



Gene Set Enrichment Analysis

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Outline

- Description of the experimental setting
- Defining gene sets
- Description of the original GSEA algorithm
 - proposed by Mootha et al (2003)
- Our approach + some extensions

Experiments/Data

- there are n samples
- for each sample G different genes are measured
- the resultant data are stored in a matrix \mathbf{X} ($G \times n$)
- a univariate, per gene, statistic can be computed, \mathbf{x} , ($G \times 1$)
 - often a t-test comparing two groups, but we can pretty much deal with anything

Differential Expression

- Usual approach is to
 1. find the set of differentially expressed genes [those with extreme values of the univariate statistic, \mathbf{x}]
 2. use a Hypergeometric calculation to identify those gene sets with too many (sometimes too few) differentially expressed genes

Differential Expression

- dividing genes into two groups
 - differentially expressed
 - not differentially expressed

is somewhat artificial

- p -value correction methods don't really do what we want
 - they seldom change the ranking (and shouldn't) so they might change the location of the cut
 - but the artificial distinction remains
- favors finding groups enriched for some genes whose expression changes a lot

A Different Approach

- a different approach is to make use of all of the genes not just the DE ones
- we recommend only using the non-specific filtering methods
- we will attempt to find gene sets where there are potentially small but coordinated changes in gene expression
- an obvious situation is one where genes in a gene set all show small but consistent change in a particular direction

Gene Sets

- can be obtained from biological motivations: GO, KEGG etc
- from experimental observations: DE genes reported in some paper
- predefined sets from the published literature etc
- regions of synteny; cytochrome bands

Gene Sets

- the **GSEABase** package in BioC provides substantial infrastructure for holding and manipulating Gene Sets
- they can have values associated with the genes
 - weights
 - +/- 1 to indicate positive or negative regulation
- a collection of gene sets does not need to be exhaustive or disjoint

Gene Sets

- the mapping from a set of entities (genes) to a collection of gene sets can be represented as a bipartite graph
 - one set of nodes are the genes
 - the other are the gene sets
- this mapping can be represented by an incidence matrix, **A** ($C \times G$)

Gene Sets

- the elements of \mathbf{A} , $\mathbf{A}[i,j]=1$ if gene j is in gene set i , it is 0 otherwise
- the row sums represent the number of genes in each gene set
- the column sums represent the number of gene sets a gene is in
- if two rows are identical (for a given set of genes) then the two gene sets are aliased (in the usual statistical sense)
- other patterns can cause problems and need some study

Gene Sets

- the simplest transformation is to use
$$\mathbf{z} = \mathbf{Ax}$$
- \mathbf{x} is the vector of t-statistics (or alternatives)
- so that \mathbf{z} is a C-vector, and in this case represents the per gene set sums of the selected test statistics
- we are interested in large or small \mathbf{z} 's
- potentially adjusted for the number of entities in the gene set (size)
 - often division by the square root of the number of genes in the gene set

Other Properties

- there is a certain amount of robustness to being correct about the mapping
- a strong signal may be detected even if not all genes in a gene set are identified
- there is also tolerance to some genes being incorrectly associated with the gene set
- this is in contrast to the usual method of differential expression - there we identify particular genes and hence are more subject to errors in annotation

Gene Set Enrichment (Original)

- For each gene set S , a Kolmogorov-Smirnov running sum is computed
- The assayed genes are ordered according to some criterion (say a two sample t -test; or signal-to-noise ratio SNR).
- Beginning with the top ranking gene the running sum increases when a gene in set S is encountered and decreases otherwise
- The enrichment score (ES) for a set S is defined to be the largest value of the running sum.

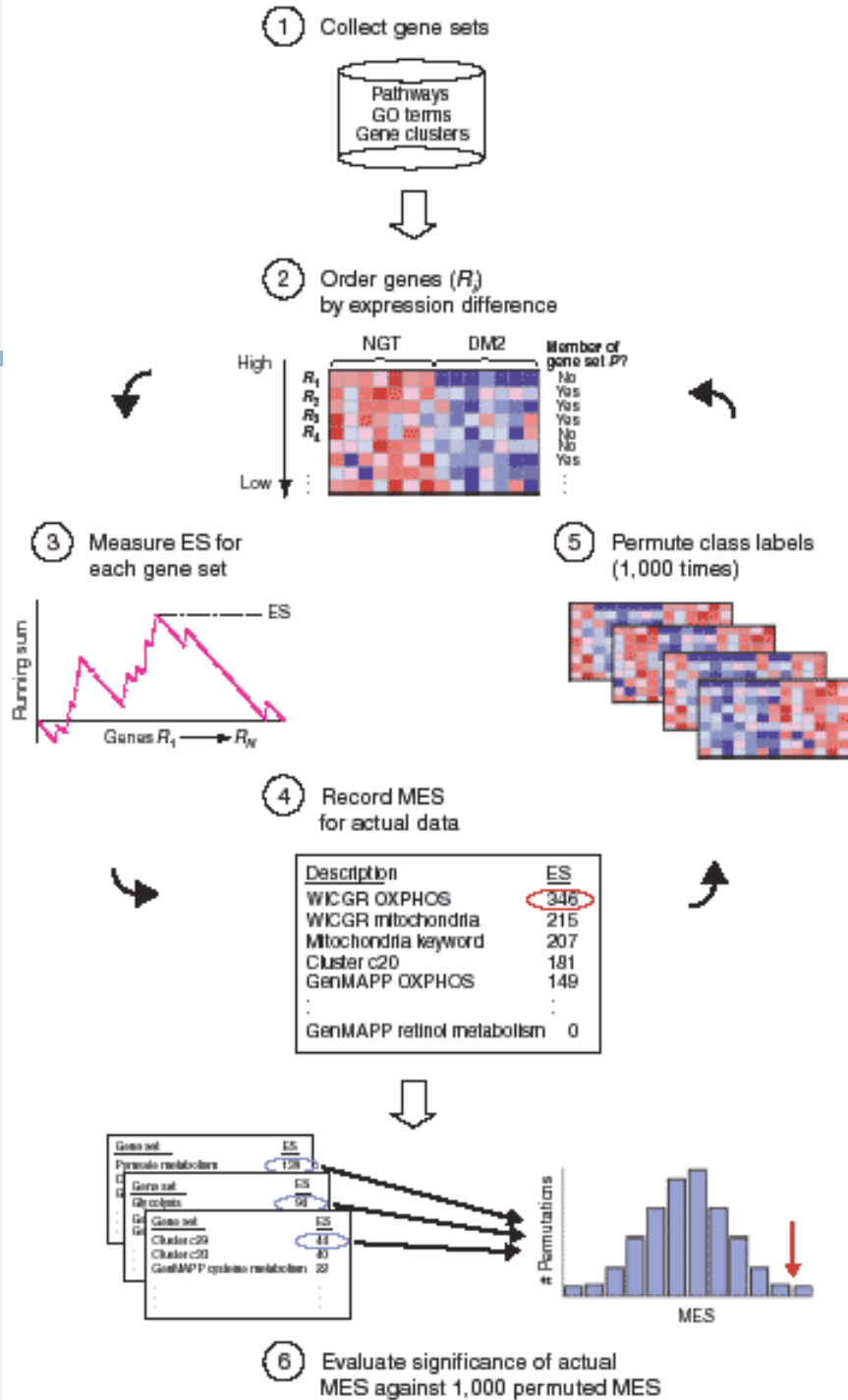
Gene Set Enrichment(Original)

- The maximal ES (MES), over all sets S under consideration is recorded.
- For each of B permutations of the class label, ES and MES values are computed.
- The observed MES is then compared to the B values of MES that have been computed, via permutation.
- This is a single p -value for all tests and hence needs no correction (on the other hand you are testing only one thing).

From Mootha *et al*

ES=enrichment score
for each gene
= scaled K-S dist

A set called OXPHOS
got the largest ES score,
with $p=0.029$ on 1,000
permutations.

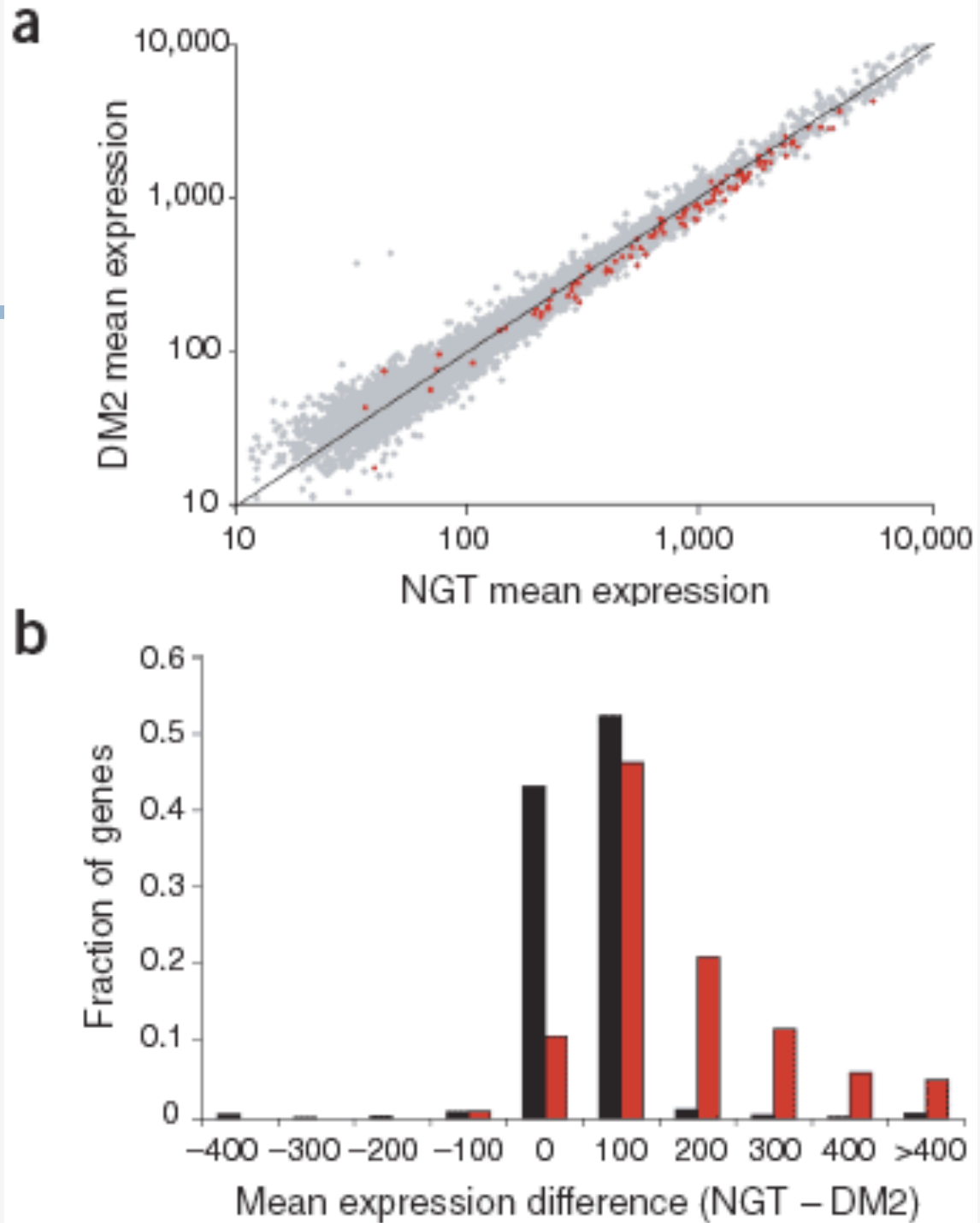


OXPHOS

Other

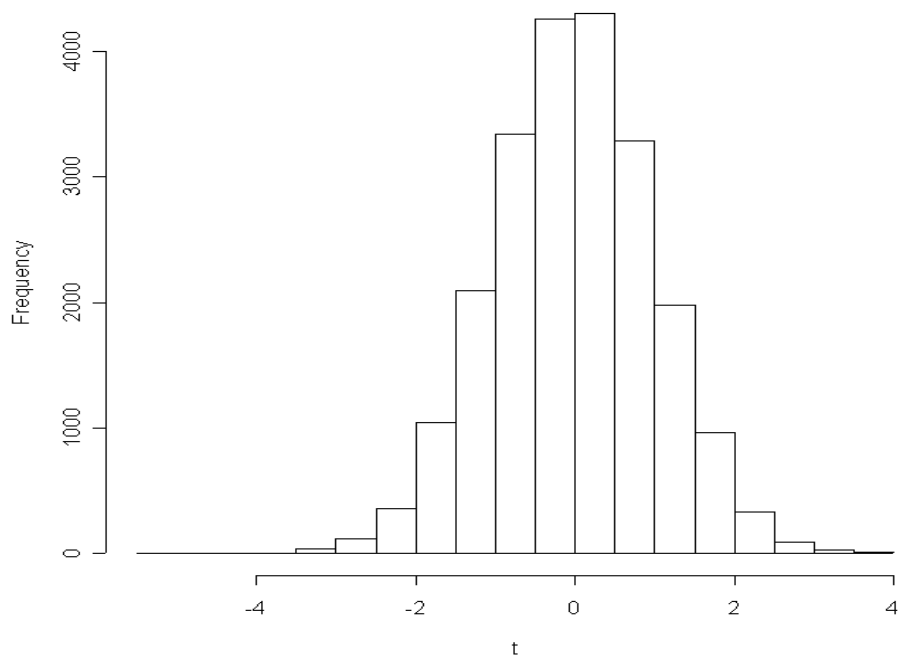
(A small difference
for many genes)

All genes
OXPHOS

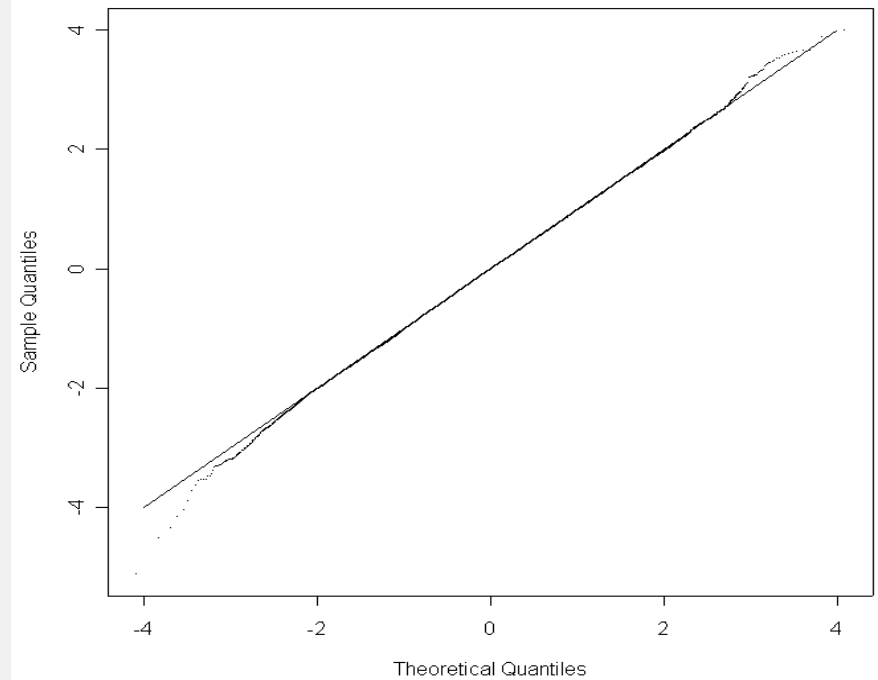


Mootha's ts are approx normal

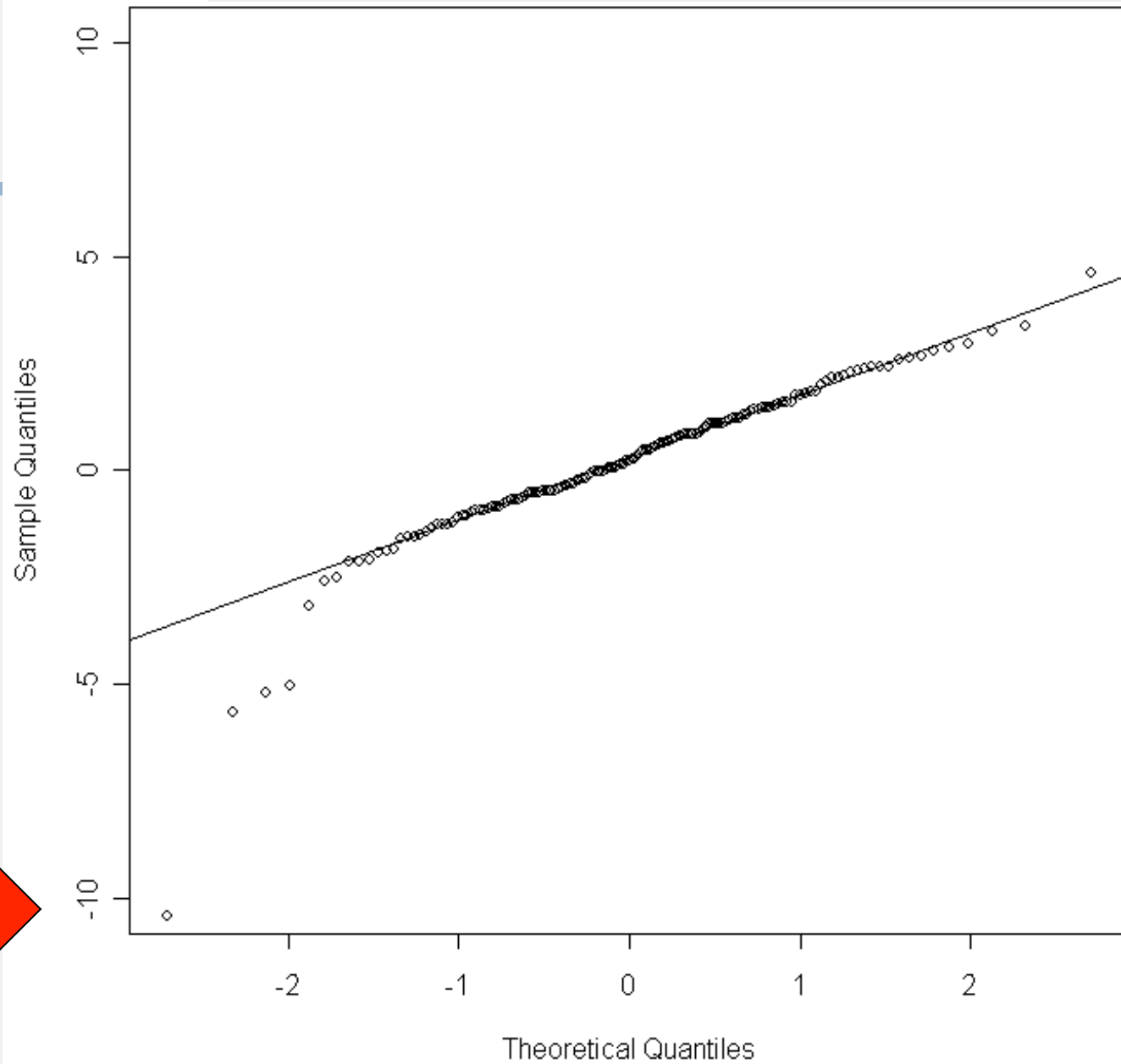
Histogram of t



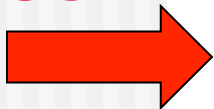
Normal Q-Q Plot for t



Normal qq-plot of $\Sigma t/\sqrt{n}$



OXPHOS



Gene Sets: Distribution

- so what might be sensible
- if n (the number of samples) is large-ish and we use a t -test to compare two groups
- and if H_0 : *no difference between the group means* is true, for all genes
- then the elements of \mathbf{x} are approximately t with $n-1$ df (for large n this is approximately $N(0,1)$)
- so that the elements of \mathbf{z} are sums of $N(0,1)$ and if we divide by the square root of the row sums of \mathbf{A} we are back at $N(0,1)$ [sort of]

Gene Sets: Distribution

- the problem is that that relies on the assumption of independence between the elements of \mathbf{x} , which does not hold
- but it does give some guidance and a qq-plot of the \mathbf{z} 's can be quite useful (as we saw above)

Summary Statistic

- one choice is to use:

$$T = \frac{\sum X}{\sqrt{n}}$$

- a second is to use the regression:

$$Y_i = \alpha + \beta 1_{i \in GS} + \varepsilon_i$$

Gene Sets: Reference Distribution

- an alternative is to generate many \mathbf{x} 's from a reference distribution
- one distribution of interest is to go back to the original expression data and either permuting the sample labels or bootstrapping can be used to provide a reference distribution

Comparisons

- you can test whether for a given gene set is the observed test statistic unusual
- or test whether any of the observed gene set statistics are unusually large with respect to the entire reference distribution

Extensions

- there is no need to compute sums over gene sets
 - you could use medians, any other statistic, such as a sign test
- the regression approach can be extended to
 - include covariates/multiple gene sets
 - use residuals (both for gene sets and for samples)

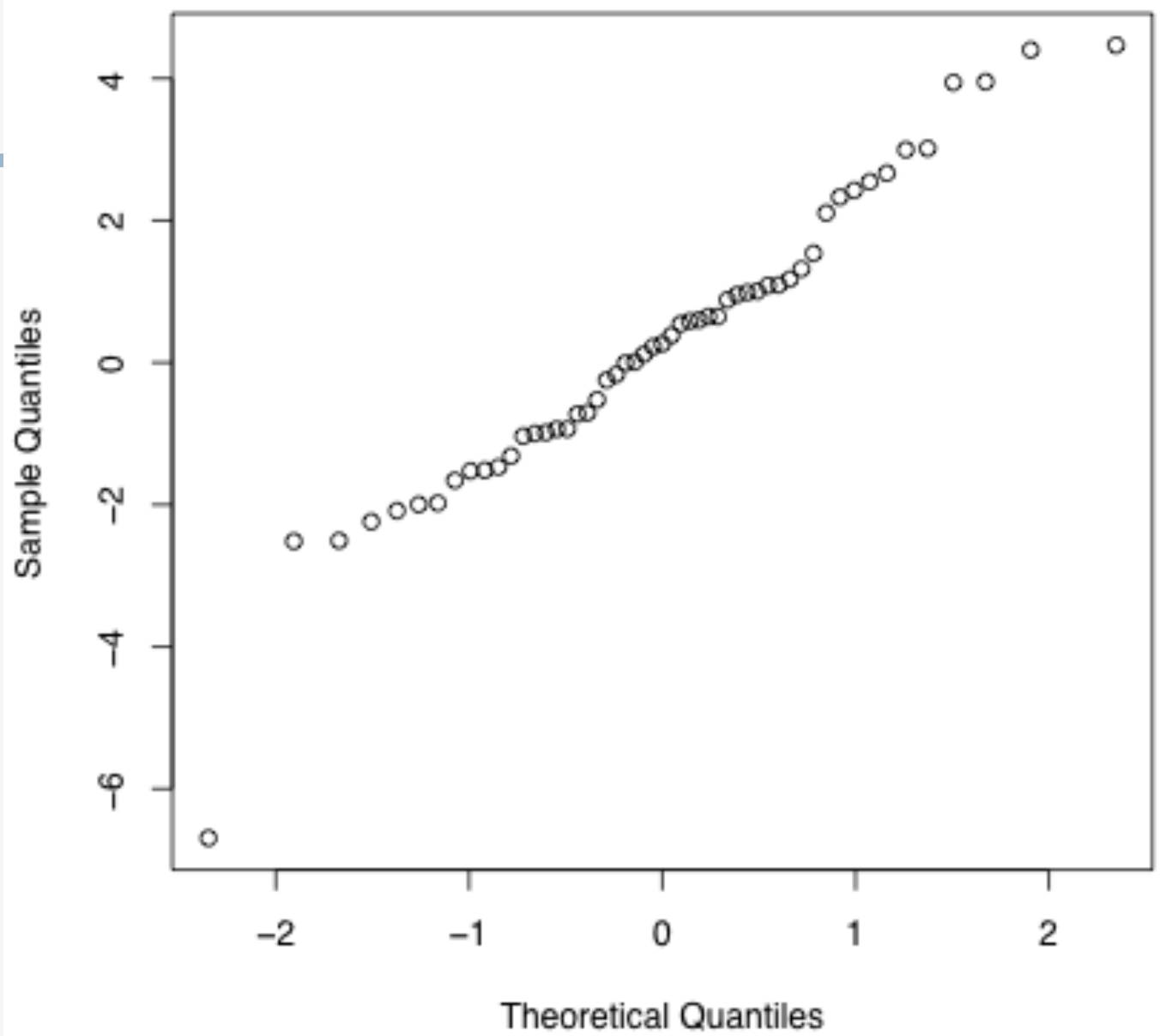
Example: ALL Data

- samples on patients with ALL were assayed using HGu95Av2 GeneChips
- we were interested in comparing those with BCR/ABL (basically a 9;22 translocation) with those that had no cytogenetic abnormalities (NEG)
- 37 BCR/ABL and 42 NEG
- non-specific filter left us with 2526 probe sets

Example: ALL Data

- we then mapped the probes to KEGG pathways
- the mapping to pathways is via LocusLink ID
 - we have a many-to-one problem and solve it by taking the probe set with the most extreme t -statistic
- this left 556 genes
- much of the reduction is due to the lack of pathway information (but there is also substantial redundancy on the chip)
- then I decided to ignore gene sets with fewer than 5 members

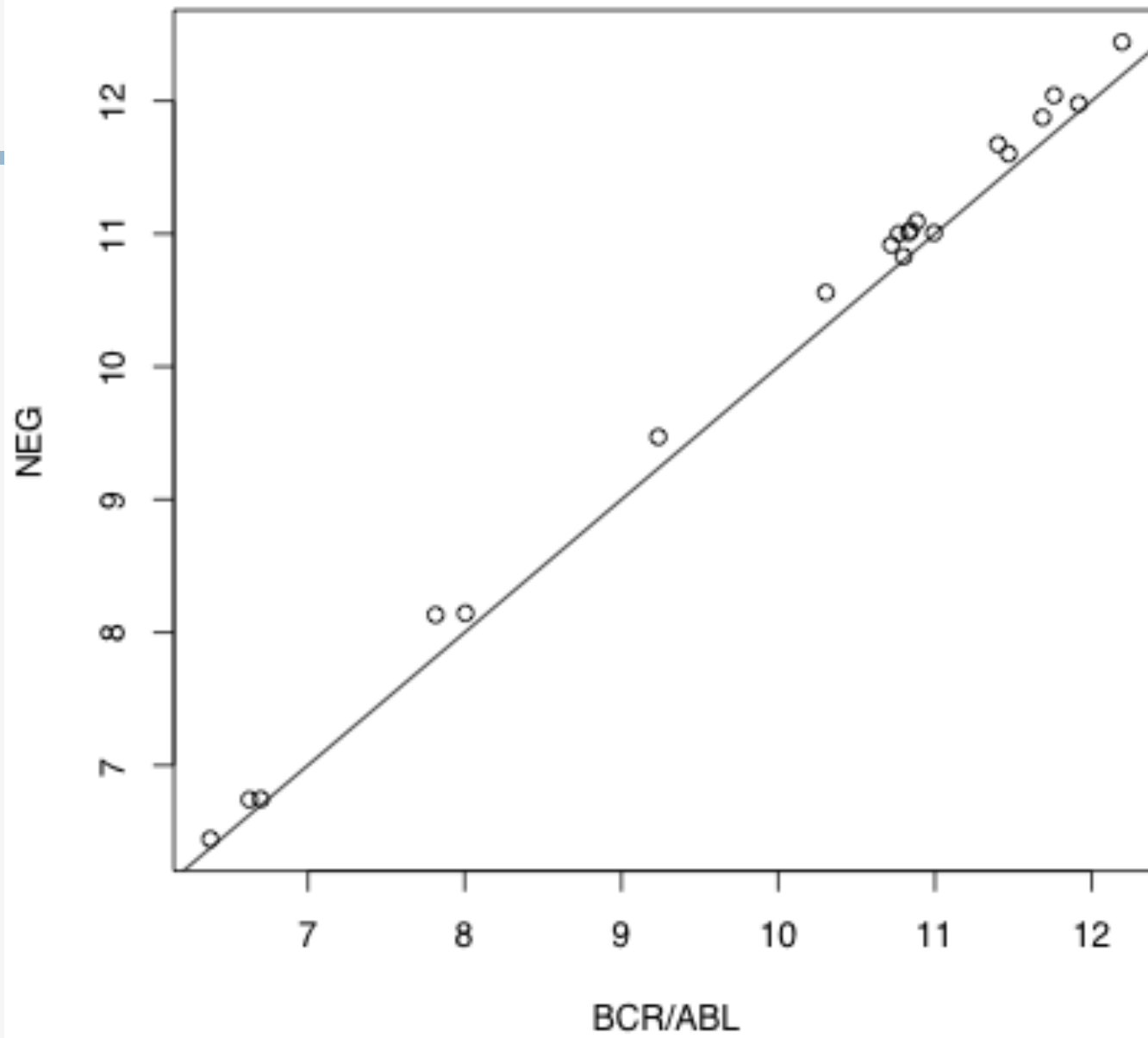
Normal Q-Q Plot



Which Gene Sets

- so the qq-plot looks interesting and identifies at least one gene set that is different
- we identify it (Ribosome), and create a plot that shows the two group means (BCR/ABL and NEG)
- if all points are below or above the 45 degree line that should be interesting

Ribosome
Overall: -6.692



Ribosome

- the mean expression of genes in this **pathway** seem to be higher in the NEG group
- unfortunately the result is spurious - sex needs to be accounted for
 - the groups are not balanced by sex
 - and there is a ribosomal gene encoded on the Y chromosome

Alternative: Permutation Test

- $B=5000$, $p=0.05$
- $NEG > BCR/ABL$
 - Ribosome
- $BCR/ABL > NEG$
 - Cytokine-cytokine receptor interaction
 - MAPK signaling pathway
 - Complement and coagulation cascades
 - TGF-beta signaling pathway
 - Apoptosis
 - Neuroactive ligand-receptor interaction
 - Huntington's disease
 - Prostaglandin and leukotriene metabolism

Recap

- basic idea is to make use of all genes
- summarize per gene data \mathbf{X} ($G \times n$) to \mathbf{x} ($G \times 1$)
 - $\mathbf{x} = f_1(\mathbf{X})$
- use predefined gene sets
 - these define a bipartite graph \mathbf{A} ($C \times G$)
- summarize the relationship between the gene sets and the per gene summary stats
 - $\mathbf{z} = f_2(\mathbf{A}, \mathbf{x})$

Recap

- the summaries of the data, \mathbf{X} , f_1 , can be any test statistic
 - doesn't really need to be 1 dimensional
- the transformations (\mathbf{A}, \mathbf{x}) , f_2 , can be sums, or many other things (medians, sign tests etc)

Some other extensions

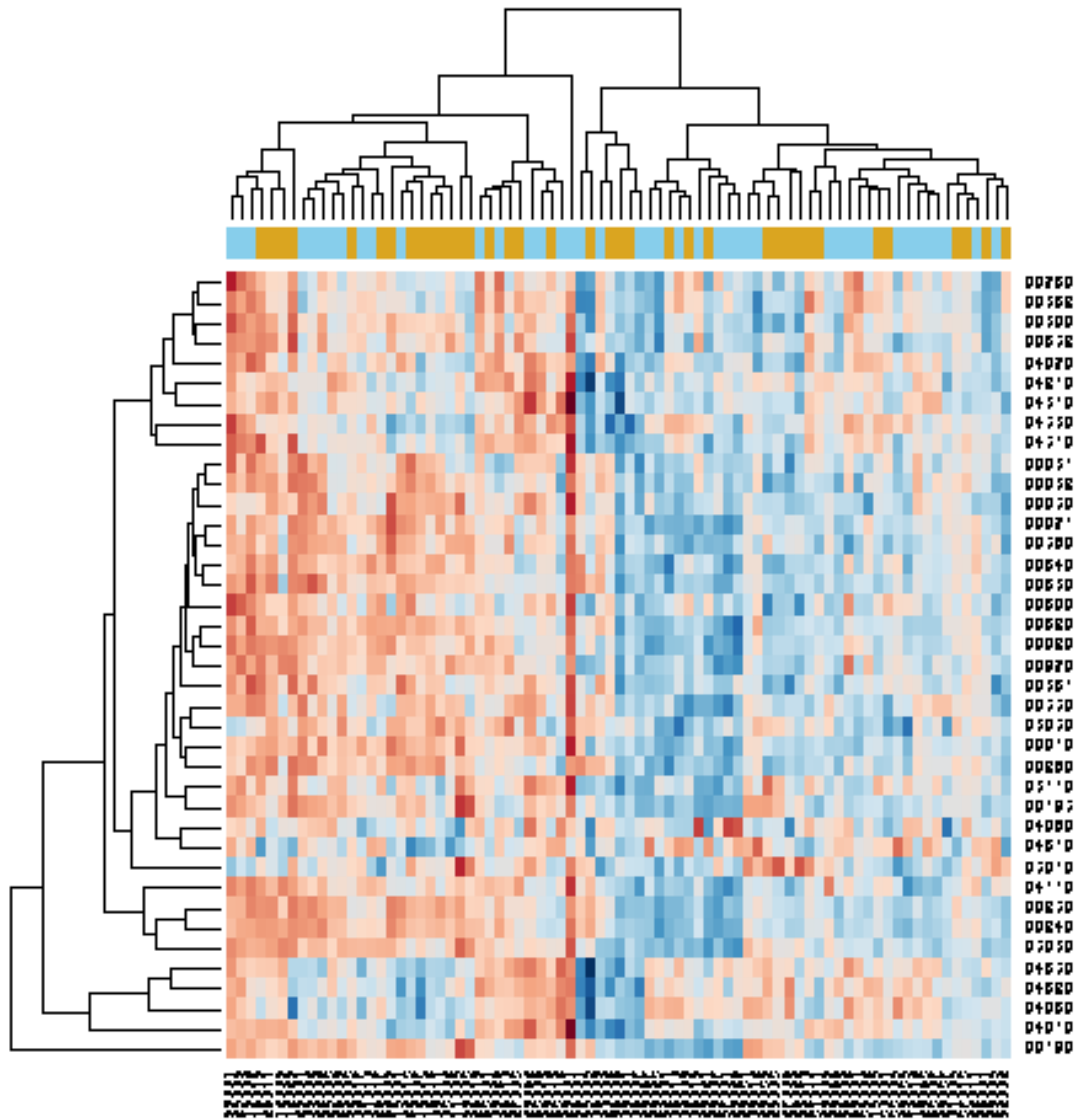
- gene sets might be a better way to do meta-analysis
- one of the fundamental problems with meta-analysis on gene expression data is the gene matching problem
- even technical replicates on the same array do not show similar expression patterns

Extensions: Meta-analysis

- if instead we compute per gene set effects these are sort of independent of the probes that were used
- matching is easier and potentially more biologically relevant
- the problem of adjustment still exists; how do we make two gene sets with different numbers of expression estimates comparable

Extensions

- you can do per array computations
- residuals are one of the most underused tools for analyzing microarrays
- we first filter genes for variability
- next standardize on a per gene basis - subtract the median divide by MAD
- now $X^* = AX$, is a $C \times n$ array, one entry for each gene set for each sample



References

- there is a rich body of literature
- my two main contributions are

Gene set enrichment analysis using linear models and diagnostics. Oron AP, Jiang Z, Gentleman R. Bioinformatics. 2008 Nov 15;24(22):2586-91. Epub 2008 Sep 11.

Extensions to gene set enrichment. Jiang Z, Gentleman R. Bioinformatics. 2007 Feb 1;23(3):306-13. Epub 2006 Nov 24.

Acknowledgements

- Terry Speed (also some slides are his)
- Arden Miller
- Vincent Carey
- Michael Newton
- Kasper Hansen
- Jerry Ritz
- Sabina Chiaretti
- Sandrine Dudoit