



# Introduction to Pathway Analysis

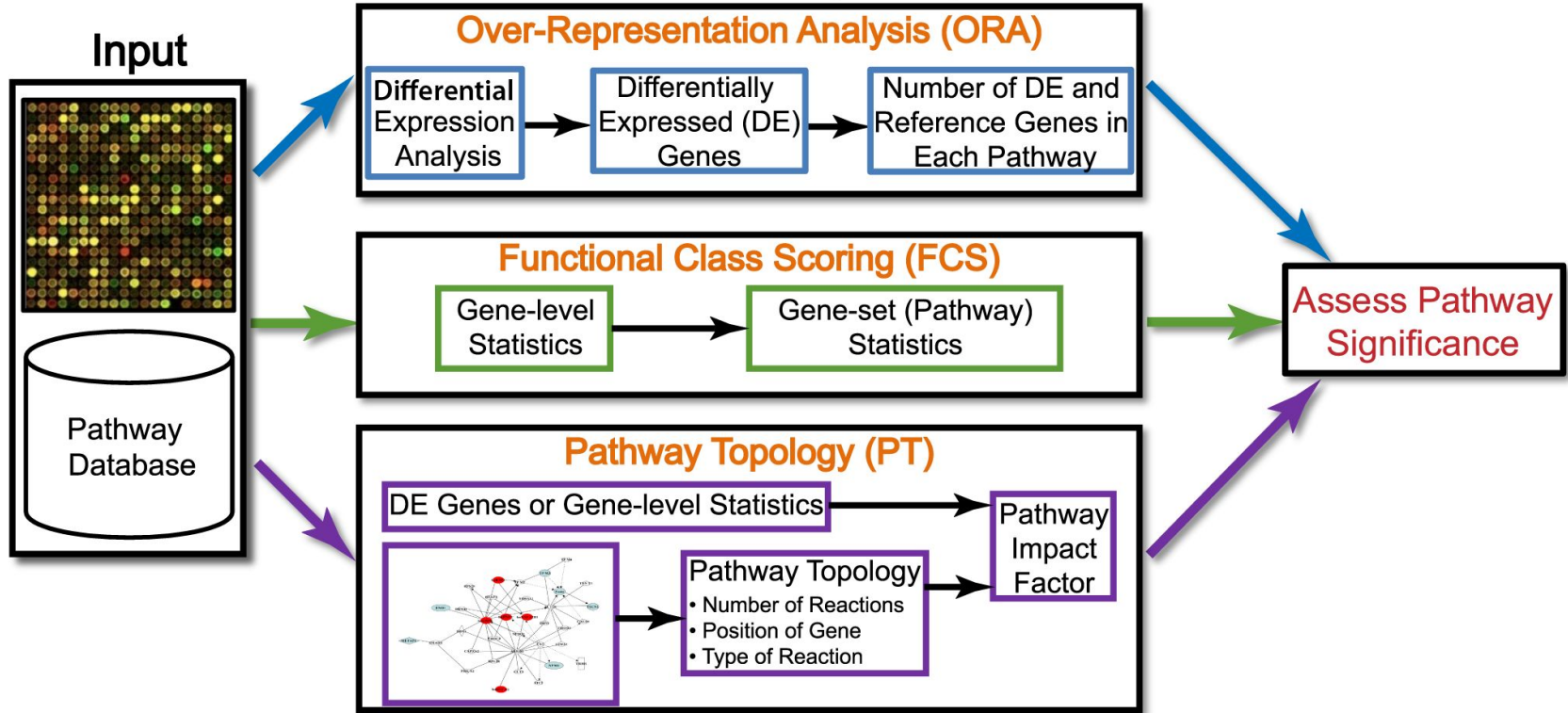
The CCDL

# Why pathway analysis?

“...one may be left with a long list of statistically significant genes without any unifying biological theme. Interpretation can be daunting and ad hoc, being dependent on a biologist's area of expertise.”

- [Subramanian et al. PNAS. 2005.](#)

# Functional Pathway Analysis



# Today we'll cover three types of pathway analysis

## Over-representation analysis (ORA)

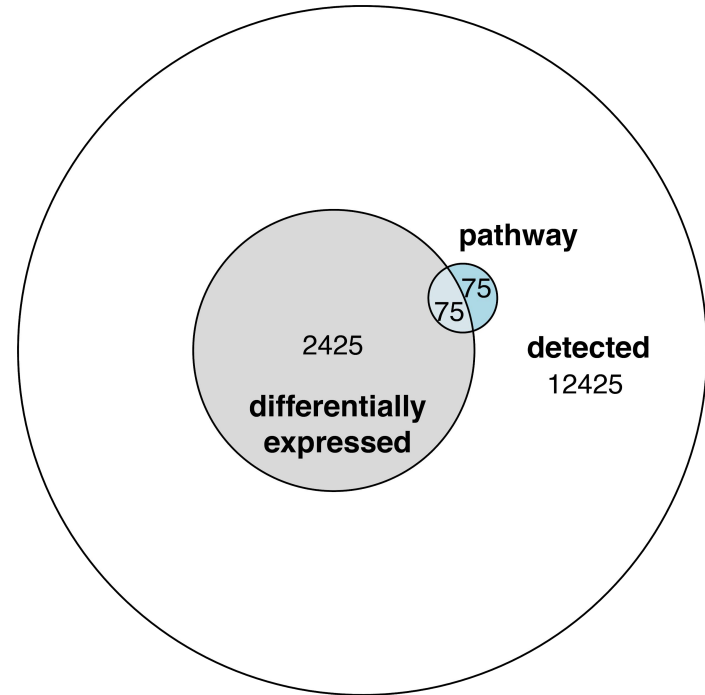
I have a list of genes from my analysis and I'm interested in if genes from a pathway are represented in that list more than I would expect by chance.

### ✓ Pros

- Simple
- Computationally inexpensive to compute p-values

### ! Cons

- Requires arbitrary thresholds and ignores any statistics associated with a gene
- Assumes independence of genes and pathways



# Today we'll cover three types of pathway analysis

## Gene Set Enrichment Analysis (GSEA)

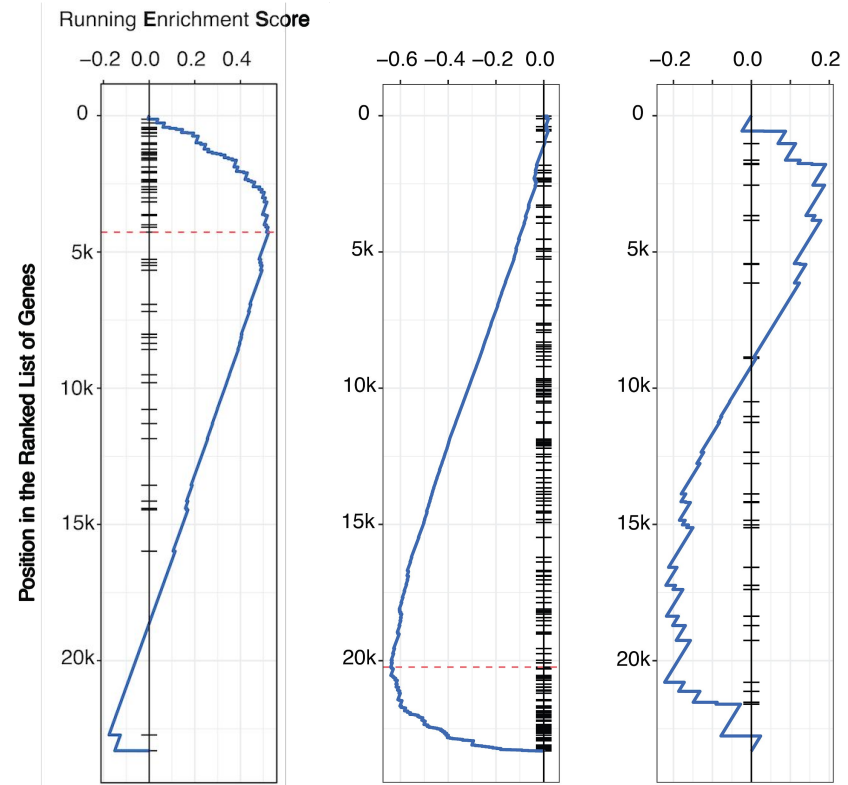
I have a gene-level statistics from a two-group comparison, and I would like to know if there are coordinated changes in pathway that are unlikely to be detected by looking at differentially expressed genes alone.

### ✓ Pros

- Includes *all* genes (no arbitrary threshold!)
- Attempts to measure coordination of genes

### ⚠ Cons

- Permutations can be expensive
- Does not account for pathway overlap
- Two-group comparisons not always appropriate/feasible

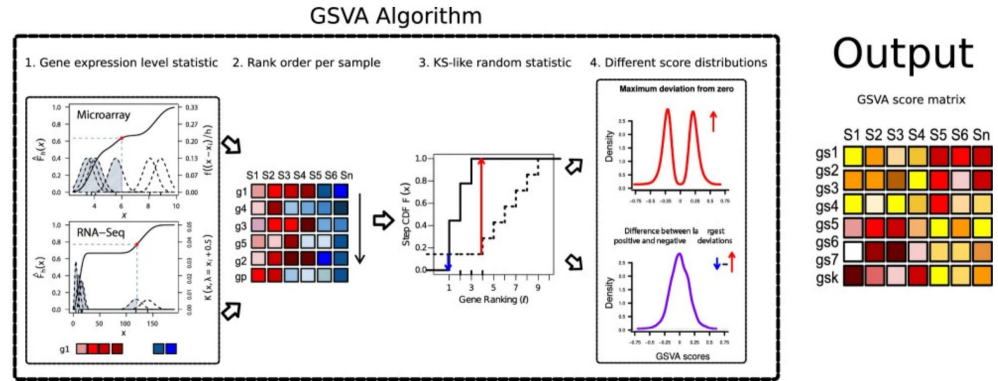


[Subramanian et al. PNAS. 2005.](#)

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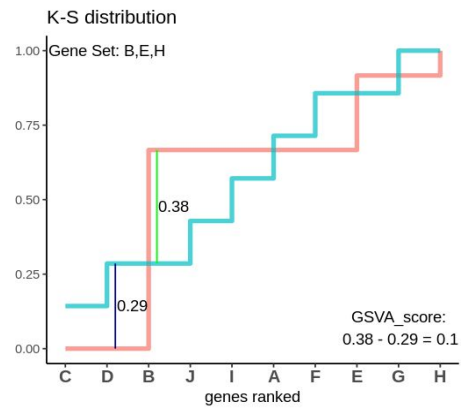
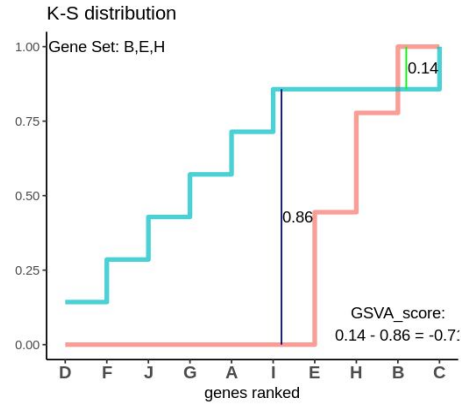
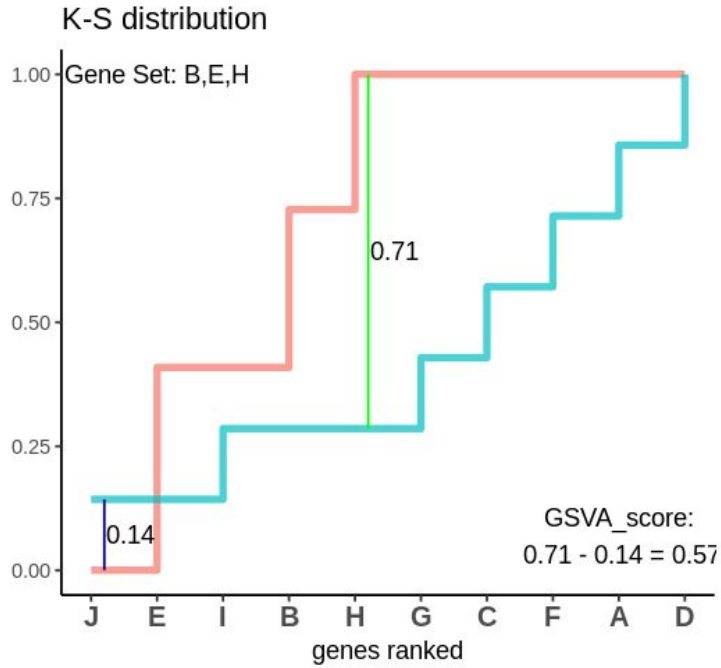
## Gene Set Variation Analysis (GSVA)

I don't have two groups to compare, so I want pathway-level scores on a per-sample basis that tell me if genes in the pathway are over- or under-expressed relative to the overall population.



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## Gene Set Variation Analysis (GSVA)



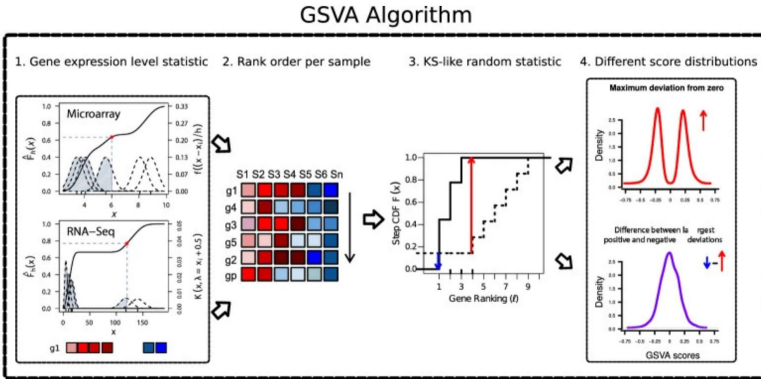
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## Gene Set Variation Analysis (GSVA)

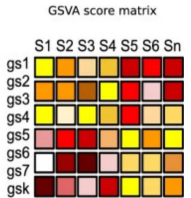
I don't have two groups to compare, so I want pathway-level scores on a per-sample basis that tell me if genes in the pathway are over- or under-expressed relative to the overall population.

### ✓ Pros

- Does not require two groups to compare upfront
- Normally distributed scores



## Output



### ⚠ Cons

- Scores are not a good fit for gene sets that contain genes that go up AND down
- Method doesn't assign statistical significance itself
- Recommended sample size  $n > 10$

[Hänzelmann, Castelo, and Guinney. BMC Bioinformatics. 2013.](#)



# Resources

Guangchuang Yu. [\*clusterProfiler: universal enrichment tool for functional and comparative study.\*](#)

Harvard Chan Bioinformatics Core Training. [\*Intro to DGE: Functional analysis.\*](#)

Saksham Malhotra. [\*Decoding Gene Set Variation Analysis.\*](#)

[refine.bio examples](#) includes pathway analysis examples for microarray and RNA-seq

[Molecular Signatures Database \(MSigDB\)](#)