A beginners guide to solving biological problems in R

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Course material:
http://logic.sysbiol.cam.ac.uk/teaching/Rcourse/

Original slides by Ian Roberts and Robert Stojnić

Day 1 schedule

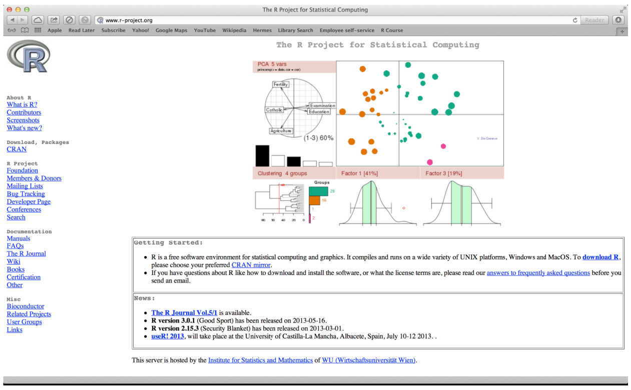
1. Introduction to R and its environment
2. Data structures
3. Data analysis example
4. Programming techniques
5. Statistics

Introduction to R and its environment

1

What’s R?

- A statistical programming environment
  - based on S
  - Suited to high level data analysis
- Open source & cross platform
- Extensive graphics capabilities
- Diverse range of add-on packages
- Active community of developers
- Thorough documentation



The R Project for Statistical Computing

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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:

- **The R Journal Vol.5/1** is available.
- **R version 3.0.1** (Good Sport) has been released on 2013-05-16.
- **R version 2.15.3** (Security Blanket) has been released on 2013-03-01.
- **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).

www.r-project.org

Various platforms supported

- Release 3.0.1 (May 2013)
  - Base package
  - Contributed packages (general purposes extras)
  - ~4700 available packages
- Download from http://www.stats.bris.ac.uk/R/
- Windows, Mac and Linux versions available

- Executed using command line, or a graphical user interface (GUI)
- On this course, we use the RStudio GUI (www.rstudio.com)
- Everything you need is installed on the training machines
- If you are using your own machine, download both R and RStudio

Getting Started

- R is a program which, once installed on your system, can be launched and is immediately ready to take input directly from the user
- There are two ways to launch R:
  1) From the command line (particularly useful if you're quite familiar with Linux)
  2) As an application called RStudio (very good for beginners)

Prepare to launch R
From command line

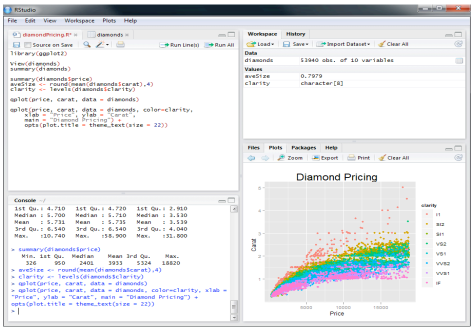
- To start R in Linux we need to enter the Linux console (also called Linux terminal and Linux shell)
- To start R, at the prompt simply type:

```
$ R
```

- If R doesn't print the welcome message, call us to help!

Prepare to launch R
Using RStudio

- To launch RStudio, find the RStudio icon in the menu bar on the left of the screen and double-click



```
library(ggplot2)

View(diamonds)
summary(diamonds)

summary(diamonds$price)
aveSize <- round(mean(diamonds$carat),4)
clarity <- levels(diamonds$clarity)

qplot(price, carat, data = diamonds)

qplot(price, carat, data = diamonds, color=clarity,
    xlab = "Price", ylab = "Carat",
    main = "Diamond Pricing") +
    opts(plot.title = theme_text(size = 22))
```

```
 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)
> qplot(price, carat, data = diamonds)
> qplot(price, carat, data = diamonds, color=clarity, xlab =
"Price", ylab = "Carat", main = "Diamond Pricing") +
opts(plot.title = theme_text(size = 22))
> |
```

Data

```
diamonds          53940 obs. of 10 variables
```

Values

```
aveSize           0.7979
clarity           character[8]
```

The Working Directory (wd)

- Like many programs R has a concept of a working directory (wd)
- It is the place where R will look for files to execute and where it will save files, by default
- For this course we need to set the working directory to the location of the course scripts
- At the command prompt in the terminal or in RStudio console type:

```
> setwd("R_course/Day_1_scripts")
```

- Alternatively in RStudio use the mouse and browse to the directory location
- Tools → Set Working Directory → Choose Directory...

Basic concepts in R
command line calculation

- The command line can be used as a calculator. Type:

```
> 2 + 2
[1] 4

> 20/5 - sqrt(25) + 3^2
[1] 8

> sin(pi/2)
[1] 1
```

- Note: The number in the square brackets is an indicator of the position in the output. In this case the output is a 'vector' of length 1 (i.e. a single number). More on vectors coming up...

Basic concepts in R
variables

- A variable is a letter or word which takes (or contains) a value. We use the assignment 'operator', `<-`

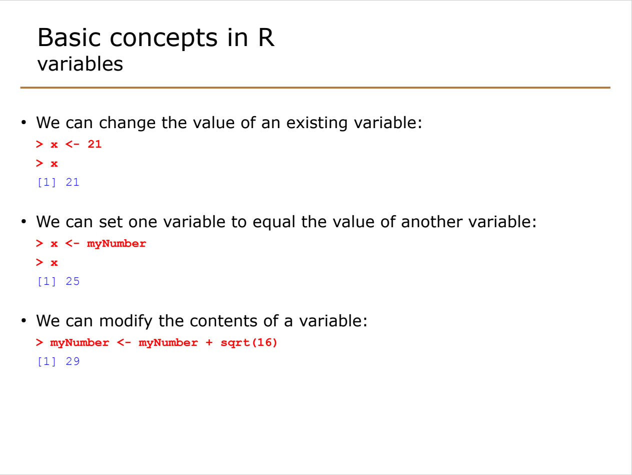
```
> x <- 10
> x
[1] 10
> myNumber <- 25
> myNumber
[1] 25
```

- We can perform arithmetic on variables:

```
> sqrt(myNumber)
[1] 5
```

- We can add variables together:

```
> x + myNumber
[1] 35
```



Basic concepts in R
variables

- We can change the value of an existing variable:

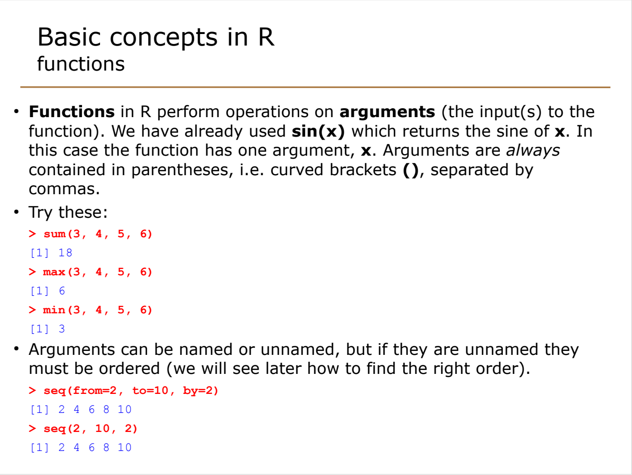
```
> x <- 21
> x
[1] 21
```

- We can set one variable to equal the value of another variable:

```
> x <- myNumber
> x
[1] 25
```

- We can modify the contents of a variable:

```
> myNumber <- myNumber + sqrt(16)
[1] 29
```



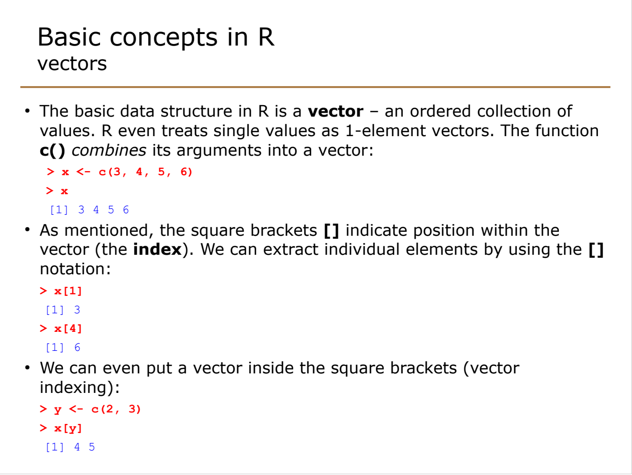
Basic concepts in R
functions

- **Functions** in R perform operations on **arguments** (the input(s) to the function). We have already used **sin(x)** which returns the sine of **x**. In this case the function has one argument, **x**. Arguments are *always* contained in parentheses, i.e. curved brackets **()**, separated by commas.
- Try these:

```
> sum(3, 4, 5, 6)
[1] 18
> max(3, 4, 5, 6)
[1] 6
> min(3, 4, 5, 6)
[1] 3
```

- Arguments can be named or unnamed, but if they are unnamed they must be ordered (we will see later how to find the right order).

```
> seq(from=2, to=10, by=2)
[1] 2 4 6 8 10
> seq(2, 10, 2)
[1] 2 4 6 8 10
```



Basic concepts in R
vectors

- The basic data structure in R is a **vector** – an ordered collection of values. R even treats single values as 1-element vectors. The function **c()** *combines* its arguments into a vector:

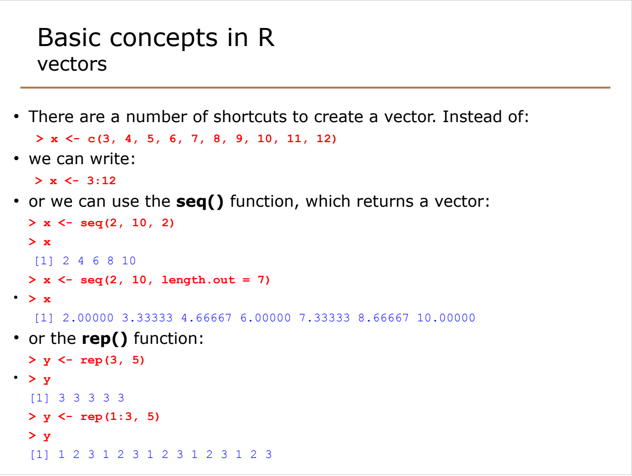
```
> x <- c(3, 4, 5, 6)
> x
[1] 3 4 5 6
```

- As mentioned, the square brackets **[]** indicate position within the vector (the **index**). We can extract individual elements by using the **[]** notation:

```
> x[1]
[1] 3
> x[4]
[1] 6
```

- We can even put a vector inside the square brackets (vector indexing):

```
> y <- c(2, 3)
> x[y]
[1] 4 5
```



Basic concepts in R
vectors

- There are a number of shortcuts to create a vector. Instead of:

```
 > x <- c(3, 4, 5, 6, 7, 8, 9, 10, 11, 12)
```

- we can write:

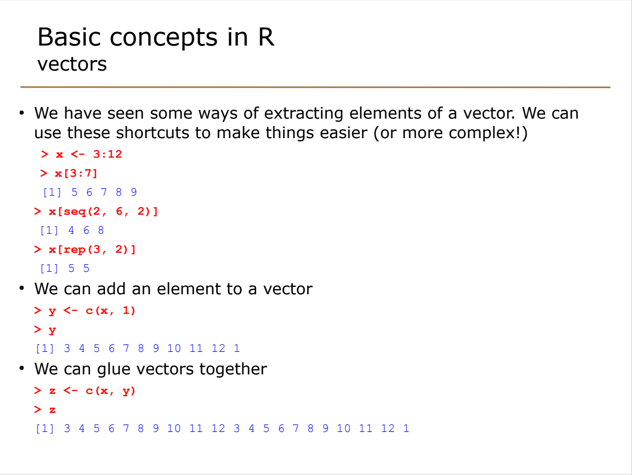
```
 > x <- 3:12
```

- or we can use the **seq()** function, which returns a vector:

```
> x <- seq(2, 10, 2)
> x
 [1] 2 4 6 8 10
> x <- seq(2, 10, length.out = 7)
• > x
 [1] 2.00000 3.33333 4.66667 6.00000 7.33333 8.66667 10.00000
```

- or the **rep()** function:

```
> y <- rep(3, 5)
• > y
[1] 3 3 3 3 3
> y <- rep(1:3, 5)
> y
[1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
```



Basic concepts in R
vectors

- We have seen some ways of extracting elements of a vector. We can use these shortcuts to make things easier (or more complex!)

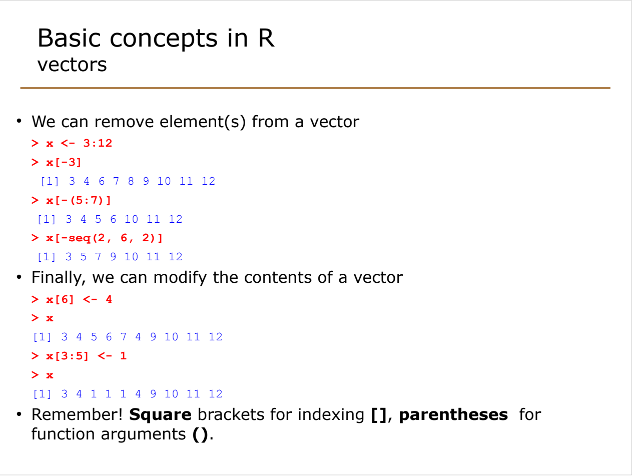
```
 > x <- 3:12
 > x[3:7]
  [1] 5 6 7 8 9
> x[seq(2, 6, 2)]
 [1] 4 6 8
> x[rep(3, 2)]
 [1] 5 5
```

- We can add an element to a vector

```
> y <- c(x, 1)
> y
[1] 3 4 5 6 7 8 9 10 11 12 1
```

- We can glue vectors together

```
> z <- c(x, y)
> z
[1] 3 4 5 6 7 8 9 10 11 12 3 4 5 6 7 8 9 10 11 12 1
```



Basic concepts in R
vectors

- We can remove element(s) from a vector

```
> x <- 3:12
> x[-3]
  [1] 3 4 6 7 8 9 10 11 12
> x[-(5:7)]
 [1] 3 4 5 6 10 11 12
> x[-seq(2, 6, 2)]
 [1] 3 5 7 9 10 11 12
```

- Finally, we can modify the contents of a vector

```
> x[6] <- 4
> x
[1] 3 4 5 6 7 4 9 10 11 12
> x[3:5] <- 1
> x
[1] 3 4 1 1 1 4 9 10 11 12
```

- Remember! **Square** brackets for indexing **[]**, **parentheses** for function arguments **()**.

Basic concepts in R
vector arithmetic

- When applying all standard arithmetic operations to vectors, application is element-wise

```
> x <- 1:10
> y <- x*2
> y
[1] 2 4 6 8 10 12 14 16 18 20
> z <- x^2
> z
[1] 1 4 9 16 25 36 49 64 81 100
```

- Adding two vectors

```
> y + z
[1] 3 8 15 24 35 48 63 80 99 120
```

- If vectors are not the same length, the shorter one will be recycled:

```
> x + 1:2
[1] 2 4 4 6 6 8 8 10 10 12
```

- But be careful if the vector lengths aren't factors of each other:

```
> x + 1:3
```

Basic concepts in R
Character vectors and naming

- All the vectors we have seen so far have contained numbers, but we can also store strings in vectors – this is called a **character** vector.

```
> gene.names <- c("Pax6","Beta-actin","FoxP2","Hox9")
```

- We can name elements of vectors using the **names** function, which can be useful to keep track of the meaning of our data:

```
> gene.expression <- c(0,3.2,1.2,-2)
> gene.expression
[1]  0.0  3.2  1.2 -2.0
> names(gene.expression)<-gene.names
> gene.expression
      Pax6 Beta-actin      FoxP2       Hox9
       0.0        3.2        1.2       -2.0
```

- We can also use the **names** function to get a vector of the names of an object:

```
> names(gene.expression)
[1] "Pax6"       "Beta-actin" "FoxP2"      "Hox9"
```

Exercise: genes and genomes

- Let's try some vector arithmetic. Here are the genome lengths and number of protein coding genes for several model organisms:

Species	Genome size (Mb)	Protein coding genes
Homo sapiens	3,102	20,774
Mus musculus	2,731	23,139
Drosophila melanogaster	169	13,937
Caenorhabditis elegans	100	20,532
Saccharomyces cerevisiae	12	6,692

- Create **genome.size** and **coding.genes** vectors to hold the data in each column using the **c** function. Create a **species.name** vector and use this vector to name the values in the other two vectors.

Exercise: genes and genomes

- Let's assume a coding gene has an average length of 1.5 kilobases. On average, how many base pairs of each genome is made of coding genes? Create a new vector to record this called **coding.bases**.

- What percentage of each genome is made up of protein coding genes? Use your **coding.bases** and **genome.size** vectors to calculate this. (See earlier slides for how to do division in R.)

- How many times more bases are used for coding in the human genome compared to the yeast genome? How many times more bases are in the human genome in total compared to the yeast genome? Look up indices of your vectors to find out.

Answers to genome exercise

- Creating vectors:

```
> genome.size<-c(3102,2731,169,100,12)
> coding.genes<-c(20774,23139,13937,20532,6692)
> species.name<-c("H. sapiens","M. musculus","D. melanogaster","C. elegans","S.
cerevisiae")
> names(genome.size)<-species.name
> names(coding.genes)<-species.name
```

- To calculate the number of coding bases, we need to use the same scale as we used for genome size: 1.5 kilobases is 0.0015 Megabases.

```
> coding.bases<-coding.genes*0.0015
> coding.bases
     H. sapiens     M. musculus D. melanogaster      C. elegans   S. cerevisiae
        31.1610         34.7085         20.9055         30.7980         10.0380
```

Answers to genome exercise

- To calculate the percentage of coding bases in each genome:

```
> coding.pc<-coding.bases/genome.size*100
> coding.pc
     H. sapiens     M. musculus D. melanogaster      C. elegans   S. cerevisiae
       1.004545        1.270908       12.370118       30.798000       83.650000
```

- To compare human to yeast:

```
> coding.bases[1]/coding.bases[5]
H. sapiens
  3.104304
> genome.size[1]/genome.size[5]
H. sapiens
     258.5
```

- Note that if a new vector is created using a named vector, the names are usually carried across to the new vector. Sometimes this is what we want (as for **coding.pc**) but sometimes it is not (when we are comparing human to yeast). We can remove names by setting them to the special NULL value:

```
> names(coding.pc)<-NULL
> coding.pc
[1]  1.004545  1.270908 12.370118 30.798000 83.650000
```

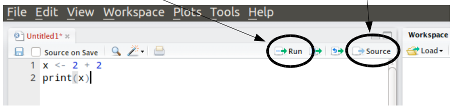
Writing scripts with Rstudio

Typing lots of commands directly to R can be tedious. A better way is to write the commands to a file and then load it into R.

- Click on **File -> New** in Rstudio
- Type in some R code, e.g.

```
x <- 2 + 2
print(x)
```

- Click on **Run** to execute the **current line**, and **Source** to execute the **whole script**



```
x <- 2 + 2
print(x)
```

Sourcing can also be performed manually with `source("myScript.R")`

Getting Help

- To get help on any R function, type `?` followed by the function name. For example:

```
> ?seq
```

- This retrieves the syntax and arguments for the function. You can see the default order of arguments here. The help page also tells you which **package** it belongs to.
- There will typically be example usage, which you can test using the **example** function:

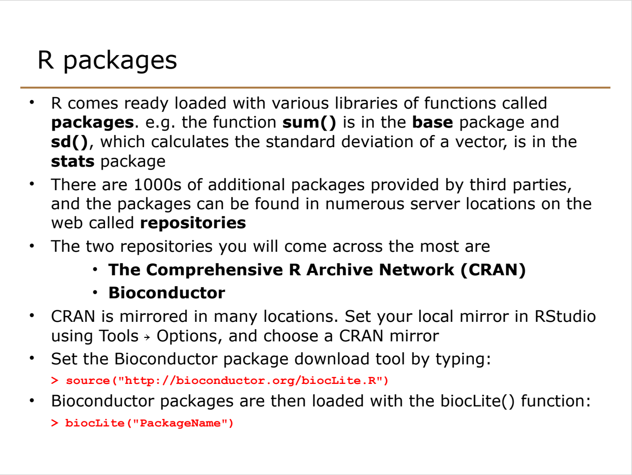
```
> example(seq)
```

- If you can't remember the exact name type `??` followed by your guess. R will return a list of possibles

```
> ??rint
```

Interacting with the R console

- R console symbols
  - ; end of line
    - Enables multiple commands to be placed on one line of text
  - # comment
    - indicates text is a comment and not executed
  - + command line wrap
    - R is waiting for you to complete an expression
- Ctrl-c or escape to clear input line and try again
- Ctrl-l to clear window
- Press q to leave help (using R from the terminal)
- Use the TAB key for command auto completion
- Use up and down arrows to scroll through the command history



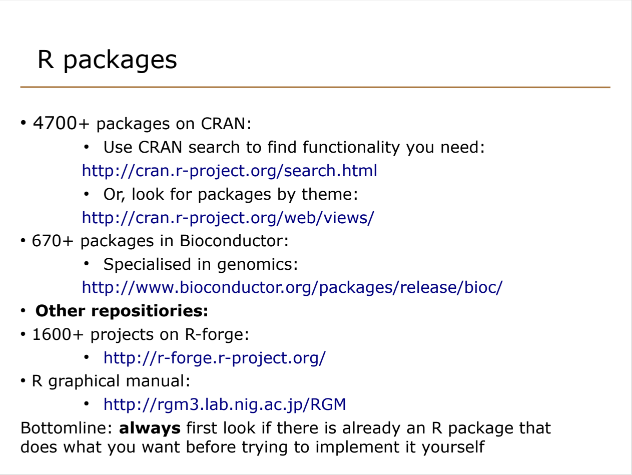
R packages

- R comes ready loaded with various libraries of functions called **packages**. e.g. the function **sum()** is in the **base** package and **sd()**, which calculates the standard deviation of a vector, is in the **stats** package
- There are 1000s of additional packages provided by third parties, and the packages can be found in numerous server locations on the web called **repositories**
- The two repositories you will come across the most are
  - **The Comprehensive R Archive Network (CRAN)**
  - **Bioconductor**
- CRAN is mirrored in many locations. Set your local mirror in RStudio using Tools → Options, and choose a CRAN mirror
- Set the Bioconductor package download tool by typing:

```
> source("http://bioconductor.org/biocLite.R")
```

- Bioconductor packages are then loaded with the biocLite() function:

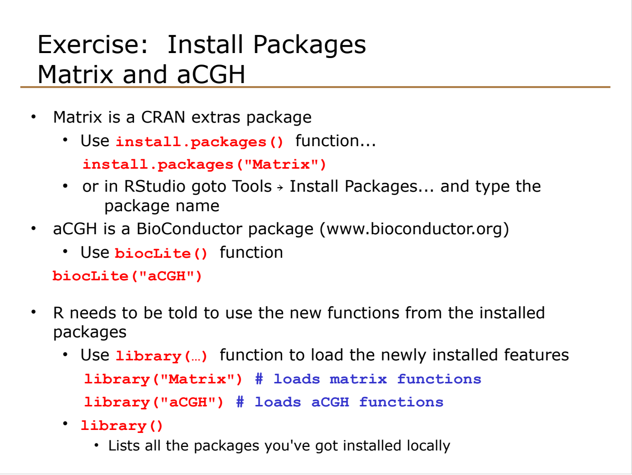
```
> biocLite("PackageName")
```



R packages

- 4700+ packages on CRAN:
  - Use CRAN search to find functionality you need:
    http://cran.r-project.org/search.html
  - Or, look for packages by theme:
    http://cran.r-project.org/web/views/
- 670+ packages in Bioconductor:
  - Specialised in genomics:
    http://www.bioconductor.org/packages/release/bioc/
- **Other repositiories:**
- 1600+ projects on R-forge:
  - http://r-forge.r-project.org/
- R graphical manual:
  - http://rgm3.lab.nig.ac.jp/RGM

Bottomline: **always** first look if there is already an R package that does what you want before trying to implement it yourself



Exercise: Install Packages Matrix and aCGH

- Matrix is a CRAN extras package
  - Use `install.packages()` function…
    `install.packages("Matrix")`
  - or in RStudio goto Tools → Install Packages… and type the package name
- aCGH is a BioConductor package (www.bioconductor.org)
  - Use `biocLite()` function

```
biocLite("aCGH")
```

- R needs to be told to use the new functions from the installed packages
  - Use `library(…)` function to load the newly installed features

```
library("Matrix") # loads matrix functions
library("aCGH") # loads aCGH functions
```

  - `library()`
    - Lists all the packages you've got installed locally

Data structures

2

R is designed to handle experimental data

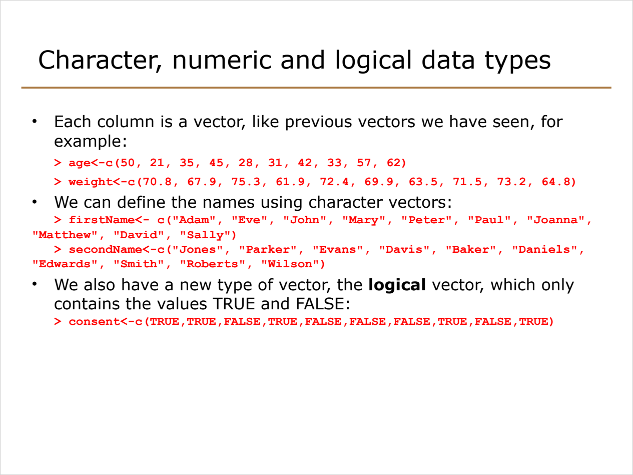
- Although the basic unit of R is a vector, we usually handle data in **data frames**.
- A data frame is a set of observations of a set of variables – in other words, the outcome of an experiment.
- For example, we might want to analyse information about a set of patients. To start with, let's say we have ten patients and for each one we know their name, sex, age, weight and whether they give consent for their data to be made public.

The patients data frame

We are going to create a data frame called 'patients', which will have ten rows (observations) and seven columns (variables). The columns must all be equal lengths.

```
   First_Name Second_Name      Full_Name    Sex Age Weight Consent
1        Adam       Jones     Adam Jones   Male  50   70.8    TRUE
2         Eve      Parker     Eve Parker Female  21   67.9    TRUE
3        John       Evans     John Evans   Male  35   75.3   FALSE
4        Mary       Davis     Mary Davis Female  45   61.9    TRUE
5       Peter       Baker    Peter Baker   Male  28   72.4   FALSE
6        Paul     Daniels   Paul Daniels   Male  31   69.9   FALSE
7      Joanna     Edwards Joanna Edwards Female  42   63.5   FALSE
8     Matthew       Smith  Matthew Smith   Male  33   71.5    TRUE
9       David     Roberts  David Roberts   Male  57   73.2   FALSE
10      Sally      Wilson   Sally Wilson Female  62   64.8    TRUE
```

Let's see how we can construct this from scratch.



Character, numeric and logical data types

- Each column is a vector, like previous vectors we have seen, for example:

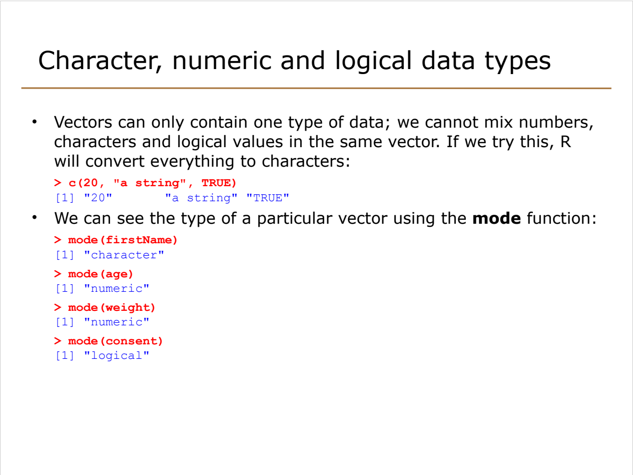
```
> age<-c(50, 21, 35, 45, 28, 31, 42, 33, 57, 62)
> weight<-c(70.8, 67.9, 75.3, 61.9, 72.4, 69.9, 63.5, 71.5, 73.2, 64.8)
```

- We can define the names using character vectors:

```
   > firstName<- c("Adam", "Eve", "John", "Mary", "Peter", "Paul", "Joanna",
"Matthew", "David", "Sally")
   > secondName<-c("Jones", "Parker", "Evans", "Davis", "Baker", "Daniels",
"Edwards", "Smith", "Roberts", "Wilson")
```

- We also have a new type of vector, the **logical** vector, which only contains the values TRUE and FALSE:

```
> consent<-c(TRUE,TRUE,FALSE,TRUE,FALSE,FALSE,FALSE,TRUE,FALSE,TRUE)
```



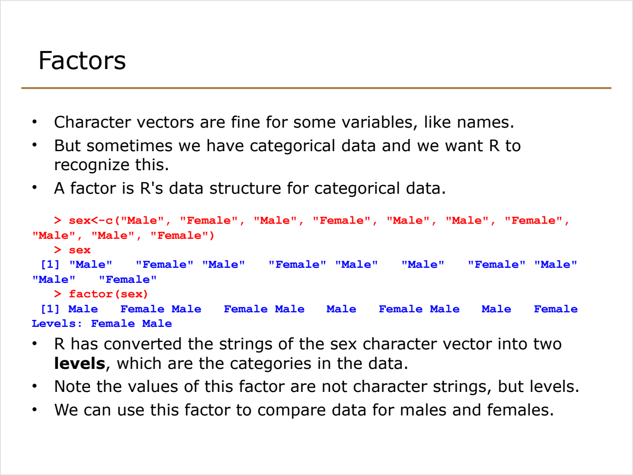
Character, numeric and logical data types

- Vectors can only contain one type of data; we cannot mix numbers, characters and logical values in the same vector. If we try this, R will convert everything to characters:

```
> c(20, "a string", TRUE)
[1] "20"        "a string" "TRUE"
```

- We can see the type of a particular vector using the **mode** function:

```
> mode(firstName)
[1] "character"
> mode(age)
[1] "numeric"
> mode(weight)
[1] "numeric"
> mode(consent)
[1] "logical"
```

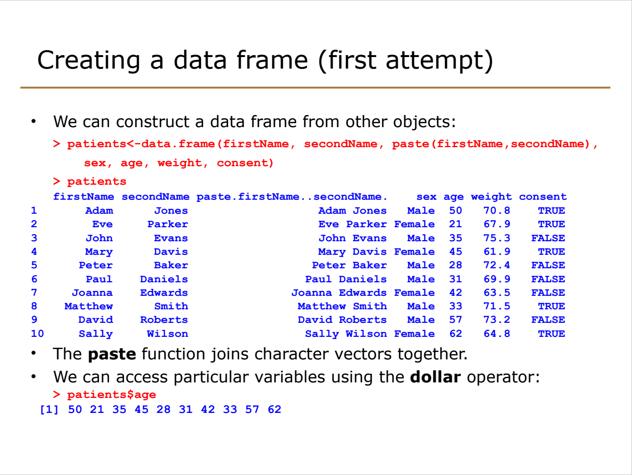


Factors

- Character vectors are fine for some variables, like names.
- But sometimes we have categorical data and we want R to recognize this.
- A factor is R's data structure for categorical data.

```
   > sex<-c("Male", "Female", "Male", "Female", "Male", "Male", "Female",
"Male", "Male", "Female")
   > sex
 [1] "Male"   "Female" "Male"   "Female" "Male"   "Male"   "Female" "Male"
"Male"   "Female"
   > factor(sex)
 [1] Male   Female Male   Female Male   Male   Female Male   Male   Female
Levels: Female Male
```

- R has converted the strings of the sex character vector into two **levels**, which are the categories in the data.
- Note the values of this factor are not character strings, but levels.
- We can use this factor to compare data for males and females.



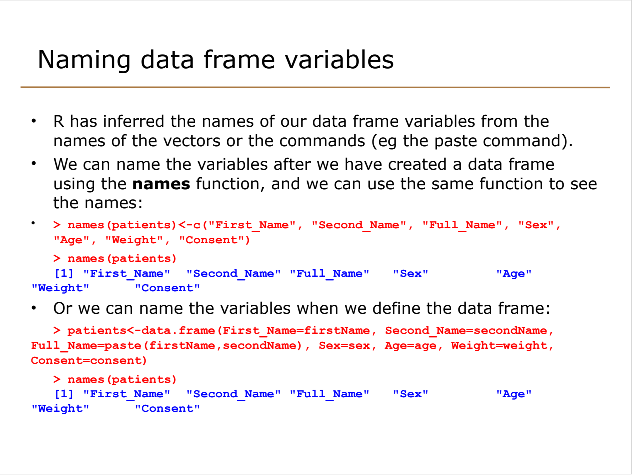
Creating a data frame (first attempt)

- We can construct a data frame from other objects:

```
   > patients<-data.frame(firstName, secondName, paste(firstName,secondName),
       sex, age, weight, consent)
   > patients
   firstName secondName paste.firstName..secondName.    sex age weight consent
1       Adam      Jones                   Adam Jones   Male  50   70.8    TRUE
2        Eve     Parker                   Eve Parker Female  21   67.9    TRUE
3       John      Evans                   John Evans   Male  35   75.3   FALSE
4       Mary      Davis                   Mary Davis Female  45   61.9    TRUE
5      Peter      Baker                  Peter Baker   Male  28   72.4   FALSE
6       Paul    Daniels                 Paul Daniels   Male  31   69.9   FALSE
7     Joanna    Edwards               Joanna Edwards Female  42   63.5   FALSE
8    Matthew      Smith                Matthew Smith   Male  33   71.5    TRUE
9      David    Roberts                David Roberts   Male  57   73.2   FALSE
10     Sally     Wilson                 Sally Wilson Female  62   64.8    TRUE
```

- The **paste** function joins character vectors together.
- We can access particular variables using the **dollar** operator:

```
   > patients$age
 [1] 50 21 35 45 28 31 42 33 57 62
```



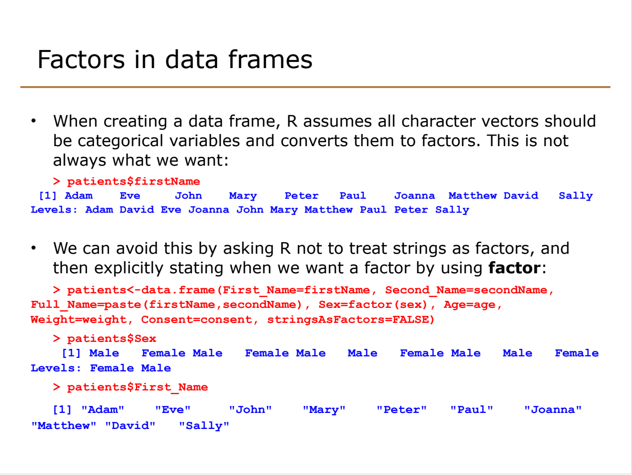
Naming data frame variables

- R has inferred the names of our data frame variables from the names of the vectors or the commands (eg the paste command).
- We can name the variables after we have created a data frame using the **names** function, and we can use the same function to see the names:
-

```
   > names(patients)<-c("First_Name", "Second_Name", "Full_Name", "Sex",
   "Age", "Weight", "Consent")
   > names(patients)
   [1] "First_Name"  "Second_Name" "Full_Name"   "Sex"         "Age"
"Weight"      "Consent"
```

- Or we can name the variables when we define the data frame:

```
   > patients<-data.frame(First_Name=firstName, Second_Name=secondName,
Full_Name=paste(firstName,secondName), Sex=sex, Age=age, Weight=weight,
Consent=consent)
   > names(patients)
   [1] "First_Name"  "Second_Name" "Full_Name"   "Sex"         "Age"
"Weight"      "Consent"
```



Factors in data frames

- When creating a data frame, R assumes all character vectors should be categorical variables and converts them to factors. This is not always what we want:

```
   > patients$firstName
 [1] Adam    Eve     John    Mary    Peter   Paul    Joanna  Matthew David   Sally
Levels: Adam David Eve Joanna John Mary Matthew Paul Peter Sally
```

- We can avoid this by asking R not to treat strings as factors, and then explicitly stating when we want a factor by using **factor**:

```
   > patients<-data.frame(First_Name=firstName, Second_Name=secondName,
Full_Name=paste(firstName,secondName), Sex=factor(sex), Age=age,
Weight=weight, Consent=consent, stringsAsFactors=FALSE)
   > patients$Sex
    [1] Male   Female Male   Female Male   Male   Female Male   Male   Female
Levels: Female Male
   > patients$First_Name

   [1] "Adam"    "Eve"     "John"    "Mary"    "Peter"   "Paul"    "Joanna"
"Matthew" "David"   "Sally"
```

Matrices

matrix(..., ncol=…, nrow=…)

- Data frames are R's speciality, but R also handles matrices:

```
> e <- matrix(1:10, nrow=5, ncol=2)
> e
     [,1] [,2]
[1,]    1    6
[2,]    2    7
[3,]    3    8
[4,]    4    9
[5,]    5   10
> f ← matrix(1:10, nrow=2, ncol=5)
> f
     [,1] [,2] [,3] [,4] [,5]
[1,]    1    3    5    7    9
[2,]    2    4    6    8   10
> f %*% e
     [,1] [,2]
[1,]   95  220
[2,]  110  260
```

The `%*%` operator is the matrix multiplication operator, not the standard multiplication operator.

Lists

list(name1=obj1, name2=obj2, …)

- We have seen that vectors can only hold data of one type. How can we store data of multiple types? Or vectors of different lengths in one object?
- We can use lists. A list can contain objects of any type.

```
a <- 1:10
b <- matrix(runif(100),ncol=10,nrow=10)
c <- data.frame(a, month.name[1:10])

myList<-list( ls.obj.1=a, ls.obj.2=b,ls.obj.3=c )
summary(myList)
names(myList)
```

- We can use the dollar syntax to access list items (in fact, a data frame is a special type of list):

```
myList$ls.obj.1
```

- We can also use `myList[[1]]` to get the first item in the list.
- (For the curious: this double indexing is necessary because lists are in fact just like vectors – they can only contain one type of object. But one of the types they can contain is a list. So any list like the above is actually a list of lists; the first element `myList[1]` is a list containing a vector, and so we need double indexing to actually get the vector.)

Indexing data frames and matrices

Special cases:
a[*i*,] i-th row
a[,*j*] j-th column

- You can index multidimensional data structures like matrices and data frames using commas. If you don't provide an index for either rows or columns, all of the rows or columns will be returned.

```
 object [ rows , columns ]

> e[1,2]
[1] 6

> e[1,]
[1] 1 6

> patients[1,2]
[1] "Jones"

> patients[1,]
  First_Name Second_Name  Full_Name  Sex Age Weight Consent
1       Adam       Jones Adam Jones Male  50   70.8    TRUE
```

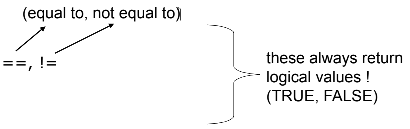
Advanced indexing

- As values in R are really vectors, so indices are actually vectors, and can be numeric or logical:

```
> s <- letters[1:5]
> s[c(1,3)]
[1] "a" "c"
> s[c(TRUE, FALSE, TRUE, FALSE, FALSE)]
[1] "a" "c"
> a<-1:5
> a<3
[1]  TRUE  TRUE FALSE FALSE FALSE
> s[a<3]
[1] "a" "b"
> s[a>1 & a<3]
[1] "b”
> s[a==2]
[1] "b"
```

Operators

- arithmetic
  +, -, *, /, ^
- comparison
  <, >, =<, >=, ==, !=
- logical
  !, &, |, xor



(equal to, not equal to)

these always return logical values ! (TRUE, FALSE)

Exercise

- Create a data frame called **my.patients** using the instructions in the slides. Change the data if you like.
- Check you have created the data frame correctly by loading the original version from this file in the *Day_1_scripts* folder using **source**:

```
> source("05_patients.R")
```

- Remake your data frame with three new variables: country, continent, and height. Make up the data. Make country a character vector but continent a factor.
- Try the **summary** function on your data frame. What does it do? How does it treat vectors (numeric, character, logical) and factors? (What does it do for matrices?)
- Use logical indexing to select the following patients:
  - Patients under 40
  - Patients who give consent to share their data
  - Men who weight as much or more than the average European male (70.8 kg)

Logical indexing answers

- Patients under 40:

```
> patients[patients$Age<40,]
```

- Patients who give consent to share their data:

```
> patients[patients$Consent==TRUE,]
```

- Men who weigh as much or more than the average European male (70.8 kg):

```
> patients[patients$Sex=="Male" & patients$Weight<=70.8,]
```

R for data analysis

3

3 steps to Basic data analysis

1. Reading in data
   - read.table()
   - read.csv(), read.delim()

2. Analysis
   - Manipulating & reshaping the data
   - Any maths you like
   - Plotting the outcome
     - High level plotting functions (covered tomorrow)

3. Writing out results
   - write.table()
   - write.csv()

A simple walkthrough
Exemplifies 3 steps to R analysis

- 50 neuroblastoma patients were tested for NMYC gene copy number by interphase nuclei FISH
  - Amplification of NMYC correlates with worse prognosis
  - We have count data
    - Numbers of cells per patient assayed
      - For each we have NMYC copy number relative to base ploidy
- We need to determine which patients have amplifications
  - (i.e >33% of cells show NMYC amplification)

Step 1.
Read in the data

Patient	Nuclei	NB_Amp	NB_Nor	NB_Del
1	42	0	34	8
2	40	3	30	7
3	56	6	50	0
4	42	5	37	0
5	32	1	30	1
6	70	10	53	7
7	65	3	58	4
8	40	4	31	5
9	60	0	54	6
10	61	0	57	4
11	43	13	29	1

This data is a tab delimited text file
Each row is a record, each column is a field
Columns are separated by tabs in the text.

We need to read in the results table and assign it to an object (rawData)

```
rawData <- read.delim("08_NBcountData.txt")
rawData[1:10,]     # View the first 10 rows to ensure import is OK
                   # Note data frame contains a patient index column
```

If the data had been comma separated values, then sep=","

```
read.csv("08_NBcountData.csv")
```

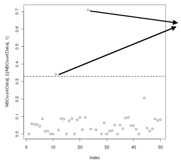
`?read.table` for a full list of arguments

08_NBcountData.R
(script commands)

08_NBcountData.txt
(data file)

Step 2.
Analysis (reshaping data & maths)

- Our analysis involves identifying patients with > 33% NB amplification
  - `prop <- rawData$NB_Amp / rawData$Nuclei # create an index of results`
  - `amp <- which(prop > 0.33) # Get sample names of amplified patients`
- We can plot a simple chart of the % NB amplification
  - `plot(prop, ylim=c(0,1.2))`
  - `abline(h=0.33,lwd=1.5,lty=2)`



These 2 samples are amplified (11 & 23)

Step 3.
Outputting the results

- We write out a data frame of results (patients > 33% NB amplification) as a 'comma separated values' text file
  - `write.csv(rawData[amp,],file="selectedSamples.csv") # Export table, file name = selectedSamples.csv`
    - Files are directly readable by Excel and Calc

- Its often helpful to double check where the data has been saved
  - Use get working directory function
    - `getwd() # print working directory`

Data analysis exercise:
Which samples are near normal?

- Patients are near normal if:

(NB_Amp/Nuclei <0.33 & NB_Del ==0)

- Modify the condition in our previous code to find these patients

- Write out a results file of the samples that match these criteria, and open it in a spreadsheet program

08_NBcountData.R
(script commands)

Solution to NB normality test
Basic data analysis

```
> norm <- which( prop < 0.33 & rawData$NB_Del==0)
> norm

[1] 3  4  7 15 20 24 36 37 42 47


> write.csv(rawData[norm,],"My_NB_output.csv")
```

R programming techniques

4

Basic R 'Built-in' functions for working with objects

- R has many built-in functions for doing simple calculations on objects. Start with a random sample of 15 numbers from 1 to 100 and try the functions below.

```
> x<-sample(100,15)
```

- Arithmetic with vectors
  - Min / Max value number in a series

```
min(x) ; max(x)
```

  - Sum of values in a series

```
sum(x)
```

  - Average estimates (mean / median)

```
mean(x) ; median(x)
```

  - Range of values in a series

```
range(x)
```

  - Variance

```
var(x)
```

- Arithmetic with vectors
  - Rank ordering

```
rank(x)
```

  - Quantiles

```
quantile(x); boxplot(x)
```

  - Square Root

```
sqrt(x)
```

  - Standard deviation

```
sd(x)
```

  - Trigonometry functions

```
tan(x) ; cos(x) ; sin(x)
```

Basic R 'Built-in' functions for working with data frames

- We have seen before how we can get the **names** of our variables, but for dataframes and matrices we can also get these names with **colnames**, and the names of the rows with **rownames**:

```
> names(patients)
[1] "First_Name"  "Second_Name" "Full_Name"   "Sex"      "Age"      "Weight"      "Consent"
> colnames(patients)
[1] "First_Name"  "Second_Name" "Full_Name"   "Sex"      "Age"      "Weight"      "Consent"
> rownames(patients)
[1] "1"  "2"  "3"  "4"  "5"  "6"  "7"  "8"  "9"  "10"
```

We can get the numbers of rows or columns with **nrow** and **ncol**:

```
> nrow(patients)
[1] 10
> ncol(patients)
[1] 7
```

We can also find the length of a vector or a list with **length**, although this may give confusing results for some structures, like data frames:

```
> length(c(1,2,3,4,5))
[1] 5
> length(patients)
[1] 7
> length(patients$Age)
[1] 10
```

Remember, a data frame is a list of variables, so its length is the number of variables. The length of one of the variable vectors (like Age) is the number of observations.

Basic R 'Built-in' functions for working with data frames

We can add rows or columns to a data frame using **rbind** and **cbind**:

```
 > newpatient<-c("Kate","Lawson","Kate Lawson","Female","35","62.5","TRUE")
 > rbind(patients,newpatient)
   First_Name Second_Name      Full_Name    Sex Age Weight Consent
1        Adam       Jones     Adam Jones   Male  50   70.8    TRUE
2         Eve      Parker     Eve Parker Female  21   67.9    TRUE
3        John       Evans     John Evans   Male  35   75.3   FALSE
4        Mary       Davis     Mary Davis Female  45   61.9    TRUE
5       Peter       Baker    Peter Baker   Male  28   72.4   FALSE
6        Paul     Daniels   Paul Daniels   Male  31   69.9   FALSE
7      Joanna     Edwards Joanna Edwards Female  42   63.5   FALSE
8     Matthew       Smith  Matthew Smith   Male  33   71.5    TRUE
9       David     Roberts  David Roberts   Male  57   73.2   FALSE
10      Sally      Wilson   Sally Wilson Female  62   64.8    TRUE
11       Kate      Lawson    Kate Lawson Female  35   62.5    TRUE
> cbind(patients,10:1)
```

- We can also remove rows and columns:

```
df[-1,] # remove first row
df[,-1] # remove first column
```

Basic R 'Built-in' functions for working with data frames

Sorting a vector with **sort**:

```
> sort(patients$Second_Name)
[1] "Baker"   "Daniels" "Davis"   "Edwards" "Evans"   "Jones"   "Parker"  "Roberts" "Smith"
 "Wilson"
```

Sorting a data frame by one variable with **order**:

```
> order(patients$Second_Name)
[1]  5  6  4  7  3  1  2  9  8 10
> patients[order(patients$Second_Name),]
   First_Name Second_Name      Full_Name    Sex Age Weight Consent
5       Peter       Baker    Peter Baker   Male  28   72.4   FALSE
6        Paul     Daniels   Paul Daniels   Male  31   69.9   FALSE
4        Mary       Davis     Mary Davis Female  45   61.9    TRUE
7      Joanna     Edwards Joanna Edwards Female  42   63.5   FALSE
3        John       Evans     John Evans   Male  35   75.3   FALSE
1        Adam       Jones     Adam Jones   Male  50   70.8    TRUE
2         Eve      Parker     Eve Parker Female  21   67.9    TRUE
9       David     Roberts  David Roberts   Male  57   73.2   FALSE
8     Matthew       Smith  Matthew Smith   Male  33   71.5    TRUE
10      Sally      Wilson   Sally Wilson Female  62   64.8    TRUE
```

The R workspace

- The objects we have been making are created in the R workspace.
- When we load a package, we are loading that package's functions and data sets into our workspace.
- You can see what is in your workspace with **ls**:

```
> ls()
```

- You can attach data frames to your workspace and then refer to the variables directly:

```
> attach(patients)
> Full_Name
```

- You can remove objects from the workspace with **rm**:

```
> x<-1:5
[1] 1 2 3 4 5
> rm(x)
> x
Error: object 'x' not found
```

- You can remove everything by giving **rm** a list of all the objects returned by **ls**:

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

Introducing loops

- Many programming languages have ways of doing the same thing many times, perhaps changing some variable each time. This is called **looping**.
- Loops are not used in R so often, because we can usually achieve the same thing using vector calculations.
- For example, to add two vectors together, we do not need to add each pair of elements one by one, we can just add the vectors.
- But there are some situations where R functions can not take vectors as input. For example, **read.csv** will only load one file at a time.
- What if we had ten files to load in, all ending in the same extension (like *.csv*)?

Introducing loops

- We could do this:

```
> colony<-data.frame()     # Start with empty data frame

> colony1<-read.csv("11_CFA_Run1Counts.csv")
> colony2<-read.csv("11_CFA_Run2Counts.csv")
> colony3<-read.csv("11_CFA_Run3Counts.csv")
...
> colony10<-read.csv("11_CFA_Run10Counts.csv")

> colony<-rbind(colony1,colony2,colony3,...,colony10)
```

But this will be boring to type, difficult to change, and prone to error.

- As we are doing the same thing 10 times, but with a different file name each time, we can use a **loop** instead.

LOOPS
Commands & flow control

- R has two basic types of loop:

  for loop: run some code on every value in a vector

  while loop: run some code while some condition is true
- Here are two simple examples of these loops:

```
for (f in 1:10) {
   print(f)
}

f <- 1
while ( f <= 10 ) {
   print(f)
   f <- f + 1
}
```

when this condition is false the loop stops

LOOPS
Commands & flow control

- Here's how we might use a **for** loop to load in our CSV files.
- We can look up files containing a particular substring in their name using the **dir** function:

```
dir(pattern="Counts.csv")
[1] "11_CFA_Run1Counts.csv" "11_CFA_Run2Counts.csv"
  "11_CFA_Run3Counts.csv"
```

- So we can load all the files using a **for** loop as follows:

```
colony<-data.frame()
countfiles<-dir(pattern=”Counts.csv”)
for (file in countfiles) {
   t<-read.csv(file)
   colony<-rbind(colony,t)
}
```

- Here, we use a temporary variable **t** to store the data in each file, and then add that data to the main **colony** data frame.

Conditional branching
Commands & flow control

- Use an **if** statement for any kind of condition testing.
- Different outcomes can be selected based on a condition within brackets.

```
if (condition) {
… do this …
} else {
… do something else …
}
```

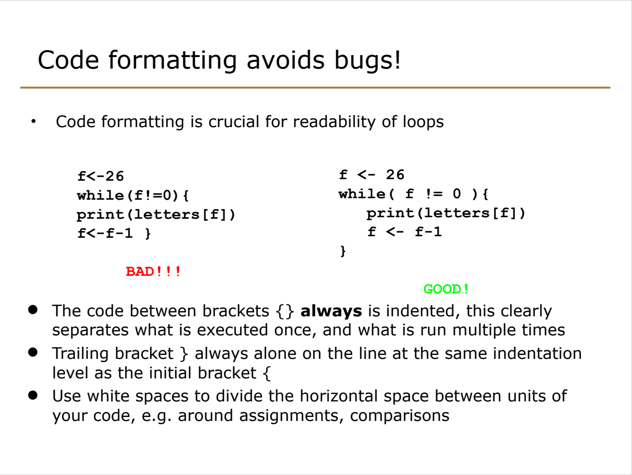
  - condition is any logical value, and can contain multiple conditions
    - e.g. `(a==2 & b <5),` this is a compound conditional argument

Conditional branching
Commands & flow control

For example, if we were writing a script to load an unknown set of files, using the **for** loop we wrote before, we might want to warn the user if we can't find any files with the pattern we are searching for. Here's how we can use an **if** statement to test for this:

```
colony<-data.frame()
countfiles<-dir(pattern="Counts.csv")

if (length(countfiles) == 0) {
    stop("No Counts.csv files found!")
} else {
    for (file in countfiles) {
        t<-read.csv(file)
        colony<-rbind(colony,t)
    }
}
```

The **stop** function outputs the error message and quits.



Code formatting avoids bugs!

- Code formatting is crucial for readability of loops

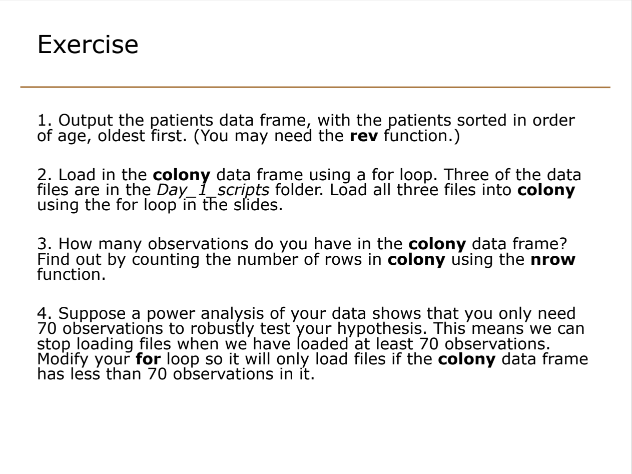
```
f<-26
while(f!=0){
print(letters[f])
f<-f-1 }
```

BAD!!!

```
f <- 26
while( f != 0 ){
   print(letters[f])
   f <- f-1
}
```

GOOD!

- The code between brackets {} **always** is indented, this clearly separates what is executed once, and what is run multiple times
- Trailing bracket } always alone on the line at the same indentation level as the initial bracket {
- Use white spaces to divide the horizontal space between units of your code, e.g. around assignments, comparisons



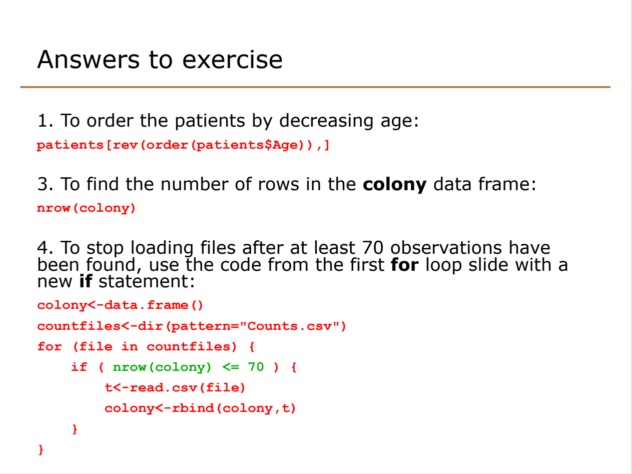
Exercise

1. Output the patients data frame, with the patients sorted in order of age, oldest first. (You may need the **rev** function.)

2. Load in the **colony** data frame using a for loop. Three of the data files are in the *Day_1_scripts* folder. Load all three files into **colony** using the for loop in the slides.

3. How many observations do you have in the **colony** data frame? Find out by counting the number of rows in **colony** using the **nrow** function.

4. Suppose a power analysis of your data shows that you only need 70 observations to robustly test your hypothesis. This means we can stop loading files when we have loaded at least 70 observations. Modify your **for** loop so it will only load files if the **colony** data frame has less than 70 observations in it.



Answers to exercise

1. To order the patients by decreasing age:

```
patients[rev(order(patients$Age)),]
```

3. To find the number of rows in the **colony** data frame:

```
nrow(colony)
```

4. To stop loading files after at least 70 observations have been found, use the code from the first **for** loop slide with a new **if** statement:

```
colony<-data.frame()
countfiles<-dir(pattern="Counts.csv")
for (file in countfiles) {
    if ( nrow(colony) <= 70 ) {
        t<-read.csv(file)
        colony<-rbind(colony,t)
    }
}
```

Statistics

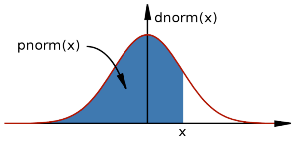
5

Built-in support for statistics

- R is a statistical programming language
  - Classical statistical tests are built-in
  - Statistical modeling functions are built-in
  - Regression analysis is fully supported
  - Additional mathematical packages are available
    - MASS, Waves, sparse matrices, etc

Pseudo-random numbers and distributions

- mostly commonly used distributions are built-in, functions have stereotypical names, e.g. for normal distribution:
  - pnorm - cumulative distribution for x
  - qnorm - inverse of pnorm (from probability gives x)
  - dnorm - distribution density
  - rnorm - random number from normal distribution



- available for variety of distributions: punif (uniform), pbinom (binomial), pnbinom (negative binomial), ppois (poisson), pgeom (geometric), phyper (hyper-geometric), pt (T distribution), pf (F distribution) ...

Two sample tests
Basic data analysis

- Comparing 2 variances
  - Fisher's F test

`var.test()`

- Comparing 2 sample means with normal errors
  - Student's t test

`t.test()`

- Comparing 2 means with non-normal errors
  - Wilcoxon's rank test

`wilcox.test()`

- Comparing 2 proportions
  - Binomial test

`prop.test()`

- Correlating 2 variables
  - Pearson's / Spearman's rank correlation

`cor.test()`

- Testing for independence of 2 variables in a contingency table
  - Chi-squared

`chisq.test()`

  - Fisher's exact test

`fisher.test()`

Comparison of 2 data sets example
Basic data analysis

- Men, on average, are taller than women.
  - The steps
    1. Determine whether variances in each data series are different
       - Variance is a measure of sampling dispersion, a first estimate in determining the degree of difference
         - *Fisher's F test*
    2. Comparison of the mean heights.
       - Determine probability that mean heights really are drawn from different sample populations
         - *Student's t test, Wilcoxon's rank sum test*

1. Comparison of 2 data sets
Fisher's F test

- Read in the data file into a new object, heightData

```
heightData<-read.csv("10_heightData.csv",header=T)
```

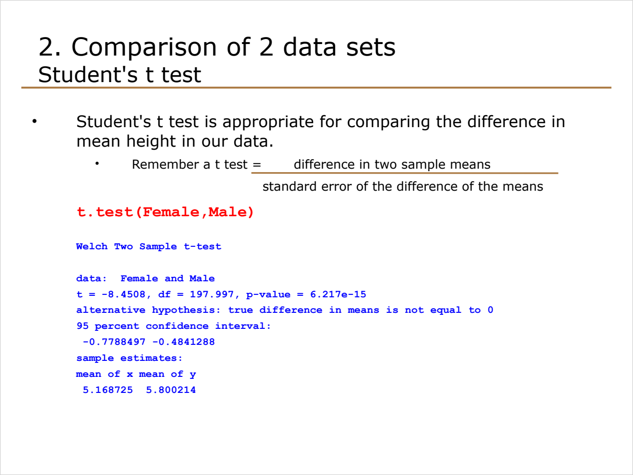
- **attach** the data frame so we don't have to refer to it by name all the time:

```
attach(heightData)
```

- Do the two sexes have the same variance?

```
var.test(Female,Male)
```

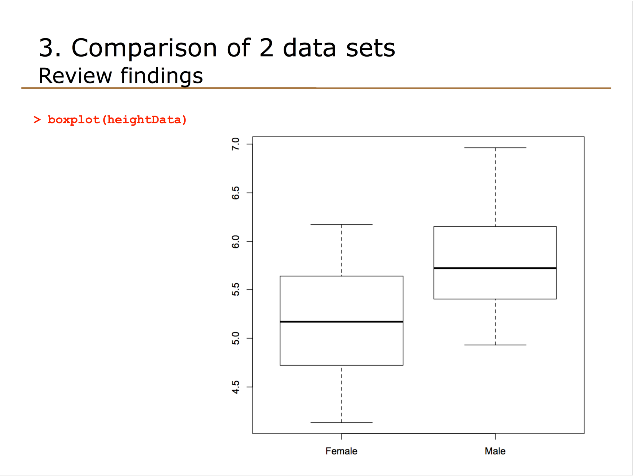
```
F test to compare two variances

data:  Female and Male
F = 1.0073, num df = 99, denom df = 99, p-value = 0.9714
alternative hypothesis: true ratio of variances is not equal to 1
95 percent confidence interval:
 0.6777266 1.4970241
sample estimates:
ratio of variances
          1.00726
```



2. Comparison of 2 data sets
Student's t test

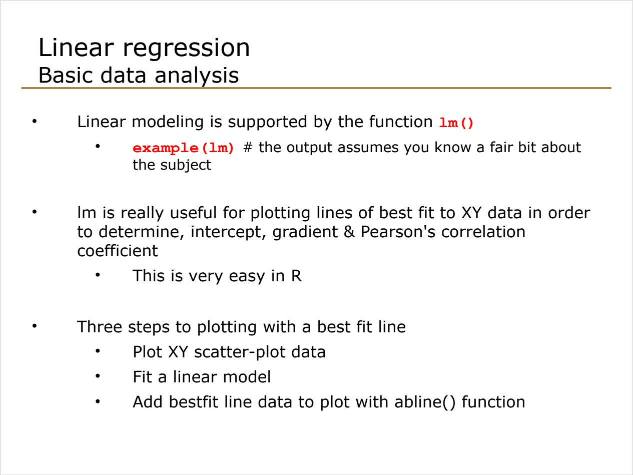
- Student's t test is appropriate for comparing the difference in mean height in our data.
  - Remember a t test = difference in two sample means / standard error of the difference of the means

```
t.test(Female,Male)

Welch Two Sample t-test

data:  Female and Male
t = -8.4508, df = 197.997, p-value = 6.217e-15
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
 -0.7788497 -0.4841288
sample estimates:
mean of x mean of y
 5.168725  5.800214
```



3. Comparison of 2 data sets
Review findings

```
> boxplot(heightData)
```



Linear regression
Basic data analysis

- Linear modeling is supported by the function `lm()`
  - `example(lm)` # the output assumes you know a fair bit about the subject

- lm is really useful for plotting lines of best fit to XY data in order to determine, intercept, gradient & Pearson's correlation coefficient
  - This is very easy in R

- Three steps to plotting with a best fit line
  - Plot XY scatter-plot data
  - Fit a linear model
  - Add bestfit line data to plot with abline() function

Modelling formulae

- R has a very powerful formula syntax for describing statistical models.
- Suppose we had two explanatory variables **x** and **z** and one response variable **y**.
- We can describe a relationship between, say, **y** and **x** using a tilde **~**, placing the response variable on the left of the tilde and the explanatory variables on the right:

```
> y~x
```

- It is very easy to extend this syntax to do multiple regressions, ANOVAs, to include interactions, and to do many other common modelling tasks. For example:

```
> y~x       # If x is continuous, this is linear regression
> y~x       # If x is categorical, this is ANOVA
> y~x+z     # If x and z are continuous, this is multiple regression
> y~x+z     # If x and z are categorical this is a two-way ANOVA
> y~x+z+x:z # : is the symbol for the interaction term
> y~x*z     # * is a shorthand for x+z+x:z
```

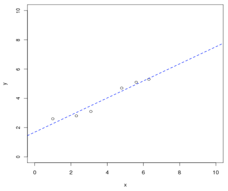
Typical linear regression analysis
Basic data analysis

X	Y
1.0	2.6
2.3	2.8
3.1	3.1
4.8	4.7
5.6	5.1
6.3	5.3

```
> x<-c(1, 2.3, 3.1, 4.8, 5.6, 6.3)
> y<-c(2.6, 2.8, 3.1, 4.7, 5.1, 5.3)
> plot(y~x, xlim=c(0,10),ylim=c(0,10))
```

Note formula notation (y is given by x)

```
> myModel<-lm(y~x)
> abline(mvModel,lty=2,lwd=1.5,col="blue")
```



Get the coefficients of the fit from:
`summary.lm(myModel)` **and**
`coef(myModel)`
`resid(myModel)`
`fitted(myModel)`

Get QC of fit from
`plot(myModel)`

Find out about the fit data from
`names(myModel)`

The linear model object
Basic data analysis

- Summary data describing the linear fit is given by
  - summary.lm(myModel)

```
> summary.lm(myModel)

Call:
lm(formula = y ~ x)ˀ

Residuals:
       1        2        3        4        5        6 
 0.33159 -0.22785 -0.39520  0.21169  0.14434 -0.06458 

Coefficients:
            Estimate Std. Error t value Pr(>|t|)   
(Intercept)  1.68422    0.29056   5.796   0.0044 **
x            0.58418    0.06786   8.608   0.0010 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1 

Residual standard error: 0.3114 on 4 degrees of freedom
Multiple R-squared: 0.9488,   Adjusted R-squared: 0.936 
F-statistic:  74.1 on 1 and 4 DF,  p-value: 0.001001
```

Y intercept

Gradient

Good fit: would happen 1 in 1000 by chance

R^2 , with pValue

Exercise
The coin toss

To learn how the distribution functions work, try simulating tossing a fair coin 100 times and then show that it is fair.

1) We can model a coin toss using the binomial distribution. Use the **rbinom** function to generate a sample of 100 coin tosses. Look up the binomial distribution help page to find out what arguments this function needs.
2) How many heads or tails were there in your sample? You can do this in two ways; either select the number of successes using indices, or convert your sample to a factor and get a summary of the factor.
3) If we toss a coin 50 times, what is the probability that we get exactly 25 heads? What about 25 heads or less? Use **dbinom** and **pbinom** to find out.
4) The argument to **pbinom** is a vector, so try calculating the probabilities for getting any number of coin tosses from 0 to 50 in fifty trials using **dbinom**. Plot these probabilities using **plot**. Does this plot remind you of anything?

Coin toss answers

- To simulate a coin toss, give **rbinom** a number of observations, the number of trials for each observation, and a probability of success:

```
> coin.toss<-rbinom(100, 1, 0.5)
```

- Because we only specified one trial per observation, we either have an outcome of 0 or 1 successes. To get the number of successes, use indices or a factor to look up the number of 1s in the **coin.toss** vector (your numbers will vary):

```
> length(coin.toss[coin.toss==1])
[1] 50
> summary(factor(coin.toss))
 0  1
50 50
```

Coin toss answers

The probability of getting exactly 25 heads from 50 observations of a fair coin:

```
> dbinom(25, 50, 0.5)
```

The probability of getting 25 heads or less from 50 observations of a fair coin:

```
> pbinom(25, 50, 0.5)
```

The probabilities for getting all numbers of coin tosses from 0 to 50 in fifty trials:

```
> dbinom(0:50, 50, 0.5)
```

To plot this distribution, which should resemble a normal distribution:

```
> plot(dbinom(0:50, 50, 0.5))
```

Exercise
Linear modelling example

Mice have varying numbers of babies in each litter. Does the size of the litter affect the average brain weight of the offspring? We can use linear modelling to find out. (This example is taken from John Maindonald and John Braun's book *Data Analysis and Graphics Using R* (CUP, 2003), p140-143.)

1) Install and load the **DAAG** package. The **litters** data frame is part of this package. Take a look at it. How many variables and observations does it have? Does **summary** tell you anything useful? What about **plot**?
2) Are any of the variables correlated? Look up the **cor.test** function and use it to test for relationships.
3) Use **lm** to calculate the regression of brain weight on litter size, brain weight on body weight, and brain weight on litter size and body weight together.
4) Look at the coefficients in your models. How is brain weight related to litter size on its own? What about in the multiple regression? How would you interpret this result?

Linear modelling answers

- To install and load the package and look at **litters**:

```
> install.packages("DAAG")
> library(DAAG)
> litters
> summary(litters)
> plot(litters)
```

- To calculate correlations between variables:

```
> attach(litters)
> cor.test(brainwt, lsize)
> cor.test(bodywt, lsize)
> cor.test(brainwt, bodywt)
```

Linear modelling answers

- To calculate the linear models:

```
> lm(brainwt~lsize)
Call:
lm(formula = brainwt ~ lsize)

Coefficients:
(Intercept)        lsize
   0.447000    -0.004033
> lm(brainwt~bodywt)
Call:
lm(formula = brainwt ~ bodywt)

Coefficients:
(Intercept)       bodywt
    0.33555      0.01048
```

```
> lm(brainwt~lsize+bodywt)
Call:
lm(formula = brainwt ~ lsize + bodywt)

Coefficients:
(Intercept)        lsize       bodywt
     0.17825      0.00669      0.02431
```

Interpretation: brain weight decreases as litter size increases, but brain weight increases proportional to body weight (when bodywt is held constant, the lsize coefficient is positive: 0.00669). This is called 'brain sparing'; although the offspring get smaller as litter size increases, the brain does not shrink as much as the body.

End of Day 1