

# **Genome Analysis Workshop MOLB 7621**

**version 0.2**

**Jay Hesselberth**

March 04, 2015



# Contents

<b>Genome Analysis Workshop</b>	<b>1</b>
Announcements	1
Course Description	1
Contents	1
Syllabus	1
General Information	1
PDF Content	2
Instructor Information	2
Schedule	2
Location	2
Course Description	2
Course Credits	2
Texts and Reading Materials	2
Course Objectives	2
Canvas	3
Assessment	3
Grading Criteria	3
Specific Dates / Material to be Covered	3
Class list	6
Class 1 : Class Introduction	6
Goals	6
Class Overview	6
Linux installations	6
Terminal and text editors	6
Cluster access	7
Running jobs on the cluster	7
Shell and Python Programming	7
First Quiz : Reading	8
Class 2 : The command-line	9
Things to fix from last class	9
Goals	9
Unix Philosophy	9
Navigating In the Terminal	9
Getting Help In The Terminal	10
Getting Help : Exercises	10
Getting Help Outside The Terminal	10
Other Commands In The Terminal	10
Questions	10
Other Commands In The Terminal (Answers)	10
Word Counts (wc)	11

Less (is More)	11
Terminal History	11
Tab-Completion	11
Directory Shortcuts	11
Make and remove directories	12
Moving and copying files	12
echo	13
Variables	13
sudo	13
other commands	13
Scripts	13
Scripts : Commenting	14
Pipes	14
Class 3 : The command-line (part 2)	15
Goals	15
wget	15
cut	15
Sort	15
Sort Questions	15
Sort Example	16
Question:	16
Sort Example (part 2)	16
Sort Exercise	16
uniq	16
Redirection of output	16
Compressed Files	17
Pipes	17
Application 1	17
Application 2	17
In Class Exercises - Class 3	17
Class 4 : grep and awk	19
Goals	19
grep	19
Exercises	19
awk	20
basic principles	20
awk program structure	20
awk BEGIN	20
filtering	20
program structure	21
in-class exercise	21
awk program structure (actions)	21

in-class exercise	21
awk continued	21
awk special variables	22
using awk to count lines with NR	22
using FS and OFS	22
regular expressions	22
advanced awk	22
In Class Exercises - Class 4	22
review	22
Exercises	23
Class 5 : BEDTools	24
Goals	24
Cluster etiquette	24
Example commands on the cluster	24
The queueing system	24
Killing jobs	25
Other cluster-specific commands	25
BEDTools	26
Goals	26
BEDTools Overview	26
BEDTools Utility	26
BEDTools Commands	26
BEDTools Documentation	26
BEDTools intersect	27
Example Files	27
intersect	27
intersect -wa	28
intersect -wo	28
intersect exercise	28
intersect -c	28
intersect -v	28
Intersect Summary	28
Exercises	29
Other Reading	29
Intersect Bam	29
Intersect Strand	29
Closest	29
Genomecov	29
ENCODE	30
ENCODE Project Cell Lines	30
Experiments	30
Common File Formats	30

References	30
How to Access ENCODE Data	30
Chromatin Immunoprecipitation Overview	30
ChIP-seq analysis workflow	34
ChIP-seq data	34
Peak calling	34
Identify sequence motifs in enriched regions	34
Class 6 : Genomic Data Vignette: ChIP-seq	34
Goals	34
ENCODE	35
ENCODE Project Cell Lines	35
Experiments	35
Common File Formats	35
References	35
How to Access ENCODE Data	35
Chromatin Immunoprecipitation Overview	35
ChIP-seq analysis workflow	38
ChIP-seq data	38
Peak calling	38
Identify sequence motifs in enriched regions	38
Class 7 : BEDTools vignette	38
Goals	38
Overview	38
Annotations	39
Signal	39
Slop	39
Make windows	39
Map signal to the annotations	40
Grouping the data	40
Code	40
Plotting	41
Exercises	41
Class 8 : R : Getting Started	42
Setting up web access to tesla	42
Goals	42
Data	42
RStudio	42
Loading data in R	42
DataFrame	43
print	43
libraries	43
R paths	44

Writing and running R scripts in RStudio	44
ggplot2	44
ggplot2 syntax	44
googling with ggplot2	44
answer	44
ggplot2 documentation	45
DataFrame	45
Histograms	45
Exercises	45
Class 9 : R : Manipulation & Plotting	46
Goals	46
dplyr	46
dplyr example	46
Exercises with dplyr	47
Combining with ggplot	47
ggplot histograms	47
Exercises	47
Practice	47
Class 10 : R : DataFrames & Plotting	49
Goals	49
data.frames	49
Extracting From data.frames	49
Named rows in data.frames	49
Boolean Operations on Columns	50
Excercises for data.frames	50
Rmarkdown	50
Github	50
Class 11 : R : Simple statistics and ggplot2	50
Goals	50
Statistics in R	50
Student t-test	51
Fisher's Exact Test	51
Wilcoxon's Test	51
Kolmogorov-Smirnov	51
Plot the distribution!	51
Exercises	51
ggplot manipulations	51
R Model Syntax	51
Let's Normalize	52
Exercises	52
Problem Sets	52
Problem Set 1	52

Reading	52
Problem 1	52
Problem 2	53
Problem 3	54
Problem Set Submission	54
Problem Set 2	55
Overview	55
Problem 1	55
Problem 2	55
Problem 3	55
Problem Set Submission	55
Problem Set 3	56
Overview	56
Problem 1	56
Problem 2	56
Problem Set Submission	56
Problem Set 4	57
Overview	57
Problem 1	57
Problem 2	57
Problem Set Submission	57
Problem Set 5	59
Overview	59
Problem 1	59
Problem 2	59
Problem Set Submission	59
Problem Set : KEYS	60
Problem Set 1 : KEY	60
Problem Set 2 : KEY	64
Problem Set 3 : KEY	67
Problem Set 4 : KEY	69
Problem Set Keys	70
Problem Set Submission	70
Miscellaneous	71
Reference: list of commands	71
cd	71
cp	71
ctrl+c	71
cut	71
echo	71
head	71
grep	72

less	72
ls	72
man	72
mkdir	72
mv	72
rm	73
sort	73
tail	73
tar	73
uniq	73
wget	74
zless	74
Redirection (>> and >)	74
Credits	75
Spring 2015	75
Spring 2014	75
Sample Data files	75
BED format	75
FASTA format	75
FASTQ format	75
ENCODE data	75
Getting Started	76
VPN on Windows computers	76
How to fix internet/VPN issue in VirtualBox	76
Programming Tips & Tricks	76
Overview	76
Learn to type	76
Learn to type funny characters	77
Learn hot keys for window management	77
Learn to use a terminal text editor	77



# Genome Analysis Workshop

**Instructor:** Jay Hesselberth <[jay.hesselberth@gmail.com](mailto:jay.hesselberth@gmail.com)>  
**Website:** <http://hesselberthlab.github.io/workshop>  
**Next offered:** Spring 2015  
**Course Number:** MOLB 7621  
**Crosslistings** STBB 7621  
:  
**Last updated:** March 04, 2015

## Announcements

- **Class size is limited to 20. We anticipate being full, so register early.**
- **Tuition waivers** are available for Postdocs and PRAs. You also need to fill out a [non-degree application](#).

## Course Description

The Genome Analysis Workshop is a hands-on tutorial of skills needed to process large genomics data sets and visualize their results. The class is taught from the standpoint of a biologist with practical goals (e.g. to interpret the results of a sequencing-based experiment and gain biologically meaningful insight).

We focus on working in the Linux environment, with emphasis on command-line tools, Python programming and the R statistical computing environment. We use publicly available next-generation DNA sequencing data from the ENCODE project to illustrate standard approaches for manipulating sequencing data, aligning sequences to a reference genome, generating coverage plots and displaying them in the UCSC Genome Browser. We will cover specific analyses used in ENCODE project including ChIP-seq, DNase I footprinting, mRNA-seq and genome sequencing to identify single nucleotide and structural variants.

## Contents

## Syllabus

## General Information

**Title:** Genome Analysis Workshop  
**Course** MOLB 7621  
**Number:**  
**Cross listings:** STBB 7621  
**Semester:** Spring 2015  
**Homepage:** <http://hesselberthlab.github.io/workshop>  
**Instructor:** Jay Hesselberth  
**Organization** University of Colorado School of Medicine  
:  
**Address:** Department of Biochemistry and Molecular Genetics RC1 South 10104 Aurora CO 80045

**Copyright:** 2014,2015 Jay R. Hesselberth All Rights Reserved.

**Last updated:** March 04, 2015

## PDF Content

The course content is available as a combined PDF: [PDF Download](#)

## Instructor Information

Instructor	Email
Jay Hesselberth	<a href="mailto:jay.hesselberth@gmail.com">jay.hesselberth@gmail.com</a>
Sally Peach	<a href="mailto:sally.peach@gmail.com">sally.peach@gmail.com</a>
Charlotte Siska	<a href="mailto:charlotte.siska@ucdenver.edu">charlotte.siska@ucdenver.edu</a>
Kyle Smith	<a href="mailto:kyle.s.smith@ucdenver.edu">kyle.s.smith@ucdenver.edu</a>

## Schedule

Tue & Thurs, 1:00 - 3:00 PM. TA office hours until 3:30 PM *See specific dates.*

## Location

Health Sciences Library, Computer Teaching Lab 2

## Course Description

**The Genome Analysis Workshop is a hands-on tutorial of skills needed to process large genomics data sets and visualize their results. The class is taught from the standpoint of biologist with practical goals (e.g. to interpret the results of a sequencing-based experiment and gain biologically meaningful insight).**

We focus on working in the Linux environment, with emphasis on Linux command-line tools, Python programming and the R statistical computing environment. We use publicly available next-generation DNA sequencing data from the ENCODE project to illustrate standard approaches for manipulating sequencing data, aligning sequences to a reference genome, generating coverage plots and displaying them in the UCSC Genome Browser. We will cover specific analyses used in ENCODE project including ChIP-seq, DNase I footprinting, mRNA-seq and genome sequencing to identify single nucleotide and structural variants.

## Course Credits

This is a 3 credit course.

## Texts and Reading Materials

1. **Required:** A Quick Guide to Organizing Computational Biology Projects [PubMed ID 19649301](#)
2. **Required:** Command Line Crash Course <http://cli.learncodethehardway.org/book/>
3. **Required:** ggplot2: Elegant Graphics for Data Analysis <http://ggplot2.org/book/>
4. **Required:** Learn Python the Hard Way, <http://learnpythonthehardway.org/book/>

## Course Objectives

- Learn to manipulate large sequencing data sets with Linux command line tools and Python programming.
- Learn to manipulate and visualize data with the R statistical computing environment.

# Genome Analysis Workshop

- Learn workflows for ENCODE experiments including ChIP-seq, DNaseI footprinting, mRNA-seq and variant detection.
- Learn to visualize data in the UCSC Genome Browser

## Canvas

The course has a Canvas page <sup>1</sup> where announcements are made and problem sets are uploaded. You need to login to see Announcements and Problem Sets.

## Assessment

Progress of individual students will be assessed during the daily exercise session, weekly problem sets, as well as a final project.

## Grading Criteria

- 50% participation
- 40% problem sets (10 sets, 4% each)
- 10% final project

## Specific Dates / Material to be Covered

Class number	Date	Topic	Problem Set
Class 1	2015 Jan 27 Tues	Introduction to VM, Linux and the shell	
Class 2	2015 Jan 29 Thurs	Linux / Utilities	<b>PS1 due</b> (2015 Feb 2 5:00 PM)
Class 3	2015 Feb 3 Tues	Linux / Utilities	
Class 4	2015 Feb 5 Thurs	grep and awk	<b>PS2 due</b> (2015 Feb 9 5:00 PM)
Class 5	2015 Feb 10 Tues	BEDTools	
Class 6	2015 Feb 12 Thurs	Analysis vignette: ChIP-seq	<b>PS3 due</b> (2015 Feb 16 5:00 PM)
Class 7	2015 Feb 17 Tues	BEDTools	
Class 8	2015 Feb 19 Thurs	Genome Browser	<b>PS4 due</b> (2015 Feb 23 5:00 PM)
Class 9	2015 Feb 24 Tues	R Data & Plotting	
Class 10	2015 Mar 3 Tues	R Data & Plotting	** No Problem Set **
Class 11	2015 Mar 5 Thurs	R Data & Plotting	

## Genome Analysis Workshop

Class 12	2015 Mar 10 Tues	R Data & Plotting	<b>PS5 due</b> (2015 Mar 9 5:00 PM)
Class 13	2015 Mar 12 Thurs	R Data & Plotting	
Class 14	2015 Mar 24 Tues	R Data & Plotting	<b>PS6 due</b> (2015 Mar 16 5:00 PM)
	** No Class Mar 16-20 **	** Spring Break **	
Class 15	2015 Mar 26 Thurs	Python	
Class 16	2015 Mar 31 Tues	Python	
Class 17	2015 Apr 2 Thurs	Python	
Class 18	2015 Apr 7 Tues	Python	<b>PS7 due</b> (2015 Apr 6 5:00 PM)
Class 19	2015 Apr 9 Thurs	Python	
Class 20	2015 Apr 14 Tues	Python	<b>PS8 due</b> (2015 Apr 13 5:00 PM)
Class 21	2015 Apr 16 Thurs	mRNA-seq	
Class 22	2015 Apr 21 Tues	mRNA-seq	<b>PS9 due</b> (2015 Apr 20 5:00 PM)
Class 23	2015 Apr 23 Thurs	Exome analysis	
Class 24	2015 Apr 28 Tues	Exome analysis	<b>PS10 due</b> (2015 Apr 27 5:00 PM)
Class 25	2015 Apr 30 Thurs	Final Projects	
Class 26	c26-date	Final Projects	



## Class list

### Class 1 : Class Introduction

**Class date:** 2015 Jan 27 Tues

**Last updated:** March 04, 2015

## Goals

1. Class overview
2. Get the VM running
3. Overall goals for the Classes

## Class Overview

Each class is 2 hours. We intend to spend the first 60 min going through exercises that demonstrate how specific tools are useful in bioinformatics. During the remaining hour, we expect you to work through exercises, asking for help when you get stuck.

We will record the first 60 minutes using Panopto Screen Capture, and these recordings will be available in Canvas. We have found that simply watching someone work in a terminal (move around, open up text editors, write and execute simple programs) can be a very effective way to get started with programming. Hopefully these movies will serve the same purpose.

Each week, we will have 1 take home quiz, due the following Tuesday at 5 PM.

## Linux installations

The PCs in the library have Virtual Box installed with a minimal Linux installation. If you have your own PC laptop, you can install Virtual Box and any standard Linux distribution (Ubuntu or Mint). If you have a Mac laptop, you can do the same, or just use the native terminal.

In any case, we will create logins on our compute cluster (amc-tesla) and all your work will be done through that.

## Terminal and text editors

When you open a Terminal, you also launch a shell process, typically a bash process. At the prompt, you can type things that bash understands, and it will do them. The shell has its own language, which you will learn over the course of the class. It also runs executable files that it can find on the PATH (i.e. the set of directories that contain executables).

You can find what is on your PATH by typing:

```
$ echo $PATH
```

The PATH is one of several environment variables that are created when you login. You can see all of these with:

```
$ env
```

One important program in the PATH is *vim*. You will use this program to keep notes and write small programs.

You can run *vim* from the terminal prompt:

```
$ vim filename.txt
```

You should notice that the prompt will disappear and you will be in a *vim* session.

Now press the *i* key to enter *insert* mode, and start typing. Press *ESC* to exit *insert* mode.

To quit a *vim* session, you need to:

1. enter *command mode* with the colon key
2. write the file
3. quit the program

This can be accomplished by typing:

```
:wq <enter>
```

Practice using *vim* with this tutorial <sup>2</sup>.

## Cluster access

We have set up accounts for the class on our departmental cluster. We will set up your accounts at the end of class and reset your passwords:

```
# the -X flag starts an X11 connection
$ ssh -X username@amc-tesla.ucdenver.pvt

...
# once you are logged in, test your X11 connection with
$ xeyes
```

## Running jobs on the cluster

First you will grab a single CPU from the queueing system so that you can work without affecting the head node. We use `qlogin` for this:

```
jhessel@amc-tesla ~
$ qlogin

Job <492536> is submitted to queue <interactive>.
<<ssh X11 forwarding job>>
<<Waiting for dispatch ...>>
<<Starting on compute00>>

jhessel@compute00 ~
$
```

## Note

The host in the prompt changed from `amc-tesla` to `compute00`.

You can now execute long-running processes without worry of affecting the cluster. Type `exit` to return back to your head node login.

## Shell and Python Programming

It is important that you learn a few new computer languages. Others have developed very good guides to teach you these languages, and we are going to use those in the class. We expect you to begin taking these classes immediately.

You will spend a lot of time going through these online classes, both in scheduled class time, and outside of class time. Instead of focusing on teaching you these languages, we will focus on helping you get through all of the frustrating problems that come up when you're learning the languages.

We will spend the first ~2 weeks learning shell <sup>3</sup> and all the things you have access to within the shell.

2

OpenVim <http://www.openvim.com/>

3

The Command Line Crash Course <http://cli.learncodethehardway.org/book/>

After learning the shell, we will begin learning R and several packages within R.

Finally, we will begin learning Python <sup>4</sup>. The Python language allows you to do more sophisticated things that would be possible in shell or R, but would be considerably more clunky.

### ***First Quiz : Reading***

Computational biology projects inevitably accrue a lot of files. For the first quiz, you'll need to read a paper <sup>5</sup> and be able to put a set of files in the correct places. We require that you use this scheme for all of your projects in and out of the class.

---

<sup>4</sup>

Learn Python the Hard Way <http://learnpythonthehardway.org/book/>

<sup>5</sup> A Quick Guide to Organizing Computational Biology Projects (2009) PLoS Comput. Biol. William S. Noble <http://dx.plos.org/10.1371/journal.pcbi.1000424>

## Class 2 : The command-line

**Class date:** 2015 Jan 29 Thurs  
**Last updated:** March 04, 2015

### Things to fix from last class

Do this on tesla:

```
$ cp ~jhessel/.vimrc ~
```

This will give line numbers (and a bunch of other stuff) when you run vim.

### Goals

1. The bash shell
2. continue learning to navigate within the terminal
3. understand the linux philosophy (small tools that do one thing well)
4. understand how to apply some common linux utilities to files
5. vim to edit files

### Unix Philosophy

The Unix philosophy<sup>6</sup> Works well for bioinformatics:

- Make each program do one thing well.
- Make every program a filter.
- Choose portability over efficiency.
- Store data in flat text files.
- Small is beautiful.
- Use software leverage to your advantage.
- Use shell scripts to increase leverage and portability.

### Navigating In the Terminal

When you start the terminal, you will be in your home directory.

In Linux home is represented as ~ and also as \$HOME.

We will often show commands preceded with a '\$' as you see in your terminal.

Try this in the terminal:

```
$ pwd
```

pwd stands for "print working directory"

Change to another directory:

```
$ cd /tmp/
```

See what's in that directory:

```
$ ls
```

Show more information:

```
$ ls -lh
```

The `-lh` part are flags for the `ls` command.

These can also be separated like:

```
$ ls -l -h
```

### Getting Help In The Terminal

How can you find out the arguments that `ls` accepts (or expects):

```
$ man ls
```

and use spacebar to go through the pages. `man` is short for "manual" and can be used on most of the commands that we will learn.

In other linux software, it is common to get help by using:

```
$ <program> -h
```

or:

```
$ <program> --help
```

Which of these works for `ls`?

### Note

If you see an error message, read it carefully. It may seem cryptic, but it is designed to inform you what went wrong.

### Getting Help : Exercises

- use `man` to find out how to list files so that the most recently modified files are listed last.  
(This is common when you're working on something and only care about the most recently modified files)

- use google to find the same thing. how else can you sort the output of `ls`?

### Getting Help Outside The Terminal

Use `google`. Useful sites include:

- [stackexchange.com](http://stackexchange.com)
- [biostars.org](http://biostars.org)
- [seqanswers.com](http://seqanswers.com)

In many cases, if you receive an error, you can copy-paste it into google and find some info.

### Other Commands In The Terminal

Use the `man` command to determine what `head` does.

Use `head` on the file `/vol1/opt/data/lamina.bed`

Use `tail` to see the end of the file.

### Questions

- By default, `head` and `tail` show 10 lines. How can you see 13 lines?

### Other Commands In The Terminal (Answers)

```
$ man head  
$ head /vol1/opt/data/lamina.bed  
$ tail /vol1/opt/data/lamina.bed  
$ head -n 13 /vol1/opt/data/lamina.bed
```

## Word Counts (wc)

Exercise:

- use **wc** to determine how many **lines** are in /vol1/opt/data/lamina.bed
- use **wc** to determine how many **words** are in /vol1/opt/data/lamina.bed

## Less (is More)

To view a large file, use less:

```
less /vol1/opt/data/lamina.bed
```

You can forward-search in the file using "/"

You can backward-search in the file using "?"

You can see info about the file (including number of lines) using "ctrl+g"

You can exit **less** using "q"

## Terminal History

Press the up arrow in the terminal.

Up and down arrows will allow you to scroll through your previous commands.

This is useful when running similar commands or when remembering what you have done previously.

You can type the start of a command and then up-arrow and it will cycle through commands that start with that prefix.

## Tab-Completion

The bash shell has several built-in utilities for expediting typing.

Type the following where [TAB] means the Tab key on the keyboard:

```
$ cd /opt/bio-w[TAB]
```

Then hit tab. And:

```
$ ls /opt/bio-w[TAB]
```

This will work for any file path and for any programs:

```
$ hea[TAB]
```

What happens if you do:

```
$ he[TAB][TAB]
```

or:

```
$ heaaa[TAB][TAB]
```

## Directory Shortcuts

We have already used the `cd` command to change directories. And we have used the `~` shortcut for home.

```
$ cd ~  
$ ls ~
```

We can also move to or see what's in the parent directory with:

```
$ ls ..  
$ cd ..
```

Or 3 directories up with:

```
$ ls ../../..  
$ cd ../../..
```

To explicitly see the current directory:

```
$ ls ./
```

We can go 2 directories up with:

```
$ cd ../../
```

Here, we can remember that "." is the current directory and .. is one directory up. What does this do:

```
$ ls ./*
```

you can go to the last directory with:

```
$ cd -
```

and switch back and forth by using that repeatedly.

### Make and remove directories

```
$ mkdir ~/tmp # OK  
$ mkdir ~/tmp/asdf/asdf # ERROR  
$ mkdir -p ~/tmp/asdf/asdf # OK
```

What does -p do?

Remove directories:

```
$ rm ~/tmp/asdf # ERROR  
$ rm -r ~/tmp/asdf/asdf # OK
```

What does the -r flag do?

### Warning

Be careful with *rm -r* and *rm -rf*. You can accidentally remove entire directories that you didn't intend to.

### Moving and copying files

*mv [source] [dest]:*

```
$ touch /tmp/asdf  
$ mv /tmp/asdf ~  
$ ls -lhtr ~/
```

In-class excercise:

1. make a directory called /tmp/moveable
2. move that directory to ~
3. copy that directory to /tmp/subdir/

## echo

echo means "print":

```
$ echo "hello world"
```

and you can use it to see **bash** variables:

```
$ echo $HOME
```

```
$ echo $HISTFILE
```

## Variables

We will start covering programming in the next classes, but variables are a key component of programming.

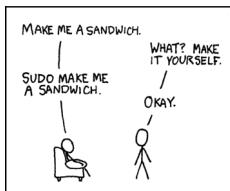
You can do:

```
# define a variable called "important"  
$ important=/vol1/opt/data/lamina.bed
```

```
# "dereference" (refer to) the variable with a dollar-sign  
$ ls -lh $important
```

## sudo

sudo lets you run commands as root (the super-user). You won't be able to do this on tesla, but you should be able to run sudo on the virtual machine.



```
$ apt-get install cowsay  
$ sudo apt-get install cowsay
```

## other commands

excercise:

use man to determine the function of:

- wget
- uniq

How many records are present for each chromosome in /vol1/opt/data/lamina.bed (assume it is sorted by chromosome)?

## Scripts

A script is simply a series of commands that you save in a file. You will need to write scripts to complete the homework.

Put this text:

```
$ ls /vol1/opt/
```

Into the file `run.sh` by opening vim pasting that text then saving the file.

You can then run it as:

```
$ bash run.sh
```

And you should see the same output as if you ran `ls /vol1/opt` directly.

Scripts will be more useful when you have a series of commands you want to run in series.

For example, a pipeline where you:

1. run quality control on some ChIP-seq reads
2. align reads to a reference genome
3. find peaks (binding sites)
4. annotate the binding sites.

a script will provide a record of what you have done.

### **Scripts : Commenting**

For the homework, it is helpful to us if you comment your scripts.

Comments are not read by the shell, but they tell us (and you) what you were trying to do. You can comment your code using the "#" symbol.

```
# list all files in the /tmp/ directory ordered so that most recently
# changed appear last
$ ls -lhtr /tmp/
```

### **Pipes**

Since Linux is made of small utilities, we often want to chain them together. We will cover this in detail next class, but the idea is that each program takes data, modifies it, and sends it to the next.

We can see lines 5-10 of a file with:

```
$ head /vol1/opt/data/lamina.bed | tail -n 5
```

## Class 3 : The command-line (part 2)

**Class date:** 2015 Feb 3 Tues  
**Last updated:** March 04, 2015

### Goals

1. learn additional linux utilities (cut, sort, uniq, less, wget)
2. understand redirects (>, <) and how to combine tools with pipes (|)

### wget

fetch a file from the web with wget:

```
# N.B.: All common data will be on tesla in /vol1/opt/data

$ cd # go $HOME
$ wget http://hesselberthlab.github.io/workshop/_downloads/states.tab
$ wget http://hesselberthlab.github.io/workshop/_downloads/lamina.bed
$ wget http://hesselberthlab.github.io/workshop/_downloads/t_R1.fastq.gz
```

### cut

The cut command allows you to extract certain columns of a file:

```
# cut columns 1-4
$ cut -f 1,2,3,4

# cut columns 1-4 and 7-10
$ cut -f 1-4,7-10 /vol1/opt/data/states.tab

# cut first column 1
$ cut -f 1 /vol1/opt/data/lamina.bed

# output all columns after the 1st
$ cut -f 2- /vol1/opt/data/lamina.bed
```

### Sort

You will often want to sort your data.

Have a look at

```
$ man sort
```

The main flag is -k to indicate which column to sort on.

You will also sometimes use -u to get unique entries.

### Sort Questions

How do you:

1. sort by a particular column? (-k 4)
2. sort as a number (-k4n)
3. sort as a general number (1e-3 < 0.05) (-k4g)
4. change the default delimiter (-t ".")
5. sort by 2 columns (-k1,1 -k2,2n)
6. sort in reverse as a number (-k1rn)

## 7. get unique entries (-u)

If you know all these, you'll know 99% of what you'll use sort for.

### Sort Example

BED files have columns *chrom* [tab] *start* [tab] *end* [tab] ...

Sort by chrom, then by start (a lot of tools will require this)

```
$ sort -k1,1 -k2,2n /vol1/opt/data/lamina.bed > /tmp/sorted.bed
```

This tells it to sort the chromosome [column 1] as a character and the start [column 2] as a number.

### Question:

What happens if you omit the *n* ?

### Sort Example (part 2)

What if we want to sort by Income **descending** in the 3rd column?

```
$ sort -t$'\t' -k3,3rg /vol1/opt/data/states.tab > /tmp/sorted.out  
$ head /tmp/sorted.out
```

### Sort Exercise

Print out the 10 states (1st column, contains spaces) with the highest income (3rd column) from states.tab using sort and piping to cut.

Or, use cut and pipe to sort to do the same.

### uniq

The uniq command allows you to get and count unique entries

```
# remove duplicate lines  
$ cut -f 1 /vol1/opt/data/lamina.bed | uniq  
  
# show duplicate lines  
$ cut -f 1 /vol1/opt/data/lamina.bed | uniq -d  
  
# count unique entries:  
$ cut -f 1 /vol1/opt/data/lamina.bed | uniq -c
```

### Important

uniq assumes that file is sorted by the column of interest.

Use sort to sort the data before uniq-ing it.

### Redirection of output

To send the output of a command (or a file) to another file, use the > operator:

```
$ cut -f 1 /vol1/opt/data/lamina.bed | uniq -c > output.txt  
$ head output.txt
```

To append the output of a command (or a file) to another file, use the >> operator:

```
$ echo "last line" >> output.txt  
$ tail output.txt
```

## Compressed Files

The most common way to uncompress single files is gunzip:

```
$ gunzip /vol1/opt/data/t_R1.fastq.gz
```

And re-zip the file with gzip:

```
$ gzip /vol1/opt/data/t_R1.fastq
```

But if we just want to stream the uncompressed data without changing the file

```
$ zless /vol1/opt/data/t_R1.fastq.gz
```

## Pipes

We probably want to do something with the file as we uncompress it

```
$ zless /vol1/opt/data/t_R1.fastq.gz | head
```

We already know the head command prints the first -n lines.

Try piping the output to some other commands (tail|echo|cowsay).

## Application 1

Use pipes () chained together to look see which transcription factor binding sites are the most common in a set of putative sites from ENCODE.

- data file available from http (wget)
- compressed BED format (zless)
- TF name in 4th column (cut)
- count frequency (uniq -c) after sorting (sort)
- sort resulting frequencies so most common are first (sort -rn)
- show top 10 (head)

## Application 2

Note that we are using the variable FILE for the long file name

```
# BED format file of transcription factor binding sites
FILE=http://bit.ly/tfbs-x

wget --quiet -O - $FILE \
  | zless \
  | head -n 7000 \
  | cut -f 4 \
  | sort \
  | uniq -c \
  | sort -k1,1rn \
  | head -n 10
```

Let's go through this line by line ...

## In Class Exercises - Class 3

1. To learn about piping (), use cowsay to:
  - a. show your current working directory
  - b. show the number of lines in /vol1/opt/data/lamina.bed
  - c. show the most recently modified file/dir in \$HOME

## Genome Analysis Workshop

2. write a bash script that you can run to list only the 2 most recently modified files in a given directory (using what you've learned in this class)
3. make that script executable (use google to learn how to do this).
4. With head, you can see the first line of a file with head -n1. How can you see all of a file *except* the first line. (use google)
5. Without using your history, how few keystrokes can you use to run the following command (must work from any directory)?

```
$ ls /vol1/opt/data/lamina.bed
```

6. How few keystrokes can you do 5. using your history?

## Class 4 : grep and awk

**Class date:** 2015 Feb 5 Thurs  
**Last updated:** March 04, 2015

### Goals

1. Review
2. remember BED format (chr, start, end)
3. learn grep and awk basics to filter and manipulate text

### grep

Use [grep\(1\)](#) to identify lines in a file that match a specified pattern.

To find any instance of *chr5* in the lamina.bed file

```
# grep [pattern] [filename]
$ grep chr5 /vol1/opt/data/lamina.bed | head
```

To find all lines that start with a number sign:

```
# The caret (^) matches the beginning of the line
# FYI dollar sign ($) matches the end
$ grep '^#' /vol1/opt/data/lamina.bed
```

To find any line that *does not* start with "chr":

```
# the -v flag inverts the match (grep "not" [pattern])
$ grep -v '^chr' /vol1/opt/data/lamina.bed
```

Beware of using grep to find patterns that might be partial matches:

```
# this will match chr1, chr10, chr11 etc.
$ grep chr1 /vol1/opt/data/lamina.bed | cut -f1 | uniq
```

You can find exact matches that are split on words with the -w flag:

```
# this will only match chr1
$ grep -w chr1 /vol1/opt/data/lamina.bed | cut -f1 | uniq
```

Beware of using grep to search for numbers:

```
# finds all strings that match `100`
$ grep 100 /vol1/opt/data/lamina.bed | head -n 20

# better, but doesn't look at numeric value
$ grep -w 100 /vol1/opt/data/lamina.bed | head -n 20
```

### Tip

If you're trying to find numeric values in a file, use awk instead:

```
$ awk '$2 == 500' /vol1/opt/data/lamina.bed
```

### Exercises

1. use grep to identify lines in lamina.bed where the second field (start) begins with 100.
2. use grep to identify lines in lamina.bed where the third field (end) ends with 99 .

3. use grep with its -w flag to count the number of 'chr1' records in lamina.bed.
4. use grep to count how many fastq records are in the /vol1/opt/data/t\_R1.fastq.gz file (fastq records begin with an '@' symbol)
5. use grep to count the number of fastq records in /vol1/opt/data/SP1.fq.gz

## awk

<http://en.wikipedia.org/wiki/AWK>

AWK is an interpreted **programming language** designed for text processing and typically used as a data extraction and reporting tool. It is a standard feature of most Unix-like operating systems.

Named after authors **A** ho, **W** einberger & **K** ernighan

### This is programming

## basic principles

1. awk operates on each line of a text file
2. in an awk program, \$1 is an alias for the 1st column, \$2 for the 2nd, etc.
3. awk can filter lines by a pattern

## awk program structure

- **BEGIN** runs before the program starts
- **END** runs after the program runs through all lines in the file
- **PATTERN** and **ACTIONS** check and execute on each line.

```
awk 'BEGIN {} (PATTERN) { ACTIONS } END {}' some.file.txt
```

## awk BEGIN

You can use **BEGIN** without a file. We just do one thing then exit:

```
awk 'BEGIN { print 12 * 12 }'
```

Same with **END**:

```
awk 'END { print 12 * 13 }'  
# then type ctrl+d so it knows it's not getting more input.
```

## filtering

A simple and powerful use of awk is lines that match a pattern or meet set of criteria. Here, we match (and implicitly print) only lines where the first column is chr12:

```
awk '($1 == "chr12")' /vol1/opt/data/lamina.bed
```

We can also filter on start position using '&&' which means 'and':

```
awk '($1 == "chr12" && $2 < 9599990)' /vol1/opt/data/lamina.bed
```

## Important

- = and == are not the same thing, and are frequently mixed up.
- = is the assignment operator == tests for equality != tests for inequality.

### program structure

```
awk '$1 == "chr12" && $2 < 9599990' /vol1/opt/data/lamina.bed
```

### Important

- when we are checking as a character ("chr12") we need the quotes.
- when we are checking as a number (9599990) can not use quotes.
- can't use commas (e.g. 9,599,990) in numbers

### in-class exercise

we will do the first of these together.

- how many regions (lines) in lamina.bed have a start less than 1,234,567 on any chromosome?
- how many regions in lamina.bed have a start less than 1,234,567 on chromosome 8?
- how many regions (lines) in lamina.bed have a start between 50,000 and 951,000
- how many regions in lamina.bed overlap the interval **chr12:5,000,000-6,000,000** ?

### Important

the last question is not trivial and understanding it will be useful

### awk program structure (actions)

print total bases covered on chromosome 13:

```
awk '$1 == "chr13" { coverage = coverage + $3 - $2 }  
END { print coverage }' /vol1/opt/data/lamina.bed
```

### Important

- the entire awk program must be wrapped in quotes. Nearly always best to use** single quotes ('') on the outside.
- coverage is a variable that stores values; we don't use** a \$ to access it like we do in bash or like we do for the \$1, \$2, ... columns

### in-class exercise

below is how we find coverage for chr13.

```
awk '$1 == "chr13" { coverage += $3 - $2 }  
END{ print coverage }' /vol1/opt/data/lamina.bed
```

how can we find the total coverage for all chromosomes **except** 13?

### awk continued

The \$0 variable contains the entire line.

multiple patterns

```
awk '$3 >= 5000 { print $0"\tGREATER" }  
$3 < 5000 { print $0"\tLESS" }' \/  
/vol1/opt/data/states.tab
```

remember we can simply filter to the lines > 5000 with:

```
awk '$3 >= 5000' /vol1/opt/data/states.tab
```

## awk special variables

1. we know \$1, \$2, ... for the column numbers
2. NR is a special variable that holds the line number
3. NF is a special variable that holds the number of fields in the line
4. FS and OFS are the (F)ield and (O)output (F)ield (S)eparators i.e. the delimiters (default is any space character)

## using awk to count lines with NR

```
$ wc -l /vol1/opt/data/lamina.bed  
$ awk 'END { print NR }' /vol1/opt/data/lamina.bed
```

## using FS and OFS

Let's convert lamina.bed to comma-delimited but only for chr12

remember FS is the input separator and OFS is the output delimiter

```
$ awk 'BEGIN{FS="\t"; OFS=","}  
($1 == "chr12"){ print $1,$2,$3 }' /vol1/opt/data/lamina.bed
```

## regular expressions

we won't cover these in detail, but you can match on *regular expressions*.

The following finds lines containing chr2 (chr2, chr20, chr21) in the first column

```
$ awk '$1 ~ /chr2/' /vol1/opt/data/lamina.bed
```

Often we can get by without *regular expressions* but they are extremely powerful and available in nearly all programming languages.

## advanced awk

You can do a lot more with awk, here are several resources:

- <http://www.hcs.harvard.edu/~dholland/computers/awk.html>
- <http://doc.infosnel.nl/quickawk.html>
- <http://www.catonmat.net/download/awk.cheat.sheet.pdf>

## In Class Exercises - Class 4

we will do the first 2 of these together.

1. use NR to print each line of *lamina.bed* preceded by it's line number
2. use NF to see how many columns are in each row of *states.tab*

## review

- \$1, \$2, \$3 (default sep is space)

- adjust sep with: OFS="t"; FS=","
- \$0 # entire line

```
BEGIN {}
(match) { coverage += $3 - $2 }
END { print coverage }
```

- NR is line number; NF is number of fields;
- BEGIN {} filter { action } END { }

### Exercises

1. are there any regions in *lamina.bed* with start > end?
2. what is the total coverage [sum of (end - start)] of regions on chr13 in *lamina.bed*?
3. what is the mean value (4th column) on chromome 3 of *lamina.bed*
4. print out only the header and the entry for colorado in *states.tab*
5. what is the (single-number) sum of all the incomes for *states.tab* with illiteracy rate less than 0.1? greater than 2?
6. use NR to filter out the header from *lamina.bed* (hint: what is NR for the header?)

## Class 5 : BEDTools

**Class date:** 2015 Feb 10 Tues  
**Last updated:** March 04, 2015

### Goals

1. Learn to run scripts on the cluster via the queuing system
2. Learn about genomic data types and where to get data
3. Start to use bedtools to analyze genomic data

### Cluster etiquette

There are some specific rules you need to know when you're operating in a cluster environment.

```
digraph cluster { "YOU" [shape=box]; "amc-tesla" [shape=box]; "filesystem" [shape=box]; "compute nodes" [shape=box]; "YOU" -> "amc-tesla"; "amc-tesla" -> "filesystem"; "amc-tesla" -> "compute nodes"; }
```

### Important

**DO NOT** run jobs on the head node (amc-tesla). The head node is the brains of the cluster and it can easily be overextended. Use qlogin instead.

### Example commands on the cluster

Find the size of the file system:

```
$ df -h
```

Find how much space you have allocated:

```
$ quota -h
```

### The queueing system

The cluster uses a queueing system that will run jobs that you submit to it. You can write a small test script to see how the system works. First, write this into a run.sh file:

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

#BSUB -J sleeper
#BSUB -e %J.err
#BSUB -o %J.out

sleep 20
```

The #BSUB lines are comments, but are read by the bsub program to identify features associated with your job.

- -J sets the job's name
- %J is a unique job ID that is set when you run the job.
- -e and -o set the filenames for stderr and stdout from the job

Now you can submit the script to the queuing system. As soon as you submit it, you can check on its progress:

```
$ bsub < run.sh  
$ bjobs
```

After the job finishes, you should see two new files that end *.out* and *.err*; these stdout and stderr from the running job. Look at the contents of those files so you know what is in each one.

### Killing jobs

Sometimes you need to kill your jobs. You can kill specific jobs using their job ID numbers, obtained from checking `bjobs`:

```
$ bkill <jobid>
```

You can also kill **all** of your jobs at once:

```
$ bkill 0
```

### Warning

`bkill 0` is dangerous – it will wipe out all of your jobs. If you have long-running jobs that you forgot about, you will kill them too if you are not careful!

### Other cluster-specific commands

```
$ bhosts      # hosts in the cluster  
$ man bhosts # bsub man page  
$ bqueues     # available queues  
$ lsload      # check load values for all hosts
```

## BEDTools

### Goals

1. Introduce the BEDTools suite of tools.
2. Understand why using BEDTools is needed.
3. Practice common operations on BED files with BEDTools.

### BEDTools Overview

BEDTools will be one of the tools with the best return on investment. For example, to extract out **all genes that overlap a CpG island**:

```
$ bedtools intersect -u -a genes.hg19.bed.gz -b cpg.bed.gz > genes-in-islands.bed
```

*intersect* is a bedtools tool. It follows a common pattern in bedtools that the query file is specified after the **-a** flag and the *subject* file after the **-b** flag

### BEDTools Utility

Finding all overlaps between a pair of BED files naively in python would look like:

```
for a in parse_bed('a.bed'):
    for b in parse_bed('b.bed'):
        if overlaps(a, b):
            # do stuff
```

If '*a.bed*' has 10K entries and '*b.bed*' has 100K entries, this would involve checking for overlaps **1 billion times**. That will be slow.

BEDTools uses an indexing scheme that reduces the number of tests dramatically.

### Note

See the original BEDTools paper for more information:  
<http://bioinformatics.oxfordjournals.org/content/26/6/841.full>

- Fast: faster than *intersect* code you will write
- Terse: syntax is terse, but readable
- Formats: handles BED, VCF and GFF formats (gzip'ed or not)
- Special Cases: handles stranded-ness, 1-base overlaps, abutted intervals, etc. (likely to be bugs if you do code in manually)

### BEDTools Commands

To see all available BEDTools commands, type

```
$ bedtools
```

The most commonly used BEDtools are:

- *intersect*
- *genomecov*
- *closest*
- *map*

### BEDTools Documentation

The BEDTools documentation is quite good and ever improving.

See the documentation for [intersect](#) with:

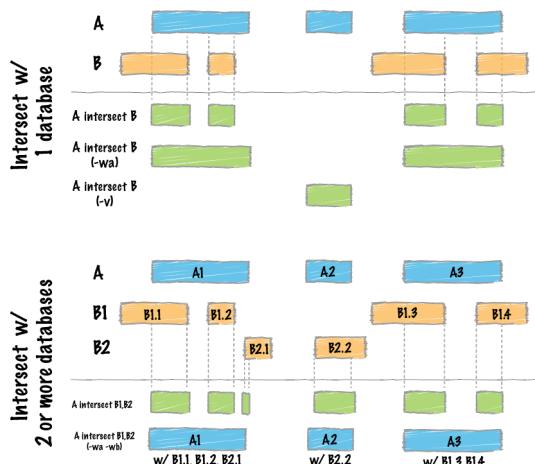
```
$ bedtools intersect
```

## The online HTML help is also good and includes pictures:

<https://bedtools.readthedocs.org/en/latest/content/tools/intersect.html>

### BEDTools intersect

Have a browser window open to [BEDTools intersect documentation](#). It will likely be the BEDTools function that you use the most. It has a lot of options.



"-v" means (like grep) include all intervals from *-a* that do not overlap intervals in *-b*

### Example Files

```
$ cat a.bed
chr1    10  20  a1  1   +
chr1    100 200 a2  2   -
$ cat b.bed
chr1    20  30  b1  1   +
chr1    90  101 b2  2   -
chr1    100 110 b3  3   +
chr1    200 210 b4  4   +
```

What will happen if you intersect those files? For example, the *a.bed* region *chr1:100-200* overlaps:

```
chr1:90-101
chr1:100-110
```

from *b.bed*

### intersect

intersect with default arguments means **extract chunks of `‐a` that overlap regions in `‐b`**

```
$ bedtools intersect -a a.bed -b b.bed
chr1    100 101 a2  2   -
chr1    100 110 a2  2   -
```

Here is the original interval from *a.bed*:

```
chr1      100      200      a2      2      -
```

And the overlapping intervals from *b.bed*:

```
chr1      90      101      b2      2      -
chr1      100     110      b3      3      +
```

### **intersect -wa**

Often, we want the *entire interval from -a if it overlaps any interval in -b*

```
$ bedtools intersect -a a.bed -b b.bed -wa
chr1  100  200  a2  2  -
chr1  100  200  a2  2  -
```

We can get that uniquely with (-u)

### **intersect -wo**

We can see which intervals in -b are associated with -a

```
$ bedtools intersect -a a.bed -b b.bed -wo
chr1  100  200  a2  2  -  chr1  90  101  b2  2  -  1
chr1  100  200  a2  2  -  chr1  100  110  b3  3  +  10
```

### **intersect exercise**

What happens if you reverse the arguments? E.g. instead of:

```
-a a.bed -b b.bed
```

use:

```
-b a.bed -a b.bed
```

Try that with no extra flags, with -u, -wa, -wo.

How does it compare to the original?

### **intersect -c**

We can count overlaps for each interval in -a with those in -b with

```
$ bedtools intersect -a a.bed -b b.bed -c
chr1  10  20  a1  1  +  0
chr1  100 200  a2  2  -  2
```

This is our original *a.bed* with an **additional column indicating number of overlaps** with *b.bed*

### **intersect -v**

Extract intervals in *a.bed* that do not overlap any interval in *b.bed*

```
$ bedtools intersect -a a.bed -b b.bed -v
chr1  10  20  a1  1  +
```

Extract intervals in *b.bed* that do not overlap any interval in *a.bed*

```
$ bedtools intersect -a b.bed -b a.bed -v
chr1  20  30  b1  1  +
chr1  200 210  b4  4  +
```

### **Intersect Summary**

- fragments of *a* that overlap *b*: *intersect -a a.bed -b b.bed*
- complete regions of *a* that overlap *b*: *intersect -a a.bed -b b.bed -u*
- intervals of *b* as well as *a*: *intersect -a a.bed -b b.bed -wo*
- number of times each *a* overlaps *b*: *intersect -a a.bed -b b.bed -c*

- intervals of *a* that do not overlap *b*: *intersect -a a.bed -b b.bed -v*

## Exercises

Use the *cpg.bed.gz* and *genes.hg19.bed.gz* files for the following exercises:

1. Extract the CpG islands that touch any gene [24611]
2. Extract CpG islands that do not touch any gene [7012]
3. Extract (uniquely) all of each CpG Island that touches any gene [21679]
4. Extract CpG's that are completely contained within a gene (look at the help for a flag to indicate that you want the fraction of overlap to be 1 (for 100 %). [10714]
5. Report genes that overlap any CpG island. [16908]
6. Report genes that overlap more than 1 CpG Island (use -c and awk). [3703].

## Note

as you are figuring these out, make sure to pipe the output to less or head

## Other Reading

- Check out the online [documentation](#).
- A [tutorial](#) by the author of BEDTools

## Intersect Bam

We have seen that *intersect <bedtools:intersect>* takes *-a* and *-b* arguments. It can also intersect against an alignment BAM file by using *-abam* in place of *-a*

e.g:

```
$ bedtools intersect \
    -abam experiment.bam \
    -b target-regions.bed \
    > on-target.bam
```

## Intersect Strand

From the [help](#), one can see that *intersect* can consider strand. For example if both files have a strand field then

```
$ bedtools intersect -a a.bed -b b.bed -s
```

Will only consider as overlapping those intervals in *a.bed* that have the same strand as *b.bed*.

## Closest

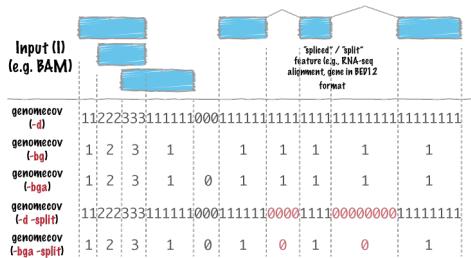
with *intersect* we can only get overlapping intervals. *closest* reports the nearest interval even if it's not overlapping.

Example: report the nearest CpG to each gene as long as it is within 5KB.

```
bedtools closest \
    -a genes.hg19.bed.gz \
    -b cpg.bed.gz -d \
    | awk '$NF <= 5000'
```

## Genomecov

Get coverage of intervals in BED by BAM



Usually want the last option `-bg -split`

## ENCODE

The Human Genome Project was finished, giving us a list of human genes and their locations. Unfortunately, we still had no idea how they were regulated. If only there was an [ENCylopedia Of Dna Elements...](#)

Advantages: massive amounts of information on key cell lines, reproducible experiments, public data access, technology development.

## ENCODE Project Cell Lines

Tier 1: GM12878 (EBV-transformed lymphoblast), K562 (CML lymphoblast), H1-hESC

Tier 2: HeLa-S3 (cervical cancer), HepG2 (liver carcinoma), HUVEC (umbilical vein)

Tier 2.5: SKNSH (neuroblastoma), IMR90 (lung fibroblast), A549 (lung carcinoma), MCF7 (breast carcinoma), LHCN (myoblast), CD14+, CD20+

[link](#) (this page also has very useful links to cell culture protocols)

## Experiments

1. ChIP-seq: Histone marks, transcription factors
2. Chromatin structure: DNaseI-seq, FAIRE, 5C/Hi-C
3. RNA expression: mRNA-seq, GENCODE gene predictions
4. Data Integration: Segway / ChromHMM integration of functional data

## Common File Formats

- FASTQ: Raw sequencing data. [\[link\]](#) <<http://maq.sourceforge.net/fastq.shtml>>
- SAM/BAM: Aligned sequence data [\[link\]](#) <<http://samtools.github.io/hts-specs/SAMv1.pdf>>
- Bed/bigBed: List of genomic regions [\[link\]](#) <<http://genome.ucsc.edu/FAQ/FAQformat.html#format1>>
- Bedgraph/Wig/bigWig: Continuous signal [\[link\]](#) <<http://genome.ucsc.edu/goldenPath/help/bedgraph.html>>

Many other formats are described on this page

## References

Completion of the entire project, and a ton of papers: [Nature](#), [Genome Research](#), [Genome Biology](#),

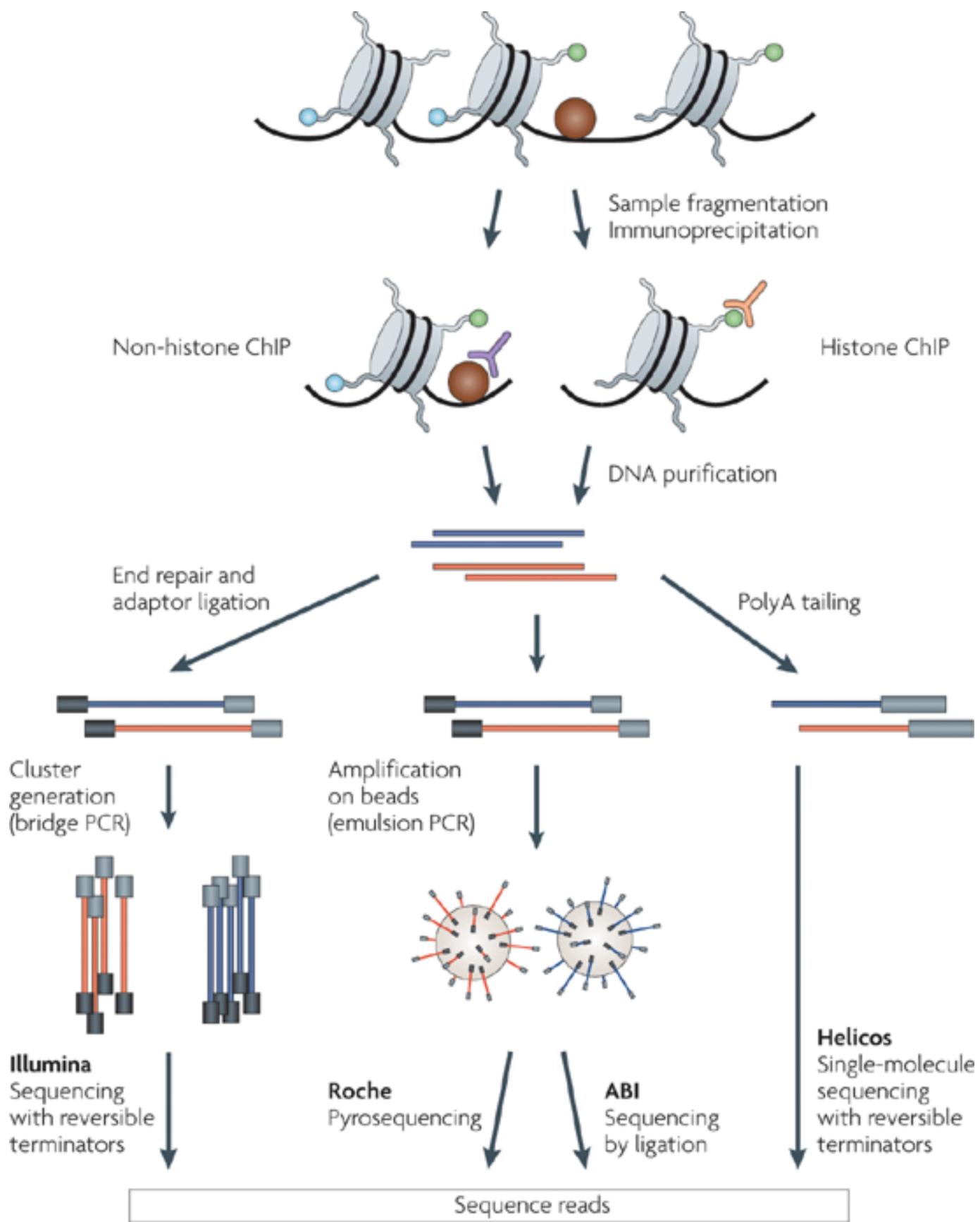
## How to Access ENCODE Data

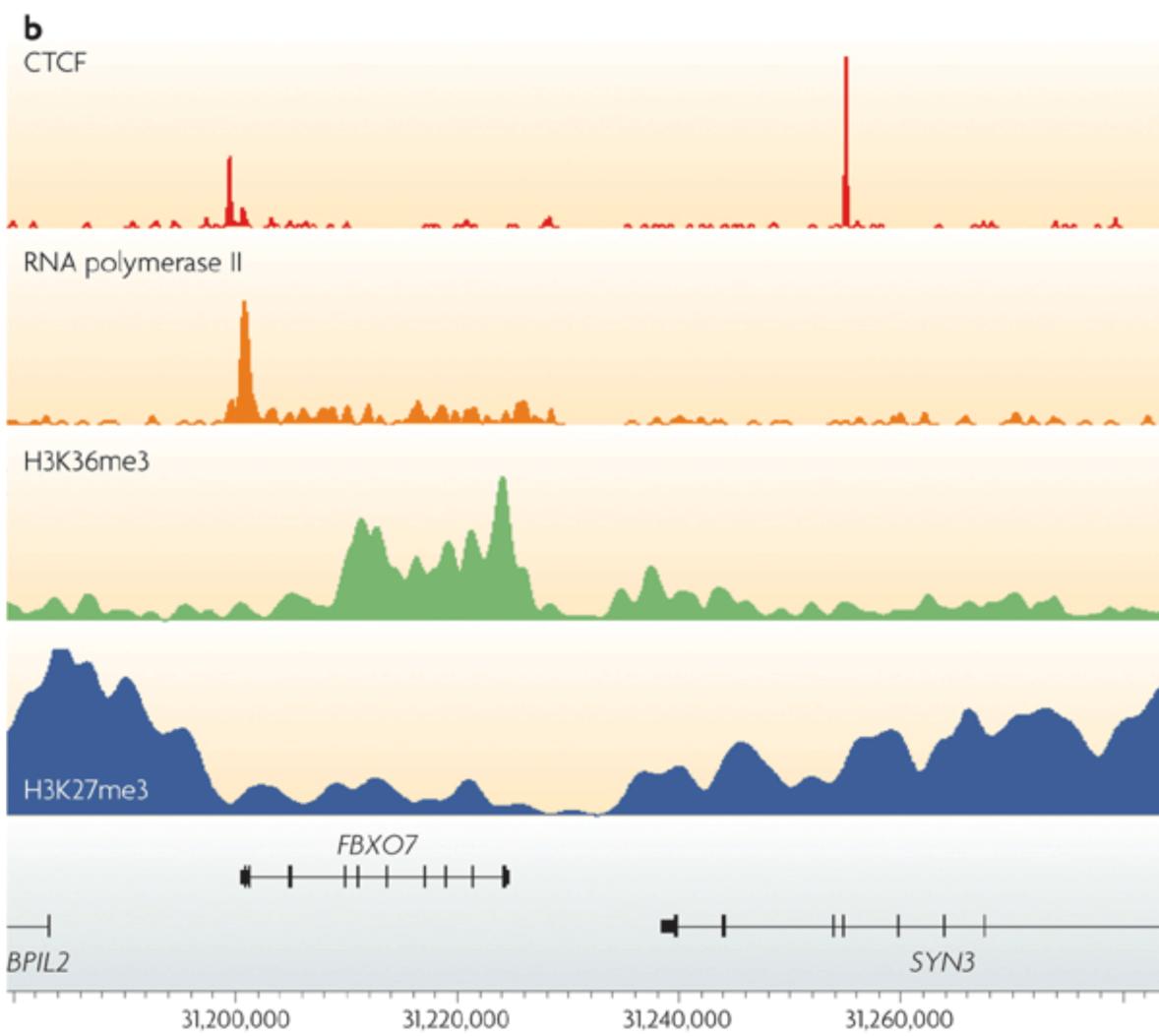
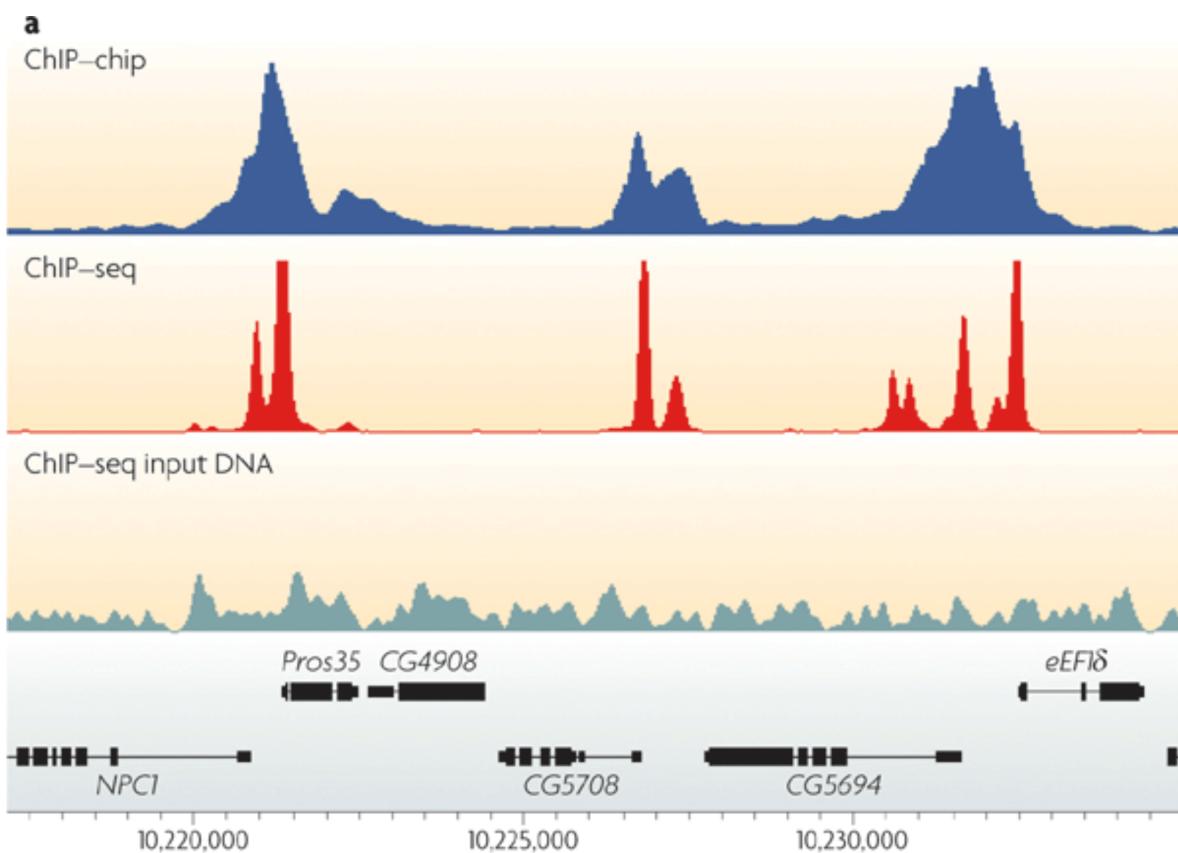
The [ENCODE project page](#) is the portal to all of the ENCODE data.

## Chromatin Immunoprecipitation Overview

## Genome Analysis Workshop

Chromatin Immunoprecipitation is used to determine where a protein of interest binds on a chromatin template [Park\_Chipseq].





## ChIP-seq analysis workflow

A general workflow for visualizing ChIP-seq data (and many other types of data) is:

Operation	File formats
Align reads to reference genome	FASTQ ~> BAM
Generate coverage plots	BAM ~> bedGraph
Call peaks	BAM ~> BED
Make binary files for UCSC display	bedGraph ~> bigWig, BED ~> bigBed
Identify motifs	BED ~> FASTA ~> TXT / HTML

## ChIP-seq data

Look at some human ChIP-seq data <sup>7</sup>.

(We'll talk more in depth about ChIP-Seq workflows in the future, but for now, just a brief introduction to a few commands you can use to work on ChIP-Seq data (and pset3)).

## Peak calling

There are several available software packages for identifying regions enriched in your IP experiment (i.e. peaks). We will use macs2 here.

```
# minimal macs2 command
$ macs2 callpeak --treatment <aln.bam> --name <exp.name> [options]
```

## Identify sequence motifs in enriched regions

You can use meme <sup>8</sup> to identify over-represented motifs in groups of sequences (e.g. sequences covered by ChIP peaks).

Use the *bedtools getfasta* command to fetch fasta sequences.

Note: meme looks at both strands of a DNA sequence by default.

```
$ bedtools getfasta -fi <ref.fa> -bed <peaks.bed> -fo peaks.fa
$ meme -nmotifs 5 -minw 6 -maxw 20 -dna <peaks.fa>
```

## Class 6 : Genomic Data Vignette: ChIP-seq

**Class date:** 2015 Feb 12 Thurs

**Last updated:** March 04, 2015

## Goals

1. Learn workflows for analyzing ChIP-seq data
2. Derive DNA sequence motifs from ChIP-seq peaks

## ENCODE

The Human Genome Project was finished, giving us a list of human genes and their locations. Unfortunately, we still had no idea how they were regulated. If only there was an [ENCYclopedia Of Dna Elements...](#)

Advantages: massive amounts of information on key cell lines, reproducible experiments, public data access, technology development.

## ENCODE Project Cell Lines

Tier 1: GM12878 (EBV-transformed lymphoblast), K562 (CML lymphoblast), H1-hESC

Tier 2: HeLa-S3 (cervical cancer), HepG2 (liver carcinoma), HUVEC (umbilical vein)

Tier 2.5: SKNSH (neuroblastoma), IMR90 (lung fibroblast), A549 (lung carcinoma), MCF7 (breast carcinoma), LHCN (myoblast), CD14+, CD20+

[link](#) (this page also has very useful links to cell culture protocols)

## Experiments

1. ChIP-seq: Histone marks, transcription factors
2. Chromatin structure: DNaseI-seq, FAIRE, 5C/Hi-C
3. RNA expression: mRNA-seq, GENCODE gene predictions
4. Data Integration: Segway / ChromHMM integration of functional data

## Common File Formats

- FASTQ: Raw sequencing data. [\[link\]](#) <<http://maq.sourceforge.net/fastq.shtml>>
- SAM/BAM: Aligned sequence data [\[link\]](#) <<http://samtools.github.io/hts-specs/SAMv1.pdf>>
- Bed/bigBed: List of genomic regions [\[link\]](#) <<http://genome.ucsc.edu/FAQ/FAQformat.html#format1>>
- Bedgraph/Wig/bigWig: Continuous signal [\[link\]](#) <<http://genome.ucsc.edu/goldenPath/help/bedgraph.html>>

Many other formats are described on this [page](#)

## References

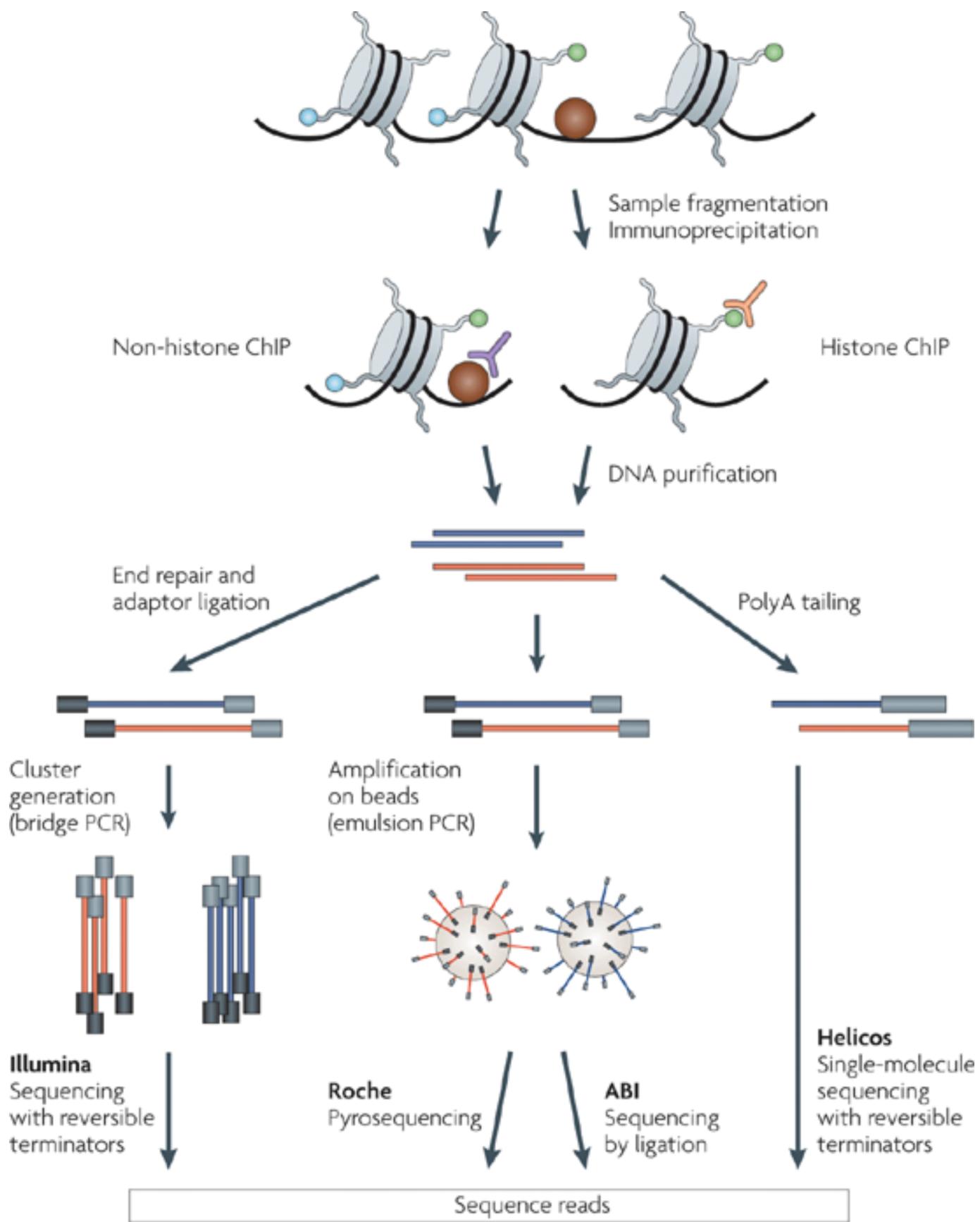
Completion of the entire project, and a ton of papers: [Nature](#), [Genome Research](#), [Genome Biology](#),

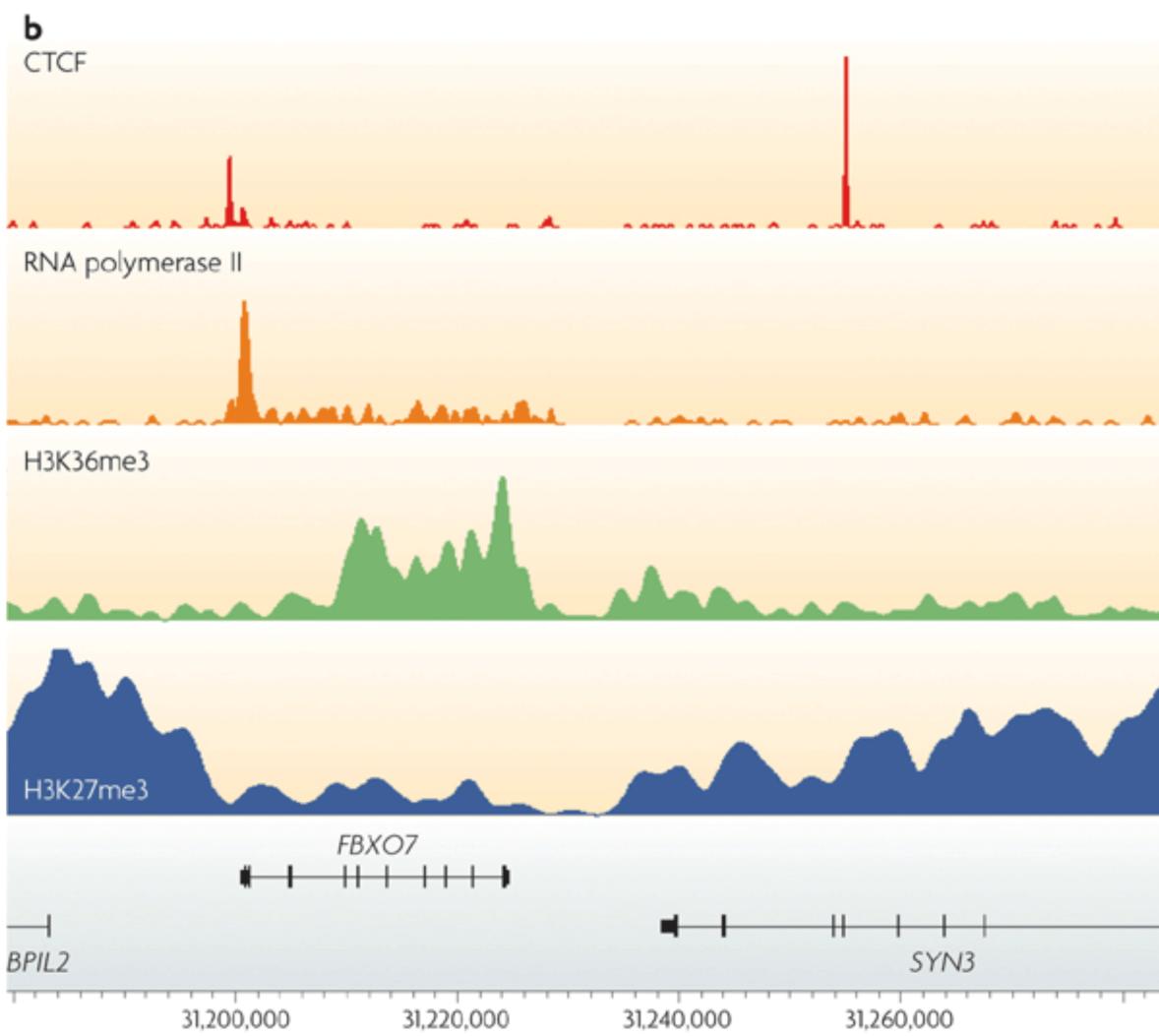
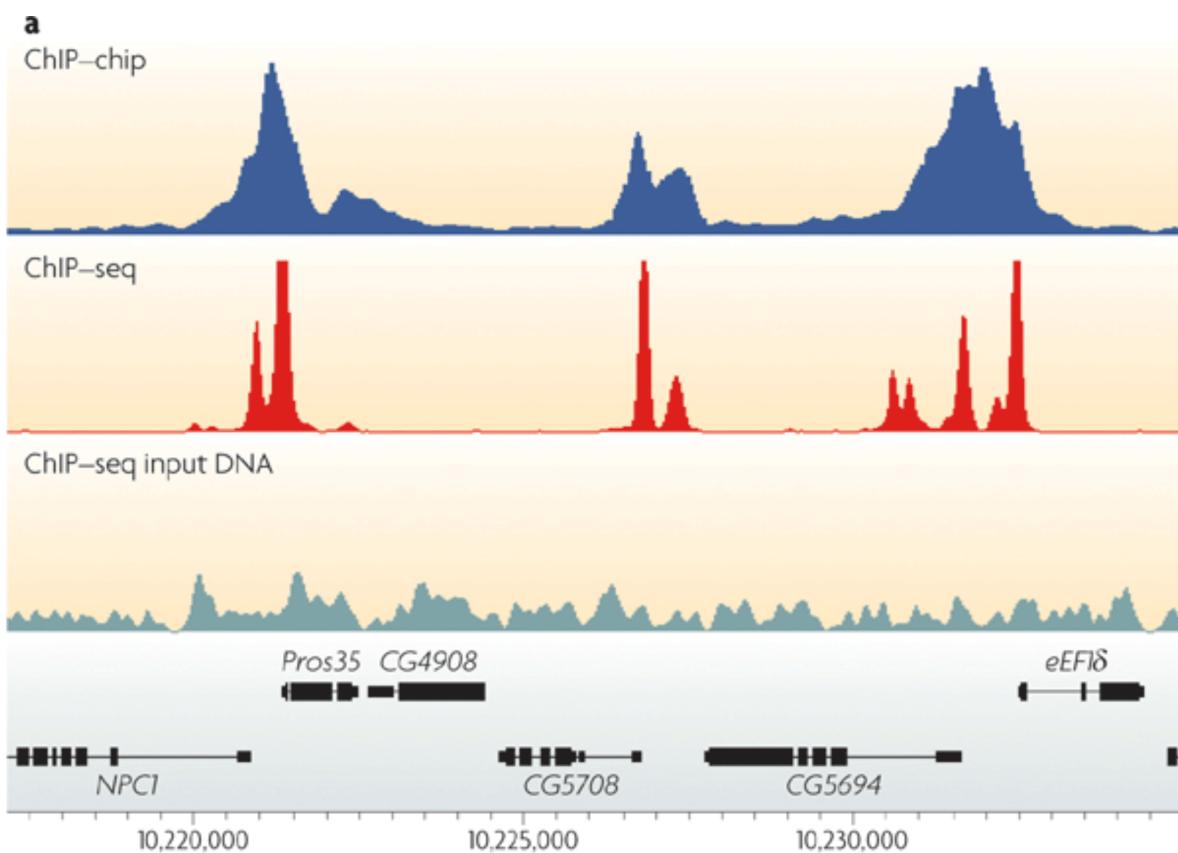
## How to Access ENCODE Data

The [ENCODE project page](#) is the portal to all of the ENCODE data.

## Chromatin Immunoprecipitation Overview

Chromatin Immunoprecipitation is used to determine where a protein of interest binds on a chromatin template [\[Park\\_Chipseq\]](#).





## ChIP-seq analysis workflow

A general workflow for visualizing ChIP-seq data (and many other types of data) is:

Operation	File formats
Align reads to reference genome	FASTQ ~> BAM
Generate coverage plots	BAM ~> bedGraph
Call peaks	BAM ~> BED
Make binary files for UCSC display	bedGraph ~> bigWig, BED ~> bigBed
Identify motifs	BED ~> FASTA ~> TXT / HTML

## ChIP-seq data

Look at some human ChIP-seq data <sup>9</sup>.

(We'll talk more in depth about ChIP-Seq workflows in the future, but for now, just a brief introduction to a few commands you can use to work on ChIP-Seq data (and pset3)).

## Peak calling

There are several available software packages for identifying regions enriched in your IP experiment (i.e. peaks). We will use macs2 here.

```
# minimal macs2 command
$ macs2 callpeak --treatment <aln.bam> --name <exp.name> [options]
```

## Identify sequence motifs in enriched regions

You can use meme <sup>10</sup> to identify over-represented motifs in groups of sequences (e.g. sequences covered by ChIP peaks).

Use the *bedtools getfasta* command to fetch fasta sequences.

Note: meme looks at both strands of a DNA sequence by default.

```
$ bedtools getfasta -fi <ref.fa> -bed <peaks.bed> -fo peaks.fa
$ meme -nmotifs 5 -minw 6 -maxw 20 -dna <peaks.fa>
```

## Class 7 : BEDTools vignette

**Class date:** 2015 Feb 17 Tues

**Last updated:** March 04, 2015

## Goals

1. Go over BEDTools analysis vignette to show how to combine different tools

There are several more vignettes [here](#). Looking over these may be helpful to understand some of the questions on [Problem Set 4](#).

## Overview

A common situation in genomic analysis is to examine how signals (e.g., coverage in a ChIP-seq experiment) relate to genome annotations (e.g., transcription start sites).

This simple process can be broken down into several individual steps:

9

Genome Browser Session <http://goo.gl/WfJxcM>

10

MEME <http://meme.nbcr.net/meme/>

1. Obtain genome annotations (we will use TSS)
2. Obtain signals in bedGraph format (we will use Pol II ChIP-seq)
3. Use *slop* to create regions to examine [optional]
4. Break the regions into windows with *makewindows*.
5. *map* data onto the windows.
6. Calculate a summary statistic for each window with *groupby*.

## Annotations

First we need some annotations. Let's get some transcription start sites (TSSs) from a BED file of gene annotations.

```
$ genes=/vol1/opt/data/refGene.bed.gz
$ tssbed=tss.bed

# need to grab the start or end based on the strand field
$ zcat $genes | awk '$6 == "+"' | awk 'BEGIN {OFS="\t"} {print $1,$2,$2+1}' > $tssbed
$ zcat $genes | awk '$6 == "-"' | awk 'BEGIN {OFS="\t"} {print $1,$3,$3+1}' > $tssbed

# we made a bed file, so now we sort it.
$ bedSort $tssbed $tssbed
$ gzip $tssbed
# rename it for future use
$ tssbed=tss.bed.gz
```

## Signal

The signal we will use is human Pol II ChIP-seq signal. The file is:

```
signal=/vol1/opt/data/encode/wgEncodeBroadHistoneHelaS3Pol2bStdSig.bigWig
```

bigWig is a compressed form of bedGraph. You can either convert to bedGraph and compress:

```
bigWigToBedGraph <wig file> <bedgraph>
gzip <bedgraph>
```

Or you can use it in a stream with:

```
<(bigWigToBedGraph <wigfile> stdout)
```

## Slop

Each of the TSS regions we made is 1 base in size. We need to make these bigger so that we can examine coverage within a larger region. Let's make them 2 kb with the *slop* command:

```
$ chromsize=/vol1/opt/data/hg19.chrom.sizes
$ sloped=tss.slop.2000.bed
$ bedtools slop -b 2000 -i $tssbed -g $chromsize > $sloped
```

Inspect the slop'd file and make sure you understand how it is different from the input BED file. Check the new widths of each region using awk.

## Make windows

Now that we have a 2 kb window around each TSS, we can break the regions up into *windows* and examine signal in each. The idea here is that we would like to see a peak of signal at the middle window, and it should decrease as we move away from TSS.

```
$ windowbed=tss.slop.2000.5bp.windows.bed
$ bedtools makewindows -b $windowbed -w 5 -i srcwinnum \
| sort -k1,1 -k2,2n \
```

```
| tr "\_" "\t" \
> $windowbed
```

Note that each window now has a unique number, and the relative positions of each window of a given number are the same. For example, all the windows labeled "1" are 2000 bases upstream of the original annotated TSS.

What would the signal look like if we didn't do this?

### Map signal to the annotations

Now we can map the signal to each of the windows that we made.

```
$ signalmap=signal.map.bg
$ bedtools map -a $windowbed \
  -b <(bigWigToBedGraph $signal stdout) \
  -c 4 -o mean -null 0 \
> $signalmap
```

- the `-c` flag specifies the values to operate on.
- the `-o` flag specifies the operation to perform (e.g. mean)
- What does `-null 0` do? Why is it important?

Inspect this file and make sure you know what it looks like.

### Grouping the data

Now we can calculate summary statistics on the mapped data with `groupby`:

```
# Note that the mapped data has to be sorted by window number
$ sort -t$'\t' -k5,5n $signalmap \
| bedtools groupby \
-i - \
-g 5 -c 6 -o sum \
> output.tab
```

- the `-g` flag specifies the column to group on
- the `-c` flag specifies the values to aggregate.
- the `-o` flag specifies the operation to perform (e.g. sum)

Inspect the output so you know what it looks like.

### Code

```
1 #! /usr/bin/env bash
2
3 #BSUB -J bedtools.vignette
4 #BSUB -o vignette.%J.out
5 #BSUB -e vignette.%J.err
6
7 genes=/vol1/opt/data/encode/refGene.bed.gz
8 tss=tss.bed
9
10 # make annotations
11 zcat $genes | awk '$6 == "+"' \
12   | awk 'BEGIN {OFS="\t"} {print $1,$2,$2+1}' > $tssbed
13 zcat $genes | awk '$6 == "-"' \
14   | awk 'BEGIN {OFS="\t"} {print $1,$3,$3+1}' > $tssbed
15
16 bedSort $tssbed $tssbed
```

```

17 gzip $tssbed
18 # rename after gzipping
19 tss=tss.bed.gz
20
21 signal=/vol1/opt/data/endcode/wgEncodeBroadHistoneHelaS3Pol2bStdSig.bigWig
22
23 chromsize=/vol1/opt/data/hg19/hg19.chrom.sizes
24 sloped=tss.slop.2000.bed
25
26 # make slop
27 bedtools slop -b 2000 -i $tssbed -g $chromsize > $sloped
28
29 # make windows
30 windowbed=tss.slop.2000.5bp.windows.bed
31 bedtools makewindows -b $sloped -w 5 -i srcwinnum \
32   | sort -k1,1 -k2,2n \
33   | tr "\_" "\t" \
34   > $windowbed
35
36 # map data
37 signalmap=signal.map.bg
38 bedtools map -a $windowbed \
39   -b <(bigWigToBedGraph $signal stdout) \
40   -c 4 -o mean -null 0 \
41   > $signalmap
42
43 # group data
44 sort -t$'\t' -k5,5n $signalmap \
45   | bedtools groupby \
46     -i - \
47     -g 5 -c 6 -o sum \
48   > output.tab
49
50 # inspect output.tab to see how to plot the data

```

## Plotting

Now we'll make a plot with R. Navigate to:

<http://amc-tesla.ucdenver.pvt/rstudio>

and login with your tesla credentials. You should see R Studio open up. Navigate to the directory where you did your work with the *Files* menu on the lower right, and then set the working directory with *More --> Set as Working Directory*.

Now make a plot!

```

> library(ggplot2)
> col.names <- c('window', 'signal')
> df <- read.table('output.tab', col.names=col.names)

# coerce to numbers
> df$signal <- as.double(df$signal)
> qplot(window, signal, data = df)

```

## Exercises

Try to generalize this approach to other types of data. How would you:

1. Look at Pol II distribution near transcription termination sites (TTSS). Write this program and run it. (easy)

2. Analyze the distribution of H3K36me3 ChIP-seq signal relative to coding exons? Download some H3K36me3 data and [bed12tobed6](#).
3. Analyze the distribution of H3K36me3 ChIP-seq relative to introns? Use the -n option in [makewindows](#).
4. Determine levels of evolutionary conservation surrounding different types of non-coding variants? (medium). Use dbSnp and phyloP scores.

## Class 8 : R : Getting Started

**Class date:** 2015 Feb 19 Thurs

**Last updated:** March 04, 2015

### Setting up web access to tesla

You can access html and images via the web if you:

1. Create a *public\_html* directory in your \$HOME:

```
$ mkdir $HOME/public_html
```

2. Copy content into the *public\_html* directory.

3. Make sure that permissions of your \$HOME are accessible by all:

```
$ chmod 755 $HOME
```

4. Access tesla on campus, or via VPN at the URL:

```
http://amc-tesla/~username
```

```
http://amc-tesla.ucdenver.pvt/~username
```

### Goals

1. Startup RStudio
2. Learn to navigate in RStudio
3. load the ggplot2 library
4. Learn to load external data

### Data

The data file we will use is:

```
/vol1/opt/data/expr-geno-covs.txt
```

Copy this to a working directory e.g. \$HOME/class/class-8

### RStudio

RStudio is a useful interface for R, encapsulates the R prompt, data views and plotting in a single User Interface.

Navigate to <http://amc-sandbox.ucdenver.pvt/rstudio> and enter your tesla credentials.

Note you need to use VPN if you are off campus.

### Loading data in R

The main function for loading data is `read.delim()`:

```
# dfx is a data.frame. look at it with ``summary`` and ``head``
> dfx <- read.delim('lamina.bed')
> is.data.frame(dfx)
```

If the column names are specified in a header line (begins with #), then you can load them as the column names with:

```
# this uses the names in the comment line as header names
> dfx <- read.delim('lamina.bed', header=TRUE)
```

You can also specify headers explicitly with:

```
> bedfilename <- '/vol1/opt/data/lamina.bed'
> colnames <- c('chrom', 'start', 'end', 'name', 'score', 'strand')
> dfx <- read.delim(bedfilename, col.names=colnames)
```

df is short for *data frame*, the basic R data structure <- is the R assignment operator, can also use =

```
> df <- read.delim('expr-geno-covs.txt')
> summary(df)
> head(df)
> head(df$expression)
```

The most common ways to read in data in R are:

```
read.csv('file.csv')
read.delim('file.txt')
```

These take common arguments. You can get help on a function in R with:

```
?read.delim
?head
```

### DataFrame

The read() functions load a data.frame. In a data.frame, everything is read into memory.

- R figures out the types of each column e.g. numeric/character/factor
- each column of the data.frame is accessed by \$ e.g df\$genotype

Access the column data with the \$ character:

```
> dfx$chrom
```

And some functions allow you to refer to columns by name:

```
> subset(dfx, chrom == 'chr1')
```

There are several data sets that are built-in to R, including:

```
> mtcars    # Motor Trend Cars Road Tests
> baseball # in ``library(plyr)``

# see all built-in data sets
> library(help = "datasets")
```

### print

```
> print("hello world")
```

### libraries

```
> library(ggplot2)
# or
> library('ggplot2')
```

## R paths

get/set working directory

```
> getwd()                      # print
> setwd('C:\whatever\path\' ) # on windows
> setwd('/vol1/opt/data/')   # on linux
```

## Writing and running R scripts in RStudio

Open up a new R Script with *File* --> *New File* --> *R Script*. You should see a new editor window open in the top left.

You can write a simple R script like:

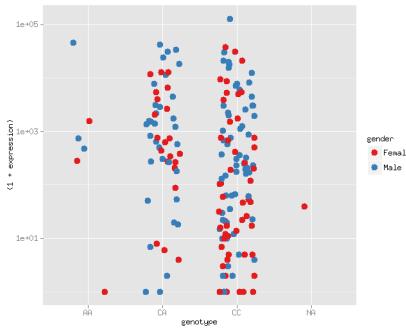
```
plot(rnorm(1000))
```

And then save the file as *plot.R*.

You can then highlight all of the lines, or just select the *plot* line, and run that portion of the program by pressing the *Run* button in the window, or with *<Cmd>-<Return>* on Macs and *<Ctrl>-<Return>* on Windows.

## ggplot2

We will learn to use it to create plots like this



## ggplot2 syntax

```
library(ggplot2)
dfx <- read.delim('expr-geno-covs.txt')

ggplot(dfx, aes(x=genotype, y=expression)) +
  geom_point()
```

*aes()* stands for **aesthetics**, which specifies the coordinates, colors, size, etc from these columns in the data.frame.

```
aes(x=genotype, y=expression, color=gender)
```

*geom\_point()* means plot these as points, could be *geom\_line()* or a number of other *geoms*.

## googling with ggplot2

Use google to find how to change the y-scale on this plot to log10

```
library(ggplot2)
dfx = read.delim('expr-geno-covs.txt')

ggplot(dfx, aes(x=genotype, y=expression)) +
  geom_point()
```

## answer

```
library(ggplot2)
dfx <- read.delim('expr-geno-covs.txt')

ggplot(dfx, aes(x=genotype, y=expression)) +
  geom_point() +
  scale_y_log10()
```

You can find a lot of info for ggplot2 with some googling.

### ggplot2 documentation

The ggplot2 docs are very good: <http://docs.ggplot2.org/current/>

Look at the `geom_point()` documentation and change the color of the plot above so that males and females are color'ed differently.

### DataFrame

In a data.frame, we read everything into memory

- R figures out if it is int/character/numeric
- each column of the data.frame is accessed by \$ e.g `df$genotype`

### Histograms

One of the simplest things to do in R, without ggplot is to look at a histogram of your data:

```
hist(dfx$expression)
# or
hist(log(dfx$expression))
```

You can make these look a lot nicer with ggplot2.

`hist()` does not work with ggplot, you'll have to use the ggplot2 machinery for that:

```
gp <- ggplot(dfx, aes(x=log10(expression)))
gp + geom_histogram()
```

Try to figure out how to overlay a density plot with `geom_density()`.

You can also make effective separate visualizations with `facet_grid()` and `facet_wrap()`. You need to specify a formula to determine how to separate:

```
gp <- ggplot(df, aes(x=genotype, y=expression)) + geom_point()

# separate by gender
gp + facet_grid(~ gender)

# separate by gender and condition
gp + facet_grid(condition ~ gender)
```

### Exercises

1. Make a histogram using ggplot and separate cases from controls by changing fill color and symbol type.
2. Make a histogram using ggplot and separate cases from controls using `facet_grid()`, `facet_wrap()`.

## Class 9 : R : Manipulation & Plotting

**Class date:** 2015 Feb 24 Tues  
**Last updated:** March 04, 2015

### Goals

1. Begin to manipulate data with dplyr and tidyr

### dplyr

dplyr provides several methods:

- summarise()
- filter()
- select()
- mutate()
- arrange()
- group\_by()

dplyr also provides an operator called `%>%` that allows you to string manipulations together.

By combining these functions together, you can quickly and easily manipulate data.frames and generate useful data summaries.

```
> summary <- df %>% select() %>% group_by() %>% summarize()
```

### dplyr example

```
dfx <- read.table('expr-geno-covs.txt', header=TRUE)

# calculate some simple stats with dplyr
grouped <- group_by(dfx, condition, genotype)
summarize(grouped, count = n(), mean.age = mean(age))

# even better
dfx %>%
  group_by(condition, genotype) %>%
  summarize(count = n(), mean.age = mean(age))
```

Summarize transcription factor binding site peaks in:

```
# columns 1-4 from merged peak calls
/vol1/opt/data/encode/peaks.bed.gz
```

```
> library(dplyr)
> colnames = c('chrom', 'start', 'end', 'name')
> bedfilename = 'peaks.bed.gz'

# use ``gzfile`` to load gzipped data
> peaks <- read.table(gzfile(bedfilename), col.names=colnames)

> peaks %>%
  group_by(name) %>%
  mutate(peak.width = end - start) %>%
  filter(peak.width > 500) %>%
  summarize(count = n(), mean.width = mean(peak.width)) %>%
  arrange(desc(count))
```

- `n()` is a special function for counting observations
- assign the summary to a new data.frame

## Exercises with dplyr

1. Use `dplyr` to calculate the mean age of smokers grouped by gender and smoking status. Plot the result.
2. Make a plot of age by expression faceted by genotype. Fit a linear model through these curves (`use geom_smooth`) on the plot.
3. Load the peaks BED file and find the 10 factors that have the largest range in peak width. Inspect a `geom_boxplot()` or `geom_violin()` to support your answer (also add individual points to the plot with `geom_jitter()`).

## Combining with ggplot

We had mean expression by condition and genotype as:

```
dfx %>% group_by(condition, genotype) \  
  %>% summarize(count=n(), mean.expr=mean(expression)) \  
  %>%
```

We can add to that expression (after typing '`library(ggplot2)`')

```
%>% ggplot(aes(x=genotype, y=expression)) \  
  + geom_histogram(stat='identity')
```

how can we change the color of all the bars to 'red'? [Hint, it's not `color = 'red'`]

## ggplot histograms

Since `expr-genotype-covs.txt` is already in long format, we can use it directly in `ggplot`:

```
ggplot(covs, aes(x=expression)) +  
  geom_histogram() +  
  scale_x_log10()
```

## Exercises

1. Adjust this:

```
ggplot(covs, aes(x=expression)) +  
  geom_histogram() +  
  scale_x_log10()
```

- to color by genotype
  - and to split plots (`facet_wrap`) by condition (case/control)
  - to color by age > 60 vs. <= 60 (use row selection stuff from start of class to make a new column named, e.g. `is_old`)
1. Figure out how to move overlapping points so categorical data is viewable (hint: look at `geom_jitter()` or the `position` argument to `geom_point()`)
  2. Load a BED file (e.g. `lamina.bed`) and calculate the mean length of regions on each chromosome in the BED file with `dplyr`. Plot the result as a bar plot with `ggplot2`.

## Practice

Practice `dplyr` and `tidyverse` with `swirl`:

```
> library(swirl)  
> install_from_swirl("Getting_and_Cleaning_Data")  
> swirl()
```



## Class 10 : R : DataFrames & Plotting

**Class date:** 2015 Mar 3 Tues  
**Last updated:** March 04, 2015

### Goals

1. Look at data.frames in more detail
2. Practice manipulating data.frames
3. more dplyr and ggplot

### data.frames

Read in a data.frame, view the first few lines and extract a single column

```
# assumes workshop repo is in $HOME/devel
setwd("~/devel/workshop/content/misc/data")

# load expr data
expr.df <- read.delim('expr-geno-covs.txt', header=TRUE)

head(expr.df)
head(expr.df$genotype)

# or
head(expr.df[, "genotype"])

# using dplyr
expr.df %>% select(genotype) %>% head()
```

To extract multiple selected columns:

```
head(expr.df[,c("genotype", "expression")])

# using dplyr
expr.df %>% select(genotype, expression) %>% head()
```

### Extracting From data.frames

The order is rows, columns. By having nothing before the comma:

```
head(expr.df[,c("genotype", "expression")])
```

We extract all rows. We can manually extract the first 5 rows and our 2 columns as:

```
expr.df[1:5, c("genotype", "expression")]

# using dplyr
expr.df %>% select(genotype, expression) %>% head(5)
```

### Named rows in data.frames

In *expr-geno-covs.txt*, there is a sample column, we may wish to use that as the row id:

```
expr.df = read.delim('expr-geno-expr.df.txt', row.names='sample_id')
head(expr.df)

expr.df[c('sample1', 'sample14'),]

# or with row numbers:
covs[c(1, 4),]
```

### Boolean Operations on Columns

```
is_old <- covs$age > 65
```

now `is_old` is a list of TRUE / FALSE values. We can extract only those > 65 as:

```
old <- covs[is_old,]  
# same as:  
old <- covs[covs$age > 65,]  
  
# using dplyr  
is_old <- covs %>% filter(age > 65)
```

We can combine selections with '&' for and and '|' for or

```
old_with_disease = (covs$age > 65) & (covs$condition == "case")  
old = covs[old_with_disease,]
```

```
# with subset  
old <- subset(covs, age > 65)  
  
# select on membership  
genos <- c('AC', 'CA')  
hets <- subset(covs, genotype %in% genos)
```

### Excercises for data.frames

Remember for combining expressions, you can create a variable for each, `is_AA`, `is_CC` and then combine after. **Do these using both dplyr and "old" R.**

1. How many people have genotype 'CC'
2. How many people have genotype 'CC' or 'AA'?
3. How many people have genotype of 'CC' or 'AA' and are under 65 years old.
4. How many *females* have genotype of 'CC' or 'AA' and are under 65 years old.

### Rmarkdown

We'll go over a Rmarkdown, a simple markup language that can be used within RStudio to generate nice looking documents combining plots and text.

### Github

Some may also find it useful to link their RStudio projects with Github accounts.

### Class 11 : R : Simple statistics and ggplot2

**Class date:** 2015 Mar 5 Thurs  
**Last** March 04, 2015  
**updated:**

### Goals

1. Simple statistics
2. ggplot2 manipulations

### Statistics in R

R provides a number of builtin statistics. Let's go over some using expr-geno-covs.txt. First, load the dataframe.

### Student t-test

Student t-test is used to determine if two sets of values are significantly different. - Assumptions are data has a normal distribution and is continuous.

### Fisher's Exact Test

Contingency tables are matrices that describe the frequency of variables. - Determine if variable "a" occurs at a higher frequency compared to variable "b". - Fisher's Exact Test is used to determine the statistical significance.

From our data, let's see if there is difference in frequency of former smokers between males and females.

### Wilcoxon's Test

This test is used on two groups of data where the samples are somehow related. Unfortunately, there is no example in df so we will have to use other data. - Use `immer`; describes the yield of barley of 30 different locations in two different years.

### Kolmogorov-Smirnov

This test determines if two distributions of data are similar. - Data does not have to have a normal distribution. - Data can be used continuous or discrete (count data).

### Plot the distribution!

Does the distribution make sense with our results??

### Exercises

1. Use `t.test()` determine whether there are significant expression differences between in `expr-geno-covs.txt` using conditions instead of gender.

### ggplot manipulations

Once you make a plot that you like, you can save it with:

```
# ggsave uses the last plot by default and learns format from the file
# suffix
> ggsave('myplot.pdf')
```

Often you will have observations of two variables that are both continuous data. How can you examine the relationships between these?

Use `geom_boxplot()` with the group aesthetic:

```
> gp <- ggplot(df, aes(x = age, y = expression) > gp + geom_boxplot()
```

Or you can plot the data and fit a curve or linear model to examine the overall relationship:

```
# plot a smoothed, possibly curvy, line
> gp + geom_point() + geom_smooth()

# fit and plot a straight line
> gp + stat_smooth(method='lm')
```

### R Model Syntax

You can also fit and examine a linear model:

```
> model <- lm(expression ~ genotype + condition + gender, data = df)
> summary(model)
```

```
# look at diagnostic plots  
> plot(model)
```

What assumptions can we make from the diagnostics? What do you suggest we do to fix it? - don't look ahead it's cheating >:(

### Let's Normalize

If we normalize the data, the diagnostics might fit. - Log that data.

But wait, there are 0 values - get values that do not exist. What on earth do we do??! - Filter out values. - After normalizing, replace again with 0.

How would you replace values with 0?

- MAJOR NOTE: you don't need to know this stuff for the homework, I'm just having fun.
- Also! knowing some of these tricks may make your life easier with your own work.

### Exercises

1. Does adding age to the existing model (expression ~ genotype + condition + gender) change the significance of the other variables?
2. How does removing condition from the model affect the significance of genotype and vice-versa?

## Problem Sets

Problem Sets for the Genome Analysis Workshop class (MOLB 7621):

### Problem Set 1

**Due date:** 2015 Feb 2 5:00 PM

### Reading

For this problem set, you'll need to read Bill Noble's paper on organizing computational biology projects <sup>11</sup> and be able to put a set of files in the correct places. You should adopt this scheme for all of your projects in and out of class.

Computational biology projects inevitably accrue a lot of files, and this paper is a great way to organize all of this information. As you read in the paper, organization of projects is important for remembering what you did, and reanalyzing data when changes are made.

This quiz will also test your ability to do simple tasks on the command line. You will need to take the tutorial to learn the necessary tools <sup>12</sup>.

You will use the command line tools discussed in the tutorial (e.g. mkdir, cd, ls, mv) to create the directory structure, move files into place and check whether everything looks ok.

### Problem 1

Make a directory structure as outlined in the paper. The directories should be nested under a common project directory, with directories for data, results and documentation (doc). You should also create dated folders with today's date so you know where to put the dated data and results. Finally, keep a list of the commands that you used to create the directory (**10 points**).

<sup>11</sup> A Quick Guide to Organizing Computational Biology Projects (2009) PLoS Comput. Biol. William S. Noble <http://dx.plos.org/10.1371/journal.pcbi.1000424>

<sup>12</sup> The Command Line Crash Course <http://cli.learncodethehardway.org/book/>

**Note**

Name your directories with YYYY-MM-DD format (year-month-date, e.g. 2014-01-10). If you do this, the directories will sort chronologically using the directory name.

**Avoid MM-DD-YY, they will not sort chronologically by name.**

**Problem 2**

Download the the following data table: **states.tab**. Then move the data file to the appropriate *dated* directory.

```
$ wget http://hesselberthlab.github.io/workshop/_downloads/states.tab
```

You need to create a run.sh shell script that runs the following code, and writes the output into a results directory with the current date (**10 points**).

You should copy the following code block into a file using *vim*. You will need to change the '???' characters in the file to correspond to the path you want to write the results in (hint: it should include today's date).

```

1 #! /usr/bin/env bash
2 #
3 # run script for quiz 1
4
5 # these are bash flags the print out variables that get set when you
6 # run the script.
7 set -o nounset -o pipefail -o errexit -x
8
9 # You will need to change the '???' strings below.
10 #
11 # define the project variable here. this should be the full path to
12 # your project directory, i.e. the directory at the top of the
13 # results/data/doc directories.
14
15 project=???
16
17 # fill in the date here
18 date=???
19
20 # these refer to the data file that you moved into place
21 data=$project/data/$date
22 datafile=$data/states.tab
23
24 # these refer to the place where you will write the results of the
25 # "analysis"
26 results=$project/results/$date
27 resultsfile=$results/result.tab
28
29 # if the directory doesn't exist, make it
30 if [[ ! -d $results ]]; then
31     mkdir -p $results
32 fi
33
34 # Note how we are using redirects here. The first ">" writes a file,
35 # and overwrites existing data. The following ">>" append data to the
36 # existing file
37
38 echo "here is our starting data ... "
39 cat $datafile > $resultsfile
40 echo
```

```
41
42 echo "here are the states sorted by population size ..."
43 sort -k2n $datafile >> $resultsfile
44 echo
45
46 echo "here are the states with the highest number of murders ..."
47 sort -k6n $datafile | head -n 10 >> $resultsfile
```

Then, save the above text in a run.sh script in your results directory. To run the file, use:

```
$ bash run.sh
```

If this ran correctly, you should see a new results.tab file in the results directory you specified in the run.sh script. If you don't see the file, double check the path you specified, and make sure you're looking in the right spot. If it's in a different spot than you intended. remove the results file you wrote, update the program and run it again.

### Problem 3

Finally you need to create a log of what you did in the root of the results directory to summarize the key points of your analysis (**5 points**). For example:

**Captain's log, star date 2014-07-16**

-----  
After examining the results.tab file, learned that Alaska has the highest income per person. Something must be wrong ...

### Problem Set Submission

Submit your problem set as a tar file to Canvas ([Problem Set Submission](#)).

## Problem Set 2

**Due date:** 2015 Feb 9 5:00 PM

### Overview

For this quiz you will use the tools we learned about in the last several classes, focusing on manipulating text files with Linux command line tools.

### Note

Continue to use the organization scheme that we learned about in [Problem Set 1](#). Part of our evaluation will include whether you are developing good organizational habits.

In the solution for all of the problems below, print out the region(s) as they appear in the BED file, with additional columns at the end. e.g.:

```
chr12 <tab> 1234 <tab> 5678 <tab> 0.93 <tab> my-extra-info
```

These should be separated by tabs (a \t character), **not spaces**, unless otherwise indicated.

Combine awk with the other utilities you have learned. Create a run.sh file that executes the commands for each problem and writes out each result in a dated directory.

### Problem 1

1. What is the region with the largest start position (2nd column) on any chromosome in *lamina.bed*? (**5 points**)
2. What is the region with the largest end position on chrY in *lamina.bed*? Report this region in the format: chr12:1234-5678" (**5 points**)

### Problem 2

1. What is the longest region (end - start) in *lamina.bed*? (**5 points**) Report as:

```
chrom <tab> start <tab> end <tab> value <tab> region_length
```

2. What is the longest region (end - start) in *lamina.bed* with a value (4th column) greater than 0.9 on chr13. Report the header (1st line) in *lamina.bed* as well as the region (**5 points**).

### Problem 3

1. What are the regions that overlap this interval in *lamina.bed*: chr12:5,000,000-6,000,000? Report regions so that they are ordered by descending value (4th column), and the columns are separated by commas rather than tabs (**5 points**).
2. What is the average value (4th column) of those regions from *lamina.bed* that overlap the region (**5 points**): chr12:5,000,000-6,000,000?

### Problem Set Submission

Submit your problem set by posting the path to your tar file on the Canvas page. ([Problem Set Submission](#)).

## Problem Set 3

**Due date:** 2015 Feb 16 5:00 PM

### Overview

For this quiz you will analyze ChIP-seq data.

Use the provided *ENCODE data* for these problems. All of the data are available on the amc-tesla cluster at:

/vol1/opt/data

Note that the FASTA sequence for hg19 is available in the same directory hg19.fa. That is a big file, so do not copy it anywhere; just use it out of that directory.

### Problem 1

Transcription factors bind specific DNA sequences. In this exercise, you will determine the specific DNA sequence bound by an abundant human transcription factor called CTCF.

Use the CTCF peak calls in the ENCODE data to derive a binding motif for the CTCF transcription factor. **(10 points)** You will need to:

1. Select out the peaks on chr22 (otherwise meme will take a long time to run)
2. create FASTA sequence from the peak calls from the hg19 genome.
3. use MEME to identify motifs from these FASTA sequences. The motifs in the meme output will be ranked according to their significance (i.e. how often this motif would occur in random sequence).

**Report the top 5 high scoring motifs, and compare your most significant motif with what is already known about CTCF binding sites.**

### Problem 2

Use BEDtools to intersect peaks calls from clustered transcription factor binding sites (TFBS) with clustered DNase I peaks. **(20 points)**

1. Identify transcription factor binding sites that do not overlap with DNase I hypersensitive sites.
  1. What transcription factors are represented in these regions? Analyze the BED file output to get this answer. Only analyze the first 500 FASTA records to speed up runtime.
2. Do the converse: identify DNase I hypersensitive sites that do not have corresponding transcription factor peak calls.
  1. What motifs are enriched in this set of hypersensitive sites on chr22? Only analyze the first 500 FASTA records to speed up runtime.
  2. How would you use existing tools to identify similar motifs, ideally ones that are previously associated with a transcription factor?

**Report the factors in the peaks and the top 5 high scoring motifs from each meme analysis.**

### Problem Set Submission

Submit your problem set as a tar file to Canvas ([Problem Set Submission](#)).

## Problem Set 4

**Due date:** 2015 Feb 23 5:00 PM

### Overview

For this quiz you will use BEDTools and command line tools to make some simple summary files and will use ggplot2 to make some plots.

### Problem 1

1. Examine Pol II distribution in a 5 kb window around transcription termination sites (TTSs) at a resolution of 50 bp? Make a plot of the result and save it as a PDF. (**15 points**)
2. Pick one other factor you might expect to find at a TTS and analyze its distribution. You can find bigWig files of ChIP-seq experiments done for a variety of factors at UCSC <sup>13</sup> and the ENCODE <sup>14</sup> website. Make a plot of the result and save it as a PDF. (**15 points**)

### Problem 2

1000 genomes BED file is at:

```
/vol1/opt/data/hg19/1000genomes/snp138.bed.gz
```

- Examine the relationship between SNP density and gene structure. Use 1000Genomes SNP data in BED format, and assume that each SNP in the file is the same in terms of quality (**20 points**).
  - Calculate SNP density (SNPs per bp) for first exons of refGene annotations. (hint: use the -n option of [bed12tobed6](#))
  - Do the same for the introns of each gene. (hint: use [subtract](#) with BED files for genes and exons)
  - Make a histogram of the results (use `geom_hist()`).
  - Add text annotations to the plot with `geom_text()` and `ggtitle`. E.g. how many SNPs were examined? How many exon / intron regions?

### Problem Set Submission

Submit your problem set as a tar file to Canvas ([Problem Set Submission](#)).

<sup>13</sup> ENCODE Project at UCSC Encyclopedia of DNA Elements at UCSC <http://genome.ucsc.edu/ENCODE/>  
<sup>14</sup> Current ENCODE data <https://www.encodeproject.org/>



## Problem Set 5

**Due date:** 2015 Mar 9 5:00 PM

### Overview

For the following problems, generate a Rmarkdown document that explains the workflow and generates the appropriate plots.

### Problem 1

Use BEDTools, dplyr and ggplot2 to analyze some ENCODE data (**25 points**).

1. Load the peaks BED file (*peaks.chr22.bed.gz*) and find the 10 factors that have the largest range in peak width. Inspect a `geom_boxplot()` or `geom_violin()` to support your answer (also add individual points to the plot with `geom_jitter()`).

You should calculate a *min.width* and *max.width* for each transcription factor in the BED file. Then the *range* is the difference between the *max.width* and *min.width*.

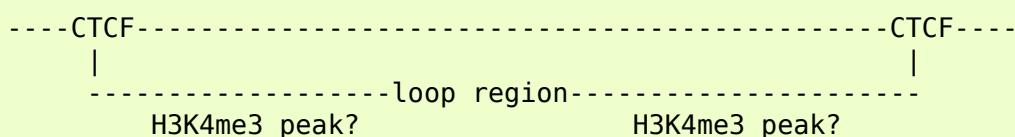
Hint: you can `summarize()` to calculate the *min.width* and *max.width*, and then `mutate()` the summary to add a *range* column.

2. Repeat the above, but only examine peaks do not overlap with any other peak (hint: use the `-c` argument to bedtools `intersect` to find these).

### Problem 2

Use ggplot2 and BEDTools to analyze the following ENCODE data (**25 points**).

Examine ENCODE ChIA-PET and ChIP-seq data to determine what histone marks are enriched in high confidence CTCF-mediated chromatin loops:



1. Download ENCODE ChIA-PET/CTCF and histone ChIP data for the K562 cell line.
2. Determine which histone marks are present in the regions defined by CTCF Chia-PET boundaries (as a control, determine which are outside) use bedtools `intersect` and other bedtools
3. Generate summary statistics for which histone marks are enriched in CTCF loops. use dplyr and ggplot2 for this.

### Problem Set Submission

Submit your problem set as a tar file to Canvas ([Problem Set Submission](#)).

**Problem Set : KEYS**

Problem Set Keys for the Genome Analyais Workshop (MOLB 7621):

**Problem Set 1 : KEY**

Here is bash script that reads in a data table and writes a results.tab file:

```

1 #! /usr/bin/env bash
2 #
3 # run script for quiz 1
4
5 # these are bash flags the print out variables that get set when you
6 # run the script.
7 set -o nounset -o pipefail -o errexit -x
8
9 # You will need to change the '????' strings below.
10 #
11 # define the project variable here. this should be the full path to
12 # your project directory, i.e. the directory at the top of the
13 # results/data/doc directories.
14
15 project="$HOME/problem-set-1"
16
17 # fill in the date here
18 date="2014-02-12"
19
20 # these refer to the data file that you moved into place
21 data=$project/data/$date
22 datafile=$data/states.tab
23
24 # these refer to the place where you will write the results of the
25 # "analysis"
26 results=$project/results/$date
27 resultsfile=$results/result.tab
28
29 # if the directory doesn't exist, make it
30 if [[ ! -d $results ]]; then
31     mkdir -p $results
32 fi
33
34 # Note how we are using redirects here. The first ">" writes a file,
35 # and overwrites existing data. The following ">>" append data to the
36 # existing file
37
38 echo "here is our starting data ..." > $resultsfile
39 cat $datafile >> $resultsfile
40 echo >> $resultsfile
41
42 echo "here are the states sorted by population size ..." \
43     >> $resultsfile
44 sort -k2n $datafile >> $resultsfile
45 echo >> $resultsfile
46
47 echo "here are the states with the highest number of murders ..." >> \
48     $resultsfile
49
50 grep 'Name' $datafile | sort -k6gr $datafile | \
51     head -n 10 >> $resultsfile
52 echo >> $resultsfile
53

```

The output directory structure should look similar to:

```
.
`-- problem-set-1
  |-- data
  |   `-- 2014-02-20
  |       |-- states.tab
  |-- doc
  |-- results
  |   |-- 2014-02-20
  |       |-- results.tab
  |       |-- run.sh
  |-- summary.txt
```

The output of this script looks like this:

	here is our starting data ...	"Population"	"Income"	"Illiteracy"	"Life Exp"	"M"
1	Name	"Population"	"Income"	"Illiteracy"	"Life Exp"	"M"
2	"Alabama"	3615	3624	2.1	69.05	15.1
3	"Alaska"	365	6315	1.5	69.31	11.3
4	"Arizona"	2212	4530	1.8	70.55	7.8
5	"Arkansas"	2110	3378	1.9	70.66	10.1
6	"California"	21198	5114	1.1	71.71	10.3
7	"Colorado"	2541	4884	0.7	72.06	6.8
8	"Connecticut"	3100	5348	1.1	72.48	3.1
9	"Delaware"	579	4809	0.9	70.06	6.2
10	"Florida"	8277	4815	1.3	70.66	10.7
11	"Georgia"	4931	4091	2	68.54	13.9
12	"Hawaii"	868	4963	1.9	73.6	6.2
13	"Idaho"	813	4119	0.6	71.87	5.3
14	"Illinois"	11197	5107	0.9	70.14	10.3
15	"Indiana"	5313	4458	0.7	70.88	7.1
16	"Iowa"	2861	4628	0.5	72.56	2.3
17	"Kansas"	2280	4669	0.6	72.58	4.5
18	"Kentucky"	3387	3712	1.6	70.1	10.6
19	"Louisiana"	3806	3545	2.8	68.76	13.2
20	"Maine"	1058	3694	0.7	70.39	2.7
21	"Maryland"	4122	5299	0.9	70.22	8.5
22	"Massachusetts"	5814	4755	1.1	71.83	3.3
23	"Michigan"	9111	4751	0.9	70.63	11.1
24	"Minnesota"	3921	4675	0.6	72.96	2.3
25	"Mississippi"	2341	3098	2.4	68.09	12.5
26	"Missouri"	4767	4254	0.8	70.69	9.3
27	"Montana"	746	4347	0.6	70.56	5
28	"Nebraska"	1544	4508	0.6	72.6	2.9
29	"Nevada"	590	5149	0.5	69.03	11.5
30	"New_Hampshire"	812	4281	0.7	71.23	3.3
31	"New_Jersey"	7333	5237	1.1	70.93	5.2
32	"New_Mexico"	1144	3601	2.2	70.32	9.7
33	"New_York"	18076	4903	1.4	70.55	10.9
34	"North_Carolina"	5441	3875	1.8	69.21	11.1
35	"North_Dakota"	637	5087	0.8	72.78	1.4
36	"Ohio"	10735	4561	0.8	70.82	7.4
37	"Oklahoma"	2715	3983	1.1	71.42	6.4
38	"Oregon"	2284	4660	0.6	72.13	4.2
39	"Pennsylvania"	11860	4449	1	70.43	6.1
40	"Rhode_Island"	931	4558	1.3	71.9	2.4
41	"South_Carolina"	2816	3635	2.3	67.96	11.6
42	"South_Dakota"	681	4167	0.5	72.08	1.7
43	"Tennessee"	4173	3821	1.7	70.11	11
44	"Texas"	12237	4188	2.2	70.9	12.2
45	"Utah"	1203	4022	0.6	72.9	4.5
46						137

47	"Vermont"	472	3907	0.6	71.64	5.5	57.1	1
48	"Virginia"	4981	4701	1.4	70.08	9.5	47.8	
49	"Washington"	3559	4864	0.6	71.72	4.3	63.5	
50	"West_Virginia"	1799	3617	1.4	69.48	6.7	41.6	
51	"Wisconsin"	4589	4468	0.7	72.48	3	54.5	
52	"Wyoming"	376	4566	0.6	70.29	6.9	62.9	
53								
54	here are the states sorted by population size ...							
55	Name	"Population"	"Income"	"Illiteracy"	"Life Exp"	"M		
56	"Alaska"	365	6315	1.5	69.31	11.3	66.7	1
57	"Wyoming"	376	4566	0.6	70.29	6.9	62.9	1
58	"Vermont"	472	3907	0.6	71.64	5.5	57.1	1
59	"Delaware"	579	4809	0.9	70.06	6.2	54.6	1
60	"Nevada"	590	5149	0.5	69.03	11.5	65.2	1
61	"North_Dakota"	637	5087	0.8	72.78	1.4	50.3	
62	"South_Dakota"	681	4167	0.5	72.08	1.7	53.3	
63	"Montana"	746	4347	0.6	70.56	5	59.2	155
64	"New_Hampshire"	812	4281	0.7	71.23	3.3	57.6	
65	"Idaho"	813	4119	0.6	71.87	5.3	59.5	120
66	"Hawaii"	868	4963	1.9	73.6	6.2	61.9	0
67	"Rhode_Island"	931	4558	1.3	71.9	2.4	46.4	
68	"Maine"	1058	3694	0.7	70.39	2.7	54.7	16
69	"New_Mexico"	1144	3601	2.2	70.32	9.7	55.2	
70	"Utah"	1203	4022	0.6	72.9	4.5	67.3	137
71	"Nebraska"	1544	4508	0.6	72.6	2.9	59.3	
72	"West_Virginia"	1799	3617	1.4	69.48	6.7	41.6	
73	"Arkansas"	2110	3378	1.9	70.66	10.1	39.9	
74	"Arizona"	2212	4530	1.8	70.55	7.8	58.1	
75	"Kansas"	2280	4669	0.6	72.58	4.5	59.9	1
76	"Oregon"	2284	4660	0.6	72.13	4.2	60	44
77	"Mississippi"	2341	3098	2.4	68.09	12.5	41	
78	"Colorado"	2541	4884	0.7	72.06	6.8	63.9	
79	"Oklahoma"	2715	3983	1.1	71.42	6.4	51.6	
80	"South_Carolina"	2816	3635	2.3	67.96	11.6	37.8	
81	"Iowa"	2861	4628	0.5	72.56	2.3	59	140
82	"Connecticut"	3100	5348	1.1	72.48	3.1	56	
83	"Kentucky"	3387	3712	1.6	70.1	10.6	38.5	
84	"Washington"	3559	4864	0.6	71.72	4.3	63.5	
85	"Alabama"	3615	3624	2.1	69.05	15.1	41.3	
86	"Louisiana"	3806	3545	2.8	68.76	13.2	42.2	
87	"Minnesota"	3921	4675	0.6	72.96	2.3	57.6	
88	"Maryland"	4122	5299	0.9	70.22	8.5	52.3	
89	"Tennessee"	4173	3821	1.7	70.11	11	41.8	
90	"Wisconsin"	4589	4468	0.7	72.48	3	54.5	
91	"Missouri"	4767	4254	0.8	70.69	9.3	48.8	
92	"Georgia"	4931	4091	2	68.54	13.9	40.6	6
93	"Virginia"	4981	4701	1.4	70.08	9.5	47.8	
94	"Indiana"	5313	4458	0.7	70.88	7.1	52.9	
95	"North_Carolina"	5441	3875	1.8	69.21	11.1	38.5	
96	"Massachusetts"	5814	4755	1.1	71.83	3.3	58.5	
97	"New_Jersey"	7333	5237	1.1	70.93	5.2	52.5	
98	"Florida"	8277	4815	1.3	70.66	10.7	52.6	
99	"Michigan"	9111	4751	0.9	70.63	11.1	52.8	
100	"Ohio"	10735	4561	0.8	70.82	7.4	53.2	12
101	"Illinois"	11197	5107	0.9	70.14	10.3	52.6	
102	"Pennsylvania"	11860	4449	1	70.43	6.1	50.2	
103	"Texas"	12237	4188	2.2	70.9	12.2	47.4	
104	"New_York"	18076	4903	1.4	70.55	10.9	52.7	
105	"California"	21198	5114	1.1	71.71	10.3	62.6	
106								

```
107 here are the states with the highest number of murders ...
108 "Alabama"      3615      3624      2.1      69.05      15.1      41.3
109 "Georgia"      4931      4091      2        68.54      13.9      40.6
110 "Louisiana"    3806      3545      2.8      68.76      13.2      42.2
111 "Mississippi"  2341      3098      2.4      68.09      12.5      41
112 "Texas"        12237     4188      2.2      70.9       12.2      47.4
113 "South_Carolina" 2816      3635      2.3      67.96      11.6      37.8
114 "Nevada"        590       5149      0.5      69.03      11.5      65.2
115 "Alaska"         365       6315      1.5      69.31      11.3      66.7
116 "Michigan"      9111      4751      0.9      70.63      11.1      52.8
117 "North_Carolina" 5441      3875      1.8      69.21      11.1      38.5
118
```

**Problem Set 2 : KEY**

Here is bash script that reads in a data table and writes a results.tab file:

```

1 #! /usr/bin/env bash
2 #
3 # run script for quiz 2 key
4
5 # these are bash flags the print out variables that get set when you
6 # run the script.
7 # set -o nounset -o pipefail -o errexit -x
8
9 # XXX: note: this will be person-dependent
10 project="$HOME/devel/bio-workshop/workshop-problem-sets/problem-set-2"
11
12 # fill in the date here
13 date="2014-02-12"
14 data=$project/data/$date
15 datafile=$data/lamina.bed
16
17 results=$project/results/$date
18 resultsfile=$results/result.tab
19
20 # if the results directory doesn't exist, make it
21 if [[ ! -d $results ]]; then
22     mkdir -p $results
23 fi
24
25 # Problem 1.1
26 # What is the region with the largest start position (2nd column) on any
27 # chromosome in lamina.bed?
28
29 echo "Answer 1.1" > results.tab
30
31 sort -k2,2nr $datafile \
32     | head -n 1 >> results.tab
33
34 # blank line
35 echo >> results.tab
36
37 # Problem 1.2
38 # What is the region with the largest end position on chrY in lamina.bed?
39 # Report this region in the format: chr12:1234-5678
40
41 echo "Answer 1.2" >> results.tab
42
43 awk '$1 == "chrY"' $datafile \
44     | sort -k2,2nr \
45     | head -n 1 \
46     | awk 'BEGIN {OFS=""} {print $1,":",$2,"-", $3}' \
47     >> results.tab
48
49 echo >> results.tab
50
51 # Problem 2.1
52 # What is the longest region (end - start) in lamina.bed?
53
54 echo "Answer 2.1" >> results.tab
55
56 awk 'BEGIN {OFS="\t"} {print $1, $2, $3, $4, $3-$2}' $datafile \
57     | sort -k5,5nr \

```

```

58      | head -n 1 \
59      >> results.tab
60
61 echo >> results.tab
62
63 # Problem 2.2
64 # What is the longest region (end - start) in lamina.bed with a value (4th
65 # column) greater than 0.9 on chr13. Report the header (1st line) in
66 # lamina.bed as well as the region
67 echo "Answer 2.2" >> results.tab
68
69 # print header line
70 head -n 1 $datafile >> results.tab
71
72 awk 'BEGIN {OFS="\t"} {print $1, $2, $3, $4, $3-$2}' $datafile \
73 | awk '$1 == "chr13" && $4 > 0.9' \
74 | sort -k5,5nr \
75 | head -n 1 \
76 >> results.tab
77
78 echo >> results.tab
79
80 # Problem 3.1
81 # What are the regions that overlap this interval in lamina.bed:
82 # chr12:5,000,000-6,000,000? Report regions so that they are ordered by
83 # descending value (4th column), and the columns are separated by commas
84 # rather than tabs
85
86 echo "Answer 3.1" >> results.tab
87
88 awk '$1 == "chr12" && $2 >= 5e6 && $3 <= 6e6' $datafile \
89 | sort -k4,4gr \
90 | awk 'BEGIN {OFS=","} {print $1,$2,$3,$4}' \
91 >> results.tab
92
93 echo >> results.tab
94
95 # Problem 3.2
96 # What is the average value (4th column) of those regions from lamina.bed
97 # that overlap the region (5 points): chr12:5,000,000-6,000,000?
98
99 echo "Answer 3.2" >> results.tab
100
101 # sum: total of all counts in lines
102 # NR: number of rows that have data
103 # i.e. sum / NR = average
104 awk '$1 == "chr12" && $2 >= 5e6 && $3 <= 6e6' $datafile \
105 | awk '{sum += $4} END {print sum / NR}' \
106 >> results.tab
107
108 echo >> results.tab
109

```

The output of this script looks like this:

1 Answer 1.1			
2 chr1	245647839	247066405	0.948487326246934
3			
4 Answer 1.2			
5 chrY:15475619-19472504			
6			

## Genome Analysis Workshop

```
7 Answer 2.1
8 chr9      38415459      70832281      0.888429752066116      32416822
9
10 Answer 2.2
11 #chrom      start      end      value
12 chr13      66889029      72179378      0.91458944281525      5290349
13
14 Answer 3.1
15 chr12,5294045,5393088,0.923076923076923
16
17 Answer 3.2
18 0.923077
19
```

**Problem Set 3 : KEY**

Here is bash script that runs the analysis:

```

1 #! /usr/bin/env bash
2
3 #BSUB -J ps5
4 #BSUB -o ps5.%J.out
5 #BSUB -e ps5.%J.err
6
7 # run script for Problem Set 5 key
8
9 set -o nounset -o pipefail -o errexit -x
10
11 # XXX this will be user-specific
12 project="$HOME/devel/bio-workshop/_test/problem-set-keys/problem-set-5"
13
14 # input files
15 data="/vol1/opt/data"
16 fasta="$data/hg19.fa"
17 dnase_bed="$data/wgEncodeRegDnaseClusteredV2.bed.gz"
18 ctcf_bed="$data/wgEncodeUwTfbsHelas3CtcfStdPkRep1.narrowPeak.gz"
19 clustered_tfbs_bed="$data/wgEncodeRegTfbsClusteredV3.bed.gz"
20
21 result="$project/results/2014-03-03"
22
23 if [ ! -d $result ]; then
24     mkdir -p $result
25 fi
26
27 # output files
28 ctcf_fasta="$result/ctcf.chr22.fa"
29 ctcf_chr22_bed="$result/ctcf.chr22.bed"
30 tfbs_no_dhs="$result/tfbs_no_dhs.bed"
31 tfbs_no_dhs_summary="$result/tfbs_no_dhs.summary.tab"
32 dhs_no_tfbs="$result/dhs_no_tfbs.bed"
33 dhs_no_tfbs_chr22="$result/dhs_no_tfbs.chr22.bed"
34 dhs_no_tfbs_fasta="$result/dhs_no_tfbs.chr22.fa"
35 problem_1_motifs="$result/problem_1_motifs"
36 problem_2_motifs="$result/problem_2_motifs"
37
38 # Problem 1.1, 1.2
39 zcat $ctcf_bed | awk '$1 == "chr22"' > $ctcf_chr22_bed
40 bedtools getfasta -fi $fasta -bed $ctcf_chr22_bed -fo $ctcf_fasta
41
42 if [[ ! -d $problem_1_motifs ]]; then
43     meme -nmotifs 5 -dna -minw 6 -maxw 20 -maxsize 100000000 \
44         -o $problem_1_motifs $ctcf_fasta
45 fi
46
47 # Problem 2
48 # Identify transcription factor binding peaks that do not overlap with
49 # DNase I hypersensitive sites
50
51 bedtools intersect -a $clustered_tfbs_bed -b $dnase_bed -v -sorted \
52     > $tfbs_no_dhs
53
54 # What transcription factors are represented in these peaks?
55 cut -f4 $tfbs_no_dhs \
56     | sort \
57     | uniq -c \

```

```
58      | sort -k1nr \
59      > $tfbs_no_dhs_summary
60
61 # identify DNase I hypersensitive sites that do not have corresponding
62 # transcription factor peak calls
63 bedtools intersect -a $dnase_bed -b $clustered_tfbs_bed -v -sorted \
64      > $dhs_no_tfbs
65
66 # What motifs are enriched in this set of hypersensitive sites?
67 awk '$1 == "chrY" && ($3 - $2 > 8)' < $dhs_no_tfbs > $dhs_no_tfbs_chr22
68 bedtools getfasta -fi $fasta -bed $dhs_no_tfbs_chr22 -fo $dhs_no_tfbs_fasta
69
70 if [[ ! -d $problem_2_motifs ]]; then
71     meme -nmotifs 10 -dna -minw 6 -maxw 20 -maxsize 100000000 \
72         -o $problem_2_motifs $dhs_no_tfbs_fasta
73 fi
```

**Problem Set 4 : KEY**

Here is bash script that runs the analysis:

## Problem Set Keys

Past keys at [Problem Set : KEYS](#)

## Problem Set Submission

In general, we want one run.sh file that includes all of the code necessary to run the problem set. This run.sh file should create any new directories (ex. a dated directory in the results folder), perform commands (ex. awk, cut, etc), and output results into a well-named file (ex. > \$results/\$date/problem1.txt). For each problem set, you should also create a log file summarizing your results.

Once your problem set is complete, you will need to create a tar file. Specify the root of your project directory and create a tar file of the whole directory like this (change STUDENTID to your student ID):

```
$ projectdir=$HOME/project  
$ tar -cvf STUDENTID-pset1.tar $projectdir
```

On the specific Problem Set assignment page at the Canvas site [15](#), click the Submit Assignment button on the top right and paste the full path to the tarfile (/vol3/home/username/.../foo.tar) in the text box. Click the Submit Assignment button below the text box to complete the submission.

Auditors can e-mail their path to the TAs here: [Instructor Information](#).

## Miscellaneous

General content not tied to specific classes.

### Reference: list of commands

#### cd

change directories:

```
$ cd /tmp/  
$ cd ~ # chage to home directory  
$ cd /vol1/opt/  
$ cd - # change to previous directory
```

#### cp

copy files and directories:

```
$ touch /tmp/asdf  
$ cp /tmp/asdf ~ # copy to home
```

must use -r for directories:

```
$ mkdir /tmp/adir  
$ cp -r /tmp/adir ~
```

#### ctrl+c

interrupt a running process:

```
$ head  
<ctrl+c>
```

#### cut

extract columns from a file:

```
$ cut -f 1-3 /vol1/opt/data/lamina.bed  
$ cut -f 1,3 /vol1/opt/data/lamina.bed  
$ cut -f 1 /vol1/opt/data/lamina.bed  
  
# use comma as delimiter instead of default tab  
$ cut -f 1-3 -d , /path/to/some.csv  
  
# keep all columns after the 1st:  
$ cut -f 2- /vol1/opt/data/lamina.bed
```

#### echo

print some text:

```
$ echo "hello world" | cowsay
```

#### head

show the start of a file:

```
$ head /vol1/opt/data/lamina.bed  
  
# show the first 4 lines  
$ head -n 4 /vol1/opt/data/lamina.bed
```

## grep

To find any instance of *chr5* in the *lamina.bed* file

```
# grep [pattern] [filename]
$ grep chr5 /vol1/opt/data/lamina.bed | head
```

To find all lines that start with a number sign:

```
# The caret (^) matches the beginning of the line
# FYI dollar sign ($) matches the end
$ grep '^#' /vol1/opt/data/lamina.bed
```

To find any line that *does not* start with "chr":

```
# the -v flag inverts the match (grep "not" [pattern])
$ grep -v '^chr' /vol1/opt/data/lamina.bed
```

Find exact matches that are split on words with the *-w* flag:

```
$ grep -w chr1 /vol1/opt/data/lamina.bed | cut -f1 | uniq
```

## less

page through a file:

```
$ less /vol1/opt/data/lamina.bed
```

use "/", "?" to search forward, backward. 'q' to exit.

use '[space]' to go page by page.

## ls

list files and directories:

```
$ ls /tmp/
# show current directory
$ ls

# show current directory (2)
$ ls .

# list files with most recently modified last
$ ls -lhtr

# list files in temp ordered by modification date
$ ls -lhtr /tmp/
```

## man

show the manual entry for a command:

```
$ man head
```

## mkdir

make a directory:

```
$ mkdir /tmp/aa
```

make sub-directories, too:

```
$ mkdir -p /tmp/aaa/bbb/
```

## mv

move a file or directory:

```
$ touch /tmp/aa  
$ mv /tmp/aa /tmp/bb
```

### rm

remove a file or directory:

```
$ touch /tmp/asdf  
$ rm /tmp/asdf  
  
# use -r to remove directory  
$ mkdir /tmp/asdf  
$ rm -r /tmp/asdf
```

### sort

sort a file by selected columns:

```
$ sort -k1n /vol1/opt/data/lamina.bed
```

sort a BED file by chromosome (1st column) as character and then by start (2nd column) as number:

```
$ sort -k1,1 -k2,2n /vol1/opt/data/lamina.bed
```

sort by 4th column as a general number, including scientific notation showing largest numbers first:

```
$ sort -k4,4rg /vol1/opt/data/lamina.bed | head
```

use literal tab ('\t') as the delimiter (default is whitespace):

```
$ sort -t$'\t' -k4,4rg /vol1/opt/data/lamina.bed | head
```

Sometimes we want to get uniq entries with sort -u:

```
$ cut -f 1 /vol1/opt/data/lamina.bed | sort -u
```

will print out the uniq chromosomes represent in the BED file.

### tail

show the end of a file:

```
$ tail /vol1/opt/data/lamina.bed  
# show the last 4 lines  
$ tail -n 4 /vol1/opt/data/lamina.bed
```

### tar

create or untar a .tar.gz file:

```
# -c create -z compress (.gz) -v verbose -f the name  
$ tar -czvf some.tar.gz /tmp/*  
  
# -x untar  
$ tar -zxvf some.tar.gz
```

### uniq

show or count unique or non-unique entries in a file:

```
# count number of times each chromosome appears.  
$ cut -f 1 /vol1/opt/data/lamina.bed | uniq -c  
  
# get non unique entries  
$ cut -f 2 /vol1/opt/data/lamina.bed | uniq -d
```

## Important

`uniq` assumes that the file is `sort-ed` first! To test this, run `uniq` on an unsorted file. What happens?

## wget

get a file from the web:

```
$ wget http://ucd-bioworkshop.github.io/_downloads/states.tab
```

## zless

like less, but for compressed files:

```
$ zless /vol1/opt/data/t_R1.fastq.gz
```

## Redirection (>> and >)

send output to a file:

```
# start a new file
$ echo "hello" > file.txt

# overwrite that file
$ echo "hello!" > file.txt

# append to the file
$ echo "world" >> file.txt
```

## Credits

Several people have contributed to the development and execution of this course:

### Spring 2015

Sally Peach Kyle Smith Charlotte Siska

### Spring 2014

Brent Pedersen Sally Peach Eric Nguyen

## Sample Data files

We will use several example data files throughout the class.

### BED format

Data in BED format contains region information (e.g. single nucleotides or megbase regions) in a simple format <sup>16</sup>:

**Download a sample BED file: lamina.bed**

### FASTA format

FASTA format just contains DNA sequence data; no quality scores:

```
>cluster_2:UMI_ATTCCG      # record name; starts with '>'  
TTTCCGGGGCACATAATCTCAGCCGGCGC  # DNA sequence
```

**Download a sample FASTA file: sample.fa**

### FASTQ format

FASTQ format contains DNA sequence data with quality scores:

```
@cluster_2:UMI_ATTCCG      # record name; starts with '@'  
TTTCCGGGGCACATAATCTCAGCCGGCGC  # DNA sequence  
+  
9C;=;=<9@4868>9:67AA<9>65<=>591  # empty line; starts with '+'  
# phred-scaled quality scores
```

**Download a sample FASTQ file: SP1.fq**

### ENCODE data

All encode data are available at <https://genome.ucsc.edu/ENCODE/downloads.html>

For Problem Set 3, you will need these files on the amc-tesla cluster, available in:

```
/vol1/opt/data
```

Experiment	Target	Cell line	Replicate	File Type	File name
ChIP-seq	Histone H3 Lysine 4 trimethyl (H3K4me3)	HeLa-S3	1	FASTQ	wgEncodeBroadHistoneHeLa s3H3k4me3StdRawDataRep1.fastq.gz
ChIP-seq	CTCF	HeLa-S3	1	narrowPeak	wgEncodeUwT fbsHelas3CtcfStdPkRep1.narrowPeak.gz

Merged TFBS ChIP-seq	all	all	n/a	BED	wgEncodeReg TfbsClustered V3.bed.gz
Merged DNase I hypersensitive sites	all	all	n/a	BED	wgEncodeReg DnaseClustere dV2.bed.gz

## Getting Started

### VPN on Windows computers

Several people have had problems using VPN while on Windows computers with VirtualBox running LinuxMint OS.

Here are Erin Baschal's notes on Mike Campbell's solutions.

### How to fix internet/VPN issue in VirtualBox

Open Network and Sharing Center

Windows 8 - right click on network icon at bottom of screen

Change adapter settings (on left)

Virtual Box Host Adapter (right click)

Properties

Uncheck TCP/IPv6

Double click TCP/IPv4

Use the following DNS server addresses:

140.226.189.35 132.194.70.65

Advanced, DNS tab

Append these DNS suffixes:

ucdenver.pvt

## Programming Tips & Tricks

### Overview

Several things influence how effectively you learn to program, and how well you program once you have mastered the basic ideas. A major issue is your efficiency in actually *using* a computer, and not programming *per se*.

For example, the longer you spend searching for a particular key to type, or surfing around with your mouse, the less time you spend writing, running and debugging programs. Here are several pointers to help you become more efficient at using computers, independent of learning programming languages.

### Learn to type

Hunting and pecking is inefficient, and prevents you from spending your valuable time efficiently. If you're looking at your keyboard, you're *not* looking at the screen, reading and debugging code. Once you have the typing basics down, you should be typing 40-50 words per minute, without ever looking at the keyboard.

There are several good, modern tools <sup>17</sup> to help you master touch-typing.

### Learn to type funny characters

In programming you use a variety of characters that you don't always use typing other kinds of documents. Learn the locations of the following by heart:

- Number sign (for commenting): #
- Dollar sign (for variables): \$
- Underscore (for variable naming): \_
- Parentheses: ()
- Brackets: []
- Curly brackets: {}
- Tilde (i.e. the squiggle; for going \$HOME): ~
- Math symbols ("+", "-", "\*", "/", "=")

### Learn hot keys for window management

**The mouse is your enemy.** Yes, it revolutionized the computer-human interaction. But the more time you spend using your mouse, the less time you spend with your hands on the keyboard and doing useful things.

You can do most things with your keyboard. There are several hot keys you should learn that will maximize your productivity on the computer by minimizing your use of the mouse:

- <Alt>-<Tab> : Flip through windows quickly and effortlessly without ever touching your mouse.
- <Ctrl>-<Page Up/Down> : Switch between Terminal windows on Linux

### Tip

#### Launch your most-used apps automatically during login.

For example, automatically launch four terminal windows and a browser window, without having to click.

### Learn to use a terminal text editor

There are two types of nerds in this world:

- vim users
- emacs users

I'm a vim-user. I can't even log out of an emacs session.

Learning a terminal text editor like vim increases productivity substantially, because it allows you to:

- run the editor within an existing terminal, without opening a new window
- work on multiple documents simultaneously
- syntax highlight your code
- manipulate blocks of text quickly and efficiently

You can run vim from the terminal prompt:

```
$ vim filename.txt
```

To quit a vim session, you need to:

16  
17

BED documentation <http://genome.ucsc.edu/FAQ/FAQformat.html#format1>

- Touch Typing Tutorial : <http://www.typingweb.com/>
- Typing IO : Language specific typing practice <http://typing.io/>

1. enter *command mode* with the colon key
2. write the file
3. quit the program

This can be accomplished by typing:

```
:wq <enter>
```

In your copious spare time, and after you have mastered the basics of shell, Python and R programming, you should take a tutorial <sup>18</sup> on using a terminal text editor.

