

Proteome Discoverer 2.2 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 Proteome Discoverer™ application, refer to either the *Proteome Discoverer User Guide* or the Help available in the Proteome Discoverer application.

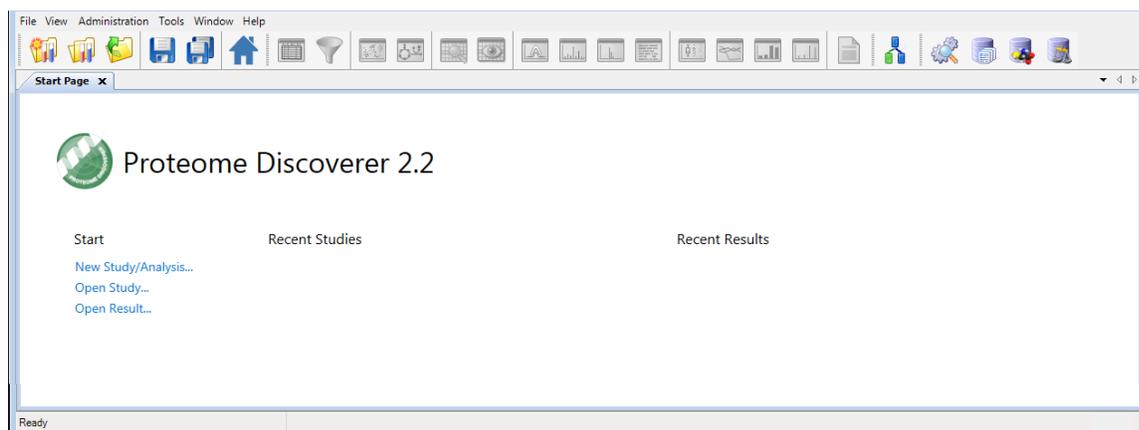
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❖ To open the Proteome Discoverer application

From the Start menu, choose **Programs > Thermo Proteome Discoverer 2.2**, or click the **Proteome Discoverer** icon, , on your desktop.

The Proteome Discoverer Start Page opens.



Configuring the Search Engine Parameters

If you use the Sequest™ HT and MascotSM search engines, you can configure certain search parameters for them before you begin your search. This guide provides an example that uses the Sequest HT search engine. For information on setting the parameters, refer to the *Proteome Discoverer User Guide*.

Configuring Temporary Files Parameters

The Proteome Discoverer application generates many temporary files until it creates the final result file, especially when experiments involve label-free and precursor ion quantification. If the input raw data files and the temporary result files generated by your experiment might be too large for the default directory on your hard drive, you can select another drive and folder for storing the temporary files.

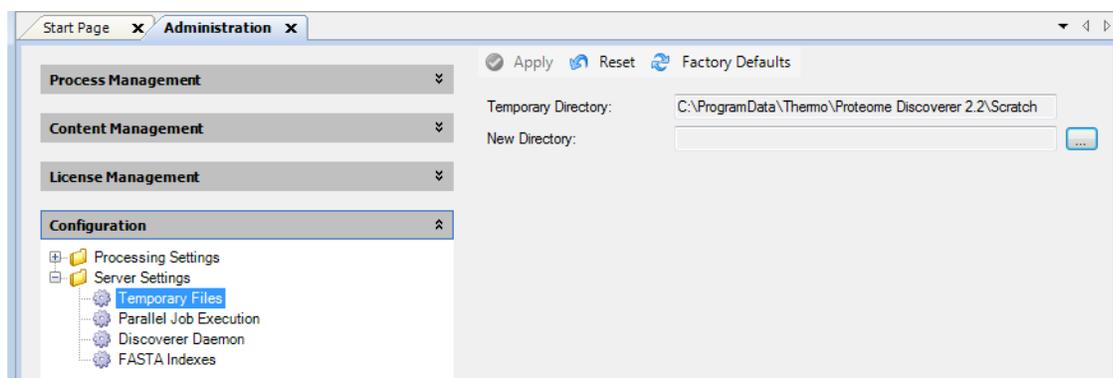
By default, the application stores temporary files in the C:\ProgramData\Thermo\Proteome Discoverer 2.2\Scratch directory.

❖ To select a temporary folder

1. In the main menu, choose **Administration > Configuration**.
2. In the left pane, under Server Settings, click **Temporary Files**.
3. In the New Directory box, type the drive, path, and name of the folder where you want to store the temporary files; or browse to the location of the folder and click **OK**.

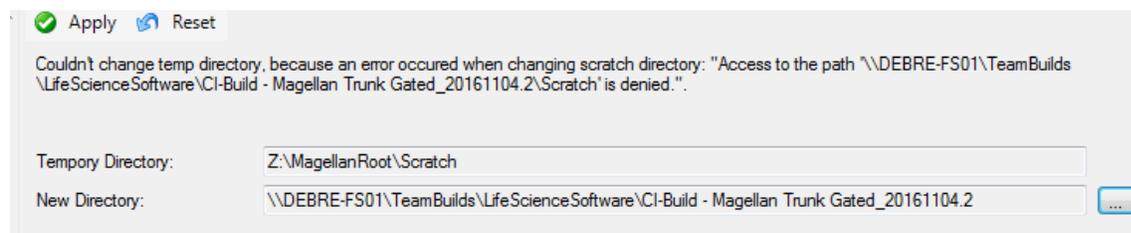
The new folder must be named Scratch. If it is not, the application creates a subfolder named Scratch.

IMPORTANT Ensure that the new temporary folder location is accessible and that you have write permission. Read/write performance must be high to prevent data processing from slowing down. Use local drives for storage, as storing on USB drives or network shares can cause slow performance.



4. Restart the Proteome Discoverer application.

If a problem occurs during the initialization of the temporary folder, the application displays an error message in the Temporary Files pane, as shown in the next figure. When you use an invalid Scratch folder as the current directory, job processing fails.



Downloading the FASTA Files

Before conducting a search with the **Sequest HT** search engine, you must add a FASTA file to the Proteome Discoverer application.

Before conducting a search with the **Mascot** search engine, you must add a FASTA file to the server that is running Mascot.

You can download FASTA files from two sources:

- Download a controlled protein database directly from the ProteinCenter™ database service and add it to a FASTA file. These controlled databases provide the latest information about proteins, peptides, and genes. They also give you the ability to select proteins of a specified taxonomy to download and use as a FASTA file.
- 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

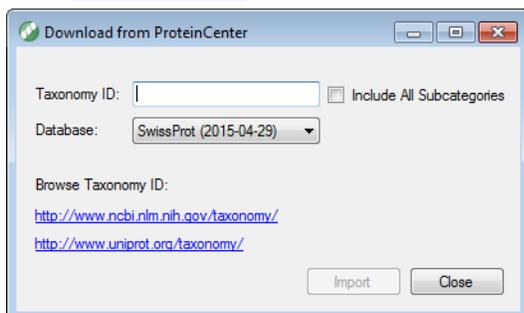
1. Configure the Proteome Discoverer application for protein annotation only if you need to use a server other than the default server. For instructions, refer to the *Proteome Discoverer User Guide*.
2. Choose **Administration > Maintain FASTA Files**, or click the **Maintain FASTA Files** icon, .

The Administration page appears with the FASTA Files view.

3. Click  **Check for Updates**.

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

4. Click **FASTA Files** under Content Management in the Configuration view to return to the FASTA Files view. The Download icon on the FASTA Files view becomes available.
5. Click  **Download** to open the Download from ProteinCenter dialog box.



6. 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.

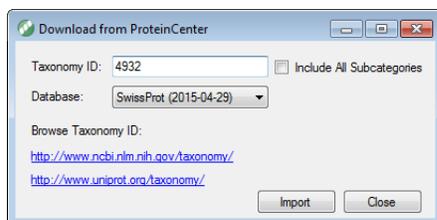
Taxonomy identifier number

Taxonomy - <i>Saccharomyces cerevisiae</i> (Baker's yeast) (SPECIES)	
Taxonomy navigation	> <i>Saccharomyces</i>
Map to	Choose one All lower taxonomy nodes (318)
	UniProtKB (589,444)
	Format
Mnemonic	YEASX
Taxon identifier	4932
Scientific name	<i>Saccharomyces cerevisiae</i>
Common name	Baker's yeast
Synonym	-
Other names	<ul style="list-style-type: none"> > ATCC 18824 > CBS 1171 > Candida robusta > NRRL Y-12632 > <i>Saccharomyces cerevisiae</i> More >
Rank	SPECIES
Lineage	<ul style="list-style-type: none"> > cellular organisms > Eukaryota > Opisthokonta > Fungi > Dikarya > Ascomycota > saccharomyceta > <i>Saccharomycotina</i> > <i>Saccharomycetes</i> > <i>Saccharomycetales</i> > <i>Saccharomycetaceae</i> > <i>Saccharomyces</i>
Strains	> 07173

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

The default is SwissProt.

This figure shows the completed Download from ProteinCenter dialog box.



d. Click **Import**.

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

- 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.

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

In this figure, the *Saccharomyces cerevisiae* (4932) species database has been downloaded from the SwissProt database.

Name	Protein Database	Taxonomy ID	Version	File Size [kB]	# Sequences	# Residues	Status	Last Modified	Update Available
ipi.HUMAN.v3.87.f...	Custom			49876	91464	36355611	Availa... 09/27/2011		<input type="checkbox"/>
PAQC_Database.f...	Custom			14158	13611	10493882	Availa... 02/06/2014		<input type="checkbox"/>
Canis lupus famili...	SwissProt	9615	2014-07-09	922	857	381701	Availa... 11/10/2014		<input checked="" type="checkbox"/>

Database downloaded from ProteinCenter

❖ To download a FASTA file from sources other than ProteinCenter

- Choose **Administration > Maintain FASTA Files**, or click the **Maintain FASTA Files** icon, .
The Administration page appears with the FASTA Files view.
- Click **Add**.
- In the Open dialog box, 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 **Abort**.
When you see “Completed” in the Execution State column, the database has finished downloading.
- 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**.

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.

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

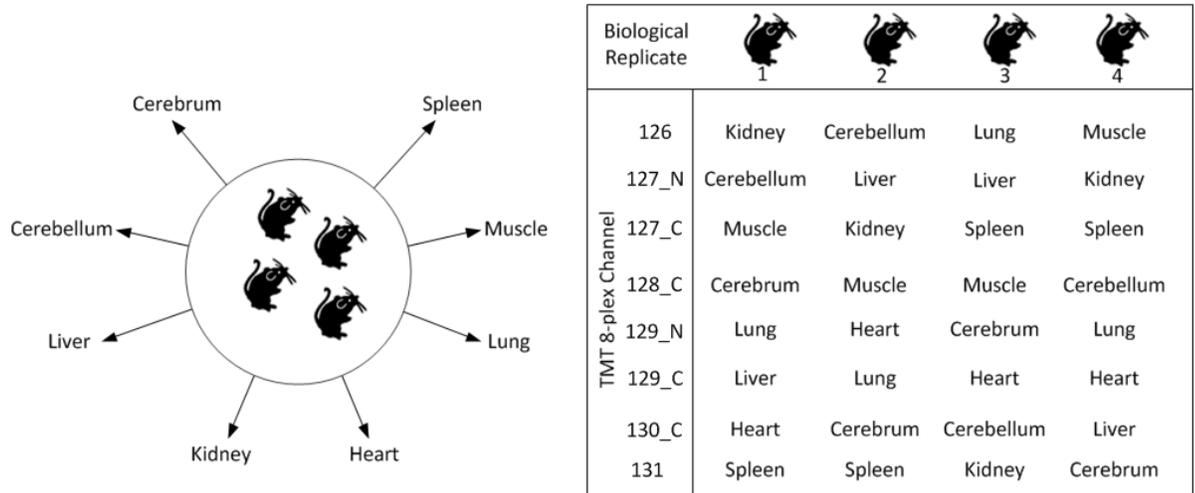
Four mice (replicates) were sacrificed and dissected. Individual organs of interest from them were homogenized, and the proteins were extracted from them and labeled. Then the differentially labeled organ-specific proteomes were mixed together if they came from the same mouse. Each pooled mouse sample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

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

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

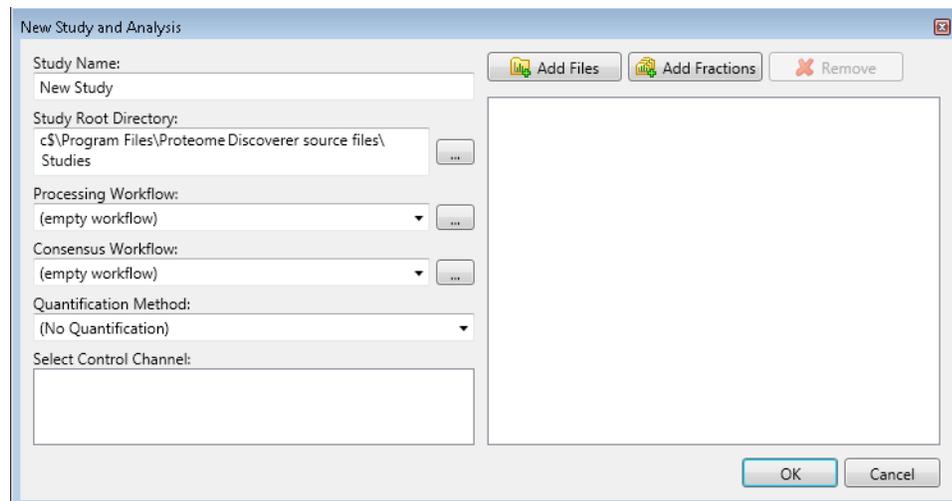
The following figure shows how different tissue samples are distributed over four biological mouse replicates.



❖ To create a study

1. On the Start Page, click **New Study/Analysis**, 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 specify only the name of the study and a root directory where you save the study. Optionally, you can specify a processing workflow, a consensus workflow, and a quantification method.

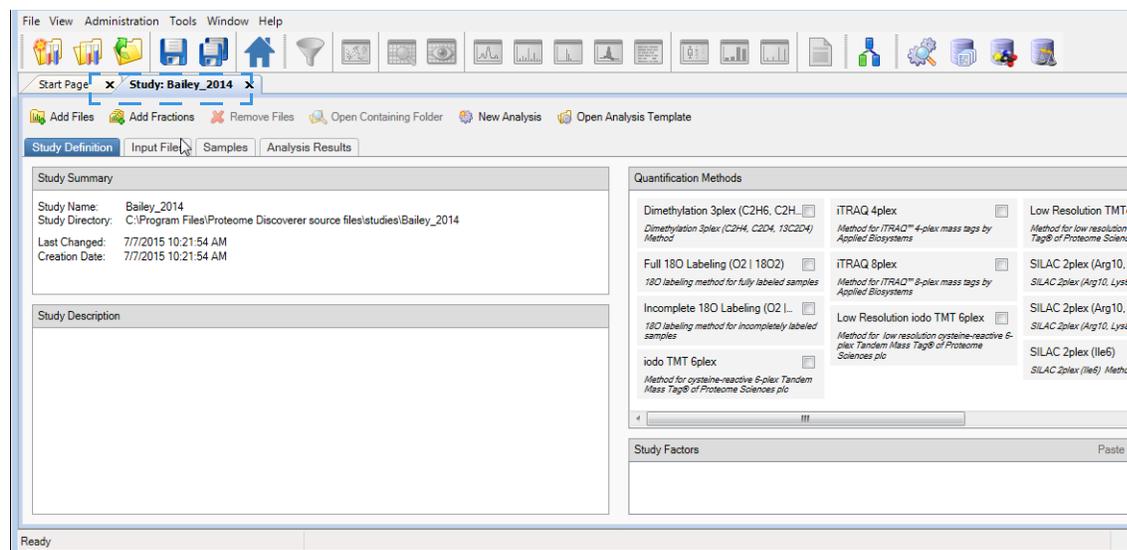
2. In the Study Name box, specify the mandatory study name.

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 part for the name of the new study.

3. In the Study Root Directory box, specify the folder where you will store the study folder:
 - a. Click the Browse button (...) to open the Select Folder dialog box.
 - b. 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). It appends .pdStudy 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.

See these topics:

- [Adding a Description](#)
- [Adding a Quantification Method to the Study](#)
- [Adding the Study Factors](#)
- [Adding the Input Files](#)
- [Specifying the Quantification Method for Multiple Input Files](#)
- [Setting the Factor Values for the Samples](#)
- [Setting Values for Multiple Samples at the Same Time](#)
- [Saving a Study](#)

(Optional) Add a description of the study by typing it in the Study Description area of the Study Definition page.

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, .

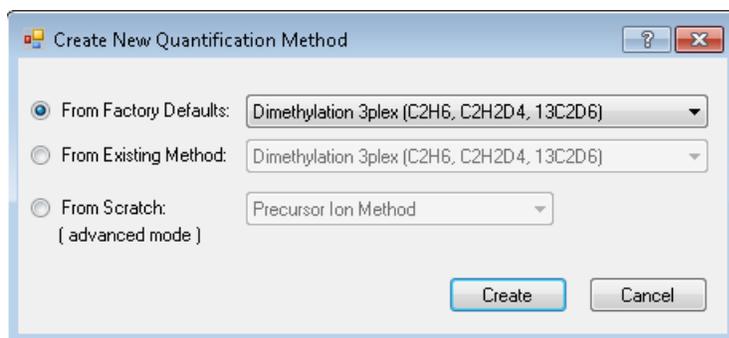
The Quantification Methods view opens. It lists all of the available methods for both precursor ion and reporter ion quantification.

2. Click  **Add**.

Adding a Description

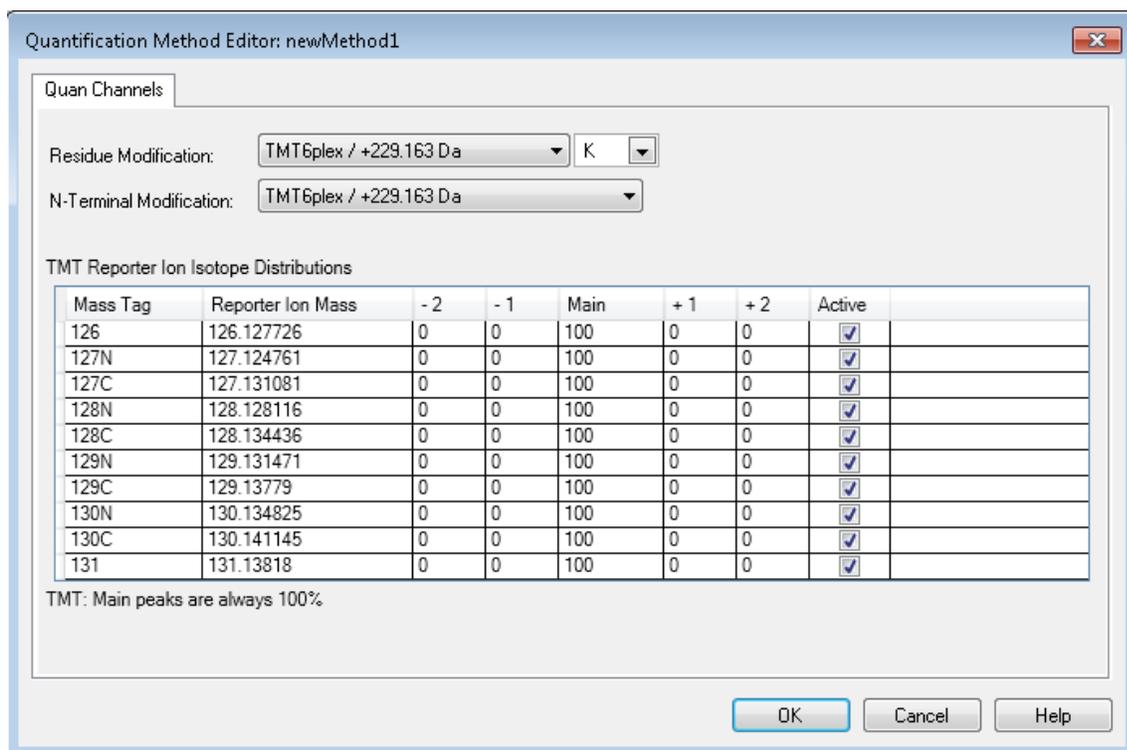
Adding a Quantification Method to the Study

The Create New Quantification Method dialog box opens.



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.

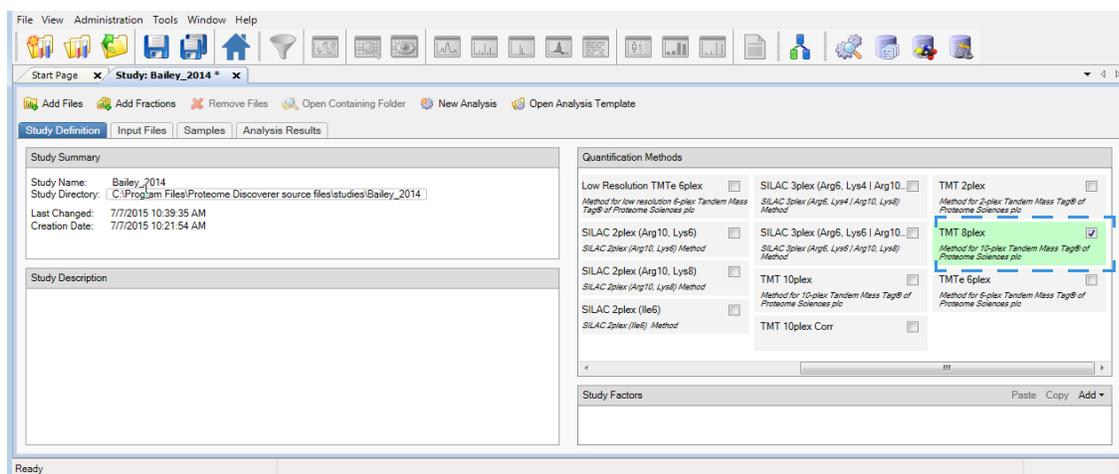
Status	Method Name	Description	Is Active
✓	Dimethylation 3plex (C2H6, C2H2D4, 13C2D6)	Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method	✓
✓	Full 18O Labeling (O2 18O2)	18O labeling method for Fully labeled samples	✓
✓	Incomplete 18O Labeling (O2 O18O + 18O2)	18O labeling method for incompletely labeled samples	✓
✓	Iodo TMT 6plex	Method for cysteine-reactive 6-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	iTRAQ 4plex	Method for iTRAQ™ 4-plex mass tags by Applied Biosystems	✓
✓	iTRAQ 4plex (Thermo Scientific Instruments)	Method for iTRAQ™ 4-plex mass tags by Applied Biosystems optimized for Ther...	✓
✓	iTRAQ 8plex	Method for iTRAQ™ 8-plex mass tags by Applied Biosystems	✓
✓	iTRAQ 8plex (Thermo Scientific Instruments)	Method for iTRAQ™ 8-plex mass tags by Applied Biosystems optimized for Ther...	✓
✓	SILAC 2plex (Arg10, Lys6)	SILAC 2plex (Arg10, Lys6) Method	✓
✓	SILAC 2plex (Arg10, Lys8)	SILAC 2plex (Arg10, Lys8) Method	✓
✓	SILAC 2plex (Ile6)	SILAC 2plex (Ile6) Method	✓
✓	SILAC 3plex (Arg6, Lys4 Arg10, Lys8)	SILAC 3plex (Arg6, Lys4 Arg10, Lys8) Method	✓
✓	SILAC 3plex (Arg6, Lys6 Arg10, Lys8)	SILAC 3plex (Arg6, Lys6 Arg10, Lys8) Method	✓
✓	TMT 10plex	Method for 10-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	TMT 2plex	Method for 2-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	TMT 8plex	Method for 8-plex Tandem Mass Tag® of Proteome Sciences plc	✓
✓	TMT 6plex	Method for 6-plex Tandem Mass Tag® of Proteome Sciences plc	✓

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, as shown.



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*.

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

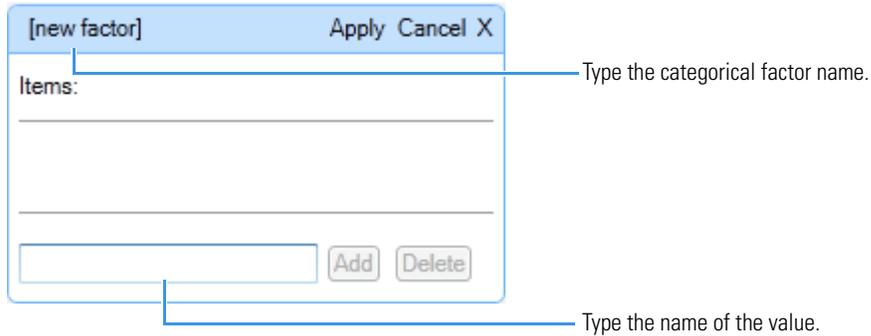
A factor is a single biological or technical parameter that you control, for example, genotype, diet, environmental stimulus, age, column length, spray voltage, or collision energy.

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.

Adding the Study Factors

❖ **To add categorical study factors**

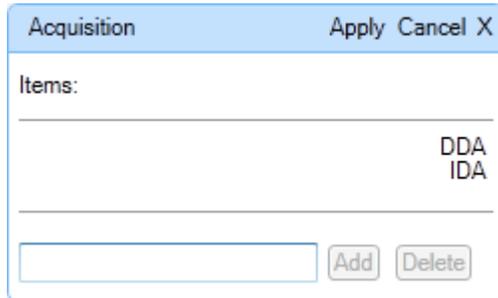
1. Add the first categorical (non-numeric) factor (Acquisition in the example), as follows:
 - a. In the Study Factors area of the Study Definition page, choose **Add > Categorical Factor**.
The categorical factor dialog box appears, and [new factor] is highlighted.



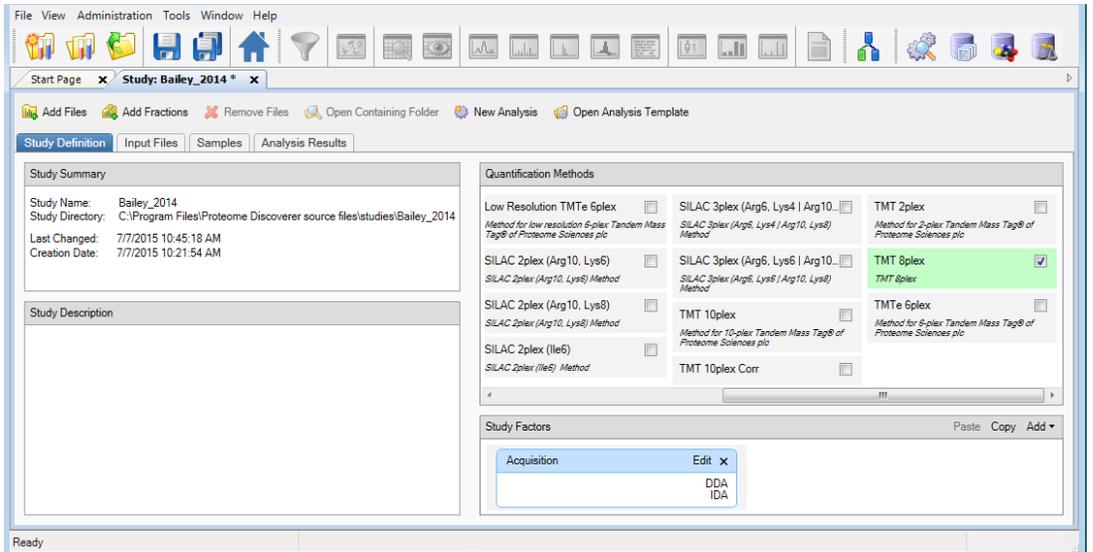
- b. Type a name over [new factor] for the new categorical factor, for example, **Acquisition**. (See the next figure.)

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 button, 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**.
The completed categorical factor dialog box follows. In this example, the second acquisition method is data-dependent acquisition (**DDA**).

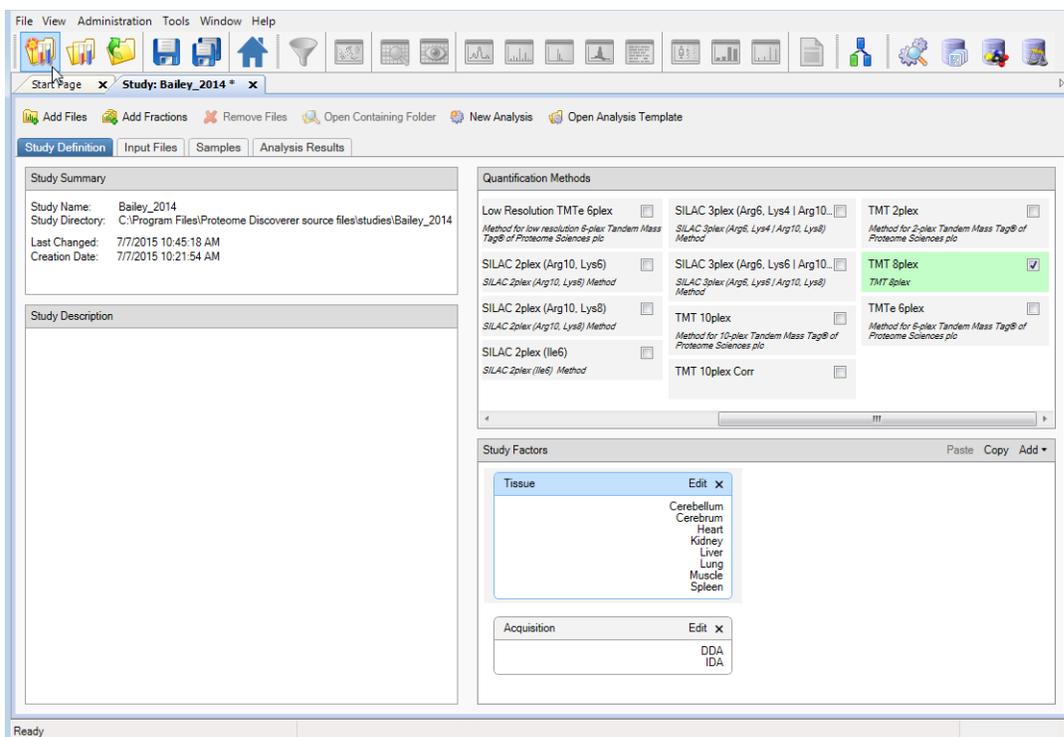


- e. Click **Apply** in the categorical factor dialog box.
Confirm that the Study Definition page resembles this figure.



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 categorical factor dialog box appears, and [new factor] is highlighted.
 - b. Type a new name over [new factor], for example, **Tissue**.
 - c. In the box to the left of the Add button, 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 this 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, also refer to the *Proteome Discoverer User Guide*.

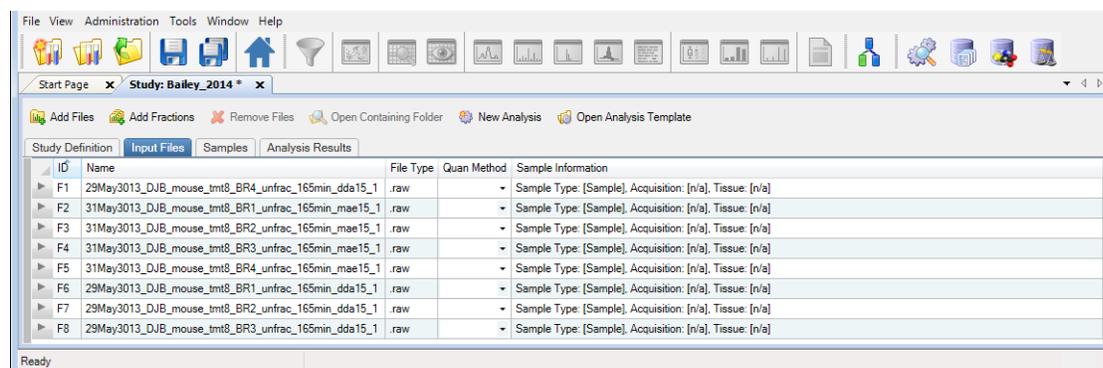
❖ To add a single input file or multiple unrelated input files

Drag the input file or files from Windows Explorer to the Input Files page.

–or–

1. Click  **Add Files**.
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.

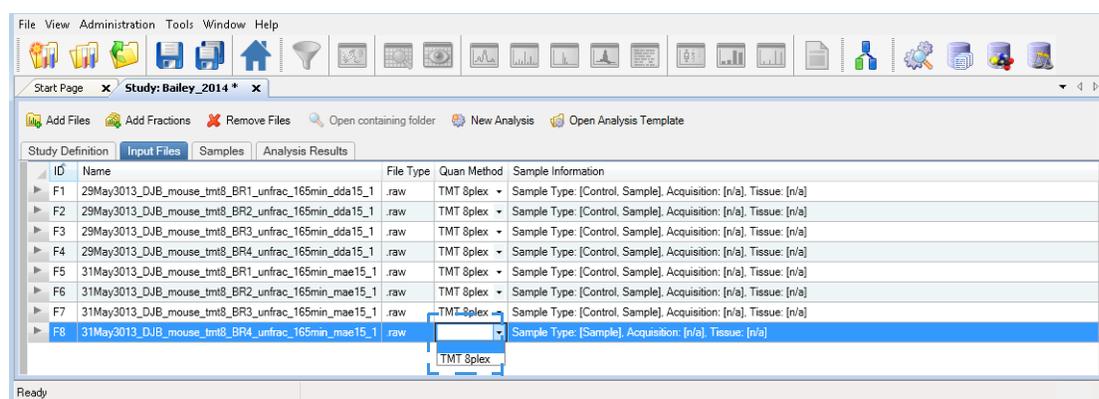


Specifying the Quantification Method for Multiple Input Files

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 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 control is the channel that was used to label the mouse liver tissues.

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.

Click the red arrow to close a file entry.

Click the gray arrow to expand a single file entry.

ID	Name	Date Modified	Size
F1.1	C:\Program Files\Proteome Discoverer source files\studies\Bailey_2014\input_files\29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1.raw	5/29/2014 8:45:18 PM	328754564 [Byte]
F2	31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_1.raw		
F3	31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_1.raw		
F4	31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_1.raw		
F5	31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_1.raw		
F6	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1.raw		
F7	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1.raw		
F8	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1.raw		

❖ 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).

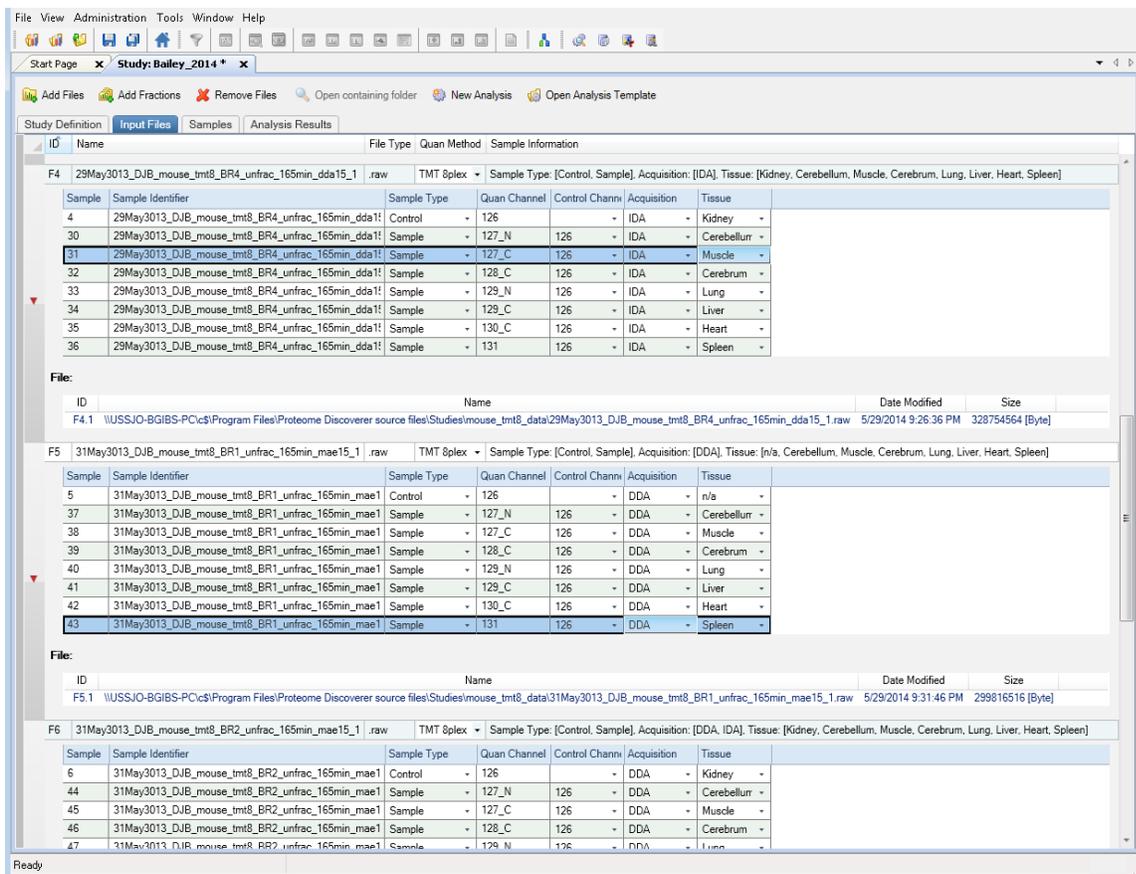
4. For the second factor (in the example, Tissue), set the value for each sample in each raw data file by selecting the down arrow in the factor column and then selecting the value from the list.

This figure shows the process for the second factor, Tissue.

ID	Name	File Type	Quan Method	Sample Information		
F1	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	.raw	TMT 8plex	Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, n/a]		
Sample	Sample Identifier	Sample Type	Quan Channel	Control Chann	Acquisition	Tissue
1	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Control	126		IDA	Kidney
9	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	127_C	126	IDA	Cerebellum
10	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	127_N	126	IDA	Muscle
11	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	128_C	126	IDA	Cerebrum
12	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	129_C	126	IDA	Lung
13	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	129_N	126	IDA	Liver
14	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	130_C	126	IDA	Heart
15	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	Sample	131	126	IDA	n/a

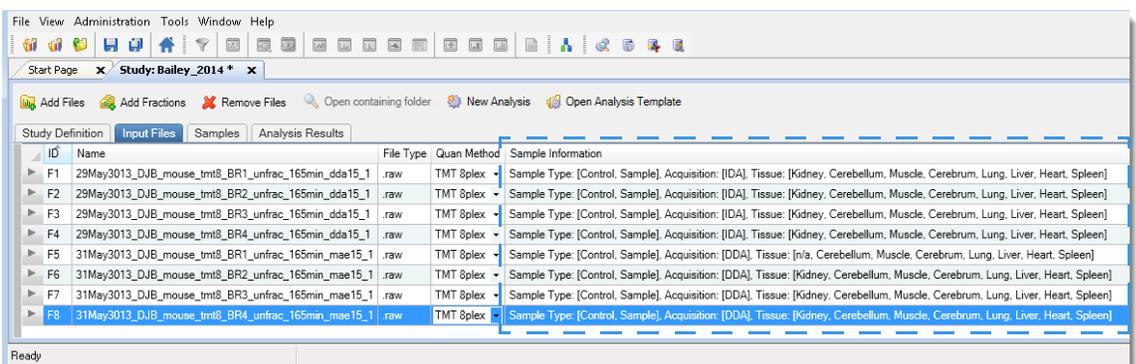
- Set the same values in the Acquisition and the Tissue columns for the rest of the samples.
- After you finish setting the factor values for each sample, confirm that the Input Files page resembles the next figure. (In the example data set, you must set 128 factor values for eight files, eight samples each, and 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*.



- Click the red down arrow next to each sample to close the file entry.

The Sample Information column summarizes the information about the samples contained in a file.



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

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

Sample	Sample Identifier	Sample Type	Acquisition	Tissue
S1	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Control	IDA	Kidney
S2	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Control	IDA	Kidney
S3	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Control	IDA	Kidney
S4	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Control	IDA	Kidney
S5	31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae1	Control	DDA	n/a
S6	31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae1	Control	DDA	Kidney
S7	31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Control	DDA	Kidney
S8	31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Control	DDA	Kidney
S9	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Cerebellum
S10	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Muscle
S11	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Cerebrum
S12	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Lung
S13	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Liver
S14	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Heart
S15	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_ddd1!	Sample	IDA	Spleen
S16	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Cerebellum
S17	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Muscle
S18	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Cerebrum
S19	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Lung
S20	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Liver
S21	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Heart
S22	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_ddd1!	Sample	IDA	Spleen
S23	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Cerebellum
S24	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Muscle
S25	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Cerebrum
S26	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Lung
S27	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Liver
S28	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Heart
S29	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_ddd1!	Sample	IDA	Spleen
S30	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Cerebellum
S31	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Muscle
S32	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Cerebrum
S33	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Lung
S34	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Liver
S35	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Heart
S36	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_ddd1!	Sample	IDA	Spleen

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

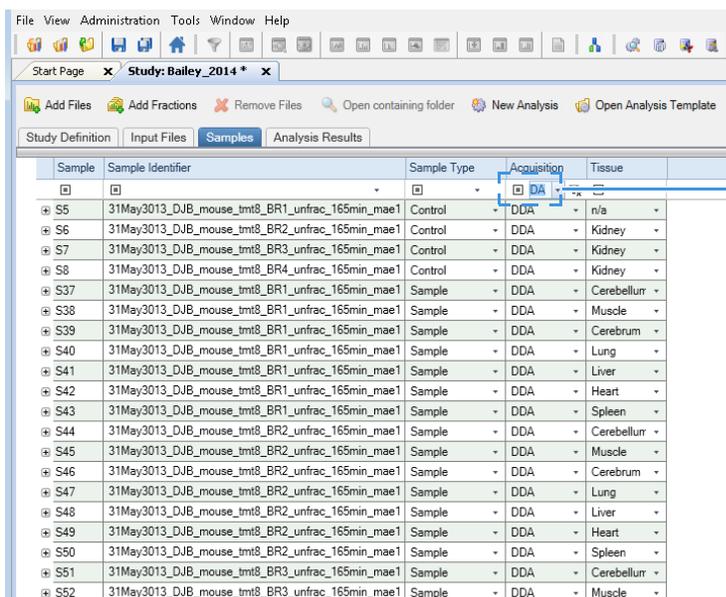
Use the following procedure to change values for multiple samples on the Samples page.

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

1. Click the **Samples** tab.
2. If necessary, click the **Contains** icon, , 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.



Type **dda** in the filter box next to the Contains icon.

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 multi-cell 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.
8. 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. An asterisk (*) in the tab after the study name indicates a change in the study that you must save.

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.

You can set an option to have the application save studies automatically when you click  **Run**. 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  **Reprocess** 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  **Show Details**.

❖ 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. Before you click  **Run**, 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**.

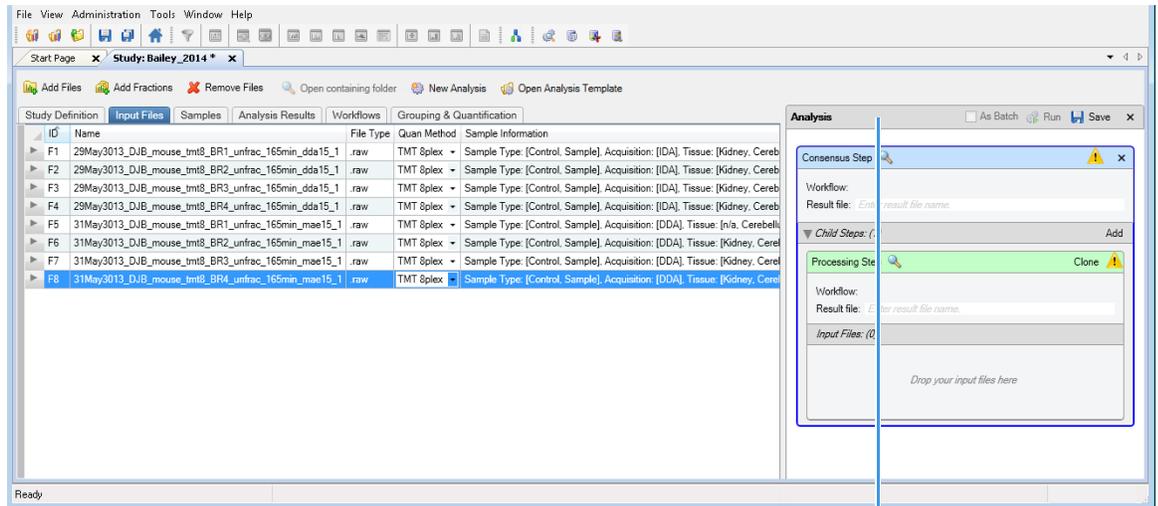
Creating an Analysis

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

❖ To create an analysis

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

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

Follow these topics:

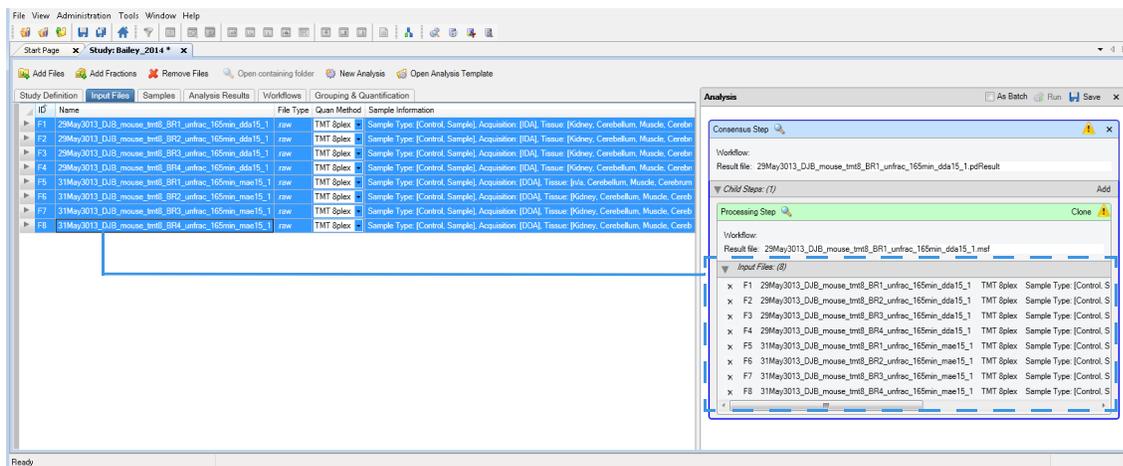
- [Adding Input Files to an Analysis](#)
- [Creating the Workflows](#)
- [Saving an Analysis](#)
- [Grouping Samples and Ratios](#)
- [Performing a Search in Individual Mode](#)
- [Performing a Search in Batch Mode](#)

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 area of the Processing Step box in the Analysis window.

The input files are listed in the Input Files area of the Processing Step box.



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. For detailed information on creating workflows, refer to the *Proteome Discoverer User Guide*.

❖ To create the processing workflow

1. Click the **Workflows** tab, to open the Workflow Editor.

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

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 create a processing workflow for reporter ion quantification, drag these nodes to the Workflow Tree pane:
 - Spectrum Files node
 - Spectrum Selector node
 - A search engine node (for example, Sequest HT or Mascot)
 - Reporter Ions Quantifier node
 - PSM Validator node, Target Decoy PSM Validator node, or Percolator node
 - To create a processing workflow for precursor ion quantification, drag these nodes to the Workflow Tree pane:
 - Spectrum Files node
 - Spectrum Selector node
 - A search engine node (for example, Mascot or Sequest HT)
 - Minora Feature Detector node
 - Fixed Value PSM Validator node, Target Decoy PSM Validator node, or Percolator node
 - To create a processing workflow for label-free quantification, drag these nodes to the Workflow Tree pane:
 - Spectrum Files RC node
 - Spectrum Selector node

- Minora Feature Detector node
 - A search engine node (for example, Mascot or Sequest HT)
 - Fixed Value PSM Validator node, Target Decoy PSM Validator node, or Percolator node
4. Connect the nodes together, as needed.

In the 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 2.

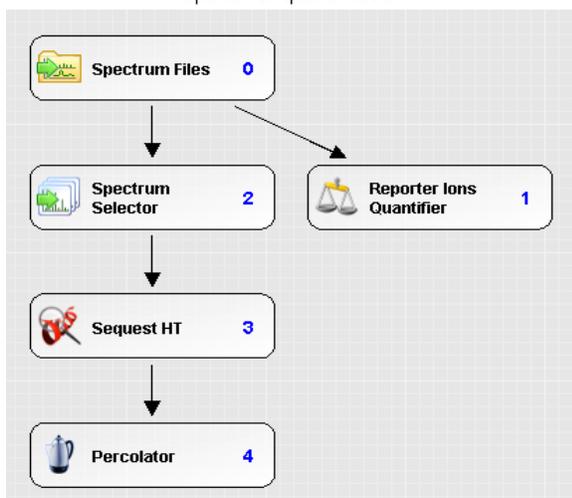
- Set the N-Terminal Modification parameter under Dynamic Modifications (Peptide Terminus), for example, **TMT6plex/+229.163 Da**.
- Set a Dynamic Modification parameter, for example, **TMT6plex /+229.163 Da (K)**.
- Set a Static Modification parameter, for example, **Carbamidomethyl/+57.021 Da (C)**.

This figure shows the parameter settings.

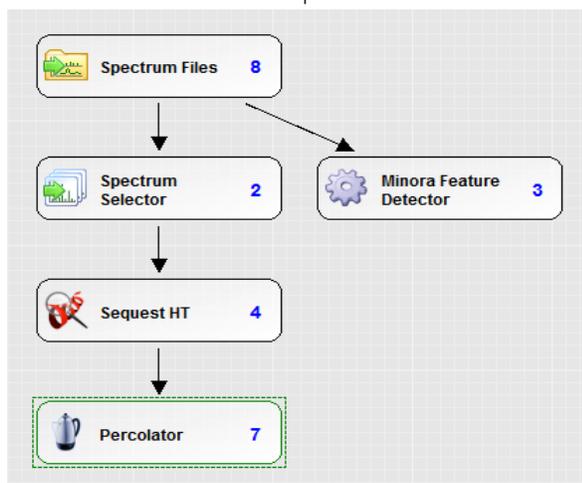
Parameters	
Show Advanced Parameters	
1. Input Data	
Protein Database	Swissprot2.fasta
Enzyme Name	Trypsin (Full)
Max. Missed Cleavage Sites	2
Min. Peptide Length	6
Max. Peptide Length	144
2. Tolerances	
Precursor Mass Tolerance	10 ppm
Fragment Mass Tolerance	0.6 Da
Use Average Precursor Mass	False
Use Average Fragment Mass	False
3. Spectrum Matching	
Use Neutral Loss a Ions	True
Use Neutral Loss b Ions	True
Use Neutral Loss y Ions	True
Use Flanking Ions	True
Weight of a Ions	0
Weight of b Ions	1
Weight of c Ions	0
Weight of x Ions	0
Weight of y Ions	1
Weight of z Ions	0
4. Dynamic Modifications	
Max. Equal Modifications Per Peptide	3
1. Dynamic Modification	TMT6plex / +229.163 Da (K)
2. Dynamic Modification	None
3. Dynamic Modification	None
4. Dynamic Modification	None
5. Dynamic Modification	None
6. Dynamic Modification	None
5. Dynamic Modifications (peptide terminus)	
1. N-Terminal Modification	TMT6plex / +229.163 Da (N-Terminus)
2. N-Terminal Modification	None
3. N-Terminal Modification	None
1. C-Terminal Modification	None
2. C-Terminal Modification	None
3. C-Terminal Modification	None

The following figures show three examples of the processing workflow—for reporter ion quantification, precursor ion quantification, and label-free quantification.

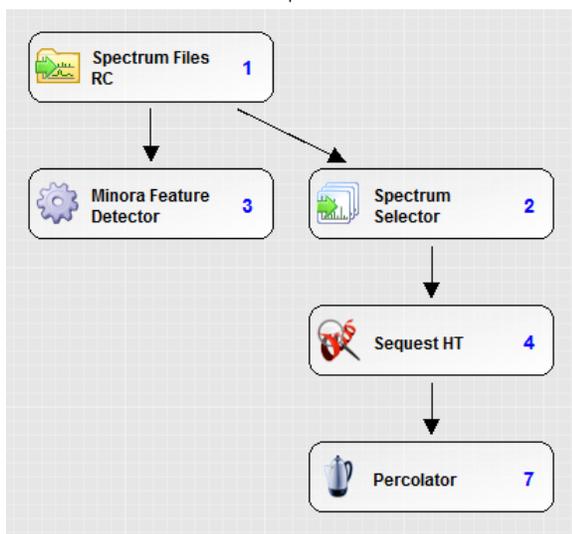
Reporter ion quantification



Precursor ion quantification



Label-free quantification



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  Save .
 - 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 (click  Save Common , 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 with an exclamation mark in the upper right corner of the Processing Step box () indicates that either the workflow or the node parameters are not set up correctly. Point to the yellow triangle to display information 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.
2. Create the appropriate consensus workflow in the Workflow Tree pane of the Workflow Editor.
 - To create a consensus workflow for reporter ion quantification, drag these nodes to the Workflow Tree pane:

– MSF Files node	– Protein Scorer node
– PSM Grouper node	– Protein Grouping node
– Peptide Validator node	– Reporter Ions Quantifier node
– Peptide and Protein Filter node	
 - To create a consensus workflow for precursor ion quantification, drag these nodes to the Workflow Tree pane:

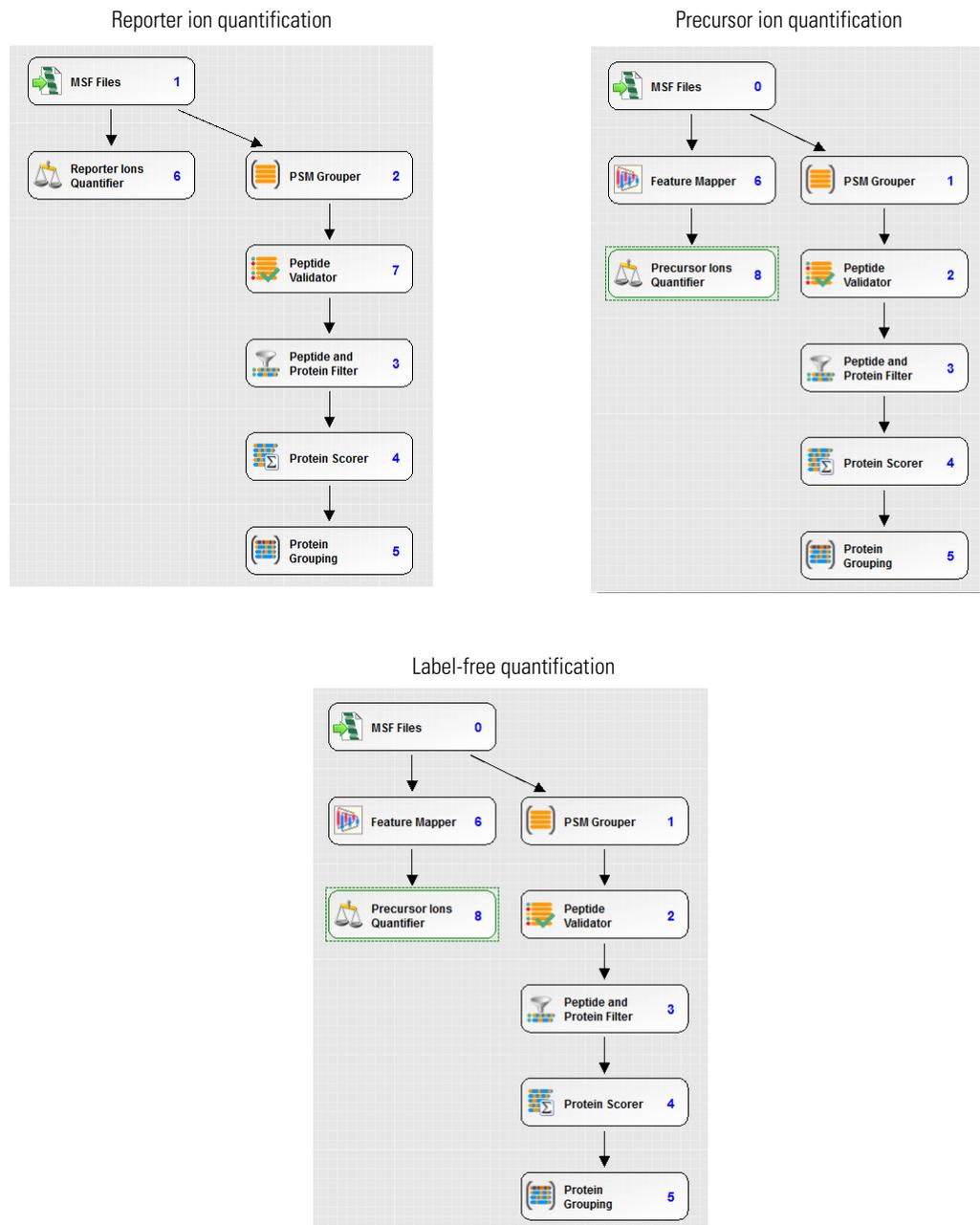
– MSF Files node	– Protein Scorer node
– PSM Grouper node	– Protein Grouping node
– Peptide Validator node	– Feature Mapper node
– Peptide and Protein Filter node	– Precursor Ions Quantifier node
 - To create a consensus workflow for label-free quantification, drag these nodes to the Workflow Tree pane:

– MSF Files node	– Protein Scorer node
– PSM Grouper node	– Protein Grouping node
– Peptide Validator node	– Feature Mapper node
– Peptide And Protein Filter node	– Precursor Ions Quantifier node

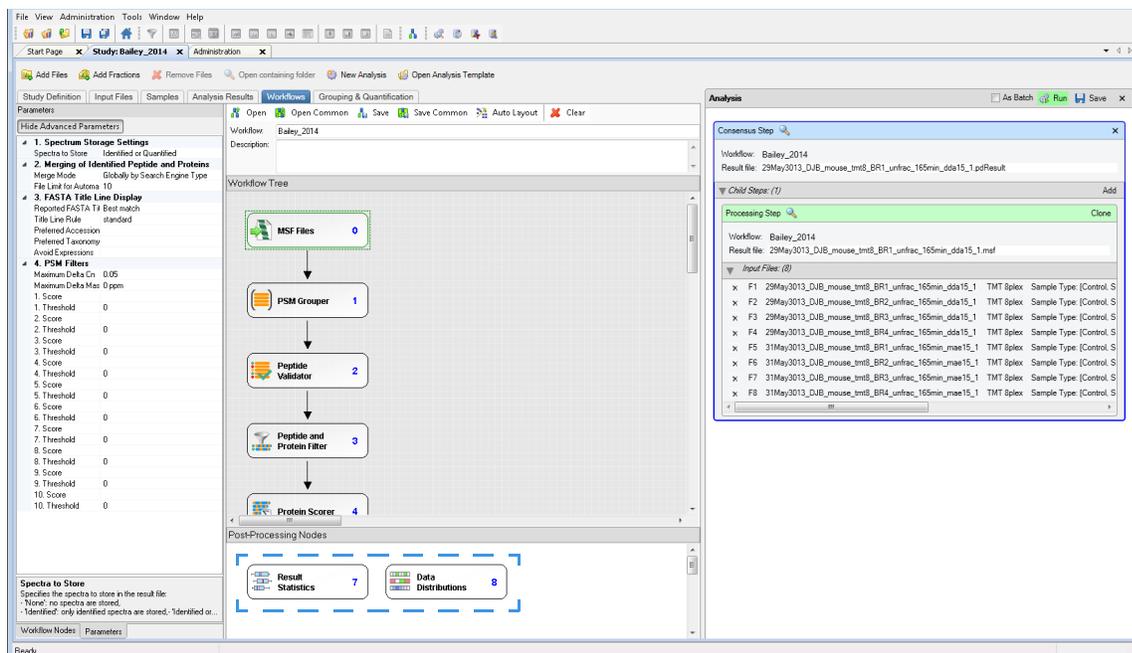
The example workflow also includes the following nodes in the Post-Processing Nodes area:

 - Result Statistics node
 - Data Distributions node
3. Connect the nodes together, as needed.
4. Set the appropriate parameters for each node.

The following figures show three examples of the consensus workflow—for reporter ion quantification, precursor ion quantification, and label-free quantification.



The next figure shows an example of the consensus workflow that includes the Result Statistics node and the Data Distributions node.



5. (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 Save .
 - 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 with an exclamation mark in the upper right corner of the Consensus Step box () indicates that either the workflow or the node parameters are not set up correctly. Point to the yellow triangle to display information 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 Save .
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.

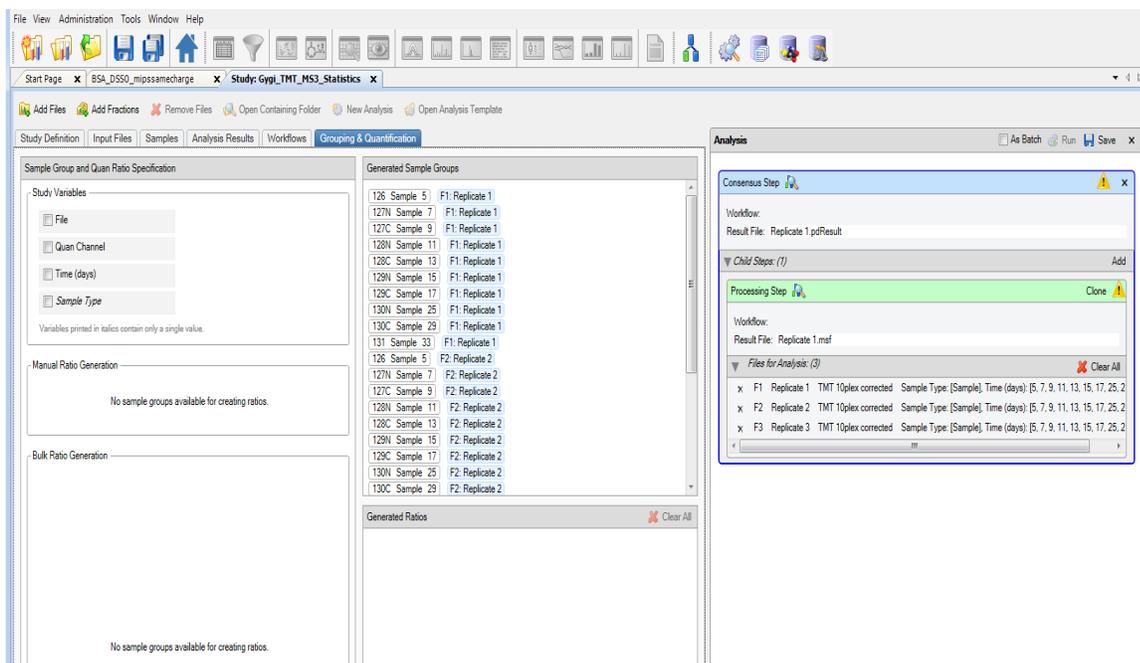
Grouping Samples and Ratios

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. This topic uses an example for reporter ion quantification. For equivalent information about precursor ion quantification and label-free quantification, refer to the *Proteome Discoverer User Guide*.

❖ To open the Grouping & Quantification page

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

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



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 in this guide, select the **Time (days)** check box to indicate that the samples and quantification ratios are grouped by time point.

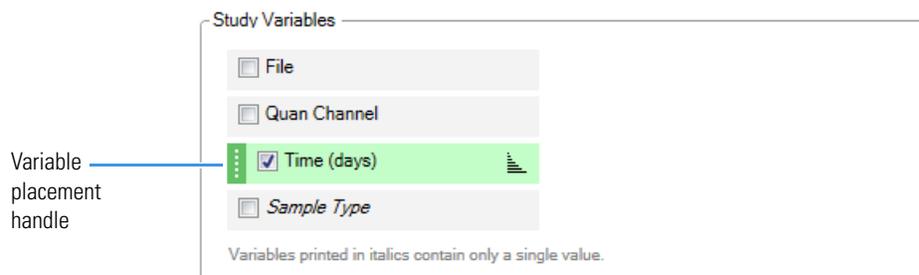
After you select the study factors, the Generated Sample Groups area displays the generated sample groups. When performing the quantification, the application calculates abundance values for each sample and averages the abundance values of all samples in a sample group.

The order of the 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. Point to the placement handle.

White up and down arrows now appear on the handle.

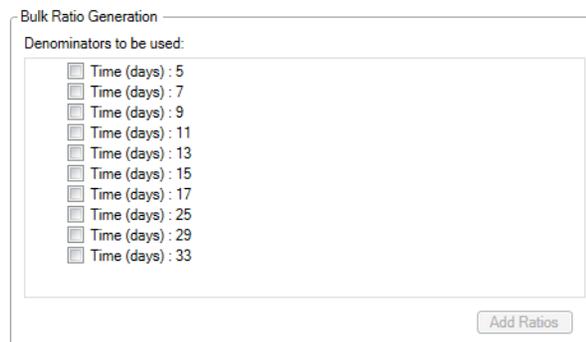
c. Drag the cursor up or down to move the variable to its new place in the list of variables, or click the up or down arrow to move the study factor.

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 **Time (days): 5**.

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.



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

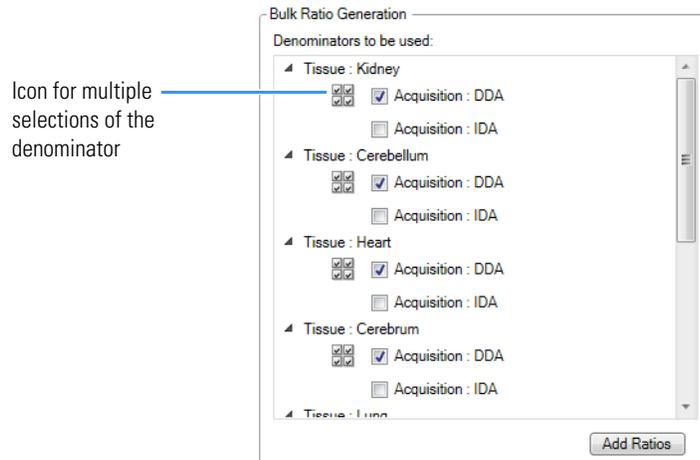
5. (Optional) To select the same study factor for all the denominators, do the following:

a. Point to 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.

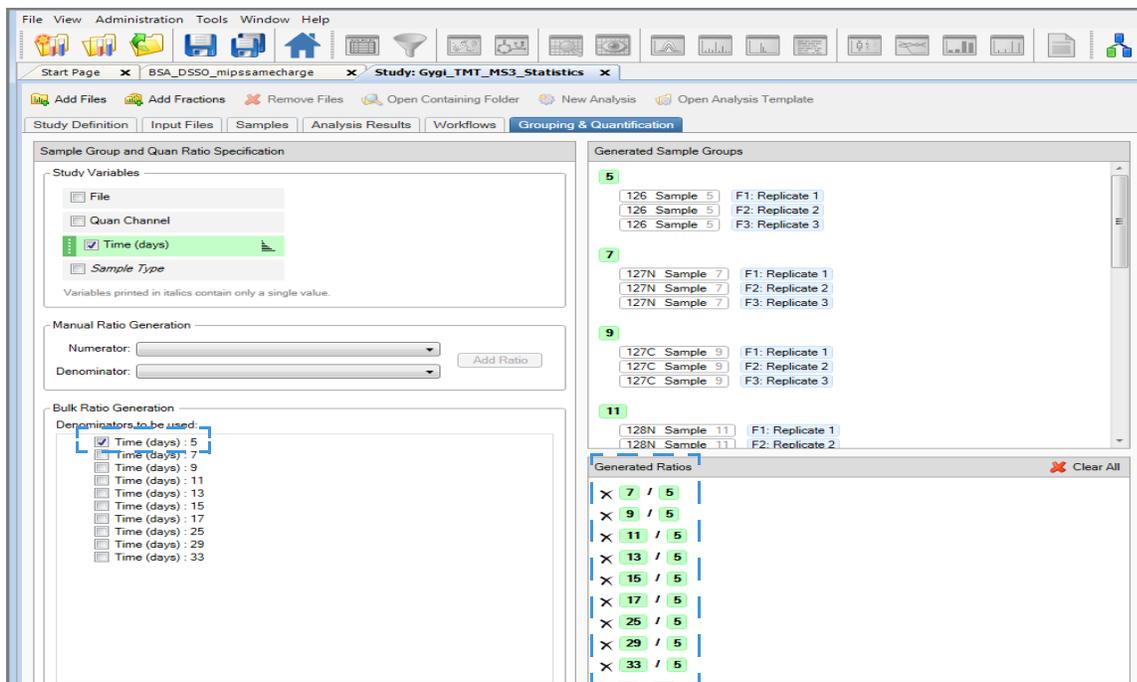
The application selects the same study factor for all denominators.



6. Click **Add Ratios**.

The application generates all possible ratios against the selected denominator values and adds them to the Generated Ratios area.

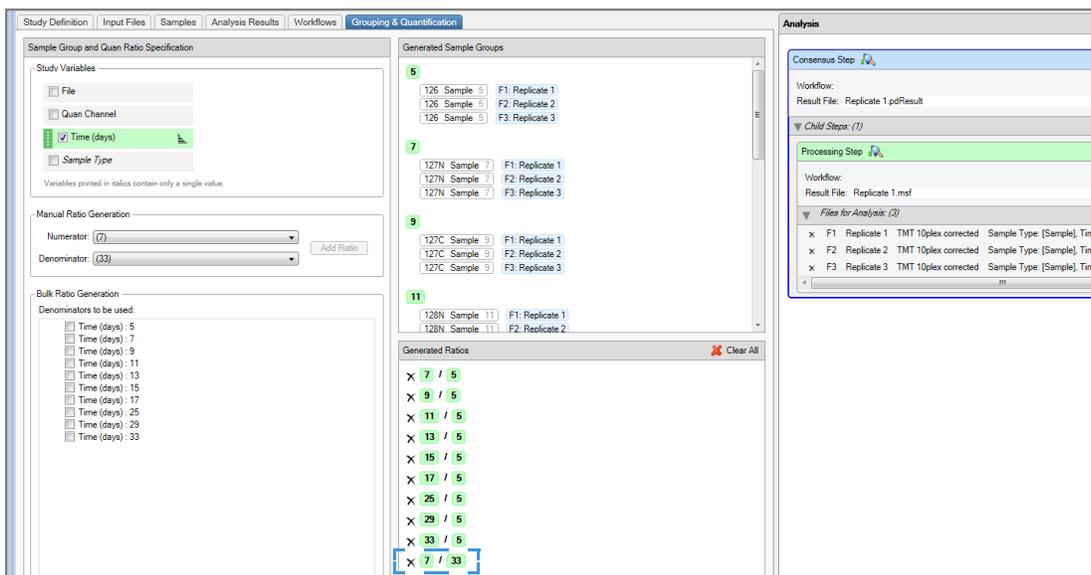
This figure shows the generated quantification ratios and ratio groups in the Generate Ratios area after selecting Time (days) as the study variable to group by and 5 as the denominator to use.



❖ **To generate custom quantification ratios manually**

1. On the Study: *Study_name* page, click the **Grouping & Quantification** tab.
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 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 **Time (days)** check box to indicate that the samples and quantification ratios are grouped by time point.
3. In the Manual Ratio Generation area, select the numerator value from the Numerator list, for example, 7.
4. Select the denominator value from the Denominator list, for example, 33.
5. Click **Add Ratio**.

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



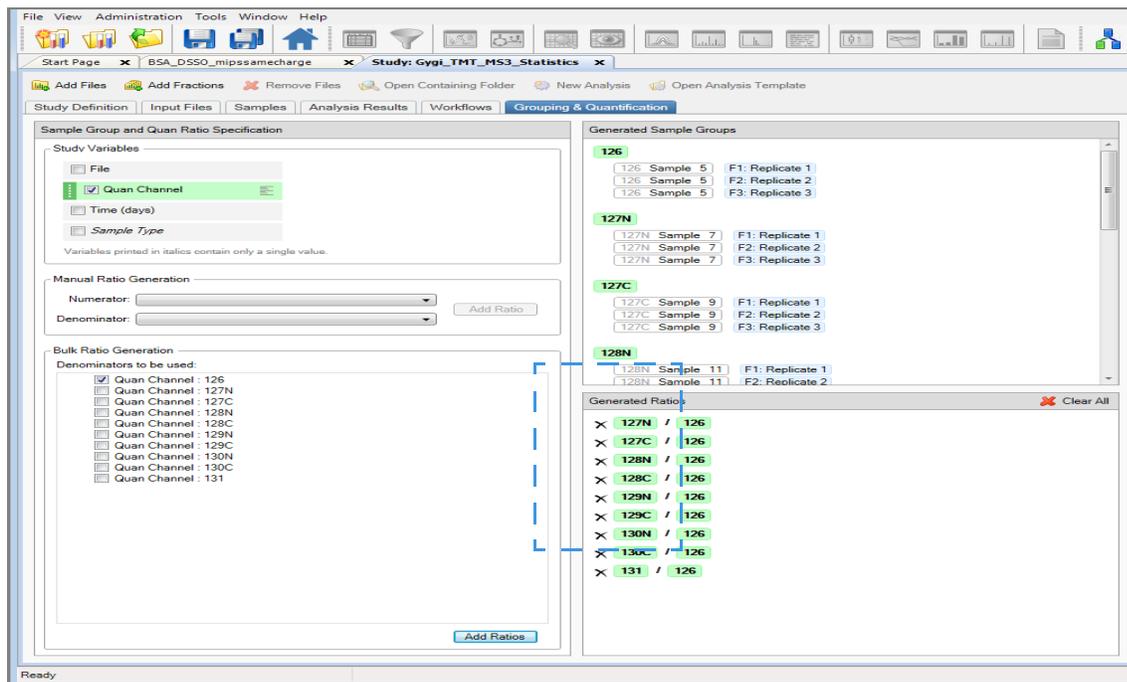
❖ **To generate custom quantification ratios based on channels**

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 25.

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.

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

❖ **To perform a search in individual mode**

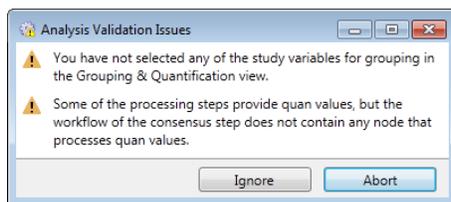
In the upper right corner of the Analysis window, click  **Run**.

The application validates the analysis setup before it starts processing and, if it finds errors, issues error or warning messages in the Analysis Validation Issues box (see the next figure). 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.

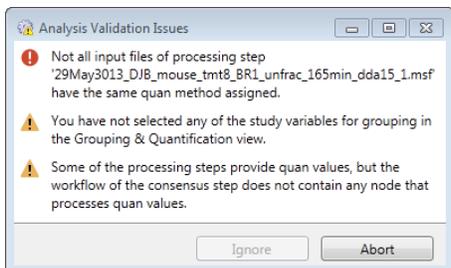
Performing the Search

Performing a Search in Individual Mode

You can ignore warnings that 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 examples of warnings.

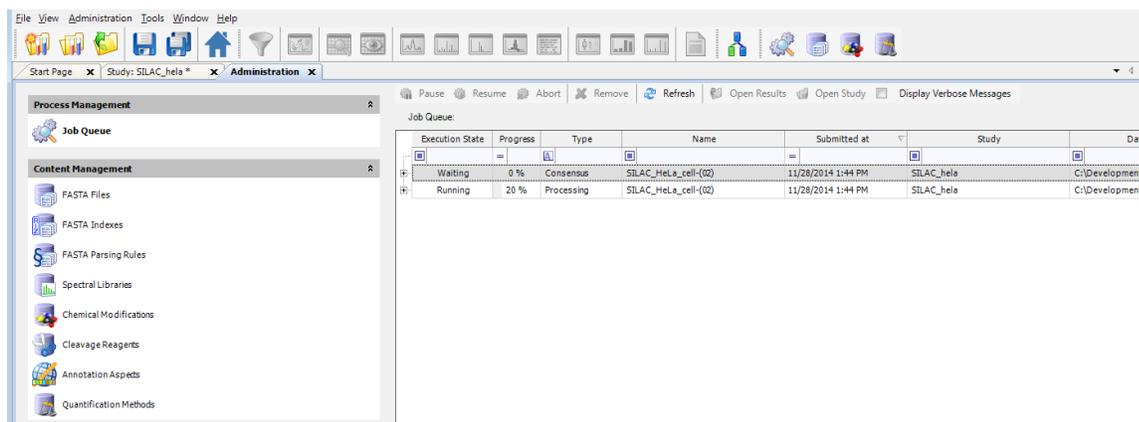


You cannot ignore validation errors, which are marked with a red exclamation mark (see the next figure). You must resolve them.



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

After the application validates the analysis, it begins processing it. The job queue opens so that you can monitor the progress of the job.



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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