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# The R Bioc Book

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## Preface

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### Who is this book for?

- People who want to learn data science
  - People who want to teach data science
  - People who want to learn how to teach data science
  - People who want to learn how to learn data science
- 

### Why this book?

This book is a collection of resources for learning R and Bioconductor. It is meant to be largely self-directed, but for those looking to teach data science, it can also be used as a guide for structuring a course. Material is a bit variable in terms of difficulty, prerequisites, and format which is a reflection of the organic creation of the material.

Students are encouraged to work with others to learn the material. Instructors are encouraged to use the material to create a course that is tailored to the needs of their students and to spend lots of time in 1:1 and small groups to support students in their learning. See below for additional thoughts on adult learning and how it relates to this material.

---

### Adult learners

Adult Learning Theory, also known as Andragogy, is the concept and practice of designing, developing, and delivering instructional experiences for adult learners. It is based on the belief that adults learn differently than children, and thus, require distinct approaches to engage, motivate, and retain information (Center 2016). The term was first introduced by Malcolm Knowles, an American educator who is known for his work in adult education (Knowles, Holton, and Swanson 2005).

One of the fundamental principles of Adult Learning Theory is that adults are self-directed learners. This means that we prefer to take control of our own learning process and set personal goals for themselves. We are motivated by our desire to solve problems or gain knowledge to improve our lives (see Figure 1). As a result, educational content for adults should be relevant and applicable to real-life situations. Furthermore,

adult learners should be given opportunities to actively engage in the learning process by making choices, setting goals, and evaluating their progress.

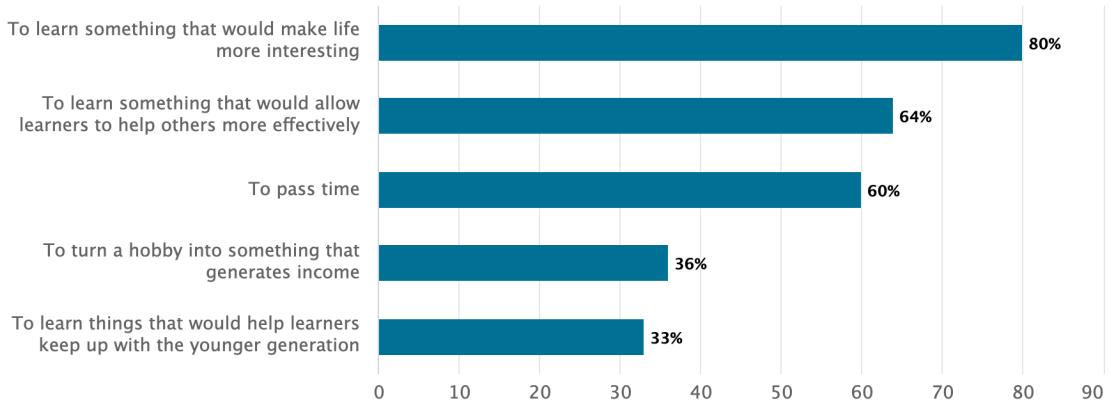


Figure 1: Why do adults choose to learn something?

Another key aspect of Adult Learning Theory is the role of experience. We bring a wealth of experience to the learning process, which serves as a resource for new learning. We often have well-established beliefs, values, and mental models that can influence our willingness to accept new ideas and concepts. Therefore, it is essential to acknowledge and respect our shared and unique past experiences and create an environment where we all feel comfortable sharing our perspectives.

To effectively learn as a group of adult learners, it is crucial to establish a collaborative learning environment that promotes open communication and fosters trust among participants. We all appreciate and strive for a respectful and supportive atmosphere where we can express our opinions without fear of judgment. Instructors should help facilitate discussions, encourage peer-to-peer interactions, and incorporate group activities and collaboration to capitalize on the collective knowledge of participants.

Additionally, adult learners often have multiple responsibilities outside of the learning environment, such as work and family commitments. As a result, we require flexible learning opportunities that accommodate busy schedules. Offering a variety of instructional formats, such as online modules, self-paced learning, or evening classes, can help ensure that adult learners have access to education despite any time constraints.

Adult learners benefit from a learner-centered approach that focuses on the individual needs, preferences, and interests of each participant can greatly enhance the overall learning experience. In addition, we tend to be more intrinsically motivated to learn when we have a sense of autonomy and can practice and experiment (see Figure 2) with new concepts in a safe environment.

Understanding Adult Learning Theory and its principles can significantly enhance the effectiveness of teaching and learning as adults. By respecting our autonomy, acknowledging our experiences, creating a supportive learning environment, offering flexible learning opportunities, and utilizing diverse teaching methods, we can better cater to the unique needs and preferences of adult learners.

In practice, that means that we will not be prescriptive in our approach to teaching data science. We will not tell you what to do, but rather we will provide you with a variety of options and you can choose what works best for you. We will also provide you with a variety of resources and you can choose where

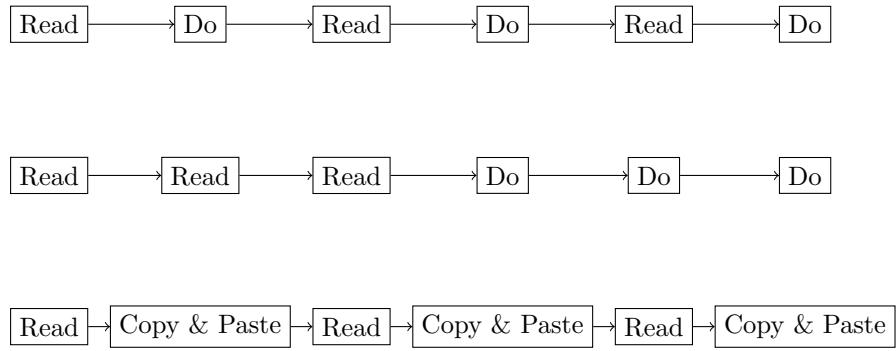


Figure 2: How to stay stuck in data science (or anything). The “Read-Do” loop tends to deliver the best results. Too much reading between doing can be somewhat effective. Reading and simply copy-paste is probably the least effective. When working through material, experiment. Try to break things. Incorporate your own experience or applications whenever possible.

to focus your time. Given that we cannot possibly cover everything, we will provide you with a framework for learning and you can fill in the gaps as you see fit. A key component of our success as adult learners is to gain the confidence to ask questions and problem-solve on our own.

**Part I**

**Introduction**

# 1

---

## *Introducing R and RStudio*

---

---

### **Questions**

- What is R?
  - Why use R?
  - Why not use R?
  - Why use RStudio and how does it differ from R?
- 

### **Learning Objectives**

- Know advantages of analyzing data in R
  - Know advantages of using RStudio
  - Be able to start RStudio on your computer
  - Identify the panels of the RStudio interface
  - Be able to customize the RStudio layout
- 

### **1.1 Introduction**

In this chapter, we will discuss the basics of R and RStudio, two essential tools in genomics data analysis. We will cover the advantages of using R and RStudio, how to set up RStudio, and the different panels of the RStudio interface.

---

### **1.2 What is R?**

R([https://en.wikipedia.org/wiki/R\\_\(programming\\_language\)](https://en.wikipedia.org/wiki/R_(programming_language))) is a programming language and software environment designed for statistical computing and graphics. It is widely used by statisticians, data scientists, and researchers for data analysis and visualization. R is an open-source language, which means it is free

to use, modify, and distribute. Over the years, R has become particularly popular in the fields of genomics and bioinformatics, owing to its extensive libraries and powerful data manipulation capabilities.

The R language is a dialect of the S language, which was developed in the 1970s at Bell Laboratories. The first version of R was written by Robert Gentleman and Ross Ihaka and released in 1995 (see [this slide deck](#) for Ross Ihaka's take on R's history). Since then, R has been continuously developed by the R Core Team, a group of statisticians and computer scientists. The R Core Team releases a new version of R every year.

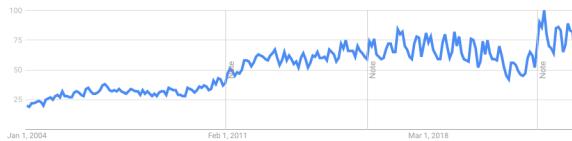


Figure 1.1: Google trends showing the popularity of R over time based on Google searches

---

### 1.3 Why use R?

There are several reasons why R is a popular choice for data analysis, particularly in genomics and bioinformatics. These include:

1. **Open-source:** R is free to use and has a large community of developers who contribute to its growth and development. [What is “open-source”?](#)
2. **Extensive libraries:** There are thousands of R packages available for a wide range of tasks, including specialized packages for genomics and bioinformatics. These libraries have been extensively tested and are available for free.
3. **Data manipulation:** R has powerful data manipulation capabilities, making it easy (or at least possible) to clean, process, and analyze large datasets.
4. **Graphics and visualization:** R has excellent tools for creating high-quality graphics and visualizations that can be customized to meet the specific needs of your analysis. In most cases, graphics produced by R are publication-quality.
5. **Reproducible research:** R enables you to create reproducible research by recording your analysis in a script, which can be easily shared and executed by others. In addition, R does not have a meaningful graphical user interface (GUI), which renders analysis in R much more reproducible than tools that rely on GUI interactions.
6. **Cross-platform:** R runs on Windows, Mac, and Linux (as well as more obscure systems).
7. **Interoperability with other languages:** R can interact with FORTRAN, C, and many other languages.
8. **Scalability:** R is useful for small and large projects.

I can develop code for analysis on my Mac laptop. I can then install the *same* code on our 20k core cluster and run it in parallel on 100 samples, monitor the process, and then update a database (for example) with

R when complete.

---

## 1.4 Why not use R?

- R cannot do everything.
  - R is not always the “best” tool for the job.
  - R will *not* hold your hand. Often, it will *slap* your hand instead.
  - The documentation can be opaque (but there is documentation).
  - R can drive you crazy (on a good day) or age you prematurely (on a bad one).
  - Finding the right package to do the job you want to do can be challenging; worse, some contributed packages are unreliable.]{}  
• R does not have a meaningfully useful graphical user interface (GUI).
- 

## 1.5 R License and the Open Source Ideal

R is free (yes, totally free!) and distributed under GNU license. In particular, this license allows one to:

- Download the source code
  - Modify the source code to your heart’s content
  - Distribute the modified source code and even charge money for it, but you must distribute the modified source code under the original GNU license]{}  
This license means that R will always be available, will always be open source, and can grow organically without constraint.
- 

## 1.6 RStudio

RStudio is an integrated development environment (IDE) for R. It provides a graphical user interface (GUI) for R, making it easier to write and execute R code. RStudio also provides several other useful features, including a built-in console, syntax-highlighting editor, and tools for plotting, history, debugging, workspace management, and workspace viewing. RStudio is available in both free and commercial editions; the commercial edition provides some additional features, including support for multiple sessions and enhanced debugging

### 1.6.1 Getting started with RStudio

To get started with RStudio, you first need to install both R and RStudio on your computer. Follow these steps:

1. Download and install R from the [official R website](#).
2. Download and install RStudio from the [official RStudio website](#).
3. Launch RStudio. You should see the RStudio interface with four panels.

### 1.6.2 The RStudio Interface

RStudio's interface consists of four panels (see Figure 1.2):

- **Console** This panel displays the R console, where you can enter and execute R commands directly. The console also shows the output of your code, error messages, and other information.
- **Source** This panel is where you write and edit your R scripts. You can create new scripts, open existing ones, and run your code from this panel.
- **Environment** This panel displays your current workspace, including all variables, data objects, and functions that you have created or loaded in your R session.
- **Plots, Packages, Help, and Viewer** These panels display plots, installed packages, help files, and web content, respectively.

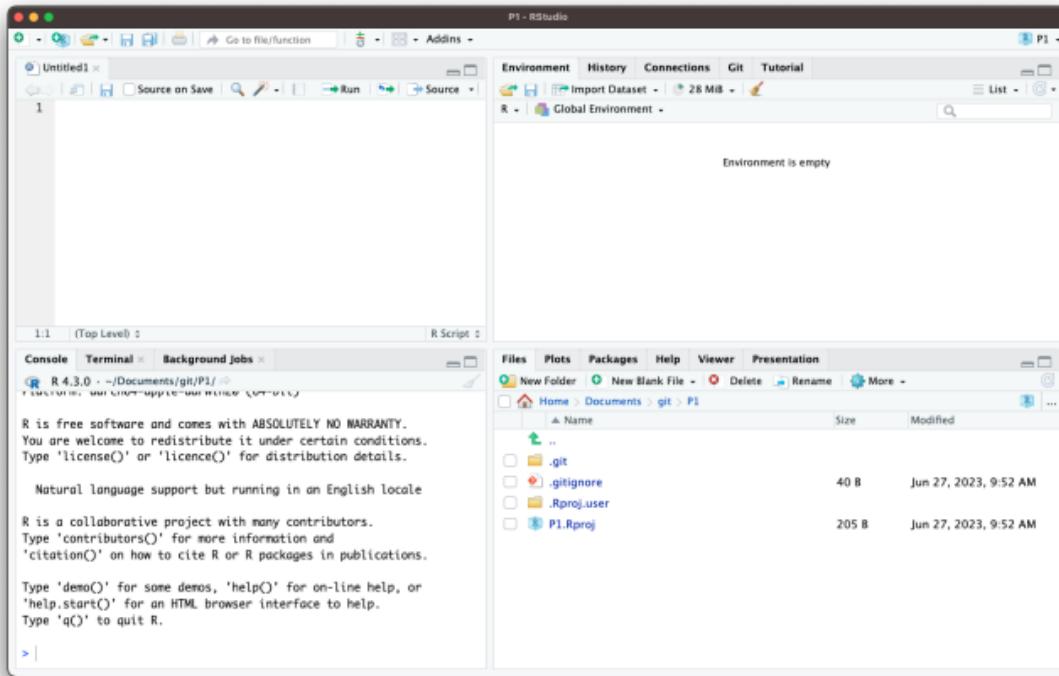


Figure 1.2: The RStudio interface. In this layout, the **source** pane is in the upper left, the **console** is in the lower left, the **environment** panel is in the top right and the **viewer/help/files** panel is in the bottom right.

### Do I need to use RStudio?

No. You can use R without RStudio. However, RStudio makes it easier to write and execute R code, and it provides several useful features that are not available in the basic R console. Note that the only part of RStudio that is actually interacting with R directly is the console. The other panels are simply providing a GUI that enhances the user experience.

### Customizing the RStudio Interface

You can customize the layout of RStudio to suit your preferences. To do so, go to **Tools > Global Options > Appearance**. Here, you can change the theme, font size, and panel layout. You can also resize the panels as needed to gain screen real estate (see Figure 1.3).

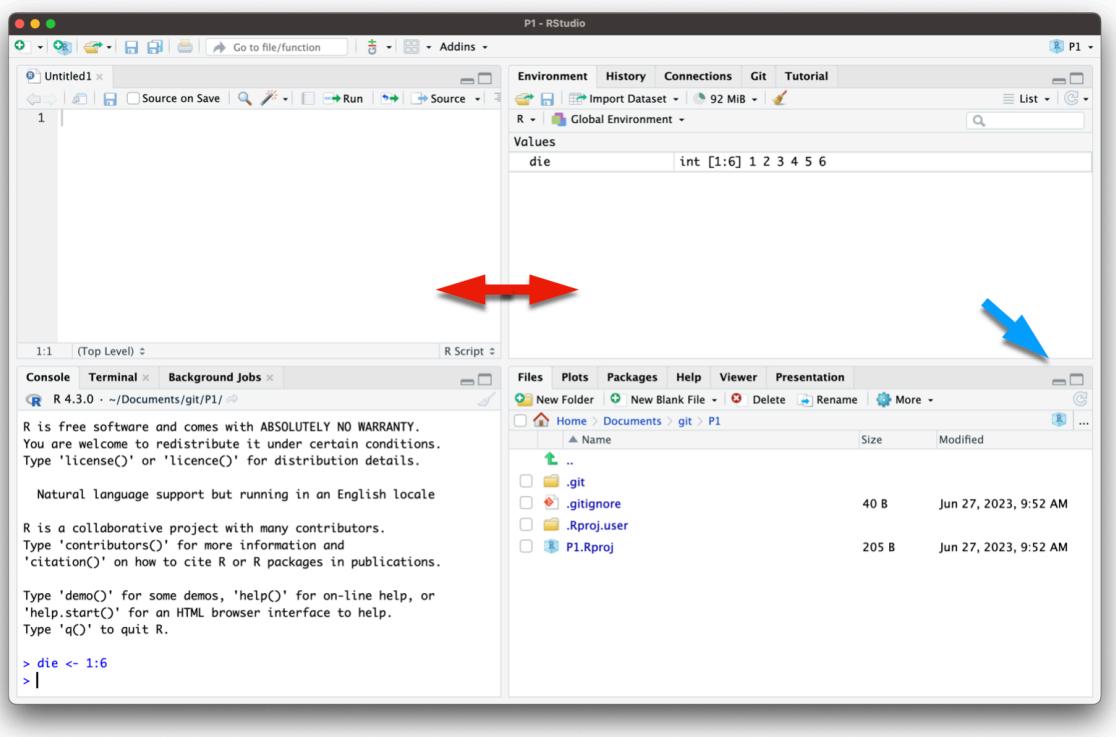


Figure 1.3: Dealing with limited screen real estate can be a challenge, particularly when you want to open another window to, for example, view a web page. You can resize the panes by sliding the center divider (red arrows) or by clicking on the minimize/maximize buttons (see blue arrow).

In summary, R and RStudio are powerful tools for genomics data analysis. By understanding the advantages of using R and RStudio and familiarizing yourself with the RStudio interface, you can efficiently analyze and visualize your data. In the following chapters, we will delve deeper into the functionality of R, Bioconductor, and various statistical methods to help you gain a comprehensive understanding of genomics data analysis.

# 2

---

## *R mechanics*

---

---

### 2.1 Learning objectives

- Be able to start R and RStudio
  - Learn to interact with the R console
  - Know the difference between expressions and assignment
  - Recognize valid and invalid R names
  - Know how to access the R help system
  - Know how to assign values to variables, find what is in R memory, and remove values from R memory
- 

### 2.2 Starting R

How to start R depends a bit on the operating system (Mac, Windows, Linux) and interface. In this course, we will largely be using an Integrated Development Environment (IDE) called *RStudio*, but there is nothing to prohibit using R at the command line or in some other interface (and there are a few).

---

### 2.3 *RStudio: A Quick Tour*

The RStudio interface has multiple panes. All of these panes are simply for convenience except the “Console” panel, typically in the lower left corner (by default). The console pane contains the running R interface. If you choose to run R outside RStudio, the interaction will be *identical* to working in the console pane. This is useful to keep in mind as some environments, such as a computer cluster, encourage using R without RStudio.

- Panes
- Options
- Help
- Environment, History, and Files

## 2.4 Interacting with R

The only meaningful way of interacting with R is by typing into the R console. At the most basic level, anything that we type at the command line will fall into one of two categories:

1. Assignments

```
x = 1  
y <- 2
```

2. Expressions

```
1 + pi + sin(42)
```

```
[1] 3.225071
```

The assignment type is obvious because either the The <- or = are used. Note that when we type expressions, R will return a result. In this case, the result of R evaluating  $1 + \pi + \sin(42)$  is 3.225071.

The standard R prompt is a “>” sign. When present, R is waiting for the next expression or assignment. If a line is not a complete R command, R will continue the next line with a “+”. For example, typing the following with a “Return” after the second “+” will result in R giving back a “+” on the next line, a prompt to keep typing.

```
1 + pi +  
sin(3.7)
```

```
[1] 3.611757
```

R can be used as a glorified calculator by using R expressions. Mathematical operations include:

- Addition: +
- Subtraction: -
- Multiplication: \*
- Division: /
- Exponentiation: ^
- Modulo: %%

The  $^$  operator raises the number to its left to the power of the number to its right: for example  $3^2$  is 9. The modulo returns the remainder of the division of the number to the left by the number on its right, for example 5 modulo 3 or  $5 \% 3$  is 2.

### 2.4.1 Expressions

```
5 + 2  
28 %% 3
```

```
3^2
5 + 4 * 4 + 4 ^ 4 / 10
```

Note that R follows order-of-operations and groupings based on parentheses.

```
5 + 4 / 9
(5 + 4) / 9
```

## 2.4.2 Assignment

While using R as a calculator is interesting, to do useful and interesting things, we need to assign *values* to *objects*. To create objects, we need to give it a name followed by the assignment operator `<-` (or, entirely equivalently, `=`) and the value we want to give it:

```
weight_kg <- 55
```

`<-` is the assignment operator. Assigns values on the right to objects on the left, it is like an arrow that points from the value to the object. Using an `=` is equivalent (in nearly all cases). Learn to use `<-` as it is good programming practice.

Objects can be given any name such as `x`, `current_temperature`, or `subject_id` (see below). You want your object names to be explicit and not too long. They cannot start with a number (`2x` is not valid but `x2` is). R is case sensitive (e.g., `weight_kg` is different from `Weight_kg`). There are some names that cannot be used because they represent the names of fundamental functions in R (e.g., `if`, `else`, `for`, see [here](#) for a complete list). In general, even if it's allowed, it's best to not use other function names, which we'll get into shortly (e.g., `c`, `T`, `mean`, `data`, `df`, `weights`). When in doubt, check the help to see if the name is already in use. It's also best to avoid dots `(.)` within a variable name as in `my.dataset`. It is also recommended to use nouns for variable names, and verbs for function names.

When assigning a value to an object, R does not print anything. You can force to print the value by typing the name:

```
weight_kg
```

```
[1] 55
```

Now that R has `weight_kg` in memory, which R refers to as the “global environment”, we can do arithmetic with it. For instance, we may want to convert this weight in pounds (weight in pounds is 2.2 times the weight in kg).

```
2.2 * weight_kg
```

```
[1] 121
```

We can also change a variable's value by assigning it a new one:

```
weight_kg <- 57.5
2.2 * weight_kg
```

```
[1] 126.5
```

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

```
weight_lb <- 2.2 * weight_kg
```

and then change weight\_kg to 100.

```
weight_kg <- 100
```

What do you think is the current content of the object weight\_lb, 126.5 or 220?

You can see what objects (variables) are stored by viewing the Environment tab in Rstudio. You can also use the ls() function. You can remove objects (variables) with the rm() function. You can do this one at a time or remove several objects at once. You can also use the little broom button in your environment pane to remove everything from your environment.

```
ls()  
rm(weight_lb, weight_kg)  
ls()
```

What happens when you type the following, now?

```
weight_lb # oops! you should get an error because weight_lb no longer exists!
```

---

## 2.5 Rules for Names in R

R allows users to assign names to objects such as variables, functions, and even dimensions of data. However, these names must follow a few rules.

- Names may contain any combination of letters, numbers, underscore, and ":"
- Names may not start with numbers, underscore.
- R names are case-sensitive.

Examples of valid R names include:

```
pi  
x  
camelCaps  
my_stuff  
MY_Stuff  
this.is.the.name.of.the.man  
ABC123  
abc1234asdf  
.hi
```

## 2.6 Resources for Getting Help

There is extensive built-in help and documentation within R. A separate page contains a collection of [additional resources](#).

If the name of the function or object on which help is sought is known, the following approaches with the name of the function or object will be helpful. For a concrete example, examine the help for the print method.

```
help(print)
help('print')
?print
```

If the name of the function or object on which help is sought is *not* known, the following from within R will be helpful.

```
help.search('microarray')
RSiteSearch('microarray')
apropos('histogram')
```

There are also tons of online resources that Google will include in searches if online searching feels more appropriate.

I strongly recommend using `help("newfunction")` for all functions that are new or unfamiliar to you.

There are also many open and free resources and reference guides for R.

- [Quick-R](#): a quick online reference for data input, basic statistics and plots
- R reference card [PDF](#) by Tom Short
- Rstudio [cheatsheets](#)

# 3

---

## Up and Running with R

In this chapter, we’re going to get an introduction to the R language, so we can dive right into programming. We’re going to create a pair of virtual dice that can generate random numbers. No need to worry if you’re new to programming. We’ll return to many of the concepts here in more detail later.

To simulate a pair of dice, we need to break down each die into its essential features. A die can only show one of six numbers: 1, 2, 3, 4, 5, and 6. We can capture the die’s essential characteristics by saving these numbers as a group of values in the computer. Let’s save these numbers first and then figure out a way to “roll” our virtual die.

---

### 3.1 The R User Interface

The RStudio interface is simple. You type R code into the bottom line of the RStudio console pane and then click Enter to run it. The code you type is called a *command*, because it will command your computer to do something for you. The line you type it into is called the *command line*.

When you type a command at the prompt and hit Enter, your computer executes the command and shows you the results. Then RStudio displays a fresh prompt for your next command. For example, if you type `1 + 1` and hit Enter, RStudio will display:

```
> 1 + 1  
[1] 2  
>
```

You’ll notice that a [1] appears next to your result. R is just letting you know that this line begins with the first value in your result. Some commands return more than one value, and their results may fill up multiple lines. For example, the command `100:130` returns 31 values; it creates a sequence of integers from 100 to 130. Notice that new bracketed numbers appear at the start of the second and third lines of output. These numbers just mean that the second line begins with the 14th value in the result, and the third line begins with the 25th value. You can mostly ignore the numbers that appear in brackets:

```
> 100:130  
[1] 100 101 102 103 104 105 106 107 108 109 110 111 112  
[14] 113 114 115 116 117 118 119 120 121 122 123 124 125  
[25] 126 127 128 129 130
```

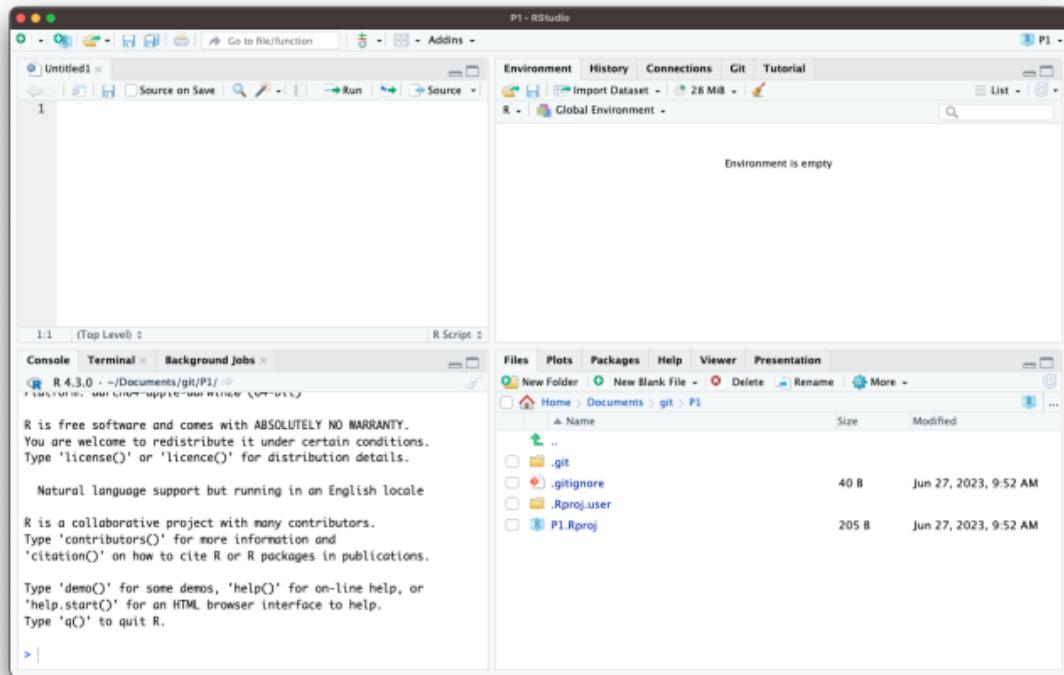


Figure 3.1: Your computer does your bidding when you type R commands at the prompt in the bottom line of the console pane. Don't forget to hit the Enter key. When you first open RStudio, the console appears in the pane on your left, but you can change this with **File > Tools > Global Options** in the menu bar.

 Tip

The colon operator (:) returns every integer between two integers. It is an easy way to create a sequence of numbers.

 When do we compile?

In some languages, like C, Java, and FORTRAN, you have to compile your human-readable code into machine-readable code (often 1s and 0s) before you can run it. If you've programmed in such a language before, you may wonder whether you have to compile your R code before you can use it. The answer is no. R is a dynamic programming language, which means R automatically interprets your code as you run it.

If you type an incomplete command and press Enter, R will display a + prompt, which means R is waiting for you to type the rest of your command. Either finish the command or hit Escape to start over:

```
> 5 -  
+  
+ 1  
[1] 4
```

If you type a command that R doesn't recognize, R will return an error message. If you ever see an error message, don't panic. R is just telling you that your computer couldn't understand or do what you asked it to do. You can then try a different command at the next prompt:

```
> 3 % 5  
Error: unexpected input in "3 % 5"  
>
```

 Tip

Whenever you get an error message in R, consider googling the error message. You'll often find that someone else has had the same problem and has posted a solution online. Simply cutting-and-pasting the error message into a search engine will often work

Once you get the hang of the command line, you can easily do anything in R that you would do with a calculator. For example, you could do some basic arithmetic:

```
2 * 3  
[1] 6  
4 - 1  
[1] 3  
# this obeys order-of-operations  
6 / (4 - 1)
```

```
[1] 2
```

**💡 Tip**

R treats the hashtag character, #, in a special way; R will not run anything that follows a hashtag on a line. This makes hashtags very useful for adding comments and annotations to your code. Humans will be able to read the comments, but your computer will pass over them. The hashtag is known as the *commenting symbol* in R.

**❗ Cancelling commands**

Some R commands may take a long time to run. You can cancel a command once it has begun by pressing **ctrl + c** or by clicking the “stop sign” if it is available in Rstudio. Note that it may also take R a long time to cancel the command.

### 3.1.1 An exercise

That's the basic interface for executing R code in RStudio. Think you have it? If so, try doing these simple tasks. If you execute everything correctly, you should end up with the same number that you started with:

1. Choose any number and add 2 to it.
2. Multiply the result by 3.
3. Subtract 6 from the answer.
4. Divide what you get by 3.

```
10 + 2
```

```
[1] 12
```

```
12 * 3
```

```
[1] 36
```

```
36 - 6
```

```
[1] 30
```

```
30 / 3
```

```
[1] 10
```

---

## 3.2 Objects

Now that you know how to use R, let's use it to make a virtual die. The : operator from a couple of pages ago gives you a nice way to create a group of numbers from one to six. The : operator returns its results

as a **vector** (we are going to work with vectors in more detail), a one-dimensional set of numbers:

```
1:6  
## 1 2 3 4 5 6
```

That's all there is to how a virtual die looks! But you are not done yet. Running `1:6` generated a vector of numbers for you to see, but it didn't save that vector anywhere for later use. If we want to use those numbers again, we'll have to ask your computer to save them somewhere. You can do that by creating an R *object*.

R lets you save data by storing it inside an R object. What is an object? Just a name that you can use to call up stored data. For example, you can save data into an object like `a` or `b`. Wherever R encounters the object, it will replace it with the data saved inside, like so:

```
a <- 1  
a  
  
[1] 1  
a + 2  
  
[1] 3
```

### What just happened?

1. To create an R object, choose a name and then use the less-than symbol, `<`, followed by a minus sign, `-`, to save data into it. This combination looks like an arrow, `<-`. R will make an object, give it your name, and store in it whatever follows the arrow. So `a <- 1` stores 1 in an object named `a`.
2. When you ask R what's in `a`, R tells you on the next line.
3. You can use your object in new R commands, too. Since a previously stored the value of 1, you're now adding 1 to 2.

### Assignment vs expressions

Everything that you type into the R console can be assigned to one of two categories:

- Assignments
- Expressions

An expression is a command that tells R to do something. For example, `1 + 2` is an expression that tells R to add 1 and 2. When you type an expression into the R console, R will evaluate the expression and return the result. For example, if you type `1 + 2` into the R console, R will return 3. Expressions can have “side effects” but they don’t explicitly result in anything being added to R memory.

```
5 + 2  
[1] 7  
28 %% 3  
[1] 1
```

```
3^2
[1] 9
5 + 4 * 4 + 4 ^ 4 / 10
[1] 46.6
```

While using R as a calculator is interesting, to do useful and interesting things, we need to assign values to objects. To create objects, we need to give it a name followed by the assignment operator `<-` (or, entirely equivalently, `=`) and the value we want to give it:

```
weight_kg <- 55
```

So, for another example, the following code would create an object named die that contains the numbers one through six. To see what is stored in an object, just type the object's name by itself:

```
die <- 1:6
die
```

```
[1] 1 2 3 4 5 6
```

When you create an object, the object will appear in the environment pane of RStudio, as shown in Figure 3.2. This pane will show you all of the objects you've created since opening RStudio.

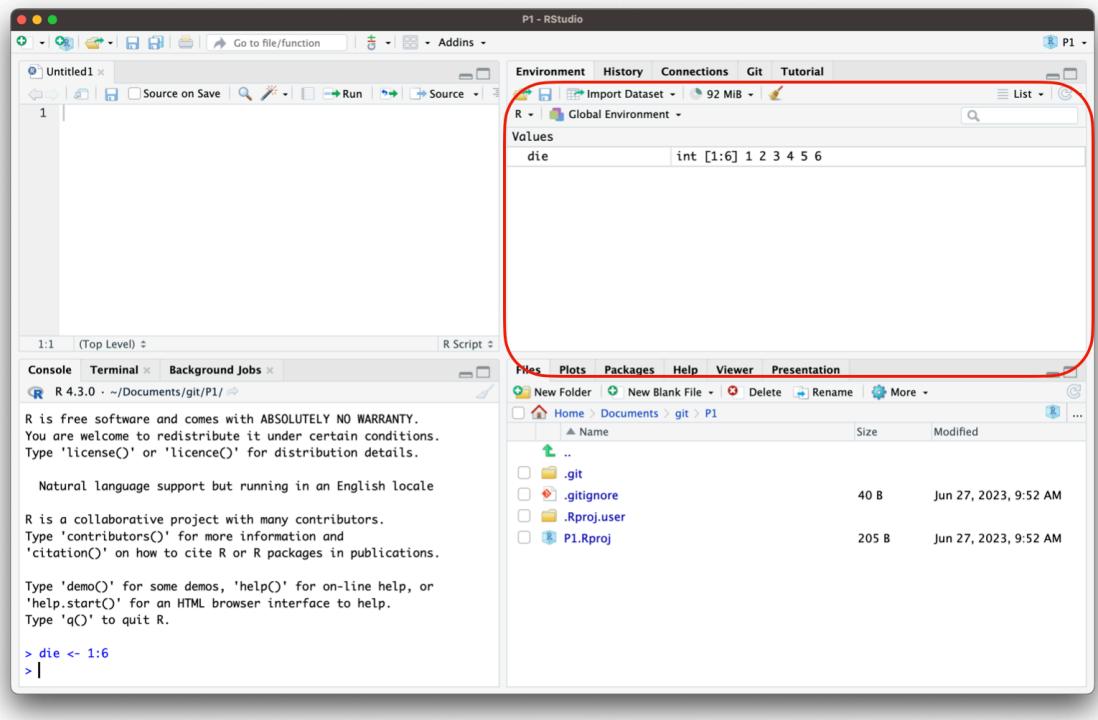


Figure 3.2: Assignment creates an object in the environment pane.

You can name an object in R almost anything you want, but there are a few rules. First, a name cannot start with a number. Second, a name cannot use some special symbols, like ^, !, \$, @, +, -, /, or \*:

Good names	Names that cause errors
a	1trial
b	\$
FOO	^mean
my_var	2nd
.day	!bad

### ⚠ Capitalization matters

R is case-sensitive, so name and Name will refer to different objects:

```
> Name = 0
> Name + 1
[1] 1
> name + 1
Error: object 'name' not found
```

The error above is a common one!

Finally, R will overwrite any previous information stored in an object without asking you for permission. So, it is a good idea to *not* use names that are already taken:

```
my_number <- 1
```

```
my_number
```

```
[1] 1
```

```
my_number <- 999
```

```
my_number
```

```
[1] 999
```

You can see which object names you have already used with the function `ls`:

```
ls()
```

Your environment will contain different names than mine, because you have probably created different objects.

You can also see which names you have used by examining RStudio's environment pane.

We now have a virtual die that is stored in the computer's memory and which has a name that we can use to refer to it. You can access it whenever you like by typing the word `die`.

So what can you do with this die? Quite a lot. R will replace an object with its contents whenever the object's name appears in a command. So, for example, you can do all sorts of math with the die. Math isn't so helpful for rolling dice, but manipulating sets of numbers will be your stock and trade as a data scientist. So let's take a look at how to do that:

```
die - 1
[1] 0 1 2 3 4 5
die / 2
[1] 0.5 1.0 1.5 2.0 2.5 3.0
die * die
[1] 1 4 9 16 25 36
```

R uses *element-wise execution* when working with a *vector* like `die`. When you manipulate a set of numbers, R will apply the same operation to each element in the set. So for example, when you run `die - 1`, R subtracts one from each element of `die`.

When you use two or more vectors in an operation, R will line up the vectors and perform a sequence of individual operations. For example, when you run `die * die`, R lines up the two `die` vectors and then multiplies the first element of vector 1 by the first element of vector 2. R then multiplies the second element of vector 1 by the second element of vector 2, and so on, until every element has been multiplied. The result will be a new vector the same length as the first two {Figure 3.3}.

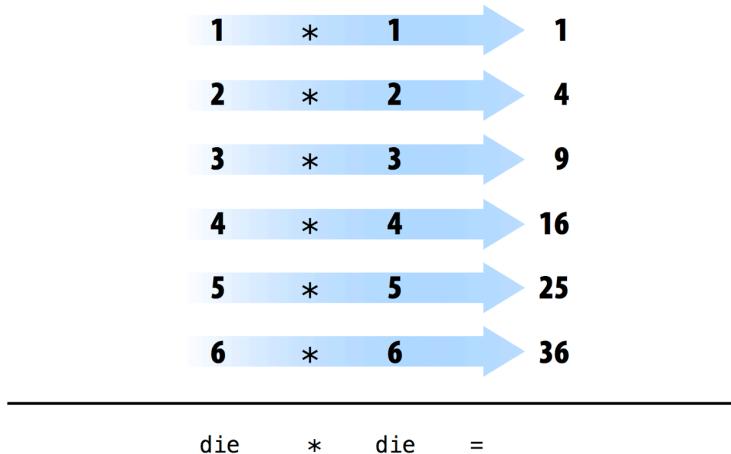


Figure 3.3: “When R performs element-wise execution, it matches up vectors and then manipulates each pair of elements independently.”

If you give R two vectors of unequal lengths, R will repeat the shorter vector until it is as long as the longer vector, and then do the math, as shown in Figure 3.4. This isn’t a permanent change—the shorter vector will be its original size after R does the math. If the length of the short vector does not divide evenly into the length of the long vector, R will return a warning message. This behavior is known as *vector recycling*, and it helps R do element-wise operations:

```
1:2
```

```
[1] 1 2
```

```
1:4
```

```
[1] 1 2 3 4
```

```
die
```

```
[1] 1 2 3 4 5 6
```

```
die + 1:2
```

```
[1] 2 4 4 6 6 8
```

```
die + 1:4
```

Warning in die + 1:4: longer object length is not a multiple of shorter object length

```
[1] 2 4 6 8 6 8
```

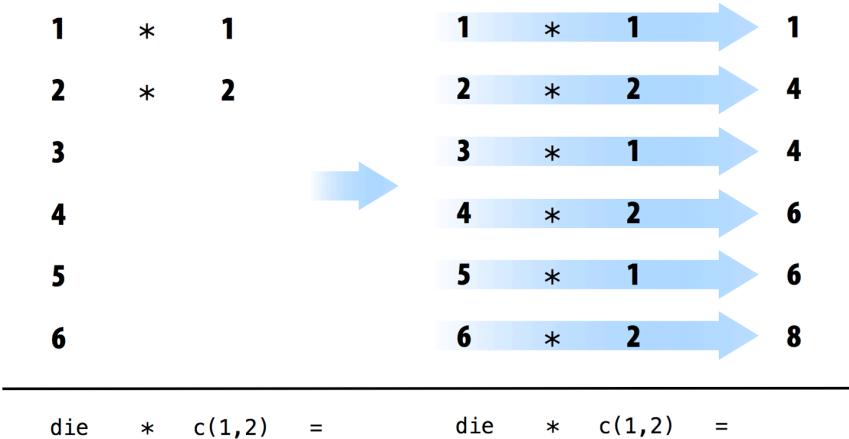


Figure 3.4: “R will repeat a short vector to do element-wise operations with two vectors of uneven lengths.”

Element-wise operations are a very useful feature in R because they manipulate groups of values in an orderly way. When you start working with data sets, element-wise operations will ensure that values from one observation or case are only paired with values from the same observation or case. Element-wise operations also make it easier to write your own programs and functions in R.

**!** Element-wise operations are not matrix operations

It is important to know that operations with vectors are not the same that you might expect if you are expecting R to perform “matrix” operations. R can do inner multiplication with the `%%%` operator and outer multiplication with the `%o%` operator:

```
# Inner product (1*1 + 2*2 + 3*3 + 4*4 + 5*5 + 6*6)
die %*% die
# Outer product
die %o% die
```

Now that you can do math with your die object, let's look at how you could "roll" it. Rolling your die will require something more sophisticated than basic arithmetic; you'll need to randomly select one of the die's values. And for that, you will need a *function*.

---

### 3.3 Functions

R has many functions and puts them all at our disposal. We can use functions to do simple and sophisticated tasks. For example, we can round a number with the `round` function, or calculate its factorial with the `factorial` function. Using a function is pretty simple. Just write the name of the function and then the data you want the function to operate on in parentheses:

```
round(3.1415)
```

```
[1] 3
factorial(3)
```

```
[1] 6
```

The data that you pass into the function is called the function's *argument*. The argument can be raw data, an R object, or even the results of another R function. In this last case, R will work from the innermost function to the outermost Figure 3.5.

```
mean(1:6)
```

```
[1] 3.5
mean(die)
```

```
[1] 3.5
round(mean(die))
```

```
[1] 4
```

Returning to our die, we can use the `sample` function to randomly select one of the die's values; in other words, the `sample` function can simulate rolling the die.

The `sample` function takes *two* arguments: a vector named `x` and a number named `size`. `sample` will return `size` elements from the vector:

```
sample(x = 1:4, size = 2)
```

```
[1] 3 2
```

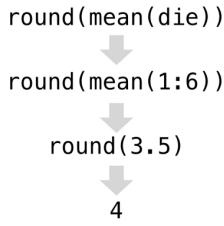


Figure 3.5: “When you link functions together, R will resolve them from the innermost operation to the outermost. Here R first looks up die, then calculates the mean of one through six, then rounds the mean.”

To roll your die and get a number back, set `x` to `die` and sample one element from it. You’ll get a new (maybe different) number each time you roll it:

```
sample(x = die, size = 1)
```

```
[1] 2
```

```
sample(x = die, size = 1)
```

```
[1] 5
```

```
sample(x = die, size = 1)
```

```
[1] 2
```

Many R functions take multiple arguments that help them do their job. You can give a function as many arguments as you like as long as you separate each argument with a comma.

You may have noticed that I set `die` and `1` equal to the names of the arguments in `sample`, `x` and `size`. Every argument in every R function has a name. You can specify which data should be assigned to which argument by setting a name equal to data, as in the preceding code. This becomes important as you begin to pass multiple arguments to the same function; names help you avoid passing the wrong data to the wrong argument. However, using names is optional. You will notice that R users do not often use the name of the first argument in a function. So you might see the previous code written as:

```
sample(die, size = 1)
```

```
[1] 2
```

Often, the name of the first argument is not very descriptive, and it is usually obvious what the first piece of data refers to anyways.

But how do you know which argument names to use? If you try to use a name that a function does not expect, you will likely get an error:

```
round(3.1415, corners = 2)
## Error in round(3.1415, corners = 2) : unused argument(s) (corners = 2)
```

If you’re not sure which names to use with a function, you can look up the function’s arguments with `args`.

To do this, place the name of the function in the parentheses behind args. For example, you can see that the round function takes two arguments, one named x and one named digits:

```
args(round)
```

```
function (x, digits = 0)
NULL
```

Did you notice that args shows that the digits argument of round is already set to 0? Frequently, an R function will take optional arguments like digits. These arguments are considered optional because they come with a default value. You can pass a new value to an optional argument if you want, and R will use the default value if you do not. For example, round will round your number to 0 digits past the decimal point by default. To override the default, supply your own value for digits:

```
round(3.1415)
```

```
[1] 3
```

```
round(3.1415, digits = 2)
```

```
[1] 3.14
```

```
# pi happens to be a built-in value in R
pi
```

```
[1] 3.141593
```

```
round(pi)
```

```
[1] 3
```

You should write out the names of each argument after the first one or two when you call a function with multiple arguments. Why? First, this will help you and others understand your code. It is usually obvious which argument your first input refers to (and sometimes the second input as well). However, you'd need a large memory to remember the third and fourth arguments of every R function. Second, and more importantly, writing out argument names prevents errors.

If you do not write out the names of your arguments, R will match your values to the arguments in your function by order. For example, in the following code, the first value, die, will be matched to the first argument of sample, which is named x. The next value, 1, will be matched to the next argument, size:

```
sample(die, 1)
```

```
[1] 2
```

As you provide more arguments, it becomes more likely that your order and R's order may not align. As a result, values may get passed to the wrong argument. Argument names prevent this. R will always match a value to its argument name, no matter where it appears in the order of arguments:

```
sample(size = 1, x = die)
```

```
[1] 2
```

### 3.3.1 Sample with Replacement

If you set `size = 2`, you can *almost* simulate a pair of dice. Before we run that code, think for a minute why that might be the case. `sample` will return two numbers, one for each die:

```
sample(die, size = 2)
```

```
[1] 5 1
```

I said this “almost” works because this method does something funny. If you use it many times, you’ll notice that the second die never has the same value as the first die, which means you’ll never roll something like a pair of threes or snake eyes. What is going on?

By default, `sample` builds a sample *without replacement*. To see what this means, imagine that `sample` places all of the values of `die` in a jar or urn. Then imagine that `sample` reaches into the jar and pulls out values one by one to build its sample. Once a value has been drawn from the jar, `sample` sets it aside. The value doesn’t go back into the jar, so it cannot be drawn again. So if `sample` selects a six on its first draw, it will not be able to select a six on the second draw; six is no longer in the jar to be selected. Although `sample` creates its sample electronically, it follows this seemingly physical behavior.

One side effect of this behavior is that each draw depends on the draws that come before it. In the real world, however, when you roll a pair of dice, each die is independent of the other. If the first die comes up six, it does not prevent the second die from coming up six. In fact, it doesn’t influence the second die in any way whatsoever. You can recreate this behavior in `sample` by adding the argument `replace = TRUE`:

```
sample(die, size = 2, replace = TRUE)
```

```
[1] 2 1
```

The argument `replace = TRUE` causes `sample` to sample *with replacement*. Our jar example provides a good way to understand the difference between sampling with replacement and without. When `sample` uses replacement, it draws a value from the jar and records the value. Then it puts the value back into the jar. In other words, `sample` *replaces* each value after each draw. As a result, `sample` may select the same value on the second draw. Each value has a chance of being selected each time. It is as if every draw were the first draw.

Sampling with replacement is an easy way to create *independent random samples*. Each value in your sample will be a sample of size one that is independent of the other values. This is the correct way to simulate a pair of dice:

```
sample(die, size = 2, replace = TRUE)
```

```
[1] 4 4
```

Congratulate yourself; you’ve just run your first simulation in R! You now have a method for simulating the result of rolling a pair of dice. If you want to add up the dice, you can feed your result straight into the `sum` function:

```
dice <- sample(die, size = 2, replace = TRUE)
dice
```

```
[1] 2 3
```

```
sum(dice)
```

```
[1] 5
```

What would happen if you call dice multiple times? Would R generate a new pair of dice values each time? Let's give it a try:

```
dice
```

```
[1] 2 3
```

```
dice
```

```
[1] 2 3
```

```
dice
```

```
[1] 2 3
```

The name dice refers to a *vector* of two numbers. Calling more than once does not change the favlue. Each time you call dice, R will show you the result of that one time you called sample and saved the output to dice. R won't rerun `sample(die, 2, replace = TRUE)` to create a new roll of the dice. Once you save a set of results to an R object, those results do not change.

However, it *would* be convenient to have an object that can re-roll the dice whenever you call it. You can make such an object by writing your own R function.

---

### 3.4 Writing Your Own Functions

To recap, you already have working R code that simulates rolling a pair of dice:

```
die <- 1:6
dice <- sample(die, size = 2, replace = TRUE)
sum(dice)
```

```
[1] 7
```

You can retype this code into the console anytime you want to re-roll your dice. However, this is an awkward way to work with the code. It would be easier to use your code if you wrapped it into its own function, which is exactly what we'll do now. We're going to write a function named roll that you can use to roll your virtual dice. When you're finished, the function will work like this: each time you call roll(), R will return the sum of rolling two dice:

```
roll()
## 8

roll()
## 3
```

```
roll()  
## 7
```

Functions may seem mysterious or fancy, but they are *just another type of R object*. Instead of containing data, they contain code. This code is stored in a special format that makes it easy to reuse the code in new situations. You can write your own functions by recreating this format.

### 3.4.1 The Function Constructor

Every function in R has three basic parts: a name, a body of code, and a set of arguments. To make your own function, you need to replicate these parts and store them in an R object, which you can do with the `function` function. To do this, call `function()` and follow it with a pair of braces, `{}`:

```
my_function <- function() {}
```

This function, as written, doesn't do anything (yet). However, it is a valid function. You can call it by typing its name followed by an open and closed parenthesis:

```
my_function()
```

```
NULL
```

function will build a function out of whatever R code you place between the braces. For example, you can turn your dice code into a function by calling:

```
roll <- function() {  
  die <- 1:6  
  dice <- sample(die, size = 2, replace = TRUE)  
  sum(dice)  
}
```

#### Indentation and readability

Notice each line of code between the braces is indented. This makes the code easier to read but has no impact on how the code runs. R ignores spaces and line breaks and executes one complete expression at a time. Note that in other languages like python, spacing is extremely important and part of the language.

Just hit the Enter key between each line after the first brace, `{`. R will wait for you to type the last brace, `}`, before it responds.

Don't forget to save the output of `function` to an R object. This object will become your new function. To use it, write the object's name followed by an open and closed parenthesis:

```
roll()
```

```
[1] 5
```

You can think of the parentheses as the “trigger” that causes R to run the function. If you type in a function’s name *without* the parentheses, R will show you the code that is stored inside the function. If you type in

the name *with* the parentheses, R will run that code:

```
roll
```

```
function() {  
  die <- 1:6  
  dice <- sample(die, size = 2, replace = TRUE)  
  sum(dice)  
}  
roll()
```

```
[1] 10
```

The code that you place inside your function is known as the *body* of the function. When you run a function in R, R will execute all of the code in the body and then return the result of the last line of code. If the last line of code doesn't return a value, neither will your function, so you want to ensure that your final line of code returns a value. One way to check this is to think about what would happen if you ran the body of code line by line in the command line. Would R display a result after the last line, or would it not?

Here's some code that would display a result:

```
dice  
1 + 1  
sqrt(2)
```

And here's some code that would not:

```
dice <- sample(die, size = 2, replace = TRUE)  
two <- 1 + 1  
a <- sqrt(2)
```

Again, this is just showing the distinction between expressions and assignments.

---

### 3.5 Arguments

What if we removed one line of code from our function and changed the name die to bones (just a name—don't think of it as important), like this?

```
roll2 <- function() {  
  dice <- sample(bones, size = 2, replace = TRUE)  
  sum(dice)  
}
```

Now I'll get an error when I run the function. The function **needs** the object bones to do its job, but there is no object named bones to be found (you can check by typing `ls()` which will show you the names in the environment, or memory).

```
roll12()
## Error in sample(bones, size = 2, replace = TRUE) :
##   object 'bones' not found
```

You can supply bones when you call roll12 if you make bones an argument of the function. To do this, put the name bones in the parentheses that follow function when you define roll12:

```
roll12 <- function(bones) {
  dice <- sample(bones, size = 2, replace = TRUE)
  sum(dice)
}
```

Now roll12 will work as long as you supply bones when you call the function. You can take advantage of this to roll different types of dice each time you call roll12.

Remember, we're rolling pairs of dice:

```
roll12(bones = 1:4)
```

```
[1] 4
roll12(bones = 1:6)
```

```
[1] 7
roll12(1:20)
```

```
[1] 19
```

Notice that roll12 will still give an error if you do not supply a value for the bones argument when you call roll12:

```
roll12()
## Error in sample(bones, size = 2, replace = TRUE) :
##   argument "bones" is missing, with no default
```

You can prevent this error by giving the bones argument a default value. To do this, set bones equal to a value when you define roll12:

```
roll12 <- function(bones = 1:6) {
  dice <- sample(bones, size = 2, replace = TRUE)
  sum(dice)
}
```

Now you can supply a new value for bones if you like, and roll12 will use the default if you do not:

```
roll12()
```

```
[1] 10
```

You can give your functions as many arguments as you like. Just list their names, separated by commas, in the parentheses that follow function. When the function is run, R will replace each argument name in the

function body with the value that the user supplies for the argument. If the user does not supply a value, R will replace the argument name with the argument's default value (if you defined one).

To summarize, function helps you construct your own R functions. You create a body of code for your function to run by writing code between the braces that follow function. You create arguments for your function to use by supplying their names in the parentheses that follow function. Finally, you give your function a name by saving its output to an R object, as shown in Figure 3.6.

Once you've created your function, R will treat it like every other function in R. Think about how useful this is. Have you ever tried to create a new Excel option and add it to Microsoft's menu bar? Or a new slide animation and add it to Powerpoint's options? When you work with a programming language, you can do these types of things. As you learn to program in R, you will be able to create new, customized, reproducible tools for yourself whenever you like.

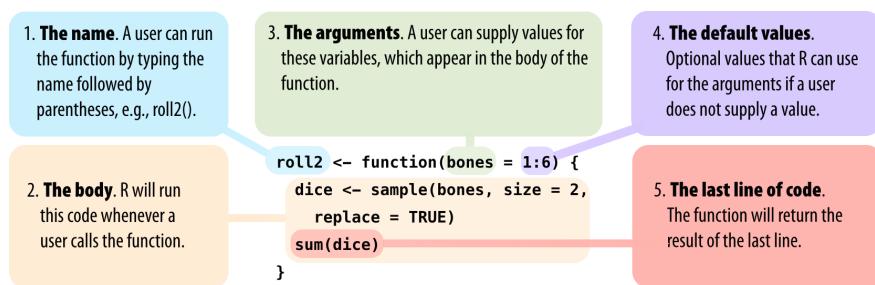


Figure 3.6: “Every function in R has the same parts, and you can use function to create these parts. Assign the result to a name, so you can call the function later.”

## 3.6 Scripts

Scripts are code that are saved for later reuse or editing. An R script is just a plain text file that you save R code in. You can open an R script in RStudio by going to **File > New File > R script** in the menu bar. RStudio will then open a fresh script above your console pane, as shown in Figure 3.7.

I strongly encourage you to write and edit all of your R code in a script before you run it in the console. Why? This habit creates a reproducible record of your work. When you're finished for the day, you can save your script and then use it to rerun your entire analysis the next day. Scripts are also very handy for editing and proofreading your code, and they make a nice copy of your work to share with others. To save a script, click the scripts pane, and then go to **File > Save As** in the menu bar.

RStudio comes with many built-in features that make it easy to work with scripts. First, you can automatically execute a line of code in a script by clicking the Run button at the top of the editor panel.

R will run whichever line of code your cursor is on. If you have a whole section highlighted, R will run the highlighted code. Alternatively, you can run the entire script by clicking the Source button. Don't like

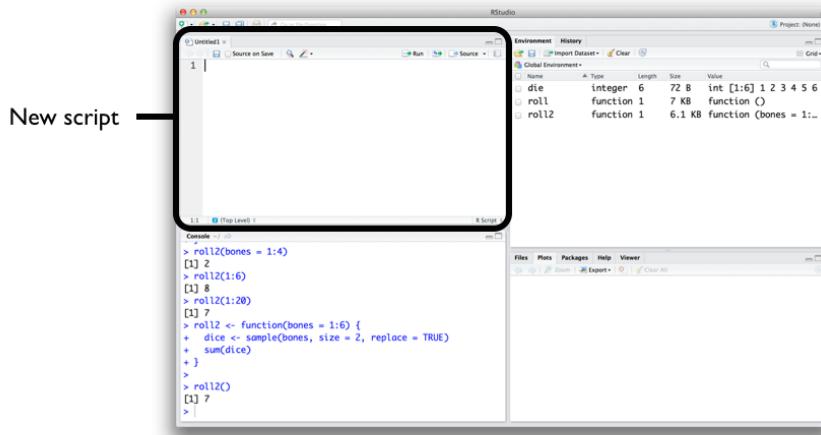


Figure 3.7: “When you open an R Script (File > New File > R Script in the menu bar), RStudio creates a fourth pane (or puts a new tab in the existing pane) above the console where you can write and edit your code.”

clicking buttons? You can use Control + Return as a shortcut for the Run button. On Macs, that would be Command + Return.

If you’re not convinced about scripts, you soon will be. It becomes a pain to write multi-line code in the console’s single-line command line. Let’s avoid that headache and open your first script now before we move to the next chapter.

### Tip

#### Extract function

RStudio comes with a tool that can help you build functions. To use it, highlight the lines of code in your R script that you want to turn into a function. Then click Code > Extract Function in the menu bar. RStudio will ask you for a function name to use and then wrap your code in a function call. It will scan the code for undefined variables and use these as arguments.

You may want to double-check RStudio’s work. It assumes that your code is correct, so if it does something surprising, you may have a problem in your code.

## 3.7 Summary

We’ve covered a lot of ground already. You now have a virtual die stored in your computer’s memory, as well as your own R function that rolls a pair of dice. You’ve also begun speaking the R language.

The two most important components of the R language are objects, which store data, and functions, which manipulate data. R also uses a host of operators like `+`, `-`, `*`, `/`, and `<-` to do basic tasks. As a data scientist, you will use R objects to store data in your computer's memory, and you will use functions to automate tasks and do complicated calculations.

# 4

---

## *Packages and more dice*

---

We now have code that allows us to roll two dice and add the results together. To keep things interesting, let's aim to weight the dice so that we can fool our friends into thinking we are lucky.

First, though, we should prove to ourselves that our dice are fair. We can investigate the behavior of our dice using two powerful and general tools;

- Simulation (or repetition or repeated sampling)
- Visualization

For the repetition part of things, we will use a built-in R function, `replicate`. For visualization, we are going to use a convenient plotting function, `qplot`. However, `qplot` does not come built into R. We must install a *package* to gain access to it.

---

### 4.1 Packages

R is a powerful language for data science and programming, allowing beginners and experts alike to manipulate, analyze, and visualize data effectively. One of the most appealing features of R is its extensive library of packages, which are essential tools for expanding its capabilities and streamlining the coding process.

An R package is a collection of reusable functions, datasets, and compiled code created by other users and developers to extend the functionality of the base R language. These packages cover a wide range of applications, such as data manipulation, statistical analysis, machine learning, and data visualization. By utilizing existing R packages, you can leverage the expertise of others and save time by avoiding the need to create custom functions from scratch.

Using others' R packages is incredibly beneficial as it allows you to take advantage of the collective knowledge of the R community. Developers often create packages to address specific challenges, optimize performance, or implement popular algorithms or methodologies. By incorporating these packages into your projects, you can enhance your productivity, reduce development time, and ensure that you are using well-tested and reliable code.

#### 4.1.1 `install.packages`

To install an R package, you can use the `install.packages()` function in the R console or script. For example, to install the popular data manipulation package “`dplyr`,” simply type `install.packages("dplyr")`.

This command will download the package from the Comprehensive R Archive Network (CRAN) and install it on your local machine. Keep in mind that you only need to install a package once, unless you want to update it to a newer version.

In our case, we want to install the `ggplot2` package.

```
install.packages('ggplot2')
```

#### 4.1.2 library

After installing an R package, you will need to load it into your R session before using its functions. To load a package, use the `library()` function followed by the package name, such as `library(dplyr)`. Loading a package makes its functions and datasets available for use in your current R session. Note that you need to load a package every time you start a new R session.

```
library(ggplot2)
```

Now, the functionality of the `ggplot2` package is available in our R session.

##### 💡 Installing vs loading packages

The main thing to remember is that you only need to install a package once, but you need to load it with `library` each time you wish to use it in a new R session. R will unload all of its packages each time you close RStudio.

#### 4.1.3 Finding R packages

Finding useful R packages can be done in several ways. First, browsing CRAN (<https://cran.r-project.org/>) and Bioconductor (more later, <https://bioconductor.org>) are an excellent starting points, as they host thousands of packages categorized by topic. Additionally, online forums like Stack Overflow and R-bloggers can provide valuable recommendations based on user experiences. Social media platforms such as Twitter, where developers and data scientists often share new packages and updates, can also be a helpful resource. Finally, don't forget to ask your colleagues or fellow R users for their favorite packages, as they may have insights on which ones best suit your specific needs.

---

## 4.2 Are our dice fair?

Well, let's review our code.

```
roll12 <- function(bones = 1:6) {  
  dice = sample(bones, size = 2, replace = TRUE)  
  sum(dice)  
}
```

If our dice are fair, then each number should show up equally. What does the sum look like with our two

dice?

Figure 4.1: In an ideal world, a histogram of the results would look like this

Read the help page for `replicate` (i.e., `help("replicate")`). In short, it suggests that we can repeat our dice rolling as many times as we like and `replicate` will return a *vector* of the sums for each roll.

```
rolls = replicate(n = 100, roll2())
```

What does rolls look like?

## head(10113)

`length(rolls)`

mean(ro

summary(

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
2.00	5.00	7.00	7.06	9.00	12.00

This looks like it roughly agrees with our sketched out ideal histogram in Figure 4.1. However, now that we've loaded the `ggplot` function from the `ggplot2` package, we can make a histogram of the data themselves.

```
qplot(rolls, binwidth=1)
```

Warning: `qplot()` was deprecated in ggplot2 3.4.0.

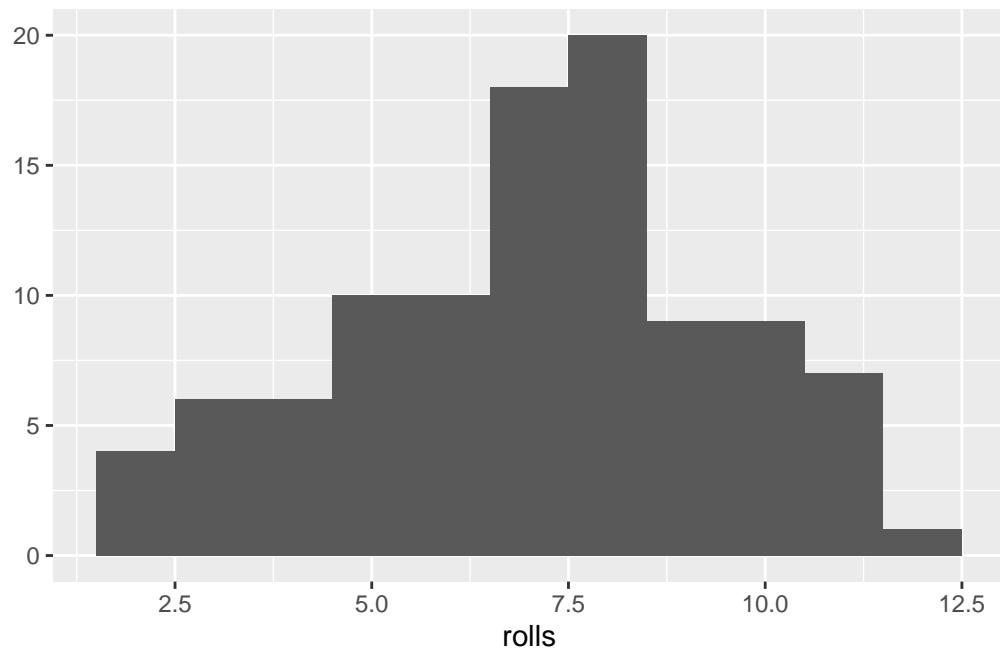


Figure 4.2: Histogram of the sums from 100 rolls of our fair dice

How does your histogram look (and yours will be different from mine since we are sampling random values)? Is it what you expect?

What happens to our histogram as we increase the number of replicates?

```
rolls = replicate(n = 100000, roll12())
qplot(rolls, binwidth=1)
```

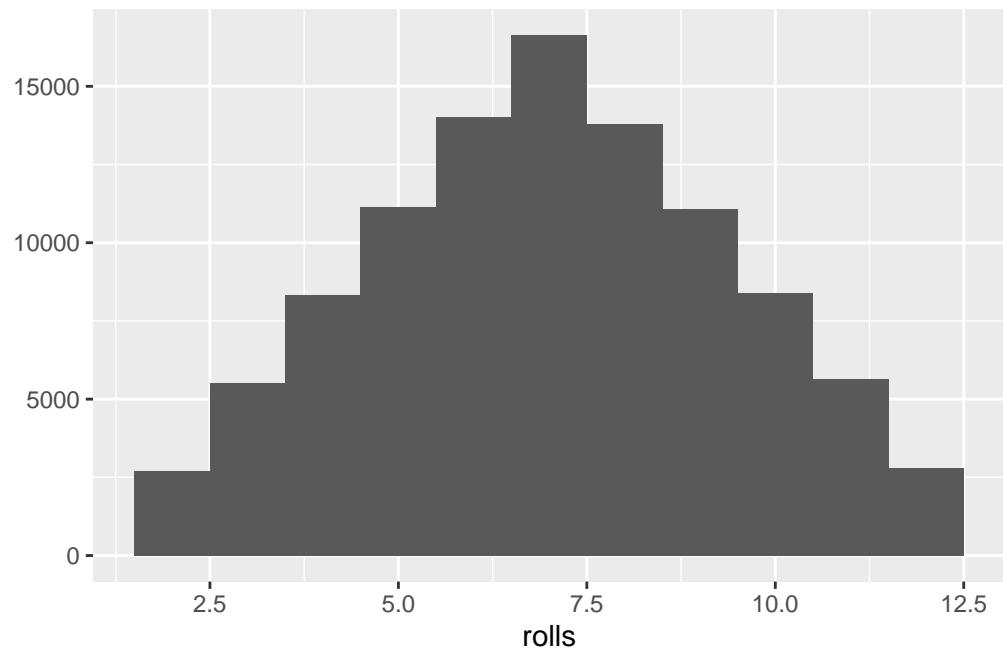


Figure 4.3: Histogram with 100000 rolls much more closely approximates the pyramidal shape we anticipated

---

### 4.3 Bonus exercise

How would you change the roll12 function to weight the dice?

## **Part II**

# **Overview of R Data Structures**

Welcome to the section on R data structures! As you begin your journey in learning R, it is essential to understand the fundamental building blocks of this powerful programming language. R offers a variety of data structures to store and manipulate data, each with its unique properties and capabilities. In this section, we will cover the core data structures in R, including:

- Vectors
- Matrices
- Lists
- Data.frames

By the end of this section, you will have a solid understanding of these data structures, and you will be able to choose and utilize the appropriate data structure for your specific data manipulation and analysis tasks.

In each chapter, we will delve into the properties and usage of each data structure, starting with their definitions and moving on to their practical applications. We will provide examples, exercises, and active learning approaches to help you better understand and apply these concepts in your work.

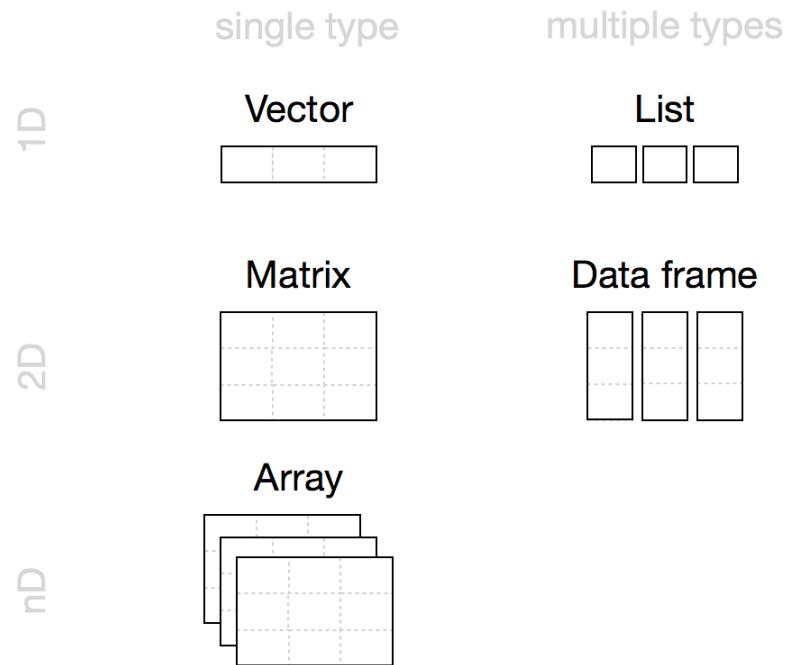


Figure 4.4: A pictorial representation of R's most common data structures are vectors, matrices, arrays, lists, and dataframes. Figure from [Hands-on Programming with R](#).

## Chapter overview

- **Vectors** : In this chapter, we will introduce you to the simplest data structure in R, the vector. We will cover how to create, access, and manipulate vectors, as well as discuss their unique properties and limitations.
- **Matrices** Next, we will explore matrices, which are two-dimensional data structures that extend vectors. You will learn how to create, access, and manipulate matrices, and understand their usefulness in mathematical operations and data organization.
- **Lists** The third chapter will focus on lists, a versatile data structure that can store elements of different types and sizes. We will discuss how to create, access, and modify lists, and demonstrate their flexibility in handling complex data structures.
- **Data.frames** Finally, we will examine data.frames, a widely-used data structure for organizing and manipulating tabular data. You will learn how to create, access, and manipulate data.frames, and understand their advantages over other data structures for data analysis tasks.
- **Arrays** While we will not focus directly on the array data type, which are multidimensional data structures that extend matrices, they are very similar to matrices, but with a third dimension.

As you progress through these chapters, we encourage you to practice the examples and exercises provided, engage in discussion, and collaborate with your peers to deepen your understanding of R data structures. This solid foundation will serve as the basis for more advanced data manipulation, analysis, and visualization techniques in R.

# 5

---

## Vectors

---

### 5.1 What is a Vector?

A vector is the simplest and most basic data structure in R. It is a one-dimensional, ordered collection of elements, where all the elements are of the same data type. Vectors can store various types of data, such as numeric, character, or logical values. Figure 5.1 shows a pictorial representation of three vector examples.

Index	1	2	3	4	5	6	7
Vector	3	7	10	NA	932	127	-3
Vector	TRUE	FALSE	FALSE	TRUE	TRUE	FALSE	NA
Vector	“Cat”	“Dog”	“A”	“C”	“T”	NA	“G”
Names (Optional)	“H”	“I”	“L”	“Z”	“This”	“That”	“Other”

Figure 5.1: “Pictorial representation of three vector examples. The first vector is a numeric vector. The second is a ‘logical’ vector. The third is a character vector. Vectors also have indices and, optionally, names.”

In this chapter, we will provide a comprehensive overview of vectors, including how to create, access, and manipulate them. We will also discuss some unique properties and rules associated with vectors, and explore their applications in data analysis tasks.

In R, even a single value is a vector with length=1.

```
z = 1  
z
```

```
[1] 1
```

```
length(z)
```

```
[1] 1
```

In the code above, we “assigned” the value 1 to the variable named z. Typing z by itself is an “expression” that returns a result which is, in this case, the value that we just assigned. The length method takes an R object and returns the R length. There are numerous ways of asking R about what an object represents, and length is one of them.

Vectors can contain numbers, strings (character data), or logical values (TRUE and FALSE) or other “atomic” data types Table 5.1. *Vectors cannot contain a mix of types!* We will introduce another data structure, the R list for situations when we need to store a mix of base R data types.

Table 5.1: Atomic (simplest) data types in R.

Data type	Stores
numeric	floating point numbers
integer	integers
complex	complex numbers
factor	categorical data
character	strings
logical	TRUE or FALSE
NA	missing
NULL	empty
function	function type

## 5.2 Creating vectors

Character vectors (also sometimes called “string” vectors) are entered with each value surrounded by single or double quotes; either is acceptable, but they must match. They are always displayed by R with double quotes. Here are some examples of creating vectors:

```
# examples of vectors
c('hello','world')
```

```
[1] "hello" "world"
```

```
c(1,3,4,5,1,2)
```

```
[1] 1 3 4 5 1 2
```

```
c(1.12341e7,78234.126)
```

```
[1] 11234100.00    78234.13
```

```
c(TRUE, FALSE, TRUE, TRUE)

[1] TRUE FALSE TRUE TRUE

# note how in the next case the TRUE is converted to "TRUE"
# with quotes around it.

c(TRUE, 'hello')

[1] "TRUE"  "hello"
```

We can also create vectors as “regular sequences” of numbers. For example:

```
# create a vector of integers from 1 to 10
x = 1:10
# and backwards
x = 10:1
```

The seq function can create more flexible regular sequences.

```
# create a vector of numbers from 1 to 4 skipping by 0.3
y = seq(1, 4, 0.3)
```

And creating a new vector by concatenating existing vectors is possible, as well.

```
# create a sequence by concatenating two other sequences
z = c(y, x)
z
```

```
[1] 1.0 1.3 1.6 1.9 2.2 2.5 2.8 3.1 3.4 3.7 4.0 10.0 9.0 8.0 7.0
[16] 6.0 5.0 4.0 3.0 2.0 1.0
```

### 5.3 Vector Operations

Operations on a single vector are typically done element-by-element. For example, we can add 2 to a vector, 2 is added to each element of the vector and a new vector of the same length is returned.

```
x = 1:10
x + 2

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

If the operation involves two vectors, the following rules apply. If the vectors are the same length: R simply applies the operation to each pair of elements.

```
x + x

[1] 2 4 6 8 10 12 14 16 18 20
```

If the vectors are different lengths, but one length a multiple of the other, R reuses the shorter vector as needed.

```
x = 1:10
y = c(1,2)
x * y
```

```
[1] 1 4 3 8 5 12 7 16 9 20
```

If the vectors are different lengths, but one length *not* a multiple of the other, R reuses the shorter vector as needed *and* delivers a warning.

```
x = 1:10
y = c(2,3,4)
x * y
```

```
Warning in x * y: longer object length is not a multiple of shorter object
length
```

```
[1] 2 6 12 8 15 24 14 24 36 20
```

Typical operations include multiplication (“\*”), addition, subtraction, division, exponentiation (“^”), but many operations in R operate on vectors and are then called “vectorized”.

Be aware of the recycling rule when working with vectors of different lengths, as it may lead to unexpected results if you’re not careful.

## 5.4 Logical Vectors

Logical vectors are vectors composed on only the values TRUE and FALSE. Note the all-upper-case and no quotation marks.

```
a = c(TRUE, FALSE, TRUE)
```

```
# we can also create a logical vector from a numeric vector
# 0 = false, everything else is 1
b = c(1,0,217)
d = as.logical(b)
d
```

```
[1] TRUE FALSE TRUE
# test if a and d are the same at every element
all.equal(a,d)
```

```
[1] TRUE
# We can also convert from logical to numeric
as.numeric(a)
```

```
[1] 1 0 1
```

### 5.4.1 Logical Operators

Some operators like `<`, `>`, `==`, `>=`, `<=`, `!=` can be used to create logical vectors.

```
# create a numeric vector
x = 1:10
# testing whether x > 5 creates a logical vector
x > 5

[1] FALSE FALSE FALSE FALSE FALSE TRUE TRUE TRUE TRUE TRUE

x <= 5

[1] TRUE TRUE TRUE TRUE TRUE FALSE FALSE FALSE FALSE FALSE

x != 5

[1] TRUE TRUE TRUE TRUE FALSE TRUE TRUE TRUE TRUE TRUE

x == 5

[1] FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE FALSE
```

We can also assign the results to a variable:

```
y = (x == 5)
y

[1] FALSE FALSE FALSE FALSE TRUE FALSE FALSE FALSE FALSE FALSE
```

## 5.5 Indexing Vectors

In R, an index is used to refer to a specific element or set of elements in a vector (or other data structure). [R uses `[` and `]` to perform indexing, although other approaches to getting subsets of larger data structures are common in R.

```
x = seq(0,1,0.1)
# create a new vector from the 4th element of x
x[4]

[1] 0.3
```

We can even use other vectors to perform the “indexing”.

```
x[c(3,5,6)]

[1] 0.2 0.4 0.5

y = 3:6
x[y]

[1] 0.2 0.3 0.4 0.5
```

Combining the concept of indexing with the concept of logical vectors results in a very power combination.

```
# use help('rnorm') to figure out what is happening next
myvec = rnorm(10)

# create logical vector that is TRUE where myvec is >0.25
gt1 = (myvec > 0.25)
sum(gt1)

[1] 5

# and use our logical vector to create a vector of myvec values that are >0.25
myvec[gt1]

[1] 0.8240789 0.8929529 0.7250657 0.2619124 0.7640435
# or <=0.25 using the logical "not" operator, "!"
myvec[!gt1]

[1] -1.0136903 0.1378304 -0.2494574 -0.1022404 -0.2366834
# shorter, one line approach
myvec[myvec > 0.25]

[1] 0.8240789 0.8929529 0.7250657 0.2619124 0.7640435
```

---

## 5.6 Named Vectors

Named vectors are vectors with labels or names assigned to their elements. These names can be used to access and manipulate the elements in a more meaningful way.

To create a named vector, use the names() function:

```
fruit_prices <- c(0.5, 0.75, 1.25)
names(fruit_prices) <- c("apple", "banana", "cherry")
print(fruit_prices)

apple banana cherry
0.50    0.75    1.25
```

You can also access and modify elements using their names:

```
banana_price <- fruit_prices["banana"]
print(banana_price)

banana
0.75
```

```
fruit_prices["apple"] <- 0.6
print(fruit_prices)
```

```
apple banana cherry
0.60    0.75    1.25
```

---

## 5.7 Character Vectors, A.K.A. Strings

R uses the paste function to concatenate strings.

```
paste("abc", "def")
```

```
[1] "abc def"
paste("abc", "def", sep="THISSEP")
```

```
[1] "abcTHISSEPdef"
paste0("abc", "def")
```

```
[1] "abcdef"
## [1] "abcdef"
paste(c("X", "Y"), 1:10)
```

```
[1] "X 1"   "Y 2"   "X 3"   "Y 4"   "X 5"   "Y 6"   "X 7"   "Y 8"   "X 9"   "Y 10"
paste(c("X", "Y"), 1:10, sep=" ")
```

```
[1] "X_1"   "Y_2"   "X_3"   "Y_4"   "X_5"   "Y_6"   "X_7"   "Y_8"   "X_9"   "Y_10"
```

We can count the number of characters in a string.

```
nchar('abc')
```

```
[1] 3
nchar(c('abc', 'd', 123456))
```

```
[1] 3 1 6
```

Pulling out parts of strings is also sometimes useful.

```
substr('This is a good sentence.', start=10, stop=15)
```

```
[1] " good "
```

Another common operation is to replace something in a string with something (a find-and-replace).

```
sub('This', 'That', 'This is a good sentence.')
```

```
[1] "That is a good sentence."
```

When we want to find all strings that match some other string, we can use grep, or “grab regular expression”.

```
grep('bcd',c('abcdef','abcd','bcde','cdef','defg'))
[1] 1 2 3
grep('bcd',c('abcdef','abcd','bcde','cdef','defg'),value=TRUE)
[1] "abcdef" "abcd"   "bcde"
```

Read about the grep1 function (?grep1). Use that function to return a logical vector (TRUE/FALSE) for each entry above with an a in it.

---

## 5.8 Missing Values, AKA “NA”

R has a special value, “NA”, that represents a “missing” value, or *Not Available*, in a vector or other data structure. Here, we just create a vector to experiment.

```
x = 1:5
x
[1] 1 2 3 4 5
length(x)
[1] 5
is.na(x)
[1] FALSE FALSE FALSE FALSE FALSE
x[2] = NA
x
[1] 1 NA 3 4 5
```

The length of x is unchanged, but there is one value that is marked as “missing” by virtue of being NA.

```
length(x)
[1] 5
is.na(x)
[1] FALSE TRUE FALSE FALSE FALSE
```

We can remove NA values by using indexing. In the following, is.na(x) returns a logical vector the length of x. The ! is the logical *NOT* operator and converts TRUE to FALSE and vice-versa.

```
x[!is.na(x)]
```

```
[1] 1 3 4 5
```

---

## 5.9 Exercises

1. Create a numeric vector called temperatures containing the following values: 72, 75, 78, 81, 76, 73.

```
temperatures <- c(72, 75, 78, 81, 76, 73, 93)
```

2. Create a character vector called days containing the following values: “Monday”, “Tuesday”, “Wednesday”, “Thursday”, “Friday”, “Saturday”, “Sunday”.

```
days <- c("Monday", "Tuesday", "Wednesday", "Thursday", "Friday", "Saturday", "Sunday")
```

3. Calculate the average temperature for the week and store it in a variable called average\_temperature.

```
average_temperature <- mean(temperatures)
```

4. Create a named vector called weekly\_temperatures, where the names are the days of the week and the values are the temperatures from the temperatures vector.

```
weekly_temperatures <- temperatures  
names(weekly_temperatures) <- days
```

5. Create a numeric vector called ages containing the following values: 25, 30, 35, 40, 45, 50, 55, 60.

```
ages <- c(25, 30, 35, 40, 45, 50, 55, 60)
```

6. Create a logical vector called is\_adult by checking if the elements in the ages vector are greater than or equal to 18.

```
is_adult <- ages >= 18
```

7. Calculate the sum and product of the ages vector.

```
sum_ages <- sum(ages)  
product_ages <- prod(ages)
```

8. Extract the ages greater than or equal to 40 from the ages vector and store them in a variable called older\_ages.

```
older_ages <- ages[ages >= 40]
```

# 6

## Matrices

A *matrix* is a rectangular collection of the same data type (see Figure 6.1). It can be viewed as a collection of column vectors all of the same length and the same type (i.e. numeric, character or logical) OR a collection of row vectors, again all of the same type and length. A *data.frame* is also a rectangular array. All of the columns must be the same length, but they **may be** of *different* types. The rows and columns of a matrix or data frame can be given names. However these are implemented differently in R; many operations will work for one but not both, often a source of confusion.

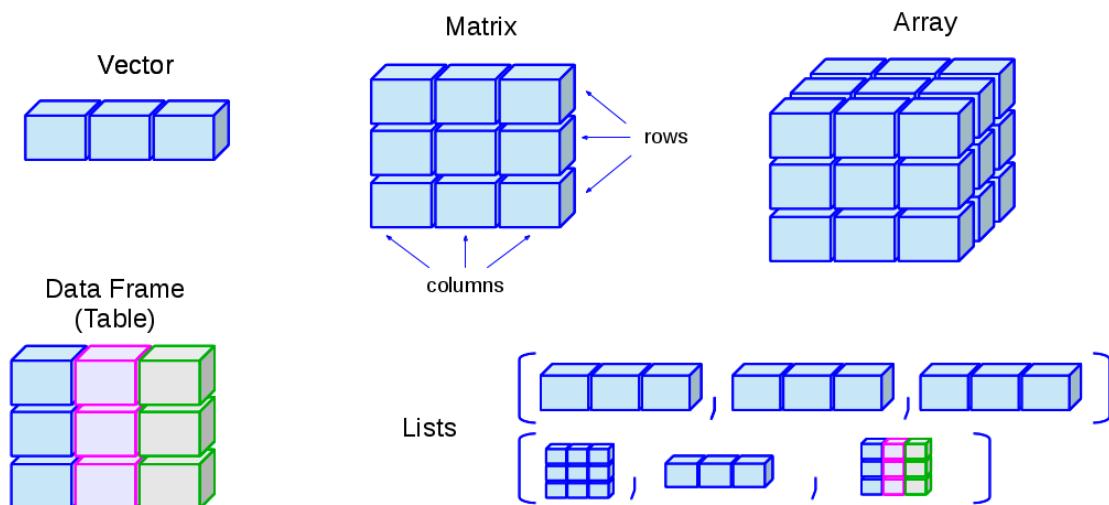


Figure 6.1: A matrix is a collection of column vectors.

### 6.1 Creating a matrix

There are many ways to create a matrix in R. One of the simplest is to use the `matrix()` function. In the code below, we'll create a matrix from a vector from 1:16.

```
mat1 <- matrix(1:16, nrow=4)
mat1
```

```
[ ,1] [ ,2] [ ,3] [ ,4]
[1,]    1     5     9    13
[2,]    2     6    10    14
[3,]    3     7    11    15
[4,]    4     8    12    16
```

The same is possible, but specifying that the matrix be “filled” by row.

```
mat1 <- matrix(1:16, nrow=4, byrow = TRUE)
mat1
```

```
[ ,1] [ ,2] [ ,3] [ ,4]
[1,]    1     2     3     4
[2,]    5     6     7     8
[3,]    9    10    11    12
[4,]   13    14    15    16
```

Notice the subtle difference in the order that the numbers go into the matrix.

We can also build a matrix from parts by “binding” vectors together:

```
x <- 1:10
y <- rnorm(10)
```

Each of the vectors above is of length 10 and both are “numeric”, so we can make them into a matrix. Using `rbind` binds rows (**r**) into a matrix.

```
mat <- rbind(x,y)
mat
```

```
[ ,1]      [ ,2]      [ ,3]      [ ,4]      [ ,5]      [ ,6]      [ ,7]
x 1.000000 2.00000000 3.000000 4.0000000 5.000000 6.000000 7.0000000
y 1.067529 0.07571403 -0.364383 -0.4984749 -1.154545 -1.259663 -0.4787461
[ ,8]      [ ,9]      [ ,10]
x 8.000000 9.00000000 10.000000
y 1.226782 -0.03506617 -1.085638
```

The alternative to `rbind` is `cbind` that binds columns (**c**) together.

```
mat <- cbind(x,y)
mat
```

```
      x          y
[1,] 1  1.06752879
[2,] 2  0.07571403
[3,] 3 -0.36438304
[4,] 4 -0.49847495
[5,] 5 -1.15454461
[6,] 6 -1.25966295
```

```
[7,] 7 -0.47874610  
[8,] 8 1.22678244  
[9,] 9 -0.03506617  
[10,] 10 -1.08563803
```

Inspecting the names associated with rows and columns is often useful, particularly if the names have human meaning.

```
rownames(mat)
```

```
NULL
```

```
colnames(mat)
```

```
[1] "x" "y"
```

We can also change the names of the matrix by assigning *valid* names to the columns or rows.

```
colnames(mat) = c('apples','oranges')  
colnames(mat)
```

```
[1] "apples" "oranges"
```

```
mat
```

	apples	oranges
[1,]	1 1.06752879	
[2,]	2 0.07571403	
[3,]	3 -0.36438304	
[4,]	4 -0.49847495	
[5,]	5 -1.15454461	
[6,]	6 -1.25966295	
[7,]	7 -0.47874610	
[8,]	8 1.22678244	
[9,]	9 -0.03506617	
[10,]	10 -1.08563803	

Matrices have dimensions.

```
dim(mat)
```

```
[1] 10 2
```

```
nrow(mat)
```

```
[1] 10
```

```
ncol(mat)
```

```
[1] 2
```

## 6.2 Accessing elements of a matrix

Indexing for matrices works as for vectors except that we now need to include both the row and column (in that order). We can access elements of a matrix using the square bracket [ indexing method. Elements can be accessed as `var[r, c]`. Here, `r` and `c` are vectors describing the elements of the matrix to select.

### ! Important

The indices in R start with one, meaning that the first element of a vector or the first row/column of a matrix is indexed as one.

This is different from some other programming languages, such as Python, which use zero-based indexing, meaning that the first element of a vector or the first row/column of a matrix is indexed as zero.

It is important to be aware of this difference when working with data in R, especially if you are coming from a programming background that uses zero-based indexing. Using the wrong index can lead to unexpected results or errors in your code.

```
# The 2nd element of the 1st row of mat  
mat[1, 2]
```

```
oranges  
1.067529
```

```
# The first ROW of mat  
mat[, 1]
```

```
apples oranges  
1.000000 1.067529
```

```
# The first COLUMN of mat  
mat[, 1]
```

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

```
# and all elements of mat that are > 4; note no comma  
mat[mat > 4]
```

```
[1] 5 6 7 8 9 10  
## [1] 5 6 7 8 9 10
```

### 🔥 Caution

Note that in the last case, there is no “,”, so R treats the matrix as a long vector (`length=20`). This is convenient, sometimes, but it can also be a source of error, as some code may “work” but be doing something unexpected.

We can also use indexing to exclude a row or column by prefixing the selection with a - sign.

```
mat[, -1]      # remove first column

[1] 1.06752879 0.07571403 -0.36438304 -0.49847495 -1.15454461 -1.25966295
[7] -0.47874610 1.22678244 -0.03506617 -1.08563803

mat[-c(1:5), ] # remove first five rows

  apples     oranges
[1,]       6 -1.25966295
[2,]       7 -0.47874610
[3,]       8  1.22678244
[4,]       9 -0.03506617
[5,]      10 -1.08563803
```

---

### 6.3 Changing values in a matrix

We can create a matrix filled with random values drawn from a normal distribution for our work below.

```
m = matrix(rnorm(20), nrow=10)
summary(m)
```

V1	V2
Min. : -1.00782	Min. : -1.3058
1st Qu.: -0.05989	1st Qu.: -0.3886
Median : 0.38975	Median : 0.3012
Mean   : 0.24393	Mean   : 0.2810
3rd Qu.: 0.55160	3rd Qu.: 0.9771
Max.   : 1.03408	Max.   : 1.7060

Multiplication and division works similarly to vectors. When multiplying by a vector, for example, the values of the vector are reused. In the simplest case, let's multiply the matrix by a constant (vector of length 1).

```
# multiply all values in the matrix by 20
m2 = m*20
summary(m2)
```

V1	V2
Min. : -20.156	Min. : -26.115
1st Qu.: -1.198	1st Qu.: -7.772
Median : 7.795	Median : 6.024
Mean   : 4.879	Mean   : 5.620
3rd Qu.: 11.032	3rd Qu.: 19.543
Max.   : 20.682	Max.   : 34.120

By combining subsetting with assignment, we can make changes to just part of a matrix.

```
# and add 100 to the first column of m
m2[,1] = m2[,1] + 100
# summarize m
summary(m2)
```

V1	V2
Min. : 79.84	Min. :-26.115
1st Qu.: 98.80	1st Qu.: -7.772
Median :107.80	Median : 6.024
Mean :104.88	Mean : 5.620
3rd Qu.:111.03	3rd Qu.: 19.543
Max. :120.68	Max. : 34.120

A somewhat common transformation for a matrix is to transpose which changes rows to columns. One might need to do this if an assay output from a lab machine puts samples in rows and genes in columns, for example, while in Bioconductor/R, we often want the samples in columns and the genes in rows.

```
t(m2)
```

[,1]	[,2]	[,3]	[,4]	[,5]	[,6]	[,7]
[1,] 110.858518	106.50114	109.08903	95.822400	102.673212	97.51182	111.08978
[2,] 5.611247	-26.11541	32.06543	6.599318	-3.634014	34.12030	-13.59299
[,8]	[,9]	[,10]				
[1,] 120.68152	79.84366	114.71482				
[2,] 23.85726	-9.15092	6.43664				

## 6.4 Calculations on matrix rows and columns

Again, we just need a matrix to play with. We'll use `rnorm` again, but with a slight twist.

```
m3 = matrix(rnorm(100,5,2),ncol=10) # what does the 5 mean here? And the 2?
```

Since these data are from a normal distribution, we can look at a row (or column) to see what the mean and standard deviation are.

```
mean(m3[,1])
```

```
[1] 6.341979
```

```
sd(m3[,1])
```

```
[1] 2.207763
```

```
# or a row
```

```
mean(m3[1,])
```

```
[1] 5.966922
```

```
sd(m3[1,])
```

```
[1] 2.680003
```

There are some useful convenience functions for computing means and sums of data in **all** of the columns and rows of matrices.

```
colMeans(m3)
```

```
[1] 6.341979 5.144662 3.912735 5.022973 5.050446 4.885206 5.162700 5.007736
[9] 5.104758 4.230469
```

```
rowMeans(m3)
```

```
[1] 5.966922 4.423758 5.317974 4.156230 4.986355 5.383235 5.615903 4.186546
[9] 5.278553 4.548189
```

```
rowSums(m3)
```

```
[1] 59.66922 44.23758 53.17974 41.56230 49.86355 53.83235 56.15903 41.86546
[9] 52.78553 45.48189
```

```
colSums(m3)
```

```
[1] 63.41979 51.44662 39.12735 50.22973 50.50446 48.85206 51.62700 50.07736
[9] 51.04758 42.30469
```

We can look at the distribution of column means:

```
# save as a variable
cmeans = colMeans(m3)
summary(cmeans)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
3.913	4.916	5.037	4.986	5.135	6.342

Note that this is centered pretty closely around the selected mean of 5 above.

How about the standard deviation? There is not a `colSD` function, but it turns out that we can easily apply functions that take vectors as input, like `sd` and “apply” them across either the rows (the first dimension) or columns (the second) dimension.

```
csds = apply(m3, 2, sd)
summary(csds)
```

Min.	1st Qu.	Median	Mean	3rd Qu.	Max.
1.382	1.926	1.959	2.085	2.333	2.762

Again, take a look at the distribution which is centered quite close to the selected standard deviation when we created our matrix.

## 6.5 Exercises

### 6.5.1 Data preparation

For this set of exercises, we are going to rely on a dataset that comes with R. It gives the number of sunspots per month from 1749-1983. The dataset comes as a ts or time series data type which I convert to a matrix using the following code.

Just run the code as is and focus on the rest of the exercises.

```
data(sunspots)
sunspot_mat <- matrix(as.vector(sunspots), ncol=12, byrow = TRUE)
colnames(sunspot_mat) <- as.character(1:12)
rownames(sunspot_mat) <- as.character(1749:1983)
```

### 6.5.2 Questions

- After the conversion above, what does sunspot\_mat look like? Use functions to find the number of rows, the number of columns, the class, and some basic summary statistics.

```
ncol(sunspot_mat)
nrow(sunspot_mat)
dim(sunspot_mat)
summary(sunspot_mat)
head(sunspot_mat)
tail(sunspot_mat)
```

- Practice subsetting the matrix a bit by selecting:

- The first 10 years (rows)
- The month of July (7th column)
- The value for July, 1979 using the rowname to do the selection.

```
sunspot_mat[1:10, ]
sunspot_mat[, 7]
sunspot_mat['1979', 7]
```

- These next few exercises take advantage of the fact that calling a univariate statistical function (one that expects a vector) works for matrices by just making a vector of all the values in the matrix. What is the highest (max) number of sunspots recorded in these data?

```
max(sunspot_mat)
```

- And the minimum?

```
min(sunspot_mat)
```

3. And the overall mean and median?

```
mean(sunspot_mat)  
median(sunspot_mat)
```

4. Use the `hist()` function to look at the distribution of all the monthly sunspot data.

```
hist(sunspot_mat)
```

5. Read about the `breaks` argument to `hist()` to try to increase the number of breaks in the histogram to increase the resolution slightly. Adjust your `hist()` and `breaks` to your liking.

```
hist(sunspot_mat, breaks=40)
```

6. Now, let's move on to summarizing the data a bit to learn about the pattern of sunspots varies by month or by year. Examine the dataset again. What do the columns represent? And the rows?

```
# just a quick glimpse of the data will give us a sense  
head(sunspot_mat)
```

7. We'd like to look at the distribution of sunspots by month. How can we do that?

```
# the mean of the columns is the mean number of sunspots per month.  
colMeans(sunspot_mat)  
  
# Another way to write the same thing:  
apply(sunspot_mat, 2, mean)
```

8. Assign the month summary above to a variable and summarize it to get a sense of the spread over months.

```
monthmeans = colMeans(sunspot_mat)  
summary(monthmeans)
```

9. Play the same game for years to get the per-year mean?

```
ymeans = rowMeans(sunspot_mat)  
summary(ymean)
```

10. Make a plot of the yearly means. Do you see a pattern?

```
plot(ymean)  
# or make it clearer  
plot(ymean, type='l')
```

# 7

---

## Data Frames

---

While R has many different data types, the one that is central to much of the power and popularity of R is the `data.frame`. A `data.frame` looks a bit like an R matrix in that it has two dimensions, rows and columns. However, `data.frames` are usually viewed as a set of columns representing variables and the rows representing the values of those variables. Importantly, a `data.frame` may contain *different* data types in each of its columns; matrices **must** contain only one data type. This distinction is important to remember, as there are *specific* approaches to working with R `data.frames` that may be different than those for working with matrices.

---

### 7.1 Learning goals

- Understand how `data.frames` are different from matrices.
  - Know a few functions for examining the contents of a `data.frame`.
  - List approaches for subsetting `data.frames`.
  - Be able to load and save tabular data from and to disk.
  - Show how to create a `data.frames` from scratch.
- 

### 7.2 Learning objectives

- Load the yeast growth dataset into R using `read.csv`.
  - Examine the contents of the dataset.
  - Use subsetting to find genes that may be involved with nutrient metabolism and transport.
  - Summarize data measurements by categories.
- 

### 7.3 Dataset

The data used here are borrowed directly from the [fantastic Bioconductor tutorials](#) and are a cleaned up version of the data from [Brauer et al. Coordination of Growth Rate, Cell Cycle, Stress Response, and Metabolic](#)

[Activity in Yeast \(2008\) Mol Biol Cell 19:352-367](#). These data are from a gene expression microarray, and in this paper the authors examine the relationship between growth rate and gene expression in yeast cultures limited by one of six different nutrients (glucose, leucine, ammonium, sulfate, phosphate, uracil). If you give yeast a rich media loaded with nutrients except restrict the supply of a single nutrient, you can control the growth rate to any rate you choose. By starving yeast of specific nutrients you can find genes that:

1. Raise or lower their expression in response to growth rate. Growth-rate dependent expression patterns can tell us a lot about cell cycle control, and how the cell responds to stress. The authors found that expression of >25% of all yeast genes is linearly correlated with growth rate, independent of the limiting nutrient. They also found that the subset of negatively growth-correlated genes is enriched for peroxisomal functions, and positively correlated genes mainly encode ribosomal functions.
2. Respond differently when different nutrients are being limited. If you see particular genes that respond very differently when a nutrient is sharply restricted, these genes might be involved in the transport or metabolism of that specific nutrient.

The dataset can be downloaded directly from:

- [brauer2007\\_tidy.csv](#)

We are going to read this dataset into R and then use it as a playground for learning about data.frames.

---

## 7.4 Reading in data

R has many capabilities for reading in data. Many of the functions have names that help us to understand what data format is to be expected. In this case, the filename that we want to read ends in .csv, meaning comma-separated-values. The `read.csv()` function reads in .csv files. As usual, it is worth reading `help('read.csv')` to get a better sense of the possible bells-and-whistles.

The `read.csv()` function can read directly from a URL, so we do not need to download the file directly. This dataset is relatively large (about 16MB), so this may take a bit depending on your network connection speed.

```
options(width=60)

url = paste0(
  'https://raw.githubusercontent.com',
  '/bioconnector/workshops/master/data/brauer2007_tidy.csv'
)
ydat <- read.csv(url)
```

Our variable, `ydat`, now “contains” the downloaded and read data. We can check to see what data type `read.csv` gave us:

```
class(ydat)  
[1] "data.frame"
```

---

## 7.5 Inspecting `data.frames`

Our `ydat` variable is a `data.frame`. As I mentioned, the dataset is fairly large, so we will not be able to look at it all at once on the screen. However, R gives us many tools to inspect a `data.frame`.

- Overviews of content
  - `head()` to show first few rows
  - `tail()` to show last few rows
- Size
  - `dim()` for dimensions (rows, columns)
  - `nrow()`
  - `ncol()`
  - `object.size()` for power users interested in the memory used to store an object
- Data and attribute summaries
  - `colnames()` to get the names of the columns
  - `rownames()` to get the “names” of the rows—may not be present
  - `summary()` to get per-column summaries of the data in the `data.frame`.

```
head(ydat)  
  
symbol systematic_name nutrient rate expression  
1   SFB2          YNL049C  Glucose  0.05    -0.24  
2 <NA>          YNL095C  Glucose  0.05     0.28  
3   QRI7          YDL104C  Glucose  0.05    -0.02  
4   CFT2          YLR115W  Glucose  0.05    -0.33  
5   SSO2          YMR183C  Glucose  0.05     0.05  
6   PSP2          YML017W  Glucose  0.05    -0.69  
                                bp  
1       ER to Golgi transport  
2   biological process unknown  
3 proteolysis and peptidolysis  
4      mRNA polyadenylation*  
5           vesicle fusion*  
6   biological process unknown  
                                mf  
1   molecular function unknown  
2   molecular function unknown  
3 metalloendopeptidase activity  
4           RNA binding  
5       t-SNARE activity
```

```
6     molecular function unknown
tail(ydat)

  symbol systematic_name nutrient rate expression
198425  DOA1          YKL213C   Uracil  0.3    0.14
198426  KRE1          YNL322C   Uracil  0.3    0.28
198427  MTL1          YGR023W   Uracil  0.3    0.27
198428  KRE9          YJL174W   Uracil  0.3    0.43
198429  UTH1          YKR042W   Uracil  0.3    0.19
198430 <NA>          YOL111C   Uracil  0.3    0.04
                                         bp
198425  ubiquitin-dependent protein catabolism*
198426  cell wall organization and biogenesis
198427  cell wall organization and biogenesis
198428  cell wall organization and biogenesis*
198429  mitochondrion organization and biogenesis*
198430           biological process unknown
                                         mf
198425      molecular function unknown
198426  structural constituent of cell wall
198427      molecular function unknown
198428      molecular function unknown
198429      molecular function unknown
198430      molecular function unknown

dim(ydat)

[1] 198430      7

nrow(ydat)

[1] 198430

ncol(ydat)

[1] 7

colnames(ydat)

[1] "symbol"        "systematic_name" "nutrient"
[4] "rate"          "expression"       "bp"
[7] "mf"

summary(ydat)

  symbol      systematic_name      nutrient
Length:198430  Length:198430  Length:198430
Class :character Class :character Class :character
Mode  :character Mode  :character Mode  :character
```

```

      rate      expression      bp
Min. :0.0500  Min. :-6.500000 Length:198430
1st Qu.:0.1000 1st Qu.:-0.290000 Class :character
Median :0.2000 Median : 0.000000 Mode  :character
Mean   :0.1752 Mean   : 0.003367
3rd Qu.:0.2500 3rd Qu.: 0.290000
Max.  :0.3000  Max.  : 6.640000

      mf
Length:198430
Class :character
Mode  :character

```

In RStudio, there is an additional function, `View()` (note the capital “V”) that opens the first 1000 rows (default) in the RStudio window, akin to a spreadsheet view.

```
View(ydat)
```

---

## 7.6 Accessing variables (columns) and subsetting

In R, `data.frames` can be subset similarly to other two-dimensional data structures. The `[` in R is used to denote subsetting of any kind. When working with two-dimensional data, we need two values inside the `[ ]` to specify the details. The specification is `[rows, columns]`. For example, to get the first three rows of `ydat`, use:

```
ydat[1:3, ]
```

```

symbol systematic_name nutrient rate expression
1 SFB2      YNL049C Glucose 0.05    -0.24
2 <NA>      YNL095C Glucose 0.05     0.28
3 QRI7      YDL104C Glucose 0.05    -0.02

      bp
1 ER to Golgi transport
2 biological process unknown
3 proteolysis and peptidolysis

      mf
1 molecular function unknown
2 molecular function unknown
3 metalloendopeptidase activity

```

Note how the second number, the `columns`, is blank. R takes that to mean “all the columns”. Similarly, we

can combine rows and columns specification arbitrarily.

```
ydat[1:3, 1:3]
```

```
symbol systematic_name nutrient
1 SFB2 YNL049C Glucose
2 <NA> YNL095C Glucose
3 QRI7 YDL104C Glucose
```

Because selecting a single variable, or column, is such a common operation, there are two shortcuts for doing so *with data.frames*. The first, the \$ operator works like so:

```
# Look at the column names, just to refresh memory
colnames(ydat)
```

```
[1] "symbol"      "systematic_name" "nutrient"
[4] "rate"        "expression"     "bp"
[7] "mf"
```

```
# Note that I am using "head" here to limit the output
head(ydat$symbol)
```

```
[1] "SFB2" NA      "QRI7" "CFT2" "SSO2" "PSP2"
```

```
# What is the actual length of "symbol"?
```

```
length(ydat$symbol)
```

```
[1] 198430
```

The second is related to the fact that, in R, data.frames are also lists. We subset a list by using [ ] notation. To get the second column of ydat, we can use:

```
head(ydat[[2]])
```

```
[1] "YNL049C" "YNL095C" "YDL104C" "YLR115W" "YMR183C"
[6] "YML017W"
```

Alternatively, we can use the column name:

```
head(ydat[["systematic_name"]])
```

```
[1] "YNL049C" "YNL095C" "YDL104C" "YLR115W" "YMR183C"
[6] "YML017W"
```

### 7.6.1 Some data exploration

There are a couple of columns that include numeric values. Which columns are numeric?

```
class(ydat$symbol)
```

```
[1] "character"
```

```
class(ydat$rate)
```

```
[1] "numeric"
class(ydat$expression)
```

```
[1] "numeric"
```

Make histograms of: - the expression values - the rate values

What does the table() function do? Could you use that to look at the rate column given that that column appears to have repeated values?

What rate corresponds to the most nutrient-starved condition?

### 7.6.2 More advanced indexing and subsetting

We can use, for example, logical values (TRUE/FALSE) to subset data.frames.

```
head(ydat[ydat$symbol == 'LEU1', ])
```

	symbol	systematic_name	nutrient	rate	expression	bp
NA	<NA>	<NA>	<NA>	NA	NA	<NA>
NA.1	<NA>	<NA>	<NA>	NA	NA	<NA>
NA.2	<NA>	<NA>	<NA>	NA	NA	<NA>
NA.3	<NA>	<NA>	<NA>	NA	NA	<NA>
NA.4	<NA>	<NA>	<NA>	NA	NA	<NA>
NA.5	<NA>	<NA>	<NA>	NA	NA	<NA>
	mf					
NA	<NA>					
NA.1	<NA>					
NA.2	<NA>					
NA.3	<NA>					
NA.4	<NA>					
NA.5	<NA>					

```
tail(ydat[ydat$symbol == 'LEU1', ])
```

	symbol	systematic_name	nutrient	rate	expression
NA.47244	<NA>	<NA>	<NA>	NA	NA
NA.47245	<NA>	<NA>	<NA>	NA	NA
NA.47246	<NA>	<NA>	<NA>	NA	NA
NA.47247	<NA>	<NA>	<NA>	NA	NA
NA.47248	<NA>	<NA>	<NA>	NA	NA
NA.47249	<NA>	<NA>	<NA>	NA	NA
	bp	mf			
NA.47244	<NA>	<NA>			
NA.47245	<NA>	<NA>			
NA.47246	<NA>	<NA>			
NA.47247	<NA>	<NA>			
NA.47248	<NA>	<NA>			
NA.47249	<NA>	<NA>			

What is the problem with this approach? It appears that there are a bunch of NA values. Taking a quick look at the symbol column, we see what the problem.

```
summary(ydat$symbol)
```

Length	Class	Mode
198430	character	character

Using the `is.na()` function, we can make filter further to get down to values of interest.

```
head(ydat[ydat$symbol == 'LEU1' & !is.na(ydat$symbol), ])
```

	symbol	systematic_name	nutrient	rate	expression
1526	LEU1	YGL009C	Glucose	0.05	-1.12
7043	LEU1	YGL009C	Glucose	0.10	-0.77
12555	LEU1	YGL009C	Glucose	0.15	-0.67
18071	LEU1	YGL009C	Glucose	0.20	-0.59
23603	LEU1	YGL009C	Glucose	0.25	-0.20
29136	LEU1	YGL009C	Glucose	0.30	0.03
			bp		
1526		leucine biosynthesis			
7043		leucine biosynthesis			
12555		leucine biosynthesis			
18071		leucine biosynthesis			
23603		leucine biosynthesis			
29136		leucine biosynthesis			
			mf		
1526		3-isopropylmalate dehydratase activity			
7043		3-isopropylmalate dehydratase activity			
12555		3-isopropylmalate dehydratase activity			
18071		3-isopropylmalate dehydratase activity			
23603		3-isopropylmalate dehydratase activity			
29136		3-isopropylmalate dehydratase activity			

Sometimes, looking at the data themselves is not that important. Using `dim()` is one possibility to look at the number of rows and columns after subsetting.

```
dim(ydat[ydat$expression > 3, ])
```

```
[1] 714    7
```

Find the high expressed genes when leucine-starved. For this task we can also use `subset` which allows us to treat column names as R variables (no \$ needed).

```
subset(ydat, nutrient == 'Leucine' & rate == 0.05 & expression > 3)
```

	symbol	systematic_name	nutrient	rate	expression
133768	QDR2	YIL121W	Leucine	0.05	4.61
133772	LEU1	YGL009C	Leucine	0.05	3.84
133858	BAP3	YDR046C	Leucine	0.05	4.29
135186	<NA>	YPL033C	Leucine	0.05	3.43

135187	<NA>	YLR267W	Leucine 0.05	3.23
135288	HXT3	YDR345C	Leucine 0.05	5.16
135963	TPO2	YGR138C	Leucine 0.05	3.75
135965	YRO2	YBR054W	Leucine 0.05	4.40
136102	GPG1	YGL121C	Leucine 0.05	3.08
136109	HSP42	YDR171W	Leucine 0.05	3.07
136119	HXT5	YHR096C	Leucine 0.05	4.90
136151	<NA>	YJL144W	Leucine 0.05	3.06
136152	MOH1	YBL049W	Leucine 0.05	3.43
136153	<NA>	YBL048W	Leucine 0.05	3.95
136189	HSP26	YBR072W	Leucine 0.05	4.86
136231	NCA3	YJL116C	Leucine 0.05	4.03
136233	<NA>	YBR116C	Leucine 0.05	3.28
136486	<NA>	YGR043C	Leucine 0.05	3.07
137443	ADH2	YMR303C	Leucine 0.05	4.15
137448	ICL1	YER065C	Leucine 0.05	3.54
137451	SFC1	YJR095W	Leucine 0.05	3.72
137569	MLS1	YNL117W	Leucine 0.05	3.76

bp

133768		multidrug transport
133772		leucine biosynthesis
133858		amino acid transport
135186		meiosis*
135187		biological process unknown
135288		hexose transport
135963		polyamine transport
135965		biological process unknown
136102		signal transduction
136109		response to stress*
136119		hexose transport
136151		response to dessication
136152		biological process unknown
136153		<NA>
136189		response to stress*
136231	mitochondrion organization and biogenesis	
136233		<NA>
136486		biological process unknown
137443		fermentation*
137448		glyoxylate cycle
137451		fumarate transport*
137569		glyoxylate cycle
		mf
133768	multidrug efflux pump activity	
133772	3-isopropylmalate dehydratase activity	
133858	amino acid transporter activity	
135186	molecular function unknown	

```

135187      molecular function unknown
135288      glucose transporter activity*
135963      spermine transporter activity
135965      molecular function unknown
136102      signal transducer activity
136109      unfolded protein binding
136119      glucose transporter activity*
136151      molecular function unknown
136152      molecular function unknown
136153          <NA>
136189      unfolded protein binding
136231      molecular function unknown
136233          <NA>
136486      transaldolase activity
137443      alcohol dehydrogenase activity
137448      isocitrate lyase activity
137451 succinate:fumarate antiporter activity
137569      malate synthase activity

```

---

## 7.7 Aggregating data

Aggregating data, or summarizing by category, is a common way to look for trends or differences in measurements between categories. Use aggregate to find the mean expression by gene symbol.

```
headaggregate(ydat$expression, by=list( ydat$symbol), mean))
```

```

Group.1           x
1   AAC1  0.52888889
2   AAC3 -0.21628571
3   AAD10 0.43833333
4   AAD14 -0.07166667
5   AAD16  0.24194444
6   AAD4 -0.79166667

# or
headaggregate(expression ~ symbol, mean, data=ydat))
```

```

symbol  expression
1   AAC1  0.52888889
2   AAC3 -0.21628571
3   AAD10 0.43833333
4   AAD14 -0.07166667
5   AAD16  0.24194444
6   AAD4 -0.79166667
```

## 7.8 Creating a data.frame from scratch

Sometimes it is useful to combine related data into one object. For example, let's simulate some data.

```
smoker = factor(rep(c("smoker", "non-smoker"), each=50))
smoker_numeric = as.numeric(smoker)
x = rnorm(100)
risk = x + 2*smoker_numeric
```

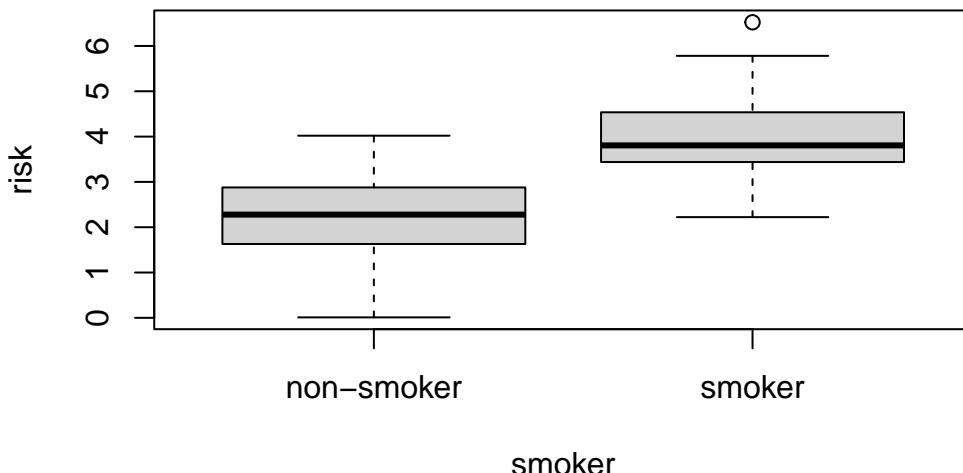
We have two variables, risk and smoker that are related. We can make a data.frame out of them:

```
smoker_risk = data.frame(smoker = smoker, risk = risk)
head(smoker_risk)
```

```
smoker      risk
1 smoker 3.056532
2 smoker 3.558159
3 smoker 4.158129
4 smoker 2.981418
5 smoker 4.052849
6 smoker 3.976635
```

R also has plotting shortcuts that work with data.frames to simplify plotting

```
plot(risk ~ smoker, data=smoker_risk)
```



## 7.9 Saving a data.frame

Once we have a data.frame of interest, we may want to save it. The most portable way to save a data.frame is to use one of the write functions. In this case, let's save the data as a .csv file.

```
write.csv(smoker_risk, "smoker_risk.csv")
```

# 8

---

## Factors

---

### 8.1 Factors

A factor is a special type of vector, normally used to hold a categorical variable—such as smoker/nonsmoker, state of residency, zipcode—in many statistical functions. Such vectors have class “factor”. Factors are primarily used in Analysis of Variance (ANOVA) or other situations when “categories” are needed. When a factor is used as a predictor variable, the corresponding indicator variables are created (more later).

Note of caution that factors in R often *appear* to be character vectors when printed, but you will notice that they do not have double quotes around them. They are stored in R as numbers with a key name, so sometimes you will note that the factor *behaves* like a numeric vector.

```
# create the character vector
citizen<-c("uk", "us", "no", "au", "uk", "us", "us", "no", "au")
```

```
# convert to factor
citizenf<-factor(citizen)
citizen
```

```
[1] "uk" "us" "no" "au" "uk" "us" "us" "no" "au"
```

```
citizenf
```

```
[1] uk us no au uk us us no au
```

```
Levels: au no uk us
```

```
# convert factor back to character vector
as.character(citizenf)
```

```
[1] "uk" "us" "no" "au" "uk" "us" "us" "no" "au"
```

```
# convert to numeric vector
as.numeric(citizenf)
```

```
[1] 3 4 2 1 3 4 4 2 1
```

R stores many data structures as vectors with “attributes” and “class” (just so you have seen this).

```
attributes(citizenf)
```

```
$levels
```

```
[1] "au" "no" "uk" "us"  
$class  
[1] "factor"  
class(citizenf)  
[1] "factor"  
# note that after unclassing, we can see the  
# underlying numeric structure again  
unclass(citizenf)
```

```
[1] 3 4 2 1 3 4 4 2 1  
attr(,"levels")  
[1] "au" "no" "uk" "us"
```

Tabulating factors is a useful way to get a sense of the “sample” set available.

```
table(citizenf)
```

```
citizenf  
au no uk us  
2 2 2 3
```

## **Part III**

# **Exploratory data analysis**

Imagine you're on an adventure, about to embark on a journey into the unknown. You've just been handed a treasure map, with the promise of valuable insights waiting to be discovered. This map is your data set, and the journey is exploratory data analysis (EDA).

As you begin your exploration, you start by getting a feel for the terrain. You take a broad, bird's-eye view of the data, examining its structure and dimensions. Are you dealing with a vast landscape or a small, confined area? Are there any missing pieces in the map that you'll need to account for? Understanding the overall context of your data set is crucial before venturing further.

With a sense of the landscape, you now zoom in to identify key landmarks in the data. You might look for unusual patterns, trends, or relationships between variables. As you spot these landmarks, you start asking questions: What's causing that spike in values? Are these two factors related, or is it just a coincidence? By asking these questions, you're actively engaging with the data and forming hypotheses that could guide future analysis or experiments.

As you continue your journey, you realize that the map alone isn't enough to fully understand the terrain. You need more tools to bring the data to life. You start visualizing the data using charts, plots, and graphs. These visualizations act as your binoculars, allowing you to see patterns and relationships more clearly. Through them, you can uncover the hidden treasures buried within the data.

EDA isn't a linear path from start to finish. As you explore, you'll find yourself circling back to previous points, refining your questions, and digging deeper. The process is iterative, with each new discovery informing the next. And as you go, you'll gain a deeper understanding of the data's underlying structure and potential.

Finally, after your thorough exploration, you'll have a solid foundation to build upon. You'll be better equipped to make informed decisions, test hypotheses, and draw meaningful conclusions. The insights you've gained through EDA will serve as a compass, guiding you towards the true value hidden within your data. And with that, you've successfully completed your journey through exploratory data analysis.

# 9

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## *Introduction to dplyr: mammal sleep dataset*

---

The dataset we will be using to introduce the *dplyr* package is an updated and expanded version of the mammals sleep dataset. Updated sleep times and weights were taken from V. M. Savage and G. B. West. A quantitative, theoretical framework for understanding mammalian sleep<sup>1</sup>.

---

### 9.1 Learning goals

- Know that *dplyr* is just a different approach to manipulating data in *data.frames*.
  - List the commonly used *dplyr* verbs and how they can be used to manipulate *data.frames*.
  - Show how to aggregate and summarize data using *dplyr*
  - Know what the piping operator, `|>`, is and how it can be used.
- 

### 9.2 Learning objectives

- Select subsets of the mammal sleep dataset.
  - Reorder the dataset.
  - Add columns to the dataset based on existing columns.
  - Summarize the amount of sleep by categorical variables using `group_by` and `summarize`.
- 

### 9.3 What is *dplyr*?

The *dplyr* package is a specialized package for working with *data.frames* (and the related *tibble*) to transform and summarize tabular data with rows and columns. For another explanation of *dplyr* see the *dplyr* package vignette: [Introduction to dplyr](#)

---

<sup>1</sup>A quantitative, theoretical framework for understanding mammalian sleep. Van M. Savage, Geoffrey B. West. Proceedings of the National Academy of Sciences Jan 2007, 104 (3) 1051-1056; DOI: [10.1073/pnas.0610080104](https://doi.org/10.1073/pnas.0610080104)

---

## 9.4 Why Is dplyr useful?

dplyr contains a set of functions—commonly called the dplyr “verbs”—that perform common data manipulations such as filtering for rows, selecting specific columns, re-ordering rows, adding new columns and summarizing data. In addition, dplyr contains a useful function to perform another common task which is the “split-apply-combine” concept.

Compared to base functions in R, the functions in dplyr are often easier to work with, are more consistent in the syntax and are targeted for data analysis around data frames, instead of just vectors.

---

---

## 9.5 Data: Mammals Sleep

The msleep (mammals sleep) data set contains the sleep times and weights for a set of mammals and is available in the dagdata repository on github. This data set contains 83 rows and 11 variables. The data happen to be available as a dataset in the ggplot2 package. To get access to the msleep dataset, we need to first install the ggplot2 package.

```
install.packages('ggplot2')
```

Then, we can load the library.

```
library(ggplot2)
data(msleep)
```

As with many datasets in R, “help” is available to describe the dataset itself.

```
?msleep
```

The columns are described in the help page, but are included here, also.

column name	Description
name	common name
genus	taxonomic rank
vore	carnivore, omnivore or herbivore?
order	taxonomic rank
conservation	the conservation status of the mammal
sleep_total	total amount of sleep, in hours
sleep_rem	rem sleep, in hours
sleep_cycle	length of sleep cycle, in hours
awake	amount of time spent awake, in hours
brainwt	brain weight in kilograms
bodywt	body weight in kilograms

## 9.6 dplyr verbs

The dplyr verbs are listed here. There are many other functions available in dplyr, but we will focus on just these.

dplyr verbs	Description
select()	select columns
filter()	filter rows
arrange()	re-order or arrange rows
mutate()	create new columns
summarise()	summarise values
group_by()	allows for group operations in the “split-apply-combine” concept

---

## 9.7 Using the dplyr verbs

The two most basic functions are `select()` and `filter()`, which selects columns and filters rows respectively. What are the equivalent ways to select columns without dplyr? And filtering to include only specific rows?

Before proceeding, we need to install the dplyr package:

```
install.packages('dplyr')
```

And then load the library:

```
library(dplyr)
```

Attaching package: 'dplyr'

The following objects are masked from 'package:stats':

  filter, lag

The following objects are masked from 'package:base':

  intersect, setdiff, setequal, union

### 9.7.1 Selecting columns: `select()`

Select a set of columns such as the name and the sleep\_total columns.

```
sleepData <- select(msleep, name, sleep_total)
head(sleepData)
```

```
# A tibble: 6 x 2
  name          sleep_total
  <chr>        <dbl>
1 Cheetah      12.1
2 Owl monkey   17
3 Mountain beaver 14.4
4 Greater short-tailed shrew 14.9
5 Cow          4
6 Three-toed sloth 14.4
```

To select all the columns *except* a specific column, use the “-” (subtraction) operator (also known as negative indexing). For example, to select all columns except name:

```
head(select(msleep, -name))
```

```
# A tibble: 6 x 10
  genus     vore order  conservation sleep_total sleep_rem sleep_cycle awake
  <chr>    <chr> <chr>    <chr>        <dbl>       <dbl>       <dbl> <dbl>
1 Acinonyx carni Carnivo~ lc           12.1       NA       NA     11.9
2 Aotus      omni Primates <NA>        17         1.8      NA      7
3 Aplodontia herbi Rodentia nt          14.4       2.4      NA     9.6
4 Blarina    omni Soricom~ lc           14.9       2.3     0.133    9.1
5 Bos        herbi Artioda~ domesticated 4          0.7     0.667   20
6 Bradypus   herbi Pilosa  <NA>        14.4       2.2     0.767   9.6
# i 2 more variables: brainwt <dbl>, bodywt <dbl>
```

To select a range of columns by name, use the “:” operator. Note that dplyr allows us to use the column names without quotes and as “indices” of the columns.

```
head(select(msleep, name:order))
```

```
# A tibble: 6 x 4
  name          genus     vore order
  <chr>        <chr>    <chr> <chr>
1 Cheetah      Acinonyx carni Carnivora
2 Owl monkey   Aotus     omni  Primates
3 Mountain beaver Aplodontia herbi Rodentia
4 Greater short-tailed shrew Blarina   omni  Soricomorpha
5 Cow          Bos       herbi Artiodactyla
6 Three-toed sloth Bradypus  herbi Pilosa
```

To select all columns that start with the character string “sl”, use the function starts\_with().

```
head(select(msleep, starts_with("sl")))
```

```
# A tibble: 6 x 3
  sleep_total sleep_rem sleep_cycle
  <dbl>       <dbl>       <dbl>
1 12.1        NA        NA
2 17          1.8       NA
```

```

3      14.4      2.4      NA
4      14.9      2.3     0.133
5       4       0.7     0.667
6      14.4      2.2     0.767

```

Some additional options to select columns based on a specific criteria include:

1. `ends_with()` = Select columns that end with a character string
2. `contains()` = Select columns that contain a character string
3. `matches()` = Select columns that match a regular expression
4. `one_of()` = Select column names that are from a group of names

### 9.7.2 Selecting rows: `filter()`

The `filter()` function allows us to filter rows to include only those rows that *match* the filter. For example, we can filter the rows for mammals that sleep a total of more than 16 hours.

```
filter(msleep, sleep_total >= 16)
```

```

# A tibble: 8 x 11
  name   genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr>  <chr> <chr> <chr> <chr>        <dbl>      <dbl>      <dbl> <dbl>
1 Owl    mo~ Aotus omni Prim~ <NA>         17        1.8      NA     7
2 Long-n~ Dasy~ carni Cing~ 1c          17.4       3.1     0.383   6.6
3 North ~ Dide~ omni  Dide~ 1c          18        4.9     0.333   6
4 Big    br~ Epte~ inse~ Chir~ 1c          19.7       3.9     0.117   4.3
5 Thick-~ Lutr~ carni Dide~ 1c          19.4       6.6      NA     4.6
6 Little~ Myot~ inse~ Chir~ <NA>         19.9       2       0.2     4.1
7 Giant ~ Prio~ inse~ Cing~ en          18.1       6.1      NA     5.9
8 Arctic~ Sper~ herbi Rode~ 1c          16.6       NA      NA     7.4
# i 2 more variables: brainwt <dbl>, bodywt <dbl>

```

Filter the rows for mammals that sleep a total of more than 16 hours *and* have a body weight of greater than 1 kilogram.

```
filter(msleep, sleep_total >= 16, bodywt >= 1)
```

```

# A tibble: 3 x 11
  name   genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr>  <chr> <chr> <chr> <chr>        <dbl>      <dbl>      <dbl> <dbl>
1 Long-n~ Dasy~ carni Cing~ 1c          17.4       3.1     0.383   6.6
2 North ~ Dide~ omni  Dide~ 1c          18        4.9     0.333   6
3 Giant ~ Prio~ inse~ Cing~ en          18.1       6.1      NA     5.9
# i 2 more variables: brainwt <dbl>, bodywt <dbl>

```

Filter the rows for mammals in the Perissodactyla and Primates taxonomic order. The `%in%` operator is a logical operator that returns TRUE for values of a vector that are present *in* a second vector.

```
filter(msleep, order %in% c("Perissodactyla", "Primates"))

# A tibble: 15 x 11
  name   genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr> <chr> <chr> <chr> <chr>       <dbl>      <dbl>      <dbl> <dbl>
1 Owl   m~ Aotus omni Prim~ <NA>        17        1.8      NA     7
2 Grivet Cerc~ omni Prim~ lc          10        0.7      NA    14
3 Horse  Equus herbi Peri~ domesticated  2.9        0.6      1     21.1
4 Donkey Equus herbi Peri~ domesticated  3.1        0.4      NA    20.9
5 Patas~ Eryt~ omni Prim~ lc          10.9       1.1      NA   13.1
6 Galago Gala~ omni Prim~ <NA>        9.8        1.1      0.55  14.2
7 Human   Homo omni Prim~ <NA>        8         1.9      1.5    16
8 Mongo~ Lemur herbi Prim~ vu          9.5        0.9      NA   14.5
9 Macaque~ Maca~ omni Prim~ <NA>      10.1       1.2      0.75  13.9
10 Slow ~ Nyct~ carni Prim~ <NA>      11         NA      NA    13
11 Chimp~ Pan   omni Prim~ <NA>      9.7        1.4      1.42  14.3
12 Baboon Papio omni Prim~ <NA>      9.4        1       0.667 14.6
13 Potto   Pero~ omni Prim~ lc          11         NA      NA    13
14 Squir~ Saim~ omni Prim~ <NA>      9.6        1.4      NA   14.4
15 Brazi~ Tapi~ herbi Peri~ vu          4.4        1       0.9   19.6
# i 2 more variables: brainwt <dbl>, bodywt <dbl>
```

You can use the boolean operators (e.g. >, <, >=, <=, !=, %in%) to create the logical tests.

---

## 9.8 “Piping” with |>

It is not unusual to want to perform a set of operations using dplyr. The pipe operator |> allows us to “pipe” the output from one function into the input of the next. While there is nothing special about how R treats operations that are written in a pipe, the idea of piping is to allow us to read multiple functions operating one after another from left-to-right. Without piping, one would either 1) save each step in set of functions as a temporary variable and then pass that variable along the chain or 2) have to “nest” functions, which can be hard to read.

Here’s an example we have already used:

```
head(select(msleep, name, sleep_total))

# A tibble: 6 x 2
  name           sleep_total
  <chr>          <dbl>
1 Cheetah        12.1
2 Owl monkey     17
3 Mountain beaver 14.4
4 Greater short-tailed shrew 14.9
5 Cow             4
```

```
6 Three-toed sloth      14.4
```

Now in this case, we will pipe the msleep data frame to the function that will select two columns (name and sleep\\_total) and then pipe the new data frame to the function head(), which will return the head of the new data frame.

```
msleep |>
  select(name, sleep_total) |>
  head()

# A tibble: 6 x 2
  name          sleep_total
  <chr>        <dbl>
1 Cheetah       12.1
2 Owl monkey    17
3 Mountain beaver 14.4
4 Greater short-tailed shrew 14.9
5 Cow            4
6 Three-toed sloth 14.4
```

You will soon see how useful the pipe operator is when we start to combine many functions.

Now that you know about the pipe operator (|>), we will use it throughout the rest of this tutorial.

### 9.8.1 Arrange Or Re-order Rows Using arrange()

To arrange (or re-order) rows by a particular column, such as the taxonomic order, list the name of the column you want to arrange the rows by:

```
msleep |> arrange(order) |> head()
```

```
# A tibble: 6 x 11
  name    genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr>   <chr> <chr> <chr> <chr>        <dbl>     <dbl>     <dbl> <dbl>
1 Tenrec  Tenr~ omni  Afro~ <NA>         15.6      2.3      NA     8.4
2 Cow     Bos    herbi Arti~ domesticated  4         0.7      0.667   20
3 Roe de~ Capr~ herbi Arti~ lc           3         NA       NA     21
4 Goat    Capri  herbi Arti~ lc           5.3      0.6      NA     18.7
5 Giraffe Gira~ herbi Arti~ cd           1.9      0.4      NA     22.1
6 Sheep   Ovis   herbi Arti~ domesticated 3.8      0.6      NA     20.2
# i 2 more variables: brainwt <dbl>, bodywt <dbl>
```

Now we will select three columns from msleep, arrange the rows by the taxonomic order and then arrange the rows by sleep\_total. Finally, show the head of the final data frame:

```
msleep |>
  select(name, order, sleep_total) |>
  arrange(order, sleep_total) |>
  head()
```

```
# A tibble: 6 x 3
  name      order    sleep_total
  <chr>     <chr>        <dbl>
1 Tenrec    Afrosoricida   15.6
2 Giraffe   Artiodactyla   1.9
3 Roe deer  Artiodactyla   3
4 Sheep     Artiodactyla   3.8
5 Cow       Artiodactyla   4
6 Goat      Artiodactyla   5.3
```

Same as above, except here we filter the rows for mammals that sleep for 16 or more hours, instead of showing the head of the final data frame:

```
msleep |>
  select(name, order, sleep_total) |>
  arrange(order, sleep_total) |>
  filter(sleep_total >= 16)
```

```
# A tibble: 8 x 3
  name      order    sleep_total
  <chr>     <chr>        <dbl>
1 Big brown bat Chiroptera   19.7
2 Little brown bat Chiroptera 19.9
3 Long-nosed armadillo Cingulata 17.4
4 Giant armadillo  Cingulata  18.1
5 North American Opossum Didelphimorphia 18
6 Thick-tailed opossum Didelphimorphia 19.4
7 Owl monkey      Primates    17
8 Arctic ground squirrel Rodentia 16.6
```

For something slightly more complicated do the same as above, except arrange the rows in the sleep\_total column in a descending order. For this, use the function desc()

```
msleep |>
  select(name, order, sleep_total) |>
  arrange(order, desc(sleep_total)) |>
  filter(sleep_total >= 16)
```

```
# A tibble: 8 x 3
  name      order    sleep_total
  <chr>     <chr>        <dbl>
1 Little brown bat Chiroptera 19.9
2 Big brown bat Chiroptera 19.7
3 Giant armadillo  Cingulata 18.1
4 Long-nosed armadillo Cingulata 17.4
5 Thick-tailed opossum Didelphimorphia 19.4
6 North American Opossum Didelphimorphia 18
7 Owl monkey      Primates    17
8 Arctic ground squirrel Rodentia 16.6
```

## 9.9 Create New Columns Using `mutate()`

The `mutate()` function will add new columns to the data frame. Create a new column called `rem_proportion`, which is the ratio of `rem sleep` to total amount of sleep.

```
msleep |>
  mutate(rem_proportion = sleep_rem / sleep_total) |>
  head()
```

```
# A tibble: 6 x 12
  name   genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr>  <chr> <chr> <chr> <chr>       <dbl>      <dbl>      <dbl> <dbl>
1 Cheetah Acin~ carni Carn~ 1c          12.1       NA        NA     11.9
2 Owl     mo~ Aotus omni Prim~ <NA>       17         1.8       NA      7
3 Mounta~ Aplo~ herbi Rode~ nt          14.4       2.4       NA     9.6
4 Greate~ Blar~ omni Sori~ lc          14.9       2.3      0.133    9.1
5 Cow     Bos   herbi Arti~ domesticated 4          0.7      0.667    20
6 Three~~ Brad~ herbi Pilo~ <NA>       14.4       2.2      0.767    9.6
# i 3 more variables: brainwt <dbl>, bodywt <dbl>, rem_proportion <dbl>
```

You can add many new columns using `mutate` (separated by commas). Here we add a second column called `bodywt_grams` which is the `bodywt` column in grams.

```
msleep |>
  mutate(rem_proportion = sleep_rem / sleep_total,
        bodywt_grams = bodywt * 1000) |>
  head()
```

```
# A tibble: 6 x 13
  name   genus vore  order conservation sleep_total sleep_rem sleep_cycle awake
  <chr>  <chr> <chr> <chr> <chr>       <dbl>      <dbl>      <dbl> <dbl>
1 Cheetah Acin~ carni Carn~ 1c          12.1       NA        NA     11.9
2 Owl     mo~ Aotus omni Prim~ <NA>       17         1.8       NA      7
3 Mounta~ Aplo~ herbi Rode~ nt          14.4       2.4       NA     9.6
4 Greate~ Blar~ omni Sori~ lc          14.9       2.3      0.133    9.1
5 Cow     Bos   herbi Arti~ domesticated 4          0.7      0.667    20
6 Three~~ Brad~ herbi Pilo~ <NA>       14.4       2.2      0.767    9.6
# i 4 more variables: brainwt <dbl>, bodywt <dbl>, rem_proportion <dbl>,
#   bodywt_grams <dbl>
```

Is there a relationship between `rem_proportion` and `bodywt`? How about `sleep_total`?

### 9.9.1 Create summaries: `summarise()`

The `summarise()` function will create summary statistics for a given column in the data frame such as finding the mean. For example, to compute the average number of hours of sleep, apply the `mean()` function to the column `sleep_total` and call the summary value `avg_sleep`.

```
msleep |>
  summarise(avg_sleep = mean(sleep_total))
```

```
# A tibble: 1 x 1
  avg_sleep
  <dbl>
1     10.4
```

There are many other summary statistics you could consider such `sd()`, `min()`, `max()`, `median()`, `sum()`, `n()` (returns the length of vector), `first()` (returns first value in vector), `last()` (returns last value in vector) and `n_distinct()` (number of distinct values in vector).

```
msleep |>
  summarise(avg_sleep = mean(sleep_total),
            min_sleep = min(sleep_total),
            max_sleep = max(sleep_total),
            total = n())
```

```
# A tibble: 1 x 4
  avg_sleep min_sleep max_sleep total
  <dbl>      <dbl>      <dbl>   <int>
1     10.4       1.9       19.9    83
```

## 9.10 Grouping data: group\_by()

The `group_by()` verb is an important function in dplyr. The `group_by` allows us to use the concept of “split-apply-combine”. We literally want to split the data frame by some variable (e.g. taxonomic order), apply a function to the individual data frames and then combine the output. This approach is similar to the `aggregate` function from R, but `group_by` integrates with dplyr.

Let's do that: split the `msleep` data frame by the taxonomic order, then ask for the same summary statistics as above. We expect a set of summary statistics for each taxonomic order.

```
msleep |>
  group_by(order) |>
  summarise(avg_sleep = mean(sleep_total),
            min_sleep = min(sleep_total),
            max_sleep = max(sleep_total),
            total = n())
```

```
# A tibble: 19 x 5
  order      avg_sleep min_sleep max_sleep total
  <chr>        <dbl>      <dbl>      <dbl>   <int>
1 Afrosoricida    15.6      15.6      15.6     1
2 Artiodactyla     4.52       1.9       9.1      6
3 Carnivora       10.1       3.5      15.8     12
```

4 Cetacea	4.5	2.7	5.6	3
5 Chiroptera	19.8	19.7	19.9	2
6 Cingulata	17.8	17.4	18.1	2
7 Didelphimorphia	18.7	18	19.4	2
8 Diprotodontia	12.4	11.1	13.7	2
9 Erinaceomorpha	10.2	10.1	10.3	2
10 Hyracoidea	5.67	5.3	6.3	3
11 Lagomorpha	8.4	8.4	8.4	1
12 Monotremata	8.6	8.6	8.6	1
13 Perissodactyla	3.47	2.9	4.4	3
14 Pilosa	14.4	14.4	14.4	1
15 Primates	10.5	8	17	12
16 Proboscidea	3.6	3.3	3.9	2
17 Rodentia	12.5	7	16.6	22
18 Scandentia	8.9	8.9	8.9	1
19 Soricomorpha	11.1	8.4	14.9	5

# 10

---

## *Case Study: Behavioral Risk Factor Surveillance System*

---

### **10.1 A Case Study on the Behavioral Risk Factor Surveillance System**

The Behavioral Risk Factor Surveillance System (BRFSS) is a large-scale health survey conducted annually by the Centers for Disease Control and Prevention (CDC) in the United States. The BRFSS collects information on various health-related behaviors, chronic health conditions, and the use of preventive services among the adult population (18 years and older) through telephone interviews. The main goal of the BRFSS is to identify and monitor the prevalence of risk factors associated with chronic diseases, inform public health policies, and evaluate the effectiveness of health promotion and disease prevention programs. The data collected through BRFSS is crucial for understanding the health status and needs of the population, and it serves as a valuable resource for researchers, policy makers, and healthcare professionals in making informed decisions and designing targeted interventions.

In this chapter, we will walk through an exploratory data analysis (EDA) of the Behavioral Risk Factor Surveillance System dataset using R. EDA is an important step in the data analysis process, as it helps you to understand your data, identify trends, and detect any anomalies before performing more advanced analyses. We will use various R functions and packages to explore the dataset, with a focus on active learning and hands-on experience.

---

### **10.2 Loading the Dataset**

First, let's load the dataset into R. We will use the `read.csv()` function from the base R package to read the data and store it in a data frame called `brfss`. Make sure the CSV file is in your working directory, or provide the full path to the file.

First, we need to get the data. Either download the data from [THIS LINK](#) or have R do it directly from the command-line (preferred):

```
download.file('https://raw.githubusercontent.com/seandavi/ITR/master/BRFSS-subset.csv',
              destfile = 'BRFSS-subset.csv')
```

```
path <- file.choose()      # look for BRFSS-subset.csv  
stopifnot(file.exists(path))  
brfss <- read.csv(path)
```

---

### 10.3 Inspecting the Data

Once the data is loaded, let's take a look at the first few rows of the dataset using the `head()` function:

```
head(brfss)
```

```
Age    Weight    Sex Height Year  
1 31 48.98798 Female 157.48 1990  
2 57 81.64663 Female 157.48 1990  
3 43 80.28585 Male 177.80 1990  
4 72 70.30682 Male 170.18 1990  
5 31 49.89516 Female 154.94 1990  
6 58 54.43108 Female 154.94 1990
```

This will display the first six rows of the dataset, allowing you to get a feel for the data structure and variable types.

Next, let's check the dimensions of the dataset using the `dim()` function:

```
dim(brfss)
```

```
[1] 20000      5
```

This will return the number of rows and columns in the dataset, which is important to know for subsequent analyses.

---

### 10.4 Summary Statistics

Now that we have a basic understanding of the data structure, let's calculate some summary statistics. The `summary()` function in R provides a quick overview of the main statistics for each variable in the dataset:

```
summary(brfss)
```

Age	Weight	Sex	Height
Min. :18.00	Min. : 34.93	Length:20000	Min. :105.0
1st Qu.:36.00	1st Qu.: 61.69	Class :character	1st Qu.:162.6
Median :51.00	Median : 72.57	Mode :character	Median :168.0
Mean :50.99	Mean : 75.42		Mean :169.2
3rd Qu.:65.00	3rd Qu.: 86.18		3rd Qu.:177.8

```
Max.    :99.00   Max.    :278.96   Max.    :218.0
NA's    :139     NA's    :649      NA's    :184
Year
Min.    :1990
1st Qu.:1990
Median  :2000
Mean    :2000
3rd Qu.:2010
Max.    :2010
```

This will display the minimum, first quartile, median, mean, third quartile, and maximum for each numeric variable, and the frequency counts for each factor level for categorical variables.

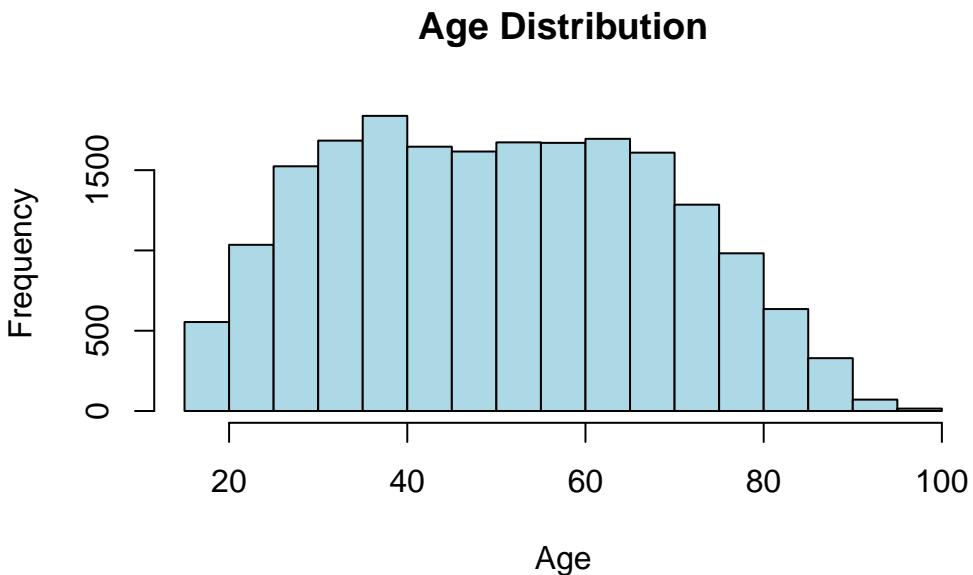
---

## 10.5 Data Visualization

Visualizing the data can help you identify patterns and trends in the dataset. Let's start by creating a histogram of the Age variable using the `hist()` function.

This will create a histogram showing the frequency distribution of ages in the dataset. You can customize the appearance of the histogram by adjusting the parameters within the `hist()` function.

```
hist(brfss$Age, main = "Age Distribution",
     xlab = "Age", col = "lightblue")
```



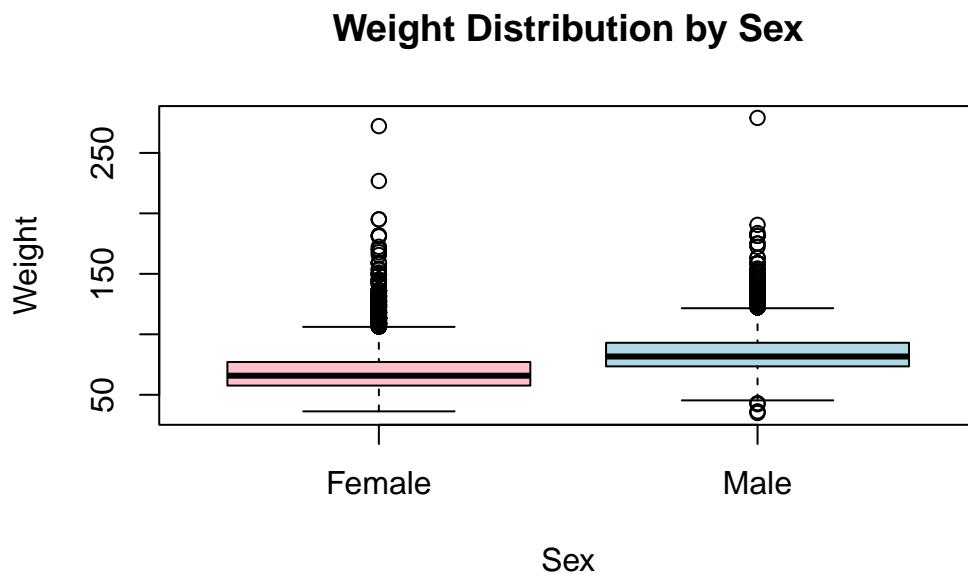
💡 What are the options for a histogram?

The `hist()` function has many options. For example, you can change the number of bins, the color of the bars, the title, and the x-axis label. You can also add a vertical line at the mean or median, or add a normal curve to the histogram. For more information, type `?hist` in the R console.

More generally, it is important to understand the options available for each function you use. You can do this by reading the documentation for the function, which can be accessed by typing `?function_name` or `help("function_name")` in the R console.

Next, let's create a boxplot to compare the distribution of Weight between males and females. We will use the `boxplot()` function for this. This will create a boxplot comparing the weight distribution between males and females. You can customize the appearance of the boxplot by adjusting the parameters within the `boxplot()` function.

```
boxplot(brfss$Weight ~ brfss$Sex, main = "Weight Distribution by Sex",
       xlab = "Sex", ylab = "Weight", col = c("pink", "lightblue"))
```



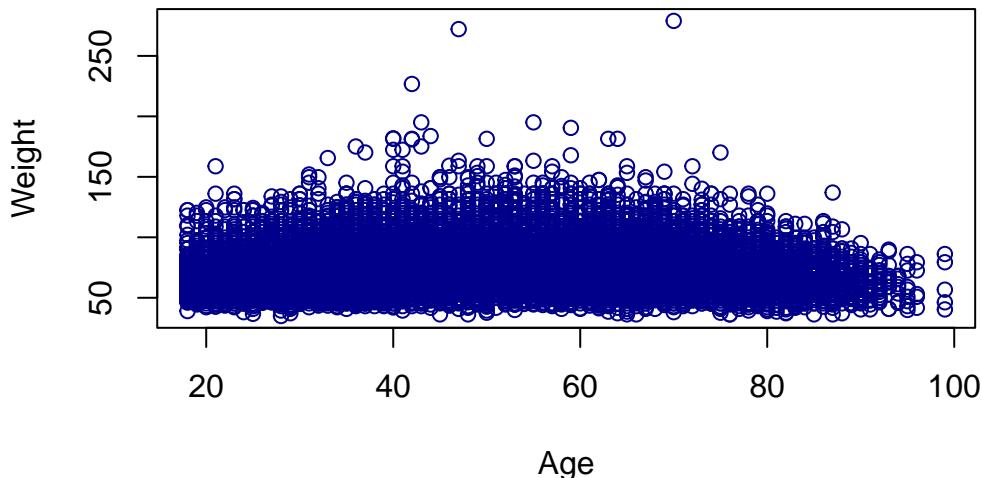
## 10.6 Analyzing Relationships Between Variables

To further explore the data, let's investigate the relationship between age and weight using a scatterplot. We will use the `plot()` function for this:

This will create a scatterplot of age and weight, allowing you to visually assess the relationship between these two variables.

```
plot(brfss$Age, brfss$Weight, main = "Scatterplot of Age and Weight",
     xlab = "Age", ylab = "Weight", col = "darkblue")
```

## Scatterplot of Age and Weight



To quantify the strength of the relationship between age and weight, we can calculate the correlation coefficient using the `cor()` function:

This will return the correlation coefficient between age and weight, which can help you determine whether there is a linear relationship between these variables.

```
cor(brfss$Age, brfss$Weight)
```

```
[1] NA
```

Why does `cor()` give a value of NA? What can we do about it? A quick glance at `help("cor")` will give you the answer.

```
cor(brfss$Age, brfss$Weight, use = "complete.obs")
```

```
[1] 0.02699989
```

---

### 10.7 Exercises

1. What is the mean weight in this dataset? How about the median? What is the difference between the two? What does this tell you about the distribution of weights in the dataset?

```
mean(brfss$Weight, na.rm = TRUE)
```

```
[1] 75.42455
```

```
median(brfss$Weight, na.rm = TRUE)
```

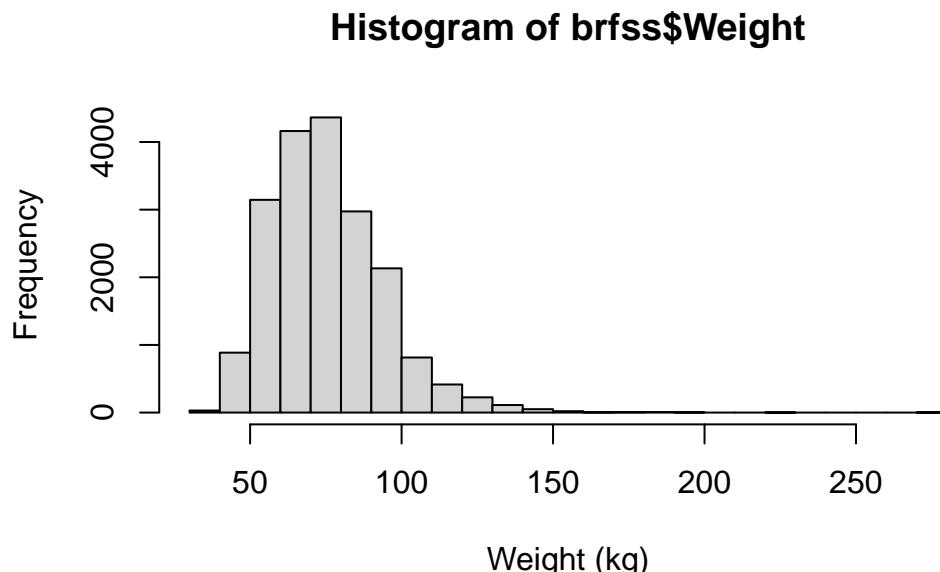
```
[1] 72.57478
```

```
mean(brfss$Weight, na.rm=TRUE) - median(brfss$Weight, na.rm = TRUE)
```

```
[1] 2.849774
```

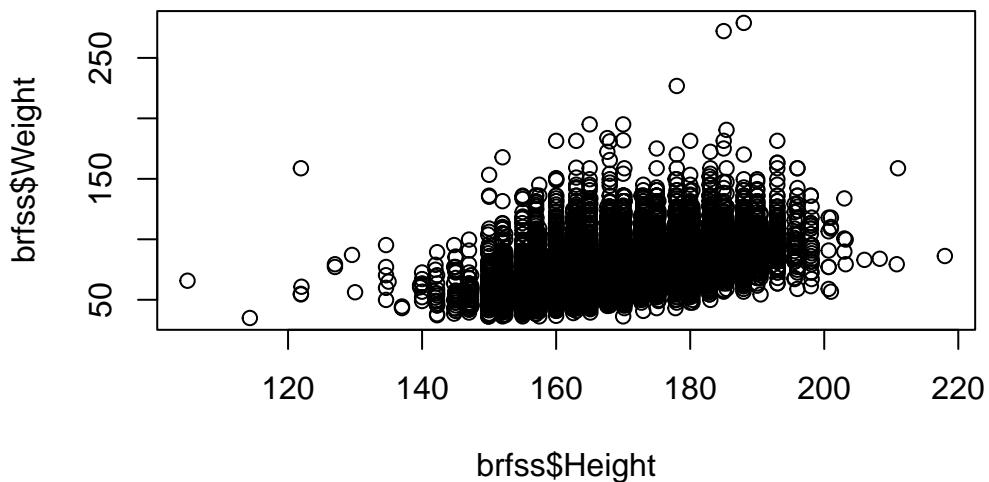
- Given the findings about the `mean` and `median` in the previous exercise, use the `hist()` function to create a histogram of the weight distribution in this dataset. How would you describe the shape of this distribution?

```
hist(brfss$Weight, xlab="Weight (kg)", breaks = 30)
```



- Use `plot()` to examine the relationship between height and weight in this dataset.

```
plot(brfss$Height, brfss$Weight)
```



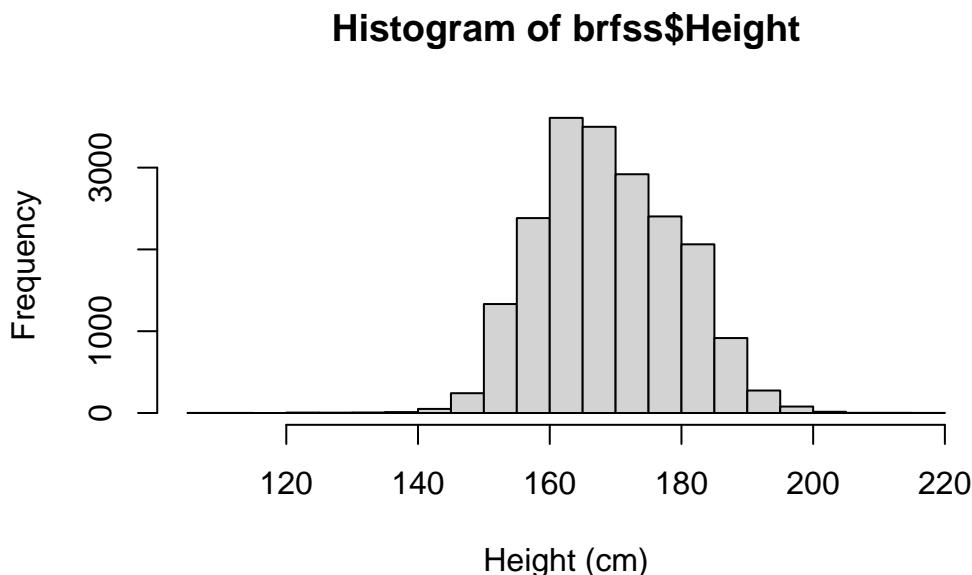
4. What is the correlation between height and weight? What does this tell you about the relationship between these two variables?

```
cor(brfss$Height, brfss$Weight, use = "complete.obs")
```

```
[1] 0.5140928
```

5. Create a histogram of the height distribution in this dataset. How would you describe the shape of this distribution?

```
hist(brfss$Height, xlab="Height (cm)", breaks = 30)
```



---

## 10.8 Conclusion

In this chapter, we have demonstrated how to perform an exploratory data analysis on the Behavioral Risk Factor Surveillance System dataset using R. We covered data loading, inspection, summary statistics, visualization, and the analysis of relationships between variables. By actively engaging with the R code and data, you have gained valuable experience in using R for EDA and are well-equipped to tackle more complex analyses in your future work.

Remember that EDA is just the beginning of the data analysis process, and further statistical modeling and hypothesis testing will likely be necessary to draw meaningful conclusions from your data. However, EDA is a crucial step in understanding your data and informing your subsequent analyses.

---

---

## 10.9 Learn about the data

Using the data exploration techniques you have seen to explore the brfss dataset.

- `summary()`
- `dim()`
- `colnames()`

- head()
- tail()
- class()
- View()

You may want to investigate individual columns visually using plotting like `hist()`. For categorical data, consider using something like `table()`.

---

## 10.10 Clean data

R read Year as an integer value, but it's really a factor

```
brfss$Year <- factor(brfss$Year)
```

---

## 10.11 Weight in 1990 vs. 2010 Females

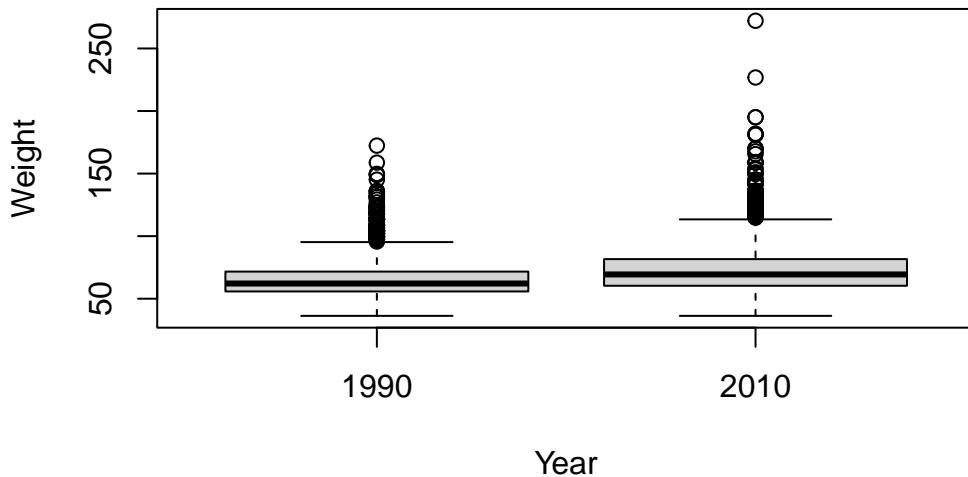
- Create a subset of the data

```
brfssFemale <- brfss[brfss$Sex == "Female",]  
summary(brfssFemale)
```

Age	Weight	Sex	Height
Min. :18.00	Min. : 36.29	Length:12039	Min. :105.0
1st Qu.:37.00	1st Qu.: 57.61	Class :character	1st Qu.:157.5
Median :52.00	Median : 65.77	Mode :character	Median :163.0
Mean :51.92	Mean : 69.05		Mean :163.3
3rd Qu.:67.00	3rd Qu.: 77.11		3rd Qu.:168.0
Max. :99.00	Max. :272.16		Max. :200.7
NA's :103	NA's :560		NA's :140
Year			
1990:5718			
2010:6321			

- Visualize

```
plot(weight ~ Year, brfssFemale)
```



- Statistical test

```
t.test(Weight ~ Year, brfssFemale)
```

Welch Two Sample t-test

```
data: Weight by Year
t = -27.133, df = 11079, p-value < 2.2e-16
alternative hypothesis: true difference in means between group 1990 and group 2010 is not equal to 0
95 percent confidence interval:
-8.723607 -7.548102
sample estimates:
mean in group 1990 mean in group 2010
64.81838      72.95424
```

---

## 10.12 Weight and height in 2010 Males

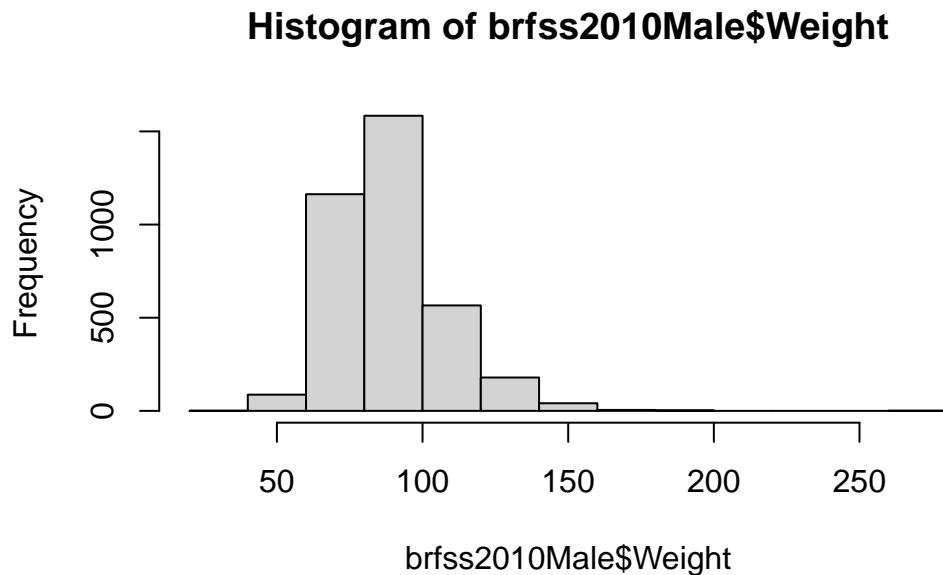
- Create a subset of the data

```
brfss2010Male <- subset(brfss, Year == 2010 & Sex == "Male")
summary(brfss2010Male)
```

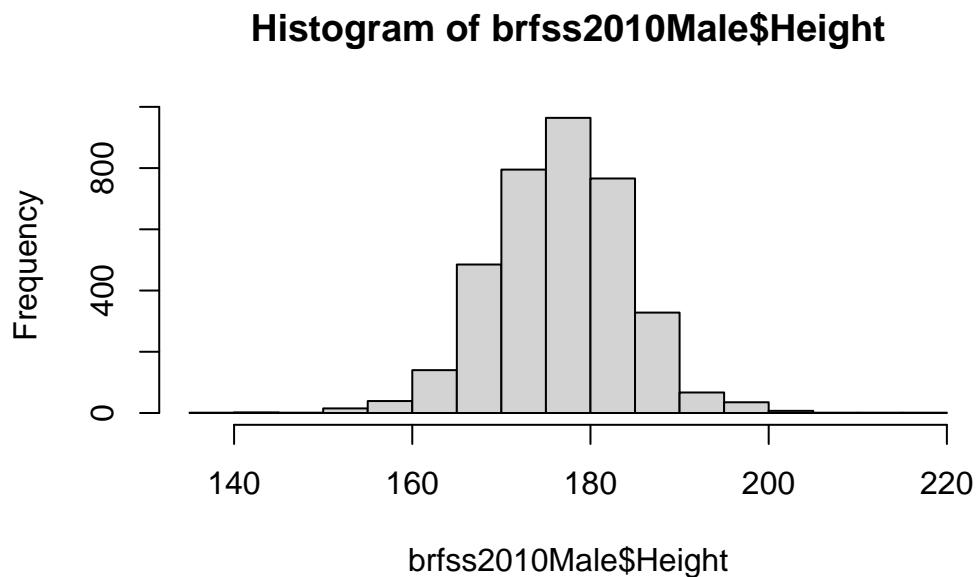
Age	Weight	Sex	Height	Year
Min. :18.00	Min. : 36.29	Length:3679	Min. :135	1990: 0
1st Qu.:45.00	1st Qu.: 77.11	Class :character	1st Qu.:173	2010:3679
Median :57.00	Median : 86.18	Mode :character	Median :178	
Mean :56.25	Mean : 88.85		Mean :178	
3rd Qu.:68.00	3rd Qu.: 99.79		3rd Qu.:183	
Max. :99.00	Max. :278.96		Max. :218	
NA's :30	NA's :49		NA's :31	

- Visualize the relationship

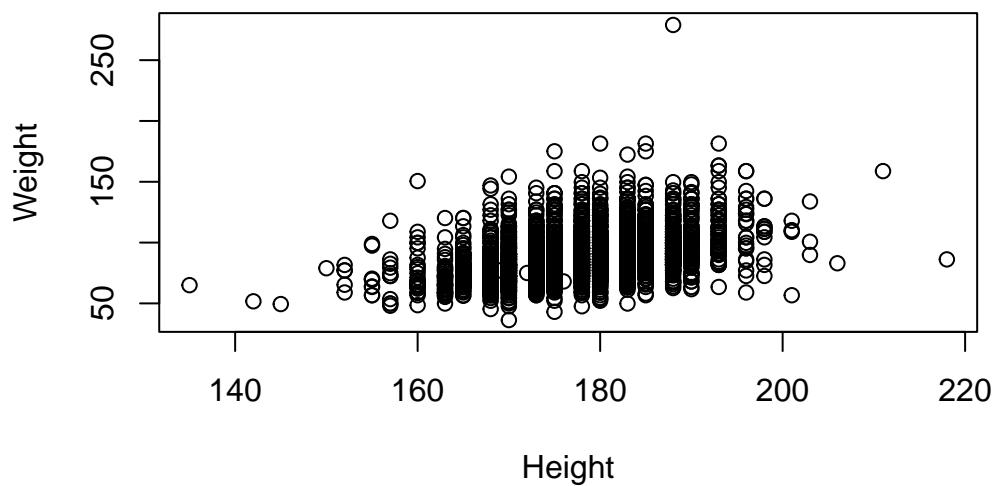
```
hist(brfss2010Male$Weight)
```



```
hist(brfss2010Male$Height)
```



```
plot(Weight ~ Height, brfss2010Male)
```



- Fit a linear model (regression)

```
fit <- lm(Weight ~ Height, brfss2010Male)
fit
```

Call:

```
lm(formula = Weight ~ Height, data = brfss2010Male)
```

Coefficients:

(Intercept)	Height
-86.8747	0.9873

Summarize as ANOVA table

```
anova(fit)
```

Analysis of Variance Table

Response: Weight

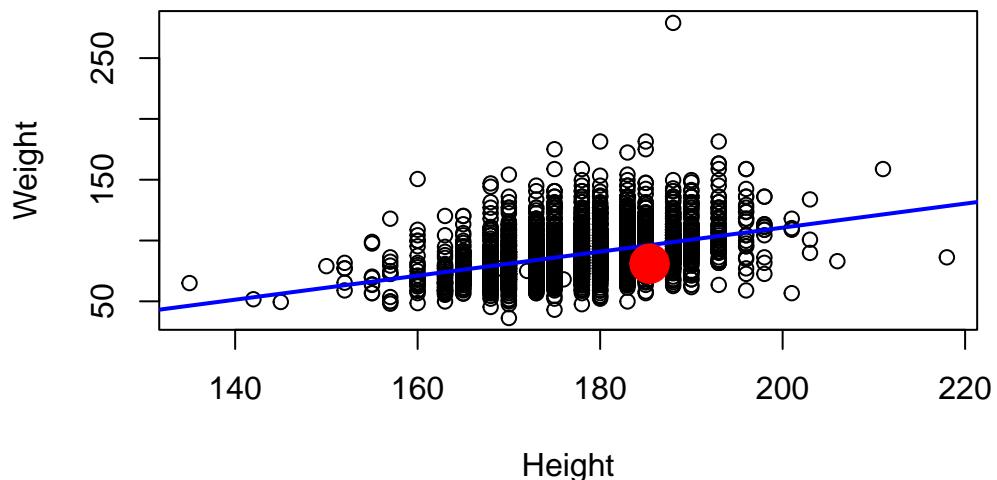
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Height	1	197664	197664	693.8	< 2.2e-16 ***
Residuals	3617	1030484	285		

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

- Plot points, superpose fitted regression line; where am I?

```
plot(Weight ~ Height, brfss2010Male)
abline(fit, col="blue", lwd=2)
# Substitute your own weight and height...
points(73 * 2.54, 178 / 2.2, col="red", cex=4, pch=20)
```



- Class and available ‘methods’

```
class(fit)                      # 'noun'  
methods(class=class(fit))       # 'verb'
```

- Diagnostics

```
plot(fit)  
# Note that the "plot" above does not have a ".lm"  
# However, R will use "plot.lm". Why?  
?plot.lm
```

## **Part IV**

# **Statistics and Machine Learning**

# 11

---

## *Working with distribution functions*

Which values do pnorm, dnorm, qnorm, and rnorm return? How do I remember the difference between these?

I find it helpful to have visual representations of distributions as pictures. It is difficult for me to think of distributions, or differences between probability, density, and quantiles without visualizing the shape of the distribution. So I figured it would be helpful to have a visual guide to pnorm, dnorm, qnorm, and rnorm.

Table 11.1: Functions for the normal distribution

Function	Input	Output
pnorm	x	$P(X < x)$
dnorm	x	$f(x)$ , or the height of the density curve at x
qnorm	q, a quantile from 0 to 1	x such that $P(X < x) = q$
rnorm	n	n random samples from the distribution

---

### 11.1 pnorm

This function gives the probability function for a normal distribution. If you do not specify the mean and standard deviation, R defaults to standard normal. Figure 11.1

```
pnorm(q, mean = 0, sd = 1, lower.tail = TRUE, log.p = FALSE)
```

The R help file for pnorm provides the template above. The value you input for q is a value on the x-axis, and the returned value is the area under the distribution curve to the left of that point.

Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.  
i Please use `linewidth` instead.

This function gives the probability function for a normal distribution. If you do not specify the mean and standard deviation, R defaults to standard normal.

pnorm(q, mean = 0, sd = 1, lower.tail = TRUE, log.p = FALSE) The R help file for pnorm provides the template above. The value you input for q is a value on the x-axis, and the returned value is the area under the distribution curve to the left of that point.

The option lower.tail = TRUE tells R to use the area to the left of the given point. This is the default, so will

remain true even without entering it. In order to compute the area to the right of the given point, you can either switch to `lower.tail = FALSE`, or simply calculate `1-pnorm()` instead. This is demonstrated below.

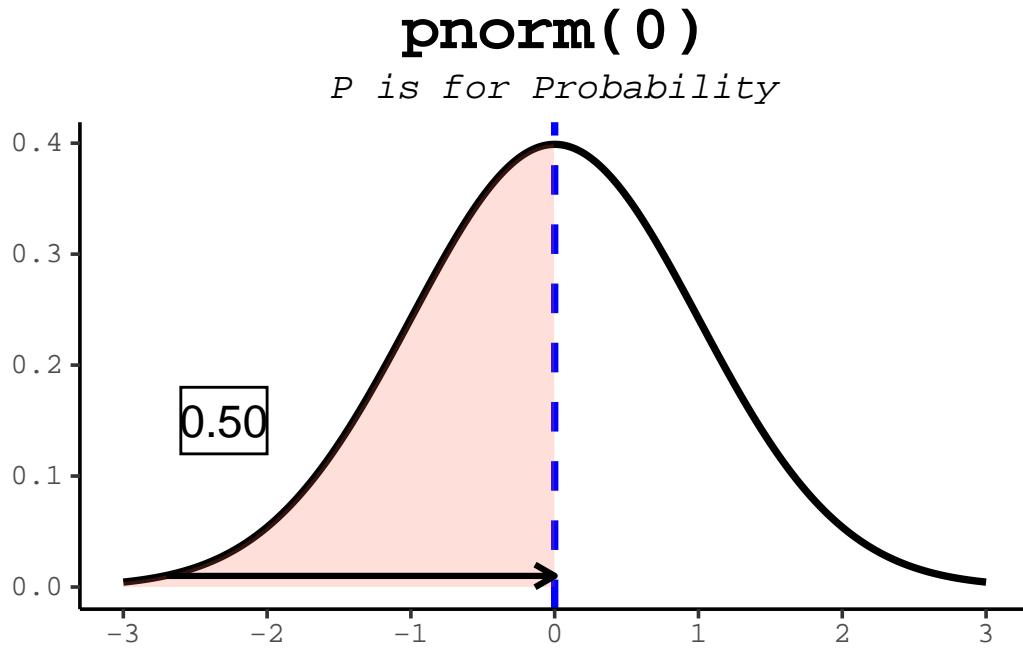


Figure 11.1: The `pnorm` function takes a quantile (value on the x-axis) and returns the area under the curve to the left of that value.

The option `lower.tail = TRUE` tells R to use the area to the left of the given point. This is the default, so will remain true even without entering it. In order to compute the area to the right of the given point, you can either switch to `lower.tail = FALSE`, or simply calculate `1-pnorm()` instead.

## 11.2 dnorm

This function calculates the probability density function (PDF) for the normal distribution. It gives the probability density (height of the curve) at a specified value ( $x$ ).

## 11.3 qnorm

This function calculates the quantiles of the normal distribution. It returns the value ( $x$ ) corresponding to a specified probability ( $p$ ). It is the inverse of the `pnorm` function.

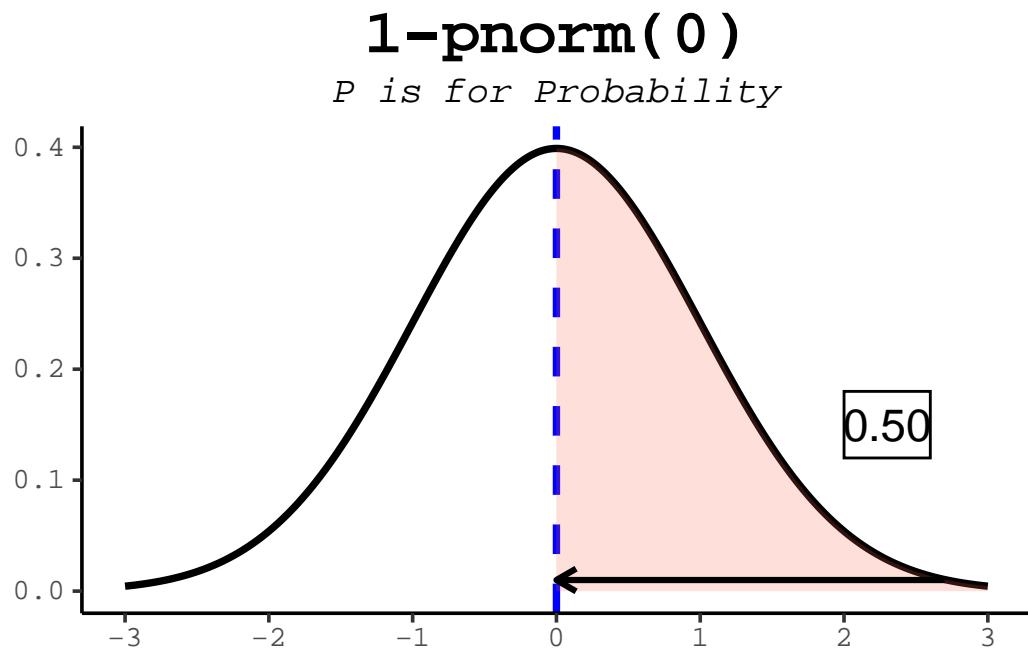


Figure 11.2: The pnorm function takes a quantile (value on the x-axis) and returns the area under the curve to the left of that value.

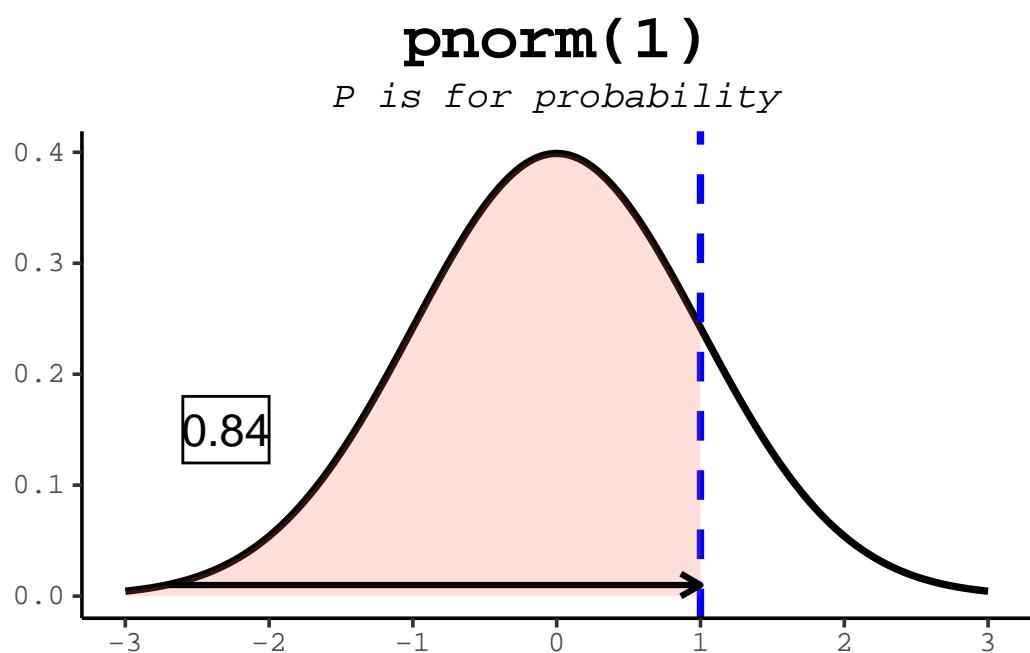


Figure 11.3: The pnorm function takes a quantile (value on the x-axis) and returns the area under the curve to the left of that value.

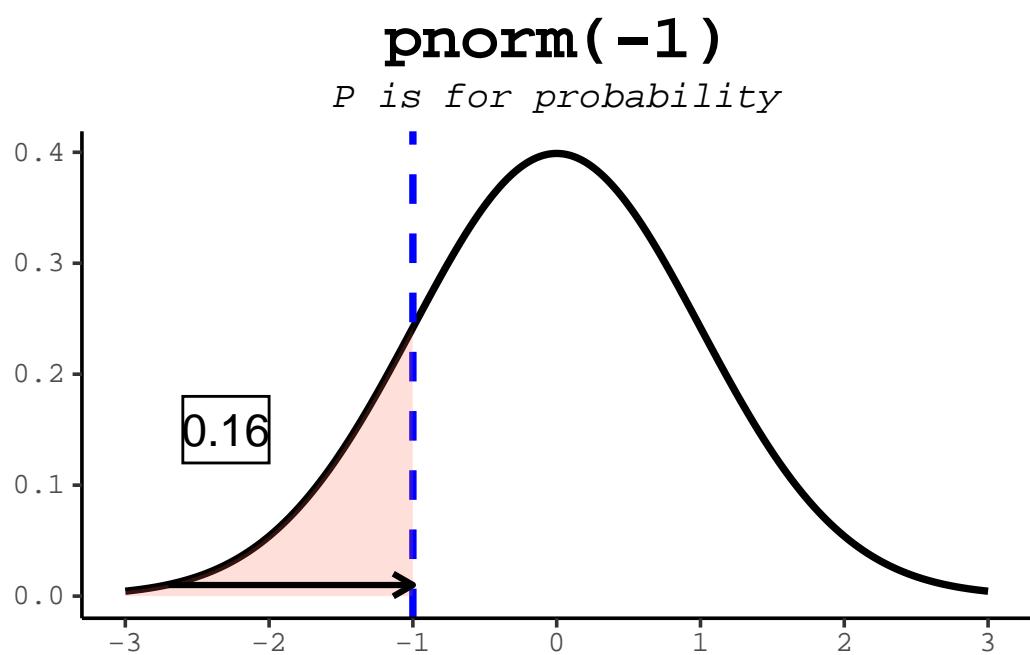


Figure 11.4: The pnorm function takes a quantile (value on the x-axis) and returns the area under the curve to the left of that value.

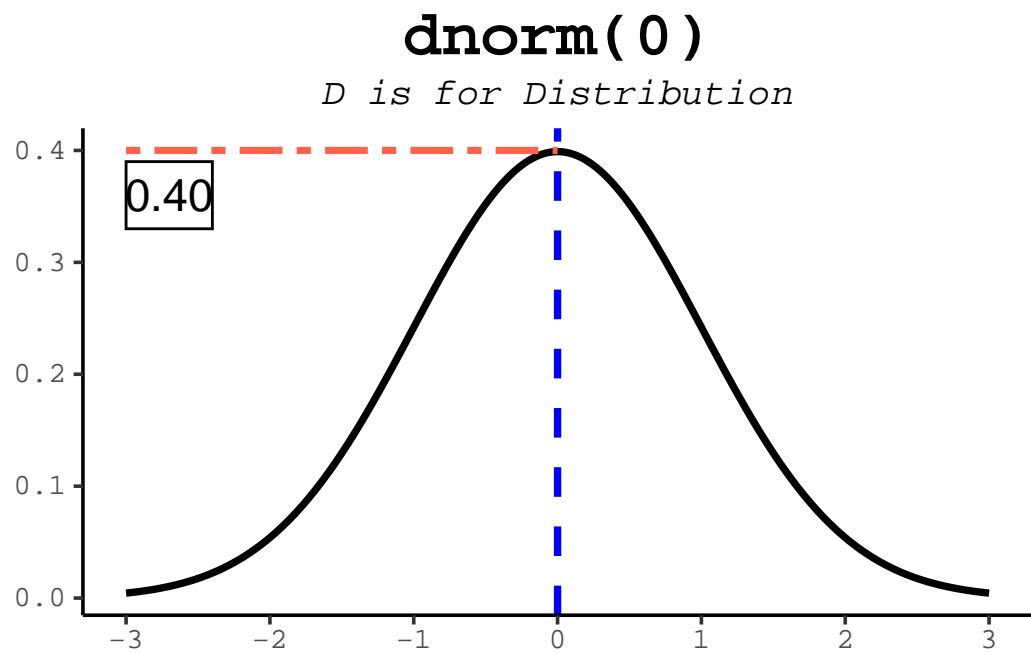


Figure 11.5: The `dnorm` function returns the height of the normal distribution at a given point.

---

## 11.4 rnorm

```
print(r1)
```

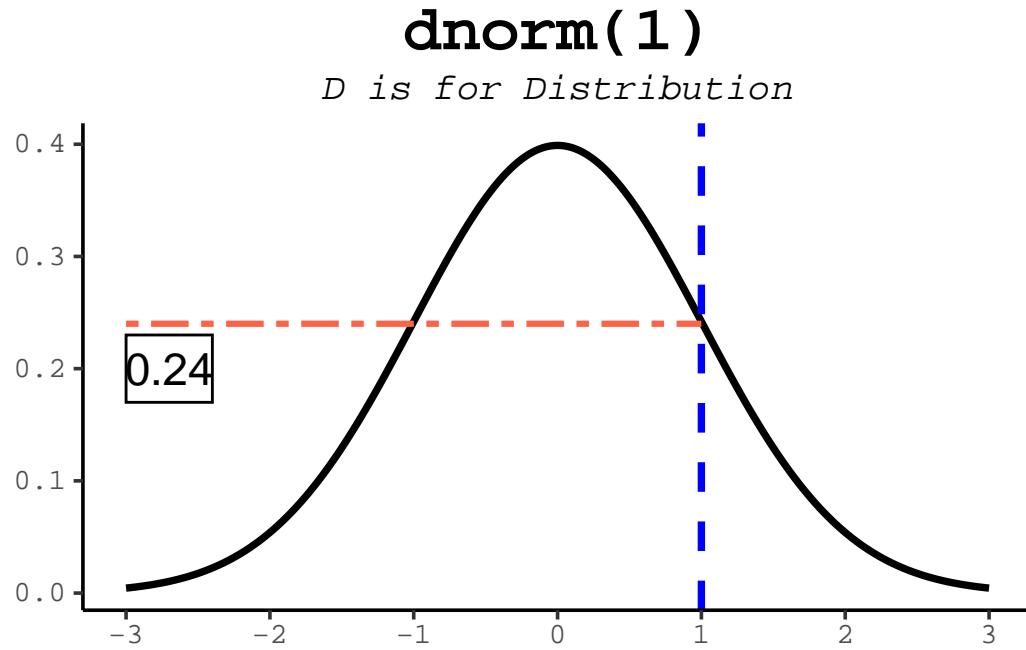


Figure 11.6: The `dnorm` function returns the height of the normal distribution at a given point.

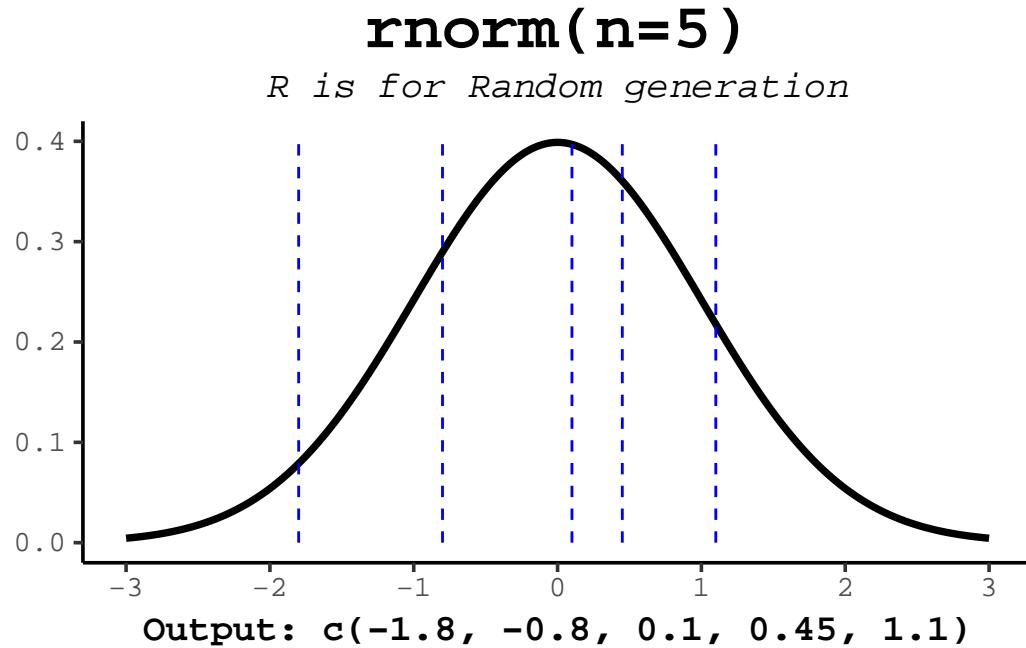


Figure 11.13: The `rnorm` function takes a number of samples and returns a vector of random numbers from the normal distribution (with mean=0, sd=1 as defaults)

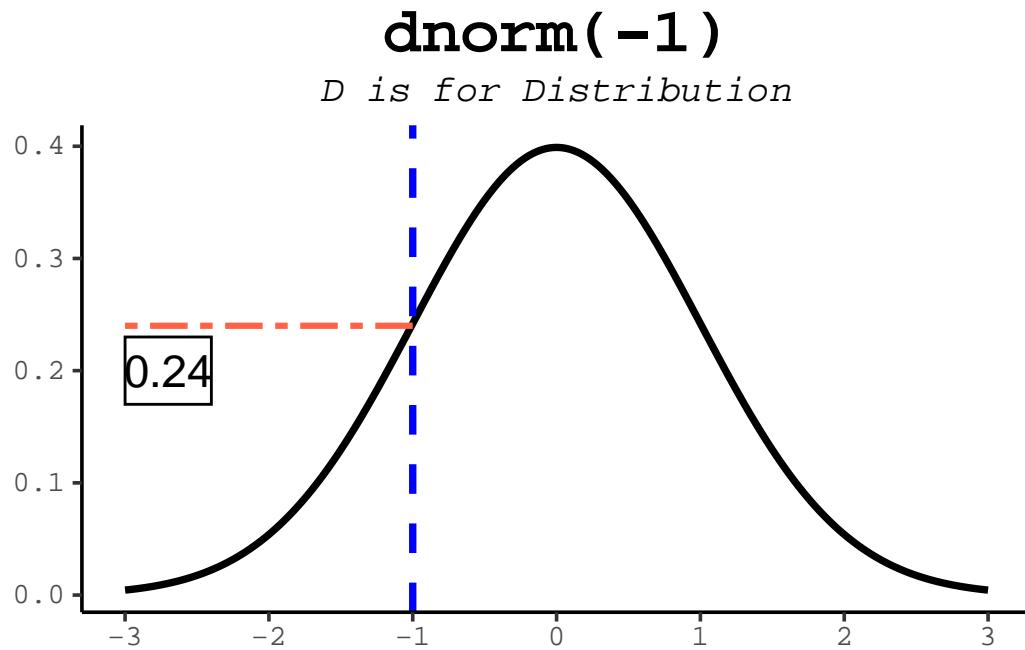


Figure 11.7: The `dnorm` function returns the height of the normal distribution at a given point.

## 11.5 IQ scores

Normal Distribution and its Application with IQ

The normal distribution, also known as the Gaussian distribution, is a continuous probability distribution characterized by its bell-shaped curve. It is defined by two parameters: the mean ( $\mu$ ) and the standard deviation ( $\sigma$ ). The mean represents the central tendency of the distribution, while the standard deviation represents the dispersion or spread of the data.

The IQ scores are an excellent example of the normal distribution, as they are designed to follow this distribution pattern. The mean IQ score is set at 100, and the standard deviation is set at 15. This means that the majority of the population (about 68%) have an IQ score between 85 and 115, while 95% of the population have an IQ score between 70 and 130.

- What is the probability of having an IQ score between 85 and 115?

```
pnorm(115, mean = 100, sd = 15) - pnorm(85, mean = 100, sd = 15)
```

- What is the 90th percentile of the IQ scores?

```
qnorm(0.9, mean = 100, sd = 15)
```

- What is the probability of having an IQ score above 130?

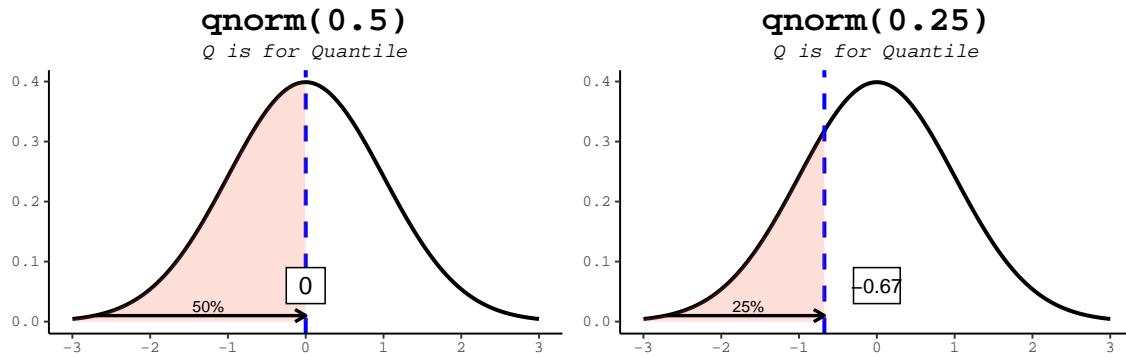


Figure 11.8: The qnorm function is the inverse of the pnorm function in that it takes a probability and gives the quantile.

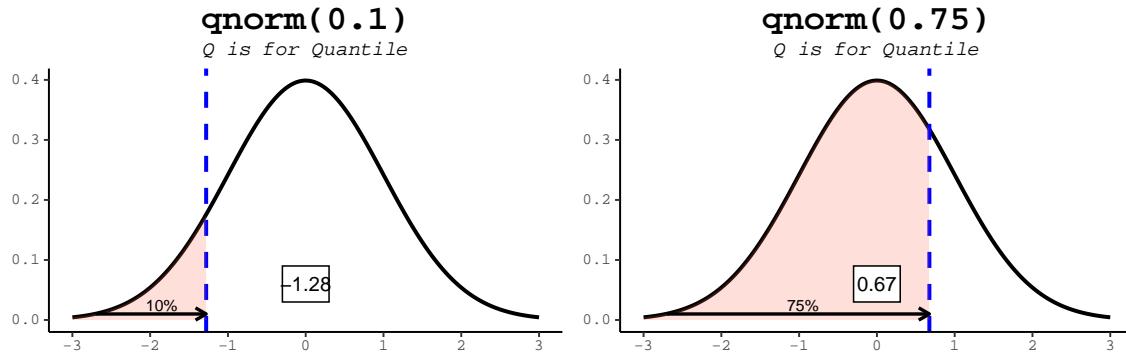


Figure 11.10: The qnorm function is the inverse of the pnorm function in that it takes a probability and gives the quantile.

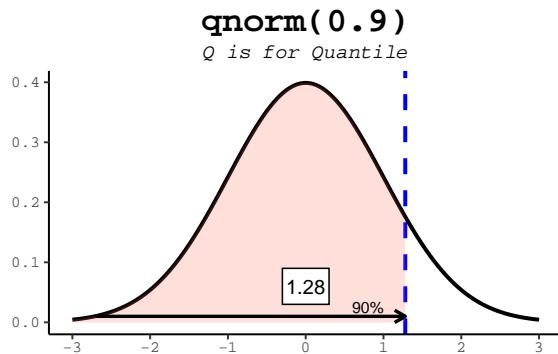


Figure 11.12: The qnorm function is the inverse of the pnorm function in that it takes a probability and gives the quantile.

```
1 - pnorm(130, mean = 100, sd = 15)
```

- What is the probability of having an IQ score below 70?

```
pnorm(70, mean = 100, sd = 15)
```

# 12

---

## *The t-statistic and t-distribution*

---

### 12.1 Background

The t-test is a [statistical hypothesis test](#) that is commonly used when the data are normally distributed (follow a normal distribution) if the value of the population standard deviation were known. When the population standard deviation is not known and is replaced by an estimate based on the data, the test statistic follows a Student's t distribution.

T-tests are handy hypothesis tests in statistics when you want to compare means. You can compare a sample mean to a hypothesized or target value using a one-sample t-test. You can compare the means of two groups with a two-sample t-test. If you have two groups with paired observations (e.g., before and after measurements), use the paired t-test.

A t-test looks at the t-statistic, the t-distribution values, and the degrees of freedom to determine the statistical significance. To conduct a test with three or more means, we would use an analysis of variance.

The distribution that the t-statistic follows was described in a famous paper (Student 1908) by “Student”, a pseudonym for [William Sealy Gosset](#).

---

### 12.2 The Z-score and probability

Before talking about the t-distribution and t-scores, let's review the Z-score, its relation to the normal distribution, and probability.

The Z-score is defined as:

$$Z = \frac{x - \mu}{\sigma} \quad (12.1)$$

where  $\mu$  is the population mean from which  $x$  is drawn and  $\sigma$  is the population standard deviation (taken as known, not estimated from the data).

The probability of observing a Z score of  $z$  or greater can be calculated by  $pnorm(z, \mu, \sigma)$ .

For example, let's assume that our “population” is known and it truly has a mean 0 and standard deviation 1. If we have observations drawn from that population, we can assign a probability of seeing that observation

by random chance *under the assumption that the null hypothesis is TRUE.*

```
zscore = seq(-5, 5, 1)
```

For each value of zscore, let's calculate the p-value and put the results in a data.frame.

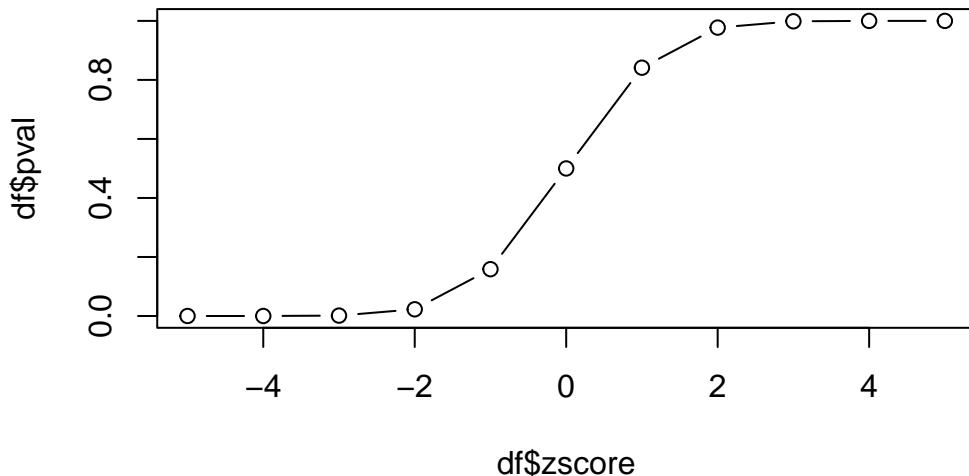
```
df = data.frame(  
  zscore = zscore,  
  pval   = pnorm(zscore, 0, 1)  
)  
df
```

	zscore	pval
1	-5	2.866516e-07
2	-4	3.167124e-05
3	-3	1.349898e-03
4	-2	2.275013e-02
5	-1	1.586553e-01
6	0	5.000000e-01
7	1	8.413447e-01
8	2	9.772499e-01
9	3	9.986501e-01
10	4	9.999683e-01
11	5	9.999997e-01

Why is the p-value of something 5 population standard deviations away from the mean (zscore=5) nearly 1 in this calculation? What is the default for pnorm with respect to being one-sided or two-sided?

Let's plot the values of probability vs z-score:

```
plot(df$zscore, df$pval, type='b')
```



This plot is the *empirical* cumulative density function (cdf) for our data. How can we use it? If we know the z-score, we can look up the probability of observing that value. Since we have constructed our experiment to follow the standard normal distribution, this cdf also represents the cdf of the standard normal distribution.

### 12.2.1 Small diversion: two-sided pnorm function

The `pnorm` function returns the “one-sided” probability of having a value at least as extreme as the observed  $x$  and uses the “lower” tail by default. Let’s create a function that computes two-sided p-values.

1. Take the absolute value of  $x$
2. Compute `pnorm` with `lower.tail=FALSE` so we get lower p-values with larger values of  $x$ .
3. Since we want to include both tails, we need to multiply the area (probability) returned by `pnorm` by 2.

```
twosidedpnorm = function(x, mu=0, sd=1) {
  2*pnorm(abs(x), mu, sd, lower.tail=FALSE)
}
```

And we can test this to see how likely it is to be 2 or 3 standard deviations from the mean:

```
twosidedpnorm(2)
```

```
[1] 0.04550026
```

```
twosidedpnorm(3)
```

```
[1] 0.002699796
```

---

### 12.3 The *t*-distribution

We spent time above working with z-scores and probability. An important aspect of working with the normal distribution is that we MUST assume that we know the standard deviation. Remember that the Z-score is defined as:

$$Z = \frac{x - \mu}{\sigma}$$

The formula for the *population* standard deviation is:

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (xi - \mu)^2} \quad (12.2)$$

In general, the population standard deviation is taken as “known” as we did above.

If we do not but only have a *sample* from the population, instead of using the Z-score, we use the t-score defined as:

$$t = \frac{x - \bar{x}}{s} \quad (12.3)$$

This looks quite similar to the formula for Z-score, but here we have to *estimate* the standard deviation, *s* from the data. The formula for *s* is:

$$s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (xi - \bar{x})^2} \quad (12.4)$$

Since we are estimating the standard deviation from the data, this leads to extra variability that shows up as “fatter tails” for smaller sample sizes than for larger sample sizes. We can see this by comparing the *t-distribution* for various numbers of degrees of freedom (sample sizes).

We can look at the effect of sample size on the distributions graphically by looking at the densities for 3, 5, 10, 20 degrees of freedom and the normal distribution:

```
library(dplyr)
library(ggplot2)
t_values = seq(-6, 6, 0.01)
df = data.frame(
```

```

value = t_values,
t_3   = dt(t_values,3),
t_6   = dt(t_values,6),
t_10  = dt(t_values,10),
t_20  = dt(t_values,20),
Normal= dnorm(t_values)
) |>
  tidy::gather("Distribution", "density", -value)
ggplot(df, aes(x=value, y=density, color=Distribution)) +
  geom_line()

```

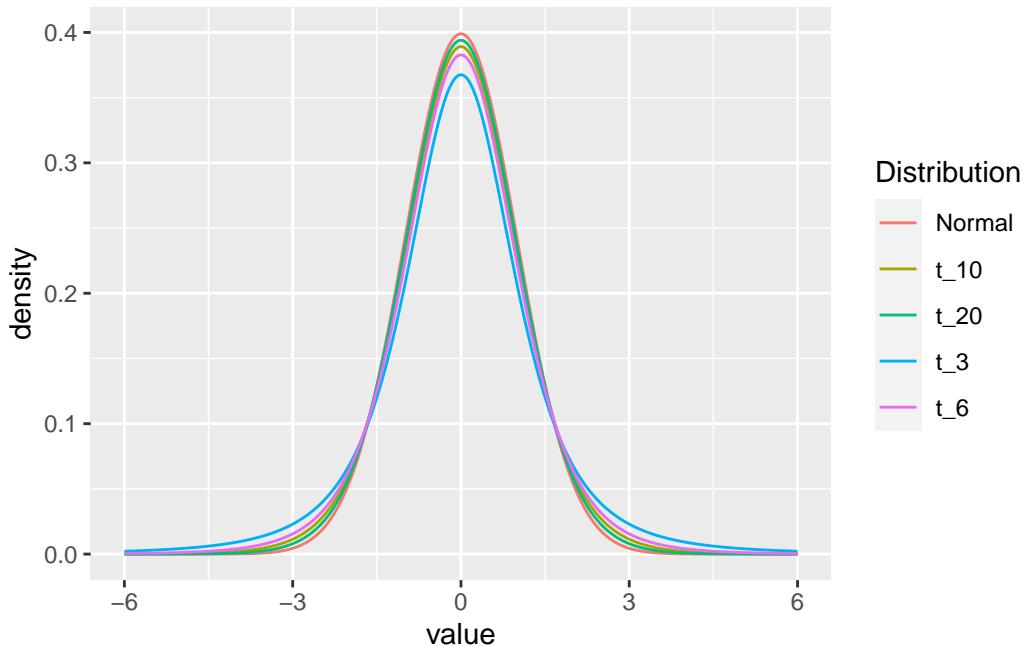


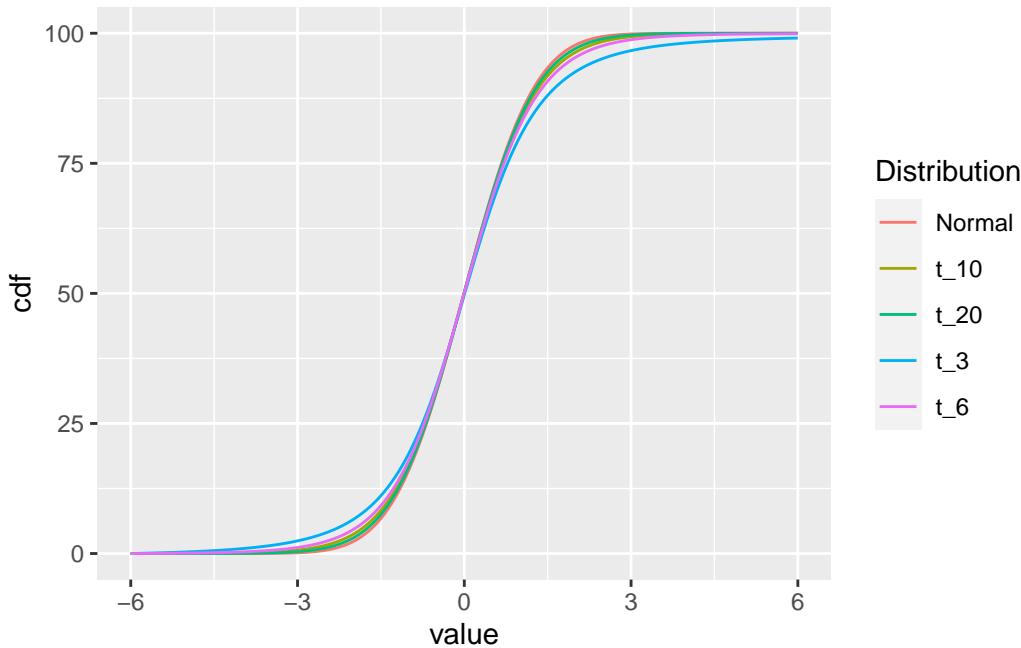
Figure 12.1: *t*-distributions for various degrees of freedom. Note that the tails are fatter for smaller degrees of freedom, which is a result of estimating the standard deviation from the data.

The `dt` and `dnorm` functions give the density of the distributions for each point.

```

df2 = df |>
  group_by(Distribution) |>
  arrange(value) |>
  mutate(cdf=cumsum(density))
ggplot(df2, aes(x=value, y=cdf, color=Distribution)) +
  geom_line()

```



### 12.3.1 p-values based on Z vs t

When we have a “sample” of data and want to compute the statistical significance of the difference of the mean from the population mean, we calculate the standard deviation of the sample means (standard error).

$$z = \frac{x - \mu}{\sigma / \sqrt{n}}$$

Let's look at the relationship between the p-values of Z (from the normal distribution) vs t for a **sample** of data.

```
set.seed(5432)
samp = rnorm(5, mean = 0.5)
z = sqrt(length(samp)) * mean(samp) #simplifying assumption (sigma=1, mu=0)
```

And the p-value if we assume we know the standard deviation:

```
pnorm(z, lower.tail = FALSE)
```

```
[1] 0.02428316
```

In reality, we don't know the standard deviation, so we have to estimate it from the data. We can do this by calculating the sample standard deviation:

```
ts = sqrt(length(samp)) * mean(samp) / sd(samp)
pnorm(ts, lower.tail = FALSE)
```

```
[1] 0.0167297
pt(ts,df = length(samp)-1, lower.tail = FALSE)
[1] 0.0503001
```

### 12.3.2 Experiment

When sampling from a normal distribution, we often calculate p-values to test hypotheses or determine the statistical significance of our results. The p-value represents the probability of obtaining a test statistic as extreme or more extreme than the one observed, under the null hypothesis.

In a typical scenario, we assume that the population mean and standard deviation are known. However, in many real-life situations, we don't know the true population standard deviation, and we have to estimate it using the sample standard deviation (Equation 12.4). This estimation introduces some uncertainty into our calculations, which affects the p-values. When we include an estimate of the standard deviation, we switch from using the standard normal (*z*) distribution to the *t*-distribution for calculating p-values.

What would happen if we used the normal distribution to calculate p-values when we use the sample standard deviation? Let's find out!

1. Simulate a bunch of samples of size *n* from the standard normal distribution
2. Calculate the p-value distribution for those samples based on the normal.
3. Calculate the p-value distribution for those samples based on the normal, but with the *estimated* standard deviation.
4. Calculate the p-value distribution for those samples based on the *t*-distribution.

Create a function that draws a sample of size *n* from the standard normal distribution.

```
zf = function(n) {
  samp = rnorm(n)
  z = sqrt(length(samp)) * mean(samp) / 1 #simplifying assumption (sigma=1, mu=0)
  z
}
```

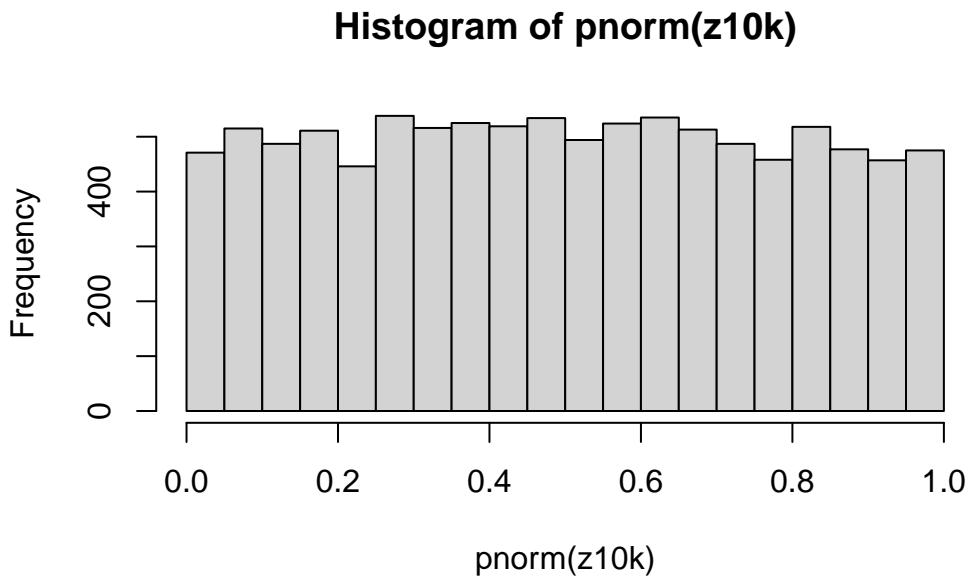
And give it a try:

```
zf(5)
```

```
[1] 0.7406094
```

Perform 10000 replicates of our sampling and *z*-scoring. We are using the assumption that we know the population standard deviation; in this case, we do know since we are sampling from the standard normal distribution.

```
z10k = replicate(10000,zf(5))
hist(pnorm(z10k))
```

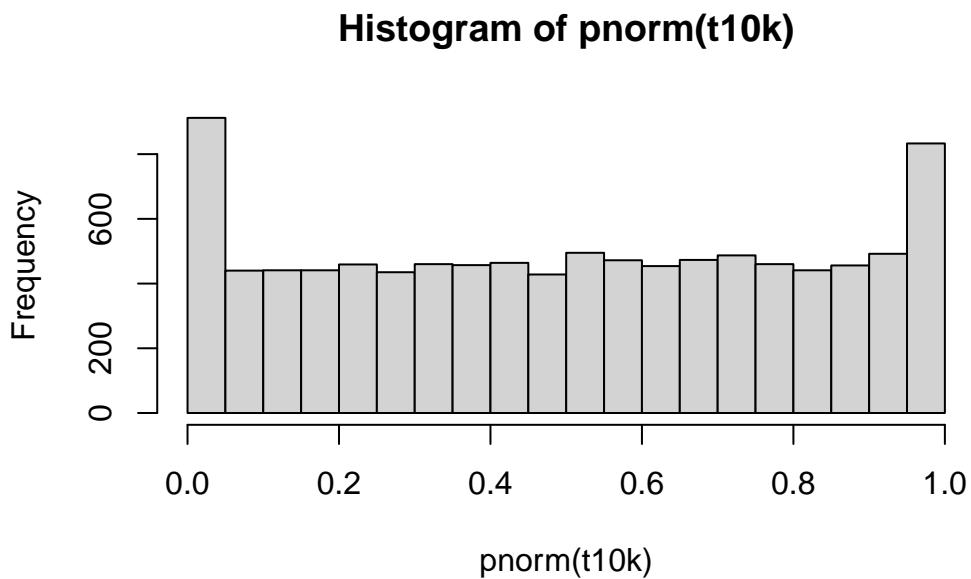


And do the same, but now creating a t-score function. We are using the assumption that we *don't* know the population standard deviation; in this case, we must estimate it from the data. Note the difference in the calculation of the t-score (*ts*) as compared to the z-score (*z*).

```
tf = function(n) {
  samp = rnorm(n)
  # now, using the sample standard deviation since we
  # "don't know" the population standard deviation
  ts = sqrt(length(samp)) * mean(samp) / sd(samp)
  ts
}
```

If we use those t-scores and calculate the p-values based on the normal distribution, the histogram of those p-values looks like:

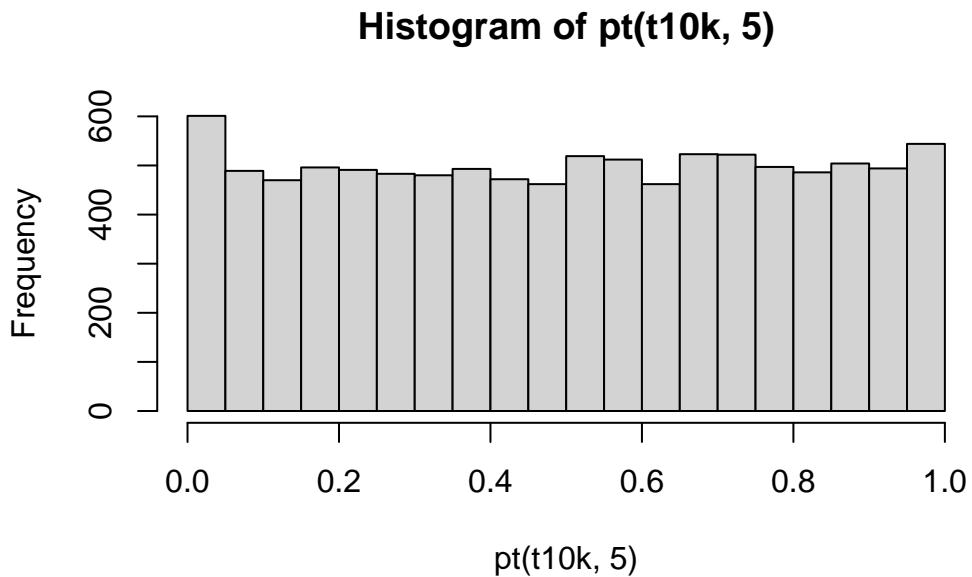
```
t10k = replicate(10000, tf(5))
hist(pnorm(t10k))
```



Since we are using the normal distribution to calculate the p-values, we are, in effect, assuming that we know the population standard deviation. This assumption is incorrect, and we can see that the p-values are not uniformly distributed between 0 and 1.

If we use those t-scores and calculate the p-values based on the t-distribution, the histogram of those p-values looks like:

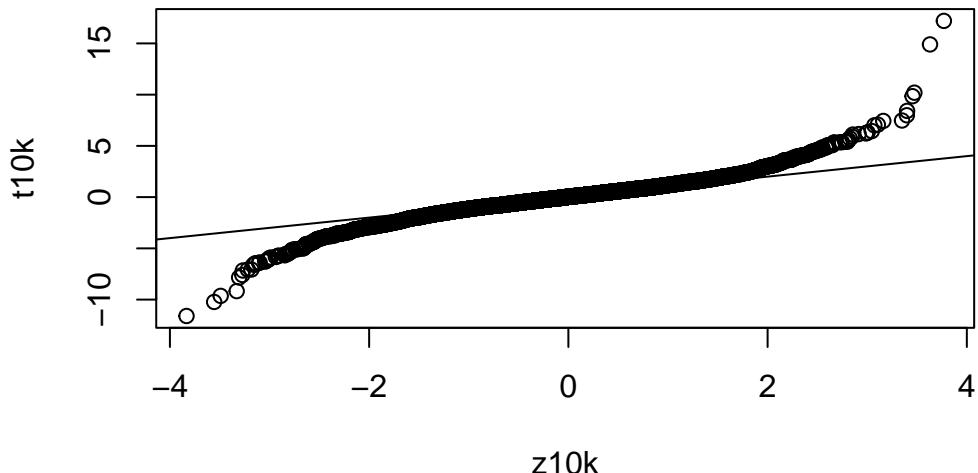
```
hist(pt(t10k, 5))
```



Now, the p-values are uniformly distributed between 0 and 1, as expected.

What is a qqplot and how do we use it? A qqplot is a plot of the quantiles of two distributions against each other. If the two distributions are identical, the points will fall on a straight line. If the two distributions are different, the points will deviate from the straight line. We can use a qqplot to compare the *t*-distribution to the normal distribution. If the *t*-distribution is identical to the normal distribution, the points will fall on a straight line. If the *t*-distribution is different from the normal distribution, the points will deviate from the straight line. In this case, we can see that the *t*-distribution is different from the normal distribution, as the points deviate from the straight line. What would happen if we increased the sample size? The *t*-distribution would approach the normal distribution, and the points would fall closer and closer to the straight line.

```
qqplot(z10k, t10k)
abline(0,1)
```



---

## 12.4 Summary of *t*-distribution vs normal distribution

The *t*-distribution is a family of probability distributions that depends on a parameter called degrees of freedom, which is related to the sample size. The *t*-distribution approaches the standard normal distribution as the sample size increases but has heavier tails for smaller sample sizes. This means that the *t*-distribution is more conservative in calculating p-values for small samples, making it harder to reject the null hypothesis. Including an estimate of the standard deviation changes the way we calculate p-values by switching from the standard normal distribution to the *t*-distribution, which accounts for the uncertainty introduced by estimating the population standard deviation from the sample. This adjustment is particularly important for small sample sizes, as it provides a more accurate assessment of the statistical significance of our results.

---

## 12.5 *t.test*

### 12.5.1 One-sample

We are going to use the `t.test` function to perform a one-sample *t*-test. The `t.test` function takes a vector of values as input that represents the sample values. In this case, we'll simulate our sample using the `rnorm` function and presume that our “effect-size” is 1.

```
x = rnorm(20,1)
# small sample
# Just use the first 5 values of the sample
t.test(x[1:5])
```

One Sample t-test

```
data: x[1:5]
t = 0.97599, df = 4, p-value = 0.3843
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
-1.029600 2.145843
sample estimates:
mean of x
0.5581214
```

In this case, we set up the experiment so that the null hypothesis is true (the true mean is not zero, but actually 1). However, we only have a small sample size that leads to a modest p-value.

Increasing the sample size allows us to see the effect more clearly.

```
t.test(x[1:20])
```

One Sample t-test

```
data: x[1:20]
t = 3.8245, df = 19, p-value = 0.001144
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
0.3541055 1.2101894
sample estimates:
mean of x
0.7821474
```

### 12.5.2 two-sample

```
x = rnorm(10,0.5)
y = rnorm(10,-0.5)
t.test(x,y)
```

Welch Two Sample t-test

```
data: x and y
t = 3.4296, df = 17.926, p-value = 0.003003
alternative hypothesis: true difference in means is not equal to 0
95 percent confidence interval:
0.5811367 2.4204048
```

```
sample estimates:
mean of x  mean of y
0.7039205 -0.7968502
```

### 12.5.3 from a data.frame

In some situations, you may have data and groups as columns in a data.frame. See the following data.frame, for example

```
df = data.frame(value=c(x,y),group=as.factor(rep(c('g1','g2'),each=10)))
df
```

	value	group
1	1.12896674	g1
2	-1.26838101	g1
3	1.04577597	g1
4	1.69075585	g1
5	0.18672204	g1
6	1.99715092	g1
7	1.15424947	g1
8	0.37671442	g1
9	-0.09565723	g1
10	0.82290783	g1
11	-1.48530261	g2
12	-1.29200440	g2
13	-0.18778362	g2
14	0.59205742	g2
15	-2.10065248	g2
16	-0.29961560	g2
17	-0.38985115	g2
18	-2.47126235	g2
19	-0.63654380	g2
20	0.30245611	g2

R allows us to perform a t-test using the formula notation.

```
t.test(value ~ group, data=df)
```

```
Welch Two Sample t-test

data: value by group
t = 3.4296, df = 17.926, p-value = 0.003003
alternative hypothesis: true difference in means between group g1 and group g2 is not equal to 0
95 percent confidence interval:
0.5811367 2.4204048
sample estimates:
mean in group g1 mean in group g2
0.7039205      -0.7968502
```

You read that as value **is a function of** group. In practice, this will do a t-test between the values in g1 vs g2.

#### 12.5.4 Equivalence to linear model

```
t.test(value ~ group, data=df, var.equal=TRUE)
```

Two Sample t-test

```
data: value by group
t = 3.4296, df = 18, p-value = 0.002989
alternative hypothesis: true difference in means between group g1 and group g2 is not equal to 0
95 percent confidence interval:
0.5814078 2.4201337
sample estimates:
mean in group g1 mean in group g2
0.7039205 -0.7968502
```

This is *equivalent to*:

```
res = lm(value ~ group, data=df)
summary(res)
```

Call:

```
lm(formula = value ~ group, data = df)
```

Residuals:

Min	1Q	Median	3Q	Max
-1.9723	-0.5600	0.2511	0.5252	1.3889

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	0.7039	0.3094	2.275	0.03538 *
groupg2	-1.5008	0.4376	-3.430	0.00299 **
---				
Signif. codes:	0 ***	0.001 **	0.01 *	0.05 .
	'	'	'	'

Residual standard error: 0.9785 on 18 degrees of freedom

Multiple R-squared: 0.3952, Adjusted R-squared: 0.3616

F-statistic: 11.76 on 1 and 18 DF, p-value: 0.002989

## 12.6 Power calculations

The power of a statistical test is the probability that the test will reject the null hypothesis when the alternative hypothesis is true. In other words, the power of a statistical test is the probability of not making a Type II error. The power of a statistical test depends on the significance level (alpha), the sample size, and the effect size.

The `power.t.test` function can be used to calculate the power of a one-sample t-test.

Looking at `help("power.t.test")`, we see that the function takes the following arguments:

- `n` - sample size
- `delta` - effect size
- `sd` - standard deviation of the sample
- `sig.level` - significance level
- `power` - power

We need to supply four of these arguments to calculate the fifth. For example, if we want to calculate the power of a one-sample t-test with a sample size of 5, a standard deviation of 1, and an effect size of 1, we can use the following command:

```
power.t.test(n = 5, delta = 1, sd = 1, sig.level = 0.05)
```

Two-sample t test power calculation

```
n = 5
delta = 1
sd = 1
sig.level = 0.05
power = 0.2859276
alternative = two.sided
```

NOTE: n is number in \*each\* group

This gives a nice summary of the power calculation. We can also extract the power value from the result:

```
power.t.test(n = 5, delta = 1, sd = 1,
             sig.level = 0.05, type='one.sample')$power
```

```
[1] 0.4013203
```



Tip

When getting results from a function that don't look "computable" such as those from `power.t.test`, you can use the `$` operator to extract the value you want. In this case, we want the power value from the result of `power.t.test`.

How would you know what to extract? You can use the `names` function or the `str` function to see

the structure of the result. For example:

```
names(power.t.test(n = 5, delta = 1, sd = 1,
                    sig.level = 0.05, type='one.sample'))
[1] "n"           "delta"        "sd"           "sig.level"    "power"
[6] "alternative" "note"         "method"

# or
str(power.t.test(n = 5, delta = 1, sd = 1,
                  sig.level = 0.05, type='one.sample'))
```

```
List of 8
$ n           : num 5
$ delta       : num 1
$ sd          : num 1
$ sig.level   : num 0.05
$ power       : num 0.401
$ alternative: chr "two.sided"
$ note        : NULL
$ method      : chr "One-sample t test power calculation"
- attr(*, "class")= chr "power.htest"
```

Alternatively, we may know a lot about our experimental system and want to calculate the sample size needed to achieve a certain power. For example, if we want to achieve a power of 0.8 with a standard deviation of 1 and an effect size of 1, we can use the following command:

```
power.t.test(delta = 1, sd = 1, sig.level = 0.05, power = 0.8, type = "one.sample")
```

One-sample t test power calculation

```
n = 9.937864
delta = 1
sd = 1
sig.level = 0.05
power = 0.8
alternative = two.sided
```

The `power.t.test` function is convenient and quite fast. As we've seen before, though, sometimes the distribution of the test statistics is now easily calculated. In those cases, we can use simulation to calculate the power of a statistical test. For example, if we want to calculate the power of a one-sample t-test with a sample size of 5, a standard deviation of 1, and an effect size of 1, we can use the following command:

```
sim_t_test_pval <- function(n = 5, delta = 1, sd = 1, sig.level = 0.05) {
  x = rnorm(n, delta, sd)
  t.test(x)$p.value <= sig.level
}
pow = mean(replicate(1000, sim_t_test_pval()))
pow
```

```
[1] 0.405
```

Let's break this down. First, we define a function called `sim_t_test_pval` that takes the same arguments as the `power.t.test` function. Inside the function, we simulate a sample of size `n` from a normal distribution with mean `delta` and standard deviation `sd`. Then, we perform a one-sample t-test on the sample and return a logical value indicating whether the p-value is less than the significance level. Next, we use the `replicate` function to repeat the simulation 1000 times. Finally, we calculate the proportion of simulations in which the p-value was less than the significance level. This proportion is an estimate of the power of the one-sample t-test.

Let's compare the results of the `power.t.test` function and our simulation-based approach:

```
power.t.test(n = 5, delta = 1, sd = 1, sig.level = 0.05, type='one.sample')$power
```

```
[1] 0.4013203
```

```
mean(replicate(1000, sim_t_test_pval(n = 5, delta = 1, sd = 1, sig.level = 0.05)))
```

```
[1] 0.414
```

---

## 12.7 Resources

See the [pwr package](#) for more information on power calculations.

# 13

## *K-means clustering*

### 13.1 History of the k-means algorithm

The k-means clustering algorithm was first proposed by Stuart Lloyd in 1957 as a technique for pulse-code modulation. However, it was not published until 1982. In 1965, Edward W. Forgy published an essentially identical method, which became widely known as the k-means algorithm. Since then, k-means clustering has become one of the most popular unsupervised learning techniques in data analysis and machine learning.

K-means clustering is a method for finding patterns or groups in a dataset. It is an unsupervised learning technique, meaning that it doesn't rely on previously labeled data for training. Instead, it identifies structures or patterns directly from the data based on the similarity between data points (see Figure 13.1).

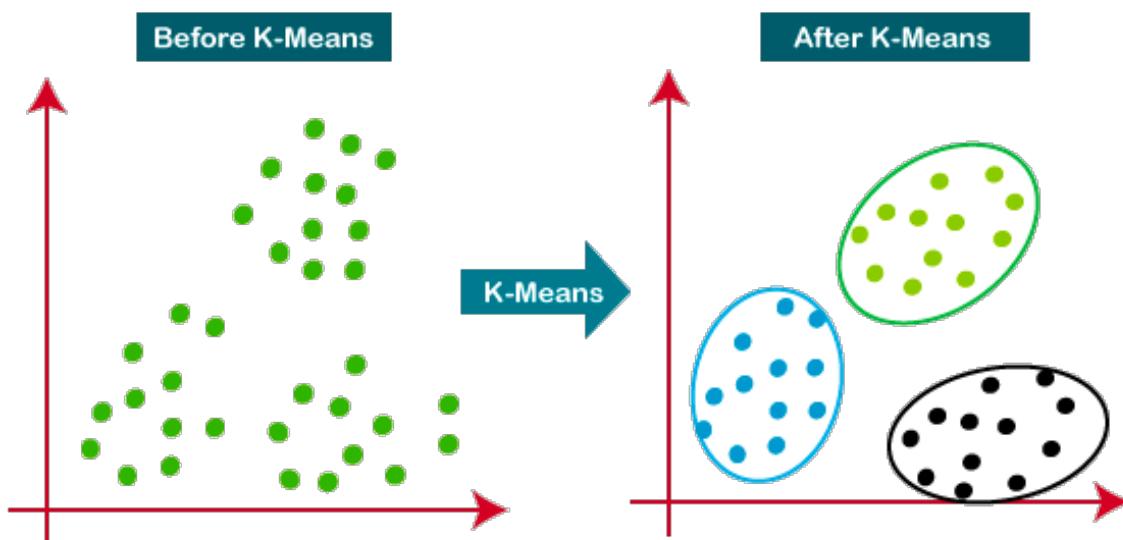


Figure 13.1: K-means clustering takes a dataset and divides it into  $k$  clusters.

In simple terms, k-means clustering aims to divide a dataset into  $k$  distinct groups or clusters, where each data point belongs to the cluster with the nearest mean (average). The goal is to minimize the variability within each cluster while maximizing the differences between clusters. This helps to reveal hidden patterns

or relationships in the data that might not be apparent otherwise.

---

## 13.2 The k-means algorithm

The k-means algorithm follows these general steps:

1. Choose the number of clusters  $k$ .
2. Initialize the cluster centroids randomly by selecting  $k$  data points from the dataset.
3. Assign each data point to the nearest centroid.
4. Update the centroids by computing the mean of all the data points assigned to each centroid.
5. Repeat steps 3 and 4 until the centroids no longer change or a certain stopping criterion is met (e.g., a maximum number of iterations).

The algorithm converges when the centroids stabilize or no longer change significantly. The final clusters represent the underlying patterns or structures in the data. Advantages and disadvantages of k-means clustering

---

## 13.3 Pros and cons of k-means clustering

Compared to other clustering algorithms, k-means has several advantages:

- **Simplicity and ease of implementation** The k-means algorithm is relatively straightforward and can be easily implemented, even for large datasets.
- **Scalability** The algorithm can be adapted for large datasets using various optimization techniques or parallel processing.
- **Speed** K-means is generally faster than other clustering algorithms, especially when the number of clusters  $k$  is small.
- **Interpretability** The results of k-means clustering are easy to understand, as the algorithm assigns each data point to a specific cluster based on its similarity to the cluster's centroid.

However, k-means clustering has several disadvantages as well:

- **Choice of  $k$**  Selecting the appropriate number of clusters can be challenging and often requires domain knowledge or experimentation. A poor choice of  $k$  may yield poor results.
- **Sensitivity to initial conditions** The algorithm's results can vary depending on the initial placement of centroids. To overcome this issue, the algorithm can be run multiple times with different initializations and the best solution can be chosen based on a criterion (e.g., minimizing within-cluster variation).

- **Assumes spherical clusters** K-means assumes that clusters are spherical and evenly sized, which may not always be the case in real-world datasets. This can lead to poor performance if the underlying clusters have different shapes or densities.
- **Sensitivity to outliers** The algorithm is sensitive to outliers, which can heavily influence the position of centroids and the final clustering result. Preprocessing the data to remove or mitigate the impact of outliers can help improve the performance of k-means clustering.

Despite limitations, k-means clustering remains a popular and widely used method for exploring and analyzing data, particularly in biological data analysis, where identifying patterns and relationships can provide valuable insights into complex systems and processes.

---

## 13.4 An example of k-means clustering

### 13.4.1 The data and experimental background

The data we are going to use are from DeRisi, Iyer, and Brown (1997). From their abstract:

DNA microarrays containing virtually every gene of *Saccharomyces cerevisiae* were used to carry out a comprehensive investigation of the temporal program of gene expression accompanying the metabolic shift from fermentation to respiration. The expression profiles observed for genes with known metabolic functions pointed to features of the metabolic reprogramming that occur during the diauxic shift, and the expression patterns of many previously uncharacterized genes provided clues to their possible functions.

These data are available from NCBI GEO as [GSE28](#).

In the case of the baker's or brewer's yeast *Saccharomyces cerevisiae* growing on glucose with plenty of aeration, the diauxic growth pattern is commonly observed in batch culture. During the first growth phase, when there is plenty of glucose and oxygen available, the yeast cells prefer glucose fermentation to aerobic respiration even though aerobic respiration is the more efficient pathway to grow on glucose. This experiment profiles gene expression for 6400 genes over a time course during which the cells are undergoing a [diauxic shift](#).

The data in deRisi et al. have no replicates and are time course data. Sometimes, seeing how groups of genes behave can give biological insight into the experimental system or the function of individual genes. We can use clustering to group genes that have a similar expression pattern over time and then potentially look at the genes that do so.

Our goal, then, is to use kmeans clustering to divide highly variable (informative) genes into groups and then to visualize those groups.

## 13.5 Getting data

These data were deposited at NCBI GEO back in 2002. GEOquery can pull them out easily.

```
library(GEOquery)
gse = getGEO("GSE28")[[1]]
class(gse)
```

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

GEOquery is a little dated and was written before the SummarizedExperiment existed. However, Bioconductor makes a conversion from the old ExpressionSet that GEOQuery uses to the SummarizedExperiment that we see so commonly used now.

```
library(SummarizedExperiment)
gse = as(gse, "SummarizedExperiment")
gse
```

```
class: SummarizedExperiment
dim: 6400 7
metadata(3): experimentData annotation protocolData
assays(1): exprs
rownames(6400): 1 2 ... 6399 6400
rowData names(20): ID ORF ... FAILED IS_CONTAMINATED
colnames(7): GSM887 GSM888 ... GSM892 GSM893
colData names(33): title geo_accession ... supplementary_file
  data_row_count
```

Taking a quick look at the colData(), it might be that we want to reorder the columns a bit.

```
colData(gse)$title
```

```
[1] "diauxic shift timecourse: 15.5 hr" "diauxic shift timecourse: 0 hr"
[3] "diauxic shift timecourse: 18.5 hr" "diauxic shift timecourse: 9.5 hr"
[5] "diauxic shift timecourse: 11.5 hr" "diauxic shift timecourse: 13.5 hr"
[7] "diauxic shift timecourse: 20.5 hr"
```

So, we can reorder by hand to get the time course correct:

```
gse = gse[, c(2,4,5,6,1,3,7)]
```

### 13.6 Preprocessing

In gene expression data analysis, the primary objective is often to identify genes that exhibit significant differences in expression levels across various conditions, such as diseased vs. healthy samples or different time points in a time-course experiment. However, gene expression datasets are typically large, noisy, and contain numerous genes that do not exhibit substantial changes in expression levels. Analyzing all genes in the dataset can be computationally intensive and may introduce noise or false positives in the results.

One common approach to reduce the complexity of the dataset and focus on the most informative genes is to subset the genes based on their standard deviation in expression levels across the samples. The standard deviation is a measure of dispersion or variability in the data, and genes with high standard deviations have more variation in their expression levels across the samples.

By selecting genes with high standard deviations, we focus on genes that show relatively large changes in expression levels across different conditions. These genes are more likely to be biologically relevant and involved in the underlying processes or pathways of interest. In contrast, genes with low standard deviations exhibit little or no change in expression levels and are less likely to be informative for the analysis. It turns out that applying filtering based on criteria such as standard deviation can also increase power and reduce false positives in the analysis (Bourgon, Gentleman, and Huber 2010).

To subset the genes for analysis based on their standard deviation, the following steps can be followed: Calculate the standard deviation of each gene's expression levels across all samples. Set a threshold for the standard deviation, which can be determined based on domain knowledge, data distribution, or a specific percentile of the standard deviation values (e.g., selecting the top 10% or 25% of genes with the highest standard deviations). Retain only the genes with a standard deviation above the chosen threshold for further analysis.

By subsetting the genes based on their standard deviation, we can reduce the complexity of the dataset, speed up the subsequent analysis, and increase the likelihood of detecting biologically meaningful patterns and relationships in the gene expression data. The threshold for the standard deviation cutoff is rather arbitrary, so it may be beneficial to try a few to check for sensitivity of findings.

```
sds = apply(assays(gse)[[1]], 1, sd)
hist(sds)
```

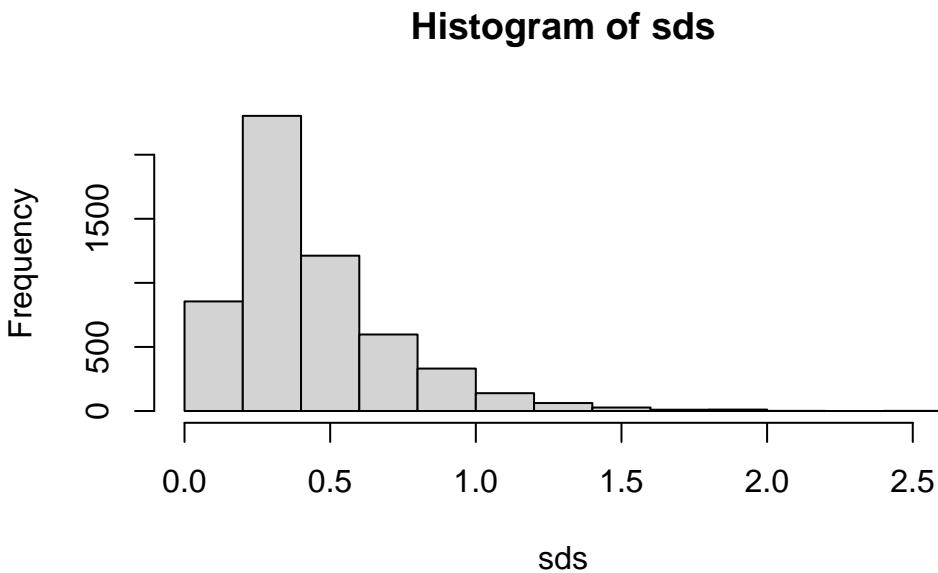


Figure 13.2: Histogram of standard deviations for all genes in the deRisi dataset.

Examining the plot, we can see that the most highly variable genes have an  $\text{sd} > 0.8$  or so (arbitrary). We can, for convenience, create a new `SummarizedExperiment` that contains only our most highly variable genes.

```
idx = sds>0.8 & !is.na(sds)
gse_sub = gse[idx,]
```

## 13.7 Clustering

Now, `gse_sub` contains a subset of our data.

The `kmeans` function takes a matrix and the number of clusters as arguments.

```
k = 4
km = kmeans(assays(gse_sub)[[1]], 4)
```

The `km` `kmeans` result contains a vector, `km$cluster`, which gives the cluster associated with each gene. We can plot the genes for each cluster to see how these different genes behave.

```
expression_values = assays(gse_sub)[[1]]
par(mfrow=c(2,2), mar=c(3,4,1,2)) # this allows multiple plots per page
```

```
for(i in 1:k) {
  matplot(t(expression_values[km$cluster==i, ]), type='l', ylim=c(-3,3),
          ylab = paste("cluster", i))
}
```

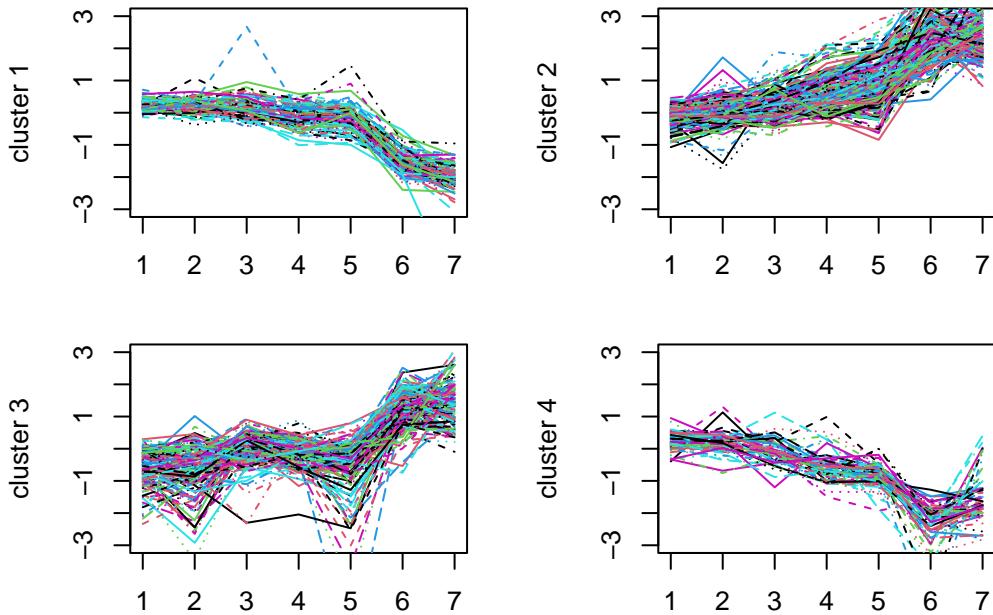


Figure 13.3: Gene expression profiles for the four clusters identified by k-means clustering. Each line represents a gene in the cluster, and each column represents a time point in the experiment. Each cluster shows a distinct trend where the genes in the cluster are potentially co-regulated.

Try this with different size k. Perhaps go back to choose more genes (using a smaller cutoff for sd).

## 13.8 Summary

In this lesson, we have learned how to use k-means clustering to identify groups of genes that behave similarly over time. We have also learned how to subset our data to focus on the most informative genes.

# 14

---

## *Machine Learning*

---

### 14.1 What is Machine Learning?

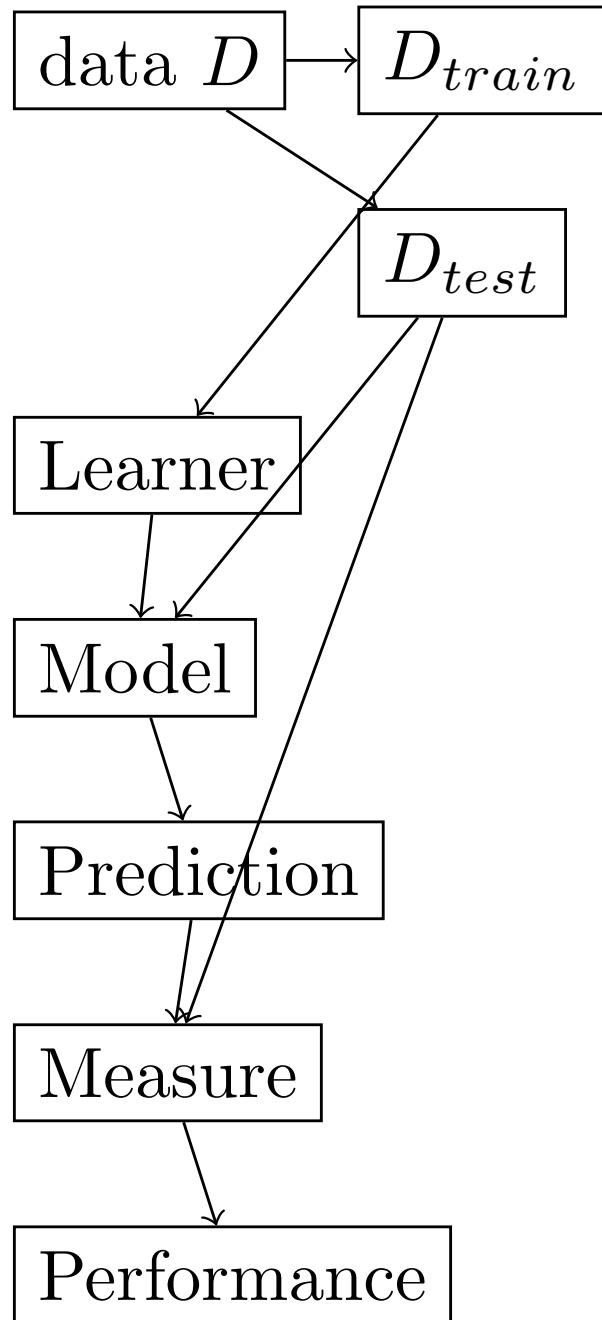
Machine learning is a subfield of artificial intelligence that focuses on the development of algorithms and models that enable computers to learn and make decisions or predictions without explicit programming. It has emerged as a powerful tool for solving complex problems across various industries, including health-care, finance, marketing, and natural language processing. This chapter provides an overview of machine learning, its types, key concepts, applications, and challenges.

Machine learning in biology is a really broad topic. Greener et al. (2022) present a nice overview of the different types of machine learning methods that are used in biology. Libbrecht and Noble (2015) also present an early review of machine learning in genetics and genomics.

---

### 14.2 Classes of Machine Learning

```
\usetikzlibrary{fit, graphs, matrix, positioning}
\begin{tikzpicture}[node distance=2cm]
\graph [grow right sep, branch down=1cm, simple, nodes={draw, rectangle}] {
  Data[as=data \$D\$] --> {dtrain[as=$D_{train}\$], dtest[as=$D_{test}\$]};
  dtrain -> Learner;
  dtest -> Model;
  Learner -> Model;
  Model -> Prediction;
  dtest -> Measure;
  Prediction -> Measure;
  Measure -> Performance;
};
\end{tikzpicture}
```



#### 14.2.1 Supervised learning

Supervised learning is a type of machine learning where the model learns from labeled data, i.e., input-output pairs, to make predictions. It includes tasks like regression (predicting continuous values) and clas-

sification (predicting discrete classes or categories).

#### 14.2.2 Unsupervised learning

Unsupervised learning involves learning from unlabeled data, where the model discovers patterns or structures within the data. Common unsupervised learning tasks include clustering (grouping similar data points), dimensionality reduction (reducing the number of features or variables), and anomaly detection (identifying unusual data points).

#### 14.2.3 Other types of machine learning

Reinforcement learning is a type of machine learning where an agent learns to make decisions based on interactions with an environment. The agent receives feedback in the form of rewards or penalties and adjusts its actions to maximize the cumulative reward over time.

##### Terminology and Concepts

- **Data** Data is the foundation of machine learning and can be structured (tabular) or unstructured (text, images, audio). It is usually divided into training, validation, and testing sets for model development and evaluation.
- **Features** Features are the variables or attributes used to describe the data points. Feature engineering and selection are crucial steps in machine learning to improve model performance and interpretability.
- **Models and Algorithms** Models are mathematical representations of the relationship between features and the target variable(s). Algorithms are the methods used to train models, such as linear regression, decision trees, and neural networks.
- **Hyperparameters and Tuning** Hyperparameters are adjustable parameters that control the learning process of an algorithm. Tuning involves finding the optimal set of hyperparameters to improve model performance.
- **Evaluation Metrics** Evaluation metrics quantify the performance of a model, such as accuracy, precision, recall, F1-score (for classification), and mean squared error, R-squared (for regression).

- Supervised
- Classification
- Regression
- Unsupervised
- “Clustering”
- Dimensionality reduction

```
set.seed(123)
sinsim <- function(n, sd=0.1) {
  x <- seq(0, 1, length.out=n)
  y <- sin(2*pi*x) + rnorm(n, 0, sd)
  return(data.frame(x=x, y=y))
}
```

```

dat <- sinsim(100,0.25)
library(ggplot2)
library(patchwork)
p_base <- ggplot(dat,aes(x=x,y=y)) +
  geom_point(alpha=0.7) +
  theme_bw()
p_lm <- p_base +
  geom_smooth(method="lm", se=FALSE, alpha=0.6, formula = y ~ x)
p_lmsin <- p_base +
  geom_smooth(method="lm",formula=y~sin(2*pi*x), se=FALSE, alpha=0.6)
p_loess_wide <- p_base +
  geom_smooth(method="loess",span=0.5, se=FALSE, alpha=0.6, formula = y ~ x)
p_loess_narrow <- p_base +
  geom_smooth(method="loess",span=0.1, se=FALSE, alpha=0.6, formula = y ~ x)
p_lm + p_lmsin + p_loess_wide + p_loess_narrow + plot_layout(ncol=2) +
  plot_annotation(tag_levels = 'A') &
  theme(plot.tag = element_text(size = 8))

```

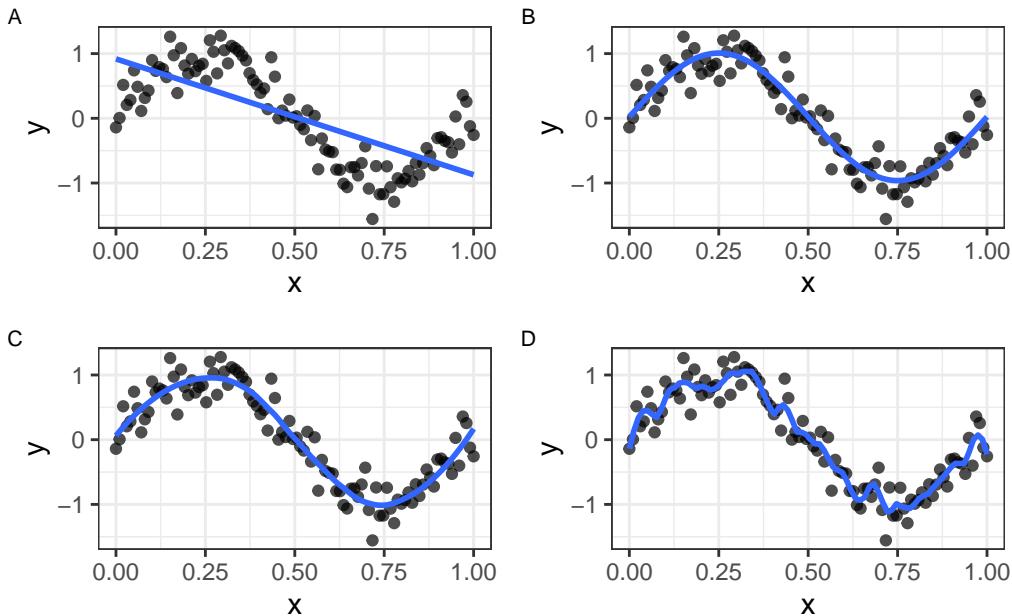


Figure 14.1: Data simulated according to the function  $f(x) = \sin(2\pi x) + N(0, 0.25)$  fitted with four different models. A) A simple linear model demonstrates *underfitting*. B) A linear model with a sin function ( $y = \sin(2\pi x)$ ) and C) a loess model with a wide span (0.5) demonstrate *good fits*. D) A loess model with a narrow span (0.1) is a good example of *overfitting*.

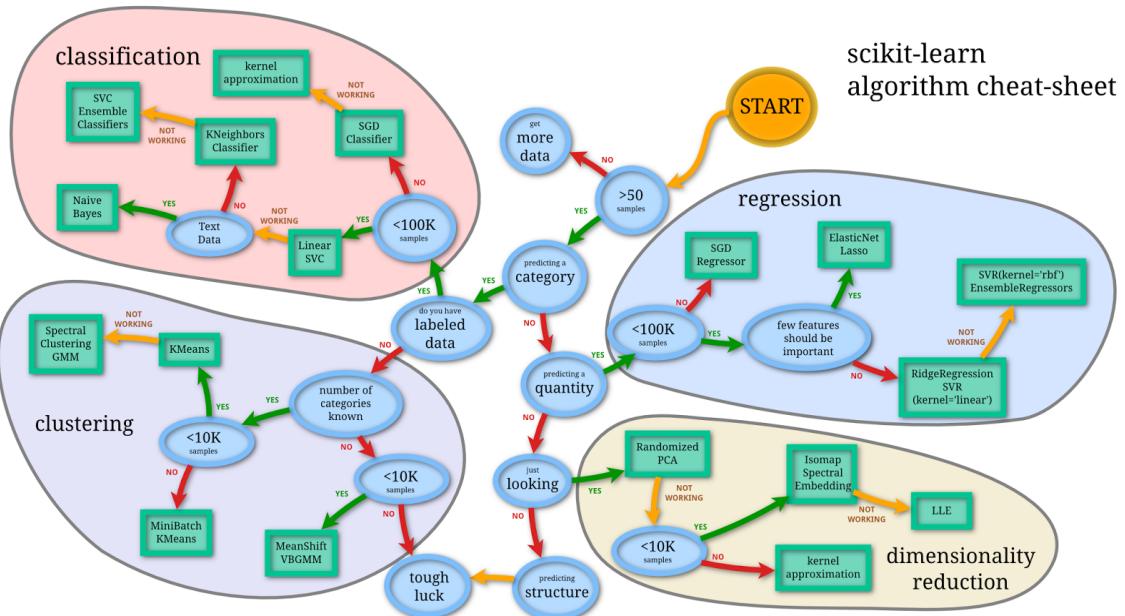


Figure 14.2: A simple view of machine learning according the sklearn.

## 14.3 Specific methods

### 14.3.1 Linear regression

In **statistics**, **linear regression** is a **linear** approach for modelling the relationship between a **scalar** response and one or more explanatory variables (also known as **dependent and independent variables**). The case of one explanatory variable is called ***simple linear regression***; for more than one, the process is called ***multiple linear regression***. This term is distinct from **multivariate linear regression**, where multiple **correlated** dependent variables are predicted, rather than a single scalar variable.

In linear regression, the relationships are modeled using [linear predictor functions](#) whose unknown model parameters are [estimated](#) from the [data](#). Such models are called [linear models](#). Most commonly, the [conditional mean](#) of the response given the values of the explanatory variables (or predictors) is assumed to be an [affine function](#) of those values; less commonly, the conditional [median](#) or some other [quantile](#) is used. Like all forms of [regression analysis](#), linear regression focuses on the [conditional probability distribution](#) of the response given the values of the predictors, rather than on the [joint probability distribution](#) of all of these variables, which is the domain of [multivariate analysis](#).

Linear regression was the first type of regression analysis to be studied rigorously, and to be used extensively in practical applications. This is because models which depend linearly on their unknown parameters are easier to fit than models which are non-linearly related to their parameters and because the statistical properties of the resulting estimators are easier to determine.

### 14.3.2 K-nearest Neighbor

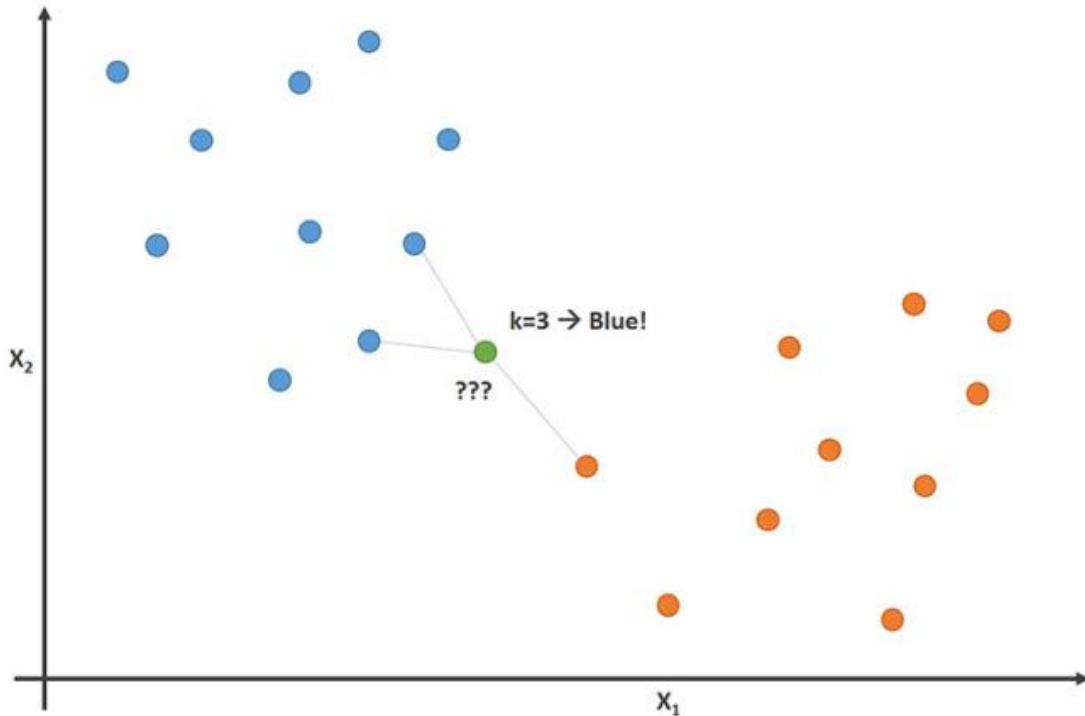


Figure 14.3: **Figure.** The k-nearest neighbor algorithm can be used for regression or classification.

The **k-nearest neighbors algorithm (k-NN)** is a [non-parametric supervised learning](#) method first developed by [Evelyn Fix](#) and [Joseph Hodges](#) in 1951, and later expanded by [Thomas Cover](#). It is used for [classification](#) and [regression](#). In both cases, the input consists of the  $k$  closest training examples in a [data set](#).

The k-nearest neighbor (k-NN) algorithm is a simple, yet powerful, supervised machine learning method used for classification and regression tasks. It is an instance-based, non-parametric learning method that stores the entire training dataset and makes predictions based on the similarity between data points. The underlying principle of the k-NN algorithm is that similar data points (those that are close to each other in multidimensional space) are likely to have similar outcomes or belong to the same class.

Here's a description of how the k-NN algorithm works:

1. Determine the value of  $k$ : The first step is to choose the number of nearest neighbors ( $k$ ) to consider when making predictions. The value of  $k$  is a user-defined hyperparameter and can significantly impact the algorithm's performance. A small value of  $k$  can lead to overfitting, while a large value may result in underfitting.
2. Compute distance: Calculate the distance between the new data point (query point) and each data point in the training dataset. The most common distance metrics used are Euclidean, Man-

hattan, and Minkowski distance. The choice of distance metric depends on the problem and the nature of the data.

3. Find k-nearest neighbors: Identify the k data points in the training dataset that are closest to the query point, based on the chosen distance metric.
4. Make predictions: Once the k-nearest neighbors are identified, the final step is to make predictions. The prediction for the query point can be made in two ways:
  - a. For classification, determine the class labels of the k-nearest neighbors and assign the class label with the highest frequency (majority vote) to the query point. In case of a tie, one can choose the class with the smallest average distance to the query point or randomly select one among the tied classes.
  - b. For regression tasks, the k-NN algorithm follows a similar process, but instead of majority voting, it calculates the mean (or median) of the target values of the k-nearest neighbors and assigns it as the prediction for the query point.

The k-NN algorithm is known for its simplicity, ease of implementation, and ability to handle multi-class problems. However, it has some drawbacks, such as high computational cost (especially for large datasets), sensitivity to the choice of k and distance metric, and poor performance with high-dimensional or noisy data. Scaling and preprocessing the data, as well as using dimensionality reduction techniques, can help mitigate some of these issues.

- In *k*-NN *classification*, the output is a class membership. An object is classified by a plurality vote of its neighbors, with the object being assigned to the class most common among its *k* nearest neighbors (*k* is a positive **integer**, typically small). If *k* = 1, then the object is simply assigned to the class of that single nearest neighbor.
- In *k*-NN *regression*, the output is the property value for the object. This value is the average of the values of *k* nearest neighbors.

*k*-NN is a type of **classification** where the function is only approximated locally and all computation is deferred until function evaluation. Since this algorithm relies on distance for classification, if the features represent different physical units or come in vastly different scales then **normalizing** the training data can improve its accuracy dramatically.

Both for classification and regression, a useful technique can be to assign weights to the contributions of the neighbors, so that the nearer neighbors contribute more to the average than the more distant ones. For example, a common weighting scheme consists in giving each neighbor a weight of  $1/d$ , where  $d$  is the distance to the neighbor.

The neighbors are taken from a set of objects for which the class (for *k*-NN classification) or the object property value (for *k*-NN regression) is known. This can be thought of as the training set for the algorithm, though no explicit training step is required.

---

## 14.4 Penalized regression

Adapted from <http://www.sthda.com/english/articles/37-model-selection-essentials-in-r/153-penalized-regression-essentials-ridge-lasso-elastic-net/>.

Penalized regression is a type of regression analysis that introduces a penalty term to the loss function in order to prevent overfitting and improve the model's ability to generalize. Remember that in regression, the *loss* function is the sum of squares Equation 14.1.

$$L = \sum_{i=0}^n (\hat{y}_i - y_i)^2 \quad (14.1)$$

In Equation 14.1,  $\hat{y}_i$  is the predicted output,  $y_i$  is the actual output, and  $n$  is the number of observations. The goal of regression is to minimize the loss function by finding the optimal values of the model parameters or coefficients. The model parameters are estimated using the training data. The model is then evaluated using the test data. If the model performs well on the training data but poorly on the test data, it is said to be overfit. Overfitting occurs when the model learns the training data too well, including the noise, and is not able to generalize well to new data. This is a common problem in machine learning, particularly when there are a large number of predictors compared to the number of observations, and can be addressed by penalized regression.

The two most common types of penalized regression are Ridge Regression (L2 penalty) and LASSO Regression (L1 penalty). Both Ridge and LASSO help to reduce model complexity and prevent over-fitting which may result from simple linear regression. However, the choice between Ridge and LASSO depends on the situation and the dataset at hand. If feature selection is important for the interpretation of the model, LASSO might be preferred. If the goal is prediction accuracy and the model needs to retain all features, Ridge might be the better choice.

#### 14.4.1 Ridge regression

Ridge regression shrinks the regression coefficients, so that variables, with minor contribution to the outcome, have their coefficients close to zero. The shrinkage of the coefficients is achieved by penalizing the regression model with a penalty term called L2-norm, which is the sum of the squared coefficients. The amount of the penalty can be fine-tuned using a constant called lambda ( $\lambda$ ). Selecting a good value for  $\lambda$  is critical. When  $\lambda=0$ , the penalty term has no effect, and ridge regression will produce the classical least square coefficients. However, as  $\lambda$  increases to infinite, the impact of the shrinkage penalty grows, and the ridge regression coefficients will get close zero. The loss function for Ridge Regression is:

$$L = \sum_{i=0}^n (\hat{y}_i - y_i)^2 + \lambda \sum_{j=0}^k \beta_j^2 \quad (14.2)$$

Here,  $\hat{y}_i$  is the predicted output,  $y_i$  is the actual output,  $\beta_j$  represents the model parameters or coefficients, and  $\lambda$  is the regularization parameter. The second term,  $\lambda \sum \beta_j^2$ , is the penalty term where all parameters are squared and summed. Ridge regression tends to shrink the coefficients but doesn't necessarily zero them.

Note that, in contrast to the ordinary least square regression, ridge regression is highly affected by the scale of the predictors. Therefore, it is better to standardize (i.e., scale) the predictors before applying the ridge regression (James et al. 2014), so that all the predictors are on the same scale. The standardization of a predictor  $x$ , can be achieved using the formula  $x' = \frac{x}{sd(x)}$ , where  $sd(x)$  is the standard deviation of  $x$ . The

consequence of this is that, all standardized predictors will have a standard deviation of one allowing the final fit to not depend on the scale on which the predictors are measured.

One important advantage of the ridge regression, is that it still performs well, compared to the ordinary least square method (see Equation 14.1), in a situation where you have a large multivariate data with the number of predictors ( $p$ ) larger than the number of observations ( $n$ ). One disadvantage of the ridge regression is that, it will include all the predictors in the final model, unlike the stepwise regression methods, which will generally select models that involve a reduced set of variables. Ridge regression shrinks the coefficients towards zero, but it will not set any of them exactly to zero. The LASSO regression is an alternative that overcomes this drawback.

#### 14.4.2 LASSO regression

LASSO stands for *Least Absolute Shrinkage and Selection Operator*. It shrinks the regression coefficients toward zero by penalizing the regression model with a penalty term called L1-norm, which is the sum of the absolute coefficients. In the case of LASSO regression, the penalty has the effect of forcing some of the coefficient estimates, with a minor contribution to the model, to be exactly equal to zero. This means that, LASSO can be also seen as an alternative to the subset selection methods for performing variable selection in order to reduce the complexity of the model. As in ridge regression, selecting a good value of  $\lambda$  for the LASSO is critical. The loss function for LASSO Regression is:

$$L = \sum_{i=0}^n (\hat{y}_i - y_i)^2 + \lambda \sum_{j=0}^k |\beta_j| \quad (14.3)$$

Similar to Ridge,  $\hat{y}_i$  is the predicted output,  $y_i$  is the actual output,  $\beta_j$  represents the model parameters or coefficients, and  $\lambda$  is the regularization parameter. The second term,  $\lambda \sum |\beta_j|$ , is the penalty term where the absolute values of all parameters are summed. LASSO regression tends to shrink the coefficients and can zero out some of them, effectively performing variable selection.

One obvious advantage of LASSO regression over ridge regression, is that it produces simpler and more interpretable models that incorporate only a reduced set of the predictors. However, neither ridge regression nor the LASSO will universally dominate the other. Generally, LASSO might perform better in a situation where some of the predictors have large coefficients, and the remaining predictors have very small coefficients. Ridge regression will perform better when the outcome is a function of many predictors, all with coefficients of roughly equal size (James et al. 2014).

Cross-validation methods can be used for identifying which of these two techniques is better on a particular data set.

#### 14.4.3 Elastic Net

Elastic Net produces a regression model that is penalized with both the L1-norm and L2-norm. The consequence of this is to effectively shrink coefficients (like in ridge regression) and to set some coefficients to zero (as in LASSO).

#### 14.4.4 Classification and Regression Trees (CART)

[Decision Tree Learning](#) is supervised learning approach used in statistics, data mining and machine learning. In this formalism, a classification or regression decision tree is used as a predictive model to draw conclusions about a set of observations. Decision trees are a popular machine learning method used for both classification and regression tasks. They are hierarchical, tree-like structures that model the relationship between features and the target variable by recursively splitting the data into subsets based on the feature values. Each internal node in the tree represents a decision or test on a feature, and each branch represents the outcome of that test. The leaf nodes contain the final prediction, which is the majority class for classification tasks or the mean/median of the target values for regression tasks.

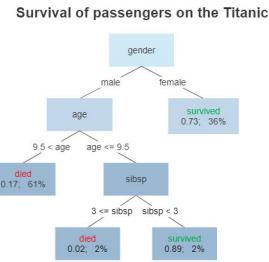


Figure 14.4: An example of a decision tree that performs classification, also sometimes called a classification tree.

Here's an overview of the decision tree learning process:

- Select the best feature and split value: Start at the root node and choose the feature and split value that results in the maximum reduction of impurity (or increase in information gain) in the child nodes. For classification tasks, impurity measures like Gini index or entropy are commonly used, while for regression tasks, mean squared error (MSE) or mean absolute error (MAE) can be used.
- Split the data: Partition the dataset into subsets based on the chosen feature and split value.
- Recursion: Repeat steps 1 and 2 for each subset until a stopping criterion is met. Stopping criteria can include reaching a maximum tree depth, a minimum number of samples per leaf, or no further improvement in impurity.
- Prune the tree (optional): To reduce overfitting, decision trees can be pruned by removing branches that do not significantly improve the model's performance on the validation dataset. This can be done using techniques like reduced error pruning or cost-complexity pruning.

Decision trees have several advantages, such as:

- **Interpretability** They are easy to understand, visualize, and explain, even for non-experts.
- **Minimal data preprocessing** Decision trees can handle both numerical and categorical data, and they are robust to outliers and missing values.
- **Non-linear relationships** They can capture complex non-linear relationships between features and the target variable.

However, decision trees also have some drawbacks:

- **Overfitting** They are prone to overfitting, especially when the tree is deep or has few samples per leaf. Pruning and setting stopping criteria can help mitigate this issue.
- **Instability** Small changes in the data can lead to different tree structures. This can be addressed by using ensemble methods like random forests or gradient boosting machines (GBMs).
- **Greedy learning** Decision tree algorithms use a greedy approach, meaning they make locally optimal choices at each node. This may not always result in a globally optimal tree.

Despite these limitations, decision trees are widely used in various applications due to their simplicity, interpretability, and ability to handle diverse data types.

#### 14.4.5 RandomForest

**Random forests** or **random decision forests** is an [ensemble learning](#) method for [classification](#), [regression](#) and other tasks that operates by constructing a multitude of [decision trees](#) at training time. For classification tasks, the output of the random forest is the class selected by most trees. For regression tasks, the mean or average prediction of the individual trees is returned. Random decision forests correct for decision trees' habit of [overfitting](#) to their [training set](#). Random forests generally outperform [decision trees](#), but their accuracy is lower than gradient boosted trees [[citation needed](#)]. However, data characteristics can affect their performance.

The first algorithm for random decision forests was created in 1995 by [Tin Kam Ho](#) using the [random subspace method](#), which, in Ho's formulation, is a way to implement the "stochastic discrimination" approach to classification proposed by Eugene Kleinberg.

An extension of the algorithm was developed by [Leo Breiman](#) and [Adele Cutler](#), who registered "Random Forests" as a [trademark](#) in 2006 (as of 2019[\[update\]](#), owned by [Minitab, Inc.](#)). The extension combines Breiman's "bagging" idea and random selection of features, introduced first by Ho and later independently by Amit and [Geman](#) in order to construct a collection of decision trees with controlled variance.

Random forests are frequently used as "blackbox" models in businesses, as they generate reasonable predictions across a wide range of data while requiring little configuration.

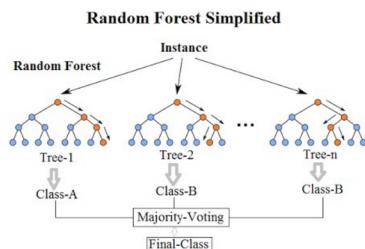


Figure 14.5: Random forests or random decision forests is an ensemble learning method for classification, regression and other tasks that operates by constructing a multitude of decision trees at training time.

# 15

## Machine Learning 2

### 15.1 Practical Machine Learning with R and mlr3

The `mlr3` R package is a modern, object-oriented machine learning framework in R that builds on the success of its predecessor, the `mlr` package. It provides a flexible and extensible platform for handling common machine learning tasks such as data preprocessing, model training, hyperparameter tuning, and model evaluation Figure 15.1. The package is designed to simplify the process of creating and deploying complex machine learning pipelines.

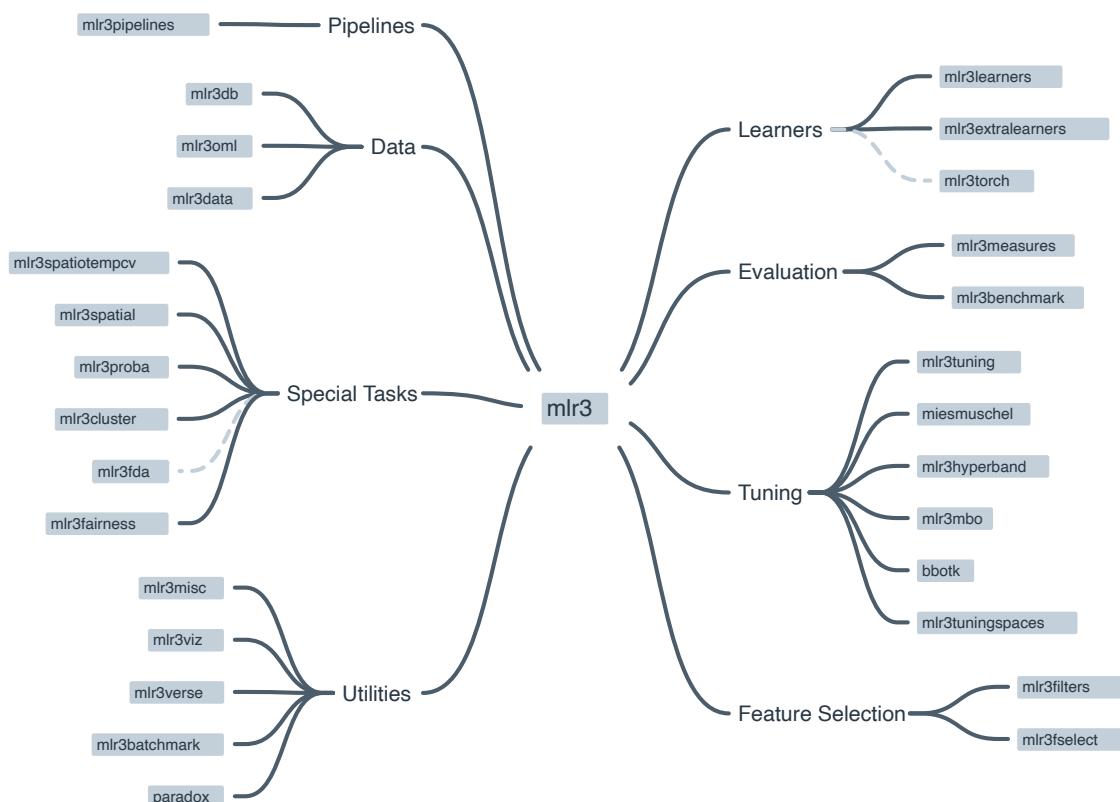


Figure 15.1: The `mlr3` ecosystem.

### 15.1.1 Key features of *mlr3*

- **Task abstraction** *mlr3* encapsulates different types of learning problems like classification, regression, and survival analysis into “Task” objects, making it easier to handle various learning scenarios.
- **Modular design** The package follows a modular design, allowing users to quickly swap out different components such as learners (algorithms), measures (performance metrics), and resampling strategies.
- **Extensibility** Users can extend the functionality of *mlr3* by adding custom components like learners, measures, and preprocessing steps via the R6 object-oriented system.
- **Preprocessing** *mlr3* provides a flexible way to preprocess data using “PipeOps” (pipeline operations), allowing users to create reusable preprocessing pipelines.
- **Tuning and model selection** *mlr3* supports hyperparameter tuning and model selection using various search strategies like grid search, random search, and Bayesian optimization.
- **Parallelization** The package allows for parallelization of model training and evaluation, making it suitable for large-scale machine learning tasks.
- **Benchmarking** *mlr3* facilitates benchmarking of multiple algorithms on multiple tasks, simplifying the process of comparing and selecting the best models.

You can find more information, including tutorials and examples, on the official *mlr3* GitHub repository<sup>1</sup> and the *mlr3* book<sup>2</sup>.

---

## 15.2 The *mlr3* workflow

The *mlr3* package is designed to simplify the process of creating and deploying complex machine learning pipelines. The package follows a modular design, which means that users can quickly swap out different components such as learners (algorithms), measures (performance metrics), and resampling strategies. The package also supports parallelization of model training and evaluation, making it suitable for large-scale machine learning tasks.

The *mlr3* workflow consists of the following steps:

1. Load data
2. Create a task to define the learning problem.
3. Split data into training and test sets.
4. Choose a learner object to specify the learning algorithm.
  - a. Train the model on the training set.
  - b. Predict the target variable for the test set.
5. Assess the performance of the model.
6. Interpret the model.

---

<sup>1</sup><https://github.com/mlr-org/mlr3>

<sup>2</sup><https://mlr3book.mlro.org/>

The following sections describe each of these steps in detail.

### 15.2.1 Tasks

Tasks are objects that contain the (usually tabular) data and additional metadata to define a machine learning problem. The meta-data is, for example, the name of the target variable for supervised machine learning problems, or the type of the dataset (e.g. a spatial or survival task). This information is used by specific operations that can be performed on a task.

Tasks are objects that contain the (usually tabular) data and additional meta-data to define a machine learning problem. The meta-data is, for example, the name of the target variable for supervised machine learning problems, or the type of the dataset (e.g. a *spatial* or *survival* task). This information is used by specific operations that can be performed on a task.

There are a number of [Task Types](#) that are supported by `mlr3`. To create a task from a `data.frame()`, `data.table()` or `Matrix()`, you first need to select the right task type:

- **Classification Task:** The target is a label (stored as character or factor) with only relatively few distinct values → [TaskClassif](#).
- **Regression Task:** The target is a numeric quantity (stored as integer or numeric) → [TaskRegr](#).
- **Survival Task:** The target is the (right-censored) time to an event. More censoring types are currently in development → [mlr3proba::TaskSurv](#) in add-on package [mlr3proba](#).
- **Density Task:** An unsupervised task to estimate the density → [mlr3proba::TaskDens](#) in add-on package [mlr3proba](#).
- **Cluster Task:** An unsupervised task type; there is no target and the aim is to identify similar groups within the feature space → [mlr3cluster::TaskClust](#) in add-on package [mlr3cluster](#).
- **Spatial Task:** Observations in the task have spatio-temporal information (e.g. coordinates) → [mlr3spatiotempcv::TaskRegrST](#) or [mlr3spatiotempcv::TaskClassifST](#) in add-on package [mlr3spatiotempcv](#).
- **Ordinal Regression Task:** The target is ordinal → [TaskOrdinal](#) in add-on package [mlr3ordinal](#) (still in development).

### 15.2.2 Learners

Objects of class [Learner](#) provide a unified interface to many popular machine learning algorithms in R. They consist of methods to train and predict a model for a [Task](#) and provide meta-information about the learners, such as the hyperparameters (which control the behavior of the learner) you can set.

The base class of each learner is [Learner](#), specialized for regression as [LearnerRegr](#) and for classification as [LearnerClassif](#). Other types of learners, provided by extension packages, also inherit from the [Learner](#) base class, e.g. [mlr3proba::LearnerSurv](#) or [mlr3cluster::LearnerClust](#).

All Learners work in a two-stage procedure:

- **Training stage:** The training data (features and target) is passed to the Learner's `$train()` function which trains and stores a model, i.e. the relationship of the target and features.

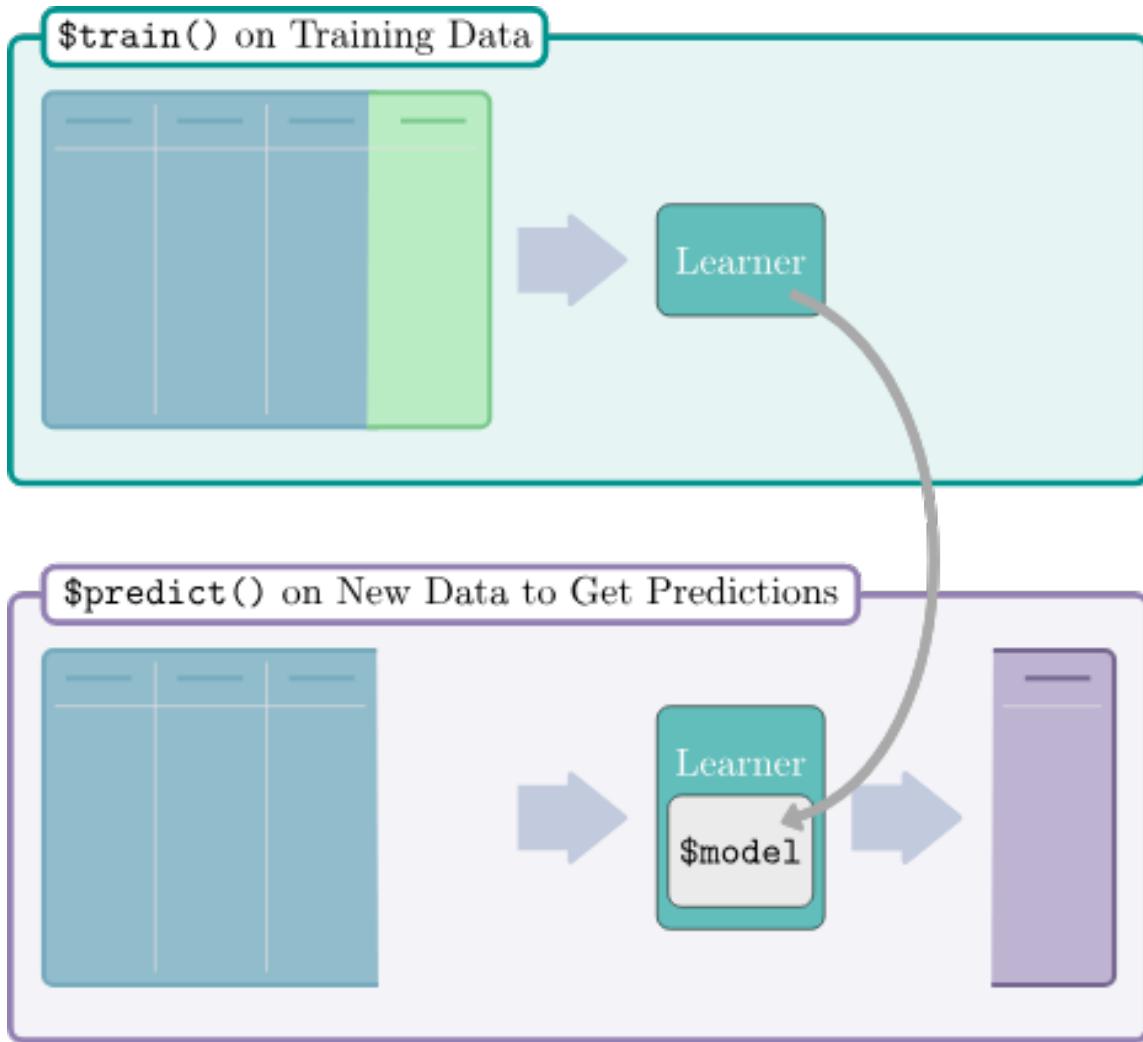


Figure 15.2: Two stages of a learner. Top: data (features and a target) are passed to an (untrained) learner. Bottom: new data are passed to the trained model which makes predictions for the ‘missing’ target column.

- **Predict stage:** The new data, usually a different slice of the original data than used for training, is passed to the `$predict()` method of the Learner. The model trained in the first step is used to predict the missing target, e.g. labels for classification problems or the numerical value for regression problems.

There are a number of [predefined learners](#). The `mlr3` package ships with the following set of classification and regression learners. We deliberately keep this small to avoid unnecessary dependencies:

- `classif.featureless`: Simple baseline classification learner. The default is to always predict the label that is most frequent in the training set. While this is not very useful by itself, it can be used as a “[fallback learner](#)” to make predictions in case another, more sophisticated, learner failed for some reason.
- `regr.featureless`: Simple baseline regression learner. The default is to always predict the mean of the target in training set. Similar to `mlr_learners_classif.featureless`, it makes for a good “[fallback learner](#)”
- `classif.rpart`: Single classification tree from package `rpart`.
- `regr.rpart`: Single regression tree from package `rpart`.

This set of baseline learners is usually insufficient for a real data analysis. Thus, we have cherry-picked implementations of the most popular machine learning method and collected them in the `mlr3learners` package:

- Linear (`regr.lm`) and logistic (`classif.log_reg`) regression
- Penalized Generalized Linear Models (`regr.glmnet`, `classif.glmnet`), possibly with built-in optimization of the penalization parameter (`regr.cv_glmnet`, `classif.cv_glmnet`)
- (Kernelized) k-Nearest Neighbors regression (`regr.kknn`) and classification (`classif.kknn`).
- Kriging / Gaussian Process Regression (`regr.km`)
- Linear (`classif.lda`) and Quadratic (`classif.qda`) Discriminant Analysis
- Naive Bayes Classification (`classif.naive_bayes`)
- Support-Vector machines (`regr.svm`, `classif.svm`)
- Gradient Boosting (`regr.xgboost`, `classif.xgboost`)
- Random Forests for regression and classification (`regr.ranger`, `classif.ranger`)

More machine learning methods and alternative implementations are collected in the [mlr3extralearners repository](#).

### 15.2.3 `mlr3` Workflow

1. Load data
2. Split data into training and test sets
3. Create a `task`.
4. Choose a `learner`. See `mlr_learners`.
5. Train
6. Predict
7. Assess
8. Interpret

### 15.3 Setup

```
library(mlr3verse)
library(GEOquery)
library(mlr3learners) # for knn
library(ranger) # for randomforest
set.seed(789)
```

---

### 15.4 Example 1: cancer types

In this exercise, we will be classifying cancer types based on gene expression data. The data we are going to access are from Brouwer-Visser et al. (2018).

#### 15.4.1 Data Preparation

Use the [GEOquery](#) package to fetch data about [GSE103512](#).

```
library(GEOquery)
gse = getGEO("GSE103512")[[1]]
```

The first step, a detail, is to convert from the older Bioconductor data structure (GEOquery was written in 2007), the ExpressionSet, to the newer SummarizedExperiment.

```
library(SummarizedExperiment)
se = as(gse, "SummarizedExperiment")
```

Examine two variables of interest, cancer type and tumor/normal status.

```
with(colData(se), table(`cancer.type.ch1`, `normal.ch1`))

normal.ch1
cancer.type.ch1 no yes
      BC     65   10
      CRC    57   12
      NSCLC  60    9
      PCA    60    7
```

Before embarking on a machine learning analysis, we need to make sure that we understand the data. Things like missing values, outliers, and other problems can cause problems for machine learning algorithms.

In R, plotting, summaries, and other exploratory data analysis tools are available. PCA analysis, clustering, and other methods can also be used to understand the data. It is worth spending time on this step, as it can save time later.

### 15.4.2 Feature selection and data cleaning

While we could use all genes in the analysis, we will select the most informative genes using the variance of gene expression across samples. Other methods for feature selection are available, including those based on correlation with the outcome variable.

#### ! Feature selection

Feature selection should be done on the training data only, not the test data to avoid overfitting.

Remember that the `apply` function applies a function to each row or column of a matrix. Here, we apply the `sd` function to each row of the expression matrix to get a vector of stan

```
sds = apply(assay(se, 'exprs'), 1, sd)
## filter out normal tissues
se_small = se[order(sds, decreasing = TRUE)[1:200],
             colData(se)$characteristics_ch1.1=='normal: no']
# remove genes with no gene symbol
se_small = se_small[rowData(se_small)$Gene.Symbol!='', ]
```

To make the data easier to work with, we will use the opportunity to use one of the `rowData` columns as the rownames of the data frame. The `make.names` function is used to make sure that the rownames are valid R variable names and unique.

```
## convert to matrix for later use
dat = assay(se_small, 'exprs')
rownames(dat) = make.names(rowData(se_small)$Gene.Symbol)
```

We also need to transpose the data so that the rows are the samples and the columns are the features in order to use the data with `mlr3`.

```
feat_dat = t(dat)
tumor = data.frame(tumor_type = colData(se_small)$cancer.type.ch1, feat_dat)
```

This is another good time to check the data. Make sure that the data is in the format that you expect. Check the dimensions, the column names, and the data types.

### 15.4.3 Creating the “task”

The first step in using `mlr3` is to create a `task`. A task is a data set with a target variable. In this case, the target variable is the cancer type. The `mlr3` package provides a function to convert a data frame into a task. These tasks can be used with any machine learning algorithm in `mlr3`.

```
tumor$tumor_type = as.factor(tumor$tumor_type)
task = as_task_classif(tumor, target='tumor_type')
```

Here, we randomly divide the data into 2/3 training data and 1/3 test data.

```
set.seed(7)
train_set = sample(task$row_ids, 0.67 * task$nrow)
```

```
test_set = setdiff(task$row_ids, train_set)
```

**!** Important

Training and testing on the same data is a common mistake. We want to test the model on data that it has not seen before. This is the only way to know if the model is overfitting.

#### 15.4.4 K-nearest-neighbor

The first model we will use is the k-nearest-neighbor model. This model is based on the idea that similar samples have similar outcomes. The number of neighbors to use is a parameter that can be tuned. We'll use the default value of 7, but you can try other values to see how they affect the results. In fact, mlr3 provides the ability to tune parameters automatically, but we won't cover that here.

##### 15.4.4.1 Create the learner

In mlr3, the machine learning algorithms are called learners. To create a learner, we use the `lrn` function. The `lrn` function takes the name of the learner as an argument. The `lrn` function also takes other arguments that are specific to the learner. In this case, we will use the default values for the arguments.

```
learner = lrn("classif.kknn")
```

You can get a list of all the learners available in mlr3 by using the `lrn()` function without any arguments.

```
lrn()
```

```
<DictionaryLearner> with 46 stored values
Keys: classif.cv_glmnet, classif.debug, classif.featureless,
       classif.glmnet, classif.kknn, classif.lda, classif.log_reg,
       classif.multinom, classif.naive_bayes, classif.nnet, classif.qda,
       classif.ranger, classif.rpart, classif.svm, classif.xgboost,
       clust.agnes, clust.ap, clust.cmeans, clust.cobweb, clust.dbscan,
       clust.diana, clust.em, clust.fanny, clust.featureless, clust.ff,
       clust.hclust, clust.kkmeans, clust.kmeans, clust.MBatchKMeans,
       clust.mclust, clust.meanshift, clust.pam, clust.SimpleKMeans,
       clust.xmeans, regr.cv_glmnet, regr.debug, regr.featureless,
       regr.glmnet, regr.kknn, regr.km, regr.lm, regr.nnet, regr.ranger,
       regr.rpart, regr.svm, regr.xgboost
```

##### 15.4.4.2 Train

To train the model, we use the `train` function. The `train` function takes the task and the row ids of the training data as arguments.

```
learner$train(task, row_ids = train_set)
```

Here, we can look at the trained model:

```
# output is large, so do this on your own
learner$model
```

#### 15.4.4.3 Predict

Lets use our trained model works to predict the classes of the **training** data. Of course, we already know the classes of the training data, but this is a good way to check that the model is working as expected. It also gives us a measure of performance on the training data that we can compare to the test data to look for overfitting.

```
pred_train = learner$predict(task, row_ids=train_set)
```

And check on the test data:

```
pred_test = learner$predict(task, row_ids=test_set)
```

#### 15.4.4.4 Assess

In this section, we can look at the accuracy and performance of our model on the training data and the test data. We can also look at the confusion matrix to see which classes are being confused with each other.

```
pred_train$confusion
```

		truth			
response	BC	CRC	NSCLC	PCA	
BC	42	0	0	0	
CRC	0	40	0	0	
NSCLC	1	0	44	0	
PCA	0	0	0	35	

This is a multi-class confusion matrix. The rows are the true classes and the columns are the predicted classes. The diagonal shows the number of samples that were correctly classified. The off-diagonal elements show the number of samples that were misclassified.

We can also look at the accuracy of the model on the training data and the test data. The accuracy is the number of correctly classified samples divided by the total number of samples.

```
measures = msrs(c('classif.acc'))
pred_train$score(measures)
```

```
classif.acc
0.9938272
```

```
pred_test$confusion
```

		truth			
response	BC	CRC	NSCLC	PCA	
BC	22	0	0	0	
CRC	0	17	1	0	
NSCLC	0	0	15	0	
PCA	0	0	0	25	

```
pred_test$score(measures)
```

```
classif.acc
0.9875
```

Compare the accuracy on the training data to the accuracy on the test data. Do you see any evidence of overfitting?

#### 15.4.5 Classification tree

We are going to use a classification tree to classify the data. A classification tree is a series of yes/no questions that are used to classify the data. The questions are based on the features in the data. The classification tree is built by finding the feature that best separates the data into the different classes. Then, the data is split based on the value of that feature. The process is repeated until the data is completely separated into the different classes.

##### 15.4.5.1 Train

```
# in this case, we want to keep the model
# so we can look at it later
learner = lrn("classif.rpart", keep_model = TRUE)

learner$train(task, row_ids = train_set)
```

We can take a look at the model.

```
learner$model

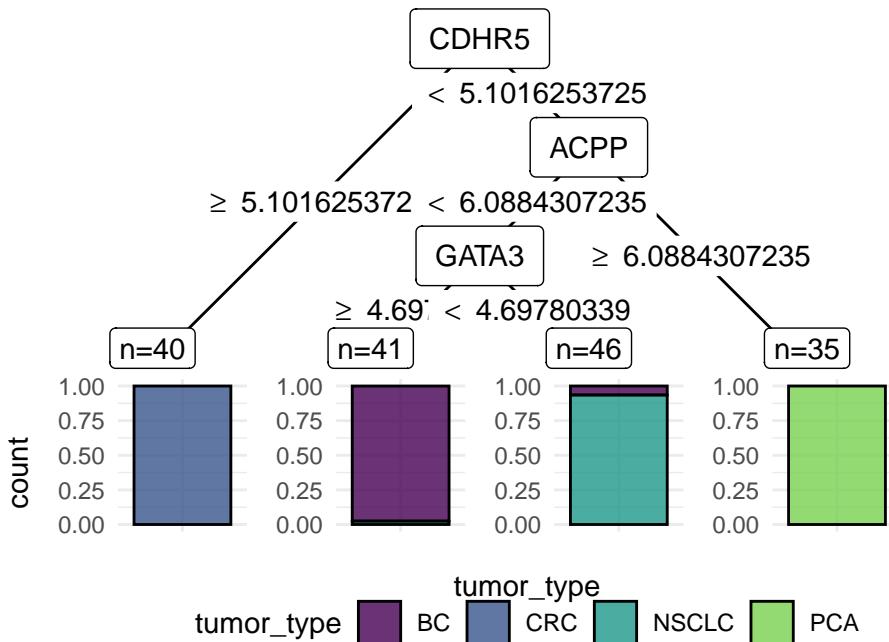
n= 162

node), split, n, loss, yval, (yprob)
 * denotes terminal node

1) root 162 118 NSCLC (0.26543210 0.24691358 0.27160494 0.21604938)
  2) CDHR5>=5.101625 40    0 CRC (0.00000000 1.00000000 0.00000000 0.00000000) *
  3) CDHR5< 5.101625 122   78 NSCLC (0.35245902 0.00000000 0.36065574 0.28688525)
     6) ACPP< 6.088431 87    43 NSCLC (0.49425287 0.00000000 0.50574713 0.00000000)
        12) GATA3>=4.697803 41    1 BC (0.97560976 0.00000000 0.02439024 0.00000000) *
        13) GATA3< 4.697803 46    3 NSCLC (0.06521739 0.00000000 0.93478261 0.00000000) *
     7) ACPP>=6.088431 35    0 PCA (0.00000000 0.00000000 0.00000000 1.00000000) *
```

Decision trees are easy to visualize if they are small. Here, we can see that the tree is very simple, with only two splits.

```
autoplot(learner)
```



#### 15.4.5.2 Predict

Now that we have trained the model on the *training* data, we can use it to predict the classes of the training data and the test data. The `$predict` method takes a task and produces a prediction based on the *trained* model, in this case, called `learner`.

```
pred_train = learner$predict(task, row_ids=train_set)
```

Remember that we split the data into training and test sets. We can use the trained model to predict the classes of the test data. Since the *test* data was not used to train the model, it is not “cheating” like what we just did where we did the prediction on the *training* data.

```
pred_test = learner$predict(task, row_ids=test_set)
```

#### 15.4.5.3 Assess

For classification tasks, we often look at a confusion matrix of the *truth* vs the *predicted* classes for the samples.

**! Important**

Assessing the performance of a model should **always** be **reported** from assessment on an independent test set.

```
pred_train$confusion
```

truth

response	BC	CRC	NSCLC	PCA
BC	40	0	1	0
CRC	0	40	0	0
NSCLC	3	0	43	0
PCA	0	0	0	35

- What does this confusion matrix tell you?

We can also ask for several “measures” of the performance of the model. Here, we ask for the accuracy of the model. To get a complete list of measures, use `msr()`.

```
measures = msrs(c('classif.acc'))
pred_train$score(measures)
```

```
classif.acc
0.9753086
```

- How does the accuracy compare to the confusion matrix?
- How does this accuracy compare to the accuracy of the k-nearest-neighbor model?
- How about the decision tree model?

```
pred_test$confusion
```

response	BC	CRC	NSCLC	PCA
BC	20	0	1	0
CRC	0	17	3	0
NSCLC	2	0	12	0
PCA	0	0	0	25

```
pred_test$score(measures)
```

```
classif.acc
0.925
```

- What does the confusion matrix in the *test* set tell you?
- How do the assessments of the *test* and *training* sets differ?

### 💡 Overfitting

When the assessment of the test set is worse than the evaluation of the training set, the model may be *overfit*. How to address overfitting varies by model type, but it is a sign that you should pay attention to model selection and parameters.

#### 15.4.6 RandomForest

```
learner = lrn("classif.ranger", importance = "impurity")
```

#### 15.4.6.1 Train

```
learner$train(task, row_ids = train_set)
```

Again, you can look at the model that was trained.

```
learner$model
```

Ranger result

Call:

```
ranger::ranger(dependent.variable.name = task$target_names, data = task$data(), probability = self$predic
```

Type:	Classification
Number of trees:	500
Sample size:	162
Number of independent variables:	192
Mtry:	13
Target node size:	1
Variable importance mode:	impurity
Splitrule:	gini
OOB prediction error:	0.62 %

For more details, the mlr3 random forest approach is based on the ranger package. You can look at the ranger documentation.

- What is the OOB error in the output?

Random forests are a collection of decision trees. Since predictors enter the trees in a random order, the trees are different from each other. The random forest procedure gives us a measure of the “importance” of each variable.

```
head(learner$importance(), 15)
```

CDHR5	TRPS1.1	FABP1	EPS8L3	KRT20	EFHD1	LGALS4	TRPS1
4.791870	3.918063	3.692649	3.651422	3.340382	3.314491	2.952969	2.926175
SFTPB	SFTPB.1	GATA3	GATA3.1	TMPRSS2	MUC12	POF1B	
2.805811	2.681004	2.344603	2.271845	2.248734	2.207347	1.806906	

More “important” variables are those that are more often used in the trees. Are the most important variables the same as the ones that were important in the decision tree?

If you are interested, look up a few of the important variables in the model to see if they make biological sense.

#### 15.4.6.2 Predict

Again, we can use the trained model to predict the classes of the training data and the test data.

```
pred_train = learner$predict(task, row_ids=train_set)
```

```
pred_test = learner$predict(task, row_ids=test_set)
```

#### 15.4.6.3 Assess

```
pred_train$confusion
```

truth	BC	CRC	NSCLC	PCA
BC	43	0	0	0
CRC	0	40	0	0
NSCLC	0	0	44	0
PCA	0	0	0	35

```
measures = msrs(c('classif.acc'))  
pred_train$score(measures)
```

```
classif.acc
```

```
1
```

```
pred_test$confusion
```

truth	BC	CRC	NSCLC	PCA
BC	22	0	0	0
CRC	0	17	0	0
NSCLC	0	0	16	0
PCA	0	0	0	25

```
pred_test$score(measures)
```

```
classif.acc
```

```
1
```

---

## 15.5 Exercise: Predicting age from DNA methylation

We will be building a regression model for chronological age prediction, based on DNA methylation. This is based on the work of [Jana Naue et al. 2017](#), in which biomarkers are examined to predict the chronological age of humans by analyzing the DNA methylation patterns. Different machine learning algorithms are used in this study to make an age prediction.

It has been recognized that within each individual, the level of [DNA methylation](#) changes with age. This knowledge is used to select useful biomarkers from DNA methylation datasets. The [CpG sites](#) with the highest correlation to age are selected as the biomarkers (and therefore features for building a regression model). In this tutorial, specific biomarkers are analyzed by machine learning algorithms to create an age prediction model.

The data are taken from [this tutorial](#).

```
library(data.table)
meth_age = rbind(
  fread('https://zenodo.org/record/2545213/files/test_rows_labels.csv'),
  fread('https://zenodo.org/record/2545213/files/train_rows.csv')
)
```

Let's take a quick look at the data.

```
head(meth_age)
```

	RPA2_3	ZYG11A_4	F5_2	HOXC4_1	NKIRAS2_2	MEIS1_1	SAMD10_2	GRM2_9	TRIM59_5
1:	65.96	18.08	41.57	55.46	30.69	63.42	40.86	68.88	44.32
2:	66.83	20.27	40.55	49.67	29.53	30.47	37.73	53.30	50.09
3:	50.30	11.74	40.17	33.85	23.39	58.83	38.84	35.08	35.90
4:	65.54	15.56	33.56	36.79	20.23	56.39	41.75	50.37	41.46
5:	59.01	14.38	41.95	30.30	24.99	54.40	37.38	30.35	31.28
6:	81.30	14.68	35.91	50.20	26.57	32.37	32.30	55.19	42.21
	LDB2_3	ELOVL2_6	DDO_1	KLF14_2	Age				
1:	56.17	62.29	40.99	2.30	40				
2:	58.40	61.10	49.73	1.07	44				
3:	58.81	50.38	63.03	0.95	28				
4:	58.05	50.58	62.13	1.99	37				
5:	65.80	48.74	41.88	0.90	24				
6:	70.15	61.36	33.62	1.87	43				

As before, we create the task object, but this time we use `as_task_regr()` to create a regression task.

- Why is this a regression task?

```
task = as_task_regr(meth_age, target = 'Age')
```

```
set.seed(7)
train_set = sample(task$row_ids, 0.67 * task$nrow)
test_set = setdiff(task$row_ids, train_set)
```

### 15.5.1 Linear regression

We will start with a simple linear regression model.

```
learner = lrn("regr.lm")
```

#### 15.5.1.1 Train

```
learner$train(task, row_ids = train_set)
```

When you train a linear regression model, we can evaluate some of the diagnostic plots to see if the model is appropriate (Figure 15.3).

```
par(mfrow=c(2,2))
plot(learner$model)
```

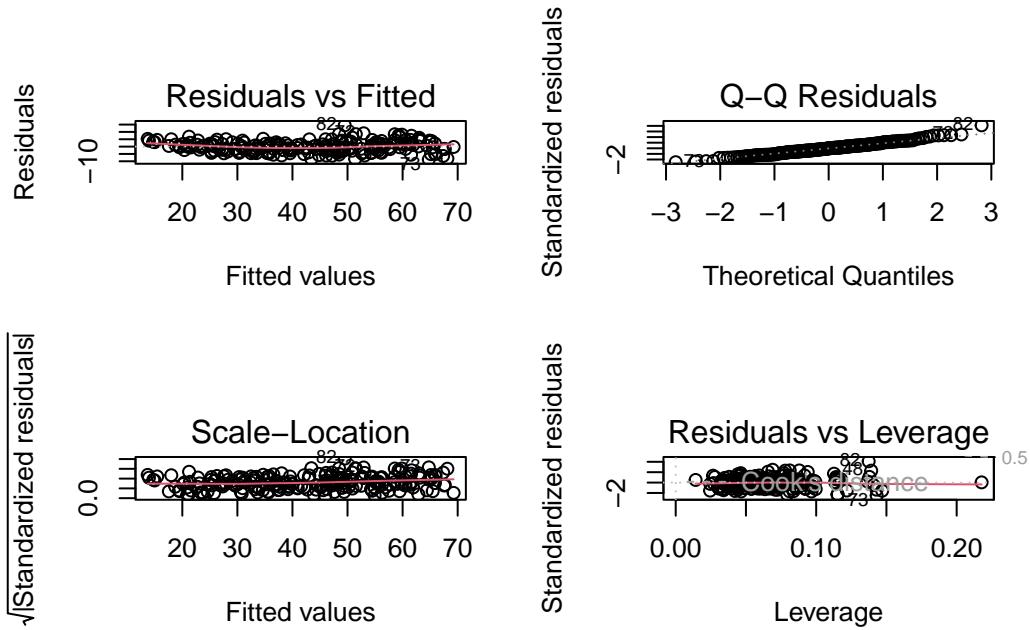


Figure 15.3: Regression diagnostic plots. The top left plot shows the residuals vs. fitted values. The top right plot shows the normal Q-Q plot. The bottom left plot shows the scale-location plot. The bottom right plot shows the residuals vs. leverage.

#### 15.5.1.2 Predict

```
pred_train = learner$predict(task, row_ids=train_set)

pred_test = learner$predict(task, row_ids=test_set)
```

#### 15.5.1.3 Assess

```
pred_train
```

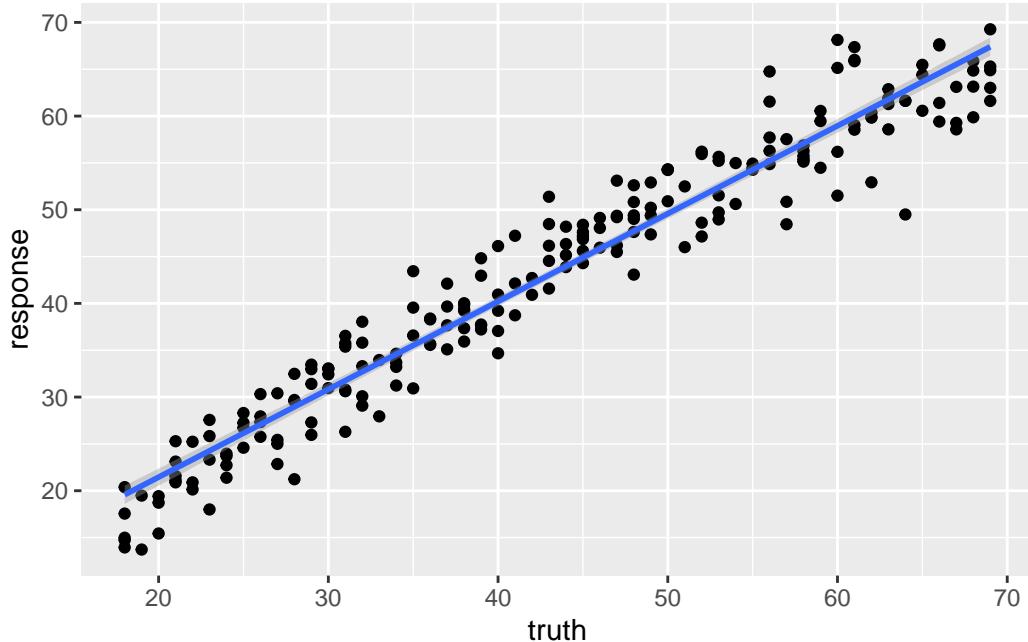
```
<PredictionRegr> for 209 observations:
  row_ids  truth response
    298     29 31.40565
    103     58 56.26019
    194     53 48.96480
  ---
    312     48 52.61195
    246     66 67.66312
```

```
238     38 39.38414
```

We can plot the relationship between the truth and response, or predicted value to see visually how our model performs.

```
library(ggplot2)
ggplot(pred_train,aes(x=truth, y=response)) +
  geom_point() +
  geom_smooth(method='lm')

`geom_smooth()` using formula = 'y ~ x'
```



We can use the r-squared of the fit to roughly compare two models.

```
measures = msrs(c('regr.rsq'))
pred_train$score(measures)
```

```
regr.rsq
0.9376672
```

```
pred_test
```

```
<PredictionRegr> for 103 observations:
  row_ids truth response
    4      37 37.64301
    5      24 28.34777
    7      34 33.22419
```

```
---
  306     42 41.65864
  307     63 58.68486
  309     68 70.41987
pred_test$score(measures)

regr.rsq
0.9363526
```

### 15.5.2 Regression tree

```
learner = lrn("regr.rpart", keep_model = TRUE)
```

#### 15.5.2.1 Train

```
learner$train(task, row_ids = train_set)

learner$model

n= 209

node), split, n, deviance, yval
      * denotes terminal node

1) root 209 45441.4500 43.27273
  2) ELOVL2_6< 56.675 98  5512.1220 30.24490
    4) ELOVL2_6< 47.24 47   866.4255 24.23404
      8) GRM2_9< 31.3 34   289.0588 22.29412 *
      9) GRM2_9>=31.3 13   114.7692 29.30769 *
    5) ELOVL2_6>=47.24 51   1382.6270 35.78431
    10) F5_2>=39.295 35   473.1429 33.28571 *
    11) F5_2< 39.295 16   213.0000 41.25000 *
  3) ELOVL2_6>=56.675 111  8611.3690 54.77477
    6) ELOVL2_6< 65.365 63   3101.2700 49.41270
    12) KLF14_2< 3.415 37   1059.0270 46.16216 *
    13) KLF14_2>=3.415 26   1094.9620 54.03846 *
    7) ELOVL2_6>=65.365 48   1321.3120 61.81250 *
```

What is odd about using a regression tree here is that we end up with only a few discrete estimates of age. Each “leaf” has a value.

#### 15.5.2.2 Predict

```
pred_train = learner$predict(task, row_ids=train_set)
```

```
pred_test = learner$predict(task, row_ids=test_set)
```

### 15.5.2.3 Assess

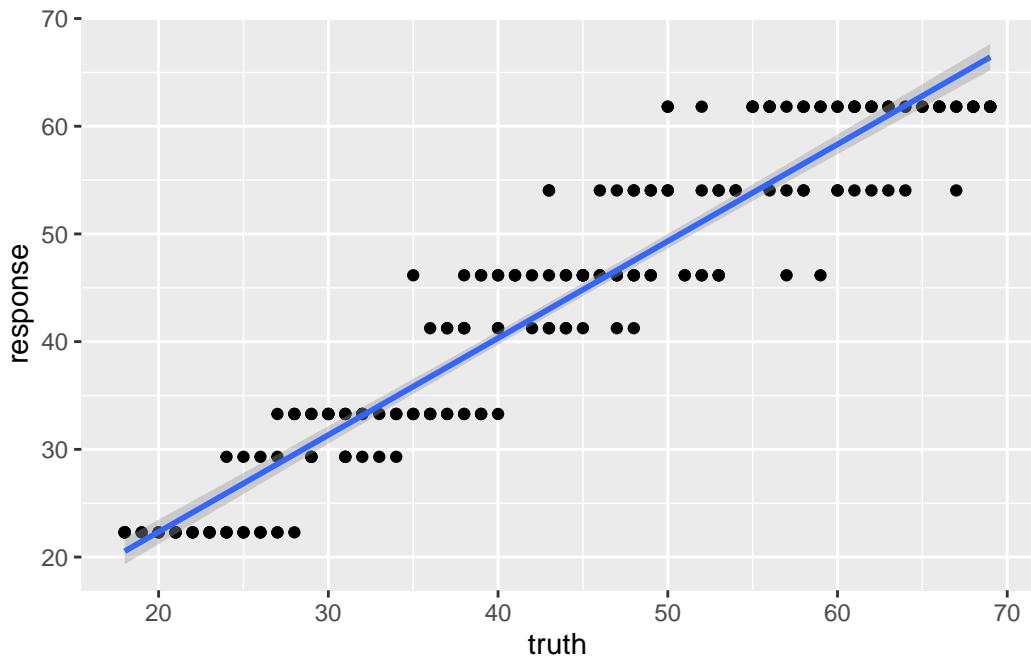
```
pred_train
```

<PredictionRegr> for 209 observations:

row_ids	truth	response
298	29	33.28571
103	58	61.81250
194	53	46.16216
---		
312	48	54.03846
246	66	61.81250
238	38	41.25000

We can see the effect of the discrete values much more clearly here.

```
library(ggplot2)
ggplot(pred_train,aes(x=truth, y=response)) +
  geom_point() +
  geom_smooth(method='lm')
`geom_smooth()` using formula = 'y ~ x'
```



And the r-squared values for this model prediction shows quite a bit of difference from the linear regression above.

```
measures = msrs(c('regr.rsq'))
pred_train$score(measures)

  regr.rsq
0.8995351

pred_test

<PredictionRegr> for 103 observations:
  row_ids  truth response
    4      37 41.25000
    5      24 33.28571
    7      34 33.28571
  ---
  306     42 46.16216
  307     63 61.81250
  309     68 61.81250

pred_test$score(measures)

  regr.rsq
0.8545402
```

### 15.5.3 RandomForest

Randomforest is also tree-based, but unlike the single regression tree above, randomforest is a “forest” of trees which will eliminate the discrete nature of a single tree.

```
learner = lrn("regr.ranger", mtry=2, min.node.size=20)
```

#### 15.5.3.1 Train

```
learner$train(task, row_ids = train_set)
```

```
learner$model
```

Ranger result

Call:

```
ranger::ranger(dependent.variable.name = task$target_names, data = task$data(), case.weights = task$weights)
```

Type:	Regression
Number of trees:	500
Sample size:	209
Number of independent variables:	13
Mtry:	2

```
Target node size: 20
Variable importance mode: none
Splitrule: variance
OOB prediction error (MSE): 18.84172
R squared (OOB): 0.9137554
```

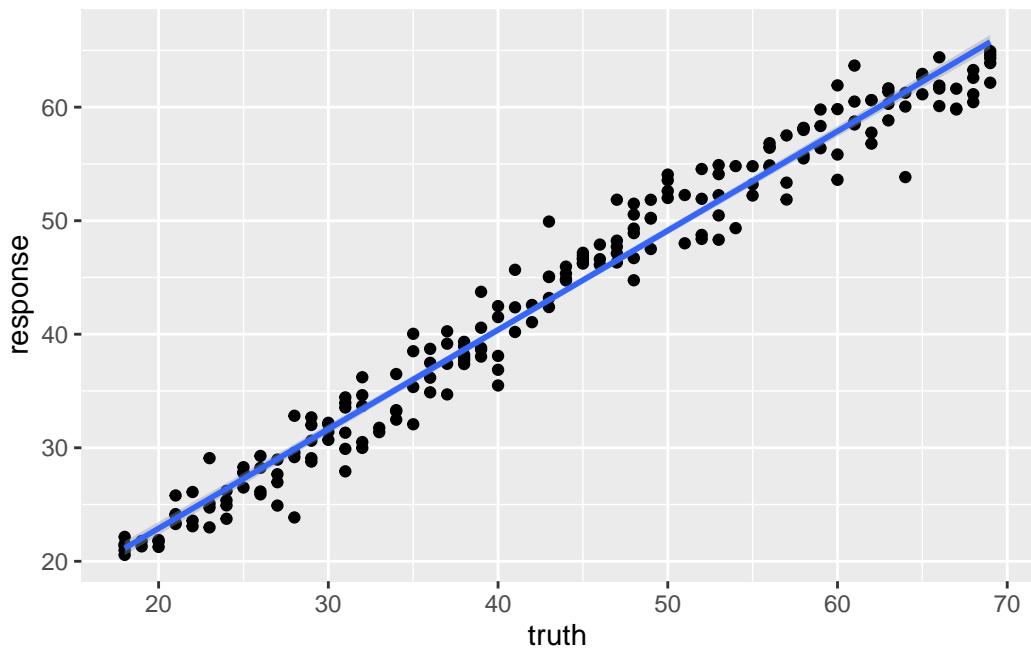
### 15.5.3.2 Predict

```
pred_train = learner$predict(task, row_ids=train_set)
pred_test = learner$predict(task, row_ids=test_set)
```

### 15.5.3.3 Assess

```
pred_train
```

```
<PredictionRegr> for 209 observations:
  row_ids truth response
    298    29 30.62154
    103    58 57.99398
    194    53 48.32491
    ---
    312    48 51.49846
    246    66 64.39315
    238    38 38.18038
ggplot(pred_train,aes(x=truth, y=response)) +
  geom_point() +
  geom_smooth(method='lm')
`geom_smooth()` using formula = 'y ~ x'
```



```
measures = msrs(c('regr.rsq'))  
pred_train$score(measures)
```

```
regr.rsq  
0.9609739
```

```
pred_test
```

```
<PredictionRegr> for 103 observations:  
  row_ids truth response  
    4      37 37.79631  
    5      24 29.18371  
    7      34 33.26780  
---  
  306     42 40.27428  
  307     63 58.26534  
  309     68 63.15481
```

```
pred_test$score(measures)
```

```
regr.rsq  
0.9208186
```

---

## 15.6 Expression prediction from histone modification data

In this little set of exercises, you will be using histone marks near a gene to predict its expression.

$$y = h_1 + h_2 + h_3 + \dots \quad (15.1)$$

We will try a couple of different approaches:

1. Penalized regression
2. RandomForest

### 15.6.1 The Data

The data in this

```
fullFeatureSet <- read.table("http://seandavi.github.io/ITR/expression-prediction/features.txt");
```

What are the column names of the predictor variables?

```
colnames(fullFeatureSet)
```

```
[1] "Control"    "Dnase"       "H2az"        "H3k27ac"     "H3k27me3"   "H3k36me3"
[7] "H3k4me1"    "H3k4me2"    "H3k4me3"    "H3k79me2"   "H3k9ac"      "H3k9me1"
[13] "H3k9me3"   "H4k20me1"
```

These are going to be predictors combined into a model. Some of our learners will rely on predictors being on a similar scale. Are our data already there?

To perform centering and scaling by column, we can convert to a matrix and then use `scale`.

```
par(mfrow=c(1, 2))
scaled_features <- scale(as.matrix(fullFeatureSet))
boxplot(fullFeatureSet, title='Original data')
boxplot(scaled_features, title='Centered and scaled data')
```

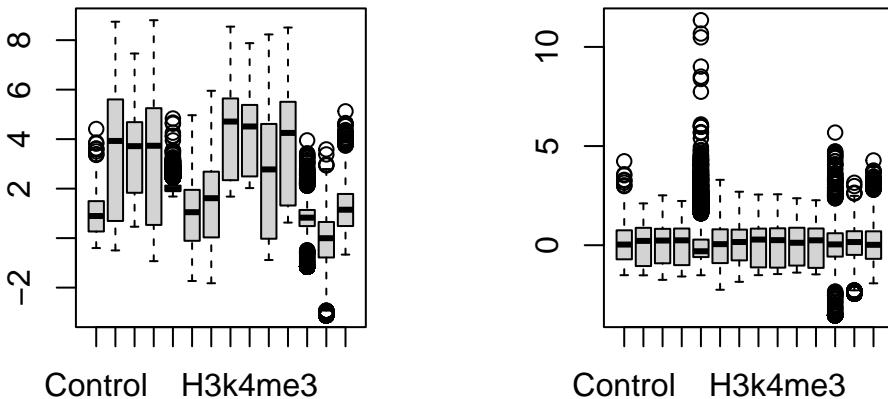


Figure 15.4: Boxplots of original and scaled data.

There is a row for each gene and a column for each histone mark and we can see that the data are centered and scaled by column. We can also see some patterns in the data (see Figure 15.5).

```
sampled_features <- fullFeatureSet[sample(nrow(scaled_features), 500),]
library(ComplexHeatmap)
```

```
Loading required package: grid
=====
ComplexHeatmap version 2.16.0
Bioconductor page: http://bioconductor.org/packages/ComplexHeatmap/
Github page: https://github.com/jokergoo/ComplexHeatmap
Documentation: http://jokergoo.github.io/ComplexHeatmap-reference
```

If you use it in published research, please cite either one:  
 - Gu, Z. Complex Heatmap Visualization. *iMeta* 2022.  
 - Gu, Z. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. *Bioinformatics* 2016.

The new `InteractiveComplexHeatmap` package can directly export static complex heatmaps into an interactive Shiny app with zero effort. Have a try!

This message can be suppressed by:  
`suppressPackageStartupMessages(library(ComplexHeatmap))`  
 ======  
`Heatmap(sampled_features, name='histone marks', show_row_names=FALSE)`

Warning: The input is a data frame-like object, convert it to a matrix.

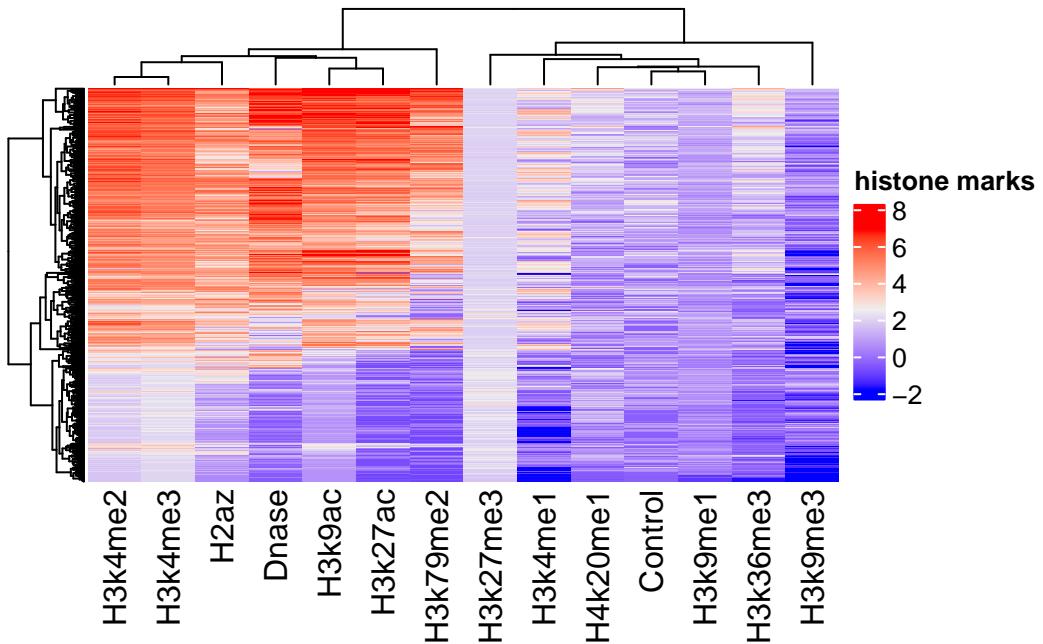


Figure 15.5: Heatmap of 500 randomly sampled rows of the data. Columns are histone marks and there is a row for each gene.

Now, we can read in the associated gene expression measures that will become our “target” for prediction.

```
target <- scan(url("http://seandavi.github.io/ITR/expression-prediction/target.txt"), skip=1)
# make into a data frame
exp_pred_data <- data.frame(gene_expression=target, scaled_features)
```

And the first few rows of the target data frame using:

```
head(exp_pred_data, 3)
```

	gene_expression	Control	Dnase	H2az
ENSG00000000419.7.49575069	6.082343	0.7452926	0.7575546	1.0728432
ENSG00000000457.8.169863093	2.989145	1.9509786	1.0216546	0.3702787
ENSG00000000938.7.27961645	-5.058894	-0.3505542	-1.4482958	-1.0390775
	H3k27ac	H3k27me3	H3k36me3	H3k4me1
ENSG00000000419.7.49575069	1.0950440	-0.5125312	1.1334793	0.4127984
ENSG00000000457.8.169863093	0.7142157	-0.4079244	0.8739005	1.1649282
ENSG00000000938.7.27961645	-1.0173283	1.4117293	-0.5157582	-0.5017450
	H3k4me2	H3k4me3	H3k79me2	H3k9ac
ENSG00000000419.7.49575069	1.2136176	1.1202901	1.5155803	1.2468256
ENSG00000000457.8.169863093	0.6456572	0.6508561	0.7976487	0.5792891
ENSG00000000938.7.27961645	-0.1878255	-0.6560973	-1.3803974	-1.0067972
	H3k9me1	H3k9me3	H4k20me1	

```
ENSG00000000419 7.49575069 0.1426980 1.185622 1.9599992
ENSG00000000457 8.169863093 0.3630902 1.014923 -0.2695111
ENSG00000000938 7.27961645 0.6564520 -1.370871 -1.8773178
```

### 15.6.2 Create task

```
exp_pred_task = as_task_regr(exp_pred_data, target='gene_expression')
```

Partition the data into test and training sets. We will use  $\frac{1}{3}$  and  $\frac{2}{3}$  of the data for testing.

```
split = partition(exp_pred_task)
```

### 15.6.3 Linear regression

```
learner = lrn("regr.lm")
```

#### 15.6.3.1 Train

```
learner$train(exp_pred_task, split$train)
```

#### 15.6.3.2 Predict

```
pred_train = learner$predict(exp_pred_task, split$train)
pred_test = learner$predict(exp_pred_task, split$test)
```

#### 15.6.3.3 Assess

```
pred_train
```

```
<PredictionRegr> for 5789 observations:
  row_ids      truth response
    1   6.082343 5.139251
    2   2.989145 2.909552
    7   5.838076 4.563759
  ---
  8543  9.016443 6.141272
  8583  7.475697 2.543423
  8618 10.049236 5.523896
```

For the training data:

```
measures = msrs(c('regr.rsq'))
pred_train$score(measures)
```

```
regr.rsq
0.7495474
```

And the test data:

```
pred_test$score(measures)
```

```
regr.rsq
0.7526609
```

#### 15.6.4 Penalized regression

Recall that we can use penalized regression to select the most important predictors from a large set of predictors. In this case, we will use the `glmnet` package to perform penalized regression, but we will use the `mlr` interface to `glmnet` to make it easier to use.

```
learner = lrn("regr.cv_glmnet", nfolds=10, alpha=1)
```

##### 15.6.4.1 Train

```
learner$train(exp_pred_task)

measures = msrs(c('regr.rsq'))
pred_train$score(measures)

regr.rsq
0.7495474
```

In the case of the penalized regression, we can also look at the coefficients of the model.

```
coef(learner$model1)
```

```
15 x 1 sparse Matrix of class "dgCMatrix"
      s1
(Intercept) 0.10173828
Control     -0.06138687
Dnase       1.15560095
H2az        0.25382598
H3k27ac    .
H3k27me3   -0.17000065
H3k36me3   0.67803937
H3k4me1    -0.06934505
H3k4me2    .
H3k4me3    0.22513201
H3k79me2   1.47587175
H3k9ac     0.51449187
H3k9me1    -0.11580672
H3k9me3    -0.17270444
H4k20me1   .
```

Note that the coefficients are all zero for the histone marks that were not selected by the model. In this case, we can use the model not to predict new data, but to help us understand the data.

## 15.7 Cross-validation

```
as.data.table(mlr_resamplings)
```

	key	label	params	iters
1:	bootstrap	Bootstrap	ratio,repeats	30
2:	custom	Custom Splits		NA
3:	custom_cv	Custom Split	Cross-Validation	NA
4:	cv	Cross-Validation	folds	10
5:	holdout	Holdout	ratio	1
6:	insample	Insample Resampling		1
7:	loo	Leave-One-Out		NA
8:	repeated_cv	Repeated Cross-Validation	folds,repeats	100
9:	subsampling	Subsampling	ratio,repeats	30

**Part V**

**Bioconductor**

## 16

---

### *Accessing and working with public omics data*

---

# 17

---

## *The data*

---

The data we are going to access are from [this paper](#).

Background: The tumor microenvironment is an important factor in cancer immunotherapy response. To further understand how a tumor affects the local immune system, we analyzed immune gene expression differences between matching normal and tumor tissue. Methods: We analyzed public and new gene expression data from solid cancers and isolated immune cell populations. We also determined the correlation between CD8, FoxP3 IHC, and our gene signatures. Results: We observed that regulatory T cells (Tregs) were one of the main drivers of immune gene expression differences between normal and tumor tissue. A tumor-specific CD8 signature was slightly lower in tumor tissue compared with normal of most (12 of 16) cancers, whereas a Treg signature was higher in tumor tissue of all cancers except liver. Clustering by Treg signature found two groups in colorectal cancer datasets. The high Treg cluster had more samples that were consensus molecular subtype 1/4, right-sided, and microsatellite-unstable, compared with the low Treg cluster. Finally, we found that the correlation between signature and IHC was low in our small dataset, but samples in the high Treg cluster had significantly more CD8+ and FoxP3+ cells compared with the low Treg cluster. Conclusions: Treg gene expression is highly indicative of the overall tumor immune environment. Impact: In comparison with the consensus molecular subtype and microsatellite status, the Treg signature identifies more colorectal tumors with high immune activation that may benefit from cancer immunotherapy.

In this little exercise, we will:

1. Access public omics data using the GEOquery package
  2. Get an opportunity to work with another SummarizedExperiment object.
  3. Perform a simple unsupervised analysis to visualize these public data.
- 

### 17.1 GEOquery to multidimensional scaling

The first step is to install the R package [GEOquery](#). This package allows us to access data from the Gene Expression Omnibus (GEO) database. GEO is a public repository of omics data.

```
BiocManager::install("GEOquery")
```

GEOquery has only one commonly used function, `getGEO()` which takes a GEO accession number as an argument. The GEO accession number is a unique identifier for a dataset.

Use the [GEOquery](#) package to fetch data about [GSE103512](#).

```
library(GEOquery)
gse = getGEO("GSE103512")[[1]]
```

The first step, a detail, is to convert from the older Bioconductor data structure (GEOquery was written in 2007), the ExpressionSet, to the newer SummarizedExperiment.

```
library(SummarizedExperiment)
se = as(gse, "SummarizedExperiment")
```

- What is the class of se?
- What are the dimensions of se?
- What are the dimensions of the assay slot of se?
- What are the dimensions of the colData slot of se?
- What variables are in the colData slot of se?

Examine two variables of interest, cancer type and tumor/normal status. The with function is a convenience to allow us to access variables in a data frame by name (rather than having to do dataframename\$variable\_name).

```
with(colData(se), table(`cancer.type.ch1`, `normal.ch1`))
```

	normal.ch1
cancer.type.ch1	no yes
BC	65 10
CRC	57 12
NSCLC	60 9
PCA	60 7

Filter gene expression by variance to find most informative genes. It is common practice to filter genes by standard deviation or some other measure of variability and keep the top X percent of them when performing dimensionality reduction. There is not a single right answer to what percentage to use, so try a few to see what happens.

In the example code, I chose to use the top 500 genes by standard deviation.

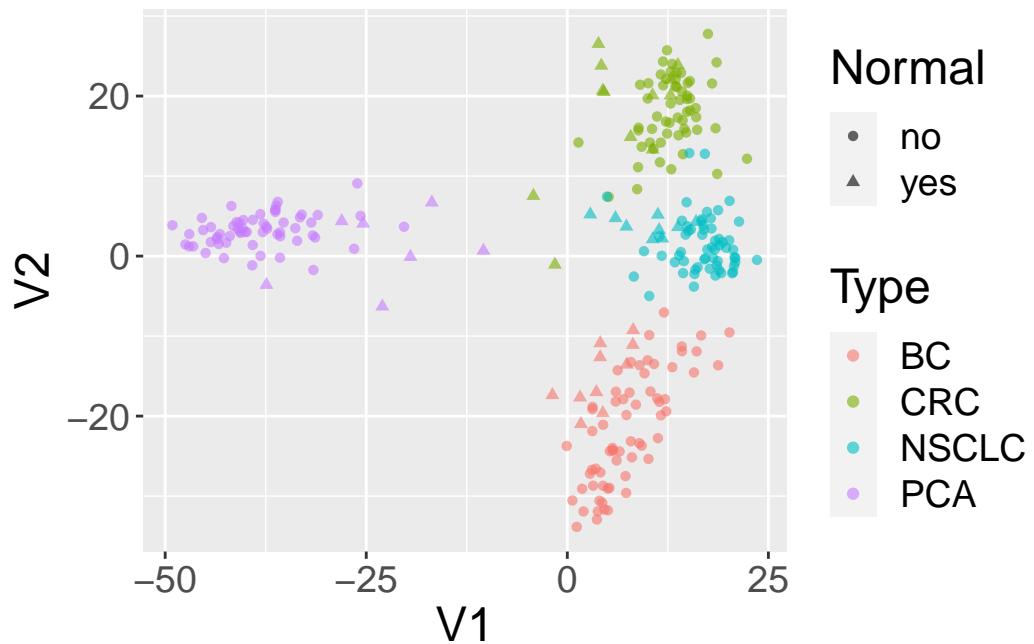
```
sds = apply(assay(se, 'exprs'), 1, sd)
dat = assay(se, 'exprs')[order(sds, decreasing = TRUE)[1:500], ]
```

Perform **multidimensional scaling** and prepare for plotting. We will be using ggplot2, so we need to make a data.frame before plotting.

```
mdsvals = cmdscale(dist(t(dat)))
mdsvals = as.data.frame(mdsvals)
mdsvals$Type=factor(colData(se)[, 'cancer.type.ch1'])
mdsvals$Normal = factor(colData(se)[, 'normal.ch1'])
```

And do the plot.

```
library(ggplot2)
ggplot(mdsvals, aes(x=V1,y=V2,shape=Normal,color=Type)) +
  geom_point( alpha=0.6) + theme(text=element_text(size = 18))
```



- What do you see?

# 18

---

## *Introduction to SummarizedExperiment*

---

The `SummarizedExperiment` class is used to store rectangular matrices of experimental results, which are commonly produced by sequencing and microarray experiments. Each object stores observations of one or more samples, along with additional meta-data describing both the observations (features) and samples (phenotypes).

A key aspect of the `SummarizedExperiment` class is the coordination of the meta-data and assays when subsetting. For example, if you want to exclude a given sample you can do for both the meta-data and assay in one operation, which ensures the meta-data and observed data will remain in sync. Improperly accounting for meta and observational data has resulted in a number of incorrect results and retractions so this is a very desirable property.

`SummarizedExperiment` is in many ways similar to the historical `ExpressionSet`, the main distinction being that `SummarizedExperiment` is more flexible in its row information, allowing both `GRanges` based as well as those described by arbitrary `DataFrames`. This makes it ideally suited to a variety of experiments, particularly sequencing based experiments such as RNA-Seq and ChIP-Seq.

```
BiocManager::install('airway')
BiocManager::install('SummarizedExperiment')
```

---

### 18.1 Anatomy of a `SummarizedExperiment`

The `SummarizedExperiment` package contains two classes: `SummarizedExperiment` and `RangedSummarizedExperiment`.

`SummarizedExperiment` is a matrix-like container where rows represent features of interest (e.g. genes, transcripts, exons, etc.) and columns represent samples. The objects contain one or more assays, each represented by a matrix-like object of numeric or other mode. The rows of a `SummarizedExperiment` object represent features of interest. Information about these features is stored in a `DataFrame` object, accessible using the function `rowData()`. Each row of the `DataFrame` provides information on the feature in the corresponding row of the `SummarizedExperiment` object. Columns of the `DataFrame` represent different attributes of the features of interest, e.g., gene or transcript IDs, etc.

`RangedSummarizedExperiment` is the “child” of the `SummarizedExperiment` class which means that all the methods on `SummarizedExperiment` also work on a `RangedSummarizedExperiment`.

The fundamental difference between the two classes is that the rows of a `RangedSummarizedExperiment`

object represent genomic ranges of interest instead of a DataFrame of features. The RangedSummarizedExperiment ranges are described by a GRanges or a GRangesList object, accessible using the `rowRanges()` function.

Figure 18.1 displays the class geometry and highlights the vertical (column) and horizontal (row) relationships.

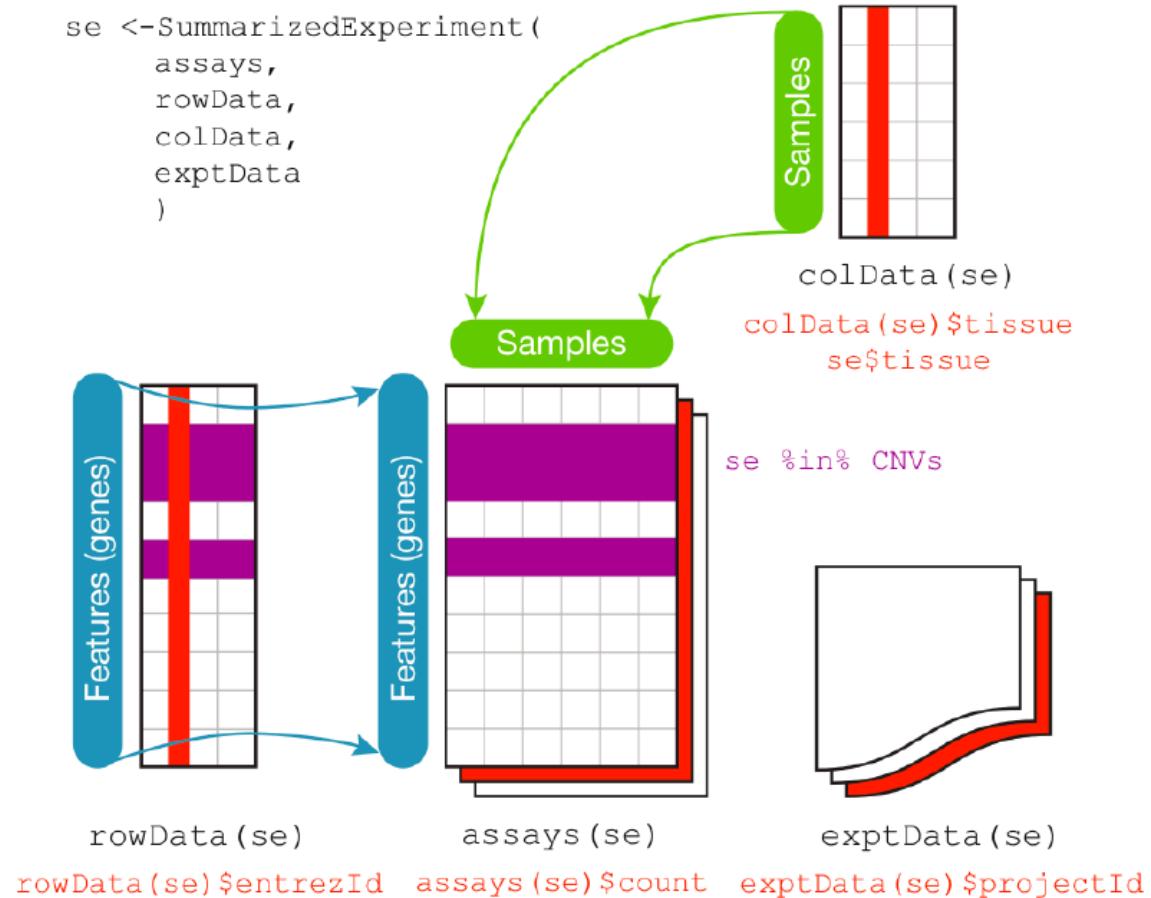


Figure 18.1: Summarized Experiment. There are three main components, the `colData()`, the `rowData()` and the `assays()`. The accessors for the various parts of a complete `SummarizedExperiment` object match the names.

### 18.1.1 Assays

The airway package contains an example dataset from an RNA-Seq experiment of read counts per gene for airway smooth muscles. These data are stored in a `RangedSummarizedExperiment` object which contains 8 different experimental and assays 64,102 gene transcripts.

Loading required package: airway

```
library(SummarizedExperiment)
data(airway, package="airway")
se <- airway
se

class: RangedSummarizedExperiment
dim: 63677 8
metadata(1): ''
assays(1): counts
rownames(63677): ENSG00000000003 ENSG00000000005 ... ENSG00000273492
  ENSG00000273493
rowData names(10): gene_id gene_name ... seq_coord_system symbol
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(9): SampleName cell ... Sample BioSample
```

To retrieve the experiment data from a SummarizedExperiment object one can use the assays() accessor. An object can have multiple assay datasets each of which can be accessed using the \$ operator. The airway dataset contains only one assay (counts). Here each row represents a gene transcript and each column one of the samples.

```
assays(se)$counts
```

	SRR1039508	SRR1039509	SRR1039512	SRR1039513	SRR1039516	SRR1039517	SRR1039520	SRR1039521
ENSG00000000003	679	448	873	408	1138	1047	770	572
ENSG00000000005	0	0	0	0	0	0	0	0
ENSG00000000419	467	515	621	365	587	799	417	508
ENSG00000000457	260	211	263	164	245	331	233	229
ENSG00000000460	60	55	40	35	78	63	76	60
ENSG00000000938	0	0	2	0	1	0	0	0
ENSG00000000971	3251	3679	6177	4252	6721	11027	5176	7995
ENSG00000001036	1433	1062	1733	881	1424	1439	1359	1109
ENSG00000001084	519	380	595	493	820	714	696	704
ENSG00000001167	394	236	464	175	658	584	360	269

### 18.1.2 ‘Row’ (regions-of-interest) data

The rowRanges() accessor is used to view the range information for a RangedSummarizedExperiment. (Note if this were the parent SummarizedExperiment class we’d use rowData()). The data are stored in a GRangesList object, where each list element corresponds to one gene transcript and the ranges in each GRanges correspond to the exons in the transcript.

```
rowRanges(se)
```

```
GRangesList object of length 63677:
$ENSG00000000003
GRanges object with 17 ranges and 2 metadata columns:
  seqnames      ranges strand | exon_id      exon_name
          ...     ...    ... | ...

```

```

<Rle>      <IRanges>   <Rle> | <integer>    <character>
[1] X 99883667-99884983 - | 667145 ENSE00001459322
[2] X 99885756-99885863 - | 667146 ENSE00000868868
[3] X 99887482-99887565 - | 667147 ENSE00000401072
[4] X 99887538-99887565 - | 667148 ENSE00001849132
[5] X 99888402-99888536 - | 667149 ENSE00003554016
...
...     ...     ...
[13] X 99890555-99890743 - | 667156 ENSE00003512331
[14] X 99891188-99891686 - | 667158 ENSE00001886883
[15] X 99891605-99891803 - | 667159 ENSE00001855382
[16] X 99891790-99892101 - | 667160 ENSE00001863395
[17] X 99894942-99894988 - | 667161 ENSE00001828996
-----
seqinfo: 722 sequences (1 circular) from an unspecified genome

...
<63676 more elements>
```

### 18.1.3 ‘Column’ (sample) data

Sample meta-data describing the samples can be accessed using `colData()`, and is a `DataFrame` that can store any number of descriptive columns for each sample row.

```
colData(se)
```

`DataFrame` with 8 rows and 9 columns

	SampleName	cell	dex	albut	Run	avgLength
	<factor>	<factor>	<factor>	<factor>	<factor>	<integer>
SRR1039508	GSM1275862	N61311	untrt	untrt	SRR1039508	126
SRR1039509	GSM1275863	N61311	trt	untrt	SRR1039509	126
SRR1039512	GSM1275866	N052611	untrt	untrt	SRR1039512	126
SRR1039513	GSM1275867	N052611	trt	untrt	SRR1039513	87
SRR1039516	GSM1275870	N080611	untrt	untrt	SRR1039516	120
SRR1039517	GSM1275871	N080611	trt	untrt	SRR1039517	126
SRR1039520	GSM1275874	N061011	untrt	untrt	SRR1039520	101
SRR1039521	GSM1275875	N061011	trt	untrt	SRR1039521	98
	Experiment	Sample	BioSample			
	<factor>	<factor>	<factor>			
SRR1039508	SRX384345	SRS508568	SAMN02422669			
SRR1039509	SRX384346	SRS508567	SAMN02422675			
SRR1039512	SRX384349	SRS508571	SAMN02422678			
SRR1039513	SRX384350	SRS508572	SAMN02422670			
SRR1039516	SRX384353	SRS508575	SAMN02422682			
SRR1039517	SRX384354	SRS508576	SAMN02422673			
SRR1039520	SRX384357	SRS508579	SAMN02422683			
SRR1039521	SRX384358	SRS508580	SAMN02422677			

This sample metadata can be accessed using the \$ accessor which makes it easy to subset the entire object by a given phenotype.

```
# subset for only those samples treated with dexamethasone
se[, se$dex == "trt"]

class: RangedSummarizedExperiment
dim: 63677 4
metadata(1): ''
assays(1): counts
rownames(63677): ENSG00000000003 ENSG00000000005 ... ENSG00000273492
ENSG00000273493
rowData names(10): gene_id gene_name ... seq_coord_system symbol
colnames(4): SRR1039509 SRR1039513 SRR1039517 SRR1039521
colData names(9): SampleName cell ... Sample BioSample
```

#### 18.1.4 Experiment-wide metadata

Meta-data describing the experimental methods and publication references can be accessed using `metadata()`.

```
metadata(se)
```

```
[[1]]
Experiment data
  Experimenter name: Himes BE
  Laboratory: NA
  Contact information:
  Title: RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates
  URL: http://www.ncbi.nlm.nih.gov/pubmed/24926665
  PMIDs: 24926665

  Abstract: A 226 word abstract is available. Use 'abstract' method.
```

Note that `metadata()` is just a simple list, so it is appropriate for *any* experiment wide metadata the user wishes to save, such as storing model formulas.

```
metadata(se)$formula <- counts ~ dex + albut
```

```
metadata(se)
```

```
[[1]]
Experiment data
  Experimenter name: Himes BE
  Laboratory: NA
  Contact information:
  Title: RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates
  URL: http://www.ncbi.nlm.nih.gov/pubmed/24926665
  PMIDs: 24926665
```

Abstract: A 226 word abstract is available. Use 'abstract' method.

```
$formula
counts ~ dex + albut
```

---

## 18.2 Common operations on SummarizedExperiment

### 18.2.1 Subsetting

- [ Performs two dimensional subsetting, just like subsetting a matrix or data frame.

```
# subset the first five transcripts and first three samples
se[1:5, 1:3]
```

```
class: RangedSummarizedExperiment
dim: 5 3
metadata(2): '' formula
assays(1): counts
rownames(5): ENSG000000000003 ENSG000000000005 ENSG00000000419
             ENSG00000000457 ENSG00000000460
rowData names(10): gene_id gene_name ... seq_coord_system symbol
colnames(3): SRR1039508 SRR1039509 SRR1039512
colData names(9): SampleName cell ... Sample BioSample
```

- \$ operates on colData() columns, for easy sample extraction.

```
se[, se$cell == "N61311"]

class: RangedSummarizedExperiment
dim: 63677 2
metadata(2): '' formula
assays(1): counts
rownames(63677): ENSG00000000003 ENSG00000000005 ... ENSG00000273492
                 ENSG00000273493
rowData names(10): gene_id gene_name ... seq_coord_system symbol
colnames(2): SRR1039508 SRR1039509
colData names(9): SampleName cell ... Sample BioSample
```

### 18.2.2 Getters and setters

- rowRanges() / (rowData(), colData(), metadata())

```
counts <- matrix(1:15, 5, 3, dimnames=list(LETTERS[1:5], LETTERS[1:3]))

dates <- SummarizedExperiment(assays=list(counts=counts),
```

```
rowData=DataFrame(month=month.name[1:5], day=1:5))
```

```
# Subset all January assays
dates[ rowData(dates)$month == "January", ]
```

```
class: SummarizedExperiment
dim: 1 3
metadata(0):
assays(1): counts
rownames(1): A
rowData names(2): month day
colnames(3): A B C
colData names(0):
```

- assay() versus assays() There are two accessor functions for extracting the assay data from a SummarizedExperiment object. assays() operates on the entire list of assay data as a whole, while assay() operates on only one assay at a time. assay(x, i) is simply a convenience function which is equivalent to assays(x)[[i]].

```
assays(se)
```

```
List of length 1
```

```
names(1): counts
```

```
assays(se)[[1]][1:5, 1:5]
```

	SRR1039508	SRR1039509	SRR1039512	SRR1039513	SRR1039516
ENSG00000000003	679	448	873	408	1138
ENSG00000000005	0	0	0	0	0
ENSG00000000419	467	515	621	365	587
ENSG00000000457	260	211	263	164	245
ENSG00000000460	60	55	40	35	78

```
# assay defaults to the first assay if no i is given
```

```
assay(se)[1:5, 1:5]
```

	SRR1039508	SRR1039509	SRR1039512	SRR1039513	SRR1039516
ENSG00000000003	679	448	873	408	1138
ENSG00000000005	0	0	0	0	0
ENSG00000000419	467	515	621	365	587
ENSG00000000457	260	211	263	164	245
ENSG00000000460	60	55	40	35	78

```
assay(se, 1)[1:5, 1:5]
```

	SRR1039508	SRR1039509	SRR1039512	SRR1039513	SRR1039516
ENSG00000000003	679	448	873	408	1138
ENSG00000000005	0	0	0	0	0
ENSG00000000419	467	515	621	365	587
ENSG00000000457	260	211	263	164	245

ENSG00000000460	60	55	40
-----------------	----	----	----

35	78		
----	----	--	--

### 18.2.3 Range-based operations

- `subsetByOverlaps()` `SummarizedExperiment` objects support all of the `findOverlaps()` methods and associated functions. This includes `subsetByOverlaps()`, which makes it easy to subset a `SummarizedExperiment` object by an interval.

In the next code block, we define a region of interest (or many regions of interest) and then subset our `SummarizedExperiment` by overlaps with this region.

```
# Subset for only rows which are in the interval 100,000 to 110,000 of
# chromosome 1
roi <- GRanges(seqnames="1", ranges=100000:1100000)
sub_se = subsetByOverlaps(se, roi)
sub_se

class: RangedSummarizedExperiment
dim: 74 8
metadata(2): '' formula
assays(1): counts
rownames(74): ENSG00000131591 ENSG0000177757 ... ENSG00000272512
  ENSG00000273443
rowData names(10): gene_id gene_name ... seq_coord_system symbol
colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
colData names(9): SampleName cell ... Sample BioSample
dim(sub_se)

[1] 74 8
```

## 18.3 Constructing a SummarizedExperiment

Often, `SummarizedExperiment` or `RangedSummarizedExperiment` objects are returned by functions written by other packages. However it is possible to create them by hand with a call to the `SummarizedExperiment()` constructor. The code below is simply to illustrate the mechanics of creating an object from scratch. In practice, you will probably have the pieces of the object from other sources such as Excel files or csv files.

Constructing a `RangedSummarizedExperiment` with a `GRanges` as the `rowRanges` argument:

```
nrows <- 200
ncols <- 6
counts <- matrix(runif(nrows * ncols, 1, 1e4), nrows)
rowRanges <- GRanges(rep(c("chr1", "chr2"), c(50, 150)),
  IRanges(floor(runif(200, 1e5, 1e6)), width=100),
```

```
        strand=sample(c("+", "-"), 200, TRUE),
        feature_id=sprintf("ID%03d", 1:200))
colData <- DataFrame(Treatment=rep(c("ChIP", "Input"), 3),
                     row.names=LETTERS[1:6])

SummarizedExperiment(assays=list(counts=counts),
                     rowRanges=rowRanges, colData=colData)
```

```
class: RangedSummarizedExperiment
dim: 200 6
metadata(0):
assays(1): counts
rownames: NULL
rowData names(1): feature_id
colnames(6): A B ... E F
colData names(1): Treatment
```

A `SummarizedExperiment` can be constructed with or without supplying a `DataFrame` for the `rowData` argument:

```
SummarizedExperiment(assays=list(counts=counts), colData=colData)
```

```
class: SummarizedExperiment
dim: 200 6
metadata(0):
assays(1): counts
rownames: NULL
rowData names(0):
colnames(6): A B ... E F
colData names(1): Treatment
```

# 19

---

## *EDA with PCA*

---

### 19.1 Introduction

In this tutorial, we will use the GEOquery package to download a dataset from the Gene Expression Omnibus (GEO) and perform some exploratory data analysis (EDA) using principal components analysis (PCA).

---

### 19.2 Downloading data from GEO

The GEOquery package can be used to download data from GEO. The `getGEO` function takes a GEO accession number as an argument and returns a list of `ExpressionSet` objects. The `[[1]]` at the end of the `getGEO` call is used to extract the first (and only) `ExpressionSet` object from the list.

Historically, it was not uncommon for GEO datasets to contain multiple separate experiments. In those cases, the `[[1]]` would need to be replaced with the index of the experiment of interest. However, it is now uncommon for GEO datasets to contain multiple experiments, but the `[[1]]` is still needed to extract the `ExpressionSet` object from the list.

```
library(GEOquery)
library(SummarizedExperiment)
```

`ExpressionSet` objects are a type of Bioconductor object that is used to store gene expression data. The `as` function can be used to convert the `ExpressionSet` object to a `SummarizedExperiment` object, which is a newer Bioconductor object that is used to store gene expression data. The `SummarizedExperiment` object is preferred over the `ExpressionSet` object so we immediately convert the `ExpressionSet` object to a `SummarizedExperiment`.

```
gse <- getGEO("GSE30219")[[1]]
Found 1 file(s)
GSE30219_series_matrix.txt.gz
se <- as(gse, "SummarizedExperiment")
```

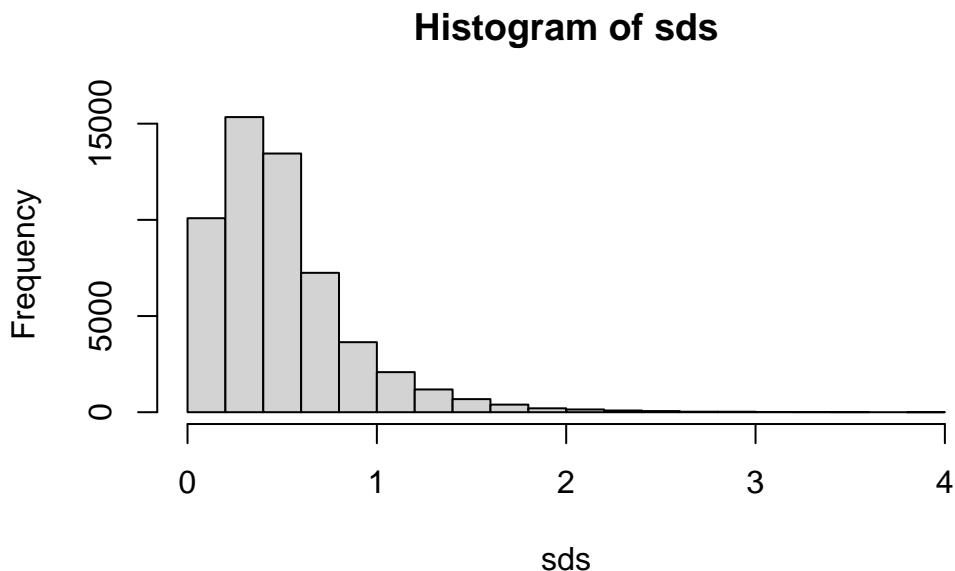
### 19.3 Filtering genes

When performing PCA, it is common to filter to the most variable genes before performing the PCA. Limiting genes to the most variable genes can help to reduce the computational burden of the PCA.

We can calculate the standard deviation of each gene using the `apply` function. The `apply` function takes a matrix as the first argument and a 1 or 2 to indicate whether the function should be applied to the rows or columns of the matrix. The `sd` function calculates the standard deviation of a vector and is performed on each row of the matrix.

A histogram of the standard deviations is not that useful, but it is easy to make.

```
sds = apply(assay(se, 'exprs'), 1, sd)
hist(sds)
```



Here, we produce a subset of the `SummarizedExperiment` object that contains only the 500 most variable genes. We'll use this subset for the rest of the tutorial. Feel free to revisit the number of genes you choose to keep and see how it affects the PCA.

```
sub_se = se[order(sds, decreasing = TRUE)[1:500], ]
```

## 19.4 PCA

PCA is a method for dimensionality reduction. It is a linear transformation that finds the directions of maximum variance in a dataset and projects it onto a new subspace with equal or fewer dimensions than the original one. The orthogonal axes (principal components) of the new subspace can be interpreted as the directions of maximum variance given the constraint that the new feature axes are orthogonal to each other.

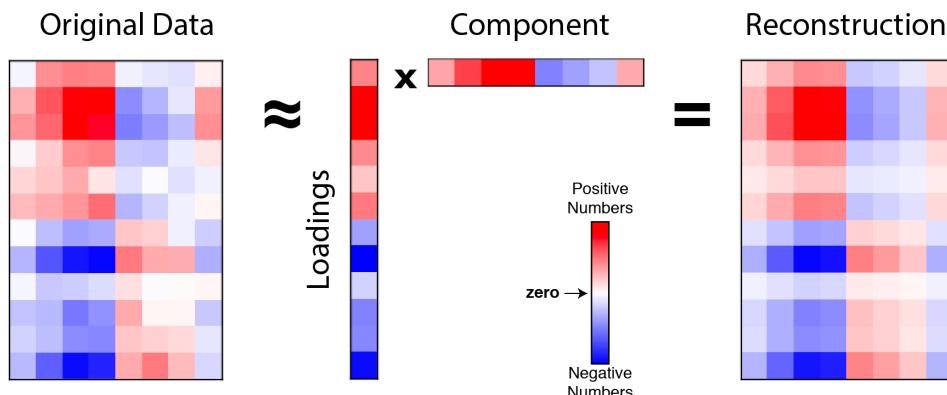


Figure 19.1: The matrix decomposition of the first PC and how we can use it to construct the dimensionally-reduced dataset.

```
# read the help for prcomp here to see what the arguments are
# ?prcomp
pca = prcomp(t(assay(sub_se, 'exprs')))
```

The PCA algorithm results in a rotation matrix that can be used to transform the original data into the new subspace. The rotation matrix is stored in the rotation slot of the `prcomp` object and represents the *loadings* of each gene for each principle component. The `prcomp` function also stores the coordinates of the samples in the new subspace in the `x` slot, which represents the locations of the samples in principle component space.

```
str(pca)
```

```
List of 5
 $ sdev    : num [1:307] 27.01 22.78 13.46 10.43 9.35 ...
 $ rotation: num [1:500, 1:307] -0.1091 0.0598 -0.0474 -0.0513 -0.0903 ...
 ...- attr(*, "dimnames")=List of 2
 ... .$. : chr [1:500] "209125_at" "209988_s_at" "223678_s_at" "218835_at" ...
 ... .$. : chr [1:307] "PC1" "PC2" "PC3" "PC4" ...
 $ center  : Named num [1:500] 6.86 5.56 7.99 10.39 7.82 ...
```

```
..- attr(*, "names")= chr [1:500] "209125_at" "209988_s_at" "223678_s_at" "218835_at" ...
$ scale   : logi FALSE
$ x       : num [1:307, 1:307] 0.571 -3.528 5.289 -31.486 1.273 ...
..- attr(*, "dimnames")=List of 2
... $ : chr [1:307] "GSM748053" "GSM748054" "GSM748055" "GSM748056" ...
... $ : chr [1:307] "PC1" "PC2" "PC3" "PC4" ...
- attr(*, "class")= chr "prcomp"
```

The prcomp function also centers the data by default. The centering values are stored in the center slot. The x slot contains the coordinates of the samples in the new subspace. The

We can plot the samples using the first two PCs as the x and y axes.

```
plot(pca$x[, 1], pca$x[, 2], pch=20)
```

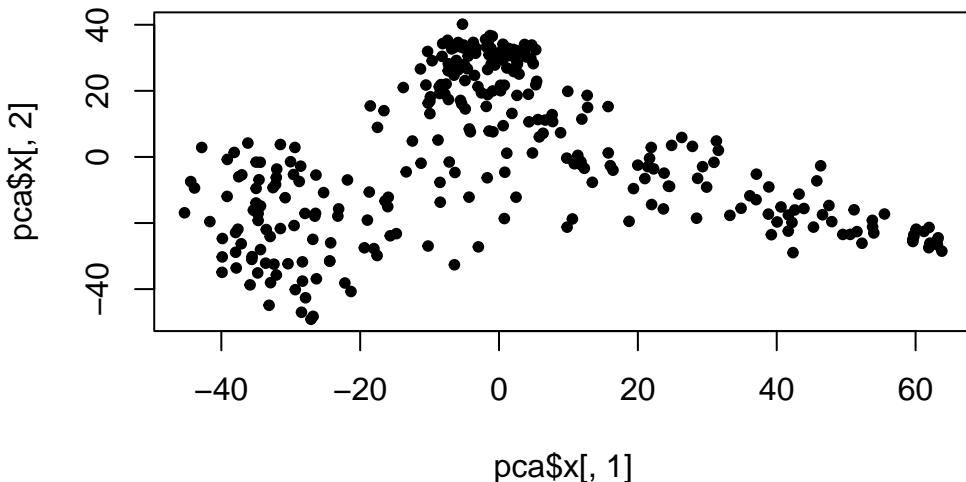


Figure 19.2: PCA plot of samples in the first two PCs.

If we use ALL the PCs, we can perform a matrix multiplication to get the original data back.

```
orig_data = pca$rotation %*% t(pca$x) + pca$center
orig_data[1:5, 1:5]
```

	GSM748053	GSM748054	GSM748055	GSM748056	GSM748057
209125_at	4.400830	4.349534	3.661922	10.289194	3.354648
209988_s_at	3.078285	3.079797	3.467936	3.447669	3.141168
223678_s_at	11.389715	10.637554	5.956832	9.311594	10.467811

```
218835_at    13.541261 12.944545  9.725079 12.744177 12.896846
201820_at     5.056486  5.030666  4.986433 11.284134  5.132626
```

Compare to the original data:

```
assay(sub_se, 'exprs')[1:5,1:5]
```

```
GSM748053 GSM748054 GSM748055 GSM748056 GSM748057
209125_at   4.400830  4.349534  3.661922 10.289194  3.354648
209988_s_at 3.078285  3.079797  3.467936  3.447669  3.141168
223678_s_at 11.389715 10.637554  5.956831  9.311594 10.467811
218835_at   13.541261 12.944545  9.725079 12.744177 12.896846
201820_at     5.056486  5.030666  4.986433 11.284134  5.132626
```

And the same thing, but using only the first 3 PCs:

```
orig_data_3pcs = pca$rotation[,1:3] %*% t(pca$x[,1:3]) + pca$center
orig_data_3pcs[1:5,1:5]
```

```
GSM748053 GSM748054 GSM748055 GSM748056 GSM748057
209125_at   4.302207  5.319141  4.660544  9.765880  4.368261
209988_s_at 3.509123  4.372072  4.644271  4.552902  4.251617
223678_s_at 12.637489 11.381274 10.702921  9.672075 11.985243
218835_at   14.587942 13.535895 12.807482 12.279706 14.037817
201820_at     5.300632  6.248694  5.741837 10.170421  5.391310
```

## 19.5 Variance explained

Often, we want to know how much of the variance in the data is explained by each PC. The `pca` object has a slot called `sdev` that represents the standard deviation of the principle component. Variance is the square of `sdev`, so we can calculate the variance by squaring `sdev`.

```
var_explained = pca$sdev ^ 2
```

The total variance is just the sum of all the variances:

```
tot_variance = sum(var_explained)
```

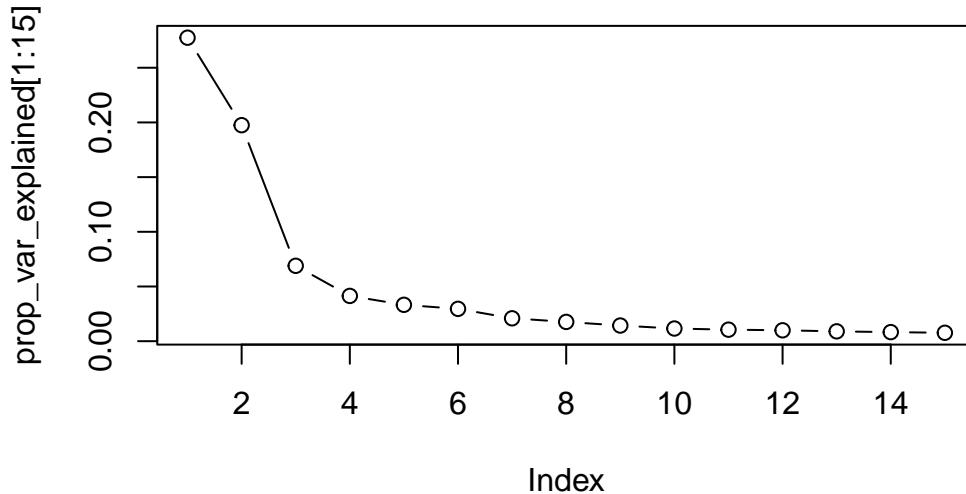
And the proportion of the variance explained by each PC is then

```
prop_var_explained = var_explained/tot_variance
head(prop_var_explained)
```

```
[1] 0.27748451 0.19747527 0.06892190 0.04138543 0.03325101 0.02956515
```

If we plot the `prop_var_explained`, it is called a scree plot and can help us to choose an appropriate number of PCs to “keep” in order to reduce the dimensionality.

```
plot(prop_var_explained[1:15], type='b')
```



Examine the plot. How many PCs would you keep?

---

## 19.6 Add PCs to our SummarizedExperiment object

Recall that the `x` matrix stored in the `pca` object represent the coordinates of the samples in the new subspace. We can look at the first five rows and columns of the `x` matrix to see what it looks like.

```
pca$x[1:5, 1:5]
```

	PC1	PC2	PC3	PC4	PC5
GSM748053	0.5712872	34.105929	15.208118	-4.738482	3.4101384
GSM748054	-3.5283223	24.664382	7.632319	-11.746590	0.1405872
GSM748055	5.2892621	21.841834	9.092392	-3.022823	11.7810845
GSM748056	-31.4864198	3.762922	-5.332805	9.695495	-8.8944295
GSM748057	1.2726085	30.640997	10.562888	6.294335	7.1601434

So, PC components for each sample are in columns and samples are in rows. For `colData`, the samples are also in rows. So, we can join the PC values to the `SummarizedExperiment`, `sub_se`, for later use and for comparison to other sample metadata.

```
# We can use cbind to join the PC values to the colData  
# note that the names of the rows are the same for both  
colData(sub_se) = cbind(colData(sub_se), pca$x)
```

We now have the PCs stored conveniently with our SummarizedExperiment.

```
colnames(colData(sub_se))
```

```
[1] "title"  
[2] "geo_accession"  
[3] "status"  
[4] "submission_date"  
[5] "last_update_date"  
[6] "type"  
[7] "channel_count"  
[8] "source_name_ch1"  
[9] "organism_ch1"  
[10] "characteristics_ch1"  
[11] "characteristics_ch1.1"  
[12] "characteristics_ch1.2"  
[13] "characteristics_ch1.3"  
[14] "characteristics_ch1.4"  
[15] "characteristics_ch1.5"  
[16] "characteristics_ch1.6"  
[17] "characteristics_ch1.7"  
[18] "characteristics_ch1.8"  
[19] "characteristics_ch1.9"  
[20] "characteristics_ch1.10"  
[21] "molecule_ch1"  
[22] "extract_protocol_ch1"  
[23] "label_ch1"  
[24] "label_protocol_ch1"  
[25] "taxid_ch1"  
[26] "hyb_protocol"  
[27] "scan_protocol"  
[28] "description"  
[29] "data_processing"  
[30] "platform_id"  
[31] "contact_name"  
[32] "contact_laboratory"  
[33] "contact_department"  
[34] "contact_institute"  
[35] "contact_address"  
[36] "contact_city"  
[37] "contact_zip.postal_code"  
[38] "contact_country"  
[39] "supplementary_file"
```

```
[40] "data_row_count"
[41] "age.at.surgery.ch1"
[42] "disease.free.survival.in.months.ch1"
[43] "follow.up.time..months..ch1"
[44] "gender.ch1"
[45] "histology.ch1"
[46] "pm.stage.ch1"
[47] "pn.stage.ch1"
[48] "pt.stage.ch1"
[49] "relapse..event.1..no.event.0..ch1"
[50] "status.ch1"
[51] "tissue.ch1"
[52] "PC1"
[53] "PC2"
[54] "PC3"
[55] "PC4"
[56] "PC5"
[57] "PC6"
[58] "PC7"
[59] "PC8"
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[61] "PC10"
[62] "PC11"
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```

```
[86] "PC35"  
[87] "PC36"  
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[131] "PC80"
```

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```

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```

```
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```
[316] "PC265"
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[353] "PC302"
[354] "PC303"
[355] "PC304"
[356] "PC305"
[357] "PC306"
[358] "PC307"
```

## 19.7 Variable relationships

Looking at relationships between variables can be a really useful way of generating hypotheses, performing quality control, and suggesting areas to focus in analysis. One common approach to looking at a few variables and their relationships is the “pairs” plot.

The GGally package has a function called ggpairs that can be used to generate a pairs plot for a few variables.

```
library(GGally)
```

Take a look at [this website](#) and examine some variable relationships in the colData(sub\_se). When working with ggplot (and ggpairs), you’ll likely want to convert the colData() to a data.frame first. See Figure 19.3 for an example.

```
ggpairs(as.data.frame(colData(sub_se)), columns=c("PC1", "PC2", "PC3", "gender.ch1"))
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

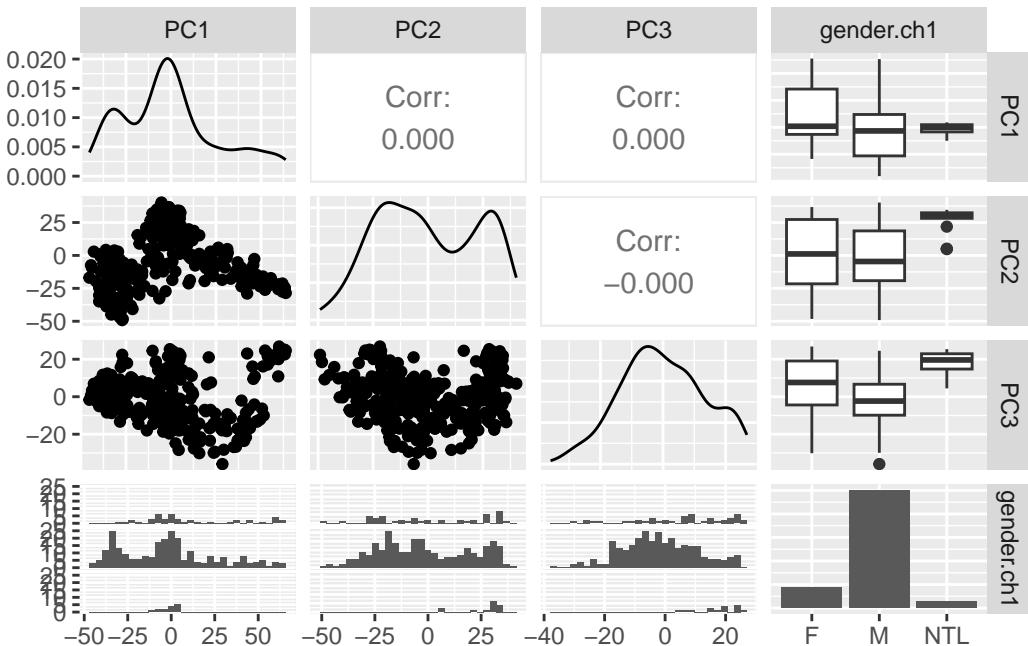


Figure 19.3: A pairs plot of a few variables.

The ggpairs function is very flexible and plays well with ggplot. Therefore, you can add aes() to the ggpairs

function to add colors, etc. to the plot (see Figure 19.4). Look at other variables that you might want to include and style the plot to your liking.

```
ggpairs(as.data.frame(colData(sub_se)), columns=c("PC1", "PC2", "PC3", "gender.ch1")),
       aes(color=gender.ch1, alpha=0.5))
```

```
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
`stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```

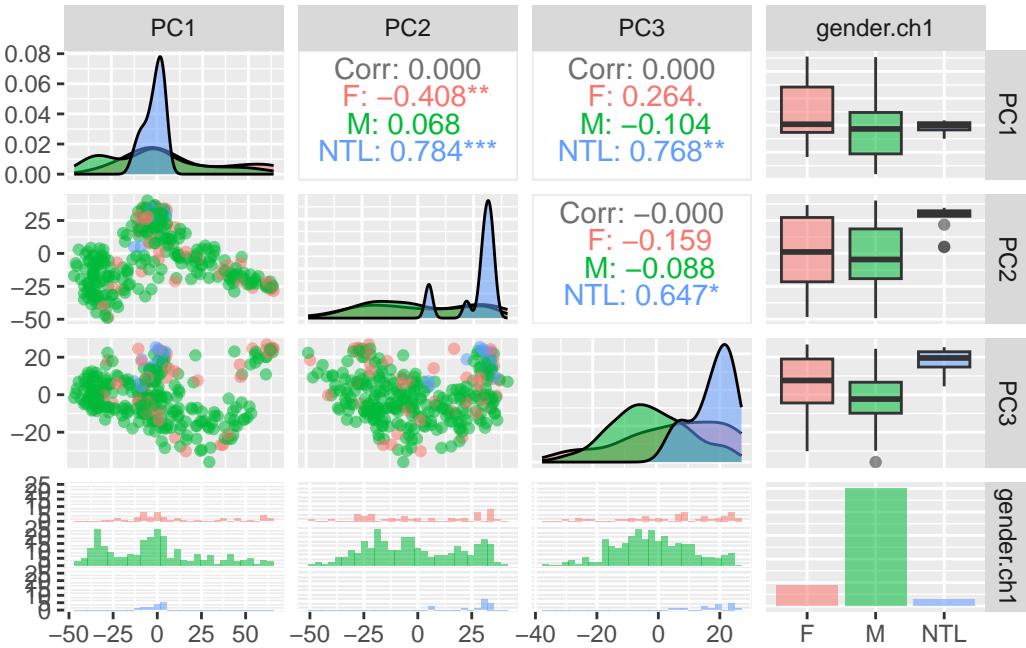


Figure 19.4: A pairs plot colored by a variable of interest.

# 20

---

## *Genomic ranges and features*

---

### 20.1 Introduction

Genomic ranges are a way of describing regions on the genome (or any other linear object, such as a transcript, or even a protein). This functionality is typically found in the [GenomicRanges](#) package (Lawrence et al. 2013a).

```
library(GenomicRanges)
```

The Bioconductor [GenomicRanges](#) package is a comprehensive toolkit designed to handle and manipulate genomic intervals and variables systematized on these intervals. Developed by Bioconductor, this package simplifies the complexity of managing genomic data, facilitating the efficient exploration, manipulation, and visualization of such data. GenomicRanges aids in dealing with the challenges of genomic data, including its massive size, intricate relationships, and high dimensionality.

The GenomicRanges package in Bioconductor covers a wide range of use cases related to the management and analysis of genomic data. Here are some key examples:

- **Genomic Feature Manipulation** The GenomicRanges and GRanges classes can be used to represent and manipulate various genomic features such as genes, transcripts, exons, or single-nucleotide polymorphisms (SNPs). Users can query, subset, resize, shift, or sort these features based on their genomic coordinates.
- **Genomic Interval Operations** The GenomicRanges package provides functions for performing operations on genomic intervals, such as finding overlaps, nearest neighbors, or disjoint intervals. These operations are fundamental to many types of genomic data analyses, such as identifying genes that overlap with ChIP-seq peaks, or finding variants that are in close proximity to each other.
- **Alignments and Coverage** The GAlignments and GAlignmentPairs classes can be used to represent alignments of sequencing reads to a reference genome, such as those produced by a read aligner. Users can then compute coverage of these alignments over genomic ranges of interest, which is a common task in RNA-seq or ChIP-seq analysis.
- **Annotation and Metadata Handling** The metadata column of a GRanges object can be used to store various types of annotation data associated with genomic ranges, such as gene names, gene biotypes, or experimental scores. This makes it easy to perform analyses that integrate genomic coordinates with other types of biological information.
- **Genome Arithmetic** The GenomicRanges package supports a version of “genome arithmetic”, which includes set operations (union, intersection, set difference) as well as other operations (like coverage,

complement, or reduction) that are adapted to the specificities of genomic data.

- **Efficient Data Handling** The CompressedGRangesList class provides a space-efficient way to represent a large list of GRanges objects, which is particularly useful when working with large genomic datasets, such as whole-genome sequencing data.

The GenomicRanges package in Bioconductor uses the S4 class system (see Table 20.1), which is a part of the methods package in R. The S4 system is a more rigorous and formal approach to object-oriented programming in R, providing enhanced capabilities for object design and function dispatch.

Table 20.1: Classes within the GenomicRanges package. Each class has a slightly different use case.

Class Name	Description	Potential Use
GRanges	Represents a collection of genomic ranges and associated variables.	ChIPSeq peaks, CpG islands, etc.
GRangesList	Represents a list of GenomicRanges objects.	transcript models (exons, introns)
RangesList	Represents a list of Ranges objects.	
IRanges	Represents a collection of integer ranges.	used mainly to <i>build</i> GRanges, etc.
GPos	Represents genomic positions.	SNPs or other single nucleotide locations
GAlignments	Represents alignments against a reference genome.	Sequence read locations from a BAM file
GAlignmentPairs	Represents pairs of alignments, typically representing a single fragment of DNA.	Paired-end sequence alignments

In the context of the GenomicRanges package, the S4 class system allows for the creation of complex, structured data objects that can effectively encapsulate genomic intervals and associated data. This system enables the package to handle the complexity and intricacy of genomic data.

For example, the GenomicRanges class in the package is an S4 class that combines several basic data types into a composite object. It includes slots for sequence names (seqnames), ranges (start and end positions), strand information, and metadata. Each slot in the S4 class corresponds to a specific component of the genomic data, and methods (see Table 20.2 and Table 20.3) can be defined to interact with these slots in a structured and predictable way.

Table 20.2: Methods for accessing, manipulating single objects

Method	Description
<b>length</b>	Returns the number of ranges in the GRanges object.
<b>seqnames</b>	Retrieves the sequence names of the ranges.
<b>ranges</b>	Retrieves the start and end positions of the ranges.
<b>strand</b>	Retrieves the strand information of the ranges.
<b>elementMetadata</b>	Retrieves the metadata associated with the ranges.
<b>seqlevels</b>	Returns the levels of the factor that the seqnames slot is derived from.

Method	Description
<b>seqinfo</b>	Retrieves the Seqinfo (sequence information) object associated with the GRanges object.
<b>start, end, width</b>	Retrieve or set the start or end positions, or the width of the ranges.
<b>resize</b>	Resizes the ranges.
<b>subset, [ , [ , \$</b>	Subset or extract elements from the GRanges object.
<b>sort</b>	Sorts the GRanges object.
<b>shift</b>	Shifts the ranges by a certain number of base pairs.

The S4 class system also supports inheritance, which allows for the creation of specialized subclasses that share certain characteristics with a parent class but have additional features or behaviors.

The S4 system's formalism and rigor make it well-suited to the complexities of bioinformatics and genomic data analysis. It allows for the creation of robust, reliable code that can handle complex data structures and operations, making it a key part of the GenomicRanges package and other Bioconductor packages.

Table 20.3: Methods for comparing and combining multiple GenomicRanges-class objects

Method	Description
<b>findOverlaps</b>	Finds overlaps between different sets of ranges.
<b>countOverlaps</b>	Counts overlaps between different sets of ranges.
<b>subsetByOverlaps</b>	Subsets the ranges based on overlaps.
<b>distanceToNearest</b>	Computes the distance to the nearest range in another set of ranges.

## 20.2 The GRanges class

To get going, we can construct a GRanges object by hand as an example.

The GRanges class represents a collection of genomic ranges that each have a single start and end location on the genome. It can be used to store the location of genomic features such as contiguous binding sites, transcripts, and exons. These objects can be created by using the GRanges constructor function. The following code just creates a GRanges object from scratch.

```
gr <- GRanges(
  seqnames = Rle(c("chr1", "chr2", "chr1", "chr3"), c(1, 3, 2, 4)),
  ranges = IRanges(101:110, end = 111:120, names = head(letters, 10)),
  strand = Rle(strand(c("-", "+", "*", "+", "-")), c(1, 2, 2, 3, 2)),
  score = 1:10,
  GC = seq(1, 0, length=10))
gr
```

GRanges object with 10 ranges and 2 metadata columns:

```
> grl = exonsBy(TxDb.Hsapiens.UCSC.hg19.knownGene, "tx", use.names=TRUE); grl
GRangesList of length 82960:
$uc001aaa.3
GRanges with 3 ranges and 3 metadata columns:
  seqnames      ranges strand | exon_id exon_name exon_rank
  <Rle>      <IRanges> <Rle> | <integer> <character> <integer>
[1] chr1 [11874, 12227]    + |     1      <NA>      1
[2] chr1 [12613, 12721]    + |     3      <NA>      2
[3] chr1 [13221, 14409]    + |     5      <NA>      3
```

GRangesList  
(list of GRanges)  
length(grl)  
grl[1:3]  
shift(grl, 1)  
range(grl)

```
$uc010nxq.1
GRanges with 3 ranges and 3 metadata columns:
  seqnames      ranges strand | exon_id exon_name exon_rank
  <Rle>      <IRanges> <Rle> | <integer> <character> <integer>
[1] chr1 [11874, 12227]    + |     1      <NA>      1
[2] chr1 [12595, 12721]    + |     2      <NA>      2
[3] chr1 [13403, 14409]    + |     6      <NA>      3
```

GRanges  
grl[[2]]  
grl[["uc010nxq.1"]]

```
$uc010nxr.1
GRanges with 3 ranges and 3 metadata columns:
  seqnames      ranges strand | exon_id exon_name exon_rank
  <Rle>      <IRanges> <Rle> | <integer> <character> <integer>
[1] chr1 [11874, 12227]    + |     1      <NA>      1
[2] chr1 [12646, 12697]    + |     4      <NA>      2
[3] chr1 [13221, 14409]    + |     5      <NA>      3
...
<82957 more elements>
---
seqinfo: 93 sequences (1 circular) from hg19 genome
```

Two kinds of fun!  
introns =  
psetdiff(range(grl), grl)  
  
grr = unlist(grl)  
## transform grr, then...  
grl = relist(grr, grl)

'flesh'      'skeleton'

Figure 20.1: The structure of a GRangesList, which is a list of GRanges objects. While the analogy is not perfect, a GRangesList behaves a bit like a list. Each element in the GRangesList is a GRanges object. A common use case for a GRangesList is to store a list of transcripts, each of which have exons as the regions in the GRanges.

```

seqnames      ranges strand |      score        GC
              <Rle> <IRanges> <Rle> | <integer> <numeric>
a      chr1    101-111     - |      1  1.000000
b      chr2    102-112     + |      2  0.888889
c      chr2    103-113     + |      3  0.777778
d      chr2    104-114     * |      4  0.666667
e      chr1    105-115     * |      5  0.555556
f      chr1    106-116     + |      6  0.444444
g      chr3    107-117     + |      7  0.333333
h      chr3    108-118     + |      8  0.222222
i      chr3    109-119     - |      9  0.111111
j      chr3    110-120     - |     10  0.000000
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

```

This creates a GRanges object with 10 genomic ranges. The output of the GRanges show() method separates the information into a left and right hand region that are separated by | symbols (see Figure 20.2). The genomic coordinates (seqnames, ranges, and strand) are located on the left-hand side and the metadata columns are located on the right. For this example, the metadata is comprised of score and GC information, but almost anything can be stored in the metadata portion of a GRanges object.

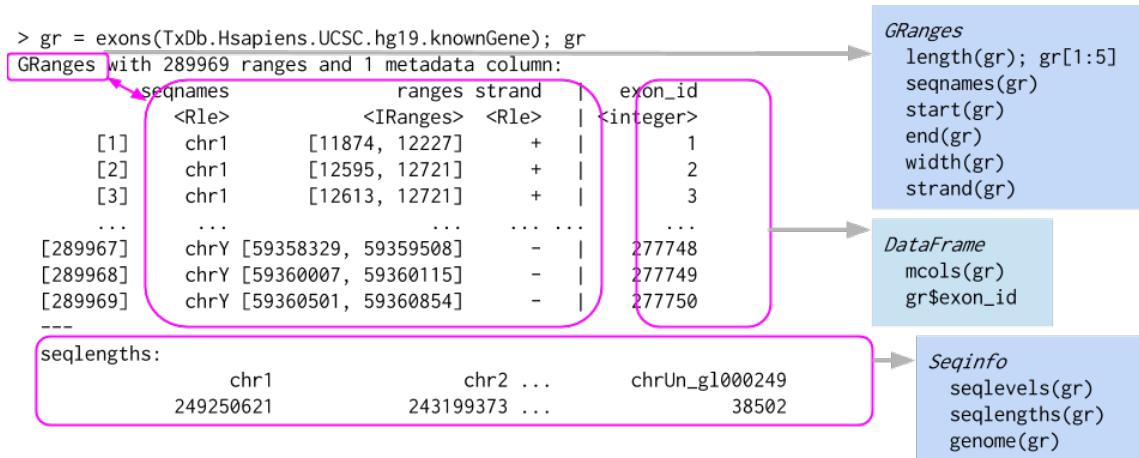


Figure 20.2: The structure of a GRanges object, which behaves a bit like a vector of ranges, although the analogy is not perfect. A GRanges object is composed of the “Ranges” part the lefthand box, the “metadata” columns (the righthand box), and a “seqinfo” part that describes the names and lengths of associated sequences. Only the “Ranges” part is required. The figure also shows a few of the “accessors” and approaches to subsetting a GRanges object.

The components of the genomic coordinates within a GRanges object can be extracted using the seqnames, ranges, and strand accessor functions.

```
seqnames(gr)
```

```
factor-Rle of length 10 with 4 runs
Lengths:    1     3     2     4
Values : chr1 chr2 chr1 chr3
Levels(3): chr1 chr2 chr3
ranges(gr)
```

```
IRanges object with 10 ranges and 0 metadata columns:
      start      end      width
      <integer> <integer> <integer>
a       101      111       11
b       102      112       11
c       103      113       11
d       104      114       11
e       105      115       11
f       106      116       11
g       107      117       11
h       108      118       11
i       109      119       11
j       110      120       11
```

```
strand(gr)
```

```
factor-Rle of length 10 with 5 runs
Lengths: 1 2 2 3 2
Values : - + * + -
Levels(3): + - *
```

Note that the GRanges object has information to the “left” side of the | that has special accessors. The information to the right side of the |, when it is present, is the metadata and is accessed using `mcols()`, for “metadata columns”.

```
class(mcols(gr))
```

```
[1] "DFrame"
attr(,"package")
[1] "S4Vectors"
```

```
mcols(gr)
```

```
DataFrame with 10 rows and 2 columns
      score        GC
      <integer> <numeric>
a       1  1.000000
b       2  0.888889
c       3  0.777778
d       4  0.666667
e       5  0.555556
f       6  0.444444
g       7  0.333333
```

```

h      8  0.222222
i      9  0.111111
j     10  0.000000

```

Since the class of `mcols(gr)` is DFrame, we can use our DataFrame approaches to work with the data.

```
mcols(gr)$score
```

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

We can even assign a new column.

```
mcols(gr)$AT = 1 - mcols(gr)$GC
gr
```

GRanges object with 10 ranges and 3 metadata columns:

seqnames	ranges	strand	score	GC	AT	
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>	<numeric>
a	chr1	101-111	-	1	1.000000	0.000000
b	chr2	102-112	+	2	0.888889	0.111111
c	chr2	103-113	+	3	0.777778	0.222222
d	chr2	104-114	*	4	0.666667	0.333333
e	chr1	105-115	*	5	0.555556	0.444444
f	chr1	106-116	+	6	0.444444	0.555556
g	chr3	107-117	+	7	0.333333	0.666667
h	chr3	108-118	+	8	0.222222	0.777778
i	chr3	109-119	-	9	0.111111	0.888889
j	chr3	110-120	-	10	0.000000	1.000000

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

Another common way to create a GRanges object is to start with a data.frame, perhaps created by hand like below or read in using `read.csv` or `read.table`. We can convert from a data.frame, when columns are named appropriately, to a GRanges object.

```
df_regions = data.frame(chromosome = rep("chr1", 10),
                        start=seq(1000, 10000, 1000),
                        end=seq(1100, 10100, 1000))
as(df_regions, 'GRanges') # note that names have to match with GRanges slots
```

GRanges object with 10 ranges and 0 metadata columns:

seqnames	ranges	strand	
	<Rle>	<IRanges>	<Rle>
[1]	chr1	1000-1100	*
[2]	chr1	2000-2100	*
[3]	chr1	3000-3100	*
[4]	chr1	4000-4100	*
[5]	chr1	5000-5100	*
[6]	chr1	6000-6100	*
[7]	chr1	7000-7100	*

```
[8] chr1 8000-8100 *
[9] chr1 9000-9100 *
[10] chr1 10000-10100 *
-----
seqinfo: 1 sequence from an unspecified genome; no seqlengths
## fix column name
colnames(df_regions)[1] = 'seqnames'
gr2 = as(df_regions, 'GRanges')
gr2
```

GRanges object with 10 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	1000-1100	*
[2]	chr1	2000-2100	*
[3]	chr1	3000-3100	*
[4]	chr1	4000-4100	*
[5]	chr1	5000-5100	*
[6]	chr1	6000-6100	*
[7]	chr1	7000-7100	*
[8]	chr1	8000-8100	*
[9]	chr1	9000-9100	*
[10]	chr1	10000-10100	*

```
-----
seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

GRanges have one-dimensional-like behavior. For instance, we can check the length and even give GRanges names.

```
names(gr)
```

```
[1] "a" "b" "c" "d" "e" "f" "g" "h" "i" "j"
```

```
length(gr)
```

```
[1] 10
```

### 20.2.1 Subsetting GRanges objects

While GRanges objects look a bit like a `data.frame`, they can be thought of as vectors with associated ranges. Subsetting, then, works very similarly to vectors. To subset a GRanges object to include only second and third regions:

```
gr[2:3]
```

GRanges object with 2 ranges and 3 metadata columns:

	seqnames	ranges	strand		score	GC	AT
	<Rle>	<IRanges>	<Rle>		<integer>	<numeric>	<numeric>
b	chr2	102-112	+		2	0.888889	0.111111

```
c      chr2    103-113      + |      3  0.777778  0.222222
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

That said, if the GRanges object has metadata columns, we can also treat it like a two-dimensional object kind of like a data.frame. Note that the information to the left of the | is not like a data.frame, so we *cannot* do something like gr\$seqnames. Here is an example of subsetting with the subset of one metadata column.

```
gr[2:3, "GC"]
```

```
GRanges object with 2 ranges and 1 metadata column:
  seqnames      ranges strand |      GC
  <Rle> <IRanges> <Rle> | <numeric>
b     chr2    102-112      + |  0.888889
c     chr2    103-113      + |  0.777778
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

The usual head() and tail() also work just fine.

```
head(gr, n=2)
```

```
GRanges object with 2 ranges and 3 metadata columns:
  seqnames      ranges strand |      score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
a     chr1    101-111      - |        1  1.000000  0.000000
b     chr2    102-112      + |        2  0.888889  0.111111
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

```
tail(gr, n=2)
```

```
GRanges object with 2 ranges and 3 metadata columns:
  seqnames      ranges strand |      score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
i     chr3    109-119      - |        9  0.111111  0.888889
j     chr3    110-120      - |       10  0.000000  1.000000
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

## 20.2.2 Interval operations on one GRanges object

### 20.2.2.1 Intra-range methods

The methods described in this section work *one-region-at-a-time* and are, therefore, called “intra-region” methods. Methods that work across all regions are described below in the [Inter-range methods](#) section.

The GRanges class has accessors for the “ranges” part of the data. For example:

```
## Make a smaller GRanges subset
g <- gr[1:3]
start(g) # to get start locations

[1] 101 102 103

end(g) # to get end locations

[1] 111 112 113

width(g) # to get the "widths" of each range

[1] 11 11 11

range(g) # to get the "range" for each sequence (min(start) through max(end))
```

GRanges object with 2 ranges and 0 metadata columns:

	seqnames	ranges	strand
	<Rle>	<IRanges>	<Rle>
[1]	chr1	101-111	-
[2]	chr2	102-113	+

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

The GRanges class also has many methods for manipulating the ranges. The methods can be classified as intra-range methods, inter-range methods, and between-range methods. Intra-range methods operate on each element of a GRanges object independent of the other ranges in the object. For example, the flank method can be used to recover regions flanking the set of ranges represented by the GRanges object. So to get a GRanges object containing the ranges that include the 10 bases *upstream* of the ranges:

```
flank(g, 10)
```

GRanges object with 3 ranges and 3 metadata columns:

	seqnames	ranges	strand	score	GC	AT
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>	<numeric>
a	chr1	112-121	-	1	1.000000	0.000000
b	chr2	92-101	+	2	0.888889	0.111111
c	chr2	93-102	+	3	0.777778	0.222222

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

Note how flank pays attention to “strand”. To get the flanking regions *downstream* of the ranges, we can do:

```
flank(g, 10, start=FALSE)
```

GRanges object with 3 ranges and 3 metadata columns:

	seqnames	ranges	strand	score	GC	AT
	<Rle>	<IRanges>	<Rle>	<integer>	<numeric>	<numeric>
a	chr1	91-100	-	1	1.000000	0.000000
b	chr2	113-122	+	2	0.888889	0.111111

```
c      chr2    114-123      + |      3  0.777778  0.222222
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

Other examples of intra-range methods include `resize` and `shift`. The `shift` method will move the ranges by a specific number of base pairs, and the `resize` method will extend the ranges by a specified width.

```
shift(g, 5)
```

```
GRanges object with 3 ranges and 3 metadata columns:
  seqnames      ranges strand |      score       GC       AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a      chr1    106-116      - |      1  1.000000  0.000000
  b      chr2    107-117      + |      2  0.888889  0.111111
  c      chr2    108-118      + |      3  0.777778  0.222222
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

```
resize(g, 30)
```

```
GRanges object with 3 ranges and 3 metadata columns:
  seqnames      ranges strand |      score       GC       AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a      chr1    82-111      - |      1  1.000000  0.000000
  b      chr2    102-131     + |      2  0.888889  0.111111
  c      chr2    103-132     + |      3  0.777778  0.222222
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

The [GenomicRanges](#) help page ?"intra-range-methods" summarizes these methods.

### 20.2.2.2 Inter-range methods

Inter-range methods involve comparisons between ranges in a single GRanges object. For instance, the `reduce` method will align the ranges and merge overlapping ranges to produce a simplified set.

```
reduce(g)
```

```
GRanges object with 2 ranges and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
[1]   chr1    101-111      -
[2]   chr2    102-113      +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

The `reduce` method could, for example, be used to collapse individual overlapping coding exons into a single set of coding regions.

Sometimes one is interested in the gaps or the qualities of the gaps between the ranges represented by your GRanges object. The `gaps` method provides this information:

```
gaps(g)
```

```
GRanges object with 2 ranges and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
[1]   chr1     1-100    -
[2]   chr2     1-101    +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

In this case, we have not specified the lengths of the chromosomes, so Bioconductor is making the assumption (incorrectly) that the chromosomes end at the largest location on each chromosome. We can correct this by setting the seqlengths correctly, but we can ignore that detail for now.

The disjoin method represents a GRanges object as a collection of non-overlapping ranges:

```
disjoin(g)
```

```
GRanges object with 4 ranges and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
[1]   chr1     101-111   -
[2]   chr2      102      +
[3]   chr2     103-112   +
[4]   chr2      113      +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

The coverage method quantifies the degree of overlap for all the ranges in a GRanges object.

```
coverage(g)
```

```
RleList of length 3
$chr1
integer-Rle of length 111 with 2 runs
  Lengths: 100 11
  Values : 0 1

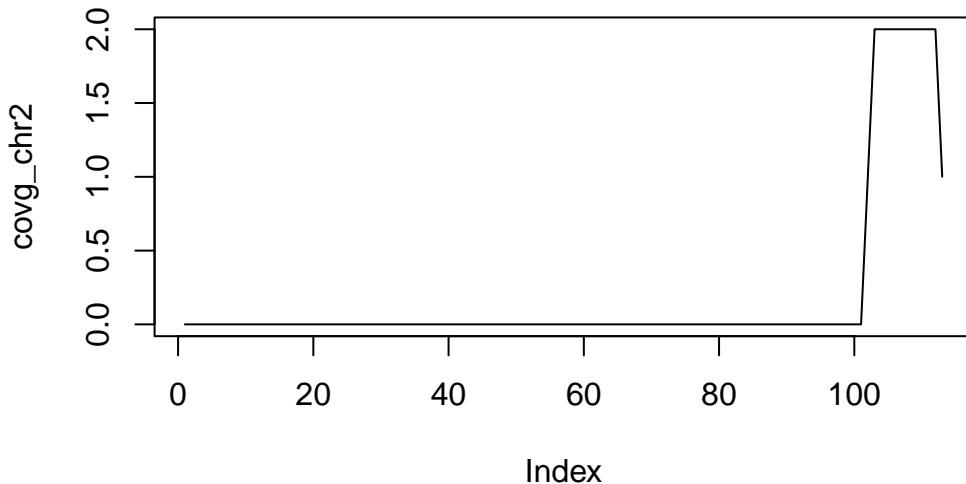
$chr2
integer-Rle of length 113 with 4 runs
  Lengths: 101 1 10 1
  Values : 0 1 2 1

$chr3
integer-Rle of length 0 with 0 runs
  Lengths:
  Values :
```

The coverage is summarized as a list of coverages, one for each chromosome. The Rle class is used to

store the values. Sometimes, one must convert these values to numeric using `as.numeric`. In many cases, this will happen automatically, though.

```
covg = coverage(g)
covg_chr2 = covg[['chr2']]
plot(covg_chr2, type='l')
```



See the [GenomicRanges](#) help page ?"intra-range-methods" for more details.

### 20.2.3 Set operations for GRanges objects

Between-range methods calculate relationships between different GRanges objects. Of central importance are `findOverlaps` and related operations; these are discussed below. Additional operations treat GRanges as mathematical sets of coordinates; `union(g, g2)` is the union of the coordinates in `g` and `g2`. Here are examples for calculating the union, the intersect and the asymmetric difference (using `setdiff`).

```
g2 <- head(gr, n=2)
union(g, g2)
```

```
GRanges object with 2 ranges and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
[1]   chr1    101-111     -
[2]   chr2    102-113     +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

```
intersect(g, g2)
```

```
GRanges object with 2 ranges and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
```

```
[1] chr1 101-111 -
[2] chr2 102-112 +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
setdiff(g, g2)

GRanges object with 1 range and 0 metadata columns:
  seqnames      ranges strand
  <Rle> <IRanges> <Rle>
[1]     chr2      113      +
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

There is extensive additional help available or by looking at the vignettes in at the [GenomicRanges](#) pages.

```
?GRanges
```

There are also many possible methods that work with GRanges objects. To see a complete list (long), try:

```
methods(class="GRanges")
```

## 20.3 GRangesList

Some important genomic features, such as spliced transcripts that are comprised of exons, are inherently compound structures. Such a feature makes much more sense when expressed as a compound object such as a GRangesList. If we think of each transcript as a set of exons, each transcript would be summarized as a GRanges object. However, if we have multiple transcripts, we want to somehow keep them separate, with each transcript having its own exons. The GRangesList is then a list of GRanges objects that. Continuing with the transcripts thought, a GRangesList can contain all the transcripts and their exons; each transcript is an element in the list.

Whenever genomic features consist of multiple ranges that are grouped by a parent feature, they can be represented as a GRangesList object. Consider the simple example of the two transcript GRangesList below created using the GRangesList constructor.

```
gr1 <- GRanges(
  seqnames = "chr1",
  ranges = IRanges(103, 106),
  strand = "+",
  score = 5L, GC = 0.45)
gr2 <- GRanges(
  seqnames = c("chr1", "chr1"),
  ranges = IRanges(c(107, 113), width = 3),
  strand = c("+", "-"),
  score = 3:4, GC = c(0.3, 0.5))
```

The gr1 and gr2 are each GRanges objects. We can combine them into a “named” GRangesList like so:

```
gr1 <- GRangesList("txA" = gr1, "txB" = gr2)
gr1
```

```
GRangesList object of length 2:
$txA
GRanges object with 1 range and 2 metadata columns:
  seqnames      ranges strand |      score        GC
  <Rle> <IRanges> <Rle> | <integer> <numeric>
 [1]    chr1    103-106     + |       5      0.45
 -----
 seqinfo: 1 sequence from an unspecified genome; no seqlengths

$txB
GRanges object with 2 ranges and 2 metadata columns:
  seqnames      ranges strand |      score        GC
  <Rle> <IRanges> <Rle> | <integer> <numeric>
 [1]    chr1    107-109     + |       3      0.3
 [2]    chr1    113-115     - |       4      0.5
 -----
 seqinfo: 1 sequence from an unspecified genome; no seqlengths
```

The show method for a GRangesList object displays it as a named list of GRanges objects, where the names of this list are considered to be the names of the grouping feature. In the example above, the groups of individual exon ranges are represented as separate GRanges objects which are further organized into a list structure where each element name is a transcript name. Many other combinations of grouped and labeled GRanges objects are possible of course, but this example is a common arrangement.

In some cases, GRangesLists behave quite similarly to GRanges objects.

### 20.3.1 Basic *GRangesList* accessors

Just as with GRanges object, the components of the genomic coordinates within a GRangesList object can be extracted using simple accessor methods. Not surprisingly, the GRangesList objects have many of the same accessors as GRanges objects. The difference is that many of these methods return a list since the input is now essentially a list of GRanges objects. Here are a few examples:

```
seqnames(gr1)

RleList of length 2
$txA
factor-Rle of length 1 with 1 run
  Lengths: 1
  Values : chr1
  Levels(1): chr1

$txB
factor-Rle of length 2 with 1 run
```

```
Lengths:      2
Values : chr1
Levels(1): chr1
ranges(gr1)

IRangesList object of length 2:
$txA
IRanges object with 1 range and 0 metadata columns:
      start      end      width
      <integer> <integer> <integer>
[1]     103     106         4

$txB
IRanges object with 2 ranges and 0 metadata columns:
      start      end      width
      <integer> <integer> <integer>
[1]     107     109         3
[2]     113     115         3
strand(gr1)
```

```
RleList of length 2
$txA
factor-Rle of length 1 with 1 run
  Lengths: 1
  Values : +
  Levels(3): + - *
  
$txB
factor-Rle of length 2 with 2 runs
  Lengths: 1 1
  Values : + -
  Levels(3): + - *
```

The length and names methods will return the length or names of the list and the seqlengths method will return the set of sequence lengths.

```
length(gr1)
```

```
[1] 2
```

```
names(gr1)
```

```
[1] "txA" "txB"
```

```
seqlengths(gr1)
```

```
chr1
```

```
NA
```

## 20.4 Relationships between region sets

One of the more powerful approaches to genomic data integration is to ask about the relationship between sets of genomic ranges. The key features of this process are to look at overlaps and distances to the nearest feature. These functionalities, combined with the operations like `flank` and `resize`, for instance, allow pretty useful analyses with relatively little code. In general, these operations are *very* fast, even on thousands to millions of regions.

### 20.4.1 Overlaps

The `findOverlaps` method in the `GenomicRanges` package is a very useful function that allows users to identify overlaps between two sets of genomic ranges.

Here's how it works:

- **Inputs** The function requires two `GRanges` objects, referred to as query and subject.
- **Processing** The function then compares every range in the query object with every range in the subject object, looking for overlaps. An overlap is defined as any instance where the range in the query object intersects with a range in the subject object.
- **Output** The function returns a `Hits` (see `?Hits`) object, which is a compact representation of the matrix of overlaps. Each entry in the `Hits` object corresponds to a pair of overlapping ranges, with the query index and the subject index.

Here is an example of how you might use the `findOverlaps` function:

```
# Create two GRanges objects
gr1 <- gr[1:4]
gr2 <- gr[3:8]
gr1

GRanges object with 4 ranges and 3 metadata columns:
  seqnames      ranges strand |   score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a     chr1    101-111    - |       1  1.000000  0.000000
  b     chr2    102-112    + |       2  0.888889  0.111111
  c     chr2    103-113    + |       3  0.777778  0.222222
  d     chr2    104-114    * |       4  0.666667  0.333333
  -----
  seqinfo: 3 sequences from an unspecified genome; no seqlengths

gr2

GRanges object with 6 ranges and 3 metadata columns:
  seqnames      ranges strand |   score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  c     chr2    103-113    + |       3  0.777778  0.222222
```

```

d    chr2  104-114    * |      4  0.666667  0.333333
e    chr1  105-115    * |      5  0.555556  0.444444
f    chr1  106-116    + |      6  0.444444  0.555556
g    chr3  107-117    + |      7  0.333333  0.666667
h    chr3  108-118    + |      8  0.222222  0.777778
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

# Find overlaps
overlaps <- findOverlaps(query = gr1, subject = gr2)
overlaps

Hits object with 7 hits and 0 metadata columns:
  queryHits subjectHits
  <integer>   <integer>
[1]       1         3
[2]       2         1
[3]       2         2
[4]       3         1
[5]       3         2
[6]       4         1
[7]       4         2
-----
queryLength: 4 / subjectLength: 6

```

In the resulting overlaps object, each row corresponds to an overlapping pair of ranges, with the first column giving the index of the range in gr1 and the second column giving the index of the overlapping range in gr2.

If you are interested in only the queryHits or the subjectHits, there are accessors for those (ie., queryHits(overlaps)). To get the actual ranges that overlap, you can use the subjectHits or queryHits as an index into the original GRanges object.

Spend some time looking at these results. Note how the strand comes into play when determining overlaps.

```
gr1[queryHits(overlaps)]
```

```

GRanges object with 7 ranges and 3 metadata columns:
  seqnames    ranges strand |      score        GC        AT
  <Rle>    <IRanges>  <Rle> | <integer> <numeric> <numeric>
a    chr1  101-111    - |      1  1.000000  0.000000
b    chr2  102-112    + |      2  0.888889  0.111111
b    chr2  102-112    + |      2  0.888889  0.111111
c    chr2  103-113    + |      3  0.777778  0.222222
c    chr2  103-113    + |      3  0.777778  0.222222
d    chr2  104-114    * |      4  0.666667  0.333333
d    chr2  104-114    * |      4  0.666667  0.333333
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths

```

```
gr2[subjectHits(overlaps)]
```

GRanges object with 7 ranges and 3 metadata columns:

seqnames	ranges	strand	score	GC	AT
e	chr1 105-115	*	5	0.555556	0.444444
c	chr2 103-113	+	3	0.777778	0.222222
d	chr2 104-114	*	4	0.666667	0.333333
c	chr2 103-113	+	3	0.777778	0.222222
d	chr2 104-114	*	4	0.666667	0.333333
c	chr2 103-113	+	3	0.777778	0.222222
d	chr2 104-114	*	4	0.666667	0.333333

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

As you might expect, the countOverlaps method counts the regions in the second GRanges that overlap with those that overlap with each element of the first.

```
countOverlaps(gr1, gr2)
```

```
a b c d
1 2 2 2
```

The subsetByOverlaps method simply subsets the query GRanges object to include *only* those that overlap the subject.

```
subsetByOverlaps(gr1, gr2)
```

GRanges object with 4 ranges and 3 metadata columns:

seqnames	ranges	strand	score	GC	AT
a	chr1 101-111	-	1	1.000000	0.000000
b	chr2 102-112	+	2	0.888889	0.111111
c	chr2 103-113	+	3	0.777778	0.222222
d	chr2 104-114	*	4	0.666667	0.333333

-----  
seqinfo: 3 sequences from an unspecified genome; no seqlengths

In some cases, you may be interested in only one hit when doing overlaps. Note the select parameter. See the help for findOverlaps

```
findOverlaps(gr1, gr2, select="first")
```

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

```
findOverlaps(gr1, gr2, select="first")
```

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

The %over% logical operator allows us to do similar things to findOverlaps and subsetByOverlaps.

```
gr2 %over% gr1
[1] TRUE TRUE TRUE FALSE FALSE FALSE
gr1[gr1 %over% gr2]

GRanges object with 4 ranges and 3 metadata columns:
  seqnames      ranges strand |   score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a     chr1    101-111    - |       1  1.000000  0.000000
  b     chr2    102-112    + |       2  0.888889  0.111111
  c     chr2    103-113    + |       3  0.777778  0.222222
  d     chr2    104-114    * |       4  0.666667  0.333333
  -----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

#### 20.4.2 Nearest feature

There are a number of useful methods that find the nearest feature (region) in a second set for each element in the first set.

We can review our two GRanges toy objects:

```
g
GRanges object with 3 ranges and 3 metadata columns:
  seqnames      ranges strand |   score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a     chr1    101-111    - |       1  1.000000  0.000000
  b     chr2    102-112    + |       2  0.888889  0.111111
  c     chr2    103-113    + |       3  0.777778  0.222222
  -----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```

```
gr
GRanges object with 10 ranges and 3 metadata columns:
  seqnames      ranges strand |   score      GC      AT
  <Rle> <IRanges> <Rle> | <integer> <numeric> <numeric>
  a     chr1    101-111    - |       1  1.000000  0.000000
  b     chr2    102-112    + |       2  0.888889  0.111111
  c     chr2    103-113    + |       3  0.777778  0.222222
  d     chr2    104-114    * |       4  0.666667  0.333333
  e     chr1    105-115    * |       5  0.555556  0.444444
  f     chr1    106-116    + |       6  0.444444  0.555556
  g     chr3    107-117    + |       7  0.333333  0.666667
  h     chr3    108-118    + |       8  0.222222  0.777778
  i     chr3    109-119    - |       9  0.111111  0.888889
  j     chr3    110-120    - |      10  0.000000  1.000000
```

- ```
-----
seqinfo: 3 sequences from an unspecified genome; no seqlengths
```
- nearest: Performs conventional nearest neighbor finding. Returns an integer vector containing the index of the nearest neighbor range in subject for each range in x. If there is no nearest neighbor NA is returned. For details of the algorithm see the man page in the IRanges package (?nearest).
  - precede: For each range in x, precede returns the index of the range in subject that is directly preceded by the range in x. Overlapping ranges are excluded. NA is returned when there are no qualifying ranges in subject.
  - follow: The opposite of precede, follow returns the index of the range in subject that is directly followed by the range in x. Overlapping ranges are excluded. NA is returned when there are no qualifying ranges in subject.

Orientation and strand for precede and follow: Orientation is 5' to 3', consistent with the direction of translation. Because positional numbering along a chromosome is from left to right and transcription takes place from 5' to 3', precede and follow can appear to have 'opposite' behavior on the + and - strand. Using positions 5 and 6 as an example, 5 precedes 6 on the + strand but follows 6 on the - strand.

The table below outlines the orientation when ranges on different strands are compared. In general, a feature on \* is considered to belong to both strands. The single exception is when both x and subject are \* in which case both are treated as +.

|    | x |  | subject |  | orientation                                           |
|----|---|--|---------|--|-------------------------------------------------------|
| a) | + |  | +       |  | --->                                                  |
| b) | + |  | -       |  | NA                                                    |
| c) | + |  | *       |  | --->                                                  |
| d) | - |  | +       |  | NA                                                    |
| e) | - |  | -       |  | <---                                                  |
| f) | - |  | *       |  | <---                                                  |
| g) | * |  | +       |  | --->                                                  |
| h) | * |  | -       |  | <---                                                  |
| i) | * |  | *       |  | ---> (the only situation where * arbitrarily means +) |

```
res = nearest(g, gr)
res
```

```
[1] 5 4 4
```

While nearest and friends give the index of the nearest feature, the distance to the nearest is sometimes also useful to have. The distanceToNearest method calculates the nearest feature as well as reporting the distance.

```
res = distanceToNearest(g, gr)
res
```

```
Hits object with 3 hits and 1 metadata column:
  queryHits subjectHits |  distance
  <integer>    <integer> | <integer>
[1]          1            5 |         0
```

```
[2]      2      4 |      0
[3]      3      4 |      0
-----
queryLength: 3 / subjectLength: 10
```

---

## 20.5 Plyranges

Plyranges is a Bioconductor package that provides a grammar of genomic data manipulation. It's a toolset for performing operations on genomic intervals (or ranges) and associated annotations in the R programming language. The package extends the functionality of the GenomicRanges package, another Bioconductor package designed to manage and manipulate genomic interval data. The `plyranges` package provides a grammar for manipulating genomic ranges (Lee, Cook, and Lawrence 2019). It is similar to the `dplyr` package for data frames.

The Plyranges package is designed to address several challenges that arise in the analysis of genomic data:

- **Manipulating Genomic Intervals** Genomic intervals (e.g., gene locations, variant locations, etc.) are a fundamental data type in bioinformatics. Plyranges provides a simple and consistent set of operations for manipulating these intervals, such as finding overlaps, nearest neighbors, or shifting and resizing intervals.
- **Working with Genomic Annotations** Genomic intervals often have associated annotations (e.g., gene names, variant alleles, etc.). Plyranges provides tools for manipulating these annotations along with their associated intervals.
- **Integration with the Tidyverse** The Tidyverse is a collection of R packages designed for data science. Plyranges uses a similar syntax and integrates well with these packages, making it easier for users familiar with the Tidyverse to work with genomic data.
- **Performance** Working with genomic data often involves large datasets. Plyranges is built on the Bioconductor ranges infrastructure and is designed to be efficient and performant, even when working with large genomic datasets.

```
library(plyranges)
```

Table 20.4: The verbs in the `plyranges` package.

| Category       | Verb             | Description                                                       |
|----------------|------------------|-------------------------------------------------------------------|
| Aggregate      | summarize()      | Aggregate over column(s)                                          |
|                | disjoin_ranges() | Aggregate column(s) over the union of end coordinates             |
|                | reduce_ranges()  | Aggregate column(s) by merging overlapping and neighboring ranges |
| Modify (Unary) | mutate()         | Modifies any column                                               |
|                | select()         | Select columns                                                    |
|                | arrange()        | Sort by columns                                                   |
|                | stretch()        | Extend range by fixed amount                                      |

| Category           | Verb                     | Description                      |
|--------------------|--------------------------|----------------------------------|
| Modify<br>(Binary) | shift_(direction)        | Shift coordinates                |
|                    | flank_(direction)        | Generate flanking regions        |
|                    | %intersection%           | Row-wise intersection            |
|                    | %union%                  | Row-wise union                   |
|                    | compute_coverage         | Coverage over all ranges         |
|                    | %setdiff%                | Row-wise set difference          |
|                    | between()                | Row-wise gap range               |
|                    | span()                   | Row-wise spanning range          |
|                    | join_overlap_*           | Merge by overlapping ranges      |
|                    | join_nearest             | Merge by nearest neighbor ranges |
| Merge              | join_follow              | Merge by following ranges        |
|                    | join_precedes            | Merge by preceding ranges        |
|                    | union_ranges             | Range-wise union                 |
|                    | intersect_ranges         | Range-wise intersect             |
|                    | setdiff_ranges           | Range-wise set difference        |
|                    | complement_ranges        | Range-wise set complement        |
| Operate            | anchor_direction()       | Fix coordinates at direction     |
|                    | group_by()               | Partition by column(s)           |
|                    | group_by_overlaps()      | Partition by overlaps            |
| Restrict           | filter()                 | Subset rows                      |
|                    | filter_by_overlaps()     | Subset by overlap                |
|                    | filter_by_non_overlaps() | Subset by no overlap             |
|                    |                          |                                  |

## 20.6 Gene models

The TxDb package provides a convenient interface to gene models from a variety of sources. The TxDb.Hsapiens.UCSC.hg38.knownGene package provides access to the UCSC knownGene gene model for the hg19 build of the human genome.

```
library(TxDb.Hsapiens.UCSC.hg38.knownGene)
txdb <- TxDb.Hsapiens.UCSC.hg38.knownGene
```

The transcripts function returns a GRanges object with the transcripts for all genes in the database.

```
tx <- transcripts(txdb)
```

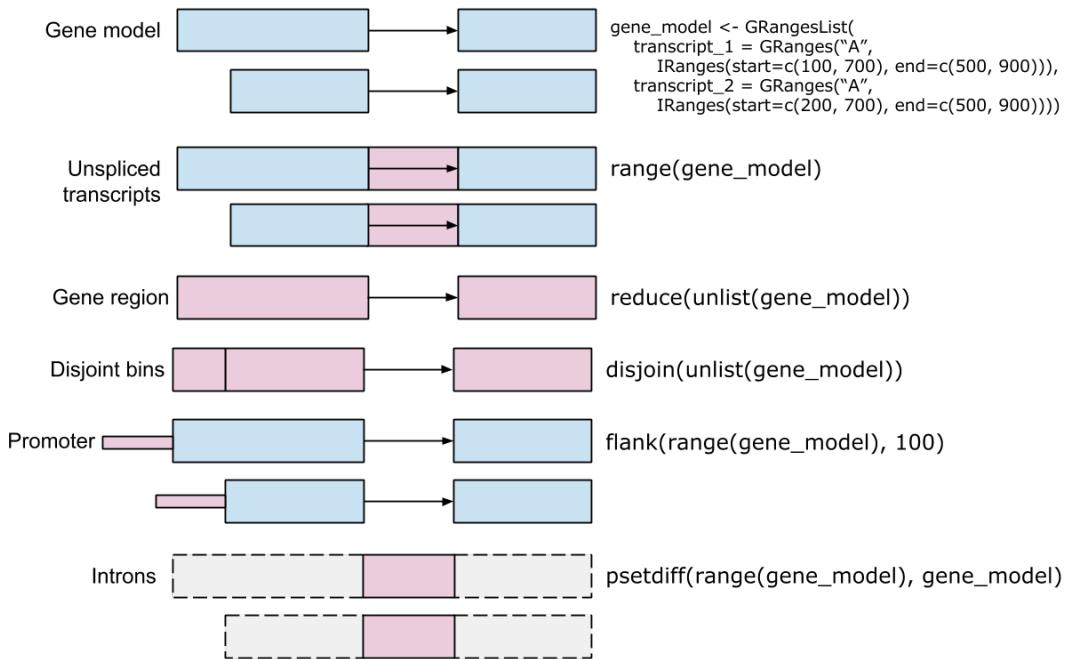


Figure 20.3: A graphical representation of range operations demonstrated on a gene model.

## 20.7 Session Info

```
sessionInfo()
```

```
R version 4.3.0 (2023-04-21)
Platform: aarch64-apple-darwin20 (64-bit)
Running under: macOS Ventura 13.1
```

```
Matrix products: default
BLAS:  /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib; LAPACK version 3

locale:
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8

time zone: America/New_York
tzcode source: internal

attached base packages:
```

```
[1] stats4      stats       graphics   grDevices  utils       datasets  methods  
[8] base
```

other attached packages:

```
[1] TxDb.Hsapiens.UCSC.hg38.knownGene_3.17.0  
[2] GenomicFeatures_1.52.1  
[3] AnnotationDbi_1.62.2  
[4] Biobase_2.60.0  
[5] plyranges_1.19.0  
[6] GenomicRanges_1.52.0  
[7] GenomeInfoDb_1.36.1  
[8] IRanges_2.34.1  
[9] S4Vectors_0.38.1  
[10] BiocGenerics_0.46.0  
[11] knitr_1.43
```

loaded via a namespace (and not attached):

```
[1] KEGGREST_1.40.0           SummarizedExperiment_1.30.2  
[3] rjson_0.2.21              xfun_0.39  
[5] lattice_0.21-8            vctrs_0.6.3  
[7] tools_4.3.0               bitops_1.0-7  
[9] generics_0.1.3            curl_5.0.1  
[11] parallel_4.3.0           tibble_3.2.1  
[13] fansi_1.0.4              RSQLite_2.3.1  
[15] blob_1.2.4               pkgconfig_2.0.3  
[17] Matrix_1.5-4.1           dbplyr_2.3.2  
[19] lifecycle_1.0.3           GenomeInfoDbData_1.2.10  
[21] stringr_1.5.0             compiler_4.3.0  
[23] progress_1.2.2            Rsamtools_2.16.0  
[25] Biostrings_2.68.1          codetools_0.2-19  
[27] htmltools_0.5.5           RCurl_1.98-1.12  
[29] yaml_2.3.7                pillar_1.9.0  
[31] crayon_1.5.2              BiocParallel_1.34.2  
[33] DelayedArray_0.26.6        cachem_1.0.8  
[35] tidyselect_1.2.0           digest_0.6.31  
[37] stringi_1.7.12            dplyr_1.1.2  
[39] restfulr_0.0.15           biomaRt_2.56.1  
[41] fastmap_1.1.1             grid_4.3.0  
[43] cli_3.6.1                 magrittr_2.0.3  
[45] S4Arrays_1.0.4             XML_3.99-0.14  
[47] utf8_1.2.3                rappdirs_0.3.3  
[49] filelock_1.0.2             prettyunits_1.1.1  
[51] bit64_4.0.5                rmarkdown_2.23  
[53] XVector_0.40.0             httr_1.4.6  
[55] matrixStats_1.0.0           bit_4.0.5  
[57] hms_1.1.3                  png_0.1-8
```

```
[59] memoise_2.0.1           evaluate_0.21
[61] BiocIO_1.10.0          BiocFileCache_2.8.0
[63] rtracklayer_1.60.0     rlang_1.1.1
[65] glue_1.6.2             DBI_1.1.3
[67] xml2_1.3.5            rstudioapi_0.14
[69] jsonlite_1.8.7         R6_2.5.1
[71] MatrixGenerics_1.12.2  GenomicAlignments_1.36.0
[73] zlibbioc_1.46.0
```

## 21

---

### *ATAC-Seq with Bioconductor*

This workshop employs Bioconductor GenomicRanges and GenomicFeatures infrastructure to perform basic import of aligned reads followed by quality control of an ATAC-Seq dataset. It will also examine how to use fragment length (insert size) to isolate chromatin compartments of interest such as putative nucleosome-free regions. Moving on to a multi-sample analysis, peak regions (called separately) will inform similarities between samples. Statistical identification of differential accessibility will provide a second measure of sample differences and serve as potential regions of interest for enrichment analysis and biological hypothesis generation. Data visualization throughout using the Integrated Genome Viewer (IGV) can make findings more intuitive, so students are encouraged to export datasets from R in formats compatible with IGV.

---

## **Overview**

---

---

### **Pre-requisites**

This workshop assumes:

- A working and up-to-date version of R
  - Basic knowledge of R syntax
  - Familiarity with the [GenomicRanges](#) package and range manipulations
  - Familiarity with BAM files and their contents
- 

---

### **Participation**

After a very brief review of ATAC-Seq and chromatin accessibility, students will work independently to follow this workflow. Additional materials are provided as links at the end of the workshop for those wanting deeper exposure. Additional materials include alignment from FASTQ files and peak calling.

---

---

### **R / Bioconductor packages used**

- [Rsamtools](#)
- [GenomicRanges](#)
- [GenomicFeatures](#)
- [GenomicAlignments](#)
- [rtracklayer](#)
- [heatmaps](#)
- [TxDb.Hsapiens.UCSC.hg19.knownGene](#)

---

## Time outline

An example for a 45-minute workshop:

| Activity                                  | Time           |
|-------------------------------------------|----------------|
| Introduction                              | 15m            |
| Independent work                          | 2-3hr          |
| Additional exercises (optional, external) | up to 12 hours |

---

## Learning goals

- Describe how to import sequence alignments in BAM format into R
  - Relate fragment size to genomic characteristics such as nucleosome occupancy and open chromatin.
  - Perform basic alignment manipulations in R to enrich ATAC-seq data for chromatin characteristics.
  - Gain familiarity with the IGV genome browser and examining data in genomic context.
  - Visualize summaries of genomic signal using profile plots and heatmaps.
- 

## Learning objectives

- Load and save genomic data in BAM and BigWig formats [GenomicAlignments and rtracklayer].
  - Perform basic QC plots from ATAC-Seq data.
  - Isolate nucleosome-free and mononucleosome regions from ATAC-seq data.
  - Install and use IGV to visualize data in genomic context.
  - Create profile plots using the heatmaps package.
- 

### 21.1 Background

Chromatin accessibility assays measure the extent to which DNA is open and accessible. Such assays now use high throughput sequencing as a quantitative readout. DNase assays, first using microarrays(Crawford, Davis, et al. 2006) and then DNase-Seq (Crawford, Holt, et al. 2006), requires a larger amount of DNA and is labor-indensive and has been largely supplanted by ATAC-Seq (Buenrostro et al. 2013).

The Assay for Transposase Accessible Chromatin with high-throughput sequencing (ATAC-seq) method maps chromatin accessibility genome-wide. This method quantifies DNA accessibility with a hyperactive Tn5 transposase that cuts and inserts sequencing adapters into regions of chromatin that are accessible.

High throughput sequencing of fragments produced by the process map to regions of increased accessibility, transcription factor binding sites, and nucleosome positioning. The method is both fast and sensitive and can be used as a replacement for DNase and MNase. A schematic of the protocol is given in Figure @ref(fig:protocol).

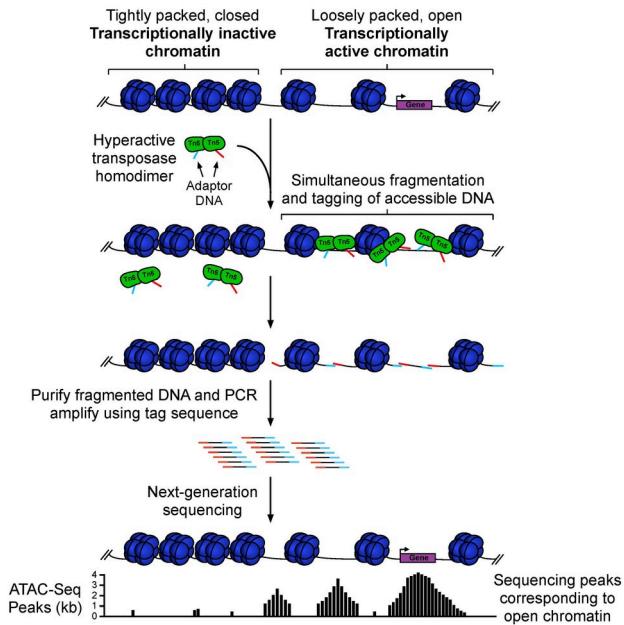


Figure 21.1: Schematic overview of ATAC-Seq protocol. Figure from Wikipedia.

An early review of chromatin accessibility assays (Tsompana and Buck 2014) compares the use cases, pros and cons, and expected signals from each of the most common approaches (Figure @ref(fig:chromatinAssays)).

The first manuscript describing ATAC-Seq protocol and findings outlined how ATAC-Seq data “line up” with other datatypes such as ChIP-seq and DNase-seq (Figure @ref(fig:greenleaf)). They also highlight how fragment length correlates with specific genomic regions and characteristics (Buenrostro et al. 2013, fig. 3).

Buenrostro et al. provide a detailed protocol for performing ATAC-Seq and quality control of results (Buenrostro et al. 2015). Updated and modified protocols that improve on signal-to-noise and reduce input DNA requirements have been described.

### 21.1.1 Informatics overview

ATAC-Seq protocols typically utilize paired-end sequencing protocols. The reads are aligned to the respective genome using bowtie2, BWA, or other short-read aligner. The result, after appropriate manipulation, often using samtools, results in a BAM file. Among other details, the BAM format includes columns for:

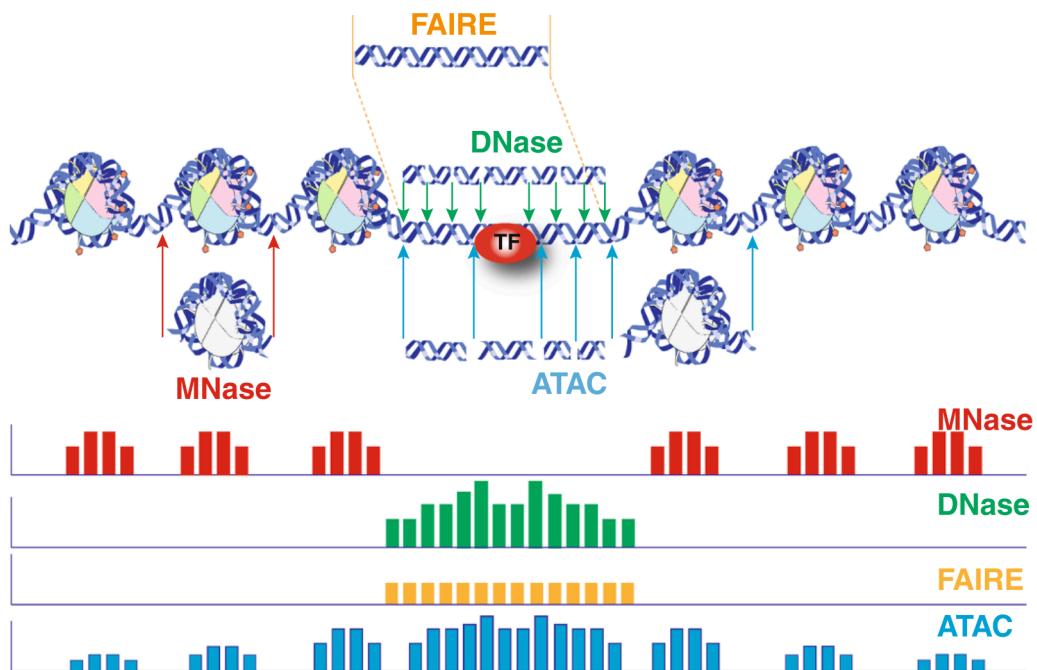


Figure 21.2: Chromatin accessibility methods, compared. Representative DNA fragments generated by each assay are shown, with end locations within chromatin defined by colored arrows. Bar diagrams represent data signal obtained from each assay across the entire region. The footprint created by a transcription factor (TF) is shown for ATAC-seq and DNase-seq experiments.

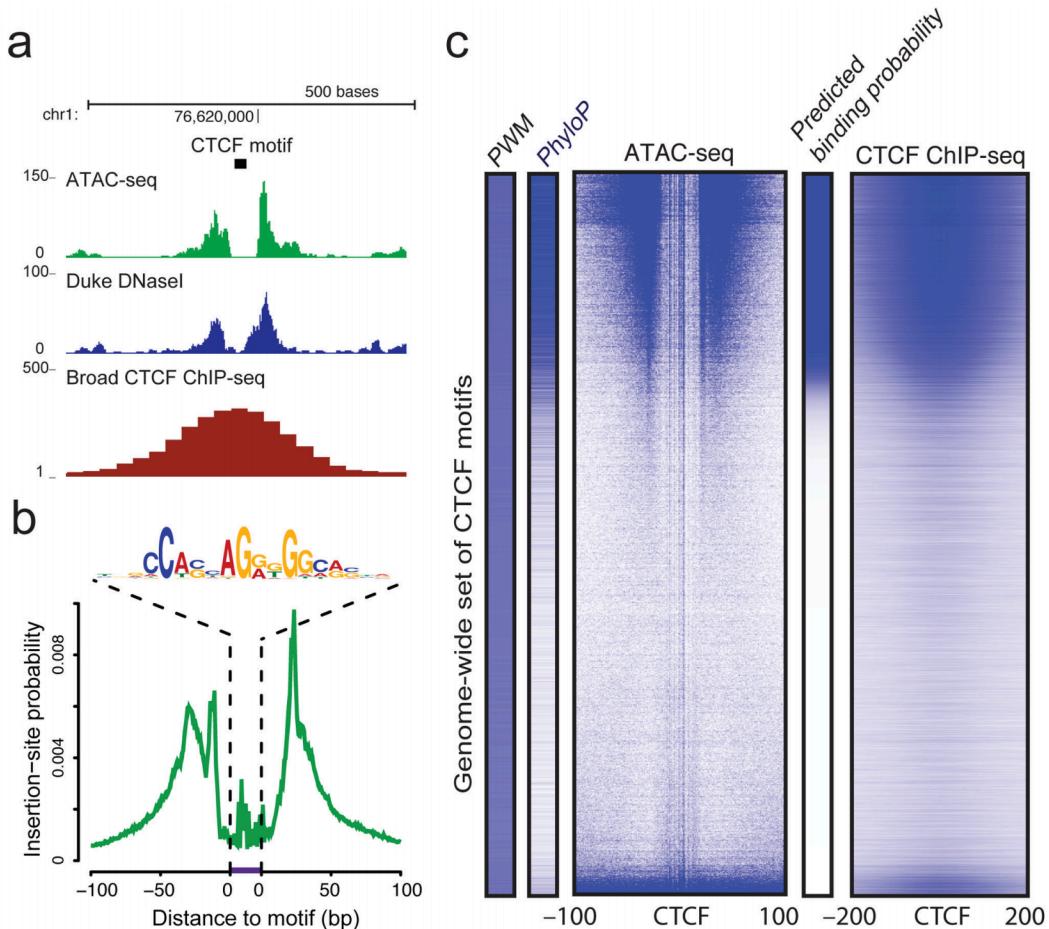


Figure 21.3: Multimodal chromatin comparisons. From (Buenrostro et al. 2013), Figure 4. (a) CTCF footprints observed in ATAC-seq and DNase-seq data, at a specific locus on chr1. (b) Aggregate ATAC-seq footprint for CTCF (motif shown) generated over binding sites within the genome (c) CTCF predicted binding probability inferred from ATAC-seq data, position weight matrix (PWM) scores for the CTCF motif, and evolutionary conservation (PhyloP). Right-most column is the CTCF ChIP-seq data (ENCODE) for this GM12878 cell line, demonstrating high concordance with predicted binding probability.

```
knitr::include_graphics('images/bam_shot.png')
```

Figure 21.4: A BAM file in text form. The output of `samtools view` is the text format of the BAM file (called SAM format). Bioconductor and many other tools use BAM files for input. Note that BAM files also often include an index .bai file that enables random access into the file; one can read just a genomic region without having to read the entire file.

- sequence name (chr1)
  - start position (integer)
  - a *CIGAR* string that describes the alignment in a compact form
  - the sequence to which the pair aligns
  - the position to which the pair aligns
  - a bit flag field that describes multiple characteristics of the alignment
  - the sequence and quality string of the read
  - additional tags that tend to be aligner-specific

Duplicate fragments (those with the *same* start and end position of other reads) are marked and likely discarded. Reads that fail to align “properly” are also often excluded from analysis. It is worth noting that most software packages allow simple “marking” of such reads and that there is usually no need to create a special BAM file before proceeding with downstream work.

After alignment and BAM processing, the workflow can switch to *Bioconductor*.

### 21.1.2 Working with sequencing data in Bioconductor

The *Bioconductor* project includes several infrastructure packages for dealing with ranges (sequence name, start, end, +/- strand) on sequences (Lawrence et al. 2013b) as well as capabilities with working with Fastq files directly (Morgan et al. 2016).

Table 21.2: Commonly used Bioconductor and their high-level use cases.

| Package                        | Use cases                                                             |
|--------------------------------|-----------------------------------------------------------------------|
| <code>Rsamtools</code>         | low level access to FASTQ, VCF, SAM, BAM, BCF formats                 |
| <code>GenomicRanges</code>     | Container and methods for handling genomic regions                    |
| <code>GenomicFeatures</code>   | Work with transcript databases, gff, gtf and BED formats              |
| <code>GenomicAlignments</code> | Reader for BAM format                                                 |
| <code>rtracklayer</code>       | import and export multiple UCSC file formats including BigWig and Bed |

As noted in the previous section, the output of an ATAC-Seq experiment is a BAM file. As paired-end sequencing is a commonly-applied approach for ATAC-Seq, the `readGAlignmentPairs` function is the appropriate method to use.

---

## 21.2 Data import and quality control

```
library(GenomicAlignments)
```

Reading a paired-end BAM file looks a bit complicated, but the following code will:

1. Read the included BAM file.
2. Include read pairs only (`isPaired = TRUE`)
3. Include properly paired reads (`isProperPair = TRUE`)
4. Include reads with mapping quality  $\geq 1$
5. Add a couple of additional fields, `mapq` (mapping quality) and  `isize` (insert size) to the default fields.

```
greenleaf = readGAlignmentPairs(
  'data/Sorted_ATAC_21_22.bam',
  param = ScanBamParam(
    mapqFilter = 1,
    flag = scanBamFlag(
      isPaired = TRUE,
      isProperPair = TRUE),
    what = c("mapq", " isize")))
```

*Exercise:* What is the class of `greenleaf`? *Exercise:* Use the `GenomicAlignments::first()` accessor to get the first read of the pair as a `GAlignments` object. Save the result as a variable called `g1_first_read`. Use

the `mcols` accessor to find the “metadata columns” of `gl_first_read`. *Exercise:* How many read pairs map to each chromosome?

We can make plot of the number of reads mapping to each chromosome.

```
library(ggplot2)
library(dplyr)
chromCounts = table(seqnames(greenleaf)) %>%
  data.frame() %>%
  dplyr::rename(chromosome=Var1, count = Freq)
```

To keep things small, the example BAM file includes only chromosomes 21 and 22.

```
ggplot(chromCounts, aes(x=chromosome, y=count)) +
  geom_bar(stat='identity') +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))
```

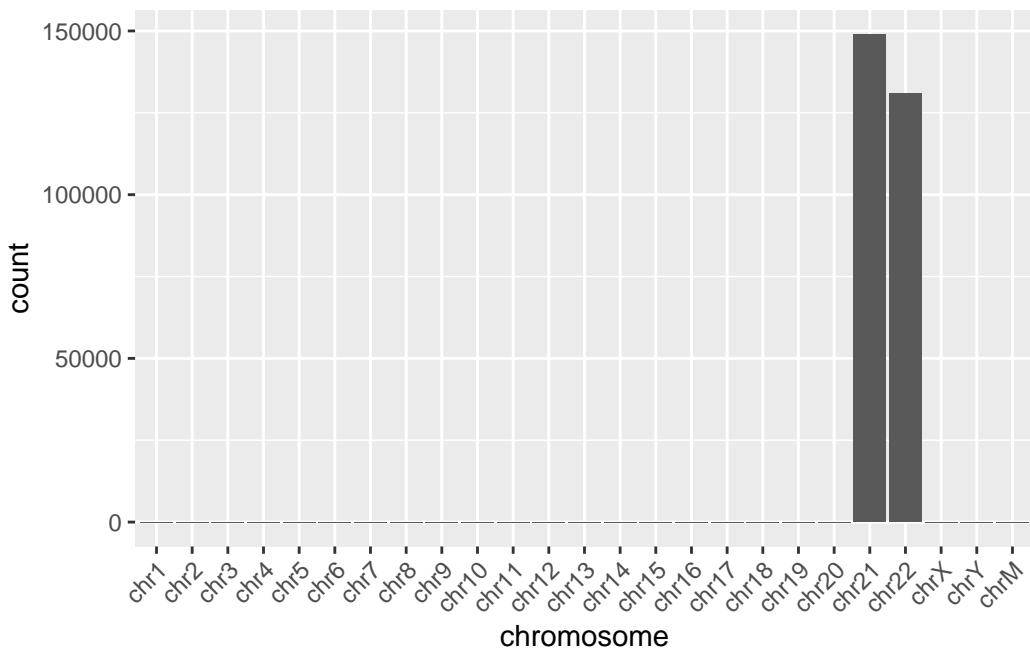


Figure 21.5: Reads per chromosome. In our example data, we are using only chromosomes 21 and 22.

Normalizing by the chromosome length can yield the reads per megabase which should crudely be similar across all chromosomes.

```
chromCounts = chromCounts %>%
  dplyr::mutate(readsPerMb = (count/(seqlengths(greenleaf)/1e6)))
```

And show a plot. For two chromosomes, this is a little underwhelming.

```
ggplot(chromCounts, aes(x=chromosome, y=readsPerMb)) +
  geom_bar(stat='identity') +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  theme_bw()
```

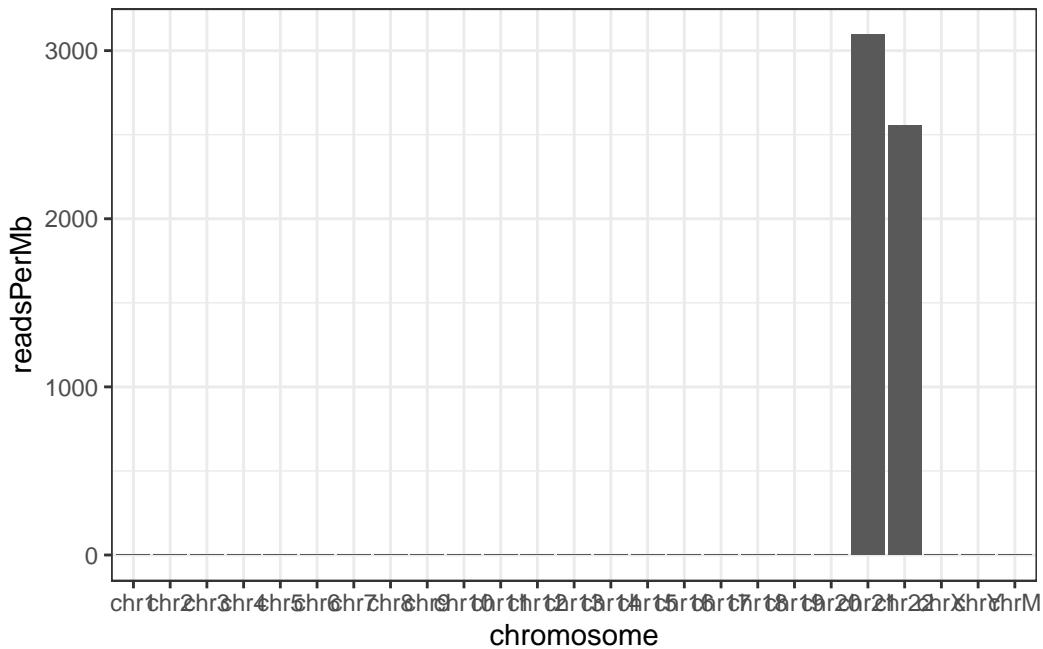


Figure 21.6: Read counts normalized by chromosome length. This is not a particularly important plot, but it can be useful to see the relative contribution of each chromosome given its length.

### 21.2.1 Coverage

The coverage method for genomic ranges calculates, for each base, the number of overlapping features. In the case of a BAM file from ATAC-Seq converted to a GAlignmentPairs object, the coverage gives us an idea of the extent to which reads pile up to form peaks.

```
cvg = coverage(greenleaf)
class(cvg)
```

```
[1] "SimpleRleList"
attr(,"package")
[1] "IRanges"
```

The coverage is returned as a SimpleRleList object. Using names can get us the names of the elements of the list.

```
names(cvg)
```

```
[1] "chr1"  "chr2"  "chr3"  "chr4"  "chr5"  "chr6"  "chr7"  "chr8"  "chr9"
```

```
[10] "chr10" "chr11" "chr12" "chr13" "chr14" "chr15" "chr16" "chr17" "chr18"
[19] "chr19" "chr20" "chr21" "chr22" "chrX" "chrY" "chrM"
```

There is a name for each chromosome. Looking at the chr21 entry:

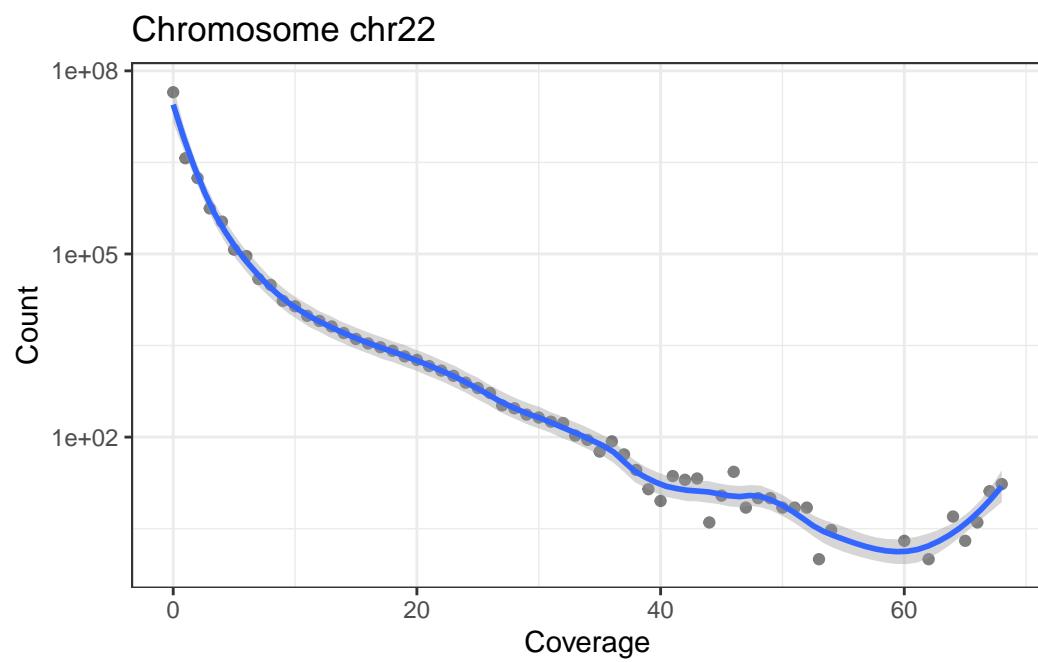
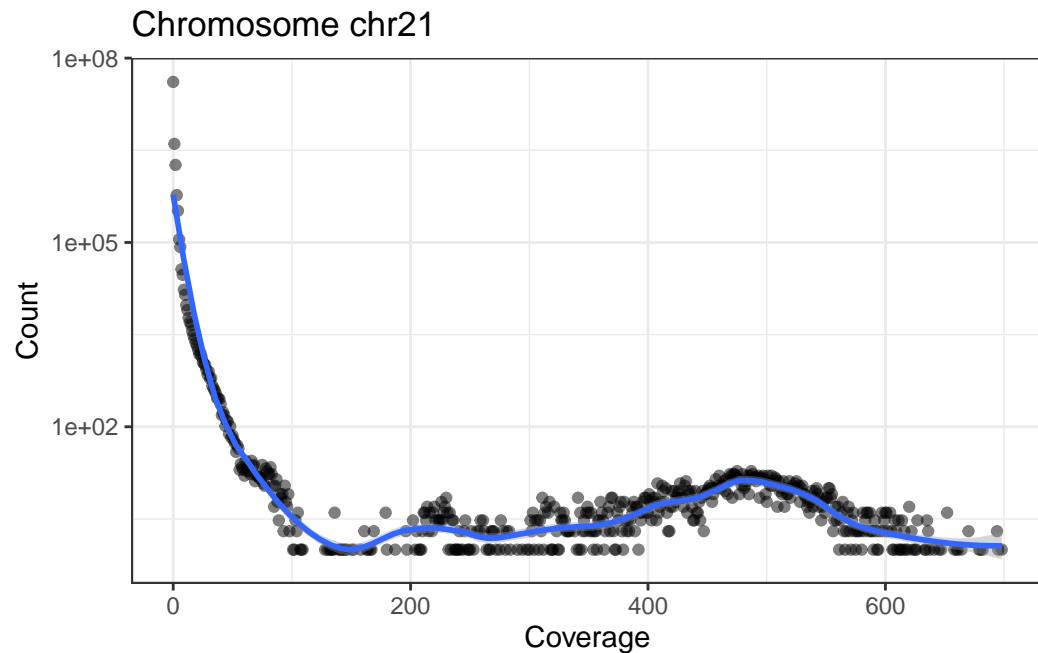
```
cvg$chr21
```

```
integer-Rle of length 48129895 with 397462 runs
Lengths: 9411376      50      11      50 ...      36      14      28    10806
Values :      0       2       0       2 ...       1       2       1       0
```

we see that each chromosome is represented as an Rle, short for run-length-encoding. Simply put, since along the chromosome there are many repeated values, we can recode the long vector as a set of (length: value) pairs. For example, if the first 9,410,000 base pairs have 0 coverage, we encode that as (9,410,000: 0). Doing that across the chromosome can very significantly reduce the memory use for genomic coverage.

The following little function, `plotCvgHistByChrom` can plot a histogram of the coverage for a chromosome.

```
plotCvgHistByChrom = function(cvg, chromosome) {
  library(ggplot2)
  cvgcounts = as.data.frame(table(cvg[[chromosome]]))
  cvgcounts[,1] = as.numeric(as.character(cvgcounts[,1]))
  colnames(cvgcounts) = c('Coverage', 'Count')
  ggplot(cvgcounts, aes(x=Coverage, y=Count)) +
    ggtitle(paste("Chromosome", chromosome)) +
    geom_point(alpha=0.5) +
    geom_smooth(span=0.2) +
    scale_y_log10() +
    theme_bw()
}
for(i in c('chr21', 'chr22')) {
  print(plotCvgHistByChrom(cvg, i))
}
```



### 21.2.2 Fragment Lengths

The first ATAC-Seq manuscript (Buenrostro et al. 2013) highlighted the relationship between fragment length and nucleosomes (see Figure @ref{fig:flgreenleaf}).

```
knitr::include_graphics('images/greenleaf_atac.png')
```

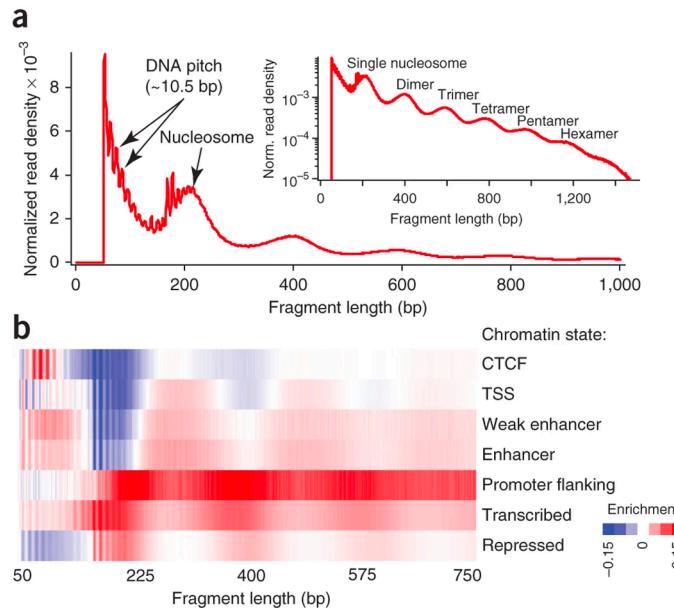


Figure 21.7: Relationship between fragment length and nucleosome number.

Remember that we loaded the example BAM file with insert sizes (`isize`). We can use that “column” to examine the fragment lengths (another name for insert size). Also, note that the insert size for the first read and the second are the same (absolute value). Here, we will use `first`.

```
GenomicAlignments::first(greenleaf)
mcols(GenomicAlignments::first(greenleaf))
class(mcols(GenomicAlignments::first(greenleaf)))
head(mcols(GenomicAlignments::first(greenleaf))$isize)
fraglengths = abs(mcols(GenomicAlignments::first(greenleaf))$isize)
```

We can plot the fragment length density (histogram) using the `density` function.

```
plot(density(fraglengths, bw=0.05), xlim=c(0,1000))
```

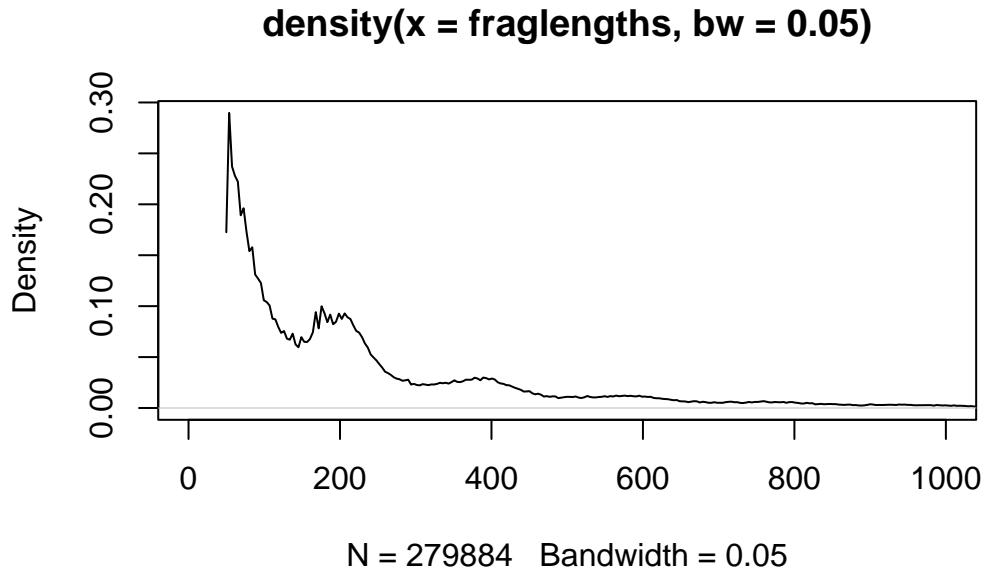
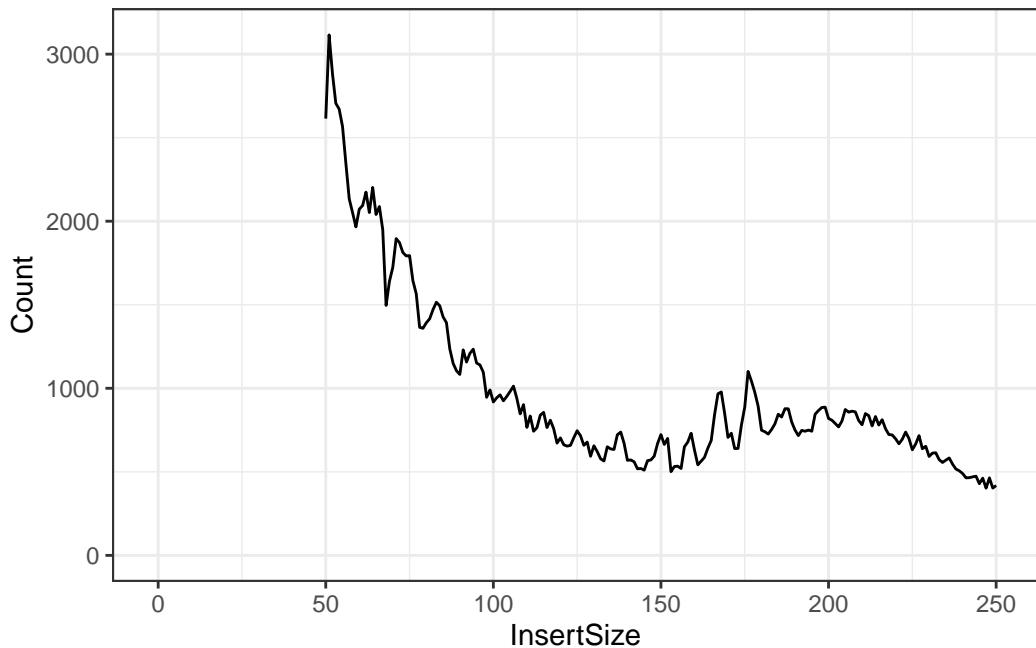


Figure 21.8: Fragment length histogram.

*Exercise:* Adjust the `xlim`, `bw`, and try `log="y"` in the plot to highlight features present in figure ??.

And for fun, the `ggplot2` version:

```
library(dplyr)
library(ggplot2)
fragLenPlot <- table(fraglengths) %>% data.frame %>% rename(InsertSize = fraglengths,
  Count = Freq) %>% mutate(InsertSize = as.numeric(as.vector(InsertSize)),
  Count = as.numeric(as.vector(Count))) %>% ggplot(aes(x = InsertSize, y = Count)) +
  geom_line()
print(fragLenPlot + theme_bw() + lims(x=c(-1,250)))
```



Knowing that the nucleosome-free regions will have insert sizes shorter than one nucleosome, we can isolate the read pairs that have that characteristic.

```
g1_nf = greenleaf[mcols(GenomicAlignments)::first(greenleaf))$isize<100]
```

And the mononucleosome reads will be between 187 and 250 base pairs for insert size/fragment length.

```
g1_mn = greenleaf[mcols(GenomicAlignments)::first(greenleaf))$isize>187 &
mcols(GenomicAlignments)::first(greenleaf))$isize<250 ]
```

Finally, we expect nucleosome-free reads to be enriched near the TSS while mononucleosome reads should not be. We will use the [heatmaps](#) package to take a look at these two sets of reads with respect to the tss of the human genome.

```
library(TxDb.Hsapiens.UCSC.hg19.knownGene)
proms = promoters(TxDb.Hsapiens.UCSC.hg19.knownGene, 250, 250)
seqs = c('chr21', 'chr22')
seqlevels(proms, pruning.mode='coarse') = seqs # only chromosome 21 and 22
```

Take a look at the [heatmaps](#) package vignette to learn more about the heatmaps package capabilities.

```
library(heatmaps)
g1_nf_hm = CoverageHeatmap(proms, coverage(g1_nf), coords=c(-250, 250))
label(g1_nf_hm) = "NucFree"
scale(g1_nf_hm)=c(0,10)
plotHeatmapMeta(g1_nf_hm)
```

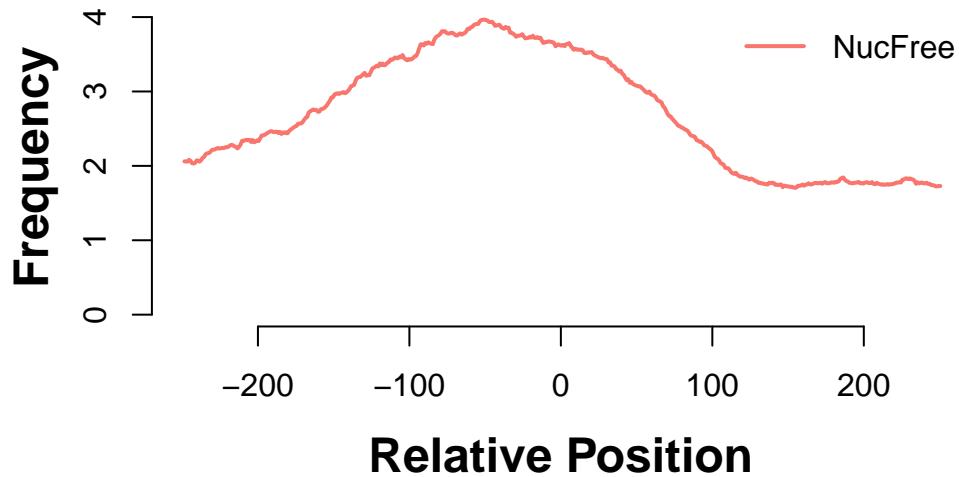


Figure 21.9: Enrichment of nucleosome free reads just upstream of the TSS.

```
g1_mn_hm = CoverageHeatmap(proms, coverage(g1_mn), coords=c(-250, 250))
label(g1_mn_hm) = "MonoNuc"
scale(g1_mn_hm)=c(0, 10)
plotHeatmapMeta(g1_mn_hm)
```

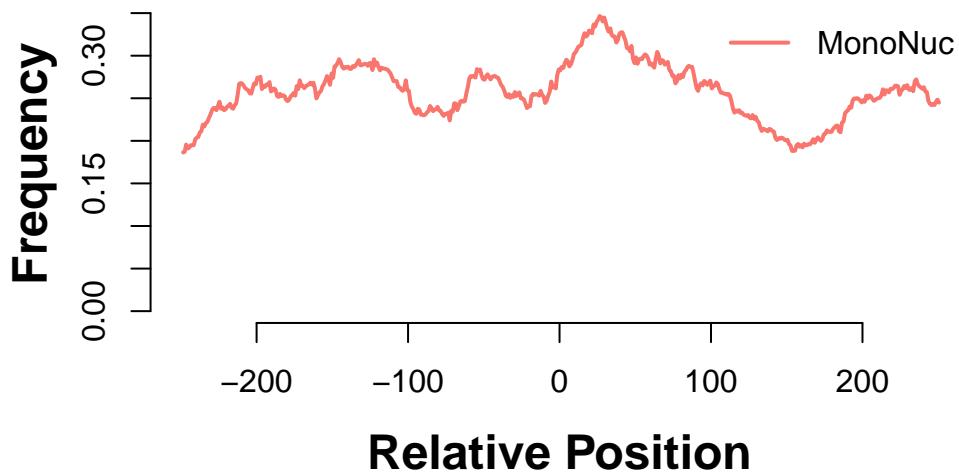


Figure 21.10: Depletion of nucleosome free reads just upstream of the TSS.

```
plotHeatmapList(list(g1_mn_hm, g1_nf_hm))
```

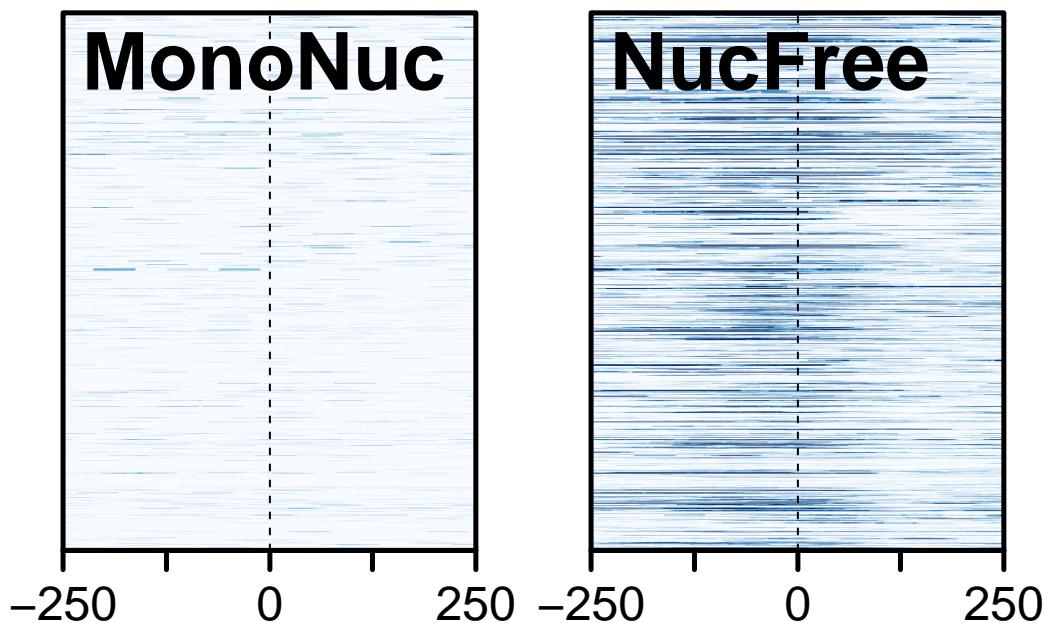


Figure 21.11: Comparison of signals at TSS. Mononucleosome data on the left, nucleosome-free on the right.

---

### 21.3 Viewing data in IGV

Install IGV from [here](#).

We export the greenleaf data as a BigWig file.

```
library(rtracklayer)
export.bw(coverage(greenleaf), 'greenleaf.bw')
```

*Exercise:* In IGV, choose hg19. Then, load the greenleaf.bw file and explore chromosomes 21 and 22. *Exercise:* Export the nucleosome-free portion of the data as a BigWig file and examine that in IGV. Where do you expect to see the strongest signals?

---

### 21.4 Additional work

For those working extensively on ATAC-Seq, there is a great workflow/tutorial available from Thomas Carroll:

[https://rockefelleruniversity.github.io/RU\\_ATAC\\_Workshop.html](https://rockefelleruniversity.github.io/RU_ATAC_Workshop.html)

Feel free to work through it. In addition to the work above, there is also the [ATACseqQC](#) package vignette that offers more than just QC. At least a couple more packages are available in *Bioconductor*.

---

## 21.5 Expression prediction

```
k562_exp = read.table('https://www.encodeproject.org/files/ENCFF913LYH/@download/ENCFF913LYH.tsv',
  header = TRUE)

download.file(
  "https://ftp.ebi.ac.uk/pub/databases/gencode/Gencode_human/release_29/gencode.v29.annotation.gtf.gz",
  "gencode.v29.annotation.gtf.gz")

library(GenomicFeatures)
txdb = GenomicFeatures::makeTxDbFromGFF("gencode.v29.annotation.gtf.gz", format="gtf")

gr_genes = genes(txdb)
gr_promoters = promoters(txdb, upstream=1000, downstream=1000)

rownames(k562_exp) = k562_exp$gene_id
```

Match the gene names in the expression data to the gene names in the TxDb object.

```
k562_exp_matched = k562_exp[names(gr_genes),]

atac_bed <- readr::read_tsv("https://www.encodeproject.org/files/ENCFF333TAT/@download/ENCFF333TAT.bed.gz")
colnames(atac_bed)[1:6] <- c("chrom", "start", "end", "name", "score", "strand")

library(GenomicRanges)
gr_atac = makeGRangesFromDataFrame(atac_bed)
n = distanceToNearest(gr_genes, gr_atac)
```

---

## Appendix

### Session info

```
R version 4.3.0 (2023-04-21)
Platform: aarch64-apple-darwin20 (64-bit)
Running under: macOS Ventura 13.1
```

```
Matrix products: default
BLAS: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRblas.0.dylib
LAPACK: /Library/Frameworks/R.framework/Versions/4.3-arm64/Resources/lib/libRlapack.dylib; LAPACK version 3
```

```
locale:  
[1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8  
  
time zone: America/New_York  
tzcode source: internal  
  
attached base packages:  
[1] stats4    stats     graphics  grDevices  utils      datasets  methods  
[8] base  
  
other attached packages:  
[1] rtracklayer_1.60.0  
[2] heatmaps_1.24.0  
[3] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2  
[4] GenomicFeatures_1.52.1  
[5] AnnotationDbi_1.62.2  
[6] dplyr_1.1.2  
[7] ggplot2_3.4.2  
[8] GenomicAlignments_1.36.0  
[9] Rsamtools_2.16.0  
[10] Biostrings_2.68.1  
[11] XVector_0.40.0  
[12] SummarizedExperiment_1.30.2  
[13] Biobase_2.60.0  
[14] MatrixGenerics_1.12.2  
[15] matrixStats_1.0.0  
[16] GenomicRanges_1.52.0  
[17] GenomeInfoDb_1.36.1  
[18] IRanges_2.34.1  
[19] S4Vectors_0.38.1  
[20] BiocGenerics_0.46.0  
[21] knitr_1.43  
  
loaded via a namespace (and not attached):  
[1] DBI_1.1.3                 bitops_1.0-7            biomaRt_2.56.1  
[4] rlang_1.1.1                magrittr_2.0.3           compiler_4.3.0  
[7] RSQLite_2.3.1              mgcv_1.8-42             png_0.1-8  
[10] fftwtools_0.9-11          vctrs_0.6.3             stringr_1.5.0  
[13] pkgconfig_2.0.3            crayon_1.5.2            fastmap_1.1.1  
[16] dbplyr_2.3.2              labeling_0.4.2          utf8_1.2.3  
[19] rmarkdown_2.23             bit_4.0.5               xfun_0.39  
[22] zlibbioc_1.46.0           cachem_1.0.8            jsonlite_1.8.7  
[25] progress_1.2.2             blob_1.2.4              DelayedArray_0.26.6  
[28] BiocParallel_1.34.2        jpeg_0.1-10             tiff_0.1-11  
[31] parallel_4.3.0             prettyunits_1.1.1        R6_2.5.1  
[34] stringi_1.7.12            RColorBrewer_1.1-3       Matrix_1.5-4.1
```

|                       |                     |                         |
|-----------------------|---------------------|-------------------------|
| [37] splines_4.3.0    | tidyselect_1.2.0    | rstudioapi_0.14         |
| [40] abind_1.4-5      | yaml_2.3.7          | EBImage_4.42.0          |
| [43] codetools_0.2-19 | curl_5.0.1          | lattice_0.21-8          |
| [46] tibble_3.2.1     | withr_2.5.0         | KEGGREST_1.40.0         |
| [49] evaluate_0.21    | BiocFileCache_2.8.0 | xml2_1.3.5              |
| [52] pillar_1.9.0     | filelock_1.0.2      | KernSmooth_2.23-21      |
| [55] generics_0.1.3   | RCurl_1.98-1.12     | hms_1.1.3               |
| [58] munsell_0.5.0    | scales_1.2.1        | glue_1.6.2              |
| [61] tools_4.3.0      | BiocIO_1.10.0       | locfit_1.5-9.8          |
| [64] XML_3.99-0.14    | grid_4.3.0          | plotrix_3.8-2           |
| [67] colorspace_2.1-0 | nlme_3.1-162        | GenomeInfoDbData_1.2.10 |
| [70] restfulr_0.0.15  | cli_3.6.1           | rappdirs_0.3.3          |
| [73] fansi_1.0.4      | S4Arrays_1.0.4      | gttable_0.3.3           |
| [76] digest_0.6.31    | rjson_0.2.21        | htmlwidgets_1.6.2       |
| [79] farver_2.1.1     | memoise_2.0.1       | htmltools_0.5.5         |
| [82] lifecycle_1.0.3  | httr_1.4.6          | bit64_4.0.5             |

## MACS2

The MACS2 package is a commonly-used package for calling peaks. Installation and other details are available<sup>1</sup>.

```
pip install macs2
```

---

<sup>1</sup><https://github.com/taoliu/MACS>

---

## References

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- Bourgon, Richard, Robert Gentleman, and Wolfgang Huber. 2010. “Independent Filtering Increases Detection Power for High-Throughput Experiments.” *Proceedings of the National Academy of Sciences* 107 (21): 9546–51. <https://doi.org/10.1073/pnas.0914005107>.
- Brouwer-Visser, Jurriaan, Wei-Yi Cheng, Anna Bauer-Mehren, Daniela Maisel, Katharina Lechner, Emilia Andersson, Joel T. Dudley, and Francesca Milletti. 2018. “Regulatory T-Cell Genes Drive Altered Immune Microenvironment in Adult Solid Cancers and Allow for Immune Contextual Patient Subtyping.” *Cancer Epidemiology, Biomarkers & Prevention* 27 (1): 103–12. <https://doi.org/10.1158/1055-9965.EPI-17-0461>.
- Buenrostro, Jason D, Paul G Giresi, Lisa C Zaba, Howard Y Chang, and William J Greenleaf. 2013. “Transposition of Native Chromatin for Fast and Sensitive Epigenomic Profiling of Open Chromatin, DNA-binding Proteins and Nucleosome Position.” *Nature Methods* 10 (12): 1213–18. <https://doi.org/10.1038/nmeth.2688>.
- Buenrostro, Jason D, Beijing Wu, Howard Y Chang, and William J Greenleaf. 2015. “ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide.” *Current Protocols in Molecular Biology / Edited by Frederick M. Ausubel ... [Et Al.]* 109 (January): 21.29.1–9. <https://doi.org/10.1002/0471142727.mb2129s109>.
- Center, Pew Research. 2016. “Lifelong Learning and Technology.” *Pew Research Center: Internet, Science & Tech.* <https://www.pewresearch.org/internet/2016/03/22/lifelong-learning-and-technology/>.
- Crawford, Gregory E, Sean Davis, Peter C Scacheri, Gabriel Renaud, Mohamad J Halawi, Michael R Erdos, Roland Green, Paul S Meltzer, Tyra G Wolfsberg, and Francis S Collins. 2006. “DNase-chip: A High-Resolution Method to Identify DNase I Hypersensitive Sites Using Tiled Microarrays.” *Nature Methods* 3 (7): 503–9. <http://www.ncbi.nlm.nih.gov/pubmed/16791207?dopt=AbstractPlus>.
- Crawford, Gregory E, Ingeborg E Holt, James Whittle, Bryn D Webb, Denise Tai, Sean Davis, Elliott H Margulies, et al. 2006. “Genome-Wide Mapping of DNase Hypersensitive Sites Using Massively Parallel Signature Sequencing (MPSS).” *Genome Research* 16 (1): 123–31. <http://www.ncbi.nlm.nih.gov/pubmed/16344561?dopt=AbstractPlus>.
- DeRisi, J. L., V. R. Iyer, and P. O. Brown. 1997. “Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale.” *Science (New York, N.Y.)* 278 (5338): 680–86. <https://doi.org/10.1126/science.278.5338.680>.
- Greener, Joe G., Shaun M. Kandathil, Lewis Moffat, and David T. Jones. 2022. “A Guide to Machine Learning for Biologists.” *Nature Reviews Molecular Cell Biology* 23 (1): 40–55. <https://doi.org/10.1038/s41580-021-00407-0>.
- Knowles, Malcolm S., Elwood F. Holton, and Richard A. Swanson. 2005. *The Adult Learner: The Definitive Classic in Adult Education and Human Resource Development*. 6th ed. Amsterdam ; Boston: Elsevier.
- Lawrence, Michael, Wolfgang Huber, Hervé Pagès, Patrick Aboyou, Marc Carlson, Robert Gentleman, Martin T. Morgan, and Vincent J. Carey. 2013a. “Software for Computing and Annotating Genomic Ranges.” *PLoS Computational Biology* 9 (8): e1003118. <https://doi.org/10.1371/journal.pcbi.1003118>.
- Lawrence, Michael, Wolfgang Huber, Hervé Pagès, Patrick Aboyou, Marc Carlson, Robert Gentleman,

- Martin T Morgan, and Vincent J Carey. 2013b. "Software for Computing and Annotating Genomic Ranges." *PLoS Computational Biology* 9 (8): e1003118. <https://doi.org/10.1371/journal.pcbi.1003118>.
- Lee, Stuart, Dianne Cook, and Michael Lawrence. 2019. "Plyranges: A Grammar of Genomic Data Transformation." *Genome Biology* 20 (1): 4. <https://doi.org/10.1186/s13059-018-1597-8>.
- Libbrecht, Maxwell W., and William Stafford Noble. 2015. "Machine Learning Applications in Genetics and Genomics." *Nature Reviews Genetics* 16 (6): 321–32. <https://doi.org/10.1038/nrg3920>.
- Morgan, Martin, Herve Pages, V Obenchain, and N Hayden. 2016. "Rsamtools: Binary Alignment (BAM), FASTA, Variant Call (BCF), and Tabix File Import." *R Package Version* 1 (0): 677–89.
- Student. 1908. "The Probable Error of a Mean." *Biometrika* 6 (1): 1–25. <https://doi.org/10.2307/2331554>.
- Tsompana, Maria, and Michael J Buck. 2014. "Chromatin Accessibility: A Window into the Genome." *Epi-genetics & Chromatin* 7 (1): 33. <https://doi.org/10.1186/1756-8935-7-33>.

# A

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## *Appendix*

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### A.1 Data Sets

- BRFSS subset
  - ALL clinical data
  - ALL expression data
- 

### A.2 Swirl

The following is from the [swirl website](#).

The swirl R package makes it fun and easy to learn R programming and data science. If you are new to R, have no fear.

To get started, we need to install a new package into R.

```
install.packages('swirl')
```

Once installed, we want to load it into the R workspace so we can use it.

```
library('swirl')
```

Finally, to get going, start swirl and follow the instructions.

```
swirl()
```

# B

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## *Additional resources*

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- [Base R Cheat Sheet](#)

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