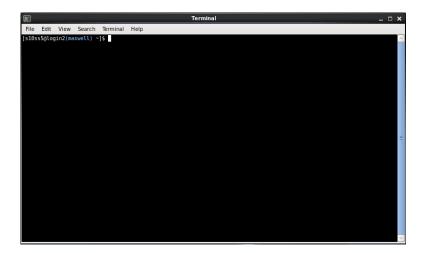




What We're Going To Do

- Why Unix?
- Cloud Computing
- Connecting to AWS
- Introduction to Unix Commands



Etiquette

- PowerPoint interspersed with Challenges
- Ask me questions
- Ask demonstrators
- Work together
- Cheat!



Unix/Linux Command Reference



File Commands

ls - directory listing

ls -al - formatted listing with hidden files

cd dir - change directory to dir

cd - change to home

pwd - show current directory

mkdir dir - create a directory dir

rm file - delete file

rm -r dir - delete directory dir

rm -f file - force remove file

rm -rf dir - force remove directory dir *

cp file1 file2 - copy file1 to file2

cp -r dir1 dir2 - copy dir1 to dir2; create dir2 if it doesn't exist

mv file1 file2 - rename or move file1 to file2 if file2 is an existing directory, moves file1 into directory file2

In -s file link - create symbolic link link to file

touch file - create or update file

cat > file - places standard input into file

more file - output the contents of file head file - output the first 10 lines of file

tail file - output the last 10 lines of file tail -f file - output the contents of file as it

grows, starting with the last 10 lines

Process Management

ps - display your currently active processes top - display all running processes

kill pid - kill process id pid

killall proc - kill all processes named proc *

bg - lists stopped or background jobs; resume a stopped job in the background

fg - brings the most recent job to foreground **fg** n - brings job n to the foreground

File Permissions

chmod octal file - change the permissions of file to octal, which can be found separately for user, group, and world by adding:

- 4 read (r)
- 2 write (w)
- 1 execute (x)

Examples:

chmod 777 - read, write, execute for all

chmod 755 - rwx for owner, rx for group and world For more options, see man chmod.

SSH

ssh user@host - connect to host as user

ssh -p port user@host - connect to host on port port as user

ssh-copy-id user@host - add your key to host for user to enable a keyed or passwordless login

Searching

grep pattern files - search for pattern in files grep -r pattern dir - search recursively for pattern in dir

command | grep pattern - search for pattern in the output of command

locate file - find all instances of file

System Info

date - show the current date and time

cal - show this month's calendar uptime - show current uptime

w - display who is online

whoami - who you are logged in as

finger user - display information about user

uname -a - show kernel information

cat /proc/cpuinfo - cpu information cat /proc/meminfo - memory information

man command - show the manual for command

df - show disk usage

du - show directory space usage

free - show memory and swap usage

whereis app - show possible locations of app which app - show which app will be run by default

Compression

tar cf file.tar files - create a tar named file.tar containing files

tar xf file.tar - extract the files from file.tar tar czf file.tar.gz files - create a tar with Gzip compression

tar xzf file.tar.gz - extract a tar using Gzip tar cjf file.tar.bz2 - create a tar with Bzip2 compression

tar xjf file.tar.bz2 - extract a tar using Bzip2 gzip file - compresses file and renames it to

gzip -d file.gz - decompresses file.gz back to

Network

ping host - ping host and output results whois domain - get whois information for domain

dig domain - get DNS information for domain

dig -x host - reverse lookup host

wget file - download file

wget -c file - continue a stopped download

Installation

Install from source:

./configure

make

make install

dpkg -i pkg.deb - install a package (Debian)

rpm -Uvh pkg.rpm - install a package (RPM)

Shortcuts

Ctrl+C - halts the current command

Ctrl+Z - stops the current command, resume with

fg in the foreground or bg in the background

Ctrl+D - log out of current session, similar to exit

Ctrl+W - erases one word in the current line Ctrl+U - erases the whole line

Ctrl+R - type to bring up a recent command

!! - repeats the last command

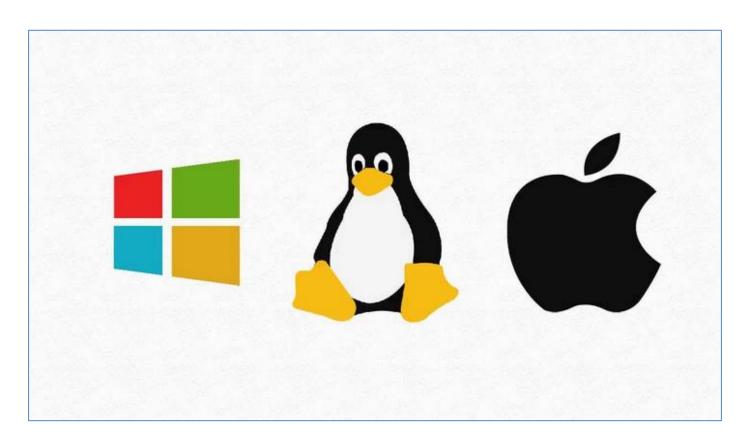
exit - log out of current session

* use with extreme caution.



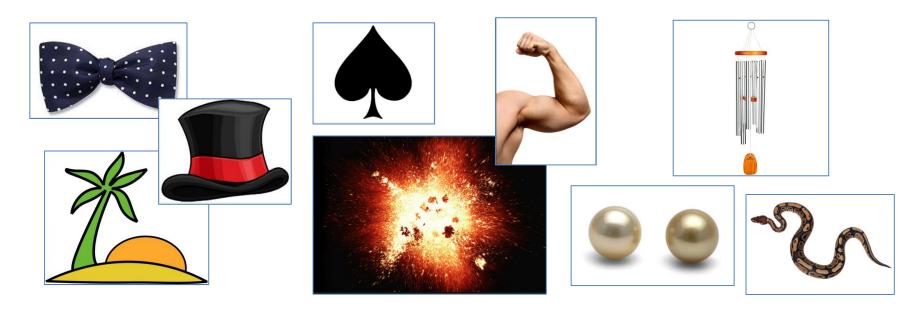
What is Unix?

Operating System



Why Unix?

- Bioinformatics software designed to run on Unix platforms.
- Large amounts of data.
- Much faster than your Windows PC.



How Can We Use Unix?

- Linux computers or servers.
- Compute clusters.
- The cloud.
 - What we're going to use this week



So What is Cloud Computing?









Cloud Computing Solutions





Google Compute Engine

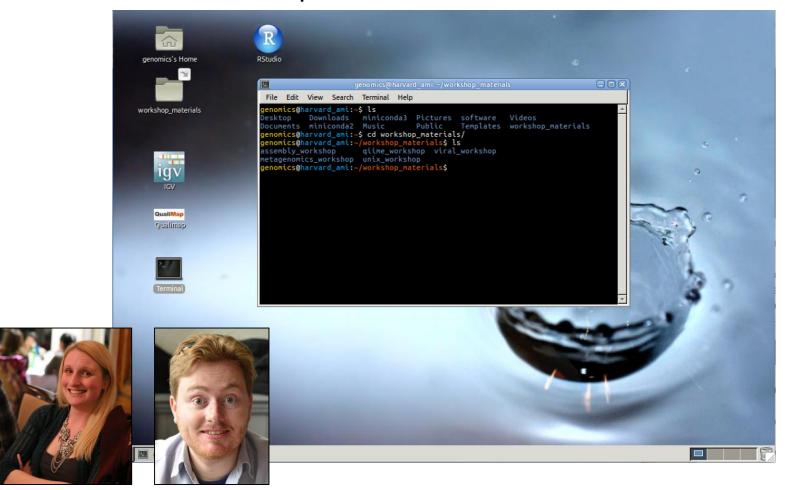




AWS "Availability Zones" and Data Centres

How it Works

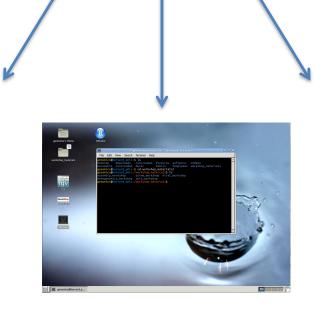
AMI ("Amazon Machine Image")
Base computer with all data and software



How it Works









Own copy of the AMI = Instance (Virtual Machine or VM)

Terminology

- Creating an instance buying a brand new computer with software already installed.
- Starting an instance turning that computer on.
- Stopping an instance turning that computer off.
- Terminating an instance setting that computer on fire and throwing it out of the window.

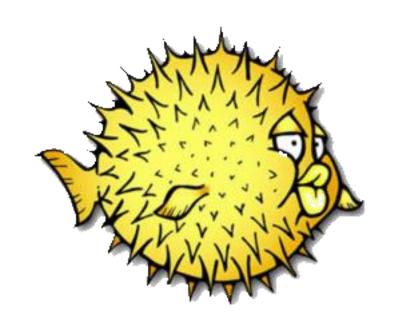
The Rules

- Only create one instance each.
- Stop your instance at the end of each day (unless you have software running).
- Name your instance (with YOUR name! No Bruce Waynes please)
- Only start or stop your own instance.
- Only terminate your own instance.

Connecting to Your Instance



Remote Desktop Software e.g. X2Go

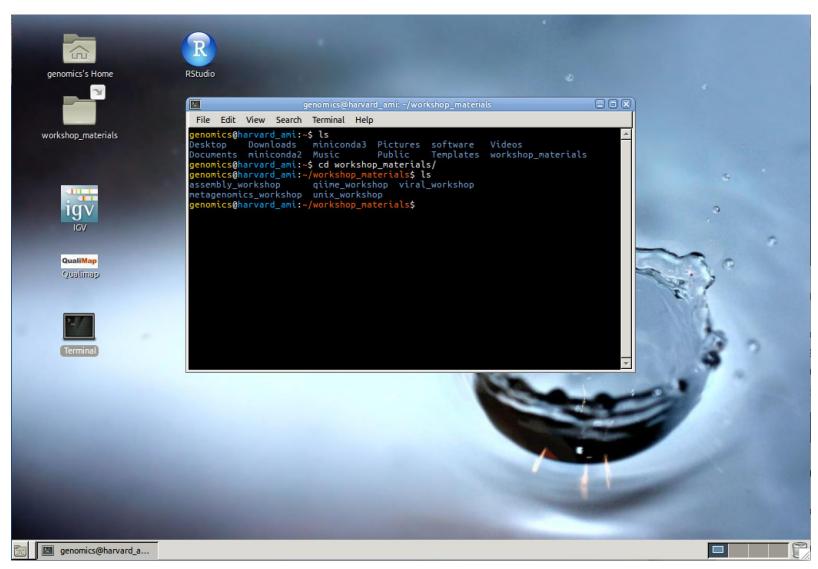


Secure Shell – "SSH" e.g. SSH or PuTTY

Now What?!

 You're each going to create, start and connect to your own instance.

INSERT LIVE DEMO

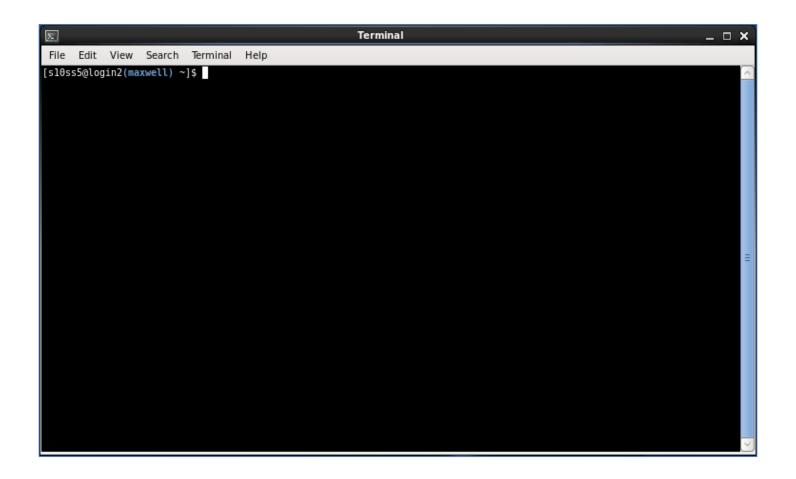


You're now connected to your instance and you're ready to learn some Unix!

Any Questions So Far?

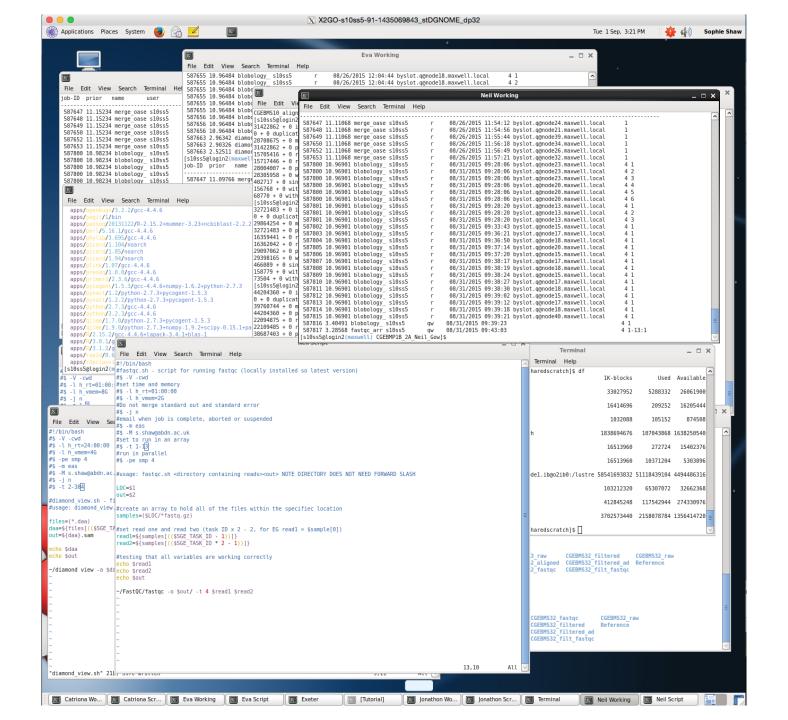


The Terminal Window



The Command Line, The Shell, The Prompt

Where you see this "\$" followed by text, I want you to type the text on your command line



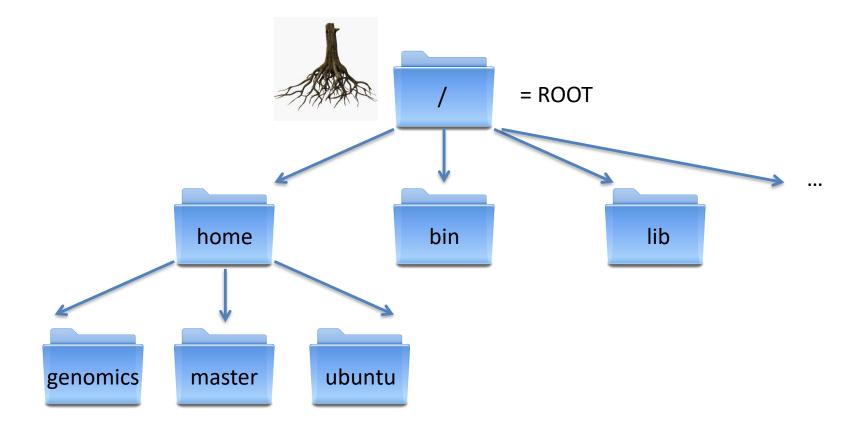
Location is Important

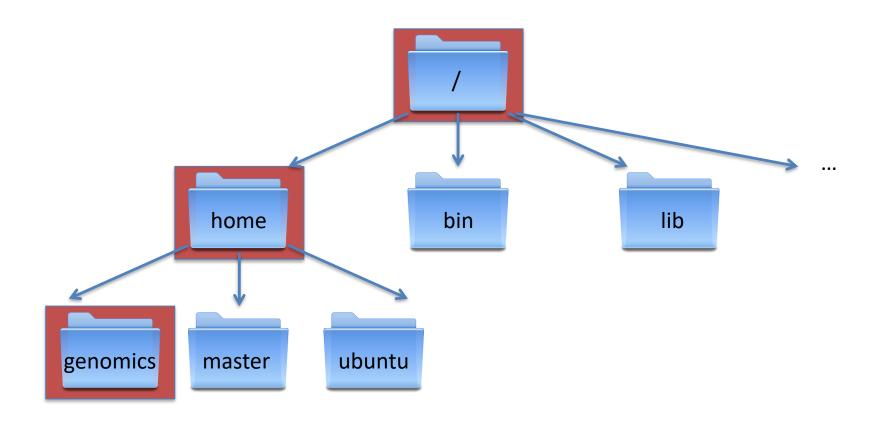
First Task – Where am I?

\$ pwd

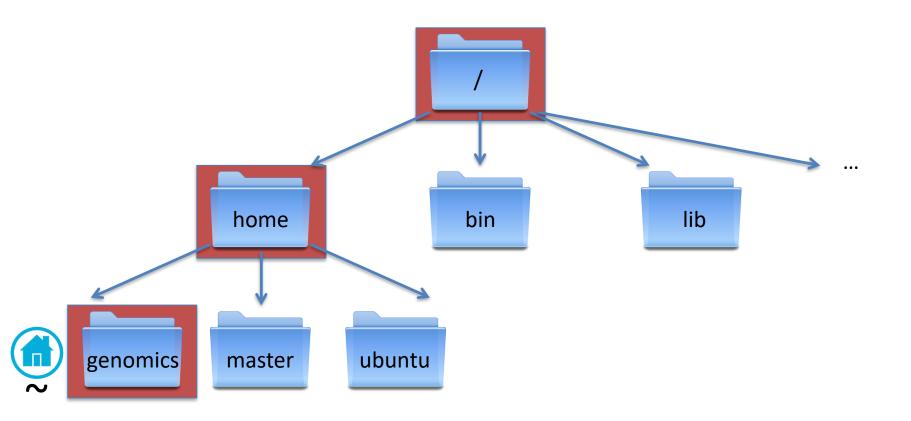
```
genomics@harvard_ami:~$ pwd
/home/genomics
genomics@harvard_ami:~$
```

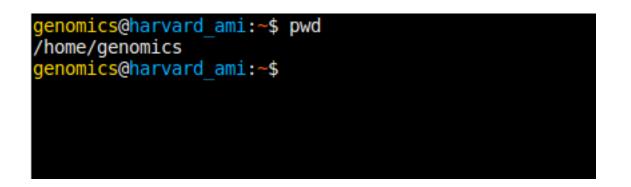
This is your "present working directory"





```
genomics@harvard_ami:~$ pwd
/home/genomics
genomics@harvard_ami:~$
```





This location is also known as your Home Directory

Tilde is shorthand for Home:

~

Now let's create some directories and files

Make a directory

\$ mkdir Data

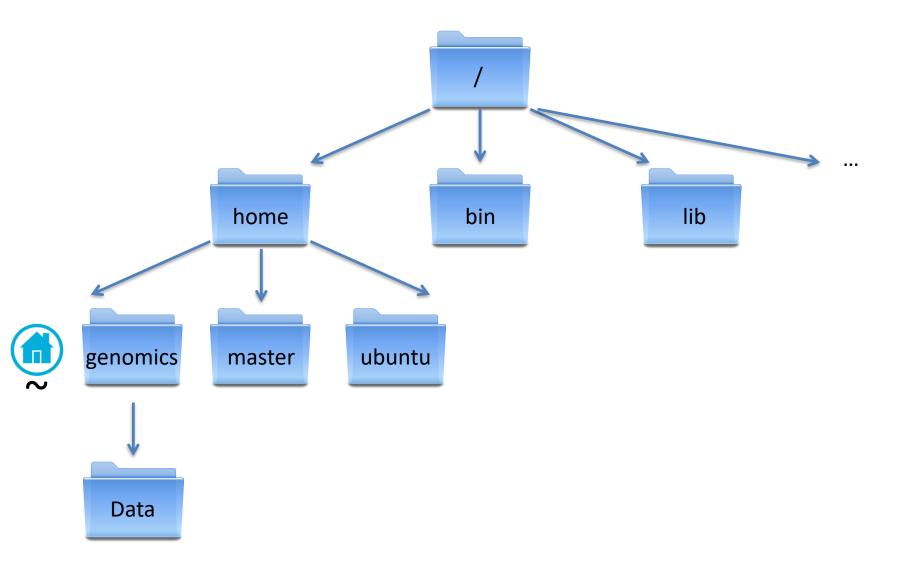
Change into this directory

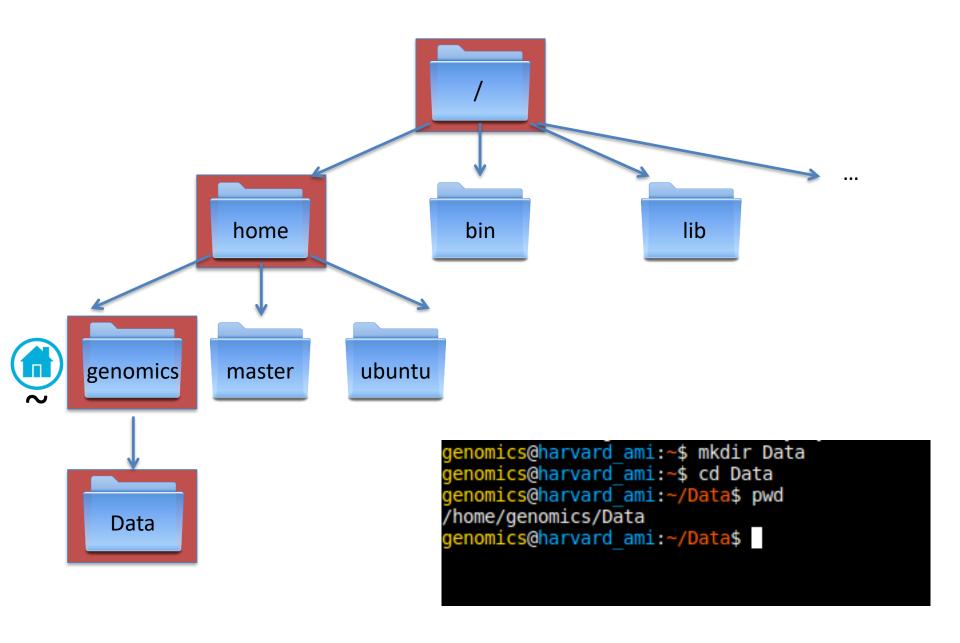
\$ cd Data

Now what is your present working directory?

NOTE! Directory names (and file names for the matter) can not contain spaces.

Underscores are often used instead if you want to separate words.





Now let's create some directories and files

Make an empty file



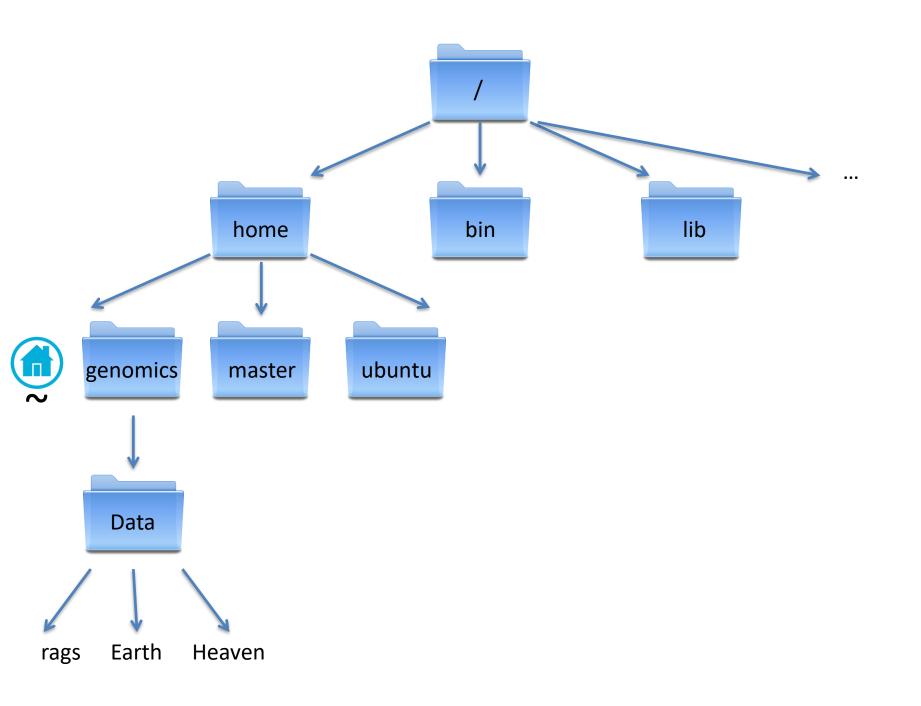
And another two

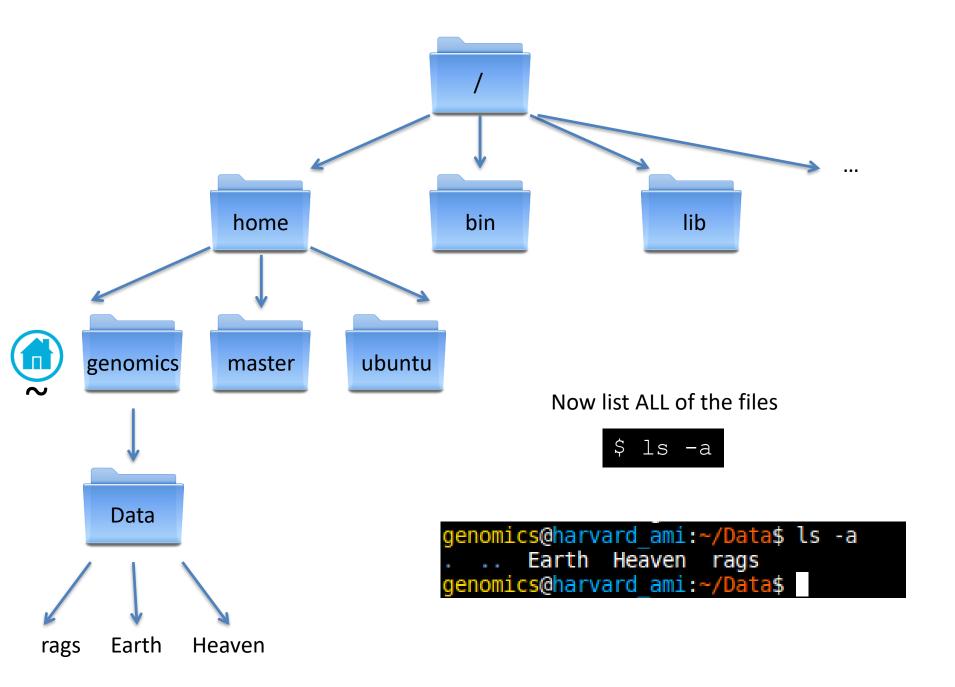
```
$ touch Earth Heaven
```

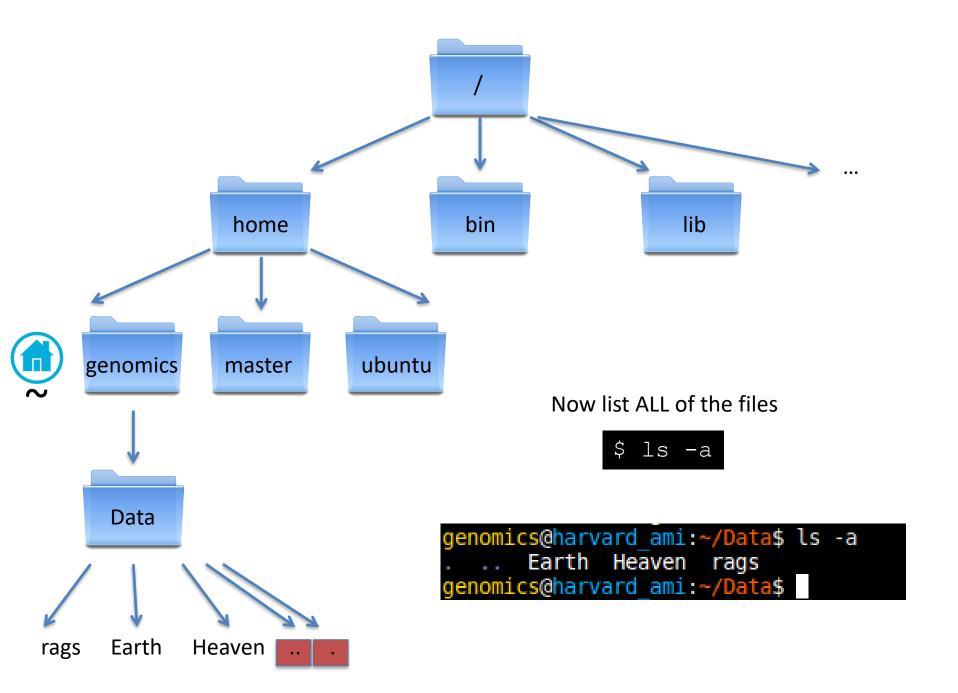
Now let's list the contents of the current directory (Data)

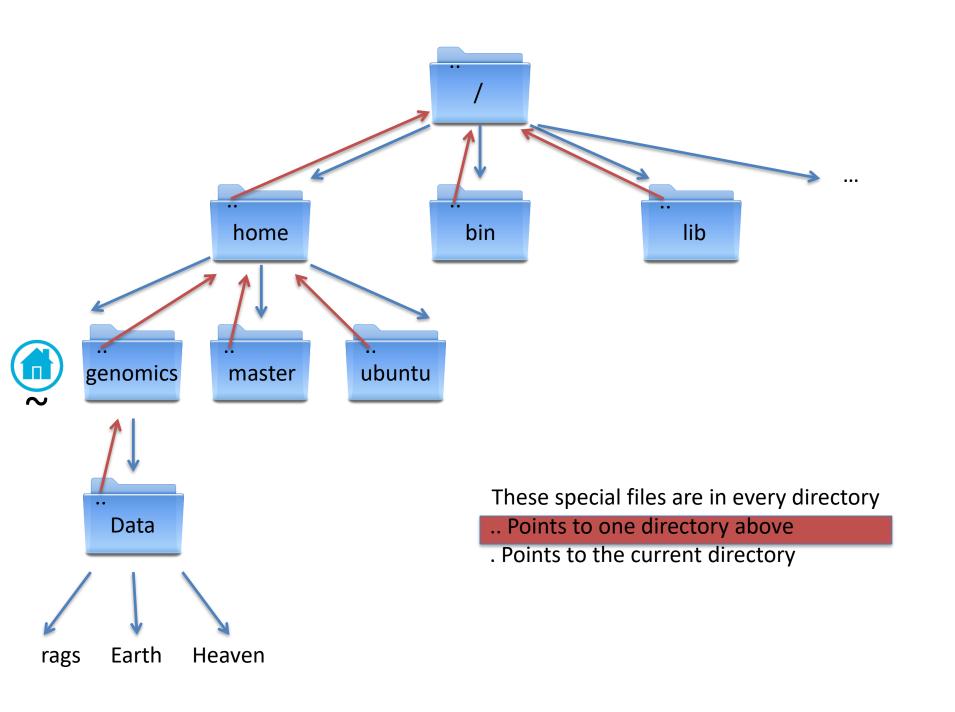
```
$ ls
```

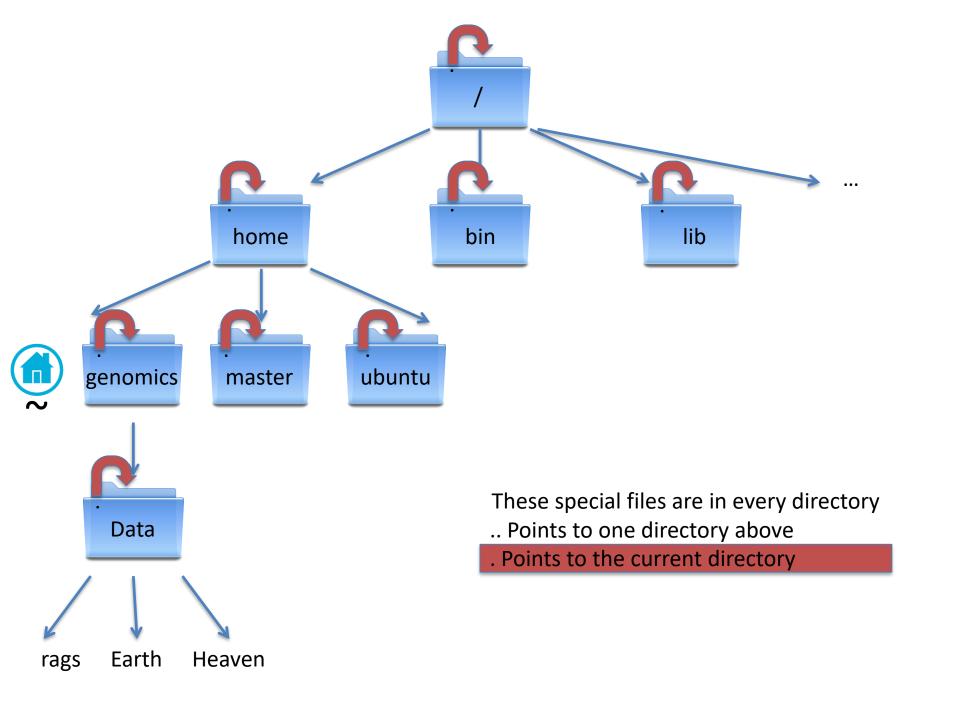
```
genomics@harvard_ami:~/Data$ touch rags
genomics@harvard_ami:~/Data$ touch Earth Heaven
genomics@harvard_ami:~/Data$ ls
Earth Heaven rags
genomics@harvard_ami:~/Data$
```



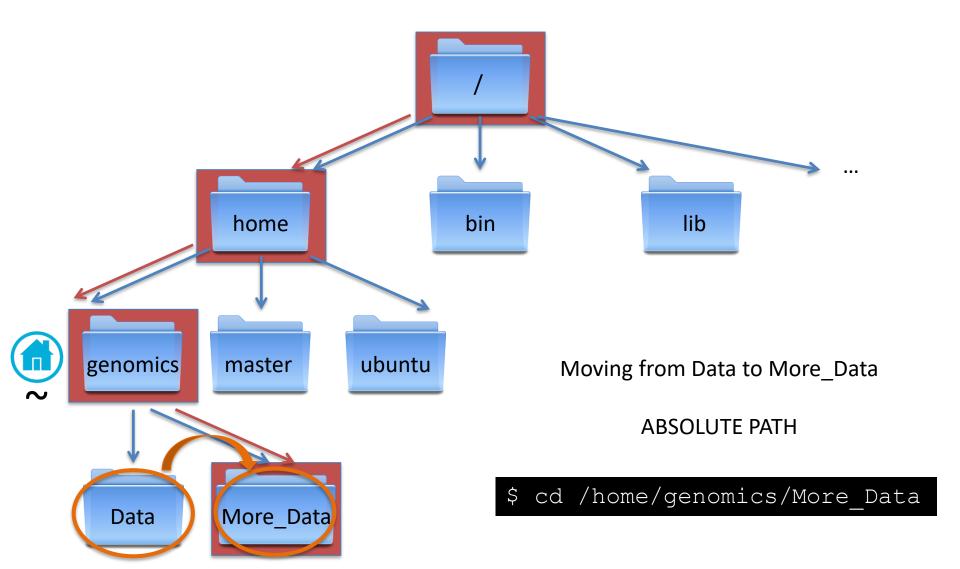


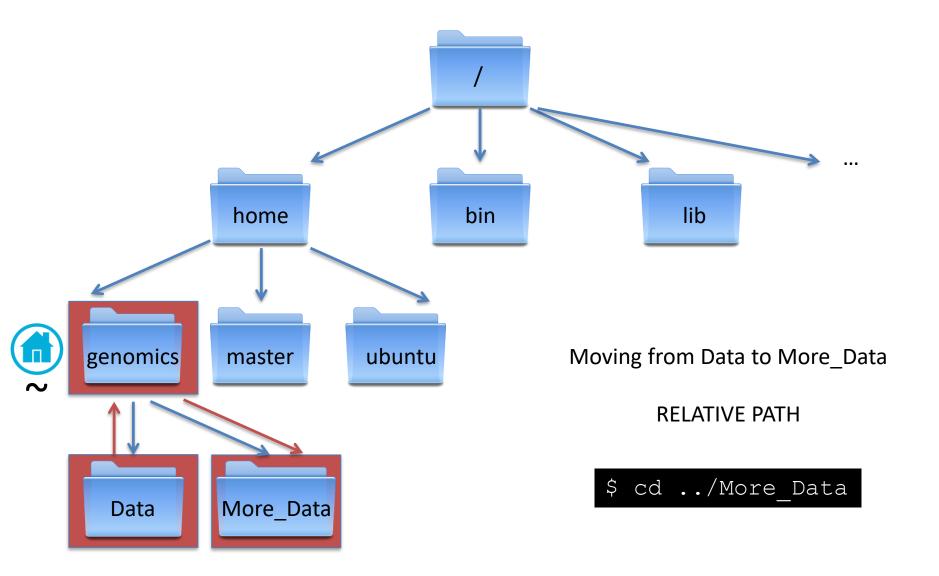


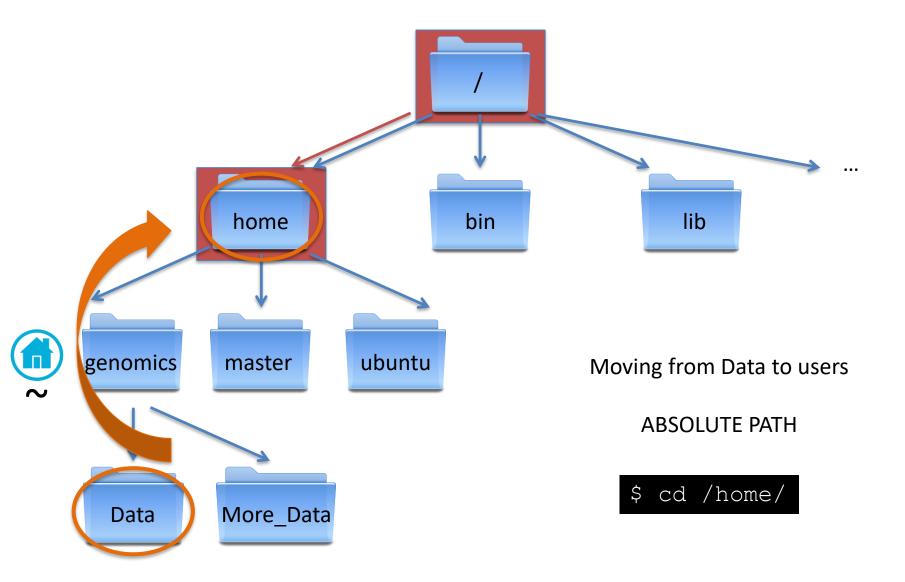


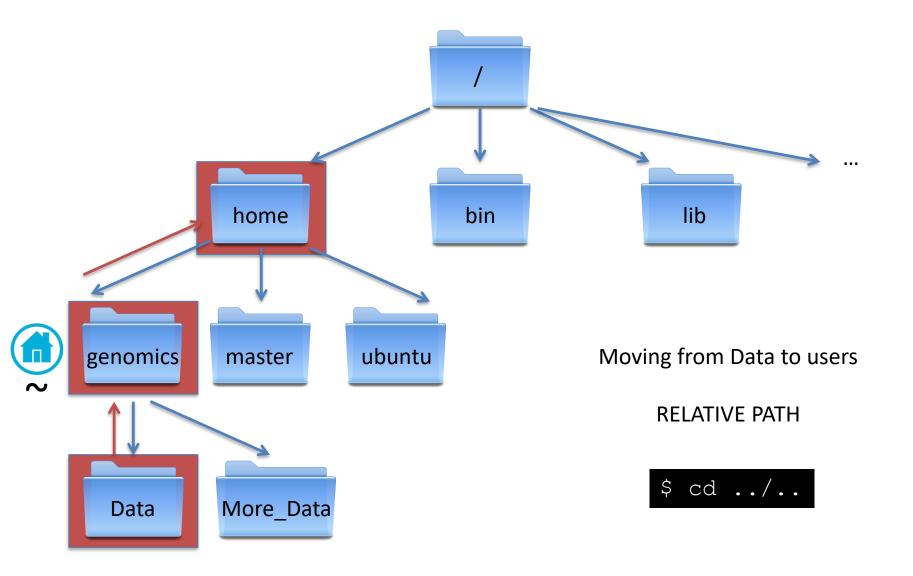


ABSOLUTE AND RELATIVE PATHS: GETTING FROM ONE PLACE TO ANOTHER









Let's put this to practice

Where am I right now? (Should be the Data directory)



Change to the directory above



Let's list the contents of the Data directory

```
$ ls ./Data
```

Let's put this to practice

Where am I right now? (Should be the Data directory)



Change to the directory above



Let's list the contents of the Data directory



CHALLENGE 1!

- 1. Move into the Data directory and list the contents of your home directory
- 2. In Data, make a new directory and move into this location
- From this new directory, move into your home directory IN ONE COMMAND and check your location

Challenge 1!

1. Move into the Data directory and list the contents of your home directory

2. In Data, make a new directory and move into this location

```
$ mkdir new
$ cd new
```

From this new directory, move into your home directory IN ONE COMMAND and check your location

```
$ cd ../.. OR $ cd /home/genomics OR $ cd ~ OR $ cd
$ pwd
```

If You're Typing, You're Doing Something Wrong!

Tab complete is a nice trick to save you typing paths

For this examples we are going to list everything in directory /var/run

Start by typing:

```
$ ls /
```

Followed by tab twice quickly

```
genomics@harvard ami:~$ ls /
                lib/
bin/
                                 root/
                                                  usr/
                lib64/
boot/
                                 run/
                                                  var/
                lost+found/
                                                  vmlinuz
dev/
                                 sbin/
                                                  vmlinuz.old
                media/
etc/
                                 snap/
home/
                mnt/
                                 srv/
initrd.img
                opt/
                                 sys/
initrd.img.old
                proc/
                                 tmp/
genomics@harvard ami:~$ ls
```

This shows the contents of the root directory

If You're Typing, You're Doing Something Wrong!

Now type:

Followed by tab once. The path to the /var/ directory has filled in.

Now type:

Followed by tab once. The path to the /var/run/ directory has filled in.

Tab complete will fill in paths, save you time in typing and prevent typos!

If You're Typing, You're Doing Something Wrong!

Two more tricks for less typing!

* Represents a special character For example:

\$ ls /home/genomics/*.txt

Will list everything in my home directory ending .txt

The up arrow can be used to re-run commands

Press your up arrow and see

If you want all of these commands listed, simply type

\$ history

Any Questions So Far?



Binary programs

These are all programs installed on the Unix machine.

They can be found in /bin

\$ ls /bin

```
enomics@harvard ami:~$ ls /bin/
bash
                    chmod
                                    hciconfig
                                                                    pwd
                                                                                                systemd-tty-ask-password-agent
                                                 mv
btrfs
                    chown
                                    hostname
                                                 nano
                                                                    rbash
                                                                                                tailf
btrfs-calc-size
                    chvt
                                                                    readlink
                                    ip
                                                                                                tar
btrfsck
                    ср
                                     iournalctl
                                                nc.openbsd
                                                                    red
                                                                                                tempfile
btrfs-convert
                    cpio
                                     kbd mode
                                                 netcat
                                                                                                touch
                                                                    rm
btrfs-debug-tree
                    dash
                                    kill
                                                 netstat
                                                                    rmdir
                                                                                                true
btrfs-find-root
                                                 networkctl
                                                                                                udevadm
                    date
                                     kmod
                                                                    rnano
btrfs-image
                    dd
                                     less
                                                 nisdomainname
                                                                    run-parts
                                                                                                ulockmgr server
btrfs-map-logical
                    df
                                    lessecho
                                                 ntfs-3q
                                                                    sed
                                                                                                umount
btrfs-select-super
                    dir
                                    lessfile
                                                 ntfs-3g.probe
                                                                    setfacl
                                                                                                uname
btrfs-show-super
                    dmesq
                                                 ntfs-3q.secaudit
                                    lesskey
                                                                    setfont
                                                                                                uncompress
btrfstune
                    dnsdomainname
                                                 ntfs-3g.usermap
                                    lesspipe
                                                                                                unicode start
                                                                    setupcon
                                                 ntfscat
btrfs-zero-log
                    domainname
                                    ln
                                                                                                vdir
                                                 ntfscluster
bunzip2
                    dumpkeys
                                    loadkevs
                                                                    sh.distrib
                                                                                                vmmouse detect
busybox
                    echo
                                     login
                                                 ntfscmp
                                                                    sleep
                                                                                                wdctl
                                    loginctl
                                                 ntfsfallocate
bzcat
                    ed
                                                                    SS
                                                                                                which
bzcmp
                                    lowntfs-3q
                                                ntfsfix
                                                                    static-sh
                                                                                                whiptail
                    egrep
bzdiff
                     false
                                    ls
                                                 ntfsinfo
                                                                                                vpdomainname
                                                                    stty
bzearep
                     fgconsole
                                    lsblk
                                                 ntfsls
                                                                    su
                                                                                                zcat
                                    lsmod
                                                 ntfsmove
bzexe
                     fgrep
                                                                    sync
                                                                                                zcmp
                                                                                                zdiff
bzfgrep
                     findmnt
                                    mkdir
                                                 ntfstruncate
                                                                    systemctl
bzgrep
                     fsck.btrfs
                                    mkfs.btrfs
                                                ntfswipe
                                                                    systemd
                                                                                                zegrep
bzip2
                     fuser
                                    mknod
                                                 open
                                                                    systemd-ask-password
                                                                                                zfgrep
bzip2recover
                    fusermount
                                                                                                zforce
                                    mktemp
                                                 openvt
                                                                    systemd-escape
bzless
                     getfacl
                                    more
                                                 pidof
                                                                    systemd-hwdb
                                                                                                zgrep
                                                                                                zless
bzmore
                    grep
                                    mount
                                                 ping
                                                                    systemd-inhibit
                                                                    systemd-machine-id-setup
cat
                    gunzip
                                    mountpoint
                                                 pina6
                                                                                               zmore
chacl
                                                 plymouth
                    gzexe
                                                                    systemd-notify
                                                                                                znew
                                    mτ
                                                                    systemd-tmpfiles
chgrp
                    gzip
                                    mt-gnu
```

These include pwd, mkdir, ls ...

Every binary program has a manual

To view the manual page, type man followed by the name of the program



Open the manual page for Is

Scroll through (enter) and find the options for: long listing format, human-readable sizes and sort by modification time

Exit the manual page (type q) and give these Is options a go in your Data directory



Every binary program has a manual

To view the manual page, type man followed by the name of the program



Open the manual page for Is

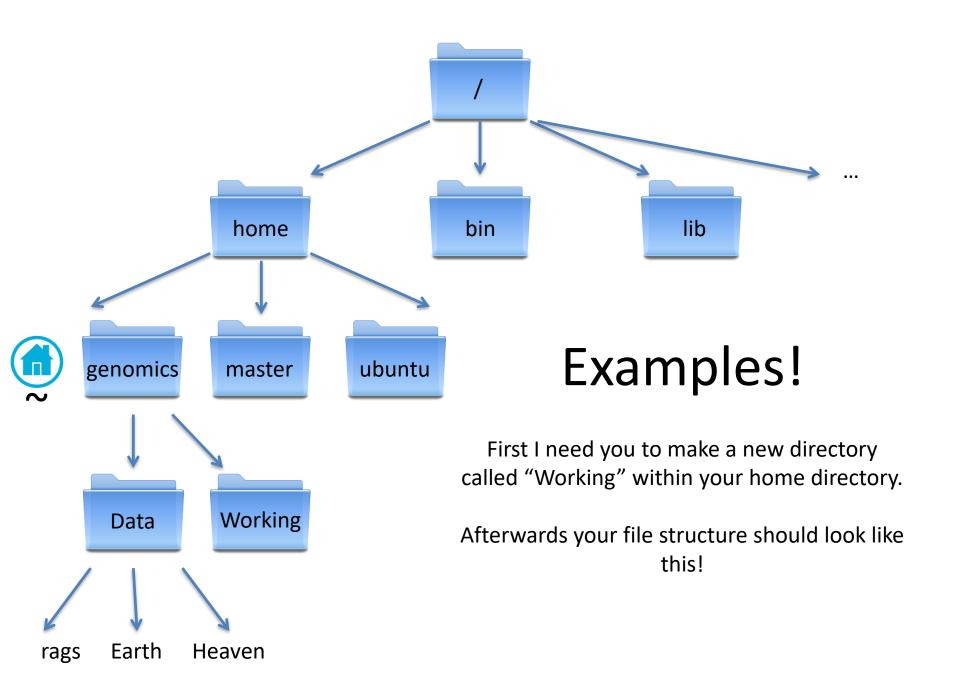
\$ man ls

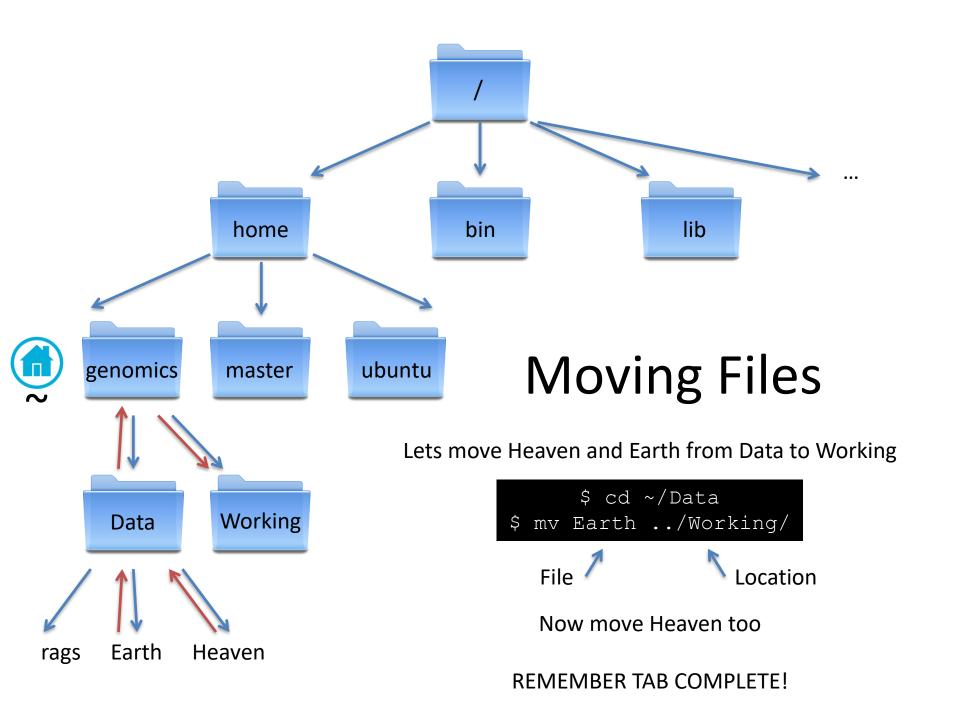
Scroll through (enter) and find the options for: long listing format (-I), human-readable Sizes (-h) and sort by modification time (-t)

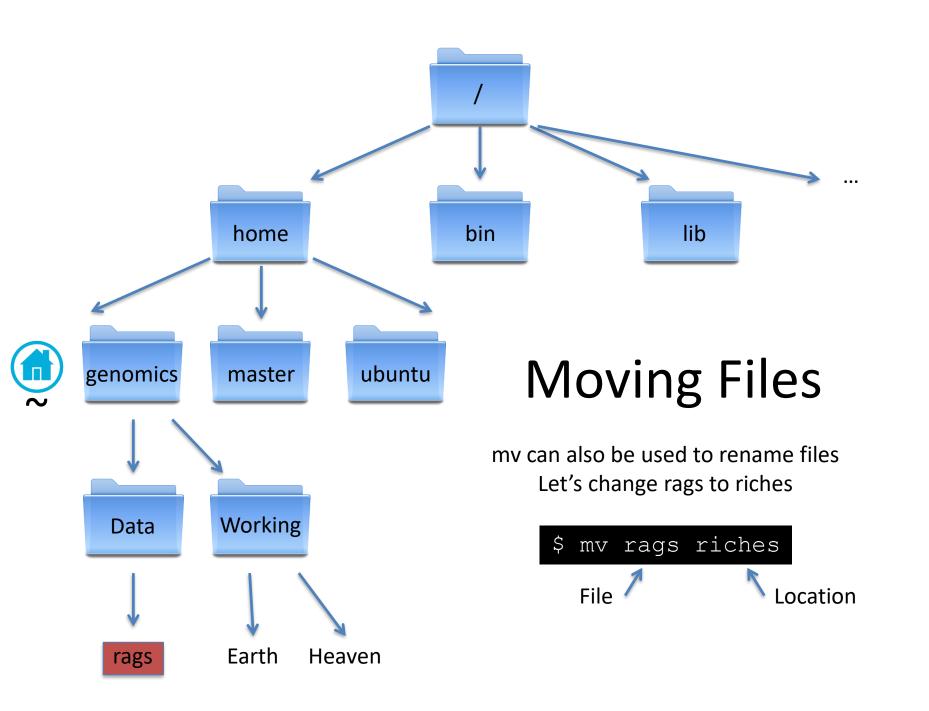
Exit the manual page (type q) and give these Is options a go in your Data directory

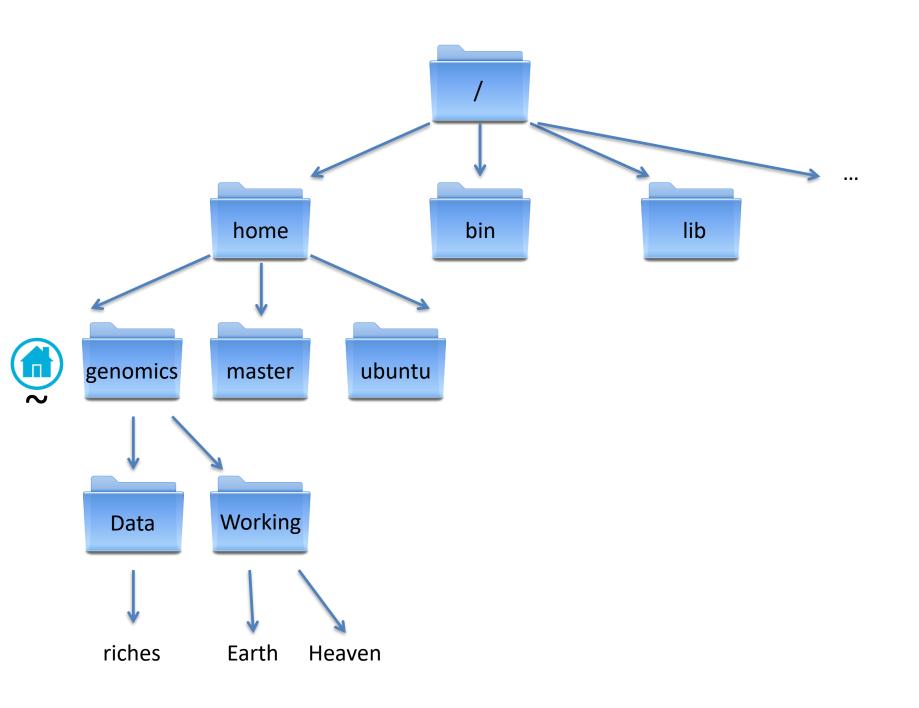


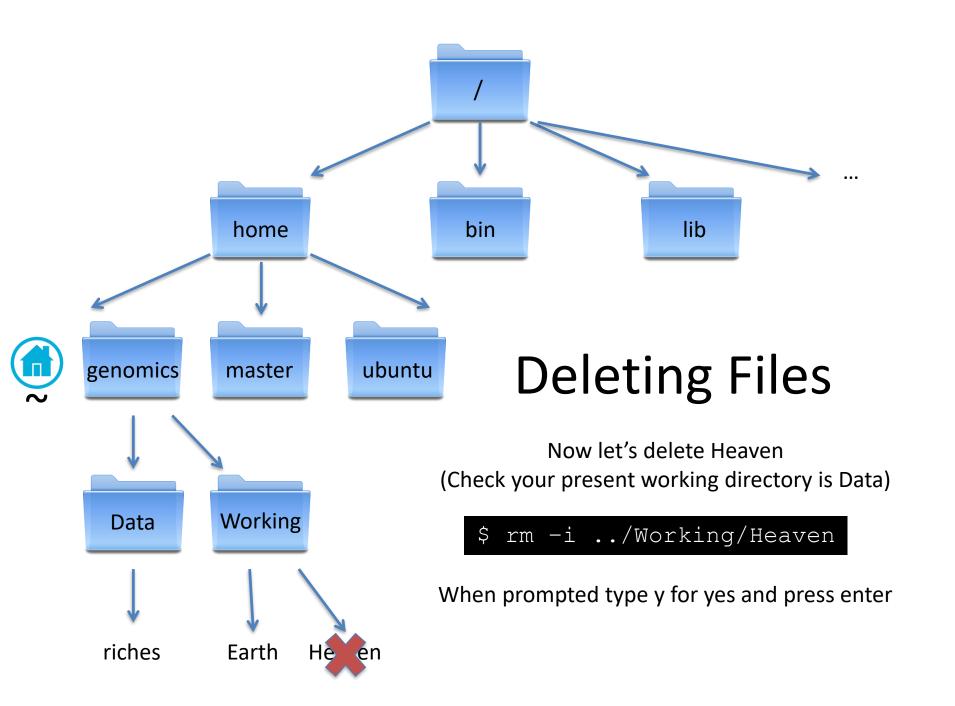


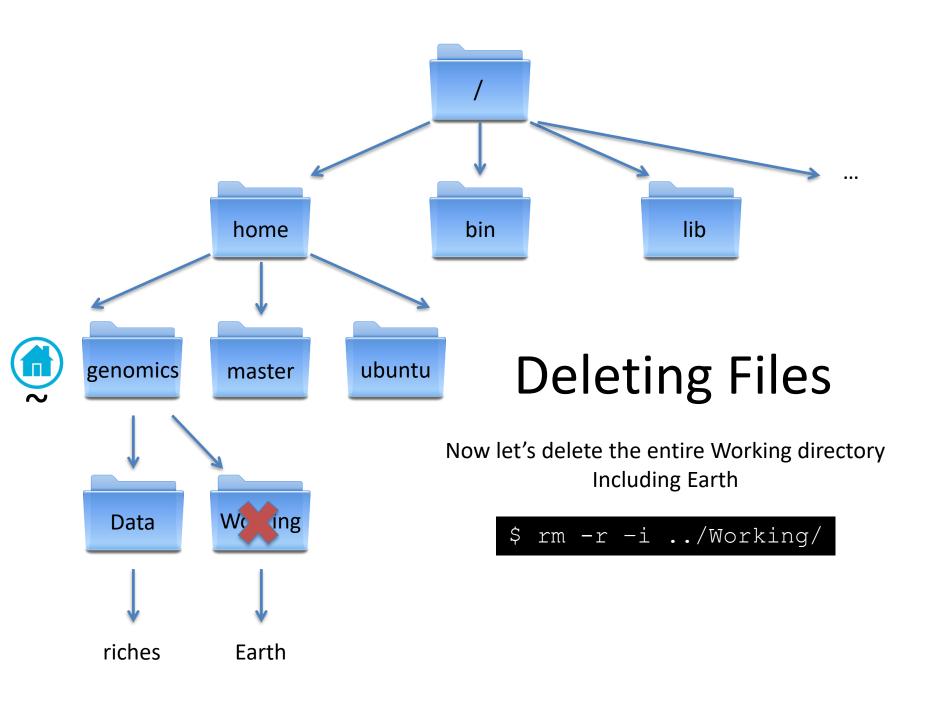


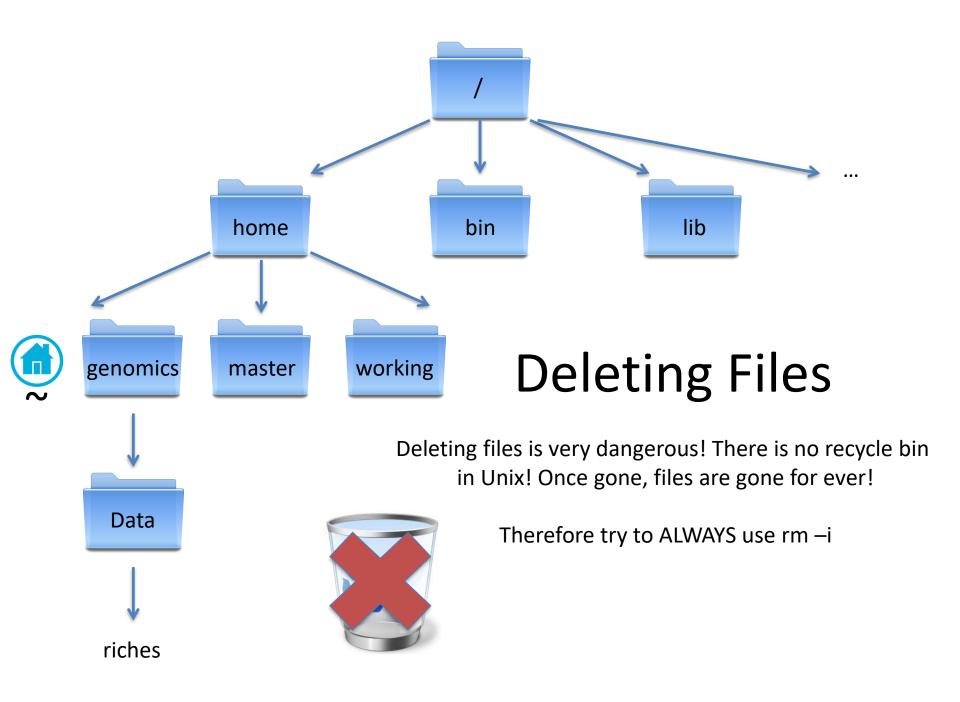


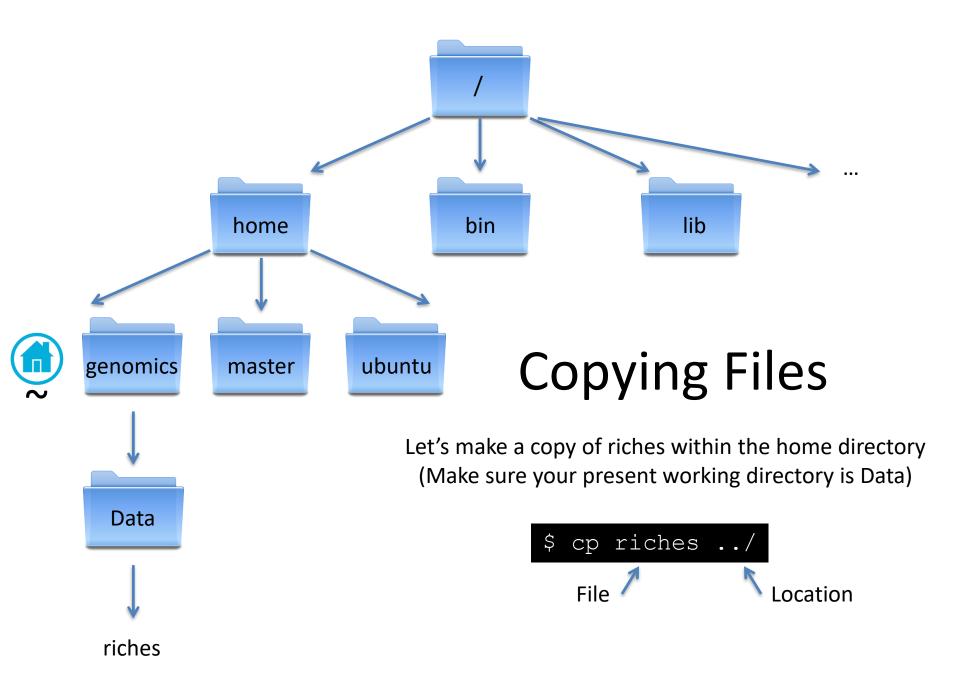


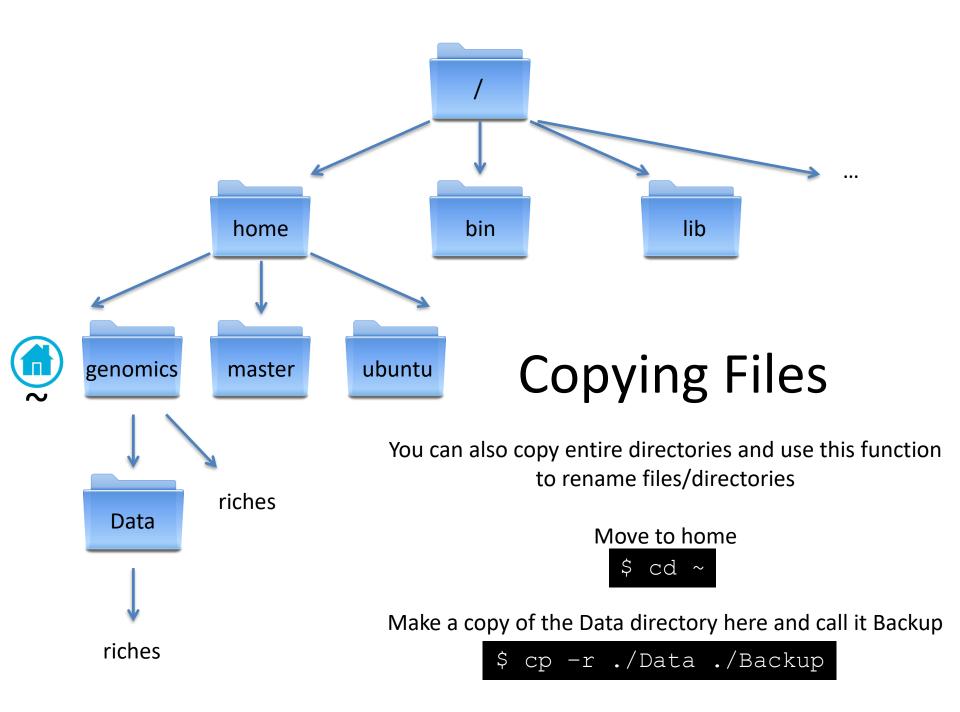


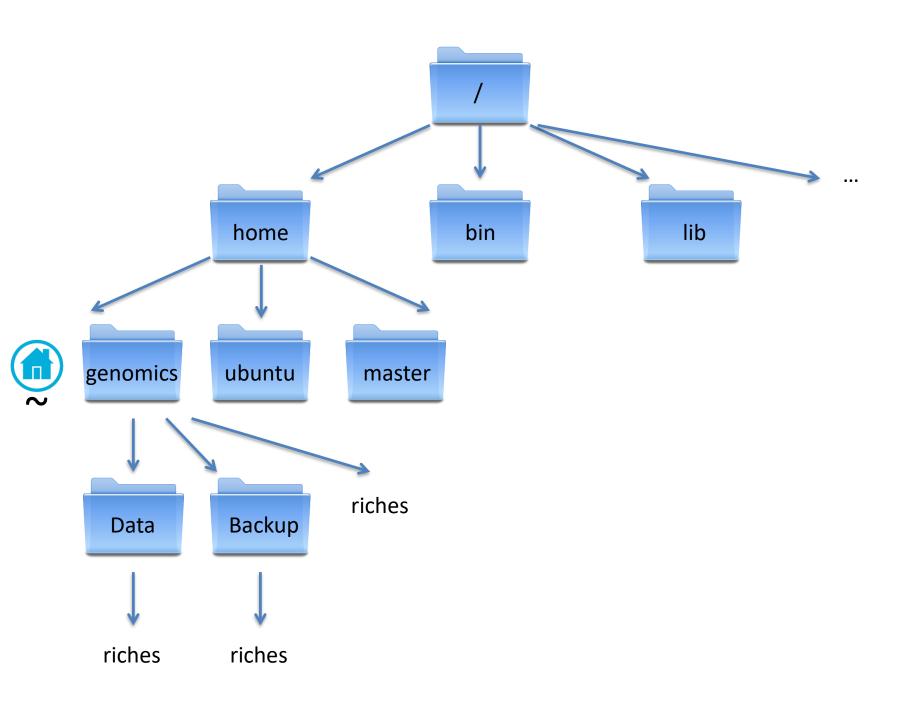












Typical File Sizes



One Sequencing Sample on the Illumina NextSeq 3,000,000 reads = 1 Gb

But typically you will sequence more than one sample You may have different patients, different locations, replicates etc...

The size of the sequencing data file can easily become 100s of Gb

(or even bigger depending on the sequencer used)

Archived/Compressed Files

Commonly, people will compress large files so that they are easier to store or share Here's an example:

sequences.tar.gz

.tar – means that it is a tape archive .gz – means that it is gzipped

These can be used alone or in combination

To uncompress A Tar Archive

\$ tar -xvf <filename>
(x = extract, v = verbose, t = all files)

A Gzipped file

\$ gunzip <filename>

A Gzipped Tar archive

\$ tar -xzvf <filename>

Any Questions So Far?



Challenge 2!

1. Change to the unix_workshop directory at the following path:

```
$ cd ~/workshop_materials/unix_workshop
```

You should find a compressed directory:

```
Sequences.tar
```

- 2. Make a copy of this file in a Backup directory
 - 3. Un archive the directory
 - 4. Unzip the read files
- 4. Rename the unarchived files sequence_1.fq and sequence_2.fq
 - 5. Delete the original .tar file

```
tar gunzip
cp mv
rm –i mkdir
cd
```

Challenge 2!

1. Change to the unix_workshop directory at the following path:

```
$ cd ~/workshop_materials/unix_workshop
```

2. Make a copy of the Sequences.tar file in a Backup directory

```
$ mkdir Backup
$ cp Sequences.tar ./Backup
```

3. Un archive the directory

```
$ tar -xvf Sequences.tar
```

4. Unzip the read files

```
$ gunzip Sequences/E_coli_Sequence1.fq.gz
$ gunzip Sequences/E_coli_Sequence2.fq.gz
$ gunzip Sequences/E_coli_Sequence*
```

OR

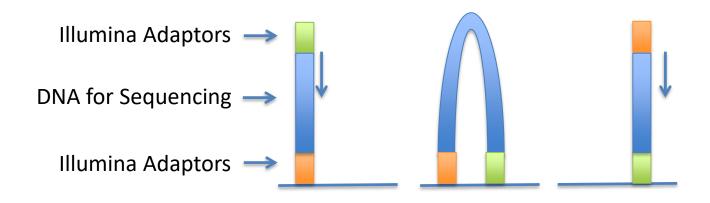
4. Rename the unarchived files – sequence 1.fq and sequence 2.fq

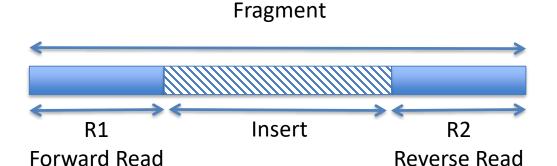
```
$ mv Sequences/E_coli_Sequence1.fq Sequences/sequence_1.fq
$ mv Sequences/E_coli_Sequence2.fq Sequences/sequence_2.fq
```

5. Delete the original .tar file

```
$ rm Sequences.tar
```

Paired Reads





An example: 300 bp paired end reads with a 700 bp fragment size R1 = 300 bp, R2 = 300 bp, Insert = 100bp

Looking at File Contents

head	tail	more	less	cat
Shows the top lines of a file	Shows the bottom lines of a file	Shows the file one full screen at a time	Shows the file one full screen at a time	Shows an entire file all at once
-n specifies the number of lines (default 10)	-n specifies the number of lines (default 10)	Enter to scroll one line Space to scroll a page q to quit	Enter to scroll one line Space to scroll a page q to quit / to search	Ctrl + C to stop

Use these command line programs to look at the sequence files

Let's put this to use

```
genomics@harvard ami:~/workshop materials/unix workshop$ cd Sequences
genomics@harvard ami:~/workshop materials/unix workshop/Sequences$ head sequence_1.fq
@E.-371320/1
GCTGGTCAGCCAGGATAAAACCACCACTGACCCGATGGCGGTTGTTGACTGGATCAACATGTTTGCACTGGCAGTGAACGAAGAACGCTGCTGG
CGGTCGCGTGGTGACTGCGCCGACTAACGGTGCGGGGATTATCCCGGCAGTTCTGGCGTACTACGACAAGTTTATCCGCGAAGTGAACGCTAA
CTCACTGGCTCGTTACCTGCTGGTAGCCAGCGCCATTGGTACTCTTTATAAGATGAAC
#
????,BBBDDD<BD?<FGFFGFCFFIIHIHIHIGIIDHGIIDIIIIIGIFHHHIHHHIHIIII-HHDIIIHIHIFIHHHFIGIHHHH:HGI=G
HHHHFGIBEGHHHHHBFH=HHHFEEFGGEGHGDEBFG?FIFEG:8EBEEDG:GEEBGGGGGGGG(GEGGGGE?FECGFFCGFDFGFGEFEE??GG?
GCGE*FG?:6E/FGCCEEHC:FGF-:G?6GCGGAAGGG6G)EGEC:GGFE'G;GC?G8</pre>
```

Fastq File Format:

Header
Sequence
Second Header (often +)

Phred Quality Score

Lot's of analysis software like paired reads to be in the same order

Use head to check that the top three headers are in the same order in sequence 1.fq and sequence 2.fq

Sequencing Stats

How many reads?

Count the number of lines

\$ wc -1 sequence_1.fq

742640 lines THEREFORE 185660 reads

Are there the same number of reverse reads?

How about just counting the header lines?

\$ grep -c "@E" sequence_1.fq
219153

BUT the numbers from the two programs don't match?!

How about with this

\$ grep -c "^@E" sequence_1.fq

185660

[^] matches this pattern at the start of the line – this is an example of a regular expression

Any Questions So Far?



AND NOW FOR A BRIEF SEGWAY INTO SCRIPTS...

Shell Scripts

Imagine you have a complicated command to run. Take this as an example:

```
ref_map.pl -o ./stacks_gsnap/ -T 4 -O ./popmap -B middleton2_
radtags -b 1 -s ./aligned_gsnap/s13_an_01.bam -s ./aligned_
gsnap/s13_an_02.bam -s ./aligned_gsnap/s13_an_03.bam -s
./aligned_gsnap/s13_an_04.bam -s ./aligned_gsnap/s13_an_05.bam
-s ./aligned_gsnap/s13_an_06.bam -s ./aligned_gsnap/s13_an_07
.bam -s ./aligned_gsnap/s13_an_08.bam -s ./aligned_gsnap/s13_
fw_01.bam -s ./aligned_gsnap/s13_fw_02.bam -s ./aligned_gsnap/
s13_fw_03.bam -s ./aligned_gsnap/s13_fw_04.bam -s ./aligned_
gsnap/s13_fw_05.bam -s ./aligned_gsnap/s13_fw_06.bam -s
./aligned_gsnap/s13_fw_07.bam -s ./aligned_gsnap/s13_fw_08.bam
```

But what if you make a mistake?

Or want to run this command 10 times?

You have to type it out every time 🙁

Shell Scripts

Instead we can put this command inside a script.

Then it can easily be edited and ran multiple times

To understand shell scripts, we're going to look at a few topics:

- Shell scripting languages
 - Text editors
 - How to write a script
 - How to run a script

Scripts make a great record of what you've done, when and with what. You should also aim to keep a computational biology lab book.

What is a Shell Script?

A computer program designed to be run by the Unix shell, the command line interpreter.

There are various types of shell scripts. These are scripting languages.

Today we are going to look at bash

First, let's run a simple bash command:

\$ echo Hello World

\$ echo Hello World
Hello World

Try using echo with a different phrase

Text Editors

These are pieces of software which can be used to write your script.

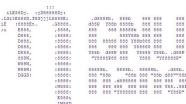
Think of them as Unix versions of Notepad.

Some have an interactive user interface – E.G. gedit

Some work from within the command line – E.G. nano, vim, emacs

Today we are going to work with nano but have a play around with the others when you have a chance. Emacs and vim are notoriously difficult to use for the first time, so look up a cheat sheet.





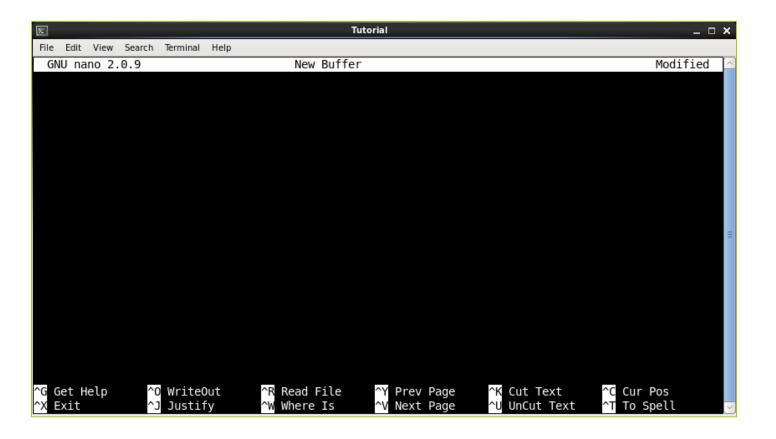




Your First Script

Let's start by opening nano

\$ nano



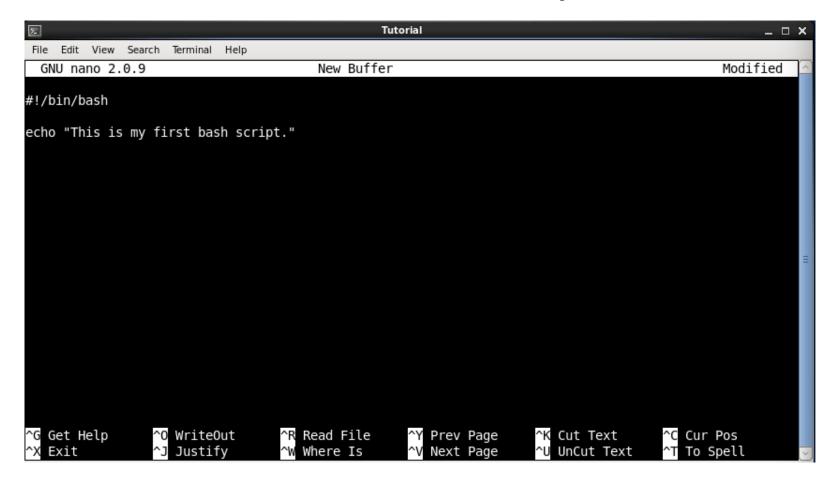
Key Nano Commands

Ctrl + O – This saves the file. You will be asked for a file name.

Type the name and press enter.

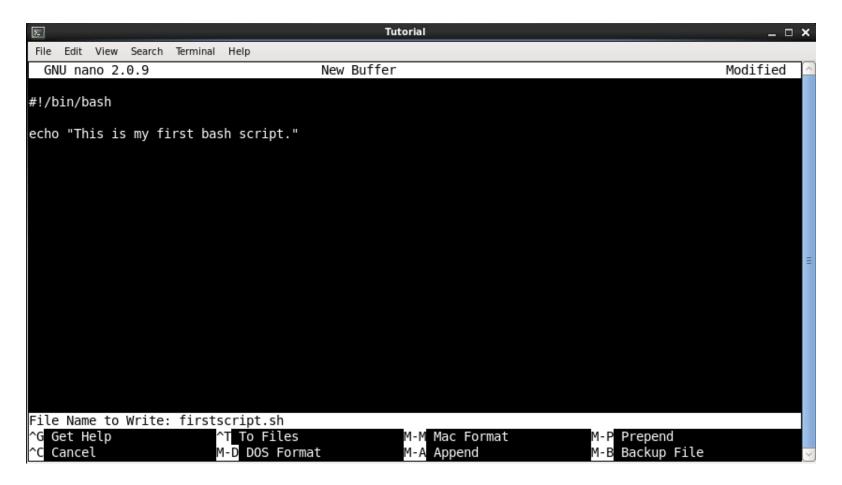
Ctrl + X – This exits nano. If the file is unsaved, you will be asked at this point if you'd like to save it.

Your First Script



#!/bin/bash tells the computer that this script is in the language bash. It always needs to go at the top of any bash script.

Your First Script



Then use Ctrl + O to save and give the file the name firstscript.sh.

Then use Ctrl + X to exit.

Now Run Your Script

Simply Type:

\$ bash firstscript.sh

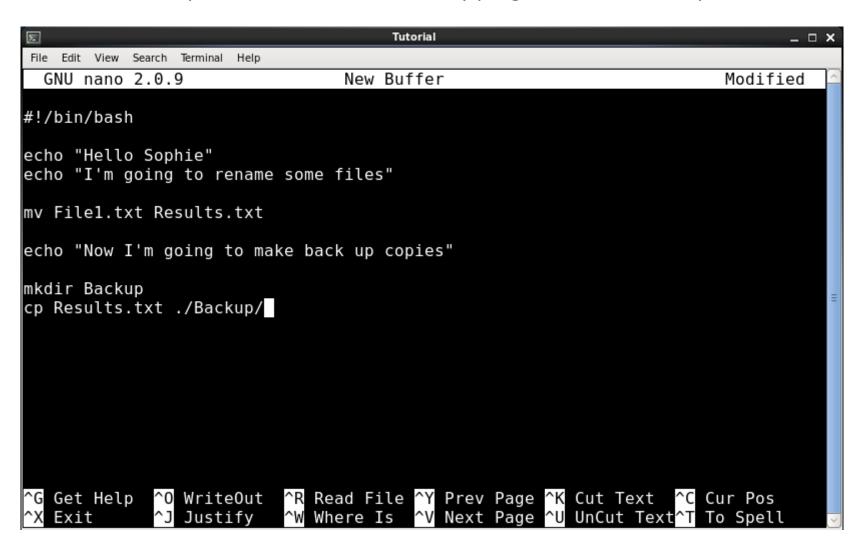
Reopen the same script:

\$ nano firstscript.sh

Change the phrase, save it and run the script again

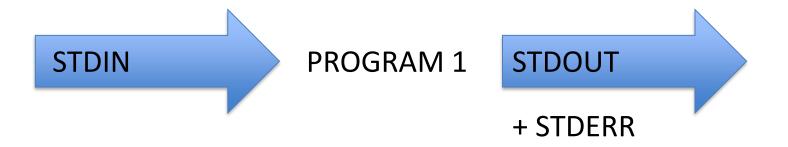
Bash Scripts

Bash scripts can be used to run binary programs like cd, mv, cp etc...

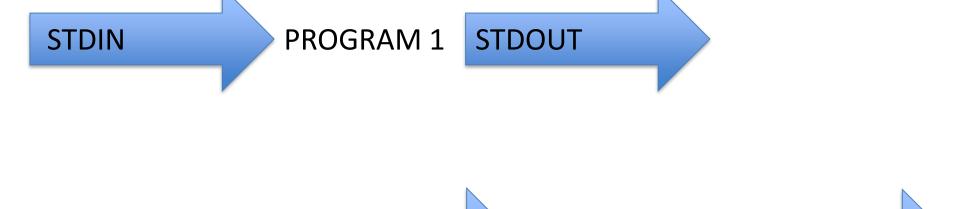


PIPELINES (TIME DEPENDING)

Pipelines



Pipelines



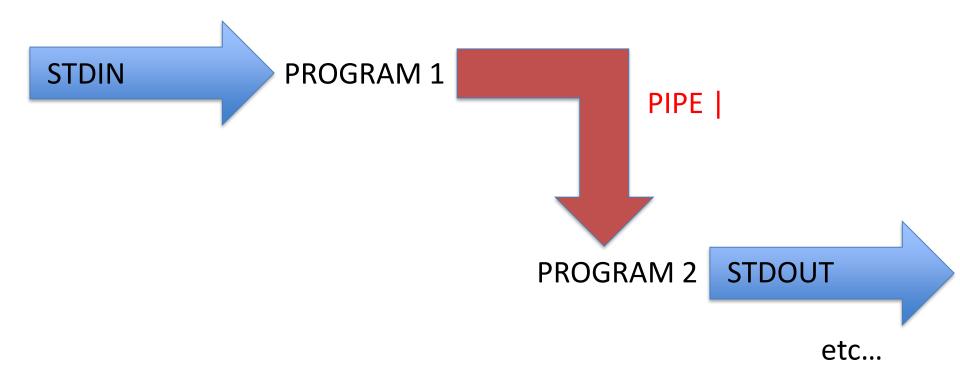
STDIN

PROGRAM 2

etc...

STDOUT

Pipelines



Let's put this to practice: Building Pipelines

Count the number of files and folders in your home directory

Let's build the first part of the pipeline, listing the files:

Number 1

```
$ ls -1 /home/genomics/
```

PIPE this into wc —I to count the number of lines: (i.e. the number of files and folders)

Letter l

```
$ ls -1 /home/genomics/ | wc -1
```

Let's put this to practice: Building Pipelines

How many base pairs in first sequence?

Firstly let's get the top two lines of the sequence file:

Now let's PIPE this into tail to get just the sequence line

```
$ head -n 2 sequence_1.fq | tail -n 1
```

Finally PIPE this into word count of characters to count the base pairs

```
$ head -n 2 sequence_1.fq | tail -n 1 | wc -c
```

Is the first reverse read the same length?

Within the Unix Workshop directory you should find a file called scientists.txt

\$ cd ~/workshop_materials/unix_workshop/

Take a look at the contents

\$ more scientists.txt

First	Last	DOB	
Charles	Darwin	12 February 1809	
Marie	Curie	07 November 1867	
Stephen	Hawking	08 January 1942	
Rosalind	Franklin	25 July 1920	
Isaac	Newton	04 January 1643	
Richard	Dawkins	26 March 1941	

\$ cat scientists.txt | cut -f 1



Now take a look at the original file

\$ more scientists.txt

\$ cat scientists.txt | cut -f 1,3

First	DOB
Charles	12 February 1809
Marie	07 November 1867
Stephen	08 January 1942
Rosalind	25 July 1920
Issac	04 January 1643
Richard	26 March 1941

\$ cat scientists.txt | cut -f 1 | sort

Charles
First
Isaac
Marie
Richard
Rosalind
Stephen

What if you wanted to keep the sorted list?

```
$ cat scientists.txt | cut -f 1 | sort > newfile.txt
```



Looking at the Saccharomyces cerevisiae gff3 file

GFF = general feature format

This is a file which lists all of the genome features, their coordinates, and info about them (genes, tRNAs, exons etc...)

```
##gff-version
##sequence-region I 1 230218
##seauence-reaion
                   II 1 813184
##sequence-region
                   IV 1 1531933
##sequence-region
##sequence-region
                   IX 1 439888
##sequence-region
##sequence-region.
##sequence-region
                  VI 1 270161
##sequence-region
                   VII 1 1090940
##sequence-region
                   VIII 1 562643
##sequence_region
                   X 1 745751
##sequence=region
##sequence-region
##sequence-region
                  XIII 1 924431
##sequence-region
                  XIV 1 784333
##sequence-region
##sequence-region XVI 1 948066
#!genome-build SGD R64-1-1
#!genome-version R64-1-1
#!genome-date 2011-09
#!genome-build-accession GCA_000146045.2
#!genebuild-last-updated 2011-12
                                                                        ID=chromosome:I;Alias=BK006935.2
               chromosome
                               649
                                                               ID=gene:YAL069W;biotype=protein_coding;description=Dubious open reading frame%3B unlikely to encode a functional protein%2C based on available experimenta
 and comparative sequence data [Source:SGD%3BAcc:S000002143];gene_id=YAL069W;logic_name=sgd
                                                                       ID=transcript:YAL069W;Parent=gene:YAL069W;Name=YAL069W;biotype=protein_coding;transcript_id=YAL069W
       ensembl exon
                               649
                                                               Parent=transcript:YAL069W;Name=YAL069W.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL069W.1;rank=1
                                                               ID=CDS:YAL069W;Parent=transcript:YAL069W;protein_id=YAL069W
       ensembl CDS
                                                               ID=gene:YAL068W-A;biotype=protein_coding;description=Dubious open reading frame%3B unlikely to encode a functional protein%2C based on available experimen
       ensembl gene
tal and comparative sequence data%3B
                                    identified by gene-trapping%2C microarray-based expression analysis%2C and genome-wide homology searching [Source:SGD%3BAcc:S000028594];gene_id=YAL068W-A;logic_name=sqd
                                                                       ID=transcript:YAL068W-A;Parent=gene:YAL068W-A;Name=YAL068W-A;biotype=protein_coding;transcript_id=YAL068W-A
                               792
                                                               Parent=transcript:YAL068W-A;Name=YAL068W-A.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL068W-A.1;rank=1
                       538
                       538
                                                               ID=CDS:YAL068W-A:Parent=transcript:YAL068W-A:protein_id=YAL068W-A
                      1807
                                                               ID=gene:YAL068C;Name=PAU8;biotype=protein_coding;description=Protein of unknown function%3B member of the seripauperin multigene family encoded mainly in
subtelomeric regions [Source:SGD%3BAcc:S000002142];gene_id=YAL068C;logic_name=sgd
                                                                       ID=transcript:YAL068C;Parent=gene:YAL068C;Name=PAU8;biotype=protein_coding;transcript_id=YAL068C
       ensembl exon 1807
                                                               Parent=transcript:YAL068C;Name=YAL068C.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL068C.1;rank=1
                                                               ID=CDS:YAL068C;Parent=transcript:YAL068C;protein_id=YAL068C
```

```
##gff-version 3
##sequence-region I 1 230218
##sequence-region
##sequence-region
                  III 1 316620
                  IV 1 1531933
##sequence-region
##sequence-region
                  IX 1 439888
##sequence-region
##sequence-region
                  VI 1 270161
##sequence-region
##sequence-region
##sequence-region VIII 1 562643
##sequence-region X 1 745751
##sequence-region XI 1 666816
##sequence-region
##sequence-region XIII 1 924431
##sequence-region XIV 1 784333
##sequence-region XV 1 1091291
##sequence-region XVI 1 948066
#!aenome-build SGD R64-1-1
#!genome-version R64-1-1
#!genome-date 2011-09
#!genome-build-accession GCA_000146045.2
#!genebuild-last-updated 2011-12
                                      230218 .
                                                                      ID=chromosome:I:Alias=BK006935.2
       ensembligene 335
                                                              ID=gene:YAL069W;biotype=protein_coding;description=Dubious open reading frame%3B unlikely to encode a functional protein%2C based on available experimenta
 and comparative sequence data [Source:SGD%3BAcc:S000002143];gene_id=YAL069W;logic_name=sgd
                            335
                                                                     ID=transcript:YAL069W;Parent=gene:YAL069W;Name=YAL069W;biotype=protein_coding;transcript_id=YAL069W
       ensembl transcript
       ensembl exon 335
                                                              Parent=transcript:YAL069W;Name=YAL069W.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL069W.1;rank=1
       ensembl CDS
                                                              ID=CDS:YAL069W;Parent=transcript:YAL069W;protein_id=YAL069W
       ensembligene 538
                              792
                                                              ID=gene:YAL068W-A;biotype=protein_coding;description=Dubious open reading frame%3B unlikely to encode a functional protein%2C based on available experimen
tal and comparative sequence data%3B identified by gene_trapping%2C microarray-based expression analysis%2C and genome-wide homology searching [Source:SGD%3BAcc:S909028594];gene_id=YAL068W-A;logic_name=sqd
       ensembl transcript
                                                                      ID=transcript:YAL068W-A;Parent=gene:YAL068W-A;Name=YAL068W-A;biotype=protein_coding;transcript_id=YAL068W-A
                                                              Parent=transcript:YAL068W-A;Name=YAL068W-A.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL068W-A.1;rank=1
       ensembliexon 538
       ensembl CDS
                                                              ID=CDS:YAL068W-A;Parent=transcript:YAL068W-A;protein_id=YAL068W-A
       ensembl gene 1807 2169
                                                              ID=gene:YAL068C;Name=PAU8;biotype=protein_coding;description=Protein of unknown function%3B member of the seripauperin multigene family encoded mainly in
subtelomeric regions [Source:SGD%3BAcc:S000002142];gene_id=YAL068C;logic_name=sgd
                               1807
                                                                      ID=transcript:YAL068C;Parent=gene:YAL068C;Name=PAU8;biotype=protein_coding;transcript_id=YAL068C
       ensembl exon 1807
                              2169
                                                              Parent=transcript:YAL068C;Name=YAL068C.1;constitutive=1;ensembl_end_phase=0;ensembl_phase=0;exon_id=YAL068C.1;rank=1
       ensembl CDS 1807
                                                              ID=CDS:YAL068C;Parent=transcript:YAL068C;protein_id=YAL068C
```

Lines that start # are comments – just run information

Column 1 = Chromosome

Column 3 = Feature/Type e.g. gene, chromosome, exon

Column 4 = Start Location

Column 5 = Stop Location

1. In the Unix workshop directory you should find a gff3 file.

\$ cd ~/Unix_Workshop/Challenge5

Saccharomyces_cerevisiae.R64-1-1.85.gff3.gz

- 2. Unzip the file.
- 3. How many feature entries are there?
- 4. List and count all the different types of features
 - 5. Which chromosome is the longest?

Hints!

- Use head to work with 10 lines whilst testing what your pipe does!
 - This is a tab delimited file with a column layout.
 - Google "gff3 format" to find out what each of the columns are.
 - Remember that cat opens an entire file at once.
- There are a number of info lines at the start which begin with a hash. Look into grep with invert matches to skip these.
 - Cut can be used to isolate certain columns. You'll want the field option.
 - The programs sort and uniq may be helpful.
 - Sort must be used before uniq.
 - Uniq has a counting option.
 - Sort uses the key option to sort by a column.

more	gunzip	head	uniq
ср	mv	grep	WC
rm —i	mkdir	cut	
cd	cat	sort	man

- 1. Find the gff3 file.
 - 2. Unzip the file.

```
$ gunzip Saccharomyces_cerevisiae.R64-1-1.85.gff3.gz
```

3. How many feature entries are there?

```
$ cat Saccharomyces_cerevisiae.R64-1-1.85.gff3 | grep -v "^#" | wc -l
28926
```

4. List and count all the different types of features (type on one line)

```
$ cat Saccharomyces_cerevisiae.R64-1-1.85.gff3 | grep -v "^#" | cut -f 3 | sort | uniq -c | sort -n
```

5. Which chromosome is the longest? (type on one line)

```
cat Saccharomyces_cerevisiae.R64-1-1.85.gff3 | grep -v "^#"
cut -f 1,3,4,5 | grep chromosome | sort -n -k 4
```

Chromosome IV 1531933 bp

Any Questions So Far?

