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.

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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, *(M)*, on your desktop.

The Proteome Discoverer Start Page opens.

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Ready														

Configuring the Search Engine Parameters

Downloading the FASTA Files If you intend to conduct a search with the Sequest[™] HT and MascotSM 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*.

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.

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, S 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, *M* Download .

The Download from ProteinCenter dialog box appears.

🕥 Download fr	rom ProteinCenter	- • •
Taxonomy ID:		Include All Subcategories
Database:	SwissProt (2015-04-29)	•
Browse Taxono	omy ID:	
http://www.nc	bi.nlm.nih.gov/taxonomy/	
http://www.uni	prot.org/taxonomy/	
		Import Close

- 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, **Q**.
- 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 - Taxonomy navigation Map to UniProtKB (589,444)	Saccharomyces	• All lower taxonomy nodes (318)
			ΥΕΔΟΥ
Taxonomy		Taxon identifier ⁱ	4932
identifier		Scientific name ⁱ	Saccharomyces cerevisiae
number		Common name i	Baker's yeast
Humber		Synonym ⁱ	
		Other names ⁱ	> ATCC 18824 > CBS 1171 > Candida robusta > NRRL Y-12632 > Saccaromyces cerevisiae More »
		Rank ⁱ	SPECIES
		Lineage ⁱ	> cellular organisms > Eukaryota > Opisthokonta > Fungi > Dikarya > Ascomycota > Saccharomyceta > Saccharomycetaes > Saccharomycetales > Saccharomycetaee > Saccharomycetae > Saccharomycetaee > Saccharomycetaee > Saccharomycetaee > Saccharomycetae > Sacchar
		Strains ⁱ	> 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 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.

🕥 Download fr	om ProteinCenter	
Taxonomy ID:	4932	Include All Subcategories
Database:	SwissProt (2015-04-29)	•
Browse Taxono	my ID:	
http://www.ncb	i.nlm.nih.gov/taxonomy/	
http://www.unip	prot.org/taxonomy/	
		Import Close

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, and icon, and icon, and icon, and icon, and icon, and

💠 Add 孋 Download 🚎 Update 💥 Remove 🚫 Cancel 🛛 🍣 Refresh 👹 Check for Updates Protein Database Taxonomy ID Name Version File Size [kB] # Sequences # Residues Status Last Modified Update Available ▶ ipi.HUMAN.v3.87.f... 91464 36355611 Availa... 09/27/2011 Custom 49876 PAOC Database.f... Custom 14158 13611 10493882 Availa... 02/06/2014 Canis lupus famili... SwissProt 9615 2014-07-09 922 857 381701 Availa... 11/10/2014

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

Database downloaded

from ProteinCenter

SwissProt database.

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

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.

✤ 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, ز

The New Study and Analysis dialog box opens.

New Study and Analysis	
Study Name: New Study	Add Files Add Fractions Kernove
Study Root Directory: c\$\Program Files\Proteome Discoverer source files\ Studies	
Processing Workflow: (empty workflow)	
Consensus Workflow: (empty workflow)	
Quantification Method: (No Quantification)	
Select Control Channel:	
	OK Cancel

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.

File View Administration Tools Window Help						
Start Page × Study: Bailey_2014 ×	Start Page 🗙 Study: Bailey_2014 🗙					
Remove Files 🤬 Add Fractions 💥 Remove Files 😡 Open Containing Folder 🕘 New Analysis 🎲 Open Analysis Template						
Study Summary	Quantification Methods					
Study Name: Bailey_2014 Study Directory: C:Program Files/Proteome Discoverer source files/studies/Bailey_2014 Last Changed: 7/7/2015 10:21:54 AM	Dimethylation 3plex (C2H6, C2H Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method	iTRAQ 4plex Method for iTRAQ [™] 4-plex mass tags by Applied Blosystems	Low Resolution TMTe 6plex Method for low resolution 6-plex Tande Tag® of Proteome Sciences plo	em Ma:		
Creation Date: 7/7/2015 10:21:54 AM	Full 18O Labeling (O2 18O2)	iTRAQ 8plex Method for iTFRAQ" 8-plex mass tags by Applied Biosystems	SILAC 2plex (Arg10, Lys6) SILAC 2plex (Arg10, Lys6) Method			
Study Description	Incomplete 180 Labeling (O2 180 labeling method for incompletely labeled samples	Low Resolution iodo TMT 6plex Method for Iow resolution cystelne-reactive S- plex Tandem Mass Tag® of Proteome Solences plo	SILAC 2plex (Arg10, Lys8) SILAC 2plex (Arg10, Lys8) Method			
	iodo TMT 6plex		SILAC 2plex (IIe6) SILAC 2plex (IIe6) Method			
	A III			Þ		
	Study Factors		Paste Copy A	dd -		
Ready						

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
scriptionYou can optionally 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 the Add icon, 💠 Add .

Description Adding a Quantification

Method to the

Study

The Create New Quantification Method dialog box now appears.

ſ	🖳 Create New Quantifica	tion Method
	En oreate New Quartance	
	From Factory Defaults:	Dimethylation 3plex (C2H6, C2H2D4, 13C2D6)
	From Existing Method:	Dimethylation 3plex (C2H6, C2H2D4, 13C2D6)
	From Scratch: (advanced mode)	Precursor Ion Method 💌
		Create Cancel

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

The Quantification Method Editor opens.

Residue Modification: TMT6plex / +229.163 Da K N-Terminal Modification: TMT6plex / +229.163 Da								
Mass Tag	Reporter Ion Mass	-2	- 1	Main	+ 1	+ 2	Active	
126	126.127726	0	0	100	0	0		
127N	127.124761	0	0	100	0	0	J	
127C	127.131081	0	0	100	0	0	J	
128N	128.128116	0	0	100	0	0	J	
128C	128.134436	0	0	100	0	0	J	-
129N	129.131471	0	0	100	0	0	J	
129C	129.13779	0	0	100	0	0	J	
130N	130.134825	0	0	100	0	0	J	
130C	130.141145	0	0	100	0	0	J	
131	131.13818	0	0	100	0	0	J	
MT: Main peaks	; are always 100%							

- 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	
 Image: A start of the start of	Dimethylation 3plex (C2H6, C2H2D4, 13C2D6)	Dimethylation 3plex (C2H4, C2D4, 13C2D4) Method	v	
 Image: A start of the start of	Full 180 Labeling (02 1802)	180 labeling method for fully labeled samples	v	
 Image: A second s	Incomplete 180 Labeling (02 0180 + 1802)	180 labeling method for incompletely labeled samples	~	
 Image: A second s	iodo TMT 6plex	Method for cysteine-reactive 6-plex Tandem Mass Tag® of Proteome Sciences plc	v	
 Image: A start of the start of	iTRAQ 4plex	Method for iTRAQ [™] 4-plex mass tags by Applied Biosystems	v	
 Image: A second s	iTRAQ 4plex (Thermo Scientific Instruments)	Method for iTRAQ [™] 4-plex mass tags by Applied Biosystems optimized for Ther	v	
 Image: A second s	iTRAQ 8plex	Method for iTRAQ™ 8-plex mass tags by Applied Biosystems	V	
 Image: A start of the start of	iTRAQ 8plex (Thermo Scientific Instruments)	Method for iTRAQ [™] 8-plex mass tags by Applied Biosystems optimized for Ther	v	
 Image: A second s	SILAC 2plex (Arg10, Lys6)	SILAC 2plex (Arg10, Lys6) Method	v	
 Image: A second s	SILAC 2plex (Arg10, Lys8)	SILAC 2plex (Arg10, Lys8) Method	~	
 Image: A set of the set of the	SILAC 2plex (Ile6)	SILAC 2plex (Ile6) Method	v	
 Image: A second s	SILAC 3plex (Arg6, Lys4 Arg10, Lys8)	SILAC 3plex (Arg6, Lys4 Arg10, Lys8) Method	v	
 Image: A second s	SILAC 3plex (Arg6, Lys6 Arg10, Lys8)	SILAC 3plex (Arg6, Lys6 Arg10, Lys8) Method	v	
 Image: A second s	TMT 10plex	Method for 10-plex Tandem Mass Tag® of Proteome Sciences plc	V	
 Image: A second s	TMT 2plex	Method for 2-plex Tandem Mass Tag® of Proteome Sciences plc	v	
 Image: A second s	TMT 8plex	Method for 8-plex Tandem Mass Tag® of Proteome Sciences plc	v	
~	TMTe 6plex	Method for 6-plex Tandem Mass Tag® of Proteome Sciences plc	V	

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.

File View Administration Tools Window Help							
Start Page × Study: Bailey_2014 * ×	Start Page X Study: Bailey_2014 * X						
🙀 Add Files 🙀 Add Fractions 🧩 Remove Files 😡 Open Containing Folder 🖏 New Analysis 🎲 Open Analysis Template Study Definition Input Files Samples Analysis Results							
Study Summary	Quantification Methods						
Study Name: Bailey_2014 Study Directory: C: C:Proglam Files/Protecome Discoverer source files/studies/Bailey_2014 Last Changed: 77/2015 10 39 35 AM	Low Resolution TMTe 6plex SILAC 3plex (Arg6, Lys4 Arg10	lex Tandem Mass Tag® of noes plo					
Creation Date: 7/7/2015 10:21:54 AM	SILAC 2plex (Arg10, Lys6) SILAC 3plex (Arg6, Lys6 Arg10. TMT 8plex SILAC 3plex (Arg10, Lys6) Method SILAC 3plex (Arg6, Lys6 Arg10, Lys8) Method Free Arg10, Lys8 Method Free Arg10, Lys8 Method Free Arg10, Lys8 Arg10,	vlex Tandem Mass Tag® of noes plo					
Study Description	SILAC 2plex (Arg10, Lys8) TMT 10plex TMTe 6plex						
	SILAC 2plex (lle6) Method for 10-plex Tandem Mass Tag® of Proteome Sciences plc Proteome Sciences plc	ex Tandem Mass Tag® of noes plo					
	SILAC 2plex (Ile6) Method TMT 10plex Corr						
	*						
	Study Factors	Paste Copy Add -					
Ready							

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]	Apply Cancel X	
Items:		Type the categorical factor name.
	Add Delete	
		Type the name of the value.

[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.

Acquisition	Apply Cancel X
Items:	
	DDA IDA
	Add Delete

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

Confirm that the Study Definition page resembles the next figure.

View Administration Tools Window Help) 🚹 🗟 🐻 🛛	
art Page × Study: Bailey_2014 * ×			•
Add Files 🚳 Add Fractions 🐹 Remove Files 😡 Open Containing Folder 🎨 New Analysis 🌾	Open Analysis Template		
dy Definition Input Files Samples Analysis Results			
udy Summary	Quantification Methods		
udy Name: Bailey_2014	Low Resolution TMTe 6plex	SILAC 3plex (Arg6, Lys4 Arg10	TMT 2plex
udy Directory: C:\Program Files\Proteome Discoverer source files\studies\Bailey_2014 ist Changed: 7/7/2015 10:45:18 AM	Method for low resolution 6-plex Tandem Mass Tag® of Proteome Sciences plc	SILAC 3plex (Arg6, Lys4 Arg10, Lys8) Method	Method for 2-plex Tandem Mass Tag® of Proteome Sciences plc
eation Date: 7/7/2015 10:21:54 AM	SILAC 2plex (Arg10, Lys6)	SILAC 3plex (Arg6, Lys6 Arg10	TMT 8plex
	SILAC 2plex (Arg10, Lys8) Method	SILAC 3plax (Arg6, Lys6 Arg10, Lys8) Method	TMT 8plax
udy Description	SILAC 2plex (Arg10, Lys8) SILAC 2plex (Arg10, Lys8) Method	TMT 10plex	TMTe 6plex Method for 6-plex Tandem Mass Tag® of Protecting Sciences plo
	SILAC 2plex (Ile6)	Proteome Solences plo	
	SILAC 2plex (Ile6) Method	TMT 10plex Corr	
	4		
	Shudu Fastar	U	Parts Carry
	Study Factors		Faste Copy A
	Acquisition	Edit 🗙	
		DDA	
		10/1	

- 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.

A 🕼 🚱 🔒 🗿 👚 💎 🔯 📖 🖾 📖	
art Fage × Study: Bailey_2014 * ×	
Add Files 🛛 Add Fractions 🛛 💥 Remove Files 🔍 Open Containing Folder 🛯 🎲 New Analysis	Open Analysis Template
dy Definition Input Files Samples Analysis Results	
udy Summary	Quantification Methods
udy Name: Bailey_2014 udy Directory: C:\Program Files\Proteome Discoverer source files\studies\Bailey_2014 st Changed: 7/7/2015 10:45:18 AM	Low Resolution TMTe Gplex SILAC 3plex (Arg6, Lys4 Arg10
eation Date: 7/7/2015 10:21:54 AM	SILAC 2plex (Arg10, Lys6) SILAC 3plex (Arg6, Lys6 Arg10, TMT 8plex SILAC 2plex (Arg10, Lys8) Method SILAC 3plex (Arg10, Lys8) Arg10, Lys8) TMT 8plex
udy Description	SILAC 2plex (Arg10, Lys8) TMT 10plex Method for 10-plex Tandem Mass Tags or Photomer Solares ple Photomer Solares
	SILAC 2plex (ille6) TMT 10plex Corr
	4
	Study Factors Paste Copy
	Tissue Edit x
	Cerebellum Cerebellum Kidney Liver Liver
	Acquisition Edit x
	DDA

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, 🔛 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, ..., Fn. The Proteome Discoverer application adds each file as a single study file.

File View Administration Tools Window Help								
Start Page 🗙 Study: Bailey_2014 * 🗙 👻 🕇								
🙀 Add Files 🍓 Add Fractions 💥 Remove Files 😡 Open Containing Folder 🚳 New Analysis 🌍 Open Analysis Template								
Study Definition Input Files Samples Analysis Results								
⊿ ID Name	File Type Quan Method	Sample Information						
F1 29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1	.raw -	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F2 31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_1	1 .raw -	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F3 31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_*	1 .raw -	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F4 31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_*	1 .raw 👻	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F5 31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_1	1.raw •	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F6 29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1	.raw •	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F7 29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1	.raw •	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
F8 29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1	.raw •	Sample Type: [Sample], Acquisition: [n/a], Tissue: [n/a]						
кеаду								

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.

ile View Administration Tools Window Help							
Add Files 🔐 Add Fractions 💥 Remove Files 🔍 Open containing fo	er 🚷 New Analysis 🍯 Open Analysis Template						
Study Definition Input Files Samples Analysis Results							
ID Name File Ty	Quan Method Sample Information						
F1 29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
F2 29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1 .raw	TMT 8plex - Sample Type: [Control, Sample], Acquisition: [n/a]. Tissue: [n/a]						
F3 29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
F4 29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
F5 31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_1 .raw	TMT 8plex - Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
► F6 31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
F7 31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_1 .raw	TMT 8plex + Sample Type: [Control, Sample], Acquisition: [n/a], Tissue: [n/a]						
F8 31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_1 .raw	Sample Type: [Sample]. Acquisition: [n/a], Tissue: [n/a]						
	TMT Selex						
Ready							

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.

	d Files 🧰	🤰 Add Fractions 🛛 🐹 Remove Files 🛛 🔍 Open containi	ng folder 🛛 🎨 New	Analysis 🔞 (Open Analysis Te	emplate		
tudy	Definition	Input Files Samples Analysis Results						
1	D Name	Fi	e Types Quan Meth	od Sample Info	rmation			
F	1 29May	3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1 .ra	W TMT 8plex	 Sample Typ 	e: [Control, Sam	ple], Acquisition:	[IDA], Tissue: [I	Gdney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, n/a]
	Sample	Sample Identifier	Sample Type	Quan Channel	Control Channe	Acquisition	Tissue	
	1	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Control +	126	•	IDA -	Kidney +	
	9	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	127_C	126 -	IDA -	Cerebellurr +]
	10	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	127_N	126 -	IDA -	Muscle +]
	11	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	128_C	126 -	IDA -	Cerebrum +	
	12	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	129_C	126 -	IDA -	Lung -	
	13	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	129_N	126 -	IDA -	Liver +	
	14	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	130_C	126 -	IDA +	Heart +	
	15	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	Sample +	131	126 -	IDA +	n/a 🔹	
E	ile:						n/a	
	10		N				Cerebellum	Data Marife d
	E1.1 (C-IProgram Files/Protoama Discoverer source files/Studies/m	Name Ivame	w2012 DIR m	use test? DD1	unfrao 165min	Cerebrum	Date Modified Size
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E	2 29May	3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1 .ra	w TMT 8plex	 Sample Typ 	e: [Control, Sam	ple], Acquisition:	Kidney	a]
F	3 29May	3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1 .ra	w TMT 8plex		e: [Control, Sam	ple], Acquisition:	Liver	a]
F	4 29May	3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1 .ra	w TMT 8plex	 Sample Typ 	e: [Control, Sam	ple], Acquisition:	Lung	[a]
F	5 31May	3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_1 .ra	w TMT 8plex	+ Sample Typ	e: [Control, Sam	ple], Acquisition:	Muscle	a)
F	6 31May	3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_1 .ra	w TMT 8plex	 Sample Typ 	e: [Control, Sam	ple], Acquisition:	Spleen	[a]
F	7 31May	3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_1 .ra	w TMT 8plex	+ Sample Typ	e: [Control, Sam	ple], Acquisition:		a]
E	8 31May	3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_1 .ra	w TMT Splex	 Sample Typ 	e: [Control, Sam	ple], Acquisition:	[n/a], Tissue: [n	/a]

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*.

rt P	age >	Study: Bailey 2014 * ×					
1	Files	🤐 Add Fractions 🛛 Remove Files 🔍 Open containin	ig tolder 🐏 New	v Analysis 🛛 🕼	Open Analysis	s l'emplate	
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	Sample	s Sample Identifier	Sample Type	Quan Channel	Control Char	Inni Acquisition	Tissue
	4	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1	Control	126		- IDA -	Kidney -
r	30	29May3013_DJB_mouse_tmt8_BR4_untrac_165min_dda1	Sample	12/_N	126	• IDA •	Cerebellurr +
L	31	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1	Sample	12/_C	126	* IDA *	Muscle •
	32	29May3013_UJB_mouse_tmt8_BR4_unfrac_165min_dda1	Sample	128_C	126	* IDA *	Cerebrum •
	33	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1	Sample	129_N	126	* IDA *	Lung -
	34	29May3U13_LUB_mouse_tmt8_BR4_unfrac_165min_dda1	Sample	129_C	126	* IDA *	Liver -
	35	25maysurs_LUB_mouse_tmts_BH4_untrac_165min_dda1	Sample	- 13U_C	126	- IDA -	Heart -
	36	29May3013_DJB_mouse_tmts_BR4_unfrac_165min_dda1	Sample	131	126	• IDA •	Spleen -
	ID F4.1	WUSSJO-BGIBS-PC\c\$\Program Files\Proteome Discoverer s	ource files\Studies\	nouse_tmt8_data	29May3013_	DJB_mouse_tmt8	BR4_unfrac_165m
F5	ID F4.1 31Ma Sample 5 37 38 39 40 41	UUSLO BOBS PC-DPogan FilesPoteone Discover a w0013.0.0Lmmaat_med_BR1_umfaat_16mm_met15_1 m Somple toming 1396vp1013.0.DLmmaat_med_BR1_umfaat_16mm_met1 1396vp1013.0.DLmmaat_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1 1396vp1013.0.DLmmaat_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1_BR1_umfaat_16mm_met1_BR1_umfaat_18mm_met1_BR1_umfaat_	w TMT 8plex Sample Type Control Sample Sample Sample Sample Sample Sample	mouse_tmt8_data Sample Typi Quan Channel 126 127_N 127_C 128_C 129_N 129_N 129_C	29May3013_1 =: [Control, Sau Control Char 126 126 126 126 126	DJB_mouse_tmt8, imple], Acquisition: DDA - DDA - DD	BR4_unfrac_165m (DDA). Tissue: (n/a n/a - Cerebellurr - Muscle - Cerebrum - Lung - Liver -
-5	ID F4.1 31Ma Sample 5 37 38 39 40 41 42	ULSS/0-66085-PCc8Progam FilesProtome Discovers a y0013_D.QL musau_tmdBR1_unftsc_165mi_mes15_1 / m Sample Identifie 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1 31May/013_D.QL musau_tmdBR1_unftsc_165mi_mas1	w TMT 8plex Sample Type Control Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Quan Channel 126 127_N 127_C 128_C 129_N 129_N 129_C 130_C	29May3013_ :: [Control, Sai Control Chai 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 mmple]. Acquisition • DDA • • DDA •	BR4_unfrac_165m (DDA), Tissue: (n/a Tissue n/a - Cerebellur - Muscle - Cerebellur - Lung - Lung - Liver - Heart -
	ID F4.1 31Ma Sample 5 37 38 39 40 41 42 43	ULSJ.0-BGIBS-PC-GP Program Files/Proteome Discovers as y2013_D/B_mouse_tmdBP1_unfrac_165min_mex15_1 / as Sample identifier 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1 31May/013_DB_mouse_tmdB_BP1_unfrac_165min_mex1	V TMT Splex Sample Type Control Sample Sample Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Quan Channel 126 127_N 127_C 128_C 129_N 129_C 130_C	29May3013_ :: [Control, Sai Control Chai 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 Imple]. Acquisition Imple]. Acquisition DDA	BR4_unfrac_165m (DDA), Trissue: (n/a Trissue n/a - Cerebellur - Muscle - Cerebrum - Ling - Ling - Heart - Spleen -
F5	ID F4.1 31Ma Sample 5 37 38 39 40 41 42 43 IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	UUSLO BGBS PCcBPogan FilesProteome Discover a 9/0013.DLR provate_tmdL_BR1_undtec_166mir_met/51_1 / rz 5 Sample Identifie 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prova 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prov 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prov 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prov 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prov 1 39/wp0101.DLR provate_tmdL_BR1_undtec_166mir_prov	w TMT 8plex Sample Type Control Sample Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Sample Type Quan Channel 126 127_C 128_C 129_N 129_N 129_C 130_C 131	129May3013_1 Control, Sai Control Chai 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 mmple). Acquisition - DDA - -	BR4_unfrac_165m (DDA). Tissue: [n/a n/a - Cerebellur - Muscle - Cerebrum - Lung - Liver - Heart - Spleen -
-5	ID F4.1 31Ma 5 37 38 39 40 41 42 43 ID ID	ULSS/0-66/85-PCc8Progam FilesProtome Discovers a y0/312_0/B_moxae_tmdBR1_unfac_155mie_mea15_1 / m Sample Identifier 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1 31May/0313_0.B_moxae_tmdBR1_unfac_155mie_mea1	w TMT Splex Sample Type Control Sample Sample Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Sample Type Quan Channel 126 127_C 128_C 129_N 129_C 130_C	29May3013_ : [Control, Sai Control Chai 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 Imple]. Acquisition Imple Acquisition DDA DDA DDA DDA DDA DDA DDA DD	BR4_unfrac_165m (DDA), Tissue: (n/a Tissue n/a • Cerebellur • Muscle • Cerebrum • Cerebrum • Liver • Heart • Spleen •
5	ID F4.1 31Ma Sample 5 37 38 39 40 41 42 43 ID F5.1	USS-0-8085-PC-dProgan Files/Potence Discover a \$2003.D.00, mose, mol, BRL, unke, 166m, mol 51, 1 /rz 30%/031.D.00, mose, mol, BRL unke, 166m, mol 51 30%/031.D.00, mose, mol, BRL unke, 166m, mol 51 30%/031.D.00, mose, mol, BRL unke, 166m, mol 53 30%/031.D.00, mose, mol, BRL unke, 166m, mol 13 30%/031.D.00, mose, mol BRL unke, 10000, mol 14 30%/031.D.00, mose, mol BRL unke, 10000, mol 14 30%/031.D.00, mose, mol 14 30%/031.D.0	w TMT Splex Sample Type Control Sample Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Sample Type Quan Channel 126 127_N 127_C 128_C 129_C 130_C 131	29May2013_1 : [Control, Sar Control Char 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 mple], Acquisition	BR4_unfrac_165m (DDA), Tissue: (n/a Tissue n/a - Cerebellur - Muscle - Cerebellur - Muscle - Cerebellur - Heart - Spleen -
-1	ID F4.1 31Ma Sample 5 37 38 39 40 41 42 43 ID F5.1	ULSS-0-6085-PC-dPhogan FilesProtome Discovers a y0013_DUB_mouse_tmdBR1_unfac_165mis_meat5_1 / m Sample Identifie 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_165mis_meat 31May/013_DB_mouse_tmdBR1_unfac_ 31May/013_DB_mouse_tmdBR1_unfac_	w TMT Splex Sample Type Control Sample Sample Sample Sample Sample Sample Sample	nouse_tmt8_data Sample Type Quan Channel 126 127_N 128_C 127_C 128_C 128_D_C 129_C 130_C 131	129May2013_1 : [Control, Sar Control Char 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 mple]. Acquisition DDA DDA DDA DDA DDA DDA DDA DD	BR4_unfrac_165m (DDA), Tissue: (n/a Tissue n/a Cerebellur Cerebellur Cerebellur Liver Heart Spleen BR1_unfrac_165m
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5	ID F4.1 31Ma 5 37 38 39 40 41 42 43 40 41 42 43 40 41 42 43 40 41 42 43 40 41 42 43 40 40 41 40 40 40 40 40 40 40 40 40 40	UUSL/0-B0BS-PCcdPhogan Files/Poteone Discovers as y0013_DUL_moset_med_BR1_winks_168m_mest[s]_in_ 3 Sample Isonities 3 Sampl	w TMT Spiex Sample Type Control - Sample Sam	with the second secon	29May2013_ : [Control. Sat Control Char 126 126 126 126 126 126 126 126	DJB_mouse_imt8 DJB_mouse_imt8 Acquisition Imple] Acquisition DDA	BR4_unfrac_165m (DDA) Tissue (n/m Tissue n/m All - Cerebellur - Muscle - Cerebrum - Liver - Heatt - Spleen - BR1_unfrac_165m (DDA IDA) Tissue Tissue
5	ID F4.1 31Ma 5 37 38 39 40 41 42 43 40 41 42 43 40 41 42 43 40 41 42 43 40 41 42 43 40 41 40 41 40 40 40 40 40 40 40 40 40 40	ULSLO-BGIBS-PCcBProgram Files/Proteome Discovers as y2013_DUB_musue_tmdl_BR1_unfrac_165min_meat5_1 / nz isongle lossnite 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_meat 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR1_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_BR2_unfrac_165min_trans1 31May/013_DB_mouse_tmdl_	w TMT Splex Sample Type Control Sample Type Control Sample Sample Sample Sample Sample Sample Sample Sample Sample Sample Sample Sample Sample Control Control	Sample Typy Quan Channel Sample Typy Quan Channel 126 127_N 127_N 128_C 128_C 128_C 130_C 131 131 13 Sample Typy Quan Channel Quan Channel Quan Channel 126 128_N	29May3013_ 2 [Control. Sat Control Char 126 126 126 126 126 126 126 126	DJB_mouse_imt8 nosis_mouse_imt8 nosis_mouse_imt8 nosis_mouse_imt8 DDA	BR4_unfrac_165m (DDA) Tissue: [n/a Tissue n/a - Cerebelum - Muscia - Cerebrum - Lung - Lung - Liver - Heart - Spleen - BR1_unfrac_165m (DDA, IDA). Tissue Tissue Con - tissue
5	ID F4.1 31Ma Sample 5 37 38 39 40 41 42 43 40 41 42 43 10 F5.1 31Ma 5 5 37 38 39 40 41 41 42 43 40 41 42 43 40 41 42 43 5 5 5 5 5 5 5 5 5 5 5 5 5	UUSLO BGBS PCcdPhogan Files/Poteone Discovers 15 9/0013_DUL_moset_med_BR1_unite_166m_mest[1] /m 3/0014_DUL_moset_med_BR1_unite_166m_mest[3] 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/0007013_DB_moset_med_BR1_unite_166m_mest 3/000703_DB_moset_med_BR2_unite_166m_mest	w TMT Splex Sample Type Control Sample Control Sample Sample Sample Sample Control Sample Samp	mouse_tmt8_data Sample Typy Quan Channel 126 127_N 127_N 128_C 128_C 130_C 131	29May3013_ 2 [Control. Sai Control Char 126 126 126 126 126 126 126 126	DJB_mouse_tmt8 DJB_mouse_tmt8 Acquisition Inno DDA	BR4_unfac_165m (DDA) Tissue n/a - 1 Cerebellur - Nuucle - Cerebellur - Heart - Spleen - BR1_unfac_165m (DDA (DA) Tissue (DDA (DA) Tissue Tissue KSdney - Cerebellur -
-5	ID F4.1 31Ma 5 37 38 39 40 41 42 43 Her: ID F5.1 31Ma Sample 6 44 45 5 5 5 5 5 5 5 5 5 5 5 5 5	UUSLO-BGBS-PCcBProgram Files/Proteome Discovers as y2013_DUB_musac_tmdl_BR1_unftac_165mic_meat5_1 / ra Sample Identifie 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac_165mic_meat 31May/013_DB_musac_tmdl_BR1_unftac 31May/013_DB_musac_tmdl_BR2_unftac 31May/013_DB_musac_tmdl_BR2_unftac 31May/013_DB_musac_tmdl_BR2_unftac.165mic_meat 31May/013_DB_musac_tmdl_BR2_unftac.165mic_meat 31May/013_DB_musac_tmdl_BR2_unftac.165mic_meat 31May/013_DB_musac_tmdl_BR2_unftac.165mic_meat	w TMT Splex Sample Type Control Sample	mouse_tmt8_data Sample Typy Quan Channel 126 127_N 127_C 128_C 130_C 131 Vame ousse_tmt8_data 128_C 133_ Vame Quan Channel 128_C 131	29May3013_ 20ntrol. Sas Control Char 126 126 126 126 126 126 126 126	DJB_mouse_imit8 mmkel_Acquisition: non-point DDA	BR4_unfrac_165m (DDA) Tissue (n/s Tissue n/s - Cerebelur - Muscle - Cerebrum - Lung - Lung - Lung - Lung - Lung - BR1_unfrac_165m (DDA, IDA) Tissue Tissue Tissue Tissue Muscle -

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).

Start Pa	nge 🗙 Study: Bailey_2014 * 🗙 🔤	taining folde	r 🕘 New Ar	alysis 🦪 Open Analysis Template	•
Study D	efinition Input Files Samples Analysis Results				
١Ď	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, Spleen]	
▶ F2	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1	.raw	TMT Splex •	Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
► F3	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1	.raw	TMT Splex •	Sample Type: [Control, Sample], Acquisition: [IDA]. Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
▶ F4	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1	.raw	TMT Splex •	Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
▶ F5	31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_	I .raw	TMT 8plex 🔹	Sample Type: [Control, Sample], Acquisition: [DDA], Tissue: [n/a, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
▶ F6	31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_	I .raw	TMT Splex •	Sample Type: [Control, Sample], Acquisition: [DDA]. Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
▶ F7	31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_	I .raw	TMT Splex •	Sample Type: [Control, Sample], Acquisition: [DDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
► F8	31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_	I .raw	TMT 8plex 💌	Sample Type: [Control, Sample]. Acquisition. (DDA). Tissue: [Kidney. Cerebellum, Muscle, Cerebrum, Lung, Liver, Heart, Spleen]	
► F8	31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_	l .raw	TMT Splex •	Sample Type [Control, Sample] Acquisition (DUN); Taske: [Notine]; Cetexenian; Nuode: Cetexinii, Lung, Liver, Heart, Spleni) Sample Type [Control, Sample]. Acquisition: [DDA], Taske: [Notine]; Cetexenian; Musde: Cetexinii, Lung, Liver, Heart, Spleni)	

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

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

View Adm	inistration Tools Window Help									
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Start Page >	x Study: Bailey_2014 * x									
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Study Definition Input Files Stamples Analysis Results										
Sample	Sample Identifier	Sample Type	A	cquisition	Tissue					
۲	• •		. 6		• •					
⊕ 51	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Control	- 11	DA .	Kidney +					
	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Control	+ II	DA -	Kidney -					
⊛ S3	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Control	- 1	DA -	- Kidney -					
⊕ 54	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1!	Control	- 1	DA .	Kidney •					
⊕ S5	31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae1	Control	* E	IDA .	r/a •					
⊕ S6	31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae1	Control	- 0	IDA 🔹	- Kidney -					
⊕ \$7	31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Control	- 0	IDA •	- Kidney -					
⊕ \$8	31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Control	* E	IDA 🔹	- Kidney -					
⊕ S9	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	· •	DA -	- Cerebellurr -					
	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	- 1	DA -	- Muscle -					
⊕ S11	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	· - 1	DA •	Cerebrum +					
	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	*	DA -	- Lung -					
⊛ \$13	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	- 1	DA -	- Liver -					
	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	- 1	DA -	Heart -					
⊕ \$15	29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1!	Sample	· •	DA •	- Spleen -					
	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	- 1	DA -	- Cerebellurr -					
 S17 	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	- 1	DA •	- Muscle -					
	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	·	DA •	- Cerebrum -					
⊕ \$19	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	· •	DA -	- Lung -					
	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	- 1	DA -	Liver -					
⊕ S21	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	· - 1	DA •	Heart -					
	29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda1!	Sample	* II	DA .	Spleen -					
❀ \$23	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	- 1	DA -	- Cerebellurr -					
 S24 	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	- 1	DA .	Muscle +					
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❀ S26	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	- 1	DA -	- Lung -					
 £ 527 	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	- 1	DA 🖣	Liver +					
 • S28 	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	* II	AC •	Heart -					
€ \$29	29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda1!	Sample	- 1	A ,	- Spleen -					
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 • 531 	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1!	Sample	* 1	DA .	Muscle •					
	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1!	Sample	- II	A .	Cerebrum •					
	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1!	Sample	- 1	DA -	- Lung -					
	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda1!	Sample	- 1	DA -	Liver +					
G35	29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15	Sample	· 11	DA -	Heart v					
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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, **I**, 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.

Sample Identifier	Sample Type		Acquisition	Tissue	
• •	٠	*	• DA • 7,		
31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae1	Control	*	DDA +	n/a •	
31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae1	Control	-	DDA -	Kidney -	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Control		DDA -	Kidney -	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Control	-	DDA -	Kidney +	
31May3013_UJB_mouse_tmt8_BR1_unfrac_165min_mae1	Sample	*	DDA •	Cerebellurr •	
31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae1	Sample	*	DDA •	Muscle •	
31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae1	Sample	*	DDA +	Cerebrum *	
simayouis_usB_mouse_tmt8_BR1_untrac_165min_mae1	Sample	*	UUA +	Lung *	
31May3013_DJB_mouse_tmt8_BR1_untrac_165min_mae1	Sample	•	DDA +	Liver -	
31May3013_DJB_mouse_tmt0_DR1_unfrac_100min_mae1	Sample	•	DUA +	Heart +	
31May3013_DJB_mouse_units_BR1_unitac_165min_mae1	Sample	•	DUA *	Spieen •	
21May2013_DJB_mouse_tmt9_BR2_unitac_165min_mae1	Sample		DDA *	Musele -	
21May2013_DJB_mouse_tmt8_BR2_unfrac_165min_mae1	Sample	•	004 *	Corohoura	
31May3013 DIB mouse tmt8 BB2 unfrac 165min mae1	Camelo		DDA -	Lung -	
31May3013 DIB mouse tmt8 BB2 unfrac 165min mae1	Sample		DDA -	Line -	
31May3013 DJB mouse tmt8 BB2 unfrac 165min mae1	Sample		DDA +	Heart -	
31May3013 DJB mouse tmt8 BB2 unfrac 165min mae1	Sample		DDA +	Soleen -	
31May3013 DJB mouse tmt8 BR3 unfrac 165min mae1	Sample		DDA -	Cerebellurr •	
31May3013 DJB mouse tmt8 BR3 unfrac 165min mae1	Sample		DDA -	Muscle -	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Sample	*	DDA +	Cerebrum +	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Sample		DDA -	Lung -	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Sample		DDA -	Liver -	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Sample		DDA -	Heart -	
31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae1	Sample		DDA -	Spleen -	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample		DDA -	Cerebellurr +	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample	*	DDA -	Muscle •	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample	*	DDA -	Cerebrum +]
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample	*	DDA -	Lung -	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample		DDA +	Liver -	
31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae1	Sample		DDA -	Heart -	
				1	
	Generation of the second	① ① ① ① ① ① ② ① ① ② ○ ① ① ○ ○ ○ ① ○	① ① ① ①	3 - 0 + 0 N 3 - 0 - 0 N 0 N 11449/0312, DLB_movae_Intell_BRIT_undres_156mm_man1 Cotad - 0.0.A - 0.0.A 11449/0312, DLB_movae_Intell_BRIT_undres_156mm_man1 Cotad - 0.0.A - 0.0.A 11449/0312, DLB_movae_Intell_BRIT_undres_156mm_man1 Cotad - 0.0.A -	① ① ○

- 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.

You can save a study manually or automatically.

- 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, $|\mathbb{T}_{\mathbf{x}}|$

Saving a Study

You can save a study manually at any time. A change in a study that requires yo

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, 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 the **Reprocess** icon, Steps (to open both the process), 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, 🔒 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. At some point before you click the Run icon, 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 the New Analysis icon, 🐏 New Analysis.

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

File View Administration Tools Window Help			
Start Page X Study: Bailey_2014 * X			▼ 4 Þ
K Add Files 🤐 Add Fractions 🎉 Remove Files 🔍 Open containing fold	der 🔮 New Analysis 🕼 Open Analysis Template		
Study Definition Input Files Samples Analysis Results Workflows	Grouping & Quantification	Analysis	🗌 As Batch 🎯 Run 📙 Save 🗙
ID Name File Type	e 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, Cerebn	Consensus Step	A x
F2 29May3013_DJB_mouse_tmt8_BR2_unfrac_165min_dda15_1 .raw	TMT 8plex + Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebn		
F3 29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda15_1 .raw	TMT 8plex + Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebr	Workflow:	
F4 29May3013_DJB_mouse_tmt8_BR4_unfrac_165min_dda15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [IDA], Tissue: [Kidney, Cerebellum, Muscle, Cerebn	Result file: Enter result :	s name.
F5 31May3013_DJB_mouse_tmt8_BR1_unfrac_165min_mae15_1 .raw	TMT 8plex • Sample Type: [Control, Sample]. Acquisition: [DDA], Tissue: [n/a, Cerebellum, Muscle, Cerebrum	Thild Steps: (1)	Add
F6 31May3013_DJB_mouse_tmt8_BR2_unfrac_165min_mae15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [DDA], Tissue: [Kidney, Cerebellum, Muscle, Cereb		
F7 31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mae15_1 .raw	TMT 8plex V Sample Type: [Control, Sample], Acquisition: [DDA], Tissue: [Kidney, Cerebellum, Muscle, Cereb	Processing Step 🔍	Clone 🔼
F8 31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mae15_1 .raw	TMT 8plex Sample Type: [Control, Sample], Acquisition: [DDA], Tissue: [Kidney, Cerebellum, Muscle, Cereb	Workflow	
		Result file: Fater arm	file came
		Input Files: (0)	
			Drop your input files here
Ready			
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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, \bigcirc , 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.

Para	ameters		
]ੈ⊉↓ │ Show Advanced Par	rameters	
4	1. Input Data		*
	Protein Database	Swissprot2.fasta	
	Enzyme Name	Trypsin (Full)	
	Max. Missed Cleavage Sites	2	
	Min. Peptide Length	6	
	Max. Peptide Length	144	
4	2. Tolerances		
	Precursor Mass Tolerance	10 ppm	
	Fragment Mass Tolerance	0.6 Da	
	Use Average Precursor Mas:	False	
	Use Average Fragment Mass	False	
4	3. Spectrum Matching		
	Use Neutral Loss a lons	True	Ξ
	Use Neutral Loss b lons	True	
	Use Neutral Loss y lons	True	
	Use Flanking lons	True	
	Weight of a lons	0	
	Weight of b lons	1	
	Weight of c lons	0	
	Weight of x lons	0	
	Weight of y lons	1	
	Weight of z lons	0	
4	4. Dynamic Modification	\$	
	Max. Equal Modifications Pe	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	
4	5. Dynamic Modification	s (peptide terminus)	
	 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	Ŧ

This figure shows a complete example of the processing workflow.

File View Administration Tools Window Help				
Start Page X Study: Bailey_2014 * X				• 4
Add Files 🚓 Add Fractions 💥 Remove Files	S Q Open containing folder S New Analysis G Open Analysis Template			
Study Definition Input Files Samples Analy	ysis Results Workflows Grouping & Quantification		Analysis	🗌 As Batch 🎲 Run 🛃 Save 🗙
Data Inst.	📕 👔 Upen 👩 Upen Lommon 🚡 Save 👩 Save Common 🖓 Auto Layout 👗 Clear			
all Spectrum Files	Workflow:		Consensus Step 🔍	<u> </u>
Construct & Control Datained	Description	^	Workflow:	
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Spectrum Selector	Workflow Tree		= 0.127 (0.000 (d))	
Spectrum Procession			₩ CANO Steple: (1)	Agg
Noise Peak Filter			Processing Step 🔍	Clone 🔔
Non-Fragment Filter	Spectrum Files 0		Madellerer	
Spectrum Grouper			Result file: 29May3013 DJB mouse tmt8 BB1 unfr	rac 165min dda15 1 msf
🥪 Spectrum Normalizer			- Innut Films (P)	
🧔 Top N Peaks Filter			White rises (b)	
Spectrum Filters	Event Detector 1 A Reporter Ions 4 Spectrum		x F1 29May3013_DJB_mouse_tmt8_BR1_unfra	c_165min_dda15_1 TMT 8plex Sample Type: [Control, S
💓 Scan Event Filter	Quantifier 4 Selector 2		x F2 29May3013_DJB_mouse_tmt8_BR2_unfra	c_165min_dda15_1 TMT 8plex Sample Type: [Control, S
💓 Spectrum Confidence Filter			x F3 29May3013_DJB_mouse_tmt8_BR3_unfra	c_165min_dda15_1 IMI 8plex Sample Type: [Control, S
💓 Spectrum Properties Filter	+ +		x F4 29May3013_DJB_mouse_tmt8_BR4_unfra	c_165min_dda15_1 TMT Splex Sample Type: [Control, S
Sequence Database Search			x F5 31May3013_DJB_mouse_tmt8_BR1_unfra	c_165min_mae15_1 TMT 8plex Sample Type: [Control, S
Mascot	Area Detector 5 Sequest HT 3		× P6 31May3013_D3B_mouse_tmts_BR2_unita	5_165min_mae15_1 TMT spiex Sample Type: [Control, S
🤣 PMI-Byonic			x F7 31May3013_D3B_mouse_tmt8_BR3_unitra	5_165min_mae15_1_TMT splex_Sample Type: [Control, S
🤣 PMI-Preview			X P8 31May3013_D3B_mouse_tmto_BR4_unira	5_165min_mae15_1 TM1 opiex Sample Type: [Control, S
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Spectral Library Search	Percolator 6			
😿 MSPepSearch	•			
PSM Validation				
I Fixed Value PSM Validator				
Percolator				
🐁 Target Decoy PSM Validator				
Quantification				
🖧 Precursor Ions Area Detector				
A Precursor Ions Quantifier				
💩 Reporter Ions Quantifier				
😑 Data Export				
🭻 Spectrum Exporter				
Workflow Nodes Parameters	<	+		
Ready				

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, 🛔 Save .

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

- 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, 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 containing an exclamation mark in the upper right corner of the Processing Step box (1) 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.

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dy Definition Input Files Samples Analysis Re	esults Workflows Grouping & Quantification		Analysis	🗌 As Batch 🥳 Run 📙 Sa
neters	🦹 Open 🎇 Open Common 👗 Save 🔣 Save Common 💥 Auto Layout 🛛 其 Clear			
e Advanced Parameters	Workflow: Raieu 2014		Consensus Step	
Spectrum Storage Settings Spectra to Store Identified or Quantified	Description:	*	Workflow: Bailey_2014	
Merce Mode Blobalu bu Search Engine Tupe		Ŧ	Result file: 29May3013_DJB_mouse_tmt8_BR1_unfrac_165min_dda1	15_1.pdResult
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2. Score			x F3 29May3013_DJB_mouse_tmt8_BR3_unfrac_165min_dda	15_1 TMT Splex Sample Type: [Cor
2. Threshold 0			× F4 29Mav3013 DJB mouse tmt8 BR4 unfrac 165min dda	15 1 TMT 8plex Sample Type: (Cor
3. Score	+		v E5 21Mau/2012 D IR moure trat? PP1 upfrag 165min mai	a15.1 TMT Poley Sample Tune (Cor
3. Threshold 0				15_1 THT Opex Sample Type [Col
4. Score 4. Threshold 0	Peptide 2		X F6 31May3013_03B_mouse_tmts_BH2_untrac_160min_mail	alb_1 IMT spiex Sample Type: [Con
5 Score	(*** Validator =)		x F7 31May3013_DJB_mouse_tmt8_BR3_unfrac_165min_mail	e15_1 TMT Splex Sample Type: [Cor
5. Threshold 0			x F8 31May3013_DJB_mouse_tmt8_BR4_unfrac_165min_mas	e15_1 TMT 8plex Sample Type: [Cor
6. Score			<	
6. Threshold 0				
7. Score	Septide and			
B. Score	Protein Filter			
8. Threshold 0				
9. Score				
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		E		
ctra to Store tiles the spectra to store in the result file: net: no spectra are stored,	Result 7 Statistics 7 Distributions 8			

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, 👗 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 containing an exclamation mark in the upper right corner of the Consensus Step box (1) 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, 📙 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.

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.

	- Study Variables
	Tile
	Quan Channel
Variable placement handle	Tissue
	Acquisition
	Sample Type
	Variables printed in italics contain only a single value.

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, **F**.
 - 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.

Bulk Ratio Generation	
Denominators to be used:	
 Tissue : Kidney Tissue : Cerebellum Tissue : Heart Tissue : Cerebrum Tissue : Lung Tissue : Liver Tissue : Muscle Tissue : Spleen 	
	Add Ratios

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).

	Bulk Ratio Generation							
	Denominators to be used:							
lcon for multiple selection of the denominator	Denominators to be used:	A III						
	Acquisition : IDA Acquisition : IDA Tissue : Cerebrum Acquisition : IDA Acquisition : IDA Acquisition : IDA Acquisition : IDA	Ratios						
	Add	nauos						

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.

Autority A study, bancy_2014 A Automisti autor		
add Files 🤹 Add Fractions 💥 Remove Files 😡 Op	en Containing Folder 💮 New Analysis 🎲 Open Analysis Template	Analysis
Sample Group and Quan Ratio Specification	Generated Sample Groups	
Sample Group and Quan Ratio Specification - Study Variables	Generated Sample Groups DDA Kidney 126 Sample DDA 126 Sample DDA 126 Sample DDA 127 Sample DDA 128 Sample DDA 129 Sample DDA 126 Sample DDA 127 Sample DDA 127 Sample DDA 127N Sample DDA 127C S	Consensus Step ▲ Workflow: Result File: 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 pdResult ▼ Child Steps: (1) Ad Processing Step ▲ Workflow: Clone Result File: 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 mdR Workflow: Result File: 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 mdF Result File: 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 TMT8 x F3 31May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_mae15_1 TMT8 x F3 31May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_mae15_1 TMT8 x F5 31May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_mae15_1 TMT8 x F6 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 TMT8 x F6 29May3013_DJ8_mouse_tmt8_BR4_unfrac_165min_dda15_1 TMT8 x F6 29May3013_DJ8_mouse_tmt8_BR1_unfrac_165min_dda15_1 TMT8 x F8 29May3013_DJ8_mouse_tmt8_BR1_unfrac_165min_dda15_1 TMT8 x F8 29May3013_DJ8_mouse_tmt8_BR3_unfrac_165min_dda15_1
I Tissue : Spleen ▲ Acquisition : IDA	X IDA Cerebellum / IDA Kidney X IDA Heart / IDA Kidney	
Tissue : Cerebellum	X IDA Cerebrum / IDA Kidney X IDA Lung / IDA Kidney X IDA Liver / IDA Kidney	

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, 💥 Clear All, 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.

File View Administration Tools Window Help		
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Start Page X Study: Bailey_2014 * X Workflow Editor X Workflow Editor X Administration	m X WorkflowEditor X 29Mey3013_DJB_mouse_tmt8_BR1_unfrac_165min_dde15_1 X	- 4 ⊅
🙀 Add Files 🍓 Add Fractions 💢 Remove Files 📢 Open Containing Folder 🏐 New Analysis 🍏 Op	en Analysis Template	
Study Definition Input Files Samples Analysis Results Workflows Grouping & Quantification		Analysis 🖂 As Batch 💣 Run 🕌 Save 🗙
Sample Group and Quan Ratio Specification	Generated Sample Groups	
Study Variables	6 of 8 sample groups not used (*) in any ratio definition.	Consensus Step 💫 🗙
Sody Vinites So	6 dl auroja grupo not und (*) in ny najo definito. 6 dl auroja grupo not und (*) in ny najo definito. 7 200 (*) 100	Consens Spir A × Videbar: Off. Spiraterials: Extended Analation: Solar: Application: Spiraterials: Solar: Application: Spiraterials: Solar: Application: Spiraterials: Solar: Application: Spiraterial: Spirateria
(Addition)		

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, 💥 Clear All, 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.

Start Page × Study: Bailey_2014 * × WorkflowEditor × WorkflowEditor × 29May	013_DJB_mouse_tmt8_BR1_unfrac_165min_dda15_1 x test x ExampleTMT x		
Latrings X Study table, 2014 X Workflow Editor X Workflow Editor X 2014 J 4d4 Files Ad4 Fractors X More Reverse File Ad4 Fractors New Analysis J 4d4 Files Ad4 Fractors X More Reverse File New Analysis New Analysis J 4d4 Files Ad4 Fractors X More Reverse File New Analysis New Analysis State Y Y New Analysis New Analysis New Analysis State Y Y New Analysis New Analysis State Y New Analysis New A	Quen Analyses Template Open Analyses Template Template Open Ana	Acadyan As Both Converses Ster FA, Violifion: Conf. Comprehension, Ethensel Annotation, Quan Beach Re: 2016;(0)(1),Quance, prid, Bell, unitsc., 105m, doi:10,107m, doi:10,10	Run 🛃 Sa
Validational in India control only a single value.	Territ Sande Loo, Gendellan II v 2016;00(1),00(1	Wear/Feet dB > F1 2886,0013,038,mone_stml,0884,writes,166m,utd015,1 TMT Bdes, Sample Type Sample > F2 3186,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes, Sample Type Sample > F3 3186,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes, Sample Type Sample > F3 3186,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes, Sample Type Sample > F3 3186,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes, Sample Type Sample > F4 3186,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes, Sample Type Sample > F4 2886,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes > F4 2886,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes > F4 2886,2013,038,mone_stml,0874,writes,166m,utd01,1 TMT Bdes > Sample Type Sample > mT	, Acquinition: [D , Acquinition: [] [] , Acquinition: [] [] , Acquinition: [] [] , Acquinition: [] , Acquinition: [] , Acquinition: []

* 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.

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dy Cultifue (Inset Files) Bandes (Analysis Files) Volutilions) Company & Constitutions) and Cana Redo Specification Constraints Cons	Concents Sample Group P 101 Sample DDL Form #1 (2004)(211), 200, means, pril. (2014), and pril. (201	Analyses Conserves ther AA Conserves ther AA Web/Sec: Conf. Comparison on Enhanced Annotation, Date Reveal Reveal Network (Non-Addition) Ministry Conf. Comparison on Enhanced Annotation, Date Reveal Reveal Network (Non-Addition) VEX.State (7) Processing State (A) With Reveal Reveal Reveal Annotation (Non-Addition) Reveal Reveal Reveal Reveal Annotation (Non-Addition) Reveal Reveal Reveal Reveal Reveal Annotation (Non-Addition) Y (2014) 2010; Diagnoona and Bill Andreveal (Non-Addition) Y (2014) 2010; Diagnoona (Non-Bill Andreveal (Non-Addition) Y (2014) 2014; Diagnoona (Non-Bill Andreveal (Non-Addition) <th>As Batch, Proc. (*) Sec end and Mit Spice Secret Type (Secret), Accuston (r) Mit Spice Secret Type (Secret), Accu</th>	As Batch, Proc. (*) Sec end and Mit Spice Secret Type (Secret), Accuston (r) Mit Spice Secret Type (Secret), Accu

* 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 the **Run** icon, 🔐 Run

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.

Performing the Search

Performing a Search in Individual Mode



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).

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Job Queue		Execution State	Progress	; Туре	Name	Submitted at	∇ Study	Data
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tent Management 2		Waiting	0 %	Consensus	SILAC_HeLa_cell-(02)	11/28/2014 1:44 PM	SILAC_hela	C:\Development1
FASTA Files	۲	Running	20 %	Processing	SILAC_HeLa_cell-(02)	11/28/2014 1:44 PM	SILAC_hela	C:\Development1
FASTA Indexes								
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Processing Settings Of Massol MSF Files MSF Files Protein Center Protein Center Server Settings Server Settings PASTA Indexes	4							×
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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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