Network-based Enrichment Analysis

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Collaborators

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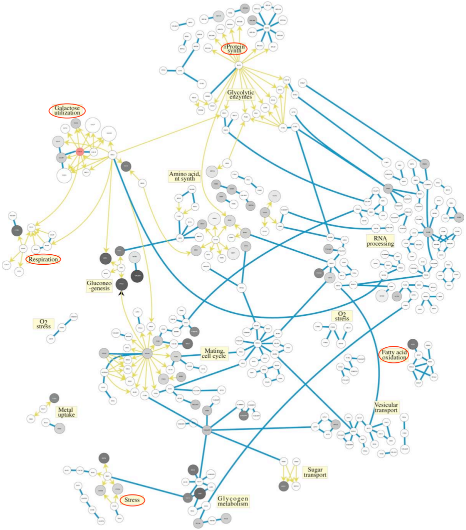
George Michailidis

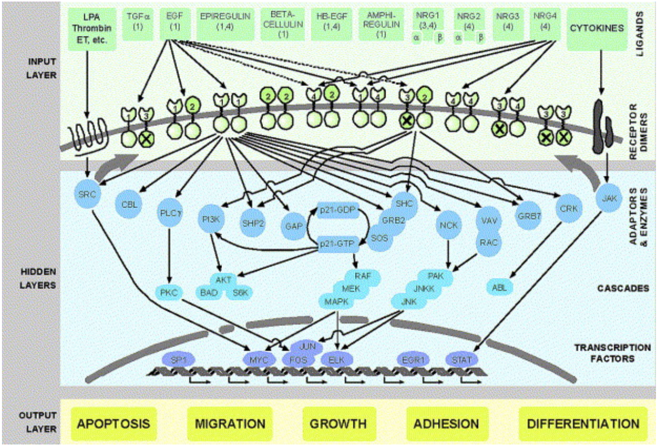
Yeast GAL Pathway[1]

- Physical-interaction network
- Nodes: genes
- Edges: DNA binding, protein-protein interaction
- Highly interconnected groups of genes have common biological function



[1] Ideker et al. Science. 2001

ERBB Signaling Network[2]



[2] Yarden & Sliwkowski Nat. Rev. Mol. Cell Biol. 2001

Pathway Enrichment Analysis

Scientific Question: whether a genetic/metabolic pathway is involved in responding to changes in environmental conditions or in specific cell functions.

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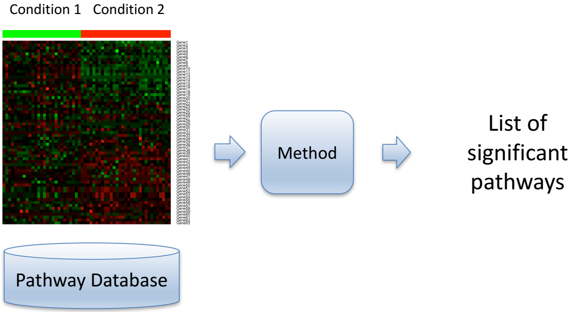
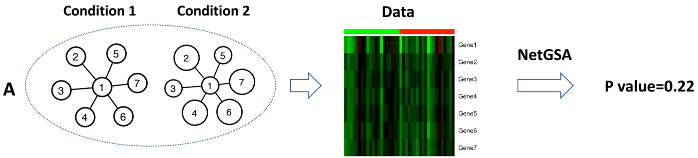


Pathway Enrichment Analysis

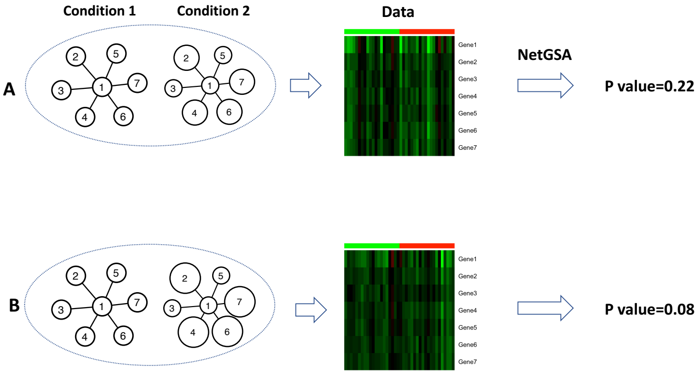
- Reduce the complexity.
- More explanatory power.

Toy Example



Condition 1
Condition 2
A
Data
Gene1
Gene2
Gene3
Gene4
Gene5
Gene6
Gene7
NetGSA
P value=0.22

Toy Example


Condition 1
Condition 2
Data
A
B
1
2
3
4
5
6
7
Gene1
Gene2
Gene3
Gene4
Gene5
Gene6
Gene7
NetGSA
P value=0.22
P value=0.08

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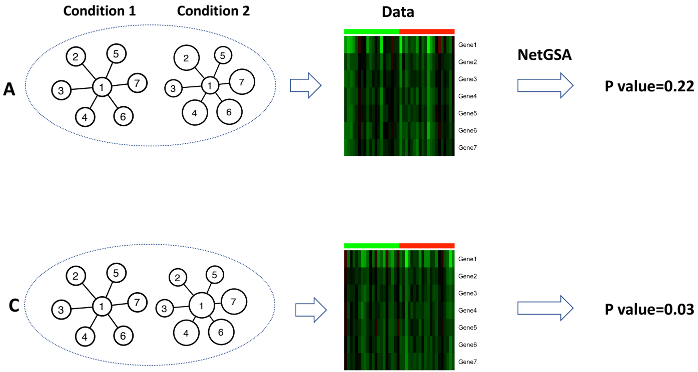
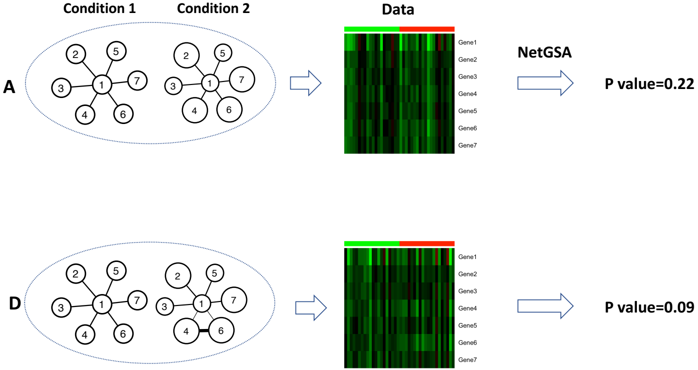

Condition 1
Condition 2
Data
A
NetGSA
P value=0.22
C
P value=0.03
Gene1
Gene2
Gene3
Gene4
Gene5
Gene6
Gene7

Toy Example


Condition 1
Condition 2
Data
A
D
NetGSA
P value=0.22
P value=0.09
Gene1
Gene2
Gene3
Gene4
Gene5
Gene6
Gene7

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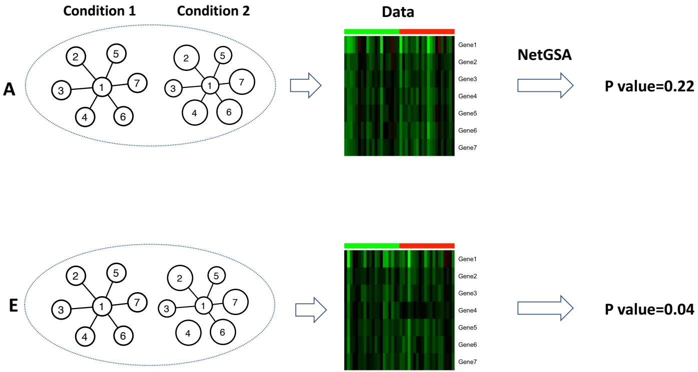

Condition 1
Condition 2
Data
A
2
5
3
1
7
4
6
Gene1
Gene2
Gene3
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Gene6
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P value=0.22
E
P value=0.04

What Drives Pathway Significance?

- Mean expression levels of all genes.
- Gene position: hub gene?
- Gene-gene interactions.

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NetGSA captures all three factors!

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- Assume the network A is known.

Linear Recursive Equations

$$\begin{aligned} X_1 + \lambda_{12} X_2 + \lambda_{13} X_3 &= \gamma_1 \\ X_2 + \lambda_{23} X_3 &= \gamma_2 \\ X_3 &= \gamma_3 \end{aligned}$$

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- $Y = \Lambda\gamma + \varepsilon$.

Linear Mixed Effects Model

- Matrix representation: letting $\mathbf{Y} \in \mathbb{R}^{np \times 1}$,

$$\mathbf{Y} = (\Psi\beta + \Pi\mathcal{G}) + \mathcal{E}$$

where β and $\mathcal{G}$ are fixed and random effect parameters and

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- Design matrices Ψ and Π are defined as functions of Λ.

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- Estimation of variance components $\sigma_\gamma^2, \sigma_\varepsilon^2$ can be done using restricted maximum likelihood (REML).

Inference

- Let ℓ be an arbitrary linear combination (contrast vector). Consider a test of the form:

$$H_0 : \ell\beta = 0 \quad \textit{vs.} \quad H_1 : \ell\beta \neq 0.$$

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- Use a *t*-test to test the significance of each hypothesis separately.

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- NetGSA combines pathway membership with the influence matrix, which also allows us to test for changes in the network.

Incomplete Network Information

$$
\begin{array}{ccccccc}
1 & 2 & 3 & 4 & 5 & 6 & \\
\end{array}
$$

$$
A = \left(\begin{array}{cccccc}
\cdot & ? & 1 & 0 & ? & 0 \\
? & \cdot & ? & ? & 0 & ? \\
1 & ? & \cdot & ? & 0 & 0 \\
0 & ? & ? & \cdot & ? & 1 \\
? & 0 & 0 & ? & \cdot & ? \\
0 & ? & 0 & 1 & ? & \cdot
\end{array}\right)
\begin{array}{c} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \end{array}
$$

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- Lacking condition/disease-specific alterations in interactions.

Incomplete Network Information

$$A = \begin{array}{cc} \begin{matrix} 1 & 2 & 3 & 4 & 5 & 6 \end{matrix} & \\ \begin{pmatrix} \cdot & ? & 1 & 0 & ? & 0 \\ ? & \cdot & ? & ? & 0 & ? \\ 1 & ? & \cdot & ? & 0 & 0 \\ 0 & ? & ? & \cdot & ? & 1 \\ ? & 0 & 0 & ? & \cdot & ? \\ 0 & ? & 0 & 1 & ? & \cdot \end{pmatrix} & \begin{matrix} 1 \\ 2 \\ 3 \\ 4 \\ 5 \\ 6 \end{matrix} \end{array}$$

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- Consistent estimation of network requires fewer observations, depending on the available external information.

Efficient Computation for Large Networks

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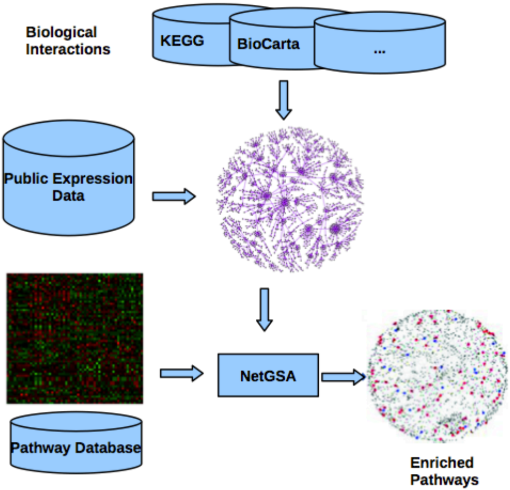
- Integrative analysis of multiple Omics data can be done using a permutation test.

A Flowchart for NetGSA



Introduction

The NetGSA Model

Extensions of NetGSA

Applications

Pathway Topology-based Methods

Competitive null:

- SPIA (Tarca et al. ’09)
- camera (Wu and Smyth, ’12)
- PathNet (Dutta, et al. ’12)

Self-contained null:

- topologyGSA (Massa et al. ’10)
- DEGraph (Jacob et al. ’12)
- NetGSA (Ma et al. ’16)

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- RNA-seq data for 2598 genes (TCGA '12).
- 403 ER positive samples; 117 ER negative samples.
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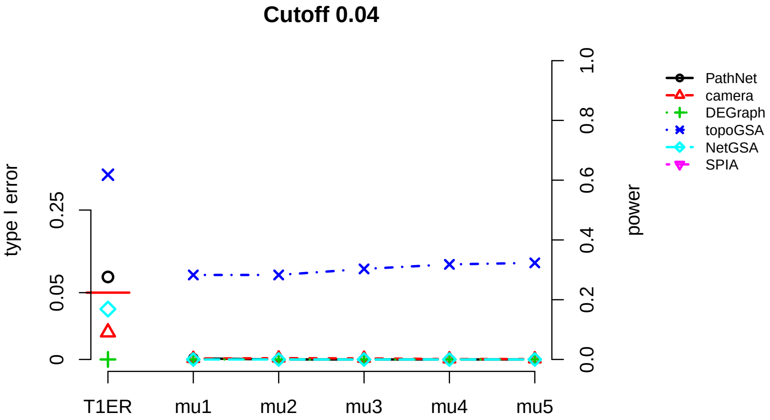

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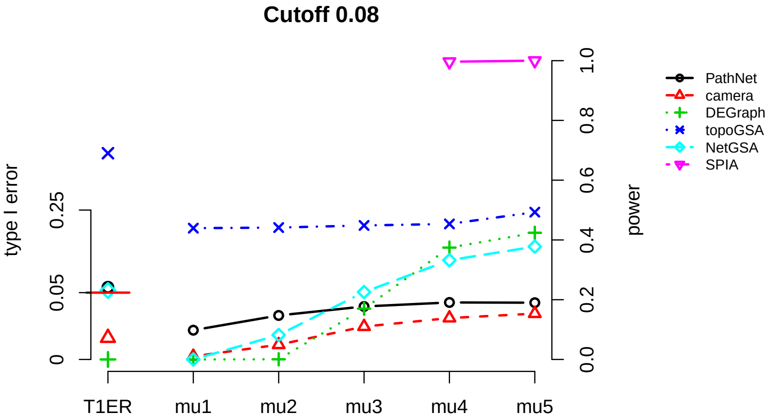

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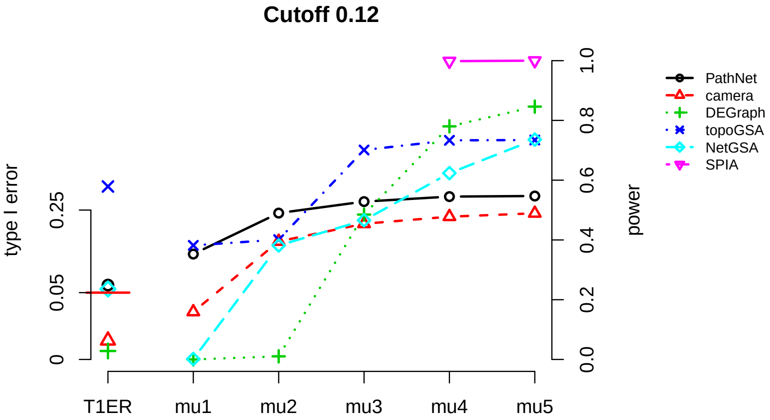

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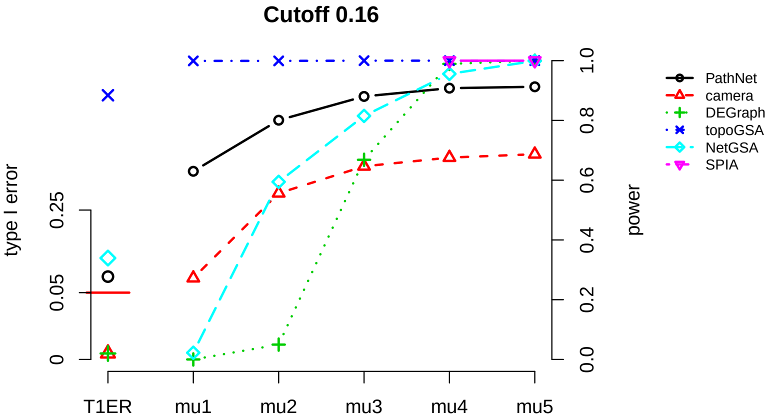

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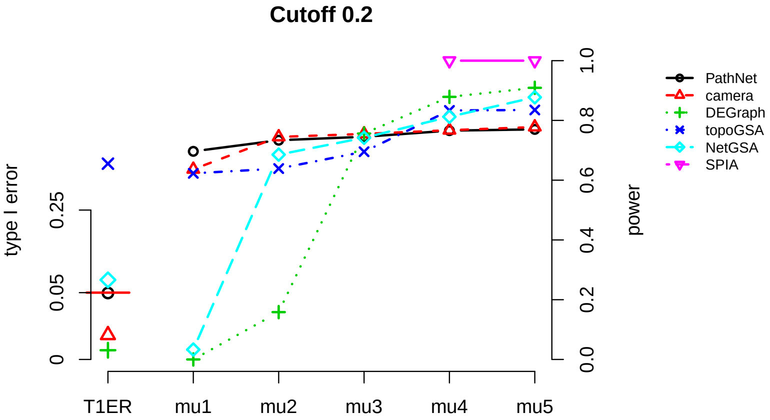

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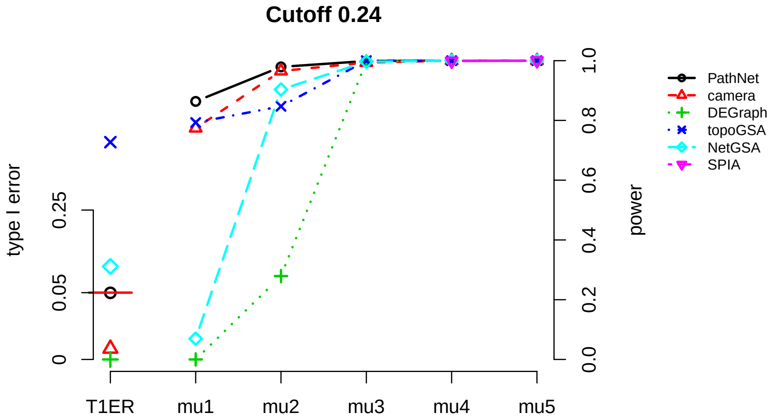

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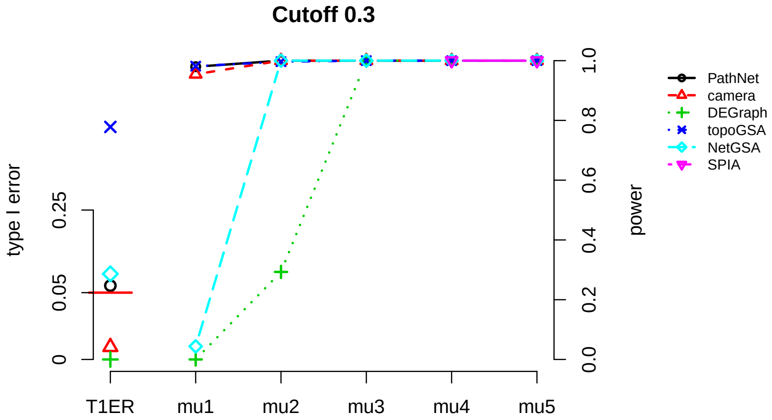

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What if we permute the samples?

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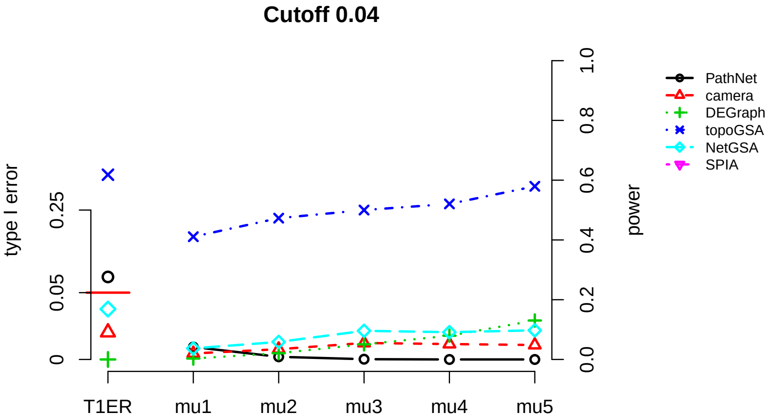

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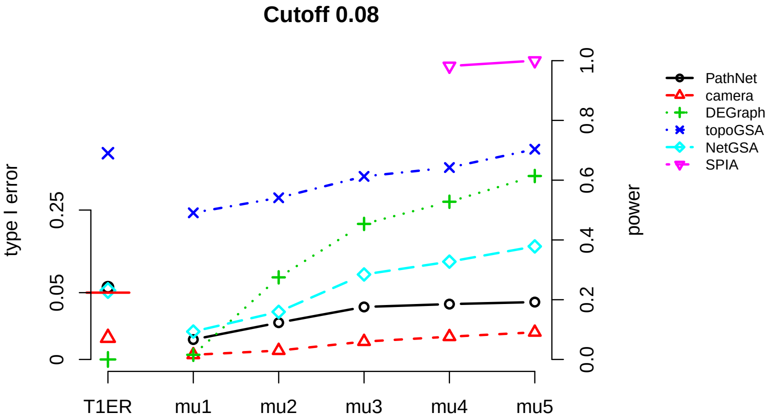

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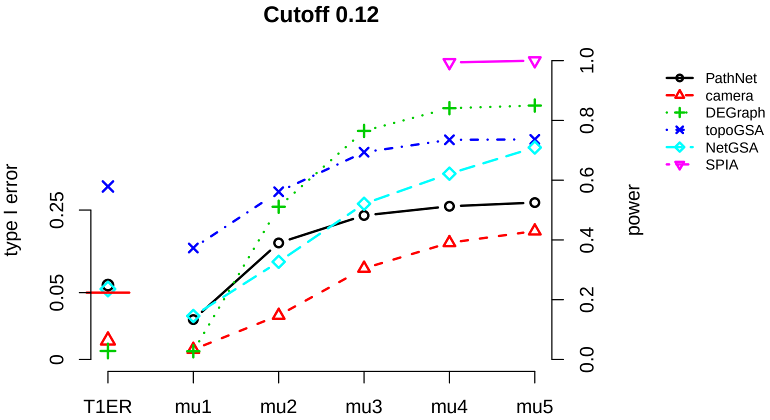

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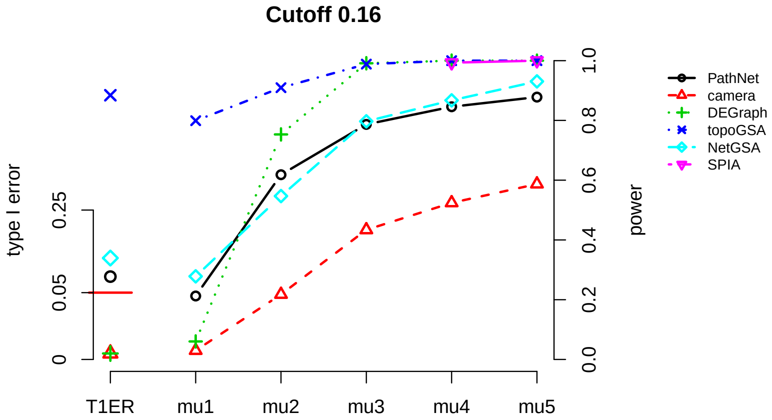

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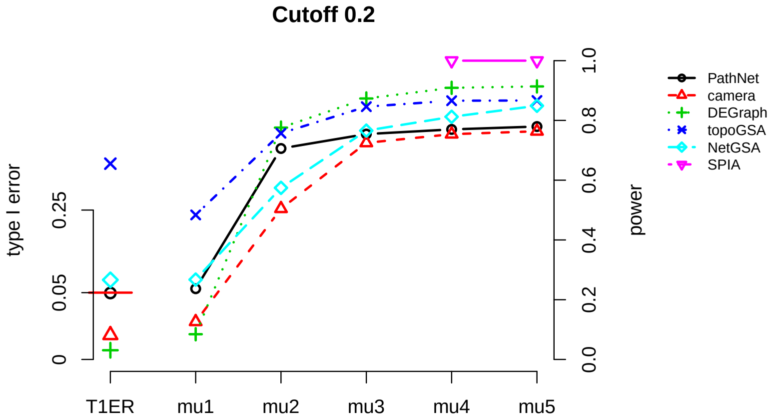

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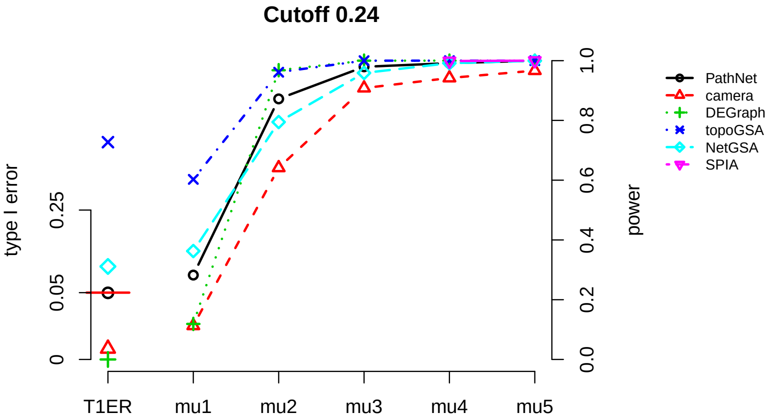

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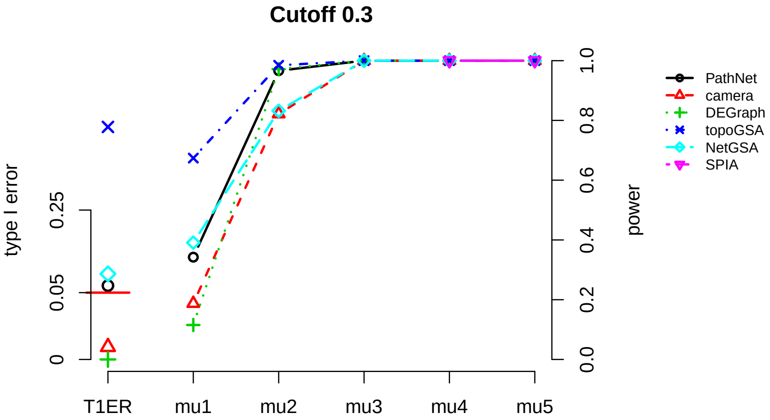

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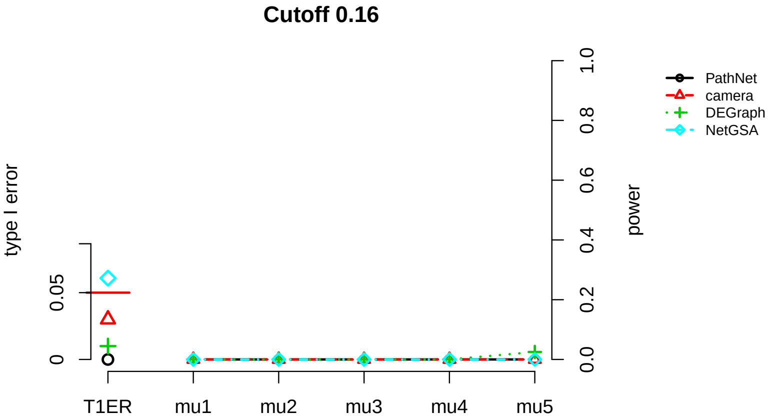

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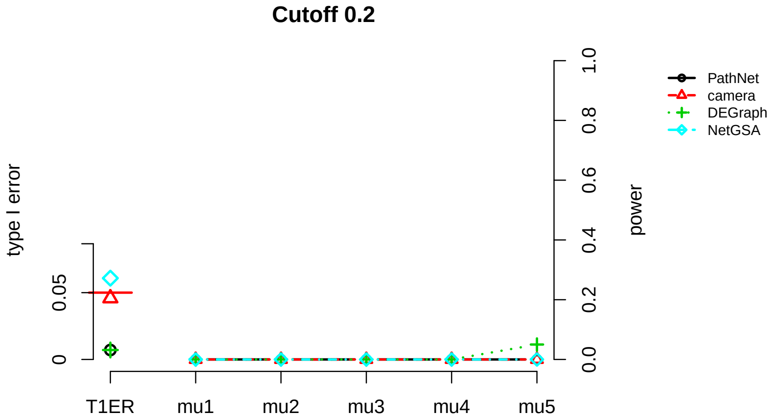

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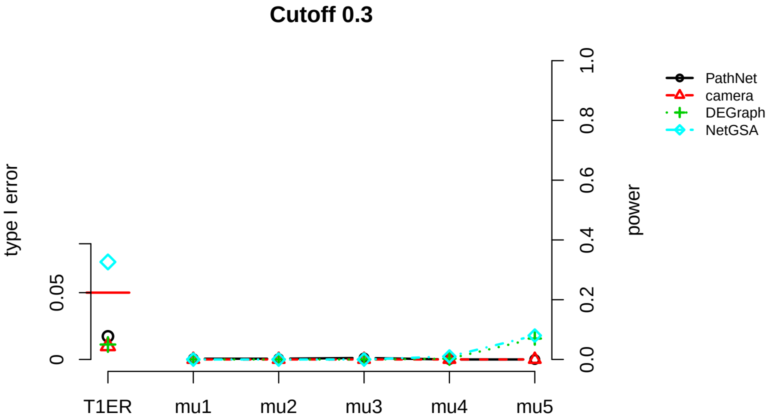

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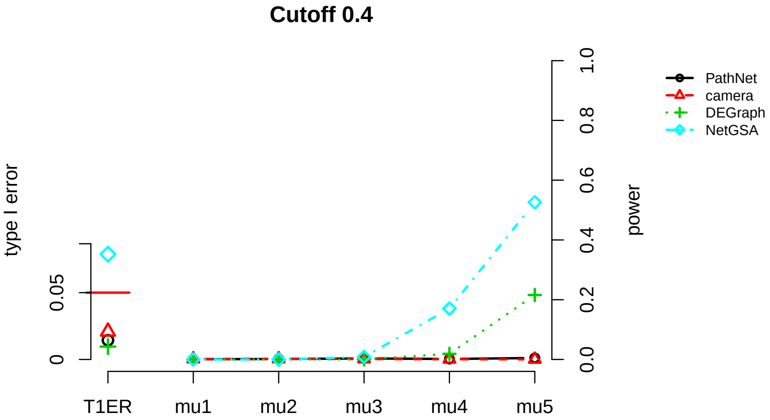

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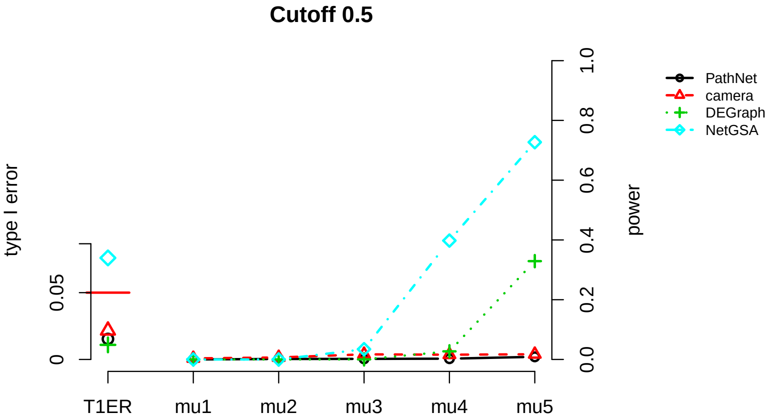

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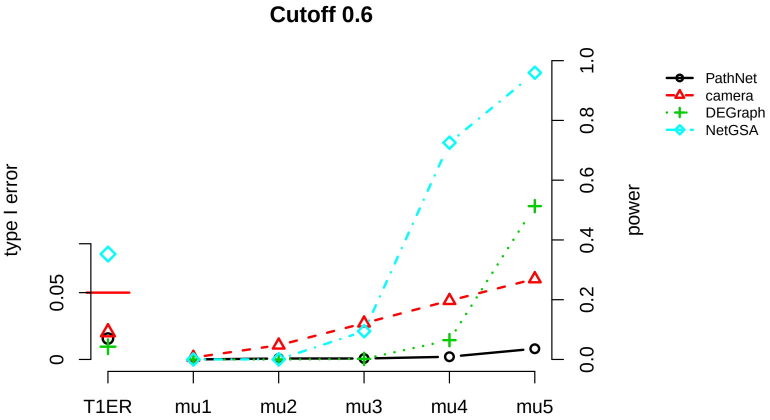

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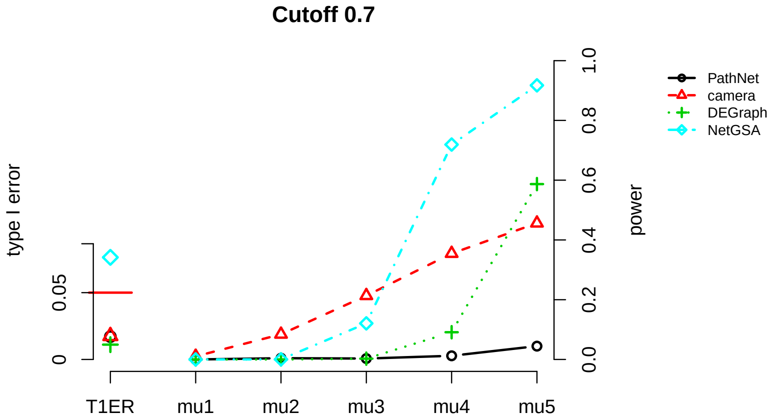

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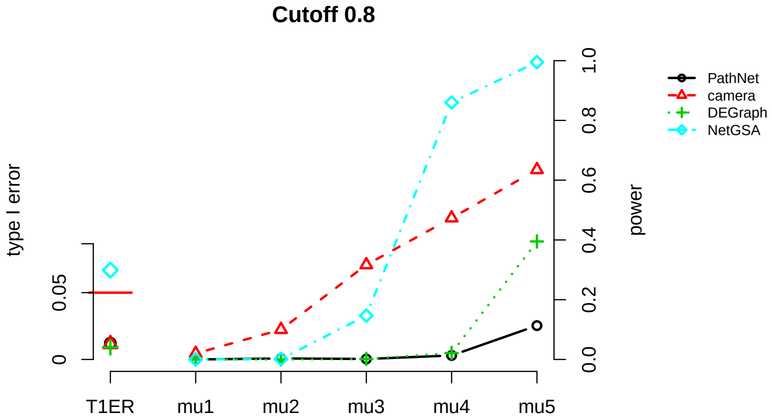
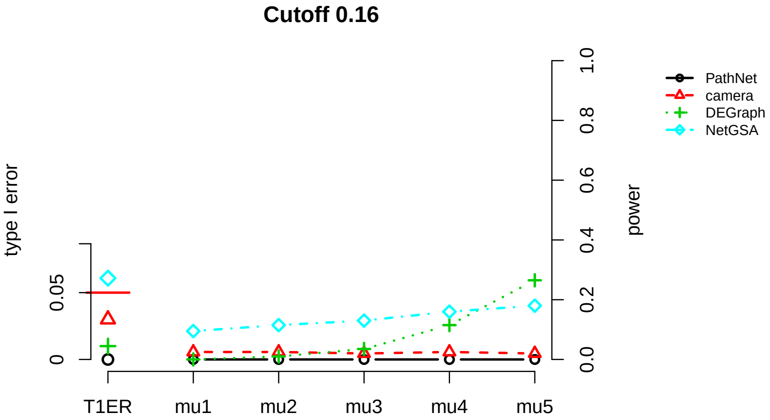


Analysis of Metabolomic Data

What if we permute the samples?

Analysis of Metabolomic Data



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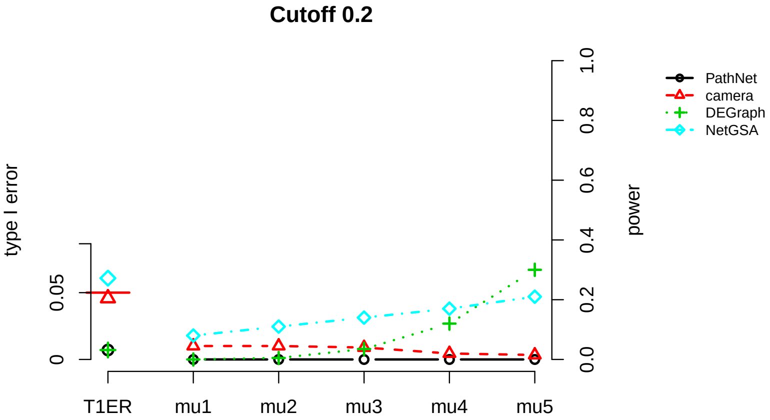

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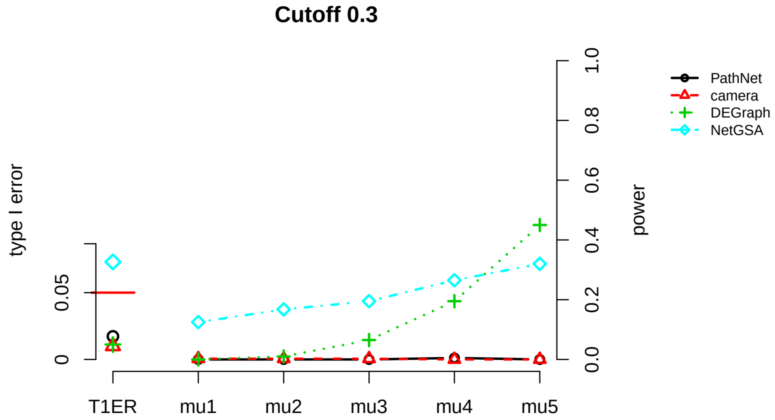

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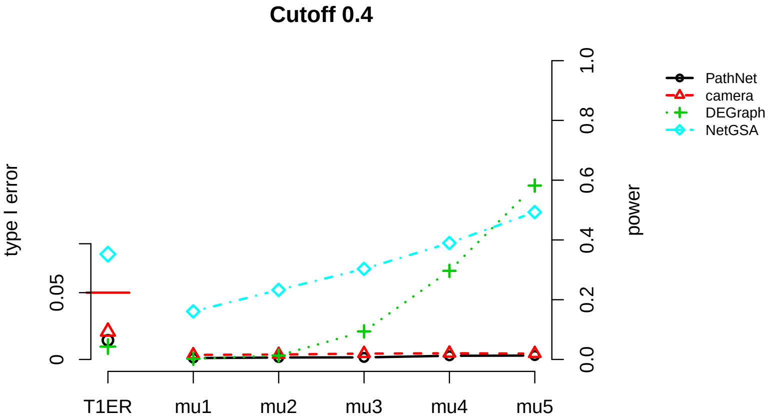

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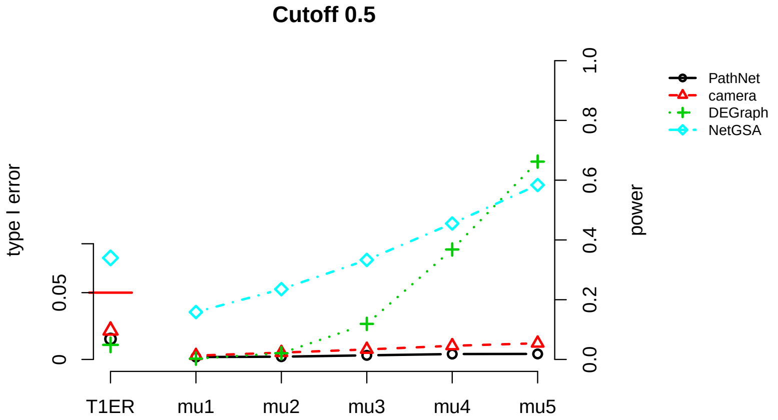

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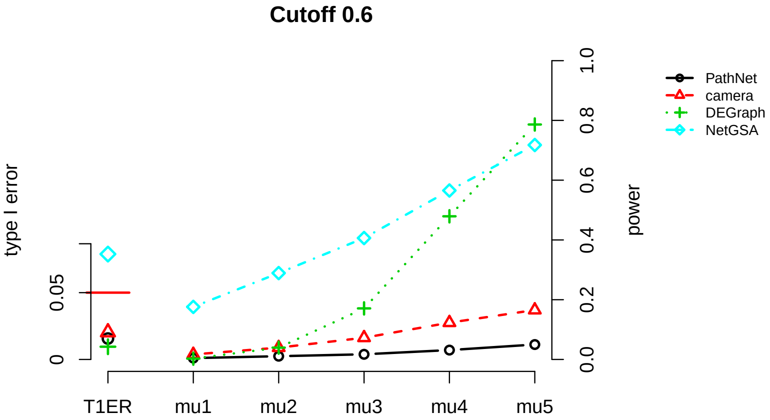

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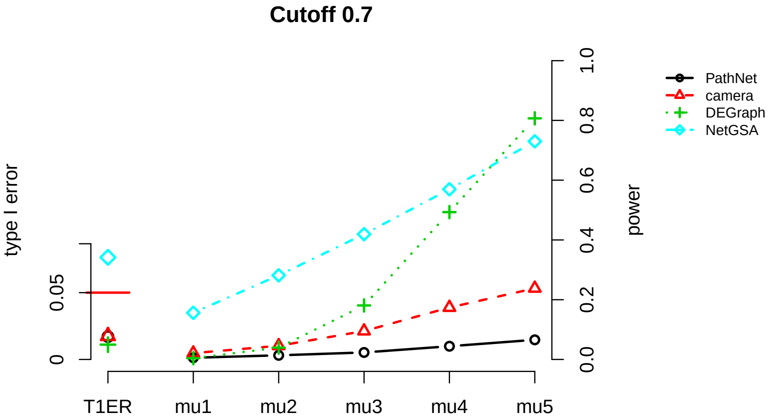

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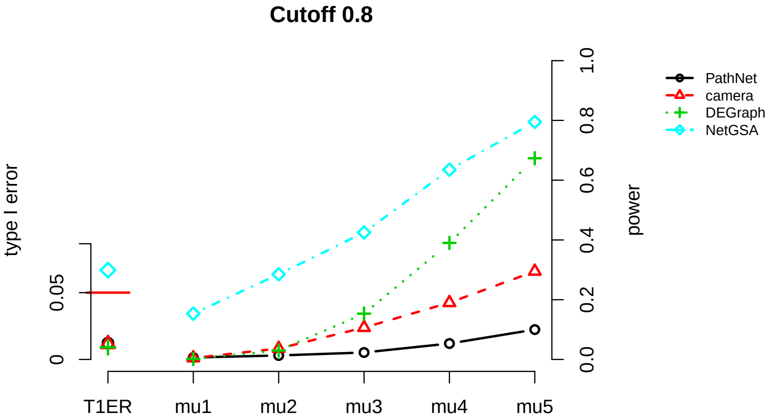

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- topologyGSA can have inflated type I errors (and is very slow!).
- SPIA may not work if the mean signal is very small.

Implementation

R-package:

- netgsa
- New version that implements the HE regression method will be released soon.

Source code: `https://github.com/drjingma/netgsa`

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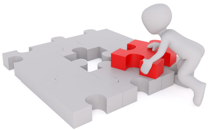

- NetGSA tests for pathway enrichment by incorporating the pathway topology.
- NetGSA can leverage existing network information and expression data.
- Caveat:
  - null hypothesis.
  - sample sizes.