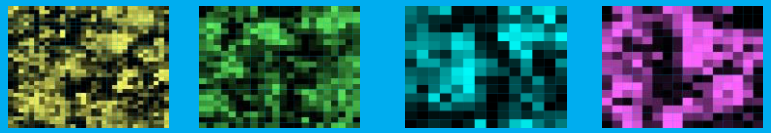
Gene Set Enrichment Analysis (GSEA) Part II



Network Analysis in Systems Biology

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A simple example: Ranking the data

- Suppose we have two classes of data, one may be from (A) muscle biopsies of patients not having diabetes, and (B) with diabetes.
- Take the simple data set:

Class A:

Gene	Sample 1	Sample 2
A	1.0	1.0
B	1.2	1.1
C	0.5	0.6
D	0.7	0.4
E	0.2	0.4

Class B:

Gene	Sample 3	Sample 4
A	2.0	1.9
B	0.6	0.7
C	0.1	0.2
D	2.2	2.0
E	0.3	0.2

- Now rank the genes according to descending differential expression across the classes:

	Class A		Class B	
	Sample 1	Sample 2	Sample 3	Sample 4
D	0.7	0.4	2.2	2.0
A	1.0	1.0	2.0	1.9
E	0.2	0.4	0.3	0.2
C	0.5	0.6	0.1	0.2
B	1.2	1.1	0.6	0.7

A simple example: Generating the running sum

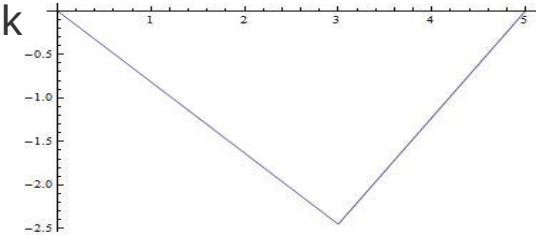
- We would like to test whether the set of genes {B, C}, which we know belongs to a particular biological category, play a significant role in the difference between the two classes of data.
- We next move down the list of genes shown in the previous table and record a running sum. If we encounter a gene which is not in our test set then we add: $-\sqrt{\frac{G}{N-G}}$

  If we encounter a gene which is in our set then we add: $\sqrt{\frac{N-G}{G}}$

  Where N is the number of genes in the microarray, and G is the number of genes in our test set.

A simple example: The supremum of the running sum

- If we calculate this running sum for the ranked data shown in the last table we obtain a walk

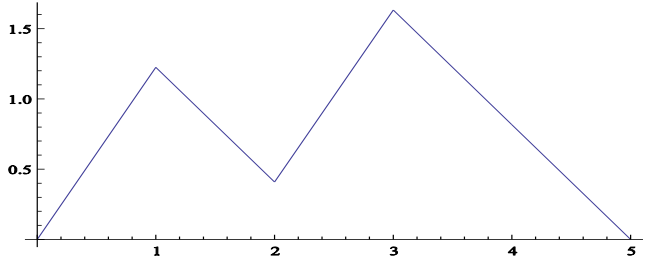


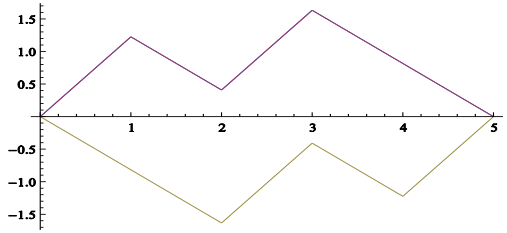
- Note the similarity to the Brownian Bridge.
- The supremum of this walk gives a quantification of the significance of the gene set. If it is significantly positive then the genes are significantly positively differentially expressed.
- If the supremum is significantly negative then the gene set is significantly negatively differentially expressed.
- The significance of the supremum is rated against similar random walks which have been constructed from the data by randomly permuting the class labels.

A simple example: Estimating the significance

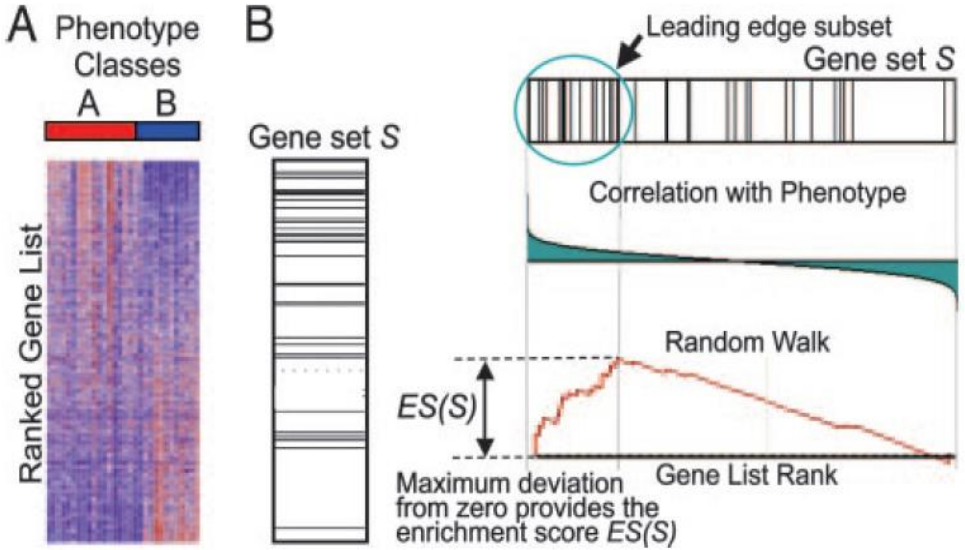
- We permute the class labels of the data – this preserves the correlation structure of the data while randomizing with respect to class
- Repeat the running sum process to produce a new walk.
- Repeat the randomization process many times.
- The number of times that the actual supremum is larger than the random gives an estimate of the significance of the result.
- In the example, the supremum of -2.5 from the data is larger than all the randomized suprema – so we would conclude that the set {B, C} is significant.
- Finally, correct for multiple hypothesis testing

	Class A		Class B	
Gene	Sample 1	Sample 3	Sample 4	Sample 2
A	1.0	2.0	1.9	1.0
B	1.1	0.6	0.7	1.2
C	0.6	0.1	0.2	0.5
D	0.4	2.2	2.0	0.7
E	0.4	0.3	0.2	0.2



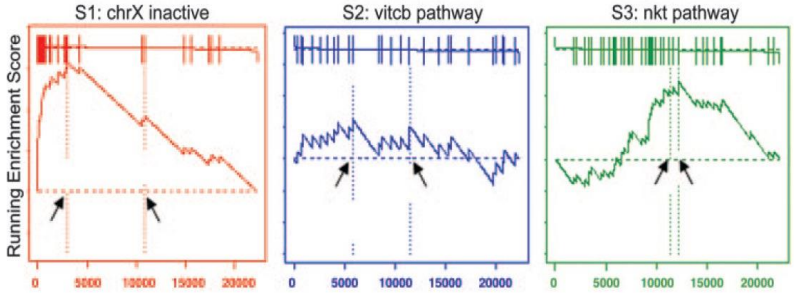


Summary of GSEA



A. Subramanian et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. PNAS, **102**, 43 (2005)

An example from the literature



The distribution of three gene sets, from the C2 functional collection, in the list of genes in the male/female lymphoblastoid cell line example ranked by their correlation with gender: S1, a set of chromosome X inactivation genes; S2, a pathway describing vitamin c import into neurons; S3, related to chemokine receptors expressed by T helper cells.

A. Subramanian et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. PNAS, **102**, 43 (2005)

An example of GSEA in the literature

- In: “PGC – -responsive genes involved in oxidative phosphorylation are coordinately down-regulated in human diabetes” Mootha, V. K. *et al* 2003 *Nat. Genet*. **34** 267-27 The authors tested expression in muscle samples from 43 age-matched males. 17 had normal glucose tolerance, 8 with impaired glucose tolerance, and 18 had type II diabetes.
- The authors identified a set of genes associated with oxidative phosphorylation to be significant
- Each individual gene in the set was only down-regulated by a small amount, but the down-regulation was well coordinated across the members of the set.
- They could use the biological mechanism of oxidative phosphorylation to investigate the biological mechanism of the disease.

Conclusion

- GSEA is a statistical test which can identify sets of genes, belonging to a particular biological category, which play an important role in distinguishing between two classes of gene expression data.
- The test is particularly sensitive as small changes which are coordinated across the set can be detected.
- The test helps reveal the biological mechanisms responsible for the difference between the two classes because the test set has an *a priori* biological theme.