ShiftedIonsFinder (Version 1.1.6) User's Manual



#### <Introduction>

In mass spectrometry-based metabolomics, it is important that the elemental composition is estimated from the accurate mass values of all the detected peaks and that the annotation of a compound is performed by comparing its analytical data with that of authentic chemicals or compounds in public databases. However, when the intensity of a detected peak is not within an appropriate range, a deviation (error) from the accurate mass occurs, and it may not be possible to estimate the elemental composition accurately. This problem tends to occur in particular when analyzing biological samples, because the quantities of the compounds contained in a sample differ widely. A technique for solving this problem is to label the biological sample externally with a stable isotope and estimate the elemental composition at a very reliable level using as an index the number of stable isotopes incorporated in the compound.

The ShiftedIonsFinder is an analytical tool (GUI program) for finding peaks having specified mass differences by comparing the mass spectra in two data sets obtained using chromatography-accurate mass spectrometry. This tool enables the user to select candidate labeled peaks by comparing a sample that is externally labeled with a stable isotope with an unlabeled sample. It is also easy to select peaks that may have been modified by hydration, glycosylation, or acylation. Since the search results can be output in Excel or text file format, the user can perform further analysis or edit the list as needed.

The tool is compatible with the output file of the LC-MS analytical tool for metabolomics, PowerGet, which is available at KOMICS (http://www.kazusa.or.jp/komics/). Since it prepares text files that follow a prescribed format, it also supports analyzed data produced by other software programs. Therefore, the user can perform a peak search using this software alone after performing peak picking, peak alignment, and database searching using other types of software. Furthermore, because the search is basically performed using only accurate mass values, the tool is also compatible with analysis data produced using other (chromatography) mass spectrometers, and thus its use is not limited only to LC-MS.

The tool was previously called Metaboloblast, but was renamed ShiftedIonsFinder upon general publication.

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#### <Search System Overview>

In this section, the process flow according to which this application software searches for peaks that have specified mass differences is described. The data that are the object of comparison are called the import data, and are called "basic samples" or "shifted samples" depending on the respective application. Furthermore, the data that describe the specified mass difference are called "element data." The three parameters that are set and used in the search are the multiple (Max. Fold), the mass-to-charge ratio (m/z) difference threshold (m/z Dif), and the retention time difference threshold (RT Dif).

For example, when searching for peaks labeled with a stable isotope, the unlabeled sample is the basic sample, the sample externally labeled with a stable isotope is the shifted sample, and the mass difference between the principal isotope and the stable isotope used in labeling is the element data. The multiple is the estimated maximum number of stable isotope atoms incorporated in the compound, and the m/z Dif and RT Dif are the allowable limits of error.

#### - Search Process Flow -

- 1. The hypothetical m/z value (Mass<sub>hyp</sub>, M<sub>bn,f</sub>) is calculated by adding the m/z value of the selected element data to the m/z value of ions (M<sub>bn</sub>) present in the basic sample. Mass<sub>hyp</sub> is calculated for each respective value from zero to the value set by the multiple (Max.Fold, F).
- 2. A search is performed to determine whether the calculated  $Mass_{hyp}$  is present in the shifted sample ( $M_{sn}$ ).
- 3. Filters are applied using the m/z Dif (m or m') and RT Dif (t) values that were set.
- 4. The search results are displayed.



Overview of the search process flow

#### <Setup>

In this section, the operating environment and methods for installing and uninstalling this application software are described.

# - Operating Environment -

The application software was developed using the Java programming language. The Java Runtime Environment (JRE) is required to run a Java program. The JRE must be installed in PC environments where it has not already been installed or where an earlier version than JRE version 1.6.0\_10 set forth in this specification is installed. The recommended environment is as follows:

OS:Windows XP or above, MacOS X (ver. 10.9 was used for an operation check)CPU:Intel Core 2 Duo equivalent or aboveMemory:512 MB or above (depending on processed files)Java:JRE Version 1.6.0\_10 or above

#### - Installing ShiftedIonsFinder -

The application software is installed by decompressing the downloaded compressed file and locating the installation folder called "ShiftedIonsFinder" on a local drive (any location). The installation folder contains the following folders.

config:	This is a folder for storing basic information. A file called "atom.txt" (editable
	by Element Manager), which contains the element data used in the search, is
	saved in this folder.
doc:	This is a folder for storing the manual and the templates used in the creation of
	import data and created import data. However, created import data do not
	necessarily have to be saved in this folder, but can be saved in any location.
img:	Images such as the ShiftedIonsFinder logo are stored in this folder.
lib:	Programs required for startup are stored in this folder.
launch.bat:	Starts up ShiftedIonsFinder for a Windows user when double-clicked.
launch.command:	Starts up ShiftedIonsFinder for a Macintosh user when double-clicked.

#### - Uninstalling ShiftedIonsFinder -

The application software is uninstalled by deleting the installation folder that was located on a local drive (any location).

#### <Preparing Import Data Used in Search>

In this section, the preparation of data for use in this application software is described. The import data used in the search function in this application software must be prepared as a text file (tab-delimited format). Further, the values of "Ave. mass (detected)" and "Ave. R.T. (min)" must be prepared as items used in computation, where Ave. mass (detected) is the m/z value of the detected ions, and Ave. R.T. (min) is the retention time. The names of these columns must not be changed because calculations in this application software are performed based on their recognition. In analyses such as in-fusion analysis, an arbitrary numeric value must be entered for Ave. R.T. (min), even when there is no retention time. As a precaution, please make sure that the two import data to be compared are in the same format. Details for preparing data when either PowerGet or other programs are used are given separately below.

#### - When Using PowerGet -

Please refer to the PowerGet manual for details on using PowerGet. An example of outputting data from PowerGet is described below, as well as a precautionary note.

- 1. From the [File] menu, select [Output...], and on the [List] tab, check all boxes in [Alignment:] and [Samples:].
- 2. Select [Each Sample] from [Group by:], and click the [Save As...] button to output the file.
- 3. Open the output file in Excel, and save it as a tab-delineated text file to any location. We recommend that you do not delete columns not used in the analysis at this stage, but wait until after the search is finished. The data preparation is now complete.

Note; The name or order of the columns on PowerGet is sometimes changed with upgrade.

#### - When Not Using PowerGet -

As described above, the Ave. mass (detected) and Ave. R.T. (min) columns are required. A recommended template is provided in the form of an Excel file in the data folder. It is recommended that you use this template when performing this operation for the first time.

- 1. Open the recommended template file in the data folder.
- 2. Enter values in the corresponding columns of the template.
- Output the template to any location as a tab-delineated text file. We recommend that you do not delete columns not used in analysis at this stage, but wait until after the search is finished. The data preparation is now complete.

#### <Memory Setup and Startup>

In this section, the memory set up and startup procedures are described.

#### - Memory Setup -

A large amount of memory is used if a large quantity of data is processed. You can set the memory size in accordance with the PC environment. In the initial setup, the maximum memory is set to 1 GB (-Xmx1G).

- 1. In a text editor, open "launch.bat" located in the installation folder.
- 2. Overwrite the maximum memory size set in the -Xmx option, and save.



#### - Starting Up ShiftedIonsFinder for a Windows User-

1. Double click "launch.bat" in the installation folder. After the splash screen is shown, the main frame is displayed.

## - Starting Up ShiftedIonsFinder for a Macintosh User-

1. Double click "launch.command" in the installation folder. After the splash screen is shown, the main frame is displayed.

\*If it doesn't work when you double click "launch.command".

- 1. Open the "terminal". [Application] > [Utilities] > [terminal]
- 2. Enter "chmod +x launch.command" and perform it.
- 3. Double click "launch.command" in the installation folder. After the splash screen is shown, the main frame is displayed.



#### <Screen Configuration>

In this section, the screen configuration is described.

#### - Configuration of Main Frame -

ShiftedIonsFinder	
File Tool Help	
2)Basic Sample	(3)
Shifted Sample	
m/z Dif	
0.005 unit ppm 0.3	

(1) Menu bar: This is the menu for file management and other tasks.

(2) Search conditions setup panel: This is the panel where search conditions are set.

(3) Data display tab panel: This is the tab panel where import data and search results are displayed.

#### - Configuration of Menu Bar -

[File]

[Import]: Menu for importing import data used in the search.

\* Details are described in a separate section.

[Export]: Menu for writing search results.

\* Details are described in a separate section.

[Element Manager]: Menu for managing element data used in the search.

\* Details are described in a separate section.

[Exit]: Menu for quitting ShiftedIonsFinder.

# [Tool]

[Join Files]: Menu for joining multiple search results.

\* Details are described in a separate section.

# [Help]

[Open Manual]: Menu for displaying manual of ShiftedIonsFinder.

[About]: Menu for displaying version information of ShiftedIonsFinder.

8			1
Basic Sampl	е ———		
	(1) 12C-unlabeled_M	edicago_Leaf.txt	
Shifted Sam	ple		
(2)13C-labeled	_Medicago_Leaf.txt		
Element	Max.Fold	(5) <sub>Add</sub>	(6) Remove
	5	Ę	
m/z Dif		RT Dif	(9)
0.005	unit ppm	0.3	START

#### -Configuration of Search Conditions Setup Panel-

- (1) Space where the file name of the basic sample that was input is displayed.
- (2) Space where the file name of the shifted sample that was input is displayed.
- (3) Selection menu for setting element data.
- (4) Box in which the value of multiple is entered.
- (5) Button for adding (3) and (4).
- (6) Button for deleting (3) and (4).
- (7) Box in which the value of m/z Dif is entered.
- (8) Box in which the value of RT Dif is entered.
- (9) Button for starting search.
- \* (1) and (2) are not displayed before input.
- \* Details are described in a separate section.

#### - Configuration of Data Display Tab Panel -



- (1) Tab that displays the basic sample information that was input.
- (2) Tab that displays the shifted sample information that was input.
- (3) Tab that displays the shifted sample data that was input.
- (4) Tab that displays the search results data.
- \* Details are described in a separate section.

#### <Import Data Input>

In this section, we describe how to input import data.

# - Import Data Input -

1. From the [File] menu, select [File Import].

File	Tool	Help
Fil	e Impo	ort
Fil	e Expo	ort
Ele	ement	Manager
Exi	t	

2. The file import dialog [File Import] is displayed.

🙀 File Import	<b>X</b>
Basic Sample	
(1)	(2)
Select File	Browse
Shifted Sample	
(1)	(2)
Select File	Browse
( <mark>3)</mark>	(4)
OK	Cancel

- (1) Box in which the import data file name is displayed.
- (2) Button for displaying the file selection dialog.
- (3) Button for confirming input of the selected import data.
- (4) Cancel button.
- 3. Click the [Browse...] button.
- 4. The file selection dialog is displayed. Select the import data to be used, and click the [Open] button. If you want to import multiple shifted samples, please select all shifted samples to analyze.

🕅 Open	
Look In: 📋	doc 💽 🙆 🔝 👰
12C-unla	beled_Medicago_Leaf.txt
13C-labe	led_Medicago_Leaf.bt
File Name:	12C-unlabeled_Medicago_Leaf.txt
Files of Type:	Text File (*.bd)
	<u>Open</u> Cancel

5. When the import data has been selected, the file name of the selected import data file is displayed in the [File Import] dialog.

File Import	<b>—</b>
Basic Sample	
12C-unlabeled_Medicago_Leaf.txt	Browse
Shifted Sample	
13C-labeled_Medicago_Leaf.txt	Browse
OK Cancel	

6. Click the [OK] button to complete the input of the import file. The basic sample and shifted sample data that were imported are respectively displayed on the basic sample information tab and shifted sample information tab.

K ShiftedIonsFinder										x
File Tool Help										
Basic Sample		Basic : 12	C-unlabeled_I	ledicago_Lea	af.txt Shifted	: 13C-labeled	_Medicago_L	eaf.txt		
12C-unlabeled_Medicago_Leaf.txt	•	No.	Ave. R.T. (	Ave, mas	Ave. mas	Ave. Inten	Pref.loniz	Ave. MS/M	(EX-HR2)	
Shifted Sample	1	0	5 104595	124 5839	123 5766	11964 07	[Actual +		0	
13C-labeled Medicago Leaf.txt		1	5.153748	129.5761	128.5688	14246.18	[Actual +		0	5
Elament May Fold		2	5.156407	182.9851	181.9778	30174.30	[Actual +		0	
Element Max.Fold Add Remove		3	5.159229	199.0294	198.0221	14813.03	[Actual +		0	
C 5		4	5.181392	133.0972	132.0899	30963.87	[Actual +	yes	1	
	n	5	5.183884	148.1162	147.1089	23520.45	[Actual +		0	
	U	6	5.185717	147.1128	129.0790	390585.9	[Actual +	yes	1	
		7	5.187665	130.0863	129.0790	37127.95	[Actual +	yes	1	
		8	5.193313	289.1618	288.1545	3582.513	[Actual +		3	
		9	5.234062	295.1498	294.1426	4822.191	[Actual +		1	
		10	5.240654	309.1655	308.1583	51311.33	[Actual +	yes	2	
		11	5.241616	310.1689	309.1616	4371.371	[Actual +		3	
m/z Dif		12	5.251558	146.0812	145.0739	23205.06	[Actual +		1	
		13	5.312194	166.0697	165.0625	8075.626	[Actual +		0	
0.005 unit ppm 0.3 01/41		14	5.313463	164.0740	163.0667	234304.0	[Actual +	yes	1	
		15	5.313858	165.0773	164.0700	11685.56	[Actual +		1	۷

The basic sample information tab

K ShiftedIonsFinder								
File Tool Help								
Basic Sample	Basic	: 12C-unlabeled_M	ledicago_Le:	af.txt Shifted	: 13C-labeled	_Medicago_L	.eaf.txt	
12C-uniabeled_medicago_Leai.txt	No.	Ave. R.T. (	Ave. mas	Ave. mas	Ave. Inten	Pref.loniz	Ave. MS/M	
Shifted Sample	0	5.148228	152.9975	151.9902	724.1895	[Actual +		A I
13C-labeled_Medicago_Leaf.txt	1	5.158530	132.0036	130.9963	22403.65	[Actual +		
Element Max Fold	2	5.173913	179.0703	178.0630	18613.77	[Actual +		
Add Remove	3	5.185799	153.1329	135.0990	287067.5	[Actual +	yes	
C 5	4	5.186463	136.1064	135.0991	22461.09	[Actual +		
T III	5	5.187222	152.1296	151.1223	55886.37	[Actual +	yes	
	6	5.212068	163.1328	162.12562	8893.542	[Actual +		
	7	5.240314	321.2058	320.1985	5143.968	[Actual +		
	8	5.262686	136.0943	135.0870	5944.791	[Actual +		
	9	5.3108137	169.0907	168.0835	40845.75	[Actual +		
	10	5.311773	170.0941	169.0868	256975.3	[Actual +	yes	
[]	11	5.312288	172.0899	171.0826	10617.23	[Actual +		
m/z Dif	12	5.312499	106.0684	105.0611	43289.72	[Actual +	yes	
START	13	5.338829	258.8994	189.9047	10540.83	[Actual +		V
	Impor	tData						

The shifted sample information tab

#### <Management of Element Data>

The element data can be customized as needed. In this section, we describe how element data are edited, added, and deleted.

#### - Editing Element Data -

Element data information is managed in a file called "atom.txt" in the [config] folder in the installation folder. Although you can edit "atom.txt" directly, you can also edit it by using Element Manager.

1. From the [File] menu, select [Element Manager].



2. The element data management dialog [Element Manager] is displayed.



- (1) Column that displays the element names of the element data.
- (2) Column that displays the mass differences of the element data.
- (3) Button for adding element data.
- (4) Button for deleting element data.
- (5) Button for confirming and saving edited contents.
- (6) Cancel button.
- 3. To edit a cell of the element data, double click it.

🕅 Element	Manager	<b>—</b>
Element	m/z Dif	Add
С	1.0033554	
S	1.9957951111	Delete
N	0.9970347926	
0	2.004246177	
	OK Cancel	

4. After editing, press the Enter key on the keyboard to confirm, and click the [OK] button to save the edited contents. Please note that if the same element name already exists, the edited contents will not be accepted.

# - Adding Element Data -

- 1. From the [File] menu, select [Element Manager].
- 2. The element data management dialog [Element Manager] is displayed.
- 3. Click the [Add] button to display the dialog for adding new element data [Add Element Data].

🕅 Add Element Data
Element :
m/z Dif :
OK Cancel

4. Enter the data to be added, and click the [OK] button to update the [Element Manager] table information. Please note that if the same element name already exists, the edited contents will not be accepted.

🕅 Add Elemen	🕅 Ele	ement M	anager	×	
Element :	Glucose	Elem	nent	m/z Dif	Add
				1.0033554	
m/z Dif :	162.0528234			1.9957951111	Delete
		N		0.9970347926	
	OK Cancel			2.004246177	
OK				162.0528234	
				OK Cancel	

5. Click the [OK] button in the [Element Manager] dialog to confirm and save the edited contents.

# - Deleting Element Data -

- 1. From the [File] menu, select [Element Manager].
- 2. The element data management dialog [Element Manager] is displayed.
- 3. Select the item to be deleted, and click the [Delete] button.

Element	m/z Dif	Add
С	1.0033554	
S	1.9957951111	Delete
N	0.9970347926	$\square$
0	2.004246177	
Glucose	162.0528234	

4. A dialog to confirm deletion is displayed.

Delete	<b>—</b>
?	Delete Data : Glucose, 162.0528234
	Yes No

5. Click the [Yes] button to update the [Element Manager] table information.

📉 Element M	anager	×
Element	m/z Dif	bbA
С	1.0033554	
S	1.9957951111	Delete
N	0.9970347926	
0	2.004246177	
		_
	OK Cancel	J

6. Click the [OK] button in the [Element Manager] dialog to confirm and save the edited contents.

#### <Search Conditions Setup>

In this section, we describe how to set the values of the element data, multiple, m/z Dif, and RT Dif. When you want to use multi-element data for the search, click the [Add] button to increase the selection menu for setting the element data and the multiple.

#### - Setting the Element Data -

Select any element from the [Element] selection menu on the search conditions setup panel. "C" is set as the default.

Г	Shifted Sample			
	13C-labeled_M	edicago_Leaf.txt		
	Element	Max.Fold		Add Remove
l	C	5	4	
l	С		P	
	S		Y	
	N			
	0			

#### - Setting the Max.Fold -

In the search conditions setup panel, enter any value in the [Max. Fold] box. Integer values from zero to the value set as the multiple are used in calculating  $Mass_{hyp}$  in the direction of addition. It is set to "5" by default, but an appropriate value must be entered depending on the selected element, assuming that the number of that element can be incorporated. We recommend that you use a certain degree of margin when setting a numeric value. After editing, press the Enter key on the keyboard to confirm and save the edited contents.

Shifted Sample	)	
13C-labeled_N	ledicago_Leaf.txt	
Element	Max.Fold	Add Remove
С	5	

#### - Setting the m/z Dif -

In the search conditions setup panel, enter any value in the [m/z Dif] box. It is set to "0.005 unit" by default, but an appropriate value must be entered depending on analysis conditions, such as the performance of the mass spectrometer. Additionally, the units (unit or ppm) can be changed by clicking the [unit] or [ppm] button.

m/z Dif		
0.005	unit	ppm

# - Setting the RT Dif -

In the search conditions setup panel, enter any value in the [RT Dif] box. It is set to "0.3" by default, but an appropriate value must be entered depending on analysis conditions, such as the chromatography conditions.



#### <Search Execution and Search Results Format>

In this section, we describe how the search is executed and how to read the search results.

#### - Search Execution Method -

After the required setup is complete, start the search process by clicking the [Start] button in the search conditions setup panel. When the process is started, a dialog that shows the state of progress is displayed.

Start	xt	Shifted : Shoot_13C_labeling.txt	33.01 %	

When the process is finished, the search results are displayed on the [Output] tab of the data display tab panel. The flow of the search, including setup and related functions performed up to this point, is shown below.

```
Importing of import data

↓

Selection of element data

↓

Setting Max.Fold

↓

Setting m/z Dif

↓

Setting RT Dif

↓

Start of search by clicking the [Start] button

↓

Display of search results data on [result: the name of selected element data] tab of data display tab

panel
```

#### - Search Results Format -

Peaks that match the search conditions are associated with both the basic and the shifted sample. These peaks are displayed in the search results; the order of the displayed data is 1) basic sample; 2) associated information; and 3) shifted sample from left to right. Associated information is as follows.

Lag(times):	Value of multiple of the search hit.
RT Difference:	Difference in elution time of the peak of the basic sample and the peak of the
	shifted sample. Units are minutes (min).
m/z Difference (u):	Difference in the $m/z$ value of the peak of the shifted sample and Mass <sub>hyp</sub> .
	Units are unified atomic mass units (u).

m/z Difference (ppm): Difference in the m/z value of the peak of the shifted sample and Mass<sub>hyp</sub>. Units are ppm.

4 million of the																			
File Tool Help																			
Basic Sample	Basic:	12C-unlabeled	Medicago_Le	aftit SP	2-labele	1 Medicago	Leaton			- 12	21					(3)			
12C-unlabeled_Medicago_Leaftst	-				•/			1 March 199			-/		-			_(0)	1	10/00/00/00/00	
Philled Parcels	No.	Ave. R.T. (	Ave. mas	Ave. mas.	Ave, Inten	Pretioniz_	Ave. MS/M	[EX+HR2]	C:Lag0.	RT Offere_	m/z Offer	mzOffer_	NO.	Ave. R.T. (	Ave mas	Ave. mas_	Ave inten	Prefioniz	Ave. MS/M.
sinter sample	2	5.156407	182.9851	181.9778	30174.30	[Actual +		0	0	0.4434386	-0.0000106	-0.0579282	82	5.599845	182.9851	181.9778	20583.35	(Actual +	
13C-labeled_Medicago_Leaf.td	3	5.159229	199.0294	198.0221	14813.03	lactual •		0	0	0.4308520	-0.0000204	-0.1024974	$\overline{n}$	5.590081	199.0294	198.0221	8585.570	(Actual •	
Element Max.Fold Add. Remove		5.183884	148,1162	147,1089	23520.45	Lectual +		0	5	0.0019145	-0.0000564	-0.3683073	3	5.185799.	153 1329	135.0990	28/06/.5	(Actual +	yes
C 5 4		6 106717	147 1190	100 0700	200605.0	TANON +	100.0	1	6	0.0016050	0.00000114	0.0740261	6	5 107222	152 1226	161 1922	56006 37	(Americal +	100
1	13	5 312194	155 0597	185 0825	8075 628	Lactural +	140	0	2	0.8651026	0.0004243	2 59 39424	108	6 177299	168.0780	167.0696	29459 27	(Artual +	1940
	13	5.312194	166.0697	165.0625	8075.626	FActual +		ō	3	0.8683140	0.0003992	2.3610146	199	6.180508	169.0802.	168.0729	394098.2	(Actual +	ves
	14	5.313463	164.0740	163.0667	234304.0	[Actual +	185	1	5	-0.0026501	-0.0000090	-0.0532258	9	5.3108137	169.0907	168.0835	40845 75	(Actual +	2006
	15	5.313858	165.0773	164.0700	11685.58	[Actual +		1	4	-0.0030452	0.0000141	0.0833872	9	5.3108137	169.0907	168.0835	40845.75	(Actual +	_
	15	5.313858	165.0773	164.0700	11685.56	[Adual +		1	5	-0.0020854	0.0000082	0.0482086	10	5.311773.	170.0941	169.0868	256975.3	(Actual +	yes
	16	5.334600	258.8993	257.8921	10690.98	[Adual + _		0	0	0.0042289	0.0000201	0.0776363	13	5.338829	258.8994	189 9047	10540.83	(Actual +	_
	17	5.352551	140.0108	139.0036	48046.094	[Actual +		0	4	-0.0050791	0.0000098	0.0680441	15	5.347472_	144.0243	143.0170	41888.29	(Actual +	
	17	5.352551	140.0108	139.0036	48046.094	[Actual +		0	0	0.5256087	-0.0001257	-0.8977873	131	5.878160.	140.0107	139.0034	39031.02	(Actual +	_
	21	5.357708	100.9506	105,9433	120020.04	Inclual +		0	0	-0.0062370	0.0000019	0.0772204	10	5.3514/1_	100 9505	100.9433	2692.940	(Actual +	
	22	5 367058	100.0110	180 2046	1200511	Tartual +		0	0	-0.0001613	0.0000160	0.0939093	18	5 350706	100 01 10	189 0047	70583.72	(Arthiol A	_
	23	6.348979	274 8732	205.8785	128745 3	lactual +		0	0	-0.0050405	0.0000131	0.0476583	20	6 362830	274 8732	205 9785	71409.68	(Actual +	_
	24	5.369631	276.8713	207.8766	25563 33.	IActual +		0	0	-0.0094675	0.0000089	0.0321449	19	5.360163	276.8713.	207.8766.	14056.64	(Actual +	
	25	5.373326	358.8345	289.8398	71864.28	[Adual +		0	0	-0.0084054	-0.0000098	-0.0273106	23	5.384920	358.8345	289 8398	39070.28	(Actual +	
	26	5.375187	444.7940	375.7993	22381.14	[Actual + _		0	0	-0.0097399	-0.0000958	-0.2176288	24	5.365447_	444.7939	443.7866	9381.185	(Actual +	
	27	5.375724_	350.8326	291.8379	19046.75	[Actual +		0	0	-0.0117522	-0.0000160	-0.0443419	22	5.363962	360.8326	291.8379_	9389.707	(Actual +	_
	28	5.377092	442,7958	373.8011	61507.44	[Actual +		0	0	-0.0083987	-0.0000316	-0.0713547	26	5.368694	442,7958	373.8011_	31189.17	(Actual +	
	29	5.379739	156.0767	155.0095	88742.84	[Actual +		1	5	-0.0116261	-0.0000035	-0.0217265	25	5.368113	161.0935	160.0852	25468.41	(Actual +	
	30	5.381050	526.7571	525.7498	60002.39	Actual +		1	0	-0.0089971	0.0000050	0.0094920	28	5.372053	526.7571	457,7624_	28494.09	(Actual + _	_
	31	5.381969	610.7184	541.7237	33829.71	[Actual +		0	0	-0.0099076	0.0000402	0.0658241	29	5.372061	610.7184	541.7237	15/56.57	(Actual +	
	32	5.38.3094	528.7551	459.7004	230/4.43	Indian .		1	0	-0.0140332	0.0000401	0.0750385	21	5.309800.	528.7552	409.7000.	11806.93	(Actual *	_
	40	5.394936	208.1050	206.0077	1093163	(ACIUM +	100.0	0	0	-0.0118465	-0.00000057	-0.0212580	40	5.363660	200.1000	200.0977_	13032.03	(ACIDS) .	1000
	53	5 398771	124 9226	123 0163	289437.0	IActual +	VPS	0	0	0.0171119	-0.0000032	-0.0256158	37	5 381659	124 92256	123 9153	141951.9	(Actual +	Ves
	54	5 399768	207 8892	206 6819	14031 93	TActual +		0	0	-0.0193367	-0.0000100	-0.0481025	33	5 380431	207 8892	206 8819	5318 689	IActual +	-
	56	5.400091	294 8434	293.8361	20420 30	IActual +		0	0	-0.0169820	-0.0000450	-0 1526234	41	5.383109	294 8433	293.8350	7912.107	(Actual +	
	58	5.400828	206.8858	205.8785	754015.37	[Actual +	yes	0	1	-0.0203974	0.0000306	0.1471938	33	5.380431	207.8892	206.8819	5318.689	(Actual +	
	58	5.400828	206.8858	205.8785	754015.37	(Actual +	yes	0	0	-0.0192521	0.0000047	0.0227178	34	5.381576.	206 8858	205.8785	346922.3	(Actual +	Yes
	50	5.400896	208.8840	207.8767	156812.2	[Actual +		0	0	-0.0188404	-0.0000180	-0.0861722	38	5.382056	208.8840	207.8767	71067.78	(Actual +	
	61	5,401603	201.0887	200.0815	27165.30	[Actual +		2	0	-0.0200246	-0.0000061	-0.0303349	35	5.381579	201.0857	200.0815	25919.26	[Actual +	
	62	5.401700	290.8471	289.8398	715810.9	[Actual +	yes	0	0	-0.0181236	-0.0000049	-0.0168473	43	5.383576	290.8471	289.8398.	305776.6	(Actual +	yes
	63	5.401897	292.8453	291.8380	198699.8	[Actual +	yes	0	0	-0.0186129	-0.0000175	-0.0597585	42	5.383284	292.8452	291.8380	83455.65	(Actual +	_
	08	5.402253	460.7678	459.7606	80075.58	Adual +		1	0	-0.0142170	-0.0000712	-0.1545247	50	5.388036	480.7878	459.7605	29/53.92	(Actual +	
	50	5.402000	275 2046	275 7003	55022.25	Elichual +		0		0.0133270	0.00000152	0.1400000	20	5 202416	176 0055	275 7002	21410.72	(Actual +	
	75	5.403967	625 5924	625 6851	8052452	lactual +		0	0	-0.01/25/49	-0.0000101	-0.0161164	47	5 387702	626 /0924	625 6851	27683.50	(Actual +	_
	73	5.404360	542,7309	541,7237	1933444	(Actual +	ves	0	0	-0.0164128	0.0000544	0.1196592	49	5.387947	542,7310	541 7237	66580.34	(Actual +	
	74	5.404453	628.6904	627.6831	42003.00	FActual +		0	0	-0.0158817	-0.0000907	-0.1442681	62	5.388592	628 6903	627.68308	13537.18	(Actual +	_
	75	5.404456	458.7697	457.7825	197826.7	[Actual +		0	0	-0.0172706	-0.0000310	-0.0675720	45	5.307177_	459.7697	457.7624	73539.25	(Actual +	
	76	5.404805	374.8084	373.8011	159455.8	[Adual +		0	0	-0.0195092	-0.0000063	-0.0168086	44	5.385296	374.8084	373.8011	50115.07_	(Actual +	-
	n	5.405089	544.7291	543.7218	91483.19	[Adual +		0	0	-0.0170049	-0.0000382	-0.0701266	51	5.388084	544 7290	543.7217_	32598.59	(Actual +	
	78	5.405099	796.5134	795.6061	34235.68	Actual +		19	0	-0.0150907	-0.0000041	-0.0051468	57	5.390009	796.6134	795.6081	7938.494	(Actual +	_
	80	5.406607	710.6537	709.6464	62278.40	[Actual +		4	0	-0.0194061	0.0000593	0.0834443	46	5.387201.	710.6538	709.6465	18279.52	(Actual +	
	81	5.406543	794.6153	/93.5080	43497.85	Indual +		15	0	-0.0187302	-0.0000153	-0.0205131	48	5.387913.	/94.6153	/93.6090_	12106.65	(Actual +	-
	62	5.400058	402.7000	401.7087	12440.04	Inclual +		40		-0.0173810	-0.0000732	-0.1081793	04	0.369277	402 7059	401.7687	0330.7097	(versal +	_
	54	5.408231	878.5767	ZAR 644"	28868.42	[ACU24 +		49	0	-0.0190440	-0.0000244	0.0277722	53	5.389187	249.05107	241.0445	F015.449	ACTURE + _	
	60	5.419736	997 1166	926 s083	10667 10	Lactural +		1	0	0.2305071	0.0000523	0.0733879	95	5.660240	007 1163	226 1000	11104.35	(Actual +	_
	100	5.477064	175 1190	174 1114	172740.9	factual +	VEC	1	5	-0.01172+7	0.00006452	0.2187240	63	5.465224	180 1357	179 1294	20915.44	(Artual +	
1999 1997 1997 1997 1997 1997 1997 1997	102	5.438490	180.0866	179.0793	4780190	lactual +	100	1	6	0.8031400	0.0000439	0.2371647	261	0 201630	185 1034	167.0696	101268.9	IActual +	_
m/z Dif	110	5.580061	146.0812	145.0739	20244 77	[Adual +		1	5	0.7058913	-0.0000915	-0.6055672	284	6 285952	151.0979	150.0905	17918.44	[Actual +	
PTADT.	445	E COODDEC	122 5026	+20.0062	0040 410	These and a		0	0	0.4405525	0.0000500	0.0006796	1	E 469520	122.0022	*20.0082	29 60460	This areas	

- (1) Column that displays the information of the basic sample that was hit in the search. If there are multiple hits, the same information is displayed in different rows, since a row is created for each hit.
- (2) Column that displays associated information.
- (3) Column that displays the information of the shifted sample that was hit in the search.

#### <Join the search result>

The "Join" function combines into a table the multiple search results in which the same basic sample is used. Thus, you can check multiple search results simultaneously. Moreover, the output file of previous search results and the current search result can be joined. In this section, we describe how to join the search results.



Overview of "Join" function

1. From the [Tool] menu, select [Join Files].



2. The join dialog [Join] is displayed.

Basic Sa	mpe	
12C-u	nlabeled_Medicago_Leaf.txt	Browse
	Result Data List	
	[C] 13C-labeled_Medicago_Leaf	.txt
🕅 Join		
Basic Sampe		
(1) Select File	(2) Browse	
Result Data List		
(3)		
	(4) Reset	
Add File List		
(5)		
	(6)	
	(7) Remove	
(8) OK	(9) Cancel	

(1) Box in which the common basic sample name used for joining is displayed.

\*If the basic sample has already been imported for search, this name is displayed.

- (2) Button for displaying the file selection dialog.
- (3) Space where the file names of the search result are displayed.\*If the search is not performed, no file name is displayed.
- (4) Button for resetting the selected search results.
- (5) Space where the file names of the selected search results that were processed previously are displayed.

\*The files selected here should be prepared as a text file (tab-delimited format) in advance.

- (6) Button for displaying the file selection dialog.
- (7) Button for removing the selected search results from (5).
- (8) Button for joining all selected (blue highlight) search results.
- (9) Cancel button.
- 3. Click the [Browse...] button to select the basic sample. If there is already a basic sample in the box, skip this and the next steps.
- 4. The file selection dialog is displayed. Select the import data to be used, and click the [Open] button.

📉 Open	
Look <u>i</u> n: 👔 1	1.1.5.2 💽 🕋 💼 👰
Config	
adoc 🚞	
img	
lib	
File <u>N</u> ame:	
Files of Type:	Text File (*.bd)
	Qpen Cancel

- 5. Click the [Add] button to select the search results that were prepared previously as needed. If you do not need these search results, skip this and the next steps.
- 6. The file selection dialog is displayed. Select the search results to be used, and click the [Open] button.

M Open		
Look <u>I</u> n: 🗎	1.1.5.2	
Config		
File <u>N</u> ame:		
Files of <u>T</u> ype:	(Text File (*.bt)	▼
		<u>Open</u> Cancel

7. After selecting all the files that you want to join, click the [OK] button to start the join process.

#### <Output of Search Results >

In this section, we describe how to output search results. The output file is in Excel format (.xlsx) or text format (.txt). Output information is as follows.

SettingData:	The name of import data and search conditions
BasicSample:	The peak list of import data for BasicSample
(No <sup>*1</sup> )_ImportData:	The peak list of import data for ShiftedSample
$(No^{*1})_{result} (Element^{*2}):$	The Search results

\*<sup>1</sup>: The number of imported files

\*<sup>2</sup>: The name of selected Elements

# - Output as Excel Format -

1. From the [File] menu, select [Export].



2. A dialog to select export file type is displayed. Select [Excel File] and click the [OK] button.



3. The file selection dialog is displayed. After entering the output file name, click the [Save] button to output the search results file. The output information is recorded in each tab in an Excel file.

🕌 Save		×
Look <u>I</u> n: 🗎	1.1.5.2	
📄 config		
doc 📄		
img		
ID	.+	
iaunch.ba	1	
File Name:		
1 no <u>r.</u> anno.		
Files of <u>T</u> ype:		•
		Save Cancel

# - Output as Text Format -

1. From the [File] menu, select [Export].



2. A dialog to select export file type is displayed. Select [Text File] and click the [OK] button.



3. The file selection dialog is displayed. After selecting the folder name, click the [Save] button to output the search results file. The output information is recorded as text files in the folder.

🛓 Save				×
Look <u>I</u> n: 👔 1	1.1.5.2	•		
Config				
doc 🦳				
img				
lib				
launch.bat				
File Neme:				
File <u>N</u> ame.				
Files of <u>T</u> ype:				•
			<u>S</u> ave	Cancel

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