Tutorial (5.10.15 version)

1. Workflow. The workflow for metabolite identification using Dns-library is shown below (Figure 1).

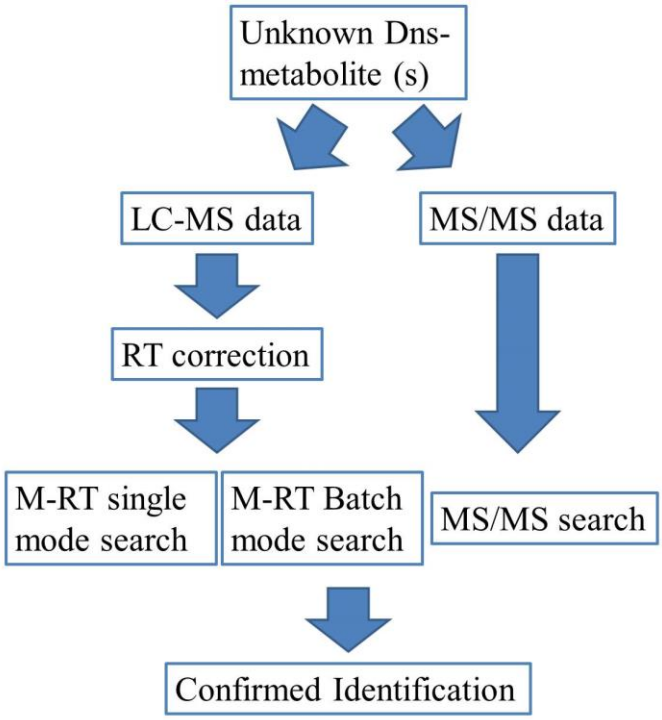

Figure 1. Workflow for M-RT search and MS/MS search.

2. Dns-library database. The current Dns-library consists of 273 unique metabolites with a total of 315 entries. The Dns-library view on the sidebar lists all these Dns-metabolites with their mz and normalized RT information. Figure 2 shows a screenshot of the Dns-library database. The user can view the HMDB number, monoisotopic molecular mass, mz_light, normalized or corrected RT for each of the Dns-metabolite standards from the table. In addition, the hyperlinks for each Dns-metabolite to HMDB and KEGG databases are provided. These databases provide detailed biological information about the metabolite.

#	HMDB No.	Name	Monoisotopic molecular mass	mz_light	Corrected RT	HMDB link	KEGG link	Show detail
1	HMDB00001	1_Methylhistidine	169.0851	403.1434	2.17	Link	Link	Detail
2	HMDB00002	1-3_Diaminopropane	74.0844	308.1427	2.63	Link	Link	Detail
3	HMDB00002	1-3_Diaminopropane - multi-tags	74.0844	271.0583	20.49	Link	Link	Detail
4	HMDB00020	p-Hydroxyphenylacetic acid	152.0473	386.1057	16.91	Link	Link	Detail
5	HMDB00021	Iodotyrosine	306.9705	387.5436	23.88	Link	Link	Detail
6	HMDB00022	3_Methoxytyramine	167.0946	317.6056	25.49	Link	Link	Detail
7	HMDB00045	Adenosine monophosphate	347.0631	581.1214	1.75	Link	Link	Detail
8	HMDB00050	Adenosine	267.0968	501.1551	3.94	Link	Link	Detail
9	HMDB00051	Ammonia	17.0266	251.0849	5.82	Link	Link	Detail
10	HMDB00056	Beta-Alanine	89.0477	323.1060	7.24	Link	Link	Detail
11	HMDB00064	Creatine	131.0695	365.1278	3.02	Link	Link	Detail
12	HMDB00070	D-Pipecolic acid	129.0790	363.1373	13.23	Link	Link	Detail
13	HMDB00085	Deoxyguanosine	267.0968	501.1551	8.49	Link	Link	Detail
14	HMDB00087	Dimethylamine	45.0578	279.1162	15.07	Link	Link	Detail
15	HMDB00089	Cytidine	243.0855	477.1438	5.87	Link	Link	Detail
16	HMDB00089	Cytidine - H2O	243.0855	459.1333	7.38	Link	Link	Detail
17	HMDB00095	Cytidine monophosphate	323.0519	557.1102	1.88	Link	Link	Detail
18	HMDB00095	Cytidine monophosphate - Isomer	323.0519	557.1102	2.87	Link	Link	Detail
19	HMDB00099	L-Cystathionine	222.0674	345.0920	13.34	Link	Link	Detail
20	HMDB00099	L-Cystathionine - Isomer	222.0674	345.0920	13.69	Link	Link	Detail
21	HMDB00101	Deoxyadenosine	251.1018	485.1602	8.72	Link	Link	Detail
22	HMDB00112	Gamma-Aminobutyric acid	103.0633	337.1216	7.79	Link	Link	Detail
23	HMDB00112	Gamma-Aminobutyric acid - H2O	103.0633	319.1144	13.57	Link	Link	Detail

Figure 2. Screenshot of a partial Dns-library table.

The user can click the “Show Detail” button, which guides the user to a page with more detailed information about the dansyl labeled metabolite (Figure 3). An LC-MS chromatogram and MS/MS spectrum are provided on this page. These data were collected using pure standard compound and can be used to compare with the user's experimental data. Details on the preparation of the Dns-standards can be found in the materials and methods part of the paper.

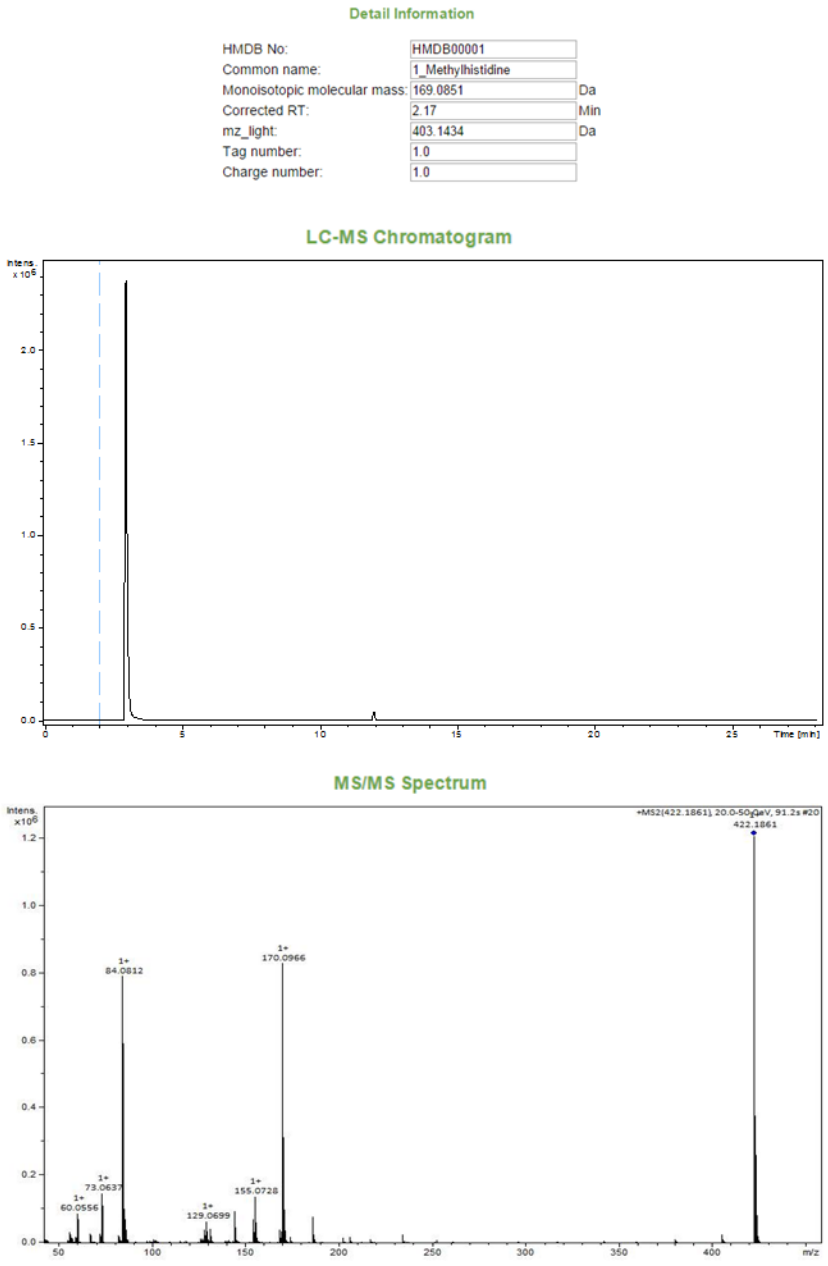

Figure 3. Screenshot of the "Show detail" page.

3. **M-RT single mode search.** M-RT single mode search allows a user to search the Dns-library by submitting a single metabolite feature with its RT and mass (M+H). Also, a calibration file needs to be submitted to correct the retention time of the single metabolite feature. Figure 4 shows the screenshot of the single mode search.

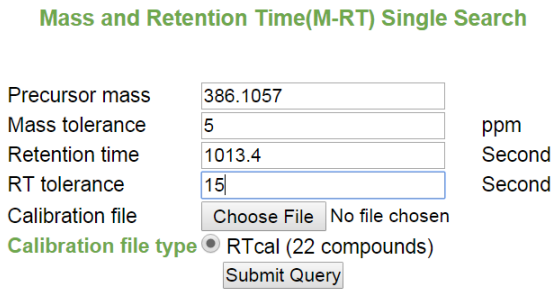

Figure 4. M-RT single mode search parameters.

There are six search parameters.

1) **Precursor Mass.** The user needs to input the precursor mass of the metabolite feature.
2) **Mass tolerance.** The user needs to define a mass tolerance for the precursor mass search. 5 ppm is normally used for data collected using high resolution MS such as TOF and FT (10 ppm or higher may be used for very low abundance peaks). If the experiment is performed using a low resolution MS instrument, a larger mass tolerance should be considered.
3) **Retention time.** The user needs to input the retention time of the metabolite feature.
4) **RT tolerance.** The user needs to define a retention time tolerance for the M-RT search;15 seconds is normally used. If no close matches are found, a wider retention time window should be considered with caution. For LC with lower retention time precision, a larger RT tolerance may be used.
5) **Calibration file.** A calibration file needs to be uploaded to adjust the retention time of the metabolite feature to match the retention time of metabolites in the Dns-library. The template of the calibration file is shown in the "**user example**". The user needs to download the template and fill in the retention time information for each of the calibration standards used in the calibration file. The retention time has a unit of second.

6) **Calibration file type.** In the current Dns-library RT correction method, a 22 Dns-standards file is used. We will include other types of the calibration files for different applications in the future.

7) **Submit query.** Once all the parameters have been set, the user can click on the "submit query" to start the M-RT single mode search.

4. M-RT batch mode search. M-RT batch search mode allows a user to search the Dns-library using the entire dansyl-labeled LC-MS file. Figure 5 shows the screenshot of the batch mode search.

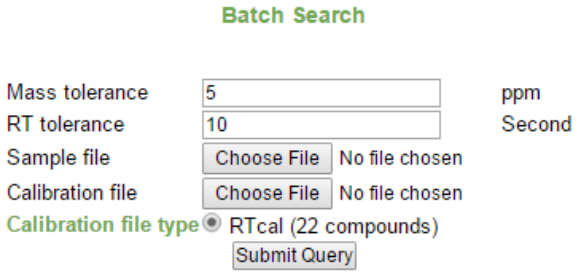

Figure 5. M-RT batch mode search parameters.

The parameters include:

1) **Mass tolerance.** The user needs to define a mass tolerance for the precursor mass search. 5 ppm is normally used for data collected using high resolution MS such as TOF and FT (10 ppm or higher may be used for very low abundance peaks). If the experiment is performed using a low resolution MS instrument, a larger mass tolerance should be considered.

2) **RT tolerance.** The user needs to define a retention time tolerance for the M-RT search;15 seconds is normally used. If no close matches are found, a wider retention time

window should be considered with caution. For LC with lower retention time precision, a larger RT tolerance may be used.

3) **Sample file.** A sample file needs to be uploaded onto the website for batch mode search. The sample file is the metabolite-intensity matrix after processing the raw LC-MS data in IsoMS, Iso-Align, and Zero-fill.
4) **Calibration file.** A calibration file needs to be uploaded for adjusting the retention time of the metabolite feature to match with the retention time of the metabolites in the Dns-library. The template of the calibration file is shown in the "**user example**". The user needs to download this template and fill in the retention time information for each of the calibration standards used in the calibration file. The retention time has a unit of second.
5) **Calibration file type.** In the current Dns-library RT correction method, a 22-Dns-standards file is used. We will include other types of the calibration files for different applications in the future.
6) **Submit query.** Once all the parameters have been set, the user can click on the "submit query" to start the M-RT batch mode search.

5. MS/MS search. The MS/MS search function allows a user to identify a dansyl labeled metabolite using MS/MS information. Figure 6 shows the screenshot of the MS/MS search function.

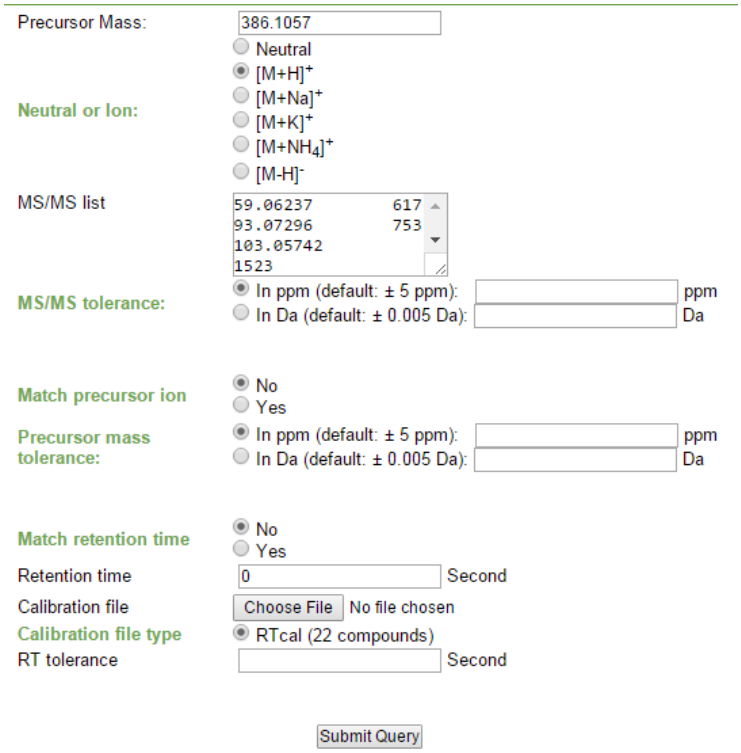

Figure 6. MS/MS search parameters.

The parameters include:

1) **Precursor mass.** The user needs to input the precursor mass of the metabolite feature.
2) **Neutral or ion.** The user can define the type of the precursor mass. It can be either an M+H ion or a neutral mass.
3) **MS/MS list.** The user needs to input a list of MS/MS fragment ion masses with their associated intensities.
4) **MS/MS tolerance.** The user needs to set a mass tolerance for the MS/MS fragment ions to perform the matching with the MS/MS information in the Dns-library.
5) **Match precursor ion.** The user has the option of defining the precursor ion mass for MS/MS search. If this option is enabled, only the Dns-metabolites that match with the precursor mass will be further used to compare the MS/MS fragment ions. If this option is disabled, the MS/MS match is performed on all 273 Dns-metabolites.

6) **Precursor mass tolerance.** The user needs to define a mass tolerance for the precursor mass search. 5 ppm is normally used for data collected using high resolution MS such as TOF and FT (10 ppm or higher may be used for very low abundance peaks). If the experiment is performed using a low resolution MS instrument, a larger mass tolerance should be considered.
7) **Match retention time.** The user has the option of including RT for MS/MS search. If this option is on, only the Dns-metabolites that match with the retention time will be further used to compare the MS/MS fragment ions. If this option is off, the MS/MS match is performed on all 273 Dns-metabolites.
8) **RT tolerance.** The user needs to define a retention time tolerance for the M-RT search;15 seconds is normally used. If no close matches are found, a wider retention time window should be considered with caution. For LC with lower retention time precision, a larger RT tolerance may be used.
9) **Calibration file.** A calibration file needs to be uploaded for adjusting the retention time of the metabolite feature to be consistent with the retention time of metabolites in the Dns-library. The template of the calibration file is shown in the "**user example**". The user needs to download that template and fill in the retention time information for each of the calibration standard used in the calibration file. The retention time has a unit of second.
10) **Calibration file type.** In the current Dns-library RT correction method, a 22-Dns-standards file is used. We will include other types of the calibration files for different applications in the future.

6. M-RT search result display. Figure 8 shows the screenshot of the M-RT search result. The search result table is similar to the Dns-library table with several extra columns.

Search Result

#	Input mass	Input RT	Calibrated RT	HMDB No.	Name	Monoisotopic molecular mass	mz_light	Library RT	Mass error	RT error	HMDB link	KEGG link	Show detail
1	375.0785	2.05	1.94	HMDB00224	O-Phosphoethanolamine	141.0191	375.0774	2.02	0.0011	0.08	Link	Link	Detail
2	359.0743	2.31	2.18	HMDB00251	Taurine	125.0147	359.0730	2.24	0.0013	0.06	Link	Link	Detail
3	403.1443	2.32	2.19	HMDB00001	1_Methylhistidine	169.0851	403.1434	2.17	0.0009	0.02	Link	Link	Detail
4	403.1443	2.32	2.19	HMDB00479	3_methyl-histidine	169.0851	403.1434	2.01	0.0009	0.18	Link	Link	Detail
5	408.1708	2.61	2.47	HMDB00517	L-Arginine	174.1117	408.1700	2.44	0.0008	0.03	Link	Link	Detail
6	343.0781	2.64	2.50	HMDB00965	Hypotaurine	109.0197	343.0781	2.47	0.0000	0.03	Link	Link	Detail
7	351.1124	2.81	2.67	HMDB00128	Guanidoacetic acid	117.0538	351.1121	2.74	0.0003	0.07	Link	Link	Detail
8	366.1132	3.09	2.94	HMDB00168	L-Asparagine	132.0535	366.1118	3.0	0.0014	0.06	Link	Link	Detail
9	422.1861	3.21	3.06	HMDB00670	Homo-L-arginine	188.1273	422.1856	3.0	0.0005	0.06	Link	Link	Detail
10	359.1547	3.28	3.14	HMDB01861	3_Methylhistamine	125.0953	359.1536	3.27	0.0011	0.13	Link	Link	Detail

Figure 8. Screenshot of M-RT search result.

7. MS/MS search result display. Figure 9 shows the screenshot of the M-RT search result. The search result table is similar to the Dns-library table with several extra columns.

Search Result

#	Input mass	Input RT	Calibrated RT	HMDB No.	Name	Monoisotopic molecular mass	mz_light	Library RT	Mass error	RT error	HMDB link	KEGG link	MS/MS score ▲	Show detail
7	581.1214	NA	NA	HMDB00045	Adenosine monophosphate	347.0631	581.1214	1.75	0.0000	NA	Link	Link	1.00	Detail
155	581.1214	NA	NA	HMDB01341	ADP	427.0294	661.0877	1.49	79.9663	NA	Link	Link	0.74	Detail
142	581.1214	NA	NA	HMDB01044	2'-Deoxyguanosine 5'-monophosphate	347.0631	581.1214	5.57	0.0000	NA	Link	Link	0.52	Detail
151	581.1214	NA	NA	HMDB01173	5'-Methylthioadenosine	297.0896	531.1479	6.97	49.9735	NA	Link	Link	0.19	Detail
8	581.1214	NA	NA	HMDB00050	Adenosine	267.0968	501.1551	3.94	79.9663	NA	Link	Link	0.18	Detail
293	581.1214	NA	NA	HMDB60003	Isovanillic acid	168.0423	402.1006	15.69	179.0208	NA	Link	Link	0.16	Detail
21	581.1214	NA	NA	HMDB00101	Deoxyadenosine	251.1018	485.1602	8.72	95.9612	NA	Link	Link	0.14	Detail
146	581.1214	NA	NA	HMDB01069	2_Phenylaminoadenosine	358.1390	592.1973	8.73	11.0759	NA	Link	Link	0.04	Detail
18	581.1214	NA	NA	HMDB00095	Cytidine monophosphate - Isomer	323.0519	557.1102	2.87	24.0112	NA	Link	Link	0.03	Detail

Figure 9. Screenshot of MS/MS search.