Agilent 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
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](http://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).

   ![Select Reference METLIN PCD/PCDL dialog box](image)

   **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.

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

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<tr>
<th>4</th>
<th>Option: Select compounds when Fellow Pathway Members is the Selection Mode.</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Click Fellow Pathway Members.</td>
</tr>
<tr>
<td>b</td>
<td>Review the updated list of compounds in the Pathway List.</td>
</tr>
<tr>
<td>c</td>
<td>Select the first eleven (11) compounds in the Pathway List.</td>
</tr>
<tr>
<td>d</td>
<td>Click Select Highlighted.</td>
</tr>
<tr>
<td>e</td>
<td>Select one or more pathways, with or without filtering the pathway list using custom search text. Select the first fifteen (15) pathways.</td>
</tr>
<tr>
<td>f</td>
<td>Click Select Highlighted.</td>
</tr>
<tr>
<td>g</td>
<td>Review the list of compounds in the Compounds List. Go to step 6 on page 9</td>
</tr>
</tbody>
</table>

### Detailed Instructions

- **Option:** Select compounds when Fellow Pathway Members is the Selection Mode.

  - **Step 1:** Click Fellow Pathway Members.
  - **Step 2:** Review the updated list of compounds in the Pathway List.
  - **Step 3:** Select the first eleven (11) compounds in the Pathway List.
  - **Step 4:** Click Select Highlighted.

  - **Additional Options:**
    - **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).
    - **Compounds:** Selected pathways and compounds in the list views are highlighted using a background color.
    - **Add Compounds:** Compounds are added to the Compound List each time you click Select Highlighted or Select All.

### Comments

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).
- **Compounds:** Selected pathways and compounds in the list views are highlighted using a background color.
- **Add Compounds:** Compounds are added to the Compound List each time you click Select Highlighted or Select All.
5 **Option:** Select compounds when **Reaction Partners** is the **Selection Mode**.

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<tr>
<td>a</td>
<td>Click <strong>Clear All</strong> in the Compound List pane to start a new compound list.</td>
<td>- <strong>Reaction Partners</strong> is not an option when WikiPathways is the Pathway Data, Source.</td>
</tr>
<tr>
<td>b</td>
<td>Click <strong>Reaction Partners</strong>.</td>
<td>- After you click <strong>Select Highlighted</strong> or <strong>Select All</strong> the original selection in the Pathways List is no longer highlighted. For this illustration the eleven compounds were re-selected to show the correlation.</td>
</tr>
<tr>
<td>c</td>
<td>Review the updated list of compounds in the Pathway List.</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>Select the first eleven (11) compounds in the Pathway List.</td>
<td></td>
</tr>
<tr>
<td>e</td>
<td>Click <strong>Select Highlighted</strong>.</td>
<td></td>
</tr>
<tr>
<td>f</td>
<td>Review the list of compounds in the Compound List.</td>
<td></td>
</tr>
</tbody>
</table>

6 **Select compounds using custom Search Text entry when any Selection Mode is selected.**

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<tr>
<td>a</td>
<td>Type <strong>tryptophan</strong> for the <strong>Search Text</strong>.</td>
<td>- Pathways can be filtered by several text searches. Click <strong>Select Highlighted</strong> or <strong>Select All</strong> after each search to build your custom compound database based on the selected pathways.</td>
</tr>
<tr>
<td>b</td>
<td>Review the list of pathways in the Compounds List. The number of pathways is smaller than when viewed in step 3 on page 7.</td>
<td></td>
</tr>
</tbody>
</table>
Review unresolved compounds in your Compound List.

- Click **View Unresolved**.
- Review the list of unresolved compounds in the **Unresolved Compound Viewer** dialog box.
- Click **OK**.
- Review the list of compounds in the Compound List.
- 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.

Select the preferred source of the final database compound names.

- Click **BioCyc/MetaCyc** as the **Prefer Compound Names from** source.
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</table>
| 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**.  
  e Click **OK** after you have reviewed the summary information describing your custom PCD/PCDL. | • 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. |
| 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 **X** 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.

[Image: BioCyc Database Collection]

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:


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


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.
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.