

# TRAINER'S MANUAL

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## Introduction to Command-Line & Scripting For Bioinformatics

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## Workshop Information

## The Trainers

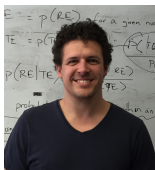
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## Welcome

Thank you for your attendance & welcome to the Introduction to Command Line & Shell Scripting Workshop. This is an offering by the University of Adelaide, Bioinformatics Hub which is a centrally funded initiative from the Department of Vice-Chancellor (Research), with the aim of assisting & enabling researchers in their work. Training workshops & seminars such as this one are an important part of this initiative. The Bioinformatics Hub itself has a web-page at <http://www.adelaide.edu.au/bioinformatics-hub/>, and to be kept up to date on upcoming events and workshops, please join the internal Bioinformatics mailing list on <http://list.adelaide.edu.au/mailman/listinfo/bioinfo>.

The Bioinformatics Hub has just started a Twitter account, so please follow us on (<https://twitter.com/UofABioinfoHub/>). We also have an active Slack team for discussing Bioinformatics questions with the local community. Slack teams do require an invitation to join, so please email the Hub on [bioinf\\_hub@adelaide.edu.au](mailto:bioinf_hub@adelaide.edu.au) to join the community. All are welcome.

Today's workshop has been put together based on previous material and courses prepared by Dr Stephen Bent (*University of Queensland*), with generous technical support & advice provided by Dr Nathan Watson-Haigh (*ACPFG*) and Dr Dan Kortschak (*Adelaide University, Adelson Research Group*). We hope it will be useful in enabling you to continue and to advance your research.

## Course Summary

In today's workshop, the morning session will be spent introducing you to the basic tools and concepts required for data handling. In the afternoon session we'll develop these skills to a more advanced level, with progress in both sessions being made at your own pace. Some people may finish early today, but the majority of you probably won't. The VMs you use will be active for the next few weeks should you wish to continue working through the material after the workshop

The majority of data handling and analysis required in the field of bioinformatics uses the *command line*, alternatively known as the terminal or the *bash shell*. This is a text-based interface in which commands must be typed, as opposed to the Graphical User Interfaces (aka GUIs) that most of us have become accustomed to. Being able to access your computer using these tools enables you to more fully utilise the power & capabilities of your machine, for both Linux & Mac operating systems, and to a lesser extent will even enable you to dig deeper on a Windows system.

Whilst some of the tools we cover today may appear trivial, they will be used on a

daily basis once you begin working in the field. These basic tools will also be very useful for writing what are known as *shell scripts*, which we will begin to cover in the afternoon session. These are essentially simple programs that utilise the inbuilt functions of the shell, and are used to automate processes such as de-multiplexing read libraries, or aligning reads to the genome. A knowledge of this simple type of programming is also essential for accessing the high-performance computing resources as offered by eResearch SA ([www.ersa.edu.au](http://www.ersa.edu.au)).

## Providing Feedback

While we endeavour to deliver a workshop with quality content and documentation in a venue conducive to an exciting, well run hands-on workshop with a bunch of knowledgeable and likable trainers, we know there are things we could do better.

Whilst we want to know what didn't quite hit the mark for you, what would be most helpful and least depressing, would be for you to provide ways to improve the workshop. i.e. constructive feedback. After all, if we knew something wasn't going to work, we wouldn't have done it or put it into the workshop in the first place!

Clearly, we also want to know what we did well! This gives us that "feel good" factor which will see us through those long days and nights in the lead up to such hands-on workshops!

With that in mind, we'll provide some really high-tech mechanisms through which you can provide anonymous feedback during the workshop:

1. Some empty ruled pages at the back of this handout. Use them for your own personal notes or for writing specific comments/feedback about the workshop as it progresses.
2. An online post-workshop evaluation survey. We'll ask you to complete this before you leave. If you've used the blank pages at the back of this handout to make feedback notes, you'll be able to provide more specific and helpful feedback with the least amount of brain-drain!

## Document Structure

We have provided you with an electronic copy of the workshop's hands-on tutorial documents. We have done this for two reasons: 1) you will have something to take away with you at the end of the workshop, and 2) you can save time (mis)typing commands on the command line by using copy-and-paste.

*We advise you to use Acrobat Reader to view the PDF. This is because it properly supports some features we have implemented to ensure that copy-and-paste of commands works as expected. This includes the appropriate copy-and-paste of special characters like tilde and hyphens as well as skipping line numbers for easy copy-and-paste of whole code blocks.*



While you could fly through the hands-on sessions doing copy-and-paste, you will learn more if you use the time saved from not having to type all those commands, to understand what each command is doing!

The commands to enter at a terminal look something like this:

```
1 tophat --solexa-quals -g 2 --library-type fr-unstranded -j \  
  annotation/Danio_rerio.Zv9.66.spliceSites -o tophat/ZV9_2cells \  
  genome/ZV9 data/2cells_1.fastq data/2cells_2.fastq
```

The following styled code is not to be entered at a terminal, it is simply to show you the syntax of the command. You must use your own judgement to substitute in the correct arguments, options, filenames etc

```
tophat [options]* <index_base> <reads_1> <reads_2>
```

The following icons are used in the margin, throughout the documentation to help you navigate around the document more easily:



Important



For reference



Follow these steps



Questions to answer



Warning - STOP and read



Bonus exercise for fast learners



Advanced exercise for super-fast learners

## Computer Setup



We will all be working on our own computers today, and will be accessing Virtual Machines running the Ubuntu operating system on the Nectar Research Cloud (<http://nectar.org.au/>). The software client No Machine which you will have already installed, enables us to access these machines in a familiar Desktop style, even though the majority of our time will be spent within the terminal.

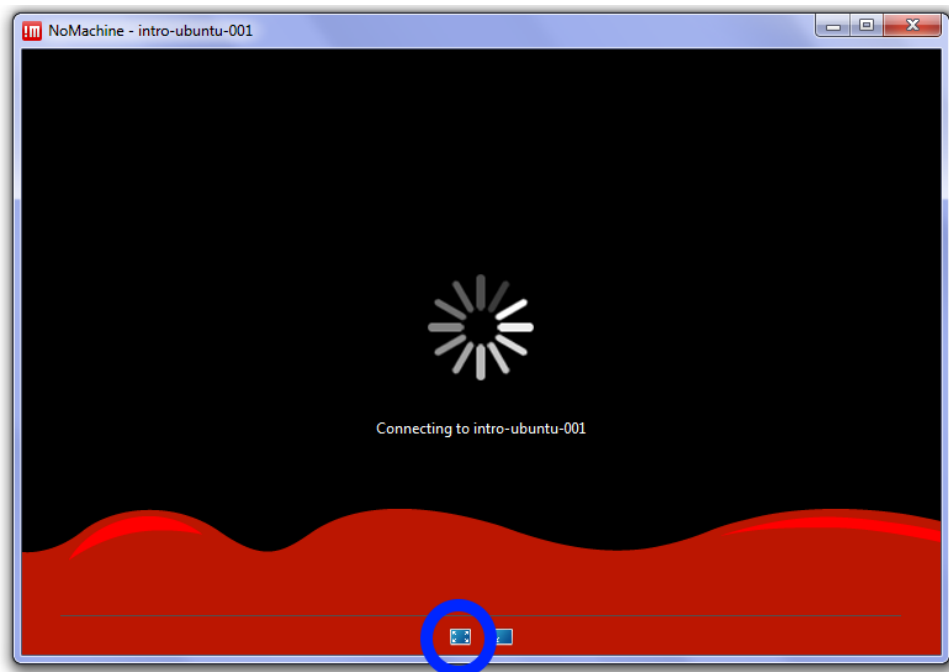


If you did not install NoMachine prior to today's workshop, the correct installers can be found at:

- <http://tinyurl.com/lcn3ns8> (Windows)
- <http://tinyurl.com/mnm23ew> (Mac)



No Machine session files will be provided to each attendee which have been pre-configured to enable easy access to these machines. These machines are effectively dual-core machine with 8GB of RAM & 70GB of hard drive space. Whilst relatively small, this will be more than enough to become familiar with the important concepts for the day. To begin today's session, simply click (or double-click) on the No Machine session file that you have been given. The desktop from the Virtual Machine (VM) that you have connected to will appear on the No Machine client.





While this is connecting, select the button circled in blue above. This will resize NoMachine to be full screen to feel more like you are physically located at the VM. If any security warnings appear, ignore them & continue connecting. This may take several attempts and if you can't connect after 3 or 4 times, call a tutor over.

If you forgot to set the client to full screen, hover your mouse over the top-right corner and click where the 'page' folds over. This will enable you to resize the client again, and can also be used to disconnect from the VM.

## The Ubuntu Desktop



Now that you are connected, you will notice we are in a standard graphical environment. The default Desktop in Ubuntu is Unity, but what we are seeing is known as the Gnome Desktop. It's easier to load this via NoMachine, but the two variants are not dissimilar. As many of us are used to seeing, there are click-able icons on the desktop, and drop-down menus.

Although we won't be using it today, Ubuntu has an built-in Office Suite of programs which you can access from the *Applications > Office* menu item. This is where links can be found to open Document Viewer (a .pdf viewer), Libre Office Calc (Excel-like), Libre Office Writer (Word-like) & other standard members of Office Program Suites.

The two main interfaces we will be using today are the **terminal** and a text editor named **gedit**. They will appear as icons on the desktop, but can also be accessed from the drop-down menus.

### Firefox

**Firefox** can also be accessed from the terminal using the command **firefox &**, by clicking the desktop icon, or from the drop-down menu in the top left.



NB: Firefox has recently been a little unstable on these machines. If you can't open firefox using any of the above methods it's just a small glitch as it shuts down, so call a tutor over & they will help get the browser back up & running.



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# Introducing The Command Line

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## Initial Goals

1. Gain familiarity and confidence within the Linux command-line environment
2. Learn how to navigate directories, as well as to copy, move & delete the files within them
3. Look up the name of a command needed to perform a specified task

## Background

Command-line tools are the mainstay of analysis of large biological data sets. Good candidate examples for command-line analysis are:

- Manual inspection of fastq, bam & sam files from NGS pipelines
- Automating similar analyses across multiple datasets
- Manipulations of data which are repetitive or laborious to perform manually
- Any analysis that is different from what is available in programs with graphical interfaces (i.e. GUIs).
- Job submission to HPC clusters

Today we'll explore a few commands to help you gain a little familiarity with some important ones, and to enable you to find help when you're working by yourself. We don't expect you to remember all the commands & options from today. The important thing is to become familiar with the basic syntax for commands, how to put them together, and where to look for help when you're unsure.



## Finding your way around



Firstly we need to open a terminal, so either click on the terminal icon on the desktop, or go to *Applications > Accessories > Terminal* on the drop-down menu at the top-left of your screen. You will notice the text `trainee@intro-bioinf-XXX:~$` where `trainee` is the username that we have assigned to you, and `intro-bioinf-XXX` indicates which virtual machine you have been assigned to. The tilde represents your current directory (explained later), whilst the dollar sign just indicates the end of the address & the beginning of where you will type commands.



The terminal has a library of commands which are built into it at the time of installing the operating system, and which are part of the Bourne-again Shell, or *bash*. (Historically, it's a replacement for the earlier Bourne Shell, written by Stephen Bourne, so the name is actually a hilarious pun.) We'll explore a few of these commands below, and the word shell will often be used interchangeably with the terminal window. Our apologies to any purists. If you've ever heard of the phrase *shell scripts*, this refers to a series of these commands strung together into a text file which is then able to be executed as a single command.

### Where are we?



Type the command `pwd` in the terminal and you will see the output `/home/trainee` appear.

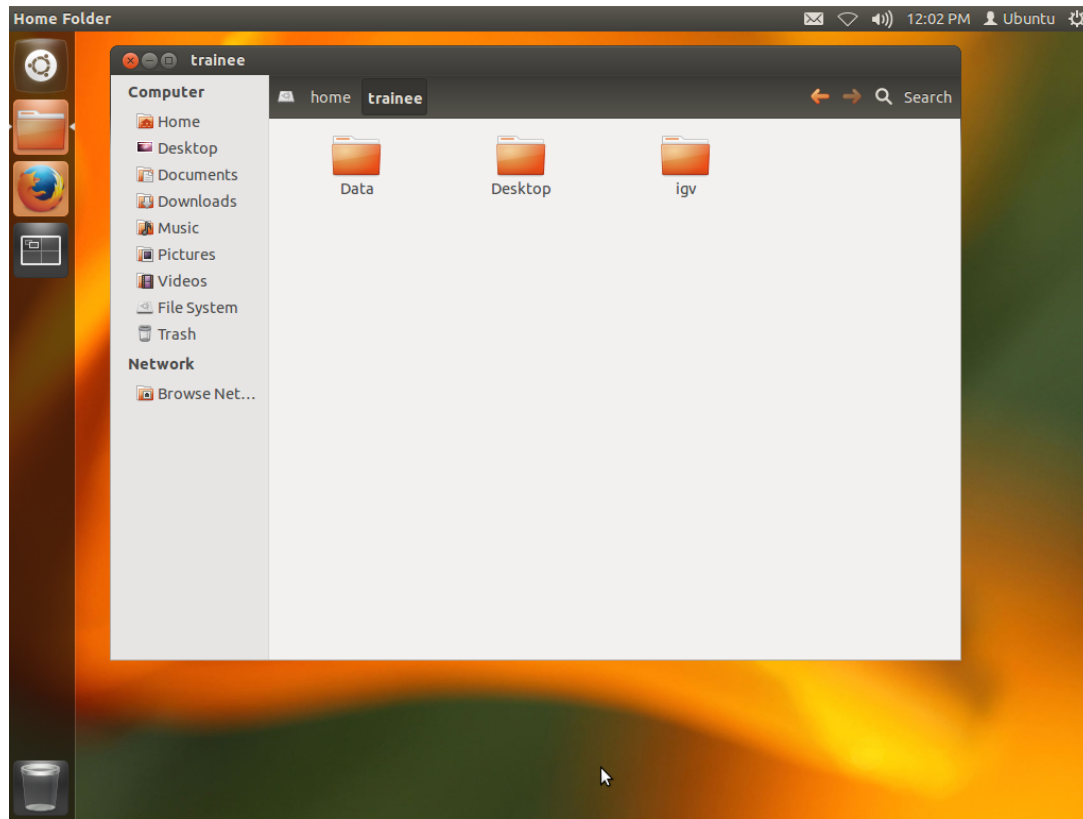
```
1 | pwd
```



The command `pwd` is what we use for printing the current (i.e. working) directory. Printing in this context means to print some information to the terminal, as opposed to a physical printer. This style of printing harks back to the days when laser printers were not commonplace. Printing information to the terminal itself is what we refer to as printing to the *standard output* or `stdout`.



The directory path that appeared (`/home/trainee`) is what will be referred to as your *home directory* for the remainder of the workshop. This is also the information that the tilde (`~`) represents as a shorthand version, so whenever you see the tilde in a directory path, this is interpreted as meaning `/home/trainee`. The *working directory* simply refers to the directory on the computer where you are currently looking, and can be thought of as being the room you are in. We are very familiar with this concept graphically, as seen in the following image. Instead of using a graphical view, we simply have a text-based view.



## The Linux File System



In the above command, the home directory began with a slash, i.e. `/`. On a Linux-based system, this is considered to be the **root directory** of the file system. Windows users would be more familiar with seeing `C:\` as the root of the file system, and this is a very important difference in the two directory structures. Note also that whilst Windows uses the backslash (`\`) to indicate a new directory, a Linux-based system uses the forward slash (`/`), or more commonly just referred to simply as “slash”, marking another but very important difference between the two.



Although we haven't directly discovered it yet, a Linux-based file system such as Ubuntu or Mac OS-X is also *case-sensitive*, whilst Windows is not. For example, the command `PWD` is completely different to `pwd` and if `PWD` is the name of a command which has been defined in your shell, you will get completely different results than from the intended `pwd` command.

Spaces are also highly important in Linux, so take note of them where-ever they appear in the given commands.

## Relative vs Absolute Paths



Also note that in your output from `pwd` the resulting path started with the slash, i.e. `/home/trainee`. This indicates that it is an **absolute path** as it began with the root of the file system “/”. If the slash was missing, it would refer to a sub-directory of the current working directory, and this is what we refer to as a **relative path**. This is an important point which will hopefully become more clear throughout the session.

## Changing Directories



The command `pwd` is an example of a *command* that is built into the shell. Another built-in command is `cd` which we use to change directory. This changes the directory we are looking in, just like clicking our way through a directory structure in the familiar graphical style we all know well. No matter where we are in a file system, we can move up a directory in the hierarchy by using the command

```
1 | cd ..
```

The string “`..`” is the convention for “*one directory above*”, whilst a single dot represents the current directory.



Enter the above command and notice that the location immediately to the left of the `$` is now given as `/home`. This is also what will be given as the output if we enter the command `pwd`. Note that this given directory is because your personal home folder is within the folder `/home` which contains all of the home folders for all users on the computer. If we now enter `cd ..` one more time we will be in the root directory of the file system. Try this and print the working directory again. As detailed earlier, the output should be the root directory given as `/`.



We can change back to your personal home folder by entering one of either:

```
1 | cd /home/trainee
```

or

```
1 | cd ~
```

or even just

```
1 | cd
```

We can also move through multiple directories in one command by separating them with the forward slash “/”. For example, we could also get to the root directory from our home

directory by typing

```
1 | cd ../../
```



Using the above process, return to your home directory `/home/trainee`.

## Looking at the Contents of a Directory



There is another built-in command “`ls`” that we can use to list the contents of a directory. Enter the `ls` command as it is and it will list the contents of the current directory.

```
1 | ls
```



Alternatively, we can specify which directory we wish to view the contents of, without having to change into that directory. We simply type the `ls` command, followed by a space, then the directory we wish to view the contents of. To look at the contents of the root directory of the file system (i.e. `/`), we simply add that directory after the command `ls`.

```
1 | ls /
```

Here you can see a whole raft of directories which contain the vital information for the computer’s operating system. Among them should be the `/home` directory which is one level above your own home directory, and where the home directories for all users are located, as mentioned earlier.



Try to think of two ways we could inspect the contents of the `/home` directory from your own home directory.

```
ls /home  
or  
ls ../
```



Notice that there are two entries in the `/home` directory. One is your home directory (`/home/trainee`), whilst the other is the default home directory that came with the VM (`/home/ubuntu`) before we set up your home directory for today’s workshop. Also note that if we ever refer to this higher-level directory, we will write it as `/home`, whereas your personal home directory is always referred to without the preceding forward-slash, and will also be written in normal text font instead of the font we specifically use for code.

## A Handy Hint



When working in the terminal, you can scroll through your previous commands by using the up arrow to go backward, and the down arrow to move forward. This can be a big time saver if you've typed a long command with a simple typo, or if you have to do a series of similar commands.

## Going Even Deeper



So far, the commands we have used were given either without the use of any subsequent arguments, e.g. `pwd` & `ls`, or with a specific directory as the second argument, e.g. `cd ../` & `ls /home`. Many commands have the additional capacity to specify different options as to how they perform, and these options are often specified between the command name, and the file being operated on. Options are commonly a single letter prefaced with a single dash, or a word prefaced with two dashes. The `ls` command can be given with the option `-l` specified between the command & the directory. This options gives the output in what is known as *long listing* format.



Inspect the contents of your home directory using the long listing format. Please make sure you can tell the difference between the characters `l` & `1`.

```
1 | ls -l ~
```

The above will give a few lines of output & the first line should be something similar to

```
drwxr-xr-x 2 trainee trainee 4096 mmm dd hh:mm Desktop
```

where `mmm dd hh:mm` are time and date information.



The important thing to notice is that the word `Desktop` at the end of the line will be coloured **blue**, indicating that it is a directory. The letter 'd' at the beginning of the initial string of codes `drwxr-xr-x` also indicates this fact. Formally, these letters are known as flags which identify key attributes about each file or directory. We can ignore the fine detail in the rest of these flags until this afternoon (or see the bonus section), but the values `rw` simply refer to who is able to read, write or execute the contents of the file or directory. These are very helpful attributes for data security & protection against malicious software.

The entries `trainee trainee` respectively refer to who is the owner of the directory (or file) & to which group it belongs. Again, this information won't be particularly relevant to us today, so we can ignore this until later in our programming careers. In brief, on an Ubuntu system you could have every member of your lab group as an individual user, whilst making every member part of a group. File access permissions can then be applied

to any file based on who created it, or whether they are a member of your lab group. Finally, the value 4096 is the size of the directory structure in bytes, whilst the date & time refer to when the directory was created.



The flags we saw at the beginning of the entries above have a clearly defined structure. The first entry shows the file type and for most common files, this entry will be the “-” seen above. The next entries are three triplets which refer to 1) the file’s owner, 2) the group they belong to & 3) all users.

In some other entries you’ll come across, the name of the file will be in **green**, indicating that it is a file not a directory. There will also be a ‘-’ instead of a ‘d’ at the beginning of the initial string of flags. The remainder of the information is essentially the same as for the directories we’ve already seen.



There are many more options that we could specify to give a slightly different output from the `ls` command. Two particularly helpful ones are the options `-h` and `-R`. We could have specified the previous command as

```
1 | ls -l -h ~
```

This will change the file size to “*human-readable*” format, whilst leaving the remainder of the output unchanged. Try it & you will notice that where we initially saw 4096 bytes, the size is now given as 4.0K. This can be particularly helpful for larger files, as most NGS files are very large indeed and seeing a file size in gigabytes will be much more informative.

The option `-R` tells the `ls` command to look through each directory recursively. If we enter

```
1 | ls -l -R ~
```

the output will be given in two sections. The first is what we have seen previously, but following that will be the contents of the directory `/home/trainee/Desktop`. It should become immediately clear that the output from setting this option can get very large & long depending on which directory you start from. It’s probably not a good idea to enter `ls -l -R /` as this will print out the entire contents of your file system.

In the case of the `ls` command we can actually specify all the above options together in the command

```
1 | ls -lhR ~
```

This can often save some time, but it is worth noting that not all programmers write their commands in such a way that this convention can be followed. The built-in shell commands are usually fine with this, but many NGS data processing functions do not accept this convention.



## Don't Panic!!!

It's easy for things to go wrong when working in the command-line, but if you've accidentally set something running which you need to exit or if you can't see the command prompt, there are some simple options for stopping a process & getting you back on track. Some options to try are:

<b>Ctrl-c</b>	kill the current job. This is usually the first port of call when things go wrong.	Also see <b>man</b>
<b>Ctrl-d</b>	end of input. Sometimes <b>Ctrl-c</b> doesn't work but this does.	

**kill** or **man killall** for details on how to kill a process.

## Exploring Commands In More Detail



In order to help us find what options are able to be specified, every command built-in to the shell has a manual, or a help page which can take some time to get familiar with. These help pages are displayed using the pager known as **less** which essentially turns the terminal window into a text viewer so we can display text in the terminal window, but with no capacity for us to edit the text.



To display the help page for **ls** enter the command

```
1 | man ls
```

As beforehand, the space between the two is important & in the first word we are invoking the command **man** which then looks for the *manual* associated with the command **ls**. To navigate through the manual page, we need to know a few shortcuts which are part of the **less** pager..



Although we can navigate through the **less** pager using up & down arrows on our keyboards, some helpful shortcuts are:

<enter>	go down one line
<spacebar>	go down one page (i.e. a screenful)
<b>b</b>	go up (i.e. <b>b</b> ackwards one page)
<	go to the beginning of the document
>	go to the end of the document
<b>q</b>	exit (i.e. <b>q</b> uit the page)



Look through the manual page for the **ls** command. How could we give the directory contents in long listing format, sorted by file size?

```
ls -l -S  
or  
ls -lS
```

Many software tools create “*hidden*” files by starting their name with a dot. By default, these files won’t be displayed using **ls** How could we make **ls** display these files as well as the main set of visible files.

```
ls -a
```





We can actually find out more about the `less` pager by calling it's own `man` page. Type the command

```
1 | man less
```

and the complete page will appear. This can look a little overwhelming, so try pressing `h` which will take you to a summary of the shortcut keys within `less`. There are a lot of them, so try out a few to jump through the file.

A good one to experiment with would be to search for patterns within the displayed text by prefacing the pattern with a slash. Try searching for a common word like “*the*” or “*to*” to see how the function behaves, then try searching for something a bit more useful, like the word “*move*”.

## Accessing Manuals or Help Pages



As well as entering the command `man` before the name of a command you need help with, you can often just enter the name of the command with the options `-h` or `--help` specified. Note the convention of a single hyphen which indicates an individual letter will follow, or a double-hyphen which indicates that a word will follow. Unfortunately the methods can vary a little from command to command, so if one method doesn't get you the manual, just try one of the others.

Sometimes it can take a little bit of looking to find something and it's important to be realise we won't break the computer or accidentally launch a nuclear bomb when we look around. It's very much like picking up a piece of paper to see what's under it. If you don't find something at first, just keep looking and you'll find it eventually.



Try accessing the manual for the command `man` all three ways. Was there a difference in the output depending on how we asked to view the manual?

Yes. Accessing the manual using `man man` displayed it a page at a time using `less`. The other two options just printed the entire contents all at once.

Could we access the help page for the command `ls` all three ways?

No. The option `-h` gives the output in human readable format. However, the other two methods work.

## Some Useful Commands



So far we have explored the commands `pwd`, `cd`, `ls` & `man` as well as the pager `less`. Inspect the `man` pages for the commands in the following table & fill in the appropriate fields. Have a look at the useful options & try to understand what they will do if specified when invoking the command.

Command	Description of function	Useful options
<code>man</code>	Display on-line manual	-k (search for keywords if you don't know the command)
<code>pwd</code>	Print working directory, i.e show where you are	none commonly used
<code>ls</code>	List contents of a directory	-a, -h, -l, -S, -t, -R
<code>cd</code>	Change directory	(scroll down in <code>man</code> builtins to find <code>cd</code> )
<code>mv</code>		-b, -f, -u
<code>cp</code>		-b, -f, -u
<code>rm</code>		-r (careful...)
<code>rmdir</code>		
<code>mkdir</code>		-p
<code>cat</code>		
<code>less</code>		
<code>wc</code>		-l
<code>head</code>		-n# (e.g., -n100)
<code>tail</code>		-n# (e.g., -n100)
<code>echo</code>		-e
<code>cut</code>		-d, -f, -s
<code>sort</code>		
<code>uniq</code>		



Sometimes the side effects of a command can also be useful. We can use `touch` to create an empty file using the command string `touch filename`. Look at the `man` page for the command `touch` & see if you can find this functionality described in the help page.

## Tab auto-complete



A very helpful & time-saving tool in the command line is the ability to automatically complete a command, file or directory name using the `<tab>` key. Try typing

```
1 | ls /home/tr <tab>
```

where `<tab>` represents the tab key.



Notice how the word `trainee` is completed automatically! This functionality will automatically fill as far as it can until conflicting options are reached. In this case, there was only one option so it was able to complete all the way to the end of the file path. This enables us to quickly enter long file paths without the risk of typos. Using this trick will save you an enormous amount of time trying to find why something doesn't work. The most common error we'll see today will be mistakes in file paths caused by people not taking advantage of this trick.



Now enter

```
1 | ls ~/D <tab>
```

and it will look like the auto-complete is not working. This is because there are two possibilities & it doesn't know which you want. Hit the tab twice and both will appear in the terminal, then choose one. As well as directory paths, you can use this to auto-complete filenames.



This technique can be used to also find command names. Type in `he` followed by two strike of the `<tab>` key and it will show you all of the commands that being with the string `he`, such as `head`, `help` or any others that may be installed on your computer. If we'd hit the `<tab>` key after typing `hea`, then the command `head` would have auto-completed, although clearly this wouldn't have saved you any typing.

## Putting It All Together

Now we can use some the above commands to perform something useful.



First, let's create a personal directory under `/home/trainee` with your first name as the directory name. **Where you see *firstname* below, use your actual firstname.**

```
1 | cd ~
2 | mkdir firstname
```

Now we can change into this directory.

```
1 | cd firstname
```

Create an empty text file.

```
1 | touch hello.txt
```

We can read it, but it won't have anything in it yet.

```
1 | cat hello.txt
```

Obviously nothing was printed to your terminal in the previous line because the file is empty. Let's write something to the file, then try reading it again.

```
1 | echo 'Hello' >> hello.txt
2 | cat hello.txt
```

We can find a whole lot of information about the file.

```
1 | wc hello.txt
```



In the previous line, what do the three numbers represent? **The lines, word & bytes (or characters)**



When we added the word `Hello` to our file, we used the symbol `>>` which actually wrote the word to the end of the file. As the file was completely empty, this placed the word in the first line. This is a VERY handy short-cut for writing information to the end of a file



Now let's add more to the file.

```
1 | echo 'It's me' >> hello.txt
2 | cat hello.txt
3 | wc hello.txt
```



For most of the above commands we could have used the auto-complete feature of the bash terminal. Did you remember this trick?



In the `~/Documents/trainingData` directory, you will find the file `pair1.fq`. We can copy this to our newly created directory using the command

```
1 | cp ~/Documents/trainingData/pair1.fq ~/firstname/pair1.fq
```

Next we will rename the file

```
1 | mv ~/firstname/pair1.fq ~/firstname/myReads1.fq
```

We can look at the first 5 lines of the file using

```
1 | head -n5 ~/firstname/myReads1.fq
```

Or we can look at the last 10 lines of the file using

```
1 | tail -n10 ~/firstname/myReads1.fq
```

We could even page through the file using `less`. (Remember to hit `q` to exit the pager.)

```
1 | less ~/firstname/myReads1.fq
```

We can even find how many lines there are in the file by using

```
1 | wc -l ~/firstname/myReads1.fq
```

We don't need the file anymore, so now we can delete it. What a waste!!!

```
1 | rm ~/firstname/myReads1.fq
```



Did we need to enter the full file path in the above commands, or could we have saved ourselves some effort by changing into the `~/firstname` directory?

Yes, we could have changed into the `~/firstname` directory & just entered the file name.



---

# Regular Expressions

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## Introduction

Regular expressions are a powerful & flexible way of searching for text strings amongst a large document or file. Most of us are familiar with searching for a word within a file, but regular expressions allow us to search for these with more flexibility, particularly in the context of genomics. Instead of searching strictly for a word or text string, we can search using less strict matching criteria. For example, we could search for a sequence that is either AGT or ACT by using the patterns `A[GC]T` or `A(G|C)T`. These two patterns will search for an A, followed by either a G or C, then followed strictly by a T. Similarly a match to ANNT can be found by using the patterns `A[AGCT][AGCT]T` or `A[AGCT]{2}T`.

Whilst the bash shell has a great capacity for searching a file to matches to regular expressions, this is where languages like *perl* and *python* offer a great degree more power. The commands `awk` & `sed` which we will look at later today also use regular expressions to great effect.

## The command `grep`

The built-in command which searches using regular expressions in the terminal is `grep`. This function searches a file or input on a line-by-line basis, making patterns split across lines more difficult to find, which is one place that a programming language like Python or Perl would become preferable.. The `man grep` page contains more detail on regular expressions under the `REGULAR EXPRESSIONS` header (scroll down a few pages). As can be seen in the `man` page, the command follows the form

```
grep [OPTIONS] 'pattern' filename
```

The option `-E` is preferable as it it stand for Extended, which we can think of as “Easier”. As well as the series of conventional numbers and characters that we are familiar with, we can match to characters with special meaning, as we saw above where enclosing the two letters in brackets gave the option of matching either. The `-E` option opens up the full set of wild-card characters, and can also be called simply by using `egrep` instead of `grep -E`.



Special Character	Meaning
\w	match any letter or digit, i.e. a word character
\s	match any white space character, includes spaces, tabs & end-of-line marks
\d	match any digit from 0 to 9
.	matches any single character
+	matches one or more of the preceding character (or pattern)
*	matches zero or more of the preceding character (or pattern)
?	matches zero or one of the preceding character (or pattern)
{x} or {x,y}	matches x or between x and y instances of the preceding character
^	matches the beginning of a line (when not inside square brackets)
\$	matches the end of a line
()	contents of the parentheses treated as a single pattern
[]	matches any one of the characters inside the brackets
[^]	matches anything other than any of the characters in the brackets
—	either the string before or the string after the "pipe" (use parentheses)
\	don't treat the following character in the way you normally would. This is why the first three entries in this table started with a backslash, as this gives them their "special" properties, whereas placing a backslash before a '.' symbol will enable it to function as an actual dot/full-stop.

## Pattern Searching

In this section we'll learn the basics of using the **grep** command & what forms the output can take. In your `~/Documents/trainingData` directory you can find the file **words**. This is simply a text file with a different word on every line.



Change into this directory & page through the first few lines of the file to get an idea about what it contains. **Make sure you're in the correct directory** or else **grep**

won't be able to find the file `words`.

Now let's try a few searches to get a feel for the basic syntax of the command. Using the previous table of special characters, try to describe what you're searching for on your notes **BEFORE** you enter the command. Do the results correspond with what you expected to see?

```
1 | grep -E 'fr..ol' words
1 | grep -E 'fr.[jsm]ol' words
1 | grep -E 'fr.[^jsm]ol' words
1 | grep -E 'fr..ol$' words
1 | grep -E 'fr.+ol$' words
1 | grep -E 'cat|dog' words
1 | grep -E '^w.+(cat|dog)' words
```



In the above, we were changing the pattern to extract different results from the files. Now we'll try a few different options to change the output, whilst leaving the pattern unchanged. If you're unsure about some of the options, don't forget to consult the `man` page.

```
1 | grep -E 'louse' words
1 | grep -Ew 'louse' words
1 | grep -Ewn 'louse' words
1 | grep -EwC2 'louse' words
1 | grep -c 'louse' words
```

## Pattern Matching in DNA sequences



Now we can have a look through the GBS-Seq file (`pair1.fq`) that we copied & renamed earlier. Before we search the file, we should know what this data actually is. These are a small subset of 100bp reads from a GBS-Seq experiment & are from the first sample in a set of paired reads. Although we can't tell this directly from this file, the sequences were obtained from a rabbit affected by *calicivirus*.



If you inspect the data using the `head` command, you'll notice that the file is in sets of four lines, as defined by the fastq format.

```
1 | head pair1.fq
```

The first line contains a *sequence identifier*, the second line is the *sequence* itself, the third line is simply the '+' symbol as a placeholder & the final line is a set of characters which correspond to *quality scores* for each base. We'll be looking at this file structure in much more detail next week, so that's all we need to know for today's session.



As these reads are from the first sample in a set of paired reads, the final value in the sequence identifier is `_1`. How could we search for a pattern just to extract the sequence identifiers?

```
grep -E '^@._1$' pair1.fq
```

Reads from the second in the set of paired reads would have an identifier that ends with the pattern `_2`. There are no reads in the file `pair1.fq` that are the second pair, so what would you expect to see if you changed the `_1` in the previous query to `_2`?

If they didn't include the EOL symbol, they'll get results...



Now we'll try searching for some sequences, so try the following search string. Does the output match what you expected?

```
grep -En 'TGCAGGCTCT' pair1.fq
```

This should give lines 774, 3382, 3758, 7326, 7594 & 9566 along with the sequence information and matching fragment highlighted in red.



For the following search string

```
grep -En 'TGCAGGCTCT.+(GA){2}.+A{3}' pair1.fq
```

What do the following components of the pattern match to:

`','`

This matches an unspecified length of any character, until the next key match is found.

`(GA){2}`

This matches to two repeats of the pattern GA.

`A{3}`

This matches three 'A's in a row.

How could you find the sequence identifier for the above match?

```
grep -EnC1 'TGCAGGCTCT.+(GA){2}.+A{3}' pair1.fq or
```

```
grep -EnB1 'TGCAGGCTCT.+(GA){2}.+A{3}' pair1.fq
```



---

# More Tips And Tricks

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## Text In the Terminal

We can display a line of text in `stdout` by using the command `echo`. The most simple function that people learn to write in most languages is called `Hello World` and we'll do the same thing today.



```
1 | echo 'Hello World'
```

That's pretty amazing isn't it & you can make the terminal window say anything you want without meaning it.

```
1 | echo 'This computer will self destruct in 10 seconds!'
```



There are a few subtleties about text which are worth noting. Inspect the `man echo` page & note the effects of the `-e` option. This allows you to specify tabs, new lines & other special characters by using the backslash to signify these characters. This is an important concept & the use of a backslash to “escape” normal meaning of a character is very common. Try the following three commands & see what effects these special characters have.



```
1 | echo 'Hello\tWorld'
2 | echo -e 'Hello\tWorld'
3 | echo -e 'Hello\nWorld'
```

## Redirection Using The Pipe Symbol



A very common process in the command line is to take the output of one process and send it to another. For example, we might want to cut a column from a `csv` file with categorical information, and then send that field to have the different categories counted by another tool. This is where the concept of `stdout` becomes more important.



By default, most command-line tools print their output to `stdout` but instead, we can send this to another tool using the pipe (`—`) symbol. This is exactly like putting a pipe on the output of one process, and routing it to the *standard input* or `stdin` of another. We can use this to build up a series or chain of processes in a single line. A trivial example might be match a word segment.

```
1 | echo -e 'Hello\nWorld' | egrep 'Hell'
```



**Note that we didn't give a file to `egrep` to work with in the above command.** It took the `stdout` from the command `echo` as it's input via `stdin`. This is how the pipe

will work on virtually all occasions.



As another simple example, we could take some long output from the `ls` command & send it to the pager `less`.

```
1 | ls -lh /usr/bin | less
```

Page through the output until you get bored, then hit `q` to quit.

## Sending Output To A File



So far, the only output we have seen has been in the terminal, i.e `stdout`. Similar to the pipe command, we can redirect the output of a command **to a file** instead of to standard output, and we do this using the greater than symbol (`>`), which we can almost envisage as an arrow.



To see this in action, we'll collect a list of the sequence identifiers in our set of RAD-seq reads & write these to a file, we could use the command as above, but instead of directing the output to the terminal, we could write this to a file.

```
1 | cd ~/Documents/trainingData
2 | grep -E '^@.+_1' pair1.fq > SeqIDs.txt
3 | less SeqIDs.txt
```



This is similar to the `>>` trick we used earlier, but using only a single `>` symbol will directly overwrite any existing file with the new information. When using the command line, there is no **Undo** command. Once we do that, it can't be undone.

Once you've had a quick look at the file, exit the `less` pager and delete the file using the `rm` command.

```
1 | rm SeqIDs.txt
```

## Downloading Data



The terminal makes obtaining data from any publicly available sources very easy using the command `wget`, once you know the location of a file. Let's find a genome to download by opening Firefox & heading to `ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/`. Here we can see a list of all the Bacteria which have genome information available. Open one of the folders to have a look at the contents, or if you'd like scroll down to everyone's favourite probiotic `Lactobacillus acidophilus 30SC.uid63605`. We'll download the *General Feature Format* file with the suffix `.gff` file which contains information about any identified genomic features. If you've chosen `Lactobacillus`, we'll download the file `NC_015214.gff`. Right-click on the file & select *Copy Link Location*. If you're not already

in your personal directory, i.e. `~/firstname` then change into this directory and type the command `wget`. Once you've typed this command, right-click and paste the contents of the clipboard to give the following (apologies for the small font):

```
1 wget ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/Lactobacillus_acidophilus_30SC_uid63605/NC_015214.gff
```

This will download the selected file into your current working directory. Don't forget to have a look at the `man` page to make sure you understand how the command works.



The first 5 lines of this file is what we refer to as a *header*, which contains important information about how the file was generated in a standardised format. Many file formats have these structures at the beginning but for our purposes today we don't need to use any of this information so we can move on. Have a look at the beginning of the file to see what it looks like.

```
1 head NC_015214.gff
```

Notice the header lines begin with one or two hash symbols, and the remainder of the file contains information about the genomic features. The first feature is annotated as a *region* in the third field, whilst the second feature is annotated as a *gene*.



How many features are contained in this file?

```
wc -l NC_015214.gff
```

This will give 4355, but the first 5 lines are header lines. To count the non-header lines you could try several things:

```
grep -vc '^#' NC_015214.gff
```

or

```
grep -c '^[^#]' NC_015214.gff
```



As mentioned above, this file contains multiple features such as *regions*, *genes*, *CDSs*, *exons* or *tRNAs*. If we wanted to find how many regions are annotated in this file we could use the processes we've learned above:

```
grep -c 'region' NC_015214.gff
```

If we wanted to count how many genes are annotated, the first idea we might have would be to do something similar using a search for the pattern '*gene*'.





Do you think this is the number of genes? Try searching for the number of coding DNA sequences using the same approach (i.e. CDS) & then add the two numbers? Is this more than the total number of features we found earlier? Can you think of a way around this using regular expressions? **Note that some of the occurrences of the word *gene* appears in many lines which are not genes. We need to restrict the search to one of the tab-separated fields by including a white-space character in the search. The command:**

```
grep -En '\sgene\s' NC_015214.gff | wc -l
```

**will give a much different result as now we are searching for the word *gene* surrounded by white-space.**

Alternatively, there is a command `cut` available. Call the manual page (`man cut`) and inspect the option `-f`. The information about the types of features annotated is in the third field. Try to think of a way of searching this field alone.

```
cut -f3 NC_015214.gff | grep -c 'gene' Or even more accurately egrep -v '^#' NC_015214.gff | cut -f3 | egrep -c 'gene' although it gives the same results in this instance
```

A similar question to a few pages back would be how many *types* of features are in this file? The commands `cut`, along with `sort` and `uniq` may prove to be useful when answering this **`egrep -v '^#' NC_015214.gff | cut -f3 | sort | uniq | wc -l`**

## Working With Compressed Files



The file we have downloaded using `wget` was in simple text format. The suffix `.gff` really just denoted a specific structure within the text file so that we know where to look within the file for certain information. Most software that uses a file in this format will manually check for the correct structure first. However, when working in the bioinformatics field, you will often see or require compressed files. This is a common enough concept for even Windows and MAC users, but a few specific compression types are commonly used in bioinformatics. The main differences are in the compression algorithms used, and that is far beyond the scope of what most of us need to worry about.

The most common types of compression you will see are:

Suffix	Compress	Extract	Useful Arguments
.zip	zip	unzip	-d, -c, -f
.gz	gzip	gunzip zcat	-d, -c, -f
.tar.gz	tar	tar	-x, -v, -f, -z
.bz2	bzip2	bunzip2	

Often, files you download this way will be compressed (tar: tape archive) and archived (zipped). If you see file name suffixes like .tar, .zip, .gz, and/or .bz2, among others, that is what these are. To explore what these command-line options do, please check the **man** pages.



Just to demonstrate how we do this on the command line, we could compress one of our fastq files

```
1 | cd ~/Documents/trainingData
2 | gzip pair1.fq
```



How would we now decompress (or extract) this file?

**gunzip pair1.fq.gz**

How could we have kept our compressed file, along with the decompressed output?

**gunzip -k pair1.fq.gz**

Why would we use **zcat** instead of **gunzip -c**?

**It's less typing, and it's easier to read back**

---

# The Tools sed & awk

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## sed: The Stream Editor



One very useful command in the terminal is **sed**, which is short for *stream editor*. Instead of the **man** page for **sed** the **info sed** page is larger but a little easier to digest. This is a very powerful command which can be a little overwhelming at first. If using this for your own scripts & you can't figure something out, remember "Google is your friend" & sites like [www.stackoverflow.com](http://www.stackoverflow.com) are full of people wrestling with similar problems to you. You can be certain you're not the first person to be stumped by a problem & these are great places to start looking for help. Even advanced programmers use Google & Stack Overflow to find solutions.

For today, there are two key **sed** functionalities that we want to introduce.

1. Using **sed** to alter the contents of a file/input;
2. Using **sed** to print regions of a file

### Altering a file or other input

**sed** uses *regular expressions* that we have come across under the **grep** section, and we can use these to replace strings or characters within a text string. The command works in the form

```
sed SCRIPT INPUT
```

and the script section is where all the action happens. Input can be given to **sed** as either a file, or just as a text stream via **stdin** using the *pipe* symbol that we have already introduced.



In the following example the script section begins with an 's' to indicate that we are going to make a substitution. The beginning of the first pattern (i.e. the *regexp* we are searching for) is denoted with the backslash, with the identical delimiter indicating the replacement pattern, and this is in turn completed with the same delimiter. Try this simple example from the link <http://www.grymoire.com/Unix/Sed.html> which is a very detailed & helpful resource about the usage **sed**. Here we are sending the input to the command via the pipe, so no 'INPUT' section is required:

```
1 echo Sunday | sed 's/day/night/'
```

Here you are passing **sed** the string Sunday, and **sed** takes day and turns it into night. **sed** will only replace the first instance of the string on any line, so try:

```
1 echo Sundayday | sed 's/day/night/'
```

Note that it only replaced the first instance of day and left the second. However, you can make it 'global', where it switches every instance by using the 'g' option at the end of the pattern like this:

```
1 | echo Sundayday | sed 's/day/night/g'
```

You can also 'capture' parts of the pattern in parentheses and access that in the second part of the regular expression (what you are switching to) using \1, \2, etc., to denote the number of the captured string, in the order they were captured. If you want to match 'ATGNNNTGA', where N is any base, and just output these three bases you could try the following:

```
1 | echo 'ATGCCAGTA' | sed -r 's/ATG(.{3})GTA/\1/g'
```

Or if we needed to replace those three bases with an expanded repeat of them, you could do the following where we capture the undefined string between ATG & GTA, and expand it:

```
1 | echo 'ATGCCAGTA' | sed -r 's/ATG(.{3})GTA/ATG\1\1\1GTA/g'
```

The \1 take the contents of the first parenthesis and uses it in the substitution, even though you don't know what the bases are. Note that the '-r' option was set for these operations, which turns on extended regular expression capabilities. This can be a powerful tool & multiple parentheses can also be used:

```
1 | echo 'ATGCCAGTA' | sed -r 's/(ATG)(.{3})(GTA)/\3\2\2\1/g'
```

In this last command we switched the order of the first & last triplet, and expanded the middle unknown string twice. Note how quickly this starts to look confusing though! Taking care to be clear when writing these types of procedures can be an important idea when you have to go back & re-read your code a year or two later. (Yes this will happen a lot!!!)



The use of backslashes to delineate each section of the script used by sed is the most common convention, but we are not restricted to it. We could have used any 'wild-card' type character to follow the 's', such as '\*' ':' or '%' although this must be consistent across all sections of the script, and should only be used if the backslash itself is part of the search or replacement string.

## Displaying a region from a file

The command `sed` can also be used to replicate some of the functionality of the `head` & `grep` commands, but with a little more power at your fingertips. By default `sed` will print the entire input stream it receives, but setting the option `-n` will turn this off. Try this by adding an `n` immediately after the `-r` in one of the above lines & you will notice you receive no output. This is useful if we wish to restrict our output to a subset of lines within a file, and by including a `p` at the end of the script section, only the section matching the results of the script will be printed.

Make sure you are in the correct directory & we can look through the file *pair1.fq* again.

```
1 | sed -n '1,10p' pair1.fq
```

This will print the first 10 lines, like the `head` command will by default. However, we could now print any range of lines we choose. Try this by changing the script to something interesting like `'101,112p'`. We could also restrict the range to specific lines by using the `sed` increment operator `'~'`.

```
1 | sed -n '1~4p' pair1.fq
```

This will print every 4th line, beginning at the first, effectively giving just the sequence identifiers from this set of reads. We could use this just to extract the sequences themselves, or the quality scores.



The previous command prints every 4th line, but isn't restricted to any range within the file. How could we restrict the output to just the first 10 identifiers?

We could pipe the output to another `sed` command or to `head`.

```
sed -n '1~4p' pair1.fq | sed -n '1,10 p'
```

```
sed -n '1~4p' pair1.fq | head -n10
```

This also gives us a simple way of counting the reads in the file. Try to think of a way of doing this using the pipe symbol.

```
sed -n '1~4p' pair1.fq | wc -l
```

or

```
sed -n '1~4p' pair1.fq | egrep -c '^@'
```



We can also make `sed` operate like `grep` by making it only print the lines which match a pattern.

```
1 | sed -rn '/TGCAGGCTCT.+(GA){2}.+/ p' pair1.fq
```

Note however, that the line numbers are not present in this output.

## Some Important Concepts

Before moving on to *awk*, we need to quickly recap two of the most widely used techniques in programming:

1. The **for** loop
2. Logical tests using an **if** statement

### For Loops



A **for** loop is what we use to cycle through an input one item at a time. As a simple example, we could print each number from a set of numbers.

```
1 | for i in 1 2 3; do (echo -e $i); done
```



In the above code, the fragment before the semi-colon asked the program to cycle through the values 1, 2 & 3, letting the variable ‘i’ take each value in order of appearance. First *i* = 1, then *i* = 2 & finally *i* = 3. If you’re wondering why we chose ‘i’, it just seemed like a sensible choice for an integer.

After that was the instruction on what to do for each value. Note that the value of the variable ‘i’ was *prefaced by the dollar sign (\$)*. *This is how the bash shell knows it is a variable, not the letter ‘i’.* The command **done** then finished the **do** command. All commands like **do**, **if** or **case** have completing statements, which respectively are **done**, **fi** & **esac**.

Another important concept which was glossed over in the previous paragraph is that of a *variable*. These are essentially just ‘*placeholders*’ which have a value that can change (hence the name). In the above loop, the same operation was performed on the variable *i*, but the value changed from 1 to 2 to 3. Variables in shell scripts can hold numbers or text strings and don’t have to be formally defined as in some other languages.



An alternate approach could be to make a breathtaking claim about some files. Here we’ll use the variable called ‘f’, which seems sensible for a filename.

```
1 | cd ~/Documents/trainingData
2 | for f in $(ls); do (echo -e "The file $f exists"); done
```

Note, that we’ve also assigned the output from the command *ls* to this variable, by using the *\$( )* syntax. The use of double quotes for the **echo** command also allows us to refer to the values held by *f*. Single quotes at this point would only return the characters ‘\$f’.

## If Statements

If statements are those which only have a binary ‘yes’ or ‘no’ response. For example, we could specify things like:

- `if (i<1) then do something`, or
- `if (fileName==bob.txt) then do something else`



Notice that in the second if statement, there was a double equals sign. This is the programmers way of saying *compare* the first argument with the second argument. A single equals sign is generally interpreted by a program as *assign* the value of the first argument to be the second argument. This use of ‘double operators’ is very common, notably you will see `&&` to represent the command ‘*and*’, and `||` to represent ‘*or*’. A final useful trick to be aware of is the use of an exclamation mark to reverse a command. A good example of this is the use of the command ‘`!=`’ as the representation of *not equal to* in a logical test.

## awk: A command and a language

Moving on to **awk**, this is a very useful tool which can be used either as a command, as well as functioning as it’s own language. We’ll just use it as a command today, and it is extremely useful for dealing with tab- or comma-separated files, such as we often see in biological data.



The basic structure of an **awk** command is:

```
awk `<pattern>` file
```

**awk** will then search the file and output any line containing the regular expression pattern (kind of like **grep**). With **awk**, you can also do:

```
\texttt{awk `{<code>}`} file}
```

where you can put a little **awk** program in the curly braces. You can specify values from different columns of the file as `$1`, `$2`, etc., (or you can use `$0` for the whole line).



We have that \*.gff file and we’ve already looked at it a little, so let’s pull out some particular features! Make your terminal as wide as the screen, then change into the appropriate directory & enter

```
1 awk '{if ($3=="rRNA") print $0}' NC_015214.gff
```

Here we’ve specified that the third field must be an rRNA, so this will give us all of the ribosomal RNAs annotated in the file.



We could make it a little more complex and just look for genes in a given region

```
1 awk '{if ( ($3=="gene") && ($4 > 10000) && ($4 < 20000) ) print $0}' \
    NC_015214.gff
```



In the above code, `$3=='gene'` asks for the entry in the third field to be “gene.” The next two fragments request for the values in the fourth field (i.e. `$4`) to be between 10000 & 20000. Thus we have found all the features annotate as genes in a 10,000bp region of this genome. Notice that each these three commands were enclosed in a pair of brackets within an outer pair of brackets. This gave a command of the form:

```
( (Condition1) && (Condition2) && (Condition3) )
```

After this came the fragment `print $0` which asked `awk` to print the entire line if the 3 conditions are true. You’ve just written (& hopefully understood) a computer program!



Another example (what does this do?):

```
1 awk '{if (($5 - $4 > 1000) && ($3 == "gene")) print $0}' NC_015214.gff
```

If you don’t want to output all of the columns, you can specify which ones to output. While we’re at it, let’s save the output as a file:

```
1 awk '{if (($5 - $4 > 1000) && ($3 == "gene")) print $1, $2, $4, $5, \
    $9}' NC_015214.gff > awkout.txt
```



The command `awk` has a pretty serious set of in-built commands which can be used in the code sections as above. Although it looks a little overwhelming, there is a detailed page <http://www.grymoire.com/Unix/Awk.html> which gives a rundown on the full capabilities of the language. One command that we may find helpful is `length`, which counts the number of characters in a line.

In your `trainingData` directory you’ll find another file `seqData.fastq`. This is a small set of reads from some mRNA pulled down by Immunoprecipitation, and they’ve been processed to have varying lengths.



Can you think of a way to combine `sed` and `awk` to create a new file `lengths.txt` which contains the length of every read in the file `seqData.fastq`?

```
sed -n '2~4p' seqData.fastq | awk '{print length($1)}' > lengths.txt
```



---

# Writing Scripts

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Sometimes we need to perform repetitive tasks on multiple files, or need to perform complex series of tasks and writing the set of instructions as a script is a very powerful way of performing these tasks. They are also an excellent way of ensuring the commands you have used in your research are retained for future reference. Keeping copies of all electronic processes to ensure reproducibility is a very important component of any research. Writing scripts requires an understanding of several key concepts which form the foundation of much computer programming, so let's walk our way through a few of them.

## Shell Scripts

Now that we've been through just some of the concepts & tools we can use when writing scripts, it's time to tackle one of our own where we can bring it all together.



Every bash shell script begins with what is known as a *shebang*, which we would commonly recognise as a hash sign followed by an exclamation mark, i.e `#!`. This is immediately followed by `/bin/bash`, which tells the interpreter to run the command `bash` in the directory `/bin`. This opening sequence is vital & tells the computer how to respond to all of the following commands. As a string this looks like:

```
#!/bin/bash
```



The hash symbol generally functions as a comment character in scripts. Sometimes we can include lines in a script to remind ourselves what we're trying to do, and we can preface these with the hash to ensure the interpreter doesn't try to run them. It's presence as a comment here, followed by the exclamation mark, is specifically looked for by the interpreter but beyond this specific occurrence, comment lines are generally ignored by scripts & programs.

## Some Example Scripts

Let's now look at some simple scripts. These are really just examples of some useful things you can do & may not really be the best scripts from a technical perspective. Hopefully they give you some pointers so you can get going

## A Simple Example to Start



**Don't try to enter these commands directly in the terminal!!!** They are designed to be placed in a script which we will do after we've inspected the contents of the script. First, just have a look through the script & make sure you understand what the script is doing.

Also remember that any long lines of code are automatically broken into new lines on your page by the '\ ' character. We don't to enter this character when we create our script. Note that the line numbers on the left of the code don't change when this happens, e.g. line 9.

```
1 #!/bin/bash
2
3 # First we'll declare some variables with some text strings
4 ME='Put your name here'
5 MESSAGE='This is your first script'
6
7 # Now well place these variables into a command to get some output
8 echo -e "Hello ${ME}\n${MESSAGE}\nWell Done!"
```



Firstly, you may notice some lines that begin with the # character. These are *comments* which have no impact on the execution of the script, but are written so you can understand what you were thinking when you wrote it. If you look at your code 6 months from now, there is a very strong chance that you won't recall exactly what you were thinking, so these comments can be a good place just to explain something to the future version of yourself. There is a school of thought which says that you write code primarily for humans to read, not for the computer to understand.



In the above script, there are two variables. Although we have initially set them to be one value, they are still variables. What are their names? **ME & MESSAGE**



Using the text editor *gedit*, enter the above code into a file setting your actual name as the ME variable, and save it as **wellDone.sh** in your **~/firstname** folder.



You'll notice that once you've done this, the text will become colour coded based on whether the text is a comment, a variable or a shell command. Scripts are usually saved with the **.sh** suffix and this colour coding can be very helpful with writing scripts.

Another coding style which can be helpful is the enclosing of each variable name in curly braces every time the value is called. Whilst not being strictly required, this can make it easy for you to follow in the future when you're looking back. Variables have also been names using strictly upper-case letters. This is another optional coding style, but can also

make things clear for you as you look back through your work. Most command line tools use strictly lower-case names, so this is another reason the upper-case variable names can be helpful.



Unfortunately, this script cannot be executed yet but we can easily enable execution of the code inside the script. If you recall the flags from earlier which denoted the read/write/execute permissions of a file, all we need to do is set the execute permission for this file. First we'll look at the files in the folder using `ls -l` and note these triplets should be `rw-` for the user & the group you belong to. To make this script executable, enter the following

```
1 | cd ~/firstname
2 | chmod +x wellDone.sh
3 | ls -l
```



Notice that the third flag in the triplet has now become an `x`. This indicates that we can now execute the file in the terminal. As a security measure, Linux doesn't allow you to execute a script from within the same directory so to execute it enter the following:

```
1 | ./wellDone.sh
```

### Making a Small Change



Now let's change the variable `ME` in the script to read as

```
1 | ME=$1
```

and save this as `wellDone2.sh`. You'll now need to set the execute permission again.

```
1 | chmod +x wellDone2.sh
```



This time we have set the script to *receive input from stdin* (i.e. the terminal), and we will need to supply a value, which will then be placed in the variable `ME`. Choose whichever random name you want and enter the following

```
1 | ./wellDone2.sh Boris
```



As you can imagine, this style of scripting can be useful for iterating over multiple objects. A trivial example, which builds on a now familiar concept would be to try the following.

```
1 | for n in Boris Fred; do (./wellDone2.sh $n); done
```

## A Script That Does Something Relevant

```
1  #!/bin/bash
2  FILE=$1
3
4  # Find all of the possible read lengths in the supplied file
5  LENGTHS=$(sed -n '2..4p' ${FILE} | awk '{print length($1)}' | sort | uniq)
6
7  # Print some column headings
8  echo -e "Read length\tTotal Number"
9
10 # Now for each possible length, count how many reads there are of that \
    length
11 for L in ${LENGTHS}
12 do
13     COUNT=$(sed -n '2..4p' ${FILE} | awk '{print length($1)}' | grep -c \
        ${L})
14     echo -e "${L}\t${COUNT}"
15 done
```



Now use the text editor `gedit` to write this script & save it as `findLengths.sh` in your home folder.



Note that after the shebang, there was a variable `FILE` which took the value `$1`. This means that after we call the script, we need to specify a filename to assign to the variable `FILE`. For example, we could run the script as:

```
./findLengths.sh ~/Documents/trainingData/seqData.fastq.
```

This would execute the remainder of the script on that file.



Where will the script send the output to? **stdout.**

How would we write the output to a file? **Use the `>` symbol to assign it to a filename**



Now we can make the script executable & run it on the file `seqData.fastq`.

```
1  chmod +x findLengths.sh
2  ./findLengths.sh ~/Documents/trainingData/seqData.fastq > lengths.txt
```



Inspect the file using `gedit`, or the `less` pager. Did it look like you expected? Is this a tab-separated file, or a comma or space separated file?



Try running it on the `pair1.fq` & `pair2.fq` files.

Is that what you expected? *All the reads should have been the same length...*



## A more complicated script

Here's a more complicated script with some more formal procedures. This is a script which will extract only the CDS features from the .gff file we have been working with, and export them to a separate file. Look through each line carefully & make write down your understanding of what each line is asking the program to do.

```
1  #!/bin/bash
2
3  # Declare some helpful variables
4  FILEDIR=~/.nectar-workshop-template/examples/intro_ubuntu_2016/files
5  FILENAME=NC_015214.gff
6  OUTFILE=NC_015214_CDS.txt
7
8  # Make sure the directory exists
9  if [ -d ${FILEDIR} ];
10 then
11     echo Changing to ${FILEDIR}
12     cd ${FILEDIR}
13 else
14     echo Cannot find directory ${FILEDIR}
15     exit 1
16 fi
17
18 # If the file exists, extract the important CDS data
19 if [ -a ${FILENAME} ];
20 then
21     echo Extracting CDS data from ${FILEDIR}/${FILENAME}
22     echo "SeqID Source Start Stop Strand Tags" > ${OUTFILE}
23     awk '{if (($3=="CDS")) print $1, $2, $4, $5, $7, $9}' ${FILENAME} >> \
        ${OUTFILE}
24 else
25     echo Cannot find ${FILENAME}
26     exit 1
27 fi
```

Notice that this time we didn't require a file to be given to the script. We defined it within the script, as we did for the output file.



The directory & file checking stages were of the form if [...]. This is a curious command that checks for the presence of something. The options -d & -a specify a directory or file respectively.



Will the above script generate a tab, comma or space delimited text file?

It will be space delimited. We could have specified tab delimited by inserting “\t” between each field.



Open the `gedit` text editor & save the blank file in your directory as `extract_CDS.sh`. Now write this above script into the editor, but *taking care to use the directory where you have the .gff file stored in the appropriate place*. Once you have written the script, save it & close it. Now make it executable and run it.

## Some Challenges

Now that we have written our first couple of scripts, the challenge for the rest of the session will be to create your own script from scratch. You can use the `gedit` text editor for this & it will automatically colour code as you go, which can be very helpful for seeing where you are as you write code.



The restriction site for the RAD-Seq dataset we have is TGCAG. In the `/trainingData` folder, we have both pairs of reads. Your challenge is to write a script to find:

1. How many reads there are in each .fq file
2. How many reads begin with the restriction site
3. Output this into a new file, with appropriate column headings.

The .fq files we have were processed by a program that has done strange things to the identifiers. Each piece of information is separated by underscores, which should be colons, as specified by the defined fastq format [https://en.wikipedia.org/wiki/FASTQ\\_format#Illumina\\_sequence\\_identifiers](https://en.wikipedia.org/wiki/FASTQ_format#Illumina_sequence_identifiers). The digit indicating which member of a pair the sequence belongs to should also be of a different format, as seen in the given definition.

Can you write a script to change these identifiers back to the correct format? You can omit the machine identifier, or just make a name up if you feel like it.

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