









#### Goals of the course

- To help you <u>perform</u> your research through instruction in the core components of data collection, organization, manipulation, analysis, interpretation and presentation.
- Provide a <u>broad coverage</u> of the core components of modern biological statistics
- Provide you with the computational tools necessary to carry out your work - <u>namely R and affiliated tools</u>
- This is a <u>practical course</u> and we will learn by doing

## Why do we need statistics?

- We almost never know the world perfectly, but still want to draw conclusions or make decisions
- We need to estimate underlying parameters from samples of data
- Sometimes we need to test hypotheses using data
- Other times we need to more succinctly summarize and/or visualize large amounts of data
- There are well known mathematical rules that help us do both
- Statistics can be done by hand, but computers let us do most of the mathematics quickly

## Why do we need statistics?

We want to turn data into conclusions about the world

- experimental design
- point estimates and confidence intervals
- hypothesis testing
- data reduction of highly dimensional data

<u>need</u>: a firm understanding of **probability**, **sampling** and **distributions** 

How do we use statistics?

We need to work together.

## Data, observations and variables

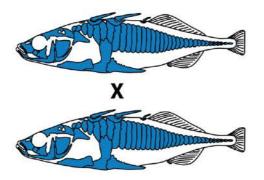
## A biological example to get us started

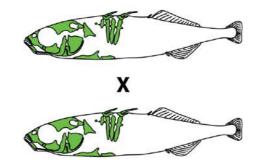
- EXAMPLE Say you perform an experiment on two different strains of stickleback fish, one from an ocean population (RS) and one from a freshwater lake (BP) by making them microbe free. Microbes in the gut are known to interact with the gut epithelium in ways that lead to a proper maturation of the immune system.
- EXPERIMENTAL SETUP You decide to carry out an experiment by treating multiple fish from each strain so that some of them have a conventional microbiota, and some of them are inoculated with only one bacterial species. You then measure the levels of gene expression in the stickleback gut using RNA-seq. Because you have a suspicion that the sex of the fish might be important, you track it too.
- GETTING THE DATA READY TO ANALYZE How should the data set be organized to best analyze it? What are the properties of the variables, and why does that matter?

## A biological example to get us started

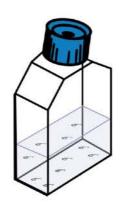
# Oceanic Line (Rabbit Slough)

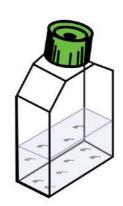
## Freshwater Line (Boot Lake)



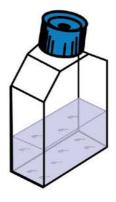


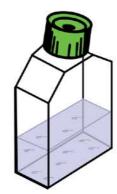
Germ-free





Conventional Microbiota





Term	Definition	Example
Measurement	A single piece of recorded information reflecting a characteristic of interest (e.g. length of a leaf, pH of a water aliquot mass of an individual, number of individuals per quadrat etc)	Protein content of the milk of a single female koala
Observation	A single measured sampling or experimental unit (such as an individual, a quadrat, a site etc)	A small quantity of milk from a single koala
Population	All the possible observations that could be measured and the unit of which wish to draw conclusions about (note a statistical population need not be a viable biological population)	The milk of all female koalas
Sample	The (representative) subset of the population that are observed	A small quantity of milk collected from 15 captive female koalas <sup>a</sup>
Variable	A set of measurements of the same type that comprise the sample. The characteristic that differs (varies) from observation to observation	The protein content of koala milk.

#### Data set rules of thumb (aka Tidy Data)

- Store a copy of data in nonproprietary software and hardware formats, such as plain ASCII text (aka a flat file)
- Leave an uncorrected file when doing analyses
- Use descriptive names for your data files and variables
- Include a header line with descriptive variable names
- Maintain effective metadata about the data
- When you add observations to a database, add rows
- When you add variables to a database, add columns, not rows
- A column of data should contain only one data type

### Repeatable science rules of thumb (aka Tidy Data)

- Use a scripting program like R for analysis, and
- Make your data and analyses freely available and understandable
- Use R Markdown for documentation and dissemination
- Use Git and GitHub to collaborate and distribute your files
- Know the basics of command line tools on your computer





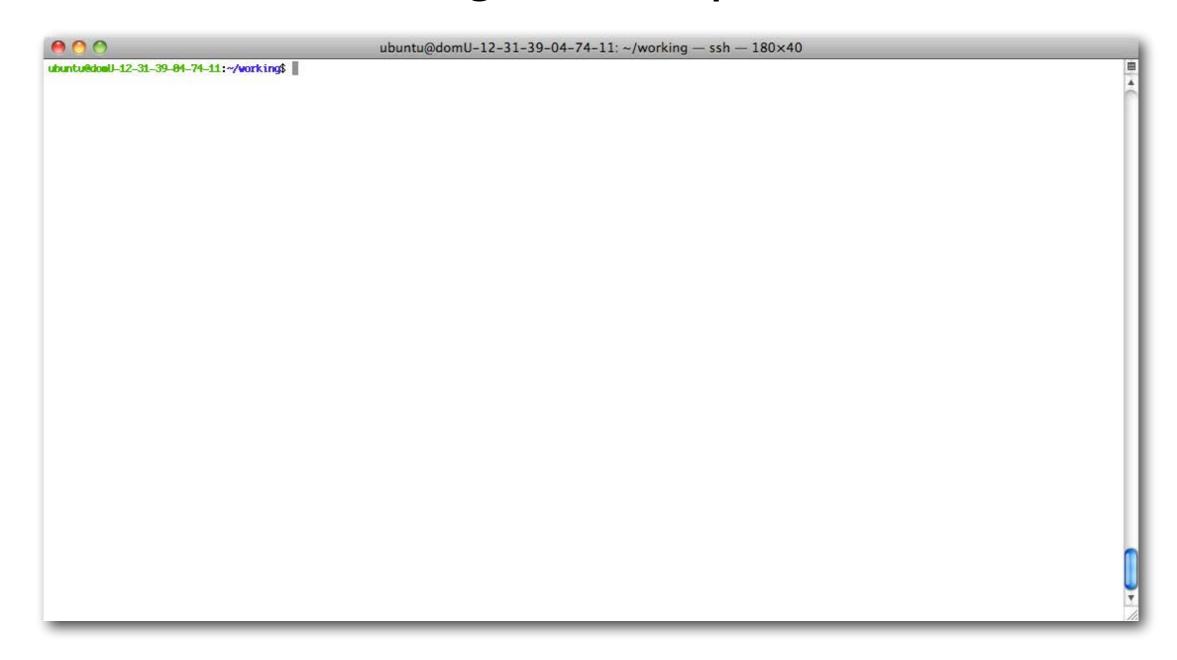






#### Unix and Unix-like environments:

#### Convenient handling and manipulation of data files



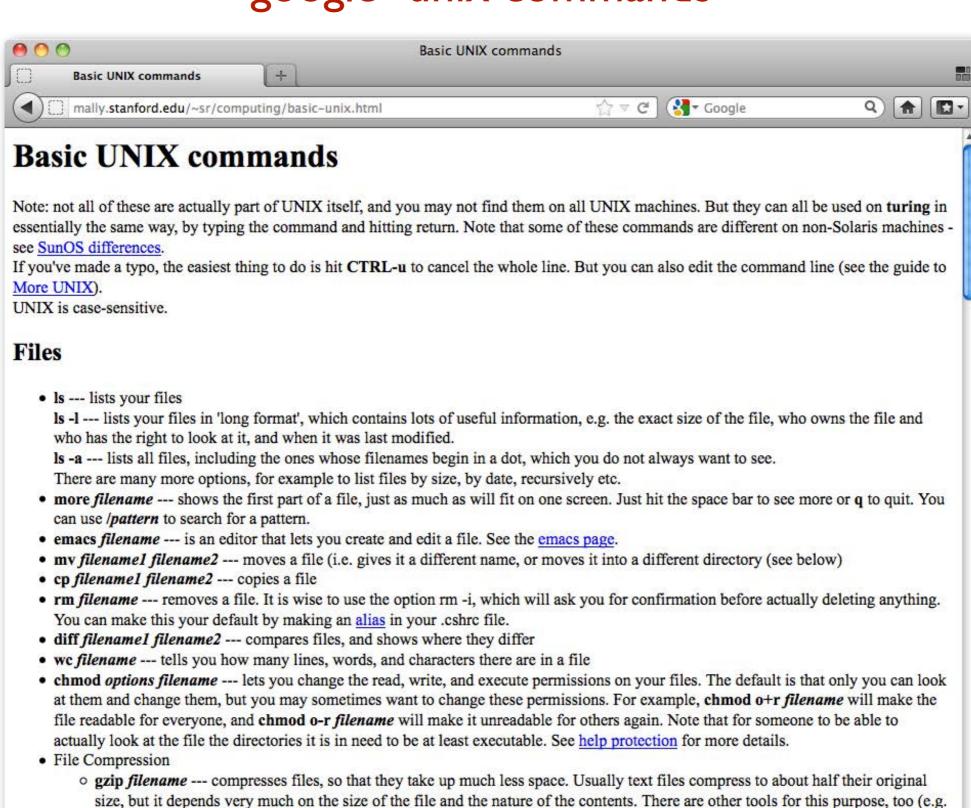
- Apple OS X Macs
- Linux workstations and servers
- Virtual Machines
- Google's Android phones

#### The Terminal Window

```
ubuntu@ip-10-4-230-31: ~/working — ssh — 141×44
ubuntu@ip-10-4-230-31:~/working$ ls -la ~/
total 444
drwxr-xr-x 18 ubuntu ubuntu
                             4096 2012-01-09 22:50 .
drwxr-xr-x 6 root root
                             4096 2011-11-14 17:12 ...
-rw----- 1 ubuntu ubuntu
                             3757 2012-03-12 12:11 .bash_history
-rw-r--r-- 1 ubuntu ubuntu
                             220 2011-05-18 10:00 .bash_logout
                             3581 2011-11-14 17:16 .bashrc
-rw-r--r-- 1 ubuntu ubuntu
                               21 2011-11-14 12:25 bin -> ../../usr/proftpd/bin
lrwxrwxrwx 1 root
                    root
drwxrwxr-x 2 ubuntu ubuntu
                             4096 2011-11-14 17:16 .byobu
drwxrwxr-x 4 ubuntu ubuntu
                             4096 2011-11-14 14:33 .cabal
drwx----- 3 ubuntu ubuntu
                             4096 2011-11-14 11:37 .cache
                               20 2011-11-14 12:23 conf -> ../../usr/nginx/conf
lrwxrwxrwx 1 root root
                              992 2011-11-14 17:12 configure_freenx.sh
-rwxrwxrwx 1 ubuntu ubuntu
drwx----- 3 ubuntu ubuntu
                             4096 2012-01-08 22:52 .emacs.d
                               21 2011-11-14 12:25 etc -> ../../usr/proftpd/etc
lrwxrwxrwx 1 root root
drwxr-xr-x 2 ubuntu ubuntu
                             4096 2011-11-14 12:51 .fontconfig
drwx----- 2 ubuntu ubuntu
                             4096 2011-11-14 14:18 .gconf
drwxr-xr-x 3 root
                    root
                             4096 2011-11-14 16:58 .gem
                             4096 2011-11-14 14:51 .gnupg
drwx----- 2 ubuntu ubuntu
                               20 2011-11-14 12:23 html -> ../../usr/nginx/html
lrwxrwxrwx 1 root
                    root
                               25 2011-11-14 12:25 include -> ../../usr/proftpd/include
lrwxrwxrwx 1 root
                    root
drwxrwxr-x 4 ubuntu ubuntu
                             4096 2011-11-28 17:49 install
                             4096 2011-11-14 12:27 .lein
drwxrwxr-x 3 ubuntu ubuntu
-rw----- 1 ubuntu ubuntu
                               65 2011-11-14 13:07 .lesshst
                               21 2011-11-14 12:25 lib -> ../../usr/proftpd/lib
lrwxrwxrwx 1 root
                    root
                               25 2011-11-14 12:25 libexec -> ../../usr/proftpd/libexec
lrwxrwxrwx 1 root
                    root
lrwxrwxrwx 1 root
                    root
                               20 2011-11-14 12:23 logs -> ../../usr/nginx/logs
drwxrwxr-x 2 ubuntu ubuntu
                             4096 2011-11-14 12:51 .m2
drwxrwxr-x 2 ubuntu ubuntu
                             4096 2011-11-14 14:18 .matplotlib
                             2964 2012-01-09 22:50 .mysql_history
-rw----- 1 ubuntu ubuntu
                             675 2011-11-14 17:16 .profile
-rw-r--r-- 1 ubuntu ubuntu
drwxr-xr-x 2 root
                    root
                             4096 2011-11-14 12:25 sbin
-rw-rw-r-- 1 ubuntu ubuntu
                                0 2011-11-14 11:37 .screenrc
                               23 2011-11-14 12:25 share -> ../../usr/proftpd/share
lrwxrwxrwx 1 root root
                             4096 2011-11-14 11:33 .ssh
drwx----- 2 ubuntu ubuntu
-rw-rw-r-- 1 ubuntu ubuntu 338416 2012-01-09 16:12 stacks-0.998.tar.az
                             4096 2011-11-14 13:44 .subversion
drwxrwxr-x 3 ubuntu ubuntu
                                0 2011-11-14 11:38 .sudo_as_admin_successful
-rw-r--r-- 1 ubuntu ubuntu
                             4096 2012-01-09 04:42 tmp
drwxrwxr-x 2 ubuntu ubuntu
                               21 2011-11-14 12:25 var -> ../../usr/proftpd/var
lrwxrwxrwx 1 root root
-rw----- 1 ubuntu ubuntu
                             7522 2012-01-09 20:35 .viminfo
                             12 2012-01-08 22:49 working -> /mnt/working
lrwxrwxrwx 1 ubuntu ubuntu
-rw----- 1 ubuntu ubuntu
                             196 2011-12-12 12:04 .Xauthority
ubuntu@ip-10-4-230-31:~/working$
```

#### Obtain a cheat sheet

#### google "unix commands"

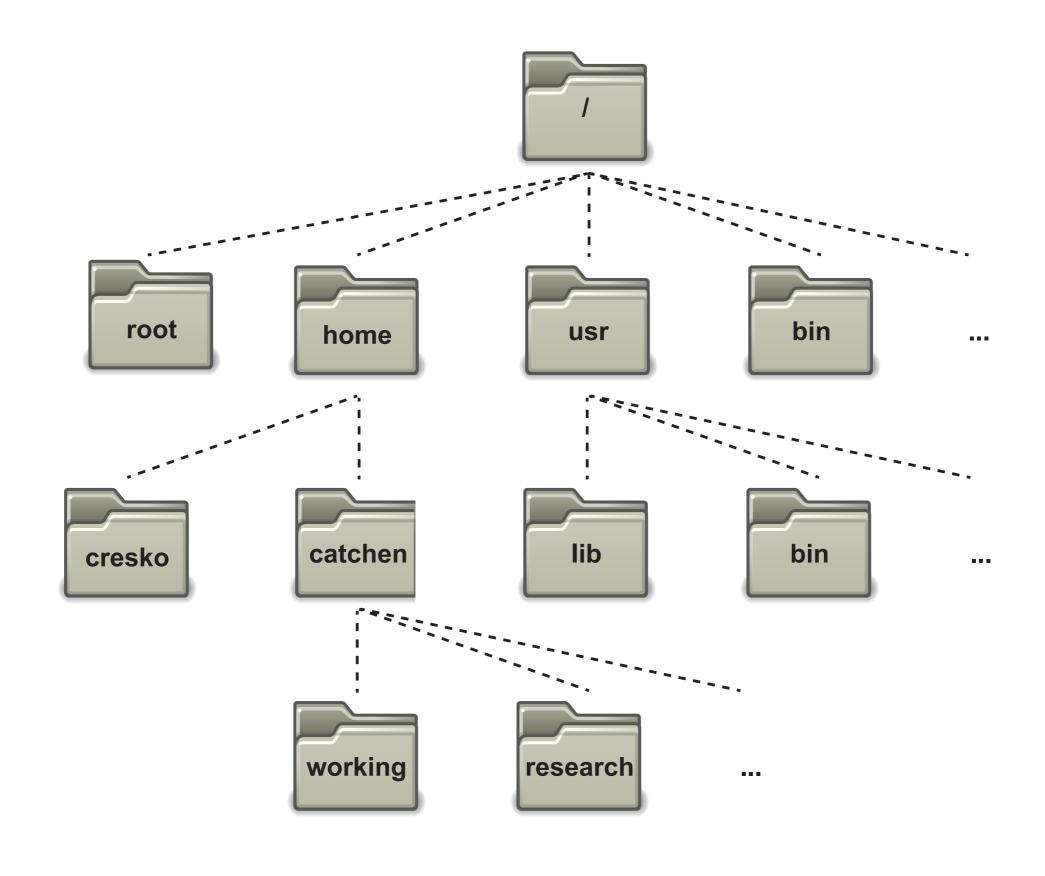


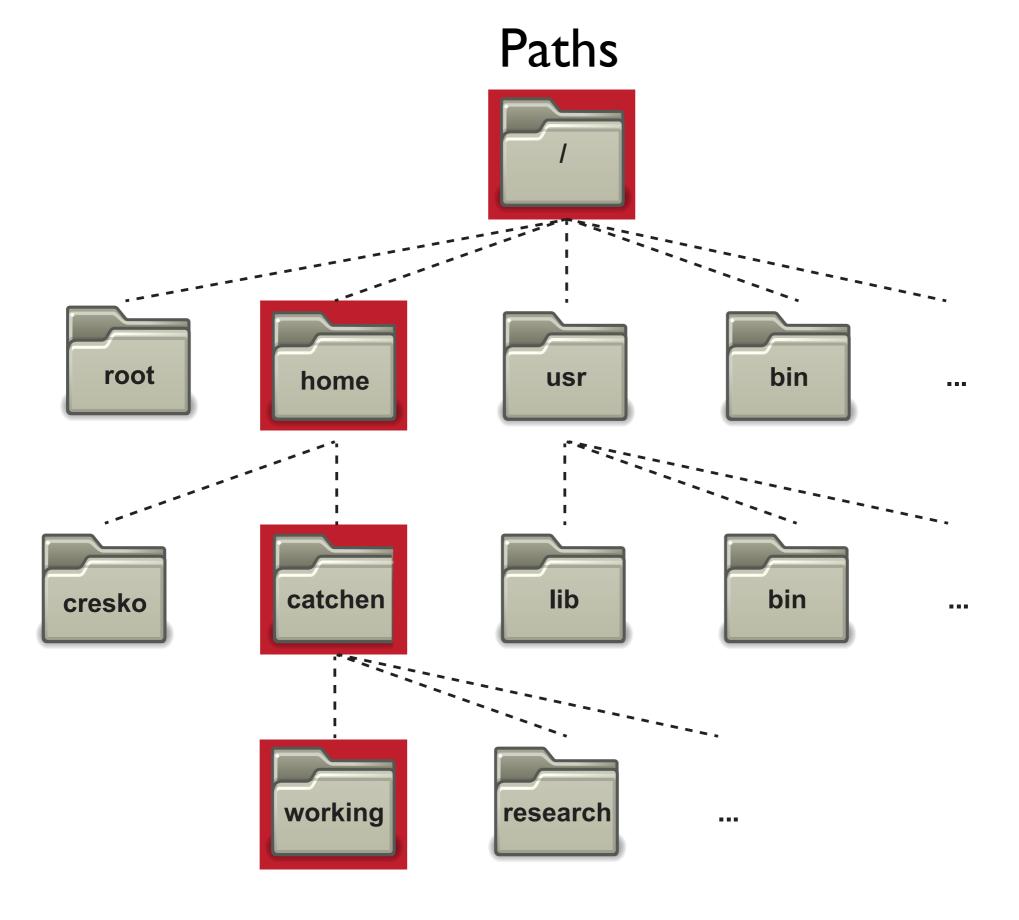
compress), but gzip usually gives the highest compression rate. Gzip produces files with the ending '.gz' appended to the original

filename.

aunzin filanama .... uncompresses files compressed by azin

## In UNIX everything is a file organized in a hierarchy





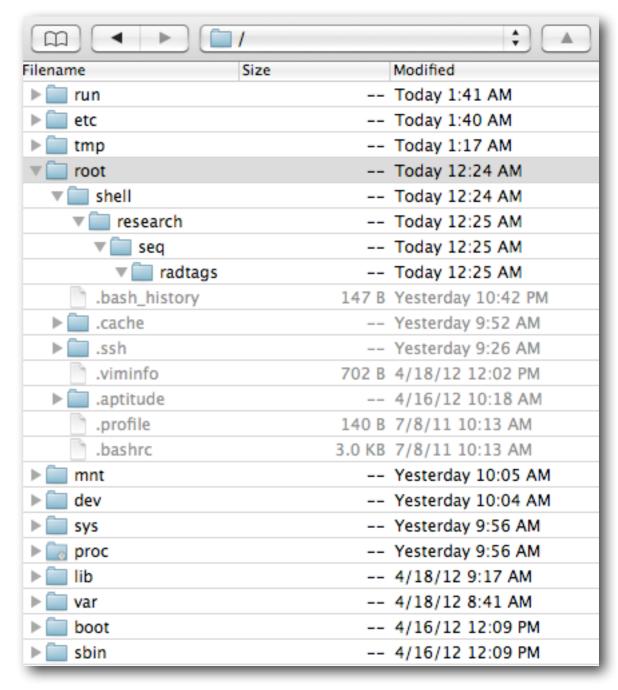
/home/catchen/working

#### Paths, cont

This shell view of the nested directories shell, research, seq, and radtags.....

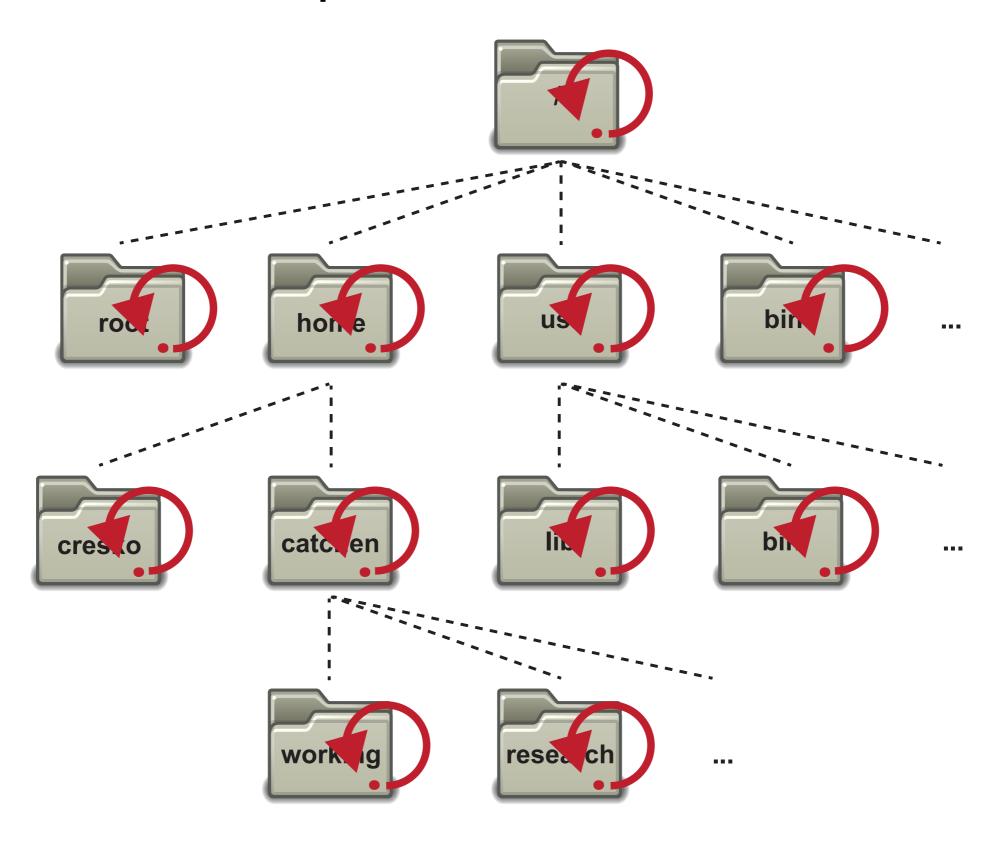
```
root@ubuntu:~# mkdir shell
root@ubuntu:~# cd shell
root@ubuntu:~/shell# mkdir research
root@ubuntu:~/shell#ls
research
root@ubuntu:~/shell# cd research
root@ubuntu:~/shell/research# mkdir sea
root@ubuntu:~/shell/research# ls
root@ubuntu:~/shell/research# cd seq
root@ubuntu:~/shell/research/seq# mkdir radtags
root@ubuntu:~/shell/research/seq# cd radtags/
root@ubuntu:~/shell/research/sea/radtags#ls
root@ubuntu:~/shell/research/sea/radtags# ls -la
total 8
drwxr-xr-x 2 root root 4096 2012-06-25 00:25 .
drwxr-xr-x 3 root root 4096 2012-06-25 00:25 ...
root@ubuntu:~/shell/research/sea/radtags# pwd
/root/shell/research/sea/radtaas
root@ubuntu:~/shell/research/sea/radtaas# ||
```

.... is equivalent to this GUI view of the same directories

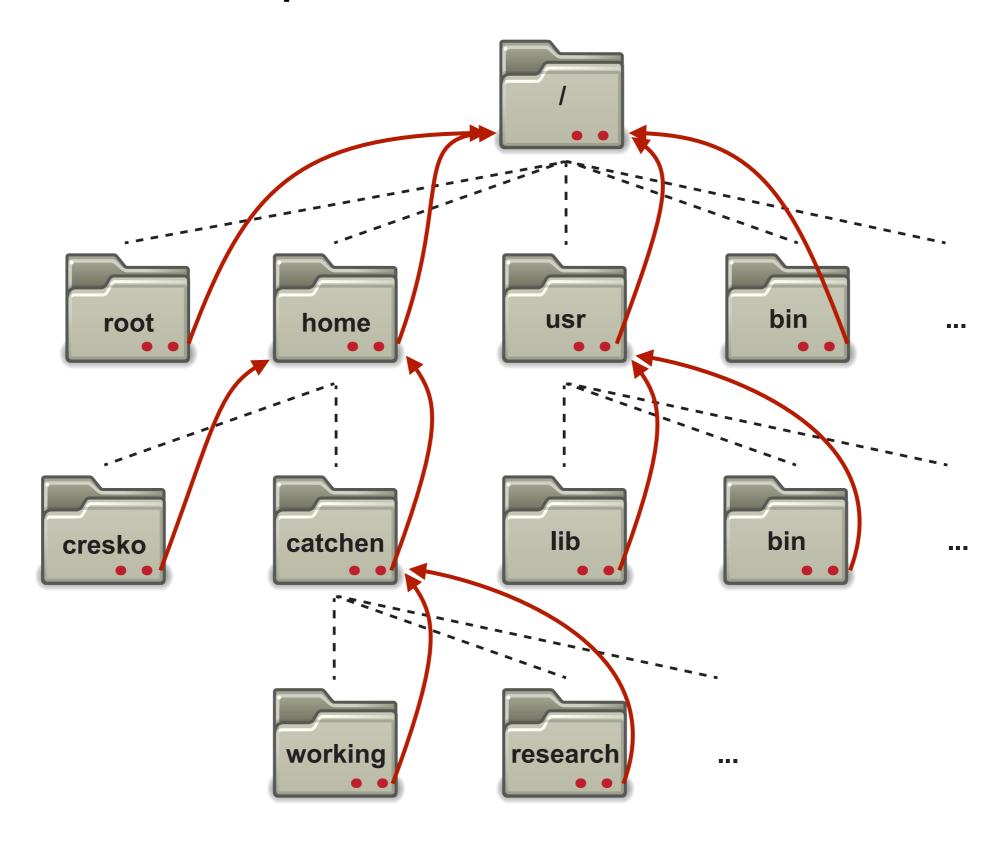


And the radtags directory is uniquely identified by its path: /root/shell/research/seq/radtags

## Special files -- 'dot'



## Special files -- 'dot dot'

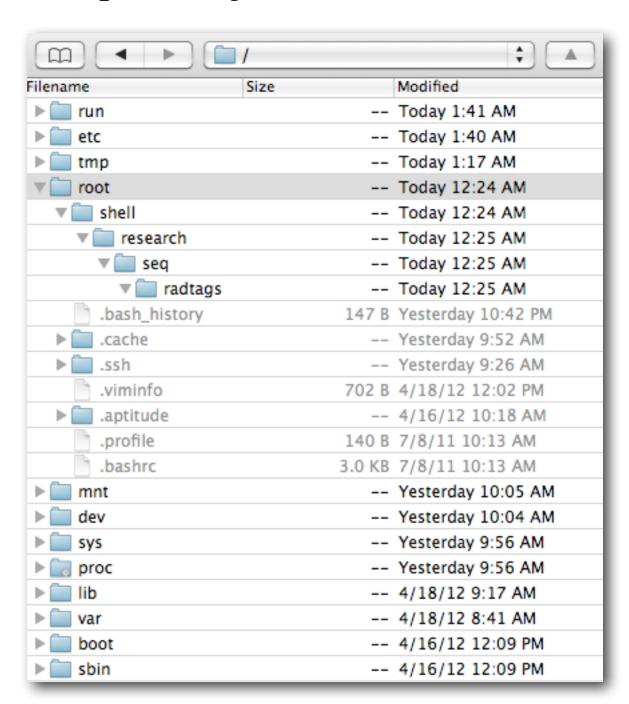


#### Relative and absolute paths

/root/shell/research/seq/radtags

#### Create a series of directories under shell:

```
root@ubuntu:~# mkdir shell
root@ubuntu:~# cd shell
root@ubuntu:~/shell# mkdir research
root@ubuntu:~/shell# ls
research
root@ubuntu:~/shell# cd research
root@ubuntu:~/shell/research# mkdir seq
root@ubuntu:~/shell/research# ls
seq
root@ubuntu:~/shell/research# cd seq
root@ubuntu:~/shell/research/seq# mkdir radtags
root@ubuntu:~/shell/research/seq# cd radtags/
root@ubuntu:~/shell/research/seq/radtags# ls
root@ubuntu:~/shell/research/seq/radtags# is -la
total 8
drwxr-xr-x 2 root root 4096 2012-06-25 00:25 .
drwxr-xr-x 3 root root 4096 2012-06-25 00:25 ...
root@ubuntu:~/shell/research/seq/radtags# pwd
/root/shell/research/seq/radtags
root@ubuntu:~/shell/research/sea/radtags# ||
```



```
% ls . % ls ../../
```

% cd ~/ % cd shell/research % pwd

## Why use the command line?

- The commands work almost identically across platforms
- You can even use them on a large computer cluster like Talapas
- It is incredibly powerful particularly for repeated actions
- It allows you to do thousands of 'clicks' with single commands

### Getting used to the power of the command line

- Get a terminal application up and running on your computer
- Make sure that you can display the path to your current directory
- List the files in the current directory
- Use arguments to your list command to show all files in long form
- Move through your directories by changing your location them
- Make a new directory and put an existing text file in it
- Use the command 'less' to see the contents of the file
- Create a new file in the directory using 'nano'
- Copy the files that you made into a new directory
- Delete the files and then the directory



#### Why use R?



- Good general scripting tool for statistics and mathematics
- Powerful and flexible and free
- Runs on all computer platforms
- New enhancements coming out all the time
- Superb data management & graphics capabilities
- Reproducibility can keep your scripts to see exactly what was done
- Can embed your R analyses in dynamic, polished files using R markdown
- You can write your own functions
- Lots of online help available
- Can use a nice GUI front end such as Rstudio



- # AN INTRODUCTION TO GETTING STARTED WITH THE BASICS IN R
- # Anything that follows '#' symbol (aka hash) is just for humans
- # You can add notes to yourself in your scripts
- # A little later we will run a better way to embed code in R Markdown scripts
- # You can run commands through the terminal, console or scripts
- # Let's start with a simple command that shows the
- # immediate results but the data are not stored.

$$(4+3)*2^2$$

- # A better way to do this is to assign variables
- # Variables are assigned values using the "<-" operator.
- # Variable names must begin with a letter, but other than that, just # About anything goes. Do keep in mind that R is case sensitive.

# These are no good

- # Arithmetic operations can be performed easily on functions # as well as numbers.
- # Try the following, and then your own.
- # Note that the last of these 'log' is a built in function # of R, and therefore the object of the function needs # to be put in parentheses
- # These parentheses will be important, and we'll come back to # them later when we add arguments after the object in the # parentheses

# the outcome of calculations can be assigned to new variables as # well, and the results can be checked using the 'print' command

```
y <- 67
print(y)

x <- 124
z <- (x*y)^2
print(z)</pre>
```

## STRINGS

```
# Variables and operations can be performed on characters as well.
# Note that characters need to be set off by quotation marks to
# differentiate them from numbers.
```

```
# The c stands for 'concatenate'.
# note that we are using the same variable names as we did
# previously, which means that we're overwriting our previous
# assignment.
```

# A good rule of thumb is to use new names for each variable, and make them short but still descriptive

```
x <- "I Love"
print (x)
y <- "Biostatistics"
print (y)
z <- c(x,y)
print (z)</pre>
```

- # The variable z is now what is called a list of character values.
- # Sometimes we would like to treat the characters as if they were # units for subsequent calculations.
- # These are called factors, and we can redefine our character # variables as factors.
- # This might seem a bit strange, but it's important for statistical # analyses where we might want to see the mean or variance for two # different treatments.

# Note that factor levels are reported alphabetically

# In general R thinks in terms of vectors (a list of characters,
# factors or numerical values) and it will benefit any R user to
# try to write programs with that in mind, as it will simplify most
# things.

# Vectors can be assigned directly using the 'c()' function and then entering the exact values.

$$x <-c(2,3,4,2,1,2,4,5,10,8,9)$$
  
print(x)

## BASIC STATISTICS OF DATA

```
# Many functions exist to operate on vectors.
# Combine these with your previous variable to see what happens.
# Also, try to find other functions (e.g. standard deviation).
mean(x)
```

```
mean(x)
median(x)
var(x)
sum(x)
length(x)
sample(x, replace = T)
```

- # Getting Help on any function is very easy just type a question # mark and the name of the function.
- # There are functions for just about anything within R and it is # easy enough to write your own functions if none already exist to # do what you want to do.
- # In general, function calls have a simple structure: a function # name, a set of parentheses and an optional set of parameters to # send to the function.
- # Help pages exist for all functions that, at a minimum, explain # what parameters exist for the function.
- # Help can be accessed a few ways try them :

```
help(mean)
?mean
example(mean)
demo(mean)
#Note, not all functions have demos
help.search("mean")
apropos ("mea")
args(mean)
```

```
# Creating vector of new data by entering it by hand can be a
# drag. However, it is also very easy to use functions such as
# 'seq' and 'sample'.
# Try the examples below Can you figure out what the three
# arguments in the parentheses mean?
# Try varying the arguments to see what happens. Don't go too
# crazy with the last one or your computer might slow way down.
seq 1 < - seq(0.0, 10.0, by = 0.1)
 print(seq 1)
seq 2 \le seq(10.0, 0.0, by = -0.1)
```

print(seq 2)

print(seq square)

seq square <- (seq 2)\*(seq 2)

seq square new <- (seq 2)^2

print(seq square new)

```
# Here is a way to create your own data sets that are random
# samples.
# Again, play around with the arguments in the parentheses to see
# what happens.

x <- rnorm (10000, 0, 10)
y <- sample (1:10000, 10000, replace = T)</pre>
```

```
plot(x,y)
plot(xy)
hist(x)
```

xy < - cbind(x,y)

```
# You've probably figured out that y from the last example is
# drawing numbers with equal probability.
# What if you want to draw from a distribution?
# Again, play around with the arguments in the parentheses to see what happens.
```

```
x <-rnorm(1000, 0, 100)
print (x)
hist(x, xlim = c(-50,50))
hist(x, xlim = c(-500,500))
curve(5000*dnorm(x, 0, 100), xlim = c(-50,50), add=TRUE)
# dnorm() generates the probability density, which can be plotted
# using the curve() function.
```

# Note that is curve is added to the plot using add=TRUE

# VISUALIZING DATA

```
# So far you've been visualizing just the list of output numbers
# Except for the last example where I snuck in a 'hist' function.
# You can also visualize all of the variables that you've created using the
# 'plot' function (as well as a number of more sophisticated plotting
# functions).
# Each of these is called a 'high level' plotting function, which sets the
# stage
# 'Low level' plotting functions will tweak the plots and make them
# beautiful
# What do you think that each of the arguments means for the plot function?
# A cool thing about R is that the options for the arguments make sense.
# Try adjusting an argument and see if it works
```

```
seq_1 <- seq(0.0, 10.0, by = 0.1)
plot (seq_1, xlab="space", ylab ="series 1", type = "p", col = "red")</pre>
```

# You can also combine plots together into a single figure.

```
seq_1 <- seq(0.0, 10.0, by = 0.1)
  print(seq_1)
seq_2 <- seq(10.0, 0.0, by = -0.1)
  print(seq_2)

par(mfrow=c(2,2))
  plot (seq_1, xlab="time", ylab ="p in population 1", type = "p", col = 'red')
  plot (seq_2, xlab="time", ylab ="p in population 2", type = "p", col = 'green')
  plot (seq_square, xlab="time", ylab ="p2 in population 2", type = "p", col = 'blue')
  plot (seq_square_new, xlab="time", ylab ="p in population 1", type = "l", col = 'yellow')</pre>
```

# The first line of the lower script tells R that you are going to create a composite figure # that has two rows and two columns. Can you tell how?

# Now, modify the cody to add two more variables and add one more row of two panels.

```
# As above for the normal distribution, data can be generated by being sampled from
# nearly any distribution and then visualized.
# Below I'm having you use the 'histogram' function. What does it do?
# Binomial Distribution
# function rbinom takes three parameters
  1. The number of observations to generate
  2. The number of trials for each observation
  3. Probability of a success
b \leftarrow rbinom(n=100, size=20, prob=0.5)
hist(b)
#10 successes (out of 20 trials) is the most frequent outcome
# This kind of statement can be run in one line as well, which is sometimes easier.
hist(rbinom(n=100, size=20, prob=0.5))
```

DATA FRAMES = DATA SETS IN R

```
# As you have seen, in R you can generate your own random data set drawn from
# nearly any distribution very easily. Often we will want to use collected data.
# Now, let's make a dummy dataset to get used to dealing with data frames
# Set up three variables (habitat, temp and elevation) as vectors
habitat <- factor(c("mixed", "wet", "wet", "wet", "dry", "dry", "dry", "mixed"))</pre>
temp < c(3.4, 3.4, 8.4, 3, 5.6, 8.1, 8.3, 4.5)
elevation \leftarrow c(0, 9.2, 3.8, 5, 5.6, 4.1, 7.1, 5.3)
# create a data frame where vectors become columns
mydata <- data.frame(habitat, temp, elevation)</pre>
# append row names to the data frame
row.names(mydata) <- c("Reedy Lake", "Pearcadale", "Warneet", "Cranbourne",
"Lysterfield", "Red Hill", "Devilbend", "Olinda")
```

```
# A strength of R is being able to import data from an external source
# Create the same table that you did above in a spreadsheet like Excel.
# Export it to comma separated and tab separated text files for importing into R.
# The first will read in a comma-delimited file, whereas the second is a tab-delimited.
# In both cases the header and row.names arguments indicate that there is a header row
# and row label column. Not that the name of the file by itself will have R look in the
# CWD, whereas a full path can also be used.

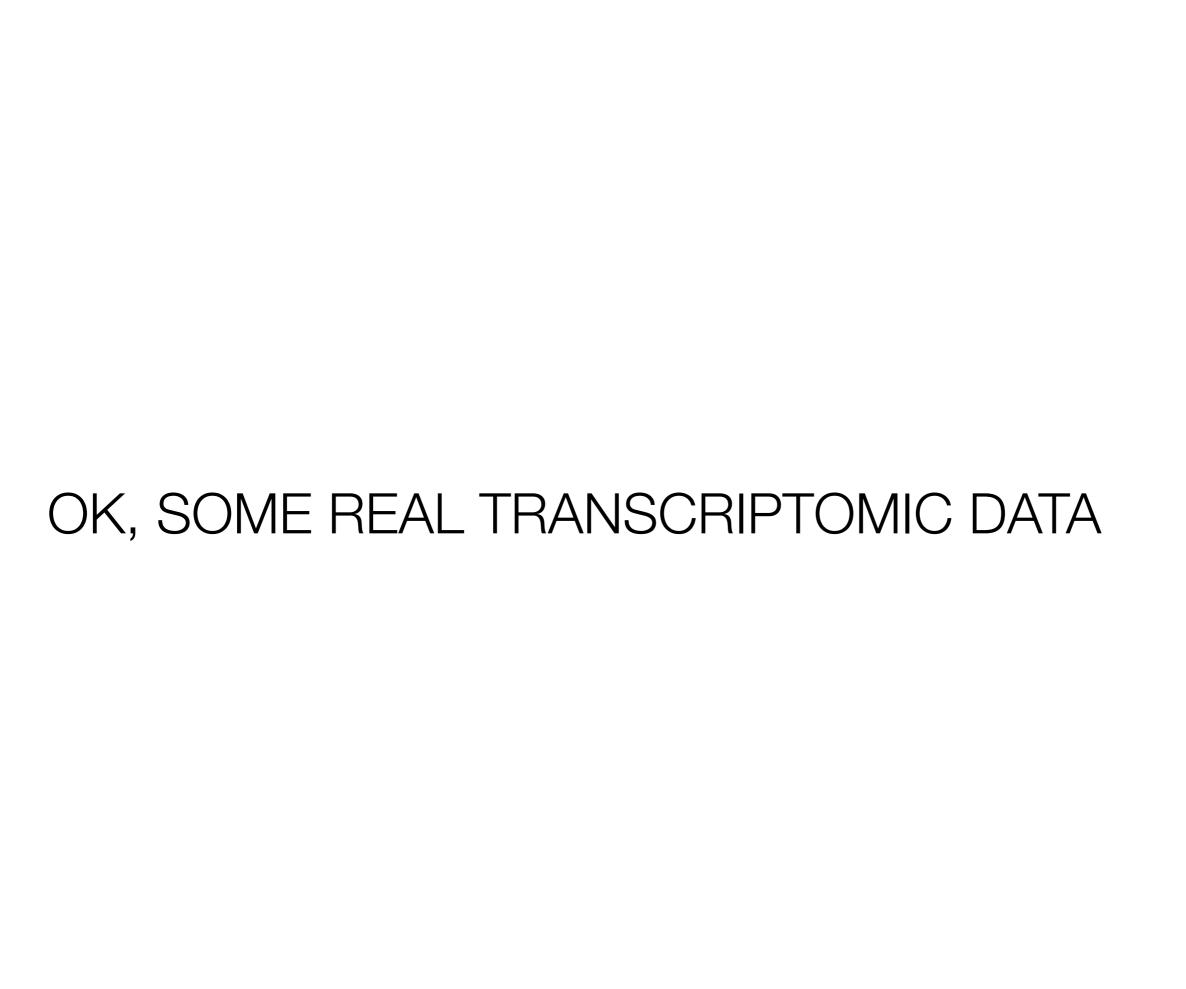
YourFile <- read.table('yourfile.csv', header=T, row.names=1, sep=',')
YourFile <- read.table('yourfile.txt', header=T, row.names=1, sep='\t')</pre>
```

```
# Exporting a data frame as a comma-delimited or tab-delimited file
```

```
write.table(YourFile, "yourfile.csv", quote=F, row.names=T, sep=",")
write.table(YourFile, "yourfile.txt", quote=F, row.names=T, sep="\t")
```

## INDEXING IN DATA FRAMES

```
# Next up - indexing just a subset of the data
# This is a very important idea in R, that you can analyze just a subset of the data.
# This is analyzing only the data in the file you made that has the factor value 'mixed'.
print (YourFile[,2])
print (YourFile$temp)
print (YourFile[2,])
plot (YourFile$temp, YourFile$elevation)
# You can perform operations on particular levels of a factor
# Calculating the mean of the 'mixed' and 'gipps' levels of habitat. Note that the first
# argument is the numerical column vector, and the second is the factor column vector.
# The third is the operation. Reversing the first two does not work (the one below).
tapply(YourFile$temp, YourFile$habitat, mean)
tapply(YourFile$temp, YourFile$habitat, var)
```



### Initial examination of RNA-seq data

- Examine the text file: GacuRNAseq.csv
- How many many rows and columns are there?
- How many different variables are there?
- What are the general types of variables?
- Now let's read the data file into R and analyze it

```
# This exercise will help you get used to reading in and manipulating genomic data files
# First off, remember to set your working directory to find your file correctly
RNAseq_Data <- read.table('GacuRNAseq.csv', header=T, sep=',')</pre>
# OK, now let's look at the data
print (RNAseq Data)
# Whoa. that's a lot of data
# This is a little easier
head (RNAseq Data)
tail (RNAseq Data)
# How many columns are there? How many variables?
# Write down what you think are the characteristics of each
# How do we look at a subset of the data?
# Can you see how we can do the same thing different ways?
print (RNAseq Data[,2])
print (RNAseq Data[1,])
print (RNAseq Data[1,2])
print (RNAseq Data$ENSGACG0000000010)
print (RNAseq Data$ENSGACG000000010>45.0)
```

#### # OK, let's try some summary stats and figures

```
summary1 <- summary(RNAseq_Data $ENSGACG00000000003)
print (summary1)

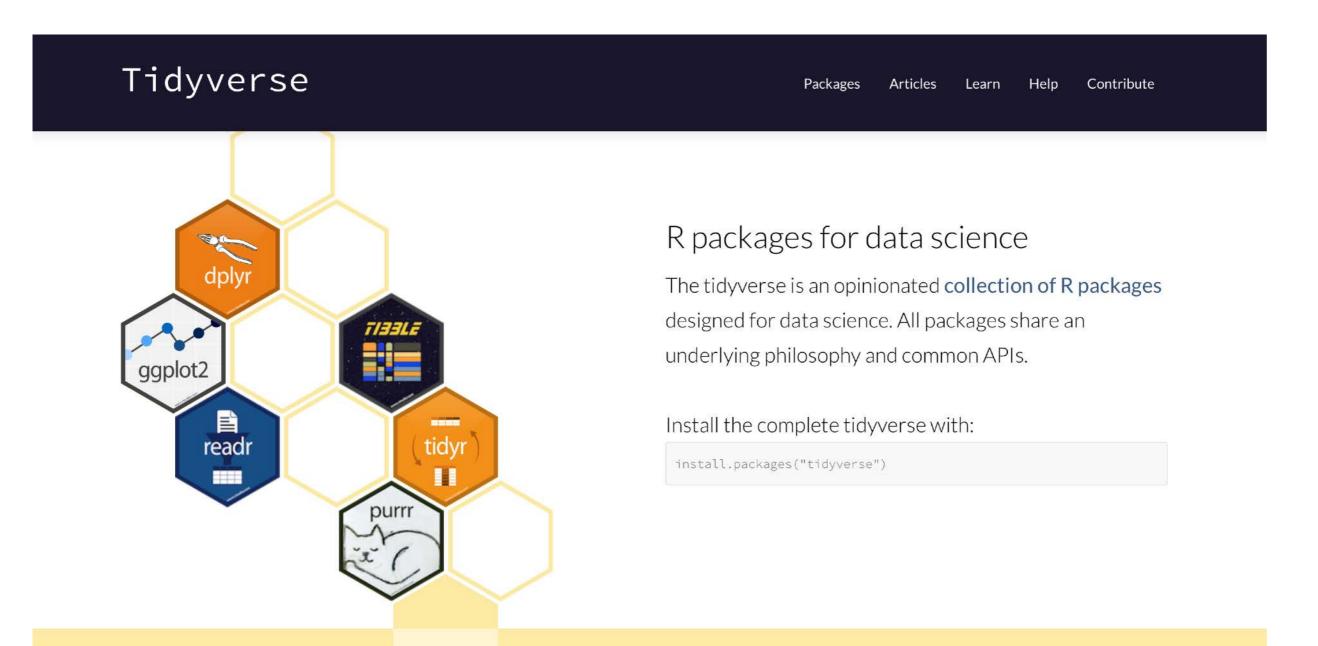
hist(RNAseq_Data $ENSGACG0000000003)

boxplot(RNAseq_Data$ENSGACG00000000003)
boxplot(RNAseq_Data$ENSGACG0000000003~RNAseq_Data$Population)

plot(RNAseq_Data $ENSGACG0000000003, RNAseq_Data$ENSGACG0000000003)

boxplot(RNAseq_Data $ENSGACG0000000003~RNAseq_Data$Treatment, col = "red", ylab = "Expression Level", xlab = "Treatment level", border = "orange", main = "Boxplot of variation in gene expression across microbiota treatments")</pre>
```

# The Tidyverse <a href="https://www.tidyverse.org">https://www.tidyverse.org</a>



```
# Hadley Wickham and others have written R packages to modify data
# These packages do many of the same things as base functions in R
# However, they are specifically designed to do them faster and more easily
# Wickham also wrote the package GGPlot2 for elegant graphics creations
# GG stands for 'Grammar of Graphics'
```

```
install.packages("tidyverse")
library(tidyverse)
```