Newton *et al.*'s "Random-set methods identify distinct aspects of the enrichment signal in gene-set analysis"

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Presentation goals

1. Describe *Newton et al.*'s flexible approach to gene set enrichment based on random sets
2. Compare empirical & theoretical properties of random set methods with those of SAFE/GSEA

Analysis of gene expression microarray study

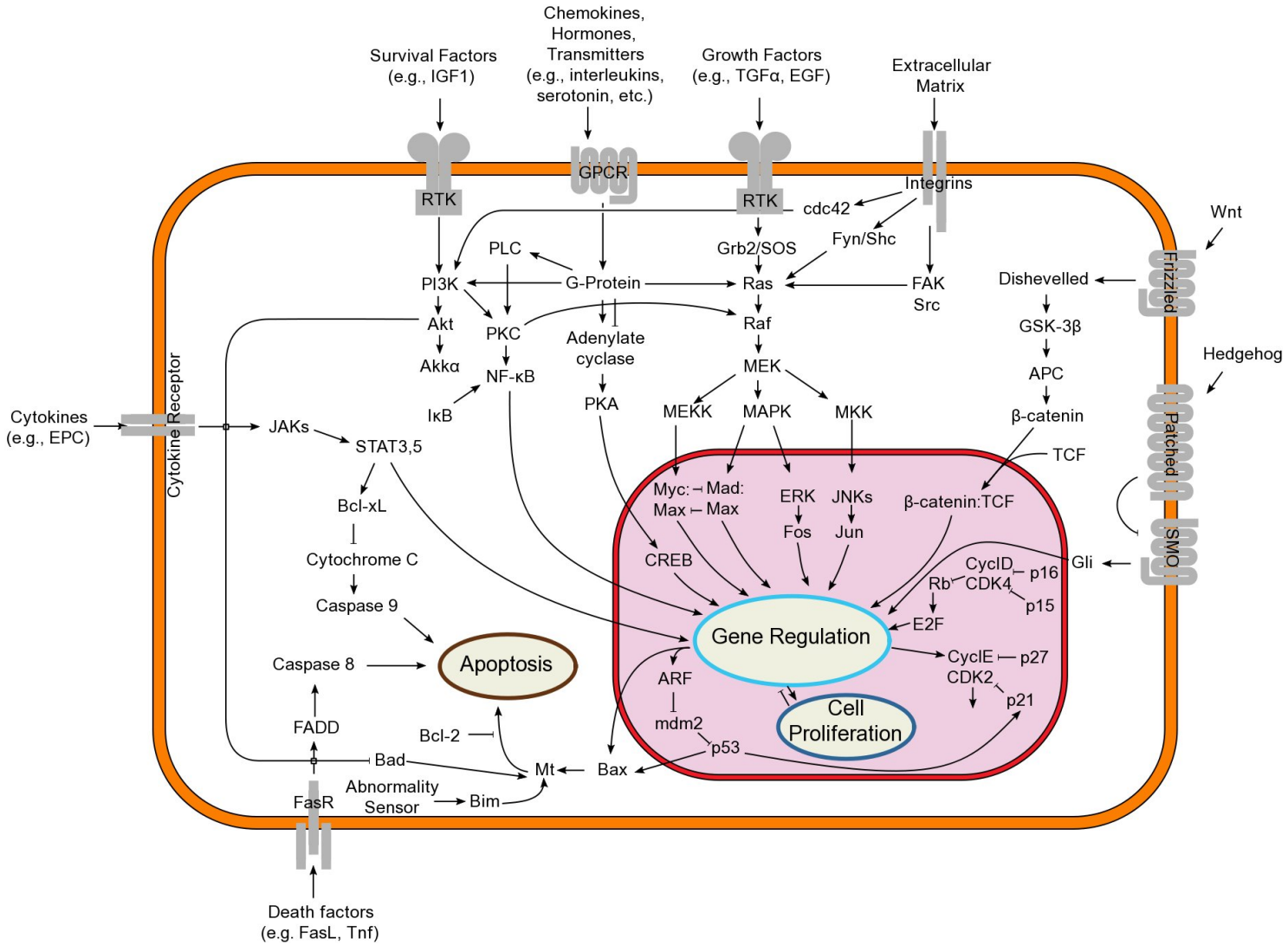
How to extract biological information from microarray results

- Identify differentially expressed genes among, for example, two classes of subjects
- Assess for related biological functions of gene products
- Gene set enrichment can be useful to identify shared biology among differentially expressed genes

Definitions

- Gene set: a collection of genes whose products are known to share biological function
  - Examples include genes whose products participate in a single known cellular signaling cascade
  - For present purposes, we'll focus on gene sets in the Gene Ontology database
- Gene set enrichment: over-representation of differential expression signal in a given gene set

Cell signaling pathways as examples of GO sets



Two existing approaches to gene set enrichment

Selection

- Choose a short list of genes with 'most altered' expression levels
- Evaluate, via Fisher's exact test (or similar test) intersection of short list and functional GO sets to get a score per GO set
- A GO set score is high if far more than expected short list genes are in the GO set

SAFE/GSEA permutation

- Retain information on all genes & permute gene labels to measure significance of set-level statistics from gene-level statistics

Limitations of above approaches

Limitations of selection approach

- Enrichment results depend on selection stringency
- Gives equal weight to genes at both 'ends' of the short list

Limitations of SAFE/GSEA permutation approach

- Computational burden, since it uses microarray data themselves, rather than results of DE analysis

Overview of *Newton et al.*'s approach

- Borrows from both SAFE/GSEA and selection approaches to combine GO set-level statistics (like SAFE/GSEA) but calibrate them like Fisher's exact test calibrates the intersection of a functional GO set and a short list of genes
- Calibration is conditional on DE analysis results since *Newton et al.* consider set-level statistics that would be achieved by random sets of genes.
- *Newton et al.* derive formulas for mean and variance of this conditional distribution of gene set scores
  - Hence, Monte Carlo methods may not be needed

Random-set enrichment scoring

- Let $g \in G$ index the genes
- Denote by $\{s_g\}$ the collection of scores for the genes
  - s_g could be indicator of being on the short list of DE genes
  - Alternatively, could be a more quantitative statistic

Consider a single category C containing m genes

- Consider unstandardized enrichment scores $\bar{X} = \frac{1}{m}\sum_{g \in C} s_g$ as random variables
  - Randomness arises from nature of assignment of genes to be in C
  - Recall that we want to compare the observed gene set scores to those we would see for hypothetical sets
- Treat C as though it were drawn uniformly at random (without replacement) from the $\binom{G}{m}$ possible sets

Extension to more general quantitative gene scores

- $\bar{X}$'s distribution becomes intractable when we consider more general quantitative scores, but we can avoid MC methods with formulas for the first two moments
- Conditional on gene-level scores,

$$\mathbb{E}\bar{X} = \frac{\sum_{g=1}^{G} s_g}{G} \tag{1}$$

$$var(\bar{X}) = \frac{1}{m}\left(\frac{G-m}{G-1}\right)\left\{\left(\frac{\%sum_{g=1}^{G} s_g^2}{G}\right) - \left(\frac{\sum_{g=1}^{G} s_g}{G}\right)^2\right\} \tag{2}$$

- Consider $Z = \frac{\bar{X}-\mathbb{E}\bar{X}}{\sigma}$
  - Z has mean zero & variance 1 under H_0: C is not enriched in DE genes

Random-set enrichment scoring

Represent

Gene	1	2	3	⋯			⋯	G	
Selected	0	1	0	⋯	1	1	⋯	1	n
In category	1	1	1	⋯	1	0	⋯	0	m
In both		1			1				x

		Selected: yes	Selected: no	
C		x	$m-x$	m
not C		$n-x$	$G-n-m+x$	$G-m$
		n	$G-n$	G

Permute

Permuted	1	0	1	⋯	0	0	⋯	0	n
In category	1	1	1	⋯	1	0	⋯	0	m
In both	1		1						X

implies $X \sim$ Hypergeometric

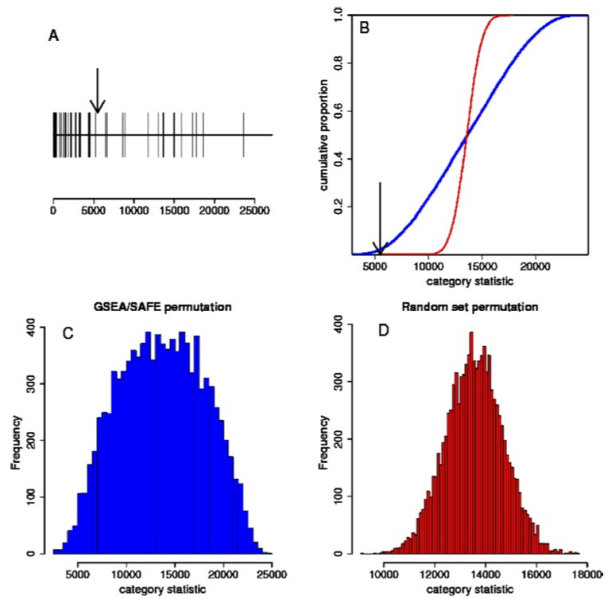
Generalize

Gene score	s_1	s_2	s_3	⋯			⋯	s_G	
In category	0	1	0	⋯	1	0	⋯	0	m
Combined		s_2			s_g				x

permuting, $X/m \sim (\mu, \sigma^2)$

- Proceed from first table to second by permuting (entries in) either of the two rows
- Then generalize to quantitative s_g, where we can still calculate mean & variance

Random sets v. SAFE/GSEA



- Panel A: rank plot of probe set correlation scores
  - $m = 48$ probe sets for a single GO category, GO:0019883
  - arrow marks the mean rank
- Random sets method shuffles the labels that are already in GO:0019883
- SAFE/GSEA shuffles labels on original chip data
- Category statistic, for this example, is rank of correlation scores, but we could use other category statistics
- SAFE p-value: 0.02; random sets p-value: $< 10^{-10}$

Two strategies with random sets

Strategy 1: Selection

- Start with a short list of extremely altered genes
- Ask if there is over-representation of in a GO category

Strategy 2: Averaging

- Averages gene-level evidence across all genes in the GO category

Comparing selection to averaging

- Each approach has a domain of superiority; neither is always preferred

Statistics

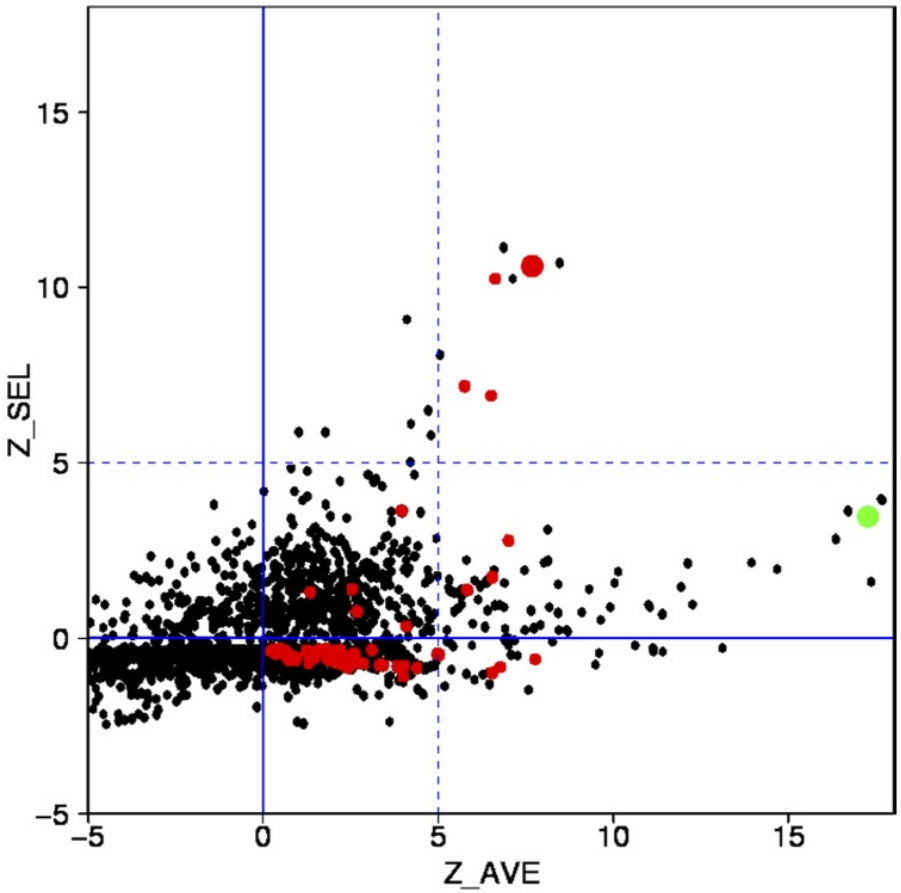
$$\bar{X}_{ave} = \frac{1}{m}\sum_{g \in C} s_g, \bar{X}_{sel} = \frac{1}{m}\sum_{g \in C} \mathbb{1}_{[s_g > k]} \tag{3}$$

Comparing GO categories

We frame the problem as a test of the null hypothesis that C is not enriched.

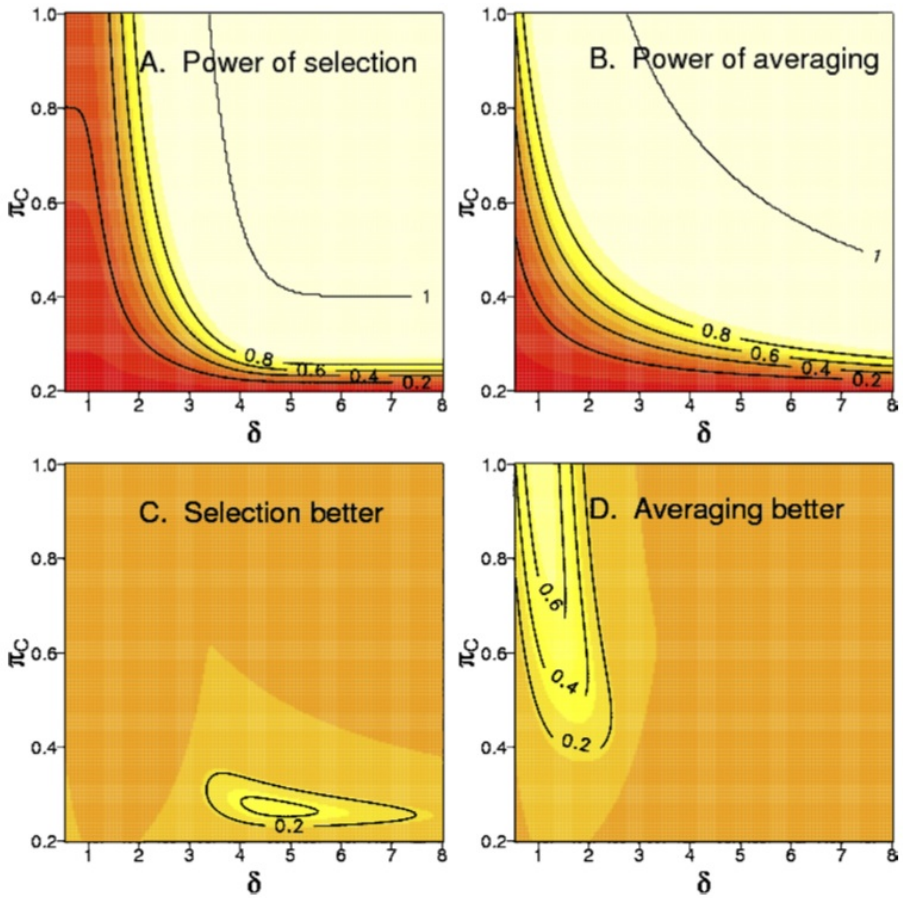
- Suppose that each gene g is either truly DE ($I_g = 1$) or not ($I_g = 0$) between two states
- Let $\pi = \frac{1}{G} \sum_{g=1}^{G} I_g$ be the fraction of genes that are truly DE
- Category C (with m genes) contains a fraction $\pi_C = \frac{1}{m} \sum_{g \in C} I_g$ of DE genes
- Hence, we write $H_0 : \pi_C = \pi$ and $H_1 : \pi_C > \pi$
- We note that enrichment can then be defined by the quantity $\pi_C - \pi$.

Averaging v. Selection



- Each point is a single GO category
- 2761 GO categories plotted (each with $m \geq 10$)
- Significant correlation between Z_{ave} and Z_{sel}
- But many categories are outliers in only one method

Averaging v. Selection: Power comparison



- Consider one category with 20 genes
- $\pi = 0.20$
- red means low power
- Both methods increase in power as effect size increases or enrichment increases

Averaging v. Selection: Power comparison

Averaging

- A test based on $\bar{X}_{ave}$ has sampling distribution $N(\delta\pi_C, \frac{1}{m})$
- Hence, power of level-α test is $1 - \Phi(\tau_{ave})$, where

$$\tau_{ave} = z_\alpha - \sqrt{m}(\pi_C - \pi)\delta \tag{4}$$

Selection

- With a normal approximation, power for $\bar{X}_{sel}$ is $1 - \Phi(\tau_{sel})$, where

$$z_\alpha \frac{\sigma(\pi)}{\sigma(\pi_C)} - \sqrt{m}(\pi_C - \pi)[\Phi(k) - \Phi(k - \delta)]/\sigma(\pi_C) \tag{5}$$

- k is a function of π, δ and α^* and chosen to give a DFDR-controlled gene list at level α^*

Conclusions

- Random-set methods offer a more flexible approach than SAFE/GSEA and enable detection of distinct aspects of enrichment signal
- Within random-set methods, both selection and averaging strategies have regions of superiority that depend on enrichment, effect size δ, and number of genes in the GO category of interest

References

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