

Canadian Bioinformatics Workshops

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English French

RNA-Seq Module 0 Introduction to Cloud Computing

Malachi Griffith and Obi Griffith Modification of slides from Francis Ouellette Informatics for RNA-seq Analysis May 28-30, 2018







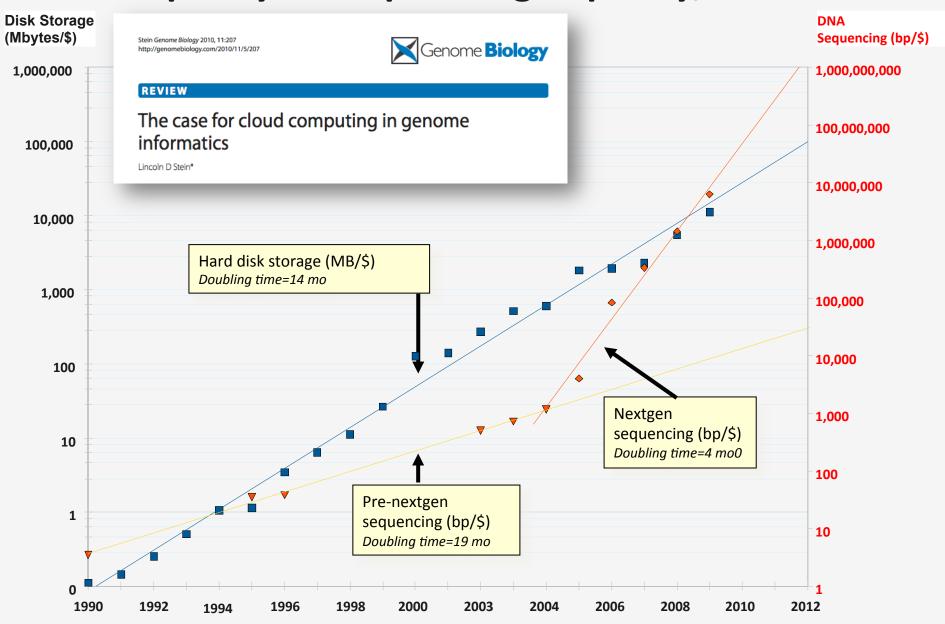
Learning objectives of the course

- Module 0: Introduction to Cloud Computing
- Module 1: Introduction to RNA Sequencing
- Module 2: Alignment and Visualization
- Module 3: Expression and Differential Expression
- Module 4: Alignment Free Expression Estimation
- 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

- Introduction to cloud computing concepts
- Introduction to cloud computing providers
- Use the Amazon EC2 console to create an instance for each student
 - Will be used for many hands-on tutorials throughout the course
- How to log into your cloud instance

Disk Capacity vs Sequencing Capacity, 1990-2012



About DNA and computers

- We hit the \$1000 genome* in ~2016
 - 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?

Cloud computing providers

- Amazon AWS
 - https://aws.amazon.com/
- Google cloud
 - https://cloud.google.com/
- Digital ocean
 - https://www.digitalocean.com/
- Microsoft Azure
 - https://azure.microsoft.com/en-us/
- More...

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:





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

Key AWS concepts and terminology

- AWS Amazon Web Services. A collection of cloud computing services provided by Amazon.
- EC2 Elastic Compute. An AWS service that allows you to configure and rent computers to meet your compute needs on an as needed basis.
- EBS Elastic Block Storage. A data storage solution that allows you to rent disk storage and associate that storage with your compute resources. EBS volumes are generally backed by SSD devices.

Key AWS concepts and terminology

- S3 Simple storage service. Cheaper than EBS and allows for storage of larger amounts of data with some drawbacks compared to EBS. S3 volumes store data as objects that are accessed by an API or command line interface or other application designed to work with S3. EBS volumes on the other hand can be mounted as if they were a local disk drive associated with the Instance.
- SSD Solid state drive. A particular type of storage hardware that is generally faster and more expensive than traditional hard drives.

Key AWS concepts and terminology

- HDD Hard disk drive. A particular type of storage hardware that is generally cheaper and larger but slower than SSD. HDD drives are traditional hard drives that access data on a spinning magnetic disk.
- Ephemeral storage Also known as Instance Store storage. Data storage associated with an EC2 instance that is local to the host computer. This storage does not persist when the instance is stopped or terminated. In other words, anything you store in this way will be lost if the system is stopped or terminated. Instance store volumes may be backed by SSD or HDD devices.

What is a Region?

- An AWS Region is set of compute resources that Amazon maintains (like the Data Center image shown before)
- Each Region corresponds to a physical warehouse of compute hardware (computers, storage, networking, etc.).
- At the time of writing there are 14 regions: (US East (N.Virginia), US East (Ohio), US West (Oregon), US West (N. California), Canada (Central), EU (Ireland), EU (Frankfurt), EU (London), Asia Pacific (Singapore), Asia Pacific (Sydney), Asia Pacific (Seoul), Asia Pacific (Tokyo), Asia Pacific (Mumbai) and South America (Sao Paulo).
- When you are logged into the AWS EC2 console you are always operating in one of these regions.

What is a Region?

- Current region shown in the upper right corner of console
- It is important to pay attention to what region you are using for several reasons.
 - When you create an EC2 instance (EBS volume, etc) in one region you won't see it in another region.
 - The cost to use many AWS resources varies by region.
 - The region may influence network performance when you are accessing the instance, especially if you need to transfer large amounts of data in or out.
 - Billing is tracked separately for each region
 - Generally you should choose a region that is close to you or your users. But cost is also a consideration.

What is difference between the 'Start', 'Stop', 'Reboot', and 'Terminate' (Instance States)?

- Start turn on an EC2 instance that you have previously created
- Stop turn off an EC2 instance that you have previously created
- Reboot restart an EC2 instance
- Terminate permanently stop and destroy an EC2 instance. Any associated EBS volumes may also be destroyed at this time depending on configuration

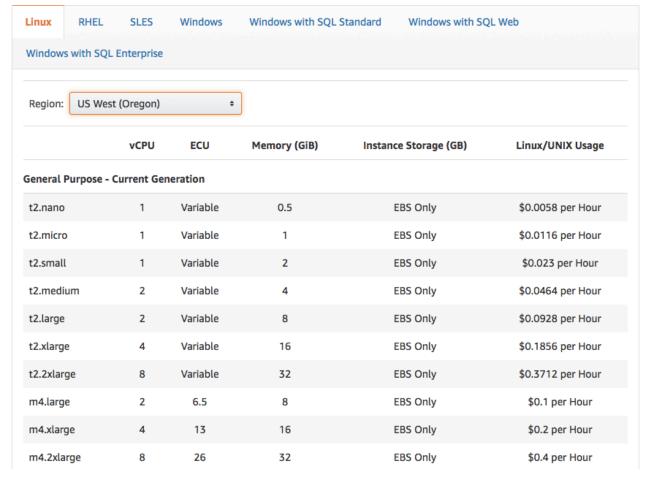
What is an AMI/snapshot?

- AMI (Amazon Machine Image) a template that specifies how to launch EC2 instances
 - Root volume with operating system (OS), pre-installed applications, etc
 - Launch permissions the determine who can use the AMI
 - Specification of (data) volumes to attach when launched
- You can create an AMI for any instance you have created/configured
- AMI can be made public for sharing (region-specific)
- Creating an AMI involves creating a snapshot of the root and any attached volumes. You will be charged to store this snapshot.

I can not log into my EC2 instance, what might have gone wrong?

- Is your instance running?
- Are you providing the correct path to your key file?
- Is it the correct key file?
- Have you set the permissions for your key file correctly?
- Did you specify a valid user for your AMI (e.g., ubuntu)?
- Did you specify the correct IP address?
- Does the Security Group for the instance allow access for your connection protocol (e.g., SSH) and location?

How much does it cost to use AWS EC2 resources?



Data transfer (GB): In: free or \$0.01; Out: free, \$0.01 or \$0.02 EBS storage (GB/Month): \$0.10

S3 storage (GB/Month): \$0.023 standard, \$0.0125 infrequent access, or \$0.004 glacier

Why am I still getting a monthly bill?

- Generally you get an accounting of usage and cost on a 30 day cycle
 - Pricing is per instance-hour (now instance-second!) consumed for each instance type.
 - Also charges for storage, transfers, etc
- Be aware of regions!
- Even when an instance is stopped, storage for root or other EBS volumes persist
- Creating AMIs/snapshots requires storage
- Explore the billing and cost management tools of AWS to track your spending, set warnings, etc

Amazon AWS documentation

https://github.com/griffithlab/rnaseq_tutorial/wiki/Introto-AWS-Cloud-Computing

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

In this workshop:

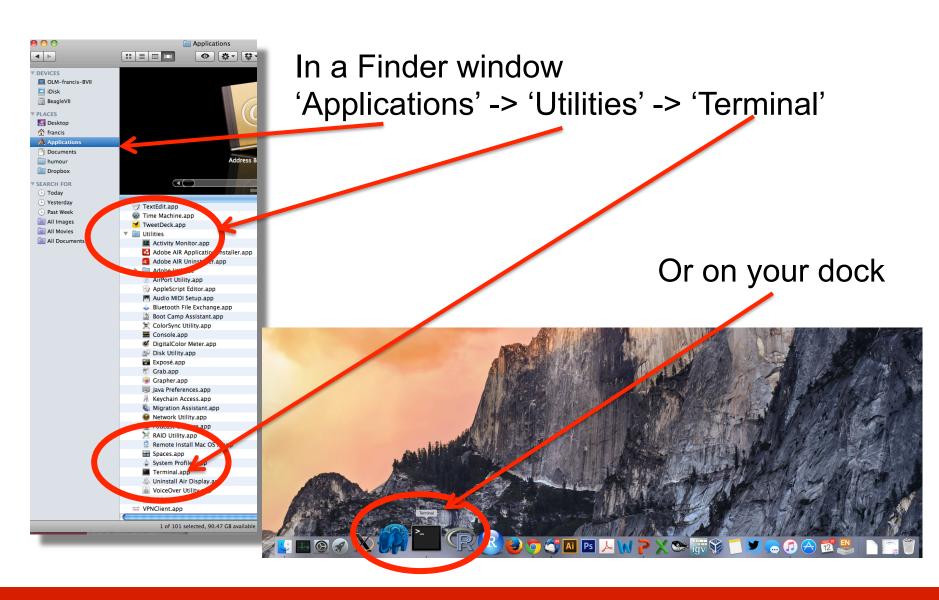
- Some tools (data) are
 - on your computer
 - on the web
 - on the cloud.
- You will become efficient at traversing these various spaces, and finding resources you need, and using what is best for you.
- There are different ways of using the cloud:
 - Command line (like your own very powerful Unix box)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

Things we have set up:

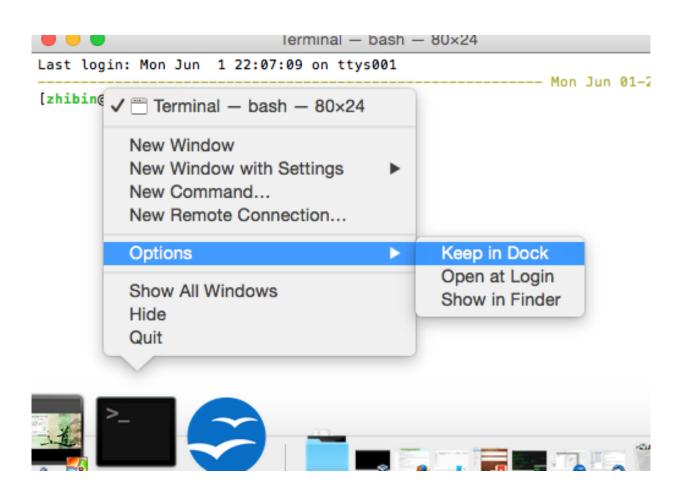
- Loaded data files to a web server
- We brought up an Ubuntu (Linux) instance, and loaded a whole bunch of software for NGS analysis.
- We will clone this and create separate instances for everybody in the class.
- We've simplified the security: you basically all have the same login and file access,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

Logging into Amazon AWS

Opening a 'terminal session' on a Mac



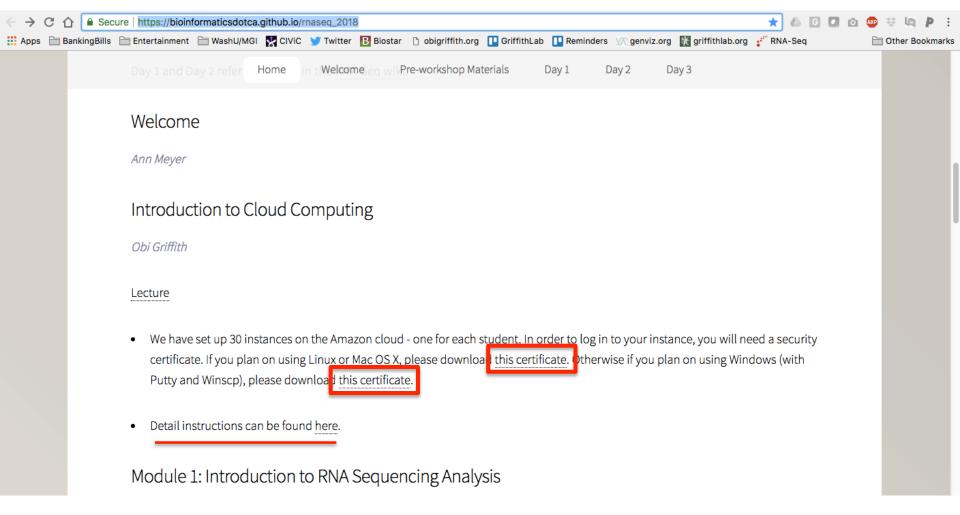
Add the terminal App to your dock



Creating a working directory on your Mac called 'cbw'

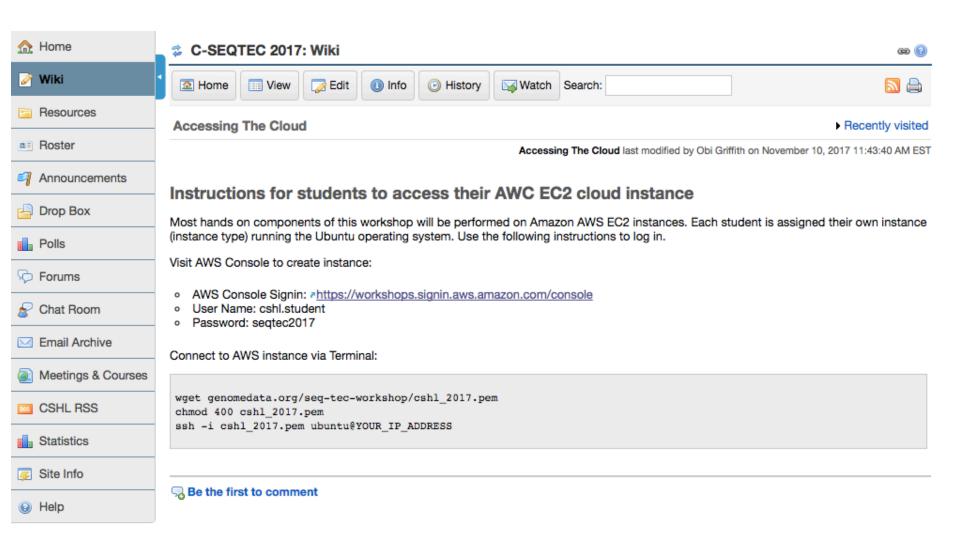
```
obis-air:~ ogriffit$ pwd
/Users/ogriffit
obis-air:~ ogriffit$ ls
Applications
               Desktop
                                Dropbox
                                               Movies
                                                                Public
                                                                               gittemp
                                                                                               temp
Attachments
                               Google Drive
                                               Music
                Documents
                                                                bin
                                                                               igv
Box Sync
               Downloads
                                Library
                                               Pictures
                                                                               ncbi
                                                                git
obis-air:~ ogriffit$ mkdir cshl
obis-air:~ ogriffit$ cd cshl
obis-air:cshl ogriffit$ ls -la
total 0
            2 ogriffit staff
                                  68 Nov 13 22:18 .
drwxr-xr-x
                                                                 mkdir cbw
drwxr-xr-x+ 58 ogriffit staff
                               1972 Nov 13 22:18 ...
obis-air:cshl ogriffit$
                                                                 cd cbw
```

Go to course wiki, "Accessing the cloud" page Download the certificate



https://bioinformaticsdotca.github.io/rnaseq 2018

Go to course wiki, "Accessing the cloud" page



Login to AWS console

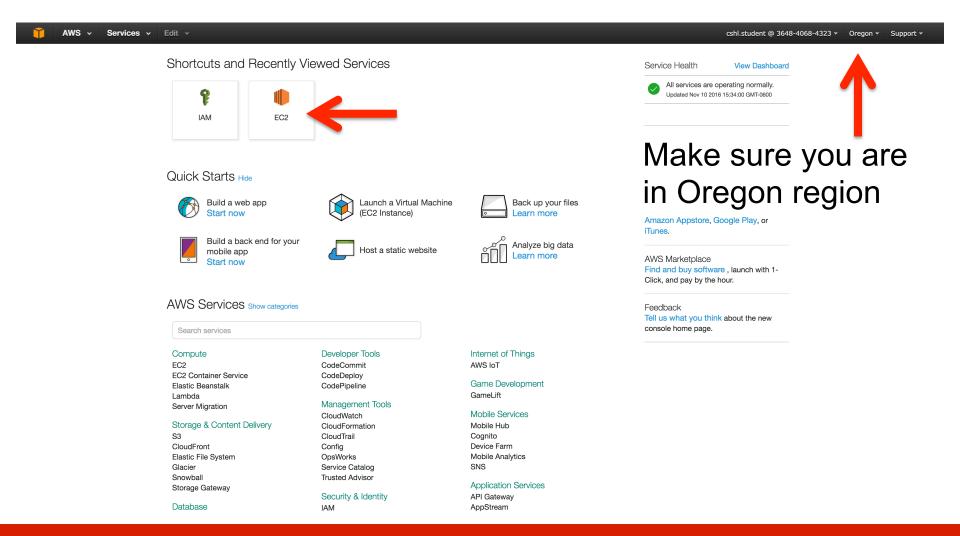


Coming Soon: Changes to Multi-Factor Authentication (MFA)
 Entry of an MFA security code for IAM users will move from this sign-in page to a subsequent page

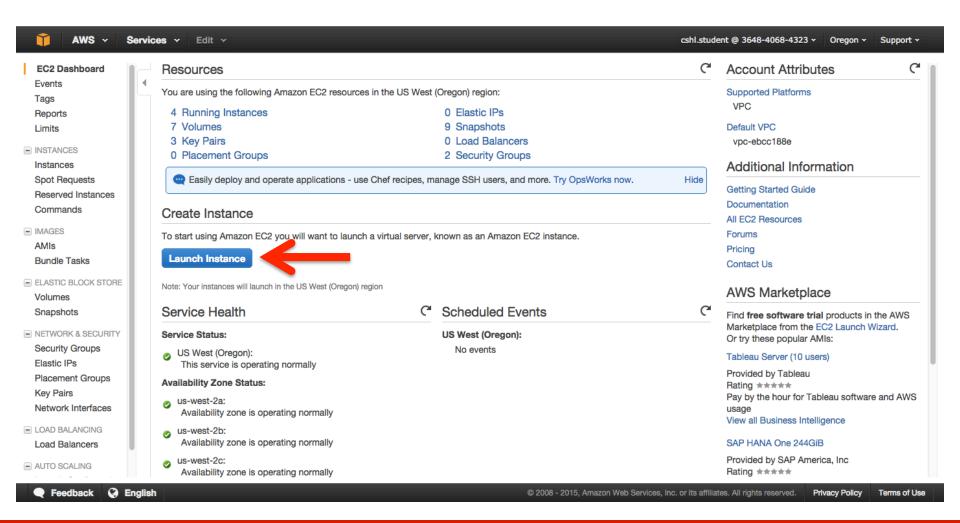
Account:	364840684323
User Name:	cshl.student
Password:	•••••
	☐ I have an MFA Token (more info)
	Sign In
	Sign-in using root account credentials

https://workshops.signin.aws.amazon.com/console

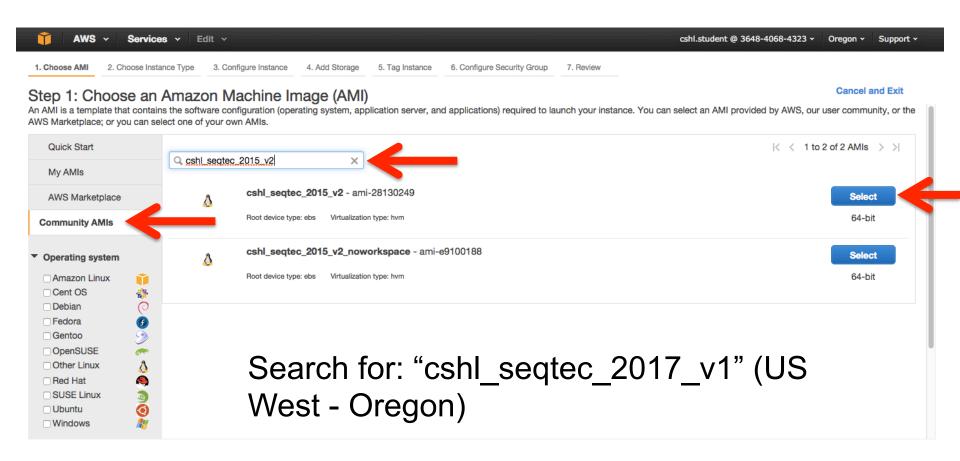
Select "EC2" service



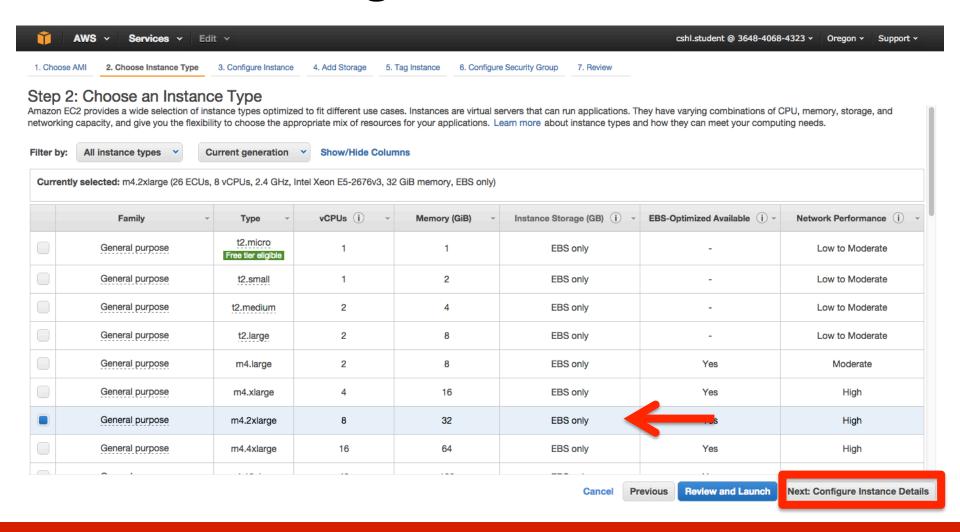
Launch a new Instance



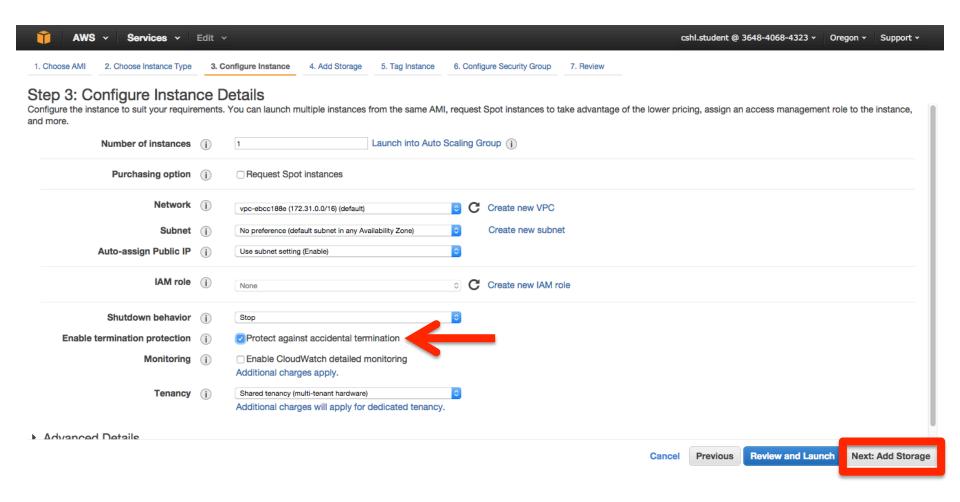
Choose an AMI – Find the CSHL SEQTEC 2017 AMI in the Community AMIs



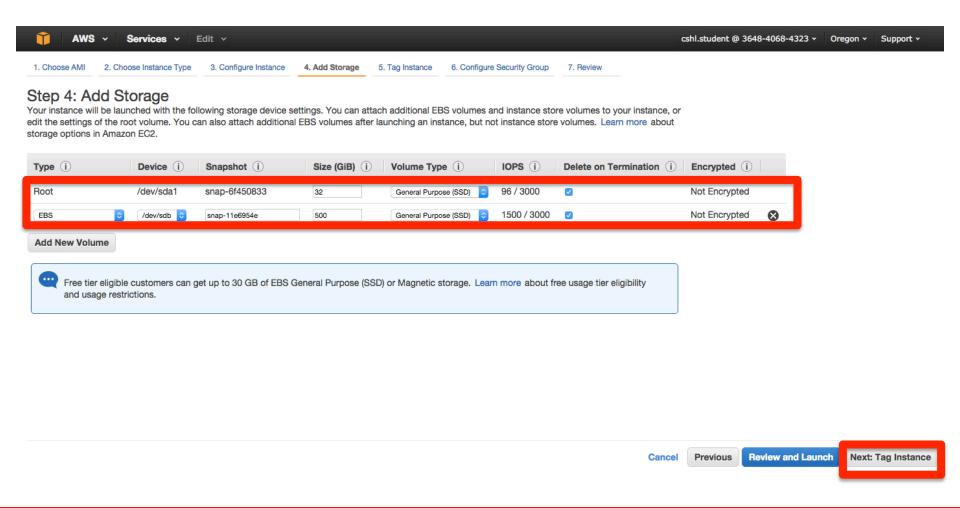
Choose "m4.2xlarge" instance type, then "Next: Configure Instance Details".



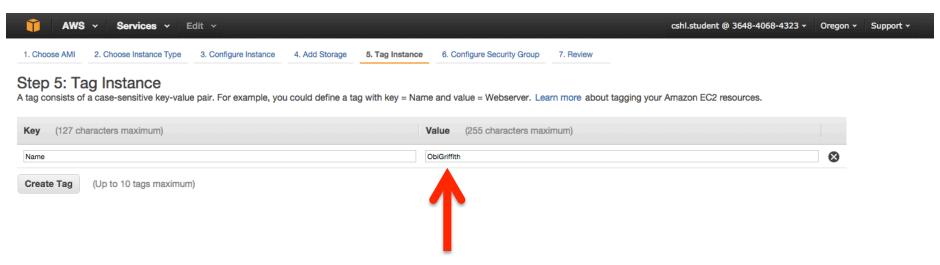
Select "Protect against accidental termination", then "Next: Add Storage".



You should see "snap-xxxxxxxx" (32GB) and "snap-xxxxxxx" (250GB) as the two storage volumes selected. Then, "Next: Tag Instance"



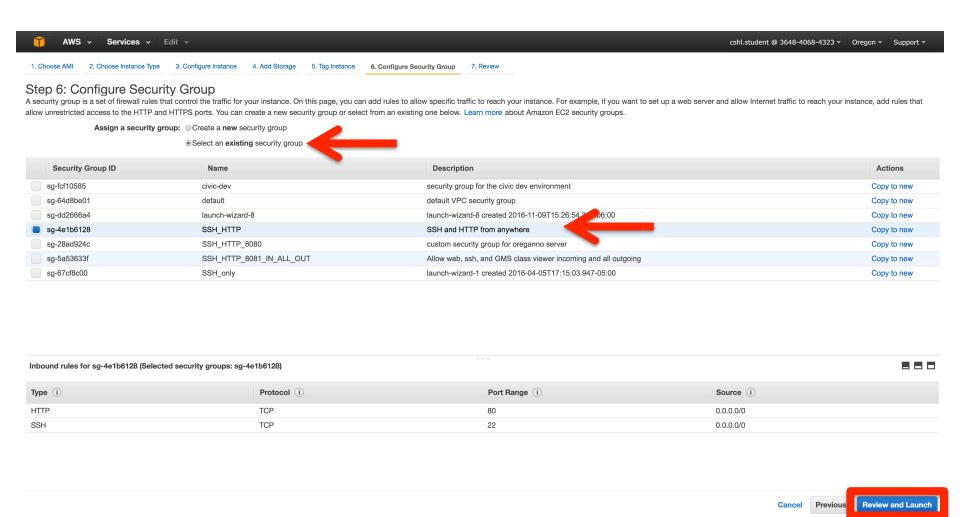
Create a tag like "Name=ObiGriffith" [use your own name]. Then hit "Next: Configure Security Group".



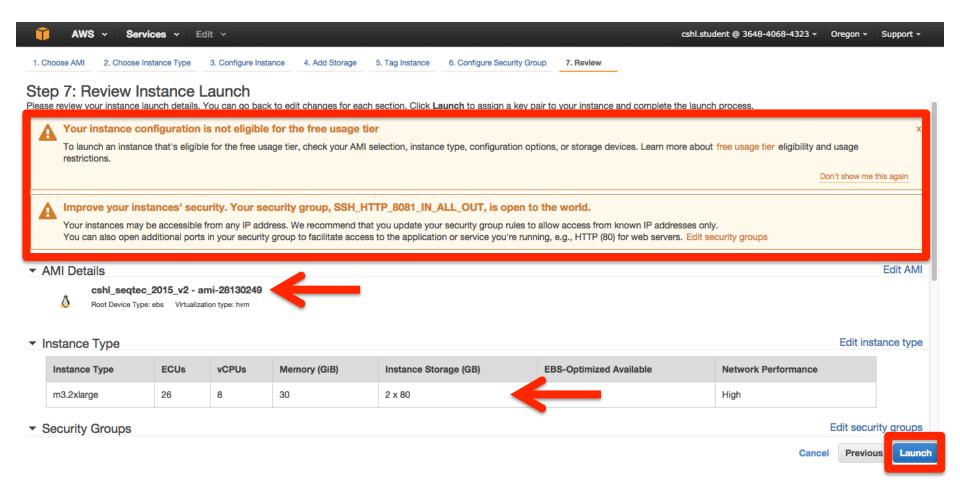
Important: Don't forget to name your instance! (FirstnameLastname)



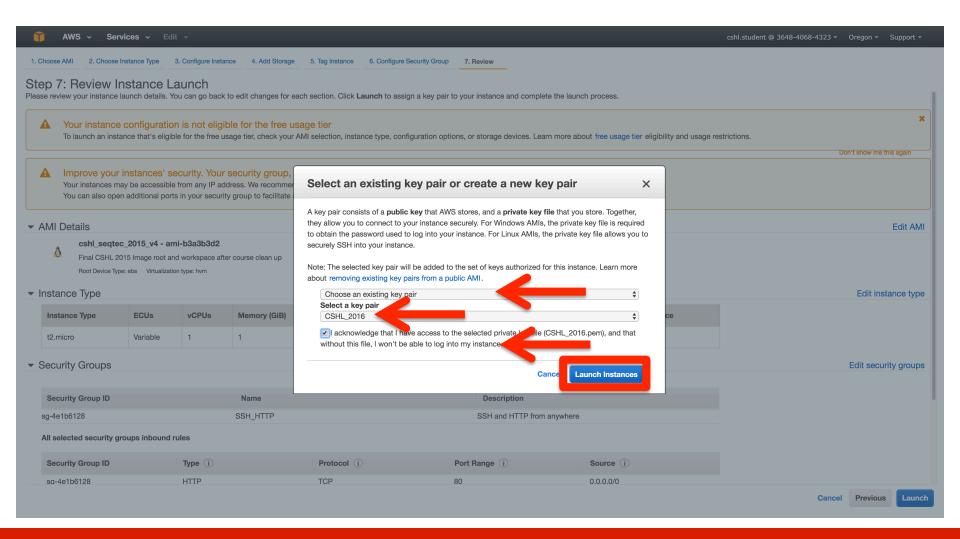
Select an Existing Security Group, choose "SSH_HTTP". Then hit "Review and Launch".



Review the details of your instance, note the warnings, then hit Launch



Choose an existing key pair: "cshl_2017" and then Launch.



View Instances to see your new instance spinning up!



Services v

cshl.student @ 3648-4068-4323 v

Oregon v

Launch Status



Your instances are now launching

The following instance launches have been initiated: i-45e4089f View launch log



Get notified of estimated charges

Create billing alerts to get an email notification when estimated charges on your AWS bill exceed an amount you define (for example, if you exceed the free usage tier).

How to connect to your instances

Your instances are launching, and it may take a few minutes until they are in the running state, when they will be ready for you to use. Usage hours on your new instances will start immediately and continue to accrue until you stop or terminate your instances.

Click View Instances to monitor your instances' status. Once your instances are in the running state, you can connect to them from the Instances screen. Find out how to connect to your instances.

- Here are some helpful resources to get you started
- · How to connect to your Linux instance
- Amazon EC2: User Guide
- · Learn about AWS Free Usage Tier

Amazon EC2: Discussion Forum

While your instances are launching you can also

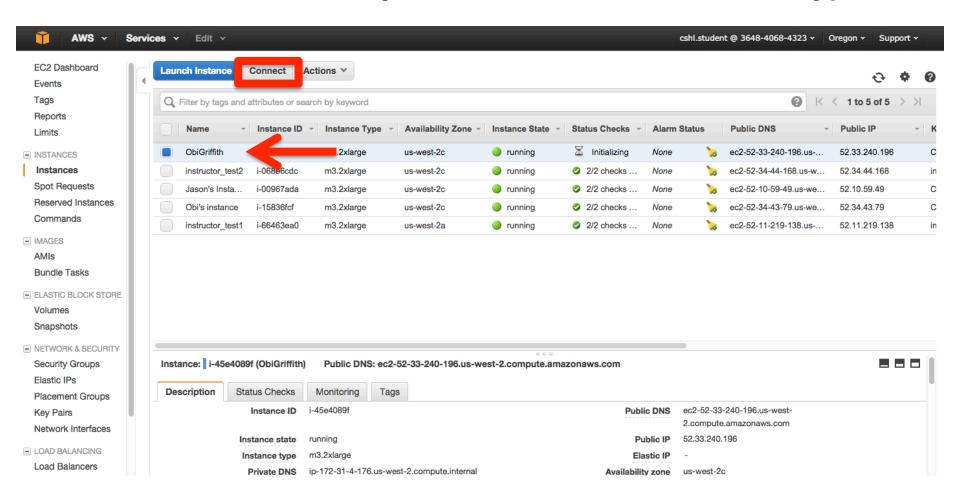
Create status check alarms to be notified when these instances fail status checks. (Additional charges may apply)

Create and attach additional EBS volumes (Additional charges may apply)

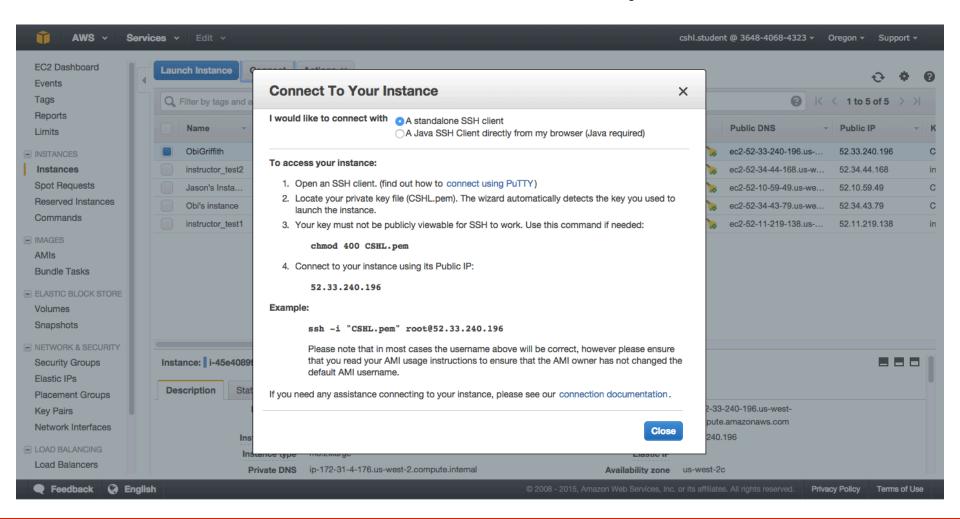
Manage security groups

View Instances

Find YOUR instance, select it, and then hit connect for instructions on how to connect (It may take some time for your instance to be ready)



Take note of your Public DNS/IP and the instructions on changing permissions for the key file (Note, we will login as ubuntu NOT root)



Use 'wget' at command line to download pem file

cd ~/cshl wget genomedata.org/seq-tec-workshop/cshl_2017.pem

Viewing the 'key' file once downloaded

cat CBWNY.pem

----END RSA PRIVATE KEY----obis-air:cshl ogriffit\$

Changing file permissions of your 'key' file (Mac/Linux)

Is -I (long listing)

```
drwx----+ 67 ogriffit staff 2278 22 May 21:25 ../
-rw-r--r--@ 1 ogriffit staff 1696 22 May 21:31 CBWNY.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 "r" for the the file owner only

Logging into your instance

Mac/Linux

cd ~/cbw chmod 600 CBWNY.pem ssh -i CBWNY.pem ubuntu@[YOUR PUBLIC IP]

Copying files from AWS to your computer (using a web browser)



Index of /workspace

<u>Name</u>	Last modified	Size Description
Parent Directory		-
Homo sapiens/	2015-11-13 06:45	-
README.txt	2014-06-17 23:53	5.3K
bam-demo/	2015-11-14 21:03	-
data/	2015-11-13 01:39	-
scratch/	2015-11-13 19:43	-
tools/	2015-11-13 01:54	-

Apache/2.4.7 (Ubuntu) Server at 52.10.8.86 Port 80

http://[YOUR PUBLIC DNS OR IP]/

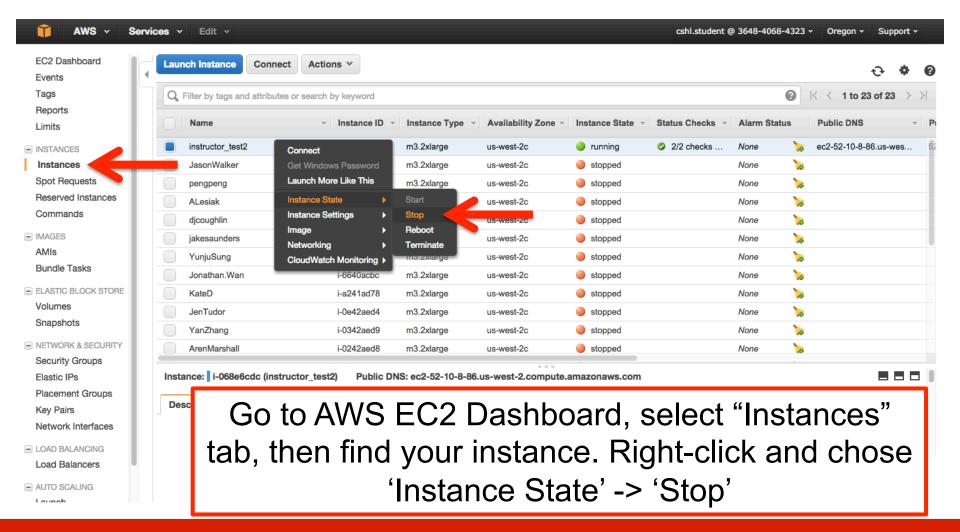
Logging out of your instance

Mac/Linux – simply type exit

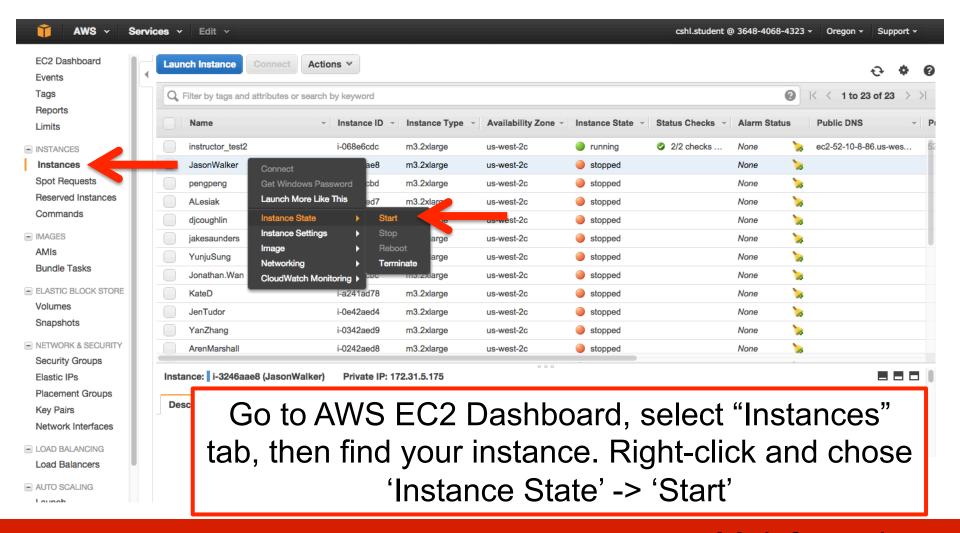
exit

Note, this disconnects the terminal session (ssh connection) to your cloud instance. But, your cloud instance is still running! See next slide for how to stop your instance.

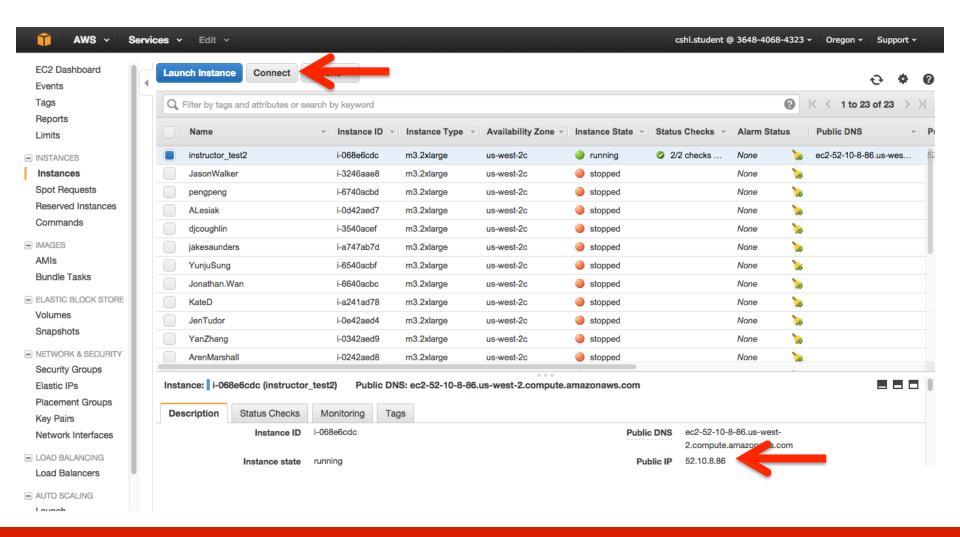
When you are done for the day you can "Stop" your instance – Don't Terminate!



Next morning, you can "Start" your instance again



When you restart your instance you will need to find your new Public DNS or IP address. Select your instance and "Connect" or look in Description tab. Then go back to instructions for "Logging into your instance"



So, at this point:

- Your Mac desktop is ready for the workshop
- If it is not, you know where to get the information you need
- You know how to login to AWS
- The next step is to login to your linux machine on AWS and learn the basics of a linux command line

We are on a Coffee Break & Networking Session