

Gene ontology, gene set over-representation analyses and public databases: Peeking into functional roles of gene sets

NGS analysis for gene regulation and epigenomics

Physalia 2021

GO over-representation analyses: what

- An ontology is a **formal representation** of a **body of knowledge** **within a given domain**

GO over-representation analyses: what

- **An ontology term** primarily consists of:
 - A definition of a concept
 - A representation of this concept
 - A formal naming of this concept

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=== Example term ===

:id:	GO:0000016
:name:	lactase activity
:ontology:	molecular_function
:def:	"Catalysis of the reaction: lactose + H2O=D-glucose + D-galactose." [EC:3.2.1.108]
:synonym:	"lactase-phlorizin hydrolase activity" BROAD [EC:3.2.1.108]
:synonym:	"lactose galactohydrolase activity" EXACT [EC:3.2.1.108]
:xref:	EC:3.2.1.108
:xref:	MetaCyc:LACTASE-RXN
:xref:	Reactome:20536
:is_a:	GO:0004553 ! hydrolase activity, hydrolyzing O-glycosyl compounds

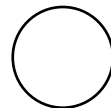
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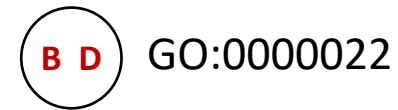
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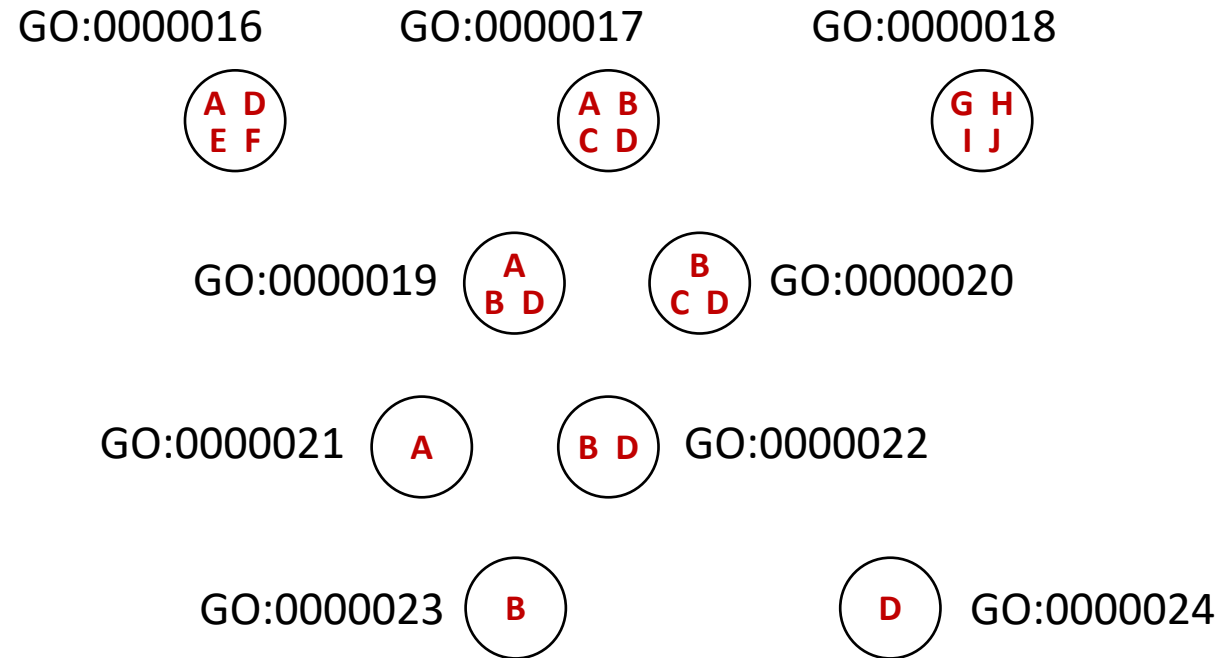
- **An ontology term** can be further enriched with additional information:
 - Elements can be annotated to individual terms



GO over-representation analyses: what

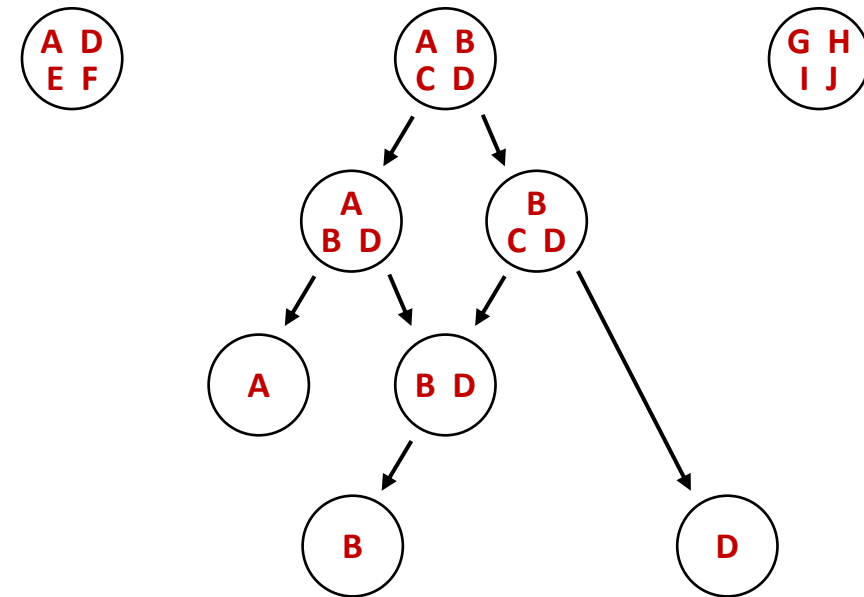
- **An ontology term** can be further enriched with additional information:

- Elements can be associated to individual terms
- Elements can be associated to multiple terms



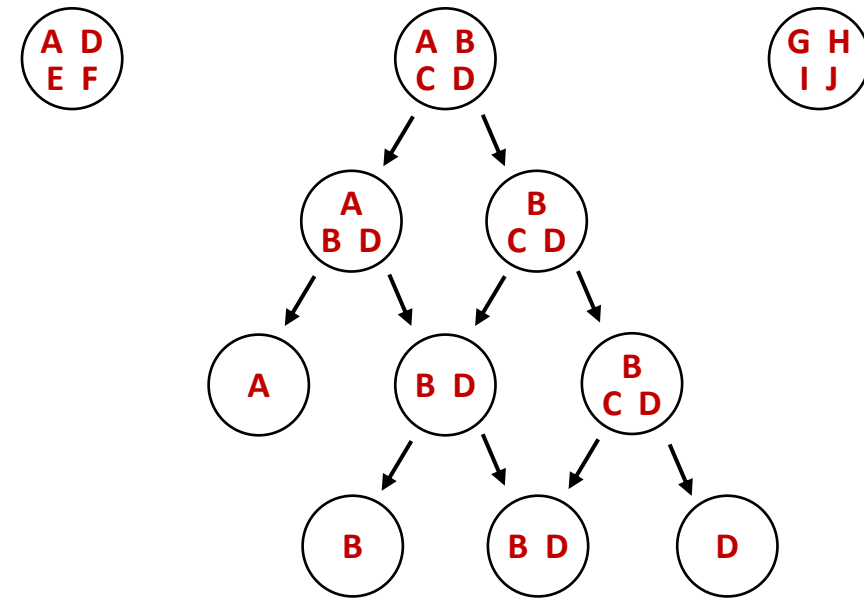
GO over-representation analyses: what

- **Ontology terms** are (loosely) hierarchically ordered in a graph structure:
 - Terms are nodes
 - Relationships between the terms are edges between the nodes



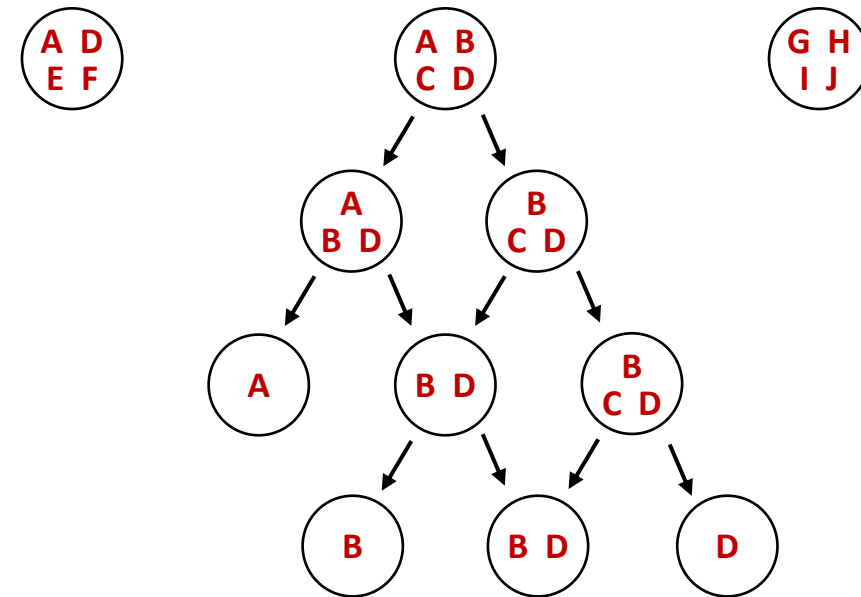
GO over-representation analyses: what

- **Ontology terms** can contain identical sets of elements



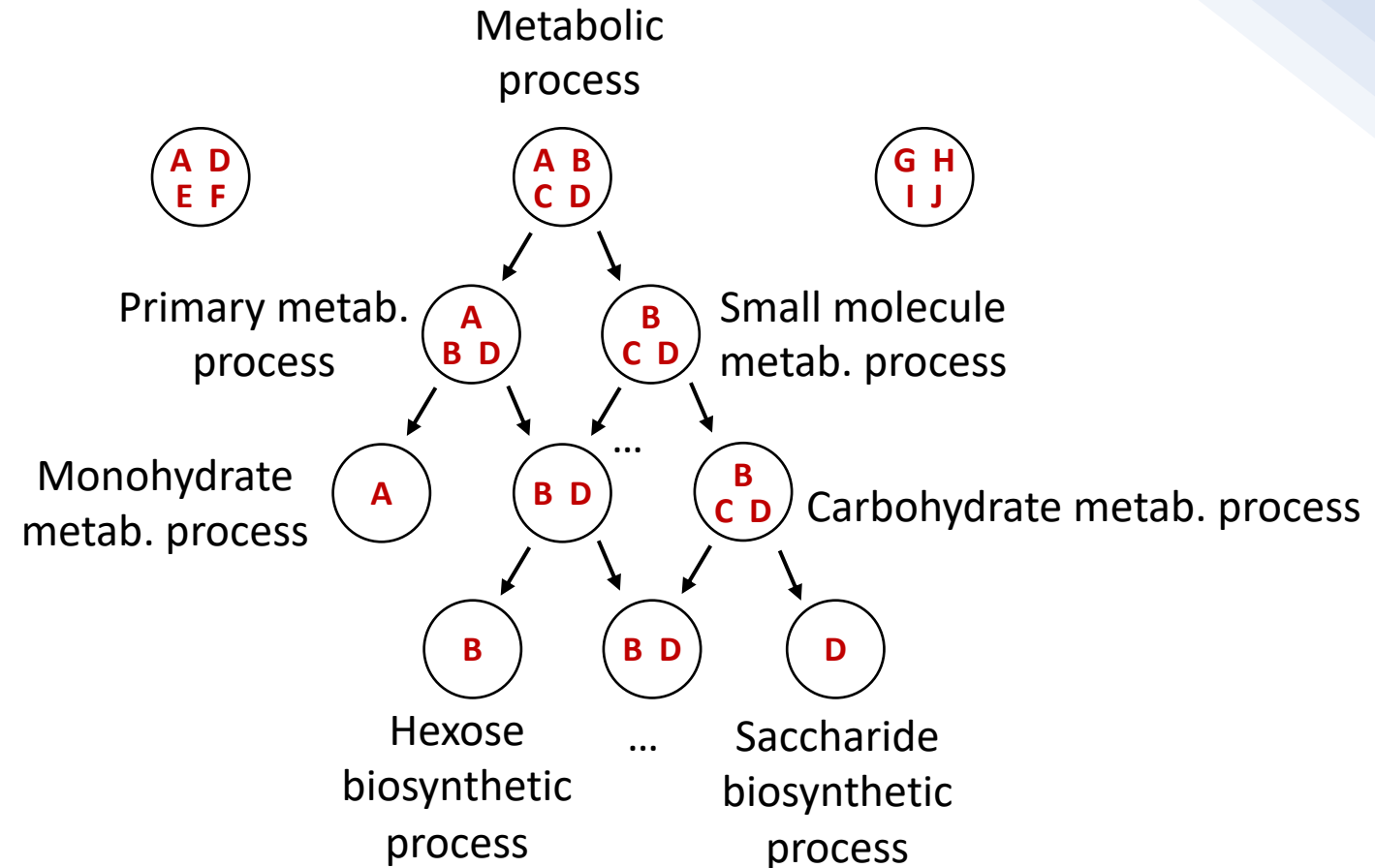
GO over-representation analyses: what

- In our case, the Gene Ontology (GO) describes the **current state** of knowledge of the three main biological domains



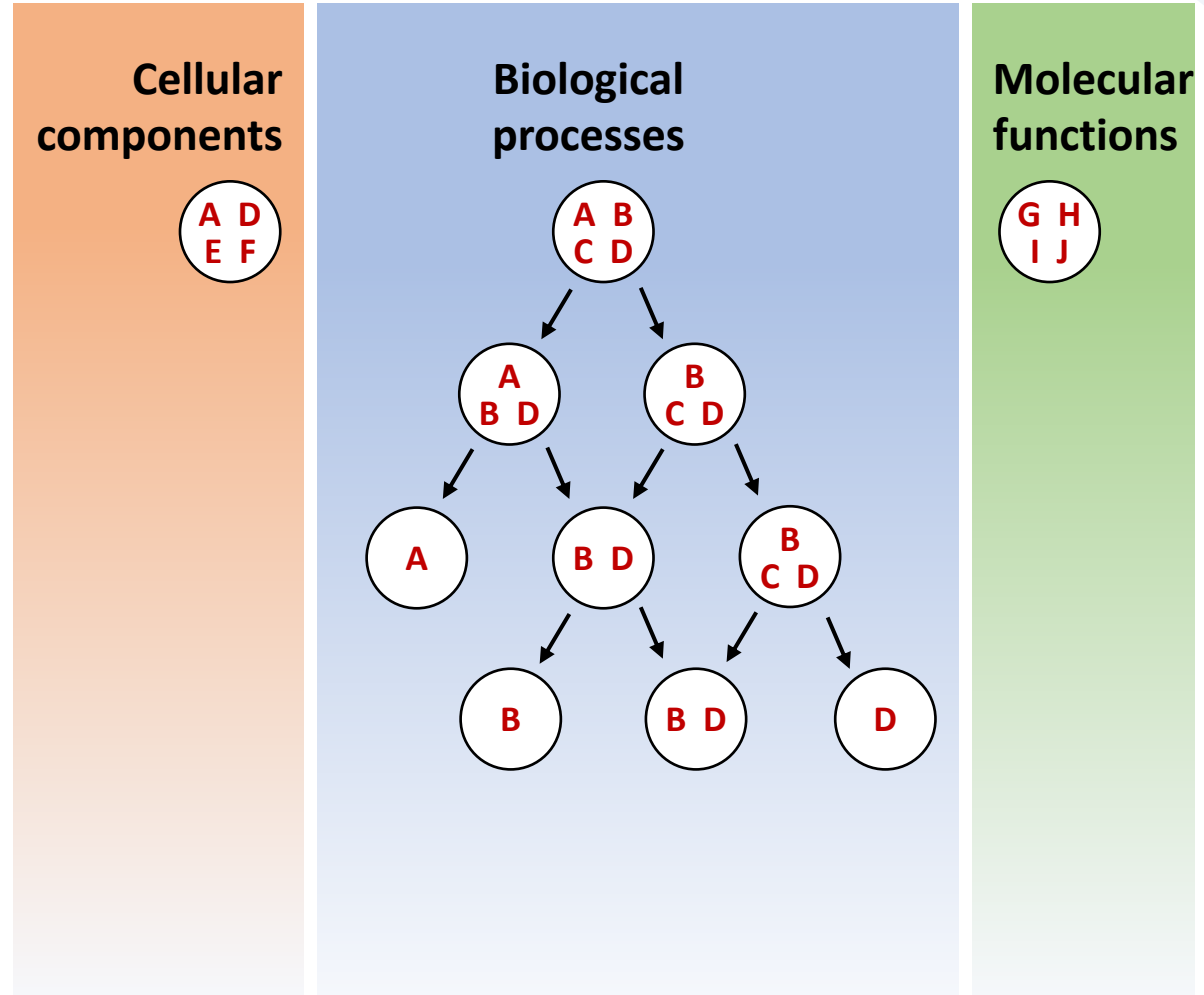
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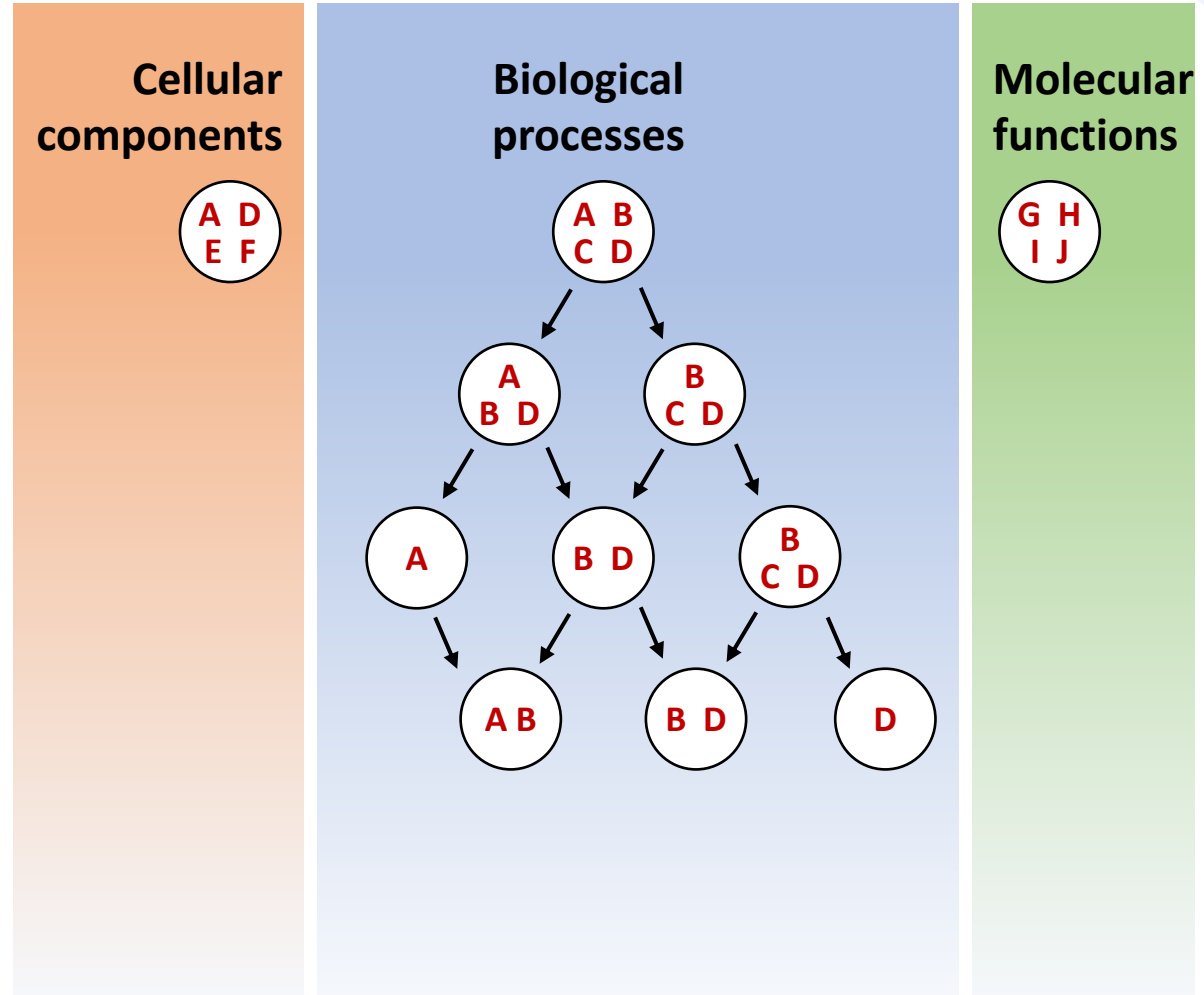
GO over-representation analyses: what

- Gene Ontology (GO) is divided in three domains
 - Biological Processes (BP)
 - Cellular Components (CC)
 - Molecular Functions (MF)



GO over-representation analyses: what

- The Gene Ontology (GO) is a dynamic, frequently updated database

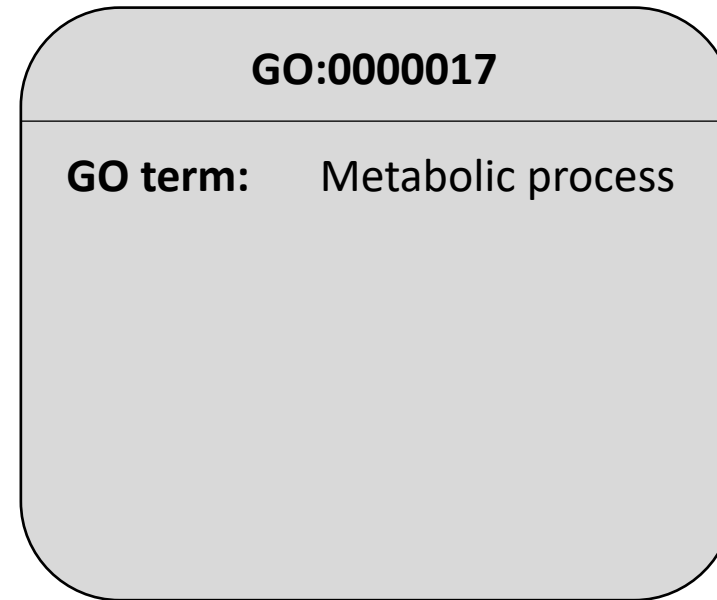
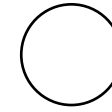


GO over-representation analyses: what

IMPORTANT:

A GO term (e.g. GO:0000017)
is different from its annotations
(i.e. the association of some
genes to this term)

GO:0000017

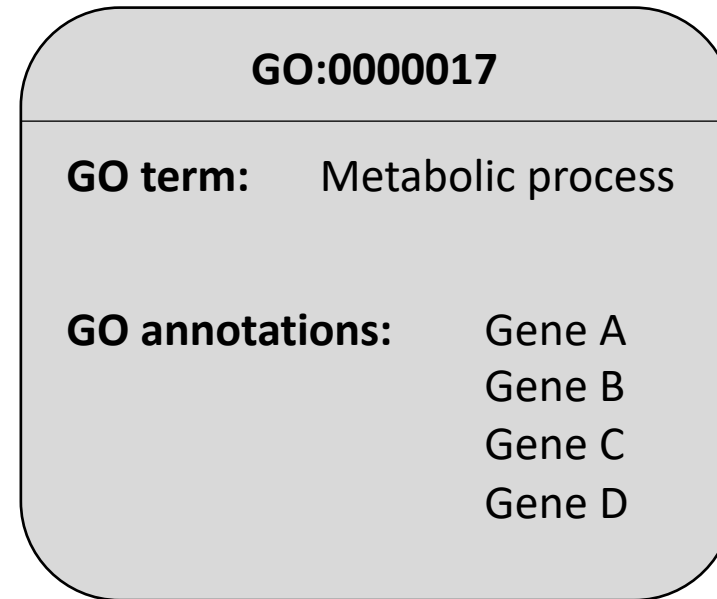


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GO over-representation analyses: what

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- External providers manage GO term annotations

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 - Mouse annotations are provided by MGI (Mouse Genome Informatics)
 - C. elegans annotations are provided by Wormbase
 - Yeast annotations are provided by SGD (Saccharomyces Genome Database)

GO over-representation analyses: what

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KNOW YOUR ORGANISM!

Official GO database

- GO Consortium is the provider of official Gene Ontology.



About the GO

Mission Statement: The mission of the GO Consortium is to develop an up-to-date, comprehensive, computational model of biological systems, from the molecular level to larger pathways, cellular and organism-level systems.

The Gene Ontology resource provides a computational representation of our current scientific knowledge about the functions of genes (or, more properly, the protein and non-coding RNA molecules produced by genes) from many different organisms, from humans to bacteria. It is widely used to support scientific research, and has been cited in tens of thousands of publications.

Official GO database

- GO Consortium is the provider of official Gene Ontology.
- Additional refined gene ontologies exist, either from GO Consortium or from independent providers, e.g. :
 - “Slim”-ed versions
 - Non-model organisms

Downloading official GO database

- Versioned database
- Easy access to entire database
- OBO format

July 2, 2018 (2020-12-08) Dataset Open Access

Gene Ontology Data Archive

 Carbon, Seth;  Mungall, Chris;

Archival bundle of GO data release.

Uploaded on December 11, 2020

July 2, 2018 (2020-11-17) Dataset Open Access

Gene Ontology Data Archive

 Carbon, Seth;  Mungall, Chris;

Archival bundle of GO data release.

Uploaded on November 20, 2020

July 2, 2018 (2020-10-09) Dataset Open Access

Gene Ontology Data Archive

 Carbon, Seth;  Mungall, Chris;

Archival bundle of GO data release.

Uploaded on October 12, 2020

July 2, 2018 (2020-09-10) Dataset Open Access

Gene Ontology Data Archive

 Carbon, Seth;  Mungall, Chris;

Archival bundle of GO data release.

Uploaded on September 16, 2020

Downloading official GO database

wget <http://purl.obolibrary.org/obo/go.obo>

- OBO format

```
1  > head -n 100 go.obo
2  format-version: 1.2
3  data-version: releases/2020-12-08
4  ontology: go
5
6  [Term]
7  id: GO:0000001
8  name: mitochondrion inheritance
9  namespace: biological_process
10 def: "The distribution of mitochondria, including the mitochondrial genome, into daughter cells after mitosis or meiosis, mediated by interactions between mitochondria and the cytoskeleton." [GOC:mcc, PMID:10873824, PMID:11389764]
11 synonym: "mitochondrial inheritance" EXACT []
12 is_a: GO:0048308 ! organelle inheritance
13 is_a: GO:0048311 ! mitochondrion distribution
14
15 [Term]
16 id: GO:0000002
17 name: mitochondrial genome maintenance
18 namespace: biological_process
19 def: "The maintenance of the structure and integrity of the mitochondrial genome; includes replication and segregation of the mitochondrial chromosome." [GOC:ai, GOC:vw]
20 is_a: GO:0007005 ! mitochondrion organization
```

Downloading GO annotations

- Also versioned

All most recent annotations for individual species available at:

<http://current.geneontology.org/annotations/>

Downloading GO annotations

```
Parent
..

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aspgd.gpad.gz
aspgd.gpi.gz
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zfin.gaf.gz
zfin.gpad.gz
zfin.gpi.gz
```


Downloading GO annotations

- Also versioned

E.g. most recent annotation for yeast (SGD is the provider):

<http://current.geneontology.org/annotations/sgd.gaf.gz>

Downloading GO annotations

- GAF format:

It's in the name: **GO Annotation Format**

Downloading GO annotations

- GAF format:

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!gaf-version: 2.1
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!Generated by GO Central
!
!Date Generated by GOC: 2020-12-09
!
!Header from source association file:
!=====
!Generated by GO Central
!
!Date Generated by GOC: 2020-12-08
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!From: Saccharomyces Genome Database (SGD)
!URL: https://www.yeastgenome.org/
!Contact Email: sgd-helpdesk@lists.stanford.edu
!Funding: NHGRI at US NIH, grant number U41-HG001315
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!Created on Mon Dec 7 11:33:04 2020.
!generated-by: PANTHER
!date-generated: 2020-12-07
!PANTHER version: v.15.0.
!GO version: 2020-11-17.
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!Documentation about this header can be found here: https://github.com/geneontology/go-site/blob/master/docs/gaf_validation.md
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SGD S000004103 HOG1 GO:0004707 PMID:10805732 IDA F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0005516 PMID:27421986 IPI UniProtKB:P06787 F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
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SGD S000004103 HOG1 GO:0045944 PMID:12743037 IDA P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
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Downloading GO annotations

- GAF format

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!Generated by GO Central
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!Date Generated by GOC: 2020-12-08
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!URL: https://www.yeastgenome.org/
!Contact Email: sgd-helpdesk@lists.stanford.edu
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DB object symbol

Peeking into functional roles of gene sets

Downloading GO annotations

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!Header from sgd source association file:
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SGD S000004103 HOG1 GO:0006972 PMID:7681220 IMP P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0007231 PMID:7681220 IMP P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0016241 PMID:16874103 IMP P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0033262 PMID:23178807 IDA P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0045944 PMID:12743037 IDA P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
...
```

GO term

Peeking into functional roles of gene sets

Downloading GO annotations

- GAF format

```
!gaf-version: 2.1
!
!Generated by GO Central
!
!Date Generated by GOC: 2020-12-09
!
!Header from source association file:
!=====
!Generated by GO Central
!
!Date Generated by GOC: 2020-12-08
!
!Header from sgd source association file:
!=====
!Date: 20201207
!From: Saccharomyces Genome Database (SGD)
!URL: https://www.yeastgenome.org/
!Contact Email: sgd-helpdesk@lists.stanford.edu
!Funding: NHGRI at US NIH, grant number U41-HG001315
!
!=====
!
!Header copied from paint_sgd_valid.gaf
!=====
!Created on Mon Dec 7 11:33:04 2020.
!generated-by: PANTHER
!date-generated: 2020-12-07
!PANTHER version: v.15.0.
!GO version: 2020-11-17.
!
!=====
!
!Documentation about this header can be found here: https://github.com/geneontology/go-site/blob/master/docs/gaf_validation.md
!
...
SGD S000004103 HOG1 GO:0003682 PMID:124308369 IDA F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0004707 PMID:10805732 IDA F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0005516 PMID:27421986 IPI UniProtKB:P06787 F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006468 PMID:10805732 IDA P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006468 PMID:12743037 IDA P Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
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Ref. for evidence

Peeking into functional roles of gene sets

Downloading GO annotations

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!
!Header from sgf source association file:
!=====
!Date: 20201207
!From: Saccharomyces Genome Database (SGD)
!URL: https://www.yeastgenome.org/
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...
SGD S000004103 HOG1 GO:0003682 PMID:2450831 IDA Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0004707 PMID:1080573 IDA Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0005516 PMID:2742198 IPI UniProtKB:P06787 F Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006468 PMID:1080573 IDA Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006468 PMID:1274303 IDA Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006468 PMID:2317880 IDA Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
SGD S000004103 HOG1 GO:0006972 PMID:7681220 IMP Mitogen-activated protein kinase involved in osmoregulation YLR113W|SSK3|mitogen-activated protein kinase HOG1
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Type of evidence

Peeking into functional roles of gene sets

GO over-representation analyses: when

- When you have a **defined** set of tens to hundreds of genes

GO over-representation analyses: when

- When you have a **defined** set of tens to hundreds of genes
 - Genes significantly over-expressed (e.g. fold-change > 2 and adj. p-value < 0.01) in one condition vs a control
 - Genes whose promoter is bound by a specific combination of transcription factors

GO over-representation analyses: when

- When you have a defined set of tens to hundreds of genes
- Thousands are probably too many genes...

GO over-representation analyses: why

Essentially, to know whether GO terms are over-enriched in a specific list of genes

- To get an idea of the functional/structural role of your set of genes
- To bring a piece of evidence that your treatment triggers some BP/MF/CC
- To know how much of the genes involved in a specific BP/MF/CC are present in your set of interest.

GO over-representation analyses: how

- Finding over-represented GO terms in a given set of genes is one of the most common tasks in genomics.

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- It usually relies on a straightforward Fisher test:

for all the genes annotated in an ontology (e.g. all the genes annotated within the Biological Processes namespace), it tests the independence between:

→ These genes belonging to a gene set of interest (e.g. over-expressed genes)

And

→ These genes being annotated to a GO term (e.g. genes annotated to the GO:0000017 term)

GO over-representation analyses: how

- Finding over-represented GO terms in a given set of genes is one of the most common tasks in genomics.
- It usually relies on a straightforward Fisher test
- Think about it in terms of contingency tables

GO over-representation analyses: how

UNIVERSE = All Yeast genes annotated in the Biological Processes (5067 genes)	Genes over-expressed <i>in an assay</i> (152)	Genes over-expressed <i>in an assay</i>
Genes annotated <i>in GO:0006836</i> (243)	89	154
Genes annotated <i>in GO:0006836</i>	63	$5067 - 89 - 154 - 63$

→ Sum = number
of genes in
GO:0006836 (243)



↓
Sum = number of
genes over-expressed
in an assay (152)



Total sum = number
of genes in BP
(5067)

GO over-representation analyses: how

UNIVERSE = All Yeast genes annotated in the Biological Processes (5067 genes)	Genes over-expressed <i>in an assay</i> (152)	Genes over-expressed <i>in an assay</i>
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➔ Now repeat that for the 44,945 GO terms in the GO database.....

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- ➔ Now repeat that for the 44,945 GO terms in the GO database.....
- ➔ AND DON'T FORGET TO CORRECT FOR MULTIPLE TESTING
(because testing 44,945 times is multiple testing...)

GO over-representation analyses: how

- Fortunately, there are many tools already out there to efficiently perform these calculations
- Some web-based, some with programmatic access
- They function with a range of “autonomy”. Some need you to download the GO database, the GO annotations, or are doing all the work for you in the background

Programs to run GO over-representation analyses: gProfiler

g:Profiler

[News](#)[Archives](#)[Beta](#)[API](#)[R client](#)[FAQ](#)[Docs](#)[Contact](#)[Cite g:Profiler](#)[Services using g:P](#)[List of organisms](#)

g:Profiler has been updated with new data from Ensembl.

[Show more...](#)[Close](#)

g:GOST

Functional profiling

g:Convert

Gene ID conversion

g:Orth

Orthology search

g:SNPense

SNP id to gene name

Query

Upload query

Upload bed file

Input is whitespace-separated list of genes ?

Run query

random example

mixed query example

Options

Organism: ?

Homo sapiens (Human)

☐ Ordered query ?

☐ Run as multiquery ?

Advanced options ▼

Data sources ▼

Bring your data (Custom GMT) ▼

2020/01/13

Jacques Serizay

Peeking into functional roles of gene sets

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Programs to run GO over-representation analyses: gProfiler

- Also available in R!
- Simple, but many optional parameters to optimize your search

```
gprofiler2::gost(  
  geneList,  
  organism = 'scerevisiae'  
)
```

Staying up-to-date...



✧ Ontologies

- [PANTHER™ GO slim](#) (version 16.0, based on GO release 2020-11-16, released 2020-12-01)
 - 3336 total terms
 - 2235 biological process terms
 - 543 cellular component terms
 - 558 molecular function terms
- PANTHER™ Protein Class (version 16.0, released 2020-12-01)
 - 210 total terms
- [Gene Ontology](#) (from GO database released 2020-10-09, DOI: 10.5281/zenodo.4081749)
 - 47308 total terms
 - 12103 molecular function terms
 - 30816 biological process terms
 - 4389 cellular component terms

Staying up-to-date...

g:Profiler

NewsArchivesBetaAPIR clientFAQDocsContactCite g:ProfilerServices using g:PList of organisms

g:GOSt

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g:Profiler has been updated with new data from Ensembl.

The database versions in this release are:

- Ensembl 101
- Ensembl Genomes 48
- Wormbase ParaSite 14

Changes in g:GOSt

There are now evidence codes available for Human Protein Atlas (HPA) results.

There is now an option to exclude evidence codes from query results. When the "No evidence codes" option is chosen, the gene-term matrix will not be shown and for large queries, the results will be somewhat faster.

Published on Tue Oct 20 2020

g:Profiler has been updated with new data from Ensembl.

The database versions in this release are:

- Ensembl 100
- Ensembl Genomes 47
- Wormbase ParaSite 14

Published on Wed Jul 22 2020

g:Profiler has been updated with new data from Ensembl.

The database versions in this release are:

- Ensembl 99
- Ensembl Genomes 46
- Wormbase ParaSite 14

There have been many minor UI improvements and small feature enhancements. One of the larger updates is the overhaul of our g:GOSt multiquery interface. Comparing the enrichment of two or more gene lists should be easier and less cluttered.

There is a new API endpoint for accessing the metadata about available species. It provides the names, ID-s and available namespaces for all our organisms. The endpoint is documented on our [API page \(https://biit.cs.ut.ee/gprofiler/page/apis\)](https://biit.cs.ut.ee/gprofiler/page/apis).

It's now possible to export high-DPI (300 or 600) images of your results for use in presentations or publications.

We have updated the documentation of our [r client \(https://biit.cs.ut.ee/gprofiler_beta/page/r\)](https://biit.cs.ut.ee/gprofiler_beta/page/r) and created an instructional vignette (<https://cran.r-project.org/web/packages/gprofiler2/vignettes/gprofiler2.html>).

Published on Mon Mar 09 2020

2020/01/13

Jacques Serizay



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Staying up-to-date...

GREAT predicts functions of cis-regulatory regions.

Many coding genes are well annotated with their biological functions. Non-coding regions typically lack such annotation. GREAT assigns biological meaning to a set of non-coding genomic regions by analyzing the annotations of the nearby genes. Thus, it is particularly useful in studying cis functions of sets of non-coding genomic regions. Cis-regulatory regions can be identified via both experimental methods (e.g. [ChIP-seq](#)) and by computational methods (e.g. [comparative genomics](#)). For more see our [Nature Biotech Paper](#).

News

-  Aug. 19, 2019: GREAT version 4 [adds support for human hg38 assembly and updates ontology datasets for all supported assemblies](#).
-  Sep. 8, 2018: GREAT has served over 1 million job submissions.
- Oct. 23, 2017: GREAT is moved to a VM to eliminate proxy errors.
- June 22, 2017: GREAT hardware upgrade to meet increasing submission volume.
- Nov. 16, 2015: The [GREAT user help forums](#) are frozen.
- Feb. 15, 2015: GREAT version 3 [switches to Ensembl genes, adds support for zebrafish danRer7 and mouse mm10 assemblies, and adds new ontologies](#).
- Apr. 3, 2012: GREAT version 2 [adds new annotations to human and mouse ontologies and visualization tools for data exploration](#).
- Feb. 18, 2012: The [GREAT user help forums](#) are opened.
- May 2, 2010: GREAT version 1 is launched, concurrent to [Nature Biotechnology publication](#) (reprint, Faculty of 1000 "Must Read"). [How to Cite GREAT?](#)

[More news items...](#)

Species Assembly

- ☐ Human: GRCh38 ([UCSC hg38, Dec. 2013](#))
- ☐ Human: GRCh37 ([UCSC hg19, Feb. 2009](#))
- ☐ Mouse: GRCm38 ([UCSC mm10, Dec. 2011](#))
- ☐ Mouse: NCBI build 37 ([UCSC mm9, Jul. 2007](#))

[Can I use a different species or assembly?](#)

Staying up-to-date...

The DAVID Knowledgebase (DAVID 6.8, Current version available at <https://david.ncifcrf.gov>)

Main Annotation Sources

Data Sources	Release / Download Date	DAVID Update Date
ENSEMBL	Mar 2016	May 2016
ENTREZ	May 2016	May 2016
UNIPROT	May 2016	May 2016

Secondary Sources

Data Sources	Release / Download Date	DAVID Update Date
AFFYMETRIX	Jun 2015	May 2016
AGILENT	Dec 2013	May 2016
BBID	Sep 2009	May 2016
BIOCARTA	Nov 2014	May 2016
CGAP_EST_QUARTILE	Oct 2006	May 2016
CGAP_SAGE_QUARTILE	Oct 2006	May 2016
COG_ONTOLOGY	Sep 2009	May 2016
GENE ONTOLOGY	Apr 2016	May 2016
GNF_U133A_QUARTILE	Oct 2006	May 2016
KEGG	Dec 2015	May 2016
UCSC_TFBS	Sep 2009	May 2016
UP_SEQ_FEATURE	Sep 2009	May 2016
UP_TISSUE	Sep 2009	May 2016
ZFIN_ANATOMY	Sep 2009	May 2016

Staying up-to-date...

GOrilla News

March. 8th 2013

- Added option to supply an e-mail address to which a link to the results will be sent.

December 3rd 2012

- Try our new tool [miTEA](#) for miRNA target enrichment analysis

October. 29th 2012

- Added option to supply a name for the analysis which will appear in the results page

May. 28th 2012

- A false discovery rate (FDR) column added to the results table
- Maximum input size increased to 1MB

Dec. 29th 2010

- GOrilla has been moved to a new and faster server

Aug. 30th 2010

- The GOrilla GO database is now automatically updated weekly
- The analysis results can now be exported to [REViGO](#) for further visualization

Feb. 7th 2010

- You can now run all 3 GO ontologies (Process, Function and Cellular component) in a single run.
(We thank Ben Gordon for the idea)
- GO and gene files were updated

Oct. 15th 2009

- GOrilla now supports Danio rerio (Zebrafish)
- GO and gene files were updated

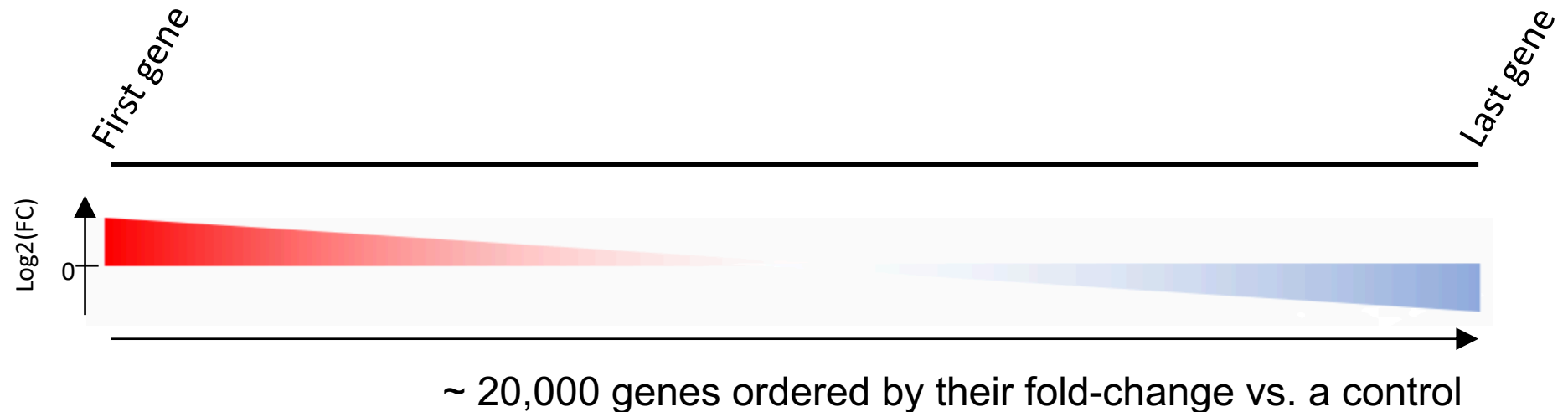
PEERING INTO FUNCTIONAL ROLES OF GENE SETS

What if I don't have a gene set of interest?

- Sometimes, you cannot really decide what is significant or not
- You don't like the idea of taking the top 100 genes differently expressed genes
- How to set a threshold for your genes? $FC > 2$? $FC > 5$? ???

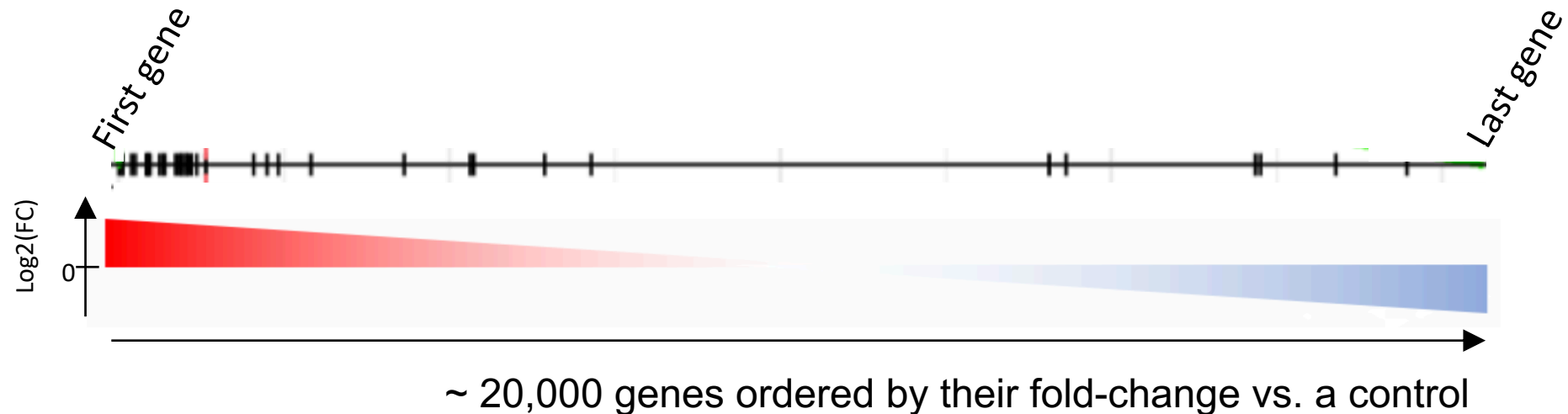
Gene Set Enrichment Analysis: cutoff-free functional analysis of ranked lists of genes

- GSEA (Gene Set Enrichment Analysis) uses a ranked list of genes as input



Gene Set Enrichment Analysis: cutoff-free functional analysis of ranked lists of genes

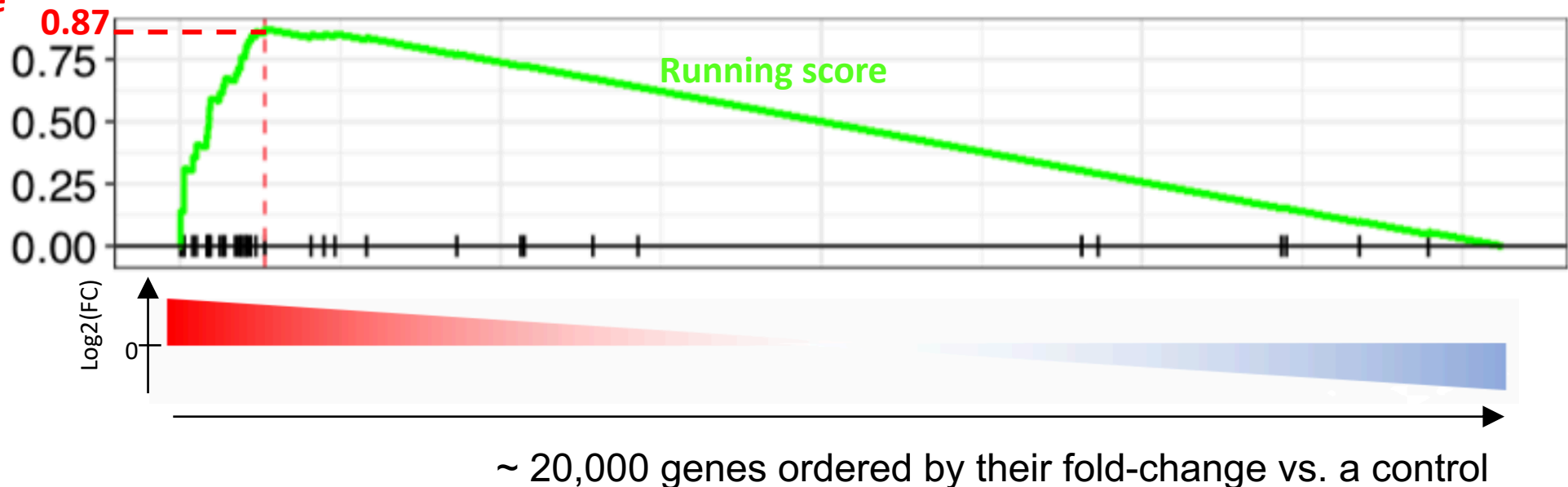
- Within this list, it flags the genes belonging to a gene set (e.g. genes annotated in “centriole assembly” GO term)



Gene Set Enrichment Analysis: cutoff-free functional analysis of ranked lists of genes

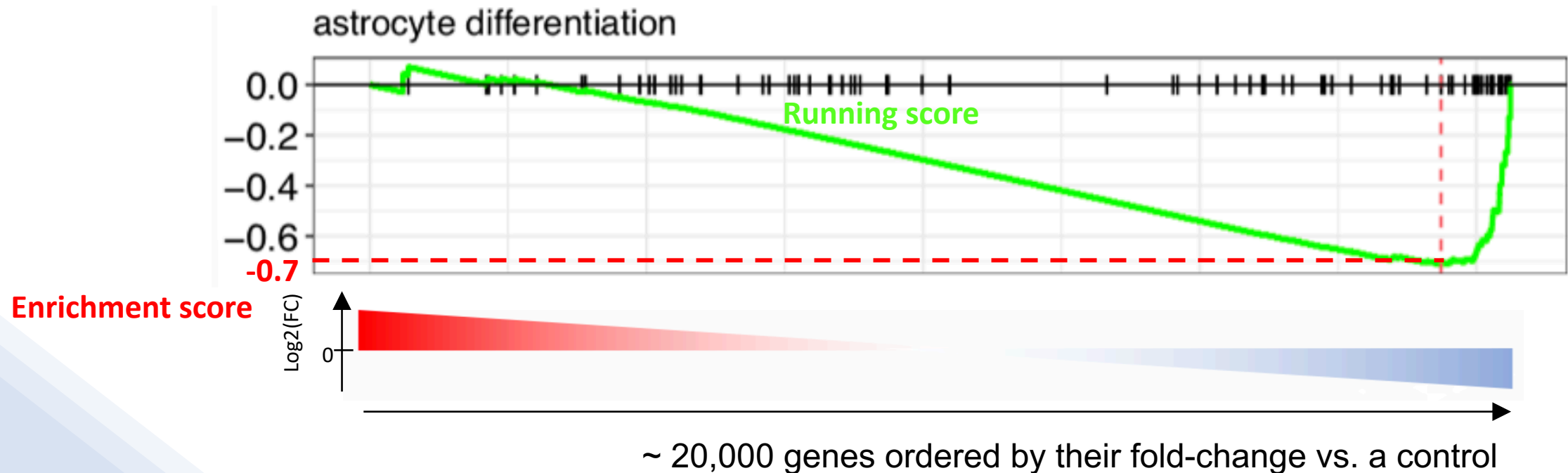
- Based on the distribution of the flagged genes, it computes a “running score” and an “enrichment score”

Enrichment score



Gene Set Enrichment Analysis: cutoff-free functional analysis of ranked lists of genes

- It can also find negative enrichment scores (indicated a depletion of genes of interest in the top of a ranked list)



How to perform GSEA?

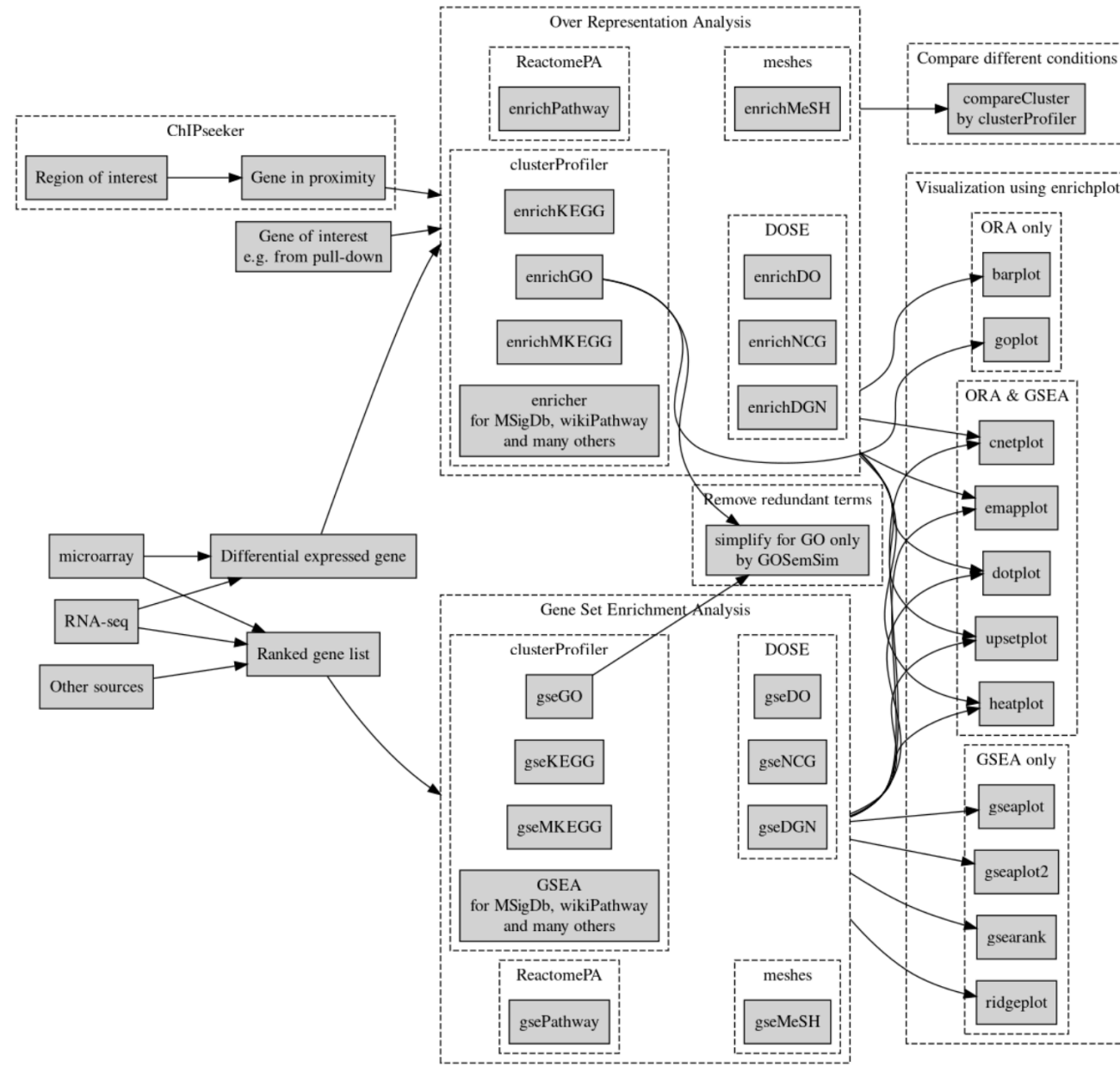
- Original software: in JAVA
 - I never managed to use it...

How to perform GSEA?

- Original software: GUI in JAVA
 - I never managed to use it...
- Since then: many programmatic implementations notably in R
 - clusterProfiler is my personal favorite
 - Based on fgsea, the original GSEA implementation in R
 - Very complete and extensive doc
 - Nice visualization outputs
 - Well-integrated with GO ecosystem and other databases (disease ontology, Reactome, ...)

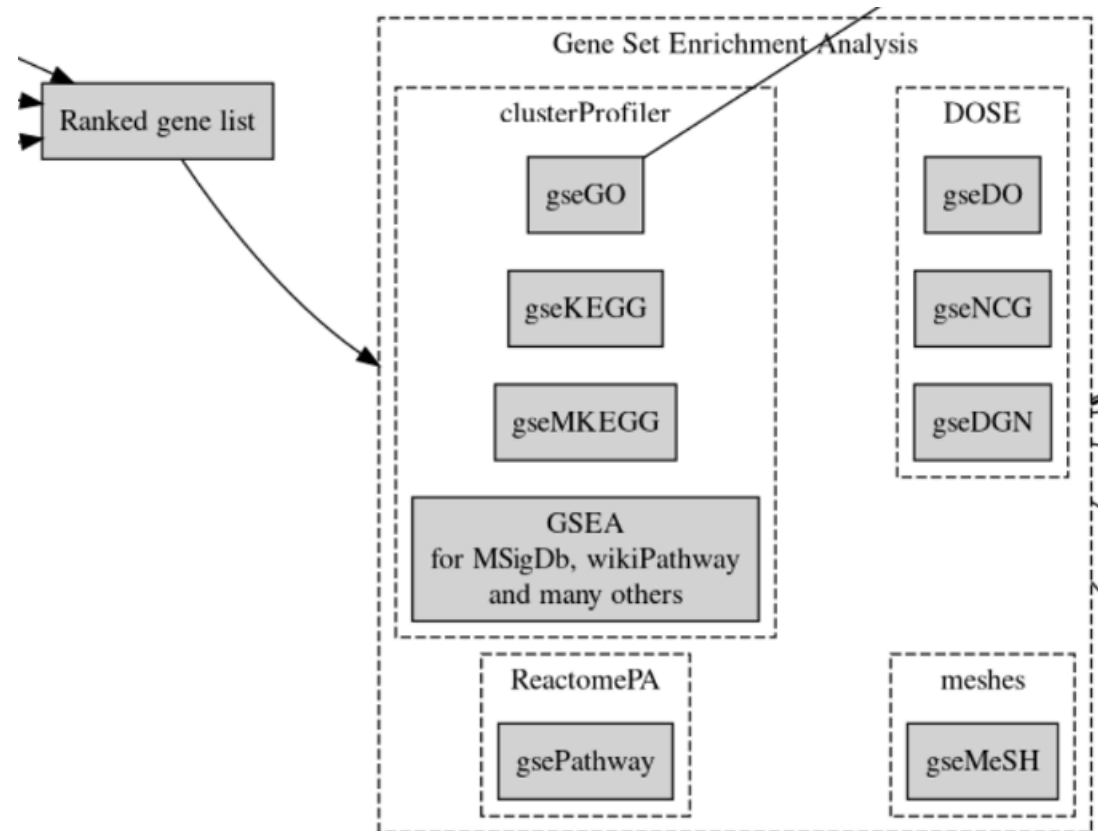
clusterProfiler

- clusterProfiler is a rich set of tools to assess and visualize enrichment of a set of genes of interest compared to different databases



clusterProfiler

- clusterProfiler provides multiple `gse*()` functions, based on the type of gene sets you want to use



clusterProfiler

- clusterProfiler provides multiple gse*() functions, based on the type of gene sets you want to use
- gseGO() compares your ranked list of genes to the reference up-to-date GO annotations

```
clusterProfiler::gseGO(  
  rankedGeneLit,  
  keyType = "ENSEMBL",  
  OrgDb = org.Sc.sgd.db::org.Sc.sgd.db  
)
```

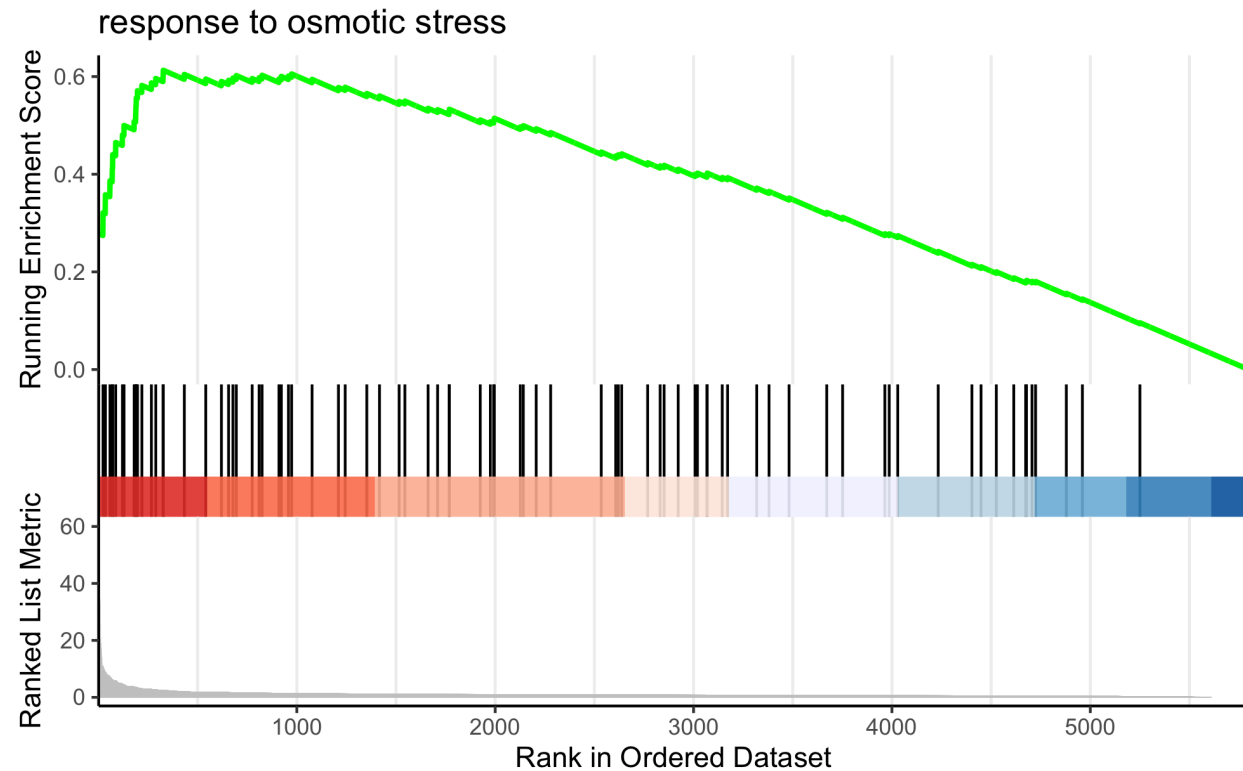
clusterProfiler

- clusterProfiler provides plotting functions to check the results for a given GO term

```
TERM <- "response to osmotic stress"
enrichplot::gseaplot2(
  gsea_results,
  title = TERM,
  geneSetID = which(gsea_results@result$Description == TERM)
)
```

clusterProfiler

- clusterProfiler provides plotting functions to check the results for a given GO term



Peeking into functional roles of gene sets

Resources

- *Ten Quick Tips for Using the Gene Ontology*, Blake PLoS Comp. Biol. 2013

Tip 1: Know the Source of the GO Annotations You Use

- *clusterProfiler: universal enrichment tool for functional and comparative study*, Guangchuang Yu (<http://yulab-smu.top/clusterProfiler-book/>)