

SIB  
Swiss Institute of  
Bioinformatics

# Enrichment analysis

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# The Bioinformatics Core Facility at SIB



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## Welcome to **BCF-SIB**



### About **BCF-SIB**

The Bioinformatics Core Facility (BCF) is a research and service group within the [SIB Swiss Institute of Bioinformatics](#). Our core competence and activities reside in the interface between biomedical sciences, statistics and computation, particularly in the application of high-throughput omics technologies, such as RNA/DNA-sequencing and microarrays, in molecular research and to problems of clinical importance, such as development of cancer biomarkers. The BCF offers consulting, teaching and training, data analysis support / services, and research collaborations for both academic and industrial partners. We are involved in consulting for several industrial partners in the area of statistical aspects of clinical biomarker development.

<https://bcf.sib.swiss>

- Teaching and training
- Biostatistics support
- Collaboration



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Bioinformatics

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## Mauro Delorenzi & Frédéric Schütz's group

In the Bioinformatics Core Facility (BCF), we promote trans-disciplinary collaborations between research teams in medicine, molecular biology, genetics, genomics, statistics, and bioinformatics...

<https://www.sib.swiss/mauro-delorenzi-frederic-schutz-group>

# Schedule

- **9:00 - 10:30**
- Recall:
  - a. Differential expression
  - b. Statistical tests
- Exercise
- **10:30 - 10:45** break
- **10:45 - 12:30**
- Method of gene set enrichment analysis
- Exercise
- **12:30 - 13:30** lunch break
- **13:30 - 15:30**
- Ontologies and sources of gene sets
- Exercise
- **15:30 - 15:45** break
- **15:45 - 16:50**
- Visualization of enrichment results
- Exercise
- **16:50 - 17:00** Feedback and end of day

# Credits: 0.25 ECTS

- Please provide results of exercises 2, 3 & 4 plus answers and R code for an additional exercise in a document (eg 1 Word with figures and 1 script file, or 1 file generated from Rmarkdown)
- Sign up for credit here by adding your name to the Google doc.
- Send answers to [tania.wyss@sib.swiss](mailto:tania.wyss@sib.swiss) by the end of next week.

# First, tell us about yourself !

- What organism are you working on? What type of data are you analyzing?
- Write your name and some keywords about yourself and/or your research into the Google doc, to share about yourself.



Photo by National Cancer Institute, Unsplash



Photo by Scott Graham, Unsplash

# Questions and Exercises

- Feel free to interrupt with questions by asking them directly or raising your hand.
- Can also use the chat or Q&A in Google doc, Isabelle and I will answer.
- Exercises in R:
  - We will try to debug as much as possible
  - **We are happy if you share your results!**
  - Computational power on RStudio cloud is limited, might crash

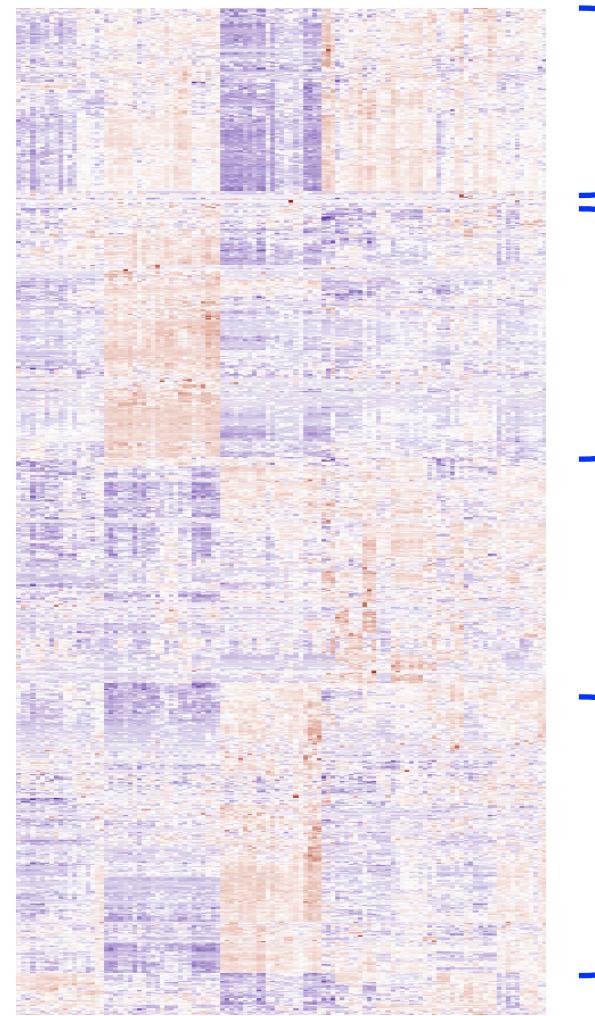


# Course material

- Via website :
- <https://sib-swiss.github.io/enrichment-analysis-training/>
- **Feedback** via Moodle, survey at the end of the day (link sent by Patricia)
- Additional links and answers to questions added to [Google doc](#)

# 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

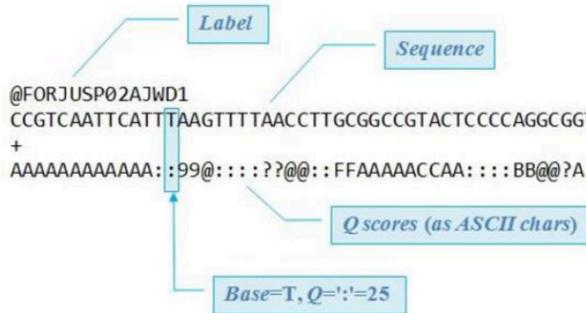


IVY GAP: <https://glioblastoma.alleninstitute.org/>

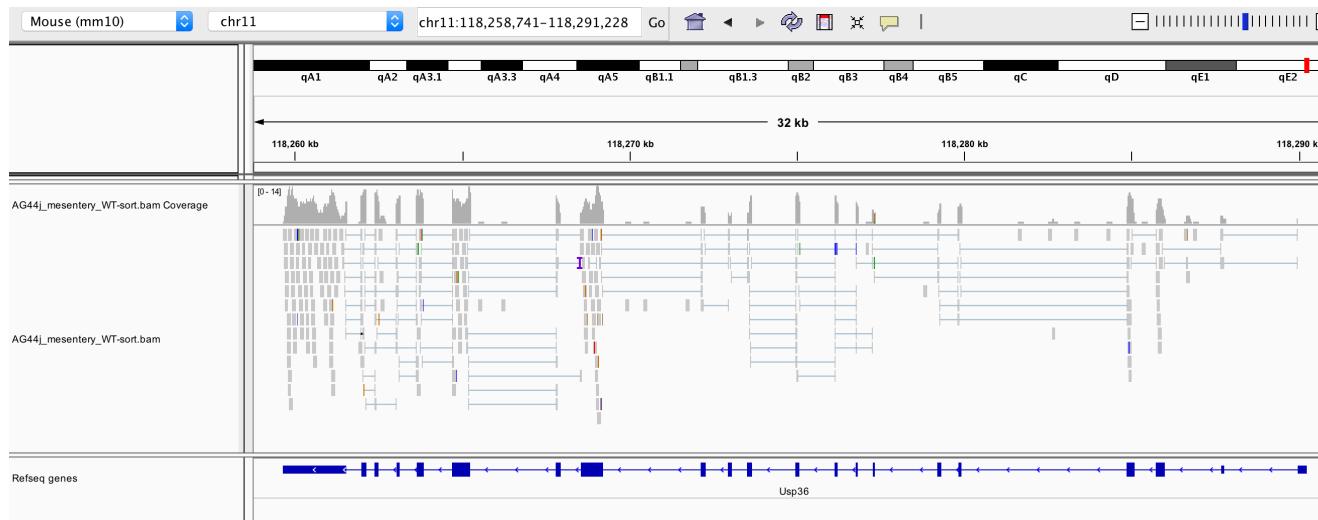
# Typical RNA sequencing analysis workflow

fastq file:

```
@HWI-M01141:63:A4NDL:1:1101:14849:1418 1:N:0:TATAGCGAGACACCGT  
NACGAAGGGTCAAGCGTTACTCGGAATTACTGGCGTAAGCGTGCCTAGGTGGTTT/  
+  
#>>>A??FAA1BGGEGGAAFGCA@BFF1D2BCF/EEG/DBEE/E?GAEEFGAEAFGJ  
@HWI-M01141:63:A4NDL:1:1101:13802:1421 1:N:0:TATAGCGAGACACCGT  
NACGGAGGGTCAAGCGTTAACCGAATTACTGGCGTAAGCGCACGCAGCGGTGTTT/  
+  
#>>AAABBBABBGGGGGGGG?FHGGGGGGHHHHHHHHGGGGH  
@HWI-M01141:63:A4NDL:1:1101:15928:1426 1:  
NACGTAGGGTCCAGCGTTAACCGAATTACTGGCGTAAA/  
+  
#>>AABFB@FBGGGGGGGGGGHGGGGFHHHHHHHGGGGH  
@HWI-M01141:63:A4NDL:1:1101:14861:1431 1:  
NACGAAGGGTCAAGCGTTACTCGGAATTACTGGCGTAAA/  
+  
#>>AAAABBFABGGGGGGCEGHGGEFFHHHHHHGGGGH  
@HWI-M01141:63:A4NDL:1:1101:15264:1465 1:  
NACGTAGGGTGCAGCGTTCTCGGAATTACTGGCGTAAA/  
+  
@FORJUSP02AJWD1  
CCGTCAATTCACTTAAGTTTAACCTGCGGCCGTACTCCCAGGCCT  
+  
AAAAAAAAAAAAA::99@:::?:@:::FFAAAAACAA:::BB@?A?  
+  
Base=T, Q=':'=25
```



Filter quality  
Align to ref. genome



count reads  
→ per gene

Downstream  
statistical analysis:  
R: import  
counts table

# Differential gene expression analysis

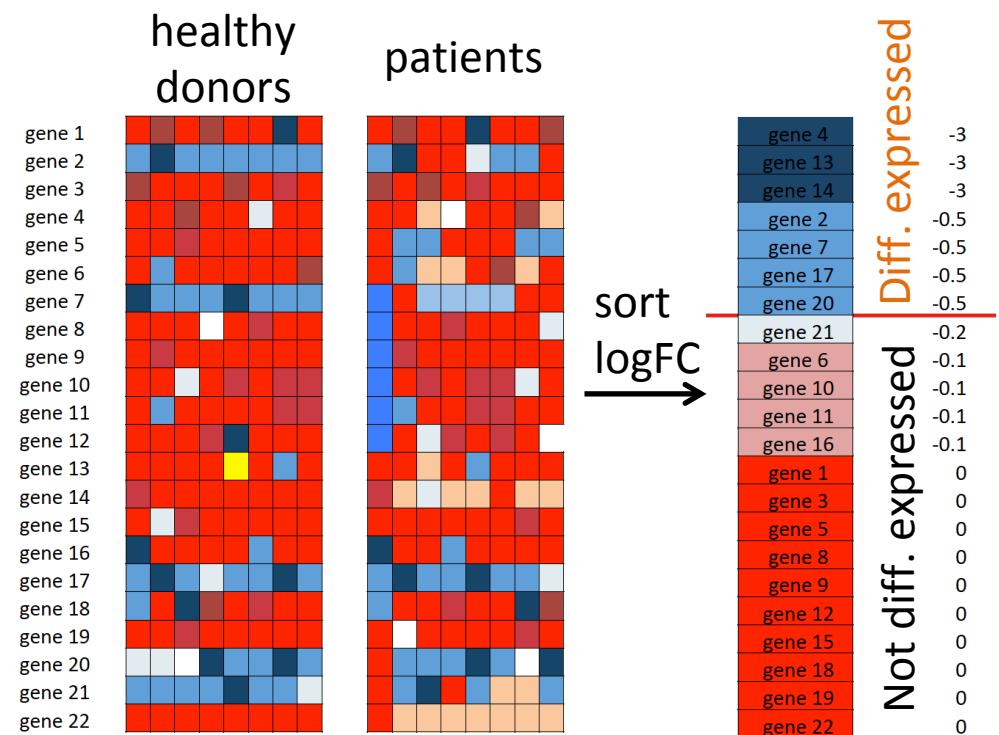
- Comparing 2 groups:

For each gene  $i$ , is there a **difference** in expression between control and patients?

- Fold change in genomics:

$$\log_2 \text{of ratios} = \log \text{fold change}$$

$$\log(\pi_{i1}/\pi_{i2}) = \log(\pi_{i1}) - \log(\pi_{i2})$$



# Differential gene expression analysis

- Comparing 2 groups:

For each gene  $i$ , is there a significant difference in mean expression between control and patients?

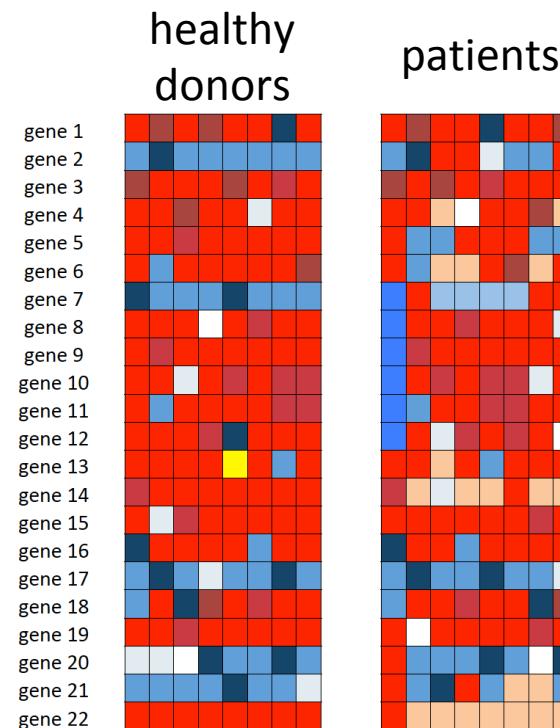
- T-test:

$H_0$ : Healthy donors and patients have similar gene  $i$  expression

$$H_{0i} : \pi_{i1} = \pi_{i2}$$

$H_1$ : Healthy donors and patients don't have a similar gene  $i$  expression

$$H_{1i} : \pi_{i1} \neq \pi_{i2}$$



# T-test in R

```
> t.test(grp1, grp2, paired = F)
```

Welch Two Sample t-test

data: grp1 and grp2

t = -6.3689, df = 8.9195, p-value = 0.0001352

alternative hypothesis: true difference in means is not equal to 0

95 percent confidence interval:

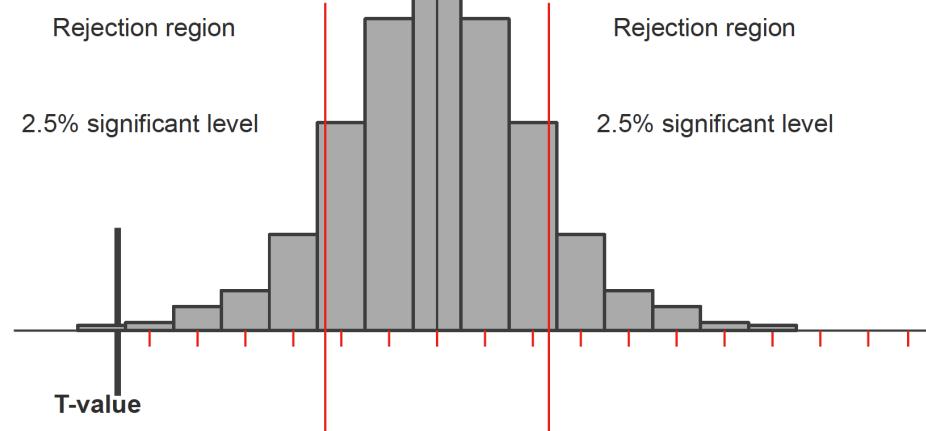
-8.908753 -4.234104

sample estimates:

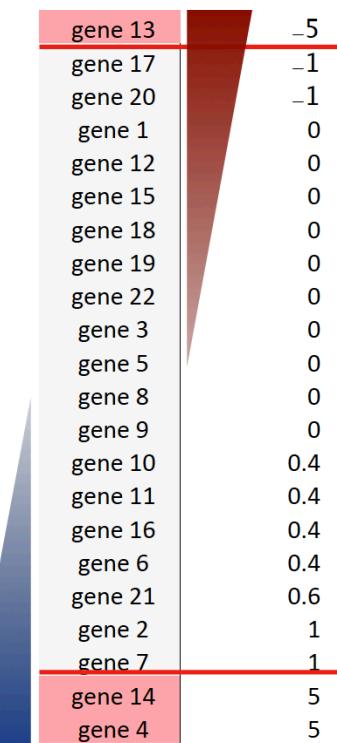
mean of x mean of y

6.00000 12.57143

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2 \left( \frac{1}{n_1} + \frac{1}{n_2} \right)}}$$



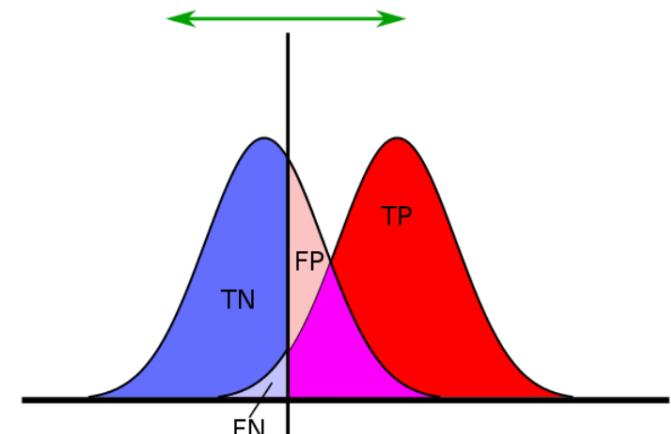
sort based  
on T-statistic



# What does $p < 0.05$ mean?

- It means that we suspect that the difference observed is not due to chance alone
- It means that if we repeat an experiment 20 times, we would reject the null hypothesis once because of random error

Decision Truth \ Decision	$H_0$ not rejected (negative)	$H_0$ Rejected (positive)
$H_0$ is true (no signal in the data)	 specificity True negative TN	 Type I error False Positive $\alpha$
$H_0$ is false (there is something to find)	 Type II error False Negative $\beta$	 Power $1 - \beta$ ; sensitivity True Positive TP



# P-value adjustment: what is it?

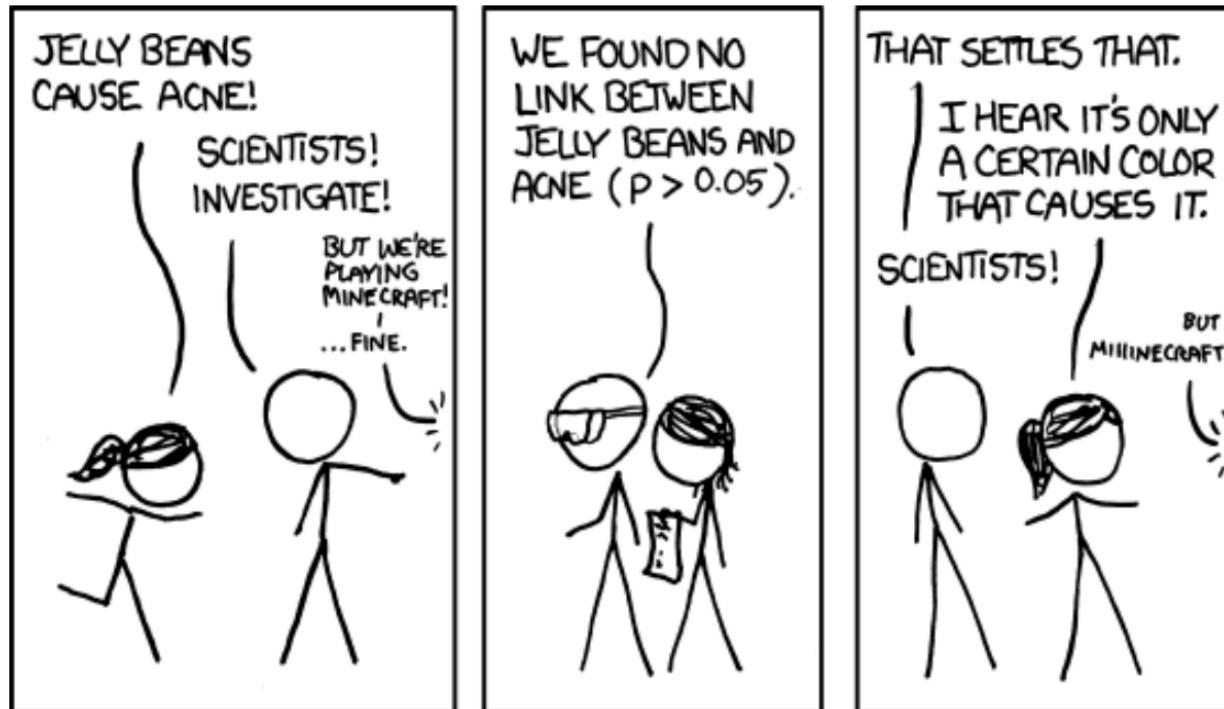


Photo by Patrick Fore on Unsplash

Cartoon: <https://xkcd.com/882/>

Paper on p-value adjustment: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6099145/>

WE FOUND NO  
LINK BETWEEN  
PURPLE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
BROWN JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
PINK JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
BLUE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
TEAL JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
SALMON JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
RED JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
TURQUOISE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
MAGENTA JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
YELLOW JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
GREY JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
TAN JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
CYAN JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND A  
LINK BETWEEN  
GREEN JELLY  
BEANS AND ACNE  
( $P < 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
MAUVE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  

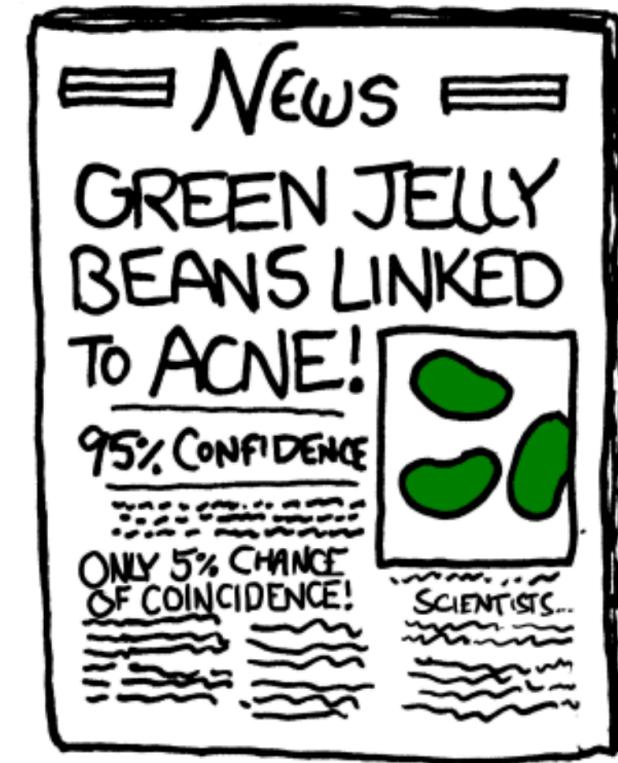

WE FOUND NO  
LINK BETWEEN  
BEIGE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
LILAC JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
BLACK JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
PEACH JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  


WE FOUND NO  
LINK BETWEEN  
ORANGE JELLY  
BEANS AND ACNE  
( $P > 0.05$ ).  

# Methods of p-value adjustment

- **Bonferroni:** the alpha level is divided by the total number of tests
- if we run  $k=20$  tests:  
 $0.05/k = 0.05/20=0.0025$

Good for small number of tests  
but too conservative for  
thousands of genes

- **Benjamini-Hochberg procedure (BH to control FDR)**
- Rank the p-values from smallest to largest, adjust less and less as the p-values get larger:

$$p\text{-value}_1 * n/1$$

$$p\text{-value}_2 * n/2$$

$$p\text{-value}_k * n/k$$

$n$ = number of genes

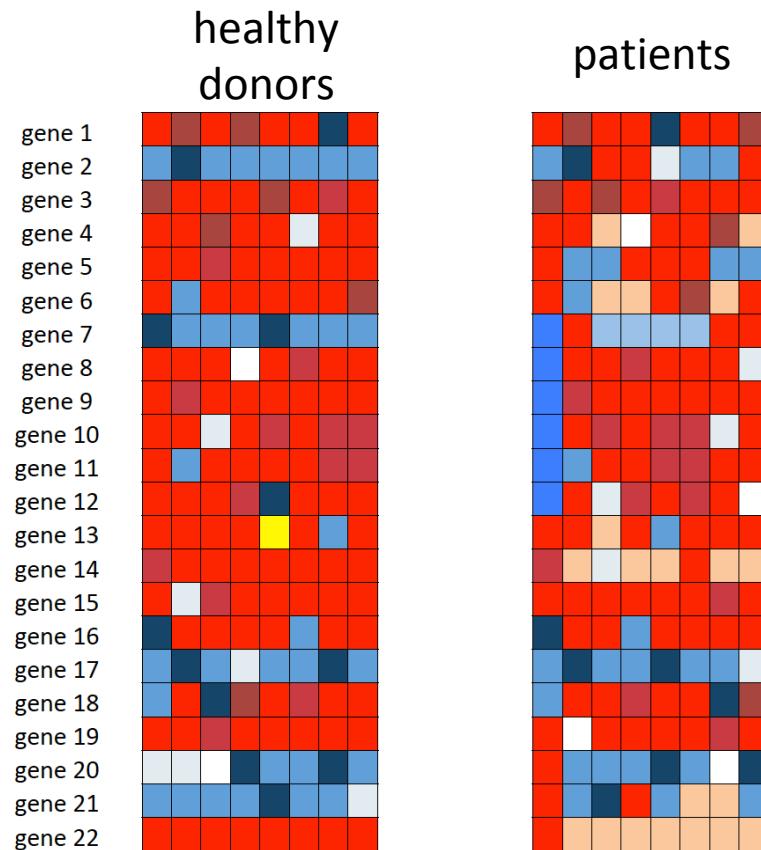
$k$ = rank number

# Differential gene expression analysis using R

- Bioconductor

<https://bioconductor.org/>

- Several packages :
    - limma: t-test
    - DESeq2: Wald test
    - edgeR: exact test



# RStudio tour

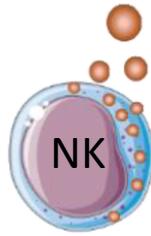
The screenshot shows the RStudio interface with the following components:

- Code Editor:** Displays an R script titled "ea\_2020\_script.R" with the following content:

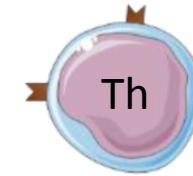
```
1 ##### Enrichment analysis course, SIB, June 26th 2020
2
3
4 # Enrichment analysis, SIB course, June 26th 2020
5
6 # load the packages needed for the R exercise
7 library(clusterProfiler)
8 library(org.Hs.eg.db)
9 library(pathview)
10 # library(biomaRt)
11
12 # Some reminders about the usage of R:
13
```

- Environment Browser:** Shows the global environment with objects like "convert\_ens\_sy..." (15991 obs. of 3 variables), "GO\_NK\_Th" (Large gseaResult (98.1 Mb)), and "NK\_vs\_Th" (20485 obs. of 6 variables).
- File Browser:** Shows the contents of a project named "project" in the Cloud. The files listed are:

Name	Size	Modified
.RData	23.6 MB	Jun 22, 2020, 2:36 P
.Rhistory	2.6 KB	Jun 22, 2020, 2:36 P
adaptive_immune_response_ge...	11.5 KB	Jun 20, 2020, 12:54
adaptive_immune_response_ge...	23.3 KB	Jun 20, 2020, 12:54
EA_2020_Exercise_1.R	1019 B	Jun 20, 2020, 1:28 A
ea_2020_script.R	5.1 KB	Jun 20, 2020, 1:26 A
gseGO_Nk_vs_Th_results.rds	5.1 MB	Jun 20, 2020, 1:22 A
NK_vs_Th_diff_gene_exercise_1...	1.2 MB	Jun 19, 2020, 10:17
project.Rproj	205 B	Jun 23, 2020, 11:37



# Recap and exercise 1



- Differential gene expression analysis typically involves calculating fold change, running a statistical test to compare gene expression between 2 conditions, and adjusting the p-value.
- **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):
  - Is the gene CPS1 significantly differentially expressed between NK and Th cells?
  - How many genes are up-regulated and down-regulated in NK after BH adjustment?
  - Is the gene CPS1 still significant after BH adjustment?

ensembl_gene_id	symbol	logFC	t	P.Value
ENSG00000000003	TSPAN6	-5.6436044	-4.6721285	4.26E-05
ENSG00000000419	DPM1	-0.1818981	-1.1018308	0.27801982
ENSG00000000457	SCYL3	0.49698737	1.49103508	0.14486907
ENSG00000000460	C1orf112	1.1217991	1.44589945	0.15705988
ENSG00000000938	FGR	10.6706873	7.21234165	1.98E-08

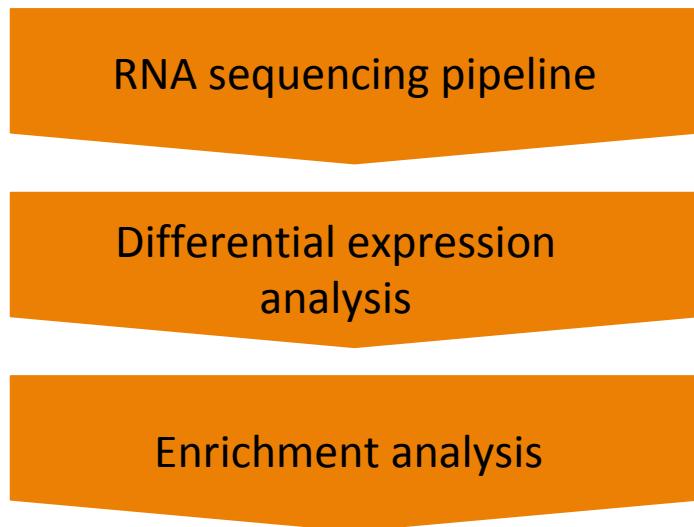
Positive logFC = higher in NK  
Negative logFC = lower in NK

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>

# Once we have identified DE genes, what do we do?



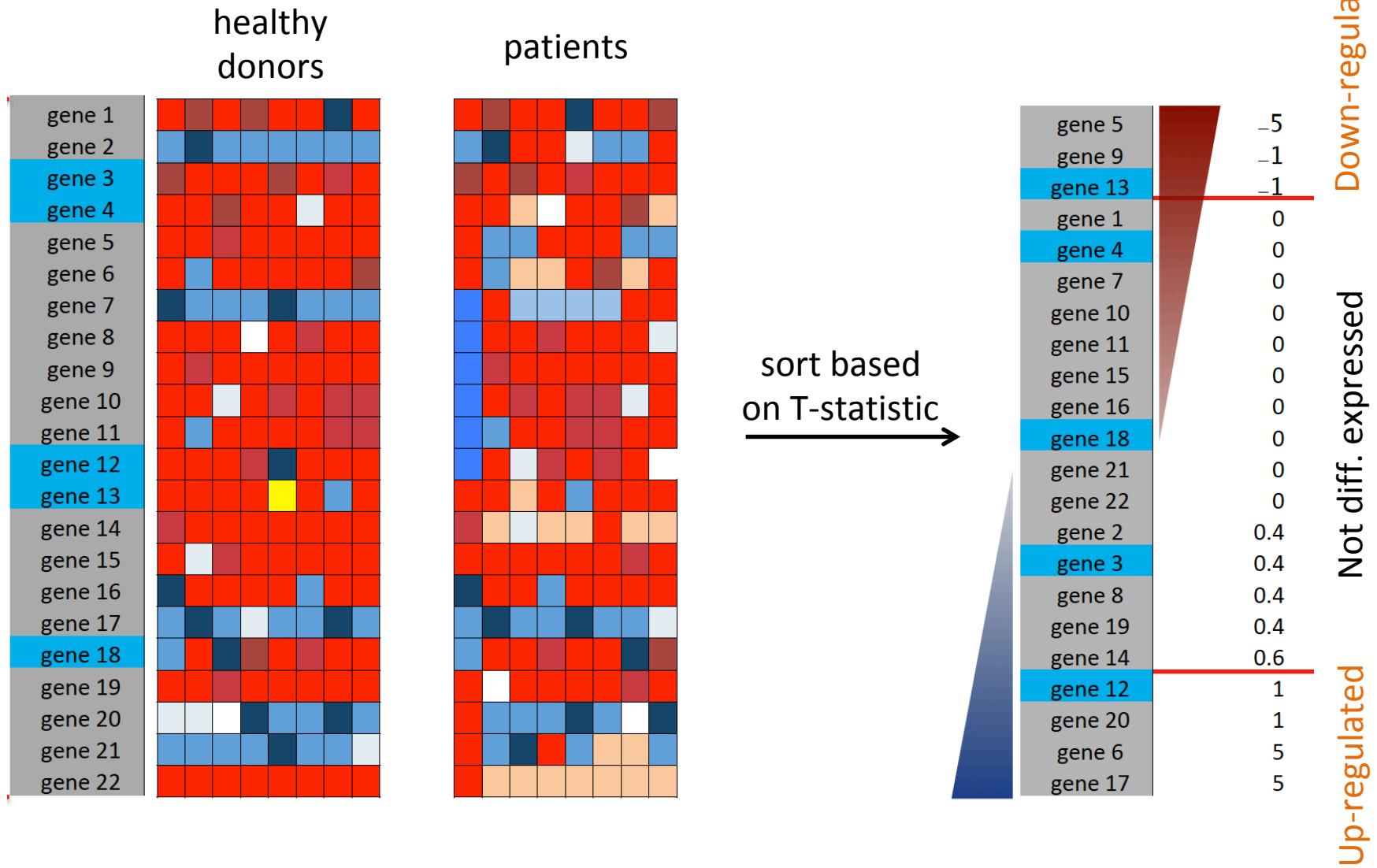
Several methods available, *e.g.*:

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

**Goal:** to gain biologically-meaningful insights from long gene lists

- test if differentially expressed genes are enriched in genes associated with a particular function
- approaches: test a small number of gene sets, or a large collection of gene sets

# Are the genes belonging to the blue set differentially expressed?



# Fisher's exact test in R

```
> cont.table<-matrix(c(2,3,5,12), 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:

0.1012333 18.7696686

sample estimates:

odds ratio

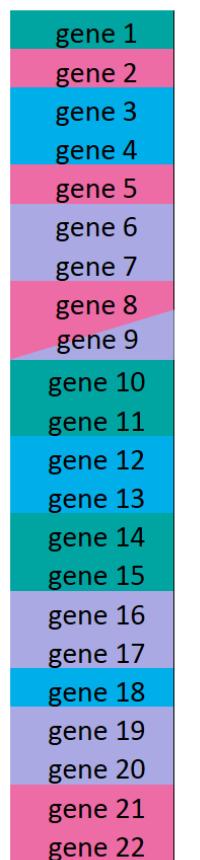
1.56456

2x2 count table	Differentially expressed	Not Differentially expressed	total
blue	2	3	5
Not blue	5	12	17
total	7	15	22

$$2/7 = \\ 0.29$$

$$3/15 = \\ 0.20$$

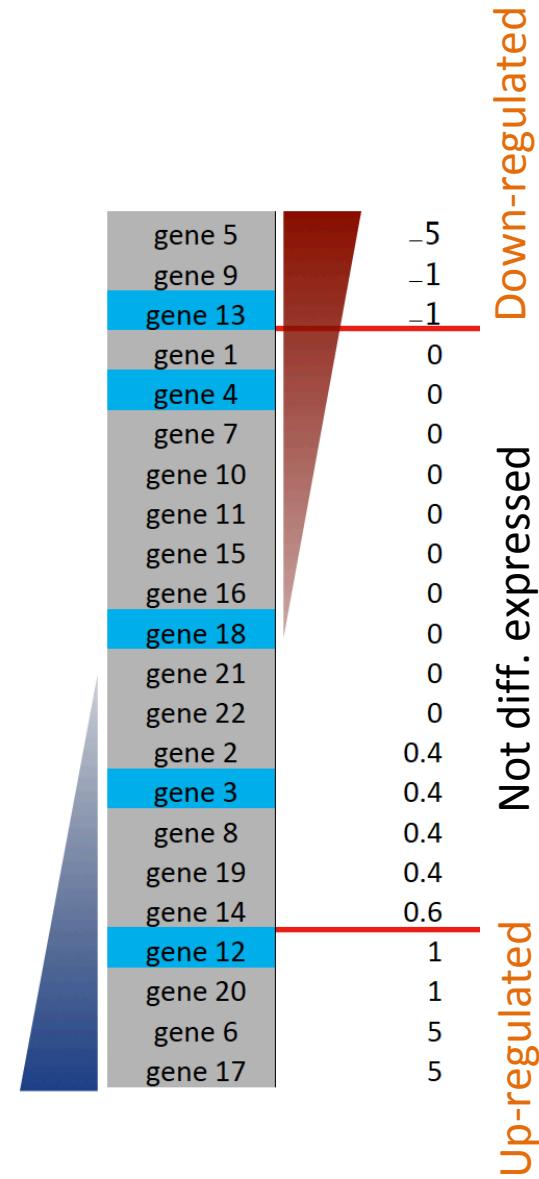
# Which gene sets are differentially expressed?



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

⇒ Multiple tests need **p-value adjustment**.

⇒ But Fisher test is **threshold-based**.

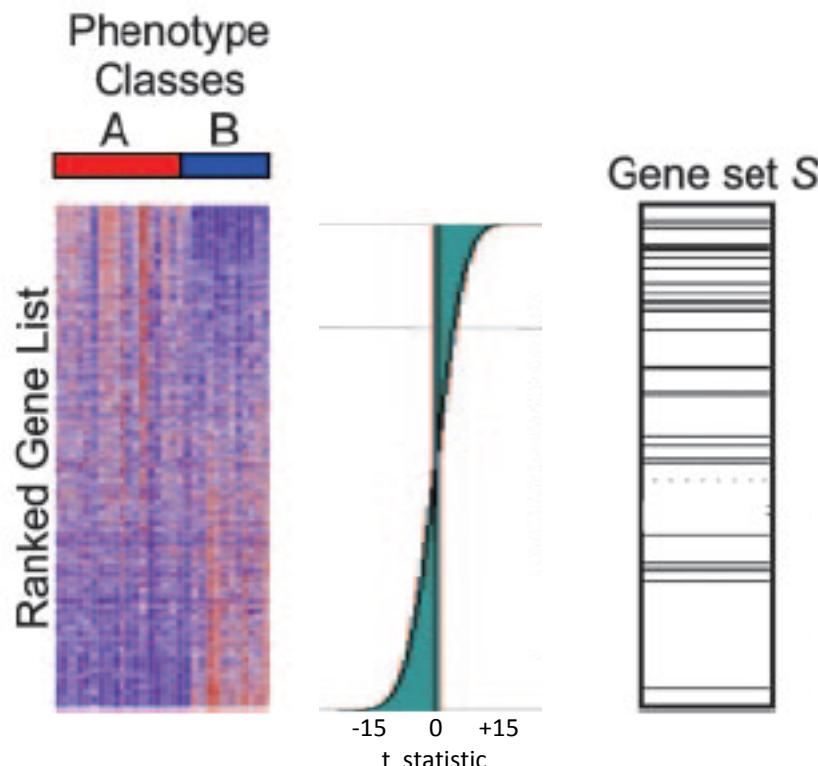


# Gene set enrichment analysis (GSEA)

- 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)
- Threshold-free: the whole list of genes detected in the RNA sequencing experiment is used.
- 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.

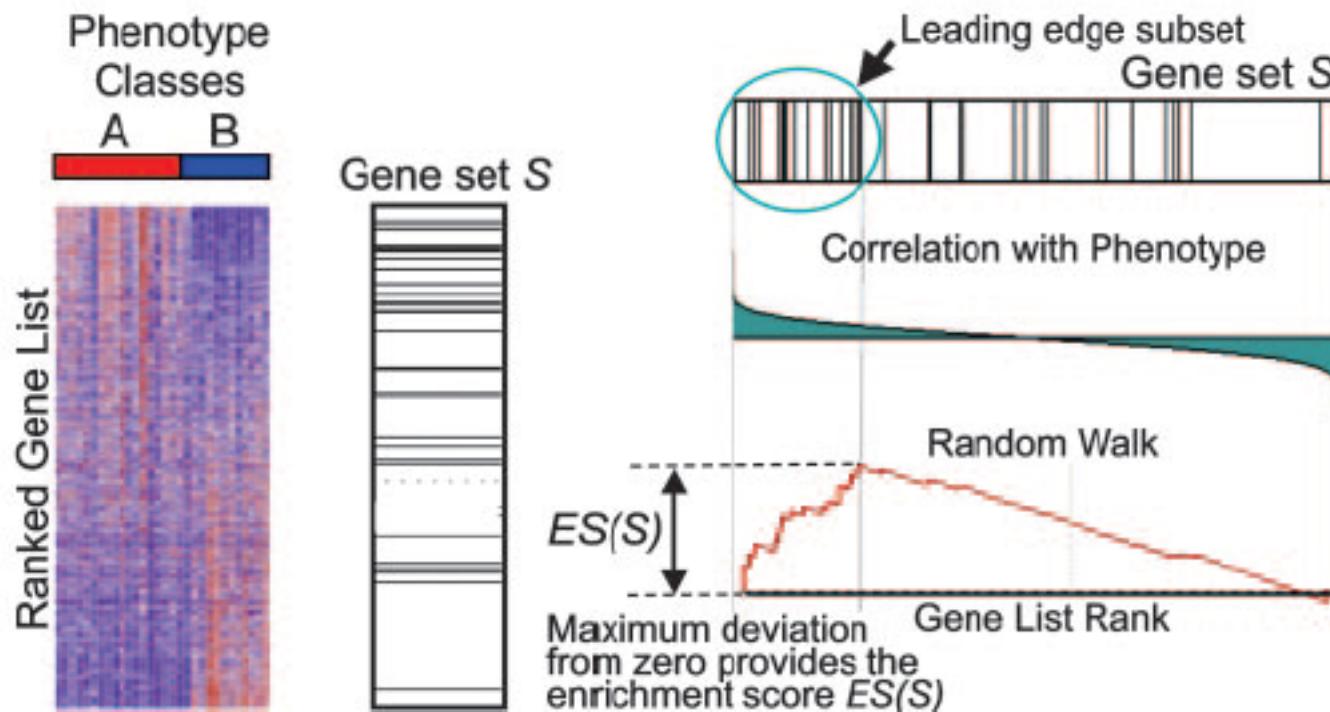
# Method of GSEA

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)

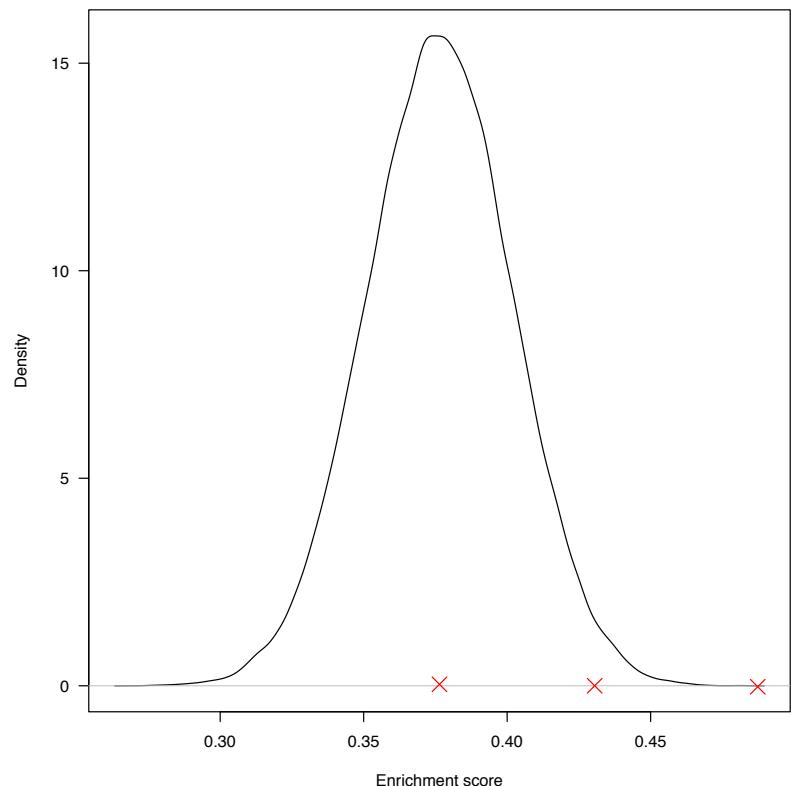
# Method of GSEA



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.)

# 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



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

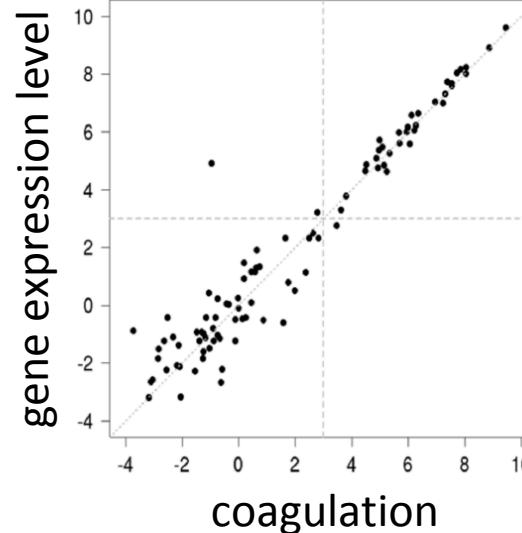
Do not forget: p-value  
adjustment if more than 1  
gene set is tested!

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

# Apply GSEA to any type of data or score

- Use t-statistic from paired t-test
- Use F statistic of one way or two way ANOVA
- Use p-value of linear model

	Adj. p-value of LM
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1



GSEA for linear model implemented in `romer()` function of the `limma` package

# GSEA using R: one possibility among many

## clusterProfiler

platforms all rank 36 / 2041 support 10 / 14 in Bioc 10 years  
build ok updated before release dependencies 123

DOI: [10.18129/B9.bioc.clusterProfiler](https://doi.org/10.18129/B9.bioc.clusterProfiler) [f](#) [t](#)

statistical analysis and visualization of functional profiles for genes and gene clusters

Bioconductor version: Release (3.13)

This package implements methods to analyze and visualize functional profiles (GO and KEGG) of gene and gene clusters.

Author: Guangchuang Yu [aut, cre, cph] , Li-Gen Wang [ctb], Erqiang Hu [ctb], Meijun Chen [ctb], Giovanni Dall'Olio [ctb] (formula interface of compareCluster)

Maintainer: Guangchuang Yu <guangchuangyu at gmail.com>

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

Built-in GO and KEGG (see later)

- 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:\[10.1089/omi.2011.0118\]](https://doi.org/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 enrichment analysis with 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)
```

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

```
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",  
  ...  
)
```

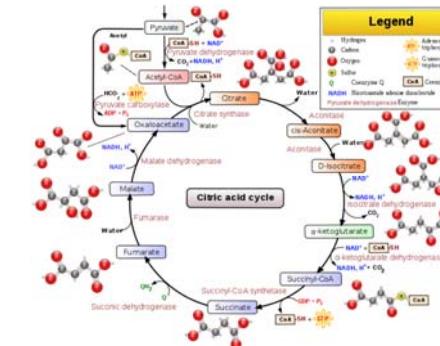
enricher(): similar to Fisher's exact test, for user defined gene list and gene set annotations (package clusterProfiler)

```
enricher(  
  gene,  
  pvalueCutoff = 0.05,  
  pAdjustMethod = "BH",  
  universe,  
  minGSSize = 10,  
  maxGSSize = 500,  
  qvalueCutoff = 0.2,  
  TERM2GENE,  
  TERM2NAME = NA  
)  
) Eg genes that are markers of cell clusters of single-cell RNA seq
```

# 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. Remember to use p-value adjustment if multiple Fisher tests are used.
- Exercise 2: use functions of clusterProfiler and data provided in Ex. 1
  - Is the adaptive immune response gene set significantly enriched in genes up-regulated in NK vs Th?
  - How many GO gene sets are significant after GSEA (use minGSSize=30) ?
  - Is the adaptive immune response gene set significant? Up-reg. or down-reg.?
  - Are the majority of gene sets rather up-regulated or down-regulated?

# What is a gene set?

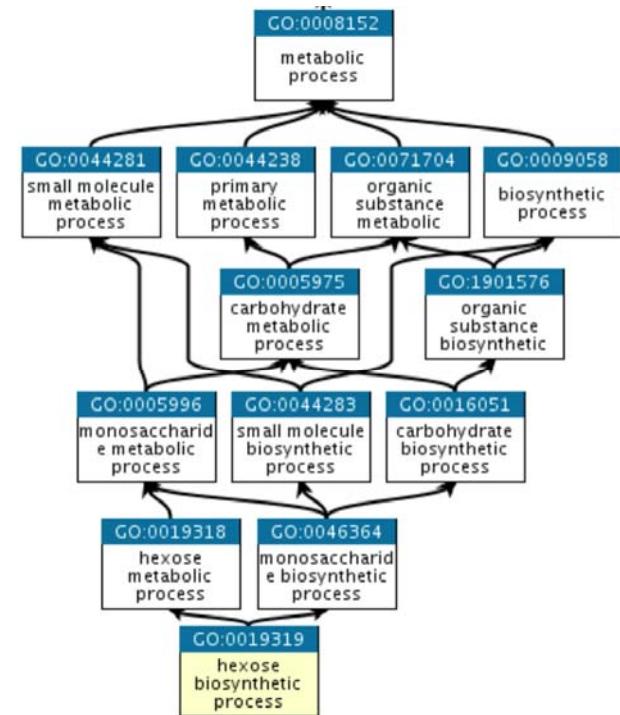


[https://en.wikipedia.org/wiki/Citric\\_acid\\_cycle](https://en.wikipedia.org/wiki/Citric_acid_cycle)

- 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 genes 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)

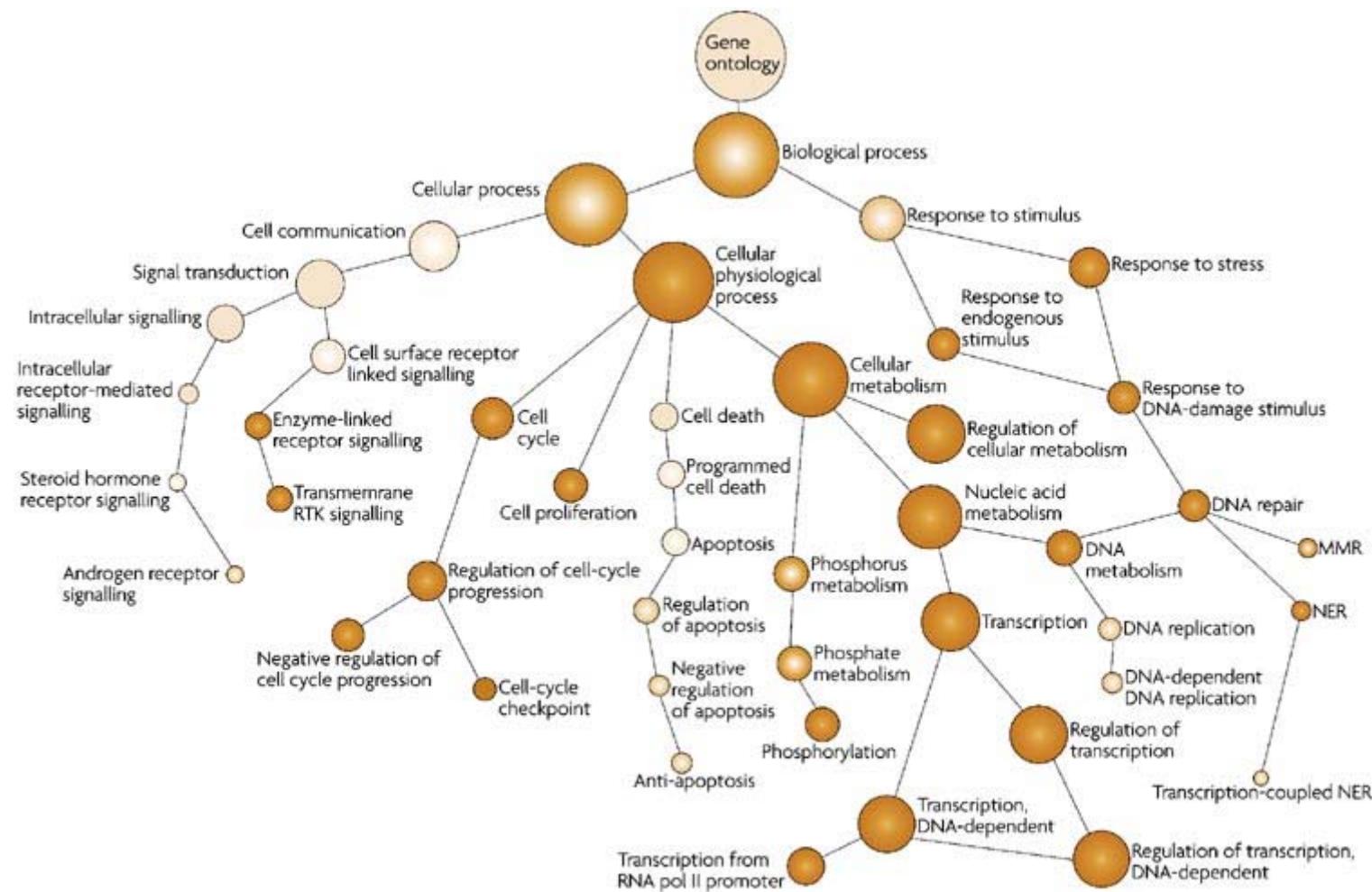


# Gene ontology

**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.

# Gene ontology



# KEGG

<https://www.genome.jp/kegg/>



## KEGG PATHWAY Database

Wiring diagrams of molecular interactions, reactions and relations

[KEGG2](#) [PATHWAY](#) [BRITE](#) [MODULE](#) [KO](#) [GENES](#) [DISEASE](#) [DRUG](#) [COMPOUND](#)

Select prefix

map

Organism

Enter keywords

Go

Help

[ [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](#).

# Reactome

<https://reactome.org/>



The screenshot shows the Reactome website homepage. At the top left is the Reactome logo. To its right is a navigation bar with links: About, Content, Docs, Tools, Community, and Download. Below the navigation bar is a search bar with the placeholder text "e.g. O95631, NTN1, signaling by EGFR, glucose" and a "Go!" button. The main content area features four large blue rounded squares, each containing a white icon and a corresponding text label below it. From left to right, the icons represent: a pathway browser (a tree-like structure), analysis tools (a bar chart), ReactomeFIViz (two overlapping circles), and documentation (a document with three lines of text).

Pathway Browser	Analysis Tools	ReactomeFIViz	Documentation
			
Pathway Browser	Analysis Tools	ReactomeFIViz	Documentation
Visualize and interact with Reactome biological pathways	Merges pathway identifier mapping, over-representation, and expression analysis	Designed to find pathways and network patterns related to cancer and other types of diseases	Information to browse the database and use its principal tools for data analysis

# MSigDB

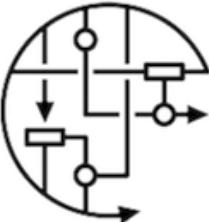
<https://www.gsea-msigdb.org/gsea/msigdb/index.jsp>

- |           |  |   |
|-----------|--|---|
| <b>H</b>  | <b>hallmark gene sets</b> are coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes. | <b>C5</b> <b>ontology gene sets</b> consist of genes annotated by the same ontology term.   |
| <b>C1</b> | <b>positional gene sets</b> for each human chromosome and cytogenetic band.  | <b>C6</b> <b>oncogenic signature gene sets</b> defined directly from microarray gene expression data from cancer gene perturbations.      |
| <b>C2</b> | <b>curated gene sets</b> from online pathway databases, publications in PubMed, and knowledge of domain experts.   | <b>C7</b> <b>immunologic signature gene sets</b> represent cell states and perturbations within the immune system.                        |
| <b>C3</b> | <b>regulatory target gene sets</b> based on gene target predictions for microRNA seed sequences and predicted transcription factor binding sites.                    | <b>C8</b> <b>cell type signature gene sets</b> curated from cluster markers identified in single-cell sequencing studies of human tissue. |
| <b>C4</b> | <b>computational gene sets</b> defined by mining large collections of cancer-oriented microarray data.   |   |

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4707969/>

# WikiPathways

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



search

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**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

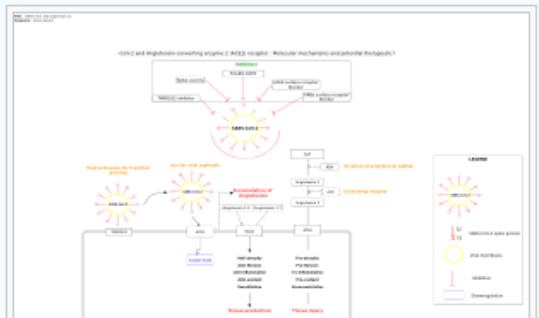
You can search by:

- Pathway name (*Apoptosis*)
- Gene or protein name (*p53*)

**Browse**

**Today's Featured Pathway**

SARS-CoV-2 and angiotensin-converting enzyme 2 receptor: molecular mechanisms (*Homo sapiens*)



SARS-CoV-2 and angiotensin-converting enzyme 2 receptor: molecular mechanisms

# GSEA of other gene sets in R

ClusterProfiler: GSEA for 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")
```

Import a .gmt file of gene sets and convert to format needed for clusterProfiler

```
read.gmt(gmtfile)

> 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

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

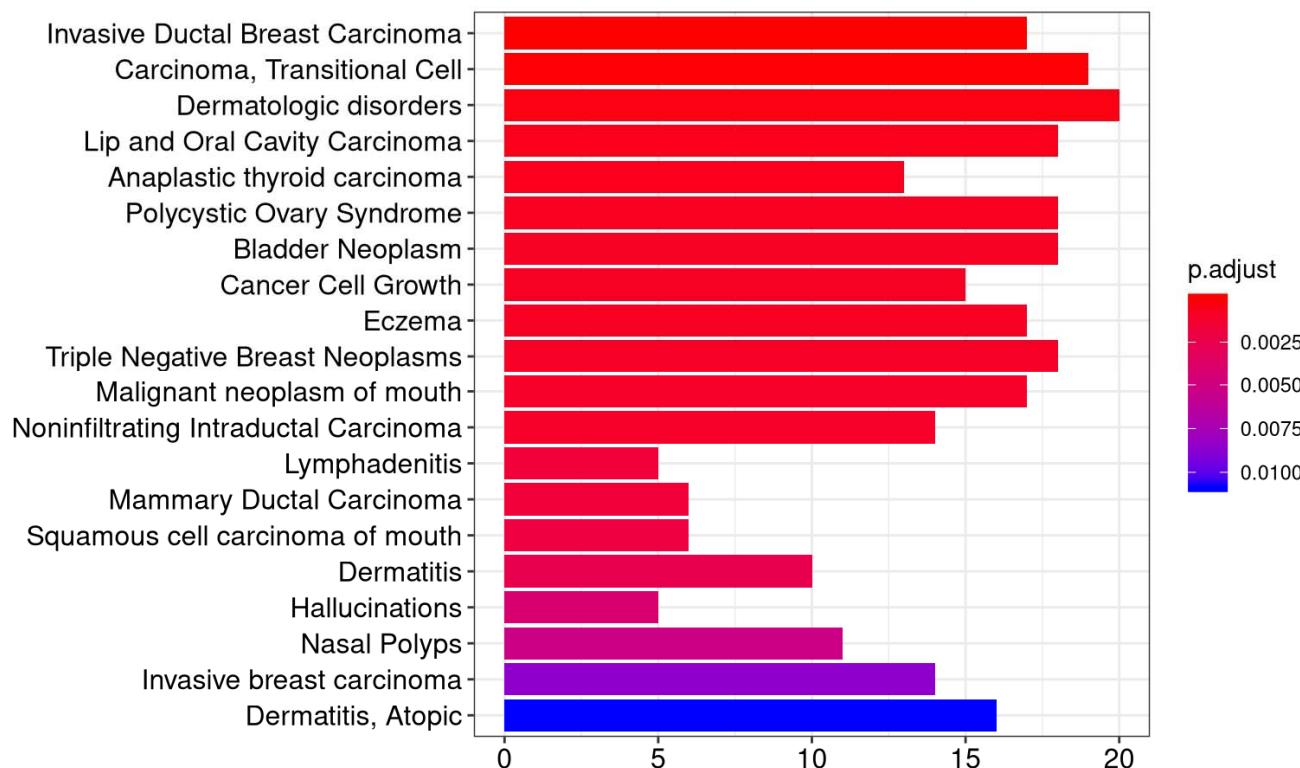
# Recap and exercise 3

- 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 3: use functions of clusterProfiler and data provided in Ex. 1, and hallmark gene sets downloaded from MSigDB
  - First convert the gene symbols to EntrezID to perform a GSEA of KEGG pathways (with argument minGSSize=30).
  - Are the majority of gene sets rather up-regulated or down-regulated?
  - Is there a KEGG immune-related gene set coming up? Is there a KEGG Natural killer gene set coming up?
  - If you want to see which genes are included in one of the built-in KEGG pathways, where could you find this information?
  - Import the hallmark gene sets and run a GSEA. How many significant gene sets are there?

# Visualization of Functional Enrichment Results

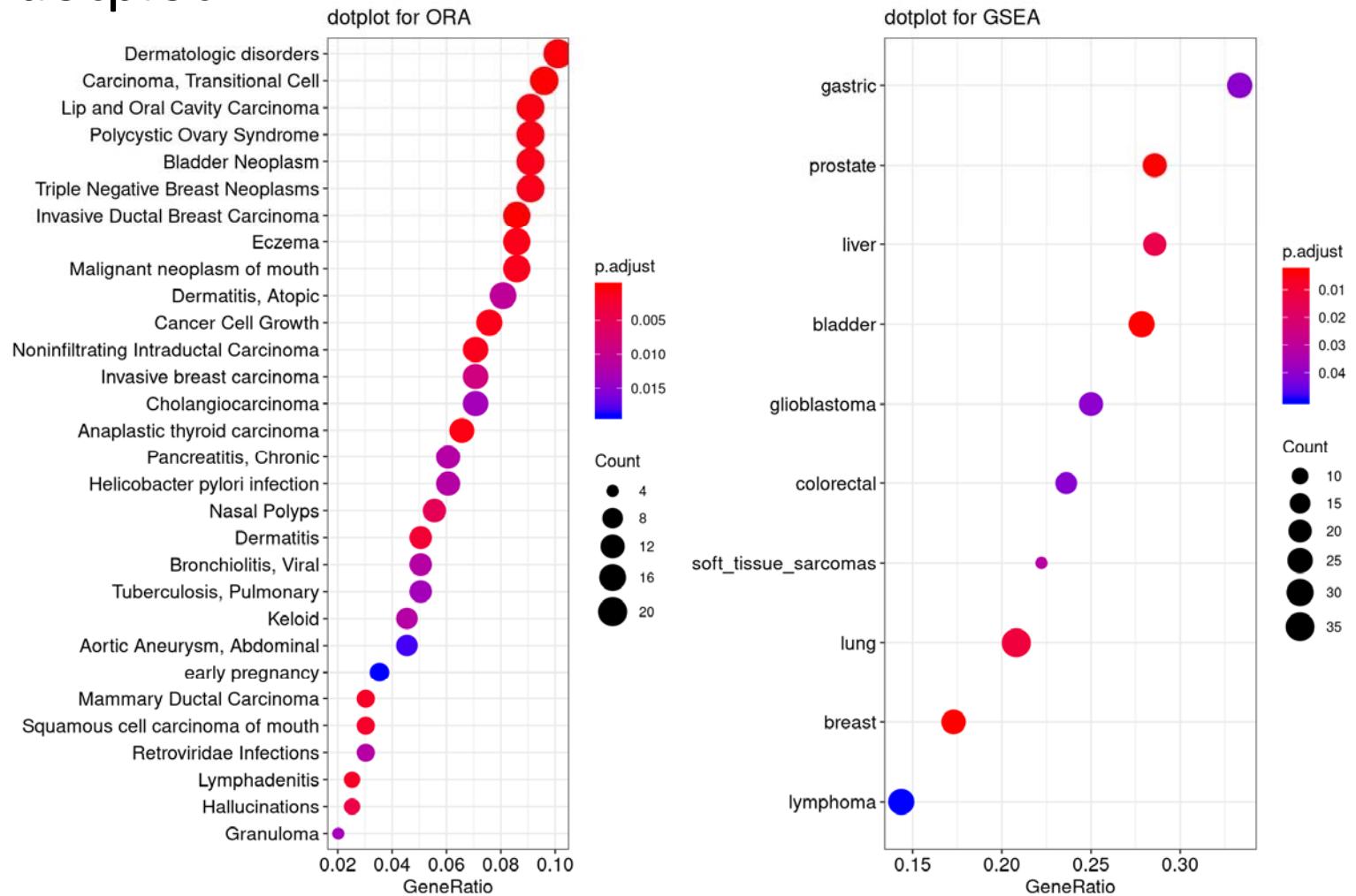
- **barplot**

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



# Visualization of Functional Enrichment Results

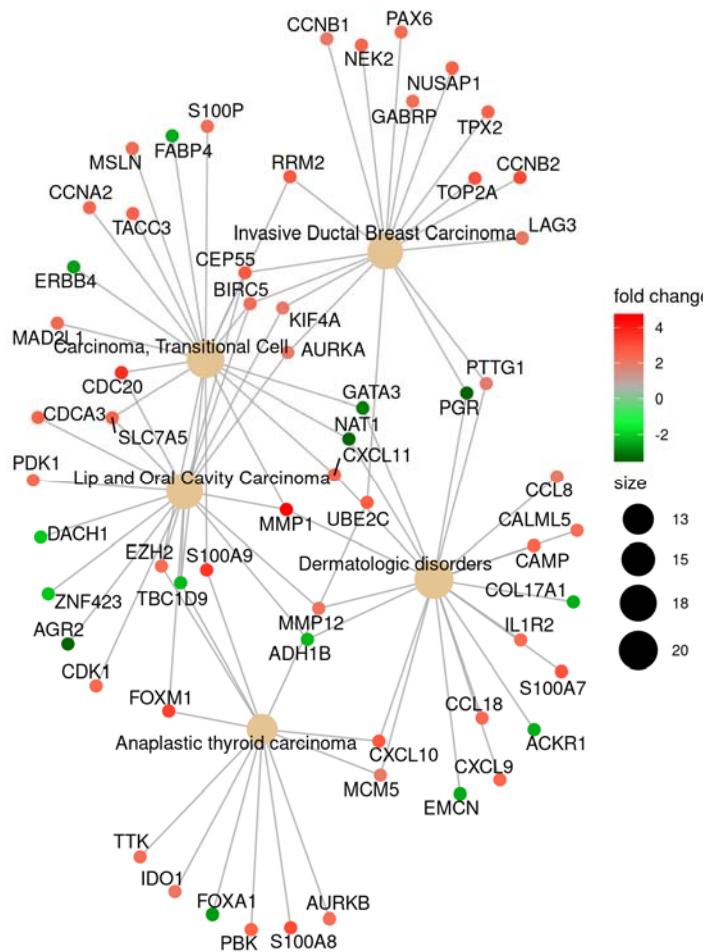
- **dotplot**



# Visualization of Functional Enrichment Results

- cnetplot

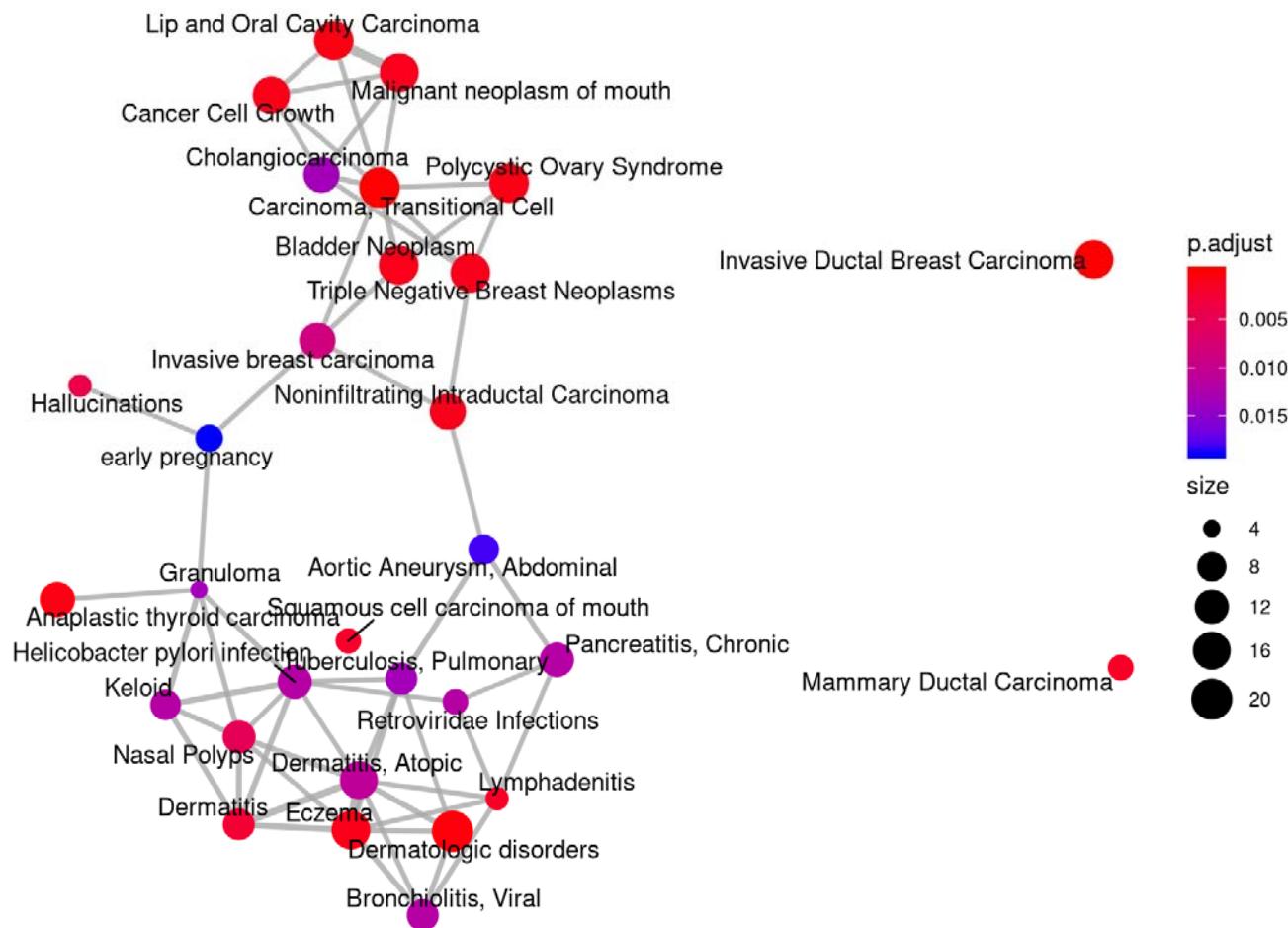
```
cnetplot(ego, categorySize="pvalue", foldChange=geneList)
```



# Visualization of Functional Enrichment Results

- Enrichment map

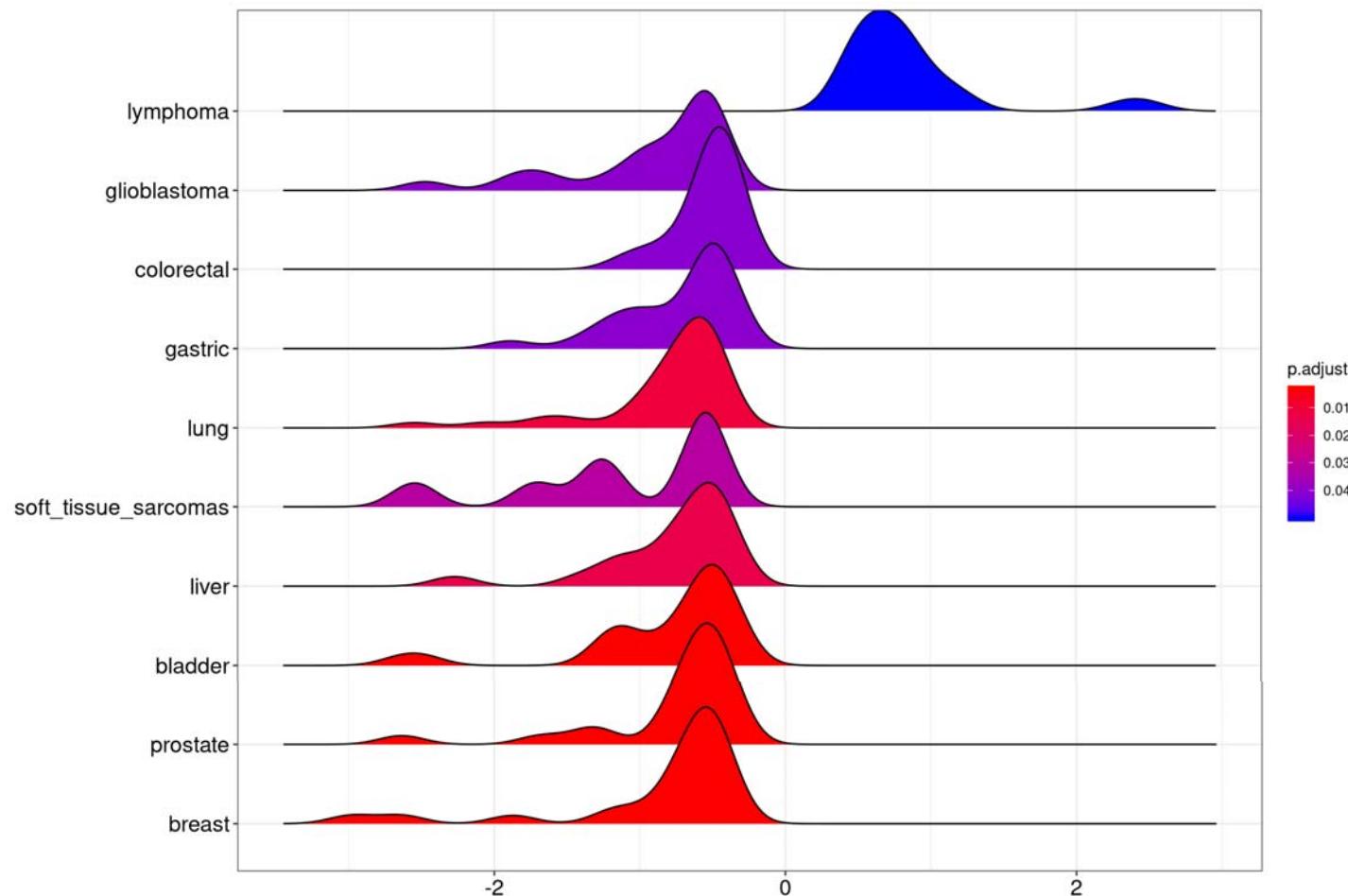
emapplot(ego)



# Visualization of Functional Enrichment Results

- Ridgeplot

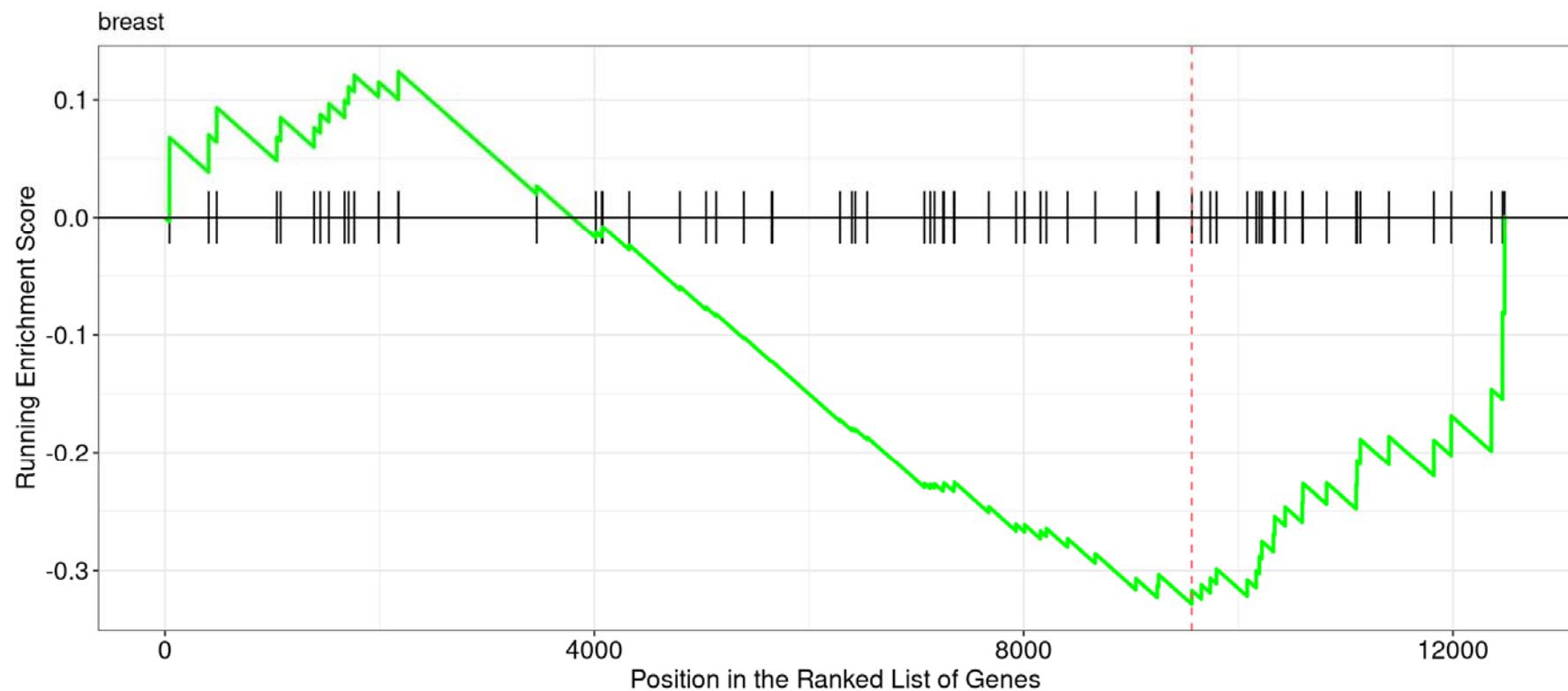
```
ggo <- gseGO(gl, ont="BP")
ridgeplot(ggo)
```



# Visualization of Functional Enrichment Results

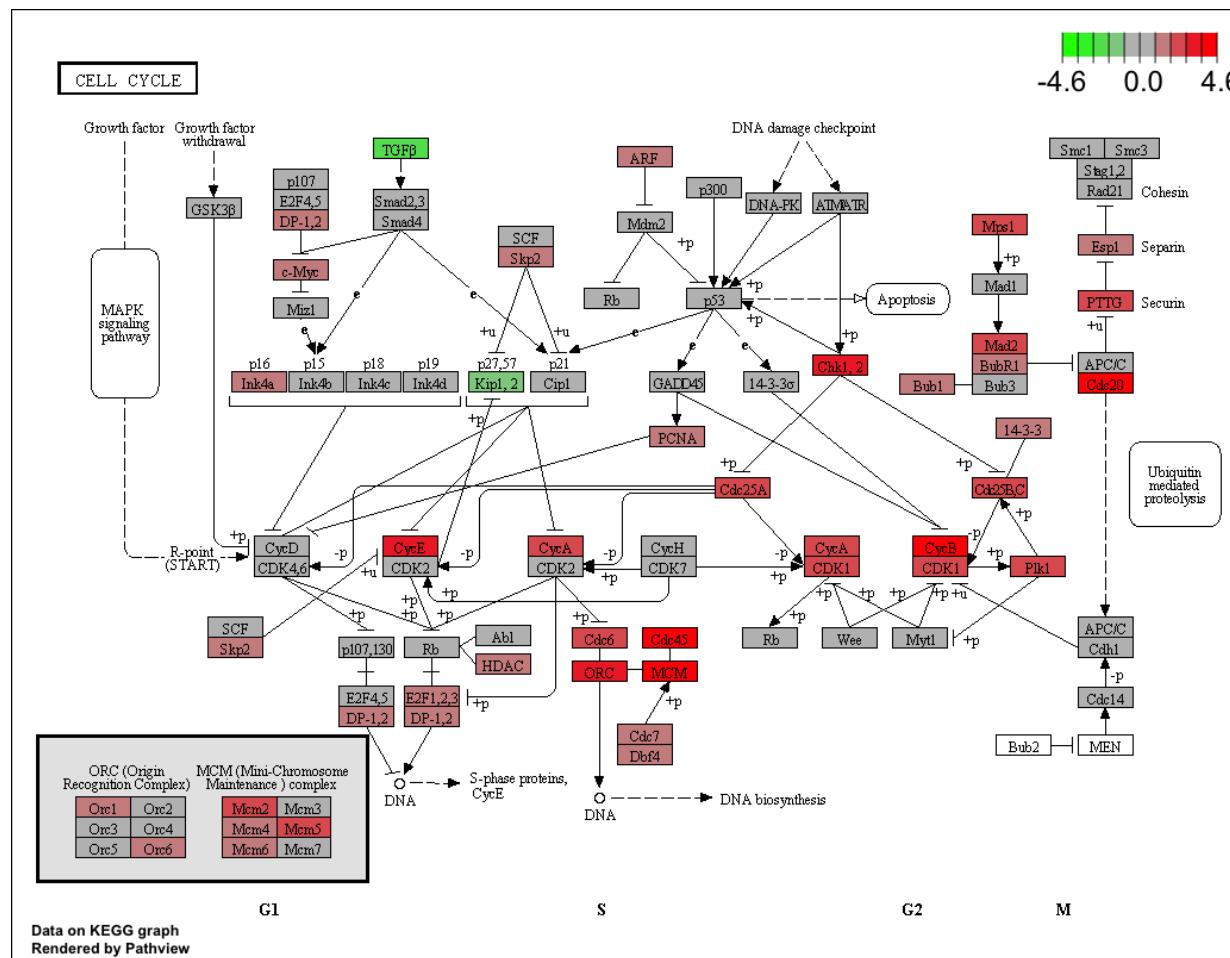
- visualizing GSEA result

```
gseaplot(h_NK_vs_Th, geneSetID =  
"BREAST", title=" BREAST")
```

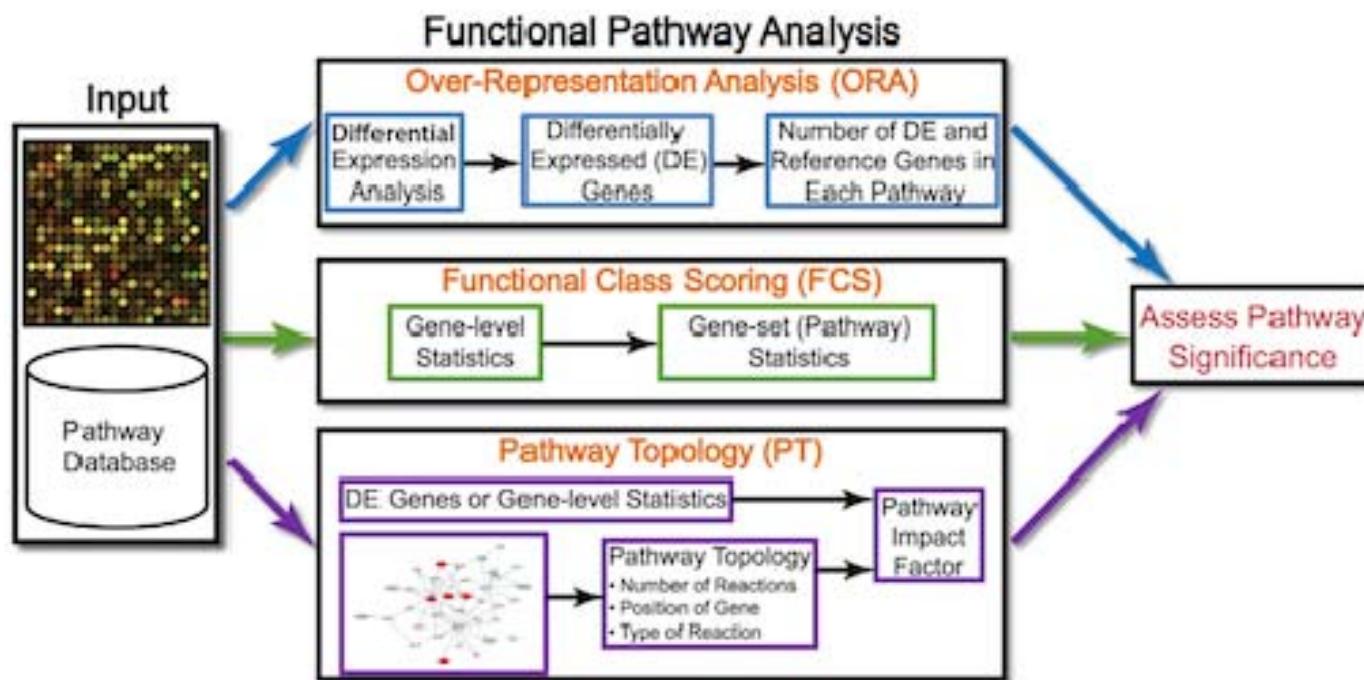


# Visualization of Functional Enrichment Results

- pathview



# Functional analysis



# 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 <sub>NDE</sub>	P <sub>PERT</sub>	P <sub>G</sub>	P <sub>FDR</sub>	P <sub>FWER</sub>	Status
Focal adhe..4510	0.0001	0.0000	0.0000	0.000000	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.

$$P_{NDE} = P(X \geq N_{DE} | H_0)$$

$P_{PERT}$ : probability to observe a larger perturbation than observed

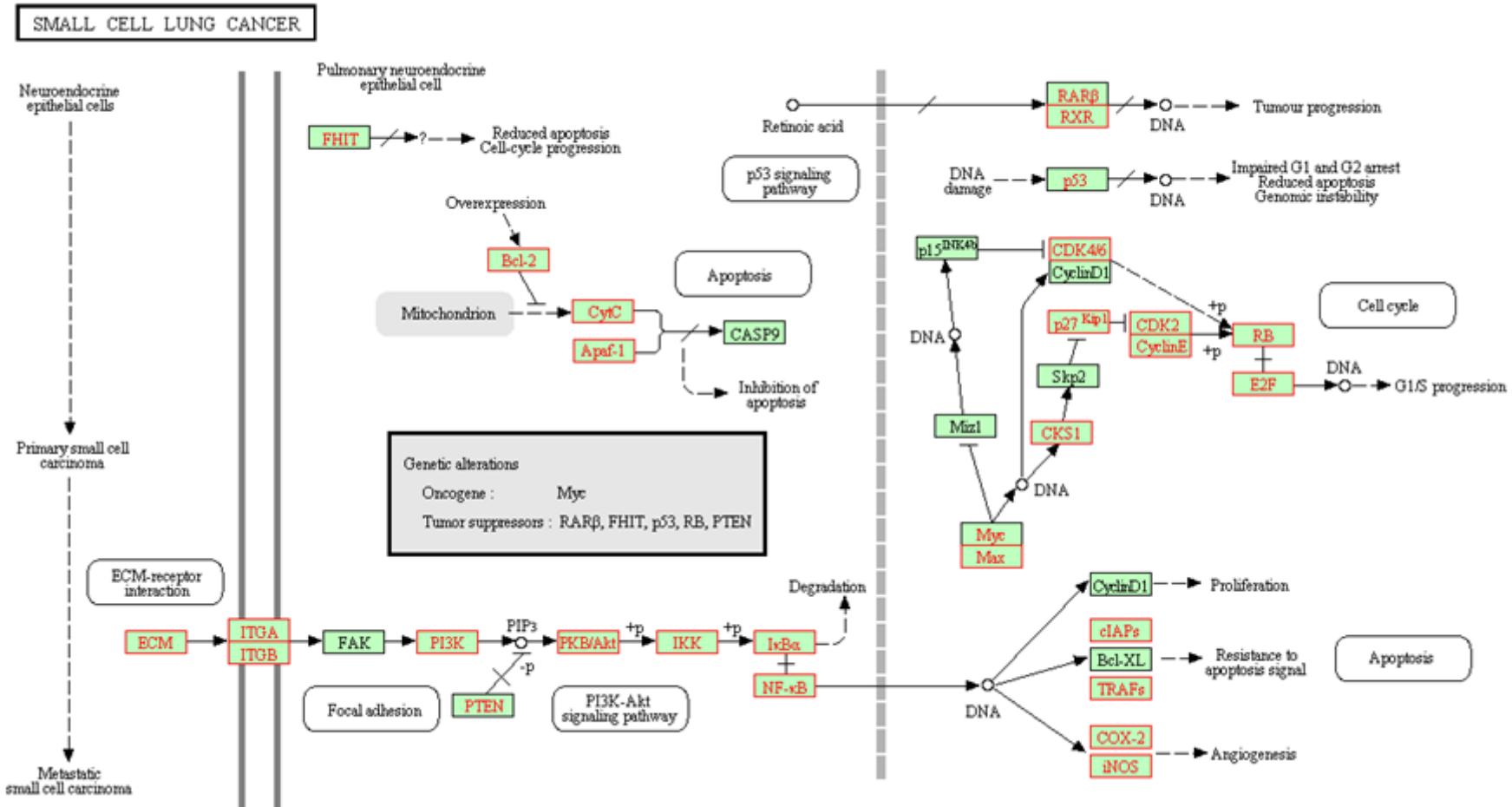
$P_G$ : combination of  $P_{NDE}$  and  $P_{PERT}$

$P_{FDR}$ : adjusted FDR p-value

$P_{FWER}$ : adjusted FDR p-value (more conservative)

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

# Functional analysis: Pathway topology tools



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

# Additional resources for functional analysis

The screenshot shows the g:Profiler website interface. At the top, there is a navigation bar with links: News, Archives, Beta, API, R client, FAQ, Docs, Contact, Cite g:Profiler, and a menu icon. A blue banner at the top of the main content area states: "g:Profiler has been updated with new data from Ensembl." with "Show more..." and "Close" buttons. Below this, there is a horizontal navigation bar with four tabs: "g:GOSt Functional profiling" (highlighted in orange), "g:Convert Gene ID conversion", "g:Orth Orthology search", and "g:SNPense SNP id to gene name". The main content area has two sections: "Query" (highlighted in orange) and "Upload query" and "Upload bed file". A note says "Input is whitespace-separated list of genes" with a help icon. To the right, there is a "Options" section with "Organism:" dropdown set to "Homo sapiens (Human)", two checkboxes for "Ordered query" and "Run as multiquery", and three buttons: "Advanced options", "Data sources", and "Bring your data (Custom GMT)".

<https://biit.cs.ut.ee/gprofiler/gost>

# Additional resources for functional analysis



The screenshot shows the DAVID Bioinformatics Resources 6.8 homepage. At the top, there's a blue header bar with the DAVID logo on the left and the text "DAVID Bioinformatics Resources 6.8" and "Laboratory of Human Retrovirology and Immunoinformatics (LHRI)" on the right. Below the header is a navigation menu with links: Home, Start Analysis, Shortcut to DAVID Tools, Technical Center, Downloads & APIs, Term of Service, About DAVID, and About LHRI. The main content area has a light gray background and features a section titled "Overview".

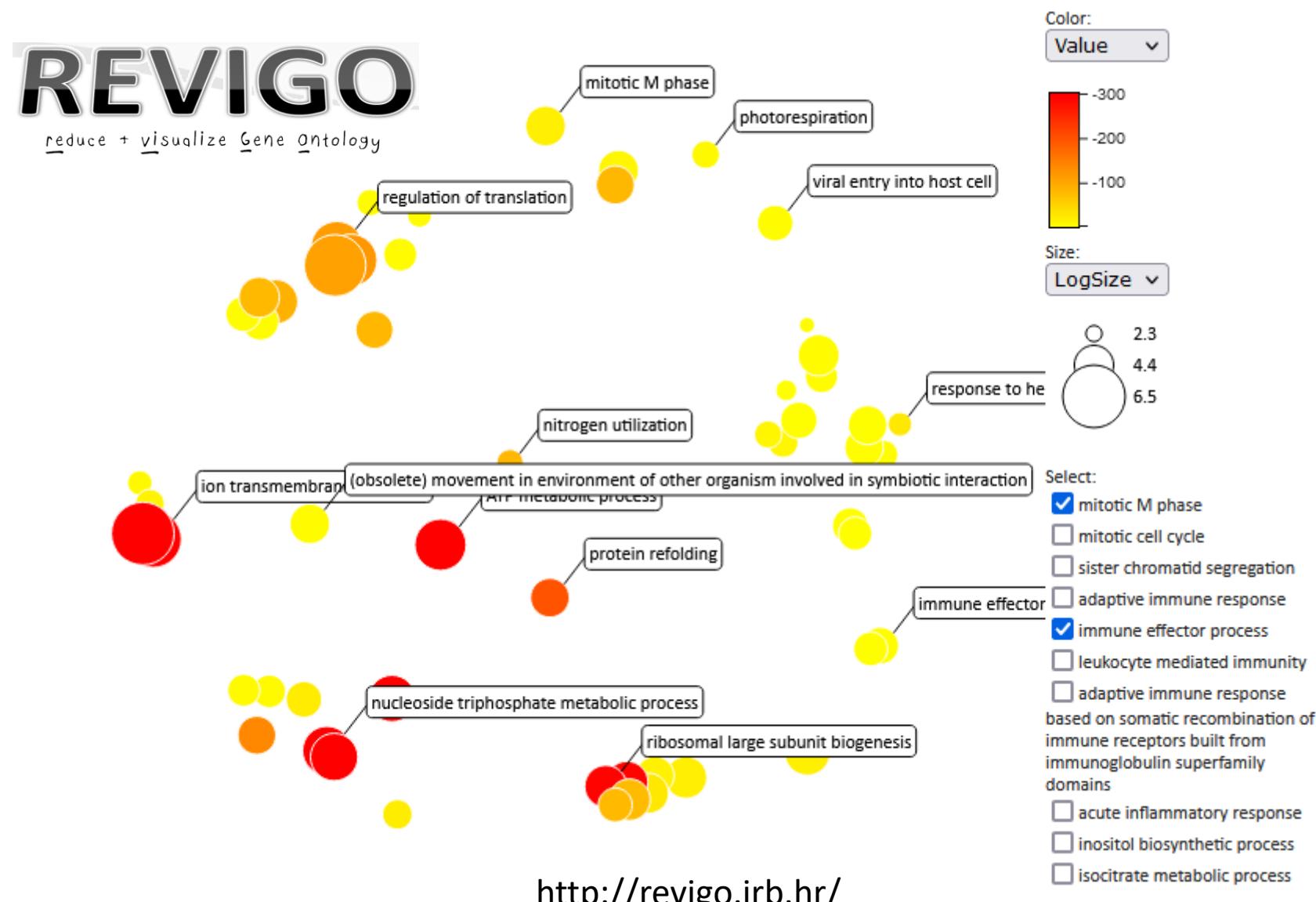
## Overview

The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.8 comprises a full Knowledgebase update to the sixth version of our original web-accessible programs. DAVID now provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes. For any given gene list, DAVID tools are able to:

- Identify enriched biological themes, particularly GO terms
- Discover enriched functional-related gene groups
- Cluster redundant annotation terms
- Visualize genes on BioCarta & KEGG pathway maps
- Display related many-genes-to-many-terms on 2-D view.
- Search for other functionally related genes not in the list
- List interacting proteins
- Explore gene names in batch
- Link gene-disease associations
- Highlight protein functional domains and motifs
- Redirect to related literatures
- Convert gene identifiers from one type to another.

<https://david.ncifcrf.gov/home.jsp>

# Additional resources for functional analysis



# Additional resources for functional analysis

- gProfiler - <http://biit.cs.ut.ee/gprofiler/index.cgi>
- DAVID - <http://david.abcc.ncifcrf.gov/tools.jsp>
- clusterProfiler - <http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html>
- GeneMANIA - <http://www.genemania.org/>
- GenePattern - <http://www.broadinstitute.org/cancer/software/genepattern/> (need to register)
- WebGestalt - <http://bioinfo.vanderbilt.edu/webgestalt/> (need to register)
- AmiGO - <http://amigo.geneontology.org/amigo>
- ReviGO (visualizing GO analysis, input is GO terms) - <http://revigo.irb.hr/>
- WGCNA - <http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork>
- GSEA - <http://software.broadinstitute.org/gsea/index.jsp>
- SPIA - <https://www.bioconductor.org/packages/release/bioc/html/SPIA.html>
- GAGE/Pathview - <http://www.bioconductor.org/packages/release/bioc/html/gage.html>

# Recap and Exercise 4

- We have seen several types of visualization methods of functional enrichment results

## Exercise 4: create the following figures:

- barplot of  $-\log_{10}(p\text{-value})$  of top 10 GO p-values
- GSEA plot for HALLMARK MTORC1 SIGNALING
- pathview map for KEGG Natural Killer mediated cytotoxicity (optional: with non-significant genes in grey)

# Some links

- Contact **Tania** if you wish to discuss enrichment analysis of your data more specifically:
  - [tania.wyss@sib.swiss](mailto:tania.wyss@sib.swiss)
- Contact the head of the Bioinformatics Core Facility if you need more extensive biostatistics support:
  - [mauro.delorenzi@sib.swiss](mailto:mauro.delorenzi@sib.swiss)

Links :

limma (for gene expression analysis and also includes functions for enrichment analysis):

<https://www.bioconductor.org/packages/devel/bioc/vignettes/limma/inst/doc/usersguide.pdf>

edgeR:

<https://www.bioconductor.org/packages/release/bioc/vignettes/edgeR/inst/doc/edgeRUsersGuide.pdf>

DESeq2:

<http://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html>

clusterProfiler:

<https://yulab-smu.github.io/clusterProfiler-book/>

bioconductor, introduction and structure

[https://ivanek.github.io/analysisOfGenomicsDataWithR/02\\_IntroToBioc\\_html.html](https://ivanek.github.io/analysisOfGenomicsDataWithR/02_IntroToBioc_html.html)

online tool for overrepresentation analysis

<http://www.pantherdb.org/>

# Credits: 0.25 ECTS

- Please provide results of exercises 2, 3 & 4 and answers to the following questions in a document:
  - Perform GSEA of the NK vs Th data using the Reactome gene sets downloaded on the MSigDB website (use minGSSize=30)
  - How many gene sets are significantly enriched? Generate an ordered barplot of the NES of all genesets, and generate a barcode plot for the gene set with the lowest NES
- Sign up for credit by adding your name to the [Google doc](#)
- Send results to [tania.wyss@sib.swiss](mailto:tania.wyss@sib.swiss) by the end of next week.

Thank you for your attention!

Please fill in the **feedback** available on the Moodle page!

We thank Linda Dib for providing course material