Swiss Institute of
Bioinformatics

BIOLOGY-INFORMED MULTIOMICS DATA INTEGRATION AND VISUALIZATION

Enrichment analysis

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June 16-17, 2025

ELIXIR SWITZERLAND

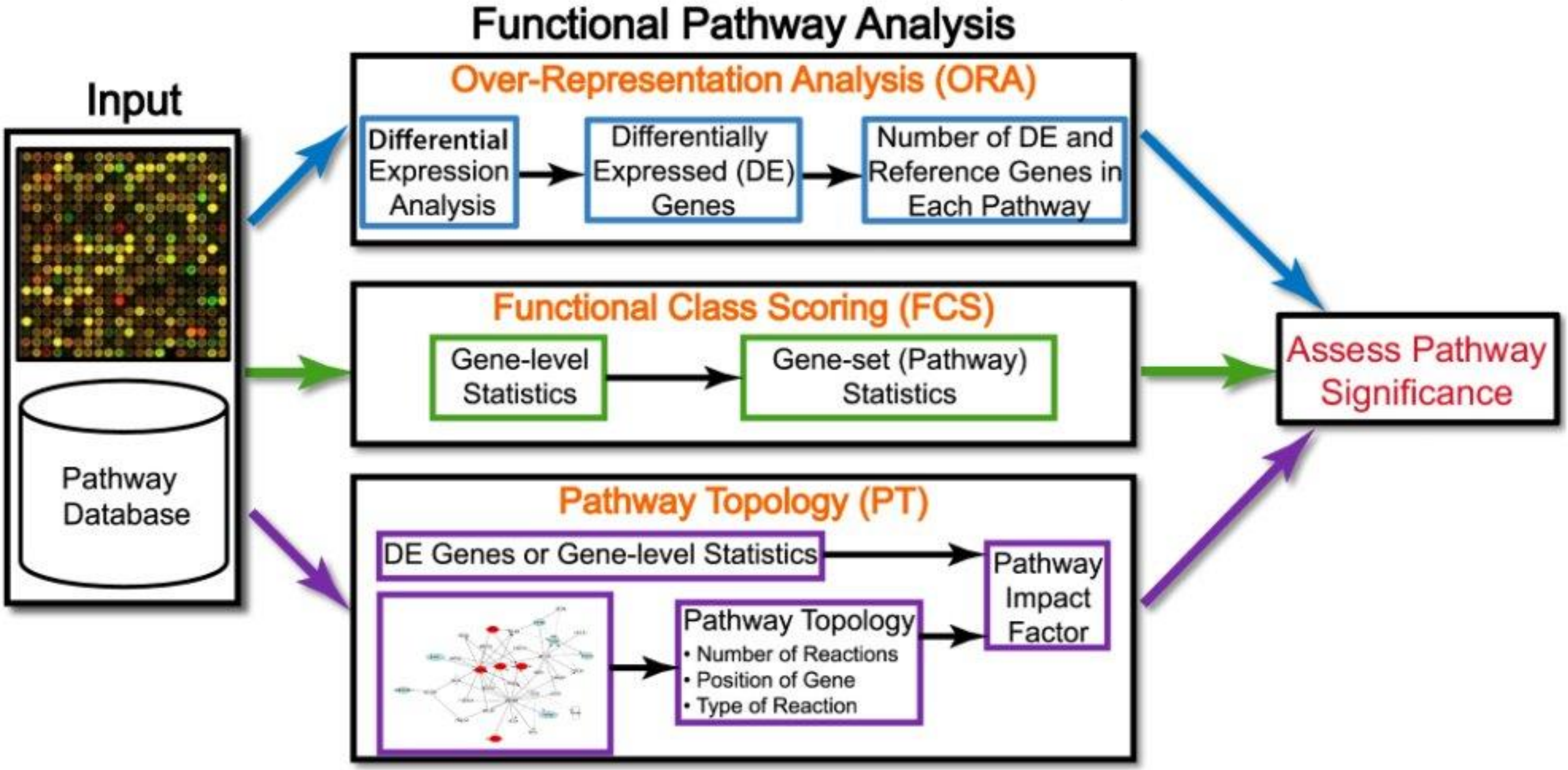
Learning objectives

What is Enrichment analysis?

Distinguish between different ways to do it.

Challenges and Limitations of methods.

Overview of functional analysis



Functional Pathway Analysis

Input

Pathway Database

Over-Representation Analysis (ORA)

Differential Expression Analysis → Differentially Expressed (DE) Genes → Number of DE and Reference Genes in Each Pathway

Functional Class Scoring (FCS)

Gene-level Statistics → Gene-set (Pathway) Statistics

Pathway Topology (PT)

DE Genes or Gene-level Statistics → Pathway Impact Factor

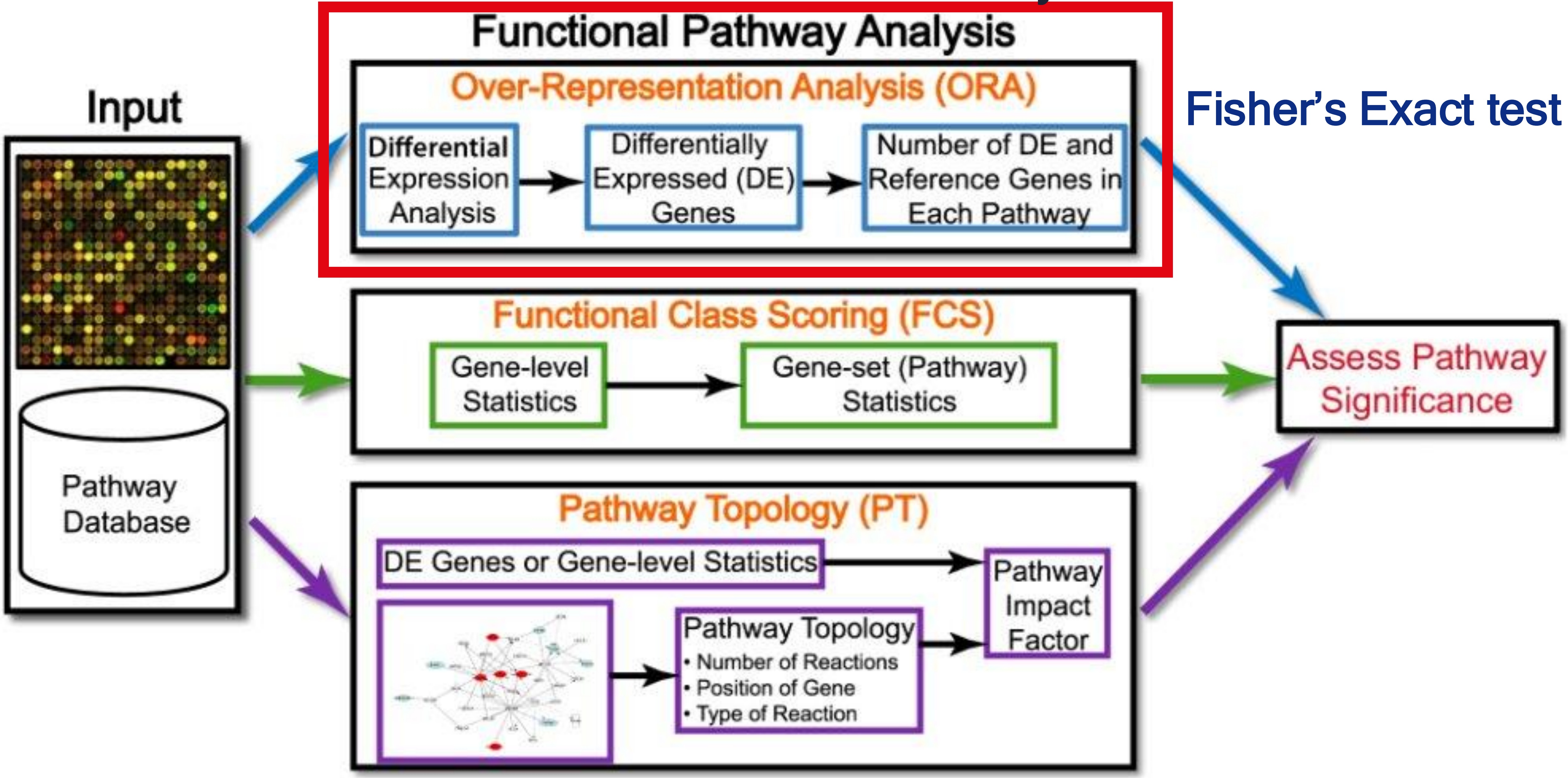
Pathway Topology → Pathway Impact Factor

- Number of Reactions
- Position of Gene
- Type of Reaction

Assess Pathway Significance

https://doi.org/10.1371/journal.pcbi.1002375

Overview of functional analysis: ORA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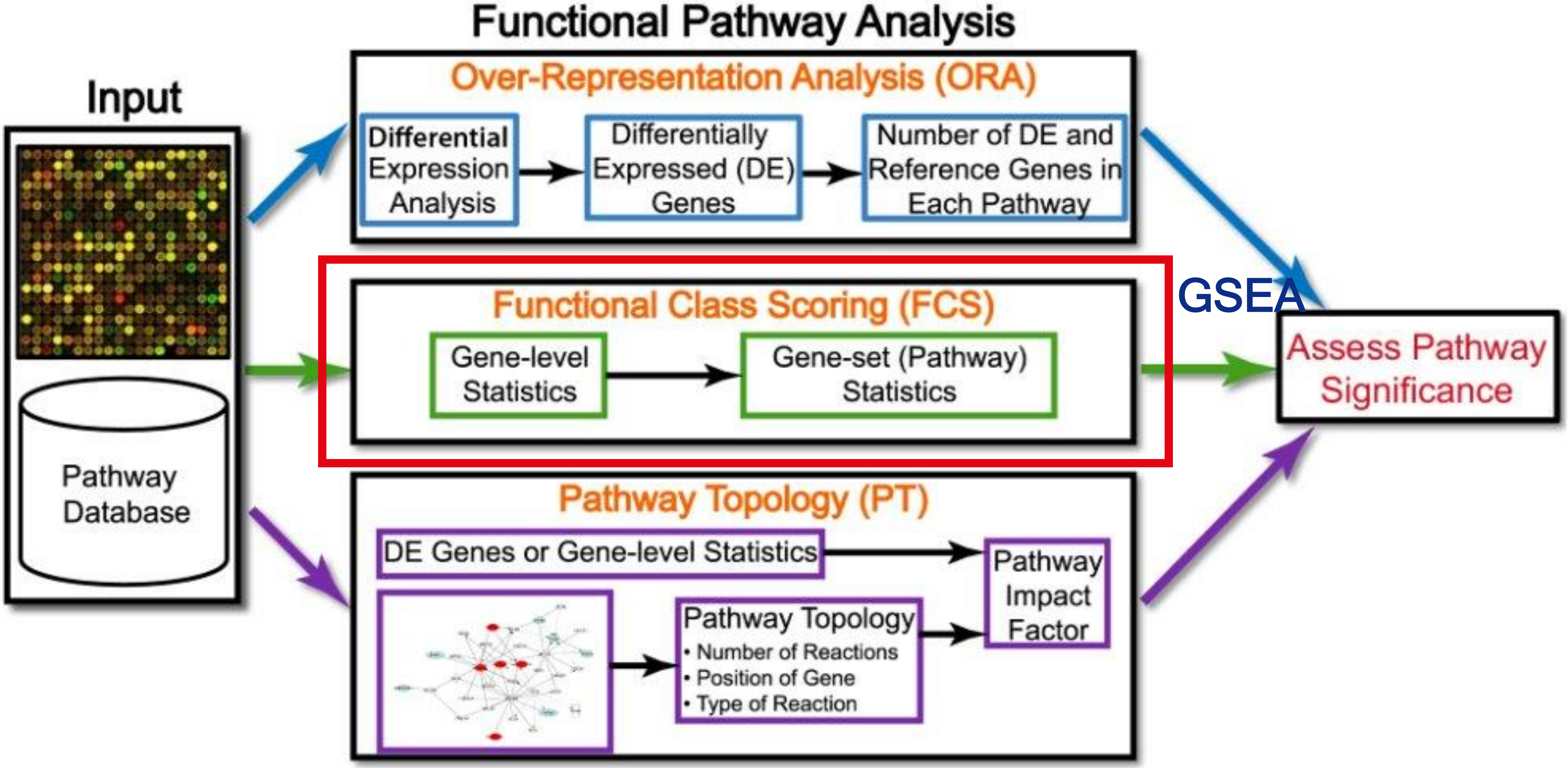
Fisher's Exact test

https://doi.org/10.1371/journal.pcbi.1002375

Overview of functional analysis: FCS



Functional Pathway Analysis

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GSEA

Pathway Topology (PT)

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Pathway Topology
- Number of Reactions
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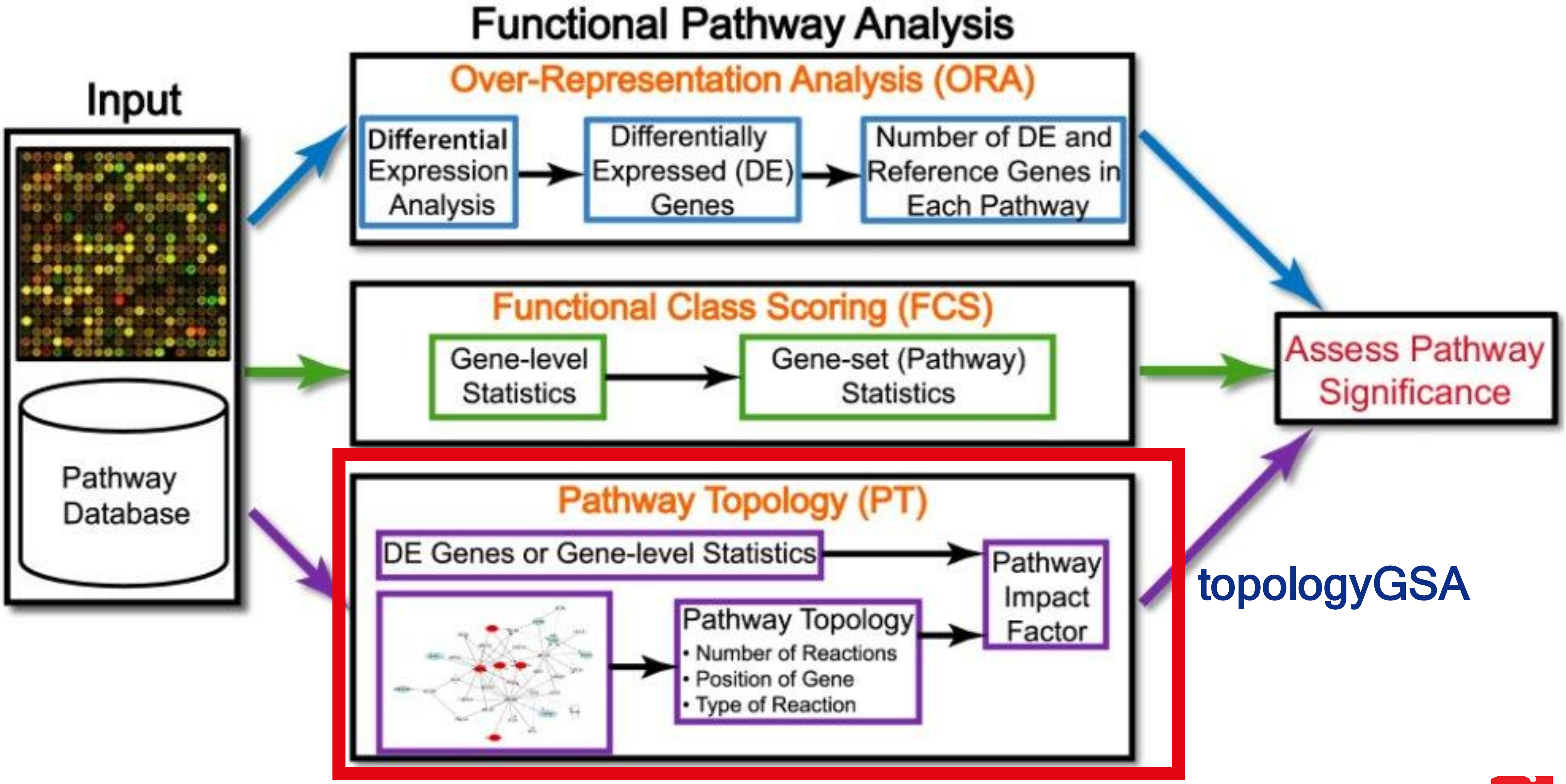
- Type of Reaction

→ Pathway Impact Factor

Assess Pathway Significance

https://doi.org/10.1371/journal.pcbi.1002375

Overview of functional analysis: PT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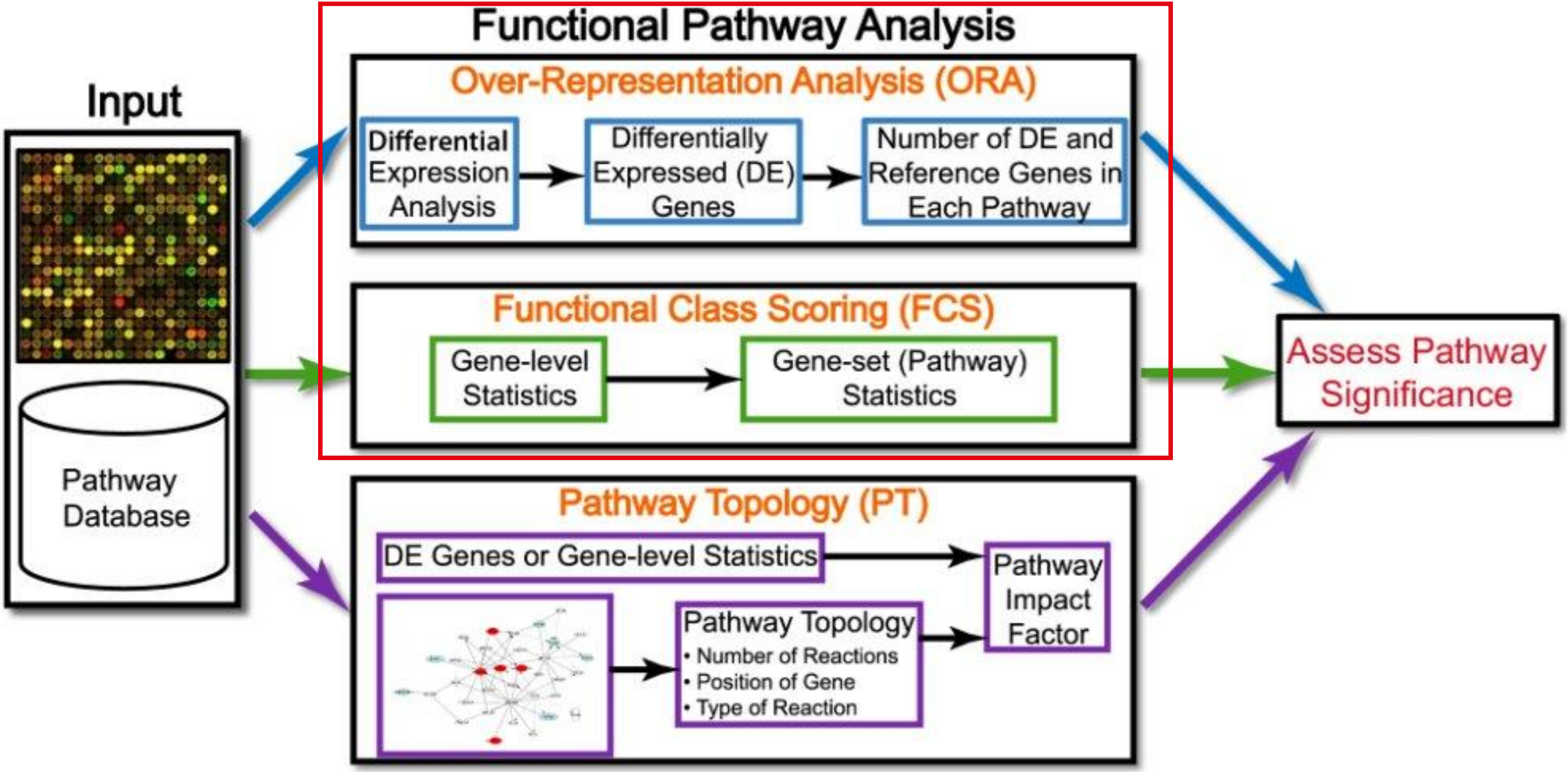
topologyGSA

https://doi.org/10.1371/journal.pcbi.1002375

Overview of functional analysis: ORA & FCS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Goal: To gain biologically meaningful insights from long gene lists

Over-representation analysis (ORA)

Statistically evaluates the fraction of genes in a particular pathway found among the set of genes showing changes in expression.

1. Select a list of genes with certain threshold (FDR <= 0.05)
2. For each pathway, count input genes that are part of the pathway
3. Repeat for an appropriate background list of genes
4. Every pathway is tested for over- or under-representation in the list of input genes

The most commonly used tests are based on the hypergeometric, chi-square, or binomial distribution

Over-representation analysis (ORA)

Gene1	0.051
Gene2	0.05001
Gene 3	0.049
Gene 4	0.001
Gene 5	0.023
Gene 6	0.04
Gene 7	0.01
Gene 8	0.0501
Gene 9	0.2
Gene 10	0.051
Gene 11	0.05
Gene 12	0.49
Gene 13	0.03
Gene 14	0.01
Gene 15	0.052
Gene 16	0.9

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pvalue <= 0.05

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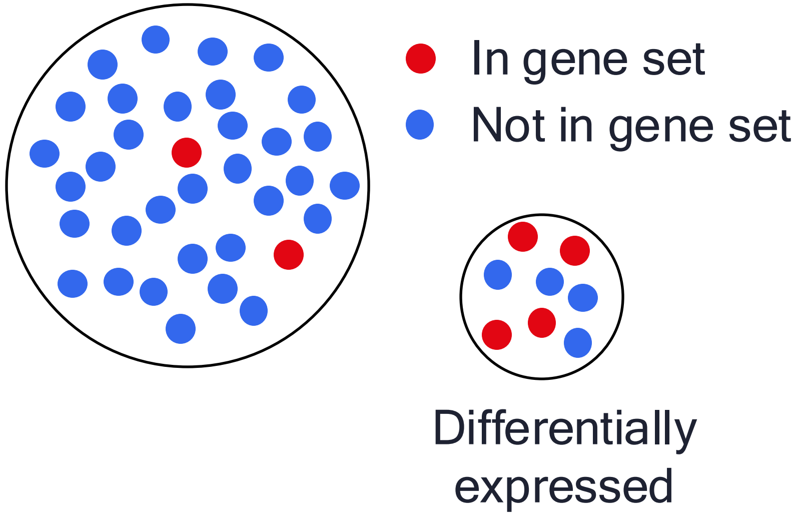
Fisher's test



H_0: The proportion of genes in the gene set is the same for both groups

H_a: The proportion of genes in the gene set is higher in the differentially expressed group

Problems with ORA

Cutoff? 0.051?

Treat all genes equally

Each gene is independent of other

Each pathway is independent of each other

Functional class scoring (FCS)

The hypothesis of FCS is that although large changes in individual genes can have significant effects on pathways, weaker but coordinated changes in sets of functionally related genes (i.e., pathways) can also have significant effects

1. Rank the genes
2. Perform gene-level statistics in a pathway
3. Calculate pathway level-statistics: - Kolmogorov-Smirnov statistic

Over-representation analysis (ORA)

Gene1	0.051	10
Gene2	0.05001	12
Gene 3	0.049	11
Gene 4	0.001	8
Gene 5	0.023	2
Gene 6	0.04	3
Gene 7	0.01	1
Gene 8	0.0501	3
Gene 9	0.2	-10
Gene 10	0.051	-3
Gene 11	0.05	-8
Gene 12	0.49	-19
Gene 13	0.03	-3
Gene 14	0.01	-2
Gene 15	0.052	-1
Gene 16	0.9	-4

Over-representation analysis (ORA)

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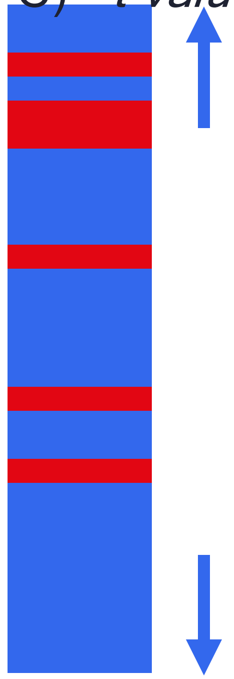
Gene 12	0.49	-19
Gene 13	0.03	-3
Gene 14	0.01	-2
Gene 15	0.052	-1
Gene 16	0.9	-4

Gene set enrichment analysis (GSEA)

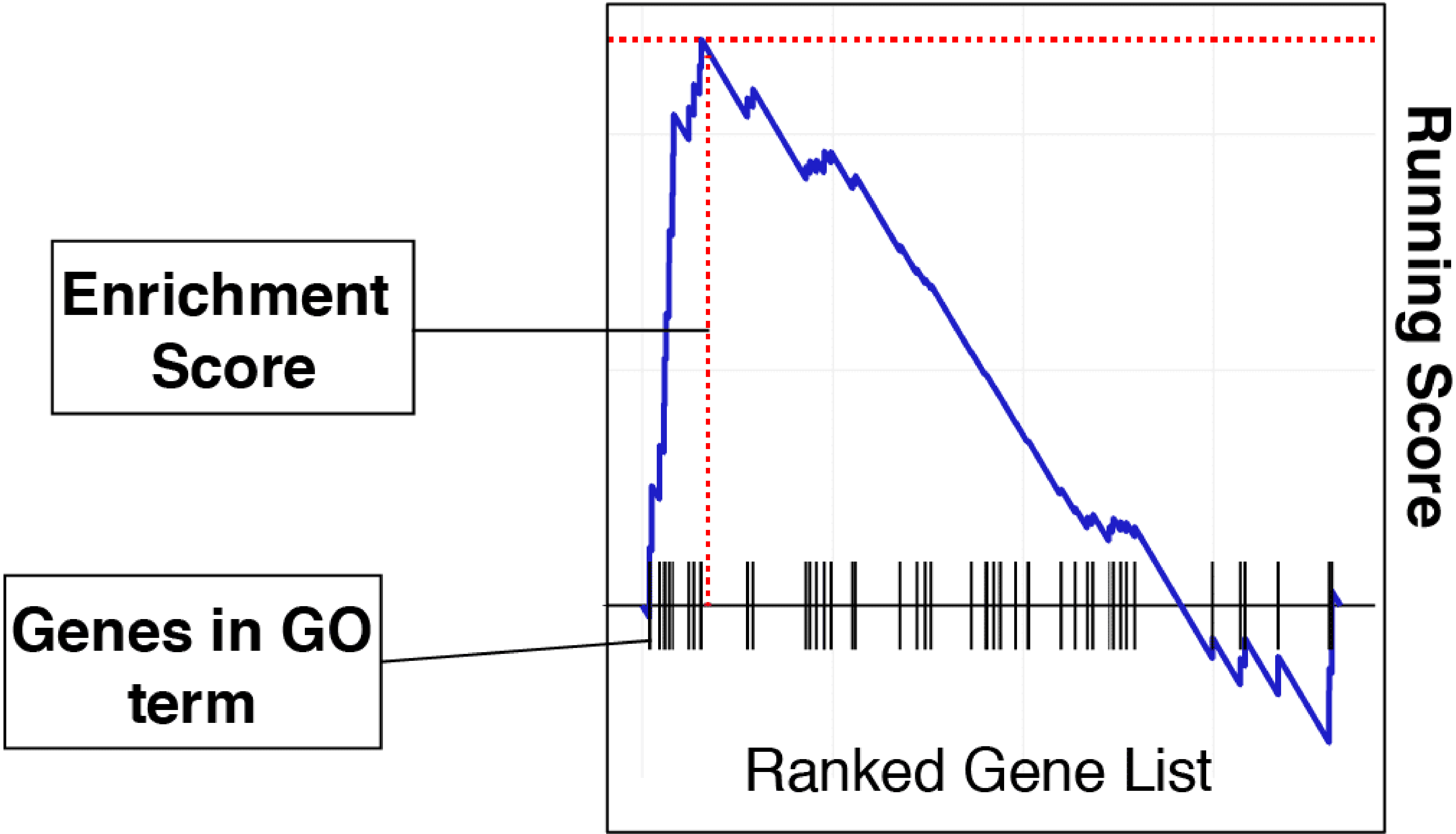
Genes ranked by test statistic
or
log2(FC) * *t-value*



H_0: Genes in set are randomly distributed over ranked list

H_a: Genes in set are not randomly distributed over the ranked list

Functional class scoring (FCS)

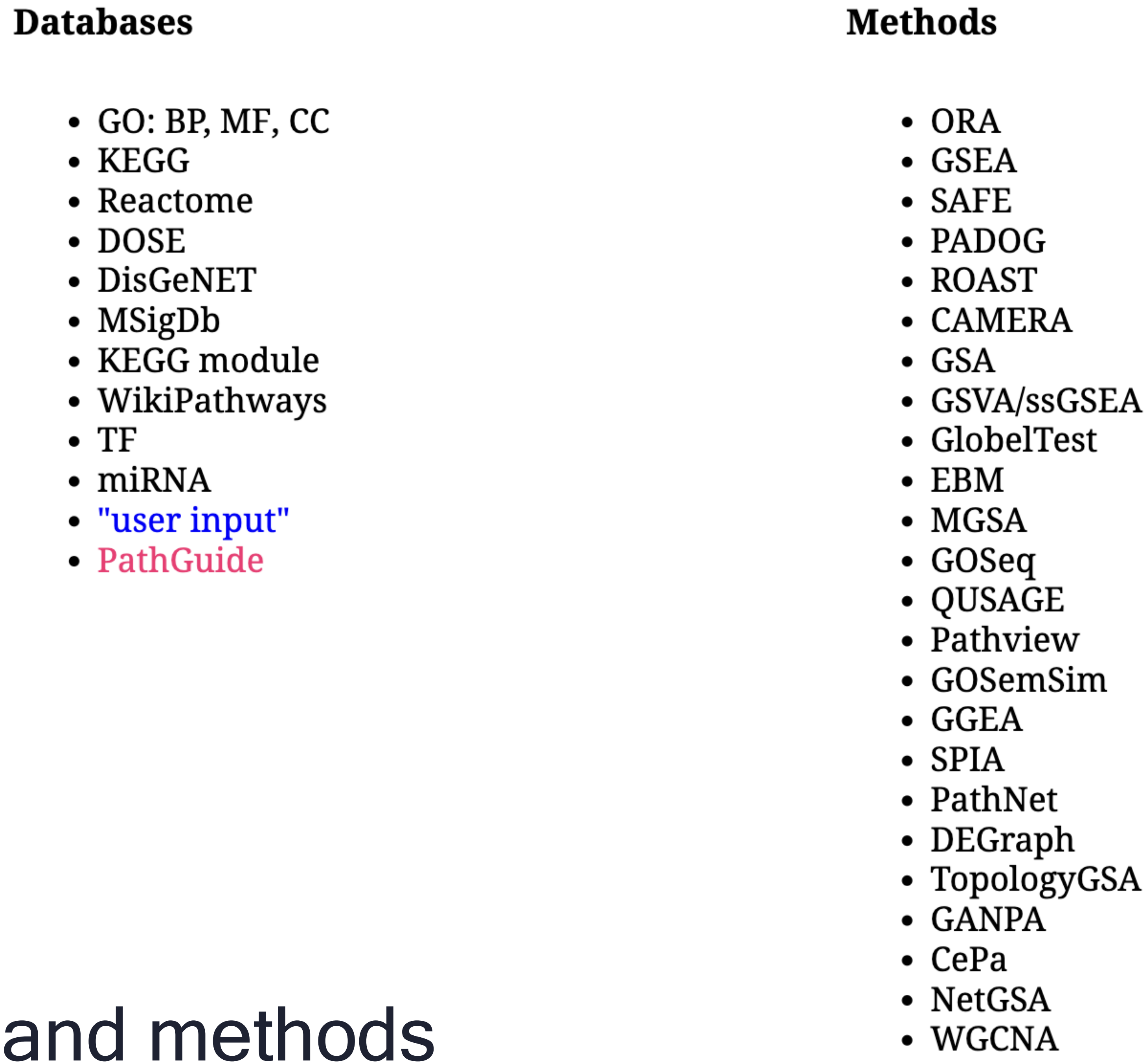


Problems with FCS

Each gene is independent of other

Each pathway is independent of each other

Databases and methods



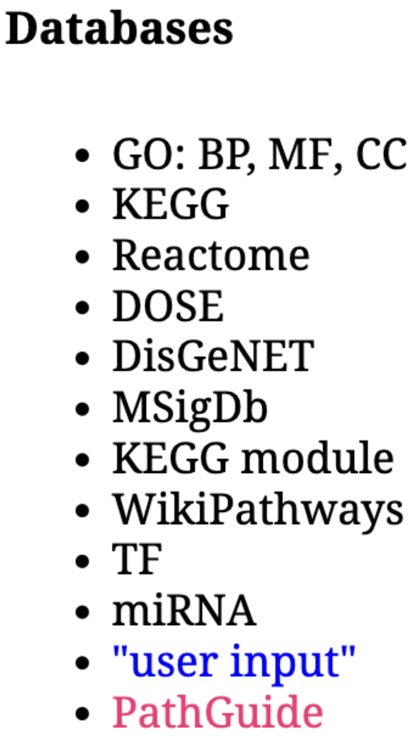
Databases

- GO: BP, MF, CC
- KEGG
- Reactome
- DOSE
- DisGeNET
- MSigDb
- KEGG module
- WikiPathways
- TF
- miRNA
- "user input"
- PathGuide

Methods

- ORA
- GSEA
- SAFE
- PADOG
- ROAST
- CAMERA
- GSA
- GSVA/ssGSEA
- GlobelTest
- EBM
- MGSA
- GOSeq
- QUSAGE
- Pathview
- GOSemSim
- GGEA
- SPIA
- PathNet
- DEGraph
- TopologyGSA
- GANPA
- CePa
- NetGSA
- WGCNA

Databases and methods



Databases

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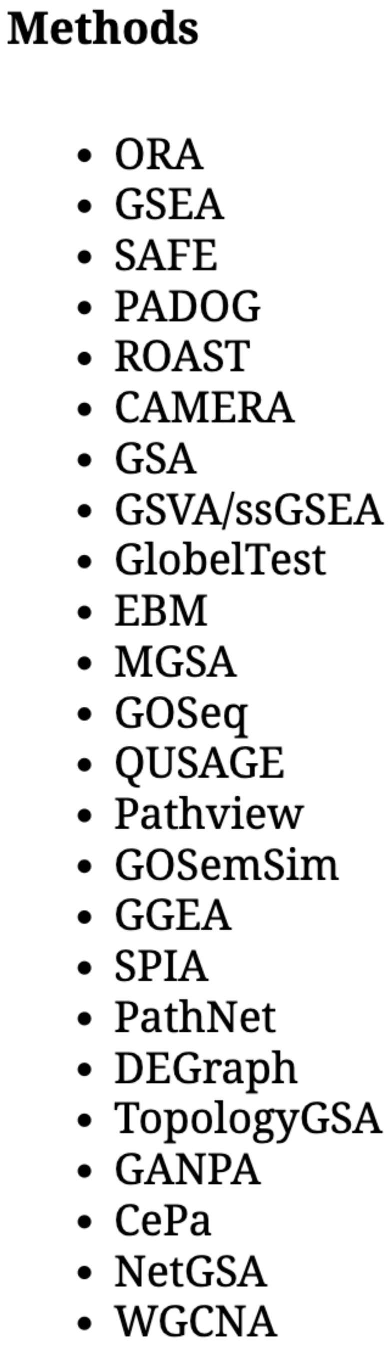

Methods

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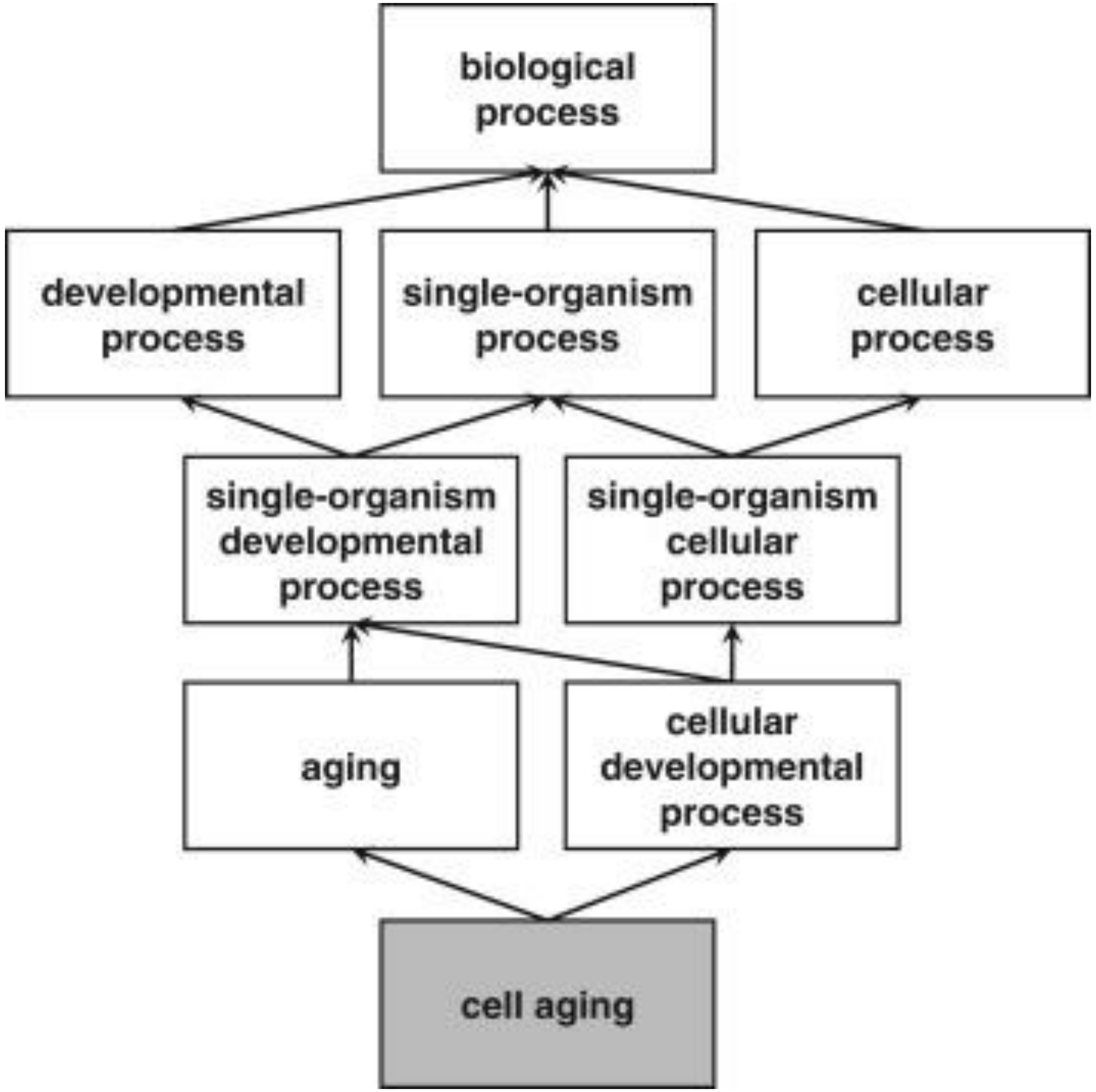
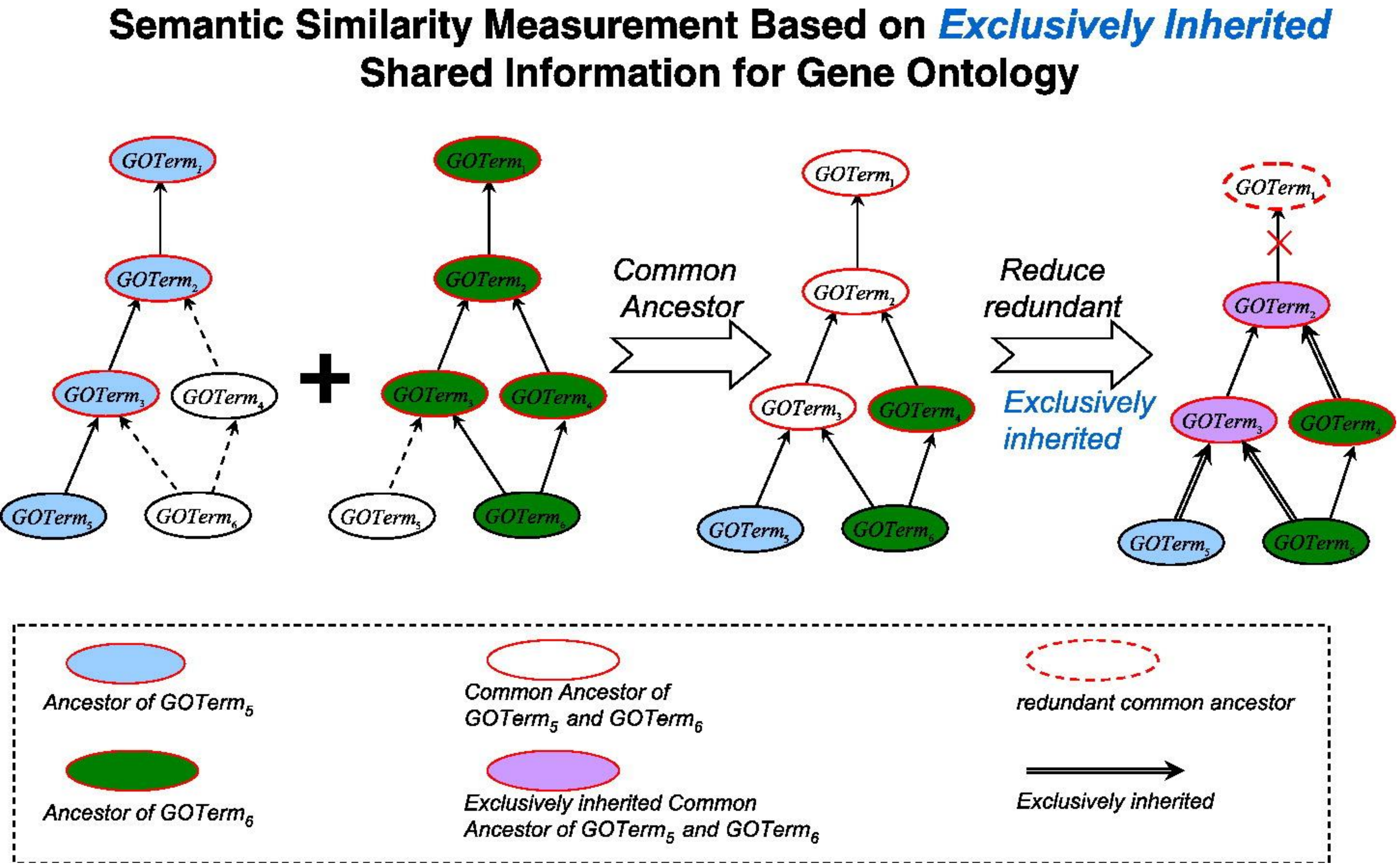
Problems with databases:

Low resolution

Gene Ontology: the world's largest source of information on the functions of genes


biological process
developmental process
single-organism process
cellular process
single-organism developmental process
single-organism cellular process
aging
cellular developmental process
cell aging

The GO contains many terms that are highly similar or overlapping in meaning (e.g., "cell cycle" and "mitosis").


Semantic Similarity Measurement Based on Exclusively Inherited Shared Information for Gene Ontology
$GOTerm_1$
$GOTerm_2$
$GOTerm_3$
$GOTerm_4$
$GOTerm_5$
$GOTerm_6$
+
Common Ancestor
Reduce redundant
Exclusively inherited
Ancestor of $GOTerm_5$
Ancestor of $GOTerm_6$
Common Ancestor of $GOTerm_5$ and $GOTerm_6$
Exclusively inherited Common Ancestor of $GOTerm_5$ and $GOTerm_6$
redundant common ancestor
Exclusively inherited

"exclusively inherited" refers to the subset of shared information that is **unique to the two terms being compared** ($GOTerm_5$ and $GOTerm_6$) and **not inherited by other unrelated terms**.

Illustration of Semantic Similarity Measurement for Gene Ontology Terms Using Exclusively Inherited Shared Information

https://www.sciencedirect.com/science/article/pii/S0378111914014887

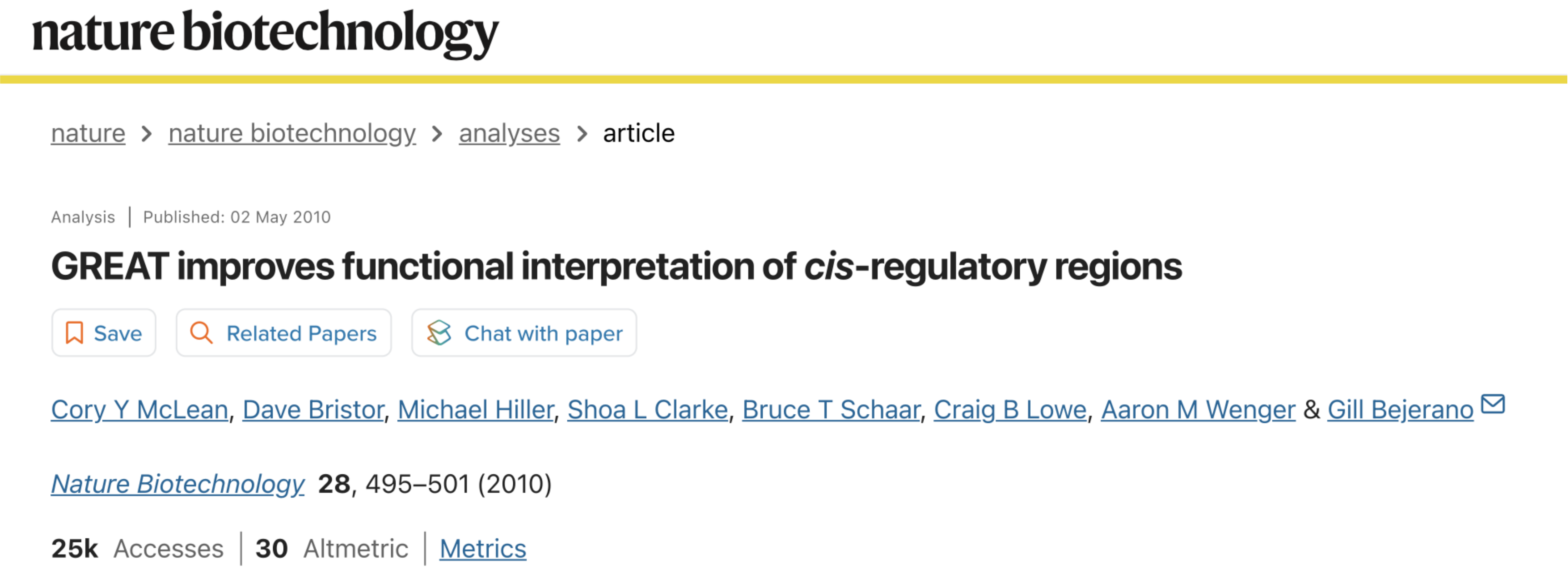
Making your own database

database_seeds

$paper1_day1
Gene1, Gene2, Gene3, Gene4

$paper2_day2
Gene3, Gene4, Gene5, Gene6

GREAT



nature biotechnology

nature › nature biotechnology › analyses › article

Analysis | Published: 02 May 2010

GREAT improves functional interpretation of *cis*-regulatory regions

Cory Y McLean, Dave Bristor, Michael Hiller, Shoa L Clarke, Bruce T Schaar, Craig B Lowe, Aaron M Wenger & Gill Bejerano

Nature Biotechnology **28**, 495–501 (2010)

25k Accesses | **30** Altmetric | Metrics

https://www.nature.com/articles/nbt.1630

GREAT

- GREAT helps determine whether these regions are linked to **gene regulation**

- **Handles distal regulatory elements:** GREAT accounts for **long-range gene regulation**, making it more effective for studying enhancers and other non-coding regions

- **Chromatin conformation capture techniques** (e.g., Hi-C, ChIA-PET) that reveal **long-range interactions** between enhancers and promoters.

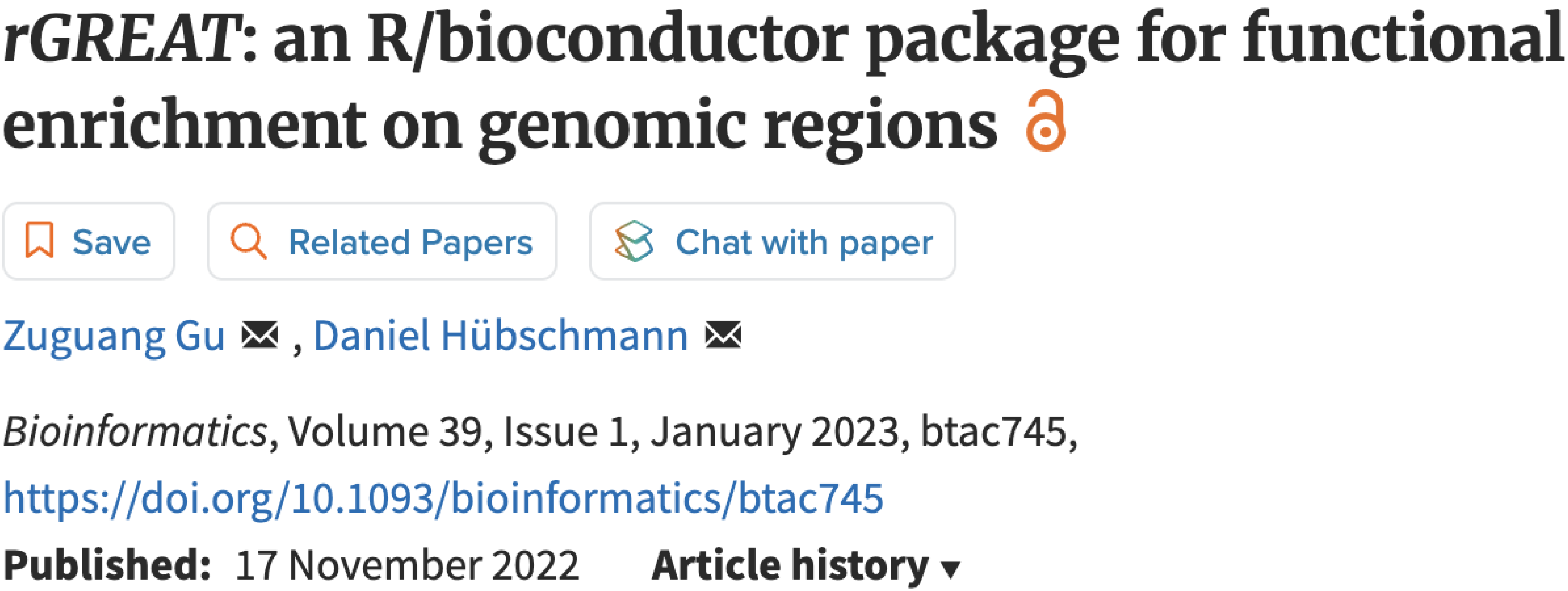
- **Epigenetic markers** such as histone modifications (e.g., H3K27ac) that indicate active regulatory regions.

GREAT

It mostly works with the mouse and human genome

Annotations are not open access

rGREAT

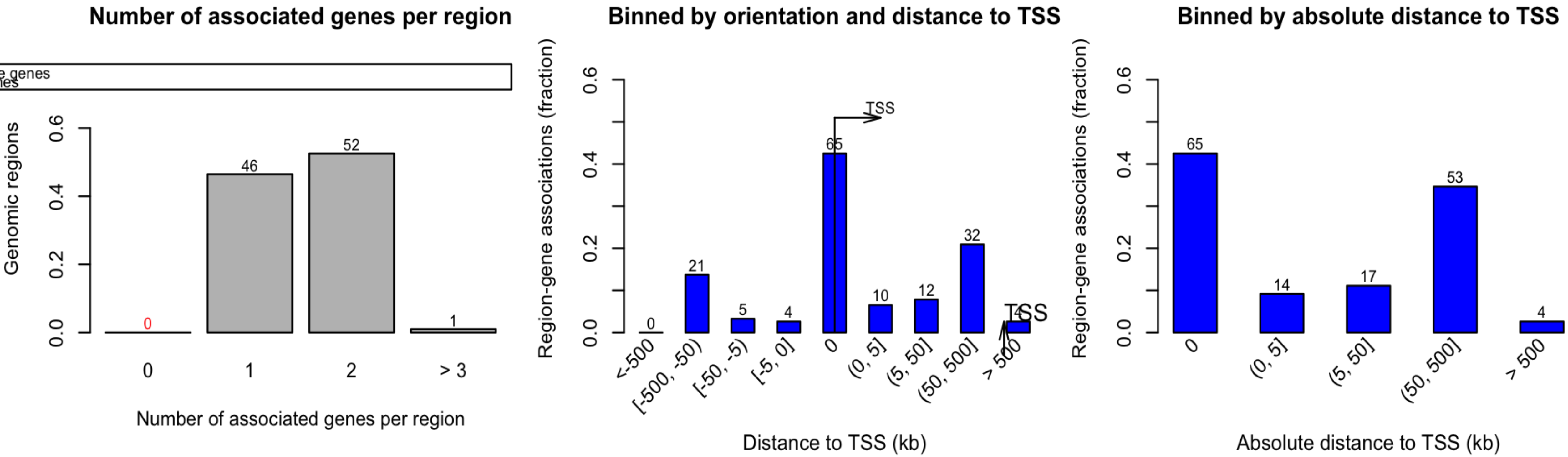


rGREAT: an R/bioconductor package for functional enrichment on genomic regions

Zuguang Gu ✉ , Daniel Hübschmann ✉

Bioinformatics, Volume 39, Issue 1, January 2023, btac745,
https://doi.org/10.1093/bioinformatics/btac745
Published: 17 November 2022 **Article history** ▾

Example plot



Summary

Three types of methods for enrichment analysis:

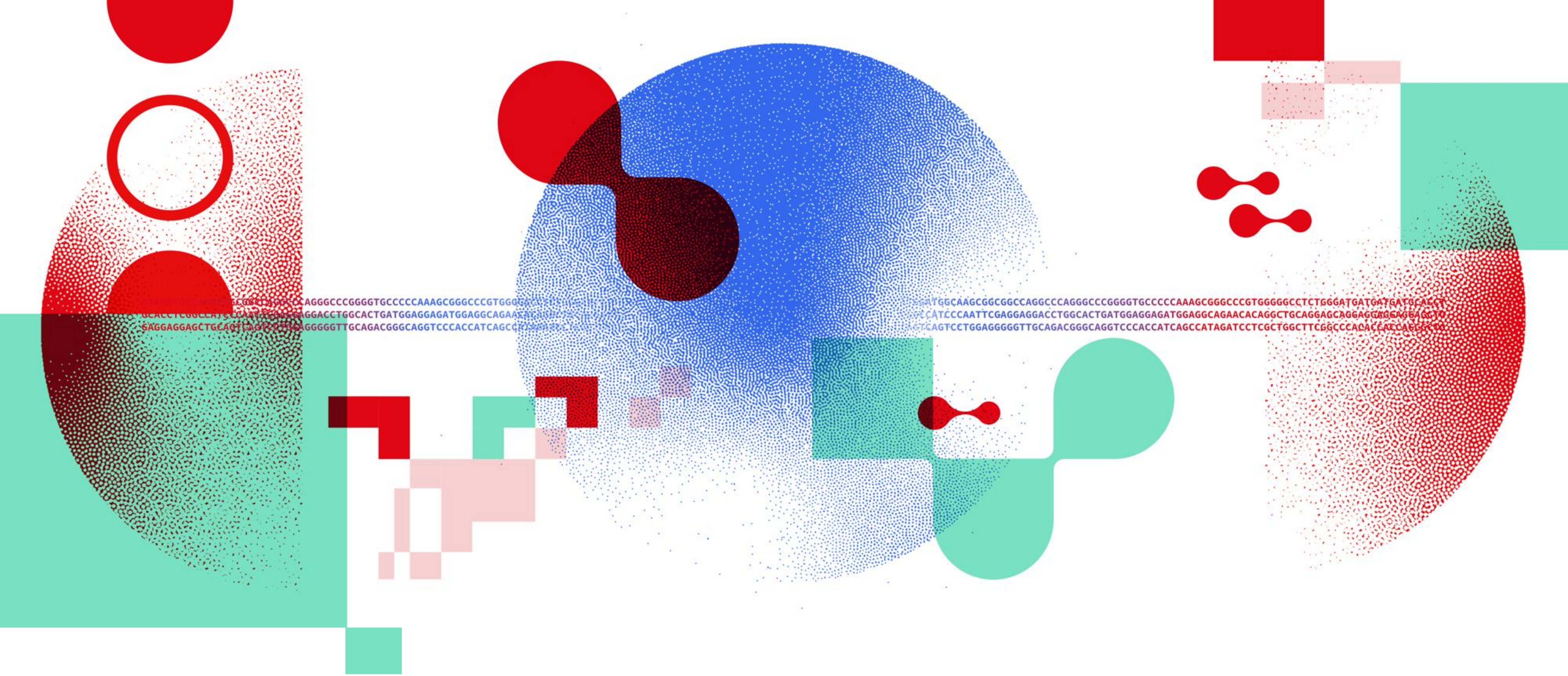
1. ORA
2. FCS
3. Pathway Topology

Databases problem

GO semantic similarity

GREAT/ rGREAT for analysis of Genomic Ranges

Exercise 7



Thank you

DATA SCIENTISTS FOR LIFE

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