

# Gene Set Enrichment – Introduction

Martin Morgan ([mtmorgan@fredhutch.org](mailto:mtmorgan@fredhutch.org))  
Fred Hutchinson Cancer Research Center  
Seattle, WA, USA

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# Objective

Is expression of genes in a gene set associated with experimental condition?

- ▶ E.g., Are there unusually many up-regulated genes in the gene set?

Many methods, a recent review is Kharti et al., 2012.

- ▶ Over-representation analysis (ORA) – are differentially expressed (DE) genes in the set more common than expected?
- ▶ Functional class scoring (FCS) – summarize statistic of DE of genes in a set, and compare to null
- ▶ Pathway topology (PT) – include pathway knowledge in assessing DE of genes in a set

# What is a gene set?

**Any** *a priori* classification of 'genes' into biologically relevant groups

- ▶ Members of same biochemical pathway
- ▶ Proteins expressed in identical cellular compartments
- ▶ Co-expressed under certain conditions
- ▶ Targets of the same regulatory elements
- ▶ On the same cytogenic band
- ▶ ...

Sets do not need to be...

- ▶ *exhaustive*
- ▶ *disjoint*

# Collections of gene sets

## Gene Ontology ([GO](#)) Annotation (GOA)

- ▶ CC Cellular Components
- ▶ BP Biological Processes
- ▶ MF Molecular Function

## Pathways

- ▶ [MSigDb](#)
- ▶ [KEGG](#) (no longer freely available)
- ▶ [reactome](#)
- ▶ [PantherDB](#)
- ▶ ...

# Collections of gene sets

E.g., [MSigDb](#)

- ▶ c1 Positional gene sets – chromosome & cytogenic band
- ▶ c2 Curated Gene Sets from online pathway databases, publications in PubMed, and knowledge of domain experts.
- ▶ c3 motif gene sets based on conserved cis-regulatory motifs from a comparative analysis of the human, mouse, rat, and dog genomes.
- ▶ c4 computational gene sets defined by mining large collections of cancer-oriented microarray data.
- ▶ c5 GO gene sets consist of genes annotated by the same GO terms.
- ▶ c6 oncogenic signatures defined directly from microarray gene expression data from cancer gene perturbations.
- ▶ c7 immunologic signatures defined directly from microarray gene expression data from immunologic studies.

# Work flow

1. Experimental design
2. Sequencing, quality assessment, alignment
3. Differential expression

and then...

4. Perform gene set enrichment analysis
5. Adjust for multiple comparisons

## Approach 1: hypergeometric tests

1. Classify each gene as 'differentially expressed' DE or not, e.g., based on  $p < 0.05$
2. Are DE genes in the set more common than DE genes not in the set?
3. Fisher hypergeometric test, *GOstats*
  - ▶ Conditional hypergeometric to accommodate GO DAG, *GOstats*
  - ▶ But: artificial division into two groups (DE vs. not DE)

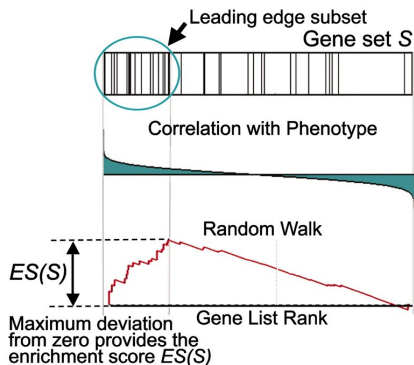
	In gene set?	
	Yes	No
DE	$k$	$K$
Not DE	$n - k$	$N - K$

`fisher.test()`

## Approach 2: enrichment score

Mootha et al., 2003; modified  
Subramanian et al., 2005.

1. Sort genes by log fold change
2. Calculate running sum: incremented when gene in set, decremented when not.
3. Maximum of the running sum is enrichment score  $ES$ ; large  $ES$  means that genes in set are toward top of list.
4. Permuting subject labels for significance



Subramanian et al., 2005, fig 1.

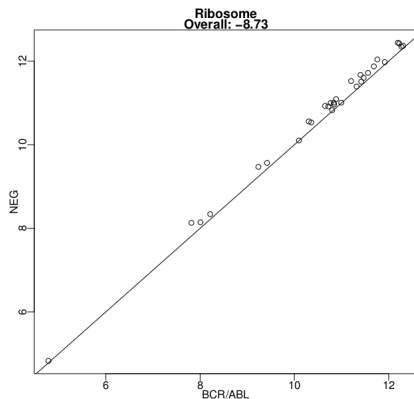


## Approach 3: category $t$ -test

E.g., Jiang & Gentleman, 2007;

### Category

1. Summarize  $t$  (or other) statistic across genes in each set
2. Test for significance by permuting the subject labels
3. Much more straight-forward to implement



Expression in NEG vs BCR/ABL samples for genes in the 'ribosome' KEGG pathway; [Category](#) vignette.

# Competitive versus self-contained null hypothesis

Goemann & Bühlmann, 2007

- ▶ Competitive null: The genes in the gene set do not have stronger association with the subject condition than other genes. (Approach 1, 2)
- ▶ Self-contained null: The genes in the gene set do not have any association with the subject condition. (Approach 3)
- ▶ Probably, self-contained null is closer to actual question of interest
- ▶ Permuting subjects (rather than genes) is appropriate

## Approach 4: linear models

E.g., Hummel et al., 2008, *GlobalAncova*

- ▶ Colorectal tumors have good ('stage II') or bad ('stage III') prognosis. Do genes in the p53 pathway (*just one gene set!*) show different activity at the two stages?
- ▶ Linear model incorporates covariates – sex of patient, location of tumor

*limma*

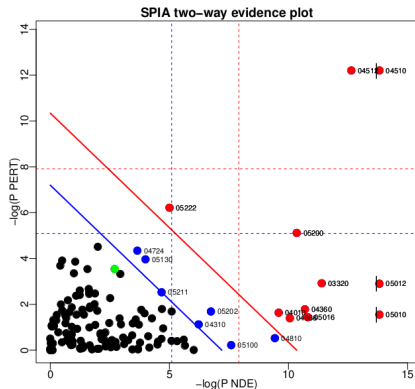
- ▶ Majewski et al., 2010 `romer` and Wu & Smythe 2012 `camera` for enrichment (competitive null) linear models
- ▶ Wu et al., 2010: `roast`, `mroast` for self-contained null linear models

## Approach 5: pathway topology

- ▶ Incorporate pathway topology (e.g., interactions between gene products) into significance testing

E.g., Tarca et al., 2009, *SPIA*

- ▶ Signaling Pathway Impact Analysis
- ▶ Combined evidence: pathway over-representation  $P_{NDE}$ ; unusual signaling  $P_{PERT}$  (equation 1 of Tarca et al.)



Evidence plot, colorectal cancer.

Points: pathway gene sets.

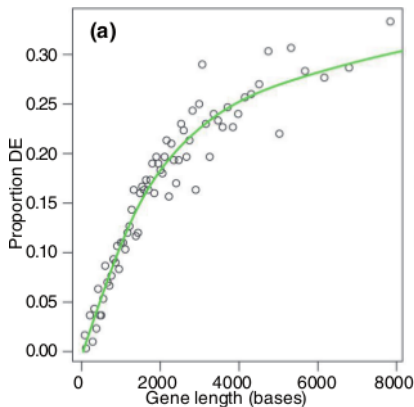
Significant after Bonferroni (red) or FDR (blue) correction.

## Approach 6: issues with sequence data?

- ▶ All else being equal, long genes receive more reads than short genes
- ▶ Per-gene  $P$  values proportional to gene size

E.g., Young et al., 2010, *goseq*

- ▶ Hypergeometric, weighted by gene size
- ▶ Substantial differences
- ▶ Better: read depth??



DE genes vs. transcript length.

Points: bins of 300 genes. Line:

fitted probability weighting function.

## Approach 7: *de novo* discovery

- ▶ So far: analogous to supervised machine learning, where pathways are known in advance
- ▶ What about unsupervised discovery?

Example: Langfelder & Horvath, WGCNA

- ▶ Weighted correlation network analysis
- ▶ Described in Langfelder & Horvath, 2008

## Representing gene sets in R

- ▶ Named `list()`, where names of the list are sets, and each element of the list is a vector of genes in the set.
- ▶ `data.frame()` of set name / gene name pairs
- ▶ *GSEABase* – input from standard file formats, representation as formal classes.

# Conclusions

## Gene set enrichment classifications

- ▶ Kharti et al: Over-representation analysis; functional class scoring; pathway topology
- ▶ Goemann & Bühlmann: Competitive vs. self-contained null

## Selected *Bioconductor* Packages

Approach	Packages
Hypergeometric Enrichment	<i>GOstats</i> , <i>topGO</i>
Category <i>t</i> -test	<i>limma::romer</i>
Linear model	<i>Category</i>
Pathway topology	<i>GlobalAncova</i> , <i>GSEAIm</i> , <i>limma::roast</i>
Sequence-specific	<i>SPIA</i>
	<i>goseq</i>



## References

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Partly based on a presentation by Simon Anders, CSAMA 2010<sup>1</sup>.

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<sup>1</sup>[http://marray.economia.unimi.it/2009/material/lectures/L8\\_Gene\\_Set\\_Testing.pdf](http://marray.economia.unimi.it/2009/material/lectures/L8_Gene_Set_Testing.pdf)

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