

# MassHunter VistaFlux Software

# **Quick Start Guide**

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

Getting Started 6 Software User Interfaces 7 MassHunter Pathways to PCDL 7 MassHunter PCDL Manager 8 MassHunter Profinder 11 Omix Premium 18 Definitions 27 Basic Qualitative Flux Analysis Workflow 30 What is Batch Isotopologue Extraction? 54 What is label incorporation? 57 MassHunter VistaFlux Software Installation 58 Acknowledgments and Citations 61

# 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 PCDLThe workflow begins with your experiment design. Pathways to<br/>PCDL facilitates the creation of a custom personal compound<br/>database and library (PCDL) from metabolites present in<br/>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.





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.

Help

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.

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.

MassHunter PCDL Manager

	Settings	Tools	Help	
	Figure 5	Pathways to	PCDL M	enu bar
Settings	Launches t can specify to exclude when you a	the <b>Pathwa</b> a reference compound are using th	<b>ys to PC</b> ce METL s if the c ne KEGG	<b>DL Settings</b> dialog box where you IN database and choose whether organism has no related enzymes database.
Tools	Provides o culled from and WikiPa files. Access license.	ptions to u n popular d athways. Yo ss to KEGG	pdate th latabase ou can al pathwa	e local copy of pathway content s such as BioCyc/MetaCyc, KEGG, lso import BioCyc tarball and zip y content requires a separate
Help	Provides a - <i>Quick Sto</i> version.	link to the art Guide a	<i>MassHu</i> and infor	enter Pathways to PCDL Software rmation about the software
	2. Display I	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.

**MassHunter PCDL Manager** 

MassHunter PCDL Man	ager for Metabolom	ics - C:\Mase	Menu	Bar	WFG.cdb											×
Eind Compounds		P		a a Ha			_		Action 1	<b>Fabs</b>						
				00108	ar					1		_ [	Actio	n Dana		
Single Search	Batch Search	Batch Sur	mmary	Edit Co	mpounds	Spectral	Search	B	rowse Spectra	Ed	it Spectra		Actio			_
Mass									Molecule	: Structure	MOL Te	đ		<u> </u>		
©	[M+H]+ <ul> <li>Neutral</li> </ul>	© [M-H]∙	Form	ula:					Q		но					
Mass tolerance:	10.0 💿 ppm 🔘	mDa	Na	me:							no					
Detection firm			No	tes:								$\rightarrow$		4.0		
Retention time	De autor		IUF	AC:										//~		
	Require										0	//	//	4		
RT tolerance: 0.1	min				Action	Dana										
lon search mode					ACTION	raite								VOH		
Include neutrals			Up	tions,	parame	ters, and	views									
Include anions			associ	ated w	ith sear	ching, vie	ewing,	and	Notes	Endogenor	us Metabo	te			~	
Include cations			editing	compo	ounds a	nd spectr	a. Con	tent		http://dbk	.ch.umist.a	c.uk/Exact	Masses.htm			
			char	iges b	ased on	the tab s	elected	1.		Compound	in the pat	nway is FUN	(fumarate)			
									1						-	
														D 1/ 1		-
Print/Copy in Summa	ary Format	Single S	earch Res	ults: 20	hits							U	ompound	<b>Kesults</b>	'ane	_
Compound	l Name	Formula	Mass	Anion	Cation	RT (min)	CAS	ChemSp	pider MET	'LIN H	MP	KEGG	LMP	IUPAC Name	Spectra	<b>_</b>
Hydrogen Ion		н	1.00783							HM	DB59597	<u>C00080</u>			0	
Water		H20	18.01057				7732-18-5		3194	HM	DB02111	C01328			0	11
Carbon dioxide		CO2	43.98983				<u>124-38-9</u>		<u>3199</u>	HM	DB01967	C00011			0	Ξ
Fumaric acid		C4H4O4	116.01096			0.634	<u>110-17-8</u>		3242	HM	DB00134	C00122			1	
Succinic acid		C4H6O4	118.02661		(m)		110-15-6		<u>114</u>	HM	DB00254	<u>C00042</u>	LMFA01170043		4	
Oxaloacetate		C4H4O5	C	ompo	ound R	esults 1	able		<u>123</u>	HM	DB00223	C00036	LMFA01170120		1	11
L-Malic acid		C4H605	Liste	Lists the compounds that most the			4593	<u>1 HM</u>	UB00156	<u>C00149</u>			3	-		
				Lists the compounds that meet the			119	HM	DB00208	<u>C00026</u>			3			
Oxoglutaric acid		COHEOS	2,000		ooorob a	ritorio			2200		0000070	000417			0	
Oxoglutaric acid Aconitic acid		C6H6O6	Liot		search o	riteria.			3300	HM	DB00072	C00417			6	
Oxoglutaric acid Aconitic acid threo-Isocitric acid		C6H6O6 C6H8O7	192.02700		search o	riteria.	77.02.0		<u>3300</u> 6364	HMI HMI	DB00072 DB01874	C00417 C00451			6 0	

**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.

File	Edit	View	PCDL	Links	Help

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*.

**MassHunter PCDL Manager** 

- **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:

#### Button **Equivalent Command PCDL Manager Toolbar** Find **PCDL > Find Compounds** 4 File > Print Results H File > Export Results (from Batch Summary tab) File > New PCDL 2 File > Open PCDL -X Auto-size Columns, no equivalent command 挡 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.



- 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.

**MassHunter Profinder** 



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 <b>Plot Display Options</b> 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.
<b>Results Modes</b>	Select the display mode to use in your results windows.

Button	Equivalent Command
Ľ	File > New Project
<b>6</b>	File > Open Project
	File > Save Project
醖	File > Add/Remove Sample Files
щ	View > Compound Groups
<b>(</b>	View > Compound Details
Δ	View > Chromatogram Results
Щ	View > MS Spectrum Results
ш	View > Isotopologue Results
A	Edit > Color by Sample Group

**Profinder Toolbar** 

**MassHunter Profinder** 

Button	Equivalent Command
<u>W</u> izards ▼	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
4 -	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.

Aut	omatic	ally Show Colu	mns 🛗			
Group	₹₽	RT (Tgt) 🛛 🕫	RT (med) 🛛 🖶	Found ∀+	Missed 🖓 🛱	%RSD (Te
	1	11.772	11.767	16	0	
	2	13.807	13.798	16	0	
	4	6.864	6.863	16	0	
	5	0.37	0.37	16	0	
	6	5.401	5.404	16	0	
	з	12.308	12.304	16	0	
	7	0.862	0.853	16	0	
	13	13.377	13.375	16	0	
	14	1.814	1.812	16	0	
	15	12.205	12.205	16	0	
	16	12.052	12.051	16	0	
	17	13.886	13.886	16	0	
	18	8.999	9	16	0	
	19	7.515	7.51	16	0	
	20	11 105	11 105	16	0	

**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.

	Germound Details						
i	Automatically Sh	ow Colum	ins 💾				
Î	File / 🖓 🛱	Flag 🔽 🛱	Score (Tg マ+⊨	Score (MFE)	7-Þ	Area	V
	1-1_Control_000.d		99.82		80	1941	300
	1-2_Control_000.d		99.86	4	7.9	2385	771
	1-3_Control_000.d		99.77	7	1.4	1539	023
	1-4_Control_000.d		99.99	5	1.6	2826	550
	2-1_Infected_000.d		99.84		80	2256	079
	2-2_Infected_000.d		99.98		80	3353	059
	2-3_Infected_000.d		99.82		80	2234	317
	2-4_Infected_000.d		99.83	1	.00	2507	199
	3-1_Control_250.d		99.54	5	4.7	1670	758
	3-2_Control_250.d		99.29	5	9.9	1204	019
	3-3_Control_250.d		99.73	6	9.6	1708	888
	3-4_Control_250.d		99.65	5	7.5	1659	736
	4-1_Infected_250.d		99.41	7	7.1	1700	606
	4-2_Infected_250.d		99.73		98	2499	697

**MassHunter Profinder** 



**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.

Omix Premium

**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.

**Omix Premium** 



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.

<u>F</u> ile	<u>E</u> dit	Inser	t <u>L</u> ayo	out	<u>D</u> ata	<u>V</u> isualization
<u>V</u> iew	<u>A</u> na	lysis	<u>E</u> xtras	Wi	indow	<u>H</u> elp

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

**Omix Premium** 

- 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.







### **Omix Premium Toolbars**

Button	Equivalent Command
~	Edit > Cut
	Edit > Paste
	File > Import > Import Network from File
1	File > Export > Export Network File
	File > Print
5	File > Save Image
	Utility Toolbar
¢∳	Visualization > Quick Visualizer
8	Visualization > Visualize by Scripting (OVL)
9	Layout > Edit Layout Patterns
$\bigcirc$	Edit > Custom Shapes
-><	Layout > Motif Stamps > Manage Motif Stamps
2	Extras > Document Extensions
E	Window > Sidebars > Component View
	Window > Sidebars > Property Editor
1>	Window > Sidebars > Log Messages
	Window > Sidebars > Data Manager
*	Layout > Automatic Layout

Button	Equivalent Command
	Visibility and Detail Toolbar
Ehuk	View > Show Rulers
##	View > Show Grid
€	View > Visualize Properties on Demand
6	View > Hide Metabolites
	View > Hide Reactions
0	View > Hide Cofactors
∕∕≋	View > Hide Effector Edges
6	View > Hide Flux edges
5	View > Hide Pathways
CMP	View > Hide Compartments
	View > Hide Node Items
10	View > Hide Connection Edges
a	View > Hide Graphical Items
<b>.</b>	View > Appearance of Metabolite Labels
a,	View > Appearance of reaction Labels
<u>5</u>	View > Appearance of Pathway Labels
<b>E</b> ,	View > Appearance of Compartment Labels
<b>.</b>	View > Appearance of Node Item Labels

Button	Equivalent Command
	View > Hide Comments
	View > Appearance of Reversibility
-2	View > Hide Coefficients
	View > Hide Item Accessories
<b>B</b>	Visualization > Visualize Chemical Structures
	Zoom Toolbar
11	View > Zoom > Zoom 1:1
Ð	View > Zoom > Zoom In
	View > Zoom > Zoom Out
Q	View > Zoom > Zoom Selection
Q	View > Zoom > Zoom Diagram
	Edit Toolbar
٩	Edit > Network View Mode
131	Edit > Select
	Insert > Insert Metabolite > Insert Metabolite
	Insert > Insert Reaction > Insert Reaction
1.	Insert > Insert Flux Edge
1/2	Insert > Insert Effector Edge
5	Insert > Insert Pathway > Insert Pathway

Button	Equivalent Command
CMP	Insert > Insert Compartment > Insert Compartment
	Graphics Toolbar
	Insert > Insert Node Item
14	Insert > Insert Connection Edge
A	Insert > Insert Text Item
<b>A</b>	Insert > Insert Graphical Item
	Insert > Insert Image
MK	Insert > Insert Chart
<b>_</b>	Insert > Insert Comment
	Select Toolbar
-	Edit > Delete
GRP	Edit > Group Items
GRP	Edit > Ungroup Items
GRP	Edit > Ungroup All
	Edit > Mirror Horizontally
	Edit > Mirror Vertically
	Edit > Rotate 90° Clockwise
	Edit > Rotate 90° Counter-Clockwise
<u>=1</u>	Edit > Stack Before (applies to graphics items)

Button	Equivalent Command
E	Edit > Stack Behind (applies to graphics items)
	Edit > Duplicate Metabolite Node
100	Edit > Invert Edge Direction
₹	Edit > Invert Reaction
\$	Add Spline Point, no equivalent command
-	Delete Spline Point, no equivalent command
1	Line, no equivalent command
(	Curve with one control point, no equivalent command
ہے	Curve with two control points, no equivalent command
Z	Angular Join, no equivalent command
₽	Axis Parallel Segments, no equivalent command
V.	Smooth Join, no equivalent command
$\bigcup$	Symmetric Join, no equivalent command
₩	Edit > Align with Grid
	MassVisualizer Toolbar
8	Visualization > Agilent MassVisualizer > Show Abundance Changes
	Visualization > Agilent MassVisualizer > Show Quilt Plots
1	Visualization > Agilent MassVisualizer > Show Bar Charts

Button	Equivalent Command
<b>\$</b>	Visualization > Agilent MassVisualizer > Show Background Information
2	Visualization > Agilent MassVisualizer > Reload Visualization
	Visualization > Stop icon
	Visualization > Back icon
	Visualization > Play icon
	Visualization > Slider bar
٢	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.

Definitions

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.
lsotopologue	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.
lsotopomer	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.

Definitions

# **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.	<ul> <li>Double-click the Pathways to PCDL icon icon located on your desktop, or click Start &gt; All Programs &gt; Agilent</li> <li>MassHunter Workstation &gt; Pathways to PCDL &gt; Pathways to PCDL.</li> </ul>	<ul> <li>The first time you run Pathways to PCDL you are prompted to set a reference METLIN database folder and filename. This is <i>optional</i>, you can click Cancel in the Select Reference METLIN PCD/PCDL dialog box.</li> </ul>		
2 Select the source to search for pathways data.	<ul> <li>Select BioCyc/MetaCyc for the Source under Pathway Data.</li> </ul>	The Pathways to PCDL converter supports pathway content from BioCyc/MetaCyc, KEGG, and		
	Pathways to PCDL         Settings       Tools         Help         Pathway Data         Source       BioQyc/MetaQyc         Organism/Database       Add/Remove         All Organisms	WikiPathways databases. You must have a license, user name and password, to obtain content from the KEGG database.		

**Create an initial PCDL from pathways content** 

Steps		Detailed Instructions	Comments
3	Select an organism to filter the pathways from the data source.	<ul> <li>Select Homo sapiens from the Organism/Database list.</li> </ul>	
		Pathways to PCDL Settings Tools Help Pathway Data Source BioCyc/MetaCyc  Organism/Database All Organisms Escherichia coli Homo sapiens Murinae Streptococcus	e Homo sapiens
4	Choose a selection mode.	Click Pathway Names for the Selection Mode.      Selection Mode     Pathway Names     Fellow Pathway Members     Reaction Pathway	<ul> <li>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.</li> </ul>
5	Add one or more pathways to your Selected Pathways List.	<ul> <li>a Type TCA for the Search Text.</li> <li>b Click the row containing the T cycle pathway.</li> <li>c Click Select Highlighted to more pathway to the Selected Pathway.</li> <li>d Repeat steps a through c to consider adding pathways sources for y compound database if you are creating your own experiment.</li> </ul>	<ul> <li>In cases when the number of pathways, or compounds, displayed in the <i>Pathways or Compounds List</i> in the display pane is large, you can reduce the number of pathways, or compounds, by using search text</li> <li>The compounds associated with the pathway are moved the <i>Selected Pathways List</i> immediately after you click Select Highlighted, or Select All.</li> </ul>

Pathways to PCDL										×
Settings Tools Help										
Pathway Data		Selection Mode	Prefer	Compound Names from						
Source BioCyc/MetaCyc -		Pathway Names	Me	taCyc						
Organism/Database	Add/Remove	Fellow Pathway Members	O ME	ETLIN						
Homo sapiens	•	Reaction Partners		Create PCD/PCDL				View	Unresolved	
Search Text		Select Highlighted	201	Inique Resolved Co	omnounde				Cle	ear Al
TCA	Clear	Select All	200	Shique Resolved O	Calastian	Catal			# -4	
1 Pathways				Organism	Mode	ID	Name		Cmpd	Del.
ID Name	e	# of Member C	6	Homo sapiens	Pathway	PWY66-398	TCA cycle		20	×
PWY66-398 TCA	cycle	20								
•		•								

**Create an initial PCDL from pathways content** 

Steps	Detailed Instructions	Comments
<b>6</b> Create your custom PCDL.	<ul> <li>a Click Create PCD/PCDL.</li> <li>b Select the folder to save your PCD/PCDL database.</li> <li>c Type the name for your PCD/PCDL database in File name, Target_01.</li> <li>d Click Save.</li> </ul>	<ul> <li>Your PCDL is created from the compounds within the <i>Selected Pathways List</i>.</li> <li>The default folder for a custom PCDL is C:\MassHunter\PCDL.</li> </ul>
Save As		
() → I → Computer → OSDisk (C:) → MassHunter → PCDL →	▼ 4 Search PCDL P	
Orazpize - New falder		
Organize *       New Folder         Configuration       Name         Interpretation       Archive         Interpretation       Mass List Files         Interpretation       Interpretation         Interpretation       Interpretation      <	Date modified         Type         Size           4/12/2016 10/28 PM         File folder         4/12/2016 11/14 PM         COB File         380 KB           4/12/2016 11/14 PM         COB File         138 KB         20/2/2016 12/24 PM         File folder           4/12/2016 4:14 PM         COB File         138 KB         20/2/2016 12/24 PM         COB File         138 KB           2/22/2016 12/24 PM         COB File         1,121,596 KB         4/13/2016 10/06 AM         COB File         1,121,596 KB           4/12/2016 4:44 PM         COB File         1,221,596 KB         4/12/2016 4:44 PM         COB File         23,068 KB           10/19/2014 4:44 PM         COB File         23,068 KB         *         *	
	e Click OK. Target_01cdb has been created. 18 compounds were based on your reference METLIN PCD/PCDL 2 compounds were created from other sources. OK	<ul> <li>Summary information describing your custom PCDL is displayed after you Save the PCDL.</li> </ul>

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 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.





Steps	Detailed Instructions	Comments		
1 Start PCDL Manager.	<ul> <li>Double-click the PCDL Manager icon         Iccated on your desktop, or click     </li> <li>Start &gt; All Programs &gt; Agilent &gt;         MassHunter Workstation &gt; PCDL     </li> <li>Manager.</li> </ul>	• 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.	<ul> <li>a Click File &gt; New PCDL.</li> <li>b Select General for Select the PCDL type.</li> <li>c Type a new name for Enter the new PCDL name.</li> <li>d Enter a useful description of the current PCDL for Enter a description of this PCDL.</li> <li>e Click Create to make a copy of the compound database.</li> </ul>	<ul> <li>The Create new PCDL dialog box immediately opens and has information completed for the open compound database.</li> <li>Creating frequent copies of your PCDL saves time returning to an earlier version of your target metabolite database.</li> </ul>		

Generate a target metabolite PCDL

1040			I	Det	ailed	Ins	tructior	IS		Comments
Create New PCDL PCDL path: C:\MassHu Select an existing PCDL: Select the PCDL type: Enter the new PCDL name: Enter a description of this PCDL: TCA Cycle after removing unintered	Inter\PCDL 01-TCA Empty Leucine Metin_ Metin_ Sufias I anget General Taarget sting compounds	_Cycle_WF s Degradatic AMRT_PCDL Ljuids_AM_ Peptides_AI Peptides_AI 	G on - Fluc DL PCDL AM_P CDL AM_P CDL AM_P CDL AM_P CDL Cance	x T E						<ul> <li>When you create a new PCDL, the original PCDL is closed and the new PCDL is automatically opened in PCDL Manager.</li> <li>In the example, the original PCDL file name is appended with numbers "_xx" to simplify restoring a previous copy.</li> </ul>
View the compoun personal compoun Musham PCDL Manger - CMMusham PCDL Tan ER ER Yew ECDL Less Help	nds in you Id databas 9et.02.cdb	r Se	i	a ( b (	Click Click	the Find	Single : I Compo	Search tab. ounds on the	toolbar.	<ul> <li>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</li> </ul>
▶ Find Compounds ⊘ ↓ ▶ Pind Compounds ⊘ ↓ ▶ Pind Compounds ⊘ ↓ ▶ Pind Pind Pind Pind Pind Pind Pind Pind	Summary Edit Com Formula: Name: Notes: IUPAC:	pounde	Spectral Se	arch	Browse	Spectra Molecule:	Edit Spectre Structure MOL Text			<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> </ul>
Indic Compands Batch Search Ba	Summary Edit Com Pomula: Name: Notes: NuPAC: CAS: OrenSpider:	pounde	Spectral Se	varch	Browse	Spectra Molecule:	Edit Spectra Structure MOL Tesd	1		<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>
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Prof. Copy in Summary Femal     Compound Semi     Hexits      Prof. Copy in Summary Femal     Compound Semi     Hexits     Prof. Copy in Summary Femal     Compound Semi     Hexits in Semi     Hexits	Same / Edit Carri Remain / Remain / Re	Mee         2           100783         1           18 01657         1           18 01657         1           116 0166         1           1200627         134 0252           134 0252         1           132 00627         132 00270           132 00270         122 00270           132 00270         120 00270           132 00270         243 00270           132 00270         243 00270           132 00270         243 00270	Vion I		RT (we) 0.654 0.059 1.015	CAS           772-18-3           100-15           228-50-7           228-50-7           228-50-7           238-5	ChenSpider	LUPAC Name	Spectra           0           0           1           3           6           6           6           6           6           6           6           0           0	<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>
Prof.Copy in Sense / Sens	Summery Edit Com Formular Notes: UPAC CarrSpider ComSpider Formular H H H H H H CarrSpider ComSp	bounds		erch Cetom (************************************	RT (em) 0.6544 0.039 1.015	Spectra           Molecule:           Q           Notes:	Chersodar Soctare ACL Teal	IUPAC Name	Spectra 0 0 1 4 4 1 3 3 6 6 6 6 6 6 0 0 3 0 0	<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>
Prof. Copy in Sumary format Corporation Prof. Copy in Sumary format Prof. Copy in Sum	Same / Edit Can Particle / Particle / Parti	Mee         J           100783         J           116 0105         J           118 01057         J           118 01057         J           118 01057         J           118 01057         J           119 0057         J           119 0057         J           120 0057         J           120 0057         J           522 90505         S2 90505           522 90505 <td< td=""><td></td><td></td><td>RT ere)</td><td>Spectra Molecule: CAS 7732-18-5 100-15-6 328-52-7 328-52-7 599-1</td><td>Data Spectra           Otem Spectra         I           I         I</td><td>LUPAC Name</td><td>Spectra           0           0           1           3           6           0           2           6           6           7           3           6           7           8           9           10           2           6           7           8           9           10           2           6           10           2           6           10           10           10           11           12           13           14           15           16           17           18           19           10           10           11           12           13           14           15           16           17           18           19           10           10     <!--</td--><td><ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul></td></td></td<>			RT ere)	Spectra Molecule: CAS 7732-18-5 100-15-6 328-52-7 328-52-7 599-1	Data Spectra           Otem Spectra         I           I         I	LUPAC Name	Spectra           0           0           1           3           6           0           2           6           6           7           3           6           7           8           9           10           2           6           7           8           9           10           2           6           10           2           6           10           10           10           11           12           13           14           15           16           17           18           19           10           10           11           12           13           14           15           16           17           18           19           10           10 </td <td><ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul></td>	<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>
P Frid Compounds  P Frid P P Frid P P P P P P P P P P P P P P P P P P P	Summery Edit Com Henness UMPAC Can Spoker Con Spok	Depends Mass / 1 100733   100733   1100733   1100735   1100735   1100735   1100735   1100735   1100735   1100735   1100755   11007555   11007555   11007555   110075555   110075555555555555555555555555555555555	vien 1	Cation	RT (ren)	CAS	Che Speter      Deen Spider      Oren Spider      Spider	IUPAC Nene	Spectra           0           0           1           3           6           0           6           7           8           9           6           7           7           8           9           6           7           7           7           8           9           6           7           7           7           8           9           6           7           7           7           7           7           7           8           9           6           7           7           8           9           9           9           10           10           11           12           13           14           15           16	<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>
P Frot Corporator (a) (b) (c) (c) (c) (c) (c) (c) (c) (c) (c) (c	Same / Edit Can Nexe:	Mee         J           100733         1           116 0105         2           116 0105         1           113 01057         1           114 02152         1           115 02056         1           120 0627         1           120 0627         5           120 0627         5           5         227020           443 0829         1           120 0627         5           5         5           5         5           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1           5         1			RT (inc) 0.654 0.422 0.554	Spectra Modecule:	Edit Specia           Stoclare         MOL Teal           Oten Speder         I           I	LUPAC Name	Spectra           0           0           0           1           3           0           6           6           6           0           3           0           3           0           6           6           0           3           0           2           3           0           2	<ul> <li>PCDL.</li> <li>Twenty compounds are in the BioCyc/MetaCyc, homo sapiens, TCA cycle pathway.</li> <li>If the compound database is large, searching the database without narrowing search criteria can take a long time.</li> </ul>

**Generate a target metabolite PCDL** 

Steps	De	ailed	Ins	tructio	ns Comments
4 Remove uninteresting compounds.	a b c d	Click editin datab Click Click <i>Resul</i> Shift- <i>Comp</i> the co incluc the ta Click	lick <b>PCDL &gt; Allow Editing</b> to enable diting of the personal compound atabase. lick the <b>Edit Compounds</b> tab. lick <b>Hydrogen Ion</b> in the <i>Compounds</i> <i>esults Table</i> . hift-click <b>Carbon dioxide</b> in the <i>ompounds Results Table</i> to select the compounds between, and iccluding, the selected compounds in the table. lick <b>Delete Selected</b> from the <i>Edit</i> <i>cctions</i> .		<ul> <li>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.</li> <li>Compounds in the <i>compounds</i> in the <i>compounds</i> in the <i>database</i> can be added and removed using the <i>Edit actions</i> only when Allowing Editing is marked in the menu</li> <li>For the TCA cycle in the example, eleven compounds are interesting and nine compounds are interesting and nine compounds are metabolically</li> </ul>
Image: Source FCBL Manager - CMAasherer FCBL/Target,82.cdb           Die Ein Seen 2001. Links Juhr           I) Frank Compounds (2) III )         III III IIII IIII IIIIIIIIIIIIIIIIIII	ipectral Search Edit actions	Browse	Spectra Molecule:	Edit Spect Structure MOL	significant, some are not observable, or observed based on the acquisition settings, in the mass spectrometer. For the
1874C Mass 43 59983 CAS: 124-38-9 RT.  Oben1gder Formula: 002 Ion type 9 Nacrai Prom Cation Cation Cation	Add Ne Save As I Update Sek	r cov cted 3ed Delete the	selected co	mpound from th Endogenous Meta http://dbk.ch.umia Compound in the p	co co co co co co co co co co
Pert-Cay in Sumary Fernat Single Search Results: 20 hits					
Compound Name Formula Mass A	nion Cation	RT (min)	CAS	ChemSpider	IUPAC Name Spectra
Hydrogen Ion H 1.00783					0
Water H2O 18.01057			7732-18-5		0
Carbon dioxide CO2 43.98983		0.00	124-38-9		0
Fumaric acid C4H4O4 116.01095		0.634	110-17-8		
Suconic and C4H604 118.02661	E E	-	110-15-6	-	4
Unite and CAUCOR 132,0058/			340-92-7		
0 - Hello - He		0.00	220 50 7	-	
Uxogutano acio USH605 146.02152		0.423	528-50-7		3

- 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.

**Generate a target metabolite PCDL** 

Steps Detailed Instructions		Comments	
5 Add a batch of new compounds from another PCDL.	<ul> <li>a Click File &gt; Open PCDL.</li> <li>b Click the file name for the METLIN PCDL (or your own PCDL) in the Open PCDL dialog box.</li> <li>c Click Open.</li> <li>d Click the Batch Search tab.</li> <li>e Enter the masses for the new compounds into the Masses table</li> <li>f Click Ignore for Retention times.</li> <li>a Click Find Compounds on the toolbar</li> </ul>	<ul> <li>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.</li> </ul>	



• 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*.



- 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.

Generate a target metabolite PCDL

Steps	Detailed Instructions	Comments
Compounds Appended	I Click No in the Compounds Appended dialog box.	<ul> <li>Click No unless you are sure you want to close your current PCDL and open the target PCDL.</li> <li>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.</li> </ul>
	<ul> <li>m Click the next mass in the Masses table in the Action Pane.</li> <li>n Repeat steps i through m for each mass.</li> <li>o Click Yes in the Compounds Appended dialog box when you have appended the last compound to the target PCDL.</li> <li>p Click the Single Search tab.</li> <li>q Click Find Compounds on the toolbar to view the compounds in the target PCDL.</li> </ul>	
<ul> <li>6 Add retention times and additional identifiers to your target metabolite list.</li> <li><i>Note:</i> 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.</li> </ul>	<ul> <li>a Click the Edit Compounds tab.</li> <li>b Click PCDL &gt; Allow Editing.</li> <li>c Click on a compound row in the <i>Compound Results Table</i>.</li> <li>d Add retention times for each compound per your acquisition method development.</li> <li>e Add any useful, additional compound information at this time.</li> <li>f Click PCDL &gt; Allow Editing to disable further editing.</li> </ul>	<ul> <li>The compound highlighted in the <i>Compound Results Table</i> is the compound that is being edited in the <i>Action Pane</i> for the <b>Edit Compounds</b> tab.</li> <li>You can add retention times using a mass list file. See "Exercise 8. Add retention times to a PCDL" in the <i>MassHunter Personal Compound Database and Library Manager - Quick Start Guide</i>.</li> </ul>
7 Save your target metabolite compound list.	<ul> <li>Click File &gt; Exit to close PCDL Manager.</li> </ul>	<ul> <li>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.     </li> </ul>

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

**Create a Profinder Project** 

Steps	Detailed Instructions	Comments
1 Start Profinder.	<ul> <li>Click the Profinder icon pour desktop, or click Start &gt; All Programs</li> <li>Agilent &gt; MassHunter Workstation</li> <li>Profinder B.08.00.</li> </ul>	• The first time you start Profinder it displays in full screen. Subsequent program launches remember the screen size from your prior session.
<ol> <li>Begin your Profinder project by adding sample files.</li> </ol>	<ul> <li>Click File &gt; Add/Remove Sample</li> <li>Files, or click</li> <li>Add/Remove Sample Files on the</li> </ul>	<ul> <li>If you click File &gt; New Project after starting Profinder, you are automatically prompted to add</li> </ul>
Agilent MassHunter Profinder B.08.00 -	toolbar.	sample files.
File Edit View Method Wizards He		Sample Files after launching
Open Project		Profinder, Profinder starts a new
Save Project		project as if you clicked <b>File &gt; New</b>
Save Project as		Project.
Add/Remove Sample Files		
Export as CEF		
Export as CSV		
Export as Detailed CSV		
Export as Profinder Archive		
Exit		

**3** Add files to the **Add Sample Files** dialog box.

a Click Add file(s) to display the Open file dialog box.

ile selection and disp	lay order		
Show/Hide	Samples	Polarity	Sample Group

**Create a Profinder Project** 

Steps		Detailed Instructions	Comments
Openfile		<ul> <li>b Browse to the folder containing your sample files.</li> <li>c Select all of the sample data files for your Profinder project.</li> <li>d Click Open.</li> </ul>	<ul> <li>Selected sample data files are highlighted in the <b>Open file</b> dialog box.</li> <li>A Profinder project contains all of the sample data files from your experiment - replicate samples for each condition (group) in your</li> </ul>
My Documents	CS Gin Flux           CS_Gin_0_5hr_Ad           CS_Gin_0_5hr_Bd           CS_Gin_0hr_Ad           CS_Gin_0hr_Ad           CS_Gin_0hr_Bd           CS_Gin_1hr_Ad           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_1hr_Cd           CS_Gin_3hr_Ad           CS_Gin_3hr_Bd           CS_Gin_8hr_Ad           CS_Gin_8hr_Ad           CS_Gin_8hr_Cd           CS_Gin_8hr_Cd		<ul> <li>experiment.</li> <li>You can click Add Sample Files, to add additional sample data files to your project and rerun feature extraction.</li> </ul>
Computer Network	File name:     "CS_Gin_0_5hr_f       Files of type:     Data Files (*.d)	\d" "CS_Gin_0_5hr_B.d" "CS_Gin_ ▼ Open	

- 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.

**Create a Profinder Project** 

#### Steps

#### **Detailed Instructions**

Show/Hide	Samples	Polarity	Sample Group
<b>V</b>	CS_GIn_0_5hr_A.d	Negative	0.5 hr
<b>V</b>	CS_GIn_0_5hr_B.d	Negative	0.5 hr
<b>V</b>	CS_GIn_0_5hr_C.d	Negative	0.5 hr
<b>V</b>	CS_GIn_0hr_A.d	Negative	0.0 hr
<b>V</b>	CS_GIn_0hr_B.d	Negative	0.0 hr
<b>V</b>	CS_Gln_0hr_C.d	Negative	0.0 hr
<b>V</b>	CS_GIn_1hr_A.d	Negative	1.0 hr
<b>V</b>	CS_GIn_1hr_B.d	Negative	
<b>V</b>	CS_GIn_1hr_C.d	Negative	
<b>V</b>	CS_Gln_3hr_A.d	Negative	An Copy
<b>V</b>	CS_GIn_3hr_B.d	Negative	& Paste
	CS_Gln_3hr_C.d	Negative	Fill Down
<b>V</b>	CS_GIn_8hr_A.d	Negative	
<b>V</b>	CS_Gin_8hr_B.d	Negative	
1	CS Gin 8hr C d	Negative	

#### 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.

Show/Hide	Samples	Polarity	Sample Group	4
<b>V</b>	CS_Gin_0hr_A.d	Negative	0.0 hr	
V	CS_Gln_0hr_B.d	Negative	0.0 hr	
<b>V</b>	CS_Gln_0hr_C.d	Negative	0.0 hr	
V	CS_Gln_0_5hr_A.d	Negative	0.5 hr	
	CS_Gln_0_5hr_B.d	Negative	0.5 hr	
V	CS_Gln_0_5hr_C.d	Negative	0.5 hr	ſ
	CS_Gin_1hr_A.d	Negative	1.0 hr	
V	CS_GIn_1hr_B.d	Negative	1.0 hr	
<b>V</b>	CS_Gln_1hr_C.d	Negative	1.0 hr	
<b>V</b>	CS_Gln_3hr_A.d	Negative	3.0 hr	
<b>V</b>	CS_Gin_3hr_B.d	Negative	3.0 hr	
	CS_Gln_3hr_C.d	Negative	3.0 hr	
<b>V</b>	CS_Gin_8hr_A.d	Negative	8.0 hr	
	CS_Gin_8hr_B.d	Negative	8.0 hr	
<b>V</b>	CS Gln 8hr C.d	Negative	8.0 hr	

- 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 a Click Batch Isotopologue Extraction. · Batch Isotopologue Extraction is 1 Select the Batch Isotopologue b Click Next. Extraction workflow to apply to optimal with data that has been acquired in profile mode and vour samples. supports only LC/MS acquired data. x Unlike the other batch feature extraction wizards, target retention Feature Extraction Workflow Select the feature extraction algorithm to run times are required for this workflow. Select algorithm Batch Molecular Feature Extraction Batch Recursive Feature Extraction I for small molecules / peptides for large molecules (intact proteins) Batch Targeted Feature Extraction Batch Isotopologue Extraction Help Previous Next Finish Cancel 2 Review and edit the parameters for a Click the lon Species tab to make · Click Help to activate online Help the Batch Isotopologue Extraction specific for the current tab in the changes to the parameters associated wizard. algorithm. with the charge carrier, charge state, and isotopic labeling. · Click Cancel to stop editing the **b** Click the **Extraction** tab to make algorithm parameters. Any changes changes to the parameters associated made to the algorithm parameters with the chromatographic and mass are not saved. spectral extraction. c Click the lon Qualification tab to make changes to the parameters associated with feature matching, isotopologue thresholds, and coelution.

**Run Batch Isotopologue Extraction** 

Steps	<b>Detailed Instructions</b>	Comments
		Ion Species Extraction Ion Qualification Chromatogram smoothing
Batch Isotopologue Extraction Step 1 of 1: Isotopologue - Extraction	n 🕞 ction Parameters 🙀	Smooth EIC before integration Smoothing function: Gaussian Function width: Gaussian width: 5000 points
Target Source (*.cdb, csv) C:\MassHunter\PCDL\default.csv		Ion abundance criterion
Default ion species		Use peak core area     20.00 % Peak height
Positive         Negative           - electron         +electron           +H         +H           +Na         +HC00           +K         +CH3C00           +NH4         +CF3C00           Custom         Custom		Extraction data format  Prefer profile data  Display raw mass spectrum plots  Display isotopologue spectrum plots  Ion Species   Extraction   Ion Qualification   Match tolerance
Charge state		Masses: +/- 15.00 ppm + 2.00 mDa
Labeling © 2H Isotope purity @ 13C	99.00 %	Heterston times: +/- 0.20 minutes Isotopologue ion thresholds Anchor ion height >= 250 counts Sum of ion heights >= 1000 counts
© 15N		Condution threshold
Help	Next Finish Cancel	

- **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.

Operation in Progress: 15 data files in parallel processing	
CS_Gin_8hr_C.d: Process targets	
	Cancel

- 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.

**Run Batch Isotopologue Extraction** 



**Create a Profinder Archive** 

### **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
<ul> <li>Select Export as Profinder Archive from the File menu.</li> <li>Agilent MassHunter Profinder B.08.00 - File Edit View Method Wizards Cc</li> <li>New Project</li> <li>Open Project</li> <li>Save Project as</li> <li>Add/Remove Sample Files</li> <li>Export as CEF</li> <li>Export as CSV</li> <li>Export as Profinder Archive</li> </ul>	<ul> <li>Click File &gt; Export as Profinder Archive.</li> </ul>	<ul> <li>Alternate export options include:</li> <li>Export as CEF- features saved to a file format used to exchange data between Agilent software.</li> <li>Export as CSV - average Mass and RT with actual feature abundance for each sample</li> <li>Export as Detailed CSV - average Mass and RT with all actual feature values for each sample</li> </ul>
Exit		

- **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.

Export to Profinder Archive (.PFA)
Export contents <ul> <li>Only compounds visible in Compound Groups table</li> <li>All compounds</li> </ul>
Export destination Export file path (.PFA)
C:\MassHunter\Data\Flux Example WFG\VistaFlux_WFG.pfa
<u>Q</u> K Cancel

### 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	
<ul> <li>Select Save Project from the menu bar.</li> <li>Agilent MassHunter Profinder B.08.00</li> <li>File Edit View Method Wizards H</li> <li>New Project</li> <li>Open Project</li> <li>Save Project</li> <li>Save Project as</li> </ul>			• Click Fi	le > Save P	roject.	<ul> <li>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.</li> </ul>		
2	Type the file name and save your project.			<ul> <li>a Type the name to use for your project file in the Save as dialog box.</li> <li>b Click Save.</li> </ul>			<ul> <li>The Save As dialog box does not appear when you click Save Project if you previously saved your project.</li> <li>Click File &gt; Save Project as if you want to save your project using a page file page.</li> </ul>	
	Recent Places	Save yr     Name       Name     Profinder-Gin-JDH2.m       Desktop     Save as type:		Date modified Type S 4/4/2016 8:58 PM File folder 4/4/2016 8:58 PM File folder 4/15/2016 12:51 File folder 3/51/2016 10:49 PROFINDER File Address State Stat		5,146 KB	<ul> <li><i>Note:</i> Profinder project files can be one GB or more in file size.</li> <li><i>Note:</i> Remember to include the original sample data when you share a Profinder project.</li> </ul>	
	Network					Save Cancel Help		

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

Visualize 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			etailed Instructions	Comments	
1	Start Omix Premium.	•	Double-click the <b>Omix Premium</b> icon located on your desktop, or click <b>Start &gt; All Programs &gt; Omix</b> <b>Premium &gt; Omix Premium</b> .		
2	2 Open the template network diagram.		Click <b>Open</b> in the <i>Document Area</i> . Click <b>Open other Document</b> . Navigate to the folder containing the VistaFlux example data, then click the <b>TCA-IDH2.omx</b> template network diagram in the <b>Open File</b> dialog box. Click <b>Open</b>	<ul> <li>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.</li> </ul>	
		d	Click <b>Open</b> .		



Steps			Detailed Instructions			Comments	
3	Import the example P results.	rofinder	a Click b Navig exam CS-G file in box. c Click	Data > Open Dat ate to the folder ple data, then clic In-IDH2.pfa Profi the Open Data S Open.	a Table. containing the :k the nder Archive ource dialog	• 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) a	is the control.	• Double group	e-click on the che 1 under the <i>Cont</i>	eck box for <i>rol</i> column.	<ul> <li>The Data Manager window is opened along the bottom of the Display Area.</li> </ul>	
Da	ta Manager					<ul> <li>If the Data Manager window is</li> </ul>	
4	MS Sample Groups	2 - 1 8	\$		already open you can click the		
	Mean Value	Visible	Control	Sample Groups	Color	Open Data Table 🙋 button, a	
	Peaks     Standard Er	V		0 hr		small button located at the	
	Peaks 2			0.5 hr		bottom-left of the Data Manager	
	Peaks - 3			1 hr		window	
<b>.</b>	CS-Gln-IDH2.pfa 🛛 🖊			and stationer de la company			
5	Close the Data manag	ger window.	Click I click t the to windo	Data > Show Dat he Data Manage olbar, to close the w.	a Manager, or Button on Data Manager	<ul> <li>Click Data &gt; Show Data Manager, or click the Data Manager button on the toolbar, to open and close the Data Manager window at any time.</li> </ul>	

<ul> <li>Add visualization of abundance changes changes to your network diagram.</li> <li>a Click the Show Abundance Changes button from the toolbar, or click Visualization &gt; Agilent MassVisualizer &gt; Show Abundance Changes.</li> <li>b Mark Show metabolite abundance fold change (vs. control).</li> <li>c Mark Show Iabeling incorporation (ratio of labeled/unlabeled isotopologue abundances).</li> <li>d Mark Show fractional labeling %.</li> <li>e Click Individual sample group.</li> <li>f Click OK.</li> <li>g Adjust the parameters and become familiar with the results shown on the network diagram.</li> </ul> Abundance Change Visualization Abundance Change (vs. control) <ul> <li>e Mark Show fractional labeling %.</li> <li>e Click Individual sample group.</li> <li>f Click OK.</li> <li>g Adjust the parameters and become familiar with the results shown on the network diagram.</li> </ul> Abundance Change Visualization Visualization (sho of labeled/unlabeled coveries of the Abundance Change parameters i viewed in the Abundance Change familiar with the results shown on the network diagram. Show metabolite dunge (vs. control) <ul> <li>e otor by log/Edd tange</li> <li>e otor by log/Edd tange</li> <li>f Click OK.</li> <li>g Abundance Change Visualization</li> </ul> Preferences Preventage range: <ul> <li>o or of utargeted metabolites:</li> <li>f Show metabolite abundance</li> <li>o of or utargeted metabolites:</li> <li>f Show metabolite abundance</li> <li>o of utargeted metabolites:</li> <li>f Show factoral labeling %.</li> <li>o of or utargeted metabolites:</li> <li>f Show factoral labeling %.</li> <li>o of utargeted metabolites:</li> <li>f Ute raw abundances:</li> <li>o of utargeted metab</li></ul>	ieps	Detailed Instructions	Comments		
Abundance Change Visualization          Preferences         ② Show metabolite change (vs. control)         ④ color by % change         ○ color by % change         ○ color by % change         ○ color by log2(fold change)         ⑦ Auto-scale color range to largest change         Color coding:         ② Show labeling incorporation (ratio of labeled/unlabeled isotopologue abundance)         ⑦ Auto-scale color range to largest incorporation         Color coding:         ② Show fractional labeling %         Color coding:         ② Show fractional labeling %         Color coding:         ③ Alti-angle group         All sample group         All sample groups         Abundance values         ③ Use raw abundances         ④ Use raw abundances	Add visualization of abundance changes to your network diagram	<ul> <li>a Click the Show Abundance Changes</li> <li>button from the toolbar, or click Visualization &gt; Agilent MassVisualizer &gt; Show Abundance Changes.</li> <li>b Mark Show metabolite abundance fold change (vs. control).</li> <li>c Mark Show labeling incorporation (ratio of labeled/unlabeled isotopologue abundances).</li> <li>d Mark Show fractional labeling %.</li> <li>e Click Individual sample group.</li> <li>f Click OK.</li> <li>g Adjust the parameters and become familiar with the results shown on the network diagram.</li> </ul>	<ul> <li>Visualize up to three different summary statistics within the metabolite node:         <ul> <li>maximum metabolite abundance change per group versus the control</li> <li>label incorporation per group</li> <li>fractional labeling change per group versus control</li> </ul> </li> <li>Abundance changes cannot be used with <i>Draw structures on the</i> <i>metabolites</i>.</li> <li>Abundance change parameters are viewed in the Abundance Change Visualization dialog box.</li> <li>See the MassHunter VistaFlux Software - Workflow Guide for a</li> </ul>		
Preferences   Image: 0   Im	Abundance Change Visualization	? <b></b> )	detailed overview of the Abundance		
Show metabolite change (vs. control) <ul> <li>color by % change</li> <li>color by log2(fold change)</li> </ul> <ul> <li>Auto-scale color range to largest change</li> </ul> Color coding: <li>Edit</li> Percentage range: <	Preferences		Change Visualization.		
<ul> <li>color by % change</li> <li>color by log2(fold change)</li> <li>Auto-scale color range to largest change</li> <li>Color coding:</li> <li>Edt</li> <li>Percentage range: &lt;</li> <li>0 &gt;&gt;</li> <li>Show labeling incorporation (ratio of labeled/unlabeled isotopologue abundance)</li> <li>Auto-scale color range to largest incorporation</li> <li>Color coding:</li> <li>Edit</li> <li>Range:</li> <li>0</li> <li>max</li> <li>Show fractional labeling %</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Color coding:</li> <li>Edit</li> <li>Color coding:</li> <li>Edit</li> <li>Color indicating urmeasurable:</li> <li>Edit</li> </ul>	Show metabolite change (vs. control)				
○ color by log2(fold change)   ☑ Auto-scale color range to largest change   Color coding:   □	Color by % change				
Image: Auto-scale color range to largest change   Color coding: Edit   Percentage range: 0   Image: 0	color by log2(fold change)				
Color coding: Edit   Percentage range: <	Auto-scale color range to largest change				
Percentage range: <	Color coding:	Edit			
Show labeling incorporation (ratio of labeled/unlabeled isotopologue abundance)   Image:   Color coding:   Range:   0   max   Show fractional labeling %   Color coding:   Edit   Range:   0%   100%   Scaling   Other colors   Color individual sample group   All sample groups   Other colors Color indicating unmeasurable:   Edit   Abundance values   Use raw abundances   We natural isotope corrected abundances	Percentage range: <	0 >			
Auto-scale color range to largest incorporation   Color coding:   Range:   0   max     Show fractional labeling %   Color coding:   Range:   0%   100%     Scaling   Other colors   Color of untargeted metabolites:   Edit   Abundance values   Use raw abundances   Wate natural isotope corrected abundances	Show labeling incorporation (ratio of labeled/u	nlabeled isotopologue abundance)			
Color coding:       Edit         Range:       0       max         Ø       Show fractional labeling %         Color coding:       Edit         Range:       0%       Edit         Scaling       Other colors         Ø       Individual sample group         All sample groups       Color of untargeted metabolites:       Edit         Abundance values       Other colors       Color indicating unmeasurable:         Ø       Use raw abundances       Edit	Auto-scale color range to largest incorpo	ration			
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Show fractional labeling %         Color coding:       Edit         Range:       0%       100%         Scaling       Other colors       Color of untargeted metabolites:         Individual sample group       Color of untargeted metabolites:       Edit         All sample groups       Color indicating unmeasurable:       Edit         Abundance values       Edit       Color indicating unmeasurable:       Edit         Use raw abundances       Use natural isotope corrected abundances       Edit       Edit	Range: 0	max			
Color coding:       Edit         Range:       0%       100%         Scaling       0ther colors       Color of untargeted metabolites:         Individual sample groups       Color of untargeted metabolites:       Edit         All sample groups       Color indicating unmeasurable:       Edit         Abundance values       Edit       Color indicating unmeasurable:       Edit         Use raw abundances       Use natural isotope corrected abundances       Edit       Edit	Show fractional labeling %				
Range:     0%     100%       Scaling     Other colors       Individual sample group     Other colors       All sample groups     Color of untargeted metabolites:       Abundance values     Color indicating unmeasurable:       Use raw abundances     Use natural isotope corrected abundances	Color coding:	Edit			
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All sample groups     Color indicating unmeasurable:       Abundance values     Edit       Use raw abundances     Use natural isotope corrected abundances	Individual sample group	Color of untargeted metabolites: Edit			
Abundance values	All sample groups	Color indicating upmeasurable:			
<ul> <li>Use raw abundances</li> <li>Use natural isotope corrected abundances</li> </ul>	Abundance values				
Use natural isotope corrected abundances	Use raw abundances				
	Output isotope corrected abundances				

Steps	Detailed Instructions	Comments	
7 Add visualization of quilt plots to your network diagram.	<ul> <li>a Click the Show Quilt Plots button from the toolbar, or click Visualization &gt; Agilent MassVisualizer &gt; Show Quilt Plots.</li> <li>b Mark Show quilt plots of isotopologue abundance.</li> <li>c Click Individual sample group.</li> <li>d Click OK.</li> <li>e Adjust the parameters and become familiar with the results shown on the network diagram.</li> </ul>	<ul> <li>Visualize the isotopologue abundances, by individual or all sample groups, in quilt plots next to the metabolite nodes.</li> <li>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.</li> </ul>	
Quilt Plots      Preferences      Show quilt plots of isotopologue abundance      Individual sample group      All sample groups      Appearance      Color coding for isotopologue quilt plots:      Edit      Show sample group names      Show colored sample group markers		<ul> <li>the Quilt Plots dialog box.</li> <li>See the MassHunter VistaFlux Software - Workflow Guide for a detailed overview of the Quilt Plots.</li> </ul>	
Scale Factor: 1.0			
Statistical visualization Visualize statistical significance:	e		

Steps			Detailed Instructions		Comments	
8	8 Add visualization of bar charts to your network diagram.		<ul> <li>a Click the Show Bar Charts button from the toolbar, or click Visualization</li> <li>&gt; Agilent MassVisualizer &gt; Show Bar Charts.</li> </ul>		<ul> <li>Visualize various isotopologue summaries in bar charts displayed next to the metabolite nodes.</li> <li>Bar chart parameters are viewed in</li> </ul>	
<ul> <li>You can view bar charts containing:</li> <li>metabolite abundance</li> <li>label incorporation</li> <li>fractional labeling</li> <li>isotopologue histograms</li> </ul>		<ul> <li>b Mark Show bar charts.</li> <li>c Click Individual sample group.</li> <li>d Click OK.</li> <li>e Adjust the parameters and become familiar with the results shown on the network diagram.</li> </ul>		the	<ul> <li>the Bar Charts dialog box. The dialog box parameters are identical for Metabolite abundance and Fractional labeling.</li> <li>See the MassHunter VistaFlux Software - Workflow Guide for a detailed overview of the Bar Charts.</li> </ul>	
	Bar Charts	2	Bar Charts	2 E	Bar Charts	
	Preferences		Preferences	P	references	
	Show bar charts		Show bar charts	5	Show bar charts	
Metabolite abundance			Metabolite abundance		Metabolite abundance	

Freierences	Ficicicies	Freierences
☑ Show bar charts	Show bar charts	Show bar charts
Metabolite abundance	Metabolite abundance	Metabolite abundance
Label incorporation	Label incorporation	Label incorporation
Fractional labeling	Fractional labeling	Fractional labeling
Isotopologue histograms	Isotopologue histograms	<ul> <li>Isotopologue histograms</li> </ul>
Sample groups:	Sample groups:	Sample groups:
Individual sample group	<ul> <li>Individual sample group</li> </ul>	<ul> <li>Individual sample group</li> </ul>
All sample groups	All sample groups	All sample groups
Abundance values	Abundance values	Abundance values
Plot raw abundance	Plot raw abundance	Plot raw abundance
Plot natural isotope corrected abundance	Plot natural isotope corrected abundance	Plot natural isotope corrected abundance
Appearance	Normalize values to 100%	Normalize values to 100%
Bar color:	Appearance	Show standard error
	Color by total label incorporation	OK Cancel
OK Cancel	Color individual isotopologues	
	Color of the unlabeled portion:	
	Color of the labeled portion:	
	OK Cancel	

Steps	Detailed Instructions	Comments	
9 Save your Omix Premium project.	<ul> <li>a Click File &gt; Save As.</li> <li>b Navigate to the folder to save your Omix Premium network in the Save Omix Network Model dialog box.</li> <li>c Enter a descriptive name for the File name.</li> <li>d Click Save.</li> </ul>	<ul> <li>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.</li> <li>When your Omix Premium document has unsaved changes an asterisk appears at the end of the file name in the title bar</li> </ul>	

**Visualize your results in Omix Premium** 

# 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.

#### What is Batch Isotopologue Extraction?

**Visualize your results in Omix Premium** 



**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 <sup>12</sup>C. However, <sup>13</sup>C, also stable, is not nearly as naturally abundant as <sup>12</sup>C; <sup>13</sup>C has a natural occurrence of 1.1% of <sup>12</sup>C. For simplicity, naturally occurring <sup>13</sup>C is considered to be negligible; therefore, the mass of the naturally occurring four-<sup>12</sup>C molecule is *m* (represented as <sup>12</sup>C<sub>4</sub>), and there are no positional differences among the isotopes of the carbon atoms.

When a single <sup>13</sup>C atom is substituted for one <sup>12</sup>C atom, four locations are possible where the <sup>13</sup>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 <sup>13</sup>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  ${}^{13}C$ 

#### What is Batch Isotopologue Extraction?

**Visualize your results in Omix Premium** 

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. Pathways to PCDL, Version B.07.00

# **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, 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<sup>®</sup>, 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.

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