**Precursor Matching Processing Program**

**General Notes:**

* All input files must be in .csv format
* If you have never used a terminal before, see instructions on last page

**Step 1: Formatting RawConverter Output:**

1. Open up a terminal, navigate to the folder where the program is housed, and type:

python s01\_RawConverter\_Formatting\_wScan.py

1. The first step will prompt you to input your working directory. This is the path to a folder where you would like all results exported to.
2. Input path to RawConverter .MS2 output file
3. Enter tissue type
4. Enter trial number
5. Intermediate messages will appear. At the end of the analysis you will see a table with the formatted spectra information, as well as a dialog that says “analysis complete”

**Step 2: Formatting Spectra List (optional):**

\*if you would like to pre-process your spectra to remove unwanted peaks, this is where that can be done.

1. Open up a terminal, navigate to the folder where the program is housed, and type:

python s02\_filtered\_extract.py

1. Follow onscreen prompts as before
2. All output data will appear in the working directory specified

**Step 3: Precursor Matching:**

1. Open up a terminal, navigate to the folder where the program is housed, and type:
   1. python s03\_precursor\_matching.py
2. Follow onscreen prompts as before
3. Enter path to ion and precursor lists. Examples are in the distribution folder
4. Here the working directory will store a lot of intermediate data
5. All output data will appear in the output directory specified
6. Repeat this process for each tissue and replicate (15x total)

**Step 4: Charge Matching:**

This step matches the charge between the matched ion and the target ion

1. Open up a terminal, navigate to the folder where the program is housed, and type:
   1. python s04\_format\_list.py
2. Follow onscreen prompts as before
3. The working directory is a folder containing all final output files from step 3 (and only these files)
4. All output data will appear in the output directory specified

**Suggested Values:**

* Intensity cutoff, 100
* MS1 precursor error, 10ppm

**Troubleshooting:**

***Converting to .csv format:***

The easiest way to accomplish this is to open the file of interest in Excel. From there go to:

File > Save As > CSV (Comma delimited) (\*.csv)

Please note, .csv files can only be one sheet. If you have multiple sheets in your Excel file, all but the first sheet will be lost when converting file types.

***Getting file path:***

(Windows) In File Explorer, highlight the file or folder of interest. In the top “home” toolbar, click copy path. The path is now copied to the clipboard.

Note: when adding path to terminal prompt, make sure no quotations around path

***Error message:***

An error message will occur if the files you are trying to access are open. Close all necessary files prior to running.

You will also likely get an error message if you do not follow the template formatting. Please see all sample data in the supplied folder so the templates match.

***Running a program through the terminal:***

My favorite way to run a homebuilt program is through Anaconda. Anaconda provides a terminal and houses all necessary python packages. Anaconda also is cross platform, and commands are generally universal across operating systems.

1. Install anaconda: <https://www.anaconda.com/products/individual>
2. From the search bar, search for the anaconda prompt:

Graphical user interface, application

Description automatically generated

1. Open the anaconda prompt:

Shape, rectangle

Description automatically generated

1. Navigate to the folder where the program is stored. For example, if the path to my folder is C:\Users\lawashburn\documents\LiP-MS:

I see that I am already in the folder C:\Users\lawashburn (this is what is written on the screen when you first open anaconda)

I can change the directory to documents by typing:

cd documents

Press enter

And then I can change the directory again to the LiP-MS folder by typing:

cd LiP-MS

It should look like this:

Text

Description automatically generated

1. From here we can jump into step 1 of the first set of instructions by typing:

python LiP-MS\_v3pt1.py

***General anaconda/command prompt notes:***

* To rerun the last command, simple press the up button and the last command will appear, and press enter to run