Annotation Enrichment Analysis - An Alternative Method for Evaluating the Functional Properties of Gene Sets

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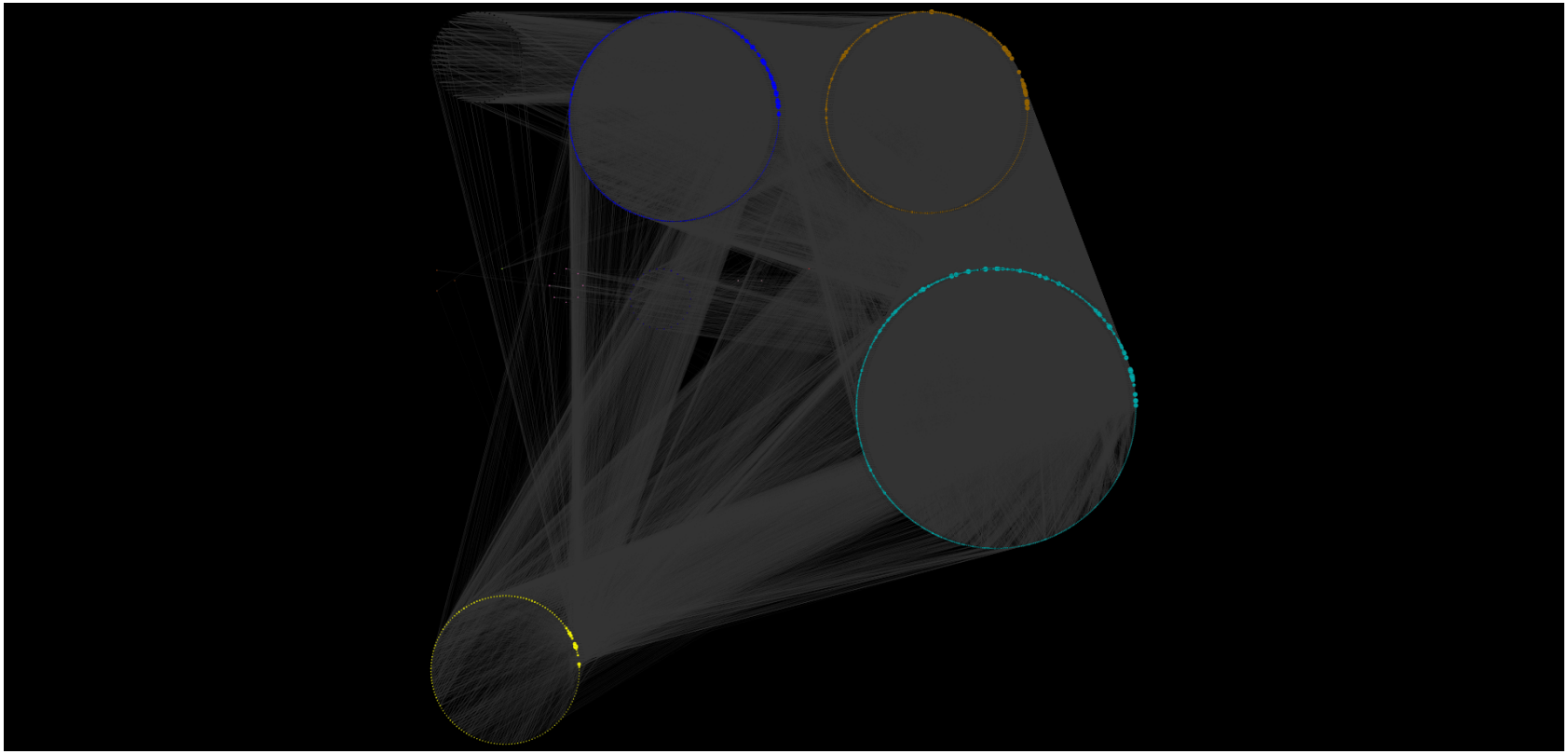
Overview

- Recent high-throughput methods (microarray, RNA-Seq, etc) made it easy to produce large datasets comparing samples in different conditions.
- The end result of many of these analyses, however, is often a large list of genes that are associated with one condition or the other.
- Numerous tools have been developed to look for "enrichment" in these resulting gene sets for genes associated with a particular known pathway or functional annotation.
- These methods (GSEA, etc) often use statistics which make some assumptions about the distribution of annotations which may not be valid.
- What are the effects of these assumptions the resulting interpretation?
- Can we do better?

Example motivation: T. cruzi co-expression network

One example of where this kind of enrichment analysis could be useful is for determining possible roles for clusters of co-expressed genes.



T. cruzi co-expression network modules detected by WGCNA

Background

Gene Ontology (GO)

What is GO?

> “The Gene Ontology is a controlled vocabulary, a set of standard terms—words and phrases—used for indexing and retrieving information. In addition to defining terms, GO also defines the relationships between the terms, making it a structured vocabulary.”

- geneontology.org

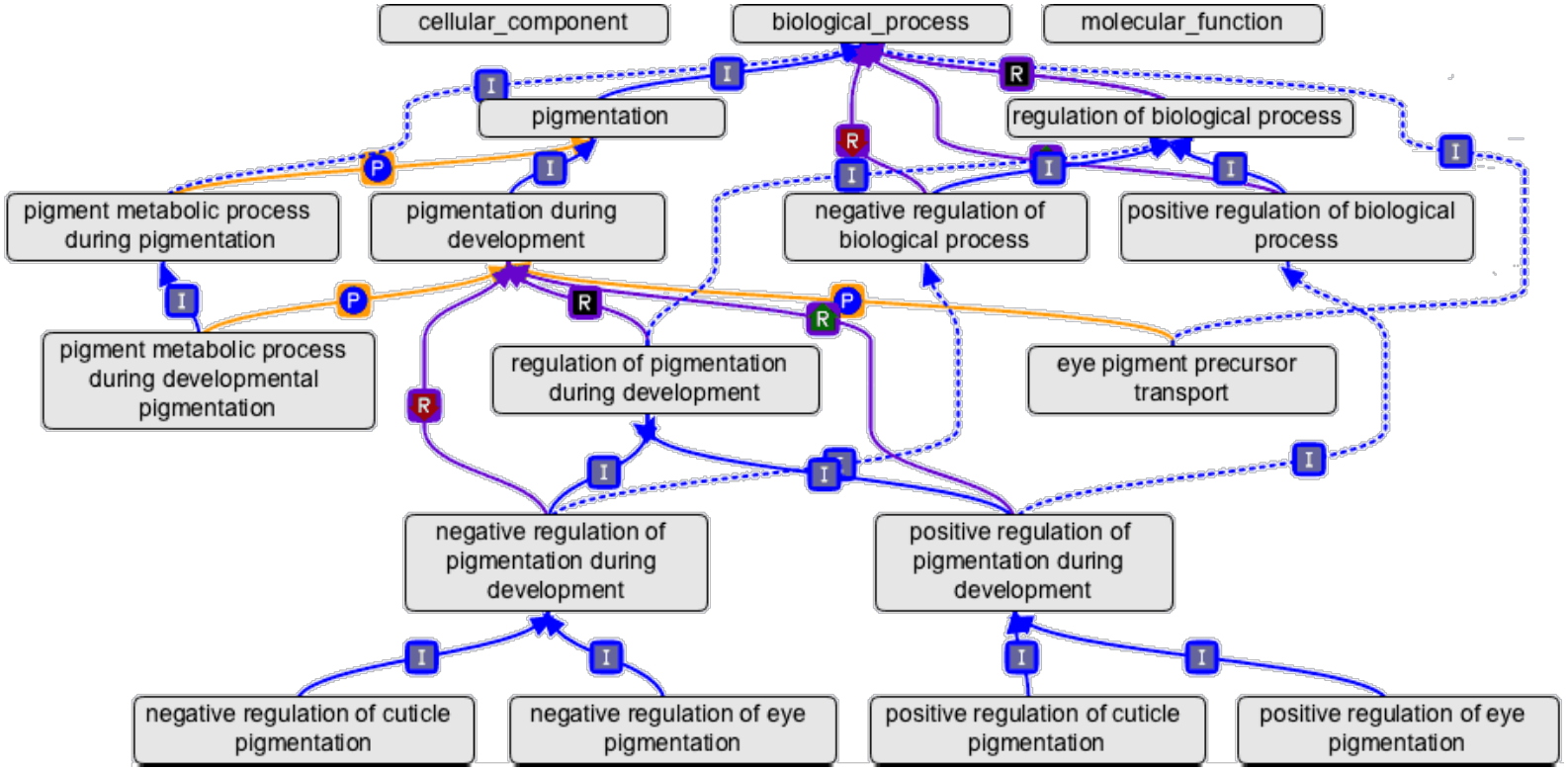
Gene Ontology (GO)

What is GO?

- Provides a common language to describe features of genes from all different species.
- GO database includes two main parts:
  - Ontologies
  - Gene annotations
- Includes three separate ontologies relating to:
  - Location (cellular component)
  - Process/pathway involved in (biological process)
  - Specific function (molecular function)
- Each ontology is represented by a directed acyclic graph (DAG).
- Two different types of relationships exist between nodes:
  - is-a, and
  - part-of
- Deeper levels in the ontology correspond to more specific descriptions.
- Maintained and developed by a consortium of scientists (Gene Ontology Consortium)

Gene Ontology (GO)

Gene Ontology structure



(source: http://www.geneontology.org/GO.ontology.structure.shtml)

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Many functional enrichment tools exist

Table 1. List of 68 enrichment tools

Enrichment tool name	Year of release	Key statistical method	Category
FunSpec	2002	Hypergeometric	Class I
Onto-express	2002	Fisher's exact; hypergeometic; binomial; chi-square	Class I
EASE	2003	Fisher's exact (modified as EASE score)	Class I
FatiGO/FatiWise/FatiGO+	2003	Fisher's exact	Class I
FuncAssociate	2003	Fisher's exact	Class I
GARBAN	2003	Hypergeometric	Class I
GeneMerge	2003	Hypergeometric	Class I
GoMiner	2003	Fisher's exact	Class I
MAPPFinder	2003	*Z*-score; hypergeometric	Class I
CLENCH	2004	Hypergeometric; chi-square; binomial	Class I
GO::TermFinder	2004	hypergeometric	Class I
GOAL	2004	Permutation	Class I
GOArray	2004	Hypergeometric; *Z*-score; permutation	Class I
GOStat	2004	Fisher's exact; chi-squre	Class I
GoSurfer	2004	Chi-square	Class I
OntologyTraverser	2004	Hypergeometric; Fisher's exact	Class I
THEA	2004	Hypergeometric	Class I
BiNGO	2005	Hypergeometric; binomial	Class I
FACT	2005	Adopt GeneMerge and GO::TermFinder statistical modules	Class I
gfinder	2005	Fisher's exact	Class I
Gobar	2005	Hypergeometric	Class I

Huang et al. (2009) Table 1

Common statistics used for enrichment analysis

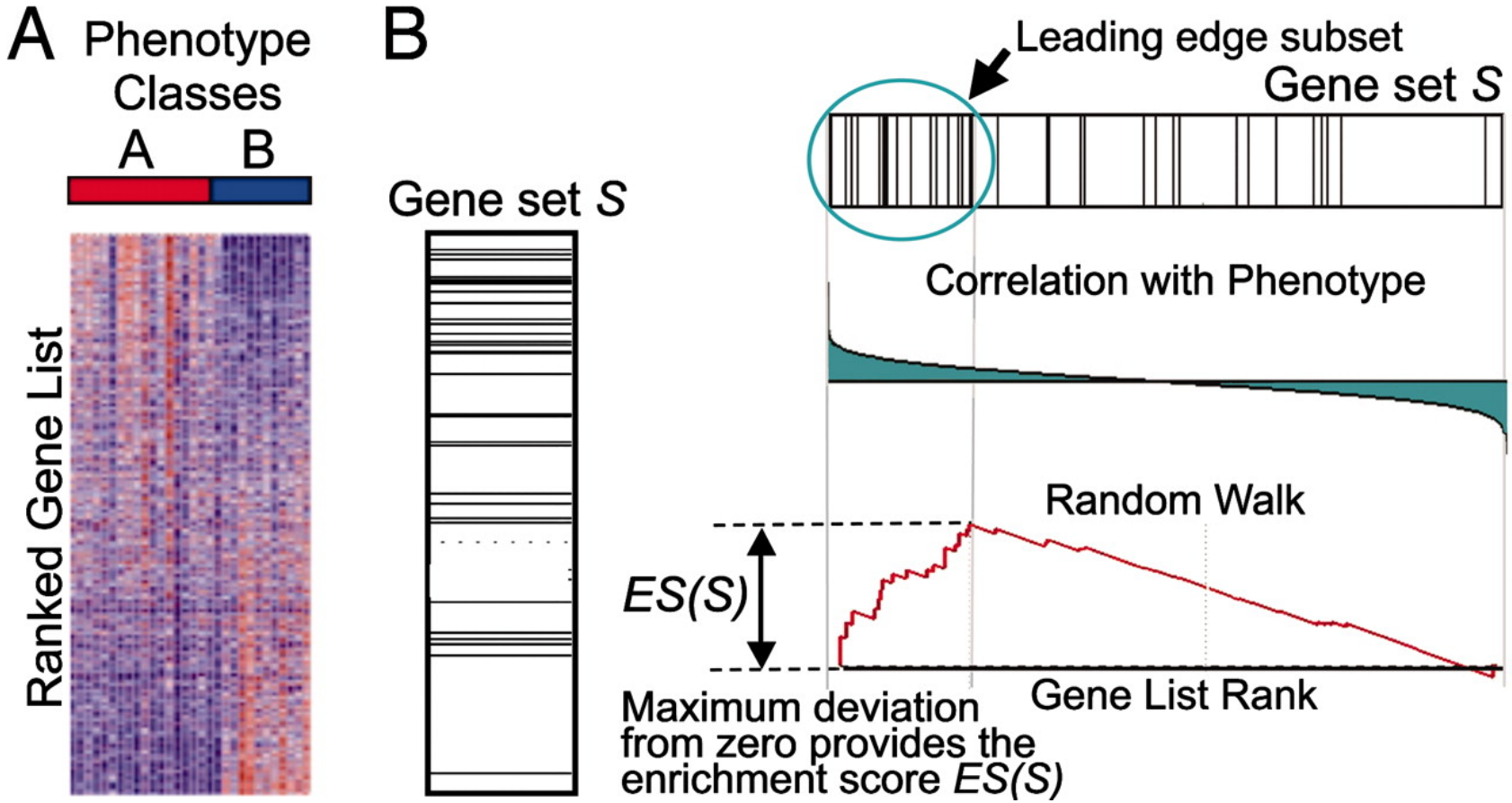
- Fisher's Exact Test (FET)
- Binomial test
- Hypergeometric test
- Chi-squared test

All of these methods assume that, under the null hypothesis, genes are equally likely to be selected.

Gene Set Enrichment Analysis (GSEA)

- Most popular tool for enrichment analysis
- Uses variant of Kolmogorov–Smirnov test
  - Compares distributions of two samples
  - Null hypothesis: the samples were drawn from the same distribution
- Looks for enrichment in genes with a known property (e.g. GO annotation) at the top of a list of genes ranked by differential expression, etc.

Gene Set Enrichment Analysis (GSEA)



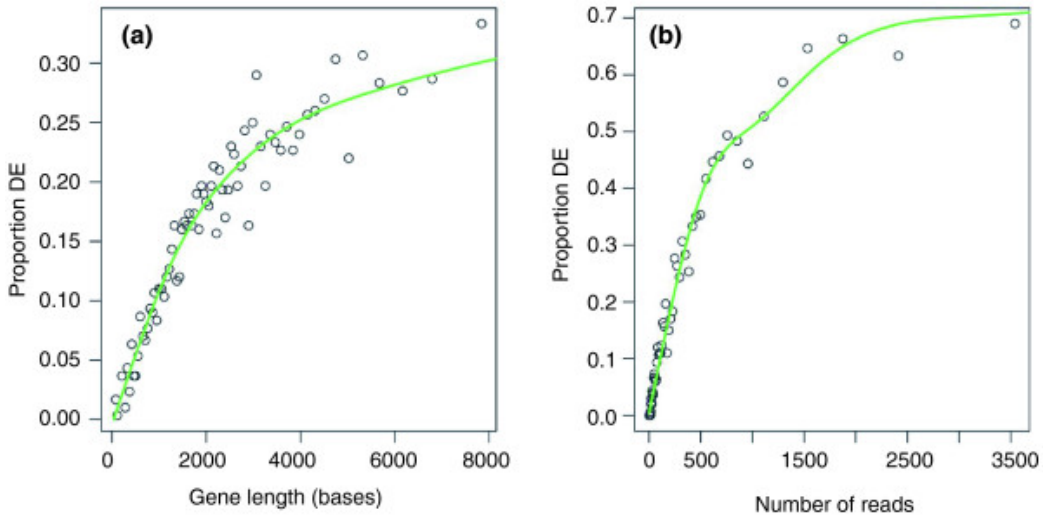
Subramanian et al. (2005) Figure 1

GOstat

- Beißbarth & Speed (2004)
- Computes frequencies of all GO terms in two sets of genes:
  - Experiment set
  - Reference set (e.g. entire GO db)
- Uses χ^2 and Fisher's Exact test to look for terms which are enriched in either gene set with respect to the other.
- Performs multiple testing correction using either Holm or Benjamini and Hochberg correction.
- Website: http://gostat.wehi.edu.au/
- Not to be confused with "GOstats", a Bioconductor package for working with GO and microarray data...

GOSeq

- Young et al. (2010) notice biases between gene length, number of reads, and differential expression.
- They show that GO categories also show a length bias (many categories have significantly longer or shorter genest than expected by chance), which indicates that enrichment results could in turn be skewed by DE length bias.
- GOSeq corrects for the length bias by random sampling of a fitted distribution based on length and DE proportion.



Young et al. (2010) Figure 2

Fisher's Exact Test (FET)

- The most common test statistic used for functional enrichment
- Considers the overlap between experiment gene set and set of genes with some known functional annotation.

	Math. Mag.	Science	
math	5	0	$R_1 = 5$
biology	1	4	$R_2 = 5$
	$C_1 = 6$	$C_2 = 4$	$N = 10.$

Computing P_{cutoff} gives

$$P_{\text{cutoff}} = \frac{5!^2\, 6!\quad 4!}{10!\,(5!\quad 0!\quad 1!\quad 4!)} = 0.0238,$$

and the other possible matrices and their Ps are

$$\begin{bmatrix} 4 & 1 \\ 2 & 3 \end{bmatrix}_P = 0.2381$$
$$\begin{bmatrix} 3 & 2 \\ 3 & 2 \end{bmatrix}_P = 0.4762$$
$$\begin{bmatrix} 2 & 3 \\ 4 & 1 \end{bmatrix}_P = 0.2381$$
$$\begin{bmatrix} 1 & 4 \\ 5 & 0 \end{bmatrix}_P = 0.0238,$$

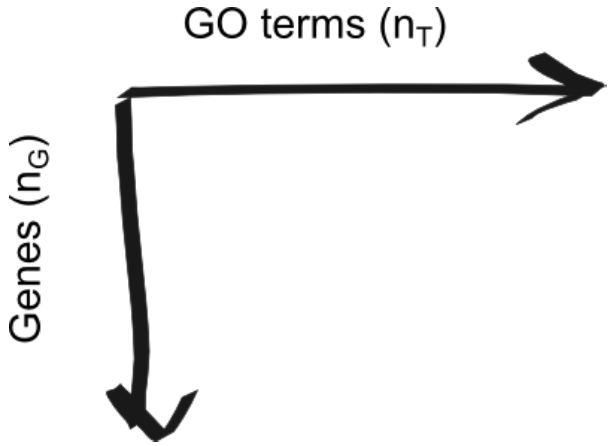
(source: http://mathworld.wolfram.com/FishersExactTest.html)

Results

Gene ontology characteristics

Gene-term graph

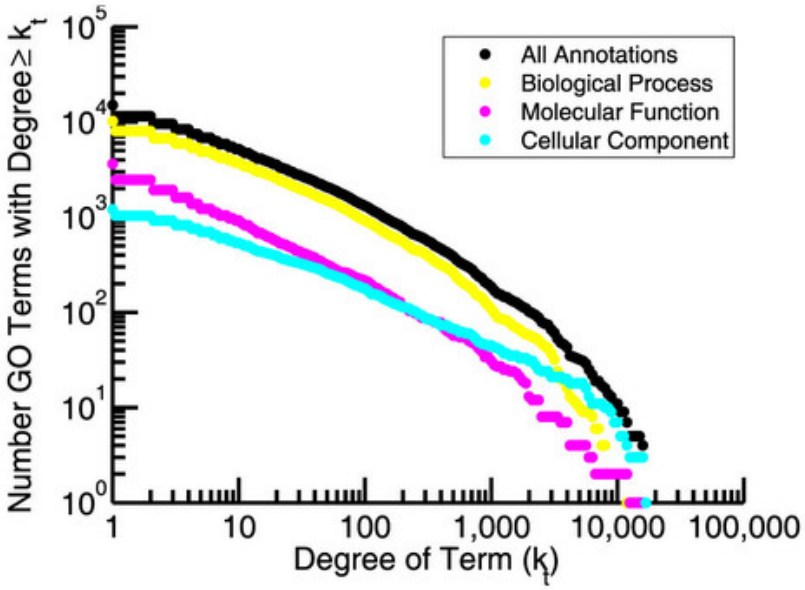
- Downloaded all human gene-term associations from the Gene Ontology website.
- Constructed a gene/annotation graph, represented by an $n_G \times n_T$ adjacency matrix



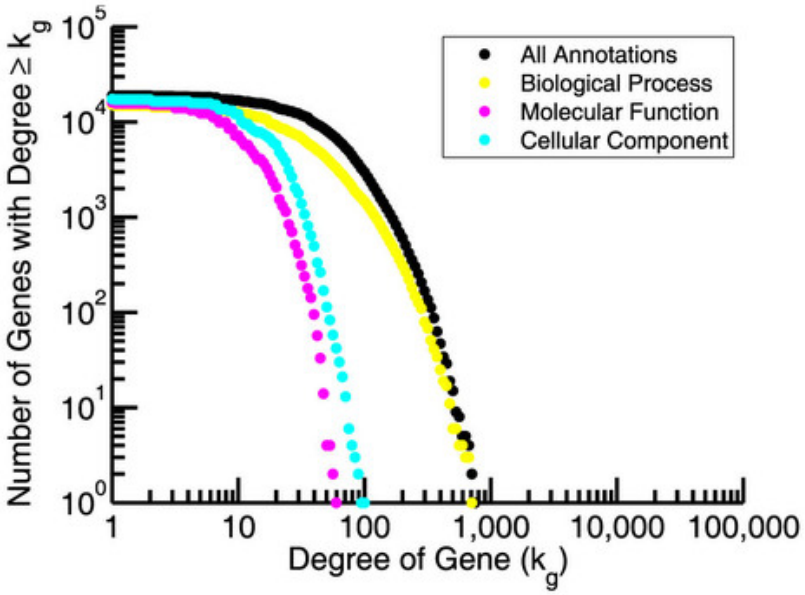
- n_G - number of genes
- n_T - number of GO terms
- $A_{ij} = 1$ - Gene i is annotated with term j
- $A_{ij} = 0$ - Gene i is not annotated with term j

Gene ontology characteristics

Gene and term degree distributions



(a) Term Degree Distribution



(b) Gene Degree Distribution

- Biological Process terms dominate the human annotations.
- Degree of term (k_t) distribution is "heavy-tailed"; most terms are associated with only a few genes, but some terms are used for a huge number of genes.

Gene ontology characteristics

Biological Process

The remainder of the results are based on the biological process ontology:

- 656,783 annotations
- 15,213 genes (avg: 43.2 annotations)
- 10192 terms (avg: 64.4 annotations)

Question: What is the effect of annotation database properties on functional enrichment analysis?

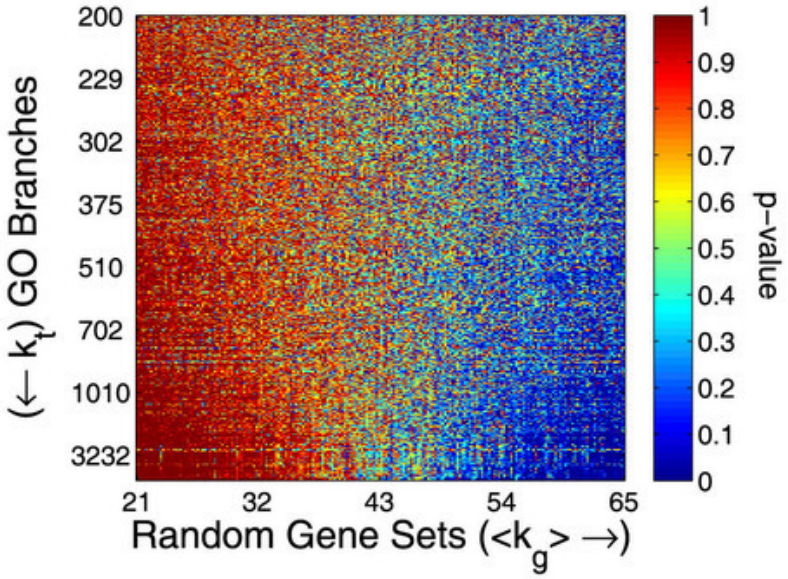
Experiment design:

- Created 200 random gene sets:
  - N_g=200 genes in each set (a "typical" gene set size)
  - Varied number of annotations (M_g)
  - Determined FET enrichment score for each of the 10192 BP GO terms

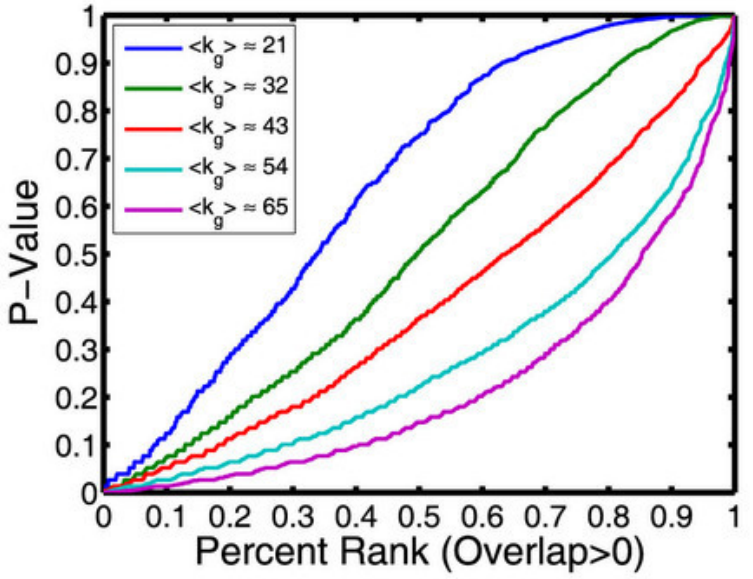
Results

- Number of unique annotations $\propto$ GO enrichment significance!

Random gene set enrichment scores



(a) Fisher's Exact Test (GO Branches)



(b) Distribution of Fisher's Exact Test Results

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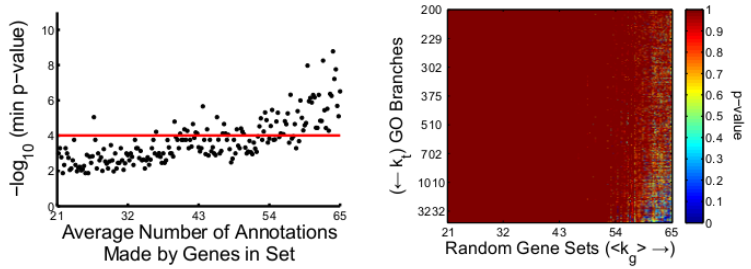
Perhaps the problem isn't quite so bad after correcting for multiple testing...

- Multiple testing correction alone is not enough to deal with this bias, although it does seem to severely reduce the problem.



(a)Min Value of FET (b)FDR q-value

FIG. S2: (a) The minimum p-value estimated by FET across all GO branches for each of the 200 random gene-sets. (b) Q-values associated with the FDR-corrected significance of GO terms in 200 randomly generated gene sets. The terms are ordered based on how many genes are annotated to the term (k_t) and the gene sets are ordered based on total the number of annotations (M_g) made by the 200 genes in that set. Note that although we tested all terms, only the 200 with the highest number of annotations are shown.

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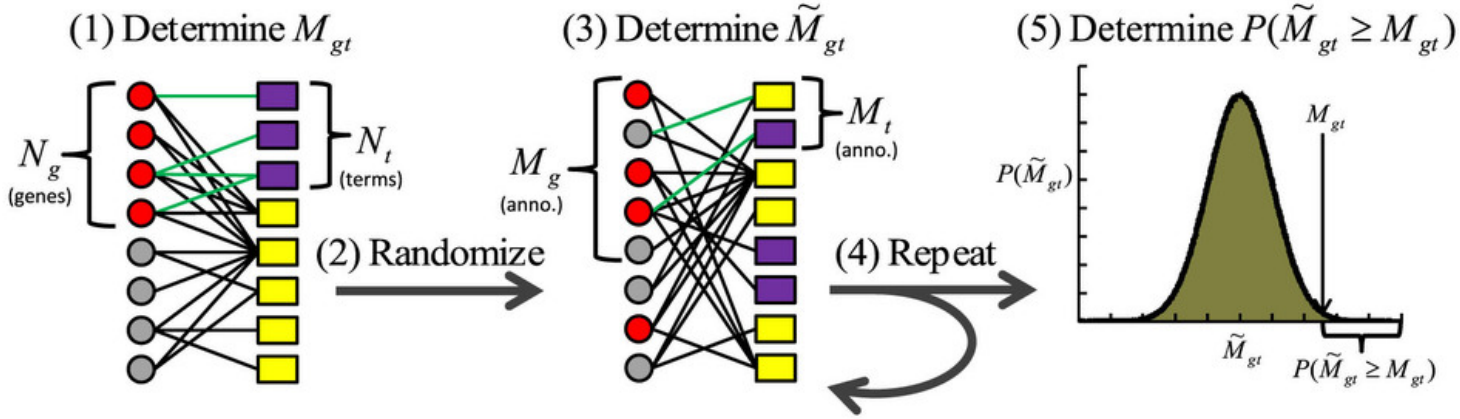
Annotation Enrichment Analysis (AEA)



(1) Determine number of annotations between signature and branch.
(2) Randomize order of genes and terms, preserving original connections.
(3) Determine, $\tilde{M}_{gt}$, the number of annotations between top random genes and the top random terms.
(4) Repeat steps (2)-(3) to build distributions of values.
(5) Determine probability of getting M_{gt} or more annotations between a signature and branch based on this distribution.

Gene in signature
Gene not in signature
Term in branch
Term not in branch

N_g - number of genes in signature
M_g - number of annotations to signature
N_t - number of terms in branch
M_t - number of annotations to branch
M_{gt} - number of annotations between signature and branch
$\tilde{M}_{gt}$ - number of annotations between top random genes and random terms

EXAMPLE:

$N_g = 4;\ M_g = 12$

$N_t = 3;\ M_t = 4;\ M_{gt} = 4$

$\tilde{M}_{gt} = 2$

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Question: Does this bias also affect biologically relevant sets of genes?

Overview

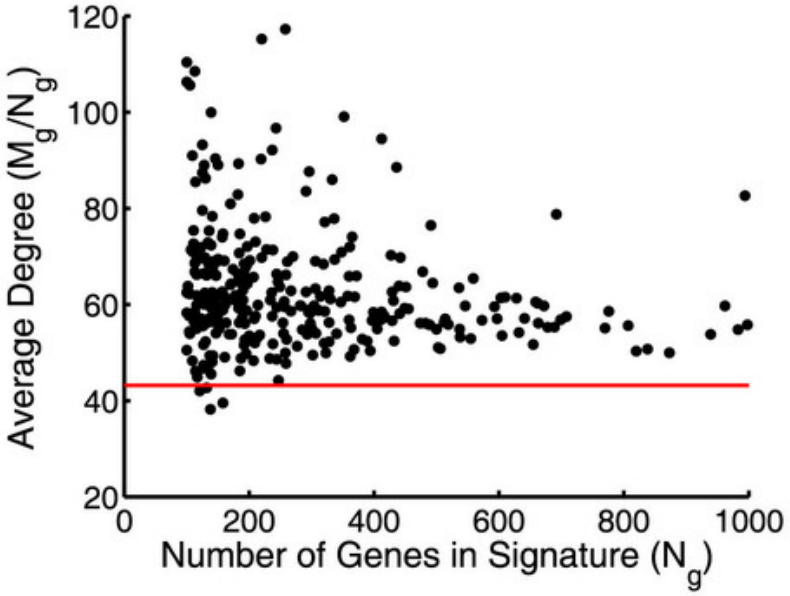
- So far, we've seen how AEA can correct for biases in the distribution of GO terms and annotation coverage across genes.
- Does this have any impact on downstream biological interpretations?

Experiment design:

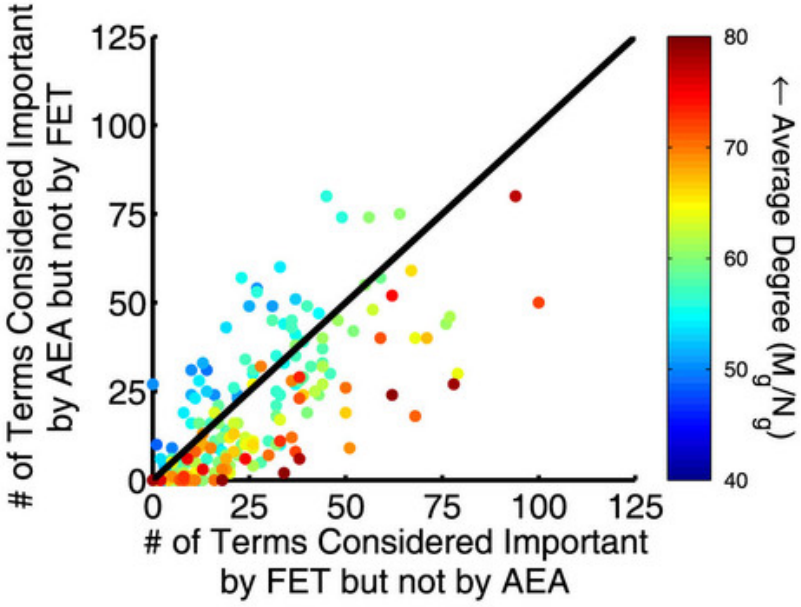
- Downloaded all expression signatures from Gene Signatures Database (GeneSigDB) which contain $100 <= n <= 1000$ genes which are annotated with a term in the BP ontology (total=309)
- First, plotted average number of annotations per gene set and compared it what would be expected for random sets of genes (verify presence of bias.)
- Next, measured enrichment in each set using FET and AEA and looked at properties of genes deemed significant in one measure but not the other.

GeneSigDB Signatures

FET enrichment bias towards well-annotated genes is also present in biological datasets:



(a) Signature Properties



(b) FET vs. AEA on Signatures

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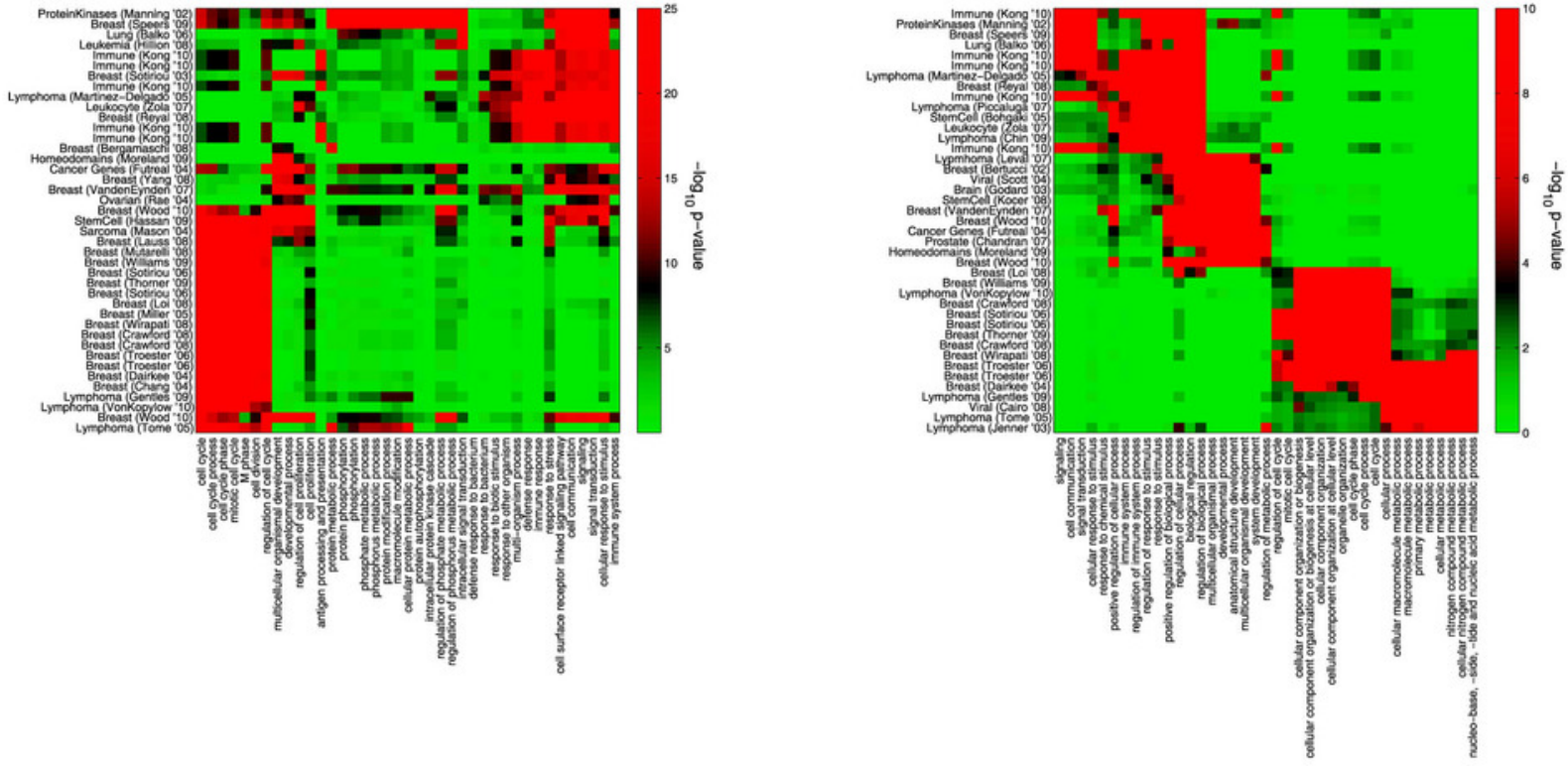
Question: Does AEA provide any additional biological insights?

Experiment design:

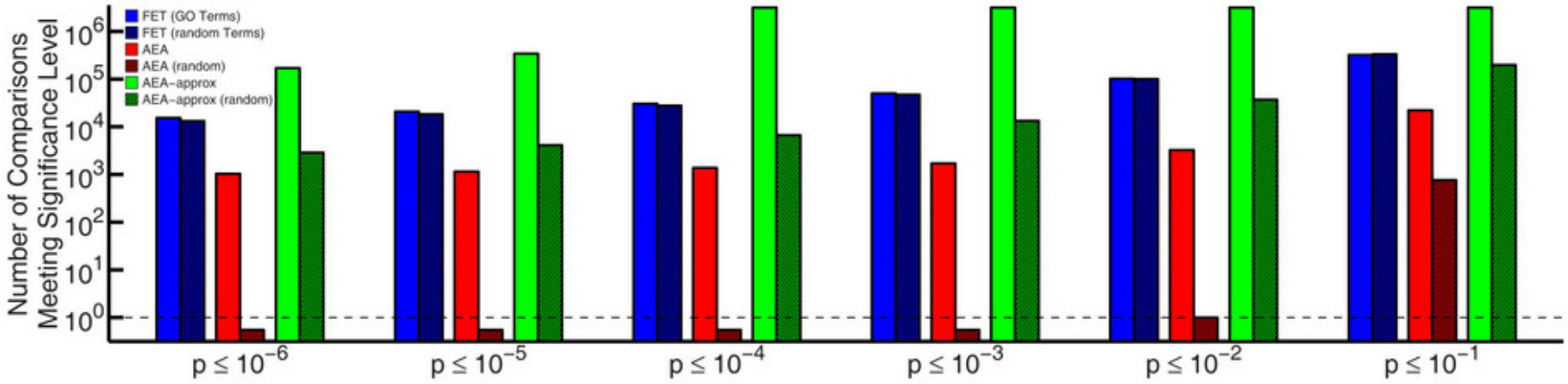
- For FET and AEA, each:
  - Selected ~40 GeneSigDB signatures with the most significant enrichment scores.
  - Select 40 GO terms with most significant enrichment scores across all signatures.
- Performed hierarchical clustering.

Functional enrichment clusters (FET vs. AEA)



- rows = gene signatures
- columns = GO terms

Real and random term-signature comparisons



- Created random term sets with same number of unique genes annotated as found in real GO branches
- Measured FET/AEA enrichment in random and real go branches
- FET find similar numbers of "significant" term-signature associations in the real and random branches!

Conclusions

Conclusions

- Biases exist in GO and other annotation databases.
- These biases can affect the performance of statistics such as FET in predicting significant enrichment.
- Annotation Enrichment Analysis (AEA) accounts for these biases and is able is not as prone to detecting spurious enrichments.

Limitations

- Performance of AEA only compared with Fisher's Exact Test (FET); how does the performance compare to other GO methods?
- Only looked at Biological Process ontology -- is the picture the same for the other GO sub-ontologies? Other annotation databases?
- Currently only implemented in C++ (R bindings would be nice.)
- Does not take into account any of the addition information about the members of the experimental gene set (e.g. DE fold-change, p-value, etc)

Beyond the Gene Ontology...

Input data

Choose an input file to upload. Separate each gene symbol with a new line. For a quantitative set, add a comma and the level of membership of that gene between 0 and 1 after each gene symbol.

Browse... No file selected.

Or paste in a list of gene symbols optionally followed by a comma and levels of membership between 0 and 1 with each gene separated by a new line. Try a regular example or an example of a quantitative set.

0 gene(s) entered

Enter a brief description for the list in case you want to share it. (Optional)

Please acknowledge Enrichr in your publications by citing the following reference:
Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A. Enrichr: interactive and collaborative

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