An Introduction to R

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Microarray Analysis using R **and Bioconductor**
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Plan

Plan

Today's topics

- ▶ The command line interface is your friend
- ▶ Reading/writing code (you will have to teach yourself programming, through practice)
- ▶ Today, I will concentrate on **data** (create and manipulate)
- ▶ R - the environment and the language

What is R?

- An <u>interactive</u> statistical environment
- A programming language
- A language and associated tools for reproducible research (these slides for example)

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- An <u>interactive</u> statistical environment
- A programming language
- A language and associated tools for reproducible research (these slides for example)

- Open source and cross platform (GNU/Linux, Windows, Mac and others)
- Stable (currently 3.0) and development versions.
- Extensive graphics capabilites
- Diverse range of add-on packages
- Active community of developers
- Thorough documentation

The R Project for Statistical Computing
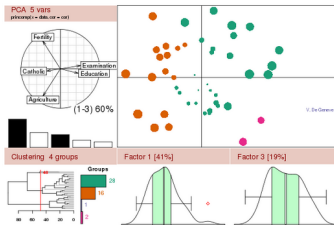
Getting Started:

- R is a free software environment for statistical computing and graphics. It compiles and runs on a wide variety of UNIX platforms, Windows and MacOS. To **download R**, please choose your preferred CRAN mirror.
- If you have questions about R like how to download and install the software, or what the license terms are, please read our answers to frequently asked questions before you send an email.

News:

- **R version 3.0.0** (Masked Marvel) has been released on 2013-04-03.
- **R version 2.15.3** (Security Blanket) has been released on 2013-03-01.
- **The R Journal Vol.4/2** is available.
- **useR! 2012**, took place at Vanderbilt University, Nashville Tennessee, USA, June 12-15, 2012.
- **useR! 2013**, will take place at the University of Castilla-La Mancha, Albacete, Spain, July 10-12 2013. .
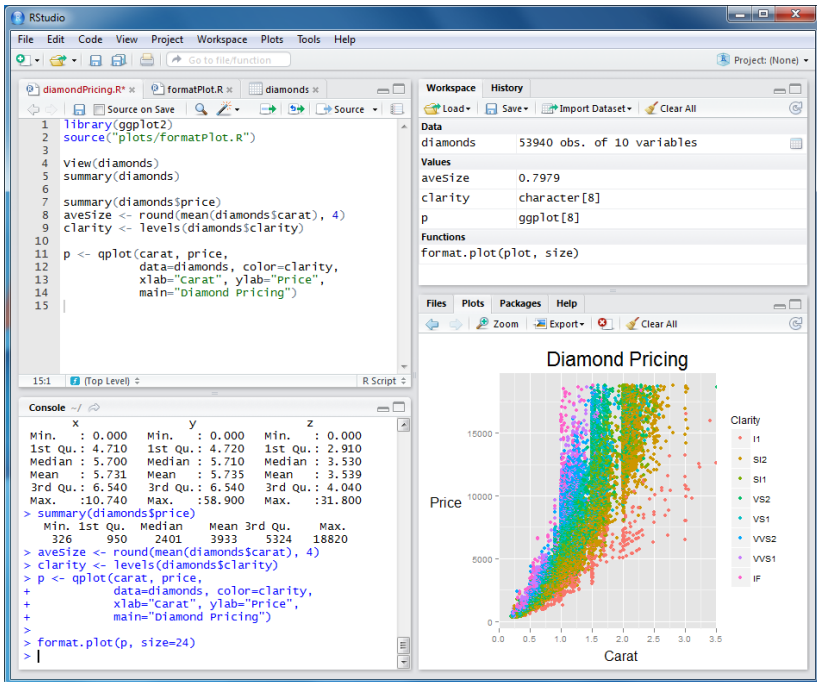
This server is hosted by the Institute for Statistics and Mathematics of WU (Wirtschaftsuniversität Wien).

What is needed:

- The R console
- An editor

We will use:

- RStudio IDE

RStudio

File Edit Code View Project Workspace Plots Tools Help

Go to file/function
Project: (None)

diamondPricing.R* ×   formatPlot.R ×   diamonds ×

Source on Save   Source

```
1  library(ggplot2)
2  source("plots/formatPlot.R")
3
4  View(diamonds)
5  summary(diamonds)
6
7  summary(diamonds$price)
8  aveSize <- round(mean(diamonds$carat), 4)
9  clarity <- levels(diamonds$clarity)
10
11 p <- qplot(carat, price,
12            data=diamonds, color=clarity,
13            xlab="Carat", ylab="Price",
14            main="Diamond Pricing")
15 |
```

15:1   (Top Level)   R Script

Console ~/

```
         x                y                z
 Min.   : 0.000   Min.   : 0.000   Min.   : 0.000
 1st Qu.: 4.710   1st Qu.: 4.720   1st Qu.: 2.910
 Median : 5.700   Median : 5.710   Median : 3.530
 Mean   : 5.731   Mean   : 5.735   Mean   : 3.539
 3rd Qu.: 6.540   3rd Qu.: 6.540   3rd Qu.: 4.040
 Max.   :10.740   Max.   :58.900   Max.   :31.800
> summary(diamonds$price)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
    326     950    2401    3933    5324   18820
> aveSize <- round(mean(diamonds$carat), 4)
> clarity <- levels(diamonds$clarity)
> p <- qplot(carat, price,
+            data=diamonds, color=clarity,
+            xlab="Carat", ylab="Price",
+            main="Diamond Pricing")
>
> format.plot(p, size=24)
> |
```

Workspace   History

Load   Save   Import Dataset   Clear All

Data
diamonds   53940 obs. of 10 variables
Values
aveSize   0.7979
clarity   character[8]
p   ggplot[8]
Functions
format.plot(plot, size)

Files Plots Packages Help

Zoom   Export   Clear All

Diamond Pricing

Clarity
I1
SI2
SI1
VS2
VS1
VVS2
VVS1
IF

Plan

Hello woRld

```
> 5

[1] 5

> 2 + 2

[1] 4

> sin(pi/2)

[1] 1
```

```
> x <- 1  ## a variable
> x

[1] 1

> x = 2  ## overwrite the content of x
> x

[1] 2

> y <- length(x)  ## calling a function
> y

[1] 1

> y + 2

[1] 3
```

```
> ## just a comment
> ls()

[1] "x" "y"

> rm(y)
> ls()

[1] "x"

> rm(list = ls())
> ls()

character(0)
```

The working directory

```
> getwd()

[1] "/home/lgatto/Documents/Teaching/Rteaching/RIntro_1day"

> setwd("/home/lgatto/tmp")
> getwd()

[1] "/home/lgatto/tmp"
```

(or use the GUI in RStudio)

Functions: `fname(argument)`

```
> floor(2.3)

[1] 2

> sum(3, 4, 10)

[1] 17

> max(3, 10, 1, -0.2)

[1] 10

> mean(3, 4, 5, 6)  ## !

[1] 3
```

Getting help

- ▶ Just ask!
- ▶ `help.start()` and the HTML help button in the Windows GUI.
- ▶ `help` and `?`: `help("sin")` or `?sin`.
- ▶ `??`, `help.search`, `apropos`.
- ▶ Online manuals and mailing lists.
- ▶ Vignettes

- ▶ Local R user groups

Exercise 1:
In the interactive R console, calculate the following expressions,
where x and y have values -0.25 and 2 respecively. Then store
the result in a new variable and print its content.

```
> x + cos(pi/y)
```

Same, as above, but writing the code in an R source code file using
the editor. Then, clean your working environment (delete all your
variables) and execute the content of that file.

New functions: print to explicitly print to the console and
source to execute the content of a file.

Plan

Atomic vectors

```
> 1

[1] 1

> c(1, 4, 7, 10)  ## concatenate

[1]  1  4  7 10

> c(0.001, 1000, 9, -1)

[1]  1e-03  1e+03  9e+00 -1e+00
```

A `vector` contains an indexed set of values

- index starts at 1
- all items are of the same *kind* (see later)

Back to our *mean* issue . . .

```
> mean(3, 4, 5, 6)  ## 4 arguments

[1] 3

> mean(c(3, 4, 5, 6))  ## 1 argument

[1] 4.5
```

Functions to create vectors: constructors with default values

```
> vector(mode = "numeric", length = 4)

[1] 0 0 0 0

> numeric(4)

[1] 0 0 0 0
```

Functions to create vectors: seq

```
> 1:5

[1] 1 2 3 4 5

> seq(from = 1, to = 10, by = 2)

[1] 1 3 5 7 9

> seq(from = 1, to = 10, length.out = 4)

[1]  1  4  7 10
```

More functions to create vectors: `rep`

```
> rep(1, 5)

[1] 1 1 1 1 1

> rep(1:3, 2)

[1] 1 2 3 1 2 3

> rep(1:3, each = 2)

[1] 1 1 2 2 3 3
```

Arguments by position or name

```
> (z1 <- seq(from = 1, to = 10, by = 2))

[1] 1 3 5 7 9

> z2 <- seq(1, 10, 2)
> z3 <- seq(to = 10, by = 2, from = 1)
> identical(z1, z2) ## returns a logical

[1] TRUE

> identical(z1, z3)

[1] TRUE
```

Subsetting

The [operator

```
> x <- 1:10
> x[4:5]

[1] 4 5

> x[seq(1, 10, 3)]

[1]  1  4  7 10

> x[c(7, 1)]

[1] 7 1
```

Negative indices in [

```
> x <- 1:10
> x[-(1:5)]  ## ? -1:5

[1]  6  7  8  9 10

> x[-seq(1, 10, 3)]

[1] 2 3 5 6 8 9
```

Out of range indices

```
> x <- 1:5
> x[5:6]

[1]  5 NA

> x[0:1]

[1] 1
```

Replacement with [

```
> (x <- 1:10)

 [1]  1  2  3  4  5  6  7  8  9 10

> x[1] <- 100
> head(x)

[1] 100   2   3   4   5   6

> x[1:5] <- 0
> x[4:8]

[1] 0 0 6 7 8
```

Vectorised arithmetic

```
> x <- 1:5
> y <- 5:1
> x

[1] 1 2 3 4 5

> y

[1] 5 4 3 2 1

> x + y

[1] 6 6 6 6 6

> x^2

[1]  1  4  9 16 25
```

Vectorised arithmetic: recycling rule

```
> x <- 1:10
> x + 1:2

 [1]  2  4  4  6  6  8  8 10 10 12

> x + 1:3

Warning:  longer object length is not a multiple of
shorter object length

 [1]  2  4  6  5  7  9  8 10 12 11
```

Modes and types

```
> a <- 10
> a <- "10"
> a <- b
> a <- "b"
```

Modes and types

```
> a <- 10
> a <- "10"
> a <- b
> a <- "b"
```

modes

- ► logical, numeric and character
- ► mode()

types

- ► logical, integer, double, character
- ► typeof()

class

- ► logical, integer, numeric, character and many more
- ► class()

```
> x <- 1; y <- "1"; z <- as.integer(x)
> class(x)

[1] "numeric"

> class(y)

[1] "character"

> class(z)

[1] "integer"
```

```
> x <- 1; y <- "1"; z <- as.integer(x)
> x + z

[1] 2

> x + y

Error:  non-numeric argument to binary operator

> x == z

[1] TRUE
```

Exercise 2:
Create vectors i, l, s and d of type `integer`, `logical`, `character` and `double` respectively.

Hints
For example, use `sample` to create a sequence of integers, the built-in `letters` character variable, `runif` to generate doubles and a logical operator (==, >, <=, ...) to create logicals.

See `Exercise-02.R` for a solution.

Matrices are 2-dimensional vectors

```
> m <- matrix(1:12, nrow = 4, ncol = 3)
> m

     [,1] [,2] [,3]
[1,]    1    5    9
[2,]    2    6   10
[3,]    3    7   11
[4,]    4    8   12

> dim(m)

[1] 4 3

> ncol(m)  ## and also nrow(m)

[1] 3
```

What if I don't get the data or dimensions right?

```
> matrix(1:11, 4, 3)

Warning:  data length [11] is not a sub-multiple or
multiple of the number of rows [4]

     [,1] [,2] [,3]
[1,]    1    5    9
[2,]    2    6   10
[3,]    3    7   11
[4,]    4    8    1

> matrix(1:12, 3, 3)

     [,1] [,2] [,3]
[1,]    1    4    7
[2,]    2    5    8
[3,]    3    6    9
```

Subsetting matrices

```
> dim(m)

[1] 4 3

> m[3:4, 2:3]

     [,1] [,2]
[1,]    7   11
[2,]    8   12

> m[1, ]

[1] 1 5 9

> m[, 1]

[1] 1 2 3 4
```

Arrays are n-dimensional vectors

```
> array(1:16, dim = c(2, 4, 2))

, , 1

     [,1] [,2] [,3] [,4]
[1,]    1    3    5    7
[2,]    2    4    6    8

, , 2

     [,1] [,2] [,3] [,4]
[1,]    9   11   13   15
[2,]   10   12   14   16
```

Lists are ordered set of arbitrary R objects.

```
> ll <- list(a = 1:3, b = letters[1:2])
> ll

$a
[1] 1 2 3

$b
[1] "a" "b"

> ll[[1]]

[1] 1 2 3

> ll$b

[1] "a" "b"
```

Dataframes are 2-dimensional list.

```
> dfr <- data.frame(type = c("A", "A", "B", "B"),
+                   time = rnorm(4))
> dfr

  type    time
1    A -0.8326
2    A -0.6693
3    B  0.8840
4    B -1.5347
```

```
> dfr[1, ]

  type     time
1    A -0.8326

> dfr[1, "time"]

[1] -0.8326

> dfr$time

[1] -0.8326 -0.6693  0.8840 -1.5347
```

Names

We have seen that function arguments have names, and named our `data.frame` columns. We can also name `matrix`/`data.frame` columns and rows, dimensions, and vector items.

```
> x <- c(a = 1, b = 2)
> x

a b
1 2

> names(x)

[1] "a" "b"
```

```
> M <- matrix(c(4, 8, 5, 6, 4, 2, 1, 5, 7), nrow=3)
> colnames(M) <- c(2005, 2006, 2007)
> rownames(M) <- c("plane", "bus", "boat")
> M

      2005 2006 2007
plane    4    6    1
bus      8    4    5
boat     5    2    7

> M[c("plane", "boat"), "2005"]

plane  boat
   4     5
```

```
> M <- matrix(c(4, 8, 5, 6, 4, 2, 1, 5, 7), nrow=3)
> dimnames(M) <- list(year =
+                     c(2005, 2006, 2007),
+                     "mode of transport" =
+                     c("plane", "bus", "boat"))
> M

      mode of transport
year   plane bus boat
  2005     4   6    1
  2006     8   4    5
  2007     5   2    7
```

Subsetting with numbers, characters, logicals

```
> x <- 1:5
> names(x) <- letters[1:5]
> x[c(1, 3)]

a c
1 3

> x[c("a", "c")]

a c
1 3

> x[c(TRUE, FALSE, TRUE, FALSE, FALSE)]

a c
1 3
```

Factors represent categorical data

```
> gender_char <- sample(c("M", "F"), 10, replace = TRUE)
> gender_fac <- factor(gender_char)
> gender_fac

 [1] M F F M M F M F M F
Levels: F M
```

Special values

```
> NULL
> is.null()
> NA
> NaN
> is.na()
> Inf
> -Inf
> is.infinite()
```

What are the mode and types of these?

Exercise 3:

How to store microarray data?

- ▶ What information do we want to store?
- ▶ How to store these individual pieces of information?
- ▶ How to store these together?

The paste function

```
> paste("A", "B", "C", sep = "-")

[1] "A-B-C"

> paste0("A", "B", "C")

[1] "ABC"
```

Normally distributed data

```
> rnorm(3)

[1] -0.02259  0.49107  0.30299

> rnorm(5, mean = 10, sd = 2)

[1]  6.867 10.826 10.558 10.136 13.307
```

The expression data

```
> expdata <- matrix(rnorm(200), nrow = 50, ncol = 4)
> dimnames(expdata) <-
+   list(features = paste0("gene", 1:nrow(expdata)),
+        samples = paste0("sample", 1:ncol(expdata)))
> head(expdata)

        samples
features  sample1 sample2  sample3 sample4
  gene1 -0.94173 -1.7384  1.43717 -0.2806
  gene2  0.88005  0.5234 -1.10106  0.7453
  gene3  0.08752 -1.5153 -0.14784 -0.6938
  gene4 -1.81514 -0.6759  0.32819 -0.9653
  gene5 -0.14522  0.2399 -0.87107 -0.2792
  gene6 -0.09888 -0.7751 -0.04283  0.2212
```

See Exercise-03.R

Sample description

```
> smdata <- data.frame(feature = colnames(expdata),
+                      group = c("ctrl", "ctrl",
+                        "cond1", "cond1"),
+                      replicate = rep(1:2, each = 2))
> smdata

  feature group replicate
1 sample1  ctrl         1
2 sample2  ctrl         1
3 sample3 cond1         2
4 sample4 cond1         2
```

See Exercise-03.R

Feature description

```
> fmdata <- data.frame(feature = rownames(expdata),
>                       description = ...)
```

See Exercise-03.R

The complete experiment

```
> marray <- list(
>    expression = expdata,
>    featuremeta = fmdata,
>    samplemeta = smdata)
```

See Exercise-03.R

Plan

Exercise 4:

- ▶ Reproduce the data structure of the previous exercise using the `MAdata1.csv`, `smeta1.csv` and `fmeta1.csv` files.
- ▶ Produce figures to explore the data.
- ▶ Count and visualise the differentially expressed genes in three microarray result data.

Data IO

  read.table creates a `data.frame` from a spreadsheet file.

  write.table writes a `data.frame`/`matrix` to a spreadsheet (tsv, csv).

Specialised data formats often have specific i/o functionality (microarray CEL files see later)

    save writes an binary representation of `R` objects to a file (cross-platform).

    load load a binary `R` file from disk.

See `Exercise-04.R`

Plotting

- ▶ scatter plots with `plot` and `smoothScatter`
- ▶ boxplots with `boxplot`,
- ▶ histograms with `hist`
- ▶ heatmaps with `heatmap`

See `Exercise-04.R`

Programming

- ► Flow control: `for` (and `while`) loops
- ► Conditions: `if`, (`if else`) and `else`
- ► (The `apply` family of functions)

See `Exercise-04.R`

Optional Exercise 5:

- ▶ Combine gene expression results from multiple files into one matrix and visualise the results.
- ▶ Extract some genes of interest from a table and subset the original data.

New functions: `lapply`, `unlist`, `unique`, `match` and `strsplit`.

See `Exercise-05.R`
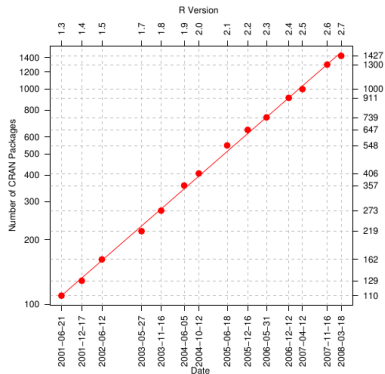
Plan

Packages

- ▶ Primary mechanism to distribute R software is via <u>packages</u>.
- ▶ <u>Packages</u> are installed in <u>libraries</u> (directories) on your had disk, and they are loaded with the `library` function.
- ▶ There are <u>software</u>, <u>data</u> and <u>annotation</u> packages.
- ▶ The **Comprehensive R Archive Network** (`CRAN`) is the main package repository. It provides an automatic build framework for package authors.
- ▶ The **Bioconductor** project manages its own CRAN-style repository.
- ▶ **R-forge** – https://r-forge.r-project.org/

Bioconductor 671 reviewed packages (2.12)

CRAN 4262 packages

R-forge 1453 projects

19th June 2012

Finding packages

- BiocViews – `http://bioconductor.org/packages/release/BiocViews.html`.
- CRAN Task Views – `http://cran.r-project.org/web/views/`.

Package installation

- From within R , using `install.packages` - takes care of dependencies.
- Update all installed packages with `update.packages`.
- For Bioconductor packages, use `biocLite`:

```
source("http://www.bioconductor.org/biocLite.R")
## or, if you have already done so in the past
library("BiocInstaller")
biocLite("packageName")
```

Getting information about packages

- CRAN/Bioconductor/R-forge web pages
- Documentation

```r
help(package = "Biobase")
```

- Vignettes (mandatory for Bioconductor packages)

```r
vignette(package = "Biobase")
```

```r
vignette("Bioconductor", package = "Biobase")
```

- Demos

```r
demo("lattice", package = "lattice")
```

Plan

The Bioconductor project

Bioconductor[1] provides tools for the analysis and comprehension of high-throughput genomic data. Bioconductor uses the R statistical programming language, and is open source and open development.

- ► Good to get *things* done.
- ► Good to programming (as in engineering).
- ► Excellent for bioinformatics.
- ► Community support.
- ► Reproducible research.

---

[1] http://bioconductor.org/

Bioconductor provides

- dedicated statistical methodologies
- that work *out-of-the-box* on specialised data structures (objects)
- including relevant annotation
- and come with extensive documentation

The eSet class

Higher order objects

When the data to be stored is more complex, special objects are created to store and handle it in a specialised manner. These higher order objects are constructed using the data types we have seen so far as building blocks.

Let's look at how microarray data is handled in Bioconductor - the eSet structure.

(The eSet model has been re-used for other technologies.)

```
> library("Biobase")
> data(sample.ExpressionSet)
> sample.ExpressionSet

ExpressionSet (storageMode: lockedEnvironment)
assayData: 500 features, 26 samples
  element names: exprs, se.exprs
protocolData: none
phenoData
  sampleNames: A B ... Z (26 total)
  varLabels: sex type score
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: hgu95av2
```

```
> class(sample.ExpressionSet)

[1] "ExpressionSet"
attr(,"package")
[1] "Biobase"

> slotNames(sample.ExpressionSet)

[1] "experimentData"    "assayData"
[3] "phenoData"         "featureData"
[5] "annotation"        "protocolData"
[7] ".__classVersion__"


> `?`(class, ExpressionSet)
```

assayData expression values in identical sized matrices.

phenoData sample annotation in `AnnotatedDataFrame`.

featureData feature annotation in `AnnotatedDataFrame`.

experimentData description of the experiment as a `MIAME` object (see `?MIAME` for more details).

annotation type of chip as a `character`.

protocolData scan dates as a `character`.

The `assayData` slot

Stored the expression data of the assay.

```
> exprs(sample.ExpressionSet)[1:4, 1:3]

                    A       B       C
AFFX-MurIL2_at   192.74  85.753  176.76
AFFX-MurIL10_at   97.14 126.196   77.92
AFFX-MurIL4_at    45.82   8.831   33.06
AFFX-MurFAS_at    22.54   3.601   14.69

> dim(sample.ExpressionSet)

Features  Samples
    500       26
```

The phenoData **slot**

stores the meta data about the samples.

```
> phenoData(sample.ExpressionSet)

An object of class 'AnnotatedDataFrame'
  sampleNames: A B ... Z (26 total)
  varLabels: sex type score
  varMetadata: labelDescription
```

The `featureData` slot
stores the meta data about the feautres.

```
> featureData(sample.ExpressionSet)

An object of class 'AnnotatedDataFrame': none
NULL
```

AnnotatedDataFrame

consists of a collection of samples and the values of variables
measured on those samples. There is also a description of each
variable measured. `AnnotatedDataFrame` associates a
`data.frame` with its metadata.

```
> head(pData(sample.ExpressionSet))

     sex    type score
A Female Control  0.75
B   Male    Case  0.40
C   Male Control  0.73
D   Male    Case  0.42
E Female    Case  0.93
F   Male Control  0.22
```

Subsetting `ExpressionSet` instances

It is reasonable to expect that subsetting operations work also for higher order objects.

```
> sample.ExpressionSet[1:10, 1:2]

ExpressionSet (storageMode: lockedEnvironment)
assayData: 10 features, 2 samples
  element names: exprs, se.exprs
protocolData: none
phenoData
  sampleNames: A B
  varLabels: sex type score
  varMetadata: labelDescription
featureData: none
experimentData: use 'experimentData(object)'
Annotation: hgu95av2
```

Plan

A typical Bioconductor script for microarray data analysis.

- ▶ Getting data
- ▶ Import data into R using dedicated infrastructure
- ▶ Analyse
- ▶ Save script, plots and objects

Using a subset of the tg-gates data

- `E-MTAB-800`: transcription profiling by array of rat liver and kidney after exposure to approximately 130 chemicals collected from repeat dosing studies[2]
- Downloaded and unzipped `E-MTAB-800.raw.1.zip`
- Using only a subset of files below.

Exercise 6:

Loading libraries

```
library("Biobase")
library("affy")
```

Reading data

```
rawdata <- ReadAffy(filenames = flnms)
```

Normalisation

```
eset <- rma(rawdata)
```

See Exercise-06.R

References

General

- W. N. Venables, D. M. Smith and the R Development Core Team, An Introduction to R (get it with `help.start()`)
- R. Gentleman, R Programming for Bioinformatics, CRC Press, 2008
- Plenty of free documentation on the `R` web page and elsewhere.

Bioconductor

- Gentleman et al., *Bioconductor: open software development for computational biology and bioinformatics*, Genome Biol. 2004; 5:(10)R80
- *Bioconductor Case Studies*, 2008, Springer.

References

Plotting

- We have covered base graphics, not lattice and ggplot2.
- Lattice: Multivariate Data Visualization with R, Deepayan Sarkar (2008)
- ggplot2: Elegant Graphics for Data Analysis, Hadley Wickham (2010)
- http://gallery.r-enthusiasts.com/allgraph.php
- R Graphics manual:
  http://rgm3.lab.nig.ac.jp/RGM/r_image_list
- http://www.cookbook-r.com/Graphs/ (ggplot2)

```
toLatex(sessionInfo())
```

- ▶ R Under development (unstable) (2013-04-05 r62500),
  x86_64-unknown-linux-gnu
- ▶ Locale: LC_CTYPE=en_GB.UTF-8, LC_NUMERIC=C,
  LC_TIME=en_GB.UTF-8, LC_COLLATE=en_GB.UTF-8,
  LC_MONETARY=en_GB.UTF-8, LC_MESSAGES=en_GB.UTF-8,
  LC_PAPER=C, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C,
  LC_MEASUREMENT=en_GB.UTF-8, LC_IDENTIFICATION=C
- ▶ Base packages: base, datasets, graphics, grDevices, methods,
  parallel, stats, utils
- ▶ Other packages: Biobase 2.21.0, BiocGenerics 0.7.2, knitr 1.2
- ▶ Loaded via a namespace (and not attached): digest 0.6.3,
  evaluate 0.4.3, formatR 0.7, stringr 0.6.2, tools 3.1.0

- Parts of these slides are based on the *Beginners guide to solving biological problems in R* course[3], University of Cambridge.
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Thank you for your attention