

# Gene Set Enrichment – Introduction

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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 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
- ▶ Issues with sequence data?
- ▶ Issues with single-cell data?

# 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](#)
- ▶ [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*; *limma*::goana()

...

- Conditional hypergeometric to accommodate GO DAG, *GOstats*
- But: artificial division into two groups (DE vs. not DE)

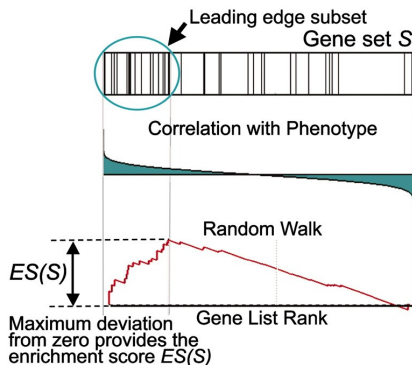
|        | In gene set? |         |
|--------|--------------|---------|
|        | Yes          | No      |
| DE     | $k$          | $K$     |
| Not DE | $n - k$      | $N - K$ |

`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



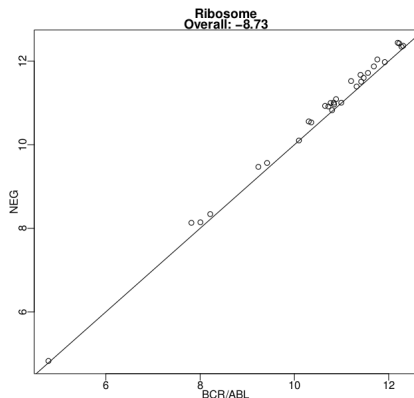
Subramanian et al., 2005, fig 1.

## 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. Distinguishing more from less important sets. (Approach 1, 2)
- ▶ Self-contained null: The genes in the gene set do not have any association with the subject condition. Assessing individual sets. (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*

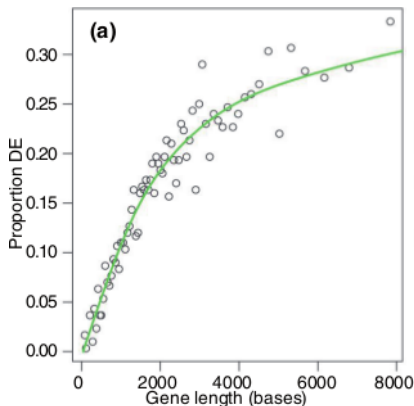
- ▶ Majewski et al., 2010 `romer()` and Wu & Smythe 2012 `camera()` for enrichment (competitive null) linear models
- ▶ Wu et al., 2010: `roast()`, `mroast()`, (and `fry()` – efficient) for self-contained null linear models

## Approach 5: 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 6: *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

# Issues with single-cell data?

- ▶ Often, projections into reduced dimensions.
- ▶ Not genes *per se*, but weightings.
- ▶ An open issue, with opportunities for new methods!

# 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.

# Benchmarks

A recent [tweet](#) from Levi Waldron provides a nice summary.

- ▶ *GSEABenchmarkR* for running benchmarks
- ▶ Self-contained tests often call random gene sets significant
- ▶ Hypergeometric test performs relatively well!

# 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 (see [biocViews](#))

| Approach                  | Packages                                                                         |
|---------------------------|----------------------------------------------------------------------------------|
| Hypergeometric Enrichment | <i>GOstats</i> , <i>topGO</i> , <i>limma</i> ::goana()<br><i>limma</i> ::romer() |
| Category <i>t</i> -test   | <i>Category</i>                                                                  |
| Linear model              | <i>GlobalAncova</i> , <i>GSEAlm</i> , <i>limma</i> ::fry()                       |
| Pathway topology          | <i>SPIA</i>                                                                      |
| Sequence-specific         | <i>goseq</i>                                                                     |
| Visualization             | <i>pathview</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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