

Compound Discoverer 3.0 Stable Isotope Labeling Tutorial

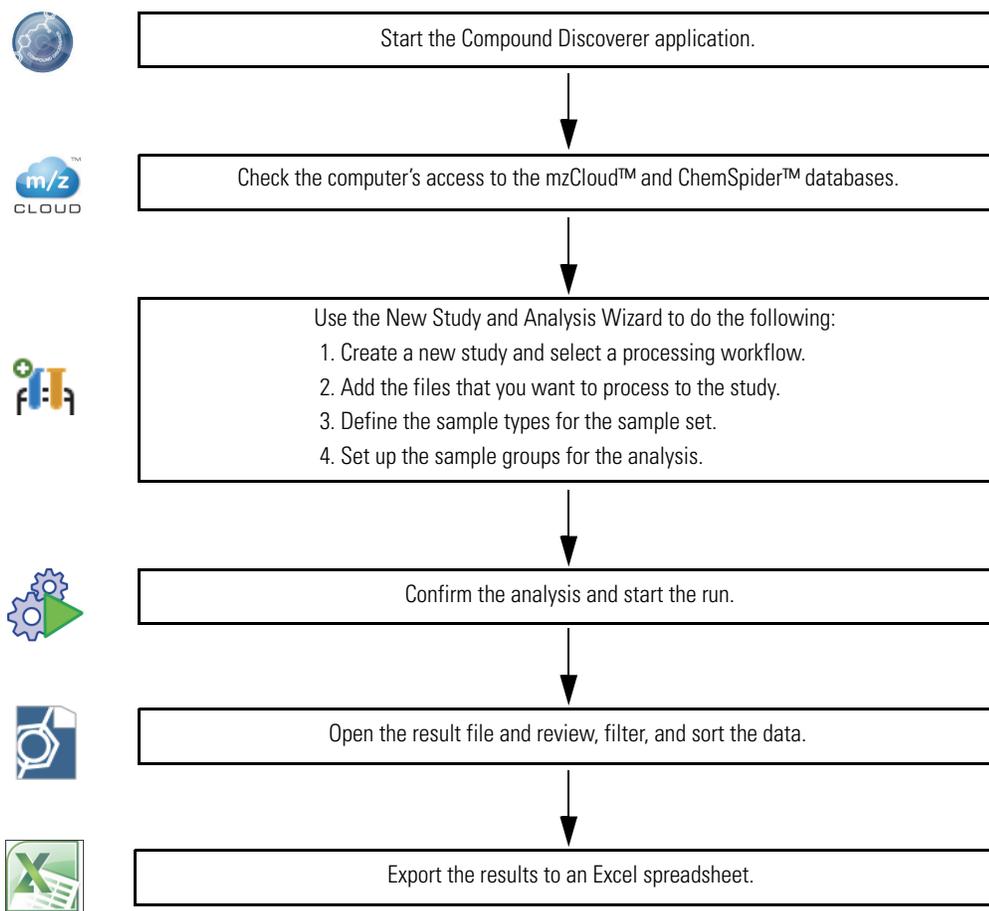
To familiarize yourself with using the Thermo Compound Discoverer™ 3.0 application to detect compounds labeled with a stable isotope such as carbon-13, follow the topics in this tutorial to set up a study and an analysis, process a set of example Xcalibur™ RAW files, review the result file produced by the analysis, and export the results to a Microsoft™ Excel™ spreadsheet.

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Overview

In the Compound Discoverer application, data processing—the analysis of a set of raw data files to extract information about the sample set—takes place within the study environment. This figure shows the tutorial's workflow.



To create a practice study, use the example Xcalibur RAW files that are located on the Compound Discoverer key-shaped USB key in the following folder:

Example Studies\Stable Isotope Labeling

The Stable Isotope Labeling folder contains the following files:

Name	Type
Blank_01.raw	RAW File
Ecoli_12C_01.raw	RAW File
Ecoli_12C_02.raw	RAW File
Ecoli_12C_03.raw	RAW File
Ecoli_12C_AcquireX_ID_01.raw	RAW File
Ecoli_12C_AcquireX_ID_02.raw	RAW File
Ecoli_12C_AcquireX_ID_03.raw	RAW File
Ecoli_12C_AcquireX_ID_04.raw	RAW File
Ecoli_13C_01.raw	RAW File
Ecoli_13C_02.raw	RAW File
Ecoli_13C_03.raw	RAW File
Stable Isotope Labeling.cdResult	CDRESULT File
Stable Isotope Labeling.cdResultView	CDRESULTVIEW File
Stable Isotope Labeling.cdStudy	CDSTUDY File

Starting the Application

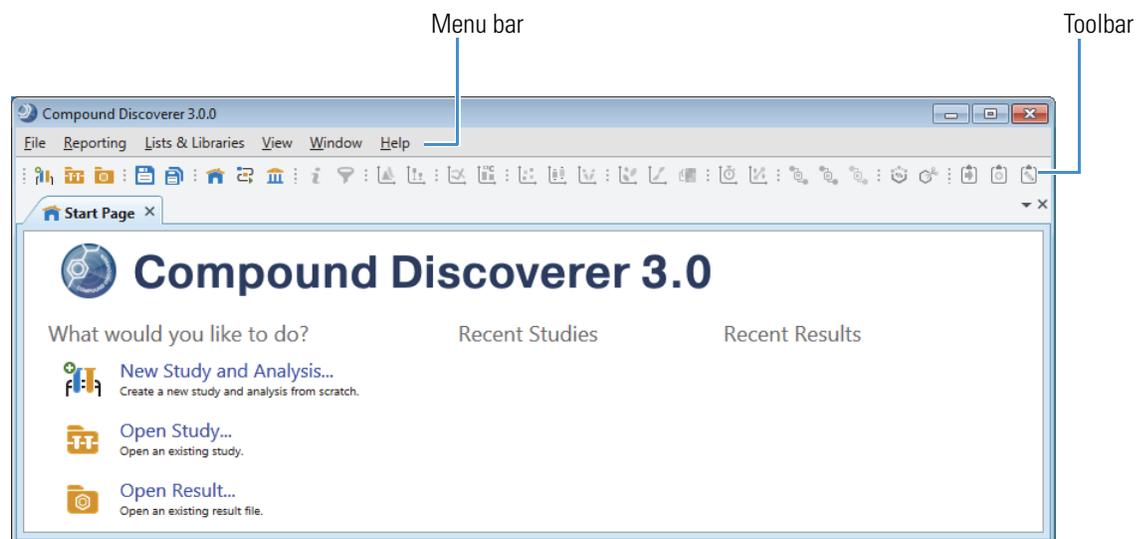
❖ To start the application

- From the taskbar, choose **Start > All Programs (or Programs) > Thermo Compound Discoverer 3.0**.

–OR–

- From the computer desktop, double-click the **Compound Discoverer** icon, .

The application opens to the Start page.



Accessing Help

The application provides Help for the views, tabbed pages, and dialog boxes.

❖ To open the Help topic for a specific view, tabbed page, or dialog box

- Open the view, tabbed page, or dialog box.
- On the computer keyboard, press the F1 key.



Checking the Computer's Access to the External Databases

This tutorial uses a processing workflow that uses online mass spectral databases to identify the unknown compounds. To use any of the processing workflows that use the online databases, such as mzCloud™ and ChemSpider™, your processing computer must have unblocked access to these databases on the Internet.

❖ To verify that your computer has access to the external mass spectral databases

1. From the menu bar, choose **Help > Communication Tests**.
2. Click the **mzCloud** tab and click **Run Tests**. When the tests are complete, go to the next step.
3. Click the **ChemSpider** tab and click **Run Tests**.

If your computer has an Internet connection, but these tests fail, leave the Communication Test window open and press the F1 key to open the Help. Then, follow the instructions to troubleshoot the communication failure.

Go to the next topic “[Setting Up a New Study and a New Analysis](#).”

Setting Up a New Study and a New Analysis

Make sure to copy the Xcalibur RAW files to an appropriate folder on your processing computer. See “[Overview](#)” on page 1.

Follow these topics to create a new study and a new analysis:

1. [Setting Up the Study Folders](#)
2. [Selecting the Processing Workflow](#)
3. [Adding the Input Files to the Study](#)
4. [Defining the Sample Types](#)
5. [Setting Up the Sample Groups](#)
6. [Modifying the Processing Workflow](#)

Each time you create a new study, the application creates a new study folder with the same name and stores the study file (.cdStudy) in the new folder. When you first install the Compound Discoverer application, you must set up a top-level folder for the study folders.

❖ To name the new study and set up the top-level folder

1. From the menu bar, choose **File > New Study and Analysis**.
The New Study and Analysis Wizard opens.
2. Click **Next** to open the Study Name and Processing Workflow page.

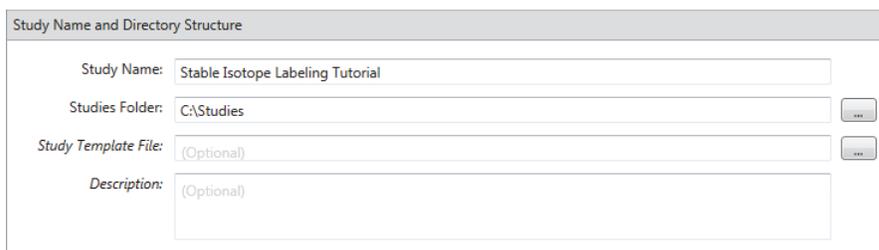
The first time you open the wizard, the top-level folder for storing the study folders is undefined.

Name the study.

Select the top-level folder for storing the study folders.

Setting Up the Study Folders

3. In the Study Name box, name the study.
 - For a stable isotope label study with the (Ecoli) example data set, type **Stable Isotope Labeling Tutorial**.
 - For a study that includes your own Xcalibur RAW files, type an appropriate name.
4. Select the folder where you want to store your Compound Discoverer study folders as follows:
 - a. Click the browse icon, , next to the Studies Folder box. Browse to your local disk drive or a location on your local network.
 - b. Click **New Folder** to create a new folder and name the folder **Studies**.



After you select or create a top-level folder, stay on this wizard page and go to the next topic, [“Selecting the Processing Workflow.”](#)

When you complete the wizard, the application creates the Stable Isotope Labeling Tutorial.cdStudy file, stores the study file in the Stable Isotope Labeling Tutorial folder, and stores the Stable Isotope Labeling Tutorial folder in the Studies folder.

Drive:\Studies\Stable Isotope Labeling Tutorial\ Stable Isotope Labeling Tutorial.cdStudy file

When you run the analysis in this tutorial, the application stores the result file (.cdResult) in the Stable Isotope Labeling Tutorial folder.

Selecting the Processing Workflow

In the Compound Discoverer application, the processing method that interprets the raw data is called a processing workflow (.cdProcessingWF). The application provides defined processing workflows for several applications including stable isotope labeling experiments.

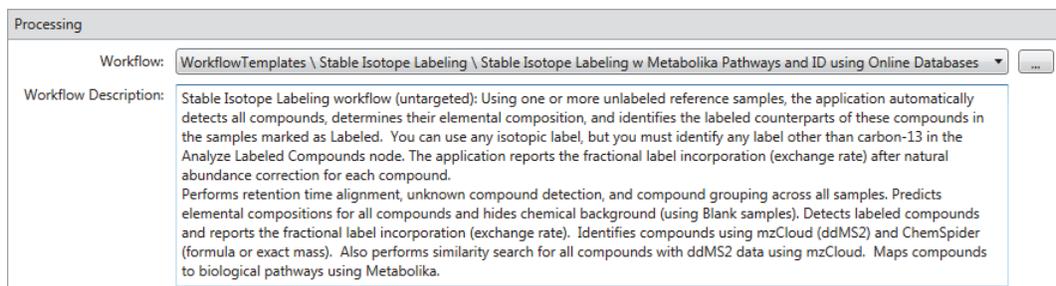
This tutorial uses a defined processing workflow that searches the mzCloud and ChemSpider databases to identify the unlabeled compounds detected in the sample files. It uses the Analyze Labeled Compounds node to detect the isotopologues of these compounds. This workflow also maps compounds to their biological pathways by using the local Metabolika pathway files.

❖ To select the processing workflow

1. Under Processing, select the following processing workflow from the Workflow list:

Stable Isotope Labeling w Metabolika Pathways and ID using Online Databases

A description of the processing workflow appears in the Workflow Description box.



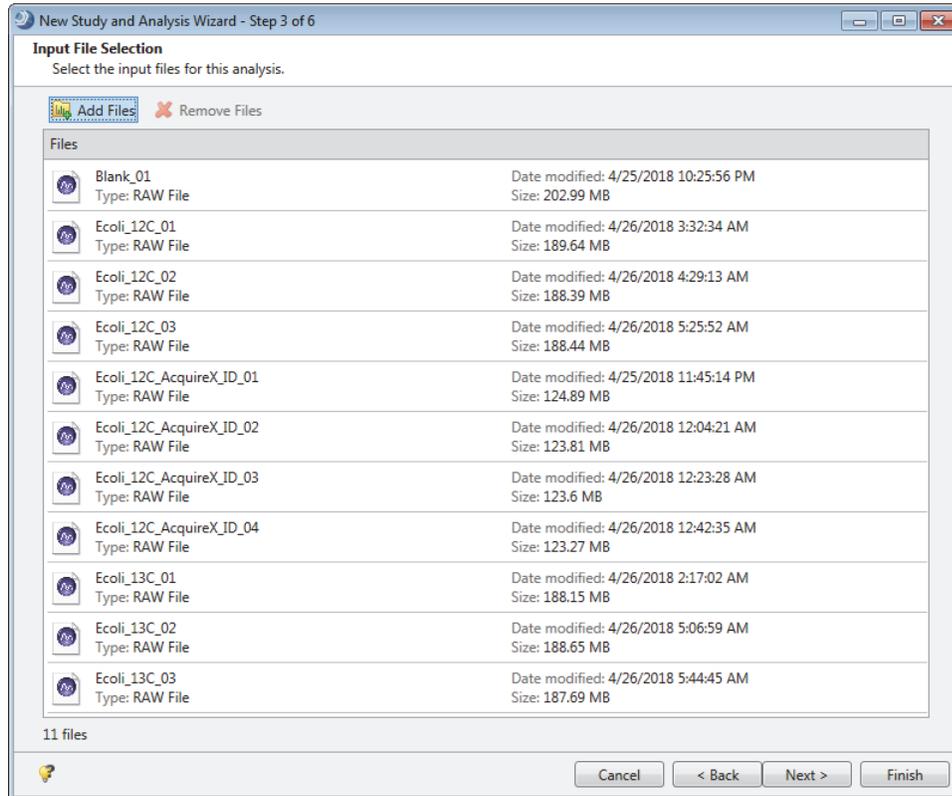
2. Read the description.

Click **Next** to open the Input File Selection page of the wizard.

Adding the Input Files to the Study

❖ To add input files to the study

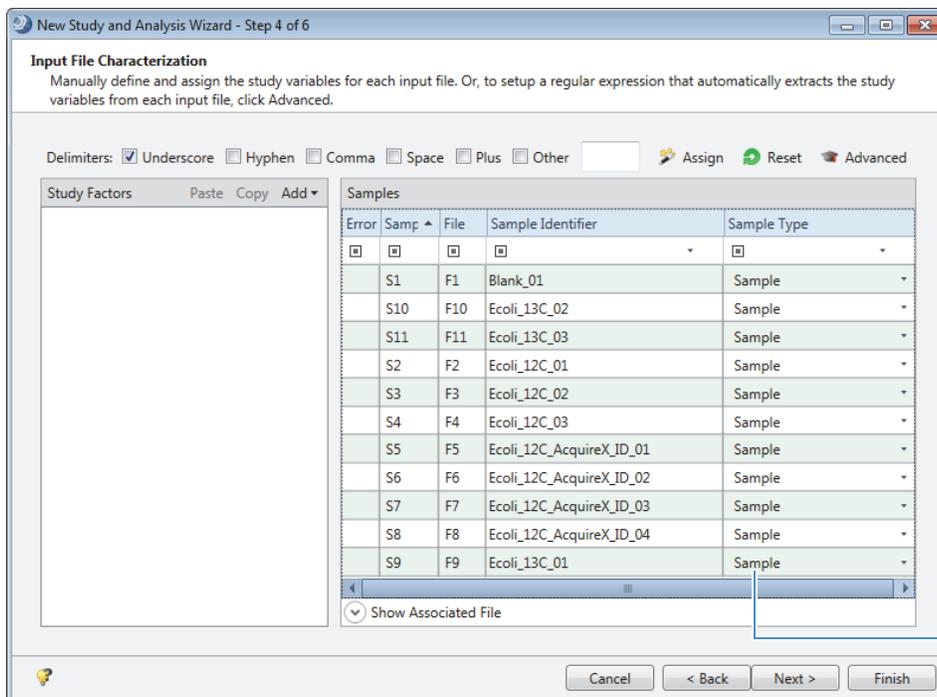
1. On the Input File Selection page, click **Add Files**.
2. In the Add Files dialog box, browse to the folder where you copied the Xcalibur RAW files.
3. Select all the Xcalibur RAW files in this folder and click **Open**.



Click **Next** to open the Input File Characterization page of the wizard.

Defining the Sample Types

This figure shows the newly added samples in the Samples area. By default, the application assigns Sample as the Sample Type to new samples.



Default Sample Type

Table 1 describes the sample types for the Stable Isotope Labeling data set.

Table 1. Sample types

Sample type	Application use
Sample	Detects the unlabeled compounds in the sample.
Blank	Marks the background compounds.
Identification Only	Does not report the chromatographic peak areas for the compounds. Uses the sample's fragmentation scans for component identification.
Labeled	Determines the isotopic label incorporation.

To set up the sample types, follow these procedures:

- [To assign the Blank sample type](#)
- [To define the samples to use for compound identification](#)
- [To define the labeled samples for the detection of labeled compounds](#)

❖ To assign the Blank sample type

Note When you select the appropriate delimiters, the application assigns the Blank sample type to files named Blank or files with Blank in the file name.

In the command bar, click **Assign**.



The application assigns the Blank sample type to the Blank.raw file.

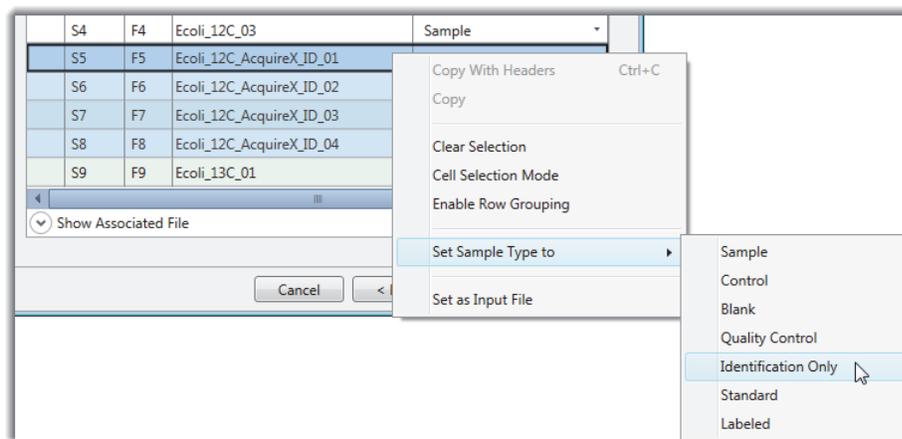
❖ To define the samples to use for compound identification

Use the SHIFT key to select the four Acquire_X_ID files. Then, right-click the selected rows and choose **Set Sample Type To > Identification Only** (Figure 1).

Tip To select a row, you can click any column but the Sample Type column.

Note The Acquire_X_ID.raw files contain the data-dependent fragmentation scans for the input file set.

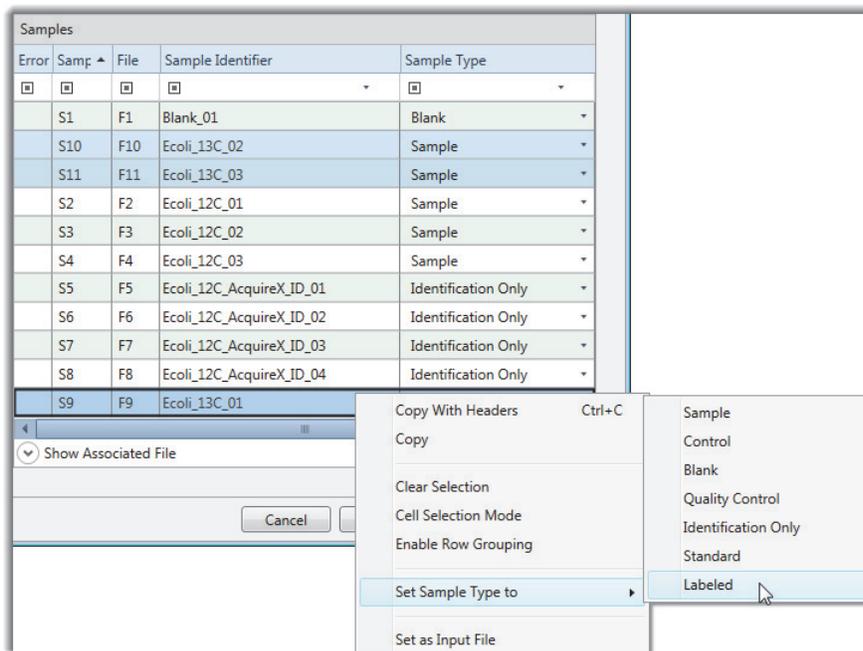
Figure 1. Defining the samples to be used for Identification Only



❖ To define the labeled samples for the detection of labeled compounds

Use the CTRL key to select the files with 13C in their file name. Then, right-click the selected rows and choose **Set Sample Type To > Labeled** (Figure 2).

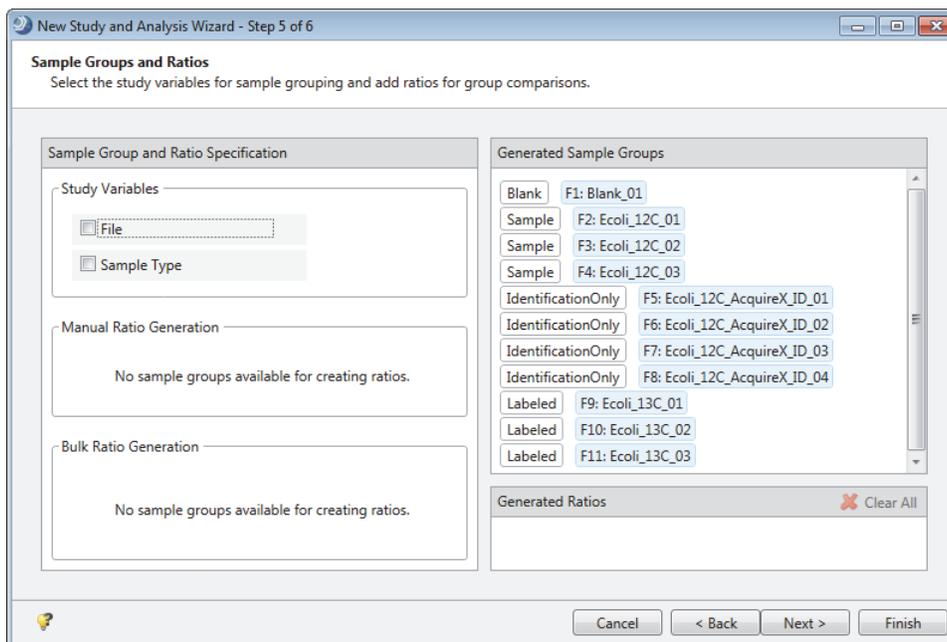
Figure 2. Defining the labeled samples



Click **Next** to open the Sample Groups and Ratios page of the wizard.

Use the Sample Groups and Ratios page to set up the sample groups and ratios for a differential analysis.

Setting Up the Sample Groups



Tip The example data for this tutorial does not include time as a study variable. To set up the study factors for a metabolic flux experiment, follow the embedded wizard Help or press the F1 key to access the Help system.

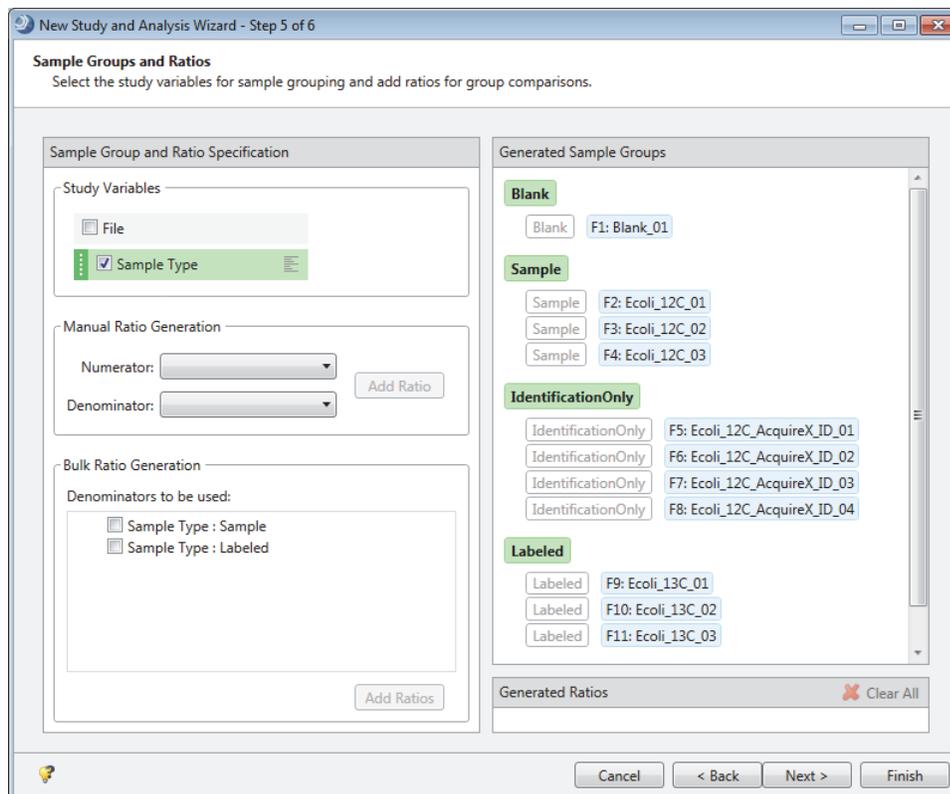
❖ To set up the sample groups

In the Study Variables area, select the **Sample Type** check box.

The sample groups—Blank, Sample, Identification Only, and Labeled—appear in the Generated Sample Groups area.

Note For the example data set, grouping the samples by sample type makes reviewing the data in the result tables easier. If you are setting up a metabolic flux study for your own data set, use the Sample Groups and Ratio page to set up ratios for a differential analysis.

Figure 3. Sample groups by sample type

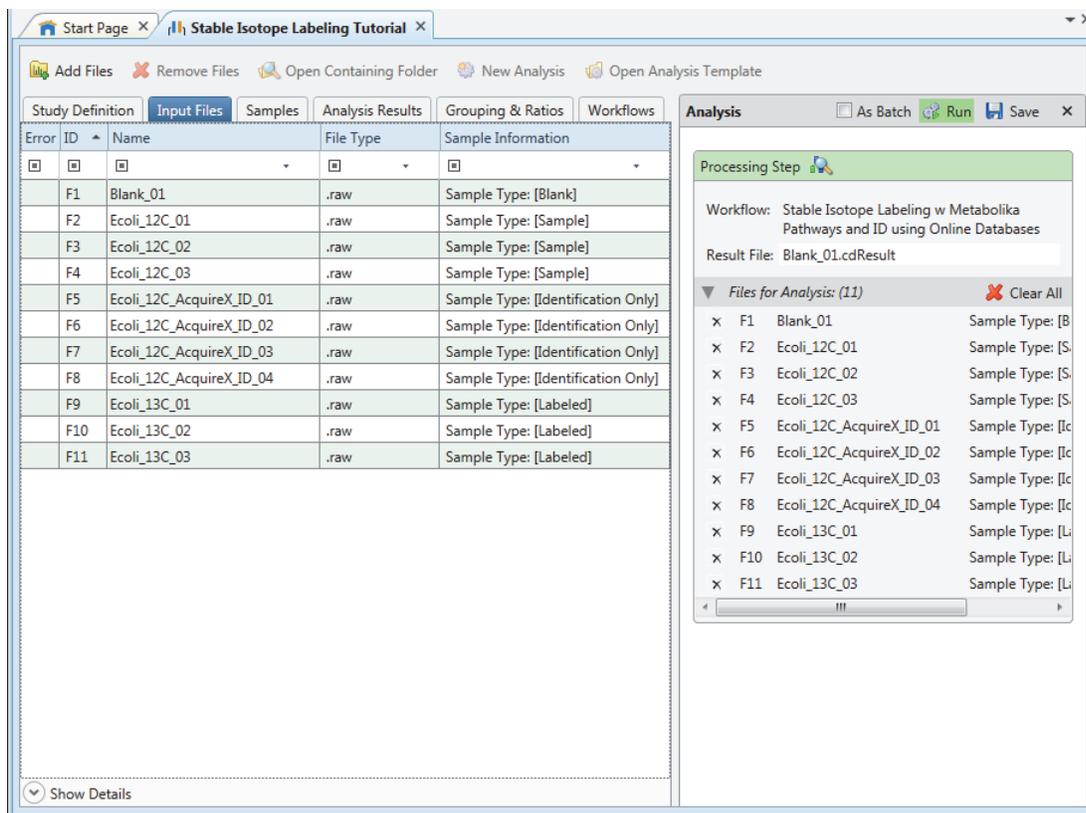


4. Click **Finish** to save the study and close the wizard.

The tabbed study page and the analysis that you set up with the wizard open.

The Analysis pane lists the 11 input files in the example data set. The analysis is set up to combine the processed results from these files into one result file—that is, the As Batch check box is clear and the Result File name is available for editing.

This figure shows the Analysis pane with a list of files for analysis.



Modifying the Processing Workflow

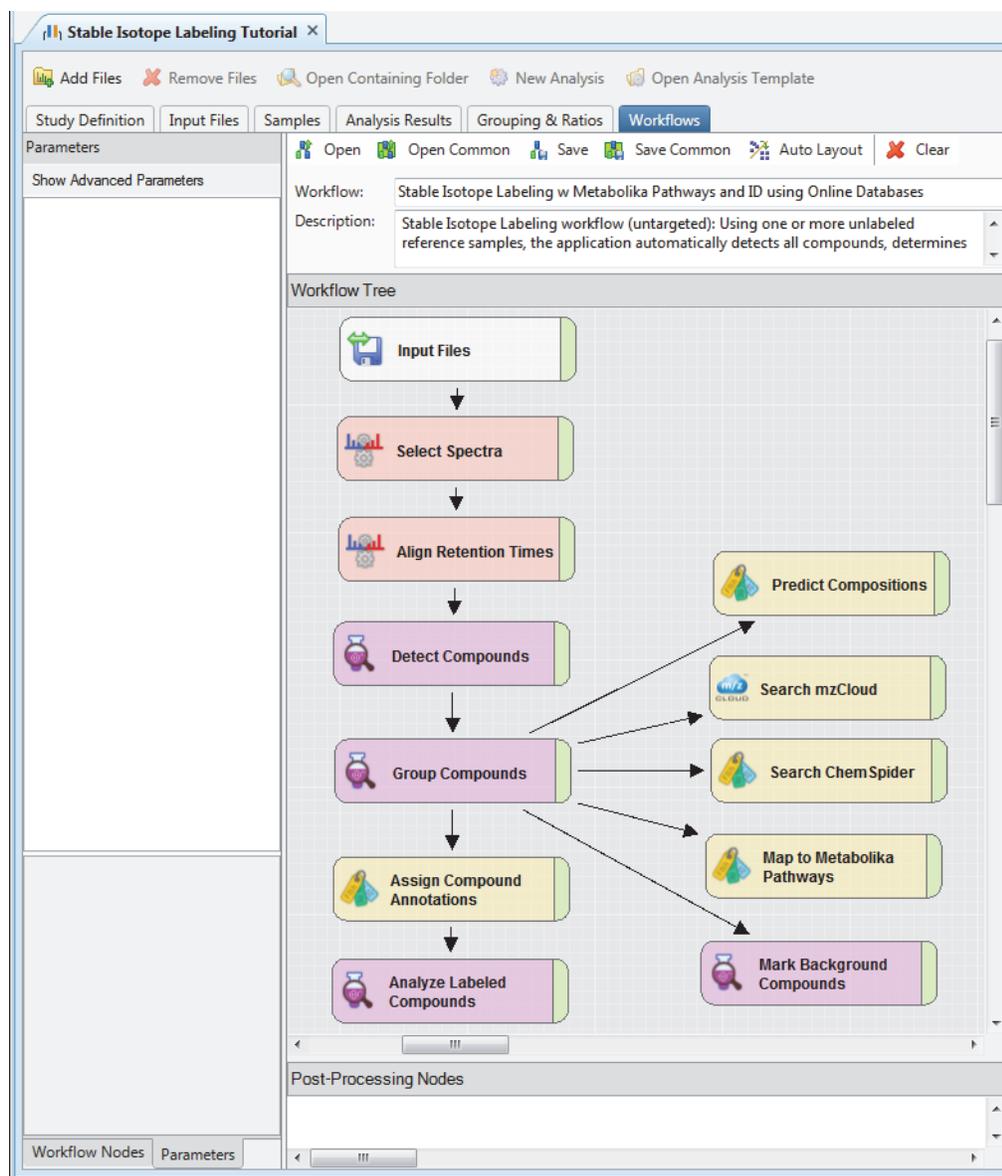
Before submitting the analysis to the job queue, review the processing workflow and make changes as needed.

❖ To review the processing workflow

1. Click the **Workflows** tab to open the Workflows page.
2. To review the parameter settings for a workflow node, select the node in the Workflow Tree pane.

The Parameters page for the selected node appears on the left.

This figure shows the processing workflow in the Workflow Tree pane.



3. Open the parameter settings for the Detect Compounds node. If you are not using the example data set, check the Min. Peak Intensity setting against the suggested setting for your data set (Table 2).

The minimum peak intensity setting defines the base peak intensity for compound detection. For this tutorial, change the setting to 100 000, as the raw data files were acquired with an Orbitrap ID-X™ mass spectrometer (see Table 2).

Minimum Peak Intensity

The screenshot shows the 'Parameters of Detect Compounds' dialog box. The 'Min. Peak Intensity' parameter is highlighted with a blue selection bar and is set to 100000. Other parameters include Mass Tolerance (5 ppm), Intensity Tolerance (30%), S/N Threshold (3), Ions ([2M+ ACN+ H]+1; [2M+ ACN+ H]+2), Min. Element Counts (C H), and Max. Element Counts (C90 H190 Br3 Cl4 K2 N10 O10). The 'Workflow Tree' pane in the background shows the 'Detect Compounds' node highlighted.

Table 2 lists the recommended range for the Min. Peak Intensity parameter. The optimal setting depends on the sensitivity of the mass spectrometer.

Table 2. Recommended minimum peak intensity range

Mass spectrometer	Minimum peak intensity (chromatographic peak height)
Q Exactive™, Q Exactive Plus™, Q Exactive HF	500 000 to 1 000 000
Orbitrap Fusion™, Orbitrap Lumos, Orbitrap ID-X	50 000 to 100 000
Exactive™, Exactive Plus™, Orbitrap Elite™, Orbitrap Velos Pro™	100 000 to 500 000
LTQ Orbitrap XL™, LTQ Orbitrap Velos™	25 000 to 100 000

4. Open the parameter settings for the Group Compounds node.

In the selected processing workflow, the RT Tolerance has been set to 0.5 min. This setting is slightly wider than the default setting of 0.2 min. For this tutorial, do not change the node settings.

The Group Compounds node creates the MSn tree that is saved to the result file and used by the search nodes and the Predict Compositions node.

Parameters of 'Group Compounds'	
Show Advanced Parameters	
▲ 1. Compound Consolidation	
Mass Tolerance	5 ppm
RT Tolerance [min]	0.5
▲ 2. Fragment Data Selection	
Preferred Ions	[M+H] ⁺ 1; [M-H] ⁻ 1

5. Open the parameter settings for the Predict Compositions node. For the example data set, keep the default settings.

The Predict Compositions node predicts the elemental compositions for compounds without hits from the search nodes.

Parameters of 'Predict Compositions'	
Show Advanced Parameters	
▲ 1. Prediction Settings	
Mass Tolerance	5 ppm
Min. Element Counts	C H
Max. Element Counts	C90 H190 Br3 Cl4 N10 O18 P3 S5
Min. RDBE	0
Max. RDBE	40
Min. H/C	0.1
Max. H/C	4
Max. # Candidates	10
▲ 2. Pattern Matching	
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
Use Dynamic Recalibration	True
▲ 3. Fragments Matching	
Use Fragments Matching	True
Mass Tolerance	5 ppm
S/N Threshold	3

6. Open the parameter settings for the Analyze Labeled Compounds node.

For the example data set, keep the default settings. For a different data set, enter the appropriate isotope for the Label Element parameter.

Parameters of 'Analyze Labeled Compounds'	
Show Advanced Parameters	
1. Label Settings	
Label Element	[13]C
Max. Exchange	25
Source Efficiency [%]	100
2. Pattern Analysis	
Mass Tolerance [ppm]	5 ppm
Intensity Tolerance [%]	30
Intensity Threshold [%]	0.1
S/N Threshold	3
3. General Settings	
Mark Irregular Exchange	True
Exclude Blanks	True
Hide Unprocessed	True

If necessary, customize this setting for your own data set.

Hides the compounds without formulas in the Compounds result table.

7. Open the parameter settings for the Search ChemSpider node.

In the selected template, three out of 596 databases are selected. For this tutorial, do not change the selection.

Parameters of 'Search ChemSpider'	
Show Advanced Parameters	
1. Search Settings	
Database(s)	BioCyc; Human Metabolome Database; KEGG
Search Mode	By Formula or Mass
Mass Tolerance	5 ppm
Max. # of results per compound	100
Max. # of Predicted Compositions to be searched per Compound	3

8. Open the parameter settings for the Search mzCloud node.

The node is set up to search the entire mzCloud database and run an Identity search and a Similarity search. For this tutorial, do not change the settings.

Parameters of 'Search mzCloud'	
Show Advanced Parameters	
1. Search Settings	
Compound Classes	All
Match Ion Activation Type	True
Match Ion Activation Energy	Match with Tolerance
Ion Activation Energy Tolerance	20
Apply Intensity Threshold	True
Identity Search	HighChem HighRes
Similarity Search	Similarity Forward
Match Factor Threshold	50

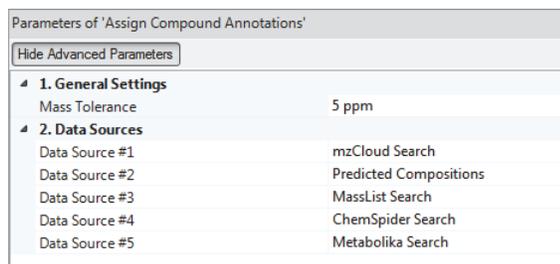
9. Open the parameter settings for the Map to Metabolika Pathways node.

For this tutorial, do not change the settings.

Parameters of 'Map to Metabolika Pathways'	
Show Advanced Parameters	
1. Search Settings	
Metabolika Pathways	\(3R)-linalool biosynthesis.metabolika
Search Mode	By Formula or Mass
2. By Mass Search Settings	
Mass Tolerance	5 ppm
3. By Formula Search Settings	
Max. # of Predicted Compositions to be searched per Compound	3
4. Display Settings	
Max. # Pathways in 'Pathways' column	20

10. Open the parameter settings for the Assign Compound Annotations node.

For the example data set, do not change the settings.



Parameters of 'Assign Compound Annotations'	
<input type="button" value="Hide Advanced Parameters"/>	
1. General Settings	
Mass Tolerance	5 ppm
2. Data Sources	
Data Source #1	mzCloud Search
Data Source #2	Predicted Compositions
Data Source #3	MassList Search
Data Source #4	ChemSpider Search
Data Source #5	Metabolika Search

Tip If you are working with your own data set and the analysis does not identify the correct isotopologues, consider changing Data Source #1 to a custom mass list for your analytes and reprocessing the analysis.

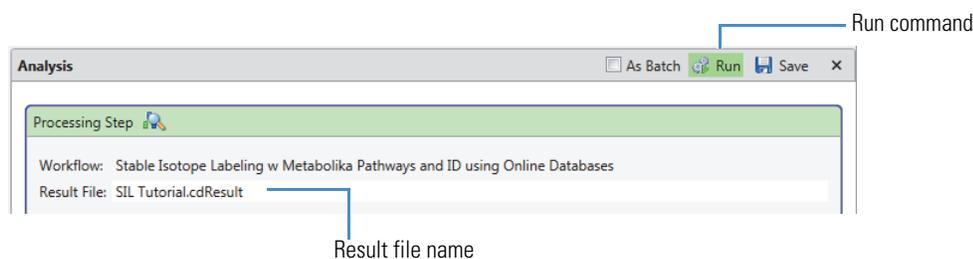
11. (Optional) Save the modified processing workflow with a new name and location.

For the example data set and analysis, you are ready to start the processing run.

Tip If you modified the analysis and the Run button is unavailable, remedy the issues listed in the Current Workflow Issues pane on the Workflows page. If the Caution symbol remains, point to it and remedy other analysis errors, for example, no input files in the Files for Analysis area of the Analysis pane.

❖ To submit the analysis to the job queue

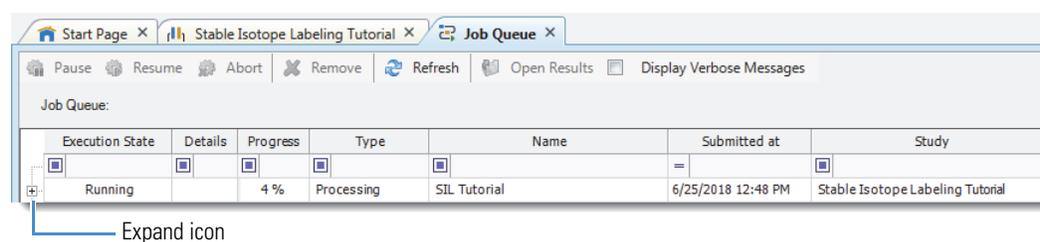
1. To create one result file for the input file set, leave the **As Batch** check box clear.
By default, the application uses the name of the first input file for the file name of the result file.
2. In the Result File box, rename the result file **SIL Tutorial**.



3. Click **Run**.

The Job Queue page opens.

4. To view the processing messages, click the expand icon, , to the left of the job row.



Note During the run, the Search ChemSpider node generates warning messages that you can ignore. Warning messages have a yellow background.

(23): Search ChemSpider Warning 110 results were not persisted as they were unparsable for Compound Discoverer.
(23): Search ChemSpider Warning 23 results were not persisted as they were unparsable for Compound Discoverer.

5. Before or after the run is completed, save the study by choosing **File > Save All** in the menu bar. Leave the Job Queue page open and go to [“Reviewing the Analysis Results.”](#)

Reviewing the Analysis Results

Follow these topics to review the analysis results.

- [Opening the Result File](#)
- [Default Result Page Layout](#)
- [Applying the Stable Isotope Labeling Layout](#)
- [Reviewing the Exchange Rates](#)
- [Reviewing the Labeling Status](#)
- [Working with the Trend Chart View](#)
- [Working with the Isotopologues Distribution Chart](#)
- [Viewing the Metabolika Pathways for a Compound](#)

For more information about a specific result table or view, select the table or view to make it active, and then press the F1 key. The Compound Discoverer application provides F1 Help for all the views that you access from the View menu and all the result tables.

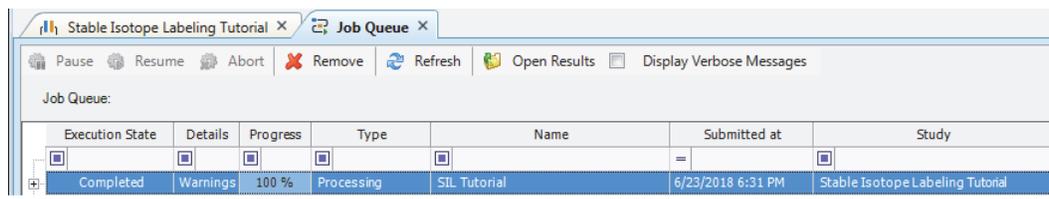
Opening the Result File

You can open a result file from multiple locations: the Job Queue page, the Analysis Results page of a study, the Compound Discoverer Start Page, or the menu bar.

Note For this tutorial, you can create a result file by setting up and running an analysis with the example data set. Or, you can open the result file—Stable Isotope Labeling—in the same folder where you found the example data set.

❖ To open the result file generated by the analysis

When the run is completed, double-click the run on the Job Queue page.



Default Result Page Layout

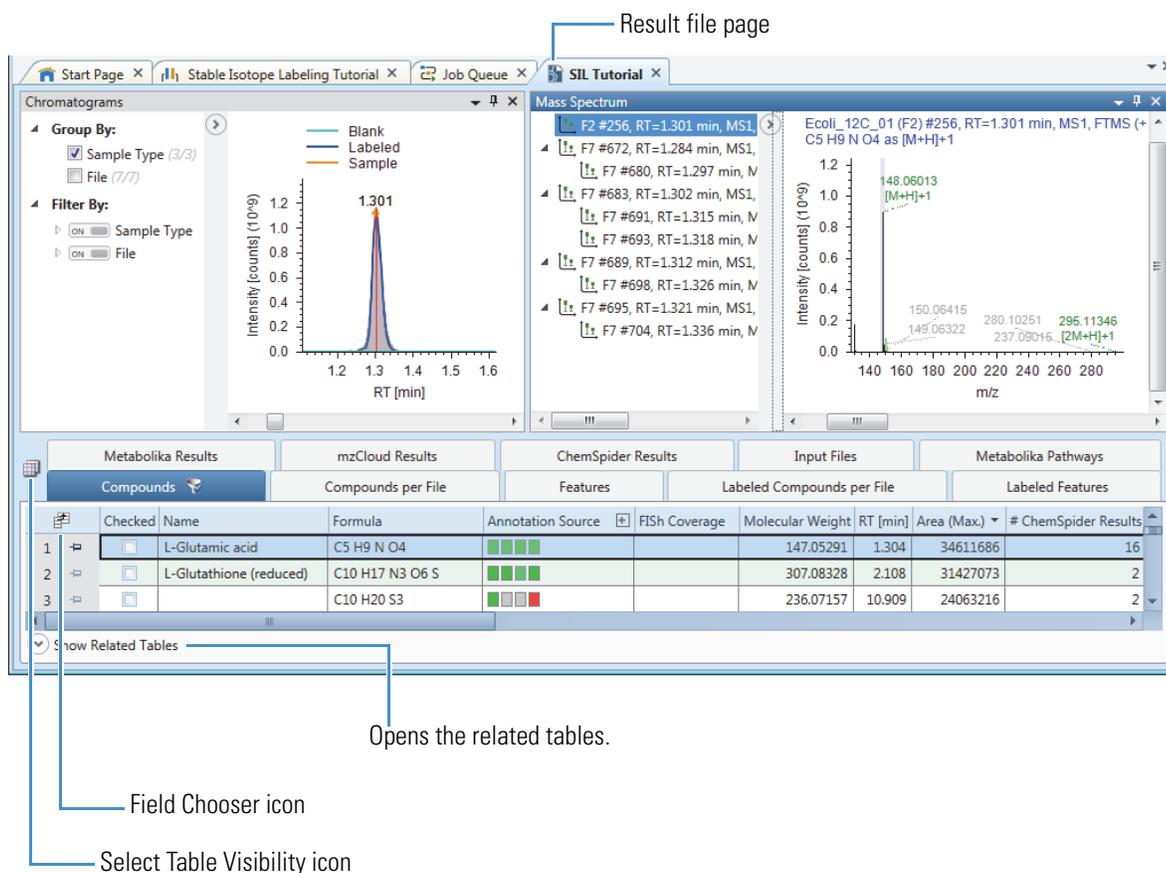
The result file opens as a tabbed document. By default, the Chromatograms view opens in the upper left, the Mass Spectrum view opens in the upper right, and a set of tabbed result tables opens in the bottom half of the page. The Compounds table is the active table. The detected compounds are listed in descending order of the maximum chromatographic peak area [Area (Max.)] across the input files. The Chromatograms and Mass Spectrum views are populated with data for the first compound in the table. The Mass Spectrum view displays the MS1 scan in the spectrum tree that is closest to the apex of the compound's chromatographic peak. The spectrum tree to the left includes the MS1 scans and the fragmentation scans for the preferred ions that were acquired within the following retention time window:

apex of the chromatographic peak for the selected compound \pm the peak's full width at half maximum (FWHM)

Note If the data set does not include data-dependent MS2 scans within the retention time window but does include AIF scans within this window, the spectrum tree includes the AIF scans.

Figure 4 shows the factory default layout for the SIL Tutorial.cdResult file.

Figure 4. Default result file layout



Tip You can hide or display any of the columns in a result table or any of the result tables in a result file.

To hide or display columns for a result table, click the table's field chooser icon (🔍). Then, in the Field Chooser dialog box, select or clear the check boxes for the columns that you want to display or hide, respectively.

To hide or display result tables, click the Select Table Visibility icon (📄). Then, in the Select Visible Tables dialog box, select or clear the check boxes for the tables that you want to display or hide, respectively.

The screen captures in this tutorial do not display all the tables and columns that are visible with the default layout.

Table 3 describes the main result tables that the selected processing workflow produces. In the active Compounds table, sorted by the Area (Max.) column, the first row displays the compound with the largest chromatographic peak area (found in one of the input files). Because the selected processing workflow includes the Mark Background Compounds node and the Analyze Labeled Compounds node, the Compounds tab has a filter icon with a check mark (🔍). The compounds that the analysis identified as background compounds are marked as background compounds in both the blank and non-blank samples and are hidden from the table. In addition, the compounds without a formula are hidden from the table.

Table 3. Main tables and some of the related tables for the selected processing workflow

Result table	Description
Visible main (top-level) tables	
Compounds	Lists all the compounds that the analysis detected, grouped by their molecular weight and retention time (MW×RT) dimensions, across all the sample input files ^a . This table also displays the background compounds and the compounds without a formula when you turn off the filter.
Compounds per File	Lists all the compounds that the analysis detected across all the “sample input files” on a per file basis. Does not list compounds that the Fill Gaps node detected by filling a full gap.
Features	Lists all the features (ions with the same mass-to-charge values and retention time) that the analysis detected across all the “sample” input files on a per file basis.
Labeled Features	Lists the ions that the analysis detected across all the “labeled” input files on a per file basis. The <i>m/z</i> column displays the <i>m/z</i> value of the unlabeled parent ion.
Labeled Compounds per File	Lists the labeled compounds, by input file, that the analysis detected across all the “labeled input files ^b .”
Metabolika Results	Lists the mapped compounds.
mzCloud Results	Lists the mzCloud search results for the detected compounds.
ChemSpider Results	Lists the ChemSpider search results for the detected compounds.
Input Files	Describes the input files that the application processed to create the result file.
Metabolika Pathways	Lists the Metabolika Pathways that contain at least one of the detected compounds.
Visible tables related to the Compounds table (tables for individual compounds)	
Structure Proposals	Displays your structure proposals for the compound selected in the Compounds table. Initially, this table is empty.
Compounds per File	Displays information about the selected compound on a per file basis.
Predicted Compositions	Displays the predicted compositions for the selected compound.
Labeled Compounds per File	Displays information about the isotopologues of the selected compound on a per file basis.
Metabolika Results	Displays the search results from the mapped Metabolika pathways for the selected compound.
mzCloud Results	Displays the mzCloud results for the selected compound.
ChemSpider Results	Displays the ChemSpider results for the selected compound.
Metabolika Pathways	Displays the mapped Metabolika pathways for the selected compound.
Visible table related to the Features table and the Labeled Features tables	
Chromatogram Peaks	Describes the chromatographic peak for the selected feature.
Visible table related to the Input Files table	
File Alignments	Describes the alignment for the input file selected in the Input File table.

^a Sample input files refers to input files that have been assigned the Sample, Control, or Standard sample type.

^b Labeled input files refers to input files that have been assigned the Labeled sample type.

The application comes with the factory default layout and four named layouts: Identification, Quantification, Statistics and Stable Isotope Labeling Layout. When reviewing the results of a stable isotope labeling analysis, apply the Stable Isotope Labeling layout.

Reviewing the Exchange Rates

❖ To apply the Stable Isotope Labeling layout to the current result file

From the application menu bar, choose **Window > Apply Layout > Stable Isotope Labeling**.

Applying the Stable Isotope Labeling layout does the following:

- Hides the following columns in the Compounds table: #Metabolika Pathways, Metabolika Pathways, Ave. Exchange, and FISh Coverage.
- Opens the Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views as tabbed views on the bottom right of the page.
- Selects the Rel. Exchange data property for the Trend Chart view.

Review the details about the detected compounds in the result tables.

❖ To review the relative exchange rates for a compound across the input file set

1. In the Compounds table, select the compound of interest.
2. Scroll to the Rel. Exchange [%] column.
3. To view the input file names, click the expand icon (⊕) next to the column heading. Or, right-click the Compounds table and choose **Expand All Column Headers** to expand all the table's column headers.

This figure shows the relative exchange rate for L-glutamic acid in each input file. The relative exchange rate for the labeled samples is 98%.

Checked	Name	Formula	Molecular Weight	RT [min]	Labeling Status	Rel. Exchange [%]					
						Ecol_12C_01.raw (F2)*	Ecol_12C_02.raw (F3)	Ecol_12C_03.raw (F4)	Ecol_13C_01.raw (F9)	Ecol_13C_02.raw (F10)	Ecol_13C_03.raw (F11)
1	L-Glutamic acid	C5 H9 N O4	147.05291	1.304	■■■■■	0	0	0	98	98	98

❖ To view the exchange rate for each isotopologue of a compound

1. In the main Compounds table, select the compound of interest.
2. To display the related tables for the selected compound, below the Compounds table, click **Hide Related Tables**.
3. Click the **Labeled Compounds per File** tab to make it the active table.
4. Scroll to the Exchange Rate [%] column.
5. To view the isotopologues, click the expand icon (⊕) next to the column heading.

This figure shows the exchange rates in the labeled (F9, F10, and F11) and unlabeled (F2, F3, and F4) samples. The exchange rates for the labeled samples are 92% for the $^{13}\text{C}_5\text{H}_9\text{NO}_4$ isotopologue and 7% for the $^{13}\text{C}_4\text{CH}_9\text{NO}_4$ isotopologue of L-glutamic acid.

The Exchange Rate [%] column contains 25 subcolumns because the analysis specified a maximum exchange rate of 25 for any of the detected compounds. Irrelevant subcolumns for unprocessed elemental compositions have a gray background. Subcolumns for isotopologues have a pink to red background that turns darker as the exchange rate increases.

Hide Related Tables																																			
Labeled Compounds per File																																			
Checked	Molecular Weight	RT [min]	# Adducts	Area	Max. Exchange	Avg. Exchange	Rel. Exchange [%]	Status	Exchange Rate [%]										Study File ID																
									0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1	147.05291	1.303	4	34611686	5	0.0	0	■	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F2	
2	147.05291	1.305	4	34065737	5	0.0	0	■	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F3
3	147.05291	1.304	4	33454584	5	0.0	0	■	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F4
4	147.05291	1.305	4	33101588	5	4.9	98	■	0	0	0	0	0	7	92																			F11	
5	147.05291	1.303	4	33065036	5	4.9	98	■	0	0	0	0	0	7	92																			F10	
6	147.05291	1.303	4	32389096	5	4.9	98	■	0	0	0	0	0	7	92																				F9
7	147.05291	1.301	3	2160	5	0.1	1	■	98	0	0	2	0	0																				F1	

Reviewing the Labeling Status

❖ To view the exchange rates for the adducts of a compound in a specific input file

1. In the related Labeled Compounds per File table, select the input file of interest.
2. Below the related Labeled Compounds per File table, click **Show Related Tables**.
3. Click the **Labeled Features** tab to make it the active table.

This figure shows the relative amounts (by chromatographic peak area) of the labeled adduct ions that the analysis detected for L-glutamic acid in input file F11 (a labeled sample).

Ion	Molecular Weight	m/z	RT [min]	Area	Max. Exchange	Rel. Exchange [%]	Exchange Rate [%]													
							0	1	2	3	4	5	6	7	8	9	10			
1 → [M+H] ⁺ +1	147.05316	148.06043	1.305	27610405	5	98	0	0	0	0	7	92								
2 → [M+H-H ₂ O] ⁺ +1	147.05316	130.04987	1.304	5397625	5	98	0	0	0	0	7	92								
3 → [M+H-NH ₃] ⁺ +1	147.05316	131.03389	1.303	49576	5	96	0	0	0	0	21	79								
4 → [2M+H] ⁺ +1	147.05316	295.11359	1.305	43982	5	100	0	0	1	0	0	99								

SFit [%]	Study File ID
49	F11
81	F11
57	F11
100	F11

The Labeling Status column in the Compounds table and the Status column in the Labeled Compounds per File table provide information about the quality of the analysis.

- Red—Indicates a contaminating mass in an unlabeled sample.
- Blue—Indicates an irregular exchange rate for a labeled sample.
- Orange—Indicates a low fit between the measured and fitted isotope patterns.
- Gray—Indicates the absence of isotopologues for the detected compound.

A contaminating mass in an unlabeled sample is more problematic than an irregular exchange rate for a labeled sample.

❖ To investigate a contaminating mass in an unlabeled sample

1. In the Compounds table for the example result file, sort the compounds in descending order by Area (Max.).
2. Select **row 4** (Cuauhtemone).
3. Click the expand icon for the Labeling Status column.

Because you grouped the samples by sample type (Figure 3), the samples are also grouped by sample type in the Labeling Status column.

Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	Labeling Status
<input type="checkbox"/>	Cuauhtemone	C ₁₅ H ₂₄ O ₃		252.17215	10.771	14071337	<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>Ecoli_12C_01.raw (F2) *</p> <p>Ecoli_12C_02.raw (F3)</p> <p>Ecoli_12C_03.raw (F4)</p> </div> <div style="width: 45%;"> <p>Ecoli_13C_01.raw (F9)</p> <p>Ecoli_13C_02.raw (F10)</p> <p>Ecoli_13C_03.raw (F11)</p> </div> </div>

■ Unlabeled samples ■ Labeled samples

4. Below the Compounds table, click **Show Related Tables**.
5. To display the information for the compound selected in the Compounds table by input file, click the **Labeled Compounds per File** tab.
6. In the Labeled Compounds per File table, click the expand icon for the Exchange Rate [%] column

This figure shows the labeling status for Cuauhtemone in the Compounds table, and the exchange rate per input file in the Labeled Compounds per File table. In the Compounds table, the red status for input files F2, F3, and F4 indicates the presence of a contaminating mass in the unlabeled samples—F2, F3, and F4. In the Labeled Compounds Per File table, the Exchange Rate [%] column shows that the contaminating mass is possibly a compound with a mass of M+4.

Hide Related Tables																													
Structure Proposals			Compounds per File			Predicted Compositions			Labeled Compounds per File			Metabolika Results			mzCloud Results			ChemSpider											
	Molecular Weight	RT [min]	Area	Max. Exchange	Avg. Exchange	Rel. Exchange [%]	Status	Exchange Rate [%]											Study File ID										
								0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1	252.17215	10.806	1407133	15	4.0	27	■	0	0	0	0	99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F1
2	252.17215	10.797	1801443	15	4.0	27	■	1	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F3
3	252.17215	10.798	1808484	15	3.9	26	■	2	0	0	0	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F4
4	252.17215	10.805	1779693	15	4.0	27	■	1	0	0	0	97	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F2
5	252.17215	10.774	1753128	15	4.0	27	■	1	0	0	0	94	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F9
6	252.17215	10.798	1757971	15	4.0	27	■	2	0	0	1	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F10
7	252.17215	10.797	1801720	15	4.0	26	■	2	0	0	1	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	F11

Working with the Trend Chart View

When you apply the Stable Isotope Labeling layout, the Trend Chart view opens as a hidden view below the Isotopologues Distribution Chart view.

Use the Trend Chart view to compare the relative exchange rate [%] for each compound by input file, sample group, or study variable (for example, the time points in a metabolic flux study). When you select a single compound in the Compounds table, you can view its distribution as a box-and-whiskers plot or as a trend line plot. When you select multiple compounds in the Compounds table, the application automatically displays the distribution for each compound as a trend line plot.

Note The example data set does not include metabolic flux samples.

Follow these procedures:

- [To view a trendline plot for a compound](#)
- [To view a box-and-whiskers plot for a compound](#)

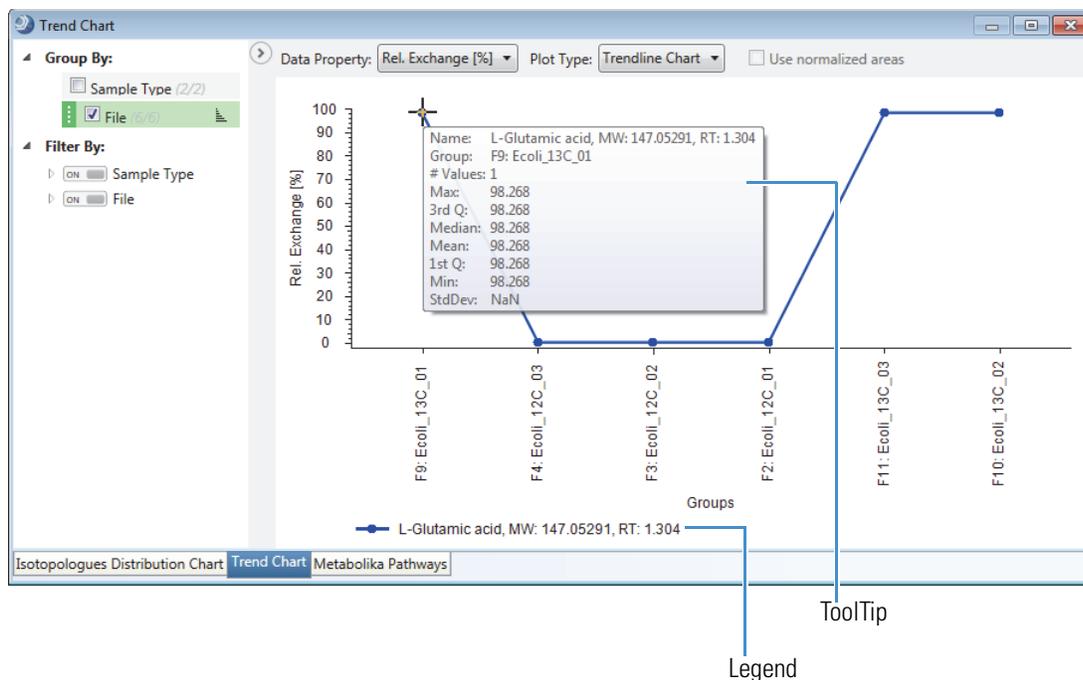
❖ To view a trendline plot for a compound

1. To sort the main Compounds table in descending order by area, click the Area (Max.) column heading. For the example data set, L-glutamic acid sorts to the top of the table.

Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	Labeling Status	Rel. Exchange [%]													
							EcolL_12C_01.raw (F2)*	EcolL_12C_02.raw (F3)	EcolL_12C_03.raw (F4)	EcolL_13C_01.raw (F9)	EcolL_13C_02.raw (F10)	EcolL_13C_03.raw (F11)								
L-Glutamic acid	C5 H9 N O4	■■■■	147.05291	1.304	34611686	■■■■ ■■■■	0	0	0	98	98	98								

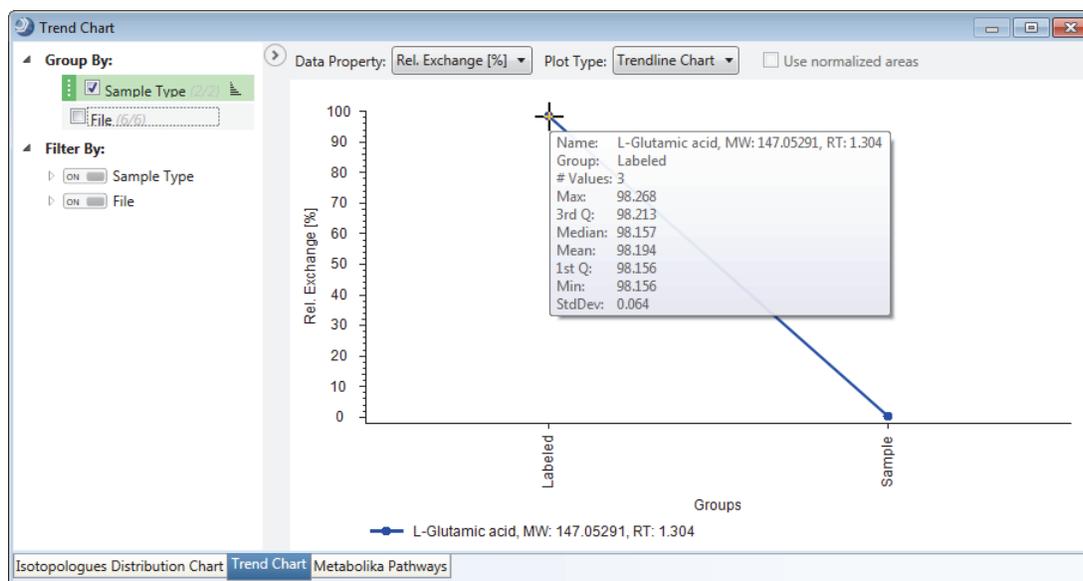
2. In the Compounds table, select **L-Glutamic Acid**.
3. In the set of tabbed views to the right of the result table, click the **Trend Chart** tab. The Trend Chart view displays a trendline plot for the relative exchange rate per input file.
4. Right-click the chart and choose **Show Legend**.
5. To display a ToolTip with descriptive statistics, point to a data point.

This figure shows the trendline plot for L-glutamic acid with the samples grouped by input file.



- To view the samples grouped by sample type, in the left pane, under Group By, clear the **File** check box and select the **Sample Type** check box.

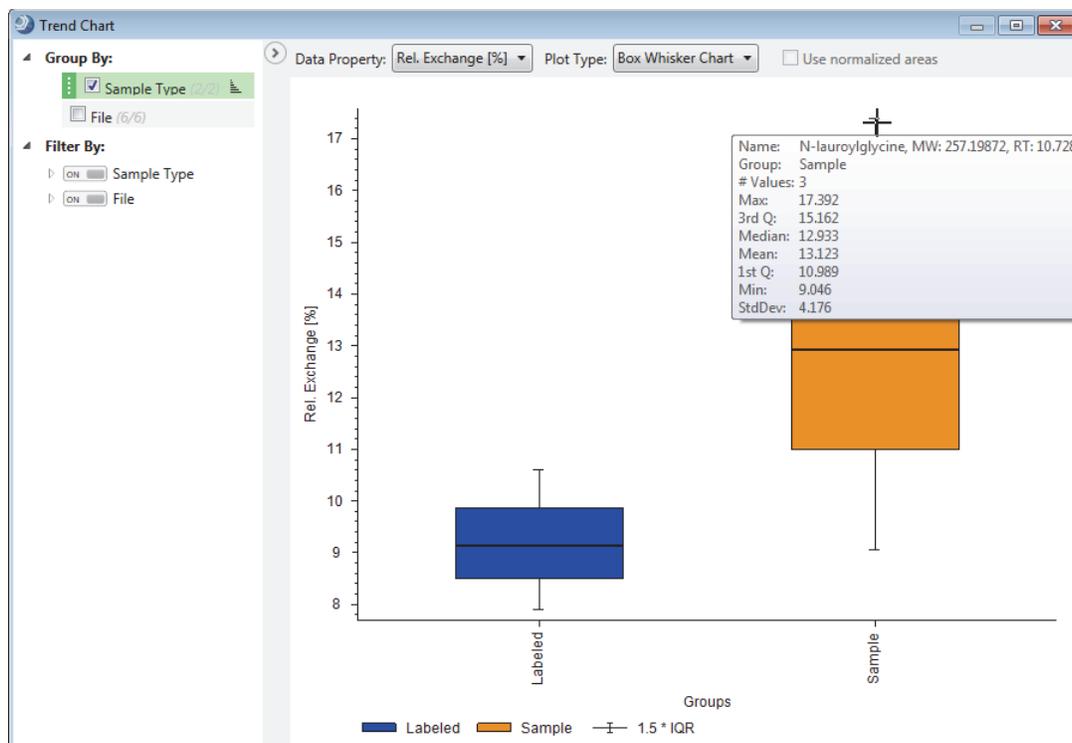
This figure shows the trendline plot for L-glutamic acid with the samples grouped by sample type (labeled versus unlabeled).



❖ **To view a box-and-whiskers plot for a compound**

- In the Compounds table (sorted in descending order by Area (Max.)), select **row 29** (N-lauroylglycine).
- To view the samples grouped by sample type, in the left pane, under Group By, clear the **File** check box and select the **Sample Type** check box.
- In the Plot Type list, select **Box Whiskers** chart.
- Right-click the chart and choose **Show Legend**.
- To display a ToolTip with descriptive statistics, point to a whisker.

This figure shows the box-and-whiskers plot for N-lauroylglycine with the samples grouped by sample type.



Working with the Isotopologues Distribution Chart

Use the Isotopologues Distribution Chart view to visualize the distribution of a compound's isotopologues.

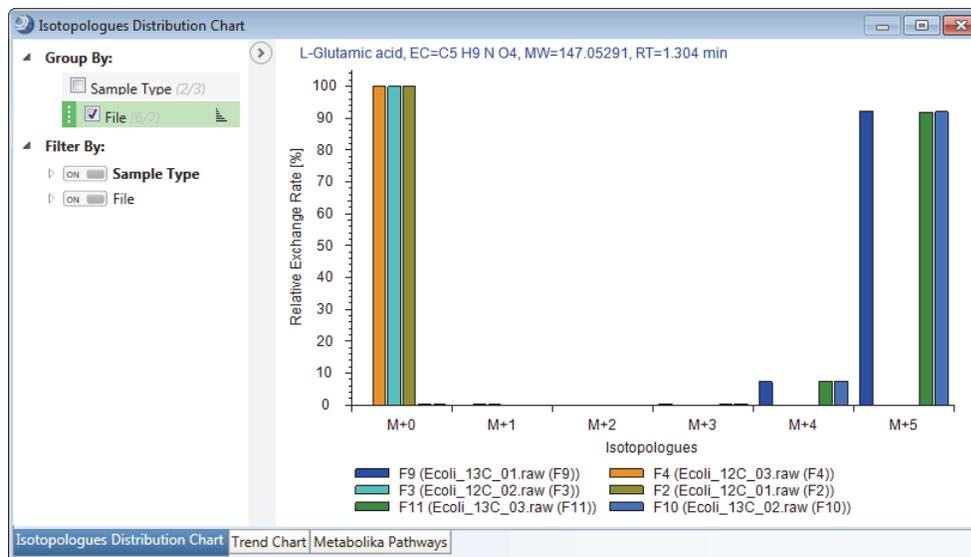
❖ To view the distribution of a compound's isotopologues

1. Apply the Stable Isotope Labeling layout to the result file (see [“Applying the Stable Isotope Labeling Layout”](#) on page 16).

The Isotopologues Distribution Chart view opens to the right of the result tables.

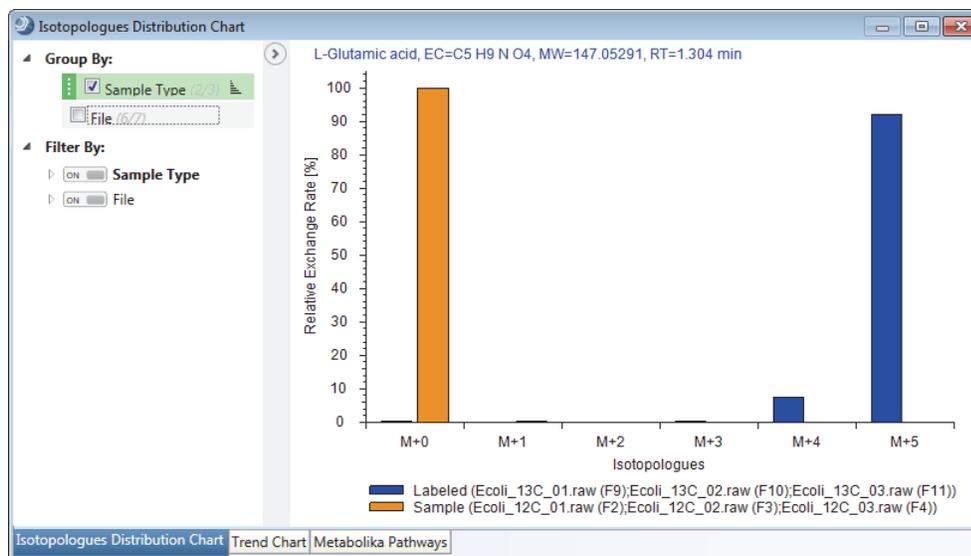
2. In the Compounds table, select a compound of interest.

This figure shows the distribution for L-glutamic acid with the samples grouped by input file.



3. To group the samples by Sample Type, under Group By, clear the File check box and select the Sample Type check box.

This figure shows the isotopologue distribution for L-glutamic acid with the samples grouped by sample type.



Viewing the Metabolika Pathways for a Compound

The Map to Metabolika Pathways node (in the selected processing workflow) returns a set of mapped pathways for each detected compound.

❖ To view the Metabolika pathways that include a selected compound

1. Apply the Stable Isotope Labeling layout to the result file (see “Applying the Stable Isotope Labeling Layout” on page 16).

The Isotopologues Distribution Chart, Trend Chart, and Metabolika Pathways views open as tabbed views on the bottom right of the page.

2. In the example result file, sort the Compounds table by the Area (Max.) column in descending order.
3. Select **row 1** (L-glutamic acid).
4. Click **Show Related Tables** to display the related tables for the selected compound.
5. To view a Metabolika pathway that includes this compound, do the following:
 - a. Click the **Metabolika Pathways** tab to make it the active table.
 - b. For this tutorial, scroll down to **row 93**—the **L-glutamate degradation IX (via 4-aminobutanoate)** pathway and select it.

This figure shows the selected Metabolika pathways file.

Hide Related Tables			
Structure Proposals		Compounds per File	
Metabolika Results		mzCloud Results	
		ChemSpider Results	
		Labeled Compounds per File	
		Metabolika Pathways	
<input type="checkbox"/>	Checked	Pathway Name	# Referenced Compounds
<input type="checkbox"/>	<input type="checkbox"/>	L-glutamate degradation VIII (to propanoate)	4
<input type="checkbox"/>	<input type="checkbox"/>	L-tyrosine degradation IV (to 4-methylphenol)	4
<input type="checkbox"/>	<input type="checkbox"/>	Superpathway of dTDP-glucose-derived antibiotic building blocks b	4
<input type="checkbox"/>	<input type="checkbox"/>	Superpathway of L-phenylalanine and L-tyrosine biosynthesis	4
<input type="checkbox"/>	<input checked="" type="checkbox"/>	L-glutamate degradation IX (via 4-aminobutanoate)	3
<input type="checkbox"/>	<input type="checkbox"/>	Superpathway of GDP-mannose-derived O-antigen building blocks	3
<input type="checkbox"/>	<input type="checkbox"/>	Superpathway of rosmarinic acid biosynthesis	3

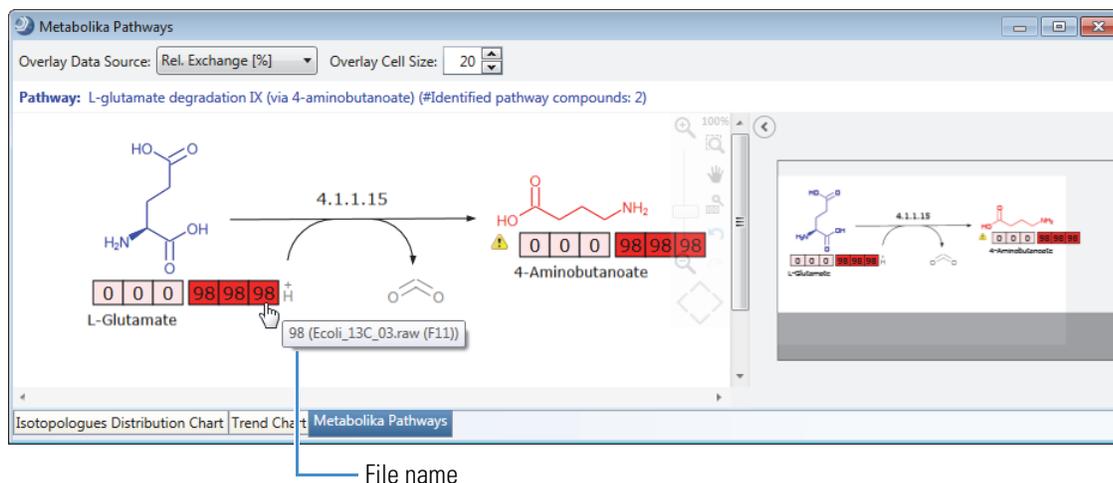
- c. In the tabbed views to the right, click the **Metabolika Pathways** tab.

The mapped pathway appears in the Metabolika Pathways view. The Stable Isotope Labeling layout automatically selects Rel. Exchange [%] as the overlay data source with an overlay cell size of 10 pixels.

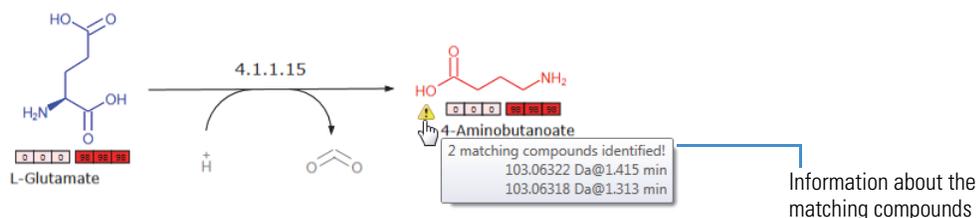
The structure for the compound that you selected in the Compounds table is blue, the structures for other detected compounds are red, and the structures for undetected compounds in the pathway are black.

6. To enlarge the overlaid data, increase the value in the Overlay Cell Size box (Range: 6 to 30 pixels in width).
7. To view the file name for a specific value, point to the value.

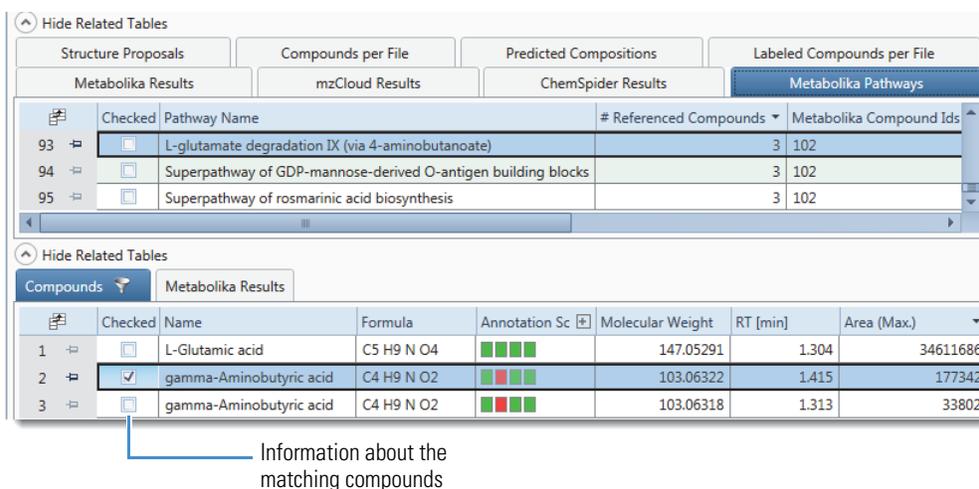
This figure shows the selected Metabolika pathway with an overlay of the relative exchange [%] data for the selected compound—L-glutamate. The overlay cell size has been increased to 20 pixels. A Caution symbol next to a compound indicates that the analysis found multiple matches.



8. To view information about the matching compounds for a structure with multiple matches, point to the Caution symbol.



9. To keep only the appropriate explanation for the structure, mark the incorrect explanation as a background compound as follows:
 - a. Open the related Compounds table.



- b. Open the Field Chooser dialog box for the related Compounds table and select the check box for the Background column.

The Background column appears in the related Compounds table.

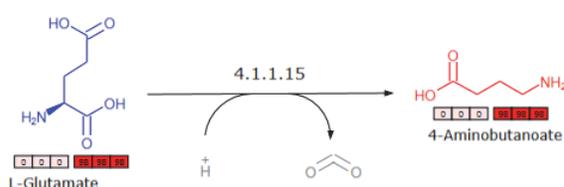
c. To mark a compound as a background compound, select its check box in the Background column.

Hide Related Tables											
Compounds Metabolika Results											
	Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	Metab	Match	MS2	Background
1	<input type="checkbox"/>	L-Glutamic acid	C5 H9 N O4	■■■■	147.05291	1.304	34611686	102	100.8	■	<input type="checkbox"/>
2	<input type="checkbox"/>	gamma-Aminobutyric acid	C4 H9 N O2	■■■■	103.06322	1.415	177342	500		■	<input type="checkbox"/>
3	<input type="checkbox"/>	gamma-Aminobutyric acid	C4 H9 N O2	■■■■	103.06318	1.313	33802	500		■	<input checked="" type="checkbox"/>

The background compound disappears from the table.

Hide Related Tables											
Compounds Metabolika Results											
	Checked	Name	Formula	Annotation Source	Molecular Weight	RT [min]	Area (Max.)	Metab	Match	MS2	Background
1	<input type="checkbox"/>	L-Glutamic acid	C5 H9 N O4	■■■■	147.05291	1.304	34611686	102	100.8	■	<input type="checkbox"/>
2	<input type="checkbox"/>	gamma-Aminobutyric acid	C4 H9 N O2	■■■■	103.06322	1.415	177342	500		■	<input type="checkbox"/>

In the Metabolika pathways view, the Caution symbol below the structure disappears (and the structure remains red).



Exporting the Analysis Results

Using the Result Filters to Select the Compounds of Interest

To create a report for your records, filter the compounds table to display only the compounds of interest, and then export the results using the appropriate format.

Follow these procedures to filter the Compounds table and export the results:

- Using the Result Filters to Select the Compounds of Interest
- Exporting the Results to a Spreadsheet

The analysis detected a total of 3155 compounds, including 1232 hidden compounds that were marked as background compounds or compounds without a formula. To reduce the number of compounds to export, filter the table or select the check boxes for the compounds of interest.

Note Pointing to the vertical scroll bar on the right displays a ToolTip with the number of compounds in the table.

Rel. Exchange [%]											
0	0	0	98	98	98						
0	0	0	98	98	98						
10	8	8	13		0						
27	27	26	27	27	26						

Compounds grouped by molecular weight and retention time
1923 of 3155 items shown (1232 filtered out)

Follow either of these procedures:

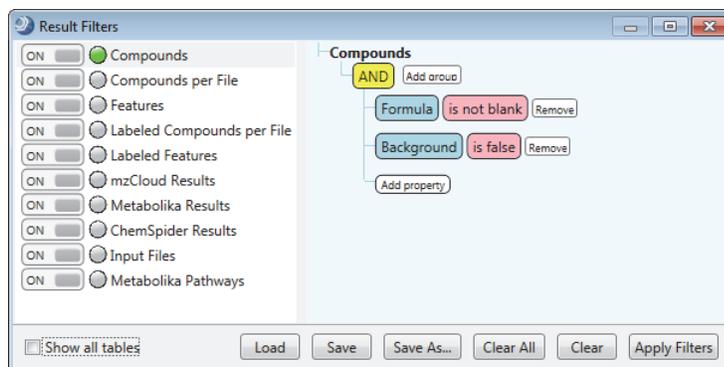
- To reduce the number of compounds to export by filtering the Compounds table
- To filter the Compounds table by the checked compounds

❖ To reduce the number of compounds to export by filtering the Compounds table

- Click the **Compounds** tab to make it the active table.
- From the application menu bar, choose **View > Result Filters**.

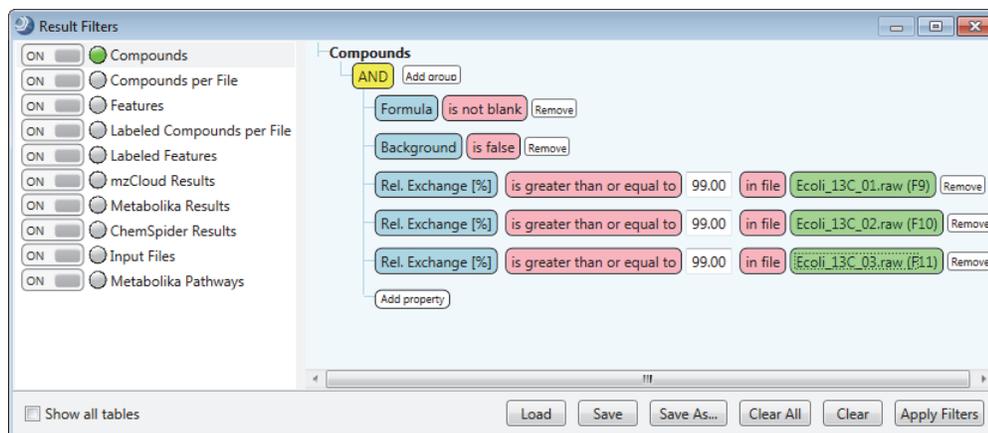
The Result Filters view opens as a floating window. Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the filter for the Compounds table already includes a filter for background compounds and a filter for components without a formula.

This figure shows the default filters for the example result file.



3. On the right side of the Result Filters view, set up filters for the relative exchange rate as follows:
 - a. Click **Add Property**, and then select **Rel. Exchange [%]** from the list.
 - b. In the pink relation list, select **Is Greater Than or Equal To**.
 - c. In the value box next to the relation list, type **99**.
 - d. In the pink condition list, select **In File**.
 - e. In the Green sample list, select one of the labeled input files.
 - f. Repeat steps [step 3a](#) through [step 3e](#) to add a filter for all three labeled input files.

This figure shows the filter set.



4. Click **Apply Filters**.

The applied filter set reduces the number of displayed rows in the Compounds table to 90.
5. To undo the relative exchange filters, click **Remove** to their right. Then, click **Apply Filters** again.

The Compounds table contains the original set of compounds.

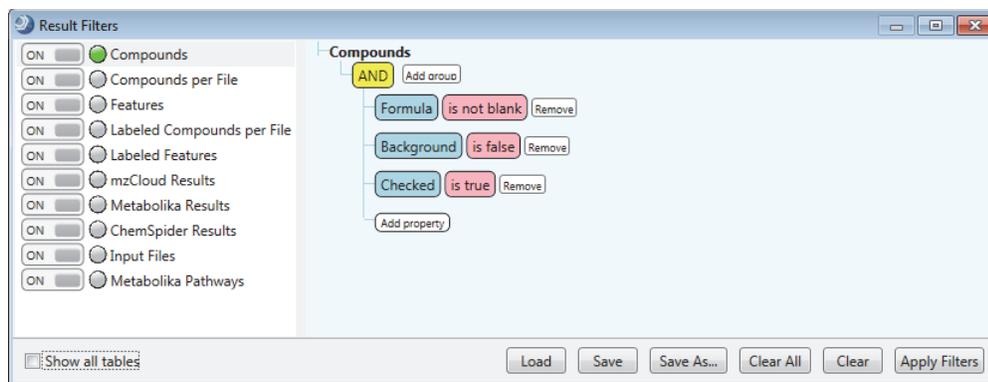
❖ **To filter the Compounds table by the checked compounds**

1. If the Compounds table is not the active table, click its tab to make it active.
2. Manually select the check boxes for the compounds of interest.
3. From the menu bar, choose **View > Result Filters**.

Because the processing workflow included the Mark Background Compounds node and the Analyze Labeled Compounds node, the Compounds table is currently filtered by two properties—Background and Formula.

4. Click **Add Property** and select **Checked**.

This figure shows the filter set.



5. Click **Apply Filters**.

The Compounds table displays only the selected compounds.

6. To undo the Checked filter, click **Remove** to its right. Then, click **Apply Filters** again.

The Compounds table contains the original set of compounds.

Exporting the Results to a Spreadsheet

Before exporting the results to a spreadsheet, filter the results table as described in “Using the Result Filters to Select the Compounds of Interest” on page 24 or select the check boxes for the compounds of interest.

To create a report, follow these procedures as needed:

- To check the number of table rows
- To display the table columns that you want to export
- To sort the rows
- To export the filtered and sorted results to an Excel™ spreadsheet

❖ **To check the number of table rows**

Point to the vertical scroll bar to the right of the compounds table.

A ToolTip appears with the row count.

❖ **To display the table columns that you want to export**

Open the Field Chooser box and select the check boxes for the columns of interest and clear the other check boxes.

❖ **To sort the rows**

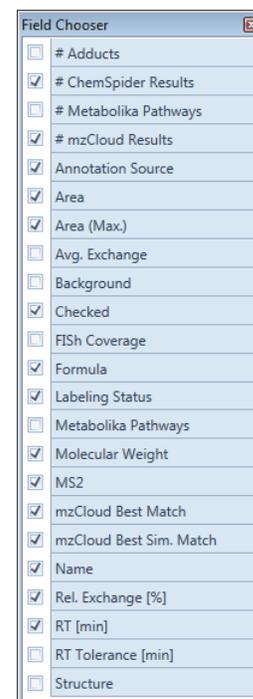
Click the column heading that you want to sort by.

❖ **To export the filtered and sorted results to an Excel™ spreadsheet**

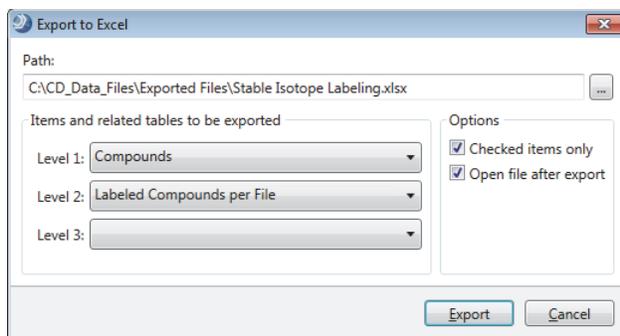
1. Right-click the Compounds table and choose **Export > Export to Excel**.

The Export to Excel dialog box opens.

2. Check the file name and location in the Path box. Then, change the file name and location as appropriate.



3. In the Options area, select the **Checked Items Only** and **Open File After Export** check boxes.



4. Click **Export**.

The Excel spreadsheet opens.

Opens the related data table.

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