:WIKIPATHWAYS"          664
#>  9 C3     "MIR:MIRDB"               2377
```

https://cran.r-project.org/web/packages/msigdbr/index.html

WikiPathways

https://www.wikipathways.org/index.php/WikiPathways



Log in / create account

page | discussion | view source | history

search

- Help
- About us
- Contact us
- Report a bug
- How to cite
- Quality control
- Development
- WikiPathways Blog
- Curation Events

download

- Download files
- Other file formats
- Web service API
- WikiPathways RDF
- Embed code

activity

- Browse pathways
- Recent changes
- New releases
- New pathways
- Edit pathways

Share your pathway knowledge in the fight against COVID-19

ACCESS **the rapidly growing collection of COVID-19 pathways,** ***CONTRIBUTE*** **your time and domain knowledge about pathway biology as a pathway author, and** ***USE*** **these pathways in your research.**

Welcome to WikiPathways

WikiPathways is a database of biological pathways maintained by and for the scientific community.

Read about our 12-year journey so far and official exit from beta or our 2021 NAR paper

Find Pathways

Search

Search

You can search by:

- Pathway name *(Apoptosis)*
- Gene or protein name *(p53)*
- Any page content *(cancer)*

Browse

Browse by species and category

Get Pathways

Download

Growth

Today's Featured Pathway

COVID-19, thrombosis and anticoagulation (Homo sapiens)

COVID-19, thrombosis and anticoagulation

Curator of the Week

Kristina Hanspers (Gladstone Institutes)

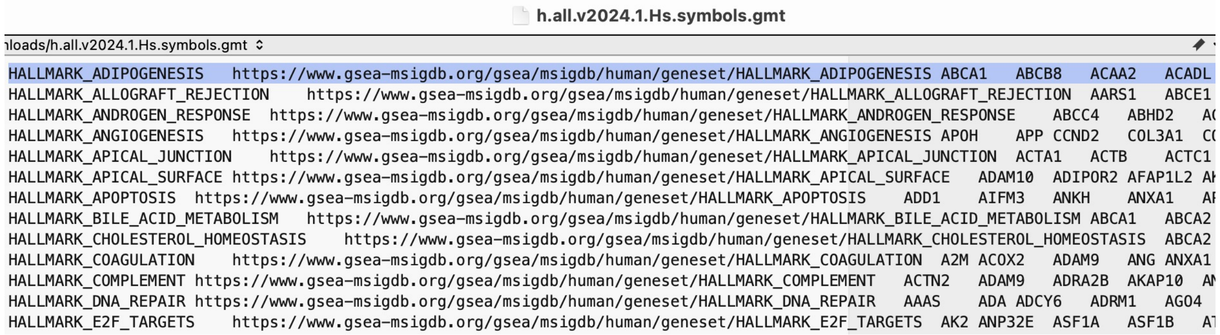
GSEA of other gene sets in R

KEGG: ClusterProfiler built-in function for GSEA of KEGG pathways

```
gseKEGG(geneList, organism = "hsa", keyType = "kegg", exponent = 1,
  nPerm = 1000, minGSSize = 10, maxGSSize = 500,
  pvalueCutoff = 0.05, pAdjustMethod = "BH", verbose = TRUE,
  use_internal_data = FALSE, seed = FALSE, by = "fgsea")
```

User-defined gene set collection: Import a .gmt file of gene sets and convert to TERM2GENE data frame needed for clusterProfiler: `read.gmt(gmtfile)`

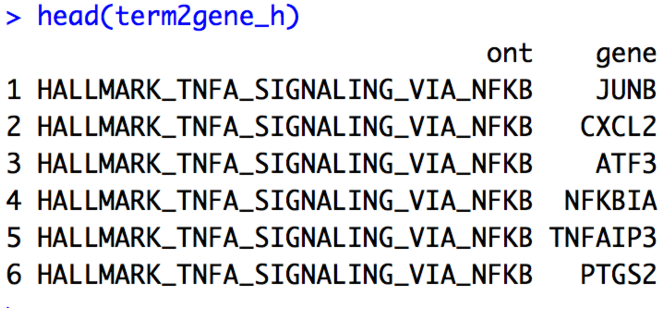
Converts a gmt text file with 1 gene set per line to a 2-column data frame:



h.all.v2024.1.Hs.symbols.gmt

nloads/h.all.v2024.1.Hs.symbols.gmt ⌃

```
HALLMARK_ADIPOGENESIS   https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_ADIPOGENESIS ABCA1   ABCB8   ACAA2   ACADL
HALLMARK_ALLOGRAFT_REJECTION    https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_ALLOGRAFT_REJECTION  AARS1   ABCE1
HALLMARK_ANDROGEN_RESPONSE  https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_ANDROGEN_RESPONSE    ABCC4   ABHD2   AC
HALLMARK_ANGIOGENESIS   https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_ANGIOGENESIS APOH    APP CCND2   COL3A1  CC
HALLMARK_APICAL_JUNCTION    https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_APICAL_JUNCTION  ACTA1   ACTB    ACTC1
HALLMARK_APICAL_SURFACE https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_APICAL_SURFACE   ADAM10  ADIPOR2 AFAP1L2 AK
HALLMARK_APOPTOSIS  https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_APOPTOSIS    ADD1    AIFM3   ANKH    ANXA1   AF
HALLMARK_BILE_ACID_METABOLISM   https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_BILE_ACID_METABOLISM ABCA1   ABCA2
HALLMARK_CHOLESTEROL_HOMEOSTASIS    https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_CHOLESTEROL_HOMEOSTASIS  ABCA2
HALLMARK_COAGULATION    https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_COAGULATION  A2M ACOX2   ADAM9   ANG ANXA1
HALLMARK_COMPLEMENT https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_COMPLEMENT   ACTN2   ADAM9   ADRA2B  AKAP10  AN
HALLMARK_DNA_REPAIR https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_DNA_REPAIR   AAAS    ADA ADCY6   ADRM1   AGO4
HALLMARK_E2F_TARGETS    https://www.gsea-msigdb.org/gsea/msigdb/human/geneset/HALLMARK_E2F_TARGETS  AK2 ANP32E  ASF1A   ASF1B   AT
```



```
> head(term2gene_h)
                               ont    gene
1 HALLMARK_TNFA_SIGNALING_VIA_NFKB    JUNB
2 HALLMARK_TNFA_SIGNALING_VIA_NFKB   CXCL2
3 HALLMARK_TNFA_SIGNALING_VIA_NFKB    ATF3
4 HALLMARK_TNFA_SIGNALING_VIA_NFKB  NFKBIA
5 HALLMARK_TNFA_SIGNALING_VIA_NFKB TNFAIP3
6 HALLMARK_TNFA_SIGNALING_VIA_NFKB   PTGS2
```

Conversion of gene ID types with clusterProfiler (or biomaRt package)

```
bitr(geneID, fromType, toType, OrgDb, drop = TRUE)
```

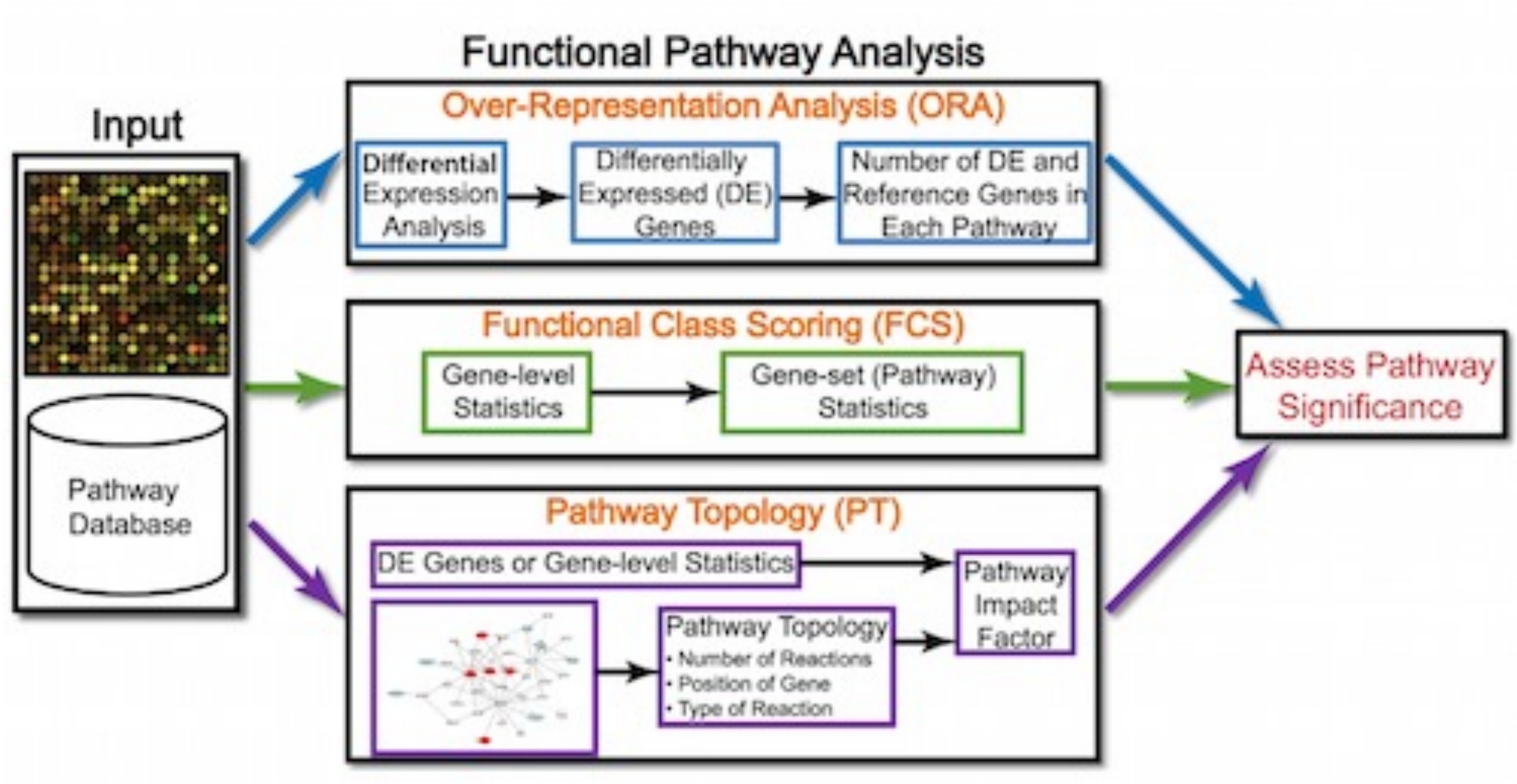
biomaRt: https://bioconductor.org/packages/release/bioc/html/biomaRt.html

Recap and exercise 4

- We have seen how to perform GSEA using the built-in GO gene sets. Please perform GSEA with the built-in KEGG pathways, as well as with the hallmark gene sets obtained from MSigDB.

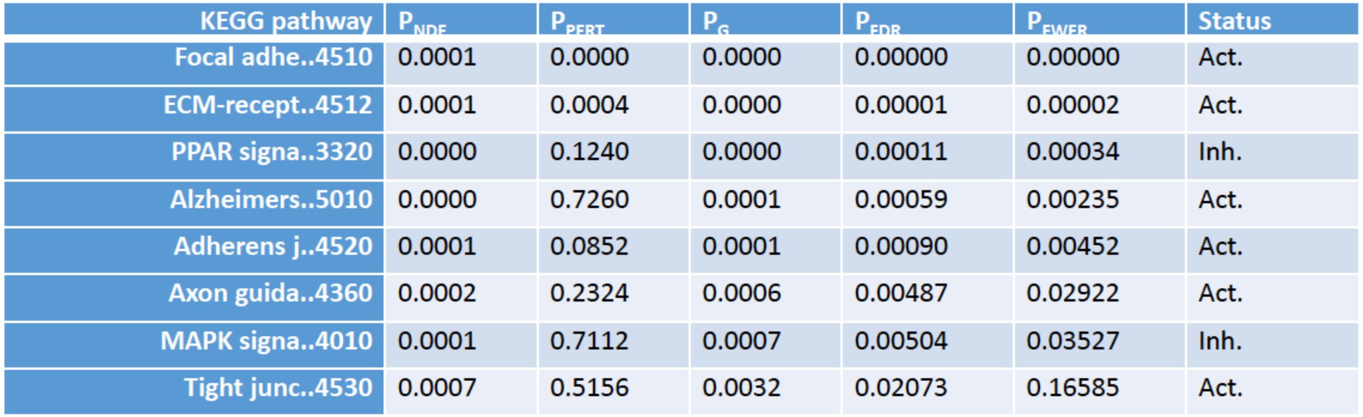
- Exercise 4: use functions of clusterProfiler and data provided in Ex. 1, and hallmark gene sets downloaded from MSigDB
  - First convert the gene symbols to EntrezID, then perform a GSEA of KEGG pathways (with argument minGSSize=30).
  - Explore the results. Is there an immune-related gene set coming up? Is there a Natural killer gene set coming up?
  - Using msigdbr, obtain a TERM2GENE data.frame of the Hallmark gene sets and run a GSEA. How many significant gene sets are there?

Enrichment/functional analysis - summary


Functional Pathway Analysis
Input
Pathway
Database
Over-Representation Analysis (ORA)
Differential Expression Analysis
Differentially Expressed (DE) Genes
Number of DE and Reference Genes in Each Pathway
Functional Class Scoring (FCS)
Gene-level Statistics
Gene-set (Pathway) Statistics
Pathway Topology (PT)
DE Genes or Gene-level Statistics
Pathway Topology
• Number of Reactions
• Position of Gene
• Type of Reaction
Pathway Impact Factor
Assess Pathway Significance

Functional analysis: **Pathway topology tools**

Signaling pathway impact analysis (SPIA)
Identification of dys-regulated pathways: taking into account gene interaction information + fold changes and adjusted p-values from differential expression analysis

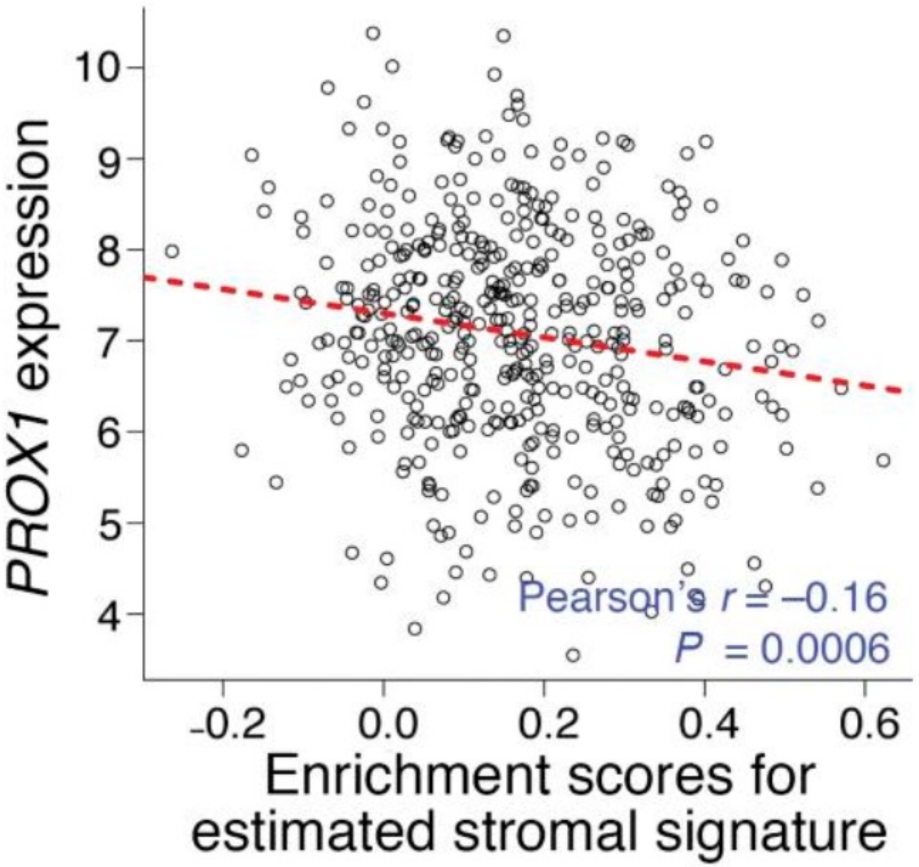


KEGG pathway	P_{NDE}	P_{PERT}	P_G	P_{FDR}	P_{FWER}	Status
Focal adhe..4510	0.0001	0.0000	0.0000	0.00000	0.00000	Act.
ECM-recept..4512	0.0001	0.0004	0.0000	0.00001	0.00002	Act.
PPAR signa..3320	0.0000	0.1240	0.0000	0.00011	0.00034	Inh.
Alzheimers..5010	0.0000	0.7260	0.0001	0.00059	0.00235	Act.
Adherens j..4520	0.0001	0.0852	0.0001	0.00090	0.00452	Act.
Axon guida..4360	0.0002	0.2324	0.0006	0.00487	0.02922	Act.
MAPK signa..4010	0.0001	0.7112	0.0007	0.00504	0.03527	Inh.
Tight junc..4530	0.0007	0.5156	0.0032	0.02073	0.16585	Act.

$P_{NDE} = P(X \geq N_{DE} \mid H_0)$
P_{PERT}: probability to observe a larger perturbation than observed
P_G: combination of P_{NDE} and P_{PERT}
P_{FDR}: adjusted FDR p-value
P_{FWER}: adjusted FDR p-value (more conservative)

https://bioconductor.org/packages/release/bioc/html/SPIA.html

Single-sample gene set variation analysis



GSVA:
https://bioconductor.org/packages/release/bioc/html/GSVA.html

https://www.jci.org/articles/view/129558

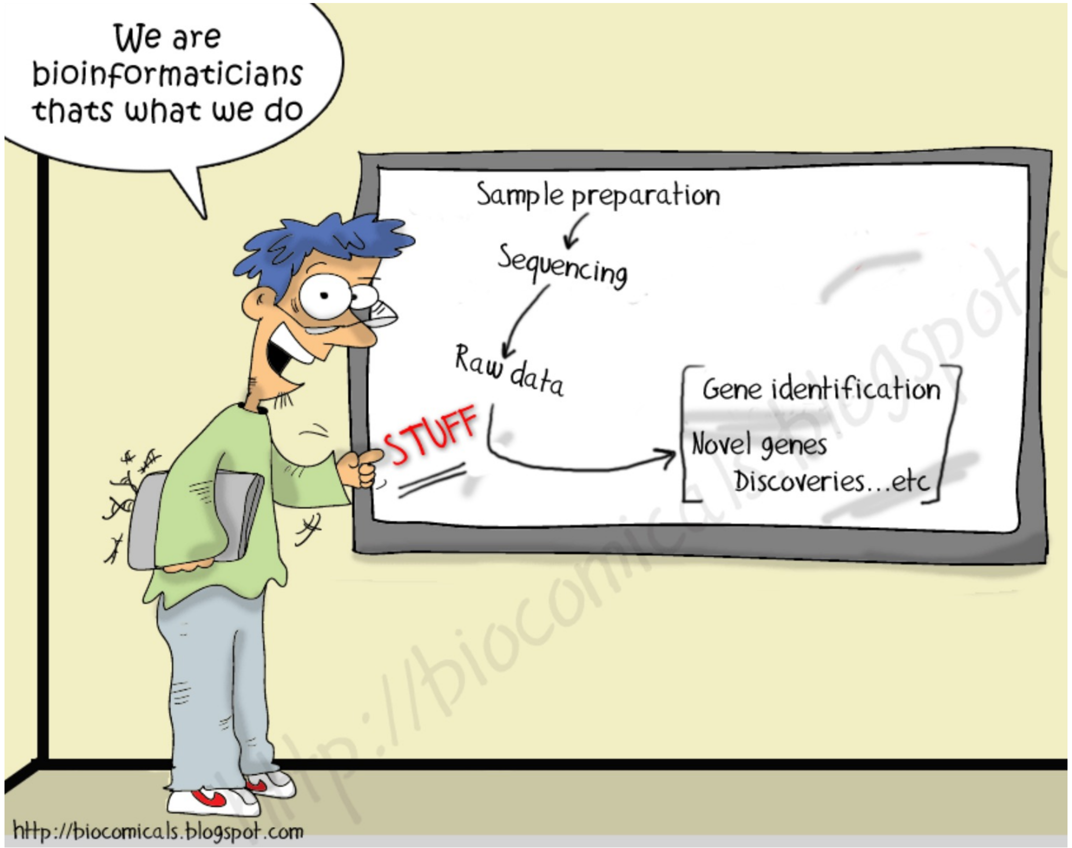
Credits: 0.25 ECTS

- Please provide answers and R code for an additional exercise (eg 1 Word with answers and figures and 1 script file, or 1 file generated from Rmarkdown)

https://sib-swiss.github.io/enrichment-analysis-training/exercises/#extra-exercise-for-ects-credits

- Sign up for credit by adding your name to the google Doc file (email sent by course organizer)
- Send answers to tania.wyss@sib.swiss within 1 week

Thank you for your attention!



Please fill in the feedback sent by the course organizer.

We thank Isabelle Dupanloup and Linda Dib for providing course material.