

10x Genomics Chromium Single Cell Gene Expression

Cell Ranger 6.1 (latest), printed on 09/09/2021

Installing Cell Ranger

In this tutorial, you will learn how to:

- Login to a Remote Computer
- Setup a Directory
- Download and Extract Cell Ranger
- Add Cell Ranger to Your \$PATH
- Perform a Sitecheck
- Request a Sitecheck Review
- Perform a Testrun

Login to a Remote Computer

1. Begin this tutorial by logging in to your remote Linux server. The procedure varies based on the machine, but most likely involves using an `ssh` command.

```
ssh user.name@jaberwocky
```

2. Sometimes you are prompted for a password:

```
user.name@jaberwocky's password:
```

When you type in your password, usually no characters are displayed. This is normal, just keep typing.

After logging in, the command prompt looks similar to this:

```
[user.name@jaberwocky ~]$
```

Cell Ranger pipelines must run on a Linux system that meets these minimum requirements (<https://support.10xgenomics.com/single-cell-gene-expression/software/overview/system-requirements>). It is common to run Cell Ranger on High Performance Computing clusters (HPCs) or stand alone servers. These resources may be available for you to use through your institution. There are also cloud computing options through could service providers such as:

- Amazon Web Services (AWS) (<https://aws.amazon.com/>)
- Google Cloud (<https://cloud.google.com/>)

If you are in the U.S. and part of an academic or non-profit institution, you may also be able to get time on a proposal based resource such as:

- Jetstream (<https://www.jetstream-cloud.org/>)
- Cyverse (<https://atmo.cyverse.org/application/images>)
- XSEDE (<https://portal.xsede.org/#/guest>)

Most institutional servers and services have a system administrator tasked with the upkeep of the machine. If available, work with your administrator to log in, identify an appropriate directory to run your analysis, and efficiently allocate system resources for running Cell Ranger.

Sometimes HPC systems have designated compute or interactive nodes for users to log in and monitor output. If available, make sure that Cell Ranger processes do not take up excessive resources and slow down your shared head node for other users.

Note: For the rest of this tutorial, commands are listed for you to copy/paste directly into your command prompt. We alternate between showing you the commands and the expected output.

Setup a Directory

1. We call our directory the **yard**. Make a new directory with the following command:

```
mkdir yard
```

2. To keep track of where the **yard** is located, use the **pwd** command (print working directory):

```
pwd
```

The yard is located here: `/mnt/home/user.name/yard` . Yours may be located in a different location, depending on your system.

3. Go into that directory using the command, **cd** (change directory), and make a folder using **mkdir apps** to install Cell Ranger.

```
cd /mnt/home/user.name/yard
mkdir apps
cd apps
```

Download and Extract Cell Ranger

1. Go to the Cell Ranger Download page (</single-cell-gene-expression/software/downloads/latest>), fill out the "10x Genomics End User Software License Agreement" information and copy the download command from the subsequent page. Paste the entire command onto your command line. Parts of the download command change periodically, so copying the same command seen here will not work. Be sure to copy the whole command from the Downloads page.

```
3vJ-zASLuXito0RvrXyZgM~zNRRFwGjvr98o0xkKZ6EzGXmunA08aPlgRMG6J9t1b02NMg__&Key-Pair-Id=APKAI7S6A5RYOXBWRPDA"
```

2. Some systems do not have the command, `curl` (client url) installed. An alternative command is `wget` .

Note: You do not need to do this if the `curl` command above worked for you.

```
QaEWBGiJ6hrnCsbPdjaVFhOcRd-6mtV-zg2WTt1kVcd8UjA4GAQ42mm1SIw5xgTkWc-~cW__&Key-Pair-Id=APKAI7S6A5RYOXBWRPDA"
```

The output looks similar to this:

```
. % Total      % Received % Xferd  Average Speed   Time    Time       Time  Current
.                  Dload  Upload   Total     Spent    Left  Speed
100  954M  100  954M    0     0  59.6M      0  0:00:16  0:00:16 --:--:-- 56.1M
```

3. List the contents of the apps directory to see the Cell Ranger "tarball" (archive of files).

```
ls -l
```

The output looks similar to this:

```
cellranger-3.1.0.tar.gz
```

4. Unpack the tarball using the **tar** command:

```
tar -zxvf cellranger-3.1.0.tar.gz
```

The output looks similar to this:

```
cellranger-3.1.0/  
cellranger-3.1.0/cellranger-tiny-fastq/  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L001_I1_001.fastq.gz  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L002_R1_001.fastq.gz  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L001_R1_001.fastq.gz  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L002_R2_001.fastq.gz  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L002_I1_001.fastq.gz  
cellranger-3.1.0/cellranger-tiny-fastq/3.0.0/tinygex_S1_L001_R2_001.fastq.gz  
...  
... wait for the command to finish ...
```

A large amount of information is printed to the screen as it unpacks. When it's finished, a directory containing the cellranger executable and all of its dependencies (except bcl2fastq (/single-cell-gene-expression/software/overview/system-requirements)) is included.

The Illumina software, bcl2fastq (https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software/downloads.html) (version 2.2.0) is required for `cellranger mkfastq`. If you know that you will not need to demultiplex and generate FASTQ files, you do not need to install bcl2fastq. But, if you do need bcl2fastq, download (https://support.illumina.com/sequencing/sequencing_software/bcl2fastq-conversion-software/downloads.html) then follow these install instructions (<https://kb.10xgenomics.com/hc/en-us/articles/360001618231-How-to-troubleshoot-installing-bcl2fastq->).

5. List the contents of the Cell Ranger directory:

```
ls -l
```

The output looks similar to this:

```
cellranger-3.1.0  
cellranger-3.1.0.tar.gz
```

Add Cell Ranger to Your \$PATH

1. Cell Ranger is now installed. To run it requires entering the path (https://astrobiomike.github.io/unix/modifying_your_path) to the executable command on the command line. We want to be able to run the Cell Ranger by simply entering the word `cellranger` on the command line. To accomplish this, we add it to your `$PATH` variable. Go into the `cellranger-3.1.0` directory and enter `pwd`.

```
cd cellranger-3.1.0  
pwd
```

The output looks something like this:

```
/mnt/home/user.name/yard/apps/cellranger-3.1.0
```

2. Then use the `export` command to add it into the `$PATH` variable.

```
export PATH=/mnt/home/user.name/yard/apps/cellranger-3.1.0:$PATH
```

3. Then enter `which cellranger` to see if we have successfully added `cellranger` to the `$PATH`.

```
which cellranger
```

The output looks similar to this:

```
~/yard/apps/cellranger-3.0.2/cellranger
```

Note, that the "tilde" symbol (<https://unix.stackexchange.com/questions/34196/why-was-chosen-to-represent-the-home-directory>), `~` is the same as `$HOME`, which is the same as `/mnt/home/user.name/`

4. For convenience, you may want to add this command to your `.bashrc`, a special script (<https://unix.stackexchange.com/questions/129143/what-is-the-purpose-of-bashrc-and-how-does-it-work>) that runs every time you login to your system.
5. Now you can enter `cellranger` on the command line from any directory to see the usage statement.

```
cellranger
```

The beginning of the usage statement looks something like this:

```
/mnt/home/user.name/yard/apps/cellranger-3.1.0/cellranger-cs/3.1.0/bin
cellranger (3.1.0)
Copyright (c) 2019 10x Genomics, Inc. All rights reserved.
```

The usage statement contains a list of cellranger commands. We will run two of them now.

- `sitecheck`
- `testrun`

Perform a Sitecheck

1. The purpose of `sitecheck` is to check your system to make sure it meets the system requirements (</single-cell-gene-expression/software/overview/system-requirements>) for running the cellranger pipeline. Run the command and use the `>` symbol to direct the output to a file.

```
cellranger sitecheck > sitecheck.txt
```

2. Now use the `less` command to take a look at this file. Use the `up` and `down` arrow keys to scroll through the file. Or `spacebar` to scroll down by page. Press the `q` key on the keyboard to quit out of the `less` program.

```
less sitecheck.txt
```

We will take a look at the following sections of the sitecheck file to see if they meet the minimum requirements for running Cell Ranger:

- CPU Cores
- Memory Total
- Disk Space
- Global File Limit
- User Limits:
 - open files
 - max user processes

3. Scroll down until you find the parts in the file with the **CPU Cores** information:

```
less sitecheck.txt
...
=====
CPU Cores
grep -c processor /proc/cpuinfo
-----
64
=====
...
```

Running Cell Ranger requires at least 8 CPUs, preferably 16 (</single-cell-gene-expression/software/overview/system-requirements>), and at least 64GB of RAM, preferably 128 (</single-cell-gene-expression/software/overview/system-requirements>). We have 64 CPU available.

The **Memory Total** looks something like this:

```
...
=====
Memory Total
grep MemTotal /proc/meminfo | cut -d ':' -f 2 | sed 's/^[ \t]*///'
-----
264132348 kB
=====
...
```

We need to convert from kB to GB. A quick Google search (https://www.google.com/search?ei=hW15XdbKMpKt-gSn17mADw&q=264132348+kB+to+GB&oq=264132348+kB+to+GB&gs_l=psy-ab.3..33i299l2.3073.3245..3494...0.2..0.144.201.1j1.....0....1..gws-wiz.....0i71.cQrr-EWW_Oo&ved=0ahUKewiWob274cnkAhWSlp4KHadrDvAQ4dUDCAs&uact=5) does that for us. So, we have 264 GB RAM available, which meets the minimum requirements.

The **Disk Space** looks something like this:

```
...
=====
Disk Space
df -Ph | awk '{print $2, $3, $4}'
-----
Size Used Avail
126G 72K 126G
126G 204K 126G
20G 9.9G 9.7G
50G 1.6G 45G
16T 16T 413G
40T 28T 13T
61G 50G 12G
...
```

The **Global File Limit** looks similar to this:

```
...
=====
Global File Limit
cat /proc/sys/fs/file-max,nr
-----
26216544
26624 0 262144
=====
...
```

This value 26216544 meets the minimum requirements of 10k per GB RAM,
 $10,000 * 264 \text{ GB} = 2,640,000 < 26216544$.

The next section is **User Limits**, also called `ulimits`.

```
...
=====
User Limits
bash -c 'ulimit -a'
-----
core file size          (blocks, -c) 0
data seg size           (kbytes, -d) unlimited
scheduling priority     (-e) 0
file size               (blocks, -f) unlimited
pending signals         (-i) 1031684
max locked memory       (kbytes, -l) 64
max memory size         (kbytes, -m) unlimited
open files              (-n) 1024
pipe size               (512 bytes, -p) 8
POSIX message queues    (bytes, -q) 819200
real-time priority      (-r) 0
stack size              (kbytes, -s) 8192
cpu time                (seconds, -t) unlimited
max user processes      (-u) 1031684
virtual memory          (kbytes, -v) unlimited
file locks              (-x) unlimited
=====
...
```

Note that this machine does meet the max user processes (</single-cell-gene-expression/software/overview/system-requirements>) requirement of 64 times the number of CPUs (1031684). $64 * 64 = 4096 < 1031684$. But, it does not meet the max open file (</single-cell-gene-expression/software/overview/system-requirements>) requirement of 16000. Some accounts have permission to adjust user limits using the **ulimit -n** command.

```
ulimit -n 16000
```

This account does not have permission to change user limits.

```
-bash: ulimit: open files: cannot modify limit: Operation not permitted
```

In this case, contact your system administrator and ask them to adjust it.

Request a Sitecheck Review

Our software support team can review your `sitecheck` results. Send it to us using `cellranger upload` from the command line.

```
cellranger upload your.email@email.com sitecheck.txt
```

The output looks something like this:

```
/mnt/home/user.name/yard/apps/cellranger-3.1.0/cellranger-cs/3.1.0/bin
cellranger upload (3.1.0)
Copyright (c) 2019 10x Genomics, Inc. All rights reserved.
-----

Getting upload information...
Uploading file...
Size: 35,903 bytes
100% [=====>] 35,903 5.71Mb/s eta 0m 0s
Upload complete!
```

You will receive an email back from support asking what you would like us to do with the file. Reply to that email, and we will review it for you.

Perform a Testrun

Once the user limit for open files is adjusted, run `cellranger testrun`.

```
cellranger testrun --id=check_install
```

The output looks something like this:

```
/mnt/home/user.name/yard/apps/cellranger-3.1.0/cellranger-cs/3.1.0/bin
cellranger testrun (3.1.0)
Copyright (c) 2019 10x Genomics, Inc. All rights reserved.
-----

Running Cell Ranger in test mode...
...
```

This process takes about five minutes to run and completes with the following statement:

```
Pipestance completed successfully!
2019-09-11 15:49:00 Shutting down.
Saving pipestance info to "check_install/check_install.mri.tgz"
```

This indicates a successful `testrun` and, by extension, a successful Cell Ranger install.

Other Tutorials in this Series

- [Running cellranger mkfastq](/single-cell-gene-expression/software/pipelines/latest/using/tutorial_fq) (/single-cell-gene-expression/software/pipelines/latest/using/tutorial_fq)

- [Running cellranger count \(/single-cell-gene-expression/software/pipelines/latest/using/tutorial_ct\)](/single-cell-gene-expression/software/pipelines/latest/using/tutorial_ct)
- [Running cellranger aggr \(/single-cell-gene-expression/software/pipelines/latest/using/tutorial_ag\)](/single-cell-gene-expression/software/pipelines/latest/using/tutorial_ag)
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