**Single Cell RNA Sequencing Worksheet: Cell Ranger Count**

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FastQ Files

**Cell Ranger**

Filtered Count Matrix

In this tutorial, you will be taking a single cell RNA-sequencing dataset and running it through the Cell Ranger pipeline. Cell Ranger performs alignment, filtering, and unique molecular identifier and barcode counting. It then outputs several files, including a count matrix which we can then analyze in R using software called Seurat.

\*Because this can take a while to run, you will get Cell Ranger running and then be given a finished count matrix to analyze in Seurat.

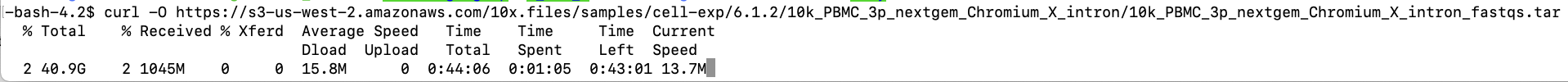
1. On the AWS, mkdir a directory called day8. cd to day8. Make a directory called e\_and\_o inside of the day8 directory.
2. Here is the path to the sbatch script you will edit and use called cellrangerCount\_sbatch:

/scratch/Shares/public/sread2023/scripts/day8/

rsync this script to your day8 directory.

1. Open cellrangerCount\_sbatch in vim. You will need to edit the error and output file path, the path to the transcriptome directory, and the path to the fastq directory. You will also need to set the ntasks and local cores equal to 34.
2. Path to transcriptome directory:
3. Path to the directory with the fastq’s:

Note: Many single cell sequencing data sets are publicly available from 10X genomics. Information about this particular data set can be found at: <https://www.10xgenomics.com/resources/datasets/10k-human-pbmcs-3-v3-1-chromium-x-with-intronic-reads-3-1-high> .

These fastq’s were downloaded using the following curl command which can be found on the 10x website:

1. \*MAY\*DELETE\*Save and exit vim. Before running the script, we need to add cellranger to our $PATH variable. This allows us to use the cellranger command from any directory:

export PATH=/scratch/Shares/dowell/temp/ChrisO/workshop/cellranger-7.1.0:$PATH

1. Now, run the sbatch script



1. Check and see if the job is running

