Enrichment analyses and results contextualisation with Knowledge Graphs

Summer School Multi-omics Data Analysis and Integration

Enrichment analyses

- [*Gene sets, pathways, metabolites*] <u>*enrichment*</u> analyses

↓

over-representation

García-Campos, M.A. et al. 2015. Pathway Analysis: State of the Art. Front Physiol

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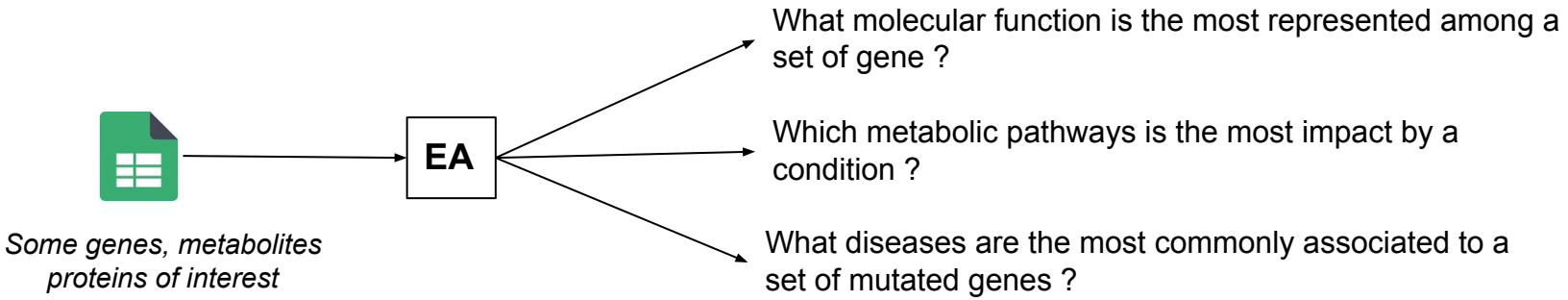
- [*Gene sets, pathways, metabolites*] *enrichment* analyses

over-representation

- Give directions for results interpretation



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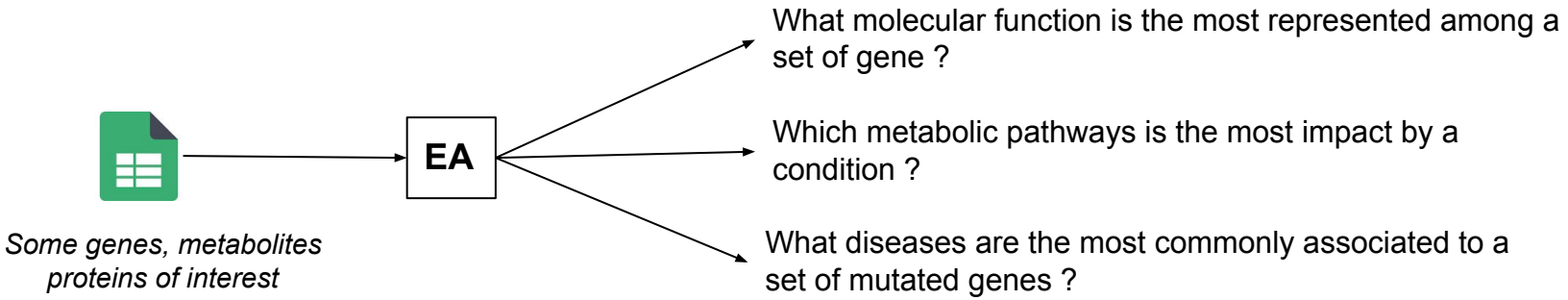

- Families of approaches:
  - Over-Representation Analysis (ORA)
  - Functional Class Scoring (eg. GSEA)
  - Topology-based methods

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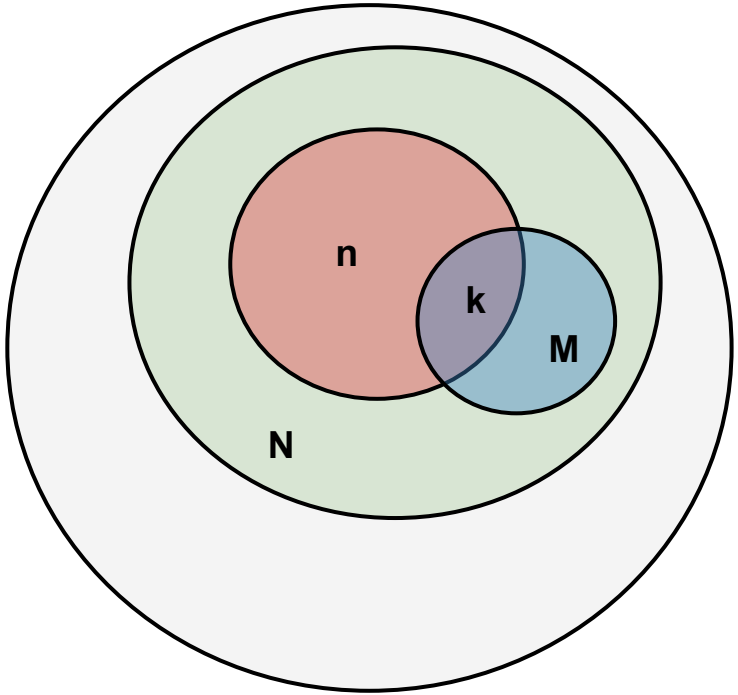
ORA: Over-Representation Analysis

What does an ORA ? it compare **overlap** between **sets**.



- Sets of genes, proteins, metabolites, organisms, etc.
  - a Universe (size = **N**) - *or background set*
  - a set of interest (size = **n**)
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  - an overlap **k**

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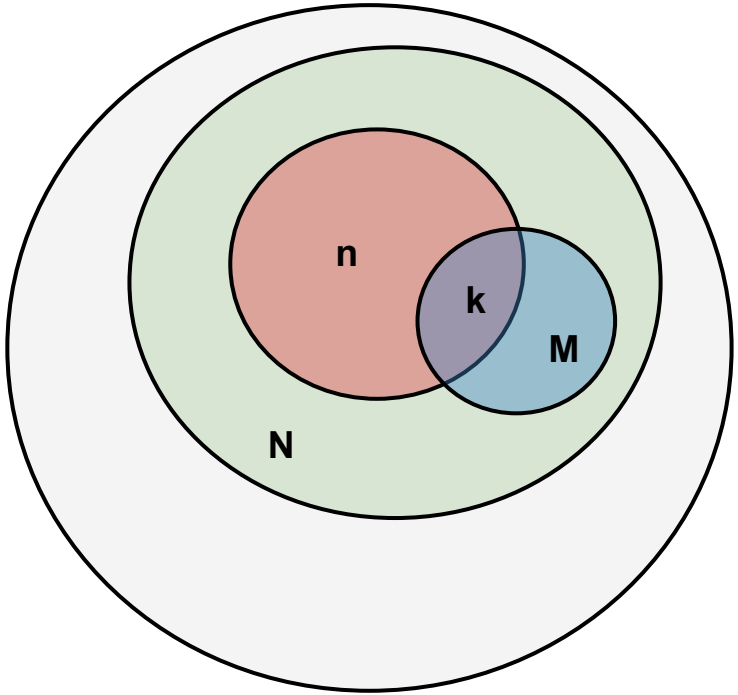

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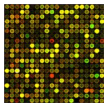
In a classic RNA-seq analysis



Experiment → **DE analysis** → **GO terms**

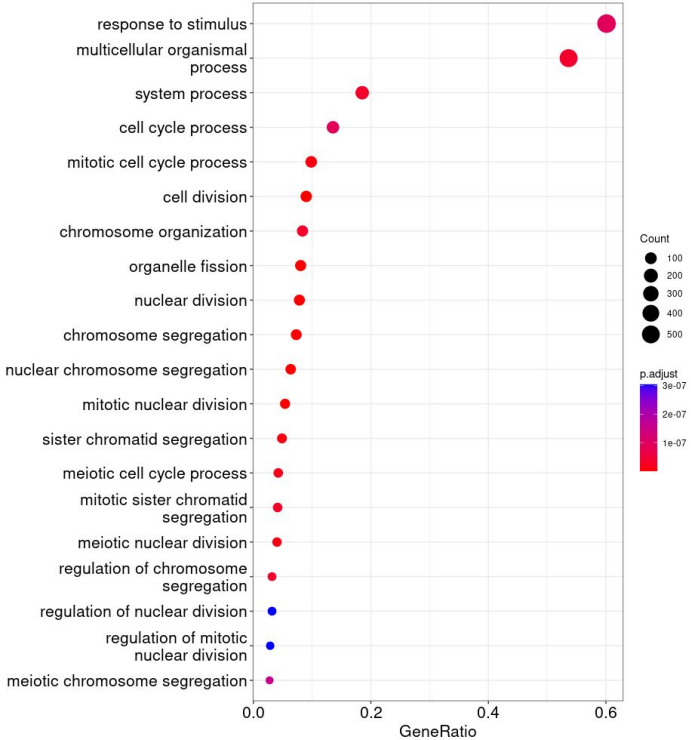
GENEONTOLOGY
Unifying Biology

  - **N** genes measured in the assay
  - **n** genes differentially expressed
  - **M** genes annotated to a GO term of interest
  - an overlap **k**

ORA: A practical example (1)

TCGA-BRCA: 5 Normal .vs. 5 Tumor samples → GDE analysis → 1068 DE genes

R packages for ORA: clusterProfiler, Enrichr



GENEONTOLOGY Unifying Biology

Standard GO (*Biological processes*) Enrichment analysis

```
ego <- enrichGO(gene = DE.set,
      universe = universe,
      OrgDb = HS.annotation,
      ont = "BP",
      keyType = "SYMBOL",
      minGSSize = 1,
      maxGSSize = 100000,
      pAdjustMethod = "BH")
```

https://master.bioconductor.org/packages/release/bioc/vignettes/TCGAbiolinks/inst/doc/analysis.html

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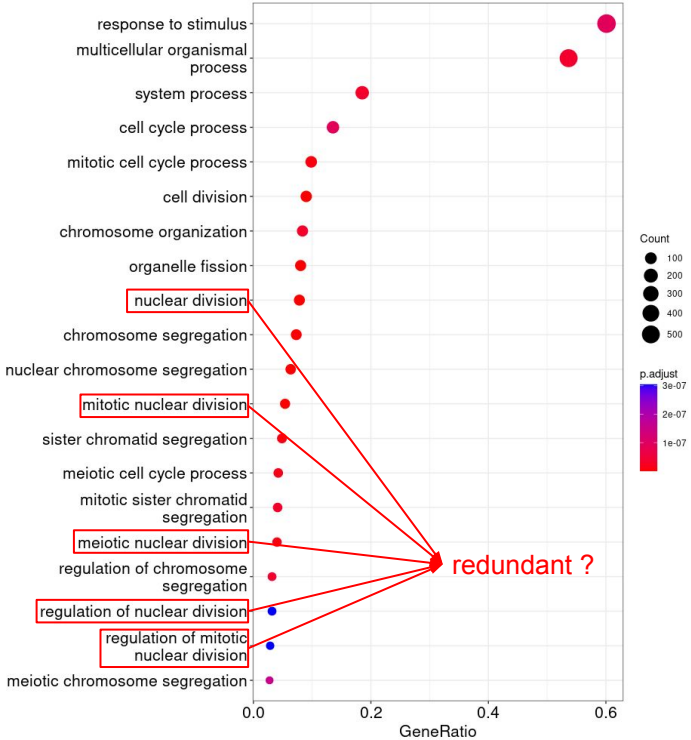

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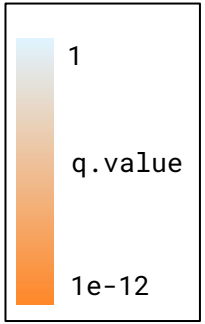
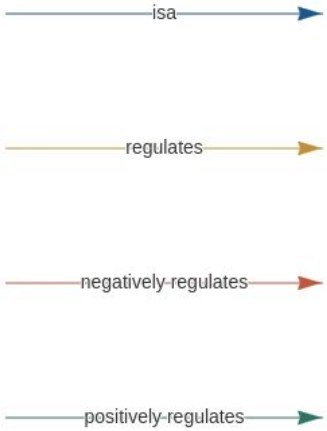
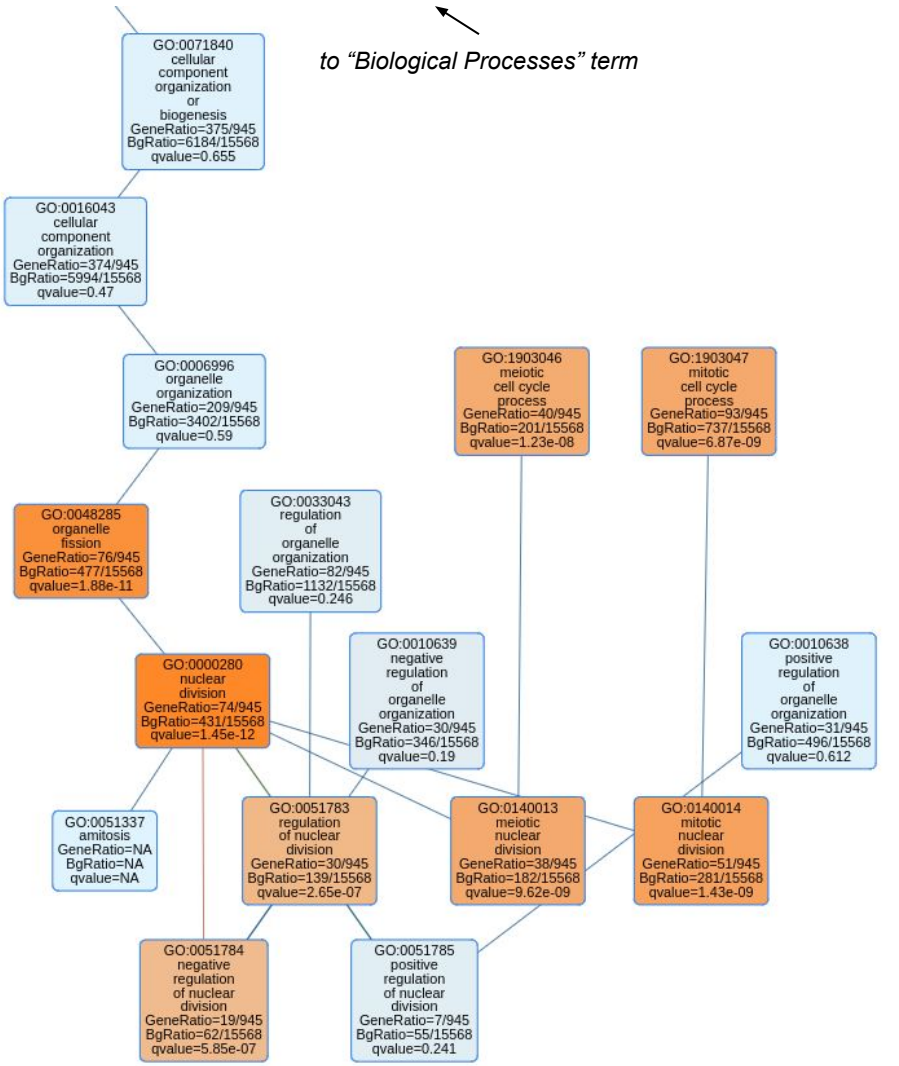
ORA: A practical example (1) - DAG view

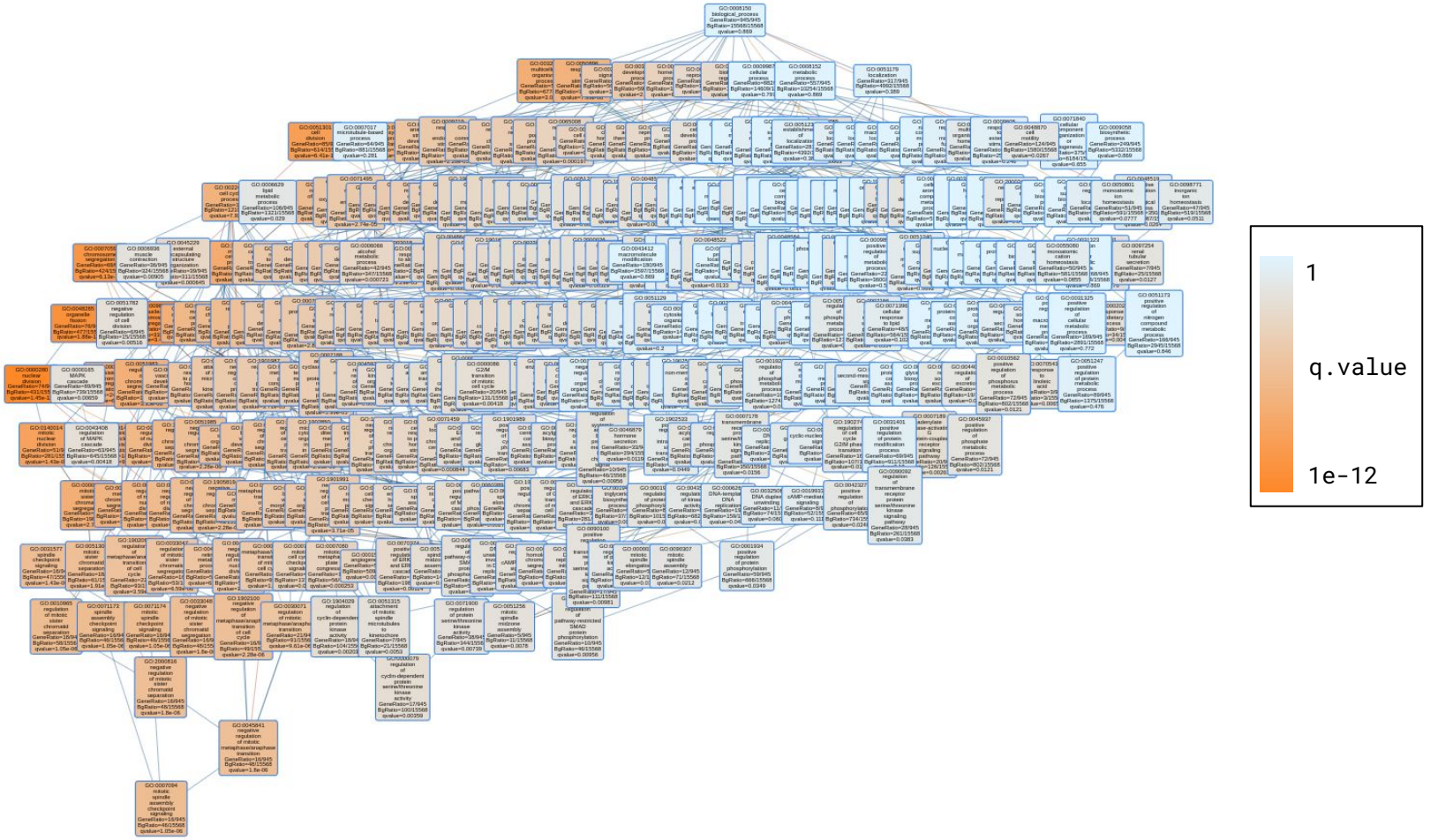
The Gene Ontology in a DAG (**Directed Acyclic** Graph)







ORA: A practical example (1) - a broader DAG view



ORA: A practical example (2)

TCGA-BRCA: 5 Normal .vs. 5 Tumor samples → GDE analysis → 1068 DE genes

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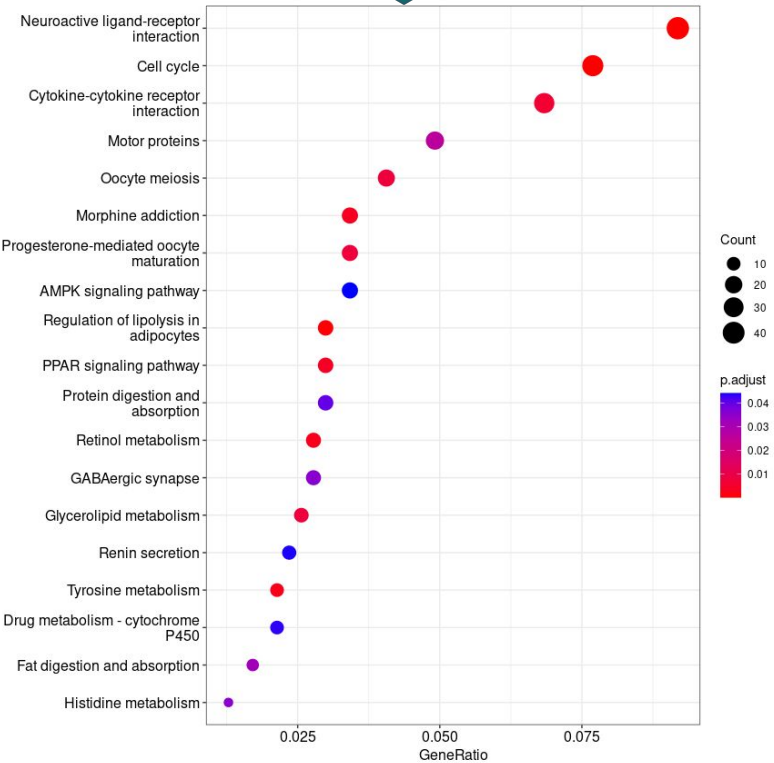

Standard KEGG (*Pathway*) Enrichment analysis

```
ekegg <- enrichKEGG(gene = DE.set2,
      organism = "hsa",
      keyType = "ncbi-geneid",
      pAdjustMethod = "BH",
      universe = universe2,
      use_internal_data = FALSE)
```

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ORA: Back to the fundamentals

ID	Description	GeneRatio	BgRatio	p.value	p.adjust	q.value
GO:0006836	neurotransmitter transport	22/945	191/15568	0.002881322	0.04909408	0.04278198

2 equivalent ways of representing and computing

Wieder, C. et al. Pathway analysis in metabolomics: Recommendations for the use of over-representation analysis. PLoS Comput Biol 17

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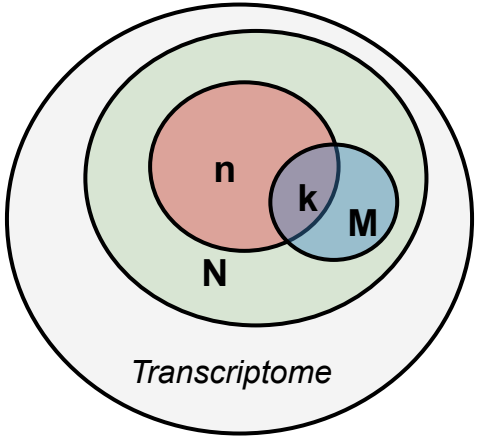

Hypergeometric distribution

$$P\left(X \geq k\right) = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

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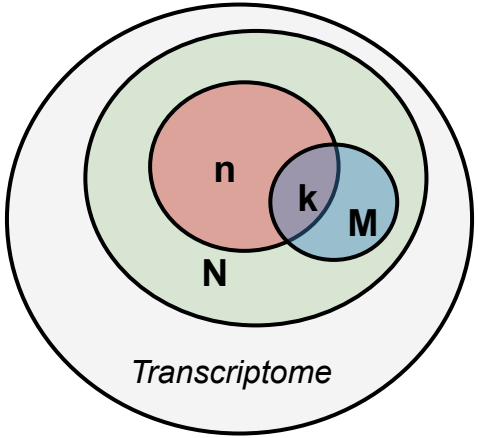

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Contingency table

	in BP set	not in BP set
in Gene set	k = 22	923
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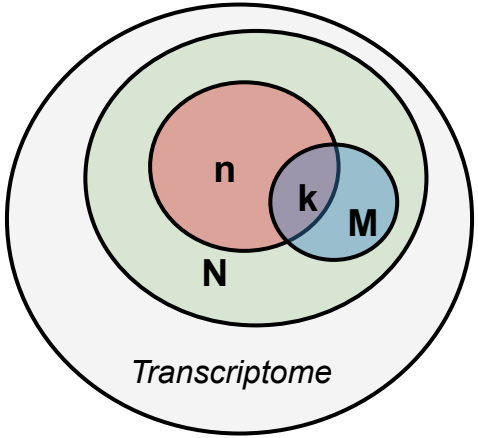

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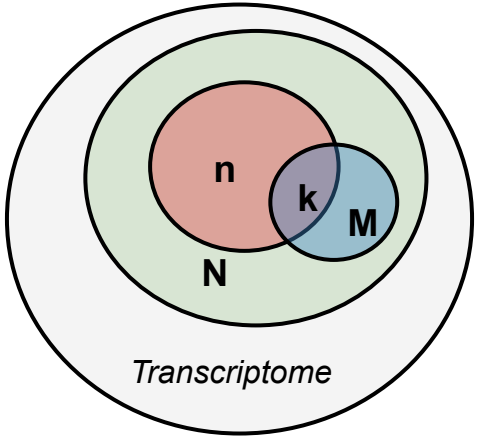

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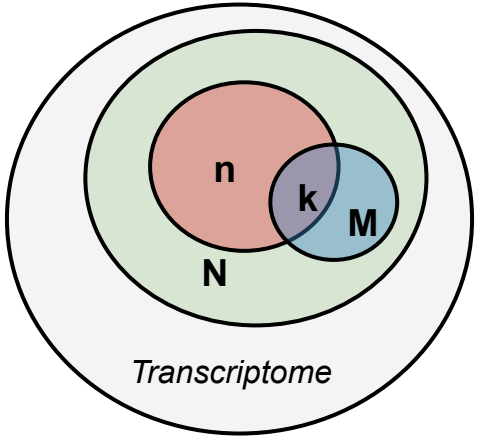

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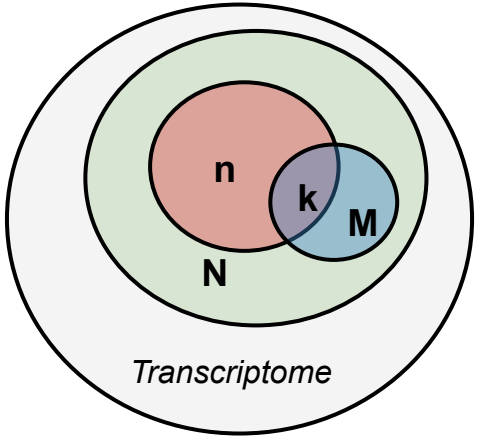

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Right-tailed fisher exact test

= *Testing Independence*

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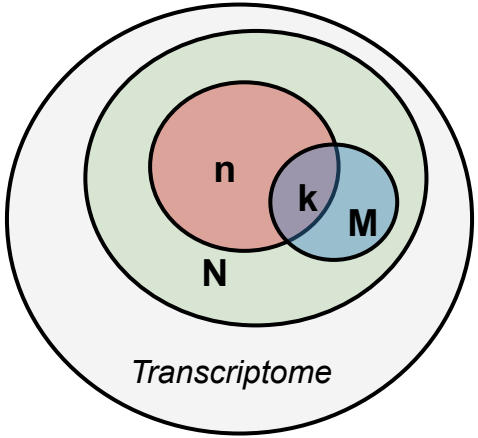

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Hypergeometric distribution → p.value=0.002881322 ← *Right-tailed fisher exact test*

= *Testing Independence*

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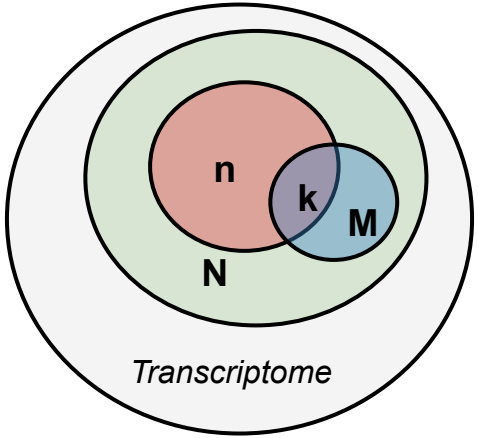

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p.value=0.002881322

Right-tailed fisher exact test

= Testing Independence

+ correction for multiple-tests

q.value

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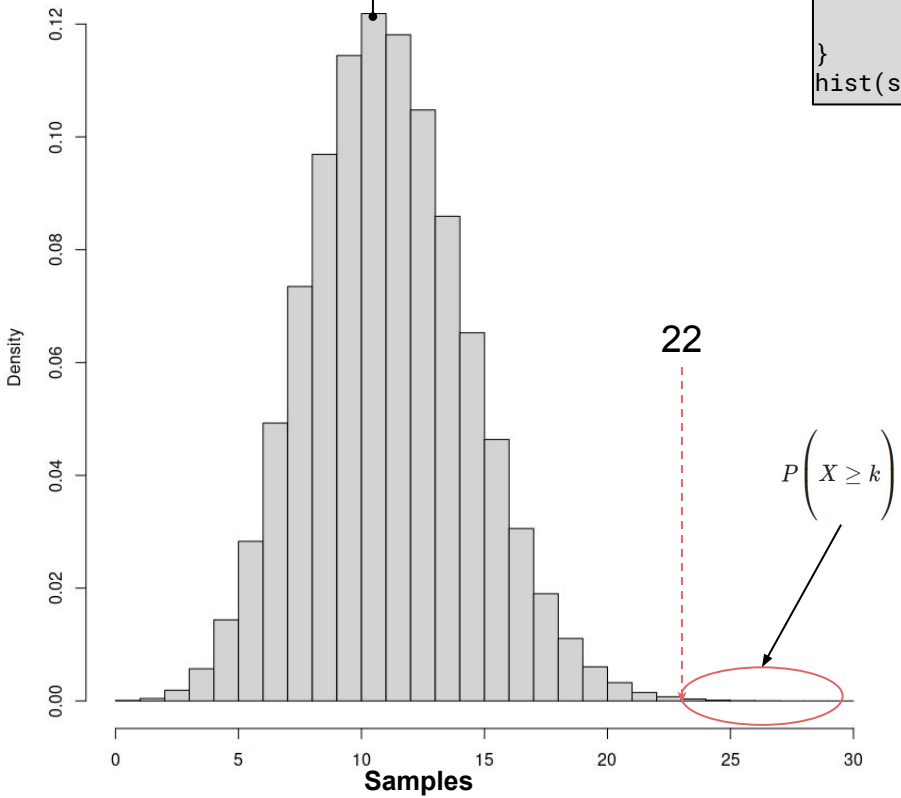
ORA: Intuition via random sampling

$$\frac{191}{15568} \times \frac{945}{15568} \times 15568 \approx 11.6$$

$P(\text{in Gene set})$ $P(\text{in BP set})$

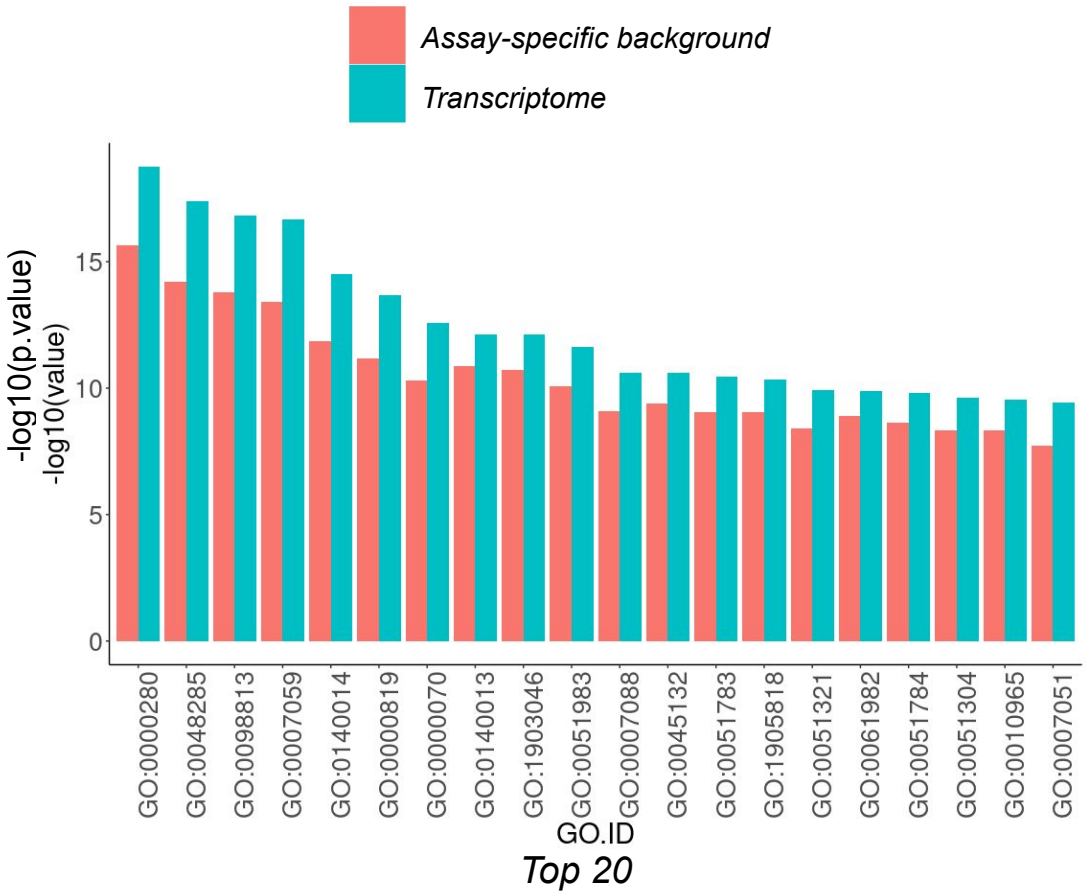



```
N_SAMPLES <- 1000000
samples <- vector(mode = "numeric", length = N_SAMPLES)
for(i in 1:N_SAMPLES){
      s <- sample(size = 945, x = universe, replace = F)
      samples[i] <- sum(s %in% GO.0006836)
}
hist(samples, freq = F, breaks = seq(0,30,1))
```

*What proportion of r**andom sets** sampled from the universe show more than 22 genes included in GO:0006836 ?*

```
estimate = 0.002816
```

ORA: The Impact of the *universe* definition



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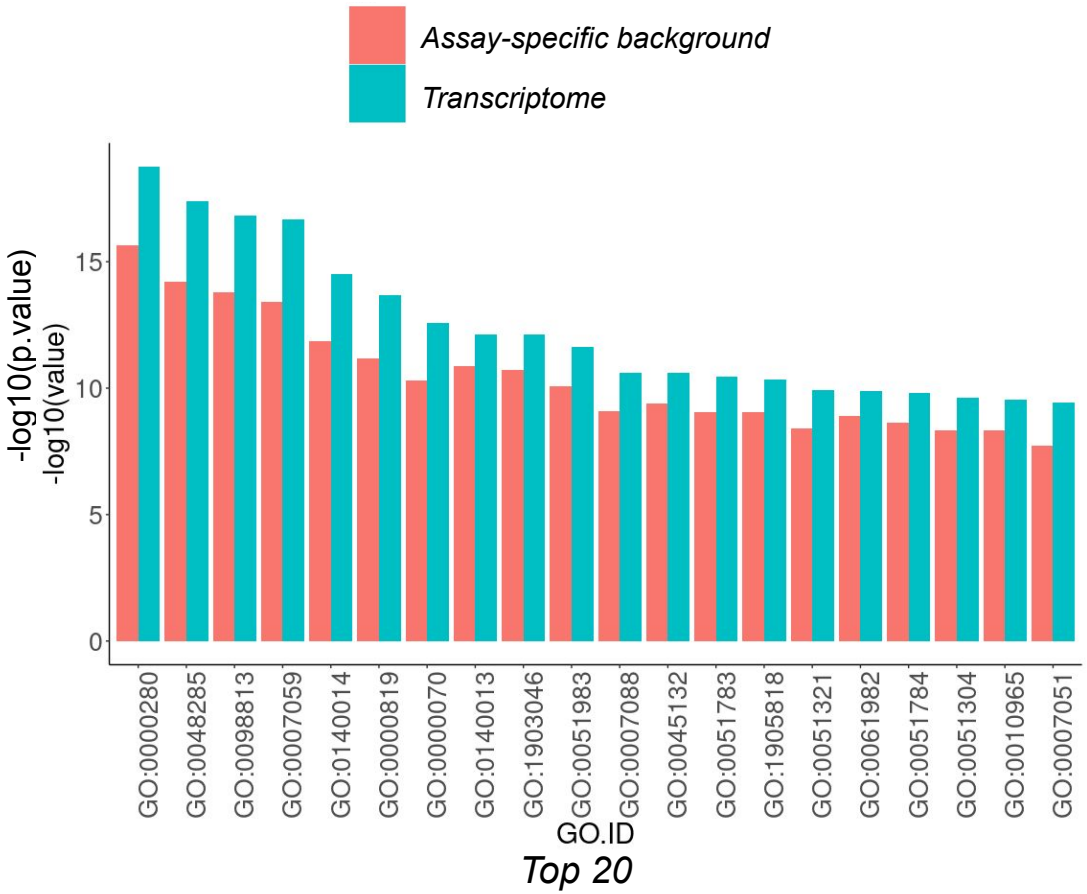


Leads to overestimation of the p-value

+

Order ~ preserved

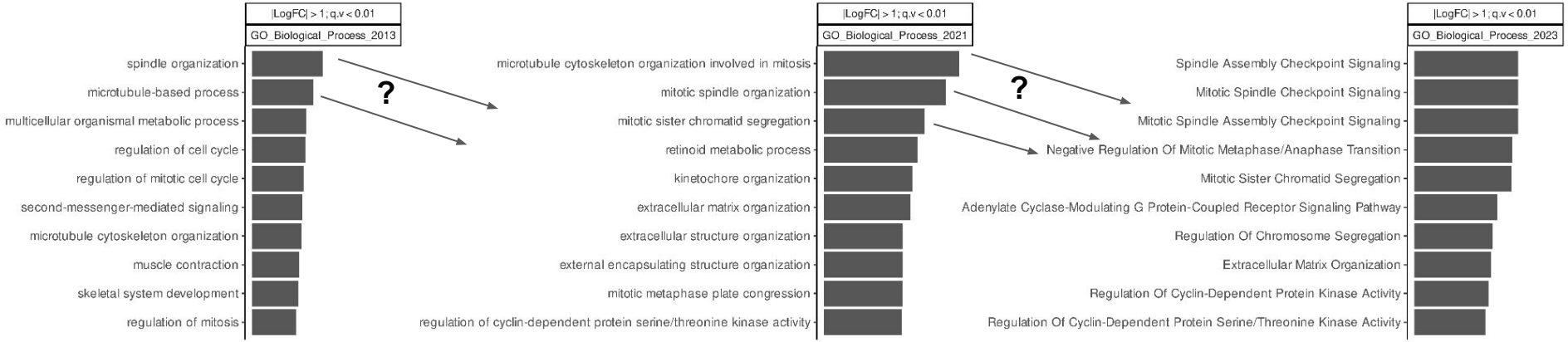
↓

Increase false positive enriched terms

133 vs 218 enriched GO terms
(q.value <= 1e.3)

ORA: Impact of the database and gene set thresholds choices

Enrichr



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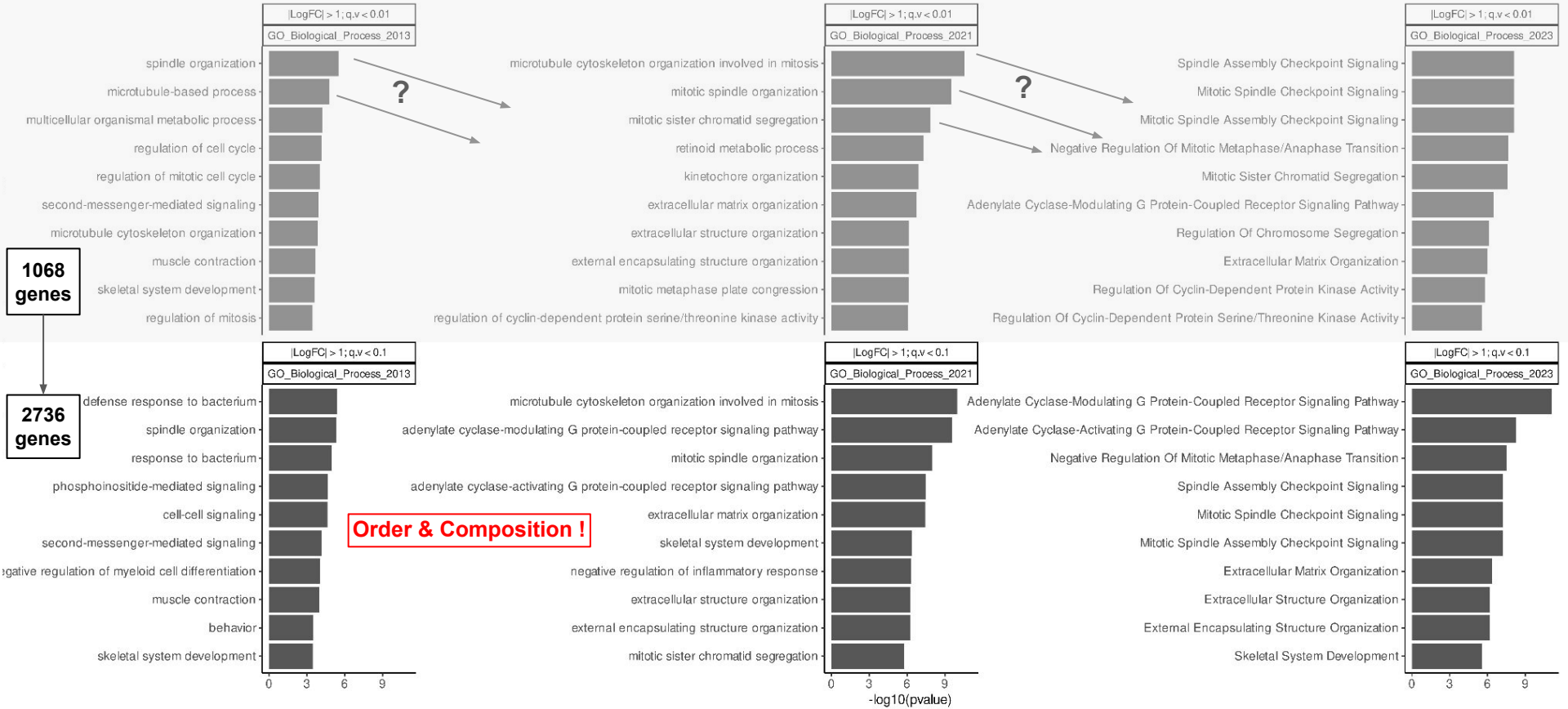

ORA: Impact of the database and gene set thresholds choices

How many significantly enriched Biological Processes ? (q.value < 0.01)

		GO BP 2013	**GO BP 2021**	**GO BP 2023**
2736 genes	**q.value < 0.1**	8	37	30
1068 genes	**q.value < 0.01**	4	44	40

Thresholds and database choices also have an impact of the number of enriched terms

ORA: Several biases

- All parameters are important
  - a universe set (size = **N**)
  - a set of interest (size = **n**)
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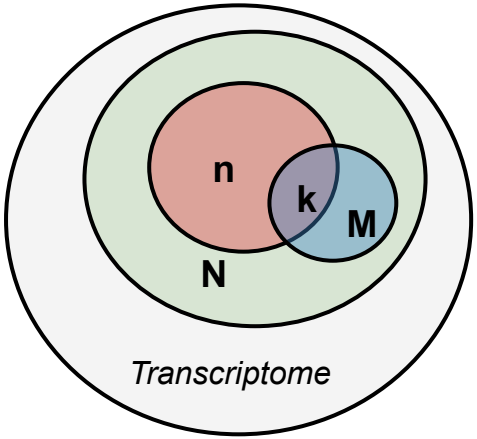
Unspecific background set can create false positives

Selection thresholds are important:
- Too large = noisy detection
- Too small = low detection

Reference database and versions can an impact of results



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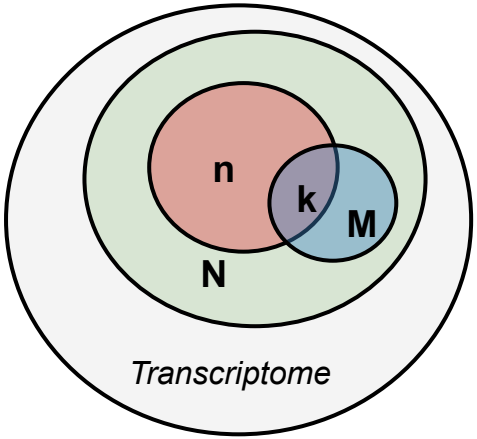


Always specify the background set, the applied thresholds and the database version → Good results = **Reproducible** results

ORA: It can be any gene sets - WGCNA example

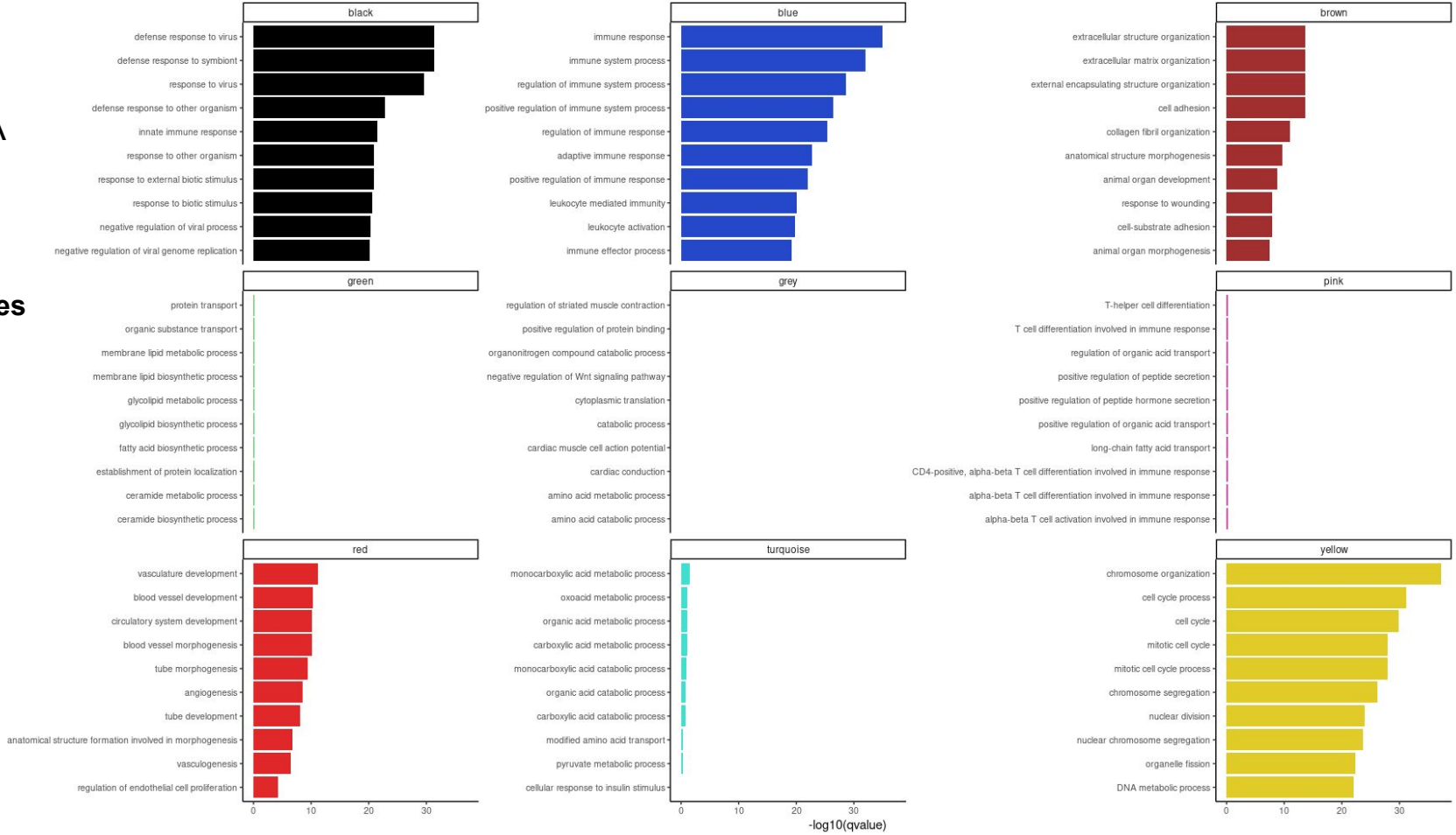
TP WGCNA

↓

9 gene modules

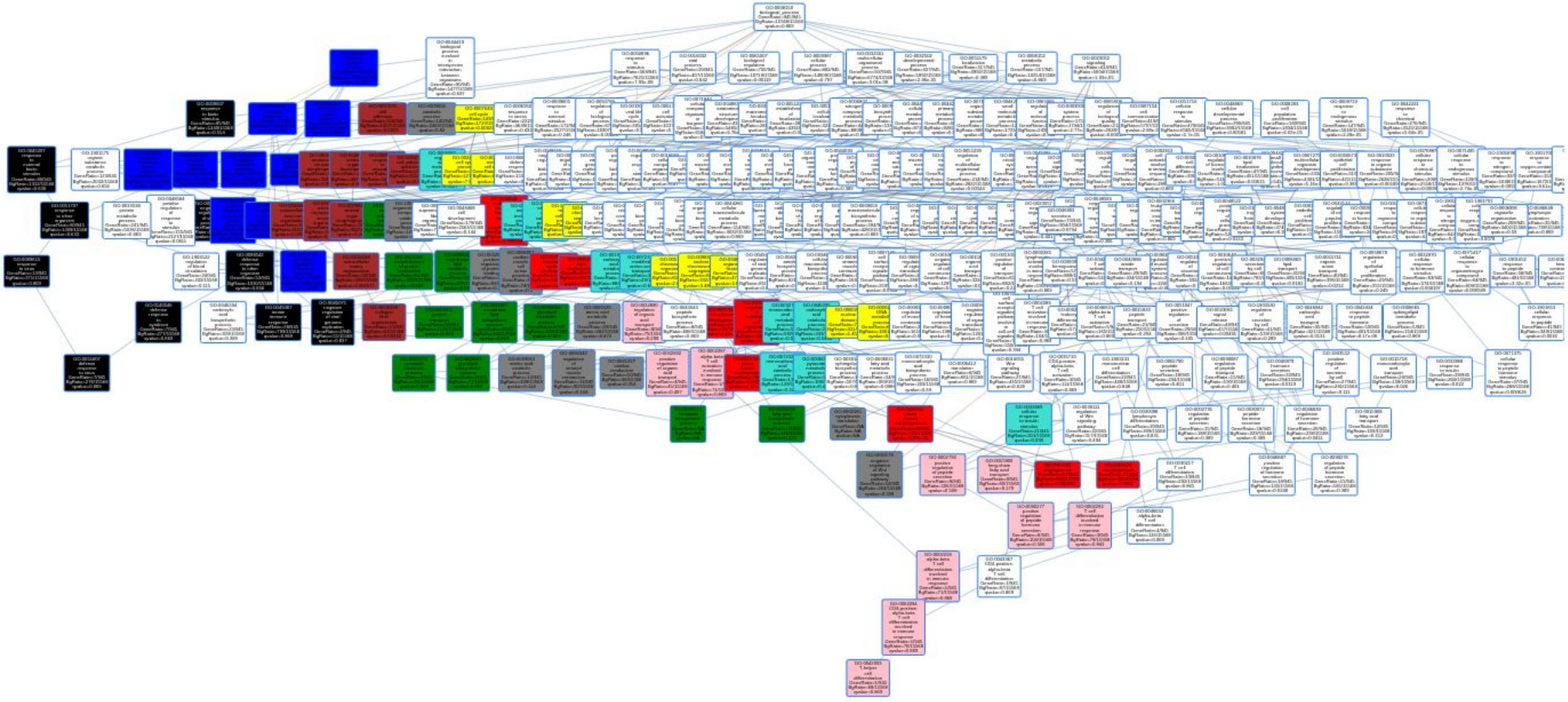
↓

Enrichment analysis on modules



ORA: It can be any gene sets - WGCNA example mapping on DAG

Mapping ofthe top 10 per modules on the GO BP DAG



GSEA: A Function scoring method

- Recall of the impact of the threshold (q.value & logFC) on the ORA results

→ **GSEA**

- All genes are not equivalent: sign and intensity of variation

Subramanian, A. et al., 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.
James H Joly et al., 2019, Differential Gene Set Enrichment Analysis: a statistical approach to quantify the relative enrichment of two gene sets, *Bioinformatics*.

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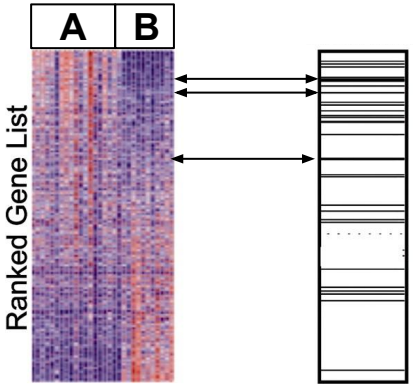

possible metrics: Log2FC, signed p-values, etc.

Genes associated with a GO term

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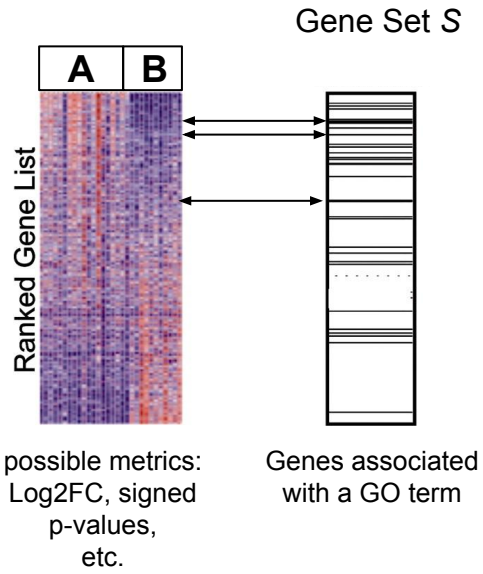
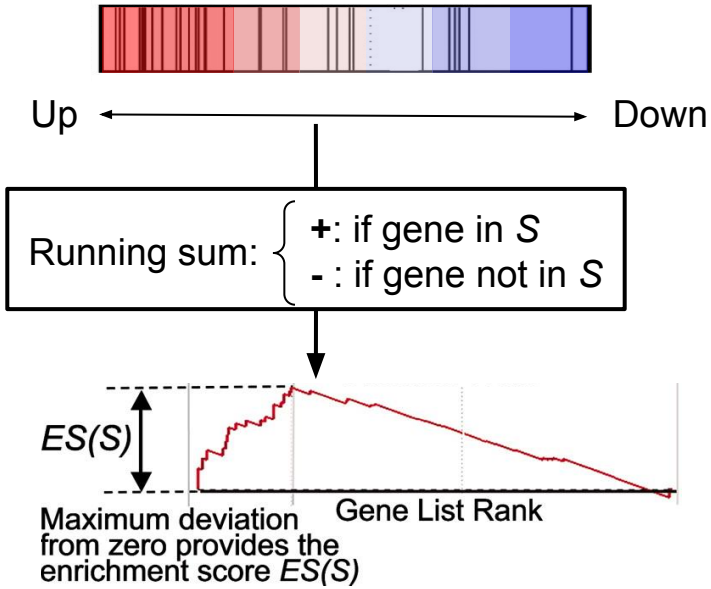


Compute the **E**nrichment **S**core (ES)



Subramanian, A. et al., 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.
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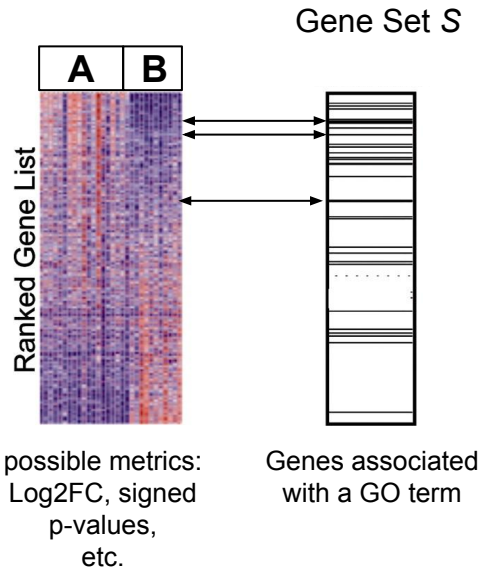
GSEA: A Function scoring method (1)

- Recall of the impact of the threshold (q.value & logFC) on the ORA results

→ **GSEA**

- All genes are not equivalent: sign and intensity of variation



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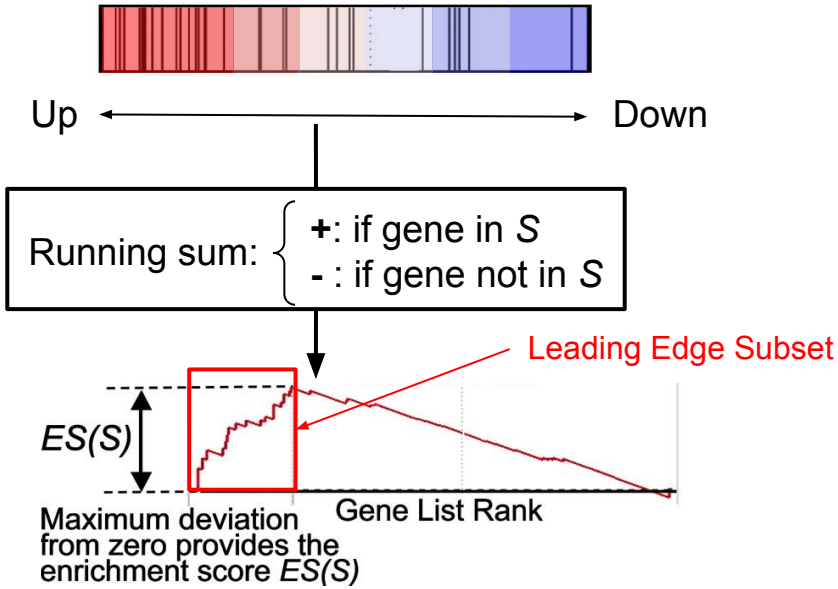

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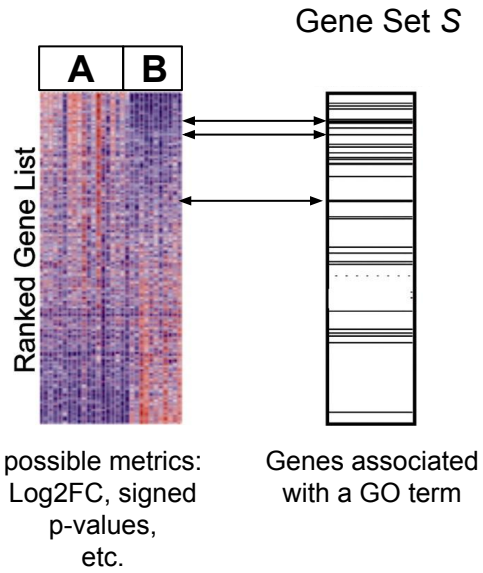

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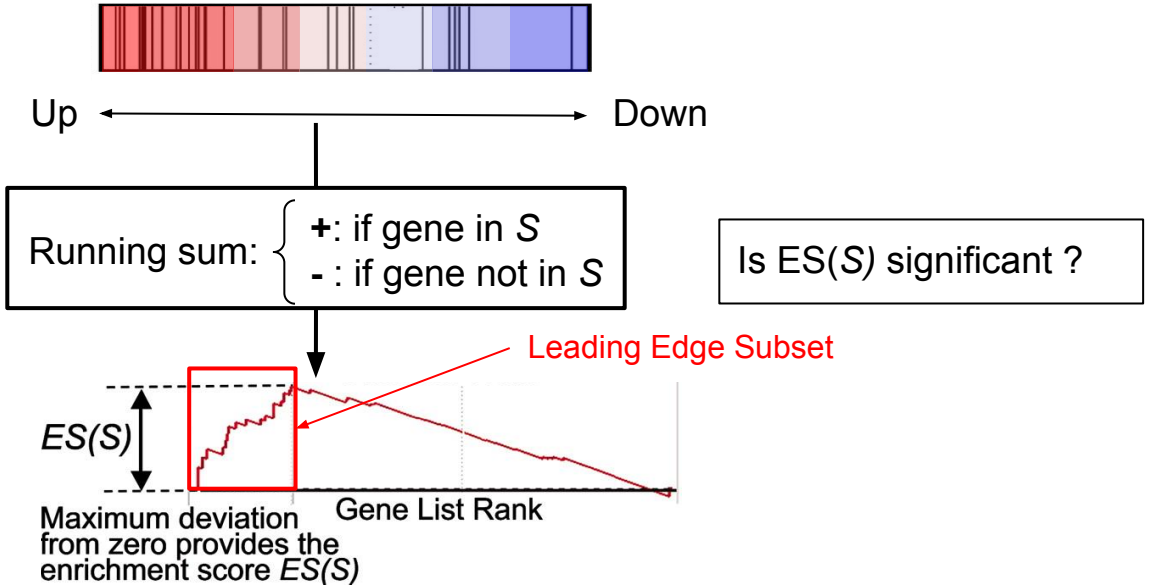

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GSEA: A Function scoring method (2)

Is ES(*S)* significant ?

- in unweighted settings (first version of GSEA): exact p-value estimation with KS-test

https://www.pathwaycommons.org/guide/primers/data_analysis/gsea/

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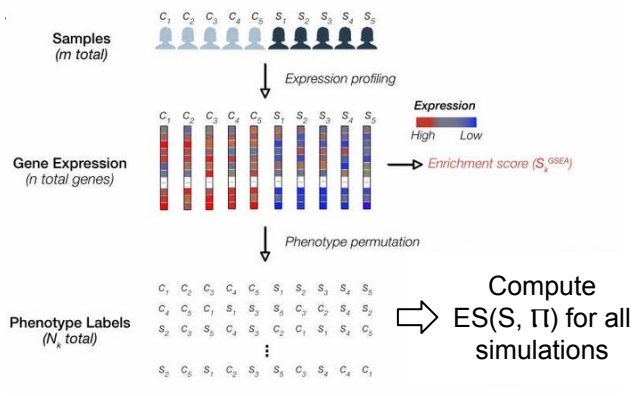

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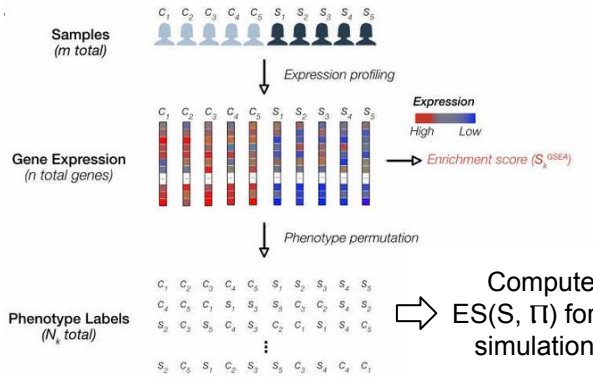
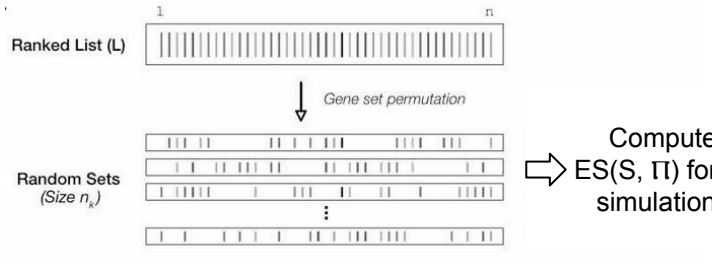


Compute ES(S, Π) for all simulations

Gene set Permutation (most common but less efficient)



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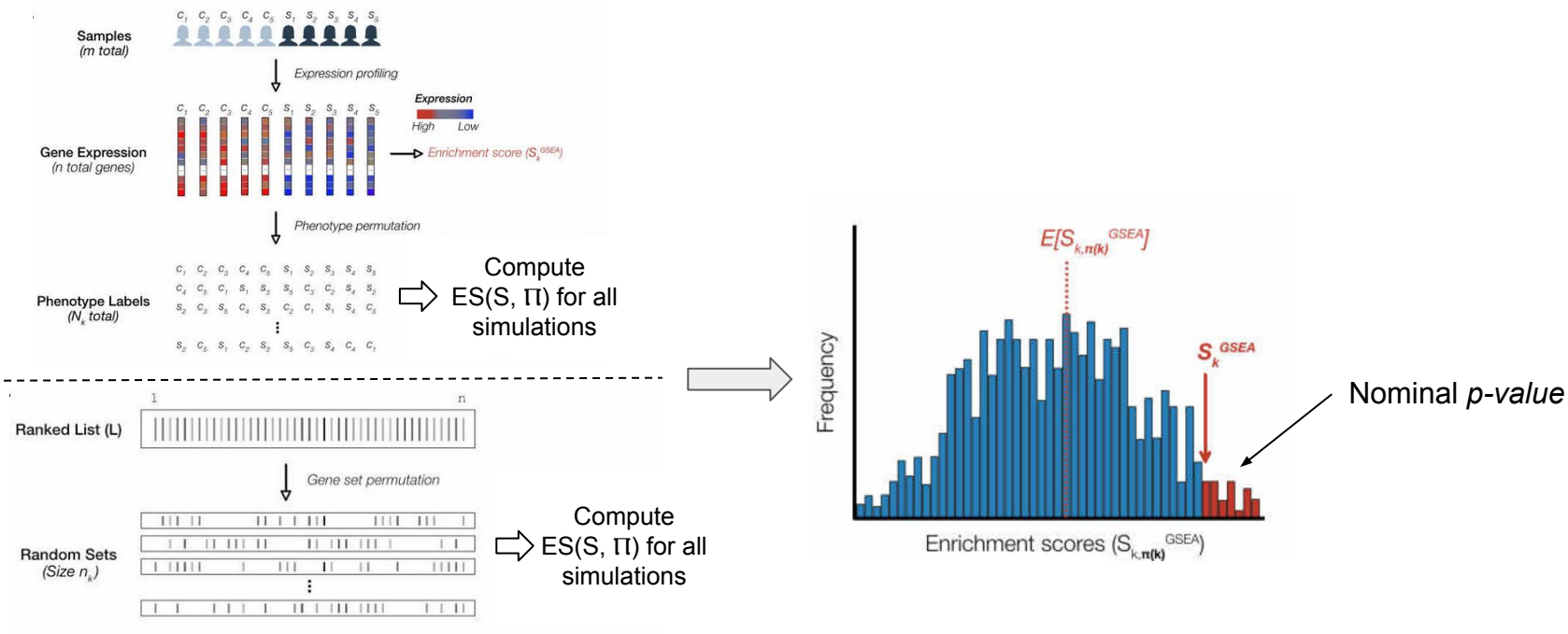


https://www.pathwaycommons.org/guide/primers/data_analysis/gsea/

GSEA: A Function scoring method (3)

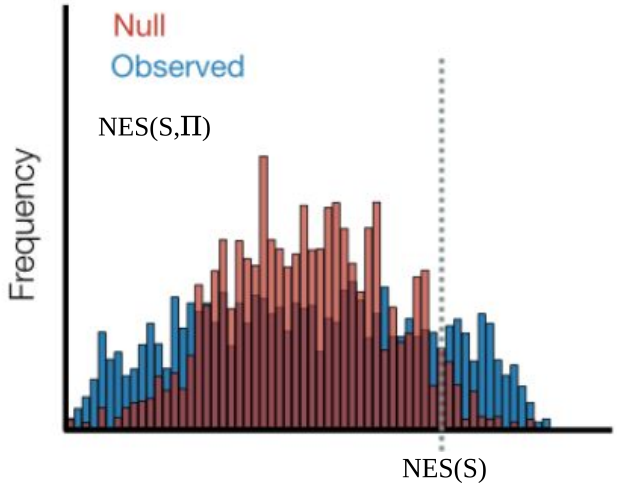
- How to account for Gene set size differences ?

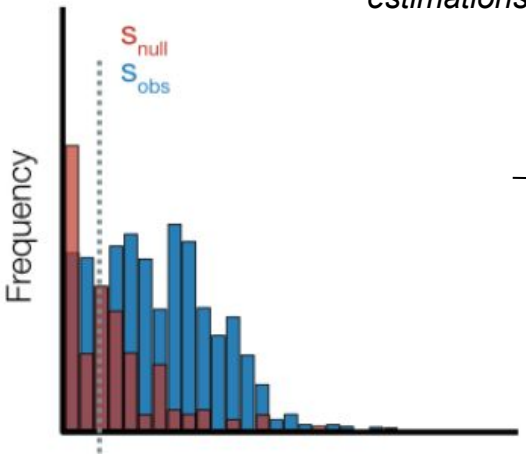
NES (**N**ormalised **E**nrichment **S**core)

$$\mathrm{NES(S)} = \frac{ES(S)}{E[ES(S,\Pi]}$$ ← Gives the direction of regulation + correct for gene set size + signed

- Multiple-test correction: FDR estimation

estimations separated by NES signs



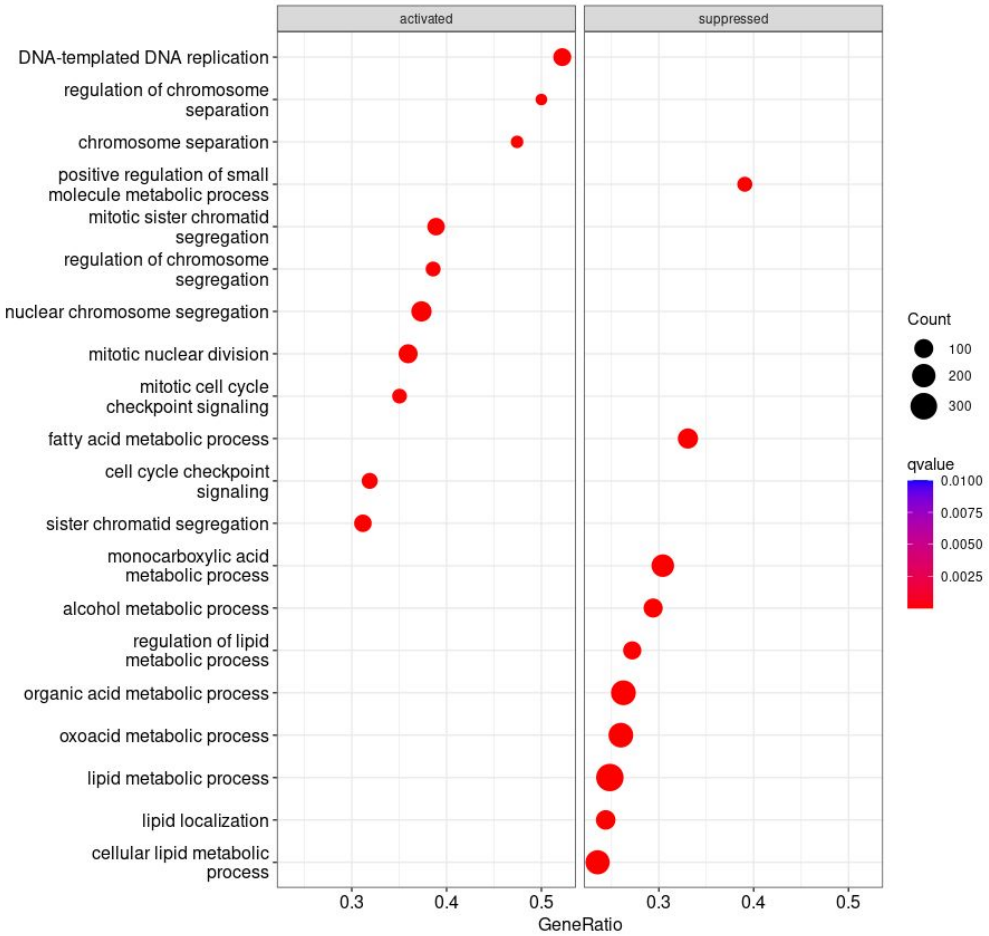


→ $F\hat{D}R$

https://www.pathwaycommons.org/guide/primers/data_analysis/gsea/

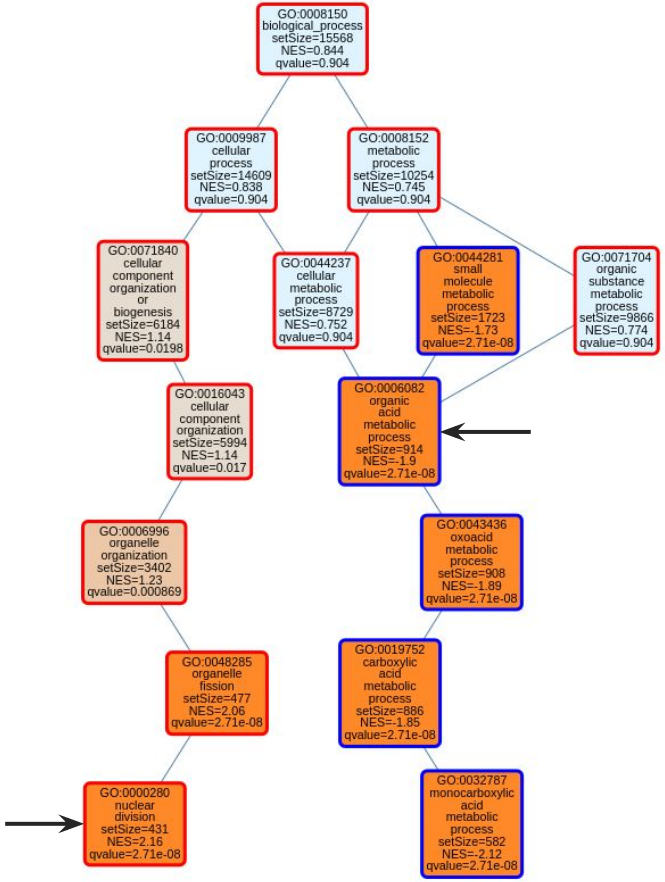
GSEA: A Function scoring method - example

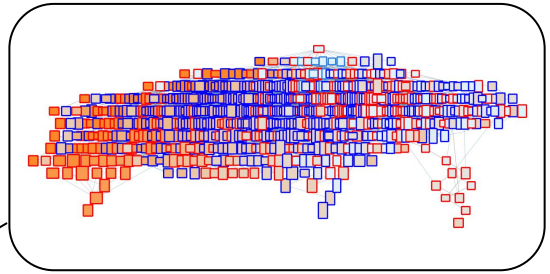
- No need of a cutoff on qvalue or LogFC, just a ranking metric !
- Results are separated between over and under expression BP
- Leading Edge subset can help to identify key actors
- However, same biases apply for database choices !

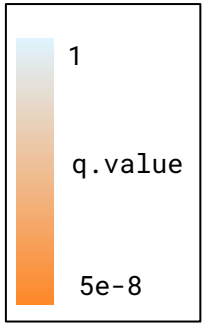


GSEA: Visualisation - example

Up and Down regulated branch of the DAG



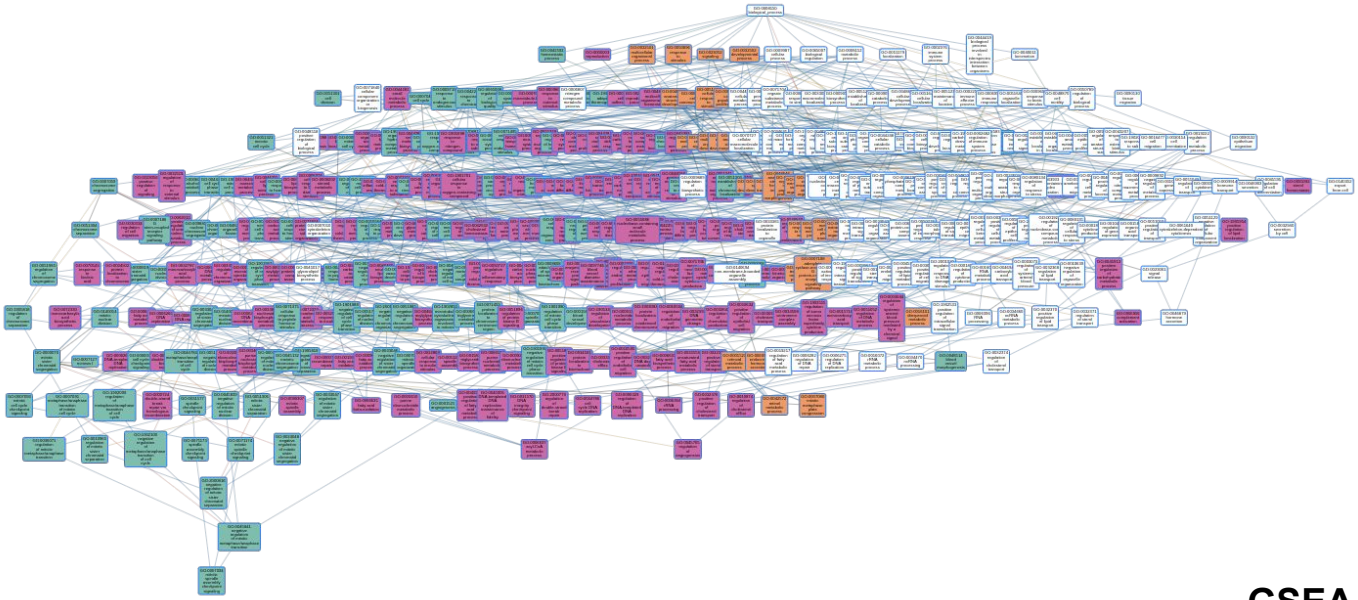




up-regulated

down-regulated

ORA vs GSEA: A visual comparison on the GO DAG graph



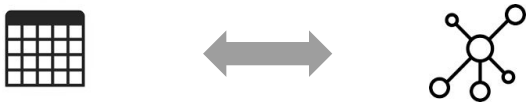
q.value < 1e-3

- **Only GSEA**
- **Both ORA and GSEA**
- **Only ORA**

GSEA is more sensitive !

Extend contextualisation with Biomedical Knowledge Graph

What is a Graph ?
A graph is defined by a set of **nodes** and **edges**



Attributes/Properties Relations/Paths

Different questions, different visualisation, different methods

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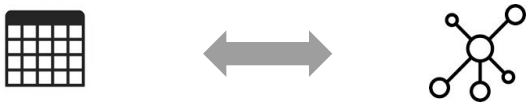


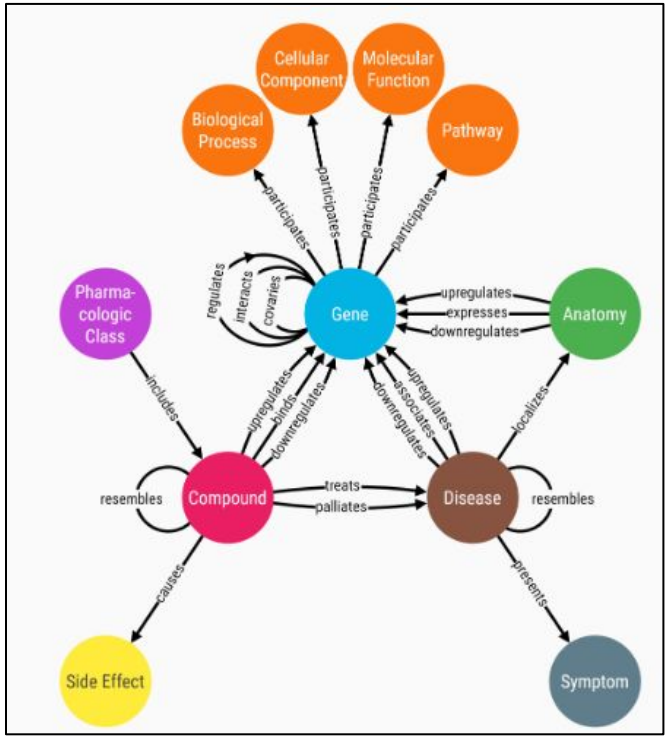
Attributes/Properties Relations/Paths

Different questions, different visualisation, different methods

What is a Biomedical Knowledge Graph ?
It's a graph describing biomedical entities and their relations

- Different Model: RDF (in Semantic Web) and LPG (Labeled Property Graph)
- Relations are stored at the individual record level
- Efficient for complex information extraction
- Examples: Hetionet, Wikidata, PharmKG, FORUM, etc.

Example of Hetionet



How to request a Knowledge Graph (in Neo4J)

neo4j Cypher

3 main clauses:

- MATCH: Specify the graph pattern
- WHERE: Add restrictions to the nodes or edges properties
- RETURN: Define what is included in the results

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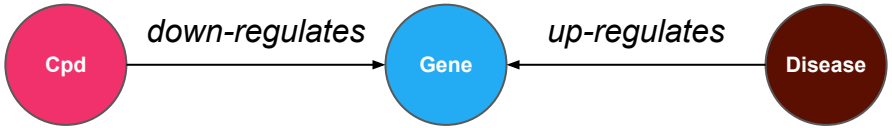



```
MATCH (c:Compound)-[r1:DOWNREGULATES_CdG]->(g:Gene)<-[r2:UPREGULATES_DuG]-(d:Disease)
WHERE g.name IN [ “BRCA1”, “BRCA2”, … ]
RETURN c, r1, g, r2, d
```

Extend contextualisation with Biomedical Knowledge Graph

TP WGCNA

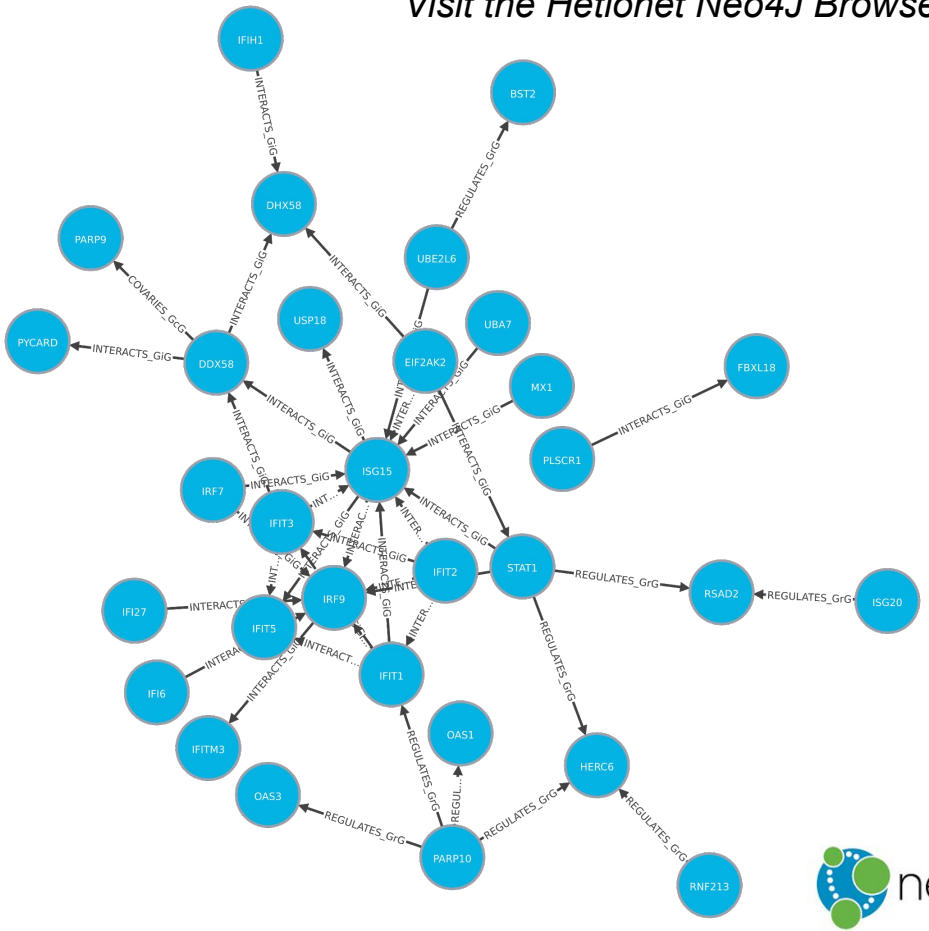
↓

Black gene module

↓

Explore relations between genes in a module

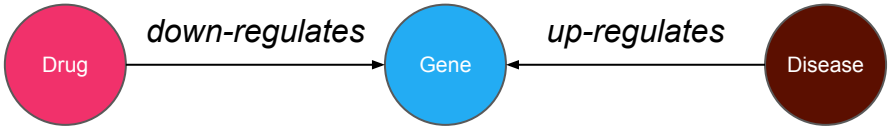
Visit the Hetionet Neo4J Browser



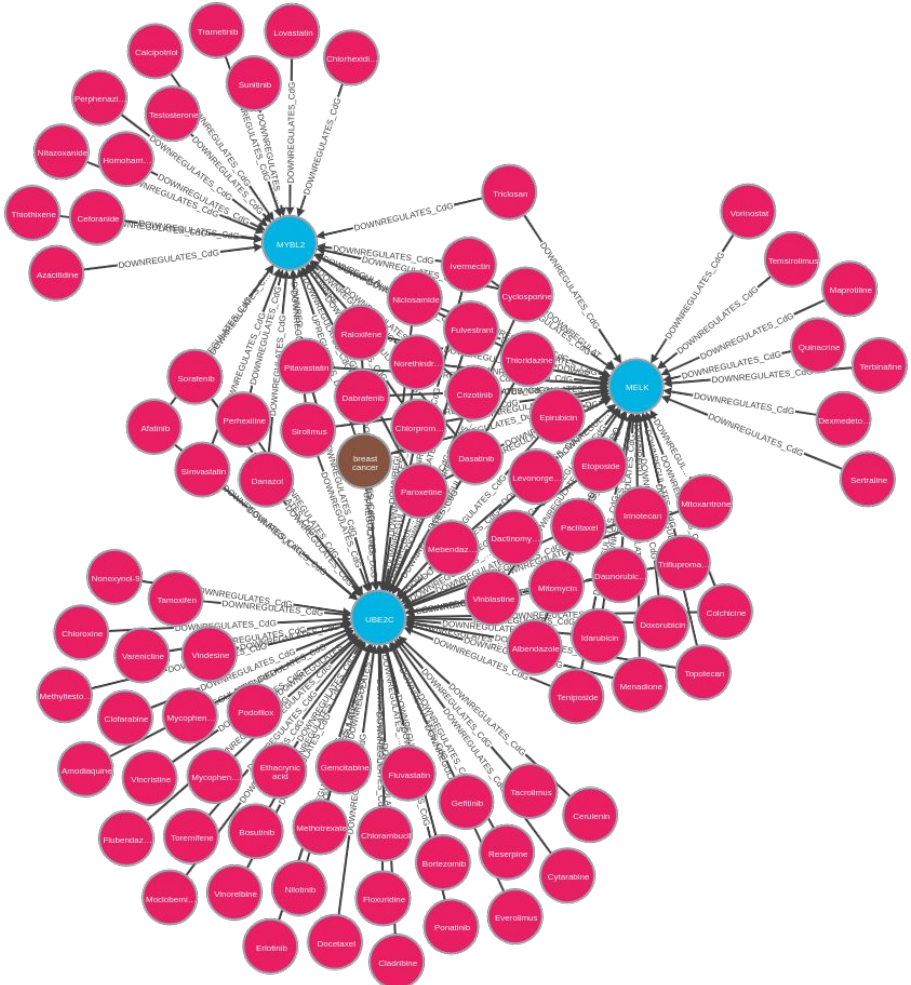
Extend contextualisation with Biomedical Knowledge Graph

A more complex path

By selecting only the up-regulated genes (LogFC > 5)



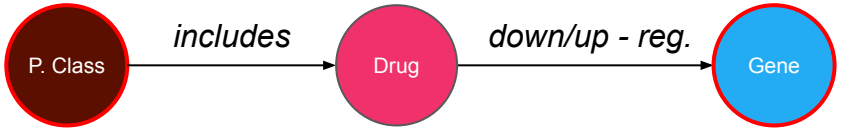
Drugs
Thioridazine
Doxorubicin
Dabrafenib
Teniposide

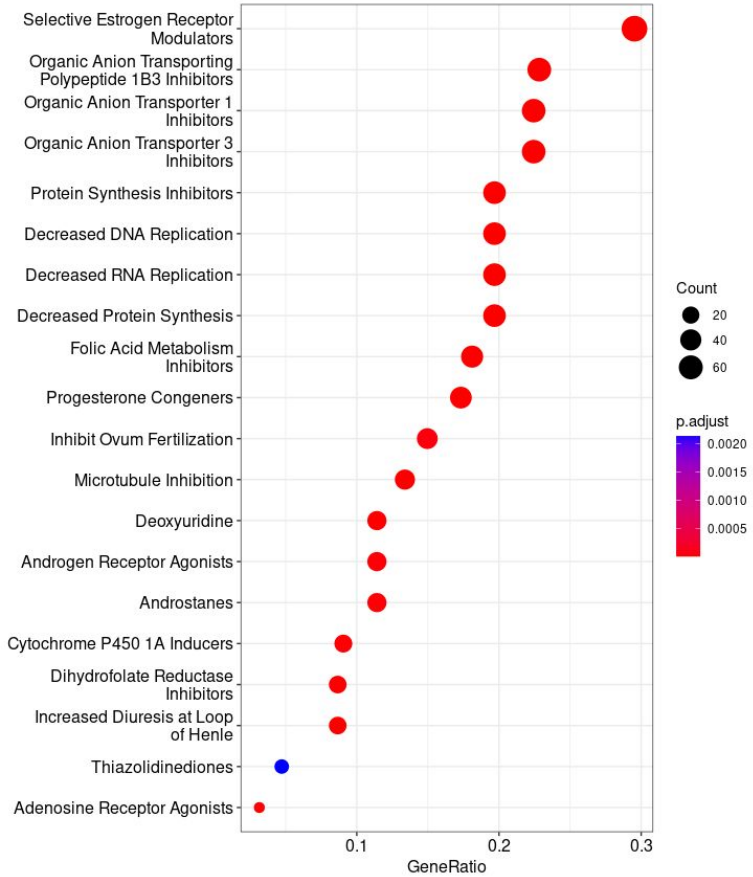


Extend contextualisation with Biomedical Knowledge Graph

Use a Biomedical Knowledge Graph to build a Enrichment custom background set

What class of drugs in enriched for their relation with the set of genes of interest ? (ORA)





Contextualisation of results: Conclusion

- Enrichment analyses (ORA or GSEA) are a powerful tool to suggest direction of interpretation and hypotheses

- ORA are simples and universals, but results can be affected by several biased: threshold, databases, universe.

- GSEA is not affected by thresholding are give more weight to the most discriminant genes

- Several biases remain:
  - Internal structure of pathway / interconnection between entities in a pathway → **Topological methods**
  - Overlap / interconnections between pathway
  - What about gene variants ?

- Biomedical KG can help to explore new connections between entities and with other entities

- They can also be used for building a custom background set.

- Build your own Biomedical KG ! Use **BioCypher**

The End

Ressources

- Enrichment analysis
  - Biblio & Resources
    - Wieder, C. et al. Pathway analysis in metabolomics: Recommendations for the use of over-representation analysis. PLoS Comput Biol 17
    - https://colab.research.google.com/drive/18pLzc_pv7Fpclotx4byYh9qMDjtnyG_u?usp=sharing
    - García-Campos, M.A. et al. 2015. Pathway Analysis: State of the Art. Front Physiol
    - Subramanian, A. et al., 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.
    - James H Joly et al., 2019, Differential Gene Set Enrichment Analysis: a statistical approach to quantify the relative enrichment of two gene sets, Bioinformatics.
    - https://www.pathwaycommons.org/guide/primers/data_analysis/gsea/
- Biomedical KG & Co.
  - LPG
    - *Hetionet*: https://het.io/about
    - *Drug Repurposing Knowledge Graph (DRKG)*: https://github.com/gnn4dr/DRKG
    - *BioKG*: https://github.com/dsi-bdi/biokg
    - PharmKG: https://academic.oup.com/bib/article/22/4/bbaa344/6042240
  - Web-Semantic
    - MetaNetX: https://www.metanetx.org/
    - Wikidata: https://www.wikidata.org/wiki/Wikidata:Main_Page
    - DisGeNeT: https://www.disgenet.org/
    - Rhea: https://www.rhea-db.org/
    - UniProt: https://www.uniprot.org/help/uniprotkb
  - Other resources
    - *Cypher Cheat Sheet*: https://neo4j.com/docs/cypher-cheat-sheet/5/auradb-enterprise/
    - *BioCypher*: https://biocypher.org/
    - Web-semantic MOOC: https://www.fun-mooc.fr/fr/cours/web-semantique-et-web-de-donnees/
    - Neo4J: https://www.youtube.com/channel/UCvze3hU6OZBkB1vkhH2lH9Q

Waagmeester, A. et al., 2020. Wikidata as a knowledge graph for the life sciences. eLife 9, e52614.