



Cold
Spring
Harbor
Laboratory

Advanced Sequencing Technologies & Applications

<http://meetings.cshl.edu/courses.html>



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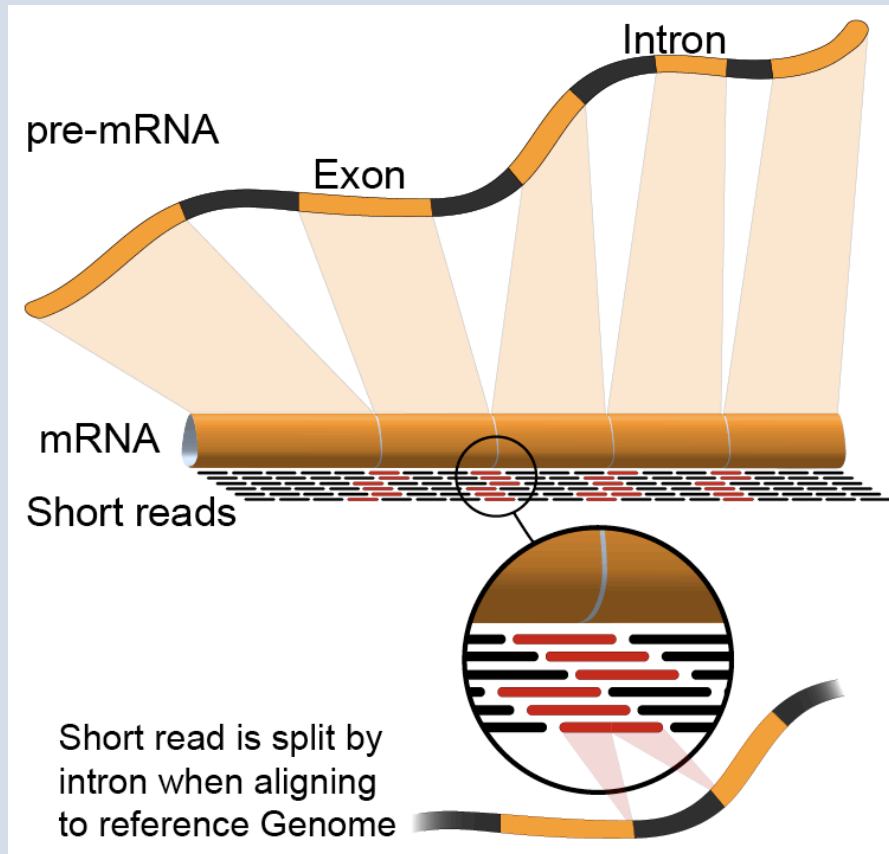
Module 1

Introduction to cloud computing

(slides modified with permission from Francis Ouellette)

Malachi Griffith, Obi Griffith, Jason Walker, Ben Ainscough
Advanced Sequencing Technologies & Applications

November 11-23, 2014



Learning objectives of the course

- **Module 1: Introduction to cloud computing**
- Module 2: Introduction to RNA sequencing
- Module 3: RNA-seq alignment and visualization
- Module 4: Expression and Differential Expression
- Module 5: Isoform discovery and alternative expression
- Tutorials
 - Provide a working example of an RNA-seq analysis pipeline
 - Run in a ‘reasonable’ amount of time with modest computer resources
 - Self contained, self explanatory, portable

Learning Objectives of module 1

- Introduction to cloud computing
- Use of the wiki in this workshop
- How to log into the cloud

Disk Capacity vs Sequencing Capacity, 1990-2012

Disk Storage
(Mbytes/\$)

Stein Genome Biology 2010, 11:207
<http://genomebiology.com/2010/11/5/207>

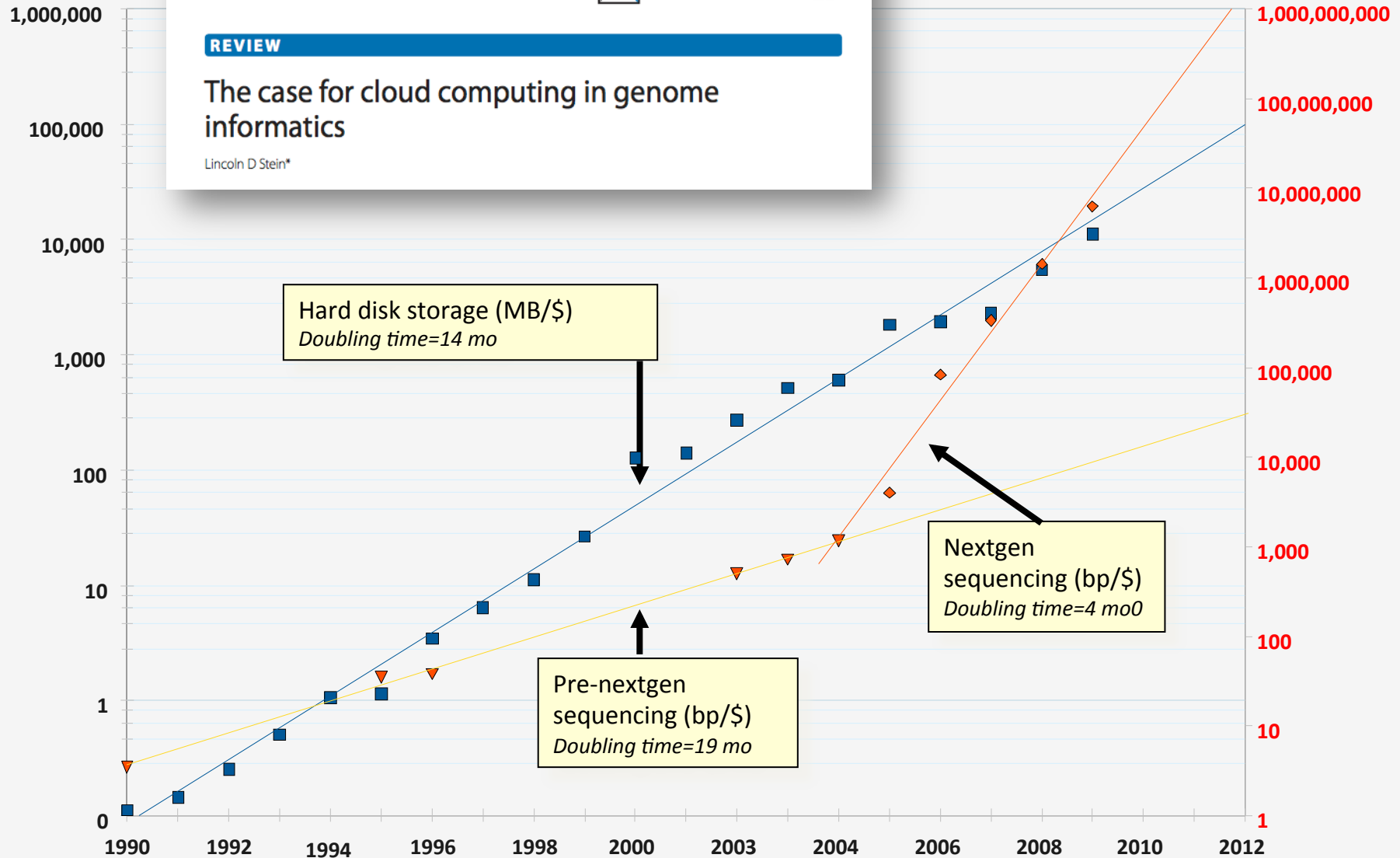


REVIEW

The case for cloud computing in genome informatics

Lincoln D Stein*

DNA
Sequencing (bp/\$)



About DNA and computers

- We'll hit the \$1000 genome during 2014-?, then need to think about the \$100 genome.
- The doubling time of sequencing has been ~5-6 months.
- The doubling time of storage and network bandwidth is ~12 months.
- The doubling time of CPU speed is ~18 months.
- The cost of sequencing a base pair will eventually equal the cost of storing a base pair

What is the general biomedical scientist to do?

- Lots of data
- Poor IT infrastructure in many labs
- Where do they go?
- Write more grants?
- Get bigger hardware?

Amazon Web Services (AWS)

- Infinite storage (scalable): S3 (simple storage service)
- Compute per hour: EC2 (elastic cloud computing)
- Ready when you are High Performance Computing
- Multiple football fields of HPC throughout the world
- HPC are expanded at one container at a time:



<http://goo.gl/7PVAI>



Some of the challenges of cloud computing:

- Not cheap!
- Getting files to and from there
- Not the best solution for everybody
- Standardization
- PHI: personal health information & security concerns
- In the USA: HIPAA act, PSQIA act, HITECH act, Patriot act, CLIA and CAP programs, etc.
 - <http://www.biostars.org/p/70204/>

Some of the advantages of cloud computing:

- We received a grant from Amazon, so supported by 'AWS in Education grant award'.
- There are better ways of transferring large files, and now AWS makes it free to upload files.
- A number of datasets exist on AWS (e.g. 1000 genome data).
- Many useful bioinformatics AMI's (Amazon Machine Images) exist on AWS: e.g. cloudbiolinux & CloudMan (Galaxy) – now one for this course!
- Many flavors of cloud available, not just AWS

In this workshop:

- Some tools (data) are
 - on your computer
 - on the web
 - on the cloud.
- You will become efficient at traversing these various spaces, and finding resources you need, and using what is best for you.
- There are different ways of using the cloud:
 1. Command line (like your own very powerful Unix box)
 2. With a web-browser (e.g. Galaxy): not in this workshop

Things we have set up:

- Loaded data files to an ftp server
- We brought up an Ubuntu (Linux) instance, and loaded a whole bunch of software for NGS analysis.
- We then cloned this, and made separate instances for everybody in the class.
- We've simplified the security: you basically all have the same login and file access, and opened ports. In your own world you would be more secure.

Amazon AWS Management Console – quick walkthrough

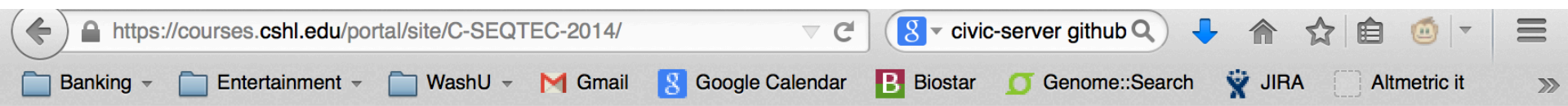
<http://aws.amazon.com/console/>

For this workshop: all on Wiki!

<https://courses.cshl.edu/portal/site/C-SEQTEC-2014/>

Login: email address

Password: password

A screenshot of a login form titled 'Login Required'. The form has two input fields: 'user id' with the value 'ogriffit@genome.' and 'password' with masked characters '.....'. There is a 'Login' button next to the password field. The form is overlaid on a background image of a DNA double helix.

Wiki

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Home

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Home last modified by Malachi Griffith on November 17, 2014 2:30:38 PM EST

Welcome

This is the wiki page for the next-generation sequencing course (Advanced Sequencing Technologies & Applications) at Cold Spring Harbor Laboratory, November, 2014. Essential course information will be posted on this site. Importantly, not only instructors and TAs but also students will be able to edit this web site. Please see information below.

Announcements

Sequencing course schedule highlights

Cold Spring Harbor Advanced Sequencing Technologies and Applications Course 2014: 10th through 23th November

► [2014 Course Schedule, v2 \(pdf\)](#)

17th November (Monday)

- 9:00am - 12noon: Assembly (Mike Schatz)
- LUNCH
- 1:00pm - 5:00pm: Structural Variant (SV) discovery (Ira Hall)
- DINNER
- RNA-seq Begins. * [RNA-seq Wiki](#)
- 7:00pm - 7:30pm: Module 0 - Introduction to Cloud Computing (Malachi Griffith)
- 7:30pm - 8:00pm: Module 0 - Introduction to Cloud Computing (Obi Griffith)
 - NOTICE: Download AWS key file here: [CSHLRNA.pem](#)
- BREAK (30 min break to troubleshoot cloud issues)
- 8:30pm - 10pm: Module 1 - Introduction to RNA-sequencing (Malachi Griffith)

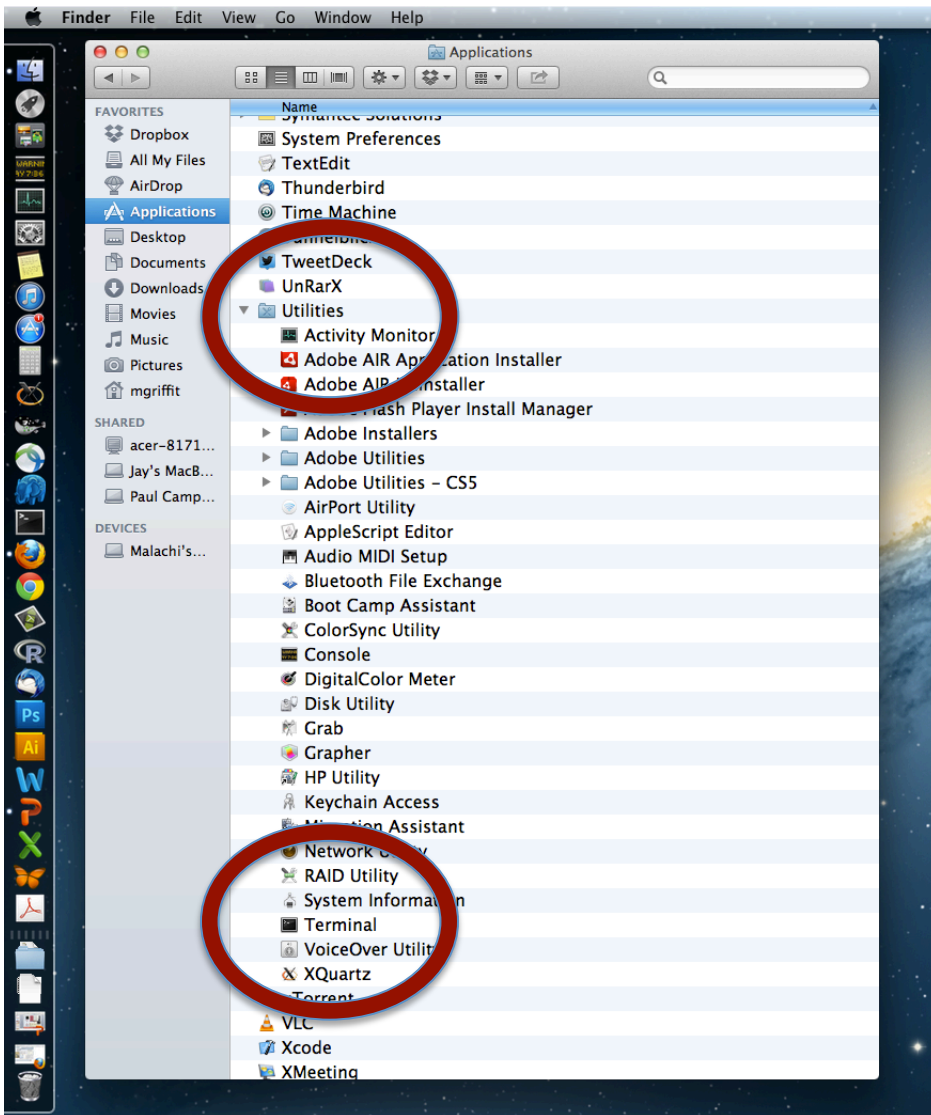
On the CSHL wiki:

- Link to public github wiki for RNAseq
- Key file for AWS

Macintosh users



Opening a 'terminal session'



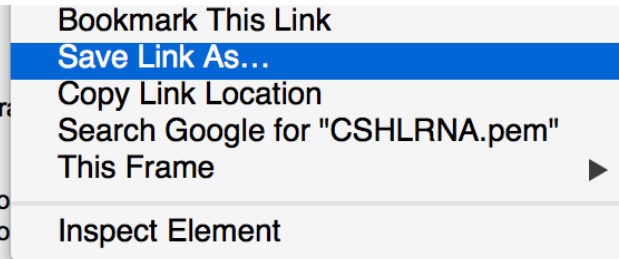
```
[mgriffit@Malachis-MacBook-Pro-2:~]$ pwd
/Users/mgriffit
[mgriffit@Malachis-MacBook-Pro-2:~]$ ls -l
total 16
drwxr-xr-x  2 mgriffit  staff   68 Jan  8 23:27 Applications
drwx-----+ 8 mgriffit  staff  272 Jun  1 15:58 Desktop
drwx-----+ 7 mgriffit  staff  238 Apr 17 22:00 Documents
drwx-----+ 12 mgriffit  staff  408 Jun  1 15:38 Downloads
drwx-----@ 12 mgriffit  staff  408 Jun  1 15:13 Dropbox
drwx-----@ 51 mgriffit  staff 1734 Feb 27 10:00 Library
drwx-----+ 4 mgriffit  staff  136 May 30 2012 Movies
drwx-----+ 4 mgriffit  staff  136 Mar  9 2012 Music
drwx-----+ 8 mgriffit  staff  272 Dec  9 21:39 Pictures
drwxr-xr-x+ 5 mgriffit  staff  170 Mar  5 2012 Public
-rwxr-xr-x  1 mgriffit  staff  594 Mar  6 2012 addSSHkey.sh
drwxr-xr-x 16 mgriffit  staff  544 Jan 17 21:42 backup
drwxr-xr-x  5 mgriffit  staff  170 May  7 18:32 git
drwxr-xr-x  6 mgriffit  staff  204 Mar  6 2012 igv
-rwxr-xr-x  1 mgriffit  staff  552 Dec  3 21:53 mac2unix
drwxr-xr-x  3 mgriffit  staff  102 Jun  1 15:55 notes
drwxr-xr-x 95 mgriffit  staff 3230 Dec 29 23:42 temp
drwxr-xr-x  3 mgriffit  staff  102 Oct 27 2012 workspace
[mgriffit@Malachis-MacBook-Pro-2:~]$ mkdir cbw
[mgriffit@Malachis-MacBook-Pro-2:~]$ cd cbw
[mgriffit@Malachis-MacBook-Pro-2:cbw]$ ls -la
total 0
drwxr-xr-x  2 mgriffit  staff   68 Jun  1 16:03 .
drwxr-xr-x+ 64 mgriffit  staff 2176 Jun  1 16:03 ..
[mgriffit@Malachis-MacBook-Pro-2:cbw]$
```

Creating a working directory on your mac

```
vpn-10-1-24-10:~ ogriffit$ pwd
/Users/ogriffit
vpn-10-1-24-10:~ ogriffit$ ls -l
total 0
drwxr-xr-x    2 ogriffit  staff    68 May 16  2013 Applications
drwxr-xr-x   331 ogriffit  staff  11254 Nov 17 14:59 Attachments
drwx-----    5 ogriffit  staff   170 Sep 29 16:32 Box Sync
drwx-----+  18 ogriffit  staff   612 Nov 12 12:34 Desktop
drwx-----+   7 ogriffit  staff   238 Jul 24 17:41 Documents
drwx-----+ 157 ogriffit  staff  5338 Nov 17 15:54 Downloads
drwx-----@  37 ogriffit  staff  1258 Nov 15 13:15 Dropbox
drwx-----@  63 ogriffit  staff  2142 Oct 18 18:27 Library
drwx-----+   4 ogriffit  staff   136 May 31  2013 Movies
drwx-----+   5 ogriffit  staff   170 Aug 24  2013 Music
drwx-----+   5 ogriffit  staff   170 Jun 30 13:39 Pictures
drwxr-xr-x+   4 ogriffit  staff   136 May 16  2013 Public
drwxr-xr-x    7 ogriffit  staff   238 Oct 22 14:32 Sync
drwxr-xr-x    3 ogriffit  staff   102 May 20 00:49 VirtualBox VMs
drwxr-xr-x   10 ogriffit  staff   340 Nov  2 17:44 bin
drwxr-xr-x   13 ogriffit  staff   442 Nov 11 01:39 git
drwxr-xr-x    6 ogriffit  staff   204 May 30  2013 igv
drwxr-xr-x    3 ogriffit  staff   102 Nov  2 17:45 lib
vpn-10-1-24-10:~ ogriffit$ mkdir cshl
vpn-10-1-24-10:~ ogriffit$ cd cshl/
vpn-10-1-24-10:cshl ogriffit$ ls -la
total 0
drwxr-xr-x    2 ogriffit  staff    68 Nov 17 16:20 .
drwxr-xr-x+  50 ogriffit  staff  1700 Nov 17 16:20 ..
vpn-10-1-24-10:cshl ogriffit$
```

Obtaining your AWS 'key' file from the wiki

- 9:00am - 12noon: Assembly (Mike Schatz)
- LUNCH
- 1:00pm - 5:00pm: Structural Variant (SV) discovery (Ira)
- DINNER
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- BREAK (30 min break to troubleshoot cloud issues)
- 8:30pm - 10pm: Module 1 - Introduction to RNA-sequencing (Malachi Griffith)



On Mac:
Control+

```
vpn-10-1-24-10:~ ogriffit$ mkdir cshl
vpn-10-1-24-10:~ ogriffit$ cd cshl/
vpn-10-1-24-10:cshl ogriffit$ ls -la
total 0
drwxr-xr-x  2 ogriffit  staff   68 Nov 17 16:20 .
drwxr-xr-x+ 50 ogriffit  staff 1700 Nov 17 16:20 ..
vpn-10-1-24-10:cshl ogriffit$ ls -la
total 8
drwxr-xr-x  3 ogriffit  staff  102 Nov 17 16:22 .
drwxr-xr-x+ 50 ogriffit  staff 1700 Nov 17 16:20 ..
-rw-r--r--@  1 ogriffit  staff 1692 Nov 17 16:22 CSHLRNA.pem
vpn-10-1-24-10:cshl ogriffit$
```

Viewing the 'key' file once downloaded

```
vpn-10-1-24-10:csHL ogriffit$ ls -la
total 8
drwxr-xr-x  3 ogriffit  staff   102 Nov 17 16:22 .
drwxr-xr-x+ 50 ogriffit  staff  1700 Nov 17 16:20 ..
-rw-r--r--@ 1 ogriffit  staff  1692 Nov 17 16:22 CSHLRNA.pem
vpn-10-1-24-10:csHL ogriffit$ cat CSHLRNA.pem
-----BEGIN RSA PRIVATE KEY-----
MIIEowIBAAKCAQEAljC42AmfGYOH8lDgDHUK3gbdFFh8UQuphRF96U0Fp0u9Jzre43U5S106vVhU
MvRNN4lmhhACKQb0aTTpZ458eDyXYUrf+1veIMxJzqN76e9/i4VN4BNJMaK9vr4jFUhM4ZH0pFKQ
9gn8T/hFicX+vNPTLVwHWqUJRNI8QLBzxW2Ba16gzAik6H9s05ahnR616jNCzj2DQ1wXT07kpoS3
X7xmcn/GvpzLteVkb800KXnPsNbg8K+W4Ca4V2ZzDdLj7YaZJ98/IYRv5HjyB0nMIIt9/Z34IuDFG
PLKgNLGT1+FLaczfbkMoUo47xHa435YKuC9L6U0PRMjyD2nNax0FJwIDAQABAOIBAD9P/Kv9qlcM
DoQEud4mfNVJ2WqZPpa/rs+MpyJx0J7kTZG7DHigUu3F0FzXXm84c1ClGFYNa7eNUf0DtBzWgPwC
tuHpuW/xszrqQ3bbjgH41zC0mlyKZMGK1CgTaSCwoNA6QgH/WGKPPRBhZNNpuwc85ncgLEifzzJ
jNpMSBGCSJ0cF9Th7Sf0orFgm7Zlowg/rNVyHg76rLWXJ3TSjc0I7VNHncaNIX88LATr0uotXwWQ
UjbnyLQWQquSsFpTeoF9RjRNcrkQlJce+9stijs0Sf1qSxsCXuDUR7Kcaxs9s7S7Uo6osggHf+AR
e4P0qhNc5JGhAJJMxLZC3TW66+ECgYEA30F2lRE/zZP+RbzhQ7tBjILUC0dXAlQPAXwAxVqTXDNA
FE/e63+q+5D7Mxp0BedU+60GjJr5Sm4PcHf0n0qZMLThlf7P78H4UYUPBxjhmgkLYAsQdBcmKaqK
FUrLB009HZFIPIvLKC0JYrX3/Nup1BbsfhGiyN0zRDgDTTrLHY3ECgYEArhHw0n8x+edb1NAPtKsc
VY8LLhodUYJLeotQnQDivN4WGwk0t960s0a7CL75yJEEFFLaqwTYWxssZEHzl4/pTqeJUXbrH/sK
0eaVzZVZ9S1nSb0SqqNmFuJuS2ASrc+MGxF38FKnYhzGoVVG6KphfMQq2fByeC9c5406yUCtdhcC
gYEAwJtqiDpuZJm0lKNL24AifKBovx0KZmFeZdu5YF7HORMHwa6U0M8vDgcyxTFAExXSKUxD08C3
uYuXsv8GEBcSVkKko+N69xsxqgRZQlsT/vn8DVgYl0K0dyE7H64bY06daHcTgpiKzynWkVkuUk5K
HU0QHa7LQFSDK+2cFLgY3kECgYAA4aB21hwQ13A/4/V91PIe4/fp8fP/lKti0pDKCPgg7dagqKKiJ
sLgDRjdTlTcyFDQDNfogExjdJQgUkP6TrZHH6ChqWqPEoEAYVqVSkkQtXAItdfR3XVSWs9dT25UR
98CKAPYhzVbqRzLiktTAjn26xYKxTuZ5JbfwWdKMZsYPDwKBgHLZZD3abGgGVghW+yZ3/7ZDqHor
sUwSKvZ4B3VT6uK02AmRDwjeHesbVSaAFyEzWWYMgaGfZGToAqJbTejov5E/GC11HlUVJyqx2he7
M5WCh3y6PKUknebocJWhn1AA80hvd+YBapX3Lq+AkfcvzKV2+IXNjRd3prARS2tUqDbm
-----END RSA PRIVATE KEY-----vpn-10-1-24-10:csHL ogriffit$
vpn-10-1-24-10:csHL ogriffit$ _
```

Changing file permissions of your 'key' file

ls -l (long listing)

```
drwx-----+ 67 francis staff 2278 22 May 21:25 ../
-rw-r--r--@ 1 francis staff 1696 22 May 21:31 CBWRNA.pem
```

 rwx : owner
 rwx : group
 rwx: world

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

Which ever way you add these 3 numbers, you know which integers were used (6 is always 4+2, 5 is 4+1, 4 is by itself, 0 is none of them etc ...)

So, when you have:

chmod 600 <file name>

It is "rw" for the the file owner **only**

Logging in to AWS

- Make sure the permissions on your certificate are secure. Use `chmod` on your downloaded certificate:

```
chmod 600 CSHLRNA.pem
```

- To log in to the node, use the `-i` command line argument to specify your certificate:

```
ssh -Y -i CSHLRNA.pem ubuntu@cshl##.dyndns.org
```



Use your assigned student #

`##` is your assigned student number. Your student number is the number on the participant list. If your number is less than 10, please add 0 in front of it. `-i` selects a file from which the public key authentication is read. `ubuntu` is the name of a user on the system you are logging into (a default user of the Ubuntu operating system).

`cshl##.dyndns.org` is the address of the linux system on Amazon that you are logging into.

Copying files from AWS to your computer

- To copy files from a node, use scp in a similar fashion (in this case to copy a file called nice_alignments.bam):

```
scp -i CSHLRNA.pem ubuntu@cshl##.dyndns.org:workspace/nice_alignments.bam .
```

- Everything created in your workspace on the cloud is also available by a web server on your cloud instance. Simply go to the following in your browser:

<http://cshl##.dyndns.org/>

So, at this point:

- Your laptop is ready for the workshop
- If it is not, you know where to get the information you need
- You know how to use the wiki for this workshop
- You know where all of the lectures are
- You have read all of the pre-lecture material
- If not, you know where the papers are, and you are a speed reader
- You know how to login to AWS

Break