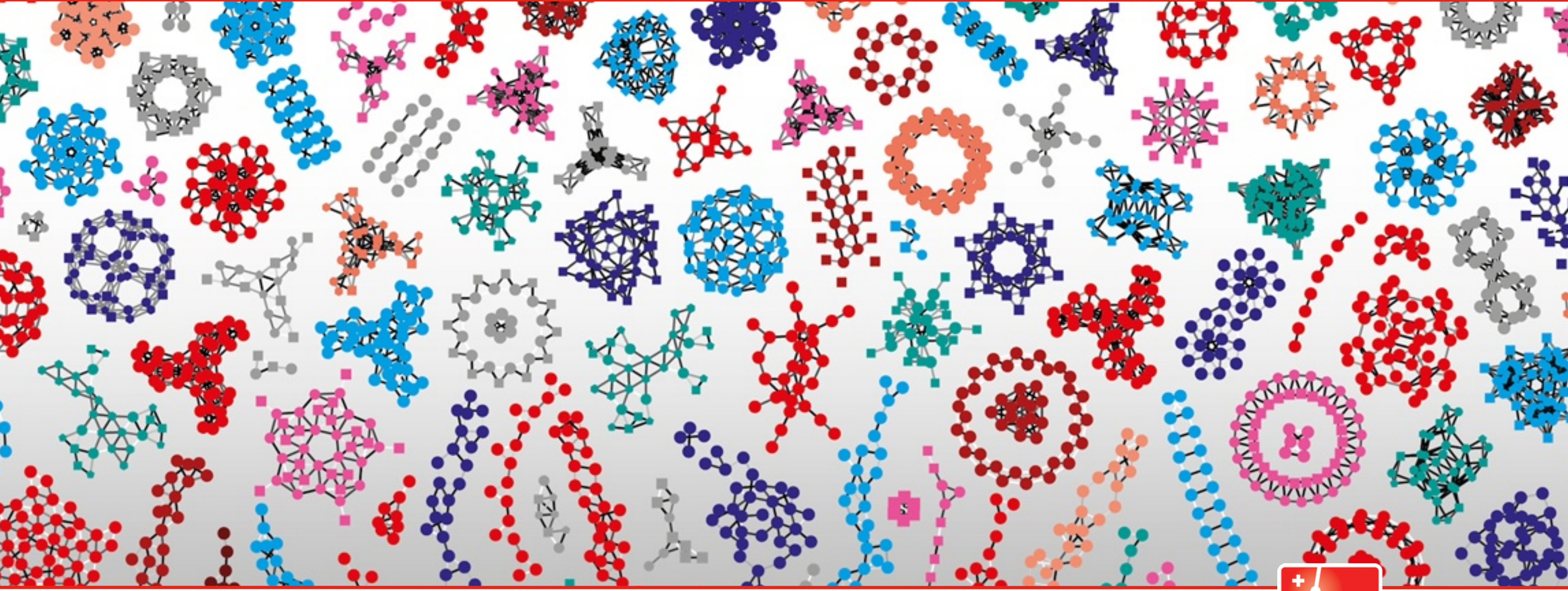


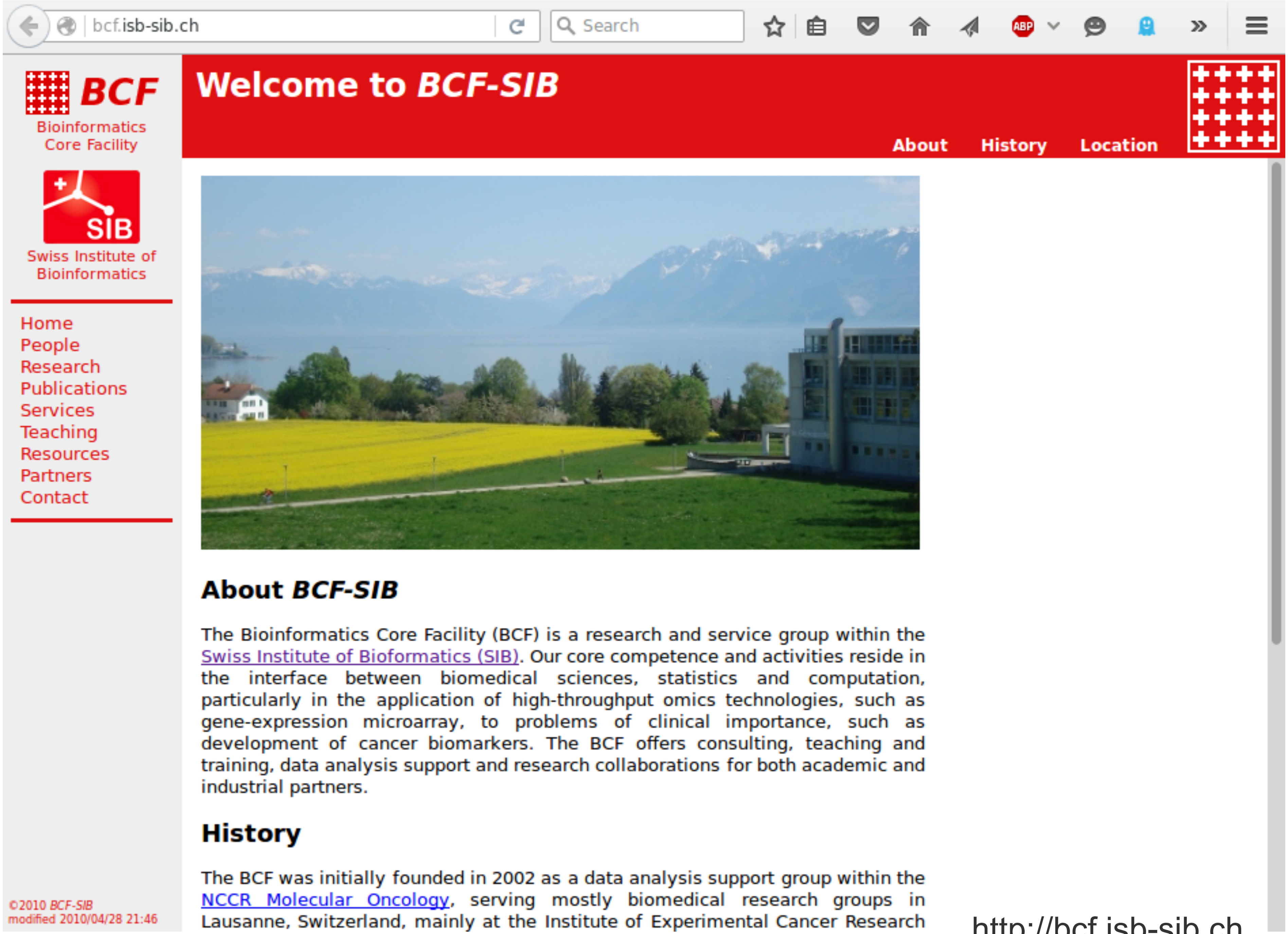
Swiss Institute of Bioinformatics

Enrichment analysis

Linda Dib

21st of march 2018

www.sib.swiss



BCF
Bioinformatics Core Facility

SIB
Swiss Institute of Bioinformatics

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- Partners
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modified 2010/04/28 21:46

Welcome to *BCF-SIB*

About History Location

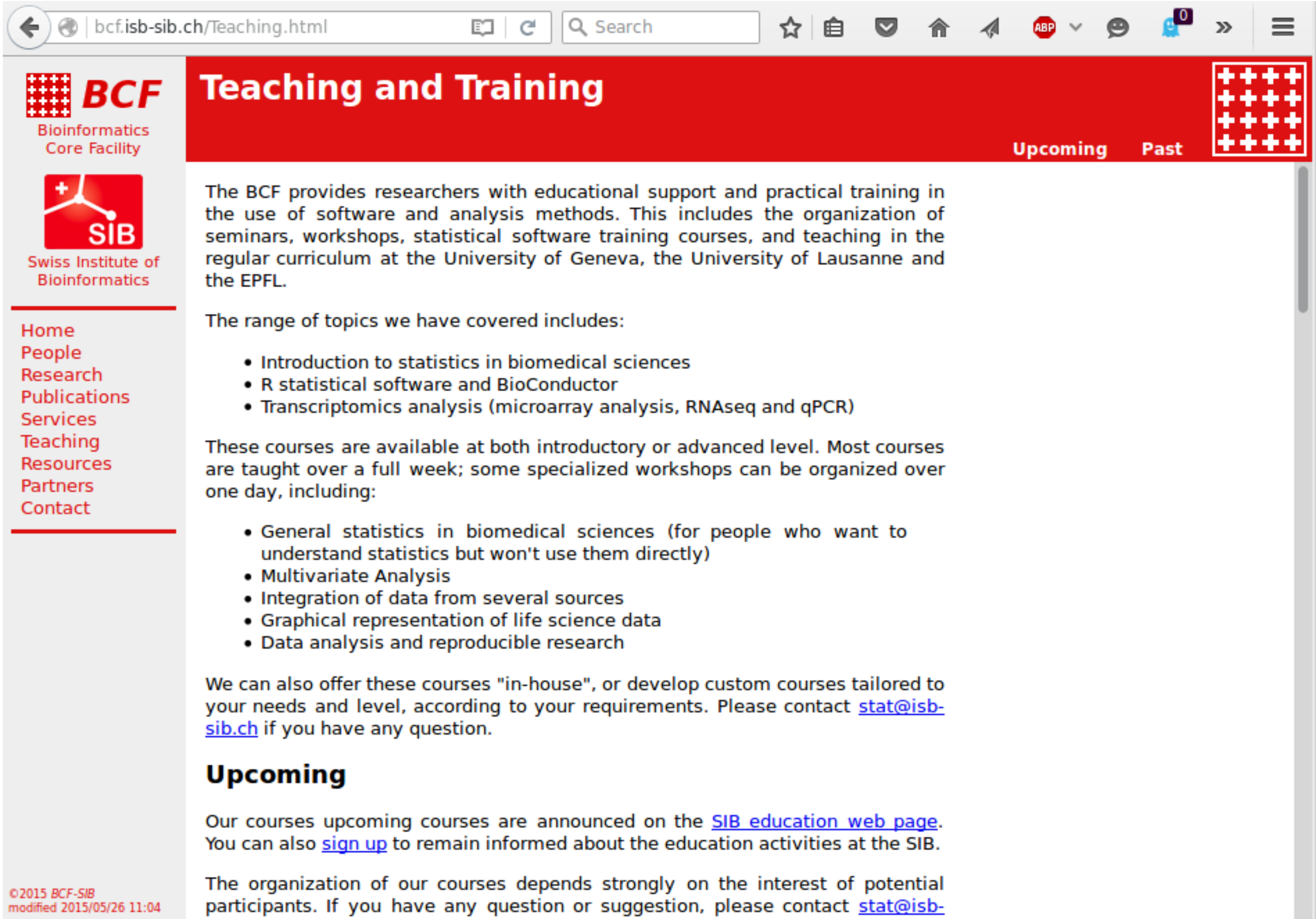
About *BCF-SIB*

The Bioinformatics Core Facility (BCF) is a research and service group within the Swiss Institute of Bioformatics (SIB). Our core competence and activities reside in the interface between biomedical sciences, statistics and computation, particularly in the application of high-throughput omics technologies, such as gene-expression microarray, to problems of clinical importance, such as development of cancer biomarkers. The BCF offers consulting, teaching and training, data analysis support and research collaborations for both academic and industrial partners.

History

The BCF was initially founded in 2002 as a data analysis support group within the NCCR Molecular Oncology, serving mostly biomedical research groups in Lausanne, Switzerland, mainly at the Institute of Experimental Cancer Research

http://bcf.isb-sib.ch



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Teaching and Training

Upcoming Past

The BCF provides researchers with educational support and practical training in the use of software and analysis methods. This includes the organization of seminars, workshops, statistical software training courses, and teaching in the regular curriculum at the University of Geneva, the University of Lausanne and the EPFL.

The range of topics we have covered includes:

- Introduction to statistics in biomedical sciences
- R statistical software and BioConductor
- Transcriptomics analysis (microarray analysis, RNAseq and qPCR)

These courses are available at both introductory or advanced level. Most courses are taught over a full week; some specialized workshops can be organized over one day, including:

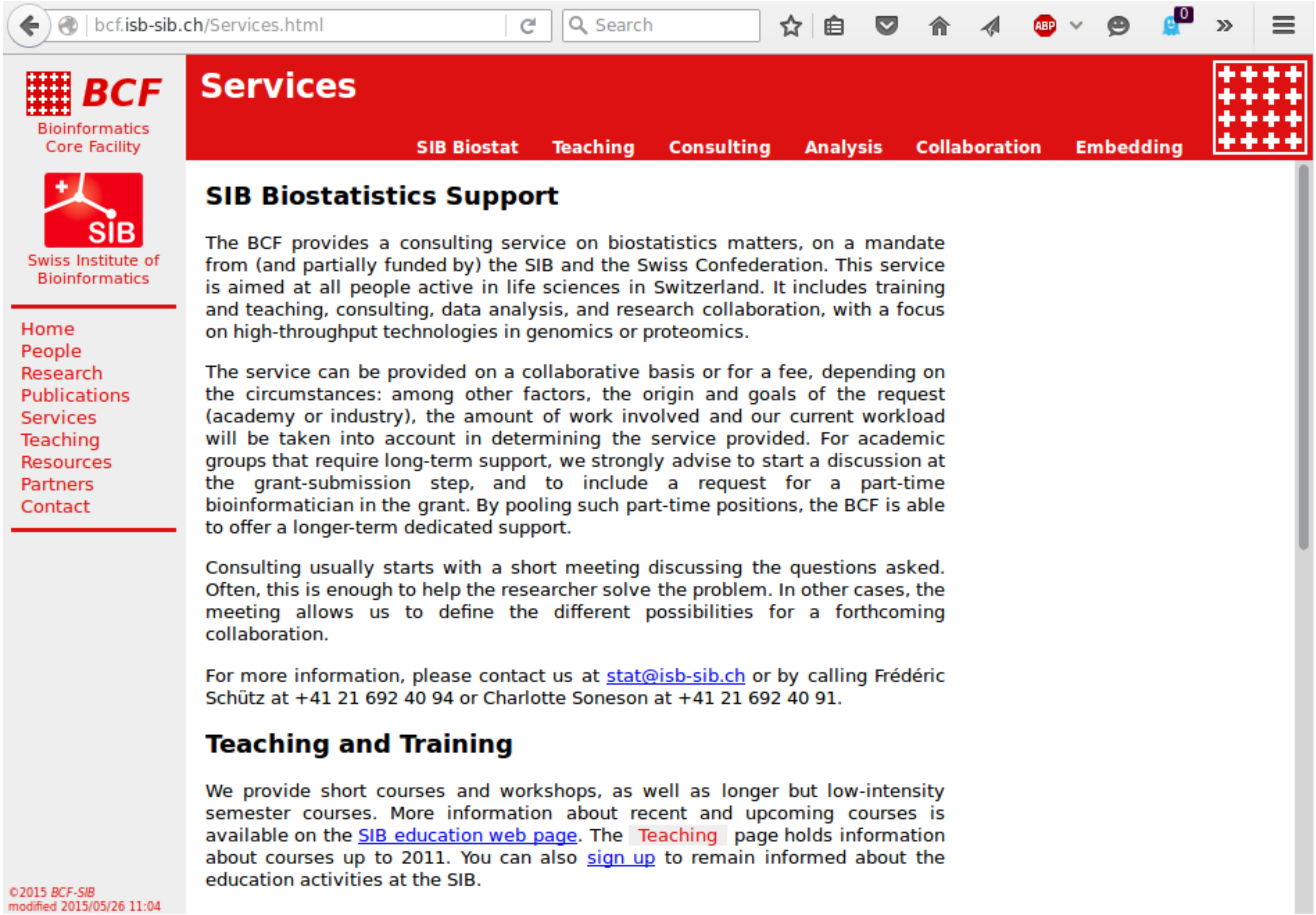
- General statistics in biomedical sciences (for people who want to understand statistics but won't use them directly)
- Multivariate Analysis
- Integration of data from several sources
- Graphical representation of life science data
- Data analysis and reproducible research

We can also offer these courses "in-house", or develop custom courses tailored to your needs and level, according to your requirements. Please contact stat@isb-sib.ch if you have any question.

Upcoming

Our courses upcoming courses are announced on the SIB education web page. You can also sign up to remain informed about the education activities at the SIB.

The organization of our courses depends strongly on the interest of potential participants. If you have any question or suggestion, please contact stat@isb-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Services

SIB Biostat · Teaching · Consulting · Analysis · Collaboration · Embedding

SIB Biostatistics Support

The BCF provides a consulting service on biostatistics matters, on a mandate from (and partially funded by) the SIB and the Swiss Confederation. This service is aimed at all people active in life sciences in Switzerland. It includes training and teaching, consulting, data analysis, and research collaboration, with a focus on high-throughput technologies in genomics or proteomics.

The service can be provided on a collaborative basis or for a fee, depending on the circumstances: among other factors, the origin and goals of the request (academy or industry), the amount of work involved and our current workload will be taken into account in determining the service provided. For academic groups that require long-term support, we strongly advise to start a discussion at the grant-submission step, and to include a request for a part-time bioinformatician in the grant. By pooling such part-time positions, the BCF is able to offer a longer-term dedicated support.

Consulting usually starts with a short meeting discussing the questions asked. Often, this is enough to help the researcher solve the problem. In other cases, the meeting allows us to define the different possibilities for a forthcoming collaboration.

For more information, please contact us at stat@isb-sib.ch or by calling Frédéric Schütz at +41 21 692 40 94 or Charlotte Soneson at +41 21 692 40 91.

Teaching and Training

We provide short courses and workshops, as well as longer but low-intensity semester courses. More information about recent and upcoming courses is available on the SIB education web page. The Teaching page holds information about courses up to 2011. You can also sign up to remain informed about the education activities at the SIB.

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http://bcf.isb-sib.ch/Services.html

Schedule

9:00	- 10:30	*Recall differential expression*
		Recall statistical tests
		Exercise
10:30	- 10:45	coffee break
10:45	- 12:30	*Threshold-based versus Threshold-free enrichment methods*
		GSEA advantages and drawbacks
		Classification of available gene enrichment methods
		Exercise
12:30	- 13:30	lunch (on your own)
13:30	- 15:30	*Generalizing enrichment*
		Exercise
15:30	- 15:45	coffee break
15:45	- 17:00	*Ontologies and enrichment*
		Exercise
17:00		end of day

Questions

Anytime, by raising your hands

Course web-page

Course page: https://edu.sib.swiss/course/view.php?id=333

Login: ea18

Password: SIB-ea18

Credits

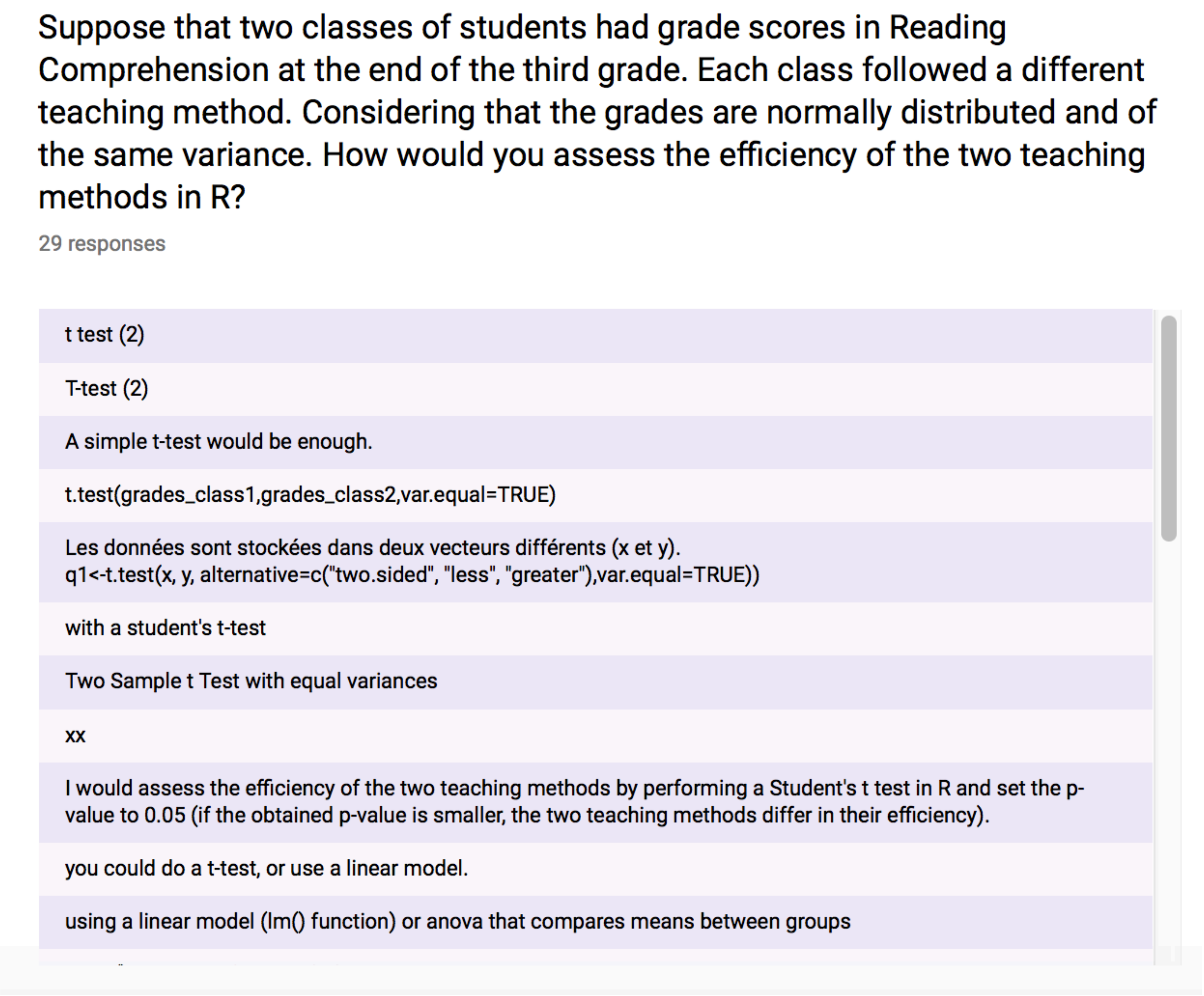
Who?

This course worth 0.25 credits

Pre-requisites

R beginner level,

Elementary statistics



Suppose that two classes of students had grade scores in Reading Comprehension at the end of the third grade. Each class followed a different teaching method. Considering that the grades are normally distributed and of the same variance. How would you assess the efficiency of the two teaching methods in R?

29 responses

t test (2)

T-test (2)

A simple t-test would be enough.

t.test(grades_class1,grades_class2,var.equal=TRUE)

Les données sont stockées dans deux vecteurs différents (x et y).
q1<-t.test(x, y, alternative=c("two.sided", "less", "greater"),var.equal=TRUE))

with a student's t-test

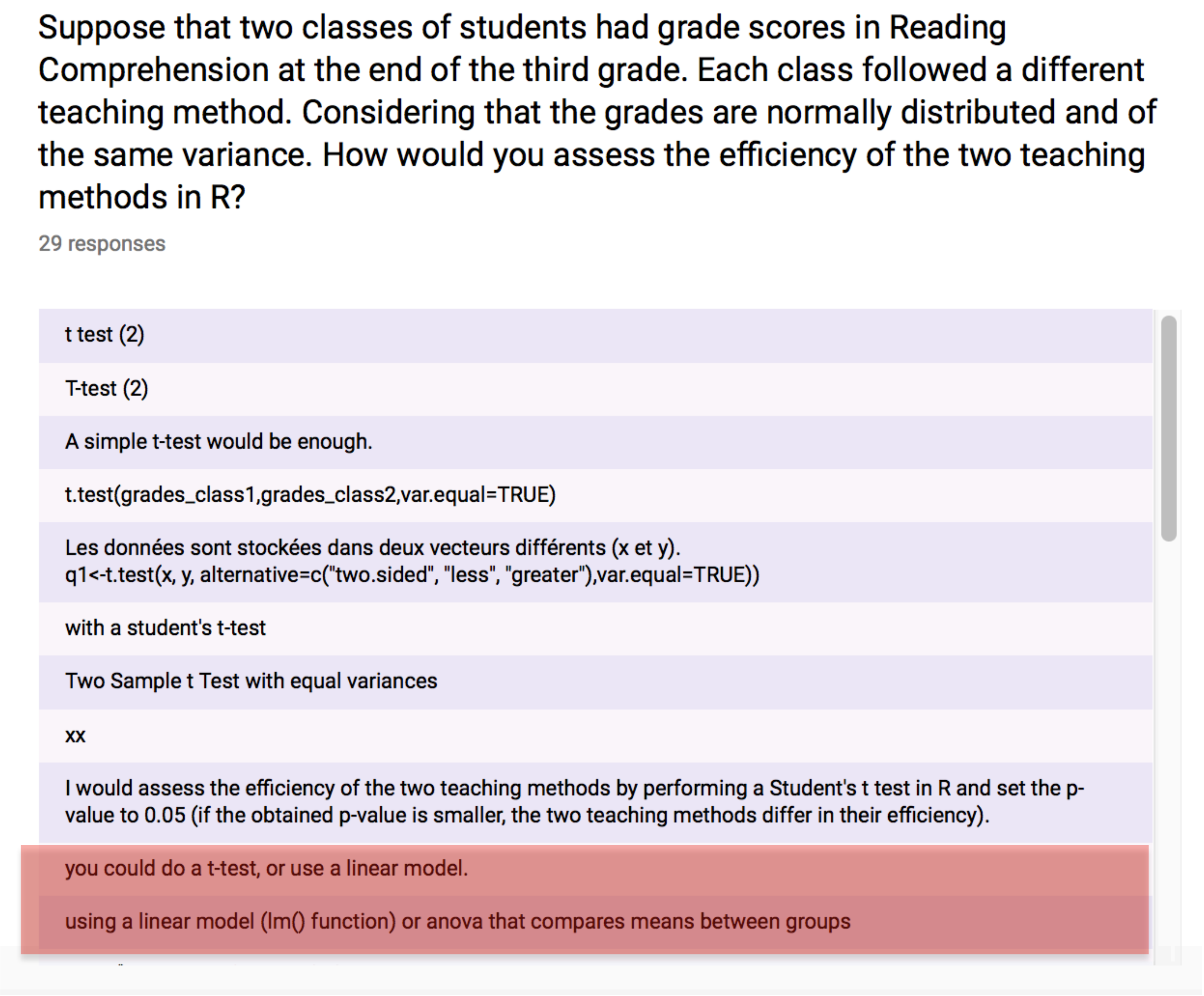
Two Sample t Test with equal variances

xx

I would assess the efficiency of the two teaching methods by performing a Student's t test in R and set the p-value to 0.05 (if the obtained p-value is smaller, the two teaching methods differ in their efficiency).

you could do a t-test, or use a linear model.

using a linear model (lm() function) or anova that compares means between groups



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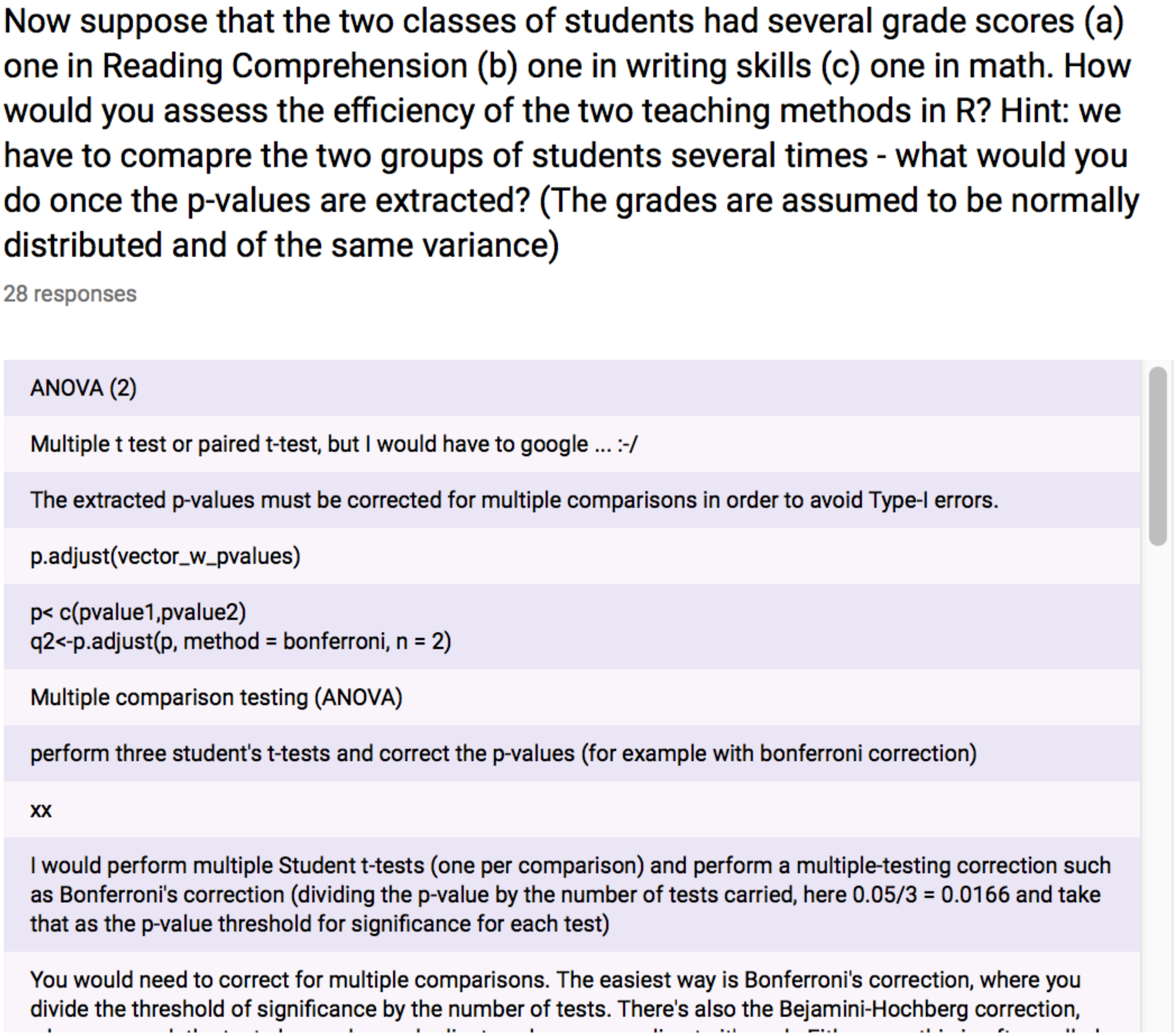


Now suppose that the two classes of students had several grade scores (a) one in Reading Comprehension (b) one in writing skills (c) one in math. How would you assess the efficiency of the two teaching methods in R? Hint: we have to comapre the two groups of students several times - what would you do once the p-values are extracted? (The grades are assumed to be normally distributed and of the same variance)

28 responses

ANOVA (2)

Multiple t test or paired t-test, but I would have to google ... :-/

The extracted p-values must be corrected for multiple comparisons in order to avoid Type-I errors.

p.adjust(vector_w_pvalues)

p< c(pvalue1,pvalue2)
q2<-p.adjust(p, method = bonferroni, n = 2)

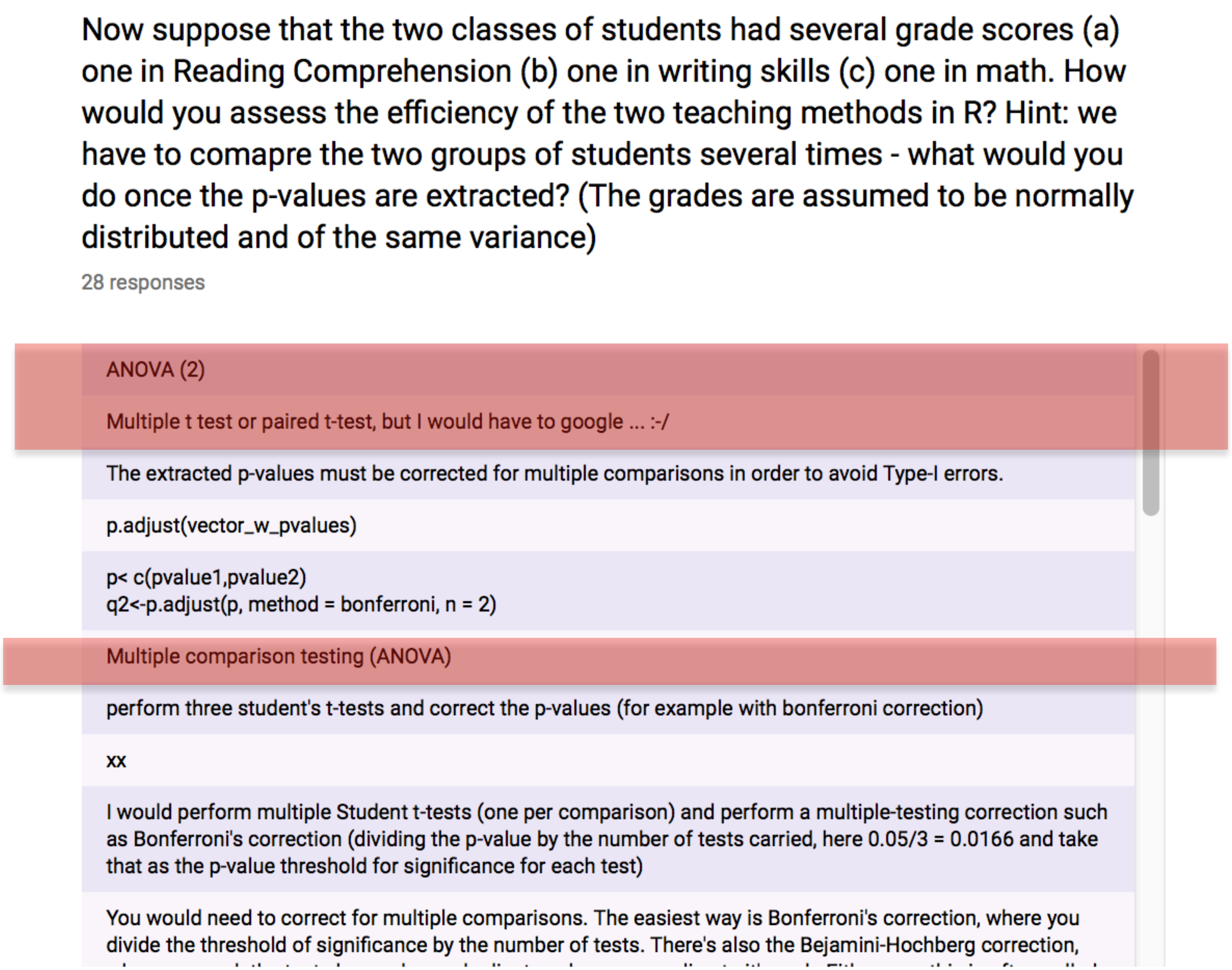
Multiple comparison testing (ANOVA)

perform three student's t-tests and correct the p-values (for example with bonferroni correction)

xx

I would perform multiple Student t-tests (one per comparison) and perform a multiple-testing correction such as Bonferroni's correction (dividing the p-value by the number of tests carried, here 0.05/3 = 0.0166 and take that as the p-value threshold for significance for each test)

You would need to correct for multiple comparisons. The easiest way is Bonferroni's correction, where you divide the threshold of significance by the number of tests. There's also the Bejamini-Hochberg correction,



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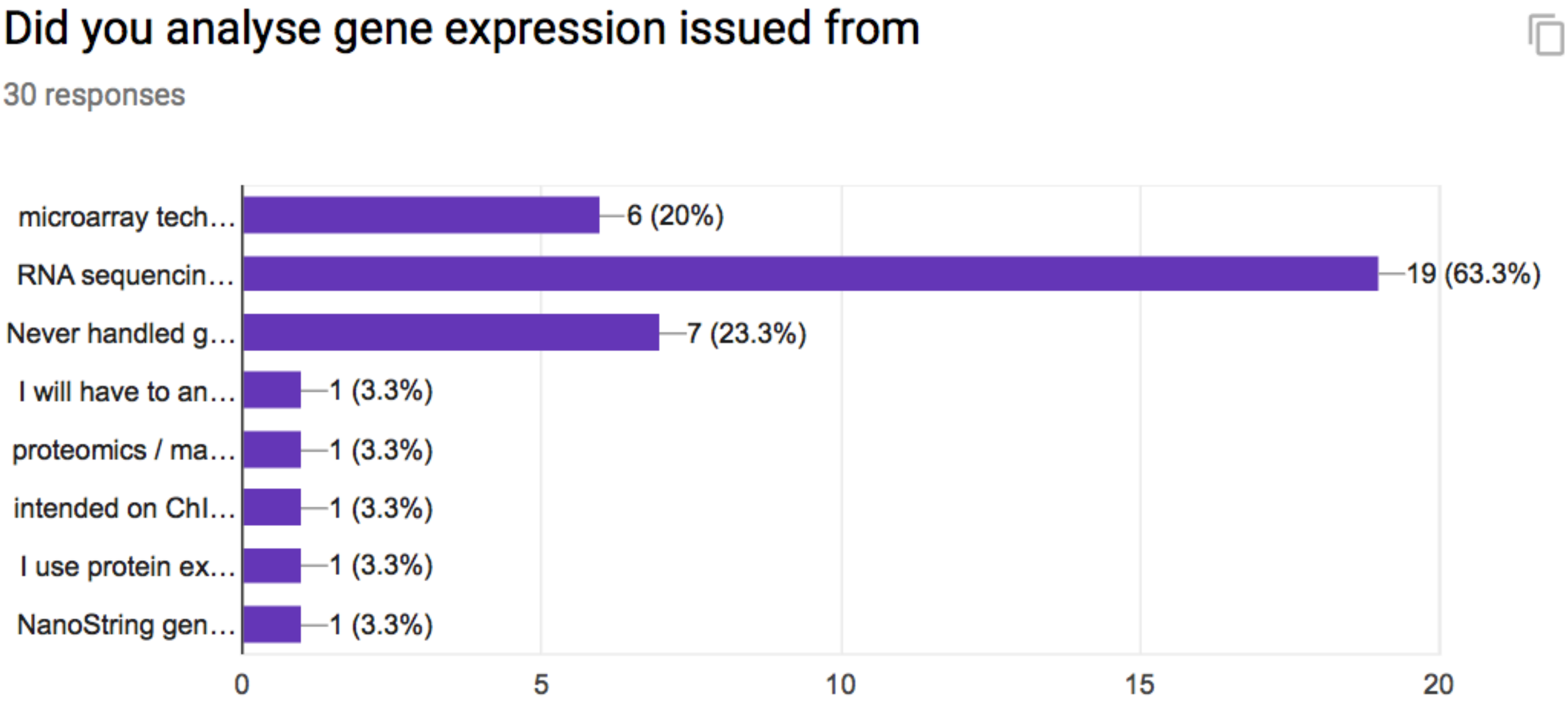


Did you analyse gene expression issued from

30 responses

RNA-seq pipeline

1. Check the quality of the reads
- FastQC
- cutAdapt to trimm

2. Map to your favorite genome
- TopHat, star, Hisat2

3. Sort , create, index bam files
- SAMTOOLS

4. Control mapping and quality
- RNAseq QC, Qualimap, noiseQC

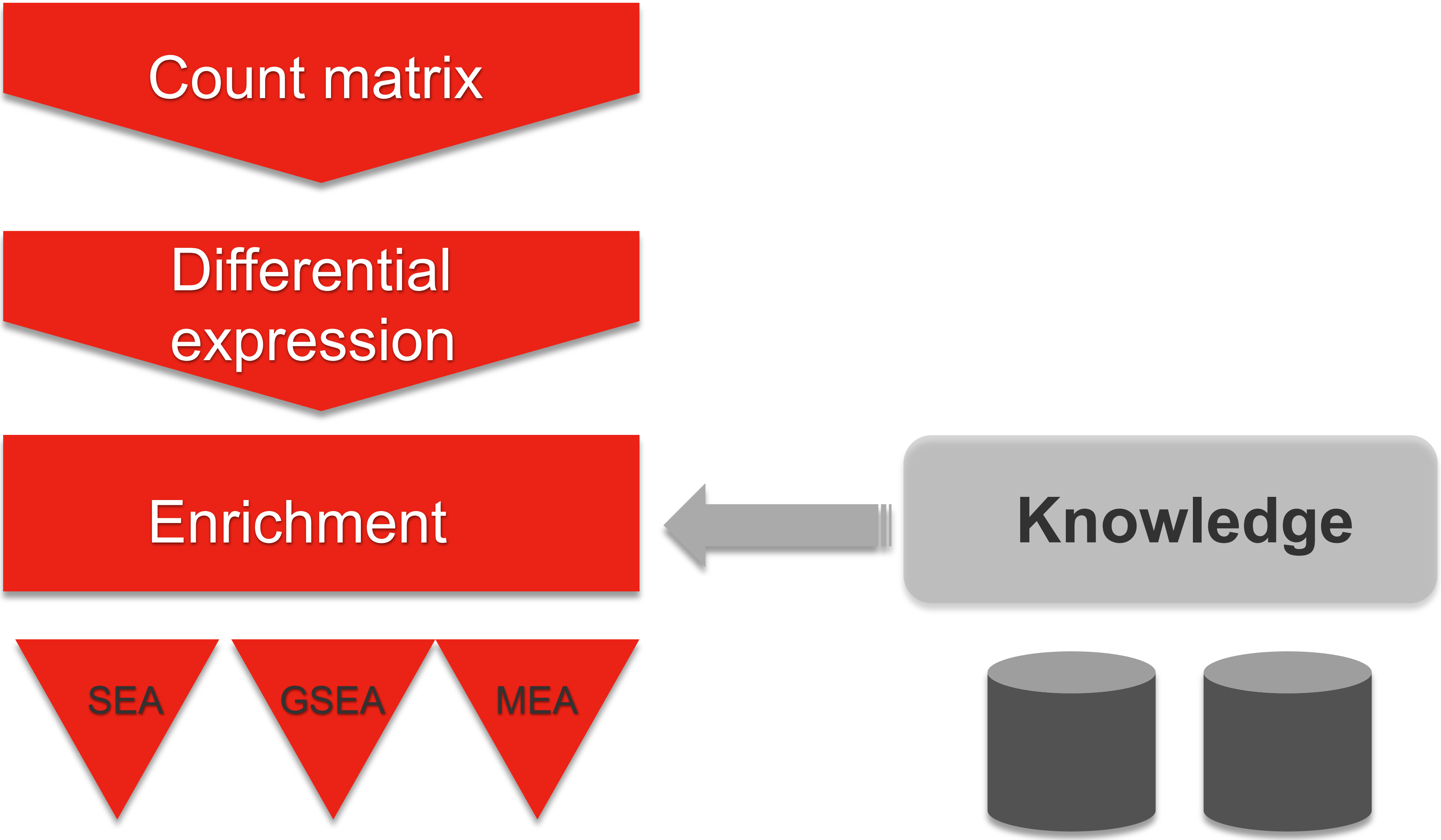
5. Generate count matrix
- summarizeOverlaps , featureCounts, tximport , htseq-count

6. Check for batch effect, normalization and correction

7. Differential expression of counts - based on statistics
- using Limma*, edgeR, DESeq2,…

8. Enrichment analysis given a phenotype - based on statistics

Overview



Count matrix

Differential expression

Enrichment

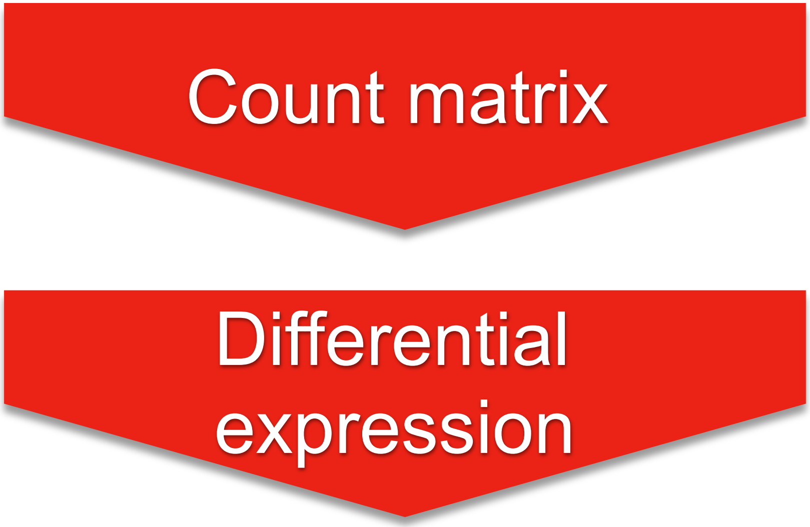
SEA

GSEA

MEA

Knowledge

Overview

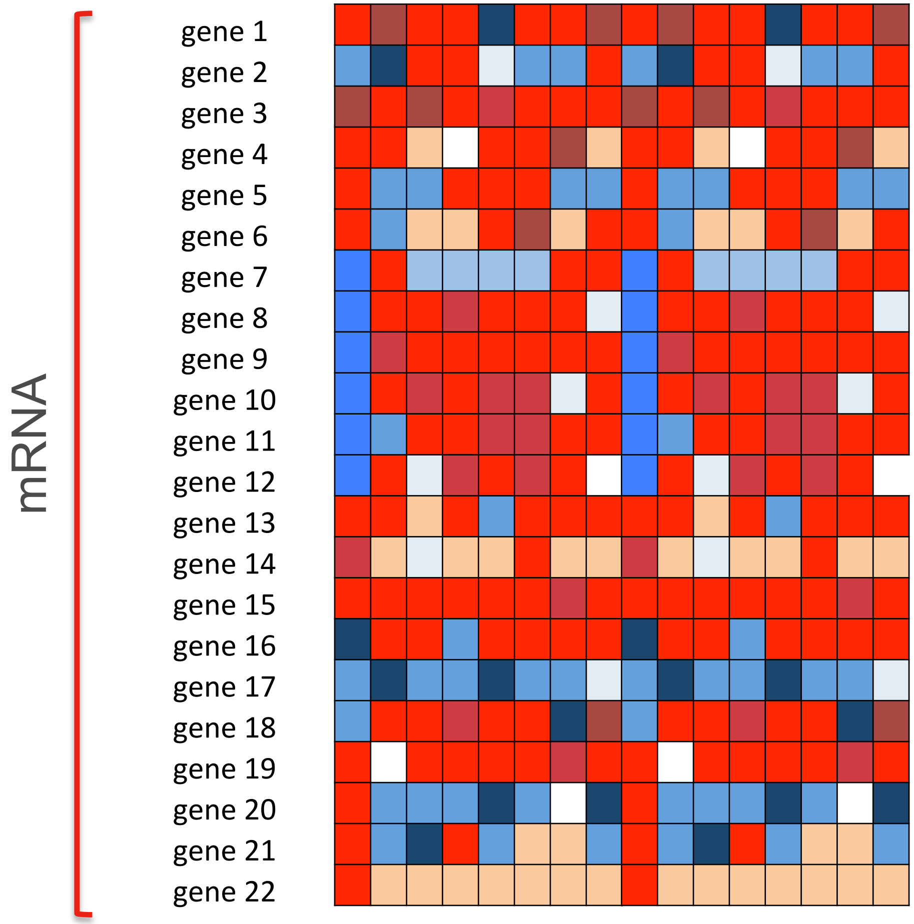


Count matrix

Differential expression

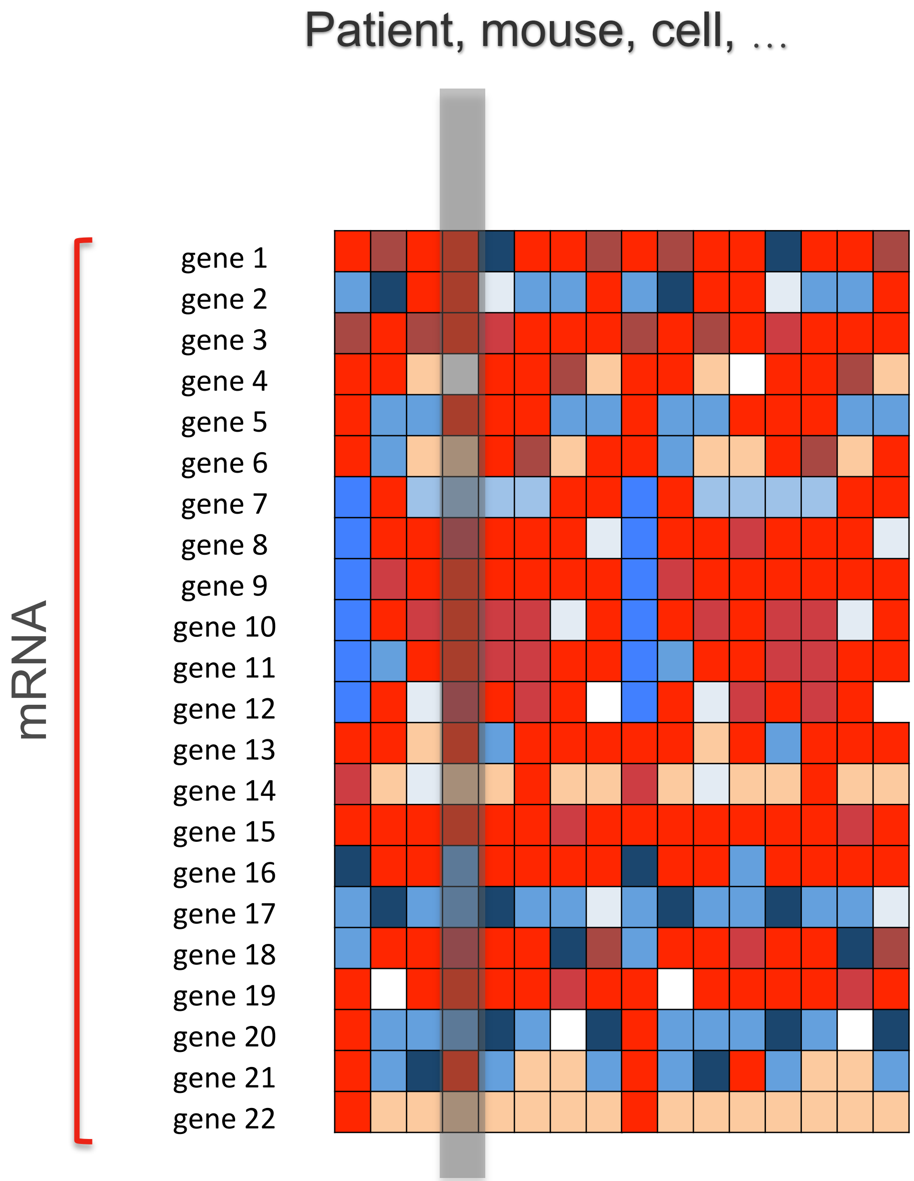
High-throughput expression data

Count matrix



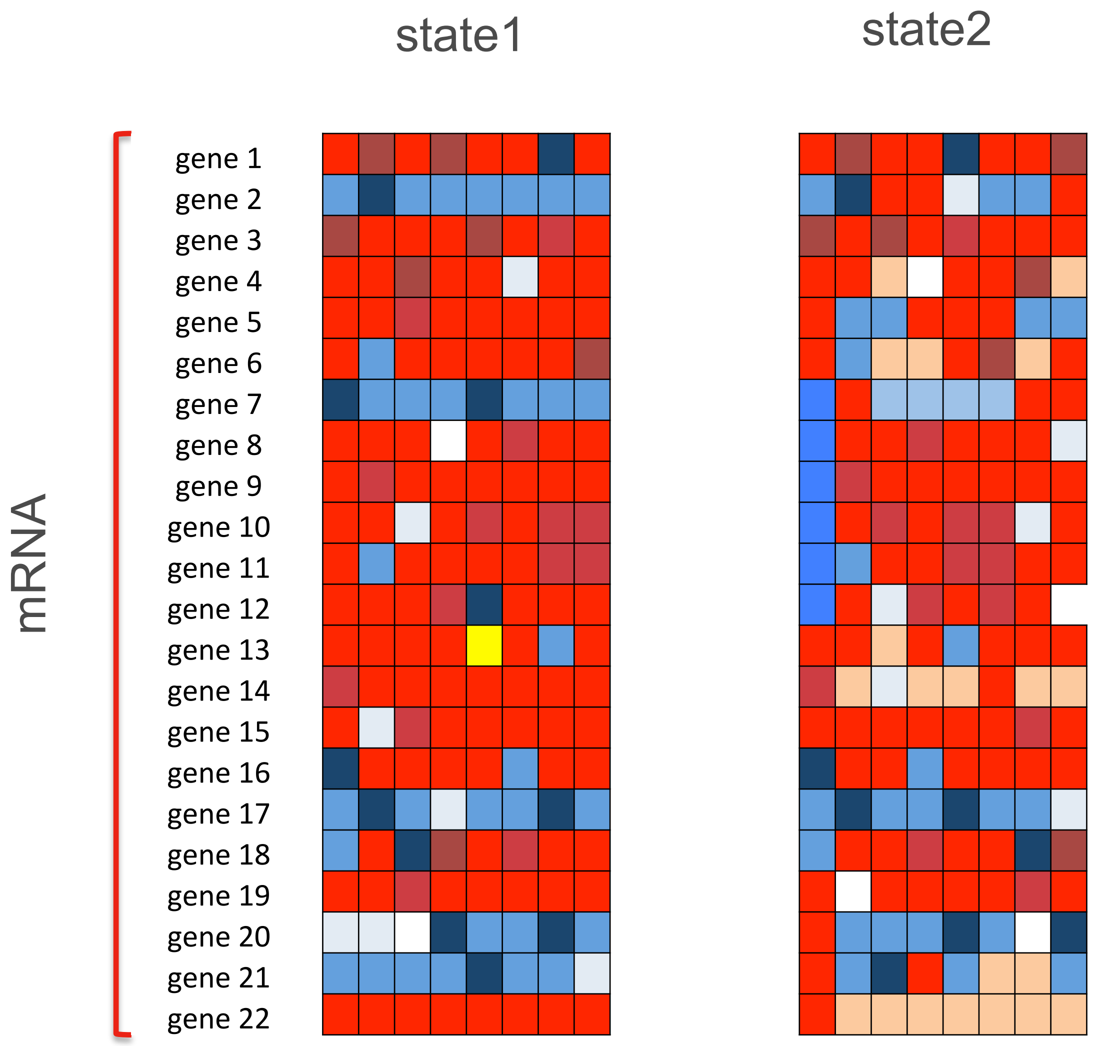
High-throughput expression data

Count matrix



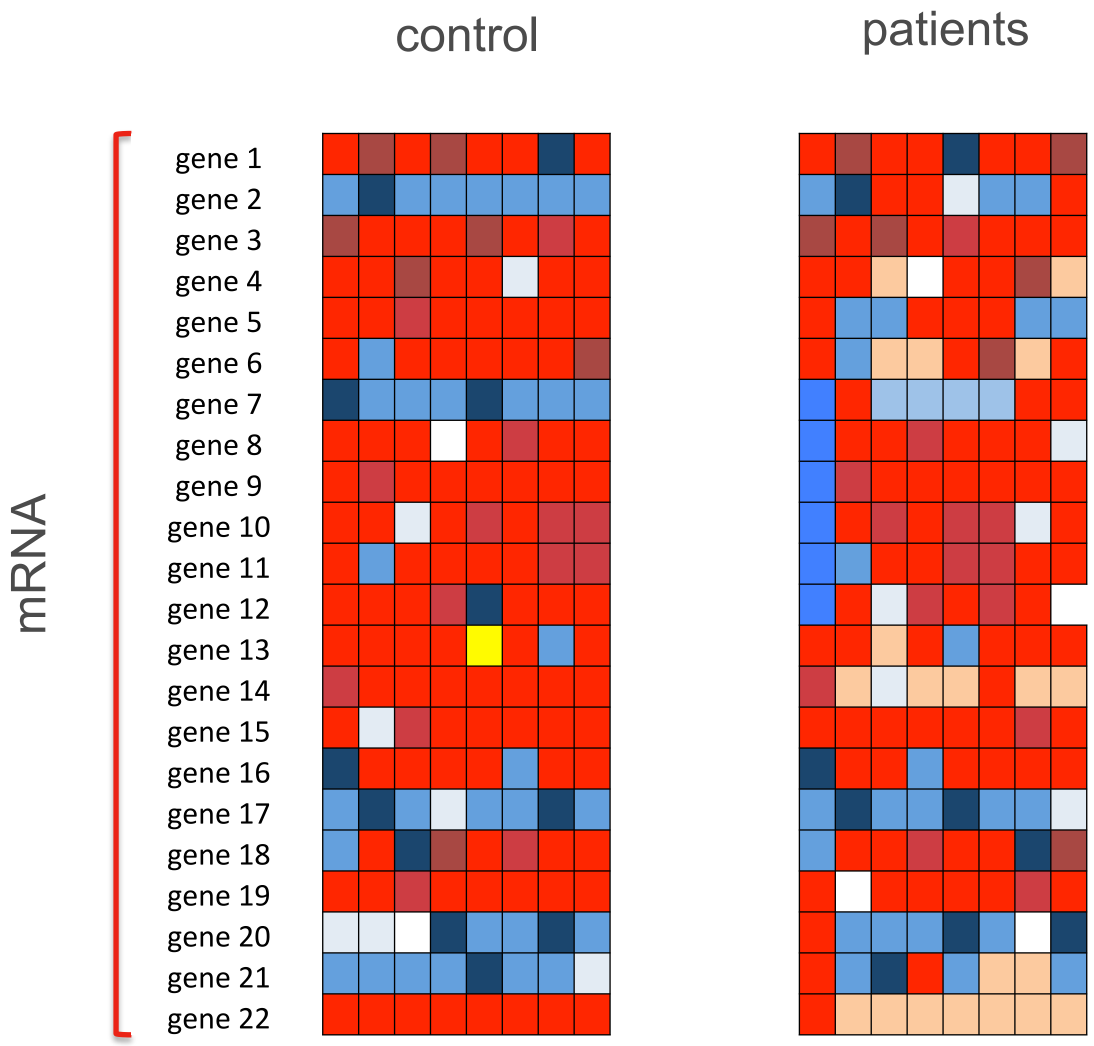
Differential expression

Comparing
two
biological
states



Differential expression

Comparing two biological states



Comparing two groups

For each gene i, is there a difference in expression between the condition1 (healthy controls) and condition2 (patients)?

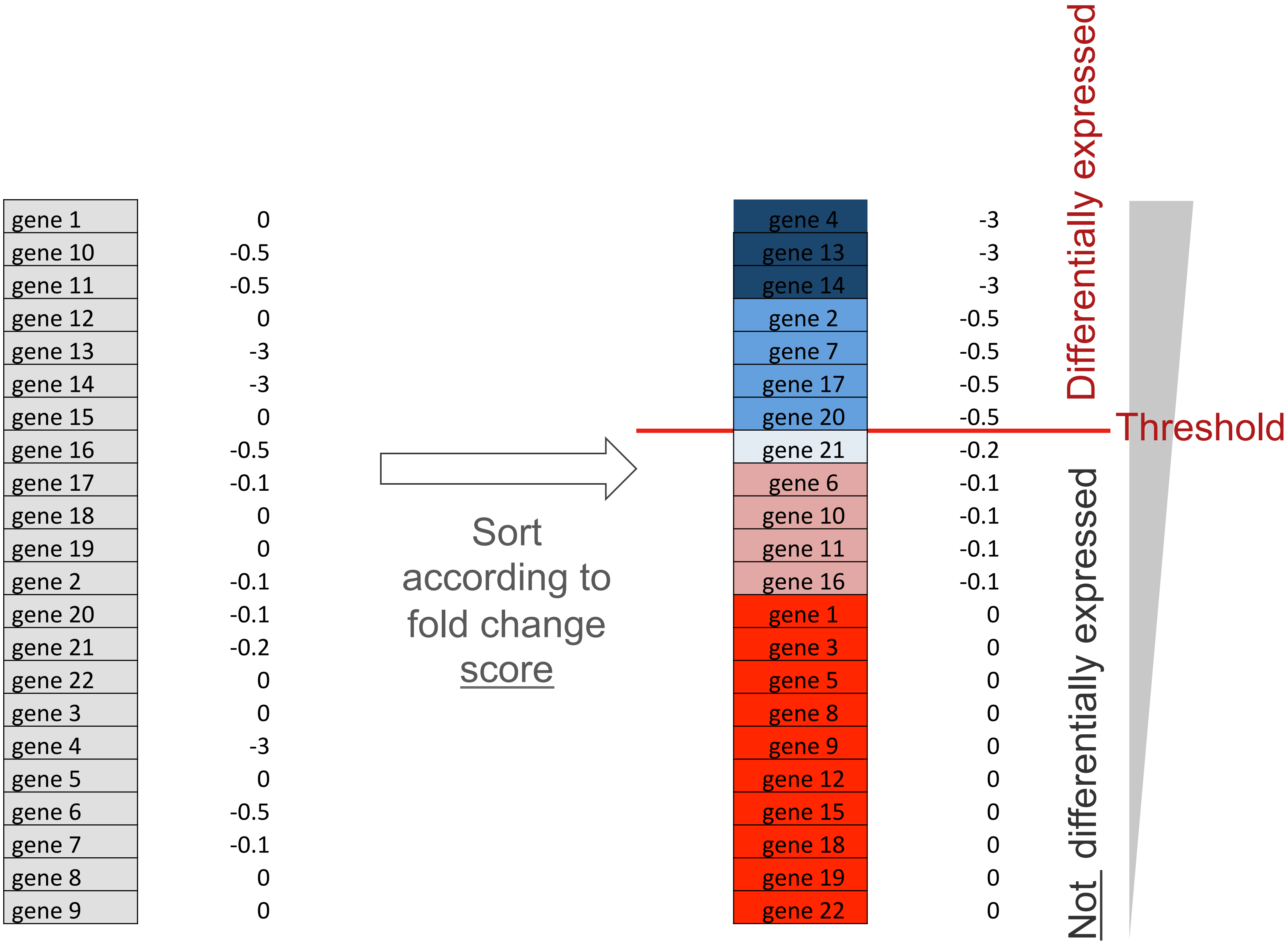
Fold change approach

$$\log(\pi_{i1}/\pi_{i2}) = \log(\pi_{i1}) - \log(\pi_{i2})$$

gene 1	0
gene 10	-0.5
gene 11	-0.5
gene 12	0
gene 13	-3
gene 14	-3
gene 15	0
gene 16	-0.5
gene 17	-0.1
gene 18	0
gene 19	0
gene 2	-0.1
gene 20	-0.1
gene 21	-0.2
gene 22	0
gene 3	0
gene 4	-3
gene 5	0
gene 6	-0.5
gene 7	-0.1
gene 8	0
gene 9	0

Sort according to fold change score

gene 4	-3
gene 13	-3
gene 14	-3
gene 2	-0.5
gene 7	-0.5
gene 17	-0.5
gene 20	-0.5
gene 21	-0.2
gene 6	-0.1
gene 10	-0.1
gene 11	-0.1
gene 16	-0.1
gene 1	0
gene 3	0
gene 5	0
gene 8	0
gene 9	0
gene 12	0
gene 15	0
gene 18	0
gene 19	0
gene 22	0



gene 1	0
gene 10	-0.5
gene 11	-0.5
gene 12	0
gene 13	-3
gene 14	-3
gene 15	0
gene 16	-0.5
gene 17	-0.1
gene 18	0
gene 19	0
gene 2	-0.1
gene 20	-0.1
gene 21	-0.2
gene 22	0
gene 3	0
gene 4	-3
gene 5	0
gene 6	-0.5
gene 7	-0.1
gene 8	0
gene 9	0

Sort according to fold change score

gene 4	-3
gene 13	-3
gene 14	-3
gene 2	-0.5
gene 7	-0.5
gene 17	-0.5
gene 20	-0.5
gene 21	-0.2
gene 6	-0.1
gene 10	-0.1
gene 11	-0.1
gene 16	-0.1
gene 1	0
gene 3	0
gene 5	0
gene 8	0
gene 9	0
gene 12	0
gene 15	0
gene 18	0
gene 19	0
gene 22	0

Differentially expressed

Threshold

Not differentially expressed

Fisher exact test

Hypergeometric

Chi-square

Binomial

T-Test

…

T-test is a statistical test that compares the mean of two states

T-test

For each gene i, is there a **significant difference** in mean expression between the condition1 (healthy controls) and condition2 (patients)?

Hypothesis testing

$\mathcal{H}_0$: Healthy controls and patients have similar **gene i** expression

$$\mathcal{H}o_i : \pi_{i1} = \pi_{i2}$$

T-test

For each gene i, is there a **significant difference** in mean expression between the condition1 (healthy controls) and condition2 (patients)?

Hypothesis testing

$\mathcal{H}_0$: Healthy controls and patients have similar **gene i** expression

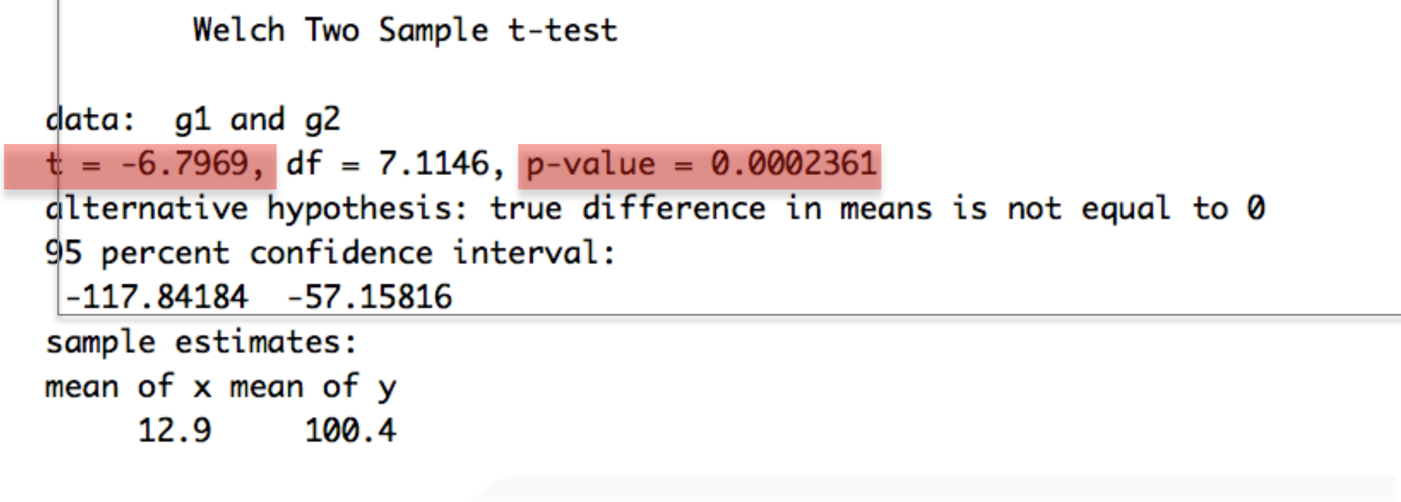
$$\mathcal{H}o_i : \pi_{i1} = \pi_{i2}$$

$\mathcal{H}_1$: Healthy controls and patients don't have a similar gene i expression

$$\mathcal{H}1_i : \pi_{i1} \neq \pi_{i2}$$

In R

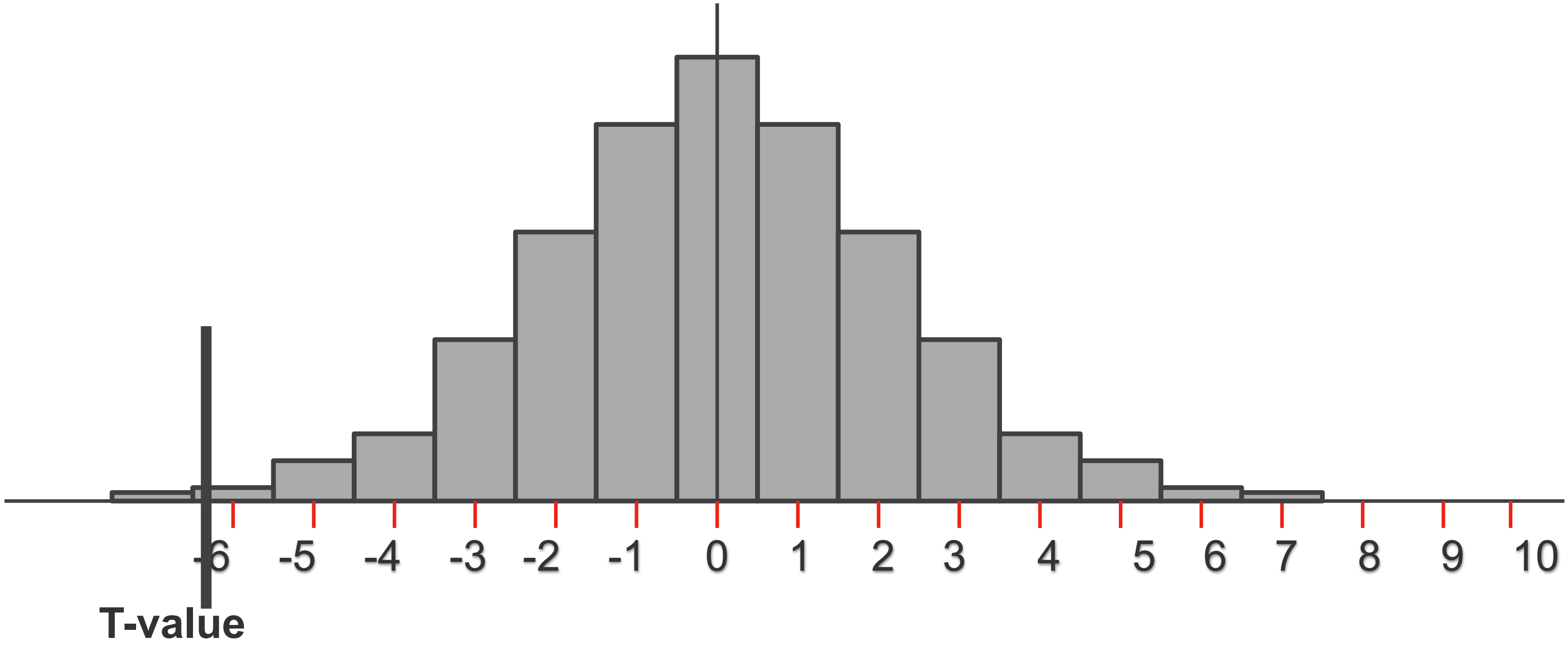
```
>?t.test
>t.test(g1,g2)
```

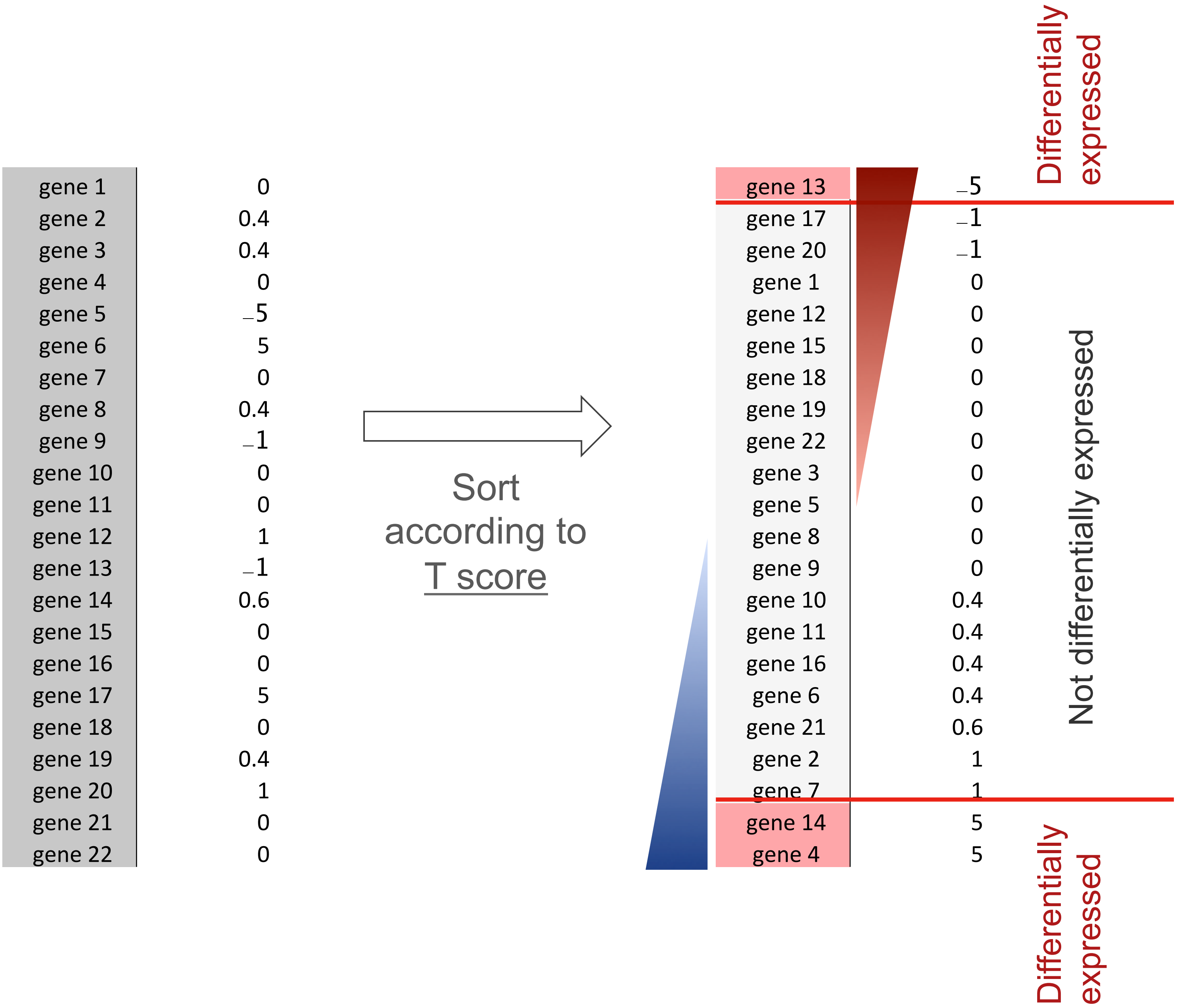


```
        Welch Two Sample t-test

data:  g1 and g2
t = -6.7969, df = 7.1146, p-value = 0.0002361
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -117.84184  -57.15816
sample estimates:
mean of x mean of y 
     12.9     100.4 
```

T-distribution with group size =8





gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	–5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	–1
gene 10	0
gene 11	0
gene 12	1
gene 13	–1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0

gene 13	–5
gene 17	–1
gene 20	–1
gene 1	0
gene 12	0
gene 15	0
gene 18	0
gene 19	0
gene 22	0
gene 3	0
gene 5	0
gene 8	0
gene 9	0
gene 10	0.4
gene 11	0.4
gene 16	0.4
gene 6	0.4
gene 21	0.6
gene 2	1
gene 7	1
gene 14	5
gene 4	5

P-value

The p-value is the probability of getting a result that is as or more extreme than the observed result, assuming that the null hypothesis is true.

The p-value reflects the magnitude of the difference between the study groups

AND

the sample size

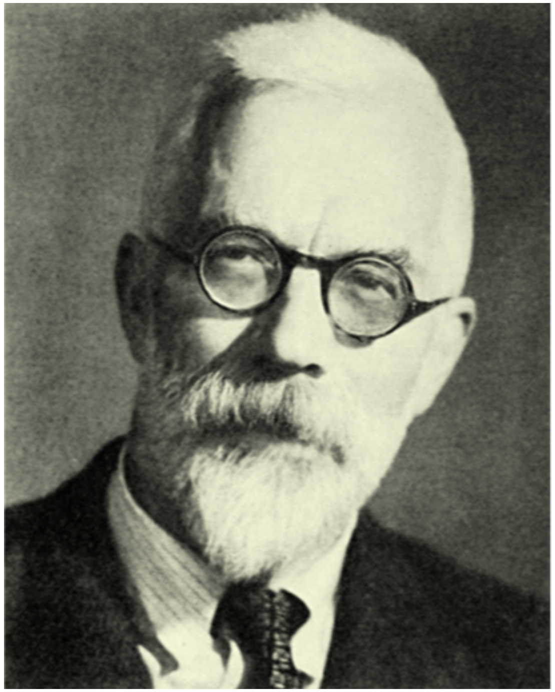
AND

the variability within each group

P-value and decision

By convention, if $p < 0.05$, then the association between the exposure and disease is considered to be "statistically significant." (e.g. we reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1))

Why 0.05?



Fisher

P-value and decision

What does $p < 0.05$ mean?

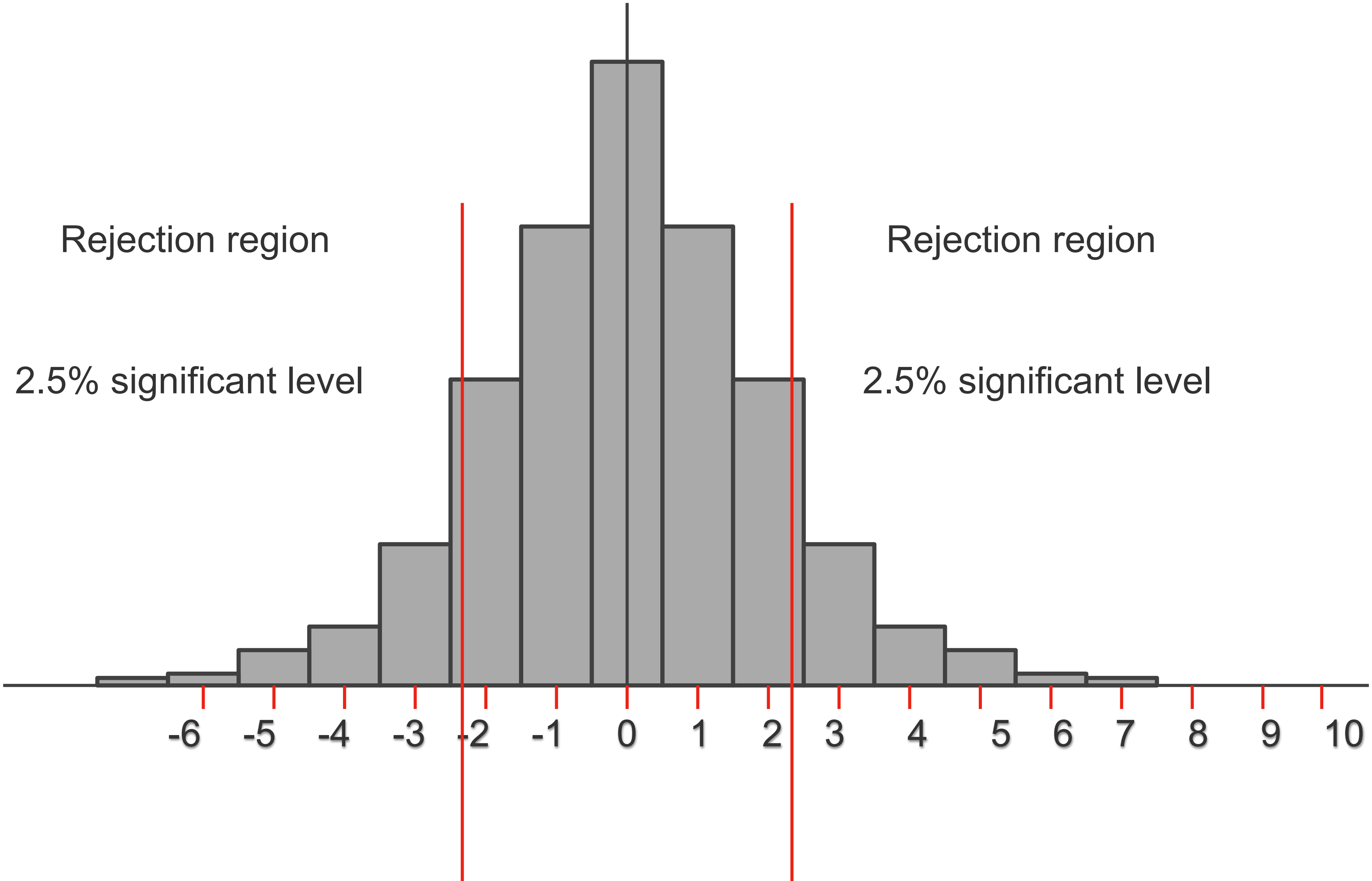
Indirectly, it means that we suspect that the magnitude of effect observed (e.g. odds ratio) is not due to chance alone

(in the absence of biased data collection or analysis)

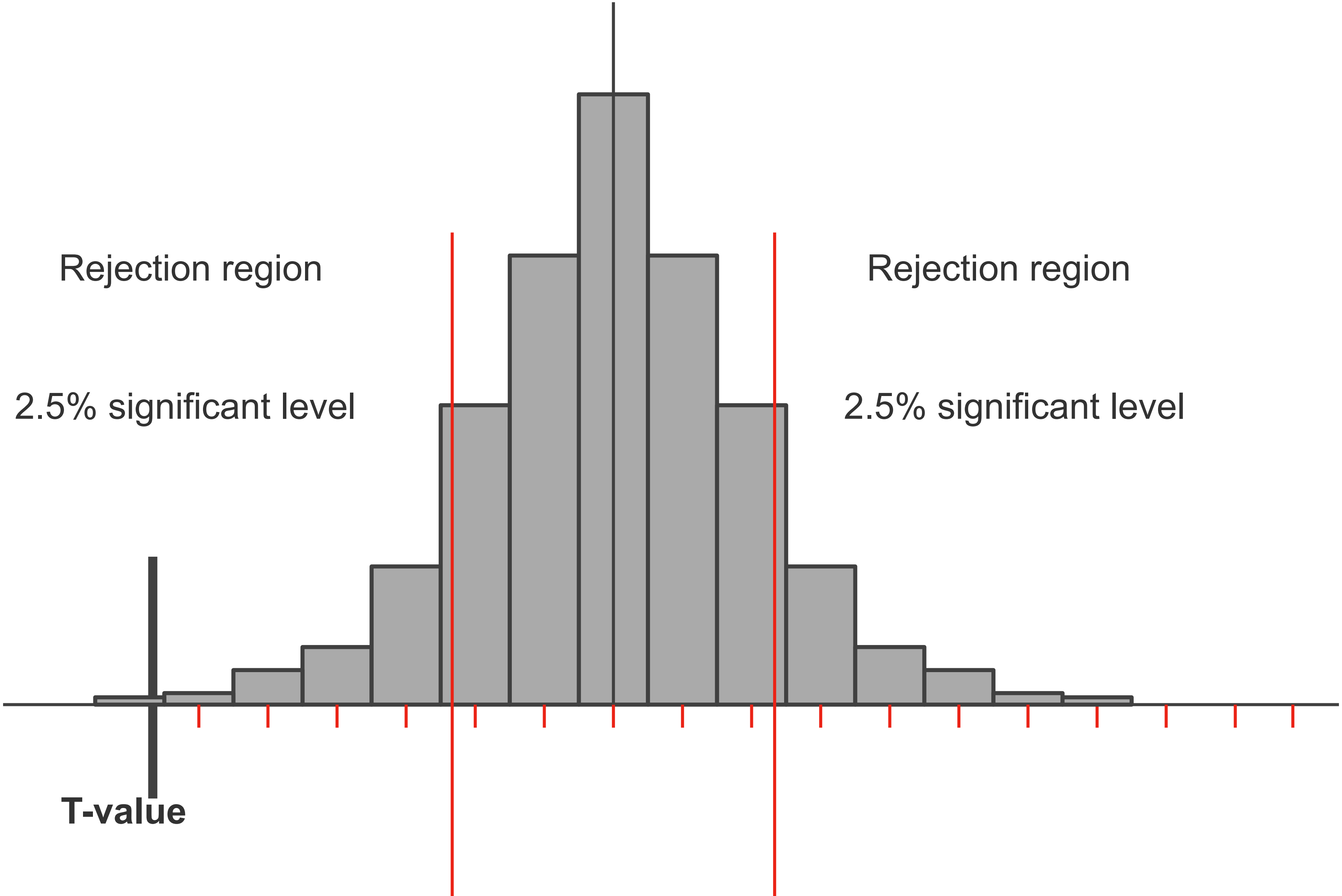
Directly, $p=0.05$ means that one test result out of twenty results would be expected to occur due to chance (random error) alone

P-value and decision

T-distribution with group size =8



P-value and decision



p-value =0.000001 & p-value =0.049

0.01 and 0.1

are also possible threshold

gene 1	1
gene 2	0.01
gene 3	1
gene 4	0.0001
gene 5	1
gene 6	0.6
gene 7	0.01
gene 8	1
gene 9	1
gene 10	0.6
gene 11	0.6
gene 12	1
gene 13	0.0001
gene 14	0.0001
gene 15	1
gene 16	0.6
gene 17	0.01
gene 18	1
gene 19	1
gene 20	0.01
gene 21	0.4
gene 22	1

Sort according to p-value

	T-score	p-value
gene 4	5	0.0001
gene 13	5	0.0001
gene 14	–5	0.0001
gene 2	5	0.01
gene 7	1	0.01
gene 17	1	0.01
gene 20	–1	0.01
gene 21	–1	0.4
gene 6	0.6	0.6
gene 10	0.4	0.6
gene 11	0.4	0.6
gene 16	0.4	0.6
gene 1	0.4	1
gene 3	0	1
gene 5	0	1
gene 8	0	1
gene 9	0	1
gene 12	0	1
gene 15	0	1
gene 18	0	1
gene 19	0	1
gene 22	0	1

Differentially expressed

P-value and decision

Decision Truth	H_0 not rejected (negative)	H_0 Rejected (positive)
H_0 is true (no signal in the data)	☺ specificity True negative TN	X Type I error False Positive α
H_0 is false (there is something to find)	X Type II error False Negative β	☺ Power $1 - \beta$; sensitivity True Positive TP

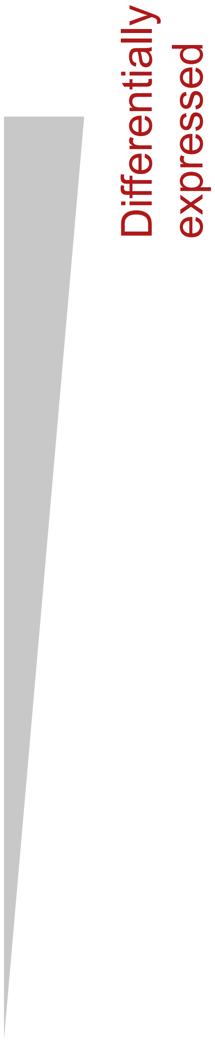
P-value and decision

gene 1	1
gene 2	0.01
gene 3	1
gene 4	0.0001
gene 5	1
gene 6	0.6
gene 7	0.01
gene 8	1
gene 9	1
gene 10	0.6
gene 11	0.6
gene 12	1
gene 13	0.0001
gene 14	0.0001
gene 15	1
gene 16	0.6
gene 17	0.01
gene 18	1
gene 19	1
gene 20	0.01
gene 21	0.4
gene 22	1

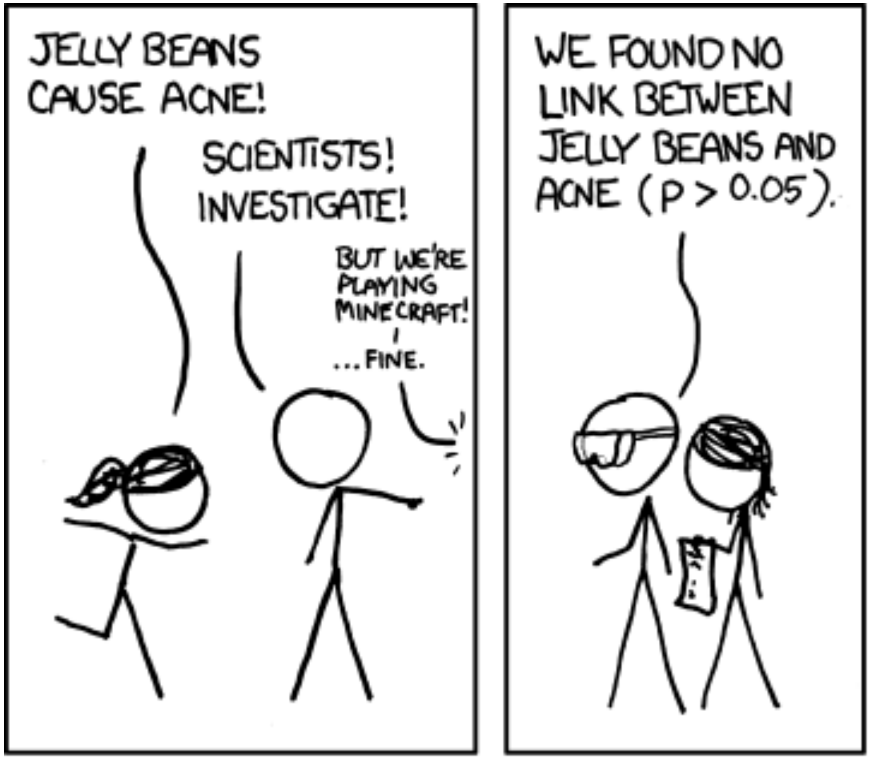
Sort according to adj. p-value

~~Sort according to p-value~~

	T-score	p-value	Adj. p-value
gene 4	5	0.0001	0.0022
gene 13	5	0.0001	0.0022
gene 14	–5	0.0001	0.0022
gene 2	5	0.01	0.19
gene 7	1	0.01	0.19
gene 17	1	0.01	0.19
gene 20	–1	0.01	0.19
gene 21	–1	0.4	1
gene 6	0.6	0.6	1
gene 10	0.4	0.6	1
gene 11	0.4	0.6	1
gene 16	0.4	0.6	1
gene 1	0.4	1	1
gene 3	0	1	1
gene 5	0	1	1
gene 8	0	1	1
gene 9	0	1	1
gene 12	0	1	1
gene 15	0	1	1
gene 18	0	1	1
gene 19	0	1	1
gene 22	0	1	1

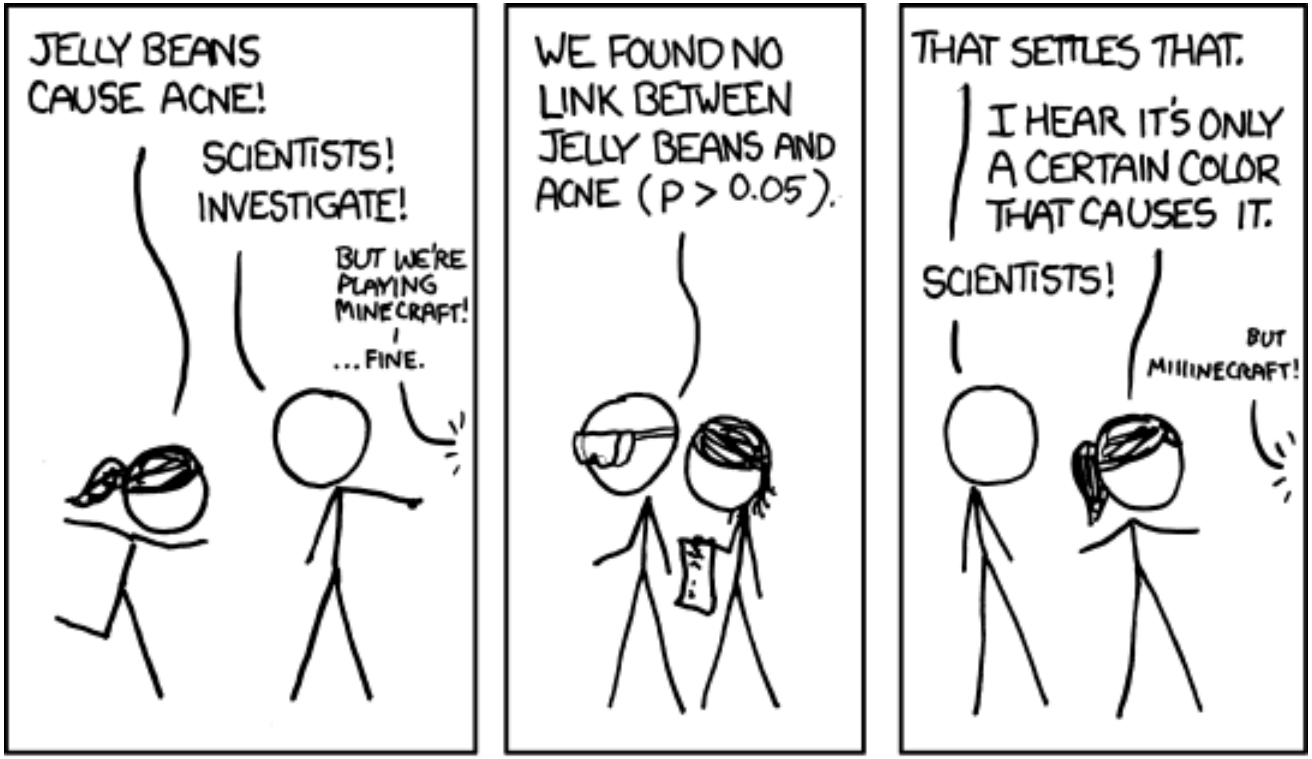


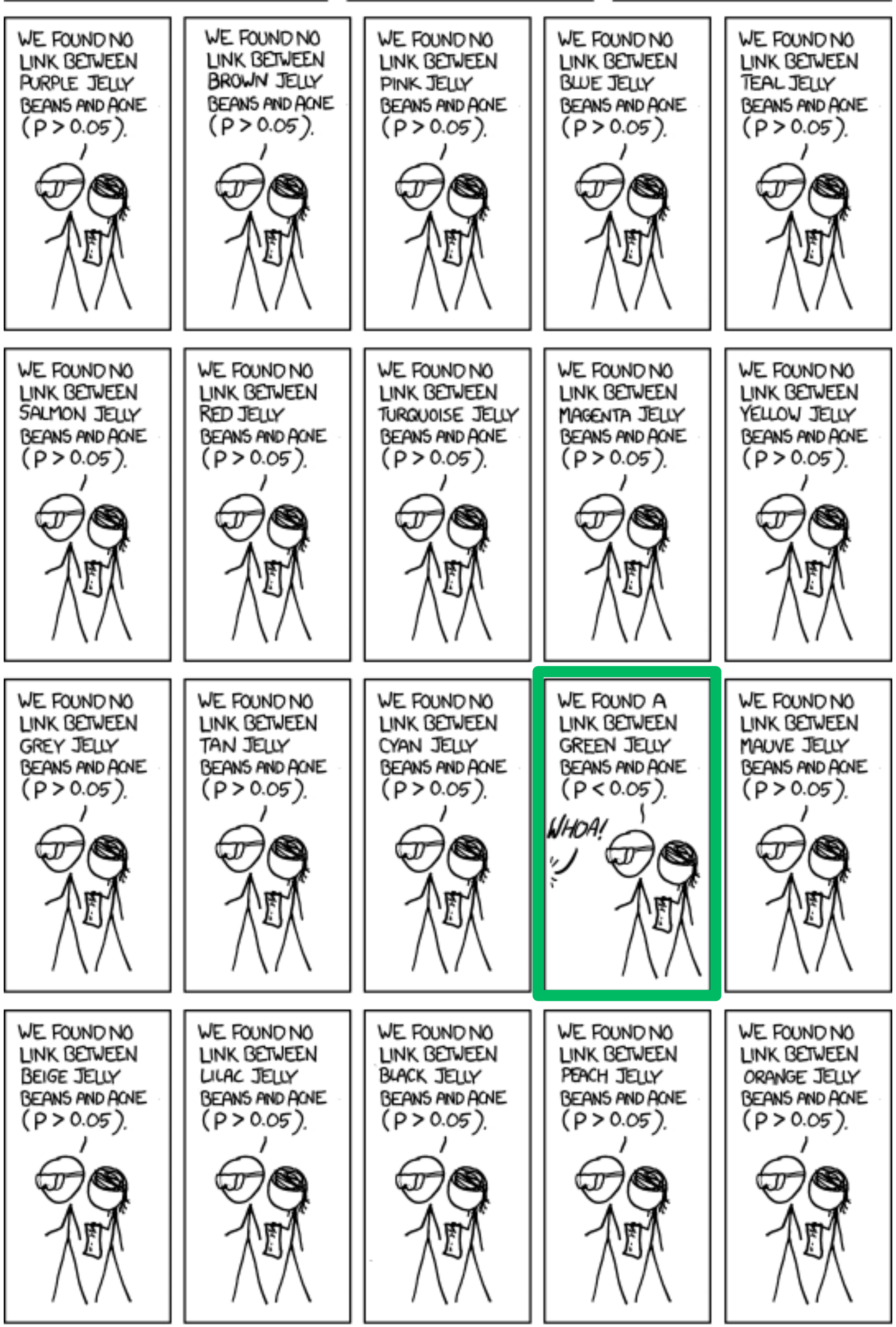
Adjusting p-value, why?

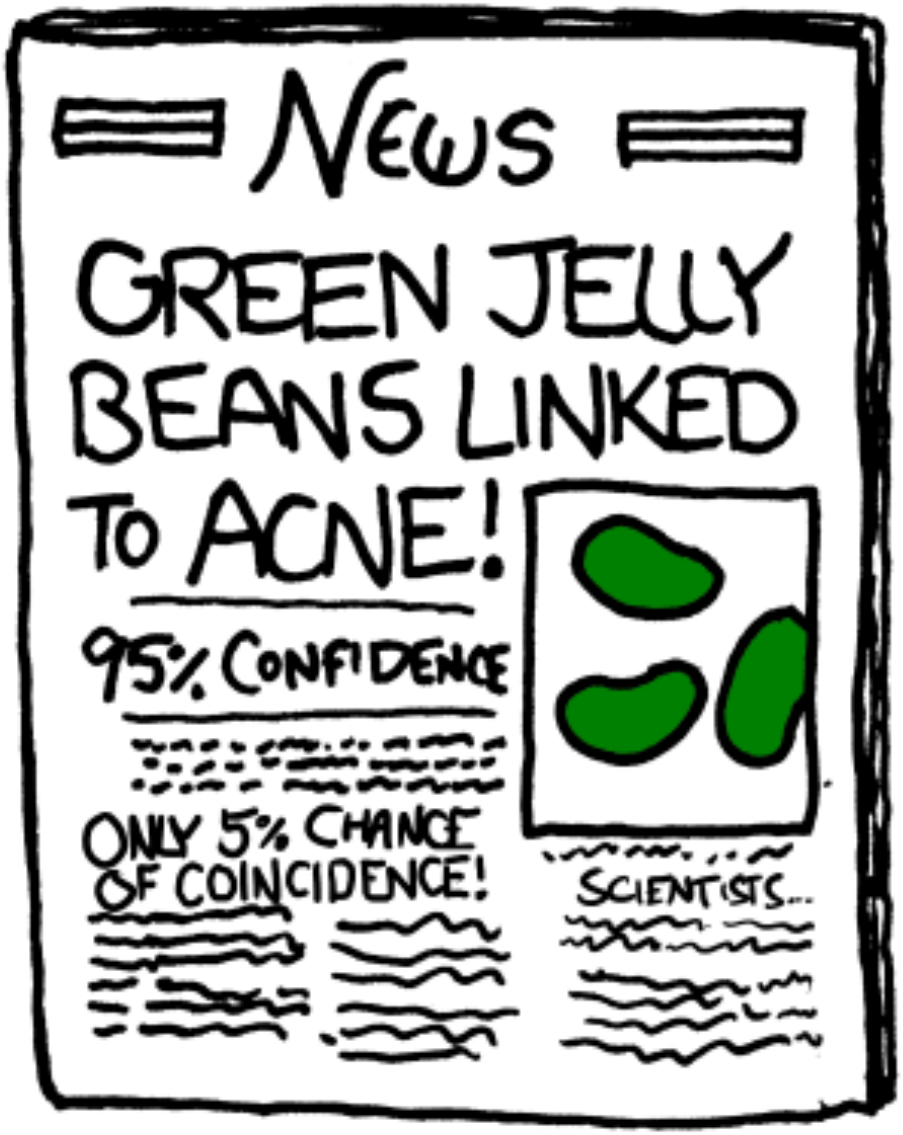

JELLY BEANS CAUSE ACNE!
SCIENTISTS! INVESTIGATE!
BUT WE'RE PLAYING MINECRAFT!
... FINE.
WE FOUND NO LINK BETWEEN JELLY BEANS AND ACNE (P > 0.05).

Significance, by Randall Munroe / XKCD http://xkcd.com/882/

Adjusting p-value, why?


JELLY BEANS CAUSE ACNE!
SCIENTISTS! INVESTIGATE!
BUT WE'RE PLAYING MINECRAFT!
... FINE.
WE FOUND NO LINK BETWEEN JELLY BEANS AND ACNE (P > 0.05).
THAT SETTLES THAT.
I HEAR IT'S ONLY A CERTAIN COLOR THAT CAUSES IT.
SCIENTISTS!
BUT MIIIINECRAFT!


WE FOUND NO LINK BETWEEN PURPLE JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN BROWN JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN PINK JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN BLUE JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN TEAL JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN SALMON JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN RED JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN TURQUOISE JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN MAGENTA JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN YELLOW JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN GREY JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN TAN JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN CYAN JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND A LINK BETWEEN GREEN JELLY BEANS AND ACNE ($P < 0.05$).
WHOA!
WE FOUND NO LINK BETWEEN MAUVE JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN BEIGE JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN LILAC JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN BLACK JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN PEACH JELLY BEANS AND ACNE ($P > 0.05$).
WE FOUND NO LINK BETWEEN ORANGE JELLY BEANS AND ACNE ($P > 0.05$).

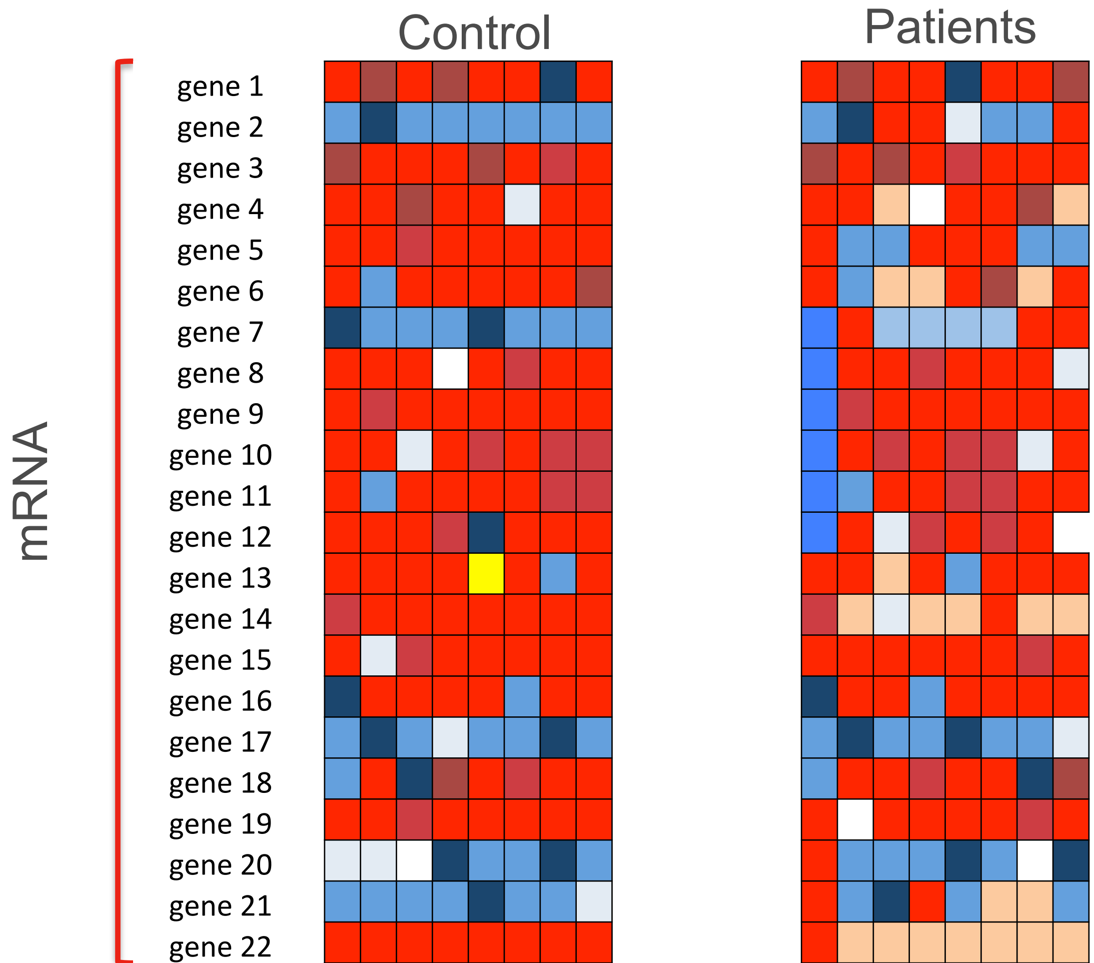

NEWS
GREEN JELLY BEANS LINKED TO ACNE!
95% CONFIDENCE
ONLY 5% CHANCE OF COINCIDENCE!
SCIENTISTS...

So, uh, we did the green study again and got no link. It was probably a—
' 'RESEARCH CONFLICTED ON GREEN JELLY BEAN/ACNE LINK; MORE STUDY RECOMMENDED!

Multiple testing correction

Experiment

- Imagine if we perform a test on each of the 10’000 genes
- None of the genes is differentially expressed



Significance level
α= 5%

Consequences:
we expect to find around 500 p-values below 0.05!

adjust the p-values to take the number of tests into account

Mutiple testing correction

FWER

Control the probability of obtaining any false positives

k is the rank

Bonferroni

α =significance level (ex: 0.05)
Change α for each test

$\alpha' = \alpha/k$,
$p_k = 1-(1-\alpha')^k$,
$p_{bonferroni} = \min(p_k, 1)$.

The probability of getting at least one significant p-value

⇒The probability of obtaining any false positive is controled.

⇒Very stringent, we may miss many true positives

Mutiple testing correction

FWER

Control the probability of obtaining any false positives

k is the rank

Bonferroni

α =significance level (ex: 0.05)
Change α for each test

$\alpha' = \alpha/k$,
$p_k = 1-(1-\alpha')^k$,
$p_{bonferroni} = \min(p_k,1)$.

The probability of getting at least one significant p-value

k	Probability (p_k)
1	0.05
5	0.23
10	0.4
20	0.64
50	0.92
100	0.99
500	1

⇒The probability of obtaining any false positive is controled.

⇒Very stringent, we may miss many true positives

Mutiple testing correction

FWER

Control the probability of obtaining any false positives

Bonferroni

α =significance level (ex: 0.05)
Change α for each test

$\alpha' = \alpha/k$,

$p_k = 1-(1-\alpha')^k$,

$p_{bonferroni} = \min(p_k,1)$.

⇒The probability of obtaining any false positive is controled.

⇒Very stringent, we may miss many true positives

k is the rank

FDR

Controls the expected number of false discoveries

Benjamini-Hochberg

Order the p-values from the smallest to the largest

$\text{q-value}_{(1)} = \text{p-value}_{(1)} \,.\, n/(n-1)$

$\text{q-value}_{(2)} = \text{p-value}_{(2)} \,.\, n/(n-2)$

$\text{q-value}_{(k)} = \text{p-value}_{(k)} \,.\, n/(n-k)$

Where
n is number of genes

Correct less and less as the p-values get larger

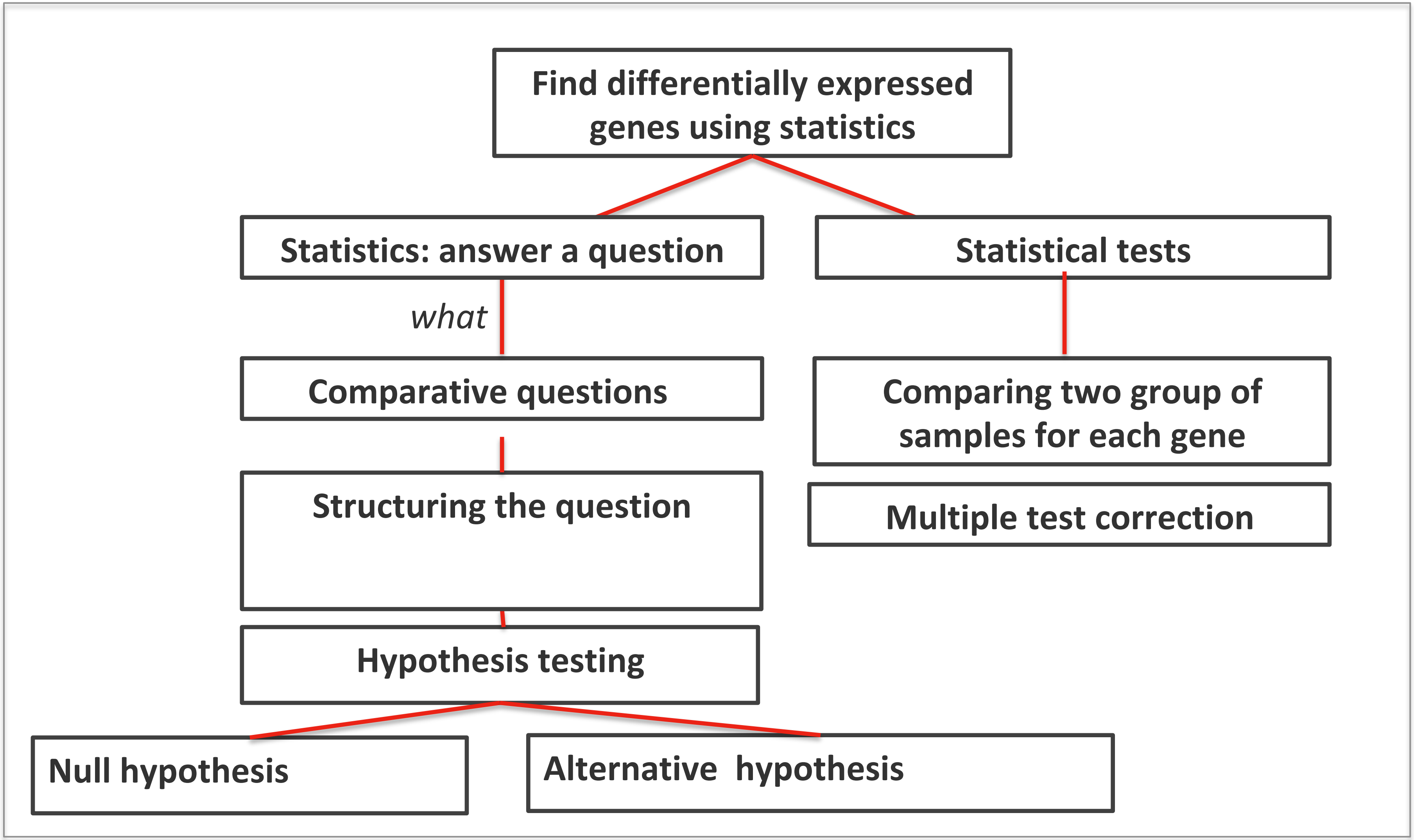
⇒Less stringent than Bonferroni.

In R:

```
>?p.adjust
>p.adjust.methods

Example
>p_bonf <- p.adjust(sort(rawp),method="bonf")
>p_bh   <- p.adjust(sort(rawp),method="BH")
>p_holm <- p.adjust(sort(rawp),method="holm")
>p_holm <- p.adjust(sort(rawp),method=p.adjust.methods)
```

Wrap up



Find differentially expressed genes using statistics

Statistics: answer a question

what

Comparative questions

Structuring the question

Hypothesis testing

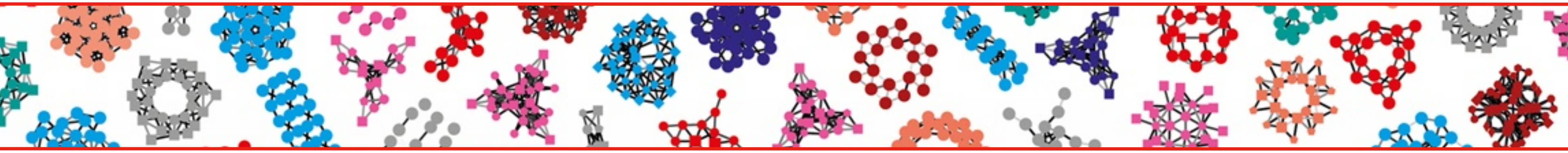
Null hypothesis

Alternative hypothesis

Statistical tests

Comparing two group of samples for each gene

Multiple test correction



EXERCICE 1: Differential expression

Analysing microarray expression of rat Affymetrix probes

Download *rat_KD.txt* from course web-page.

1. Is probe *1398751_at* differentially expressed considering a significance value of 0.01?

2. How many probes are differentially expressed considering a significance value of 0.01?

In R: solution

Get data

```
>rat <- read.table("rat_KD.txt", sep = "\t", header = T,stringsAsFactors=FALSE)
>dimnames(rat)[[1]] <- rat[,1]
```

Question1

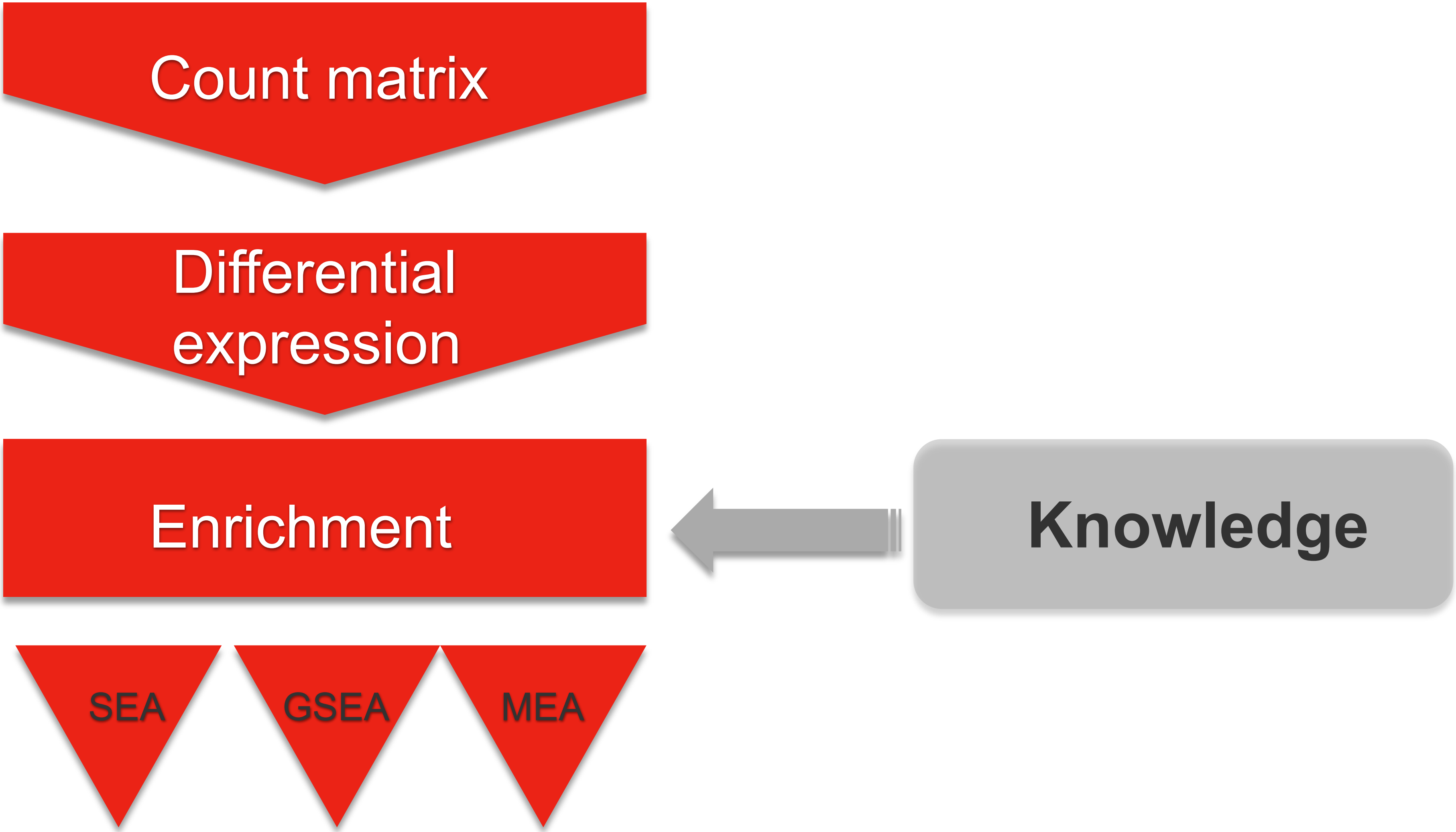
```
>rowNb<-which(rat[,1] == "1398751_at")
>v1<- t.test(rat[rowNb,2:7], rat[rowNb,8:12])
```

Question2

```
>ttestRat <- function(df, grp1, grp2) {
x = df[grp1]
y = df[grp2]
x = as.numeric(x)
y = as.numeric(y)
results = t.test(x, y)
results$p.value }

>rawp <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
>p_holm <- p.adjust(sort(rawp),method="BH")
>hist(p_holm)
```

Overview



Count matrix

Differential expression

Enrichment

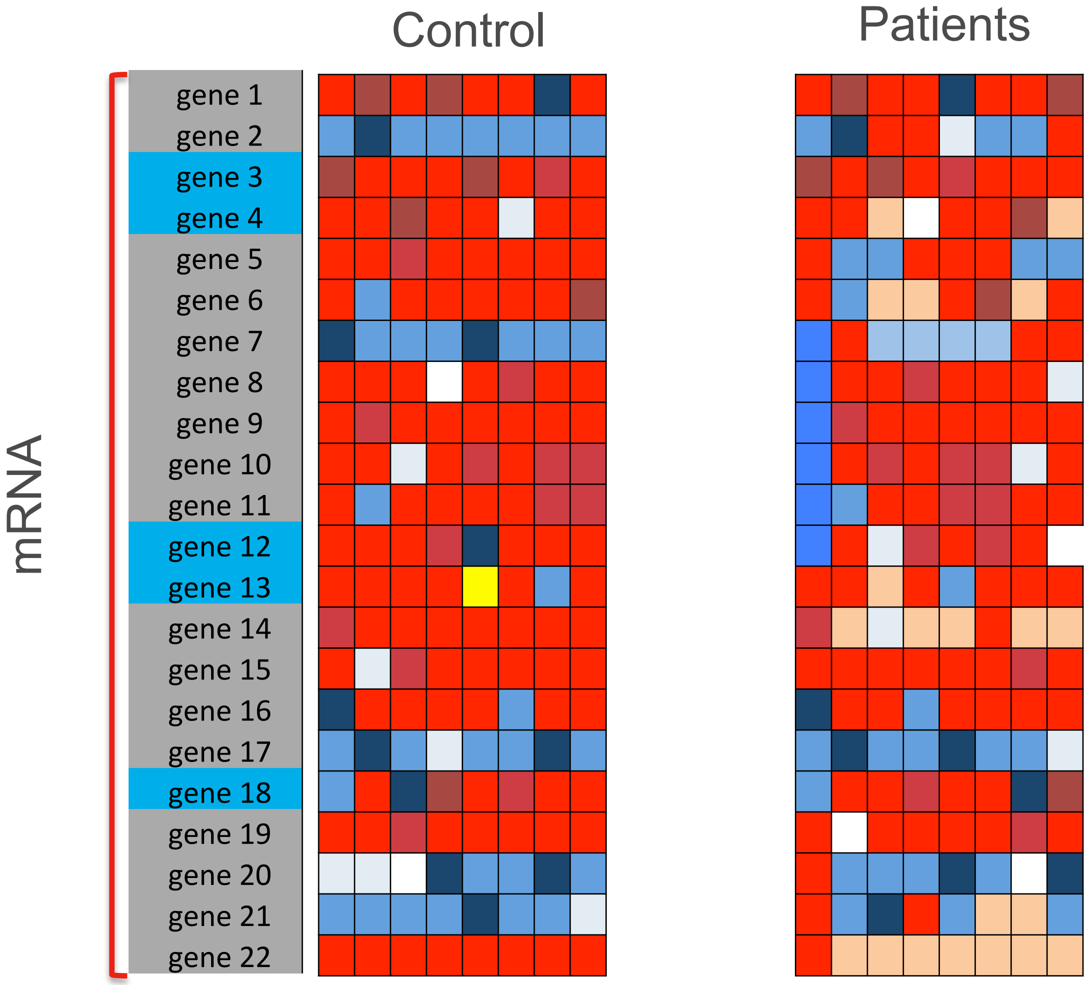
Knowledge

SEA

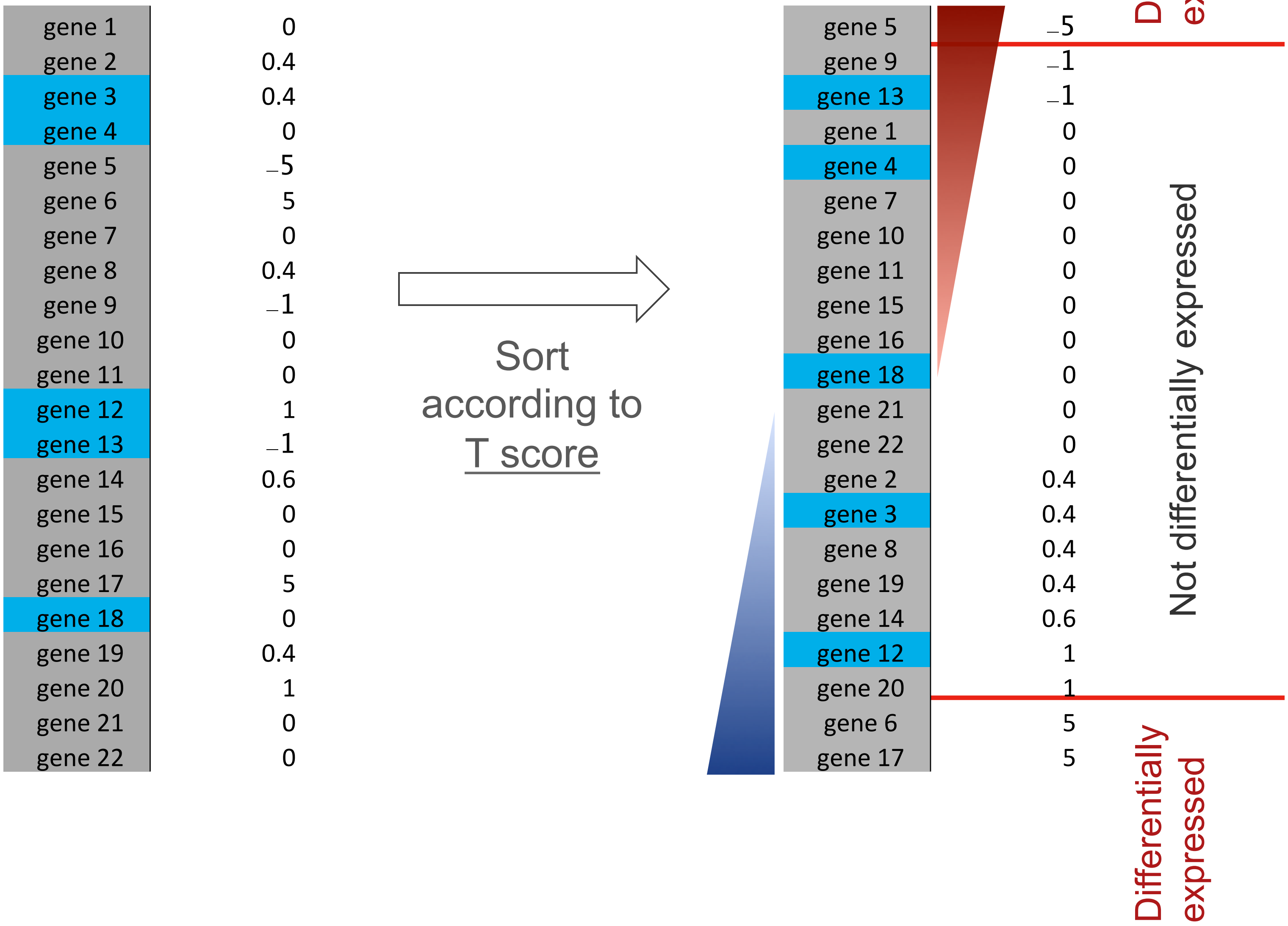
GSEA

MEA

Are genes belonging to blue set differentially expressed?



Are genes belonging to blue set differentially expressed?



gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	-5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	-1
gene 10	0
gene 11	0
gene 12	1
gene 13	-1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0

Sort according to T score

gene 5	-5
gene 9	-1
gene 13	-1
gene 1	0
gene 4	0
gene 7	0
gene 10	0
gene 11	0
gene 15	0
gene 16	0
gene 18	0
gene 21	0
gene 22	0
gene 2	0.4
gene 3	0.4
gene 8	0.4
gene 19	0.4
gene 14	0.6
gene 12	1
gene 20	1
gene 6	5
gene 17	5

Fisher exact test

2X2 count table	Differentially expressed	Not Differentially expressed	total
blue	2	3	**5**
Not blue	5	12	**17**
total	**7**	**15**	**22**

Fisher exact test

$\mathcal{H}_0$: The proportion of blue genes differentially expressed set is the same as the proportion of blue genes in non-differentially expressed

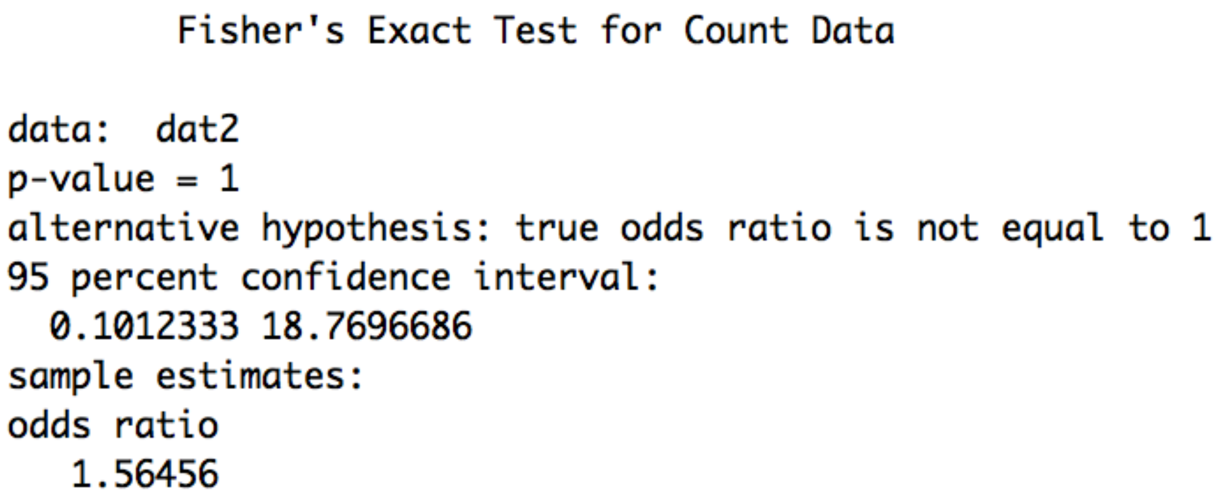
$$\mathcal{H}o: \frac{\pi_{b1}}{\pi_{D}} = \frac{\pi_{b2}}{\pi_{ND}}$$

$\mathcal{H}_1$: The proportion of blue genes differentially expressed set is not the same as the proportion of blue genes in non-differentially expressed

$$\mathcal{H}1: \frac{\pi_{b1}}{\pi_{D}} \neq \frac{\pi_{b2}}{\pi_{ND}}$$

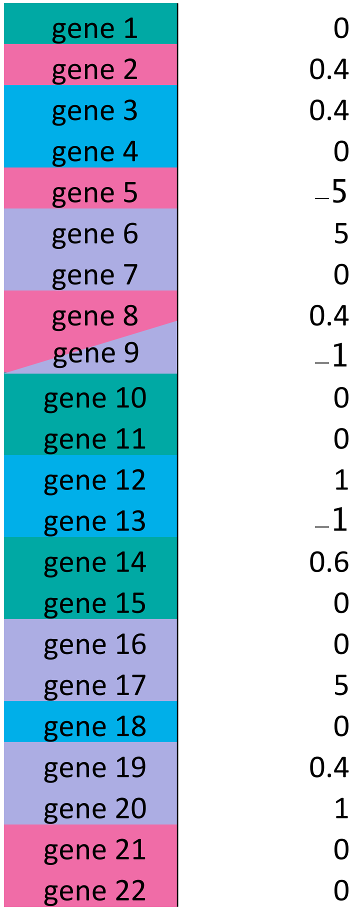
In R

```
>dat2 <- matrix(c(2,3,5,12), ncol=2)
>dat2
>fisher.test(dat2)
```



```
        Fisher's Exact Test for Count Data

data:  dat2
p-value = 1
alternative hypothesis: true odds ratio is not equal to 1
95 percent confidence interval:
  0.1012333 18.7696686
sample estimates:
odds ratio
   1.56456
```



gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	-5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	-1
gene 10	0
gene 11	0
gene 12	1
gene 13	-1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0

Which gene class
(blue, pink, purple, green)
is differentially expressed?

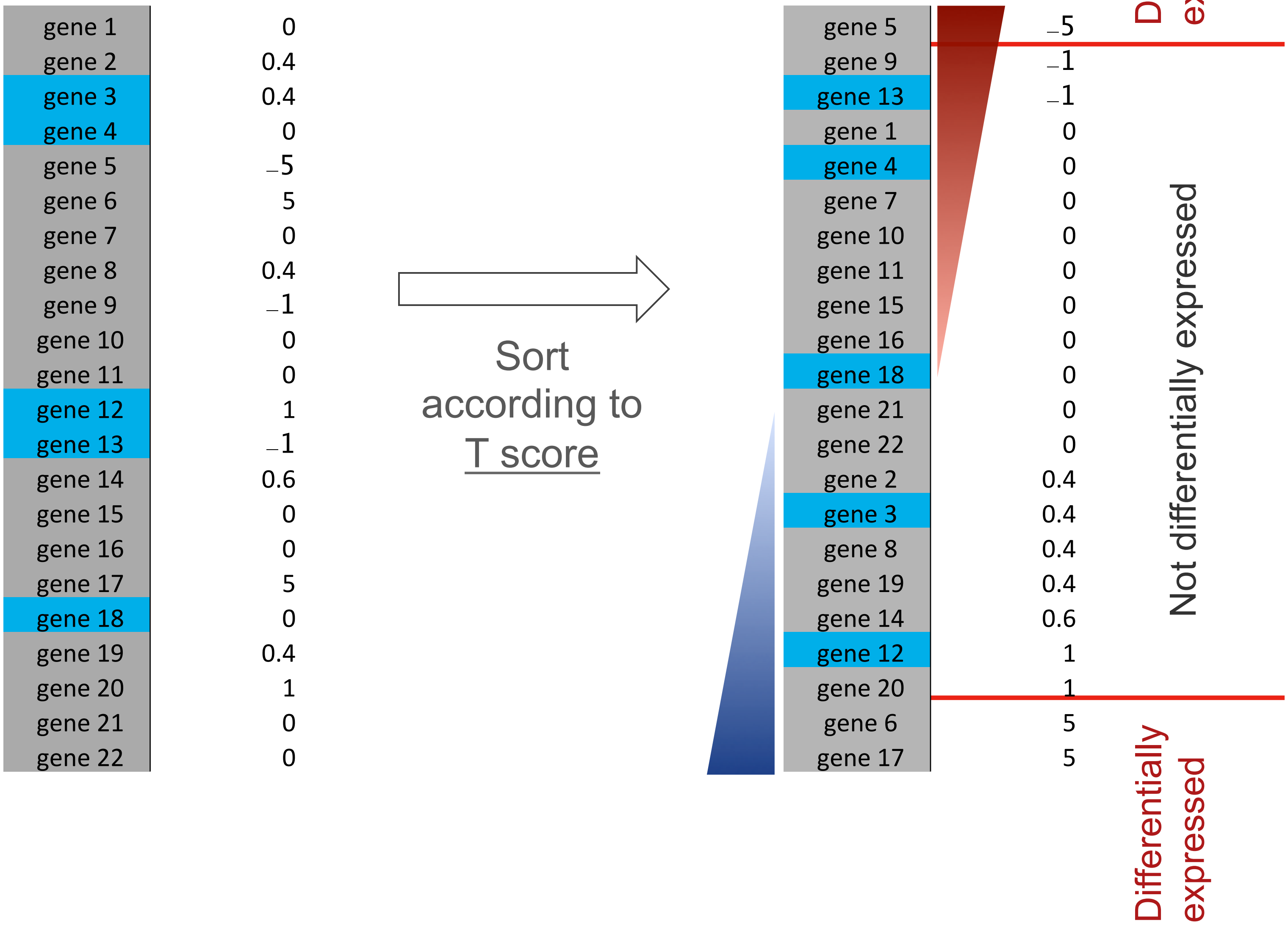
Enrichment analysis of
several phenotypes/classes:

multiple testing!

Fisher exact test

is a Threshold-based test

Are genes belonging to blue set differentially expressed?



gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	–5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	–1
gene 10	0
gene 11	0
gene 12	1
gene 13	–1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0

Sort according to T score

gene 5	–5
gene 9	–1
gene 13	–1
gene 1	0
gene 4	0
gene 7	0
gene 10	0
gene 11	0
gene 15	0
gene 16	0
gene 18	0
gene 21	0
gene 22	0
gene 2	0.4
gene 3	0.4
gene 8	0.4
gene 19	0.4
gene 14	0.6
gene 12	1
gene 20	1
gene 6	5
gene 17	5

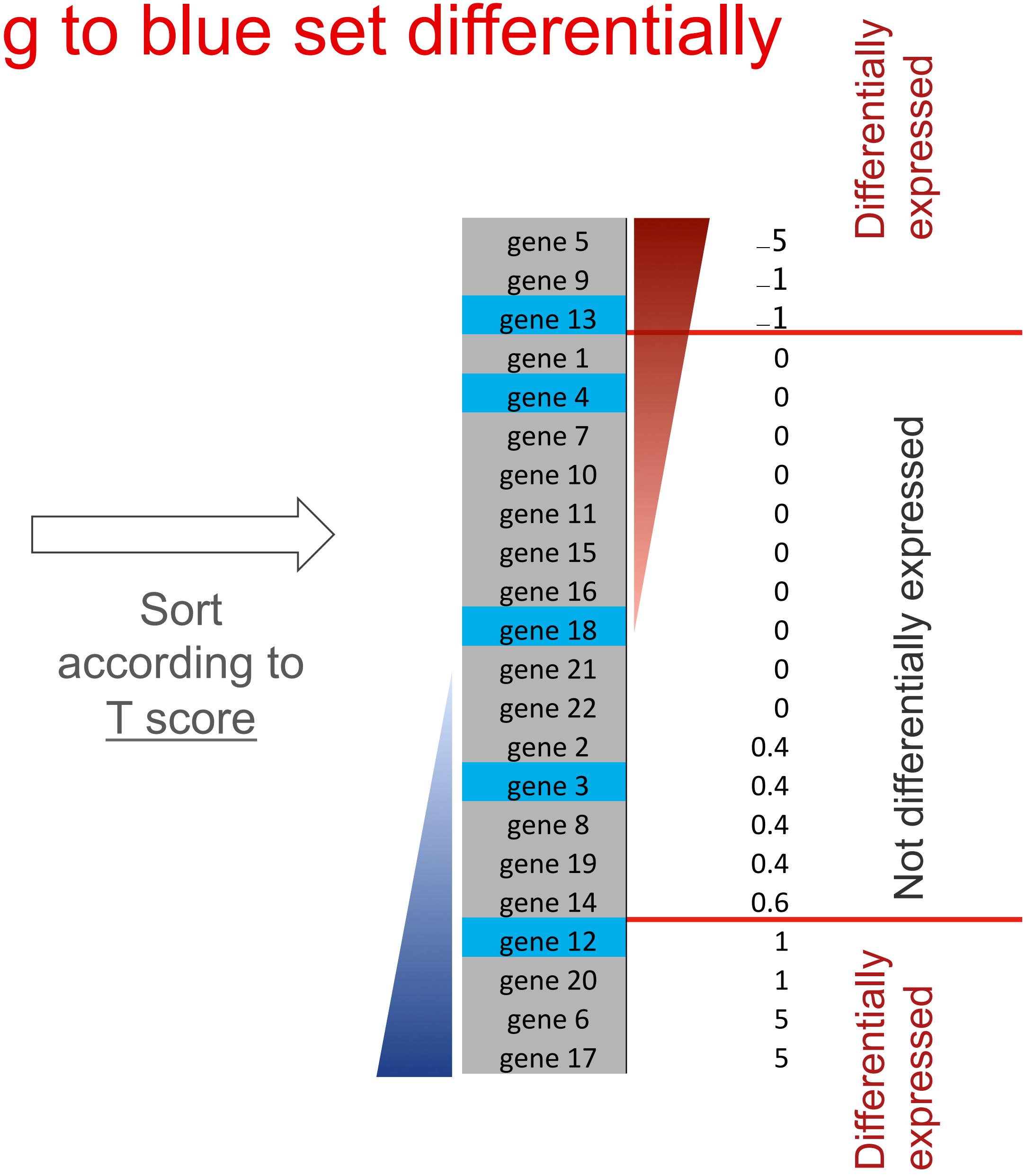
Differentially expressed

Not differentially expressed

Differentially expressed

Are genes belonging to blue set differentially expressed?

gene 1	0
gene 2	0.4
gene 3	0.4
gene 4	0
gene 5	-5
gene 6	5
gene 7	0
gene 8	0.4
gene 9	-1
gene 10	0
gene 11	0
gene 12	1
gene 13	-1
gene 14	0.6
gene 15	0
gene 16	0
gene 17	5
gene 18	0
gene 19	0.4
gene 20	1
gene 21	0
gene 22	0



Sort according to T score

gene 5	-5
gene 9	-1
gene 13	-1
gene 1	0
gene 4	0
gene 7	0
gene 10	0
gene 11	0
gene 15	0
gene 16	0
gene 18	0
gene 21	0
gene 22	0
gene 2	0.4
gene 3	0.4
gene 8	0.4
gene 19	0.4
gene 14	0.6
gene 12	1
gene 20	1
gene 6	5
gene 17	5

Threshold-free?

Kolmogorov-Smirnov-like

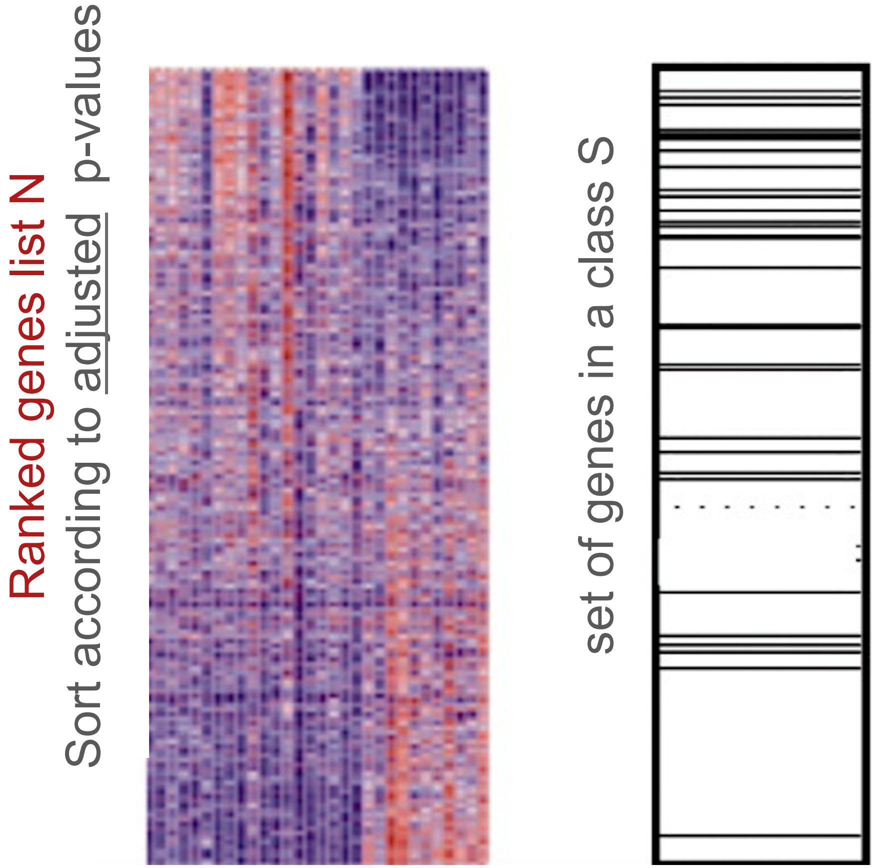
Permutation

Z-score

…

Gene Set Enrichment Analysis

Given



Ranked genes list N

Sort according to adjusted p-values

set of genes in a class S

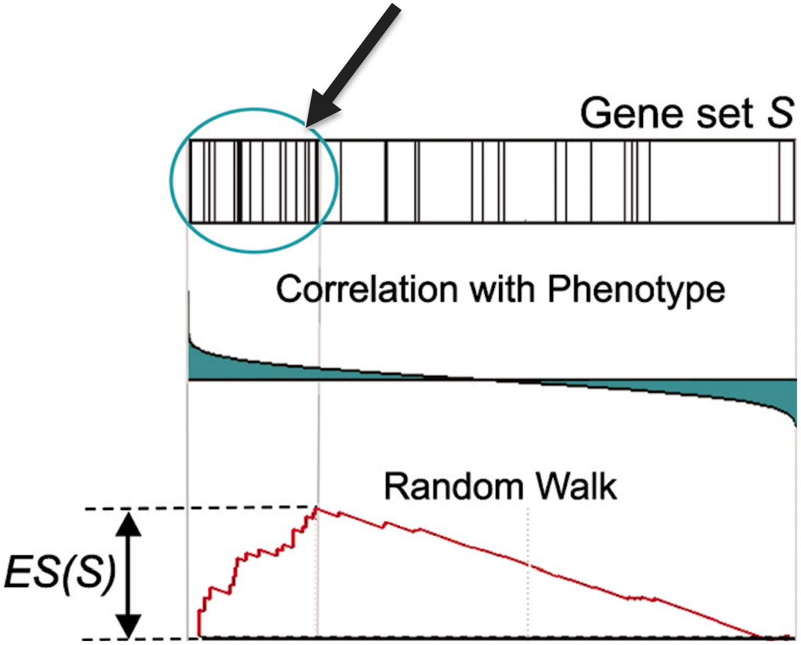
Subramanian et al. - 2005

Three steps:

Evaluate, Estimate, Adjust

Evaluate

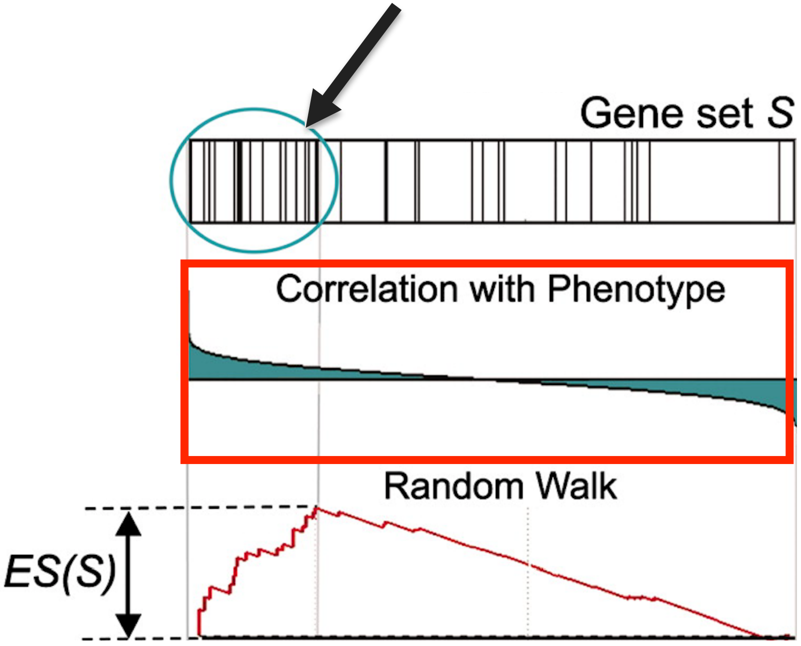
the enrichment score using a Kolmogorov-Smirnov-like score



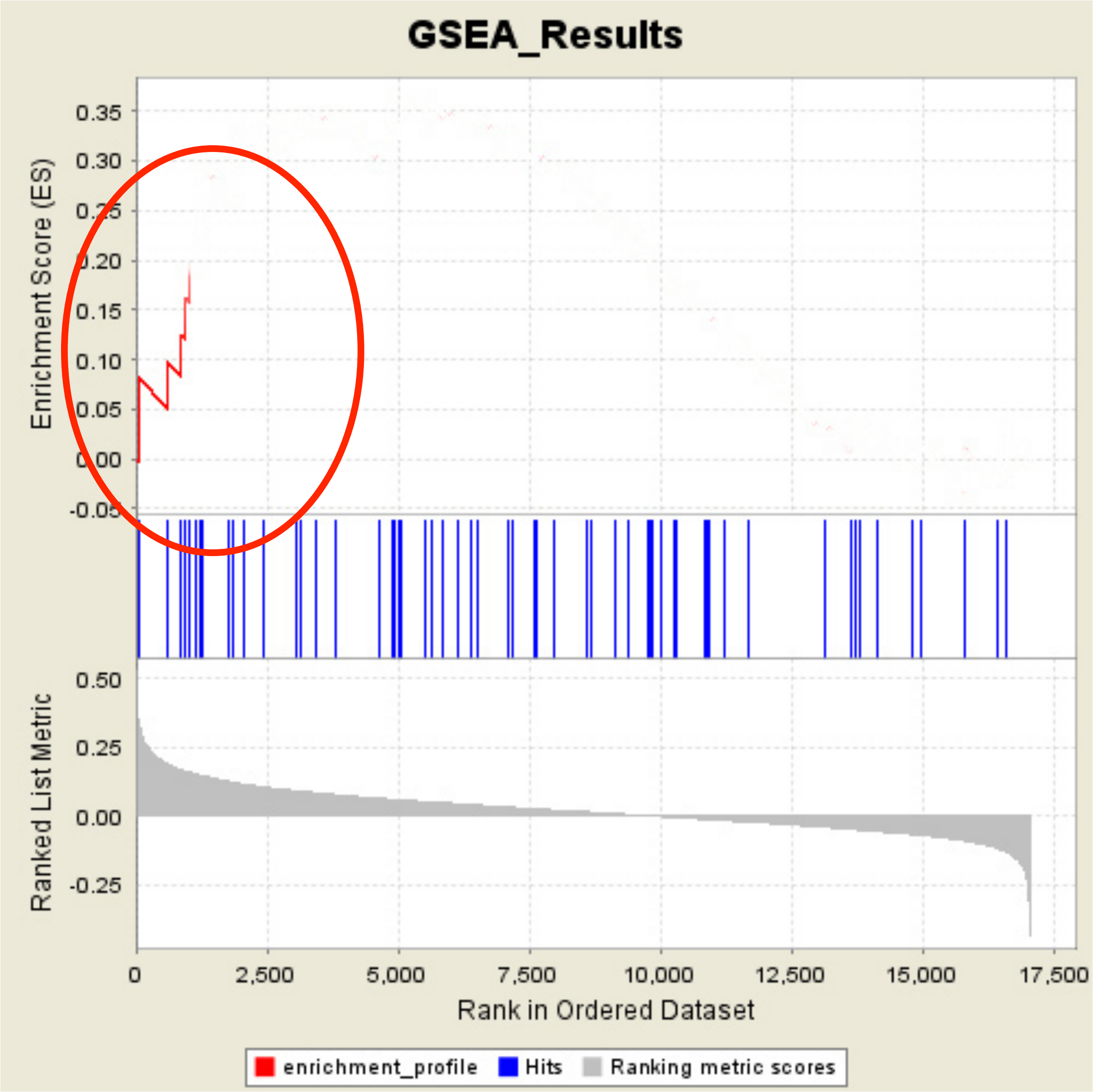
ES reflects the degree to which a set S is overrepresented at the extremes (top or bottom) of the entire ranked list L

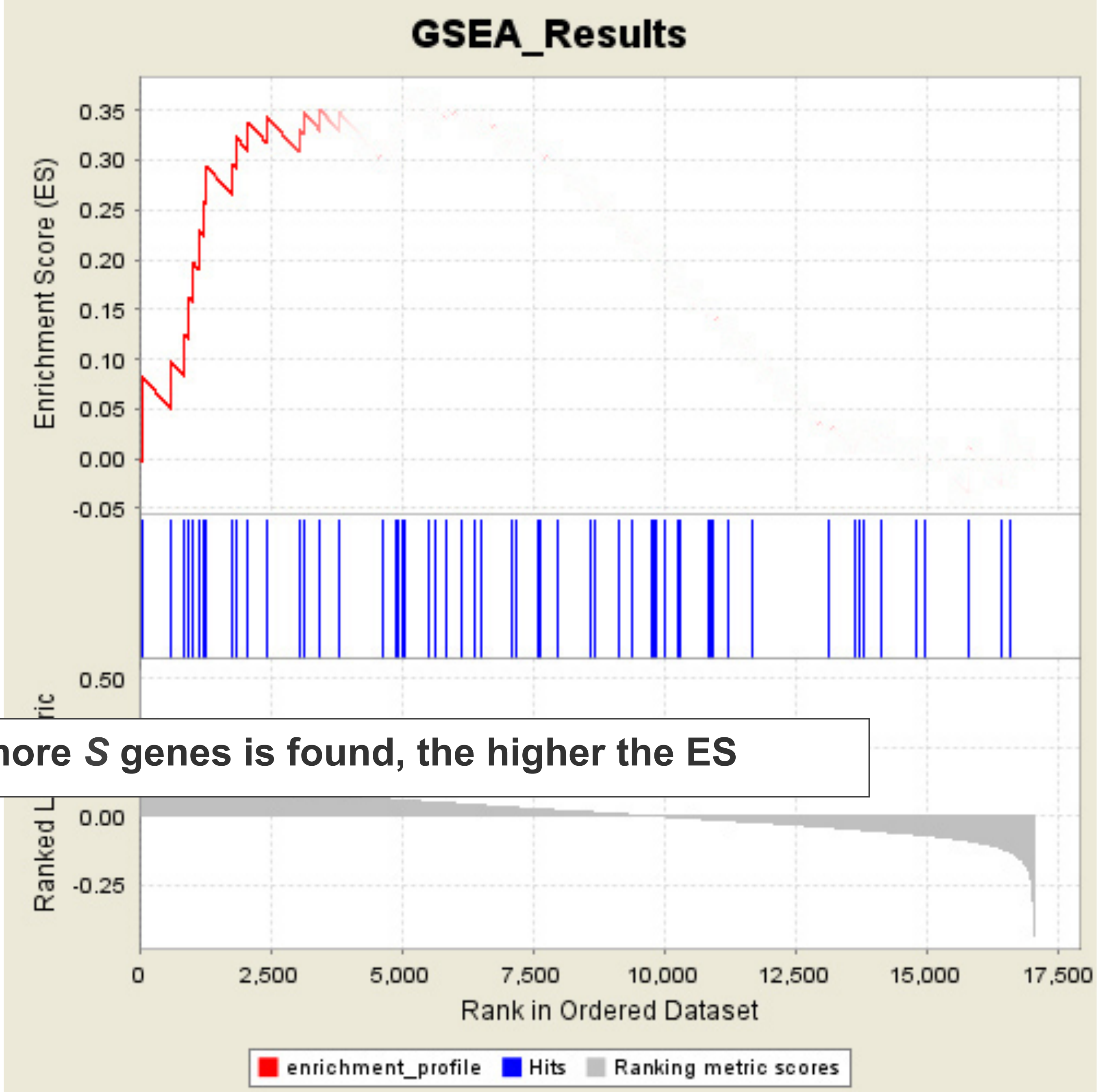
Evaluate

the enrichment score using a Kolmogorov-Smirnov-like score

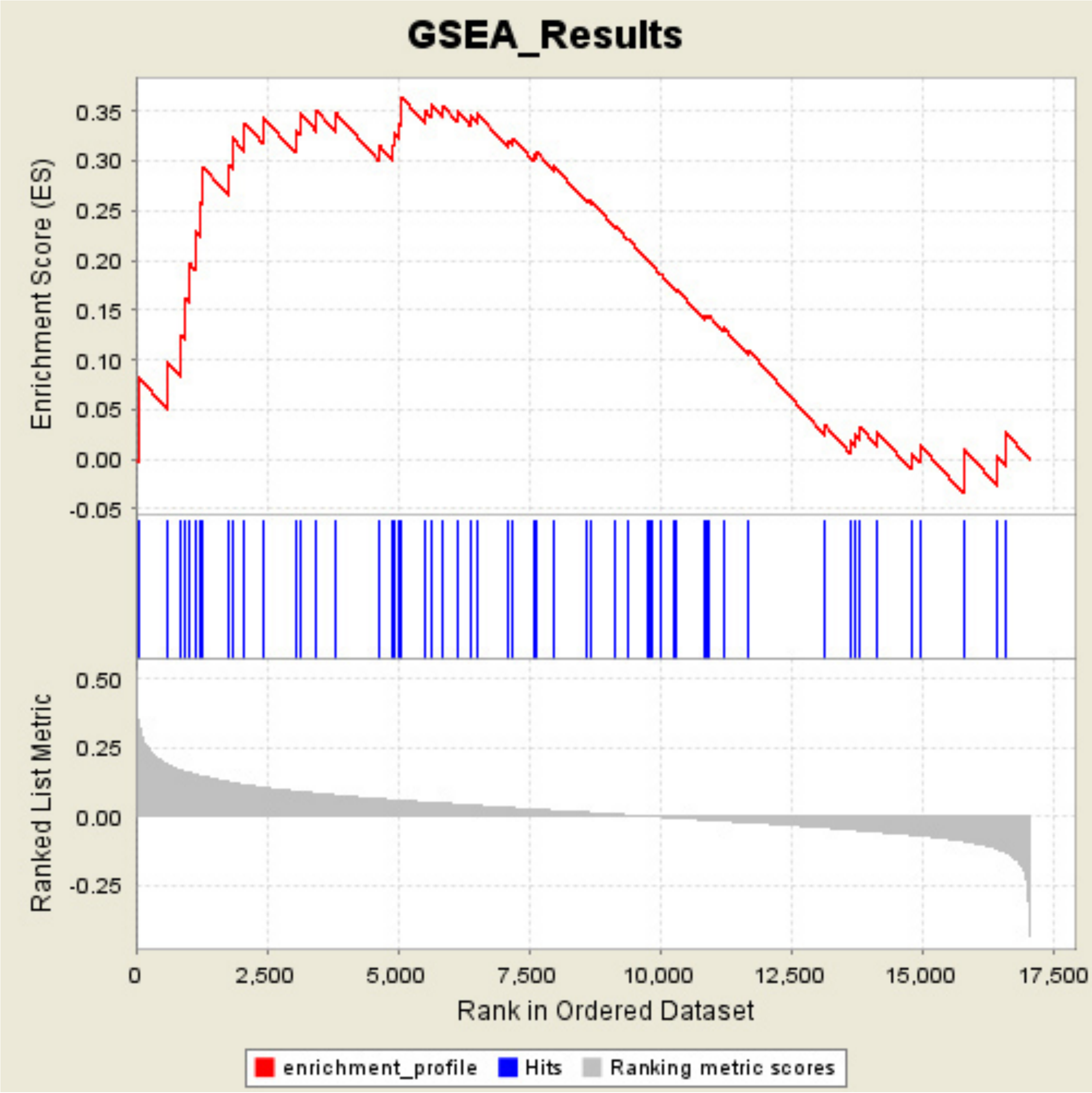


ES reflects the degree to which a set S is overrepresented at the extremes (top or bottom) of the entire ranked list L

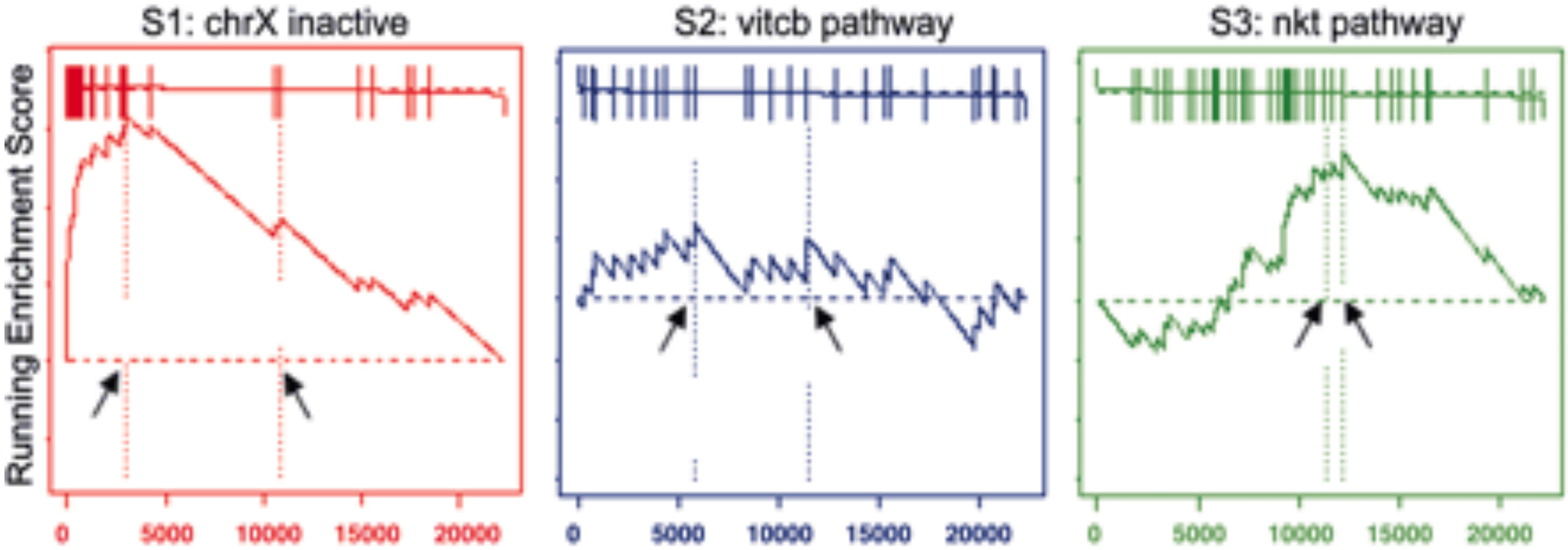

GSEA_Results
Enrichment Score (ES)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Ranked List Metric
0.50
0.25
0.00
-0.25
0
2,500
5,000
7,500
10,000
12,500
15,000
17,500
Rank in Ordered Dataset
enrichment_profile
Hits
Ranking metric scores



The more *S* genes is found, the higher the ES


GSEA_Results
Enrichment Score (ES)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
Ranked List Metric
0.50
0.25
0.00
-0.25
0
2,500
5,000
7,500
10,000
12,500
15,000
17,500
Rank in Ordered Dataset
enrichment_profile
Hits
Ranking metric scores

Different KS outcomes

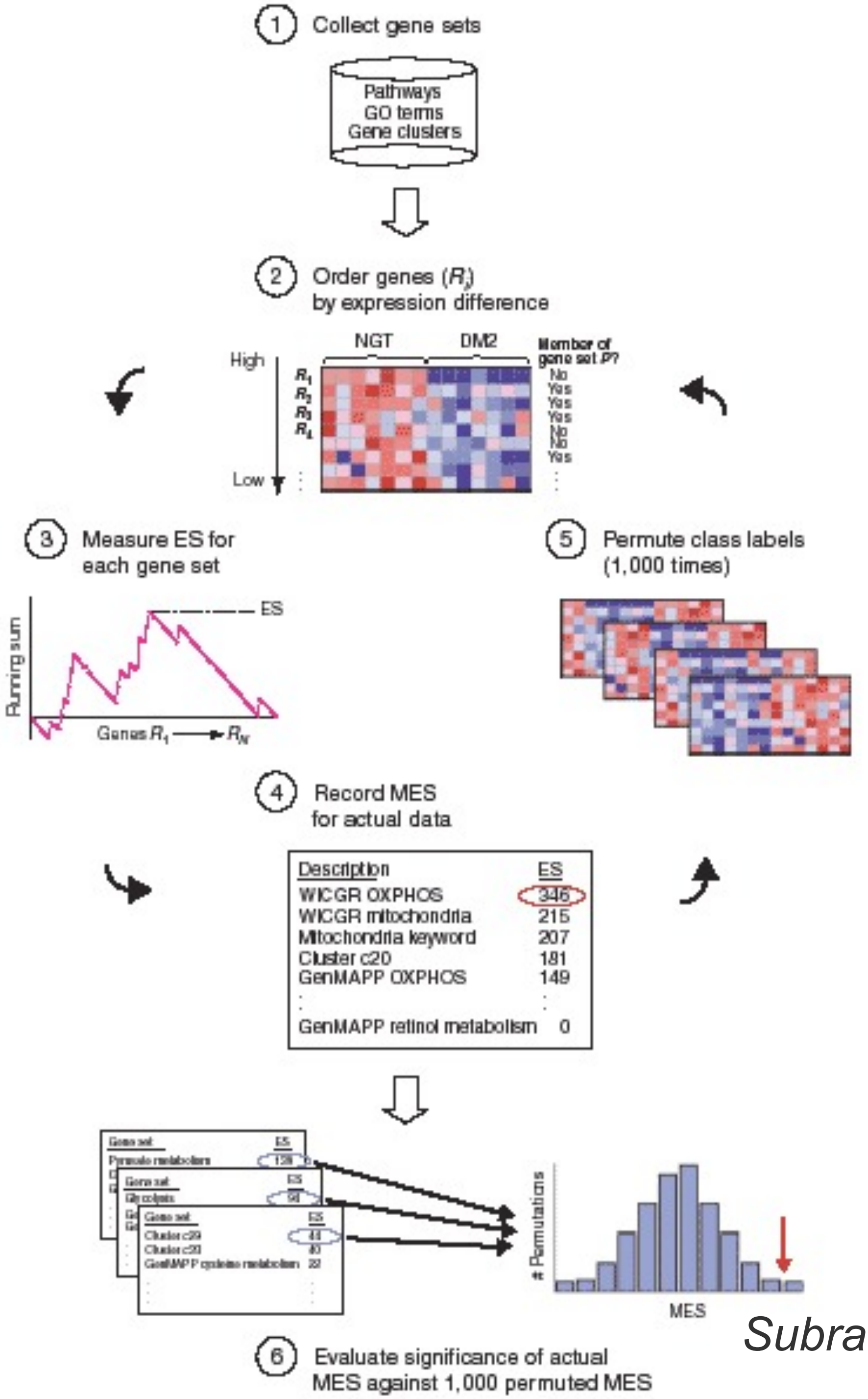


Subramanian et al. - 2005

Estimate

the statistical significance

The statistical significance (P-value) for each gene set is calculated based on permutation of genes labels



1. Collect gene sets

2. Order genes (R_i) by expression difference

3. Measure ES for each gene set

4. Record MES for actual data

Description	ES
WICGR OXPHOS	346
WICGR mitochondria	215
Mitochondria keyword	207
Cluster c20	181
GenMAPP OXPHOS	149
⋮	⋮
GenMAPP retinol metabolism	0

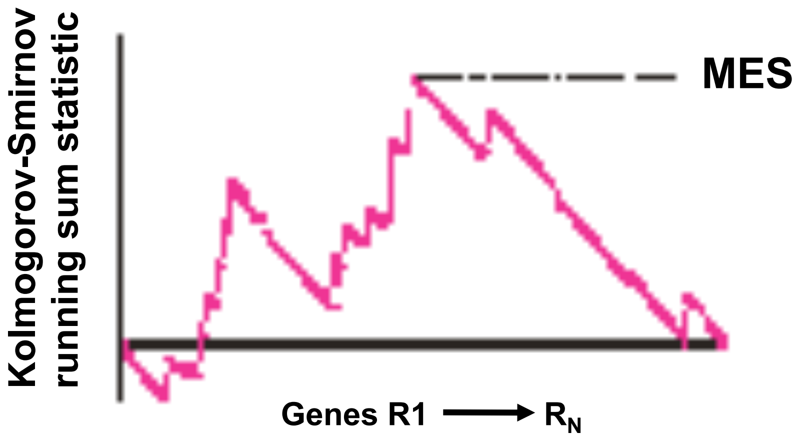
5. Permute class labels (1,000 times)

6. Evaluate significance of actual MES against 1,000 permuted MES

Subramanian et al. - 2005

Kolmogorov-Smirnoff test statistic

3 Measure ES for each gene set S



4 Record Maximum Enrichment Score (MES)

Description	ES
WICGR OXPHOS	346
WICGR mitochondria	215
Mitochondria keyword	207
Cluster c20	181
GenMAPP OXPHOS	148
-	.
-	.
GenMAPP retinol metabolism	0

if R_i is not a member of S

$$X_i = -\sqrt{\frac{G}{N-G}}$$

if R_i is a member of S

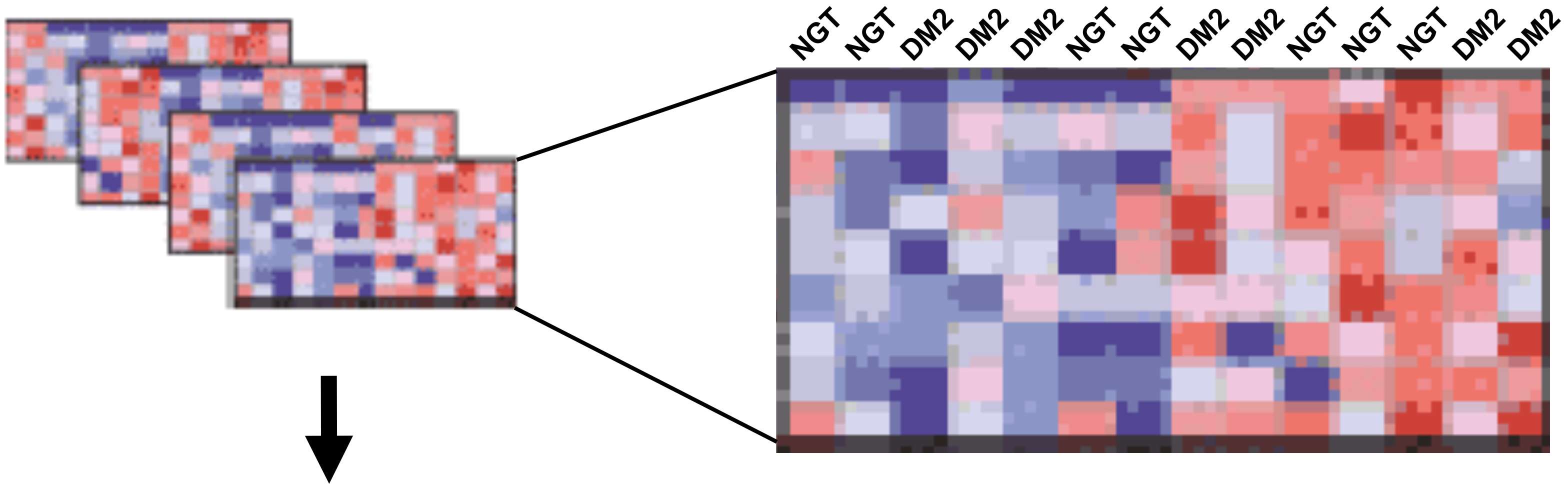
$$X_i = \sqrt{\frac{N-G}{G}}$$

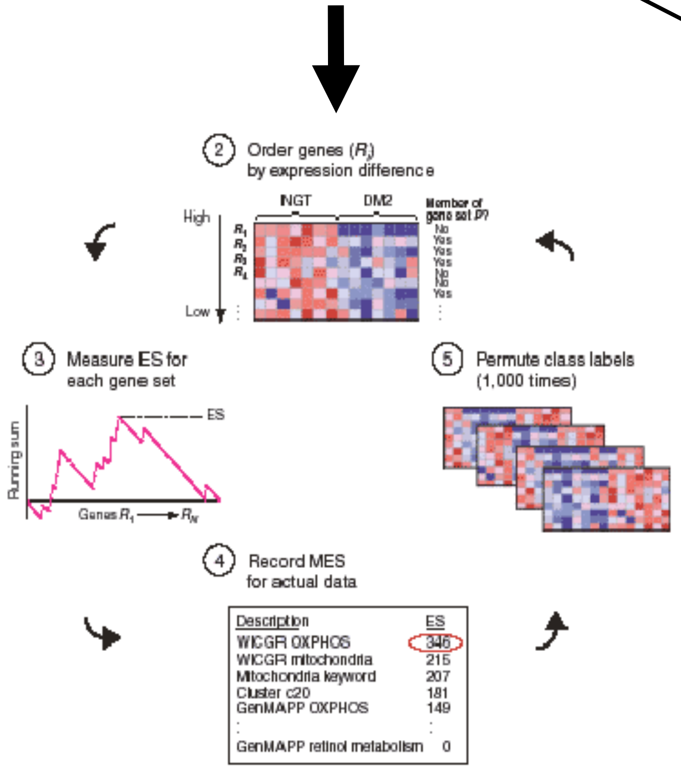
Kolmogorov-Smirnov running sum statistic : ES

$$\boxed{\text{MES}= \max_{1\le j\le N} \sum_{i=1}^{j} X_i}$$

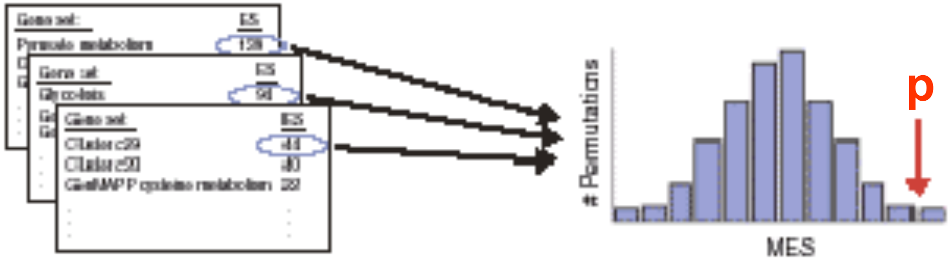
N = Number of genes
G= number of members in a gene set S
MES= Maximum Enrichment Score

⑤ Permute class labels (1000 times)





⑥ Evaluate significance of actual MES against 1000 permuted MES



$$p = \frac{\text{nb permuted MES >MES}}{\text{nb permutations}}$$

Adjust

for multiple hypothesis testing

To take into account multiple hypotheses testing of multiple gene sets

Gene Set Enrichment Analysis

Advantages

- It only requires gene set membership information to compute enrichment scores
- It considers the entire ranked list of genes
- Threshold-free model

Gene Set Enrichment Analysis

Drawbacks

Significance is measured using a permutation-based procedure: Incorporates the permutation of pathway labels

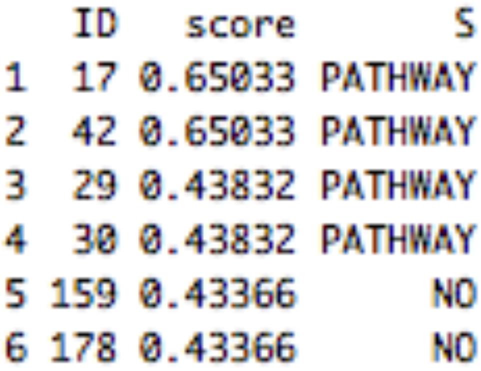
=>thereby not preserving the "biological" correlation structure of the markers

A null distribution considering samples permutation would be computationally expensive

In R

```
>source(“https://bioconductor.org/biocLite.R”)
>biocLite("clusterProfiler")
>require(clusterProfiler)

# Get data
>table<-read.csv("GSEA_data_input.csv”)
>df_case1 <- data.frame(table$gene.ID, table$scores, table$case1)
>colnames(df_case1) <- c("ID","score","S")
>head(df_case1)
```



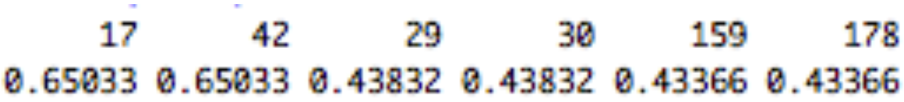
```
   ID   score       S
1  17 0.65033 PATHWAY
2  42 0.65033 PATHWAY
3  29 0.43832 PATHWAY
4  30 0.43832 PATHWAY
5 159 0.43366      NO
6 178 0.43366      NO
```

In R

```
>source(https://bioconductor.org/biocLite.R)
>biocLite("clusterProfiler")
>require(clusterProfiler)

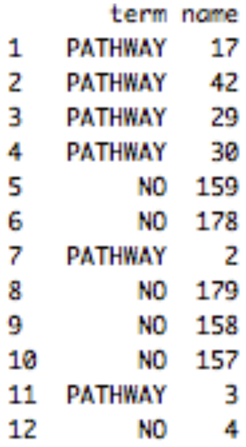
# Get data
>table<-read.csv("GSEA_data_input.csv")
>df_case1 <- data.frame(table$gene.ID, table$scores, table$case1)
>colnames(df_case1) <- c("ID","score",”PATHWAY")
>head(df_case1)

# set score (those you get from a t-test or any other statistical test)
>SCORE=df_case1$score
>names(SCORE)=df_case1$ID
>SCORE=sort(SCORE,decreasing=TRUE)
>head(SCORE)
```



```
     17      42      29      30     159     178 
0.65033 0.65033 0.43832 0.43832 0.43366 0.43366 
```

```
# get phenotype (term)
>term2gene_case1=data.frame(term=df_case1$PATHWAY,
                            name=df_case1$ID)
>head(term2gene_case1)
```



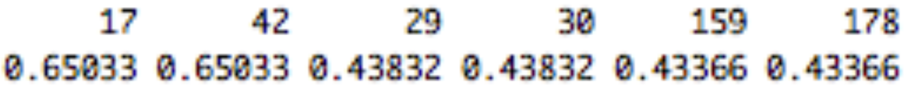
```
      term name
1  PATHWAY   17
2  PATHWAY   42
3  PATHWAY   29
4  PATHWAY   30
5       NO  159
6       NO  178
7  PATHWAY    2
8       NO  179
9       NO  158
10      NO  157
11 PATHWAY    3
12      NO    4
```

In R

```
>source(https://bioconductor.org/biocLite.R)
>biocLite("clusterProfiler")
>require(clusterProfiler)

# Get data
>table<-read.csv("GSEA_data_input.csv")
>df_case1 <- data.frame(table$gene.ID, table$scores, table$case1)
>colnames(df_case1) <- c("ID","score",”PATHWAY")
>head(df_case1)

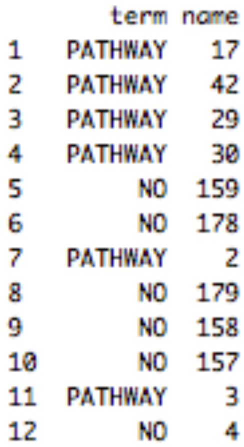
# set score (those you get from a t-test or any other statistical test)
>SCORE=df_case1$score
>names(SCORE)=df_case1$ID
>SCORE=sort(SCORE,decreasing=TRUE)
>head(SCORE)
```



```
     17      42      29      30     159     178 
0.65033 0.65033 0.43832 0.43832 0.43366 0.43366 
```

```
# get phenotype (term)
>term2gene_case1=data.frame(term=df_case1$PATHWAY,
                            name=df_case1$ID)
>head(term2gene_case1)


# run GSEA
>gsea.out_case1=GSEA(SCORE,
                     TERM2GENE=term2gene_case1,
                     nPerm=10000,
                     pvalueCutoff=1,
                     pAdjustMethod = "BH")
>gseaplot(gsea.out_case1,”PATHWAY")
```



```
      term name
1  PATHWAY   17
2  PATHWAY   42
3  PATHWAY   29
4  PATHWAY   30
5       NO  159
6       NO  178
7  PATHWAY    2
8       NO  179
9       NO  158
10      NO  157
11 PATHWAY    3
12      NO    4
```

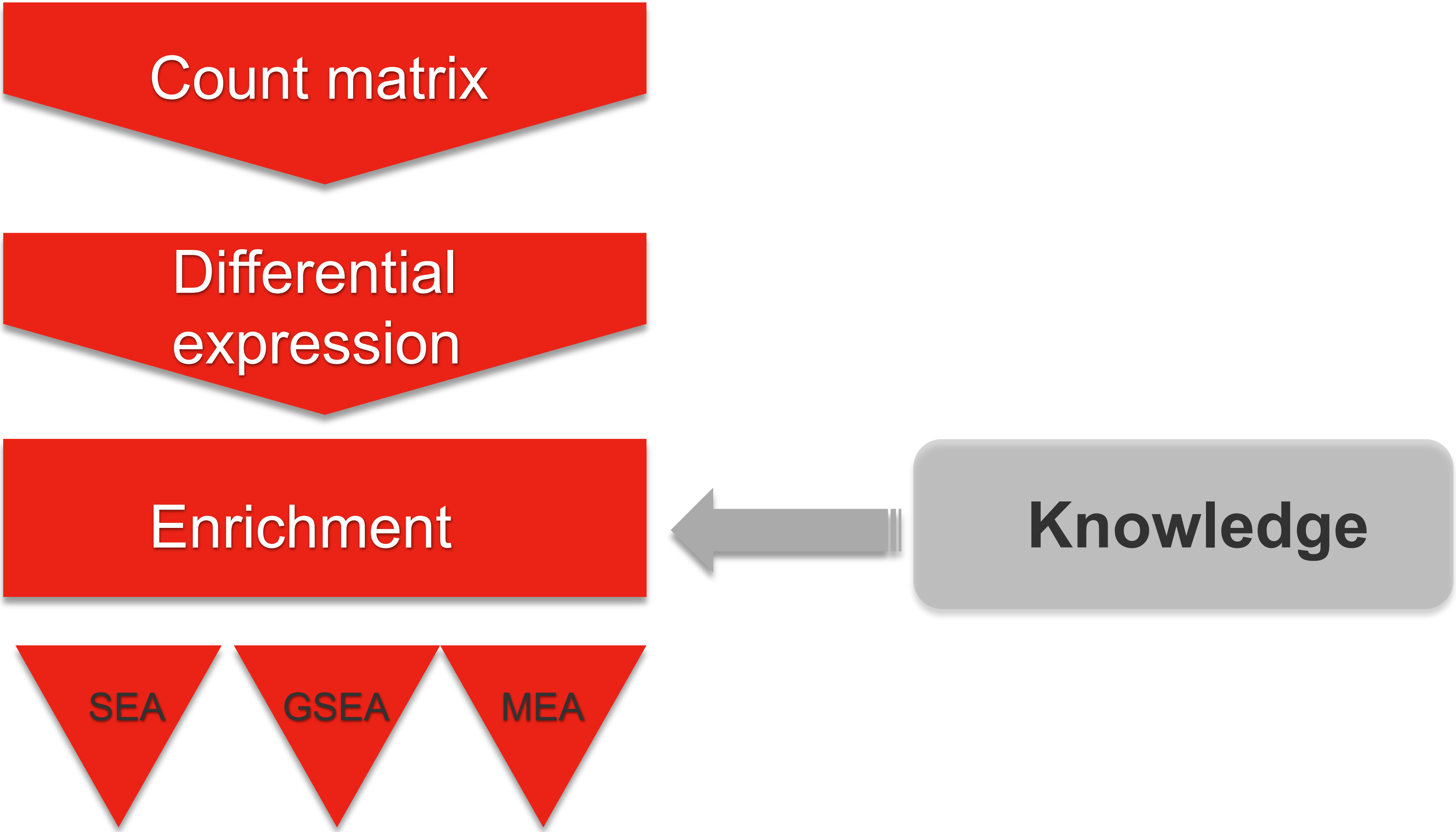
Enrichment Analysis classification

Gene Set Enrichment Analysis

Singular Enrichment Analysis

Modular Enrichment Analysis

Overview


Count matrix

Differential expression

Enrichment

Knowledge

SEA

GSEA

MEA

Gene Set Enrichment Analysis

- All genes are included in analysis
- Pairwise comparisons (e.g., disease vs. control)

⬇

No need to select list

Example

GSEA of broad institute
GSA
SAFE
GeneTrail
FatiScan

Singular Enrichment Analysis

- P-value calculated on each term from pre-selected list
- Enrichment terms are listed

Example

ClueGO
GOStat
DAVID: Provides a comprehensive set of functional annotation tools for investigators to understand biological meaning behind large list of genes
FatiGO
Marmite
Babelomics Suite: Suite of web tools for the functional profiling of genome scale experiments

Modular Enrichment Analysis

- Predetermined list of genes
- term-term or gene-gene relationships included in enrichment P-value calculation

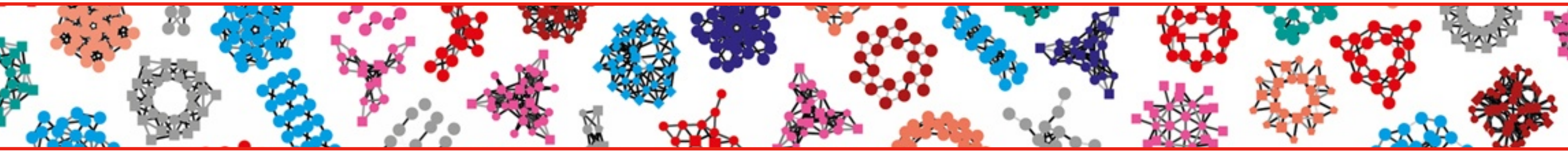
➡ Closest to nature of biological data structure

We could consider the gene-gene relationship

Example

DAVID
GOtoolBox

Suderman & Hallett 2007 Bioinformatics



EXERCICE 2

1. Using exercise 1 evaluated p-values, what is the outcome of GSEA on pathway 1 (*Pathway.csv*)?

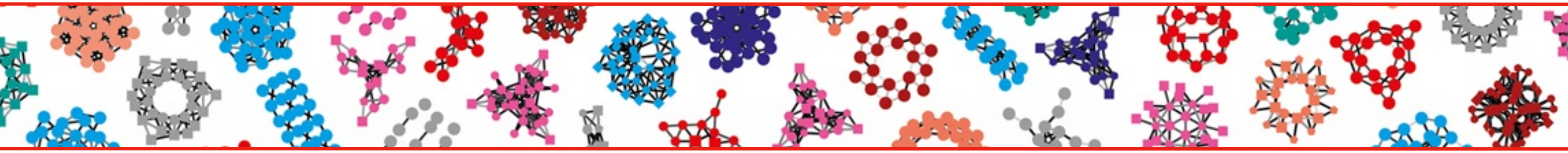
In column pathways of *Pathway.csv* designates the probes of interest (“yes” means in pathway, “no” means not in pathway)

2. Transform affymetrix probes into genes names

Given a list of Affymetrix probes, in R

```
>library("AnnotationDbi")
>library("rat2302.db")
>PROBES     <- rat$row.names
>OUT          <- select(rat2302.db,keys= PROBES, columns=c("SYMBOL", "ENTREZID", "GENENAME"))
```

3. What is the enrichment outcome on probes coding for ribosomal proteins?



Note:

If the following command line raises an error

```
>?GSEA
```

Then install

bit
AnnotationDbi
DO.db
stringi

```
biocLite("tibble”)
biocLite("clusterProfiler")
```

In R solution

```
>require(clusterProfiler)
>pathway<-read.csv(file="Pathway.csv", stringsAsFactor=FALSE, header=TRUE)

# set score (those you get from a t-test or any other statistical test)
>rawp <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
>names(rawp)        <-rat$row.names
>sortedrawp         <-sort(rawp)
>p_holm             <-p.adjust(sortedrawp,method="BH")
>names(p_holm)      <-names(sortedrawp)
>SCORE              <-p_holm
>SCORE              <-sort(SCORE,decreasing=TRUE)
>head(SCORE)

# get phenotype (term)
>term2gene          <-data.frame(term=pathway$pathways,name=pathway$row.names)
>head(term2gene)

# run GSEA
>gsea.out           <-GSEA(SCORE, TERM2GENE=term2gene, nPerm=10000, pvalueCutoff=1,
pAdjustMethod = "BH")
>gseaplot(gsea.out,”yes”)
>summary(gsea.out)
```

In R solution

```
>library("AnnotationDbi")
>library("rat2302.db")
>library(“DescTools”)

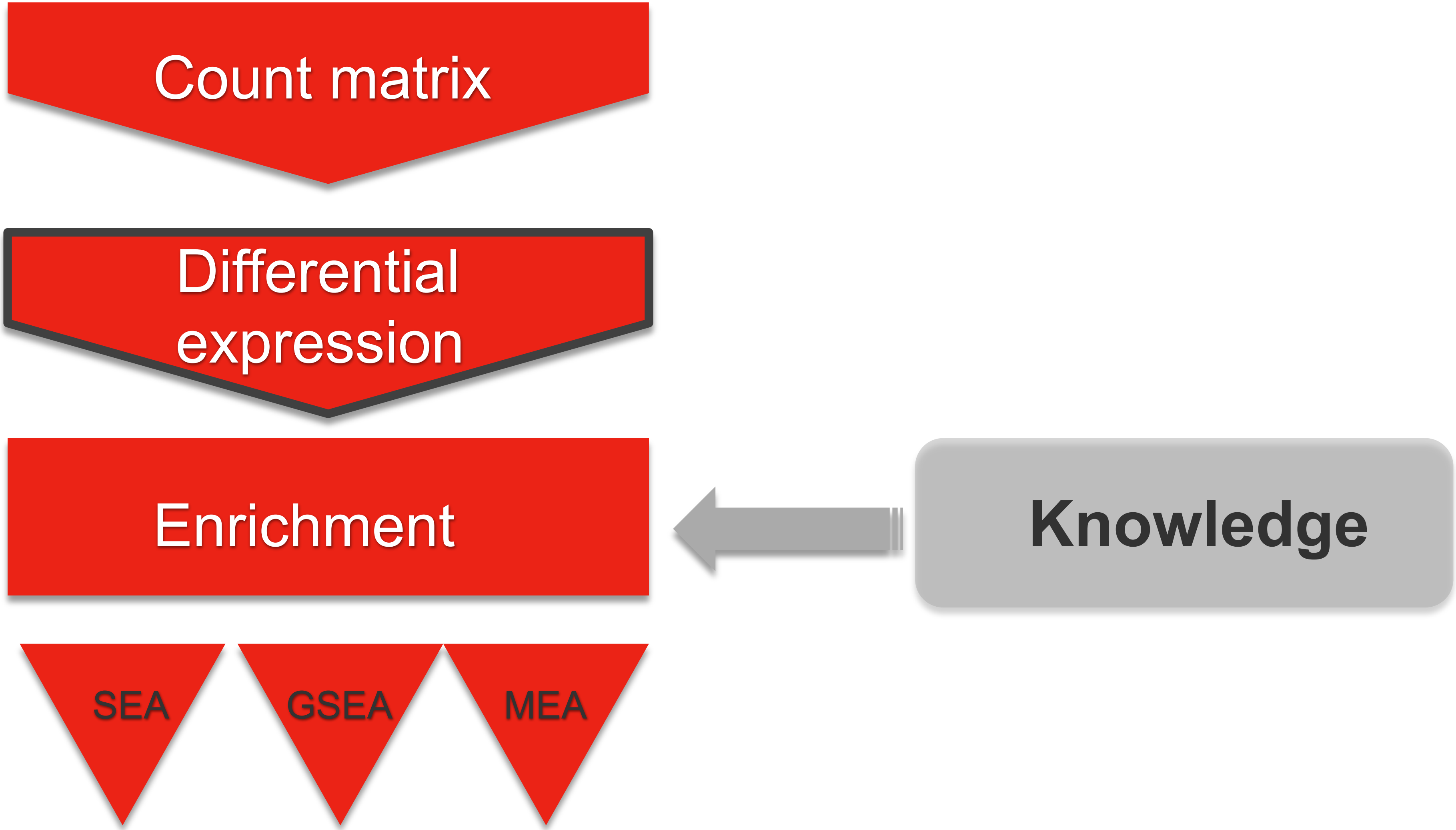
>PROBES<- rat$row.names
>OUT   <- select(rat2302.db,keys= PROBES, columns=c("SYMBOL", "ENTREZID", "GENENAME"))
>ribosomal<-OUT[ which(OUT$GENENAME %like% "ribosomal protein"),]

# set score (those you get from a t-test or any other statistical test)
>rawp <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
>names(rawp)<-rat$row.names
>sortedrawp<-sort(rawp)
>p_holm <- p.adjust(sortedrawp,method="BH")
>names(p_holm)<-names(sortedrawp)
>SCORE<-p_holm
>SCORE=sort(SCORE,decreasing=TRUE)
>head(SCORE)

# get phenotype (term)
>term2gene<-data.frame(term="no",name=rat$row.names,stringsAsFactors=FALSE)
>term2gene[which(term2gene$name %in% ribosomal$PROBEID),1]<-"yes"
>head(term2gene)

# run GSEA
>gsea.out<-GSEA(SCORE, TERM2GENE=term2gene, nPerm=10000, pvalueCutoff=1, pAdjustMethod
= "BH")
>gseaplot(gsea.out, "yes")
>summary(gsea.out)
```

Overview



Count matrix

Differential expression

Enrichment

Knowledge

SEA

GSEA

MEA

Application to any type of data!

	Adj. p-value
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1

Differentially expressed

+ Enrichment

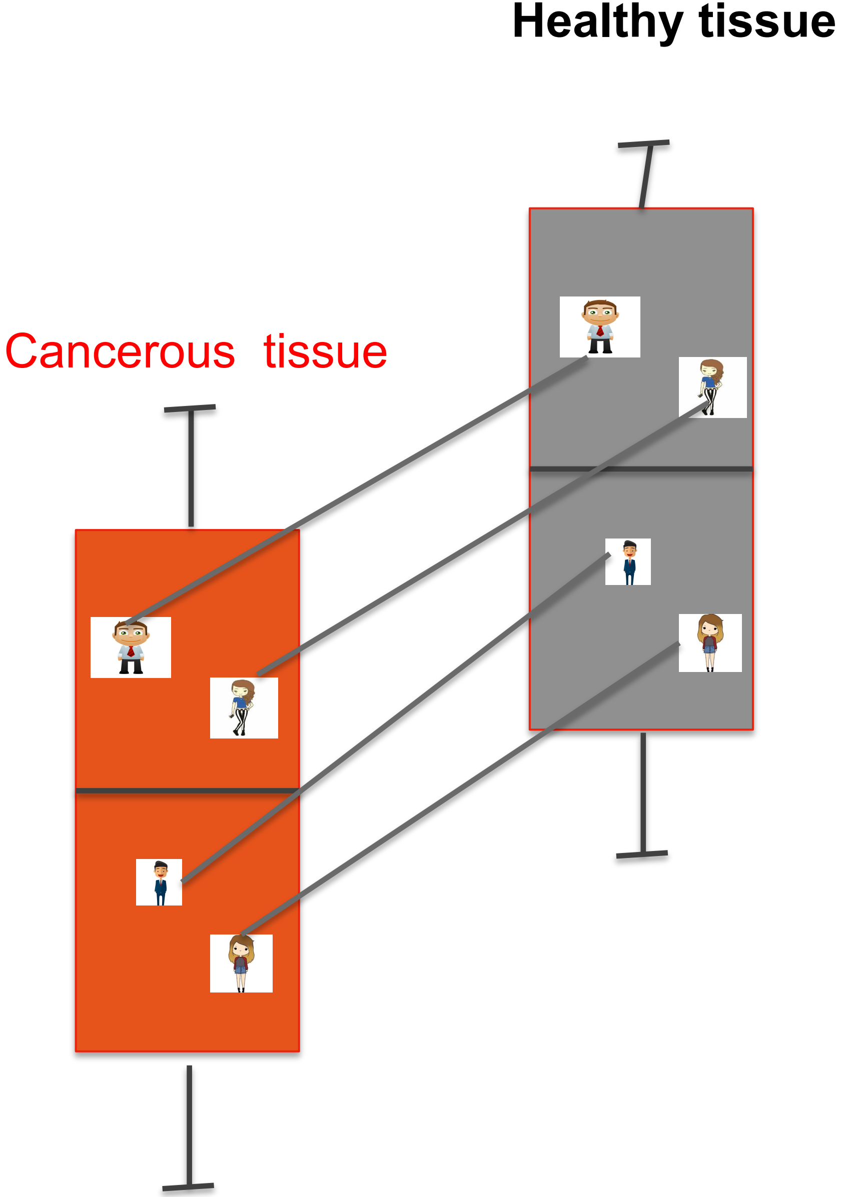
Paired data

	Paired t-test adj. p-value
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1

Differentially expressed

+

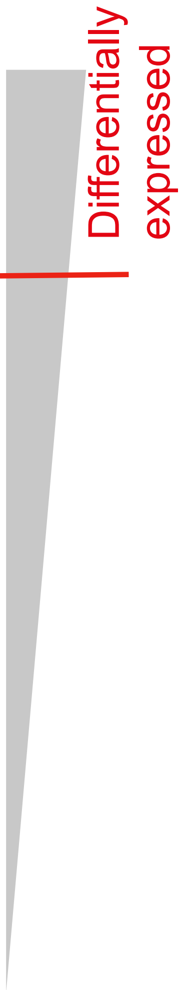
Enrichment



Paired T-test: Equivalent to testing whether the difference between the pairs is different from zero

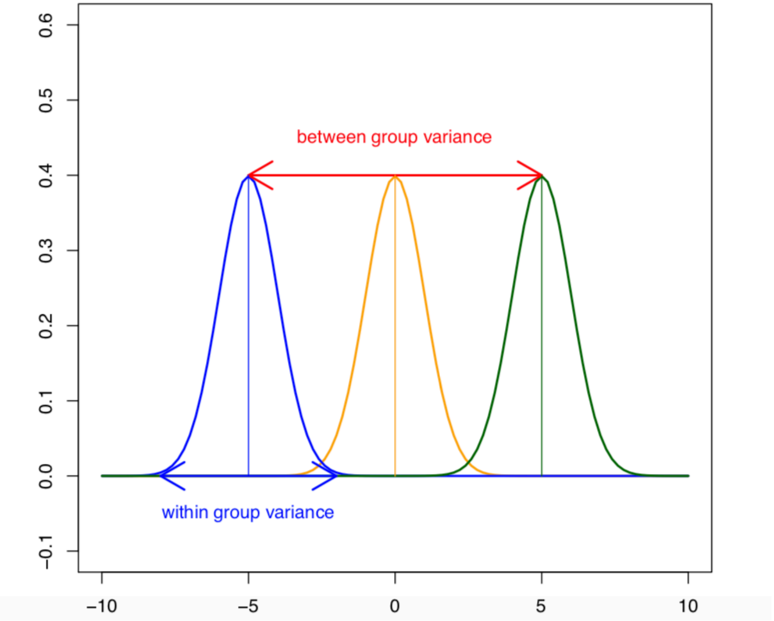
ANOVA one factor

	F score or p-value of ANOVA
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1



+ Enrichment

ANOVA
=
analysis of ~~variance~~ mean



ANOVA determines whether there are any statistically significant differences between the means of three or more independent (unrelated) groups

aov(expression~ patient type)

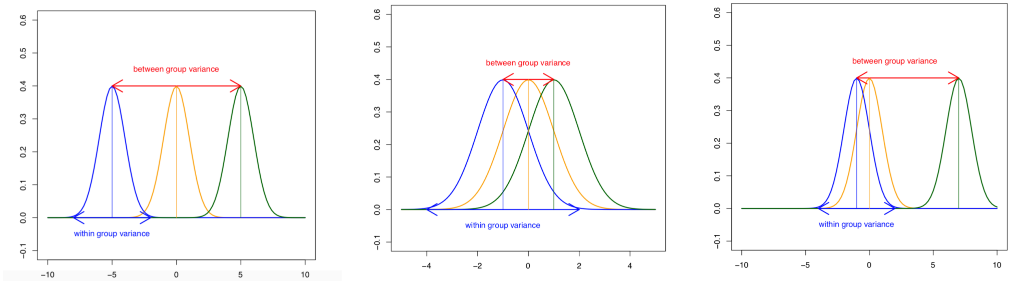
where patient type either healthy, sick without nodules, sick with nodules

ANOVA one factor

	F score or p-value of ANOVA
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1

Differentially expressed

+ Enrichment



within group variance $= SS_{error}$

between group variance $= SS_{group}$

if $SS_{group} > SS_{error}$

⇒ at least two means are different!

ANOVA two factors

	F score or p-value of ANOVA
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1

Differentially expressed

+ Enrichment

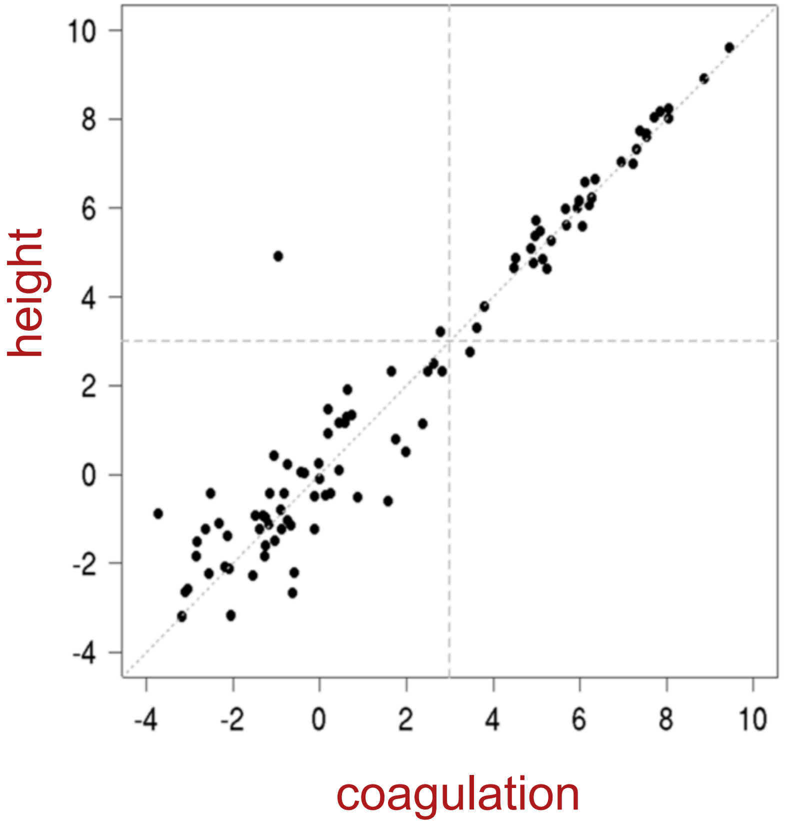
		Gender	
		Male	Female
Patient type	Healthy		
	Patient without nodular aspect		
	Patient with nodular aspect		

aov(expression ~ patient type * gender)

Linear model

	Adj. p-value of LM
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1

Differentially expressed



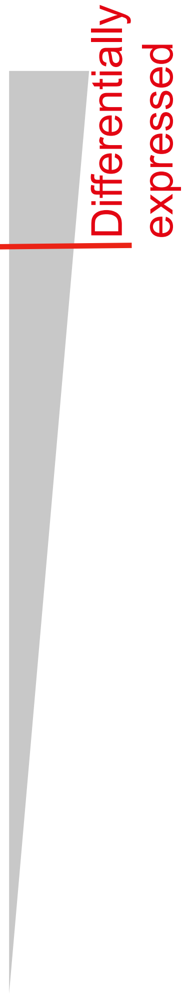
lm(expression ~ height + coagulation)

lm(expression ~ height * coagulation)

+ Enrichment

Linear model

	Adj. p-value of LM
gene 4	0.0022
gene 13	0.0022
gene 14	0.0022
gene 2	0.19
gene 7	0.19
gene 17	0.19
gene 20	0.19
gene 21	1
gene 6	1
gene 10	1
gene 11	1
gene 16	1
gene 1	1
gene 3	1
gene 5	1
gene 8	1
gene 9	1
gene 12	1
gene 15	1
gene 18	1
gene 19	1
gene 22	1



+

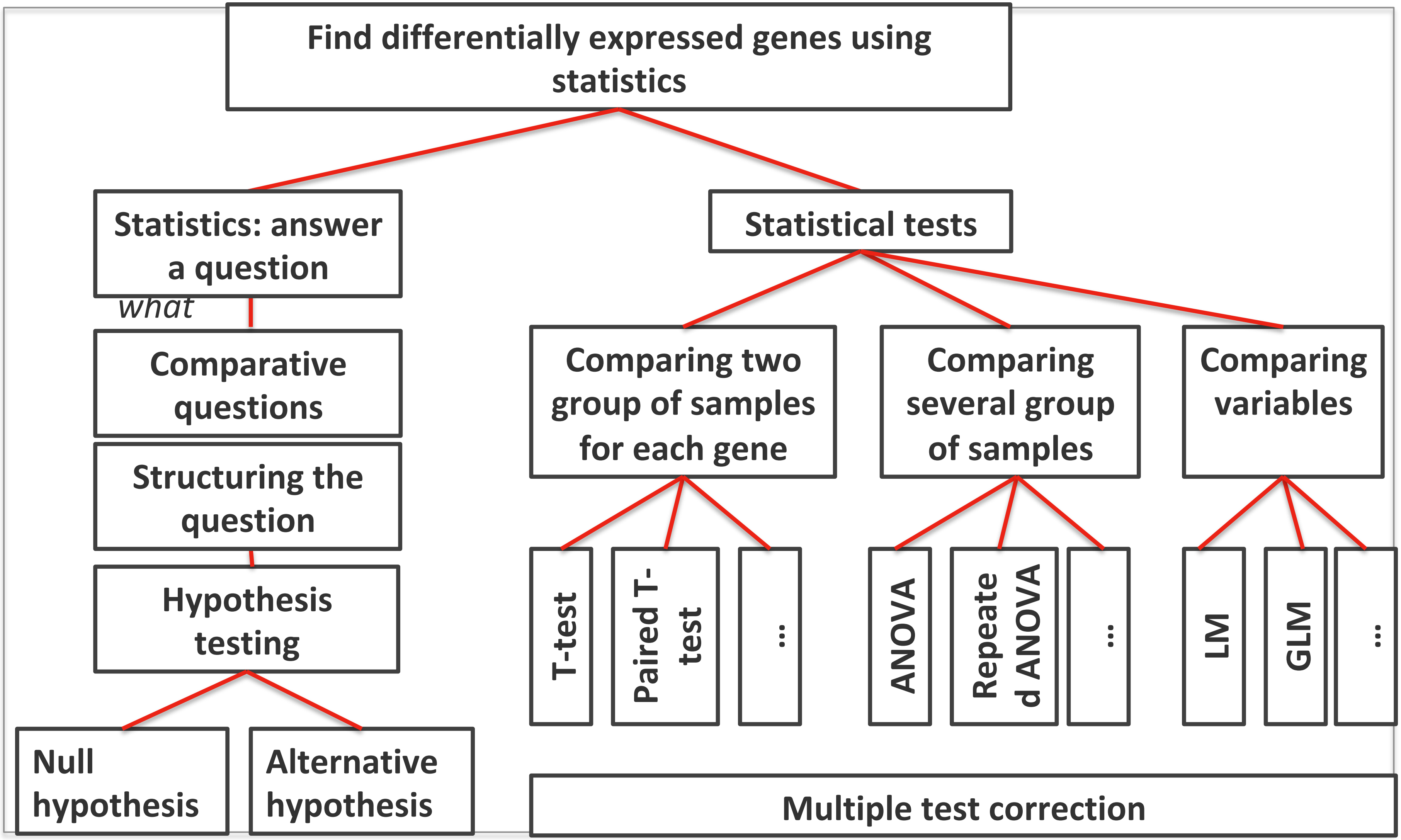
Enrichment

Rotation-based GSEA

Implemented in the “ROMER” functionality within the limma package from Bioconductor

[Langsrud, 2005; Wu et al, 2010]

Wrap up



Find differentially expressed genes using statistics

Statistics: answer a question

what

Comparative questions

Structuring the question

Hypothesis testing

Null hypothesis

Alternative hypothesis

Statistical tests

Comparing two group of samples for each gene

T-test

Paired T-test

…

Comparing several group of samples

ANOVA

Repeated ANOVA

…

Comparing variables

LM

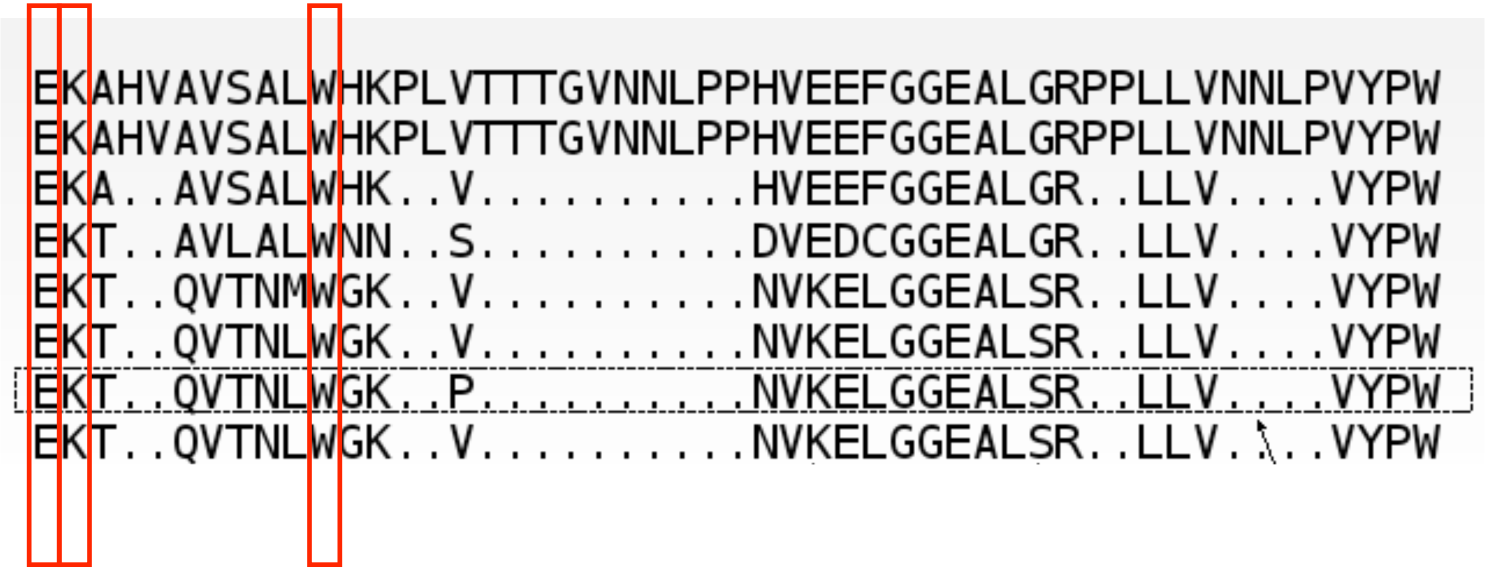
GLM

…

Multiple test correction

Application to any type of data!

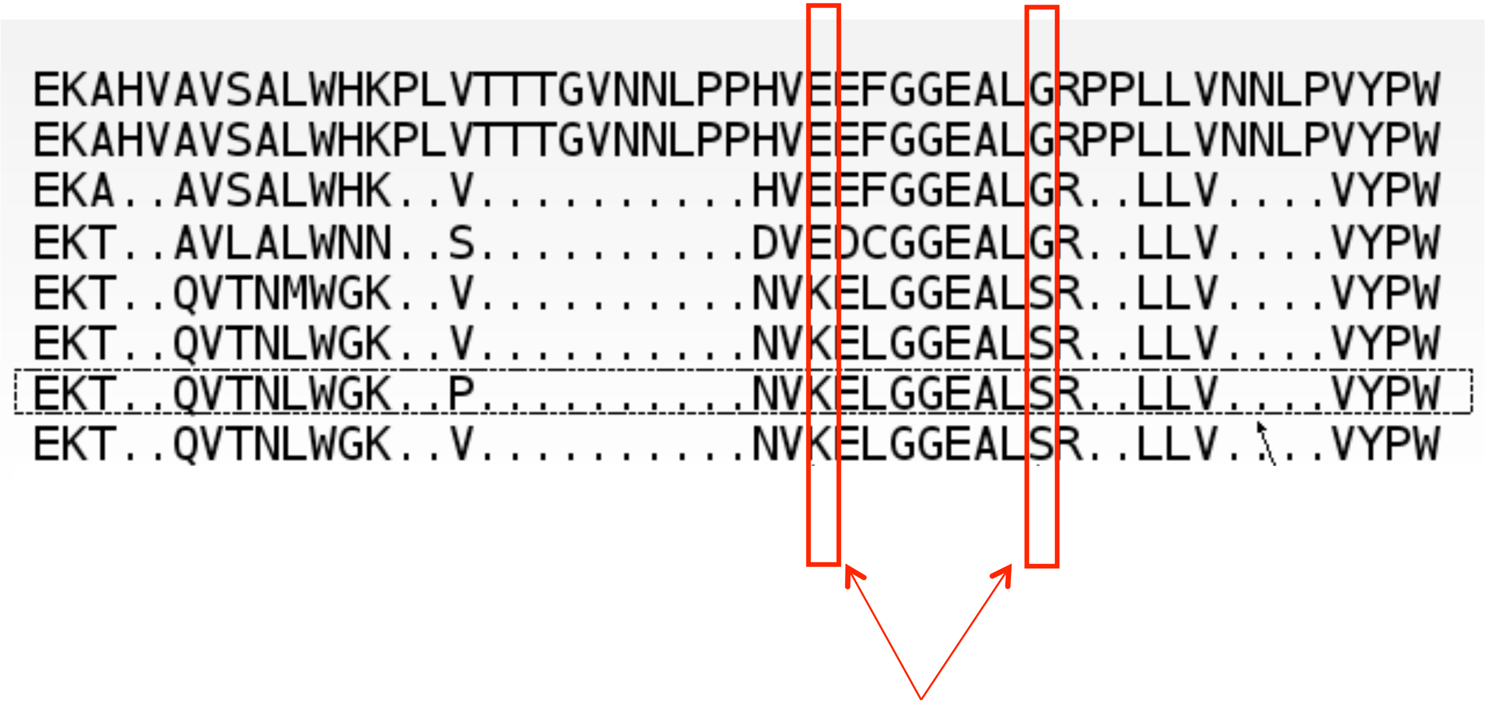
Homo sapiens
Gorilla gorilla
Macaca mulatta
Callithrix jacchus
Bos taurus
Felis caritus
Canis lupus
Myotis lifugus



```
EKAHVAVSALWHKPLVTTTGVNNLPPHVEEFGGEALGRPPLLVNNLPVYPW
EKAHVAVSALWHKPLVTTTGVNNLPPHVEEFGGEALGRPPLLVNNLPVYPW
EKA..AVSALWHK..V..........HVEEFGGEALGR..LLV....VYPW
EKT..AVLALWNN..S..........DVEDCGGEALGR..LLV....VYPW
EKT..QVTNMWGK..V..........NVKELGGEALSR..LLV....VYPW
EKT..QVTNLWGK..V..........NVKELGGEALSR..LLV....VYPW
EKT..QVTNLWGK..P..........NVKELGGEALSR..LLV....VYPW
EKT..QVTNLWGK..V..........NVKELGGEALSR..LLV....VYPW
```

Conserved sites

Application to any type of data!



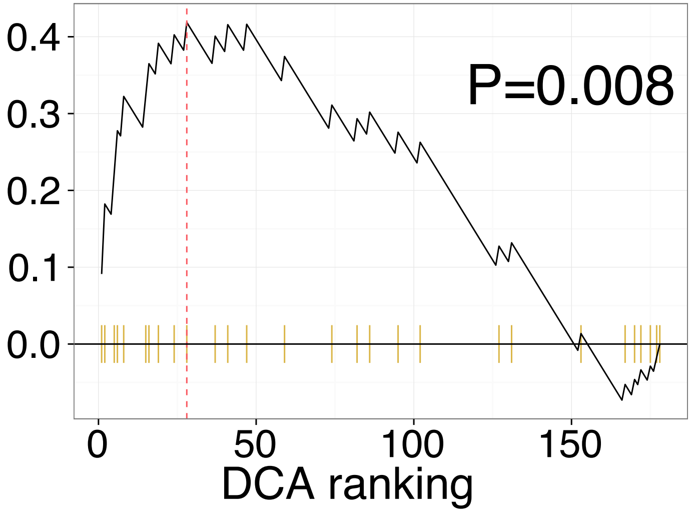
```
Homo sapiens         EKAHVAVSALWHKPLVTTTGVNNLPPHVEEFGGEALGRPPLLVNNLPVYPW
Gorilla gorilla      EKAHVAVSALWHKPLVTTTGVNNLPPHVEEFGGEALGRPPLLVNNLPVYPW
Macaca mulatta       EKA..AVSALWHK..V..........HVEEFGGEALGR..LLV....VYPW
Callithrix jacchus   EKT..AVLALWNN..S..........DVEDCGGEALGR..LLV....VYPW
Bos taurus           EKT..QVTNMWGK..V..........NVKELGGEALSR..LLV....VYPW
Felis caritus        EKT..QVTNLWGK..V..........NVKELGGEALSR..LLV....VYPW
Canis lupus          EKT..QVTNLWGK..P..........NVKELGGEALSR..LLV....VYPW
Myotis lifugus       EKT..QVTNLWGK..V..........NVKELGGEALSR..LLV....VYPW
```

coevolution

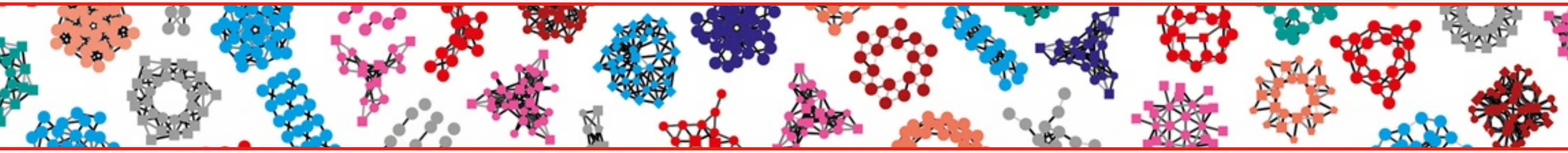
Application to any type of data!

	Coevolution score	polymorphic
position 4	0.2	no
position 13	0.2	no
position 14	0.15	no
position 2	0.15	no
position 7	0.14	yes
position 17	0.14	no
position 20	0.14	no
position 21	0.14	no
position 6	0.06	no
position 10	0.06	yes
position 11	0.06	yes
position 16	0.06	no
position 1	0.001	yes
position 3	0.001	no
position 5	0.001	yes
position 8	0.001	yes
position 9	0.001	yes
position 12	0.001	no
position 15	0.001	yes
position 18	0.001	yes
position 19	0.001	no
position 22	0.001	yes

Polymorphism in human and coevolving constraints

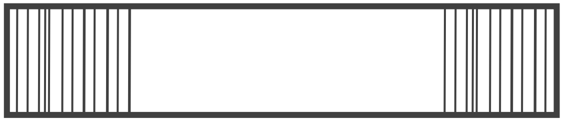


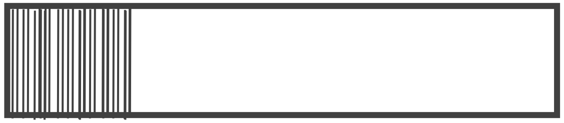
Dib *et al.* accepted

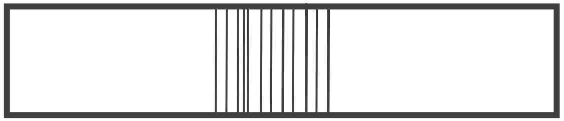


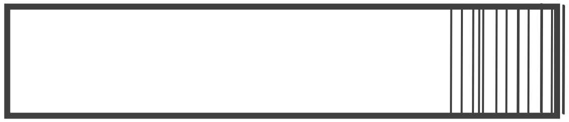
EXERCICE 3

How does GSEA deal with genes sets enrichment when they in the following configurations

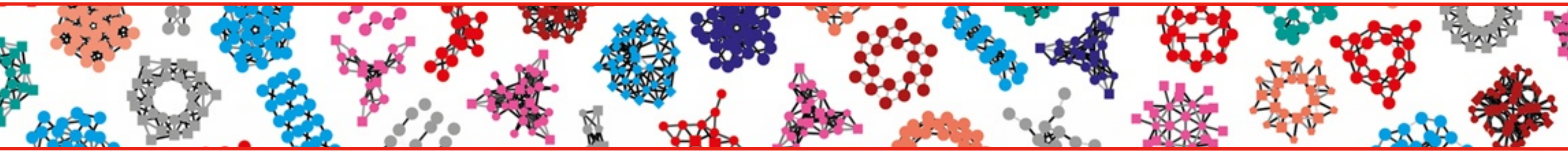








GSEA_data_input.csv



EXERCICE 3

A. Are the pathway’s genes, pinpointed in case2 of *GSEA_data_input.csv* dataset, highly differentially expressed?

Answer using

a. Fisher test and a threshold of 0.17 on scores
b. GSEA

B. Repeat question 1 using pathway association of cases 3, 4 and 5 of GSEA_data_input.csv dataset.

In R solution

```
>source(https://bioconductor.org/biocLite.R)
>biocLite("clusterProfiler")
>require(clusterProfiler)

# Get data
>table<-read.csv("GSEA_data_input.csv”)

>case2.mat=matrix(c(

  length(which(table$scores<0.17 & table$case2=="PATHWAY")),

  length(which(table$scores<0.17 & table$case2=="NO")),

  length(which(table$scores>0.17 & table$case2=="PATHWAY")),

  length(which(table$scores>0.17 & table$case2=="NO")))

  ,nrow=2)

>fisher.test(case2.mat)
```

In R solution

```
>df_case2 <- data.frame(table$gene.ID, table$scores, table$case2)
>colnames(df_case2) <- c("ID","score","S")
>head(df_case2)

>SCORE=df_case2$score
>names(SCORE)=df_case2$ID
>SCORE=sort(SCORE,decreasing=TRUE)
>head(SCORE)

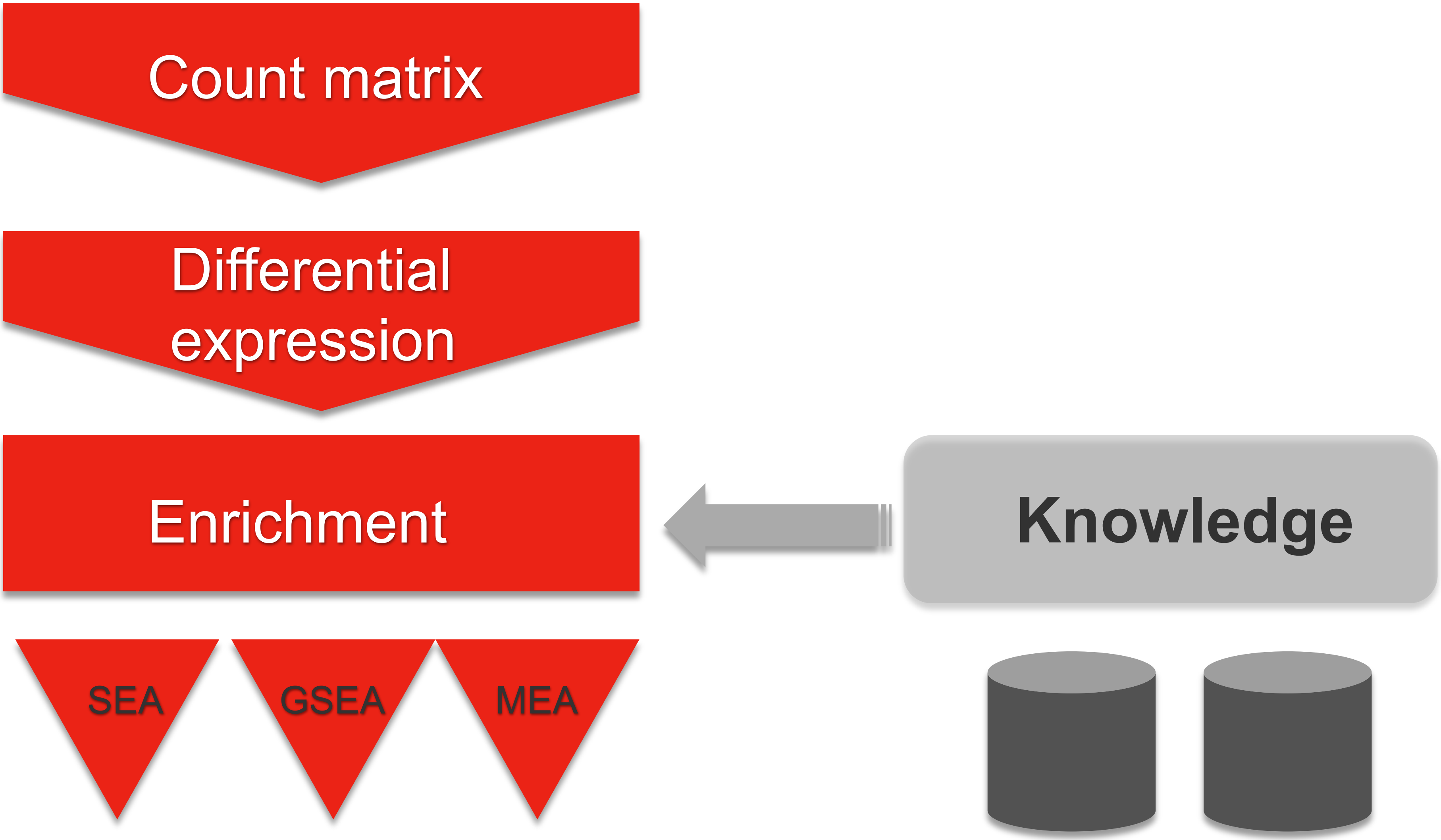
>term2gene_case2=data.frame(term=df_case2$S,name=df_case2$ID)
>head(term2gene_case2)

>gsea.out_case2<-GSEA(SCORE,
                    TERM2GENE=term2gene_case2,
                    nPerm=10000,
                    pvalueCutoff=1,
                    pAdjustMethod = "BH")

>gseaplot(gsea.out_case1,”PATHWAY”)

…
```

Overview



Count matrix

Differential expression

Enrichment

Knowledge

SEA

GSEA

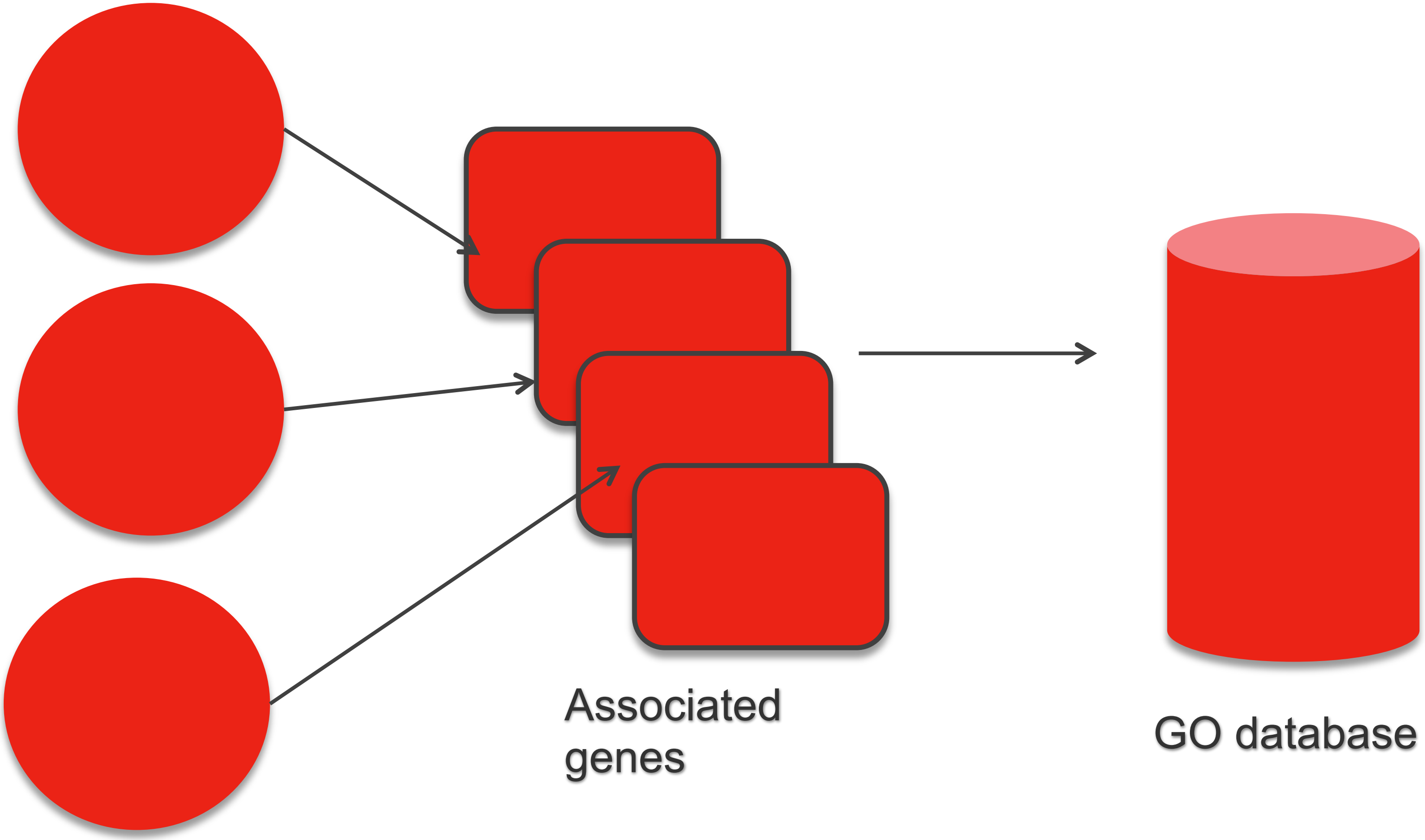
MEA

Enrichment analysis & ontologies

An ontology is a specification of the concepts & relationships that can exist in a domain of discourse.

The Gene Ontology (GO) project is an effort to provide consistent descriptions of gene products

The Gene Ontology (GO)

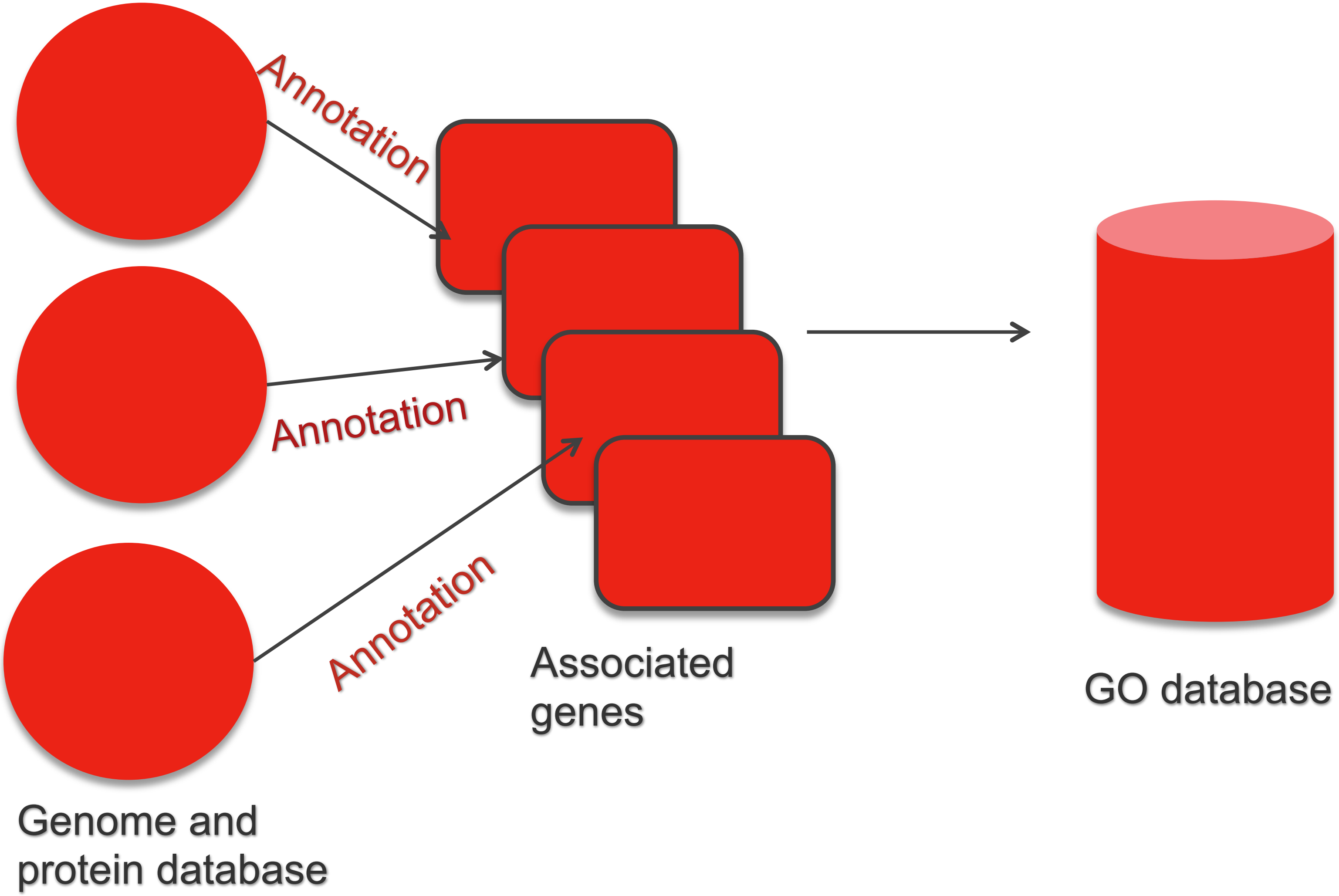


Associated genes

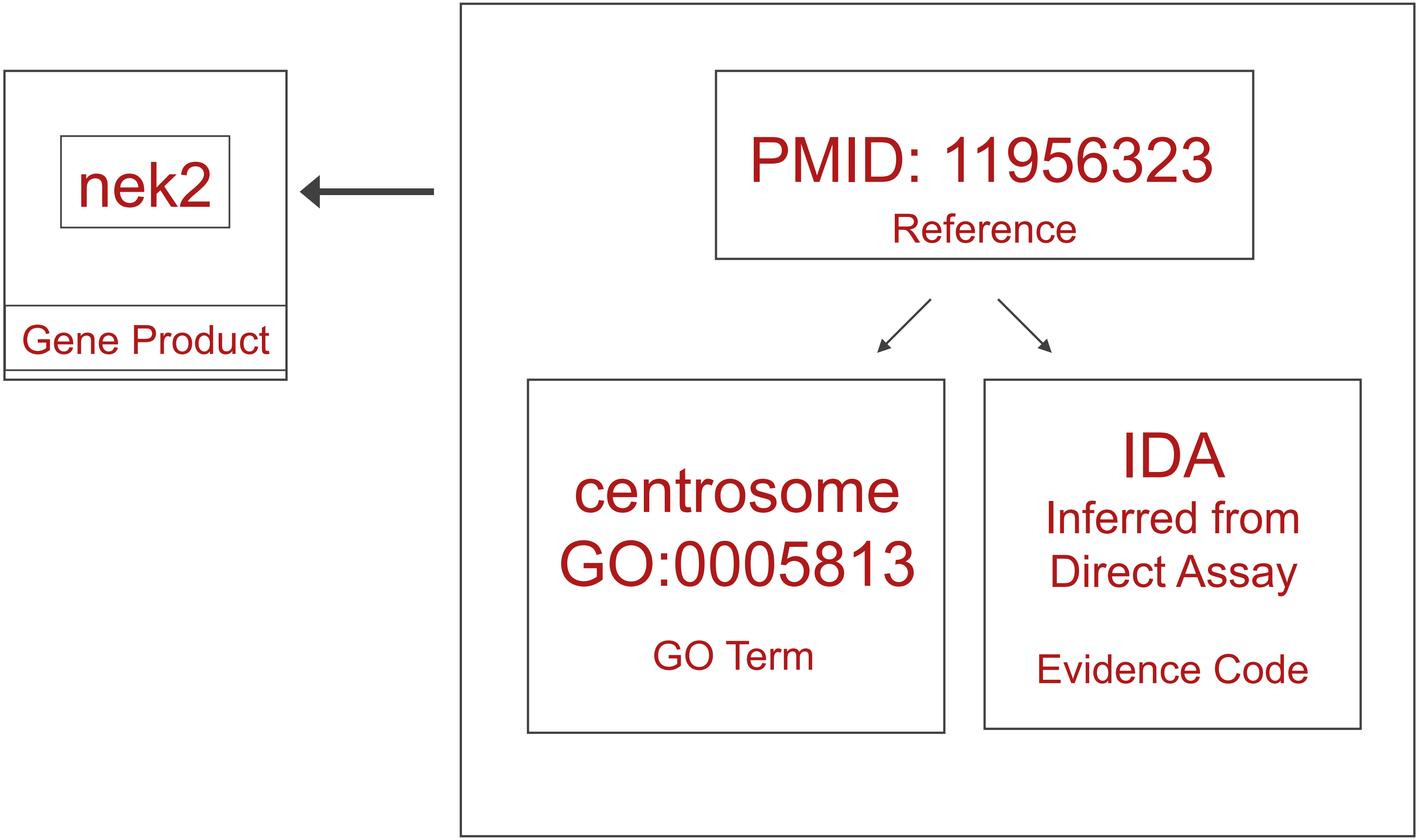
GO database

Genome and protein database

The Gene Ontology (GO)



Example Annotation



nek2

Gene Product

PMID: 11956323

Reference

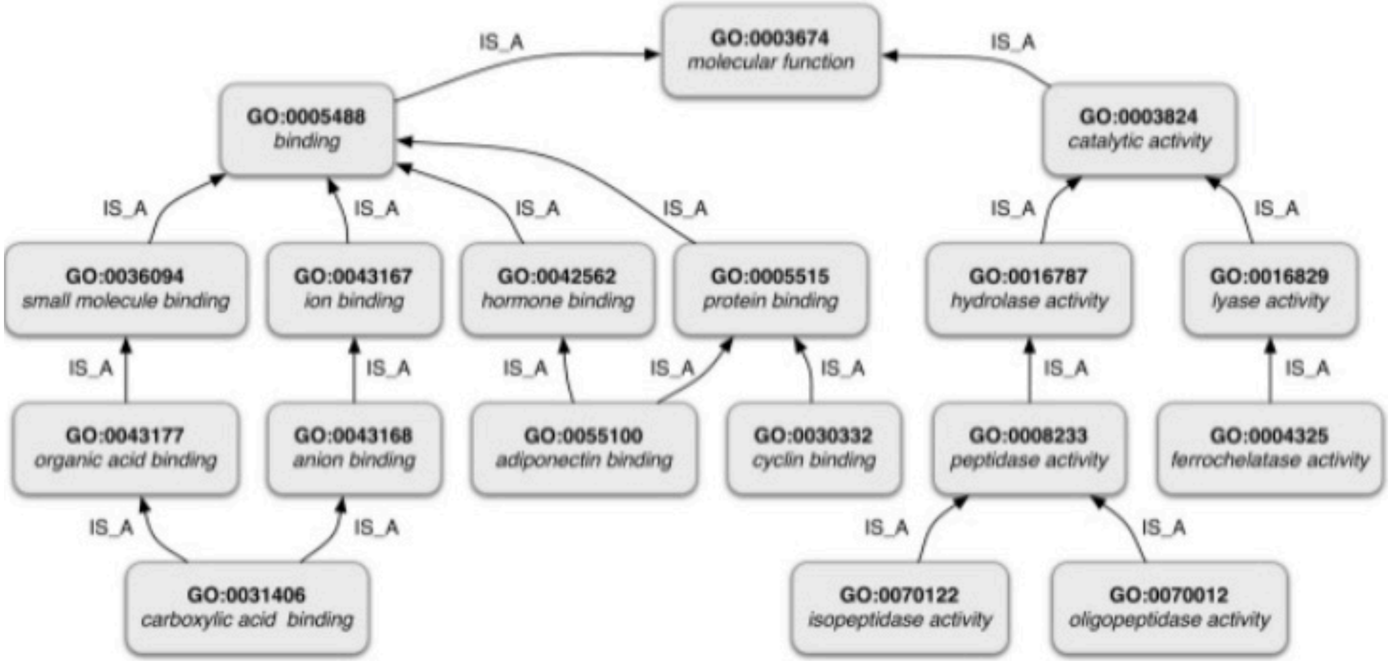
centrosome
GO:0005813

GO Term

IDA
Inferred from
Direct Assay

Evidence Code

Links between GO terms



GO domain

1. cellular component (CC)
2. biological process (BP)
3. molecular function (MF)

In enrichment several GO terms are checked

↓

multiple testing

Comparison to other GO enrichment tools (as of late 2008)

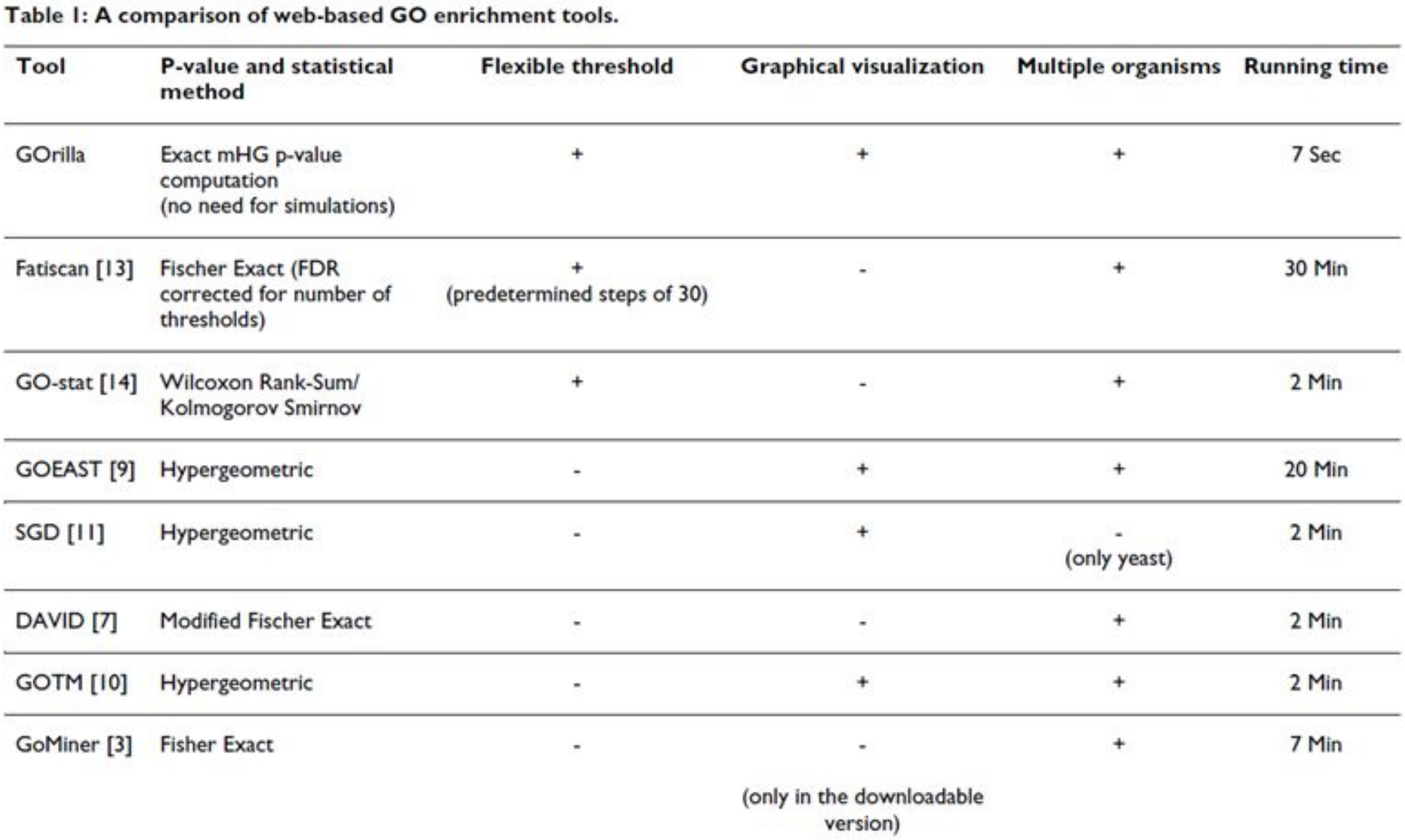


Table 1: A comparison of web-based GO enrichment tools.

Tool	P-value and statistical method	Flexible threshold	Graphical visualization	Multiple organisms	Running time
GOrilla	Exact mHG p-value computation (no need for simulations)	+	+	+	7 Sec
Fatiscan [13]	Fischer Exact (FDR corrected for number of thresholds)	+ (predetermined steps of 30)	-	+	30 Min
GO-stat [14]	Wilcoxon Rank-Sum/ Kolmogorov Smirnov	+	-	+	2 Min
GOEAST [9]	Hypergeometric	-	+	+	20 Min
SGD [11]	Hypergeometric	-	+	- (only yeast)	2 Min
DAVID [7]	Modified Fischer Exact	-	-	+	2 Min
GOTM [10]	Hypergeometric	-	+	+	2 Min
GoMiner [3]	Fisher Exact	-	- (only in the downloadable version)	+	7 Min

In R

```
>source(https://bioconductor.org/biocLite.R)
>biocLite("clusterProfiler")
>require(clusterProfiler)

# Use GSEA to evaluate the Gene set enrichment and  find an ontology that is
differentially expressed in our dataset
>? gseGO
>gsecc<- gseGO(geneList      = geneList,
              OrgDb        = org.Hs.eg.db,
              ont          = "ALL",
              nPerm        = 10000,
              pvalueCutoff = 1,
              verbose      = FALSE)

>gseaplot(gsecc, geneSetID="GO:0000779")
```

→ Ranked adj. p-value scores

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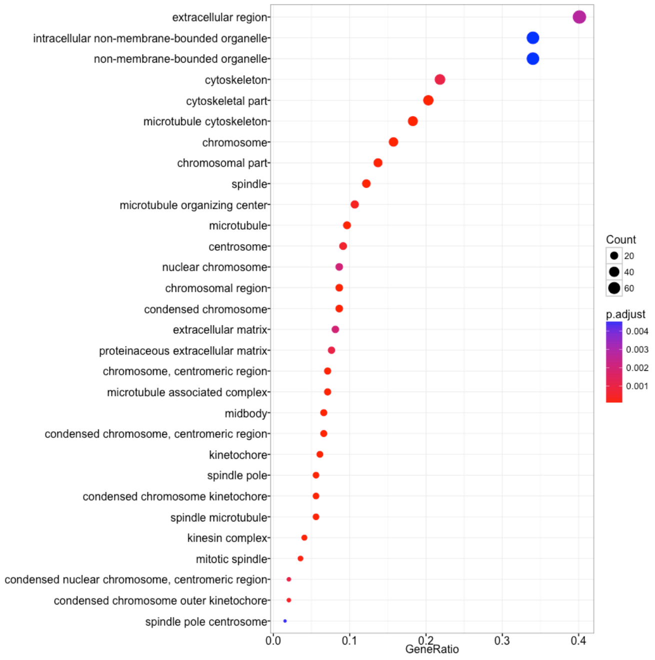
>gseaplot(gsecc, geneSetID="GO:0000779")

# Visualize
>?dotplot
>dotplot(ego, showCategory=30)
```

Ranked adj. p-value scores



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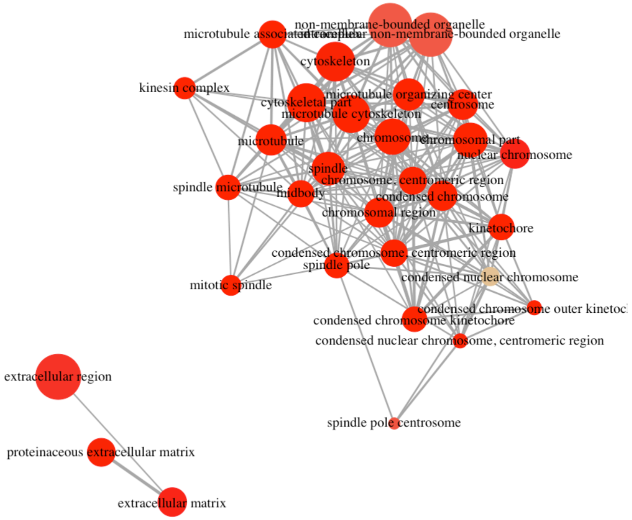
>?enrichMap
```

Ranked adj. p-value scores



non-membrane-bounded organelle
microtubule associated complex
intracellular non-membrane-bounded organelle
cytoskeleton
kinesin complex
microtubule organizing center
cytoskeletal part
centrosome
microtubule cytoskeleton
microtubule
chromosome
chromosomal part
nuclear chromosome
spindle
chromosome, centromeric region
spindle microtubule
midbody
condensed chromosome
chromosomal region
kinetochore
condensed chromosome, centromeric region
spindle pole
condensed nuclear chromosome
mitotic spindle
condensed chromosome outer kinetoc
condensed chromosome kinetochore
condensed nuclear chromosome, centromeric region
extracellular region
spindle pole centrosome
proteinaceous extracellular matrix
extracellular matrix

In R

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              verbose      = FALSE)

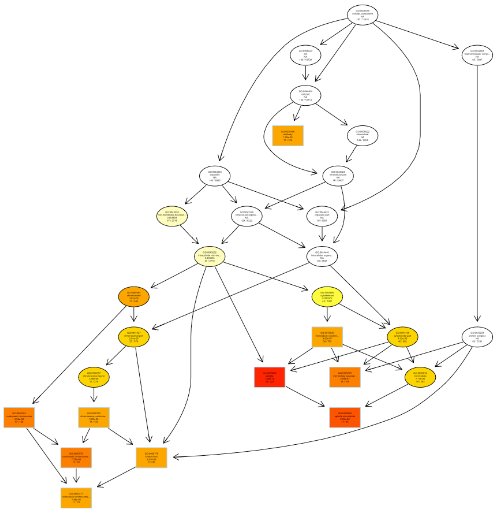
>gseaplot(gsecc, geneSetID="GO:0000779")

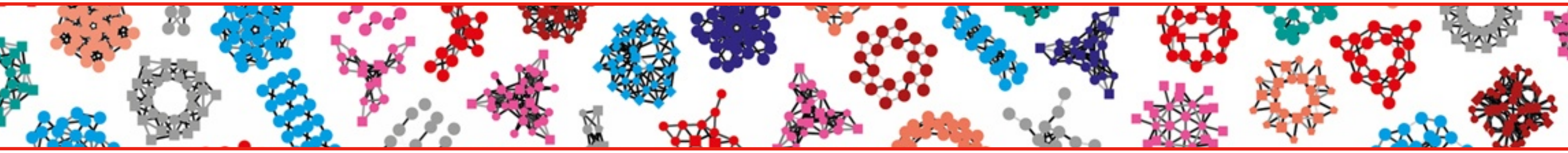
# Visualize
>?dotplot
>dotplot(ego, showCategory=30)

>?enrichMap

>plotGOgraph(ego)
```

→ Ranked adj. p-value scores





EXERCICE 4:
Enrichment and ontologies

HS_pvalues.csv is a file containing the adj. p-values issued from an ANOVA statistical test for several ENTREZ gene names.

1. Use *HS_pvalues.csv* dataset and look for GO ontologies that are enriched with a significant value
2. What are the gene names that enriched the best GO ontology?

In R

```
>require(clusterProfiler)
>library(org.Hs.eg.db)
>keytypes(org.Hs.eg.db)

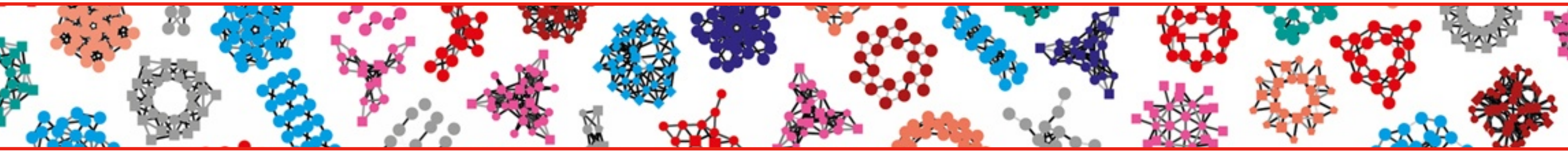
>table       <-read.csv("HS_pvalues.csv")
>SCORE       <-table$score
>names(SCORE)<-table$gene.ENTREZ.ID
>SCORE       <-sort(SCORE ,decreasing=T)

>ego <- gseGO(geneList     = SCORE,
              OrgDb        = org.Hs.eg.db,
              ont          = ”ALL",
              nPerm        = 1000,
              pvalueCutoff = 1,
              verbose      = FALSE)

>head(ego)

>gseaplot(ego, geneSetID="GO:0048518")
>dotplot(ego, showCategory=30)
>enrichMap(ego)
>plotGOgraph(ego)

#can be used on the outcome of enrichGO function
#barplot(ego, showCategory=30)
```



EXERCICE extra:
Enrichment and ontologies

1. Use *Rat_KS.txt* dataset and look for GO ontologies that are enriched with a significant value
2. What are the gene names that enriched the best GO ontology?
3. What is the enrichment outcome using KEGG database?
4. Can you distinguish up and down-regulated genes enrichments ?

In R: solution

```
>require(clusterProfiler)
>library(org.Rn.eg.db)
>keytypes(org.Rn.eg.db)
# USE RAT DATABASES AND ANNOTATION
>rat<-read.csv(”rat_KD.txt")
>PROBES<- rat$row.names
>OUT   <- select(rat2302.db,keys= PROBES, columns=c("SYMBOL", "ENTREZID",
”ENSEMBL"))
>duplicated(OUT$PROBEID)
>OUT<-OUT[-which(duplicated(OUT$PROBEID)),]
>dim(OUT)

>rawp <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
>names(rawp)        <-OUT$ENSEMBL
>sortedrawp         <-sort(rawp)
>p_holm             <-p.adjust(sortedrawp,method="BH")
>names(p_holm)      <-names(sortedrawp)
>SCORE              <-p_holm
>SCORE              <-sort(SCORE, decreasing=TRUE)
>head(SCORE)

>egoGSECC <- gseGO(geneList= SCORE,
              OrgDb        = org.Rn.eg.db,
              keyType      = 'ENSEMBL',
              ont          = "CC",
              nPerm        = 1000,
              minGSSize    = 10,
              maxGSSize    = 500,
              pvalueCutoff = 1,
              verbose      = FALSE)
>head(egoGSECC)
```

In R: solution

```
>gseaplot(egoGSECC , geneSetID="GO:0014069")
>dotplot(egoGSECC , showCategory=30)
>enrichMap(egoGSECC )
>plotGOgraph(egoGSECC )


# KEGG ONLY WORKS WITH ENTREZ ID
>rawp <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
>names(rawp)        <-OUT$ENTREZ
>sortedrawp         <-sort(rawp)
>p_holm             <-p.adjust(sortedrawp,method="BH")
>names(p_holm)      <-names(sortedrawp)
>SCORE              <-p_holm
>SCORE              <-sort(SCORE, decreasing=TRUE)
>head(SCORE)


>kk2 <- gseKEGG(geneList     = SCORE,
                organism     = 'rat',
                nPerm        = 1000,
                minGSSize    = 10,
                pvalueCutoff = 1,
                verbose      = FALSE)
>head(kk2)
```

In R: solution

```
library(gtools)
fcRat <- function(df, grp1, grp2) {
x = df[grp1]
y = df[grp2]
x = as.numeric(x)
y = as.numeric(y)
x = mean(x)
y = mean(y)
foldchange(x, y}
rawp      <- apply(rat, 1, ttestRat, grp1 = c(2:7), grp2 = c(8:12))
Fc        <- apply(rat, 1, fcRat   , grp1 = c(2:7), grp2 = c(8:12))

resExp               <- data.frame(pValues=rawp, log2FC=fc, name=OUT$ENSEMBL)
topDEGenesDetails    <- resExp[which(resExp[,1] < 0.01 & abs(resExp[,2])>2), ]
topDEGenesFC         <- resExp[which(resExp[,1] < 0.01 & abs(resExp[,2])>2),3]

mydf <- data.frame(ENSEMBL=topDEGenesFC, FC=topDEGenesDetails[,2])
mydf$group                              <- "upregulated"
mydf$group[mydf$FC < 0]                 <- "downregulated”

formula_res <- compareCluster(ENSEMBL~group,
               data           = mydf,
               fun            = "enrichGO",
               keyType        = 'ENSEMBL',
               OrgDb          = org.Rn.eg.db,
               ont            = "ALL",
               pAdjustMethod  = "BH",
               pvalueCutoff   = 0.01,
               qvalueCutoff   = 0.05,
               readable       = TRUE)
dotplot(formula_res,font.size=10)
```

Learning objectives

At the end of the course, the participants are expected to be able to:

1. identify statistical methods that could be used to pinpoint differentially expressed genes
2. determine whether a set of genes shows statistically significant differences between two classes
3. apply GSEA using R
4. distinguish available enrichment analysis methods
5. apply enrichment analysis implementations using R
6. do an Ab initio exploration of transcript data
7. determine whether the genes of a GO term have a statistically significant difference in expression.

Feedbacks
through course web-page

Thank you for your attention

SIB Swiss Institute of Bioinformatics

BCF