**G-PTMD User Manual**

Note: The current version of Morpheus search software supports “.raw” and “.mzML” files. Perl script running environment is also needed.

1. **Preparing Uniprot .xml Database**
2. Go to <http://www.uniprot.org/proteomes/> and select your target organism. Download all protein entries in XML format. Rename the .xml database as “uniprot.xml” and put it in the G-PTMD\_v1.0 folder.
3. To delete the unnecessary information contained in the downloaded .xml database and thus speed up the search, the uniprot.xml database can be trimmed by running the Perl script “xml\_trimming.pl”. A simplified database named “uniprot\_trimmed.xml” will be created. Currently, Morpheus can’t deal with modifications that are discovered on peptides with amino acids specified as ‘B’ or ‘Z’. Therefore, these proteins are deleted from the database completely. A list of deleted proteins is provided at runtime.
4. **Performing the 1st-Round Search with Wide Precursor Tolerance**
5. Open the folder “Morpheus” (with the correct data format) and double click on the Morpheus application file .
6. Upload your data files (.raw or .mzML) and the uniprot\_trimmed.xml database. After uploading the .xml database, all the modifications contained in the database will be automatically checked in the “Variable Modifications” column. Assign the “Precursor Mass Tolerance” with any value less than 1000Da. (200 Da is recommended in order to capture most interpretable modifications in a time-efficient manner.) Maximum Threads used can also be adjusted.
7. **Making a New .xml Database with the 1st-Round Search Result**
8. Move either the “PSMs.tsv” or “aggregated.PSMs.tsv” file in the output folder of the 1st-round search to the G-PTMD\_v1.0 folder.
9. Run the Perl script “xml\_AddOpenSearchResult.pl”. A “new\_uniprot.xml” file will be created that contains possible modifications detected by the first-round search. Note that only the modifications listed in the “sub\_ptmlist\_regular.txt” file will be added in the new database. More modification types you are interested in can be added in the “sub\_ptmlist.txt” file before running the Perl script.
10. **Performing the 2nd-Round Search with Normal Precursor Tolerance**
11. Open the Morpheus application again.
12. Upload your data files and the new\_uniprot.xml database. Assign the “Precursor Mass Tolerance” with a regular value (e.g., 10 ppm) and start the search.
13. Note that if a user-defined modification is added to the “sub\_ptmlist.txt” file in Step 2, it also needs to be added to the ptmlist.txt file in the Morpheus folder with the correct format, in order for Morpheus to recognize it. Set permissions for the ptmlist.txt file in the Morpheus program folder to “Read Only” to prevent Morpheus from updating this file and overwriting the ptmlist.txt.

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