



# Gene Set Enrichment Analysis

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# Outline

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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)

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- 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)

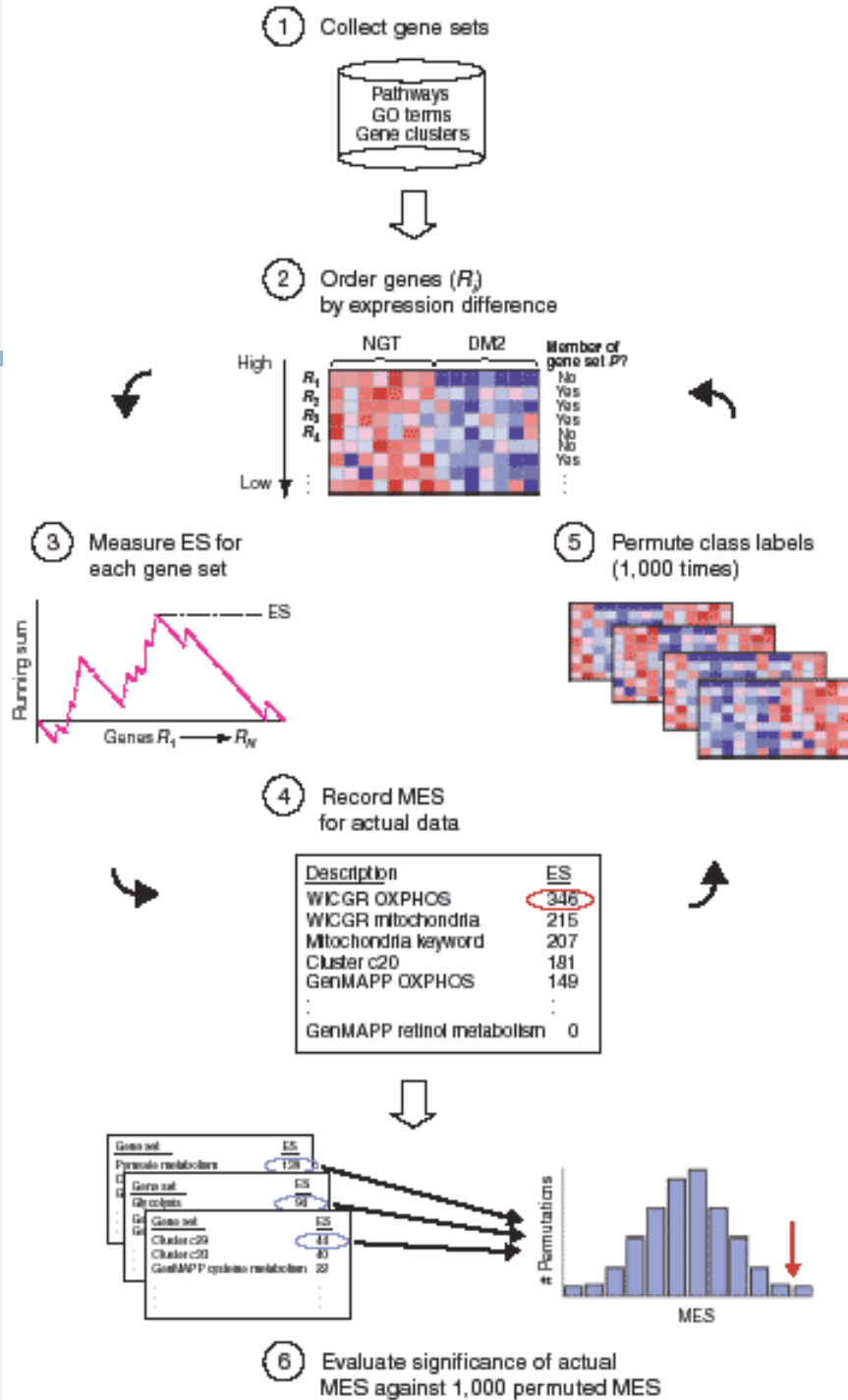
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- The maximal ES (MES), over all sets  $S$  under consideration is recorded.
- For each of  $B$  permutations of the class label, ES and MES values are computed.
- The observed MES is then compared to the  $B$  values of MES that have been computed, 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.

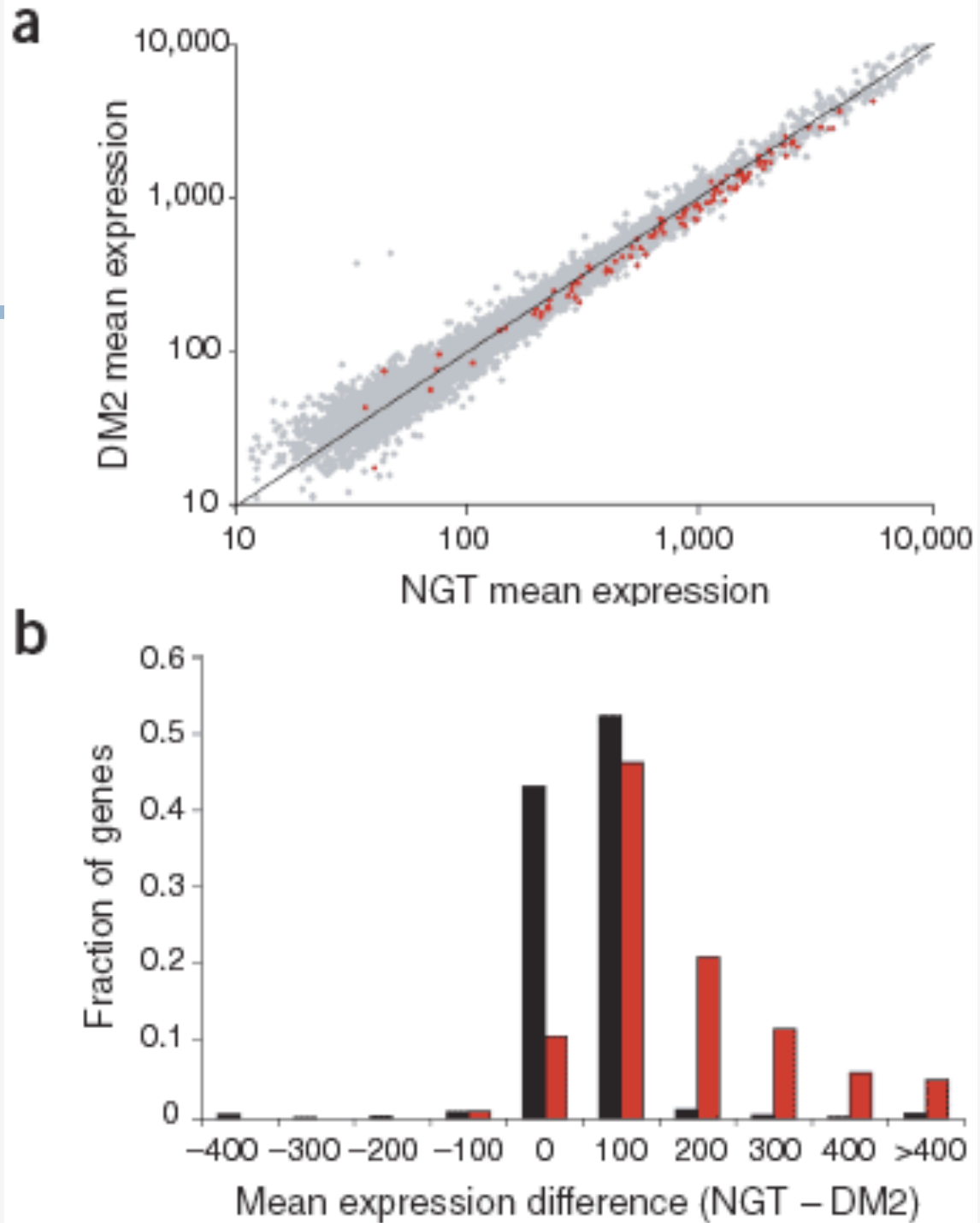


**OXPPOS**

Other

(A small difference  
for many genes)

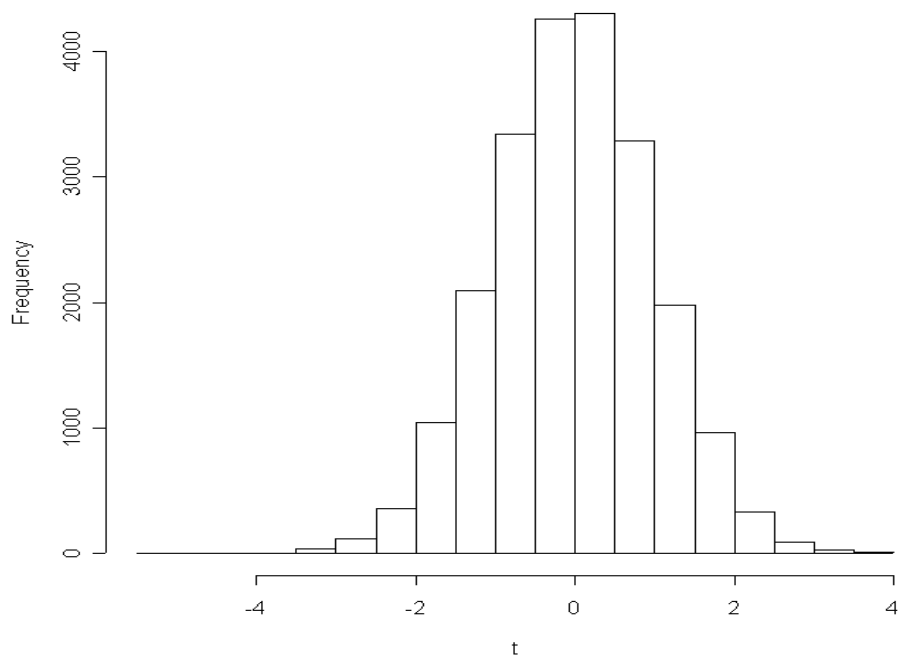
**All genes  
OXPPOS**



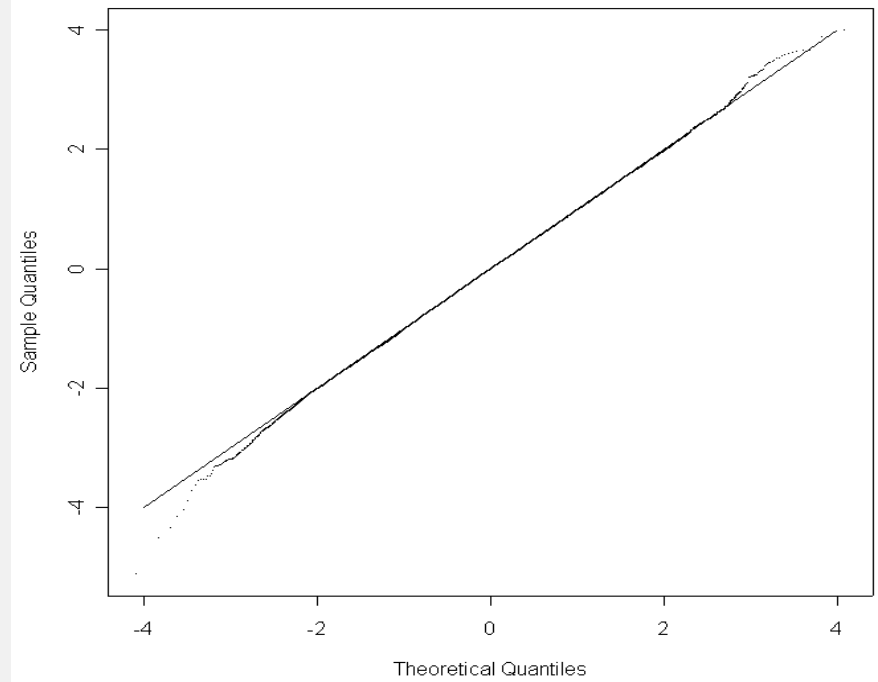


# Mootha's ts are approx normal

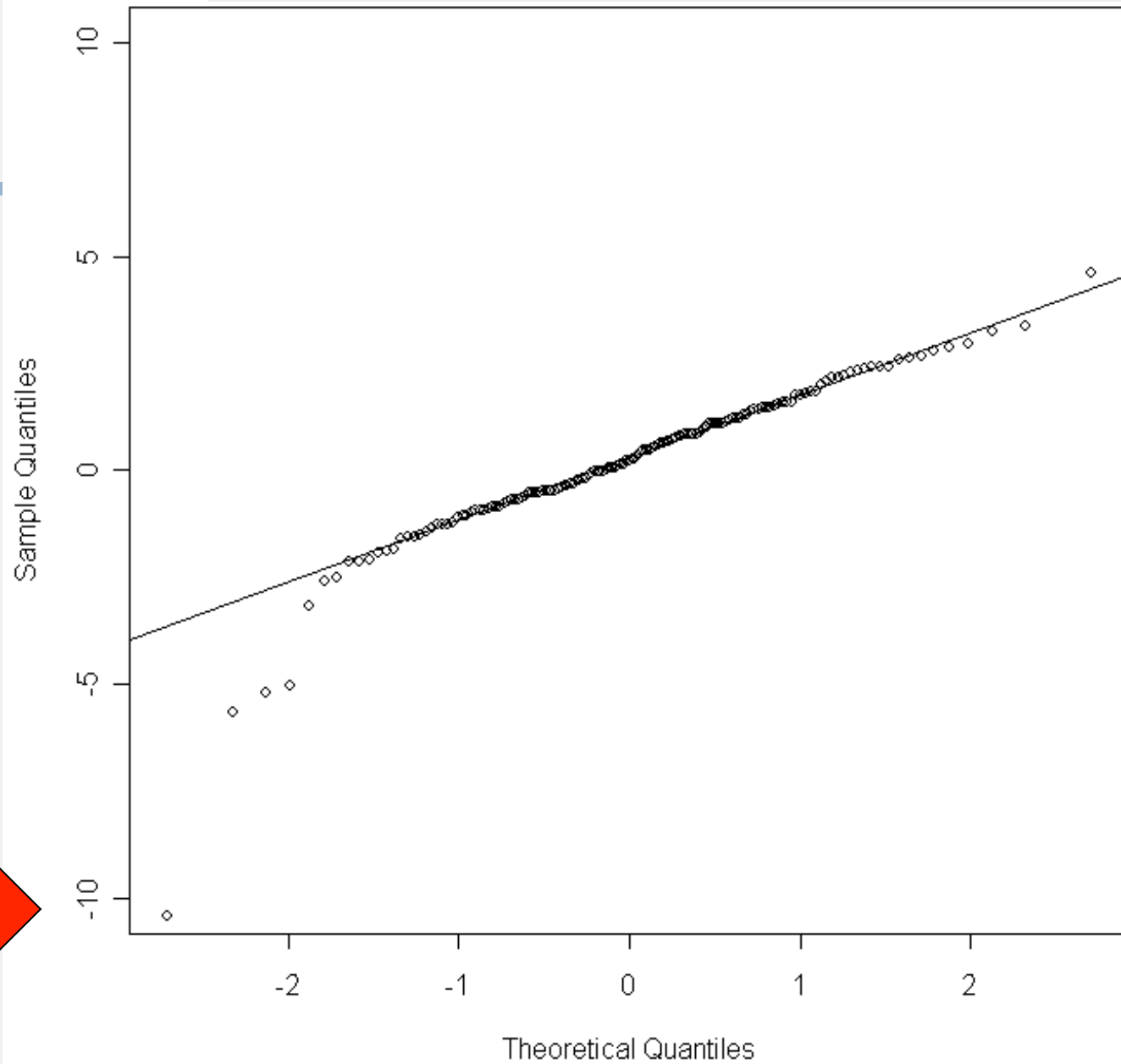
Histogram of t



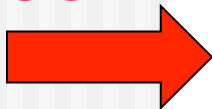
Normal Q-Q Plot for t



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



**OXPHOS**



# Gene Sets: Distribution

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- 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

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- 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

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- 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

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- 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

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- 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

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- 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

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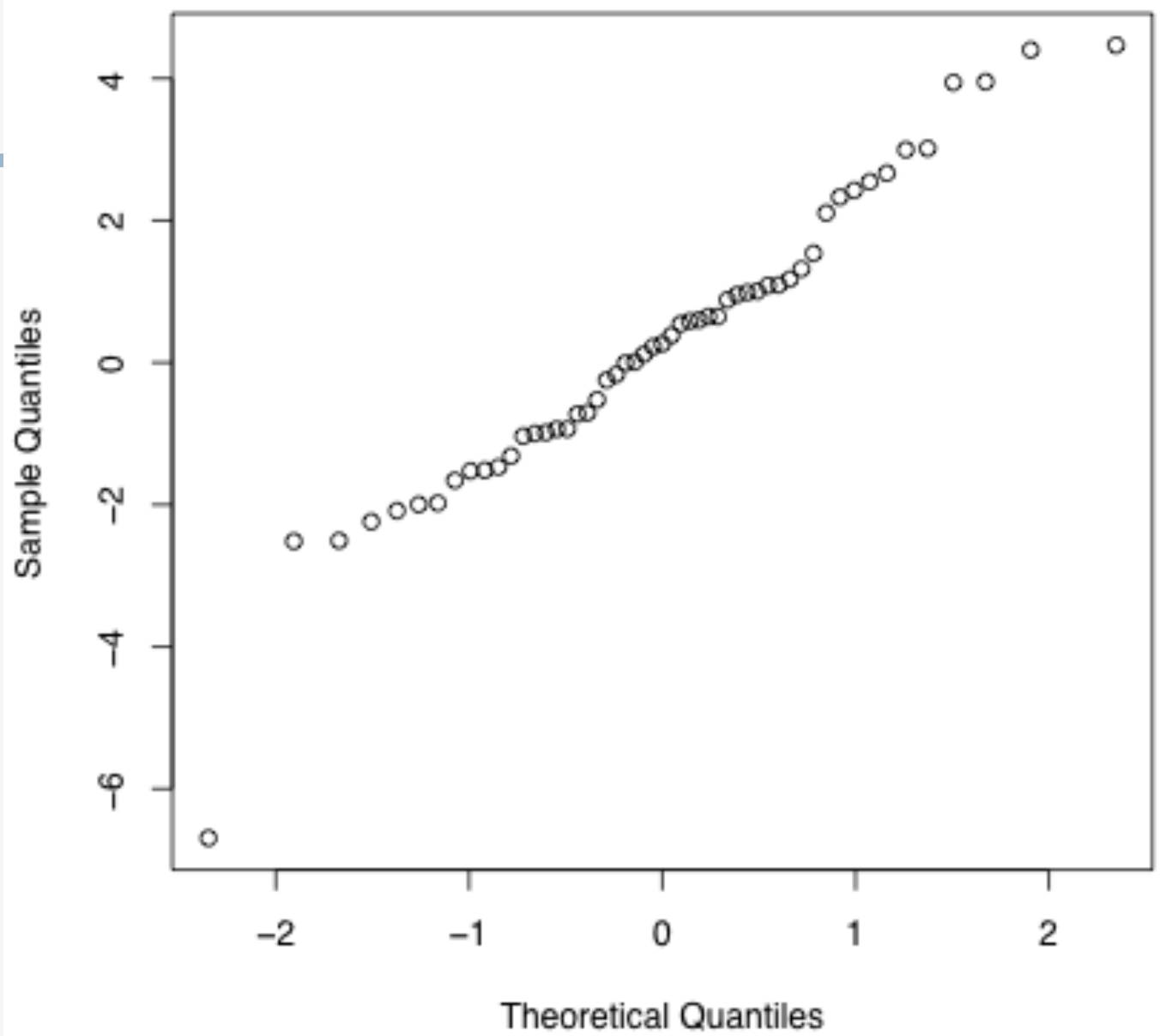
- samples on patients with ALL were assayed using HGu95Av2 GeneChips
- we were interested in comparing those with BCR/ABL (basically a 9;22 translocation) with those that had no cytogenetic abnormalities (NEG)
- 37 BCR/ABL and 42 NEG
- non-specific filter left us with 2526 probe sets

# Example: ALL Data

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- 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

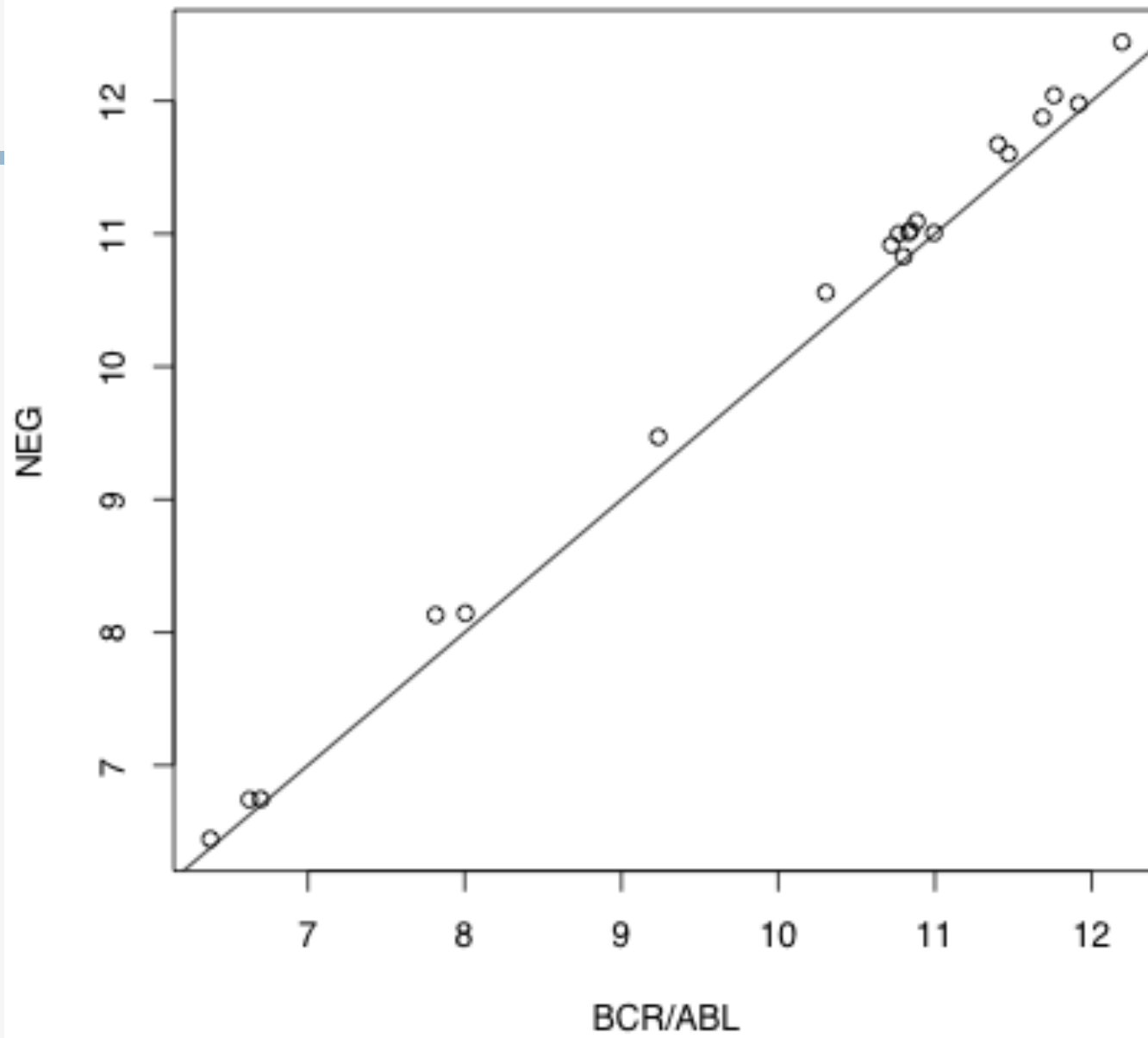


# Which Gene Sets

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- 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

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- 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

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- $B=5000$ ,  $p=0.05$
- $NEG > BCR/ABL$ 
  - 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

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- 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

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- 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

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- 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

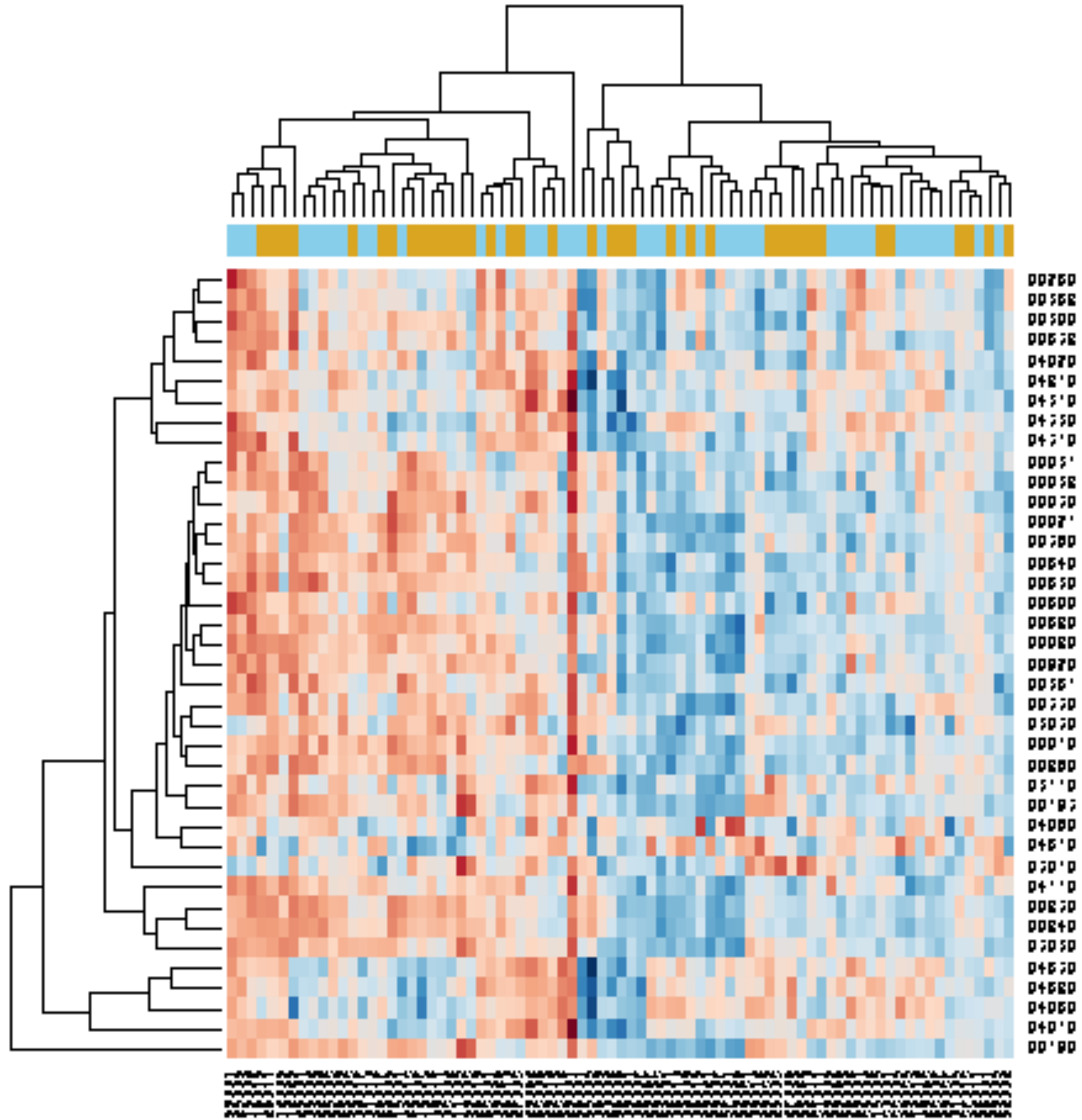
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- if instead we compute per gene set effects these are sort of independent of the probes that were used
- matching is easier and potentially more biologically relevant
- the problem of adjustment still exists; how do we make two gene sets with different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

# Extensions

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- 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

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- 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

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