MassBank
User's Manual

Version 2.4, 7 February 2012

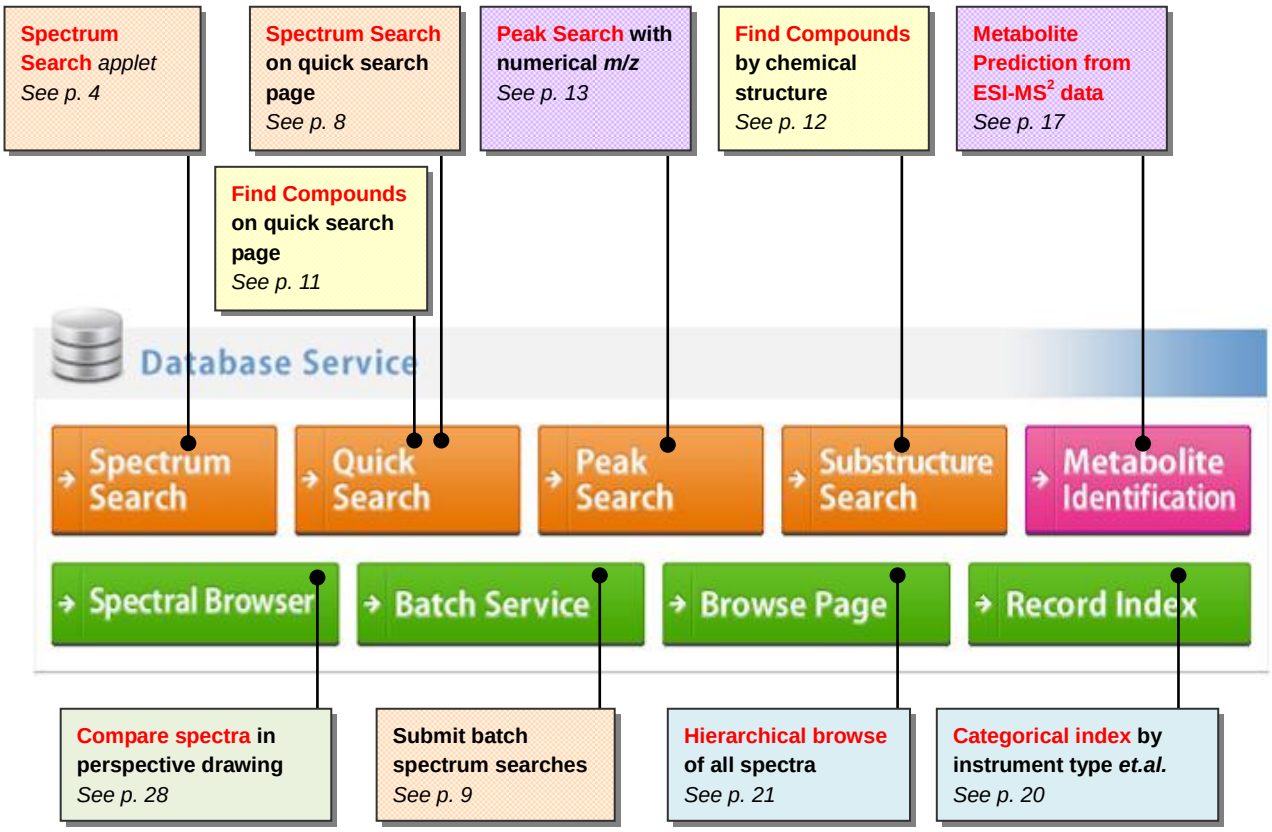
CONTENTS

1. Introduction

This manual mainly describes the operation of the MassBank database services.

1.1 Overview of the Database Services

MassBank provides the following database services.



1.2 System Requirements

Below are the system requirements for using the MassBank database services.

(1) Web Browser

We recommend using Internet Explorer 6 or higher, or Firefox 2 or higher as your browser.

(2) Web Browser Settings

Make sure that your Web browser is configured as follows.

[1] JavaScript execution enabled

[2] Popups enabled

- See the page below for instructions on how to check this (URL to allow: http://www.massbank.jp)
  http://www.microsoft.com/japan/windowsxp/using/web/sp2_popupblocker.mspx

(3) Install Java runtime environment

The Java Runtime Environment (JRE) Version 5 or higher must be installed.

- You can use the page below to check your installation status and download Java.
  http://www.java.com/en/download/

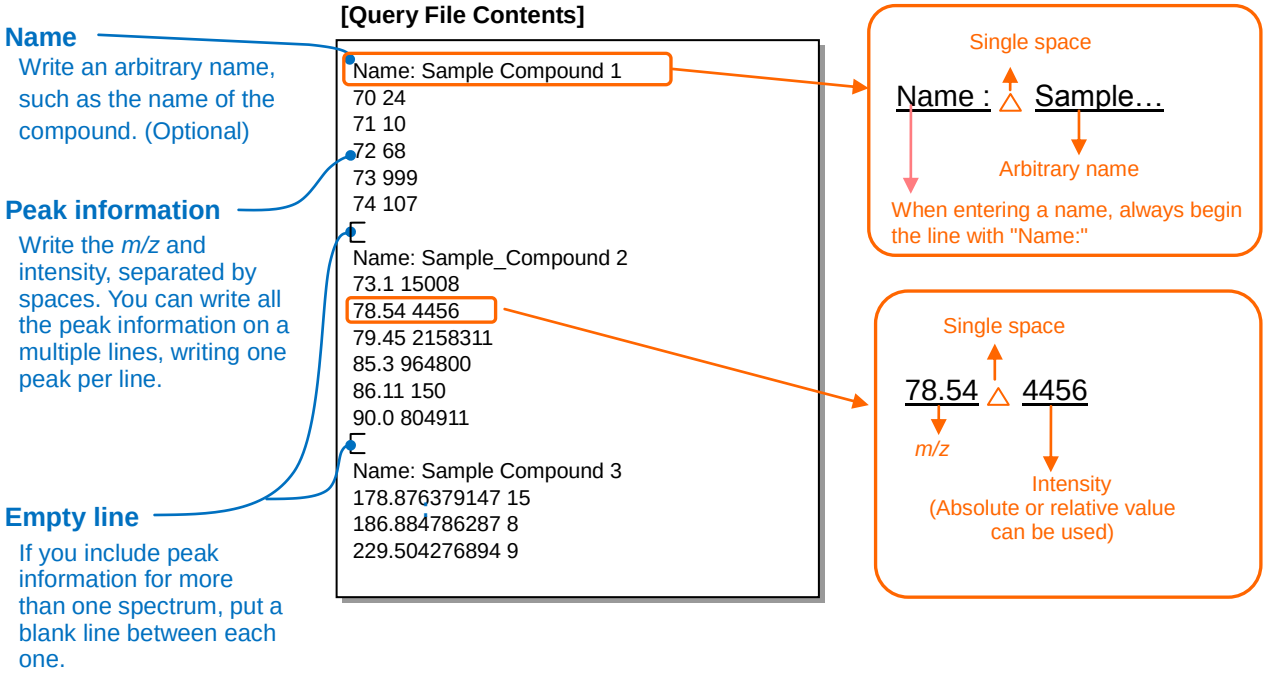
2. Searching for Similar Spectra

2.1 Spectrum Search Applet

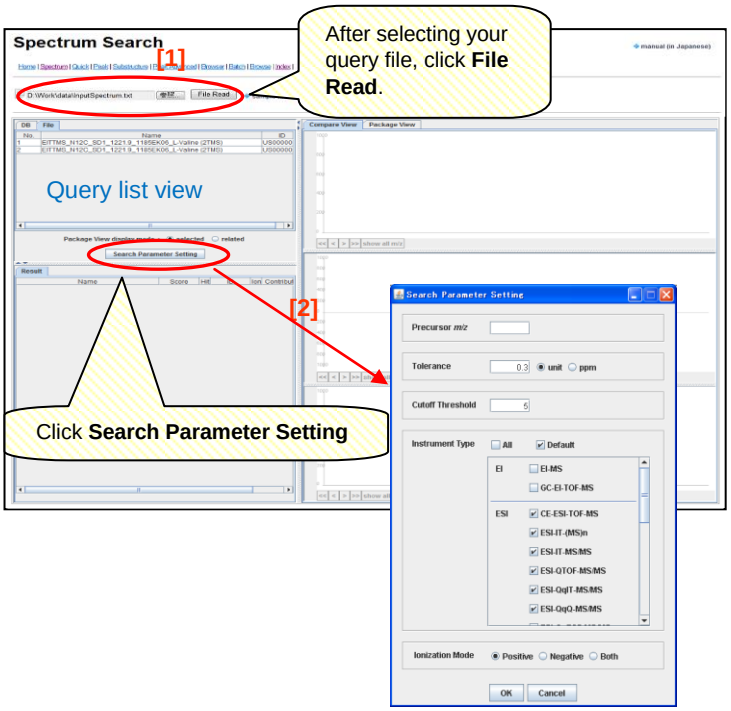
Spectrum Search enables you to perform GUI-based spectrum searches in a Web browser. Spectra in the MassBank similar to one provided by the user as a query are retrieved and displayed in a list. It is also possible to graphically compare the query spectrum with the retrieved spectrum.

(1) Prepare the query file

When performing a spectrum search with the applet, you must prepare a query file in one of the following formats.
A sample can be downloaded from http://www.massbank.jp/sample/sample.zip



(2) Load the query and configure the search parameters



Search Parameter Setting Window

[1] Load the query file

Click **Browse**, and select the query file you have prepared. Click **File Read** to load the file. After the file finishes loading, a list of queries appears.

[2] Set the search parameters

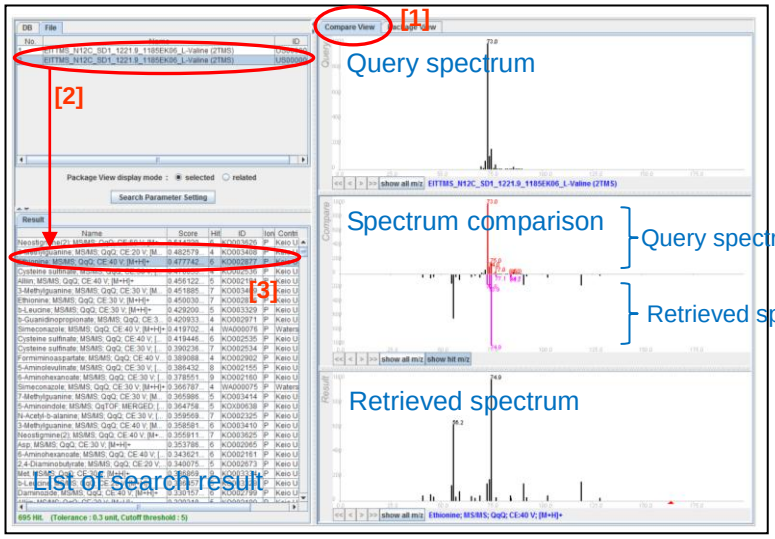
Click **Search Parameter Setting**.
The Setting window opens.

Settings

- **Precursor *m/z*** :
  Precursor ion specified by *m/z*
- **Tolerance**:
  m/z error range
- **Cutoff Threshold**:
  Relative intensity threshold
- **Instrument Type**:
  Type of instrument
- **Ionization Mode**:
  Mode of ionization

(3) Perform the search

Compare View: Compare query and retrieved spectrums one to one.



[1] Select "Compare View"

Click to select.
It is selected by default.

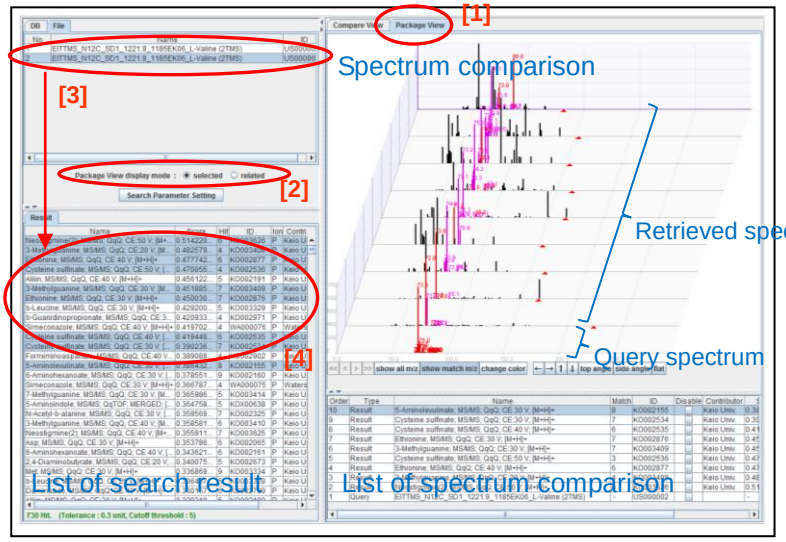
[2] Select the query spectrum

Click to select the spectrum to use in the query. As soon as it is selected, similar spectrums are retrieved.

[3] Select a retrieved spectrum

Click to select any spectrum in the list of search result.

Package View: Compare query and retrieved spectrums one to many.



[1] Select "Package View"

Click to select.

[2] Select package view display mode

Click "**selected**" or "**related**".

[3] Select the query spectrum

Click to select the spectrum to use in the query. As soon as it is selected, similar spectrums are retrieved.

[4] Select a retrieved spectrum

Click to select any spectrum in the list of search result. If you chose "**selected**" in step 2, then you can hold down Ctrl + Shift to select multiple rows. If you chose "**related**" in step 2, then only one row can be selected.

Different Display Modes in Package View

There are two display modes in Package View.

selected

Multiple spectrums can be selected from the list of search results. The selected spectrums and the query spectrum are shown in a tiled view.

related

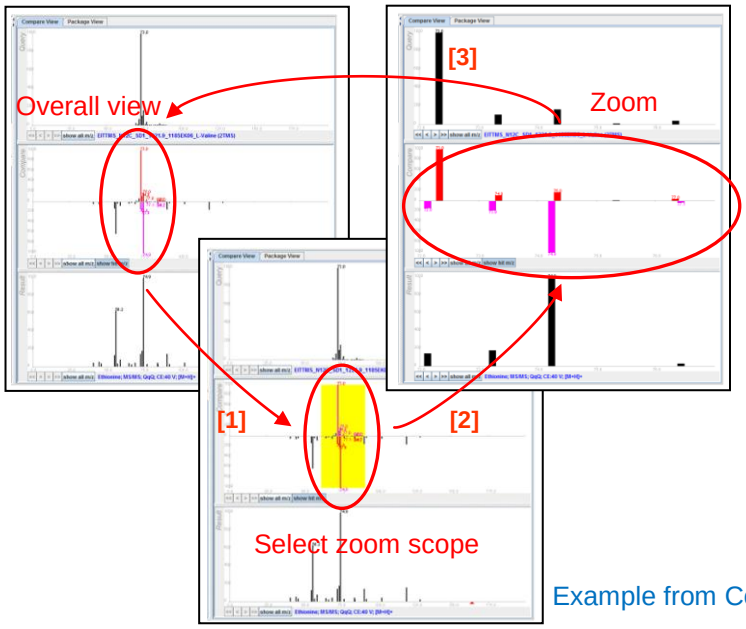
Only one spectrum can be selected from the list of search results. The selected spectrum, a spectrum with different collision energy than the selected spectrum (detected automatically), and the query spectrum are displayed in tiled view.

Peak Color in Spectrum Comparison Window

In the spectrum comparison windows in Compare View and Package View, matching peaks can be distinguished by color.

Matching m/z on query peak / *Peak*	Perfect Match	Match within Error Margin
Query Spectrum	**Red**	**Red**
Retrieved Spectrum	**Red**	**Pink**

<<Handy Feature 1: Spectrum Zoom>>



Example from Compare View

[1] Select location to zoom

Drag from the location of the start of the zoom.

[2] Set zoom position

Drop to set the location on the spectrum to zoom.

[3] End zoom

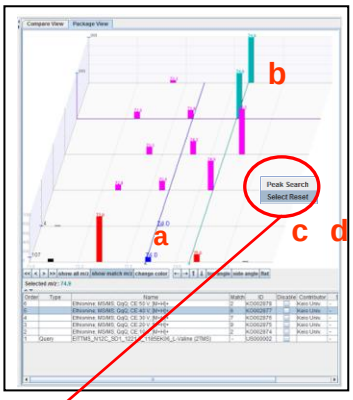
Double click on the spectrum.

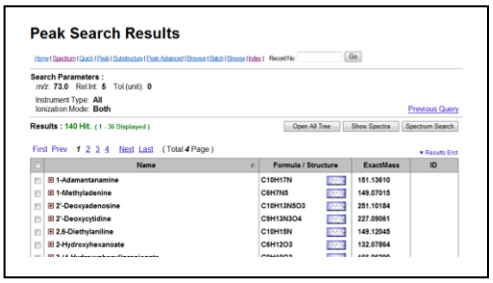
Note: You can also zoom on spectrums from the Package View.

<<Handy Feature 2: Peak Manipulation>>

Example from Compare View

Example from Package View





Peak Search Results Window

a. Highlight peak

Place the cursor over a peak. It is highlighted in blue, and the *m/z* and intensity values appear.

b. Select peak

Click on a peak. It is rendered in blue to indicate that it is selected. Up to 6 peaks can be selected.

c. Search for peaks

If you right click while one or more peaks are selected, a menu appears, and "Peak Search" can be selected. The peak search begins immediately after selection.

d. Cancel peak selection

When you right click on the spectrum, a menu appears. Chose **"Select Reset"**.

Peak Rendering Color

Highlighted: Blue

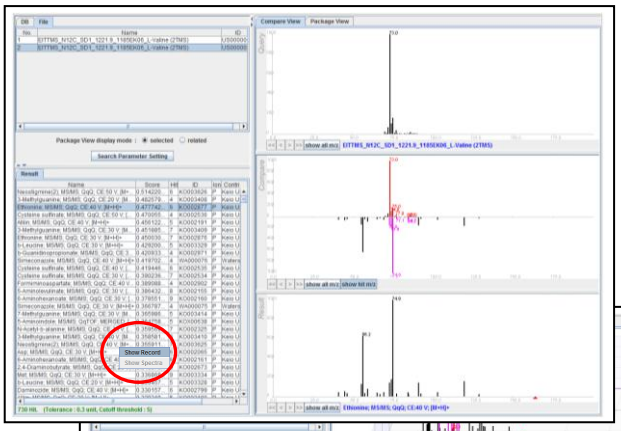
Selected: Light blue

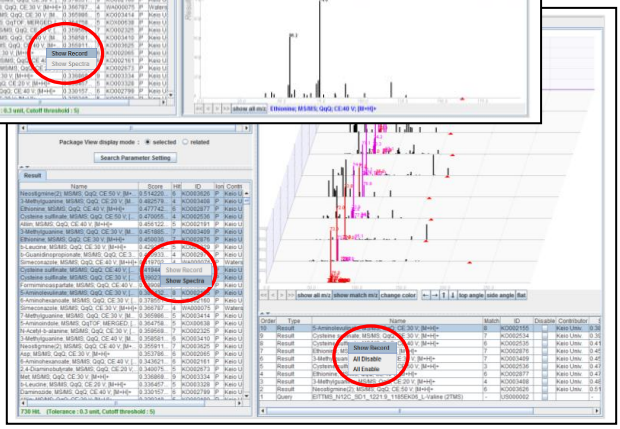
Highlighting and Selecting Peaks in Package View

When multiple spectrums are displayed in Package View, you can highlight or select the peaks in any spectrum. When you do so, if there is a peak in another spectrum with a perfectly matching *m/z*, then that peak is also highlighted or selected.

<<Handy Feature 3: Show Record>>

Example from Compare View

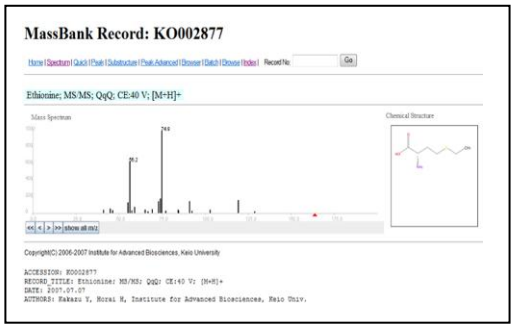




Example from Package View

a. Show record

When you right click with one spectrum selected, the "**Show Record**" command appears on the menu. Select this to view details about the spectrum.



b. Show multiple spectra

When you right click with two or more spectra selected, the "**Show Spectra**" command appears on the menu. Select this to display multiple spectra in tiled view.

<<Handy Feature 4: Spectrum Manipulation>>

Spectrum Manipulation Buttons in Compare View

<<、<、>、>>	Move display location (only when zoomed on spectrum)
show all *m/z*	Display *m/z* values of all peaks
show hit *m/z*	Display *m/z* values of matching peaks

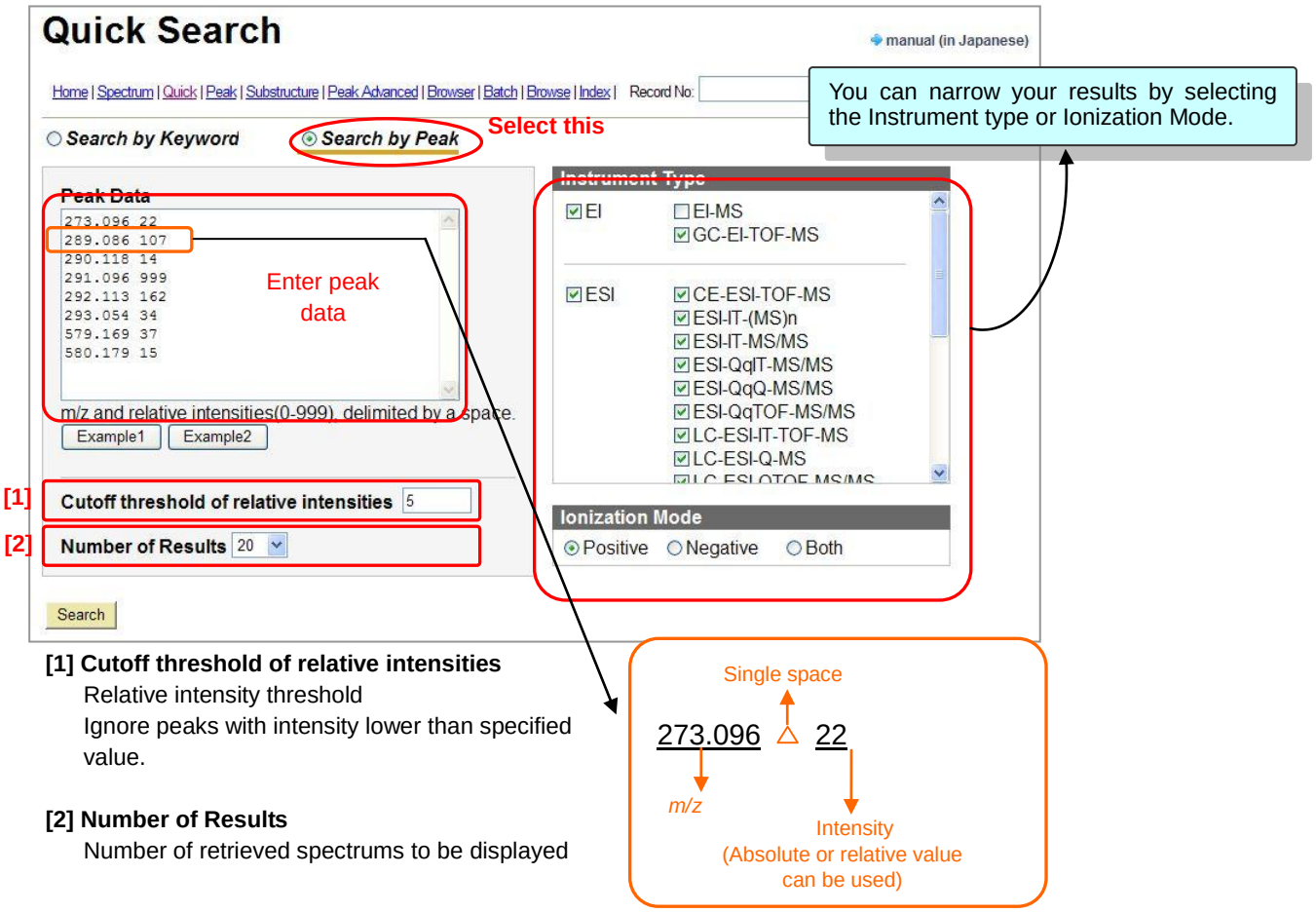
Spectrum Manipulation Buttons in Package View

<<、<、>、>>	Move display location (only when zoomed on spectrum)
show all *m/z*	Display *m/z* values of all peaks
show match *m/z*	Display *m/z* values of matching peaks
change color	Change color of entire spectrum
←、→、↑、↓	Change angle (manual manipulation)
top angle	Change angle (top perspective)
side angle	Change angle (side perspective)
flat	Change angle (all spectrums in flat view)

2.2 Spectrum Search on Quick Search Page

The Quick Search feature allows you to search for similar spectrums just using simple input.

>> Enter Search Parameters



[1] Cutoff threshold of relative intensities

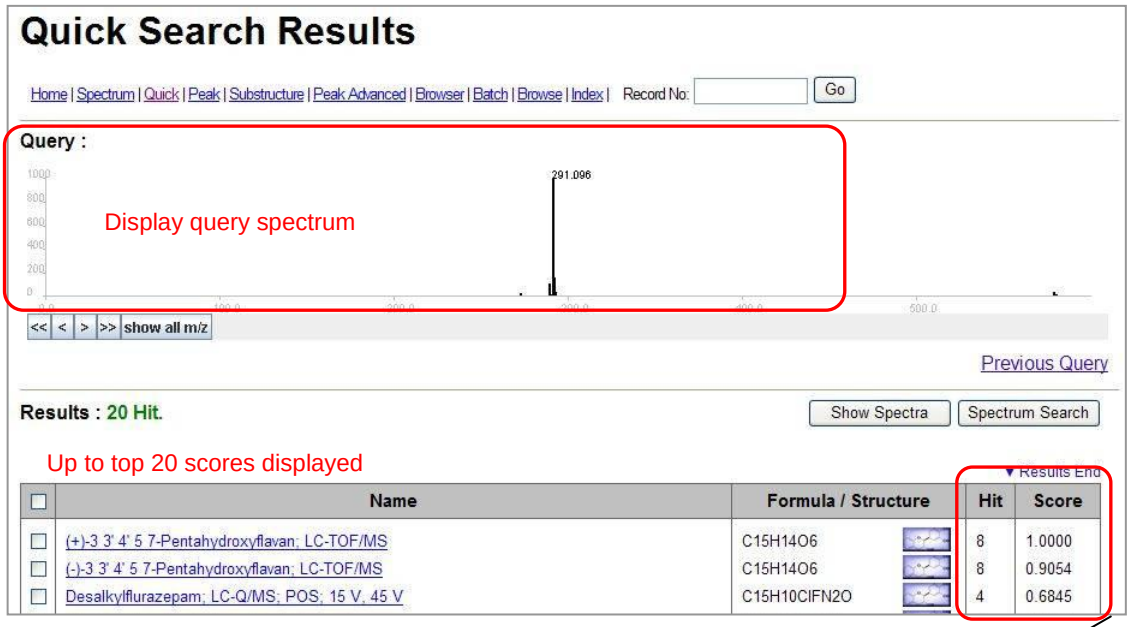
Relative intensity threshold

Ignore peaks with intensity lower than specified value.

[2] Number of Results

Number of retrieved spectrums to be displayed

>> Display Search Results



Hit: Number of peaks in the retrieved spectrums matched to those in a query data

Score: Similarity score of retrieved spectrum to a query data

2.3 Conduct Batch Spectrum Searches

Batch Service conducts a batch spectrum search, and emails you the results.

Use this in such cases as when you would like to search for a large number of spectra.

[1] Load the query file

Prepare a query file with the same format as the one described in "2.1 (1) Spectrum Search Applet". Click **Browse**, and select the query file you have prepared.

[2] Enter email address

Enter the email address to which you would like the search results sent.

Click **Submit** to perform the batch query. A message appears indicating that the query has been accepted.

Search results are sent to the specified address by e-mail. The following files are attached to the e-mail: (1) Search Results; and (2) Summary of Results.

(1) Search Results (Text Format)

```
***** MassBank Batch Service Results *****

Request Date: 2010/10/15 14:07:51 JST
# Instrument Type:
CE-ESI-TOF-MS,ESI-IT-(MS)n,ESI-IT-MS/MS,ESI-QTOF-MS/MS,ESI-QqIT-MS/MS,ESI-QqQ-MS/MS,ESI-QqTOF-MS/MS,LC-ESI-FT-MS,LC-ESI-IT-MS/MS,LC-ESI-IT-TOF-MS,LC-ESI-Q-MS,LC-ESI-QTOF-MS/MS,LC-ESI-QqQ-MS/MS,LC-ESI-TOF-MS
# Ion Mode: Negative


### Query 1 ###
# Name: Scan530        Name of a query spectrum
# Hit: 37

Top 20 List          LIst of search results (at most 20 high-scored spectra)
Accession   Title                                          Formula     Mass        Score     Hit

KNA00756    L-Aspartate; LC-ESI-FT-MS; NEG                 C4H7NO4     133.03751   0.3545    2
KNA00487    L-Aspartate; LC-ESI-FT-MS; NEG                 C4H7NO4     133.03751   0.3104    2
KNA00752    L-Serine; LC-ESI-FT-MS; NEG                    C3H7NO3     105.04259   0.3041    2
KNA00542    cis-Aconitate; LC-ESI-FT-MS; NEG               C6H6O6      174.01644   0.2955    2
KNA00475    L-Serine; LC-ESI-FT-MS; NEG                    C3H7NO3     105.04259   0.2942    2
KNA00628    L-Glutamine; LC-ESI-FT-MS; NEG                 C5H10N2O3   146.06914   0.2911    2
KNA00728    L-Homoserine; LC-ESI-FT-MS; NEG                C4H9NO3     119.05824   0.2904    2
KNA00812    Citrate; LC-ESI-FT-MS; NEG                     C6H8O7      192.027     0.2885    2
KNA00820    3-Phospho-D-glycerate; LC-ESI-FT-MS; NEG       C3H7O7P     185.99294   0.2844    2
KNA00554    L-Homoserine; LC-ESI-FT-MS; NEG                C4H9NO3     119.05824   0.2823    2
KNA00538    sn-Glycerol 3-phosphate; LC-ESI-FT-MS; NEG     C3H9O6P     172.01367   0.2756    2
KNA00527    (S)-Malate; LC-ESI-FT-MS; NEG                  C4H6O5      134.02152   0.2746    2
KNA00648    L-Threonine; LC-ESI-FT-MS; NEG                 C4H9NO3     119.05824   0.2716    2
KNA00736    L-Asparagine; LC-ESI-FT-MS; NEG                C4H8N2O3    132.05349   0.2713    2
KNA00808    cis-Aconitate; LC-ESI-FT-MS; NEG               C6H6O6      174.01644   0.2673    2
KNA00491    L-Glutamine; LC-ESI-FT-MS; NEG                 C5H10N2O3   146.06914   0.2663    2
KNA00483    L-Asparagine; LC-ESI-FT-MS; NEG                C4H8N2O3    132.05349   0.2650    2
KNA00696    4-Hydroxy-L-proline; LC-ESI-FT-MS; NEG         C5H9NO3     131.05824   0.2646    2
KNA00546    D-Gluconic acid; LC-ESI-FT-MS; NEG             C6H12O7     196.0583    0.2616    2
KNA00530    2-Oxoglutarate; LC-ESI-FT-MS; NEG              C5H6O5      146.02152   0.2587    2
```

(2) Summary of Results (HTMLformat)

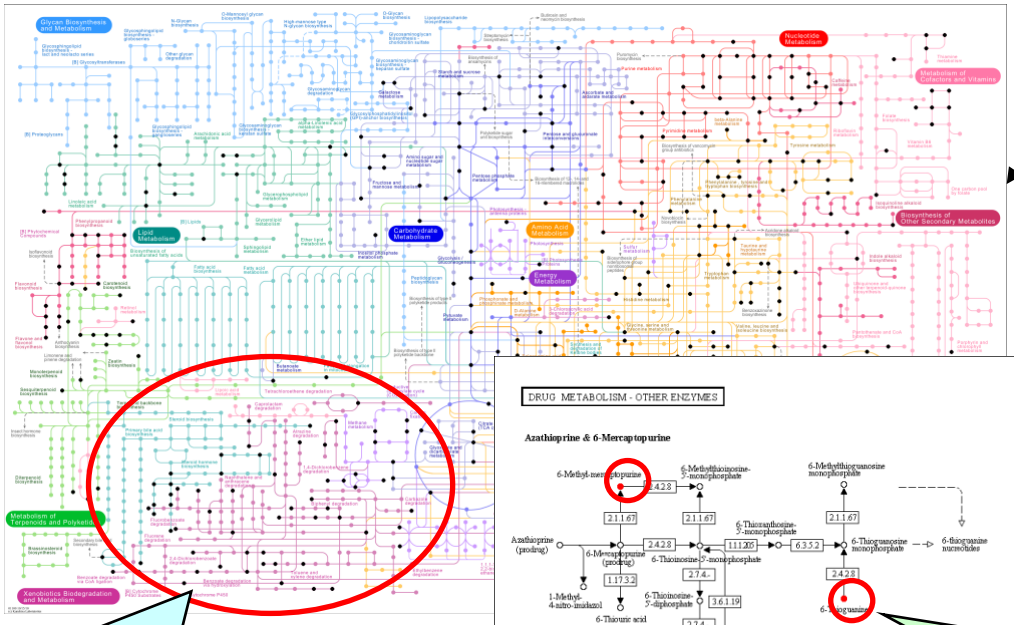
Summary Of Batch Service Results

Request Date : 2010/10/15 14:07:51 JST

Instrument Type : CE-ESI-TOF-MS,ESI-IT-(MS)n,ESI-IT-MS/MS,ESI-QTOF-MS/MS,ESI-QqIT-MS/MS,ESI-QqQ-MS/MS,ESI-QqTOF-MS/MS,LC-ESI-FT-MS,LC-ESI-IT-MS/MS,LC-ESI-IT-TOF-MS,LC-ESI-Q-MS,LC-ESI-QTOF-MS/MS,LC-ESI-QqQ-MS/MS,LC-ESI-TOF-MS
Ion Mode : Negative

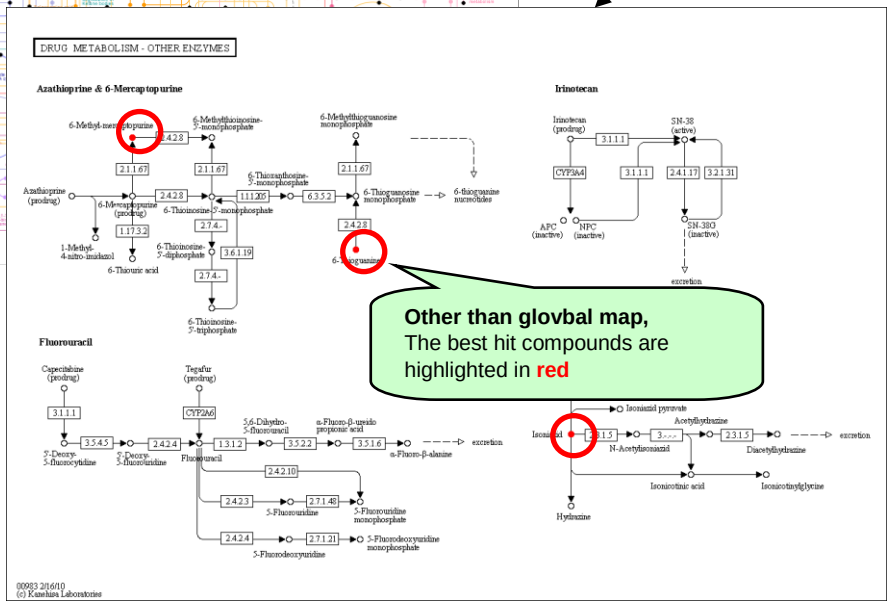
List of the best hit for each query

No.	Query Name	Score	MassBank ID	Record Title	Formula	KEGG ID	Colored Pathway Maps			
							MAP1	MAP2	MAP3	MAP4
1	Scan530	0.3545	KNA00756	L-Aspartate; LC-ESI-FT-MS; NEG	C4H7NO4	C00049	map:01100(28)	-	-	-
2	Scan531	0.9999	KO001625	Propiolate; MS/MS; QqQ; CE:10 V; [M-H]-	C3H2O2	C00804	-	-	map:00640(1)	-
3	Scan532	0.2593	KNA00475	L-Serine; LC-ESI-FT-MS; NEG	C3H7NO3	C00065	map:01100(28)	-	-	-
4	Scan534	0.3524	KNA00756	L-Aspartate; LC-ESI-FT-MS; NEG	C4H7NO4	C00049	map:01100(28)	-	-	-
5	Scan535	0.9999	KO001625	Propiolate; MS/MS; QqQ; CE:10 V; [M-H]-	C3H2O2	C00804	-	-	map:00640(1)	-
6	Scan538	No Hit Record								
7	Scan539	0.0751	UT002896	Phosphatidylethanolamine alkenyl 18:0-24:5; LC-MS/MS; Orbitrap; m/z: 804.59; [M-H]-; RT: 37.85; Exp: 3	C47H84NO7P	-	-	-	-	-
8	Scan540	0.4198	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
9	Scan542	No Hit Record								
10	Scan543	0.1230	UT002896	Phosphatidylethanolamine alkenyl 18:0-24:5; LC-MS/MS; Orbitrap; m/z: 804.59; [M-H]-; RT: 37.85; Exp: 3	C47H84NO7P	-	-	-	-	-
11	Scan544	0.4294	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
12	Scan546	0.0876	UT002244	Phosphatidylserine 18:1-22:0 / 20:0-20:1; LC-MS/MS; Orbitrap; m/z: 844.61; [M-H]-; RT: 51.49; Exp: 2	C47H80NO9P	-	-	-	-	-
13	Scan547	0.5831	PR050557	Acetylsalicylic acid; ESI-QTOF-MS/MS; MERGED; [M-H]-	C9H8O4	-	-	-	-	-
14	Scan548	0.1290	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
15	Scan550	0.3157	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
16	Scan551	0.4386	WA000541	Pentobarbital sodium salt; LC-Q/MS; NEG; 30 V	C11H17N2NaO3	-	-	-	-	-
17	Scan552	0.1185	TY000116	Baicalin; LC-ESI-IT-MS/MS; [M-H]-	C21H18O11	-	-	-	-	-
18	Scan554	0.2940	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
19	Scan555	0.1109	UT002896	Phosphatidylethanolamine alkenyl 18:0-24:5; LC-MS/MS; Orbitrap; m/z: 804.59; [M-H]-; RT: 37.85; Exp: 3	C47H84NO7P	-	-	-	-	-
20	Scan556	0.5934	PR050557	Acetylsalicylic acid; ESI-QTOF-MS/MS; MERGED; [M-H]-	C9H8O4	-	-	-	-	-
21	Scan558	No Hit Record								
22	Scan559	0.1074	UT002896	Phosphatidylethanolamine alkenyl 18:0-24:5; LC-MS/MS; Orbitrap; m/z: 804.59; [M-H]-; RT: 37.85; Exp: 3	C47H84NO7P	-	-	-	-	-
23	Scan560	0.1286	PB004142	Kaempferol; MS/MS; QqQ; CE:35 eV; [M-H]-	C15H10O6	C05903	map:01100(28)	-	-	-
24	Scan562	No Hit Record								
25	Scan563	0.2191	UT002452	Phosphatidylserine 18:0-20:1 / 18:1-20:0; LC-(MS)3; Orbitrap; m/z: 816.57/729.15; [M-H]-/[M-Ser]-; RT: 43.92; Exp: 2	C45H86NO9P	-	-	-	-	-
26	Scan564	0.4069	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-
27	Scan566	No Hit Record								
28	Scan567	0.0201	MT000095	taurochenodeoxycholate; MS/MS; IT; m/z: 498.3; [M-H]-	C26H45NO6S	C05465	-	-	-	map:00121(1)
29	Scan568	0.4064	TY000102	Cinobufotalin; LC-ESI-IT-TOF-MS; [(M+CH3COOH)-H]-	C26H34O7	-	-	-	-	-



Click a link, then the pathway map is displayed. In the pathway map, the best hit compounds of all queries are highlighted

In **global map** (map:01100), the best hit compounds are highlighted in **black**.



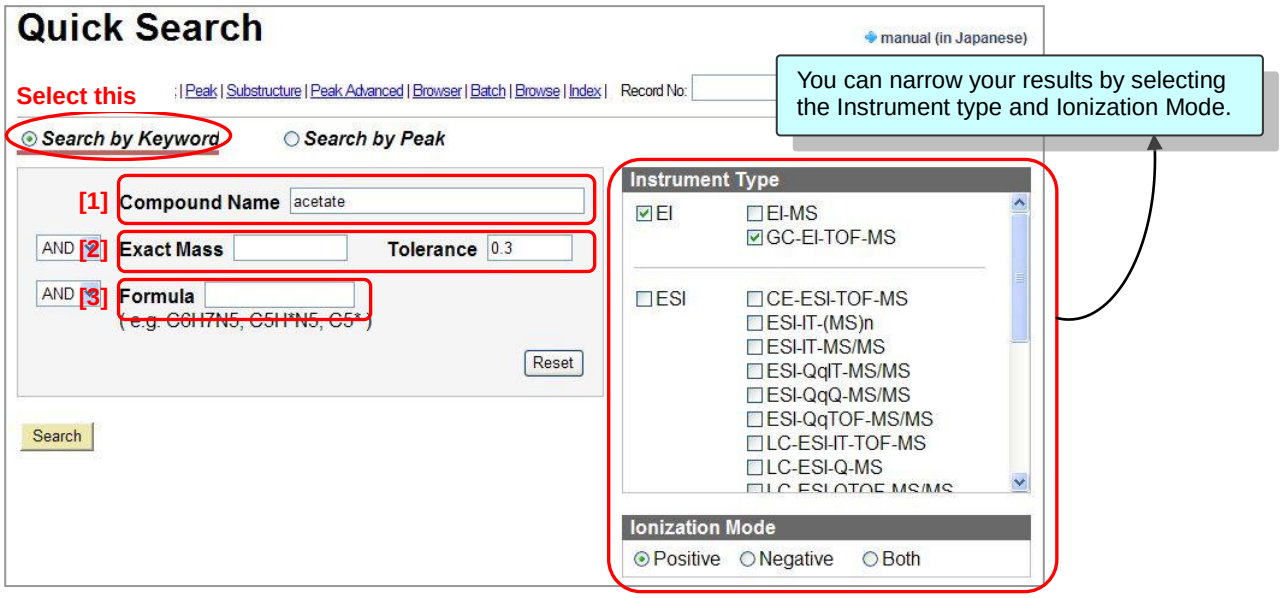
Other than glovbal map, The best hit compounds are highlighted in **red**

3. Compound Search

3.1 Find Compounds on Quick Search Page

The Quick Search feature allows you to search for compounds by compound name, molecular formula, etc.

>> Enter Search Parameters



[1] Compound Name

Enter the compound name. Names with substring matches on the string you entered are retrieved.

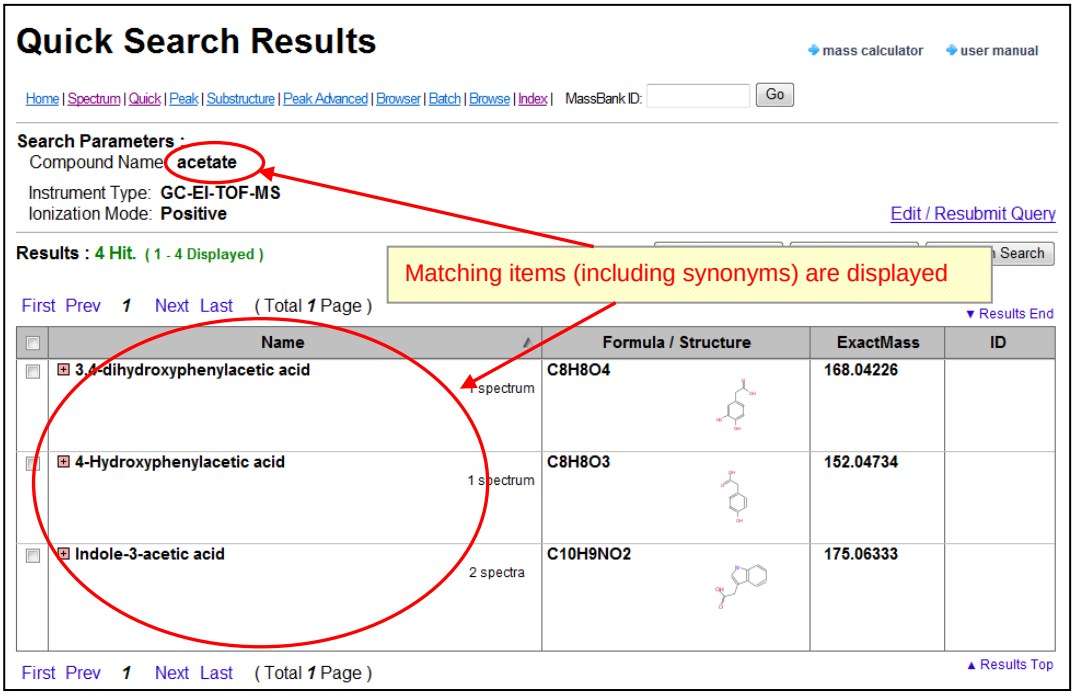
[2] Exact Mass/Tolerance

Enter the exact mass and error tolerance.

[3] Formula

Enter the molecular formula of the compound. Enter the formula starting with "C", followed by "H", and then the other letters in alphabetical order. Add wildcards ("*") to find partial matches.
Example: C5H*N5

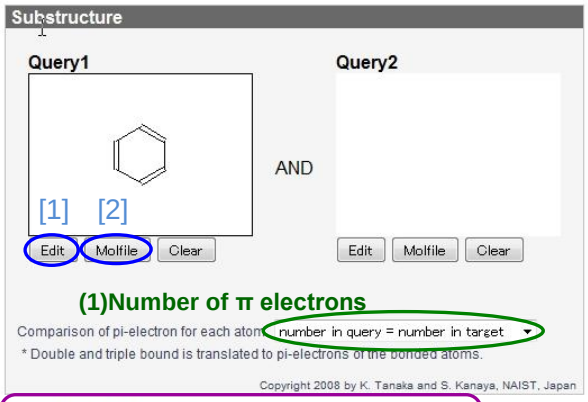
>> Display Search Results

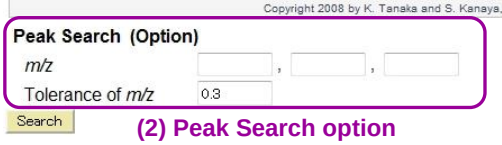


3.2 Find Compounds by Chemical Structure

The Substructure Search enables you to find compounds including the specified chemical structure.

>> Enter Search Parameters





(1) Number of π electrons

- Query structure = target structure (default)
- Query structure ≤ target structure
- No comparison

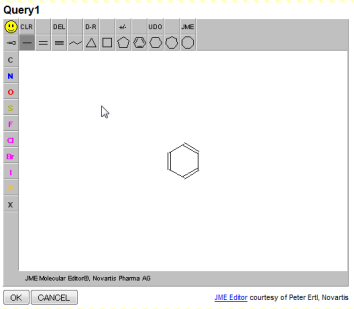
(2) Peak Search option

Specify *m/z* values to perform peak search at same time.

You can also narrow your results by selecting the Instrument type or Ionization Mode.

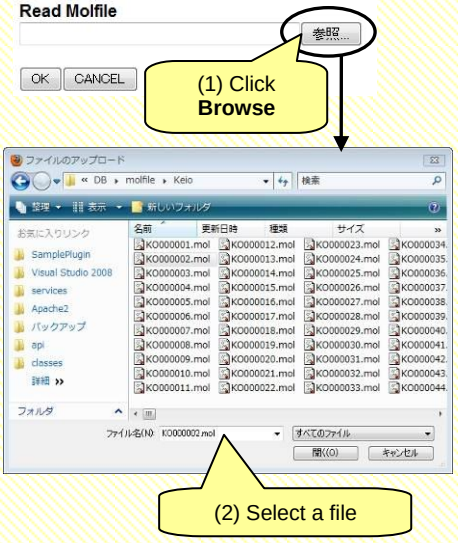
Enter query structure

[1] Write in editor



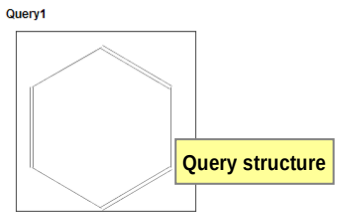
Or:

[2] Load Molfile



The loaded structure formula can be modified in the editor as well.

>> Display Search Results



Edit / Resubmit Query

Results : 48 Hit. (1 - 42 Displayed)

Open All Tree | Multiple Display | Spectrum Search

First Prev 1 2 Next Last (Total 2 Page)

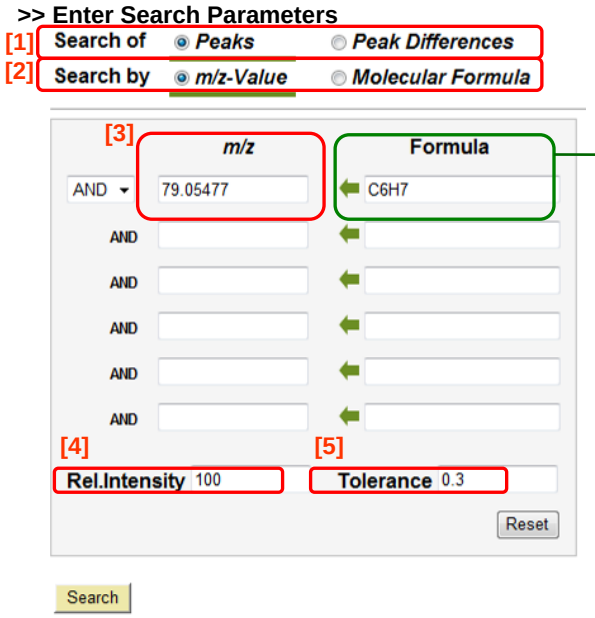
▼ Results End

	Name	Formula / Structure	ExactMass	ID
☐	⊞ (R)-(-)-Phenylephrine 1 spectrum	C9H13NO2	167.09463	
☐	⊞ 1,10-Phenanthroline 2 spectra	C12H8N2	180.06875	

4. Peak Search

4.1 Peak Search by *m/z* values

Peak Search enables you to find peaks by specifying the *m/z* or *m/z* difference as numerical values.



[1] Search of "Peaks" or "Peak Differences"
- "Peaks": search of the *m/z* value.
- "Peak Differences": search of the *m/z* differences.

[2] Search by "*m/z*-Value" or "Molecular Formula"
Select ""m/z-Value" for Peak Search by *m/z* values.

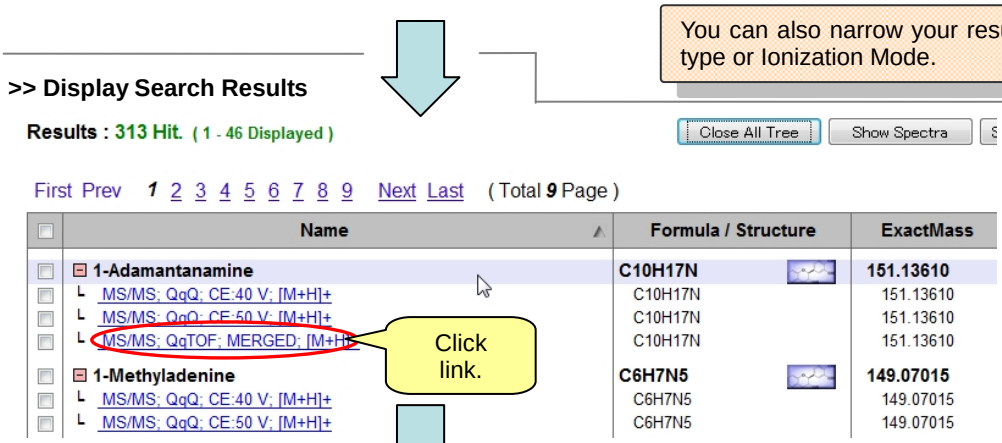
[3] *m/z*
Specify the peak *m/z* value. You can specify up to 6, and connect them via AND or OR conditions.

[4] Rel. Intensity
Ignore peaks with intensity lower than specified value. Specify a relative intensity from 1 to 999.

[5] Tolerance
Specify the *m/z* error range.

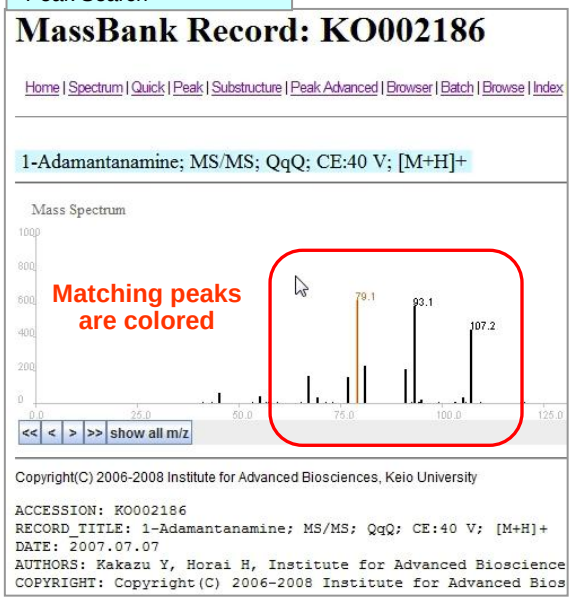
Converting from molecular formula to *m/z*
Enter a molecular formula the exact calculated mass in the *m/z* text box. The exact calculated mass is truncated to 5 decimal place.

You can also narrow your results by selecting the Instrument type or Ionization Mode.

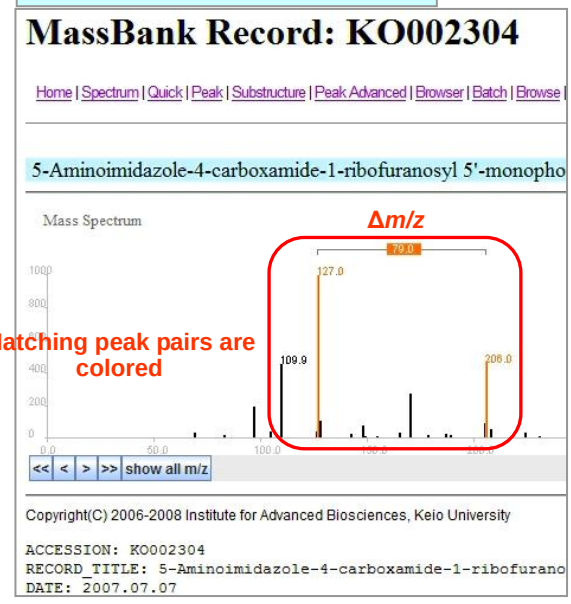


>> Spectrum View

Peak Search



Peak Difference Search

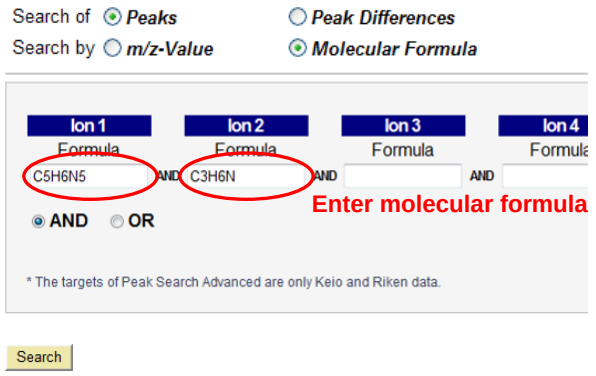


4.2 Peak Search with Molecular Formulas

Peak Search Advanced enables you to find peaks by specifying an ion or neutral loss by molecular formulas instead of a numerical *m/z* values.

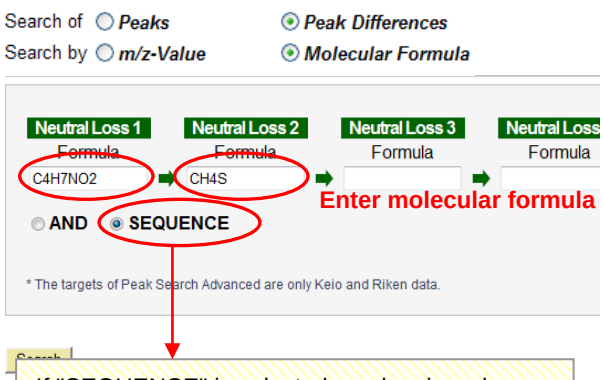
>> Enter Search Parameters

Searching via ion



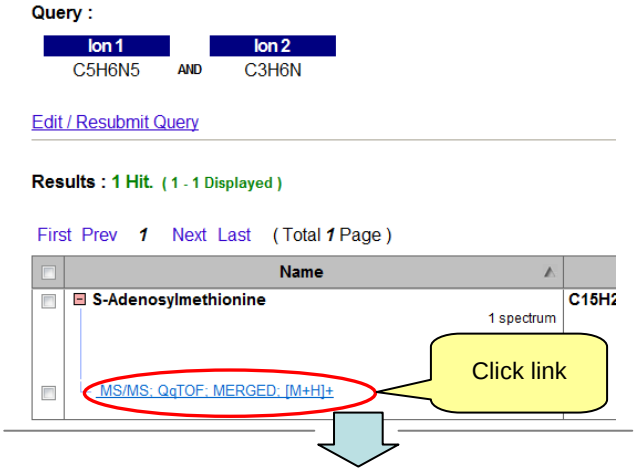
If CH3COOH is entered, it is converted internally to C2H4O2 for the search.

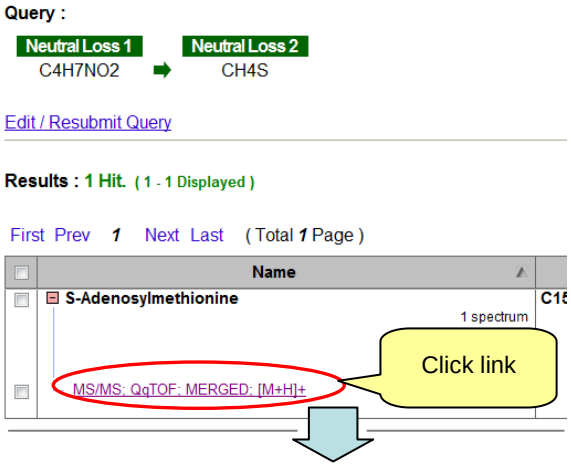
Searching via neutral loss



If "SEQUENCE" is selected, peak pairs, whose m/z difference is matched to the molecular formula, are retrieved in the order of Neutral Loss 1, 2, and 3. (In the example above, order of CH4H7NO2, then CH4S)

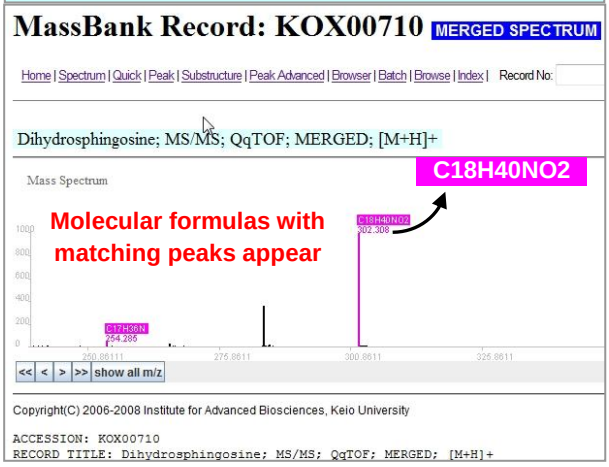
>> Display Search Results



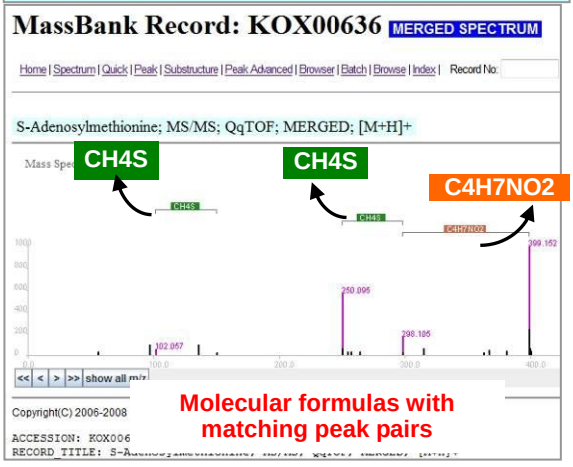


>> Spectrum View

Searching by Ion



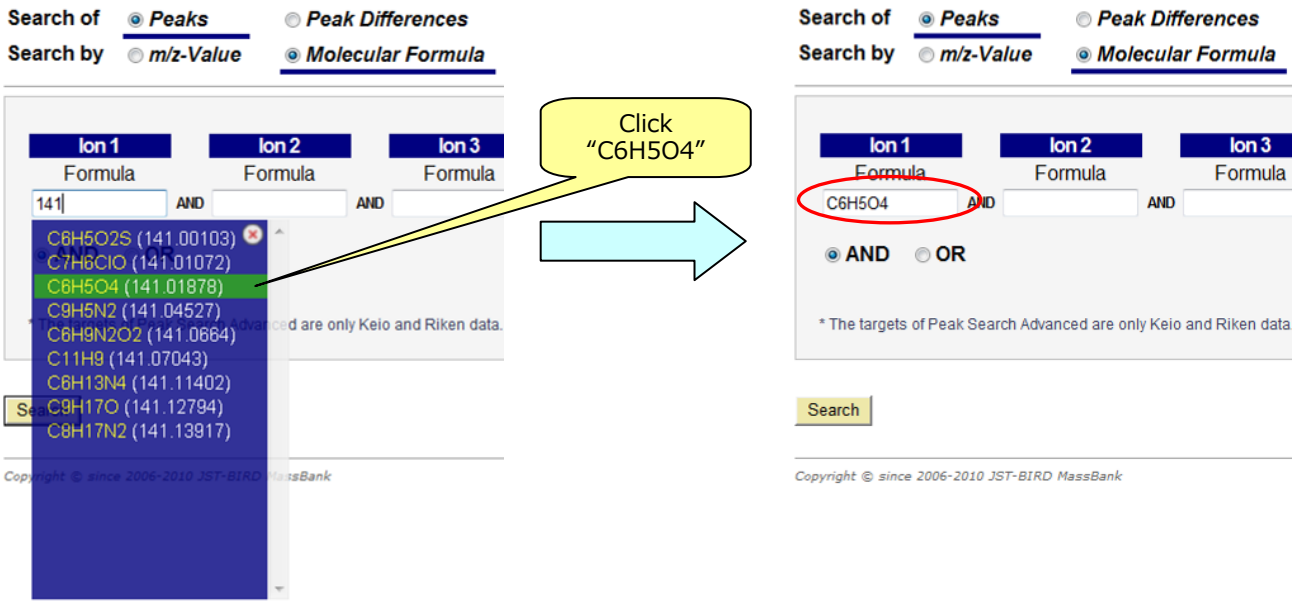
Searching by Neutral Loss



* Assist in the Input of Molecular Formulas

When *m/z* values instead of molecular formulas are entered in the "Formula" boxes, a list of candidate molecular formulas, whose exact masses are close to the entered *m/z* values, is shown in the pull-down menu.

Following example shows a case when a value, 141, was entered in a Formula box on the "Search of Peaks" and "Search by Molecular Formula" mode.



Pull-down menu shows a list of the molecular formulas, whose exact masses is starting from the value 141. No molecular formula such as 140.9 are listed because prefix string match algorithm is applied.

5. Prediction of Metabolites from ESI-MS^2 Data

5.1 Metabolite prediction by using peak-chemical substructure relationships

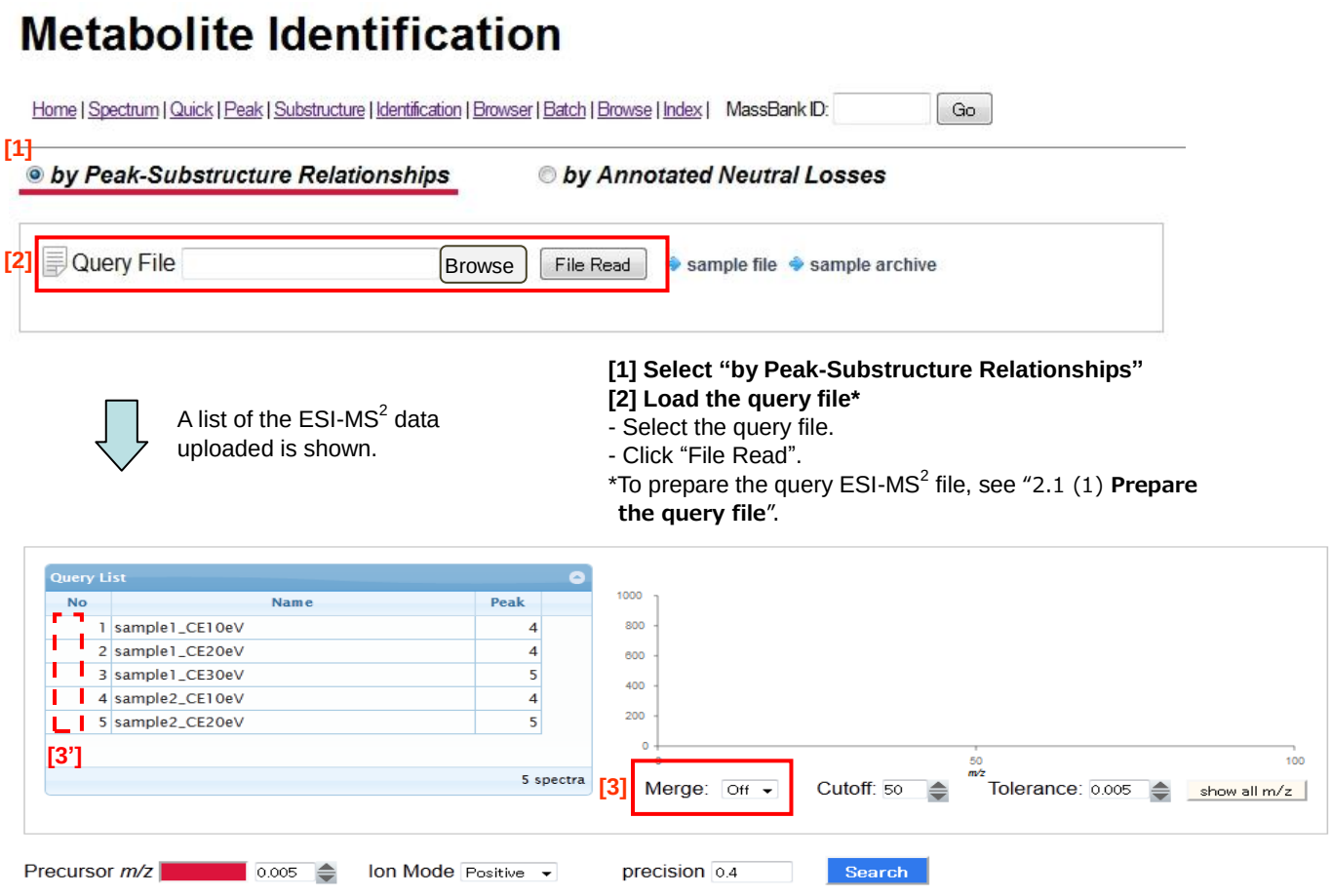
Currently this tool is adapted to predict primary metabolites and their derivatives from the query ESI-MS^2 data. MassBank has analyzed and accumulated the relationships between peaks (product ions) and chemical substructures by chemically annotating ESI-MS^2 data of primary metabolites.

The present tool elucidates possible chemical substructures of the unknown metabolite from the query ESI-MS^2 data by using the relationships that linked the observed product ions to chemical substructures. Additionally possible molecular formulae of the unknown metabolite are predicted from the precursor ion. KNApSAcK database < http://kanaya.naist.jp/knapsack_jsp/top.html > provides a list of metabolites that satisfy the predicted molecular formula with the possible chemical substructures. Finally the prediction tool outputs the list as the candidates of the unknown metabolite.

A reliable prediction is expected when (1) the query ESI-MS^2 data should be analyzed on high resolution mass analyzers, (2) the precursor ion observed is a type of "M ± H", (3) more than ten product ions with the relative intensity higher than 5 % are observed, (4) a query data that was prepared by merging two or more ESI-MS^2 data analyzed on the same unknown metabolite at different CID conditions gives a good result. The present tool automatically merges them into a single query data.

>> Input the query data and set the parameters

The following procedure shows a case when three ESI-MS^2 data analyzed on the same unknown metabolite at different CID conditions are uploaded, merged into a single query data, and predicted.

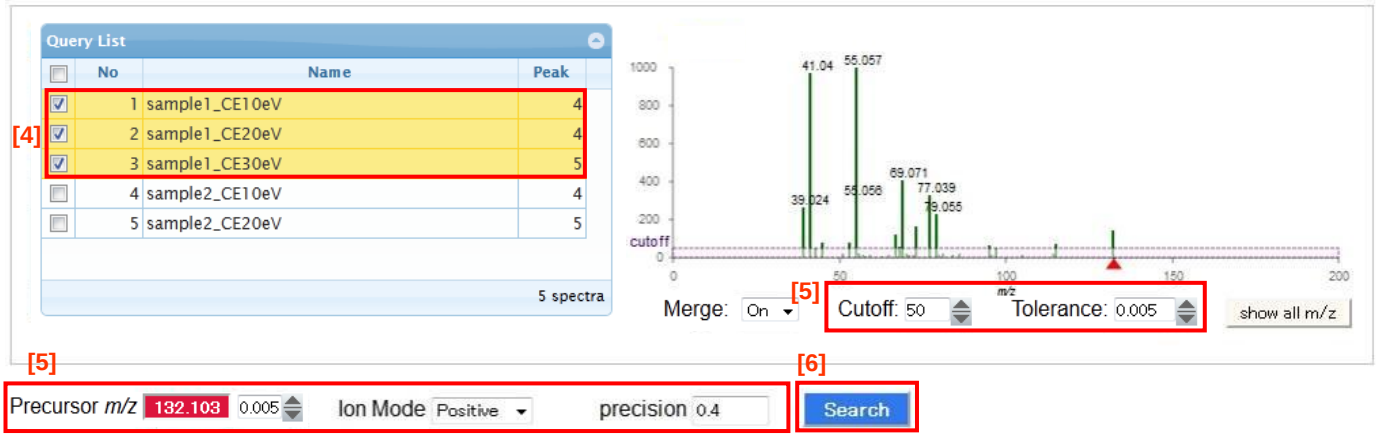


A list of the ESI-MS^2 data uploaded is shown.

[1] Select "by Peak-Substructure Relationships"
[2] Load the query file*
- Select the query file.
- Click "File Read".

*To prepare the query ESI-MS^2 file, see "2.1 (1) **Prepare the query file**".

[3] Set "Merge On"
- A check box [3'] is appeared at each data.



[4] By checking the box, select the data files of the metabolite to be identified.
- As one data is selected by checking the box, it is added and merged into the query.
- With each selection, the merged ESI-MS^2 data generated is shown in the right window

[5] Set parameters (see the table below)
- All the parameters should be set correctly.
- Input "Precursor *m/z*".

[6] Click "Search" button to start.

– Paremeters settings–

・**Merge**:
Select "ON" when two or more ESI-MS2 data are merged into the query. Check box is appeared at each data. Select the data by checking their boxes.
Leave "OFF" (default) to predict the metabolite from a single data.

・**Cutoff**:
This selects the product ions for the prediction.
Only the peaks larger than the cutoff value are considered in the prediction.
Cutoff value is indicated by the red dotted line on the merged spectra in the write window.
Precursor ion is always included in the prediction independent of the cutoff value.

・**Tolerance**:
This defines the mass accuracy of the product ions in the query data. Within the tolerance, substructure-peak relationships give possible substructures to each product ion.

・**Precursor *m/z* in the red box**:
Check whether the precursor *m/z* is correct.
When the *m/z* value is not correct, correct it manually.

・**Precursor *m/z* in the white box**:
This defines the mass accuracy of the precursor *m/z*.

・**Ion Mode**:
Select either "Positive" or "Negative".

・**Precision**:
This is the precision of an empirical relationship between a peak and a chemical substructure that is used for prediction. False positive decreases with the precision. See the next page for details.

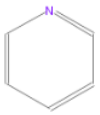
>> Search Results

Matched Formulae : 4

m/z	78.0355	80.0508	96.0458	123.0560
Formula	C5H4N	C5H6N	C5H6NO	C6H7N2O
No.	ion-pos-0013	ion-pos-0013	ion-pos-0013 ion-pos-0028	ion-pos-0013

Hit Relationships : 2

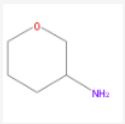
ion-pos-0013
Substructure



Formula, Precision & Recall, TP

C5H4N	0.66	0.68	27
C6H7N2O	0.67	0.1	4
C5H6N C5H4N	0.71	0.38	15

ion-pos-0028
Substructure



Formula, Precision & Recall, TP

C5H6NO	0.55	0.55	11

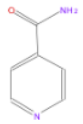
A

- ***m/z***: Product ions used for prediction
- **Formula**: Possible molecular formulae assigned to the ions
- **No**: ID numbers of the substructures predicted by the relationships between peaks and chemical substructures

* By placing the cursor on an ID number, the corresponding substructure is shown as "**A**".

Results : 3 Hit.

Isonicotineamide

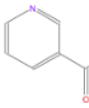


Formula : **C6H6N2O**
Exact Mass : **122.048**

DB Links
KEGG : C02421

Hit Relationship-No.
ion-pos-0013

Nicotinamide

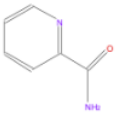


Formula : **C6H6N2O**
Exact Mass : **122.04801**

DB Links
KNApSAck : C00000209
KEGG : C00153

Hit Relationship-No.
ion-pos-0013

Picolinamide



Formula : **C6H6N2O**
Exact Mass : **122.048**

DB Links
KEGG : C01950

Hit Relationship-No.
ion-pos-0013

B

A: Possible substructures that are embedded in the structure of the unknown metabolite are predicted by using the relationships between peaks and chemical substructures.

B: Candidates of the unknown metabolite are shown. The KNApSAcK database outputs the candidates that satisfy the following conditions; they have one or more of the substructures shown in **A** and the molecular formula is the same to that predicted from the mass of the precursor ion. A substructure in the gray box in **A** is not applicable in the candidates in **B**.

Formula, Precision & Recall, TP

C5H4N	0.66	0.68	27

C5H4 precision 0.66 ⇄ recall 0.68

Formula, Precision & Recall, TP: ("C5H4N", "0.66", "0.68", "27" in the above example)

Formula: Chemical formula of a peak

Precision: Ratio of the number of ESI-MS^2 data (pyrimidine substructure & C5H4N) to that of ESI-MS^2 data (C5H4N), where among the ESI-MS^2 data (C5H4N), in which the peak C5H4N was observed, ESI-MS^2 data (pyrimidine substructure & C5H4N) are those analyzed on chemical compounds with the pyrimidine substructure.
In the above example, the ratio is 0.66.

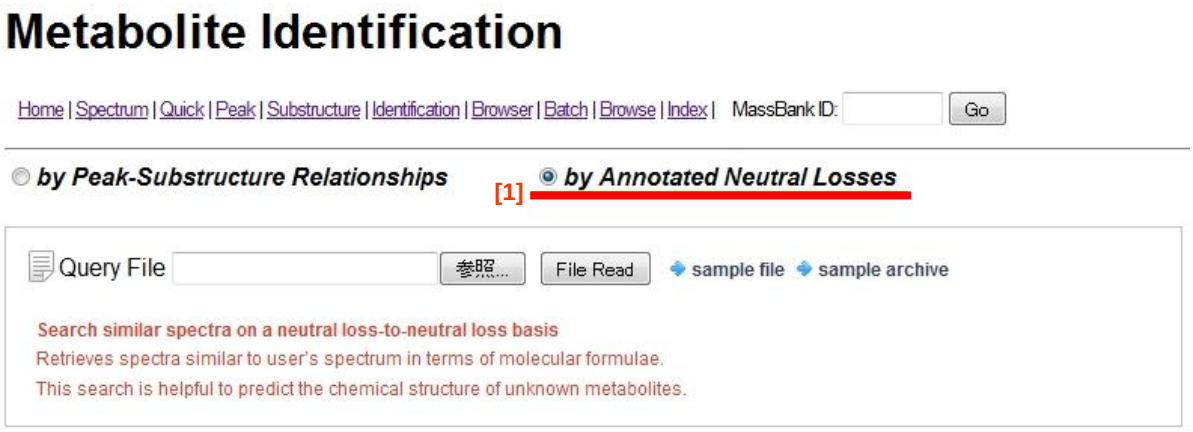
Recall: Ratio of the number of ESI-MS^2 data (pyrimidine substructure & C5H4N) to that of ESI-MS^2 data (pyrimidine substructure), where the ESI-MS^2 data (pyrimidine substructure) were analyzed on chemical compounds with the pyrimidine substructure. In the above example, the ratio is 0.68.

TP: Total number of the ESI-MS^2 data (pyrimidine substructure & C5H4N). In the above example, it is 27.

5.2 Metabolite prediction by spectrum search in terms of neutral loss

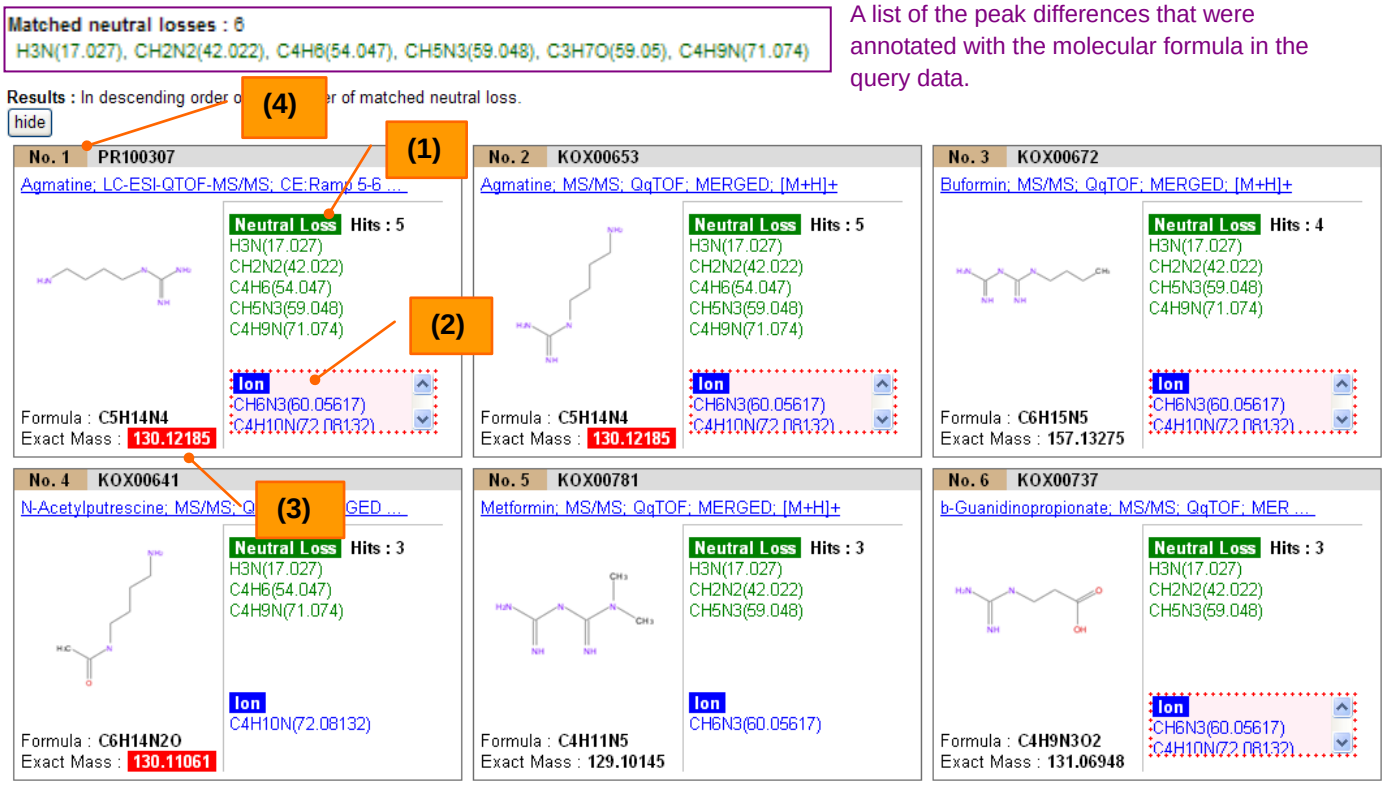
Chemical compounds are often synthesized from a common chemical compound by slight chemical modifications. Such derivatives share the chemical structure of the common chemical compound. Their mass spectra are also similar, although their similarity cannot be detected by conventional spectral similarity search. Peaks observed in the mass spectrum of one derivative are shifted by □ u from the corresponding peaks of the other one where □ is the mass difference between their chemical structures. Such a similarity is searchable by comparing not the *m/z* of the corresponding peaks but the mass difference between the corresponding peak pairs because □ is unknown. Thus this tool searches the ESI-MS^2 data in MassBank that are similar to the query one by comparing the *m/z* difference between the corresponding peak pairs. Here "**neutral loss**" is defined as the *m/z* difference between peak pairs. Search results suggest the users the common chemical compound among the target and query chemical compounds. However, this comparison has such a general difficulty that many possible peak pairs should be compared within the mass accuracy of *m/z*. To escape the difficulty, the targets in the present method are limited to the high resolution MassBank ESI-MS2 data with chemical annotation on peaks. All possible peak differences in each target data have been calculated not by *m/z* but by the assigned molecular formulae. MassBank recommends the query data that is enough high in the resolution to assign the molecular formula to peaks. All possible peak differences in the target are also calculated by the molecular formulae. Peak differences between the target and the query are compared by the molecular formulae. Additionally the present tool evaluates the matching peaks observed in the range of *m/z* 50-99.

>> Input the query data and set the parameters



[1] Select "by Annotated Neutral Losses".
[2] - **[6]** the same in the Section 5.1 "Metabolite prediction based on peak-chemical substructure relationships".

>> Search Results



* MassBank data similar to the query one are shown (max. 30 data).

(1) Neutral Loss: Here "**Neutral Loss**" is defined as the difference of the corresponding peak pairs in terms of molecular formulae. The target data are listed by a total number (**Hits**) of the molecular formulae that were matched between the query and target ESI-MS^2 data.

(2) Ion: The matching peaks ($50 < m/z < 99$) are listed by molecular formulae.

A. They are shown in a dotted red box when the target data have the maximum number of matching peaks.

B. The target data that have no matching peak are shown in a grey box. They are not shown when the "**hide**" button was clicked.

C. "Results: **NO peaks within *m/z* 50-99 in query."** is shown when no peak ($50 < m/z < 99$) was observed in the query data.

D. "Results: **NO matched ions to peaks within *m/z* 50-99 in query."** is shown when no peak ($50 < m/z < 99$) has no annotated molecular formulae.

(3) Exact Mass: Molecular mass of the target data is shown in white characters in the red box or in the grey box when it is equal to "Precursor *m/z* +1" or "Precursor *m/z* -1" in the positive or negative mode, respectively.

(4) Rank: The target data are listed by the rank which is scored by a total number of matching peak pairs with matching peaks.

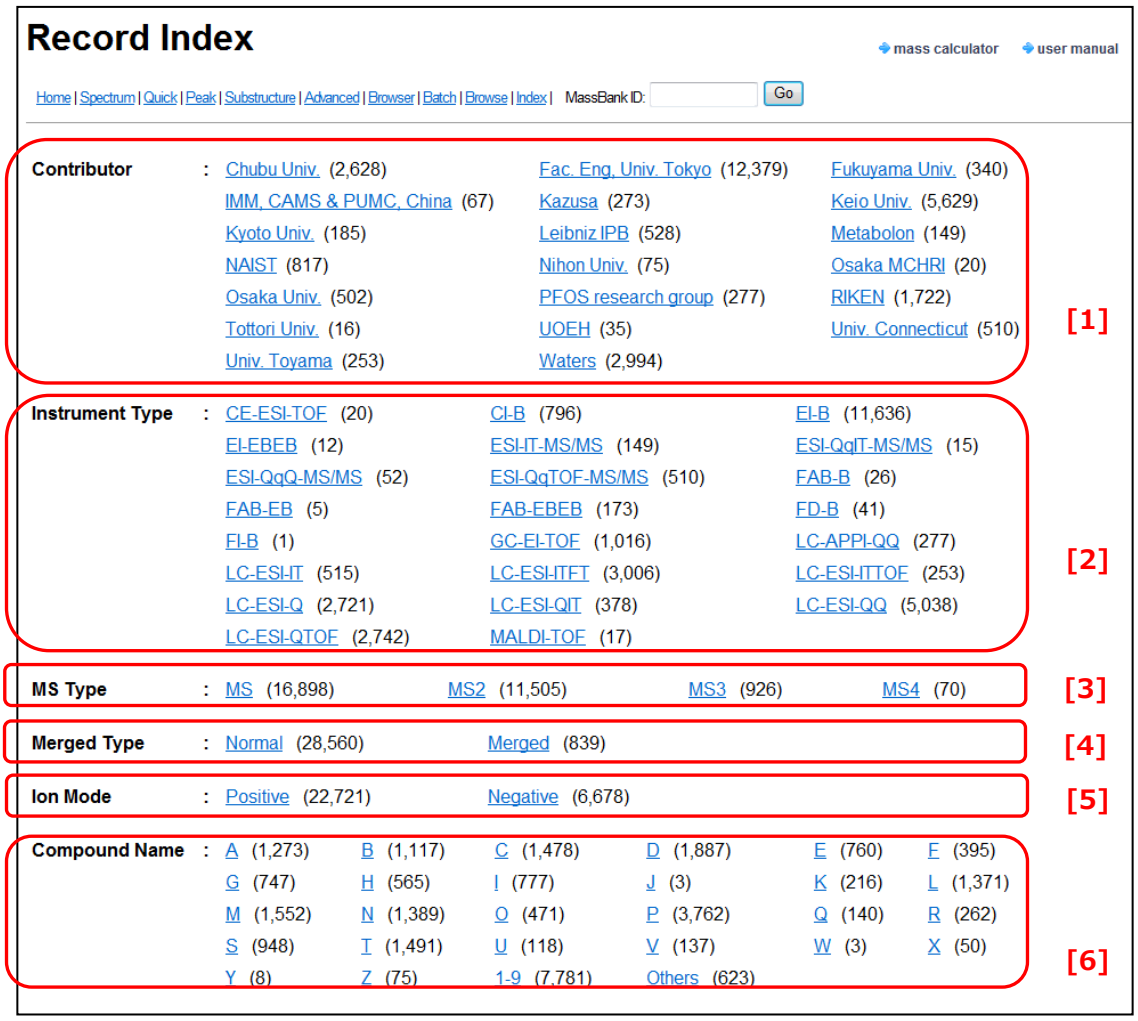
Minimum requirements that target data are matched or similar to the query are as follows.

- Target data are ranked at high.
- They have one or more matching peaks ($50 < m/z < 99$).
- Their molecular mass is equal to that of the query.

6. Browsing All Data

6.1 Categorical Index

In Record Index, all data is categorized into contributor, instrument type, MS type, merged type, ion mode and compound name categories. Click a link in this page, then the whole data in the category is displayed in the same format as other search results.



[1] Contributor
Number of data listed by contributors.

Each link is followed by the parenthesized number of spectra in the category.

[2] Instrument Type
Number of data listed by the types of chromatography and mass spectrometer.

[3] MS Type
Number of data listed by the types of mass spectrometry.

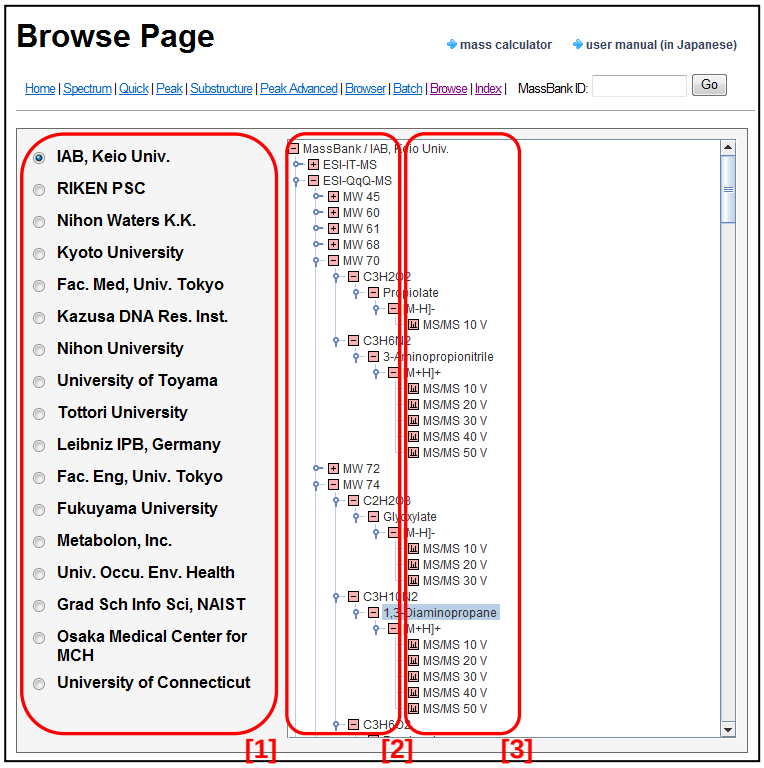
[4] Merged Type
Number of merged ESI-MS2 data and that of the other data.

[5] Ionization Mode
Number of data analyzed by positive and negative modes.

[6] Compound Name
Number of data listed by the first letters of compound names.

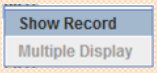
6.2 Hierarchical Browse

In Browse Page, all data are hierarchically displayed for each data provider. You can go up and down the hierarchy (tree) and find a specific data you want.



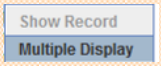
After selecting a spectrum by clicking, you can select multiple spectra by clicking with Ctrl key or Shift key.
After that, a right button click shows the following popup menu.

[case of single selection]



Click "Show Record", then the details of the MassBank Record are diplayed.

[case of multiple selection]



Click "Multiple Display", then multiple spectra are displayed in tiled view.

[1] Select Provider

Click a radio button, then display the hierarchy of the selected provider is displayed in right hand side.

[2] Browse Tree

[+] : open the lower layer.
[−] : close the lower layer.

[3] Display Spectrum

View detailed MassBank Record: Double click of ; or "Show Record" in popup menu by right button cllick when a spectrum is selected.

Display multiple spectra in a tiled view: "Mutiple Display" in popup menu by right button click when multiple spectra are selected.

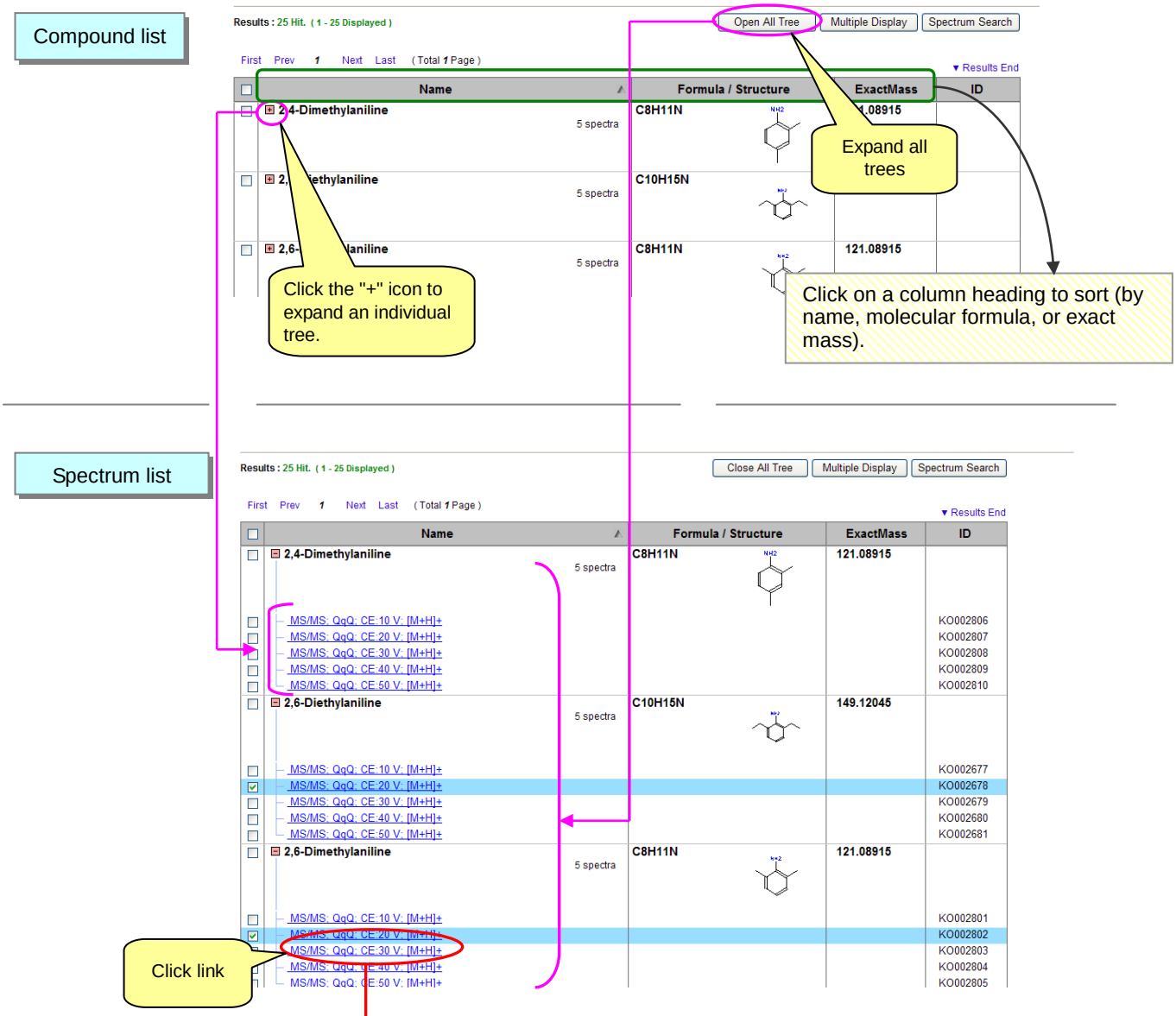
7. Others

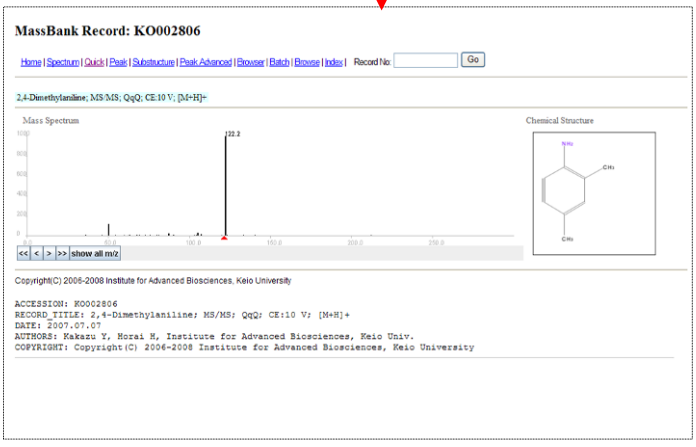
7.1 Search Results

When you conduct a search via "Quick Search", "Substructure Search", "Peak Search", "Peak Search Advanced", or "Record Index" , the search results are displayed in a common format.

<<Operation 1>>

List of Search Results

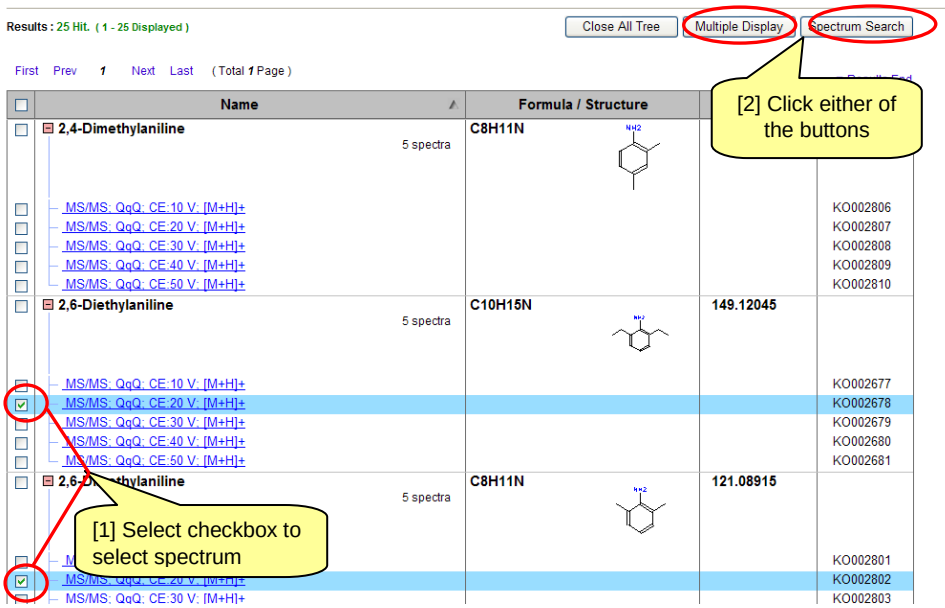




MassBank record of an individual spectral data is displayed.

<<Operation 2>>

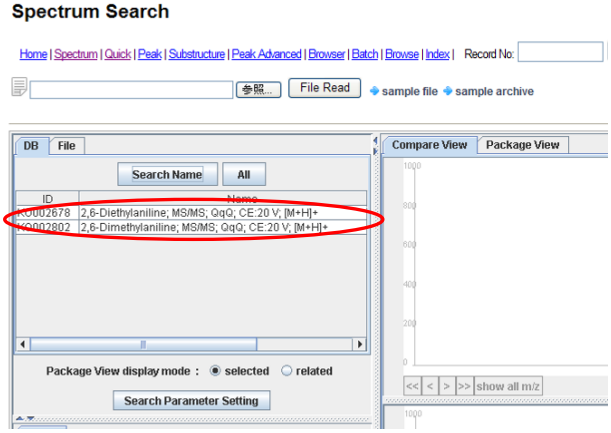
Interaction with "Multiple Display" and "Spectrum Search"



Clicking "Show Spectra"

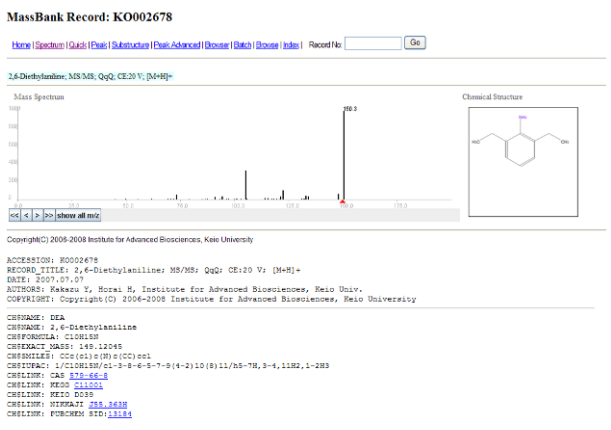
The selected spectra are displayed in tiled view.

Clicking "Spectrum Search"

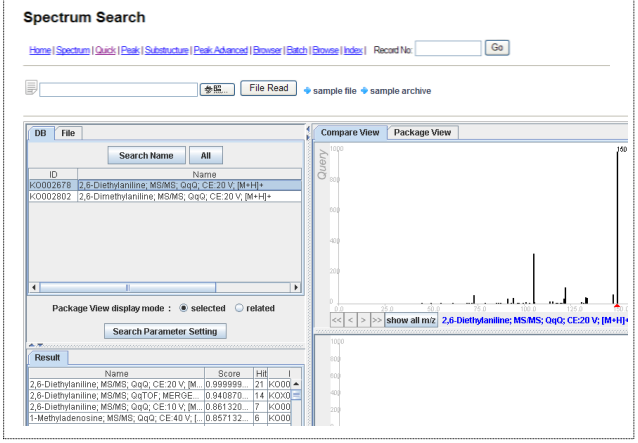


The "Spectrum Search" page opens, and the selected spectra are added to the query.

Click a button displaying a record title to show the MassBank record.

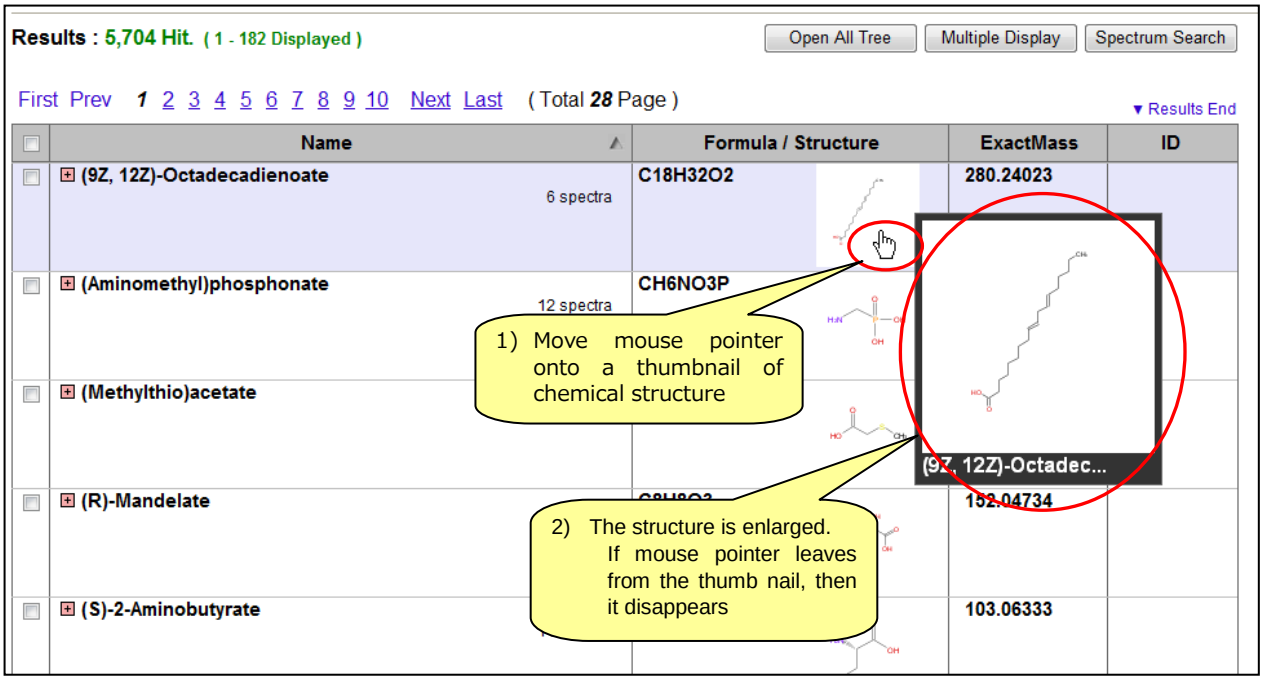


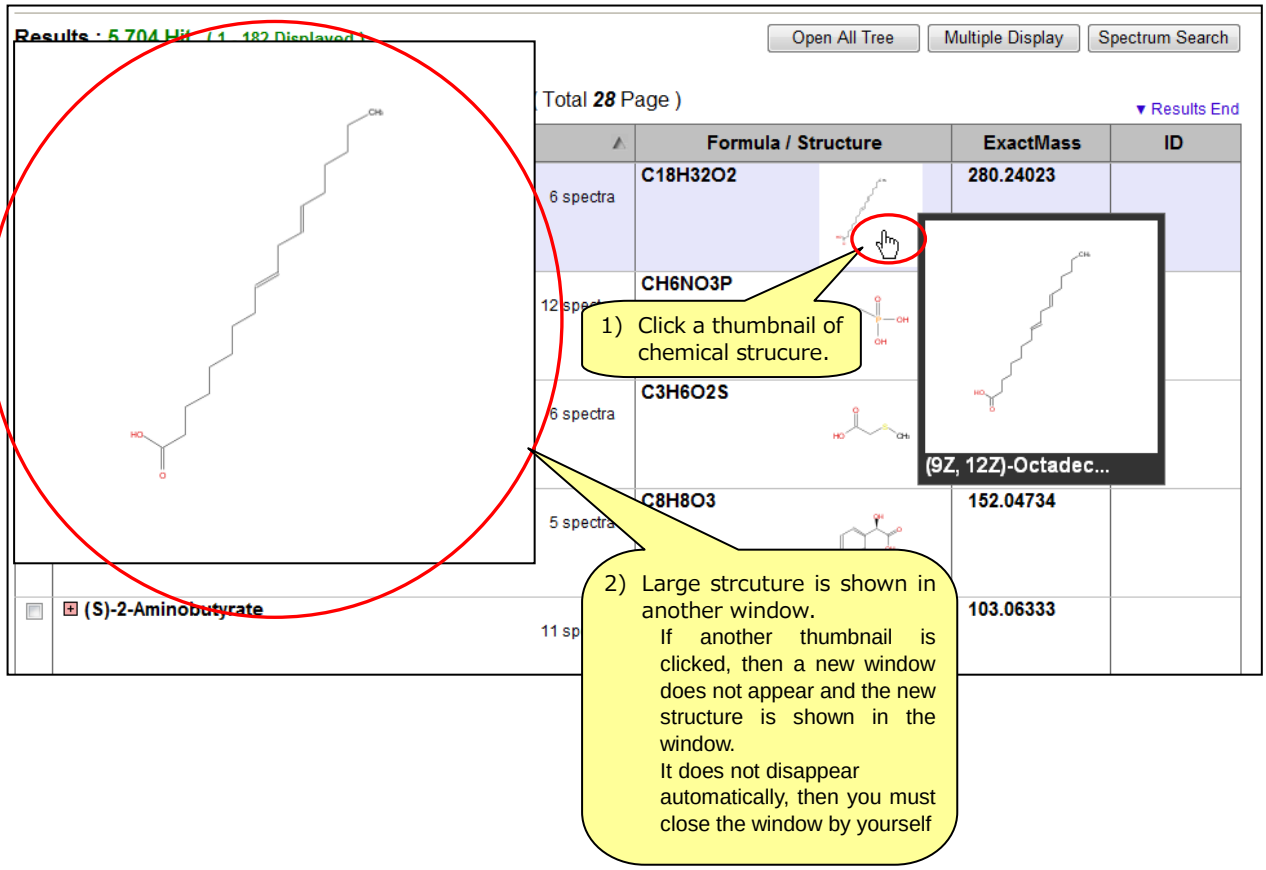
You can search with the selected spectrum as the query.



<<Operation 3>>

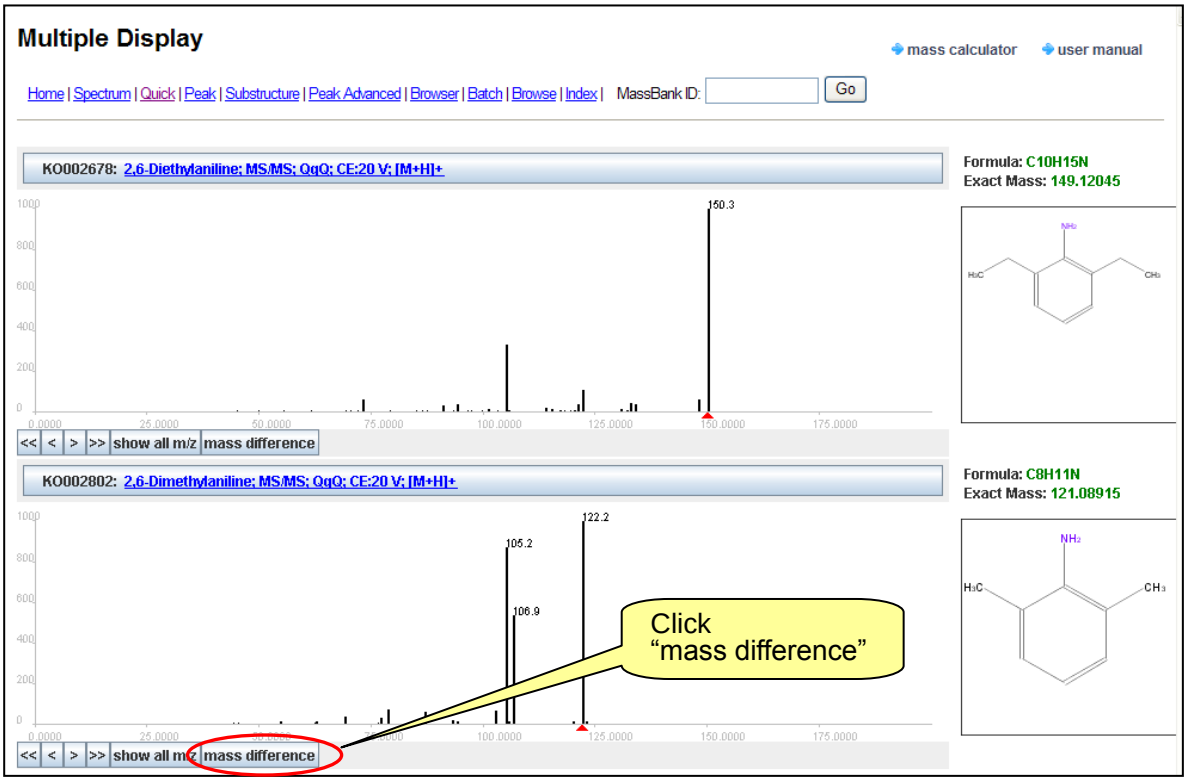
Brief display of chemical structure

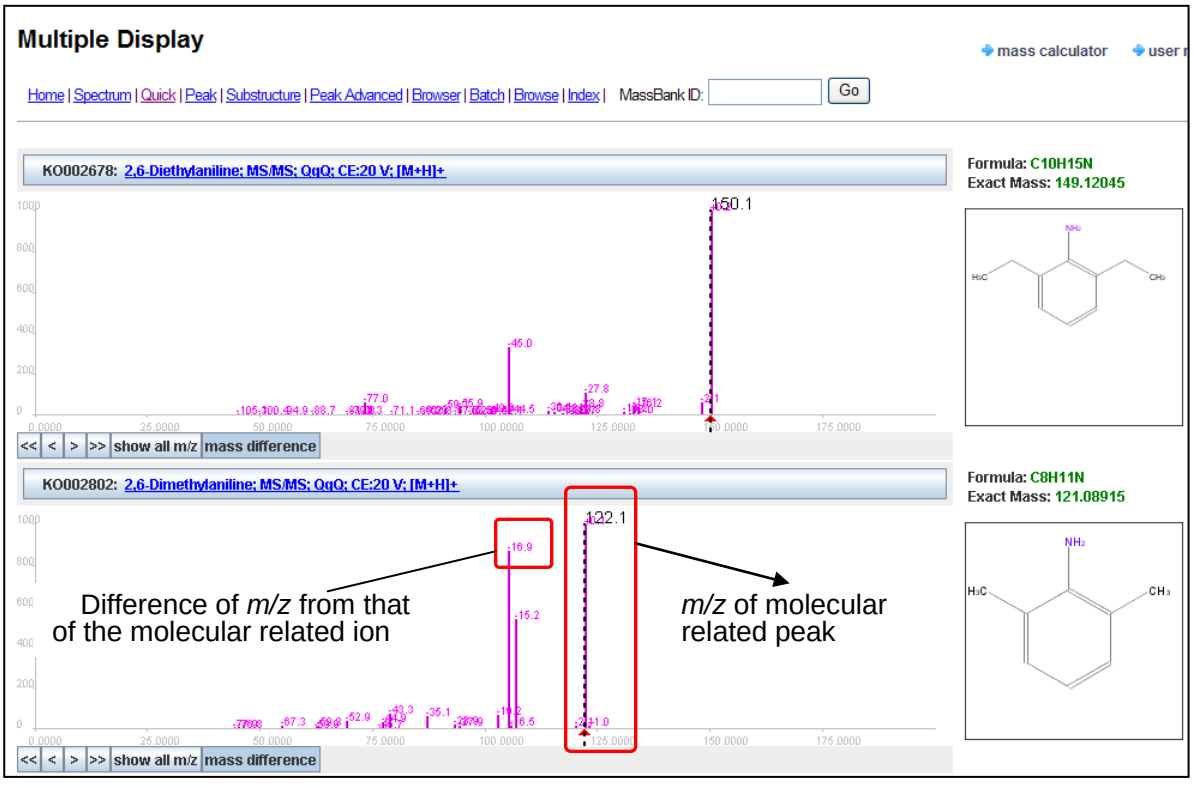




>> mass difference

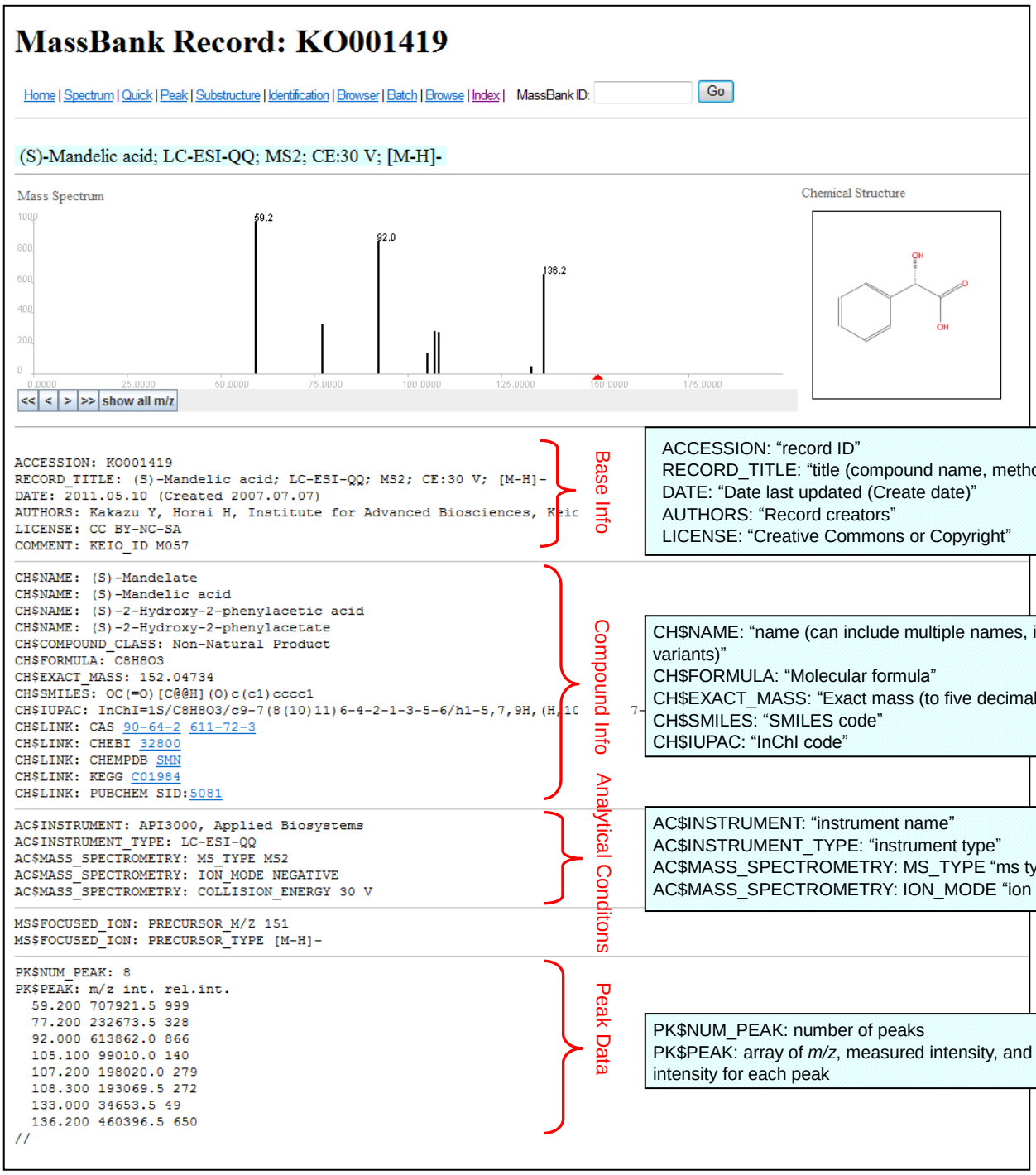
When click the "mass difference" button on the "Multiple Display" panel of "Compound and Peak Search Results", spectra display the peaks with the value of *m/z* difference from that of the molecular related ion.





7.2 MassBank Record Detailed Display

The MassBank record is the fundamental unit of data in the MassBank database. Each mass spectrum has one MassBank record. In addition to peak data, each record includes the compound information (CH$), test conditions (AC$), etc. (To see "Record Editor Manual" for more details)



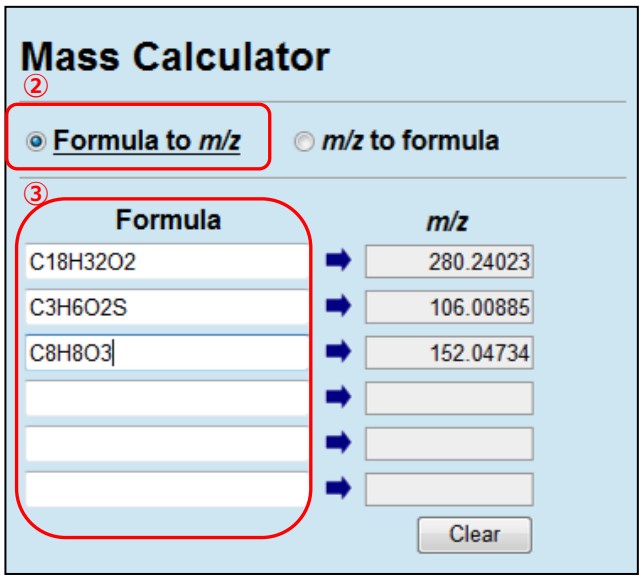
∗For details of MassBank Record, see "Record Editor Manual".

7.3 Basic Mass Calculation Tool

Mass Calculator on the upper right corner is a basic mass calculation tool that you can use everywhere in MassBank. It calculates *m/z* (*i.e.* exact mass) of the input formula or displays a list of chemical formulae corresponding to *m/z*.

[1] Click "mass calculator"
MassCalculator appears in a separate window.

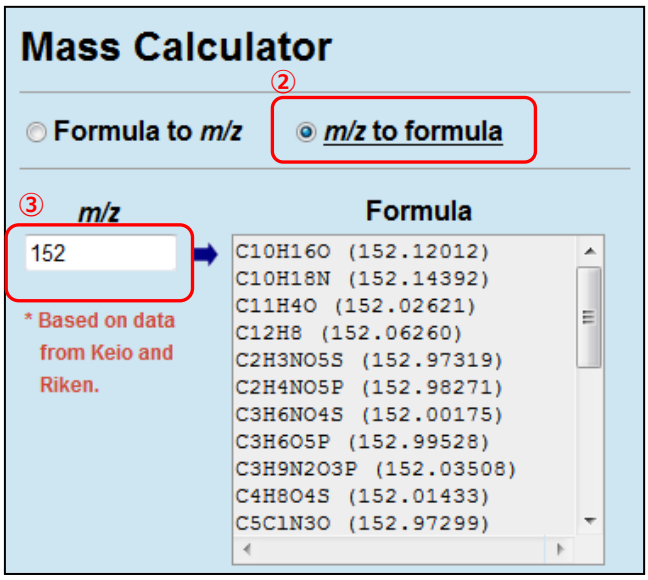
Calculation of *m/z* from chemical formula.



[1] Select "Formula to *m/z*"

[3] Input Formula
When a formula is entered, *m/z* is calculated and displayed.

Listing of chemical formulae corresponding to *m/z*.



[2] Select "*m/z* to formula"

[3] Input *m/z*
When a m/z value is entered, the chemical formulae that were observed in the MassBank annotated ESI-QTOF-MS^2 data are shown.

Chemical formulae listed are those that were actually observed in Keio and RIKEN ESI-QTOF-MS^2 data, but not possible chemical formulae.

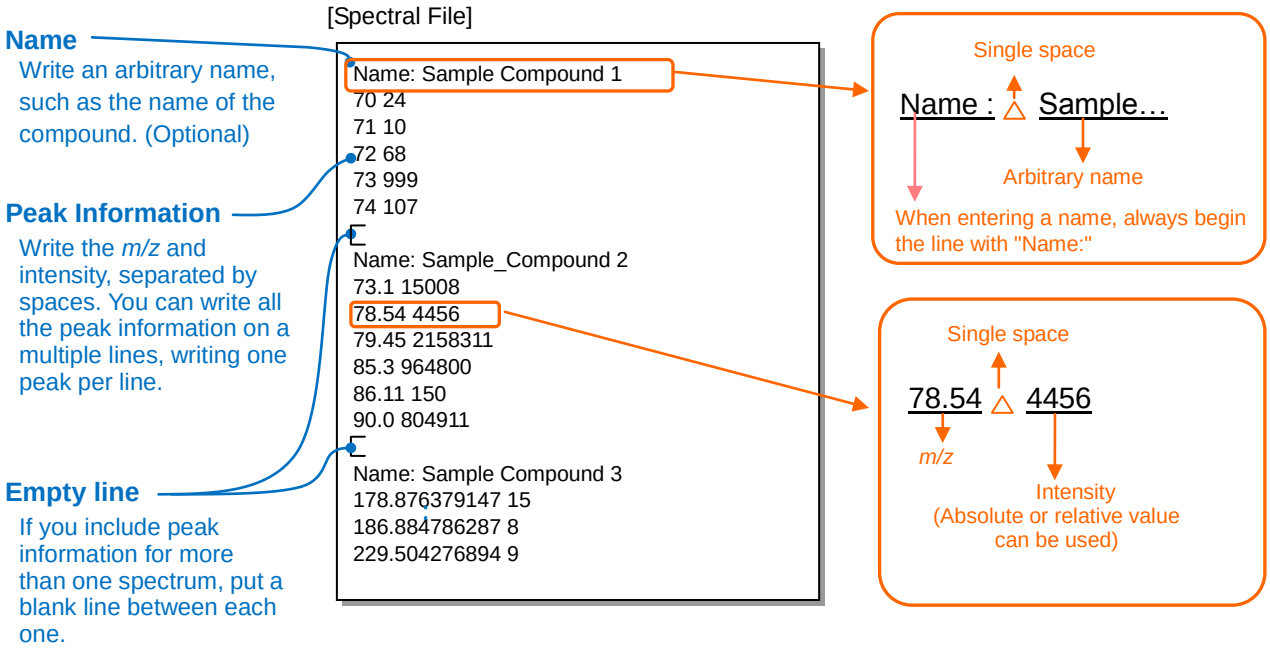
Mass Calculator window can be closed by ESC key.

7.4 Comparison Tool of User Spectra

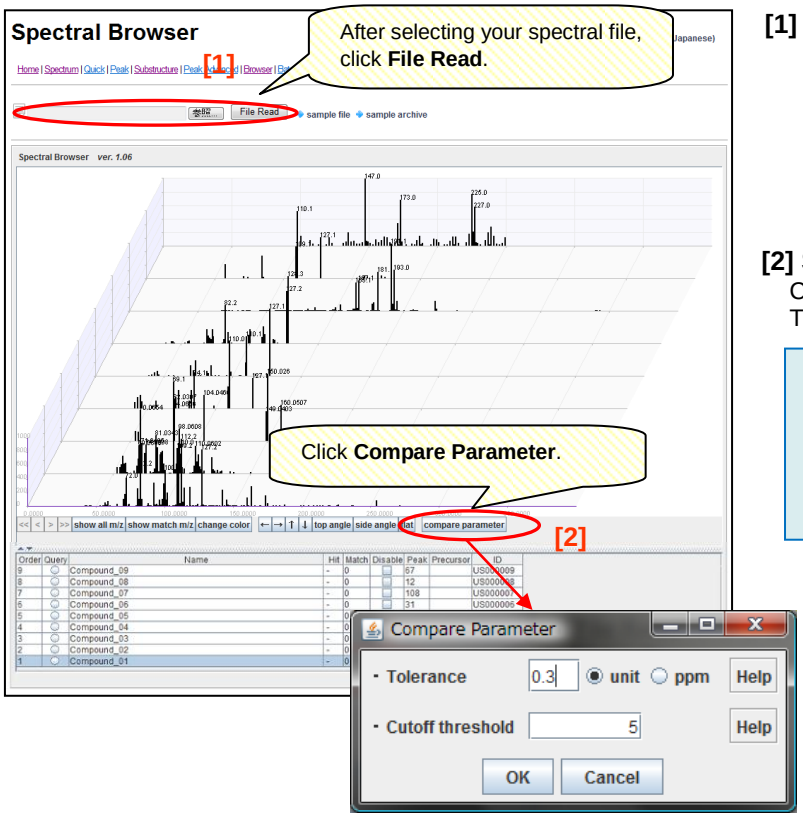
Spectral Browser is a comparison tool of spectra which reads a file which consists of a set of user spectra and displays them in perspective drawing. The upper limit of user spectra is 20. It also compare input spectra each other.

(1) Prepare the spectral file

The format of an input of Spectral Browswer, so-called a spectral file, is as follows. You can download a sample from http://www.massbank.jp/sample/sample.zip . The format of this spectral file is same as the format of a query file of Spectrum Search, then a spectral file is commonly used in Spectrum Search、Spectral Browser.



(2) Load spectral file and set the compare parameters



Compare Parameter Setting Window

[1] Load the spectral file

Click **"Browse"**, and select a spectral file. Afterwards, click **"File Read"**, then Spectral Browser reads the spectral file and display the spectra in it

[2] Set the compare parameters

Click **Compare Parameter**.
The Setting window opens.

Settings
- **Tolerance**:
  m/z error range
- **Cutoff Threshold**:
  Relative intensity threshold

(3) Perform the compare

When you specify an arbitrary spectrum as a query, then you can compare input spectra in one-to-many manner.

[1] Select query spectrum

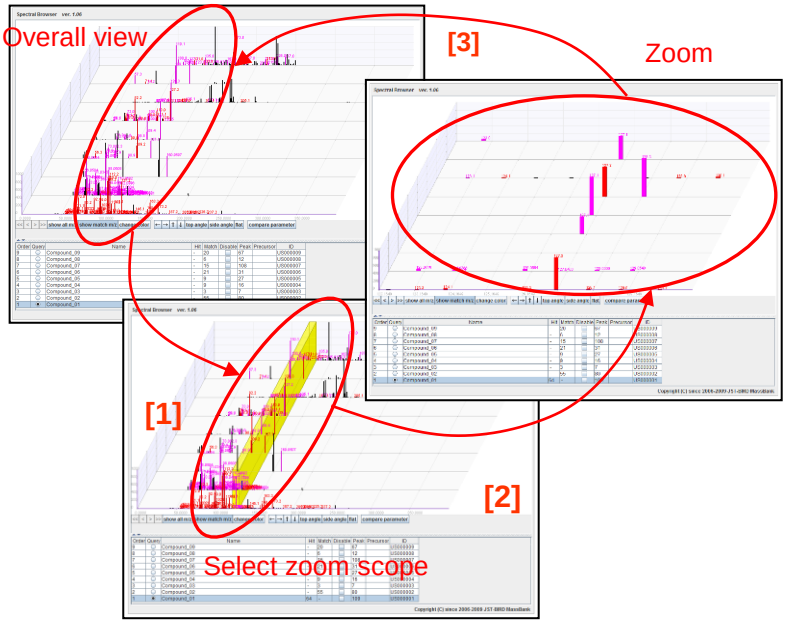
Click a radio button for selecting a query. The one-to-many comparison is suddenly executed and its results are reflected automatically.

Peak Color in Spectrum Comparison Window

In the spectrum comparison windows in Compare View and Package View, matching peaks can be distinguished by color.

Matching m/z on query peak / *Peak*	Perfect Match	Match within Error Margin
Query Spectrum	**Red**	**Red**
Retrieved Spectrum	**Red**	**Pink**

<<Handy Feature 1: Spectrum Zoom>>



[1] Select location to zoom

Drag from the location of the start of the zoom.

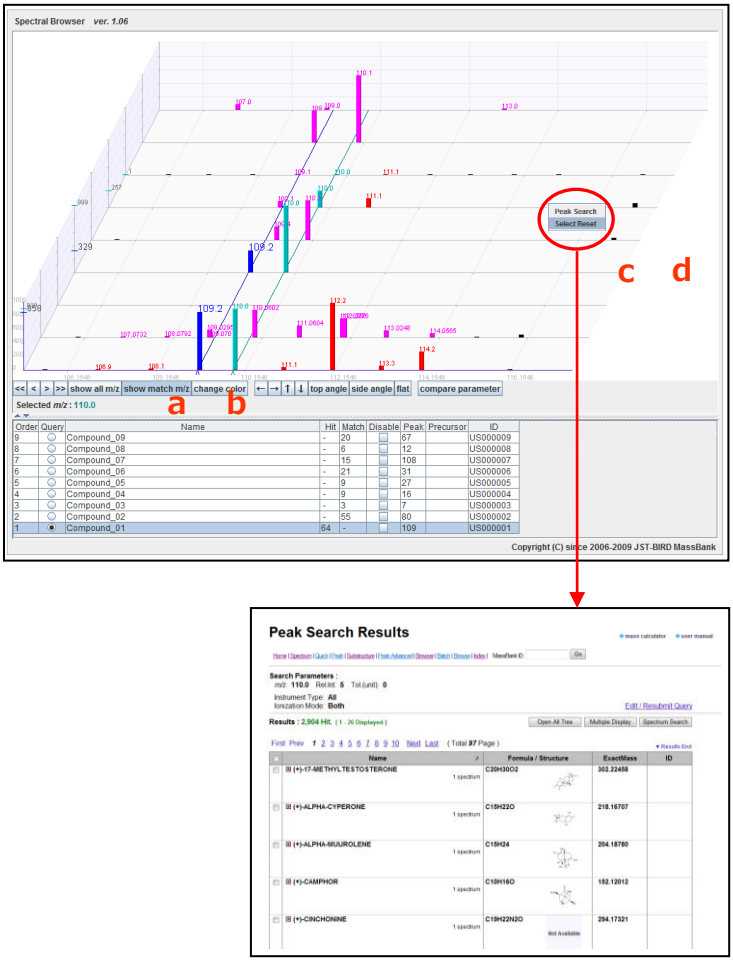
[2] Set zoom position

Drop to set the location on the spectrum to zoom.

[3] End zoom

Double click in comparison window, then return to the initial size without zooming

<< Handy Feature 2: Peak Manipulation>>



Peak Search Results Window

a Highlight peak

Place the cursor over a peak. It is highlighted in blue, and the *m/z* and intensity values appear.

b Select peak

Click on a desired peak. It is rendered in blue to indicate that it is selected. Up to 6 peaks can be selected.

Peak Rendering Color
Highlighted: Blue
Selected: Light blue

c Search for peaks

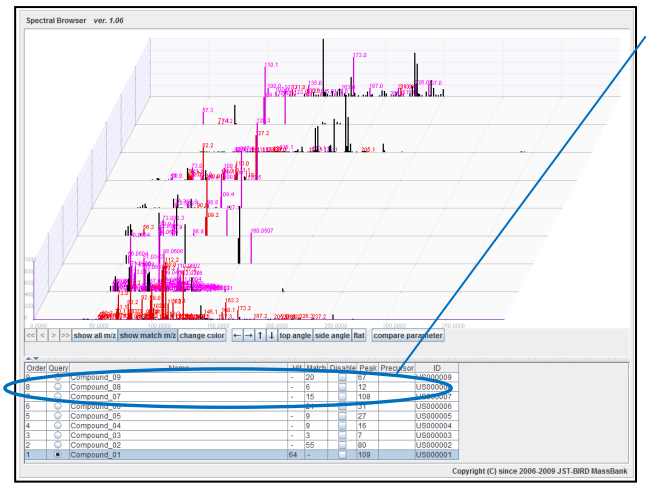
If you right click while one or more peaks are selected, a menu appears, and **"Peak Search"** can be selected. The peak search begins immediately after selection.

d Cancel peak selection

Select **"Select Reset"** from a menu popped up by a right button click in graphic area.

- Highlight & select peak in Spectral Browser -
In Spectral Browser, when you highlight or select a peak in a spectrum, peaks in other spectra are also highlighted or selected if whose *m/z* are completely equal to the *m/z* of the highlighted/selected peak.

<< Handy Feature 3: Spectrum Manipulation>>



Spectrum Manipulation Buttons

<<, <, >, >>
... Move display location (only when zoomed on spectrum

show all m/z
… Display *m/z* values of all peaks

show match m/z
… Display m/z values of matching peaks

change color
… Change color of entire spectrum

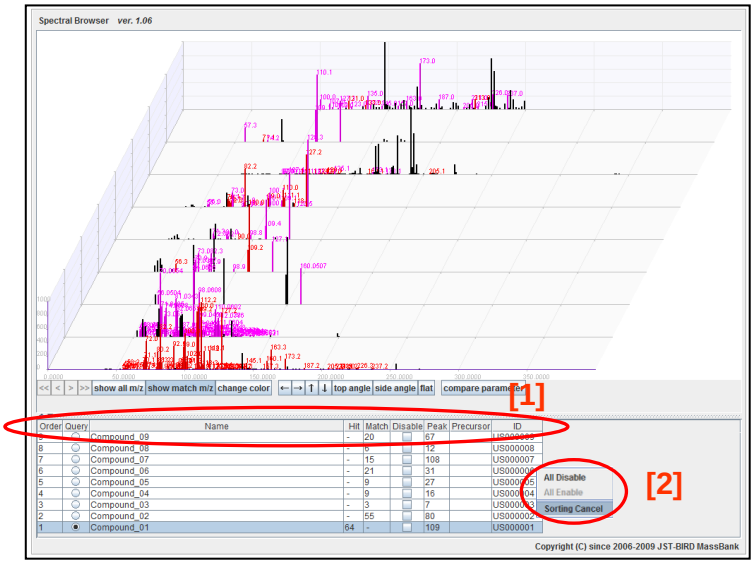
←, →, ↑, ↓
… Change angle (manual manipulation)

top angle
… Change angle (top perspective)

side angle
… Change angle (side perspective)

flat
… Change angle (all spectrums in flat view)

<< Handy Feature 4: Sort Spectra>>



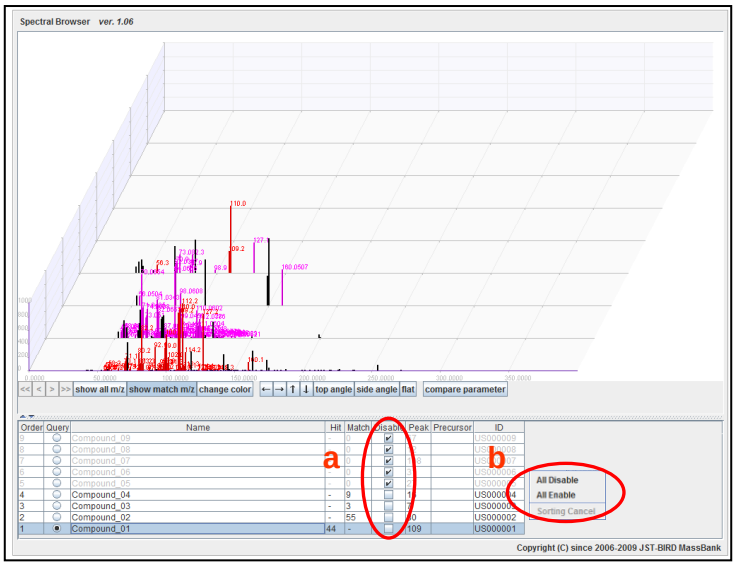
[1] Sort Spectra

Click one of header items in comparison list (*i.e.* **"Order"**, "**Query"**, **"Name", "Hit"**, **"Match", "Disable",** "**Peak", "Precusor",** and **"ID"**), then the list is sorted by the item. Consecutive clicks of an item change the order of sorting cyclically in ascending, descending and without sorting

[2] Cancel sorting

Select **"Sorting Cancel"** in a menu popped up by right button click on comparison list.

<< Handy Feature 5: Spectrum Hiding>>



a Hide/show specrum

For each item in comparison list, tick its checkbox "**Disable**", then the corresponding spectrum is hided. Untick the checkbox, then it appears again

b Hide/show all spectra

Select "**All Disable**" in a menu popped up by right button click on comparison list, then all spectra are hided. Select "**All Enable**" in the same menu, then all spectra appear again.

- Comparison window and comparison list -

Comparison window and Comparison list are operated simultaneously. For example, spectra are sorted in comparison list, the results is suddenly reflected to comparison window and spectra in comparison window are also sorted in same order.

Contact

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