

Bioinformatics and Programming

AU course: Bioinformatik og programmering - 2024

Table of contents

Welcome	1
Course description	1
Course contents	1
Lecture slides	3
Lecture recordings	5
Week 1	5
Week 2	5
Week 3	5
Week 4	6
Week 5	6
Week 6	6
Week 7	7
Week 8	7
Week 9	7
Week 10	7
Week 11	8
Week 12	8
Week 13	8
Assignments	9
I Learning Python	11
1 Preface	13
2 Before you begin	15
Install Python	15
2.1 Mac	15
2.2 Windows	15
The text editor	16
The terminal	16
Create a conda environment for the course	20
You are all set	22

3 Writing a program	23
Hello World	23
Error Messages	26
Strings	28
Comments	29
4 Dealing with values	31
Math	31
Logic	35
Variables	38
Different types of values	41
Mixed exercises	44
5 The order of events	49
Precedence of Operators	49
Statements and Expressions	51
Substitution and Reduction	52
General exercises	55
6 Course tools	65
Wax on, wax off	65
A helping hand	67
7 Controlling behavior	69
If-statement	69
Else-statement	71
Blocks of code	73
Elif-statement	73
General exercises	75
8 Organizing code	79
Functions	79
Functions can take arguments	84
Functions and variables	86
Builtin functions	88
General exercises	90
9 Values are objects	97
Methods	97
Using the Python documentation	99
String formatting	100
Indexing and slicing strings	103
General exercises	107

10 Lists of things	109
Lists	109
Indexing and slicing lists	111
General exercises	116
11 Pairs of things	117
Dictionaries	117
General exercises	119
12 Grouping values	123
Tuples	123
Tuple assignment	124
13 Iterating values	127
The for-loop	127
14 Working with files	133
Writing files	133
Reading files	135
General exercises	137
15 Structuring data	139
General exercises	146
16 Recursion	151
Recursion	151
Divide and conquer	152
17 Testing your code	155
Why test your code?	155
Basic testing	155
The project testing utility	156
18 Appendix: PyMol for BSF	159
Install Python	159
18.1 Mac	159
18.2 Windows	159
Install PyMol	160
18.3 Mac	160
18.4 Windows	160
Running PyMol	160

II Python projects	161
19 Translating ORFs	163
Project files	163
Translating a single codon	164
Splitting an open reading frame into codons	165
Translating an open reading frame	166
20 Primer analysis	167
Count the number of bases in your candidate primer	167
Compute the melting temperature	168
Reverse complement the sequence	169
Check for hairpins	169
21 Pairwise alignment	171
Filling in the dynamic programming matrix	172
Reconstructing the optimal alignment	176
22 Codon usage	181
Read an open reading frame and count its codons	183
Group codon counts by amino acid	184
Turn counts into frequencies	186
Compute the codon usage	188
23 HIV sub-groups	189
Compute the similarity of two sequences	190
Read the HIV sequences into your program	192
Compare your HIV sequence to HIV sequences of known subtype	193
Compute maximum similarity to each subtype	194
Identify the HIV subtype	195
24 Sequence trees	197
Measuring sequence distance	197
Lower triangular distance matrices	199
Generate a distance matrix	200
Clustering	201
Perform the clustering	202
25 Finding genes	207
Finding Open Reading Frames	208
Translation of open reading frames	213
Put everything together	213
26 Genome assembly	215
Read and analyze the sequencing reads	217

Compute overlaps between reads	219
Find the correct order of reads	223
Reconstruct the genomic sequence	225
III Web exercises	227
27 GWAS candidates	229
Browsing SNPs from a GWAS	230
Exploring a genomic region	231
Gathering information about genes	233
Exploring tissue expression	234
Hypotheses and Discussion	235
28 CCR5-delta32	237
Human Immunodeficiency Virus targets immune cells	237
Can specific gene variants provide resistance to HIV?	238
Aim	239
Preparation	239
Aligning protein sequences	241
Searching a database using Blast	242
GenBank sequence format	244
Putting it all together	245
Project files	246
29 MRSA	247
Sequence Retrieval	248
Database Searching using BLAST	249
Multiple Sequence Alignment using ClustalW	252
Discussion	253
Additional tools	254
30 Aardvark?	255
Build a FASTA file	257
One-click analysis	258
Explore phylogeny - part 3	262
Food for thought	263
Project files	263
31 Plasmid ORFs	265
Project files	267
32 Read mapping	269
32.1 HIFI reads	269
Project files	274

33 Neural networks	275
Project files	279
Python projects	281
Web exercises	281
Databases and resources	283
Knowledge data bases	284
Do it yourself:	284

Welcome

This is the homepage for the AU course Bioinformatics and programming (Bioinformatick og programmering). You will find all course content here. The Brightspace course page is only used for communication, and assignments.

Course description

After the course, the participants will have basic knowledge of computer methods and applications for analyzing biological sequence data and insight into principles and techniques for constructing simple programs. Participants will acquire practical experience with analyzing problems in bioinformatics and related fields and implementing programs to solve such problems using the Python programming language.

The participants must, at the end of the course, be able to:

- Apply fundamental constructs of a programming language.
- Analyse data and construct data structures for the representation of data.
- Analyse simple computational problems and construct programs for their solution.
- Describe and relate essential methods in bioinformatics analysis.
- Apply bioinformatics software to biological data.
- Judge the reliability of results obtained using Bioinformatics software.

Course contents

The course introduces programming and its practical applications in bioinformatics. The course also outlines and discusses bioinformatics algorithms, and the most common tools for bioinformatics analysis of sequence data are presented and demonstrated. The participant will acquire and train basic programming skills during the first seven weeks. The last seven weeks introduce key topics in bioinformatics, focusing on applying bioinformatical software and developing programming skills. Subjects for lectures and exercises include bioinformatics databases, sequence alignment, genome annotation, sequence evolution, and phylogenetic analysis.

Lecture slides

- Week 1
 - Monday
 - Wednesday
- Week 2
 - Monday
 - Wednesday
- Week 3
 - Monday
 - Wednesday
- Week 4
 - Monday
 - Wednesday
- Week 5
 - Monday
 - Wednesday
- Week 6
 - Monday
 - Wednesday
- Week 7
 - Monday
 - Wednesday
- Week 8
 - Monday
 - The Wednesday lecture was canceled.
- Week 9
 - Monday
 - Wednesday

- Week 10
 - Monday
 - Wednesday
- Week 11
 - Monday
 - Wednesday
- Week 12
 - Monday
 - Wednesday
- Week 13
 - Monday
 - Wednesday
- Week 14
 - Monday
 - Wednesday

Lecture recordings

Week 1

Monday

Unfortunately, the Monday recording failed :/

Wednesday

<https://youtu.be/6Kl6b3r2uJ4>

Week 2

Monday

The sound quality is terrible on this one, sorry.

<https://youtu.be/s3vKQY8rntw>

Wednesday

The sound quality is terrible on this one, sorry.

<https://youtu.be/IkQZI5XuNVU>

Week 3

Monday

https://youtu.be/injj7E0_ddI

Wednesday

https://youtu.be/CHcq4_-isTM

Week 4

Monday

<https://youtu.be/e86mF44Sgyo>

Wednesday

The sound is missing from most of this one. The mic battery ran out...

<https://youtu.be/DthrBNwxmiM>

Week 5

Monday

No recording.

Wednesday

<https://youtu.be/Oym9VWLi1ks>

Week 6

Monday

<https://youtu.be/k8EVqg2UXD8>

Wednesday

<https://youtu.be/GCeLRJCsDyU>

Week 7

Monday

<https://youtu.be/cTr7DPec4D8>

Wednesday

https://youtu.be/H7_UHCayxr4

Week 8

Monday

<https://youtu.be/9UR9uqbMSaU>

Wednesday

This lecture was canceled.

Week 9

Monday

<https://youtu.be/3HCgpZHATY>

Wednesday

<https://youtu.be/iQNMasqnQi8>

Week 10

Monday

<https://youtu.be/l4UShr240EU>

Wednesday

https://youtu.be/Cm-A_ybQ10g

Week 11

Monday

<https://youtu.be/5XwD7EWxtVY>

Wednesday

<https://youtu.be/q0usuYKaaI>

Week 12

Monday

<https://youtu.be/wEj7w4GNAkY>

Wednesday

<https://youtu.be/CHHPOKgdZ8k>

Week 13

Monday

<https://youtu.be/HpMw-CS2g88>

Assignments

Mandatory assignments are handed in through assignments in Brightspace
Information about assignments can be found under “Schedule” in the menu bar.

Part I

Learning Python

1 Preface

These lecture notes are inspired by the many books and resources I have used in this course. “Learning Python the Hard Way” and “How to Think Like a Computer Scientist” have inspired my own notes on programming. These implement the following ideas, which I think best support learning in an introduction to programming:

1. Each topic and concept is introduced so that it can be applied immediately on top of what you know.
2. The introduction of each topic covers only the most basic facts and rules required to learn the rest through practical exercises.

I would like to improve these notes as much as I can. If you find errors in exercises, poorly explained concepts, redundant information, missing items, or exercises that would work better in a different order, please let me know. You can do this by reporting an issue.

Happy coding, Kasper Munch

2 Before you begin

This chapter serves to get the practicalities out of the way so you can start programming. Read the whole chapter once carefully before you install anything

Install Python

In this course, we use the Python programming language and you need to install the Python program to run the code we will write. I have automated the installation procedure, which include a few other tools that you will also need later in the course:

2.1 Mac

1. Click the clipboard icon at the right end of the box below to copy the command to your clipboard.
2. Open your Terminal application.
3. Paste the command into the Terminal window and press Enter. You will be prompted several times for either your user password or permissions of the installed apps.

```
curl -fsSL http://munch-group.org/franklin/installers/install.sh | bash -s -- --role student --yes
```

2.2 Windows

1. Click the clipboard icon at the right end of the box below to copy the command to your clipboard.
2. Open your PowerShell application.
3. Paste the command into the PowerShell window and press Enter. You will be prompted several times to allow the app to make changes to your computer.

```
powershell -ExecutionPolicy ByPass -c "irm -useb http://munch-group.org/franklin/installers/install.sh | bash -s -- --role student --yes"
```

If you see any red text during the installation, the installation was not successful. In that case take a screenshot and send it to kaspermunch@birc.au.dk.

The text editor

You will also need a *text editor*. A text editor is where you write your Python code. For this course, we will use *Visual Studio Code* - or *VScodem* for short. You can download it from this page. If you open *VScodem*, you should see something like Figure 2.1. You may wonder why we cannot use Word to create and edit files with programming code. The reason is that a text editor made for programming, such as *VScodem*, only saves the actual characters you type. So, unlike Word, it does not silently save all kinds of formatting, like margins, boldface text, headers, etc. With *VScodem*, what you type is *exactly* in the file when you save it. In addition, where Word is made for prose, *VScodem* is made for programming and has many features that will make your programming life easier.

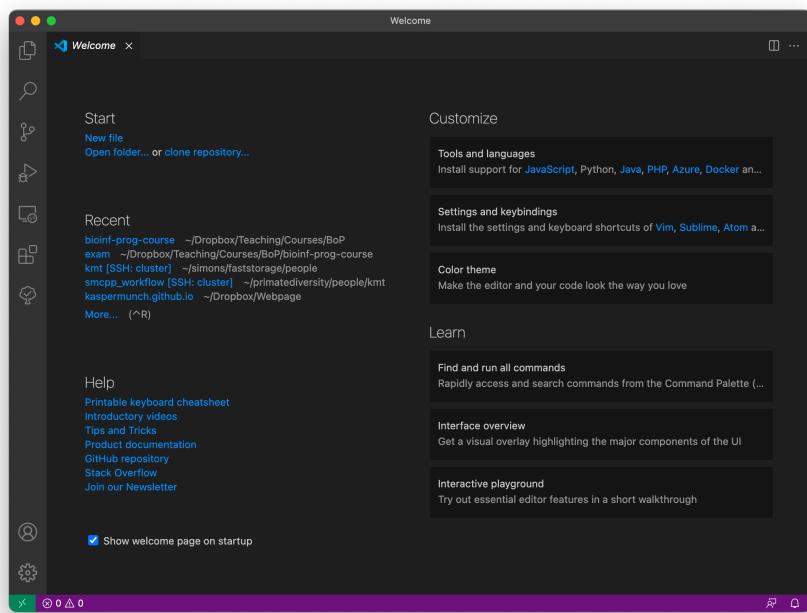


Figure 2.1: Visual Studio Code (VScodem)

The terminal

The last thing you need is a tool to make Python run the programs you write. Fortunately, that is already installed. On **OSX**, this is an application called *Terminal*. You can find it by typing “Terminal” in Spotlight Search. When you start, you will see something like Figure 2.2. You may be presented with the following text:

The default interactive shell is now zsh.
To update your account to use zsh, please run `chsh -s /bin/zsh`.
For more details, please visit <https://support.apple.com/kb/HT208050>.



Figure 2.2: The Terminal app on Mac

Do **not** update your account *after* you install Anaconda (see below). If you do, *Terminal* will not be able to find the Anaconda Python (If you did so by mistake, you change back using this command: `chsh -s /bin/bash`).

On Windows, the tool you need is called the Anaconda Powershell Prompt, which was installed along with Anaconda Python. You should be able to find it from the Start menu. Ensure you open *Anaconda Powershell Prompt* and **not** *Anaconda Powershell Prompt*. They are different programs. If you open *Anaconda Powershell Prompt*, you should see something like Figure 2.3.

What is *Anaconda Powershell Prompt* and this *Terminal* thing? Both programs are what we call *terminal emulators*. They are used to run other programs, like the ones you will write yourself. I will informally refer to both *Terminal* and *Anaconda Powershell Prompt* as “the terminal.” So if I write something like “open the terminal,” you should open *Anaconda Powershell Prompt* if you are running Windows and the *Terminal* application if you are running OS X.

The terminal is a very useful tool. However, to use it, you need to know a few basics. First of all, a terminal lets you execute commands on your computer. You type the

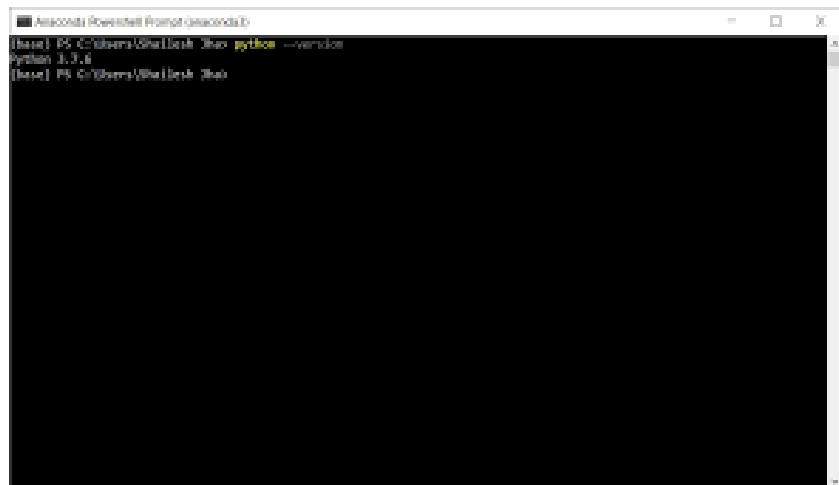


Figure 2.3: Anaconda Powershell Prompt app on Windows

command you want and then hit enter. The place where you type is called a prompt (or command prompt), and it may look a little different depending on which terminal emulator you use. In this book, we represent the prompt with the character \$. So, a command in the examples below is the line of text to the left of the \$. When you open the terminal, you'll be redirected to a folder. You can see which folder you are in by typing `pwd`, and then press Enter on the keyboard. When you press Enter, you tell the terminal to execute your written command. In this case, the command you typed tells you the path to the folder we are in. If I do it, I get:

Listing 2.1 Terminal

```
$ pwd  
/Users/kasper/programming
```

If I had been on a Windows machine, it would have looked something like this:

Listing 2.2 Terminal

```
$ cd  
C:\Users\kasper\programming
```

So, right now, I am in the `programming` folder. `/Users/kasper/programming` is the folder's path or "full address" with dashes (or backslashes on Windows) separating nested folders. So `programming` is a subfolder of `kasper`, a subfolder of `Users`. That way, you know which folder you are in and where that folder is. Let us see what is in this folder. You can use the `ls` command (l as in Lima and s as in Sierra). When I do that

and press Enter I get the following:

Listing 2.3 Terminal

```
$ ls  
notes projects
```

There seem to be two other folders, one called notes and another called projects. If you are curious about what is inside the notes folder, you can “walk” into the folder with the cd command. To use this command, you must specify which folder you want to walk into (in this case, notes). We do this by typing cd, then a space, and then the folder’s name. When I press Enter I get the following:

Listing 2.4 Terminal

```
$ cd notes  
$
```

It seems that nothing really happened, but if I run the pwd command now to see which folder I am in, I get the following:

Listing 2.5 Terminal

```
$ pwd  
/Users/kasper/programming/notes
```

Just to keep track of what is happening: before we ran the cd command, we were in the directory /Users/kasper/programming folder, and now we’re in /Users/kasper/programming/notes. This means that we can now use the ls command to see what is in the notes folder:

Again, it seems like nothing happened. Well, ls and dir do not show anything if the folder we are in is empty. So notes must be empty. Let us go back to where we came from. To walk “back” or “up” to /Users/kasper/programming, we again use the cd command, but we do not need to name a folder this time. Instead, we use the special name .. to say that we wish to go to the parent folder called programming, i.e., the folder we just came from:

When we run the pwd command, we see that we are back where we started. Let us see if the two folders are still there:

They are!

Listing 2.6 Terminal

```
$ ls  
$
```

Listing 2.7 Terminal

```
$ cd ..  
$ pwd  
/Users/kasper/programming
```

Hopefully, you can now navigate your folders and see what is in them. You will need this later to access the folders with the code you write for the exercises and projects during the course.

Action	OSX
Show current folder	pwd
List folder content	ls
Go to subfolder “notes”	cd notes
Go to parent folder	cd ..

Create a conda environment for the course

In bioinformatics, we install packages and programs to use them in our analyses and pipelines. Sometimes, however, the packages you need for one project conflict with the ones you need for other projects you work on in parallel. Such conflicts seem like an unsolvable problem. If only you could create a small insulated world for each project that only contained the packages you needed for that particular project. If each project lived in an isolated world, then there would be no such version conflicts. Fortunately, a tool lets you do just that, and its name is Conda.

“ Conda is an open-source environment management system for installing multiple versions of software packages and their dependencies and easily switching between them. ”

The small worlds that Conda creates are called “environments”. You can create as many environments as you like and then use each for a separate bioinformatics project, a course, a bachelor project, or whatever you want to insulate from everything else. Conda also downloads and installs the packages for you, ensuring that the software

Listing 2.8 Terminal

```
$ ls  
notes projects
```

packages you install in each environment are compatible. It even makes sure that packages needed by packages (dependencies) are also installed. Conda is truly awesome.

When you install Anaconda, Conda makes a single base environment for you. It is called “base”, and this is why it says “(base)” on your terminal.

In this course, you must install programs and Python libraries that could conflict with the packages you need for other courses or future projects. So, we will create an isolated Conda environment for Bioinformatics and Programming to avoid such conflicts. Conda is a program you run from the command line, like python or cd. So open your terminal (i.e., the “Terminal” program if you are on a Mac and the “Anaconda Power-shell Prompt” program if you are on Windows). Now copy/paste these command lines into the terminal *one at a time* and press return (enter) after pasting each one:

Listing 2.9 Terminal

```
conda create -y -n bioprog  
conda activate bioprog  
conda config --env --add channels conda-forge  
conda config --env --add channels sepandhaghghi  
conda config --env --add channels kaspermunch  
conda install -y 'python=3.9' pygments textual rich art bp-help
```

This command runs the Conda program and tells it to create a new environment named “bioprog” and install the packages we need in that environment. Once you hit enter on the last command, Conda works for some time and then writes a long list of packages in your terminal. These are all the packages and dependencies required in versions that fit together.

Notice how the command prompt changed from “(base)” to “(bioprog)” to show that you are now in the bioprog environment. It looks like nothing has changed, but now you can access unavailable terminal commands in the base environment. You’ll be able to learn about these later. Try this command:

```
conda deactivate
```

Notice how it again says “(base)” on your command prompt. That is because you are back in your base environment. When you start a new terminal window, you must run `conda activate bioprog` to activate the environment and access the course tools.

You are all set

Well done! You are all set to start the course. Have a cup of coffee, and look forward to your first program. While you sip your coffee, I need you to take an oath (one of three you will take during this course). Raise your right hand! (put the coffee on your left).

“ Oath 1: I swear *never* to copy and paste code examples from this book into my text editor. I will always *read* the examples and then *type* them into my editor. ”

This serves three purposes (as if one was not enough):

1. You will be fully aware of every bit of each example.
2. You will learn to write code correctly and without omissions and mistakes.
3. You will get Python “into your fingers”. It sounds silly, but it *will* get into your fingers.

3 Writing a program

Let's get you started...

Hello World

Dive in and make your first program. Create a new file in your editor (*VScode*) and save it as `hello.py`. The `.py` suffix tells your editor that this file contains Python code. As you will see, this makes your life a whole lot easier. Such a file with Python code is usually called a *script*, but we can also call it a program.

Now write *exactly* this in the file (`hello.py`):

```
print("Hello world")
```

Your editor will color your code differently, but that is unimportant. Save your file with the added code and have your first program! Of course, there is not much point in having a program if it just sits there on your computer. To run your program, do the following:

1. Open the terminal and navigate to the folder (directory) where you saved `hello.py`. Use the `cd` command to do so. If you do not remember how, reread the previous chapter.
2. Type `python hello.py` in the terminal and hit Enter.

You should see something like Figure 3.1.

This is where you shout, “It’s alive!” toss your head back, and do the insane scientist laugh.

Okay, what just happened? You wrote a program by creating a file in which you wrote one line of code. You then ran the program using Python, and it wrote (printed) Hello world in the terminal. Do not worry about the parentheses and quotes for now; just enjoy your new life as a programmer.

Maybe you wonder why we write `print` and not `write` or something else. That goes back to when computers were enormous, clunky things with no screens attached. They could only interact with the user by *printing* things on an actual physical paper printer.

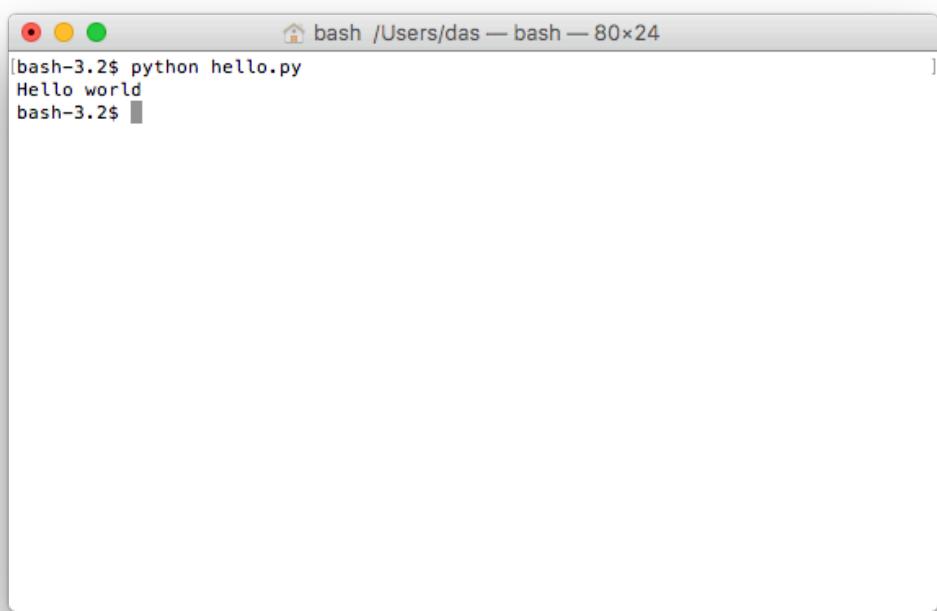


Figure 3.1: Hello world

Back then, the output you now see on the screen was printed onto a piece of paper that the programmer could then look at. These days, print shows up in the terminal, but the story should help you remember that print spits text *out of your program* just like a printer.

Now try to add another line of code like this:

```
print("Hello world")
print("I am your first program")
```

Save the file and run it again by typing `python hello.py` into your terminal and hitting Enter.

You should see this:

```
Hello world
I am your first program
```

Now your program prints Hello world and *then* prints I am your first program. The *then*-part is important. That is how a Python program works. Python (the `python` you write in front of `hello.py`) reads your `hello.py` file and then executes the code, one line after another, until it reaches the end of the file. This is essential, so reread from the beginning of the paragraph. Now, read it once more. It may seem trivial, but it is fundamental to remember that this is how Python runs your code. So here is Oath 2:

“ “ **Oath 2:** I swear to always remember that each line of code in my script is executed one after another, starting from the first line and ending at the last line. ” ”

When you write Python code, you always follow this workflow:

1. Change the code in the file.
2. Save the file.
3. Execute the code in the file using the terminal.
4. Start over.

Make sure you get the hang of this in the following exercises.

Important: The examples and exercises in this course are designed to work if you execute your scripts from their stored folder. So, you'll need to go ahead and navigate the relevant folder before you're done with your script. If your script is called `hello.py`, you must *always* execute it precisely like this: `python hello.py`. If you do it any other way, you may use a different Python version than you think. On some computers, it is possible to type `hello.py` without `python` in front of it. Don't do that. Do *not* "drag" the script file into the Terminal either. In VScode, you can press a small play icon on the top

right to execute the code. Don't do that either. There are many other things you should not do, but you get the drift.

Exercise 3-1

Try swapping the two lines of code in the file and rerun the program. What does it print now?

Exercise 3-2

Try to make the program print a greeting to yourself. Something like this:

Hello Sarah!

– if your name is Sarah, of course.

Exercise 3-3

Add more lines of code to your program to make it print something else. Can you make your program print the same thing ten times?

Error Messages

Did you get everything right with your first program, or did you get error messages when executing your code with python? Maybe you wrote the following code (adding an extra closing parenthesis):

```
print("Hello world"))
```

– and then got an error like this:

```
File "hello.py", line 1
    print('Hello world'))^
SyntaxError: invalid syntax
```

This is Python's way of telling you that the `hello.py` script has an error in line 1. If you write something that does not conform to the proper syntax for Python code, you will get a `SyntaxError`. Python will do its best to figure out where the problem is and point to it with a `^` character.

You will see many error messages in your new life as a programmer. So you must practice reading them. At first, they will be hard to decipher, but once you get used to them, they will help you quickly identify where the problem is. If there is an error message that you do not understand, the internet is your friend. Just paste the error message into Google's search field, and you will see that you are not the only one on the internet getting started on Python programming. It is okay if you do not know how to fix the problem right now, but it is essential to remember that these error messages are Python's way of helping you understand what you did wrong.

Exercise 3-4

Try to break your new shiny program and make it produce an error message when you run it. An easy way of doing this is to remove or change random characters from the program. If you run this (with a missing end-parenthesis):

```
print("Hello world"
print("I am your first program")
```

You will get this error:

```
File "hello.py", line 2
    print("I am your first program")
           ^
SyntaxError: invalid syntax
```

The `^` character tells you when your code stops making sense to Python. Sometimes, that is a bit after where you made your mistake.

Try to make other kinds of errors. Which error messages do you see? Do you always see the same error message, or are they different? Try googling the error messages you get. Can you figure out why the change you made broke the program? How many other error messages can you produce?

Strings

In programs, text values are called *strings*, and you have already used strings a lot in your first program. A string is simply a text, but we call it a string because it is a “string of characters”. In Python, we represent a string like this:

```
"this is a string"
```

or like this:

```
'Hello world'
```

That is, we take the text we wish to use as our value and put quotes around it. We can use double quotes (the first example) or single quotes (the second example). We can mix them like this:

```
print('this is "some text" with a quote')
```

– but not like this:

```
print("this is a broken string")
```

Can you see why and how handy it is to have single and double quotes? If we did not have both, we could not have text with quotes. However, you must use the same kind of quotes at each end of the string. Running the latter example gives an error message telling you that Python cannot find the quote that was supposed to end the string:

```
File "broken.py", line 1
    print("this is a broken string")
                           ^
SyntaxError: EOL while scanning string literal
```

It is Python’s way of saying, “ I reached the end of the line (EOL) without finding a matching end quote.”

Comments

You have already learned that Python reads and executes one line of code at a time until your program has no more lines of code.

However, we can make a line invisible to Python by putting a # symbol in front of it, like this:

```
# print("Hello world")
print("Greetings from your first program")
```

When you do that, Python does not read that line. It is not part of the program. Rerun the program. You will notice that now only the second line is part of your program:

```
$ python hello.py
Greetings from your first program
```

This is useful in two ways:

1. It lets you disable certain lines of your code by keeping Python from reading them. For example, see what happens if that line of code is not executed to understand how your program works.
2. It allows you to write notes in your Python code to help you remember how it works.

Lines with a # in front are called “comments” because we usually use them to write comments about our code. If you ask for help with some problem, you will often hear your instructor say: try to “comment out” in line two. When your instructor says that, it simply means that you should add a # in front of line two to see what then happens.

Exercise 3-5

What happens if I put a # in the middle of a line of code? Try it out!

Exercise 3-6

Try this:

```
# Note to self: the lines below print stuff
print("Hello world")
print("Greetings from your first program")
```

Exercise 3-7

Try this:

```
print("Hello world") # actually, everything after a # is ignored  
print("Greetings from your first program")
```

What did you learn? Which parts of each line are considered part of the program?

Exercise 3-8

Now try this:

```
print("Hello # world")  
print("Greetings from your first program")
```

What did you learn about # characters in strings? #### Exercise Try this:

```
print("Hello world"#)  
print("Greetings from your first program")
```

Did you expect this to work? Why? Why not? What error message did you get?

4 Dealing with values

This chapter is about values and variables, the two most central concepts in programming.

Math

Much programming is done to compute stuff. In Python, the usual math operations are done using these arithmetic operators:

Operator	Operation
+	plus
-	minus
/	division
//	integer division
*	multiplication
**	exponentiation
%	modulo (remainder)

You are probably quite familiar with these - except perhaps for integer division, exponentiation, and modulo. Let us take some of the operators for a spin. Remember to carefully write the whole thing in an empty file in *VScode*. Do *not* copy-paste. Then save the file as `mathandlogic.py` and run it from the terminal. Do not call your file `math.py`. It may bite you later. Just trust me on that one.

```
print("Four times two is", 4 * 2)
```

Notice how you can print more than one thing at a time if you put commas between the values you want to print? We can group computations using parentheses, just like in normal math. Try this:

```
print("10 / (2 + 3) is", 10 / (2 + 3))
print("(10 / 2) + 3 is", (10 / 2) + 3)
```

In addition to the regular math operators, there are a few extra operators that we call *comparison operators* because they are used to compare two values, e.g., two numbers.

Operator	Operation
<	less-than
>	greater-than
<=	less-than-or-equal
>=	greater-than-or-equal
==	equal
!=	not equal

Try this:

```
print("Is 5 greater than -2?", 5 > -2)
print("Is 5 greater or equal to -2?", 5 >= -2)
print("Is 5 less or equal to -2?", 5 <= -2)
print("Is 5 less than 7 - 2?", 5 < 7 - 2)
print("Is 5 equal to 7 - 2?", 5 == 7 - 2)
```

As you may have noticed, running this code and comparing things using these operators, we always produce either True or False. E.g., the following

```
print(5 < 7)
```

prints the value True because 5 is smaller than 7. True and False are special values in Python that we can use (and print if we like) just like any other Python value:

```
print(True)
print(False)
```

Exercise 4-1

Try to write and run the code below. Compare each line to what is printed when you run the code. Make sure you understand why.

```
print("I have", 25 + 30 / 6, "of something")
print("I have", 100 - 25 * 3 % 4, "of something else")

print("Is it true that 3 + 2 < 5 - 7?")
print(3 + 2.1 < 5.4 - 7)
```

```
print("3 + 2.1 is", 3 + 2.1)
print("5.4 - 7 is", 5.4 - 7)

print("Oh, that's why it's False.")
```

Exercise 4-2

An additional comparison operator even tests if something is a part of something else. That operator is called `in`. One use of it is to test if one string is part of another string. Try this to figure out how it works:

```
print("Hell" in "Hello world")
print("Hello world" in "Hello world")
print("Hello world" in "Hell")
print("lo wo" in "Hello world")
print("Artichoke" in "Hello world")
```

Exercise 4-3

Say the supermarket has chocolate bars for 7 kr. Write a small Python program (in a file called `chocolate.py`) that prints how many chocolate bars you can get for your 30 kr. You should run it like this;

For example, it could output something like this:

```
$ python chocolate.py
```

to have it print something like this:

```
I can buy 4.285714285714286 chocolate bars!
```

Exercise 4-4

We mentioned a special operator called *modulo*. Google it if you do not remember what it does. How about *integer division*. Explain both to a fellow student (or to yourself out loud).

Exercise 4-5

You obviously cannot go buy 4.3 chocolate bars in a store. You will have to settle for 4. Can you change the program you made in exercise 4-3 to print the number of bars you can buy and the change you then have left? Use the modulo and integer division operators. Something like:

I can buy 4 chocolate bars, leaving me with 2 kr in change.

Exercise 4-6

What happens if you try to run the following program?

```
print( 1 / 0 )
```

If you get an error? What kind of error? Why do you think you get that error? Do you think it makes sense?

Exercise 4-7

You probably know the Pythagorean theorem for computing a right-angled triangle's hypotenuse (the longest side). The Pythagorean theorem looks like this: $a^2 + b^2 = c^2$. Here c is the length of the hypotenuse, and a and b are the lengths of the triangle's two legs. So if we have a triangle where $a = 5$ and $b = 2$ and we want to find c^2 we can do this in Python:

```
print("The squared length of the hypotenuse is:", 5**2 + 2**2)
```

Exercise 4-8

However, we are rarely interested in the *squared* length of the hypotenuse. Can you modify the code you wrote in exercise 4-7 so you compute c instead of c^2 ? Taking the square root of a number is the same as taking that number and exponentiating it to 0.5, so the square root of x is $x^{0.5}$. Do you know of a Python operator that does exponentiation?

Logic

Now you know how to use the comparison operations to produce a True or False value. There are three additional operators that let you express more elaborate “True/False” statements than with the comparison operators alone. These are the *logical* operators: and, or and not.

Exercise 4-9

Go through the code below and see if you can figure out what each line does. Then, write the code into your editor and run it to see what happens.

```
print(2 < 3)
print(10 < 12)
print(8 > 100)
print(2 < 3 and 10 < 12)
print(8 > 100 and 2 < 3)
print(8 > 100 or 2 < 3)
print(not 8 > 100 and 2 < 3)
print(not 8 > 100 and not 2 > 3)
```

Did it do what you expected? Can you explain each line?

Exercise 4-10

When exposed to the operators and, or and not, some values are considered true and others are considered false. What happens when you put not in front of something that is considered true or false? Decide what you think and why before you write the code and try it out.

```
print(not True)
print(not False)
print(not 0)
print(not -4)
print(not 0.0000000)
print(not 3.14159265359)
print(not "apple")
print(not "")
```

From the code above, try to find out which values Python considers true and which it considers false. Can you come up with a rule?

Exercise 4-11

The logical operator `and` takes two values (the one to the left of the operator and the one to the right) and figures out whether *both* the left and the right expression are true. It boils down to this:

Left expression	Right expression	Result
True	True	True
True	False	False
False	True	False
False	False	False

Write some code to confirm that the table above is correct using Python. For example, to test the first case, do this:

```
print(True and True)
```

Exercise 4-12

Python will only do the necessary work to determine if a logical expression is true. That means that if the value left of `and` is considered false by Python, then there is no reason to look at the right value since it is already established that they are not *both* considered true. In this case the expression reduces to the *left* value. I.e. `False and True` reduces to `False`.

If Python considers the value left of `and` true, then It needs to look at the right value, too, to establish if they are both considered true. In this case, the expression reduces to the value on the right. I.e., `True and False` reduces to `False`.

A rule of thumb is that the whole expression reduces to the last value that Python needs to consider to decide if the whole expression is true or false. Use that rationale to explain how the two last combinations in exercise 4-11 are evaluated.

Exercise 4-13

Like the `and` operator, the `or` operator also takes two values. However, the `or` operator determines whether *one* of the two values is true. Thus, the `or` operator boils down to this:

Left expression	Right expression	Result
True	True	True

Left expression	Right expression	Result
True	False	True
False	True	True
False	False	False

Write some code using Python to confirm that the table above is correct. For example, to test the first case, do this:

```
print(True or True)
```

Exercise 4-14

As with the `and` operator, Python will not do any more work than absolutely necessary when evaluating an expression with 'or'. So if the value left of `or` is considered true by Python, then there is no reason to look at the right value since it is already established that at least *one* of them is considered true. In this case the expression reduces to the *left* value. I.e. `True or False` reduces to `True`.

If the value left of `or` is considered false by Python, then Python still needs to look at the right value to establish if at least one of them is considered true. In this case, the expression reduces to the right value. I.e., `False or True` reduces to `True`.

Again, the whole expression reduces to the last value that Python needs to consider to decide if the whole expression is true or false. Use that same rationale to explain to yourself how the two last combinations in exercise 4-13 are evaluated.

Exercise 4-15

Remember what you learned in exercise 4-10 about which values are considered true and which are considered false. Combine that with what you learned in exercise 4-11 and exercise 4-13 about what logical expressions reduce to `and` and see if you can figure out what is printed below and why. Use the rule-of-thumb from exercise 4-14. Decide what you think before you write the code and try it out.

```
print(True and 4)
print(0 and 7)
print(-27 and 0.5)
print(42 and 0)
print("apple" and "orange")
print("apple" and "")
print(42 or 0)
```

```
print("apple" and "")  
print("apple" or "")
```

If you were surprised by what was printed, maybe go back and look at exercise 4-11 and exercise 4-13 again.

Exercise 4-16

Recall the `in` operator from exercise 4-2? There is also an operator called `not in`. I guess you can imagine what that tests. Try it out.

Variables

“ By now, you probably feel the first signs of brain overload. If you do not take breaks, your brain may overheat and explode - we have seen that happen. One of the nice things about the brain is that it works when you rest. Archiving and understanding a lot of new information takes time, and force-feeding your brain will not help. The last part of this chapter is very important so now might be a good time for a good long break. ”

This section is about *variables* and is where the fun begins. A variable is a way of assigning a name to a value. `8700000` is just a value, but if we assign a name to it, then it gets a special meaning:

```
number_of_species = 8700000  
print(number_of_species)
```

In this case, the variable `number_of_species` represents the estimated number of eukaryotic species on the planet, which is `8700000`. So `8700000` is the *value*, and “`number_of_species`” is the variable name. Write the code above into a file and run it. Notice how this lets us refer to the *value* using the *variable name*. What appears in the terminal when you do that? Do you see `number_of_species` or `8700000`?

As you can see in the small program above, one of two different things happens when a variable name appears in Python code:

- **Assignment:** When a variable name appears to the left of an equal sign, a value is assigned to the variable. This happens in the first line where `number_of_species` is *assigned* the value `8700000`.

- **Substitution:** In all other contexts, the variable is substituted for its value. This happens in the second line where Python *substitutes* the variable name `number_of_species` for its value `8700000` and then prints that.

That is it, but let us take the example further and create another variable to which we assign the value `1200000`. That is the number of species discovered so far. Now, let us add this to the program and use it to compute the number of species we have yet to identify. Start by reading the code below super carefully. Remember that a variable is *either* assigned a value or substituted for the value it represents. For each occurrence of the variables below, determine if they are being assigned a value or if they are substituted for their value.

```
number_of_species = 8700000
number_discovered = 1200000
number_unidentified = number_of_species - number_discovered
print(number_unidentified)
```

Now write the code into a file and run it. Take some time to let it sink in that variables are extremely useful for two reasons:

1. Variables give meaning to a value. Without the variable name, the value of `1200000` could just as well be the number of people that live in Copenhagen. However, by giving the value a meaningful name, it becomes clear what it represents.
2. We can assign new values to variables (that is why they are called variables). For example, we can change the value of `number_discovered` as new species are discovered.

Your variable names can be pretty much anything, but they have to start with a letter or an underscore (`_`), and the rest of the name has to be either letters, numbers, or underscores. To be clear, a space is *not* any of those things, so do not use spaces in variable names. Above all, be careful in your choice of variable names. Variable names are case-sensitive, meaning that `count` and `Count` are different variables. Stick to lowercase variable names. That makes your code easier to read.

Exercise 4-17

For each occurrence of the variables below, determine if they are being assigned a value or if they are substituted for their value.

```
breeding_birds = 4
print(breeding_birds)
breeding_birds = 5
print(breeding_birds)
```

Exercise 4-18

For each occurrence of the variables below, determine if they are being assigned a value or if they are substituted for their value.

```
breeding_birds = 4
print(breeding_birds)
breeding_birds = breeding_birds + 1
print(breeding_birds)
```

Exercise 4-19

What happens if you take the first example in this section and swap the two lines? So, going from this:

```
number_of_species = 8700000
print(number_of_species)
```

to this:

```
print(number_of_species)
number_of_species = 8700000
```

Explain to yourself what happens in each case. What kind of error do you get with version two, and why? Remember Oath 2!

Exercise 4-20

Write the following code in a file, save it, and run it.

```
income = 45000
taxpercentage = 0.43
tax_amount = tax_percentage * income
income_after_tax = income - tax_amount
print('Income after tax is', income_after_tax)
```

You should get an error that looks a lot like this one:

```
Traceback (most recent call last):
  File "tax.py", line 3, in <module>
    tax_amount = tax_percentage * income
NameError: name 'tax_percentage' is not defined
```

It says that the error is on line 3. Can you figure out what is wrong? Hopefully, you will now appreciate how much attention to detail is required when programming. Every tiny, little symbol or character in your code is *essential*.

Different types of values

By now, you probably have a pretty good idea of what a value in Python is. So far, you have seen text like 'Banana', integers like 7, and numbers with a fractional part like 4.25.

In Python, a text value is a *type* of value called a *string*, which Python denotes as `str` (abbreviation for "string"). So 'Banana' is a string, and so is 'Banana split'. There are two types of numbers in Python. Integers (7, 42, and 3) are called `int`. Numbers with a fractional part (like 3.1254 and 4.0) are that are called `float` (an abbreviation for "floating-point number").

As I mentioned earlier, `True` and `False` are Python values too. They are called `booleans` or `bool`, named after an English mathematician called George Boole famous for his work on logic.

So the different *types* of values we know so far are:

Name	Type in Python	Examples
String	<code>str</code>	"hello", '9'
Integer	<code>int</code>	0, 2721, 9
Floating-point	<code>float</code>	1.0, 4.4322
Boolean	<code>bool</code>	<code>True</code> , <code>False</code>
None	<code>NoneType</code>	None

In case you did not notice, I added a special type at the end that can only have the value `None`. It may sound a little weird, but in programming, we sometimes need a value representing nothing or `None`. For now, just make a mental note that `None` is also a Python value.

When you do computations in Python, it is no problem to mix integers and floating-point numbers. Try this:

```
print("What is 0.5 * 2?", 0.5 * 2)
print("What is 3 / 2?", 3 / 2)
```

As you can see we can also make computations using only integers that result in floating-point numbers.

Some of the math operators not only work on numbers, but they also work on strings. That way, you can add two strings together. It is no longer math, of course - but quite handy.

```
fruit = 'Ba' + 'na' + 'na'
print(fruit)
```

Exercise 4-21

If you try to combine different types of values in ways that are not allowed in Python, you will get an error. Try each of the following weird calculations, and read each error message carefully.

```
x = 3 - '1.5'
print(x)
```

```
x = None - 4
print(x)
```

Exercise 4-22

Write these two examples and compare the resulting values of x

```
x = '9' + '4'
print(x)
```

```
x = 9 + 4
print(x)
```

Exercise 4-23

Try these two examples. What happens in each case? Does it make sense?

```
x = '72' * 3  
  
x = '72' * '3'
```

Exercise 4-24

Will this work? Use what you have learned from the other exercises and try to predict what will happen here. Then, write the code and try it out.

```
x = 'Ba' + 'na' * 2  
print(x)
```

Exercise 4-25

Sometimes, you may need to change a string to a number. You can do that like this:

```
some_value = "42"  
other_value = int(some_value)
```

Write some code that converts strings to numbers and numbers to strings. Remember that numeric values are either integers or float. Use `int`, `float` as in the example above. You will notice that only meaningful conversions work. E.g., this will not work: `number = int('four')`. To convert a number to a string, you can use `str`.

Having completed the above exercises, you should take note of the following four important points:

1. All Python values have a type. You know about strings, integers, floating-points, and booleans so far.
2. Math operators let you do cool things like concatenating two strings by adding them together.
3. The flip side of that cool coin is that Python will assume you know what you are doing if you add two strings ('4' + '4' is '44' *not* 8) or multiply a string with an integer ('4' * 4 is '4444' *not* 16).
4. You can change the type of a value, e.g., '4' to 4 or 1 to 1.0.
5. Python will throw a `TypeError` if you try to combine types values of values in ways that are not allowed.



Escape characters: An escape character is a backslash \ followed by a single character. \n and \t are the most commonly used ones.



Exercise 4-26

What do you think is printed here?

```
main_course = 'Duck a la Banana\n'
dessert = 'Banana split\n'
menu = main_course + dessert
print(menu)
```

Can you figure out what the special character \n represents?

Exercise 4-27

What do you think is printed here?

```
dish_one = 'Banana\t\tsplit'
dish_two = 'Chocolate\tcake'
print(dish_one)
print(dish_two)
```

Can you figure out what the special character \t represents?

Mixed exercises

Each chapter in the book ends with a set of mixed exercises meant to allow you to combine what you have learned so far. In this case, they are meant to train your familiarity with the following topics:

- Strings
- Math
- Logic
- Types of values
- Variables

Exercise 4-28

What happens if you try to run the following program?

```
print("What happens now?", 1 / )
```

If you get an error, why do you think you get that error?

Exercise 4-29

What happens if you try to run the following program?

```
print("What happens now?", 1 / 3
```

If you get an error, why do you think you get that error? Can you fix it? (Hint: EOF is short for End Of File)

Exercise 4-30

Determine, for each of the eight occurrences of the variable `x` below, where it is being assigned a value and when it is substituted for its value:

```
x = 1
x = x + 1
x = x + 1
x = x + 1
print(x)
```

Then, figure out what is printed and why (remember oath 2). What value does `x` represent at each occurrence in the code?

Exercise 4-31

Some comparison operators also work with strings. Consider this code:

```
print("apples" == "pears")
```

What is printed here? Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise 4-32

Consider this code:

```
print('aaaaaa' < 'b')
print('a' < 'b')
print('aa' < 'ab')
print('99' > '100')
print('four bananas' > 'one banana')
```

What is printed here? Write the code and see for yourself once you think you know. By what rule does Python decide if one string is smaller than another? You may have a clue if you have looked something up in an encyclopedia recently. Also, try to google "ASCII table".

Exercise 4-33

Consider this code:

```
print('banana' < 'Banana')
```

What is printed here? Write the code and see for yourself once you think you know.

Exercise 4-34

Do you think it is allowed to use relational operators on values of different types? Try these out and see for yourself:

```
print('Banana' > 4)

print('42' == 43) # this one is dangerous...

print(4 in '1234')
```

Practice reading this kind of error (TypeError).

Exercise 4-35

Can you use the in operator to test if this mini gene is part of the DNA string?

```
mini_gene = 'ATGTAG'  
dna_string = 'GCTATGTAGGTA'
```

Exercise 4-36

Say you have two strings "4" and "2". What happens if you add them like this: "4" + "2". Can you convert each one to integers so you get 6 when you add them? (have a look at exercise 4-25 if you do not remember).

Exercise 4-37

What happens if you run this code? Do you get an error? Do you remember why?

```
1value = 42
```

Exercise 4-38

What happens if you run this code?

```
print('Hi')  
print('Hi')  
print('Hi')
```

Compare this to what happens when you run this code:

```
print('Hi\nHi\nHi')
```

Do you remember what \n represents? What does it tell about what is added at the end every time you print something?

Exercise 4-39

Make three exercises for your fellow students. See if you can make them so they test the understanding of (almost) all you have learned so far.

5 The order of events

This chapter is about how Python interprets (or evaluates) the code you write. It has a few fancy long words that may seem foreign to you. Do not let that throw you off. They are all just fancy names for something straightforward.

Precedence of Operators

Fear not. Precedence is just a nasty word for something we have already talked about. Precedence just means that some things are done before others or, more correctly, that some operations are performed before others. You already know that multiplication is done before addition. Another way of saying that is that multiplication *takes precedence* over addition. The expression below obviously reduces to 7 in two steps:

$$1 + 3 \cdot 2$$

First, $3 \cdot 2$ reduces to 6, and then $1 + 6$ reduces to 7. If we wanted to add 1 and 3 first, we would need to enforce this by adding parentheses:

$$(1 + 3) \cdot 2$$

This is because the multiplication operator ($*$) has higher precedence than the addition operator ($+$). Here is the list of the most common operators and their precedence in Python:

Level	Category	Operators
Highest	exponent	$**$
	positive / negative	$+x, -x$
	multiplication	$*, /, //, %$
	addition	$+, -$
	relational	$!=, ==, <=, >=, <, >, \text{in}, \text{not in}$
	logical	not
	logical	and
Lowest	logical	or

Sometimes, a statement contains adjacent operators with the same precedence. In this case, Python evaluates the expression from left to right. I.e., This following expression first reduces to 0.5 * 2 and then to 1

`2 / 4 * 2`

The following one first reduces to 1 * 4 and then to 4:

`2 / 2 * 4`

If you want Python to order the operations in any other way, you need to use parentheses (E.g., `2 / (2 * 4)`).

Exercise 5-1

Look at each expression in the exercises below and use the table above to decide if it evaluates to True or False. Then, write the code and test if you were right. If not, figure out why.

`2 + 4 * 7 == 2 + (4 * 7)`

Exercise 5-2

Does this reduce to True or False?

`4 > 3 and 2 < 1 or 7 > 2`

Exercise 5-3

Does this reduce to True or False?

`4 > 3 and (2 < 1 or 7 > 2)`

Exercise 5-4

Does this reduce to True or False?

```
2 * 4 ** 4 + 1 == (2 * 4) ** (4 + 1)
```

Statements and Expressions

To talk concisely about programming (and to receive more useful help from your instructors), you need to have a bit of vocabulary. *Statements* and *expressions* are two words you need to know. Distinguishing between statements and expressions will help us discuss our code.

- A **statement** is a line of code that performs an action. Python evaluates each statement until it reaches the end of the file (remember oath 2?). `print(y * 7)` is a statement, and so is `x = 14`. They each represent a full line of code and perform an action.
- An **expression** is any code that reduces to one value. `y * 7` is an expression, and so are `y * 7 + 14 - x` and `4 > 5`.

We will talk more about how Python handles expressions in the next section, but right now, you must understand that statements *do something* while expressions are things that reduce to a value. Hopefully, this distinction will be more clear after completing the following exercises.

Exercise 5-5

Did you notice in the above examples that `print(y * 7)` is a statement and `y * 7` is an expression? Yes, expressions can be part of statements. In fact, they most often are. Similarly, expressions are often made up of other smaller expressions. E.g., `y * 7` is part of the longer expression `y * 7 + 14 - x`.

Take a look at this code:

```
x = 5
y = 20
z = (x + y) / 2 + 20
print(z * 2 + 1)
h = 2 * x - 9 * 48
print(h)
```

Write down the code on a piece of paper. Now, mark all statements and all expressions. Remember that expressions are often made up of smaller expressions so that you can find a *lot* of them. E.g. `(x + y)`, `2 + 20`, and `(x + y) / 2 + 20` are all expressions. A single variable (like `x`) is also a small expression. Discuss with a fellow student. Do you agree on what to find?

Exercise 5-6

Consider the following code:

```
greeting = 'Hello' + ' my '
print(greeting + 'friend')
```

How many statements are there in this piece of code? How many expressions?

Substitution and Reduction

Although *substitution* and *reduction* may not sound like your new best friends, they truly are! If you remember to think about your Python code in terms of substitution and reduction, then programming will make a lot of sense. Understanding and using these simple rules will allow you to read and understand any code. If you do not, you may get by for a while - only to find yourself in big trouble later when things become more complicated.

You should remember, from the section on variables in the previous chapter, that variables in Python are either assigned a value or substituted for the value they represent.

In the first two lines of code below, the variables *x* and *y* are each assigned a value. Now consider the last line in the example:

```
x = 4
y = 3
z = x * y + 8
```

Here, *x* is *substituted* by the value 4, and *y* is *substituted* by the value 3. So now the expression after the equals sign reads $4 * 3 + 8$. Because we multiply before we add, $4 * 3$ *reduces* to 12 so that the expression now reads $12 + 8$. Finally, this *reduces* to the value 20. The very *last* thing that happens is that the variable *z* is assigned the value 20.

You should do these steps every time you see an expression. You may think this is overdoing things a bit, but it is *not*. This kind of explicit thinking is what programming is all about, and it will become increasingly important as the course progresses. So make sure you make it a habit while it still seems trivial. Then, over time, it will become second nature.

Now raise your right hand and read the third and last oath out loud:

Oath 3: I now solemnly swear to consciously consider every single substitution and reduction in every Python expression that I read or write from this moment on.

This was the last of the three oaths, but it is *by far* the most important one. You can take your hand down now.

NB: You may not realize this at this point, but the last two subsections are the most important ones in the book. Go back and read them many times as you proceed through the course. If you explicitly think in terms of substitution and reduction, you will be fine. If you do not, you are entering a minefield with snowshoes on.

Exercise 5-7

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
x = 7  
y = 4 + x  
2 + x * x**2 + y - x
```

Exercise 5-8

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
a = 4  
b = a  
c = 2  
c = a + b + c
```

Exercise 5-9

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
x = 1  
x = x
```

Exercise 5-10

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
microsatellite = "GTC" * 41
```

Surprised?

Exercise 5-11

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
mini_gene = "ATG" + "GCG" + "TAA"
```

What did you do first here? Does the order of additions matter? What operations does Python perform first when operators have the same precedence? (left to right or right to left)

Exercise 5-12

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
number = 1 / 1 * 4
```

In what order are reductions made? Does the order of operations matter, and in what order does Python do the reductions?

Exercise 5-13

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a `print` statement at the end.

```
x = 4  
y = x + x
```

Exercise 5-14

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a print statement at the end.

```
x = 4  
x = x + 1
```

Exercise 5-15

Do the substitution and reduction steps with pen and paper, then run it to check yourself by inserting a print statement at the end.

```
x = 4  
x += 1
```

Compare the final value of x to that in exercise 5-14. Can you see what `+=` is a shorthand for? Nifty, right?

General exercises

The following exercises are meant to train your familiarity with the topics we have treated so far – in this case especially:

- Substitution
- Reduction
- Assignment
- Simple precedence rules
- Comparison operators
- Logical operators
- Distinction between text and numbers

Read each exercise and think hard about the questions before you code anything. *Then* write the code and try it out. Remember that it is crucial that you type it in – as super dull as it may be (remember oath one). This trains your accuracy and attention to detail, and it builds programming into your brain. Play around with each bit of code. Make small changes and see how it behaves.

There is a reason why there are lots of questions in this exercise but no answers. You are supposed to find them yourself if it takes you quite a while. That is the way you build understanding. Some of the questions may seem trivial, but do them anyway. If you only understand these concepts superficially, they will come back and bite you in the ass when things get more complicated.

Exercise 5-16

Consider this code:

`1.2 * 3 + 4 / 5.2`

What does that expression evaluate to? Try to explicitly make all the reductions on paper before you write and run the code.

Exercise 5-17

Consider this code:

`1.2 * (3 + 4) / 5.2`

What does that expression evaluate to? Try to explicitly make all the reductions on paper before you write and run the code.

Exercise 5-18

Consider this code:

`10 % 3 - 2`

What does that expression evaluate to? Try to explicitly make all the reductions on paper before you write and run the code.

Exercise 5-19

Consider this code:

`11 % (7 - 5)**2`

What does that expression evaluate to? Try to explicitly make all the reductions on paper before you write and run the code.

Exercise 5-20

Consider this code:

```
a = 5
x = 9
banana = 7
x + 4 * a > banana
```

What does the last expression evaluate to? Try to explicitly make all the substitutions and reductions on paper before writing the code. What happens if you write and run the code? Why?

Exercise 5-21

Consider this code:

```
dance = 'can'
dance = dance + dance
print("Do the", dance)
```

What is printed? Try to explicitly make all the substitutions and reductions on paper before writing the code. What happens if you write and run the code? Why?

Exercise 5-22

Consider this code:

```
foo = 30
bar = 50
baz = bar + foo
print(baz)
bar = 10
print(baz)
```

There are two print statements. The first print statement prints 80. But what about the second print statement? Does that print 80 or 40? Find out and make sure you understand why it prints what it prints. If not, reread the section on substitution.

Exercise 5-23

Consider this code:

```
1 == '1'
```

and this:

```
1 == 1.0
```

What does this reduce to? Try printing it and seeing once you think you know. If you were wrong, make sure you figure out why.

Exercise 5-24

Consider this code:

```
a = '1'  
b = '2'  
c = a + b  
print(a, b, c)  
print(a + b == 3)
```

What is printed here? Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise 5-25

Consider this code:

```
a = 1  
b = 2  
c = a + b  
print(a, b, c)  
print(a + b == 3)
```

What is printed here? Compare to exercise 5-24. Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise 5-26

Consider this code:

```
x = 4
print(x + 2 and 7)
print(x + 2 or 7)
x = -2
print(x + 2 and 7)
print(x + 2 or 7)
```

What is printed here? Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise {#sec-puzzle) 5-27

I have shuffled the statements in the code below. Put them in the right order to make the code print 100.

```
x = x + 4
print(x)
x = x * 5
x = x * x
x = 4
```

Exercise 5-28

I have shuffled the statements in the code below. Put them in the right order to make the code print the string “Banana.”

```
y = 'n'
x = 'B' + y + x
print(x)
x = 'a'
y = (x + y) * 2
```

Exercise 5-29

Make a puzzle exercise, like the two previous ones, for a fellow student.

Exercise 5-30

Remind yourself of the different types of Python values you know. For example, one of them is an integer (int). Make a list.

Exercise 5-31

You already know about several types of data values in Python. Two are integers called `int`, and decimal numbers (or floating points) called `float`. When you use an operator like `+` or `>` it produces a value. No matter what you put on either side of `>` it produces a boolean value (`bool`), `True` or `False`. For other operators, the type of value produced depends on which values the operator works on. Try this and see if you print an integer or a float (8 or `8.0`):

```
x = 4
y = 2
result = x * y
print(result)
```

Now try to replace 4 with `4.0`. What type is the result now? Try to also replace 2 with `2.0`. What type is the result now? Can you extract a rule for what the `*` operator produces depending on what types the two values have?

Exercise 5-32

In exercise 5-31, you investigated what types of values the `*` operator produces. Redo that exercise with the operators: `+`, `-`, `/`, `**`, `//`, and `%`. What are the rules for what is returned if both values are integers, one value is a float, or both values are floats?

Exercise 5-33

Make a list of all the operators you know so far in order of precedence (without looking in the notes). Then check yourself.

Exercise 5-34

What does this expression reduce to, and what type of value is it?

`3 > 2`

Exercise 5-35

What does his expression reduce to, and what type of value is it? Do all the reduction steps in your head.

`2 - 4 * 5 - 2 * 1/3`

Exercise 5-36

What does his expression reduce to, and what type of value is it? Do all the reduction steps in your head.

`3 > 2 and 2 - 4 * 5 - 2 * 9`

Exercise 5-37

What is printed here and why?:

```
print(True and "banana" or "orange")
```

Try to change the `True` value to `False` and see what happens. Can you explain it? If not, look at exercise 4-14 again.

Exercise 5-38

What does his expression reduce to? Do all the reduction steps in your head.

`0 and 1 or 2`

Exercise 5-39

What does his expression reduce to? Do all the reduction steps in your head.

`4 and 1 or 2`

Exercise 5-40

If you understood exercise 5-37, then you should also understand this one:

```
weather = 'rain'  
what_to_do = weather == 'rain' and 'watch movies' or 'go swimming'  
print(what_to_do)
```

What happens if you change 'rain' in the first line to something else (like 'sun')?

Exercise 5-41

What is the value of results once the code below has run? Do the substitutions, reductions, and assignments in your head before you run the code.

```
x = 7  
y = 13  
z = x + y  
x = 0  
result = x + y + z
```

Exercise 5-42

What is the value of results once the code below has run? Do the substitutions, reductions, and assignments in your head before you run the code.

```
x = 5  
y = x + 1  
x = y + 1  
y = x + 1  
result = x + y
```

Exercise 5-43

In the code below I have shuffled the statements. Put them in the right order to make the code print 9. To do that you must think about which values each variable will in each statement depending on the how you order the statements.

```
x = x + 1  
y = 5  
y = y - 1  
print(y)  
x = 1  
y = y * x
```

Exercise 5-44

In the code below, I have shuffled the statements. Put them in the right order to make the code print 'Mogens'

```
c = b  
print(c)  
a = b + a  
b = 'og'  
b = c + a  
c = 'M'  
a = 'ens'
```

Exercise 5-45

Make three exercises that require the knowledge of programming so far. Have your fellow students solve them.

6 Course tools

Wax on, wax off

“ Mr. Miyagi: First, wash all car. Then wax. Wax on...

Daniel: Hey, why do I have to...?

Mr. Miyagi: Ah ah! Remember deal! No questions!

Daniel: Yeah, but...

Mr. Miyagi: Hai! Wax on, right hand. Wax off, left hand. Wax on, wax off. Breathe in through nose, out of mouth. Wax on, wax off. Don't forget to breathe, very important. [walks away, still making circular motions with hands] Wax on... wax off. Wax on... wax off.

”

Seeing the sequence of substitutions and reductions in a Python expression will become natural over time. Until it does, you are in troubled waters, and if you do not practice in time, you may only realize this too late. Considering how simple this is to practice and how crucial it is to your progress, I have written a small companion program called `myiagi` where you can train this particular skill daily. The program is installed in the `conda` environment you created for this source, so make sure it is activated as described. To run the program, you execute this command in the terminal:

`myiagi`

It should look like Figure 6.1, and the simple game is as follows. The program generates a Python expression. From that expression, all the substitution and reduction steps are performed. Each substitution or reduction results in an intermediate expression until only a single Python value remains. Here is an example where the expression is `4 * y + x` and the value it reduces to is 37:

1. `4 * y + x`
2. `4 * 8 + x`
3. `24 + x`
4. `24 + 13`
5. `37`

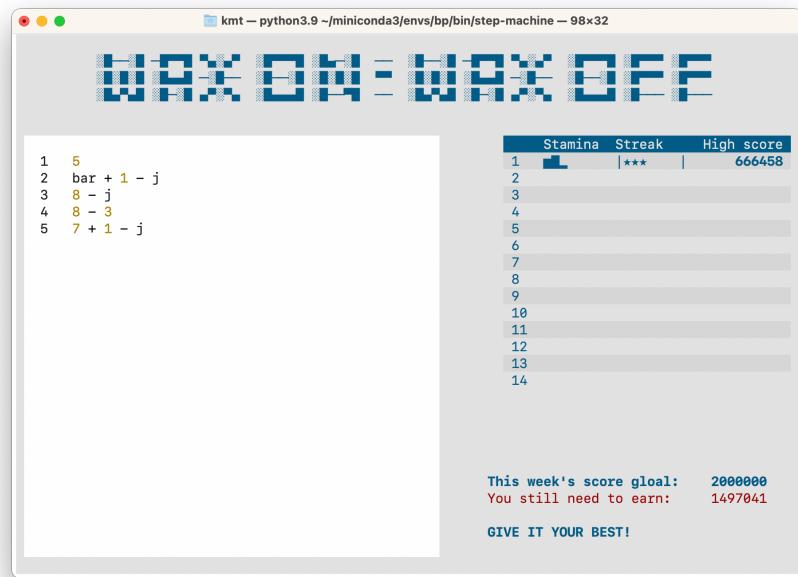


Figure 6.1: Visual Studio Code (VScode)

You do not know what values the y and x variables point to, but you can deduce it from the sequence of expressions that they are 24 and 13. In the game, you are given a series of numbered expressions in the wrong order like this:

1. $4 * y + x$
2. 37
3. $4 * 8 + x$
4. $24 + x$
5. $24 + 13$

Your task is to put them in the right order so that the original expression is at the top and the single Python value it reduces to is at the bottom. Now, you might grab line 2 by tabbing 2 on your keyboard (the number turns red so you can see it is active). Then, you move the line using the up/down arrow keys. If you move it to the bottom, the list then looks like this:

1. $4 * y + x$
2. $4 * 8 + x$
3. $24 + x$
4. $24 + 13$
5. 37

Now, you repeat this process until the order is correct (the program will let you know when it is). The fewer lines you grab to produce the right order, the more points you earn. Problems with longer lists of expressions also earn you more points. As problems become harder and include more aspects of Python, solving them also awards more points. Each week has a score goal to guide your effort. Reaching this goal ensures that you practice as much as you should. Practicing a bit every day daily is more effective than practicing a lot a few days a week. To provide an incentive, the points you earn slowly expire, so the easiest way to maintain your score is to practice a bit every day.

A helping hand

Using the `myagi`, you train your ability to read and understand Python expressions. Seeing a similar breakdown of a Python expression in your code may also be helpful. For that purpose, I have written another tool called `print-steps`. Say you have some code like the one below and need clarification on how the single value assigned to `z` is produced (here, you are probably not).

```
x = 7  
y = 5  
z = x * y + 4
```

All you need to do is then to add `# PRINT STEPS` comment to the end of the line like this:

```
x = 7  
y = 5  
z = x * y + 4 # PRINT STEPS
```

Say your file is called `myfile.py`, you would normally run the code like this:

Listing 6.1 Terminal

```
python myfile.py
```

But to see the breakdown of expressions marked by `# PRINT STEPS`, you need to run your code with the `print-steps` program instead:

The command prints the following in the terminal:

Listing 6.2 Terminal

```
print-steps myfile.py
```

```
Line 4 in test_studentfile.py:  
As written:      z = x * y + 4  
Substitution:    z = 7 * y + 4  
Substitution:    z = 7 * 5 + 4  
Reduction:       z = 35 + 4  
Reduction:       z = 39
```

You can even mark more than one line like this and have print-steps break down all of them for you:

```
x = 7  
y = 5  
z = x * y + 4 # PRINT STEPS  
k = z * 42 # PRINT STEPS
```

like this:

```
Line 3 in myfile.py:  
As written:      z = x * y + 4  
Substitution:    z = 7 * y + 4  
Substitution:    z = 7 * 5 + 4  
Reduction:       z = 35 + 4  
Reduction:       z = 39
```

```
Line 4 in myfile.py:  
As written:      k = z * 42  
Substitution:    k = 39 * 42  
Reduction:       k = 1638
```

However, it would be best if you used this helping hand sparingly. It is much better to train your ability to do this in your head with the help of Mr. Myagi. Trust me, it works.

7 Controlling behavior

This chapter is about how you make your program do different things under different circumstances. Making functionality dependent on data is what makes programs useful.

If-statement

So far, the small programs you have written run the same sequence of statements (lines). Imagine if you could control which statements were run depending on the circumstances. Then, you would be able to write more flexible and useful programs. Cue the music - and let me introduce the “if-statement”.

Write the following carefully into a file. It is a small program that monitors bus passenger status. Notice the colon ending the if-statements. Also, note that the lines below each if-statement are indented with precisely four spaces. While writing the program, figure out what the if-statement does. Then, run the code and see what happens.

```
bus_seats = 32
passengers = 20
bags = 20

print(passengers, "people ride the bus")

if bus_seats >= passengers + bags:
    print("Smiles, everyone has room for bags")

if bus_seats >= passengers:
    print('Everyone gets to sit down, no complaints')

if bus_seats < passengers:
    print('Some passengers standing, annoyed')

if bus_seats < passengers / 3:
    print("General dissatisfaction, some swearing too")
```

Try changing the values of bus_seats, passengers, and bags and see how the program executes.

You have probably realized that the if-statements control which prints statements that are evaluated. A statement nested under an if-statement is only evaluated if the expression between the if keyword and the : reduces to a value Python considers as *true*. This does not happen if the expression between the if and : reduces to a value Python considers *false*.

When asked to evaluate something as true or false, Python will interpret zero and empty values (like 0 and '') as False and all other non-zero and non-empty values as True.

Exercise 7-1

Which of the following letters are printed: A, B, C, D, E, F, G. Make up your mind before you write and run the code.

```
if 0:  
    print('A')  
  
if "Banana":  
    print('B')  
  
if 3.14159265359:  
    print('C')  
  
if False:  
    print('D')  
  
if 9 > 5 and 4 < 7:  
    print('E')  
  
if '':  
    print('F')  
  
if False or "banana":  
    print('G')
```

Exercise 7-2

What happens if you forget to write the : in the if-statement?

```
if 4 > 2  
    print('Hi!')
```

Exercise 7-3

What happens if you do not indent the code under the if-statement?

```
if 4 > 2:  
print('Hi!')
```



By now, you probably know that your text editor is intelligent regarding indentation. If you hit Enter after a statement ending with :, it will indent the next line with four spaces. Also, if you use the tab in Python code, it will produce four spaces for you.



FAQ

Q: Isn't "If" a poem by Rudyard Kipling?

A: Yes.

Else-statement

Sometimes you not only want your program to do something if an expression reduces to True, you also want it to do something *else* if it is False. It is as simple as it looks:

```
cookies = 3  
  
if cookies > 0:  
    print("Uh, I wonder if we have some milk too...")  
else:  
    print("Sigh!")
```

Remember to put a : after the else keyword. Write the code and change the value of cookies to 0.

Exercise 7-4

Test your understanding about which expressions that reduce to a True or False value. Write the code below and then see how it responds to different values of x. Try to come up with other variations yourself.

```

x = 0.0
# x = '0'
# x =
# x =
# x = not 0
# x = 'zero'

if x:
    print('x is substituted with True in the if-statement')
else:
    print('x is substituted with False in the if-statement')

```

FAQ

Q: Isn't "Else" a poem by Rudyard Kipling?

A: No.

Exercise 7-5

What do you think this code prints? Notice how you can nest if and else-statements under other if and else-statements. This way, you can make your program include only some statements when certain combinations of conditions are met. Just remember that the code below each if or else is indented by four spaces. Try to change the True/False values of milk and cookies.

```

milk = False
cookies = True
if milk:
    if cookies:
        status = 'Good times!'
    else:
        status = 'Not thirsty, thanks or asking'
else:
    if cookies:
        status = 'How does something like this happen?'
    else:
        status = 'Whatever...'

print(status)

```

Blocks of code

In the examples above, some lines are indented more than others, and you probably already have some idea of how Python interprets this. Indentation defines blocks of code. The `if` and `else` statements control each code block's evaluation when your code runs. The following three rules define individual blocks of code:

1. All statements in a code block have the *same indentation*. That is, they line up vertically.
2. A block of code *begins* at the first line of code at a line that is indented more than the one before it.
3. A block *ends* when it is followed by a less indented line or at the last line of code.

This way, a block can be nested inside another block by indenting it further to the right, as shown in Figure 7.1. Compare the example in Figure 7.1 to the code example above. Note how a colon at the end of a statement means “this applies the block of code below”. Make sure you understand which print statements are controlled by which `if` and `else` statements.

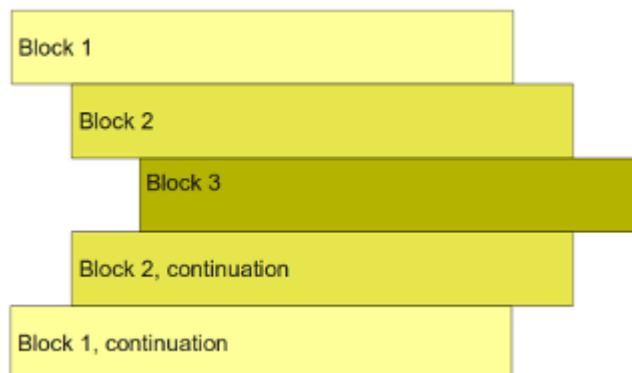


Figure 7.1: The amount of indentation defines blocks of code

Elif-statement

Say you need to test several mutually exclusive scenarios. For example, if a base is equal to A, T, C, or G. You can do that like in the example below, but it is very verbose and shifts your code further and further to the right.

```
base = 'G'  
  
if base == 'A':
```

```

    print('This is adenine')
else:
    if base == 'T':
        print('This is thymine')
    else:
        if base == 'C':
            print('This is cytosine')
        else:
            print('This is guanine')

```

This is where an `elif` statement can be helpful. It is basically short for “else if.” The correspondence is hopefully obvious if you compare it to the example below.

```

base = 'G'

if base == 'A':
    print('This is adenine')
elif base == 'T':
    print('This is thymine')
elif base == 'C':
    print('This is cytosine')
else:
    print('This is guanine')

```

Here, we put an `else`-statement at the end to capture all cases not covered by the `if`-statement and the two `elif`-statements.

Exercise 7-6

You can use logical operators (`and`, `or`, `not`) in the expressions tested in an `if`-statement. Can you change the program from exercise 7-5 so that there are no nested `if`-statements - in a way that the program still does the same? You can use `if`, `elif`, and `else` and test if, e.g., `milk` and `cookies` are true using `and`.

Exercise 7-7

The snippet of code below has three blocks with three statements. Which statements belong to which block? Which statements are executed?

```
x = 5
if x > 4:
    y = 3
    if x < 1:
        x = 2
        y = 7
        z = 1
    x = 1
z = 4
```

Exercise 7-8

Can you see four blocks of code? If not, read the three rules above again. Which statements are executed?

```
x = 5
if x > 4:
    y = 3
    if x < 1:
        x = 2
        y = 7
    else:
        x = 1
        y = 9
z = 4
```

General exercises

Exercise 7-9

Will this print You are a superstar!?

```
if -4 and 0 or 'banana' and not False:
    print("You are a super star!")
```

Exercise 7-10

Will this print You are a superstar!?

```
if -1 + 16 % 5 == 0 :  
    print("You are a super star!")
```

Exercise 7-11

Assign values to two variables, `x` and `y`. Then, write code that prints OK if (and only if) `x` is smaller than five *and* `y` is larger than five. Do it using *two* if statements:

```
x = 3 # or something else  
y = 7 # or something else  
  
# rest of code here...
```

Now solve the same problem using only *one* if statement.

Exercise 7-12

Assign values to two variables, `x` and `y`. Then, write some code that prints OK if and only if `x` is smaller than five *or* `y` is larger than five. Do it using *two* if statements:

```
x = 3 # or something else  
y = 7 # or something else  
  
# rest of code here...
```

Now solve the same problem using only *one* if statement and *one* elif statement.

Exercise 7-13

Assign values to two variables, `x` and `y`. Then, write some code that prints OK if either `x` or `y` is zero but not if both are zero (this is tricky).

```
x = 3 # or something else  
y = 7 # or something else  
  
# rest of code here...
```

Exercise 7-14

Which value of x makes the code below print Banana?

```
x =  
s = ''  
if x**2 == 16:  
    s = s + 'Ba'  
if x + 6 == 2:  
    s = s + 'na'  
if 7 == x - 3:  
    s = 'na' * 2  
else:  
    s = s + 'na'  
print(s)
```

Exercise 7-15

Make three exercises that require the knowledge of programming so far. Have your fellow students solve them.

8 Organizing code

This chapter is about organizing your code into chunks that you can call upon to perform well-defined tasks in your program.

Functions

Buckle down for the most powerful and useful thing in programming. Functions! Functions serve as mini-programs that perform small, well-defined tasks in your program.

I have started to write a song about functions:

```
print("Functions are super, Functions are cool")
print("When writing a program they are a great tool")
print("La la dim du da da di")
print("Skubi dubi dumdi di")
print("Bing di dubi dum da di")

print("Functions are used to package some code")
print("They are not so strange that your head will explode")
print("La la dim du da da di")
print("Skubi dubi dumdi di")
print("Bing di dubi dum da di")
```

I will add many more verses, and I do not want to write the entire chorus every time. So what would be more natural than to make a function named `chorus` that takes care of that for us? That way, we can write our song the way lyrics with a chorus are usually written:

```
def chorus():
    line1 = "La la dim du da da di"
    line2 = "Skubi dubi dumdi di"
    line3 = "Bing di dubi dum da di"
    return line1 + '\n' + line2 + '\n' + line3

print("Functions are super, Functions are cool")
```

```

print("When writing a program they are a great tool")
print( chorus() )

print("Functions are useful to wrap up some code")
print("They are not so strange that your head will explode")
print( chorus() )

```

First, let us break down the function definition in the top part of this code:

1. We *define* a function with the `def` keyword (which is short for “define” in case you wonder).
2. After `def`, we write the function `chorus`. We could name it something else, but like good variable names, good function names can help you remember what your code does.
3. After the name, you put two parentheses, `()`.
4. Then a colon, `:`.
5. The statements that are part of the function are nested under the `def` statement and are indented with four spaces exactly like we do under `if`-statements.
6. The `return` statement ends the function. After the `return` keyword, the expression reduces to a *value* that the function returns.

When Python runs this code, each line is executed one by one, starting from the first line (remember oath two?). So, in this case, python first executes the *definition* of the `chorus` function. The only thing that happened after Python had executed the first five lines of code was that it assigned the name `chorus` to the four indented statements. So Python now “knows” about the `chorus` function (like it “knows” about a variable `x` after we do `x = 4`).

To *use* the function, we “call” it by writing its name followed by parentheses: `chorus()`. When it comes to functions, “use”, “call” and “run” means the same thing. As you can see, we call the function twice in the rest of the code. Each time we do, the following happens:

1. When a function is *called*, each statement in the definition is executed one after the other. If you look at the function definition, you can see that our `chorus` function has four statements.
2. The first statement assigns a string value to the `line1` variable.
3. The second statement assigns a string value to the `line2` variable.
4. The third statement assigns a string value to the `line3` variable.
5. The fourth statement is a `return` statement. The expression after the `return` keyword in the final statement is reduced to a *value*, and this value is substituted for the function call. In this case, that value is the following string:

"La la dim du da da di\nSkubi dubi dumdi di\nBing di dubi dum da di"

So, the key properties of functions are:

- A function names a piece of code (some statements) just like variables name values like strings and numbers.
- We call a function by writing the function name followed by parentheses: `chorus()`. Just writing the function name will not call the function.
- When a function is *called*, it is substituted by the *value* that the function *returns* – exactly like a variable in an expression is substituted by its value. It is crucial that you remember this.

Exercise 8-1

Now that we have a chorus function, that part is out of the way, and we can concentrate on our song without worrying about remembering how many "la la"s it has and so on. Try to change the "lyrics" in the chorus a little bit. Notice how you only need to make the change in one place to change all the choruses in the song – cool, right? Without the function, you would have to rely on correctly changing the code in many different places.

Exercise 8-2

Try to delete the return statement in the chorus function (the last line in the function) and run the code again. You should see something like this:

```
Functions are super, Functions are cool
When writing a program they are a great tool
None
Functions are used to wrap up some code
They are not so strange that your head will explode
None
```

The function call (`chorus()`) is now substituted with `None`. How can that be when we did not return anything? The reason is that when you do not specify a return statement, the function returns `None` by default. This is to honour the rule that variables and a function calls are substituted by a value, and `None` is simply the value that Python uses to represent "nothing". `None` is a value denoting the lack of value. As you just saw, it represents that no value is returned from a function. It can also be assigned to a variable as a placeholder value until another value is assigned:

```
x = None  
x = 4
```

Also, `None` is considered false in a logical context:

```
print(not None)
```

Exercise 8-3

Try this variant to the `chorus` function. Go through the code *slowly* and repeat all the steps to break down what happens when a function is called. Remember also to do each substitution and reduction carefully.

```
def chorus():  
    line1 = 'La la'  
    line2 = 'Du bi du'  
    return line1 + '\n' + line2
```

Do the same for this variant:

```
def chorus():  
    line1 = 'La la'  
    line2 = 'Du bi du'  
    chrous_text = line1 + '\n' + line2  
    return chrous_text
```

and for this variant:

```
def chorus():  
    return "La la\nDu bi du"
```

Exercise 8-4

What do you think happens if you move the definition of `chorus` to the bottom of your file? Decide what you think will happen and why (maybe you remember what happens when you try to use a variable in an expression before defining it?). Then try it out.

```

print("Functions are super, Functions are cool")
print("When writing a program they are a great tool")
print(chorus())

print("Functions are useful to wrap up some code")
print("They are not so strange that your head will explode")
print(chorus())

def chorus():
    line1 = "La la dim du da da di"
    line2 = "Skubi dubi dumdi di"
    line3 = "Bing di dubi dum da di"
    return line1 + '\n' + line2 + '\n' + line3

```

Ensure you understand how the error you get relates to how Python runs your script (remember oath two?). If you still need help understanding, do the next exercise and then return to this one.

Exercise 8-5

Which error do you get here, and why? How is that similar to the error in the previous exercise?

```

print(x)
x = 7

```

Exercise 8-6

Consider the code below. Do all the substitution and reduction steps in your head. Remember that each function call is substituted by the value that the function returns. Then run it.

```

def lucky_number():
    return 7

x = lucky_number()
y = lucky_number()
twice_as_lucky = x + y
print(twice_as_lucky)

```

Now, change the code to that below. The code makes the same computation but in fewer steps. Do all the substitution and reduction steps.

```
def lucky_number():
    return 7

twice_as_lucky = lucky_number() + lucky_number()
print(twice_as_lucky)
```

Now, change the code to the one below. The code makes the exact computation but in fewer steps. Do all the substitution and reduction steps.

```
def lucky_number():
    return 7

print(lucky_number() + lucky_number())
```

Functions can take arguments

The functions we have written so far are not very flexible because they return the same thing every time they are called. Now write and run this beauty:

```
def square(number):
    squared_number = number**2
    return squared_number

result = square(3)
print(result)
```

Notice how we put a variable (`number`) between parentheses in the function *definition*. This variable is assigned the value we put between the parentheses (3) when we *call* the function. So when we call like that (`square(3)`), Python automatically makes the assignment `number = 3`.

Here is another example:

```
def divide(numerator, denominator):
    result = numerator / denominator
    return result

division_result = divide(44, 77)
print(division_result)
```

When the function call `divide(44, 77)`, these two things implicitly happen: `numerator = 44` and `denominator = 77`.

Take note of the following three important points: 1. The *values* that we pass to the function in the function call (like 3, 44, and 77) are called *arguments*. It is crucial to remember that it is *values* and not variables that are passed to functions. 2. The *variables* in the definition line of a function, like `number`, `numerator` and `denominator`, are called *parameters*. They hold the *values* passed to the function when it is called (the arguments). 3. You can define functions with any number of *parameters* if you use the same number of *arguments* when you call the function.

Exercise 8-7

Try to call your `divide` function like this `divide(77, 44)`. What does it return, and what do you learn from it? Does the order of arguments and parameters correspond?

Exercise 8-8

Try to call your `divide` function like this `divide(44)`. Do you get an error, and what do you learn from that?

Exercise 8-9

Try to call your `divide` function like this `divide(44, 77, 33)`. Do you get a different error message, and what do you learn from that?

Exercise 8-10

Read this code and do all substitution and reduction steps from beginning to end.

```
def square(x):
    return x ** 2

result = square(9) + square(5)
print("The result is:", result)
```

Now replace the line `return x ** 2` with `print(x ** 2)`. What is printed now and why?

Exercise 8-11

As described above, a `return` statement ends the function by producing the value that replaces the function call. If a function has more than one `return` statement, then the function ends when the first one is executed.

```
def assess_number(x):
    if x < 3:
        return 'quite a few'
    if x < 100:
        return 'a lot'
    return 'a whole lot'

nr_apples = 2
print(nr_apples, "apples is", assess_number(nr_apples))
```

What happens when `x` is 2, 3, 50, 200? Think about it first.

Functions and variables

A function call is the temporary little world that only exists when the function is *called* and until it *returns* its value. It did not exist *before* the function was called, and it does not exist *after* the function returns its value. By necessity, the variables defined in your function are also temporary.

This means variables defined inside a function are private to each function call. It also means that variables defined inside functions are unavailable to code *outside* the function. Running the following example should help you understand this:

```
def make_greeting():
    greeting = 'Guten tag'
    name = 'Heinz'
    message = greeting + " " + name
    return message

greeting = 'Buongiorno'
name = 'Giovanni'
print( make_greeting() )
print(greeting + " " + name)
```

Notice how Heinz and Giovanni are greeted in their native languages. This means that the variable definitions inside the function do not overwrite the Italian versions already

defined outside the function. This is because the variables defined in the function are temporary and private to the function, even if they have the same names as variables outside the function. This is why the function call `make_greeting()` in the `print` statement does not change the variables' values printed in the last line.

Now try to “comment out” the line `greeting = 'Guten tag'` and run the example again. All of a sudden, Heinz is greeted in Italian! The reason is that now Python cannot find a definition of `greeting` inside the function. It then looks outside the function for a definition and finds the Italian version.

Now try to “comment out” the line `greeting = 'Buongiorno'` and run the example again. You get an error, but which one? Python complains that it cannot find a definition of `greeting`. The reason is that once the last `print` statement is executed, the small world of the function call in the previous line no longer exists.

You should learn two rules from the above example:

1. All variables you define inside a function are *private* to the function. If a variable in a function has the same name as a variable in the main script (like `greeting` above), then these are two *separate* variables that just happen to have the same name.
2. When you use a variable like `greeting` in the function (E.g., `message = greeting + " " + name`), Python checks if the variables have been defined in the function. If that is not the case, then it will look for it outside the function. In the above example, `name` is found in the function, and `greeting` is found outside the function. It is good practice to make your functions “self-contained” in the sense that Python should not have to look outside the function for variables.

Exercise 8-12

Try this version of the example above. Now, `name` is defined as a function parameter, but it is still a function variable, just like `greeting`.

```
def make_greeting(name):
    greeting = "Guten tag"
    message = greeting + " " + name
    return message

greeting = 'Buongiorno'
name = 'Giovanni'
print( make_greeting("Heinz") )
print(greeting + " " + name)
```

Exercise 8-13

Consider the following example:

```
def double(z):
    return z * 2

x = 7
result = double(x)
print(result)
```

When the function is called (`double(x)`), the `x` is substituted by its value 7. That *value* is passed as the argument and assigned to the function parameter `z` (`z = 7`). `z` is a private function variable and does not exist before or after the function call. Does this change in any way if we use the variable name `x` instead of `z`, as shown below?

```
def double(x):
    return x * 2

x = 7
result = double(x)
print(result)
```

Do *all* substitutions and reductions for each line of code from top to bottom. Keep the sequence of events in mind and remember that a function definition is merely a template describing a mini-world that is created anew every time the function is called.

Builtin functions

So far we have only talked about functions you write yourself, but Python also has built-in functions that are already available to you. They work just like a function you would write yourself. You already know the `print` function quite well; that is an example of a function that prints something but returns `None`. There are many other useful built-in functions, but I will tell you about another two: `len` and `type`.

Exercise 8-14

Try these examples:

```
x = 'Banana'  
print("The value of variable x is of type", type(x))  
print("The value of variable x has length", len(x))
```

As you can see, `type` returns the type of the value passed as the argument, and `len` returns the length of the value passed as the argument. The `type` function is handy if you wonder what type a value has, but it is not a function we will use in this course. The `len` function, however, is your new best friend. You will see why soon enough.

Exercise 8-15

Try to change the value of the `x` in exercise 8-14 to an integer or a float and see what happens when you run it. Do you get an error? Does it make sense that not all types of values can meaningfully be said to have a length?

Exercise 8-16

What happens if you pass an empty string ("") as the argument to the `len` function?

Exercise 8-17

What is printed here? Think about it first, and then try it out. Remember to do the substitution and reduction steps.

```
return_value = print("Hello world")  
print(return_value)
```

Exercise 8-18

What is printed here? Think about it first and then try it out. Remember to do the substitution and reduction steps.

```
print(print("Hello world"))
```

General exercises

The following exercises treat the areas we have worked on in this and previous chapters. They are meant to train your familiarity with if-statements and functions. Remember that the purpose of the exercises is not to answer the questions but to train the chain of thought that *allows* you to answer them. Play around with the code for each example and see what happens if you change it a bit.

Exercise 8-19

Consider this function definition that takes a single number as the argument:

```
def square(n):
    return n**2
```

What does it do? What does it return? What number does `square(2)` then represent?

Below, I have used it in some printed expressions. Make sure you understand what each expression evaluates to. Make the explicit substitutions and reductions on paper before you run it. Remember that we substitute a function call (like `square(2)`) for the value it returns, just like a variable `x` substitutes for the value it points to.

```
print(square(3))
print(square(2 + 1))
print(square(2) * 2 + square(3))
print(square(square(2)))
print(square(2 * square(1) + 2))
```

Exercise 8-20

What does this function do? How many parameters does it have? How many statements does the function have? What does the function print? Which value does it return?

```
def power(a, b):
    print("This function computes {}**{}".format(a, b))
    return a**b

print(power(4, 2))
```

Try (possibly strange) variations of the code like the ones below better to understand the contribution of each line of code. What is the difference between return and print? What happens when Python gets to a return statement in a function? What happens when the function does not have a return statement?

Variation 1:

```
def power(a, b):
    print("This computes", a, "to the power of", b)
    print(a**b)

result = power(4, 2)
print(result)
```

Variation 2:

```
def power(a, b):
    print("This computes", a, "to the power of", b)
    return a**b

result = power(4, 2)
print(result)
```

Variation 3:

```
def power(a, b):
    print("This computes", a, "to the power of", b)
    a**b

print(power(4, 2))
```

Variation 4:

```
def power(a, b):
    return a**b
    print("This computes", a, "to the power of", b)

print(power(4, 2))
```

Exercise 8-21

Define a function called `diff`, with two parameters, `x` and `y`. The function must return the difference between the values of `x` and `y`.

Example:

```
def diff(x, y):
    ...
diff(8, 2) # should return 6
diff(-1, 2) # should return -3
```

Save the value returned from the function in a variable. Then, test if the function works correctly by comparing the result to what you know is the true difference (using `==`).

Exercise 8-22

Define a function called `all_equal` that takes five arguments and returns `True` if all five arguments have the same value and `False` otherwise. The function should work with any input, for example:

```
all_equal("Can", "Can", "Can", "Can", "Can")
all_equal(0, 0, 0, 0, 0)
all_equal(0.5, 0.5, 0.5, 0.5, 0.5)
all_equal(True, True, True, True, True)
```

Hint: You test equality with `a == b`. Now, think back to what you learned about logic. Which operator can you use to test if `a == b and b == c`?

Exercise 8-23

Define a function called `is_even`, which takes one argument and returns `True` if (and only if) this is an even number and `False` otherwise (remember the modulo operator?).

```
is_even(8) # should return True
is_even(3) # should return False
```

Exercise 8-24

Define a function called `is_odd`, which takes one argument and returns True if (and only if) the argument is an odd number and False otherwise.

```
is_odd(8) # should return False  
is_odd(3) # should return True
```

Can you complete this exercise using the `is_even` you defined in exercise 8-23? How? Why is that a good idea?

Exercise 8-25

Here is a function that should return True if given an uppercase (English) vowel and False otherwise:

```
def is_uppercase_vowel(c):  
    c == 'A' or c == 'E' or c == I or c == 'O' or c == 'U'  
  
char = 'A'  
if is_uppercase_vowel(char):  
    print(char, "is an uppercase vowel")  
else:  
    print(char, "is NOT an uppercase vowel")
```

Now you can just type the code exactly as shown and run it. Do you get what you expect? Does the code work? If not, try to figure out why. Try to print the value that the function returns. Do you know if that gives you any hints about the cause of the problem?

Exercise 8-26

Define a function called `is_nucleotide_symbol`, which takes one argument and returns True if this is either A, C, G, T, a, c, g or t, and False in any other case.

Name your parameter something sensible like `symbol`.

```
is_nucleotide_symbol("A") # should return True  
is_nucleotide_symbol("B") # should return False  
is_nucleotide_symbol("Mogens") # should return False  
is_nucleotide_symbol("") # should return False
```

Exercise 8-27

Define a function called `is_base_pair` which takes two parameters, `base1`, `base2`, and returns `True` if `base2` is the complementary of `base1`, and `False` otherwise.

```
is_base_pair("A", "G") # should return False  
is_base_pair("A", "T") # should return True  
is_base_pair("T", "A") # should return True  
is_base_pair("Preben", "A") # should return False
```

Exercise 8-28

Did you find the bug in exercise 8-25? You were supposed to find that the function did not have a return value. This makes the function return `None` by default. Do you think the `None` value is considered true or false in an if-statement?

Exercise 8-29

Define a function called `celcius2fahrenheit` that converts from celsius to Fahrenheit. You can do this because you know the linear relationship between the two. On Figure 8.1 you can see that the slope is $9 / 5$ and the intercept is 32. The function should have one parameter `celsius`. Inside the function, you should define the variables `slope` and `intercept` and give them the appropriate values. Then you can calculate the conversion to Celcius using these variables and return the result.

Exercise 8-30

Try to change your conversion function so it takes three arguments, corresponding to `celsius`, `slope` and `intercept` so you can call it like this to convert 27 degrees celsius: `conversion(37, 9 / 5, 32)`. Now you have a function that can do any linear conversion that you can put inside another function like this:

```
def celcius2fahrenheit(celsius):  
    return conversion(celsius, 9 / 5, 32)
```

Exercise 8-31

Now try to extend this to a different problem: It has been found that the height and weight of a person are related by a linear equation with $\text{slope} = 0.55$ and $\text{intercept} = -25$. Define a function called `predict_weight` which takes just one argument, the height of a person, and returns the estimated weight of the person.

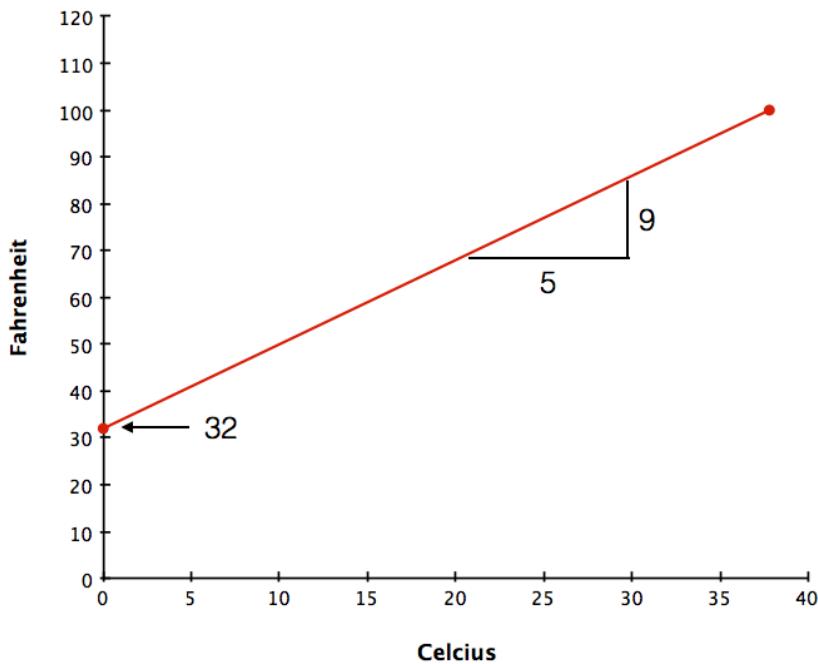


Figure 8.1: Temperature conversion

Exercise 8-32

By now you know that some of the words in your code have specific purposes. `def` defines functions, `return` returns value from a function, and `is` is a logical operator etc. Here is a list of the ones you will see in this course: `and`, `assert`, `break`, `continue`, `def`, `del`, `elif`, `else`, `False`, `for`, `from`, `if`, `import`, `in`, `is`, `not`, `or`, `pass`, `return`, `while`, `True`, `None` (you can see a full list [here](#))

These words are reserved for their special purposes in Python and you will not be allowed to assign values to them. Try this to see for yourself:

`None = 4`

or this:

`and = 2`

FAQ - Frequently Asked Questions

Q: Can function names be anything?

A: Just about. The rules that apply to variable names also apply to function names.

Good function names are lower case with underscores (_) to separate words, like in the examples above.

9 Values are objects

This chapter introduces the notion of an object, one of the most central aspects of Python. Once you catch your breath, you will love that all Python values are objects.

Methods

In Python, a value like an integer or string not only holds data. It is also packaged with a lot of useful functionality relevant to the particular type of value. When a value is packaged with such relevant functionality and meta information, programmers call it an object—and in Python, all values are objects.

The associated functionality comes in the form of *methods*. You can think of methods as functions that are packaged together with the value. For example, string values have a method called `capitalize`. Try it out:

```
x = "banana".capitalize()  
print(x)
```

To call the method on the string value, you connect the string value and the method call with a dot. So, to call a method on a value, you do the following:

1. Write the value (or a variable name that substitutes for a value).
2. Then write a ..
3. Then write the name of the method (like `capitalize`).
4. Then, write two parentheses to call the method. If the method takes any arguments other than the value it belongs to, then you write those additional values between the parentheses with commas in between, just as when you call a function.

You can see that the method call looks just like a function call, and in many ways, calling a method works much like calling a function. The difference is that when we call a function, we say: “*Hey function, capitalize this string!*”. When we call a method, we say: “*Hey string, capitalize yourself!*”

So why do we need methods? Why do we need them when we have functions? It is very handy to have some relevant and ready-to-use functionality packaged together with the data it works on. You will start to appreciate that sooner than you think.

Methods are almost always used with variables. So remember to make any substitutions and reductions required. When we put a method call after a *variable* like below, the variable is first substituted for its value, and *then* the method is called on the value. Consider the second line of this example:

```
x = "banana"  
print(x.capitalize())
```

Here, x is first substituted by "banana" and *then* the method is called on that value, like this: "banana".capitalize().

Now write and run these examples:

```
message = "Methods Are Cool"  
print(message)  
  
shout = message.upper()  
print(shout)  
  
whisper = message.lower()  
print(whisper)  
  
new_message = message.replace("Cool", "Fantastic")  
print(new_message)
```

You can see what these methods do. For example: upper returns an uppercased copy of the string.

Exercise 9-1

Write and run the following code. What do you think it does?

```
line = '\n\tSome text\n'  
print(">{}<".format(line))  
  
line = line.strip()  
print(">{}<".format(line))
```

Make sure you do the substitution and reduction steps in your head. Be especially careful about the third line of code. Also, what do you think the special \t character is?

Exercise 9-2

The string methods you have tried so far have all returned a new string. Try this example:

```
'ATGACGCGGA'.startswith('ATG')
```

and this

```
'ATGACGCGGA'.endswith('ATG')
```

What do the methods do, and what do they return?

Using the Python documentation

Now that you are well underway to becoming a programmer, you should know your way around the Python documentation. Especially the part called the Python standard library. There is a *lot* of details in there that we do not cover in this course. These are mainly tools and techniques for writing more efficient, extensible, robust, and flexible code. The parts we cover in this course are the minimal set that will allow you to write a program that can do *anything*.

Exercise 9-3

There is a string method that returns a secret agent:

```
print('7'.zfill(3))
```

You can look it up in the Python documentation.

Exercise 9-4

Browse through all the string methods to get an impression of all the functionality that is packaged with string objects.

String formatting

You have already tried string formatting in exercise 9-1. String formatting is a simple but powerful technique that lets you generate pretty strings from pre-computed values. You may have noticed that many decimals are shown every time we print a floating-point number. It is not very pretty if you are only interested in two decimals. You use the `format` method (surprise) to format a string. In its most straightforward use, `format` replaces occurrences of `{}` with the arguments that are passed to it - like this:

```
taxon = "genus:{} , species:{}".format('Homo' , 'sapiens')
```

Exercise 9-5

What happens if you try this?

```
question = "Was {} {} Swedish?".format('Carl' , 'Linneaus')
```

and this?

```
question = "Was {} Swedish?".format('Carl' , 'Linneaus')
```

and this?

```
question = "Was {} {} Swedish?".format('Carl Linneaus')
```

In the two last examples, the number of `{}` did not match the number of arguments to `format`. What happens when there are too few and when there are too many?

Exercise 9-6

Consider this code:

```
s = "{} is larger than {}".format(4, 3)
print(s)
```

What will happen if you run this code? Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise 9-7

Consider this code:

```
language = 'Python'
invention = 'sliced bread'
s = '{} is the best thing since {}'.format(language, invention)
print(s)
```

What will happen if you run this code? Write the code and see for yourself once you think you know. If you were wrong, make sure you understand why.

Exercise 9-8

Consider this code:

```
my_template = '{} is the best thing since {}'
language = 'Python'
print(my_template.format(language, 'sliced bread'))
print(my_template.format(language, 1900 + 89))
```

What will happen if you run this code? Do the substitution and reduction steps in your head.

Exercise 9-9

Think back to exercise 4-3, where you calculated how many cookies you could buy for 30 kr. The bars are 7 kr. So your program looked something like this:

```
nr_bars = 30 / 7
print('I can buy', nr_bars, 'chocolate bars!')
```

and it ran like this: python chocolate.py

I can buy 4.285714285714286 chocolate bars!

String formatting lets you rewrite the program like this:

```
nr_bars = 30 / 7
message = "I can buy {} chocolate bars!".format(nr_bars)
print(message)
```

Try to replace {} with {:.2f}. format reads the stuff after the colon in each set of curly brackets and uses it as directions for formatting the value it inserts. Try it out and see what happens if you change the number 2 to 3, 4, 5 or 10.

Exercise 9-10

See if you can find the documentation for the format function in the Python documentation. It can do wondrous things, for this course we will only try to control the number of digits and padding with spaces. Look at the examples below. Maybe you can figure out how it works.

```
pi = 3.14159265359
print("*{}*".format(pi))
print("*{:3f}*".format(pi))
print("*{:6f}*".format(pi))
print("*{:>5.3f}*".format(pi))
print("*{:>10.3f}*".format(pi))
```

Exercise 9-11

This is bonus info rather than an actual exercise. How do you think Python can figure out that adding strings is supposed to work differently than adding numbers? Remember that '1' + '2' is '12' not 3. The answer is that all values you can add with the + operator have a secret method called `__add__` that defines how adding works for that type of value:

```
s1 = "11"
s2 = "22"
n1 = 11
n2 = 22
print(s1 + s2)
print(s1.__add__(s2))
print(n1 + n2)
print(n1.__add__(n2))
```

This is one of many examples of how objects allow Python to implement functionality that fits each value type. This was to show how Python does this. Like yellow and black

stripes in nature means “don’t touch me!” – double underscores (`__`) is Python’s way of saying “do not use this!”. You are supposed to use the `+` operator, not the `__add__` method.

Indexing and slicing strings

Another feature of string objects is that they allow you to extract individual parts of the string.

Each character in a string is identified by its *index* in the string. To access a character in a list, you write brackets after the string. Between those brackets, you specify the index of the character you want. The first character has an index of 0; the second has an index of 1, and so on.

```
codon = 'ATG'  
print("first base is", codon[0])  
print("second base is", codon[1])  
print("third base is", codon[2])
```

You may wonder why the index of the first character is zero and not one. That is simply the convention in programming and is so for good reason. Over time you will begin to find this useful rather than annoying. You should think of the index as the offset from the start of the string.

That also means that the index is not the length of the string but the length minus one:

```
amino_acids = 'ARNDCQEGHILKMFPSTWYV'  
last_index = len(amino_acids)-1  
print("Last amino acid is", amino_acids[last_index])
```

If you want a sub-string from a larger string (we call that a *slice*), you specify a start index and an end index separated by a colon:

```
print(amino_acids[1:4])
```

When you run that, you can see that `amino_acids[1:4]` is substituted for 'RND', so the slicing operation produces a sub-string of `amino_acids`. You may wonder why the value at index 4 is not in the resulting sub-string. That is another programming convention: intervals are *ends exclusive*. So when you specify an interval with a start index of 1 and an end index of 4, it represents all the characters starting from 1 and up to, *but not including*, 4. So, the slice `1:4` corresponds to the characters at indexes 1, 2, and 3. The reason programmers handle intervals in this way is that it makes it easier to write clear and simple code as you will see in the exercises.

Exercise 9-12

What does this expression reduce to?

"Futterwacken"[**7**]

Exercise 9-13

What is printed here? Do all the substitution and reduction steps and compare to the exercise above.

```
s = "Futterwacken"  
print(s[7])
```

Exercise 9-14

What is printed here? Do all the substitution and reduction steps – and do it *twice*. Next week you will be happy you did.

```
dna = 'TGAC'  
i = 0  
print(dna[i])  
i = 1  
print(dna[i])  
i = 2  
print(dna[i])
```

Exercise 9-15

What do you think happens here? Make up your mind and try out the code below:

```
s = "Futterwacken"  
s[6] = 'W'
```

Did you see that coming? Strings are *immutable*, which means that you cannot change them once you have made them. If you want "FutterWacken" you need to produce a new string with that value. Try to figure out how to do that with the `replace` method of strings.

Exercise 9-16

When you do not specify a slice's start and/or end, Python will assume sensible defaults for the start and end indexes. What do you think they are? Make up your mind and try out the code below:

```
s = 'abcdefghijklmnopqrstuvwxyz'  
print(s[:11])  
print(s[11:])  
print(s[:])
```

Exercise 9-17

Find the documentation for how the slicing of strings works.

Exercise 9-18

What do you think happens when you specify an index that does not correspond to a value in the list:

```
alphabet = 'abcdefghijklmnopqrstuvwxyz'  
print(alphabet[99])
```

Read and make sure you understand the error message. You can try to Google the error message.

Exercise 9-19

Do you think you also get an error when you specify a slice where the end is too high? Try it out:

```
alphabet = 'abcdefghijklmnopqrstuvwxyz'  
print(alphabet[13:99])
```

and this:

```
alphabet = 'abcdefghijklmnopqrstuvwxyz'  
print(alphabet[10000:10007])
```

I guess that is worth remembering.

Exercise 9-20

Which character in a string named alphabet does this expression reduce to?

```
alphabet[len(alphabet)-1]
```

Exercise 9-21

Because intervals are “ends exclusive”, we can compute the length of a slice as end - start:

```
dna = "ATGAGGTCAAG"  
start = 1  
end = 4  
print("{} has length {}".format(dna[start:end], end-start))
```

Figure out what this code would look like if ends were included in intervals.

Exercise 9-22

Another advantage of “ends exclusive” intervals is that you only need one index to split a string in two:

```
s = 'Banana'  
idx = 3  
beginning = s[:idx]  
end = s[idx:]  
print(beginning + end)
```

Figure out what indexes you would need to use to split a sequence in two if ends were included in intervals.

Exercise 9-23

Did you look up the details of how slicing works in exercise 9-17? Then you should be able to explain what happens here:

```
s = 'zyxvutsrqponmlkjihgfedcba'  
print(s[::-1])
```

General exercises

Exercise 9-24

Will this print `Bananas rule!`? Do all the substitutions and reductions.

```
if 'na' * 2 == "Banana"[2:]:  
    print("Bananas rule!")
```

Exercise 9-25

Will this print `Bananas rule!`? Do all the substitutions and reductions.

```
if "{}s".format('Banana')[1:].capitalize() == 'Ananas':  
    print("Bananas rule!")
```

Exercise 9-26

Write a function called `even_string` that takes a string argument and returns True if the length of the string is an even number and False otherwise. E.g. `even_string('Pear')` should return True and `even_string('Apple')` should return False (remember the modulo operator?).

Exercise 9-27

Look at the code below and decide what is printed at the end. Then, write the code and test your prediction. If you are wrong, figure out why by revisiting the chapter about functions.

```
def enigma(x):  
    if x == 4:  
        return x  
  
result = enigma(5)  
print(result)
```

Exercise 9-28

Inspect the code below and determine why it does not print that you are a super star. Test the function using various inputs and identify the mistake.

```
def even_number(x):
    if x % 2:
        return False

if even_number(4):
    print('You are a super star!')
```

10 Lists of things

This chapter is about lists and dictionaries that are Python values that can contain other Python values. Lists and dictionaries let you build relationships between values, which is what data structures represent.

Lists

For many kinds of data, the order of things is important. Just like the order of characters is important for the meaning of the text in a string, we sometimes want to specify the order of other things because the relative order of items in the list has some meaning. It could be a grocery list where you have listed the things to buy in the order you get to them in the supermarket. This is where Python lists are helpful. When you print a list, it nicely shows all the values it contains.

```
grocery_list = ["salad", "canned beans", "milk", 'beer', 'candy']  
print(grocery_list)
```

Unlike strings that can only store the order of characters, lists can contain *any* kind of values, and you can mix different types of values in any way you like. Here is a list that contains an integer, a boolean, a string, and a list:

```
mixed_list = [42, True, 'programming', [1, 2, 3] ]
```

By now, you have probably guessed you will make a list with two square brackets. Between them, you can put values with commas in between. A list is a *container* of other values, and the value of the list itself does not depend on the values it contains. This makes sense. Otherwise, an empty list would not have a value:

```
my_list = []
```

You can add single values to the end of a list using the `append` method of lists. Try it out:

```
desserts = []
print(desserts)
desserts.append('Crepe suzette')
print(desserts)
desserts.append('Tiramisu')
print(desserts)
desserts.append('Creme brulee')
print(desserts)
```

If you have a list you want to add to the end of another list, you use the `extend` method:

```
cheeses = ['Gorgonzola', 'Emmentaler', 'Camembert']
desserts.extend(cheeses)
print(desserts)
```

Notice how `append` and `extend` *modifies* the existing list instead of producing a new list with the added element.

Exercise 10-1

Do you think this will work?

```
cheeses = ['Gorgonzola',
           'Emmentaler',
           'Camembert']
print(cheeses)
```

Surprised? Code inside parentheses, brackets, and braces can span several lines, sometimes making your code easier to read.

Exercise 10-2

You use the 'in' operator to test if a value is in a list. Try this:

```
print('Tiramisu' in desserts)
print('Meatloaf' in desserts)
```

Exercise 10-3

You can concatenate two lists to produce a new joined list. Make sure you figure out how this works before you try it. Then, experiment with changing the lists. Can you concatenate two empty lists?

```
some_list = [1, 2, 3]
another_list = [7, 8, 9]
merged_list = some_list + another_list
print(merged_list)
```

This is yet another example of how the functionality of Python objects lets them “know” how to behave under different circumstances, such as when adding two objects (see exercise 9-11).

Exercise 10-4

What do you think is printed here? Make sure you figure out how you think this works before you try it out. What does the append method return?

```
my_list = []
x = my_list.append(7)
print(x)
print(my_list)
```

Indexing and slicing lists

Now you know how to make lists, but to work with the values in lists, you must also know how to access the individual values a list contains. Fortunately, indexing lists work just like indexing strings: Each value in a list is identified by an *index* exactly like each character in a string:

```
numbers = [7, 4, 6, 2, 8, 1]
print("first value is", numbers[0])
print("second value is", numbers[1])
print("third value is", numbers[2])
```

Notice that the function `len` can also compute the length of a list. So you also get the last value in a list like this:

```
numbers = [7, 4, 6, 2, 8, 1]
last_index = len(numbers)-1
print("Last element is", numbers[last_index])
```

If you want a sub-list of values from a list (we also call that a *slice*), you specify a start index and an end index separated by a colon, just like with strings:

```
print(numbers[1:4])
```

When you run that, you can see that `numbers[1:4]` is substituted for `[4, 6, 2]`, so the slicing operation produces a new list of the specified values.

Exercise 10-5

What do these two expressions reduce to?

```
[11, 12, 13, 14, 15, 16, 17][2]
```

Exercise 10-6

What is printed here? Do all the substitution and reduction steps and compare them to the exercise above.

```
l = [11, 12, 13, 14, 15, 16, 17]
print(l[2])
```

Exercise 10-7

What is printed here? Do all the substitution and reduction steps — and do them twice. Next week, you will be happy you did.

```
numbers = [1,2,3]
i = 0
print(number[i])
i = 1
print(number[i])
i = 2
print(number[i])
```

Exercise 10-8

What do you think happens here? Make up your mind and try out the code below:

```
l = [11, 12, 13, 14, 15, 16, 17]
l[4] = "Donald"
print(l)
```

Were you surprised by what happened? Compare to exercise 9-15. Lists are not immutable like strings, and you can replace values by assigning a new value to an index in the list.

Exercise 10-9

With your knowledge of slicing, what do you think is printed below:

```
l = [11, 12, 13, 14, 15, 16, 17]
print(l[:3])
print(l[3:])
print(l[:])
```

Exercise 10-10

What do you think happens when you specify an index that does not correspond to a value in the list:

```
l = [11, 12, 13, 14, 15, 16, 17]
print(l[7])
```

Read and understand the error message. Does it ring a bell?

Exercise 10-11

Do you also get an error when you specify a slice where the end is too high? Try it out:

```
l = [11, 12, 13, 14, 15, 16, 17]
print(l[4:99])
```

I guess that is also worth remembering.

Exercise 10-12

Which value in a list named `l` does this expression reduce to?

```
l[len(l)-1]
```

Exercise 10-13

If you do not like Emmentaler, you can delete it. What does the `del` keyword do?

```
cheeses = ['Gorgonzola', 'Emmentaler', 'Camembert']
print(cheeses)
del cheeses[1]
print(cheeses)
```

Exercise 10-14

Because intervals are “ends exclusive,” we can compute the length of a slice as `end - start`:

```
l = [7, 4, 6, 2, 8, 1]
start = 1
end = 4
print("{} has length {}".format(l[start:end], end-start))
```

Consider what this code would look like if ends were included in intervals.

Exercise 10-15

Another advantage of “ends exclusive” intervals is that you only need one index to split a list in two:

```
numbers = [1, 2, 3, 4, 5, 6]
i = 3
beginning = numbers[:i]
end = numbers[i:]
print(beginning + end)
```

If ends were included in intervals, this would be more complex.

Exercise 10-16

Do *all* the substitution and reduction steps in your head (or on paper) before you write any of the following code. Think carefully and decide what you think will be printed below. Remember that the *value* of a list is a container that holds other *values* in it. Then, write the code and see if you are right. If you were not, figure out what led you to the wrong conclusion.

```
x = 'A'  
y = 'B'  
z = 'C'  
lst = [x, y, z]  
print(lst)  
  
x = 'Preben'  
print(lst) # what is printed here?  
  
lst[0] = 'Mogens'  
print(lst) # what is printed here?
```

Exercise 10-17

Do you remember this trick from string slicing?

```
l = [1, 2, 3, 4, 5]  
print(l[::-1])
```

Exercise 10-18

You can produce a list by splitting a long string into smaller parts. Think: “*Hey string, split yourself on this smaller string*”. Try these variations to figure out how it works.

```
"Homo sapiens neanderthalensis".split(" ")  
"Homo sapiens neanderthalensis".split('en')  
'ATGCTCGTAACGACACTGCACTACTACAATAG'.split()  
"1, 2, 3, 5, 3, 2, 5, 3".split(',')  
"1,2,3,5,3,2,5,3".split(',')  
'ATGCTCGTAACGACACTGCACTACTACAATAG'.split()  
"Homo sapiens neanderthalensis".split()
```

Notice that the method has a default behavior when no argument is passed to it.

Exercise 10-19

You can produce a string by joining the elements of a list (if all the elements are strings, of course). Think: “*Hey string, put yourself between all the strings in this list*”.

```
"-".join(['Homo', 'sapiens', 'neanderthalensis'])  
".".join(['Homo', 'sapiens', 'neanderthalensis'])  
"".join(['A', 'T', 'G'])
```

Notice how you can join something on an empty string. This is a very useful technique to turn a list of characters into a string.

General exercises

What does this expression reduce to? 'aaaaa', 'BaBaBa', or 'Banan'. Make up your mind, and then run the expression to check.

```
'a'.join('Banana'.split('a')[:3] * 4)[-5:]
```

11 Pairs of things

This chapter is about dictionaries that, like lists, is another Python value that can contain other Python values. Dictionaries let you build relationships between values, which is what data structures represent.

Dictionaries

Lists are useful for storing values when the order of the values is important, but they have one drawback: you can only access a value in a list using its index.

A dictionary called dict in Python, is a much more flexible data type. Like a list, a dictionary is a container for other values, but dictionaries do not store values in sequence. They work more like a database that lets you store individual *values*. When you store a value, you assign it to a *key* that you can use to access the stored value. Now, create your first dictionary:

```
person = {'name': 'Robert Redford', 'height': 179, 'job': 'Actor'}
```

This dictionary has three values ('Actor', 'Robert Redford' and 179) and each value is associated with a key. Here 'height' is the key for the value 179. So, when defining a dictionary, you should note the following:

1. You make a dictionary using braces.
2. you put key-value pairs separated by a colon between your braces.
3. Commas separate the key-value pairs.
4. To make an empty dictionary, write the braces with nothing between them: {}.

To access a value in the dictionary, you put its key in square brackets after the dictionary:

```
"{} is a {} cm {}".format(person['name'], person['height'], person['job'])
```

Here we used strings as keys, but you can also use many types of values as keys (Python will give you an error if you try to use a type that is not allowed):

```
misc_dict = {42: "Meaning of life", "pi": 3.14159, True: 7}
```

A dictionary stores key-value pairs but does not keep track of their order. So, when you print a dictionary, the order of the key-value pairs is arbitrary.

If you have a dictionary, you can add key-value pairs in this way:

```
person['job'] = 'Retired'  
person['hair'] = 'uniquely combed'  
print(person)
```

Notice that if you assign a value (71) to a key that is already in the dictionary ('age'), then the old value (70) is replaced.

Exercise 11-1

What does this expression evaluate to?

```
{'name': 'Robert Redford', 'height': 179, 'job': 'Actor'}['name']
```

Exercise 11-2

Assuming the definition of the person dictionary above, what does this expression evaluate? Compare this to the expression in the previous exercise.

```
person['name']
```

Exercise 11-3

The *in* operator also works with dictionaries. Look at what these expressions reduce to and then try to figure out what *in* does when applied to a dictionary:

```
'name' in person  
'height' in person  
'job' in person  
84 in person  
'Actor' in person  
'Robert Redford' in person
```

Exercise 11-4

Write and run this code with different values of key and read any error messages.

```
key = 3
# key = 'banana'
# key = 3.14159
# key = True
# key = {}
# key = []
d = {}
d[key] = 7
```

Are any of the values not allowed as keys?

Exercise 11-5

Do you think this will work?

```
person = {'name': 'Robert Redford',
          'height': 179,
          'job': 'Actor'}
print(person)
```

General exercises

Start by making dictionaries for (some of) the Trump family:

```
donald = {'name': 'Donald Trump', 'age': 70, 'job': 'President' }
melania = {'name': 'Melania Trump', 'age': 70, 'job': 'First lady' }
tiffany = {'name': 'Tiffany Trump', 'age': 23, 'job': 'Internet personality' }
ivanka = {'name': 'Ivanka Trump', 'age': 35, 'job': 'Top aide' }
```

Exercise 11-6

What do you think the following code produces? Do *all* of the substitution and reduction steps in your head, and only then try out the code.

```
donald['child'] = tiffany
melania['husband'] = donald

print(melania)
print(melania['husband']['child'])
```

Exercise 11-7

A dictionary can contain *any* kind of Python values, even lists or dictionaries. Consider the code below, where we add a list of ex-wives to the Trump persona. Can you see why we need to check the 'ex-wives' key before we add it to the list of ex-wives?

```
donald = {'name': 'Donald Trump', 'age': 70, 'job': 'President' }

if 'ex-wives' not in donald:
    donald['ex-wives'] = []
donald['ex-wives'].append('Marla Maples')
donald['ex-wives'].append('Ivana Trump')

print(donald)
```

Exercise 11-8

In case you wonder what the *type* of value a list is, or a dictionary, try this:

```
print("A list has type:", type([]))
print("A dictionary has type:", type({}))
```

Now the types list and dict are your friends too.

Exercise 11-9

Lists can also contain any type of value. Consider this example. What do you think the following code produces? Do *all* the substitution and reduction steps in your head, and only then try out the code.

```
trump_family = [donald, melania, ivanka, tiffany]
print(trump_family)
print(trump_family[1]['job'])
```

Exercise 11-10

Write and run this code

```
amino_acids = {}  
amino_acids['ATG'] = 'met'  
amino_acids['TCT'] = 'ser'  
amino_acids['TAC'] = 'tyr'  
  
codon = 'TCT'  
print("{} encodes {}".format(codon, amino_acids[codon]))
```



You have probably noticed that the interpretation of length is different for each type of value. In a string, it is the number of characters; in a list, it is the number of values in the list; in a dictionary, it is the number of key-value pairs. How do you think Python knows which length interpretation to use when the `len` function is called? This is where objects shine. `len(x)` returns the value that `x.__len__()` returns. So the `len` function is defined roughly like this:

```
def len(x):  
    return x.__len__()
```

Similarly, the `in` operator calls a secret `__contains__` method.



12 Grouping values

Tuples

A tuple is a sequence of values, just like a list. However, unlike a list, the elements of a tuple can not be changed. You cannot append to a tuple, either. Once a tuple is made, it is immutable (or unchangeable). To make a tuple, you just use round brackets instead of square brackets:

```
fruits = ("apple", "banana", "cherry")
```

It may seem strange that Python has both tuples and lists. One reason is that tuples are more efficient, whereas lists are more flexible. We will not use tuples often, but you must know what they are.

You can do most of the operations on a tuple that you can also do on a list. The following exercises should be easy if you remember how to do the same thing on lists:

Exercise 12-1

Find the number of elements in the `fruits` tuple using the `len` function.

Exercise 12-2

Extract the second element of the `fruits` tuple ("banana") using indexing.

Exercise 12-3

Try to change the second element of the `fruits` tuple to "apple" and see what happens. It should be something like this:

```
Traceback (most recent call last):
  File "script.py", line 2, in <module>
    fruits[3] = "apple"
TypeError: 'tuple' object does not support item assignment
```

You cannot change elements of a tuple because they are immutable (once made, they stay that way).

Tuple assignment

Python lets you assign a tuple of values to a tuple of variables like this:

```
father, mother, son = ("Donald", "Ivana", "Eric")
```

It does the same as the following three assignments:

```
father = "Donald"  
mother = "Ivana"  
son = "Eric"
```

When a tuple is made, the values are “packed” in sequence:

```
family = ("Donald", "Ivana", "Eric")
```

Using the same analogy, values can be “unpacked” using tuple assignment:

```
father, mother, son = family
```

The only requirement is that the number of variables equals the number of values in the tuple.

Once in a while, it is useful to swap the values of two variables. With conventional assignment statements, we have to use a temporary variable. For example, to swap a and b:

```
tmp = a  
a = b  
b = tmp
```

Exercise 12-4

Try this and read the error message:

```
family = ("Donald", "Ivana", "Eric")
father, mother = family
```

Exercise 12-5

Try this and read the error message:

```
family = ("Donald", "Ivana", "Eric")
father, mother, son, daughter = family
```

Compare to the error message in the previous exercise.

Exercise 12-6

Say you want to swap the values of two variables, a and b. To do that, you would need to keep one of the values in an extra variable like this:

```
temp = a
a = b
b = temp
```

Using what you have learned in this chapter, can you devise a simple and pretty way of swapping a and b in one statement? It may occur to you before you realize how it works, so make sure you can connect your solution to the rules of tuples and tuple assignment.

13 Iterating values

This chapter is about how you repeat the same code for many different values – and the many reasons why this is useful.

The for-loop

Programs often need to do repetitive things. Consider this example below, where `x` is assigned a value that is then printed:

```
x = 1
print(x)
x = 5
print(x)
x = 3
print(x)
x = 7
print(x)
```

You can see that we do the same thing four times, with the only difference being that the variable `x` takes a new value each time. Now, carefully write the alternative version below and compare what is printed to what was printed in the above example.

```
for x in [1, 5, 3, 7]:
    print(x)
```

It should be the same. What you just wrote is called a *for-loop*. It is called a for-loop because it does something *for* each of many values – in this case, for each value in our list.

The statements nested under the for-loop are run as many times as there are values in our list, and every time they are run, `x` is assigned a new value. The first time the statements are run, `x` is assigned the *first* value in the list. The second time they run `x`, the *second* value is assigned to the list. This continues until `x` has been assigned all the values in the list.

The semantics of a for-loop is as follows:

1. First, you write `for`.
2. Then, you write the name of the variable that will be assigned a new value for each iteration of the loop (`x` in the above case).
3. Then you write `in`.
4. Then you write the name of an *iterable* or an expression that reduces to one. In the above case, it was the list `[1, 5, 3, 7]`.
5. The statements nested under the `for` loop are indented with four spaces, just like with `if`-statements. These statements are executed once for every value in the *iterable*.

What is an *iterable*, you may ask? It is any Python value that knows how to serve one value at a time until there are none left. Only objects with an `__iter__` method can do this. You will get an error if you try to iterate over a value that does not have an `__iter__` method. Try the code below and see how Python complains that “‘int’ object is not iterable”:

```
for x in 4:
    print(x)
```

Try these variations of the `for`-loop above and notice how the rules 1-5 apply in each case:

```
for x in [1, 5, 3, 7]:
    print(x)

list_of_numbers = [1, 5, 3, 7]
for x in list_of_numbers:
    print(x)

for x in [1, 5] + [3, 7]:
    print(x)
```

In each case, the expression after `in` reduces to the *value* `[1, 5, 3, 7]`, which then serves as the *iterable*.

It is not only lists that are iterable. Strings are, too. Their ‘`iter`’ method of a string tells it that it should serve one character at a time. Try this:

```
for character in 'banana':
    print(character)
```

Neat, right?

In programming, you often need to iterate over integer values and sometimes quite a few (like the 250 million bases of the human chromosome one). It would be quite annoying if you had to manually make long lists of integers, so Python provides a built-in function called `range` that helps you out. It returns a special *iterator* value that lets you iterate over a specified range of numbers. Try the two examples below and compare what is printed:

```
total = 0
for number in [0, 1, 2, 3, 4, 5, 6, 7, 8, 9]:
    total += number
print(total)

total = 0
for number in range(10):
    total += number
print(total)
```

You can see that using `range` works just like using a list of numbers, but the cool thing about the `range` is that it does not return a list. It just serves one number at a time until it is done. This is also why you will not see a list if you try to print what `range` returns:

```
number_iterator = range(10)
print(number_iterator)
```

The `range` function needs three values to know which values to iterate over: “start”, “end” and “step”. It will assume sensible defaults if you do not give it all three arguments. Try this:

```
for i in range(0, 10, 1):
    print(i)

for i in range(0, 10):
    print(i)

for i in range(10):
    print(i)
```

You can see that the first and last arguments default to 0 and 1. If you give it two arguments, it will assume that they are “start” and “end”. If you only give it one argument, it will assume that it is the “end”.

Exercise 13-1

What do you think the third argument to `range` specifies? Try these variations and see if you can figure it out:

```
for i in range(0, 10, 1):
    print(i)

for i in range(0, 10, 2):
    print(i)

for i in range(0, 10, 3):
    print(i)
```

Check the documentation once you have decided.

Exercise 13-2

What will happen here:

```
for x in []:
    print(x)
```

and here:

```
for x in range(0):
    print(x)
```

and here:

```
for x in range(10, 10):
    print(x)
```

Exercise 13-3

The two examples below print the same. Make sure you understand why. Write and experiment with the code on your own.

```

list_of_words = ['one', 'two', 'three']

# example 1
for word in list_of_words:
    print(word)

# example 2
list_length = len(list_of_words)
for index in range(list_length):
    print(list_of_words[index])

```

Exercise 13-4

Finish the code below so all the even numbers go into one list, and all the odd numbers go into the other (hint: remember the modulo operator?)

```

numbers = [4, 9, 6, 7, 4, 5, 3, 2, 6]
even = []
odd = []
for n in numbers:
    # your code here ...

```

Exercise 13-5

You can put any statements under the for loop. Here, it includes an if-statement that lets you generate a list of all the a characters in banana (in case you need that).

```

result = []
fruit = 'banana'
for character in fruit:
    if character == 'a':
        result.append(character)
print(result)

```

Now change the code so you instead get the *indexes* of the 'a' characters: [1, 3, 5]. Here are some hints:

1. You need a for-loop over a list of numbers.
2. `range(len(fruit))` may be relevant numbers :-).
3. `fruit[1]` substitutes for 'a'.

Exercise 13-6

Imagine you want to throw a big party and have rented a place with space for 100 people. Now, you want to start inviting people. What kind of error do you get here, and why?

```
friends = ["Mogens", "Preben", "Berit"]
invited = []
for index in range(100):
    invited.append(friends[index])
```

Exercise 13-7

You can also put a for loop under another for loop, and the rules for each for loop are the same as those explained above. The statements nested under the for loop are indented with four spaces, just like with if statements. These statements are executed once for every value in the iterable. Think carefully about what you think is printed in the example below before you try it out.

```
for i in range(3):
    for j in range(3):
        print(i, j)
```

Make sure you understand i, j pairs are printed in the order they are.

14 Working with files

This chapter covers the bare necessities of making your program read data from a file on your computer and how to create a file to write results.

Writing files

To interact with a file on your hard disk, you need to know its name and whether you want to write to it or read from it. Then, you can use the built-in function `open` to create a file object that lets you read or write to that file. The `open` function takes two arguments: The first is a string, which gives the file's name. The second argument is also a string and should be '`w`' for "write" if you want to write to the file or '`r`' for "read" if you are going to read from the file. To keep things simple, we will assume that the file you want to open is always in the same folder (directory) as the Python script that calls the `open` function.

Exercise 14-1

Try to write the code below and run it:

```
f = open('workfile.txt', 'w')
f.write("First line\n")
f.write("Second line\n")
f.close()
```

Now open the `workfile.txt` in `VScode` and see what is in it now. It should contain:

`First` line
`Second` line

Let's break down what happened:

1. You used the `open` built-in function to open a file called "workfile.txt" in *writing* mode using the '`w`' as the second argument.

2. You then wrote the string "First line\n" to the file using the `write` method of the file object.
3. You wrote another string "Second line\n" to the file using the `write` method of the file object.
4. You closed the file using the `close` method of the file object.

Note that if you open a file for writing, a file with that name is created. If a file of that name already exists, it is overwritten.

Exercise 14-2

Close `workfile.txt` in *VScode* again and change your program above to this (removing the `\n` characters):

```
f = open('workfile.txt', 'w')
f.write("First line")
f.write("Second line")
f.close()
```

What do you think the content of `workfile.txt` is now? Decide before you open `workfile.txt` in *VScode* again and have a look. What do you think the `\n` character represents?

Exercise 14-3

Close `workfile.txt` in *VScode* and change your program above to this:

```
f = open('workfile.txt', 'w')
f.write("First line\nSecond line\n")
f.close()
```

Can you see how that is equivalent to what you did before? Open `workfile.txt` in *VScode* again and have a look.

Exercise 14-4

You can also make `print` write to a file instead of the terminal. That way, your output will end up in the file instead of the terminal. To make `print` write to a file, you need to use `print`'s `file` keyword argument to tell `print` which file to write to. The argument must be an object that represents the file you want to write to (`file=f` below). Try to write the code below and run it:

```
f = open('workfile.txt', 'w')
print("First line", file=f)
print("Second line, file=f")
f.close()
```

Compare the code to that in exercise 14-1. Notice how the strings we print end with a newline character `\n`. This is because the default behavior for `print` is to add a new line to the end of what it prints—just like when you print to the terminal.

Reading files

When you want to read from an existing file, you give the `open` function the name of that file and specify '`r`' for reading as a second argument. If the file you name does not exist, Python will tell you it does not exist (it is nice like that). Before you head into the rest of this section, make sure you redo exercise 14-1 so the content of `workfile.txt` is:

```
First line
Second line
```

Exercise 14-5

```
f = open('workfile.txt', 'r')
file_content = f.read()
print(file_content)
f.close()
```

Let's break down what happened:

1. You used the `open` built-in function to open a file called `workfile.txt` in *reading* mode using the '`r`' as the second argument.
2. You then read the file's content using the `read` method, which returns the contents as a string.
3. You printed the string.
4. You closed the file using the `close` method of the file object.

Exercise 14-6

Try to read from the file after you close it:

```
f = open('workfile', 'r')
f.close()
file_content = f.read()
```

Do you get an error? Which one? Do you understand why?

Exercise 14-7

You can use the readline method to read one line at a time. What do you think happens if you run this code:

```
f = open('workfile.txt', 'r')
line = f.readline()
print(line)
line = f.readline()
print(line)
line = f.readline()
print(line)
```

Once you decide, try it out. What is printed in the last print statement? The thing is, the file object keeps track of how much of the file it has read. Once it ends, you can read as much as you like- nothing is left. If you want to start reading from the top of the file, you can close it and open it again. Try to insert the following two statements at various places in the code above and see what happens.

```
f.close()
f = open('workfile.txt', 'r')
```

Exercise 14-8

Look at the code below and figure out for yourself what it does:

```
input_file = open('workfile.txt', 'r')
output_file = open('results.txt', 'w')

for line in input_file:
    line = line.upper()
    output_file.write(line)
```

Then, run it and open results.txt in *VScode* and see what it produced.

Were you surprised that the file object can be an iterator in a for-loop? Just like strings can iterate over characters, lists can iterate over values, dictionaries can iterate over keys, and file objects can iterate over the lines in the file.

Try to modify your code to use the `print` function instead of the `write` method (see exercise 14-4).

General exercises

Exercise 14-9

Write a function `read_file` that takes the name of a file as an argument. The function should read the content and return it. Like:

```
content_of_file = read_file('some_file.txt')
```

Exercise 14-10

Write a function that takes the name of two files as arguments. The file should read the content of the first file and write it to the second file.

```
copy_file('some_file.txt', 'other_file.txt')
```


15 Structuring data

This section will further train your ability to create such data structures using iteration. Data structures in Python are fundamental tools that allow you to organize, store, and manipulate data effectively. They provide a way to represent and manage data in a structured and organized manner, making it easier to perform various operations on the data. Python offers a variety of built-in data structures, such as lists and dictionaries, with different properties that are useful for different needs. Sometimes, a single list or dictionary is all you need. Still, sometimes you need to combine many lists and dictionaries to make an elaborate data structure. As your programming skills progress, you will find that mastering the use of different data structures is crucial for becoming a proficient Python programmer.

Exercise 15-1

Imagine you want to count how many times each nucleotide appears in a DNA string like this: 'ATGCCGATTAA'. One way to proceed with an account of this is by using a dictionary where the keys represent the different values we want to count (in this case 'A', 'T', 'C' and 'G'). The value associated with each key is the number of times we have seen that key (nucleotide). So, we want to end up with a dictionary like this one (not necessarily with key-value pairs in this order):

```
{'G': 2, 'C': 2, 'T': 3, 'A': 4}
```

Remind yourself how you assign a value to an existing key in a dictionary. Here is some code to get you going:

```
dna = 'ATGCCGATTAA'  
counts = {'G': 0, 'C': 0, 'T': 0, 'A': 0}  
for base in dna:  
    # Your code here...
```

Exercise 15-2

In exercise 15-1 we started by initializing a dictionary with a key for each nucleotide and the number zero for each key to show that we had not seen any of those nucleotides yet. *Then* we iterated over the values we wanted to count using the for-loop. This approach of course only works if we know which values we will encounter in the iteration. When counting nucleotides, this works because we know there are only four different nucleotides.

Often, however, we are faced with one or both of the following problems:

- The number of different values to count is very large. If we were to count English words, we would have to initialize a dictionary with more than 170.000 key-value pairs (which would be somewhat impractical). If we were to count numbers, this would be impossible (as there are infinitely many of those).
- We do not know what values we are going to count. It goes without saying that we can only initialize a dictionary with keys if we know what they are.

We can solve this problem by only adding keys for the values we see in the iteration. To do this, we need to change our approach from before in two ways:

1. We start with an *empty* dictionary.
2. For every value we iterate over, we check if that value is a key in our dictionary. If it is not, then we need to add it and pair it with the value 0. You can test if a key is not in a dictionary using the not in operator:

```
if number not in counts:  
    counts[number] = 0
```

Now use these hints to complete the code below so that it counts how many times each number appears in number_list.

```
counts = {}  
number_list = [13, 51, 3, 51, 6, 42, 3]  
for number in number_list:  
    # Your code here...
```

When you are done you should have a dictionary like this (possibly with key-value pairs in a different order):

```
{3: 2, 42: 1, 51: 2, 13: 1, 6: 1}
```

Exercise 15-3

The counting technique you developed in exercise 15-2 lets you count pretty much anything that can be a key in a dictionary. Try using the same approach to count the number of each thing in this list:

```
stuff = ['sofa', 42, 42, 3.14159, 'sofa', 'Dragon']
```

Exercise 15-4

Instead of counting values, we sometimes need to split the values we iterate over into categories. Here, we build a dictionary where the keys are the first letter of each word we iterate over, and the value associated with each key is a list of all the words that start with that letter. So, if we iterate over the words in this list:

```
names = ['apricot', 'banana', 'ananas', 'apple', 'cherry']
```

We should end up with this dictionary (not necessarily with key-value pairs in that order):

```
{'c': ['cherry'], 'a': ['apricot', 'ananas', 'apple'], 'b': ['banana']}
```

Now, figure out how to reorder and indent the statements below to produce code that performs this task:

```
names = ['apricot', 'banana', 'ananas', 'apple', 'cherry']
words[first_letter].append(fruit)
first_letter = fruit[0]
for fruit in names:
    words = {'a': [], 'b': [], 'c': []}
```

Exercise 15-5

Produce the same dictionary as in exercise 15-4 by reordering and indenting the following statements:

```

first_letter = fruit[0]
words = {}
words[first_letter].append(fruit)
if first_letter not in words:
    names = ['apricot', 'banana', 'ananas', 'apple', 'cherry']
    words[first_letter] = []
for fruit in names:

```

Compare the solution to that in exercise 15-4. How is it different? Is it more generic? How does the difference relate to the difference between exercise 15-1 and exercise 15-2?

Exercise 15-6

Look at this code and decide for yourself what will be printed. Do we greet one person in three languages first, or do we greet all people in one language first? Then, try it out to see if you were right.

```

greetings = ['Hi', 'Hola', 'Ciao']
names = ['Mogens', 'Preben', 'Henning']
for greeting in greetings:
    for name in names:
        print(greeting, name)

```

Exercise 15-7

Now consider the code below. How is it different from the code in exercise 15-6? How does that alter the order of what is printed?

```

greetings = ['Hi', 'Hola', 'Ciao']
names = ['Mogens', 'Preben', 'Henning']
for name in names:
    for greeting in greetings:
        print(greeting, name)

```

Exercise 15-8

Decide what you think the code below does and why you think so. Do *every* step in your head, including all the substitutions and reductions. Then write the code carefully and run it.

```

nr_list = [10, 20, 30]
combinations = []
for a in nr_list:
    for b in nr_list:
        pair = [a, b]
        combinations.append(pair)

```

The combinations list becomes:

```

[[10, 10], [10, 20], [10, 30],
 [20, 10], [20, 20], [20, 30],
 [30, 10], [30, 20], [30, 30]]

```

Here, I broke over three lines to make it fit on the page. You should print your own combinations list to ensure you got the code right.

Exercise 15-9

The code in exercise 15-8 printed all combinations of numbers in the list – including those where the two numbers are the same. Change the code above so these pairs are *not* printed. You should end up with this list:

```

[[10, 20], [10, 30], [20, 10], [20, 30], [30, 10], [30, 20]]

```

Exercise 15-10

Can you solve the same task as in exercise 15-9, by modifying the code below? You can begin by understanding why it produces the same list as that in exercise 15-8. If you need help with that, then have another look at exercise 13-7.

```

nr_list = [10, 20, 30]
combinations = []
for i in range(len(nr_list)):
    for j in range(len(nr_list)):
        pair = [nr_list[i], nr_list[j]]
        combinations.append(pair)

```

Exercise 15-11

The code in the exercise above printed all combinations of *different* numbers in the list. But you can see that each pair of numbers still appears twice if you do not take their order into account (e.g., [10, 30] are the same two numbers as [30, 10]). Change the code you wrote for the previous exercise so these pairs are not printed. You should end up with a list like this:

```
[[10, 20], [10, 30], [20, 30]]
```

Hint: the easiest way to do it is to use the value of *i* to change the range of numbers you iterate over in the second for-loop.

Exercise 15-12

Sometimes programmers (like you) work with matrices of numbers like the one below:

```
[[0, 1, 2, 3, 4],  
 [0, 1, 2, 3, 4],  
 [0, 1, 2, 3, 4],  
 [0, 1, 2, 3, 4],  
 [0, 1, 2, 3, 4]]
```

Here, I nicely wrote the list so you can see that a matrix is just *a list of lists*. When you print it looks like this:

```
[[0, 1, 2, 3, 4], [0, 1, 2, 3, 4], [0, 1, 2, 3, 4], [0, 1, 2, 3, 4], [0, 1, 2, 3, 4]]
```

Can you write some code that produces this matrix? If you let out a sigh just now, then reread the sections on lists and for-loops. You may think you absorbed all the information you could when you read it the first time, but with your practice since then, you may be able to understand it at a deeper level the second or third time you read it.

The code below produces the matrix above. There are several tricky parts that you need to make sure you understand. In line three, we add an empty list to the list of lists. In line five, we add the value of *j* to the list at index ‘*i*’ in the list. Go over this many times in your head and with pen and paper.

```
matrix = []
for i in range(5):
    matrix.append([])
    for j in range(5):
        matrix[i].append(j)
```

Exercise 15-13

If you understand how you created the matrix in exercise 15-12, you should be able to produce the matrix below using a small modification to the code from exercise 15-12.

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

Exercise 15-14

Now produce this matrix:

```
[[0, 0, 0, 0, 0],
 [0, 0, 0, 0, 0],
 [0, 0, 0, 0, 0],
 [0, 0, 0, 0, 0],
 [0, 0, 0, 0, 0]]
```

Exercise 15-15

Can you write some code that produces this matrix?:

```
[[ 0, -1, -2, -3, -4],
 [-1, 0, 0, 0, 0],
 [-2, 0, 0, 0, 0],
 [-3, 0, 0, 0, 0],
 [-4, 0, 0, 0, 0]]
```

General exercises

By now, you have learned a lot, and the general exercises, which serve to keep it all current, get more complicated. But remember: even though the code may mix lists, for-loops, and functions, the *rules* for lists, for-loops, and functions are *not* mixed. The separate simple rules for a list, a for-loop, and a function are *still* the same. If you get confused, it is time to revisit the sections about each separate topic. You may have to do that many times during the course.

Exercise 15-16

Write a function, `square_numbers`, that takes a list of numbers as argument and returns a new list with the numbers squared.

```
# write your function definition here ...
numbers = [1, 5, 3, 7]
# then you can call it like this:
squared = squared_numbers(numbers)
```

Exercise 15-17

Write a function `count_characters`, which takes a string argument and returns a dictionary with the counts of each character in the string. When you call the function like this:

```
count_characters('banana')
```

it must return (not necessarily with key-value pairs in that order):

```
{'n': 2, 'b': 1, 'a': 3}
```

The technique you should use is the one you learned in exercise 15-2. Here, we iterate over a string of characters instead of a list of numbers. Here is a bit of code to help you along.

```
def count_characters(text):
    counts = {}
    # fill in the missing code ...

    return counts
```

Exercise 15-18

Use the function you made in the previous exercise to construct the following data structure below from this list: ['banana', 'ananas', 'apple'].

```
{ 'banana': {'b': 1, 'a': 3, 'n': 2},
  'apple': {'a': 1, 'e': 1, 'p': 2, 'l': 1},
  'ananas': {'a': 3, 's': 1, 'n': 2} }
```

Here is some code to help you along:

```
my_database = {}
for word in ['banana', 'ananas', 'apple']:
    my_database[word] = # you figure this out...
```

Once you are done, what value do you think `my_database['banana']` represents? I.e., what will it be reduced to if used in an expression? And what value does `my_database['banana']['a']` represent?

Exercise 15-19

Read the code below and make sure you understand each step before you write any of it. If necessary, revisit previous sections and look in the Python documentation. Then, write and run the code—and enjoy that it was exactly what you expected.

```
def get_words(text, search_string):
    hits = []
    for word in text.split():
        if search_string in word:
            hits.append(word)
    return hits

s = 'eenie meenie minie moe'
nie_words = get_words(s, 'nie')
```

```
m_words = get_words(s, 'm')
print(' '.join(nie_words))
print(' '.join(m_words))
```

Exercise 15-20

This larger will take you through some of the most common string manipulations. A palindrome is a string that is spelled the same way, backward and forwards.

Write a function, is_palindrome, which takes one argument:

- A string.

The function must return:

- True if the string argument is a palindrome and False otherwise.

Example usage:

```
is_palindrome('abcba')
```

should return True and

```
is_palindrome('foo')
```

should return False

One approach to this is to run through s from the first to the middle character, and for each character, check if the character is equal to the character at the same index from the right rather than the left. Remember that the first character of a string is at index 0 and the last at index -1, the second character is at index 1 and the second last at index -2 and so forth.

Since you need to run through the string from the first to the middle character, you must first figure out how many characters that corresponds to. Say your palindrome is "ACTGTCA", then the number of indexes you need to loop over with a for loop is:

```
s = "ACTGTCA"
nr_indexes = len(s)//2
```

Figure out how to make `range()` return indexes to access the characters in the first half of the sequence. Then make a for loop where you iterate over the indexes you get from `range()`. Try to make the for-loop print out the first half of the characters, just to make sure you are using the right indexes.

Once you get this far, you must compare each character from the first half of the corresponding ones, starting from the other end of the palindrome. Figure out how to change each index used for the first half to the corresponding index for the other half so you can compare the relevant pairs. (You need to compare index 0 with -1, 1 with -2 and so on...)

Now, try to make the for loop print both the character from the first half and the corresponding character from the other end. If you get the indexes right, you will see that the A prints with the A from the other end, the C with the C, and so on.

Write an if-statement in the for-loop that tests whether the two corresponding characters are identical. If the string is a palindrome, then each pair is identical. So, as soon as you see a pair that is not identical, you know it is not a palindrome, and you can let your function return `False` like this:

```
if left_character != right_character:  
    return False
```

Remember that the function ends when it encounters a `return` statement.

If all pairs pass the test, the string is a palindrome, and the function should return `True` when exiting the for loop.

16 Recursion

At the time of “printing,” this chapter was unfinished. So I have added some empty pages so you can merge the chapter into the pdf later without messing up the chapter, exercise and page numbers

Recursion

PLACEHOLDER PAGE

Divide and conquer

PLACEHOLDER PAGE

Exercise 16-1

PLACEHOLDER PAGE

17 Testing your code

This chapter is about how you figure out if the code you wrote solves the problem in the way you intended. You will be surprised how often that is not the case – even for seasoned programmers.

Why test your code?

There are tons of reasons why you should test your code. Here are what I think are the two most important ones:

1. **Makes you think:** Testing forces you to slow down and think about exactly what the code is supposed to do. By deciding what tests to do before you start coding, you try to anticipate errors and cases that need to be covered by how you want to solve the problem. The notion of falsification is important in science and in coding, too. You should try to prove that your idea is wrong and consider it valid only if this process fails. So, testing motivates you to think about ways to break their code, thereby helping you solve your programming problem in a general and robust way so that it does exactly what you expect it to do.
2. **Gives you peace of mind:** Testing increases your confidence that a function you have written works as it is supposed to so that you can now stop thinking about how it is implemented and focus on using it as a component in solving a larger, more complicated problem. Having set up a series of tests also allows you to change and improve your function's implementation without worrying that it stops working the way it is supposed to. As long as it passes all the tests, it should be ok.

Testing of code is a *big* thing in programming. Professionals consistently test their code. You will do it in time, too, but in this course, you will only do the basic testing yourself. Instead, you will have access to readymade testing suites made especially for each of your programming projects.

Basic testing

Say that you are asked to make a function that takes a string argument and returns `True` if that string is a palindrome and `False` otherwise (hypothetical example). Then

you start thinking about which strings should make the function return True and which return False. Once you have defined your `is_palindrome` function, you can set up some fairly obvious tests like this:

```
print(is_palindrome('123321') == True)
print(is_palindrome('ATGGTA') == True)
print(is_palindrome('ATGATG') == False)
print(is_palindrome('XY') == False)
```

But if you keep thinking, maybe you will come up with more tests to cover all the different types of cases you may encounter:

```
print(is_palindrome('12321') == True) # uneven length
print(is_palindrome('121') == True) # uneven length
print(is_palindrome('AA') == True)
print(is_palindrome('A') == True) # single char
```

The project testing utility

To keep you focused on the programming part, each of the programming projects you will do in this course comes with a ready-made suite of tests of the functions you are asked to implement. So, for each function, you can run tests to ensure it implements the behavior it is supposed to.

Each project comes with two files that you can download from the course page. They have their names for a good reason, so do not change them. In the first project about translating DNA, they are called `translationproject.py` and `test_translationproject.py`.

To be able to test your functions, you *must* write your code in the file called `translationproject.py`. To run your code, you type this in the Terminal as usual:

Listing 17.1 Terminal

```
python translationproject.py
```

To test the functions as you complete each one, you can run the test script `test_translationproject.py` like this:

The code in `test_translationproject.py` reads your code in `translationproject.py` and performs a series of tests of each function. When you run the test script, four things may happen depending on the state of your code:

Listing 17.2 Terminal

```
python test_translationproject.py
```

Case 1: If you have not yet implemented all the functions, the test script will remind you (once for each test) that you did not implement the functions with the names required.

Listing 17.3 Terminal

```
*****
ATTENTION! The following functions are not defined:
```

```
translate_codon
split_codons
translate_orf
```

These functions are either not correctly named (spelled) or not defined at all. They will be marked as FAILED.
Check your spelling if this is not what you intend.

```
*****
```

```
Ran 16 tests in 0.000s
```

```
OK (skipped=14)
```

If you have implemented a function but misspelled its name, you will also get this type of reminder. The reminders are meant to ensure that you do not hand in the assignment with missing or misspelled function definitions.

Case 2: If a test for one of the functions you have written fails, the testing is aborted, and the script prints some information to help you understand what the problem could be. Say you wrote the function `translate_codon` wrongly so that it always returns M for some reason:

```
def translate_codon(codon):
    return 'M' # crazy
```

then you would get this message:

It is now left to you to figure out why your function returns the wrong value when called with these arguments.

Listing 17.4 Terminal

FAILED TEST CASE: test_translate_codon_2

MESSAGE:

The call:

```
translate_codon('TAA')
```

returned:

```
'M'
```

However, it should return:

```
'*'
```

```
=====
```

Ran 4 tests in 0.001s

FAILED (failures=1)

Case 3: If you defined all functions correctly and they all work the way they are supposed to, then the test script just prints:

Ran 14 tests in 0.140s

OK

18 Appendix: PyMol for BSF

Install Python

This installation procedure includes python and few other tools that you will also need later in the semester:

18.1 Mac

1. Click the clipboard icon at the right end of the box below to copy the command to your clipboard.
2. Open your Terminal application.
3. Paste the command into the Terminal window and press Enter. You will be prompted several times for either your user password or permissions of the installed apps.

```
curl -fsSL http://munch-group.org/franklin/installers/install.sh | bash -s -- --role student --yes
```

18.2 Windows

1. Click the clipboard icon at the right end of the box below to copy the command to your clipboard.
2. Open your PowerShell application.
3. Paste the command into the PowerShell window and press Enter. You will be prompted several times to allow the app to make changes to your computer.

```
powershell -ExecutionPolicy ByPass -c "irm -useb http://munch-group.org/franklin/installers/install.ps1" | iex
```

Install PyMol

18.3 Mac

1. Create a folder on your computer dedicated to the BSF course. Let's say you call it 'BSF' and put it in your 'Documents' folder.
2. Open your Terminal application, and navigate to that folder using cd (something like cd Documents and then cd BSF).
3. Once you are in the 'BSF' folder, paste the command into the Terminal window and press Enter. You will be prompted several times for either your user password or permissions of the installed apps.

```
pixi init --platform osx-64 -c conda-forge -c anaconda -c schrodinger && pixi config set
```

18.4 Windows

1. Create a folder on your computer dedicated to the BSF course. Let's say you call it 'BSF' and put it in your 'Documents' folder.
2. Open your PowerShell application, and navigate to that folder using cd (something like cd Documents and then cd BSF).
3. Once you are in the 'BSF' folder, paste the command into the PowerShell window and press Enter. You will be prompted several times to allow the app to make changes to your computer.

```
pixi init -c conda-forge -c anaconda -c schrodinger && pixi config set --local run-post
```

Running PyMol

In your BSF folder, run:

```
pixi run pymol
```

Part II

Python projects

19 Translating ORFs

This chapter is about translating DNA into protein. If bacteria can do it, so can you.

In this project you will write the code needed to translate an open reading frame (ORF) on a DNA sequence into the corresponding sequence of amino acids.

Project files

Begin by downloading the files you need for the project:

https://munch-group.org/bioinformatics/supplementary/project_files

The file `translationproject.py` is for your code. The file `test_translationproject.py` is the script you use to test the functions you write for this project (see Chapter 17).

In this project you will need a data structure that pairs each codon to the amino acid it encodes. This is an obvious use of a dictionary and at the top of `translationproject.py` I have defined such a dictionary you can use. Defining it outside the functions means that it is visible inside all your functions (unless you define *another* variable called `codon_map` inside a function). Defining variables globally to your program sometimes make sense if some value can be considered a *constant* in your program and is *never* changed.

“ It is normally very bad programming style to access variables outside functions in this way because it may have all kinds of unexpected side effects across function calls. So make it a rule for yourself that code *inside* a function should never access variables *outside* the function. The reason we define `codon_map` globally in this project is to help you understand that when Python cannot find a variable inside a function, it looks outside the function to find it. In this project functions will find `codon_map` in this way. However, as I already said, you should *never* do this yourself. The chance that you make an unexpected mistake is overwhelming.”

Translating a single codon

Write a function, `translate_codon` that takes one argument:

1. A string, which is a codon.

The function should return:

- A string of length one (one character). If the string argument is a valid codon then this letter should be an amino acid letter specified by the `codon_map` dictionary. Note that stop codons are represented by a star ('*'). If the string argument is *not* a valid codon, the function must return '?'.

Example usage:

```
translate_codon('ACG')
```

should return

```
'T'
```

Before you start coding you should always outline for yourself intuitively what you need to do to complete the task at hand. In this case want to translate, or map, between a three letter string, codon, and the corresponding one letter string for the amino acid that the codon corresponds to. Notice that the keys in the `codon_map` dictionary are in upper case, so you must make sure that the keys *you* use are also in upper case. You can translate codon into an upper case version of itself using the `upper()` method.

Try this out first:

```
codon = 'TTG'  
amino_acid = codon_map[codon]  
print(amino_acid)
```

Now write the function so it uses the string parameter as a key to look up the corresponding amino acid letter and returns this letter. Before you go on, make a function that does only that.

Before you are completely done you need to make your function handle the situation when the argument to the function is not a key in the `codon_map` dictionary. Use an if-else construct to handle the two cases. The boolean expression must test if the function argument is a key in `codon_map`. Remember that you can use the `in` operator to do this.

Splitting an open reading frame into codons

To translate an entire open reading frame into the corresponding amino acid sequence, you need to split the ORF sequence into codons. When we have done that we can translate each codon using the function `translate_codon` you just wrote.

Write a function, `split_codons`, that takes one argument:

1. A string, which is an ORF sequence

The function must return:

- A list of strings. Each string must have length 3 and must represent the non overlapping triplets in the same sequence as they appear in the string given as argument.

Example usage:

```
split_codons('ATGTATGCCTGA')
```

should return

```
['ATG', 'TAT', 'GCC', 'TGA']
```

Divide the problem into simpler tasks like above. You need to loop over the sequence to perform operations on it. Start by writing a function that prints each character:

```
def split_codons(orf):
    for i in range(len(orf)):
        print(orf[i])
```

Now try to figure out how you can modify the function to make it move over the sequence in jumps of three. Look at the documentation for the `range` function to see how you can make it iterate over numbers with increments of three like this: 0, 3, 6, 9, 12, Modify your function so that it now prints every third character.

What you want is obviously not every third character. You want three characters. I.e. every third character *and* the two characters that come right after. You can use the index in the for loop to get the corresponding codon using slicing. Modify your function so that it prints each codon.

Now all that remains is to put each codon on a list that you can return from the function. You can define a list before your for-loop so you have a list to add codons to.

Translating an open reading frame

Now you can use the two functions `split_codons` and `translate_codon` to write a function that translates an ORF into a protein sequence.

Write a function, `translate_orf`, that takes one argument:

1. A string, which is a DNA sequence.

The function must return

- A string, which is the protein sequence translated from the ORF sequence argument.

Example usage:

```
translate_orf('ATGGAGCTTANCAAATAG')
```

should return

```
'MEL?K*' 
```

20 Primer analysis

Say you have a short sequence that you want to use as a PCR primer. To evaluate if it a suitable primer, you need to know the melting temperature of the sequence. You also need to make sure that it will not fold up on itself so that it cannot bind to the DNA sequence you want to amplify.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `foldingproject.py` is an empty file where you must write your code.
- `test_foldingproject.py` is the test program that lets you test the code you write in `translationproject.py`.

Put the files in a folder dedicated to this project. On most computers you can right-click on the link and choose “Save file as...” or “Download linked file”.

Count the number of bases in your candidate primer

Before you can compute the melting temperature, you need to determine how many times each base occurs in the sequence. You can assume that the only characters in the string are A, T, G, and C.

Write a function, `count_bases`, which takes one argument:

1. A string, which is a DNA sequence.

The function must return:

- A dictionary, which in which keys are strings that represent bases and values are integers that represent the number of occurrences of each base. If a base is not found in the sequence, its count must be zero.

Example usage: If the function is called like this:

```
count_bases("ATGG")
```

then it should return (not necessarily with key-value pairs in the same order):

```
{"A": 1, "C": 0, "G": 2, "T": 1}
```

Compute the melting temperature

Knowing the base composition in your sequence, you can now calculate the melting temperature the double-stranded DNA that forms when your primer pairs up with the sequence to amplify. If the primer has less than 14 bases the formula for calculating melting temperature is:

$$Temp = (A + T) \cdot 2 + (G + C) \cdot 4$$

and if the primer has 14 bases or more it is calculated like this:

$$Temp = 64.9 + 41 \cdot (G + C - 16.4) / (A + T + G + C)$$

The A, T, C, and G in the formulas represent the numbers of A, T, C and G in the DNA primer.

You must write a function that applies these two formulas appropriately by taking the length of the primer into account.

Write a function, melting_temp, which takes one argument:

1. A string, which is a DNA sequence (your primer).

The function must return:

- A number, which represents the melting temperature of the double-stranded DNA corresponding to the DNA string given as argument.

Example usage: If the function is called like this:

```
melting_temp("ATG")
```

then it should return:

Reverse complement the sequence

It is possible that one part of the primer forms base pairs with another part of the primer to form a hairpin structure. To figure out if this can happen to your primer, you need to be able to find the reverse complement of DNA sequence. The reverse complement of a DNA sequence is one where the sequence of bases is first reversed, and then each base is replaced with its Watson-Crick complementary base.

Write a function, reverse_complement, which takes one argument:

1. A string, which is a DNA sequence.

The function must return:

- A string, which represents the reverse complement of the DNA string given as argument.

Example usage: If the function is called like this:

```
reverse_complement("AATTC")
```

then it should return:

```
"GAATT"
```

Check for hairpins

You would like to be able to determine if your primer can fold to form a hairpin with some specified minimum number of consecutive base pairs. We assume that hairpin loops are always at least four bases long and that base pairs in the hairpin can only be Watson-Crick basepairs. Here is an example of a hairpin with five basepairs and a loop of four bases (four Cs):

```
    C C  
    C     C  
    A - T  
    T - A  
    A - T  
    T - A  
    A - T
```

To test if a sequence can form a hairpin with at least four consecutive base pairs, you need to test if the sequence contains any subsequence of length four whose reverse complement is identical to another nonoverlapping subsequence. To take into account that the hairpin loop is at least four bases long, any such two subsequences must be separated by at least four bases.

Write a function, has_hairpin, which takes two arguments:

1. A string, which is a DNA sequence.
2. An integer, which represents the minimum number of consecutive base pairs in the hairpins to search for.

The function must return:

- True if the sequence contains a hairpin of at least the specified length and False otherwise.

Example usage: If the function is called like this:

```
print(has_hairpin("ATATACCCTATAT", 4))
```

then it should return:

```
True
```

This is a hard one, so I will give you a bit of help. Here is the function with some parts missing.

```
def has_hairpin(s, k):  
    looplen = 4  
    for i in range(len(s)-k+1):  
        subs = # Hint A  
        right = # Hint B  
        revcl = reverse_complement(subs)  
        if revcl in right[looplen:]:  
            return True  
    return False
```

Hint A: Here you need to extract a substring of length k starting at i. Hint B: Here you need to extract all the sequence to the right of substr.

21 Pairwise alignment

This chapter is about global pairwise alignment, and you will implement your own Needleman-Wunch algorithm.

Your task is to find an optimal alignment of two sequences. If two such sequences are roughly 140 bases long, there are as many different ways to align them as there are atoms in the visible universe. Finding an optimal alignment among those 10^{80} possibilities is a hard problem, but implementing the Needleman-Wunch algorithm will let you do it.

This project is meant to train your coding abilities and consolidate your understanding of the Needleman-Wunch algorithm. The better you understand the algorithm before you begin, the easier and more rewarding the project will be. So, re-read the book chapter about pairwise alignment and browse the lecture slides.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files - alignmentproject.py is the file where you must write your code. It already contains a function I wrote for you. `- test_alignmentproject.py` is the test program that lets you test the code you write in `alignmentproject.py`.

Put the files in a folder dedicated to this project. On most computers, you can right-click on the link and choose “Save the file as...” or “Download linked file.”

The project is split into two parts:

1. Filling in a dynamic programming matrix.
2. Reconstructing the optimal alignment by doing traceback through the dynamic programming matrix.

To help you along, the `alignmentproject.py` file already contains a function I wrote for you, so you can print your dynamic programming (DP) matrix as you gradually fill it in. You are not expected to understand how this function works. I made it as condensed as possible so it does not take up so much space in your file.

The function is named `print_dp_matrix` and takes two arguments:

1. A string, which represents a DNA sequence of some length m .
2. A string, which represents a DNA sequence of some length n .
3. A lists of lists of integers, representing a dynamic programming matrix.

The function returns:

- None, but it *prints* a nice representation of the matrix lined with the bases of the two sequences.

Filling in the dynamic programming matrix

Make a matrix

We start by making a list of lists (a matrix) with the right shape but only holds None values. We use the None values as placeholders, which you can later replace with scores. You can think of it as an empty matrix into which you can fill scores, just as we did in the lectures. If you want to align two sequences like AT and GAT, you want a matrix with three rows and four columns. Note that the matrix must have one more row than the number of bases in sequence one and one more column than the number of bases in sequence two.

Write a function, empty_matrix, that takes two arguments

1. An integer (which represents the length of sequence one + 1).
2. An integer (which represents the length of sequence two + 1).

The function must return:

- A list of lists. The number of sub-lists must be equal to the first integer argument. Each sublist must contain a number of None values equal to the second integer argument.

Example usage:

```
empty_matrix(3, 4)
```

returns a list with *three* lists each of length *four*:

```
[[None, None, None, None], [None, None, None, None], [None, None, None, None]]
```

Even though this is a list of lists, we can *think of it* as a three-by-four matrix:

```
[[None, None, None, None],  
 [None, None, None, None],  
 [None, None, None, None]]
```

If you want to print the matrix in a way that looks like the slides I showed you at the lecture, you can use the `print_dp_matrix` function (again, `None` represents empty cells):

```
G   A   T  
None None None None  
A None None None None  
T None None None None
```

Important: You can implement `empty_matrix` in a way that superficially looks ok but will cause you all kinds of grief when you start filling it in. When you create the list of lists (e.g., three as above), you must generate and add three *separate* lists. If you add the *same* list three times, you do not have three separate rows in your matrix. Instead, you have *three* references to the *same* row. You can test if you did it right this way – by changing the value of one cell to see what happens:

```
empty = empty_matrix(3, 4)  
empty[0][0] = 'Mogens'  
print(empty)
```

If this only changed *one* value like below, you are ok:

```
[['Mogens', None, None, None], [None, None, None, None], [None, None, None, None]]
```

If it changed the first value in *all* the lists, it means that all your lists are the same (which is not what you want).

```
[['Mogens', None, None, None], ['Mogens', None, None, None], ['Mogens', None, None, None]]
```

Fill the top row and left column

Now that you can make a matrix with the correct dimensions, you need to write a function that fills in the top row and the left column with multiples of the gap score. E.g., if the gap score is -2 , you want the matrix to look something like this when you print it with `print_dp_matrix`:

```
G   A   T  
0   -2   -4   -6  
A   -2   None  None  None  
T   -4   None  None  None
```

Write a function, `prepare_matrix`, which takes three arguments:

1. An integer (which represents the length of sequence one plus one)
2. An integer (which represents the length of sequence two plus one)
3. An integer, which represents the gap_score used for alignment.

The function must return:

- A list of lists. The number of sub-lists must be equal to the first integer argument. The values in the first sub-list must be multiples of the gap score given as the third argument. The first elements of the remaining sub-lists must be multiples of the gap score. All remaining elements of sub-lists must be `None`.

Example usage:

```
prepare_matrix(3, 4, -2)
```

must return:

```
[[0, -2, -4, -6], [-2, None, None, None], [-4, None, None, None]]
```

i Hint

You should call `empty_matrix` inside `prepare_matrix` to get a matrix filled with `None`.

Now, all you need to do is replace the right `None` values with *multiples* of the gap score. For example, the third element in the first sub-list is `matrix[2][0]`, for which you would need to assign the value 2 times the gap score. In the same way `matrix[3][0]` should be 3 times the gap score. So, you need to figure out which elements you should replace and which pairs of indexes you need to access those elements. Then, use `range` to generate those indexes and for-loops to loop them over.

Fill the entire matrix

Now that we can fill the top row and left column, we can start thinking about how to fill the whole matrix.

For that, we need a score matrix of match scores. In Python, that is most easily represented as a dictionary of dictionaries like this:

```
score_matrix = {'A': {'A': 2, 'T': 0, 'G': 0, 'C': 0},  
                'T': {'A': 0, 'T': 2, 'G': 0, 'C': 0},  
                'G': {'A': 0, 'T': 0, 'G': 2, 'C': 0},  
                'C': {'A': 0, 'T': 0, 'G': 0, 'C': 2}}
```

That lets you get the score for matching an A with a T like this: `score_matrix['A']['T']`. Note that the match scores are only for uppercase letters (A, T, G, C).

Write a function, `fill_matrix`, which takes four arguments:

1. A string, which represents the first sequence.
2. A string, which represents the second sequence.
3. A dictionary of dictionaries like the one shown above, which represents match scores.
4. An integer, which represents the gap score.

The function must return:

- A list of lists of integers, which represents a correctly filled dynamic programming matrix given the two sequences, the match scores, and the gap score.

Example usage: If `score_matrix` is defined as above, then

```
fill_matrix('AT', 'GAT', score_matrix, -2)
```

must return:

```
[[0, -2, -4, -6], [-2, 0, 0, -2], [-4, -2, 0, 2]]
```

If you print that matrix using `print_dp_matrix` it should look like this:

	G	A	T
0	-2	-4	-6
A	-2	0	0
T	-4	-2	0
			2

i Hint

You should call `prepare_matrix` inside `fill_matrix` to get a matrix with the top row and left column filled. Assuming `seq1` is sequence one and `seq2` is sequence two, then you can do it like this:

```
matrix = prepare_matrix(len(seq1)+1, len(seq2)+1, gap_score)
```

Now, you only need to fill out the rest. You need two nested for-loops to produce the indexes of the elements in the list of lists that you need to assign values to.

```

for i in range(1, len(seq1)+1):
    for j in range(1, len(seq2)+1):
        print(i, j) # just to see what i and j are

```

Examine this code and make sure you understand why we give those arguments to range. Each combination of i and j lets you access an element `matrix[i][j]` in `matrix` (list of lists) that you can assign a value to. The value to assign to `matrix[i][j]` (green cell on the slides) is the maximum of three values (the yellow cells on the slide):

1. The value of the cell to the left (`matrix[i][j-1]`) plus the gap score.
2. The cell above (`matrix[i-1][j]`) plus the gap score.
3. The diagonal cell (`matrix[i-1][j-1]`) plus the match score for base number i (index `i-1`) of sequence one and base number j (index `j-1`) of sequence two.

Reconstructing the optimal alignment

This is the most challenging part, so I will hold your hand here. Below is first a function that identifies which of three cells (the yellow cells on the slides) some cell (green cell on the slides) is derived from. On the slides, this is the cell pointed to by the red arrow.

```

def get_traceback_arrow(matrix, row, col, match_score, gap_score):

    # yellow cells:
    score_diagonal = matrix[row-1][col-1]
    score_left = matrix[row][col-1]
    score_up = matrix[row-1][col]

    # green cell:
    score_current = matrix[row][col]

    if score_current == score_diagonal + match_score:
        return 'diagonal'
    elif score_current == score_left + gap_score:
        return 'left'
    elif score_current == score_up + gap_score:
        return 'up'

```

Write (do not copy and paste) this into your file, and make sure that it works and that you understand exactly how it works before you proceed.

Here is a function that uses get_traceback_arrow to do the traceback. It reconstructs the alignment starting from the last column, adding columns in front as the traceback proceeds. It is big, so it breaks across three pages.

```
def trace_back(seq1, seq2, matrix, score_matrix, gap_score):

    # Strings to store the growing alignment strings:
    aligned1 = ''
    aligned2 = ''

    # The row and col index of the bottom right cell:
    row = len(seq1)
    col = len(seq2)

    # Keep stepping backwards through the matrix untill
    # we get to the top row or the left col:
    while row > 0 and col > 0:

        # The two bases we available to match:
        base1 = seq1[row-1]
        base2 = seq2[col-1]

        # The score for mathing those two bases:
        match_score = score_matrix[base1][base2]

        # Find out which cell the score in the current cell was derived from:
        traceback_arrow = get_traceback_arrow(matrix, row, col, match_score, gap_score)

        if traceback_arrow == 'diagonal':
            # last column of the sub alignment is base1 over base2:
            aligned1 = base1 + aligned1
            aligned2 = base2 + aligned2
            # next cell is the diagonal cell:
            row -= 1
            col -= 1
        elif traceback_arrow == 'up':
            # last column in the sub alignment is base1 over a gap:
            aligned1 = base1 + aligned1
            aligned2 = '-' + aligned2
            # next cell is the cell above:
            row -= 1
        elif traceback_arrow == 'left':
            # last column in the sub alignment is a gap over base2:
            aligned1 = '-' + aligned1
```

```

        aligned2 = base2 + aligned2
        # next cell is the cell to the left:
        col -= 1

    # If row is not zero, step along the top row to the top left cell:
    while row > 0:
        base1 = seq1[row-1]
        aligned1 = base1 + aligned1
        aligned2 = '-' + aligned2
        row -= 1

    # If col is not zero, step upwards in the left col to the top left cell:
    while col > 0:
        base2 = seq2[col-1]
        aligned1 = '-' + aligned1
        aligned2 = base2 + aligned2
        col -= 1

    return [aligned1, aligned2]

```

Once you have *written* it into your file, make sure you understand the correspondence to the sequences of events on the lecture slides.

Now you can write a function that performs the alignment. You get to do that yourself. It just calls `fill_matrix` and then `trace_back` to get the optimal alignment

Write a function, align, that takes four arguments:

1. A string, which represents sequence one.
2. A string, which represents sequence two.
3. A dictionary of dictionaries, which represents the match scores (as described above).
4. An integer, which represents the gap score.

The function must return:

- A list of length two. The first element of that list must be a string representing the aligned sequence one. The second element must be a string, representing the aligned sequence two.

Example usage:

```
align('ATAT', 'GATGAT', score_matrix, -2)
```

must return:

```
[ '-AT-AT' , 'GATGAT' ]
```

Once you have written that function, you can print your alignment like this:

```
alignment = align('ATAT' , 'GATGAT' , score_matrix , -2)
for s in alignment:
    print(s)
```


22 Codon usage

Codon usage bias refers to differences in the frequency of occurrence of synonymous codons in coding DNA. There are 64 different codons (61 codons encoding for amino acids plus 3 stop codons) but only 20 different translated amino acids. The overabundance in the number of codons allows many amino acids to be encoded by more than one codon. Because of such redundancy, it is said that the genetic code is degenerate. Different organisms often show particular preferences for one of the several codons that encode the same amino acid, that is, a greater frequency of one will be found than expected by chance. How such preferences arise is a much-debated area of molecular evolution.

Given an open reading frame (ORF), you must compute the codon usage. Your goal is to create a data structure where you can look up an amino acid and the frequencies of codons in that encode that amino acid the ORF. Here is a made-up example:

```
{'A': {'GCA': 0.0, 'GCC': 0.0, 'GCT': 1.0, 'GCG': 0.0},  
 'C': {'TGC': 0.0, 'TGT': 1.0},  
 'E': {'GAG': 0.2, 'GAA': 0.8},  
 'D': {'GAT': 1.0, 'GAC': 0.0},  
 'G': {'GGT': 0.3, 'GGG': 0.0, 'GGA': 0.7, 'GGC': 0.0},  
 'F': {'TTC': 0.0, 'TTT': 1.0},  
 'I': {'ATT': 1.0, 'ATC': 0.0, 'ATA': 0.0},  
 'H': {'CAC': 0.0, 'CAT': 1.0},  
 'K': {'AAG': 0.2, 'AAA': 0.8},  
 '*': {'TAG': 0.0, 'TGA': 1.0, 'TAA': 0.0}, 'M': {'ATG': 1.0},  
 'L': {'CTT': 0.0, 'CTG': 0.6, 'CTA': 0.0, 'CTC': 0.0, 'TTA': 0.4, 'TTG': 0.0},  
 'N': {'AAT': 0.5, 'AAC': 0.5},  
 'Q': {'CAA': 0.6, 'CAG': 0.4},  
 'P': {'CCT': 0.5, 'CCG': 0.0, 'CCA': 0.5, 'CCC': 0.0},  
 'S': {'TCT': 0.0, 'AGC': 0.0, 'TCG': 0.0, 'AGT': 0.5, 'TCC': 0.0, 'TCA': 0.5},  
 'R': {'CGA': 0.5, 'CGC': 0.0, 'AGA': 0.5, 'AGG': 0.0, 'CGG': 0.0, 'CGT': 0.0},  
 'T': {'ACC': 0.0, 'ACA': 0.0, 'ACG': 0.0, 'ACT': 1.0},  
 'W': {'TGG': 1.0},  
 'V': {'GTA': 0.6, 'GTC': 0.0, 'GTT': 0.2, 'GTG': 0.2},  
 'Y': {'TAT': 1.0, 'TAC': 0.0}}
```

That data structure is a dictionary with keys corresponding to amino acids (i.e. single letter strings designating an amino acid such as 'R' for arginine). The value associated

with *each* amino acid key is also a dictionary, and the keys of *this* dictionary should be the different codons that encode the amino acid. The value associated with each codon key should be a number, representing the frequency with which that codon is used to encode that amino acid. The final data structure should only include amino acids that are found in the ORF.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `sample_orfs.txt` contains open reading frame sequences you can work on.
- `codonbiasproject.py` is an empty file where you must write your code.
- `test_codonbiasproject.py` is the test program that lets you test the code you write in `codonbiasproject.py`.

Now open each file in *VScode* and have a look at what is in `sample_orfs.txt`. (Do *not* change it in any way and do not save it after viewing. If *VScode* asks you if you want to save it before closing, say *no*.) How many sequences are there in each file?

As in the translation project, you will need a data structure that pairs each codon to the amino acid it encodes. This is an obvious use of a dictionary and at the top of `codonbiasproject.py` I have defined such a dictionary you can use. Defining it outside the functions means that it is visible inside all your functions (unless you define *another* variable called `codon_map` inside a function). Defining variables globally like this sometimes make sense if some value can be considered a *constant* in your program and is *never* changed.

“ It is normally very bad programming style to access variables outside functions in this way because it may have all kinds of unexpected side effects across function calls. So make it a rule for yourself that code *inside* a function should never access variables *outside* the function. The reason we define `codon_map` globally in this project is to help you understand that when Python cannot find a variable inside a function, it looks outside the function to find it. In this project, functions will find `codon_map` in this way. However, as I already said, you should *never* do this yourself. The chance that you make an unexpected mistake is overwhelming. ”

The project is split into four parts:

1. Read an open reading frame (ORF) into your script and count the codons.
2. Group the codon counts by the amino acids the codons encode.
3. Convert counts to frequencies.
4. Build the data structure representing the codon bias information.

Read an open reading frame and count its codons

Read ORFs from a file

You can use this code to read the ORFs into your script:

```
f = open('sample_orfs.txt', 'r')
orf_list = []
for line in f:
    seq = line.strip()
    orf_list.append(seq)
f.close()
```

Try to print the list to see it. Then pick out the first ORF in the list so you can use that to test your code:

```
test_orf = orf_list[0]
```

Split the ORF into codons

You need a function that splits the ORF into codons. This one you have already implemented in the exercise about translating DNA – and if, not here it is in my version to get you started.

```
def split_codons(orf):
    codon_list = []
    for i in range(0, len(orf)-2, 3):
        codon_list.append(orf[i:i+3])
    return codon_list
```

Before you go on, make sure you understand/remember how this function works and what it returns.

Count codons in an ORF

Now you need to count the number of times each codon occurs in the ORF.

Write a function, count_codons, that take one argument:

1. A string, which represents the open reading frame.

The function must return:

- A dictionary where keys are strings representing codons and associated values are integers representing the number of times each codon occurs in the ORF given as argument.

Example usage:

```
count_codons("ATGTCATCATGA")
```

should return:

```
{'CTT': 0, 'ATG': 1, 'ACA': 0, 'ACG': 0, 'ATC': 0, 'AAC': 0,
 'ATA': 0, 'AGG': 0, 'CCT': 0, 'ACT': 0, 'AGC': 0, 'AAG': 0,
 'AGA': 0, 'CAT': 0, 'AAT': 0, 'ATT': 0, 'CTG': 0, 'CTA': 0,
 'CTC': 0, 'CAC': 0, 'AAA': 0, 'CCG': 0, 'AGT': 0, 'CCA': 0,
 'CAA': 0, 'CCC': 0, 'TAT': 0, 'GGT': 0, 'TGT': 0, 'CGA': 0,
 'CAG': 0, 'TCT': 0, 'GAT': 0, 'CGG': 0, 'TTT': 0, 'TGC': 0,
 'GGG': 0, 'TAG': 0, 'GGA': 0, 'TGG': 0, 'GGC': 0, 'TAC': 0,
 'TTC': 0, 'TCG': 0, 'TTA': 0, 'TTG': 0, 'TCC': 0, 'ACC': 0,
 'TAA': 0, 'GCA': 0, 'GTA': 0, 'GCC': 0, 'GTC': 0, 'GCG': 0,
 'GTG': 0, 'GAG': 0, 'GTT': 0, 'GCT': 0, 'TGA': 1, 'GAC': 0,
 'CGT': 0, 'GAA': 0, 'TCA': 2, 'CGC': 0}
```

– though not necessarily with key-value pairs in that order.

In the function, you should use the `split_codons` function to split the ORF into a list of codons. Then create an empty dictionary that you can populate with counts. You want *all* the possible codons to be in your dictionary. That way, the codons you do *not* find in your ORF will have a count of 0. In this case, such absence is also valuable information. To achieve this you must start by filling the dictionary with a key for each codon and give each a count of 0. You can do that by iterating over the keys in the dictionary that maps codons to amino acids. Then you must iterate over all the codons in the list of codons produced by the `split_codons` function and add counts to the dictionary as you go.

Group codon counts by amino acid

Having counted how many times each codon appears in the ORF, you need to group the counted codons by the amino acid they encode.

Write a function, `group_counts_by_amino_acid`, which takes one argument:

1. A dictionary, as that returned by `count_codons`.

The function must return:

- A dictionary of dictionaries, which pairs each amino acid with a dictionary with counts of how many times each codon is used to encode that amino acid in the ORF.

Example usage: Assuming counts is the dictionary returned by count_codons as in the previous example.

```
grouped_counts = group_counts_by_amino_acid(counts)
```

then group_counts_by_amino_acid should return:

```
{'A': {'GCA': 0, 'GCC': 0, 'GCT': 0, 'GCG': 0},  
 'C': {'TGC': 0, 'TGT': 0},  
 'E': {'GAG': 0, 'GAA': 0},  
 'D': {'GAT': 0, 'GAC': 0},  
 'G': {'GGT': 0, 'GGG': 0, 'GGA': 0, 'GGC': 0},  
 'F': {'TTC': 0, 'TTT': 0},  
 'I': {'ATT': 0, 'ATC': 0, 'ATA': 0},  
 'H': {'CAC': 0, 'CAT': 0},  
 'K': {'AAG': 0, 'AAA': 0},  
 '*': {'TAG': 0, 'TGA': 1, 'TAA': 0},  
 'M': {'ATG': 1},  
 'L': {'CTT': 0, 'CTG': 0, 'CTA': 0, 'CTC': 0, 'TTA': 0, 'TTG': 0},  
 'N': {'AAT': 0, 'AAC': 0},  
 'Q': {'CAA': 0, 'CAG': 0},  
 'P': {'CCT': 0, 'CCG': 0, 'CCA': 0, 'CCC': 0},  
 'S': {'TCT': 0, 'AGC': 0, 'TCG': 0, 'AGT': 0, 'TCC': 0, 'TCA': 2},  
 'R': {'AGG': 0, 'CGC': 0, 'CGG': 0, 'CGA': 0, 'AGA': 0, 'CGT': 0},  
 'T': {'ACC': 0, 'ACA': 0, 'ACG': 0, 'ACT': 0},  
 'W': {'TGG': 0},  
 'V': {'GTA': 0, 'GTC': 0, 'GTT': 0, 'GTG': 0},  
 'Y': {'TAT': 0, 'TAC': 0}}
```

- though not necessarily with key-value pairs in that order.

So if we pretend the resulting dictionary of dictionaries is called d, then d['S'] will be a dictionary where keys are codons encoding the 'S' amino acid and the values are the counts of those codons. That means that you can access a particular count like this:

```
d['S']['TCA']
```

In the above example, this count is 2. So the task is really just to distribute counts given as the first argument into groups for each amino acid. Depending on what you call your variables it could look something like this:

```
grouped_counts[acid][codon] = codon_counts[codon]
```

Your function should begin by defining an empty dictionary to add to. Then use a for-loop to run through all codon/amino-acid pairs and populate your dictionary of dictionaries.

Turn counts into frequencies

Now you know how many times each codon represents a certain amino acid, but we would like to know with which *frequency* a certain codon represents an amino acid. So you need to normalize the counts so they become frequencies. You do that by dividing each codon count by the total number of codons encoding the same amino acid. We split the solution to this problem in two. We first write a helper function that turns codon counts for *one* amino acid into frequencies.

Write a function, normalize_counts, which takes one argument:

1. A dictionary, where keys are strings representing codons and values are integers representing the counts of these codons.

The function must return:

- A dictionary, where keys are strings representing codons and values are floats representing the frequency at which each codon appears. That is, the count of that codon divided by the total of all codon counts in the dictionary. The frequencies for codons that encode the same amino acid must sum to one. That means that in cases where the total count is zero, the function must return None.

Example usage:

```
normalize_counts({'ATT': 8, 'ATC': 10, 'ATA': 2})
```

should return:

```
{'ATC': 0.5, 'ATA': 0.1, 'ATT': 0.4}
```

- though not necessarily with key-value pairs in that order.

Now you have solved part of the task, what remains is to now use that function to normalise the codon counts of *each* amino acid in your grouped counts:

Write a function, normalize_grouped_counts, which takes one argument:

1. A dictionary of dictionaries, as that returned by group_counts_by_amino_acid.

The function must return:

- A new dictionary of dictionaries where the values of the inner dictionaries are frequencies instead of counts as in the example in the introduction. You should not include amino acids for which there are no counted codons.

Example usage: Assuming gr_counts is the dictionary of dictionaries returned by grouped_group_counts_by_amino_acid in the example above.

```
normalize_grouped_counts(gr_counts)
```

should return:

```
{'*': {'TAA': 0.0, 'TGA': 1.0, 'TAG': 0.0},
'M': {'ATG': 1.0},
'S': {'AGC': 0.0, 'TCG': 0.0, 'TCC': 0.0, 'TCT': 0.0, 'AGT': 0.0, 'TCA': 1.0}}
```

- though not necessarily with key-value pairs in that order.

Here is some help to get you started:

```
def normalize_grouped_counts(grouped_counts):
    grouped_freqs = {}
    for aa in grouped_counts:
        counts = grouped_counts[aa]
```

Amino acids with no codon counts should *not* be part of the data structure. Remember that in this case normalize_counts returns None, so you can simply test if the return value from normalize_counts is None

Compute the codon usage

Now all that remains is to tie together the functions you have written in a final function that generates your big data structure from an ORF:

Write a function, `codon_usage`, which takes one argument:

1. A string, which is an ORF.

The function must return:

- A dictionary of dictionaries, same as that returned by `normalize_grouped_counts`.

23 HIV sub-groups

This chapter is a programming project where you will put your new programming skills to use analyzing an HIV DNA sequences.

You have now been introduced to all the programming rules you will see in this course. You now know all the building blocks required to write *any* program – literally *any*. The reason why computer geeks are good at what they do is not that they know some incomprehensible secrets. It is because they practiced, a lot. With practice, the simple rules you know now will let you write anything from first-person shooter games over jumbo jet autopilots to scripts for simple problems in bioinformatics. In the last three chapters, we will train your ability to solve bioinformatics problems by putting together all the things you have learned.

The programming project in this chapter deals with DNA sequences from HIV viruses. There are two types of HIV: HIV-1, which is by far the most common, and HIV-2, which is mostly found in West Africa. HIV-1 vira are divided into groups M, N, O, and P. The most important group M (for major) is one primarily responsible for the global epidemic. Group M is further divided into subtypes A, B, C, D, F, G, J, K, and CRFs. In this project we will look at sequences from the subtypes A, B, C, and D. You have multiple database sequences for each of these four subtypes and you have one *unknown* sequence from a patient that you need to assign to either subtype A, B, C or D. To do this you will have to write a program that *predicts* the subtype of the unknown sequence. How cool is that?

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `unknown_type.txt` contains an HIV sequence of unknown subtype
- `subtypeA.txt` contains a database of HIV sequences of subtype A
- `subtypeB.txt` contains a database of HIV sequences of subtype B
- `subtypeC.txt` contains a database of HIV sequences of subtype C
- `subtypeD.txt` contains a database of HIV sequences of subtype D

You also need to download the two project files:

- `hivproject.py` is an empty file where you must write your code.
- `test_hivproject.py` is the test program that lets you test the code you write in `hivproject.py`.

Put the files in a folder dedicated to this project. On most computers you can right-click on the link and choose “Save file as...” or “Download linked file”.

Now open each file in *VScode* and have a look at what is in the data files. (Do *not* change them in any way and do not save them after viewing. If *VScode* asks you if you want to save it before closing, say *no*.) How many sequences are there in each file?

The project is divided into the following parts:

- Compute the similarity of two sequences
- Read the HIV sequences into your program.
- Assess the similarity of your unknown HIV sequence to each of the HIV sequences with known subtype.
- Find the maximum similarity of your unknown sequence to sequences from each subtype.
- Identify the HIV subtype of your sequence as the subtype of that sequence that your sequence is most similar to.

Make sure you read the entire exercise and understand what you are supposed to do before you begin!

Compute the similarity of two sequences

We need to compare our unknown HIV sequence to all the HIV sequences of known subtypes. That way we can identify the sequence of a known subtype that is most similar to your unknown sequence. We will then assume that our unknown sequence has the same subtype as this sequence. To accomplish this we first need to write some code that compares two sequences so we can compare our HIV sequence to each of the other HIV sequences.

Compare two sequences

Write a function `sequence_similarity` that takes two arguments:

1. A string which is a DNA sequence.
2. A string of the same length as argument one, which is also a DNA sequence.

The function must return:

- A float, which is the proportion of bases that are the same in two DNA sequences.

Example usage:

```
sequence_similarity('AGTC' 'AGTT')
```

should return 0.75.

Start out defining your function like this:

```
def sequence_similarity(seq1, seq2):  
    # your code here...
```

Remember that `range(len(seq1))` generates the numbers you can use to index the string `seq1`. You can use those numbers as indexes to look up positions in both strings. You will need a for-loop in your function and a variable that keeps track of how many similarities you have seen as you iterate through the sequences.

Compare aligned sequences

All sequences, including the unknown sequence, are from the same multiple alignment. This ensures that sequence positions match up across all sequences but also means that a lot of gap characters ('-') are inserted. To compute similarities between such sequences you need to make function much like `sequence_similarity` that does not consider sequence positions where both bases are a gap ('-') characters. In other words, you must not only count the number of characters that are the same, you also need to count how many alignment columns that are "-" for both sequences. E.g. the following mini alignment has five such columns and four columns where the bases are the same. So in the following alignment, the similarity is 0.8 (4/5):

```
A-CT-A  
A-CTTA
```

Write a function `alignment_similarity` that takes two arguments:

1. A string which is a DNA sequence with gap characters.
2. A string of the same length as argument one, which is also a DNA sequence with gap characters.

The function must return:

- A float, which is the proportion of bases that are the same in two DNA sequences.

```
alignment_similarity('A-CT-A', 'A-CTTA')
```

should return 0.8.

i Hint

Use an if-statement to test if the two characters at some index are equal to '-' in both sequences. You can use an expression like this:

```
seq1[i] == '-' and seq2[i] == '-'
```

Once your function has computed both the number of identical bases and the number of alignment columns that are not both '-', you can have it return the similarity as the ratio of the two.

Read the HIV sequences into your program

To use your `alignment_similarity` function to assess similarity between your unknown sequence and the sequences of known subtype, you need to read the sequences into your program. Here is a function that will read the sequences from one of the files you downloaded into a list:

```
def read_data(file_name):
    f = open(file_name)
    sequence_list = list()
    for line in f:
        seq = line.strip()
        sequence_list.append(seq)
    f.close()
    return sequence_list
```

You can use that function to read the unknown sequence into your program:

```
unknown_list = read_data('unknown_type.txt')
```

In this case, the list only contains the one unknown HIV sequence in `unknown_type.txt`.

You also need to load the typed HIV sequences into your program. Here is a function that returns a dictionary in which the keys are subtypes ('A', 'B', 'C' and 'D') and each value is a lists of sequences with that subtype:

```
def load_typed_sequences():
    return {'A': read_data('subtypeA.txt'),
            'B': read_data('subtypeB.txt'),
            'C': read_data('subtypeC.txt'),
            'D': read_data('subtypeD.txt') }
```

If you use the function like this:

```
typed_data = load_typed_sequences()
```

then you can access the list of sequences of subtype A like this: `typed_data['A']`.

Compare your HIV sequence to HIV sequences of known subtype

To type your HIV sequence you must compare your sequence to all the database sequences to see which group has the best matching sequence.

Write a function `get_similarities` that takes two arguments:

1. A string, which is your unknown HIV sequence.
2. A list of strings, each of which is an HIV sequence of known type.

The function must return:

- A list of floats, which should be the similarities between the unknown sequence given as the first argument and the list of sequences given as the second argument.

Example usage:

```
get_similarities(unknown_list[0], typed_data['A'])
```

should return:

```
[0.8553288474061211, 0.8721742704480066,
 0.854924397221087, 0.8481709291032696,
 0.8498330281159108]
```

The function should use the function `alignment_similarity` to compare your unknown sequence (`unknown_list[0]`) to each of the sequences of some subtype. Start out like this:

```
def get_similarities(unknown, typed_sequences):
    # Your code here...
```

In your function you need to define a list that you can *append* the similarities you compute to:

```
similarities = []
```

This is the list of results that your function must return. To compute the similarity between your unknown sequence and each of the sequences of known subtype, you can use your alignment_similarity function inside a for-loop.

Compute maximum similarity to each subtype

To predict the subtype of the unknown HIV sequence you need to compare the unknown sequence to all the sequences of each of the different subtypes. The subtype of the sequence with the highest similarity to your unknown sequence is then our predicted subtype (or our best guess).

Write a function get_max_similarities that takes two arguments:

1. A string, which is your unknown HIV sequence.
2. A dictionary, like the one returned by load_typed_sequences.

The function must return:

- A dictionary, in which keys are strings representing each subtype ('A', 'B', 'C', and 'D') and values are floats representing the maximum similarity between the unknown sequence and the sequences of a subtype. The dictionary *could* look like this (it does not, you need to compute the similarities yourself.):

```
{'A': 0.89, 'B': 0.95, 'C': 0.82, 'D': 0.99}
```

To get the highest number in a list of numbers, you can use the `max` function in Python. It works like this:

```
numbers = [3, 8, 53, 12, 7]
print(max(numbers)) # prints 53
```

For example, to get the highest similarity between the unknown sequence and sequences in `typed_data['A']`:

```
subtypeA_similarities = get_similarities(unknown_list[0], typed_data['A'])
subtypeA_max = max(subtypeA_similarities)
```

Identify the HIV subtype

Now for the grand finale! You ultimately want to be able to write code like this:

```
unknown_list = read_data('unknown_type.txt')
typed_data = load_typed_sequences()
subtype = predict_subtype(unknown_list[0], typed_data)
print("Patient HIV is subtype {}".format(subtype))
```

So all you need now is the predict_subtype function.

Write a function predict_subtype that takes two arguments:

1. A string, which is your unknown HIV sequence.
2. A dictionary, like the one returned by load_typed_sequences.

The function must return:

- A string of length one (either 'A', 'B', 'C', or 'D') representing the predicted subtype of your unknown HIV sequence.

The function should use get_max_similarities to compute the dictionary of max similarities and then extract from that dictionary the key with the highest value (similarity). So the function must return 'A' if the unknown sequence is most similar to a sequence of subtype A, 'B' if the unknown sequence is most similar to a sequence of subtype B and so on.

24 Sequence trees

This chapter is about clustering sequence based on the evolutionary distance between them.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `seqdistproject.py` is an empty file where you must write your code.
- `test_seqdistproject.py` is the test program that lets you test the code you write in `translationproject.py`.

Put the files in a folder dedicated to this project. On most computers you can right-click on the link and choose “Save file as...” or “Download linked file”.

Measuring sequence distance

Clustering is based on the distances between all pairs of sequences. So before you can build your tree you must compute those distances and fill them into a table like that in the book. Here we break that task into three parts:

1. Compare two sequences
2. Make the Jukes-Cantor correction
3. Generate a (lower triangular) distance matrix

Compare two sequences

The first function you must write is one that finds the proportion of different bases between two sequences:

Write a function, `sequence_difference`, which takes two arguments:

1. A string, which represents a DNA sequence.
2. A string, which represents a DNA sequence of the same length as argument one.

The function must return:

- A float, which represents the proportion of different bases between the two sequences.

Example usage:

```
sequence_difference('AAATTAAA', 'AAAAAAA')
```

should return

0.25

Make the Jukes-Cantor correction

To take into account that some substitutions may fall on top of others you must do the Jukes-Cantor correction you read about in the book. The formula is like this:

$$K = \frac{3}{4} \ln\left(1 - \frac{4}{3} D\right)$$

Where D is the proportion of differences as returned by `sequence_diff` and K is the Jukes-Cantor corrected distance. In the top of `seqdistproject.py` it already says:

```
from math import log
```

That line makes the `log` (logarithm) builtin function from the `math` python library available to your programme. Try to find its Python documentation to see how you use it.

Write a function, `jukes_cantor`, which takes one argument:

1. A float, which represents a proportion of different bases between two sequences.

The function must return:

- A float, which represents the Jukes-Cantor corrected distance corresponding the proportion of differences given as argument.

Example usage:

```
jukes_cantor(0.1)
```

should return

0.10732563273050497

Lower triangular distance matrices

This project is all about distances between pairs (of sequences), and what would be more natural than to put all the distances in a matrix so you can look up the distance between the sequences with indexes i and j as the matrix element in row i and column j . You already know how matrices can be represented by lists of lists. E.g. a matrix like this:

can be expressed as a list of lists like this:

```
[[0.0, 0.1, 0.3, 0.3, 0.1, 0.2],  
 [0.1, 0.0, 0.2, 0.4, 0.1, 0.1],  
 [0.3, 0.2, 0.0, 0.4, 0.2, 0.3],  
 [0.3, 0.4, 0.4, 0.0, 0.2, 0.1],  
 [0.1, 0.1, 0.2, 0.2, 0.0, 0.1],  
 [0.2, 0.1, 0.3, 0.1, 0.1, 0.0]]
```

Notice how the diagonal is all zeros because these distances represent the distance of a sequence to itself. Also, notice that the part above the diagonal is a mirror the part below the diagonal (in bold). This is all a bit redundant, especially in this project where you will have to reduce the matrix as you group (or cluster) sequences together. We want something nice and lean where we only have the numbers we need – and that is the *lower triangular matrix*:

```
0.1  
0.3 0.2  
0.3 0.4 0.4  
0.1 0.1 0.2 0.2  
0.2 0.1 0.3 0.1 0.1
```

In Python this is still just a list of lists, only, each sublist now has the same length as its index in the big list (E.g. `[0.3, 0.2]` has index 2 in the list and has length 2):

```
matrix = [[],  
           [0.1],  
           [0.3, 0.2],  
           [0.3, 0.4, 0.4],  
           [0.1, 0.1, 0.2, 0.2],  
           [0.2, 0.1, 0.3, 0.1, 0.1]]
```

Here I am just writing it nicely. If you were to print that list of lists it would look like this:

```
[], [0.1], [0.3, 0.2], [0.3, 0.4, 0.4], [0.1, 0.1, 0.2, 0.2], [0.2, 0.1, 0.3, 0.1, 0.1]]
```

Say your sequences had names: A, B, C, D, E, and F, then the above data structure represents distances between each pair like this:

A					
B	0.1				
C	0.3	0.2			
D	0.3	0.4	0.4		
E	0.1	0.1	0.2	0.2	
F	0.2	0.1	0.3	0.1	0.1
A	B	C	D	E	F

There is only one drawback with this reduced representation of the full square matrix: In the full matrix you can get the distance between the sequences with indexes i and j as *both* `matrix[i][j]` and `matrix[j][i]` because the part above and below the diagonal are the same. Using the lower triangular matrix, you must always use the *largest* index first. Using the smaller one first will give you an `IndexError`. So if you want the distance between sequences with index 2 and 4, you must use the bigger index first (as the row index): `matrix[4][2]`.

Generate a distance matrix

Write a function, `lower_trian_matrix`, which takes one argument:

1. A list of strings. All strings have equal length and represent DNA sequences.

The function must return:

- A list of lists of floats, which represent the lower triangular matrix of Jukes-Cantor distances between DNA sequences given as argument.

Example usage:

```
sequences = ['TAAAAAAAAAAA',  
            'TTAAAAAAAAAA',  
            'AAAAAAAAGGG',  
            'AAAAAAAAGGGG']  
lower_trian_matrix(sequences)
```

here `lower_trian_matrix` should return:

```
[[],  
 [0.08833727674228764],  
 [0.30409883108112323, 0.4408399986765892],  
 [0.6081976621622466, 0.8239592165010822, 0.18848582121067953]]
```

You should use `sequence_difference` to compute the proportion of differences between each pair of sequences and `jukes_cantor` to produce the corrected distance to fill into the matrix.

Start by figuring out what pairs of indexes you need and then figure out how you can make two nested for-loops generate them. Remember that the length of each sublist is equal to its index in the big list.

Clustering

Now that you have the distance matrix you are ready for the actual clustering. There are three steps to that:

1. Find the pair you want to join
2. Compute the distances between the joined pair and all other elements (linkage)
3. Keep going until you only have one left

Depending on how you choose which pair to join and how you compute the new distances for the joined pair determines what kind of clustering you do. Here we will try a centroid-like linkage called WPGMA. It does not work as well as UPGMA but is a bit easier to implement (you can look up WPGMA on wikipedia).

Find the pair to join

Here you want to be able to find the pair with the smallest distance. To do that we identify the cell in the matrix with the smallest value:

Write a function, `find_lowest_cell`, which takes one argument:

1. A list of lists, which represents a lower triangular distance matrix as returned by `lower_trian_matrix`.

The function must return:

- A list of two integers, which represent the row and column index of the cell with the smallest value in the matrix.

Remember that the row index is always smaller than the column index. The two indexes tell you which two elements to join next.

Example usage: Assuming that `matrix` is the lower triangular matrix returned by `lower_trian_matrix` in the previous example, then

```
find_lowest_cell(matrix)
```

Should return

```
[1, 0]
```

Decide on a linkage method

You also need a function that computes a new distance from two original ones using the the centroid-like linkage we have decided to use.

Write a function, `link`, that takes two arguments:

1. A float, which represents a matrix element.
2. A float, which represents another matrix element.

The function must return:

- A float, which is the average of the two arguments

Example usage:

```
link(0.4, 0.2)
```

Should return:

```
0.3
```

Perform the clustering

The three functions that do the actual clustering are complicated but you should be able to follow what they do. The first one updates the table to reflect that you join a pair. The second updates the list of sequence names (labels) to reflect that you joined a pair. The last one uses the two other functions to cluster pair until there is only one cluster left.

Your task is to carefully type the code for each function and to understand what every line of code does.

Updating labels

The function `update_labels` takes three arguments:

1. A list of strings representing sequence names.
2. An integer representing the index of a sequence name.
3. An integer representing the index of another sequence name.

The function does not return anything, but it updates the list of names to reflect that you joined a pair. If your list looks like this *before* you call the function:

```
labels = ['A', 'B', 'C', 'D']
```

Then *after* you call the function like this `update_labels(labels, 1, 0)`, the list will look like this:

```
['(A,B)', 'C', 'D']
```

Here is the function:

```
def update_labels(labels, i, j):  
  
    # turn the label at first index into a combination of both labels  
    labels[j] = "({},{})".format(labels[j], labels[i])  
  
    # Remove the (now redundant) label in the first index  
    del labels[i]
```

Updating the matrix

The function `update_table` takes three arguments:

1. A list of lists, which represents a lower triangular distance matrix.
2. An integer representing the index of one of the elements to join.
3. An integer representing the index of the other element to join.

The way this function is implemented, it is assumed that the second argument is always larger than the third argument. I.e. the second argument is a row index and the third argument is a column index.

The function does not return anything, but it updates the matrix to reflect that a pair has been joined. If your matrix looks like this *before* you call the function:

```
m = [[], [0.1], [0.3, 0.4], [0.6, 0.8, 0.2]]
```

Then *after* you call the function like this `update_table(m, 1, 0)`, the matrix will look like this:

```
[], [0.35], [0.7, 0.2]]
```

Here is the function:

```
def update_table(table, a, b):  
  
    # For the lower index, reconstruct the entire row (ORANGE)  
    for i in range(0, b):  
        table[b][i] = link(table[b][i], table[a][i])  
  
    # Link cells to update the column above the min cell (BLUE)  
    for i in range(b+1, a):  
        table[i][b] = link(table[i][b], table[a][i])  
  
    # Link cells to update the column below the min cell (RED)  
    for i in range(a+1, len(table)):  
        table[i][b] = link(table[i][b], table[i][a])  
  
    # Delete cells we no longer need (lighter colors)  
    for i in range(a+1, len(table)):  
        # Remove the (now redundant) first index column entry  
        del table[i][a]  
    # Remove the (now redundant) first index row  
    del table[a]
```

The colors refer to cell colors on the slide you I showed you at the lecture.

Do the clustering

Now onto the real task, the actual clustering. The function `cluster` takes two arguments:

1. A list of strings representing DNA sequences of equal length.
2. A list of strings representing sequence names.

The function returns:

- A string representing the generated clustering.

Here is the function:

```
def cluster(sequences, names):

    table = lower_trian_matrix(sequences)
    labels = names[:]

    # Until all labels have been joined...
    while len(labels) > 1:
        # Locate lowest cell in the table
        i, j = find_lowest_cell(table)

        # Join the table on the cell co-ordinates
        update_table(table, i, j)

        # Update the labels accordingly
        update_labels(labels, i, j)

    # Return the final label
    return labels[0]
```

Here is a simple example of how you can use your new clustering code:

```
names = ['A', 'B', 'C', 'D']
sequences = ['TAAAAAAAAAAA',
             'TTAAAAAAAAAA',
             'AAAAAAAAGG',
             'AAAAAAAAGGG']  
  
tree = cluster(sequences, names)
print(tree)
```

On your own

From here on you are on your own. If you find a FASTA file with aligned (ungapped) homologous sequences, you can use the function below to read it into your program and try your code out on real-world sequences. I will leave it to you to figure out how it works.

```
def read_fasta(filename):
    f = open(filename, 'r')
    record_list = []
    header = ""
    sequence = ""
    for line in f:
        line = line.strip() ## get rid of whitespace and newline
        if line.startswith(">"):
            if header != "": ## if it is the first header
                record_list.append([header, sequence])
                sequence = ""
            header = line[1:]
        else:
            sequence += line
    record_list.append([header, sequence])
    return record_list
```

25 Finding genes

This chapter is a programming project where you will find open reading frames in the genome of a particularly virulent strain of E. coli.

In this project, you will analyze DNA to identify the open reading frames (ORFs) and the proteins they encode.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `e_coli_0157_H157_str_Sakai.fasta` contains the full genome of the *Escherichia coli* O157:H7 Sakai strain (yes the full genome).

You also need to download the two project files:

- `orfproject.py` is an empty file where you must write your code.
- `test_orfproject.py` is the test program that lets you test the code you write in `orfproject.py`.

Put all three files in a folder dedicated to this project. On most computers you can right-click on the link and choose “Save file as...” or “Download linked file”.

For your convenience, the file `orfproject.py` already contains three global *constants* (variables that must never be changed by the code). One is a dictionary `codon_map`, which maps codons to letters that represent amino acids. The other two are a string, `start_codon`, and a list, `stop_codons`. You can refer to these three variables in your code, but obviously, never change them.

The project has three parts.

1. In the first part of the project, you will write the code necessary to identify ORFs in a long genomic sequence.
2. In the second part, you will use the code you wrote on the programming project about translating DNA to translate each ORF into a protein sequence.
3. In the third part, you will use the code from parts one and two together to find candidate proteins encoded by ORFs in a genomic sequence.

Start by reading through the exercise before you do anything else. That way you have a good overview of the tasks ahead. Here is a mind map of how we split the larger problem into smaller bits and how they fit together:

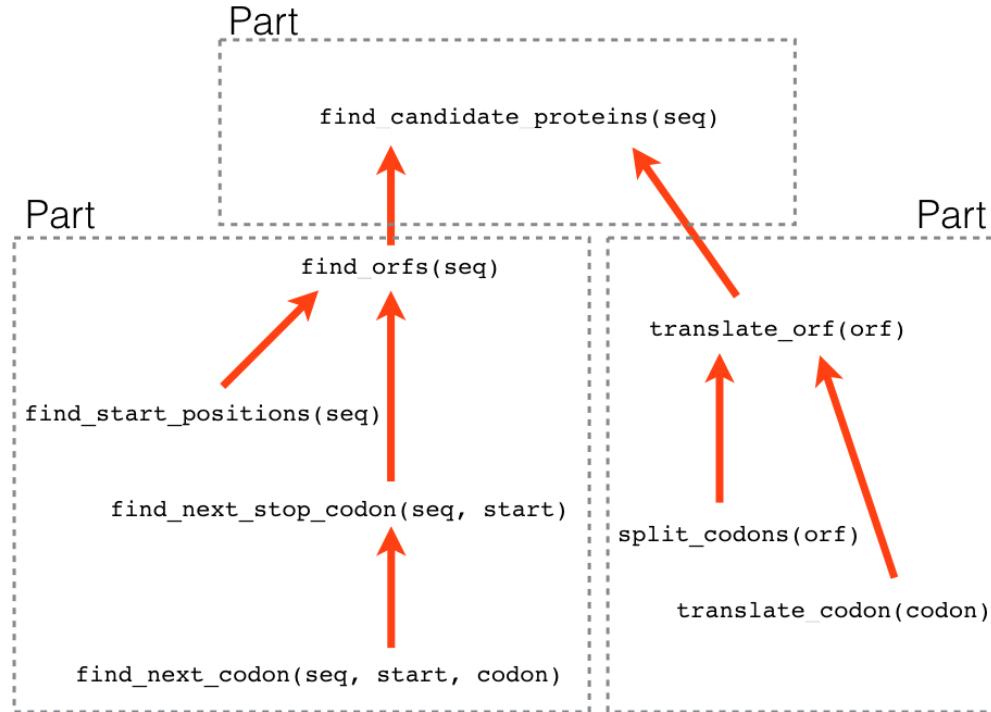


Figure 25.1: Overview

Finding Open Reading Frames

Find the start positions of ORFs in a DNA sequence

The first task is to write a function that finds all the possible positions where an ORF can begin.

Write a function, `find_start_positions`, which takes one argument:

1. A string, which is a DNA sequence.

The function must return:

- A list of integers, which represent the indexes of the first base in start codons in the DNA sequence argument.

Example usage:

```
find_start_positions('TATGCATGATG')
```

should return

[1, 5, 8]

Your function should contain a for-loop that iterates over all possible positions in a DNA string where a codon can begin. Not surprisingly, these are all the positions except for the last two. So start out with this:

```
def find_start_positions(seq):
    for i in range(len(seq) - 2):
        print(i)
```

Now, instead of just printing i, try and make it print the three bases following i using the slicing technique:

```
triplet = seq[i:i+3]
```

I.e. if your sequence is 'TATGCATGATG' it should first print 'TAT' then 'ATG' then 'TGC' and so on.

When you have this working you should change the code so that triplets are only printed if they are start codons. You can use an if-statement that tests if each triplet is equal to start_codon.

Then try and make your function print i only when i is the first base of a start codon.

Finally, modify the function so all the relevant values of i are collected in a list using the same technique as in the split_codons(orf) function, and then return this list from the function.

Finding the next occurrence of some codon in an ORF

Now that you can find where the ORFs begin in our sequence you must also be able to identify where each of these end. As you know, an ORF ends at any of three different stop codons in the same reading frame as the start codon. So, starting at the start codon of the ORF, we need to be able to find the next occurrence of some specific codon. I.e. you should look at all codons after the start codon and find the first occurrence of some specified codon. If the function does not find that codon in the string it should return None.

Write a function, `find_next_codon`, that takes three arguments:

1. A string, which is the DNA sequence.
2. An integer, which is the index in the sequence where the ORF starts.
3. A string, which is the codon to find the next occurrence of.

The function must return:

- An integer, which is the index of the first base in the next in-frame occurrence of the codon. If the function does not find that codon in the string it should return None.

Example usage:

```
find_next_codon('AAAAAATTAATTAA', 1, 'TTT')
```

should return

10

Your function should contain a for-loop that iterates over all the relevant starts of codons. Remember that no valid codon can start at the last two positions in the sequence. E.g. if the second argument is 7 and the length of the sequence is 20 then the relevant indexes are 7, 10, 13, 16.

Start by writing a function just with a for-loop that lets you print these indexes produced by range. Figure out how to make the range function iterate over the appropriate numbers.

```
def find_next_codon(seq, start, codon):  
    for idx in range( ?? ):  
        print(idx)
```

When you have that working, use the slicing technique to instead print the codons that start at each index.

Finally, add an if-statement that tests if each codon is equal to codon. When this is true, the function should return the value of idx.

Finding the first stop codon in an ORF

Now that you can find the next occurrence of any codon, you are well set up to write a function that finds the index for the beginning of the next in-frame *stop codon* in an ORF.

Write a function, `find_next_stop_codon`, that takes two arguments:

1. A string, which is the DNA sequence.
2. An integer, which is the index in the sequence where the ORF starts.

The function must return:

- An integer, which is the index of the first base in the next in-frame stop codon. If there is no in-frame stop codon in the sequence the function should return None.

Example usage:

```
find_next_stop_codon('AAAAATAGATGAAA', 2)
```

should return

5

Here is some inspiration:

1. You should define a list to hold the indexes for the in-frame stop codons we find.
2. Then we loop over the three possible stop codons to find the next in-frame occurrence of each one from the start index. You can use `find_next_codon` for this. Remember that it returns None if it does not find any. If it does find a position you can add it to your list.
3. At the end, you should test if you have any indexes in your list.
4. If you do, you should return the smallest index in the list. I.e the ones closest to the start codon.
5. If you did not find any stop codons the function must return None to indicate this.

Finding ORFs

Now you can write a function that uses `find_start_positions` and `find_next_stop_codon` to extract the start and end indexes of each ORF in a genomic sequence.

Write a function, `find_orfs`, that takes one argument:

1. A string, which is a DNA sequence.

The function should return:

- A list, which contains lists with two integers. The list returned must contain a list for each ORF in the sequence argument. These lists each contain two integers. The first integer represents the start of the ORF, the second represents the end. The function should handle both uppercase and lowercase sequences.

Example usage:

```
find_orfs("AAAATGGGTAGAATGAAATGA")
```

should return

```
[[3, 9], [13, 19]]
```

Start by using `find_start_positions` to get a list of all the start positions in sequence:

```
def find_orfs(seq):
    start_positions = find_start_positions(seq)
```

When you have that working, add a for-loop that iterates over the start positions. Inside the for-loop, you can then get the next in-frame stop codon for each start position by calling `find_next_stop_codon`. Try to print the start and end indexes you find to make sure the code does what you think.

Finally, you need to add a `[start, stop]` list for each ORF to the big list that the function returns. To append a list to a list you do write something like this:

```
orf_coordinate_list.append([start, stop])
```

Test your function. Chances are that some of the end positions you get are `None`. This is because some of the start codons were not followed by an in-frame stop codon. Add an if-statement to your function that controls that only start-stop pairs with a valid stop coordinate are added to the list of results.

Translation of open reading frames

We need to translate the reading frames we find into the proteins they may encode. So why not use the code you already wrote in the programming project where you translated open frames? Copy the content of `translationproject.py` into `orfproject.py`. Now you can use the function `translate_orf` to translate your ORFs.

Put everything together

Read in genomic sequences

The file `e_coli_O157_H157_str_Sakai.fasta` contains the genome that we want to analyze to find open reading frames. This is an especially nasty strain of *Escherichia coli* O157:H7 isolated after a massive outbreak of infection in school children in Sakai City, Japan, associated with consumption of white radish sprouts.

You can use the function below to read the genome sequence into a string.

```
def read_genome(file_name):
    f = open(file_name, 'r')
    lines = f.readlines()
    header = lines.pop(0)
    substrings = []
    for line in lines:
        substrings.append(line.strip())
    genome = ''.join(substrings)
    f.close()
    return genome
```

Now for the grand finale: Using `read_genome`, `find_orfs` and `translate_orf` you can write a function that finds all protein sequences produced by open reading frames in the genome.

Write a function, `find_candidate_proteins`, that takes one argument:

1. A string, which is a genome DNA sequence.

The function must return

- A list of strings, which each represent a possible protein sequence.

Note that this is a full genome so finding all possible proteins will take a while (~5 min.). You can start by working on the first 1000 bases:

Example usage:

```
genome = read_genome('e_coli_0157_H157_str_Sakai.fasta')
first_1000_bases = genome[:1000]
find_candidate_proteins(first_1000_bases)
```

should return

```
['MSLCGLKKESLTAASELVTCRE*', 'MKRISTTITTTTITTTITGNGAG*',  
 'MQNVFCGLPIFWKAMPGRGRWPSSLPPPSPPTTWWR*', 'MPGRGRWPPSSLPPPSPPTTWWR*',  
 'MLYPISAMPNVFLPNF*', 'MPNVFLPNF*', 'MSCMALVC*', 'MALVC*']
```

The function should call `find_orfs` to get the list of start-end pairs. For each index pair, you must then slice the ORF out of the sequence (remember that the end index represents the first base in the stop codon), translate the ORF to protein, and add it to a list of proteins that the function can return.

i Hint

To check your result note that all returned sequences should start with a start codon 'M', end with a stop codon '*' and contain no stop codons in the middle.

On your own

This is where this project ends, but *you* can continue if you like. Given a long list of candidate proteins of all sizes, what would you do to narrow down your prediction to a smaller set of very likely genes? If you have some ideas, then try them out.

- Maybe you can rank them by length? What is the expected minimum length of proteins?
- Maybe you can look for a Shine-Delgarno motif upstream of the start codon? You know how to do that from the lectures.
- You can also try to BLAST them against the proteins in Genbank. The true ones should have some homologs in other species.

26 Genome assembly

This chapter is a programming project where you will assemble a small genomic sequence from a set of short sequencing reads.

In genome assembly, many short sequences (reads) from a sequencing machine are assembled into long sequences – ultimately chromosomes. This is done by ordering overlapping reads so that they together represent genomic sequences. For example, given these three reads: AGGTCGTAG, CGTAGAGCTGGGAG, GGGAGGTTGAAA, ordering them based on their overlap like this

```
AGGTCGTAG  
CGTAGAGCTGGGAG  
GGGAGGTTGAAA
```

produces the following genomic sequence:

```
AGGTCGTAGAGCTGGGAGGTTGAAA
```

Real genome assembly is, of course, more sophisticated than what we do here, but the idea is the same. To limit the complexity of the problem, we make two simplifying assumptions:

1. There are no sequencing errors, implying that true overlaps between reads can be identified as perfectly matching overlaps.
2. No reads are nested in other reads. I.e., They are never a smaller part of another read.

The second assumption implies that overlaps are always of this type:

```
CGTAGAGCTGGGAG  
GGGAGGTTGAAA
```

and never of this type:

```
CGTAGAGCTGGGAG  
AGAGCTG
```

In this project, you will be asked to write functions that solve the problem of assembling a genomic sequence. Each function solves a small problem, and you may need to call these functions inside other functions to put together solutions to larger subproblems.

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

- `sequencing_reads.txt` contains the sequencing reads.

You also need to download the two project files:

- `assemblyproject.py` is an empty file where you must write your code.
- `test_assemblyproject.py` is the test program that lets you test the code you write in `assemblyproject.py`.

Put the files in a folder dedicated to this project. On most computers you can right-click on the link and choose “Save file as...” or “Download linked file”.

Now open each file in your editor and look at what is in `sequencing_reads.txt`. (Do *not* change it in any way, and do not save it after viewing. If your editor asks you if you want to save it before closing, say *no*.) How many sequences are there in each file?

The project is split into four parts:

1. Read and analyze the sequencing reads.
2. Compute the overlaps between reads.
3. Find the right order of reads.
4. Reconstruct the genomic sequence.

Here is an overview of the functions you will write in each part of the project and of which functions are used by other functions.

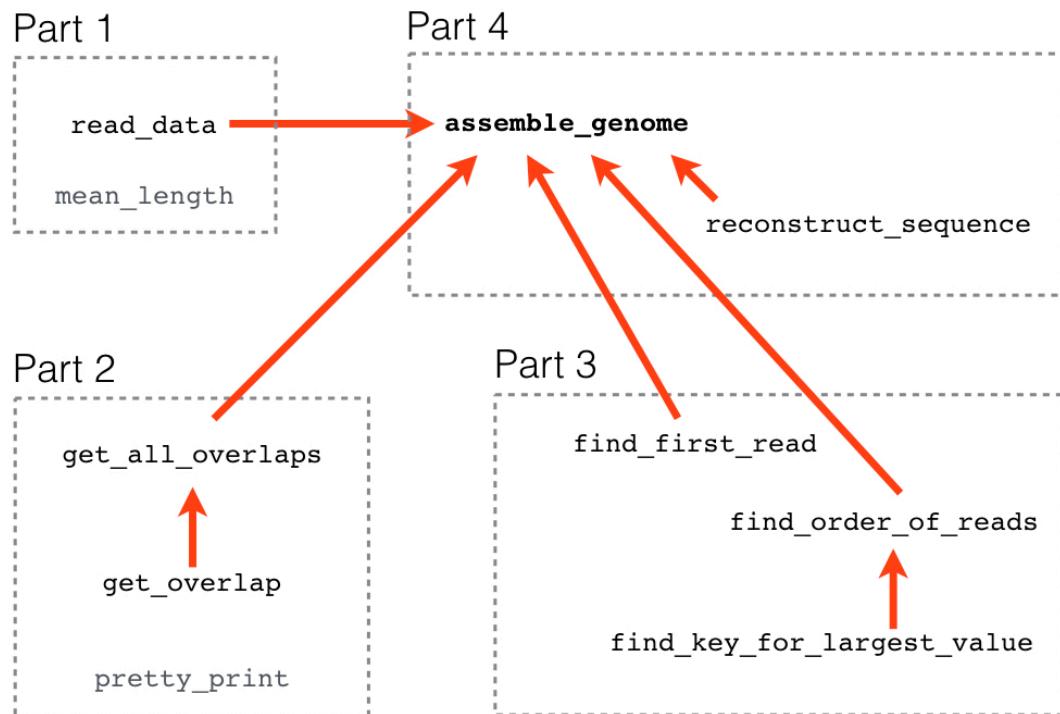


Figure 26.1: Overview

Make sure to read the entire exercise and understand what you are supposed to do before you begin!

Read and analyze the sequencing reads

The first task is to read and parse the input data. The sequence reads for the mini-assembly are in the file `sequencing_reads.txt`. The first two lines of the file look like this:

```
Read1 GGCTCCCCACGGGGTACCCATAACTTGACAGTAGATCTCGTCCAGACCCCTAGC
Read2 CTTTACCCGGAAGAGCGGGACGCTGCCCTGCGCATTCCAGGCTCCCCACGGG
```

Each line represents a read. The first field on each line is the name of the read, and the second field is the read sequence itself. So for the first line, Read1 is the name, and ATGCG... is the sequence.

Read the sequencing reads into your program

Write a function, `read_data`, that takes one argument:

1. A string, which is the name of the data file.

The function must return

- A dictionary, where the keys are the *names* of reads and the values are the associated read *sequences*. Both keys and values must be strings.

Example usage:

```
read_data('sequencing_reads.txt')
```

should return a dictionary with the following content (maybe not with key-value pairs in that order)

```
{'Read1': 'GGCTCCCCACGGGGTACCCATAACTGACAGTAGATCTCGTCCAGACCCCTAGC',
 'Read3': 'GTCTTCAGTAGAAAATTGTTTTTCTTCAAGAGGTGGAGTCGTGAACACATCAGT',
 'Read2': 'CTTTACCCGGAAGAGCGGGACGCTGCCCTGCGCGATTCAGGCTCCCACGGG',
 'Read5': 'CGATTCAGGCTCCCCACGGGGTACCCATAACTGACAGTAGATCTC',
 'Read4': 'TGCGAGGGAAGTGAAGTATTGACCCTTACCCGGAAGAGCG',
 'Read6': 'TGACAGTAGATCTCGTCCAGACCCCTAGCTGGTACGTCTCAGTAGAAAATTGTTTTCTTCCAAGAGGTGGAA'}
```

Here is some scaffolding code to get you started:

```
def read_data(file_name):
    input_file = open(file_name)
    # ...
    for line in input_file:
        # ...
    # ...

    input_file.close()
```

The `line` variable in the for loop holds each line in the file, including the `\n` newline character at the end. You can use the 'split' method of strings to split each line into the name of the read and the read sequence. You can see the documentation for that method by typing `pydoc str.split` in your terminal.

Compute the mean length of reads

After writing that function, we would like to get an idea about the length of the reads. There are often too many reads to look at manually, so we need to make a function that computes the mean length of the reads.

Write a function, `mean_length`, that takes one argument:

1. A dictionary, in which keys are read names and values are read sequences (this is a dictionary like that returned by `read_data`).

The function must return

- A float, which is the average length of the sequence reads.

One way to do this is to loop over the keys in the dictionary like this:

```
def mean_length(reads):  
    count = 0  
    total = 0  
    for name in reads:  
        seq = reads[name]  
        # ...  
        # ...  
    # ...  
    return total / count
```

Remember that you can use the `len` function to find the length of a read.

Compute overlaps between reads

The next step is to determine which reads overlap each other. We need a function that takes two read sequences and computes their overlap to do that. Remember that in the input data, none of the reads are completely nested in another read.

Compute the overlap between two reads

We know there are no sequencing errors so that the sequence match will be perfect in the overlap. To compute the overlap between the 3' (right) end of the left read with the 5' (left) end of the righthand read, you need to loop over all possible overlaps, honoring that one sequence is the left one and the other is the right one. In the for loop, start with the largest possible overlap (`min(len(left), len(right))`) and evaluate smaller and smaller overlaps until you find an exact match.

Write a function, get_overlap, that takes two arguments

1. A string, which is the lefthand read sequence.
2. A string, which is the righthand read sequence.

The function must return

- A string, which is the overlapping sequence. If there is no overlap, it should return an empty string.

Example usage:

```
s1 = "CGATTCAGGCTCCCACGGGTACCCATAACTTGACAGTAGATCTC"  
s2 = "GGCTCCCCACGGGTACCCATAACTTGACAGTAGATCTCGTCCAGACCCCTAGC"  
get_overlap(s1, s2)
```

should return the string

```
'GGCTCCCCACGGGTACCCATAACTTGACAGTAGATCTC'
```

and `get_overlap(s2, s1)`

should return the string

```
'C'
```

From these two examples, it seems that `s1` and `s2` overlap and that `s1` is the left and `s2` is the right. Treating `s2` as the left one and `s1` as the right one only gives an overlap of one base (we expect a few bases of overlap even for unrelated sequences).

Compute all read overlaps

When you have written `get_overlap`, you can use it to evaluate the overlap between all pairs of reads in both left-right and right-left orientations.

Write a function, get_all_overlaps, that takes one argument:

1. A dictionary with read data as returned by `read_data`.

The function must return

- A dictionary of dictionaries specifying the number of overlapping bases for a pair of reads in a specific left-right orientation. Computing the overlap of a read to itself is meaningless and must not be included. Assuming the resulting dictionary of dictionaries is called `d`, then `d['Read2']` will be a dictionary where keys are the names of reads that have an overlap with read 'Read2' when 'Read2' is put in the left position, and the values for these keys are the number of overlapping bases for those reads.

Example usage: assuming that `reads` is a dictionary returned by `read_data` then:

```
get_all_overlaps(reads)
```

should return the following dictionary of dictionaries (but not necessarily with the same ordering of the key-value pairs):

```
{'Read1': {'Read3': 0, 'Read2': 1, 'Read5': 1, 'Read4': 0, 'Read6': 29},  
 'Read3': {'Read1': 0, 'Read2': 0, 'Read5': 0, 'Read4': 1, 'Read6': 1},  
 'Read2': {'Read1': 13, 'Read3': 1, 'Read5': 21, 'Read4': 0, 'Read6': 0},  
 'Read5': {'Read1': 39, 'Read3': 0, 'Read2': 1, 'Read4': 0, 'Read6': 14},  
 'Read4': {'Read1': 1, 'Read3': 1, 'Read2': 17, 'Read5': 2, 'Read6': 0},  
 'Read6': {'Read1': 0, 'Read3': 43, 'Read2': 0, 'Read5': 0, 'Read4': 1}}
```

i Hint

You can use the `get_overlap` function you just made to find the overlap between a pair of reads. To generate all combinations of reads, you need two for-loops. One looping over reads in the left position, and another (inside the first one) looping over reads in the right position. Remember that we do *not* want the overlap of a read to itself, so there should be an if-statement checking that the left and right reads are not the same.

Print overlaps as a nice table

The dictionary returned by `get_all_overlaps` is a bit messy. We want to print it in a nice matrix-like format so we can better see which pairs overlap in which orientations.

This `pretty_print` function should take one argument:

1. A dictionary of dictionaries as returned by `get_all_overlaps`.

The function should *not* return anything but must *print* a matrix exactly as shown in the example below with nicely aligned and right-justified columns. The first column must hold names of reads in left orientation. The top row holds names of reads in right

orientation. The remaining cells must each have the number of overlapping bases for a left-right read pair. The diagonal corresponds to overlaps with the read itself. You must put dashes in these cells.

Example usage: assuming that `overlaps` is a dictionary of dictionaries returned by `get_all_overlaps` then:

```
pretty_print(overlaps)
```

should print exactly

	Read1	Read2	Read3	Read4	Read5	Read6
Read1	-	1	0	0	1	29
Read2	13	-	1	0	21	0
Read3	0	0	-	1	0	1
Read4	1	17	1	-	2	0
Read5	39	1	0	0	-	14
Read6	0	0	43	1	0	-

This function is hard to get completely right. So, to spare you the frustration, this one is on me:

```
def pretty_print(d):
    print('      ', end=' ')
    for j in sorted(d):
        print("{: >6}".format(j), end=' ')
    print()
    for i in sorted(d):
        print("{: >6}".format(i), end=' ')
        for j in sorted(d):
            if i == j:
                s = '      -'
            else:
                s = "{: >6}".format(d[str(i)][str(j)])
            print(s, end=' ')
    print()
```

Make sure you understand how it works. You can look up in the documentation what `"{: >6}" .format(i)` does.

Find the correct order of reads

Now that we know how the reads overlap, we can chain them pair by pair from left to right to get the order in which they represent the genomic sequence. To do this, we take the first (left-most) read and identify which read has the largest overlap at its right end. Then we take that read and find the read with the largest overlap to the right end of that - until we reach the rightmost (last) read.

Find the first read

The first thing you need to do is to identify the first (leftmost) read so we know where to start. This read is identified as the one that has no significant (>2) overlaps to its left end (it only has a good overlap when positioned to the left of other reads). In the example output from `pretty_print` above, the first read would be read 'Read4' because the 'Read4' column has no significant overlaps (no one larger than two).

We break the problem in two and first write a function that gets all the overlaps to the left end of a read (i.e., when it is in the right position):

Write a function, `get_left_overlaps`, that takes two arguments:

1. A dictionary of dictionaries as returned from `get_all_overlaps`.
2. A string, which represents the name of a read.

The function must return

- A sorted list of integers, which represent the overlaps of other reads to its left end.

Example usage: assuming that `overlaps` is a dictionary of dictionaries returned by `get_all_overlaps` then.

```
get_left_overlaps(overlaps, 'Read1')
```

should return

```
[0, 0, 1, 13, 39]
```

i Hint

Once you have made a list of left overlaps, you can use the built-in function `sorted` to make a sorted version of the list that you can return from the function.

OK, now that we have a function that can find all the overlaps to the left end of a given read, all we need to do is find the particular read with no significant (>2) overlaps to its left end.

Write a function, `find_first_read`, that takes one argument:

1. A dictionary of dictionaries as returned from `get_all_overlaps`.

The function must return

- A string containing the name of the first read.

Example usage: assuming that `overlaps` is a dictionary of dictionaries returned by `get_all_overlaps` then.

```
find_first_read(overlaps)
```

should return

```
'Read4'
```

Find the order of reads

Now that we have the first read, we can find the correct order of the reads. We want a list of the read names in the right order.

Given the first (left) read, the next read is the one that has the largest overlap to the right end of that read. We use our dictionary of overlaps to figure out which read that is. If the first read is 'Read4', then `overlaps['Read4']` is a dictionary of reads with overlap to the right end of read 'Read4'. So, to find the name of the read with the largest overlap, you must write a function that finds the key associated with the largest value in a dictionary. We do that first:

Write a function, `find_key_for_largest_value`, that takes one argument:

1. A dictionary.

The function must return the key associated with the largest value in the dictionary argument.

Having written `find_key_for_largest_value`, you can use it as a tool in the function that finds the order of reads:

Write a function, `find_order_of_reads`, that takes two arguments:

1. A string, which is the name of the first (left-most) read (that is returned by `find_first_read`).

2. A dictionary of dictionaries of all overlaps (that returned by `get_all_overlaps`).

The function must return

- A list of strings, which are read names in the order in which they represent the genomic sequence.

i Hint

You know the first read is given by the first argument to the function. You also know that you can find the next read in the chain of overlapping reads by using the `find_key_for_largest_value` function. You should keep adding reads to the chain as long as the overlap is larger than two (you can use a for loop with an if statement inside to check that the overlap is larger than 2).

Example usage: assuming that `overlaps` is a dictionary of dictionaries returned by `get_all_overlaps` then:

```
find_order_of_reads('Read4', overlaps)
```

should return:

```
['Read4', 'Read2', 'Read5', 'Read1', 'Read6', 'Read3']
```

Before you implement the function, make sure you understand why this is the right list of read names.

Reconstruct the genomic sequence

Now that you have the number of overlapping bases between reads and the correct order of the reads, you can reconstruct the genomic sequence.

Reconstruct the genomic sequence from the reads

Write a function, `reconstruct_sequence`, that takes three arguments:

1. A list of strings, which are the names of reads in the order identified by `find_order_of_reads`.
2. A dictionary, with read data as returned from `read_data`.
3. A dictionary of dictionaries with overlaps as returned from `get_all_overlaps`.

The function must return

- A string, which is the genomic sequence.

Example usage: assuming that `order` is the list of strings returned by `find_order_of_reads`, that `reads` is the dictionary returned by `read_data` and that `overlaps` is a dictionary of dictionaries returned by `get_all_overlaps` then:

```
reconstruct_sequence(order, reads, overlaps)
```

should return one long DNA string (I had to break it in three to make it fit on the page):

```
TGCGAGGGAAGTGAAGTATTGACCTTACCCGGAAGAGCGGGACGCTGCCCTGCGCGATT  
CCAGGCTCCCCACGGGTACCCATAACTTGACAGTAGATCTGTCCAGACCCCTAGCTGGTA  
CGTCTTCAGTAGAAAATTGTTTTCTTCCAAGAGGTGGAGTCGTGAACACATCAGT
```

Hint

Iterate over the reads in order and use the overlap information to extract and join the appropriate parts of the reads.

Putting the whole thing together

Now that you have written functions to handle each step, you can write one last function that uses them to complete the entire assembly.

Write a function, `assemble_genome`, that takes one argument:

1. A string, which is the name of a file with sequencing reads in the format described at the beginning of this project description.

The function must return

- A string, which is the genome assembled from the sequencing reads

Example usage:

```
assemble_genome('sequencing_reads.txt')
```

should return the assembled genome:

```
TGCGAGGGAAGTGAAGTATTGACCTTACCCGGAAGAGCGGGACGCTGCCCTGCGCGATT  
CCAGGCTCCCCACGGGTACCCATAACTTGACAGTAGATCTGTCCAGACCCCTAGCTGGTA  
CGTCTTCAGTAGAAAATTGTTTTCTTCCAAGAGGTGGAGTCGTGAACACATCAGT
```

Part III

Web exercises

27 GWAS candidates

The purpose of this exercise is to expose you to the different kinds of information that are stored in databases relevant to bioinformatics. It takes experience and skill to navigate the user interface of these databases. Here, you will see a few important ones, but there are many others. I have put a list at the end of this exercise with some additional relevant ones on Brightspace. Browse them at your heart's content.

Genome-wide association studies (GWAS) are a powerful tool in genetics and genomics research to identify genetic variants associated with diseases or traits. When a significant SNP is identified in a GWAS, it is a genomic signpost tagging an associated genomic region. Still, it only partially reveals the genes causing the disease if the genomic region identified contains several genes or genomic features. Investigating candidate disease genes near a GWAS SNP involves: Exploring the genomic region around the SNP. Studying nearby genes and regulatory elements. Evaluating their potential roles in the disease or trait of interest. A standard GWAS only includes a select subset of the SNPs in the genome. So, the most significant included SNP variant is rarely responsible for the disease. Still, it is so close to the causal one that individuals with the causal SNP variant carry it. The closer two SNPs are along the genome, the more likely they appear together like this. The further away from each other, the more likely it is that genetic recombination has removed this correlation.

Type 1 diabetes, often referred to as juvenile diabetes or insulin-dependent diabetes, is a chronic autoimmune condition that affects how the body regulates blood sugar (glucose). Unlike type 2 diabetes, which is commonly associated with lifestyle factors such as obesity and physical inactivity, type 1 diabetes is not preventable and typically develops early in life, often during childhood or adolescence. In type 1 diabetes, the immune system, usually responsible for defending the body against harmful invaders like bacteria and viruses, becomes misguided. It mistakenly identifies the insulin-producing beta cells within the pancreas as foreign threats. This misrecognition triggers an autoimmune response, leading immune cells to launch an attack on these vital beta cells. The pancreas, an organ located behind the stomach, is a key player in regulating blood sugar levels. It consists of clusters of cells called the Islets of Langerhans, which house the insulin-producing beta cells. When the immune system attacks these beta cells, it results in their destruction or severe impairment. This process is thought to involve various immune cells, such as T-cells and antibodies, which play pivotal roles in autoimmune reactions. Due to this autoimmune attack, the pancreas gradually loses its ability to produce insulin, a hormone with crucial responsibilities in maintaining proper blood sugar balance. Insulin acts as a molecular key that unlocks the doors of cells throughout

the body, allowing glucose to enter and be used as an energy source. Think of insulin as a bridge that facilitates the movement of glucose from the bloodstream into cells. Research into type 1 diabetes is ongoing, and scientists are exploring potential cures and better management strategies. However, until a cure is found, individuals with type 1 diabetes continue to lead fulfilling lives by effectively managing their condition through insulin therapy, a balanced diet, regular exercise, and closely monitoring their blood sugar levels.

Browsing SNPs from a GWAS

Start by examining a Manhattan plot or list of significant SNPs obtained from a GWAS study. We are going to select one of these SNPs and do further analysis. While doing the analysis, note the SNP identifier (rsID) and its significance level (p-value). Go to locuszoom.org and press the blue button shown on Figure 27.1:

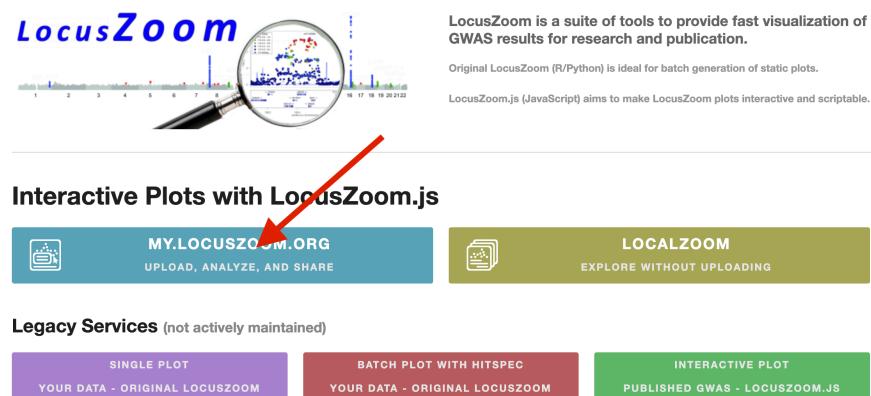


Figure 27.1: Screenshot of the LocusZoom website

Now, you should be able to search for public GWAS studies. Copy/paste “Rare Genetic Variants of Large Effect Influence Risk of Type 1 Diabetes” into the search bar. Only one result should show up. Press it. Now, you are ready to examine the Manhattan plot. You can see a lot of significant SNPs in the Manhattan plot, and you are welcome to examine them, but the one we are interested in for this exercise is the one located close to RSBN1. Click it, and it will zoom in. The SNP has an rsID of rs6679677, and its position is 113.761.186 on chromosome 1. It’s a C to A mutation. Save this information because we will use it for the rest of the exercise.

Exploring a genomic region

In this section, we are going to explore our SNP and its surrounding genomic regions to identify our candidate gene/genes for diabetes type 1. Go to the UCSC Genome Browser. In the “Human Assembly” drop-down, make sure it says “GRCh38/hg38”. That way, you access the genome assembly also used in the GWAS study. If you choose another one, the SNP and gene coordinates will be different. Now paste your SNP identifier rs6679677 into the search field where it says “Position/Search Term” and click GO. You will get a list of search results. Clicking the link in the top one (if it says rs6679677) should take you to a page centered on this SNP looking something like the one shown in Figure 27.2:

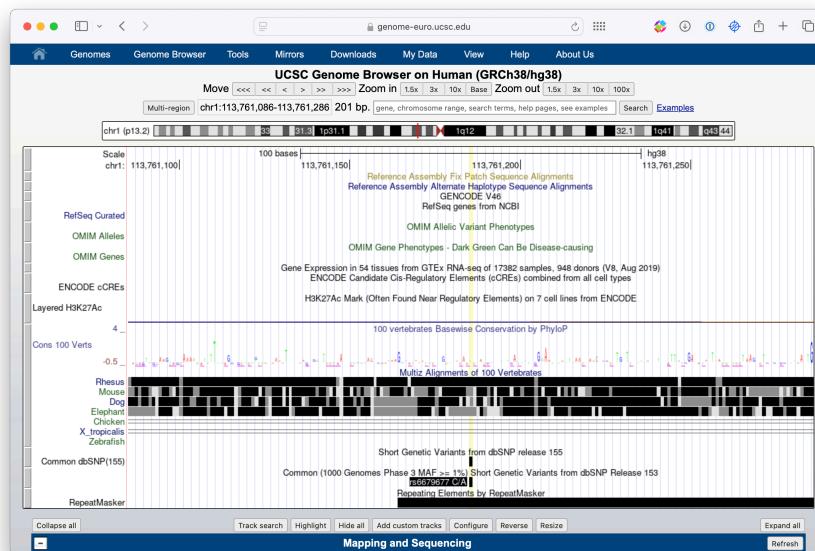


Figure 27.2: Screenshot of the UCSC genome browser

Now, you see the SNP in a window. You can play around with the zoom function until you start seeing a couple of other SNP IDs. Now we want to look at surrounding genes, so scroll down to the “Genes and Gene predictions” Section. All the options you see here are called “tracks”, and they all contain different information about the genome. If you want to look at the annotated gene, select the “GENCODE V44” select “pack” and press refresh on the right side of the section. Play around with the different settings (pack, dense, squish, all). They all give you a different amount of information. Select the one that you are most comfortable with when exploring. You might have to change it for some types of questions. “Pack” is a good place to start.

Exercise 27-1

Play around with the zoom function. To the left of where you inserted the SNP ID is the size of the window you are looking at. The more you zoom out, the more genes you will be able to see.

Exercise 27-2

Identify nearby genes, including their names, locations, and orientations. Remember that linkage means that the causal gene may not be the one closest to the GWAS SNP. Are there any genes located close to the SNP of interest? If yes. Note them down for further analysis.

Exercise 27-3

What is the genomic context (e.g., intronic, intergenic) of the SNP?

Exercise 27-4

You see the same gene multiple times in the gene track. Why do you think that is?

Exercise 27-5

Try to select other tracks such as promoters (EPDnew Promoters) and regulatory elements (ENCODE Regulation). See if there are known regulatory elements, such as enhancers or promoters, in the vicinity of the SNP.

Exercise 27-6

Are there any annotated regulatory elements near the SNP that might influence gene expression? Try to press the regulatory track. What kind of information does this track represent? (Hint: Read the description of the track).

Gathering information about genes

Now, we are ready to look at the genes we are interested in relation to our SNP. For this, we will use 3 well-known databases. The goal is to identify which genes are the potential cause of type 1 diabetes. Take your genes to each of the websites and use the information they give you to see which genes can be related to diabetes.

- NCBI Gene
- ENSEMBL
- Genecards

It's important to understand what kind of information these sites provide and their intentions. So, let us do some light exploration.

Exercise 27-7

Google "NCBI". What is it, and who administers it? Do the same with ENSEMBL. What's its function, and who administers it?

Exercise 27-8

On the NCBI Gene website, the drop-down menu shows "gene", but other databases on NCBI exist. Get yourself an overview. Do you recognize other databases? Some you don't know?

Exercise 27-9

When a gene is found in the "About this gene" section at ENSEMBL, try to press "phenotypes" if there are any. Do you see diabetes for any of the genes?

Exercise 27-10

The Genecards resource is different from the two other databases. Can you list two major differences?

Exercise 27-11

By now, you should have a feeling for the databases and their goals. NCBI and ENSEMBL store the same sequence information, so it is mostly about which site presents data in the most useful way for the task at hand. Which one is your favorite one and why? Which of the genes are related to Type 1 Diabetes? If you can't find a related gene, go back to "Accessing the UCSC Genome Browser and make initial exploration" and look at your tracks again. You may have missed it.

Exercise 27-12

With the gene you now have chosen, answer the following questions by using the 3 above databases.

1. How long is the entire of the gene (in bp or kb)?
2. How many transcripts of the gene are in NCBI's "Transcript table"?
3. Does Ensembl and NCBI show the same number of transcripts?
4. In the graphical representation of a gene, how are exons and introns depicted at ENSEMBL and NCBI?
5. At ENSEMBL, try to click on the UniprotKN identifier. This takes you to a page in the "UniProt" database. Can you retrieve the amino acid sequence? - Do that.

Exploring tissue expression

In this step, we are interested in investigating our gene's expression. Or multiple genes if we have yet to choose a specific gene. Expression analysis will help us understand the genes better. Go to the GTEx Portal (<https://www.gtexportal.org/home/>) and search for the gene/genes associated with your SNP to see their expression profiles across tissues. Explore tissue-specific gene expression data for the identified genes. In which tissues is the gene(s) expressed, and is there any tissue where its expression is notably higher? You may know "EBV-transformed lymphocytes" as "lymphocytes" cells. They are important to the immune system.

Exercise 27-13

In which tissues is the gene(s) typically expressed, and are there variations in expression levels across different tissues?

Exercise 27-14

Do the expression profiles make sense with the information you got from the other databases? Do you expect to see certain tissues with a high expression? Other with a low? Is it relevant to the disease we are investigating?

Exercise 27-15

What can you infer about the potential functional relevance of the gene(s) in the context of the disease or trait?

Hypotheses and Discussion

Based on the information gathered from UCSC, various gene databases, and GTEx, propose hypotheses about the potential role of the identified gene(s) in the disease or trait associated with the SNP. Think about the importance of tissue-specific gene expression in understanding disease mechanisms. Are there any other genes or regulatory elements near the SNP that could also be relevant to the disease or trait?

Exercise 27-16

Based on the genomic and expression data, what are your hypotheses regarding the role of the identified gene(s) in the disease or trait?

Exercise 27-17

How might tissue-specific gene expression patterns provide insights into the disease mechanism?

Exercise 27-18

Are there potential challenges or limitations in establishing causality between the gene(s) and the disease?

Exercise 27-19

Summarize your findings and discuss the significance of investigating candidate disease genes near a GWAS SNP. Reflect on the challenges and limitations of this approach in pinpointing causal genes for complex diseases.

Exercise 27-20

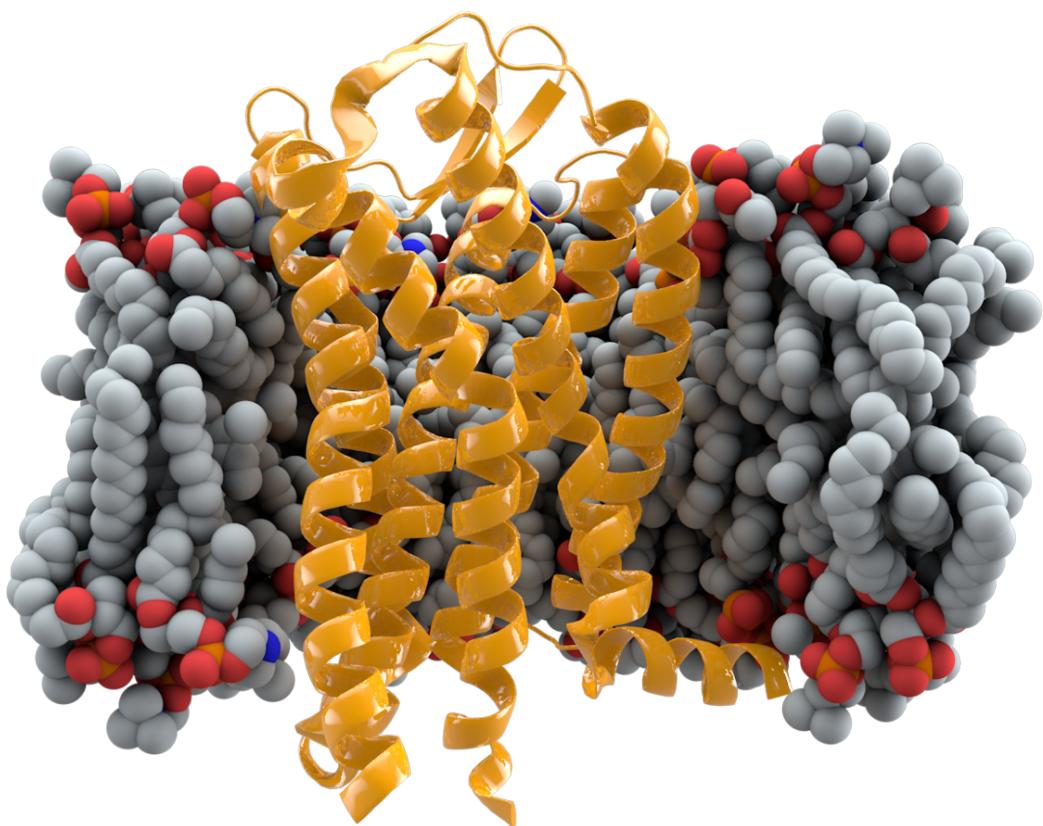
What did you learn from this exercise about the importance of exploring the genomic context and tissue-specific gene expression in GWAS follow-up?

Exercise 27-21

How might these findings inform future research or therapeutic approaches for the disease or trait?

28 CCR5-delta32

Human Immunodeficiency Virus targets immune cells



HIV (Human Immunodeficiency Virus) infects immune cells by specifically targeting and binding to certain receptors on the surface of these cells. The primary immune cells that HIV infects are CD4+ T cells (commonly known as T-helper cells), macrophages, and dendritic cells. Here's a simplified step-by-step explanation of how HIV infects immune cells: The first step in the infection process involves the attachment of HIV to immune cells. The virus carries a glycoprotein on its surface called gp120, which binds to the CD4 receptor on the surface of CD4+ T cells, macrophages, and dendritic cells. This interaction is the initial binding event between the virus and the host cell. After

binding to CD4, HIV also requires a co-receptor to gain entry into the host cell. Two common co-receptors used by the virus are CCR5 and CXCR4. Depending on the viral strain, it can use one or both of these co-receptors. This interaction between gp120 and the co-receptor triggers a conformational change in the virus, allowing it to fuse with the host cell membrane. The conformational change in the virus membrane allows it to fuse with the host cell membrane. This fusion event enables the virus to release its genetic material into the interior of the host cell. Once inside the host cell, HIV carries an enzyme called reverse transcriptase, which converts its single-stranded RNA genome into double-stranded DNA. This process is known as reverse transcription.

The newly formed viral DNA is transported into the cell's nucleus, where it is integrated into the host cell's DNA. The enzyme integrase plays a critical role in this step. Once integrated, the viral DNA becomes a permanent part of the host cell's genetic material. The integrated viral DNA is transcribed into messenger RNA (mRNA), which is then translated by the host cell's machinery to produce viral proteins and RNA. This leads to the assembly of new viral particles. New viral particles are assembled in the host cell and are released from the cell's surface in a process known as budding. The new virus particles can go on to infect other immune cells and continue the cycle of infection. As HIV continues to infect and replicate within CD4+ T cells, macrophages, and dendritic cells, the immune system's response is compromised. Over time, the gradual loss of CD4+ T cells, which are crucial for coordinating the immune response, weakens the immune system's ability to defend the body against various infections. This progressive immune system decline eventually leads to the clinical symptoms associated with AIDS (Acquired Immunodeficiency Syndrome).

Can specific gene variants provide resistance to HIV?

The CCR5 gene and its protective variant, CCR5-delta32, thus play a pivotal role in the complex interplay between human genetics and HIV. The CCR5 gene, specifically its CCR5-delta32 mutation, has garnered significant attention in the field of HIV research due to its remarkable ability to confer a degree of natural resistance against the virus. The CCR5 gene, short for "C-C chemokine receptor type 5," encodes a protein receptor found on the surface of certain immune cells, including T-cells and macrophages. This receptor acts as a gateway for HIV to enter these cells, a critical step in the virus's infection cycle. However, a naturally occurring genetic variant of CCR5 known as CCR5-delta32 possesses a mutation that renders the receptor non-functional. Individuals who inherit two copies of this mutation are notably resistant to HIV infection, as the virus struggles to enter and infect their immune cells. This extraordinary genetic resistance has raised considerable interest in the scientific and medical communities, as it offers insights into potential HIV treatment strategies and the development of innovative therapies.

The frequency of the CCR5-delta32 variant varies significantly across different populations worldwide. It is important to note that historical factors, such as population migrations, genetic bottlenecks, and the prevalence of diseases like HIV, can influence the presence and distribution of this protective mutation. Here's an overview of the frequency of the CCR5-delta32 mutation in different regions: The CCR5-delta32 mutation is most common in populations of Northern European descent, particularly in Scandinavia. In some regions of Northern Europe, such as Sweden and Finland, the mutation can be found in approximately 10-15% of the population. This high prevalence is thought to have resulted from strong selection pressures due to past epidemics, like the bubonic plague. As you move southward in Europe, the frequency of the CCR5-delta32 mutation decreases. In Southern European countries like Spain and Italy, the prevalence drops to about 5% or even lower. The CCR5-delta32 mutation is relatively rare in African, Asian, and Indigenous populations. In most cases, it occurs in less than 1% of these populations. The low frequency can be attributed to the historical lack of selective pressure from diseases like HIV in these regions.

Aim

Your goal is to use pairwise alignment to find the position of the delta32 mutation and establish if it is a deletion, insertion, or nucleotide substitution. This way, you, or other researchers, will be able to learn how the mutation changes the gene's protein-coding sequence.

The learning goals of this exercise are:

- Acquire practical experience using online alignment tools.
- Understanding how parameters in the Needleman-Wunch algorithms affects the alignment.
- Understand the different uses of DNA and protein alignment.
- Acquire practical experience using online Blast to search an NCBI sequence database.
- Getting acquainted with different file formats for storing sequence information.

Preparation

Fasta sequence format

Files containing DNA or protein sequences are just text files formatted in a particular way so that each sequence can be associated with additional information. The most simple format is called FASTA format, and files with sequences in this format are called FASTA files and are usually given either a ".fa" or ".fasta" suffix. A FASTA has two

elements for each sequence: a header line with a leading ">" character followed by one or more lines with the DNA or protein sequence (the sequence may be broken over several lines). The content of a FASTA file with two (short) sequences in it could look like this:

```
>7423344 some additional description  
AGTCCCTTGCA  
TTATTGCAATAT  
>2342134 some additional description  
GGTCCAATTGC  
AAATTGGAATA
```

The first word after the ">" in the header line is usually a sequence identifier; the rest is an additional description or information.

Aligning DNA sequences

For this exercise, you will have four fasta files each with one sequence in it:

- The full mRNA of the normal CCR5 gene: **CCR5_mRNA.fa**
- The full mRNA of the CCR5-delta32 variant: **CCR5_mRNA_delta32.fa**
- The coding sequence (CDS) of CCR5: **CCR5_CDS.fa**
- The coding sequence (CDS) of CCR5-delta32: **CCR5_CDS_delta32.fa**

You can download the files on Brightspace.

Exercise 28-1

Before we head into the actual investigation, lets begin by looking at the exon/intron structure of CCR5 by aligning the mRNAs of CCR5 to its CDS using the Needleman-Wunsch algorithm. Go to the EBI website for pairwise alignment. Under "Sequence type" you need to select "DNA" to enable alignment parameters suitable for DNA alignment. Now align the two sequences by copy/pasting the mRNA into the top input field and the CDS into the bottom one (you must include the header line with the ">" character). Leave the rest of the options with their default values.

Guided Reflections

- What are the other default values? Click "more options" to see what they are?
- Which score matrix gap penalties are used?
- Does the program use linear or affine gap scoring?
- Are gaps at the ends of the alignment scored differently than the internal gaps?

Exercise 28-2

Now click “Submit”, to get your job queued on the server. You, and the bottom part shows you have to wait a bit. When the job is completed and the output is shown, the lines beginning with “#” provide additional information about the alignment. The top part lists the options used for running the “needle” program in the terminameter values and alignment statistics.

Guided Reflections

- What gap scoring was used?
- Which substitution matrix was used?
- Where do you find the result of the alignment?
- The alignment shows differences and similarities between the two sequences. Which differences do you see between the mRNA and the CDS? Why is there a difference?

Exercise 28-3

Now, on to the actual investigation. Begin by aligning the mRNAs of CCR5 and CCR5-delta32 to each other using the Needleman-Wunsch algorithm using the default values of parameters. Look at the alignment results.

Guided Reflections

- Describe the similarities between the two sequences. How many bases are the same and where are they located?
- Describe the differences between the two sequences. Where are the differences located on the mRNA and how many bases are involved? (Hint: 5'-UTR, 3'-UTR, Coding region)

Aligning protein sequences

Exercise 28-4

Now, you will translate the coding sequence of CCR5 and CCR5-delta32 to see which effect the mutation has on the protein level. Go to the ExPasy translate website and set the “Output format” to “verbose”. Then, translate the two coding sequences, one at a time, by pasting the FASTA entries into the field (including the header line).

Guided Reflections

- What are the likely reading frames of each sequence? (number and direction)
- Which differences do you observe based on the translated sequence?

Exercise 28-5

Now go back to the EMBOSS Needle website and align the two protein sequences. Look at the results of the alignment.

Guided Reflections

- Where are the similarities between the two protein sequences?
- Where are the differences between the two protein sequences?
- How does the mutation affect the protein? (Hints: deletion, insertion, missense, nonsense, frameshift)

Searching a database using Blast

Sooty mangabay (*Cercocebus atys*) is a species of primates. Sooty mangabays also have common variants in their CCR5 gene that seem to protect them against SIV (simian immunodeficiency virus). We want to investigate how the Sooty mangabay CCR5 variants are similar or different to the gene variants found in the human CCR5.

Exercise 28-6

You can use Blast to find and investigate these sequences too. Go to the Blast website at NCBI. Clicking “Nucleotide BLAST” will take you to the search interface for the versions of Blast optimized for DNA and RNA searches. Leave the settings to their default values, but take a look around to see what options are available. “Program Selection” lists the version of Blast. We are using megablast for this.

Guided Reflections

- Click the “?” icon to learn a bit about each one. How do you think megablast and blastn may differ in the kmer sizes and thresholds they use?

Exercise 28-7

Before you start the database search, you want to limit its scope to only sequences from sooty mangabey. Set organism to sooty mangabey (“sooty mangabey (taxid:9531)”). Once you do this, it should look like Figure 28.1. Now paste in the CCR5 CDS and click the blue “BLAST” button at the bottom of the page to queue your search request (you may have to wait a bit).

The screenshot shows the BLAST search interface. In the 'Enter Query Sequence' section, the CCR5_CDS sequence is pasted into the text area. In the 'Choose Search Set' section, the 'Organism' dropdown is set to 'sooty mangabey (taxid:9531)'. Under 'Program Selection', the 'Optimize for' option is set to 'Highly similar sequences (megablast)'. At the bottom, the 'BLAST' button is visible.

Figure 28.1: BLAST search interface

Once the search results appear, they are sorted by E-value. The light blue header row lets you sort them by other criteria. “Query cover” sorts the database hits by how much of your search sequence (query) they cover.

Guided Reflections

- What does the E-value mean?
- What types of sequences does the BLAST search return?

Exercise 28-8

Since you are looking for search hits to a CDS in the other species, look for “C-C motif chemokine receptor 5 (CCR5), complete CDS” in the descriptions of search hits and pick the one with the highest percent identity. Clicking the corresponding link in the description column takes you to a local alignment of the part of your query sequence that aligns with the database sequence. In this case, it covers your entire CCR5 CDS.

Guided Reflections

- Describe the differences shown by the alignment. How many bases are different? How many gaps are there?

GenBank sequence format

Exercise 28-9

Now go back to the list of search hits by clicking the “Descriptions” link in the light blue bar above the alignment you were just looking at. See if you can find any search hits with names including “delta”. There should be a delta delta-24 allele among them. Pick the one with sequence ID AF079473.1. Inspect the alignment to the human CCR5.

Guided Reflections

- What characterizes these variants? What does the “delta” in the variant name refer to?
- Given what you now know about the human and mangabey CCR5 genes, do you expect that the delta32 mutation to have arisen before or after the common ancestor of humans and mangabeys?

Exercise 28-10

Go back to the descriptions list by clicking the “Descriptions” link in the light blue bar. On the left are tick boxes for each search hit. Mark only the two you have been looking at (IDs AF051905.1 and AF079473.1). In the “Download” dropdown list pick “GenBank” to download the two sequences in GenBank format. GenBank is a sequence entry format like Fasta, including much more information about each sequence. Open the downloaded file in VScode and see what it looks like.

Guided Reflections

- When were the sequences determined/published?
- What sequencing method was used?
- For each sequence, note another piece of information provided in the GenBank entry format.

Exercise 28-11

In the GenBank file, you can find the translated sequences of each CDS. Make a new text file in VScode and paste the two translated sequences in to create a Fasta file like this:

```
>normal
MDYQVSSPTYDIDYYTSEPCQKINVVKQIAARLLPPLYSLVFIFG
rest of the sequence

>delta24
MDYQVSSPTYDIDYYTSEPCQKINVVKQIAARLLPPLYSLVFIFG
rest of the sequence
```

Now, head back to the EBI needle website and align the two protein sequences. Make sure it is set to protein this time.

Guided Reflections

- Where is the deletion located?
- Does it just delete amino acids, and if so, how many, or does it change the reading frame or induce stop codons?
- How does the mangabey CCR5 mutation compare to that in humans?

Putting it all together

Exercise 28-12

Have a look at this scientific paper: “A Novel CCR5 Mutation Common in Sooty Mangabeys Reveals SIVsmm Infection of CCR5-Null Natural Hosts and Efficient Alternative Coreceptor Use In Vivo” and look at Figure 1 and its figure legend.

Guided Reflections

- Figure 1A shows the alignment of 6 different gene variants. What species are they from and what variants are shown?
- Figure 1B shows the alignment of the predicted protein sequence. What do the grey boxes mean? What do the arrows mean? What does the highlighted sequence mean?

- We have now looked at the sequences of different mutations. Suggest an experimental method/setup, that might show something about the functional importance of the mutations.

Project files

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

29 MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a strain of the staph bacteria that's become resistant to the antibiotics typically used to treat ordinary staph infections. Originally, the *Staphylococcus aureus* bacterium was a common cause of skin infections, pneumonia, and other medical conditions. However, its methicillin-resistant counterpart, MRSA, emerged as a strain that resists many of the conventional antibiotics. This resistance is not limited to just methicillin but extends to other antibiotics, often rendering standard treatments ineffective.

Individuals infected with MRSA can experience a range of ailments, from skin and wound infections to more severe conditions like pneumonia, bloodstream infections, and sepsis. Particularly concerning is its ability to cause life-threatening complications in older or immunocompromised people or people with chronic illnesses.

A critical factor that has amplified MRSA's resilience and adaptability is the phenomenon of horizontal gene transfer (HGT). Unlike the usual vertical transfer of genes from parent to offspring, HGT facilitates the direct exchange of genetic material between different bacterial species. This form of gene transfer has been pivotal in the rapid acquisition and spread of antibiotic resistance genes within microbial communities, including those of *Staphylococcus aureus*.

The most common mechanism of HGT involves direct cell-to-cell contact, where a donor bacterium transfers genetic material, like the SCCmec carrying the *mecA* gene, to a recipient bacterium. Less commonly, viruses that infect bacteria, known as bacteriophages, can mistakenly package bacterial DNA, including resistance genes, and transfer them to another bacterium upon subsequent infection.

MRSA's resilience stems from various genetic mutations but is prominently due to the acquisition of the *mecA* gene. This gene provides the bacteria with the ability to produce a unique protein that alters its cell wall, reducing the efficacy of even last-resort antibiotics, such as methicillin. *mecA* is believed to have been acquired through HGT and often locates to a mobile genetic element called the staphylococcal chromosomal cassette (SCCmec), allowing for its potential transfer between different strains or even species of bacteria.

Given the role of HGT in the rapid spread of resistance, it's evident that MRSA's evolution isn't merely a product of its internal genetic changes. It's deeply intertwined with

a broader microbial ecosystem, where genes, particularly those conferring survival advantages like antibiotic resistance, can be exchanged, adopted, and propagated. This understanding underscores the challenge that antibiotic resistance presents.

Figur 1. Antal MRSA-tilfælde fordelt på år, 2001-2021

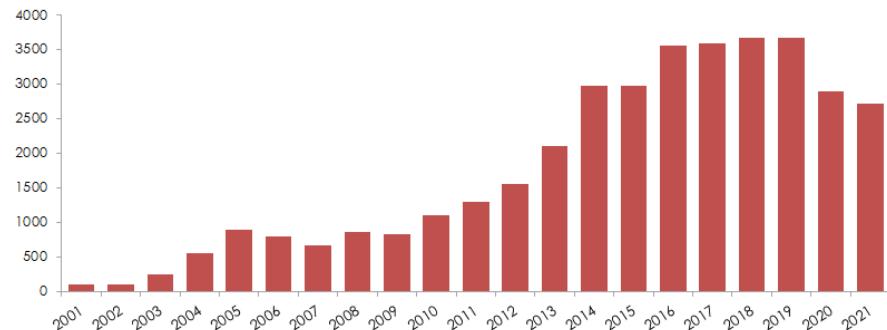


Figure 29.1: MRSA cases per year in Denmark. SSI hypothesises that COVID restrictions and effects is the main cause of the reduction in cases in later years

Investigating the origins of the *mecA* gene in MRSA provides insights into the potential sources and the evolutionary trajectory of antibiotic resistance. Your goal is to identify which organism(s) *Staphylococcus aureus* might have acquired the *mecA* gene, and what evidence supports this potential horizontal gene transfer event?

The structural learning goals of this exercise are:

- Learn how to retrieve sequences for analysis
- Build practical experience using Blast
- Acquire practical experience with multiple alignment tools.

Sequence Retrieval

Exercise 29-1

Obtain the genetic sequence for the *mecA* gene from MRSA. Go to Genbank and find a complete sequence for *mecA* in *Staphylococcus aureus*. That is, do a nucleotide search for "Staphylococcus aureus *mecA*" and select a *MecA* gene version with "complete cds". See Figure 29.2. Do not pick the complete genome. Check if MRSA is mentioned in the documentation for the result.

Exercise 29-2

Find the FASTA version of the genomic reference assembly. This will give you the genetic sequence of the gene. Keep the header of the sequence (">... Staph..., complete cds"), as we will need this later.

Exericse 29-3

Find the FASTA version of the protein sequence. There might be a link to this in "Related Information."

Database Searching using BLAST

Exercise 29-4

Go to the NCBI BLAST web portal. Use the *mecA* nucleotide sequence to search for similar sequences in the nucleotide database. Which organisms have regions with high similarity to the *mecA* gene nucleotide sequence in your query? Tip: Check the "Show results in a new window" to allow searching multiple times and compare results. Further, know that it is normal for blasting to take a few minutes. Examine the following metrics (columns):

- **E-value (Expect value):** The number of hits one can "expect" to see by chance when searching a database of a particular size. Lower E-values indicate a more significant match. Typically, E-values below 0.05 or especially 0.01 suggest a significant match, but the threshold can depend on the content and especially the size of the search. The longer a search term, the less likely it is for random matches.
- **Percent Identity:** The percentage of identical matches between the query and subject sequences over the aligned region.

Exercise 29-5

Try adjusting the number of maximum target sequences in "Algorithm Parameters" (e.g., to 1000). You can also exclude "Staphylococcus aureus" from the search results to only see other organisms.

- How did this affect the results?
- Check the taxonomy of your found organisms.
- Check the graphic summary of the results.

National Library of Medicine
National Center for Biotechnology Information

Nucleotide Nucleotide staphylococcus aureus MecA

Create alert Advanced

Species Summary 20 per page Sort by Default order Send to:

Fungi (4)
Bacteria (118,305)
Viruses (8)
Customize ...

Molecule types
genomic DNA/RNA (118,508)
mRNA (1)
Customize ...

Source databases
INSDC (GenBank) (39,730)
RefSeq (26,576)
Customize ...

Sequence Type
Nucleotide (118,728)

Genetic compartments
Plasmid (32)

Sequence length
Custom range...

Release date
Custom range...

Revision date
Custom range...

[Clear all](#)

[Show additional filters](#)

ANTIMICROBIAL RESISTANCE GENE Was this helpful?

PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA gene family

This gene family can be found in the set of reference sequences used to annotate antimicrobial resistance genes from the [National Database of Antibiotic Resistant Organisms \(NDARO\)](#).

[RefSeq genomic \(10\)](#) [RefSeq protein \(10\)](#)

[Pathogen Isolate Browser](#) [Reference Gene Catalog](#) [MicroBIGG-E](#) [Download](#)

Items: 1 to 20 of 118728 << First < Prev Page 1 of 5937 Next > Last >>

- [Staphylococcus aureus DNA, complete genome, strain: JP080](#)
1. 2,729,352 bp circular DNA
Accession: AP017922.1 Gi: 1236586396
Assembly BioProject BioSample Protein Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)
- [Staphylococcus aureus subsp. aureus strain MRSA252, complete genome](#)
2. 2,902,619 bp circular DNA
Accession: BX571856.1 Gi: 49240382
Assembly BioProject BioSample Protein PubMed Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)
- [Staphylococcus aureus strain SACI-3 MecA \(mecA\) gene, partial cds](#)
3. 271 bp linear DNA
Accession: OR255918.1 Gi: 2576870659
Protein Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)
- [Staphylococcus aureus strain SACI-5 MecA \(mecA\) gene, partial cds](#)
4. 253 bp linear DNA
Accession: OR263564.1 Gi: 2576869532
Protein Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)
- [Staphylococcus aureus strain CM21 MecA \(mecA\) gene, partial cds](#)
5. 270 bp linear DNA
Accession: KX024711.1 Gi: 1109484962
Protein Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)
- [Staphylococcus aureus strain TN/CN/1/12 MecA \(mecA\) gene, complete cds](#)
6. 2,129 bp linear DNA
Accession: KC243783.1 Gi: 441494908
Protein Taxonomy
[GenBank](#) [FASTA](#) [Graphics](#)

Figure 29.2: Screenshot

Exercise 29-6

Try the other programs (“Program Selection”). They may be slower but can find less similar matches that may uncover the potential sources of the gene.

- Did this affect the results? If so, how? (Use the taxonomy and graphic summary in your analysis.)
- What are the main differences between the programs (megablast, discontiguous megablast, and blastn).
- What do we expect from the different algorithms? It is okay to use Wikipedia or similar resources.

Exercise 29-7

Try the protein blast algorithms and compare the findings. This requires the protein sequence. Do you find the MecA gene product (i.e., protein) in other types of bacteria (i.e., not *Staphylococcus* strains)? If so, what may this imply about horizontal gene transfer of this gene? Is there a risk of MRSA “helping” completely different, and perhaps more dangerous, bacteria genera become antibiotics resistant?

Exercise 29-8

You might see multiple *Staphylococcus* strains when allowing higher maximum target sequences. What are the potential interpretations of this concerning horizontal gene transfer? Can we deduce a single origin of the gene in *Staphylococcus aureus* from these results? Remember that we see a snapshot of where the genes were present during various studies – this is ever-changing.

Exercise 29-9

According to Bloemendaal et al. (2010): “Methicillin Resistance Transfer from *Staphylococcus epidermidis* to Methicillin-Susceptible *Staphylococcus aureus* in a Patient during Antibiotic Therapy”, *mecA* may be transferred from *Staphylococcus epidermidis*. The *mecA* gene is located on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*), and they found that the SCC*mec* were almost identical in MRSA and in *S. epidermidis* in a patient.

- Does your results imply this may be true?
- If this was the case in that one patient, does it mean the *MecA* gene in MRSA always stems from *S. epidermidis*?

Multiple Sequence Alignment using ClustalW

Exercise 29-10

Choose five or more types of bacteria from your BLAST results, such as *Staphylococcus epidermidis* (include *Staphylococcus aureus*). You will be comparing the MecA gene's sequence in each of these to find relations between them.

Exercise 29-11

Go to Genbank and find nucleotide sequences for the MecA gene in each of your selected organisms. E.g., search for "Staphylococcus epidermidis MecA". You should get the (when possible) complete sequence for the gene only. Get the header of the FASTA file as well.

Exercise 29-12

Go to ClustalW at <https://www.genome.jp/tools-bin/clustalw> and input the sequences (with header first) of the *mecA* sequences from MRSA and the selected organisms. See Figure 29.3 Select DNA as you're inputting nucleotide sequences. Feel free to try the protein sequences.

Run the multiple alignments. - What does the output tell us? - How may these alignments be useful to us in determining the origin of the MecA gene?

Exercise 29-13

At the top of the output page, select a method in the tree menu (e.g., FastTree) and press Exec. This will generate a phylogram. A phylogram is a depiction of evolutionary relationships between taxa, in this case, the *mecA* gene from different types of bacteria. The branch lengths illustrate evolutionary distance and nodes from where the tree branches can be interpreted as common ancestors when we are working with vertical gene transfer. Try to imagine what the nodes could be interpreted as when we are talking about horizontal gene transfer. Hint: maybe the nodes could be interpreted as possible horizontal gene transfer events.

- What does the generated phylogram tell you?
- How could we use phylogenograms to track the potential horizontal gene transfer between the different bacteria?



Multiple Sequence Alignment by CLUSTALW

ETE3	MAFFT	CLUSTALW	PRRN
Help			
General Setting Parameters:			
Output Format: <input checked="" type="radio"/> CLUSTAL <input type="radio"/>			
Pairwise Alignment: <input checked="" type="radio"/> FAST/APPROXIMATE <input type="radio"/> SLOW/ACCURATE			
Enter your sequences (with labels) below (copy & paste): <input checked="" type="radio"/> PROTEIN <input checked="" type="radio"/> DNA			
Support Formats: FASTA (Pearson), NBRF/PIR, EMBL/Swiss Prot, GDE, CLUSTAL, and GCG/MSF			
<pre>>NG_047941.1 Staphylococcus capitis CSLAS mecA gene for PBP2a family beta-lactam-resistant peptidoglycan transpeptidase MecA, complete CDS ATGAAAAAGATAAAAATTGTCCACTTATTAAATAGTTGAGTTGCGGGTTGGTATA TATTTTATG CTTCAAAAGATAAAAGAAATTATAATAACTATTGATGCAATTGAAGATAAAAATTCAAAC</pre>			
Or give the file name containing your query <input type="text" value="Gennemse..."/> Ingen fil valgt.			
<input type="button" value="Execute Multiple Alignment"/> <input type="button" value="Reset"/>			

Figure 29.3: Screenshot

Discussion

Based on the BLAST and ClustalW results, which organisms might have been the potential source(s) for the *mecA* gene in MRSA?

- Discuss evidence of potential horizontal gene transfer events based on sequence similarity.
- Reason about the roles of the various HGT approaches in MRSA (i.e., Conjugation, Transduction, Transformation). What types are most likely between staph bacteria?
- Discuss the significance of horizontal gene transfer in the rapid emergence of antibiotic resistance.
- What are some ways we can use sequence alignments of MRSA to combat them? E.g., treatment, outbreak management (like tracking sources, spread, and evolution), and new drugs such as protein-targeting vaccines.
-

Additional tools

DTU (Technical University of Denmark) has developed a set of tools highly relevant to antibiotic resistance and MRSE specifically. These are not part of the assignment, but check them out if you are curious:

- Identification of acquired antibiotic resistance genes
- SCCmecFinder identifies SCCmec elements in sequenced *S. aureus* isolate
- Identification of acquired virulence genes
- spaTyper predicts the *S. aureus* spa type

30 Aardvark?

"What is an Aardvark?" You may have bugged you for a while. It might have kept you up at night. You might have been twisting and turning due to the mystery that is the Aardvark. But fear not. Today, you will attempt to answer this exact question. Or at least a part of the question from a phylogenetic perspective. In the process, hopefully, you will learn something about phylogeny. Let's start with a more straightforward question. "What does the Aardvark look like?", Figure 30.1.

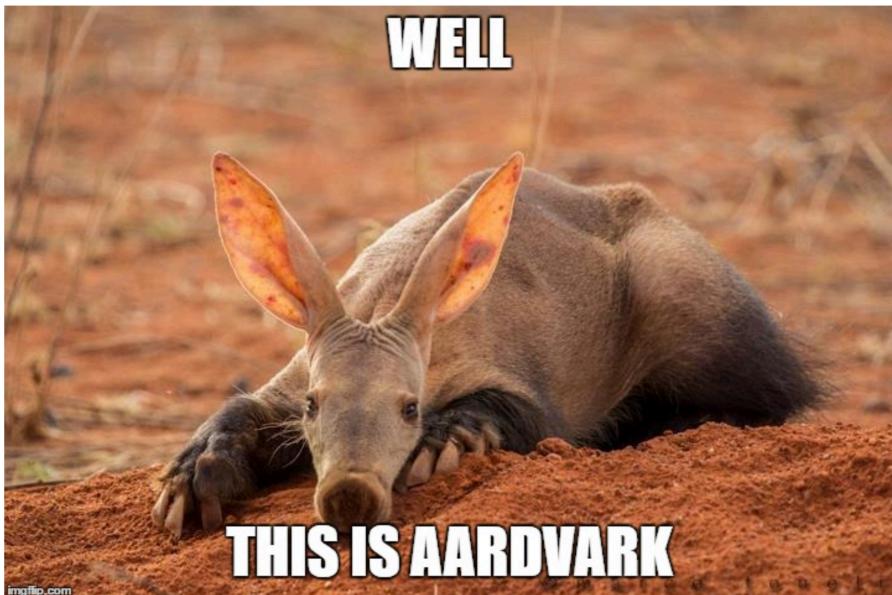


Figure 30.1: This is an Ardvaark

The Aardvark is a nocturnal mammal found in Africa, with the size of a big rottweiler. The long ears and face shape (not considering the nose) make you wonder if it is the long-lost cousin of the kangaroo. It mainly feeds on ants and termites, which might change your mind in regards to its heritage. It could be a type of anteater. Looking at the nose and considering that in Afrikaans, 'erdvark' means 'earth pig' or 'ground pig,' you might change your mind once again and consider it a type of pig. The Aardvark is truly a mystery. So, let's solve the mystery of the Aardvark's evolutionary relation to these reference animals! Feel free to look up cute pictures of these animals while you work on the exercises. I have collected a list of animals that I think of when I look at the Aardvark. Have the animals in Figure 30.2 in mind when you do the exercises.



Creative commons:

A: <https://www.flickr.com/photos/8488744@N07/513572189>
 C: <https://namibian.org/nature/mammals/insect-eaters/grants-golden-mole>
 E: <https://www.flickr.com/photos/deadeyebart/122444782>
 G: <https://www.flickr.com/photos/markdescande/31406725074>

B: <https://www.flickr.com/photos/23031163@N03/43599637522>
 D: <https://flickrriver.com/photos/blacktigersdream/37204713241/>
 F: <https://www.flickr.com/photos/corey-hayes/52372488343>

Figure 30.2: A: Aardvark, B: Grey Kangaroo, C: Golden Mole, D: Elephant Shrew, E: Red River Hog, F: Collared anteater, G: African Elephant

To uncover the mystery of the Aardvark and its relation to the reference animals, we will be looking at the evolutionary distance between the animals listed above and the Aardvark. To do so, we need to align sequences from each of the animals. Not any gene will do. We need a highly conserved gene found in all animals, that can represent to long evolutionary distances in question.

I suggest the COI (cytochrome c oxidase I) gene (also known as the COX1 gene). The COI gene is one of the most popular phylogenetic markers for evaluating evolutionary relationships. It is found in nearly all aerobic eukaryotes, where it encodes Cytochrome C Oxidase subunit 1, a protein involved in mitochondrial respiration.

COI is a part of the mitochondrial DNA, which has the added benefit that the gene is inherited from the mother and that it does not undergo recombination. Recombination would make different parts of the aligned sequences follow separate paths through the generations. Each segment of the alignment would then have its own tree, and we would end up modeling an average of many trees with a model that assumes a single tree for the entire sequence. This is obviously not good, but think about how it might bias the resulting phylogeny.

We are not the first to think this specific gene would be neat to use for phylogeny. COI has been used for DNA barcoding, which is a method for identifying and classifying species based on their genetics. This also means that this gene has been sequenced for

a lot of species, making our job more manageable. It means that we do not need to fly off to some jungle, catch animals, extract DNA, create primers for the COI gene, and sequence each one. We simply look them up in the online database.

“ Before you go on, sit back and appreciate amazing all this is. Not only is this magnificent beast encoded by strings of only four different nucleic acids that we are able to extract because they are carried by all its cells. We can also identify the sequence of these nucleic acids and we can use models their evolution to place the Aardvark in the tree of life among the other species.”

Build a FASTA file

To investigate the mystery of the Aardvark, you need to collect the sequences of the COI genes of all the reference animals in a way that makes it possible to compare all the gene sequences. A common way to do so is to use FASTA files. FASTA is a file format used for sequences of nucleotides or amino acids that is used by almost all bioinformatic software tools. A FASTA file can contain multiple sequences as long as they are formatted as follows: First, a line starting with ">" followed by an ID and a description of the sequence. This is known as the "header" and is *one line only*. The following lines are the sequence. This can take up as many lines as needed. The sequence is usually split across lines with 60-80 characters on each line. The example below has 70 characters per line. To start a new sequence in the file, simply add a new header, followed by a sequence. Like this:

```
> ID1 some kind of description
ATGTCTTCTATTAAACAGCTCTGAATCGCTTGCTGCTTCGGGAGGAAAGCCTCTGTTCCACGAGTCCT
TGCCCTATAAAACTGTCACCTACTCCGAGAAGGCAATGAGTATGTAATTATTGACAACAAAAAAACTT
GAGGCACGAGTTGATGGCTGCCCTCGGTGGTACCTCAATCCTGGTTGGCACCCCTTCCTAACGATCAG
> ID2 some other kind of description
ATGTCTTCTATTAAACAGCTCTGAATCGCTTGCTGCTTCGGGAGGAAAGCCTCTGTTCCACGAGTCCT
TGCCCTATAAAACTGTCACCTACTCCGAGAAGGCAATGAGTATGTAATTATTGACAACAAAAAAACTT
GAGGCACGAGTTGATGGCTGCCCTCGGTGGTACCTCAATCCTGGTTGGCACCCCTTCCTAACGATCAG
TTTGGTAACGCTCTGCCCTAGGTATAGCAGCATTGCCCTACCGCTTTAGTGTGGCTTGTATAATT
TGCAAGCCAAGACATTACAATTCAAATATGATTGTTGGTTATGTTCTACGGTGGCTTGTCA
ATTCTTATCTGGACTCTGGAAATGGTATGGAAACACCTTGCTGCCACTTCCT
```

Exercise 30-1

Now it is your turn! Make a FASTA file containing the nucleotide sequence of the COI (COX1) gene of all the reference animals and the Aardvark. Use the file “animals.fasta”

to insert the nucleotide sequences. You can find the nucleotide sequences in the NCBI Nucleotide database. To search for genes, select “Gene” instead of nucleotide from the drop-down menu. If you were looking for the PRDM9 gene in humans you would, you search like this:

PRDM9[Gene Name] AND Homo sapiens[Orgn]

The [Gene Name] and [Orgn] tags tells the database that “PRDM9” should be interpreted as a gene name, and “Homo sapiens” as an organism. This greatly limits your search and excludes database entries where the same words appear in other contexts. Have a quick look at the complete list of search terms available.

You are looking for COX1 and *Orycterus afer* is the Latin name for Aardvark. This guides you to the page containing the database information for the COI gene for the specific organism (animal). You can find the gene sequence in a fasta format in the section “NCBI Reference Sequences (RefSeq).” Copy the gene sequence and paste it into the animals.fasta file under the appropriate header. To help you along, here are the Latin names of the animals:

Species	Latin name
Aardvark	<i>Orycterus afer</i>
Grey Kangaroo	<i>Macropus giganteus</i>
Golden Mole	<i>Eremitalpa granti</i>
Elephant Shrew	<i>Macroscelides flavicaudatus</i>
Red River Hog	<i>Potamochoerus porcus</i>
Collared anteater	<i>Tamandua tetradactyla</i>
African Elephant	<i>Loxodonta africana</i>

We would like to find an animal that can serve as “outgroup” in our analysis. An outgroup is a sequence so distantly related animal to all your other sequences (your “ingroup”) that you can safely assume that all your ingroup sequences find a common ancestor before they find one with the outgroup sequence (Figure 30.3). The point where the outgroup attaches to the ingroup tree must then be the common ancestor of your ingroup sequences. Since all your subject animals are all live-bearing mammals, you can use the platypus (a monotreme) (*Ornithorhynchus anatinus*) as the outgroup and add it to your fasta-file.

One-click analysis

Now that you have created a FASTA file with the COI (COX1) gene of the Aardvark and all the reference animals, it is time to look into the evolutionary distances between

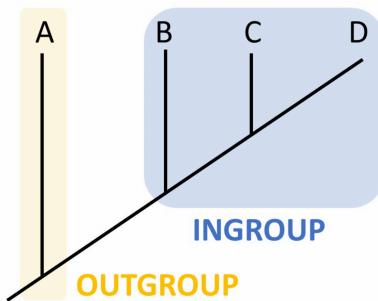


Figure 30.3: Ingroup and outgroup in a phylogeny

them. We will be using the web tool www.phylogeny.fr for this exercise. When you do your phylogenetic analyses below, you need to screengrab/save your phylogenetic trees along the way. You should paste them into a document with notes on which models and parameters used for each tree. That way you can compare them all at the end.

Exercise 30-2

Go to www.phylogeny.fr and get aquainted with the web interface. Then navigate to the “one-click” analysis under the “phylogeny analysis” tab. The “one-click” analysis runs an easy initial analysis of your sequences, automating the four steps below with default models and parameters:

1. Multiple sequences alignment of all the genes in your newly made FASTA file.
2. Curation of the aligned sequences in order to eliminate poorly aligned positions (such as trailing nucleotides in cases where one sequence is much longer than the others).
3. Building of the phylogenetic tree according to evolutionary distances calculated from the curated multiple sequence alignment.
4. Visualization of the tree.

Exercise 30-3

Now that you have navigated to the “one-click” analysis page, you can upload the FASTA file you created in the previous task. Then click submit. The result is a phylogenetic tree describing the evolutionary distances between COI genes of the different animals. Take a good look at it. Does it look anything like what you expected?

Exercise 30-4

If you want to make the tree easier to interpret or to highlight specific relations, you can change the visualization without changing the information in the tree. One way is to reroot the tree. Let's try it out. First, click on the "Reroot (outgroup)" button below the tree under the section "Select an action and click leaf or internal branch." Then click on the name "Platypus" and wait for the tree to re-render. Now, look at it again. You can also manipulate the visualization of the tree by, instead of clicking "reroot," you can click on "Flip" or "Swap."

1. What does Flip do?
2. What does Swap do?
3. What does Reroot do?

Exercise 30-5

You have played around with the tree a bit, and now you are ready address what it tells you. Start by clicking on "Reset (cancel all changes)" in the section "Select an action" and follow up by rerooting the tree with Platypus as the outgroup. Now answer the following questions:

1. How much does re-rooting change the tree?
2. How many terminal nodes (leaves) and how many internal nodes are there in the tree?
3. Which node represents the most recent common ancestor between the Aardvark and the African Elephant? Which one is the common ancestor between the Aardvark and the Elephant Shrew? Does this hold no matter whether you forced the platypus as the outgroup?
4. Which of the reference animals are closest related to the Aardvark according to this model? Does the tree look like you expected?

Different models of DNA evolution 30-6

Exercise 30-7

The "one-click" analysis uses the HKY85 substitution model to calculate the phylogenetic distances, but you can choose other models as well. Here is a rundown of the most important ones:

The **Jukes-Cantor model** is one of the simplest nucleotide substitution models. It assumes that all types of nucleotide substitutions (transitions and transversions) occur at an equal rate, meaning that there's a single rate parameter for all types of substitutions.

This is a highly simplified model and is often used as a baseline for comparison with more complex models.

The **Kimura two-parameter model** is a simple model that takes into account two types of substitutions: transitions (purine-to-purine or pyrimidine-to-pyrimidine changes) and transversions (purine-to-pyrimidine or vice versa). It assumes that transitions occur at a different rate than transversions, reflecting the fact that transitions are often more common in DNA evolution.

The **HKY85 model** is a relatively simple model that takes into account two major factors in the evolution of nucleotide sequences: transitions (purine-to-purine or pyrimidine-to-pyrimidine changes) and transversions (purine-to-pyrimidine or vice versa). It assumes that transitions and transversions occur at different rates, which makes it more biologically realistic compared to some simpler models like the Jukes-Cantor model.

The **GTR model** is a more complex and flexible model compared to HKY85. It allows for different substitution rates between all possible pairs of nucleotides, making it a highly general model. This means that it can accommodate variations in the substitution rates of all six possible types of nucleotide changes (AC, AG, AT, CG, CT, GT).

The **Hamming distance** is not a traditional substitution model used for phylogenetics. It is a simple method for comparing sequences of equal length, where it counts the number of positions at which two sequences differ (i.e., the number of substitutions needed to convert one sequence into another). It does not consider the specific types of substitutions (transitions or transversions).

What might be the reason for not always choosing the model with the largest number of parameters?

Exercise 30-8

Let's explore how the evolutionary distances change when you use other models and also how the different tree construction methods make a difference. You can do this by running the steps from the "one-step" analysis, but change the model of DNA evolution to explore how this affects your results.

You will start at step 1: Multiple sequence alignment. You need a multiple alignments of all your sequences. Lucky for you, www.phylogeny.fr has already done that in order to make the tree. Here, the MUSCLE program has been used (MUSCLE is conceptually close to ClustalW). We will not change the alignment method. To access the multiple alignments, click on the tab "3. Alignment". To see the curated part, click on the tab "4. Curation". In this tab, the alignment has been curated by the program Gblocks. Gblocks has identified the portions of the alignment suitable for distance calculation and tree-building and has underlined these portions with a dark blue box. You can read more about Gblocks in the Gblocks documentation. Go read the introduction in the Gblocks documentation. What kind of positions are excluded after curation?

Download the curated alignment by clicking on “Cured alignment in Phylip format” under the Outputs section. This gives you the equal-length aligned sequences resulting from curation by Gblocks in a phy-file. Go read the introduction in the Gblocks documentation. What kind of positions are NOT included after curation?

Exercise 30-9

Now you can see what happens if we use the same alignment same curation, but different evolutionary models to calculate the evolutionary distances. To help you calculate the evolutionary distances, www.phylogeny.fr has some nice options for calculating distance matrices for you. Let’s start by building phylogenetic trees using PhyML.

PhyML (Phylogenetic Maximum Likelihood) employs a statistical approach (maximum likelihood) to estimate the most likely tree given the input alignment. To Use PhyML, navigate to the top, tap “Online Programmes,” and choose PhyML. Here you can upload your curated alignment in phylip format (the one you downloaded from the curation tab).

PhyML now lets you choose a substitution model. A substitution model is a mathematical model that describes how genetic sequences change over time. So now you will see how these different models actually affect your tree. Try the two DNA/RNA substitution models for PhyML (HKY85 and GTR). Remember to save your tree by clicking “Tree in newick format” and save the file. These tree files can be visualised by using the program “TreeDyn” under the top tab “Online Programmes”. Do the trees formed by GTR and HKY85 look alike? What are the Aardvark’s closest relatives according to the two trees?

Explore phylogeny - part 3

Not only can you change the substitution model used in calculating the evolutionary distances. You can also use different tree-builders. You just tried PhyML, which uses a maximum likelihood approach. Next, try to use BioNJ. BioNJ stands for “Biased Neighbor Joining,” which is an adaptation of the neighbor-joining algorithm (not currently a part of www.phylogeny.fr). Neighbour Joining is a distance-based tree-building method, and BioNJ uses the same approach, with a slight bias, to avoid errors with particularly long branches. Try the three DNA/RNA substitution models for BioNJ (kimura, jukes-cantor, hamming). Remember to save your trees. Do these three trees look alike? Do they look like the trees formed by PhyML? What are the Aardvark’s closest relatives according to the three trees?

Food for thought

Okay, step back from the Aardvark for a moment. What about the other animals we are looking at? Are their relations always the same? Are some always grouped together while others are always grouped far apart? If you are particularly curious, try to add other types of elephants to the FASTA-file and see how other types of elephants group when doing phylogeny.

Now, you have looked at the Aardvark and its evolutionary relations with other animals. You have tried many different ways. Are you more confident in your knowledge about the Aardvark, or are you feeling more confused than ever? What of the animals in your analysis is the closest relative of the Aardvark? Does Wikipedia agree with you? (<https://en.wikipedia.org/wiki/Aardvark>) (Read from the Introduction, Name, and Taxonomy). Do you trust Wikipedia? See if you can find the Aardvark (*Orycteropus afer*) in NCBI's Taxonomy Browser. What does that report its taxonomy?

Project files

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

31 Plasmid ORFs

Gene prediction in bacteria is a crucial step in understanding the genetic makeup of these microorganisms. It involves identifying the locations and boundaries of genes within bacterial genomes, essential for studying bacterial physiology and pathogenicity and developing targeted therapies. The main component of bacterial genes is the open reading frame (ORFs), which translates into protein. So, identifying ORFs is the main task in “de novo” prediction of bacterial genes. The most commonly used start codon is ATG, and less frequently, GTG and TTG. Stop codons are either one of TAA, TAG, or TGA. Although the three “reading frames” on each strand allow ORFs to overlap, this is mainly a feature of highly compacted viral genomes and not often observed in bacteria. Promoter and terminator DNA motifs provide additional information usually built into hidden Markov models or neural networks. Such models also assess the coding potential, codon usage, and length of ORFs to distinguish randomly occurring pairs of start and stop codons from those representing genes.

A bacterial plasmid is a circular, double-stranded DNA molecule separate from the genome found in the cytoplasm of bacteria. It is relatively small, ranging from a few kilobases to several hundred kilobases in size, and replicates autonomously, passing to daughter cells at cell division. Although they are usually not essential to the bacterium’s survival, plasmids often carry genes that can confer selective advantages to the bacteria under certain conditions. Such conditions include exposure to antibacterial agents like the antibiotics used to combat infections, and some plasmids carry genes making the bacterium resistant to such drugs.

In this exercise, the bacterial DNA you will investigate is a plasmid extracted from an *Enterococcus faecium* strain to test if it is responsible for its documented resistance to vancomycin. Vancomycin is used to treat infections of bacteria strains resistant to other antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one such strain. Because vancomycin is considered a “last resort” antibiotic, resistance to this drug is dangerous, and it must be monitored carefully.

You will use the NCBI’s ORF Finder tool to identify ORFs in the plasmid, and then use various online tools to narrow down the ORFs to a set of likely resistance genes. You can download a fasta file with the plasmid sequence from Brightspace.

When you open up ORF Finder, start by pasting the fasta file into the “Query Sequence” field. Look at the search parameters, but do not change them yet. One parameter controls the minimum ORF length. We can change the expected genetic code, specify alternate start codons, and choose to ignore nested ORFs.

Exercise 31-1

Run ORF Finder with the default parameters.

- How many ORFs did you find?
- What is the longest and shortest ORF in nucleotides (nt)? You can click on “length” to sort them.
- To the right, there is an option to alter the tracks. Try adding the “six-frame translations”, which are located under sequence. What is most common, start codons (green) or stop codons (red)?

Exercise 31-2

You can see that a lot of the identified ORFs are tiny and that many of them overlap other ORFs. Both are possible in bacterial plasmids but are much rarer than seen here. Return to the start page and rerun the search, but this time with a minimal ORF size of 300 nucleotides (100 amino acids). - How many overlapping ORFs do we find now? Which ORFs overlap? Some may be hard to distinguish visually; you can use the table below the tracks to see the start and stop and order them based on start/stop. - There is often a strand bias in transcribed regions. If two genes are close to each other, they need the same strandedness, as RNA polymerases can otherwise collide. Which strand is most common in this plasmid? How many genes are on the majority strand?

Exercise 31-3

With this improved list of possible genes, you are now ready to investigate the putative functions of the different ORFs. Sort the ORFs by size and Blast the longest 10. You can also Blast all the ORFs if you want to. Smartblast is recommended if you want to do it quickly, and it also has multiple useful features. These include showing a phylogeny of the closest related known proteins and a list of the best significant hits. Use a table or list, such as the one below, to keep track of your findings. Answer the three questions below for each of the top ten ORFs.

1. Is the translated ORF related to a known protein? You can blast multiple at the same time. Note down the best match with a descriptive name for Putative Function. Sometimes, the best match will be a “hypothetical protein” or similar, in which case you should move on to the next one on the list.
2. What are the functions of the related proteins? Are any of them related to antibiotic resistance, and if so, which antibiotics?
3. The names of the gene homologs you find may themselves suggest resistance function. Go to the NCBI Gene database and search for the gene name to determine if you can find it in a resistance database, such as NDARO. If so, note it as a resistance gene.

ORF_Label	Length	Putative Function	Resistance?
ORF18	337	ISL3 family transposase (no strong match)	No

Exercise 31-4

Go back to the overlapping ORFs. Do both of the overlapping ORFs contain good hits?

Exercise 31-5

Conclude on your findings. Does the plasmid contain genes that confer antibiotic resistance? If so, what kinds of antibiotics does it provide resistance to?

Project files

Download the files you need for this project:

https://munch-group.org/bioinformatics/supplementary/project_files

32 Read mapping

Next-generation sequencing (NGS) has become extremely popular among geneticists and bioinformaticians in recent decades. Three of the biggest sequencing companies are Illumina, Pacific Biosciences (PacBio), and Oxford Nanopore Technologies (ONT). The three companies use different technologies to sequence DNA, and they each have pros and cons. Illumina reads are relatively short (150 bp) but are also the cheapest solution. PacBio produces long, accurate reads (~15 kb) but is more than twice as expensive as Illumina. ONT can produce extremely long reads (60 kb) but suffers from low-quality base calls.

The choice of sequencing technique is highly dependent on the specific research question, so it is worth understanding the capabilities of the methods before choosing how the DNA should be sequenced.

32.1 HiFi reads

In this exercise, you will work with PacBio HiFi reads. Currently, this sequencing technique is the most accurate of the NGS technologies. The sequencing method is shown in the below figure. The biggest strength of this sequencing technique is that the reads are read multiple times. Every time the read is read, a subread is produced, and the final HiFi read is the consensus of all the subreads. This approach eliminates most of the sequencing errors found in the subreads.

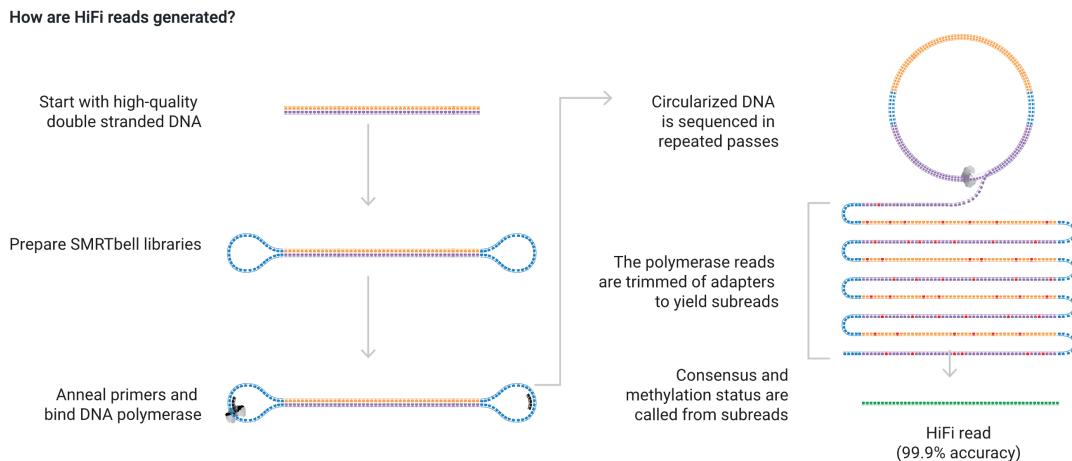


Figure 32.1: HIFI reads. © 2023 PacBio

In this exercise, you will work on a 3.5 Mb contig from an unknown chromosome and tissue. The reads are PacBio HiFi reads, and in this sample, the average read length is approximately 10 kb. We can use the IGV (Integrative Genomics Viewer) program to visualize bam files containing the reads.

Exercise 32-1

Go to the IGV download page and download the program that fits your computer. You should also download the four files found on Brightspace. Once downloaded, you are ready to load the read data in IGV. In the top panel, click Genomes > Load Genome from file and then load the file called `contig_1.fa`. Now you should be able to see that the contig is 3534 kb. Next, click on File > Load from file in the top panel and load the file called `contig_1.bam` (if you get something like “index error”, ensure that all your files are named correctly, no spaces in the file names and that they are all placed in the same folder). You can zoom in and out in the top right corner of your screen. Zoom in until you see a window size of 23 kb. If you want to go to a specific region, you can change the coordinates in the white box next to the contig name. The format is like the UCSC Genome Browse (`chr:start-end`). Drag the data track to see different areas on the contig. If you encounter problems, watch this five-minute video. Play around for five minutes to understand what you are looking at.

- What are the purple ‘I’s and the black dots,
- How does the coverage vary?
- Try to zoom in as much as you can. What information appears in the lower panel?
- Click on one of the reads; what kind of information do you see? Some of the lines are obvious, but some of them are more tricky. Find some of the tags on this page. You should focus on MM, np, rq, clipping, and QV tags:

MM: This tag denotes the probability of a cytosine being methylated when a guanine follows the cytosine. The dinucleotide CG is often referred to as a CpG site. These sites are unique due to their elevated mutation rate, but that topic will be another time.

NP: The np tag tells you how many times a read is read in the PacBio machine. If a read is read once or twice, it often results in low base qualities. In contrast, if the read is read 15-20 times, we are more confident that the base calls are correct.

QV: Denotes the quality of the base calling. It ranges from 1-93 on the Phred scale (https://genome.sph.umich.edu/wiki/Phred_scale). The tag is highly correlated with the np tag because the more a read is read, the higher the base calling quality.

RQ: This tag shows the overall quality of the entire read.

Clipping: If a proportion of the read does not fit the reference sequence, that proportion can be masked/clipped. Because of this masking, the read will look shorter in IGV.

Exercise 32-2

Go to: ptg0000011:1003200-1007274. What is the maximum coverage in this region? There is a read with a red arrowhead at the end of the reads. What does that mean (Hint: Look at the tags and compare them to the rest of the reads; one of the tags is different)? How does your conclusion relate to the length of the read that is shown? Right-click on your screen, and you will see a lot of options. Click on Quick consensus mode. Can you see a difference in some of the reads? You will see two red and green lines on one of the reads. To find out what the lines represent, try to zoom in on these positions and click on each line.

- What does the colored line represent?
- Which base is highlighted in green, and which is highlighted in red?
- As we know, there are four nucleotides. Find out which colors are related to the last two nucleotides (You should zoom out to find these).
- Now go back to ptg0000011:1003200-1007274. You have probably figured out that the colored lines represent a mismatch in the alignment. These mismatches could be rare but true mutations, but they could also be sequencing errors. Use your knowledge about the np tag and QV tag to determine whether the mismatches are due to sequencing errors or true mutations. (Hint: Compare the tags with the reads that do not have mismatches)

Exercise 32-3

Now that you are an expert in IGV, we are ready for more advanced stuff. Pack your suitcase and travel to ptg0000011:2879315-2879495. Right-click and click on Hide small indels.

- How many mismatches, insertions, and deletions do you see? You should also include the length of the indels.
- You might have noticed that some of the reads have turned white. What does the white color mean (Hint: Look at the tags)?

Exercise 32-4

In one of the previous exercises, you found positions where only one read contained the mismatch. This mismatch is often referred to as a singleton. Base quality (QV) is essential when determining whether the mismatch is a mutation or a sequencing error. In this exercise, you look at another measure called mapping quality (MAPQ). This quality measure ranges from 0 to 60, telling you how unique a read is mapped. If the mapping quality is 0, the read fits equally well in another part of the genome. In this exercise, you will also see what highly frequent single nucleotide polymorphisms (SNPs) look like. Lastly, you will learn to think critically about the coverage and understand how high coverage relates to poor mapping quality.

Go to ptg0000011:3178259–3178531.

- What is the maximum coverage in this region, and how does that compare to the values from the previous exercises?

You should be able to see four SNPs. Click on the colored bars in the coverage panel.

- What is the count for each nucleotide at the SNP position?
- What do the numbers in the parenthesis tell you?
- Try to click on five different reads. What is the MAPQ for each of these reads (write them down)?
- Compare the five values with the MAPQ of five reads found in the previous region (ptg0000011:2879315:2879495). Are they different? Which region do you trust more?

Exercise 32-5

Place your cursor on “Color alignments by,” and choose “base modification (5mc)”. What do you see?

Zoom in on one of the blue/red boxes and click some. What do they show you? (Hint: Look at the sequence. Which two bases do you see for all the blue/red colors?)

Go to ptg0000011:910,755–912,644. You might have noticed that the blue/red color represents the probability of the C being methylated. In this region, you can see that the blue color dominates and that the concentration of CpG sites is high. To see this more clearly, zoom out by clicking the minus button five times. The region looks unique, so let’s investigate it a bit more.

Exercise 32-6

Next to where you put your coordinates, there is a blue square with a red line on it. Click this symbol and move your cursor to the left side of your alignment. Your cursor should look like a “plus” sign. Click on the alignment, then move the cursor to the right side of the screen and click again. The region should now be highlighted by a red line, like in the figure. Click the red line, and click “Copy sequence”. Go to the Bast home page, paste the sequence, and press ‘BLAST’.

- Which chromosome is the sequence from?
- The sequence aligns 100 % with a gene called KDM5D. Try to explain why it is likely that the blue cluster in IGV represents a gene.

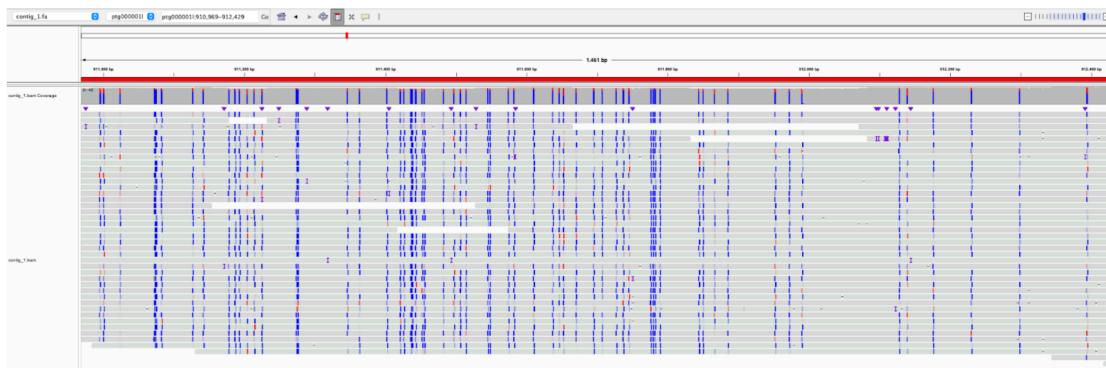


Figure 32.2: IGV screenshot

Exercise 32-7

Go to ‘ptg0000011:1,855,223-1,901,969’. You should see a region with a high number of CpGs, but the base modification differs between the reads. Discuss what causes this pattern.

Exercise 32-8

It is time for the grand finale. Go to the end destination, which is found at ptg0000011:243,727-244,325.

- Do you think the region contains a gene? Explain why/why not
- Highlight the region, and BLAST the sequence.
- Which tissue do you think the DNA comes from?
- If you have time, discover more interesting patterns in the data set.

Project files

Download the files you need for this project:

contig_1.bam
contig_1.bam.bai
contig_1.fa
contig_1.fa.fai

33 Neural networks

Machine learning comes in many forms. Whether you want to call them artificial intelligence or just models is a matter of taste. Hidden Markov models are one class of models, and neural networks are another.

At this point, you already know the basics of neural networks, especially feed-forward networks, and how we can train such a network of sigmoid neurons to work as a classifier. In this exercise, you will play with neural networks and explore how features of input data and hidden layers affect the properties and the neural network's performance.

Follow the above link into the Neural Network playground. Start by reading the text below the dashboard and look over the dashboard's components. At the top, there is a panel with dropdown menus controlling the properties of the network and selecting the type of problem to solve. We will start with an activation type of "Tanh" (similar to sigmoid activation) and a problem type of "Classification" (already set if you have not used the site before). You can pick the data set to work on in the "Data" column on the left side. Select the top left one (blue dots surrounded by orange ones), and leave the other controls as they are.

You see the network layout in the middle, with the input layer on the left and the output layer on the right. The data is a set of colored points in a two-dimensional coordinate system. The points are split into a training set and a test set. In each epoch of the training, the model parameters are changed, and the resulting change in performance is evaluated by running the model on the test data. The points included in the training data are the ones you see on the right over the output. There is a tick box in the output panel that lets you see the test data too. If you drag the "Data" slider "Ratio of training to test data" to the left, you can see how the training data set shrinks as you include more data in your test set. Leave it at 50%.

The neurons in the input layer fire if the data shows a particular feature. In the case of feature X1, the neuron produces output when the data exhibits a gradient of orange points on the left and blue points on the right. Other features trigger the other input neurons, as their thumbnails show.

Exercise 33-1

Trim the network down to a single hidden layer (-1 layer) with just two neurons, and make sure only the X1 feature is selected in the input layer. In the output on the right, the color of each point shows which class it belongs to. The background color represents the classification made by your neural network.

Press play to start the training. Pay close attention to how the weights and outputs change from their initial states. Run the network for 100-200 epochs, then stop and inspect it.

1. The two neurons in the hidden layer should produce output for different features
- try hovering over them and think about how the single feature creates these activations.
2. Do the weights or the biases differ the most? You can hover over the lines/points or look at their color.
3. What is the Test Loss noted under output?
4. Do you think adding more features, neurons, or layers will improve the classification?

Exercise 33-2

We may need more features. Try adding the input node capturing the X2 feature and run this model for 200 epochs.

1. Is the Test Loss better now (Test Loss is the loss function of the test data).
2. The Output prediction is quite different now; look at the neurons and consider how adding the X2 Feature changed the other neuron outputs.
3. It seems to have quite a different fit - will adding more neurons make it even better?

Exercise 33-3

Two neurons may not allow for enough flexibility. Add more neurons to the hidden layer and run the model for 200 epochs.

1. How many neurons must you add to gain Test Loss under > 0.005?

Exercise 33-4

Now lets see how good you are:

1. Challenge: How low can you go in total Neurons and Features and still produce a good fit (Test Loss under 0.005)? Consider which features work well with the data especially.
2. Challenge: Try creating a model that performs poorly despite having at least three features and four neurons (poorly being Test Loss over 0.1).

Exercise 33-5

The ring-shaped classification problem is relatively easy (for neural networks). Now, it is time to challenge it with a more complex problem. Pick the spiral pattern (bottom left option under Data). This pattern is much more complicated, so a Test Loss below 0.05 (rather than 0.005) is good. Using more Features and Neurons will also take longer for each epoch, so you need to be more patient. It also requires more Epochs to train, so wait for 500-1000 Epochs before deciding how well the network performs. While you wait, you can watch the Test Loss graph in the output panel.

1. Try fitting a model using all features, and decide how many neurons and layers to use. How many neurons do you need to do a good fit? Hint: You may need more than one hidden layer.
2. Look at the neurons in the last layer - does any of the neurons reflect the spiral of the data well?
3. Try removing some of the features the network uses the least - does it still perform well?
4. Summarize the changes you had to make to the network to make it fit the spiral data. Do these changes capture the non-linear pattern in the data?

Exercise 33-6

Let's wrap up with a different kind of problem - regression. In contrast to classification, we do not try to predict a discrete class but rather a specific numerical value. At the top left, click on problem type and choose "Regression". Of the datasets, pick the more difficult dataset to the right, listed as "Multi Gaussian". Include only the X1 and X2 features, but try different network architectures. Let the training run between 200 and 500 Epochs when testing your network.

1. Can you get the Test Loss below 0.005 with only a single hidden layer?
2. Does adding more hidden layers improve the model?
3. Which additional features enhance the model the most?

4. Does the addition of some features only improve models with more than one hidden layer?

Project files

Python projects

In each sections below, you will find download links to the files you need for each python project.

Translating open reading frames

Primer analysis

Pairwise global alignment

Codon usage in Streptococcus bacteria

Identifying the subtype of an HIV sequence

Clustering sequences based on distance

Finding genes in bacteria

Genome assembly

Web exercises

In each sections below, you will find download links to the files you need for each web exercise.

GWAS

None

CCR5

MRSA

None

Aardwark

ORF finding

Long reads

contig_1.bam
contig_1.bam.bai
contig_1.fa
contig_1.fa.fai

Neural networks

None

Databases and resources

1. GenBank
2. UniProt
3. PubMed
4. Ensembl
5. NCBI Gene
6. STRING
7. dbSNP
8. OMIM
9. PDB (Protein Data Bank)
10. KEGG (Kyoto Encyclopedia of Genes and Genomes)
11. Reactome
12. GO (Gene Ontology)
13. HPRD (Human Protein Reference Database)
14. COSMIC (Catalogue Of Somatic Mutations In Cancer)
15. ClinVar
16. TCGA (The Cancer Genome Atlas)
17. Pfam
18. Rfam
19. InterPro
20. GTEx (Genotype-Tissue Expression Project)
21. HGNC (HUGO Gene Nomenclature Committee)
22. FlyBase
23. WormBase
24. TAIR (The Arabidopsis Information Resource)
25. Mouse Genome Informatics (MGI)
26. RGD (Rat Genome Database)
27. PharmGKB
28. DrugBank
29. PubChem
30. dbGaP (Database of Genotypes and Phenotypes)
31. dbCAN (Carbohydrate-Active enZYmes Database)
32. CATH (Class, Architecture, Topology, Homology)
33. MEROPS (Peptidase Database)
34. BioGRID
35. IntAct
36. IUPHAR/BPS Guide to Pharmacology

37. GenAtlas
38. miRBase
39. lncRNADB
40. DGV (Database of Genomic Variants)
41. UCSC Genome Browser
42. ExPASy (Expert Protein Analysis System)
43. Swiss-Model
44. Pfam
45. SUPERFAMILY
46. EMBL-EBI InterProScan
47. WikiPathways
48. ChEMBL
49. BioCyc
50. dbVar

Knowledge data bases

Do it yourself: