Addressing Scale Uncertainty in Gene and Microbe Set Enrichment Analysis

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Review of Key Concepts

Consider as an example an 16S rRNA-seq experiment measuring D taxa in the colons of N patients:

$$\underbrace{W_{dn}}_{\substack{\text{Absolute Abundance} \\ \text{Taxa d, Patient n} \\ \text{(Unmeasured)}}} = \underbrace{W_{dn}^{\parallel}}_{\substack{\text{Composition} \\ \text{Taxa d, Patient n} \\ \text{(Measured)}}} \times \underbrace{W_{n}^{\perp}}_{\substack{\text{Scale} \\ \text{(e.g., total \# of microbes in} \\ \text{patient n's colon)} \\ \text{(Unmeasured)}}}$$

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Further consider as an example estimation of the LFC (Log Fold Change) of taxa d in patients with and without Ulcerative Colitis:

$$\underbrace{\theta_d}_{\text{LFC in Absolute Abundance}} = \underbrace{\theta_d^{\parallel}}_{\text{LFC in Composition}} + \underbrace{\theta^{\perp}}_{\text{LFC in Scale}} .$$

Review of Key Concepts

Methods like ALDEx2, DESeq2, Limma, etc. estimate LFCs using sequence count data Y:

$$f(Y) = \hat{\theta}_d$$

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Estimates $\hat{\theta}^{\perp}$ come from normalization, for example:

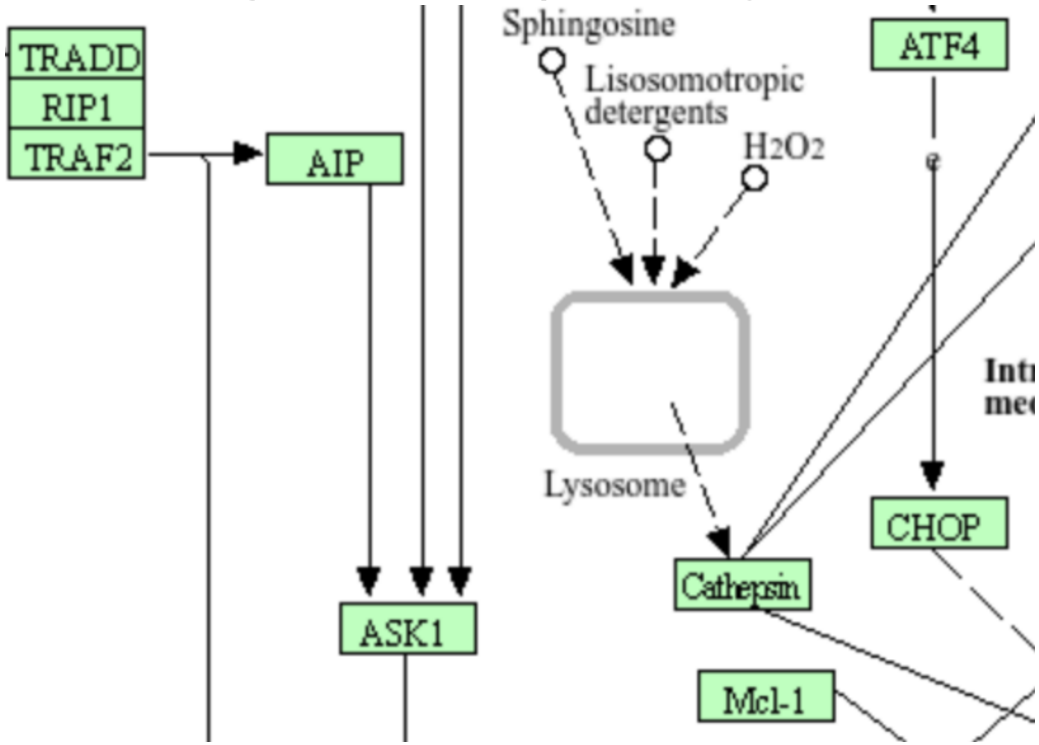
- Total Sum Scaling (TSS): $\hat{\theta}^{\perp} = 0$
- Centered Log Ratio (CLR): $\hat{\theta}^{\perp} = -\text{mean}(\hat{\theta}^{\parallel})$

Differential Set Analysis (DSA)

Rather than estimating **LFCs** of **single** genes/taxa

ASK1

What if we are interested in a **set** of genes in a pathway?



Differential Set Analysis (DSA) is used to estimate enrichment or depletion of a gene/taxa set

Key Points of this Talk

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Key Points of this Talk

1. Errors in scale assumptions (i.e., estimates $\hat{\theta}^{\perp}$, $\hat{W}^{\perp}$) inflate false positive rates in DSA
2. Errors in DSA estimates are a **non-linear** function of scale errors
3. We have developed three solutions to these errors:
    1. LFC Sensitivity Analysis
    2. LFC Sensitivity Testing
    3. Compositional Weighting Methods

Three Methods for DSA

In this presentation 3 common DSA methods will be considered

1. **Gene Set Enrichment Analysis (GSEA) with Gene Label permutations**
2. Gene Set Enrichment Analysis (GSEA) with Sample Label permutations
3. CAMERA

GSEA with Gene Label Permutations

The GSEA Algorithm Step-by-Step

1. Pick a set of genes S (e.g., the apoptosis signaling pathway):

$$S = \{\text{ASK1, CHOP, TRAF2}\}$$

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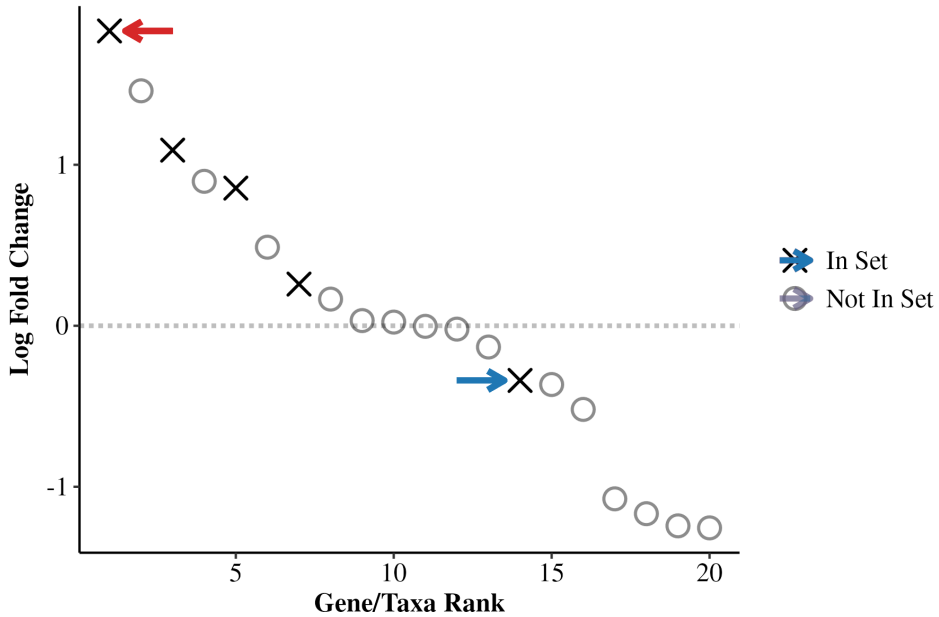
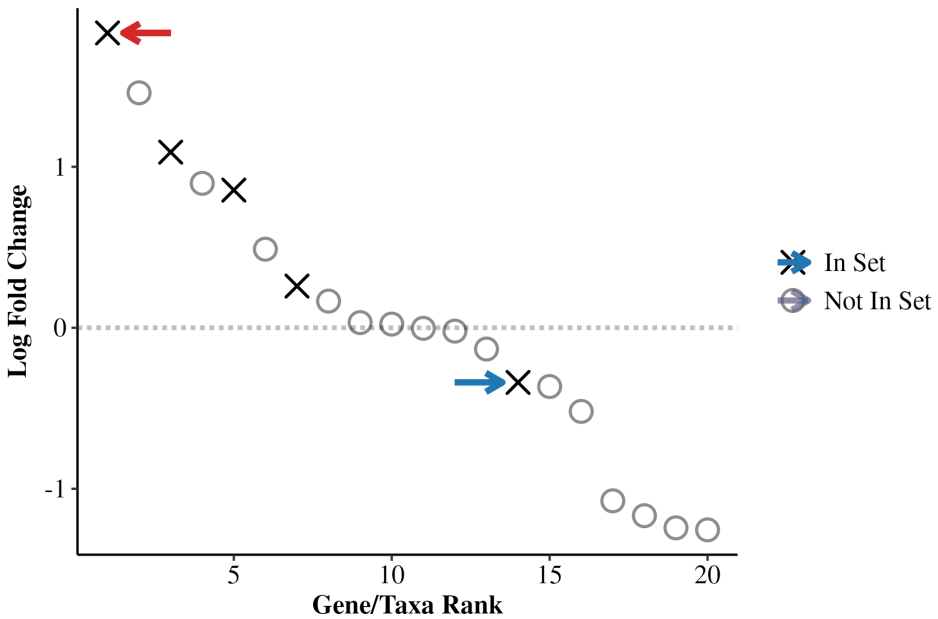
3. Order the LFCs from largest to smallest

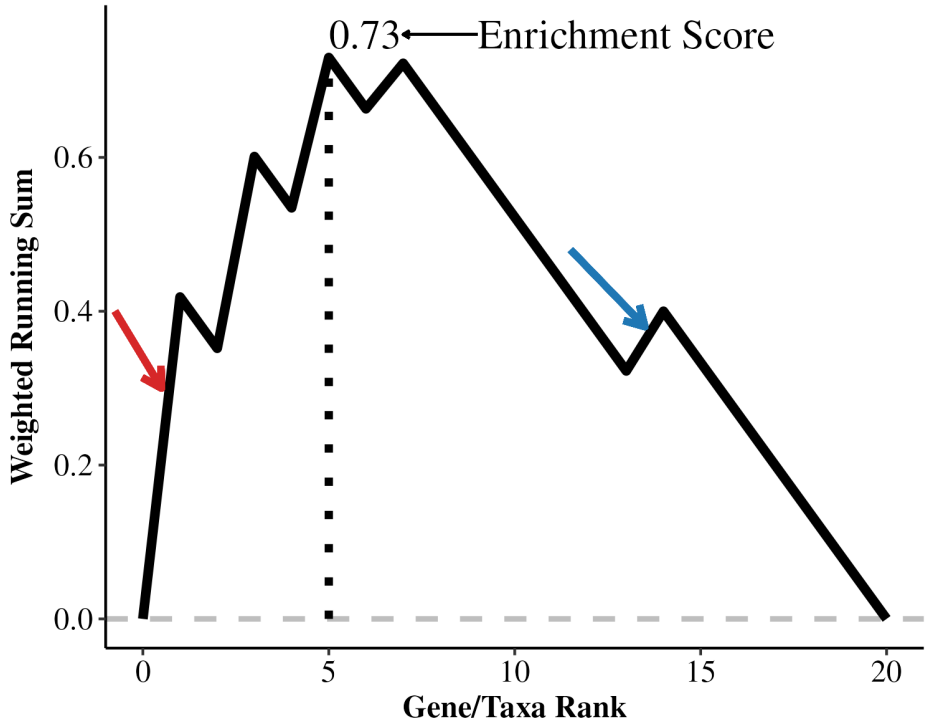


GSEA with Gene Label Permutations

The GSEA Algorithm Step-by-Step

3. Calculate a running sum **weighted** by the LFC
4. Calculate an enrichment score (max distance from 0 of **weighted** running sum)





GSEA with Gene Label Permutations

The GSEA Algorithm Step-by-Step

5. Calculate a null distribution of Enrichment Scores (ESs)

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\begin{aligned}
S &= \{\textbf{ASK1}, \textbf{CHOP}, \textbf{TRAF2}\} &&\Longrightarrow \text{ES} \\
S_1^* &= \{\textbf{ASK1}, \textbf{CHOP}, \text{B2M}\} &&\Longrightarrow \text{ES}_1^* \\
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6. Use null distribution to calculate a p-value

DSA Target Estimand

The goal of DSA is to estimate a **target estimand** ϕ_S:

$$\phi_S = \begin{cases} 1 & \text{Gene Set } S \text{ is significantly enriched} \\ -1 & \text{Gene Set } S \text{ is significantly depleted} \\ 0 & \text{Gene Set } S \text{ is not significantly changing.} \end{cases}$$

In GSEA the target estimand is a function of the **true** LFCs:

$$\phi_S = g(\theta)$$

LFC Sensitivity Analysis

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But we don't know the true LFCs, we only have **estimates**:

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LFC Sensitivity Analysis

A sensitivity analysis of how error in $\hat{\theta}^{\perp}$ affects ϕ_S

LFC Sensitivity Analysis

Error $\epsilon^{\perp}$ in our estimate of the unmeasured scale $\theta^{\perp}$:

$$\underbrace{\theta^{\perp}}_{\text{True LFC in Scale}} = \underbrace{\hat{\theta}^{\perp}}_{\text{Estimate}} + \underbrace{\epsilon^{\perp}}_{\text{Estimation Error}}$$

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How does the **true** ϕ_S change with error $\epsilon^{\perp}$?

$$\begin{aligned} \phi_s &= g(\hat{\theta}^{\parallel} + \hat{\theta}^{\perp} + \epsilon^{\perp}) \\ &= g(\hat{\theta} + \epsilon^{\perp}) \end{aligned}$$

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LFC Sensitivity Analysis Algorithm:

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2. Run GSEA with $\epsilon^{\perp} = 0$ (i.e., $\hat{\phi}_S = g(\hat{\theta})$)
3. Rerun GSEA with $\epsilon^{\perp} \neq 0$ and compare to $\epsilon^{\perp} = 0$ (i.e., $\phi_S = g(\hat{\theta} + \epsilon^{\perp})$

Interpreting Error $\epsilon^{\perp}$ and LFC Sensitivity Analysis Results

Consider error $\epsilon^{\perp} = \pm 0.5$:

1. This error corresponds to the true $\theta^{\perp}$ being $e^{0.5} = 1.65$ times lower/higher than $\hat{\theta}^{\perp}$

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2. Example results if a Gene set S is sensitive to error:

$\epsilon^{\perp} = -0.5$	$\epsilon^{\perp} = 0$	$\epsilon^{\perp} = 0.5$
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3. Example results if a Gene set S is not sensitive to error:

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