An Introduction to R

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Microarray Analysis using R and Bioconductor diXa Training – 14th May 2013

Plan

About R

Hello woRld

Data types

Basic data structures Exercise

R scripting: a complete use case

Packages

Bioconductor

The eSet class

A Bioc script

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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 interactive statistical environment
- A programming language
- ► A language and associated tools for reproducible research (these slides for example)

What is R?

- An interactive 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







About R What is R? Contributors Screenshots What's new?

Download, Packages CRAN

R Project
Foundation
Members & Donors
Mailing Lists
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Developer Page
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Search

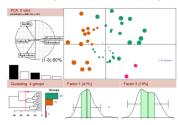
Documentation

Manuals FAQs The R Journal

Wiki Books Certification

Other Misc

Bioconductor Related Projects User Groups Links 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.

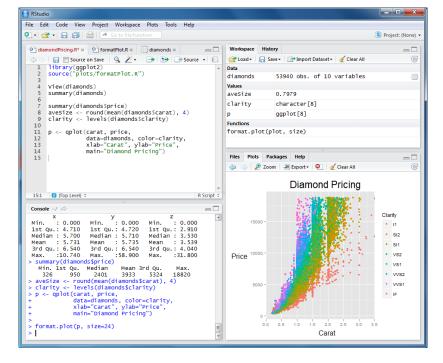
This server is hosted by the Institute for Statistics and Mathematics of WU (Wirtschaftsuniversität Wien),

What is needed:

- ► The R console
- An editor

We will use:

► RStudio IDE



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Hello woRld

- > 5
- [1] 5

[1] 1

- > 2 + 2
- [1] 4
- > sin(pi/2)

```
> 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/RIntro"
> 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 \mathbb{R} console, calculate the following expressions, where x and y have values -0.25 and 2 respectively. 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.

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Atomic vectors

```
> 1
[1] 1
> c(1, 4, 7, 10) ## concatenate
\lceil 1 \rceil \quad 1 \quad 4 \quad 7 \quad 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 ....
```

[1] 3

[1] 4.5

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

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

```
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 \leftarrow seq(from = 1, to = 10, by = 2))
```

[1] 1 3 5 7 9

[1] TRUE

[1] TRUE

> z2 <- seq(1, 10, 2)

> identical(z1, z3)

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

Subsetting

The [operator

[1] 7 1

```
> x <- 1:10
> x[4:5]
[1] 4 5
> x[seq(1, 10, 3)]
[1] 1 4 7 10
> x[c(7, 1)]
```

```
Negative indices in [
```

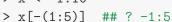
> x <- 1:10

[1] 6 7 8 9 10

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

[1] 2 3 5 6 8 9























Out of range indices

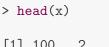
```
> x <- 1:5
> x[5:6]
[1] 5 NA
```

[1] 1

```
> x[0:1]
```

```
Replacement with [
> (x < -1:10)
```

```
[1] 1 2 3 4 5 6 7 8 9 10
> x[1] <- 100
```



> x[4:8]

> x[1:5] <- 0

[1] 0 0 6 7 8





```
Vectorised arithmetic
> x <- 1:5
> y <- 5:1
> x
[1] 1 2 3 4 5
```

[1] 5 4 3 2 1

[1] 6 6 6 6 6

[1] 1 4 9 16 25

> y

> x + y

 $> x^2$



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"</pre>
```

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] 3

```
[1] 4 3
```

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

```
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,]

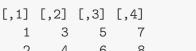
[1] 1 5 9

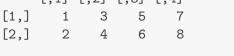
> m[, 1]

[1] 1 2 3 4

Arrays are n-dimensional vectors

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





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

, , 2



Lists are ordered set of arbitrary $\ensuremath{\mathbb{R}}$ objects.

[1] 1 2 3

[1] "a" "b"

> 11\$b

```
> 11 <- list(a = 1:3, b = letters[1:2])
> 11
$a
[1] 1 2 3
$b
[1] "a" "b"
> 11[[1]]
```

Dataframes are 2-dimensional list.

4 B 0.4293

```
> dfr[1, ]
 type time
1 A -0.1631
> dfr[1, "time"]
[1] -0.1631
> dfr$time
[1] -0.1631 0.6897 1.4115 0.4293
```

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 \leftarrow 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")</pre>
> M
     2005 2006 2007
plane 4 6 1
bus 8 4 5
boat 5 2 7
> M[c("plane", "boat"), "2005"]
```

plane boat

5

```
Subsetting with numbers, characters, logicals
> x <- 1:5
> names(x) <- letters[1:5]</pre>
> x[c(1, 3)]
ас
1 3
```

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

```
a c
1 3
```

a c 1 3

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

Factors represent categorical data

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

LIJ F M M F M M F F M 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] 1.5303 0.4423 1.7589
> rnorm(5, mean = 10, sd = 2)
[1] 10.154 7.180 10.432 8.284 11.949
```

The expression data

```
> expdata <- matrix(rnorm(200), nrow = 50, ncol = 4)
> dimnames(expdata) <-</pre>
   list(features = paste0("gene", 1:nrow(expdata)),
         samples = paste0("sample", 1:ncol(expdata)))
> head(expdata)
        samples
features sample1 sample2 sample3 sample4
   gene1 0.5510 -1.0402 0.74508 -0.2230
   gene2 -0.4729 -0.9047 0.91703 -0.4883
  gene3 -1.1166 2.0203 0.91014 0.6332
   gene4 -0.1062 0.6774 -0.05938 0.3309
   gene5 -0.9661 1.0632 1.26402 -0.1405
   gene6 0.0197 -0.0078 -1.07178 0.5440
```

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
2 sample2 ctrl
3 sample3 cond1
4 sample4 cond1
```

Feature description

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

The complete experiment

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

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A Bioc script

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 10

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

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.

Plotting

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

Programming

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

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.

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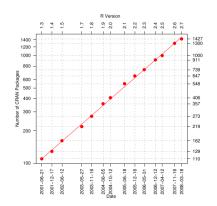
Packages

- ▶ Primary mechanism to distribute R software is via packages.
- Packages are installed in <u>libraries</u> (directories) on your had disk, and they are loaded with the <u>library</u> function.
- ▶ There are software, data and annotation 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

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

Vignettes (mandatory for Bioconductor packages)

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

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

Demos

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

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The Bioconductor project

Bioconductor¹ 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.

¹http://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

experimentData: use 'experimentData(object)'

featureData: none

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.

 $\begin{tabular}{ll} \textbf{feature Data} & \textbf{feature annotation in Annotated Data Frame}. \end{tabular}$

annotation type of chip as a character.

protocolData scan dates as a character.

The assayData slot

Features

500

Stored the expression data of the assay.

> dim(sample.ExpressionSet)

Samples

26

```
> 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
```

The phenoData slot

stores the meta data about the samples.

> phenoData(sample.ExpressionSet)

varMetadata: labelDescription

An object of class 'AnnotatedDataFrame' sampleNames: A B ... Z (26 total)

varLabels: sex type score

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
```

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- Getting data
- ▶ Import data into R using dedicated infrastructure

Save script, plots and objects

Analyse

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 studies²
- ▶ Downloaded and unzipped E-MTAB-800.raw.1.zip
- Using only a subset of files below.

²http://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-800/

Exercise 6:

Loading libraries

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

Reading data

```
rawdata <- ReadAffy(filenames = flnms)</pre>
```

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 course³, University of Cambridge.
- ▶ This work is licensed under a CC BY-SA 3.0 License
- Course web page:

https://github.com/lgatto/TeachingMaterial

Thank you for your attention



³http://www.training.cam.ac.uk/gsls/course/gsls-rintro