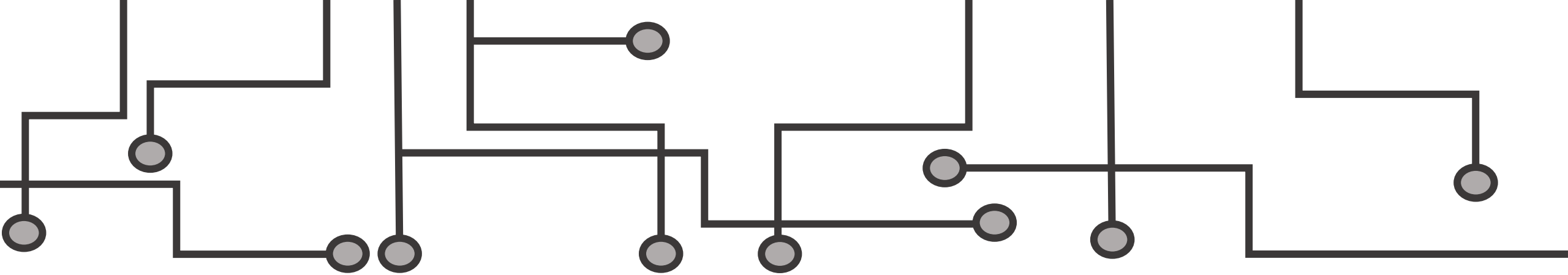

MEDICAL MICROBIOLOGY AND INFECTIOUS DISEASES CODING WORKSHOP

Presents

Introduction to BASH

INSTRUCTED BY

Grace E. Seo (MSc student)

Email: seog@myumanitoba.ca

INFORMATION FOR PARTICIPANTS

All workshops are being recorded and posted to the MMID Coding Workshop - YouTube

Please hold your questions until Q & A session

Question and Answer period will not be recorded.

LEARNING OBJECTIVES

1. ***Describe benefits of using command line interface (CLI) over graphical user interface (GUI)***
2. ***Install BASH terminal on your operating system***
3. ***Navigate, create, rename, move, and copy directory and files in terminal***
4. ***Install, execute, and troubleshoot bioinformatics program***
5. ***Write a BASH script, change permission, and execute the script***

Click to go back to the beginning

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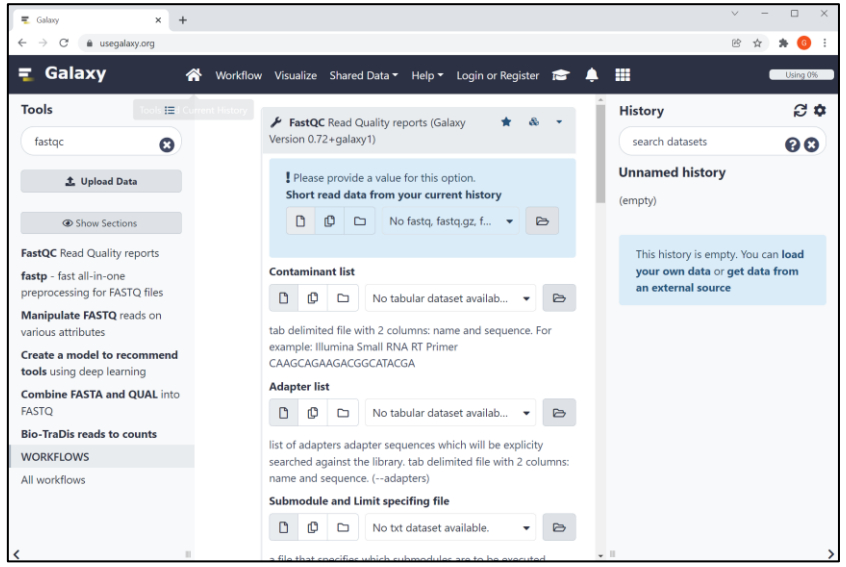
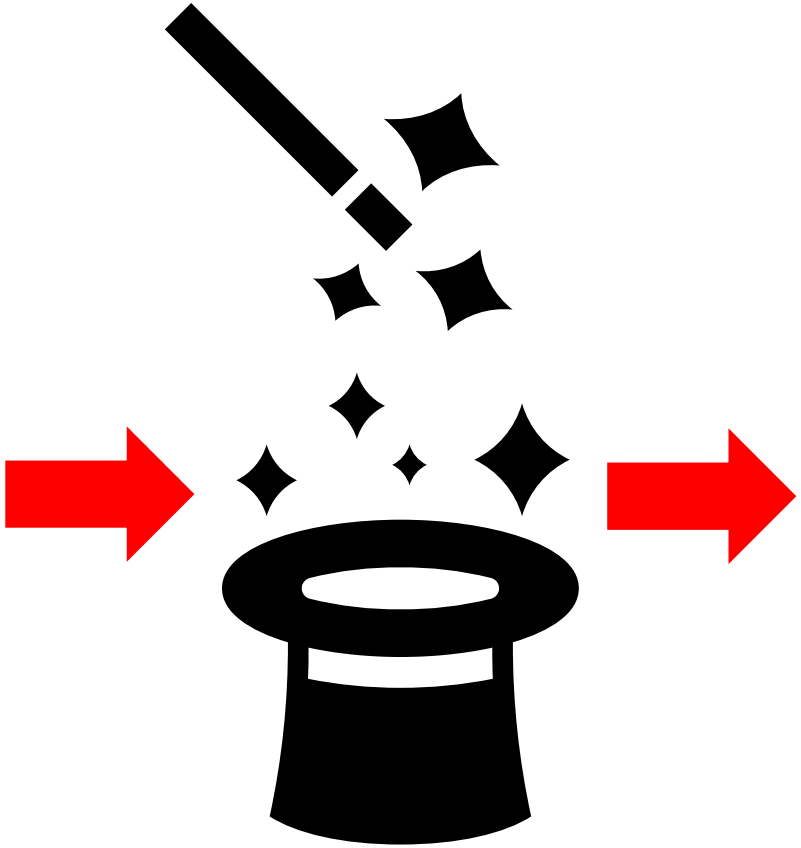
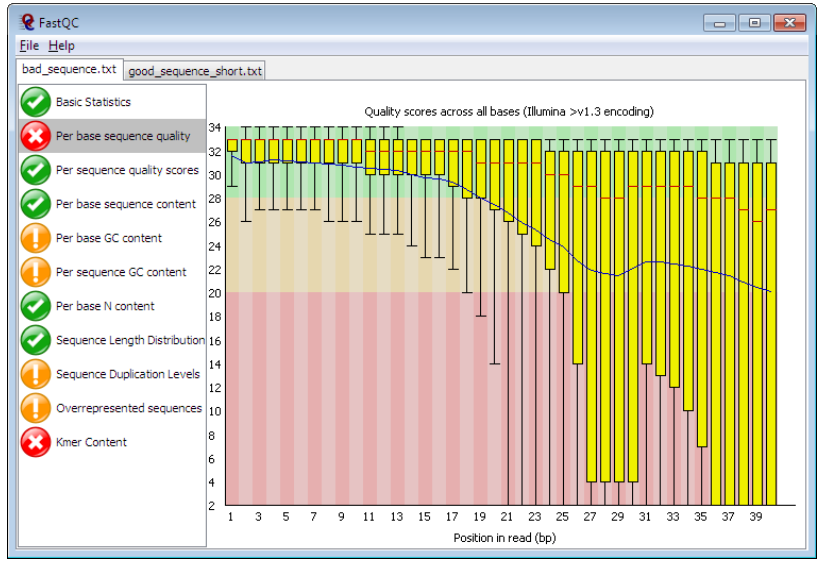
Graphical user interface (GUI) + Magic? = Result



https://usegalaxy.org/

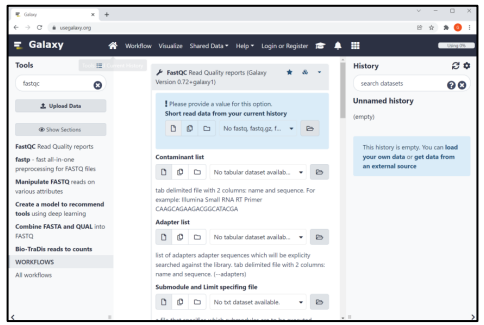




https://du-bii.github.io/module-5-Methodes-Outils/seance1_NGS/slides.html#1

Graphical user interface (GUI) → Command line interface (CLI) → Computer = Result

GUI

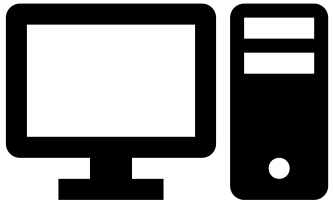


https://usegalaxy.org/

CLI

```
ubuntu@domU-12-31-39-02-88-C1: ~
File  Edit  View  Search  Terminal  Help
ubuntu@domU-12-31-39-02-88-C1:~$ fastqc -h

          FastQC - A high throughput sequence QC analysis tool

SYNOPSIS

      fastqc seqfile1 seqfile2 .. seqfileN

   fastqc [-o output dir] [--(no)extract] [-f fastq|bam|sam]
          [-c contaminant file] seqfile1 .. seqfileN

DESCRIPTION

   FastQC reads a set of sequence files and produces from each one a quality
   control report consisting of a number of different modules, each one of
   which will help to identify a different potential type of problem in your
   data.

   If no files to process are specified on the command line then the program
   will start as an interactive graphical application.  If files are provided
   on the command line then the program will run with no user interaction
   required.  In this mode it is suitable for inclusion into a standardised
   analysis pipeline.

   The options for the program as as follows:

   -h --help       Print this help file and exit

   -v --version    Print the version of the program and exit

   -o --outdir     Create all output files in the specified output directory.
                   Please note that this directory must exist as the program
                   will not create it.  If this option is not set then the
                   output file for each sequence file is created in the same
```

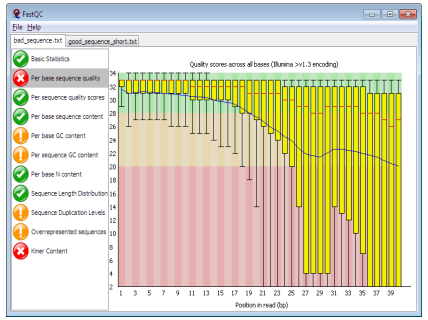
Computer



OR

https://en.wikipedia.org/wiki/Supercomputer

Result



https://du-bii.github.io/module-5-Methodes-Outils/seance1_NGS/slides.html#1

BASH (Bourne Again SHell) language

- Command line interface aka console or terminal in Unix operating system
- BASH originally written by Stephen Bourne
- Unix shell is:
  - programming language
  - command interpreter

- BASH is a default shell in Linux and most Unix-like operating systems

https://commons.wikimedia.org/wiki/File:Bash_Logo_Colored.svg

Why use command line interface (CLI)?

1. **Program is always available on CLI but might not be available on GUI**
2. Take control over your analysis (set your own parameters)
3. Analyze faster using less computer resource
4. Let computers do repetitive tasks for you
5. Automate your jobs and get them done while you are sleeping!

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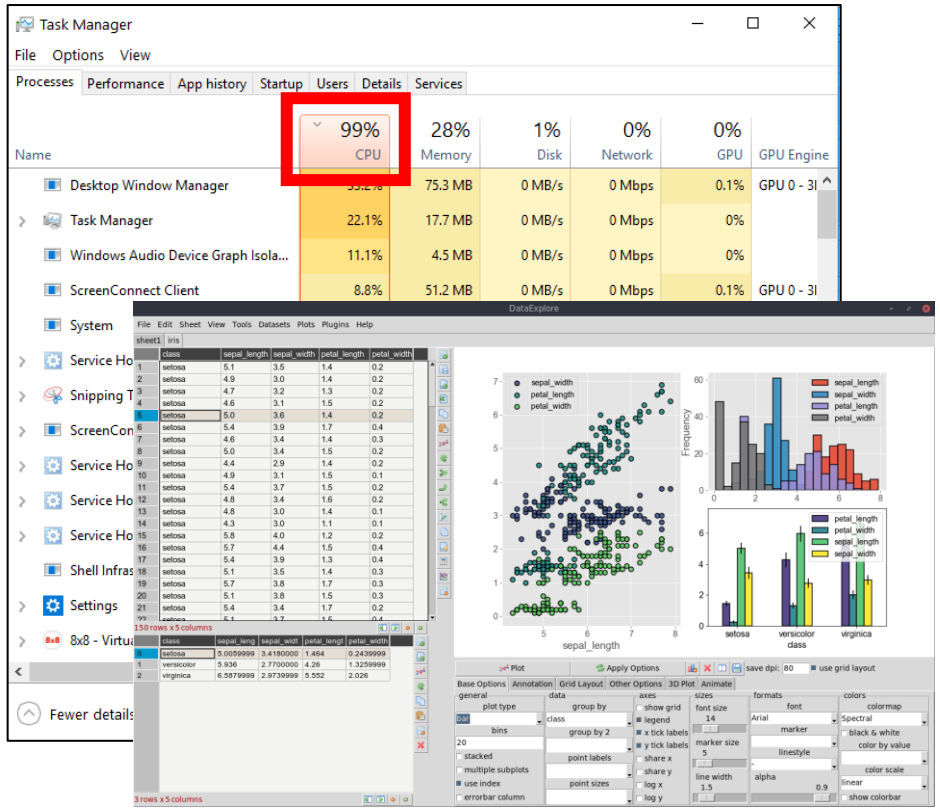
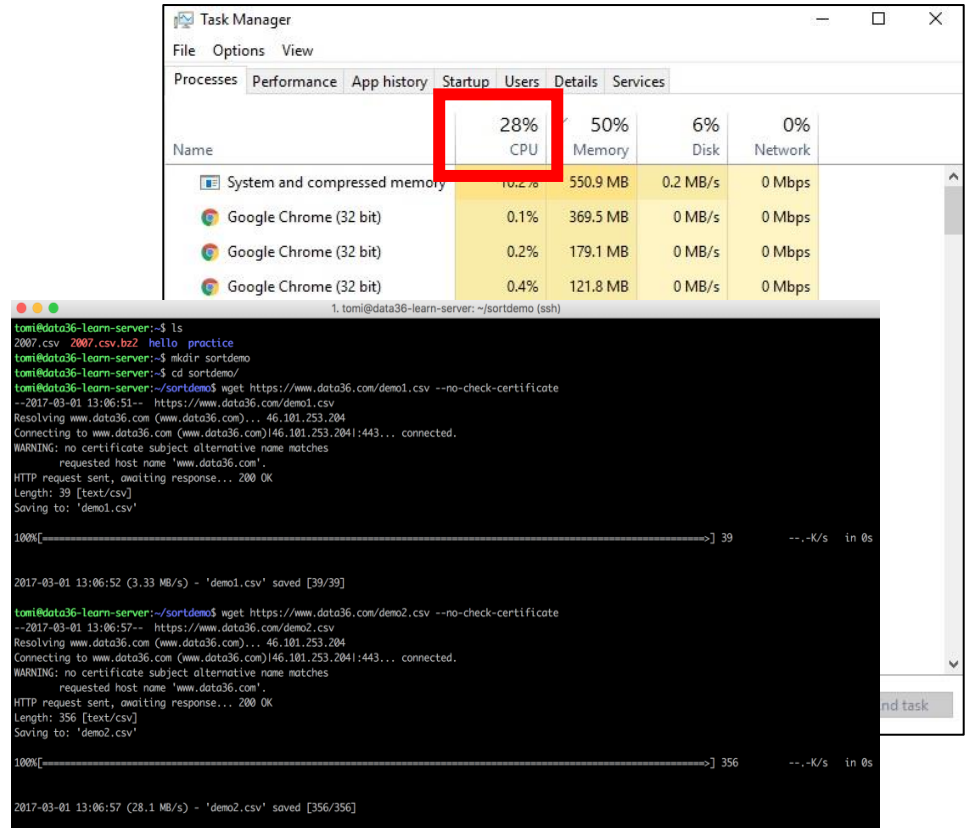


https://decisionstats.com/2015/12/25/interview-damien-farrell-python-gui-dataexplore-python-rstats-pydata/

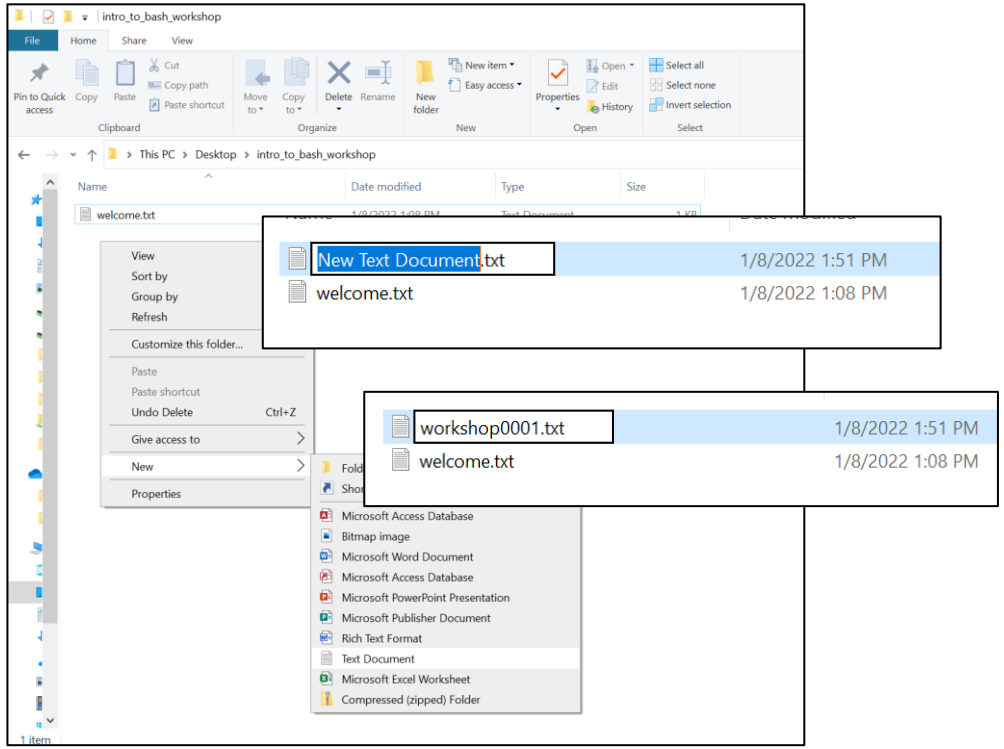
VS



https://data36.com/command-line-data-science-introduction-to-bash/

Why use command line interface (CLI)?

4. Let computers do repetitive tasks for you



VS

```
seog@Smarty:.../intro_to_bash_workshop$ ll
total 0
drwxrwxrwx 1 seog seog 512 Jan  8 13:48 ./
drwxrwxrwx 1 seog seog 512 Jan  8 13:07 ../
-rwxrwxrwx 1 seog seog  81 Jan  8 13:08 welcome.txt*
seog@Smarty:.../intro_to_bash_workshop$ touch workshop{0001..0010}.txt
seog@Smarty:.../intro_to_bash_workshop$ ll
total 0
drwxrwxrwx 1 seog seog 512 Jan  8 13:48 ./
drwxrwxrwx 1 seog seog 512 Jan  8 13:07 ../
-rwxrwxrwx 1 seog seog  81 Jan  8 13:08 welcome.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0001.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0002.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0003.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0004.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0005.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0006.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0007.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0008.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0009.txt*
-rwxrwxrwx 1 seog seog   0 Jan  8 13:48 workshop0010.txt*
seog@Smarty:.../intro_to_bash_workshop$
```

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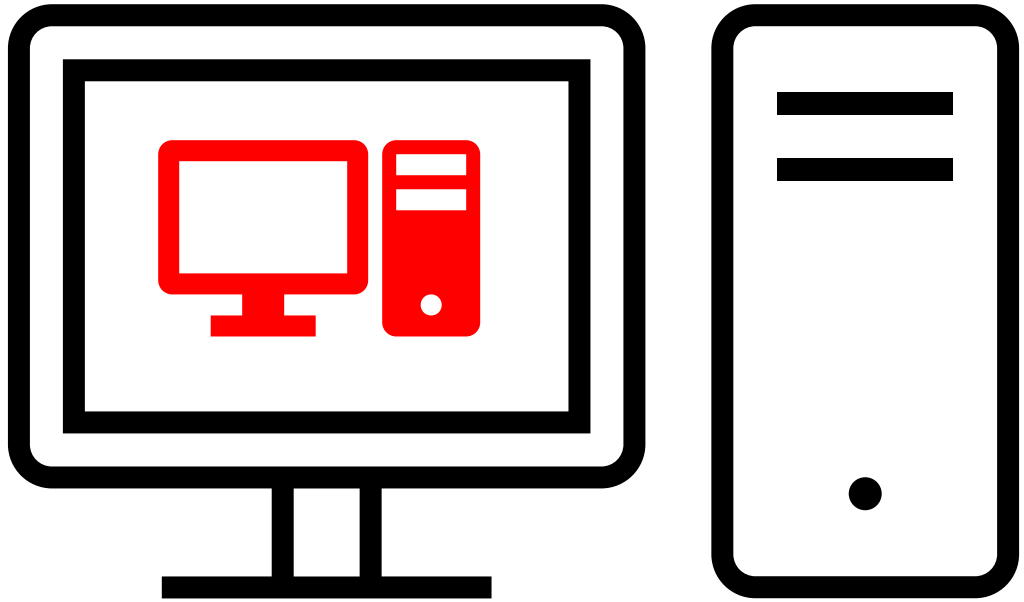
Virtual Machine (VM)

Virtual machine is a computer within your actual computer

Advantages:

- Explore and try BASH coding without affecting actual computer system
- If something happens, simply delete the virtual machine



Disadvantages:

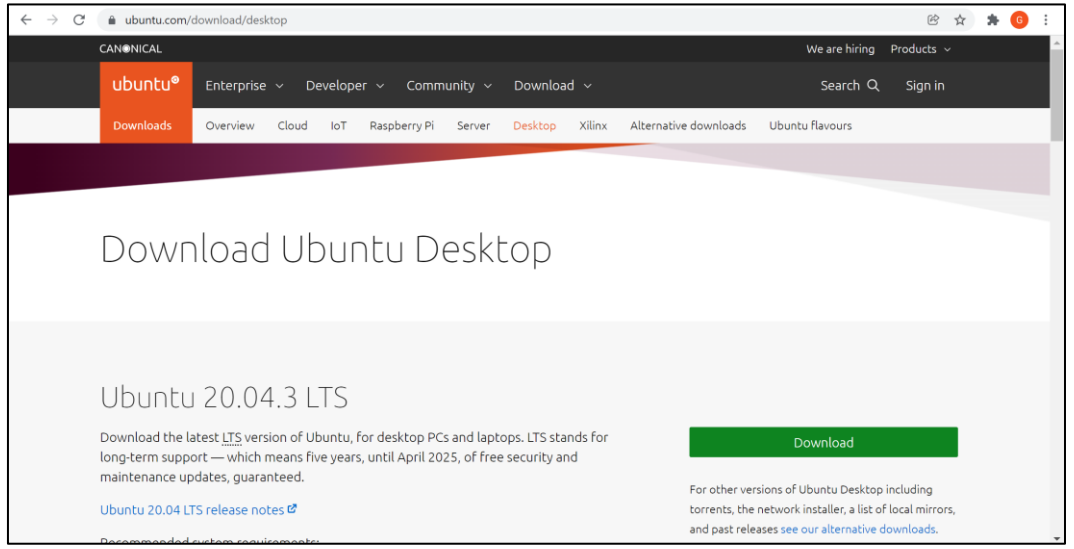
- If you are doing data analysis, you need to transfer files between computer and VM using a “shared folder”

Virtual Machine (VM)

Follow this guide to install and set-up Ubuntu VM: https://itsfoss.com/install-linux-in-virtualbox/

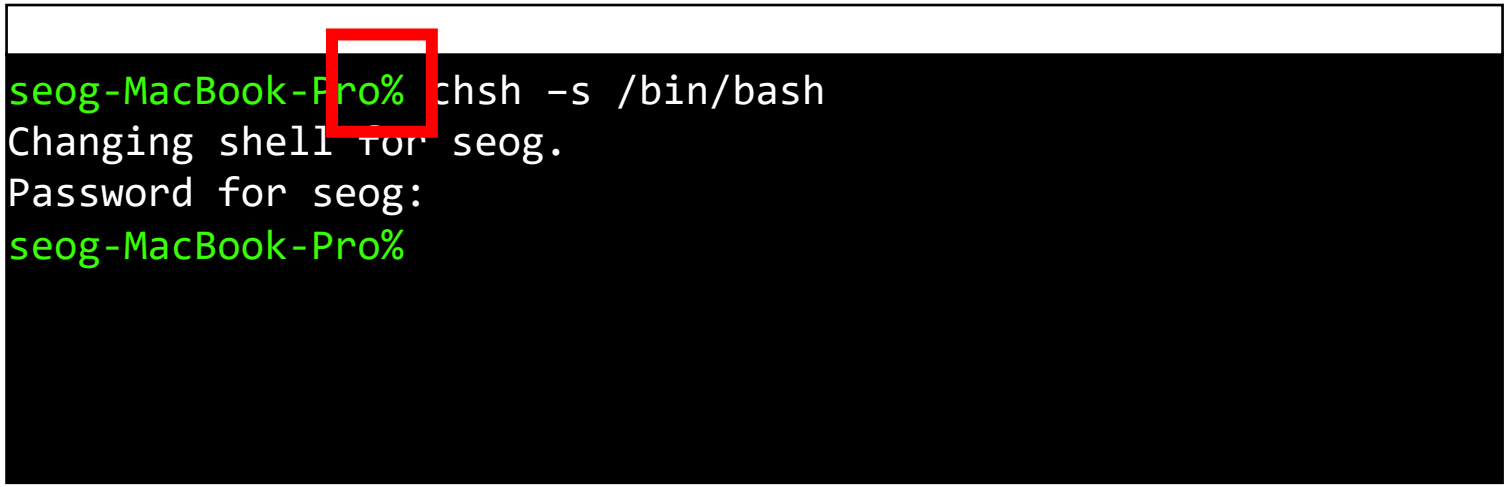
1. **Download VM by clicking the correct host**
   https://www.virtualbox.org/wiki/Downloads

2. **Download Ubuntu iso**
   https://ubuntu.com/desktop



Linux and Mac OS – default terminal App

- Open terminal app (Linux and Mac OS)
- BASH was the default shell in Mac OS until 2019 (replaced with Zsh)
- In Mac OS - To change BASH back to default shell:
  - Type the command: chsh –s /bin/bash then Restart the terminal



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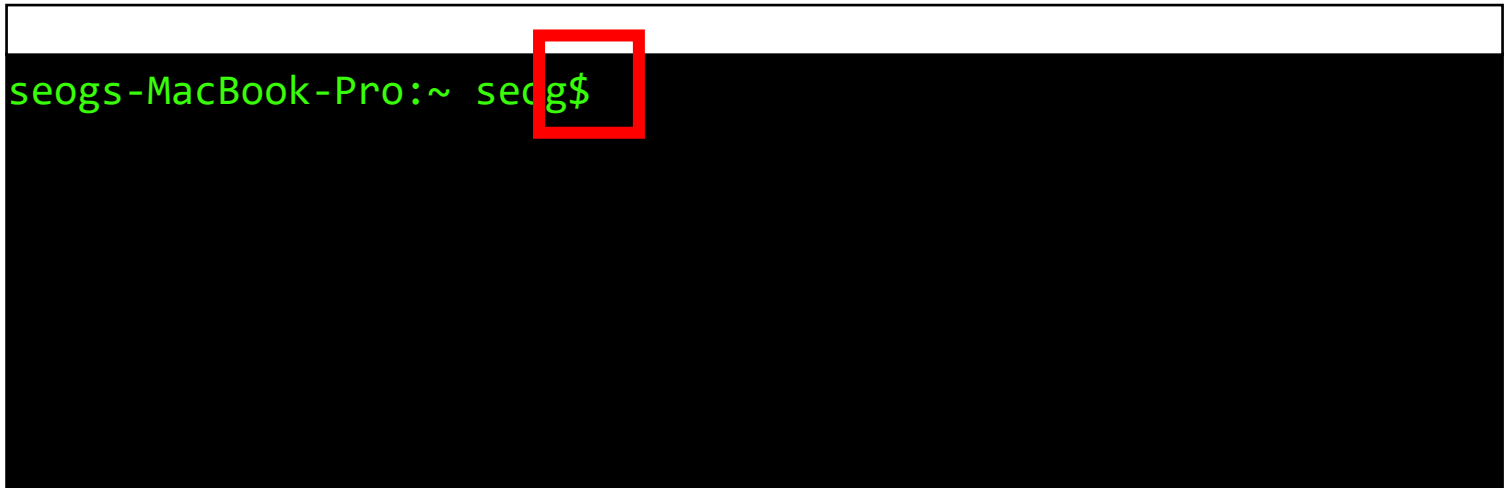
BASH prompt is $



Windows Subsystem for Linux (WSL)

To use BASH on Windows, enable WSL

Advantages:

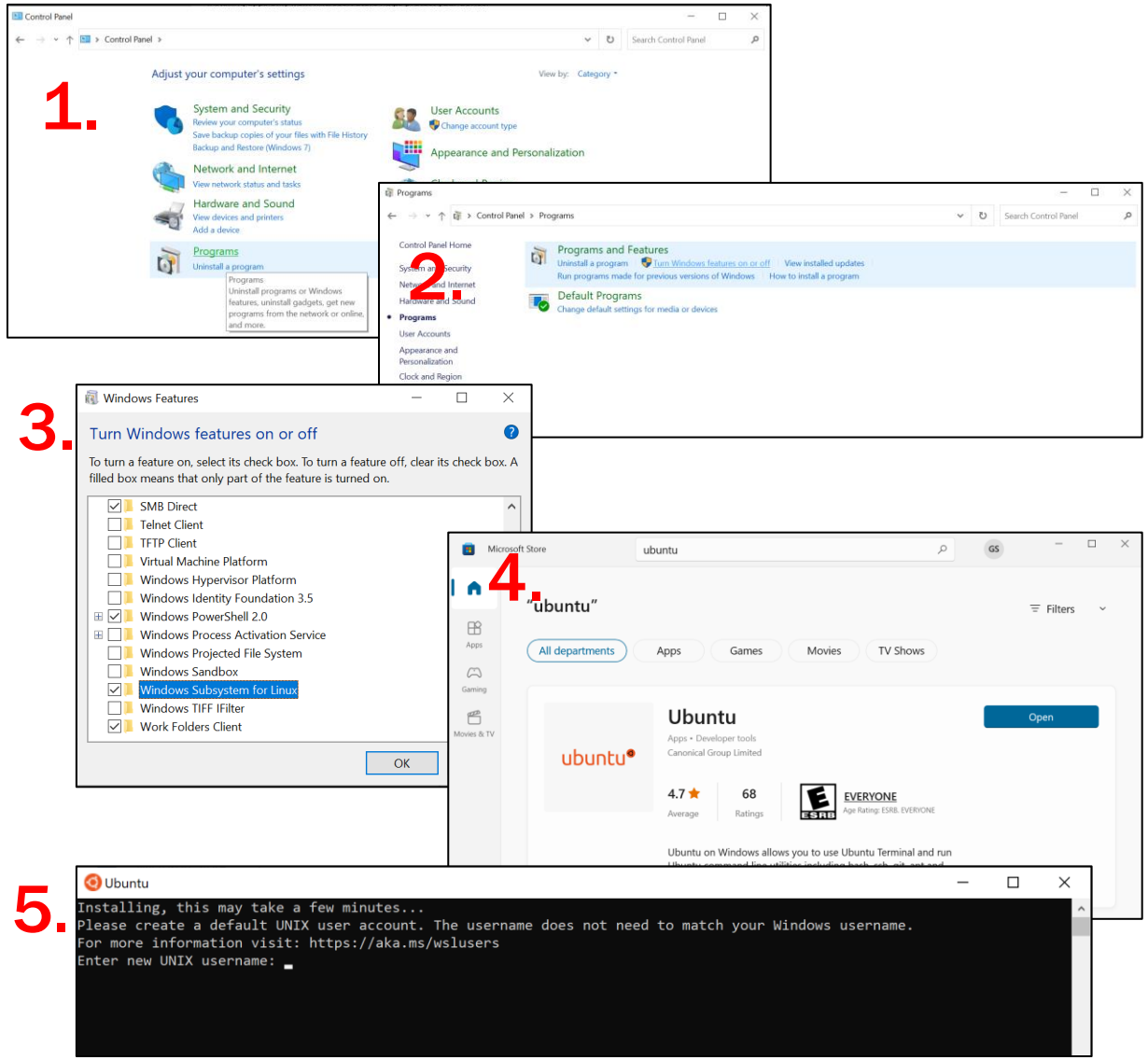
- Use BASH terminal on Windows as if you are on Linux machine.
- BASH terminal can be accessed using: PowerShell, command prompt or any other terminal programs.
- Access files and run bioinformatics program directly on your computer.

Disadvantages:

- You can't undo what you just did and there is no trash can.

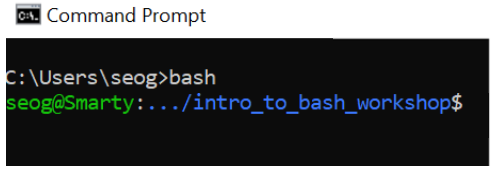
Windows Subsystem for Linux (WSL)

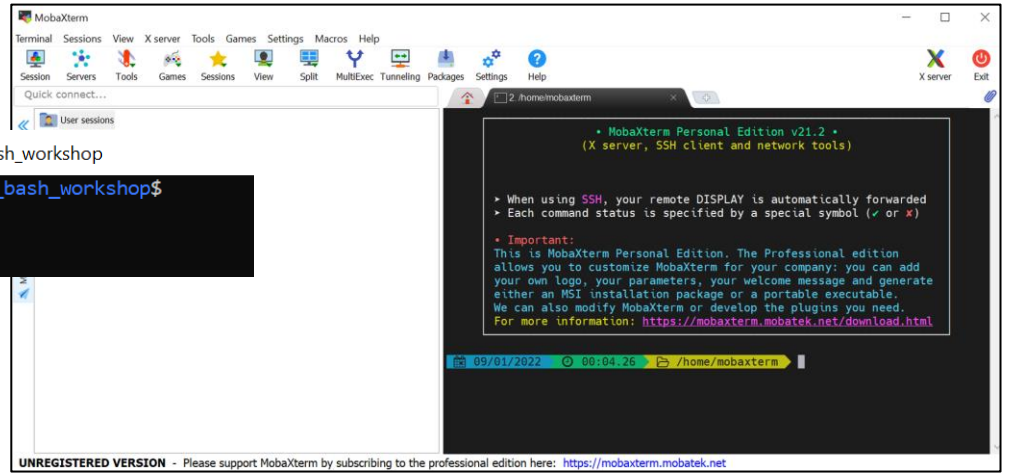
1. Open control panel from Start menu.
2. Click “Programs” → “Turn Windows features on or off”.
3. Scroll down and select “Windows Subsystem for Linux” then **restart your computer.**
4. In “Microsoft Store”, search and install “Ubuntu” from Canonical Group Limited.
5. Open Ubuntu and setup your username and password.

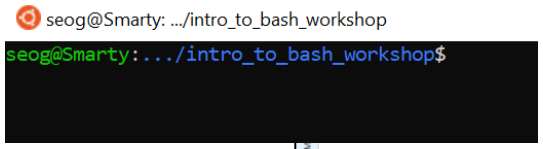


Examples of BASH terminal emulators

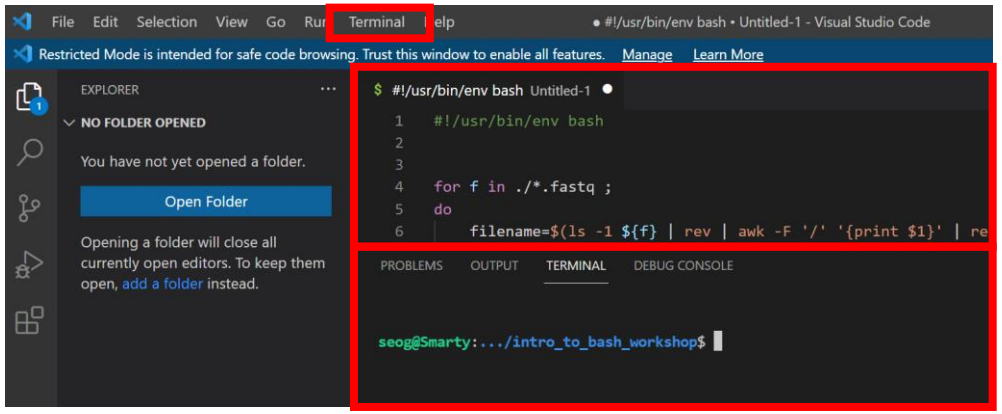
- Windows default apps
  - Command prompt
  - PowerShell







- Terminal emulator
  - Ubuntu terminal (emulator)
  - Visual Studio Code (emulator & text editor)
    - https://code.visualstudio.com/download
  - MobaXTerm (emulator & SSH client)
    - https://mobaxterm.mobatek.net/download-home-edition.html



- SSH (secure shell) client only
  - Bitvise https://www.bitvise.com/
  - PuTTy https://www.chiark.greenend.org.uk/~sgtatham/putty/latest.html

Click to go back to the beginning

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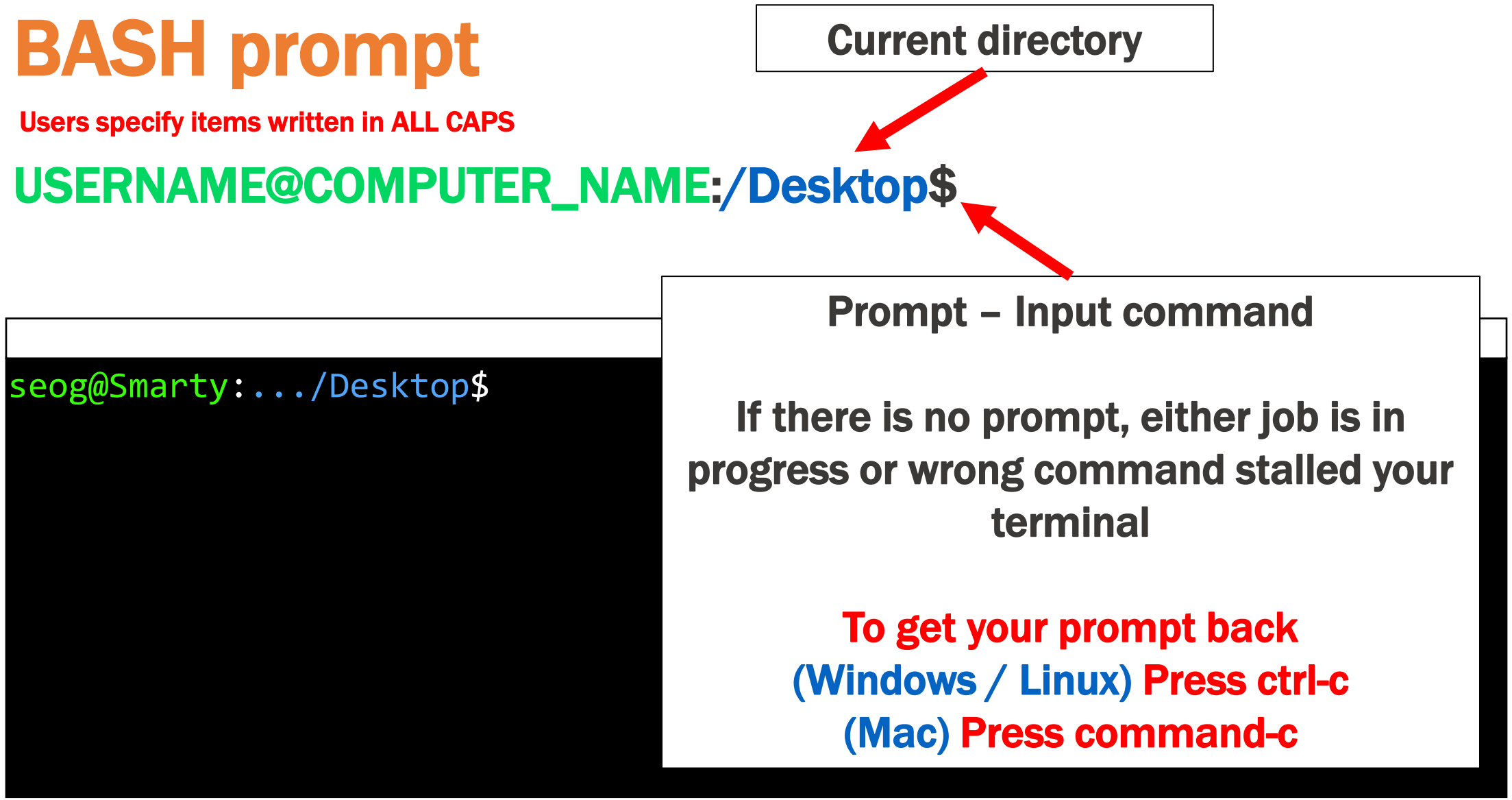
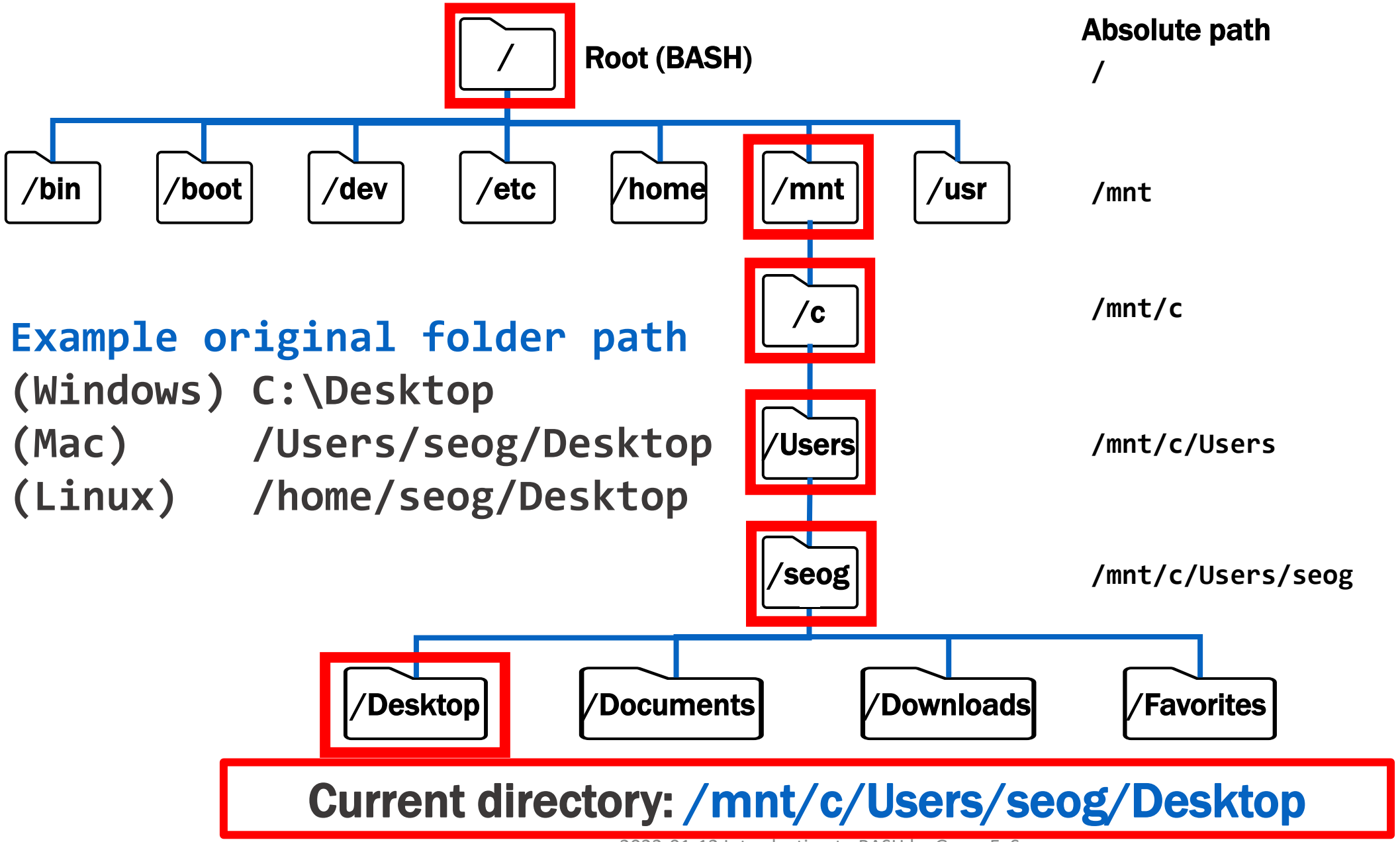
BASH prompt

Users specify items written in ALL CAPS

USERNAME@COMPUTER_NAME:/Desktop$



Example Windows Subsystem for Linux BASH folder structure



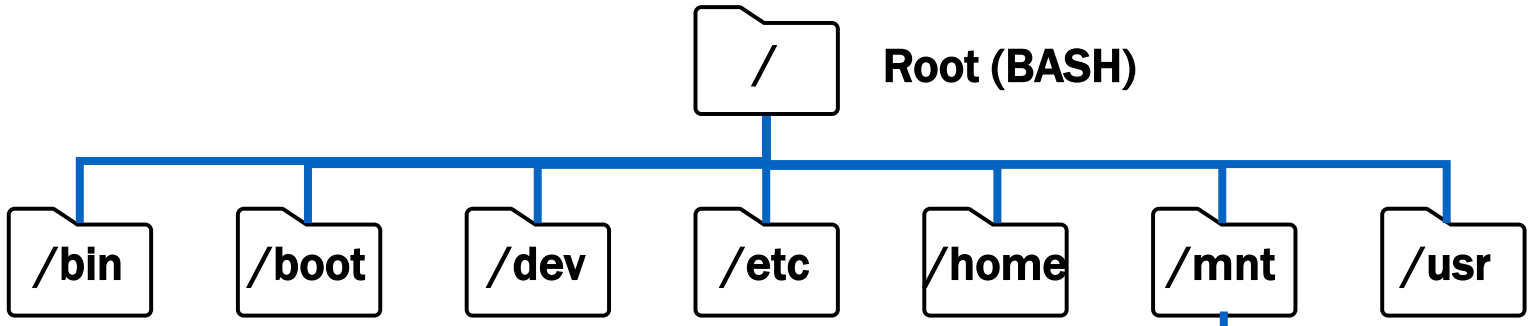
Absolute path	Relative path
/	../../../../../
/mnt	../../../../
/mnt/c	../../../
/mnt/c/Users	../../
/mnt/c/Users/seog	../
	./

Example original folder path

(Windows) C:\Desktop
(Mac) /Users/seog/Desktop
(Linux) /home/seog/Desktop

Current directory: /mnt/c/Users/seog/Desktop

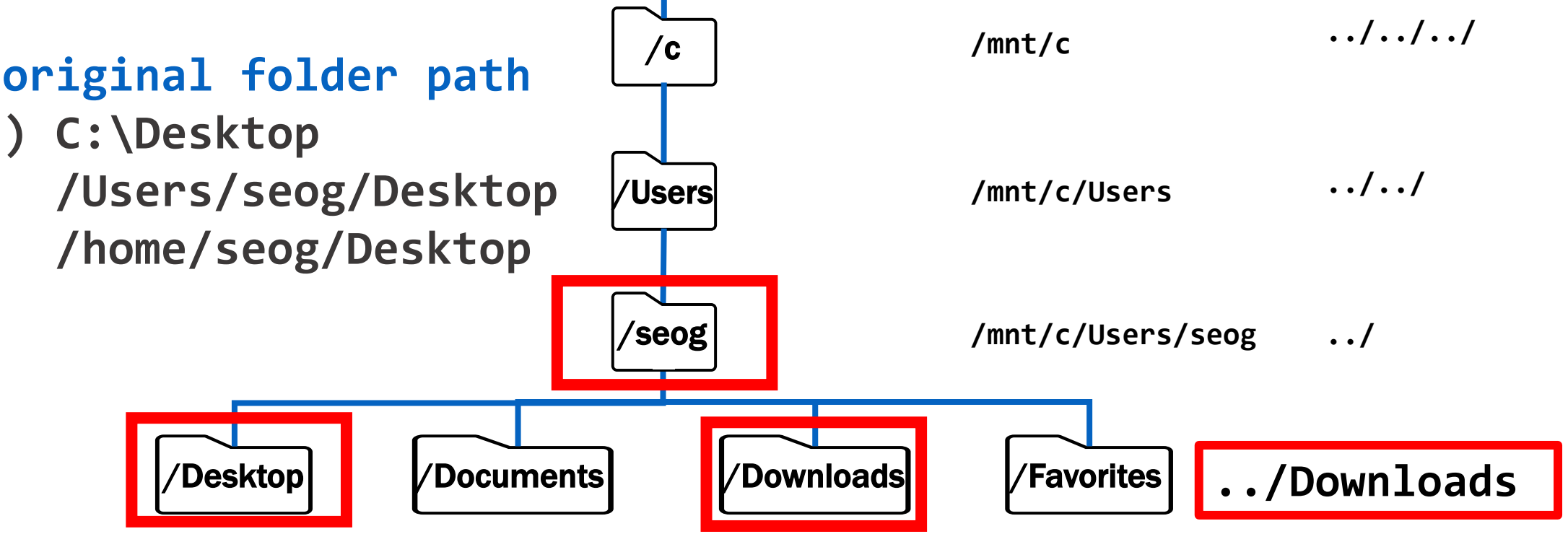
Example Windows Subsystem for Linux BASH folder structure



Absolute path	Relative path
/	../../../../../
/mnt	../../../../
/mnt/c	../../../
/mnt/c/Users	../../
/mnt/c/Users/seog	../

Example original folder path

(Windows) C:\Desktop
(Mac) /Users/seog/Desktop
(Linux) /home/seog/Desktop



Current directory: /mnt/c/Users/seog/Downloads

Navigate directories (folders)

Users specify items written in ALL CAPS

$ pwd *(print current working directory)*

$ cd /PATH *(change directory)*

```
seog@Smarty:~$ pwd
/home/seog
seog@Smarty:~$ cd /mnt/c/Users/seog/Desktop
seog@Smarty:.../Desktop$ pwd
/mnt/c/Users/seog/Desktop
seog@Smarty:.../Desktop$ cd /home
seog@Smarty:/home$ cd ../mnt/c/Users/seog/Desktop
seog@Smarty:.../Desktop$ cd ../../
seog@Smarty:.../Users$ cd seog/Desktop
seog@Smarty:.../Desktop$
```

Change highlighted part with your username

```
(WSL)    /mnt/c/Users/seog/Desktop
(Mac)    /Users/seog/Desktop
(Linux)  /home/seog/Desktop
```

Create directory and files

Users specify items written in ALL CAPS

$ mkdir NAME *(create a new folder)*

$ touch NAME *(create a new file)*

$ ls *(list files in the folder)*

$ ls -l *(list files in the folder with long details)*

```
seog@Smarty:.../Desktop$ mkdir intro_to_bash
seog@Smarty:.../Desktop$ cd intro_to_bash
seog@Smarty:.../intro_to_bash$ touch test0001.txt test0002.txt
seog@Smarty:.../intro_to_bash$ ls
test0001.txt  test0002.txt
seog@Smarty:.../intro_to_bash$ ls -l
total 0
-rwxrwxrwx 1 seog seog 0 Jan  9 23:14 test0001.txt
-rwxrwxrwx 1 seog seog 0 Jan  9 23:14 test0002.txt
seog@Smarty:.../intro_to_bash$
```

File information and permission

```
                                  File size
total 0                  Owner  Group     Date & time created     File name
-rwxrwxrwx  1  seog  seog  0  Jan   9 23:14  test0001.txt
-rwxrwxrwx  1  seog  seog  0  Jan   9 23:14  test0002.txt
```

r: readable
w: writable
x: executable

Permission ($ chmod ###)

No permission	---	---	---
Symbol:	rwx	rwx	rwx
Bit:	421	421	421
Triad:	Owner	group	others

```
$ chmod 755 test0001.txt
Owner(rwx) group(r-x) others(r-x)
```

```
$ chmod a+wxr test0001.txt
Owner(rwx) group(rwx) others(rwx)
```

https://en.wikipedia.org/wiki/File-system_permissions

Rename, move & copy directory and files

Users specify items written in ALL CAPS

$ mv OLD NEW *(move to a new location or rename)*

$ cp TARGET NEWCOPY *(copy file or folder with new name)*

```
seog@Smarty:.../intro_to_bash$ ls
test0001.txt test0002.txt
seog@Smarty:.../intro_to_bash$ mv test0001.txt renamed0001.txt ; ls
renamed0001.txt  test0002.txt
seog@Smarty:.../intro_to_bash$ cp renamed0001.txt copied0001.txt
seog@Smarty:.../intro_to_bash$ mkdir testFolder
seog@Smarty:.../intro_to_bash$ mv renamed0001.txt testFolder/
seog@Smarty:.../intro_to_bash$ ls
copied0001.txt  test0002.txt  testFolder
seog@Smarty:.../intro_to_bash$ ls -l ./testFolder
total 0
-rwxrwxrwx 1 seog seog 0 Jan  09 23:14 renamed0001.txt
seog@Smarty:.../intro_to_bash$ mv testFolder renamedFolder ; ls
copied0001.txt renamedFolder  test0002.txt
```

Semicolon is a command separator

Remove files or folders

Users specify items written in ALL CAPS

$ rm FILE *(delete file - irreversible)*

$ rm -r FOLDER *(delete folder recursively - irreversible)*

$ rmdir FOLDER* *(delete empty folders matching wildcard pattern- irreversible)*

$ mkdir pattern{0000..0005}
create 6 folders in order

```
seog@Smarty:.../intro_to_bash$ mkdir testFolder{0000..0005}
seog@Smarty:.../intro_to_bash$ ls
copied0001.txt  test0002.txt    testFolder0001  testFolder0003  testFolder0005
renamedFolder   testFolder0000  testFolder0002  testFolder0004
seog@Smarty:.../intro_to_bash$ rmdir testFolder000*
seog@Smarty:.../intro_to_bash$ ls
copied0001.txt  renamedFolder  test0002.txt
seog@Smarty:.../intro_to_bash$ rm renamedFolder/
rm: cannot remove 'renamedFolder/': Is a directory
seog@Smarty:.../intro_to_bash$ rm -r renamedFolder/ ; ls
copied0001.txt  test0002.txt
```

*** Wildcard is used to define pattern for search and matches**

Viewing files and editing text

Users specify items written in ALL CAPS

$ echo "TEXT" >> FILE *(print function and >> store output in a file)*

$ cat FILE *(concatenate files or printout file content)*

$ less FILE *(view file interactively, press **q** (quit) viewer)*

$ nano FILE *(create or edit file in terminal)*

```
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!"
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!" >> welcome.txt
seog@Smarty:.../intro_to_bash$ cat welcome.txt
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ less welcome.txt
```

Viewing files and editing text

Users specify items written in ALL CAPS

$ echo "TEXT" >> FILE *(print function and >> store output in a file)*

$ cat FILE *(concatenate files or printout file content)*

$ less FILE *(view file interactively, press **q** (quit) viewer)*

$ nano FILE *(create or edit file in terminal)*

Viewing files and editing text

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```
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!"
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!" >> welcome.txt
seog@Smarty:.../intro_to_bash$ cat welcome.txt
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ less welcome.txt
seog@Smarty:.../intro_to_bash$ nano welcome.txt
```

Viewing files and editing text

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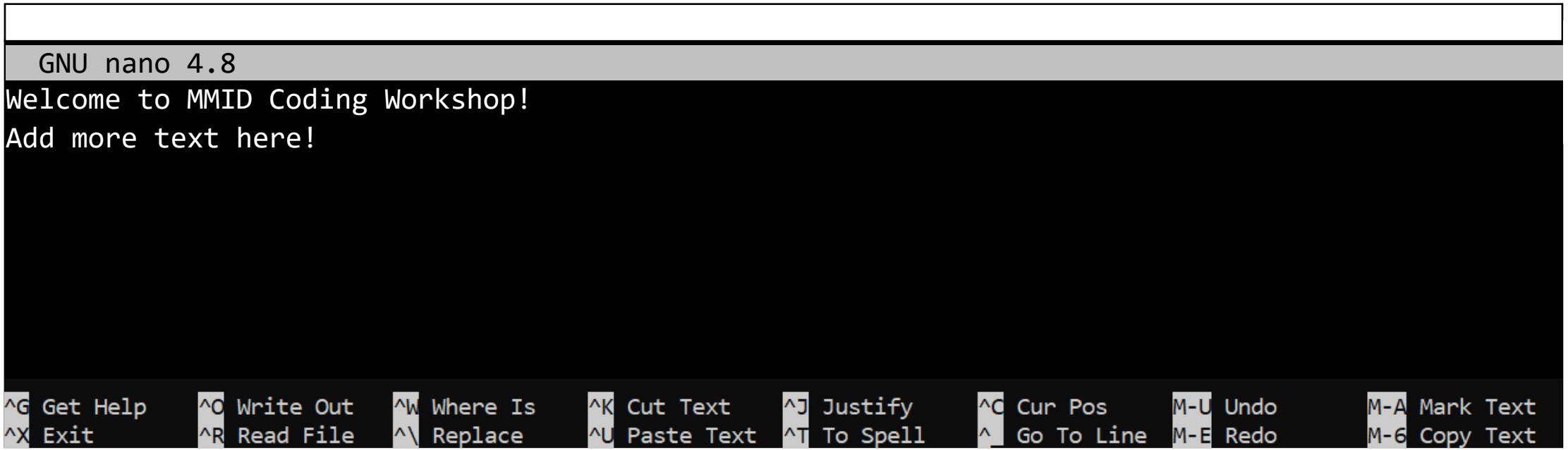
$ less FILE *(view file interactively, press q (quit) viewer)*

$ nano FILE *(create or edit file in terminal)*



Viewing files and editing text

Users specify items written in ALL CAPS

$ echo "TEXT" >> FILE *(print function and >> store output in a file)*

$ cat FILE *(concatenate files or printout file content)*

$ less FILE *(view file interactively, press q (quit) viewer)*

$ nano FILE *(create or edit file in terminal)*

```
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!"
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ echo "Welcome to MMID Coding Workshop!" >> welcome.txt
seog@Smarty:.../intro_to_bash$ cat welcome.txt
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$ less welcome.txt
seog@Smarty:.../intro_to_bash$ nano welcome.txt
seog@Smarty:.../intro_to_bash$ cat welcome.txt
Welcome to MMID Coding Workshop!
Add more text here!
```

Click to go back to the beginning

LEARNING OBJECTIVES

1. *Describe benefits of using command line interface (CLI) over graphical user interface (GUI)*
2. *Install BASH terminal on your operating system*
3. *Navigate, create, rename, move, and copy directory and files in terminal*
4. ***Install, execute, and troubleshoot bioinformatics program***
5. *Write a BASH script, change permission, and execute the script*

Necessary updates and builds

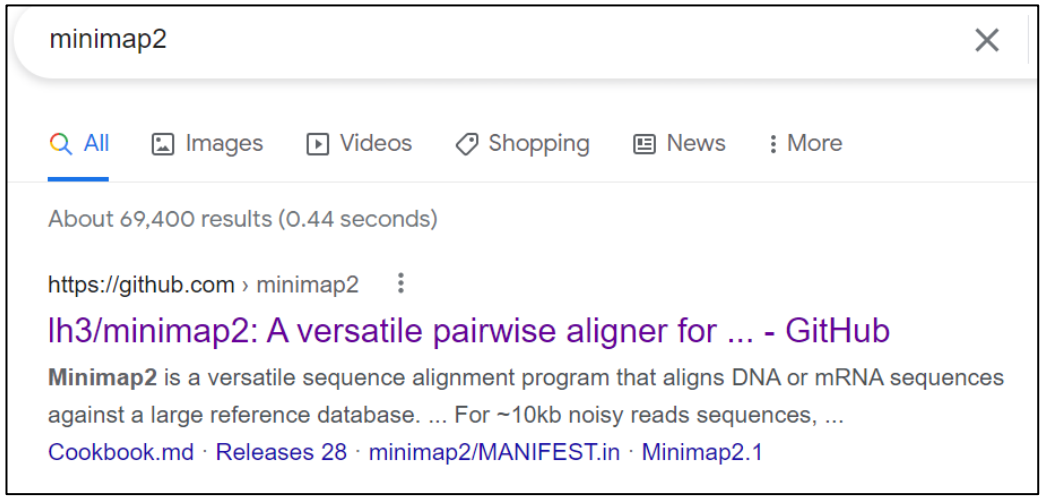
Following installations are required to ensure proper program installation

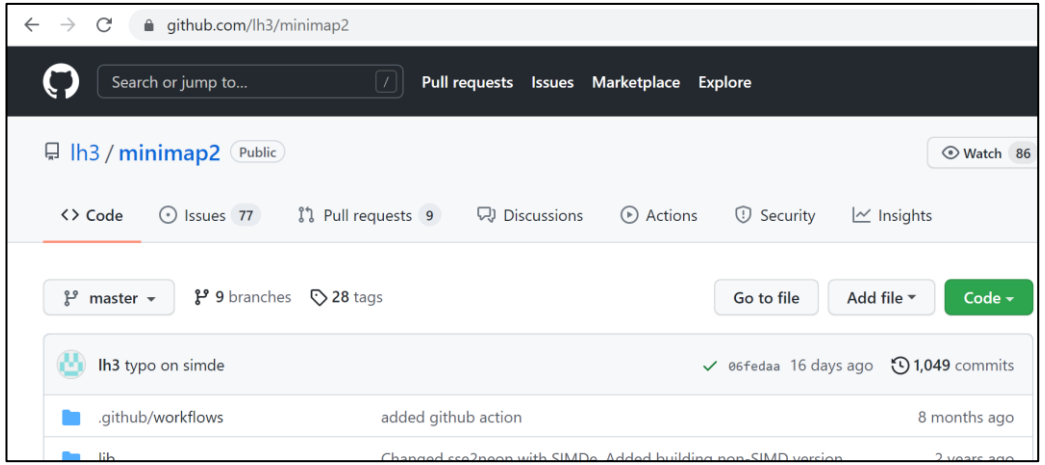
```
seog@Smarty:.../intro_to_bash$ sudo apt-get update
Get:1 http://security.ubuntu.com/ubuntu focal-security InRelease [114 kB]
Hit:2 http://archive.ubuntu.com/ubuntu focal InRelease
...
Fetched 21.2 MB in 34s (628 kB/s)
Reading package lists... Done
seog@Smarty:.../intro_to_bash$ sudo apt-get install build-essential
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  bseq.c -o bseq.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  sketch.c -o sketch.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  sdust.c -o sdust.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  options.c -o options.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  index.c -o index.o
index.c: In function ‘mm_idx_load’:
index.c:519:3: warning: ignoring return value of ‘fread’, declared with attribute warn_unused_result [-Wunused-result]
  519 |   fread(&l, 1, 1, fp);
      |   ^~~~~~~~~~~~~~~~~~~
...
z2_sse2.o ksw2_extd2_sse2.o ksw2_exts2_sse2.o ksw2_dispatch.o
ar: `u' modifier ignored since `D' is the default (see `U')
cc -g -Wall -O2 -Wc++-compat  main.o -o minimap2 -L. -lminimap2 -lm -lz -lpthread
seog@Smarty:.../intro_to_bash$
```

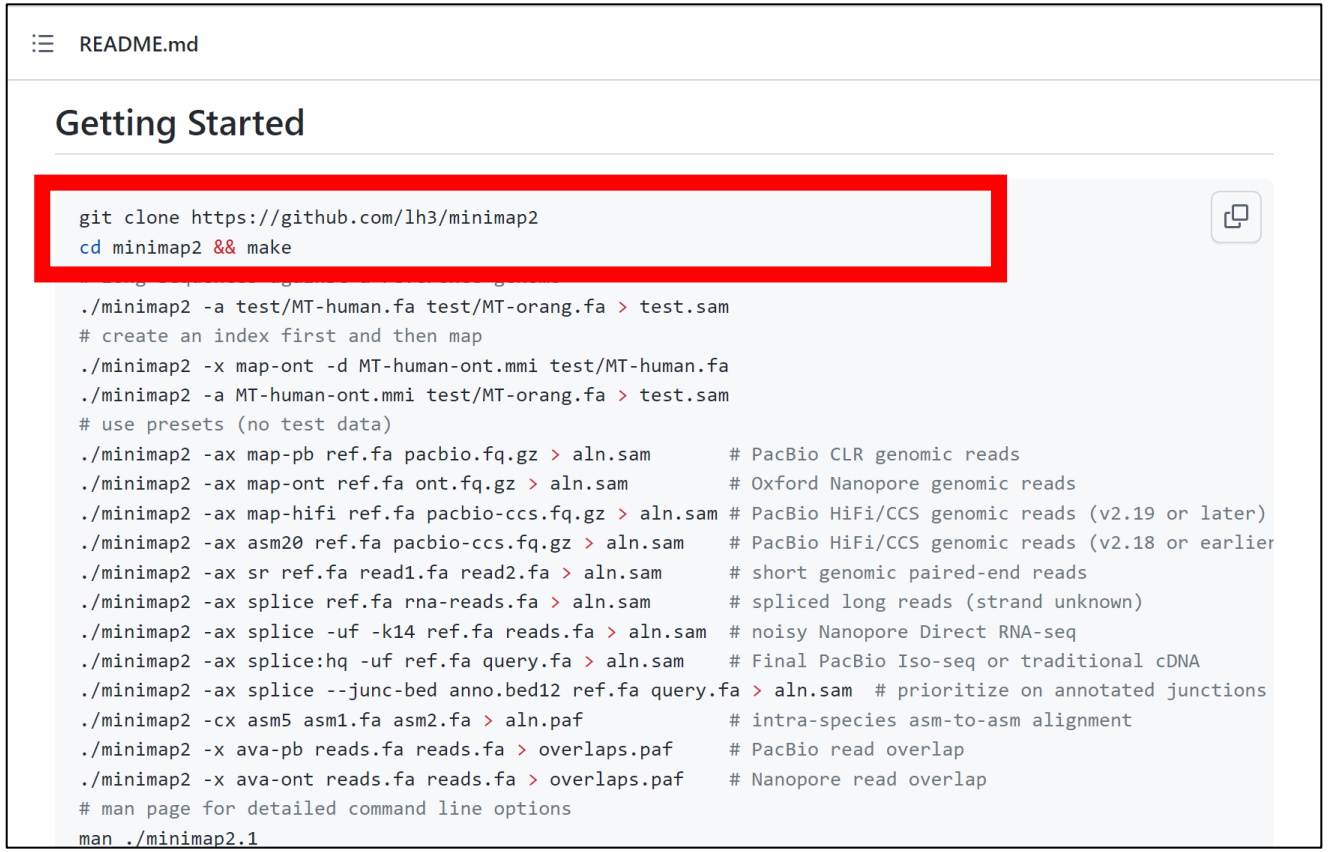
$ sudo apt-get update
$ sudo apt-get install build-essential

Program from: https://github.com/lh3/minimap2

Command line program: (i.e.) minimap2







Program from: https://github.com/lh3/minimap2

Git clone repository and make: minimap2

From README.md file find: $ git clone https://github.com/lh3/minimap2

This process clones repository to local computer for easy program installation.

```
seog@Smarty:.../intro_to_bash$ rm *
seog@Smarty:.../intro_to_bash$ git clone https://github.com/lh3/minimap2
Cloning into 'minimap2'...
remote: Enumerating objects: 5274, done.
remote: Counting objects: 100% (1359/1359), done.
remote: Compressing objects: 100% (209/209), done.
remote: Total 5274 (delta 1238), reused 1193 (delta 1149), pack-reused 3915
Receiving objects: 100% (5274/5274), 1.67 MiB | 5.71 MiB/s, done.
Resolving deltas: 100% (3806/3806), done.
Updating files: 100% (90/90), done.
seog@Smarty:.../intro_to_bash$ ls ; cd minimap2/ && make
minimap2
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  main.c -o main.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  kthread.c -o kthread.o
...
ar: `u' modifier ignored since `D' is the default (see `U')
cc -g -Wall -O2 -Wc++-compat  main.o -o minimap2 -L. -lminimap2 -lm -lz -lpthread
seog@Smarty:.../minimap2$
```

Program from: https://github.com/lh3/minimap2

Execute minimap2 within the folder

You can now use absolute or relative path to run minimap2.

i.e. when you are in minimap2 folder, run the program: $ ./minimap2

```
seog@Smarty:.../minimap2$ ls
FAQ.md          bseq.o              index.o    ksw2_dispatch.c     ksw2_ll_sse.c  main.o      options.o  sketch.o
LICENSE.txt     code_of_conduct.md  kalloc.c   ksw2_dispatch.o     ksw2_ll_sse.o  map.c       pe.c       splitidx.c
MANIFEST.in     cookbook.md         kalloc.h   ksw2_extd2_sse.c    kthread.c      map.o       pe.o       splitidx.o
Makefile        esterr.c            kalloc.o   ksw2_extd2_sse2.o   kthread.h      [illegible] python     sse2neon
Makefile.simde  esterr.o            kdq.h      ksw2_extd2_sse41.o  kthread.o      minimap2    sdust.c    test
NEWS.md         example.c           ketopt.h   ksw2_exts2_sse.c    kvec.h         [illegible] sdust.h    tex
README.md       format.c            khash.h    ksw2_exts2_sse2.o   lchain.c       misc        sdust.o
align.c         format.o            krmq.h     ksw2_exts2_sse41.o  lchain.o       misc.c      seed.c
align.o         hit.c               kseq.h     ksw2_extz2_sse.c    lib            misc.o      seed.o
bseq.c          hit.o               ksort.h    ksw2_extz2_sse2.o   libminimap2.a  mmpriv.h    setup.py
bseq.h          index.c             ksw2.h     ksw2_extz2_sse41.o  main.c         options.c   sketch.c
seog@Smarty:.../minimap2$ ./minimap2
Usage: minimap2 [options] <target.fa>|<target.idx> [query.fa] [...]
Options:
  Indexing:
    -H           use homopolymer-compressed k-mer (preferrable for PacBio)
    -k INT       k-mer size (no larger than 28) [15]
    -w INT       minimizer window size [10]
...
See `man ./minimap2.1' for detailed description of these and other advanced command-line options.
seog@Smarty:.../minimap2$
```

Program from: https://github.com/lh3/minimap2

Execute minimap2 within the folder

If you want to run minimap2 anywhere without having to assign absolute path to minimap2, copy minimap2 into one of your $PATH directory

```
seog@Smarty:.../minimap2$ echo $PATH
/usr/local/sbin:/usr/local/bin:/usr/sbin:/usr/bin:/sbin:/bin
seog@Smarty:.../minimap2$ sudo cp minimap2 /usr/local/bin/
seog@Smarty:.../minimap2$ ls -l /usr/local/bin/
total 1920
drwxr-xr-x 1 root root    4096 Jan 10 18:54 ./
drwxr-xr-x 1 root root    4096 Aug 19 16:40 ../
-rwxr-xr-x 1 root root 1403440 Jan 10 18:54 minimap2*
seog@Smarty:.../minimap2$ cd ../
seog@Smarty:.../intro_to_bash$ minimap2
Usage: minimap2 [options] <target.fa>|<target.idx> [query.fa] [...]
Options:
  Indexing:
    -H           use homopolymer-compressed k-mer (preferrable for PacBio)
    -k INT       k-mer size (no larger than 28) [15]
    -w INT       minimizer window size [10]
...
See `man ./minimap2.1' for detailed description of these and other advanced command-line options.
seog@Smarty:.../intro_to_bash$ rm -r minimap2
```

Sample fastq datasets from: https://doi.org/10.5281/zenodo.3736457

Download fastq files and reference

Download fastq files and reference file from

https://github.com/MMID-coding-workshop/2022-01-12-Introduction-to-BASH.git

```
seog@Smarty:.../intro_to_bash$ git clone https://github.com/MMID-coding-workshop/2022-01-12-Introduction-to-BASH.git
Cloning into '2022-01-12-Introduction-to-BASH'...
remote: Enumerating objects: 9, done.
remote: Counting objects: 100% (9/9), done.
remote: Compressing objects: 100% (8/8), done.
remote: Total 9 (delta 0), reused 0 (delta 0), pack-reused 0
Unpacking objects: 100% (9/9), 7.68 MiB | 3.55 MiB/s, done.
seog@Smarty:.../intro_to_bash$ ls 2022-01-12-Introduction-to-BASH/data/
1_control_18S_2019_minq7.fastq  1_control_ITS2_2019_minq7.fastq  reference_MN385595_1.fasta
seog@Smarty:.../intro_to_bash$ cd 2022-01-12-Introduction-to-BASH/
seog@Smarty:.../2022-01-12-Introduction-to-BASH$ mkdir aligned ; cd aligned
seog@Smarty:.../aligned$
```

Program from: https://github.com/lh3/minimap2

Map reads using minimap2

1. Create an index
2. Map reads against reference genome

Press TAB to auto-complete unique file/folder names to speed-up typing

```
seog@Smarty:.../aligned$ minimap2 -d ../data/reference_MN385595_1.mmi ../data/reference_MN385595_1.fasta
[M::mm_idx_gen::0.002*0.00] collected minimizers
[M::mm_idx_gen::0.004*0.00] sorted minimizers
[M::main::0.007*0.00] loaded/built the index for 1 target sequence(s)
[M::mm_idx_stat] kmer size: 15; skip: 10; is_hpc: 0; #seq: 1
[M::mm_idx_stat::0.008*0.00] distinct minimizers: 216 (100.00% are singletons); average occurrences:
1.000; average spacing: 5.671; total length: 1225
[M::main] Version: 2.24-r1122
[M::main] CMD: minimap2 -d ./data/reference_MN385595_1.mmi ./data/reference_MN385595_1.fasta
[M::main] Real time: 0.016 sec; CPU: 0.000 sec; Peak RSS: 0.002 GB
seog@Smarty:.../aligned$ ls ../data/
1_control_18S_2019_minq7.fastq  1_control_ITS2_2019_minq7.fastq  reference_MN385595_1.fasta
reference_MN385595_1.mmi
seog@Smarty:.../aligned$
```

Program from: https://github.com/lh3/minimap2

Map reads using minimap2

1. Create an index
2. Map reads against reference genome

Press TAB to auto-complete unique file/folder names to speed-up typing

```
seog@Smarty:.../aligned$ minimap2 -a ../data/reference_MN385595_1.mmi
../data/1_control_18S_2019_minq7.fastq > alignment.sam
[M::main::0.004*0.00] loaded/built the index for 1 target sequence(s)
[M::mm_mapopt_update::0.005*0.00] mid_occ = 10
[M::mm_idx_stat] kmer size: 15; skip: 10; is_hpc: 0; #seq: 1
[M::mm_idx_stat::0.006*0.00] distinct minimizers: 216 (100.00% are singletons); average occurrences:
1.000; average spacing: 5.671; total length: 1225
[M::worker_pipeline::0.218*2.15] mapped 18357 sequences
[M::main] Version: 2.24-r1122
[M::main] CMD: minimap2 -a ../data/reference_MN385595_1.mmi ../data/1_control_18S_2019_minq7.fastq
[M::main] Real time: 0.219 sec; CPU: 0.469 sec; Peak RSS: 0.019 GB
seog@Smarty:.../aligned$ ls -l
total 9232
drwxrwxrwx 1 gseo gseo    4096 Jan 10 04:02 ./
drwxrwxrwx 1 gseo gseo    4096 Jan 10 03:58 ../
-rwxrwxrwx 1 gseo gseo 9452653 Jan 10 04:02 alignment.sam*
seog@Smarty:.../aligned$
```

Troubleshooting

Troubleshooting - installation

Often when you get an error during installation, it could be due to missing dependency. Run following commands to install all required packages

```
$ sudo apt-get update
$ sudo apt-get install build-essential
```

```
seog@Smarty:.../intro_to_bash$ cd minimap2
seog@Smarty:.../minimap2$ make
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  main.c -o main.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  kthread.c -o kthread.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  kalloc.c -o kalloc.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  misc.c -o misc.o
cc -c -g -Wall -O2 -Wc++-compat  -DHAVE_KALLOC  bseq.c -o bseq.o
bseq.c:1:10: fatal error: zlib.h: No such file or directory
    1 | #include <zlib.h>
      |          ^~~~~~~~
compilation terminated.
make: *** [Makefile:41: bseq.o] Error 1
seog@Smarty:.../minimap2$
```

Troubleshooting – missing file

When you are unsure of the error message, search it online!

Someone probably had the same problem and found the solution.

```
seog@Smarty:.../aligned$ minimap2 -a ./reference_MN385595_1.mmi
../data/1_control_18S_2019_minq7.fastq > alignment.sam
[ERROR] failed to open file './reference_MN385595_1.mmi': No such file or directory
```

Check your path for each files

```
seog@Smarty:.../aligned$ minimap2 -v ./reference_MN385595_1.mmi
../data/1_control_18S_2019_minq7.fastq > alignment.sam
[ERROR] missing input: please specify a query file to map or option -d to keep the index
```

Check if proper options were used

Click to go back to the beginning

LEARNING OBJECTIVES

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BASH scripting

Create a file with .sh extension inside intro_to_bash folder

You can create it using a notepad, VS Code or use text editors on terminal

i.e. one of the following:

$ touch SCRIPT.sh

$ nano SCRIPT.sh

```
seog@Smarty:.../intro_to_bash$ touch script.sh
```

BASH scripting: add shebang

#!/usr/bin/env bash *(shebang, for recognizing which shell was used to write the script)*

C: > Users > seog > Desktop > intro_to_bash > script.sh

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

# Anything after the pound sign become comments
# shebang can also be written as: #!/bin/bash

echo "Welcome to MMID Coding Workshop!" # echo function prints message inside ""
```

```
seog@Smarty:.../intro_to_bash$ chmod 755 script.sh
seog@Smarty:.../intro_to_bash$ ./script.sh
Welcome to MMID Coding Workshop!
seog@Smarty:.../intro_to_bash$
```

BASH scripting: adding variables

Add elements to the script (scroll down) + save script & run

C: > Users > seog > Desktop > intro_to_bash > script.sh

```
echo "Welcome to MMID Coding Workshop!" # echo function prints message inside ""

# set variables – use absolute path for directories!
BASE_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH"
DATA_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH/data"
OUTPUT_DIR="$BASE_DIR/script_output"
INPUT="1_control_18S_2019_minq7.fastq"
REFERENCE="reference_MN385595_1.fasta"

echo $BASE_DIR # print out variable
```

Variables: abstract storage location where information can be saved and accessed later

```
seog@Smarty:.../intro_to_bash$ ./script.sh
Welcome to MMID Coding Workshop!
/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH
seog@Smarty:.../intro_to_bash$ echo $BASE_DIR

seog@Smarty:.../intro_to_bash$
```

BASH scripting: cd and create directory

Add elements to the script (scroll down) + save script & run

C: > Users > seog > Desktop > intro_to_bash > script.sh

```
BASE_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH"
DATA_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH/data"
OUTPUT_DIR="$BASE_DIR/script_output"
INPUT="1_control_18S_2019_minq7.fastq"
REFERENCE="reference_MN385595_1.fasta"

# echo $BASE_DIR # print out variable
cd $BASE_DIR # you can also use ${BASE_DIR}
mkdir -p "script_output" # create folder if it doesn't exist -p
echo "hi" # just random word to verify script output
pwd # print script's current working directory
```

```
seog@Smarty:.../intro_to_bash$ ./script.sh
Welcome to MMID Coding Workshop!
hi
/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH
seog@Smarty:.../intro_to_bash$
```

BASH scripting: add minimap2 jobs

Add elements to the script (scroll down) + save script & run

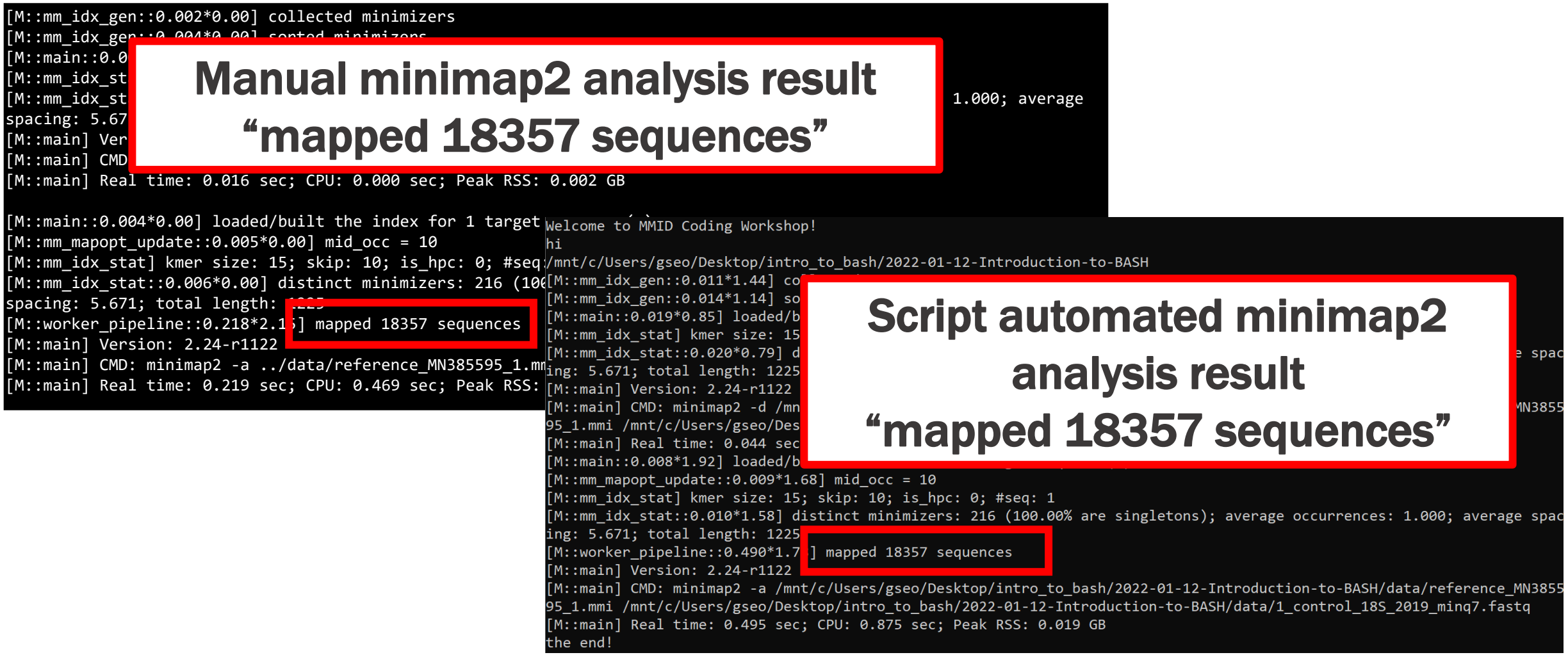
C: > Users > seog > Desktop > intro_to_bash > script.sh

```
# Run minimap2 and save output result into $OUTPUT_DIR
# Copy and paste previous command used but use variables for input files
# minimap2 -d ../data/reference_MN385595_1.mmi ../data/reference_MN385595_1.fasta
minimap2 -d $DATA_DIR/reference_MN385595_1.mmi $DATA_DIR/$REFERENCE

# minimap2 -a ../data/reference_MN385595_1.mmi ../data/1_control_18S_2019_minq7.fastq >
alignment.sam
minimap2 -a $DATA_DIR/reference_MN385595_1.mmi $DATA_DIR/$INPUT > $OUTPUT_DIR/alignment.sam

echo "the end!"
exit
```

```
seog@Smarty:.../intro_to_bash$ ./script.sh
Welcome to MMID Coding Workshop!
...
[M::main] Real time: 0.495 sec; CPU: 0.875 sec; Peak RSS: 0.019 GB
the end!
seog@Smarty:.../intro_to_bash$
```

BASH scripting: minimap2 output

Final BASH script output printed on command line interface



Complete BASH script

C: > Users > seog > Desktop > intro_to_bash > script.sh

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

# Anything after the pound sign become comments
# shebang can also be written as: #!/bin/bash

echo "Welcome to MMID Coding Workshop!" # echo function prints message inside ""

# set variables – use absolute path for directories!
BASE_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH"
DATA_DIR="/mnt/c/Users/seog/Desktop/intro_to_bash/2022-01-12-Introduction-to-BASH/data"
OUTPUT_DIR="$BASE_DIR/script_output"
INPUT="1_control_18S_2019_minq7.fastq"
REFERENCE="reference_MN385595_1.fasta"

# echo $BASE_DIR # print out variable
cd $BASE_DIR # you can also use ${BASE_DIR}
mkdir -p "script_output" # create folder if it doesn't exist -p
echo "hi" # just random word to verify script output
pwd # print script's current working directory

# Run minimap2 and save output result into $OUTPUT_DIR
# Copy and paste previous command used but use variables for input files
# minimap2 -d ../data/reference_MN385595_1.mmi ../data/reference_MN385595_1.fasta
minimap2 -d $DATA_DIR/reference_MN385595_1.mmi $DATA_DIR/$REFERENCE

# minimap2 -a ../data/reference_MN385595_1.mmi ../data/1_control_18S_2019_minq7.fastq > alignment.sam
minimap2 -a $DATA_DIR/reference_MN385595_1.mmi $DATA_DIR/$INPUT > $OUTPUT_DIR/alignment.sam

echo "the end!"
exit
```

Click to go back to the beginning

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5. *Write a BASH script, change permission, and execute the script*

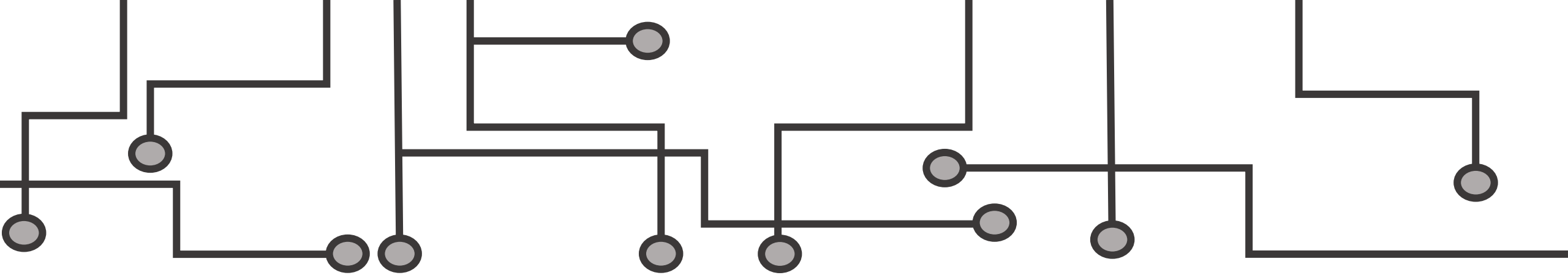
* Items are recommended

HELPFUL RESOURCES

1. ***What is CLI*** ***https://www.hostinger.com/tutorials/what-is-cli***
2. ***BASH manual:*** ***https://www.gnu.org/software/bash/manual/bash.html***
3. ***Information on Linux folder structure***
   https://www.howtogeek.com/117435/htg-explains-the-linux-directory-structure-explained/
4. ****The BASH Guide:*** ***https://guide.bash.academy/***
5. ****Learn Enough Command-Line to be dangerous (free first few chapters):***
   https://www.learnenough.com/command-line-tutorial

YouTube Videos

1. ****Joe Collins - Beginner's Guide to the Bash Terminal:***
   https://www.youtube.com/watch?v=oxuRxtrO2Ag
2. ****Traversy Media – Shell Scripting Crash Course – Beginner Level:***
   https://www.youtube.com/watch?v=v-F3YLd6oMw



THANK YOU FOR ATTENDING!

The Q&A Session will now begin.

Please make sure to fill out the Exit Survey

We value your feedback!

More questions? Please email us at
mmid.coding.workshop@gmail.com or post them to the workshop slack channel