

1 Unix for Bioinformatics

1.1 Introduction

Unix is the standard operating system on most large computer systems in scientific research, in the same way that Microsoft Windows is the dominant operating system on desktop PCs.

Unix and MS Windows both perform the important job of managing the computer's hardware (screen, keyboard, mouse, hard disks, network connections, etc...) on your behalf. They also provide you with tools to manage your files and to run application software. They both offer a graphical user interface (desktop). These desktop interfaces look different between the operating systems, use different names for things (e.g. directory versus folder) and have different images but they mostly offer the same functionality.

Unix is a powerful, secure, robust and stable operating system which allows dozens of people to run programs on the same computer at the same time. This is why it is the preferred operating system for large-scale scientific computing. It runs on all kinds of machines, from mobile phones (Android), desktop PCs... to supercomputers.

1.1.1 Why Unix?

Increasingly, the output of biological research exists as *in silico* data, usually in the form of large text files. Unix is particularly suitable for working with such files and has several powerful and flexible commands that can be used to process and analyse this data. One advantage of learning Unix is that many of the commands can be combined in an almost unlimited fashion. So if you can learn just six Unix commands, you will be able to do a lot more than just six things.

Unix contains hundreds of commands, but to conduct your analysis you will probably only need 10 or so to achieve most of what you want to do. In this tutorial we will introduce you to some basic Unix commands followed by some more advanced commands and provide examples of how they can be used in bioinformatics analyses.

1.2 Learning outcomes

This tutorial consists of two sections, **Introduction to UNIX** and **Advanced UNIX for Bioinformatics**. By the end of the first section you can expect to be able to:

- Describe why UNIX is suitable for analysing NGS data
- Know what the UNIX command line is
- Understand the UNIX directory structure and navigate around this structure
- Manipulate (move, copy and delete) files using the command line
- Look at and sort the contents of a file
- Find the unique items in a list
- Use the man command to find out more information about UNIX commands

By the end of the second section you can expect to be able to:

- Extract information from large files
- Use regular expressions to search for particular patterns in a file
- Use the AWK programming language to extract and filter information from a file
- Create a bash script to perform several tasks at once
- Use a bash loop to perform the same task several times

1.3 Sections of the Unix tutorial

Introduction to UNIX comprises the following sections:

1. [Basic unix](#)
2. [Files](#)

Advanced UNIX for Bioinformatics comprises the following sections:

3. [grep](#)
4. [awk](#)
5. [Bash scripts](#)

1.4 Authors

This tutorial was created by [Jacqui Keane](#) and [Martin Hunt](#).

1.5 Running the commands from this tutorial

You can run the commands in this tutorial either directly from the Jupyter notebook (if using Jupyter), or by typing the commands in your terminal window.

1.5.1 Running commands on Jupyter

If you are using Jupyter, command cells (like the one below) can be run by selecting the cell and clicking *Cell -> Run* from the menu above or using *ctrl Enter* to run the command. Let's give this a try by printing our working directory using the *pwd* command and listing the files within it. Run the commands in the two cells below.



```
pwd
```



```
ls -l
```

1.5.2 Running commands in the terminal

You can also follow this tutorial by typing all the commands you see into a terminal window. This is similar to the “Command Prompt” window on MS Windows systems, which allows the user to type DOS commands to manage files.

To get started, select the cell below with the mouse and then either press control and enter or choose Cell -> Run in the menu at the top of the page.



```
echo cd $PWD
```

Now open a new terminal on your computer and type the command that was output by the previous cell followed by the enter key. The command will look similar to this:



```
cd /home/manager/pathogen-informatics-training/Notebooks/Unix/
```

Now you can follow the instructions in the tutorial from here.

1.6 Cheat sheet

We've also included a [cheat sheet](#). It probably won't make a lot of sense now, but it might be a useful reminder of this module later in the tutorial.

1.7 Let's get started!

To get started with the tutorial, head to the first section: [Basic unix](#)

2 Basic Unix

2.1 The Commandline

The commandline or ‘terminal’ is an interface you can use to run programs and analyse your data. If this is your first time using one it will seem pretty daunting at first but, with just a few commands, you’ll start to see how it helps you to get things done much quicker. You’re probably more familiar with software which uses a graphical user interface, also known as a GUI.

2.2 Getting started

Before we get started, let’s check that you’re in the right place. Please click on the cell below and press the `ctrl` and `Enter` keys. If you’re not sure what this command does, don’t worry for now; we’ll explain it in more detail later.



```
echo "cd $PWD"
```

It should say something like:



```
cd /home/manager/pathogen-informatics-training/Notebooks/Unix/
```

Type whatever it said into your terminal and press `Enter`.

Then continue through the course, entering any commands that you encounter into your terminal window. Let’s start by moving into the directory called `basic`:



```
cd basic
```

However, before getting started there are some general points to remember that will make your life easier:

- Unix is case sensitive - typing `ls` is not the same as typing `LS`.
- Often when you have problems with Unix, it is due to a spelling mistake. Check that you have not missed or added a space. Pay careful attention when typing commands across a couple of lines.

2.3 Files and directories

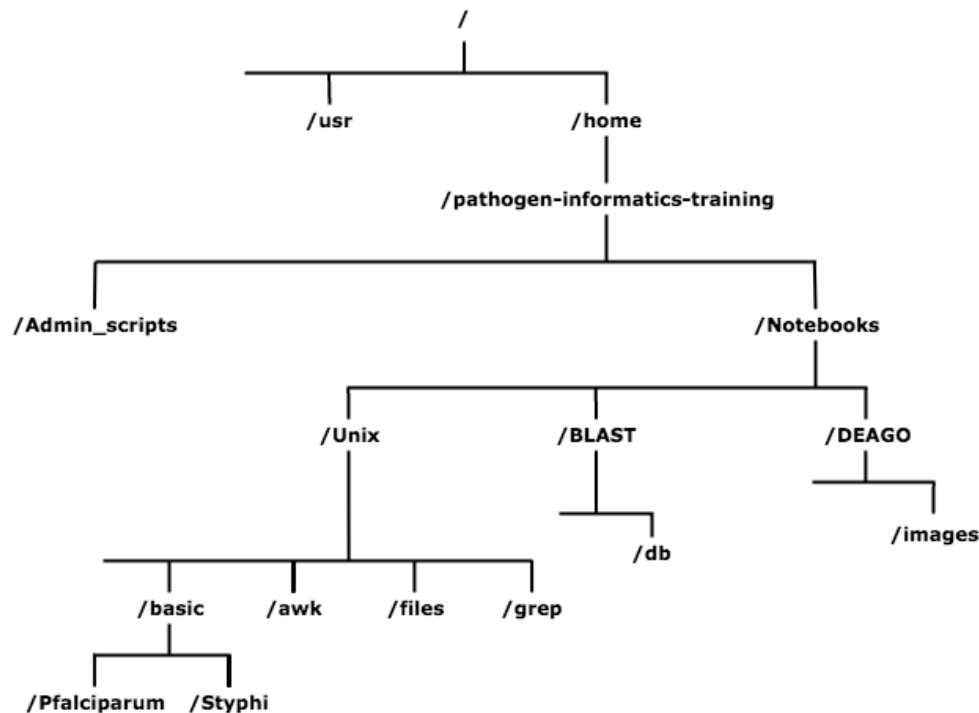
Directories are the Unix equivalent of folders on a PC or Mac. They are organised in a hierarchy, so directories can have sub-directories and so on. Directories are very useful for organising your work and keeping your account tidy - for example, if you have more than one project, you can organise the files for each project into different directories to keep them separate. You can think of directories as rooms in a house. You can only be in one room (directory) at a time. When you are in a room you can see everything in that room easily. To see things in other rooms, you have to go to the appropriate door and crane your head around. Unix works in a similar manner, moving

from directory to directory to access files. The location or directory that you are in is referred to as the current working directory.

For the file called `index.ipynb` in the `Unix` directory, the location or full pathname can be expressed as:



```
/home/pathogen-informatics-training/Notebooks/Unix/index.ipynb
```



Directory structure

2.4 pwd - find where you are

The command `pwd` stands for print working directory. A *command* (also known as a *program*) is something which tells the computer to do something. Commands are therefore often the first thing that you type into the terminal (although we'll show you some advanced exceptions to this rule later).

As described above, directories are arranged in a hierarchical structure. To determine where you are in the hierarchy you can use the `pwd` command to display the name of the current working directory. The current working directory may be thought of as the directory you are in, i.e. your current position in the file-system tree.

To find out where you are, type the following:



```
pwd
```

Remember that Unix is case sensitive, `PWD` is not the same as `pwd`.

`pwd` will list each of the folders you would need to navigate through to get from the `root` of the file system to your current directory. This is sometimes referred to as your 'absolute path' to distinguish that it gives a complete route rather than a 'relative path' which tells you how to get from one folder to another. More on that shortly.

2.5 ls - list the contents of a directory

The command `ls` stands for list. The `ls` command can be used to list the contents of a directory.

To list the contents of your current working directory type:



```
ls
```

You should see that there are 3 items in this directory.

To list the contents of a directory with extra information about the items type:



```
ls -l
```

Instead of printing out a simple list, this should have printed out additional information about each file. Note that there is a space between the command `ls` and the `-l`. There is no space between the dash and the letter `l`.

`-l` is our first example of an *option*. Many commands have options which change their behaviour but are not always required.

What do each of the columns represent?

To list all contents of a directory including hidden files and directories type:



```
ls -a -l
```

This is an example of a command which can take multiple options at the same time. Different commands take different options and sometimes (unhelpfully) use the same letter to do different things.

How many hidden files and directories are there?

Try the same command but with the `-h` option:



```
ls -alh
```

You'll also notice that we've combined `-a -l -h` into what appears to be a single `-alh` option. It's almost always ok to do this for options which are made up of a single dash followed by a single letter.

What does the `-h` option do?

To list the contents of the directory called Pfalcciparum with extra information type:



```
ls -l Pfalcciparum/
```

In this case we gave `ls` an *argument* describing the *relative path* to the directory Pfalcciparum from our current working directory. Arguments are very similar to options (and I often use the terms interchangeably) but they often refer to things which are not prefixed with dashes.

How many files are there in this directory?

2.6 Tab completion

Typing out file names is really boring and you're likely to make typos which will at best make your command fail with a strange error and at worst overwrite some of your carefully crafted analysis. *Tab completion* is a trick which normally reduces this risk significantly.

Instead of typing out `ls Pfalcciparum/`, try typing `ls P` and then press the tab character (instead of Enter). The rest of the folder name should just appear. If you have two folders with similar names (e.g. `my_awesome_scripts/` and `my_awesome_results/`) then you might need to give your terminal a bit of a hand to work out which one you want. In this case you would type `ls -l m`, when you press tab the terminal would read `ls -l my_awesome_`, you could then type `s` followed by another tab and it would work out that you meant `my_awesome_scripts/`

2.7 File permissions

Every file and directory have a set of permissions which restrict what can be done with a file or directory.

- Read (r): permission to read from a file/directory
- Write (w): permission to modify a file/directory
- Execute (x): Tells the operating system that the file contains code for the computer to run, as opposed to a file of text which you open in a text editor.

The first set of permissions (characters 2,3,4) refer to what the owner of the file can do, the second set of permissions (5,6,7) refers to what members of the Unix group can do and the third set of permissions (8,9,10) refers to what everyone else can do.

2.8 cd - change current working directory

The command `cd` stands for change directory.

The `cd` command will move you from the current working directory to another directory, in other words allow you to move up or down in the directory hierarchy.

To move into the `Styphi` directory type the following. Note, you'll remember this more easily if you type this rather than copying and pasting. Also remember that you can use tab completion to save typing all of it.

```
cd Styphi/
```



Now use the `pwd` command to check your location in the directory hierarchy and the `ls` command to list the contents of this directory.

```
pwd
ls
```



You should see that there are 3 files called: `Styphi.fa`, `Stypi.gff`, `Styphi.noseq.gff`

2.9 Tips

There are some short cuts for referring to directories:

- `.` Current directory (one full stop)
- `..` Directory above (two full stops)
- `~` Home directory (tilde)
- `/` Root of the file system (like C: in Windows)

Try the following commands, what do they do?



```
ls .
```



```
ls ..
```



```
ls ~
```

As you may remember, `ls` will only *list* what is in the directories. To move to the directory, you need to use `cd`. Try moving between directories a few times. Can you get into the `Pfalciiparum/` and then back into `Styphi/`?



2.10 cp - copy a file

The command `cp` stands for copy.

The `cp` command will copy a file from one location to another and you will end up with two copies of the file.

To copy the file `Styphi.gff` to a new file called `StyphiCT18.gff` type:



```
cp Styphi.gff StyphiCT18.gff
```

Use `ls` to check the contents of the current directory for the copied file:



```
ls
```


2.11 mv - move a file

The `mv` command stand for move.

The `mv` command will move a file from one location to another. This moves the file rather than copies it, therefore you end up with only one file rather than two. When using the command, the path or pathname is used to tell Unix where to find the file. You refer to files in other directories by using the list of hierarchical names separated by slashes. For example, the file called bases in the directory genome has the path genome/bases. If no path is specified, Unix assumes that the file is in the current working directory.

To move the file `StyphiCT18.gff` from the current directory to the directory above type:



```
mv StyphiCT18.gff ..
```

Use the `ls` command to check the contents of the current directory and the directory above to see that `StyphiCT18.gff` has been moved.



```
ls
```



```
cd ..
```



```
ls
```

2.12 rm - delete a file

The command `rm` stands for remove.

The `rm` command will delete a file permanently from your computer so take care!

To remove the copy of the *S. typhi* file, called `StyphiCT18.gff` type:



```
rm StyphiCT18.gff
```

Use the `ls` command to check the contents of the current directory to see that the file `StyphiCT18.gff` has been removed.



```
ls
```

Unfortunately there is no “recycle bin” on the command line to recover the file from, so you have to be careful.

2.13 find - find a file

The `find` command can be used to find files matching a given expression. It can be used to recursively search the directory tree for a specified name, seeking files and directories that match the given name.

To find all files in the current directory and all its subdirectories that end with the suffix `gff`:



```
find . -name *.gff
```

How many `gff` files did you find?

To find all the subdirectories contained in the current directory type:



```
find . -type d
```

How many subdirectories did you find?

Sometimes you may want to search for files based on when they were last modified or accessed. In those instances, the following two options to find come in handy:

- `-mtime` : search files by modifying date
- `-atime` : search files by last access date

With these commands, you can specify that the files you are looking for are older or newer than a given time using `+` and `-` respectively. For example, to search for files and directories that were modified more than one hour ago, you can run:



```
find . -mtime +1h
```

If instead you wanted to look for all files and directories that were modified in the last 20 minutes, you simply change the `+` to `-` and put `20m` instead of `1h`:



```
find . -mtime -20m
```

For more information, have a look in the man page for `find`.

These are just some basic examples of the `find` command but it is possible to use the following `find` options to search in many other ways. Two further examples are:

- `-size` : search files by file size
- `-user` : search files by user they belong to

2.14 Exercises

Many people panic when they are confronted with a Unix prompt! Don't! All the commands you need to solve these exercises are provided above and don't be afraid to make a mistake. If you get lost ask a demonstrator. If you are a person skilled at Unix, be patient this is only a short exercise.

To begin, open a terminal window and navigate to the `basic` directory in the `Unix` directory (remember use the Unix command `cd`) and then complete the exercise below.

1. Use the `ls` command to show the contents of the `basic` directory.
2. How many files are there in the `Pfalcciparum` directory?
3. What is the largest file in the `Pfalcciparum` directory?
4. Move into the `Pfalcciparum` directory.
5. How many files are there in the `fasta` directory?
6. Copy the file `Pfalcciparum.bed` in the `Pfalcciparum` directory into the `annotation` directory.
7. Move all the `fasta` files in the directory `Pfalcciparum` to the `fasta` directory.
8. How many files are there in the `fasta` directory?
9. Use the `find` command to find all `gff` files in the `Unix` directory, how many files did you find?
10. Use the `find` command to find all the `fasta` files in the `Unix` directory, how many files did you find?



Now go to the next part of the tutorial, [looking inside files](#).
You can also [return to the index](#).

3 Looking inside files

A common task is to look at the contents of a file. This can be achieved using several different Unix commands, `less`, `head` and `tail`. Let us consider some examples.

But first, change directory into the `Unix/files/` directory:



```
cd files
```

3.1 less

The `less` command displays the contents of a specified file one screen at a time. To test this command type the following command followed by the enter key:

```
less Styphi.gff
```



```
head Styphi.gff
```

The contents of the file `Styphi.gff` is displayed one screen at a time, to view the next screen press the space bar. As `Styphi.gff` is a large file this will take a while, therefore you may want to escape or exit from this command. To do this, press the `q` key, this kills the `less` command and returns you to the Unix prompt. `less` can also scroll backwards if you hit the `b` key. Another useful feature is the slash key, `/`, to search for an expression in the file. Try it, search for the gene with locus tag `t0038`. What is the start and end position of this gene?

3.2 head and tail

Sometimes you may just want to view the text at the beginning or the end of a file, without having to display all of the file. The `head` and `tail` commands can be used to do this.

The `head` command displays the first ten lines of a file.

To look at the beginning of the file `Styphi.gff` file type:



```
head Styphi.gff
```

The `tail` command displays the last ten lines of a file.

To look at the end of `Styphi.gff` type:



```
tail Styphi.gff
```

The amount of the file that is displayed can be increased by adding extra arguments. To increase the number of lines viewed from 10 to 25 add `-n 25` to the command:



```
tail -n 25 Styphi.gff
```

In this case you've given `tail` an argument in two parts. In this case the `-n` says that you want to specify the number of lines to show and the `25` bit tells it how many. Unlike earlier when we merged arguments like `ls -lha` together, it's not a good idea to merge multiple two part arguments together because otherwise it is ambiguous which value goes with which argument.

`-n` is such a common argument for `tail` and `head` that it even has a shorthand: `-n 25` and `-25` mean the same thing.

3.3 Saving time

Saving time while typing may not seem important, but the longer that you spend in front of a computer, the happier you will be if you can reduce the time you spend at the keyboard.

- Pressing the up/down arrows will let you scroll through previous commands entered.
- If you highlight some text, middle clicking on the mouse will paste it on the command line.
- Tab completion doesn't just work on filenames, it also works on commands. Try it by typing `tai` and pressing `tab`...

`tai`

Although tab completion works on commands and filenames, unfortunately it does not work on options or other arguments.



3.4 Getting help man

To obtain further information on any of the Unix commands introduced in this course you can use the `man` command. For example, to get a full description and examples of how to use the `tail` command type the following command in a terminal window.

```
man tail
```



There are several other useful commands that can be used to manipulate and summarise information inside files and we will introduce some of these next, `cat`, `sort`, `wc` and `uniq`.

3.5 Writing to files

So far we've been running commands and outputting the results into the terminal. That's obviously useful but what if you want to save the results to another file?

Type this:



```
head -1 Styphi.gff > first_Styphi_line.txt
```

It may look like nothing has happened. This is because the `>` character has *redirected* the output of the `head` command. Instead of writing to the *standard output* (your terminal) it sent the output into the file `first_Styphi_line.txt`. Note that tab completion works for `Styphi.gff` because it exists but doesn't work for `first_Styphi_line.txt` because it doesn't exist yet.

3.6 cat

`cat` is another way of reading files, but unlike `less` it just throws the entire contents of the file onto your standard output. Try it on `first_Styphi_line.txt`



```
cat first_Styphi_line.txt
```

We don't need `first_Styphi_line.txt` any more so delete it by typing



```
rm first_Styphi_line.txt
```

The `cat` command can also be given the names of multiple files, one after the other and it will just output the contents of all files. The order in which the files are displayed is determined by the order in which they appear in the command line. You can use this concept and the `>` symbol to join files together into a single file.

Having looked at the beginning and end of the `Styphi.gff` file you should notice that in the GFF file the annotation comes first, then the DNA sequence at the end. If you had two separate files containing the annotation and the DNA sequence, it is possible to concatenate or join the two together to make a single file like the `Styphi.gff` file you have just looked at.

For example, we have two separate files, `Styphi.noseq.gff` and `Styphi.fa`, that contain the annotation and DNA sequence, respectively for the *Salmonella typhi* CT18 genome. To join together these files type:



```
cat Styphi.noseq.gff Styphi.fa > Styphi.concatenated.gff
```

The files `Styphi.noseq.gff` and `Styphi.fa` will be joined together and written to a file called `Styphi.concatenated.gff`.

The `>` symbol in the command line directs the output of the `cat` program to the designated file `Styphi.concatenated.gff`. Use the command `ls` to check for the presence of this file.



```
ls
```

3.7 wc - counting

The command `wc` counts lines, words or characters.

There are two ways you could use it:



```
wc -l Styphi.gff
```

or



```
cat Styphi.gff | wc -l
```

Both give a similar answer. In the first example you tell `wc` the file that you want it to review (`Styphi.gff`) and pass the `-l` option to say that you're only interested in the number of lines.

In the second example you use the `|` symbol which is also known as the *pipe* symbol. This *pipes* the output of `cat Styphi.gff` into the input of `wc -l`. This means that you can also use the same `wc` tool to count other things. For example to count the number of files that are listed by `ls` type:



```
ls | wc -l
```

You can connect as many commands as you want. For example, type:



```
ls | grep ".gff" | wc -l
```

What does this command do? You will learn more about the `grep` command later in this course.

3.8 sort - sorting values

The `sort` lets you sort the contents of the input. When you sort the input, lines with identical content end up next to each other in the output. This is useful as the output can then be fed to the `uniq` command (see below) to count the number of unique lines in the input.

To sort the contents of a BED file type:

```
sort Pfalciparum.bed
```

Now type:



```
sort Pfalciparum.bed | head
```



```
sort Pfalciparum.bed | tail
```

To sort the contents of a BED file on position, type the following command.

```
sort -k 2 -n Pfalciparum.bed
```

The `sort` command can sort by multiple columns e.g. 1st column and then 2nd column by specifying successive `-k` parameters in the command. Type the following commands:



```
sort -k 2 -n Pfalciparum.bed | head
```



```
sort -k 2 -n Pfalciparum.bed | tail
```

Why not have a look at the manual for `sort` to see what these options do? Remember that you can type `/` followed by a search phrase, `n` to find the next search hit, `N` to find the previous search hit and `q` to exit.

```
man sort
```



3.9 uniq - finding unique values

The `uniq` command extracts unique lines from the input. It is usually used in combination with `sort` to count unique values in the input.

To get the list of chromosomes in the `Pfalciparum bed` file type:



```
awk '{ print $1 }' Pfalciparum.bed | sort | uniq
```

How many chromosomes are there? You will learn more about the `awk` command later in this course.

Warning: `uniq` is really stupid; it can only spot that two lines are the same if they are right next to one another. You therefore almost always want to `sort` your input data before using `uniq`.

Do you understand how this command is working? Why not try building it up piece by piece to see what it does?

```
awk '{ print $1 }' Pfalciparum.bed | less
awk '{ print $1 }' Pfalciparum.bed | sort | less
awk '{ print $1 }' Pfalciparum.bed | sort | uniq | less
```



3.10 Exercises

Open up a new terminal window, navigate to the `files` directory in the `Unix` directory and complete the following exercise:

1. Use the `head` command to extract the first 500 lines of the file `Styphi.gff` and store the output in a new file called `Styphi.500.gff`.
2. Use the `wc` command to count the number of lines in the `Pfalciparum.bed` file.

3. Use the `sort` command to sort the file `Pfalciiparum.bed` on chromosome and then gene position.
4. Use the `uniq` command to count the number of features per chromosome in the `Pfalciiparum.bed` file. Hint: use the `man` command to look at the options for the `uniq` command. Or peruse the `wc` or `grep` manuals. There's more than one way to do it!



Now go to the next part of the tutorial, [searching inside files with grep](#).
You can also [return to the index](#) or revisit the [previous section](#).

4 Searching inside files with `grep`

A common task is to extract information from large files. This can be achieved using the Unix command `grep`, which stands for “Globally search for a Regular Expression and Print”. The meaning of this acronym will become clear later, when we discuss Regular Expressions. First, we will consider simpler examples.

Before we start, change into the `Unix/grep` directory:



```
cd grep
```

4.1 Simple pattern matching

We will use a small example file (in “BED” format), which contains the expression levels of some genes. This is a column-based file, with a tab character between each column. There can be more than 10 columns, but only the first three are required to be a valid file. The file format is described in full here: <http://genome.ucsc.edu/FAQ/FAQformat#format1>. We will use the first 5 columns:

1. Sequence name
2. start position (starting from 0, not 1)
3. end position (starting from 0, not 1)
4. feature name
5. score (which is used to store the gene expression level in our examples).

Here is the contents of the first example BED file used in this course:



```
cat gene_expression.bed
```

In reality, such a file could contain 100,000s of lines, so that it is not practical to read manually. Suppose we are interested in all the genes from chromosome 2. We can find all these lines using `grep`:



```
grep chr2 gene_expression.bed
```

This has shown us all the lines that contain the string “chr2”.

We can use a pipe to then just extract the genes that are on the positive strand, using `grep` a second time:



```
grep chr2 gene_expression.bed | grep +
```

However, since `grep` is reporting a match to a string *anywhere* on a line, such simple searches can have undesired consequences. For example, consider the result of doing a similar search for all the genes in chromosome 1:



```
grep chr1 gene_expression.bed
```

Oops! We found genes in chromosome 10, because “chr1” is a substring of “chr10”.

Or consider the following file, where the genes have unpredictable names (which is not unusual for bioinformatics data).



```
cat gene_expression_sneaky.bed
```

Now we try to find genes on chromosome 1 that are on the negative strand. We put the minus sign in quotes, to stop Unix interpreting this as an option to `grep`, as opposed to the string we are searching for:



```
grep chr1 gene_expression_sneaky.bed | grep '-'
```

The extra lines are found by `grep` because of matches in columns we were not expecting to match. Remember, `grep` is reporting these lines because they each contain the strings “chr1” and “-” *somewhere*.

We need a way to make searching with `grep` more specific.

4.2 Regular expressions

Regular expressions provide the solution to the above problems. They are a way of defining more specific patterns to search for.

4.2.1 Matching the start and end of lines

First, we can specify that a match must be at the start of a line using the symbol “^”, which means “start of line”. Without the ^, we find any match to “chr1”:



```
grep chr1 gene_expression_sneaky.bed
```

However, notice the effect of searching for `^chr1` instead. Note that we put the regular expression in quotes, to avoid Unix errors. Not using quotes may or may not give an error, but it is safest to use quotes for anything but the simplest of searches.



```
grep '^chr1' gene_expression_sneaky.bed
```

Good! We have removed the match to the badly-named gene “chr11.gene1”, which is on chromosome 8. Now we want to avoid matching chromosomes 10 and 11. This can be done by also looking for a “tab” character, which is represented by writing `\t`. For technical reasons, which are beyond the scope of this course, we must also put a dollar sign before the quotes to make any search involving a tab character work.



```
grep $'^chr1\t' gene_expression_sneaky.bed
```

To find the genes on the negative strand, all that remains is to match a minus sign at the *end* of the line (so that we do not find “sneaky-gene3”). We can do this using the dollar “\$”, which means “end of line”.



```
grep $'^chr1\t' gene_expression_sneaky.bed | grep '\-$'
```

4.2.2 Wildcards and alphabets

Another special character in regular expressions is the dot: “.”. This stands for any single character. For example, this finds all matches to chromosomes 1-9, and chromosomes X and Y:



```
grep $'^chr.\t' gene_expression.bed
```

In fact, the earlier command that found all genes on chromosome 1 that are on the negative strand, could be found with a single call to `grep` instead of two calls piped together. To do this, we need a regular expression that finds lines that:

- start with chr1, then a tab character
- end with a minus
- have arbitrary characters between.

The asterisk “*” has a special meaning: it says to match any number (including zero) of whatever character is before the *. For example, the regular expression ‘AC*G’ will match AG, ACG, ACCG, etc. The simpler, improved command is:



```
grep $'^chr1\t.*-$' gene_expression_sneaky.bed
```

As well as matching any character using a dot, we can define any list of characters to match, using square brackets. For example, [12X] means match a 1, 2, or an X. This can be used to find all genes from chromosomes 1, 2 and X:



```
grep $'^chr[12X]\t' gene_expression.bed
```

Or just the autosomes may be of interest. To do this we introduce two new features:

- Ranges can be given in square brackets, for example [1-5] will match 1, 2, 3, 4 or 5.
- The plus sign “+” has a special meaning that is similar to “*”. Instead of any number of matches (including zero), it looks for at least one match. To avoid simply matching a plus sign, it must be preceded by a backslash: “\+”. For example, the regular expression ‘AC\+G’ will match ACG, ACCG, ACCCG etc (but will not match AG).

Warning: Adding a backslash is often called *escaping* (e.g. *escape the plus symbol*). Depending on the software you’re using (and the options you give it), you may need to escape the symbol to indicate that you want its special regex meaning (e.g. multiple copies of the last character please) or its literal meaning (e.g. give me a ‘+’ symbol please). If your command isn’t working as you

expect, try playing with these options and always test your regular expression before assuming it gave you the right answer.

The command to find the autosomes is:



```
grep $'^chr[0-9]\+\t' gene_expression.bed
```

4.3 Other `grep` options

The Unix command `grep` and regular expressions are extremely powerful and we have only scratched the surface of what they can do. Take a look at the manual (by typing `man grep`) to get an idea. A few particularly useful options are discussed below.

4.3.1 Counting matches

A common use-case is counting matches within files. Instead of output each matching line, the option “`-c`” tells `grep` to report the number of lines that matched. For example, the number of genes in the autosomes in the above example can be found by simply adding `-c` to the command.



```
grep -c $'^chr[0-9]\+\t' gene_expression.bed
```

4.3.2 Case sensitivity

By default, `grep` is case-sensitive. It can be useful to ignore the distinction between upper and lower case using the option “`-i`”. Suppose we have a file of sequences, and want to find the sequences that contain the string `ACGT`. It is not unusual to come across files that have a mix of upper and lower case nucleotides. Consider this FASTA file:



```
cat sequences.fasta
```

A simple search for `ACGT` will not return all the results:



```
grep ACGT sequences.fasta
```

However, making the search case-insensitive solves the problem.



```
grep -i ACGT sequences.fasta
```

4.3.3 Searching in more than one file

So far, we have restricted to searches in one file, but `grep` can be given a list of files in which to search. As an example, we are given three files called `list_example.1`, `list_example.2`, and `list_example.3`. They are simple lists of genes, for illustrative purposes. For example, the first file looks like this:



```
cat list_example.1
```

Which files contain “gene1”?



```
grep '^gene1$' list_example.1 list_example.2
```

gene1 only appears in the file `list_example.1`. The output format of `grep` has now changed, because it was given a list of files. The format is:

- filename:line_that_matches

ie, the name of the file has been added to the start of each matching line.

For convenience, there’s also a way of specifying all of the list examples:



```
echo list_example.*
```



```
grep '^gene1$' list_example.*
```

How about gene42?



```
grep '^gene42$' list_example.*
```

gene42 appears once in `list_example.2` and twice in `list_example.3`.

4.3.4 Inverting matches

By default, `grep` reports all lines that do match the regular expression. Sometimes it is useful to filter a file, by reporting lines that *do not* match the regular expression. Using the option “-v” makes `grep` “invert” the output. For example, we could exclude genes from autosomes in the BED file from earlier.



```
grep -v $'^chr[0-9]\+\t' gene_expression.bed
```

4.4 Replacing matches to regular expressions

Finally, we show how to replace every match to a regular expression with something else, using the command “`sed`”. The general form of this is:

```
sed 's/regular expression/new string/' input_file
```

This will output a new version of the input file, with each match to the regular expression replaced with “new string”. For example:



```
sed 's/^chr/chromosome/' gene_expression.bed
```

4.5 Exercises

The following exercises all use the FASTA file `exercises.fasta`. Before starting the exercises, open a new terminal and navigate to the `grep/` directory, which contains `exercises.fasta`.

Use `grep` to find the answers. Hint: some questions require you to use `grep` twice, and possibly some other Unix commands.

1. Make a `grep` command that outputs just the lines with the sequence names.
2. How many sequences are in the file?
3. Do any sequence names have spaces in them? What are their names?
4. Make a `grep` command that outputs just the lines with the sequences, not the names.
5. How many sequences contain unknown bases (an “n” or “N”)?
6. Are there any sequences that contain non-nucleotides (something other than A, C, G, T or N)?
7. How many sequences contain the 5′ cut site GCWGC (where W can be an A or T) for the restriction enzyme *AclI*?
8. Are there any sequences that have the same name? You do not need to find the actual repeated names, just whether any names are repeated. (Hint: it may be easier to first discover how many unique names there are).



Now go to the next part of the tutorial, [file processing with AWK](#). You can also [return to the index](#) or revisit the [previous section](#).

5 File processing with AWK

AWK is a programming language named after the initials of its three inventors: Alfred Aho, Peter Weinberger, and Brian Kernighan. AWK is incredibly powerful at processing files, particularly column-based files, which are commonplace in Bioinformatics. For example, BED, GFF, and SAM files.

Although long programs, put into a separate file, can be written using AWK, we will use it directly on the command line. Effectively, these are very short AWK programs, often called “one-liners”.

Before we start, change into the `Unix/awk` directory:



```
cd awk
```

5.1 Extracting columns from files

`awk` reads a file line-by-line, splitting each line into columns. This makes it easy to do simple things like extract a column from a file. We will use the following GFF file for our examples.



```
cat genes.gff
```

The columns in the GFF file are separated by tabs and have the following meanings:

1. Sequence name
2. Source - the name of the program that made the feature
3. Feature - the type of feature, for example gene or CDS
4. Start position
5. Stop position
6. Score
7. Strand (+ or -)
8. Frame (0, 1, or 2)
9. Optional extra information, in the form `key1=value1;key2=value2;...`

The score, strand, and frame can be set to `.` if it is not relevant for that feature. The final column 9 may or may not be present and could contain any number of key, value pairs.

We can use `awk` to just print the first column of the file. `awk` calls the columns `$1`, `$2`, ... etc, and the complete line is called `$0`. Try



```
awk -F"\t" '{print $1}' genes.gff
```

A little explanation is needed.

- The option `-F"\t"` was needed to tell `awk` that the columns are separated by tabs (more on this later).
- For each line of the file, `awk` does what is inside the curly brackets. In this case, we simply print the first column.

The repeated chromosome names are not nice. It is more likely to want to know just the unique names, which can be found by piping into the Unix command `sort`.



```
awk -F"\t" '{print $1}' genes.gff | sort -u
```

5.2 Filtering the input file

Similarly to `grep`, `awk` can be used to filter out lines of a file. However, since `awk` is column-based, it makes it easy to filter based on properties of any columns of interest. The filtering criteria can be added before the braces. For example, the following extracts just chromosome 1 from the file.



```
awk -F"\t" '$1=="chr1" {print $0}' genes.gff
```

There are two important things to note from the above command:

1. `$1=="chr1"` means that column 1 must be *exactly* equal to “chr1”. This means that “chr10” is not found.
2. The “`{print $0}`” part only happens when the first column is equal to “chr1”, otherwise `awk` does nothing (the line gets ignored).

Awk commands are made up of two parts, a *pattern* (e.g. `$1=="chr1"`) and an *action* (e.g. `print $0`) which is contained in curly braces. The *pattern* defines which lines the *action* is applied to.

In fact, the action (the part in curly braces) can be omitted in this example. `awk` assumes that you want to print the whole line, unless it is told otherwise. This gives a simple method of filtering based on columns.



```
awk -F"\t" '$1=="chr1"' genes.gff
```

You might remember using another of `awk`’s defaults in a previous exercise. In that example we supplied an action but no pattern. In this case, `awk` assumes that you want to apply the action to every line in the file. For example:



```
awk -F"\t" '{print $1}' genes.gff
```

Multiple patterns can be combined using “`&&`” to mean “and”. For example, to find just the genes from chromosome 1:



```
awk -F"\t" '$1=="chr1" && $3=="gene"' genes.gff
```

The entire line need not be printed (remember, if not specified, `awk` assumes a `print $0`). Suppose we want only the sources of the genes on chromosome 1:



```
awk -F"\t" '$1=="chr1" && $3=="gene" {print $2}' genes.gff | sort -u
```

Similarly to using “`&&`” for “and”, there is “`||`” to mean “or”. To find features that are repeats or made by the tool “source2”:



```
awk -F"\t" '$2=="source2" || $3=="repeat"' genes.gff
```

So far, we have only used strings for the filtering. Numbers can also be used. We could ask `awk` to return all the genes on chromosome 1 that start before position 1100:



```
awk -F"\t" '$1=="chr1" && $3=="gene" && $4 < 1100' genes.gff
```

Instead of looking for exact matches to strings, regular expressions can be used. The symbol “`~`” is used instead of “`==`”. For example, to find all the autosomes, we need to use a regular expression for matches to the first column. The regular expression is written between forward slashes.



```
awk -F"\t" '$1 ~ /^chr[0-9]+$/' genes.gff
```

Like with `grep`, matches can be inverted. `grep` has the option `-v`, but with `awk` we use “`!~`” to mean “does not match”. This inverts the previous example:



```
awk -F"\t" '$1 !~ /^chr[0-9]+$/' genes.gff
```

If we do not specify a column, `awk` looks for a match anywhere in the whole line (it assumes we wrote `$0 ~ /regex/`). So, in some sense, `awk` can be used as a replacement for `grep`:



```
awk '/repeat/' genes.gff
```

(the `-F"\t"` was omitted because the match is to the whole line, so how the columns are separated is not relevant.)



```
grep repeat genes.gff
```

However, with `awk` we can easily pull out information from the matching lines. Suppose we want to know which chromosomes have repeats. It is easy with `awk`.



```
awk -F"\t" '/repeat/ {print $1}' genes.gff | sort -u
```

Sanity checking files Never, ever trust the contents of Bioinformatics files (even if you made them!). We now have enough skills to do some basic sanity checking of a GFF file. For example, to check that every gene has been assigned a strand:



```
awk -F"\t" '$3=="gene" && !($7 == "+" || $7 == "-)' genes.gff
```

Something went wrong when this file was made: gene3 has an unknown strand.

Do the start and end coordinates of all the features make sense?



```
awk -F"\t" '$5 < $4' genes.gff
```

According to the file, this gene starts at position 10000 and ends at position 1200, which does not make sense. Also, it has no name (the final optional column is empty). We could check if there are any other genes with no name. One way to do this is to use the special variable “NF”, which is the number of columns (fields) in the current line. Since the final column is optional, each line might have 8 or 9 columns. We need to write a command that will check:

- If the feature is a gene, and if it is:
- check if the number of columns is less than 9. When there are 9 columns, check if there is a name defined.



```
awk -F"\t" '$3=="gene" && (NF<9 || $NF !~/name/)' genes.gff
```

Note the distinction between NF (the number of columns) and “\$NF” (the contents of the final column).

As promised earlier, we now consider the relevance of the option “-F“\t””, to tell awk that the columns in the input file are separated with tab characters. If we forgot to use this option, then awk will use its default behaviour, which is to separate on *any* whitespace (which usually means tabs and/or spaces). However, consider the final column of the file - it can contain whitespace, which means that messy things happen. Suppose we try to extract the optional extra final column of the file, when it is present. Compare the effect of running awk with and without “-F“\t””.



```
awk -F"\t" 'NF>8 {print $NF}' genes.gff
```



```
awk 'NF>8 {print $NF}' genes.gff
```

One more sanity check: each line should have 8 or 9 columns (remembering to use -F“\t”!)



```
awk -F"\t" 'NF<8 || NF>9' genes.gff
```

There was no output, which means that every line does indeed have 8 or 9 columns.

5.3 Changing the output

In addition to filtering, awk can be used to change the output.

Every value in a column could be changed to something else, for example suppose we want to change the source column (column number 2) to something else.



```
awk -F"\t" '{ $2="new_source"; print $0 }' genes.gff
```

This is close, but look carefully at the output. What happened? The output is not tab-separated, but is instead separated with spaces. To restore the tabs, we need to use another special variable called “OFS” (Output Field Separator), and change it before `awk` does any processing of the input file. This can be achieved by adding “`BEGIN{OFS="\t"}`”, as in the next example. Before `awk` reads any lines of the file it runs the `BEGIN` block of code, which in this case changes `OFS` to be a tab character.



```
awk -F"\t" 'BEGIN{OFS="\t"} {$2="new_source"; print $0}' genes.gff
```

5.4 Processing the data

More in-depth processing is possible. For example, we could print the length of each repeat (and then sort the results numerically)



```
awk -F"\t" '$3=="repeat" {print $5 - $4 + 1}' genes.gff | sort -n
```

Perhaps we would like to know the total length of the repeats. We need to use a variable to add up the total lengths and print the final total. In the same way that `awk` has a `BEGIN` block, it can also be given an `END` block that is only run when `awk` has finished reading all lines of the input file.



```
awk -F"\t" 'BEGIN{sum=0} $3=="repeat" \
           {sum = sum + $5 - $4 + 1} \
           END{print sum}' genes.gff
```

The total repeat length was stored in a variable called `sum`. The previous `awk` command can be broken down into three parts:

1. The `BEGIN{sum=0}` sets `sum` to zero before any lines of the file are read.
2. `awk` reads each line of the file. Each time a repeat is found, the length of that repeat is added to `sum`.
3. Once all lines of the file have been read, `awk` runs the `END` block: `END{print sum}`. This prints the value of `sum`.

In fact, the command can be shortened a little. Adding a number to a variable is so common, that there is a shorthand way to write it. Instead of

```
sum = sum + $5 - $4 + 1
```

we can use

```
sum += $5 - $4 + 1
```

to get the same result.



```
awk -F"\t" 'BEGIN{sum=0} \
           $3=="repeat" {sum += $5 - $4 + 1} \
           END{print sum}' genes.gff
```

Maybe we would like to know the mean score of the genes. We need to calculate the total score, and divide this by the number of genes. To keep track of the number of genes, we use a variable called `count`. Each time a new gene is found, 1 must be added to `count`. This could be done by writing

```
count = count + 1
```

but instead we will use the shorthand

```
count++
```



```
awk -F"\t" 'BEGIN{sum=0; count=0} \
           $3=="gene" {sum += $6; count++;} \
           END{print sum/count}' genes.gff
```

Finally, `awk` has a default behaviour that means we do not even need the `BEGIN` block. It can be completely omitted in this example because we are setting `sum` and `count` to zero. The first time `awk` sees a variable being used, it will set it to zero by default. For example, when `awk` reads the first line of the file, the piece of code

```
count++
```

tells `awk` to add 1 to `count`. However, if `awk` has not encountered the variable `count` before, it assumes it is zero (as if we had written `BEGIN{count=0}`), then adds 1 to it. The result is that `count` is equal to 1. Similar comments apply to the variable `sum`.



```
awk -F"\t" '$3=="gene" {sum += $6; count++;} \
           END{print sum/count}' genes.gff
```

If this confuses you, then be explicit and use the `BEGIN` block of code. The result is the same.

5.5 Exercises

The following exercises all use the BED file `exercises.bed`. Before starting the exercises, open a new terminal and navigate to the `awk/` directory, which contains `exercises.bed`.

Use `awk` to find the answers to the following questions about the file `exercises.bed`. Many questions will require using pipes (eg “`awk ... | sort -u`” for question 1).

1. What are the names of the contigs in the file?
2. How many contigs are there?
3. How many features are on the positive strand?
4. How many features are on the negative strand?
5. How many genes are there?
6. How many genes have no strand assigned to them (ie the final column is not there)?
7. Are any gene names repeated? (Hint: you do not need to find their names, just a yes or no answer. Consider the number of unique gene names.)
8. What is the total score of the repeats?
9. How many features are in contig-1?
10. How many repeats are in contig-1?

11. What is the mean score of the repeats in contig-1?



Now go to the next part of the tutorial, [BASH scripts](#).

You can also [return to the index](#) or revisit the [previous section](#).

6 BASH scripts

So far, we have run single commands in a terminal. However, it is useful to be able to run multiple commands that process some data and produce output. These commands can be put into a separate file (ie a script), and then run on the input data. This has the advantage of reproducibility, so that the same analysis can be run on many input data sets.

6.1 First script

It is traditional when learning a new language (in this case BASH), to write a simple script that says “Hello World!”. We will do this now.

First, open a terminal and make a new directory in your home called `scripts`, by typing

```
cd
mkdir ~/scripts
```

Next open a text editor, which you will use to write the script. What text editors are available will depend on your system. For example, `gedit` in Linux. Do not try to use a word processor, such as Word! If you don’t already have a favorite, try `gedit` by running the following command:

```
gedit &
```

Type this into the text editor:

```
echo Hello World!
```

and save this to a file called `hello.sh` in your new `scripts` directory. This script will print `Hello World!` to the screen when we run it. First, in your terminal, check that the script is saved in the correct place.



```
cd scripts
```



```
ls hello.sh
```

If everything is OK, then next try to run the script. For now, we need to tell Unix that this is a bash script, and where it is:



```
bash hello.sh
```

6.2 Setting up a scripts directory

It would be nice if all our scripts could simply be run from anywhere in the filesystem, without having to tell Unix where the script is, or that it is a BASH script. This is how the built-in commands work, such as `cd` or `ls`.

To tell Unix that the script is a BASH script, make this the first line of the script:

```
#!/usr/bin/env bash
```

and remember to save the script again. This special line at the start of the file tells Unix that the file is a bash script, so that it expects bash commands throughout the file. There is one more change to be made to the file to tell Unix that it is a program to be run (it is “executable”). This is done with the command `chmod`. Type this into the terminal to make the file executable:



```
chmod +x hello.sh
```

Now, the script can be run, but we must still tell Unix where the script is in the filesystem. In this case, it is in the current working directory, which is called “./”.



```
./hello.sh
```

The final thing to do is change our setup so that Unix can find the script without us having to explicitly say where it is. Whenever a command is typed into Unix, it has a list of directories that it searches through to look for the command. We need to add the new scripts directory to that list of directories. Try typing

```
echo $PATH
```

It returns a list of directories, which are all the places Unix will look for a command. Before we add the scripts directory to this list, check what happens if we try to run the script without telling Unix where it is:

```
hello.sh
bash: hello.sh: command not found
```

Unix did not find it! The command to run to add the scripts directory to `$PATH` is:

```
export PATH=$PATH:~/scripts/
```

If you want this change to be permanent, ie so that Unix finds your scripts after you restart or logout and login again, add that line to the end of a file called `~/.bashrc`. If you are using a Mac, then the file should instead be `~/.bash_profile`. If the file does not already exist, then create it and put that line into it.

The following command is only here so that this notebook finds scripts correctly and the remaining examples work. **Do not type the next command into your terminal.**



```
PATH=$PATH:$PWD # do not type this into your terminal!
```

Now the script works, no matter where we are in the filesystem. Unix will check the scripts directory and find the file `hello.sh`. You can be *anywhere* in your filesystem, and simply running

```
hello.sh
```

will always work. Try it now.



```
hello.sh
```


In general, when making a new script, you can now copy and edit an existing script, or make a new one like this:

```
cd ~/scripts
touch my_script.sh
chmod +x my_script.sh
```

and then open `my_script.sh` in a text editor.

6.3 Getting options from the terminal and printing a help message

Usually, we would like a script to read in options from the user, such as the name of an input file. This would mean a script can be run like this:

```
my_script.sh input_file
```

Inside the script, the parameters provided by the user are given the names `$1`, `$2`, `$3` etc (do not confuse these with column names used by `awk`!). Here is a simple example that expects the user to provide a filename and a number. The script simply prints the filename to the screen, and then the first few lines of the file (the number of lines is determined by the number given by the user).



```
cat options_example.sh
```



```
options_example.sh test_file 2
```

The options have been used by the script, but the script itself is not very readable. It is better to use names instead of `$1` and `$2`. Here is an improved version of the script that does exactly the same as the previous script, but is more readable.



```
cat options_example.2.sh
```

6.4 Checking options from the user

The previous scripts will have strange behaviour if the input is not as expected by the script. Many things could go wrong. For example:

- The wrong number of options are given by the user
- The input file does not exist.

Try running the script with different options and see what happens.

A convention with scripts is that it should output a help message if it is not run correctly. This shows anyone how the script should be run (including you!) without having to look at the code inside the script.

A basic check for this script would be to verify that two options were supplied, and if not then print a help message. The code looks like this:

```
if [ $# -ne 2 ]
then
    echo "usage: options_example.3.sh filename number_of_lines"
    echo
    echo "Prints the filename, and the given first number of lines of the file"
    exit
fi
```

You can copy this code into the start of any of your scripts, and easily modify it to work for that script. A little explanation:

- A special variable `$#` has been used, which is the number of options that were given by the user.
- The whole block of code has the form “`if [$# -ne 2] then fi`”. This only runs the code between the `then` and `fi`, if `$#` (the number of options) is not 2.
- The line `exit` simply makes the script end, so that no more code is run.



```
options_example.3.sh
```

Another check is that the input file really does exist. If it does not exist, then there is no point in trying to run any more code. This can be checked with another `if ... then ... fi` block of code:

```
if [ ! -f $filename ]

then
    echo "File '$filename' not found! Cannot continue"
    exit
fi
```

Putting this all together, the script now looks like this:



```
cat options_example.3.sh
```

Two new features have also been introduced in this file:

1. The second line is “`set -eu`”. Without this line, if any line produces an error, the script will carry on regardless to the end of the script. Using the `-e` option, an error anywhere in the file will result in the script stopping at the line that produced the error, instead of continuing. In general, it is best that the script stops at any error. The `-u` creates an error if you try to use a variable which doesn’t exist. This helps to stop typos doing bad things to your analysis.
2. There are several lines starting with a hash `#`. These lines are “comment lines” that are not run. They are used to document the code, containing explanations of what is happening. It is good practice to comment your scripts!

The above script provides a template for writing your own scripts. The general method is:

1. Tell Unix that this is a BASH script, and to stop at the first error.
2. Check if the user ran the script correctly. If not, output a message telling the user how to run the script.
3. Check the input looks OK (in this case, that the input file exists).
4. Process the input.

6.5 Using variables to store output from commands

It can be useful to run a command and put the results into a variable. Recall that we stored the input from the user in sensibly named variables:

```
filename=$1
```

The part after the equals sign could actually be any command that returns some output. For example, running this in Unix

```
wc -l filename | awk '{print $1}'
```

returns the number of lines. In case you are wondering why the command includes `| awk '{print $1}'`, check what happens with and without the pipe to `awk`:



```
wc -l options_example.3.sh
```



```
wc -l options_example.3.sh | awk '{print $1}'
```

With a small change, this can be stored in a variable and then used later.



```
filename=options_example.3.sh
line_count=$(wc -l $filename | awk '{print $1}')
echo There are $line_count lines in the file $filename
```

6.6 Repeating analysis with loops

It is common in Bioinformatics to run the same analysis on many files. Suppose we had a script that ran one type of analysis, and wanted to repeat the same analysis on 100 different files. It would be tedious, and error-prone, to write the same command 100 times. Instead we can use a loop. As an example, we will just run the Unix command `wc` on each file but instead, in reality this would be a script that runs in-depth analysis. We can run `wc` on each of the files in the directory `loop_files/` with the following command.



```
for filename in loop_files/*; do wc $filename; done
```

6.7 Exercises

1. Write a script that gets a filename from the user. If the file exists, it prints a nice human-readable message telling the user how many lines are in the file.
2. Use a loop to run the script from Exercise 1 on the files in the directory `loop_files/`.
3. Write a script that takes a GFF filename as input. Make the script produce a summary of various properties of the file. There is an example input file provided called `bash_scripts/exercise_3.gff`. Use your imagination! You could have a look back at the `awk` section of the course for inspiration. Here are some ideas you may wish to try:
 - Does the file exist?
 - How many records (ie lines) are in the file?
 - How many genes are in the file?
 - Is the file badly formatted in any way (eg wrong number of columns, do the coordinates look like numbers)?

This is the end of the tutorial. You can [return to the index](#) or revisit the [previous section](#).

You can find the answers to all exercises in this tutorial [here](#).