MetaboAnalyst 6.0

-- a unified platform for metabolomics data processing, analysis and interpretation

Peak Annotation [MS2-DDA/DIA]

2024-03-10

Module Overview

Database searching for MS2 spectra-based compound identification and results visualization

- ✓ Support single MS2 spectrum searching. This spectrum can be generated by either DDA or DIA;
- ✓ Support MS2 spectra batch searching. The accepted file format can be either MSP (from MS-DIAL) or MGF;
- ✓ Batch searching support at most 20 spectra once at a time. Users can prioritize the spectra;
- ✓ A total of 11 public MS2 reference libraries have been included. Users can choose to use one or more database options for searching;
- ✓ MS2 spectra searching supports direct searching or searching based on neutral loss;
- ✓ MS2 spectrum similarity can be evaluated based on dot-product or spectral entropy method.

Content

1. Introduction

Background

- MS2 data acquisition could be carried out independently from MS1 in DDA or DIA method.
- MS2 spectra-based compound identification is a commonly used approach for global (untargeted) metabolomics;
- There are multiple public reference libraries curated for metabolomics/exposomics community;

Data Formats

To accommodate application scenario and offer compatibility with MS2 spectra results from other popular tools. There are three formats supported:

i. Simple text file (m/z and intensity separated by tab);
ii. MGF file format (standard);
iii. MSP file format (MS-DIAL);

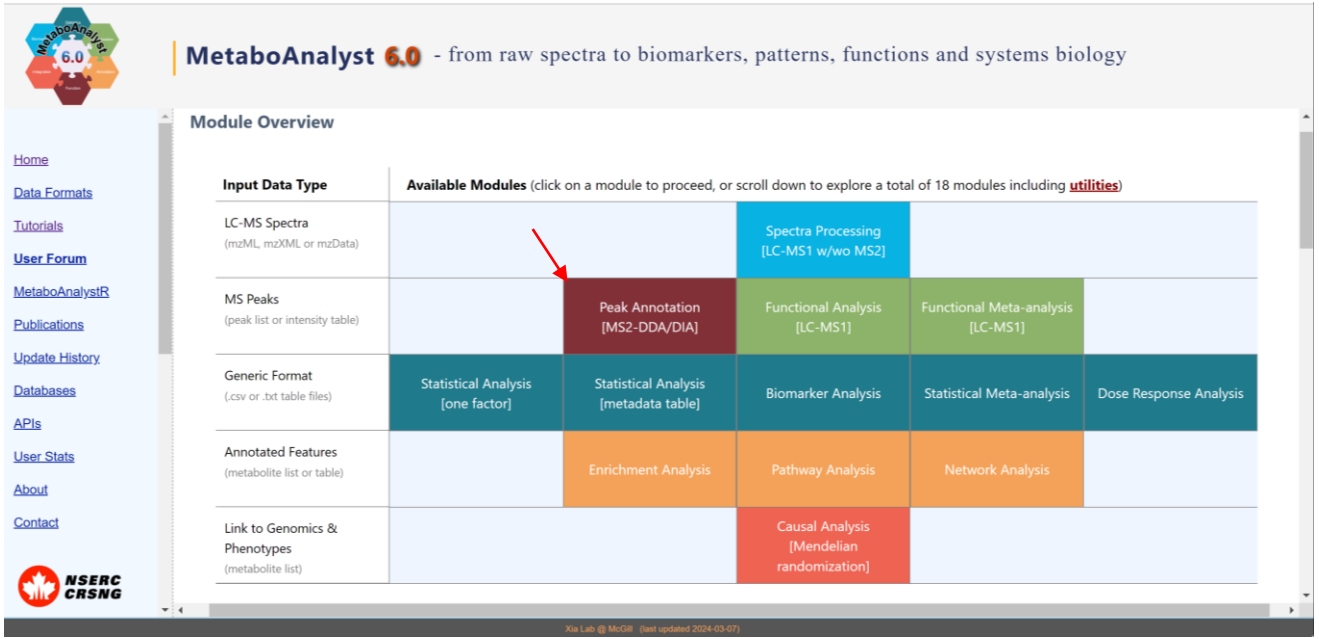
Expected Results

The MS2 spectra/spectrum searching provides results including comprehensive compound identifcation summary and visualization of the matching pattern:

i. Compound identifcation summary table;
ii. Visualization on MS2 matching pattern and annotation of fragments;

2. Choose the Module

Go to MetaboAnalyst (https://www.metaboanalyst.ca), and select the module



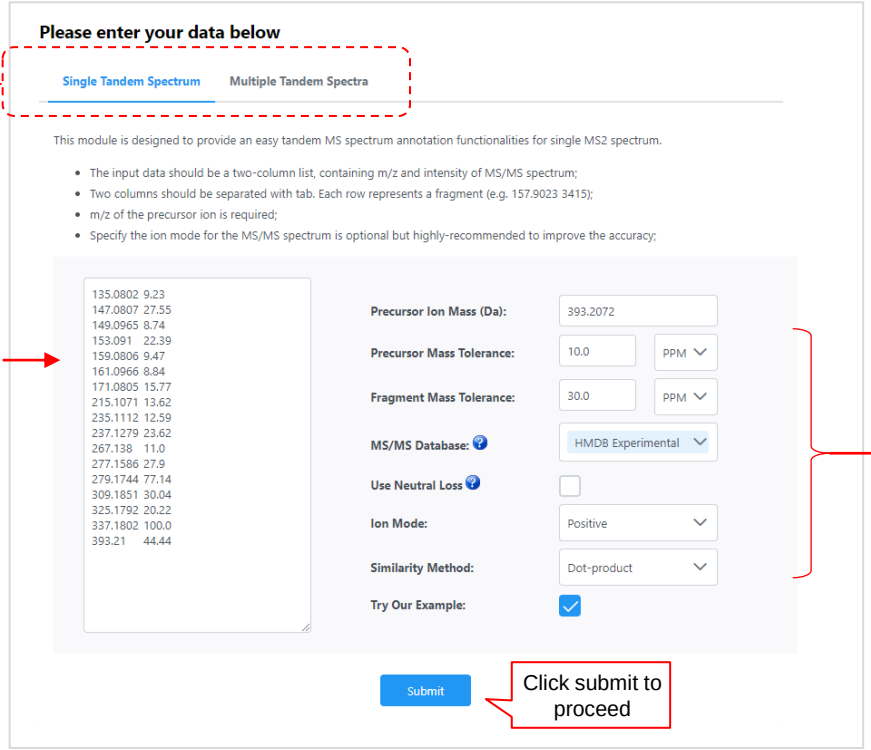
3. MS2 search of single spectrum

3.1 Single spectrum upload

At the first page, user can upload single spectrum or multiple spectra. We used the "***Single Tandem Spectrum***" at this stage.

For single spectrum uploading,

1. It should be a text containing two columns. The first column is *m/z* values, while the second column is intensity values.
2. The two columns must be separated by tab (not space).
3. It is unnecessary to normalize the intensity values, we will automatically do it.

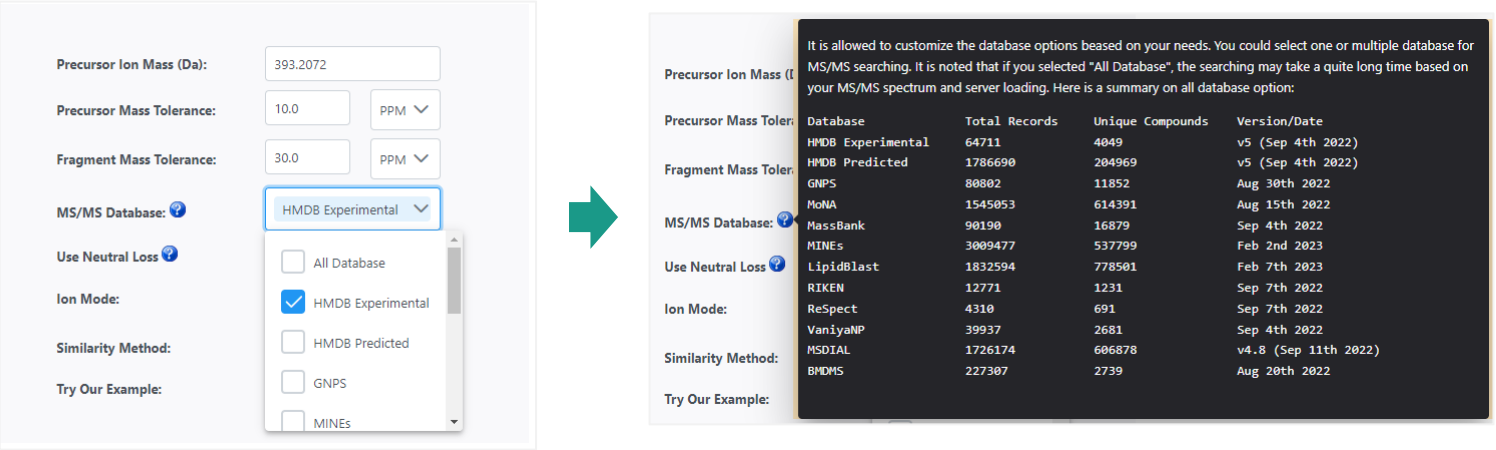


Parameters for searching,

1. **Precursor Ion Mass** is required, please input the value as precisely as possible;
2. **Tolerance**: both tolerance values are recommended to be optimized based on MS instrument'
3. **MS/MS Databases**: user could customize their database option (see 3.2 for more information);
4. **Use Neutral Loss:** user could optionally use Neutral Loss for database search by use the option. Please note, this is only encouraged for unknown new compound discovery;
5. **Similarity Method**: User could choose traditional way (dot-product) or a new strategy (spectral entropy).

3.2 MS2 spectra databases

- Users can choose one or multiple MS2 spectra data for searching.
- Different databases contain significantly different number of MS2 spectra records.
- Choose “All Database” will search the entire MS2 spectra database, but it may take significantly longer to complete.

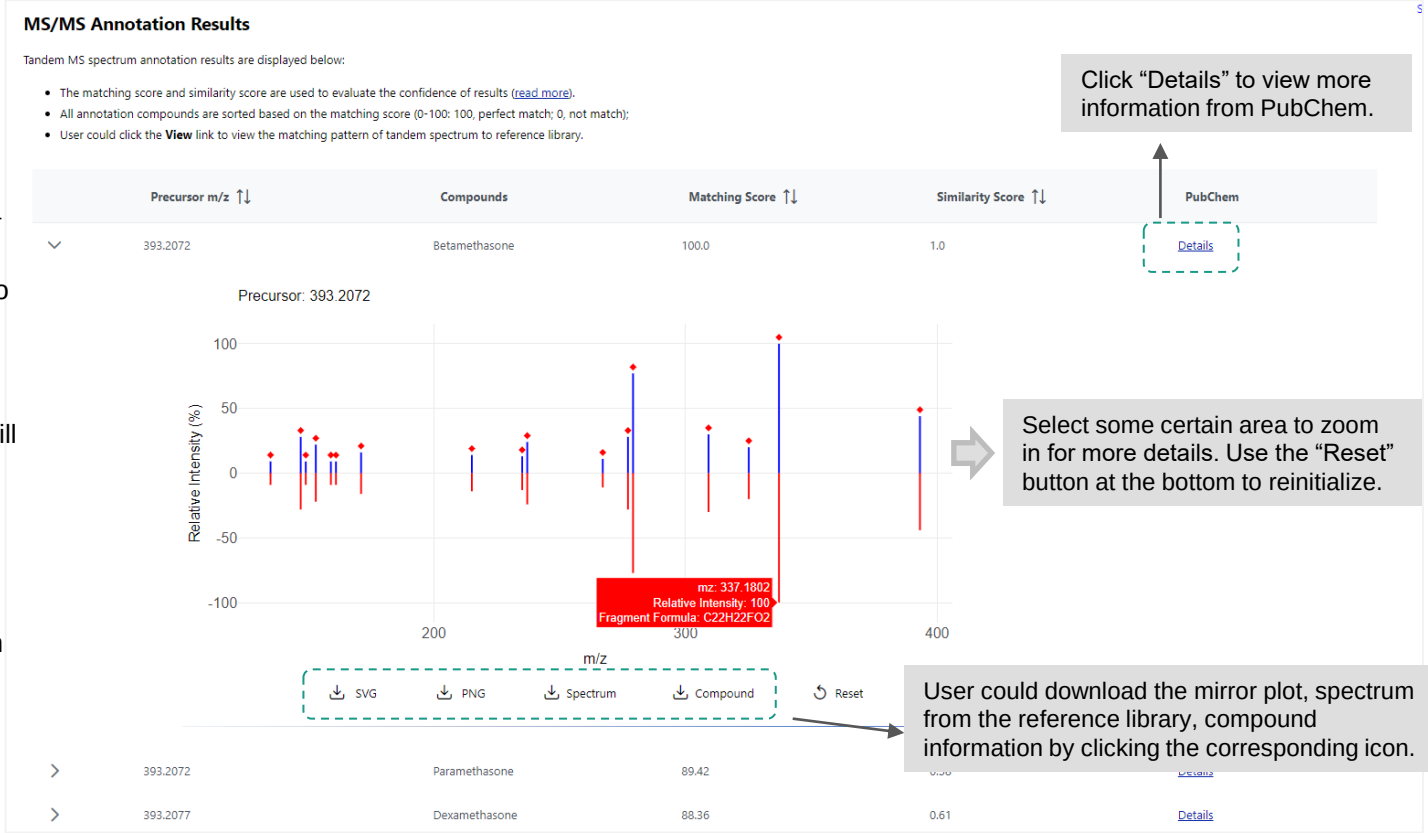


Mouse hover the help tip to view the detailed information on different database options.

3.3 MS2 spectra searching results

MS2 results explanation,

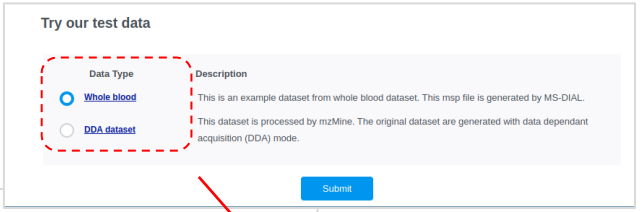
1. Database search results would be summarized as a table;
2. User could expand a row to visually explore the matching results of a MS2 spectrum;
3. Information of fragments will be automatically displayed when mouse hover the fragment;
4. The top (blue) part are users' input, while the bottom (red) parts are from the reference library;
5. All matched fragments will be marked with red diamond at the top.



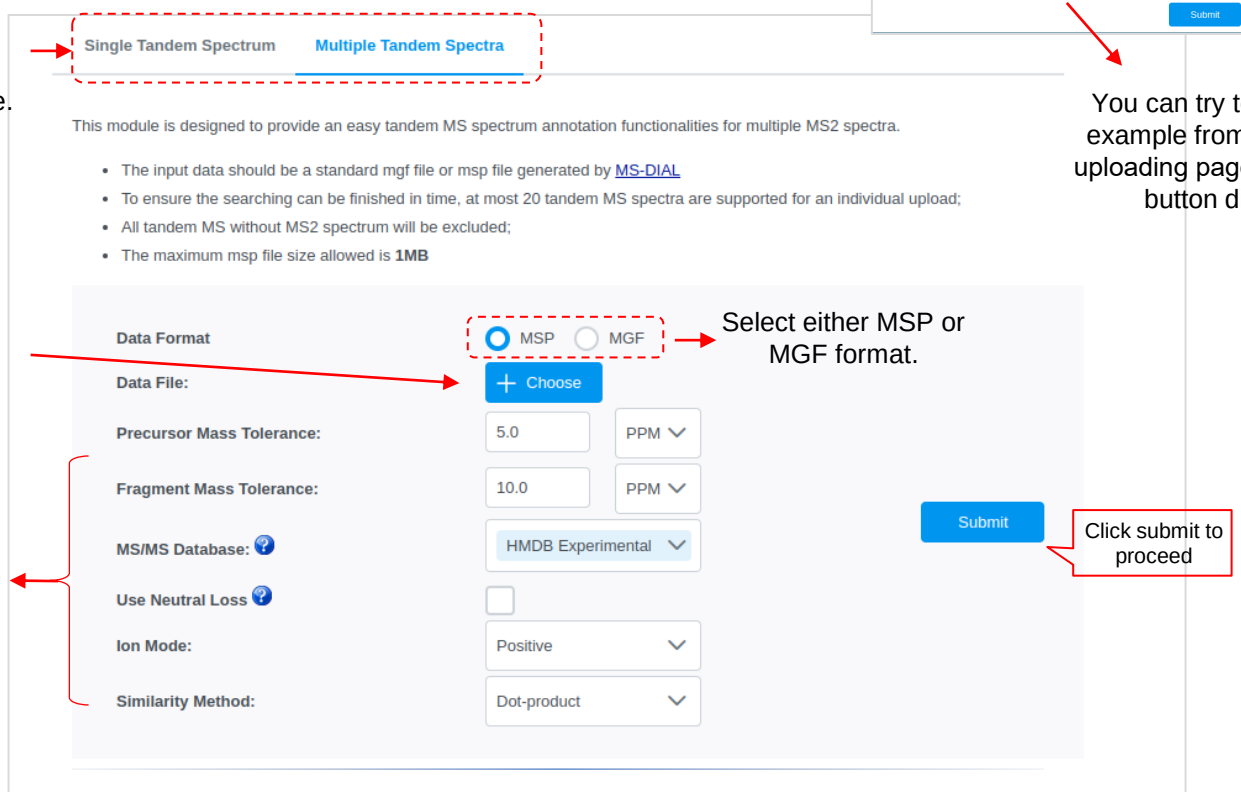
4. MS2 spectra batch search

4.1 MSP/MGF spectra upload

"***Multiple Tandem Spectra***" tab can be used to upload multiple spectra once at a time.



You can try to download either example from the bottom of the uploading page or Click "***Submit***" button directly to test.



Select either MSP or MGF format.

Click "***Choose***" button and select msp or mgf file to upload.

These parameters are the same as single tandem spectra (see 3.1 section).

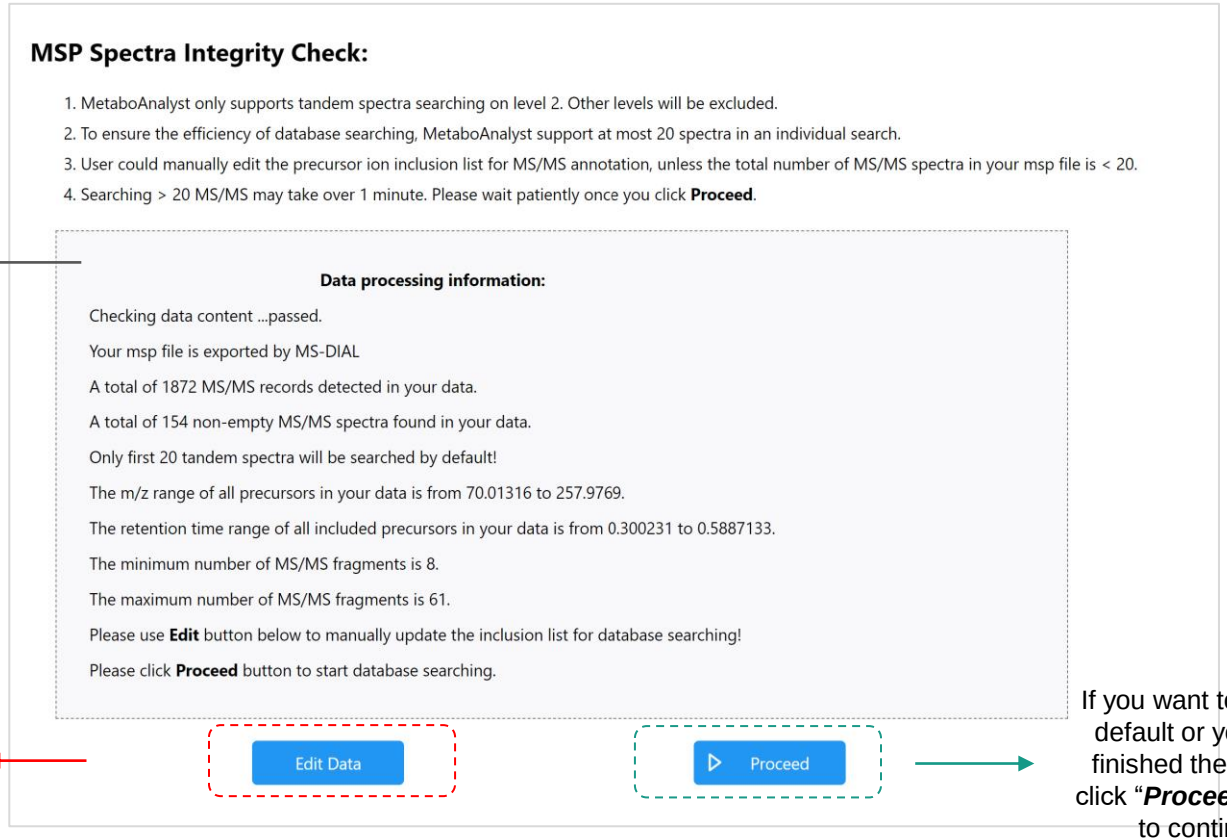
Click submit to proceed

4.2 Spectra Integrity Check

MetaboAnalyst could process your data and do an integrity check. The integrity check results are summarized here.

Public server is limited to search 20 spectra once at a time due to computing constraints.

If you msp/mgf files contains more than 20 spectra, you can Click "***Edit Data***" to customize/prioritize some features (See 4.3), or MetaboAnalyst will include the first 20 spectra.

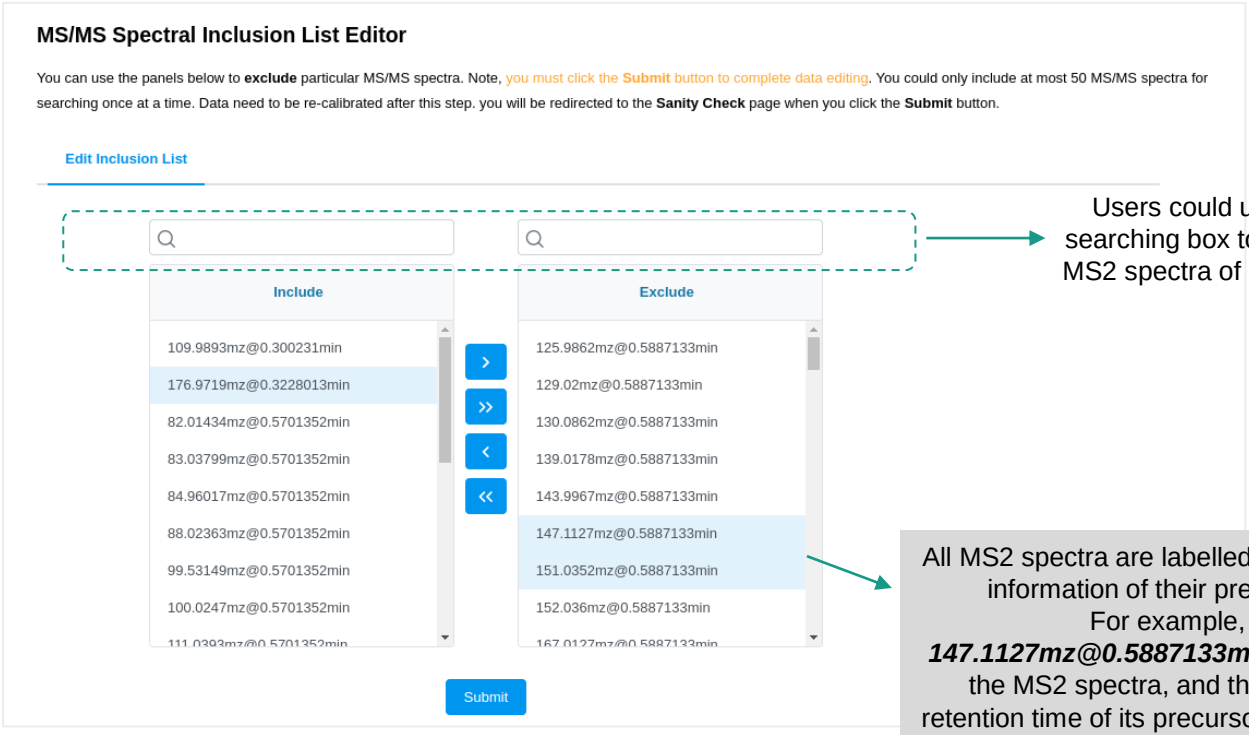


If you want to use the default or you have finished the Editing, click "***Proceed***" button to continue.

4.3 Spectra inclusion for searching

Spectra inclusion editor,

1. Since MetaboAnalyst only support at most 20 spectra searching once at a time, user could manually customize the inclusion list for MS2 database search;
2. By default, the first 20 spectra will be listed into “Include” list to be included for searching;
3. User could move MS2 spectra features between two lists by using the blue moving arrows; ›
4. Once the editing is done, Click “***Submit***” button to confirm.



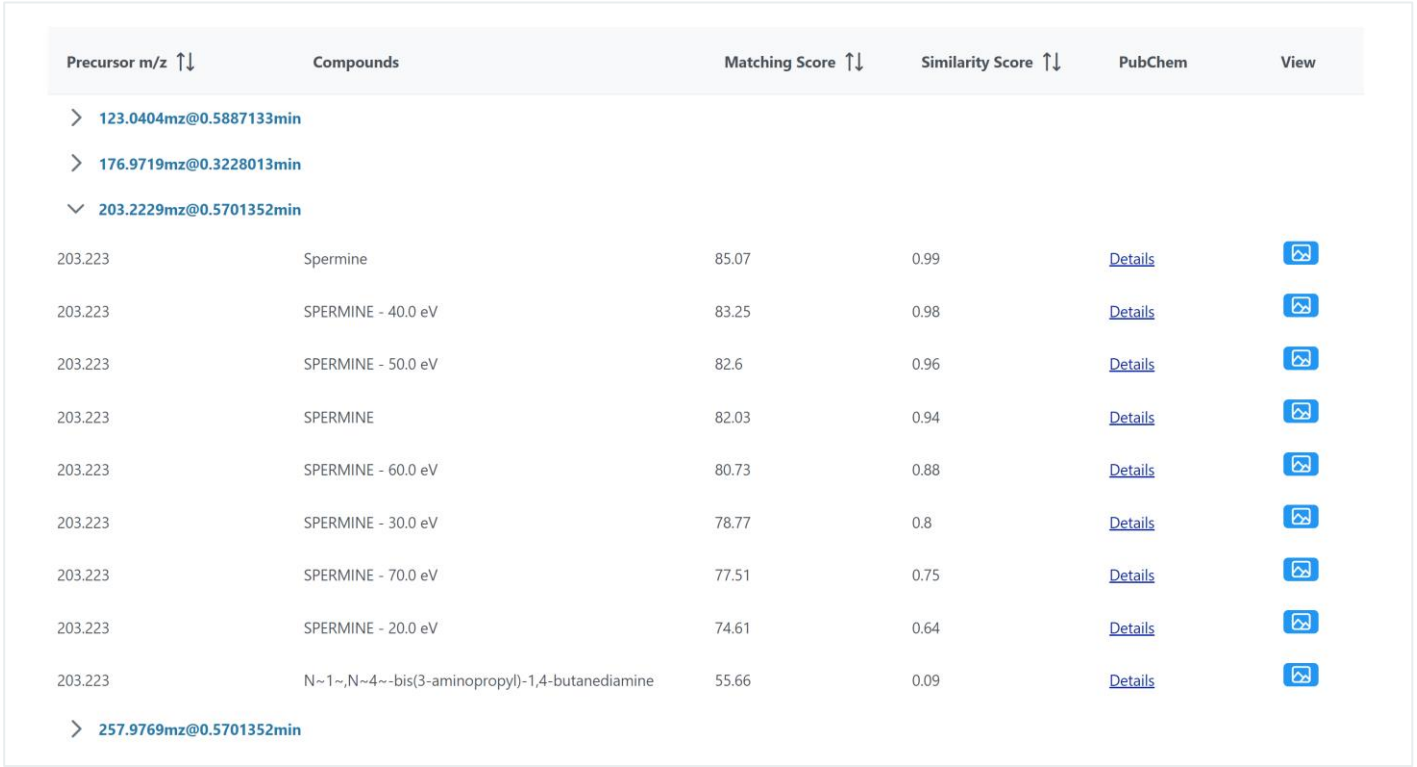
Users could use the searching box to find the MS2 spectra of interests.

All MS2 spectra are labelled based on the information of their precursors. For example, ***147.1127mz@0.5887133min*** represents the MS2 spectra, and the *m/z* and retention time of its precursos is 147.1127 and 0.5887133min.

4.4 MS2 spectra batch searching results

MS2 results explanation,

1. Database search results would be summarized as a table;
2. Each row of the table is a MS2 spectra (labelled with their corresponding precursor's information);
3. User could expand a row to check all chemical candidate;
4. Click "***Details***" to see the detailed information of this compound;
5. Click "***View***" icon to visually check the MS2 matching results from the pop-up dialog (see 4.5).

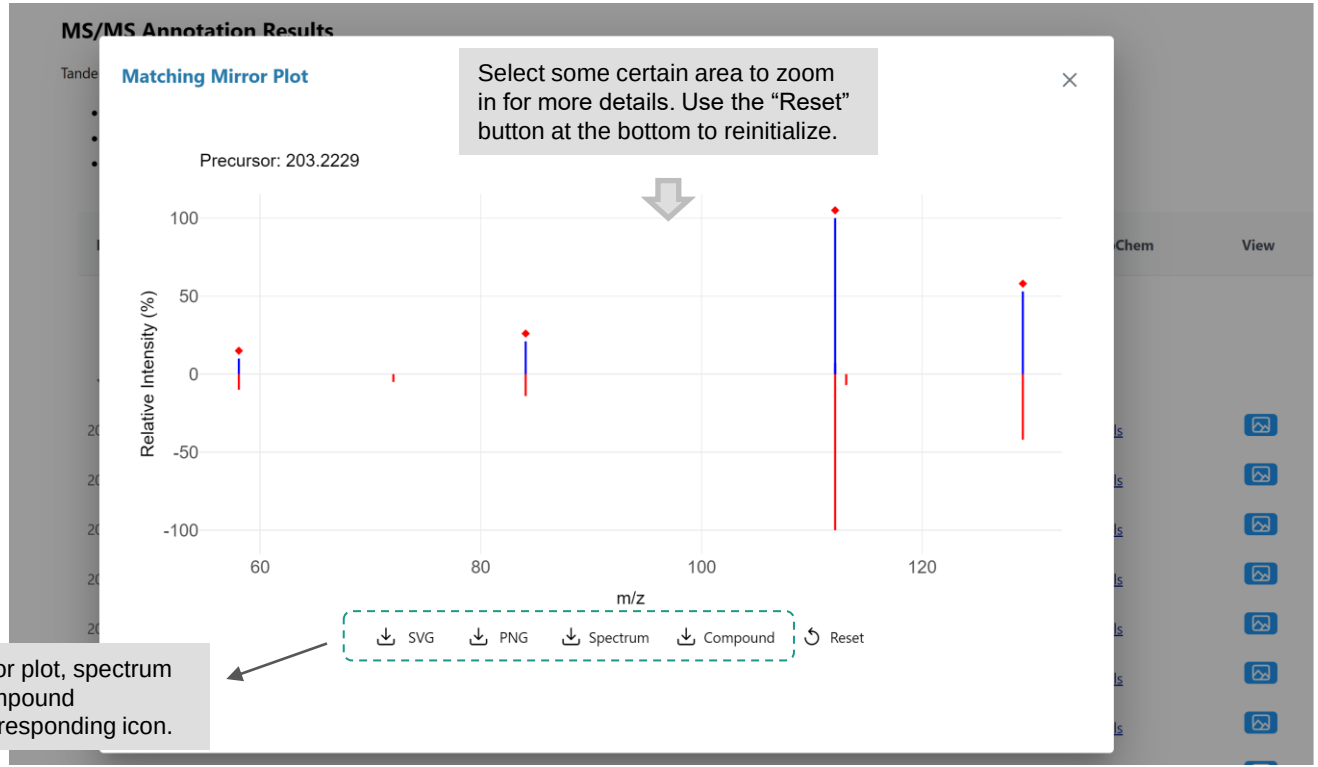


Precursor m/z	Compounds	Matching Score	Similarity Score	PubChem	View
123.0404mz@0.5887133min					
176.9719mz@0.3228013min					
203.2229mz@0.5701352min					
203.223	Spermine	85.07	0.99	Details	
203.223	SPERMINE - 40.0 eV	83.25	0.98	Details	
203.223	SPERMINE - 50.0 eV	82.6	0.96	Details	
203.223	SPERMINE	82.03	0.94	Details	
203.223	SPERMINE - 60.0 eV	80.73	0.88	Details	
203.223	SPERMINE - 30.0 eV	78.77	0.8	Details	
203.223	SPERMINE - 70.0 eV	77.51	0.75	Details	
203.223	SPERMINE - 20.0 eV	74.61	0.64	Details	
203.223	N~1~,N~4~-bis(3-aminopropyl)-1,4-butanediamine	55.66	0.09	Details	
257.9769mz@0.5701352min					

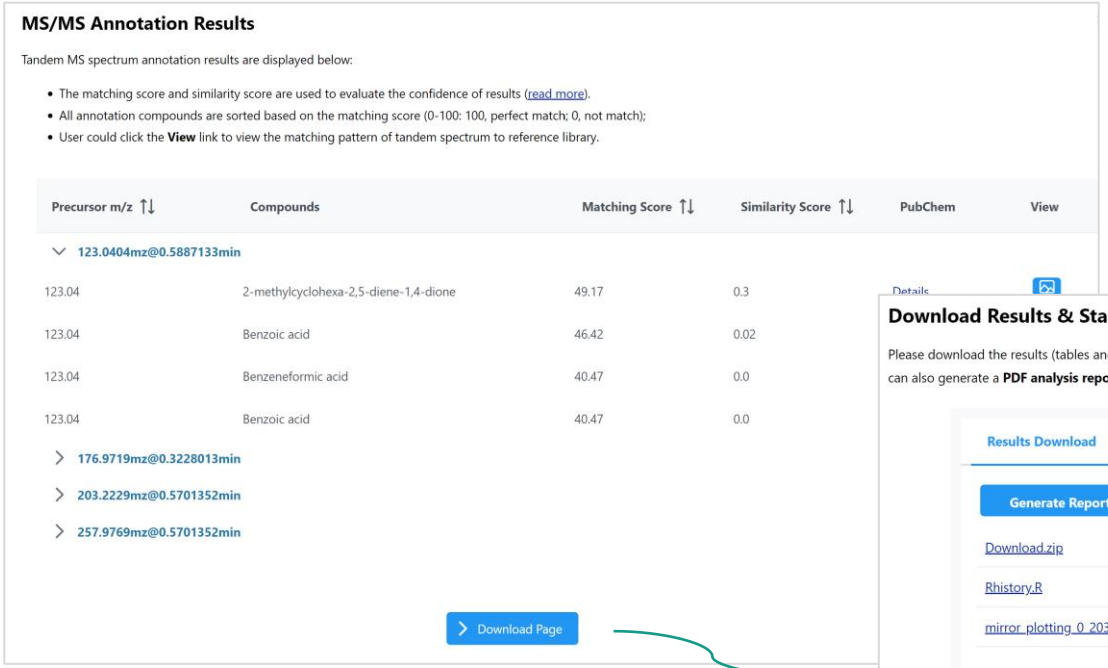
4.5 MS2 spectra visualization results

MS2 results visualization,

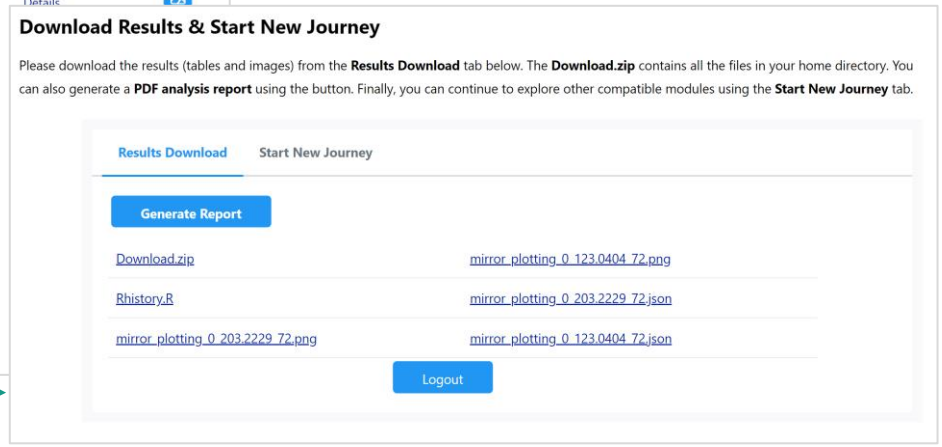
1. Information of fragments will be automatically displayed when mouse hover the fragment;
2. The top (blue) part are users' input, while the bottom (red) parts are from the reference library;
3. All matched fragments will be marked with red diamond at the top.



5. Download Results



From the bottom of the result page, click "***Download Page***" button to go to the results downloading page.



In summary

If you have any questions, please read/post into OmicsForum (www.omicsforum.ca)

Or contact us:

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- MS2 data acquisition could be carried out independently from MS1.
- Users can input MS2 spectra directly into the module by uploading a single MS2 spectrum or an MSP file containing multiple MS2 spectra.
- For single spectrum searching, users must specify the m/z value of precursors. However, for batch searching based on MSP file, users do not need to specify the precursors' *m/z*.
- MetaboAnalyst 6.0 public server processes only 20 spectra for each submission. Users can manually prioritize preferred spectra for searching.
- Following this pilot study, users can then download the R command history to annotate all MS2 spectra in the MSP/MFG file locally using the MetaboAnalystR.