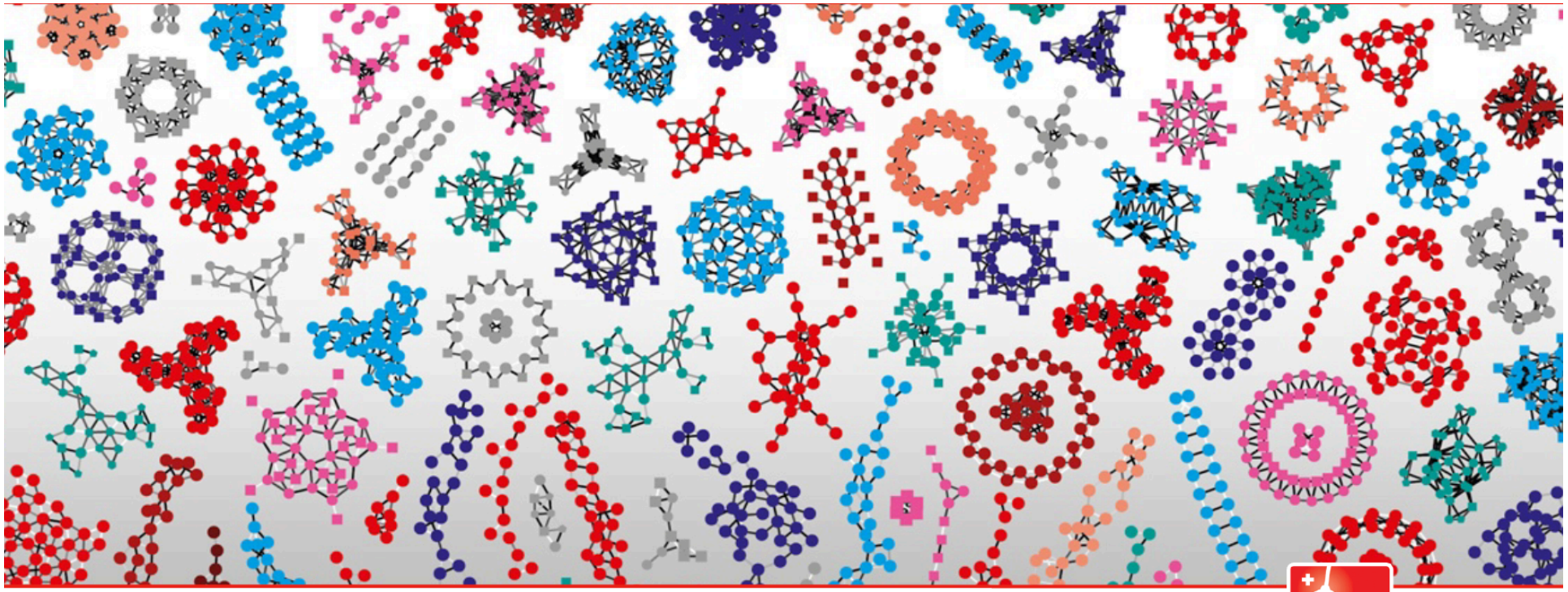

Enrichment analysis

Swiss Institute of Bioinformatics

June 24th 2022

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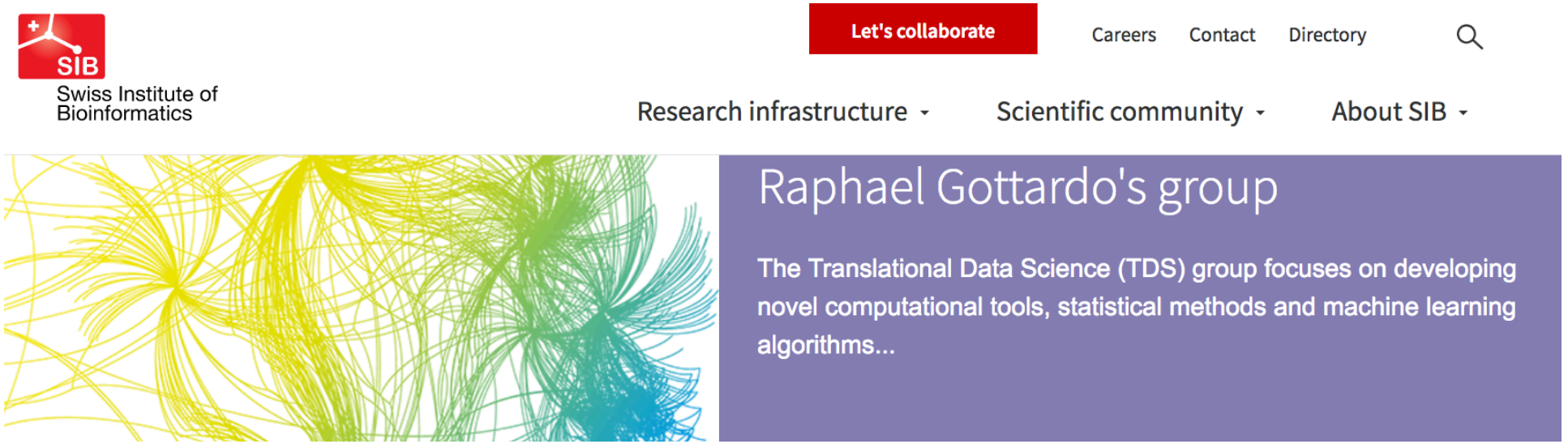
gustavo.ruizbuendia@sib.swiss

Schedule

- **9:00 - 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



https://www.sib.swiss/raphael-gottardo-group

The Translational Data Science (TDS) group focuses on developing novel computational tools, statistical methods and machine learning algorithms for the analysis of high-throughput and high-dimensional datasets generated by novel assay technologies with applications in immunology, vaccine research and immunotherapy. We collaborate with multiple research groups locally, nationally and internationally to address important immunological problems through high-dimensional modeling and data integration.

Domains of activity:
Core facility and competence center, Biostatistics, Infectious diseases, Machine learning, Mathematical modeling, Next generation sequencing, Oncology, Personalized medicine, Single-cell biology, Transcriptomics, Vaccines
For core facility service inquiry: nadine.fournier@sib.swiss

Tell us about yourself !

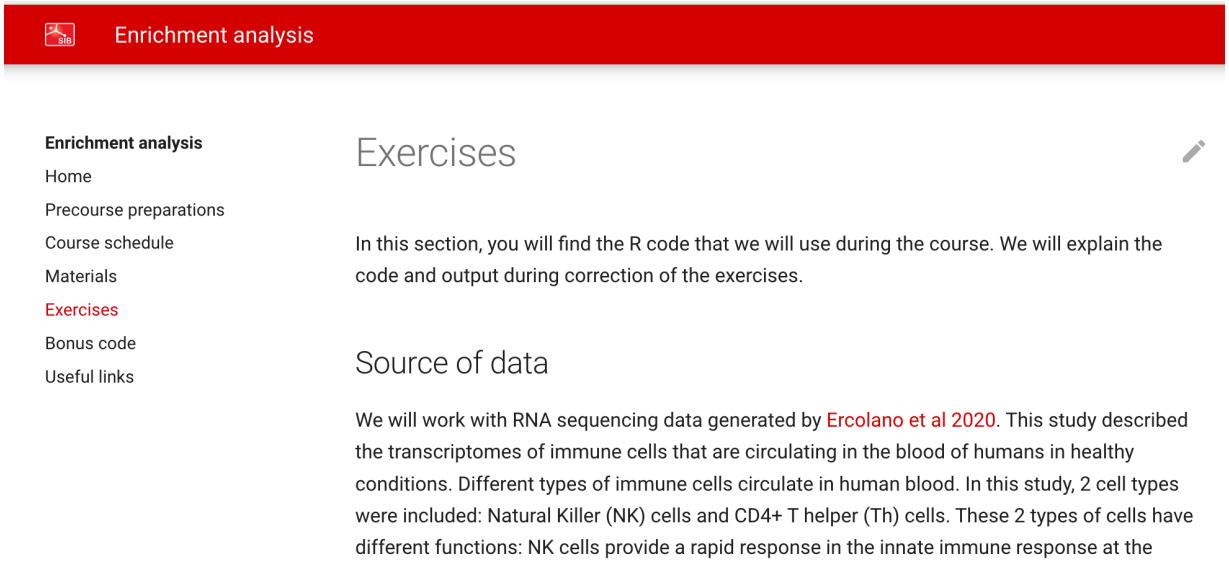
- What organism are you working on? What type of data are you analyzing?
- Write your name and some keywords about yourself and/or your research into the Google doc, to share about yourself.

Photo by National Cancer Institute, Unsplash

Photo by Scott Graham, Unsplash

Course material

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



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

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 Monique Zahn)
- Send answers to tania.wyss@sib.swiss by July 1st 2022, 11:59pm

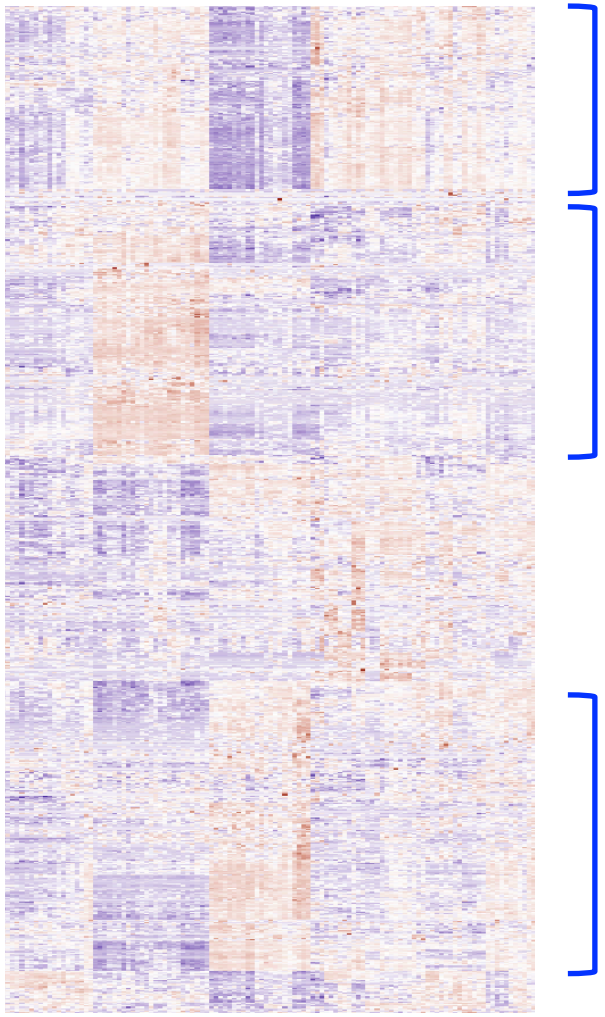
Questions and Exercises

- Feel free to interrupt with questions by asking them directly or raising your (virtual) hand.
- Use the chat or Q&A in google Doc, Gustavo and I will answer

- Exercises in R:
  - We will try to debug as much as possible
  - We are happy if you share your results or alternative code!
  - Computational power on RStudio cloud is limited, might crash

Why do we perform enrichment analysis?

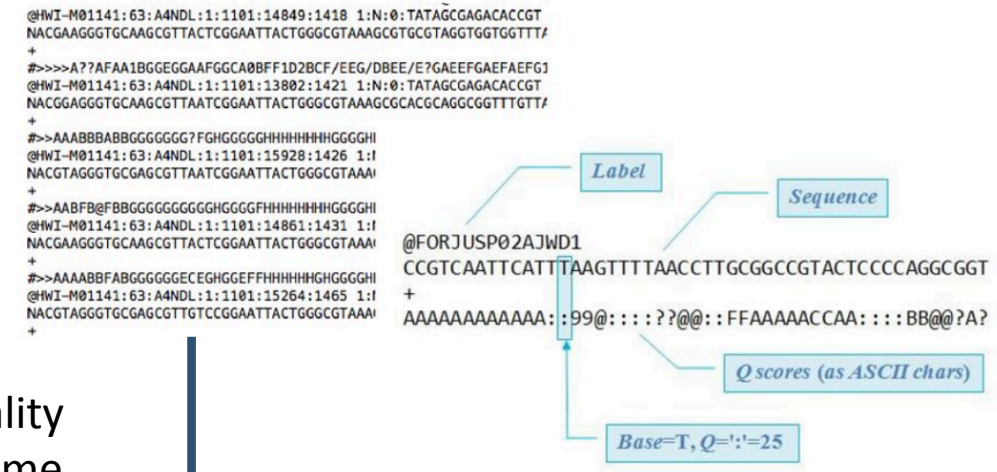
- Gene expression analysis yields hundreds to thousands of significant genes
  - We need to summarize the information provided by so many genes
  - Understand their biological relationships



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

Typical RNA sequencing analysis workflow

fastq file:



Filter quality
Align to ref. genome

count reads
per gene

Downstream statistical analysis: R: import counts table

Differential gene expression analysis

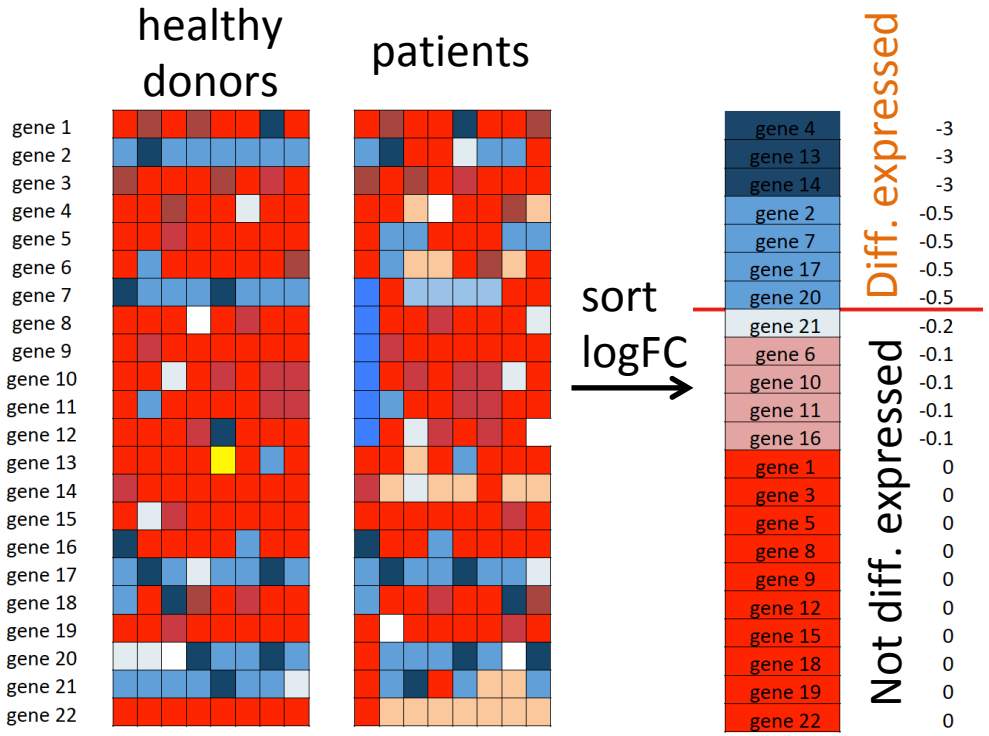
- Comparing 2 groups:

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

- Fold change in genomics:

  $\log_2$ of ratios = log 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?

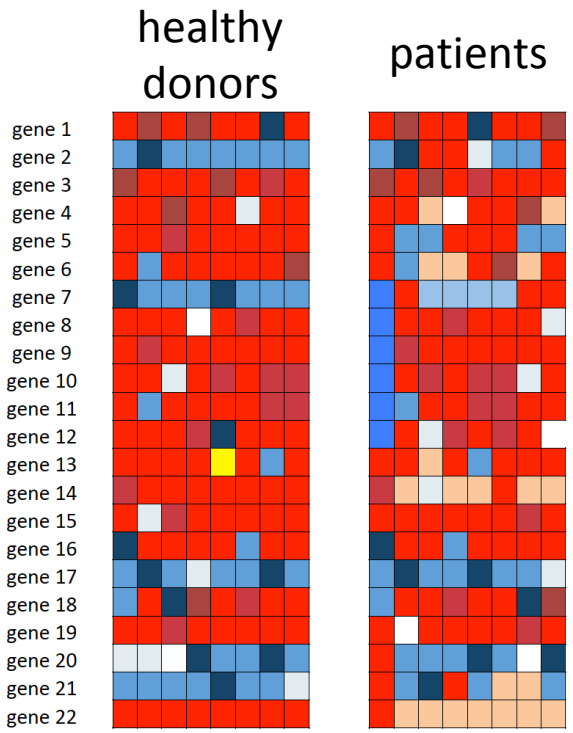
- T-test:

H0: Healthy donors and patients have similar gene I expression

H0i : $\pi i1 = \pi i2$

H1: Healthy donors and patients don't have a similar gene i expression

H1i : $\pi i1 \neq \pi i2$



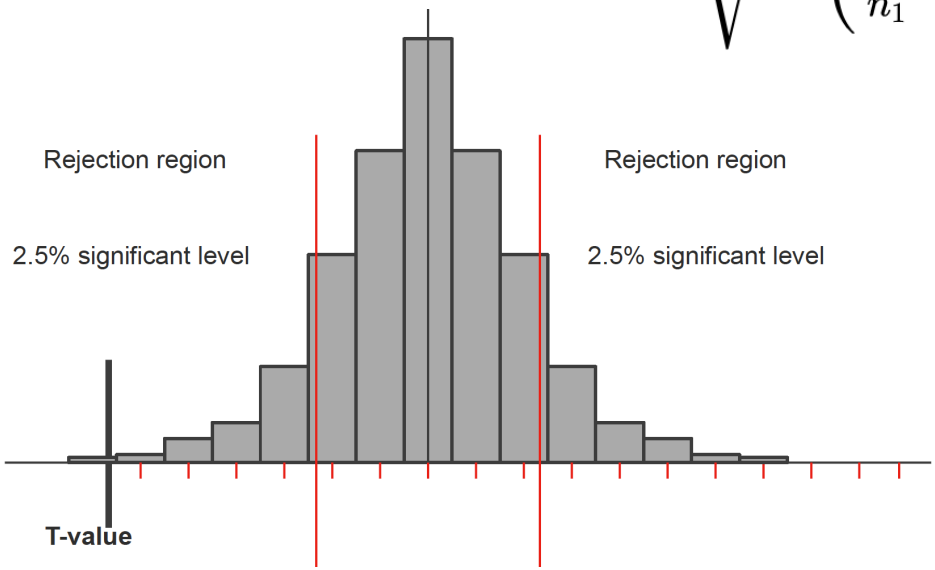
T-test in R

```
> 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

gene 13	−5
gene 17	−1
gene 20	−1
gene 1	0
gene 12	0
gene 15	0
gene 18	0
gene 19	0
gene 22	0
gene 3	0
gene 5	0
gene 8	0
gene 9	0
gene 10	0.4
gene 11	0.4
gene 16	0.4
gene 6	0.4
gene 21	0.6
gene 2	1
gene 7	1
gene 14	5
gene 4	5

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

P-value adjustment: what is it?

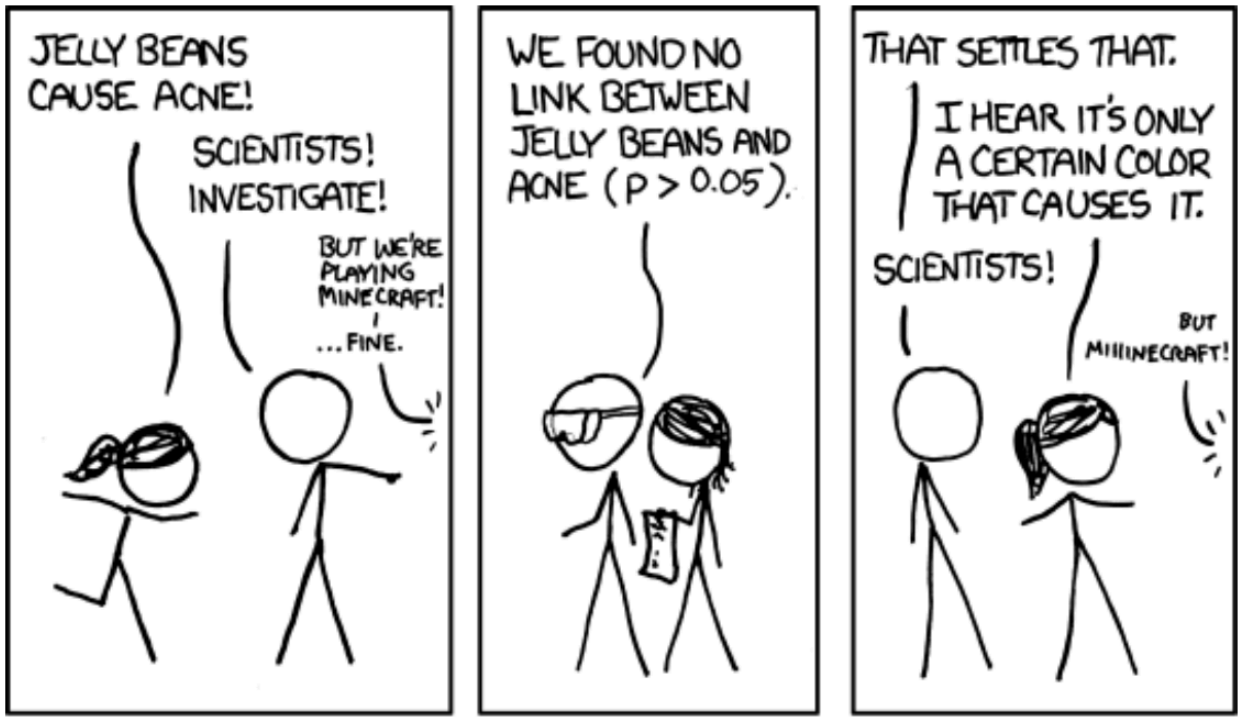
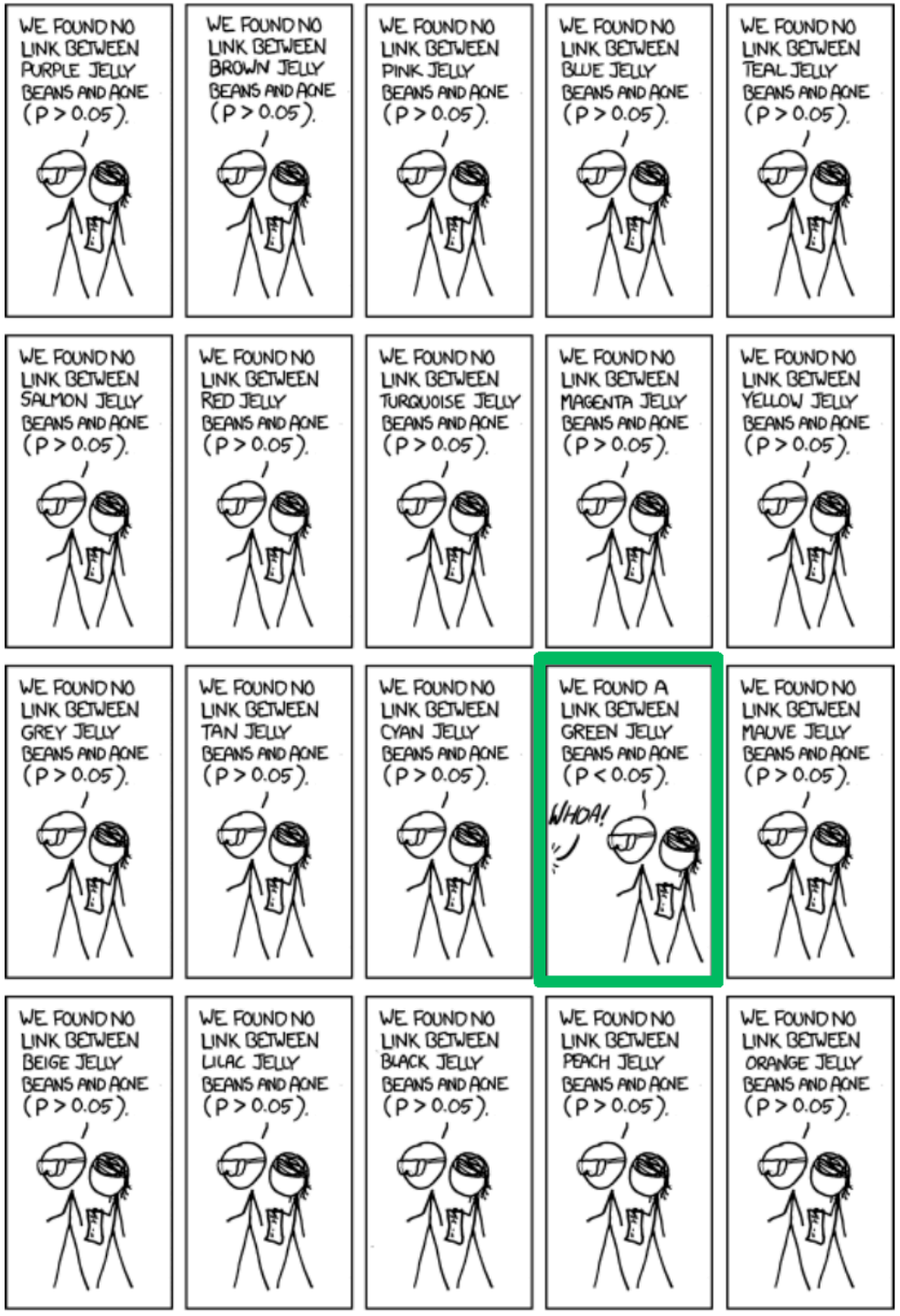
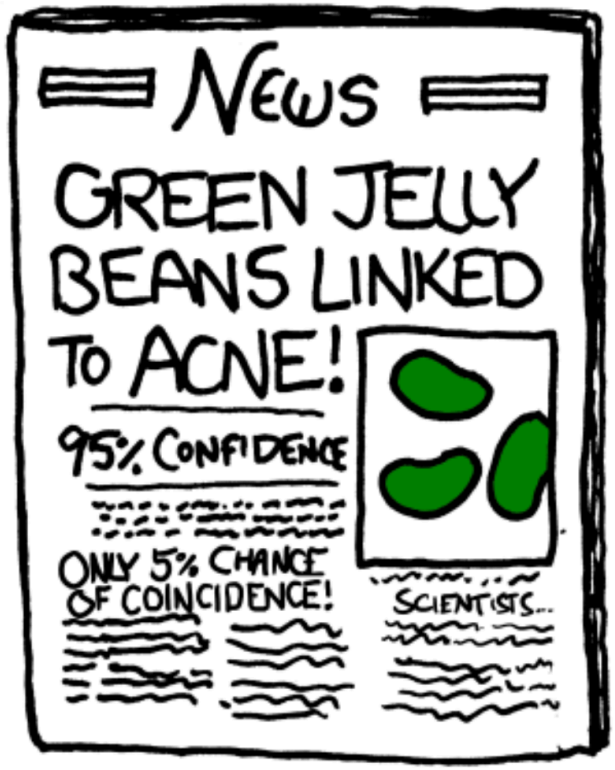


Photo by Patrick Fore on Unsplash

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).
WHOA!
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).


News
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...

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

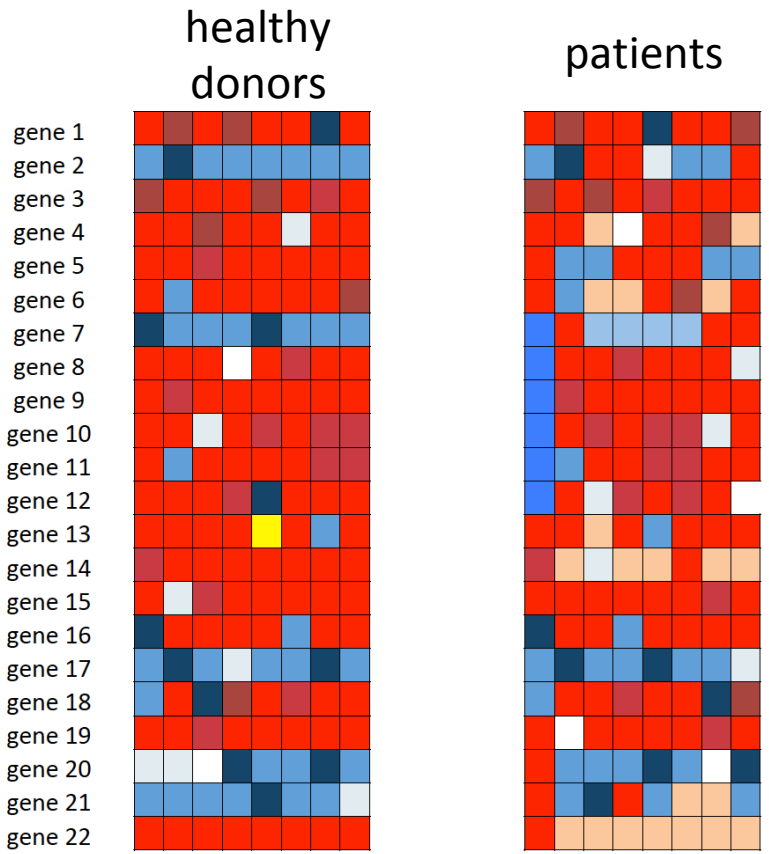
k= rank number

Differential gene expression analysis using R

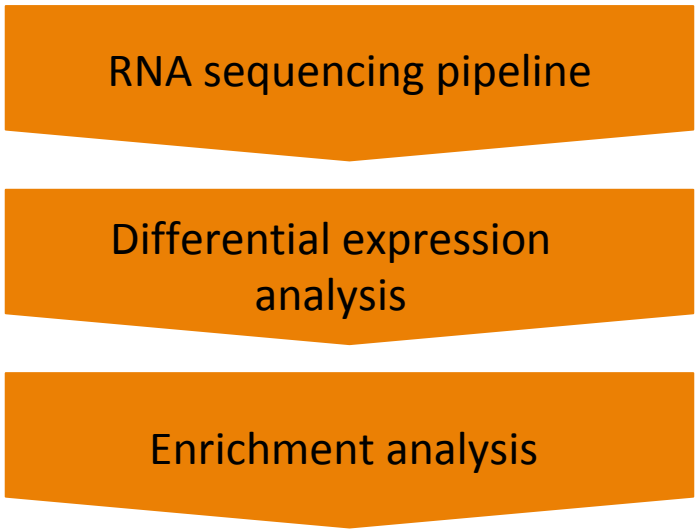
- Bioconductor

https://bioconductor.org/

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



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



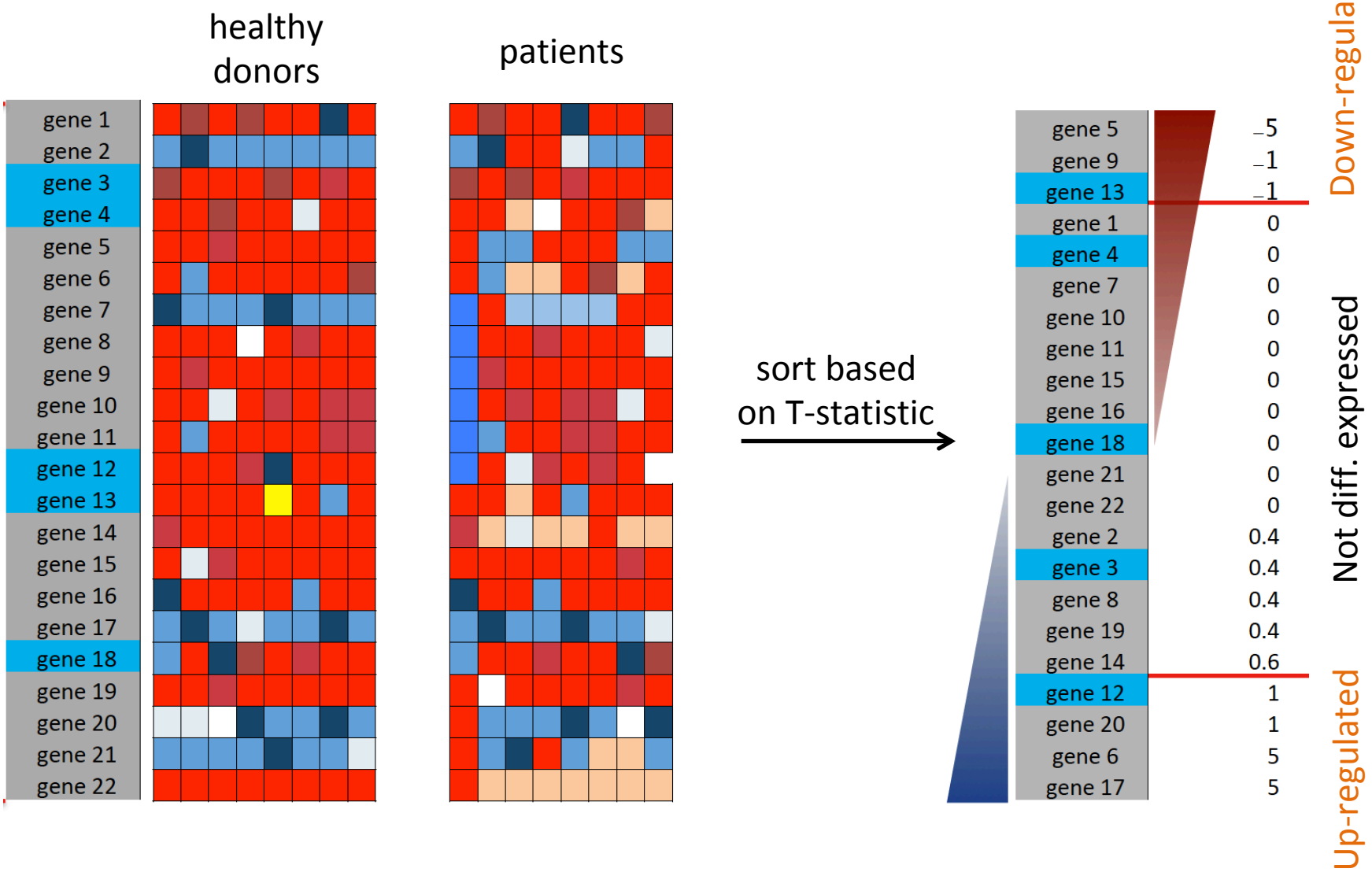
Several methods available, *e.g.*:

- 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

2X2 count table	Differentially expressed	Not Differentially expressed	total
blue	2	3	**5**
Not blue	5	12	**17**
total	**7**	**15**	**22**

contingency table

H_0: The proportion of blue genes differentially expressed is the same as the proportion of blue genes in non-differentially expressed genes

H_1: The proportion of blue genes differentially expressed is not the same as the proportion of blue genes in non-differentially expressed genes

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

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

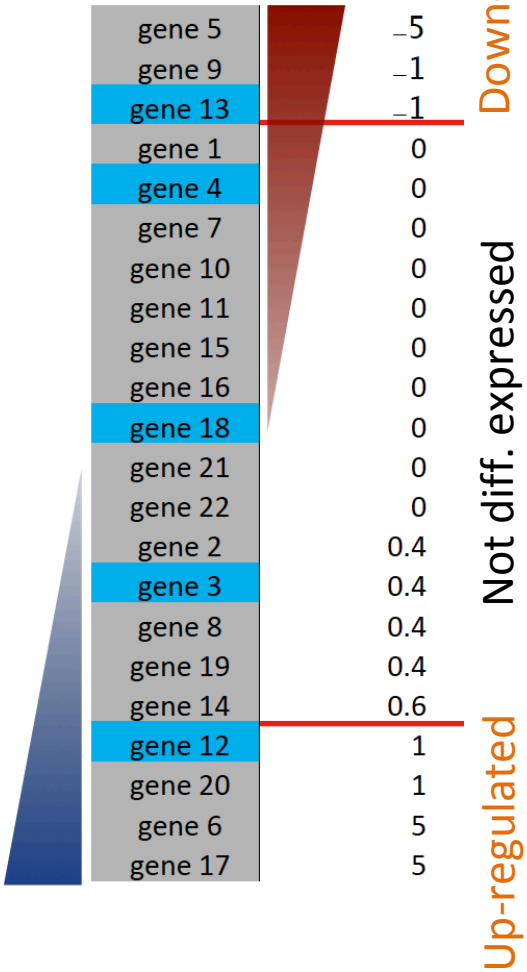
3/15 = 0.20

Which gene sets are differentially expressed?

gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	−5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	−1
gene 10	0
gene 11	0
gene 12	1
gene 13	−1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0

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

⇒Multiple tests need p-value adjustment.



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 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)
```

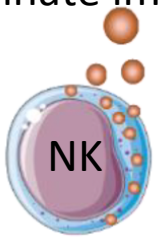
enricher(): implementation of hypergeometric test (one-sided Fisher test)
for user defined gene list and gene set annotations (package clusterProfiler)

```
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

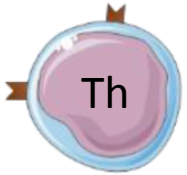
RStudio tour

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

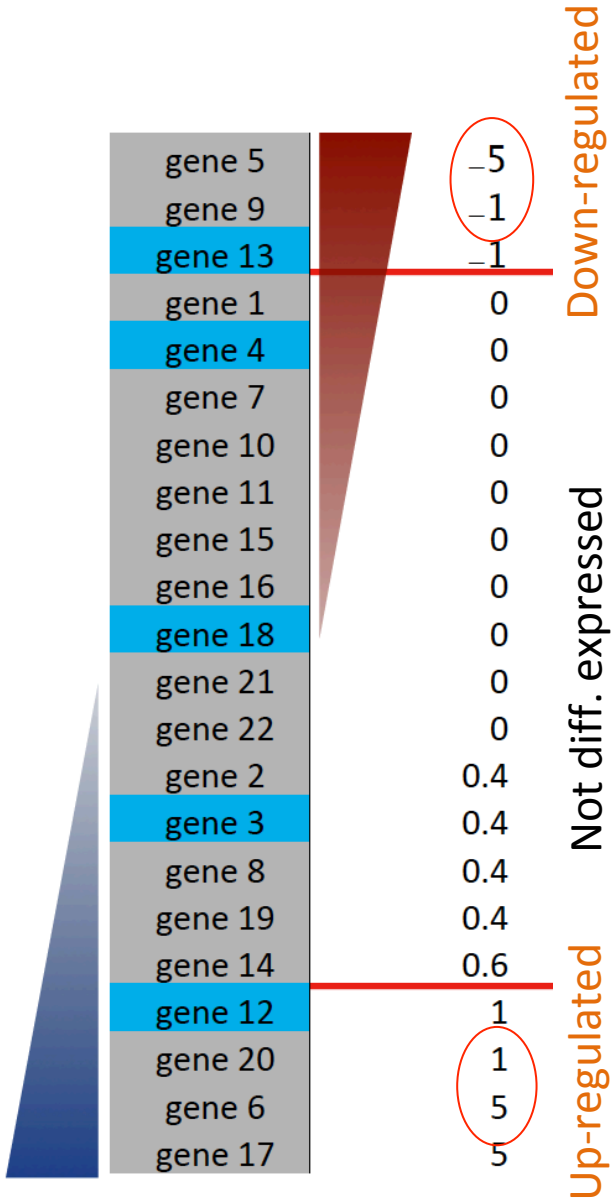
- Run a **Fisher's exact test** to determine whether genes involved in the **adaptive immune response** are over-represented among the genes up-regulated in Th cells.

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

2X2 count table	Differentially expressed	Not Differentially expressed	total
blue	2	3	**5**
Not blue	5	12	**17**
total	**7**	**15**	**22**

Contingency table with count of genes, without taking into account the magnitude of the change of each gene.



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

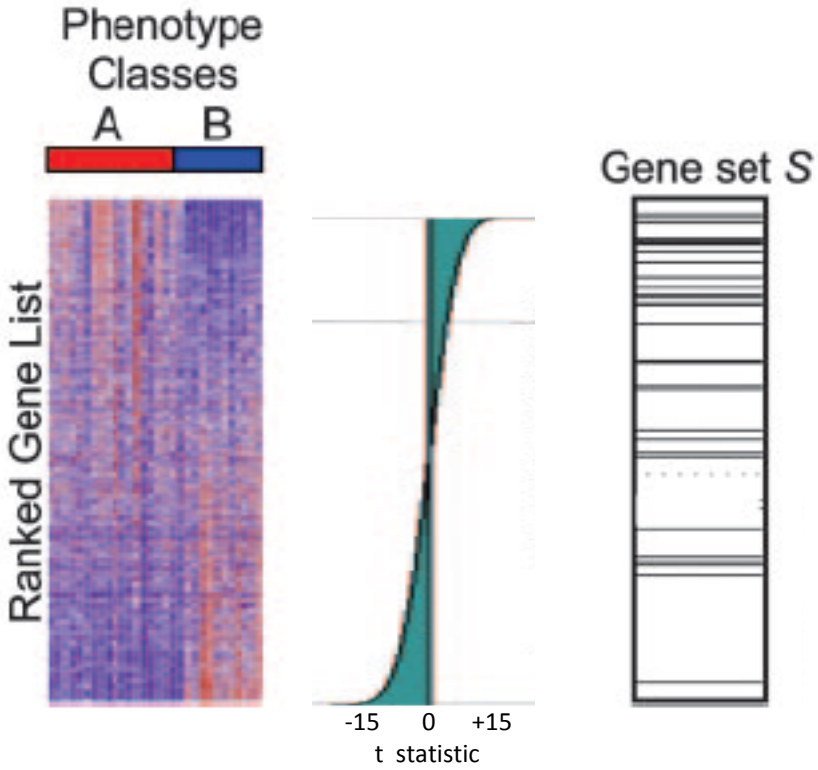
Gene set enrichment analysis (GSEA)

- **Threshold-free**: the whole list of genes detected in the RNA sequencing experiment 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

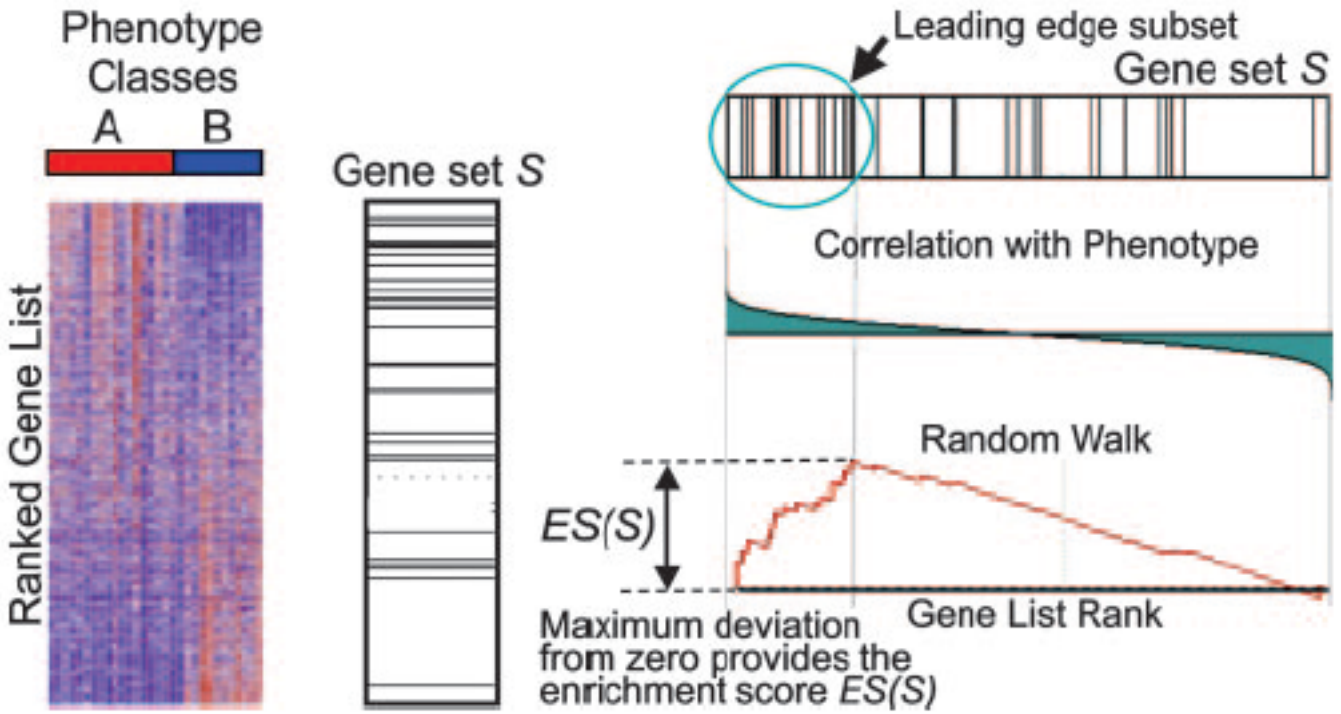
Goal: determine whether the members of a gene set S are randomly distributed throughout a ranked gene list or if they are located at the top or bottom of the ranked gene lists



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

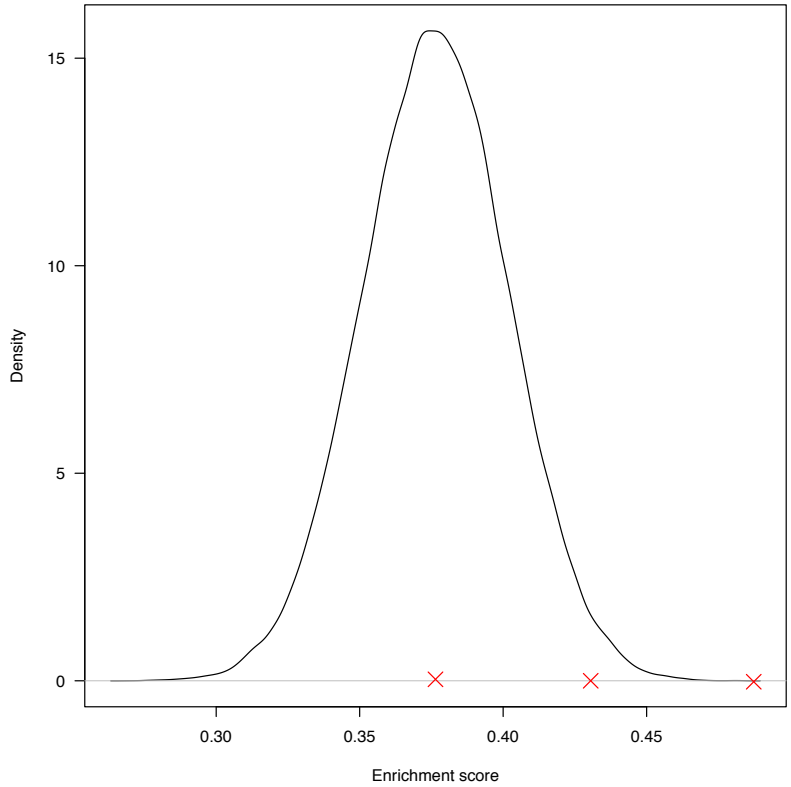
Method of GSEA



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.)

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



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

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

NES: 1 p: 0.5

NES: 1.16 p: 0.05

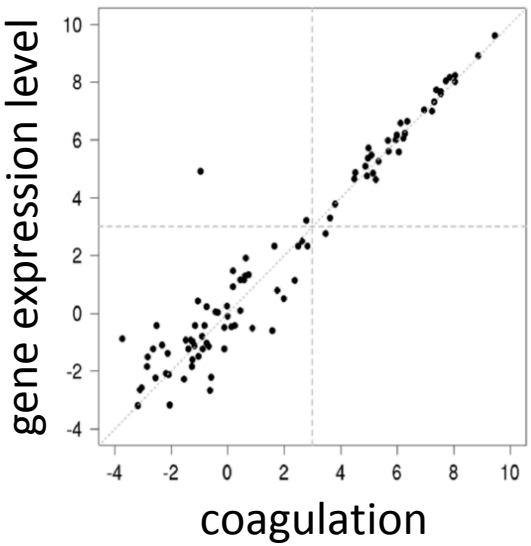
NES: 1.32 p: 0.001

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 coefficients or 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

Differentially expressed



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

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",
  ...
)
```

TERM2GENE:

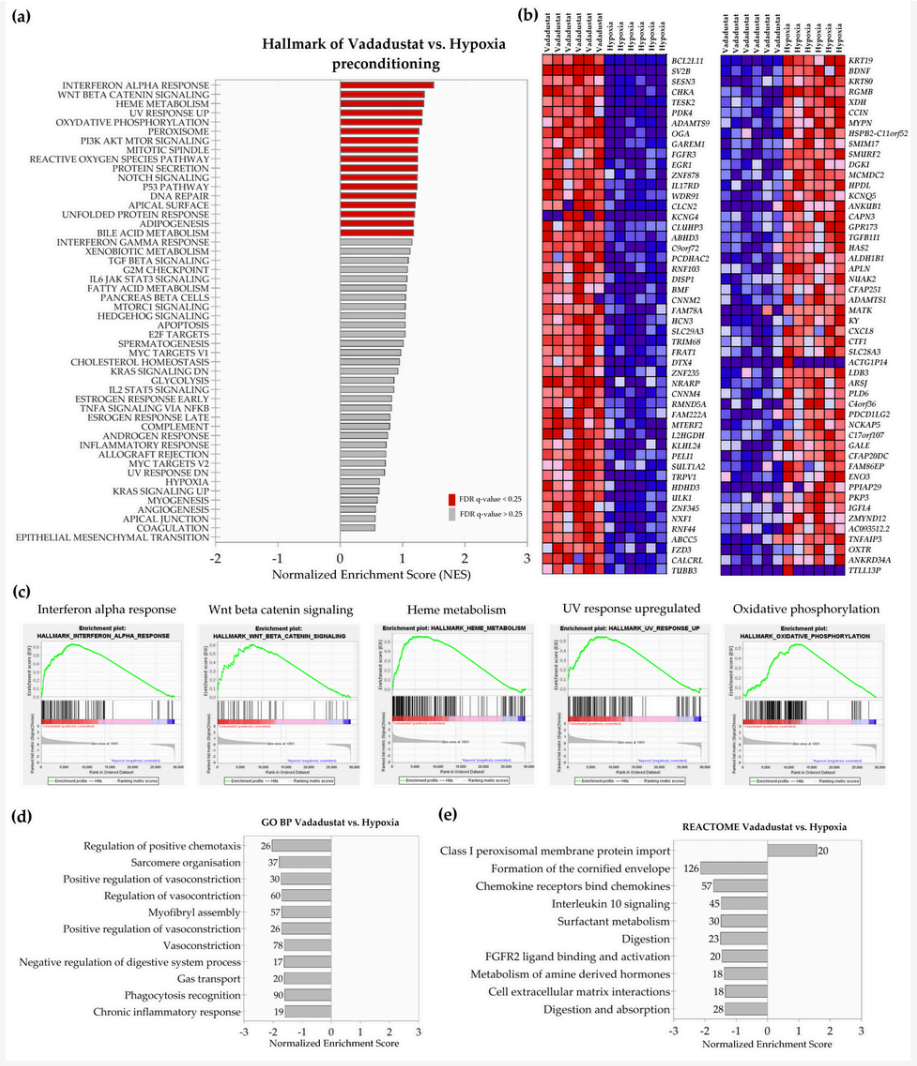
term	gene
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZC3H12A
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZNF683
GOBP_ADAPTIVE_IMMUNE_RESPONSE	ZP3
GOBP_HAIR_CELL_DIFFERENTIATION	ATOH1
GOBP_HAIR_CELL_DIFFERENTIATION	CDH23
GOBP_HAIR_CELL_DIFFERENTIATION	CLRN1

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?

How to show the results of an enrichment analysis?



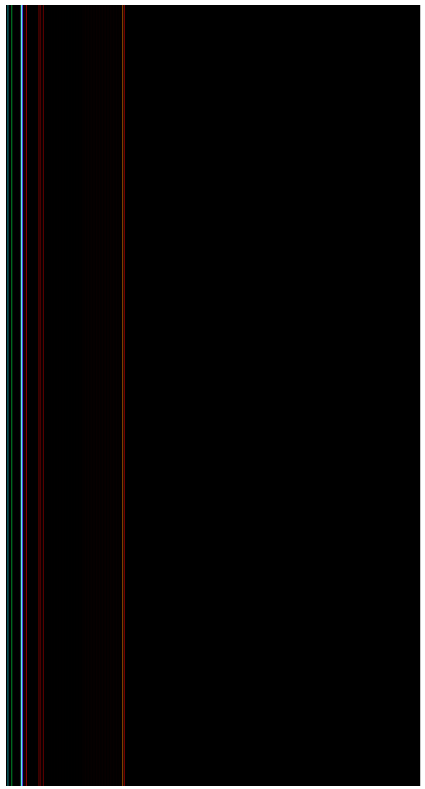
Zielniok et al 2021

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

Visualizations available in clusterProfiler

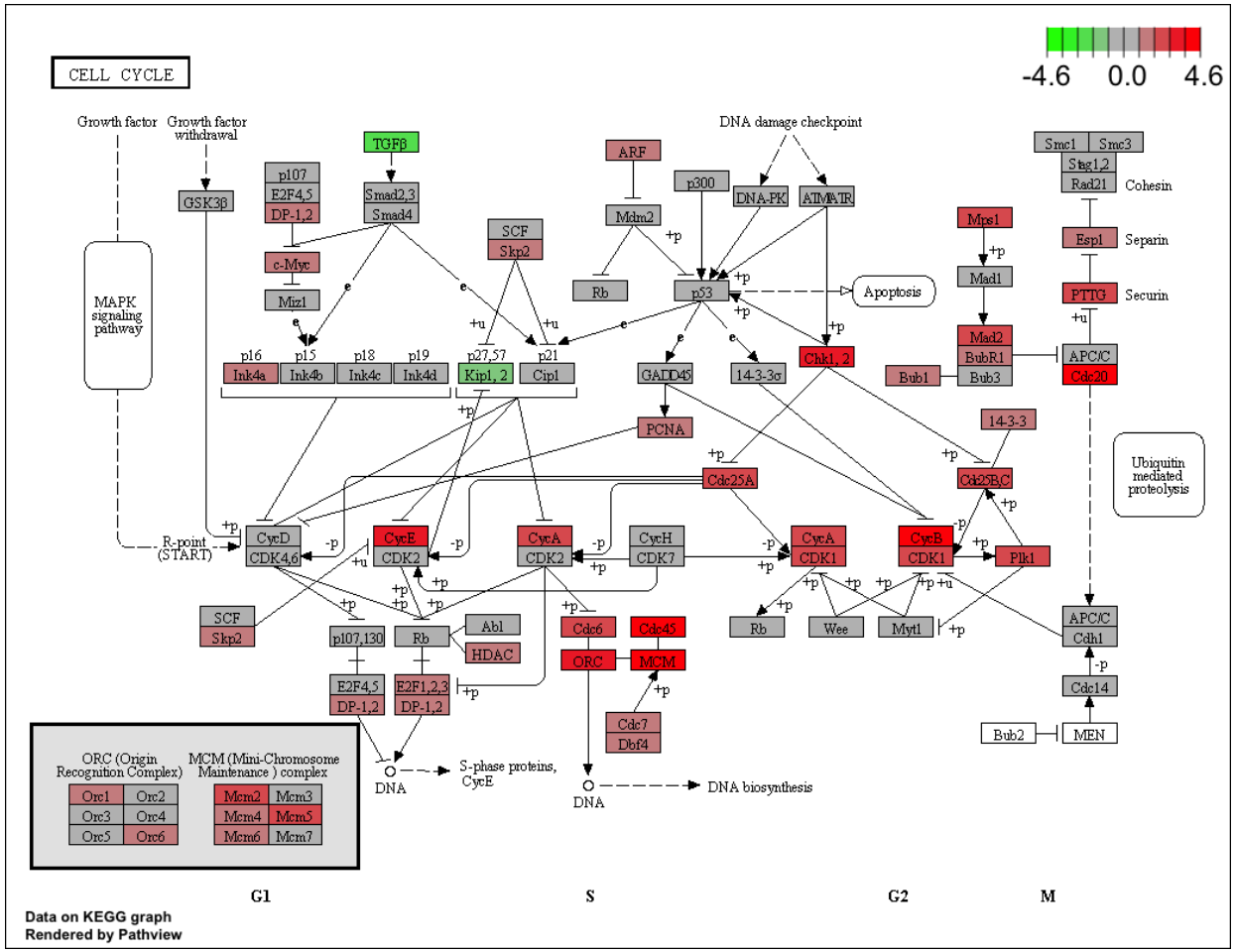
- barcode plot

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



Visualizations available - pathview package

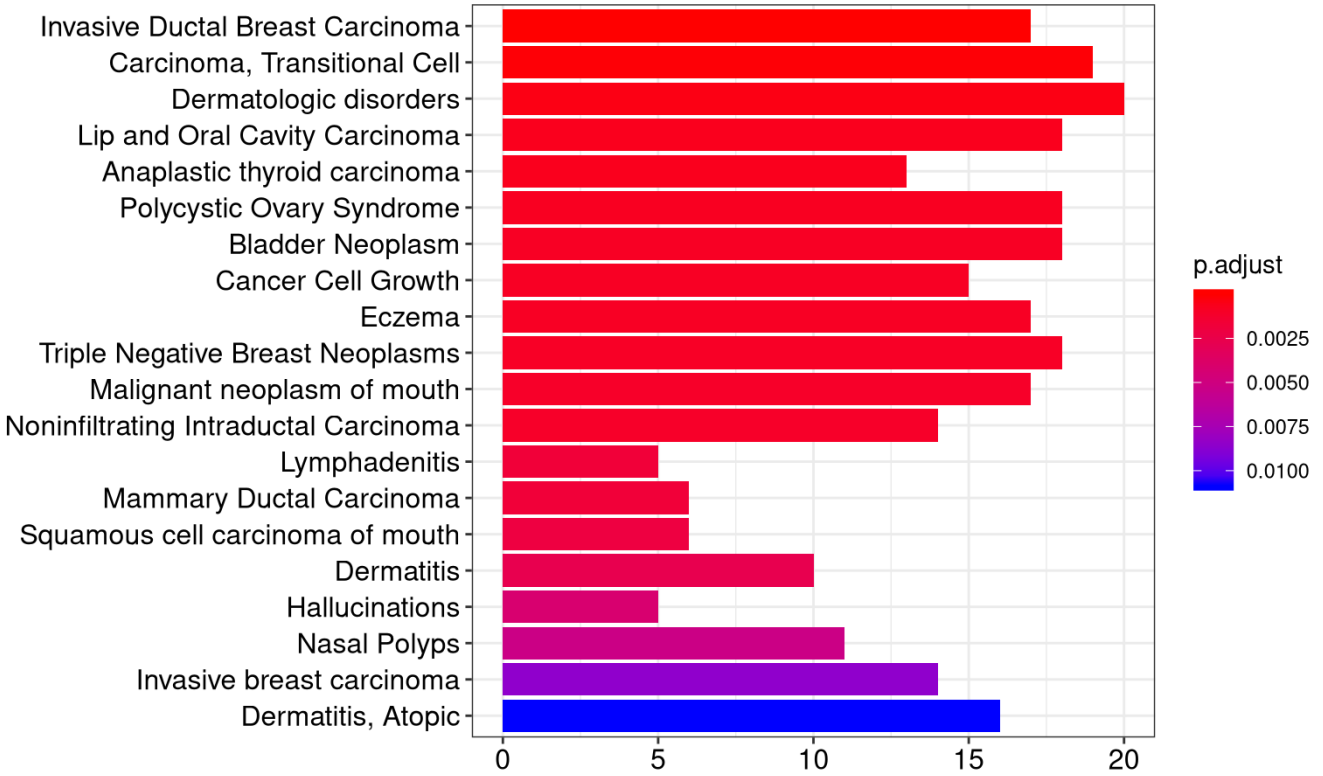
hsa04110 <- pathview(gene.data = geneList, pathway.id = "hsa04110", species = "hsa", limit = list(gene=max(abs(geneList)), cpd=1))



Visualizations available in clusterProfiler

- barplot

```
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)
```

Visualizations available in clusterProfiler

cnetplot(edox, categorySize="pvalue", foldChange=geneList)

- Gene-concept network

Visualizations available in clusterProfiler

- Enrichment map

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

Visualizations available in clusterProfiler

- Ridgeplot

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

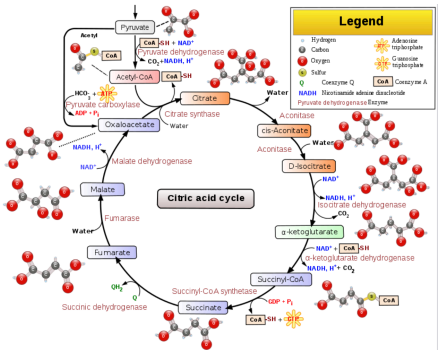
Recap and Exercise 3

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

Exercise 3: Create figures for the enrichment results:

- barplot of −log10(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

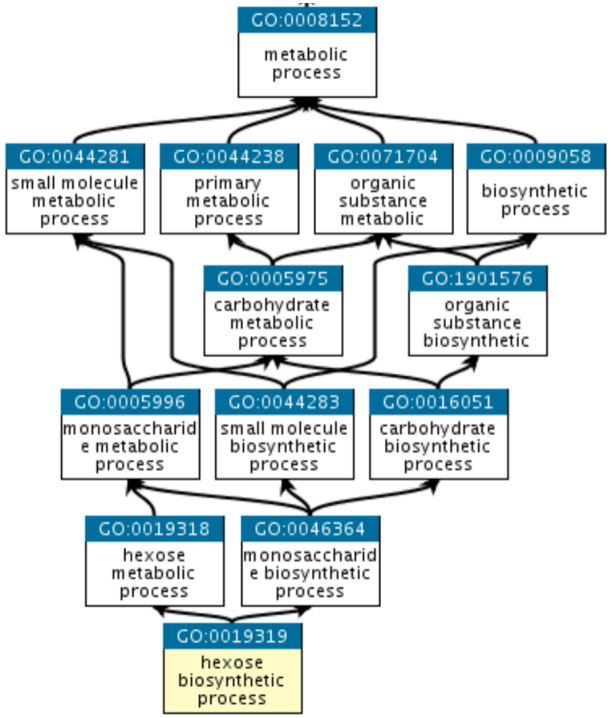
- http://geneontology.org/

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)



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

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

Gene ontology

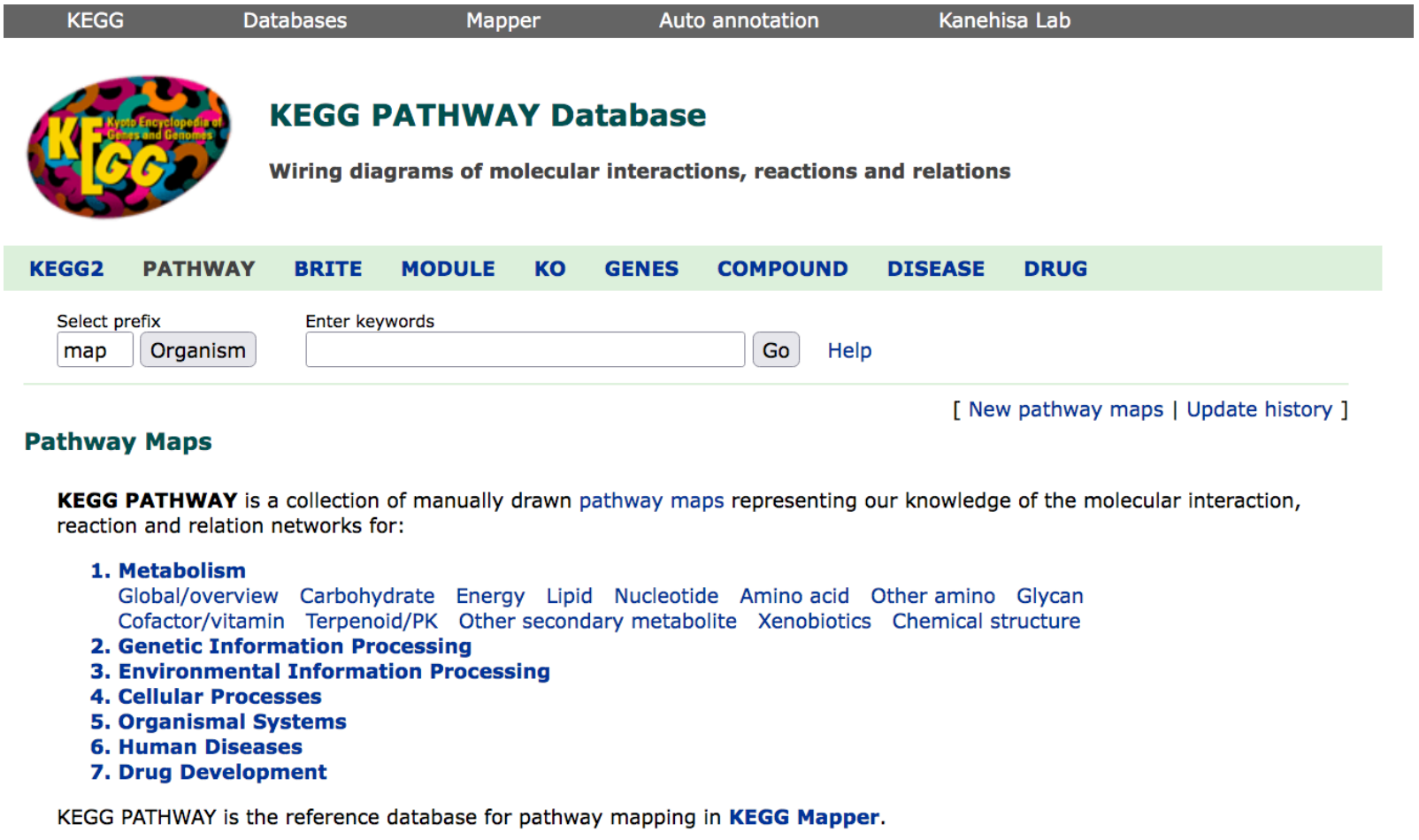
GO ontologies: GO terms organized in 3 independent controlled vocabularies

- **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

Bi-directional eg mTOR signaling

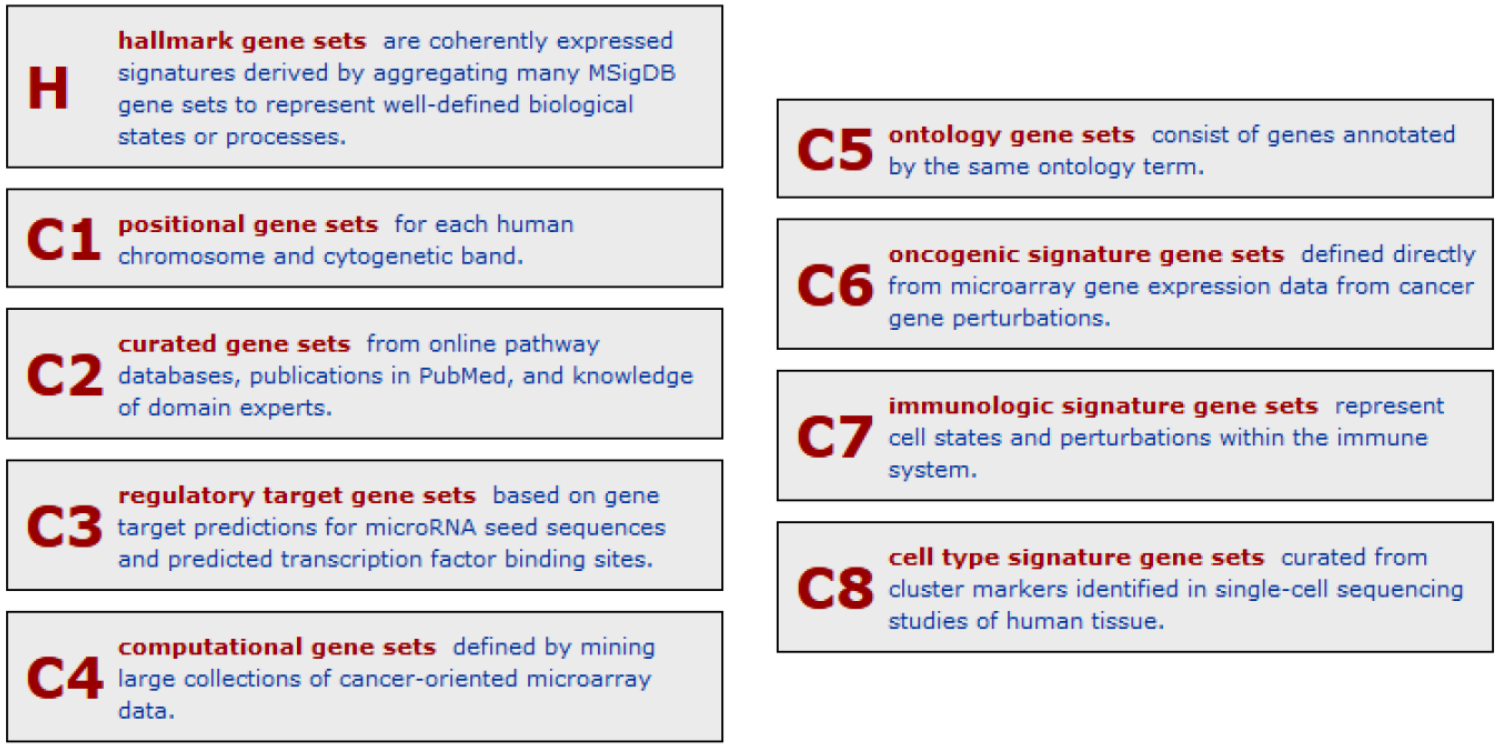


Reactome

https://reactome.org/

MSigDB

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



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/

WikiPathways

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

Bioconductor

- GO.db：A set of annotation maps describing the entire Gene Ontology assembled using data from GO
- gskb: mouse
  - mm_GO: gene sets from Gene Ontology for mouse (Mus musculus)
  - mm_location: Gene sets based on chromosomal location
  - mm_metabolic: metabolic pathways
  - mm_miRNA: Target genes of microRNAs, predicted or experimentally verified
  - mm_pathway: Currated pathways
  - mm_TF: Transcription factor target genes.
  - mm_other
- KEGG.db: A set of annotation maps for KEGG assembled using data from KEGG

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 (or biomaRt package)

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

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

Note on gene ID conversion

When converting, not all genes have a matching ID type, or some genes have duplicated IDs (eg symbols).

- A good idea is to obtain Ensembl ID-to-symbol conversion for your reference genome version:
- Download gtf from Ensembl: http://www.ensembl.org/index.html
- ftp://ftp.ensembl.org/pub/release-100/gtf/homo_sapiens/

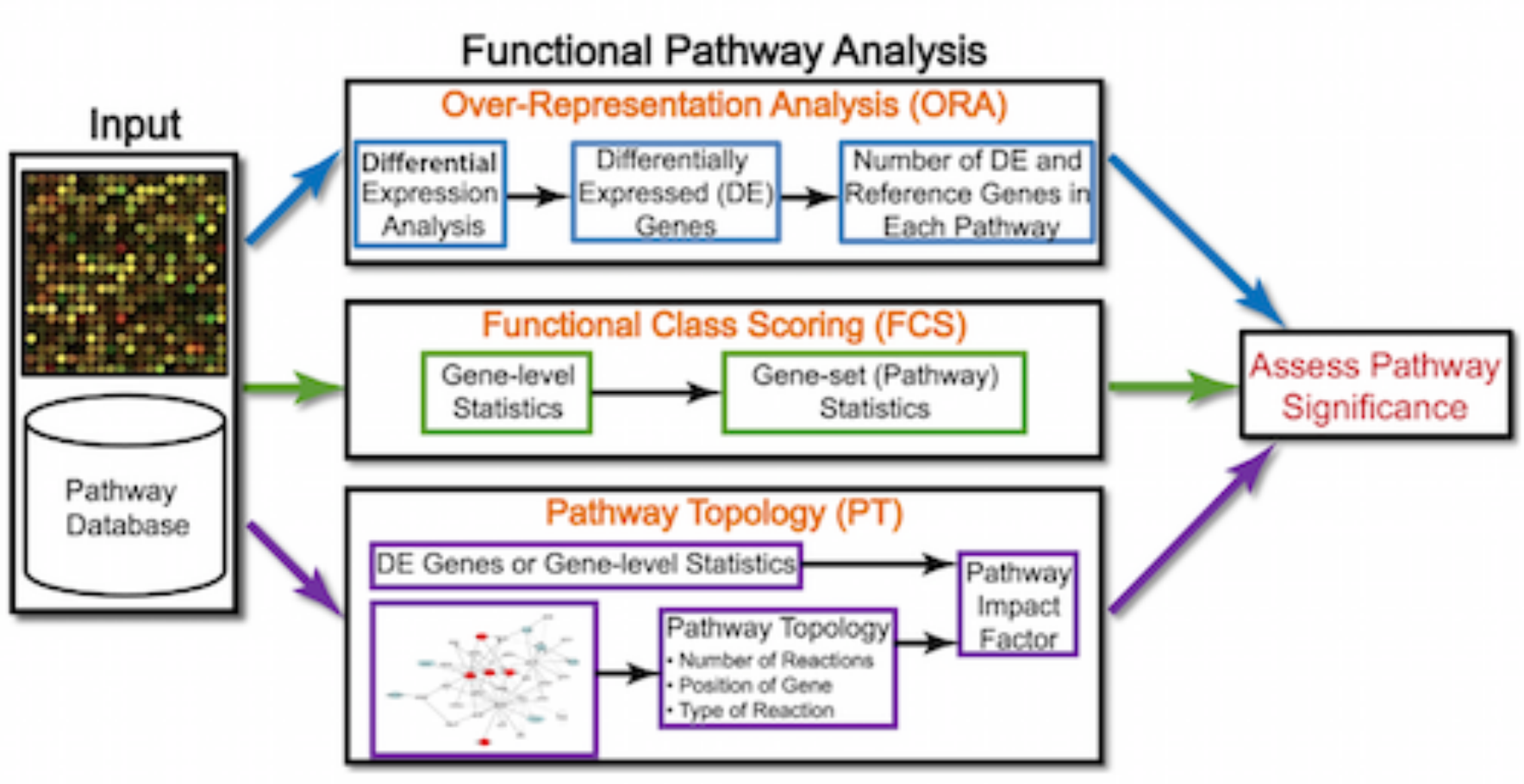
- Download as a data frame with refGenome package (archived)
- https://cran.r-project.org/web/packages/refGenome/index.html

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 to perform a GSEA of KEGG pathways (with argument minGSSize=30).
  - Explore the results. Is there a KEGG immune-related gene set coming up? Is there a KEGG Natural killer gene set coming up?
  - Import the hallmark gene sets and run a GSEA. How many significant gene sets are there?

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_{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

Some additional resources

- g:Profiler - 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

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 Monique Zahn)
- Send answers to tania.wyss@sib.swiss by July 1st 2022, 11:59pm

Thank you for your attention!

Please fill in the feedback sent by Monique Zahn.

We thank Isabelle Dupanloup and Linda Dib for providing course material