### R: Introduction to Basic Features

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Bioinformatics Core
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## Workshop aims

• To introduce the R language and environment

- To explain some programming jargon and concepts
- To use R to run some typical tasks
- To be aware of the vital importance of good programming habits

This course is hosted at: https://whg-training.github.io/whg-training-resources/programming/introduction to R/R Introduction to Basic Features.html.

This course has workshop content intended to be worked through using RStudio. The course tutor will lead work through that content during the session, but you are encouraged to work at your own pace and read ahead if you feel comfortable doing so.

The course is intended to start at the very beginning, for people with no previous programming experience, using R or otherwise. If you're finding it at too basic a level, feel free to skip ahead or use this time for something more useful to you! Some suggestions for more R material are at the end of this document.

### Installing R and RStudio

R and RStudio are available for a variety of platforms, including Windows, macOS and Linux. R is open source software. RStudio is open source software, supplemented by non-free add-ons.

- R for Windows download
- R for macOS X
- R for Linux can probably be obtained through your usual package manager, or else here
- RStudio downloads

These should already be installed and ready.

#### R: what is it?

- Powerful statistical computing environment and programming language
- Developed by Robert Gentleman and Ross Ihaka at the University of Auckland in mid 1990s originally something to teach statistics that would run on a Mac
- Full story of R's development
- Now widely used all around the world

### R: strengths and weaknesses

• Incredibly powerful and versatile statistical programming software... but where do I start?

- Features that make life easier in many ways... but potential pitfalls as well
- Open-source, free software with a strong support and development community
- Extensive additional functionality for bioinformatics and genomic data through the Bioconductor project
- Infamous for opaque error messages and help files

# Getting started with R

This workshop will introduce you to the R environment, with the aim of becoming familiar with how commands are written and structured and how to perform basic tasks associated with data analysis using R.

#### R fundamentals

R is, at its heart, a command line language like bash. Text commands are entered and text output is printed to the console. Similarly, you can write R scripts to be executed when you need them. R supports stepping through the commands you have previously entered with the up and down arrow keys, and will attempt to complete function and file names when you press the tab key, just like bash.

However, unlike bash, R also keeps data in its own internal store rather than only keeping data in memory while the command is running. R can also open new windows to display plots it has created, or show help files.

When you close R, it will ask you whether you want to save the workspace. If you say yes, then R creates a file called '.Rdata' (which is a hidden file on most operating systems by default). This file contains *all* of the data you had stored in your R session. This makes is easy to pick up where you left off, but be aware that this file can become very large if you load a lot of data into R.

### Getting started with RStudio

RStudio is an integrated development environment (IDE) that wraps around R and makes a lot of common tasks much more convenient. Launch RStudio to open a new R session.

By default, RStudio displays 4 different panels. If there's only one panel on the left, then one of them is currently hidden – you can make it visible by opening a new R script file from the 'File' menu.

#### RStudio screenshot

If you see something notably different, there's a good chance you've launched bare-bones R rather than RStudio.

#### Bottom left

The bottom left panel is R itself, in the tab 'Console'. If you were running R without RStudio, this is exactly what you would see.

The > prompt is telling you that R is ready to receive new commands. You can type R code here and press return to have R run that code. R will print any output from that command and then show the > prompt again to say it is ready for new input. Note that something may have happened 'behind the scenes' – such creating or altering a data object. It is important to know what each command has done and it was as intended.

Two useful things to know about this panel are that you can use the up and down arrows to scroll through previous commands – very useful to fix an error without retyping the entire line! – and you can use the tab key to auto-complete commands, variable names and file names.

The other tabs you don't need to use now.

Try typing a simple command into the R console (remember to press Return):

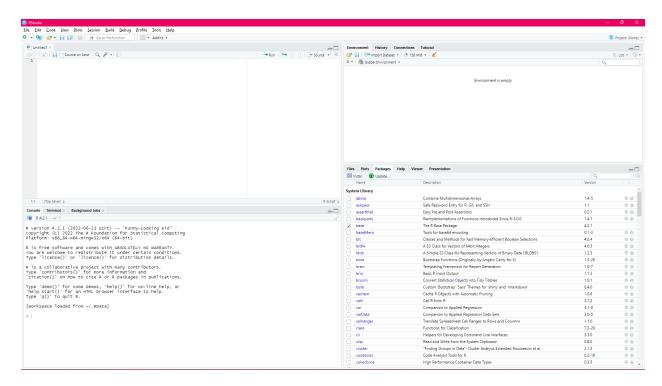


Figure 1: images/RStudio screenshot.png

#### 6 \* 7

You'll see that it will give you the answer. The [1] is a little cryptic for now, but that will be explained later. See how it shows the > prompt again afterwards.

#### Top left

The top left panel is a simple text editor (like Notepad), intended to be used to write R scripts. The editor can have multiple files open at a time.

This text editor is integrated into RStudio and has several convenient features. If you click on the green 'Run' button, it will copy the current line of code into the R window and run it. If you highlight a portion of code, the same button will copy and run that selection. There is also a 'Source' button, what will copy the entire file into the R panel and run it.

Try this by typing this line into the editor:

#### 6 \* 9

and clicking 'Run'.

#### Top right

The top right panel contains information about the current R session. The 'Environment' tab lists all the data objects currently stored in R. Each entry is an 'object', in R's terminology. The 'History' tab shows all the commands that the main R panel has executed so far. This is the same list you can browse with the arrow keys in the R panel. The other tabs here you don't need to worry about.

#### **Bottom right**

The bottom right panel is used to show a variety of things in a tabbed interface. 'Files' allows you to browse your computer's directories. 'Plots' displays any plots you have made using R in the current session. 'Help' is where R will display its help files.

### Working with the R console

R is a very interactive environment; commands can be entered into the console one by one, and each is interpreted and executed by R in real time. R is a high-level programming language, meaning that it is fairly human-readable.

If a command is not valid in the way it is constructed (its syntax), R will print an error message to the screen. These can sometimes be hard to interpret but particularly common culprits are simple typing mistakes, or errors with quotes and brackets (in the wrong place, missing, or not in pairs).

You can type the R commands directly, which is useful at first to get used to the syntax (structure) of commands. However, you can use RStudio's editor to copy commands into the console if you prefer. This way, it's easier to go back and run a command again, or change it slightly first.

If you see an error message, first double check that the command is entered exactly as shown. If you can't find the problem, please ask for help. The advantage of copying the commands into the text editor panel of RStudio means that you can save the file afterwards to have a record of what you have done and can easily re-run again in future.

To do anything useful in R, we need to use objects to hold data and perform various operations on them. The terms object, variable and data structure can all refer generally to 'objects' created in R.

Although variable is a widely used programming term and would be the preferred one in certain situations, we use 'object' throughout to refer to any of R's data structures. These include vectors, factors, matrices, data frames and lists. Don't worry about the differences between those things for now.

# Following along

The editor also understands some other R-related files. This includes a '.rmd' file, which is a special kind of R script where you can put pieces of R code in a text document.

Type the following into the console (bottom left) in RStudio:

download.file("https://whg-training.github.io/whg-training-resources/R-intro/R\_introduction\_to\_Basic\_Fe

Now go to the 'File' menu and select 'Open', then navigate to and select that file (it's likely to be in 'Downloads'). You'll see this entire document appear in the top left panel. The snippets of R code each have their own little run button (the green arrow on the right) that lets you run that section (called a 'chunk') with one click.

Surrounding a section of an .rmd file with triple backticks (" $\{r\}$ ) tells RStudio that this is a section of code. If you're copying or typing these examples into the R console you shouldn't include this decoration.

Now you have this course open you can follow it from inside RStudio. Feel free to work through the material at your own pace. As well as example code snippets to run, there are questions to think about and try your own R code out on. You're not expected to answer these questions before moving on to the next section.

#### R command syntax

The following few commands give some sense of how R stores information in objects and how simple manipulations of data can be performed. The # symbol indicates a comment. We'll sometimes use them here to show what R's output is without needing to run the command.

The first example assigns the value 1 to a new object we create and name x.

```
x <- 1
```

The assignment operator is  $\leftarrow$  and running this command creates a new object named x in R's memory. If you look in the 'Environment' panel in the top right, you'll see that an entry for x has appeared. To inspect the contents of the new object in the console, we simply type its name:

```
## Inspect the contents of the new object
x
## [1] 1
```

The # symbol is a comment. R will ignore everything after that symbol. It's being used here to show the expected output of the command.

Note that the contents of an object will be overwritten – without any warning message – if later assigned a different value:

```
x <- 4
# x now contains the value 4
x
## [1] 4</pre>
```

We can also perform operations directly on the object:

```
x * 2
## [1] 8
# x still contains the value 4
x
## [1] 4

## unless we were to re-assign the output back to the same object:
x <- x * 2
# x now contains the value 8
x
## [1] 8</pre>
```

**Question** How would you get R to calculate 3x - 2?

R is case sensitive so x and X are different:

```
X
## An error
```

Trying to run this command will produce an error message: Error: object "X" not found. Object names are chosen by the programmer – informative names are helpful for several reasons. You can use capitalization, \_ or . to separate parts of an object name but they cannot contain spaces, nor start with a number. To avoid confusion or potential issues, it is also best not to give them the same name as an R function, which have their own defined names. An object named raw\_data, raw.data, rawData (or even d.raw for minimal typing!) is fine, but trying to assign a value to a variable named raw data will give an error because R cannot parse it correctly.

Elsewhere R ignores whitespace so the following commands are equivalent:

```
x < -4+3

x < -4+3
```

Now we will assign a set of numbers to our variable x, which R will store as a vector. A vector is a series of different data values.

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

The [1] part of the output is R's way of displaying vectors. The number in square brackets is the index of the first entry in the line. If the output is long enough that it spans more than one line, this makes it easier to work out the index of entries on that line. Note that in R indices start at 1 (instead of 0 as in most other programming languages).

Question How might you create the vector 2, 4, 6, 8, 10 from the object x?

If we forget the closing bracket before pressing enter, then R can detect that the command is incomplete. It gives us a different prompt: +. This indicates that R is waiting for you to complete the command.

```
x <- c(1, 2, 3, 4, 5
## + appears as the prompt
)</pre>
```

If we can't simply continue our command, use Esc or control-C to cancel the input so far and return to the > prompt and start again.

Rstudio and some other text editors highlight different parts of the syntax in different colours and automatically close brackets and quotation marks to help eliminate typing mistakes.

### R Objects

Objects can be created in many different ways and hold different kinds of information. Unlike other programming languages, there is no need to initialise a variable or object in R (i.e. define it before first use) – it can simply be created and used directly. R also automatically decides which of its data structures and types are most appropriate for the data given, rather than being explicitly specified by the programmer.

We'll work through some examples and look at ways to access or manipulate the data contained within an object. Be aware that the type (class) of an object and data it contains (numeric, character etc) can affect how it is treated by R.

There are many shortcuts in R to avoid tedious or error-prone steps. When we created our small example vector containing the numbers 1 to 5, we issued this command:

```
x <- c(1, 2, 3, 4, 5) # the c function tells R to concatenate these 5 numbers ## equivalently we could use this command x <- 1:5 # handy if we needed a much longer vector such as 1 to 100, or 1 to 100000
```

The: symbol is special syntax that tells R to make a sequence of whole numbers from one value to another. We can also put together non-consecutive strings of numbers or a mixture

```
x2 <- c(1, 3, 5, 7, 9)
x3 <- c(1:5, 7, 9, 10:15)
```

If we need to create a sequence of numbers, the function seq is very useful.

```
seq1 <- seq(from=1, to=99, by=2)
seq2 <- seq(from=0, to=1, by=0.01)</pre>
```

You can look at all of these objects in the 'Environment' panel to see what you get when you use these commands.

**Question** How might you create a sequence of numbers from 100 to 1? It can be done with the seq function and using: syntax; can you find both ways?

### Using functions

Functions are defined blocks of code that perform a particular task. R has many hundreds of in-built functions for common tasks, and they can be used by 'calling' the function by its name. Above, we used the function seq; the function name is followed by any arguments in parentheses – the arguments determine exactly what the function does and enable it to be useful for many situations. By changing the arguments, we changed the sequence of numbers that was produced. Some arguments are required and others are optional or may have default settings defined in the function. Documentation about the usage of any R function is given in the corresponding help page:

```
help(seq)
```

help is, itself, a function that opens up the help file for a given names function. Note that the help file is opened in the lower right panel of RStudio.

### Vectors

Vectors are one-dimensional objects; in the case of the object  $\mathbf{x}$  we created earlier, it has length 5. There is an in-built R function called length that we can use to check how long any given vector object is:

```
length(x)  
## [1] 5  
## If we change what is assigned to x, the length of the vector is automatically adjusted: x <-1:10  
length(x)  
## [1] 10
```

Vectors are R's primary object type and many computations in R are highly efficient because they operate on the whole vector at once, rather than element by element.

Vectors can contain numeric or character data (or both). We can create a new vector, y, containing the letters 'a', 'b', and 'c'.

```
y <- c("a", "b", "c")
```

Note how x and y are displayed differently in the 'Environment' panel. We can also see how R has automatically treated them differently by checking the class of the objects directly:

```
class(x)
## [1] "integer"
class(y)
## [1] "character"
```

R has as family of functions as.integer, as.character, and so on to convert from one class to another.

**Question** Can you think of value you can give an object so that it's class is neither 'integer' nor 'character'?

Now try running the following command:

```
y <- c(a, b, c)
## An error
```

What do you think R has tried to do and why does it result in an error message?

### Accessing elements of a vector

Square brackets ([ and ]) are used to access specific elements or subsets of a vector, factor, matrix or data frame. Let's create a new vector as an example:

```
x <- c(1:5, 10:14)
## now extract 3rd element
x[3]
## or extract alternate elements
x[c(1, 3, 5, 7, 9)]
## or extract subset of elements
x[3:6]</pre>
```

The second and third examples above show how R can be powerful by working with vectors. Instead of having to use square brackets to extract each element of the vector, and then using c to combine them into a new vector, we use a vector *inside* the square brackets and R interprets that as an instruction to return a vector of those elements of x.

**Question** How would you return the elements of x in reverse order? How would you extract the elements of x where the index is a square number?

**Question** Can you predict what R will do in each of these cases: x[11], x[-3]?

R will decide the most appropriate way to store the data it is provided with, including when you mix different classes in a single vector. To give more examples of how data is interpreted by R, run the following and note the results.

```
x2 <- c(1:5, 6.5)
class(x2)
## [1] "numeric"
x3 <- c(1:5, 6.5, "a", "b", "c")
class(x3)
## [1] "character"</pre>
```

This gives some idea of R's internal rules. Because the way data is being handled by R is important for both performing computations correctly and the source of many error messages, it is useful to be familiar with the common data types. Some functions, such as computing a mean for example, require numeric data objects to operate on. What happens when you try to find the mean of x2 and x3?

```
mean(x2)
mean(x3)
```

### Functions and packages

There are hundreds, probably thousands, of in-built functions in R. Some you will use very often and others rarely or never. There are always several ways to do the same thing in R, using closely-related functions.

Examples of the functions we have used so far include length, mean, and class. In the case of length, the argument supplied is the name of the object we wish to find the length of. We can check the length of another vector object simply by changing the argument.

The length function is only applicable to vectors (or factors) and does not work on other data types such as matrices or data frames, which are 2-dimensional. Often, data is stored in a table format (e.g. in Excel), and commonly handled as a 'data frame' in R. We will introduce these ideas in the next section.

There are many thousands of additional functions available to R through something called 'packages'. Each package is a self-contained parcel of functions created for a specific application. For example, 'Bioconductor' is a repository of many packages related to handling data in bioinformatics. Because it would be confusing to always have all these functions available (and because some packages may end up using the same function names) you need to specifically tell R to make a package available. This is done using the <code>install.packages</code> and <code>library</code> functions:

```
## Install the package for your R environment
install.packages("ggplot2")
## Make the package available to use
library(ggplot2)
```

The first function might prompt you to choose from a list of 'mirrors', which are just different places on the internet where the packages are stored. It doesn't much matter which you choose, although choosing one close to where you currently are is recommended.

Note that when installing the package, you need to treat the package name as character data, inside double-quotes. Once the package has been installed, R is aware the package exists and can interpret the name without quotes when you use library. Installing packages is something you only need to do once per computer (in theory, at least). You must library in each new R session before trying to use any functions from the package; as this is what tells R to look in that package for function names (again, to try and handle duplicate names in different packages gracefully). If you save your R session and come back to it later, you will need to use library again to make those functions available – which packages you have used library on is not saved with the data.

### Working directory

An important concept before we get started is the working directory. Your R session is active in one particular directory on your computer – by default your 'home' directory, as defined by the operating system.

When you save an R session, the .RData file is saved there. When you start a new R session in a particular directory, any .RData file is re-opened in that session. If is also possible to load and save these session files from within RStudio.

It is useful to set the working directory to the place where you have the files you want to work with. It means you have to do less typing to load the save files and you can keep the R version of the data alongside the original files.

We need to set a working directory for the rest of the workshop. From RStudio's Session menu, select 'Set working directory', and then 'Choose directory...' Create a new directory to work in and select that. You can change the working directory using the R function setwd, but it's more convenient to use RStudio's interface.

You will also need to download two data files by entering the following commands:

```
download.file("https://whg-training.github.io/whg-training-resources/R-intro/inflammation_data.csv", ".
download.file("https://whg-training.github.io/whg-training-resources/R-intro/sample.csv", "./sample.csv
```

These will download files into your current working directory.

### Removing objects

Sometimes you are testing things out and creating lots of objects – that's fine but it's always good to start a new session when running or checking your final code to be sure previous objects do not affect it in any way. Sessions can also be cleaned up by deleting objects with the rm command:

```
rm(x3)
```

### **Break**

This is an excellent time to take a short break. Stretch your legs, get a drink.

# Workshop part 2

Earlier we introduced the R software environment, some key features of the R programming language and how to start using it. We will now do some practical exercises working with example data to perform typical tasks. First, some important acknowledgements:

The tutorial that follows is in part adapted from the Software Carpentry Foundation Programming with R, specifically the Analysing Patient Data tutorial. The Software Carpentry material is available for re-use under a Creative Commons License and we are grateful to the original authors.

### Setup

We set up our R session for this practical before the break. You can check the current working directory with the following command:

```
getwd()
```

The output (directory path) should match the location you are working in today, and this folder should also contain the files named inflammation\_data.csv and sample.csv.

### General Helpful Tips

- Enter your commands in the top-left panel of RStudio (a text editor) as this means they can be saved to keep a record of what you have done. Use the 'Run' to check that they run and do what you expect.
- To save your script, click on the disk icon in the same toolbar as the 'Run' button. Giving a filename with a .R extension, such as 'R\_course\_code.R', saves it as an R script file. The .R suffix lets RStudio know that the file is meant to be an R script.
- Any scripts you make are separate files to the session data that is saved when you close R, but RStudio will reload whateer files you had open when you restart it.

This is the usually the easiest way to work; if you need to close your session and return to it another time, it is easy to run the code again from start to finish. All of the code stored in a script can be executed in R from start to finish with the command:

```
source("script_name.R")
## This file doesn't exist so will cause an error
```

You may recognise the function name source from the button in the editor panel that does the same thing.

### Reading in data from a file

The first thing we need to do is load or read in the data from our files so it is accessible in the current R session. There are a few possible ways to do this but we will use the function read.csv because our files are saved in 'comma-separated values' or csv format. To find out details of how to use this function you can search RStudio's Help (bottom right panel) or type:

```
help(read.csv)
```

The help page shows us the arguments for this function and their default values where applicable. For example, we see sep=",", which means the fields in a row will be separated on commas, and header=TRUE, which means it is expecting the first row of the file to contain names for each column. The first file we will work with is the one named 'inflammation\_data.csv'. If we inspect this file in Excel or a text editor (by opening it directly from its location on your computer) we see there are no column names, just a large set of

numerical values. Therefore, we need to explicitly include the header argument in our command, changing it to FALSE to over-ride the default behaviour of the function. This is an example of how arguments modify a function's precise behaviour, rather than requiring two separate functions to exist for files with/without header rows.

```
inf.data <- read.csv("inflammation_data.csv", header=FALSE)</pre>
```

If you get an error here, it might be because: - This command was mistyped - The earlier command to download the file was mistyped, resulting in a file with a different name or a missing file - Your current working directory is not the one where the file was saved

You can use the file browser in RStudio to look for the file, and set your working directory to what it needs to be, or move or rename the files so the command works.

It's useful to break this command down a little more.

We have given a name for a new object, inf.data, in which to store the contents of the file 'inflammation\_data.csv'. Our object name is descriptive without being too long, shortening 'inflammation' to 'inf' for our convenience. We use the in-built R function read.csv, and provide two arguments:

- the name of the file to read in
- header=FALSE, indicating to R that our file does not have a header row

There are many other arguments to the read.csv function to further refine its behaviour but these are either optional or the default settings are fine for most situations. By running this command, R creates the object inf.data and information about it appears in the 'Environment' panel of RStudio.

Notice that, unlike the vectors we've created before, that it doesn't show all the data stored in the object. It is detailed as 60 obs. (observations) of 40 variables and if you hover the mouse pointer over the name, inf.data, it indicates that the object is a data.frame, which is how R stores tabular data by default.

If you double-click on the object name or click on the spreadsheet icon to the righthand side, the contents of the object are loaded in the top-left panel in a new tab. The columns have been automatically named by R as V1 through to V40, as column names have to start with a letter. The rows are simply numbered – the only restriction to row names is that they must be unique.

What do you think will happen if you run the command above without including the header argument? Try it by saving the contents into a new object called test and compare this to inf.data:

```
test <- read.csv("inflammation data.csv")</pre>
```

Another object named test now appears. View the contents of that object and compare it to inf.data.

This is a good example of how easily something unwanted can happen in R and the importance of checking your objects contain what you intend them to. Any mistakes can simply be corrected by re-running the command e.g. with the appropriate header argument, and overwriting any previous version of the object.

#### Two-dimensional data structures

Our original file contained rows and columns of data, and R has suitable 2-dimensional data structures to store such data: matrices and dataframes. These can both be thought of as tables of data, analogous to an Excel spreadsheet. Matrices require all columns to be of the same type, while data frames can have columns of different data types. Given that experimental data is often a mixture of numeric values (e.g. measurements) and associated descriptive information, data frames are a very commonly used data structure in R.

NB while it is possible to create a matrix object with mixed data types, R will use its internal hierarchy of data types to choose one that is applicable to all of the data – often this means numerical columns get converted to character strings, and certain functions may not perform as expected if this is not noticed, or they may produce an error message.

In this case, a data frame object has been created:

```
class(inf.data)
```

We can check how each column of data has been treated by R, e.g. for the first column:

```
class(inf.data[, 1])
```

In fact, as all the columns contain data of the same type, R could equally well store this data as a matrix object. A data frame has been created because the functions read.csv and read.table are specifically designed to deal with mixed column classes and produce data frames by default. Another function scan can be used to read in matrices, especially large ones.

It is worth noting that some functions operate on matrix objects, and so converting between classes is sometimes needed. 'matrix' is a class of data that R recognises, separate to 'integer' or 'numeric'.

```
inf.data <- as.matrix(inf.data)
class(inf.data)</pre>
```

We will continue with the matrix form of this object for now, and load a mixed dataset later. Most operations on either kind of 2-dimensional object are the same. For example, we can find the dimensions of a matrix or a data frame with the dim function.

```
dim(inf.data)
```

The output of dim is printed to the screen and shows the number of rows the object contains, followed by the number of columns (the convention is always rows, then columns but this can be hard to remember at first as there is no indication of which way round it is in the syntax).

If unsure, the functions nrow and ncol will return the number of rows or columns respectively:

```
nrow(inf.data)
ncol(inf.data)
```

This particular file suffers from the lack of any labels to annotate what data is recorded in the rows and columns. The Software Carpentry tutorial provides the following information:

"We are studying inflammation in patients who have been given a new treatment for arthritis. Each row holds the observations for just one patient. Each column holds the inflammation measured in a day, so we have a set of values in successive days."

Our object has 60 rows and 40 columns, so we infer from the information above that there are 60 patients, and 40 days.

Again we see a way for mistakes to easily creep into data analysis – here we have to rely on information given to us second-hand to know what is what. What if that information were wrong? Are there any checks we can make ourselves to be sure patients are in rows? We are not told how many patients were included so simply checking the number of rows won't help. And what if there were 50 patients and measurements taken over 50 days?

Even with careful scrutiny it would be hard to know how the data are presented (patients in rows or columns) from the data alone. We could perhaps make some plots to help us, or we might spot the zero values in the first column. Scrolling down the object display in the top-left panel or displaying the first column in the console confirms they are all zeroes, and the values in each row tend to rise across the first few columns. We may be reassured by this that the patients are indeed in the rows, since we might expect inflammation to rise over time, and an individual recording 0 on every single day might be unlikely (though not impossible).

It would be prudent to add our own row and column names to reduce the chance of making a mistake later when dealing with this data:

```
rownames(inf.data) <- paste("Patient", 1:60, sep="_")
colnames(inf.data) <- paste("Day", 1:40, sep="_")</pre>
```

This introduces the very useful and versatile function paste. It creates new vectors of strings based on concatenating its arguments. In this case, it's concatenating the character object "Patient" and a vector of numbers. paste, and R in general, will try to 'recycle' shorter vectors to match longer ones in this kind of situation. So the length 1 character vector "Patient" is turned into a length 60 vector where each element is the same word. The sep argument tells paste what character(s) to use when concatenating. By default it's a space but that can be awkward to work with later.

Note that adding row and column names does not change the size of the data object. Names are meta data attached to the underlying object, not part of the object itself. We can see them when the object is displayed.

### Accessing data

Earlier we looked at accessing elements of a one-dimensional vector object. For matrices and dataframes, a similar approach with square brackets is used:

```
object_name[rows, cols]
```

By specifying the rows and columns of interest, an object can be subset in a variety of ways to inspect or extract different parts of it.

```
inf.data[1, 1] # this pulls out the data value in the first row of the first column inf.data[30, 20] # any single entry can be extracted by specifying the row and column
```

Think about how might you select the data in the first 5 rows for the first 5 columns.

```
## If we need to select non-contiguous portions of the object, we'll need the help of c():
inf.data[c(1, 3, 5), c(10, 20)]

## If you want to display all columns for selected row(s), leave blank space after the comma:
inf.data[5, ] # All columns for row 5

## Or blank space before the comma to select all rows for given column(s):
inf.data[, 1:5] # all rows, columns 1 through 5
```

Question Can you infer what will happen with inf.data[,]? Can you produce the matrix with the patients in reverse order?

When you select a single column from a data frame or matrix, R will 'helpfully' turn it into a vector:

```
test <- inf.data[, 2] # Select the second column
class(test)</pre>
```

This can cause errors if you were expecting test to still be 2-dimensional in a later command.

We added column names to our object earlier. Rows and columns can also be accessed by name (in double-quotes) inside square brackets. These methods work for any two-dimensional class of data in R, but data frames specifically have another: \$ is a shorthand way of referring to a named column. Both alternatives in the snippet below return the same vector.

```
inf.data[, "Day_3"]
tmp <- as.data.frame(inf.data)
tmp$Day_3</pre>
```

Another wrinkle here is that the output of the two versions is slightly different. When you extract a column from a matrix, the resulting vector retains the names given to the rows. However, when you extract a column from a data frame, the resulting vector does not.

**Question** Suppose you want to determine the maximum inflammation for patient 5 across days three to seven. To do this you would extract the relevant subset from the data frame and calculate the maximum value. Which of the following lines of R code gives the correct answer?

```
    max(inf.data[5, ])
    max(inf.data[3:7, 5])
    max(inf.data[5, 3:7])
    max(inf.data[5, 3, 7])
```

### Analysing data

We can perform many simple analyses of the data by applying functions such as max, min, mean, or summary to our data object. We might want to determine the maximum value per patient or the average value per day. The following examples illustrate how this can be done extremely efficiently in R, starting with an approach that is the opposite (and definitely not recommended!).

Suppose we want to find the maximum inflammation score for each patient across the 40 days of measurements. Let's start by calculating it for patient 1.

Extracting the data for patient 1 (i.e. the first row) is the first obvious step, and perhaps we decide it makes sense to store the values for this patient in a new object:

```
inf.patient1 <- inf.data[1, ]</pre>
```

We can then calculate the maximum value for Patient 1:

```
max(inf.patient1)
```

Although this seems reasonable enough, there are several issues:

- We've created an additional object to store data that is simply a duplicate of what is already contained in our original object
- It doesn't scale well to do this for all 60 patients
- The result is output to the console and therefore hard to do anything further with

If we did continue with this approach, there would be 60 new objects (all with very similar names), a high probability of having made a typing mistake somewhere (perhaps overwriting one patient's data with another), and a large set of results that we'd have to manually write down or transfer to an Excel spreadsheet – all of which is very messy and prone to error.

We can easily dispense with the intermediate step of creating a new object:

```
# max inflammation for patient 1
max(inf.data[1, ])

# or equivalently
max(inf.data["Patient_1", ])
```

These commands are the same as extracting the data for patient 1 as we did earlier, but instead of printing to the screen or storing in a new object, the command is used directly as an argument to the function max by enclosing in the parentheses.

Commands can be nested in this way to achieve multiple steps in a single line of code; too many commands in one line though can make it harder to work out what the code is doing, as well as increase the chance of the code not doing as intended – the location of brackets becomes vital.

We'd really like a way to this for all 60 patients without duplicating the code 60 times. Loops are one option (not discussed here) but the apply function is the most efficient approach:

apply allows us to repeat a function on all of the rows (MARGIN = 1) or all of the columns (MARGIN = 2) of a data frame simultaneously:

```
max_inf_patient <- apply(inf.data, MARGIN=1, max)</pre>
```

Similarly, we could compute the average inflammation per day with a single line of code:

```
avg_inf_day <- apply(inf.data, MARGIN=2, mean)</pre>
```

Comparing these two commands will help understand the apply function, which is not intuitive but highly efficient. The arguments to apply are:

- The data object
- MARGIN, indicating whether to apply over rows (1) or columns (2)
- The name of the function to be applied (functions can sometimes be treated as objects when used as arguments for other functions)

We wanted to find the maximum inflammation score for each patient, so we looked across the rows and used the max function. To modify the command to find the average inflammation per day, we switched the MARGIN argument to 2 for columns, and gave the final argument as mean.

While the MARGIN argument is explicitly assigned above, R is equally happy to infer from the shortened command apply(inf.data, 1, max) that the 1 should be assigned to the second defined argument of apply. You can also write your own bespoke functions as required and use apply to run them over an object.

We have also solved the final issue with our initial approach by storing the results in suitably-named objects for further work.

Question What functions other than mean and max can you use with the apply function? Looking at the help files for the functions might suggest some.

```
length(max_inf_patient)
head(max_inf_patient)
```

head is a function that shows the first six elements of a vector or the first 6 rows of a data frame.

Another useful function is summary. This returns the minimum value, first quartile, median, mean, third quartile and the maximum value, all very useful information to make an initial inspection of your data.

```
summary(inf.data[, 1:4]) # for each of the first 4 days
```

Question What happens when you use summary with apply?

#### Handling data with factors

We'll next read in data from another file to illustrate a few more features of data frames and how to work with them in R. In this case the file does contain a header row and the default arguments for read.csv are appropriate for this file so we only need provide the filename:

```
data2 <- read.csv("sample.csv")
head(data2)</pre>
```

This displays the first 6 rows, and we can see immediately that we have a range of different types of data in each column. Let's see how R has treated it (you can paste the following 4 lines as one block).

```
for(i in 1:ncol(data2)) {
    print(class(data2[, i]))
}
```

Here, we've used a for loop to iterate over each column in the object data2, and print to screen the class of each column. The output tells us that columns 1:3 are treated as characters, and most of the rest are treated as integers. Column 5 is 'numeric', the basic data type in R for non-integer numbers.

Although columns 1-3 are currently characters, some functions in R that perform statistical analysis prefer a data type called 'factors'. Factors are useful when you have category data, where the character data can only come from a well defined set of possibilities. Factors can be useful when you want to make sure incorrect values don't creep in accidentally, because R will report an error if factor data tries to be changed to an illegal value.

We can specifically convert the Group column to a factor:

```
data2$Group <- as.factor(data2$Group)
class(data2$Group)</pre>
```

The as.factor function returns a factor version of a vector. However, as this doesn't change the original data2 object by itself, we need to assign that vector of factors back to the column, replacing what was there before. Indices (and by extension, the \$ syntax) in R can be used when assigning values to objects, not just when extracting them. You can see how using the \$ syntax makes this line easier to read.

**Question** If you used square brackets instead of the \$ notation for the above, how would you do it?

A very useful summary function is table:

```
table(data2$Group)
table(data2$Gender)
```

This alerts us to the fact that data in the Gender column has not been entered consistently, which you might have already spotted from viewing the object in RStudio.

To fix this, we can make sure F and M are used throughout; this involves determining which rows contain a lowercase f for example, and substituting with F. Similarly for the lowercase case m. The following code does each a different way.

```
data2$Gender <- gsub("f", "F", data2$Gender)
data2$Gender[data2$Gender=="m"] <- "M"

## checking we have modified the data as intended
table(data2$Gender)</pre>
```

The gsub function replaces the first argument "f" with the second argument "F" in the third argument data2\$Gender wherever it appears, leaving the other entries unchanged. As before, we need to assign this new vector back to the original object.

The second line shows one of R's particularly useful features. We're telling R to replace values in data2\$Gender, but only where those values equal "m".

This is one example of how there can be multiple ways of doing a particular task in R. When deciding which method to use, in general, it is better to stick with the method you understand the best as that's the method where you are least likely to make a mistake. Some mistakes will cause R to generate an error, but the more dangerous mistakes are ones that don't result in an error but result in incorrect data.

**Question** Look at the help file for the function ifelse. Can you work out how you would to the above correction using that instead of either of those methods?

#### Plotting data

Visualising data is a vital part of statistical analysis, and R's plotting capabilities are a key reason for its popularity. Here, we introduce ways to make a few simple plots. Let's go back to our first table of data and

take a look at the average inflammation over time. Recall that we already calculated these values above and saved them in an object named avg\_inf\_day. Plotting the values is done with the function plot:

```
## Default labels and settings are used but we can refine our plot with some additional arguments:
plot(avg_inf_day, main="Inflammation Scores Over Time", xlab="Day", ylab="Average_inflammation_score")
plot(avg_inf_day, main="Inflammation Scores Over Time", xlab="Day", ylab="Average_inflammation_score",

## Similarly, we could plot the data per patient:
plot(max_inf_patient)

## Here, we might decide to use a boxplot instead:
boxplot(max_inf_patient, main="Maximum Inflammation Scores", ylab="Max_inf_score")
legend("topright", legend="n=60 patients", cex=0.8) # adding a legend
```

If you run this code in RStudio from the .rmd file, the plots will be displayed in amongst the text document. If you ran them in the console, then the most recent one will be visible in the 'Plots' panel in the bottom right. It also contains a history of all the plots you've made using the console that you can flick through using the arrow buttons. In vanilla R, a new plot will replace the existing one unless you create a new window for it.

When we are happy with our plots, they can be saved to a file.

```
pdf("Inflammation_plots.pdf", onefile=T)
plot(avg_inf_day, main="Inflammation Scores Over Time", xlab="Day", ylab="Average_inflammation_score",
boxplot(max_inf_patient, main="Maximum Inflammation Scores", ylab="Max_inf_score")
legend("topright", legend="n=60 patients", cex=0.8)
dev.off()
```

This will be saved to the current working directory by default so if we check the folder, a new file named 'Inflammation\_plots.pdf' should have been created. The onefile=T argument instructs R to append additional plots to the same file and the dev.off command at the end closes the file connection. Note that the file is not updated each time you run a new plot command; it's only when the file is closed with dev.off that anything is actually written to it. You can also export plots directly to a pdf file from the RStudio 'Plot' panel.

**Question** Create some other plots based on this data using the examples as templates to work from, and save them as a new pdf file. Can you find a way of saving individual plots as .png files?

### Bioconductor

Bioconductor is a separate third-party repository of R code, specifically geared towards bioinformatics. Many of the packages in Bioconductor are also available via R's usual package installation method. However, Bioconductor has its own preferred installation mechanism which gets around some of R's more annoying limitations with version incompatibilities. In particular, the packages in Bioconductor are updated more swiftly following the release of a new version of R. To get started with Bioconductor, use the following command:

```
install.packages("BiocManager")
```

This installs the package used for managing Bioconductor tools. You can then install Bioconductor packages like this:

```
BiocManager::install("limma")
```

Loading Bioconductor packages this way is typically a longer process but more reliable. (If the console asks you if you want to update existing packages, press 'n' and 'return'.)

# Getting help

As you read the R help pages, you may not find them terribly helpful. The R help function is most useful for refreshing your memory about specific functions you have used before. Unfortunately, it is not very useful for learning the how to use the functions in the first place.

There are several online forums used by R programmers, novice and expert, to get help and advice from their peers. Searching for your R problem will often give results from one of these forums.

One page 'quick reference' documents:

- R cheat sheet
- R reference card

# Further resources and useful information

The Software Carpentry Foundation website contains many tutorials for learning a variety of programming languages, including R. There is also a series of domain-specific Data Carpentry courses, which focus on computational skills needed to handle and analyse data – tutorials are currently available for Ecology, Genomics, Geospatial Data and Social Sciences, with others in development.

- Software Carpentry lessons
- Data Carpentry lessons

In particular, the tutorials at the following links give further details and examples on some of the ideas already introduced or extend to other topics once you feel comfortable interacting with the R environment.

- Data structures
- Read and write csv files
- Factors
- Addressing data
- Best practices
- R genomics
- Geospatial data

# Epilogue: good programming habits

- 1. Break it down into small steps
  - Write code to do individual parts of your overall task and build it up into a larger script
  - This makes it easier to get right and test as you are going along
  - It is also a good way to solve errors/bugs in your code
- 2. Pay attention to details and program carefully
  - Commands must be accurate both in their syntax and what they do
  - It is easy to overwrite objects, pull out wrong subsets of data, mix up sample names etc.
  - To avoid this, you need to check carefully all the time what you are working with and what R produces after you run a command
  - Inspect object contents, make plots, test examples can all helpGood programming habits
- 3. Test your code thoroughly
  - You are the only person who can make sure it is doing what you intend it to do
  - R will give an error if a command is incorrectly specified according to the rules of the language but it can't tell you if the command you've written does not do what you think it does...
  - ... and it will go ahead and execute it regardless, because that is what you have instructed R to do!
- 4. Comment your code
  - Comments are really useful to jog your memory if you come back to some code after a while or want someone else to understand it.

# Finally...

Please let us know if you have any questions and good luck on your programming journey!

We are happy to help anytime: bioinformatics@well.ox.ac.uk  $\,$