UNIVERSITÉ DU LUXEMBOURG

LUXEMBOURG INSTITUTE OF HEALTH
RESEARCH DEDICATED TO LIFE

PhD Course

Advanced Biostatistics

Lecture 6
Advanced Topics.
Data Analysis in Transcriptomics

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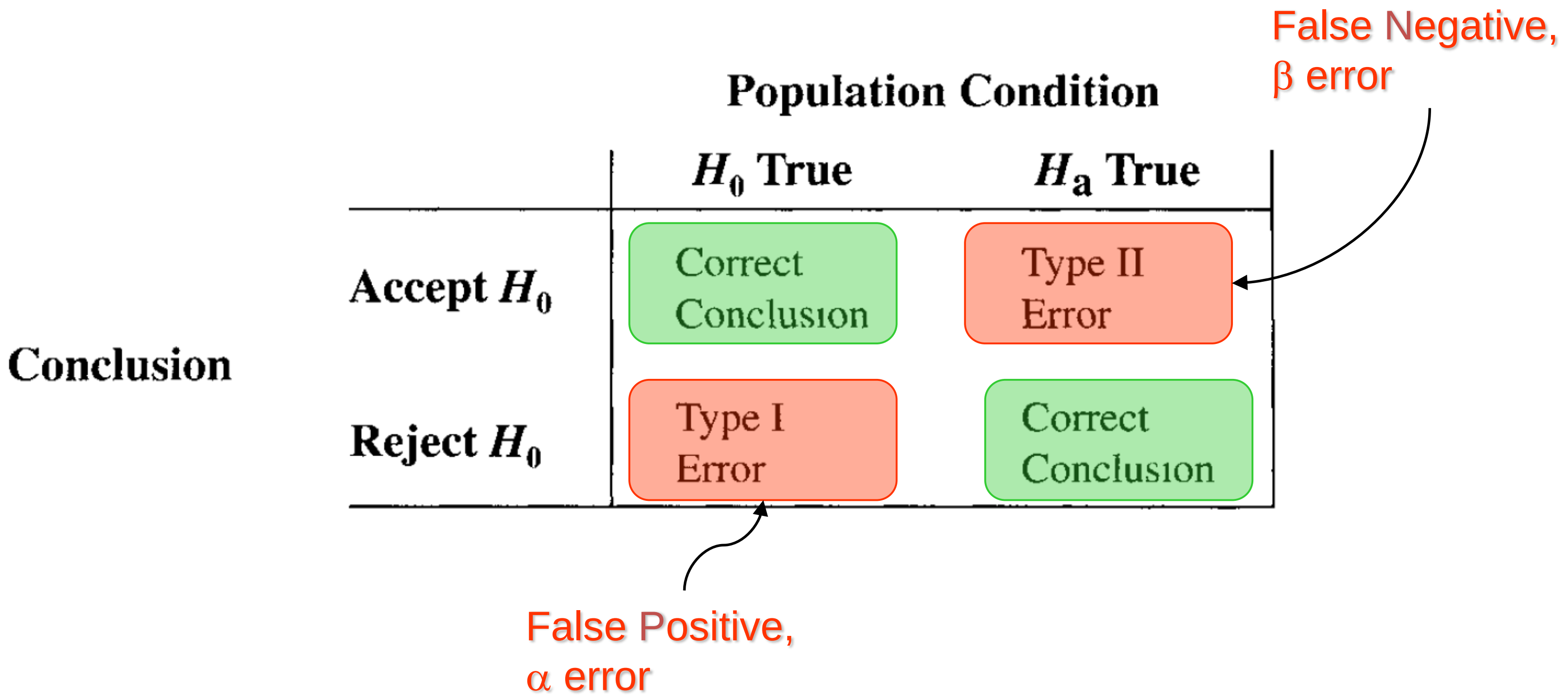
27-05-2016

Outline

- **Multiple Comparisons (L6.1)**
- **Survival analysis (L6.2)**
- **Microarray data analysis (L6.3)**
  - Principles
  - Pipeline for data analysis
  - Experiment description
  - APT import
  - QC, differential expression analysis
  - Differential expression analysis
- **RNASeq data analysis (L6.4)**
- **Enrichment analysis (L6.5)**

L6.1. Multiple Comparisons

Correct Results and Errors



		Population Condition	
		H_0 True	H_a True
Conclusion	Accept H_0	Correct Conclusion	Type II Error
	Reject H_0	Type I Error	Correct Conclusion

False Negative,
β error

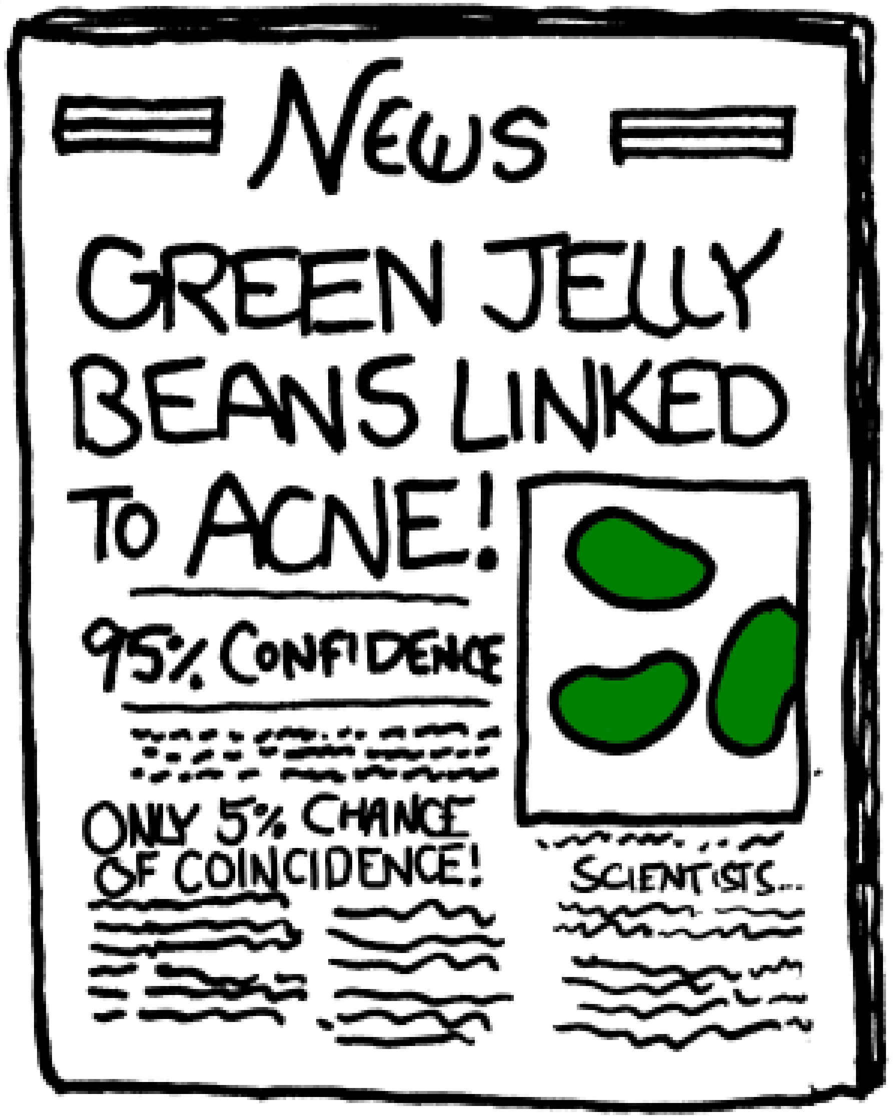
False Positive,
α error

Probability of an error in a multiple test:

$1–(0.95)^{\text{number of comparisons}}$

Differential Expression Analysis

Example


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

http://www.xkcd.com/882/

L6.1. Multiple Comparisons

False Discovery Rate

False discovery rate (FDR)

FDR control is a statistical method used in multiple hypothesis testing to correct for multiple comparisons. In a list of rejected hypotheses, FDR controls the expected proportion of incorrectly rejected null hypotheses (type I errors).

		Population Condition		
		H_0 is TRUE	H_0 is FALSE	Total
Conclusion	Accept H_0 (non-significant)	$\boldsymbol{U}$	$\boldsymbol{T}$	$m - R$
	Reject H_0 (significant)	$\boldsymbol{V}$	$\boldsymbol{S}$	R
	Total	m_0	$m - m_0$	$\boldsymbol{m}$

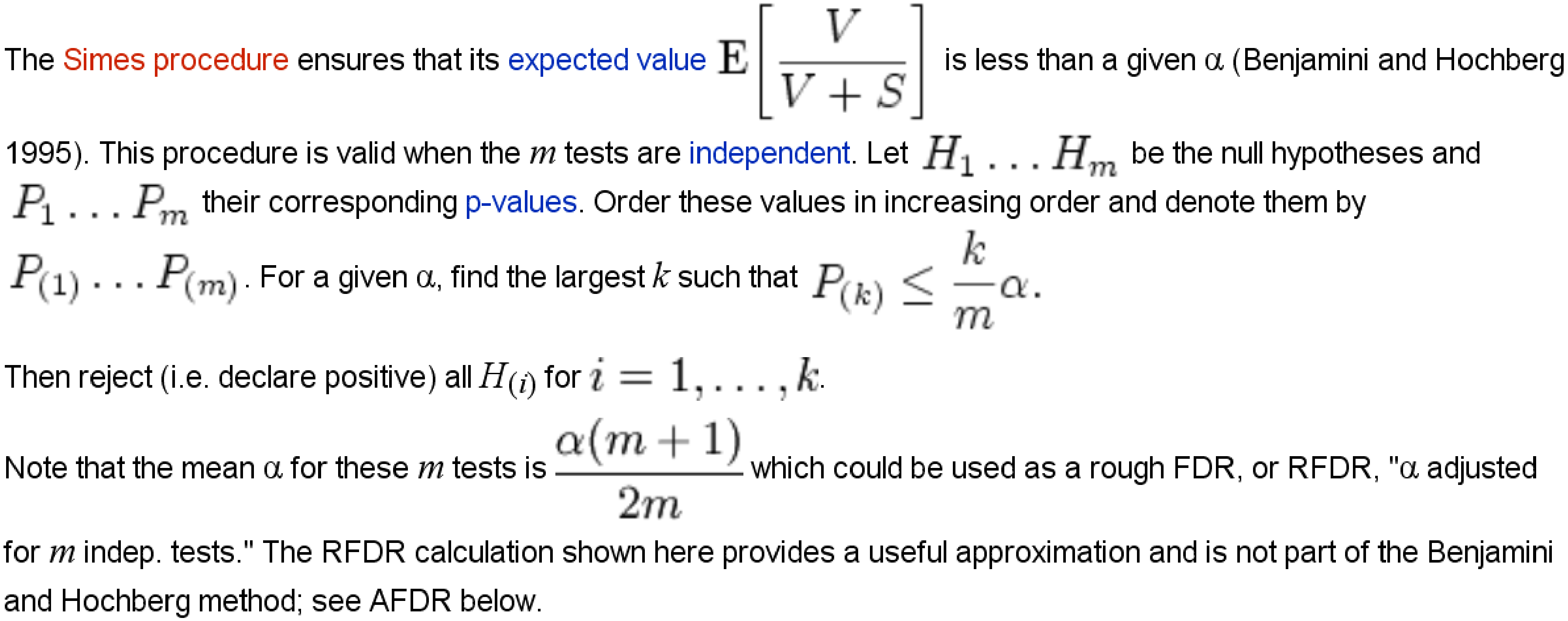
$$FDR = E\left(\frac{V}{V+S}\right)$$

L6.1. Multiple Comparisons

False Discovery Rate

Assume we need to perform *m* = 100 comparisons, and select maximum **FDR = α = 0.05**

Independent tests



The Simes procedure ensures that its expected value $\mathrm{E}\left[\frac{V}{V+S}\right]$ is less than a given α (Benjamini and Hochberg 1995). This procedure is valid when the m tests are independent. Let $H_1 \ldots H_m$ be the null hypotheses and $P_1 \ldots P_m$ their corresponding p-values. Order these values in increasing order and denote them by $P_{(1)} \ldots P_{(m)}$. For a given α, find the largest k such that $P_{(k)} \leq \frac{k}{m}\alpha.$

Then reject (i.e. declare positive) all $H_{(i)}$ for $i = 1, \ldots, k$.

Note that the mean α for these m tests is $\frac{\alpha(m+1)}{2m}$ which could be used as a rough FDR, or RFDR, "α adjusted for m indep. tests." The RFDR calculation shown here provides a useful approximation and is not part of the Benjamini and Hochberg method; see AFDR below.

L6.1. Multiple Comparisons

False Discovery Rate: Benjamini & Hochberg

Assume we need to perform *m* = 100 comparisons, and select maximum **FDR = α = 0.05**

$$FDR = E\left(\frac{V}{V+S}\right)$$

Expected value for FDR < α if $P_{(k)} \le \frac{k}{m}\alpha$

$$\frac{mP_{(k)}}{k} \le \alpha$$

```
p.adjust(pv, method=“fdr”)
```

Other Methods

Bonferroni – simple, but too stringent, not recommended

Holm – a more powerful and less stringent version of Bonferroni (ok)

L6.1. Multiple Comparisons

p-value or FDR?

Let’s generate a completely ran dom experiment (script L6.1)

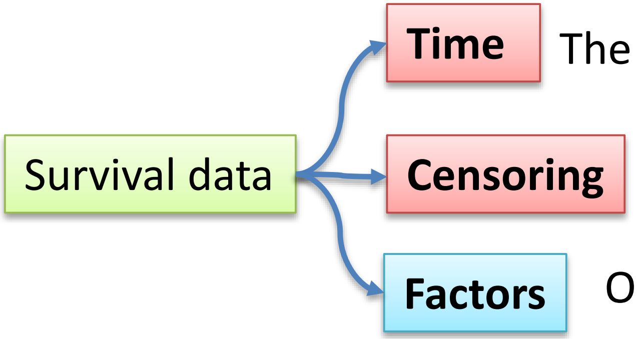
L6.2. Survival Analysis

Survival Data

Survival analysis
is a branch of statistics which deals with analysis of time to events, such as death in biological organisms and failure in mechanical systems (i.e. **reliability theory** in engineering).

Survival analysis attempts to answer questions such as:

- What is the proportion of a population which will survive past a certain time?
- Of those that survive, at what rate will they die or fail?
- Can multiple causes of death or failure be taken into account?
- How do particular circumstances or characteristics increase or decrease the probability of survival?



Survival data

Time The time person is observed alive in a study

Censoring index saying whether person died or was lost for further observation

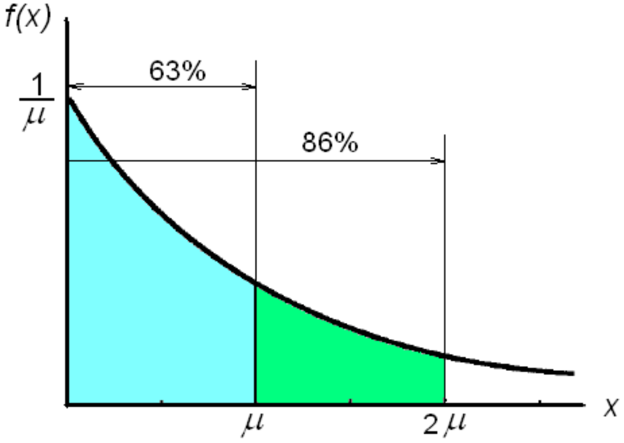
Factors Optionally: various factors affecting lifetime

http://www.partek.com/webinars/survival-analysis-partek-genomics-suite-software

L6.2. Survival Analysis

Cox's Survival Model

$$f(x) = \frac{1}{\mu} e^{-\frac{x}{\mu}} \qquad for\ x \geq 0, \mu > 0$$



$$h(t) = h_0(t) e^{\beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n}$$

h(t) – hazard function

$h_0(t)$ – basic hazard

$x_1 \ldots x_n$ – covariates (age, smoking, expression of gene X) which are time-independent!

$\beta_1 \ldots \beta_n$ – unknown parameters

$$HR = \frac{h_i(t)}{h_j(t)} = \exp\{\beta_1(x_{i1} - x_{j1}) + \ldots + \beta_n(x_{in} - x_{jn})\}$$

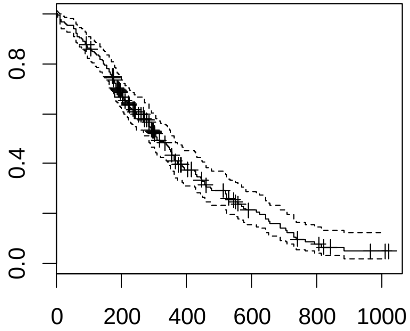
To identify significantly involved covariate: partial likelihood is calculated

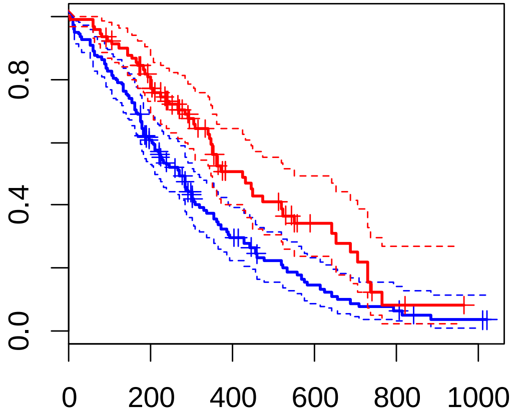
L6.2. Survival Analysis

Example: Lung

```
library(survival)
str(lung)

## create a survival object
## lung$status: 1-censored, 2-dead
sData = Surv(lung$time,event = lung$status == 2)
print(sData)

## Let's visualize it
fit = survfit(sData~1)
plot(fit)

## Let's visualize it for male/female
fit.sex = survfit(sData ~ lung$sex)
plot(fit.sex, col=c("blue","red"), conf.int = TRUE)

## Rank test for survival data
dif.sex = survdiff(sData ~ lung$sex)
dif.sex

## build Cox regression model
mod = coxph(sData ~ sex + age, data=lung)
summary(mod)
```

“event” should be:
0 – for censored
1 – for dead patients



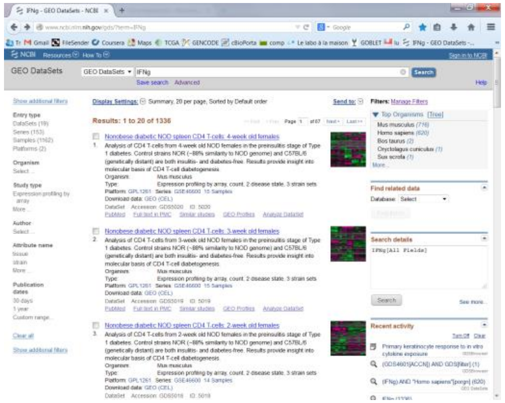


ovarian

L6.3. Microarrays

Public Repositories

GEO: http://www.ncbi.nlm.nih.gov/gds



Display Settings: Summary, 20 per page, Sorted by Default order

Send to:

Results: 1 to 20 of 1336

Page 1 of 67

Show additional filters

Entry type: DataSets (19), Series (153), Samples (1162), Platforms (2)

Organism: Select ...

Study type: Expression profiling by array, More ...

Author: Select ...

Attribute name: tissue, strain, More ...

Publication dates: 30 days, 1 year, Custom range...

Clear all

Show additional filters

1. Nonobese diabetic NOD spleen CD4 T-cells: 4-week old females
Analysis of CD4 T-cells from 4-week old NOD females in the preinsulitis stage of Type 1 diabetes. Control strains NOR (~88% similarity to NOD genome) and C57BL/6 (genetically distant) are both insulitis- and diabetes-free. Results provide insight into molecular basis of CD4 T-cell diabetogenesis.
Organism: Mus musculus
Type: Expression profiling by array, count, 2 disease state, 3 strain sets
Platform: GPL1261 Series: GSE46600 15 Samples
Download data: GEO (CEL)
DataSet Accession: GDS5020 ID: 5020
PubMed Full text in PMC Similar studies GEO Profiles Analyze DataSet

2. Nonobese diabetic NOD spleen CD4 T-cells: 3-week old females
Analysis of CD4 T-cells from 3-week old NOD females in the preinsulitis stage of Type 1 diabetes. Control strains NOR (~88% similarity to NOD genome) and C57BL/6 (genetically distant) are both insulitis- and diabetes-free. Results provide insight into molecular basis of CD4 T-cell diabetogenesis.
Organism: Mus musculus
Type: Expression profiling by array, count, 2 disease state, 3 strain sets
Platform: GPL1261 Series: GSE46600 15 Samples
Download data: GEO (CEL)
DataSet Accession: GDS5019 ID: 5019
PubMed Full text in PMC Similar studies GEO Profiles Analyze DataSet

3. Nonobese diabetic NOD spleen CD4 T-cells: 2-week old females
Analysis of CD4 T-cells from 2-week old NOD females in the preinsulitis stage of Type 1 diabetes. Control strains NOR (~88% similarity to NOD genome) and C57BL/6 (genetically distant) are both insulitis- and diabetes-free. Results provide insight into molecular basis of CD4 T-cell diabetogenesis.
Organism: Mus musculus
Type: Expression profiling by array, count, 2 disease state, 3 strain sets
Platform: GPL1261 Series: GSE46600 14 Samples
Download data: GEO (CEL)
DataSet Accession: GDS5018 ID: 5018

Filters: Manage Filters

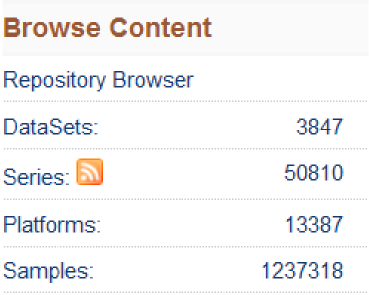
Top Organisms [Tree]: Mus musculus (716), Homo sapiens (620), Bos taurus (2), Oryctolagus cuniculus (1), Sus scrofa (1), More...

Find related data — Database: Select

Search details: IFNg[All Fields]

Search See more...

Recent activity — Turn Off Clear
Primary keratinocyte response to in vitro cytokine exposure
(GDS4601[ACCN]) AND GDS[filter] (1)
(IFNg) AND "Homo sapiens"[porgn] (620)
IFNg (1336)

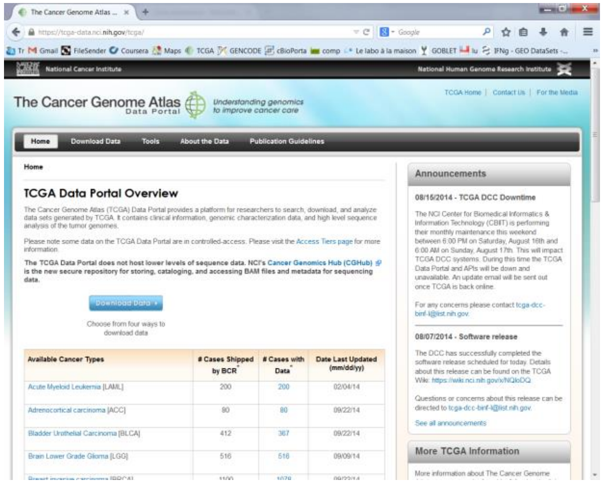


Browse Content

Repository Browser

DataSets:	3847
Series:	50810
Platforms:	13387
Samples:	1237318

TCGA: https://tcga-data.nci.nih.gov/tcga/


The Cancer Genome Atlas ...
https://tcga-data.nci.nih.gov/tcga/
National Cancer Institute
National Human Genome Research Institute
The Cancer Genome Atlas Data Portal
Understanding genomics to improve cancer care
TCGA Home | Contact Us | For the Media
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Download Data
Tools
About the Data
Publication Guidelines

Home

TCGA Data Portal Overview

The Cancer Genome Atlas (TCGA) Data Portal provides a platform for researchers to search, download, and analyze data sets generated by TCGA. It contains clinical information, genomic characterization data, and high level sequence analysis of the tumor genomes.

Please note some data on the TCGA Data Portal are in controlled-access. Please visit the Access Tiers page for more information.

The TCGA Data Portal does not host lower levels of sequence data. NCI's Cancer Genomics Hub (CGHub) is the new secure repository for storing, cataloging, and accessing BAM files and metadata for sequencing data.

Download Data ›

Choose from four ways to download data

Available Cancer Types	# Cases Shipped by BCR*	# Cases with Data*	Date Last Updated (mm/dd/yy)
Acute Myeloid Leukemia [LAML]	200	200	02/04/14
Adrenocortical carcinoma [ACC]	80	80	09/22/14
Bladder Urothelial Carcinoma [BLCA]	412	387	09/22/14
Brain Lower Grade Glioma [LGG]	516	516	09/09/14
Breast invasive carcinoma [BRCA]	1100	1078	09/22/14

Announcements

08/15/2014 - TCGA DCC Downtime

The NCI Center for Biomedical Informatics & Information Technology (CBIIT) is performing their monthly maintenance this weekend between 6:00 PM on Saturday, August 16th and 6:00 AM on Sunday, August 17th. This will impact TCGA DCC systems. During this time the TCGA Data Portal and APIs will be down and unavailable. An update email will be sent out once TCGA is back online.

For any concerns please contact tcga-dcc-binf-l@list.nih.gov.

08/07/2014 - Software release

The DCC has successfully completed the software release scheduled for today. Details about this release can be found on the TCGA Wiki: https://wiki.nci.nih.gov/x/NQloDQ

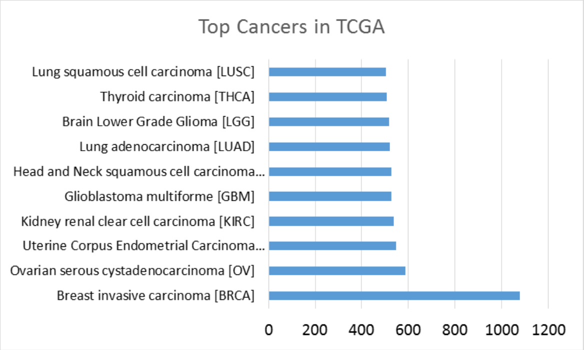
Questions or concerns about this release can be directed to tcga-dcc-binf-l@list.nih.gov.

See all announcements

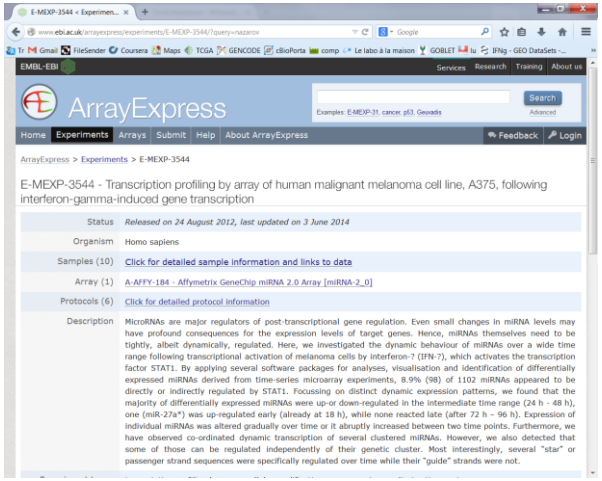
More TCGA Information

More information about The Cancer Genome

Sep 2014 – more then 10k patients



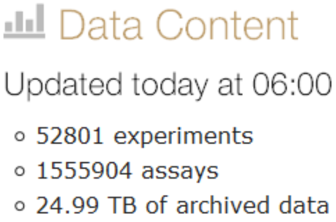
ArrayExpress: http://www.ebi.ac.uk/arrayexpress/


E-MEXP-3544 < Experimen...
www.ebi.ac.uk/arrayexpress/experiments/E-MEXP-3544/?query=nazarov
EMBL-EBI
Services Research Training About us
ArrayExpress
Search
Examples: E-MEXP-31, cancer, p53, Geuvadis
Advanced
Home Experiments Arrays Submit Help About ArrayExpress
Feedback Login

ArrayExpress > Experiments > E-MEXP-3544

E-MEXP-3544 - Transcription profiling by array of human malignant melanoma cell line, A375, following interferon-gamma-induced gene transcription

Status	*Released on 24 August 2012, last updated on 3 June 2014*
Organism	Homo sapiens
Samples (10)	Click for detailed sample information and links to data
Array (1)	A-AFFY-184 - Affymetrix GeneChip miRNA 2.0 Array [miRNA-2_0]
Protocols (6)	Click for detailed protocol information
Description	MicroRNAs are major regulators of post-transcriptional gene regulation. Even small changes in miRNA levels may have profound consequences for the expression levels of target genes. Hence, miRNAs themselves need to be tightly, albeit dynamically, regulated. Here, we investigated the dynamic behaviour of miRNAs over a wide time range following transcriptional activation of melanoma cells by interferon-? (IFN-?), which activates the transcription factor STAT1. By applying several software packages for analyses, visualisation and identification of differentially expressed miRNAs derived from time-series microarray experiments, 8.9% (98) of 1102 miRNAs appeared to be directly or indirectly regulated by STAT1. Focussing on distinct dynamic expression patterns, we found that the majority of differentially expressed miRNAs were up-or down-regulated in the intermediate time range (24 h - 48 h), one (miR-27a*) was up-regulated early (already at 18 h), while none reacted late (after 72 h – 96 h). Expression of individual miRNAs was altered gradually over time or it abruptly increased between two time points. Furthermore, we have observed co-ordinated dynamic transcription of several clustered miRNAs. However, we also detected that some of those can be regulated independently of their genetic cluster. Most interestingly, several "star" or passenger strand sequences were specifically regulated over time while their "guide" strands were not.



Data Content

Updated today at 06:00

- 52801 experiments
- 1555904 assays
- 24.99 TB of archived data

Analysis via:
http://www.cbioportal.org/public-portal/

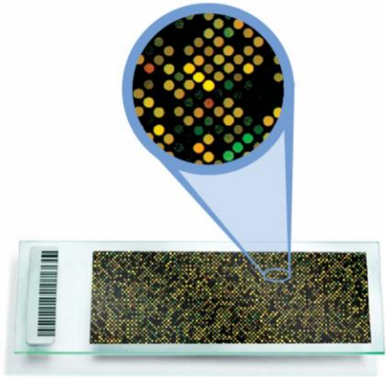
Data for our course: http://edu.sablab.net/transcript

L.6.3. Microarray Data

Types of Microarrays

Two-color Arrays (2C)

- Agilent full genome
- Thematic arrays



Pro

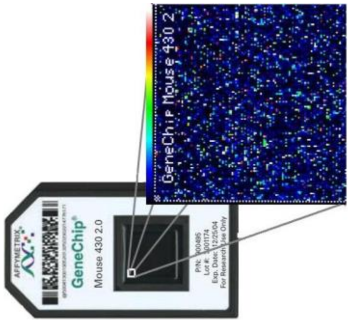
- Direct comparison
- Less sensitive to inaccuracies of spotting

Con

- Dye effects: need for “dye-swaps”
- Non-flexibility in analysis

One-color Arrays (1C)

- Affymetrix GeneChip
- Affymetrix Exon
- Affymetrix mRNA



Pro

- Flexible analysis
- High level of standardization

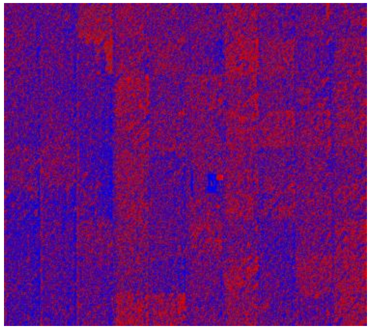
Con

- Price

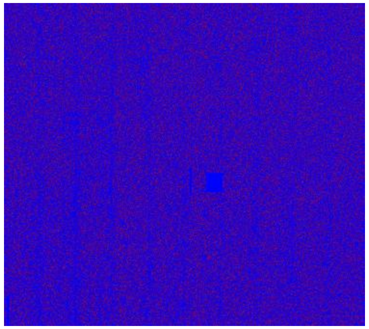
Microarray Data

One-color Arrays

Raw

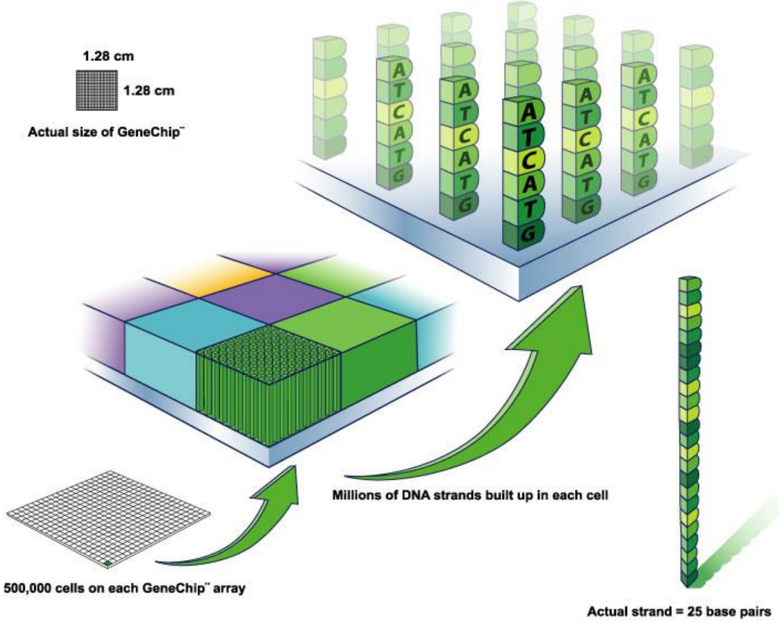


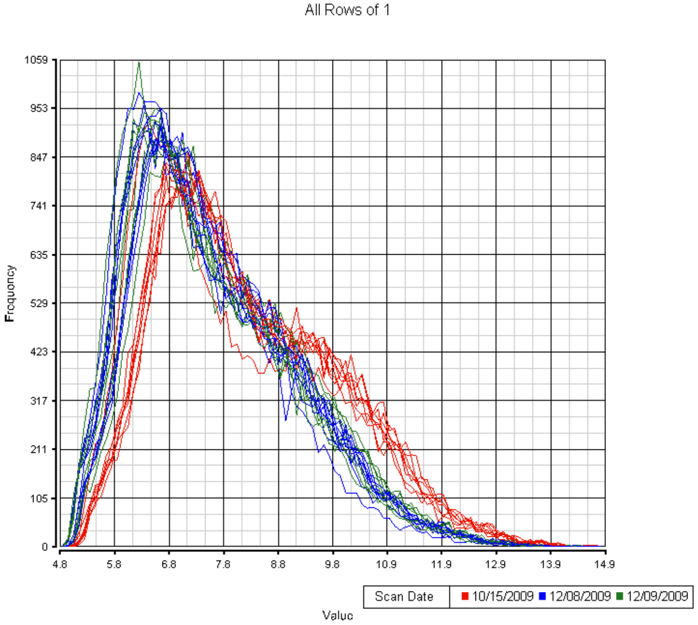
Normalized



High reproducibility and quality of spotting is required.

Affymetrix – “photolithography”-like technique


1.28 cm
1.28 cm
Actual size of GeneChip™
A
T
C
A
T
G
Millions of DNA strands built up in each cell
500,000 cells on each GeneChip™ array
Actual strand = 25 base pairs



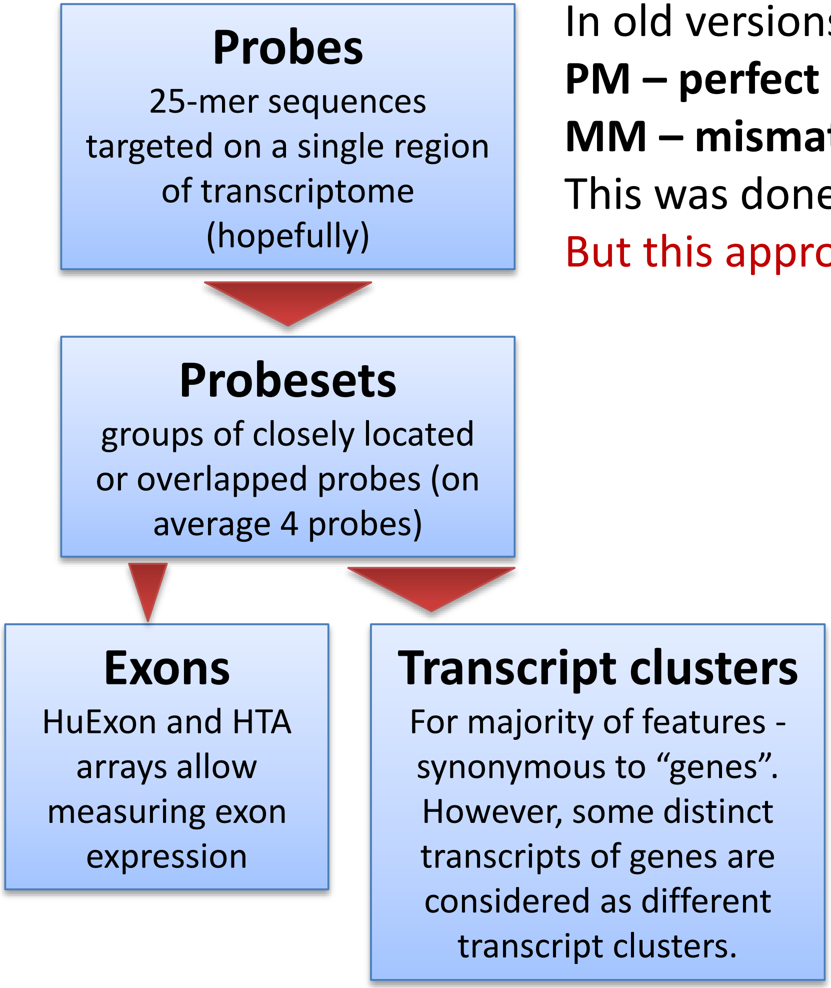
$$LogIntensity = log_2(I)$$

Background is “removed” during normalization step

Filtering may help removing uninformative features

Microarray Data

Affymetrix: Probes, Probesets and Transcript clusters



Probes

25-mer sequences targeted on a single region of transcriptome (hopefully)

Probesets

groups of closely located or overlapped probes (on average 4 probes)

Exons

HuExon and HTA arrays allow measuring exon expression

Transcript clusters

For majority of features - synonymous to "genes". However, some distinct transcripts of genes are considered as different transcript clusters.

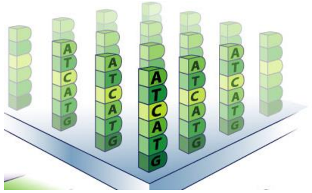
In old versions of Affy arrays (hgu95, hgu133, etc), there were:

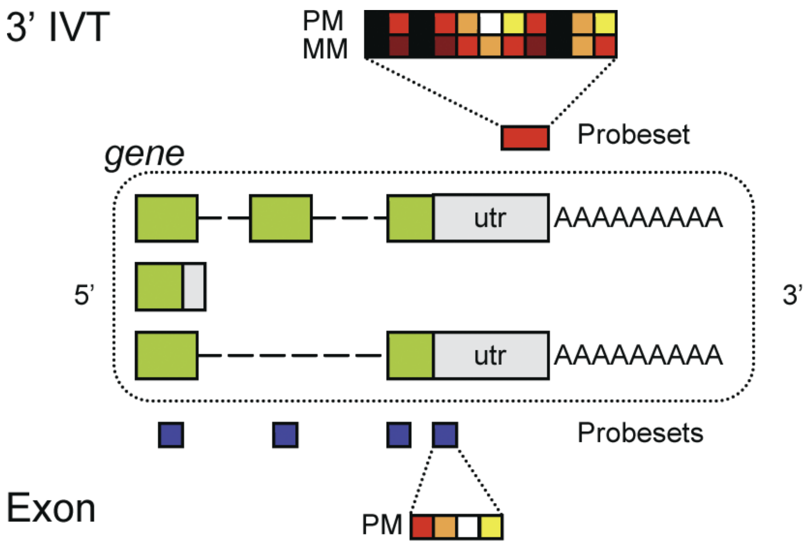
PM – perfect match probes

MM – mismatch probes (having replacement in th 13th character)

This was done for background estimation.

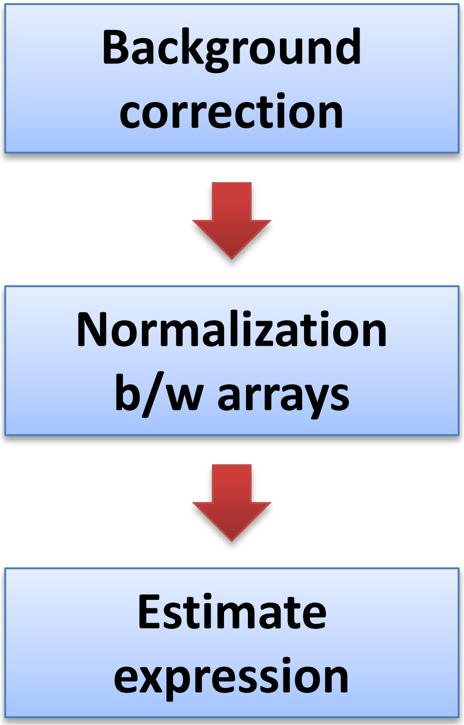
But this approach is not used now!!





Okoniewski M, Comprehensive Analysis of Affymetrix Exon Arrays Using BioConductor, PLoS CompBiol, 2008

Normalization of Affymetrix Arrays by RMA


Background correction

Normalization b/w arrays

Estimate expression

Background and signal are strictly positive.
Noise is additive in log scale:

$$PM_{ij} = \underset{\text{exponential}}{Sijn} + \overset{\text{normal}}{Bijn}$$

Quantile **normalization** b/w arrays: makes distribution of probes the same across all arrays

Probeset expression is estimated from a linear model:

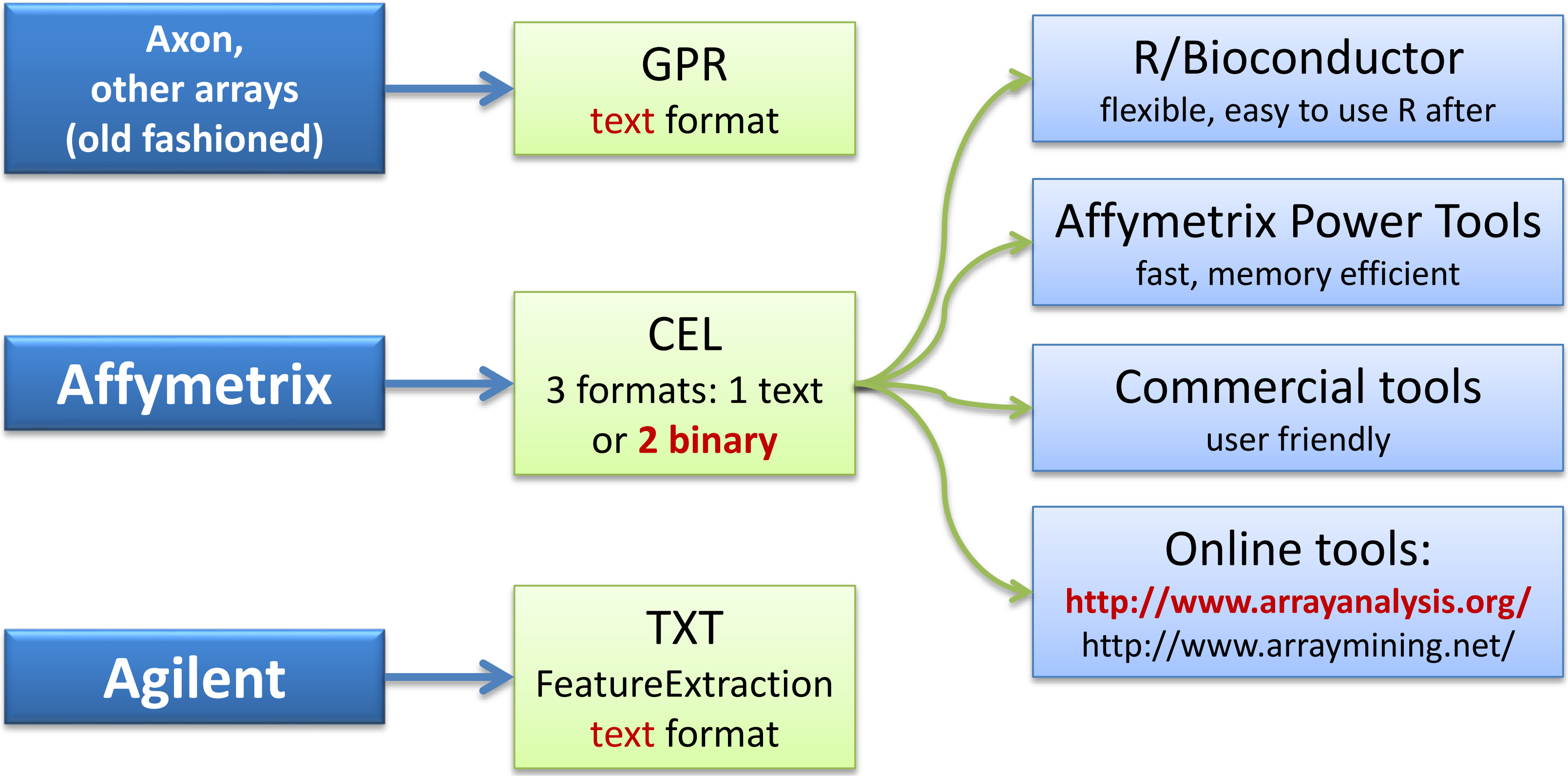
$$\underset{\text{observed}}{Y_{ijn}} = \overset{\text{expression}}{\mu_{in}} + \underset{\text{probe affinity}}{\alpha_{jn}} + \overset{\text{error with 0 mean}}{\varepsilon_{ijn}}$$

i – array
j -- probe
n -- probeset

“Median polish” helps avoid outliers effect

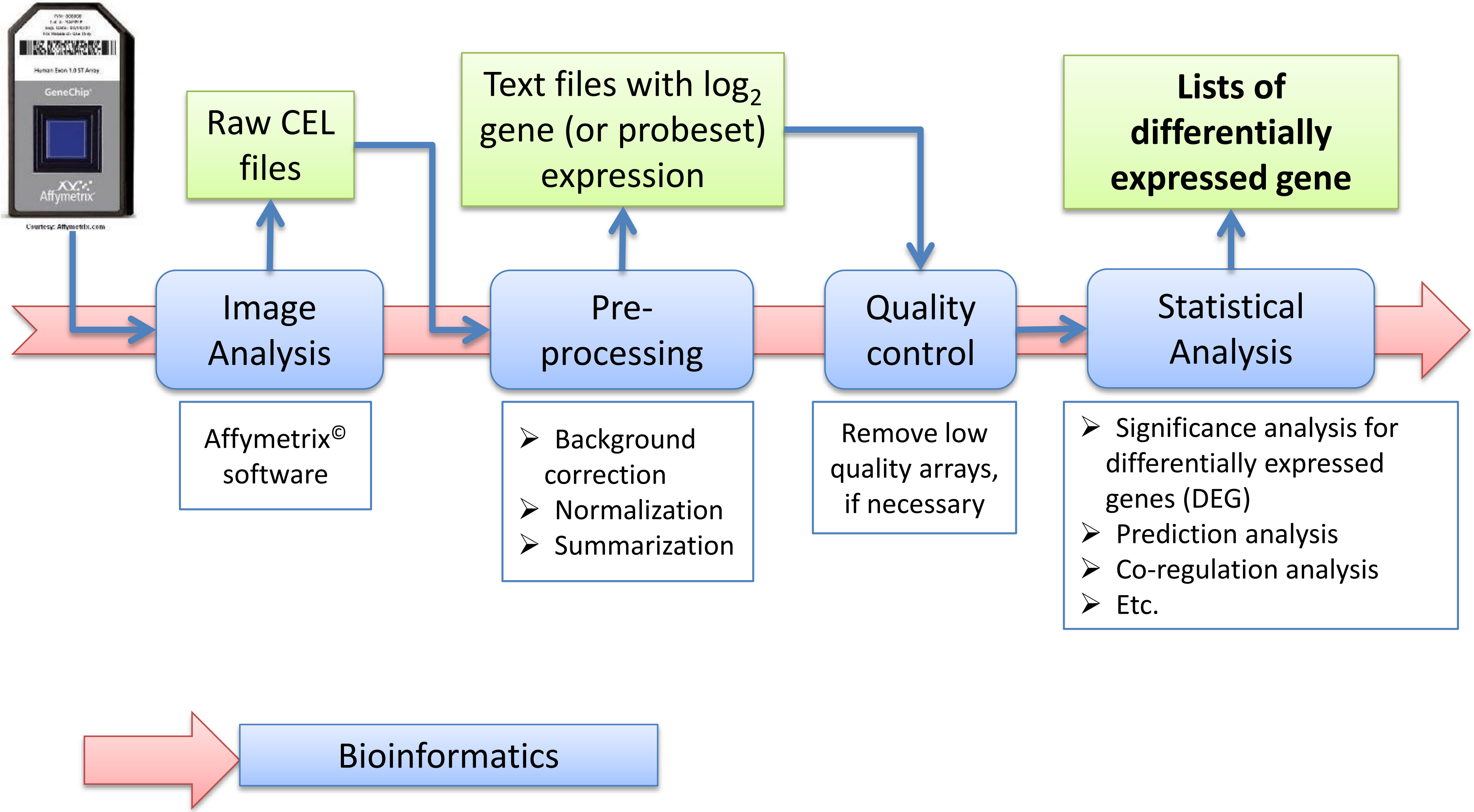
Microarray Data

File Formats



Microarray Data

Analysis Pipeline



Raw CEL files

Text files with $\log_2$ gene (or probeset) expression

Lists of differentially expressed gene

Image Analysis

Pre-processing

Quality control

Statistical Analysis

Affymetrix© software

- Background correction
- Normalization
- Summarization

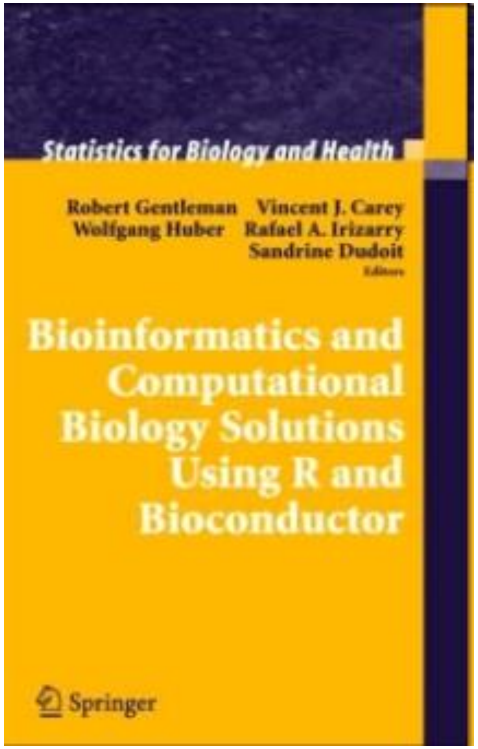
Remove low quality arrays, if necessary

- Significance analysis for differentially expressed genes (DEG)
- Prediction analysis
- Co-regulation analysis
- Etc.

Bioinformatics

L6.3. Microarray Data Analysis

R / Bioconductor

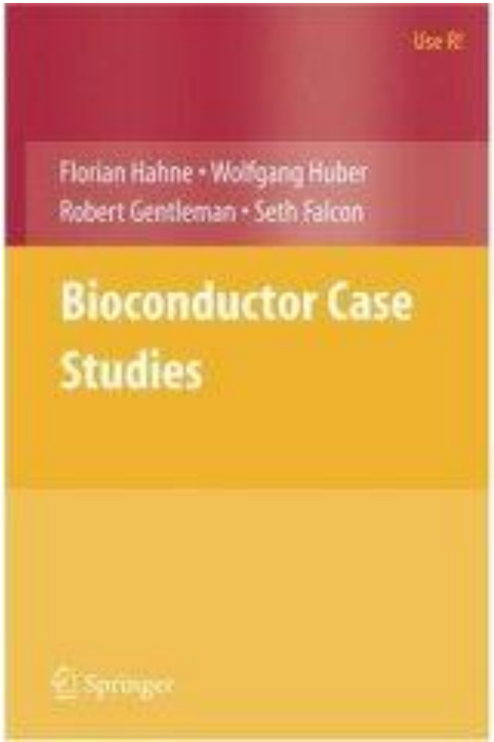


Statistics for Biology and Health

Robert Gentleman Vincent J. Carey
Wolfgang Huber Rafael A. Irizarry
Sandrine Dudoit
Editors

Bioinformatics and Computational Biology Solutions Using R and Bioconductor

Springer



Use R!

Florian Hahne · Wolfgang Huber
Robert Gentleman · Seth Falcon

Bioconductor Case Studies

Springer

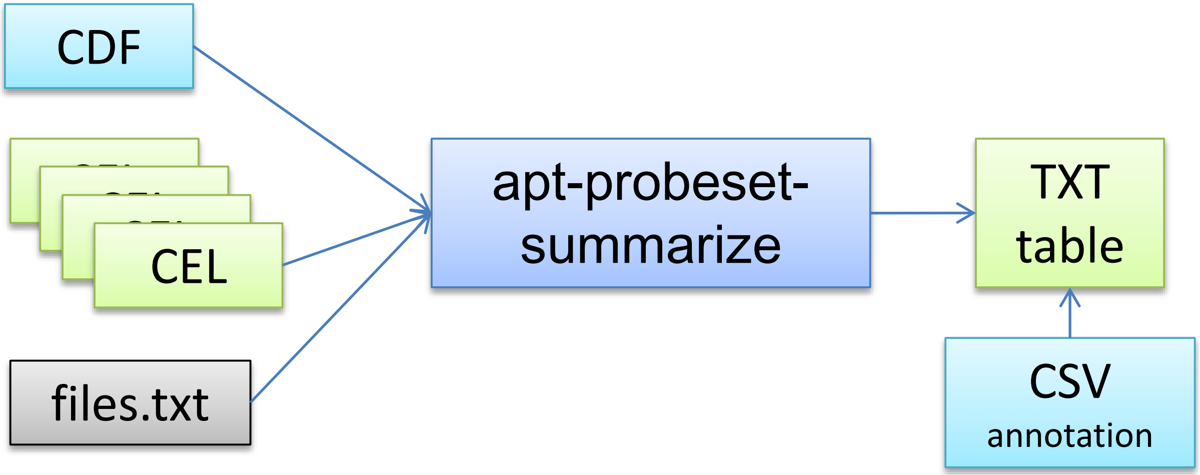
Affymetrix Power Tools

apt-probeset-summarize is a program for doing background subtraction, normalization and summarizing probe sets from Affymetrix expression microarrays. It implements analysis algorithms such as RMA, Plier, and DABG (detected above background).

The main features of **apt-probeset-summarize** not common in other implementations are: Quantile normalization using a subset (sketch) of the data which results in much smaller memory usage.

http://www.affymetrix.com/support/developer/powertools/changelog/apt-probeset-summarize.html

```
apt-probeset-summarize
    -a rma-sketch -d chip.cdf -o output-dir  --cel-files  files.txt
```



http://edu.sablab.net/data/gz/

L6.3. Microarray Data Analysis

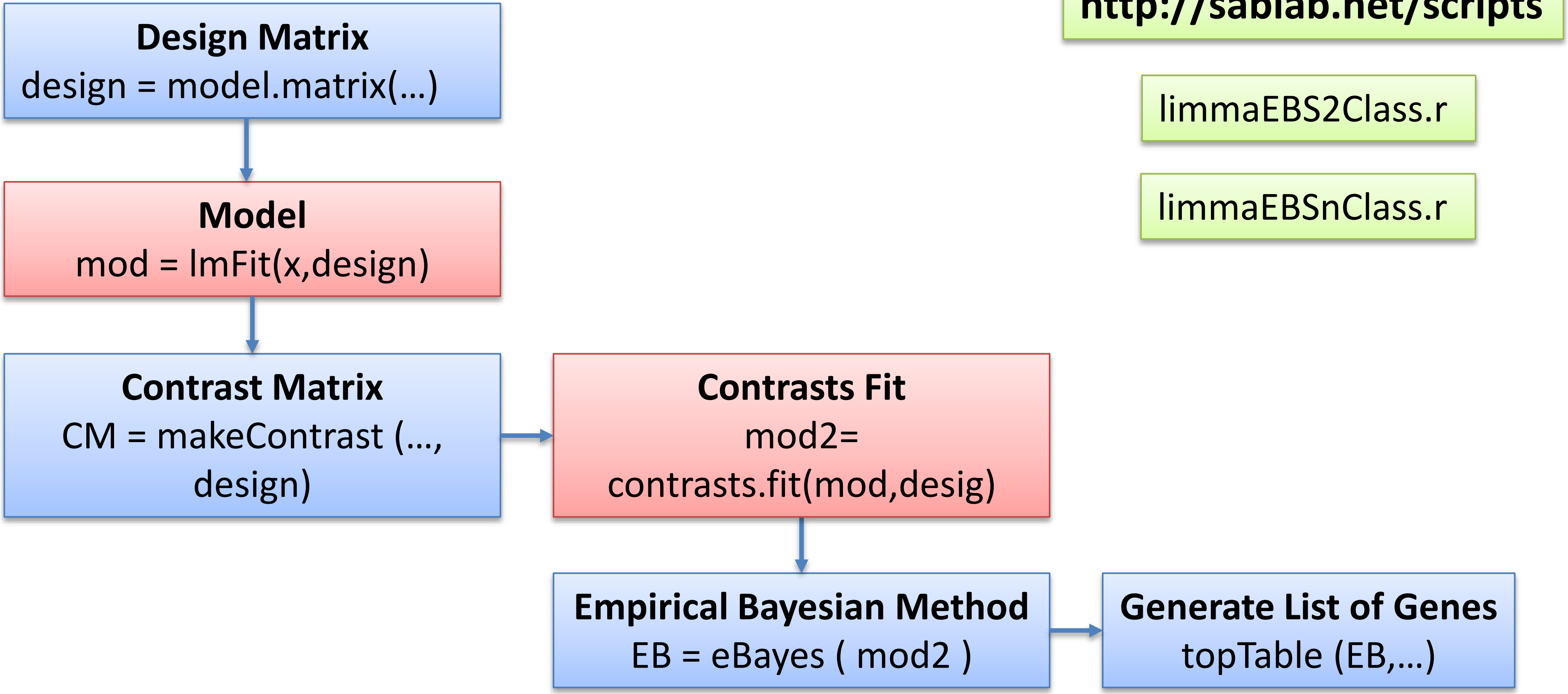
Differential Expression Analysis

Factorial design

http://sablab.net/scripts

limmaEBS2Class.r

limmaEBSnClass.r



Design Matrix
design = model.matrix(…)

Model
mod = lmFit(x,design)

Contrast Matrix
CM = makeContrast (…, design)

Contrasts Fit
mod2= contrasts.fit(mod,desig)

Empirical Bayesian Method
EB = eBayes (mod2)

Generate List of Genes
topTable (EB,…)

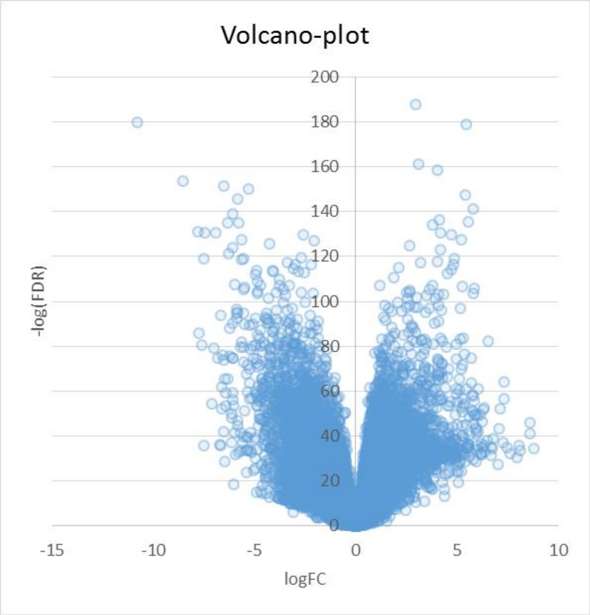
Differential Expression Analysis

http://edu.sablab.net/data/txt/lusc.zip

1. Find genes significantly differentially expressed in SCC vs normal tissue
   - apply ***limma***
   - Keep genes with FDR > 0.001
   - keep only genes with |logFC| > 2

2. Make a "volcano plot":
   -log10(FDR) vs LogFC

3. Save lists of up and down regulate genes – we shall need them



http://sablab.net/scripts

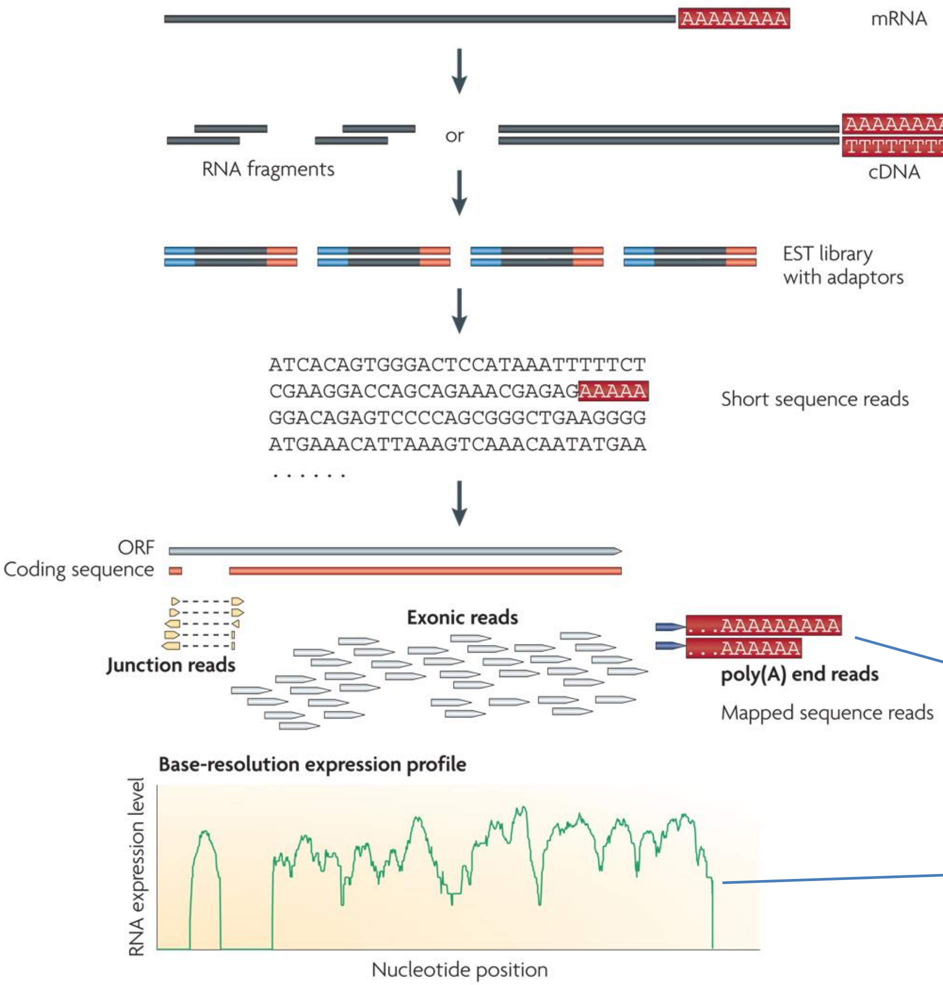
L6.3. Microarray Data Analysis

```
################################################################################
# L6.2. Import and Analysis
################################################################################
## clear memory
rm(list = ls())

##------------------------------------------------------------------------------
## L6.2.1. Loading results after APT and QC
##------------------------------------------------------------------------------

## load data after APT
## alternative: http://edu.sablab.net/data/gz/rma-sketch.summary.txt
Data = read.table("e:/data/kreis/+data+/miR.pub/cel/res/rma-sketch.summary.txt",
                  header=T, sep="\t", as.is=T)

## load sample description
## alternative: http://edu.sablab.net/data/gz/Affymetrix_miRNA2.txt
Meta= read.table("e:/data/kreis/+data+/miR.pub/cel/files.txt",
                  header=T, sep="\t", as.is=T)
str(Data)
Meta

## keep only human miRNA
Data = Data[grep("hsa-",Data$probeset_id),]

# if order of Data columns and Meta rows are the same - simply change columns
if (sum(names(Data[,-1])!= Meta[,1]) == 0) names(Data)[-1] = Meta[,2]

source("http://sablab.net/scripts/plotDataPDF.r")
x11()
plotDataPDF(Data,add.legend=T,col=rainbow(ncol(Data)))
x11()
boxplot(Data[,-1],outline=F,col=rainbow(ncol(Data)),las=2)
```

```
##------------------------------------------------------------------------------
## L6.2.2 Analysis
##------------------------------------------------------------------------------

## let's filter out miR with low expression
## and put the rest into matrix Y
thr = 3
idx.keep = logical(nrow(Data))|T
idx.keep[apply(Data[,-1],1,max)<=thr]=F
sum(idx.keep)

## Y contains now the data
Y = as.matrix(Data[idx.keep,-1])
colnames(Y) = names(Data)[-1]
rownames(Y) = sub("_st","",Data[idx.keep,1])
str(Y)

## plot heatmap of scaled data
heatmap(t(scale(t(Y))))

## plot PCA
PC = prcomp(t(Y))
## plot 3D
library(rgl)
plot3d(PC$x[,1],PC$x[,2],PC$x[,3],
       size = 2,
       col  = rainbow(ncol(Data)),
       type = "s",
       xlab = "PC1",
       ylab = "PC2",
       zlab = "PC3")
text3d(PC$x[,1]+0.5,PC$x[,2]+0.5,PC$x[,3]+0.5,colnames(Y))

## DEA
source("http://sablab.net/scripts/limmaEBS2Class.r")

idx=c(grep("T000",colnames(Y)),
      grep("T48",colnames(Y)))
res=limmaEBS2Class(Y[,idx],rownames(Y),classes=c("T00","T00","T48","T48"),
              plotTop=20)
```

RNASeq

Next Generation Sequencing: RNA-Seq



raw counts

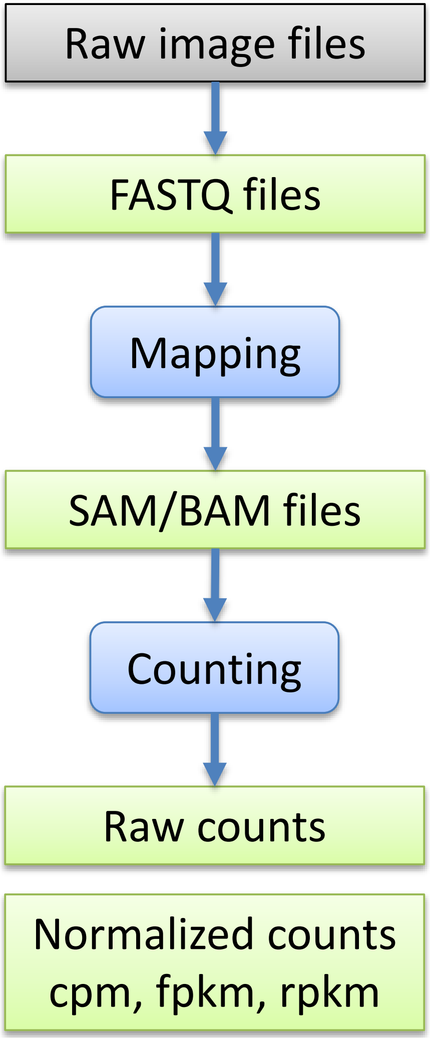
normalized counts:
CPM, fpkm, rpkm

coverage

Wang Z et al. RNA-Seq: a revolutionary tool for transcriptomics. **Nat Rev Genet. 2009**

6.4. RNA-Seq Data

File Types



```
@HWI-ST508:152:D06G9ACXX:2:1101:1160:2042 1:Y:0:ATCACG
NAAGACCGAATTCTCCAAGCTATGGTAAACATTGCACTGGCCTTTCATCTG
+
#11??+2<<<CCB4AC?32@+1@AB1**1?AB<4=4>=BB<9=>?######
```

Read – a short sequence identified in RNA-Seq experiment

Library – set (10^5 – 10^8) of reads from a single sample

```
@HD         VN:1.0 SO:coordinate
@SQ         SN:seq1 LN:5000
@SQ         SN:seq2     LN:5000
@CO         Example of SAM/BAM file format.

B7_591:4:96:693:509 73   seq1       1          99         36M        *
            0          0          CACTAGTGGCTCATTGTAAATGTGTGGTTTAACTCG
                                  <<<<<<<<<<<<<<<;<<<<<<<<<5<<<<<;:<;7
            MF:i:18    Aq:i:73    NM:i:0     UQ:i:0     H0:i:1

H1:i:0EAS54_65:7:152:368:113      73         seq1       3          99
            35M        *          0          0
            CTAGTGGCTCATTGTAAATGTGTGGTTTAACTCGT
            <<<<<<<<<<0<<<<655<<7<<<:9<<3/:<6): MF:i:18    Aq:i:66
            NM:i:0     UQ:i:0     H0:i:1     H1:i:0
```

For the list of tools see:
http://en.wikipedia.org/wiki/List_of_RNA-Seq_bioinformatics_tools

6.4. RNA-Seq Data

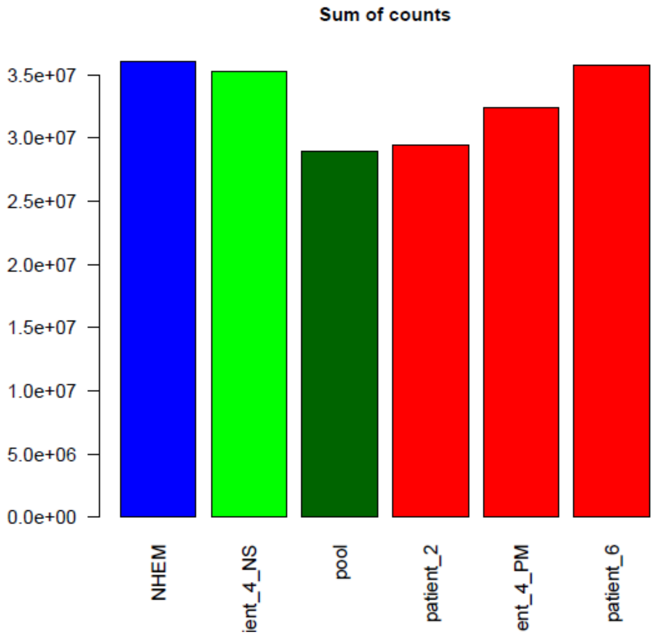
Normalization

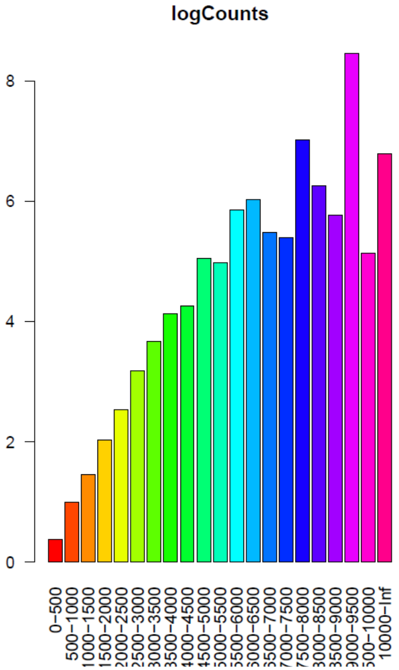
Problems:

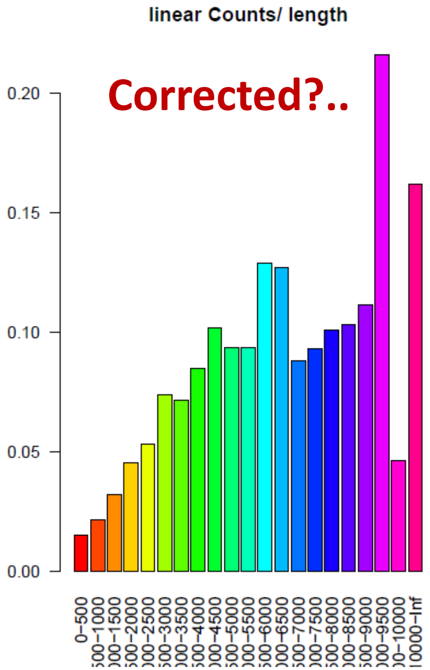
- Libraries has different size (different number of reads from samples)
- Long transcripts produce more reads

Solutions (?) :

- Accounting for library size during analysis (standard) or direct correction for it
- Correction for transcript size (but which transcript is expressed?)



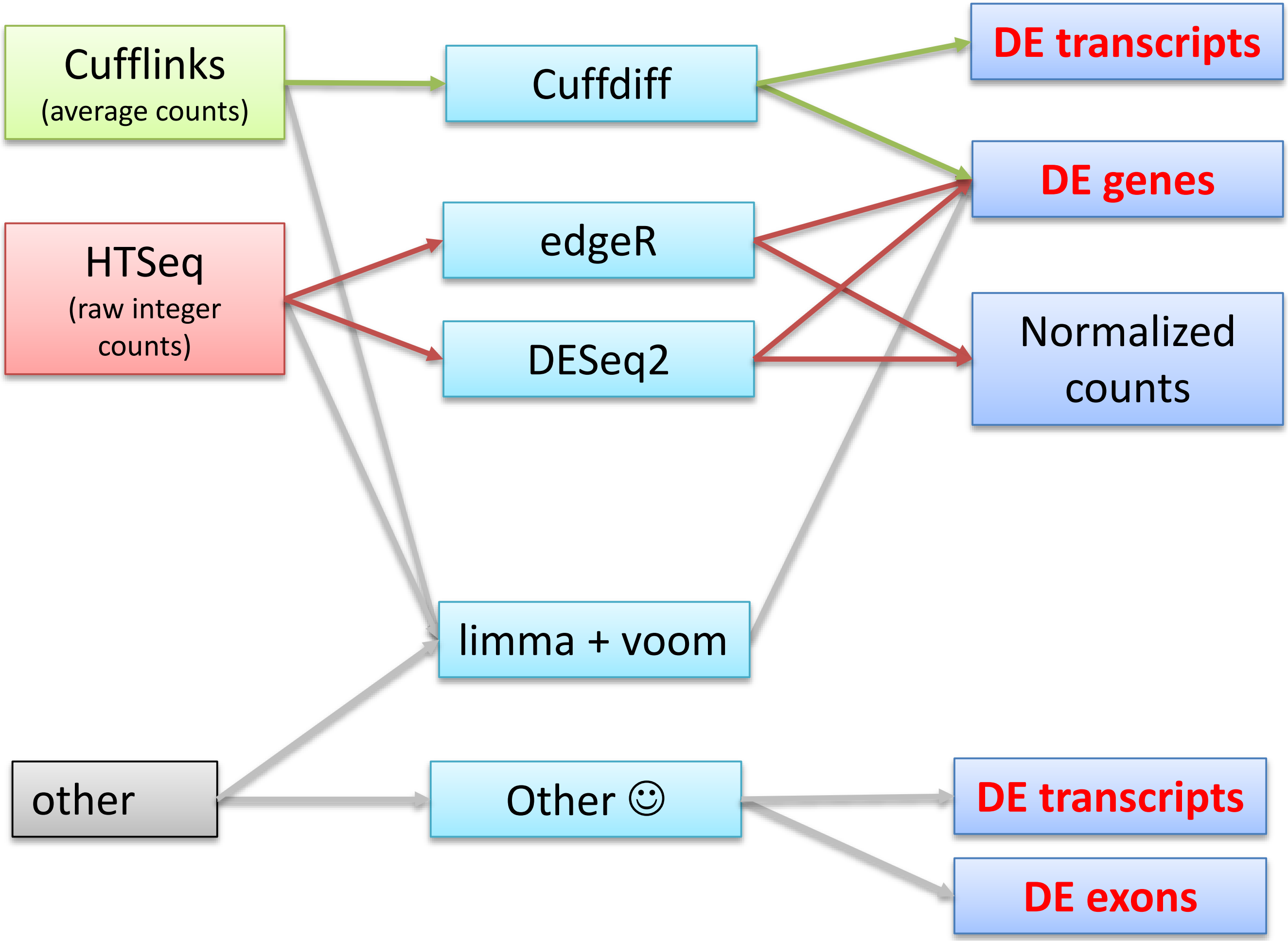




Corrected?..

6.4. RNA-Seq Data

Differential Expression Analysis



UNIVERSITÉ DU LUXEMBOURG

L6.4. RNASeq Data

LUXEMBOURG INSTITUTE OF HEALTH
RESEARCH DEDICATED TO LIFE

Differential Expression Analysis (edgeR)

htts://sablab.net/scripts

LibDEA.r

Differential Expression Analysis (DESeq2)

htts://sablab.net/scripts

LibDEA.r

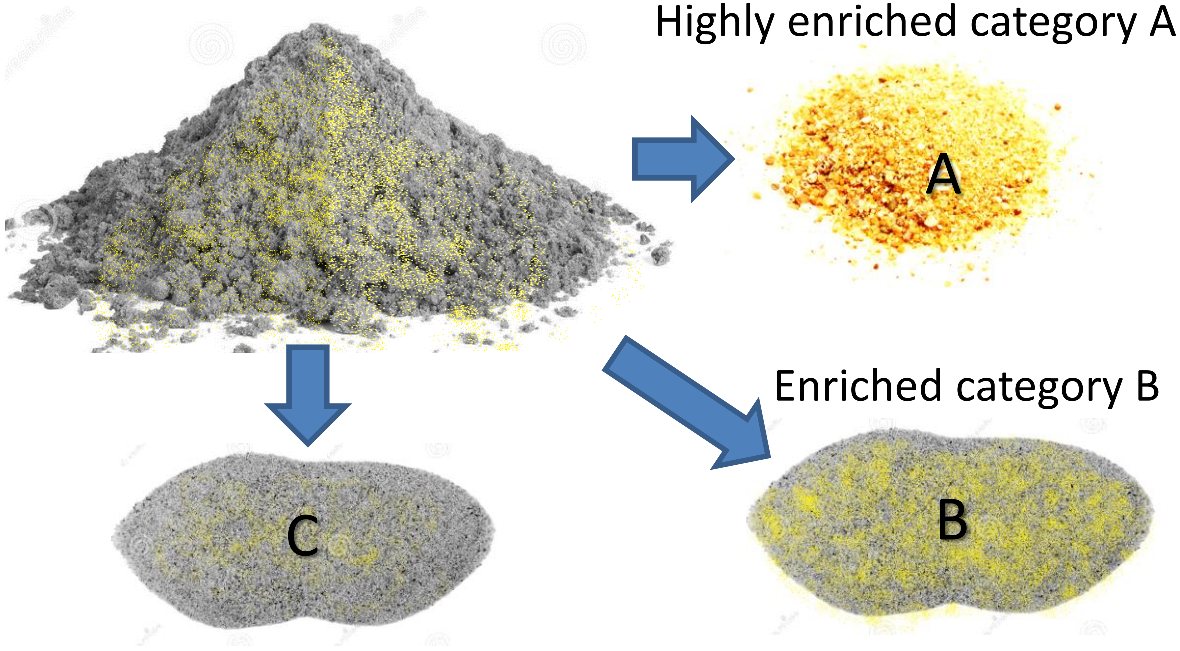
Enrichment Analysis

L6.5. Enrichment Analysis

1. Category Enrichment Analysis

Are interesting genes overrepresented in a subset corresponding to some biological process?

Highly enriched category A



Enriched category B

No enrichment in C

Method of the analysis:
Fisher’s exact test

Someone grabs “randomly” 20 balls from a box with 100x 🔴 and 100x 🟢

How surprised will you be if he grabbed

🔴🔴🔴🟢🔴🔴🔴🟢🔴🔴🔴🔴🔴🔴🔴🔴🔴🟢🔴🔴

(17 red , 3 green)

sand belongs to: http://www.dreamstime.com/photos-images/pile-sand.html ;)))

L6.5. Enrichment Analysis

1. Category Enrichment Analysis

Fisher’s exact test: based on hypergeometrical distributions

Hypergeometrical: distribution of objects taken from a “box”, without putting them back

$$P = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

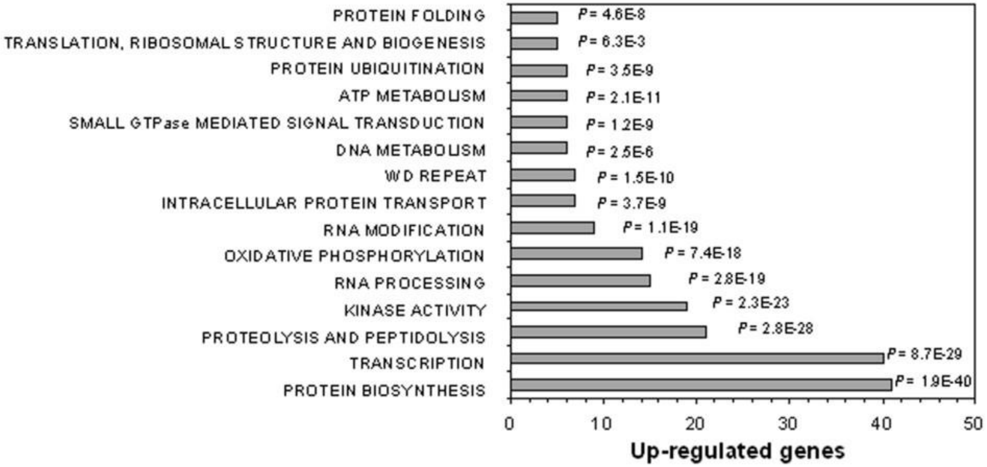
N: total number of genes

M: total number of genes annotated with this term

n: number of genes in the list

k: number of genes in the list annotated with this term

$$C_k^n = C_n^k = \binom{n}{k} = \frac{n!}{k!\,(n-k)!}$$

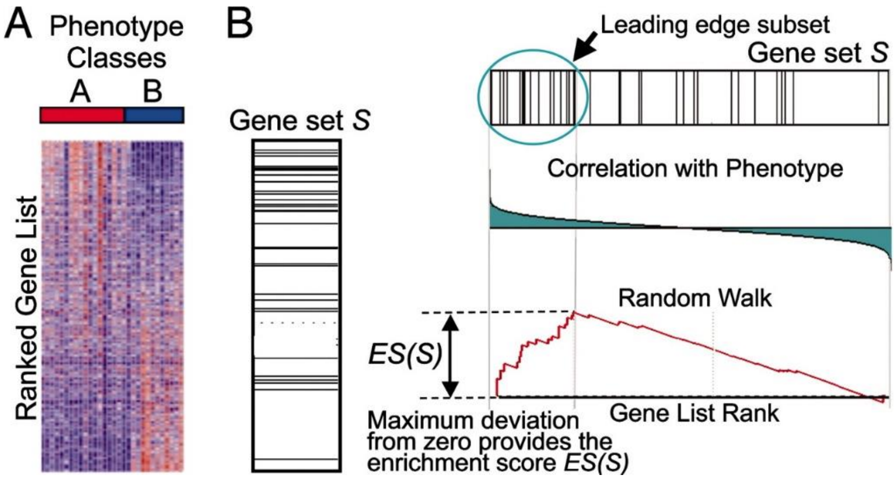


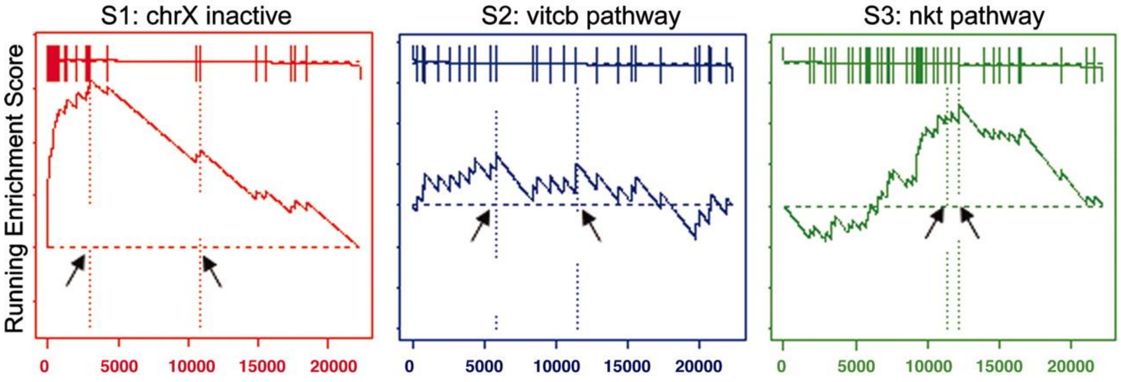
Okamoto et al. Cancer Cell International 2007 7:11 doi:10.1186/1475-2867-7-11

L6.5. Enrichment Analysis

2. Gene Set Enrichment Analysis (GSEA)

Is direction of genes in a category random?





A. Subramanian et al. PNAS **2005**,102,43

L6.5. Enrichment Analysis

Example: GO enrichment

http://edu.sablab.net/transcript

Strategy 1:

Take all DEG and use them in enrichment.

- Safe
- No additional assumptions
- Cannot distinguish ↑ and ↓ functions

Strategy 2:

Separate DEG to down- and up- regulated genes. Then perform independent enrichment by these 2 groups

- Can be biased (gene can be ↑↓)
- Assume ↑gene => ↑function
- Can distinguish ↑ and ↓ functions

Enrichr
http://amp.pharm.mssm.edu/Enrichr/enrich

BioCompendium
http://biocompendium.embl.de/

L6.5. Enrichment Analysis

LUSC Example

http://edu.sablab.net/data/txt/lusc.zip

http://amp.pharm.mssm.edu/Enrichr/

http://biocompendium.embl.de/

http://string-db.org

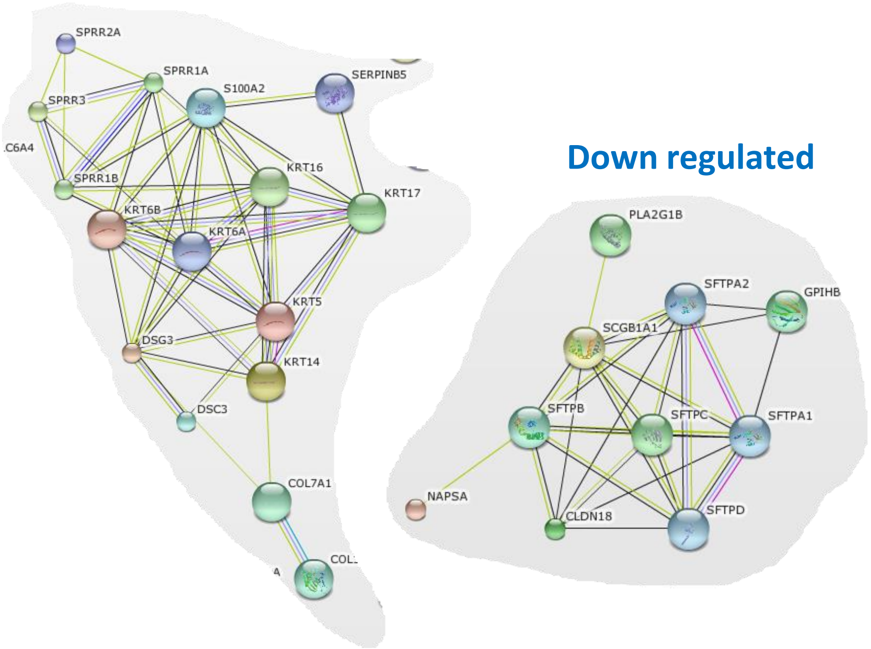
0. Prepare lists of DE genes…

1. Put up-regulated into **enrich**

3. Check: Down CMAP, Disease Signatures from GEO up,

4. Try **biocompendium**

5. Put top 100 genes into String to see PP-interactions



Up regulated

Down regulated

L6.5. Enrichment Analysis

In R

```
#########################################################################################
## enrichGOens - warpup for topGO package: enrichment analysis of GO-terms
## based on Ensembl IDs
##-----------------------------------------------------------------------------
## genes   - vector with list of ENSEBML IDs (character)
## fdr     - vector of FDR for each gene (numeric)
## fc      - vector of logFC for each gene (numeric)
## thr.fdr - significance threshold for FDR (numeric)
## thr.fc  - significance threshold for absolute logFC (numeric)
## db      - name of GO database: "BP","MF","CC" (character)
## genome  - R-package for genome annotation used. For human - 'org.Hs.eg.db' (character)
## do.sort - if TRUE - resulted functions sorted by p-value (logical)
## randomFraction - for testing only, the fraction of the genes to be randomized (numeric)
##-----------------------------------------------------------------------------
## (c)GNU GPL P.Nazarov 2014. petr.nazarov[at]crp-sante.lu
#########################################################################################
```

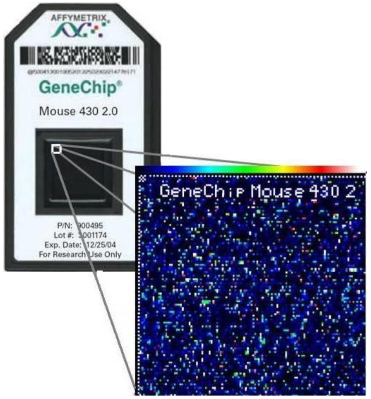
htts://sablab.net/scripts

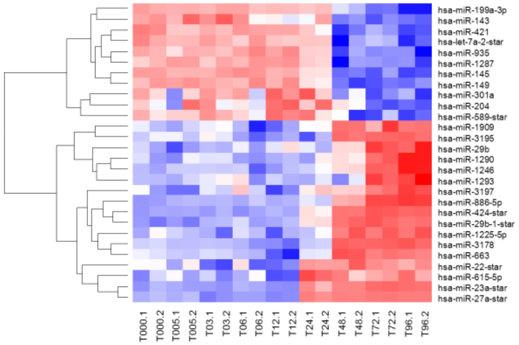
enrichGOens.r

```
enrichGOens =
function(genes,fdr,fc,thr.fdr=0.05,thr.fc=0,db="BP",genome="org.Hs.eg.db",do.sort=TRUE,
         randomFraction=0){
  ## load libraries
  if (!require(genome,character.only=TRUE)){
    cat("MESSAGE enrichGO: '",genome,"' package is not found. Installing...\n",sep="")
    source("http://bioconductor.org/biocLite.R")
    biocLite(genome)
    library(genome,character.only=TRUE)
  }
  if (!require("topGO")){
    cat("MESSAGE enrichGO: ' topGO ' package is not found. Installing...\n")
    source("http://bioconductor.org/biocLite.R")
    biocLite("topGO")
    library("topGO")
  }
  if (!exists("sortDataFrame")) source("http://sablab.net/scripts/sortDataFrame.r")
  ## prepare gene categories and score
  myGO2genes <- annFUN.org(db, mapping = "org.Hs.eg.db", ID = "ensembl")
  score = (-log10(fdr)*abs(fc))
  names(score)=genes
  score[fdr>=thr.fdr | abs(fc)<=thr.fc]=0

  ## add randomness if required, to test stability
  if (randomFraction>0){
    ## define remove probability: low score have more chances
    prob1 = 1/(1+score)
    prob1[is.na(prob1)]=0
    prob1[score == 0] = 0
    ## define add probability: high score has more chances
    prob2 = -log10(fdr)*abs(fc)
    prob2[is.na(prob2)]=0
    prob2[score > 0] = 0
    ## add and remove
    n=round(sum(score>0)*randomFraction)
    score[sample(1:length(genes),n,prob=prob2)]=1+rexp(n,1/mean(score[score>0]))
    score[sample(1:length(genes),n,prob=prob1)]=0
  }
  ## create topGOdata object
  SelectScore = function(sc){return(sc>0)} ## simple function for significance
  GOdata = new("topGOdata",  ##constructor
              ontology = db,
              allGenes = score,
              geneSelectionFun = SelectScore,
              annot = annFUN.GO2genes,
              GO2genes = myGO2genes)
  ## run testing
  resFisher = runTest(GOdata, algorithm = "classic", statistic = "fisher")
  ## transform results into a table
  enrichRes = GenTable(GOdata, classicFisher = resFisher,
              ranksOf = "classicFisher",topNodes = length(resFisher@score))
  enrichRes$classicFisher[grep("<",enrichRes$classicFisher)] = "1e-31"
  enrichRes$classicFisher = as.double(enrichRes$classicFisher)
  enrichRes$FDR = p.adjust(enrichRes$classicFisher,"fdr")
  enrichRes$Score = -log10(enrichRes$FDR)
  ## by default sorted by p-value. If needed - sort by ID
  if (!do.sort) enrichRes = sortDataFrame(enrichRes,"GO.ID") ## remove sorting
  return(enrichRes)
}
```

Questions ?

Thank you for your attention !




hsa-miR-199a-3p
hsa-miR-143
hsa-miR-421
hsa-let-7a-2-star
hsa-miR-935
hsa-miR-1287
hsa-miR-145
hsa-miR-149
hsa-miR-301a
hsa-miR-204
hsa-miR-589-star
hsa-miR-1909
hsa-miR-3195
hsa-miR-29b
hsa-miR-1290
hsa-miR-1246
hsa-miR-1293
hsa-miR-3197
hsa-miR-886-5p
hsa-miR-424-star
hsa-miR-29b-1-star
hsa-miR-1225-5p
hsa-miR-3178
hsa-miR-663
hsa-miR-22-star
hsa-miR-615-5p
hsa-miR-23a-star
hsa-miR-27a-star
T000.1
T000.2
T005.1
T005.2
T03.1
T03.2
T06.1
T06.2
T12.1
T12.2
T24.1
T24.2
T48.1
T48.2
T72.1
T72.2
T96.1
T96.2