

# Contents

1	Introduction	2
2	Starting out in R	3
	Variables	3
	Vectors	5
	Functions	5
	Lists	7
3	Working with data in a matrix	8
	Loading data	8
	Challenge - Slicing (subsetting) data	13
	A t-test or two	13
	Challenge - using t.test	14
	Plotting	15
	Challenge - Plotting data	16
	Saving plots	17
4	Working with data in a data frame	18
	Indexing data frames	19
	Factors	19
	Missing data	23
	Summarizing data frames	24
	Melting a matrix into a data frame	24
	Merging two data frames	25
5	For loops	<b>2</b> 6
6	Plotting with ggplot2	30
7	Next steps	35

# Introduction

These are the course notes for the "Introduction to R" course given by the Monash Bioinformatics Platform on the 30th of November 2015.

R is both a programming language and an interactive environment for statistics. Today we will be concentrating on R as an interactive environment.

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 $<sup>\</sup>frac{^{1} \rm http://software\text{-}carpentry.org/}{^{2} \rm https://creative commons.org/licenses/by/4.0/}$ 

# Starting out in R

Working with R is primarily text-based. The basic mode of use for R is that the user types in a command in the R language and presses enter, and then R computes and displays the result.

We will be working in RStudio<sup>1</sup>. This surrounds the *console*, where one enters commands and views the results, with various conveniences. In addition to the console, RStudio provides panels containing:

- A text editor, where R commands can be recorded for future reference.
- A history of commands that have been typed on the console.
- A list of variables, which contain values that R has been told to save from previous commands.
- A file manager.
- Help on the functions available in R.
- A panel to show plots (graphs).

Open RStudio, click on the "Console" pane, type 1+1 and press enter. R displays the result of the calculation. In this document, we will be showing such an interaction with R as shown below.

1+1

#### ## [1] 2

\* has higher precedence than +. We can use brackets if necessary ( ). Try 1+2\*3 and (1+2)\*3.

Spaces can be used to make code easier to read.

We can compare with == < > <= >=. This produces a "logical" value, TRUE or FALSE. Note the double equals, ==, for equality comparison.

There are also character strings such as "string".

#### Variables

A variable is a name for a value, such as x, current\_temperature, or subject\_id. We can create a new variable by assigning a value to it using <-

```
weight_kg <- 55
```

RStudio helpfully shows us the variable in the "Environment" pane. We can also print it by typing the name of the variable and hitting Enter (or return). In general, R will print to the console any object returned by a function or operation *unless* we assign it to a variable.

<sup>&</sup>lt;sup>1</sup>https://www.rstudio.com/

```
weight_kg
```

#### ## [1] 55

Examples of valid variables names: hello, hello\_there, hello.there, value1. Spaces aren't ok *inside* variable names. Dots (.) are ok, unlike in many other languages.

We can do arithmetic with the variable:

```
# weight in pounds:
2.2 * weight_kg
## [1] 121
```

#### Tip

We can add comments to our code using the # character. It is useful to document our code in this way so that others (and us the next time we read it) have an easier time following what the code is doing.

We can also change an object's value by assigning it a new value:

```
weight_kg <- 57.5
# weight in kilograms is now
weight_kg
## [1] 57.5</pre>
```

If we imagine the variable as a sticky note with a name written on it, assignment is like putting the sticky note on a particular value:



This means that assigning a value to one object does not change the values of other variables. For example, let's store the subject's weight in pounds in a variable:

```
weight_lb <- 2.2 * weight_kg
# weight in kg...
weight_kg

## [1] 57.5

# ...and in pounds
weight_lb

## [1] 126.5

57.5

126.5

weight_kg

weight_lb</pre>
```

and then change weight\_kg:

Since weight\_lb doesn't "remember" where its value came from, it isn't automatically updated when weight\_kg changes. This is different from the way spreadsheets work.

### Vectors

We can make vectors with c(), for example c(1,2,3), and do maths to them. c means "combine". Actually in R, values are just vectors of length one. R is obsessed with vectors.

```
myvec <- c(1,2,3)
myvec + 1

## [1] 2 3 4

myvec + myvec

## [1] 2 4 6

c(10, myvec)

## [1] 10 1 2 3</pre>
```

Access elements of a vector with [], for example myvec[1].

We will also encounter character strings, for example "hello". R also has something called "factors", which are categorical vectors, and behave very much like character vectors (think the factors in an experiment). R likes turning character vectors into factors, which is usually fine.

### **Functions**

R has various functions, such as sum( ). We can get help on a function with, eg ?sum.

```
?sum sum( c(1,2,3) )
```

Because R is a language for statistics, it has many built in statistics-related functions. We will also be loading more specialized functions from "libraries" (also known as "packages").

Functions take some number of *arguments*. Lets look at the function rep, which means "repeat", and which can take a variety of different arguments. In the simplest case, it takes a value and the number of times to repeat that value.

```
rep(42, 10)
## [1] 42 42 42 42 42 42 42 42 42 42
```

As with many functions in R, which is obsessed with vectors, the thing to be repeated can be a vector with multiple elements.

```
rep(c(1,2,3), 10)
## [1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
```

So far we have used *positional* arguments, where R determines which argument is which by the order in which they are given. We can also give arguments by *name*. For example, the above is equivalent to

```
rep(c(1,2,3), times=10)
## [1] 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3 1 2 3
```

Arguments can have default values, and a function may have many different possible arguments that make it do obscure things. For example, rep can also take an argument each=. It's typical for a function to be invoked with some number of positional arguments, which are always given, plus more rarely used arguments, typically given by name.

```
rep(c(1,2,3), each=3)
## [1] 1 1 1 2 2 2 3 3 3
rep(c(1,2,3), each=3, times=5)
## [1] 1 1 1 2 2 2 3 3 3 1 1 1 2 2 2 3 3 3 1 1 1 2 2 2 3 3
## [36] 3 1 1 1 2 2 2 3 3 3
```

## Lists

Vectors contain all the same kind of thing. Try c(42, "hello"). Lists can contain different kinds of thing.

We generally gives the things in a list names. Try list(num=42, greeting="hello"). To access named elements we use \$.

```
mylist <- list(num=42, greeting="Hello, world")
mylist$greeting
## [1] "Hello, world"</pre>
```

I'm skipping a lot of details here.

This terminology is peculiar to R. Other languages make the same distinction they may use different words for vectors and lists.

If you're not sure what sort of object you are dealing with you can use class, or for more detailed information str (structure).

# Working with data in a matrix

## Loading data

Our example data is quality measurements (particle size) on PVC plastic production, using eight different resin batches, and three different machine operators.

The data sets are stored in comma-separated values<sup>1</sup> (CSV) format. Each row is a resin batch, and each column is an operator. In RStudio, open pvc.csv and have a look at what it contains.

```
read.csv(file="data/pvc.csv", row.names=1)
```

The expression read.csv(...) is a function call<sup>2</sup> that asks R to run the function read.csv.

read.csv has two arguments<sup>3</sup>: the name of the file we want to read, and which column contains the row names. The filename needs to be a character string (or string<sup>4</sup> for short), so we put it in quotes. Assigning the second argument, row.names, to be 1 indicates that the data file has row names, and which column number they are stored in.

#### Tip

read.csv actually has many more arguments that you may find useful when importing your own data in the future. You can learn more about these options in this supplementary lesson<sup>5</sup>.

The utility of a function is that it will perform its given action on whatever value is passed to the named argument(s). For example, in this case if we provided the name of a different file to the argument file, read.csv would read it instead. We'll learn more of the details about functions and their arguments shortly.

```
dat <- read.csv(file="data/pvc.csv", row.names=1)

dat

## Alice Bob Carl
## Resin1 36.25 35.40 35.30
## Resin2 35.15 35.35 33.35
## Resin3 30.70 29.65 29.20

1reference.html#comma-separated-values-(csv)
2reference.html#function-call
3reference.html#argument
4reference.html#string</pre>
```

 $^501$ -supp-read-write-csv.html

```
## Resin4 29.70 30.05 28.65
## Resin5 31.85 31.40 29.30
## Resin6 30.20 30.65 29.75
## Resin7 32.90 32.50 32.80
## Resin8 36.80 36.45 33.15

class(dat)

## [1] "data.frame"

str(dat)

## 'data.frame': 8 obs. of 3 variables:
## $ Alice: num 36.2 35.1 30.7 29.7 31.9 ...
## $ Bob : num 35.4 35.4 29.6 30.1 31.4 ...
## $ Carl : num 35.3 33.4 29.2 28.6 29.3 ...
```

read.csv has loaded the data as a data frame. A data frame contains a collection of "things" (rows) each with a set of properties (columns) of different types.

Actually this data is a matrix. In a data frame the columns contain different types of data, but in a matrix all the elements are the same type of data. A matrix in R is like a mathematical matrix, containing all the same type of thing (usually numbers).

R often but not always lets these be used interchangably. It's also helpful when thinking about data to distinguish between a data frame and a matrix. Different operations make sense for data frames and matrices.

Data frames are very central to R, and mastering R is very much about thinking in data frames. However when we get to RNA-Seq we will be using matrices of read counts, so it will be worth our time to learn to use matrices as well.

Let us insist to R that what we have is a matrix.

```
mat <- as.matrix(dat)
class(mat)
## [1] "matrix"</pre>
```

Much better.

### Tip

Matrices can also be created de novo in various ways.

matrix converts a vector into a matrix with a specified number of rows and columns.

rbind stacks several vectors as rows one top of each other to form a matrix. Or it can stack smaller matrices on top of each other to form a larger matrix.

cbind similarly stacks several vectors as columns next to each other to form a matrix. Or it can stack smaller matrices next to each other to form a larger matrix.

#### Manipulating Data

We can see the dimensions, or shape<sup>6</sup>, of the matrix with the functions nrow and ncol:

<sup>&</sup>lt;sup>6</sup>reference.html#shape-(of-an-array)

```
nrow(mat)
## [1] 8
ncol(mat)
## [1] 3
```

This tells us that our matrix, mat, has 8 rows and 3 columns.

If we want to get a single value from the data frame, we can provide an index<sup>7</sup> in square brackets, just as we do in math:

```
# first value in mat
mat[1, 1]

## [1] 36.25

# a middle value in mat
mat[4, 2]

## [1] 30.05
```

If our matrix has row names and column names, we can also refer to rows and columns by name.

```
mat["Resin4","Bob"]
## [1] 30.05
```

An index like [4, 2] selects a single element of a data frame, but we can select whole sections as well. For example, we can select the first two operators (columns) of values for the first four resins (rows) like this:

```
mat[1:4, 1:2]
## Alice Bob
## Resin1 36.25 35.40
## Resin2 35.15 35.35
## Resin3 30.70 29.65
## Resin4 29.70 30.05
```

The slice 1:4 means, "Start at index 1 and go to index 4." It's the same as c(1,2,3,4).

The slice does not need to start at 1, e.g. the line below selects rows 5 through 8:

```
mat[5:8, 1:2]

## Alice Bob

## Resin5 31.85 31.40

## Resin6 30.20 30.65

## Resin7 32.90 32.50

## Resin8 36.80 36.45
```

 $<sup>^{7}</sup>$ reference.html#index

 $<sup>^8</sup>$ reference.html#slice

We can use vectors created with  $\mathbf{c}$  to select non-contiguous values:

```
mat[c(1,3,5), c(1,3)]

## Alice Carl

## Resin1 36.25 35.3

## Resin3 30.70 29.2

## Resin5 31.85 29.3
```

We also don't have to provide a slice for either the rows or the columns. If we don't include a slice for the rows, R returns all the rows; if we don't include a slice for the columns, R returns all the columns. If we don't provide a slice for either rows or columns, e.g. dat[, ], R returns the full matrix.

```
# All columns from row 5
mat[5, ]

## Alice Bob Carl
## 31.85 31.40 29.30

# All rows from column 2
mat[, 2]

## Resin1 Resin2 Resin3 Resin4 Resin5 Resin6 Resin7 Resin8
## 35.40 35.35 29.65 30.05 31.40 30.65 32.50 36.45
```

Now let's perform some common mathematical operations to learn about our data. When analyzing data we often want to look at partial statistics, such as the maximum value per resin or the average value per operator. One way to do this is to select the data we want to create a new temporary vector (or matrix, or data frame), and then perform the calculation on this subset:

```
# first row, all of the columns
resin_1 <- mat[1, ]
# max particle size for resin 1
max(resin_1)
## [1] 36.25</pre>
```

We don't actually need to store the row in a variable of its own. Instead, we can combine the selection and the function call:

```
# max particle size for resin 2
max(mat[2, ])
## [1] 35.35
```

R also has functions for other common calculations, e.g. finding the minimum, mean, median, and standard deviation of the data:

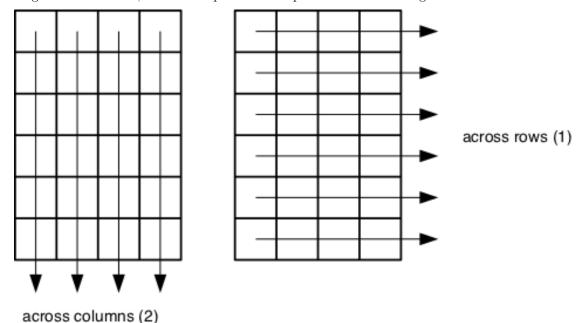
```
# minimum particle size for operator 3
min(mat[, 3])
## [1] 28.65
```

```
# mean for operator 3
mean(mat[, 3])
## [1] 31.4375

# median for operator 3
median(mat[, 3])
## [1] 31.275

# standard deviation for operator 3
sd(mat[, 3])
## [1] 2.49453
```

What if we need the maximum particle size for all resins, or the average for each operator? As the diagram below shows, we want to perform the operation across a margin of the matrix:



To support this, we can use the apply function.

### Tip

To learn about a function in R, e.g. apply, we can read its help documention by running help(apply) or ?apply.

apply allows us to repeat a function on all of the rows (MARGIN = 1) or columns (MARGIN = 2) of a matrix.

Thus, to obtain the average particle size of each resin we will need to calculate the mean of all of the rows (MARGIN = 1) of the matrix.

```
avg_resin <- apply(mat, 1, mean)</pre>
```

And to obtain the average particle size for each operator we will need to calculate the mean of all of the columns (MARGIN = 2) of the matrix.

```
avg_operator <- apply(mat, 2, mean)</pre>
```

Since the second argument to apply is MARGIN, the above command is equivalent to apply(dat, MARGIN = 2, mean). We'll learn why this is so in the next lesson.

### Tip

Some common operations have more efficient alternatives. For example, you can calculate the row-wise or column-wise means with rowMeans and colMeans, respectively.

## Challenge - Slicing (subsetting) data

We can take slices of character vectors as well:

```
animal <- c("m", "o", "n", "k", "e", "y")
# first three characters
animal[1:3]
## [1] "m" "o" "n"
# last three characters
animal[4:6]
## [1] "k" "e" "v"</pre>
```

- 1. If the first four characters are selected using the slice animal[1:4], how can we obtain the first four characters in reverse order?
- 2. What is animal[-1]? What is animal[-4]? Given those answers, explain what animal[-1:-4] does.
- 3. Use a slice of animal to create a new character vector that spells the word "eon", i.e. c("e", "o", "n").

#### Challenge - Subsetting data 2

Suppose you want to determine the maximum particle size for resin 5 across operators 2 and 3. To do this you would extract the relevant slice from the data frame and calculate the maximum value. Which of the following lines of R code gives the correct answer?

```
(a) max(dat[5, ])
(b) max(dat[2:3, 5])
(c) max(dat[5, 2:3])
(d) max(dat[5, 2, 3])
```

#### A t-test or two

R has many statistical tests built in. A classic test is the t test. Do the means of two vectors differ significantly?

```
mat[1,]
## Alice Bob Carl
## 36.25 35.40 35.30

mat[2,]
## Alice Bob Carl
## 35.15 35.35 33.35

t.test(mat[1,], mat[2,])
```

```
##
## Welch Two Sample t-test
##
## data: mat[1, ] and mat[2, ]
## t = 1.4683, df = 2.8552, p-value = 0.2427
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.271985 3.338652
## sample estimates:
## mean of x mean of y
## 35.65000 34.61667
```

Actually, this can be considered a paired sample t-test, since the values can be paired up by operator. By default t.test performs an unpaired t test. We see in the documentation (?t.test) that we can give paired=TRUE as an argument in order to perform a paired t-test.

```
t.test(mat[1,], mat[2,], paired=TRUE)

##
## Paired t-test
##
## data: mat[1,] and mat[2,]
## t = 1.8805, df = 2, p-value = 0.2008
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.330952 3.397618
## sample estimates:
## mean of the differences
## mean of the differences
##
```

## Challenge - using t.test

Can you find a significant difference between any two resins?

When we call t.test it returns an object that behaves like a list. Recall that in R a list is a miscellaneous collection of data.

```
result <- t.test(mat[1,], mat[2,], paired=TRUE)
names(result)

## [1] "statistic" "parameter" "p.value" "conf.int" "estimate"

## [6] "null.value" "alternative" "method" "data.name"

result$p.value

## [1] 0.2007814</pre>
```

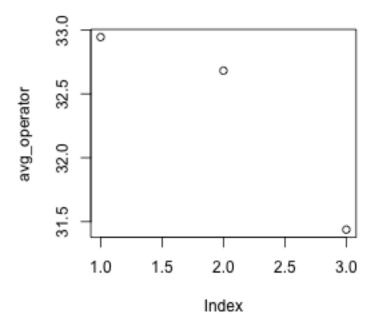
This means we can write software that uses the various results from t-test, for example performing a whole series of t-tests and reporting the significant results.

## **Plotting**

The mathematician Richard Hamming once said, "The purpose of computing is insight, not numbers," and the best way to develop insight is often to visualize data. Visualization deserves an entire lecture (or course) of its own, but we can explore a few of R's plotting features.

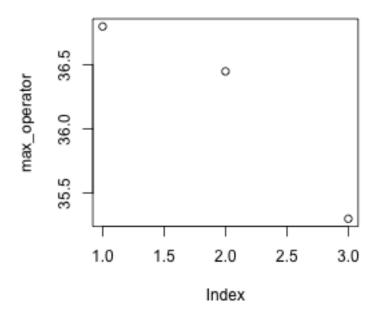
Let's take a look at the average inflammation over time. Recall that we already calculated these values above using apply(mat, 2, mean) and saved them in the variable avg\_operator. Plotting the values is done with the function plot.

plot(avg\_operator)

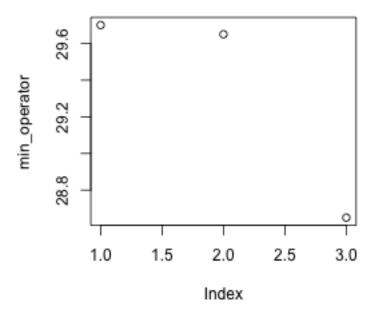


Above, we gave the function plot a vector of numbers corresponding to the average per operator across all resins. plot created a scatter plot where the y-axis is the average particle size and the x-axis is the order, or index, of the values in the vector, which in this case correspond to the 3 operators. Let's have a look at two other statistics: the maximum and minimum inflammation per operator.

```
max_operator <- apply(mat, 2, max)
plot(max_operator)</pre>
```



min\_operator <- apply(dat, 2, min)
plot(min\_operator)</pre>



## Challenge - Plotting data

Create a plot showing the standard deviation of for each operator across all resins.

## Saving plots

It's possible to save a plot as a .PNG or .PDF from the RStudio interface with the "Export" button. However if we want to automate plot making, we need to do this with R code.

Plotting in R is sent to a "device". By default, this device is RStudio. However we can temporarily send plots to a different device, such as a .PNG file (png("filename.png")) or .PDF file (pdf("filename.pdf")).

```
pdf("test.pdf")
plot(avg_resin)
dev.off()
```

dev.off() is very important. It tells R to stop outputting to the pdf device and return to using the default device. If you forget it, your interactive plots will stop appearing as expected!

# Working with data in a data frame

```
As we saw earlier, read.csv loads tabular data from a CSV file into a data frame.
diabetes <- read.csv("data/diabetes.csv")</pre>
class(diabetes)
## [1] "data.frame"
head(diabetes)
##
     subject glyhb
                     location age gender height weight frame
## 1
       S1002 4.64 Buckingham 58 female
                                                    256 large
                                              61
       S1003 4.63 Buckingham 67
                                    male
                                              67
                                                    119 large
      S1005 7.72 Buckingham 64
                                    male
                                                    183 medium
       S1008 4.81 Buckingham 34
                                    male
                                              71
                                                    190 large
## 5
       S1011 4.84 Buckingham 30
                                    male
                                              69
                                                    191 medium
      S1015 3.94 Buckingham 37
                                              59
                                    male
                                                    170 medium
colnames(diabetes)
## [1] "subject"
                  "glyhb"
                              "location" "age"
                                                    "gender"
                                                                "height"
## [7] "weight"
                  "frame"
ncol(diabetes)
## [1] 8
nrow(diabetes)
## [1] 354
    Tip
    A data frame can also be created de novo from some column vectors with data.frame. For
    data.frame(foo=c(10,20,30), bar=c("a","b","c"))
         foo bar
     ## 1 10
     ## 2 20
     ## 3 30
```

#### Tip

A data frame can have both column names (colnames) and rownames (rownames). However, the modern convention is for a data frame to use column names but not row names. Typically a data frame contains a collection of items (rows), each having various properties (columns). If an item has an identifier such as a unique name, this would be given as just another column.

## Indexing data frames

As with a matrix, a data frame can be accessed by row and column with [,].

Internally, a data frame is a list of column vectors. We can use the \$ syntax we saw with lists to access columns by name.

The data we are working with is derived from a dataset called **diabetes** in the **faraway** package. The rows are people interviewed as part of a study of diabetes prevalence. The column **glyhb** is a measurement of Glycosylated Haemoglobin. Values greater than 7 are usually taken as a positive diagnosis of diabetes. Lets add this as a column.

```
diabetes$diabetic <- diabetes$glyhb > 7.0
```

#### head(diabetes)

```
##
     subject glyhb
                     location age gender height weight
                                                         frame diabetic
## 1
                               58 female
                                              61
       S1002 4.64 Buckingham
                                                    256
                                                         large
                                                                  FALSE
## 2
       S1003 4.63 Buckingham
                               67
                                     male
                                              67
                                                    119
                                                                  FALSE
                                                         large
                                              68
## 3
       S1005 7.72 Buckingham
                               64
                                    male
                                                    183 medium
                                                                   TRUE
## 4
       S1008 4.81 Buckingham
                               34
                                    male
                                              71
                                                    190
                                                        large
                                                                  FALSE
       S1011 4.84 Buckingham
                                              69
                                                    191 medium
                                                                  FALSE
                                    male
       S1015 3.94 Buckingham
                                    male
                                              59
                                                    170 medium
                                                                  FALSE
                               37
```

### **Factors**

When R loads a CSV file, it tries to give appropriate types to the columns. Lets examine what types R has given our data.

#### str(diabetes)

```
## 'data.frame': 354 obs. of 9 variables:
## $ subject : Factor w/ 354 levels "S10000","S10001",..: 4 6 7 8 9 10 11 12 13 14 ...
## $ glyhb : num 4.64 4.63 7.72 4.81 4.84 ...
## $ location: Factor w/ 2 levels "Buckingham","Louisa": 1 1 1 1 1 1 1 1 2 2 ...
## $ age : int 58 67 64 34 30 37 45 55 60 38 ...
## $ gender : Factor w/ 2 levels "female","male": 1 2 2 2 2 2 2 1 1 1 ...
## $ height : int 61 67 68 71 69 59 69 63 65 58 ...
## $ weight : int 256 119 183 190 191 170 166 202 156 195 ...
## $ frame : Factor w/ 3 levels "large","medium",..: 1 1 2 1 2 2 1 3 2 2 ...
## $ diabetic: logi FALSE FALSE TRUE FALSE FALSE FALSE ...
```

We might have expected the text columns to be the "character" data type, but they are instead "factor"s.

```
head( diabetes$frame )
## [1] large large medium large medium medium
## Levels: large medium small
```

R uses factor data type to store a vector of *categorical* data. The different possible categories are called *levels*.

Factors can be created from character vectors with factor. We sometimes care what order the levels are in, since this can affect how data is plotted or tabulated by various functions. If there is some sort of baseline level, such as "wildtype strain" or "no treatment", it is usually given first. factor has a parameter levels= to specify the desired order of levels.

Factors can be converted back to a character vector with as.character.

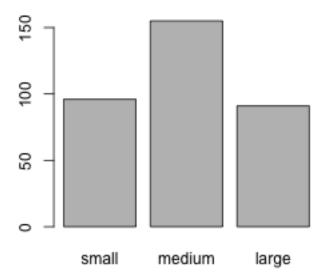
When R loaded our data, it chose levels in alphabetical order. Lets adjust that for the column diabetes\$frame.

```
diabetes$frame <- factor(diabetes$frame, levels=c("small", "medium", "large"))
head( diabetes$frame )
## [1] large large medium large medium medium
## Levels: small medium large</pre>
```

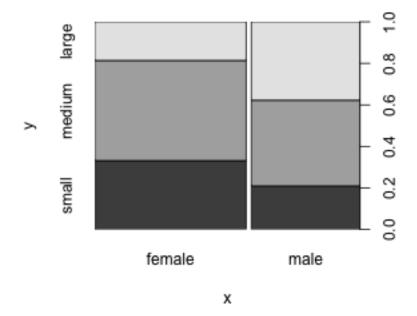
### Plotting factors

If we plot factors, we can see that R uses the order of levels in the plot.

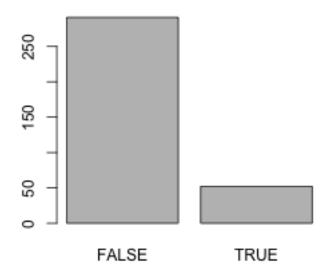
```
plot( diabetes$frame )
```



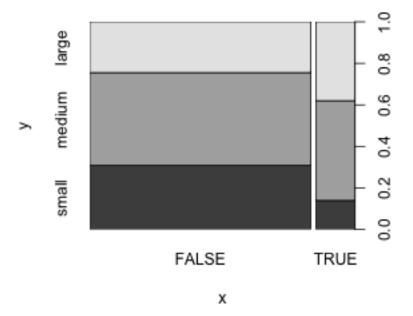
plot( diabetes\$gender, diabetes\$frame )



plot( factor(diabetes\$diabetic) )



plot( factor(diabetes\$diabetic), diabetes\$frame )



### Summarizing factors

The table function gives us the actual numbers behind the graphical summaries we just plotted.

```
table(diabetes$frame)
##
##
    small medium large
##
       96
             155
                      91
table(diabetes$diabetic, diabetes$frame)
##
##
           small medium large
##
     FALSE
              87
                     126
                            69
     TRUE
                      24
                            19
```

Fisher's Exact Test (fisher.test) or a chi-squared test (chiseq.test) can be used to show that two factors are not independent.

```
##
## Fisher's Exact Test for Count Data
##
## data: table(diabetes$diabetic, diabetes$frame)
## p-value = 0.02069
## alternative hypothesis: two.sided
```

fisher.test( table(diabetes\$diabetic, diabetes\$frame) )

#### Challenge - gender and diabetes

Do you think any association between gender and whether a person is diabetic is shown by this data set?

Why?

## Missing data

summary gives an overview of a data frame.

#### summary(diabetes)

```
##
       subject
                       glyhb
                                           location
                                                            age
##
    S10000 : 1
                          : 2.680
                                     Buckingham: 178
                                                              :19.00
                   Min.
                                                       Min.
##
    S10001:
              1
                   1st Qu.: 4.385
                                     Louisa
                                               :176
                                                       1st Qu.:35.00
    S10016 :
##
              1
                   Median : 4.840
                                                       Median :45.00
##
    S1002
                   Mean
                          : 5.580
                                                       Mean
                                                              :46.91
##
    S10020 :
                   3rd Qu.: 5.565
                                                       3rd Qu.:60.00
##
    S1003
                   Max.
                          :16.110
                                                       Max.
                                                              :92.00
              1
                   NA's
##
    (Other):348
                          :11
##
       gender
                      height
                                       weight
                                                       frame
                                                                  diabetic
##
    female:206
                  Min.
                         :52.00
                                   Min.
                                          : 99.0
                                                    small: 96
                                                                 Mode :logical
##
    male :148
                  1st Qu.:63.00
                                   1st Qu.:150.0
                                                    medium:155
                                                                 FALSE:291
##
                  Median :66.00
                                   Median :171.0
                                                    large: 91
                                                                 TRUE:52
##
                  Mean
                         :65.93
                                   Mean
                                          :176.2
                                                   NA's : 12
                                                                 NA's :11
##
                  3rd Qu.:69.00
                                   3rd Qu.:198.0
##
                 Max.
                         :76.00
                                   Max.
                                          :325.0
##
                  NA's
                         :5
                                   NA's
                                          :1
```

We see that some columns contain NAs. NA is R's way of indicating missing data. Missing data is important in statistics, so R is very careful with its treatment of this. If we try to calculate with an NA the result will be NA.

```
1 + NA

## [1] NA

mean(diabetes$glyhb)

## [1] NA
```

Many summary functions, such as mean, have a flag to say ignore NA values.

```
mean(diabetes$glyhb, na.rm=TRUE)
## [1] 5.580292
```

## Summarizing data frames

We were able to summarize the dimensions (rows or columns) of a matrix with apply. In a data frame instead of summarizing along different dimensions, we can summarize with respect to different factor columns.

We already saw how to count different levels in a factor with table.

We can use summary functions such as mean with a function called tapply, which works similarly to apply.

```
tapply(diabetes$glyhb, diabetes$frame, mean)
## small medium large
## NA NA NA
```

We obtain NAs because our data contains NAs. We need to tell mean to ignore these. Additional arguments to tapply are passed to the function given, here mean, so we can tell mean to ignore NA with

```
tapply(diabetes$glyhb, diabetes$frame, mean, na.rm=TRUE)
## small medium large
## 4.971064 5.721333 6.035795
```

The result is a vector, with names from the classifying factor.

We can summarize over several factors, in which case they must be given as a list. Two factors produces a matrix. More factors would produce a higher dimensional *array*.

tapply(diabetes\$glyhb, list(diabetes\$frame, diabetes\$gender), mean, na.rm=TRUE)

```
## small 5.042308 4.811379
## medium 5.490106 6.109464
## large 6.196286 5.929811
```

## Melting a matrix into a data frame

You may be starting to see that the idea of a matrix and the idea of a data frame with some factor columns are interchangeable. Depending on what we are doing, we may shift between these two representations of the same data.

Modern R usage emphasizes use of data frames over matrices, as data frames are the more flexible representation. Everything we can represent with a matrix we can represent with a data frame, but not vice versa.

tapply took us from a data frame to a matrix. We can go the other way, from a matrix to a data frame, with the melt function in the package reshape2.

```
library(reshape2)
```

```
averages <- tapply(diabetes$glyhb, list(diabetes$frame, diabetes$gender), mean, na.rm=TRUE)
melt(averages)</pre>
```

```
##
      Var1
             Var2
                     value
## 1 small female 5.042308
## 2 medium female 5.490106
## 3 large female 6.196286
## 4 small
             male 4.811379
## 5 medium male 6.109464
## 6 large male 5.929811
counts <- table(diabetes$frame, diabetes$gender)</pre>
melt(counts)
##
      Var1
             Var2 value
## 1 small female
                     96
## 2 medium female
## 3 large female
                     37
## 4 small
                     30
             male
## 5 medium
             male
                     59
## 6 large
             male
                     54
```

Note: The aggregate function effectively combines these two steps for you. See also the ddply function in package plyr, and the dplyr package. There are many variations on the basic idea behind apply!

## Merging two data frames

# For loops

Suppose we want to print each word in a sentence. One way is to use six print statements:

```
best_practice <- c("Let", "the", "computer", "do", "the", "work")
print_words <- function(sentence) {
    print(sentence[1])
    print(sentence[2])
    print(sentence[3])
    print(sentence[4])
    print(sentence[5])
    print(sentence[6])
}

print_words(best_practice)

## [1] "Let"
## [1] "the"
## [1] "do"
## [1] "the"
## [1] "the"
## [1] "work"</pre>
```

but that's a bad approach for two reasons:

- 1. It doesn't scale: if we want to print the elements in a vector that's hundreds long, we'd be better off just typing them in.
- 2. It's fragile: if we give it a longer vector, it only prints part of the data, and if we give it a shorter input, it returns NA values because we're asking for elements that don't exist!

#### Tip

R has a special variable, NA, for designating missing values that are Not Available in a data set. See ?NA for more details.

Here's a better approach:

```
print_words <- function(sentence) {
   for (word in sentence) {
      print(word)
   }
}

print_words(best_practice)

## [1] "Let"
## [1] "the"
## [1] "do"
## [1] "the"
## [1] "work"</pre>
```

This is shorter—certainly shorter than something that prints every character in a hundred-letter string—and more robust as well:

```
print_words(best_practice[-6])
```

```
## [1] "Let"
## [1] "the"
## [1] "computer"
## [1] "do"
## [1] "the"
```

The improved version of print\_words uses a for loop to repeat an operation—in this case, printing—once for each thing in a collection. The general form of a loop is:

```
for (variable in collection) {
  do things with variable
}
```

We can name the loop variable anything we like (with a few restrictions, e.g. the name of the variable cannot start with a digit). in is part of the for syntax. Note that the body of the loop is enclosed in curly braces { }. For a single-line loop body, as here, the braces aren't needed, but it is good practice to include them as we did.

Here's another loop that repeatedly updates a variable:

```
len <- 0
vowels <- c("a", "e", "i", "o", "u")
for (v in vowels) {
   len <- len + 1
}
# Number of vowels
len
## [1] 5</pre>
```

It's worth tracing the execution of this little program step by step. Since there are five elements in the vector vowels, the statement inside the loop will be executed five times. The first time around, len is zero (the value assigned to it on line 1) and v is "a". The statement adds 1 to the old value of len, producing 1, and updates len to refer to that new value. The next time around, v is "e" and len is 1, so len is updated to be 2. After three more updates, len is 5; since there is nothing left in the vector vowels for R to process, the loop finishes.

Note that a loop variable is just a variable that's being used to record progress in a loop. It still exists after the loop is over, and we can re-use variables previously defined as loop variables as well:

```
letter <- "z"
for (letter in c("a", "b", "c")) {
    print(letter)
}

## [1] "a"
## [1] "b"
## [1] "c"

# after the loop, letter is
letter

## [1] "c"</pre>
```

Note also that finding the length of a vector is such a common operation that R actually has a built-in function to do it called length:

```
length(vowels)
## [1] 5
```

length is much faster than any R function we could write ourselves, and much easier to read than a two-line loop; it will also give us the length of many other things that we haven't met yet, so we should always use it when we can (see this lesson to learn more about the different ways to store data in R).

#### Challenge - Using loops

1. R has a built-in function called seq that creates a list of numbers:

```
seq(3)
## [1] 1 2 3
```

Using seq, write a function that prints the first N natural numbers, one per line:

```
print_N(3)
## [1] 1
## [1] 2
## [1] 3
```

2. Write a function called total that calculates the sum of the values in a vector. (R has a built-in function called sum that does this for you. Please don't use it for this exercise.)

```
ex_vec <- c(4, 8, 15, 16, 23, 42)
total(ex_vec)
```

 $<sup>^{1}</sup>$ 01-supp-data-structures.html

```
## [1] 108
```

3. Exponentiation is built into R:

2^4

## [1] 16

Write a function called <code>expo</code> that uses a loop to calculate the same result.

expo(2, 4)

## [1] 16

# Plotting with ggplot2

Let's return to our matrix example.

```
dat <- read.csv(file="data/pvc.csv", row.names=1)
mat <- as.matrix(dat)</pre>
```

Instead of storing this data in a matrix, we could store each measurement in a row of a data frame. We can convert to this "long" form with the melt function in the library reshape2.

```
library(reshape2)
long <- melt(mat)</pre>
head(long)
##
       Var1 Var2 value
## 1 Resin1 Alice 36.25
## 2 Resin2 Alice 35.15
## 3 Resin3 Alice 30.70
## 4 Resin4 Alice 29.70
## 5 Resin5 Alice 31.85
## 6 Resin6 Alice 30.20
colnames(long) <- c("resin", "operator", "value")</pre>
head(long)
##
      resin operator value
## 1 Resin1
               Alice 36.25
## 2 Resin2
               Alice 35.15
## 3 Resin3
               Alice 30.70
## 4 Resin4
               Alice 29.70
## 5 Resin5
               Alice 31.85
## 6 Resin6
               Alice 30.20
```

This long form is often the best form for exploration and statistical testing of data.

We already saw some of R's built in plotting facilities with the function plot. A more recent and much more powerful plotting library is ggplot2. This implements ideas from a book called "The Grammar of Graphics". The syntax is a little strange, but there are plenty of examples in the online documentation<sup>1</sup>.

If ggplot2 isn't already installed, we need to install it.

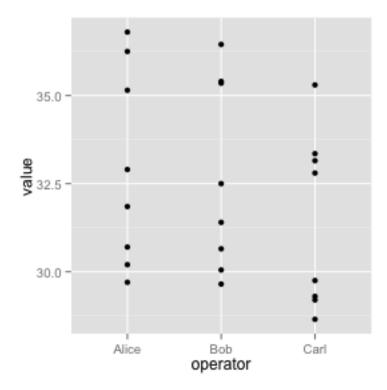
```
install.packages("ggplot2")
```

<sup>&</sup>lt;sup>1</sup>http://docs.ggplot2.org/current/

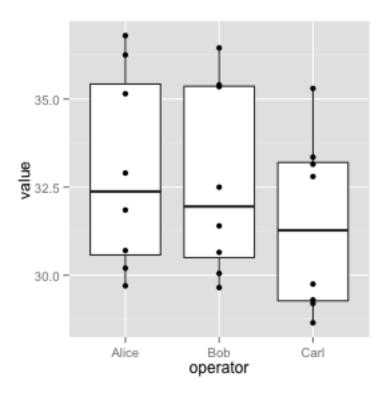
### library(ggplot2)

```
## Loading required package: methods
```

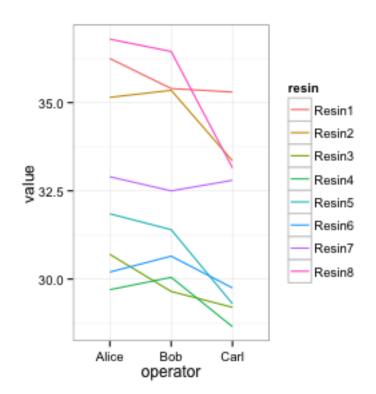
A simple example:



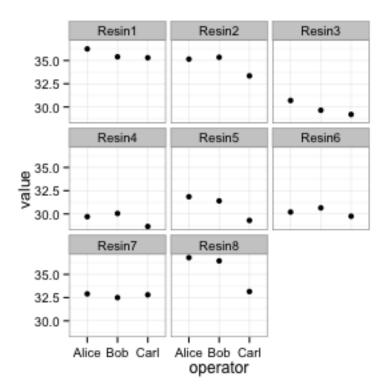
The call to ggplot sets up the basics of how we are going to represent the various columns of the data frame. We then literally add layers of graphics to this.



ggplot(long, aes(x=operator, y=value, group=resin, color=resin)) +
 geom\_line() + theme\_bw()



```
ggplot(long, aes(x=operator, y=value)) +
  facet_wrap(~ resin) + geom_point() + theme_bw()
```



ggplots can be save as we talked about above, but with one small twist to keep in mind. The act of plotting a ggplot is actually triggered when it is printed. In an interactive session we are automatically printing each value we calculate, but if you are writing a function you might need to explcitly print() the plot.

```
# Plot created but not shown.
p <- ggplot(long, aes(x=operator, y=value)) + geom_point()
# Only when we try to look at the value p is it shown
p
# Alternatively, we can explicitly print it
print(p)
# To save to a file
png("test.png")
print(p)
dev.off()</pre>
```

See also the function ggsave.

## 0.0002445

This long form is also ideal for statistical testing. Here is a one-way ANOVA.

```
oneway.test(value ~ resin, data=long)
##
## One-way analysis of means (not assuming equal variances)
##
## data: value and resin
## F = 26.605, num df = 7.0000, denom df = 6.5368, p-value =
```

A two-way ANOVA analysis would be better here. This is well beyond the scope of this course, but could be achived with something like:

```
null <- lm(value ~ operator, data=long)
alt <- lm(value ~ resin + operator, data=long)
anova(null, alt)

## Analysis of Variance Table
##
## Model 1: value ~ operator
## Model 2: value ~ resin + operator
## Res.Df RSS Df Sum of Sq F Pr(>F)
## 1 21 149.141
## 2 14 7.168 7 141.97 39.615 3.599e-08 ***
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.05 '.' 0.1 ' ' 1
```

# Next steps

We can barely touch the surface of what R has to offer today. If you want to take your skills to the next level, here are some topics to investigate:

#### Programming:

• Writing functions.

Tidying and summarizing data:

- dplyr, plyr, and tidyr packages by Hadley Wickham.
- magrittr's %>% operator for chaining together data frame manipulations.

#### Data visualization:

• ggplot2 for general plotting.

#### Statistics:

- Many statistical tests are built in to R.
- Linear models and the linear model formula syntax  $\sim$ .
- Many statistical techniques take linear models as their starting point, including edgeR which we will be using to test for differential gene expression.
- Many R function repurpose the ~ formula syntax for other ways of relating response and explanatory variables.

#### RNA-Seq:

• This will be covered in the Thursday class.