Compound Discoverer 3.3 SP2 BioCyc Tutorial

BioCyc (https://biocyc.org/) provides reconstructed metabolic networks for 20,000 sequenced organisms and access to 3,085 pathways and 18,786 metabolites.

BioCyc's informatics tools include the following:

- Pathway diagrams with chemical structures
- Zoomable, organism-specific metabolic network diagrams
- The capability of overlaying user-obtained OMICS study results (for example, fold change, peak area, or stable isotope labeling data) onto metabolic pathways

This tutorial shows you how to work with the BioCyc Pathways mapping feature in the Thermo Scientific™ Compound Discoverer™ 3.3 service pack 2 (SP2) application.

IMPORTANT To use the BioCyc Pathways mapping feature, you must set up a BioCyc account. To select a mouse (organism) database for the ZDF rats sample set that you use in this tutorial, you must set up a BioCyc account and a BioCyc subscription as described in the topic "Setup a new user account or request free trial access or purchase a subscription for an existing account" on page 5.

The first time you set up a BioCyc account, you are granted free unlimited access to all the BioCyc databases for a brief demonstration period. If you have an existing BioCyc account, you can request free access to all the BioCyc databases for a 30-day trial period. To continue your unlimited access to all the BioCyc pathway databases when this free trial period expires, you must purchase a subscription.

To map the biological pathways for the ZDF rat data set, follow these topics in order:

Note If you are using the Compound Discoverer 3.3 SP1 application instead of the Compound Discoverer 3.3 SP2 application, you must install the following configuration file:

BioCycService.config

For information about downloading this file from the LSMS Software Download and Licensing portal and installing this file on your data system computer, see the following topic: "Using the BioCyc Pathway Mapping feature in Compound Discoverer 3.3 SP1" on page 28. After you install the configuration file, you can begin this tutorial at "Copy the ZDF example files to your data processing computer" on page 3.

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Locate the ZDF example files

In this tutorial you use the same ZDF example files that Thermo Fisher Scientific originally provided for the metabolomics tutorial. If you already have these files on a local hard drive on your data processing computer, skip this topic and go to "Start the Compound Discoverer application" on page 4.

To copy the example files to a local hard drive on your data processing computer, do one of the following:

- If you do not already have the example ZDF files on the local hard drive of your data processing computer, and you do not have the key-shaped USB drive that was provided in the Compound Discoverer 3.3 software media kit, do the following:
  a. Follow the instructions in "Download the ZDF example files."
  b. Follow the instructions in "Copy the ZDF example files to your data processing computer" on page 3.
- If you have the key-shaped USB drive from the Compound Discoverer 3.3 software media kit, go to "Copy the ZDF example files to your data processing computer" on page 3.

Download the ZDF example files

If you do not already have the ZDF example files on your data processing computer and you do not have the original key-shaped USB drive that was provided in the Compound Discoverer 3.3 software media kit, follow this procedure to download these files.

❖ To download the example ZDF files

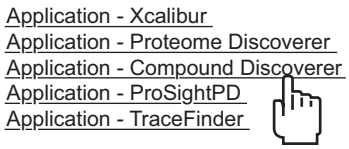
1. Go to the following URL: thermo.flexnetoperations.com

   The LSMS Software Download and Licensing Portal website opens.

2. Log in.

3. Under Software & Services at the left, click the **Product List** link.

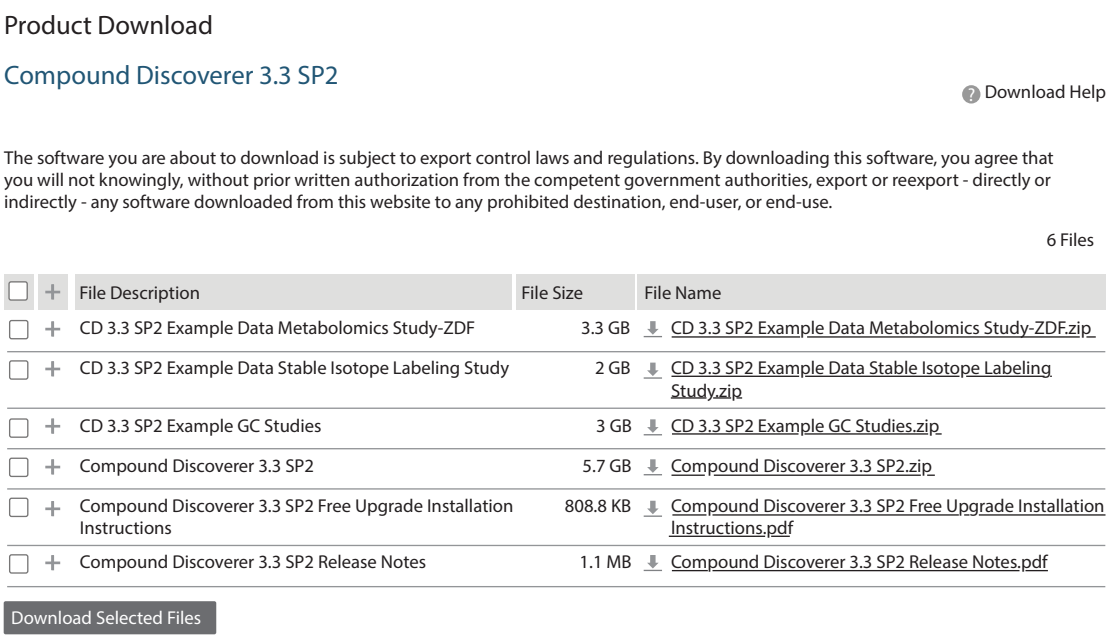
4. On the Product List page, click the **Application - Compound Discoverer** link.



5. On the Product Information page, click the **Compound Discoverer 3.3 SP2** link.

   The Product Download page for the Compound Discoverer 3.3 SP2 application opens. See Figure 1 on page 3. This page contains the compressed folders with the files for the tutorials provided with the application.

Figure 1. Product Download page for the Compound Discoverer 3.3 SP2 application



6. Download the ZDF example files by clicking ⬇ to the left of the CD 3.3 SP2 Example Metabolomics Study-ZDF.

   These items download to the Downloads folder on your data processing computer.

Copy the ZDF example files to your data processing computer

For optimal performance of the Compound Discoverer application, store study files (.cdStudy) and result files (.cdResult) on the local hard drive of your data processing computer.

Tip For most analyses, you can store the input files (Xcalibur raw data files) for an analysis on a local hard drive, an external hard drive, or a network drive, without sacrificing performance. For this tutorial, keep all the files together in the ZDF folder. If you change the relative location of the raw data files, you must resolve the location of the input files for the analysis. For more information, refer to the "Resolve the location of the input files in a study" Help topic.

❖ To copy the example files for this analysis to your data processing computer

1. Locate the files on the key-shaped USB flash drive that was provided in the software media kit or download the example files from the LSMS Software Download and Licensing Portal website as described in "Download the ZDF example files" on page 2.

   The example files for this tutorial are in the following folder on the USB flash drive:

   Example Studies\LC\Metabolomics\ZDF

2. If you downloaded the files, extract the files from the CD 3.3 SP2 Example Data Metabolomics Study-ZDF.zip compressed folder and copy the CD 3.3 SP2 Example Data Metabolomics Study-ZDF folder to a local hard drive on your data processing computer.

   The ZDF folder contains the following files: eight Xcalibur raw data files, one Compound Discoverer study file (.cdStudy), and one Compound Discoverer result file (.cdResult).

Start the Compound Discoverer application

This tutorial describes how to use review the BioCyc pathway mapping results from an analysis run on a data processing computer with the Compound Discoverer 3.3 SP2 application[1]. The Compound Discoverer 3.3 SP2 application is compatible with the Windows™ 10 and Windows 11 operating systems.

❖ To start the Compound Discoverer 3.3 SP2 application

Do one of the following:

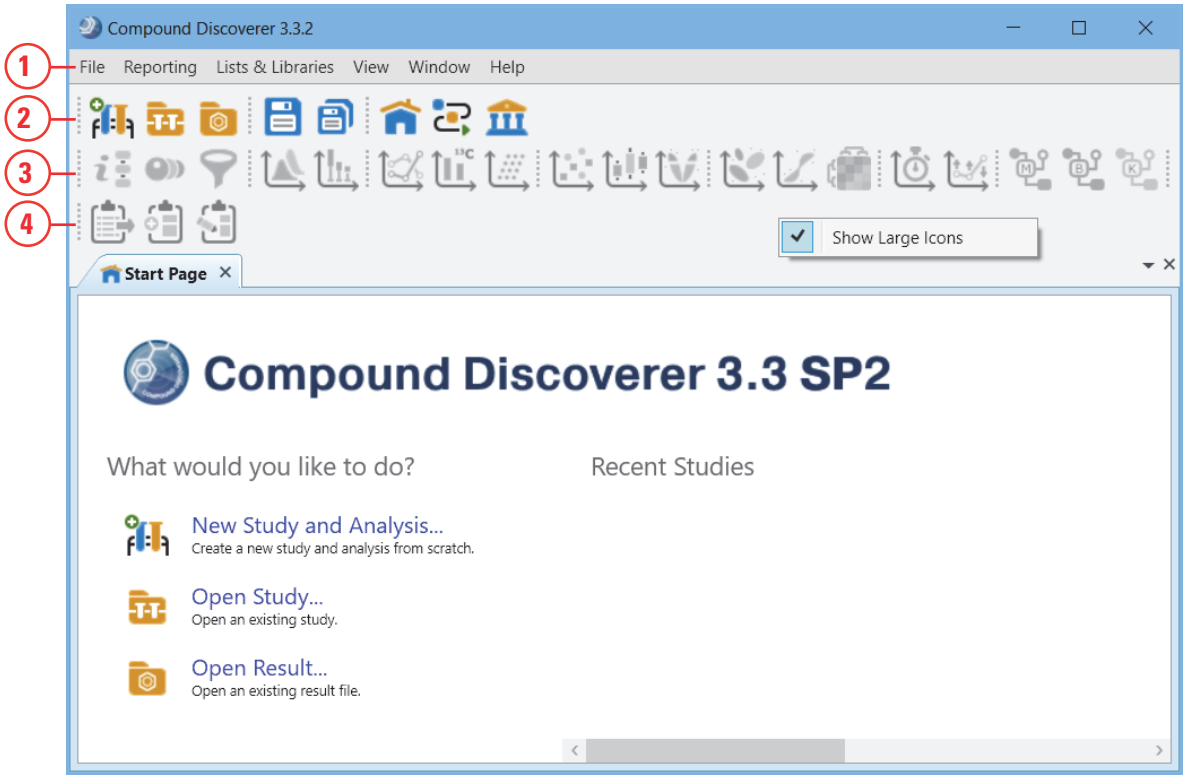
- On the Windows 10 taskbar, click **Start** . Then, choose **Thermo Compound Discoverer 3.3 > Compound Discoverer 3.3** from the Start menu.
- On the Windows 11 taskbar, click **Start** . Then, click the **Compound Discoverer 3.3** icon in the Pinned apps section or in the All Apps section of the Start menu, open the Thermo Compound Discoverer 3.3 folder, and choose **Compound Discoverer 3.3**.

–or–

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

The application opens to the Start Page.

Figure 2. Compound Discoverer window (prior to creating any studies or running any analyses)



No.	Description	No.	Description
1	Application menu bar	2	Study toolbar
3	Data review toolbar	4	Reporting toolbar

[1] After you install the required configuration file, you can also follow this tutorial for the Compound Discoverer 3.3 SP1 application.

Setup a new user account or request free trial access or purchase a subscription for an existing account

You must have a user account to use the BioCyc pathways mapping feature in the Compound Discoverer application. To access all the BioCyc databases, you must also have an organization subscription, an individual subscription, or your free 30-day trial period of full access must not have expired.

To create a user account, your data processing computer must have Internet access. The first time you set up a user account, with or without a subscription, you will have unlimited access to all the BioCyc databases for a 30-day trial period. However, if you do not have a paid subscription, you will lose access to all the BioCyc databases, except for the EcoCyc and MetaCyc databases, when your free trial period expires.

If you have an existing user account but no subscription, you can request 30 days of free access to all the BioCyc databases.

To create and activate your user account and purchase a subscription or to request a 30-day trial period of free access to all the BioCyc databases, follow these topics:

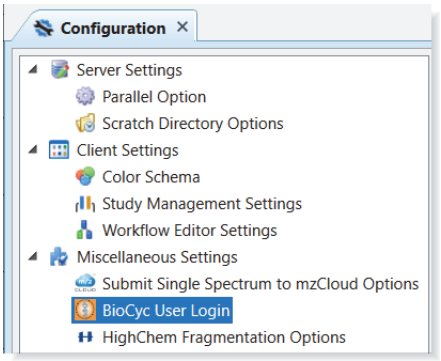
1. Open the BioCyc User Login view
2. Do the following as needed:
   - Create a new user account
   - Purchase a subscription or request a free 30-day trial period
3. Enter, test, and save your BioCyc account information

Open the BioCyc User Login view

You must validate your BioCyc user account from the BioCyc User Login view of the Compound Discoverer application.

❖ To open the BioCyc User Login view

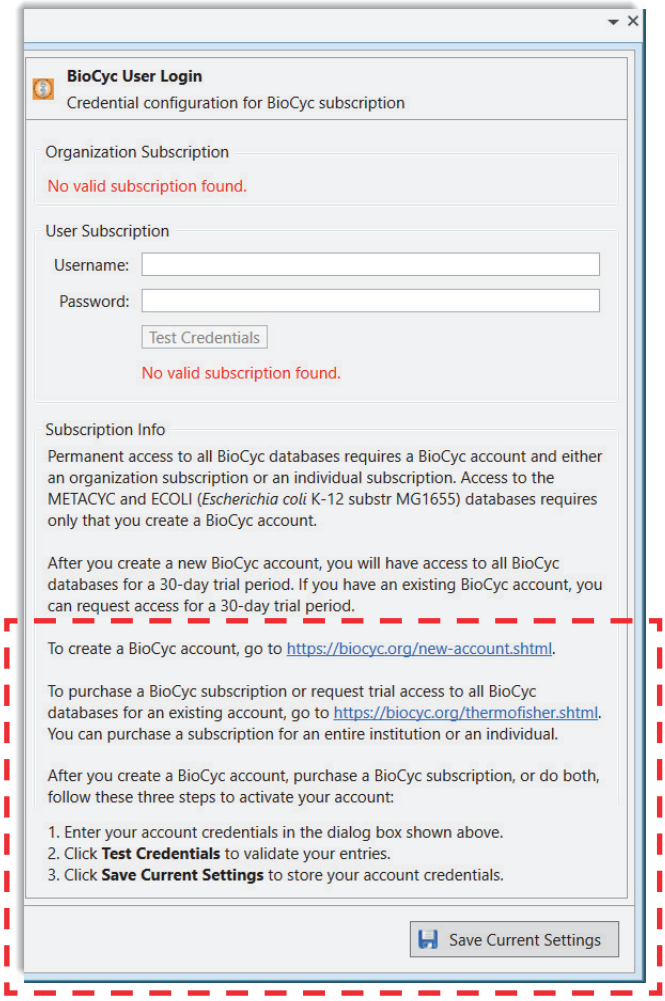
1. From the Compound Discoverer 3.3 SP2 menu bar, choose **Help > Configuration**.
2. In the navigation pane on the Configuration page, under Miscellaneous Settings, choose **BioCyc User Login**.



The BioCyc User Login view appears at the right with the following information:

- The Organization Subscription area displays the status of your organization subscription, if you have one.
- The User Subscription area displays your email address and the remaining time on your trial access period or individual subscription. See Figure 4 on page 8.
- The Subscription Information area includes instructions for creating a new account, purchasing a subscription, and requesting a free 30-day trial period for an existing account. See Figure 3.

Figure 3. BioCyc User Login view with no user account information



3. Review the current user account and subscription information in the BioCyc User Login view:
   - Review the information in the Organization Subscription area:
     - If you have an organization subscription, the Organization Subscription area displays the organization name in green.
     - If you do not have an organization subscription, the Organization Subscription area displays the following text in red: No Valid Subscription Found.

     Note You do not need an organization subscription to use the BioCyc pathways mapping feature.
   - Review the information for your user account in the User Subscription area:
     - If you have already set up a BioCyc user account—that is, you have entered and tested your credentials (email address and password) and saved the settings—the Username box displays your email address.
     - If you do not have an individual subscription or free trial access to all the BioCyc databases, the following text appears in red under User Subscription:

       No Valid Subscription Found.

Create a new user account

You must have a user account to use the BioCyc pathways mapping feature in the Compound Discoverer application. To access all the BioCyc databases, you must have one of the following: an organization subscription, an individual subscription, or your 30-day free trial access must not have expired.

❖ **To create a new BioCyc user account**

1. Click the following link in the BioCyc User Login view:

   https://biocyc.org/new-account.shtml

   The BioCyc website opens to the Create Free Account page.
2. Sign up for your account.
3. To activate your new BioCyc account, go to "Enter, test, and save your BioCyc account information."

Purchase a subscription or request a free 30-day trial period

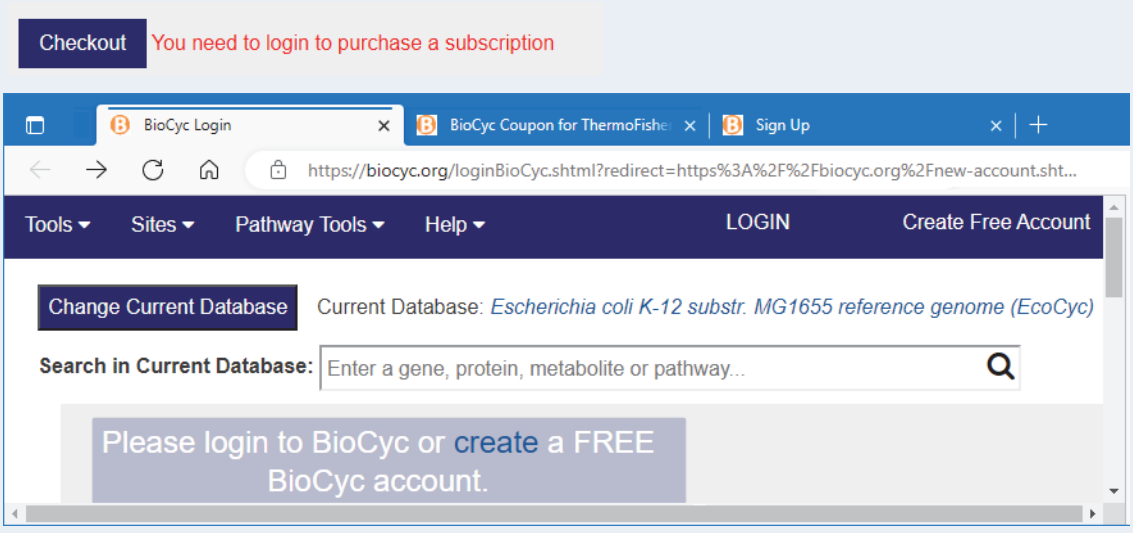
❖ **To purchase a subscription or request a free 30-day trial period for an existing account**

1. In the BioCyc User Login view, click the following link:

   https://biocyc.org/thermofisher.shtml

   The BioCyc website opens to the Welcome Thermo Fisher Customers page.
2. Do one of the following:
   - To request trial access to all the BioCyc databases, click **Request Trial Access**, and then follow the instructions on the website.
   - To purchase an individual subscription, click **Individual Subscriptions**, and then follow the instructions on the website.
   - To purchase an organization subscription, click **Institutional Subscriptions**, and then follow the instructions on the website.

Tip To purchase a subscription, you must be logged in to the BioCyc website. If you are not logged in, the following message appears when you click Checkout. Click LOGIN to log in as requested.



Enter, test, and save your BioCyc account information

After you set up your user account, test your credentials, and then save your account information.

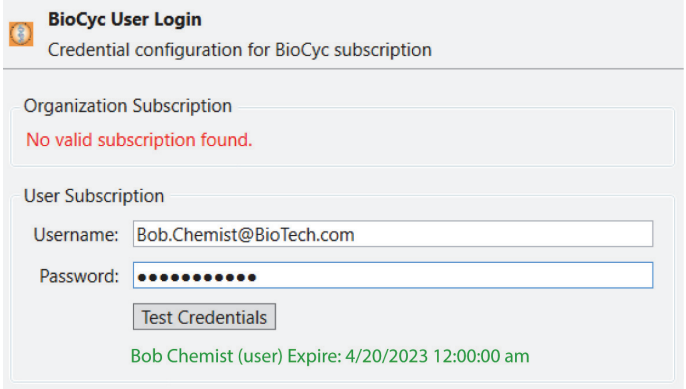
❖ To enter, test, and save your account information

1. In the User Subscription area of the BioCyc User Login view on the Configuration page, enter your user name (email address) and password.
2. Below the Password box, click **Test Credentials**.
3. At the bottom right of the view, click **Save Current Settings**.

> **IMPORTANT** If you do not click Save Current Settings to save your account information, the following issues will occur:
>
> - You will not have a valid user account the next time you restart the Compound Discoverer application, and you will have to enter your user account information again.
> - The BioCyc communication test will fail.
> - If you add the Map to BioCyc Pathways node to a processing workflow template and attempt to run an analysis with this template, the analysis will not map the BioCyc pathways.

Figure 4 shows the information for a user account with 30 days of trial access to all the BioCyc databases.

Figure 4. BioCyc User Login view with information for a new user account



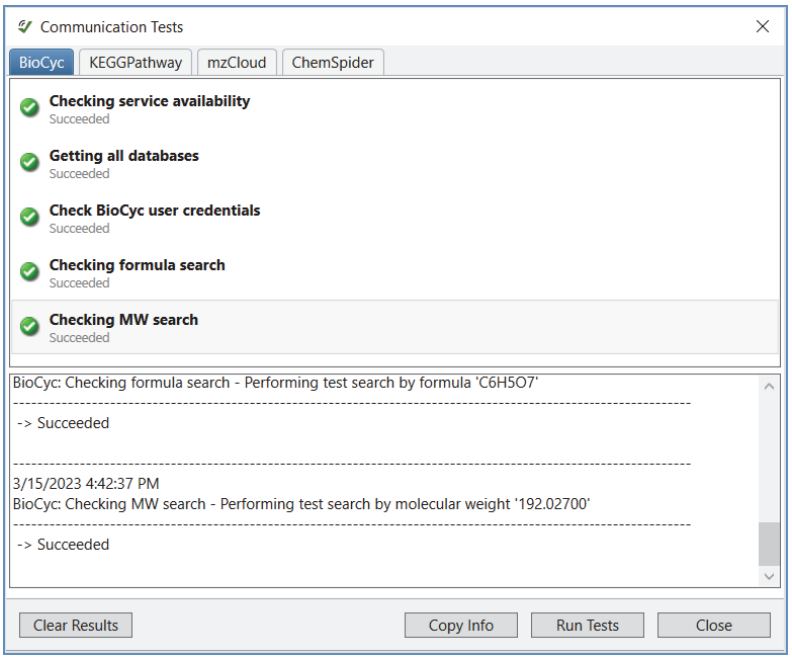
Check the computer's access to the BioCyc database

To use the processing workflow that you create in this tutorial, your data processing computer must have unblocked access to the BioCyc database on the Internet.

❖ To test the communication to the online BioCyc database

1. From the application menu bar, choose **Help > Communication Tests**.
2. Click the **BioCyc** tab, and then click **Run Tests**. See Figure 5.

Figure 5. BioCyc page of the Communication Test dialog box



3. If your computer has an Internet connection, but these tests fail, do one of the following:
   - If only the Check BioCyc User Credentials test fails, follow the instructions in "Enter, test, and save your BioCyc account information" on page 8.
   - If all the tests fail, go to step 4.
4. If all the test fail, do the following:
   a. Leave the Communication Test dialog box open.
   b. Press the **F1** key (or equivalent, for example, Fn+F1) on the computer keyboard 

.

      The Test Communication to the Online Databases Help topic opens.
   c. Click the **Troubleshoot Access to the Online Databases** link, and then follow the instructions in the Help topic to troubleshoot the communication failure.

Modify the original ZDF analysis and submit it for partial reprocessing

The processing workflow for the original analysis that was used to generate the ZDF.cdResult file did not include the Map to BioCyc Pathways node.

Because the Map to BioCyc Pathways node is downstream of the compound detection nodes, you can add it to the original processing workflow and partially reprocess the analysis result. Partial reprocessing does not trigger an analysis that involves the core compound detection nodes.

> **Note** In the processing workflow for this tutorial, the Input Files, Select Spectra, Align Retention Times, Detect Compounds, Group Compounds, and Fill Gaps nodes are core nodes.

To reprocess the original analysis result with an additional downstream workflow node, the Map to BioCyc Pathways node, follow these topics:

1. Open the original analysis for the ZDF analysis result
2. Add the Map to BioCyc Pathways node to the processing workflow
3. Submit the modified analysis to the job queue

Open the original analysis for the ZDF analysis result

In this tutorial, you partially reprocess the example ZDF.cdResult file in the example ZDF.cdStudy file after you add the Map to BioCyc Pathways workflow node to the original analysis.

Note An analysis includes the processing workflow, the grouping and ratio settings, and the list of raw data files used to generate an analysis result.

Prerequisites

You have copied the example ZDF files to a local hard drive on your data processing computer. In addition, you have done one of the following:

- Created a new BioCyc user account with temporary free access to all the BioCyc databases
- Requested a 30-day trial period of free access to all the BioCyc databases for an existing account

–or–

- Created a BioCyc user account and purchased a subscription

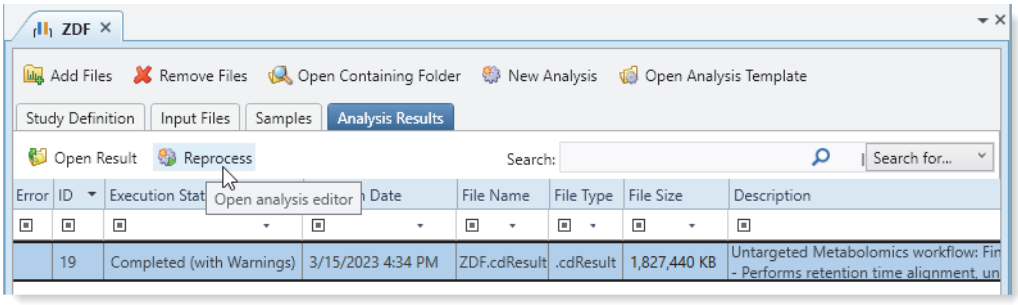
❖ To open the original analysis for reprocessing

1. From the application menu bar, choose **File > Open Study**.
2. Browse to the ZDF.cdStudy file, select it, and click **Open**.

   The study opens to the Analysis Results page of the study. There is only one analysis result in the table on this page. Because the analysis result is not selected, the commands in the Analysis Results command bar are unavailable.
3. Select the analysis result, **ZDF.cdResult**.

   The Open Result and Reprocess commands become available. See Figure 6 on page 10.
4. Click **Reprocess**.

Figure 6. Analysis Results page of the example ZDF study



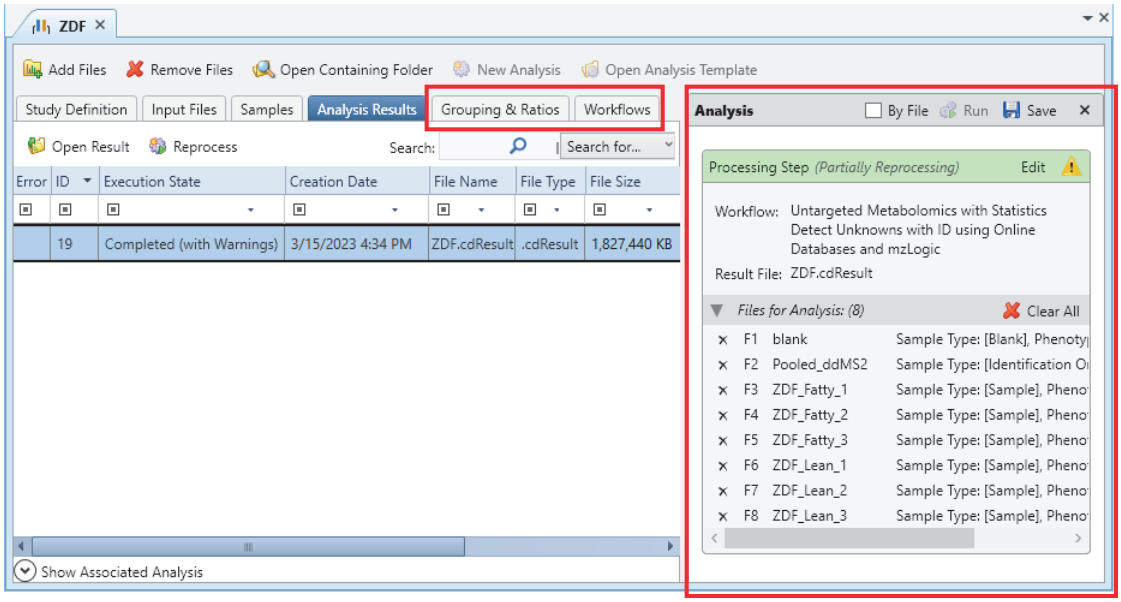
The Analysis view opens. The Analysis view consists of the Grouping and Ratios page, the Workflows page, and the Analysis pane. See Figure 7.

Note For an existing analysis, the Analysis view is also known as the analysis editor. See the tooltip for the Reprocess command in Figure 8 on page 12.

In the analysis editor, the Grouping and Ratios page, the Workflows page, and the Analysis pane contain settings from the previous analysis. The Grouping and Ratios page contains the sample groups and ratios, the Workflows page contains the processing workflow, and the Analysis pane contains the filename of the previous analysis result and the list of input files for the analysis.

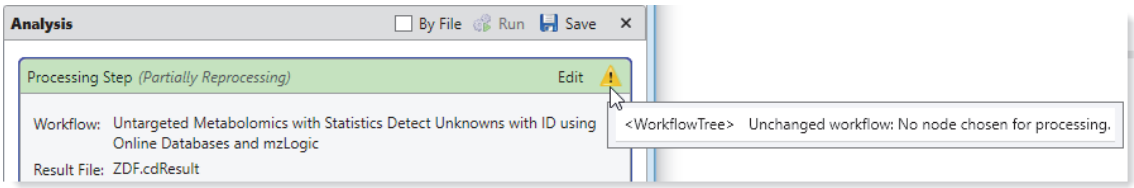
In the Analysis pane, the Run button is unavailable, the state of the Processing Step is listed as Partially Reprocessing, and there is a Caution symbol at the right of the Edit button.

Figure 7. Analysis editor for the ZDF analysis result



Pointing to the Caution symbol displays the following message:

Unchanged workflow. No node chosen for processing.



Add the Map to BioCyc Pathways node to the processing workflow

The processing workflow for the example ZDF analysis result did not include the Map to BioCyc Pathways node.

Note The original processing workflow for the ZDF analysis result was created by modifying the following predefined processing workflow template:

Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic.cdProcessingWF

Prerequisites

The analysis editor for the ZDF analysis result (ZDF.cdResult) is open, and the state of the Processing Step is Partially Reprocessing. See "Open the original analysis for the ZDF analysis result" on page 10.

❖ To add the Map to BioCyc Pathways node to the processing workflow

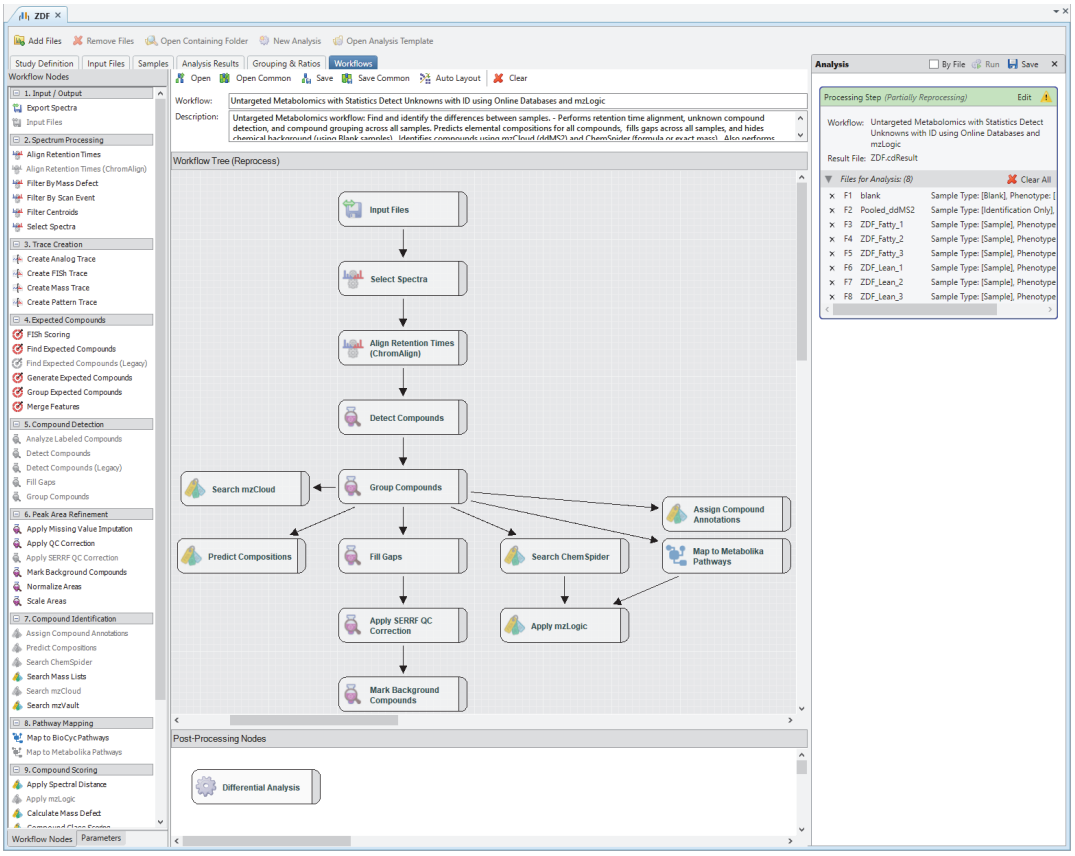
1. Click the **Workflows** tab.

   The Workflows page of the Analysis view opens.

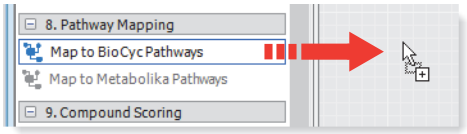
In the Workflow Tree and Post-Processing Nodes panes on the Workflows page, notice the following in Figure 8:

- All the workflow nodes have lost their distinguishing colors, and the right sides of all the nodes have a gray tab.
- To the left of the Workflow Tree pane, the Workflow Nodes subpage is open.

Figure 8. Original processing workflow for the ZDF.cdResult file (analysis result)



2. Add the Map to BioCyc Pathways node under Pathway Mapping by dragging it from the Workflow Nodes page at the left to the Workflow Tree pane.



After you drop the Map to BioCyc Pathways node in the Workflow Tree pane, the following changes occur on the Workflows page (shown in Figure 9):

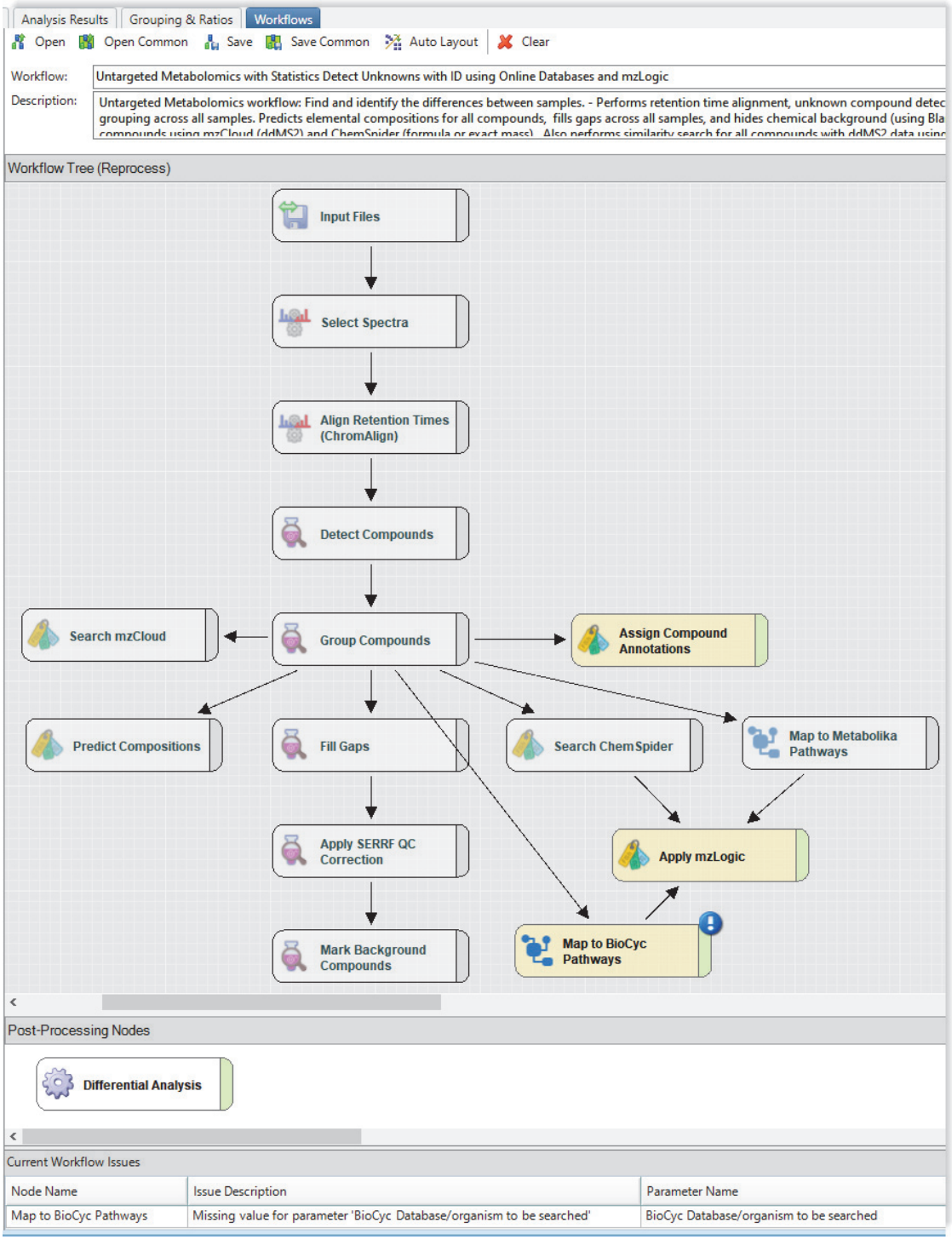
- In the Workflow Tree pane, the Group Compounds node automatically connects to the Map to BioCyc Pathways node and the Map to BioCyc Pathways node automatically connects to the Apply mzLogic node. In addition, the Apply mzLogic node and the Assign Compound Annotations node are automatically set for reprocessing, as indicated by their yellow background color and the green tab on the right side of each node.
- In the Post-Processing Nodes pane, the Differential Analysis node is automatically set for reprocessing, as indicated by the green tab on the right side of the node.
- Below the Post-Processing Nodes pane, the Current Workflow Issues table opens.

- The exclamation mark at the top-right corner of the Map to BioCyc Pathways node indicates that the node has an undefined parameter setting. This undefined parameter setting is described in the Current Workflow Issues table.

In the Analysis pane, the Source File box appears below the Result File box. The application automatically duplicates the file name of the original result file in the Result File and Source File boxes. See Figure 13 on page 16.

Figure 9 shows the modified processing workflow and the Current Workflow Issues table.

Figure 9. Changes to the processing workflow



3. In the Workflow Tree pane, click the **Map to BioCyc Pathways node** to select it.

   A dashed green border appears around the node and the Map to BioCyc Pathways page opens to the left of the Workflow Tree pane. See Figure 10.

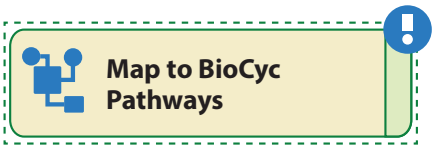
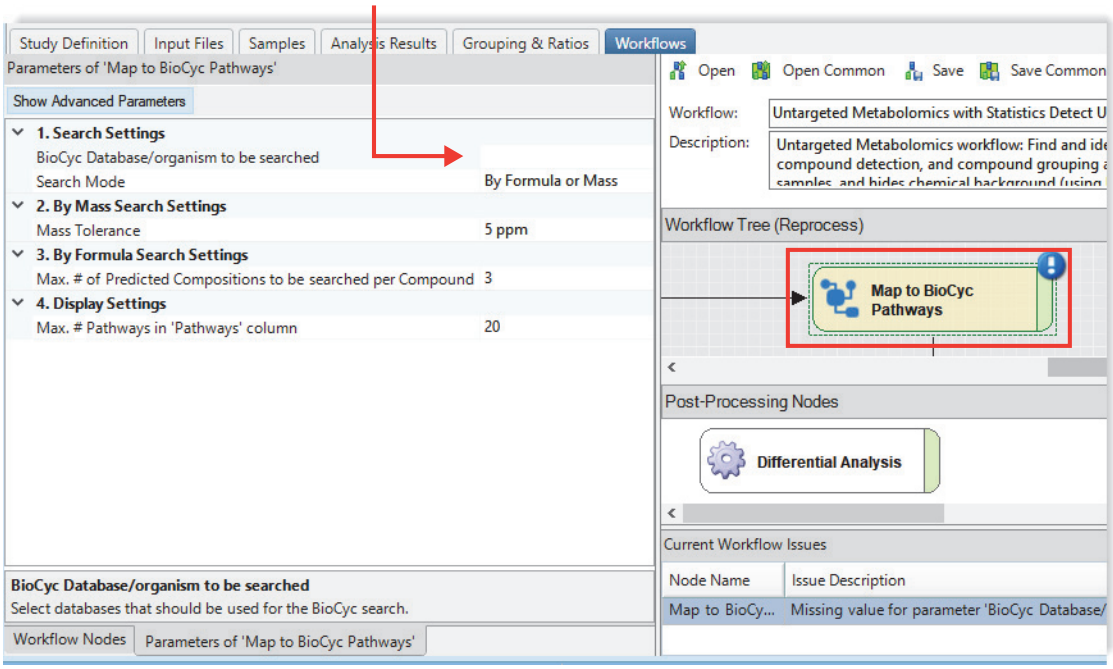


Figure 10. Parameters of Map to BioCyc Pathways page



4. On the Map to BioCyc Pathways page, select the database to be searched as follows:

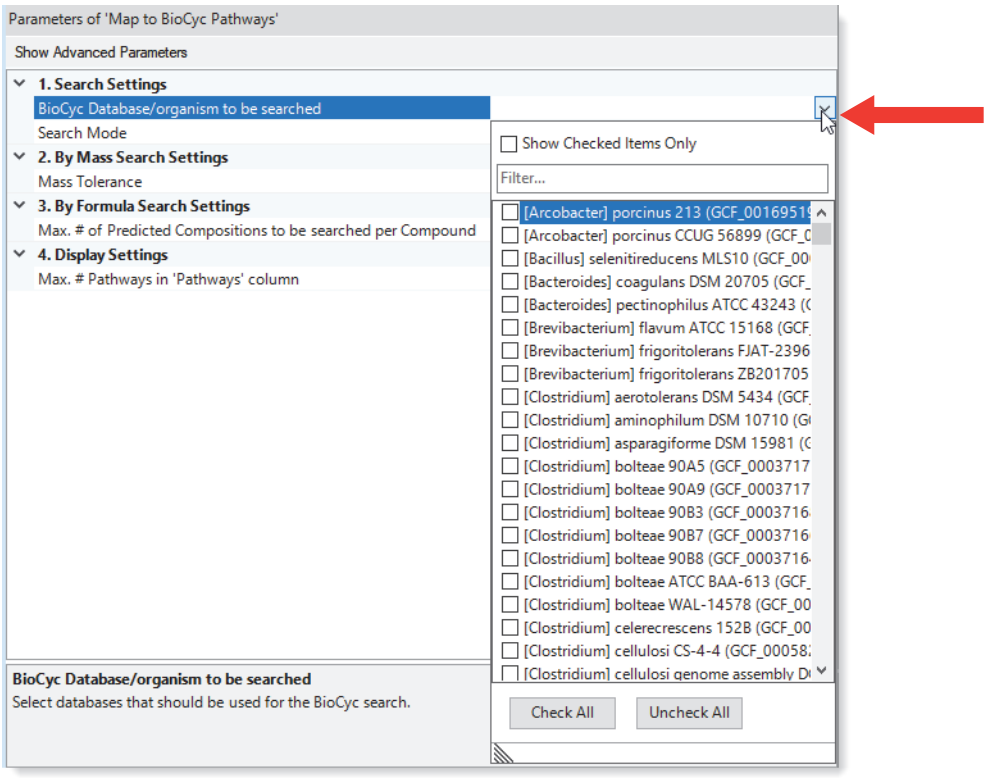
   a. Click the box to the right of the BioCyc Database/Organism to be Searched parameter.

      A down arrow icon ⌄ appears.

   b. Click the down arrow and wait a few seconds. If nothing happens, click the down arrow again.

      The dropdown list of BioCyc pathway databases appears. See Figure 11.

Figure 11. Parameter of Map to BioCyc Pathways page with a list of BioCyc Pathways to select from

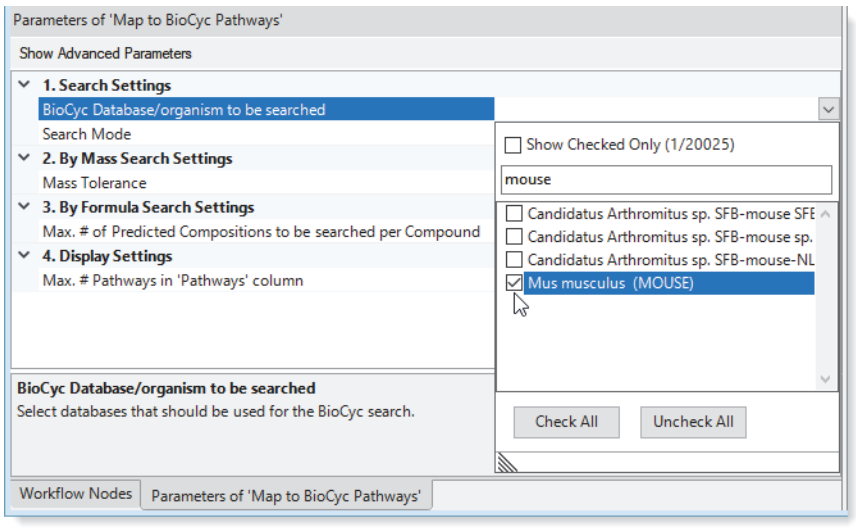


c. Type mouse in the Filter box.

   Only four databases remain in the filtered list.

d. Select the checkbox for the **Mus Musculus (MOUSE)** database.

Figure 12. Mus Musculus (Mouse) database selection



Note The Map to BioCyc Pathways node performs pathway mapping by using the exact mass, the formula, or both for all the detected compounds. After the analysis is completed, you can filter the analysis results by various parameters, for example, you can limit the mapping to compounds in the Compounds table that the Search mzCloud node identified. See Table 1 on page 28.

5. Click anywhere outside the list of databases.

   The database list closes, the Mus Musculus (Mouse) selection appears in the BioCyc Database/Organism to be Searched box, the exclamation mark disappears from the upper-right corner of the Map to BioCyc Pathways node, the Current Workflow Issues table below the Post-Processing Nodes pane closes, and the Run button in the Analysis pane becomes available.

Submit the modified analysis to the job queue

In this topic, you submit the modified analysis with the Map to BioCyc Pathways node to the job queue for partial reprocessing.

Prerequisites

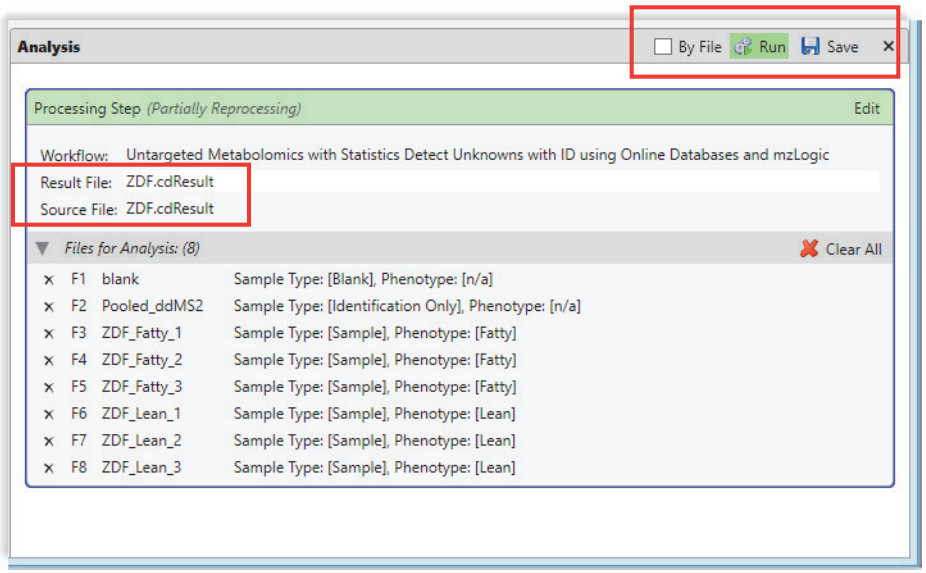
You have added the Map to BioCyc Pathways node to the original processing workflow for the example ZDF.cdResult file, and you have selected the Mus Musculus (MOUSE) database for the search.

❖ To submit the new analysis to the job queue

1. In the Analysis pane, leave the **By File** checkbox clear. (This is its default state.)

   Note When the By File checkbox is clear, the application creates one result file for the entire set of input files.

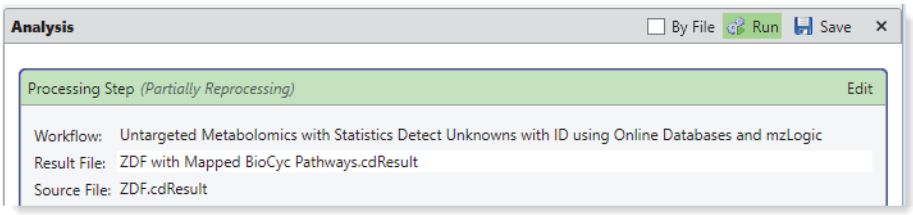
   Figure 13. Default settings for the file names in the Result File and Source File boxes



2. In the Result File box, rename the result file for the new analysis that includes the Map to BioCyc Pathways node. For example, rename the file **ZDF with Mapped BioCyc Pathways**.

   Note The filename in the Source File box is unavailable for editing. If you do not rename the ZDF.cdResult file in the Result File box, the application increments the filename to ZDF-(1).

   Figure 14. Analysis set for partial reprocessing

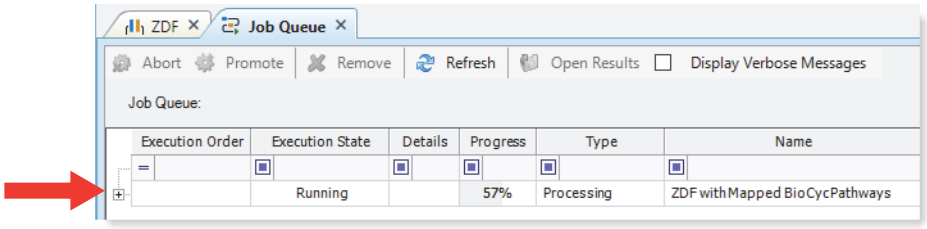


3. Click **Run**.

   The Job Queue page opens.

   The analysis maps the detected compounds to the BioCyc pathways in the selected database; applies mzLogic scoring to the annotations from the ChemSpider node, the Map to Metabolika Pathways node, and the Map to BioCyc Pathways node; performs a differential analysis; and assigns the compound annotations to the detected compounds.

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



Note During the run, the application generates several warning messages, which you can ignore. Warning messages have a yellow background.

Do not close the Job Queue page. When the run is completed, go to the next topic to "Review the BioCyc mapping results."

Review the BioCyc mapping results

There are two ways to view the results of the BioCyc pathway mapping. You can review all the mapped pathways from the main BioCyc Pathways table or you can review the mapped pathways for a compound in the main Compounds table by opening the related BioCyc Pathways table for the compound.

To review the pathway mapping results in your reprocessed analysis result, follow these topics:

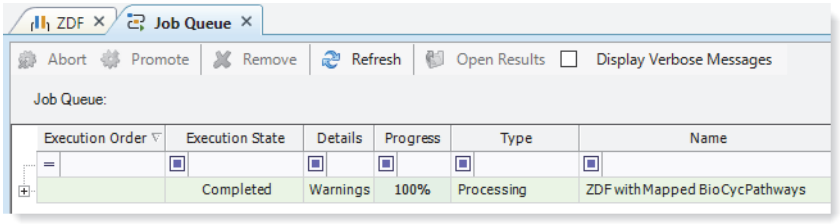
1. Open the newly reprocessed result file
2. (Optional) Review the default result page layout
3. View the mapped BioCyc pathways for the analysis
4. Display only the compounds identified by a confirmatory spectral match

Open the newly reprocessed result file

The Job Queue page lists all the jobs you have run until you remove them from the Job Queue table.

❖ **To open the result file when the job is completed**

Double-click the job on the Job Queue page.



Tip If the Job Queue page is closed, from the menu bar, choose **View > Job Queue** to open it.

You can also open a result file from the Analysis Results page of a study, from the File menu, from the Start Page, or from the File Explorer directory.

(Optional) Review the default result page layout

The factory default layout for a result file includes the following items, as they are numbered in Figure 15 on page 19:

1. A page tab with the result file name.
2. A Chromatograms view on the top left that is populated with XIC traces (across the input file set) for the compound in the first row of the active table. The view automatically zooms in to the start and end points of the chromatographic peak for the compound, and the integrated peak areas are shaded.
3. A Mass Spectrum view on the top right that is populated with the MS1 scan (for a preferred ion) that is closest to the chromatographic peak apex for the selected compound across the input files. The spectrum tree to the left includes the MS1 scans and the fragmentation scans for the preferred ions that were acquired within the following time range:

   - The chromatographic peak apex for the selected compound ± peak width at half maximum (FWHM)

   –or–

   - The start and end points of the chromatographic peak, as determined by the peak detection algorithm

   Note If the data set does not include data-dependent MS2 scans within the retention time window but does include DIA scans within this window, the spectrum tree includes the DIA scans. Your Thermo Scientific data-visualization application displays DIA or AIF in the scan headers of data-independent scans. The Compound Discoverer application displays DIA for both of these scan types.

4. A set of tabbed main tables below the two graphical views. For a metabolomics analysis, the Compounds table is the active table, and it is sorted by the Area (Max.) column—that is, the first row displays the compound with the largest chromatographic peak area (found in one of the input files).

   Note Because the selected processing workflow includes the Mark Background Compounds node, the Compounds tab has a filter icon with a blue check mark ( ).

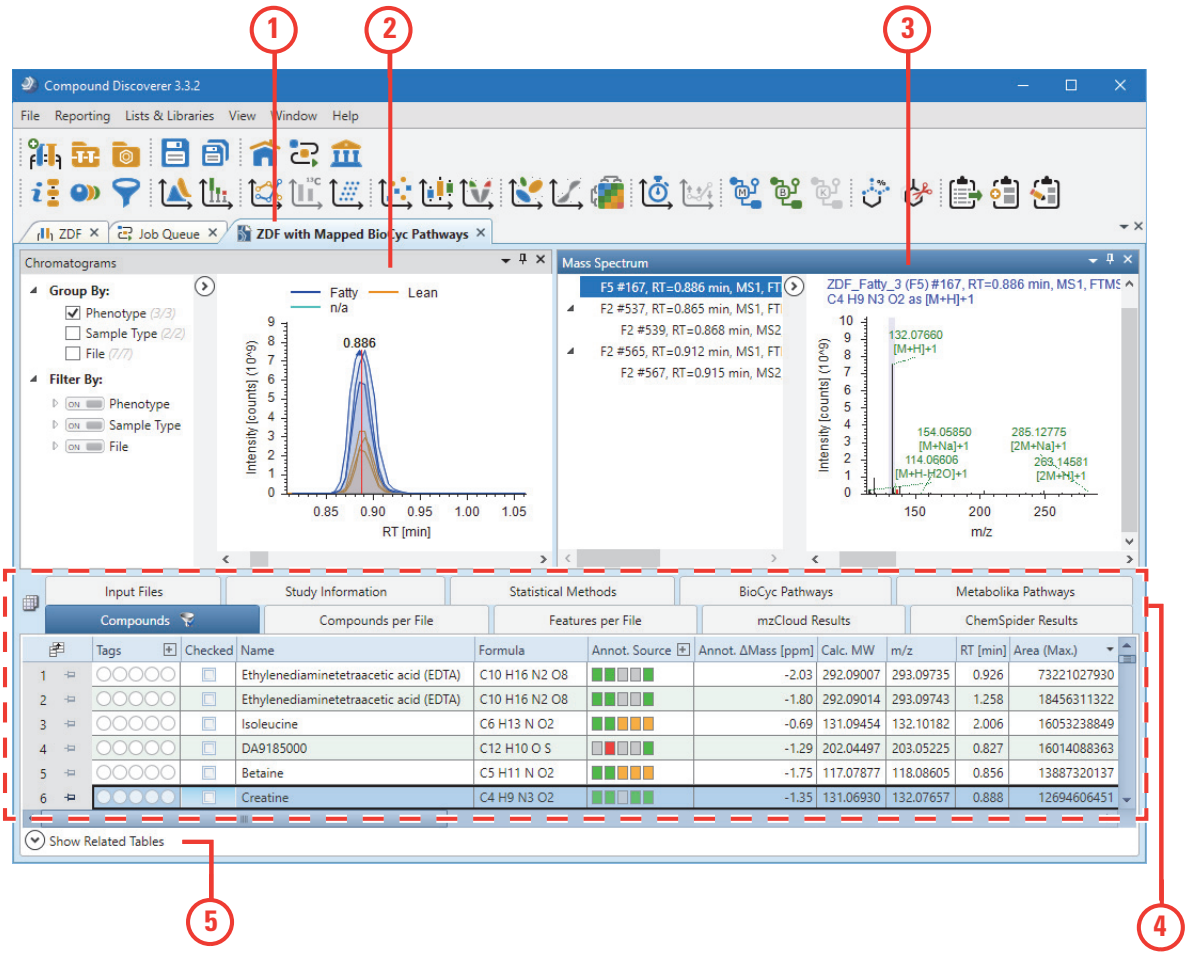
   The compounds that the analysis identified as background compounds are marked as background compounds in both the blank and non-blank samples and are hidden from the table.

5. A collapsed area for the related tables below the main tables.

Figure 15 shows the factory default layout of the views and result tables for the ZDF with Mapped BioCyc Pathways.cdResult file.

Note In Compound Discoverer 3.1 or later, the chromatographic peak area for compounds and features is reported in counts × seconds. The chromatographic peak area for a compound in each input file is the combined peak area for all its detected preferred ions (features).

Figure 15. Default result file layout (with the exception that row 6 instead of row 1 is selected in the Compounds table)

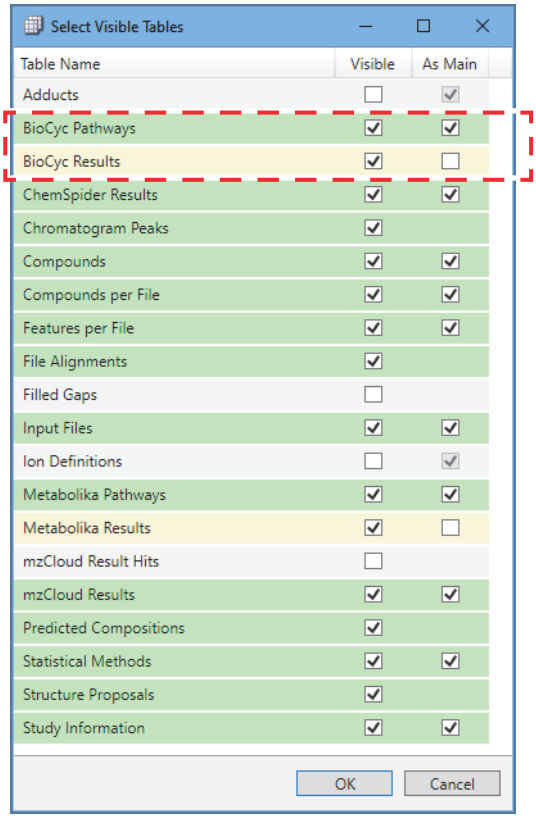


	Tags	Checked	Name	Formula	Annot. Source	Annot. ΔMass [ppm]	Calc. MW	m/z	RT [min]	Area (Max.)
1			Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		-2.03	292.09007	293.09735	0.926	73221027930
2			Ethylenediaminetetraacetic acid (EDTA)	C10 H16 N2 O8		-1.80	292.09014	293.09743	1.258	18456311322
3			Isoleucine	C6 H13 N O2		-0.69	131.09454	132.10182	2.006	16053238849
4			DA9185000	C12 H10 O S		-1.29	202.04497	203.05225	0.827	16014088363
5			Betaine	C5 H11 N O2		-1.75	117.07877	118.08605	0.856	13887320137
6			Creatine	C4 H9 N3 O2		-1.35	131.06930	132.07657	0.888	12694606451

Note Clicking the Select Visible Tables icon to the left of the main table tabs opens the Select Visible Tables dialog box where you can view a list of main and related tables that are in the result file but are currently hidden. See Figure 16.

Clicking the Field Chooser icon above the row number column opens the Field Chooser dialog box for the active table.

Figure 16. Select Visible Tables dialog box, which shows that the BioCyc Pathways table is visible as a main table and as a related table



Tip To view the related information for a specific compound in the main Compounds table, do the following:

1. Select the compound in the main table.
2. Click **Show Related Tables**.
3. In the related tables pane, open the table of interest by clicking its tab.

View the mapped BioCyc pathways for the analysis

By default, the BioCyc Pathways table is visible as a main table. In addition, it is visible as a related table for any compound that you select in the Compounds table. The BioCyc Results table is available only as a main table, and by default it is visible. See Figure 16.

Precondition

The reprocessed result file, ZDF with mapped BioCyc pathways, is open, and it is the active document in the application window. See "Open the newly reprocessed result file" on page 17.

❖ To view the BioCyc pathways that include compounds detected by the analysis

1. If you have modified the layout for the reprocessed result file, do the following:
   a. Choose **Window > Reset Layout** from the application menu bar.
   b. At the prompt, click **Yes**.

2. (Optional) Close the Chromatogram and Mass Spectrum views by clicking Close ✕ in the upper-right corner of each view.

   Closing these views provides more space for reviewing the BioCyc pathways.

3. Click the **BioCyc Pathways** tab in the set of main tables.

4. From the application menu bar, choose **View > BioCyc Pathways**.

   The BioCyc Pathways view appears to the left of the main tables.

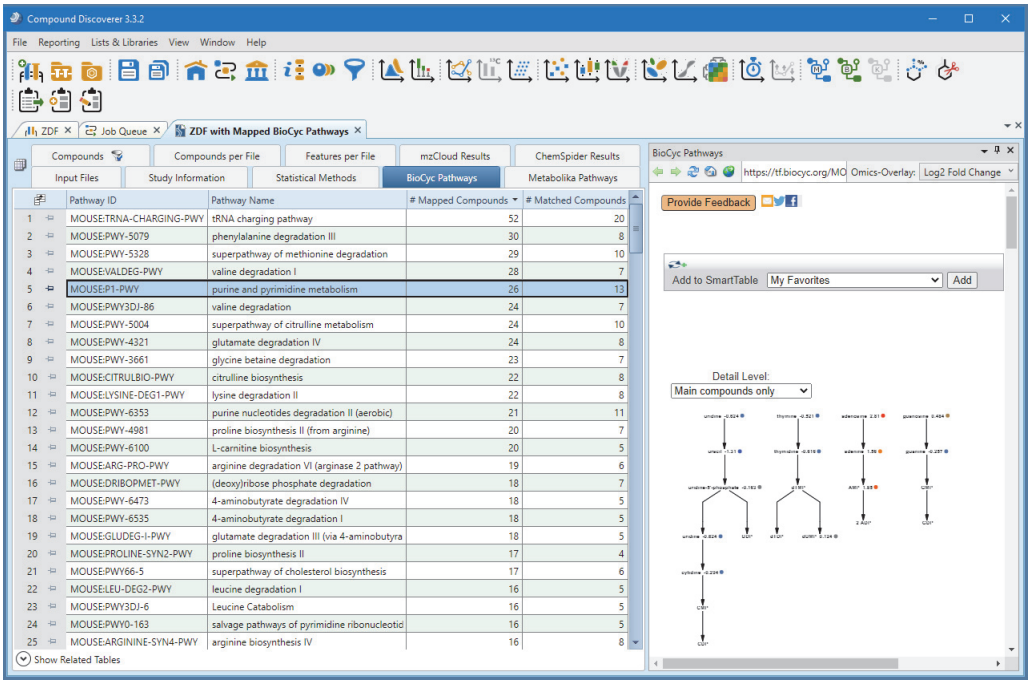
5. In the Omics-Overlay dropdown list, select **Log2 Fold Change**.

6. In the BioCyc Pathways table, select the **Purine and Pyrimidine Metabolism** pathway (row 5).

> **Note** In Figure 17, the Checked and Tags table columns are hidden to fit the figure on the page.

All the compounds in the pathway that match compounds in the main Compounds table have a color dot that indicates the fold change. The color key for these dots is below the pathways. See Figure 18.

Figure 17. Main BioCyc Pathways table and BioCyc Pathways view with a Log2 Fold Change overlay

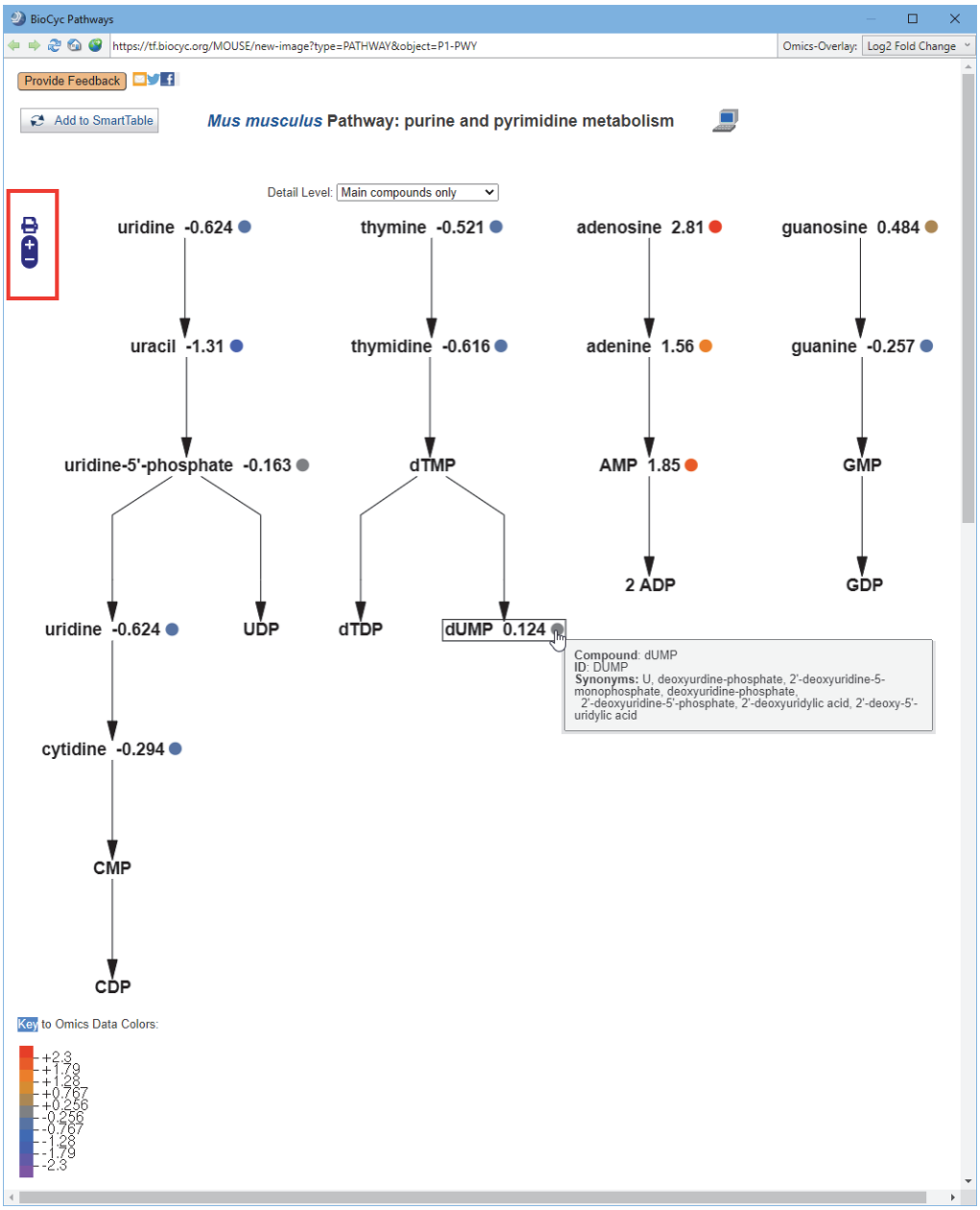


7. To review the pathways in the BioCyc Pathways view, do any of the following:

   - Drag the view to a second monitor.
   - Use the + and – buttons to enlarge or decrease the size of the pathways.
   - Use the mouse wheel to increase or decrease the size of the pathways.
   - Point to a compound in a pathway to view more information about the compound.
   - Click **Print** to print the pathways.

> **Tip** To display the + (increase size), – (decrease size), and print buttons at the left side of the view, point anywhere in the pathways area of the view.

Figure 18. Enlarged BioCyc Pathways view with color-coded compounds and the increase, decrease, and print buttons



View the mapped pathways for a specific compound in the Compounds table

Follow this topic to review the mapped BioCyc pathways for compounds of interest in the main Compounds table.

Note Not every compound in the Compounds table can be found in a metabolic pathway. The #BioCyc Pathways column in the Compounds table lists the number of pathways where the analysis found each compound, but by default, this column is not visible in the table. To view this column, you must enable it.

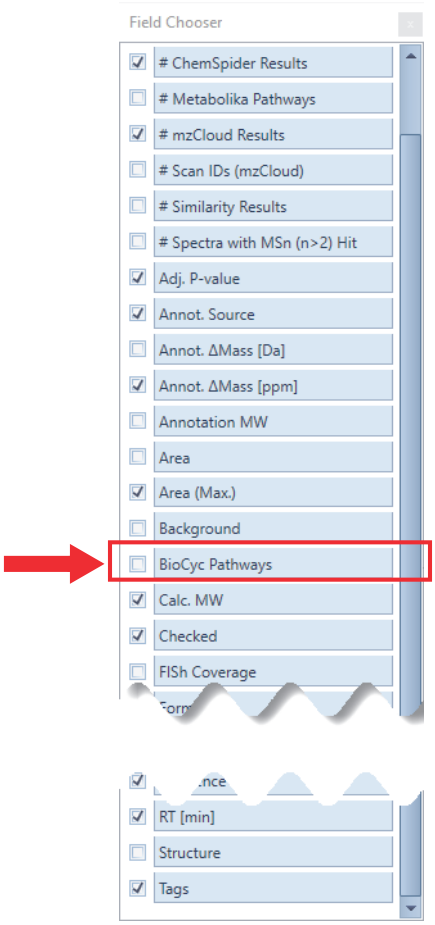
Prerequisites

The ZDF with BioCyc Mapping.cdResult file is open, and it is the active document in the application window. See "Open the newly reprocessed result file" on page 17.

❖ To view the mapped BioCyc pathways for a specific compound

1. Add the #BioCyc Pathways column to the Compounds table as follows:
   a. Click the **Compounds** tab to make the Compounds table the active main table.
   b. Click the field chooser icon in the upper-left corner of the table.

      The Field Chooser dialog box opens.

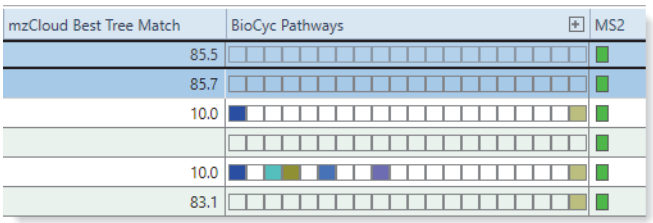


   c. Select the **BioCyc Pathways** checkbox.

      The BioCyc Pathways column appears to the right of the mzCloud Best Tree Match column and to the left of the MS2 column. See Figure 19 on page 23.

   d. Close the Field Chooser dialog box.

Figure 19. Default location of the BioCyc Pathways column in the main Compounds table



2. (Optional) Modify the layout of the columns in the Compounds table to make the BioCyc pathways easier to review as follows:

   - Right-click the Compounds table and choose **Enable Column Fixing**. Then, click the pin icons in the heading row of the following columns to move them to the left:

     i. To move the BioCyc Pathways column to the leftmost column, click the pin icon in its heading row.

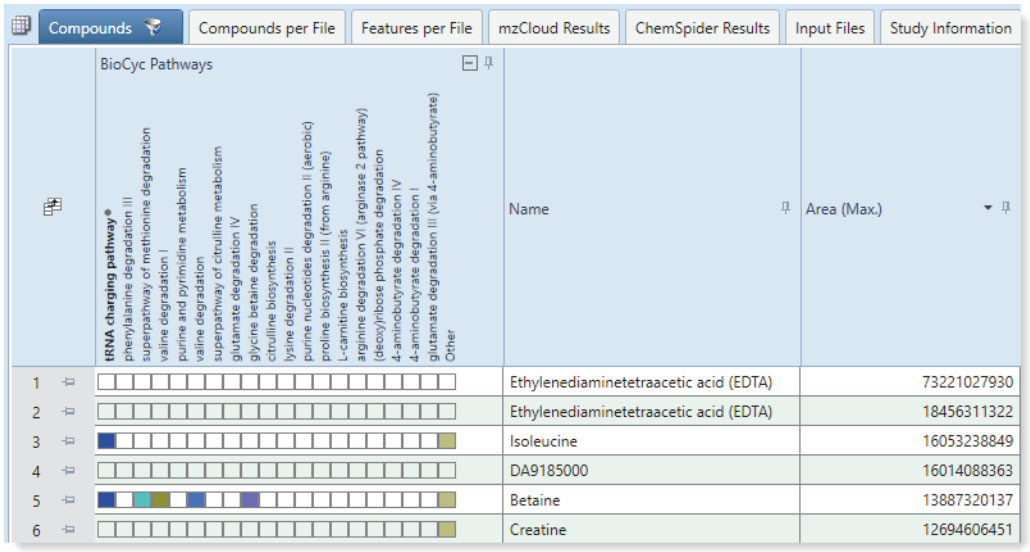
     

     ii. To move the Name column to the right of the BioCyc Pathways column, click the pin icon in its heading row.

     iii. To move the Area Max column to the right of the Name column, click the pin icon in its heading row.

   - In the BioCyc Pathways column, click the **expand** icon ⊞ to display the BioCyc Pathway names.

   Figure 20. Modified layout for the Compounds table

3. In the Compounds table, select **Creatine** [row 6 when the Compounds table is sorted in descending order by Area (Max)].

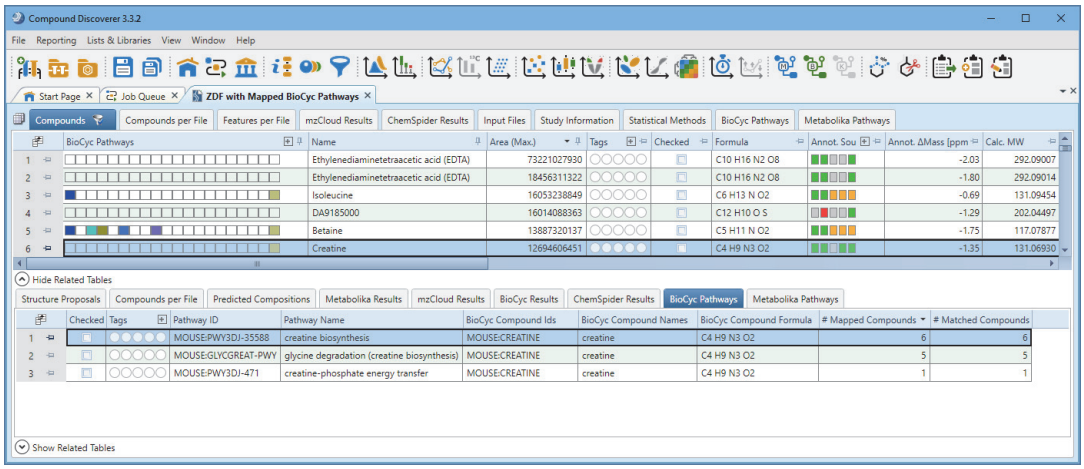
4. Open the related BioCyc Pathways table for creatine as follows:

   a. Click **Show Related Tables** below the main result tables. See Figure 15 on page 19.

   b. In the set of related table tabs for Creatine, click the **BioCyc Pathways** tab.

   c. In the related BioCyc Pathways table, select the **Creatine Biosynthesis** pathway (row 1). See Figure 21 on page 25.

Figure 21. Creatine biosynthesis pathway selected in the related BioCyc Pathways table for creatine

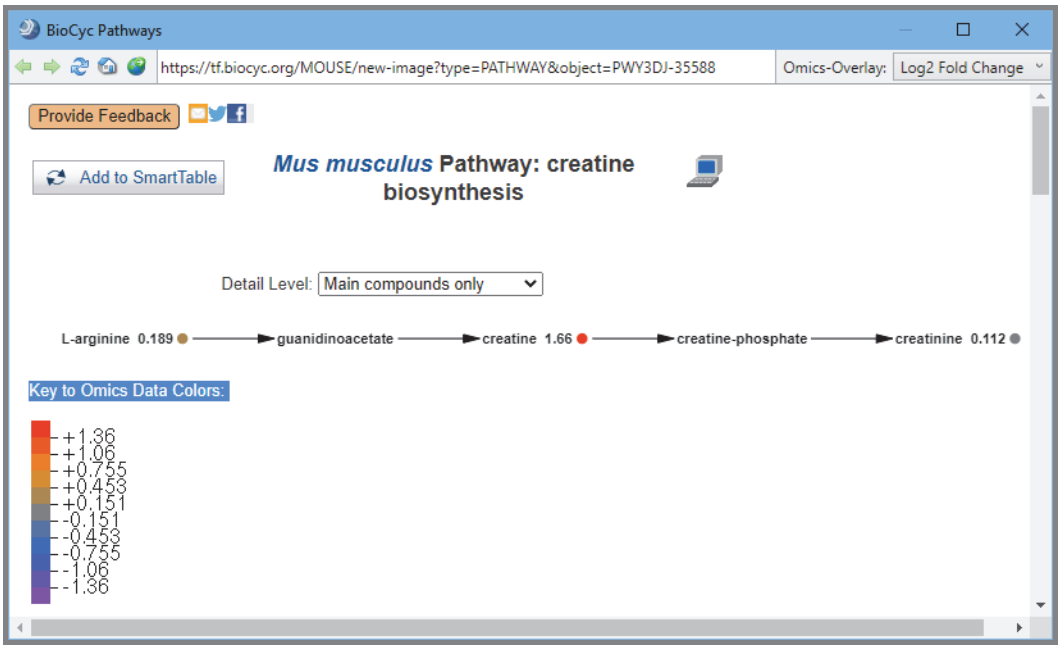


5. If you closed the BioCyc Pathways view, reopen it by choosing **View > BioCyc Pathways** in the application menu bar. Then, select **Log2 Fold Change** in the Omics-Overlay dropdown list.

   The creatine biosynthesis pathway for the Mus musculus organism appears in the BioCyc Pathways view.

6. (Optional) To enlarge the view, drag it to a second monitor.

Figure 22. Creatine biosynthesis with a Log2 Fold Change overlay



Display only the compounds identified by a confirmatory spectral match

You can filter the Compounds table to reduce the number of compounds displayed. The processing workflow contained the Mark Background Compounds node, so the background compounds that the analysis detected are already hidden.

❖ To display only the compounds that were identified by the mzCloud search

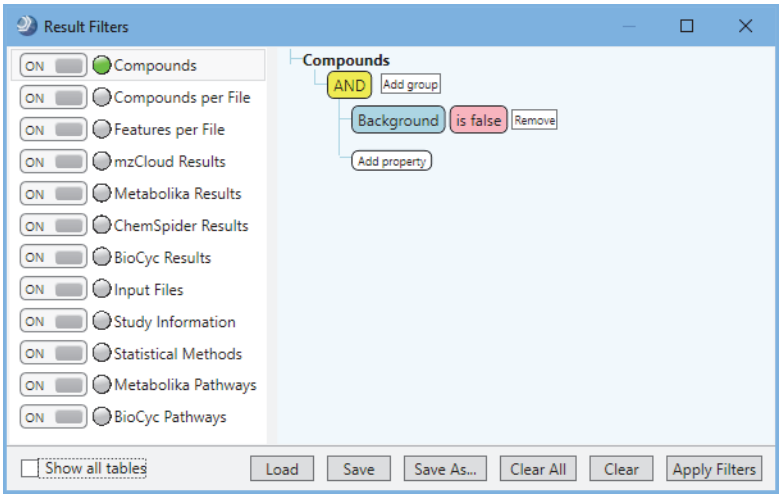
1. Return the result file to its default layout by choosing **Window > Reset Layout** in the application menu bar.

   The main Compounds table is the active result table.

2. In the application menu bar, choose **View > Result Filters**.

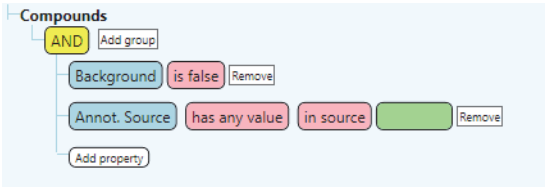
   The Result Filters view opens. The right pane is set to the Compounds table.

   Figure 23. Result Filters view for the Compounds table

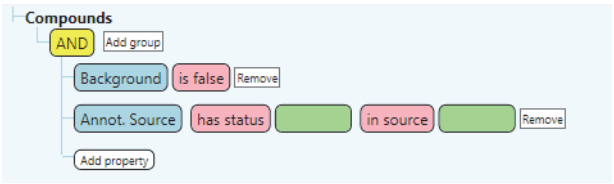


3. Set up the following filter: Annotation Source has status Full Match in source mzCloud Search as follows:

   a. Click **Add Property** and select **Annot Source** from the dropdown list.

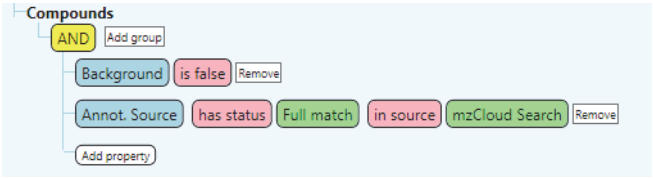


   b. Click **Has Any Value** and select **Has Status** from the dropdown list.



   c. Click the green box to the right of Has Status and select **Full Match** from the dropdown list.

   d. Click the green box to the right of In Source and select **mzCloud Search** from the dropdown list.



4. Click **Apply**, and then close the Result Filters dialog box.

   The number of visible compounds in the Compounds table has been reduced from 4199 to 139.

5. Click the **BioCyc Pathways** tab in the set of main tables.

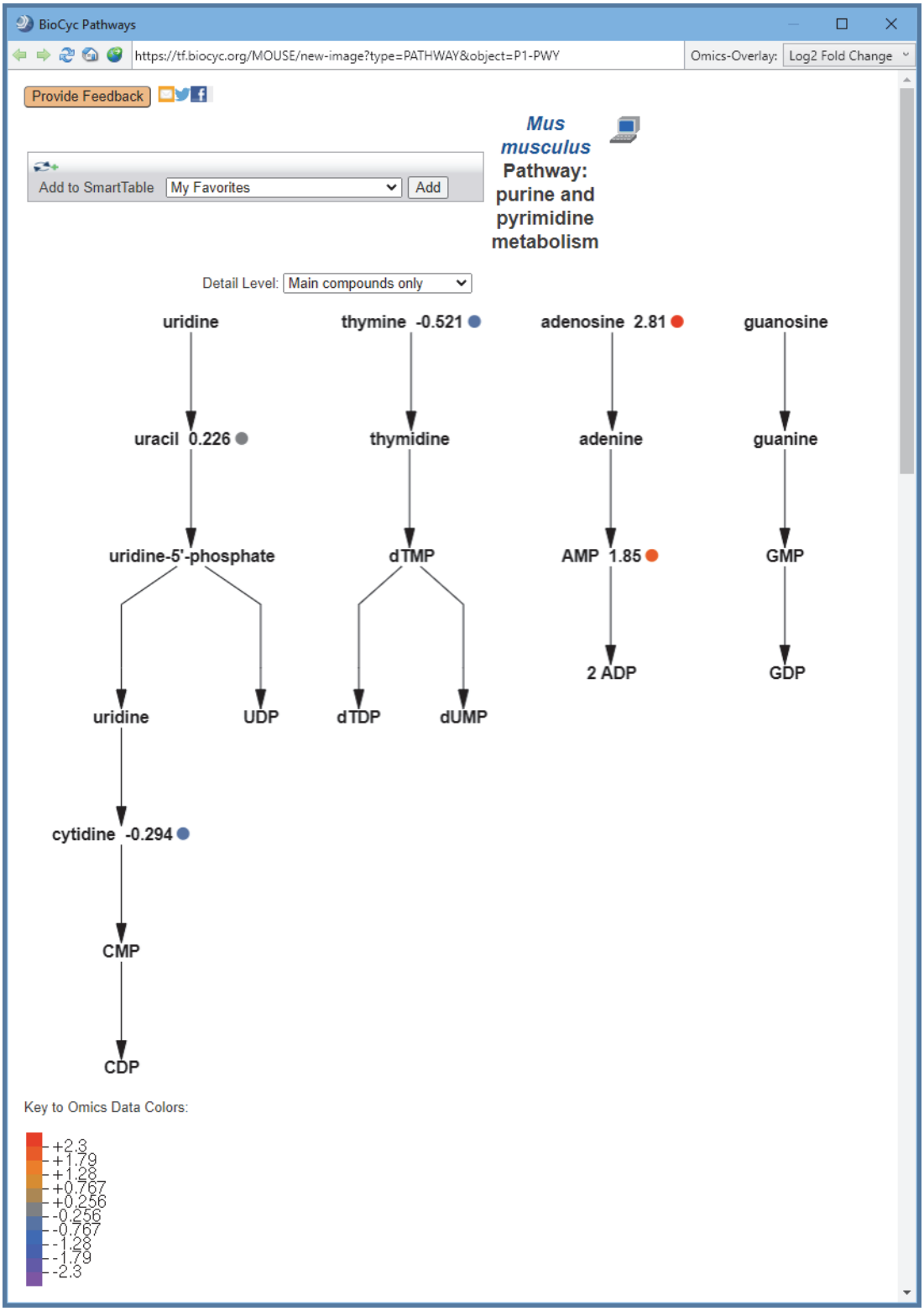
6. From the application menu bar, choose **View > BioCyc Pathways**.

   The BioCyc Pathways view appears to the left of the main tables.

7. In the Omics-Overlay dropdown list, select **Log2 Fold Change**.
8. In the BioCyc Pathways table, select the **Purine and Pyrimidine Metabolism** pathway.

   Now only the compounds that the analysis identified by searching the mzCloud mass spectral database are highlighted in the pathways, as indicated by the color-coded circles.

Figure 24. Matched compounds in the filtered main Compounds table



9. Compare the number of highlighted (color-coded circle) compounds in Figure 24 on page 27 to the number of highlighted compounds in Figure 18 on page 22.

Table 1. Comparison of the matched compounds found in the Compounds table before and after filtering out compounds that were not identified by the mzCloud search

Filtered by background compounds (4199 visible compounds)	Filtered by mzCloud identification and background compounds (139 visible compounds)
uridine	–
uracil	uracil
uridine-5'-phosphate	–
cytidine	cytidine
thymine	thymine
thymidine	–
dUMP	–
adenosine	adenosine
adenine	–
AMP	AMP
guanosine	–
quanine	–

You have completed the BioCyc tutorial for the Compound Discoverer 3.3 SP2 application.

Using the BioCyc Pathway Mapping feature in Compound Discoverer 3.3 SP1

If you are using the Compound Discoverer 3.3 SP1 application, you must download and install a configuration file to use the Map to BioCyc Pathways node.

Follow these topics:

1. Download the configuration file and the example files
2. Install the BioCyc service configuration file

Download the configuration file and the example files

❖ **To download and install the required configuration file and the Compound Discoverer 3.3.1 example files**

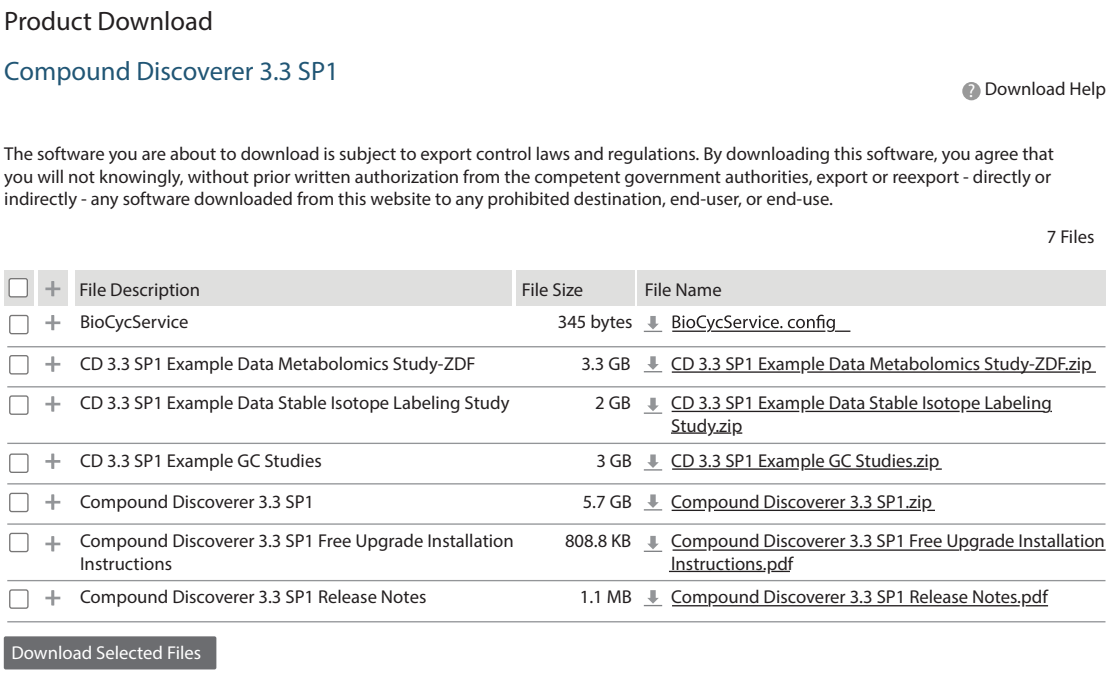
1. Go to the following URL: thermo.flexnetoperations.com

   The LSMS Software Download and Licensing Portal website opens.

2. Log in.
3. Under Software & Services at the left, click the **Product List** link.
4. On the Product List page, click the **Application - Compound Discoverer** link.
5. On the Product Information page, click the **Compound Discoverer 3.3 SP1** link.

   The Product Download page for the Compound Discoverer 3.3 SP1 application opens. This page contains BioCycService.config file and the compressed folders with the files for the tutorials provided with the application.

Figure 25. Product Download page for the Compound Discoverer 3.3 SP1 application



6. Download the following items:

   - In the File Name column, click  to the left of BioCycService.config.

     The BioCyc service configuration file downloads to the Downloads folder on your data processing computer.

   - If you do not have the example ZDF study folder, click to the left of the CD 3.3 SP1 Example Metabolomics Study.ZDF.

   The items download to the Downloads folder on your data processing computer. For information about the items in the CD 3.3 SP1 Example Metabolomics Study.ZDF and where to store them on your data processing computer, see "Locate the ZDF example files" on page 2.

Install the BioCyc service configuration file

After you download the BioCycService.config file, you must copy it to the appropriate folder on your data processing computer.

❖ To install the BioCyc service configuration file

1. Close the Compound Discoverer 3.3 SP1 application, if it is open.
2. On your data processing computer, navigate to the following folder:

   C:\Program Files\Thermo\Compound Discoverer 3.3\bin\Config

3. Rename the BioCycService.config file to BioCycService.config.bak.
4. Copy the BioCycService.config file from the Downloads folder to the Config folder.
5. Restart the Compound Discoverer 3.3 SP1 application.

After you install the configuration file, you are ready to begin this tutorial at "Copy the ZDF example files to your data processing computer" on page 3.

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