

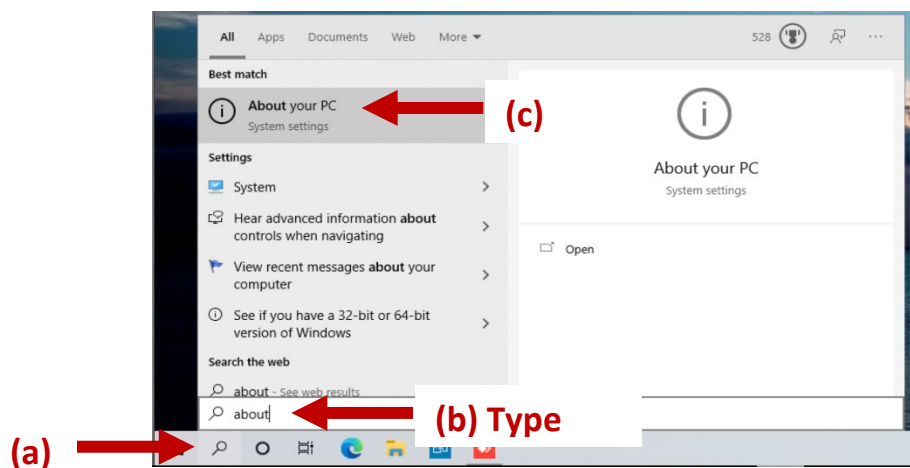
Windows Installation Guide v1

Prerequisite Installation

Of all the installation guides we've prepared, the Windows guide is by far the longest and most involved with most command line dabbling till set up. This is because the software used for ChIP-AP was never destined to run on Windows but there is a way around it if you're tech savvy enough. Fortunately for you, we think we are 😊

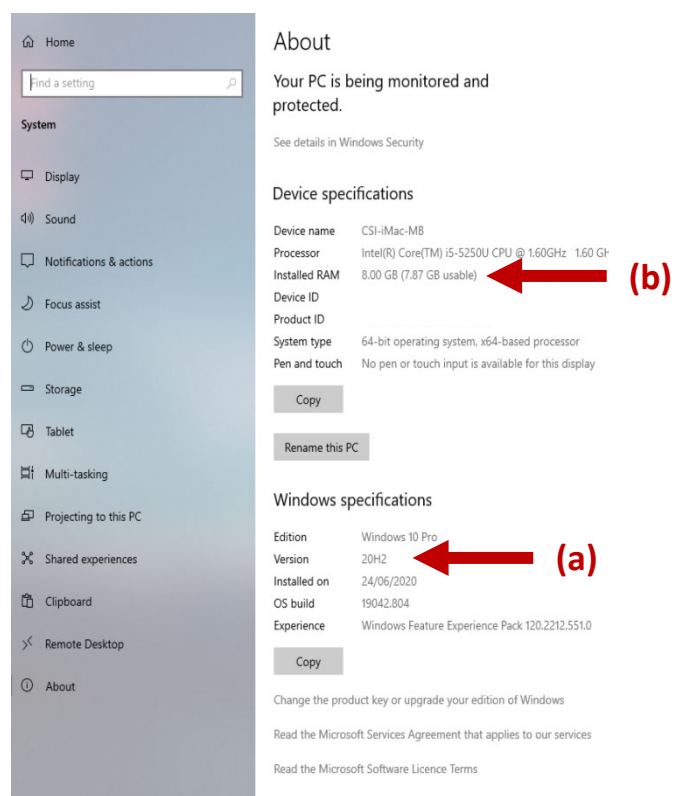
This installation guide was performed on a Windows 10 installation running v20H2, although will work with v1903 or later (Homer or Professional). Basically, as long as you have access to install the Linux Subsystem you will be fine. Older versions of Windows, like Windows 7/8, are not compatible. For this installation, you **will** need administrator access to your machine to install and make modifications as well as an active Microsoft Store Account. If you are setting it up on your own personal laptop, this should be fine as it's your usual login password. If this is a computer owned/maintained by your institute's IT department, you will either need them to set it up for you or give you temporary administrator access to set it up (which they can if they are nice).

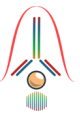
1. To check which version of Windows you are running, go to the search icon (a), and type "About" in search segment (b), and then click "About your PC" in (c).



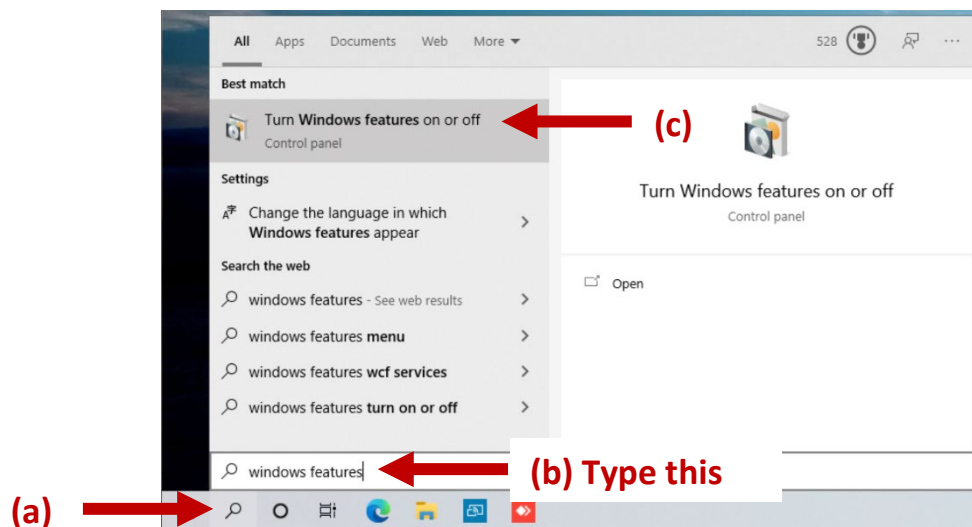
2. In the window that appears, you will see which version of Windows you are running (a). Your version needs to be 1903 or greater. In our example we are running 20H2. The 19/20 refers to the year of release (2019/2020). So, if your "year" is 2019 onwards you should be set to proceed. If not, then you need to update to the latest version of Windows 10 to continue.

You will also see how much RAM is installed, as a minimum you need 8Gb, but 16Gb or more is recommended. You will also need at least 60Gb of storage space on your SSD/HDD just to setup ChIP-AP, not including any additional space you need for your actual analysis, which we recommend as another 100Gb or so.

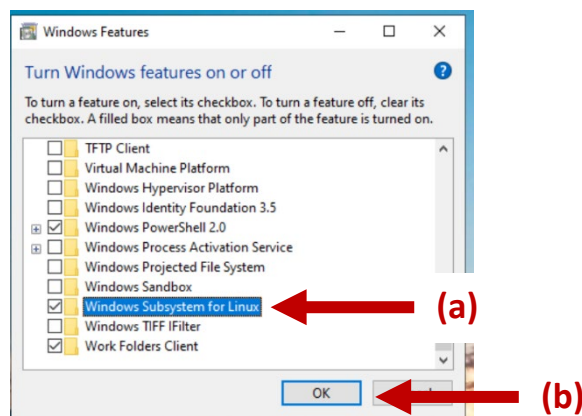




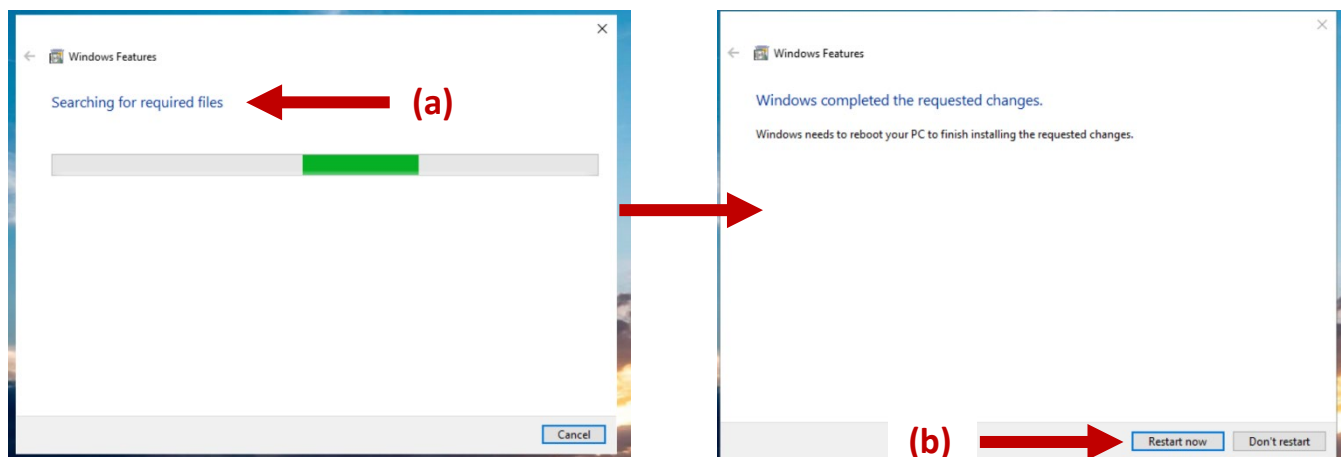
3. Now that we're sure you have the right build of Windows installed; we need to setup the Linux Subsystem component of Windows to allow us to install everything. To do this, you need to go back to the search icon (a) and search for "Windows Features" (b), and select it (c).

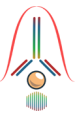


4. In the window that appears, scroll down until you find the "Windows Subsystem for Linux" option and tick the box next to it (a), then press OK (b).

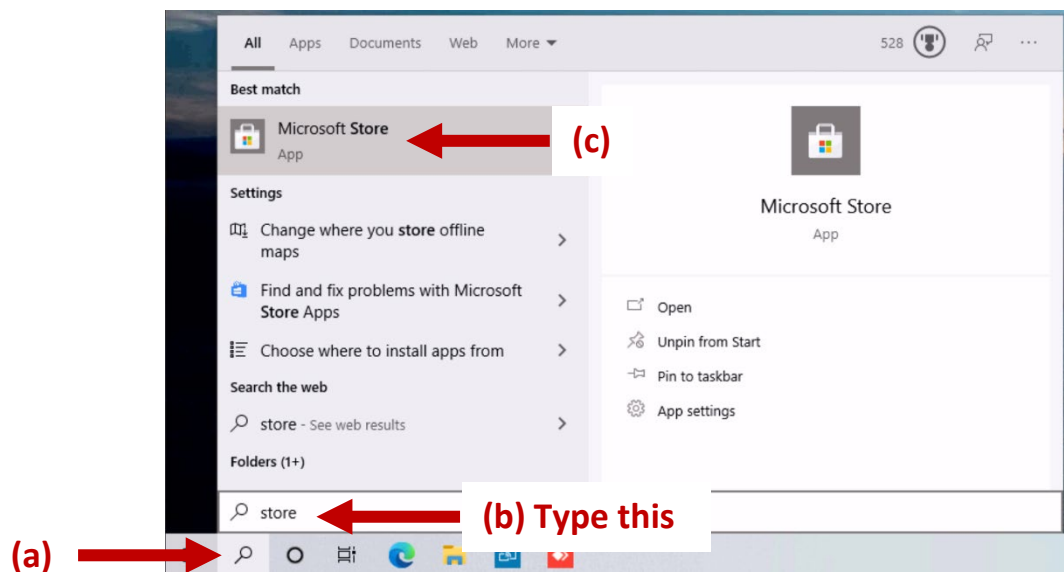


5. When you press OK, the following window will appear (a) and Windows will go through to setup what it needs to. This can take a while depending on the speed of your machine. When complete you **MUST** restart your computer before you can continue the rest of the installation process (b).

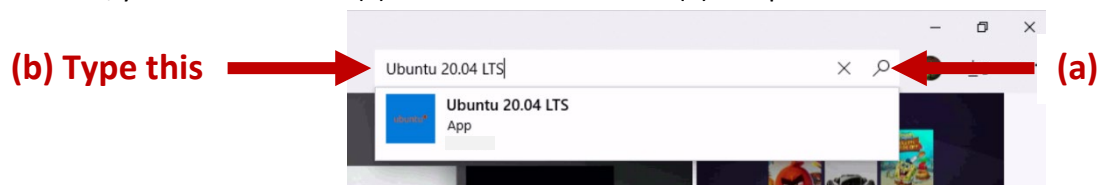




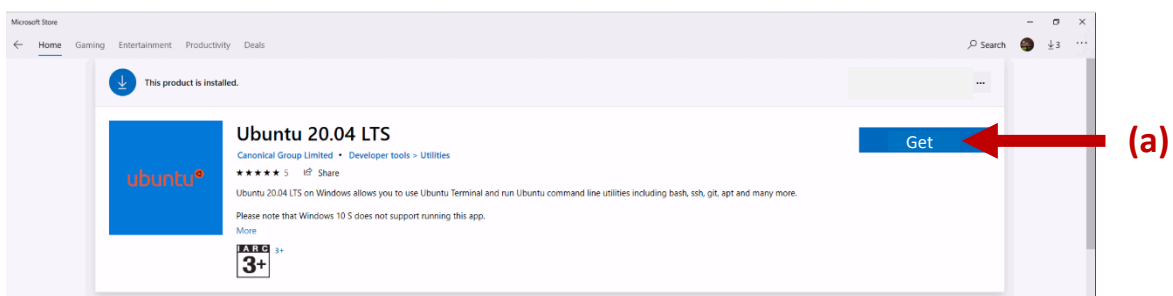
6. Alright now that we have the Linux subsystem component installed, we now need to actually install Linux as a subsystem (ok that sounds confusing but it actually is right). To do this you need to go to the search icon again (a) and search for the “Store” (b), and select it (c).



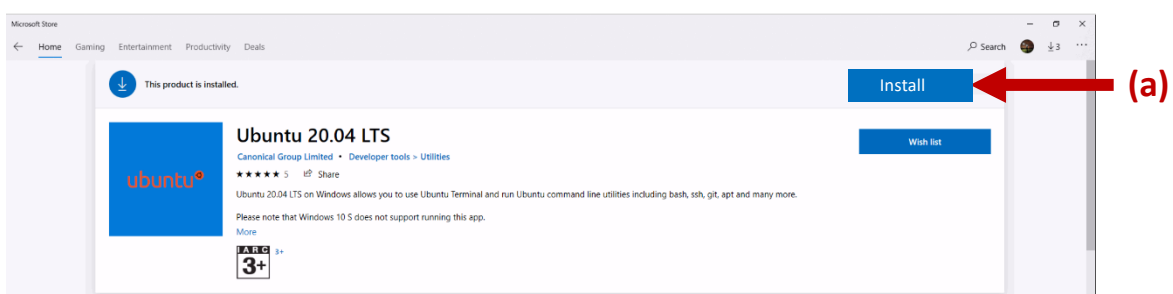
7. Once loaded, you need to search (a) for “Ubuntu 20.04 LTS” (b) and press ENTER or select the search result.

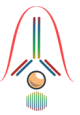


8. In the following window, you will need to “Get” this Linux distribution (a). You will then be prompted to enter your login and purchase details. This is *FREE* to download and install so there is no charge but you still need to go through the steps to get it added to your account be it add password/PIN or other means. Ubuntu 16.04/18.04 are also compatible and will work just fine.

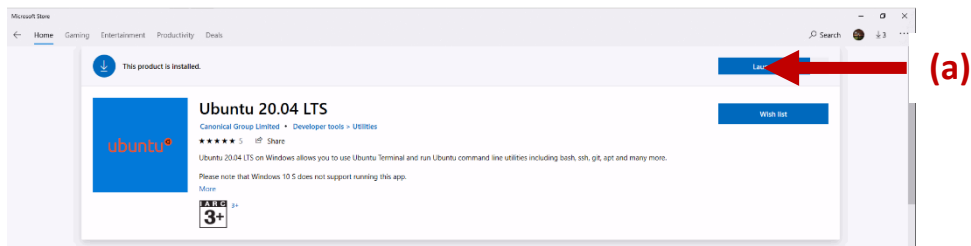


9. Once Ubuntu 20.04 LTS is added to your account you then need to click the “Install” icon (a).

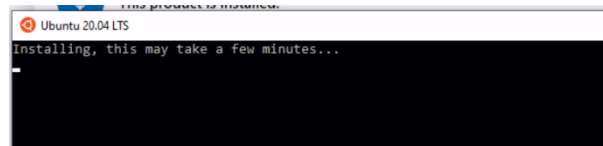




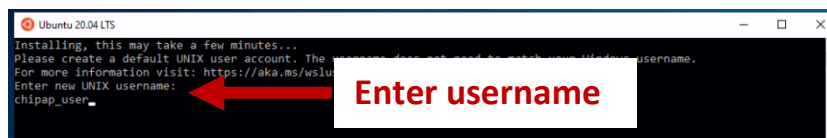
10. Finally once its installed, you need to “Launch” (a) the installation.



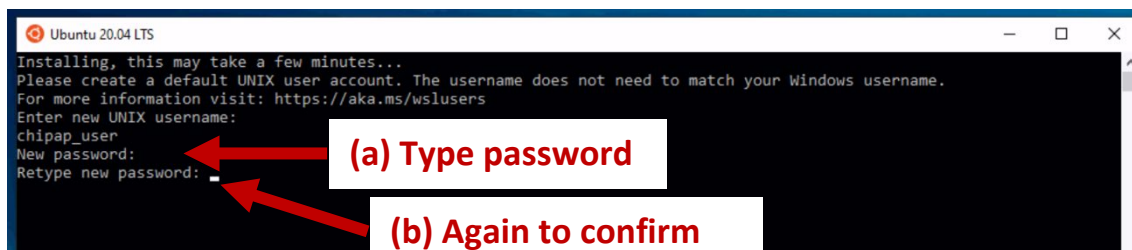
11. In the terminal window that appears, you’re first message will be “Installing, this may take a few minutes”... which it will....



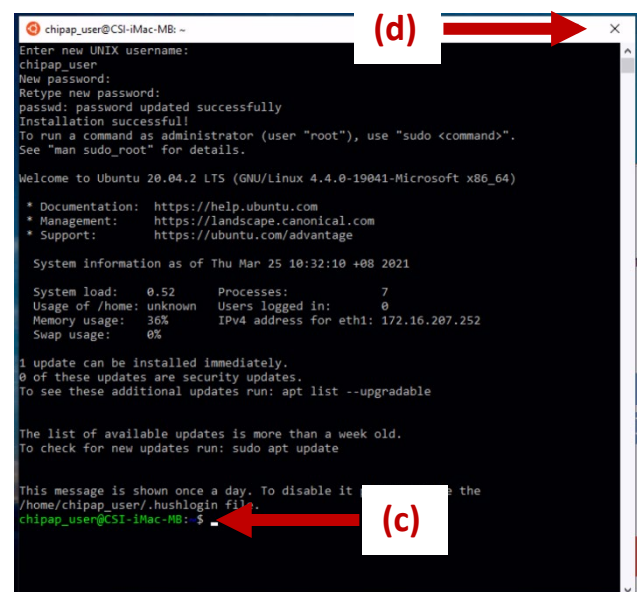
12. After a few moments you will be asked to enter a new username (which can be different than your windows login name) and then press ENTER. If after a few minutes nothing appears at all, press ENTER once, this should then bring up the following username question if it didn’t appear (for whatever reason that may happen).

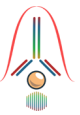


13. Next, you will be asked for a new password (a). This can be different than your windows password. When you start typing, NOTHING will appear for security reasons. So make sure you enter the password exactly as you want it. Press ENTER when done. You will be asked to re-enter the password to confirm (b). Press ENTER again when done.

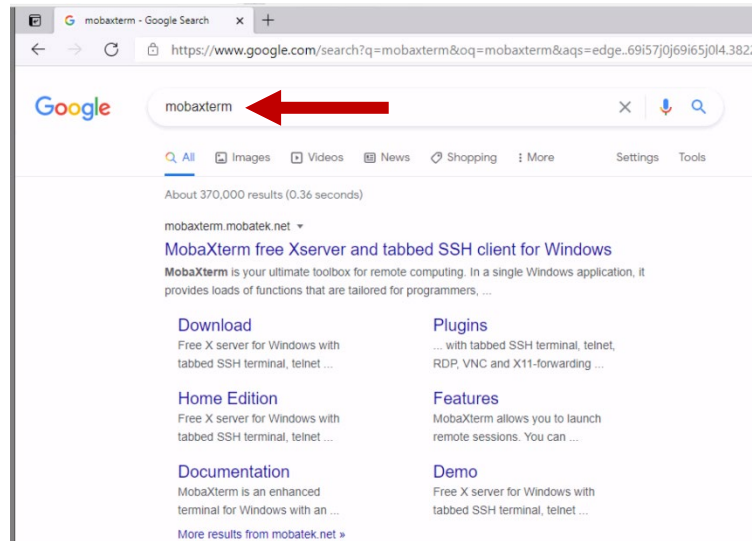


14. This now completes the Linux subsystem installation and setup. You will see information comparable to the following to validate everything worked fine. Once you see the green final prompt (c), everything is good to go and you can close the Linux terminal (d)

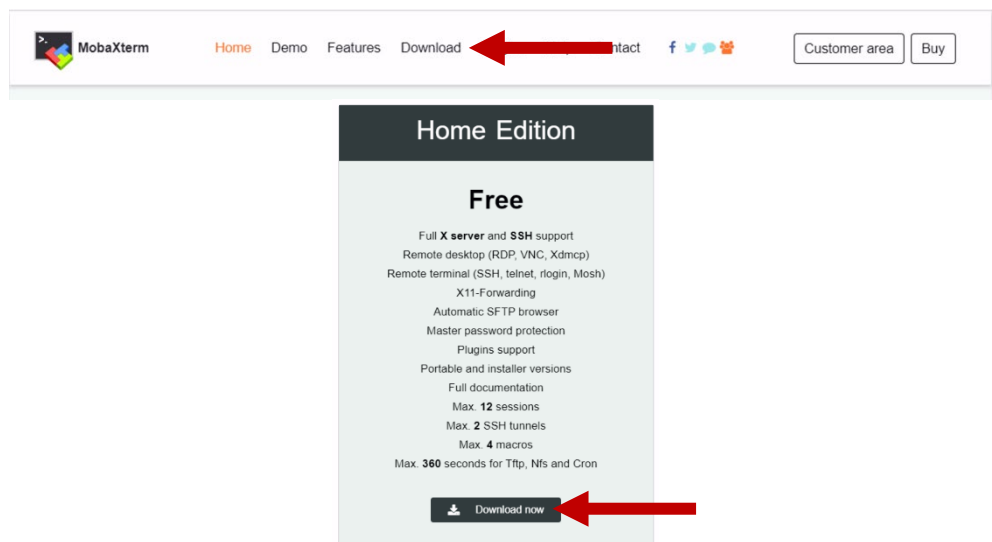




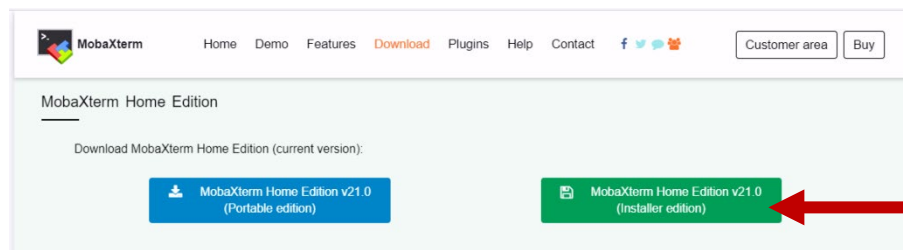
15. So, up until now, we have installed the Linux subsystem to allow us to install all the necessary programs under the hood. There are now 2 more things to install before we can get to installing ChIP-AP. The first thing to install is MobaXterm. To do this, open up your browser of choice and search for “MobaXterm”

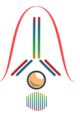


16. Next, navigate to the download section and download the “Home Edition” which is free.



17. On the next web-page, since you have administrator rights, pick the installer edition.

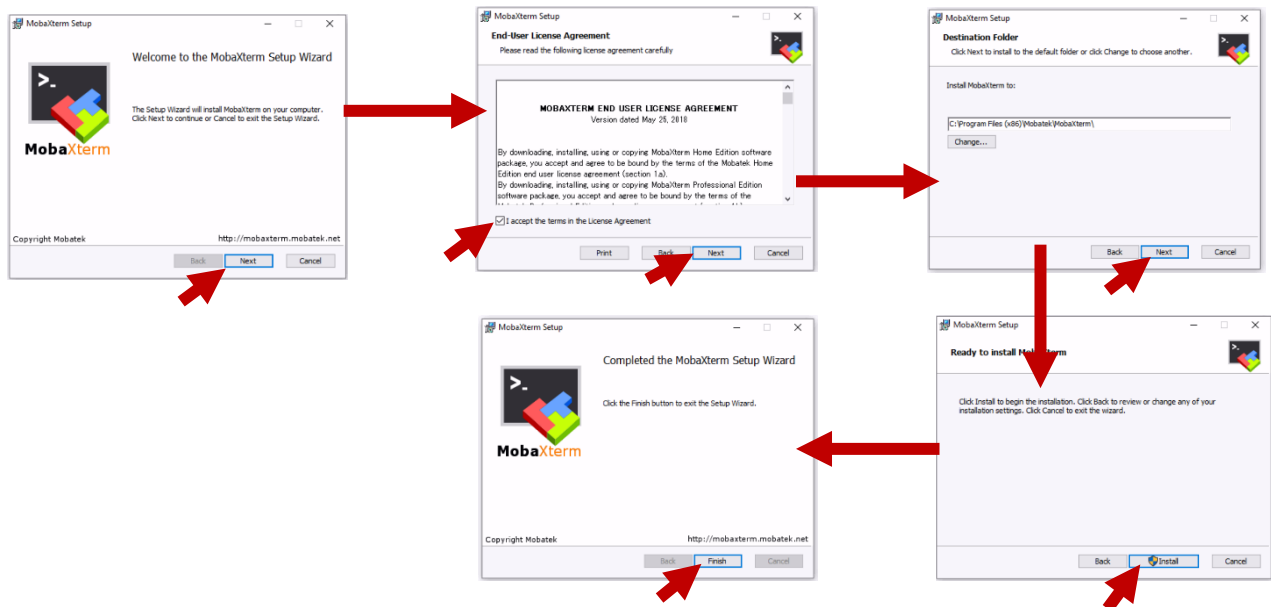




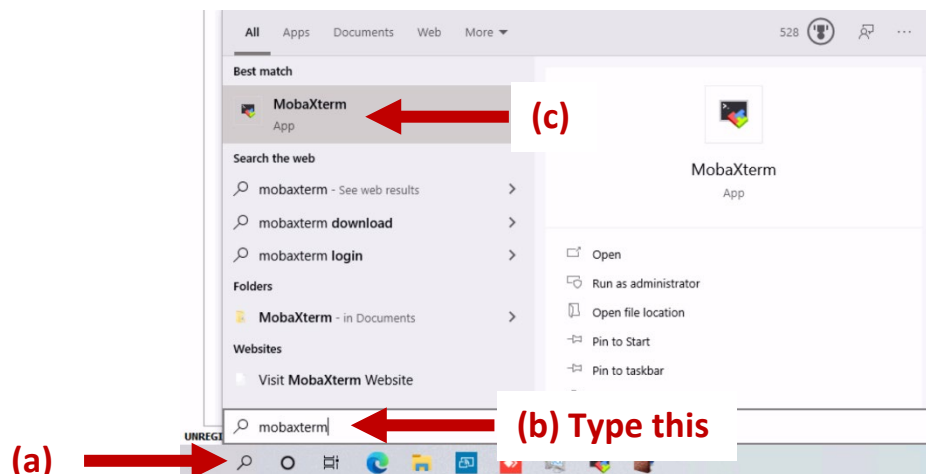
18. When you choose to download it, you will be downloading a *.zip folder. Unzip this in a location of your choosing. You will then need to run the *.msi installer.

Name	Date modified	Type	Size
MobaXterm_installer.dat	25/03/2021 10:58 AM	DAT File	15,646 KB
MobaXterm_installer_21.0.msi		Windows Installer ...	12,000 KB

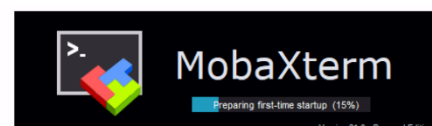
19. Next, run through the installer accepting all default settings (unless you know what you're doing and want to configure it yourself).



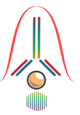
20. Next, run MobaXterm by going to the search icon (a) and search for "MobaXterm" (b) and select it (c).



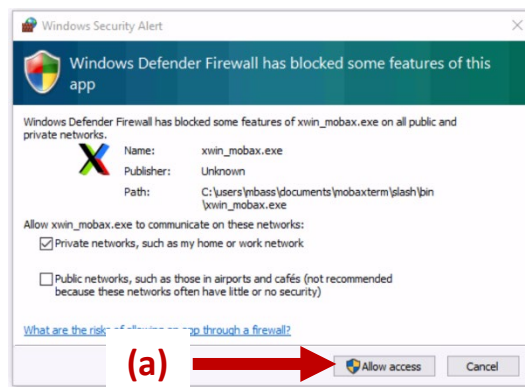
21. The first time you run MobaXterm, the initial splash screen will a little longer to set everything up.



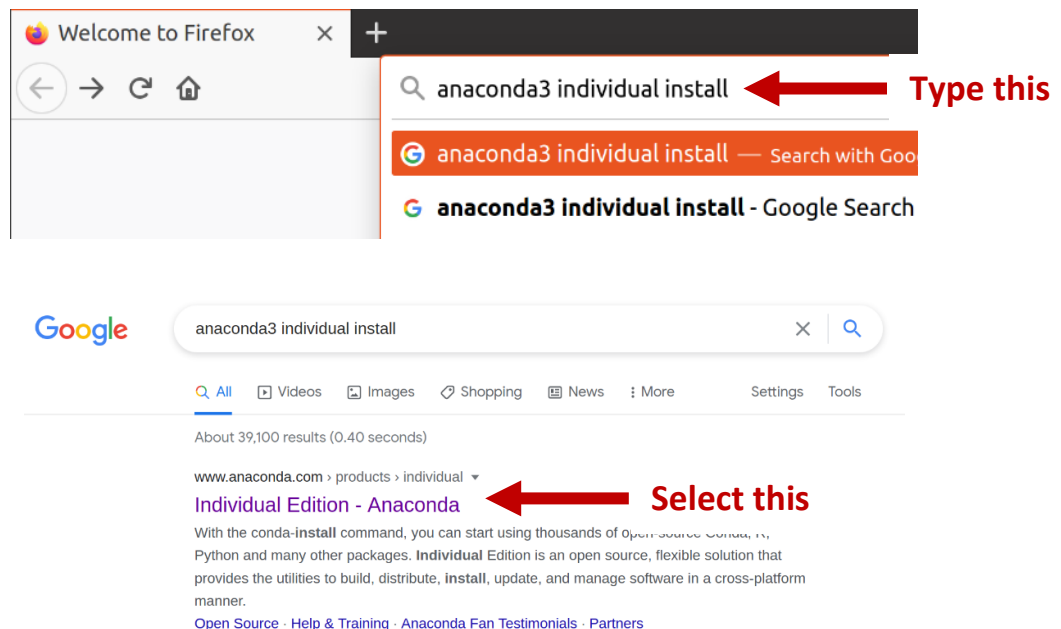
take



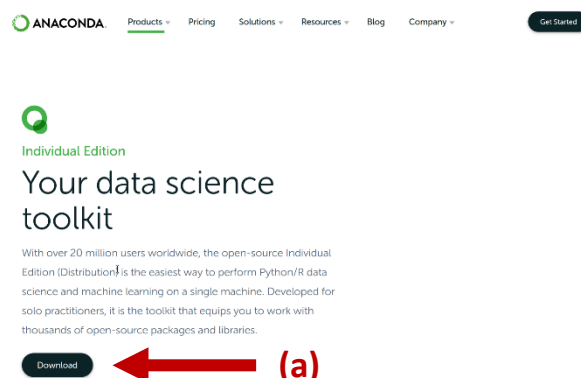
22. You will then be asked to give MobaXterm access through the firewall, which you must accept to (a).



23. Alright, the next couple steps are a little more complicated so take special care. We will need to switch between your web-browser and MobaXterm. So first, your web-browser of choice, search for “anaconda3 individual install”

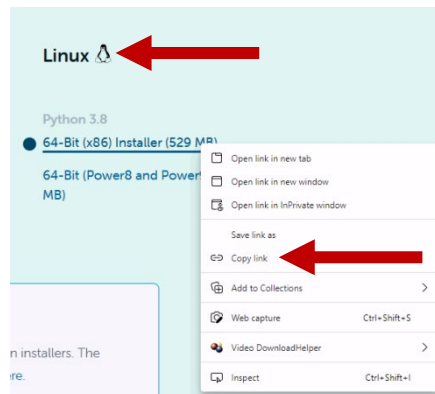


24. Next, go to the bottom of the page by clicking, “Download” (a) and get to the stage where you can select the right installer (b).

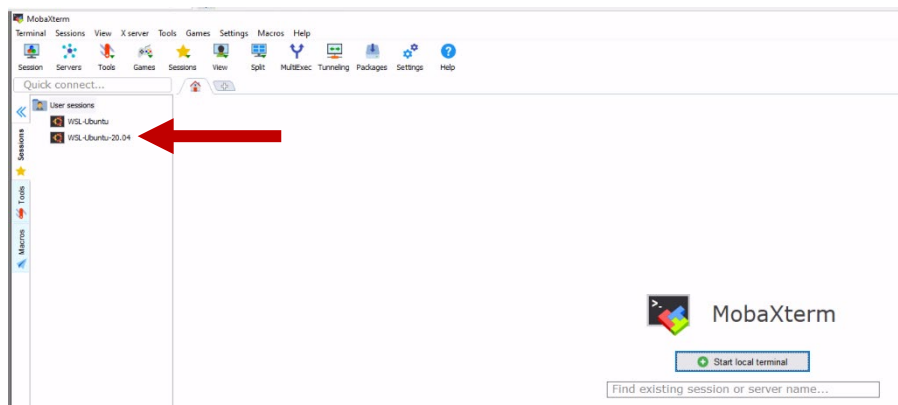




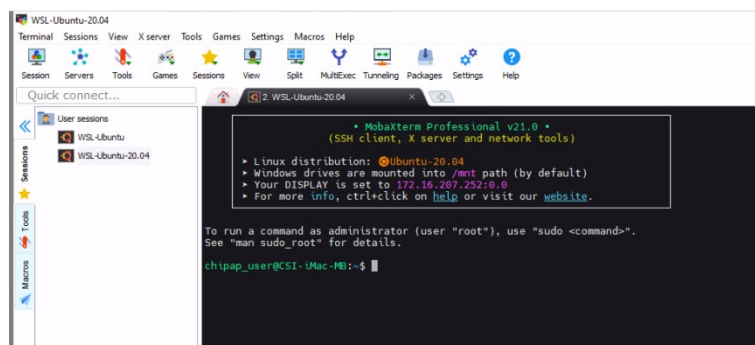
25. Next, right click on the “64-Bit (x86) Installer” under Linux and select “copy link.”

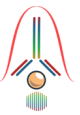


26. Now, go back to MobaXterm and double mouse click “WSL-Ubuntu-20.04” in the top left.



27. This will open the panel shown below





28. Next, type the following commands, 1 line at a time, pressing ENTER after the end of each line.

```
mkdir tools
cd tools
mkdir chipap
cd chipap
```

What you should now see is something as follows...

```

• MobaXterm Professional v21.0 •
(SSH client, X server and network tools)

> Linux distribution: Ubuntu-20.04
> Windows drives are mounted into /mnt path (by default)
> Your DISPLAY is set to 172.16.207.252:0.0
> For more info, ctrl+click on help or visit our website.

To run a command as administrator (user "root"), use "sudo <command>".
See "man sudo_root" for details.

chipap_user@CSI-iMac-MB:~$ mkdir tools
chipap_user@CSI-iMac-MB:~$ cd tools
chipap_user@CSI-iMac-MB:~/tools$ mkdir chipap
chipap_user@CSI-iMac-MB:~/tools$ cd chipap
chipap_user@CSI-iMac-MB:~/tools/chipap$

```

29. So, what we did is made a new directory (folder) named “tools”, changed directory (cd) into it. Then we made another new directory named “chipap” and then changed directory (cd) into that. The next step is to download the anaconda installation file and run it in Linux. Now, at the command prompt, type “wget” and then paste the link path from the web-browser above (right-click and go to paste). It will look something like what’s below. Now press ENTER.

```
wget https://repo.anaconda.com/archive/Anaconda3-2020.11-Linux-x86_64.sh
```

```

• MobaXterm Professional v21.0 •
(SSH client, X server and network tools)

> Linux distribution: Ubuntu-20.04
> Windows drives are mounted into /mnt path (by default)
> Your DISPLAY is set to 172.16.207.252:0.0
> For more info, ctrl+click on help or visit our website.

To run a command as administrator (user "root"), use "sudo <command>".
See "man sudo_root" for details.

chipap_user@CSI-iMac-MB:~$ mkdir tools
chipap_user@CSI-iMac-MB:~$ cd tools
chipap_user@CSI-iMac-MB:~/tools$ mkdir chipap
chipap_user@CSI-iMac-MB:~/tools$ cd chipap
chipap_user@CSI-iMac-MB:~/tools/chipap$ wget https://repo.anaconda.com/archive/Anaconda3-2020.11-Linux-x86_64.sh

```

30. The installer file will then download and give you back the command line prompt. You then need to type “bash Ana” and then press TAB on the keyboard and the terminal will auto-complete the rest of the command required so you don’t have to complete it manually. It will look as follows (below). Then press ENTER to start the installation process.

```

• MobaXterm Professional v21.0 •
(SSH client, X server and network tools)

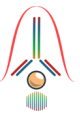
> Linux distribution: Ubuntu-20.04
> Windows drives are mounted into /mnt path (by default)
> Your DISPLAY is set to 172.16.207.252:0.0
> For more info, ctrl+click on help or visit our website.

To run a command as administrator (user "root"), use "sudo <command>".
See "man sudo_root" for details.

chipap_user@CSI-iMac-MB:~$ mkdir tools
chipap_user@CSI-iMac-MB:~$ cd tools
chipap_user@CSI-iMac-MB:~/tools$ mkdir chipap
chipap_user@CSI-iMac-MB:~/tools$ cd chipap
chipap_user@CSI-iMac-MB:~/tools/chipap$ wget https://repo.anaconda.com/archive/Anaconda3-2020.11-Linux-x86_64.sh
--2021-03-25 12:50:38-- https://repo.anaconda.com/archive/Anaconda3-2020.11-Linux-x86_64.sh
Resolving repo.anaconda.com (repo.anaconda.com)... 104.16.131.3, 104.16.130.3, 2606:4700::6810:8203, ...
Connecting to repo.anaconda.com (repo.anaconda.com)|104.16.131.3|:443... connected.
HTTP request sent, awaiting response... 200 OK
Length: 554535580 (529M) [application/x-sh]
Saving to: 'Anaconda3-2020.11-Linux-x86_64.sh'

Anaconda3-2020.11-Linux-x86_64.sh 100%[=====] 528.85M 33.8MB/s in 14s
2021-03-25 12:50:54 (36.7 MB/s) - 'Anaconda3-2020.11-Linux-x86_64.sh' saved [554535580/554535580]
chipap_user@CSI-iMac-MB:~/tools/chipap$ bash Anaconda3-2020.11-Linux-x86_64.sh

```



31. Press ENTER to view the license agreement which you must agree to for installation to proceed. To progress through each page of the agreement press the SPACE bar - read it, if you want...

```
chipap_user@CSI-iMac-MB:~/tools/chipap$ bash Anaconda3-2020.11-Linux-x86_64.sh
Welcome to Anaconda3 2020.11
In order to continue the installation process, please review the license
agreement.
Please, press ENTER to continue
>>> 
```

Once you get to the end... you must type "yes" in full and press ENTER

```
Last updated September 28, 2020

Do you accept the license terms? [yes|no]
[no] >>> 
```

32. Next, you will be asked where to install anaconda3. Unless you are an advanced user and know how to configure anaconda properly, accept defaults and press ENTER again.

```
Do you accept the license terms? [yes|no]
[no] >>> yes

Anaconda3 will now be installed into this location:
/home/chipap_user/anaconda3

- Press ENTER to confirm the location
- Press CTRL-C to abort the installation
- Or specify a different location below

[/home/chipap_user/anaconda3] >>> 
```

Anaconda will then go ahead and install itself. This will take a few minutes. Remember though, no eating in the lab while this is working!

33. When complete, you will be asked to initialize Anaconda3, type "yes" and press ENTER

```
Do you accept the license terms? [yes|no]
[no] >>> yes

Anaconda3 will now be installed into this location:
/home/chipap_user/anaconda3

- Press ENTER to confirm the location
- Press CTRL-C to abort the installation
- Or specify a different location below

[/home/chipap_user/anaconda3] >>>
PREFIX=/home/chipap_user/anaconda3
Unpacking payload ...
Collecting package metadata (current_repodata.json): done
Solving environment: done

# All requested packages already installed.

installation finished.
Do you wish the installer to initialize Anaconda3
by running conda init? [yes|no]
[no] >>> yes
```

Great! Anaconda3 is now installed. Now you **must** close and re-start MobaXterm before the next steps of actually installing CHIP-AP!!!

Please read carefully the final few lines if it says something along the lines of "you have chosen to not initialize anaconda...." You need to re-run the anaconda installation using the command below making sure to add the "-u" at the end.

```
(base) chipap_user@CSI-iMac-MB:~/tools/chipap$ bash Anaconda3-2020.11-Linux-x86_64.sh -u
```

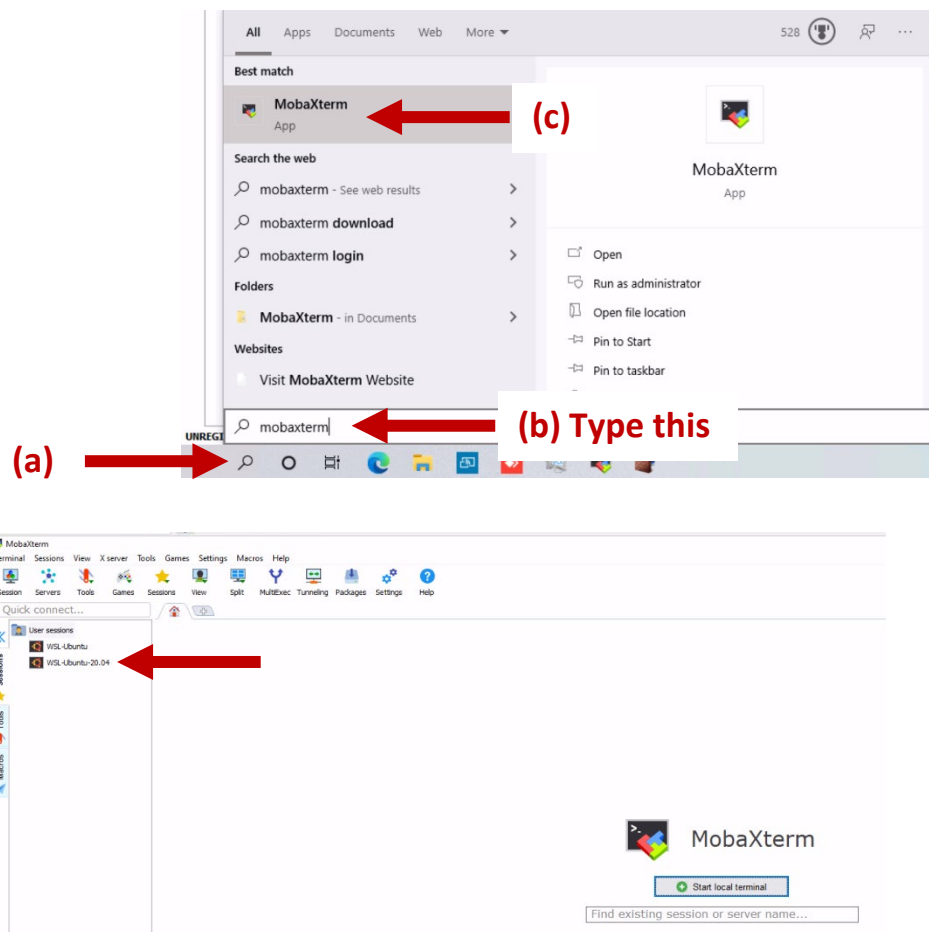
If you don't make sure to initialize anaconda post-installation, nothing down-stream of this will work.



Setting up CHIP-AP

1. Now down to the “meat” of the installation!

1.1. First thing, you need to open a new terminal of MobaXterm as before. Double click on Ubuntu 20.04.



1.2. In the terminal window, you need to type the following commands 1 per line followed by ENTER.

```
cd tools
```

```
cd chipap
```

1.3. You now need to download the latest version of CHIP-AP from our **github**(dropbox) page by typing “wget” followed by a space, then pasting the following url and press ENTER after to initiate the download.

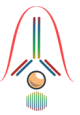
https://www.dropbox.com/s/t80w47e6n3t3ibi/chipap_installation_20210324.zip

```
(base) chipap_user@CSI-iMac-MB:~$ cd tools/  
(base) chipap_user@CSI-iMac-MB:~/tools$ cd chipap/  
(base) chipap_user@CSI-iMac-MB:~/tools/chipap$ wget https://www.dropbox.com/s/t80w47e6n3t3ibi/chipap_installation_20210324.zip?dl=0
```

This shouldn't take long to download. Next, we need to download the chipap genomes required for the run. As before, typing “wget” followed by a space, then pasting the following url and press ENTER after to initiate the download. https://www.dropbox.com/s/073mpy8aho4vjda/chipap_genomes.zip

```
(base) chipap_user@CSI-iMac-MB:~/tools/chipap$ wget https://www.dropbox.com/s/073mpy8aho4vjda/chipap_genomes.zip?dl=0
```

This WILL take a long time to download



1.4. Once the previous download has finished, enter the following command followed by ENTER.

```
sudo apt-get install unzip
```

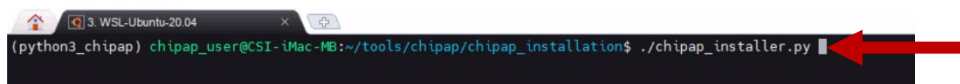
This will ask you to enter the password you set in the beginning when we installed Linux.

1.5. Next, enter the following commands 1 at a time followed by ENTER.

```
unzip chipap_installation_20210324.zip
unzip chipap_genomes.zip
mv chipap_genomes ./chipap_installation/
cd ./chipap_installation
chmod +x ./chipap_installation.py
```

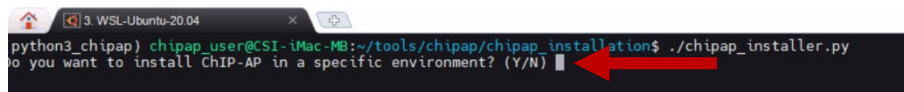
Next...type the following command and press ENTER, to start the installation process

```
./chipap_installer.py
```



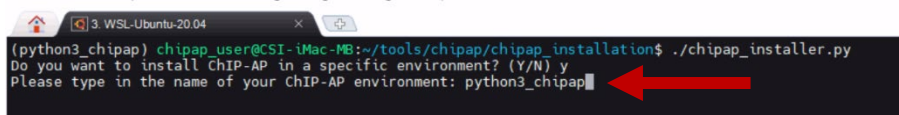
1.6. As part of the installation process, ChIP-AP will ask a couple of questions

1.6.1. The first question asked is whether you want to install ChIP-AP in its own environment. This is so ChIP-AP will sit in its own contained “capsule” and won’t be affected by other installed programs and so this will ensure that all the dependencies of ChIP-AP will be correct and met all the time. So, we highly recommend this option

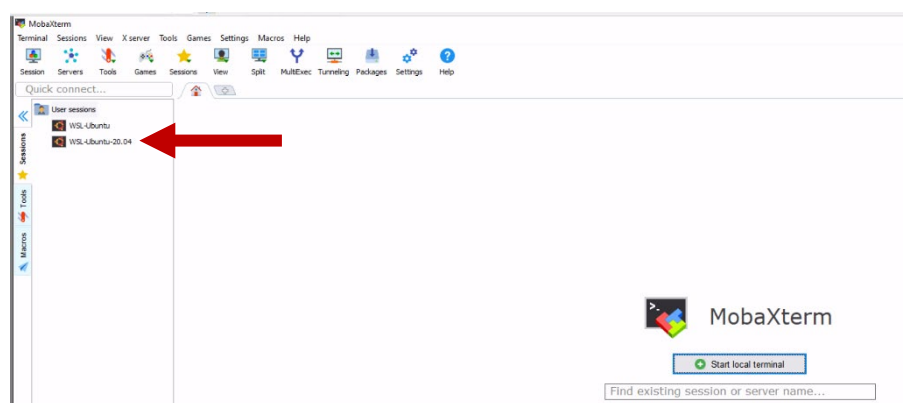


If you answer “n”, then ChIP-AP will be installed in the base environment along with its dependencies.

If answer “y”, you will be asked to name the environment and then press ENTER. A confirmation statement will be printed for you to confirm with an additional ENTER press. The installation will take a while to run as a lot of packages will need to be installed, so this is highly dependent on your internet and cpu speeds as to how long it will take. Take note of the environment name you set (we will refer to it in step 2.7 as xxxxx)



1.7. Once the installation completes, close MobaXterm and reopen it. Open up a new Ubuntu 20.04 terminal as before.





Depending on your proficiency, there are 3 ways to use ChIP-AP.

If you setup ChIP-AP in an environment in step 1.6, once you open the terminal, you will need to type the following command (below) where xxxx is the name of the environment as you defined in step 1.6 before running ChIP-AP EVERYTIME. Otherwise, this command isn't necessary.

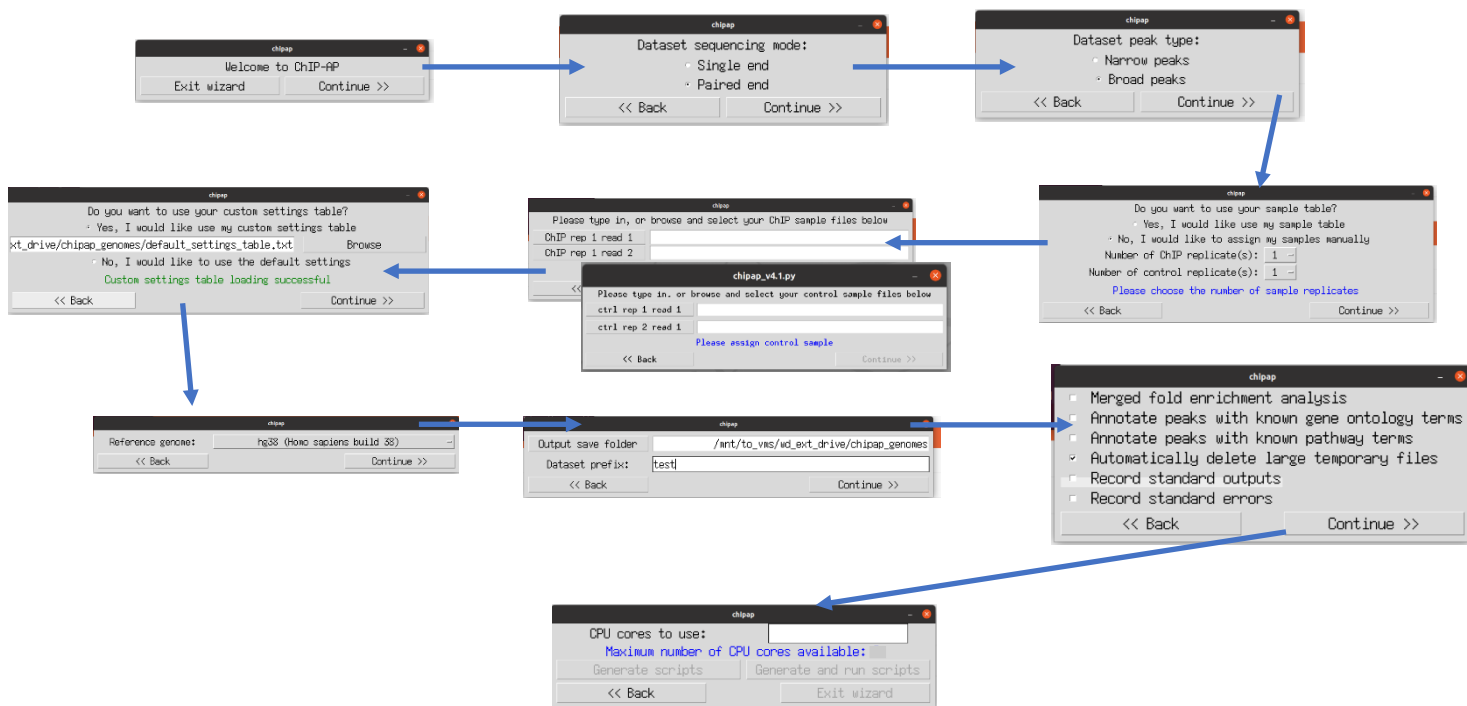
```
conda activate xxxxxx
conda activate python3_chipap
```

1.7.1. To use ChIP-AP using the command line, refer to the documentation on our github (xxx) for full details on how to setup a run with appropriate flags/parameters (For advanced/proficient users).

1.7.2. For most non-bioinformatician users of ChIP-AP, we recommend using the wizard. To use this, at the opened terminal command line type

```
chipap_wizard.py
```

and the first window of the wizard will appear (below). The wizard will ask you questions sequentially to acquire all the data it needs for a successful run. Below also is a snapshot of the windows you will see throughout the wizard process.



(... continued on next page...)



1.7.3. For users who are more experienced with ChIP-AP, we recommend using the dashboard. At the command line, simply type

```
chipap_dashboard.py
```

and the dashboard will appear (below) wherein you can fill in all required information.

The dashboard interface for chipap_v4.0.py is shown. It features a top section with 'Dataset sequencing mode' (Single end, Paired end) and 'Dataset peak type' (Narrow peaks, Broad peaks). Below this, there are two main sections: 'Sample table loading successful' and 'Custom settings table loading successful'. Each section has a 'Load' button and a text input field for the file path. The 'Sample table loading' section also has a 'Clear samples' button. Below these, there are two columns of input fields for ChIP and Ctrl samples, each with 'read 1' and 'read 2' fields. The 'Reference genome' section includes a dropdown menu for 'Reference genome' (set to hg38), a text input for 'Genome folder' (set to ~/genomes), a text input for 'Known motif file' (set to /home/js/tools/JS/browser_test/SALL4.motif), a text input for 'Output save folder' (set to /home/js/tools/JS/browser_test), and a text input for 'Dataset name' (set to 180906). There are also checkboxes for 'Merged fold enrichment analysis', 'Annotate peaks with known gene ontology terms', 'Annotate peaks with known pathway terms', 'Delete large temporary files', 'Record standard outputs', and 'Record standard errors'. At the bottom, there is a 'ChIP-AP command line' section with a text area containing the command: `chipap_v4.0.py --mode paired --peak narrow --output /home/js/tools/JS/browser_test --setname 180906 --ref hg38 --genome ~/genomes --sample_table /home/js/tools/JS/browser_test/180906/180906_sample_table.tsv --custom_setting_table /home/js/tools/JS/browser_test/180906/180906_setting_table.tsv --motif /home/js/tools/JS/browser_test/SALL4.motif --goann --pathann --deltemp --thread 8 1> /home/js/tools/JS/browser_test/180906/180906.out 2> /home/js/tools/JS/browser_test/180906/180906.err`. The bottom status bar shows 'CPU cores to use: 8' and 'ChIP-AP ready to go!' with buttons for 'Generate scripts' and 'Generate and run scripts'.

1.8. Once the run is complete, please refer to our github wiki for the tutorial on navigating and interpreting the results.

Subsequent Running of ChIP-AP

Ok so now that everything is set up to run ChIP-AP each time is relatively simple. Open a new MobaxTerm terminal window...

If you setup ChIP-AP in its own environment then you need to write the following command first

```
conda activate xxxxx
```

where xxxxx is the name of the environment you created in the installation.

The next command you need to write at the command line will start chipap depending on what mode you want

```
chipap_wizard.py
```

```
chipap_dashboard.py
```

```
chipap_v4.1.py
```