Group testing: global tests, holistic approaches

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Content of the lecture

Biological relevant information may rather be encoded in groups and not predominantly in the expression of single genes.

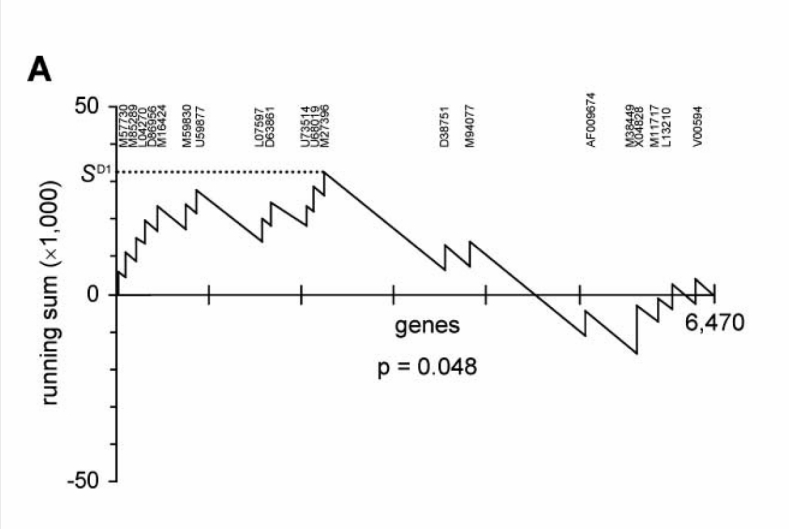
- **How to assess the relevance of groups of genes:**
  - outstanding gene expression in a specific group compared to other genes;
  - differential gene expression not of single genes but over a specific group of genes.
  - Relevance of specific pathway for biological phenomena
- **How to define gene-groups:**
  - exploratory research produces functional groups and genomic signatures: confirm the relevance of the specific group.
  - Bioinformatic algorithms can be used to define pathways and functional groups

Holistic approach

- **Differential gene expression:**
  - dividing genes into two groups: differentially expressed yes/no is artificial
  - p-value correction methods don't really do what we want
  - categories enter by *gene set enrichment* methods, where the identification of categories with too many differentially expressed genes seems to be the goal.
- **Holistic approach:**
  - Define interesting categories:
    pathway (KEGG, cMAP, BioCarta)
    molecular function, biological process, cellular component (GO)
    predefined sets from the published literature, etc
  - find categories of genes where there are potentially small but coordinated changes in gene expression
  - i.e. where genes in a category all show small but consistent change in a particular direction

Example I: **Cyclin D1 Action**

- Lamb J et al. (2003) *A mechanism of Cyclin D1 Action Encoded in the Patterns of Gene Expression in Human Cancer*, Cell, 114: 323-334
- Cyclin D1 expression signature: cyclin D1 target gene set.
- Cyclin D1 activity in Human Tumors: Does the cyclin D1 target gene set play a prominent role in different tumor entities? Being present as highly expressed genes.



The ideas behind the analysis

Problem:

Two groups of genes have to be compared with respect to gene expression: Is the gene expression in gene group A different from the expression in gene group B. **Important: Genes in both groups are different!**

Basic idea:

n_A genes in group A, n_B genes in group B

Order the genes with respect to the expression value. If there is a difference in expression level between both groups, the expression values will be separated. **The position of a value in group A will have the tendency to be in general high or low**. In case of no difference, the values will be nicely mixed.

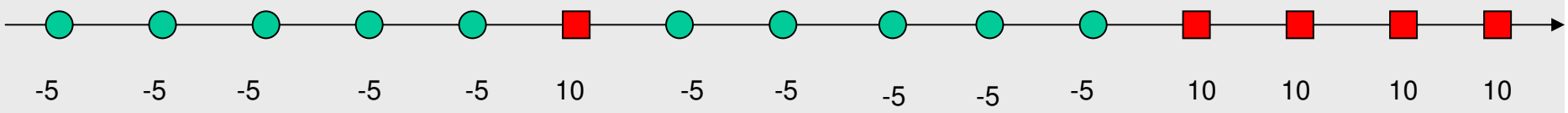
Group A: not regulated by Cyclin D1

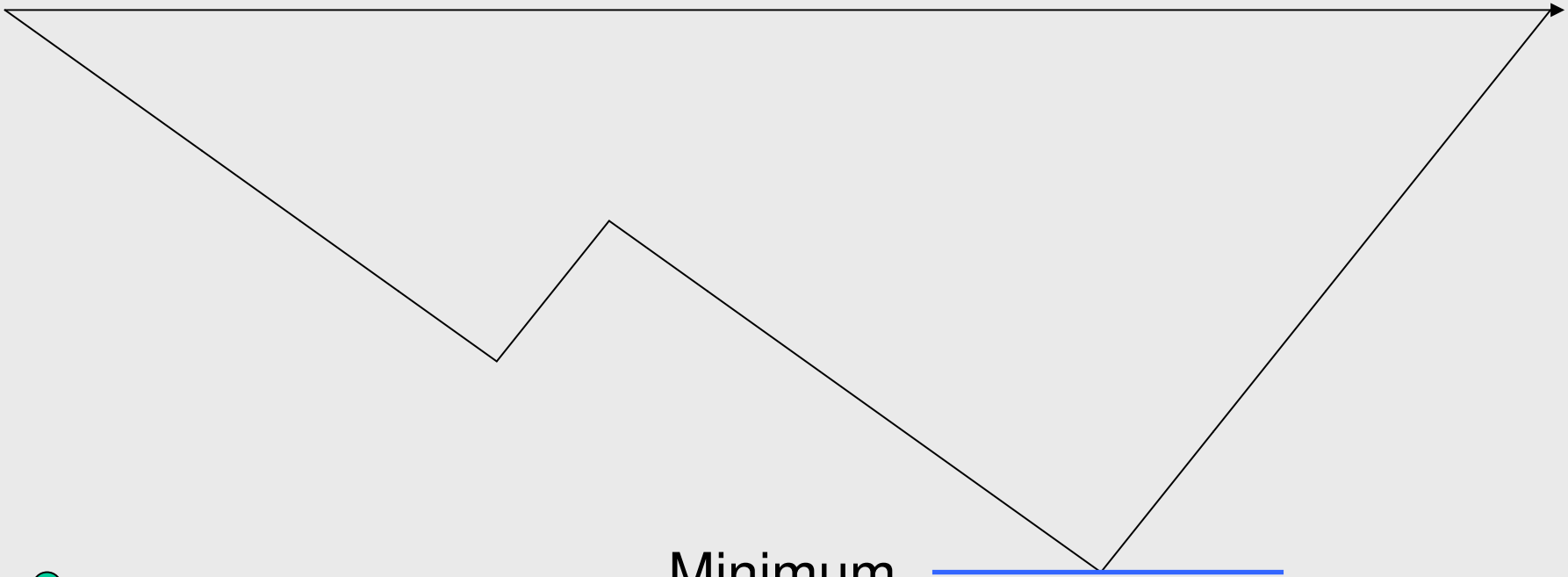
Group B: target genes

Rank of expression value

The ideas behind the analysis

Genes ordered by rank of expression





Minimum

Group A (n_A=10)

Group B (n_B=5)

Is the minimum extreme with respect to random group mixing?

The algorithm fomalized

Basic idea:

- n_A genes in group A, n_B genes in group B.
- Order the genes with respect to expression values.
- Create a vector vv of (n_A+n_B) components with value $-n_B$ at each position where a value from group A is sitting and with value n_A at each position where a value from group B is sitting.
- Calculate yy = cumsum(vv).
- Draw a line starting at (0,0) through points (i, yy[i]). The line will end in $(n_A+n_B,0)$ because $(-n_B)\cdot n_A + n_A \cdot n_B = 0$.
- Look at $M_{vv} = \max\{|\min(yy)|,\max(yy)\}$ which will be large in case of a good separation between both groups.
- Permute the vector vv to get vv*, calculate yy* and M_{vv^*}. Use permutation to calculate the distribution of M_{vv} under the Null hypothesis, determine the permutation based p-value: $p_{perm} = \#\{M_{vv^*} \geq M_{vv}\}/$ # permutations.

Example II: Colon Cancer

Study: 18 patients with UICC II colon cancer, 18 patients with UICC III colon cancer, HG-U133A, 22.283 probesets representing ~18.000 genes. Snap-frozen material, laser microdisection.

Question 1: Are there specific cancer related pathways with a more distinct differential gene expression between UICC II/III?

Gene set enrichment – Colon cancer

1407 probe sets are studied which belong to 9 cancer specific pathways.

androgen_receptor_signalling	122
apoptosis	245
cell_cycle_control	51
notch_delta_signalling	50
p53_signalling	45
ras_signalling	316
tgf_beta_signalling	100
tight_junction_signalling	425
wnt_signalling	214

Gene set enrichment – Colon cancer

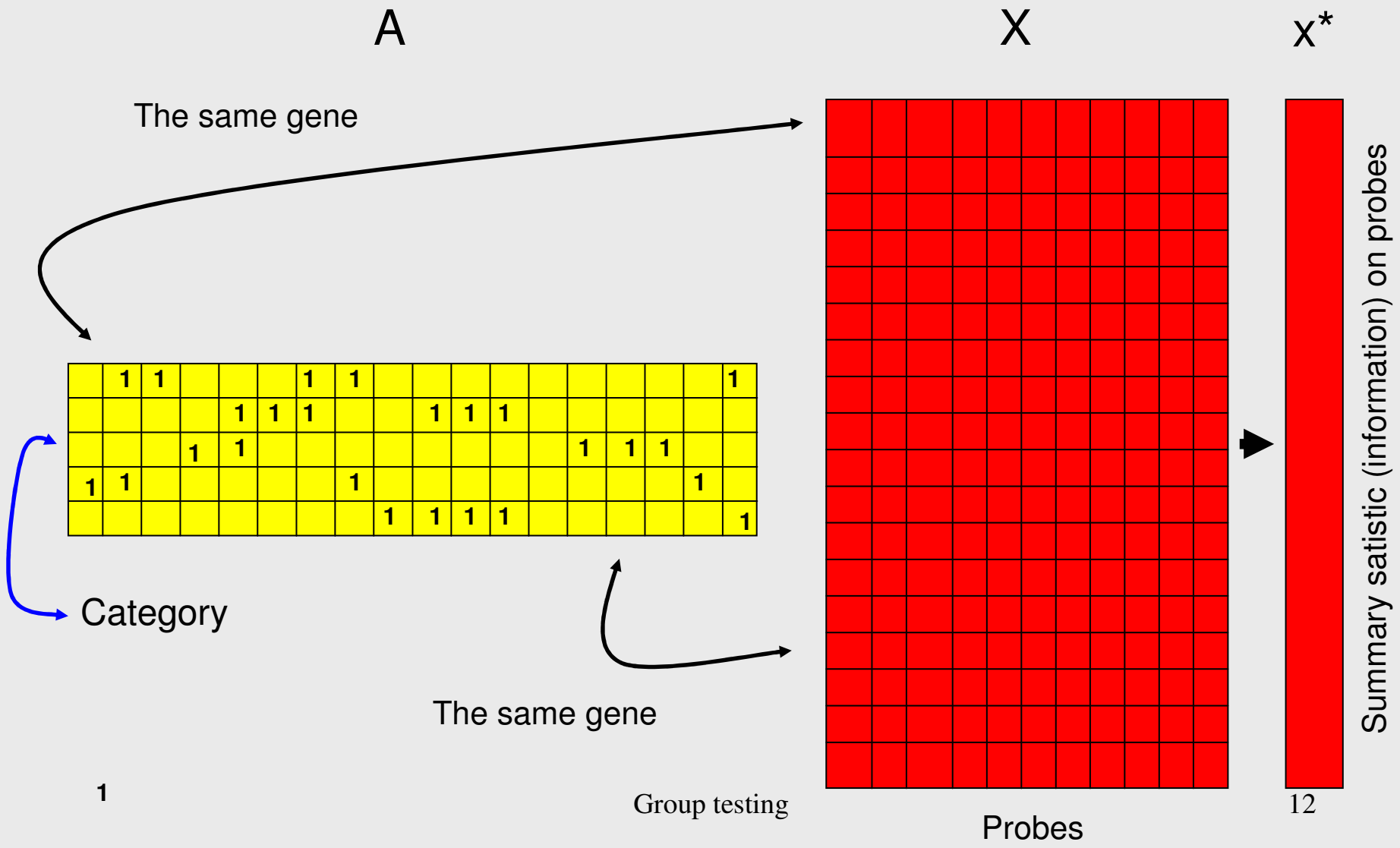
	group.A	group.B	M_{yy}	p.value
androgen_receptor_signaling	118	1289	6983	0.0568
Apoptosis	238	1169	17801	0.7438
cell_cycle_control	51	1356	10413	0.3616
notch_delta_signalling	50	1357	9010	0.6492
p53_signalling	45	1362	12390	0.0924
ras_signalling	311	1096	15486	0.6252
tgf_beta_signaling	100	1307	22615	0.0128
tight_junction_signaling	406	1001	15456	0.4414
wnt_signaling	214	1193	16318	0.8432

Restriction of the analysis to genes in cancer specific pathways

Gentleman's categories (I)

- A set of categories is merely a grouping of genes (entities)
- The groups do not need to be exhaustive or disjoint
- The mapping from a set of entities (genes) to a set of categories can be represented as a bipartite graph:
  one set of nodes are the genes
  the other are the categoies
- This mapping can be presented by an incidence matrix A (CxG)
  C: Number of categories
  G: Number of genes
- The elements of A: A[i,j] = 1 if gene j is in category i else 0
- Row sums: Number of genes in category
- Column sums: Number of categories a gene is in.

Gentleman's categories (II)



Gentleman's categories (III)

- $z = A \bullet X$ or $z = A \bullet x^*$
- z is a vector of length C, represents *per category* sum, we are interested in large or small z's
- x^* could be the vector of gene wise t-statistics between two groups, so we look for gene expression
- H_0: no difference between their means
- Components of x^* are approximately N(0,1)
- The elements of $z = A \bullet x^*$ are sums of N(0,1) [unfortunately not independent summands]
- Permutation test: Permute the columns of A. This is the same as permuting the gene labels (the labels or rows of X and x^*)

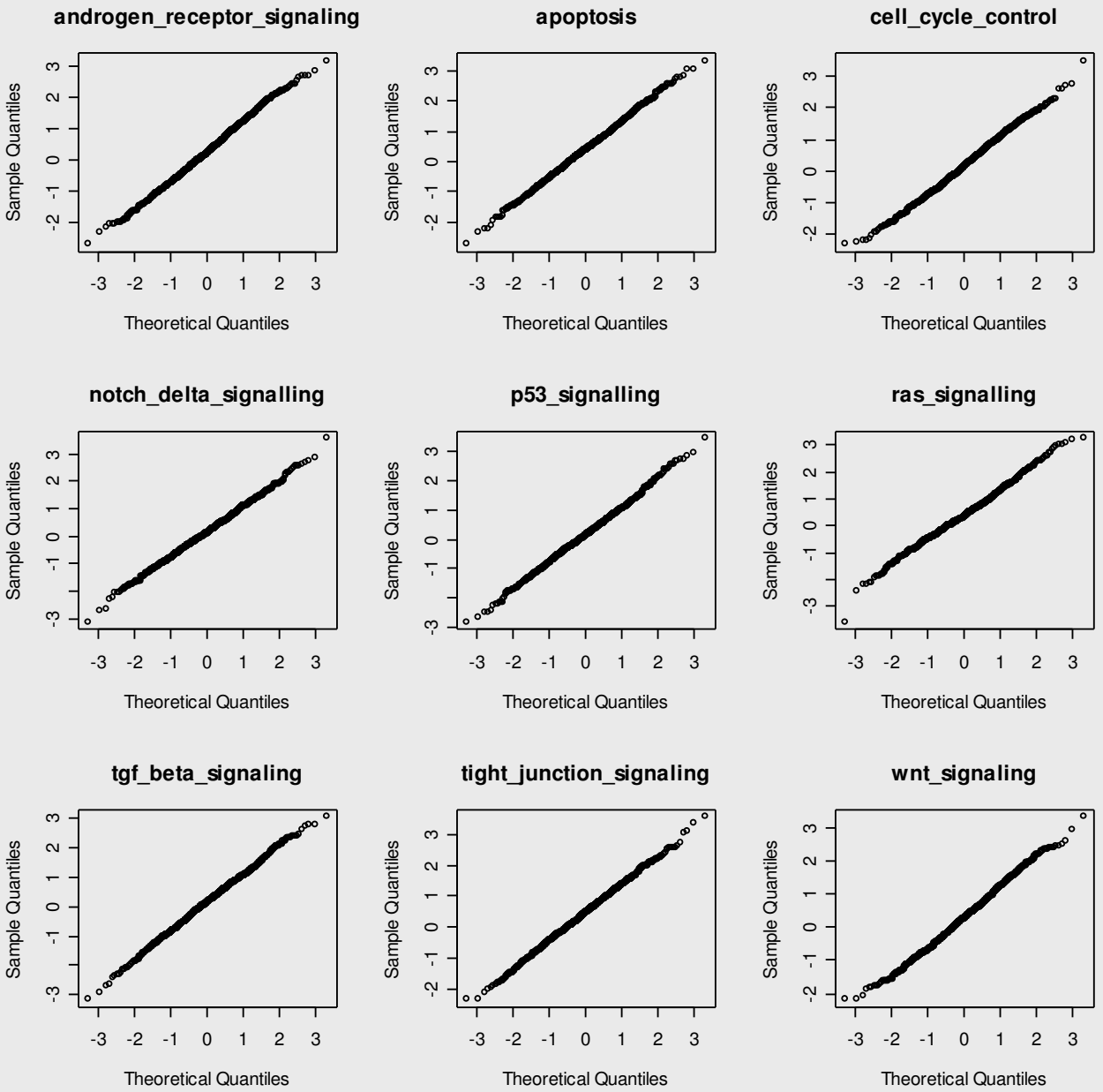
Gentleman's categories (IV)

- **Comparisons:**
  within category comparison: for a given category is the observed test statistic unusual?
  overall comparison: are any of the observed catergory statistics unusually large or small with respect to the entire reference distribution?
- **Note:** The approach is inherently multivariate, one data set gives G test statistics and these are transformed to yield C z_i's.
- The approach is well suited to fit the reasoning in a proper statistical framework.

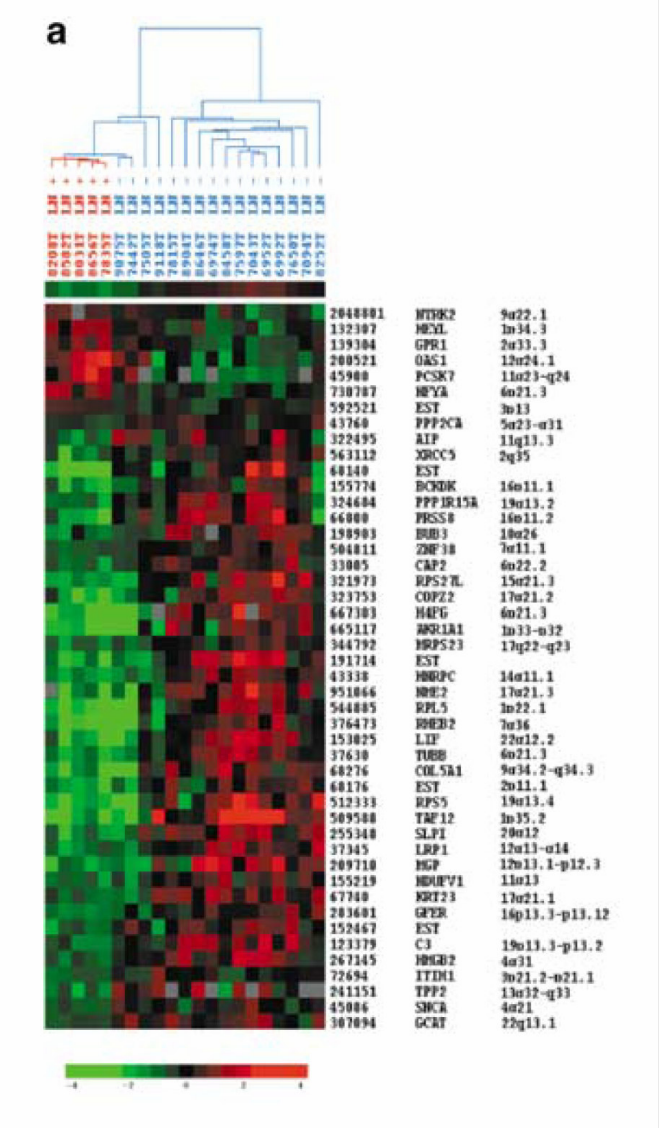
Gentleman's categories (V)

Results for the colon data:

```
Called from: Categories.results.rfc()
Browse[1]>
                             obs.values      2.5%    97.5%
androgen_receptor_signaling  2.4124695 -1.592178 2.122092
apoptosis                   -0.6270588 -1.400973 2.247003
cell_cycle_control          -0.7682091 -1.600006 1.865218
notch_delta_signalling       0.6442985 -1.663848 1.899821
p53_signalling               0.9325874 -1.675115 2.076324
ras_signalling              -0.4736045 -1.380786 2.270405
tgf_beta_signaling           1.1235767 -1.849378 2.081477
tight_junction_signaling     0.5652049 -1.347741 2.185699
wnt_signaling                1.8580599 -1.463279 2.130000
```


androgen_receptor_signaling
apoptosis
cell_cycle_control
notch_delta_signalling
p53_signalling
ras_signalling
tgf_beta_signaling
tight_junction_signaling
wnt_signaling
Sample Quantiles
Theoretical Quantiles

Example III: Lymph node metastases



Bertucci F et al. (2004) *Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters*, Oncogene 23, 1377–1391

Bertucci at al present a gene signature consisting of 46 genes which is claimed to be able to discriminate between LN- and LN+ colorectal cancer.

Is it possible to prove with new data that the signature has discriminative value. Can we reject the Nullhypothesis

$$P[Y|X] = P[Y].$$

Y : LN+/LN-
X: expression pattern of 46 genes

Goeman's Global Test

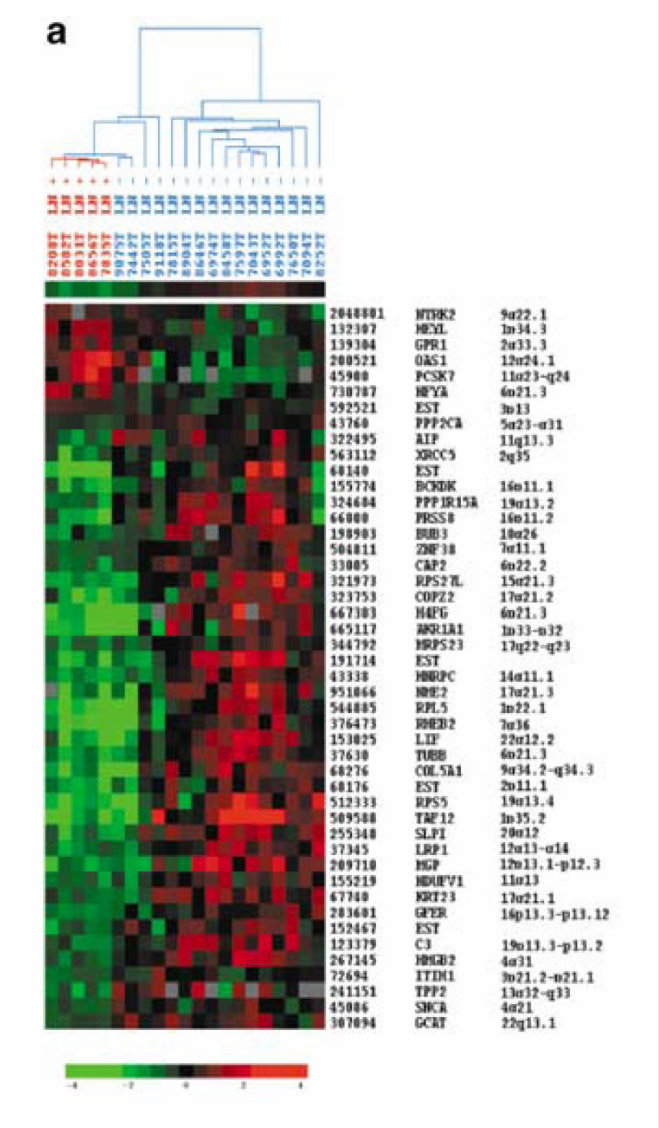
- Test if global expression pattern of a group of genes is significantly related to some outcome of interest (groups, continuous phenotype).
- If this relationship exists, then the knowledge of gene expression helps to improve the prediction of the phenotype of interest. If the prediction can not improved by knowing the gene expression then there will not be differential gene expression.
- Test statistic:

$$Q \sim (Y-\mu)'R\,(Y-\mu)$$
$$\sim \Sigma\,[X_i'(Y-\mu)]^2 \quad \text{sum over genes of the pathway}$$
$$\sim \Sigma\,\Sigma\, R_{ij}(Y_i-\mu)\,(Y_j-\mu) \text{ sum over subjects}$$

μ: Mean of phenotype,
X_{mi} Expression for gene m in subject i
R : X'X matrix of correlations between gene expression of subjects

Goeman JJ. Et al. (2003) *A global test for groups of genes: Testing association with a clinical outcome*, Bioinformatics, 20:93-99; Bioconductor package: *globaltest*

Example III: Lymph node metastases



Test is not significant (p=0.43)

No clear answer on the predictive power of the signature.

No evidence for a difference is not evidence for no difference!

Question of power

Reasons for a non-significance: bad experiment or ...?

Example IV: Colon Cancer

Study: 18 patients with UICC II colon cancer, 18 patients with UICC III colon cancer, HG-U133A, 22.283 probesets representing ~18.000 genes. Snap-frozen material, laser microdisection.

Question 2: Is there differential gene expression in the p53 signalling pathway between UICC II and UICC III colon cancer?

Goeman's Global Test – Example IV

- Test for differential gene expression in *p53 signalling* pathway
  45 probesets
- Global Test result:

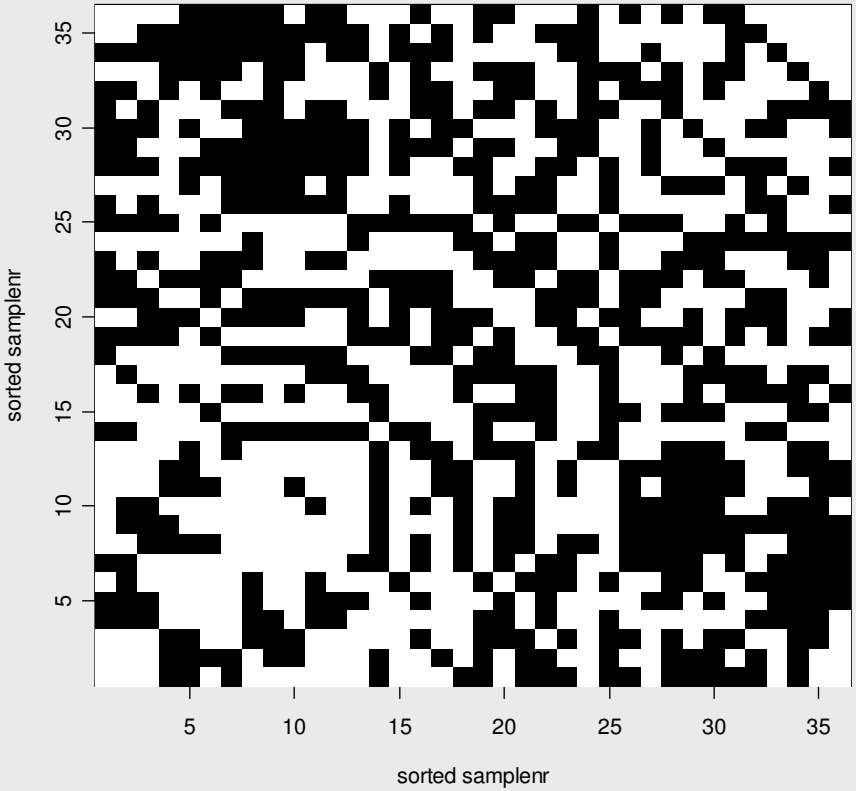
```
45 out of 45 genes used; 36 samples

p value = 0.0114
based on 10000 permutations

Test statistic Q = 11.78
with expectation EQ = 5.466
and standard deviation sdQ = 2.152 under the null hypothesis
```

- Informative plots:
  Sample plot: how good fits a sample to its phenotype
  Checkerboard: Correlation between samples
  Gene plot: Influence of single genes to test statistics

Goeman's Global Test – Example IV

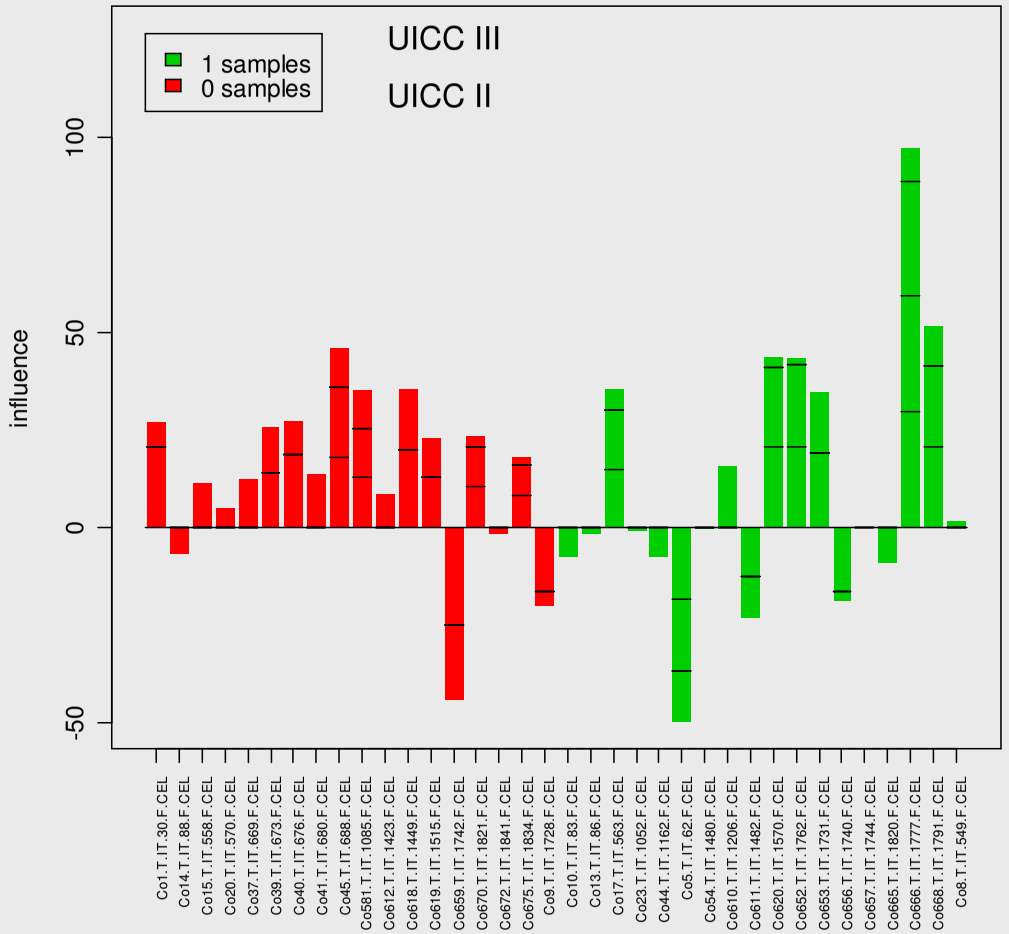
$$\Sigma \Sigma R_{ij}(Y_i-\mu)\,(Y_j-\mu)$$

Simultaneous correlation of phenotype and expression



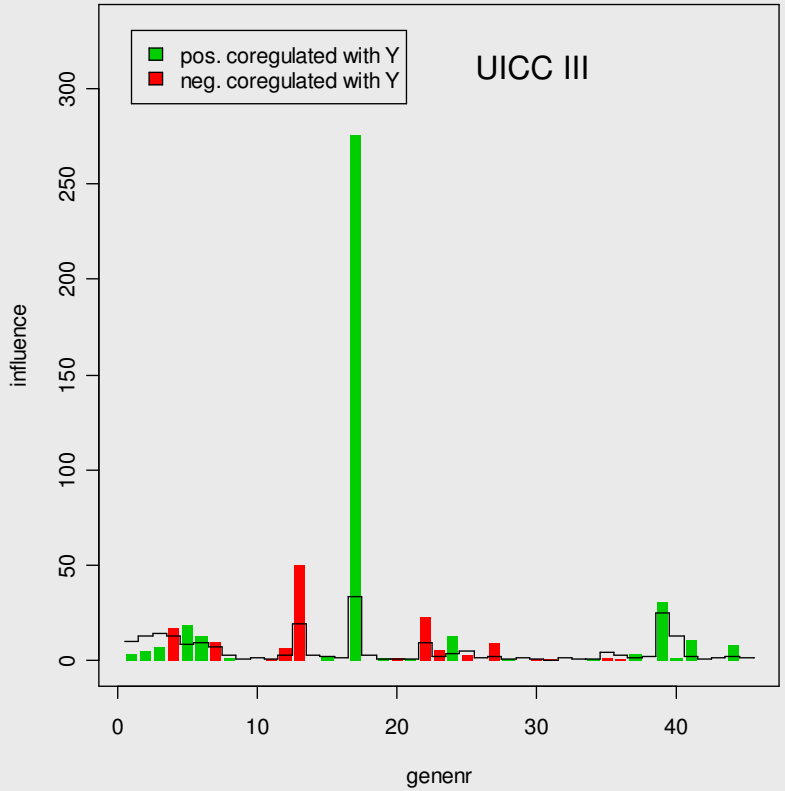
Values dichotomized around median

Goeman's Global Test – Example IV



Goeman's Global Test – Example IV

$$\Sigma [X_i'(Y-\mu)]^2$$



Summary: Two perspectives on gene groups

Question 1: Two groups of genes have to be compared with respect to gene expression: Is the gene expression in gene group A different from the expression in gene group B.

Genes of group A | Genes of group B

Question 2: Is there differential gene expression between different biological entities not in terms of single genes but with respect to a defined group of genes.