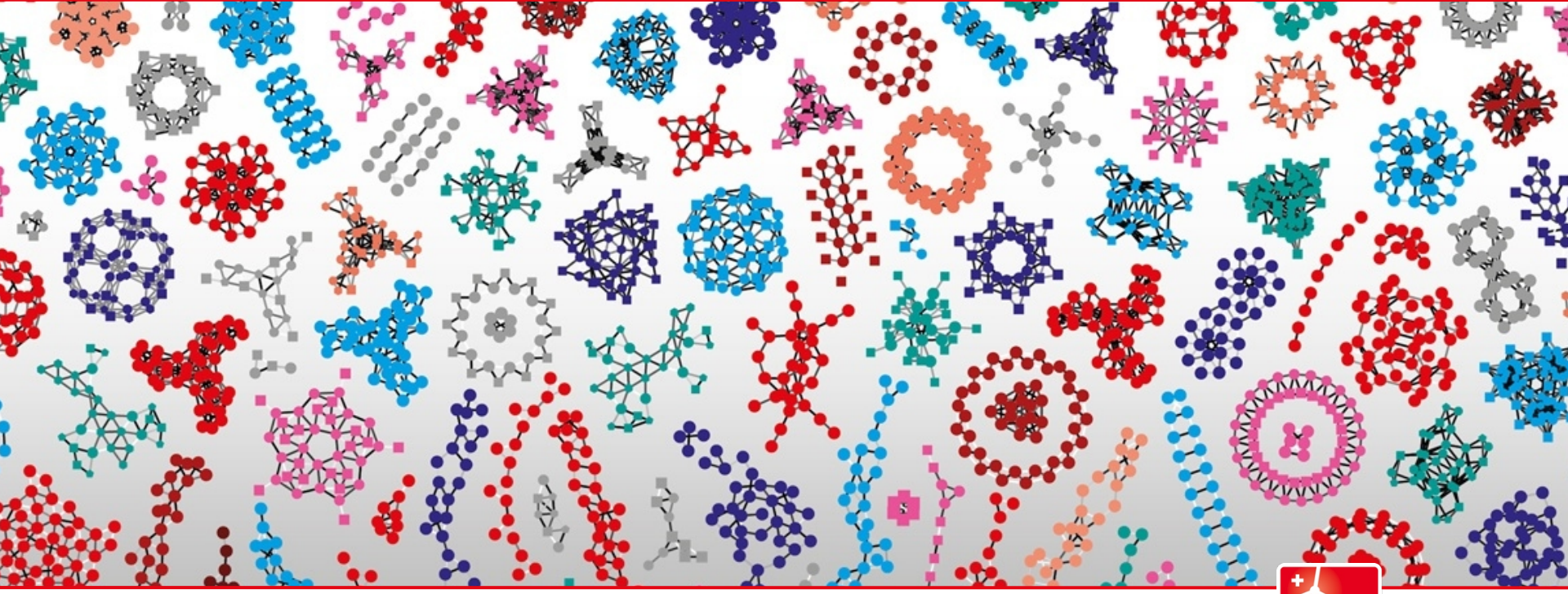

Swiss Institute of Bioinformatics

Introduction to RNA-Seq – Enrichment analysis

Wandrille Duchemin

Enrichment analysis

- **What to do with your list of differentially expressed gene ?**
- **Interpretation can be difficult, especially when many genes are DE**

Enrichment analysis

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DE genes 

ENRICHED

Biological function

Pathway

TF

...

Enrichment analysis

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DE genes

mappin g

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...

Enrichment analysis

- **What to do with your list of differentially expressed gene ?**
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DE genes 

ENRICHED

Biological function

Pathway

TF

...

Enrichment analysis - mapping

- **The goal is to regroup certain genes together in meaningful sets**
- **Genes involved in the same pathway (eg. DNA repair)**
- **Genes located in the same biological compartment**
- **Genes with a similar molecular function**
- **Genes regulated by the same transcription factor**
- **…**

Enrichment analysis - mapping

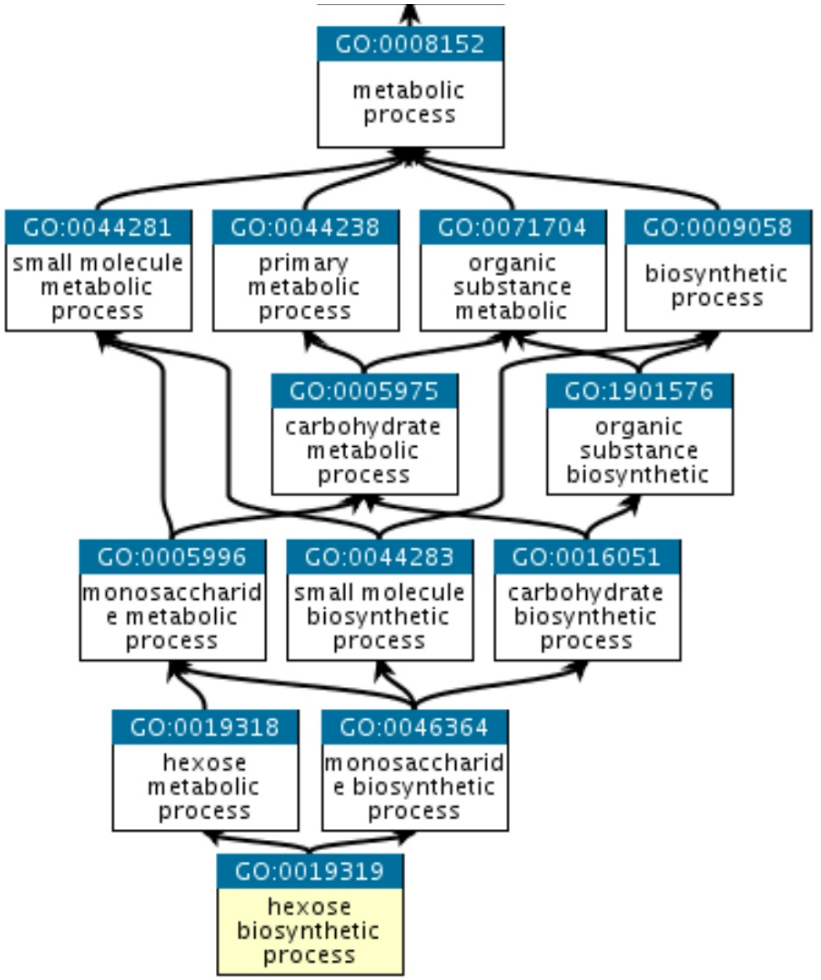
- **A few of the possibilities :**
- **Gene Ontology**
- **Reactome**
- **KEGG**
- **MSigDB**
- **Custom set**
- **…**

Enrichment analysis - mapping

Gene ontology : http://geneontology.org

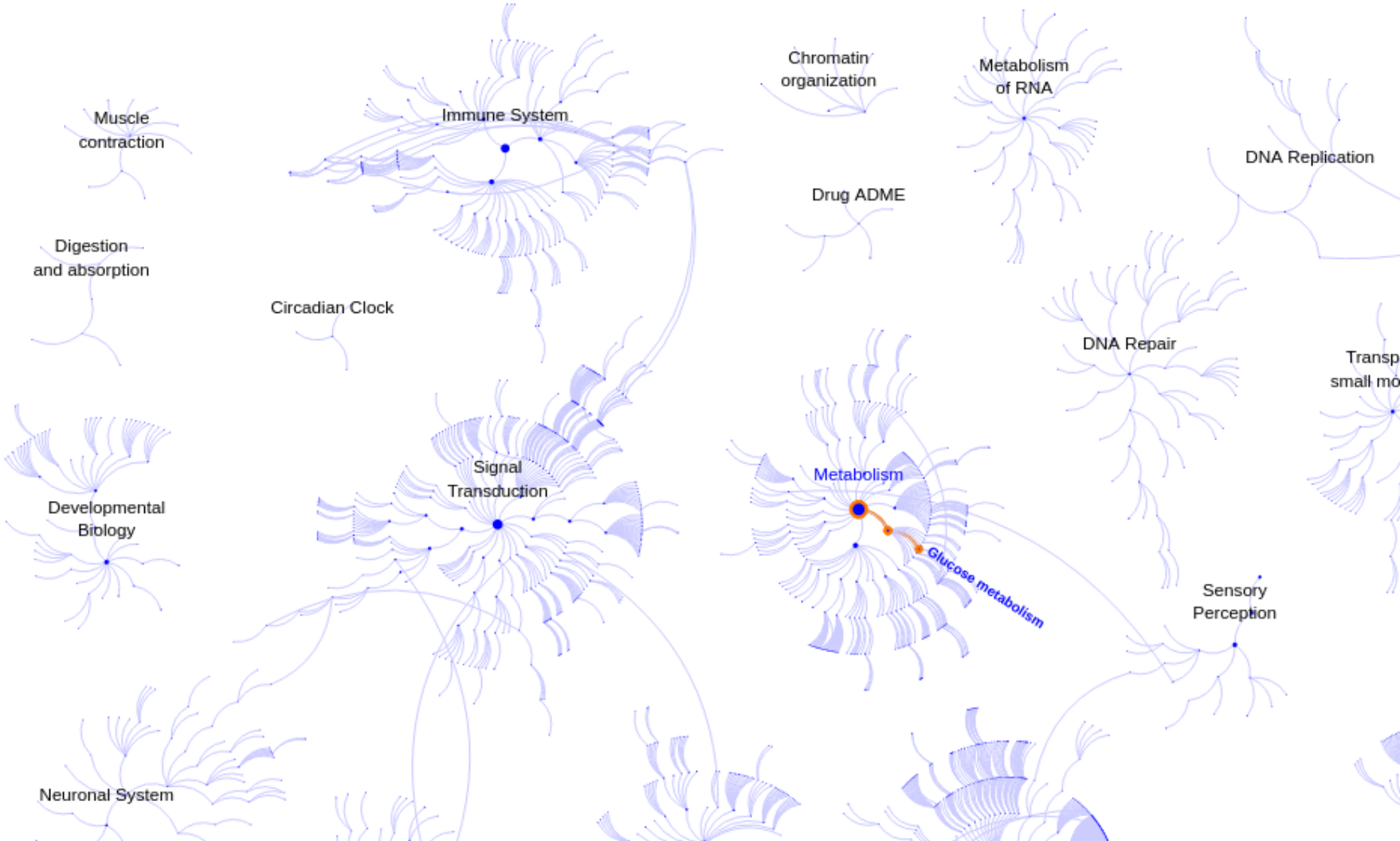
3 domains of nested terms:

- **Molecular Function**
- **Cellular Component**
- **Biological Process**



Enrichment analysis - mapping

Reactome : https://reactome.org



Enrichment analysis - mapping

MSigDB : http://www.gsea-msigdb.org/gsea/msigdb/index.jsp

Human mouse and rat only

H **hallmark gene sets** are coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes.

C1 **positional gene sets** for each human chromosome and cytogenetic band.

C2 **curated gene sets** from online pathway databases, publications in PubMed, and knowledge of domain experts.

C3 **regulatory target gene sets** based on gene target predictions for microRNA seed sequences and predicted transcription factor binding sites.

C4 **computational gene sets** defined by mining large collections of cancer-oriented microarray data.

C5 **ontology gene sets** consist of genes annotated by the same ontology term.

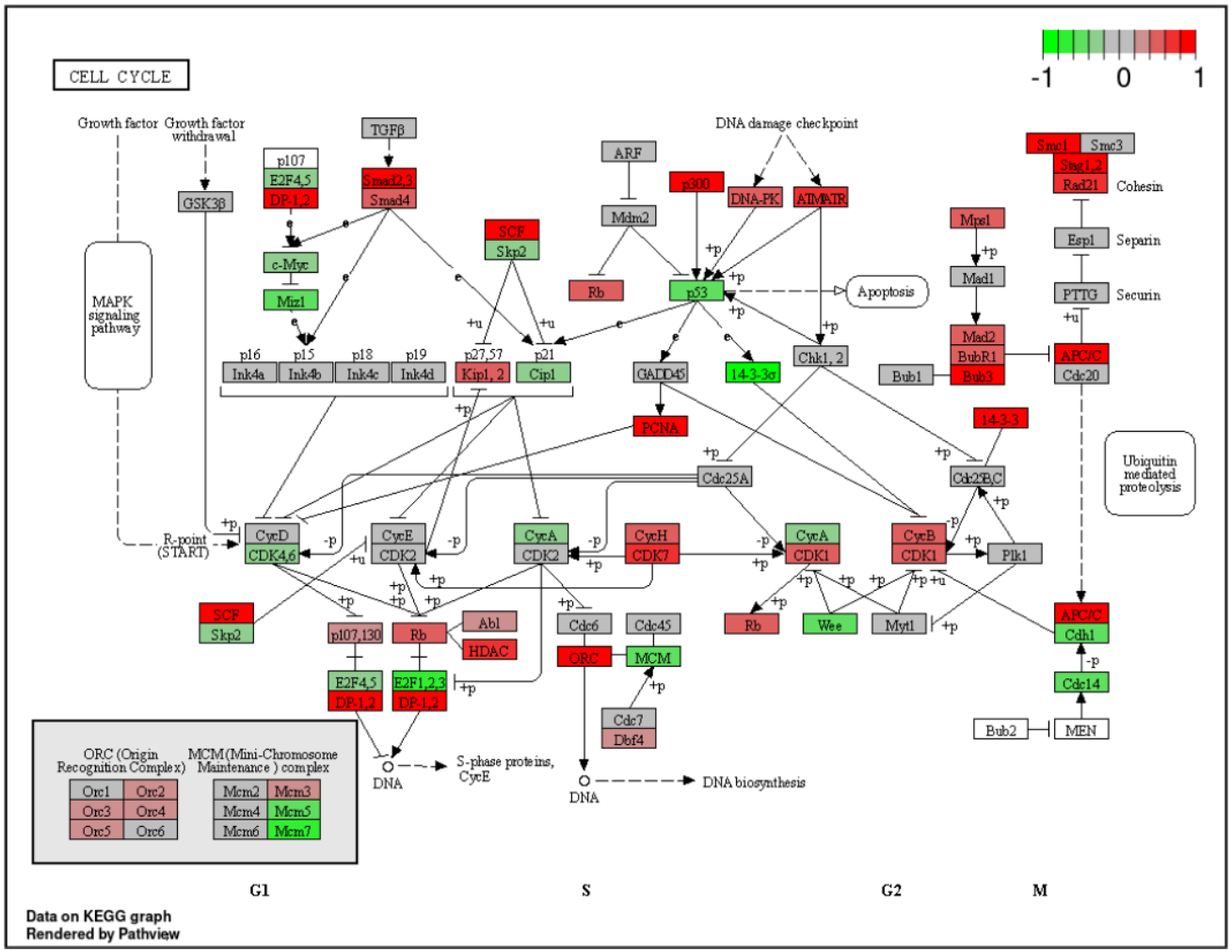
C6 **oncogenic signature gene sets** defined directly from microarray gene expression data from cancer gene perturbations.

C7 **immunologic signature gene sets** represent cell states and perturbations within the immune system.

C8 **cell type signature gene sets** curated from cluster markers identified in single-cell sequencing studies of human tissue.

Enrichment analysis - mapping

KEGG : https://www.genome.jp/kegg/ - KEGG PATHWAY



Projection of DE data
onto a KEGG pathway map
With R package pathview

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

Enrichment analysis - mapping

- **Custom gene sets :**
- **Derived from specialized litterature**
- **Tentative annotation**
- **Understudied organisms**
- **...**

Enrichment analysis – computing enrichment

- **2 possible approaches (among many!)**
- **Over-representation analysis**
- **Gene Set Enrichment Analysis**

Enrichment analysis – computing enrichment

- **2 possible approaches (among many!)**
- **Over-representation analysis**
  - **Fisher's exact test (with p-val correction)**

	DE	Not DE
in gene set	A	B
not in gene set	C	D

N = A+B+C+D **# total genes**
M = A+B **# genes in set**

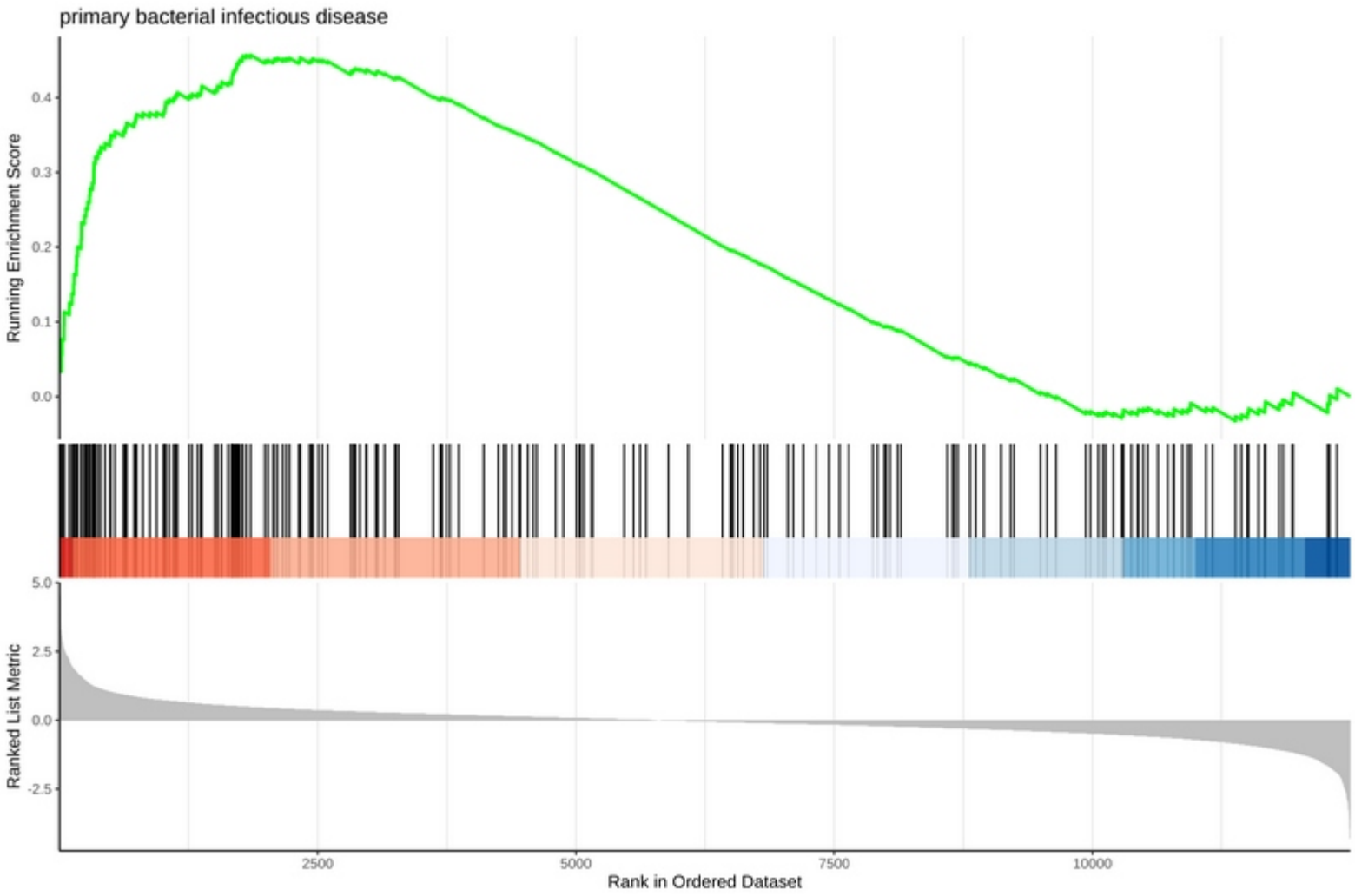
n = A+C **# DE genes**
k = A **# DE genes in set**

$$p = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

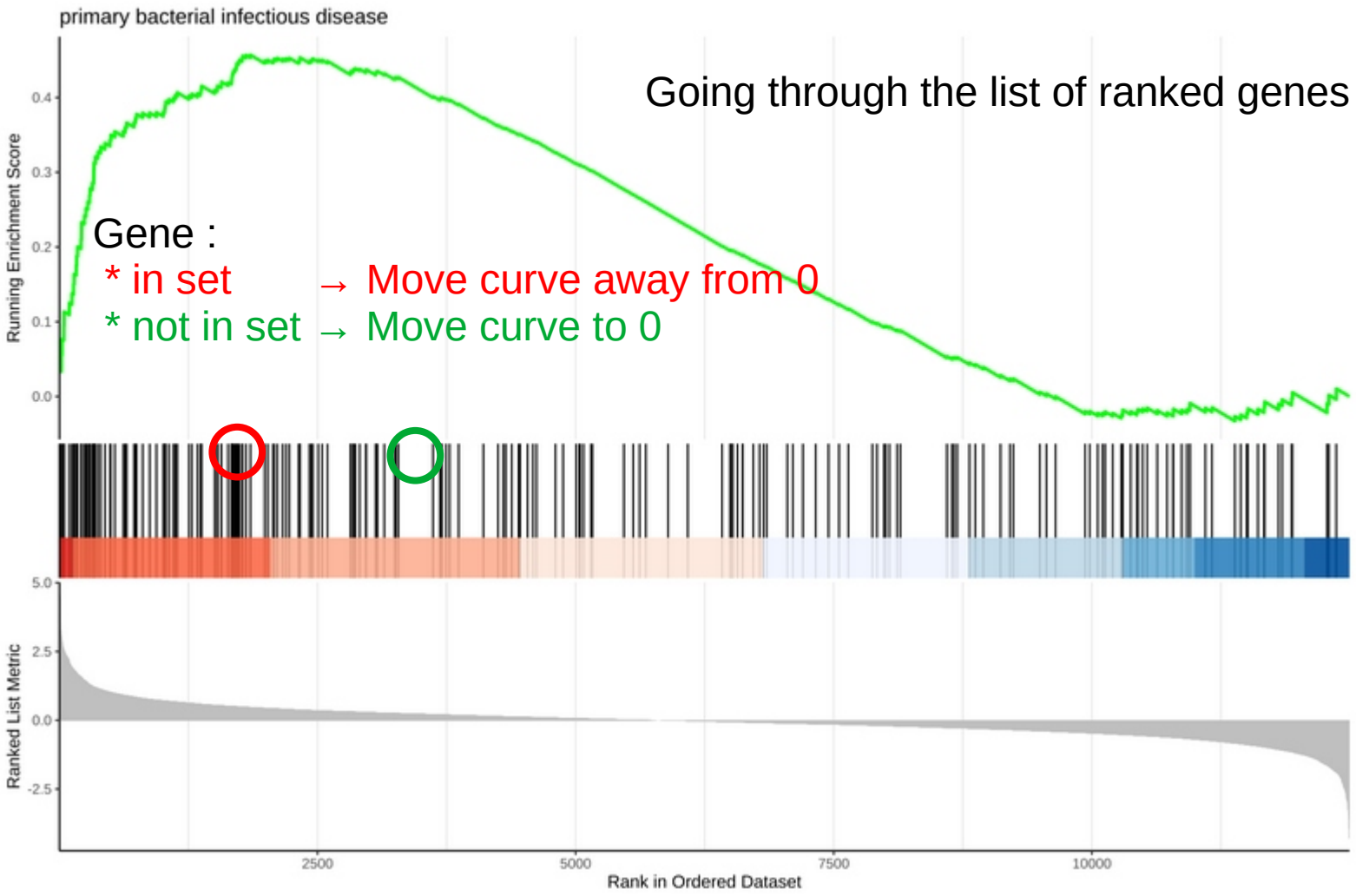
Enrichment analysis – computing enrichment

- **2 possible approaches (among many!)**
- **Gene Set Enrichment Analysis**
  - **Do not rely on 0/1 DE status, but on a continuous measurement (eg. log2FC)**
  - **Computes Enrichment Score from list of ranked genes**
  - **Estimates significance using permutations**

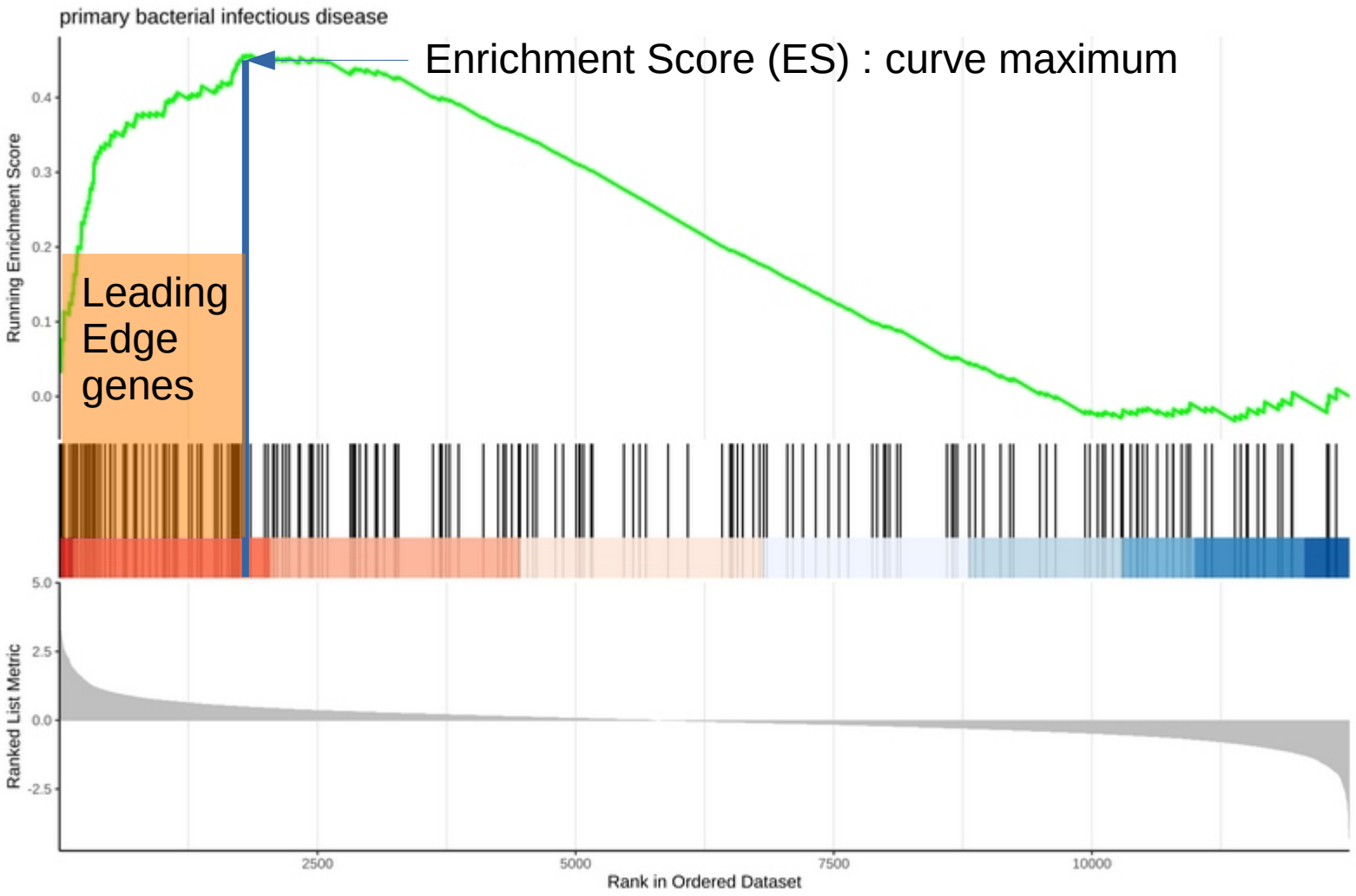
Enrichment analysis – GSEA



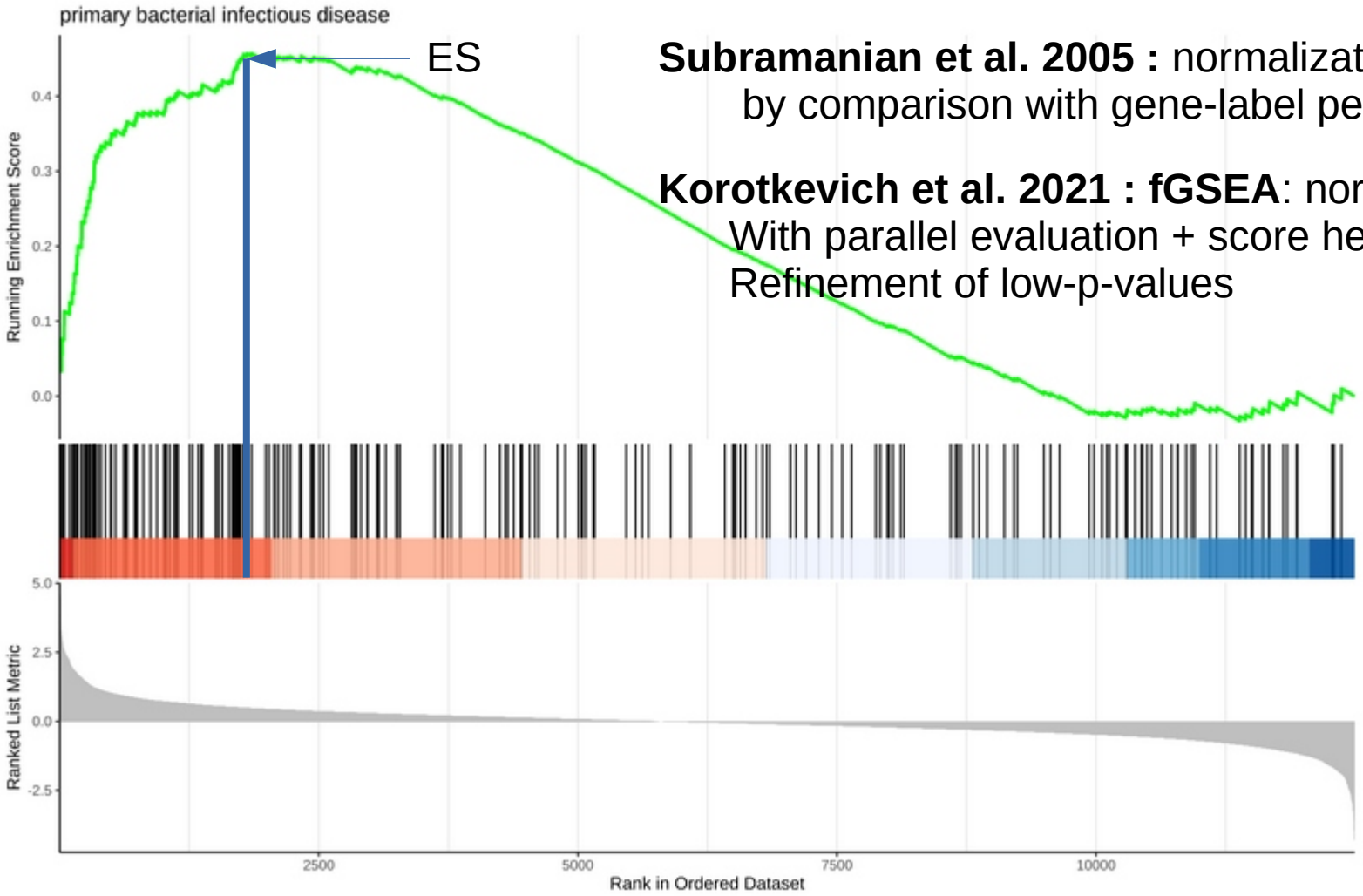
Enrichment analysis – ES



Enrichment analysis – ES



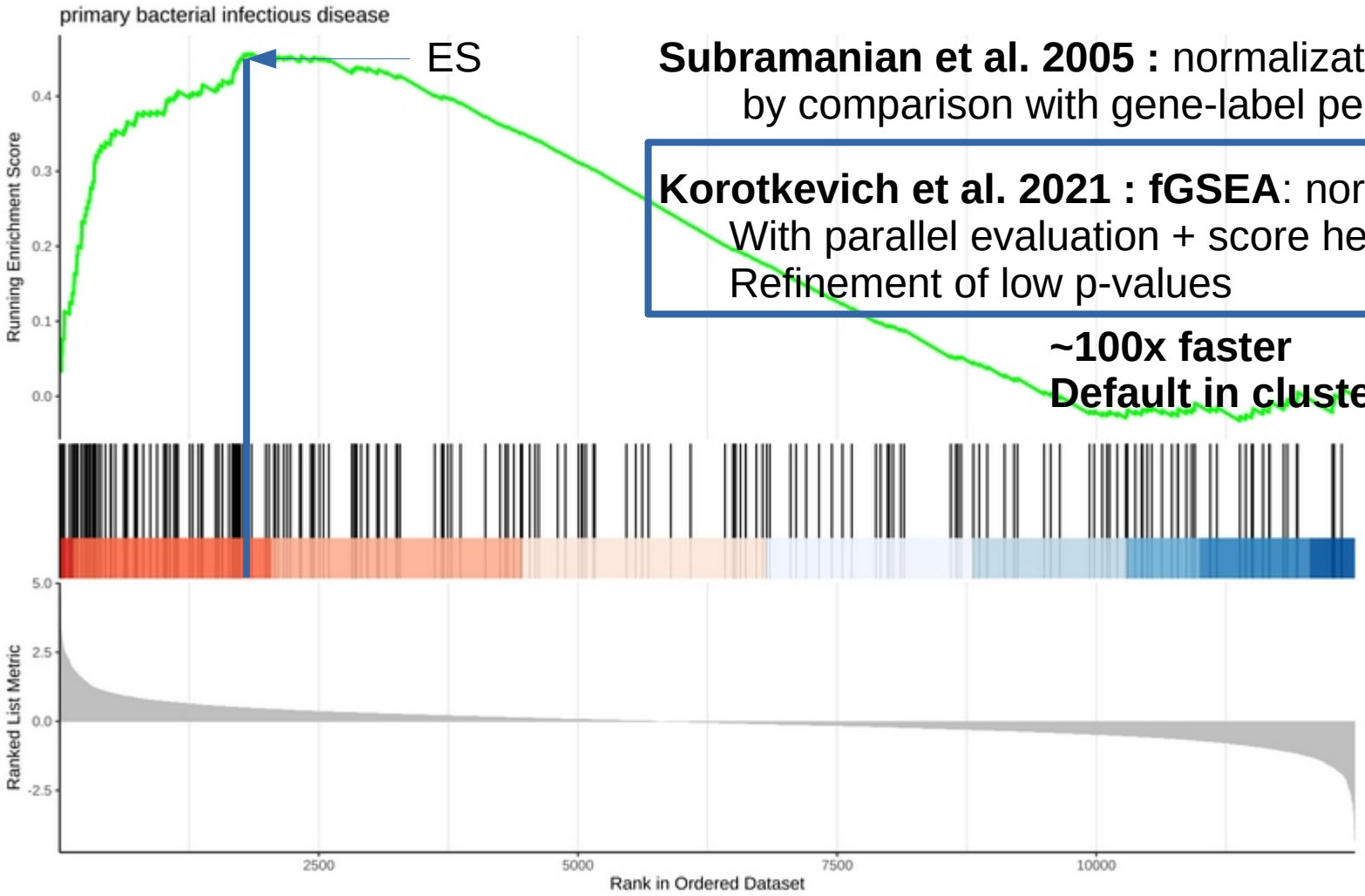
Enrichment analysis – NES



Subramanian et al. 2005 : normalization by comparison with gene-label permuted sets

Korotkevich et al. 2021 : fGSEA: normalization With parallel evaluation + score heuristic + Refinement of low-p-values

Enrichment analysis – (f)GSEA

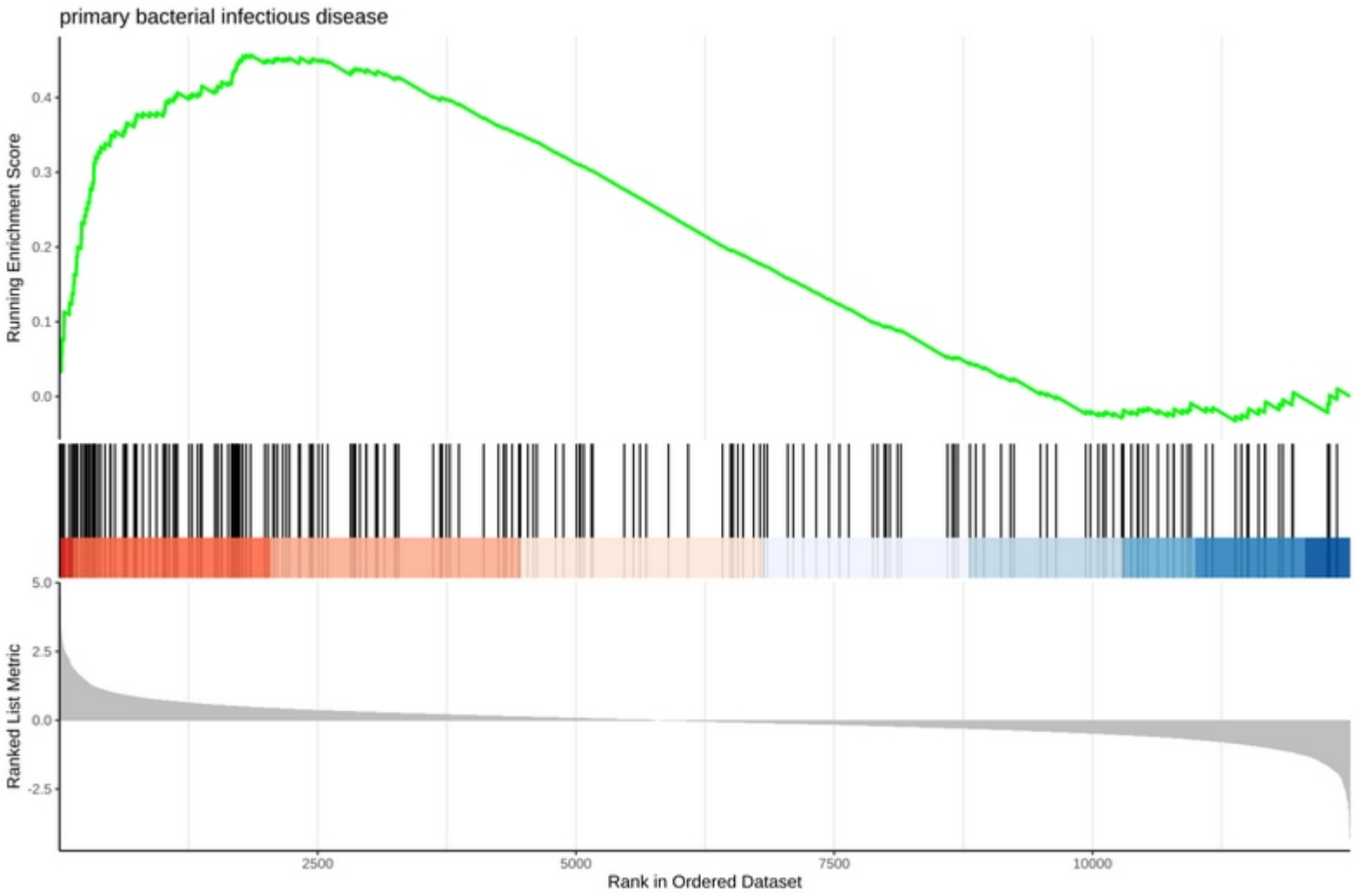


Subramanian et al. 2005 : normalization by comparison with gene-label permuted sets

Korotkevich et al. 2021 : fGSEA: normalization With parallel evaluation + score heuristic + Refinement of low p-values

~100x faster
Default in clusterProfiler

Enrichment analysis – GSEA



Enrichment Score visualized using functions from the R package enrichplot

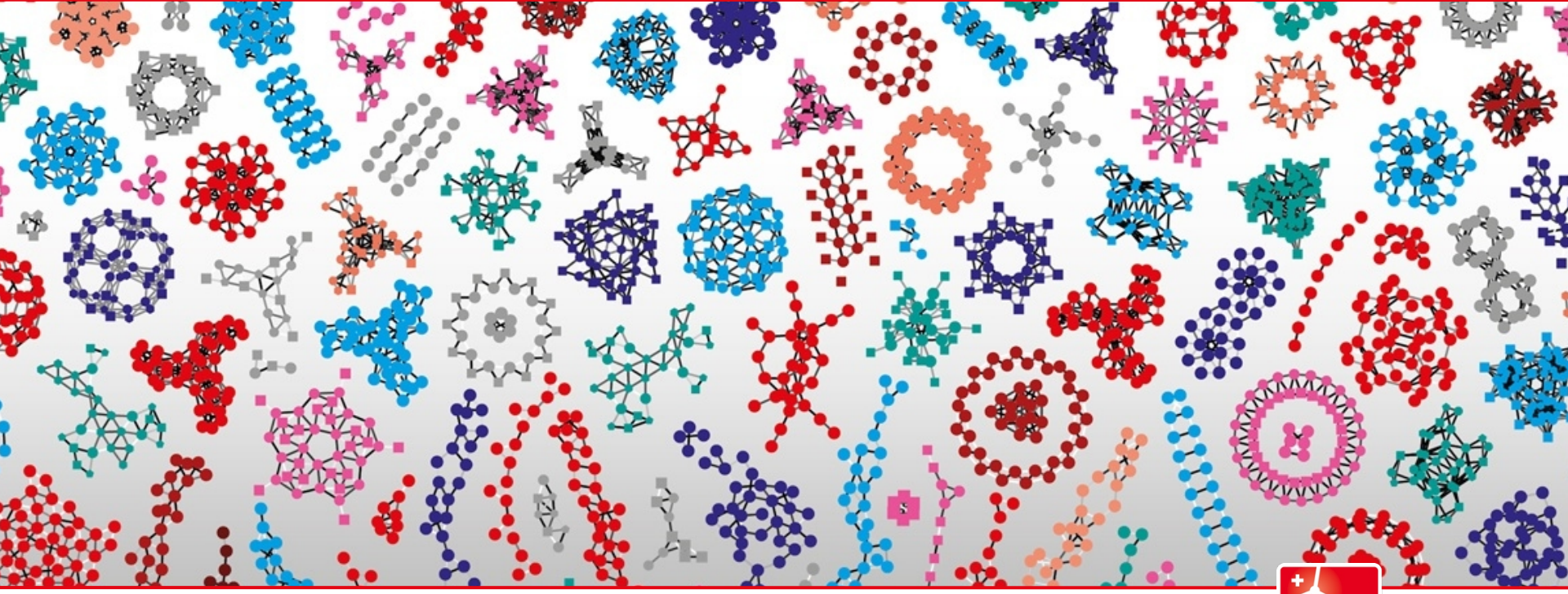
http://www.bioconductor.org/packages/release/bioc/html/enrichplot.html

Enrichment analysis – computing enrichment

- **Many more methods or implementations:**
- **Signaling Pathway Impact Analysis**
  - **https://bioconductor.org/packages/release/bioc/html/SPIA.html**
- **ISMARA**
  - **https://ismara.unibas.ch/mara/**
- **...**

Practical

- **Go to the website and follow the Enrichment practical**



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