MassHunter VistaFlux Software

Quick Start Guide

For Research Use Only. Not for use in diagnostic procedures.

What is MassHunter VistaFlux Software?

The Agilent MassHunter VistaFlux software provides you with an intuitive workflow to perform your qualitative flux analysis and visualize your results on pathways network diagrams. The MassHunter tools necessary to perform a qualitative flux analysis are referred to collectively as MassHunter VistaFlux Software and consists of four software programs. The software suite is illustrated in context of the qualitative analysis workflow in Figure 1 on page 2.

MassHunter Pathways to PCDL

The workflow begins with your experiment design. *Pathways to PCDL* facilitates the creation of a custom personal compound database and library (PCDL) from metabolites present in pathway content you cull from popular databases such as

BioCyc/MetaCyc, KEGG, and WikiPathways. You can filter and select pathways based on database, organism, and/or custom text entries to generate a preliminary PCDL that contains compounds related to your experiment design.

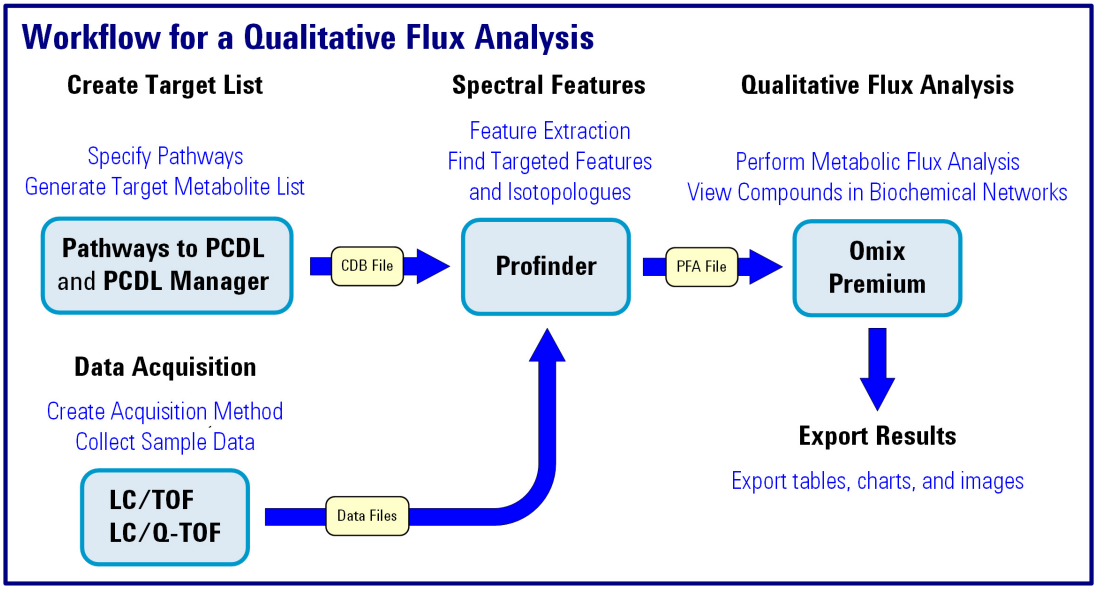


Figure 1 Illustration of a qualitative flux analysis workflow using MassHunter VistaFlux Software.

MassHunter PCDL Manager

After your preliminary PCDL is created, *PCDL Manager* helps you manage the compounds and compound content within your PCDL to create your target metabolite database used during your qualitative flux analysis. You can add new compounds manually or from existing PCDLs, remove compounds, and add additional compound information, such as formulas, identifiers, retention times, and structural information. The compounds in your target metabolite database must contain, at a minimum, identification, mass, and retention time (based on your sample data acquisition method). For identification, use one or more of the following examples - molecular formula, CAS, HMP, KEGG.

MassHunter Profinder Profinder uses your target metabolite database and extracts the target metabolites (compound features) and their isotopologues from your sample data files. Profinder is optimized to extract isotopologue compound features from large data sets and provides you with an intuitive user interface to inspect and review each compound feature across the files associated with your data set. With Profinder, you can review and compare extracted ion chromatograms, mass spectral data, and isotopologues associated with each compound feature. When your extraction method is complete you export your results as a Profinder archive file.

Omix Premium Omix Premium is used to create pathways network diagrams and view your Profinder results in the context of biochemical networks, including isotopologue results. Your Profinder archive files, which contain extracted compound features and sample group information, are imported into Omix Premium where you create customized visualizations of your qualitative flux analysis results.

What's new in 1.0?

- Extend the power of your metabolomics research by studying the metabolic network in motion.
- Speed up your analysis using an integrated workflow to process isotopologue data.
- Create target lists, extract batch isotopologues, and visualize results on pathways.
- Review and edit isotopologue results with ease using an intuitive interface.
- Interpret results and communicate with colleagues using pathway images and videos of qualitative fluxes.
- Import KEGG or BioCyc content to build pathway models.
- Create confidence in your results using t-test and ANOVA statistics on isotopologue data.

Qualitative Flux Analysis

Qualitative flux analysis comprises the steps to identify target metabolites of interest for your experiment, create visual pathways networks for your experiment, acquire accurate mass data from your samples, extract accurate isotopic metabolite information from the sample data, and then view the results on network diagrams of the pathways involved in your experiment. A qualitative flux analysis that uses VistaFlux follows the flow illustrated in Figure 2.

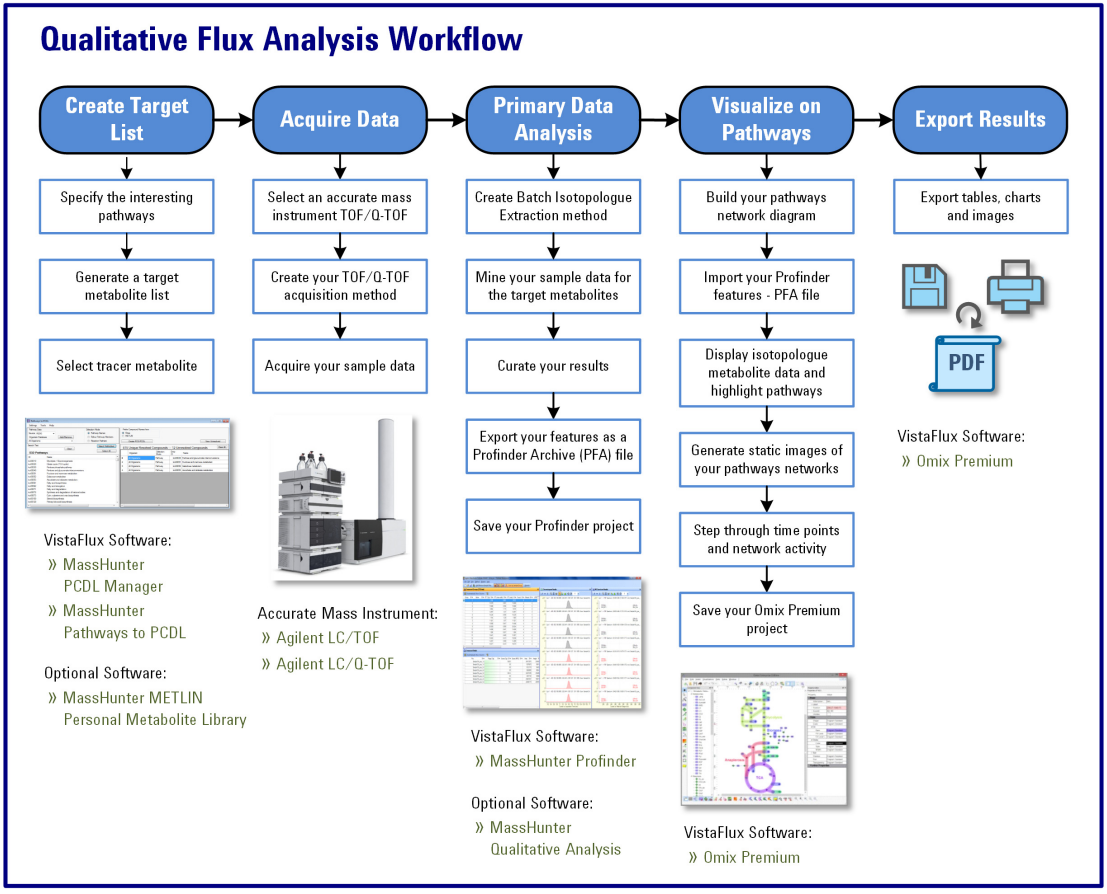


Figure 2 Workflow to perform an Agilent qualitative flux analysis

Where to Find More Information

Online Help

Press F1 To get more information about a window or dialog box, place the cursor on the window or dialog box of interest and press **F1**.

Help Menu Click **Help** or **Help > Contents**, depending on the software tool your are using, to access the contents of online Help which includes information on wizards, basic tasks, user interface, and reference information.

Click **Help** for information specific to wizards.

Documents

VistaFlux Software/Omix Premium

- MassHunter VistaFlux Software - Workflow Guide
- Agilent MassHunter VistaFlux for Qualitative Flux Analysis - Technical Overview

Pathways to PCDL

- MassHunter Pathways to PCDL Software - Quick Start Guide

PCDL Manager

- MassHunter Personal Compound Database and Library Manager - Quick Start Guide
- MS/MS Library Creation of Q-TOF LC/MS Data for MassHunter PCDL Manager - Quick Start Guide

Profinder

- MassHunter Profinder Software - Quick Start Guide
- Agilent MassHunter Profinder: Solving the Challenge of Isotopologue Extraction for Qualitative Flux Analysis - Technical Overview

Getting Started

How do I get started?

This *Quick Start Guide* helps you install VistaFlux Software, review the software user interfaces, and perform basics tasks using the files found in the *Data* folder on the installation DVD.

Figure 3 Desktop icons for MassHunter VistaFlux Software: Pathways to PCDL, PCDL Manager, Profinder, and Omix Premium

1. Install the VistaFlux Software. Follow the instructions in "MassHunter VistaFlux Software Installation" on page 58.
2. Review the *MassHunter VistaFlux Software - Workflow Guide*.
3. Review the following sections in this *Quick Start Guide*:

   "Software User Interfaces" on page 7

   "MassHunter Pathways to PCDL" on page 7

   "MassHunter PCDL Manager" on page 8

   "MassHunter Profinder" on page 11

   "Omix Premium" on page 18

   "Definitions" on page 27

   "Basic Qualitative Flux Analysis Workflow" on page 30

   "What is Batch Isotopologue Extraction?" on page 54
4. Perform your qualitative flux analysis following the"Basic Qualitative Flux Analysis Workflow" on page 30.

Software User Interfaces

A compound may be referred to as a feature, metabolite, molecular feature, element, or entity during the various steps of analysis using Agilent MassHunter software.

Help and detailed information regarding the various parameters and statistical treatments are available when you press **F1** or click **Help > Contents** from the menu bar.

MassHunter Pathways to PCDL 

The main Pathways to PCDL window consists of two parts: the Menu Bar, and Display Pane. The window areas are shown in Figure 4.

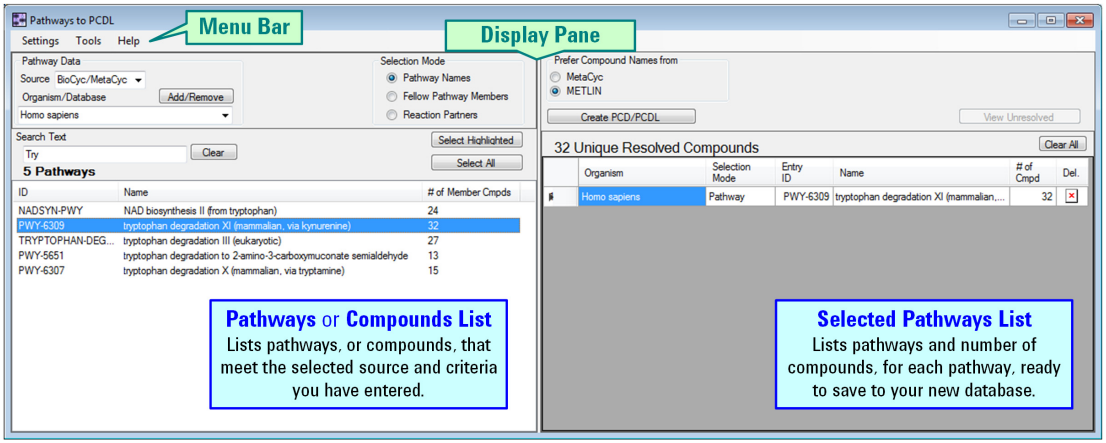


Figure 4 The main functional areas of Pathways to PCDL

1. Menu Bar

The menu bar (Figure 5 on page 8) provides actions that are used for finding pathways and creating a list of pathways from which compounds are extracted to create your PCDL.

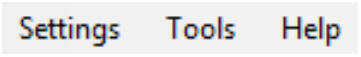


Figure 5 Pathways to PCDL Menu bar

Settings Launches the **Pathways to PCDL Settings** dialog box where you can specify a reference METLIN database and choose whether to exclude compounds if the organism has no related enzymes when you are using the KEGG database.

Tools Provides options to update the local copy of pathway content culled from popular databases such as BioCyc/MetaCyc, KEGG, and WikiPathways. You can also import BioCyc tarball and zip files. Access to KEGG pathway content requires a separate license.

Help Provides a link to the *MassHunter Pathways to PCDL Software - Quick Start Guide* and information about the software version.

2. Display Pane

The *Display Pane*, see Figure 4 on page 7, is further divided into two panes – (1) Pathways or Compound List and (2) Selected Pathways List. The *Display Pane* helps you visualize your progress as you select pathways to create your PCDL. The number of pathways and compounds that meet your criteria are shown above each table in the *Display Pane*.

MassHunter PCDL Manager 

The main PCDL Manager window consists of five parts: Menu Bar, Toolbar, Action Tabs, Action Pane, and Compound Results. The number of compounds that meet your search criteria are shown above the table in the *Compounds Results*. The window areas are shown in Figure 6 on page 9.

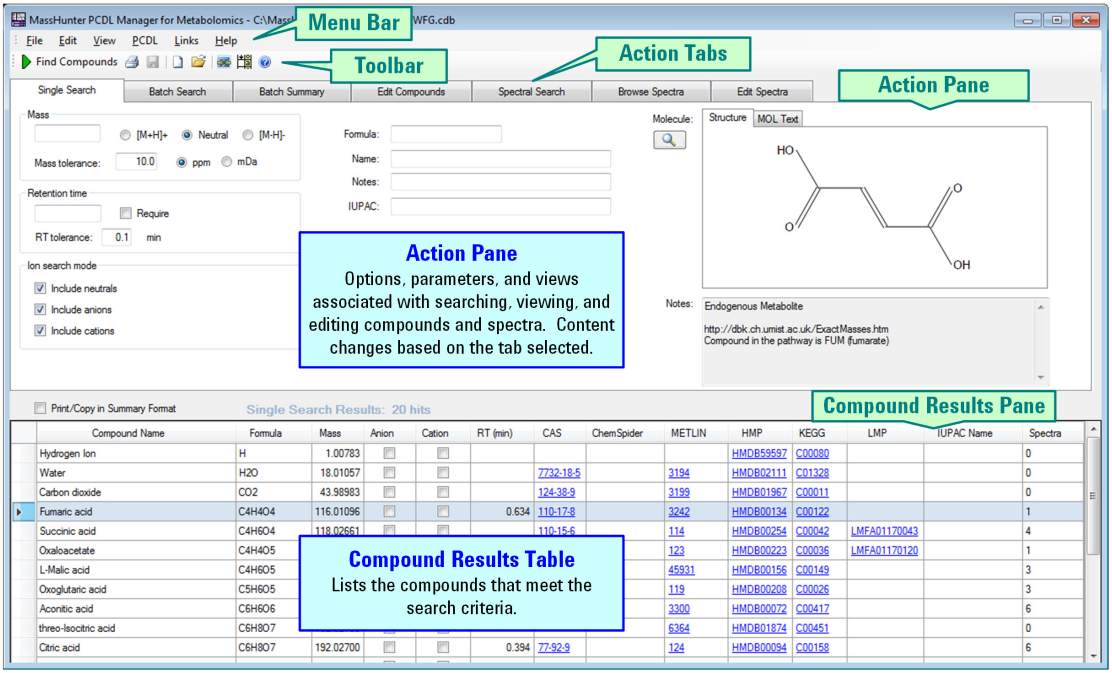


Figure 6 The main functional areas of PCDL Manager

1. Menu Bar

The menu bar (Figure 7) provides actions that are used to create your custom PCDL.

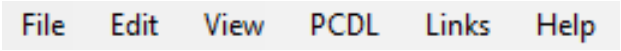


Figure 7 PCDL Manager Menu bar

File Open, close, backup, and create new PCDLs. You can also create subset PCDLs, import compounds from another PCDL, and print and export your search results.

Edit Add, delete, save as, and update compounds in your PCDL. These commands are for use with custom PCDLs when (1) **PCDL > Allow Editing** is enabled and (2) the *Edit Compounds* tab is selected in the *Action Pane*.

View Manage the columns in the compound results table in the *Compounds Results Pane* and view the structure details in a larger **Compound** window.

PCDL Search for compounds and spectra in the open PCDL, toggle the **Allow Editing** mode, update your PCDL to the current version of *PCDL Manager*, and review information about your PCDL.

Links Open your default Internet browser to the selected database.

Help Provides a link to online Help and information about the software version.

2. Toolbar

The toolbar is located below the menu bar and contains three groups of buttons for commonly performed tasks:

PCDL Manager Toolbar

Button	Equivalent Command
	**PCDL > Find Compounds**
	**File > Print Results**
	**File > Export Results** (from Batch Summary tab)
	**File > New PCDL**
	**File > Open PCDL**
	Auto-size Columns, no equivalent command
	Hide Empty Columns, no equivalent command
	**Help > Contents**

3. Action Tabs

The first four tabs (Single Search, Batch Search, Batch Summary, and Edit Compounds) provide options for you to search, view, and edit compounds in the open PCDL. The last

three tabs (Spectral Search, Browse Spectra, and Edit Spectra) provide options for you to search, browse, and edit spectra in the open PCDL.

4. Action Pane

The *Action Pane* is where you enter and select parameters, information, and options, and then you view the compound and spectra results. Content of the *Action Pane* changes based on the tab selected.

5. Compound Results Pane

Lists the compounds that meet the search criteria. This pane is not available when you select the *Spectral Search* tab.

MassHunter Profinder 

The main Profinder window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in Figure 9 on page 12.

1. Menu Bar

The menu bar (Figure 8) provides actions that you use to manage your projects, methods, display, and extract features.

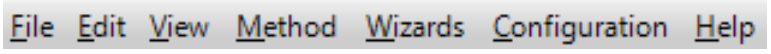


Figure 8 Profinder Menu bar

File Open, close, and save projects. You can also add or remove sample files from your project and export your results.

Edit Access to the **Copy to Clipboard** and **Color by Sample Group** operations.

View Show or hide the windows used to review the results from applying the feature extraction method to your data set (see "3. Main Window" on page 14).

Method Open and save your batch extraction methods.

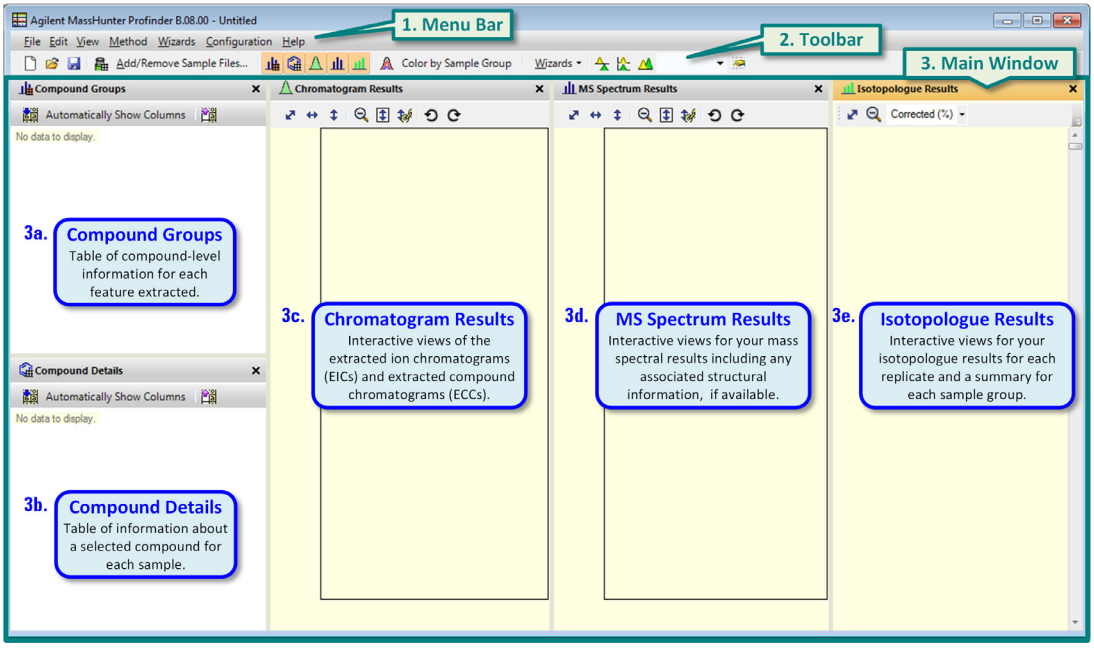


Figure 9 The main functional areas of Profinder as viewed before you begin a project.

Wizards Run one of the feature extraction algorithms. Each extraction algorithm is designed to efficiently extract the features (compounds) in your sample data files. Batch isotopologue extraction results are exported as a Profinder Archive (PFA) file and imported into Omix Premium as part of your qualitative flux analysis.

Configuration Launch the **Plot Display Options** dialog box where you can customize how chromatograms and spectra are displayed.

Help Provides a link to online Help and information about the software version.

2. Toolbar

The toolbar is located below the menu bar and contains five groups of buttons for commonly performed tasks:

Project New project, Open project, and Save project

Samples Add sample files to your new or current project.

Main Window Display or hide the various tables and results generated by Profinder, so you can increase the available display area for your review.

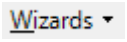
Feature Coloring Toggle the feature coloring by sample group.

Extraction Select the feature selection algorithm you want to review, edit, and apply to your data set.

Results Modes Select the display mode to use in your results windows.

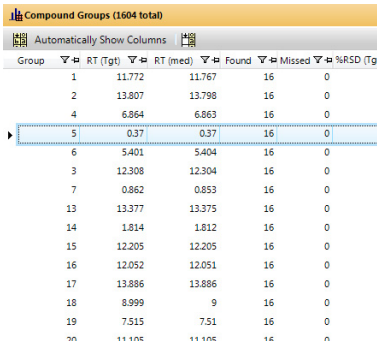
Profinder Toolbar

Button	Equivalent Command
	File > New Project
	File > Open Project
	File > Save Project
	File > Add/Remove Sample Files
	View > Compound Groups
	View > Compound Details
	View > Chromatogram Results
	View > MS Spectrum Results
	View > Isotopologue Results
	Edit > Color by Sample Group

Button	Equivalent Command
	**Wizards > Batch Molecular Feature Extraction** **Wizards > Batch Recursive Feature Extraction (small molecules / peptides)** **Wizards > Batch Recursive Feature Extraction (large molecules)** **Wizards > Batch Target Feature Extraction** **Wizards > Batch Isotopologue Extraction**
	List mode, no equivalent command
	Sample group mode, no equivalent command
	Overlaid mode, no equivalent command
	Maximum number of chromatograms or spectra to display, no equivalent command
	**Configuration > Plot Display Options**

3. Main Window

The main window, see Figure 9 on page 12, is further divided into up to five windows – (3a) Compound Groups, (3b) Compound Details, (3c) Chromatogram Results, (3d) MS Spectrum Results, and (3e) Isotopologue Results that are used to review the results from applying the feature extraction method to your data set. Each window can be floated independently to any location and size on your computer display or arranged to your preference within the main window. The various windows are described in the following paragraphs.

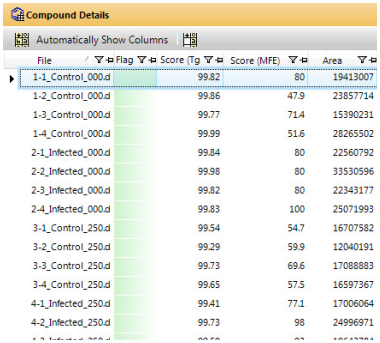


Compound Groups The data presented in Compound Groups is organized as a list of all of your extracted feature data averaged and summarized across all of the data files in your project.

The Compound Groups window shows a table of compound-level information for each feature extracted from at least one data file, if the data was extracted using Batch Molecular Feature Extraction, or for all targeted features, if the data was extracted using Batch Targeted Feature Extraction or Batch Isotopologue Extraction. Measured information is shown as the average value for the feature across all of the files where the feature was found.

A *compound group* is a single compound (feature) found in any one or more of the data files in a project. For example, if the first data file in the project yields 35 compounds, then at least 35 compound groups are in the project. If additional unique compounds are found in the other data files, then additional compound groups are created.

Information regarding the available columns are found in the online Help in the topic “Compound Groups Columns.” A list of the available columns is displayed when you right-click within the Compound Groups table, and then click **Add/Remove Columns**.

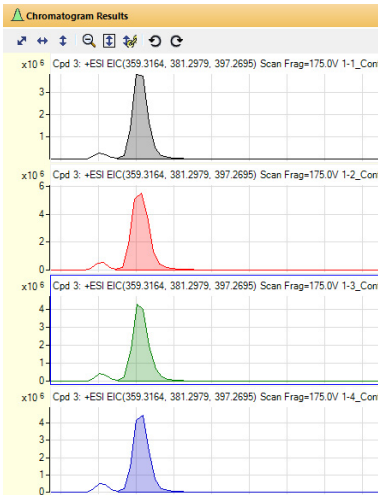


Compound Details The data presented in Compound Details is organized as a list of the appearance of a selected feature (compound) in all of the data files in your project - *feature information by data file*.

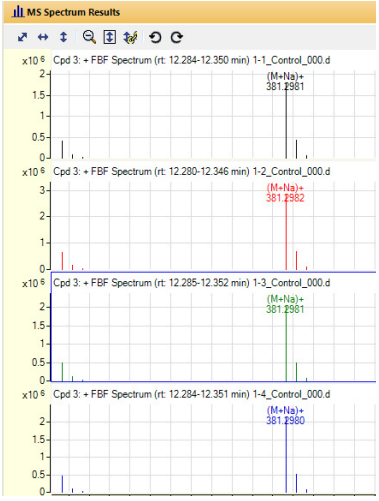
The Compound Details window shows a table of compound-level information for a single feature selected in the Compound Groups window. The quantitative information is shown for the selected feature as it is found in each data file in your project.

Information regarding the available columns are found in the online Help “Compound Details Columns.” A list of the available columns is displayed when you right-click within the Compound Details table, and then click **Add/Remove Columns**.

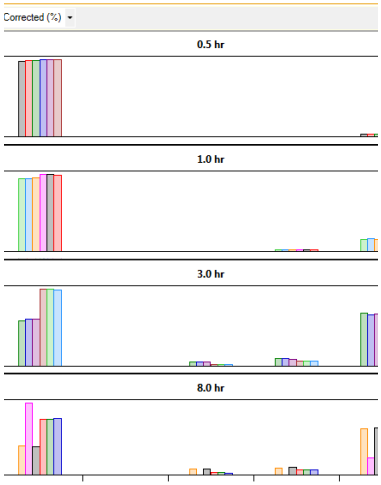
See *MassHunter Profinder Software - Quick Start Guide* for an explanation of the symbols used in the *Flags (Tgt)* column.



Chromatogram Results Chromatogram Results presents the extracted ion chromatogram (EIC) for each feature and, for isotopologue extraction results, the sum of the EICs for all of the isotopologues. For non-targeted feature extraction, the extracted compound chromatogram (ECC) is displayed for the ions contained in the molecular feature of the feature selected in the Compound Groups window. An EIC/ECC set is displayed for each data file. By default the chromatograms are displayed in an alternating cycle of ten colors to help you review the data for a particular data file as you select different features. **Color by Sample Group** displays the samples in an alternating cycle of colors based on the sample group assignment.



MS Spectrum Results MS Spectrum Results presents the averaged mass spectrum (MS) across the integrated ECC for the feature selected in the Compound Groups window for each data file. For isotopologue extraction results the mass spectra are presented across the isotopologue extraction region. By default the MS is displayed in an alternating cycle of ten colors, matched with the Chromatogram Results, to help you review the MS data for a particular data file as you select different features. **Color by Sample Group** displays the samples in an alternating cycle of colors based on the sample group assignment.



Isotopologue Results Isotopologue Results presents a sequence of charts, or a single chart, depending on the results mode selected from the toolbar.

List mode displays the isotopologue results for each sample file. The isotopologue charts are arranged in the order of your sample groups with each sample replicate displayed in an alternating cycle of ten colors, matched with the Chromatogram Results and MS Spectrum Results. The coloring can be changed to represent the sample groups by selecting **Color by Sample Group** from the toolbar.

Sample group mode displays the isotopologue results for each sample group. Each sample replicate is displayed within each group chart in an alternating cycle of ten colors, matched with the Chromatogram Results and MS Spectrum Results. The coloring can be changed to represent the sample groups by selecting **Color by Sample Group** from the toolbar.

Overlaid mode displays a single summary chart of the isotopologue results. The summary chart contains the average and standard error for each isotopologue per sample group presented in gray scale. The sample groups can be viewed in color by selecting **Color by Sample Group** from the toolbar.

The order of the appearance of the samples and sample groups is set in the **Add/Remove Sample Files** dialog box. The compound containing the isotopologues is selected in the Compound Groups window.

The chart y-axes can be scaled to raw abundances (Raw), raw abundances normalized to 100% (Raw (%)), natural isotope abundance corrected abundances (Corrected), and natural isotope abundance corrected and normalized to 100% (Corrected (%)).

Raw: The actual abundances of each isotopologue by sample data file, or average abundance of each isotopologue when the data is viewed in the summary chart.

Corrected: The abundance for each isotopologue is corrected to remove the natural isotopic contributions so that the abundance is due to the isotopic enrichment from the qualitative flux analysis.

Raw (%): The actual relative abundances of each isotopologue by sample data file, or average of each isotopologue when the data is viewed in the summary chart.

Corrected (%): The relative abundance for each isotopologue after the abundance for each isotopologue is corrected to remove the natural isotopic contributions so that the abundance is due to the isotopic enrichment from the qualitative flux analysis.

Unsaved parameter changes in Profinder

When you make a change to a parameter in Profinder, the software automatically places a change icon  (a blue triangle shape) in the wizard tab and next to the value containing the parameter where you made a change. This icon indicates that you have unsaved parameters changes and helps you remember to save the changes you have made to the method. To view the original parameter value, place your pointer over the change icon. When you save your method, the change icons disappear.

Omix Premium 

The main functional areas of Omix Premium are shown in Figure 10 on page 19. The main Omix Premium window consists of four parts: (1) the Menu Bar, (2) the Toolbar, (3) the Document Area, and (4) the Status bar. The document area can be further divided into up to five windows – (3a) Drawing Area, (3b) Component View window, (3c) Property Editor window, (3d) Data Manager window, and (3e) Log Messages window.

Most of your interaction with Omix Premium takes place in the *Drawing Area*. Each window can be floated independently to any location and size on your computer display or arranged to your preference within the Document Area.

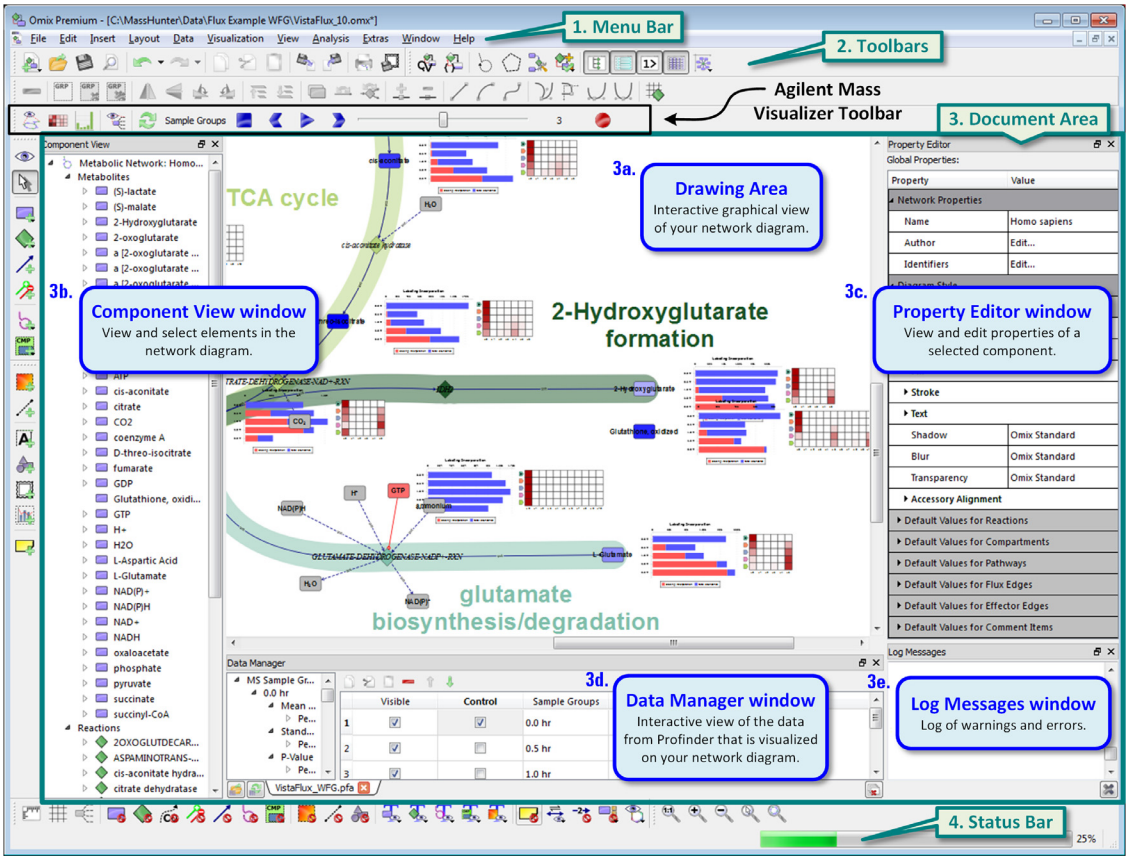


Figure 10 The main functional areas of Omix Premium

1. Menu Bar

The menu bar (Figure 11) provides actions that you use to create, edit, manage, view, and export your network diagram.

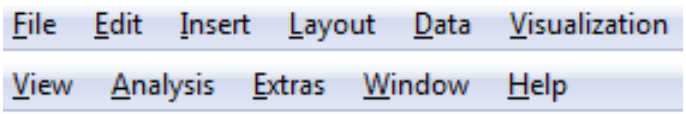


Figure 11 Omix Premium Menu bar (displayed in two rows)

File Open, close, and create new network diagrams (documents). You can also import a network and export, print, and save images of your network diagram.

Edit Copy, cut, paste, undo, and redo operations related to the network diagram you create in the *Drawing Area*. Many of the operations in the *Select* toolbar are in this menu.

Insert Access to operations in the *Edit* and *Graphics* toolbars.

Layout Options to manage your network diagram layout.

Data Load your Profinder data, and open and close the *Data Manager* window.

Visualization Access visualization features of Omix Premium and operations related to the *Agilent MassVisualizer* plug-in.

View Zoom and enable layout feature in the *Drawing Area* and access to operations in the *Visibility and Detail* toolbar.

Analysis Access to network statistics and plug-in features related to 13CFLUX2, atomic layer options, and chemical structure validity.

Extras Manage plug-ins available for Omix Premium, enable and disable document extensions, and launch the Omix Premium configuration dialog box.

Window Enable and disable any of the toolbars and switch between the windows associated with Omix Premium.

Help Provides a link to the Omix Visualization web site, update plug-ins, and information about the license and version.

2. Toolbars

There many toolbars that you can choose to show while using Omix Premium. Each toolbar is positioned below the menu bar along any side of the *Drawing Area* or each toolbar can be floated independently anywhere on your PC screen.

The commonly used toolbars are: Standard, Utility, Visibility and Detail, Zoom, Edit, Graphics, Select, and Agilent MassVisualizer. The default toolbar locations are shown in Figure 12 on page 21.

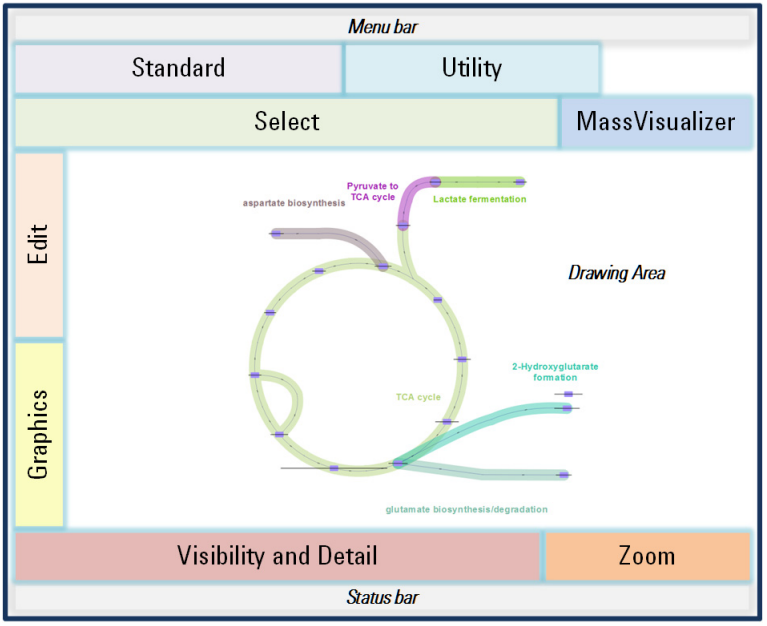


Figure 12 Default toolbar locations around the Drawing Area

Omix Premium Toolbars

Button	Equivalent Command
	Standard Toolbar
	**File > New > New Network**
	**File > Open Network**
	**File > Save**
	**Edit > Search**
	**Edit > Undo**
	**Edit > Redo**
	**Edit > Copy**

Button	Equivalent Command
	Edit > Cut
	Edit > Paste
	File > Import > Import Network from File
	File > Export > Export Network File
	File > Print
	File > Save Image
	Utility Toolbar
	Visualization > Quick Visualizer
	Visualization > Visualize by Scripting (OVL)
	Layout > Edit Layout Patterns
	Edit > Custom Shapes
	Layout > Motif Stamps > Manage Motif Stamps
	Extras > Document Extensions
	Window > Sidebars > Component View
	Window > Sidebars > Property Editor
	Window > Sidebars > Log Messages
	Window > Sidebars > Data Manager
	Layout > Automatic Layout

Button	Equivalent Command
	Visibility and Detail Toolbar
	View > Show Rulers
	View > Show Grid
	View > Visualize Properties on Demand
	View > Hide Metabolites
	View > Hide Reactions
	View > Hide Cofactors
	View > Hide Effector Edges
	View > Hide Flux edges
	View > Hide Pathways
	View > Hide Compartments
	View > Hide Node Items
	View > Hide Connection Edges
	View > Hide Graphical Items
	View > Appearance of Metabolite Labels
	View > Appearance of reaction Labels
	View > Appearance of Pathway Labels
	View > Appearance of Compartment Labels
	View > Appearance of Node Item Labels

Button	Equivalent Command
	View > Hide Comments
	View > Appearance of Reversibility
	View > Hide Coefficients
	View > Hide Item Accessories
	Visualization > Visualize Chemical Structures
	Zoom Toolbar
	View > Zoom > Zoom 1:1
	View > Zoom > Zoom In
	View > Zoom > Zoom Out
	View > Zoom > Zoom Selection
	View > Zoom > Zoom Diagram
	Edit Toolbar
	Edit > Network View Mode
	Edit > Select
	Insert > Insert Metabolite > Insert Metabolite
	Insert > Insert Reaction > Insert Reaction
	Insert > Insert Flux Edge
	Insert > Insert Effector Edge
	Insert > Insert Pathway > Insert Pathway

Button	Equivalent Command
	Insert > Insert Compartment > Insert Compartment
	Graphics Toolbar
	Insert > Insert Node Item
	Insert > Insert Connection Edge
	Insert > Insert Text Item
	Insert > Insert Graphical Item
	Insert > Insert Image
	Insert > Insert Chart
	Insert > Insert Comment
	Select Toolbar
	Edit > Delete
	Edit > Group Items
	Edit > Ungroup Items
	Edit > Ungroup All
	Edit > Mirror Horizontally
	Edit > Mirror Vertically
	Edit > Rotate 90° Clockwise
	Edit > Rotate 90° Counter-Clockwise
	Edit > Stack Before (applies to graphics items)

Button	Equivalent Command
	**Edit > Stack Behind (applies to graphics items)**
	**Edit > Duplicate Metabolite Node**
	**Edit > Invert Edge Direction**
	**Edit > Invert Reaction**
	Add Spline Point, no equivalent command
	Delete Spline Point, no equivalent command
	Line, no equivalent command
	Curve with one control point, no equivalent command
	Curve with two control points, no equivalent command
	Angular Join, no equivalent command
	Axis Parallel Segments, no equivalent command
	Smooth Join, no equivalent command
	Symmetric Join, no equivalent command
	**Edit > Align with Grid**
	MassVisualizer Toolbar
	**Visualization > Agilent MassVisualizer > Show Abundance Changes**
	**Visualization > Agilent MassVisualizer > Show Quilt Plots**
	**Visualization > Agilent MassVisualizer > Show Bar Charts**

Button	Equivalent Command
	**Visualization > Agilent MassVisualizer > Show Background Information**
	**Visualization > Agilent MassVisualizer > Reload Visualization**
	**Visualization > Stop** icon
	**Visualization > Back** icon
	**Visualization > Play** icon
	**Visualization > Slider** bar
	**Visualization > Record** icon

3.Document Area

The *Document Area* is where you visually generate your network diagram, add representations of your qualitative flux analysis results, and generate report and presentation views. You can enable the Component View, Property Editor, Data Manager, and Log Messages windows to reside around the *Drawing Area* or float them anywhere on your PC desktop. the *Drawing Area* is replaced with the *Pattern Editor* when you add patterns to help align your network diagram.

4. Status Bar

The *Status Bar* shows the progress when you load and save network diagram documents. The *Status Bar* appears blank during most activities within Omix Premium.

Definitions

Algorithm An algorithm is a set of automated, sequential mathematical tasks performed to find, filter, align, and extract features from your chromatographic/mass spectral data sets.

Compound Group A single compound that is targeted, or found, in any of the sample data files in a project. For example, if 20 compounds are found in the first data file in the project, then there are at least 20 compound groups in the project. If additional unique compounds are found in the remaining data files for your project, then additional compound groups are created.

Edge A visual representation of the connection between a reaction and a metabolite when creating a network diagram.

Feature A feature is synonymous with compound. A feature is referred to interchangeably with compound, metabolite, molecular feature, element, or entity during the various steps of analysis using Agilent MassHunter software.

Fold Change A measure of the amount of change expressed in the ratio of the amount of change from the original value versus the original value. A fold change can be positive (increasing) or negative (decreasing).

Sample Group An experimental condition, such as the time a sample was acquired after an experiment was started, assigned to replicate samples. Larger number of samples in a sample group improve the statistical significance of your qualitative flux analysis.

Isotopologue Molecules that contain the same molecular formula and structure but differ in their isotopic composition through the substitution of one or more atoms with a different isotope. The exact location of the isotope in the molecule, while important chemically, is not important in flux analysis, just the number of isotopes in the molecule.

Isotopomer Molecules that contain the same molecular formula, structure, and number of isotopes but differ in the specific atomic location of the isotopes in the molecular structure.

Labeling When an isotope of an atom is substituted for the naturally occurring atom, the resulting compound is referred to as being labeled. Metabolites in a cell can become labeled when a isotopically enriched compound is introduced to the cellular metabolism. An experiment that studies the rate that metabolites become labeled through metabolism are referred to as metabolic flux analysis or qualitative flux analysis.

Method A method is a set of parameters that are associated with the three feature extraction algorithms used by Profinder. Methods containing the parameters for the algorithms can be saved using unique file names.

Model Another name to refer to a network diagram.

Network A set of metabolite and reaction nodes that can be assembled with additional information to represent the operation biochemical system.

Network Diagram A graphical visualization of metabolite and reaction nodes, effectors, and flux edges that together represent the operation biochemical system.

Node A representation of a metabolite or reaction when you create a network diagram.

Pathway A sequence of reactions and metabolites that represent the chemical reactions that occur in a cell.

PCDL A personal compound database and library that contains necessary information compound information about your target metabolites; at a minimum the information must include identification, mass, and retention time.

Tracer A stable isotope labeled compound, referred to as a tracer, is introduced into the biological system for flux analysis. The tracer typically contains multiple atoms of ^{13}C, ^{15}N, or ^{2}H.

Wizard A wizard is a sequence of interactive steps used by Agilent MassHunter software to guide you through the steps necessary to complete an analytical task. Profinder uses a set of wizards to guide you through the parameters associated with each feature extraction algorithm.

Workflow A workflow is an Agilent document or a graphical overview that captures a sequence of steps to guide you through an analytical task. A workflow may cover more than one wizard and may include steps performed by more than one software program.

Basic Qualitative Flux Analysis Workflow

The basic qualitative analysis workflow, illustrated in Figure 2 on page 4, guides you through the steps necessary to identify target metabolites of interest for your experiment, create visual pathways networks for your experiment, acquire accurate mass data from your samples, extract accurate isotopic metabolite information from the sample data, and then view the results on network diagrams of the pathways involved in your experiment.

Pathways to PCDL

- "Create an initial PCDL from pathways content" on page 31

PCDL Manager

- "Generate a target metabolite PCDL" on page 34

Profinder

- "Create a Profinder Project" on page 39
- "Run Batch Isotopologue Extraction" on page 43
- "Create a Profinder Archive" on page 46
- "Save your Profinder project" on page 47

Omix Premium

- "Visualize your results in Omix Premium" on page 48

Create an initial PCDL from pathways content

In this task, you launch Pathways to PCDL, select a pathway, and add the metabolites to an initial PCDL. A typical workflow using Pathways to PCDL is shown in Figure 13.

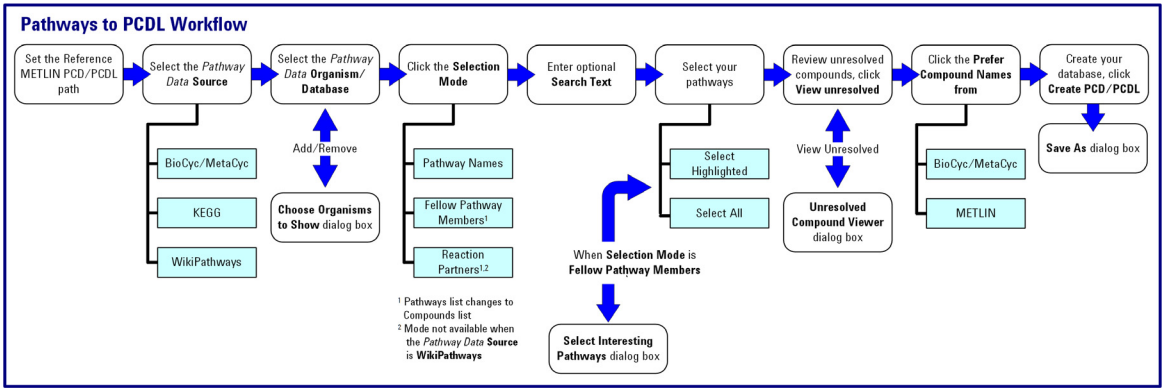
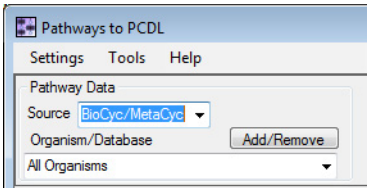
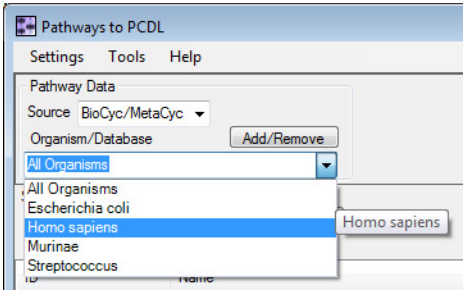
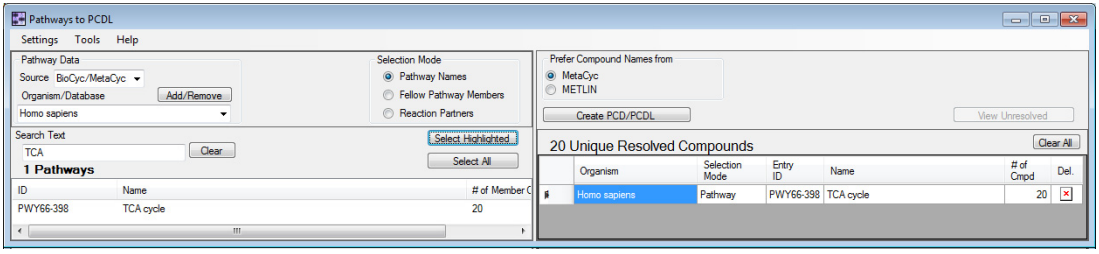


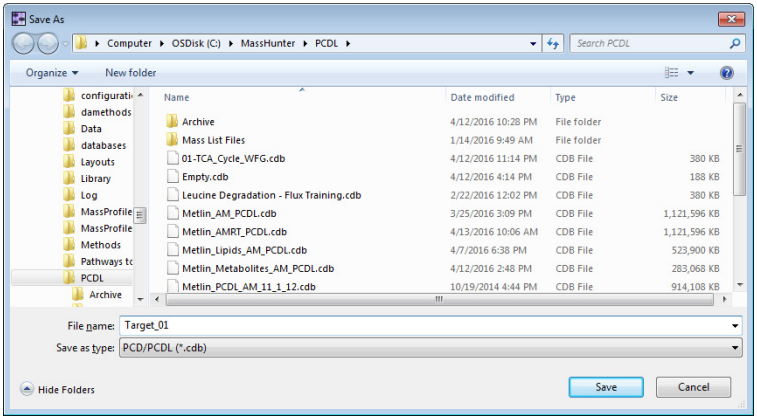
Figure 13 Typical Pathways to PCDL workflow

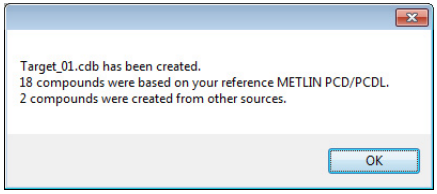
Steps	Detailed Instructions	Comments
1 Start Pathways to PCDL.	• Double-click the **Pathways to PCDL** icon located on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > Pathways to PCDL > Pathways to PCDL.**	• The first time you run Pathways to PCDL you are prompted to set a reference METLIN database folder and filename. This is *optional*, you can click **Cancel** in the **Select Reference METLIN PCD/PCDL** dialog box.
2 Select the source to search for pathways data.	• Select **BioCyc/MetaCyc** for the **Source** under Pathway Data. 	• The Pathways to PCDL converter supports pathway content from BioCyc/MetaCyc, KEGG, and WikiPathways databases. You must have a license, user name and password, to obtain content from the KEGG database.

Steps	Detailed Instructions	Comments
3 Select an organism to filter the pathways from the data source.	• Select **Homo sapiens** from the **Organism/Database** list. 	
4 Choose a selection mode.	• Click **Pathway Names** for the **Selection Mode**. 	• The selection mode provides you with an option to select compounds (metabolites) for your PCDL by association with a pathway name, pathways that have a common compound name, and reactions that have a common compound name.
5 Add one or more pathways to your *Selected Pathways List*.	**a** Type `TCA` for the **Search Text**. **b** Click the row containing the **TCA cycle** pathway. **c** Click **Select Highlighted** to move the pathway to the *Selected Pathways List*. **d** Repeat steps a through c to continue adding pathways sources for your compound database if you are creating your own experiment.	• In cases when the number of pathways, or compounds, displayed in the *Pathways or Compounds List* in the display pane is large, you can reduce the number of pathways, or compounds, by using search text • The compounds associated with the pathway are moved the *Selected Pathways List* immediately after you click **Select Highlighted**, or **Select All**.



Steps	Detailed Instructions	Comments
6 Create your custom PCDL.	**a** Click **Create PCD/PCDL**. **b** Select the folder to save your PCD/PCDL database. **c** Type the name for your PCD/PCDL database in File name, Target_01. **d** Click **Save**.	• Your PCDL is created from the compounds within the *Selected Pathways List*. • The default folder for a custom PCDL is C:\MassHunter\PCDL.
	e Click **OK**.	• Summary information describing your custom PCDL is displayed after you **Save** the PCDL.





Your initial compound database is now created.

The next step is to review the initial PCDL and create your target metabolite PCDL.

Generate a target metabolite PCDL

Generate a target list of metabolites of interest to your experiment using MassHunter PCDL Manager. In this step you edit the compound list in the initial PCDL you created using Pathways to PCDL. A typical workflow using Pathways to PCDL is shown in Figure 14.

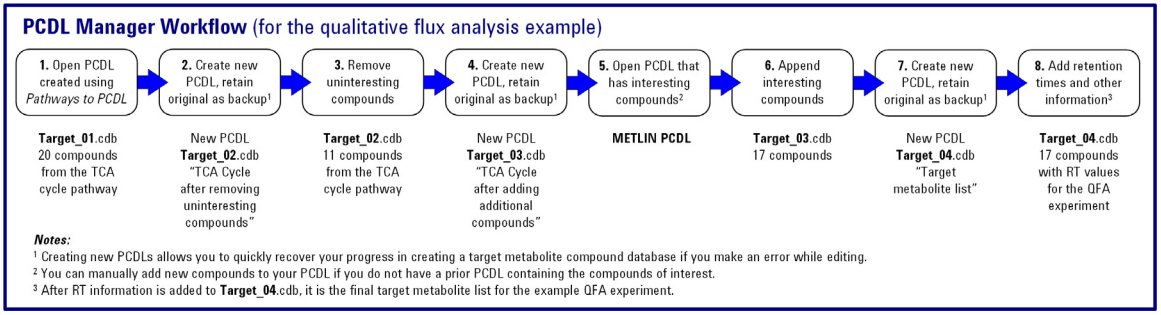
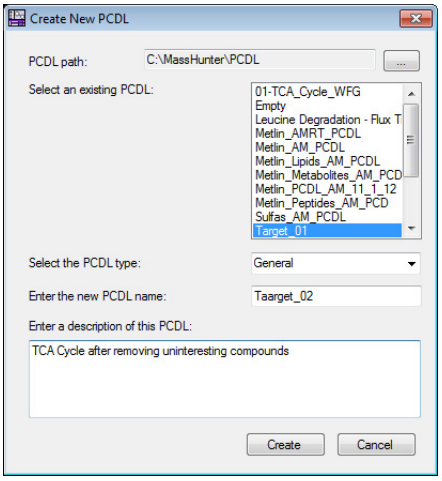
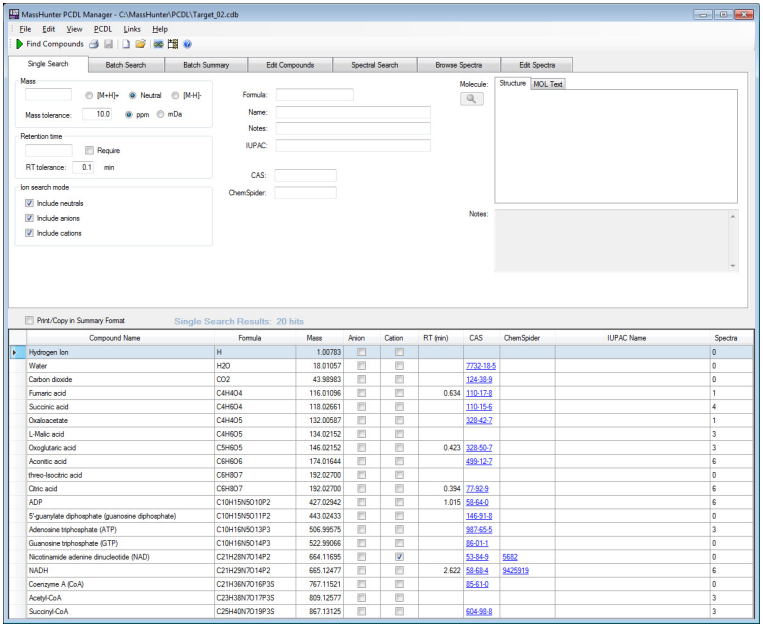


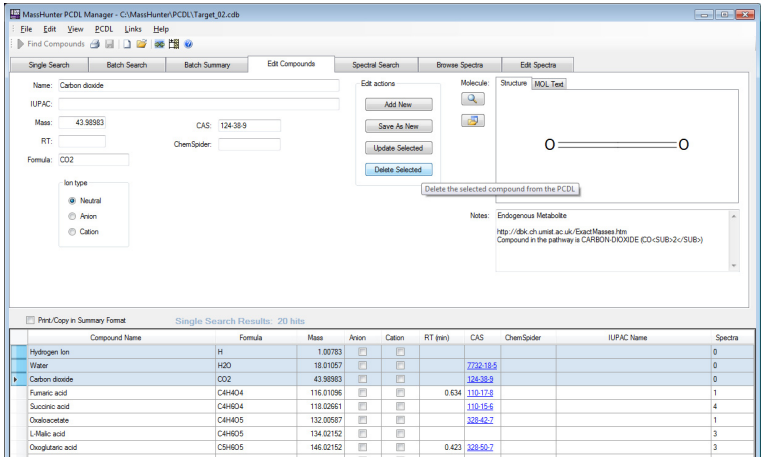
Figure 14 Typical PCDL Manager workflow

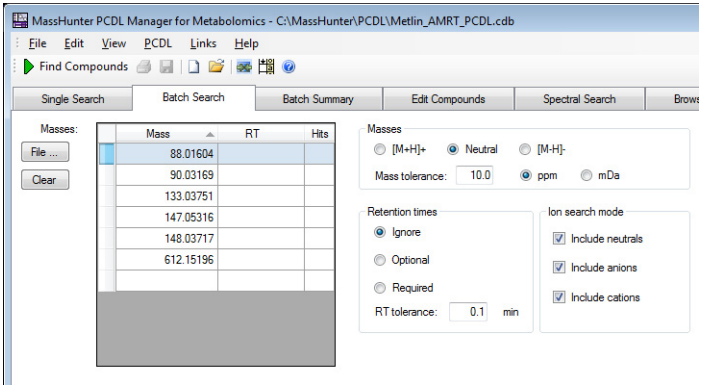
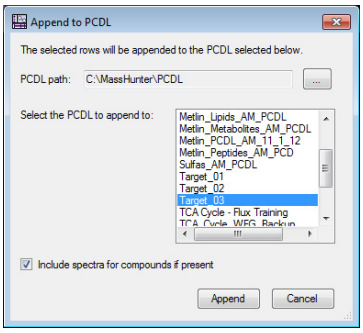
Steps	Detailed Instructions	Comments
1 Start PCDL Manager.	• Double-click the **PCDL Manager** icon located on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > PCDL Manager.**	• Edits made to your personal compound database are saved real-time to the open database and cannot be undone.
2 Make a copy of your personal compound database.	**a** Click **File > New PCDL**. **b** Select **General** for **Select the PCDL type**. **c** Type a new name for **Enter the new PCDL name**. **d** Enter a useful description of the current PCDL for **Enter a description of this PCDL**. **e** Click **Create** to make a copy of the compound database.	• The **Create new PCDL** dialog box immediately opens and has information completed for the open compound database. • Creating frequent copies of your PCDL saves time returning to an earlier version of your target metabolite database.

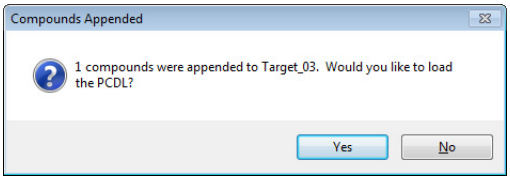
Steps	Detailed Instructions	Comments
		• When you create a new PCDL, the original PCDL is closed and the new PCDL is automatically opened in PCDL Manager. • In the example, the original PCDL file name is appended with numbers "_xx" to simplify restoring a previous copy.
3 View the compounds in your personal compound database	**a** Click the **Single Search** tab. **b** Click **Find Compounds** on the toolbar.	• You must perform some type of search after you open your PCDL to view any, or all, of the compounds you created using Pathways to PCDL. • Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway. • If the compound database is large, searching the database without narrowing search criteria can take a long time.



Steps	Detailed Instructions	Comments
4 Remove uninteresting compounds.	**a** Click **PCDL > Allow Editing** to enable editing of the personal compound database. **b** Click the **Edit Compounds** tab. **c** Click **Hydrogen Ion** in the *Compounds Results Table*. **d** Shift-click **Carbon dioxide** in the *Compounds Results Table* to select the compounds between, and including, the selected compounds in the table. **e** Click **Delete Selected** from the *Edit actions*.	• Since the databases created using Pathways to PCDL contain all of the compounds associated with the selected pathways, compounds that are not interesting your experiment can be removed. • Compounds in the database can be added and removed using the *Edit actions* only when **Allowing Editing** is marked in the menu • For the TCA cycle in the example, eleven compounds are interesting and nine compounds are removed. • While all of the pathway compounds are metabolically significant, some are not observable, or observed based on the acquisition settings, in the mass spectrometer. For the example data set hydrogen ion, water, and carbon dioxide were both uninteresting target compounds and not visible in the mass spectrometer.
	f Click **Yes** in the **Delete Compound** dialog box. **g** Click the **Single Search** tab to refresh the search results value above the *Compound Results Table*. **h** Click the **Edit Compounds** tab to return to editing the compounds. **i** Repeat these steps to remove all of the uninteresting compounds from your PCDL.	• *Reminder:* Edits made to the compound database while **Allowing Editing** is enabled are saved real-time to your open database and cannot be undone. • After you remove all of the uninteresting compounds you can make a new copy of your edited personal compound database.



Steps	Detailed Instructions	Comments
5 Add a batch of new compounds from another PCDL.	**a** Click **File > Open PCDL**. **b** Click the file name for the *METLIN PCDL* (or your own PCDL) in the **Open PCDL** dialog box. **c** Click **Open**. **d** Click the **Batch Search** tab. **e** Enter the masses for the new compounds into the *Masses* table **f** Click **Ignore** for *Retention times*. **g** Click **Find Compounds** on the toolbar	• While you can manually add new compounds to your compound database, a simple method is to append compounds to your compound database from another PCDL, such as from the Agilent METLIN PCDL.
		• When the search is complete the results are displayed in the *Compound Results Table*. The compounds results are shown for a single mass at a time; the *Compound Results Table* is refreshed when you click on a mass in the *Masses* table in the *Action Pane*.
	**h** Right-click the row containing the desired compound for the first mass in the *Masses* table. **i** Click **Append to PCDL**. **j** Select the compound database to add the selected compound for **Select the PCDL to append to** in the **Append to PCDL** dialog box. **k** Click **Append**.	• For each mass you entered, PCDL Manager marks the row for the compound that is the best match for your search criteria. When the search criteria is minimal, the is compound is based on the Compound Name and CAS ID.

Steps	Detailed Instructions	Comments
	l Click **No** in the **Compounds Appended** dialog box.	• Click **No** unless you are sure you want to close your current PCDL and open the target PCDL. • When you click **Yes** in the **Compounds Appended** dialog box the target PCDL is opened and the current PCDL, along with your progress of adding compounds to the target PCDL, is closed.
		
	m Click the next mass in the *Masses* table in the *Action Pane*. **n** Repeat steps i through m for each mass. **o** Click **Yes** in the **Compounds Appended** dialog box when you have appended the last compound to the target PCDL. **p** Click the **Single Search** tab. **q** Click **Find Compounds** on the toolbar to view the compounds in the target PCDL.	
6 Add retention times and additional identifiers to your target metabolite list. ***Note:*** *The compounds in your target compound database for qualitative flux analysis must have retention times in order to perform the batch isotopologue extraction in Profinder.*	**a** Click the **Edit Compounds** tab. **b** Click **PCDL > Allow Editing**. **c** Click on a compound row in the *Compound Results Table*. **d** Add retention times for each compound per your acquisition method development. **e** Add any useful, additional compound information at this time. **f** Click **PCDL > Allow Editing** to disable further editing.	• The compound highlighted in the *Compound Results Table* is the compound that is being edited in the *Action Pane* for the **Edit Compounds** tab. • You can add retention times using a mass list file. See "Exercise 8. Add retention times to a PCDL" in the *MassHunter Personal Compound Database and Library Manager - Quick Start Guide*.
7 Save your target metabolite compound list.	• Click **File > Exit** to close PCDL Manager.	• All of the edits made while **Allowing Editing** is enabled are saved real-time to your open database; therefore you simply exit PCDL Manager to save your final target metabolite database.

Your target metabolite database is now created.

Create a Profinder Project

In this task, you launch Profinder, select your sample files, and add the sample files to a new Profinder project. A typical Batch Isotopologue Extraction workflow using Profinder is shown in Figure 15.

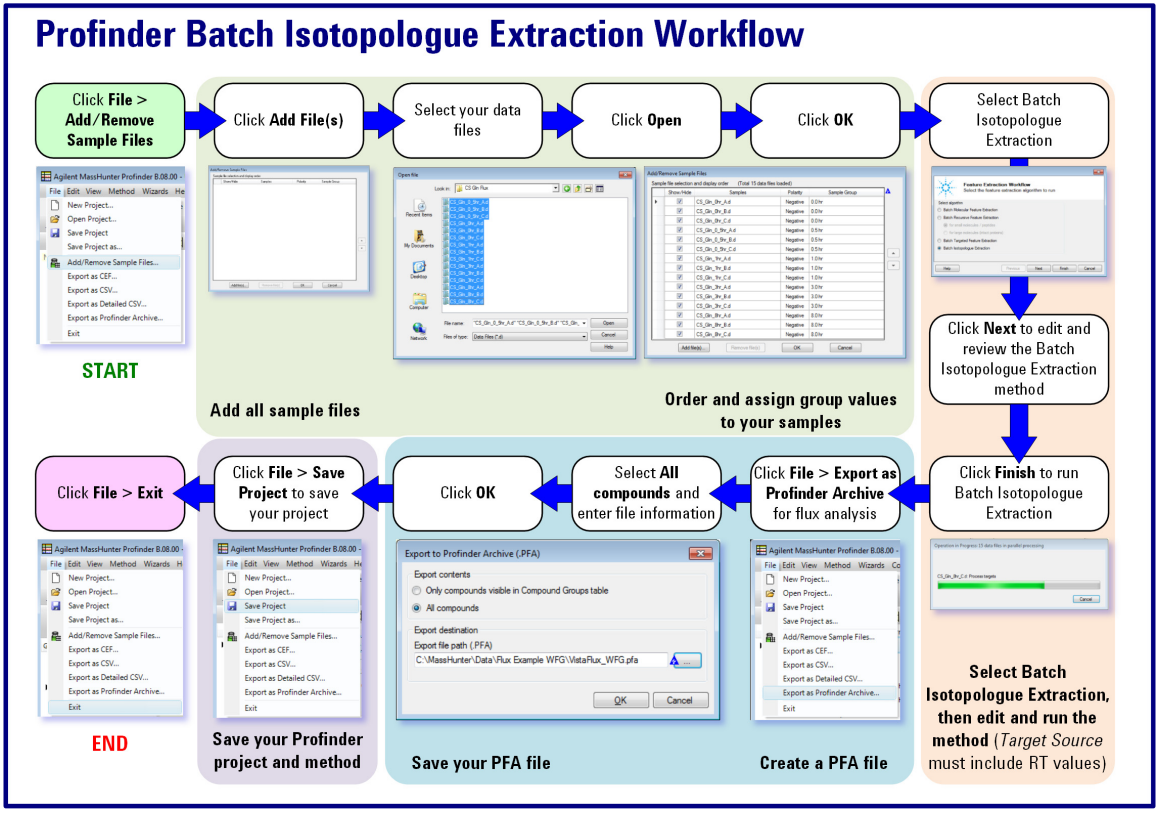
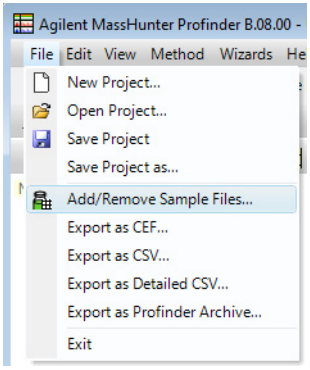
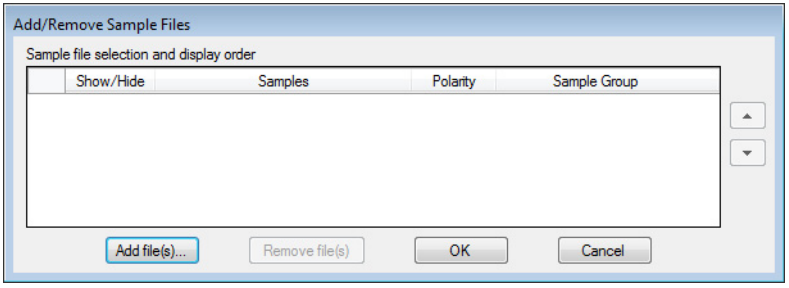
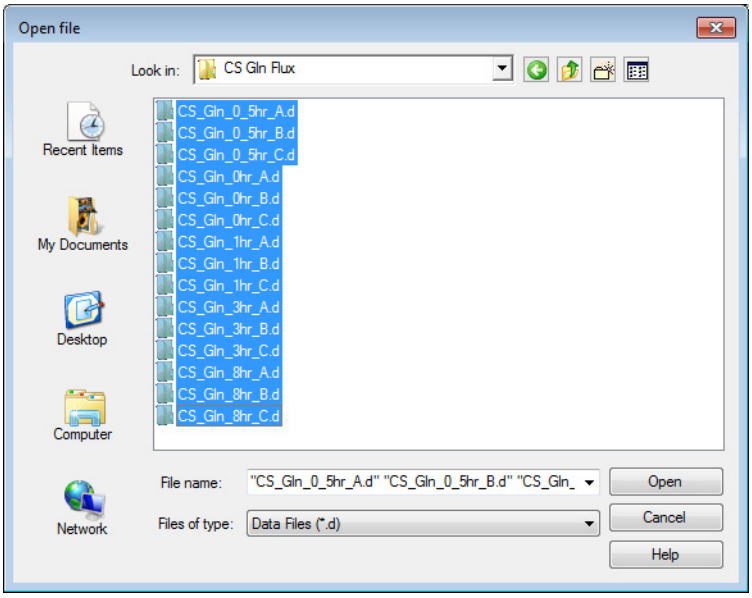
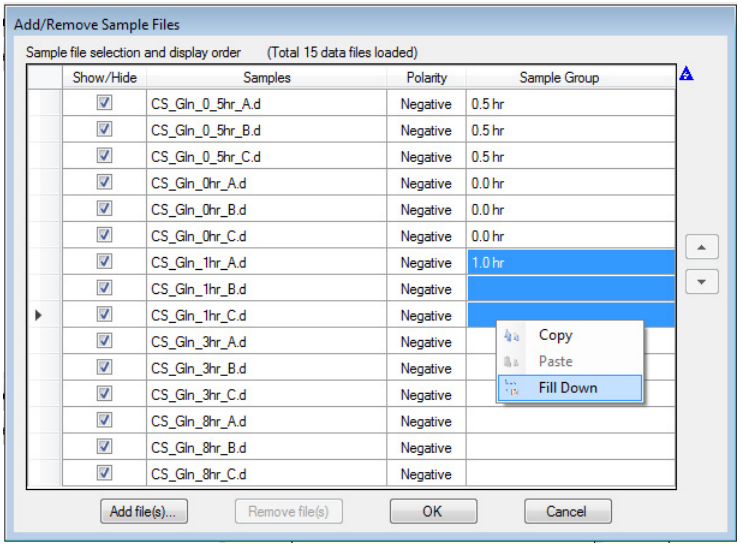
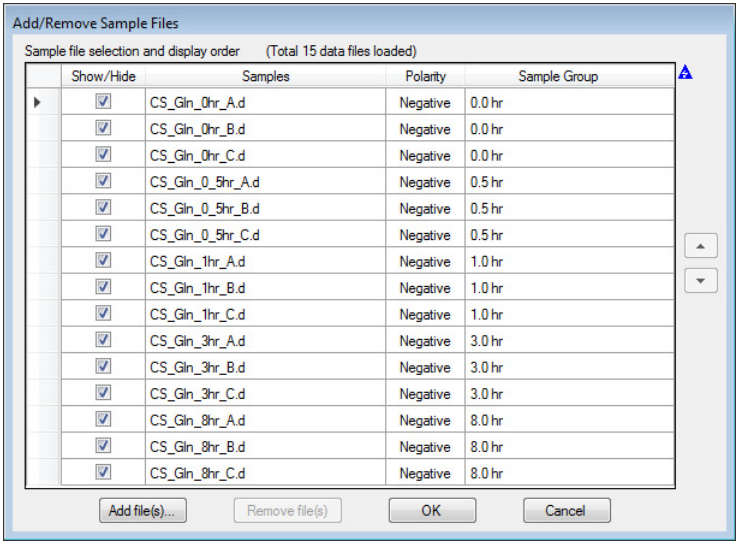


Figure 15 A typical Profinder Batch Isotopologue Extraction workflow

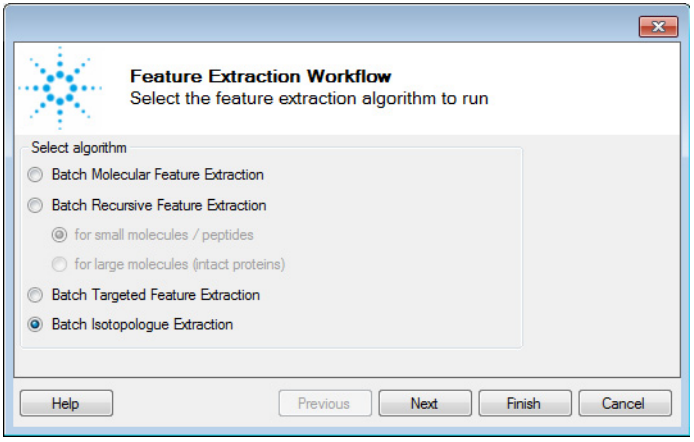
Steps	Detailed Instructions	Comments
1 Start Profinder.	• Click the **Profinder** icon on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > Profinder B.08.00**.	• The first time you start Profinder it displays in full screen. Subsequent program launches remember the screen size from your prior session.
2 Begin your Profinder project by adding sample files. 	• Click **File > Add/Remove Sample Files**, or click Add/Remove Sample Files... on the toolbar.	• If you click **File > New Project** after starting Profinder, you are automatically prompted to add sample files. • When you click **Add/Remove Sample Files** after launching Profinder, Profinder starts a new project as if you clicked **File > New Project**.
3 Add files to the **Add Sample Files** dialog box. 	**a** Click **Add file(s)** to display the **Open file** dialog box.	

Steps	Detailed Instructions	Comments
	b Browse to the folder containing your sample files. **c** Select all of the sample data files for your Profinder project. **d** Click **Open**.	• Selected sample data files are highlighted in the **Open file** dialog box. • A Profinder project contains all of the sample data files from your experiment - replicate samples for each condition (group) in your experiment. • You can click **Add Sample Files**, to add additional sample data files to your project and rerun feature extraction.
		
4 Enter Sample Group values to the sample files in the **Add Sample Files** dialog box.	**a** Click the data entry cell under the **Sample Group** column, next to the sample file name. **b** Enter the group identification text. **c** Repeat for each sample file.	

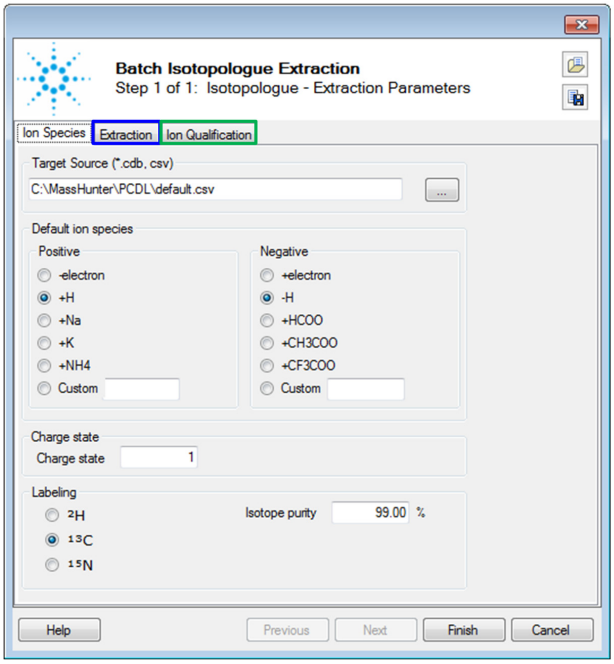
Steps	Detailed Instructions	Comments
		• When a data entry cell is selected it is highlighted using a background color. • When you enter **Sample Group** names, the entries must use identical letters, numbers, punctuation, and case in order for the grouping functions to perform properly. • Use the **Copy**, **Paste**, and **Fill Down** shortcuts (click the right mouse button) to help assign **Sample Group** values to each sample.
5 Mark the samples to add to your project.	**a** Mark the samples to add to your project in the **Show/Hide** column. **b** Click **OK**.	• All of the **Samples** in the same project must have the same polarity. If some of the Samples have a Positive **Polarity** and some have a Negative **Polarity** an error message is shown when you click **OK**. • The Feature Extraction Workflow immediately prompts you to select a feature extraction algorithm. The next steps in this *Quick Start Guide* guides you through the steps to edit and run the Batch Isotopologue Extraction algorithm.
		

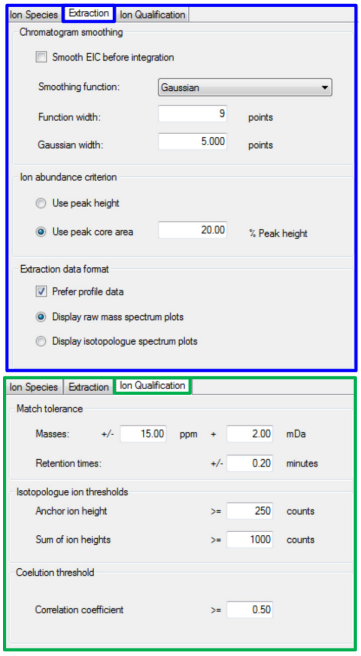
Run Batch Isotopologue Extraction

Batch Isotopologue Extraction supports only LC/MS acquired data. Batch isotopologue extraction anticipates that the target compounds may have undergone some degree of isotope labeling, to extract features from your sample data files. Batch Isotopologue Extraction is optimized to extract isotopologues for targeted features for qualitative flux analysis.

Steps	Detailed Instructions	Comments
1 Select the Batch Isotopologue Extraction workflow to apply to your samples. 	**a** Click **Batch Isotopologue Extraction**. **b** Click **Next**.	• **Batch Isotopologue Extraction** is optimal with data that has been acquired in profile mode and supports only LC/MS acquired data. • Unlike the other batch feature extraction wizards, target retention times are required for this workflow.
2 Review and edit the parameters for the Batch Isotopologue Extraction algorithm.	**a** Click the **Ion Species** tab to make changes to the parameters associated with the charge carrier, charge state, and isotopic labeling. **b** Click the **Extraction** tab to make changes to the parameters associated with the chromatographic and mass spectral extraction.	• Click **Help** to activate online Help specific for the current tab in the wizard. • Click **Cancel** to stop editing the algorithm parameters. Any changes made to the algorithm parameters are not saved.
	c Click the **Ion Qualification** tab to make changes to the parameters associated with feature matching, isotopologue thresholds, and coelution.	

Steps	Detailed Instructions	Comments

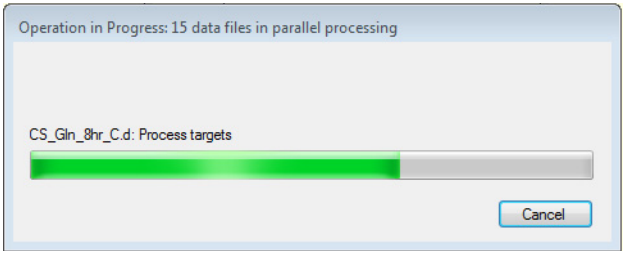




3 Run the Batch Isotopologue Extraction algorithm.

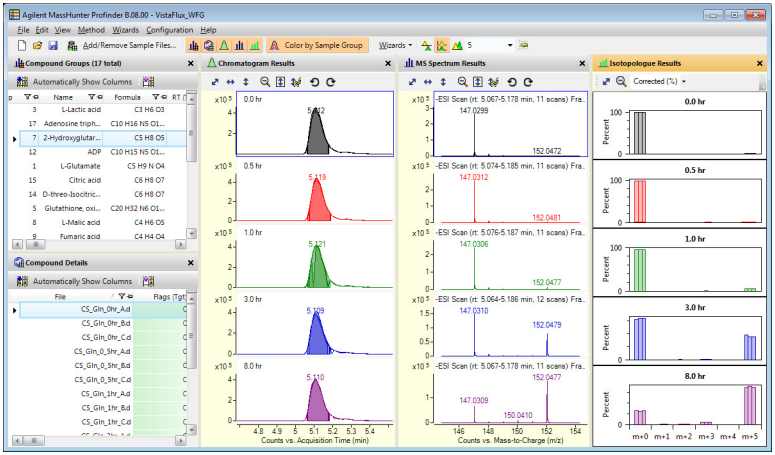
a Click **Finish** to run the extraction algorithm on your sample data.

b An **Operation in Progress** dialog box is displayed while the extraction process is running.



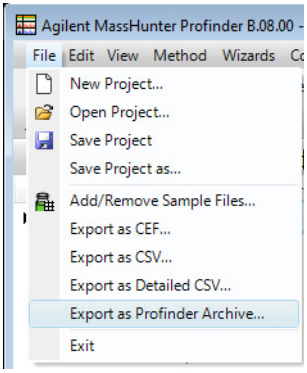
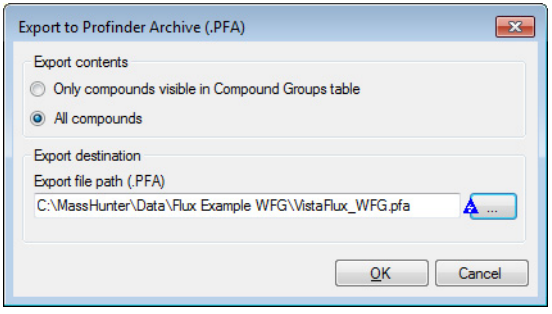
- Click **Cancel** to stop editing the algorithm parameters. Any changes made to the algorithm parameters are not saved.
- The feature extraction process can take a long time. To significantly improve performance, use an SSD, increase the amount of RAM on your PC, and use a faster processor.

Steps	Detailed Instructions	Comments
4 Review your results.	• Review your results and then export the results and save your method.	• Using the Compound Groups, Compound Details, Chromatogram Results, MS Spectrum Results, and Isotopologue Results windows, you can edit your results: delete a compound group, delete abundance from compound details window or chromatogram results window, and integrate a peak manually. More information is available in the online Help. • The next steps in this *Quick Start Guide* help you export your results and save your method.



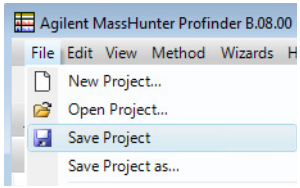
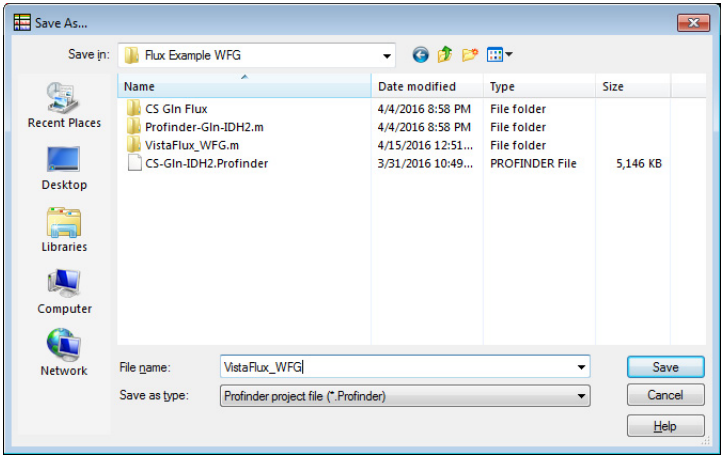
Create a Profinder Archive

Save your Profinder results as a Profinder Archive (PFA) file to visualize your results in the context of biochemical networks in Omix Premium.

Steps	Detailed Instructions	Comments
1 Select **Export as Profinder Archive** from the File menu. 	• Click **File > Export as Profinder Archive**.	• Alternate export options include: ◦ **Export as CEF**- features saved to a file format used to exchange data between Agilent software. ◦ **Export as CSV** - average Mass and RT with actual feature abundance for each sample ◦ **Export as Detailed CSV** - average Mass and RT with all actual feature values for each sample
2 Export your Profinder project as a PFA file. 	**a** Click **All compounds** under **Export contents**. **b** Enter your export destination folder, and enter your file name. **c** Click **OK**.	

Save your Profinder project

Save your Profinder project, method, and the current sample data file extraction results so that you can continue reviewing your results and the extraction method at a later time.

Steps	Detailed Instructions	Comments
1 Select **Save Project** from the menu bar. 	• Click **File > Save Project**.	• If you have not previously saved your project, **Save Project** is the same as **Save Project as** and prompts you for a name to save your project.
2 Type the file name and save your project. 	**a** Type the name to use for your project file in the **Save as** dialog box. **b** Click **Save**.	• The **Save As** dialog box does not appear when you click **Save Project** if you previously saved your project. • Click **File > Save Project as** if you want to save your project using a new file name. • ***Note:*** Profinder project files can be one GB or more in file size. • ***Note:*** Remember to include the original sample data when you share a Profinder project.

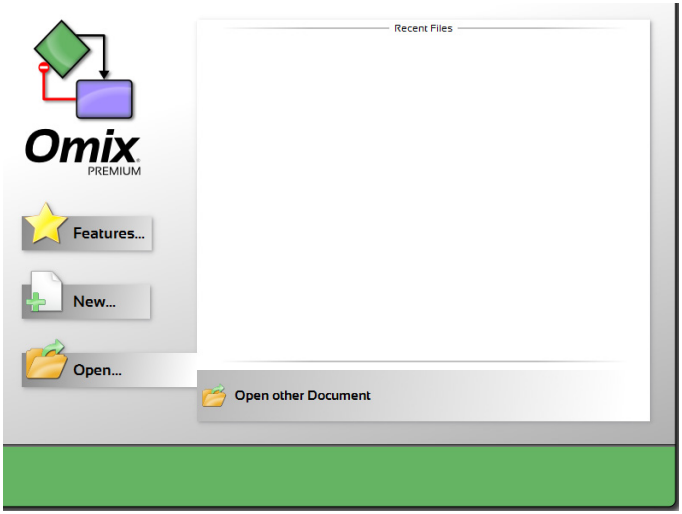
You are now ready to view your results in Omix Premium.

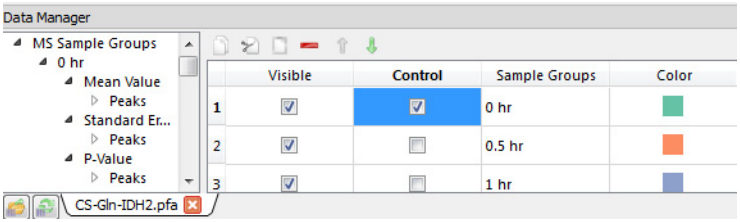
Visualize your results in Omix Premium

In this task, you launch Omix Premium, open a template network diagram, import your Profinder results, and view the results in the context of your network diagram.

The *Agilent MassVisualizer* plug-in provides you with the ability to visualize isotopologue data from Profinder; you can view absolute and relative abundances, labeling incorporation, and statistics within the context of your network diagram.

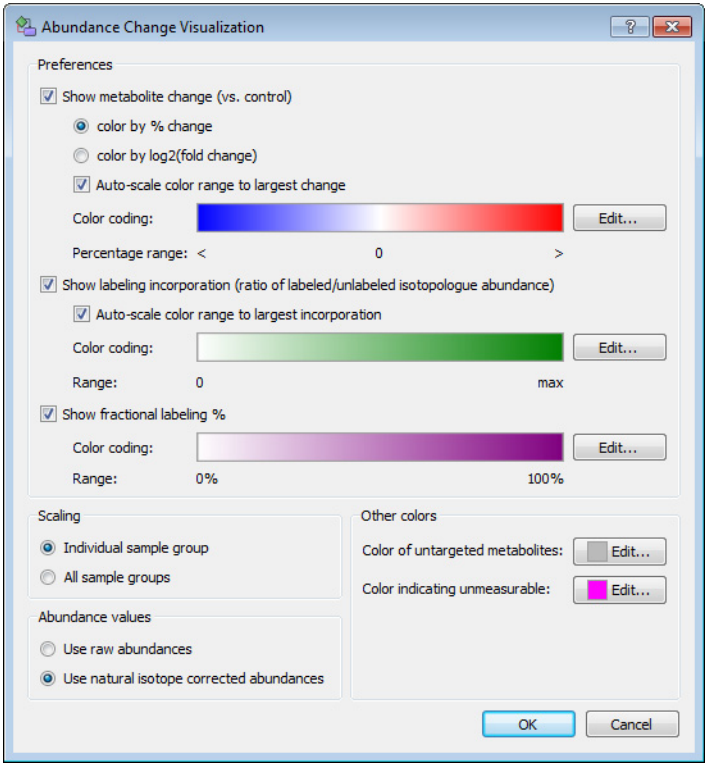
Steps	Detailed Instructions	Comments
1 Start Omix Premium.	• Double-click the **Omix Premium** icon located on your desktop, or click **Start > All Programs > Omix Premium > Omix Premium.**	
2 Open the template network diagram.	**a** Click **Open** in the *Document Area*. **b** Click **Open other Document**. **c** Navigate to the folder containing the VistaFlux example data, then click the **TCA-IDH2.omx** template network diagram in the **Open File** dialog box. **d** Click **Open**.	• When Omix Premium opens the document area is arranged to facilitate a quick means to review the software features, open a new document, and open a recent document.



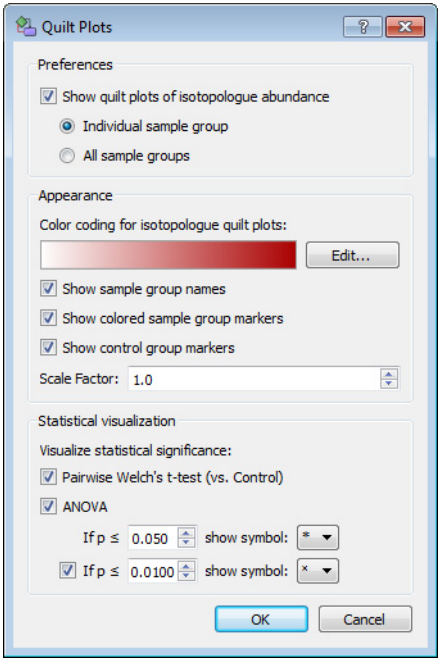
Steps	Detailed Instructions	Comments
3 Import the example Profinder results.	**a** Click **Data > Open Data Table**. **b** Navigate to the folder containing the example data, then click the **CS-Gln-IDH2.pfa** Profinder Archive file in the **Open Data Source** dialog box. **c** Click **Open**.	• Your target metabolites and isotopologue extraction results are imported into Omix Premium using a Profinder Archive (PFA) file, see "Create a Profinder Archive" on page 46.
4 Mark group 1 (0 Hr) as the control. 	• Double-click on the check box for group 1 under the *Control* column.	• The *Data Manager* window is opened along the bottom of the *Display Area*. • If the *Data Manager* window is already open you can click the **Open Data Table** button, a small button located at the bottom-left of the *Data Manager* window
5 Close the Data manager window.	• Click **Data > Show Data Manager**, or click the **Data Manager** button on the toolbar, to close the *Data Manager* window.	• Click **Data > Show Data Manager**, or click the **Data Manager** button on the toolbar, to open and close the *Data Manager* window at any time.

Steps	Detailed Instructions	Comments

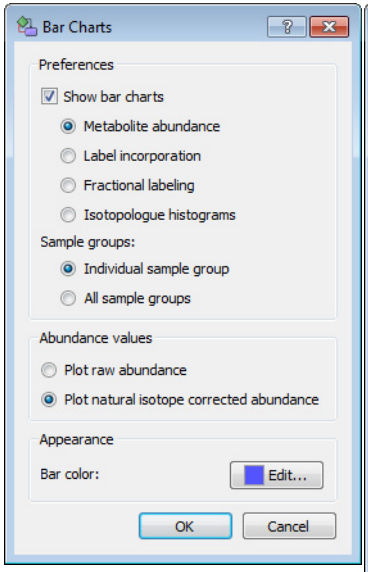
6 Add visualization of abundance changes to your network diagram.	**a** Click the **Show Abundance Changes** button from the toolbar, or click **Visualization > Agilent MassVisualizer > Show Abundance Changes**.
	b Mark **Show metabolite abundance fold change (vs. control)**.
	c Mark **Show labeling incorporation (ratio of labeled/unlabeled isotopologue abundances)**.
	d Mark **Show fractional labeling %**.
	e Click **Individual sample group**.
	f Click **OK**.
	g Adjust the parameters and become familiar with the results shown on the network diagram.
		• Visualize up to three different summary statistics within the metabolite node:
		  ◦ maximum metabolite abundance change per group versus the control
		  ◦ label incorporation per group
		  ◦ fractional labeling change per group versus control
		• Abundance changes cannot be used with *Draw structures on the metabolites*.
		• Abundance change parameters are viewed in the **Abundance Change Visualization** dialog box.
		• See the *MassHunter VistaFlux Software - Workflow Guide* for a detailed overview of the Abundance Change Visualization.

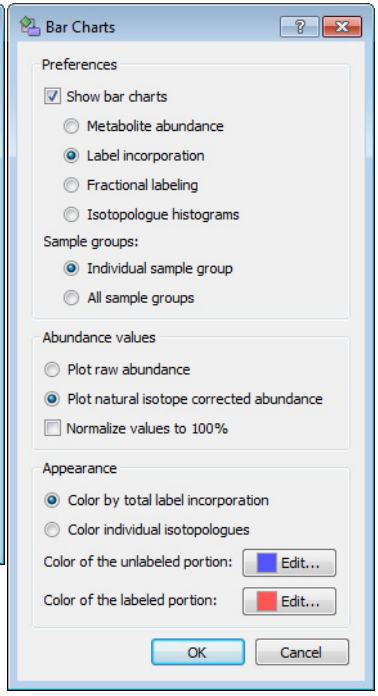


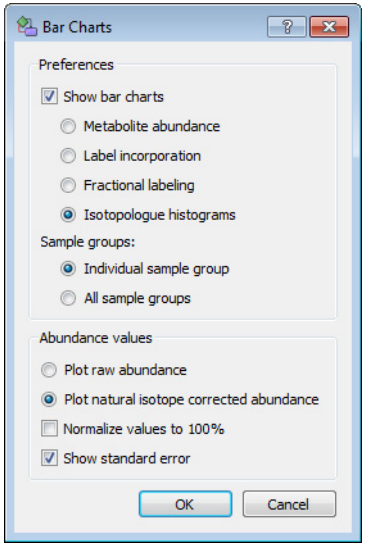
Steps	Detailed Instructions	Comments
7 Add visualization of quilt plots to your network diagram.	**a** Click the **Show Quilt Plots** button from the toolbar, or click **Visualization > Agilent MassVisualizer > Show Quilt Plots**. **b** Mark **Show quilt plots of isotopologue abundance**. **c** Click **Individual sample group**. **d** Click **OK**. **e** Adjust the parameters and become familiar with the results shown on the network diagram.	• Visualize the isotopologue abundances, by individual or all sample groups, in quilt plots next to the metabolite nodes. • Indications of statistical significance of the isotopologue abundances among the groups can be enabled and adjusted by either a pairwise Welch's t-test versus control or a one-way ANOVA of each group against every other group. • Quilt plot parameters are viewed in the **Quilt Plots** dialog box. • See the *MassHunter VistaFlux Software - Workflow Guide* for a detailed overview of the Quilt Plots.



Steps	Detailed Instructions	Comments
8 Add visualization of bar charts to your network diagram. • You can view bar charts containing: ◦ metabolite abundance ◦ label incorporation ◦ fractional labeling ◦ isotopologue histograms	**a** Click the **Show Bar Charts** button from the toolbar, or click **Visualization > Agilent MassVisualizer > Show Bar Charts**. **b** Mark **Show bar charts**. **c** Click **Individual sample group**. **d** Click **OK**. **e** Adjust the parameters and become familiar with the results shown on the network diagram.	• Visualize various isotopologue summaries in bar charts displayed next to the metabolite nodes. • Bar chart parameters are viewed in the **Bar Charts** dialog box. The dialog box parameters are identical for **Metabolite abundance** and **Fractional labeling**. • See the *MassHunter VistaFlux Software - Workflow Guide* for a detailed overview of the Bar Charts.







Steps	Detailed Instructions	Comments
9 Save your Omix Premium project.	**a** Click **File > Save As**. **b** Navigate to the folder to save your Omix Premium network in the **Save Omix Network Model** dialog box. **c** Enter a descriptive name for the **File name**. **d** Click **Save**.	• At any time during your session with Omix Premium you can save your pathways network diagram as an Omix Premium (OMX) document. Save your session with a descriptive name and a sequential number for the file name to save time when you return to prior network diagram. • When your Omix Premium document has unsaved changes an asterisk appears at the end of the file name in the title bar

What is Batch Isotopologue Extraction?

Batch isotopologue extraction is optimal with data that has been acquired in profile mode GC/Q-TOF and GC/MSD is not supported by batch isotopologue extraction. Unlike the other batch feature extraction wizards, target retention times are required for this workflow.

Isotopologue extraction uses an input CSV file or compound database file, PCD/PCDL, containing the target feature molecular formulas, mass, and/or retention time information, and anticipates that the target compound may have undergone some degree of isotope labeling. After feature extraction is performed, the extraction algorithm determines which of the possible isotopologues are actually present, measures the raw abundances of the isotopologues, and corrects the isotopologues abundances for the natural occurrence of the unlabeled ions.

Isotopes, Isotopomers, Isotopologues, and Mass Spectra

Isotopologues are molecules that contain the same molecular formula and structure but differ in their isotopic composition through the substitution of one or more atoms with a different isotope. The exact location of the isotope in the molecule, while important chemically, is not important in flux analysis, just the number of isotopes in the molecule. Isotopologues can be identified using single-stage MS.

Isotopomers are molecules that contain the same molecular formula, structure, and number of isotopes but differ in the location of the isotopes in the molecular structure. Isotopomers can be identified using advanced MS/MS techniques.

Note: Profinder finds and extracts isotopologues in your sample data; it does not find or extract isotopomers.

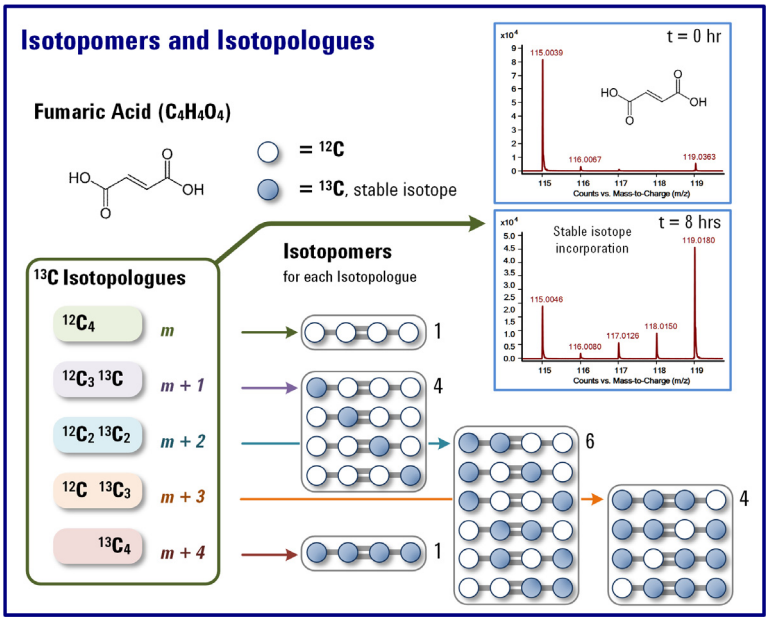


Figure 16 An illustration of how two stable carbon isotopes in a four-carbon molecule relate to isotopomers and isotopologues. Isotopologues are viewed in mass spectra during flux analyses.

A simple four carbon molecule, fumaric acid ($C_4H_4O_4$), is used to explain the relationship of isotopes to isotopomers, isotopologues, and mass spectra. The most abundant isotope of carbon is ^{12}C. However, ^{13}C, also stable, is not nearly as naturally abundant as ^{12}C; ^{13}C has a natural occurrence of 1.1% of ^{12}C. For simplicity, naturally occurring ^{13}C is considered to be negligible; therefore, the mass of the naturally occurring four-^{12}C molecule is m (represented as $^{12}C_4$), and there are no positional differences among the isotopes of the carbon atoms.

When a single ^{13}C atom is substituted for one ^{12}C atom, four locations are possible where the ^{13}C atom can be placed (isotopomers as shown in Figure 16), and each isotopomer has a mass of m + 1 ($^{12}C_3\,^{13}C$). When two of the ^{12}C atoms are replaced with ^{13}C atoms, six isotopomers are possible, and each of the doubly substituted molecules has a mass of m + 2 ($^{12}C_2\,^{13}C_2$). When three of the ^{12}C atoms are replaced with ^{13}C

atoms, four isotopomers are possible, and each isotopomer has a mass of m + 3 ($^{12}C\ ^{13}C_3$). Finally, when all four of the ^{12}C atoms are replaced with ^{13}C atoms, only a single arrangement with a mass of m + 4 ($^{13}C_4$) exists. The five different masses m, m + 1, m + 2, m + 3, and m + 4 represent the masses of the five isotopologues visible in the resulting mass spectra.

Isotopologue mining

Profinder performs isotopologue mining in two stages, an initial screening followed by refinement. The initial screening stage extracts isotopologue EICs around the target retention time range and then evaluates peak mass spectral data to find ions that match the predicted list of possible isotopologues. The refinement stage uses a self-optimizing peak finder to refine the *m/z* assignment from the profile data and then re-extracts the EICs using the new isotopologue *m/z* values, refines the start and end retention time bounds on the newly extracted EICs, and then reports both EIC peak area and summed isotopologue peak heights as the compound abundances.

What is label incorporation?

A feature in Omix Premium is to display label incorporation (L) for your target metabolites. The label incorporation for each metabolite is the sum of all non-zero isotopologue abundances divided by the m+0 isotopologue abundance as shown by the following equation:

$$L = \sum_{1}^{n} \frac{A_{m+n}}{A_{m+0}}$$

where m represents the non-labeled target metabolite, n is an integer representing the maximum number of labeled atoms observed in the target metabolite, and A is the abundance of the indicated metabolite isotopologue.

When label incorporation is visualized for a ***single sample group***, the minimum and maximum label incorporations, L_{min} and L_{max}, are searched within the current sample group.

Only those metabolites are considered that are not on the ignore list. When L=0 the metabolite is additionally ignored because L=0 has a special color; therefore, L_{min} is always > 0. L_{min} and L_{max} are then scaled to values between 0 and 1 for color coding. When L of an ignored metabolite is $0 < L < L_{min}$ it is scaled to 0, when $L > L_{max}$ it is scaled to 1. All other L values scaled between 0 and 1.

In the default color coding, from white to dark green, the whitest metabolite is $L = L_{min}$ and the most dark green colored metabolite is $L = L_{max}$. If a metabolite has no label incorporation (L=0) the no-fold-change color is taken.

When label incorporation is visualized for ***all sample groups***, the greatest label incorporation value out of all of the sample groups is assigned for each individual metabolite. Then, the same scaling is applied as described for a single sample group.

MassHunter VistaFlux Software Installation

MassHunter VistaFlux Software 1.0 contains the following components on the installation DVD:

Agilent MassHunter **Pathways to PCDL** Software B.07.00

Agilent MassHunter **PCDL Manager** B.07.00 SP2

Agilent MassHunter **Profinder** B.08.00

Omix Premium Version 1.9

Data folder containing example data and project files:

CS Gln Flux - data folder containing 15 sample data files

Profinder-Gln-IDH2.m - Profinder method folder

BioCyc metabolites.cdb - PCDL for the example data

CS-Gln-IDH2.pfa - Profinder archive file

CS-Gln-IDH2.Profinder - Profinder project file

TCA-IDH2.omx - Omix Premium project file

TCA-BioCyc-IDH2.cdb - metabolite compound database

Install MassHunter VistaFlux Software on the highest performing PC you have available. Profinder requires a PC running Windows 7 (64-bit) with at least 8GB of RAM and at least 30GB of available disk space.

Note: A PC with 16GB or more of RAM and a solid-state drive has significantly improved Profinder performance and reduction in the time it takes to extract features from large data sets.

Pathways to PCDL, Version B.07.00 

Pathways to PCDL is installed from a Setup Wizard, which you run from the main installation program.

Double-click **Pathways to PCDL.msi**, or right-click the file and then click **Install**.

PCDL Manager, Version B.07.00 SP2 

PCDL Manager is installed from a Setup Wizard, which you run from the main installation program. After you install version B.07.00, follow the instructions to install service packs SP1 and SP2.

Right-click **PCDLSetup.exe**, and then click **Run as administrator**.

Install SP1

1. Navigate to the folder **Service Packs\SP1** on the installation DVD.
2. Right-click **PCDL.B.07.00.SP1.exe**, and then click **Run as administrator**.

Install SP2.

1. Navigate to the folder **Service Packs\SP2** on the installation DVD.
2. Right-click **PCDL.B.07.00.SP1.exe**, and then click **Run as administrator**.

Profinder, Version B.08.00 

Profinder is installed from a Setup Wizard, which you run from the main installation program. If you have a prior version, uninstall Profinder before installing this newer version (see "Uninstall a prior version of Profinder" on page 59).

Right-click **ProfinderSetup.exe**, and then click **Run as administrator**.

Uninstall a prior version of Profinder If you have a prior version of Profinder installed, you must remove the prior version before installing a newer version.

1. Click **Start > Control Panel**.
2. Click **Programs and Features**.
3. Click **Agilent MassHunter Workstation Profinder Software**.
4. Click **Uninstall/Change** to uninstall Profinder.

Omix Premium, Version 1.9

Omix Premium is installed from a Setup Wizard, which you run from the main installation program.

When you start Omix Premium for the first time, you need an Internet connection and you are requested to activate the installation with a serial number.

1 Right-click **OmixPremium1.9.exe**, and then click **Run as administrator**.

2 Launch **Omix Premium** and activate your installation with your serial number.

Note: The Omix Premium serial number is located on the sleeve containing your MassHunter VistaFlux Software DVD.

- **a** Double-click the **Omix Premium** icon located on your desktop, or click **Start > All Programs > Omix Premium > Omix Premium**.
- **b** Type your serial number.
- **c** Click **Activate**. You must have an Internet connection to activate Omix Premium.
- **d** Click **Close**.

3 Optional, register your serial number by visiting http://premium.omix.bio/registerserialnumber.

Note: After you register Omix Premium you can manage your serial number and activations in your user account. Every serial number includes activations on three different computers. You can remove a computer from the list of installations every six months and use the available activation for a new computer, this helps you move Omix Premium to newer computer hardware and manage users in your laboratory.

Acknowledgments and Citations

BioCyc Pathway/Genome Databases

Includes BioCyc Pathway/Genome databases from the Bioinformatics Research Group at SRI International®, used under license.

http://www.biocyc.org/

Citation based on use of BioCyc databases or the Pathway Tools software

If you use BioCyc databases or the Pathway Tools software in your research, cite relevant publications as described on the BioCyc website:

http://biocyc.org/publications.shtml

For example, users who publish research results in scientific journals based on use of data from the EcoCyc Pathway/Genome database should cite:

Keseler et al., *Nucleic Acids Research* **39**:D583-90, 2011.

Users who publish research results in scientific journals based on use of data from most other BioCyc Pathway/Genome databases should cite:

Caspi et al., *Nucleic Acids Research* **40**:D742-53, 2012.

KEGG Database

Includes KEGG (Kyoto Encyclopedia of Genes and Genomes) databases developed by Kanehisa Laboratories.

http://www.genome.jp/kegg/

Citation based on use of KEGG Database

If you use the KEGG database in your research, cite relevant publications as described on the KEGG website:

http://www.genome.jp/kegg/kegg1.html

www.agilent.com

In this book

The *Agilent G4992AA MassHunter VistaFlux Software Quick Start Guide* presents the first steps to use the MassHunter VistaFlux Software.

This Quick Start Guide applies to MassHunter VistaFlux 1.0 and later until superseded.

For Research Use Only. Not for use in diagnostic procedures.

Revision A, May 2016

G4992-90001

Agilent Technologies