

# A Step-by-Step Guide to Using MAPLE

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## 1. Check system requirements:

- a. For enrichment analysis only: 32 or 64-bit Windows 7 or 10, high speed Internet connection, 2 GHz dual-core CPU, 2 GB of RAM, 2 GB of free disk space.
- b. Minimum for metaproteome profiling: 64-bit Windows 7, 2.5 GHz quad-core CPU, 10 GB of free disk space, 32 GB of free RAM to process a single file.
- c. Recommended for metaproteome profiling: 64-bit Windows 10, 3.5 GHz octa-core CPU, 20 GB of free disk space, 128 GB of free RAM or above to process multiple files.
- d. To avoid incomplete search due to insufficient memory, 256 GB of free RAM or more may be required for analysis of large data sets.

## 2. Check Internet connection:

- a. High-speed Internet is required for the first use of the metaproteome profiling function (Function 1) to complete the initial configuration. Once the initialization is completed, Internet is not required for future uses anymore.
- b. On the other hand, Internet is always required during the enrichment analysis (Function 2).

## 3. Check computer user account privilege:

- a. If the computer is administered by an IT administrator, please ensure that your user account has been granted with a privilege to install and run new software.

#### **4. Prepare data for analysis:**

- a. For non-Thermo raw files, please convert them into mzML files using MSConvert or vendor software before use.
- b. To analyze Thermo raw files directly without conversion, please check whether Xcalibur or MSFileReader has been installed.
- c. If neither of them has been installed or you see errors related to reading Thermo raw files, please download and install Thermo MS File Reader 3.0 SP3 (MSFileReader\_x86\_x64.exe) from Internet.
- d. Alternatively, please convert Thermo raw files into mzML files before use.

#### **5. Launch MAPLE software:**

- a. Please extract MAPLE from the zip file and double-click to launch the wizard window.

#### **6. Select a function:**

- a. Please enter a number between 1 and 5 corresponding to a function in the menu.

#### **7. If Function 1 (metaproteome profiling) is selected:**

- a. A file selection window will pop up. By default, MS data in the generic mzML format can be selected directly by clicking the file name(s).
- b. To select Thermo raw files, please ensure that the required MSFileReader has been preinstalled and tick the “Use Thermo Raw files” checkbox.
- c. Following the on-screen prompts, please enter the mass tolerances of the precursor (e.g., 10 ppm) and the fragment (e.g., 20 ppm or 0.5 Da), respectively.
- d. If this is the first use, please ensure that Internet is connected, and MAPLE will initialize and configure itself automatically. The progress will be displayed on screen. The time to completion depends on the Internet speed.
- e. The analysis will start once the operations listed above have been completed and you will see the progress of analysis on screen. The time to completion will depend on the CPU speed, the memory available, the number of files and the number of spectra of

each file. It may take a few days to analyze a set of files. Please be patient and avoid using other software consuming substantial memory .

- f. The optimized FASTA database will be output to the MS data folder.

**8. If Function 2 (enrichment analysis) is selected:**

- a. Please check Internet connection.
- b. Please right-click or Ctrl-v to paste all peptide sequences identified.
- c. Please press ENTER to add a blank line to accept the input at the end.
- d. Please right-click or Ctrl-v to paste differentially expressed peptide sequences.
- e. Please press ENTER to add a blank line to accept the input at the end.
- f. The analysis will start and will usually complete in a few minutes depending on the Internet speed and the annotation server load at the moment of analysis.
- g. The results will be output to the MAPLE folder.

**9. If Function 3 (check for updates) is selected:**

- a. The MAPLE website (<http://maple.rx.umaryland.edu/>) will be opened in web browser and you can check and download the latest version of MAPLE.

**10. If Function 4 (check for literature) is selected:**

- a. Papers related to MAPLE will be displayed in web browser.

**11. To quit MAPLE:**

- a. Please select **Function 5** and confirm exit.

**12. If you have any questions or suggestions:**

- a. Please contact Dr. Weiliang Huang ([whuang@rx.umaryland.edu](mailto:whuang@rx.umaryland.edu)).