Tutorial for MCID MS/MS Search

(Version 8.14.15 prepared by Tao Hua and Liang Li, University of Alberta)

Part I. Introduction to MCID MS/MS Search

Part II. Examples of MCID MS/MS Search

- **Part II includes the instructions for file splitting and file merging in batch mode search using a large file of > 100 spectra:**
    - **2.1. Use "MCID-split.R" to split a big MS/MS data file**
    - **2.3. Use "MCID-merge.R" to combine all the search results**

Tutorial Part I. Introduction to MCID MS/MS Search

1. **Workflow.** The workflow for metabolite identification using MCID MS/MS search is shown in Figure 1. The precursor ion mass and fragment ion masses in an experimental MS/MS spectrum are entered into the program for comparison with the library metabolites and their predicted fragment ions. A match score (fit score) is generated in the search result which can be used to judge the quality of a match.

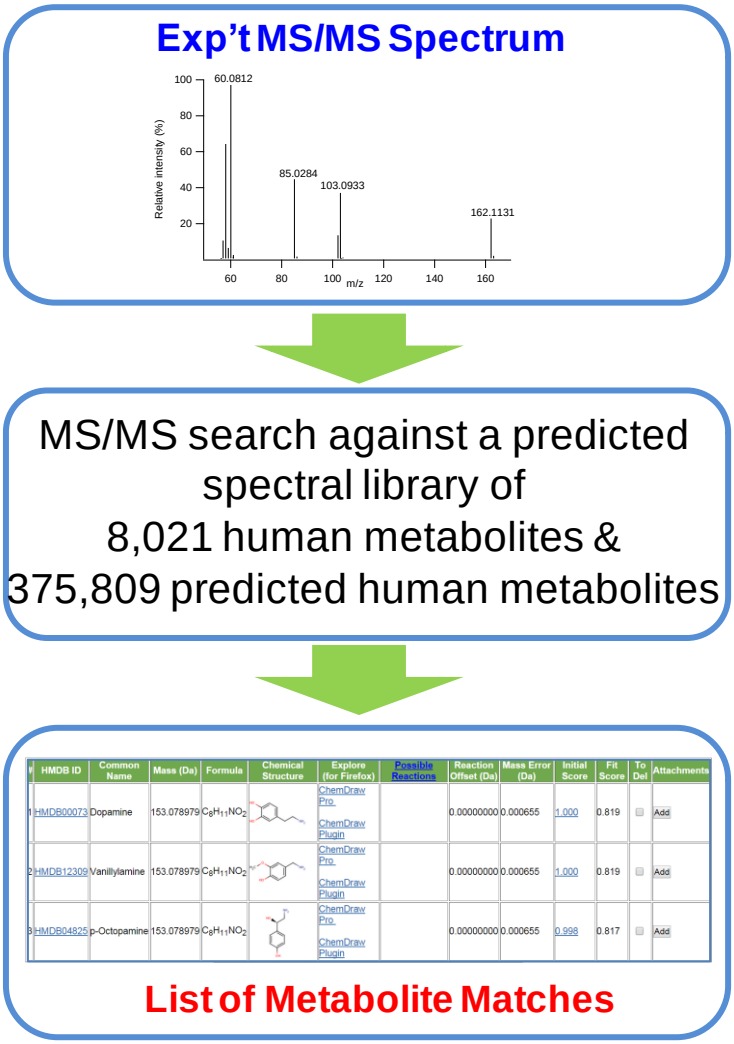


Figure 1. MCID MS/MS search workflow.

2. **MCID spectral library for MS/MS search.** The MCID database is composed of all the known endogenous human metabolites in the Human Metabolome Database (HMDB) (8,021 metabolites) and their predicted metabolic products via one metabolic reaction in the Evidence-based Metabolome Library (EML) (375,809 predicted metabolites). All the predicted MS/MS spectra are generated using *in silico* fragmentation algorithms. This spectral library is hosted at the public MCID website (www.MyCompoundID.org) and allows user to submit single experimental MS/MS spectrum or a batch of MS/MS spectra to search against the library spectra for possible match(s).
3. **MCID single-mode MS/MS search.** The MCID single-mode MS/MS search allows a user to search one experimental MS/MS data against the library spectra. Figure 2 shows the screenshot of MCID single mode MS/MS search interface.

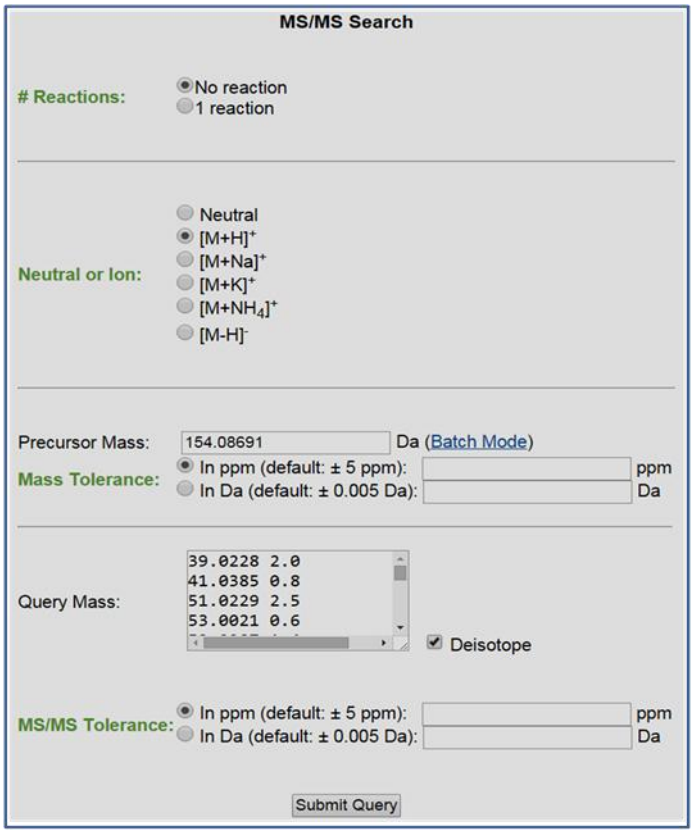


Figure 2. MCID single-mode MS/MS search interface.

a. **# Reaction.** The user needs to choose the type of library, either zero-reaction metabolite library (no reaction) or one-reaction metabolite library (one reaction).

b. **Neutral or Ion.** The user needs to define the type of precursor ion.

c. **Precursor Mass.** The user needs to input a precursor mass.

d. **Mass Tolerance.** The user needs to define a mass tolerance for the precursor mass. 0.005 Da is normally used for MS/MS data collected using high resolution MS such as TOF and FT. If the experiment is performed using a low resolution or low mass-accuracy MS instrument, a larger mass tolerance should be considered.

e. **Query Mass.** The user needs to input the list of MS/MS peaks with their intensities in this box. Once the "Deisotope" checkbox is checked, natural isotopic peaks will be excluded from the matching with the library MS/MS spectra to avoid false matching.

f. **MS/MS Tolerance.** The user needs to define a mass tolerance for the fragment ion peaks. 0.005 Da is normally used for data collected using high resolution MS such as TOF and FT. If the experiment is performed using a low resolution or low mass-accuracy MS instrument such as a triple quadrupole MS, a larger mass tolerance should be considered.

4. **MCID batch-mode MS/MS search.** The MCID batch-mode MS/MS search allows a user to search an entire experimental LC-MS/MS dataset for all the possible matches. Figure 3 shows the screenshot of the MCID batch-mode MS/MS search interface.

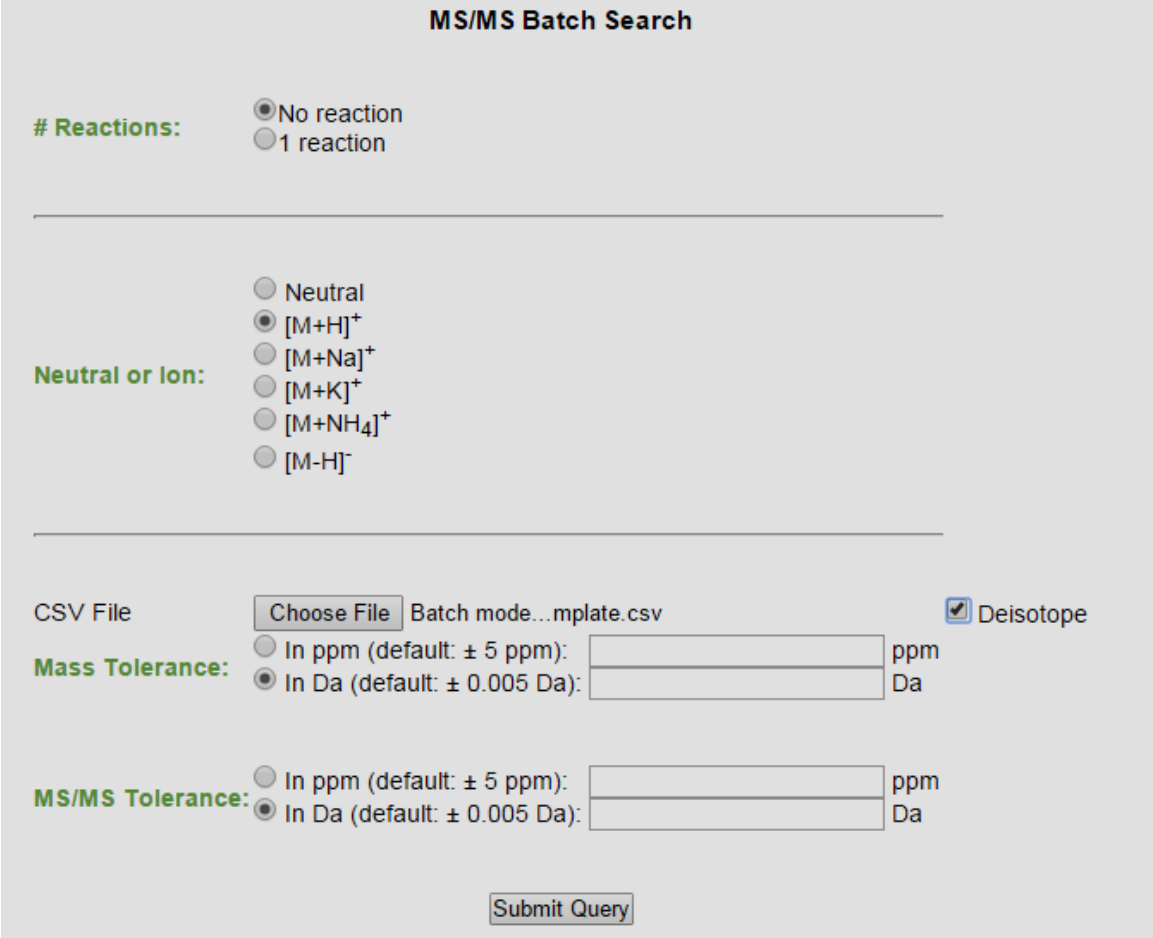


Figure 3. MCID batch-mode MS/MS search interface.

a. **# Reactions.** The user needs to choose the type of library, either zero-reaction metabolite library (no reaction) or one-reaction metabolite library (one reaction).

b. **Neutral or Ion.** The user needs to define the type of precursor ion. Usually $[M+H]^+$ is selected in a typical LC-MS/MS analysis.

c. **CSV File.** The user needs to upload a CSV file generated from LC-MS/MS analysis of a sample for batch-mode search. An example of the file format used (e.g., MSMS file example) can be downloaded from the website. The file size is limited to 100 spectra. If a large file is used, a file split program can be used to split the large file into several small files for uploading (see Instruction given in Part II, section 2).

d. **Deisotope.** Once the “Deisotope” checkbox is checked, natural isotopic peaks will be excluded from the matching with the library MS/MS spectra to avoid false matching.

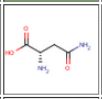
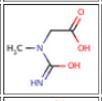
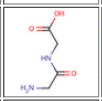
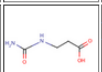
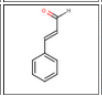
e. **Mass Tolerance.** The user needs to define a mass tolerance for the precursor mass. 0.005 Da is normally used for MS/MS data collected using high resolution MS such as TOF and FT. If the experiment is performed using a low resolution or low mass-accuracy MS instrument, a larger mass tolerance should be considered.

f. **MS/MS Tolerance.** The user needs to define a mass tolerance for the fragment MS peaks. 0.005 Da is normally used for data collected using high resolution MS such as TOF and FT. If the experiment is performed using a low resolution or low mass-accuracy MS instrument, a larger mass tolerance should be considered.

5. **Single-mode search result display.** Figure 4 shows the screenshots of the MCID MS/MS single-mode search results using L-Asparagine as an example. After MS/MS search, all the mass-matched candidates are listed in the result page shown in Figure 4A. The correct structure, L-Asparagine, has the highest fit score (0.984). To further interpret the match result, the user can click the web link in the “Initial Score” column to display another layer of the match result. For example, by clicking "1.000" in Initial Score from L-Asparagine, a new page is displayed as shown in Figure 4B. This page shows the matching quality of the predicted MS/MS spectrum against the experimental MS/MS spectrum. All the matched peaks are labeled in red and unmatched peaks are in grey. On the same page, all the experimental MS/MS peaks are listed in a table (see Figure 4C). By clicking in the “Detail” column, another page will be displayed as shown in Figure 4D. On this page, a specific experimental MS/MS peak is matched with a predicted MS/MS peak and the matched

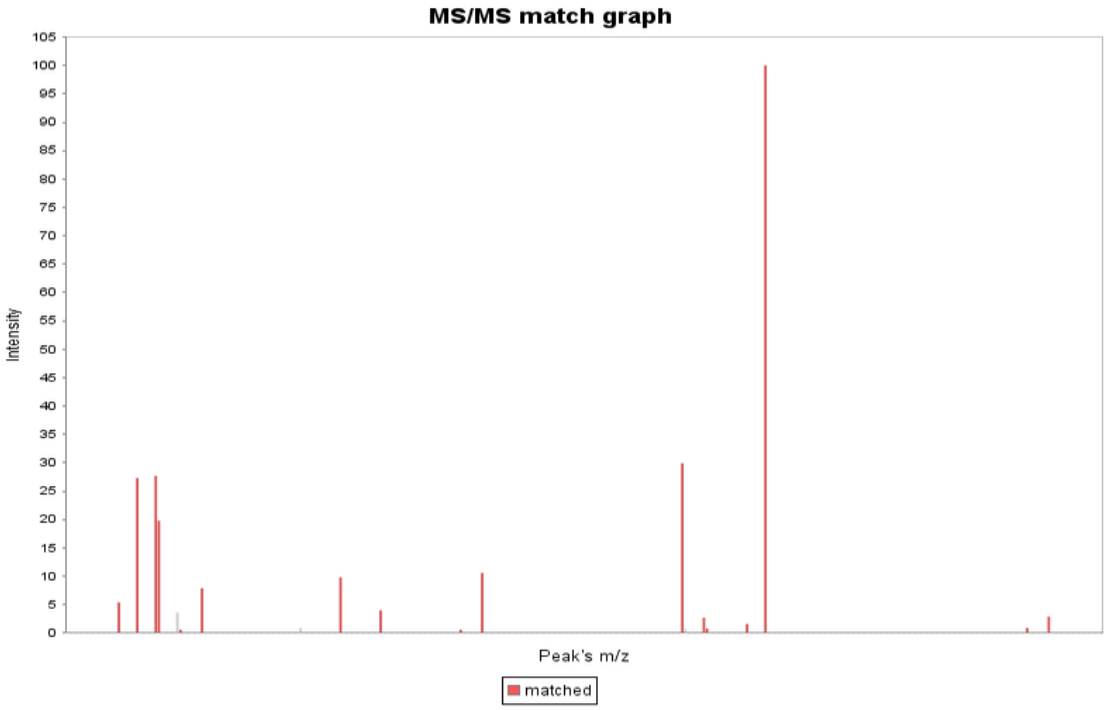
structure is displayed. The user can judge whether this matched structure is reasonable or not against the entire metabolite structure.

(A)

#	HMDB ID	Common Name	Mass (Da)	Formula	Chemical Structure	Explore (for Firefox)	Possible Reactions	Reaction Offset (Da)	Mass Error (Da)	Initial Score	Fit Score	To Del	Attachments
1	HMDB00168	L-Asparagine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	1.000	0.984		Add
2	HMDB12265	N-Carbamoylsarcosine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.943	0.928		Add
3	HMDB11733	Glycyl-glycine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.861	0.847		Add
4	HMDB00026	Ureidopropionic acid	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.483	0.475		Add
5	HMDB03441	Cinnamaldehyde	132.057515	C_9H_8O		ChemDraw Pro ChemDraw Plugin		0.00000000	-0.004015	0.062	0.061		Add

(B)

NC(=O)CC(N)C(=O)O
Initial Score= 1.000
Fit Score= 0.984



(C)

Experimental peak	Intensity	Matched simulated peaks	Detail information	Experimental peak	Intensity	Matched simulated peaks	Detail information
42.0337	5.4	1	Detail	43.0177	27.3	2	Detail
44.013	27.7	1	Detail	44.0494	19.8	1	Detail
45.0448	3.6	0		45.0523	0.6	1	Detail
46.0287	7.9	1	Detail	51.0228	0.9	0	
53.0023	9.8	1	Detail	55.0179	4.0	1	Detail
59.037	0.6	2	Detail	60.0446	10.6	1	Detail
70.0291	29.9	1	Detail	70.0656	0.7	0	
71.013	2.7	1	Detail	71.0326	0.8	1	Detail
73.029	1.6	1	Detail	74.0243	100.0	1	Detail
87.0555	0.9	1	Detail	88.0394	2.9	1	Detail

(D)

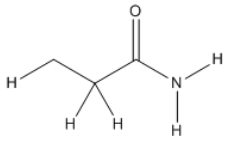
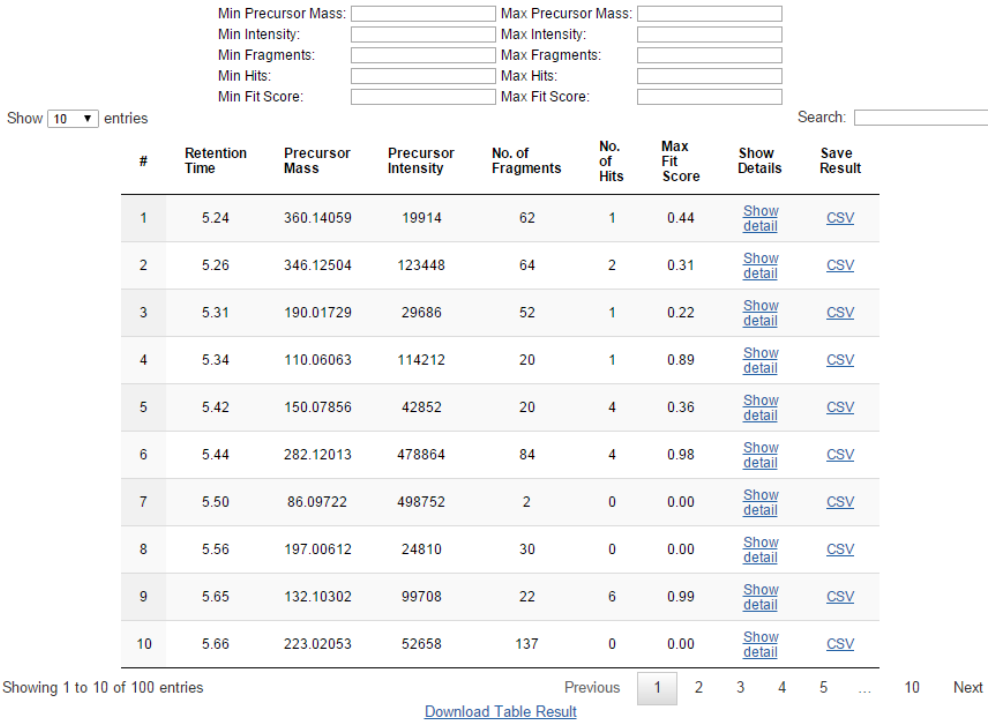
Fragment's mass	Plus or minus H's number	Simulated mass	Matched experiment mass	Mass error	Structure
71.0371	-1	70.0287	70.0291	0.0004	

Figure 4. Screenshots of single-mode MS/MS search results.

6. **Batch-mode search result display.** Figure 5 shows a screenshot of the MCID batch-mode MS/MS search result. As displayed at the top of the table, the user can further filter the search results table using precursor mass, intensity, number of fragments, number of hits, and the fit score. Also, the entire search results table can be exported as a CSV file by clicking the "Download Table Result". Figure 6 shows the screenshot of the exported search results. The web link provided at the end of each row allows the user to manually check the matching

result from the MCID website. The user merely needs to cut and paste the link name to the internet and the search result displayed for a given match will be the same as a single-spectrum search result. The user can follow the instruction given in Section 5 to interpret the search results.



Min Precursor Mass: Max Precursor Mass:
Min Intensity: Max Intensity:
Min Fragments: Max Fragments:
Min Hits: Max Hits:
Min Fit Score: Max Fit Score:

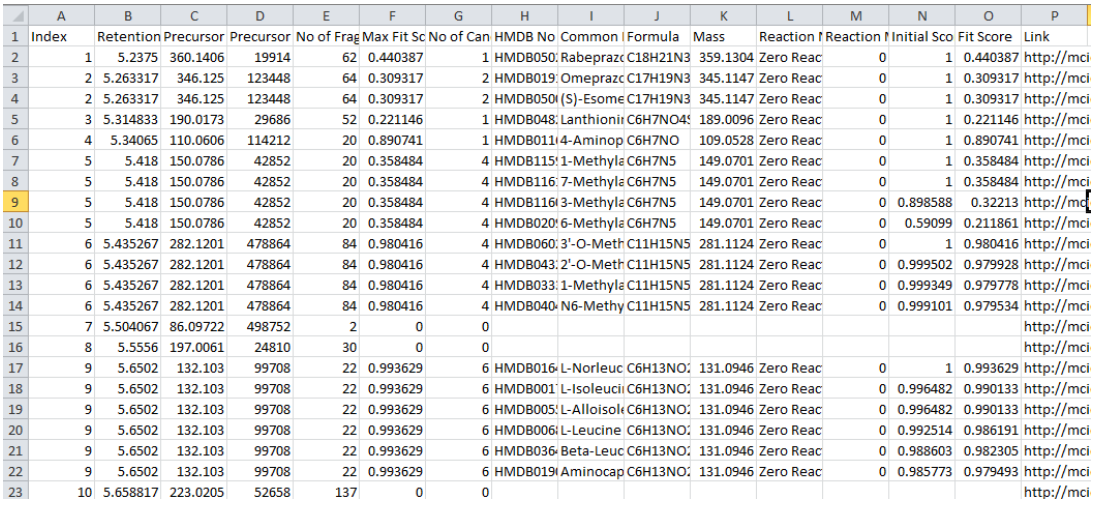
Show 10 entries Search:

#	Retention Time	Precursor Mass	Precursor Intensity	No. of Fragments	No. of Hits	Max Fit Score	Show Details	Save Result
1	5.24	360.14059	19914	62	1	0.44	Show detail	CSV
2	5.26	346.12504	123448	64	2	0.31	Show detail	CSV
3	5.31	190.01729	29686	52	1	0.22	Show detail	CSV
4	5.34	110.06063	114212	20	1	0.89	Show detail	CSV
5	5.42	150.07856	42852	20	4	0.36	Show detail	CSV
6	5.44	282.12013	478864	84	4	0.98	Show detail	CSV
7	5.50	86.09722	498752	2	0	0.00	Show detail	CSV
8	5.56	197.00612	24810	30	0	0.00	Show detail	CSV
9	5.65	132.10302	99708	22	6	0.99	Show detail	CSV
10	5.66	223.02053	52658	137	0	0.00	Show detail	CSV

Showing 1 to 10 of 100 entries Previous 1 2 3 4 5 … 10 Next

Download Table Result

Figure 5. Screenshot of batch-mode search results.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Index	Retention	Precursor	Precursor	No of Frag	Max Fit Sc	No of Can	HMDB No	Common	Formula	Mass	Reaction	Reaction	Initial Sco	Fit Score	Link
2	1	5.2375	360.1406	19914	62	0.440387	1	HMDB050	Rabeprazc	C18H21N3	359.1304	Zero Reac	0	1	0.440387	http://mci
3	2	5.263317	346.125	123448	64	0.309317	2	HMDB019	Omeprazc	C17H19N3	345.1147	Zero Reac	0	1	0.309317	http://mci
4	2	5.263317	346.125	123448	64	0.309317	2	HMDB050	(S)-Esome	C17H19N3	345.1147	Zero Reac	0	1	0.309317	http://mci
5	3	5.314833	190.0173	29686	52	0.221146	1	HMDB048	Lanthioni	C6H7NO4S	189.0096	Zero Reac	0	1	0.221146	http://mci
6	4	5.34065	110.0606	114212	20	0.890741	1	HMDB011	4-Aminop	C6H7NO	109.0528	Zero Reac	0	1	0.890741	http://mci
7	5	5.418	150.0786	42852	20	0.358484	4	HMDB115	1-Methyla	C6H7N5	149.0701	Zero Reac	0	1	0.358484	http://mci
8	5	5.418	150.0786	42852	20	0.358484	4	HMDB116	7-Methyla	C6H7N5	149.0701	Zero Reac	0	1	0.358484	http://mci
9	5	5.418	150.0786	42852	20	0.358484	4	HMDB116	3-Methyla	C6H7N5	149.0701	Zero Reac	0	0.898588	0.32213	http://mc
10	5	5.418	150.0786	42852	20	0.358484	4	HMDB020	6-Methyla	C6H7N5	149.0701	Zero Reac	0	0.59099	0.211861	http://mci
11	6	5.435267	282.1201	478864	84	0.980416	4	HMDB060	3'-O-Meth	C11H15N5	281.1124	Zero Reac	0	1	0.980416	http://mci
12	6	5.435267	282.1201	478864	84	0.980416	4	HMDB043	2'-O-Meth	C11H15N5	281.1124	Zero Reac	0	0.999502	0.979928	http://mci
13	6	5.435267	282.1201	478864	84	0.980416	4	HMDB033	1-Methyla	C11H15N5	281.1124	Zero Reac	0	0.999349	0.979778	http://mci
14	6	5.435267	282.1201	478864	84	0.980416	4	HMDB040	N6-Methy	C11H15N5	281.1124	Zero Reac	0	0.999101	0.979534	http://mci
15	7	5.504067	86.09722	498752	2	0	0									http://mci
16	8	5.5556	197.0061	24810	30	0	0									http://mci
17	9	5.6502	132.103	99708	22	0.993629	6	HMDB016	L-Norleuc	C6H13NO2	131.0946	Zero Reac	0	1	0.993629	http://mci
18	9	5.6502	132.103	99708	22	0.993629	6	HMDB001	L-Isoleucii	C6H13NO2	131.0946	Zero Reac	0	0.996482	0.990133	http://mci
19	9	5.6502	132.103	99708	22	0.993629	6	HMDB005	L-Alloisol	C6H13NO2	131.0946	Zero Reac	0	0.996482	0.990133	http://mci
20	9	5.6502	132.103	99708	22	0.993629	6	HMDB006	L-Leucine	C6H13NO2	131.0946	Zero Reac	0	0.992514	0.986191	http://mci
21	9	5.6502	132.103	99708	22	0.993629	6	HMDB036	Beta-Leuc	C6H13NO2	131.0946	Zero Reac	0	0.988603	0.982305	http://mci
22	9	5.6502	132.103	99708	22	0.993629	6	HMDB019	Aminocap	C6H13NO2	131.0946	Zero Reac	0	0.985773	0.979493	http://mci
23	10	5.658817	223.0205	52658	137	0	0									http://mci

Figure 6. Screenshot of the exported batch-mode search results.

Tutorial Part II. Examples of MCID MS/MS Search

1. An example of using MCID single-mode MS/MS search

Using L-Asparagine as an example, the MS/MS data are shown below.

```
Precursor ion(neutral): 132.0535
MS/MS list:
m/z     I %
42.0337 5.4
43.0177 27.3
44.0130 27.7
44.0494 19.8
45.0448 3.6
45.0523 0.6
46.0287 7.9
51.0228 0.9
53.0023 9.8
55.0179 4.0
59.0370 0.6
60.0446 10.6
70.0291 29.9
70.0656 0.7
71.0130 2.7
71.0326 0.8
73.0290 1.6
74.0243 100.0
75.0275 2.5
87.0555 0.9
88.0394 2.9
```

Referring to Figure 1, the user selects the spectral library as the zero-reaction library (i.e., No reaction), selects the type of precursor mass as Neutral, and enters the precursor mass (132.0535) along with the mass tolerance. In this case, the mass tolerance for the precursor mass is selected as the default (i.e., 0.005 Da). The user then enters the fragment ion masses and their corresponding intensities from the experimental MS/MS spectrum in the Query Mass box. Deisotope is selected as default to remove the ^{13}C-natural abundance peaks accompanied with the fragment ion peaks. The user enters the mass tolerance for the fragment ion masses or selects the default (0.005 Da). The user clicks the "Submit Query" to start the single-mode MS/MS search.

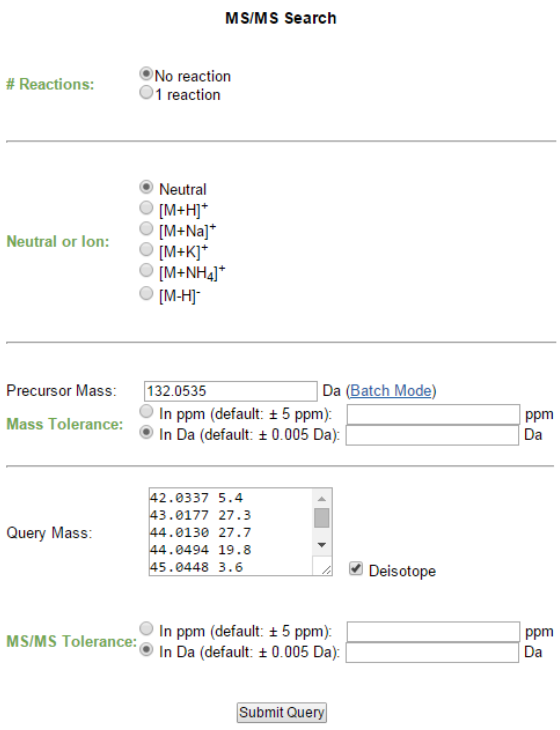


Figure 1. Screenshot of MCID single-mode MS/MS search settings.

The search result is shown in Figure 2A. To help interpret the match, the user can click the web link in the "Initial Score" column to display another layer of the match result. For example, by clicking "1.000" in Initial Score from L-Asparagine, a new page is displayed as shown in Figure 2B. This page shows the match quality of the predicted MS/MS spectrum against the experimental MS/MS spectrum. All the matched peaks are labeled in red and unmatched peaks are in grey. On the same page, all the experimental MS/MS peaks are listed in a table (see Figure 2C). By clicking in the "Detail" column, another page will be displayed as shown in Figure 2D. On this page, a specific experimental MS/MS peak is matched with a predicted MS/MS peak and the matched structure is displayed. The user can judge whether this matched structure is reasonable or not against the entire metabolite structure. The user can also follow the instruction given in Part I for more information on how to interpret the search results.

(A)

Search Result

Input Parameter Name	Parameter Value(s)
# Reactions	*0*
Ion Type	*Neutral*
Query Mass	*132.053500 Da*
Neutral Mass	*132.053500 Da*
Mass Tolerance	*0.005 Da*

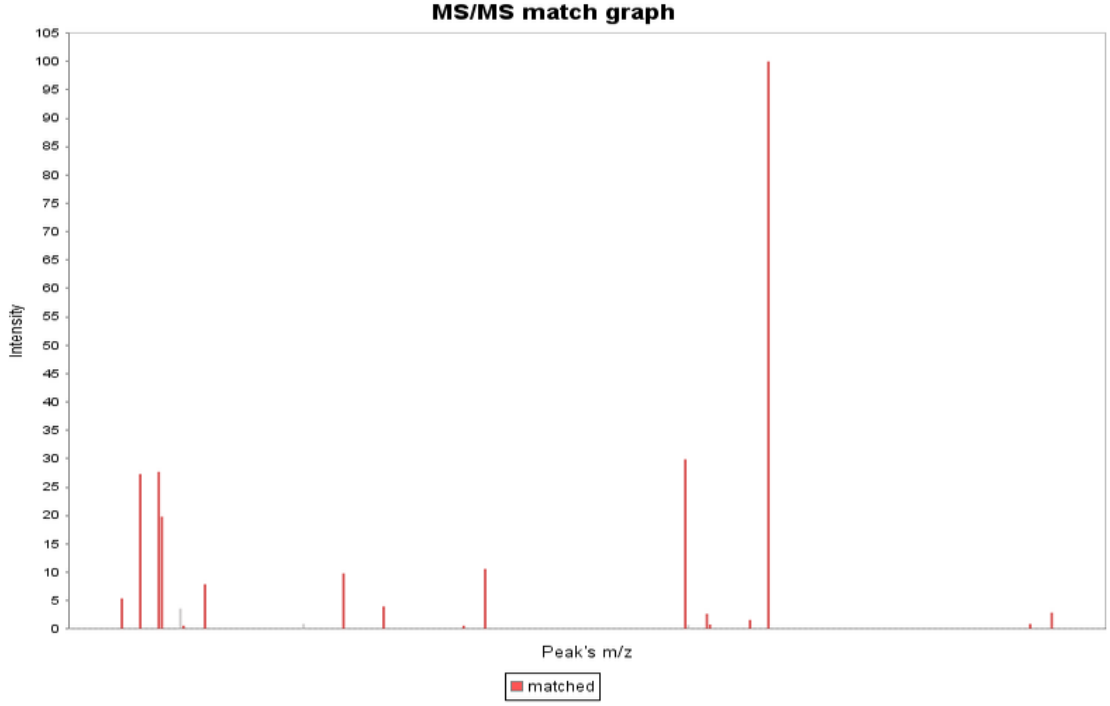
Export as CSV | Check All | Uncheck All | Delete Selected Entries | Save Attachments

#	HMDB ID	Common Name	Mass (Da)	Formula	Chemical Structure	Explore (for Firefox)	Possible Reactions	Reaction Offset (Da)	Mass Error (Da)	Initial Score	Fit Score	To Del	Attachments
1	HMDB00168	L-Asparagine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	1.000	0.984	☐	Add
2	HMDB12265	N-Carbamoylsarcosine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.943	0.928	☐	Add
3	HMDB11733	Glycyl-glycine	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.861	0.847	☐	Add
4	HMDB00026	Ureidopropionic acid	132.053493	$C_4H_8N_2O_3$		ChemDraw Pro ChemDraw Plugin		0.00000000	0.000007	0.483	0.475	☐	Add
5	HMDB03441	Cinnamaldehyde	132.057515	C_9H_8O		ChemDraw Pro ChemDraw Plugin		0.00000000	-0.004015	0.062	0.061	☐	Add

Export as CSV | Check All | Uncheck All | Delete Selected Entries | Save Attachments

(B)

NC(=O)CC(N)C(=O)O
Initial Score= 1.000
Fit Score= 0.984



(C)

Experimental peak	Intensity	Matched simulated peaks	Detail information	Experimental peak	Intensity	Matched simulated peaks	Detail information
42.0337	5.4	1	Detail	43.0177	27.3	2	Detail
44.013	27.7	1	Detail	44.0494	19.8	1	Detail
45.0448	3.6	0		45.0523	0.6	1	Detail
46.0287	7.9	1	Detail	51.0228	0.9	0	
53.0023	9.8	1	Detail	55.0179	4.0	1	Detail
59.037	0.6	2	Detail	60.0446	10.6	1	Detail
70.0291	29.9	1	Detail	70.0656	0.7	0	
71.013	2.7	1	Detail	71.0326	0.8	1	Detail
73.029	1.6	1	Detail	74.0243	100.0	1	Detail
87.0555	0.9	1	Detail	88.0394	2.9	1	Detail

(D)

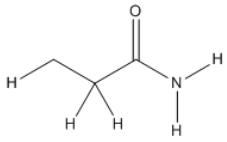
Fragment's mass	Plus or minus H's number	Simulated mass	Matched experiment mass	Mass error	Structure
71.0371	-1	70.0287	70.0291	0.0004	

Figure 2. Screenshots of single-mode MS/MS search results.

2. An example of using MCID batch-mode MS/MS search

2.1. Use "MCID-split.R" to split a big MS/MS data file

For the MCID batch-mode MS/MS search, we limit the size of the uploaded batch-mode file to 100 MS/MS spectra so that the server is not occupied for too long by a search work using a very big file. We provide an R based program, "MCID-split.R", for the user to split a big file into smaller files of up to 100 MS/MS spectra in each file. The user can download this program

from the MCID website and the latest R program from https://www.r-project.org/. To run the "MCID-split.R", the user needs to open the R program and assign the fold of "MCID-split.R" as the working folder of RGui by clicking: File → Change dir… (see Figure 3).

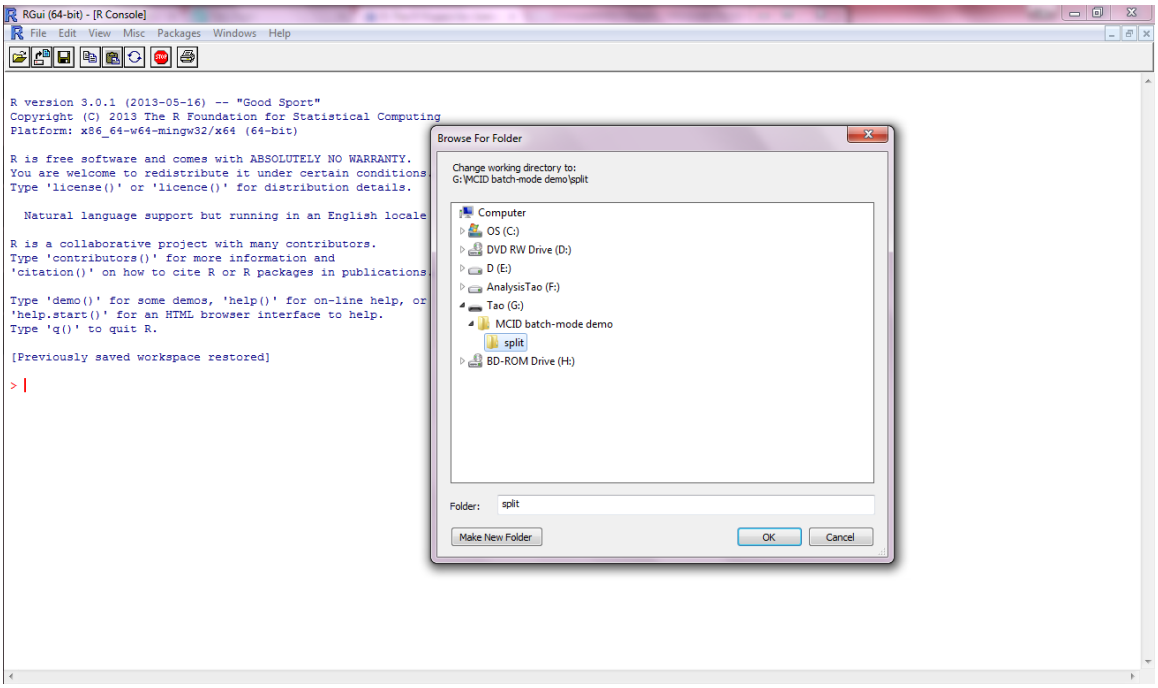


Figure 3. Screenshot of changing the working directory.

Then, the user opens the MCID-split.R script and changes the data path (data.path) (Figure 4) to the folder that contains the big file.

```
#########################################################################
# This is the setting part
data.path <- "G:/MCID batch-mode demo/split/"
file.name <- "run1.csv"
#########################################################################
```

Figure 4. Screenshot of setting the data path.

Next, the user needs to type in "source("MCID-split.R")" into the RGui and press enter to start the splitting process (see Figure 5).

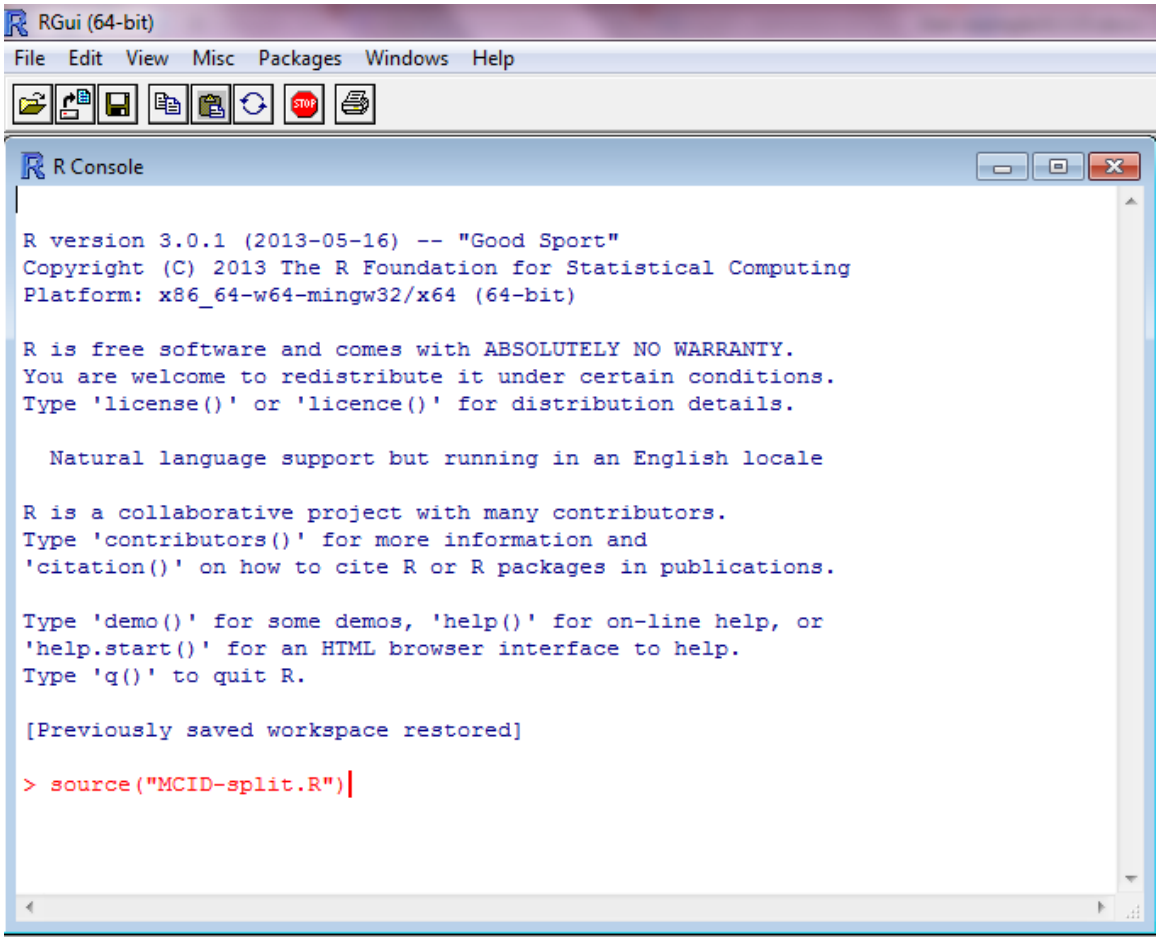
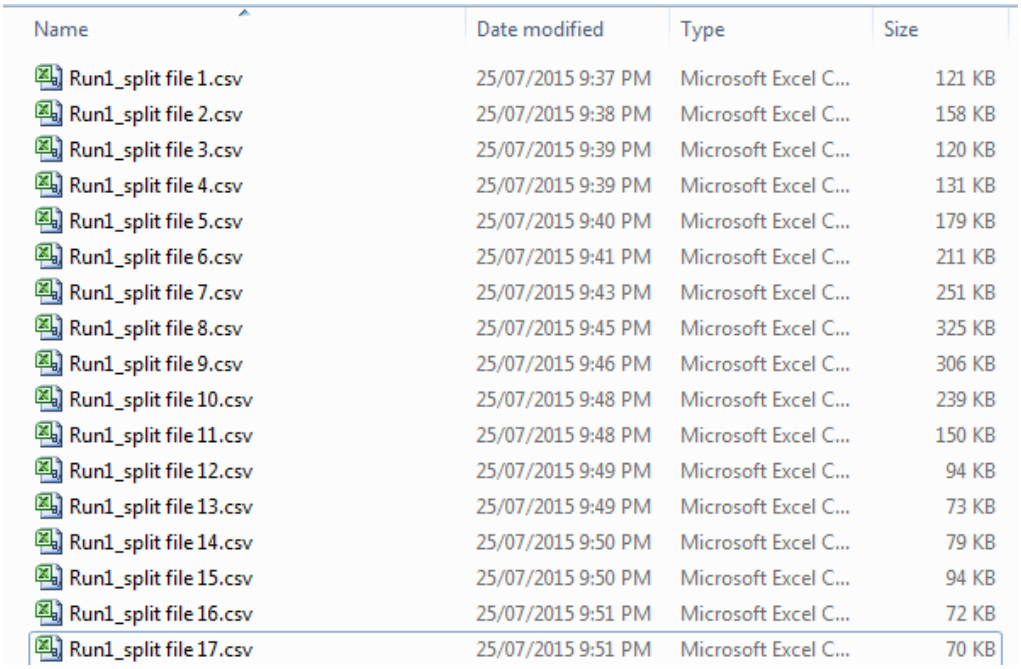


Figure 5. How to run the MCID-split.R.

After running the program, the user can find a list of small files with each containing a maximum of 100 MS/MS spectra (see Figure 6). These files are ready to be used to do batch-mode search online.



Name	Date modified	Type	Size
Run1_split file 1.csv	25/07/2015 9:37 PM	Microsoft Excel C...	121 KB
Run1_split file 2.csv	25/07/2015 9:38 PM	Microsoft Excel C...	158 KB
Run1_split file 3.csv	25/07/2015 9:39 PM	Microsoft Excel C...	120 KB
Run1_split file 4.csv	25/07/2015 9:39 PM	Microsoft Excel C...	131 KB
Run1_split file 5.csv	25/07/2015 9:40 PM	Microsoft Excel C...	179 KB
Run1_split file 6.csv	25/07/2015 9:41 PM	Microsoft Excel C...	211 KB
Run1_split file 7.csv	25/07/2015 9:43 PM	Microsoft Excel C...	251 KB
Run1_split file 8.csv	25/07/2015 9:45 PM	Microsoft Excel C...	325 KB
Run1_split file 9.csv	25/07/2015 9:46 PM	Microsoft Excel C...	306 KB
Run1_split file 10.csv	25/07/2015 9:48 PM	Microsoft Excel C...	239 KB
Run1_split file 11.csv	25/07/2015 9:48 PM	Microsoft Excel C...	150 KB
Run1_split file 12.csv	25/07/2015 9:49 PM	Microsoft Excel C...	94 KB
Run1_split file 13.csv	25/07/2015 9:49 PM	Microsoft Excel C...	73 KB
Run1_split file 14.csv	25/07/2015 9:50 PM	Microsoft Excel C...	79 KB
Run1_split file 15.csv	25/07/2015 9:50 PM	Microsoft Excel C...	94 KB
Run1_split file 16.csv	25/07/2015 9:51 PM	Microsoft Excel C...	72 KB
Run1_split file 17.csv	25/07/2015 9:51 PM	Microsoft Excel C...	70 KB

Figure 6. Screenshot of the file splitting results.

2.2. Batch-mode search parameters and results

To perform the batch-mode search, the user needs to define the reaction type (i.e., select the zero-reaction or one-reaction library), precursor ion type, precursor MS tolerance as well as MS/MS tolerance. Then, click the "Submit Query" to start the batch-mode search (see Figure 7). It takes about 2 min to complete a batch mode search with 100 MS/MS spectra using a precursor ion mass tolerance 0.005 Da. However, this search time may be longer if the server is busy to process many queries from multiple users.

Figure 8 shows a screenshot of the MCID batch-mode MS/MS search result. The user can follow the instructions in Part I to interpret the search results. As displayed at the top of the table, the user can further filter the search results table using precursor mass, intensity, number of fragments, number of hits (i.e., mass-matched candidates), and the fit score. Also, the entire search results table can be exported as a CSV file by clicking the "Download Table Result".

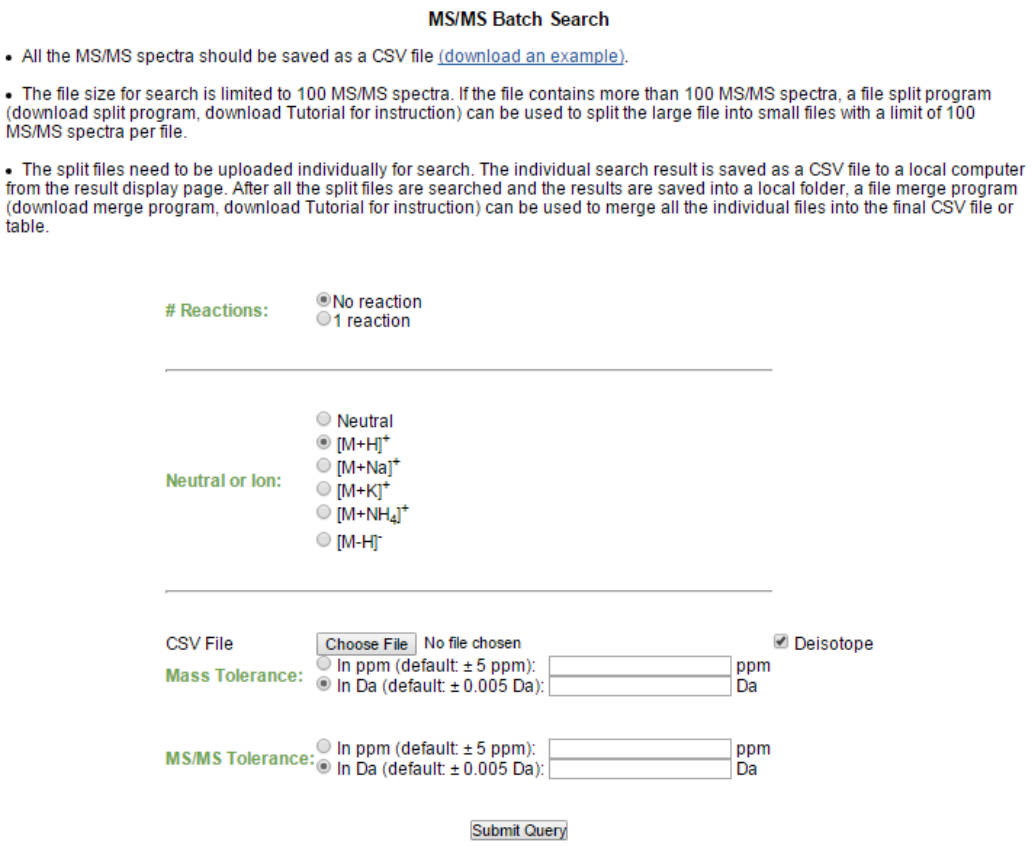


Figure 7. MCID batch-mode MS/MS search settings.

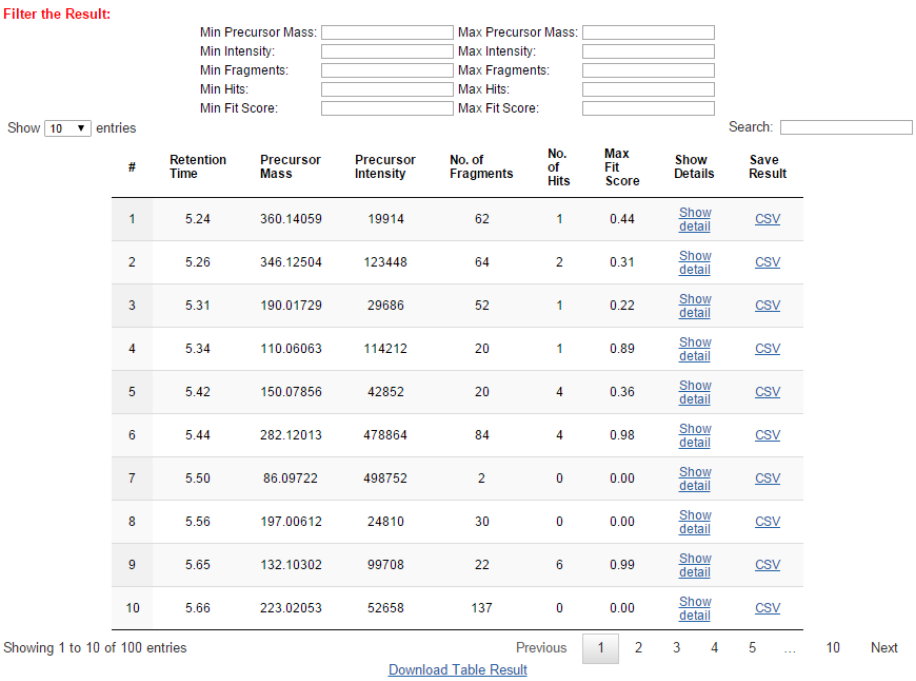


Figure 8. Screenshot of batch-mode MS/MS search results.

2.3. Use "MCID-merge.R" to combine all the search results

After all the search results in CSV are downloaded, another R program "MCID-merge.R" is used to combine all the individual search results files into one complete final results CSV table. To do so, similar to the use of "MCI-split.R", the user needs to open the RGui and assign the fold of "MCID-merge.R" as the working folder of RGui by clicking: File → Change dir… (see Figure 9).

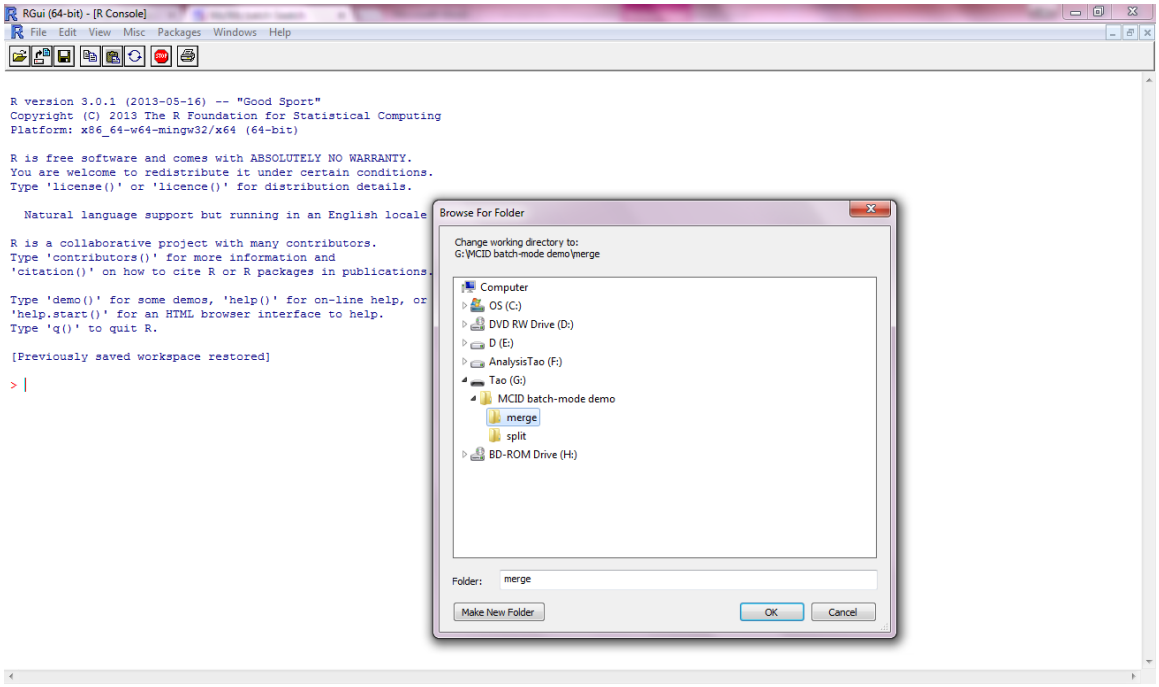


Figure 9. Screenshot of changing work directory.

Then, the user opens the MCID-merge.R script and changes the data path (data.path) (Figure 10) to the folder that contains all the search results files.

```
#########################################################################
# This is the setting part
data.path <- "G:/MCID batch-mode demo/merge/"
#########################################################################
```

Figure 10. Screenshot of data.path setting.

Next, the user needs to type in “source(“MCID-merge.R”)” into the RGui and press enter to start merging all the results files together (see Figure 11).

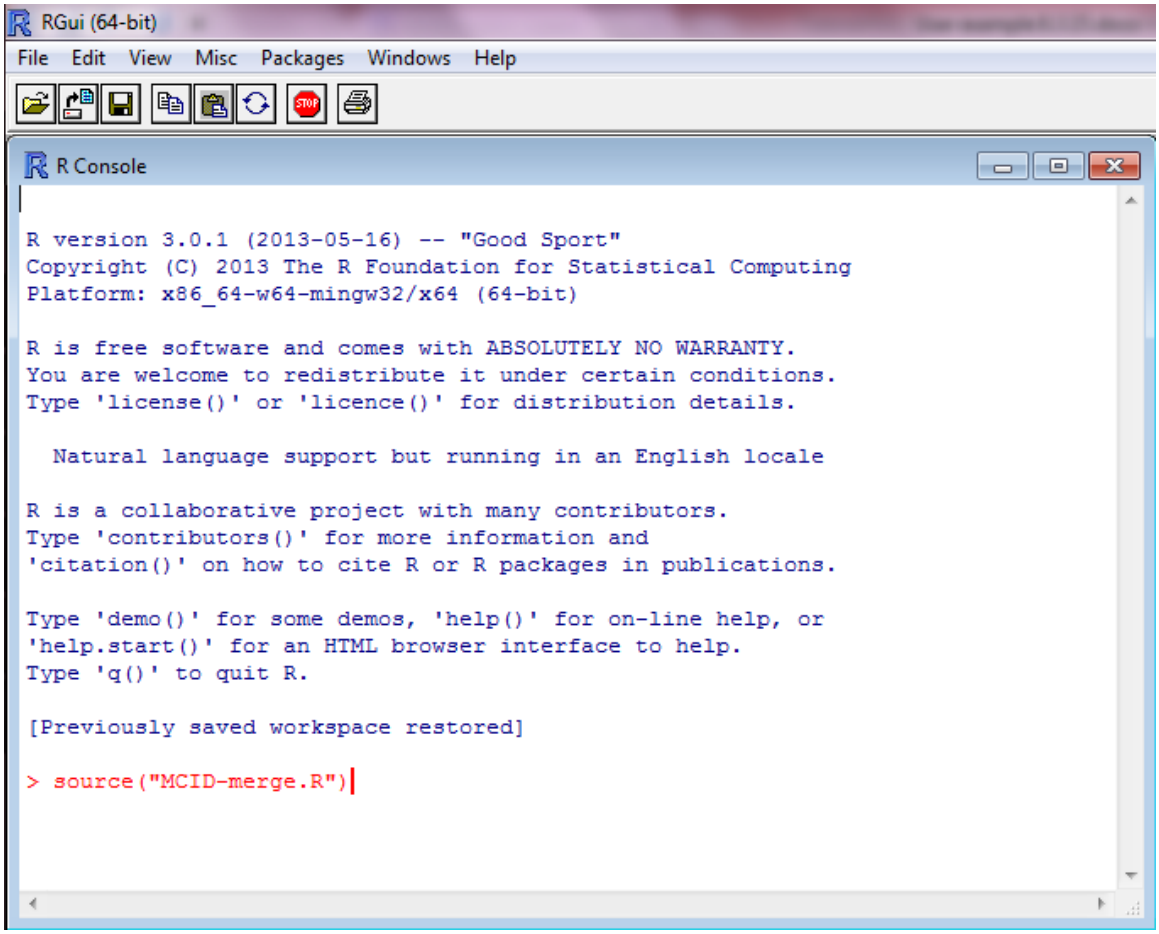
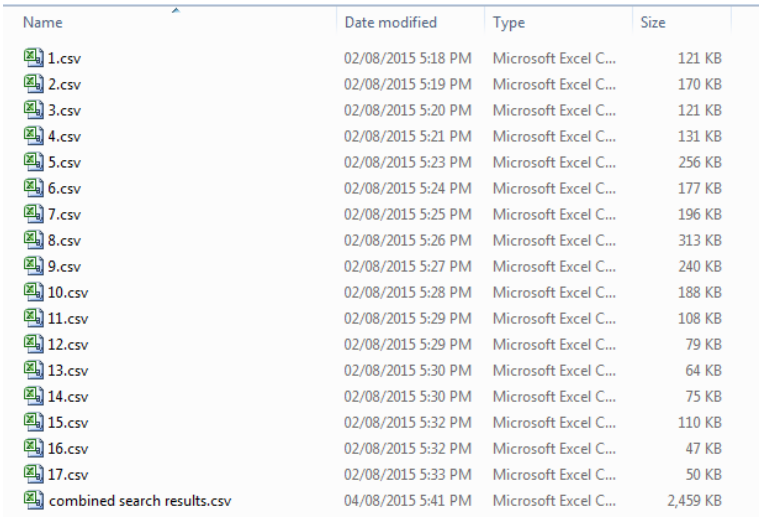


Figure 11. How to run the MCID-merge.R.

After the process is completed, a “combined search results.csv” file will be created (see Figure 12) and this file contains all the information from all the individual searches.



Name	Date modified	Type	Size
1.csv	02/08/2015 5:18 PM	Microsoft Excel C...	121 KB
2.csv	02/08/2015 5:19 PM	Microsoft Excel C...	170 KB
3.csv	02/08/2015 5:20 PM	Microsoft Excel C...	121 KB
4.csv	02/08/2015 5:21 PM	Microsoft Excel C...	131 KB
5.csv	02/08/2015 5:23 PM	Microsoft Excel C...	256 KB
6.csv	02/08/2015 5:24 PM	Microsoft Excel C...	177 KB
7.csv	02/08/2015 5:25 PM	Microsoft Excel C...	196 KB
8.csv	02/08/2015 5:26 PM	Microsoft Excel C...	313 KB
9.csv	02/08/2015 5:27 PM	Microsoft Excel C...	240 KB
10.csv	02/08/2015 5:28 PM	Microsoft Excel C...	188 KB
11.csv	02/08/2015 5:29 PM	Microsoft Excel C...	108 KB
12.csv	02/08/2015 5:29 PM	Microsoft Excel C...	79 KB
13.csv	02/08/2015 5:30 PM	Microsoft Excel C...	64 KB
14.csv	02/08/2015 5:30 PM	Microsoft Excel C...	75 KB
15.csv	02/08/2015 5:32 PM	Microsoft Excel C...	110 KB
16.csv	02/08/2015 5:32 PM	Microsoft Excel C...	47 KB
17.csv	02/08/2015 5:33 PM	Microsoft Excel C...	50 KB
combined search results.csv	04/08/2015 5:41 PM	Microsoft Excel C...	2,459 KB

Figure 12. Screenshot of the merged result.

When the "combined search results.csv" file is opened, all the information about the search results are shown (see Figure 13). The web link provided at the end of each row allows the user to manually check an individual match result from the MCID website. The user merely needs to cut and paste the link name to the internet and the search result displayed for a given match will be the same as a single-spectrum search result. The user can then follow the instruction given in Part I to interpret the search results.

Index	Retention	Precursor.	Precursor.	No.of.Fra	Max.Fit.Sc	No.of.Can	HMDB.No	Common.	Formula	Mass	Reaction.I	Reaction.I	Initial.Sco	Fit.Score	Link
1	0.035942	141.9587	19684	17	0	0				NA		NA	NA	NA	http://mcid
2	0.0445	158.003	9094	25	0	0				NA		NA	NA	NA	http://mcid
3	0.053058	159.9697	8954	29	0	0				NA		NA	NA	NA	http://mcid
4	0.311	106.9923	3560	1	0	0				NA		NA	NA	NA	http://mcid
5	0.457117	122.097	4338	23	0.645035	3	HMDB010	N-N-Dime	C8H11N	121.0891	Zero Reac	0	1	0.645035	http://mcid
5	0.457117	122.097	4338	23	0.645035	3	HMDB020	1-Phenyle	C8H11N	121.0891	Zero Reac	0	1	0.645035	http://mcid
5	0.457117	122.097	4338	23	0.645035	3	HMDB122	Phenyleth	C8H11N	121.0891	Zero Reac	0	0.957412	0.617564	http://mcid
6	0.525883	113.9648	3556	5	0	0				NA		NA	NA	NA	http://mcid
7	0.534442	158.0033	9094	16	0	0				NA		NA	NA	NA	http://mcid
8	0.534442	253.0928	2624	29	0.278611	1	HMDB000	Deoxyino	C10H12N4	252.0859	Zero Reac	0	1	0.278611	http://mcid
9	0.637575	97.96898	4714	1	0	0				NA		NA	NA	NA	http://mcid
10	0.671958	106.9928	3122	2	0	0				NA		NA	NA	NA	http://mcid
11	0.809483	338.3417	1018	4	0	0				NA		NA	NA	NA	http://mcid
12	0.8955	158.0029	9312	20	0	0				NA		NA	NA	NA	http://mcid
13	0.904058	141.9589	11380	11	0	0				NA		NA	NA	NA	http://mcid
14	0.947008	176.0147	2304	35	0	0				NA		NA	NA	NA	http://mcid
15	1.067383	97.96921	4714	1	0	0				NA		NA	NA	NA	http://mcid
16	1.075975	130.0089	2724	9	0	0				NA		NA	NA	NA	http://mcid
17	1.084533	141.9593	1610	8	0	0				NA		NA	NA	NA	http://mcid
18	1.2394	158.0032	9312	13	0	0				NA		NA	NA	NA	http://mcid
19	1.247958	90.94944	2490	2	0	0				NA		NA	NA	NA	http://mcid
20	1.256525	122.0978	4392	20	0.514689	3	HMDB010	N-N-Dime	C8H11N	121.0891	Zero Reac	0	1	0.514689	http://mcid
20	1.256525	122.0978	4392	20	0.514689	3	HMDB020	1-Phenyle	C8H11N	121.0891	Zero Reac	0	1	0.514689	http://mcid
20	1.256525	122.0978	4392	20	0.514689	3	HMDB122	Phenyleth	C8H11N	121.0891	Zero Reac	0	1	0.514689	http://mcid

Figure 13. Screenshot of the exported batch-mode search results.