Agilent G6825AA MassHunter Pathways to PCDL Software

Quick Start Guide

What is Agilent Pathways to PCDL?

Agilent MassHunter Pathways to PCDL converter is stand-alone software designed to facilitate the creation of custom Agilent PCDLs from metabolites present in pathway content culled from popular databases such as BioCyc, KEGG, and WikiPathways. You can create a personal compound database (PCD) or a personal compound database and library (PCDL) from one or more compounds (metabolites/lipids) that participate, or are associated with metabolic reactions, in selected pathway(s). The PCD/PCDL is created by finding compound names and their associated formulas in a reference METLIN database, that is based on a selected pathway(s). You can filter and select pathways based on database, organism, and/or custom text entries.

The pathway metabolites can be filtered based on their relationship with the pathway (member of the pathway or reaction partner) or through custom text entries.

The Pathways to PCDL converter currently supports pathway content from BioCyc, KEGG (with the required license), and WikiPathways. The databases created with Pathways to PCDL can be used to query acquired data in a targeted data mining workflow, the results of which can be mapped onto biological pathway maps.

Features of Pathways to PCDL

- Create a custom MassHunter METLIN compound PCD/PCDL from pathway information contained in BioCyc, KEGG, and WikiPathways. The pathways may be selected based on:
  - one or many organisms
  - custom search text entries

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    - combinations of organism and text entries
- The list of target compounds that you can extract can be filtered based on the compound being present:
    - in the selected pathway(s)
    - as a reaction partner to compounds involved in the selected pathway(s)
- The pathway PCD/PCDL that you create may be used for identification of metabolites as a source database in other MassHunter software (for example, MassHunter Qualitative Analysis and ID Browser).

Terminology

PCD: An accurate mass compound database. The database may or may not contain retention time data.

PCDL: An accurate mass compound database that also contains an MS/MS accurate mass spectral database (spectral library).

Compound Name: Many compounds are commonly known by their salts. The mass spectrometer, however, detects the anion or cation portion of the salt, rather than the neutral salt. PCD/PCDL entries may contain the familiar compound names, but the empirical formulae reflect the detectable cation or anion portion of the molecule rather than the formula of the neutral compound salt. For example, the full name Vecuronium bromide may be used for identification in the PCD/PCDL, even though the mass/formula only includes the Vecuronium cation.

METLIN database: The MassHunter METLIN PCD/PCDL (METLIN database) is one of the best-known and most-comprehensive metabolite and lipid databases in the world today. The METLIN database contains endogenous and exogenous metabolites, as well as lipids, drug metabolites, and di- and tripeptides. Each entry may include mass, chemical formula, and structure information, as well as ID numbers that link to more information about the compound, such as the CAS and ChemSpider IDs.

Where to Find More Information

Go to www.chem.agilent.com for the most current information on Agilent products.

Getting Started

How do I get started?

This *Quick Start Guide* helps you launch Pathways to PCDL, become familiar with a typical workflow, and use the software to create a custom PCD/PCDL metabolite database from a reference METLIN Metabolite PCD/PCDL database.

Start Pathways to PCDL

1 Double-click the **Pathways to PCDL** icon  located on your desktop, or click **Start > All Programs > Agilent > MassHunter Workstation > Pathways to PCDL > Pathways to PCDL**.

2 The first time you run Pathways to PCDL you are prompted to set a reference METLIN database path and filename.

a Click **Select** in the **Select Reference METLIN PCD/PCDL** dialog box (see Figure 1).

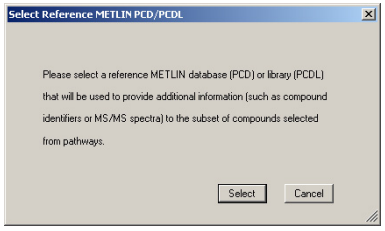


Figure 1 **Select Reference METLIN PCD/PCDL** dialog box

b Select your METLIN database in the **Please specify reference METLIN *.cdb file** dialog box (see Figure 2 on page 4).

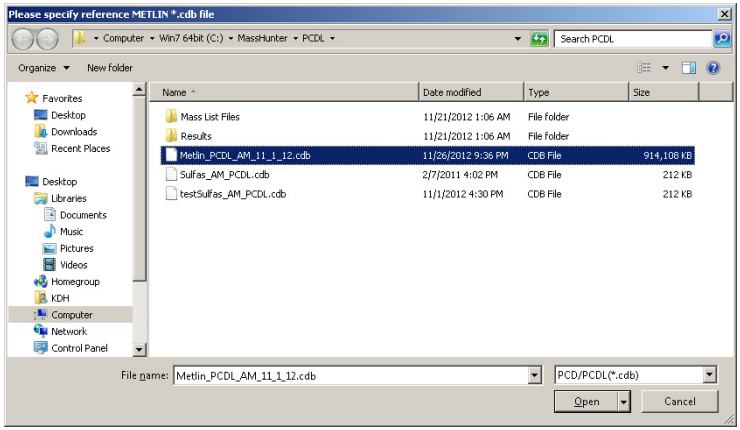


Figure 2 **Please specify reference METLIN *.cdb file** dialog box

3 Study the Pathways to PCDL Workflow shown in Figure 3.

4 Review the rest of this *Quick Start Guide* and follow the steps using your METLIN database.

Pathways to PCDL Workflow

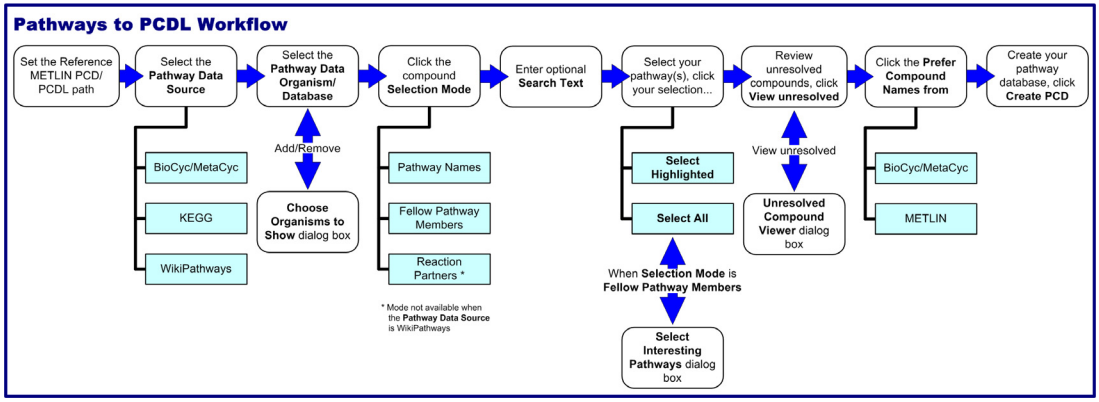


Figure 3 Typical Pathways to PCDL Workflow

User Interface

The main functional areas of Pathways to PCDL software are shown in Figure 4.

The Pathways to PCDL window consists of two parts: the Menu Bar and the Display Pane. The Display Pane is divided into the Pathway List and the Compound List to help you visualize your progress as you select pathway(s) and compound(s) to create your PCDL based on pathway metabolites. The number of pathways and compounds that meet your criteria are shown above each table.

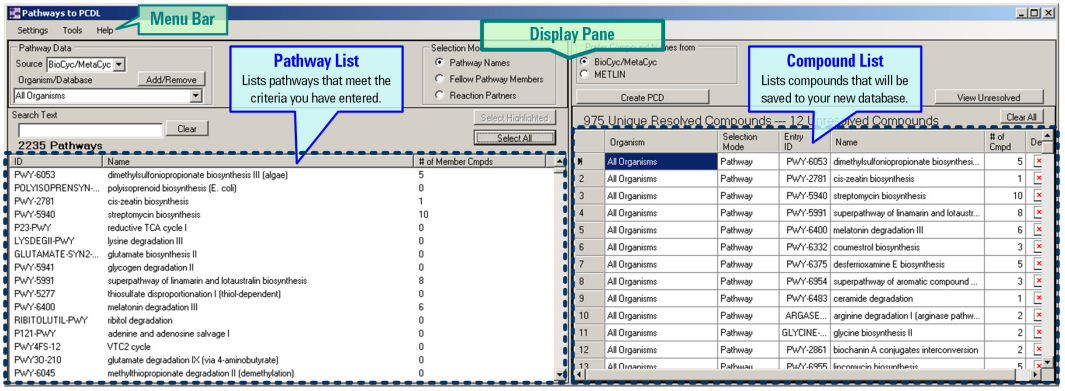


Figure 4 Screen areas of Pathways to PCDL converter

You can update the pathway databases from Tools located on the Menu Bar (see Figure 5).

This *Quick Start Guide* and the software version are available from Help located on the Menu Bar (see Figure 5).

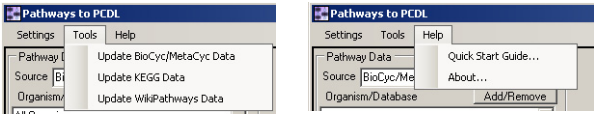
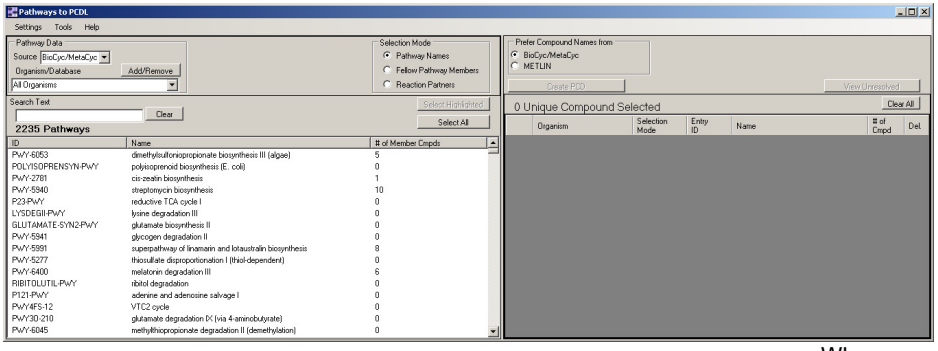
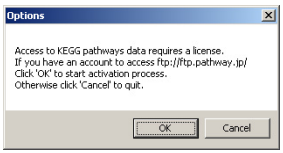
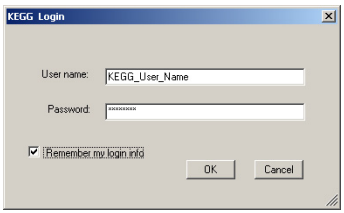


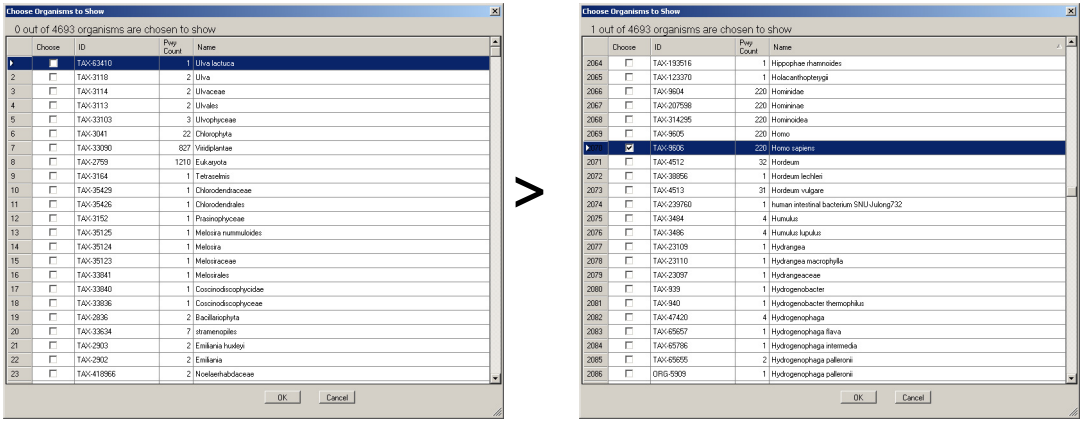
Figure 5 Tools and Help options on the Menu Bar

Creating a Custom PCDL

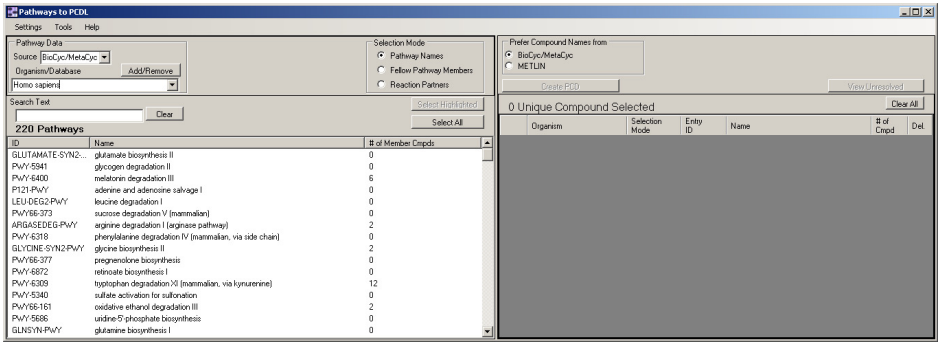
Creating a custom PCD/PCDL from metabolites present in pathway content follows a simple workflow as shown in Figure 3 on page 4.

Steps	Detailed Instructions	Comments
1 Start Pathways to PCDL.	**a** Click the **Pathways to PCDL** icon  on your desktop.	• You can also click **Start > All Programs > Agilent > MassHunter Workstation > Pathways to PCDL > Pathways to PCDL**.
2 Select **Source** under **Pathway Data**.	**a** Select **BioCyc/MetaCyc** for the **Source** under **Pathway Data**. If you select **KEGG**, go to step c on page 7. **b** Review the updated list of pathways in the Pathway List, and then go to step 3.	• The Pathways to PCDL converter supports pathway content from **BioCyc**, **KEGG**, and **WikiPathways**.
		
KEGG pathway database license:  > 	**c** Click **OK** in the **Options** dialog box. **d** Enter your license information if you select the **KEGG** pathway database. **e** Click **OK**.	• When you select the **KEGG** pathway database, you are required to enter your license **User name** and **Password** in the **KEGG Login** dialog box. • If the **User name** or **Password** is incorrect a dialog box notifies you. 

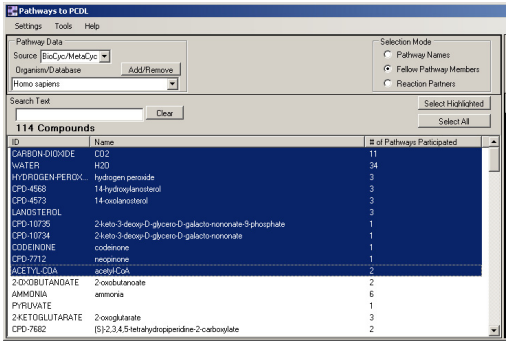
Steps	Detailed Instructions	Comments
3 Select the organism(s) you want to include within the pathway data source.	**a** Click **Add/Remove** under **Pathway Data**. **b** Click on the heading of the column to sort the organisms. For example click the **Name** column heading. **c** Mark **Homo sapiens**. Mark the check box(es) in the **Choose** column and in the row of the organism(s) that you want to include in your **Organism/Database** selection list. **d** Click **OK**.	• In the **Choose Organisms to Show** dialog box, the **Pwy Count** column is the number of pathways associated with each organism. • Each of the organisms that you select in the **Choose Organisms to Show** dialog box are available in the **Organization/Database** selection list. The selection is persistent the next time you run **Pathways to PCDL**.

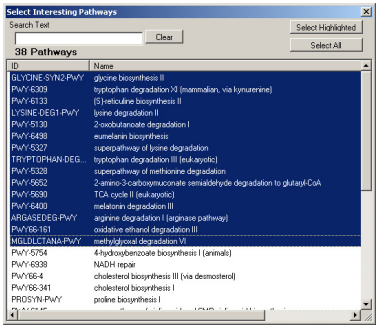


	e Select **Homo sapiens** from the **Organism/Database** list. **f** Review the updated list of pathways in the Pathway List.	• Organisms may be removed from the **Organism/Database** selection list. Click **Add/Remove** and clear the organism check boxes.



Steps	Detailed Instructions	Comments
4 *Option:* Select compounds when **Fellow Pathway Members** is the **Selection Mode**.	**a** Click **Fellow Pathway Members**. **b** Review the updated list of compounds in the Pathway List. **c** Select the first eleven (11) compounds in the Pathway List. **d** Click **Select Highlighted**.	Three options are available as the **Selection Mode**: • **Pathway Name:** Select compounds that match the pathway name(s) you select from the Pathway List view (see the last figure on the prior page). • **Fellow Pathway Members:** Select compounds from pathways related to the initial compound list that is displayed in the Pathway List view. After you click **Select Highlighted** or **Select All**, the *Fellow Pathways* are selected from the **Select Interesting Pathways** dialog box (see the figures on this page). • **Reaction Partners:** Select compounds that are related to the selected list of compounds that is displayed in the Pathway List view (see the figures on the next page). • Selected pathways and compounds in the list views are highlighted using a background color. • Compounds are added to the Compound List each time you click **Select Highlighted** or **Select All**.

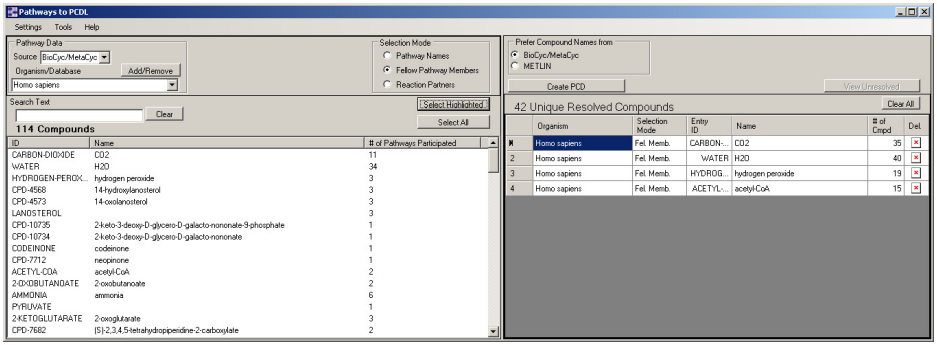




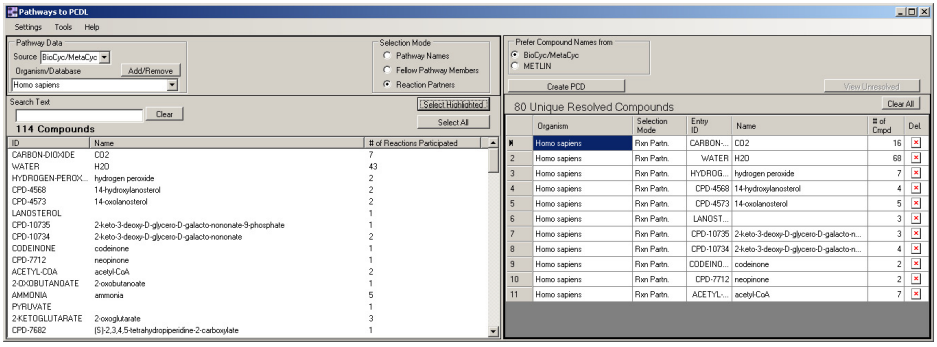
e Select one or more pathways, with or without filtering the pathway list using custom search text. Select the first fifteen (15) pathways.

f Click **Select Highlighted**.

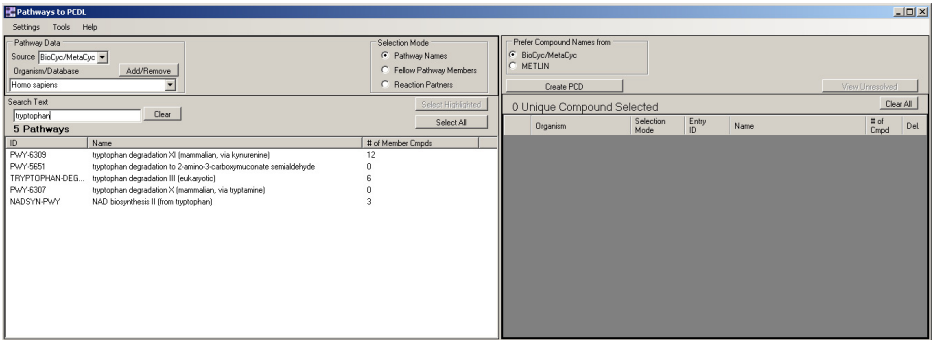
g Review the list of compounds in the Compounds List. Go to step 6 on page 9



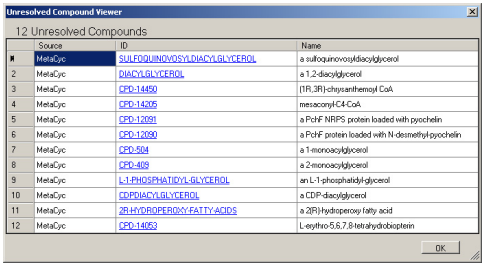
Steps	Detailed Instructions	Comments
5 *Option:* Select compounds when **Reaction Partners** is the **Selection Mode**.	**a** Click **Clear All** in the Compound List pane to start a new compound list. **b** Click **Reaction Partners**. **c** Review the updated list of compounds in the Pathway List. **d** Select the first eleven (11) compounds in the Pathway List. **e** Click **Select Highlighted**. **f** Review the list of compounds in the Compound List.	• **Reaction Partners** is not an option when WikiPathways is the **Pathway Data**, **Source**. • After you click **Select Highlighted** or **Select All** the original selection in the Pathways List is no longer highlighted. For this illustration the eleven compounds were re-selected to show the correlation.

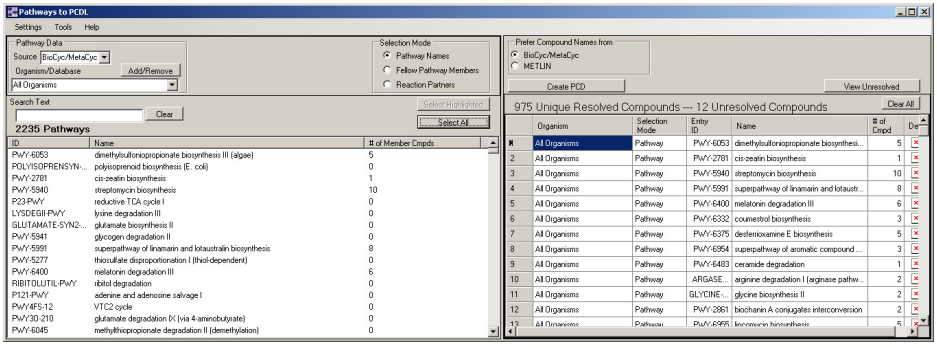


6 Select compounds using custom **Search Text** entry when any **Selection Mode** is selected.	**a** Type `tryptophan` for the **Search Text**. **b** Review the list of pathways in the Compounds List. The number of pathways is smaller than when viewed in step 3 on page 7.	• Pathways can be filtered by several text searches. Click **Select Highlighted** or **Select All** after each search to build your custom compound database based on the selected pathways.

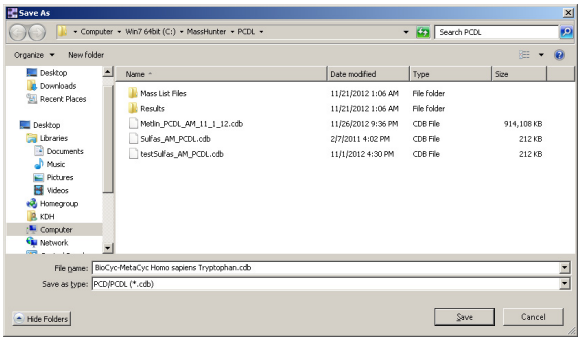
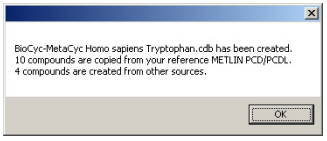


Steps	Detailed Instructions	Comments
7 Review unresolved compounds in your Compound List.	**a** Click **View Unresolved**. **b** Review the list of unresolved compounds in the **Unresolved Compound Viewer** dialog box. **c** Click **OK**. **d** Review the list of compounds in the Compound List. **e** Remove unresolved compounds from your Compound List. Mark compounds to delete under the **Del.** column.	• Pathways to PCDL provides you with an opportunity to manually review the compounds in your Compound List that may not have a strong correlation with the pathway(s) you have selected (unresolved compounds). • The **View Unresolved** button is available when you have unresolved compounds in your Compound List. • After reviewing the unresolved compounds, you may retain them in your Compound List or remove them from the Compound List by marking the compound row under the **Del.** column. When you mark the compound under the **Del.** column it is immediately removed.





8 Select the preferred source of the final database compound names.	**a** Click **BioCyc/MetaCyc** as the **Prefer Compound Names from** source.	

Steps	Detailed Instructions	Comments
9 Create your custom PCD/PCDL.	**a** Click **Create PCD**. **b** Select the folder to save your PCD/PCDL database. **c** Type the name for your PCD/PCDL database in **File name**. **d** Click **Save**.	• This step creates your custom PCD/PCDL based on the compounds involved in the pathway selection(s) you have made. • If your reference METLIN metabolite database is a PCD, your custom database does not contain MS/MS data. If your reference METLIN metabolite database is a PCDL, your custom database contains MS/MS data.
	**e** Click **OK** after you have reviewed the summary information describing your custom PCD/PCDL.	
		
10 Create another PCD/PCDL or exit Pathways to PCDL.	**a** Create another PCD by returning to "Select Source under Pathway Data." on page 6. **b** Click the close button  to exit Pathways to PCDL if you are done creating your custom PCD/PCDL database(s).	• You have completed creating your first custom PCD/PCDL database. You can create another database or exit the software.

BioCyc Pathway/Genome Databases

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

http://www.biocyc.org/

Citation based on use of BioCyc

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.

In some cases, BioCyc Pathway/Genome databases are described by other specific publications that can be found by selecting the database and then going to the Summary Statistics pages under the Tools menu. The resulting page sometimes contains a citation for that database.

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In this book

The *Agilent G6825AA MassHunter Pathways to PCDL Software - Quick Start Guide* presents the first steps to use the MassHunter Pathways to PCDL Software.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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