Documentation for Using the New Pipeline for Analysis

Step 0: Make sure you have the following files in the same folder:

Python files

* Analyzer.py
* v12-q50.py
* merger.py
* zeroscreator.py

Text Files

* genomeADalts.txt
* genomeADposns.txt
* genomewithinst.txt
* nt\_coding.txt (do not include for TSC1/TSC2)
* genomeADfreqs.txt (do not include for TSC1/TSC2)
* exonCoordData.txt (do not include for TSC1/TSC2)

Matlab Files

* Matlab\_commands\_3\_16\_GenomADupdate.m
* matlab\_input\_genomeADcoord.mat

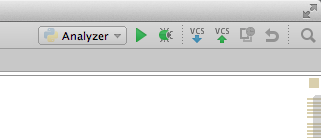
Other files

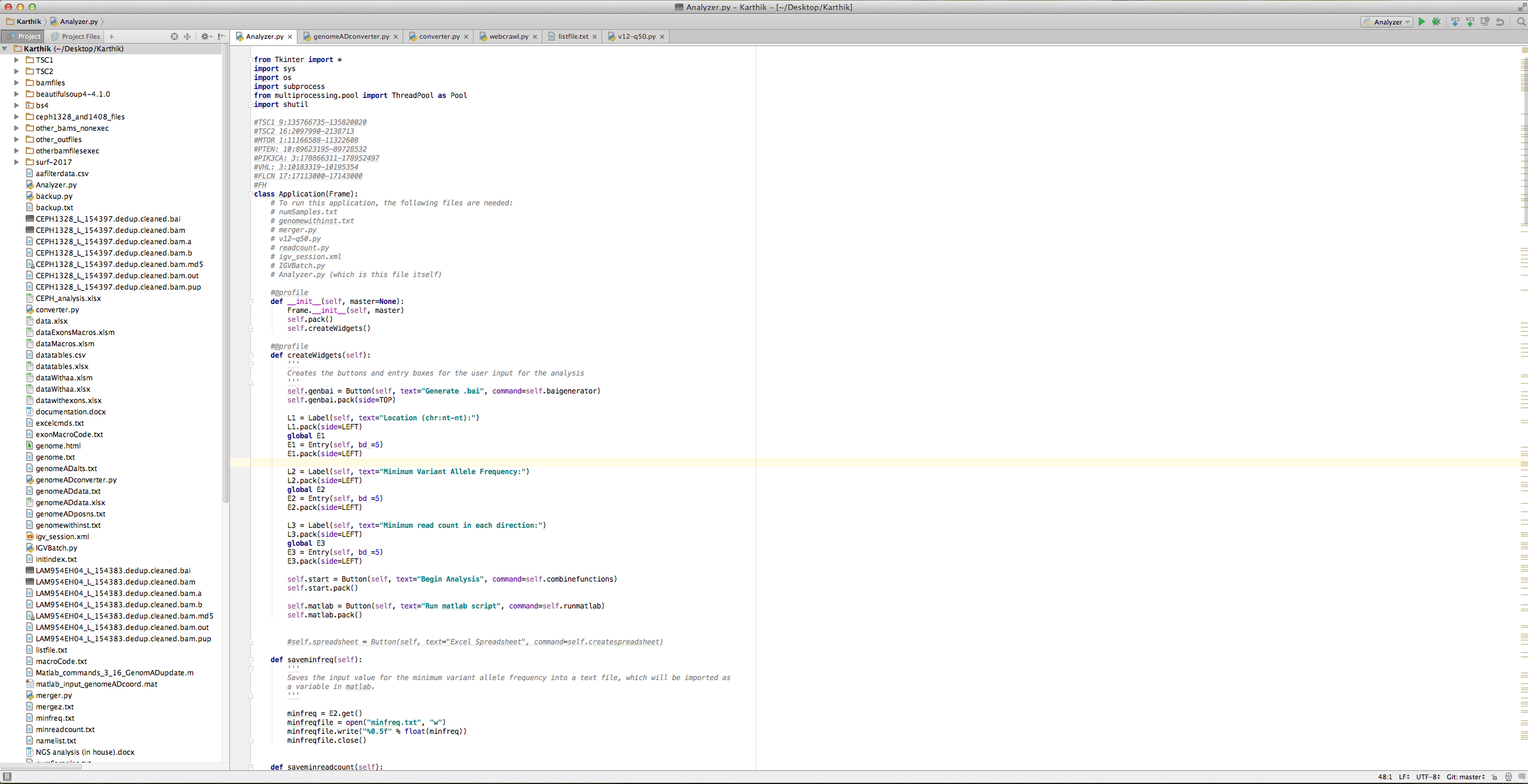
* dataWithaa.xlsm
* indelmacro.xlsm
* All .bam and .bai and .md5 files for the samples being analyzed

Now, open the Analyzer.py file using the program PyCharm CE.

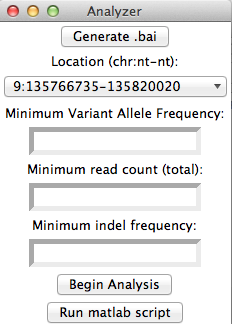
Step 1: Click the “run” button in the PyCharm Window. (Alternatively, type

“python Analyzer.py” in the Terminal.

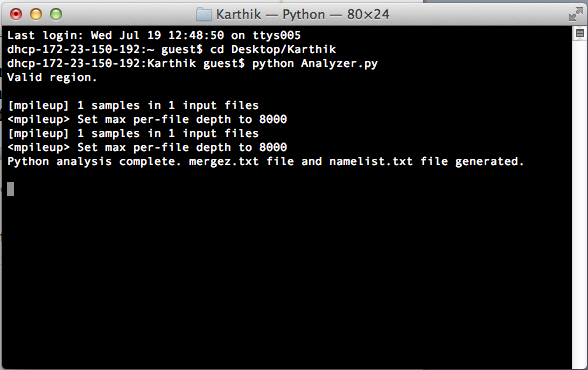




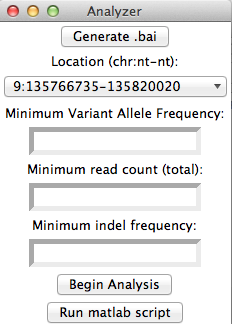
Step 2: Now, the following window should appear. Type in the location of interest for the analysis (in the form chr:nt-nt), the minimum variant allele frequency (as a decimal), and the minimum read count in each direction in the fields shown below.



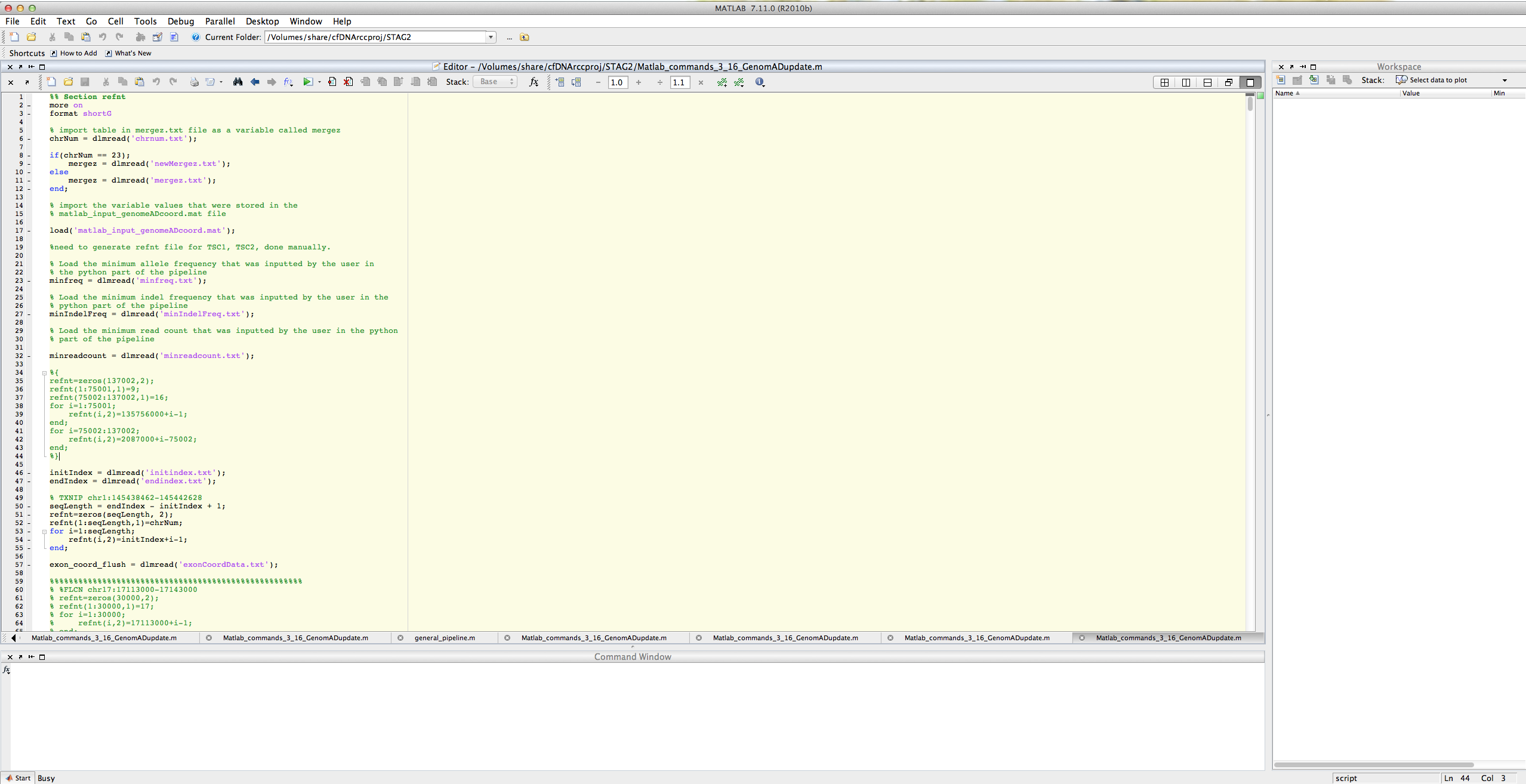
Step 3: Click “Begin Analysis.” Now the mergez.txt and namelist.txt files are being generated. When these are generated, the following will be displayed:



Step 4: Now, click “Run matlab script” in the previous window. Then, when the Matlab script has completed, then the filtered data of interest are in the files “snvdata.csv” and “formatfilterindels.csv”, which can be opened in Excel.



Step 4 (Alternate): Open the file “Matlab\_commands\_3\_16\_GenomADupdate.m” in Matlab. Then, click the “Run” button at the top of the screen. Then, click “Change Folder” if a popup comes up after clicking the run button. Wait until the bottom left-hand corner of the Matlab window does not say “Busy.”



Step 5: Open the file “snvdata.csv” in Excel. Create a new sheet in this workbook and title this sheet “aa codes.” Copy the data in the “aa codes” sheet from the dataWithaa.xlsm file and paste it in the same sheet in this snvdata.csv file. Then, click on the tab “Developer.” Select “Macros” and select “dataWithaa.xlsm!FormatAA.” Then, click “Run.” The resulting file should be formatted as desired. Now, save this file with the name “snvdata” as an xlsx file (Excel workbook).

Step 6: Open the file “formatfilterindels.csv” in Excel and click on the tab “Developer.” Then select “Macros” and select “indelmacro.xlsm!formatIndels.” Then, click “Run.” The resulting file should be formatted as desired. Now, save this file with the name “indeldata” as an xlsx file (Excel workbook).