

Exercises: Linux Bootcamp

Version 2018-10



Licence

This manual is © 2018, Simon Andrews.

This manual is distributed under the creative commons Attribution-Non-Commercial-Share Alike 2.0 licence. This means that you are free:

- to copy, distribute, display, and perform the work
- to make derivative works

Under the following conditions:

- Attribution. You must give the original author credit.
- Non-Commercial. You may not use this work for commercial purposes.
- Share Alike. If you alter, transform, or build upon this work, you may distribute the resulting work only under a licence identical to this one.

Please note that:

- For any reuse or distribution, you must make clear to others the licence terms of this work.
- Any of these conditions can be waived if you get permission from the copyright holder.
- Nothing in this license impairs or restricts the author's moral rights.

Full details of this licence can be found at http://creativecommons.org/licenses/by-nc-sa/2.0/uk/legalcode

Exercise 1: Basic Unix commands

• The figlet command draws pretty graphical representations of text you supply, something like this:



- Read the man page for figlet to work out how to use it
- Write your name
- Get your name centred in the terminal
- xcowsay is a graphical program which makes a cow say something
- Run xcowsay -t 0 "I am a graphical program"
 - Note that you can't enter more commands in the terminal until you click on the cow to make it go away
 - \circ Read the man page to find out what the <code>-t 0</code> means

Exercise 2: File system basics

- In your home directory create a folder called compare
- Move into the seqmonk_genomes/Saccharomyces cerevisiae directory in your home directory. Make sure you use tab completion to write the folder name.
 - Note that there is a space in the second folder name. How does the command line completion deal with this?
- Try the following commands and note the differences
 - o ls
 - o ls -l
 - o ls *
 - o ls -ld *
- List the contents of directories containing a 4 in their name
- Use the head command to simultaneously show the first line only of all of the I.dat files in any of the subdirectories
 - Are the chrl sequences all the same length?
- Use less to look at Mito.dat in the EF4 directory.
 - See if you can find the first rRNA gene
 - What is its position?
- Copy Mito.dat into the compare directory in your home directory
 - Use nano to edit the file
 - Change Mito to Mitochondrion in the ID and AC header lines
 - o Save the file and exit nano
 - o Rename the file from Mito.dat to Mitochondrion.txt
- Create a symlink from the original Mito.dat file (the one inside seqmonk_genomes) to the same filename in your compare directory
- Run diff on Mitochrondrion.txt and Mito.dat to see what differences it can find

Babraham

Bioinformatics -

Exercise 3: Understanding how PATH works

- Use the echo command to show what is in your current PATH (remember it needs to be called \$PATH when you use it)
 - Which directories are being searched?
- Use the which command to find where on your file system the figlet program is installed.
- Try using which -a to see if there are any other programs called figlet later in the PATH
- We have given you a second figlet program which is in /opt/figlet/figlet. Run this by providing a direct call to it (giving its full path). Check that it runs OK.
- Modify your PATH to add /opt/figlet/figlet before the current directories (use the export PATH=... function, and make sure the existing \$PATH is still included)
- Rerun which figlet to show that the version in /opt is now found first
- Run figlet from the command line and see that the /opt version is now the default.
- Use nano to edit the end of your .bashrc file to permanently add /opt/figlet to your PATH. Open a new shell to check that this still works.

Exercise 4: Redirection and Bash Loops

- Go into the ${\tt FastQ_Data}$ directory and look at one of the fastq files using less
 - \circ Less will not directly read <code>fastq.gz</code> files, so you'll need to use <code>zcat</code> on the file and then pipe the result to less
 - \circ $\;$ Now validate that one of the files can be successfully decompressed
 - Run zcat on the file, but...
 - Throw away the STDOUT output so that you just see errors or warnings
- Create a profile of the configuration files in /etc/ using the shalsum program
 - o Start by running shalsum on /etc/profile to see how it works
 - Now run it on the entire contents of /etc/ using a wildcard (rather than a loop)
 - Write the results to a file in your home directory
 - Write any errors to a different file in your home directory
 - Have a look at the errors to see why it might have failed in some cases
- Write a bash loop which will go through every .dat file in seqmonk_genomes and will count the number of lines containing rrna (case insensitive). The process will be:
 - o Move to the seqmonk genomes/Saccharomyces cerevisiae folder
 - o Work out a shell wildcard which will find all of the .dat files
 - Write a loop to iterate over these. For each one
 - Use echo to write out the name of the file plus a space (check for how to not include a newline at the end)
 - Use grep to get the lines containing "rrna" (check for case insensitive)
 - Use wc to get and print the number of lines of hit (check how to just get the line count)
 - Run the loop and save the results to a file called rrna_count.txt
- [If you have time] Convert every fastq.gz file in FastQ_Data into a fastq.bz2 file
 - o Read the file with zcat
 - o Pipe it to bzip2 (with the option to write to stdout)
 - Redirect the output to a new file with .bz2 on the end in a different folder
 - Maybe add an echo statement so you can see which file it's processing

Babraham

Bioinformatics

Exercise 5: Installing OS packages using apt

- Use apt to install the clustalw multiple alignment tool and the clustalx graphical interface
- How many additional packages were needed to satisfy the dependencies for each tool
- Use the clustalx tool to align the rRNA sequences in Align_Data/reference_sequences.txt
- You can use the apt-file program to see which files have been installed by a particular package. Use this to see what the clustalw package installed
 - o Install apt-file with apt install apt-file (as root)
 - o Build the file cache with apt-file update
 - o List the files for clustalw with apt-file listmore / clustalw
 - o Look at the directories the files are installed into

Exercise 6: Binary and script installation

- Install the Blast search tool from NCBI.
 - Find the appropriate distribution file to download from the project web site at <u>ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/</u>
 - Use the md5sum program to calculate a hash signature for the file you downloaded and compare the answer to the .md5 file on the NCBI site. If they're the same then the file download was successful
 - o Move the extracted files to /opt/blast/
 - o Add /opt/blast/bin to your PATH
 - \circ Use 1dd to see what libraries the blastn program links to
 - Go into the Align_Data folder and build a blast index of your references sequences using makeblastdb -dbtype nucl -in reference sequences.txt
 - Search your new database with the test_seq.txt sequence by using blastn -db reference_sequences.txt -query test_seq.txt
 - Which species does the test sequence most likely come from?
- Install FastQC from http://www.bioinformatics.babraham.ac.uk/projects/fastqc/
 - \circ $\;$ You will need to check that you have java installed before fastqc will run
 - Run java -version to see if you have it. If not then use apt to install the defaultjre (java runtime environment)
 - o Download the zip file into your home directory and unzip it
 - o Move the unzipped data to /opt/FastQC
 - o Change the permissions on the /opt/FastQC/fastqc launch script to be executable
 - o Check what interpreter the launch script is using and that it exists.
 - Create a symlink from /opt/FastQC/fastqc to /usr/local/bin/fastqc so that fastqc appears in your PATH (/usr/local/bin should already be there)
 - Check that the install works by performing a fastqc analysis of all of the files in FastQ_Data. Run fastqc --help if you're not sure how to run the program

Exercise 7: Installation from source code

Babraham

Bioinformatics

- We are going to be compiling from source so we need to install the basic command line toolset. Use apt to install the make te package for the OS which contains these.
- Install samtools, a library for manipulating BAM and CRAM mapped sequence files.
 - Go to <u>http://www.htslib.org/download/</u> and download the latest sourcecode for samtools
 - Extract the data from the file. Note the type of compression the tar file uses and make sure you put the appropriate switches onto your tar command
 - Move into the new directory which was created and go through the standard autotools build process. We will install samtools into /opt/samtools
 - ./configure --prefix=/opt/samtools
 - make-j 2
 - make test
 - make install
 - Note that the configure will fail because of missing dependencies (probably a couple of times!). Each time, read the error, install the missing dependency using apt and then run configure again until it completes successfully.
 - After the install has completed, add the appropriate directory to your PATH so that samtools is accessible everywhere
 - Have a look at the results of ldd on the samtools program binary, can you see the different libraries which you had to install?

Exercise 8: Installing R packages

- R is not yet installed on these systems. It should be available in the package repository under the name r-base-core. Install this package using apt
- Compilation of packages requires some additional libraries, so also use apt to install libxml2-dev and libcurl4-openssl-dev and libssl-dev
- Install the CRAN beanplot package
 - You want to install this for all users, so start an R session as root (using sudo)
 - o Use install.packages to install the package directly from CRAN
 - Check that you can load the library using library (beanplot)
 - Check it works by running:
 - beanplot(rnorm(1000), rnorm(1000)+2)
- Install the Bioconductor package GenomeGraphs.
 - Find the install instructions on the Bioconductor website
 - The install may take a while as there are a large number of dependencies
 - Make sure you can load the library using library (GenomeGraphs)
 - Make sure it works by running
 - mart <- useMart("ensembl",</pre>
 - dataset="hsapiens_gene_ensembl")
 - gene <- makeGene(id = "ENSG00000095203",</pre>
 - type="ensembl_gene_id", biomart = mart)
 - gdPlot(gene) asdas]
- Install the intensitydiff package from github
 - Download the latest release as a tar.gz from <u>https://github.com/s-andrews/intensitydiff/releases/</u>
 - Install the package (as root) using R CMD INSTALL

Exercise 9: Installing Perl modules

Babraham

- Install the Date::Calc module using the cpan program
- Try to spot the download, compile, test and install phases of the installation
- Find out how many days you've been alive with:
 - o perl -MDate::Calc -e 'print Date::Calc::Delta Days(1973, 9, 29, 2018, 11, 5)'
 - Use your own birthday for the first 3 arguments, obviously though
- Manually install the Digest::SHA1 module
 - Find the package on http://search.cpan.org
 - Download the tar.gz file
 - Extract the contents and cd into the newly created directory
 - o Go through the standard manual install
 - perl Makefile.PL
 - make

- make test.
- sudo make install
- Check it worked with
- o perl-MDigest::SHA1 -e 'print Digest::SHA1::sha1 hex(12345)'

Exercise 10: Installing Python3 packages

- Install the multiqc python package (and program) using pip3
 - Use apt to install python3-pip 0
 - Use pip3 to install multige use sudo so it's available to everyone 0
 - Run multiqc on the FastQ Data folder you ran fastqc in before 0
 - multiqc . (you need the dot at the end)
 - Look at the multiqc report.html file which is generated
- Install the ColourScience package from https://github.com/colour-science/colour
 - Download the latest release tarball
 - Uncompress it and move into the directory
 - o Run python setup.py install to install it
 - o If you're feeling ambitious, try installing it into a non-standard directory

Exercise 11: Using conda

- Install miniconda from https://conda.io/miniconda.html
 - Download the .sh script 0
 - Run it
 - Accept the default location so it installs in your home directory
 - o Allow it to add conda to your PATH, and start a new shell so it takes effect
- Add the bioconda channels to your conda install
 - o conda config --add bioconda
 - o conda config -add conda-forge
- Install the circos package into a new environment called course
 - What other packages are pulled in?
 - o Can you start the environment and get circos --version to run
 - o What do you get when you run which perl?



o ${\sf Run}\xspace$ deactivate and try which perl again

Exercise 12: Using singularity

- Install the singularity-container package using apt
 - o Check the version with singularity --version
- Go to <u>https://singularity-hub.org</u> and do a search for hello-world (the one by vsoch). Find the address for the container
- Pull the container down onto your machine, calling it hello-world.simg
- Start a shell inside the container
- List the contents of your home directory in the shell
 - o Can you see all of your files?
- List the contents of /opt in the shell
 - o Can you see the software you installed in there before?
- Look at the contents of /singularity in the container, Work out what it does?
- Run the container as an application (exit the shell and run ./hello-world.simg) do you get what you expected?

[Optional] Exercise 15: Troubleshooting

Babraham

Bioinformatics

- You have been given 4 programs called broken1, broken2, broken3 and broken4. All of which are in /usr/local/bin/. None of them currently run.
- Alter the programs or system so that you can run all of them by just calling their name

Exercise 16: Installing Linux in a VM

- You have been provided with two ISO images
 - An Ubuntu live ISO
 - A CentOS install ISO
- Pick one of these and install it into a VirtualBox VM
 - Set up a new VM
 - Give it some memory (2GB) and disk (20GB)
 - Add the ISO to the virtual DVD drive
 - \circ Start the image and step through the installation
- Once your new image is running see if you can replicate some of the exercises you ran before in your own VM instance.

[Optional] Exercise 17: Creating a cloud Linux instance

- Create an amazon AWS login for yourself if you don't have one already
- Go through the process of creating and connecting to an EC2 linux instance
 - \circ $\,$ Create a key pair and download the .pem file for it
 - o Create an EC2 instance and launch it
 - Use the key file to connect to your instance
 - \circ Try out some of the previous exercises to show that they work in this environment