

A Little Book of R for Bioinformatics 2.0

Avirl Coghlan, with contributions by Nathan L. Brouwer

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```
14 {r, echo = F, eval = F} # knitr::include_graphics(here::here("images/NCBI_accesssion_NC_001477_genban
```

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Preface to version 2.0

Welcome to *A Little Book of R for Bioinformatics 2.0!*.

This book is based on the original *A Little Book of R for Bioinformatics* by Dr. Avril Coghlan (Hereafter “ALBRB 1.0”). Dr. Coghlan’s book was one of the first and most thorough introductions to using *R* for bioinformatics and computational biology, and was generously published under the Creative Commons 3.0 Attribution License (CC BY 3.0). In addition to describing how to do bioinformatics in *R*, Coghlan provided numerous functions to facilitate important tasks, practice questions, and references to further reading.

ALBRB 1.0 was extremely useful to me when I was learning bioinformatics and computational biology. In this version of the book, which I’ll refer to as **ALBRB 2.0**, I have adapted Dr. Coghlan’s original book to suit my own teaching needs, updated it with current packages now available in *R*, added background materials from other open-access sources, and added in my original materials.

Below I’ve outlined the general types of changes I’ve made to the original book. I have tried to link back to the original content that these updates are derived from and note how changes were made. Any errors or inconsistencies should be ascribed to me, not Dr. Coghlan. If you have any feedback, please email me at brouwern@gmail.com

~Nathan Brouwer, June 2021

Changes implemented in ALBRB 2.0 by Nathan Brouwer

1. Converted the entire book to RMarkdown and published it via bookdown.
2. Added instructions for using RStudio and RStudio Cloud.
3. Updated instructions to reflect any changes in software, including changes to how the bioinformatics repository Bioconductor now works.
4. Split up chapters into smaller units.
5. Reorganized the order of some material.
6. Added biological background information by integrating information from the Open Access textbook LibreText General Biology
7. Added links to the books I am developing, *Get R Done!* and *Computational Biology for All*.
8. Moved functions written by Coghlan and datasets to my teaching package *compbio4all*.
9. Functions names changed from camelCase to snake_case
10. Functions re-written so as not to use Bioconductor to reduce/eliminate dependency of *compbio4all* on Bioconductor.
11. Changed some plotting to ggplot2 or ggpubr.
12. Added additional subheadings
13. Added vocab and function lists to the beginning of many chapters
14. At times replaced non-biological examples with biological ones.
15. Change from British to American English (Sorry! Couldn’t help myself - the spellchecker made me do it!)
16. Provided additional links to external resources.
17. Added use of *rentrez* for querying NCBI databases

Chapter 1

Downloading R

By: Avril Coghlan

Adapted, edited and expanded: Nathan Brouwer (brouwern@gmail.com) under the Creative Commons 3.0 Attribution License (CC BY 3.0).

1.1 Preface

The following introduction to *R* is based on the first part of “How to install *R* and a Brief Introduction to *R*” by Avril Coghlan, which was released under the Creative Commons 3.0 Attribution License (CC BY 3.0). For additional information see the Appendices and “Getting *R* onto your computer”.

1.2 Introduction to R

R (www.r-project.org) is a commonly used free statistics software. *R* allows you to carry out statistical analyses in an interactive mode, as well as allowing programming.

1.3 Installing R

To use *R*, you first need to install the *R* program on your computer.

1.3.1 Installing *R* on a Windows PC

These instructions will focus on installing *R* on a Windows PC. However, I will also briefly mention how to install *R* on a Macintosh or Linux computer (see below).

These steps have not been checked as of 8/13/2019 so there may be small variations in what the prompts are. Installing R, however, is basically that same as any other program. Clicking “Yes” etc on everything should work.

PROTIP: Even if you have used *R* before its good to regularly update it to avoid conflicts with recently produced software.

Minor updates of *R* are made very regularly (approximately every 6 months), as *R* is actively being improved all the time. It is worthwhile installing new versions of *R* a couple times a year, to make sure that you have a recent version of *R* (to ensure compatibility with all the latest versions of the *R* packages that you have downloaded).

To install *R* on your **Windows** computer, follow these steps:

1. Go to <https://cran.r-project.org/>
2. Under “Download and Install R”, click on the “Windows” link.
3. Under “Subdirectories”, click on the “**base**” link.
4. On the next page, you should see a link saying something like “Download *R* 4.1.0 for Windows” (or *R* X.X.X, where X.X.X gives the version of the program). Click on this link.
5. You may be asked if you want to save or run a file “R-x.x.x-win32.exe”. Choose “Save” and save the file. Then double-click on the icon for the file to run it.
6. You will be asked what language to install it in.
7. The *R* Setup Wizard will appear in a window. Click “Next” at the bottom of the *R* Setup wizard window.
8. The next page says “Information” at the top. Click “Next” again.
9. The next page says “Select Destination Location” at the top. By default, it will suggest to install *R* on the C drive in the “Program Files” directory on your computer.
10. Click “Next” at the bottom of the *R* Setup wizard window.
11. The next page says “Select components” at the top. Click “Next” again.
12. The next page says “Startup options” at the top. Click “Next” again.
13. The next page says “Select start menu folder” at the top. Click “Next” again.
14. The next page says “Select additional tasks” at the top. Click “Next” again.
15. *R* should now be installing. This will take about a minute. When *R* has finished, you will see “Completing the *R* for Windows Setup Wizard” appear. Click “Finish”.
16. To start *R*, you can do one of the following steps:
17. Check if there is an “R” icon on the desktop of the computer that you are using. If so, double-click on the “R” icon to start *R*. If you cannot find an “R” icon, try the next step instead.
18. Click on the “Start” button at the bottom left of your computer screen, and then choose “All programs”, and start *R* by selecting “R” (or *R* X.X.X, where X.X.X gives the version of *R*) from the menu of programs.
19. The *R* console (a rectangle) should pop up:

1.3.2 How to install *R* on non-Windows computers (eg. Macintosh or Linux computers)

These steps have not been checked as of 8/13/2019 so there may be small variations in what the prompts are. Installing *R*, however, is basically that same as any other program. Clicking “Yes” etc on everything should work.

The instructions above are for installing *R* on a Windows PC. If you want to install *R* on a computer that has a non-Windows operating system (for example, a Macintosh or computer running Linux, you should download the appropriate *R* installer for that operating system at <https://cran.r-project.org/> and follow the *R* installation instructions for the appropriate operating system at https://cran.r-project.org/doc/FAQ/R-FAQ.html#How-can-R-be-installed_003f.

1.4 Starting *R*

To start *R*, Check if there is an *R* icon on the desktop of the computer that you are using. If so, double-click on the *R* icon to start *R*. If you cannot find an *R* icon, try the next step instead.

You can also start *R* from the Start menu in Windows. Click on the “Start” button at the bottom left of your computer screen, and then choose “All programs”, and start *R* by selecting “R” (or *R* X.X.X, where X.X.X gives the version of *R*, e.g.. *R* 2.10.0) from the menu of programs.

Say “Hi” to *R* and take a quick look at how it looks. Now say “Goodbye”, because we will never actually do any work in this version of *R*; instead, we’ll use the **RStudio IDE (integrated development environment)**.

Chapter 2

Installing the RStudio IDE {`$installRStudio`}

By: Nathan Brouwer

The name “R” refers both to the programming language and the program that runs that language. When you download *itR** there is also a basic **GUI** (graphical user interface) that you can access via the *R* icon.

Other GUIs are available, and the most popular currently is **RStudio**. RStudio a for-profit company that is a main driver of development of R. Much of what they produce has free basic versions or is entirely free. They produce software (RStudio), cloud-based applications (**RStudio Cloud**), and web server infrastructure for business applications of R.

A brief overview of installing RStudio can be found here “Getting RStudio on to your computer”

2.1 Getting to know RStudio

For a brief overview of RStudio see “Getting started with RStudio”

A good overview of what the different parts of RStudio can be seen in the image in this tweet: <https://twitter.com/RLadiesNCL/status/1138812826917724160?s=20>

2.2 RStudio versus RStudio Cloud

RStudio and RStudio cloud work almost identically, so anything you read about RStudio will apply to RStudio Cloud. RStudio is easy to download and use, but RStudio Cloud eliminates even the minor hiccups that occur. Free accounts with RStudio Cloud allow up to 15 hours per month, which is enough for you to get a taste for using R.

Chapter 3

Installing *R* packages

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

R is a programming language, and **packages** (aka **libraries**) are bundles of software built using *R*. Most sessions using *R* involve using additional *R* packages. This is especially true for bioinformatics and computational biology.

NOTE: If you are working in an RStudio Cloud environment organized by someone else (e.g. a course instructor), they likely are taking care of many of the package management issues. The following information is still useful to be familiar with.

3.1 Downloading packages with the RStudio IDE

There is a point-and-click interface for installing *R* packages in RStudio. There is a brief introduction to downloading packages on this site: <http://web.cs.ucla.edu/~gulzar/rstudio/>

I’ve summarized it here:

1. “Click on the”Packages” tab in the bottom-right section and then click on “Install”. The following dialog box will appear.
2. In the “Install Packages” dialog, write the package name you want to install under the Packages field and then click install. This will install the package you searched for or give you a list of matching package based on your package text.

3.2 Downloading packages with the function `install.packages()`

The easiest way to install a package if you know its name is to use the *R* function `install.packages()`. Note that it might be better to call this “download.packages” since after you install it, you also have to load it!

Frequently I will include `install.packages(...)` at the beginning of a chapter the first time we use a package to make sure the package is downloaded. Note, however, that if you already have downloaded the package, running `install.packages(...)` will download a new copy. While packages do get updated from time to time, but its best to re-run `install.packages(...)` only occasionally.

We’ll download a package used for plotting called `ggplot2`, which stands for “Grammar of Graphics.” `ggplot2` was developed by Dr. Hadley Wickham, who is now the Chief Scientists for RStudio.

To download `ggplot2`, run the following command:

```
install.packages("ggplot2") # note the " "
```

Often when you download a package you'll see a fair bit of angry-looking red text, and sometime other things will pop up. Usually there's nothing of interest here, but sometimes you need to read things carefully over it for hints about why something didn't work.

3.3 Using packages after they are downloaded

To actually make the functions in package accessible you need to use the `library()` command. Note that this is *not* in quotes.

```
library(ggplot2) # note: NO " "
```

Chapter 4

Installing Bioconductor

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0), including details on install Bioconductor and common prompts and error messages that appear during installation.

4.1 Bioconductor

R **packages** (aka “libraries”) can live in many places. Most are accessed via **CRAN**, the **Comprehensive R Archive Network**. The bioinformatics and computational biology community also has its own package hosting system called Bioconductor. *R* has played an important part in the development and application of bioinformatics techniques in the 21st century. Bioconductor 1.0 was released in 2002 with 15 packages. As of winter 2021, there are almost 2000 packages in the current release!

NOTE: If you are working in an RStudio Cloud environment organized by someone else (eg a course instructor), they likely are taking care of most of package management issues, including setting up Bioconductor. The following information is still useful to be familiar with.

To interface with Bioconductor you need the BiocManager package. The Bioconductor people have put BiocManager on CRAN to allow you to set up interactions with Bioconductor. See the BiocManager documentation for more information (<https://cran.r-project.org/web/packages/BiocManager/vignettes/BiocManager.html>).

Note that if you have an old version of R you will need to update it to interact with Bioconductor.

4.2 Installing BiocManager

BiocManager can be installed using the `install.packages()` packages command.

```
install.packages("BiocManager") # Remember the " "; don't worry about the red  
text
```

Once downloaded, BiocManager needs to be explicitly loaded into your active R session using `library()`

```
library(BiocManager) # no quotes; again, ignore the red text
```

Individual Bioconductor packages can then be downloaded using the `install()` command. An essential packages is Biostrings. To do this ,

```
BiocManager::install("Biostrings")
```

4.3 The ins and outs of package installation

IMPORTANT Bioconductor has many **dependencies** - other packages which it relies on. When you install Bioconductor packages you may need to update these packages. If something seems to not be working during this process, restart R and begin the Bioconductor installation process until things seem to work.

Below I discuss the series of prompts I had to deal with while re-installing Biostrings while editing this chapter.

4.3.1 Updating other packages when downloading a package

When I re-installed Biostrings while writing this I was given a HUGE block of red text that contained something like what's shown below (this is only about 1/3 of the actual output!):

```
'getOption("repos")' replaces Bioconductor standard repositories ,
see '?repositories' for details
```

```
replacement repositories:
```

```
CRAN: https://cran.rstudio.com/
```

```
Bioconductor version 3.11 (BiocManager 1.30.16), R 4.0.5 (2021-03-31)
```

```
Old packages: 'ade4', 'ape', 'aster', 'bayestestR',
'bio3d', 'bitops', 'blogdown',
'bookdown', 'brio', 'broom', 'broom.mixed',
'broomExtra', 'bslib', 'cachem', 'callr',
'car', 'circlize', 'class', 'cli', 'cluster',
'colorspace', 'corrplot', 'cpp11', 'curl',
'devtools', 'DHARMa', 'doBy', 'dplyr', 'DT',
'e1071', 'ellipsis', 'emmeans', 'emojifont',
'extRemes', 'fansi', 'flextable', 'forecast',
'formatR', 'gap', 'gargle', 'gert', 'GGally'
```

Hidden at the bottom was a prompt: "Update all/some/none? [a/s/n]:"

It's a little vague, but what it wants me to do is type in a, s or n and press enter to tell it what to do. I almost always chose a, though this may take a while to update everything.

4.3.2 Packages “from source”

You are likely to get lots of random-looking feedback from R when doing Bioconductor-related installations. Look carefully for any prompts as the very last line. While updating Biostrings I was told: “*There are binary versions available but the source versions are later:*” and given a table of packages. I was then asked “*Do you want to install from sources the packages which need compilation? (Yes/no/cancel)*”

I almost always chose “no”.

4.3.3 More on angry red text

After the prompt about packages from source, R proceeded to download a lot of updates to packages, which took a few minutes. Lots of red text scrolled by, but this is normal.


```
~/google_backup_sync_mB24/lbrb/
~/google_backup_sync_mB24/lbrb/
trying URL 'https://cran.rstudio.com/bin/macosx/contrib/4.0/tidytree_0.3.4.tgz'
Content type 'application/x-gzip' length 198800 bytes (194 KB)
downloaded 194 KB

trying URL 'https://cran.rstudio.com/bin/macosx/contrib/4.0/tinytex_0.32.tgz'
Content type 'application/x-gzip' length 121221 bytes (118 KB)
downloaded 118 KB

trying URL 'https://cran.rstudio.com/bin/macosx/contrib/4.0/tufte_0.10.tgz'
Content type 'application/x-gzip' length 269000 bytes (262 KB)
downloaded 262 KB

trying URL 'https://cran.rstudio.com/bin/macosx/contrib/4.0/UnitProtR_2.0.7.tgz'
Content type 'application/x-gzip' length 236776 bytes (231 KB)
downloaded 231 KB

trying URL 'https://cran.rstudio.com/bin/macosx/contrib/4.0/units_0.7-2.tgz'
Content type 'application/x-gzip' length 1268870 bytes (1.2 MB)
downloaded 1.2 MB
```

4.4 Actually loading a package

Again, to actually load the Biostrings package into your active R sessions requires the `library()` command:

`library(Biostrings)`

As you might expect, there's more red text scrolling up my screen!

```
~/google_backup_sync_mB24/lbrb/
~/google_backup_sync_mB24/lbrb/
The following objects are masked from 'package:base':
  anyDuplicated, append, as.data.frame, basename, cbind, colnames, dirname,
  do.call, duplicated, eval, evalq, Filter, find, get, grep, grepl, intersect,
  is.unsorted, lapply, Map, mapply, match, mget, order, paste, pmax, pmax.int,
  pmin, pmin.int, Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
  table, tapply, union, unique, unsplit, which, which.max, which.min

Loading required package: S4Vectors
Loading required package: stats4
Attaching package: 'S4Vectors'

The following object is masked from 'package:base':
  expand.grid

Loading required package: IRanges
Loading required package: XVector
Attaching package: 'Biostrings'

The following object is masked from 'package:base':
  strsplit

> |
```

I can tell that is actually worked because at the end of all the red stuff is the R prompt of “>” and my cursor.



Chapter 5

A Brief introduction to R

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

This chapter provides a brief introduction to R. At the end of are links to additional resources for getting started with R.

5.1 Vocabulary

- scalar
- vector
- list
- class
- numeric
- character
- assignment
- elements of an object
- indices
- attributes of an object
- argument of a function

5.2 R functions

- `<-`
- `[]`
- `$`
- `table()`
- `function`
- `c()`
- `log10()`
- `help()`, `?`
- `help.search()`
- `RSiteSearch()`
- `mean()`
- `return()`
- `q()`

5.3 Interacting with R

You will type *R* commands into the RStudio **console** in order to carry out analyses in *R*. In the RStudio console you will see the R prompt starting with the symbol “>”. “>” will always be there at the beginning of each new command - don’t try to delete it! Moreover, you never need to type it.



We type the **commands** needed for a particular task after this prompt. The command is carried out by *R* after you hit the Return key.

Once you have started *R*, you can start typing commands into the RStudio console, and the results will be calculated immediately, for example:

```
2*3
```

```
## [1] 6
```

Note that prior to the output of “6” it shows “[1]”.

Now subtraction:

```
10-3
```

```
## [1] 7
```

Again, prior to the output of “7” it shows “[1]”.

R can act like a basic calculator that you type commands in to. You can also use it like a more advanced scientific calculator and create **variables** that store information. All variables created by *R* are called **objects**. In *R*, we assign values to variables using an arrow-looking function `<-` the **assignment operator**. For example, we can **assign** the value `2*3` to the variable `x` using the command:

```
x <- 2*3
```

To view the contents of any *R* object, just type its name, press enter, and the contents of that *R* object will be displayed:

```
x
```

```
## [1] 6
```

5.4 Variables in R

There are several different types of objects in *R* with fancy math names, including **scalars**, **vectors**, **matrices** (singular: **matrix**), arrays, dataframes, tables, **and** lists. The scalar** variable `x` above is one example of an *R* object. While a scalar variable such as `x` has just one element, a **vector** consists of several elements. The elements in a vector are all of the same **type** (e.g.. numbers or alphabetic characters), while **lists** may include elements such as characters as well as numeric quantities. Vectors and dataframes are the most common variables you’ll use. You’ll also encounter matrices often, and lists are ubiquitous in *R* but beginning users often don’t encounter them because they remain behind the scenes.

5.4.1 Vectors

To create a vector, we can use the `c()` (combine) function. For example, to create a vector called `myvector` that has elements with values 8, 6, 9, 10, and 5, we type:

```
myvector <- c(8, 6, 9, 10, 5) # note: commas between each number!
```

To see the contents of the variable `myvector`, we can just type its name and press enter:

```
myvector
```

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

5.4.2 Vector indexing

The `[1]` is the **index** of the first **element** in the vector. We can **extract** any element of the vector by typing the vector name with the index of that element given in **square brackets** `[...]`.

For example, to get the value of the 4th element in the vector `myvector`, we type:

```
myvector[4]
```

```
## [1] 10
```

5.4.3 Character vectors

Vectors can contain letters, such as those designating nucleic acids

```
my.seq <- c("A", "T", "C", "G")
```

They can also contain multi-letter **strings**:

```
my.oligos <- c("ATCGC", "TTTCGC", "CCCGCG", "GGGCGC")
```

5.4.4 Lists

NOTE: *below is a discussion of lists in R. This is excellent information, but not necessary if this is your very very first time using R.*

In contrast to a vector, a **list** can contain elements of different types, for example, both numbers and letters. A list can even include other variables such as a vector. The `list()` function is used to create a list. For example, we could create a list `mylist` by typing:

```
mylist <- list(name="Charles_Darwin",
               wife="Emma_Darwin",
               myvector)
```

We can then print out the contents of the list `mylist` by typing its name:

```
mylist
```

```
## $name
## [1] "Charles Darwin"
##
## $wife
## [1] "Emma Darwin"
##
## [[3]]
## [1] 8 6 9 10 5
```

The **elements** in a list are numbered, and can be referred to using **indices**. We can extract an element of a list by typing the list name with the index of the element given in double **square brackets** (in contrast to a vector, where we only use single square brackets).

We can extract the second element from `mylist` by typing:

```
mylist[[2]] # note the double square brackets [...]]
```

```
## [1] "Emma Darwin"
```

As a baby step towards our next task, we can wrap index values as in the `c()` command like this:

```
mylist[[c(2)]] # note the double square brackets [...]]
```

```
## [1] "Emma Darwin"
```

The number 2 and `c(2)` mean the same thing.

Now, we can extract the second AND third elements from `mylist`. First, we put the indices 2 and 3 into a vector `c(2,3)`, then wrap that vector in double square brackets: `[c(2,3)]`. All together it looks like this.

```
mylist[c(2,3)] # note the double brackets
```

```
## $wife
## [1] "Emma Darwin"
##
## [[2]]
## [1] 8 6 9 10 5
```

Elements of lists may also be named, resulting in a **named lists**. The elements may then be referred to by giving the list name, followed by "\$", followed by the element name. For example, `mylist$name` is the same as `mylist[[1]]` and `mylist$wife` is the same as `mylist[[2]]`:

```
mylist$name
```

```
## [1] "Emma Darwin"
```

We can find out the names of the named elements in a list by using the `attributes()` function, for example:

```
attributes(mylist)
```

```
## $names
## [1] "name" "wife" ""
```

When you use the `attributes()` function to find the named elements of a list variable, the named elements are always listed under a heading "\$names". Therefore, we see that the named elements of the list variable `mylist` are called "name" and "wife", and we can retrieve their values by typing `mylist$name` and `mylist$wife`, respectively.

5.4.5 Tables

Another type of object that you will encounter in R is a **table**. The `table()` function allows you to total up or tabulate the number of times a value occurs within a vector. Tables are typically used on vectors containing **character data**, such as letters, words, or names, but can work on numeric data data.

5.4.5.1 Tables - The basics

If we made a vector variable “nucleotides” containing the of a DNA molecule, we can use the `table()` function to produce a **table variable** that contains the number of bases with each possible nucleotides:

```
bases <- c("A", "T", "A", "A", "T", "C", "G", "C", "G")
```

Now make the table

```
table(bases)
```

```
## bases
## A C G T
## 3 2 2 2
```

We can store the table variable produced by the function `table()`, and call the stored table “bases.table”, by typing:

```
bases.table <- table(bases)
```

Tables also work on vectors containing numbers. First, a vector of numbers.

```
numeric.vecter <- c(1,1,1,1,3,4,4,4,4)
```

Second, a table, showing how many times each number occurs.

```
table(numeric.vecter)
```

```
## numeric.vecter
## 1 3 4
## 4 1 4
```

5.4.5.2 Tables - further details

To access elements in a table variable, you need to use double square brackets, just like accessing elements in a list. For example, to access the fourth element in the table `bases.table` (the number of Ts in the sequence), we type:

```
bases.table[[4]] # double brackets!
```

```
## [1] 2
```

Alternatively, you can use the name of the fourth element in the table (“John”) to find the value of that table element:

```
bases.table[["T"]]
```

```
## [1] 2
```

5.5 Arguments

Functions in R usually require **arguments**, which are input variables (i.e.. objects) that are **passed** to them, which they then carry out some operation on. For example, the `log10()` function is passed a number, and it then calculates the log to the base 10 of that number:

```
log10(100)
```

```
## [1] 2
```

There's a more generic function, `log()`, where we pass it not only a number to take the log of, but also the specific **base** of the logarithm. To take the log base 10 with the `log()` function we do this

```
log(100, base = 10)
```

```
## [1] 2
```

We can also take logs with other bases, such as 2:

```
log(100, base = 2)
```

```
## [1] 6.643856
```

5.6 Help files with `help()` and `?`

In *R*, you can get help about a particular function by using the `help()` function. For example, if you want help about the `log10()` function, you can type:

```
help("log10")
```

When you use the `help()` function, a box or web page will show up in one of the panes of RStudio with information about the function that you asked for help with. You can also use the `?` next to the function like this

```
?log10
```

Help files are a mixed bag in *R*, and it can take some getting used to them. An excellent overview of this is Kieran Healy's "How to read an *R* help page."

5.7 Searching for functions with `help.search()` and `RSiteSearch()`

If you are not sure of the name of a function, but think you know part of its name, you can search for the function name using the `help.search()` and `RSiteSearch()` functions. The `help.search()` function searches to see if you already have a function installed (from one of the *R* packages that you have installed) that may be related to some topic you're interested in. `RSiteSearch()` searches *all* *R* functions (including those in packages that you haven't yet installed) for functions related to the topic you are interested in.

For example, if you want to know if there is a function to calculate the standard deviation (SD) of a set of numbers, you can search for the names of all installed functions containing the word "deviation" in their description by typing:

```
help.search("deviation")
```

Among the functions that were found, is the function `sd()` in the `stats` package (an *R* package that comes with the base *R* installation), which is used for calculating the standard deviation.

Now, instead of searching just the packages we've have on our computer let's search all *R* packages on CRAN. Let's look for things related to DNA. Note that `RSiteSearch()` doesn't provide output within RStudio, but rather opens up your web browser for you to display the results.

```
RSiteSearch("DNA")
```

The results of the `RSiteSearch()` function will be hits to descriptions of *R* functions, as well as to *R* mailing list discussions of those functions.

5.8 More on functions

We can perform computations with R using objects such as scalars and vectors. For example, to calculate the average of the values in the vector `myvector` (i.e.. the average of 8, 6, 9, 10 and 5), we can use the `mean()` function:

```
mean(myvector) # note: no " "  
## [1] 7.6
```

We have been using built-in R functions such as `mean()`, `length()`, `print()`, `plot()`, etc.

5.8.1 Writing your own functions

NOTE: *Writing your own functions is an advanced skills. New users can skip this section.

We can also create our own functions in R to do calculations that you want to carry out very often on different input data sets. For example, we can create a function to calculate the value of 20 plus square of some input number:

```
myfunction <- function(x) { return(20 + (x*x)) }
```

This function will calculate the square of a number (`x`), and then add 20 to that value. The `return()` statement returns the calculated value. Once you have typed in this function, the function is then available for use. For example, we can use the function for different input numbers (e.g.. 10, 25):

```
myfunction(10)  
## [1] 120
```

5.9 Quitting R

To quit R either close the program, or type:

```
q()
```

5.10 Links and Further Reading

Some links are included here for further reading.

For a more in-depth introduction to R, a good online tutorial is available on the “Kickstarting R” website, cran.r-project.org/doc/contrib/Lemon-kickstart.

There is another nice (slightly more in-depth) tutorial to R available on the “Introduction to R” website, cran.r-project.org/doc/manuals/R-intro.html.

Chapter 3 of Danielle Navarro’s book is an excellent intro to the basics of R.

Chapter 6

Nucleic Acids

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Nucleic acids are the most important macromolecules for the continuity of life. They carry the genetic blueprint of a cell and carry instructions for the functioning of the cell.

6.1 DNA and RNA

The two main types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multicellular mammals. It is found in the nucleus of eukaryotes and in the organelles, chloroplasts, and mitochondria. In prokaryotes, the DNA is not enclosed in a membranous envelope.

The entire genetic content of a cell is known as its genome, and the study of genomes is genomics. In eukaryotic cells but not in prokaryotes, DNA forms a complex with histone proteins to form chromatin, the substance of eukaryotic chromosomes. A chromosome may contain tens of thousands of genes. Many genes contain the information to make protein products; other genes code for RNA products. DNA controls all of the cellular activities by turning the genes “on” or “off.”

The other type of nucleic acid, RNA, is mostly involved in protein synthesis. The DNA molecules never leave the nucleus but instead use an intermediary to communicate with the rest of the cell. This intermediary is the messenger RNA (mRNA). Other types of RNA—like rRNA, tRNA, and microRNA—are involved in protein synthesis and its regulation.

DNA and RNA are made up of monomers known as nucleotides. The nucleotides combine with each other to form a polynucleotide, DNA or RNA. Each nucleotide is made up of three components: a nitrogenous base, a pentose (five-carbon) sugar, and a phosphate group (Figure 3.5.1). Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to one or more phosphate groups.

The nitrogenous bases, important components of nucleotides, are organic molecules and are so named because they contain carbon and nitrogen. They are bases because they contain an amino group that has the potential of binding an extra hydrogen, and thus, decreases the hydrogen ion concentration in its environment, making it more basic. Each nucleotide in DNA contains one of four possible nitrogenous bases: adenine (A), guanine (G) cytosine (C), and thymine (T).

Adenine and guanine are classified as purines. The primary structure of a purine is two carbon-nitrogen rings. Cytosine, thymine, and uracil are classified as pyrimidines which have a single carbon-nitrogen ring

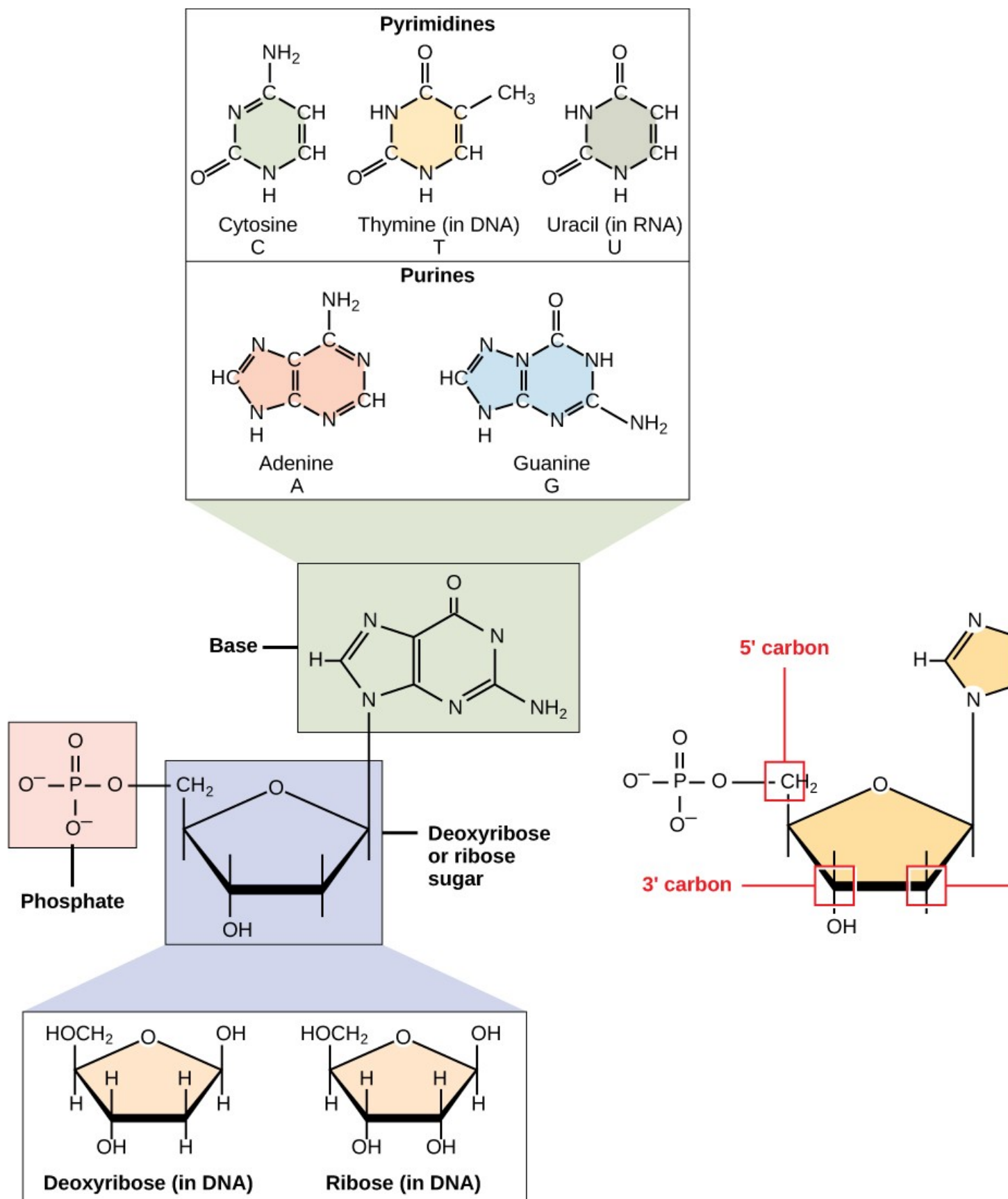


Figure 6.1: A nucleotide is made up of three components: a nitrogenous base, a pentose sugar, and one or more phosphate groups. Carbon residues in the pentose are numbered 1 through 5 (the prime distinguishes these residues from those in the base, which are numbered without using a prime notation). The base is attached to the 1 position of the ribose, and the phosphate is attached to the 5 position. When a polynucleotide is formed, the 5 phosphate of the incoming nucleotide attaches to the 3 hydroxyl group at

as their primary structure (Figure 3.5.1). Each of these basic carbon-nitrogen rings has different functional groups attached to it. In molecular biology shorthand, the nitrogenous bases are simply known by their symbols A, T, G, C, and U. DNA contains A, T, G, and C whereas RNA contains A, U, G, and C.

The pentose sugar in DNA is deoxyribose, and in RNA, the sugar is ribose (Figure 3.5.1). The difference between the sugars is the presence of the hydroxyl group on the second carbon of the ribose and hydrogen on the second carbon of the deoxyribose. The carbon atoms of the sugar molecule are numbered as 1', 2', 3', 4', and 5' (1' is read as "one prime"). The phosphate residue is attached to the hydroxyl group of the 5' carbon of one sugar and the hydroxyl group of the 3' carbon of the sugar of the next nucleotide, which forms a 5'–3' phosphodiester linkage. The phosphodiester linkage is not formed by simple dehydration reaction like the other linkages connecting monomers in macromolecules: its formation involves the removal of two phosphate groups. A polynucleotide may have thousands of such phosphodiester linkages.

6.2 DNA Double-Helix Structure

DNA has a double-helix structure (Figure 3.5.2). The sugar and phosphate lie on the outside of the helix, forming the backbone of the DNA. The nitrogenous bases are stacked in the interior, like the steps of a staircase, in pairs; the pairs are bound to each other by hydrogen bonds. Every base pair in the double helix is separated from the next base pair by 0.34 nm. The two strands of the helix run in opposite directions, meaning that the 5' carbon end of one strand will face the 3' carbon end of its matching strand. (This is referred to as antiparallel orientation and is important to DNA replication and in many nucleic acid interactions.)

Only certain types of base pairing are allowed. For example, a certain purine can only pair with a certain pyrimidine. This means A can pair with T, and G can pair with C, as shown in Figure 3.5.3. This is known as the base complementary rule. In other words, the DNA strands are complementary to each other. If the sequence of one strand is AATTGGCC, the complementary strand would have the sequence TTAACCGG. During DNA replication, each strand is copied, resulting in a daughter DNA double helix containing one parental DNA strand and a newly synthesized strand.

Art Connection

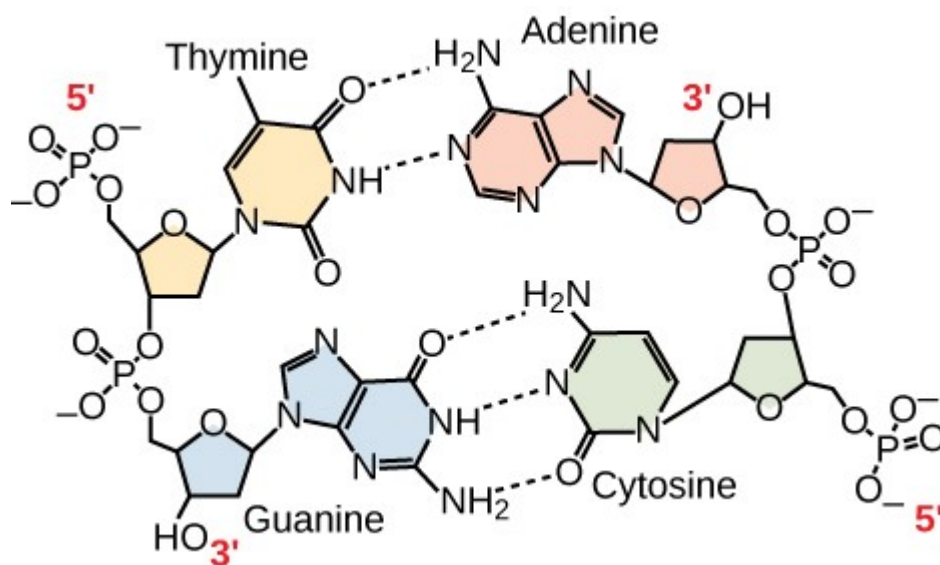


Figure 3.5.3 : In a double stranded DNA molecule, the two strands run antiparallel to one another so that one strand runs 5' to 3' and the other 3' to 5'. The phosphate backbone is located on the outside, and the bases are in the middle. Adenine forms hydrogen bonds (or base pairs) with thymine, and guanine base pairs with cytosine.

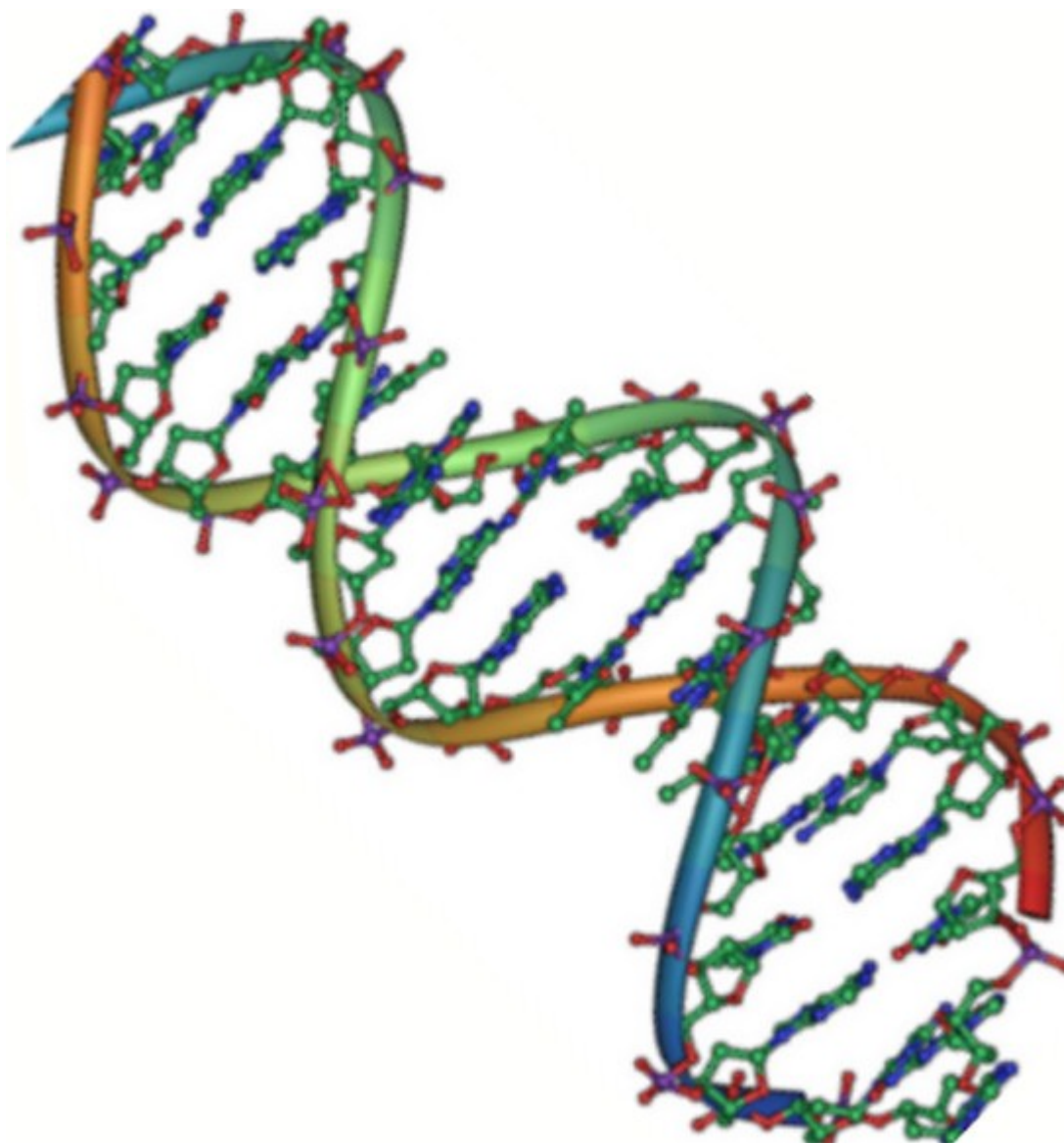


Figure 6.2: Figure 3.5.2 : Native DNA is an antiparallel double helix. The phosphate backbone (indicated by the curvy lines) is on the outside, and the bases are on the inside. Each base from one strand interacts via hydrogen bonding with a base from the opposing strand. (credit: Jerome Walker/Dennis Myts)

A mutation occurs, and cytosine is replaced with adenine. What impact do you think this will have on the DNA structure?

6.3 RNA

Ribonucleic acid, or RNA, is mainly involved in the process of protein synthesis under the direction of DNA. RNA is usually single-stranded and is made of ribonucleotides that are linked by phosphodiester bonds. A ribonucleotide in the RNA chain contains ribose (the pentose sugar), one of the four nitrogenous bases (A, U, G, and C), and the phosphate group.

There are four major types of RNA: messenger RNA (mRNA), ribosomal RNA (rRNA), transfer RNA (tRNA), and microRNA (miRNA). The first, mRNA, carries the message from DNA, which controls all of the cellular activities in a cell. If a cell requires a certain protein to be synthesized, the gene for this product is turned “on” and the messenger RNA is synthesized in the nucleus. The RNA base sequence is complementary to the coding sequence of the DNA from which it has been copied. However, in RNA, the base T is absent and U is present instead. If the DNA strand has a sequence AATTGCGC, the sequence of the complementary RNA is UUAACGCG. In the cytoplasm, the mRNA interacts with ribosomes and other cellular machinery (Figure 3.5.4).

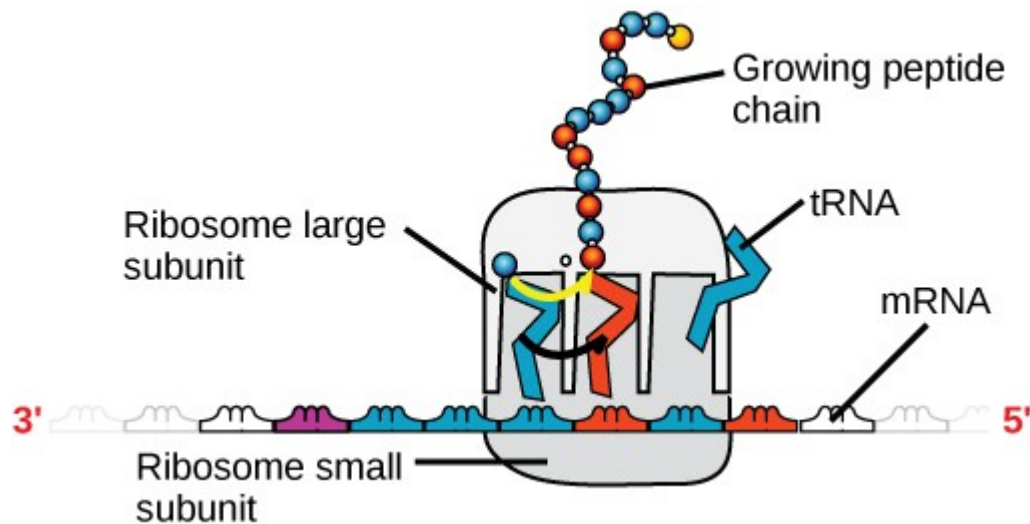


Figure 3.5.4 : A ribosome has two parts: a large subunit and a small subunit. The mRNA sits in between the two subunits. A tRNA molecule recognizes a codon on the mRNA, binds to it by complementary base pairing, and adds the correct amino acid to the growing peptide chain.

The mRNA is read in sets of three bases known as codons. Each codon codes for a single amino acid. In this way, the mRNA is read and the protein product is made. Ribosomal RNA (rRNA) is a major constituent of ribosomes on which the mRNA binds. The rRNA ensures the proper alignment of the mRNA and the ribosomes; the rRNA of the ribosome also has an enzymatic activity (peptidyl transferase) and catalyzes the formation of the peptide bonds between two aligned amino acids. Transfer RNA (tRNA) is one of the smallest of the four types of RNA, usually 70–90 nucleotides long. It carries the correct amino acid to the site of protein synthesis. It is the base pairing between the tRNA and mRNA that allows for the correct amino acid to be inserted in the polypeptide chain. microRNAs are the smallest RNA molecules and their role involves the regulation of gene expression by interfering with the expression of certain mRNA messages. Table 3.5.1 below summarizes features of DNA and RNA.

Table 3.5.1 *: Features of DNA and RNA.

	Features of DNA and RNA	
	DNA	RNA
Function	Carries genetic information	Involved in protein synthesis
Location	Remains in the nucleus	Leaves the nucleus
Structure	Double helix	Usually single-stranded
Sugar	Deoxyribose	Ribose
Pyrimidines	Cytosine, thymine	Cytosine, uracil
Purines	Adenine, guanine	Adenine, guanine

Even though the RNA is single stranded, most RNA types show extensive intramolecular base pairing between complementary sequences, creating a predictable three-dimensional structure essential for their function.

As you have learned, information flow in an organism takes place from DNA to RNA to protein. DNA dictates the structure of mRNA in a process known as transcription, and RNA dictates the structure of protein in a process known as translation. This is known as the Central Dogma of Life, which holds true for all organisms; however, exceptions to the rule occur in connection with viral infections.

Link to Learning



- To learn more about DNA, explore the Howard Hughes Medical Institute BioInteractive animations on the topic of DNA.*

6.4 Summary

Nucleic acids are molecules made up of nucleotides that direct cellular activities such as cell division and protein synthesis. Each nucleotide is made up of a pentose sugar, a nitrogenous base, and a phosphate group. There are two types of nucleic acids: DNA and RNA. DNA carries the genetic blueprint of the cell and is passed on from parents to offspring (in the form of chromosomes). It has a double-helical structure with the two strands running in opposite directions, connected by hydrogen bonds, and complementary to each other. RNA is single-stranded and is made of a pentose sugar (ribose), a nitrogenous base, and a phosphate group. RNA is involved in protein synthesis and its regulation. Messenger RNA (mRNA) is copied from the DNA, is exported from the nucleus to the cytoplasm, and contains information for the construction of proteins. Ribosomal RNA (rRNA) is a part of the ribosomes at the site of protein synthesis, whereas transfer RNA (tRNA) carries the amino acid to the site of protein synthesis. microRNA regulates the use of mRNA for protein synthesis.

Art Connections

6.5 Analysis Questions

Free Response

6.6 Glossary

deoxyribonucleic acid (DNA): double-helical molecule that carries the hereditary information of the cell

messenger RNA (mRNA): DNA that carries information from DNA to ribosomes during protein synthesis

nucleic acid: biological macromolecule that carries the genetic blueprint of a cell and carries instructions

for the functioning of the cell

nucleotide: monomer of nucleic acids; contains a pentose sugar, one or more phosphate groups, and a nitrogenous base

phosphodiester: linkage covalent chemical bond that holds together the polynucleotide chains with a phosphate group linking two pentose sugars of neighboring nucleotides

polynucleotide: long chain of nucleotides

purine: type of nitrogenous base in DNA and RNA; adenine and guanine are

purines, pyrimidine: type of nitrogenous base in DNA and RNA; cytosine, thymine, and uracil are pyrimidines

ribonucleic acid (RNA): single-stranded, often internally base paired, molecule that is involved in protein synthesis

ribosomal RNA (rRNA): RNA that ensures the proper alignment of the mRNA and the ribosomes during protein synthesis and catalyzes the formation of the peptide linkage

transcription: process through which messenger RNA forms on a template of DNA

transfer RNA (tRNA): RNA that carries activated amino acids to the site of protein synthesis on the ribosome

translation: process through which RNA directs the formation of protein

6.7 Contributors and Attributions

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Chapter 7

Proteins

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7.0.1 Skills to develop:

1. Describe the functions proteins perform in the cell and in tissues
2. Discuss the relationship between amino acids and proteins
3. Explain the four levels of protein organization
4. Describe the ways in which protein shape and function are linked

Proteins are one of the most abundant organic molecules in living systems and have the most diverse range of functions of all **macromolecules**. Proteins may be structural, regulatory, contractile, or protective; they may serve in transport, storage, or membranes; or they may be toxins or enzymes. Each cell in a living system may contain thousands of proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, **polymers of amino acids**, arranged in a linear sequence.

7.1 Types and Functions of Proteins

Enzymes, which are produced by living cells, are **catalysts** in biochemical reactions (like digestion) and are usually complex or conjugated proteins. Each enzyme is specific for the substrate (a reactant that binds to an enzyme) it acts on. The enzyme may help in breakdown, rearrangement, or synthesis reactions. Enzymes that break down their substrates are called **catabolic enzymes**, enzymes that build more complex molecules from their substrates are called **anabolic enzymes**, and enzymes that affect the rate of reaction are called **catalytic enzymes**. It should be noted that all enzymes increase the rate of reaction and, therefore, are considered to be organic catalysts. An example of an enzyme is salivary amylase, which hydrolyzes its substrate amylose, a component of starch.

Hormones are chemical-signaling molecules, usually small proteins or steroids, secreted by endocrine cells that act to control or regulate specific physiological processes, including growth, development, metabolism, and reproduction. For example, insulin is a **protein hormone** that helps to regulate the blood glucose level.

Proteins have different shapes and molecular weights; some proteins are **globular** in shape whereas others are fibrous. For example, hemoglobin is a globular protein, but collagen, found in our skin, is a fibrous protein. Protein shape is critical to its function, and this shape is maintained by many different types of

chemical bonds. Changes in temperature, pH, and exposure to chemicals may lead to permanent changes in the shape of the protein, leading to loss of function, known as **denaturation**. All proteins are made up of different arrangements of the same 20 types of **amino acids**.

7.2 Amino Acids

Amino acids are the **monomers** that make up proteins. Each amino acid has the same fundamental structure, which consists of a central carbon atom, also known as the alpha (α) carbon, bonded to an amino group (NH_2), a carboxyl group (COOH), and to a hydrogen atom. Every amino acid also has another atom or group of atoms bonded to the central atom known as the R group (Figure 3.4.1).

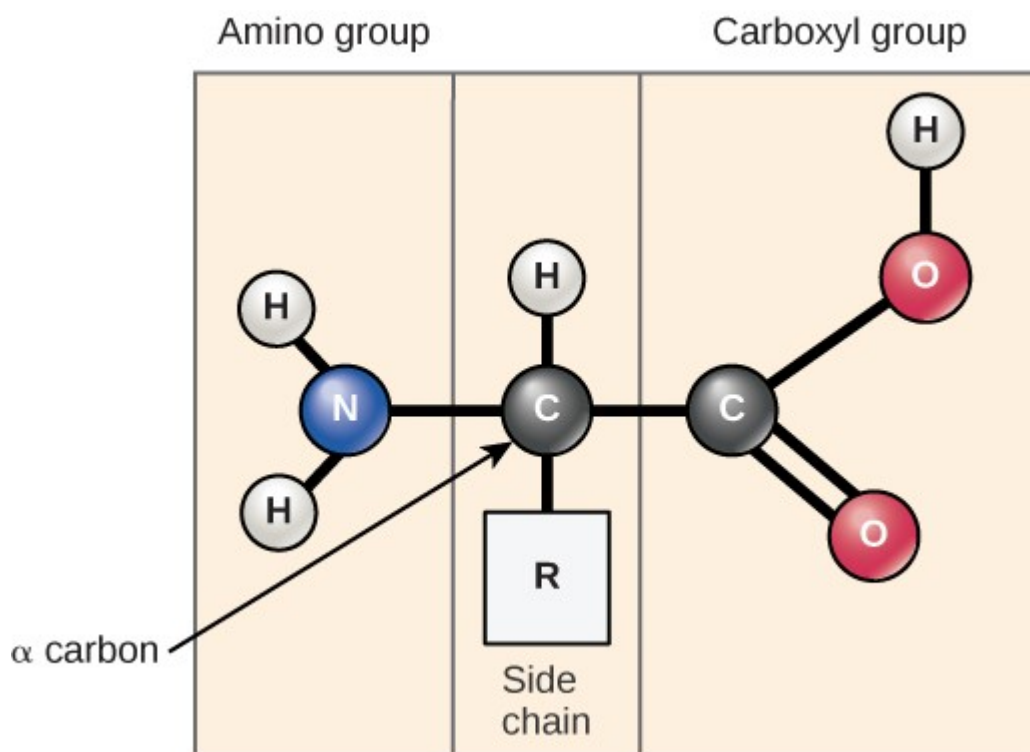


Figure 7.1: Amino acids have a central asymmetric carbon to which an amino group, a carboxyl group, a hydrogen atom, and a side chain (R group) are attached.

The name “amino acid” is derived from the fact that they contain both an amino group and carboxyl-acid-group in their basic structure. For each amino acid, the R group (or side chain) is different (Figure 3.4.2).

Which categories of amino acid would you expect to find on the surface of a soluble protein, and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?

The chemical nature of the side chain determines the nature of the amino acid (that is, whether it is **acidic**, **basic**, **polar**, or **nonpolar**). For example, the amino acid glycine has a hydrogen atom as the R group. Amino acids such as valine, methionine, and alanine are nonpolar or hydrophobic, while amino acids such as serine, threonine, and cysteine are polar and have hydrophilic side chains. The side chains of lysine and arginine are positively charged, and therefore these amino acids are also known as basic amino acids. Proline has an R group that is linked to the amino group, forming a ring-like structure. Proline is an exception to the standard structure of an amino acid since its amino group is not separate from the side chain.

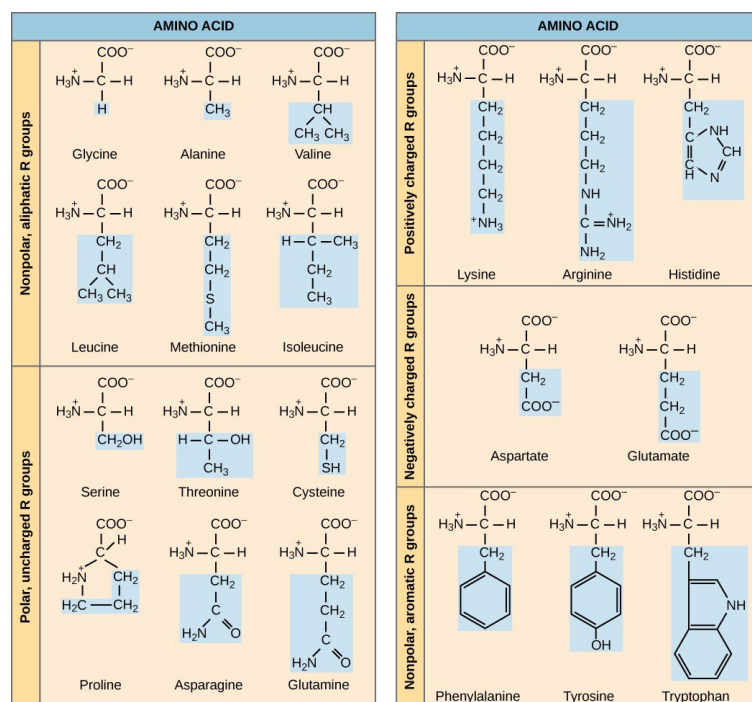


Figure 7.2: There are 20 common amino acids commonly found in proteins, each with a different R group (variant group) that determines its chemical nature.

Amino acids are represented by a single upper case letter or a three-letter abbreviation. For example, valine is known by the letter V or the three-letter code val.

The sequence and the number of amino acids ultimately determine a protein's shape, size, and function. Each amino acid is attached to another amino acid by a covalent bond, known as a **peptide bond**, which is formed by a dehydration reaction. The carboxyl group of one amino acid and the amino group of the incoming amino acid combine, releasing a molecule of water. The resulting bond is the peptide bond

The products formed by such linkages are called **peptides**. As more amino acids join to this growing chain, the resulting chain is known as a **polypeptide**. Each polypeptide has a free amino group at one end. This end is called the **N-terminal**, or the amino terminal, and the other end has a free carboxyl group, also known as the C- or carboxyl terminal. The terms polypeptide and protein are sometimes used interchangeably, a polypeptide is technically a polymer of amino acids, whereas the term protein is more formally used for a polypeptide or that have combined together, often have bound non-peptide prosthetic groups, have a distinct shape, and have a unique function.

After **protein synthesis (translation)**, most proteins are modified. These are known as **post-translational modifications**. They may undergo **cleavage**, **phosphorylation** (addition of a phosphate group), or may require the addition of other chemical groups. Only after these modifications is the protein completely functional.

7.3 Evolution Connection:

The Evolutionary Significance of Cytochrome: Cytochrome c is an important component of the electron transport chain, a part of cellular respiration, and it is normally found in the cellular organelle, the mitochondrion. This protein has a heme prosthetic group, and the central ion of the heme gets alternately reduced and oxidized during electron transfer. Because this essential protein's role in producing cellular

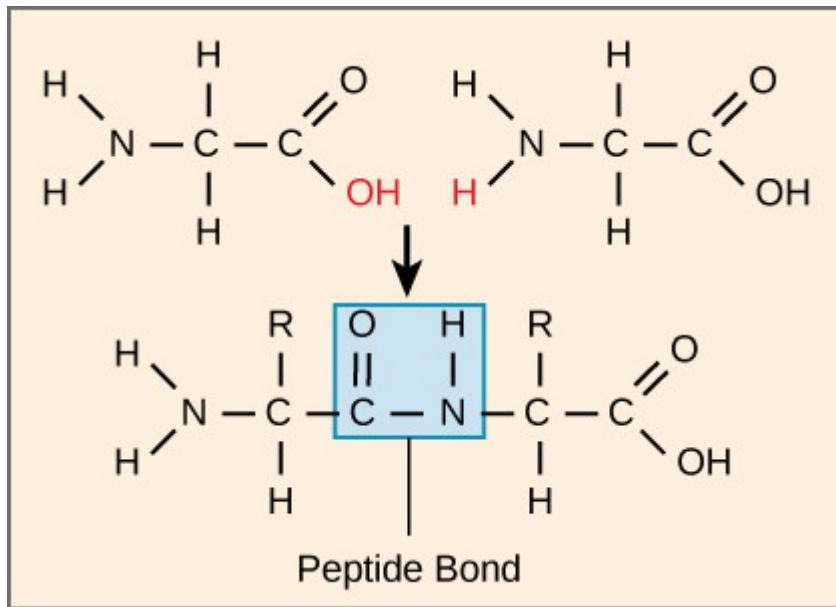


Figure 7.3: Peptide bond formation is a dehydration synthesis reaction. The carboxyl group of one amino acid is linked to the amino group of the incoming amino acid. In the process, a molecule of water is released.

energy is crucial, it has changed very little over millions of years. Protein sequencing has shown that there is a considerable amount of cytochrome c amino acid sequence **homology** among different species; in other words, evolutionary kinship can be assessed by measuring the similarities or differences among various species' DNA or protein sequences.

Scientists have determined that human cytochrome c contains 104 amino acids. For each cytochrome c molecule from different organisms that has been sequenced to date, 37 of these amino acids appear in the same position in all samples of cytochrome c. This indicates that there may have been a **common ancestor**. On comparing the human and chimpanzee protein sequences, no sequence difference was found. When human and rhesus monkey sequences were compared, the single difference found was in one amino acid. In another comparison, human to yeast sequencing shows a difference in the 44th position.

7.4 Protein Structure

The shape of a protein is critical to its function. For example, an enzyme can bind to a specific substrate at a site known as the **active site**. If this active site is altered because of local changes or changes in overall protein structure, the enzyme may be unable to bind to the substrate. To understand how the protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary.

7.4.1 Primary Structure

The unique sequence of amino acids in a polypeptide chain is its primary structure. For example, the pancreatic hormone insulin has two polypeptide chains, A and B, and they are linked together by disulfide bonds. The N terminal amino acid of the A chain is glycine, whereas the C terminal amino acid is asparagine (Figure 3.4.4). The sequences of amino acids in the A and B chains are unique to insulin.

The unique sequence for every protein is ultimately determined by the gene encoding the protein. A change in nucleotide sequence of the gene's coding region may lead to a different amino acid being added to the

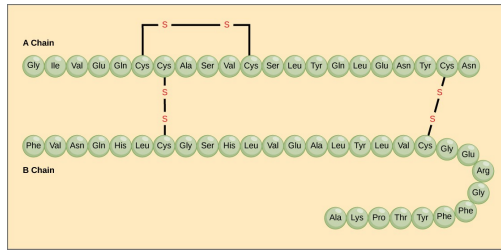


Figure 7.4: Bovine serum insulin is a protein hormone made of two peptide chains, A (21 amino acids long) and B (30 amino acids long). In each chain, primary structure is indicated by three-letter abbreviations that represent the names of the amino acids in the order they are present. The amino acid cysteine (cys) has a sulfhydryl (SH) group as a side chain. Two sulfhydryl groups can react in the presence of oxygen to form a disulfide (S-S) bond. Two disulfide bonds connect the A and B chains together, and a third helps the A chain fold into the correct shape. Note that all disulfide bonds are the same length, but are drawn different sizes for clarity.

growing polypeptide chain, causing a change in protein structure and function. In sickle cell anemia, the hemoglobin *beta* chain (a small portion of which is shown in Figure 3.4.5) has a single amino acid substitution, causing a change in protein structure and function. Specifically, the amino acid glutamic acid is substituted by valine in the beta chain. What is most remarkable to consider is that a hemoglobin molecule is made up of two alpha chains and two beta chains that each consist of about 150 amino acids. The molecule, therefore, has about 600 amino acids. The structural difference between a normal hemoglobin molecule and a sickle cell molecule—which dramatically decreases life expectancy—is a single amino acid of the 600. What is even more remarkable is that those 600 amino acids are encoded by three nucleotides each, and the mutation is caused by a single base change (point mutation), 1 in 1800 bases.

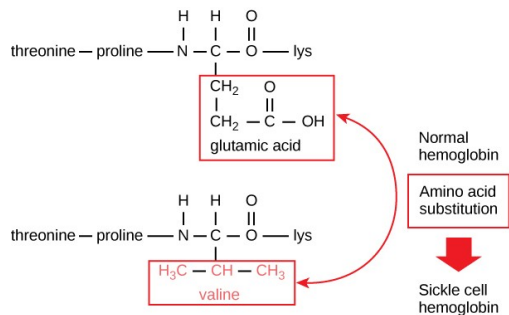


Figure 7.5: The beta chain of hemoglobin is 147 residues in length, yet a single amino acid substitution leads to sickle cell anemia. In normal hemoglobin, the amino acid at position seven is glutamate. In sickle cell hemoglobin, this glutamate is replaced by a valine.

Because of this change of one amino acid in the chain, hemoglobin molecules form long fibers that distort the biconcave, or disc-shaped, red blood cells and assume a crescent or “sickle” shape, which clogs arteries (Figure 3.4.6). This can lead to myriad serious health problems such as breathlessness, dizziness, headaches, and abdominal pain for those affected by this disease.

7.4.2 Secondary Structure

The local folding of the polypeptide in some regions gives rise to the secondary structure of the protein. The most common are the α -helix and *beta*-pleated sheet structures (Figure 3.4.7). Both structures are the α -helix structure—the helix held in shape by hydrogen bonds. The hydrogen bonds form between the oxygen atom in the carbonyl group in one amino acid and another amino acid that is four amino acids farther along the chain.

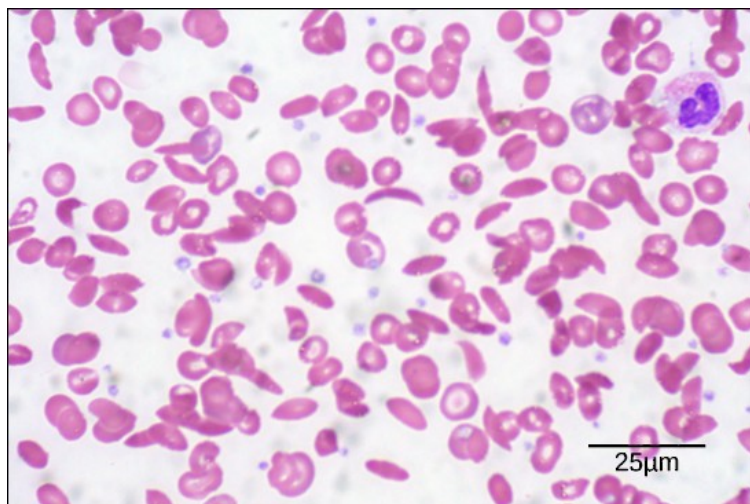


Figure 7.6: In this blood smear, visualized at 535x magnification using bright field microscopy, sickle cells are crescent shaped, while normal cells are disc-shaped. (credit: modification of work by Ed Uthman; scale-bar data from Matt Russell)

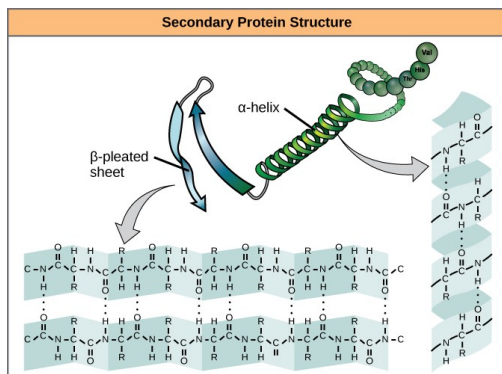


Figure 7.7: The α -helix and β -pleated sheet are secondary structures of proteins that form because of hydrogen bonding between carbonyl and amino groups in the peptide backbone. Certain amino acids have a propensity to form an α -helix, while others have a propensity to form a β -pleated sheet.

Every helical turn in an alpha helix has 3.6 amino acid residues. The R groups (the variant groups) of the polypeptide protrude out from the α -helix chain. In the *beta*-pleated sheet, the “pleats” are formed by hydrogen bonding between atoms on the backbone of the polypeptide chain. The R groups are attached to the carbons and extend above and below the folds of the pleat. The pleated segments align parallel or antiparallel to each other, and hydrogen bonds form between the partially positive nitrogen atom in the amino group and the partially negative oxygen atom in the carbonyl group of the peptide backbone. The α -helix and *beta*-pleated sheet structures are found in most globular and fibrous proteins and they play an important structural role.

7.4.3 Tertiary Structure

The unique three-dimensional structure of a polypeptide is its tertiary structure (Figure 3.4.8). This structure is in part due to chemical interactions at work on the polypeptide chain. Primarily, the interactions among R groups creates the complex three-dimensional tertiary structure of a protein. The nature of the R groups found in the amino acids involved can counteract the formation of the hydrogen bonds described for standard secondary structures. For example, R groups with like charges are repelled by each other and those with unlike charges are attracted to each other (ionic bonds). When protein folding takes place, the hydrophobic R groups of nonpolar amino acids lay in the interior of the protein, whereas the hydrophilic R groups lay on the outside. The former types of interactions are also known as hydrophobic interactions. Interaction between cysteine side chains forms disulfide linkages in the presence of oxygen, the only covalent bond forming during protein folding.

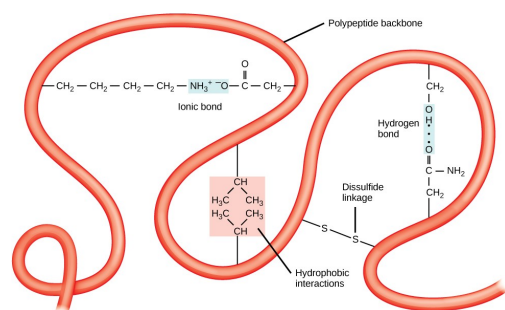


Figure 7.8: The tertiary structure of proteins is determined by a variety of chemical interactions. These include hydrophobic interactions, ionic bonding, hydrogen bonding and disulfide linkages.

All of these interactions, weak and strong, determine the final three-dimensional shape of the protein. When a protein loses its three-dimensional shape, it may no longer be functional.

7.4.4 Quaternary Structure

In nature, some proteins are formed from several polypeptides, also known as subunits, and the interaction of these subunits forms the quaternary structure. Weak interactions between the subunits help to stabilize the overall structure. For example, insulin (a globular protein) has a combination of hydrogen bonds and disulfide bonds that cause it to be mostly clumped into a ball shape. Insulin starts out as a single polypeptide and loses some internal sequences in the presence of post-translational modification after the formation of the disulfide linkages that hold the remaining chains together. Silk (a fibrous protein), however, has a *beta*-pleated sheet structure that is the result of hydrogen bonding between different chains.

The four levels of protein structure (primary, secondary, tertiary, and quaternary) are illustrated in Figure 3.4.9.

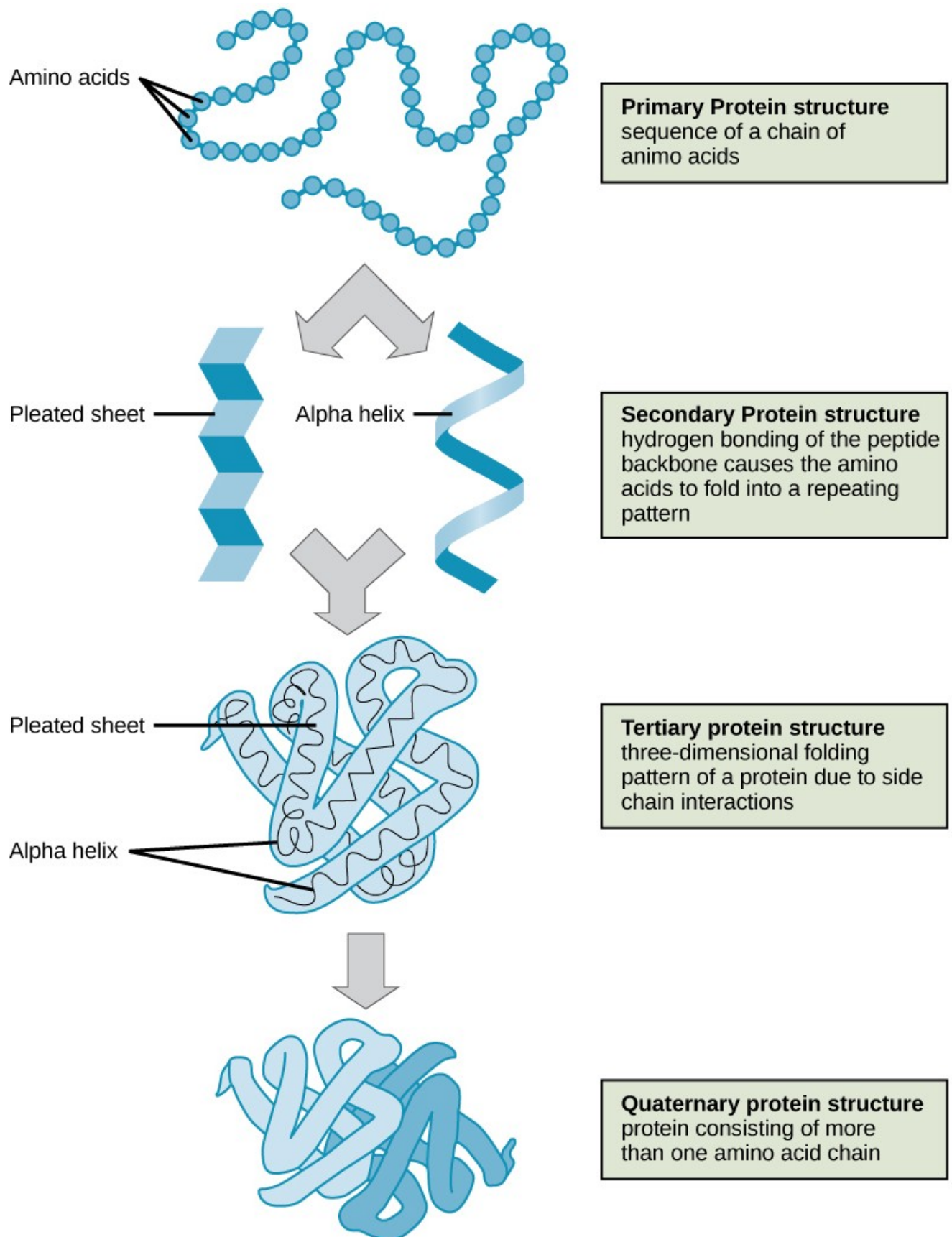


Figure 7.9: The four levels of protein structure can be observed in these illustrations. (credit: modification of work by National Human Genome Research Institute)

7.5 Denaturation and Protein Folding

Each protein has its own unique sequence and shape that are held together by chemical interactions. If the protein is subject to changes in temperature, pH, or exposure to chemicals, the protein structure may change, losing its shape without losing its primary sequence in what is known as denaturation. Denaturation is often reversible because the primary structure of the polypeptide is conserved in the process if the denaturing agent is removed, allowing the protein to resume its function. Sometimes denaturation is irreversible, leading to loss of function. One example of irreversible protein denaturation is when an egg is fried. The albumin protein in the liquid egg white is denatured when placed in a hot pan. Not all proteins are denatured at high temperatures; for instance, bacteria that survive in hot springs have proteins that function at temperatures close to boiling. The stomach is also very acidic, has a low pH, and denatures proteins as part of the digestion process; however, the digestive enzymes of the stomach retain their activity under these conditions.

Protein folding is critical to its function. It was originally thought that the proteins themselves were responsible for the folding process. Only recently was it found that often they receive assistance in the folding process from protein helpers known as chaperones (or chaperonins) that associate with the target protein during the folding process. They act by preventing aggregation of polypeptides that make up the complete protein structure, and they disassociate from the protein once the target protein is folded.

7.6 Summary

Proteins are a class of macromolecules that perform a diverse range of functions for the cell. They help in metabolism by providing structural support and by acting as enzymes, carriers, or hormones. The building blocks of proteins (monomers) are amino acids. Each amino acid has a central carbon that is linked to an amino group, a carboxyl group, a hydrogen atom, and an

R group or side chain. There are 20 commonly occurring amino acids, each of which differs in the R group. Each amino acid is linked to its neighbors by a peptide bond. A long chain of amino acids is known as a polypeptide.

Proteins are organized at four levels: primary, secondary, tertiary, and (optional) quaternary. The primary structure is the unique sequence of amino acids. The local folding of the polypeptide to form structures such as the α -helix and β -pleated sheet constitutes the secondary structure. The overall three-dimensional structure is the tertiary structure. When two or more polypeptides combine to form the complete protein structure, the configuration is known as the quaternary structure of a protein. Protein shape and function are intricately linked; any change in shape caused by changes in temperature or pH may lead to protein denaturation and a loss in function.

7.7 Art Connections

- Which categories of amino acid would you expect to find on the surface of a soluble protein, and which would you expect to find in the interior? What distribution of amino acids would you expect to find in a protein embedded in a lipid bilayer?
- Polar and charged amino acid residues (the remainder after peptide bond formation) are more likely to be found on the surface of soluble proteins where they can interact with water, and nonpolar (e.g., amino acid side chains) are more likely to be found in the interior where they are sequestered from water. In membrane proteins, nonpolar and hydrophobic amino acid side chains associate with the hydrophobic tails of phospholipids, while polar and charged amino acid side chains interact with the polar head groups or with the aqueous solution. However, there are exceptions. Sometimes, positively and negatively charged amino acid side chains interact with one another in the interior of a protein, and polar or charged amino acid side chains that interact with a ligand can be found in the ligand binding pocket.

7.8 Review Questions

The monomers that make up proteins are called 1. nucleotides 1. disaccharides 1. amino acids 1. chaperones

Answer: C

The helix and the *beta*-pleated sheet are part of which protein structure?

1. primary
2. secondary
3. tertiary
4. quaternary

Answer: B

7.9 Free Response

Explain what happens if even one amino acid is substituted for another in a polypeptide chain. Provide a specific example.

A change in gene sequence can lead to a different amino acid being added to a polypeptide chain instead of the normal one. This causes a change in protein structure and function. For example, in sickle cell anemia, the hemoglobin *beta* chain has a single amino acid substitution—the amino acid glutamic acid in position six is substituted by valine. Because of this change, hemoglobin molecules form aggregates, and the disc-shaped red blood cells assume a crescent shape, which results in serious health problems.

Describe the differences in the four protein structures.

The sequence and number of amino acids in a polypeptide chain is its primary structure. The local folding of the polypeptide in some regions is the secondary structure of the protein. The three-dimensional structure of a polypeptide is known as its tertiary structure, created in part by chemical interactions such as hydrogen bonds between polar side chains, van der Waals interactions, disulfide linkages, and hydrophobic interactions. Some proteins are formed from multiple polypeptides, also known as subunits, and the interaction of these subunits forms the quaternary structure.

7.10 Glossary

alpha-helix structure (-helix): type of secondary structure of proteins formed by folding of the polypeptide into a helix shape with hydrogen bonds stabilizing the structure

amino acid: monomer of a protein; has a central carbon or alpha carbon to which an amino group, a carboxyl group, a hydrogen, and an R group or side chain is attached; the R group is different for all 20 amino acids

beta-pleated sheet (beta-pleated): secondary structure found in proteins in which “pleats” are formed by hydrogen bonding between atoms on the backbone of the polypeptide chain

chaperone: (also, chaperonin) protein that helps nascent protein in the folding process **denaturation:** loss of shape in a protein as a result of changes in temperature, pH, or exposure to chemicals

enzyme: catalyst in a biochemical reaction that is usually a complex or conjugated protein

hormone: chemical signaling molecule, usually protein or steroid, secreted by endocrine cells that act to control or regulate specific physiological processes **peptide bond:** bond formed between two amino acids by a dehydration reaction

polypeptide: long chain of amino acids linked by peptide bonds

primary structure: linear sequence of amino acids in a protein

protein: biological macromolecule composed of one or more chains of amino acids

quaternary structure: association of discrete polypeptide subunits in a protein

secondary structure: regular structure formed by proteins by intramolecular hydrogen bonding between the oxygen atom of one amino acid residue and the hydrogen attached to the nitrogen atom of another

amino acid residue **tertiary structure**: three-dimensional conformation of a protein, including interactions between secondary structural elements; formed from interactions between amino acid side chains

7.11 Contributors and Attributions

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Chapter 8

A primer for working with vectors

By: Avril Coghlan

Adapted, edited and expanded: Nathan Brouwer (brouwer@gmail.com) under the Creative Commons 3.0 Attribution License (CC BY 3.0).

8.1 Preface

This is a modification of part of “DNA Sequence Statistics (2)” from Avril Coghlan’s *A little book of R for bioinformatics*. Most of text and code was originally written by Dr. Coghlan and distributed under the Creative Commons 3.0 license.

8.2 Vocab

- base R
- scalar, vector, matrix
- vectorized operation
- regular expressions

Chapter 9

Functions

- `seq()`
- `is()`, `is.vector()`, `is.matrix()`
- `gsub()`

9.1 Vectors in R

Variables in R include **scalars**, **vectors**, and **lists**. **Functions** in R carry out operations on variables, for example, using the `log10()` function to calculate the log to the base 10 of a scalar variable `x`, or using the `mean()` function to calculate the average of the values in a vector variable `myvector`. For example, we can use `log10()` on a scalar object like this:

```
# store value in object
x <- 100

# take log base 10 of object
log10(x)

## [1] 2
```

Note that while mathematically `x` is a single number, or a scalar, R considers it to be a vector:

```
is.vector(x)

## [1] TRUE
```

There are many “is” commands. What is returned when you run `is.matrix()` on a vector?

```
is.matrix(x)

## [1] FALSE
```

Mathematically this is a bit odd, since often a vector is defined as a one-dimensional matrix, e.g., a single column or single row of a matrix. But in *R* land, a vector is a vector, and matrix is a matrix, and there are no explicit scalars.

9.2 Math on vectors

Vectors can serve as the input for mathematical operations. When this is done *R* does the mathematical operation separately on each element of the vector. This is a unique feature of *R* that can be hard to get used to even for people with previous programming experience.

Let's make a vector of numbers:

```
myvector <- c(30,16,303,99,11,111)
```

What happens when we multiply myvector by 10?

```
myvector*10
```

```
## [1] 300 160 3030 990 110 1110
```

R has taken each of the 6 values, 30 through 111, of myvector and multiplied each one by 10, giving us 6 results. That is, what *R* did was

```
## 30*10    # first value of myvector
## 16*10    # second value of myvector
## 303*10   # ....
## 99*10
## 111*10   # last value of myvector
```

The normal order of operations rules apply to vectors as they do to operations we're more used to. So multiplying myvector by 10 is the same whether you put the 10 before or after vector. That is myvector*10 is the same as 10*myvector.

```
myvector*10
```

```
## [1] 300 160 3030 990 110 1110
```

```
10*myvector
```

```
## [1] 300 160 3030 990 110 1110
```

What happens when you subtract 30 from myvector? Write the code below.

```
myvector-30
```

```
## [1] 0 -14 273 69 -19 81
```

So, what *R* did was

```
## 30-30    # first value of myvector
## 16-30    # second value of myvector
## 303-30   # ....
## 99-30
## 111-30   # last value of myvector
```

Again, myvector-30 is a vectorized operation.

You can also square a vector

```
myvector^2
```

```
## [1] 900 256 91809 9801 121 12321
```

Which is the same as

```
## 30^2    # first value of myvector
## 16^2    # second value of myvector
## 303^2   # ....
## 99^2
## 111^2   # last value of myvector
```

Also you can take the square root of a vector using the functions `sqrt()`...

```
sqrt(myvector)
```

```
## [1]  5.477226  4.000000 17.406895  9.949874  3.316625 10.535654
```

...and take the log of a vector with `log()`...

```
log(myvector)
```

```
## [1] 3.401197 2.772589 5.713733 4.595120 2.397895 4.709530
```

...and just about any other mathematical operation. Here we are working on a separate vector object; all of these rules apply to a column in a matrix or a dataframe.

This attribute of R is called **vectorization**. When you run the code `myvector*10` or `log(myvector)` you are doing a **vectorized** operation - its like normal math with special vector-based super power to get more done faster than you normally could.

9.3 Functions on vectors

As we just saw, we can use functions on vectors. Typically these use the vectors as an input and all the numbers are processed into an output. Call the `mean()` function on the vector we made called `myvector`.

```
mean(myvector)
```

```
## [1] 95
```

Note how we get a single value back - the mean of all the values in the vector. R saw that we had a vector of multiple and knew that the mean is a function that doesn't get applied to single number, but sets of numbers.

The function `sd()` calculates the standard deviation. Apply the `sd()` to `myvector`:

```
sd(myvector)
```

```
## [1] 110.5061
```

9.4 Operations with two vectors

You can also subtract one vector from another vector. This can be a little weird when you first see it. Make another vector with the numbers 5, 10, 15, 20, 25, 30. Call this `myvector2`:

```
myvector2 <- c(5, 10, 15, 20, 25, 30)
```

Now subtract `myvector2` from `myvector`. What happens?

```
myvector-myvector2
```

```
## [1] 25 6 288 79 -14 81
```

9.5 Subsetting vectors

You can extract an **element** of a vector by typing the vector name with the index of that element given in **square brackets**. For example, to get the value of the 3rd element in the vector `myvector`, we type:

```
myvector[3]
```

```
## [1] 303
```

Extract the 4th element of the vector:

```
myvector[4]
```

```
## [1] 99
```

You can extract more than one element by using a vector in the brackets:

First, say I want to extract the 3rd and the 4th element. I can make a vector with 3 and 4 in it:

```
nums <- c(3,4)
```

Then put that vector in the brackets:

```
myvector[nums]
```

```
## [1] 303 99
```

We can also do it directly like this, skipping the vector-creation step:

```
myvector[c(3,4)]
```

```
## [1] 303 99
```

In the chunk below extract the 1st and 2nd elements:

```
myvector[c(1,2)]
```

```
## [1] 30 16
```

9.6 Sequences of numbers

Often we want a vector of numbers in **sequential order**. That is, a vector with the numbers 1, 2, 3, 4, ... or 5, 10, 15, 20, ... The easiest way to do this is using a colon

```
1:10
```

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

Note that in R `1:10` is equivalent to `c(1:10)`

```
c(1:10)
```

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

Usually to emphasize that a vector is being created I will use `c(1:10)`

We can do any number to any numbers

```
c(20:30)
```

```
## [1] 20 21 22 23 24 25 26 27 28 29 30
```

We can also do it in *reverse*. In the code below put 30 before 20:

```
c(30:20)
```

```
## [1] 30 29 28 27 26 25 24 23 22 21 20
```

A useful function in *R* is the `seq()` function, which is an explicit function that can be used to create a vector containing a sequence of numbers that run from a particular number to another particular number.

```
seq(1, 10)
```

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

Using `seq()` instead of a `:` can be useful for readability to make it explicit what is going on. More importantly, `seq` has an argument `by = ...` so you can make a sequence of number with any interval between. For example, if we want to create the sequence of numbers from 1 to 10 in steps of 1 (i.e.. 1, 2, 3, 4, ... 10), we can type:

```
seq(1, 10,
    by = 1)
```

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

We can change the **step size** by altering the value of the `by` argument given to the function `seq()`. For example, if we want to create a sequence of numbers from 1-100 in steps of 20 (i.e.. 1, 21, 41, ... 101), we can type:

```
seq(1, 101,
    by = 20)
```

```
## [1] 1 21 41 61 81 101
```

9.7 Vectors can hold numeric or character data

The vector we created above holds numeric data, as indicated by `class()`

```
class(myvector)
```

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

Vectors can also hold character data, like the genetic code:

```
# vector of character data
myvector <- c("A", "T", "G")
```

```
# how it looks
myvector
```

```
## [1] "A" "T" "G"
```

```
# what is "is"
class(myvector)
```

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

9.8 Regular expressions can modify character data

We can use **regular expressions** to modify character data. For example, change the Ts to Us

```
myvector <- gsub("T", "U", myvector)
```

Now check it out

```
myvector
```

```
## [1] "A" "U" "G"
```

Regular expressions are a deep subject in computing. You can find some more information about them [here](#).

Chapter 10

Plotting vectors in base R

By: Avril Coghlan

Adapted, edited and expanded: Nathan Brouwer (brouwern@gmail.com) under the Creative Commons 3.0 Attribution License (CC BY 3.0).

10.1 Preface

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10.2 Plotting numeric data

R allows the production of a variety of plots, including **scatterplots**, **histograms**, **piecharts**, and **boxplots**. Usually we make plots from **dataframes** with 2 or more columns, but we can also make them from two separate vectors. This flexibility is useful, but also can cause some confusion.

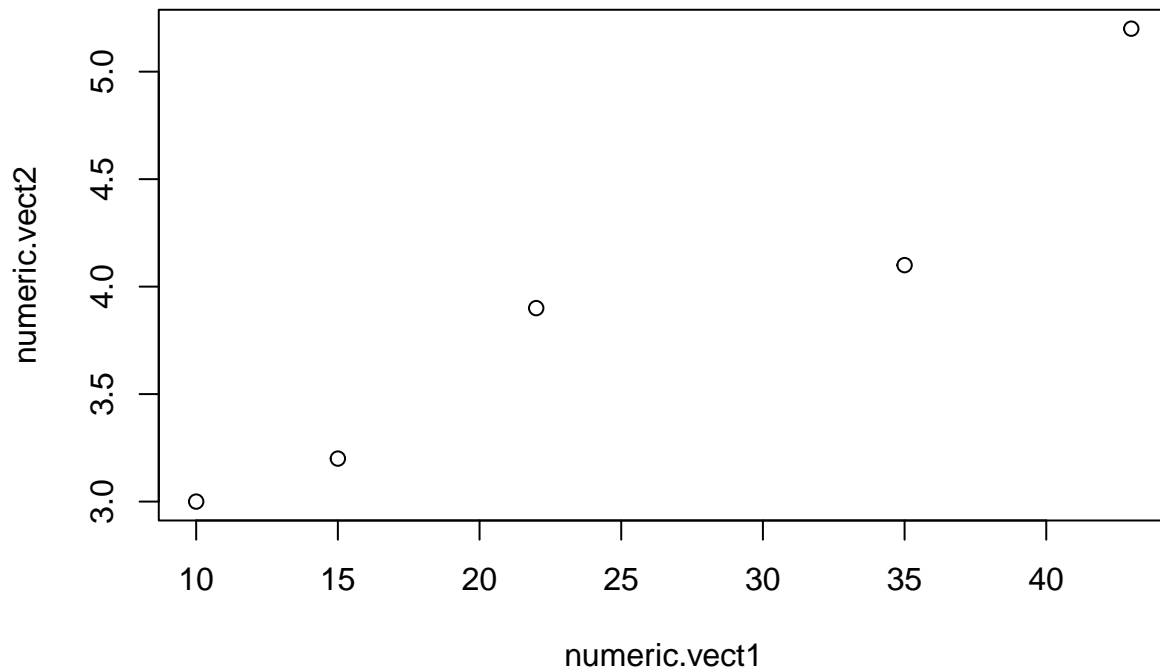
For example, if you have two equal-length vectors of numbers `numeric.vect1` and `numeric.vect2`, you can plot a **scatterplot** of the values in `myvector1` against the values in `myvector2` using the **base R** `plot()` function.

First, let’s make up some data and put it in vectors:

```
numeric.vect1 <- c(10, 15, 22, 35, 43)
numeric.vect2 <- c(3, 3.2, 3.9, 4.1, 5.2)
```

Not plot with the base R `plot()` function:

```
plot(numeric.vect1, numeric.vect2)
```

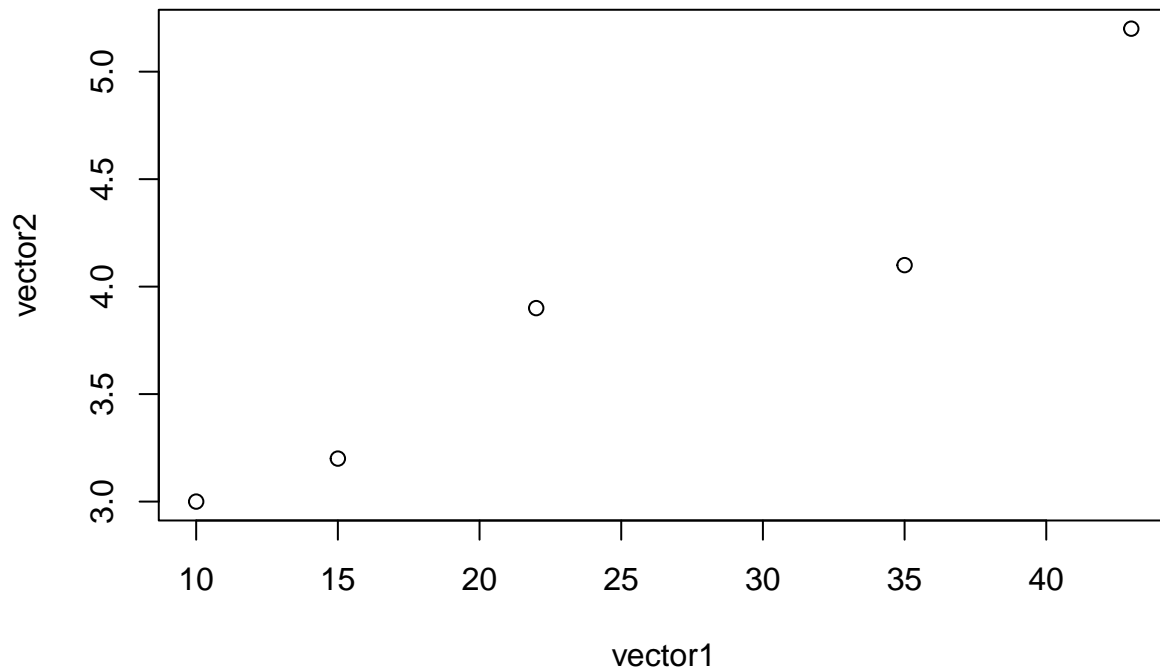


Note that there is a comma between the two vector names. When building plots from dataframes you usually see a tilde (~), but when you have two vectors you can use just a comma.

Also note the order of the vectors within the `plot()` command and which axes they appear on. The first vector is `numeric.vect1` and it appears on the horizontal x-axis.

If you want to label the axes on the plot, you can do this by giving the `plot()` function values for its optional arguments `xlab =` and `ylab =`:

```
plot(numeric.vect1, # note again the comma, not a ~
     numeric.vect2,
     xlab="vector1",
     ylab="vector2")
```

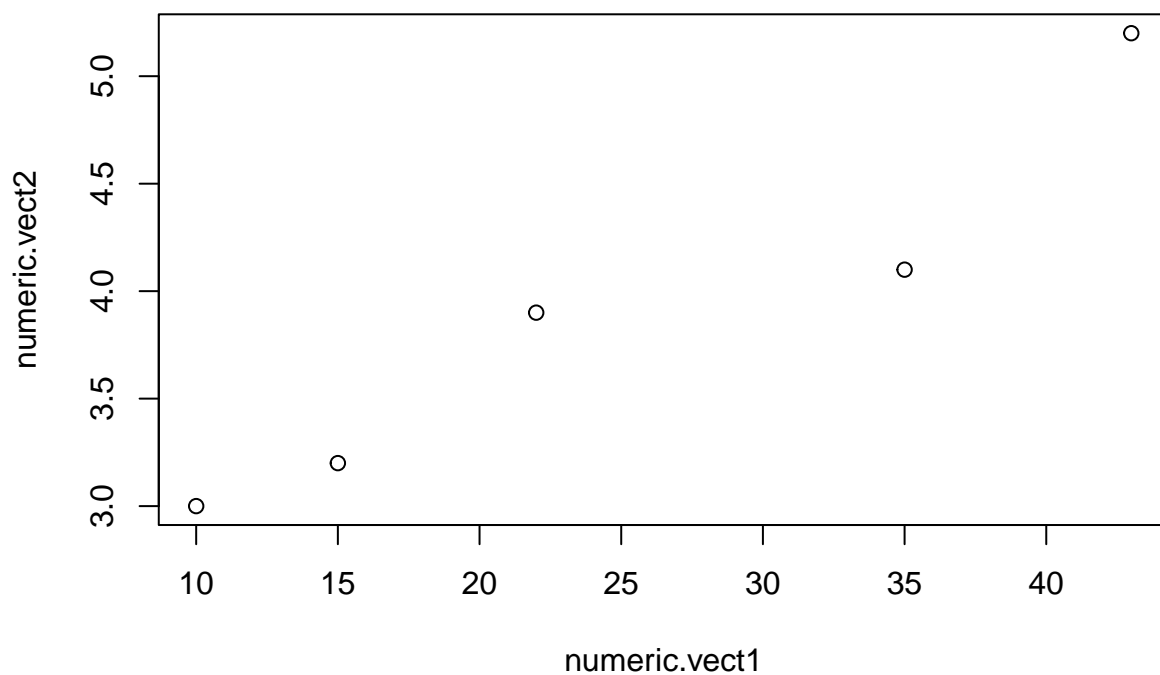



We can store character data in vectors so if we want we could do this to set up our labels:

```
mylabels <- c("numeric.vect1", "numeric.vect2")
```

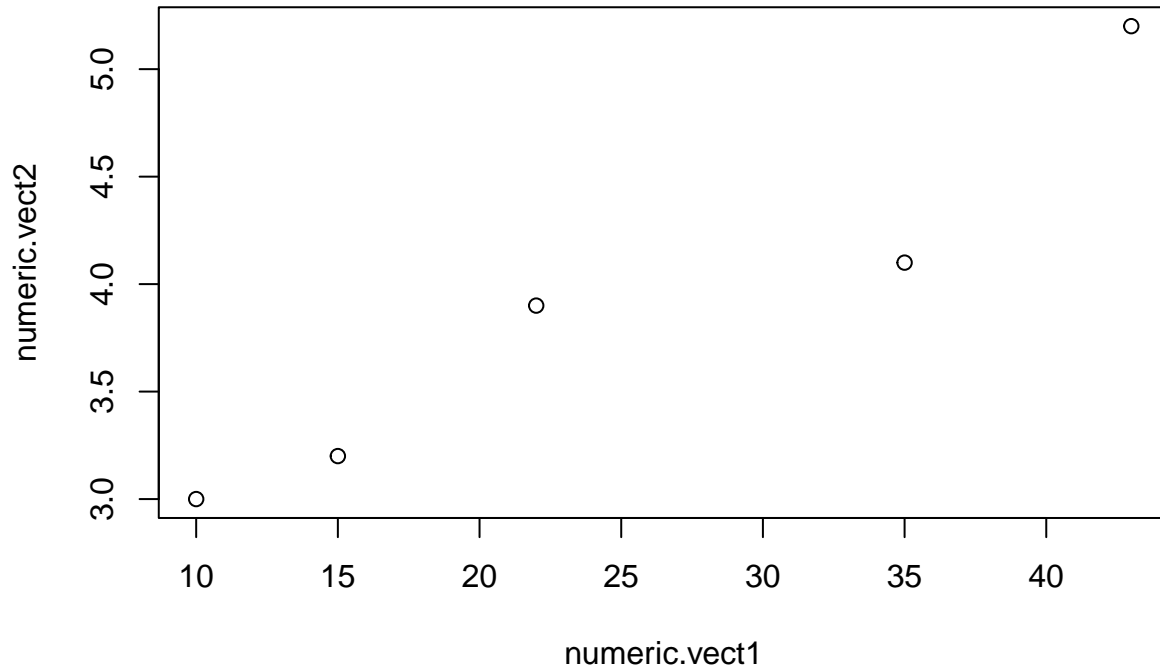
Then use bracket notation to call the labels from the vector

```
plot(numeric.vect1 ,
     numeric.vect2 ,
     xlab=mylabels[1] ,
     ylab=mylabels[2])
```



If we want we can use a tilde to make our plot like this:

```
plot(numeric.vect2 ~ numeric.vect1)
```



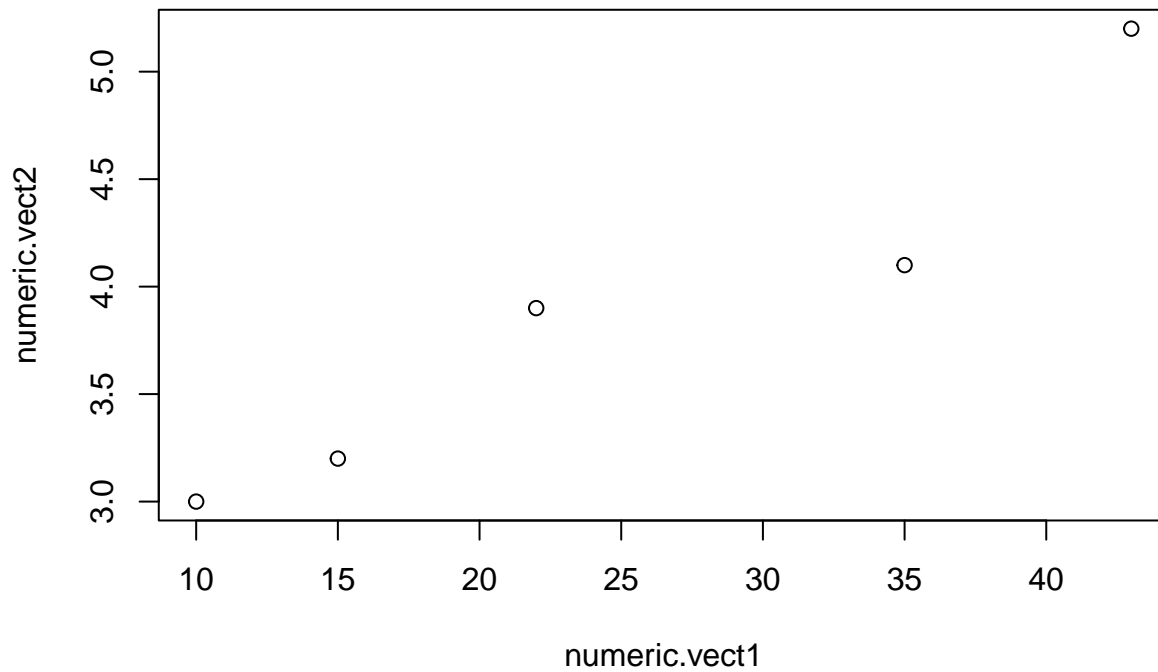
Note that now, `numeric.vect2` is on the left and `numeric.vect1` is on the right. This flexibility can be tricky to keep track of.

We can also combine these vectors into a dataframe and plot the data by referencing the data frame. First, we combine the two separate vectors into a dataframe using the `cbind()` command.

```
df <- cbind(numeric.vect1 , numeric.vect2)
```

Then we plot it like this, referencing the dataframe `df` via the `data = ...` argument.

```
plot(numeric.vect2 ~ numeric.vect1 , data = df)
```



10.3 Other plotting packages

Base R has lots of plotting functions; additionally, people have written packages to implement new plotting capabilities. The package `ggplot2` is currently the most popular plotting package, and `ggpubr` is a package which makes `ggplot2` easier to use. For quick plots we'll use base R functions, and when we get to more important things we'll use `ggplot2` and `ggpubr`.

Chapter 11

Intro to R objects

By: Nathan Brouwer

11.1 Commands used

- `<-`
- `c()`
- `length()`
- `dim()`
- `is()`

11.2 R Objects

- Everything in R is an object, works with an object, tells you about an object, etc
- We'll do a simple data analysis with a `t.test` and then look at properties of R objects
- There are several types of objects: **vectors**, **matrices**, **lists**, **dataframes**
- R objects can hold numbers, text, or both
- A typical dataframe has columns of **numeric data** and columns of text that represent **factor variables** (aka “**categorical variables**”)

11.3 Differences between objects

Different objects are used and show up in different contexts.

- Most practical stats work in R is done with **dataframes** .
- A dataframe is kind of like a spreadsheet, loaded into R.
- For the sake of simplicity, we often load data in as a **vector**. This just makes things smoother when we are starting out.
- **vectors** pop up in many places, usually in a support role until you start doing more programming.
- **matrices** are occasionally used for applied stats stuff but show up more for programming. A matrix is like a stripped-down dataframe.
- **lists** show up everywhere, but you often don't know it; many R functions make lists
- Understanding **lists** will help you efficiently work with stats output and make plots.

11.4 The Data

We'll use the following data to explore R objects.

Motulsky 2nd Ed, Chapter 30, page 220, Table 30.1. Maximal relaxation of muscle strips of old and young rat bladders stimulated w/ high concentrations of nonrepinephrine (Frazier et al 2006). Response variable is %E.max

11.5 The assignment operator “<-” makes object

The code above has made two objects. We can use several commands to learn about these objects.

- `is()`: what an object is, i.e., vector, matrix, list, dataframe
- `length()`: how long an object is; only works with vectors and lists, not dataframes!
- `dim()`: how long AND how wide and object is; doesn't work with vectors, only dataframes and matrices

11.5.1 `is()`

What is our “old.E.max” object?

```
is(old.E.max)
```

```
## [1] "numeric" "vector"
```

```
is(young.E.max)
```

```
## [1] "numeric" "vector"
```

Its a vector, containing numeric data.

What if we made a vector like this?

```
cat.variables <- c("old","old","old","old",  
                  "old","old","old","old","old")
```

And used `is()`

```
is(cat.variables)
```

```
## [1] "character" "vector" "data.frameRowLabels"
```

```
## [4] "SuperClassMethod"
```

It tells us we have a vector, containing character data. Not sure why it feels the need to tell us all the other stuff...

11.5.2 `length()`

Our vector has 9 elements, or is 9 elements long.

```
length(old.E.max)
```

```
## [1] 9
```

Note that `dim()`, for dimension, doesn't work with vectors!

```
dim(old.E.max)
```

```
## NULL
```

It would be nice if it said something like “1 x 9” for 1 row tall and 9 elements long. So it goes.

11.5.3 str()

str() stands for “structure”.

- It summarizes info about an object;
- I find it most useful for looking at lists.
- If our vector here was really really long, str() would only show the first part of the vector

```
str(old.E.max)
```

```
## num [1:9] 20.8 2.8 50 33.3 29.4 38.9 29.4 52.6 14.3
```

11.5.4 c()

- We typically use c() to gather together things like numbers, as we did to make our objects above.
- note: this is *lower case* “c”!
- Uppercase is something else
- For me, R’s font makes it hard sometimes to tell the difference between “c” and “C”
- If code isn’t working, one problem might be a “C” instead of a “c”

Use c() to combine two objects

```
old.plus.new <- c(old.E.max, young.E.max)
```

Look at the length

```
length(old.plus.new)
```

```
## [1] 17
```

Note that str() just shows us the first few digits, not all 17

```
str(old.plus.new)
```

```
## num [1:17] 20.8 2.8 50 33.3 29.4 38.9 29.4 52.6 14.3 45.5 ...
```

11.6 Debrief

We can...

- learn about objects using length(), is(), str()
- access parts of list using \$ (and also brackets)
- access parts of vectors using square brackets []
- save the output of a model / test to an object
- access part of lists for plotting instead of copying stuff

Chapter 12

NCBI: The National Center for Biotechnology Information

NOTE: The following material was partially adapted by N. Brouwer from Wikipedia. See the underlying .Rmd file for information on specific paragraphs from Wikipedia.

The **National Center for Biotechnology Information (NCBI)** is part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health (NIH). It was founded in 1988 and is approved and funded by the government of the United States. The NCBI houses a series of **databases** relevant to the basic and applied life sciences and is an important resource for **bioinformatics** tools and services. Major databases include **GenBank** for DNA sequences and **PubMed**, a **bibliographic** database for biomedical literature. All these databases are available online through the **Entrez** search engine.


In this chapter we'll briefly discuss the major databases, following up with specific details in subsequent chapters. One thing to note is that in practice people do not always adhere to the specific names of databases and other tools. For example, someone may say "I searched NCBI for sequences." It would be more accurate to say "I used Entrez to search GenBank for sequences." For in-depth details on all the features see Database resources of the National Center for Biotechnology Information

12.1 GenBank sequence database

The GenBank sequence database is an open access collection of publicly available DNA and protein sequences. If you've work with sequence data, you'll work with GenBank. GenBank is the actual database, and it can be searched several ways. For example, you can search for a sequence by its ID number (**accession number**) if you know it, or do a **BLAST search** using an actual sequence to look for similar sequences.

A key component of GenBank are the **GeneBank Records**, which are annotated summaries of sequences in the databases. For example, below is shown record for a gene pallysin from syphilis. In addition to the actual A, T, C, and Gs of the sequences, the record provides **metadata**, such as the scientific name of the organism (*Treponema pallidum*), who did the sequencing, the name of the paper where the sequence was published, and important features of the gene.

A key feature of PubMed records is that they are **hyperlinked** to other NCBI databases. For example, you can click link under the name of the paper which reported the sequence of the gene and it will take you to the PubMed record for that paper (see below). You can also click the "Run BLAST" link and you can search the database for similar sequences. This protein coded for by this particular gene has had its structure solved using x-ray crystallography, and you can see these results under "Protein 3D Structure." In a later chapter we'll get to know these records in further detail.


NCBI
Resources ☒ How To ☒

Nucleotide

Nucleotide ▼

Advanced

GenBank ▼

Saccharomyces cerevisiae TCP1-beta gene, partial cds

Rev7p (REV7) genes, complete cds

GenBank: U49845.1

[FASTA](#) [Graphics](#)

[Go to:](#) ☒

LOCUS	SCU49845	5028 bp	DNA	linear	PLN 29-OCT
DEFINITION	Saccharomyces cerevisiae TCP1-beta gene, partial cds; and Axl2p (AXL2) and Rev7p (REV7) genes, complete cds.				
ACCESSION	U49845				
VERSION	U49845.1				
KEYWORDS	.				
SOURCE	Saccharomyces cerevisiae (baker's yeast)				
ORGANISM	Saccharomyces cerevisiae Eukaryota; Fungi; Dikarya; Ascomycota; Saccharomycotina; Saccharomycetes; Saccharomycetales; Saccharomycetaceae; Saccharomyces.				
REFERENCE	1 (bases 1 to 5028)				
AUTHORS	Roemer,T., Madden,K., Chang,J. and Snyder,M.				
TITLE	Selection of axial growth sites in yeast requires Axl2p, a novel plasma membrane glycoprotein				
JOURNAL	Genes Dev. 10 (7), 777-793 (1996)				
PUBMED	8846915				
REFERENCE	2 (bases 1 to 5028)				
AUTHORS	Roemer,T.				
TITLE	Direct Submission				
JOURNAL	Submitted (22-FEB-1996) Biology, Yale University, New Haven, CT 06520, USA				
FEATURES	Location/Qualifiers				
source	1..5028				
	/organism="Saccharomyces cerevisiae"				

12.2 PubMed and PubMed Central article database

PubMed and **PubMed Central (PMC)** are databases of scientific articles related to data contained in NCBI databases. PubMed contains basic bibliographic information, the abstract, relevant links to sequences, and to the websites of the actual publishers of the papers. If the text of an article is open-access, PMC should have a copy of it. Articles in PMC contain relevant hyperlinks, such as to any sequences that are mentioned. For example, the image below show where the sequence of Tp0751 is mentioned in a paper and linked to the GenBank record we looked at above.

12.3 Entrez

<https://en.wikipedia.org/wiki/Entrez>

“Entrez is a federated search engine and web portal that allows users to search many discrete health sciences databases of the NCBI website. The name “Entrez” (a greeting meaning “Come in” in French) was chosen to reflect the spirit of welcoming the public to search the content available from NCBI.”

“Entrez Global Query is an integrated search and retrieval system that provides access to all databases simultaneously with a single query and user interface. Entrez can efficiently retrieve related sequences, structures, and references. The Entrez system can provide views of gene and protein sequences and chromosome maps. Some textbooks are also available online through the Entrez system.”

12.4 BLAST

[https://en.wikipedia.org/wiki/BLAST_\(biotechnology\)](https://en.wikipedia.org/wiki/BLAST_(biotechnology))

“BLAST (basic local alignment search tool) is an algorithm and program for comparing primary biological sequence information, such as the amino-acid sequences of proteins or the nucleotides of DNA and/or RNA sequences. A BLAST search enables a researcher to compare a subject protein or nucleotide sequence (called a query) with a library or database of sequences, and identify database sequences that resemble the query sequence above a certain threshold. For example, following the discovery of a previously unknown gene in the mouse, a scientist will typically perform a BLAST search of the human genome to see if humans carry a similar gene; BLAST will identify sequences in the human genome that resemble the mouse gene based on similarity of sequence.”

Chapter 13

Introduction to biological sequences databases

`library(compbio4all)`

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

13.1 Topics

- NCBI vs. EMBL vs. DDBJ
- annotation
- Nucleotide vs. Protein vs. EST vs. Genome
- PubMed
- GenBank Records
- FASTA file format
- FASTA header line
- RefSeq
- refining searches

13.2 Introduction

NCBI is the National Center for Biotechnology Information. The NCBI Website is the entry point to a large number of databases giving access to **biological sequences** (DNA, RNA, protein) and biology-related publications.

When scientists sequence DNA, RNA and proteins they typically publish their data via databases with the NCBI. Each is given a unique identification number known as an **accession number**. For example, each time a unique human genome sequence is produced it is uploaded to the relevant databases, assigned a unique **accession**, and a website created to access it. Sequence are also cross-referenced to related papers, so you can start with a sequence and find out what scientific paper it was used in, or start with a paper and see if any sequences are associated with it.

This chapter provides an introduction to the general search features of the NCBI databases via the interface on the website, including how to locate sequences using accession numbers and other search parameters, such as specific authors or papers. Subsequent chapters will introduce advanced search features, and how to carry out searches using R commands.

One consequence of the explosion of biological sequences used in publications is that the system of databases has become fairly complex. There are databases for different types of data, different types of molecules, data from individual experiments showing **genetic variation** and also the **consensus sequence** for a given molecule. Luckily, if you know the accession number of the sequence you are looking for – which will be our starting point throughout this book – it's fairly straightforward. There are numerous other books on bioinformatics and genomics that provide all details if you need to do more complex searches.

In this chapter we'll typically refer generically to "NCBI data" and "NCBI databases." This is a simplification, since NCBI is the name of the organization and the databases and search engines often have specific names.

13.3 Biological sequence databases

Almost published biological sequences are available online, as it is a requirement of every scientific journal that any published DNA or RNA or protein sequence must be deposited in a public database. The main resources for storing and distributing sequence data are three large databases:

1. USA: **NCBI database** (www.ncbi.nlm.nih.gov/)
2. Europe: **European Molecular Biology Laboratory (EMBL)** database (<https://www.ebi.ac.uk/ena>)
3. Japan: **DNA Database of Japan (DDBJ)** database (www.ddbj.nig.ac.jp/). These databases collect all publicly available DNA, RNA and protein sequence data and make it available for free. They exchange data nightly, so contain essentially the same data. The redundancy among the databases allows them to serve different communities (e.g. native languages), provide different additional services such as tutorials, and assure that the world's scientists have their data backed up in different physical locations – a key component of good data management!

13.4 The NCBI Sequence Database

In this chapter we will explore the **NCBI sequence database** using the accession number NC_001477, which is for the complete DEN-1 Dengue virus genome sequence. The accession number is reported in scientific papers originally describing the sequence, and also in subsequent papers that use that particular sequence.

In addition to the sequence itself, for each sequence the NCBI database also stores some additional **annotation** data, such as the name of the species it comes from, references to publications describing that sequence, information on the structure of the proteins coded by the sequence, etc. Some of this annotation data was added by the person who sequenced a sequence and submitted it to the NCBI database, while some may have been added later by a human curator working for NCBI.

13.5 The NCBI Sub-Databases

The NCBI database contains several sub-databases, the most important of which are:

- **Nucleotide database:** contains DNA and RNA sequences
- **Protein database:** contains protein sequences
- **EST database:** contains ESTs (expressed sequence tags), which are short sequences derived from mRNAs. (This terminology is likely to be unfamiliar because it is not often used in introductory biology courses. The "Expressed" of EST comes from the fact that mRNA is the result of gene expression.)
- **Genome database:** contains the DNA sequences for entire genomes
- **PubMed:** contains data on scientific publications

From the main NCBI website you can initiate a search and it will look for hits across all the databases. You can narrow your search by selecting a particular database.

13.6 NCBI GenBank Record Format

As mentioned above, for each sequence the NCBI database stores some extra information such as the species that it came from, publications describing the sequence, etc. This information is stored in the GenBank entry (aka GenBank Record) for the sequence. The GenBank entry for a sequence can be viewed by searching the NCBI database for the accession number for that sequence.

To view the GenBank entry for the DEN-1 Dengue virus, follow these steps:

1. Go to the NCBI website (www.ncbi.nlm.nih.gov).
2. Search for the accession number NC_001477.
3. Since we searched for a particular accession we are only returned a single main result which is titled “NUCLEOTIDE SEQUENCE: Dengue virus 1, complete genome.”
4. Click on “Dengue virus 1, complete genome” to go to the GenBank entry.

The GenBank entry for an accession contains a LOT of information about the sequence, such as papers describing it, features in the sequence, etc. The **DEFINITION** field gives a short description for the sequence. The **ORGANISM** field in the NCBI entry identifies the species that the sequence came from. The **REFERENCE** field contains scientific publications describing the sequence. The **FEATURES** field contains information about the location of features of interest inside the sequence, such as regulatory sequences or genes that lie inside the sequence. The **ORIGIN** field gives the sequence itself.


```
{r, echo = F, eval = F} #
knitr::include_graphics(here::here("images
#
```

The **FASTA** file format is a simple file format commonly used to store and share sequence information. When you download sequences from databases such as NCBI you usually want FASTA files.

```
## >mysequence1
## ACATGAGACAGACAGACCCCCAGAGACAGACCCCTAGACACAGAGAGAG
## TATGCAGGACAGGGTFTTTTGCCCAGGGTGGCAGTATG
```

This is what a FASTA file with two sequence looks like.

14.2 RefSeq

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to the NCBI database, and variation exists between individual organisms due to population-level variation due to previous mutations and also potential recent spontaneous mutations. There also can be some error in the sequencing process that results in differences between sequences.

There are also many different types of nucleotide sequences and protein sequences in the NCBI database. With respect to nucleotide sequences, some may be entire genomic DNA sequences, some may be mRNAs, and some may be lower quality sequences such as expressed sequence tags (ESTs, which are derived from parts of mRNAs), or DNA sequences of **contigs** from genome projects. That is, you can end up with an entry in the protein database based on sequence derived from a genomic sequence, from sequencing just the gene, and from other routes. Furthermore, some sequences may be **manually curated** by NCBI staff so that the associated entries contain extra information, but the majority of sequences are **uncurated**.

Therefore, NCBI databases often contains redundant information for a gene, contains sequences of varying quality, and contains both uncurated and curated data. As a result, NCBI has made a special database called **RefSeq (reference sequence database)**, which is a subset of the NCBI database. The data in RefSeq is manually curated, is high quality sequence data, and is non-redundant; this means that each gene (or **splice-form** / **isoform** of a gene, in the case of eukaryotes), protein, or genome sequence is only represented once.

The data in RefSeq is curated and is of much higher quality than the rest of the NCBI Sequence Database. However, unfortunately, because of the high level of manual curation required, RefSeq does not cover all species, and is not comprehensive for the species that are covered so far. To speed up searches and simplify the results in to can be very useful to just search RefSeq. However, for detailed and thorough work the full database should probably be searched and the results scrutinized.

You can easily tell that a sequence comes from RefSeq because its accession number starts with particular sequence of letters. That is, accessions of RefSeq sequences corresponding to protein records usually start with **NP__**, and accessions of RefSeq curated complete genome sequences usually start with **NC__** or **NS__**.

14.3 Querying the NCBI Database

You may need to interrogate the NCBI Database to find particular sequences or a set of sequences matching given criteria, such as:

- The sequence with accession NC_001477
- The sequences published in Nature 460:352-358
- All sequences from *Chlamydia trachomatis*
- Sequences submitted by Caroline Cameron, a syphilis researcher
- Flagellin or fibrinogen sequences
- The glutamine synthetase gene from *Mycobacterium leprae*
- Just the upstream control region of the *Mycobacterium leprae* dnaA gene
- The sequence of the *Mycobacterium leprae* DnaA protein
- The genome sequence of syphilis, *Treponema pallidum* subspp. *pallidum*
- All human nucleotide sequences associated with malaria

There are two main ways that you can query the NCBI database to find these sets of sequences. The first possibility is to carry out searches on the NCBI website. The second possibility is to carry out searches from R using one of several packages that can interface with NCBI. As of October 2019 rentrez seems to be the best package for this..

Below, I will explain how to manually carry out queries on the NCBI database.

14.4 Querying the NCBI Database via the NCBI Website (for reference)

NOTE: The following section is here for reference; you need to know its *possible* to refine searches but do not need to know any of these actual tags.

If you are carrying out searches on the NCBI website, to narrow down your searches to specific types of sequences or to specific organisms, you will need to use “**search tags**”.

For example, the search tags “[PROP]” and “[ORGN]” let you restrict your search to a specific subset of the NCBI Sequence Database, or to sequences from a particular taxon, respectively. Here is a list of useful search tags, which we will explain how to use below:

- [AC], e.g. NC_001477[AC] With a particular accession number
- [ORGN], e.g. Fungi[ORGN] From a particular organism or taxon
- [PROP], e.g. biomol_mRNA[PROP] Of a specific type (eg. mRNA) or from a specific database (eg. RefSeq)
- [JOUR], e.g. Nature[JOUR] Described in a paper published in a particular journal
- [VOL], e.g. 531[VOL] Described in a paper published in a particular journal volume
- [PAGE], e.g. 27[PAGE] Described in a paper with a particular start-page in a journal
- [AU], e.g. “Smith J”[AU] Described in a paper, or submitted to NCBI, by a particular author

To carry out searches of the NCBI database, you first need to go to the NCBI website, and type your search query into the search box at the top. For example, to search for all sequences from Fungi, you would type “Fungi[ORGN]” into the search box on the NCBI website.

You can combine the search tags above by using “AND”, to make more complex searches. For example, to find all mRNA sequences from Fungi, you could type “Fungi[ORGN] AND biomol_mRNA[PROP]” in the search box on the NCBI website.

Likewise, you can also combine search tags by using “OR”, for example, to search for all mRNA sequences from Fungi or Bacteria, you would type “(Fungi[ORGN] OR Bacteria[ORGN]) AND biomol_mRNA[PROP]” in the search box. Note that you need to put brackets around “Fungi[ORGN] OR Bacteria[ORGN]” to specify that the word “OR” refers to these two search tags.

Here are some examples of searches, some of them made by combining search terms using “AND”:

- NC_001477[AC] - With accession number NC_001477
- Nature[JOUR] AND 460[VOL] AND 352[PAGE] - Published in Nature 460:352-358
- “Chlamydia trachomatis”[ORGN] - From the bacterium Chlamydia trachomatis
- “Berriman M”[AU] - Published in a paper, or submitted to NCBI, by M. Berriman
- flagellin OR fibrinogen - Which contain the word “flagellin” or “fibrinogen” in their NCBI record
- “Mycobacterium leprae”[ORGN] AND dnaA - Which are from M. leprae, and contain “dnaA” in their NCBI record
- “Homo sapiens”[ORGN] AND “colon cancer” - Which are from human, and contain “colon cancer” in their NCBI record
- “Homo sapiens”[ORGN] AND malaria - Which are from human, and contain “malaria” in their NCBI record
- “Homo sapiens”[ORGN] AND biomol_mrna[PROP] - Which are mRNA sequences from human
- “Bacteria”[ORGN] AND srcdb_refseq[PROP] - Which are RefSeq sequences from Bacteria
- “colon cancer” AND srcdb_refseq[PROP] - From RefSeq, which contain “colon cancer” in their NCBI record

Note that if you are searching for a phrase such as “colon cancer” or “Chlamydia trachomatis”, you need to put the phrase in quotes when typing it into the search box. This is because if you type the phrase in the search box without quotes, the search will be for NCBI records that contain either of the two words “colon” or “cancer” (or either of the two words “Chlamydia” or “trachomatis”), not necessarily both words.

As mentioned above, the NCBI database contains several sub-databases, including the NCBI **Nucleotide database** and the NCBI **Protein database**. If you go to the NCBI website, and type one of the search queries above in the search box at the top of the page, the results page will tell you how many matching NCBI records were found in each of the NCBI sub-databases.

For example, if you search for “Chlamydia trachomatis[ORGN]”, you will get matches to proteins from *C. trachomatis* in the NCBI Protein database, matches to DNA and RNA sequences from *C. trachomatis* in the NCBI Nucleotide database, matches to whole genome sequences for *C. trachomatis* strains in the NCBI Genome database, and so on:

Alternatively, if you know in advance that you want to search a particular sub-database, for example, the NCBI Protein database, when you go to the NCBI website, you can select that sub-database from the drop-down list above the search box, so that you will search that sub-database.

14.5 Example: finding the sequences published in Nature 460:352-358 (for reference)

NOTE: The following section is here for reference; you need to know its *possible* to refine searches but do not need to know any of these actual tags.

For example, if you want to find sequences published in Nature 460:352-358, you can use the “[JOUR]”, “[VOL]” and “[PAGE]” search terms. That is, you would go to the NCBI website and type in the search box on the top: “Nature[JOUR] AND 460[VOL] AND 352[PAGE]”, where [JOUR] specifies the journal name, [VOL] the volume of the journal the paper is in, and [PAGE] the page number.

This should bring up a results page with “50890” beside the word “Nucleotide”, and “1” beside the word “Genome”, and “25701” beside the word “Protein”, indicating that there were 50890 hits to sequence records in the Nucleotide database, which contains DNA and RNA sequences, and 1 hit to the Genome database, which contains genome sequences, and 25701 hits to the Protein database, which contains protein sequences.

If you click on the word “Nucleotide”, it will bring up a webpage with a list of links to the NCBI sequence records for those 50890 hits. The 50890 hits are all contigs from the schistosome worm *Schistosoma mansoni*.

Likewise, if you click on the word “Protein”, it will bring up a webpage with a list of links to the NCBI sequence records for the 25701 hits, and you will see that the hits are all predicted proteins for *Schistosoma mansoni*.

If you click on the word “Genome”, it will bring you to the NCBI record for the *Schistosoma mansoni* genome sequence, which has NCBI accession NS_00200. Note that the accession starts with “NS_”, which indicates that it is a RefSeq accession.

Therefore, in Nature volume 460, page 352, the *Schistosoma mansoni* genome sequence was published, along with all the DNA sequence contigs that were sequenced for the genome project, and all the predicted proteins for the gene predictions made in the genome sequence. You can view the original paper on the Nature website at <http://www.nature.com/nature/journal/v460/n7253/abs/nature08160.html>.

Note: *Schistmosoma mansoni* is a parasitic worm that is responsible for causing **schistosomiasis**, which is classified by the WHO as a **neglected tropical disease**.

Chapter 15

Downloading NCBI sequence data by hand

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

15.1 Preface

The following chapter was originally written by Avril Coghlan. It provides brief, basic information for how to access sequences via the internet. Subsequent chapters provide more details and R code.

15.2 Retrieving genome sequence data via the NCBI website

You can easily retrieve DNA or protein sequence data by hand from the NCBI Sequence Database via its website www.ncbi.nlm.nih.gov.

Dengue DEN-1 DNA is a viral DNA sequence and its NCBI **accession number** is NC_001477. To retrieve the DNA sequence for the Dengue DEN-1 virus from NCBI, go to the NCBI website, type “NC_001477” in the Search box at the top of the webpage, and press the “Search” button beside the Search box.

(While this is the normal workflow, accessions related to well-known organisms can sometimes turn up using a direct Google search. This is the case for NC_001477 - if you Google it you can go directly to the website with the genome sequence.)

On the results page of a normal NCBI search you will see the number of hits to “NC_001477” in each of the NCBI databases on the NCBI website. There are *many* databases on the NCBI website, for example, **PubMed** and **Pubmed Central** contain abstracts from scientific papers, the **Genes** and **Genomes** database contains DNA and RNA sequence data, the **Proteins** database contains protein sequence data, and so on.

Most biologist would do this type of work by hand from within their web browser, but it can also be done by writing small programs in **scripting languages** such as **Python** or **R**. In R, the **rentrez** package is a powerful tool for intersecting with NCBI resource. In this tutorial we’ll focus on the web interface. Its good to remember, though, that almost anything done via the webpage can be automated using a computer **script**.

A challenge when learning to use NCBI resources is that there is a tremendous amount of sequence information available and you need to learn how to sort through what the search results provide. As you are looking

for the *DNA* sequence of the Dengue DEN-1 virus genome, you expect to see a hit in the NCBI **Nucleotide database**. This is indicated at the top of the page where it says “**NUCLEOTIDE SEQUENCE**” and lists “Dengue virus 1, complete genome.”

When you click on the link for the Nucleotide database, it will bring you to the record for NC_001477 in the NCBI Nucleotide database. This will contain the name and NCBI **accession** of the sequence, as well as other details such as any papers describing the sequence. If you scroll down you’ll see the sequence also.

If you need it, you can retrieve the DNA sequence for the DEN-1 Dengue virus genome sequence as a **FASTA format** sequence file in a couple ways. The easiest is just to copy and paste it into a text, .R, or other file. You can also click on “**Send to**” at the top right of the NC_001477 sequence record webpage (just to the left of the side bar; its kinda small).

After you click on **Send to** you can pick several options. and then choose “File” in the menu that appears, and then choose FASTA from the “Format” menu that appears, and click on “Create file”. The sequence will then download. The default file name is **sequence.fasta** so you’ll probably want to change it.

You can now open the FASTA file containing the DEN-1 Dengue virus genome sequence using a text editor like Notepad, WordPad, Notepad++, or even RStudio on your computer. To find a text editor on your computer search for “text” from the start menu (Windows) and usually one will come up. (Opening the file in a word processor like word isn’t recommended).

Chapter 16

Downloading sequences from UniProt by hand

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

16.1 Vocab

- RefSeq
- manual curation
- UniProt
- accession

16.2 Downloading Protein data from UniProt

In a previous vignette you learned how to retrieve sequences from the NCBI database. The NCBI database is a key database in bioinformatics because it contains essentially all DNA sequences ever sequenced.

As mentioned previously, a subsection of the NCBI database called **RefSeq** consists of high quality DNA and protein sequence data. Furthermore, the NCBI entries for the RefSeq sequences have been **manually curated**, which means that expert biologists employed by NCBI have added additional information to the NCBI entries for those sequences, such as details of scientific papers that describe the sequences.

Another extremely important manually curated database is **UniProt**, which focuses on protein sequences. UniProt aims to contain manually curated information on all known protein sequences. While many of the protein sequences in UniProt are also present in RefSeq, the amount and quality of manually curated information in UniProt is much higher than that in RefSeq.

For each protein in UniProt, the UniProt curators read all the scientific papers that they can find about that protein, and add information from those papers to the protein's UniProt entry. For example, for a human protein, the UniProt entry for the protein usually includes information about the biological function of the protein, in what human tissues it is expressed, whether it interacts with other human proteins, and much more. All this information has been manually gathered by the UniProt curators from scientific papers, and the papers in which the found the information are always listed in the UniProt entry for the protein.

Just like NCBI, UniProt also assigns an **accession** to each sequence in the UniProt database. Although the same protein sequence may appear in both the NCBI database and the UniProt database, it will have

different NCBI and UniProt accessions. However, there is usually a link on the NCBI entry for the protein sequence to the UniProt entry, and vice versa.

16.3 Viewing the UniProt webpage for a protein sequence

If you are given the UniProt accession for a protein, to find the UniProt entry for the protein, you first need to go the UniProt website, www.uniprot.org. At the top of the UniProt website, you will see a search box, and you can type the accession of the protein that you are looking for in this search box, and then click on the “Search” button to search for it.

For example, if you want to find the sequence for the chorismate lyase protein from *Mycobacterium leprae* (the bacterium which causes leprosy), which has UniProt accession Q9CD83, you would type just “Q9CD83” in the search box and press “Search”. The UniProt entry for UniProt accession Q9CD83 will then appear in your web browser.

Beside the heading “Organism” you can see the organism is given as *Mycobacterium leprae*. If you scroll down you’ll find a section **Names and Taxonomy** and beside the heading “Taxonomic lineage”, you can see “Bacteria - Actinobacteria - Actinobacteridae - Actinomycetales - Corynebacterineae - Mycobacteriaceae - Mycobacterium”.

This tells us that *Mycobacterium* is a species of bacteria, which belongs to a group of related bacteria called the Mycobacteriaceae, which itself belongs to a larger group of related bacteria called the Corynebacterineae, which itself belongs to an even larger group of related bacteria called the Actinomycetales, which itself belongs to the Actinobacteridae, which itself belongs to a huge group of bacteria called the Actinobacteria.

16.3.1 Protein function

Back up at the top under “organism” it says “Status”, which tells us the **annotation score** is 2 out of 5, that it is a “Protein inferred from homology”, which means what we know about it is derived from bioinformatics and computational tools, not lab work.

Beside the heading “Function”, it says that the function of this protein is that it “Removes the pyruvyl group from chorismate to provide 4-hydroxybenzoate (4HB)”. This tells us this protein is an enzyme (a protein that increases the rate of a specific biochemical reaction), and tells us what is the particular biochemical reaction that this enzyme is involved in. At the end of this info it says “By similarity”, which again indicates that what we know about this protein comes from bioinformatics, not lab work.

16.3.2 Protein sequence and size

Under **Sequence** we see that the sequence length is 210 amino acids long (210 letters long) and has a mass of 24,045 daltons. We can access the sequence as a FASTA file from here if we want and also carry out a BLAST search from a link on the right.

16.3.3 Other information

Further down the UniProt page for this protein, you will see a lot more information, as well as many links to webpages in other biological databases, such as NCBI. The huge amount of information about proteins in UniProt means that if you want to find out about a particular protein, the UniProt page for that protein is a great place to start.

16.4 Retrieving a UniProt protein sequence via the UniProt website

There are a couple different ways to retrieve the sequence. At the top of the page is a tab that says “Format” which brings you to a page with the FASTA file. You can copy and paste the sequence from here if you want.

To save it as a file, go to the “File” menu of your web browser, choose “Save page as”, and save the file. Remember to give the file a sensible name (eg. “Q9CD83.fasta” for accession Q9CD83), and in a place that you will remember (eg. in the “My Documents” folder).

For example, you can retrieve the protein sequences for the chorismate lyase protein from *Mycobacterium leprae* (which has UniProt accession Q9CD83) and for the chorismate lyase protein from *Mycobacterium ulcerans* (UniProt accession A0PQ23), and save them as FASTA-format files (eg. “Q9CD83.fasta” and “A0PQ23.fasta”, as described above.

You can also put the UniProt information into an online **Basket**. If you do this for both Q9CD83 and A0PQ23 you can think click on **Basket**, select both entries, and carry out a pairwise alignment by clicking on **Align**.

Mycobacterium leprae is the bacterium which causes leprosy, while *Mycobacterium ulcerans* is a related bacterium which causes Buruli ulcer, both of which are classified by the WHO as neglected tropical diseases. The *M. leprae* and *M. ulcerans* chorismate lyase proteins are an example of a pair of **homologous** (related) proteins in two related species of bacteria.

If you downloaded the protein sequences for UniProt accessions Q9CD83 and A0PQ23 and saved them as FASTA-format files (eg. “Q9CD83.fasta” and “A0PQ23.fasta”), you could read them into R using the `read.fasta()` function in the `SeqinR` R package (as detailed in another Vignette) or a similar function from another package.

Note that the `read.fasta()` function normally expects that you have put your FASTA-format files in the the **working directory** of R. For convenience so you can explore these sequences they have been saved in a special folder in the *compbio4all* package and can be accessed like this for the Leprosy sequence

```
# load compbio4all
library(compbio4all)

# locate the file within the package using system.file()
file.1 <- system.file("./extdata/Q9CD83.fasta", package = "compbio4all")

# load seqinr
library("seqinr")

# load fasta
leprae <- read.fasta(file = file.1)
lepraeseq <- leprae[[1]]
```

We can confirm

```
str(lepraeseq)
```

For the other sequence

```
# locate the file within the package using system.file()
file.2 <- system.file("./extdata/A0PQ23.fasta", package = "compbio4all")

# load fasta
ulcerans <- read.fasta(file = file.2)
ulceransseq <- ulcerans[[1]]
```


Chapter 17

Introducing FASTA Files

Adapted from Wikipedia: https://en.wikipedia.org/wiki/FASTA_format

“In bioinformatics, the FASTA format is a text-based format for representing either nucleotide sequences or amino acid (protein) sequences, in which nucleotides or amino acids are represented using single-letter codes. The format allows for sequence names and comments to precede the sequences. The format originates from the FASTA alignment software, but has now become a near universal standard in the field of bioinformatics.

“The simplicity of FASTA format makes it easy to manipulate and parse sequences using text-processing tools and scripting languages like the R programming language and Python.

“The first line in a FASTA file starts with a “>” (greater-than) symbol and holds summary information about the sequence, often starting with a unique accession number and followed by information like the name of the gene, the type of sequence, and the organism it is from.

“On the next is the sequence itself in a standard one-letter character string. Anything other than a valid character is be ignored (including spaces, tabs, asterisks, etc...).

“A multiple sequence FASTA format can be obtained by concatenating several single sequence FASTA files in a common file (also known as multi-FASTA format).

“Following the header line, the actual sequence is represented. Sequences may be protein sequences or nucleic acid sequences, and they can contain gaps or alignment characters. Sequences are expected to be represented in the standard amino acid and nucleic acid codes. Lower-case letters are accepted and are mapped into upper-case; a single hyphen or dash can be used to represent a gap character; and in amino acid sequences, U and * are acceptable letters.

“FASTQ format is a form of FASTA format extended to indicate information related to sequencing. It is created by the Sanger Centre in Cambridge.

“Bioconductor.org’s Biostrings package can be used to read and manipulate FASTA files in R

from <https://zhanglab.dcmf.med.umich.edu/FASTA/>

“FASTA format is a text-based format for representing either nucleotide sequences or peptide sequences, in which base pairs or amino acids are represented using single-letter codes. A sequence in FASTA format begins with a single-line description, followed by lines of sequence data. The description line is distinguished from the sequence data by a greater-than (“>”) symbol in the first column. It is recommended that all lines of text be shorter than 80 characters in length.”

17.1 Example FASTA file

Here is an example of the contents of a FASTA file. (If you are viewing this chapter in the form of the source .Rmd file, the `cat()` function is included just to print out the content properly and is not part of the FASTA format).

```
cat(">gi|186681228|ref|YP_001864424.1|_phycoerythrobilin:ferredoxin_
oxidoreductase
MNSERSDVTLYQPFLDYAIAYMRSRLDLEPYIPTGFESNSAVVGKGNQEEVTTTSYAFQTAKLRQIRA
AHVQGGNSLQVLNFVIFPHLNYDLPPFGADLVTLPGGHLLALDMQPLFRDDSAQAKYTEPILPIFHAHQ
QHLSWGGDFPEEAQPFSPAFWTRPQETAVVETQVFAAFKDYLKAYLDFVEQAEAVTDSQNLVAIKQAAQ
LRYLRYRAEKDPARGMFKRFYGAEWTEEYIHGFLFDLERKLTVVK")

## >gi|186681228|ref|YP_001864424.1|_phycoerythrobilin:ferredoxin
oxidoreductase
## MNSERSDVTLYQPFLDYAIAYMRSRLDLEPYIPTGFESNSAVVGKGNQEEVTTTSYAFQTAKLRQIRA
## AHVQGGNSLQVLNFVIFPHLNYDLPPFGADLVTLPGGHLLALDMQPLFRDDSAQAKYTEPILPIFHAHQ
## QHLSWGGDFPEEAQPFSPAFWTRPQETAVVETQVFAAFKDYLKAYLDFVEQAEAVTDSQNLVAIKQAAQ
## LRYLRYRAEKDPARGMFKRFYGAEWTEEYIHGFLFDLERKLTVVK
```

17.2 Multiple sequences in a single FASTA file

Multiple sequences can be stored in a single FASTA file, each on separated by a line and have its own headline.

```
cat(">LCBO_-_Prolactin_precursor_-_Bovine
MDSKGSSQKGSRLLLLLVSNLLLCQGVVSTPVCNPGPGNCQVSLRDLFDRAVMVSHYIHDLS
EMFNEFDKRYAQKGFTMALNSCHTSSLPTPEDKEQAQQTHHEVLMSLILGLLRSWNDPLYHL
VTEVRGMKGAPDAILSRAIEIEEENKRLLGEMEMIFGQVIPGAKETEPYPVWSGLPSLQTKDED
ARYSAFYNNLLHCLRRDSSKIDTYLKLLNCRIYNNNC*

>MCHU_-_Calmodulin_-_Human,_rabbit,_bovine,_rat,_and_chicken
MADQLTEEQIAEFKEAFSLFDKGDGTTITKELGTVMRSLGQNPTEAELQDMINEVDADGNGTID
FPEFLTMMARKMKDITDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDDEMIREA
DIDGDGQVNYEEFVQMMTAK*

>gi|5524211|gb|AAD44166.1|_cytochrome_b_[Elephas_maximus_maximus]
LCLYTHIGRNYYGSYLYSETWNTGIMLLITMATAFMGYVLPWQGQMSFWGATVITNLFSAIPYIGTNLV
EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYTIKDFLG
LLILLLLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
GLMPFLHTSKHRSMMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIIGQMASILYFSIILAFLPIAGX
IENY")

## >LCBO - Prolactin precursor - Bovine
## MDSKGSSQKGSRLLLLLVSNLLLCQGVVSTPVCNPGPGNCQVSLRDLFDRAVMVSHYIHDLS
## EMFNEFDKRYAQKGFTMALNSCHTSSLPTPEDKEQAQQTHHEVLMSLILGLLRSWNDPLYHL
## VTEVRGMKGAPDAILSRAIEIEEENKRLLGEMEMIFGQVIPGAKETEPYPVWSGLPSLQTKDED
## ARYSAFYNNLLHCLRRDSSKIDTYLKLLNCRIYNNNC*
##
## >MCHU - Calmodulin - Human, rabbit, bovine, rat, and chicken
## MADQLTEEQIAEFKEAFSLFDKGDGTTITKELGTVMRSLGQNPTEAELQDMINEVDADGNGTID
## FPEFLTMMARKMKDITDSEEEIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDDEMIREA
## DIDGDGQVNYEEFVQMMTAK*
##
```

```
## >gi|5524211|gb|AAD44166.1| cytochrome b [Elephas maximus maximus]
## LCLYTHIGRNIYYGSYLYSETWNTGIMLLITMATAFMGYVLPWGQMSFWGATVITNLFSAIPYIGTNLV
## EWIWGGFSVDKATLNRFFAFHFILPFTMVALAGVHLTFLHETGSNNPLGLTSDSDKIPFHPYYTIKDFLG
## LLILLLLLLLLLALLSPDMLGDPDNHMPADPLNTPLHIKPEWYFLFAYAILRSVPNKLGGVLALFLSIVIL
## GLMPFLHTSKHRSMLRPLSQALFWTLTMDLLTLTWIGSQPVEYPYTIGQMASILYFSIILAFPLIAGX
## IENY
```

17.3 Multiple sequence alignments can be stored in FASTA format

Aligned FASTA format can be used to store the output of **Multiple Sequence Alignment (MSA)**. This format contains

1. Multiple entries, each with their own header line
2. **Gaps** inserted to align sequences are indicated by `.`
3. Each spaces added to the beginning and end of sequences that vary in length are indicated by `~`

In the sample FASTA file below, the example1 sequence has a gap of 8 near its beginning. The example2 sequence has numerous `~` indicating that this sequence is missing data from its beginning that are present in the other sequences. The example3 sequence has numerous `~` at its end, indicating that this sequence is shorter than the others.

```
cat(">example1
MKALWALLLVPLLTGCLA . . . . . EGELEVTDQLPGQSDQP.WEQALNRFWDYLRWVQ
GNQARDRLEEVRQMEEEVRSKMEEQTQQIRLQAEIFQARIKGWFEPLVEDMQRQWANLME
KIQASVATNSIASTTVPLENQ
>example2
~~~~~KVQQELEPEAGWQTGQP.WEAALARFWDYLRWVQ
SSRARGHLEEMREQIQEVRVKMEEQADQIRQKAEAFQARLKSWFEPLLEDMDQRQWDGLVE
KVQAAVAT.IPTSKPVEEP~~
>example3
MRSLVVFFALAVLTGCQARSLFQAD . . . . . APQPRWEEMVDRFWQYVSELN
AGALKEKLEETAENL . . . RTSLEGRVDELTSLLAPYSQKIREQLQEVMDKIKEATAALPT
QA~~~~~")

## >example1
## MKALWALLLVPLLTGCLA . . . . . EGELEVTDQLPGQSDQP.WEQALNRFWDYLRWVQ
## GNQARDRLEEVRQMEEEVRSKMEEQTQQIRLQAEIFQARIKGWFEPLVEDMQRQWANLME
## KIQASVATNSIASTTVPLENQ
## >example2
## ~~~~~KVQQELEPEAGWQTGQP.WEAALARFWDYLRWVQ
## SSRARGHLEEMREQIQEVRVKMEEQADQIRQKAEAFQARLKSWFEPLLEDMDQRQWDGLVE
## KVQAAVAT.IPTSKPVEEP~~
## >example3
## MRSLVVFFALAVLTGCQARSLFQAD . . . . . APQPRWEEMVDRFWQYVSELN
## AGALKEKLEETAENL . . . RTSLEGRVDELTSLLAPYSQKIREQLQEVMDKIKEATAALPT
## QA~~~~~
```

17.4 FASTQ Format

Adapted from Wikipedia: https://en.wikipedia.org/wiki/FASTQ_format

“FASTQ format is a text-based format for storing both a biological sequence (usually nucleotide sequence) and its corresponding quality scores. Both the sequence letter and quality score are each encoded with a single ASCII character for brevity.

“It was originally developed at the Wellcome Trust Sanger Institute to bundle a FASTA formatted sequence and its quality data, but has recently become the de facto standard for storing the output of high-throughput sequencing instruments such as the Illumina Genome Analyzer.

“A FASTQ file normally uses four lines per sequence.

- Line 1 begins with a @ character and is followed by a sequence identifier and an optional description (like a FASTA title line).
- Line 2 is the raw sequence letters.
- Line 3 begins with a + character and is optionally followed by the same sequence identifier (and any description) again.
- Line 4 encodes the **quality values** for the sequence in Line 2 of the file, and must contain the same number of symbols as letters in the sequence.

“A FASTQ file containing a single sequence might look like this:”

```
cat( "@SEQ_ID
GATTGTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
+
!''*(((((***+))%%%)++)(%%%)%.1***-+*'))**55CCF>>>>>>>CCCCCCC65")

## @SEQ_ID
## GATTGTGGGGTTCAAAGCAGTATCGATCAAATAGTAAATCCATTTGTTCAACTCACAGTTT
## +
## !''*(((((***+))%%%)++)(%%%)%.1***-+*'))**55CCF>>>>>>>CCCCCCC65
```

“Here are the quality value characters in left-to-right increasing order of quality (ASCII):”

```
!"#$%&'()*+,-./0123456789:;<=>?@ABCDEFGHIJKLMNPOQRSTUVWXYZ[\]^_`
abcdefghijklmnopqrstuvwxyz{|}~
```

FASTQ files typically do not include line breaks and do not wrap around when they reach the width of a normal page or file.

Chapter 18

Downloading DNA sequences as FASTA files in R

This is a modification of “DNA Sequence Statistics” from Avril Coghlan’s *A little book of R for bioinformatics*.. Most of the text and code was originally written by Dr. Coghlan and distributed under the Creative Commons 3.0 license.

NOTE: There is some redundancy in this current draft that needs to be eliminated.

18.0.1 Functions

- `library()`
- `help()`
- `length`
- `table`
- `seqinr::GC()`
- `seqinr::count()`
- `seqinr::write.fasta()`

18.0.2 Software/websites

- www.ncbi.nlm.nih.gov
- Text editors (e.g. Notepad++, TextWrangler)

18.0.3 R vocabulary

- `list`
- `library`
- `package`
- `CRAN`
- `wrapper`

18.0.4 File types

- FASTA

18.0.5 Bioinformatics vocabulary

- accession, accession number

- NCBI
- NCBI Sequence Database
- EMBL Sequence Database
- FASTA file

18.0.6 Organisms and Sequence accessions

- Dengue virus: DEN-1, DEN-2, DEN-3, and DEN-4.

The NCBI accessions for the DNA sequences of the DEN-1, DEN-2, DEN-3, and DEN-4 Dengue viruses are NC_001477, NC_001474, NC_001475 and NC_002640, respectively.

According to Wikipedia

“Dengue virus (DENV) is the cause of dengue fever. It is a mosquito-borne, single positive-stranded RNA virus ... Five serotypes of the virus have been found, all of which can cause the full spectrum of disease. Nevertheless, scientists’ understanding of dengue virus may be simplistic, as rather than distinct ... groups, a continuum appears to exist.” https://en.wikipedia.org/wiki/Dengue_virus

18.0.7 Preliminaries

```
library(rentrez)
library(compbio4all)
```

18.1 DNA Sequence Statistics: Part 1

18.1.1 Using R for Bioinformatics

The chapter will guide you through the process of using R to carry out simple analyses that are common in bioinformatics and computational biology. In particular, the focus is on computational analysis of biological sequence data such as genome sequences and protein sequences. The programming approaches, however, are broadly generalizable to statistics and data science.

The tutorials assume that the reader has some basic knowledge of biology, but not necessarily of bioinformatics. The focus is to explain simple bioinformatics analysis, and to explain how to carry out these analyses using *R*.

18.1.2 R packages for bioinformatics: Bioconductor and SeqinR

Many authors have written *R* packages for performing a wide variety of analyses. These do not come with the standard *R* installation, but must be installed and loaded as “add-ons”.

Bioinformaticians have written numerous specialized packages for *R*. In this tutorial, you will learn to use some of the function in the SeqinR package to carry out simple analyses of DNA sequences. (SeqinR can retrieve sequences from a DNA sequence database, but this has largely been replaced by the functions in the package rentrez)

Many well-known bioinformatics packages for *R* are in the Bioconductor set of *R* packages (www.bioconductor.org), which contains packages with many *R* functions for analyzing biological data sets such as microarray data. The SeqinR package is from CRAN, which contains R functions for obtaining sequences from DNA and protein sequence databases, and for analyzing DNA and protein sequences.

We will also use functions from the rentrez and ape packages.

Remember that you can ask for more information about a particular *R* command by using the help() function. For example, to ask for more information about the library(), you can type:


```
help("library")
```

You can also do this

```
?library
```

18.1.3 FASTA file format

The FASTA format is a simple and widely used format for storing biological (e.g. DNA or protein) sequences. It was first used by the FASTA program for sequence alignment in the 1980s and has been adopted as standard by many other programs.

FASTA files begin with a single-line description starting with a greater-than sign > character, followed on the next line by the sequences. Here is an example of a FASTA file. (If you're looking at the source script for this lesson you'll see the `cat()` command, which is just a text display function used format the text when you run the code).

```
## >A06852 183 residues
MPRLFSYLLGVWLLLSQLPREIPGQSTNDFIKACGRELVRWLWVEICGSVSWGRTALSLEEPQLETGPPAETMPSSITKDAEILKMMI
```

18.1.4 The NCBI sequence database

The US National Centre for Biotechnology Information (NCBI) maintains the **NCBI Sequence Database**, a huge database of all the DNA and protein sequence data that has been collected. There are also similar databases in Europe, the European Molecular Biology Laboratory (EMBL) Sequence Database, and Japan, the DNA Data Bank of Japan (DDBJ). These three databases exchange data every night, so at any one point in time, they contain almost identical data.

Each sequence in the NCBI Sequence Database is stored in a separate **record**, and is assigned a unique identifier that can be used to refer to that record. The identifier is known as an **accession**, and consists of a mixture of numbers and letters.

For example, Dengue virus causes Dengue fever, which is classified as a **neglected tropical disease** by the World Health Organization (WHO), is classified by any one of four types of Dengue virus: DEN-1, DEN-2, DEN-3, and DEN-4. The NCBI accessions for the DNA sequences of the DEN-1, DEN-2, DEN-3, and DEN-4 Dengue viruses are

- NC_001477
- NC_001474
- NC_001475

- NC_002640

Note that because the NCBI Sequence Database, the EMBL Sequence Database, and DDBJ exchange data every night, the DEN-1 (and DEN-2, DEN-3, DEN-4) Dengue virus sequence are present in all three databases, but they have different accessions in each database, as they each use their own numbering systems for referring to their own sequence records.

18.1.5 Retrieving genome sequence data using rentrez

You can retrieve sequence data from NCBI directly from *R* using the `rentrez` package. The DEN-1 Dengue virus genome sequence has NCBI accession NC_001477. To retrieve a sequence with a particular NCBI accession, you can use the function `entrez_fetch()` from the `rentrez` package. Note that to be specific where the function comes from I write it as `package::function()`.

```
dengueseq_fasta <- rentrez::entrez_fetch(db = "nucleotide",
                                         id = "NC_001477",
                                         rettype = "fasta")
```

Note that the “`_`” in the name is just an arbitrary way to separate two words. Another common format would be `dengueseq.fasta`. Some people like `dengueseqFasta`, called **camel case** because the capital letter makes a hump in the middle of the word. Underscores are becoming most common and are favored by developers associated with *RStudio* and the **tidyverse** of packages that many data scientists use. I switch between “`_`” and “” as separators, usually favoring “`_`” for function names and “” for objects; I personally find camel case harder to read and to type.

Ok, so what exactly have we done when we made `dengueseq_fasta`? We have an R object `dengueseq_fasta` which has the sequence linked to the accession number “NC_001477.” So where is the sequence, and what is it?

First, what is it?

```
is(dengueseq_fasta)

## [1] "character"          "vector"              "data.frameRowLabels"
## [4] "SuperClassMethod"

class(dengueseq_fasta)

## [1] "character"
```

How big is it? Try the `dim()` and `length()` commands and see which one works. Do you know why one works and the other doesn’t?

```
dim(dengueseq_fasta)

## NULL

length(dengueseq_fasta)

## [1] 1
```

The size of the object is 1. Why is this? This is the genomics sequence of a virus, so you’d expect it to be fairly large. We’ll use another function below to explore that issue. Think about this first: how many pieces of unique information are in the `dengueseq` object? In what sense is there only *one* piece of information?

If we want to actually see the sequence we can type just `dengueseq_fasta` and press enter. This will print the **WHOLE** genomic sequence out but it will probably run off your screen.

```
dengueseq_fasta
```

This is a whole genome sequence, but its stored as single entry in a vector, so the `length()` command just tells us how many entries there are in the vector, which is just one! What this means is that the entire genomic sequence is stored in a single entry of the vector `dengueseq_fasta`. (If you’re not following along with this, no worries - its not essential to actually working with the data)

If we want to actually know how long the sequence is, we need to use the function `nchar()`.

```
nchar(dengueseq_fasta)

## [1] 10935
```

The sequence is 10935 bases long. All of these bases are stored as a single **character string** with no spaces in a single entry of our `dengueseq_fasta` vector. This isn't actually a useful format for us, so below we're going to convert it to something more useful.

If we want to see just part of the sequence we can use the `strtrim()` function. Before you run the code below, predict what the 100 means.

```
strtrim(dengueseq_fasta , 100)

## [1] ">NC_001477.1 Dengue virus 1, complete genome\
      nAGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTCGAATCGGAAGCTTGCTTAAC"
```

Note that at the end of the name is a slash followed by an `n`, which indicates to the computer that this is a **newline**; this is read by text editor, but is ignored by R in this context.

```
strtrim(dengueseq_fasta , 45)

## [1] ">NC_001477.1 Dengue virus 1, complete genome\nA"
```

After the `\n` begins the sequence, which will continue on for a LOOOOOONG way. Let's just print a little bit.

```
strtrim(dengueseq_fasta , 52)

## [1] ">NC_001477.1 Dengue virus 1, complete genome\nAGTTGTTA"
```

Let's print some more. Do you notice anything beside A, T, C and G in the sequence?

```
strtrim(dengueseq_fasta , 200)

## [1] ">NC_001477.1 Dengue virus 1, complete genome\
      nAGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTCGAATCGGAAGCTTGCTTAACGTAGTTCTAACAGT\
      nTTTTATTAGAGAGCAGATCTCTGATGAACAACCAACGGAAAAAGACGGGTGACCGTCTTTCAATATGC\
      nTGAAACGCGCGAGAAA"
```

Again, there are the `\n` newline characters, which tell text editors and wordprocessors how to display the file.

Now that we have a sense of what we're looking at let's explore the `dengueseq_fasta` a bit more.

We can find out more information about what it is using the `class()` command.

```
class(dengueseq_fasta)

## [1] "character"
```

As noted before, this is character data.

Many things in R are vectors so we can ask `R` is `vector()`

```
is.vector(dengueseq_fasta)

## [1] TRUE
```

Yup, that's true.

Ok, let's see what else. A handy though often verbose command is `is()`, which tells us what an object, well, what it is:

```
is(dengueseq_fasta)
```

```
## [1] "character"          "vector"              "data.frameRowLabels"
## [4] "SuperClassMethod"
```

There is a lot here but if you scan for some key words you will see “character” and “vector” at the top. The other stuff you can ignore. The first two things, though, tell us the `dengueseq_fasta` is a **vector** of the class **character**: that is, a **character vector**.

Another handy function is `str()`, which gives us a peak at the context and structure of an *R* object. This is most useful when you are working in the R console or with dataframes, but is a useful function to run on all *R* objects. How does this output differ from other ways we’ve displayed `dengueseq_fasta`?

```
str(dengueseq_fasta)

## chr ">NC_001477.1 Dengue virus 1, complete genome\
  nAGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTCGAATCGGAAGCTTGCTTAACGTAGTTCTA"|
  ____truncated____
```

We know it contains character data - how many characters? `nchar()` for “number of characters” answers that:

```
nchar(dengueseq_fasta)

## [1] 10935
```

18.2 OPTIONAL: Saving FASTA files

We can save our data as .fasta file for safe keeping. The `write()` function will save the data we downloaded as a plain text file.

```
write(dengueseq_fasta ,
      file="dengueseq.fasta")
```

If you do this, you’ll need to figure out where *R* is saving things, which requires an understanding *R*’s **file system**, which can take some getting used to, especially if you’re new to programming. As a start, you can see where *R* saves things by using the `getwd()` command, which tells you where on your harddrive *R* currently is using as its home base for files.

```
getwd()

## [1] "/Users/nlb24/OneDrive - University of Pittsburgh/0-books/lbrb-bk/lbrb"
```

18.3 Next steps

On their own, FASTA files in R are not directly useful. In the next lesson we’ll process our `dengueseq_fasta` file so that we can use it in analyses.

Chapter 19

Downloading DNA sequences as FASTA files in R

This is a modification of “DNA Sequence Statistics” from Avril Coghlan’s *A little book of R for bioinformatics*.. Most of the text and code was originally written by Dr. Coghlan and distributed under the Creative Commons 3.0 license.

19.1 Preliminaries

We’ll need the `dengueseq_fasta` FASTA data object, which is in the `compbio4all` package. We’ll also use the `stringr` package for cleaning up the FASTA data, which can be downloaded with `install.packages("stringr")`

```
# compbio4all, which has dengueseq_fasta
library(compbio4all)
data(dengueseq_fasta)
```

```
# stringr, for data cleaning
library(stringr)
```

19.2 Convert FASTA sequence to an R variable

We can’t actually do much with the contents of the `dengueseq_fasta` we downloaded with the `rentrez` package except read them. If we want to address some biological questions with the data we need is to convert it into a data structure *R* can work with.

There are several things we need to remove:

1. The **meta data** line `>NC_001477.1 Dengue virus 1, complete genome` (metadata is “data” about data, such as where it came from, what it is, who made it, etc.).
2. All the `\n` that show up in the file (these are the **line breaks**).
3. Put each nucleotide of the sequence into its own spot in a vector.

There are functions that can do this automatically, but

1. I haven’t found one I like, and
2. walking through this will help you understand the types of operations you can do on text data.

The first two steps involve removing things from the existing **character string** that contains the sequence. The third step will split the single continuous character string like “AGTTGTTAGTCTACGT...” into a

character vector like `c("A","G","T","T","G","T","T","A","G","T","C","T","A","C","G","T"...)` , where each element of the vector is a single character stored in a separate slot in the vector.

19.2.1 Removing unwanted characters

The second item is the easiest to take care of. *R* and many programming languages have tools called **regular expressions** that allow you to manipulate text. *R* has a function called `gsub()` which allows you to substitute or delete character data from a string. First I'll remove all those `\n` values.

The regular expression function `gsub()` takes three arguments: 1. `pattern = ...`. This is what we need it to find so we can replace it. 1. `replacement = ...`. The replacement. 1. `x = ...`. A character string or vector where `gsub()` will do its work.

We need to get rid of the `\n` so that we are left with only A, T and G, which are the actual information of the sequence. We want `\n` completely removed, so the replacement will be `" "`, which is a set of quotation marks with nothing in the middle, which means "delete the target pattern and put nothing in its place."

One thing that is tricky about regular expressions is that many characters have special meaning to the functions, such as slashes, dollar signs, and brackets. So, if you want to find and replace one of these specially designated characters you need to put a slash in front of them. So when we set the pattern, instead of setting the pattern to a slash before an `n` `\n`, we have to give it two slashes `\\n`.

Here is the regular expression to delete the newline character `\n`.

```
# note: we want to find all the \n, but need to set the pattern as \\n
dengueseq_vector <- gsub(pattern = "\\n",
                        replacement = " ",
                        x = dengueseq_fasta)
```

We can use `strtrim()` to see if it worked

```
strtrim(dengueseq_vector, 80)
```

```
## [1] ">NC_001477.1 Dengue virus 1, complete
genomeAGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTC"
```

Now for the metadata header. This is a bit complex, but the following code is going to take all the that occurs before the beginning of the sequence ("`AGTTGTTAGTC`") and delete it.

First, I'll define what I want to get rid of in an *R* object. This will make the **call** to `gsub()` a little cleaner to read

```
seq.header <- ">NC_001477.1_Dengue_virus_1,_complete_genome"
```

Now I'll get rid of the header with `gsub()`.

```
dengueseq_vector <- gsub(pattern = seq.header, # object defined above
                        replacement = " ",
                        x = dengueseq_vector)
```

See if it worked:

```
strtrim(dengueseq_vector, 80)
```

```
## [1] "
AGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTCGAATCGGAAGCTTGCTTAACGTAGTTCTAACAGTTTTTATTAG
"
```

19.2.2 Splitting unbroken strings in character vectors

Now the more complex part. We need to split up a continuous, unbroken string of letters into a vector where each letter is on its own. This can be done with the `str_split()` function (“string split”) from the `stringr` package. The notation `stringr::str_split()` mean “use the `str_split` function from the `stringr` package.” More specifically, it temporarily loads the `stringr` package and gives R access to just the `str_split` function. These allows you to call a single function without loading the whole library.

There are several arguments to `str_split`, and I’ve tacked a `[[1]]` on to the end.

First, run the command

```
dengueseq_vector_split <- stringr::str_split(dengueseq_vector,
                                             pattern = "",
                                             simplify = FALSE)[[1]]
```

Look at the output with `str()`

```
str(dengueseq_vector_split)
```

```
## chr [1:10735] "A" "G" "T" "T" "G" "T" "T" "A" "G" "T" "C" "T" "A" "C" "G"
...

```

We can explore what the different arguments do by modifying them. Change `pattern = ""` to `pattern = "A"`. Can you figure out what happened?

```
# re-run the command with "pattern = "A"
dengueseq_vector_split2 <- stringr::str_split(dengueseq_vector,
                                              pattern = "A",
                                              simplify = FALSE)[[1]]

str(dengueseq_vector_split2)
```

```
## chr [1:3427] "" "GTTGTT" "GTCT" "CGTGG" "CCG" "C" "" "G" "" "C" "GTTTCG"
...

```

And try it with `pattern = ""` to `pattern = "G"`.

```
# re-run the command with "pattern = "G"
dengueseq_vector_split3 <- stringr::str_split(dengueseq_vector,
                                              pattern = "G",
                                              simplify = FALSE)[[1]]

str(dengueseq_vector_split3)
```

```
## chr [1:2771] "A" "TT" "TTA" "TCTAC" "T" "" "ACC" "ACAA" "AACA" "TTTC" ...

```

Run this code to compare the two ways we just used `str_split` (don’t worry what it does). Does this help you see what’s up?

```
options(str = strOptions(vec.len = 10))
str(list(dengueseq_vector_split[1:20],
        dengueseq_vector_split2[1:10],
        dengueseq_vector_split3[1:10]))

## List of 3
## $ : chr [1:20] "A" "G" "T" "T" "G" "T" "T" "A" "G" "T" ...
## $ : chr [1:10] "" "GTTGTT" "GTCT" "CGTGG" "CCG" "C" "" "G" "" "C"
## $ : chr [1:10] "A" "TT" "TTA" "TCTAC" "T" "" "ACC" "ACAA" "AACA" "TTTC"
```

So, what does the `pattern = ...` argument do? For more info open up the help file for `str_split` by calling `?str_split`.

Something cool which we will explore in the next exercise is that we can do summaries on vectors of nucleotides, like this:

```
table(dengueseq_vector_split)
```

```
## dengueseq_vector_split  
##      A      C      G      T  
## 3426 2240 2770 2299
```


Chapter 20

DNA descriptive statics - Part 1

By: Avril Coghlan

Adapted, edited and expanded: Nathan Brouwer (brouwern@gmail.com) under the Creative Commons 3.0 Attribution License (CC BY 3.0).

20.1 Preface

This is a modification of “DNA Sequence Statistics (1)” from Avril Coghlan’s *A little book of R for bioinformatics..* The text and code were originally written by Dr. Coghlan and distributed under the Creative Commons 3.0 license.

20.2 Writing TODO:

- Add biology introduction
- Work on flow
- organize intial sections (intro, vocab, preliminaries)

20.3 Introduction

20.4 Vocabulary

- GC content
- DNA words
- scatterplots, histograms, piecharts, and boxplots

20.5 Functions

- `seqinr :: GC()`
- `seqinr :: count()`

20.6 Preliminaries

```
library(compbio4all)
library(seqinr)
```

20.7 Converting DNA from FASTA format

In a previous exercise we downloaded and examined DNA sequence in the FASTA format. The sequence we worked with is also stored as a data file within the `compbio4all` package and can be brought into memory using the `data()` command.

```
data("dengueseq_fasta")
```

We can look at this data object with the `str()` command

```
str(dengueseq_fasta)

## chr ">NC_001477.1 Dengue virus 1, complete genome\
nAGTTGTTAGTCTACGTGGACCGACAAGAACAGTTTCGAATCGGAAGCTTGCTTAACGTAGTTCTA"|
__truncated__
```

This isn't in a format we can work with directly so we'll use the function `fasta_cleaner()` to set it up.

```
header <- ">NC_001477.1_Dengue_virus_1_complete_genome"
dengueseq_vector <- compbio4all::fasta_cleaner(dengueseq_fasta)
```

Now check it out.

```
str(dengueseq_vector)

## chr [1:10735] "A" "G" "T" "T" "G" "T" "T" "A" "G" "T" "C" "T" "A" "C" "G"
...
```

What we have here is each base of the sequence in a separate slot of our vector.

The first four bases are "AGTT"

We can see the first one like this

```
dengueseq_vector[1]
```

```
## [1] "A"
```

The second one like this

```
dengueseq_vector[2]
```

```
## [1] "G"
```

The first and second like this

```
dengueseq_vector[1:2]
```

```
## [1] "A" "G"
```

and all four like this

```
dengueseq_vector[1:4]
```

```
## [1] "A" "G" "T" "T"
```

20.8 Length of a DNA sequence

Once you have retrieved a DNA sequence, we can obtain some simple statistics to describe that sequence, such as the sequence's total length in nucleotides. In the above example, we retrieved the DEN-1 Dengue virus genome sequence, and stored it in the vector variable `dengueseq_vector`. To obtain the length of the genome sequence, we would use the `length()` function, typing:

```
length(dengueseq_vector)
```

```
## [1] 10735
```

The `length()` function will give you back the length of the sequence stored in variable `dengueseq_vector`, in nucleotides. The `length()` function actually gives the number of **elements** (slots) in the input vector that you passed to it, which in this case is the number of elements in the vector `dengueseq_vector`. Since each element of the vector `dengueseq_vector` contains one nucleotide of the DEN-1 Dengue virus sequence, the result for the DEN-1 Dengue virus genome tells us the length of its genome sequence (ie. 10735 nucleotides long).

20.8.1 Base composition of a DNA sequence

An obvious first analysis of any DNA sequence is to count the number of occurrences of the four different nucleotides ("A", "C", "G", and "T") in the sequence. This can be done using the `table()` function. For example, to find the number of As, Cs, Gs, and Ts in the DEN-1 Dengue virus sequence (which you have put into vector variable `dengueseq_vector`, using the commands above), you would type:

```
table(dengueseq_vector)
```

```
## dengueseq_vector
##      A      C      G      T
## 3426 2240 2770 2299
```

This means that the DEN-1 Dengue virus genome sequence has 3426 As occurring throughout the genome, 2240 Cs, and so forth.

20.8.2 GC Content of DNA

One of the most fundamental properties of a genome sequence is its **GC content**, the fraction of the sequence that consists of Gs and Cs, ie. the $\%(G+C)$.

The GC content can be calculated as the percentage of the bases in the genome that are Gs or Cs. That is, $GC\ content = (number\ of\ Gs + number\ of\ Cs) / (genome\ length)$. For example, if the genome is 100 bp, and 20 bases are Gs and 21 bases are Cs, then the GC content is $(20 + 21) / 100 = 41\%$.

You can easily calculate the GC content based on the number of As, Gs, Cs, and Ts in the genome sequence. For example, for the DEN-1 Dengue virus genome sequence, we know from using the `table()` function above that the genome contains 3426 As, 2240 Cs, 2770 Gs and 2299 Ts. Therefore, we can calculate the GC content using the command:

```
(2240+2770)*100/(3426+2240+2770+2299)
```

```
## [1] 46.66977
```

Alternatively, if you are feeling lazy, you can use the `GC()` function in the `SeqinR` package, which gives the fraction of bases in the sequence that are Gs or Cs.

```
seqinr::GC(dengueseq_vector)
```

```
## [1] 0.4666977
```

The result above means that the fraction of bases in the DEN-1 Dengue virus genome that are Gs or Cs is 0.4666977. To convert the fraction to a percentage, we have to multiply by 100, so the GC content as a percentage is 46.66977%.

20.8.3 DNA words

As well as the frequency of each of the individual nucleotides (“A”, “G”, “T”, “C”) in a DNA sequence, it is also interesting to know the frequency of longer **DNA words**, also referred to as **genomic words**. The individual nucleotides are DNA words that are 1 nucleotide long, but we may also want to find out the frequency of DNA words that are 2 nucleotides long (ie. “AA”, “AG”, “AC”, “AT”, “CA”, “CG”, “CC”, “CT”, “GA”, “GG”, “GC”, “GT”, “TA”, “TG”, “TC”, and “TT”), 3 nucleotides long (eg. “AAA”, “AAT”, “ACG”, etc.), 4 nucleotides long, etc.

To find the number of occurrences of DNA words of a particular length, we can use the `count()` function from the R `SeqinR` package.

The `count()` function only works with lower-case letters, so first we have to use the `tolower()` function to convert our upper case genome to lower case

```
dengueseq_vector <- tolower(dengueseq_vector)
```

Now we can look for words. For example, to find the number of occurrences of DNA words that are 1 nucleotide long in the sequence `dengueseq_vector`, we type:

```
seqinr::count(dengueseq_vector, 1)
```

```
##
##      a      c      g      t
## 3426 2240 2770 2299
```

As expected, this gives us the number of occurrences of the individual nucleotides. To find the number of occurrences of DNA words that are 2 nucleotides long, we type:

```
seqinr::count(dengueseq_vector, 2)
```

```
##
##      aa      ac      ag      at      ca      cc      cg      ct      ga      gc      gg      gt      ta      tc      tg
##      tt
## 1108   720   890   708   901   523   261   555   976   500   787   507   440   497   832
##      529
```

Note that by default the `count()` function includes all overlapping DNA words in a sequence. Therefore, for example, the sequence “ATG” is considered to contain two words that are two nucleotides long: “AT” and “TG”.

If you type `help('count')`, you will see that the result (output) of the function `count()` is a table object. This means that you can use double square brackets to extract the values of elements from the table. For example, to extract the value of the third element (the number of Gs in the DEN-1 Dengue virus sequence), you can type:

```
denguetable_2 <- seqinr::count(dengueseq_vector, 2)
denguetable_2[[3]]
```

```
## [1] 890
```

The command above extracts the third element of the table produced by `count(dengueseq_vector, 1)`, which we have stored in the table variable `denguetable`.

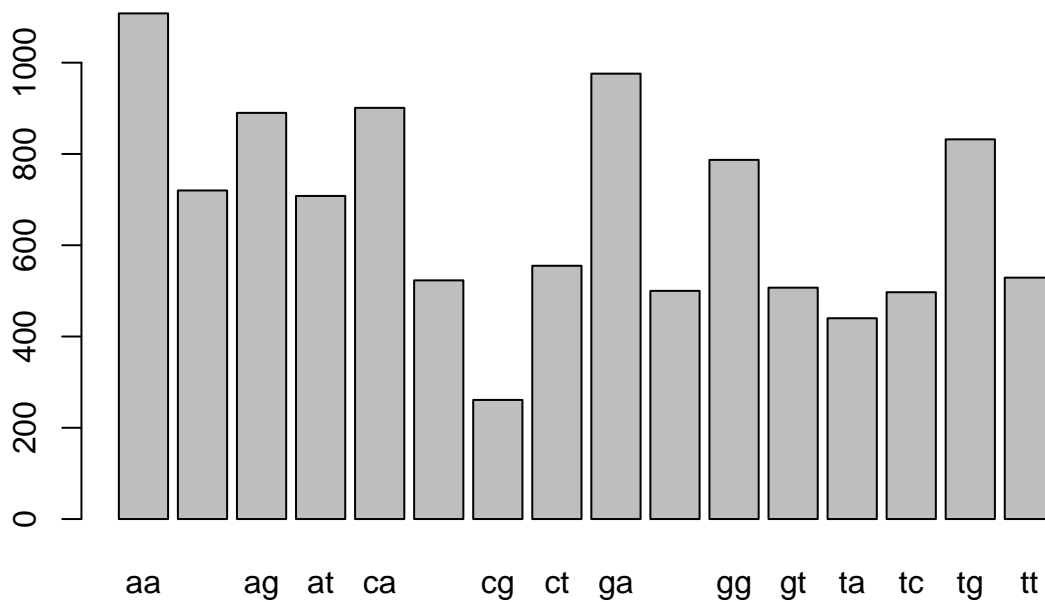
Alternatively, you can find the value of the element of the table in column “g” by typing:

```
denguetable_2[["aa"]]
```

```
## [1] 1108
```

Once you have table you can make a basic plot

```
barplot(denguetable_2)
```



We can sort by the number of words using the `sort()` command

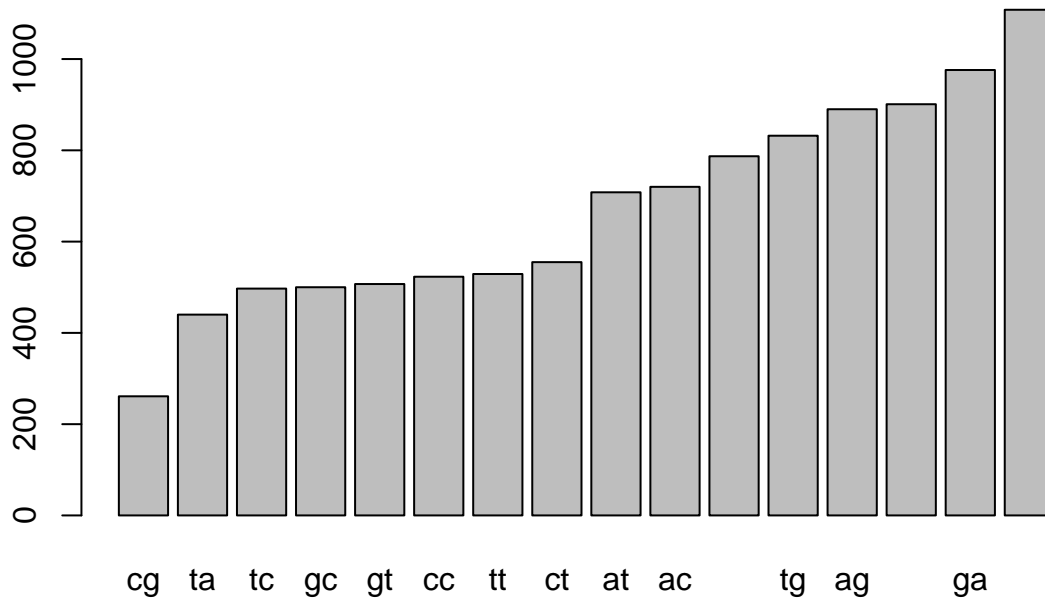
```
sort(denguetable_2)
```

```
##
##   cg   ta   tc   gc   gt   cc   tt   ct   at   ac   gg   tg   ag   ca   ga
## 261  440  497  500  507  523  529  555  708  720  787  832  890  901  976
## 1108
```

Let's save over the original object

```
denguetable_2 <- sort(denguetable_2)
```

```
barplot(denguetable_2)
```



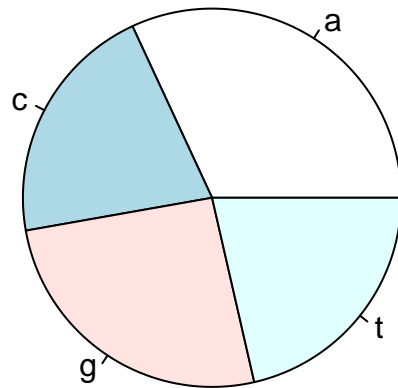
R will automatically try to optimize the appearance of the labels on the graph so you may not see all of them; no worries.

R can also make pie charts. Piecharts only really work when there are a few items being plots, like the four bases.

```
denguetable_1 <- seqinr::count(dengueseq_vector,1)
```

Make a piechart with pie()

```
pie(denguetable_1)
```



20.8.4 Summary

In this practical, have learned to use the following R functions:

`length()` for finding the length of a vector or list `table()` for printing out a table of the number of occurrences of each type of item in a vector or list. These functions belong to the standard installation of R.

You have also learnt the following R functions that belong to the SeqinR package:

`GC()` for calculating the GC content for a DNA sequence `count()` for calculating the number of occurrences of DNA words of a particular length in a DNA sequence

20.9 Acknowledgements

This is a modification of “DNA Sequence Statistics (1)” from Avril Coghlan’s *A little book of R for bioinformatics..* Almost all of text and code was originally written by Dr. Coghlan and distributed under the Creative Commons 3.0 license.

In “A little book...” Coghlan noted: “Many of the ideas for the examples and exercises for this chapter were inspired by the Matlab case studies on *Haemophilus influenzae* (www.computational-genomics.net/case_studies/haemophilus_demo.html) and Bacteriophage lambda (http://www.computational-genomics.net/case_studies/lambdaphage_demo.html) from the website that accompanies the book *Introduction to Computational Genomics: a case studies approach* by Cristianini and Hahn (Cambridge University Press; www.computational-genomics.net/book/).”

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20.9.2 Exercises

Answer the following questions, using the R package. For each question, please record your answer, and what you typed into R to get this answer.

Model answers to the exercises are given in Answers to the exercises on DNA Sequence Statistics (1).

1. What are the last twenty nucleotides of the Dengue virus genome sequence?
2. What is the length in nucleotides of the genome sequence for the bacterium *Mycobacterium leprae* strain TN (accession NC_002677)? Note: *Mycobacterium leprae* is a bacterium that is responsible for causing leprosy, which is classified by the WHO as a neglected tropical disease. As the genome sequence is a DNA sequence, if you are retrieving its sequence via the NCBI website, you will need to look for it in the NCBI Nucleotide database.
3. How many of each of the four nucleotides A, C, T and G, and any other symbols, are there in the *Mycobacterium leprae* TN genome sequence? Note: other symbols apart from the four nucleotides A/C/T/G may appear in a sequence. They correspond to positions in the sequence that are not clearly one base or another and they are due, for example, to sequencing uncertainties. For example, the symbol ‘N’ means ‘any base’, while ‘R’ means ‘A or G’ (purine). There is a table of symbols at www.bioinformatics.org/sms/iupac.html.
4. What is the GC content of the *Mycobacterium leprae* TN genome sequence, when (i) all non-A/C/T/G nucleotides are included, (ii) non-A/C/T/G nucleotides are discarded? Hint: look at the help page for the `GC()` function to find out how it deals with non-A/C/T/G nucleotides.
5. How many of each of the four nucleotides A, C, T and G are there in the complement of the *Mycobacterium leprae* TN genome sequence? Hint: you will first need to search for a function to calculate the complement of a sequence. Once you have found out what function to use, remember to use the `help()` function to find out what are the arguments (inputs) and results (outputs) of that function. How does the function deal with symbols other than the four nucleotides A, C, T and G? Are the numbers of As, Cs, Ts, and Gs in the complementary sequence what you would expect?
6. How many occurrences of the DNA words CC, CG and GC occur in the *Mycobacterium leprae* TN genome sequence?
7. How many occurrences of the DNA words CC, CG and GC occur in the (i) first 1000 and (ii) last 1000 nucleotides of the *Mycobacterium leprae* TN genome sequence? 1. How can you check that the subsequence that you have looked at is 1000 nucleotides long?

Chapter 21

Downloading protein sequences in *R*

By: Avril Coghlan.

Adapted, edited and expanded: Nathan Brouwer under the Creative Commons 3.0 Attribution License (CC BY 3.0).

21.1 Preliminaries

```
library(compbio4all)
```

21.2 Retrieving a UniProt protein sequence using rentrez

We can use `entrez_fetch()` to download protein sequences.

For example to retrieve the protein sequences for UniProt accessions Q9CD83 and A0PQ23, we type in R:

```
# sequence 1: Q9CD83
leprae_fasta <- rentrez::entrez_fetch(db = "protein",
                                     id = "Q9CD83",
                                     rettype = "fasta")

# sequence 2: OIN17619.1
ulcerans_fasta <- rentrez::entrez_fetch(db = "protein",
                                       id = "OIN17619.1",
                                       rettype = "fasta")
```

Display the contents of the lepraeseq FASTA file.

```
leprae_fasta
```

```
## [1] ">sp|Q9CD83.1|PHBS_MYCLE RecName: Full=Chorismate pyruvate-lyase;
AltName: Full=4-HB synthase; AltName: Full=p-hydroxybenzoic acid synthase\
nMTNRTLSREEIRKLDRLRLVATNGTLTRVLNVVANEEIVVDIINQQLDVAPKPELENLKIGRILQR\
nDILLKGQKSGILFVAAESLIVIDLLPTAITTYLTKTHHPIGEIMAAASRIETYKEDAQVWIGDLPCWLADY\
nGYWDLPKRAVGRRYRIAGGQPVIITTEYFLRSVFQDTPREELDRCQYSNDIDTRSGDRFVLHGRVFKNL\n\
n"
```

Let's clean these up to remove the header and new line characters using the function `fasta_cleaner()`.

```
leprae_vector <- fasta_cleaner(leprae_fasta)
ulcerans_vector <- fasta_cleaner(ulcerans_fasta)
```

Examine the output using `length()`, `class()`, and `head()`:

```
length(leprae_vector)
class(leprae_vector)
head(leprae_vector, 20)
```

Chapter 22

Changes to database entries

Sometimes databases entries not linked or database entries get removed. When revising this chapter I had to update three of the accession numbers because the UniProt accessions in the original version weren't working for me.

For example the original UniProt entry included in the chapter E3M2K8 does not currently link directly to the NCBI entry for this same protein. Instead, I had to use the NCBI accession XP_003109757.1. I'm not sure why this is.

Two accession numbers in the original version of this chapter appear to have been removed from UniProt because they were preliminary and perhaps did not meet the quality standards for UniProt. While the NCBI Gene and Protein are meant to be a record of *all* sequence, NCBI RefSeq and UniProt are carefully curated to contain information that is consistent, accurate, and represents biological reality.

Searching the UniProt data base for two accessions in the original version of this chapter, E1FUV2 (*Loa loa*) and A8NSK3(*Brugia malayi*), both yielded the result: >“This entry is obsolete... this entry was deleted. The protein sequence for this entry is available in UniParc. For previous versions of this entry, please look at its history.”

Looking at the history for the *Loa loa* version of the protein we can see it was listed as “unreviewed” prior to it being pulled and has the note “The sequence shown here is derived from an ... whole genome shotgun (WGS) entry which i preliminary data.” (<https://www.uniprot.org/uniprot/E1FUV2.txt?version=9>). Once they got around to reviewing in, the curators at UniProt must have decided that it didn't meet the standards for inclusion in the database. The information about the sequence in other databases, however, was not deleted, just the entry in UniProt.

Most proteins are not experimentally studied in a lab, let alone their structure determined or their expression levels assessed. They are therefore predicted to be real based on their similarity to proteins that have been studied in model organisms.

To track down these sequences, I used BLAST. BLASTing the well-studied model organism *C. elegans* version of the protein against the *Loa loa* genome resulted in a hypothetical protein LOAG_18175 a 93% query coverage with *C. elegans* and 44% identity. The accession number for this predicted protein in NCBI is XP_020305433.1. I similarly tracked down the accession for the *Brugia malayi* version of the protein.

Chapter 23

A complete bioinformatics workflow in R

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Chapter 24

“Worked example: Building a phylogeny in R”

24.1 Introduction

Phylogenies play an important role in computational biology and bioinformatics. Phylogenetics itself is an obligately computational field that only began rapid growth when computational power allowed the many algorithms it relies on to be done rapidly. Phylogenies of species, genes and proteins are used to address many biological issues, including

- Patterns of protein evolution
- Origin and evolution of phenotypic traits
- Origin and progression of epidemics
- Origin of evolution of diseases (eg, zoonoses)
- Prediction of protein function from its sequence
- ... and many more

The actual building of a phylogeny is a computationally intensive task; moreover, there are many bioinformatics and computational tasks the precede the construction of a phylogeny:

- genome sequencing and assembly
- computational gene prediction and annotation
- database searching and results screening
- pairwise sequence alignment
- data organization and cleaning
- multiple sequence alignment
- evaluation and validation of alignment accuracy

Once all of these steps have been carried out, the building of a phylogeny involves

- picking a model of sequence evolution or other description of evolution
- picking a statistical approach to tree construction
- evaluating uncertainty in the final tree

In this chapter we will work through many of these steps. In most cases we will pick the easiest or fastest option; in later chapters we will unpack the various options. This chapter is written as an interactive R sessions. You can follow along by opening the .Rmd file of the chapter or typing the appropriate commands into your own script. I assume that all the necessary packages have been installed and they only need to be loaded into R using the *library()* command.

This lesson walks you through an entire workflow for a bioinformatics, including

1. obtaining FASTA sequences
2. cleaning sequences
3. creating alignments
4. creating distance a distance matrix
5. building a phylogenetic tree

We’ll examine the Shroom family of genes, which produces Shroom proteins essential for tissue formation in many multicellular eukaryotes, including neural tube formation in vertebrates. We’ll examine shroom in several very different organism, including humans, mice and sea urchins. There is more than one type of shroom in vertebrates, and we’ll also look at two different Shroom genes: shroom 1 and shroom 2.

This lesson draws on skills from previous sections of the book, but is written to act as a independent summary of these activities. There is therefore review of key aspects of R and bioinformatics throughout it.

24.2 Software Preliminaires

24.2.1 Vocab

- argument
- function
- list
- named list
- vector
- named vector
- for() loop
- R console

24.2.2 R functions

- library()
- round()
- plot()
- mtext()
- nchar()
- rentrez::entrez_fetch()
- compbio4all::entrez_fetch_list()
- compbio4all::print_msa() (Coghlan 2011)
- Biostrings::AAStringSet()
- msa::msa()
- msa::msaConvert()
- msa::msaPrettyPrint()
- seqinr::dist.alignment()
- ape::nj()

A few things need to be done to get started with our R session.

24.2.3 Download necessary packages

Many *R* sessions begin by downloading necessary software packages to augment *R*’s functionality.

If you don’t have them already, you’ll need the following packages from CRAN:

1. ape
2. seqinr
3. rentrez
4. devtools

The CRAN packages can be loaded with `install.packages()`.

You'll also need these packages from Bioconductor:

1. `msa`
2. `Biostrings`

For installing packages from Bioconductor, see the chapter at the beginning of this book on this process.

Finally, you'll need this package from GitHub

1. `compbio4all`
2. `ggmsa`

To install packages from GitHub you can use the code `devtools::install_github("brouwern/combio4all")` and `devtools::install_github("brouwern/ggmsa")`

24.2.4 Load packages into memory

We now need to load up all our bioinformatics and phylogenetics software into R. This is done with the `library()` command.

To run this code just click on the sideways green triangle all the way to the right of the code.

NOTE: You'll likely see some red code appear on your screen. No worries, totally normal!

```
# github packages
library(compbio4all)

# CRAN packages
library(rentrez)
library(seqinr)
library(ape)

# Bioconductor packages
library(msa)
library(Biostrings)
```

24.3 Downloading macro-molecular sequences

We're going to explore some sequences. First we need to download them. To do this we'll use a function, `entrez_fetch()`, which accesses the **Entrez** system of database (ncbi.nlm.nih.gov/search/). This function is from the `rentrez` package, which stands for "R-Entrez."

We need to tell `entrez_fetch()` several things

1. `db = ...` the type of entrez database.
2. `id = ...` the **accession** (ID) number of the sequence
3. `rettype = ...` file type what we want the function to return.

Formally, these things are called **arguments** by R.

We'll use these settings:

1. `db = "protein"` to access the Entrez database of protein sequences
2. `rettype = "fasta"`, which is a standard file format for nucleic acid and protein sequences

We'll set `id = ...` to sequences whose **accession numbers** are:

1. NP_065910: Human shroom 3
2. AAF13269: Mouse shroom 3a
3. CAA58534: Human shroom 2
4. XP_783573: Sea urchin shroom

There are two highly conserved regions of shroom 3 1. ASD 1: aa 884 to aa 1062 in hShroom3 1. ASD 2: aa 1671 to aa 1955 in hShroom3

Normally we'd have to download these sequences by hand through pointing and clicking on GeneBank records on the NCBI website. In *R* we can do it automatically; this might take a second.

All the code needed is this:

```
# Human shroom 3 (H. sapiens)
hShroom3 <- entrez_fetch(db = "protein",
                        id = "NP_065910",
                        rettype = "fasta")
```

The output is in FASTA format; we'll use the `cat()` to do a little formatting for us:

```
cat(hShroom3)

## >NP_065910.3 protein Shroom3 [Homo sapiens]
## MMRTTDFHFKPSATLNSNTATKGRIYILEAFLEGGAPWGFTLKGGLEHGEPLIISKVEEGGKADTLSSKL
## QAGDEVVHINEVTLSRRKEAVSLVKGSYKTLRLVVRDVCCTDPGHADTGASNFVSPEHLTSGPQHRKAA
## WSGGVKLRLKHRSEPAGRPHSWHTTKSGEKQPDASMMQISQGMIGPPWHQSYHSSSTSDLSNYDHAYL
## RRSPPDQCSSQGSMELEPSGAYPPCHLSPAKSTGSIDQLSHFHNKRDSAYSSFSTSSSILEYHPHGISGR
## ERSGMDNTSARGGLLEGMRQADIRYVKTVYDTRRGVSAEYEVNSSLALLQGREARASANGQGYDKWSNI
## PRGKGVPSPSWSQQCPSLETATDNLPPKVGAPLPPARSDSYAAFRHRERPSSWSSLDQKRLCRPQANSL
## GSKSPFIEEQHLTVLEKSPENSPPVKPKHNYTQKAQPGQPLPSTIYPVPSLEPHFAQVPQPSVSSNGM
## LYPALAKESGYIAPQACNKMATIDENGNGSGRPGFAFCQPLEHDLLSPVEKKPEATAKYVPSKVHFC
## SVPNEEDASLKRHLTPQGNSPHSNERKSTHSNKPSSHPHSLKCPQAQAWQAGEDKRSSRLSEPWEQDF
## QEDHNANLWRRLEREGLGQSLSCNFGKTKSAFSSLQNPESLRRHSSLELGRGTQEGYPGGRPTCAVNTK
## AEDPGRKAAPDLGSHLDQVSYPRPEGRGTGASASFNSTDPSPPEPPAPSHPTSSLGRRGPGPGSASALQ
## GFQYQKPHCSVLEKVSKEFEQREQSGRPSVGGSGFGHNYRPHRTVSTSTSGNDFEETKAHIRFSESAEP
## LGNGEQHFKNKELKLEASRQPCGQQLSGGASDSGRGPQRPDARLLRSQSTFQLSSEPEREPEWRDRPGS
## PESPLLDAPFSRAYRNSIKDAQSRVLGATSFRRRDLELGAPVASRSWRPRPSSAHVGLRSPASASASPH
## TPRERHSVTPAEGDLARPVPPAARRGARRRLTPEQKKRSYSEPEKMNEVGIVEEAEPAPLGPQRNGMRFP
## ESSVADRRRLFERDGGKACSTLSLSCPELKQFQQSALADYIQRKTGKRPTSAAGCSLQEPGLRERAQSA
## LQPGPAALEGSGLASASSLREPSLQPRREATLLPATVAETQQAPRDRSSSFAGGRRLGERRRGDLS
## GANGGTRGTQRGDETPREPSSWGARGKMSAEDLLERSDVLGAPVHVRSSRPATADKRQDVLLGQDSG
## FGLVKDPCYLAGPGSRSLSCSERGQEEMLPFLFHLTPRWGGSGCKAIGDSSVPSECPGTLDHQRQASRTP
## CPRPPLAGTQGLVTDTRAAPLTPIGTPLPSAIPSGYCSQDQGTGRQPLPPYTPAMMHRNNGHTLTQPPGP
## RGCEGDGPEHGVVEEGTRKRVSLPQWPPPSRAKWAHAAREDSLPEESSAPDFANLKHYQKQQLSLCSTS
## DDPDTPLGAPSTPGRISLRISVLRDSPPHEDYEDEVFVRDHPKATSSPTFEPLPPPPPPPPPSQETPV
## YSMDDFPPPPPHTVCEAQLDSEDPGPRPSFNKLSKVTIARERHMPGAHVVGSQLASRLQTSIKGSEA
## ESTPPSFMVHAQLAGSLGGQPAPIQTQSLSHDPVSGTQGLEKKVSPDPQKSSDIRTEALAKEIVHQDK
## SLADILDPDSRLKTTMDLMEGLFPRDVNLLKENSVKRKAIQRTVSSSGCEGKRNEDEKAVSMLVNCPAYY
## SVSAPKAELNLIKEMPAEVNEEEQADVNEKKAELIGSLTHKLETLQEAKGSLTLDIKLNNALGEEVEA
## LISELCKPNEFDKYRMFIGDLKVVNLLLSLGRLARVENVLSGLGEDASNEERSSLYEKRKILAGQHED
## ARELKENLDRRERVVLGILANYLSEEQLQDYQHFKMKSTLLIEQRKLLDDKIKLGQEQVKCLLESLSDF
## IPKAGALALPPNLTSEPIPAGGCTFSGIFPTLTSP
```

Note the initial `>`, then the header line of `NP_065910.3 protein Shroom3 [Homo sapiens]`. After that is the amino acid sequence. The underlying data also includes the **newline character** `\n` to designate where each line of amino acids stops.

We can get the rest of the data by just changing the `id = ...` argument:

```
# Mouse shroom 3a (M. musculus)
mShroom3a <- entrez_fetch(db = "protein",
                           id = "AAF13269",
                           rettype = "fasta")

# Human shroom 2 (H. sapiens)
hShroom2 <- entrez_fetch(db = "protein",
                          id = "CAA58534",
                          rettype = "fasta")

# Sea-urchin shroom
sShroom <- entrez_fetch(db = "protein",
                        id = "XP_783573",
                        rettype = "fasta")
```

I'm going to check about how long each of these sequences is - each should have an at least slightly different length. If any are identical, I might have repeated an accession name or re-used an object name. The function `nchar()` counts of the number of characters in an *R* object.

```
nchar(hShroom3)
```

```
## [1] 2070
```

```
nchar(mShroom3a)
```

```
## [1] 2083
```

```
nchar(sShroom)
```

```
## [1] 1758
```

```
nchar(hShroom2)
```

```
## [1] 1673
```

24.4 Prepping macromolecular sequences

“90% of data analysis is data cleaning” (-Just about every data analyst and data scientist on twitter)

We have our sequences, but the current format isn't directly usable for us yet because there are several things that aren't sequence information

1. metadata (the header)
2. page formatting information (the newline character)

We can remove this non-sequence information using a function I wrote called `fasta_cleaner()`, which is in the `compbio4all` package. The function uses **regular expressions** to remove the info we don't need.

ASIDE: If we run the name of the command with out any quotation marks we can see the code:

```
fasta_cleaner
```

```
## function(fasta_object, parse = TRUE){
##
##   fasta_object <- sub("^(>)(.*?)(\\n)(.)(\\n\\n)", "\\4", fasta_object)
##   fasta_object <- gsub("\\n", "", fasta_object)
##
##   if(parse == TRUE){
##     fasta_object <- stringr::str_split(fasta_object,
##                                       pattern = "",
##                                       simplify = FALSE)
##   }
##
##   return(fasta_object[[1]])
## }
## <bytecode: 0x7ff16a0e69e0>
## <environment: namespace:compbio4all>
```

End ASIDE

Now use the function to clean our sequences; we won't worry about what `parse = ...` is for.

```
hShroom3 <- fasta_cleaner(hShroom3, parse = F)
mShroom3a <- fasta_cleaner(mShroom3a, parse = F)
hShroom2 <- fasta_cleaner(hShroom2, parse = F)
sShroom <- fasta_cleaner(sShroom, parse = F)
```

Now let's take a peek at what our sequences look like:

```
hShroom3
```

```
## [1] "
MMRTTDFHKPSATLNSNTATKGRYIYLEAFLEGGAPWGFILKGGLEHGEPLISKVEEGKADTLSSKLQAGDEVVHINEVTLSSS
"
```

24.5 Aligning sequences

We can do a **global alignment** of one sequence against another using the `pairwiseAlignment()` function from the **Bioconductor** package `Biostrings` (note that capital “B” in `Biostrings`; most R package names are all lower case, but not this one).

Let's align human versus mouse shroom:

```
align.h3.vs.m3a <- Biostrings::pairwiseAlignment(
  hShroom3,
  mShroom3a)
```

We can peek at the alignment

```
align.h3.vs.m3a
```

```
## Global PairwiseAlignmentsSingleSubject (1 of 1)
## pattern: MMRTTDFHKPSATLNSNTATKGRYIYLEAFLE...
KAGALALPPNLTSEPIPAGGCTFSGIFTLTSP
## subject: MKTPENLEEPSATPNPSRTPTERFVYLEALLE...
KAGAISLPPALTGHATPGGTSVFGGVFPTLTSP
## score: 2189.934
```

The **score** tells us how closely they are aligned; higher scores mean the sequences are more similar. Its hard to interpret the number on its own so we can get the **percent sequence identity (PID)** using the `pid()` function.

```
Biostrings::pid(align.h3.vs.m3a)
```

```
## [1] 70.56511
```

So, *shroom3* from humans and *shroom3* from mice are ~71% similar (at least using this particular method of alignment, and there are many ways to do this!)

What about human shroom 3 and sea-urchin shroom?

```
align.h3.vs.h2 <- Biostrings::pairwiseAlignment(
  hShroom3,
  hShroom2)
```

First check out the score using `score()`, which accesses it directly without all the other information.

```
score(align.h3.vs.h2)
```

```
## [1] -5673.853
```

Now the percent sequence alignment with `pid()`:

```
Biostrings::pid(align.h3.vs.h2)
```

```
## [1] 33.83277
```

So Human shroom 3 and Mouse shroom 3 are 71% identical, but Human shroom 3 and human shroom 2 are only 34% similar? How does it work out evolutionary that a human and mouse gene are more similar than a human and a human gene? What are the evolutionary relationships among these genes within the shroom gene family?

24.6 The shroom family of genes

I've copied a table from a published paper which has accession numbers for 15 different Shroom genes.

```
shroom_table <- c("CAA78718" , "X. laevis Apx" , "xShroom1" ,
  "NP_597713" , "H. sapiens APXL2" , "hShroom1" ,
  "CAA58534" , "H. sapiens APXL" , "hShroom2" ,
  "ABD19518" , "M. musculus Apxl" , "mShroom2" ,
  "AAF13269" , "M. musculus ShroomL" , "mShroom3a" ,
  "AAF13270" , "M. musculus ShroomS" , "mShroom3b" ,
  "NP_065910" , "H. sapiens Shroom" , "hShroom3" ,
  "ABD59319" , "X. laevis Shroom-like" , "xShroom3" ,
  "NP_065768" , "H. sapiens KIAA1202" , "hShroom4a" ,
  "AAK95579" , "H. sapiens SHAP-A" , "hShroom4b" ,
  "#DQ435686" , "M. musculus KIAA1202" , "mShroom4" ,
  "ABA81834" , "D. melanogaster Shroom" , "dmShroom" ,
  "EAA12598" , "A. gambiae Shroom" , "agShroom" ,
  "XP_392427" , "A. mellifera Shroom" , "amShroom" ,
  "XP_783573" , "S. purpuratus Shroom" , "spShroom") #sea urchin
```

I'll do a bit of formatting; you can ignore these details if you want

```

# convert to matrix
shroom_table_matrix <- matrix(shroom_table,
                              byrow = T,
                              nrow = 14)

# convert to dataframe
shroom_table <- data.frame(shroom_table_matrix,
                           stringsAsFactors = F)

# name columns
names(shroom_table) <- c("accession", "name.orig", "name.new")

# Create simplified species names
shroom_table$spp <- "Homo"
shroom_table$spp[grepl("laevis", shroom_table$name.orig)] <- "Xenopus"
shroom_table$spp[grepl("musculus", shroom_table$name.orig)] <- "Mus"
shroom_table$spp[grepl("melanogaster", shroom_table$name.orig)] <- "Drosophila"
shroom_table$spp[grepl("gambiae", shroom_table$name.orig)] <- "mosquito"
shroom_table$spp[grepl("mellifera", shroom_table$name.orig)] <- "bee"
shroom_table$spp[grepl("purpuratus", shroom_table$name.orig)] <- "sea_urchin"

```

Take a look:

```
shroom_table
```

##	accession		name.orig	name.new	spp
## 1	CAA78718		X. laevis Apx	xShroom1	Xenopus
## 2	NP_597713		H. sapiens APXL2	hShroom1	Homo
## 3	CAA58534		H. sapiens APXL	hShroom2	Homo
## 4	ABD19518		M. musculus Apxl	mShroom2	Mus
## 5	AAF13269	M. musculus	ShroomL	mShroom3a	Mus
## 6	AAF13270	M. musculus	ShroomS	mShroom3b	Mus
## 7	NP_065910		H. sapiens Shroom	hShroom3	Homo
## 8	ABD59319	X. laevis	Shroom-like	xShroom3	Xenopus
## 9	NP_065768		H. sapiens KIAA1202	hShroom4a	Homo
## 10	AAK95579		H. sapiens SHAP-A	hShroom4b	Homo
## 11	ABA81834	D. melanogaster	Shroom	dmShroom	Drosophila
## 12	EAA12598	A. gambiae	Shroom	agShroom	mosquito
## 13	XP_392427	A. mellifera	Shroom	amShroom	bee
## 14	XP_783573	S. purpuratus	Shroom	spShroom	sea_urchin

24.7 Downloading multiple sequences

Instead of getting one sequence at a time we can download several by accessing the “accession” column from the table

```
shroom_table$accession
```

```

## [1] "CAA78718" "NP_597713" "CAA58534" "ABD19518" "AAF13269" "AAF13270"
## [7] "NP_065910" "ABD59319" "NP_065768" "AAK95579" "ABA81834" "EAA12598"
## [13] "XP_392427" "XP_783573"

```

We can give this whole set of accessions to `entrez_fetch()`:

```

shrooms <- entrez_fetch(db = "protein",
                        id = shroom_table$accession,
                        rettype = "fasta")

```

We can look at what we got here with `cat()` (I won't display this because it is very long!)

```
cat(shrooms)
```

The current format of these data is a single, long set of data. This is a standard way to store, share and transmit FASTA files, but in *R* we'll need a slightly different format.

We'll download all of the sequences again, this time using a function from `compbio4all` called `entrez_fetch_list()` which is a **wrapper** function I wrote to put the output of `entrez_fetch()` into an *R* data format called a **list**.

```
shrooms_list <- entrez_fetch_list(db = "protein",
                                id = shroom_table$accession,
                                rettype = "fasta")
```

Now we have a list which has 14 **elements**, one for each sequence in our table.

```
length(shrooms_list)
```

```
## [1] 14
```

We now need to clean up each one of these sequences. We can do that using a simple `for()` loop:

```
for(i in 1:length(shrooms_list)){
  shrooms_list[[i]] <- fasta_cleaner(shrooms_list[[i]], parse = F)
}
```

Second to last step: we need to take each one of our sequences from our list and put it into a **vector**, in particular a **named vector**

```
# make a vector to store output
shrooms_vector <- rep(NA, length(shrooms_list))
```

```
# run the loop
for(i in 1:length(shrooms_vector)){
  shrooms_vector[i] <- shrooms_list[[i]]
}
```

```
# name the vector
names(shrooms_vector) <- names(shrooms_list)
```

Now the final step: we need to convert our named vector to a **string set** using `Biostrings::AAStringSet()`. Note the `_ss` tag at the end of the object we're assigning the output to, which designates this as a string set.

```
shrooms_vector_ss <- Biostrings::AAStringSet(shrooms_vector)
```

24.8 Multiple sequence alignment

We must **align** all of the sequences we downloaded and use that **alignment** to build a **phylogenetic tree**. This will tell us how the different genes, both within and between species, are likely to be related.

24.8.1 Building an Multiple Sequence Alignment (MSA)

We'll use the software `msa`, which implements the **ClustalW** multiple sequence alignment algorithm. Normally we'd have to download the ClustalW program and either point-and-click our way through it or use the **command line***, but these folks wrote up the algorithm in *R* so we can do this with a line of *R* code. This will take a second or two.

```
shrooms_align <- msa(shrooms_vector_ss,
                     method = "ClustalW")

## use default substitution matrix
```

24.8.2 Viewing an MSA

Once we build an MSA we need to visualize it.

24.8.2.1 Viewing an MSA in R

We can look at the output from `msa()`, but its not very helpful

```
shrooms_align

## CLUSTAL 2.1
##
## Call:
##   msa(shrooms_vector_ss, method = "ClustalW")
##
## MsaAAMultipleAlignment with 14 rows and 2252 columns
##      aln                                     names
## [1] _____ NP_065768
## [2] _____ AAK95579
## [3] _____SVFGGVFPTLT SPL_____ AAF13269
## [4] _____SVFGGVFPTLT SPL_____ AAF13270
## [5] _____CTFSGIFPTLT SPL_____ NP_065910
## [6] _____NKS—LPPPLTSSL_____ ABD59319
## [7] _____ CAA58534
## [8] _____ ABD19518
## [9] _____LT_____ NP_597713
## [10] _____ CAA78718
## [11] _____ EAA12598
## [12] _____ ABA81834
## [13] MTELQSPPGYRVQDEAPGPPSCPP... XP_392427
## [14] _____AATSSSSNGIGGPEQLNSNATSSYC XP_783573
## Con _____ Consensus
```

A function called `print_msa()` (Coghlan 2011) which I’ve put into `combio4all` can give us more informative output by printing out the actual alignment into the R console.

To use `print_msa()` We need to make a few minor tweaks though first. These are behind the scenes changes so don’t worry about the details right now. We’ll change the name to `shrooms_align_seqinr` to indicate that one of our changes is putting this into a format defined by the bioinformatics package `seqinr`.

```
class(shrooms_align) <- "AAMultipleAlignment"
shrooms_align_seqinr <- msaConvert(shrooms_align, type = "seqinr::alignment")
```

I won’t display the output from `shrooms_align_seqinr` because its very long; we have 14 shroom genes, and shroom happens to be a rather long gene.

```
print_msa(alignment = shrooms_align_seqinr,
          chunksize = 60)
```


24.8.2.2 Displaying an MSA as an R plot

I'm going to just show about 100 amino acids near the end of the alignment, where there is the most overlap across all of the sequences. This is set with the `start = ...` and `end = ...` arguments. Note that we're using the `shrooms_align` object.

```
ggmsa::ggmsa(shrooms_align, # shrooms_align, NOT shrooms_align_seqinr
  start = 2000,
  end = 2100)

## Registered S3 methods overwritten by 'ggalt':
##   method                      from
##   grid.draw.absoluteGrob      ggplot2
##   grobHeight.absoluteGrob     ggplot2
##   grobWidth.absoluteGrob      ggplot2
##   grobX.absoluteGrob          ggplot2
##   grobY.absoluteGrob          ggplot2
```

24.8.2.3 Saving an MSA as PDF

We can take a look at the alignment in PDF format if we want. In this case I'm going to just show about 100 amino acids near the end of the alignment, where there is the most overlap across all of the sequences. This is set with the `y = c(...)` argument.

```
msaPrettyPrint(shrooms_align, # alignment
  file = "shroom_msa.pdf", # file name
  y=c(2000, 2100), # range
  askForOverwrite=FALSE)
```

You can see where R is saving things by running `getwd()`

```
getwd()
```

```
## [1] "/Users/nlb24/OneDrive - University of Pittsburgh/0-books/lbrb-bk/lbrb"
```

On a Mac you can usually find the file by searching in Finder for the file name, which I set to be "shroom_msa.pdf" using the `file = ...` argument above.

24.9 Genetic distance.

Next need to first get an estimate of how similar each sequences is. The more amino acids that are identical to each other, the more similar.

Instead of similarity, we usually work in terms of *difference* or **genetic distance** (a.k.a. **evolutionary distance**). This is done with the `dist.alignment()` function.

```
shrooms_dist <- seqinr::dist.alignment(shrooms_align_seqinr,
  matrix = "identity")
```

We’ve made a matrix using `dist.alignment()`; let’s round it off so its easier to look at using the `round()` function.

```
shrooms_dist_rounded <- round(shrooms_dist ,
                               digits = 3)
```

Now let’s look at it

```
shrooms_dist_rounded
```

```
##          NP_065768 AAK95579 AAF13269 AAF13270 NP_065910 ABD59319 CAA58534
## AAK95579      0.000
## AAF13269      0.884      0.917
## AAF13270      0.897      0.917      0.000
## NP_065910      0.878      0.912      0.533      0.536
## ABD59319      0.893      0.921      0.783      0.783      0.782
## CAA58534      0.872      0.908      0.838      0.849      0.840      0.864
## ABD19518      0.866      0.912      0.834      0.846      0.838      0.855      0.548
## NP_597713      0.916      0.939      0.903      0.903      0.902      0.904      0.896
## CAA78718      0.925      0.955      0.896      0.895      0.893      0.893      0.898
## EAA12598      0.914      0.947      0.899      0.899      0.902      0.897      0.891
## ABA81834      0.938      0.943      0.935      0.934      0.936      0.940      0.935
## XP_392427      0.936      0.963      0.935      0.934      0.938      0.941      0.938
## XP_783573      0.940      0.958      0.942      0.939      0.942      0.935      0.942
##          ABD19518 NP_597713 CAA78718 EAA12598 ABA81834 XP_392427
## AAK95579
## AAF13269
## AAF13270
## NP_065910
## ABD59319
## CAA58534
## ABD19518
## NP_597713      0.900
## CAA78718      0.891      0.919
## EAA12598      0.896      0.920      0.922
## ABA81834      0.935      0.932      0.946      0.882
## XP_392427      0.934      0.927      0.947      0.878      0.923
## XP_783573      0.946      0.947      0.941      0.925      0.954      0.943
```

24.10 Phylogenetic trees (finally!)

We got our sequence, built multiple sequence alignment, and calculated the genetic distance between sequences. Now we are - finally - ready to build a phylogenetic tree.

First, we let R figure out the structure of the tree. There are **MANY** ways to build phylogenetic trees. We’ll use a common one used for exploring sequences called **neighbor joining** algorithm via the function `nj()`. Neighbor joining uses genetic distances to cluster sequences into **clades**.

```
tree <- nj(shrooms_dist)
```

24.10.1 Plotting phylogenetic trees

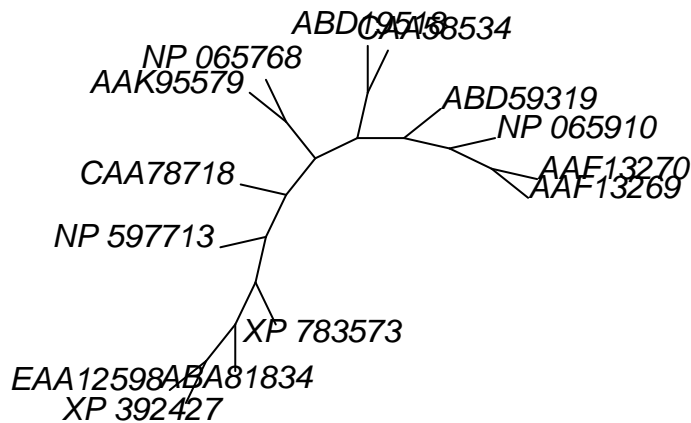
Now we’ll make a quick plot of our tree using `plot()` (and add a little label using a function called `mtext()`).

```
# plot tree
plot.phylo (tree, main="Phylogenetic Tree",
            type = "unrooted",
            use.edge.length = F)

# add label
mtext(text = "Shroom family gene tree - unrooted, no branch lengths")
```

Phylogenetic Tree

Shroom family gene tree – unrooted, no branch lengths



This is an ****unrooted tree***. For the sake of plotting we've also ignored the evolutionary distance between the sequences.

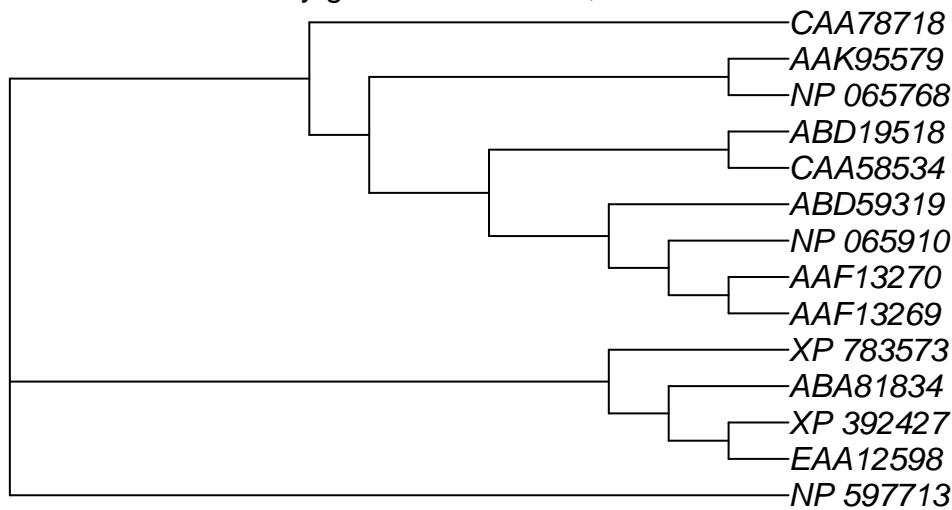
To make a rooted tree we remove `type = "unrooted"`.

```
# plot tree
plot.phylo (tree, main="Phylogenetic Tree",
            use.edge.length = F)

# add label
mtext(text = "Shroom family gene tree - rooted, no branch lengths")
```

Phylogenetic Tree

Shroom family gene tree – rooted, no branch lengths



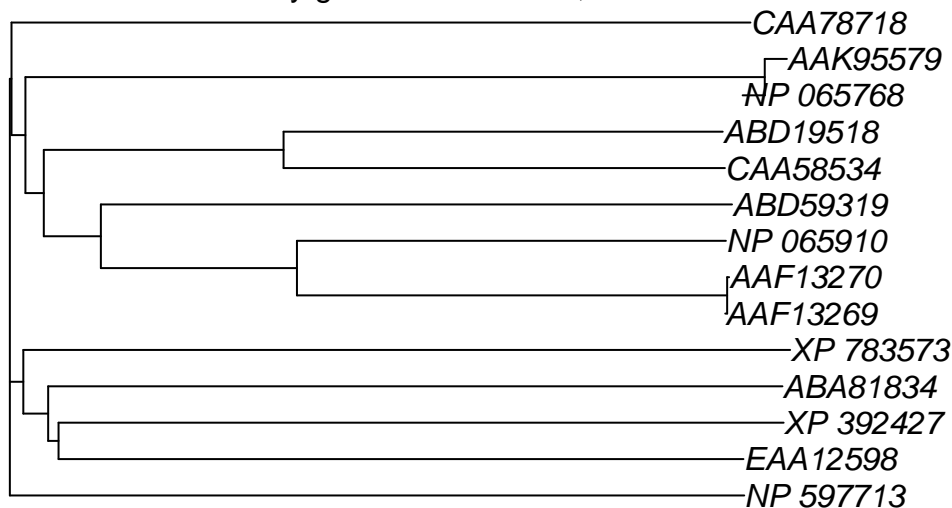
We can include information about branch length by setting `use.edge.length = ...` to `T`.

```
# plot tree
plot.phylo (tree , main="Phylogenetic Tree",
            use.edge.length = T)

# add label
mtext(text = "Shroom family gene tree – rooted, with branch lengths")
```

Phylogenetic Tree

Shroom family gene tree – rooted, with branch lengths



Some of the branches are now very short, but most are very long, indicating that these genes have been evolving independently for many millions of years.

Let's make a fancier plot. Don't worry about all the steps; I've added some more code to add some annotations on the right-hand side to help us see what's going on.

```
plot (tree , main="Phylogenetic Tree")
mtext(text = "Shroom family gene tree")
```

```

x <- 0.551
x2 <- 0.6

# label Shrm 3
segments(x0 = x, y0 = 1,
         x1 = x, y1 = 4,
         lwd=2)
text(x = x*1.01, y = 2.5, "Shrm_3", adj = 0)

segments(x0 = x, y0 = 5,
         x1 = x, y1 = 6,
         lwd=2)
text(x = x*1.01, y = 5.5, "Shrm_2", adj = 0)

segments(x0 = x, y0 = 7,
         x1 = x, y1 = 9,
         lwd=2)
text(x = x*1.01, y = 8, "Shrm_1", adj = 0)

segments(x0 = x, y0 = 10,
         x1 = x, y1 = 13,
         lwd=2)
text(x = x*1.01, y = 12, "Shrm_?", adj = 0)

segments(x0 = x, y0 = 14,
         x1 = x, y1 = 15,
         lwd=2)
text(x = x*1.01, y = 14.5, "Shrm_4", adj = 0)

segments(x0 = x2, y0 = 1,
         x1 = x2, y1 = 6,
         lwd=2)

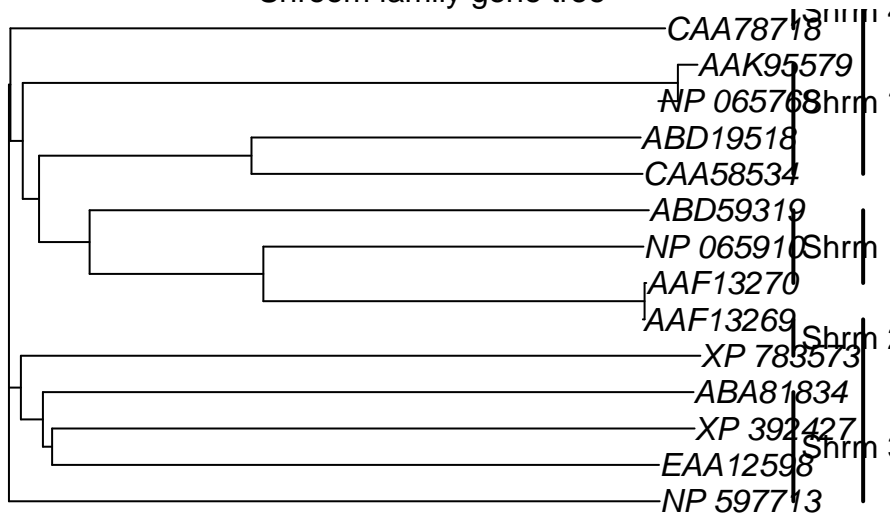
segments(x0 = x2, y0 = 7,
         x1 = x2, y1 = 9,
         lwd=2)

segments(x0 = x2, y0 = 10,
         x1 = x2, y1 = 15,
         lwd=2)

```

Phylogenetic Tree

Shroom family gene tree



Chapter 25

Logarithms in R

By Nathan Brouwer

Logging splits up multiplication into addition. So, $\log(m*n)$ is the same as $\log(m) + \log(n)$

You can check this

```
m<-10
n<-11
log(m*n)
```

```
## [1] 4.70048
```

```
log(m)+log(n)
```

```
## [1] 4.70048
```

```
log(m*n) == log(m)+log(n)
```

```
## [1] TRUE
```

Exponentiation undoes logs

```
exp(log(m*n))
```

```
## [1] 110
```

```
m*n
```

```
## [1] 110
```

The key equation in BLAST's E values is

$$u = \ln(Kmn)/\lambda$$

This can be changed to

$$[\ln(K) + \ln(mn)]/\lambda$$

We can check this

```
K <- 1
m <- 10
n <- 11
lambda <- 110

log(K*m*n)/lambda

## [1] 0.04273164

(log(K) + log(m*n))/lambda

## [1] 0.04273164

log(K*m*n)/lambda == (log(K) + log(m*n))/lambda

## [1] TRUE
```


Part I

Appendices

Appendix 01: Getting access to R

25.1 Getting Started With R and RStudio

- R is a piece of software that does calculations and makes graphs.
- RStudio is a GUI (graphical user interface) that acts as a front-end to R
- You can use R directly, but most people use a GUI of some kind
- RStudio has become the most popular GUI

The following instructions will lead you click by click through downloading R and RStudio and starting an initial session. If you have trouble with downloading either program go to YouTube and search for something like “Downloading R” or “Installing RStudio” and you should be able to find something helpful, such as “How to Download R for Windows”.

25.1.1 RStudio Cloud

TODO: Add RStudio cloud

25.1.2 Getting R onto your own computer

To get R on to your computer first go to the CRAN website at <https://cran.r-project.org/> (CRAN stands for “comprehensive R Archive Network”). At the top of the screen are three bullet points; select the appropriate one (or click the link below)

- Download R for Linux
- Download R for (Mac) OS X
- Download R for Windows

Each page is formatted slightly differently. For a current Mac, click on the top link, which as of 8/16/2018 was “R-3.5.1.pkg” or click this link. If you have an older Mac you might have to scroll down to find your operating system under “Binaries for legacy OS X systems.”

For PC select “base” or click this link.

When its downloaded, run the installer and accept the defaults.

25.1.3 Getting RStudio onto your computer

RStudio is an R interface developed by a company of the same name. RStudio has a number of commercial products, but much of their portfolio is freeware. You can download RStudio from their website www.rstudio.com/. The download page (www.rstudio.com/products/rstudio/download/) is a bit busy because it shows all of their commercial products; the free version is on the far left side of the table of products. Click on the big green DOWNLOAD button under the column on the left that says “RStudio Desktop Open Source License” (or click on this link).

This will scroll you down to a list of downloads titled “Installers for Supported Platforms.” Windows users can select the top option RStudio 1.1.456 - Windows Vista/7/8/10 and Mac the second option RStudio

1.1.456 - Mac OS X 10.6+ (64-bit). (Versions names are current of 8/16/2018).

Run the installer after it downloads and accept the default. RStudio will automatically link up with the most current version of R you have on your computer. Find the RStudio icon on your desktop or search for “RStudio” from your task bar and you’ll be read to go.

25.1.4 Keep R and RStudio current

Both R and RStudio undergo regular updates and you will occasionally have to re-download and install one or both of them. In practice I probably do this about every 6 months.

Getting started with R itself (or not)

Vocabulary

- console
- script editor / source viewer
- interactive programming
- scripts / script files
- .R files
- text files / plain text files
- command execution / execute a command from script editor
- comments / code comments
- commenting out / commenting out code
- stackoverflow.com
- the rstats hashtag

R commands

- `c(...)`
- `mean(...)`
- `sd(...)`
- `?`
- `read.csv(...)`

This is a walk-through of a very basic R session. It assumes you have successfully installed R and RStudio onto your computer, and nothing else.

Most people who use R do not actually use the program itself - they use a GUI (graphical user interface) “front end” that make R a bit easier to use. However, you will probably run into the icon for the underlying R program on your desktop or elsewhere on your computer. It usually looks like this:

ADD IMAGE HERE

The long string of numbers have to do with the version and whether is 32 or 64 bit (not important for what we do).

If you are curious you can open it up and take a look - it actually looks a lot like RStudio, where we will do all our work (or rather, RStudio looks like R). Sometimes when people are getting started with R they will accidentally open R instead of RStudio; if things don't seem to look or be working the way you think they should, you might be in R, not RStudio

25.1.4.1 R's console as a scientific calculator

You can interact with R's console similar to a scientific calculator. For example, you can use parentheses to set up mathematical statements like

```
5*(1+1)
```

```
## [1] 10
```

Note however that you have to be explicit about multiplication. If you try the following it won't work.

```
5(1+1)
```

R also has built-in functions that work similar to what you might have used in Excel. For example, in Excel you can calculate the average of a set of numbers by typing “=average(1,2,3)” into a cell. R can do the same thing except

- The command is “mean”
- You don't start with “=”
- You have to package up the numbers like what is shown below using “c(…)”

```
mean(c(1,2,3))
```

```
## [1] 2
```

Where “c(…)” packages up the numbers the way the mean() function wants to see them.

If you just do the following R will give you an answer, but its the wrong one

```
mean(1,2,3)
```

This is a common issue with R – and many programs, really – it won't always tell you when somethind didn't go as planned. This is because it doesn't know something didn't go as planned; you have to learn the rules R plays by.

25.1.4.2 Practice: math in the console

See if you can reproduce the following results

Division

```
10/3
```

```
## [1] 3.333333
```

The standard deviation

```
sd(c(5,10,15)) # note the use of "c(...)"
```

```
## [1] 5
```

25.1.4.3 The script editor

While you can interact with R directly within the console, the standard way to work in R is to write what are known as **scripts**. These are computer code instructions written to R in a **script file**. These are save with the extension **.R** but area really just a form of **plain text file**.

To work with scripts, what you do is type commands in the script editor, then tell R to **excute** the command. This can be done several ways.

First, you tell RStudio the line of code you want to run by either * Placing the cursor at the end a line of code, OR * Clicking and dragging over the code you want to run in order highlight it.

Second, you tell RStudio to run the code by * Clicking the “Run” icon in the upper right hand side of the script editor (a grey box with a green error emerging from it) * pressing the control key (“ctrl”) and then then enter key on the keyboard

The code you’ve chosen to run will be sent by RStudio from the script editor over to the console. The console will show you both the code and then the output.

You can run several lines of code if you want; the console will run a line, print the output, and then run the next line. First I’ll use the command `mean()`, and then the command `sd()` for the standard deviation:

```
mean(c(1,2,3))
```

```
## [1] 2
```

```
sd(c(1,2,3))
```

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

25.1.4.4 Comments

One of the reasons we use script files is that we can combine R code with **comments** that tell us what the R code is doing. Comments are preceded by the hashtag symbol `#`. Frequently we’ll write code like this:

```
#The mean of 3 numbers
mean(c(1,2,3))
```

If you highlight all of this code (including the comment) and then click on “run”, you’ll see that RStudio sends all of the code over console.

```
## [1] 2
```

Comments can also be placed at the *end* of a line of code

```
mean(c(1,2,3)) #Note the use of c(...)
```

Sometimes we write code and then don’t want R to run it. We can prevent R from executing the code even if its sent to the console by putting a “`#`” *infront* of the code.

If I run this code, I will get just the mean but not the sd.

```
mean(c(1,2,3))
#sd(c(1,2,3))
```

Doing this is called **commenting out** a line of code.

25.2 Help!

There are many resource for figuring out R and RStudio, including

- R’s built in “help” function
- Q&A websites like **stackoverflow.com**
- twitter, using the hashtag `#rstats`
- blogs
- online books and course materials

25.2.1 Getting “help” from R

If you are using a function in R you can get info about how it works like this

```
?mean
```

In RStudio the help screen should appear, probably above your console. If you start reading this help file, though, you don't have to go far until you start seeing lots of R lingo, like "S3 method", "na.rm", "vectors". Unfortunately, the R help files are usually not written for beginners, and reading help files is a skill you have to acquire.

For example, when we load data into R in subsequent lessons we will use a function called "read.csv"

Access the help file by typing "?read.csv" into the console and pressing enter. Surprisingly, the function that R give you the help file isn't what you asked for, but is read.table(). This is a related function to read.csv, but when you're a beginner thing like this can really throw you off.

Kieran Healy as produced a great cheatsheet for reading R's help pages as part of his forthcoming book. It should be available online at <http://socviz.co/appendix.html#a-little-more-about-r>

25.2.2 Getting help from the internet

The best way to get help for any topic is to just do an internet search like this: "R read.csv". Usually the first thing on the results list will be the R help file, but the second or third will be a blog post or something else where a usually helpful person has discussed how that function works.

Sometimes for very basic R commands like this might not always be productive but its always work a try. For but things related to stats, plotting, and programming there is frequently lots of information. Also try searching YouTube.

25.2.3 Getting help from online forums

Often when you do an internet search for an R topic you'll see results from the website www.stackoverflow.com, or maybe www.crossvalidated.com if its a statistics topic. These are excellent resources and many questions that you may have already have answers on them. Stackoverflow has an internal search function and also suggests potentially relevant posts.

Before posting to one of these sites yourself, however, do some research; there is a particular type and format of question that is most likely to get a useful response. Sadly, people new to the site often get "flamed" by impatient pros.

25.2.4 Getting help from twitter

Twitter is a surprisingly good place to get information or to find other people knew to R. Its often most useful to ask people for learning resources or general reference, but you can also post direct questions and see if anyone responds, though usually its more advanced users who engage in twitter-based code discussion.

A standard tweet might be "Hey #rstats twitter, am knew to #rstats and really stuck on some of the basics. Any suggestions for good resources for someone starting from scratch?"

25.3 Other features of RStudio

25.3.1 Ajusting pane the layout

You can adjust the location of each of RStudio 4 window panes, as well as their size.

To set the pane layout go to 1. "Tools" on the top menu 1. "Global options" 1. "Pane Layout"

Use the drop-down menus to set things up. I recommend 1. Lower left: "Console" 1. Top right: "Source" 1. Top left: "Plot, Packages, Help Viewer" 1. This will leave the "Environment..." panel in the lower right.

25.3.2 Adjusting size of windows

You can click on the edge of a pane and adjust its size. For most R work we want the console to be big. For beginners, the “Environment, history, files” panel can be made really small.

25.4 Practice (OPTIONAL)

Practice the following operations. Type the directly into the console and execute them. Also write them in a script in the script editor and run them.

Square roots

```
sqrt(42)
```

```
## [1] 6.480741
```

The date Some functions in R can be executed within nothing in the parentheses.

```
date()
```

```
## [1] "Fri Sep 17 22:24:20 2021"
```

Exponents The \wedge is used for exponents

```
42^2
```

```
## [1] 1764
```

A series of numbers A colon between two numbers creates a series of numbers.

```
1:42
```

```
## [1] 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
    24 25
```

```
## [26] 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
```

logs The default for the `log()` function is the natural log.

```
log(42)
```

```
## [1] 3.73767
```

`log10()` gives the base-10 log.

```
log10(42)
```

```
## [1] 1.623249
```

exp() raises e to a power

```
exp(3.73767)
```

```
## [1] 42.00002
```

Multiple commands can be nested

```
sqrt(42)^2
```

```
log(sqrt(42)^2)
```

```
exp(log(sqrt(42)^2))
```