Proteome Discoverer 2.1 Quick Start

This guide shows you how to create a study, an analysis, and a workflow, and how to perform a search. For complete details on how to use the Thermo Protein Discoverer™ application, refer to either the *Proteome Discoverer User Guide* or the Help available in the Proteome Discoverer application.

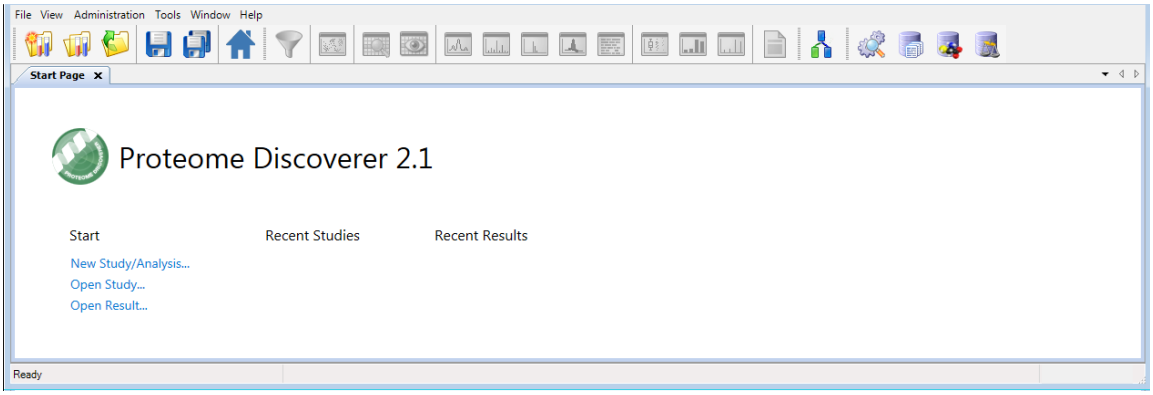
Contents

Opening Proteome Discoverer

❖ **To open the Proteome Discoverer application**

From the Start menu, choose **Programs > Thermo Proteome Discoverer 2.1**, or click the **Proteome Discoverer** icon, , on your desktop.

The Proteome Discoverer Start Page opens.



Configuring the Search Engine Parameters

If you intend to conduct a search with the Sequest™ HT and Mascot℠ search engines, you can configure certain search parameters for them before you begin your search. The example that this guide follows uses the Sequest HT search engine. For information on setting these parameters, refer to the *Proteome Discoverer User Guide*.

Downloading the FASTA Files

You must add a FASTA file to the Proteome Discoverer application before you can conduct a search with the Sequest HT. You must add a FASTA file to the server that is running Mascot before you can conduct a search with the Mascot search engine.

Revision A XCALI-97752

Thermo SCIENTIFIC

You can download FASTA files from two sources:

- You can download a controlled protein database directly from ProteinCenter and add it to a FASTA file. These controlled databases offer you access to the latest protein information. The ProteinCenter database service provides extensive information about proteins, peptides, and genes. In addition, it gives you the ability to select proteins of a specified taxonomy to download and use as a FASTA file.
- You can add a FASTA file that you download from other sources onto your hard drive and then register it.

❖ To download a FASTA file from ProteinCenter

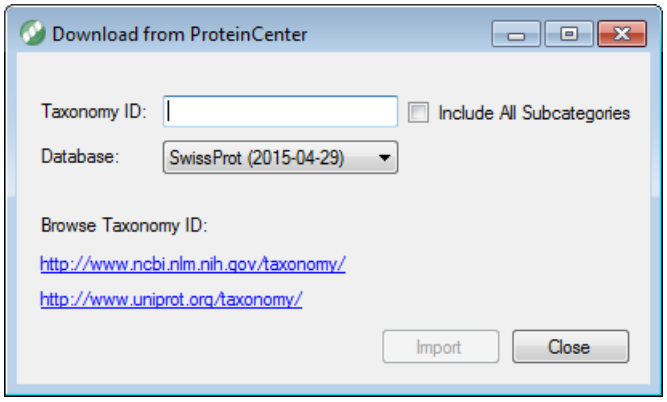
1. Configure the Proteome Discoverer application for protein annotation. For instructions, refer to the *Proteome Discoverer User Guide*.
2. Choose **Administration > Maintain FASTA Files**, or click the **Maintain FASTA Files** icon, .

   The Administration page appears with the FASTA files view.
3. Click the **Check for Updates** icon, Check for Updates .

   The Proteome Discoverer application updates the available databases in the job queue.

   The Download icon becomes available.
4. Click the **Download** icon, Download .

   The Download from ProteinCenter dialog box appears.



5. In the Taxonomy ID box, do the following:
   a. Type the taxonomy identification number of the appropriate organism-specific sequence database in ProteinCenter.

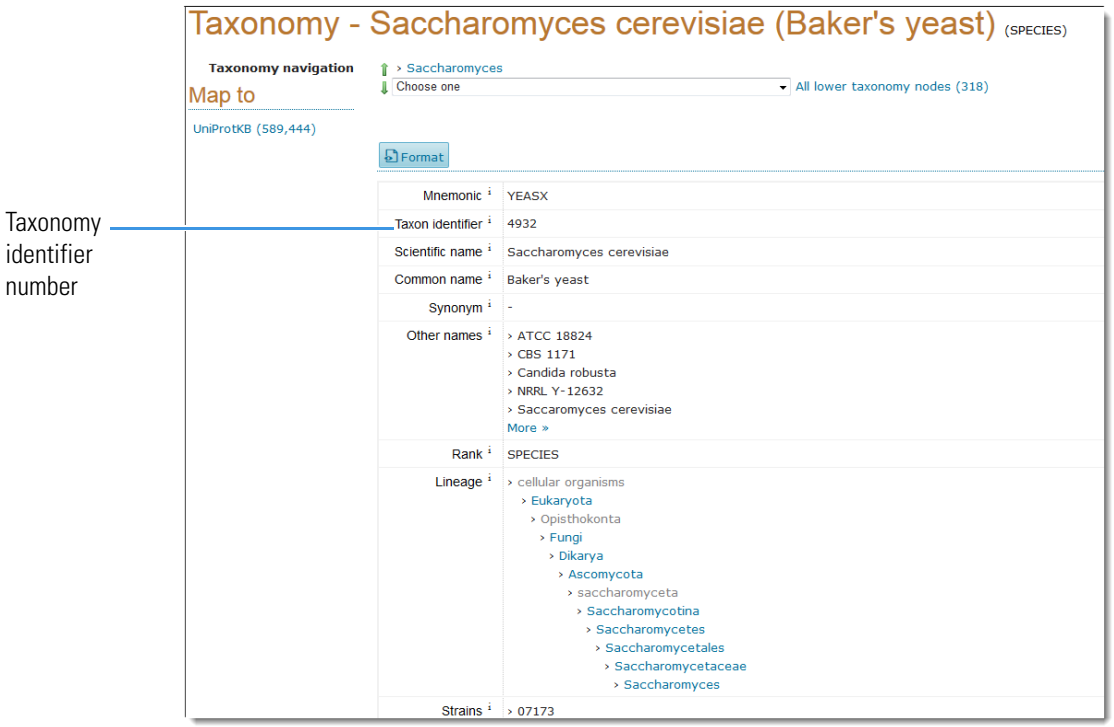
      The taxonomy identification number is a unique number identifying a biological species, a special subspecies, or a bacterial strain. You can find the organism's taxonomy identification number on the UniProt™ website (http://www.uniprot.org/taxonomy) or at the NCBI.

      i. In the box to the right of the Taxonomy menu on the UniProt website, type the name of the organism that you are interested in, for example, **Baker's yeast**.

      ii. Click the **Search** icon, .

      iii. (Optional) Under the Taxon heading, click the name of the subspecies that you are interested in, for example, **Saccharomyces cerevisiae**.

The taxonomy identification number appears on the Taxon Identifier line, as shown in this figure.

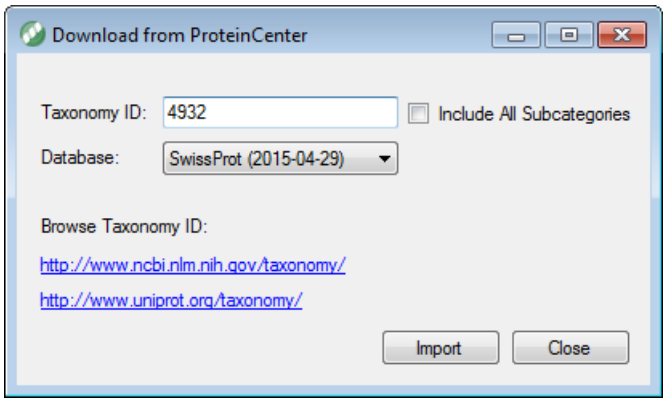


b. (Optional) To include data for a subspecies or subcategory of the selected species in the downloaded database, select the **Include All Subcategories** check box in the Download from ProteinCenter dialog box.

c. From the list in the Database box of the Download from ProteinCenter dialog box, select the name of the original source database to download the proteins from.

The default is SwissProt.

The next figure shows the completed Download from ProteinCenter dialog box.



d. Click **Import**.

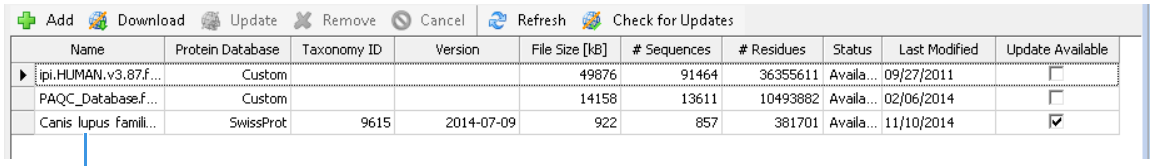
The application now displays the download as a job running in the job queue.

6. When the job queue displays “Completed” in the Execution State column, click **FASTA Files** under Content Management in the Configuration view to return to the FASTA Files view.

The downloaded database appears in the FASTA Files view. It might take several minutes to appear.

7. If you do not see the downloaded database after a few minutes, click the **Refresh** icon, Refresh .

The next figure shows the Saccharomyces cerevisiae (4932) species database downloaded from the SwissProt database.



Database downloaded from ProteinCenter

❖ To download a FASTA file from sources other than ProteinCenter

1. Choose **Administration > Maintain FASTA Files**, or click the **Maintain FASTA Files** icon, .

   The Administration page appears with the FASTA files view.

2. Click the **Add** icon, Add .

3. In the Open dialog box that appears, browse to and select the FASTA file that you want to process, and then click **Open**.

   The FASTA file that you selected appears as a job in the job queue. To cancel the addition of this file, click the **Abort** icon, Abort .

   When you see "Completed" in the Execution State column, the database has finished downloading.

4. To add another FASTA file, wait until the Execution State column indicates that the addition of the FASTA file is completed, click **FASTA Files** in the left pane of the Administration page under Content Management, and then click **Add** to add the next file.

The amount of time required to import a FASTA file depends on the file size. When the application finishes importing a FASTA file, it displays "Available" in the Status column. The FASTA file is now available to use for a protein or peptide search with the Proteome Discoverer application.

Creating a Study

The first step in using the Proteome Discoverer application is to create a study. For illustrative purposes, this section uses an example study called Bailey_2014, which uses a publicly available data set from the Chorus Project (http://chorusproject.org)[1]. This project resides under the Elution Order Algorithm project and includes data about the following:

- Two sets of biological replicates

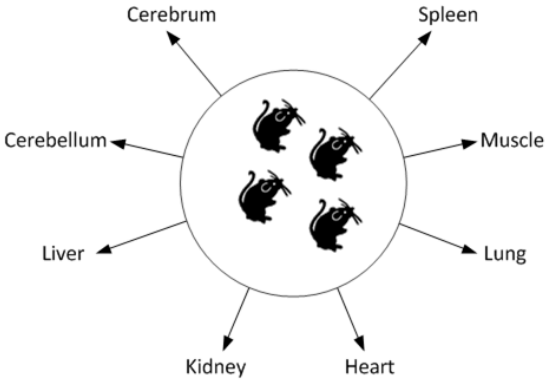
  Four mice (replicates) were sacrificed and dissected. Individual organs of interest from them were homogenized, and the proteins were extracted from them and labeled. Then the differentially labeled organ-specific proteomes were mixed together if they came from the same mouse. Each pooled mouse sample was then run twice, using different acquisition method parameters.

  For information on replicates, refer to the *Proteome Discoverer User Guide*.

- The custom TMT™ 8plex quantification method
- Label switching
- One biological factor: different tissues
- One technical factor: different acquisition methods

[1] Bailey, D.J.; McDevitt, M.T.; Westphall, M.S.; Pagliarini, D. J; Coon, J. J. Intelligent data acquisition blends targeted and discovery methods. *Journal of Proteome Research*, **2014**, 13 (4): 2152–2161.

The following figure shows how different tissue samples are distributed over four biological mouse replicates.



Biological Replicate	1	2	3	4
TMT 8-plex Channel 126	Kidney	Cerebellum	Lung	Muscle
127_N	Cerebellum	Liver	Liver	Kidney
127_C	Muscle	Kidney	Spleen	Spleen
128_C	Cerebrum	Muscle	Muscle	Cerebellum
129_N	Lung	Heart	Cerebrum	Lung
129_C	Liver	Lung	Heart	Heart
130_C	Heart	Cerebrum	Cerebellum	Liver
131	Spleen	Spleen	Kidney	Cerebrum

❖ To create a study

1. On the Start Page, click **New Study/Analysis**.

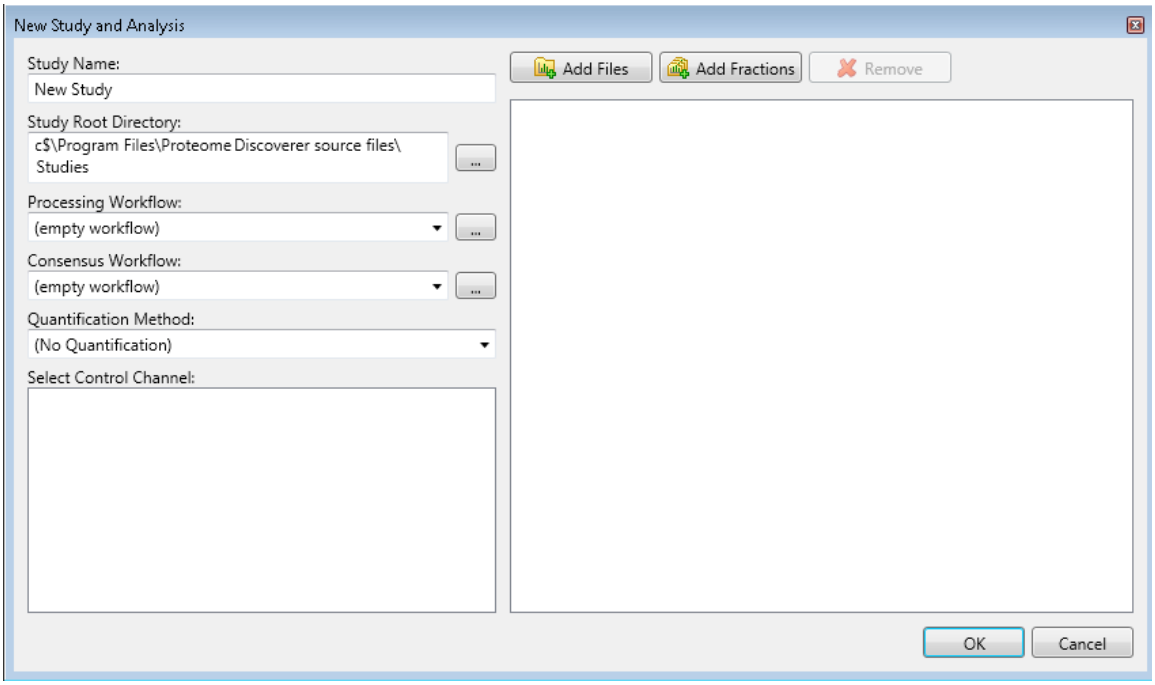
–or–

Choose **File > New Study/Analysis**.

–or–

Click the **Create New Study/Analysis** icon,  .

The New Study and Analysis dialog box opens.



In this example, you only specify the name of the study and a root directory to save the study in.

2. In the Study Name box, specify the mandatory study name.

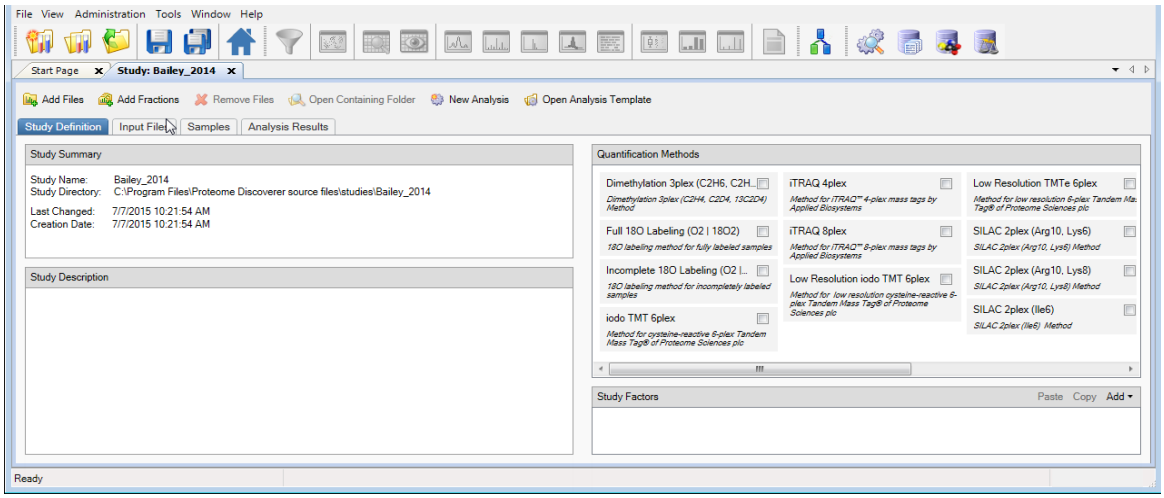
   The example uses the study name of Bailey_2014.

   The application generates a default study name by searching for the common part of the file names when you add multiple files at once and using this common part as the suggestion for the name of the new study.

3. In the Study Root Directory box, specify the folder where you will store the study folder. Click the **Browse** button (...), and in the Select Folder dialog box, specify the folder and click **Select Folder**.

4. Click **OK**.

   The application creates a new study folder in the folder that you specified as the root directory and opens a new page with the study name (Study: Bailey_2014 in the example), as shown in the next figure. It appends a .pdStudy extension to the study file name.



   On the Study Definition page, you add a description of your study, select the quantification method or methods to use in the study, and set up the new factors that describe and distinguish your samples.

Adding a Description

You can optionally add a description of the study by typing it in the Study Description area of the Study Definition page.

Adding a Quantification Method to the Study

A quantification method contains the specification of the available quantification channels. The Proteome Discoverer application currently supports precursor ion-based quantification methods and MS/MS reporter ion-based quantification methods. It also supports peak area calculation detection. You can specify a quantification method for each of the input files.

The example used in this guide uses a custom TMT 8plex method, which you must create.

❖ To create a quantification method

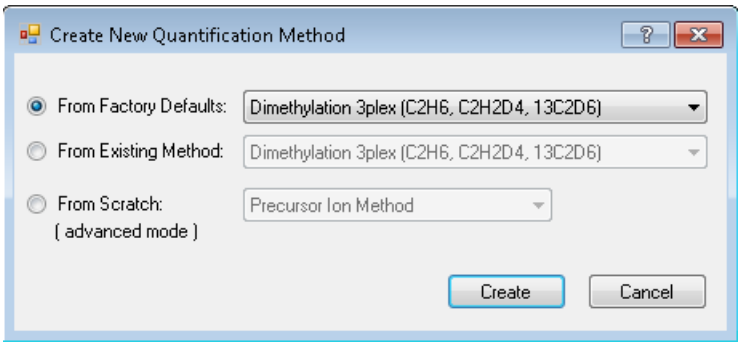
1. Choose **Administration > Maintain Quantification Methods**, or click the **Maintain Quantification Methods** icon,  .

   The Quantification Methods view opens. It lists all of the available methods for both precursor ion and reporter ion quantification.

2. Click the **Add** icon, 

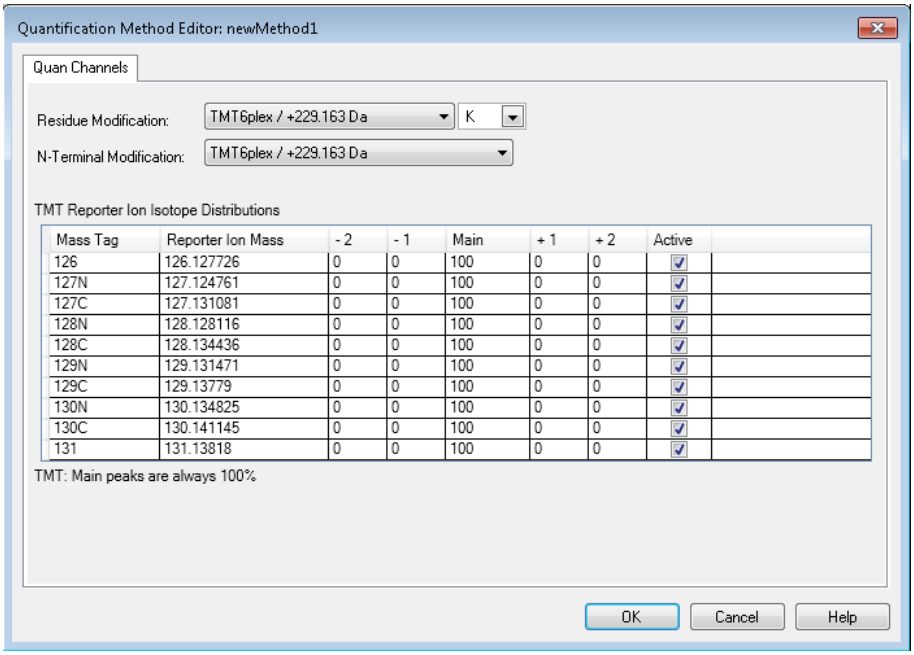
.

The Create New Quantification Method dialog box now appears.



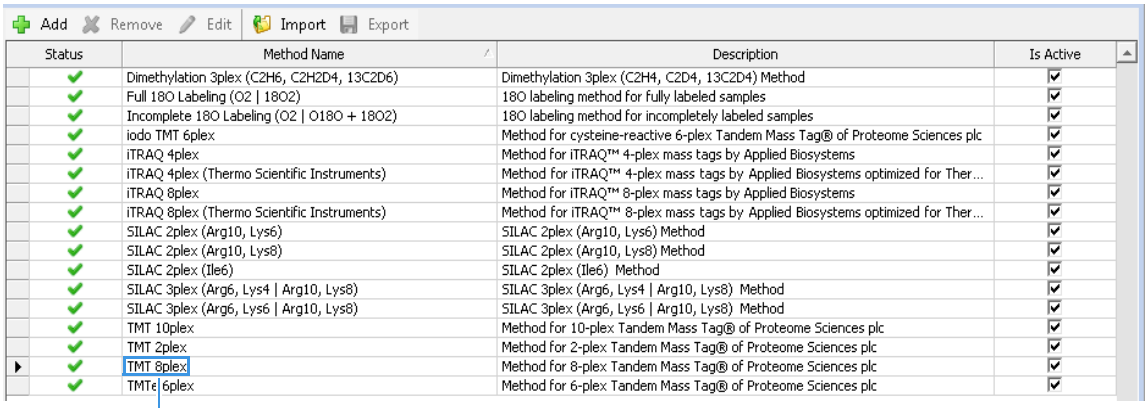
3. Select the **From Existing Method** option, and select **TMT 10plex** from the adjacent list.
4. Click **Create**.

   The Quantification Method Editor opens.



5. In the Active column to the right, clear the check boxes for the following two channels:
   - **128N**
   - **130N**
6. Click **OK**.
7. In the Save Quantification Method dialog box, type the name of the quantification method that you want to create: **TMT 8plex**.

The application adds the TMT 8plex method to the Quantification Methods view.



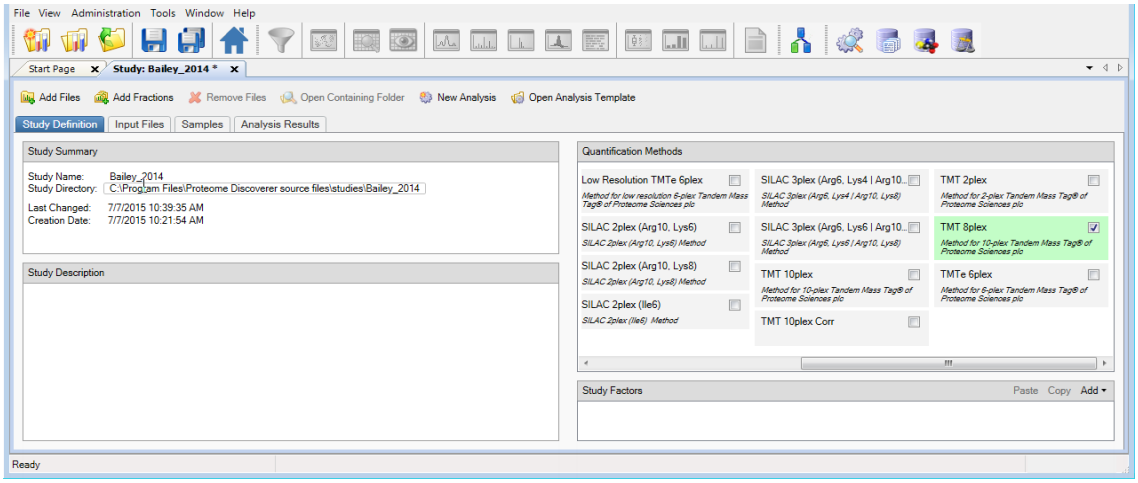
New custom TMT 8plex method in the Quantification Methods view

❖ To select the quantification method to use in the study

1. Click the **Study Definition** tab in the study if it is not already selected.
2. Select the check box corresponding to the quantification method or methods that you want to use.

   In this example, the samples are labeled with the custom TMT 8plex quantification method, so you would select the TMT 8plex check box (see the next figure).

   If the Quantification Methods pane does not include the TMT 8plex method, choose **File > Save All**, and close and reopen the study.



3. If you selected more than one quantification method in step 2, after you add the input files, specify the quantification method for each input file. For instructions, refer to the *Proteome Discoverer User Guide*.

Adding the Study Factors

In this step, you add the study factors that you want to use for your samples.

A factor is a single biological or technical parameter that you control, for example, genotype, diet, environmental stimulus, age, column length, spray voltage, or collision energy.

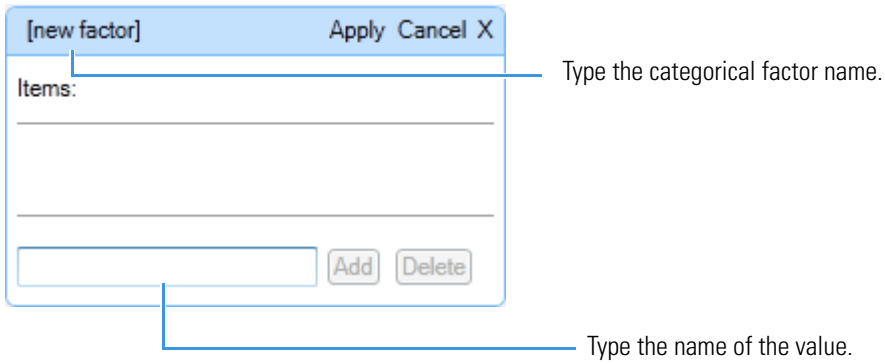
The experiment in the example dataset was performed by using eight different tissues and two different acquisition methods, so you would add two categorical factors, “Acquisition” and “Tissue,” to the study.

❖ To add categorical study factors

1. Add the first categorical (non-numeric) factor (Acquisition in the example), as follows:

   a. In the Study Factors area of the Study Definition page, choose **Add > Categorical Factor**.

   The categorical factor dialog box appears.



   [new factor] is highlighted.

   b. Type a name over [new factor] for the new categorical factor, for example, **Acquisition**. (See the next figure.)

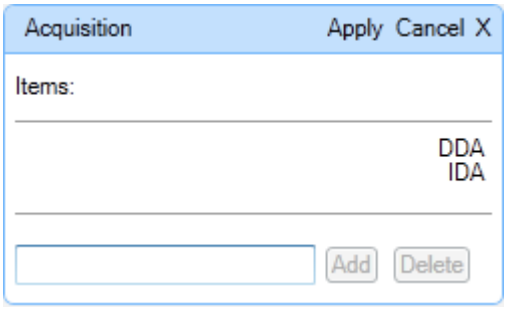
   > **Note** If the full categorical box becomes compressed, click **Edit** to restore it to its original size.

   c. In the box to the left of the Add and Delete buttons, type the name of the first value and click **Add**.

   In this example, the value is the acquisition method, and the first acquisition method is intelligent data acquisition (IDA).

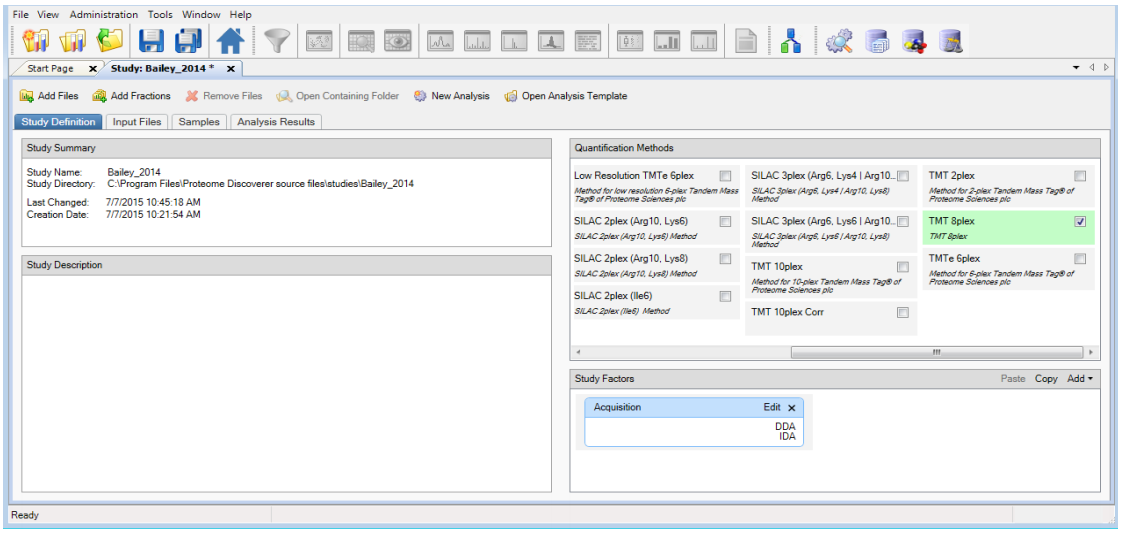
   d. In the same box, type the name of the second acquisition method and click **Add**.

   In this example, the second acquisition method is data-dependent acquisition (DDA). This figure shows the completed categorical factor dialog box.



   e. Click **Apply** in the categorical factor dialog box.

   Confirm that the Study Definition page resembles the next figure.



2. Add the second categorical factor (Tissue in the example), as follows:

   a. In the Study Factors area of the Study Definition page, choose **Add > Categorical Factor**.

      The dialog box shown previously appears.

      [new factor] is highlighted.

   b. Type a name over [new factor] for the new factor, for example, **Tissue**.

   c. In the box to the left of the Add and Delete buttons, type the name of the first type of tissue and click **Add**.

      In this example, the first type of tissue is Cerebellum.

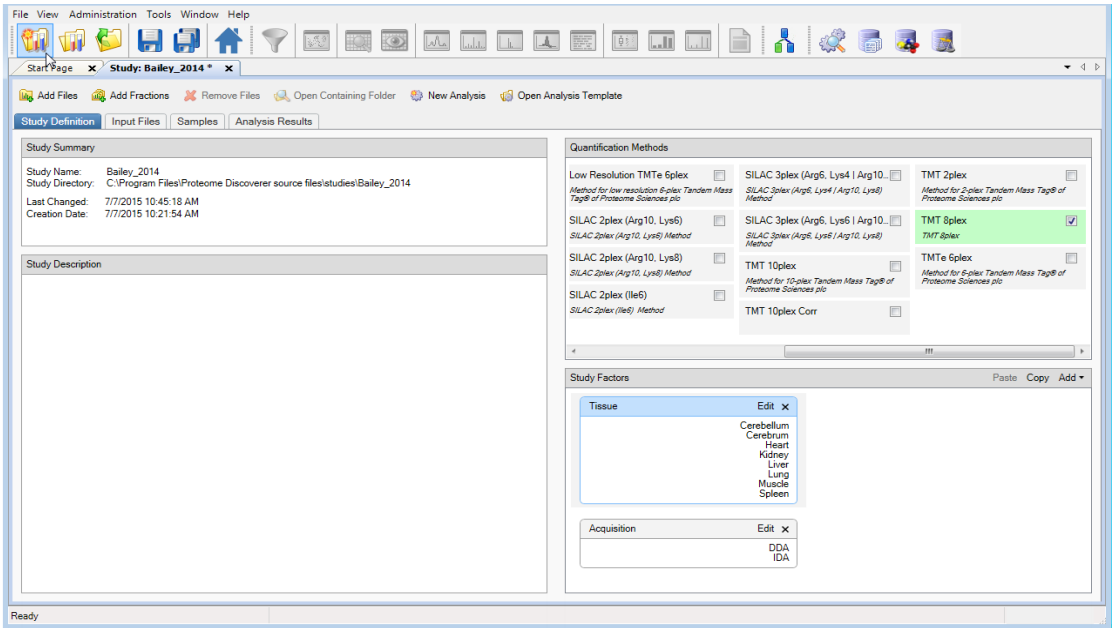
   d. In the same box, type the name of any additional types of tissue and click **Add** after each one.

      The example adds the following types of tissue to the study:

      - Cerebellum
      - Cerebrum
      - Heart
      - Kidney
      - Liver
      - Lung
      - Muscle
      - Spleen

   e. Click **Apply** in the Tissue dialog box.

      Confirm that the Study Definition page resembles the next figure.



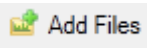
Adding the Input Files

Add the input files from the example data set to your study. (You can add input files to the study at any point.) For the types of input files supported, refer to the *Proteome Discoverer User Guide*. You can add individual input files, multiple unrelated input files, or fractions. To add fractions, refer to the *Proteome Discoverer User Guide.*

❖ To add a single input file or multiple unrelated input files

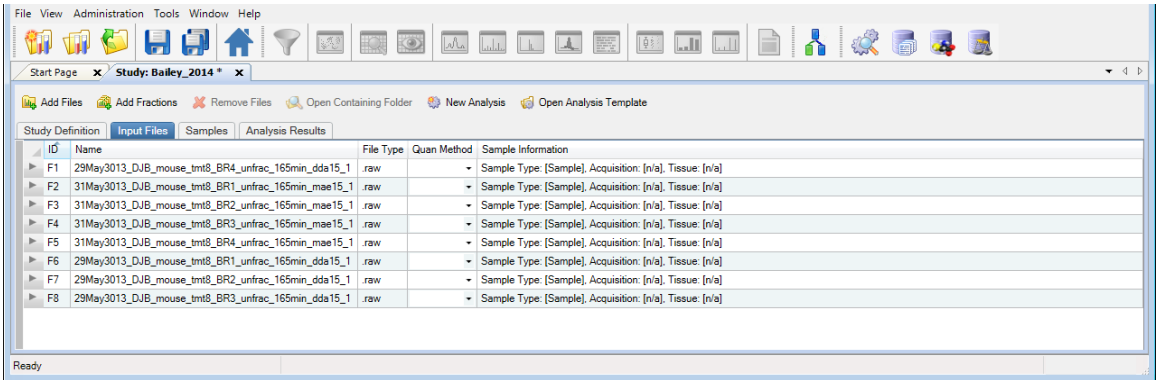
Drag the input file or files from Windows Explorer and drop them onto the Input Files page.

–or–

1. Click the **Add Files** icon, 

.
2. In the Add Files dialog box, browse to the location of the input files and select them.
3. Click **Open**.

The input files appear on the Input Files page (see the next figure). Each file on the page receives a unique identifier: F1, F2, ..., F*n*. The Proteome Discoverer application adds each file as a single study file.

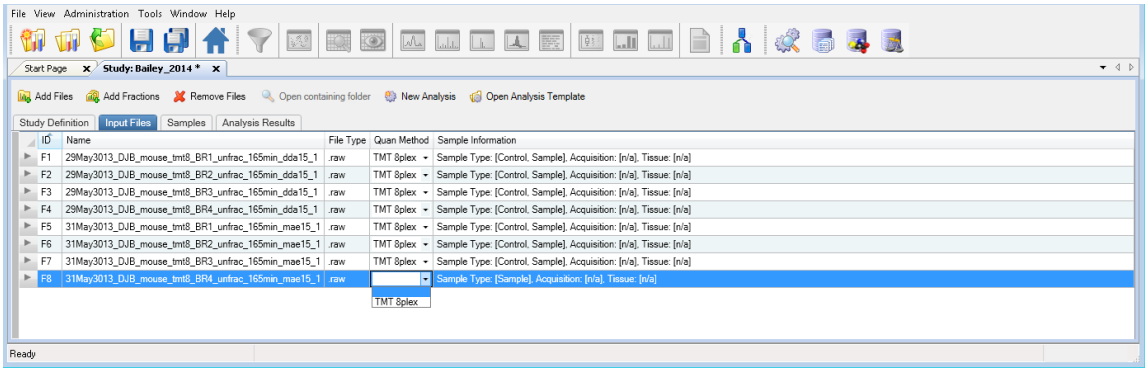


Specifying the Quantification Method for Multiple Input Files

In this step, you specify the quantification method that was used for each of the files. In the example data set, all samples are labeled with TMT 8plex.

❖ To set the quantification method for each of the input files

1. Click the **Input Files** tab if it is not already selected.
2. In each sample row, click the Quan Method column and select the quantification method (in this example, **TMT 8plex**) from the list.



Setting the Factor Values for the Samples

When you select a quantification method for a file, the application generates a sample placeholder for each quantification channel.

Each sample is associated with a sample type. Currently only quantification uses sample types. The application calculates quantification ratios from samples designated as either the “sample” or “control” sample type.

- Control sample type: A sample used as a reference sample in a quantification experiment
- Sample type: A sample not used as a reference sample
- Blank: A sample consisting only of solvent and no sample mixture
- Standard: A sample consisting of a standard quality-control peptide mixture

The Sample Type column on the Samples page of the study displays the sample type of each sample. The default sample type is Sample. If a file has samples for different quantification channels, mark one of the samples Control. Marking a sample as a control affects the scaling of quantification values. In this example, the channel that was used to label the mouse liver tissues is used as the control.

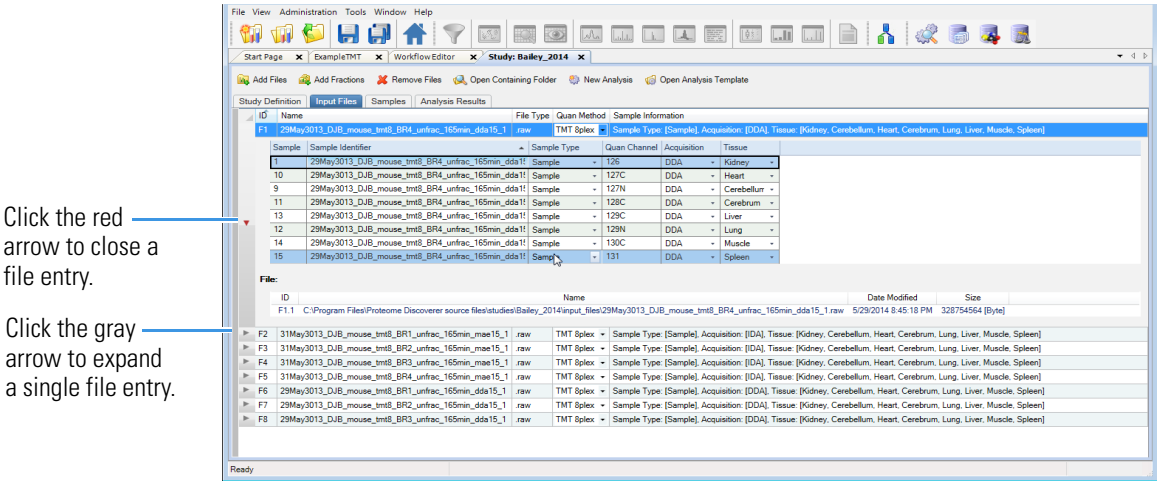
Each sample is associated with a quantification channel shown in the Quan Channel column and with values for each of the factors that you specified for your study. Previously, you specified a factor for the acquisition method used and a factor for the tissue that was extracted and labeled. You now set the correct factor values for each of the samples in the study.

Each sample has an automatically generated sample name composed of the raw data file name and the appended name of the quantification channel. You can change this name, but the name must be unique among all samples in the study.

❖ To view the samples

On the Input Files page, click the gray arrow to the left of a sample to display its constituent file entries.

A hierarchical view opens, showing the samples contained in a raw data file. For each of the raw data files in the following example, there are eight samples for the eight quantification channels of the TMT 8plex method.

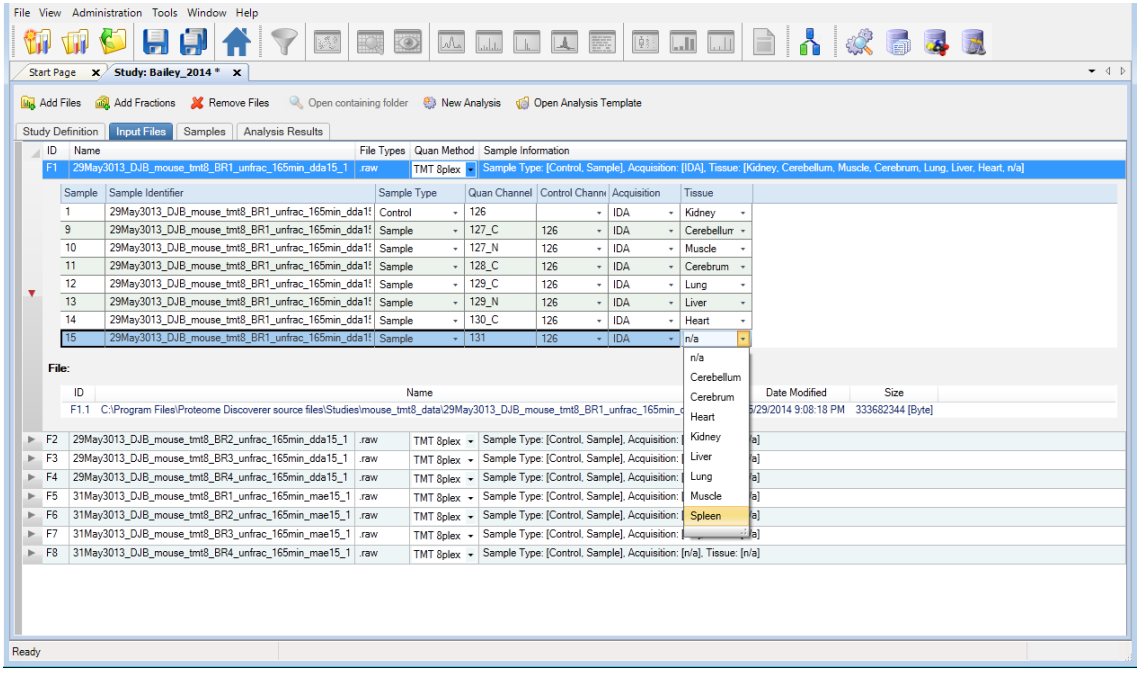


❖ To set the factor values for the samples

1. Click the **Input Files** tab if it is not already selected.
2. Click the gray arrow next to the first sample to expand the information about the sample.
3. For the first factor (in the example, Acquisition), set the value for each sample in each raw data file by selecting the down arrow in the factor column and then selecting the value from the list.

   In the example, select **IDA** in the Acquisition column (see the next figure).
4. For the second factor (in the example, Tissue), set the value for each sample in each raw data file by selecting the down arrow in the factor column and then selecting the value from the list.

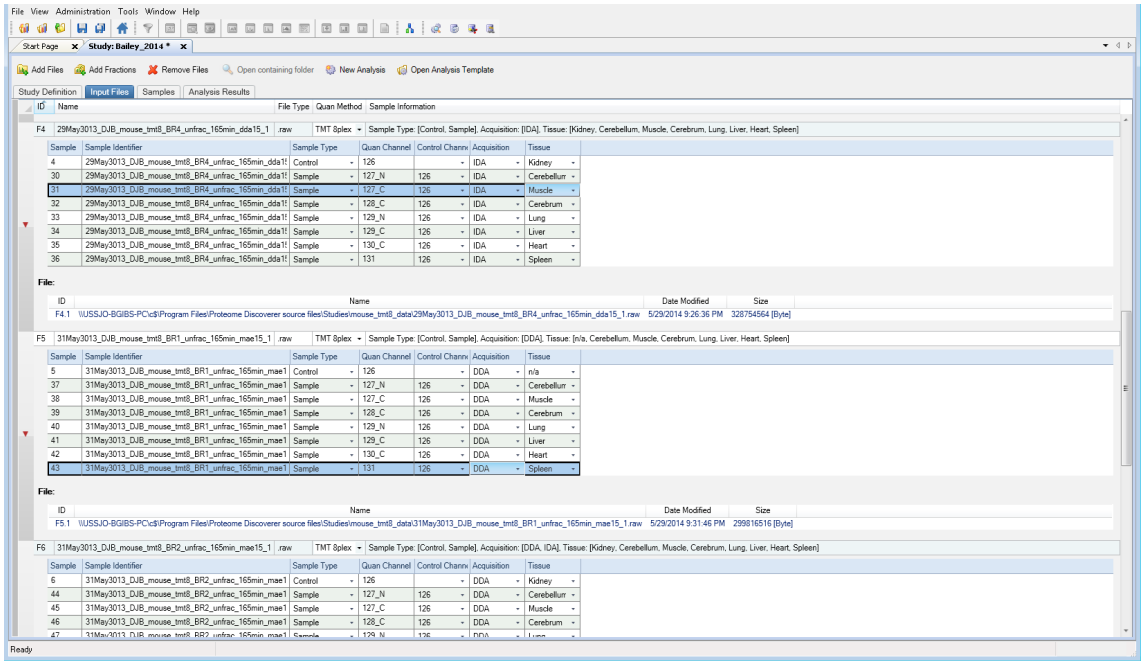
   The next figure shows this process for the Tissue factor.



5. Set the same values in the Acquisition and the Tissue columns for the rest of the samples.

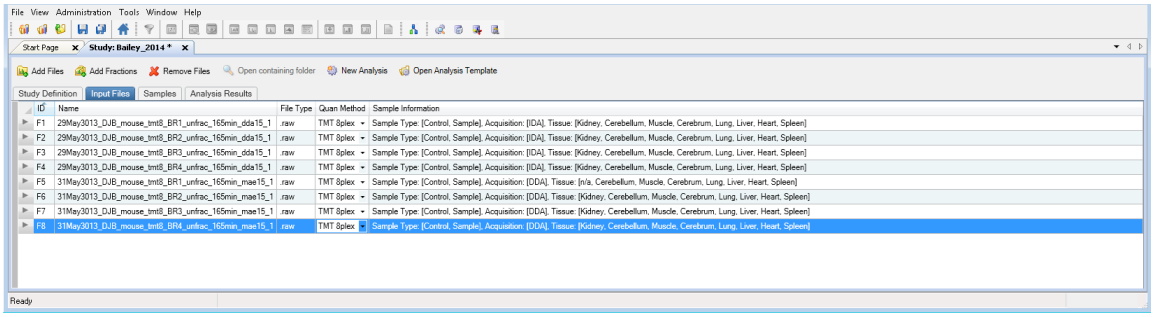
After you finish setting the factor values for each sample, confirm that the Input Files page resembles the next figure. (In the example data set, you must set 128 factor values for eight files with eight samples each with two factors each.)

Note For instructions on changing the values for factors or other study variables for multiple samples at once, refer to the *Proteome Discoverer User Guide*.



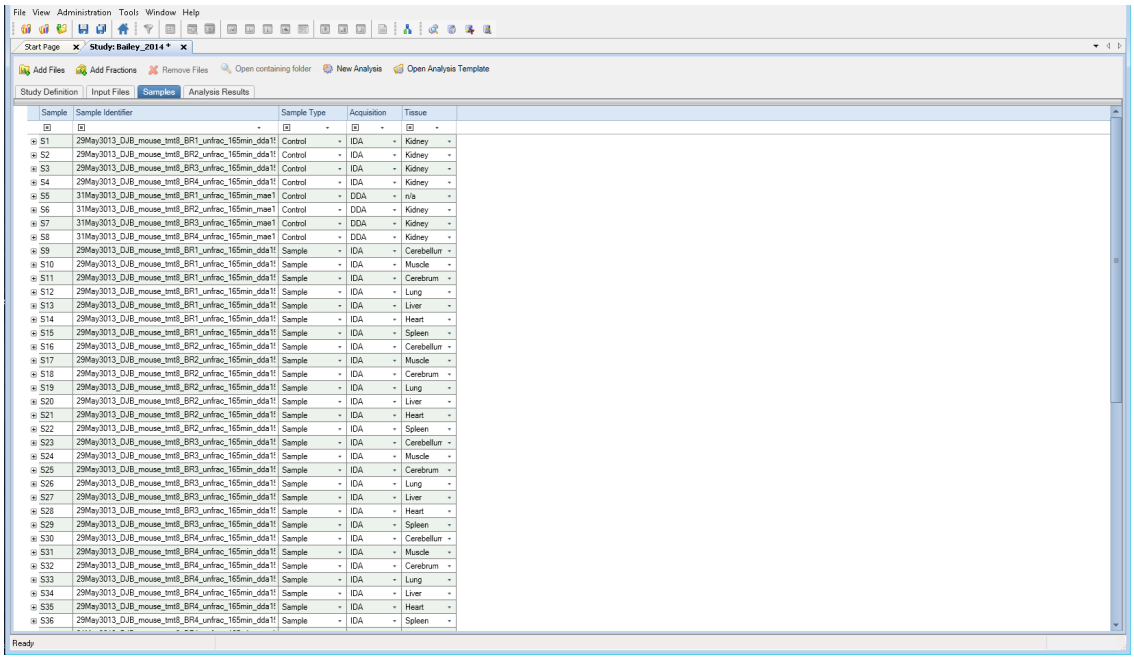
6. Click the red down arrow next to each sample to compress the information displayed.

The Sample Information column summarizes the information about the samples contained in a file (see the next figure).



7. (Optional) Click the **Samples** tab.

   The Samples page displays the same sample information as the Input files page.



Setting Values for Multiple Samples at the Same Time

On the Input Files Page

Highly multiplexed data are results obtained from processing several samples from one raw data file mixed and analyzed together in one LC/MS analysis, where isotopic and isobaric labels were used in quantification to distinguish the contribution of the individual samples. If you have highly multiplexed data, several files and samples with many study variables to set (sample type, quantification channel, study factors), or both, you can set values for study variables for multiple samples at once. You can use either the mouse or the keyboard to set these values on the Input Files page. For information on these two methods, refer to the *Proteome Discoverer User Guide*.

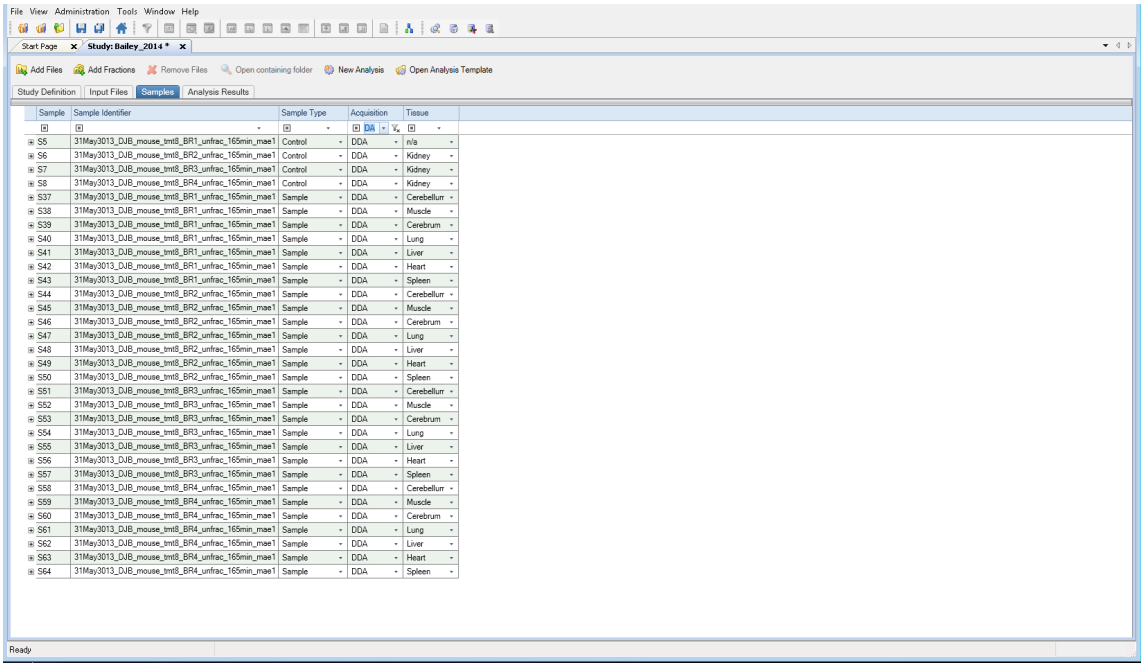
On the Samples Page

Use the following procedure to change values for multiple samples on the Samples page.

❖ **To set values for multiple samples at the same time on the Samples page**

1. Click the **Samples** tab.
2. Click the **Contains** icon, ⧈, if necessary in the Acquisition column.
3. In the box next to the icon, type the name of the filter.

For example, suppose you want to change the acquisition method for a number of samples from IDA to DDA. To display only samples that contain "DDA," type **dda** in the filter box, as shown in this figure.



4. Select the first cell to change, and then drag the cursor to select the remaining cells that you want to change.
5. Press the F2 key to enter multicell editing mode.
6. Select the new value from the list in the last cell that you selected.
7. Press the RETURN or ENTER key, or click elsewhere in the application.

To return the samples to their unfiltered state, select the **Clear All Filters** icon,  .

Saving a Study

You can save a study manually or automatically.

You can save a study manually at any time. A change in a study that requires you to save it is indicated with an asterisk (*) in the tab after the study name.

Note Studies and analyses in the Proteome Discoverer application are separate and must be saved separately. Saving a study does not save an analysis, and saving an analysis does not save a study.

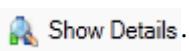
You can set an option to have the application save studies automatically when you click the Run icon, 

. This option also saves the results generated in the study. It saves the analysis containing the workflow, but when you close the study and reopen it, you must access the analysis by clicking the Analysis Results tab and then doing one of the following:

Click the **Reprocess** icon, 

, and choose **All Analysis Steps** (to open both the processing and consensus workflow) or **Last Consensus Step** (to open just the consensus workflow).

–or–

Click the **Show Details** icon, 

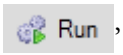
.

❖ To save a study manually

Choose **File > Save**.

The application saves the study in the *study_name*.pdStudy file in the study directory.

❖ To save a study automatically

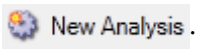
1. At some point before you click the Run icon, 

, choose **Tools > Options**.
2. In the Options dialog box, select **Study Options** in the left pane.
3. Select the **Auto Save When Starting Analysis** check box.
4. Click **OK**.

Creating an Analysis

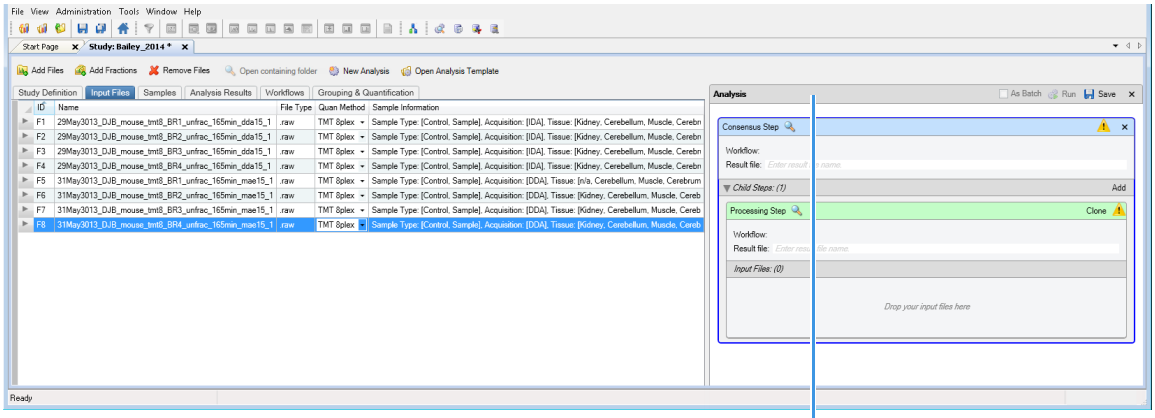
The next general step in performing a search is to create an analysis.

❖ To create an analysis

On the Study: *Study_name* page, click the **New Analysis** icon, 

.

An Analysis window opens on the right side of the Study: *Study_name* page.



Analysis window containing a Consensus Step box and a Processing Step box

An Analysis window contains the following items:

- A Consensus Step box, which represents the consensus workflow step of the data processing.
- A Processing Step box, which represents the processing workflow step of the data processing.
- A Child Steps bar, which contains an Add button to add another Processing Step box. Multiple Processing Step boxes are useful when you want to process the same data in different ways—for example, by using different nodes or different node settings.

In addition, two new tabs appear on the Study: *Study_name* page: the Workflows tab and the Grouping & Quantification tab.

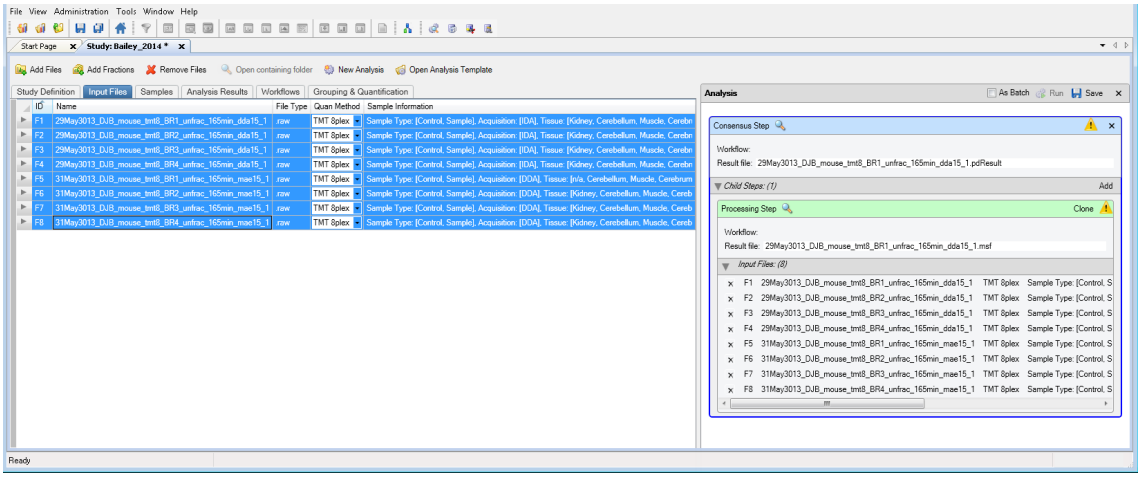
If you open an existing study that includes an Analysis window containing an existing consensus workflow, processing workflow, or both, and you want to open a new analysis, you must close the existing Analysis window and open a new Analysis window. To close the window, click **X** in the upper right corner.

Adding Input Files to an Analysis

❖ To add the input files to an analysis

Select and drag the files from the Input Files page to the Input Files box of the Processing Step box in the Analysis pane.

The input files are listed in the Input Files area of the Processing Step box (see the next figure).



Note You can remove a file from the Input Files area of the Processing Step box by clicking the X to the left of the file name.

Creating the Workflows

The next step in creating an analysis is to create the workflows to use for the processing and consensus steps. This section gives a brief overview of the steps required to create processing and consensus workflows. For detailed information on creating workflows, refer to the *Proteome Discoverer User Guide*.

❖ To create the processing workflow

1. Click the **Workflows** tab.

   Note The Workflows tab does not appear until you add or open an analysis.

   The Workflow Editor opens.

2. Click the **Show Workflow** icon,  , in the title bar of the Processing Step box to indicate that you want to create a processing workflow.

   The Workflow Nodes pane lists the nodes available for use in the processing workflow. You might need to click the Workflow Nodes tab to see this pane.

3. Create the appropriate processing workflow in the Workflow Tree pane of the Workflow Editor. To follow the example given in this guide, drag the following nodes to the Workflow Tree pane:

   - Spectrum Files node
   - Event Detector node
   - Spectrum Selector node
   - Sequest HT node
   - Percolator node
   - Precursor Ions Area Detector node

- Reporter Ions Quantifier node

4. Connect the nodes together, as needed.

   In this example, the only connections that you must make are from the Spectrum Selector node to the Sequest HT node to the Percolator node.

5. Set the appropriate parameters for each node as follows:

   a. Click the node.

   b. (Optional) Click **Show Advanced Parameters** in the Parameters pane to the left to display all parameters.

   c. Set the appropriate parameters.

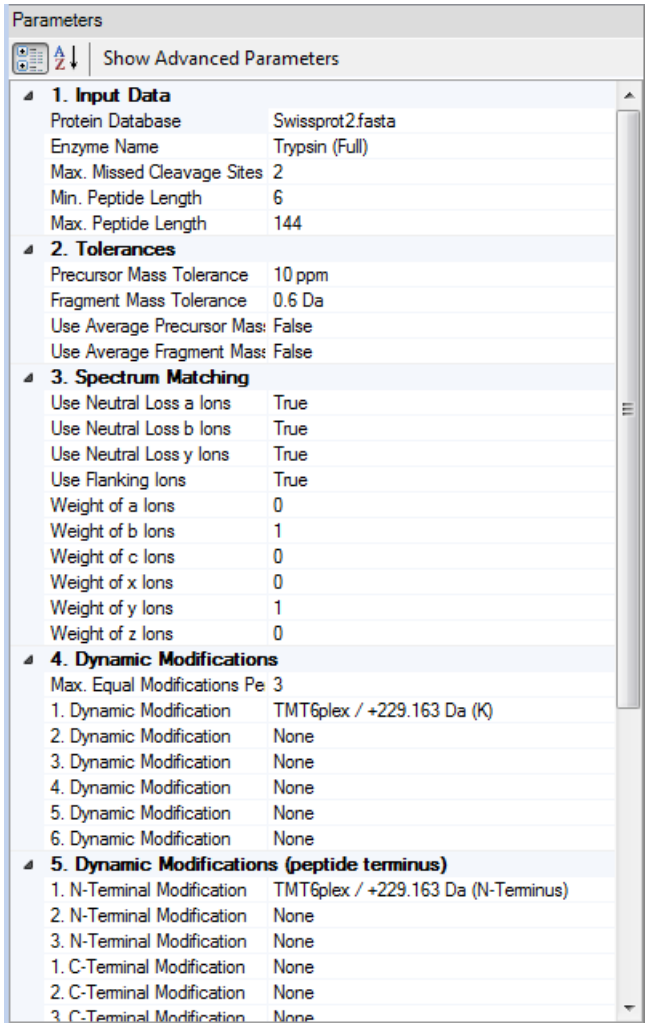
6. For this example, set the parameters of the Sequest HT node as follows:

   - Set the Protein Database parameter to an appropriate database, for example, **SwissProt**.

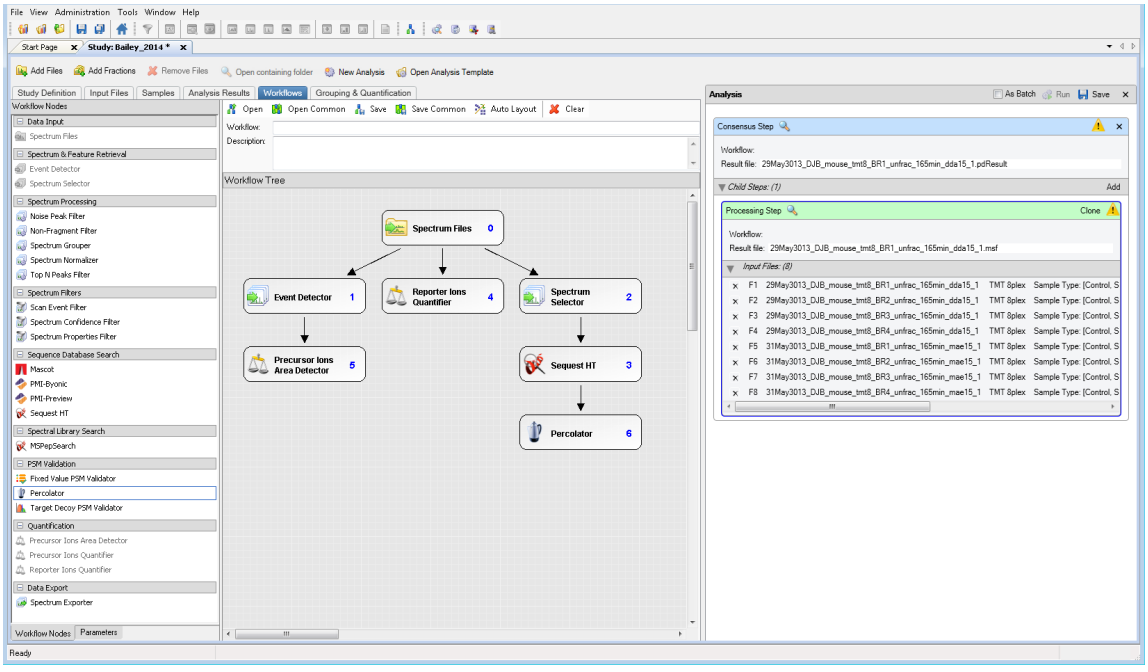
     Note You must download this database before conducting a search with Sequest HT. For instructions, see "Downloading the FASTA Files" on page 1.

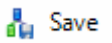
   - Set the N-Terminal Modification parameter under Dynamic Modifications (Peptide Terminus), for example, **TMT 6plex/+229.163 Da**.
   - Set a Dynamic Modification parameter, for example, **TMT6plex /+229.163 Da (K)**.
   - Set a Static Modification parameter, for example, **Carbamidomethyl/+57.021 Da (C)**.

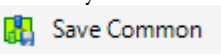
   This figure shows the parameter settings.



This figure shows a complete example of the processing workflow.



7. (Optional) Save the processing workflow:
   a. In the Workflow box above the Workflow Tree pane, type a name for the processing workflow.
   b. (Optional) In the Description box, type a brief description of the processing workflow.
   c. In the Workflow Editor, click the **Save** icon, 

   .
   d. In the Save Workflow dialog box, do the following:
      i. Select the file to save the workflow in, or type a file name in the File Name box. You can save the workflow in the study folder or in the Common Templates folder (select the **Save Common** icon, 

, in this case), or in a separate folder of workflows.
      ii. Click **Save**.

The application saves the workflow in the *file_name*.pdProcessingWF file.

Note A yellow triangle containing an exclamation mark in the upper right corner of the Processing Step box () indicates that the workflow is not set up correctly or the node parameters are not set correctly. Hold the mouse pointer over the triangle to display further details about what is missing.

❖ To create the consensus workflow

1. Click the **Show Workflow** icon,  , in the title bar of the Consensus Step box.

   The Workflow Nodes pane lists the nodes available for use in the consensus workflow. You might need to click the Workflow Nodes tab to see this pane.

   Create the appropriate consensus workflow in the Workflow Tree pane by dragging the following nodes to the Workflow Tree pane:

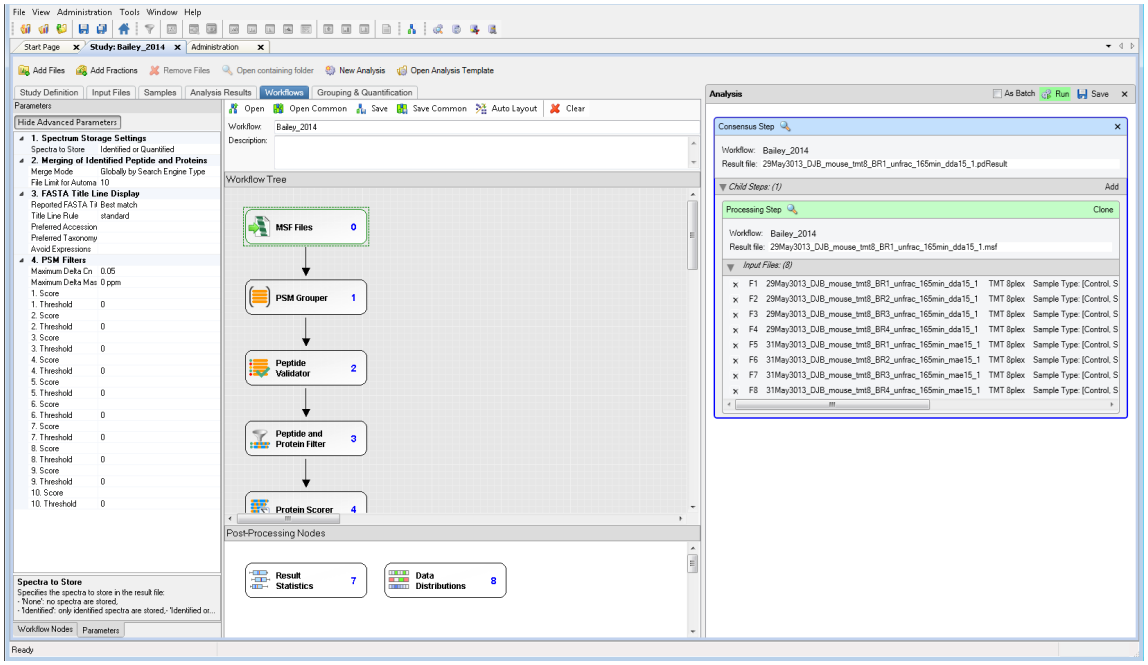
   - MSF Files node
   - PSM Grouper node
   - Peptide Validator node
   - Peptide and Protein Filter node
   - Protein Scorer node
   - Protein Grouping node
   - Peptide and Protein Quantifier node

   The example workflow includes the following nodes in the Post-Processing Nodes area:

   - Result Statistics node
   - Data Distributions node

2. Set the appropriate parameters for each node.

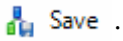
   This figure shows an example of the consensus workflow.



3. (Optional) Save the consensus workflow:

   a. In the Workflow box, type a name for the consensus workflow.

   b. (Optional) In the Description box, type a brief description of the consensus workflow.

   c. In the Workflow Editor, click the **Save** icon, 

.

   d. In the Save Workflow dialog box, do the following:

      i. Browse to the study folder, and select the file to save the workflow in, or type a file name in the File Name box.

      ii. In the Save As Type box, select **Consensus Workflow File (*.pdConsensusWF)**.

      iii. Click **Save**.

The application saves the workflow in a *file_name*.pdConsensusWF file.

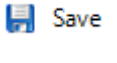
> **Note** A yellow triangle containing an exclamation mark in the upper right corner of the Consensus Step box () indicates that the workflow is not set up correctly or the node parameters are not set correctly. Hold the mouse pointer over the triangle to display details about what is missing.

Saving an Analysis

To use an analysis as a template for later reuse, you can save it as a .pdAnalysis template file.

> **Note** Studies and analyses in the Proteome Discoverer application are separate, so you must save them separately. Saving a study does not save an analysis, and saving an analysis does not save a study.

❖ To save an analysis as a template for later reuse

1. In the upper right corner of the Analysis window, click the **Save** icon, 

.

2. In the Save Analysis Template dialog box, browse to the location where you want to store the template.

3. In the File Name box, browse to the study folder, and type or select the template file name.

4. In the Save as Type box, select **Analysis Templates (*pdAnalysis)**.

5. Click **Save**.

   The application saves the analysis in a file with a .pdAnalysis extension.

   The .pdAnalysis template file saves the processing and consensus workflows. It saves neither the input files nor the study variables that were selected to group the samples and quantification ratios.

Grouping Samples and Ratios

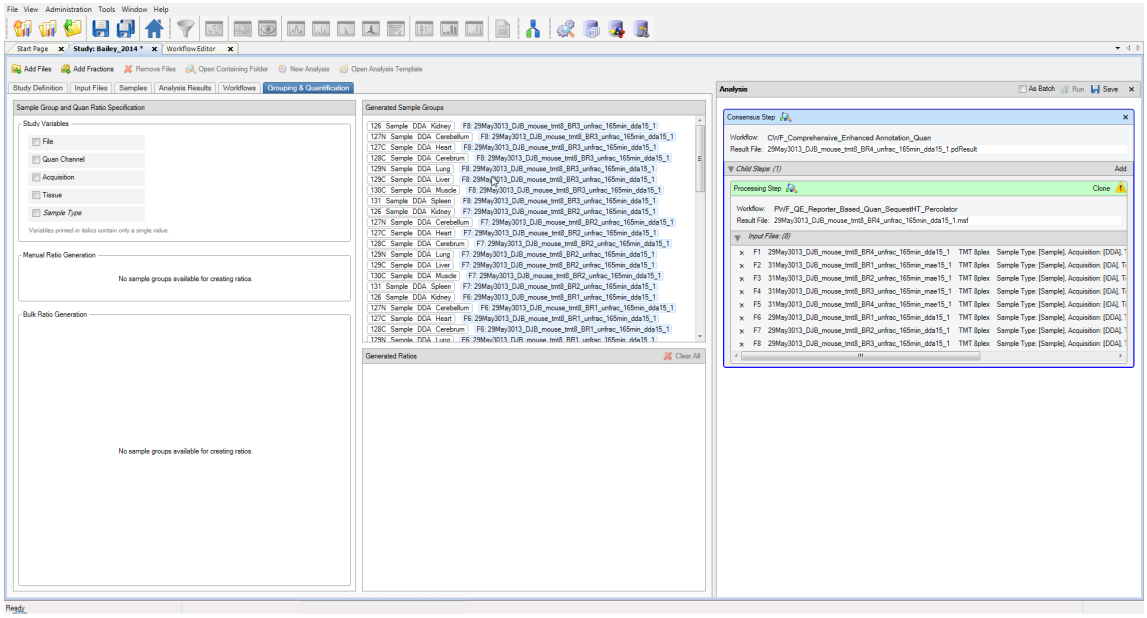
After you set up the workflow to use for the analysis, you can specify what ratios to report for the quantification and how to group your samples with respect to the specified factor values.

❖ To open the Grouping & Quantification page

1. On the Study: *Study_name* page, click the **Grouping & Quantification** tab.

   Note The Grouping & Quantification tab does not appear until you add input files that are associated with a single quantification method.

   The Grouping & Quantification page opens.



   For a description of the areas on this page, refer to the *Proteome Discoverer User Guide*.

❖ To generate custom quantification ratios semiautomatically

1. In the Study Variables area, select the check box of the study factors, or variables, that you want to use to group your samples and from which you want to draw the numerators and denominators of the ratios. For the example used throughout this document, select the **Tissue** check box to indicate that the samples and quantification ratios are grouped by the values set for the tissue factor.

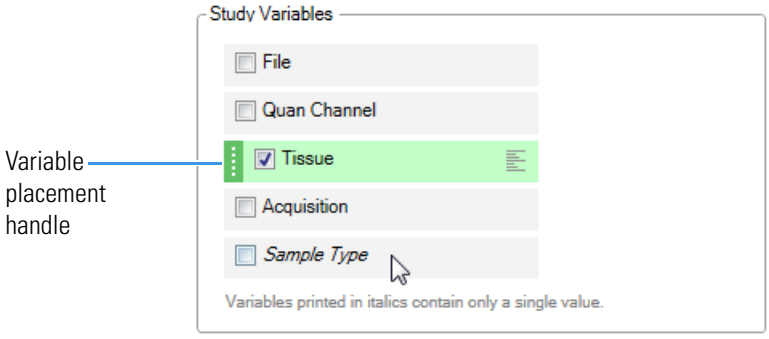
   After you select the study factors, the Generated Sample Groups area displays the generated sample groups. When performing the quantification, the application calculates abundance values for each sample and averages the abundance values of all samples in a sample group.

   The order of study factors is relevant for the semiautomatic generation of ratios.

2. (Optional) To change the placement of a study factor in the list, do the following:

   a. In the Study Variables area, select the check box for a study factor.

      A placement handle in the form of a green rectangle appears to the left of the selected check box.



b. Hold the cursor over the placement handle.

White up and down arrows now appear on the handle.

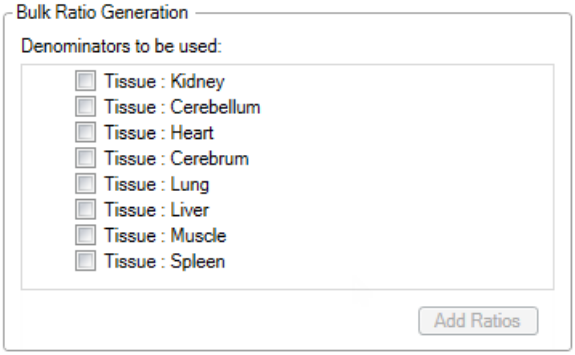
c. Drag the cursor up or down to move the variable to its new place in the list of variables, or click the up or down arrows to move the study factor.

3. (Optional) To sort the order of the study factors in the Bulk Ratio Generation area and the order of the sample groups in the Generated Sample Groups area, click one of the following to the right of each study factor in the Study Variables area.

- For descending order, click the **Sort Descending** icon, .
- For ascending order, click the **Sort Ascending** icon, .
- To leave these items unsorted, click the **No Sorting** icon, .

4. In the Bulk Ratio Generation area, select the check box for the type of tissue to use in the denominator of the ratio. For the example, select **Tissue: Kidney**.

The Bulk Ratio Generation area displays a list of the denominator values for this type of study factor. If you select only one study factor, it displays a list of the available denominator values for this factor, as shown in this figure.



If you select multiple study factors, the Bulk Ratio Generation area displays the denominator values available for each factor.

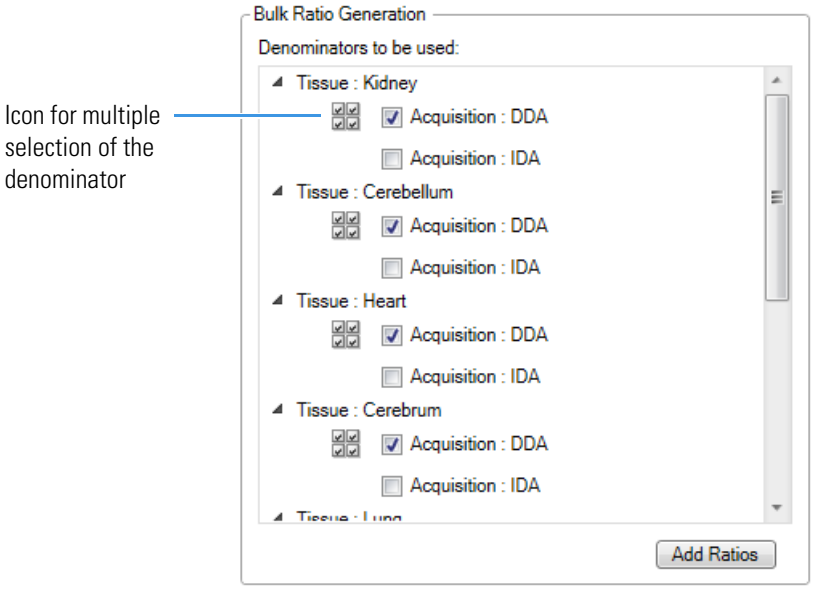
5. (Optional) To select the same study factor for all the denominators, do the following:

a. Hold the cursor over a denominator value.

An icon containing four check boxes in a square appears on the left side of that item, as shown in the next figure.

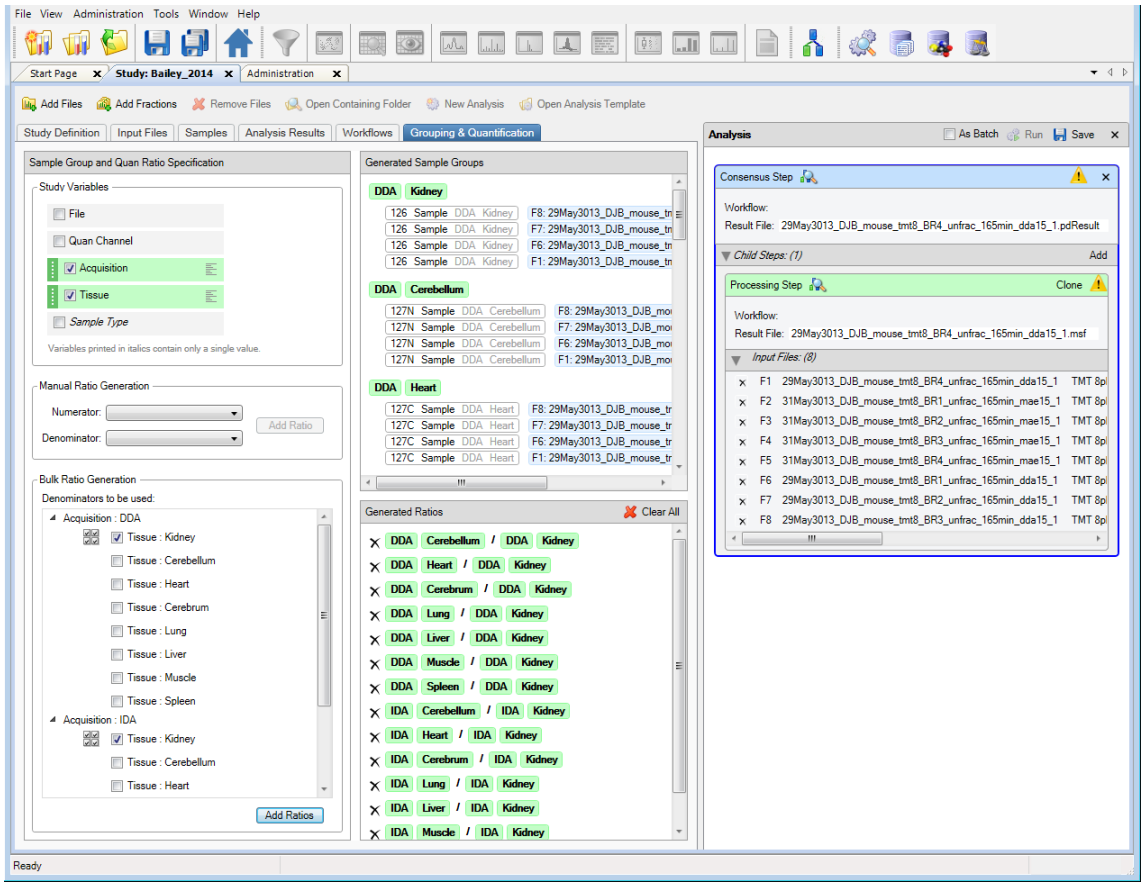
b. Click the icon.

The application selects the same study factor for all denominators (see the next figure).



6. Click **Add Ratios**.

The application generates all possible ratios against the selected denominator values and adds them to the Generated Ratios area. The next figure shows the generated quantification ratios and ratio groups in the Generate Ratios area after selecting Tissue as the study variable to group by and Kidney as the denominator to use.



7. (Optional) Select another variable or variables. In the example, select the **Acquisition** check box in the Study Variables area.

   When you add a second variable, the information in the Generated Ratios area becomes invalid and appears in a gold color.

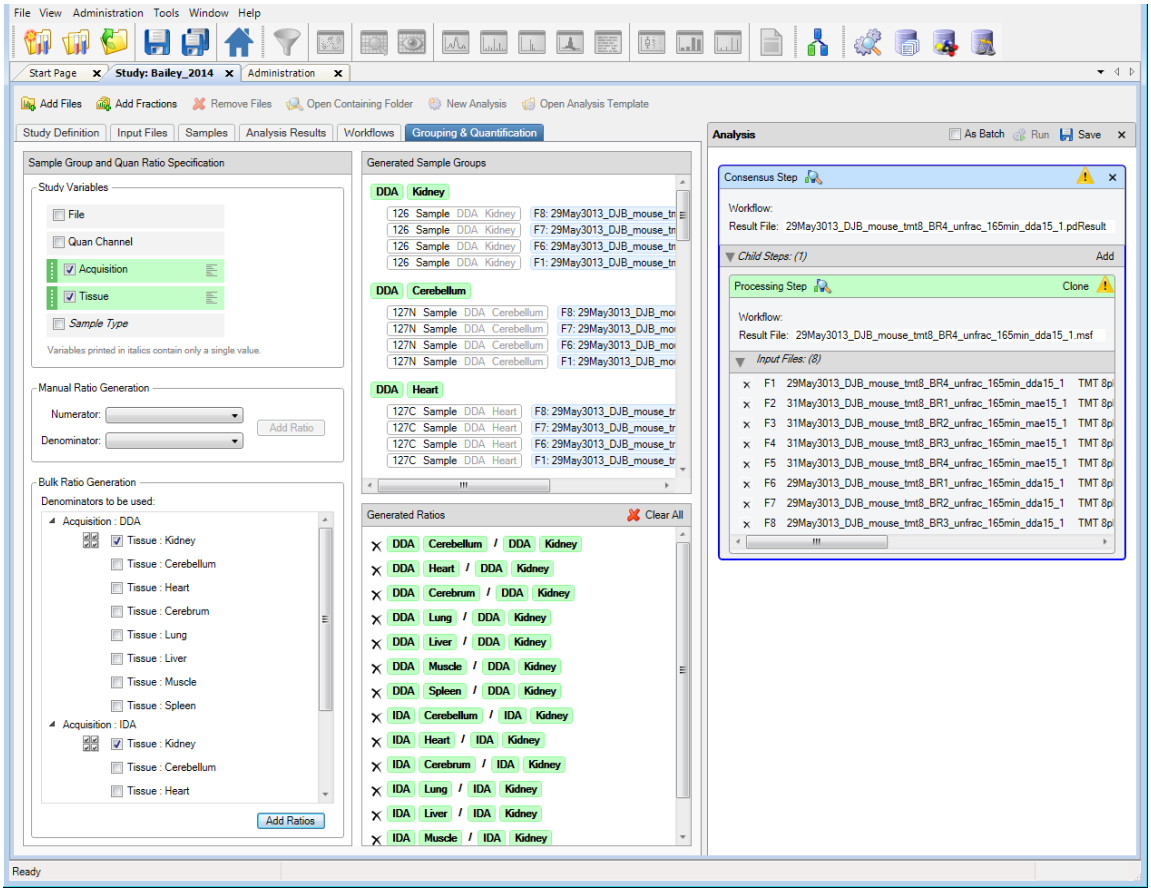
8. In the Generate Ratios area, click the **Clear All** icon, 

, to delete the previous ratios.

9. In the Bulk Ratio Generation area, select the check box for the type of tissue to use in the denominator. For the example, select **Tissue: Kidney**.

10. Click **Add Ratios**.

    The next figure shows the generated quantification ratios and ratio groups on the Generated Ratio Groups page after selecting Acquisition and Tissue as the study variables to group by.



❖ To generate custom quantification ratios manually

1. On the Study: *Study_name* page, click the **Grouping & Quantification** tab.

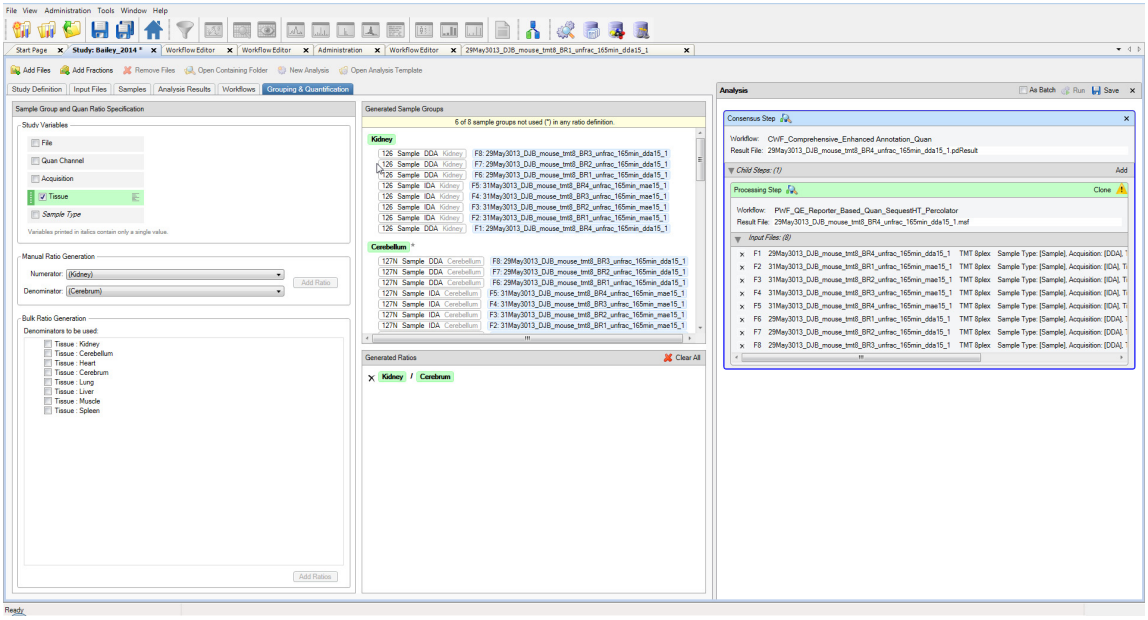
   Note The Grouping & Quantification tab does not appear until you add or open an analysis.

2. In the Study Variables area, select the check box of the study factor or factors that you want to use to group your samples and from which you want to draw the numerators and denominators of the ratios. For the example, select the **Tissue** check box to indicate that the samples and quantification ratios are grouped by the values set for the tissue factor.

3. In the Manual Ratio Generation area, select the numerator value from the Numerator list.

4. Select the denominator value from the Denominator list.
5. Click **Add Ratio**.

   The generated ratio appears in the Generated Ratios area, as shown in this figure.

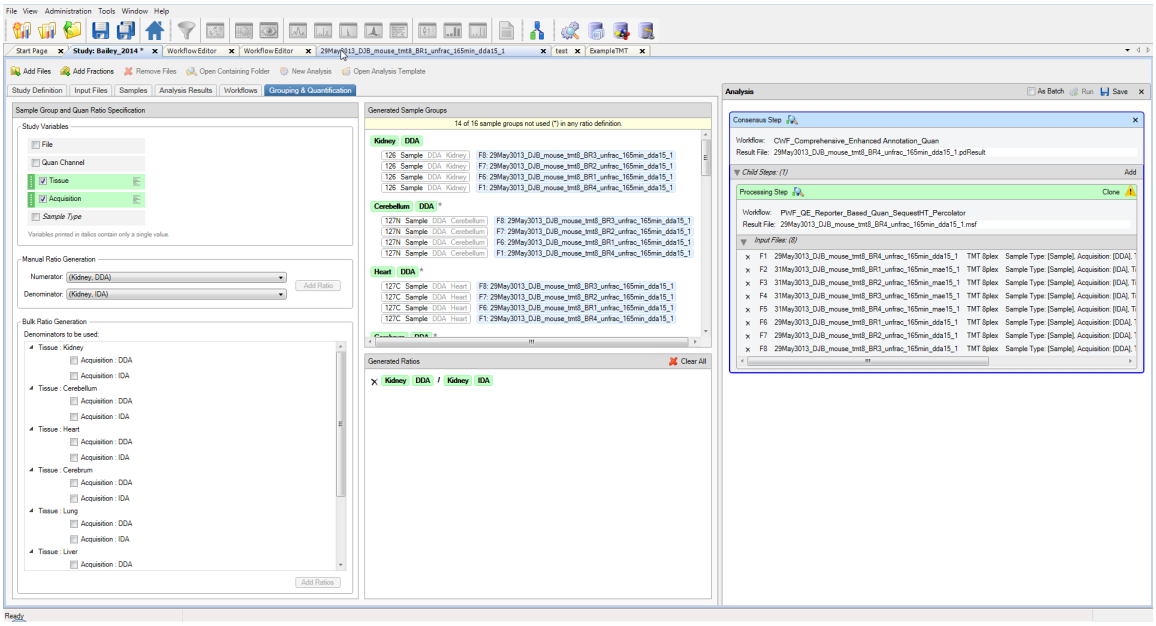


6. (Optional) Select another study factor or factors in the Study Variables area, for example, **Acquisition**.

   When you add a second factor, the information in the Generated Ratios area becomes invalid and appears in a gold color.

7. In the Generated Ratios area, click the **Clear All** icon,  , to delete the previous ratios.
8. In the Manual Ratio Generation area, select the numerator value from the Numerator list, for example, **(Kidney, DDA)**.
9. Select the denominator value from the Denominator list, for example, **(Kidney, IDA)**.
10. Click **Add Ratio**.

    The next figure shows the ratios generated in the Generated Ratios area.



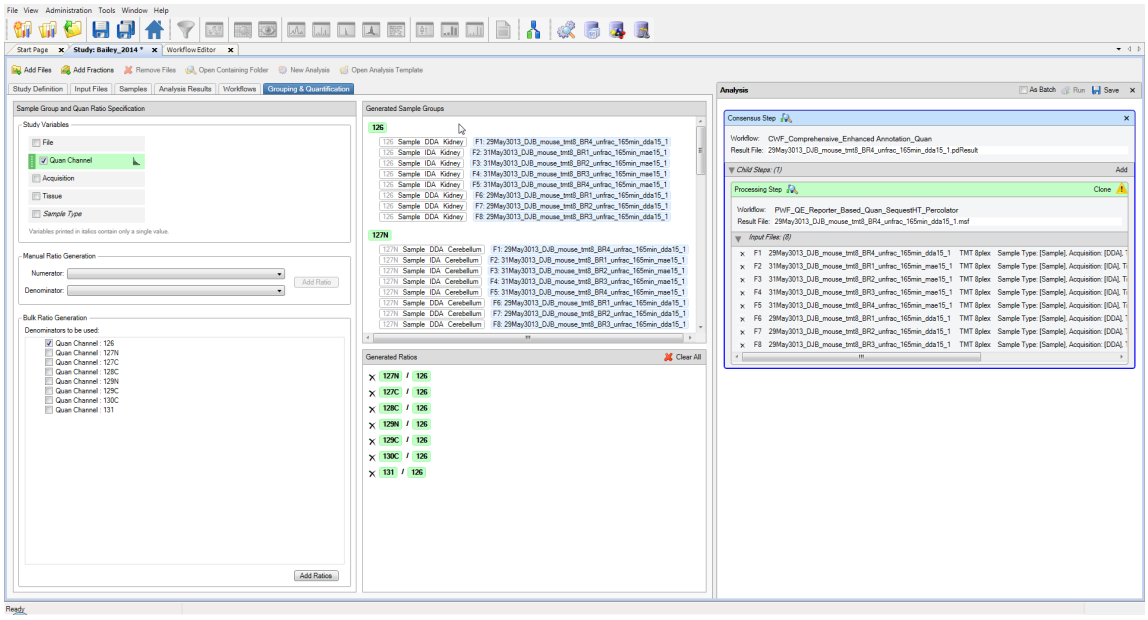
❖ To generate custom quantification ratios based on channels

1. In the Study Variables area, select the **Quan Channel** check box.
2. To generate quantification ratios semiautomatically, follow the procedure in "To generate custom quantification ratios semiautomatically" on page 23.

   –or–

   To generate quantification ratios manually, follow the procedure in "To generate custom quantification ratios manually" on page 26.

   This figure shows the ratios generated in the Generated Ratios area.



❖ To save the settings on the Grouping & Quantification page

> **Note** The application does not save the settings on the Grouping & Quantification page with a study or with an analysis. Instead, it associates the settings with search results, so you must load them from data sets that have already been processed within the study or recreate them from the beginning.

1. Click the **Analysis Results** tab of the study.
2. Select the result on the Analysis Results page, and choose either **Reprocess > All Analysis Steps** or **Reprocess > Last Consensus Step**.

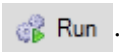
   If you select Use Results to Make New (Multi) Consensus, the Grouping and Quantification page no longer displays ratios.

Performing the Search

You can perform a search in individual mode or batch mode.

Performing a Search in Individual Mode

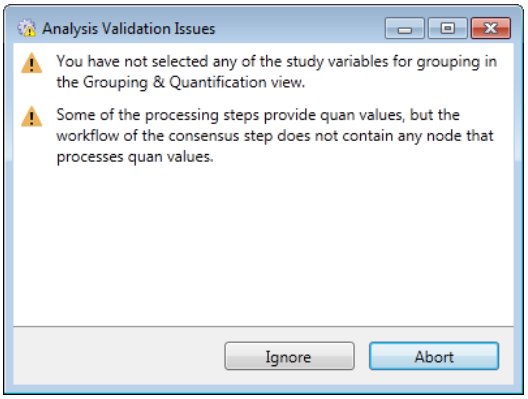
❖ To perform a search in individual mode

In the upper right corner of the Analysis window, click the **Run** icon, 

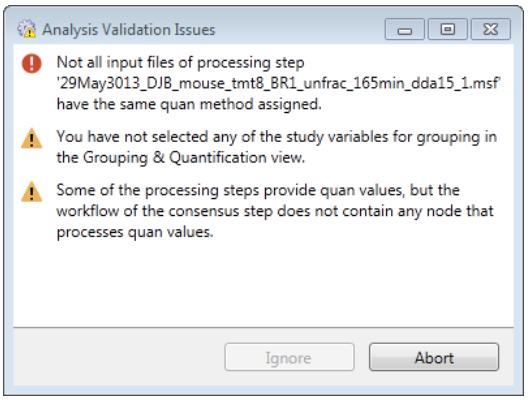
.

The application validates the analysis setup before it starts processing and issues error or warning messages in the Analysis Validation Issues box (see the next figure), if it finds errors. For example, it might issue an error message if not all the input files have the same quantification method. Or, it might issue a warning message if you added several input files with quantification but did not set any of the study variables to group your samples and quantification ratios.

You can ignore warnings, which are marked by an exclamation mark inside a yellow triangle. Because warnings are only hints that the analysis might not be set up correctly, you can click Ignore in the Analysis Validation Issues box. This figure shows an example of the warnings.

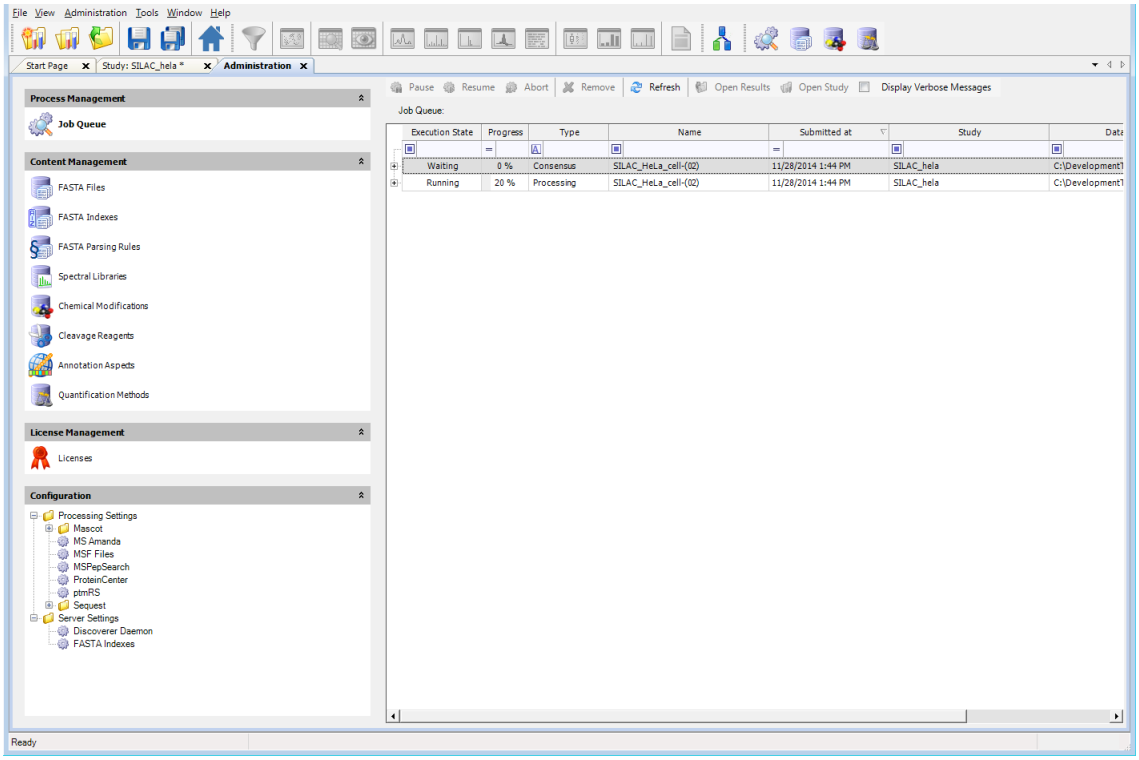


You cannot ignore validation errors, which are marked with a red exclamation mark (see the next figure). You must resolve them.



The validation cannot detect every potential problem but can check for the specific problems or inconsistencies listed in the *Proteome Discoverer User Guide*.

Once the application validates the analysis, it begins processing it. The job queue opens so that you can monitor the progress of the job (see the next figure).



The job is done when "Completed" appears in the Execution State column for the processing and consensus workflows.

The application uses the name of the first raw data file or sample as the default name of the results file as a whole.

Performing a Search in Batch Mode

You might want to process each file in a set of files with the same processing workflow and the same consensus workflow. Processing a set of files in this way is called batch mode. Batch mode is only available if there is more than one input file and if the analysis has just one processing step—that is, if there is just one Processing box in the Analysis window. For information on performing a search in bath mode, refer to the *Proteome Discoverer User Guide*.

For information on interpreting the results of the search, refer to the *Proteome Discoverer User Guide*.

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