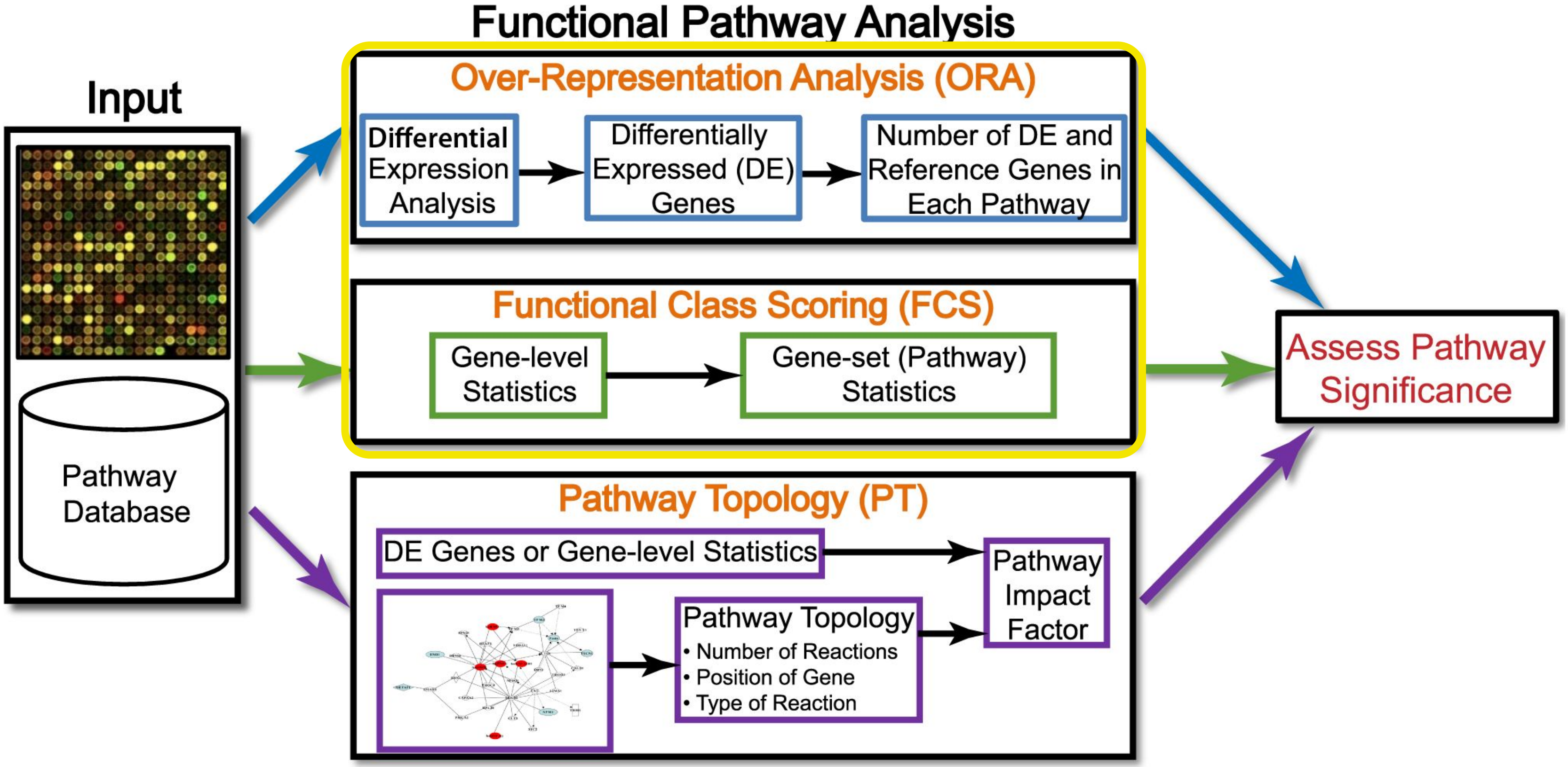
Introduction to Pathway Analysis

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

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 Topology

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

Pathway Impact Factor

Assess Pathway Significance

Khatri, Sirota, and Butte. *PLoS Comp Bio*. 2012.

Today we'll cover three types of pathway analysis

Over-representation analysis (ORA)

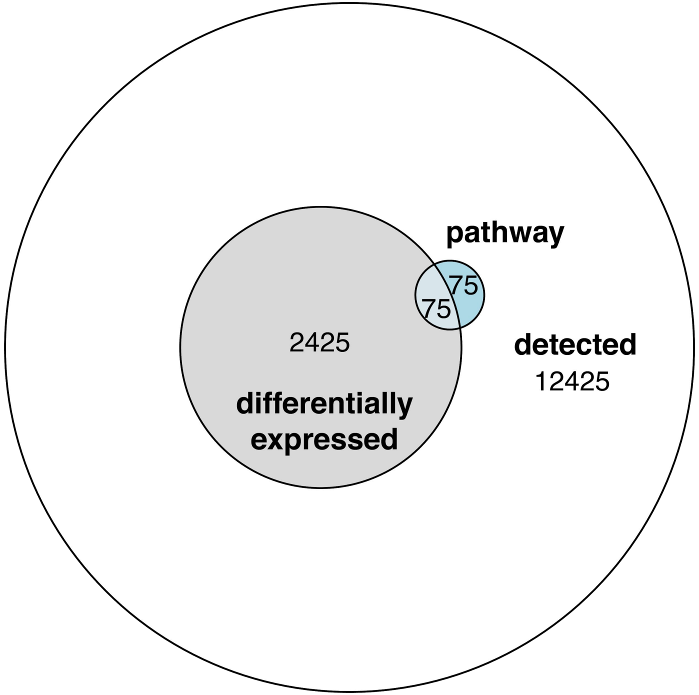
I have a list of genes from my analysis and I'm interested in if genes from a pathway are represented in that list more than I would expect by chance.

✓ **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



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Gene Set Enrichment Analysis (GSEA)

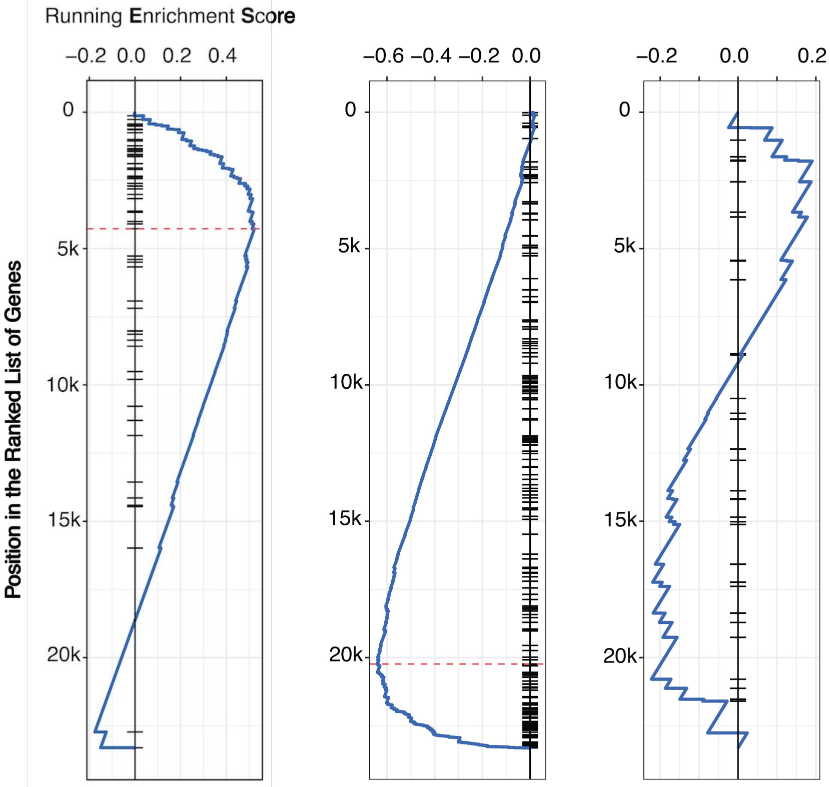
I have a gene-level statistics from a two-group comparison, and I would like to know if there are coordinated changes in pathway that are unlikely to be detected by looking at differentially expressed genes alone.

✓ **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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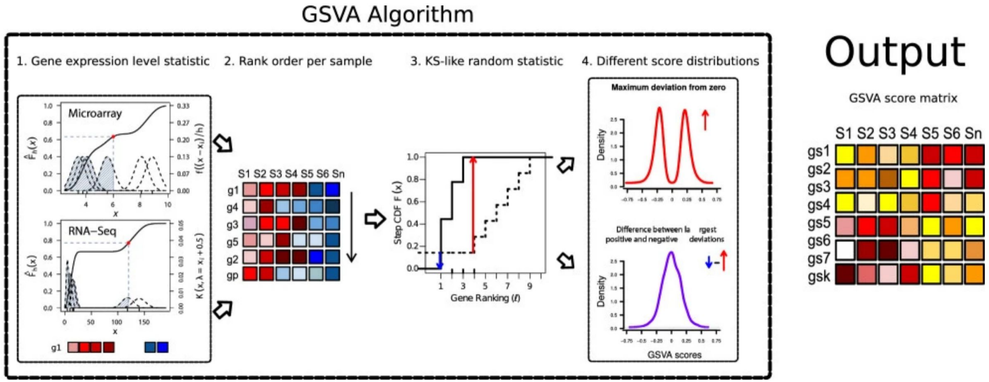
Gene Set Variation Analysis (GSVA)

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.



Hänzelmann, Castelo, and Guinney. *BMC Bioinformatics*. 2013.

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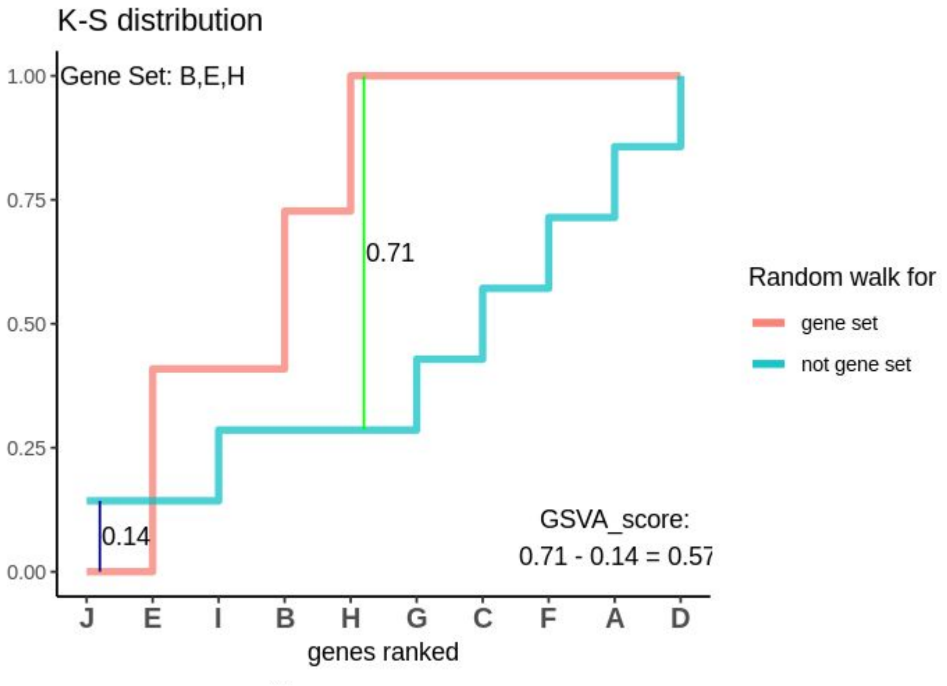

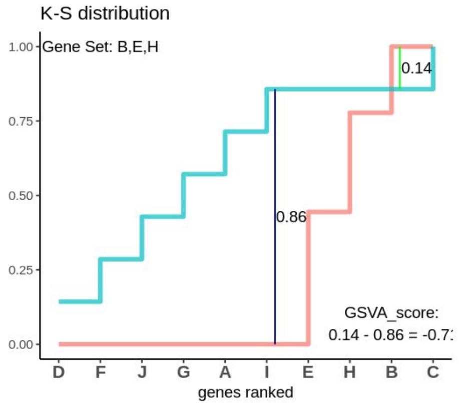

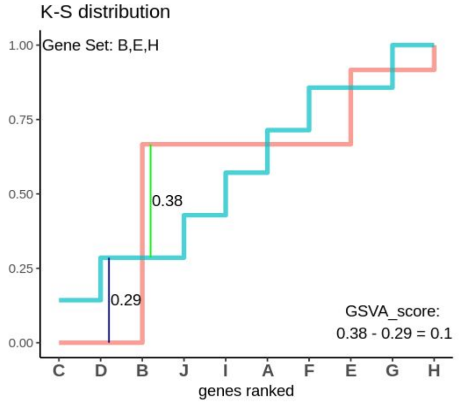

All images from Malhotra. 2018.

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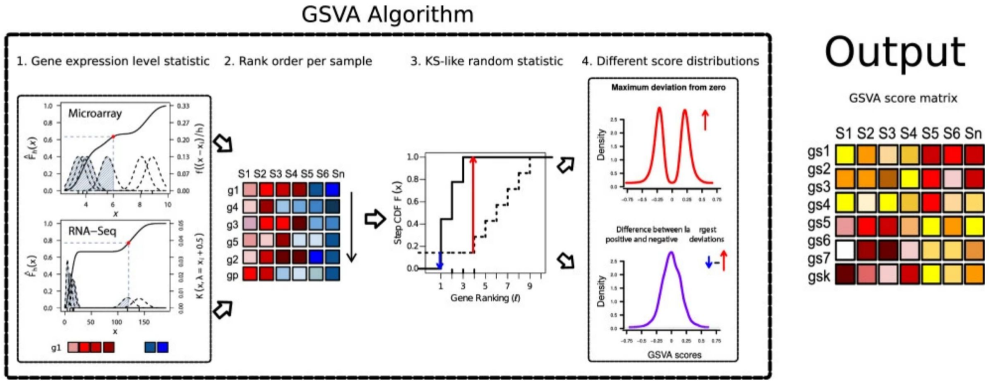

✓ Pros

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

⚠️ 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 on pathway analysis

Molecular Signatures Database (MSigDB)