

Configuring Proteome Discoverer 2.2 for Compatibility with Scaffold

Scaffold can load data from Proteome Discoverer and numerous quantitative options are supported. However, PD must be configured properly to ensure Scaffold has access to both identification and quantitative data.

To begin a few notes:

1. Scaffold reads the MSF files created by Proteome Discoverer. For version 2.2 both the MSF and the PDStudy file must be in the same directory.
2. Make sure to **Save All** in PD before loading files into Scaffold
3. When running the Daemon Scaffold needs access to the PARAMS files instead of the PDStudy file. Make sure this is in the same directory as the MSF files.

Note, at this time Scaffold does not support custom quantitative methods created in PD. Please use the default methods for iTRAQ, TMT or SILAC quantification.

External Document Resources

- [File Compatibility Matrix](#)
- [System Requirements](#)
- [Installation Guide](#)

Suggested Delta Cn and FDR Settings

Scaffold uses either the LFDR or PeptideProphet algorithms to calculate peptide probabilities. The algorithms will provide better results if they have access to all of the PSMs generated by the search engine. By default, the Delta Cn value in PD is set to 0.01. It is recommended that this setting be adjusted before results are loaded into Scaffold. Additionally, setting the FDR value to 1.0 or 100% will give Scaffold access to lower scoring PSMs.

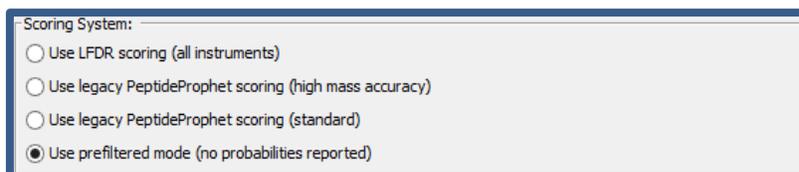
The recommended validation node for use in workflows is the Target Decoy PSM Validator. If loading PD results into Scaffold do not use the Percolator node. The maximum Delta Cn allowed by percolator is 0.1.



The Maximum Delta Cn and target FDR are set to 1. This setting is located in the Target Decoy PSM Validator node

Pre-Filtered Mode

Scaffold 4.8.1 introduced Pre-Filtered Mode which allows users to bypass Scaffold’s built in probability model and filter your data using the search engine instead. If you would like to use this option you should not set the FDR value to 1.0 as described in the section above but set the value to an acceptable FDR cutoff. If you use Pre-Filtered Mode you will not be able to filter your data in Scaffold using the Protein and Peptide dropdown menus.

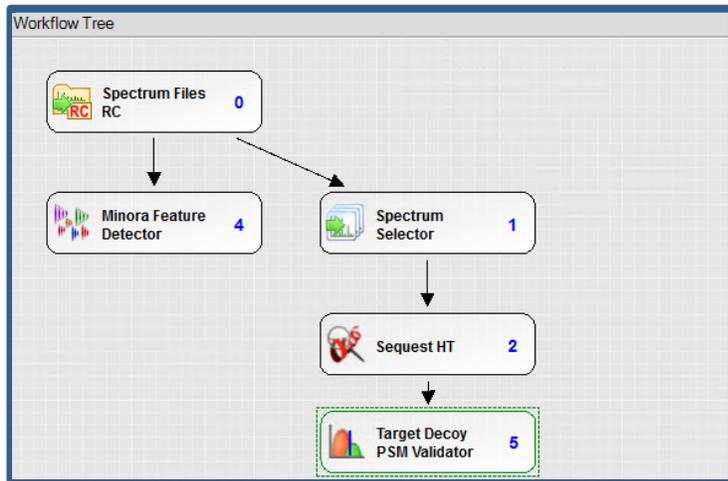


The Pre-Filtered option is found in the Scoring System section of the Load and Analyze Data step of Scaffold’s Load Data Wizard

Quantitative Workflows

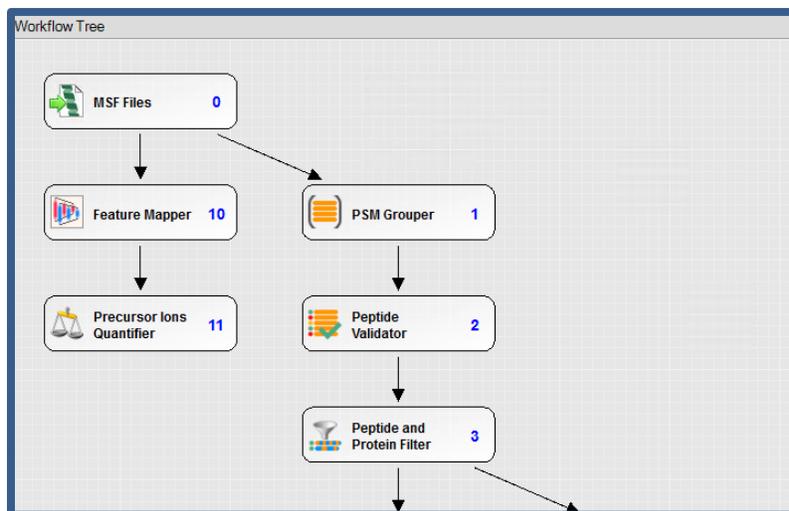
Scaffold supports multiple types of quantitative experiments when loading files from Proteome Discoverer including precursor intensity, reporter ion (iTRAQ or TMT), and precursor ion (SILAC) quantitation. Each type of experiment requires different quantitative nodes in the Processing and Consensus workflows. Changes were made for PD 2.2 so workflows are slightly different than those found in PD 2.1.

Precursor Intensity Quantitation



Parameters of 'Target Decoy PSM Validator'	
Hide Advanced Parameters	
1. Input Data	
Maximum Delta Cn	1
Maximum Rank	0
2. Decoy Database Search	
Target FDR (Strict)	1
Target FDR (Relax)	1
Concatenated FDR	False

A typical processing workflow for precursor intensity quantitation with the Target Decoy PSM validator Node FDR parameters shown. Note the Minora Feature Detector Node



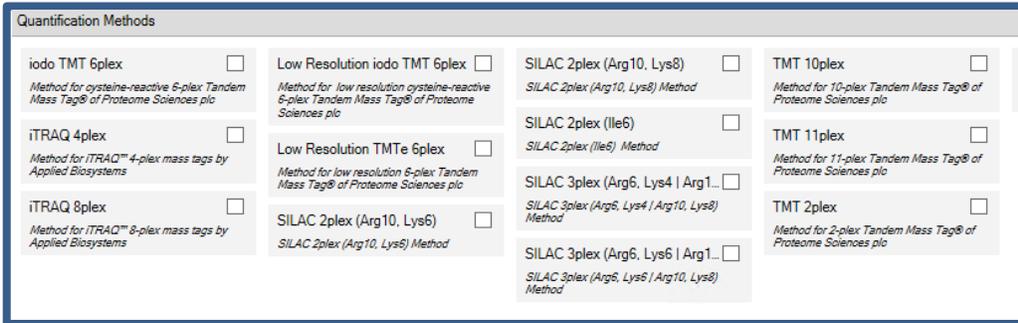
A portion of the consensus workflow for precursor intensity quantitation. Note the Precursor Ions Quantifier.

To set up the workflow above:

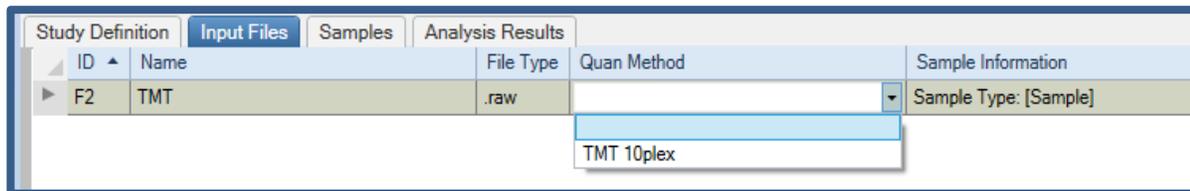
1. Use the "PWF_QE_Precursor_Quan_and_LFQ_SequestHT_Percolator" Processing workflow. Remove the Percolator node and use the Target Decoy PSM Validator node instead. Set the DeltaCN, and FDR to 1. Use which ever search engine you like.
2. Set the processing parameters such as modifications and tolerances appropriately for the data that was captured.
3. Use the "CWF_Comprehensive_Enhanced Annotation_LFQ_and_Precursor_Quan" Consensus workflow

Reporter Ion Quantiation

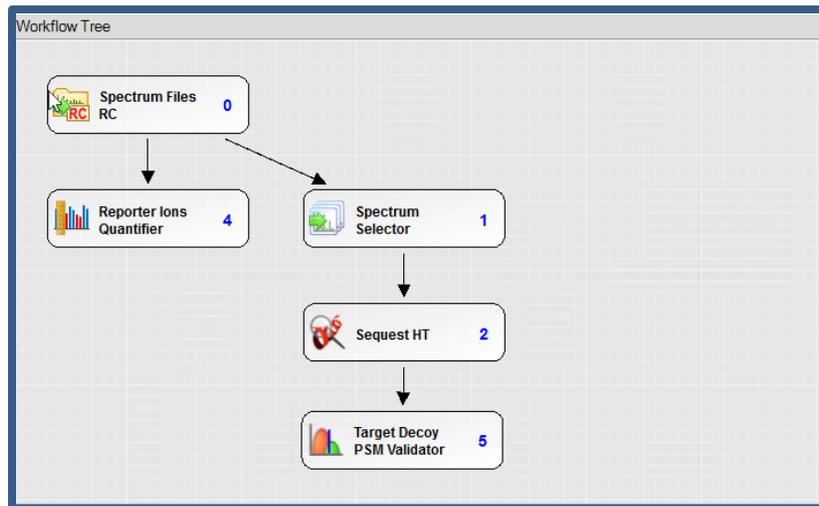
The following workflows should be used for iTRAQ or TMT experiments.



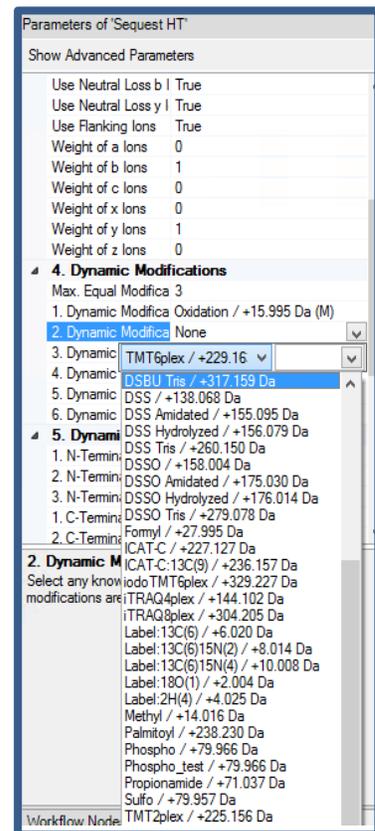
Start by selecting the proper Quant method in the Study Definition tab.

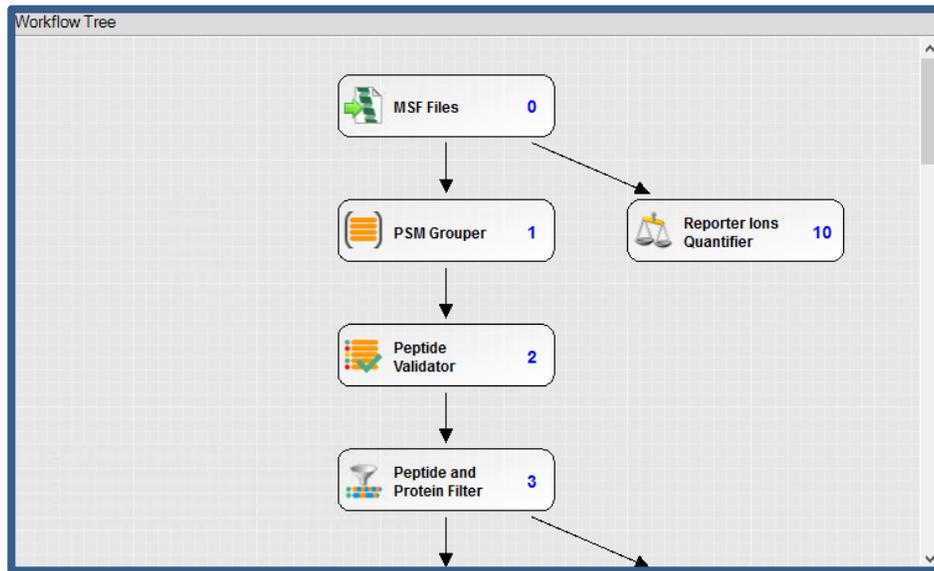


Chose the proper quant method for each file in the Input Files Tab



Example Processing workflow for TMT quantitation. Note the Reporter Ions Quantifier node. The proper modifications must be set in the search engine parameters.





A portion of the Consensus workflow for Reporter Ion Quantitation. Note the Reporter Ions Quantifier

To set up the workflow above:

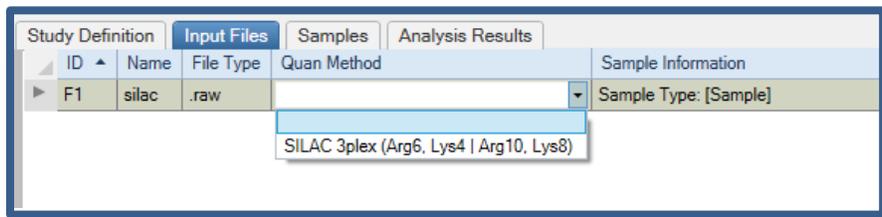
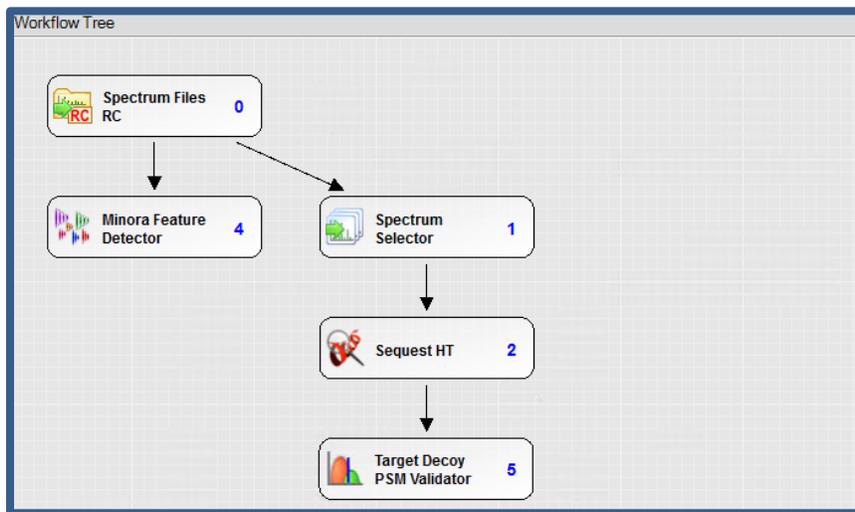
1. Use the “PWF_QE_Reporter_Based_Quan_SequestHT_Percolator” Processing workflow. Remove the Percolator node and use the Target Decoy PSM Validator node instead. Set the DeltaCN, and FDR to 1. Use which ever search engine you like.
2. Set the processing parameters such as modifications and tolerances appropriately for the data that was captured. Make sure the proper quantitative modifications are selected.
3. Use the “CWF_Comprehensive_Enhanced Annotation_Reporter_Quan” Consensus workflow
4. If using the Mascot node as opposed the Sequest HT node the iTRAQ Or TMT modifications can be set using the “From Quan Method” dropdown in the Modification Groups section of the search engine parameters. They do not need to be defined as dynamic modifications when this is done.

SILAC Quantification

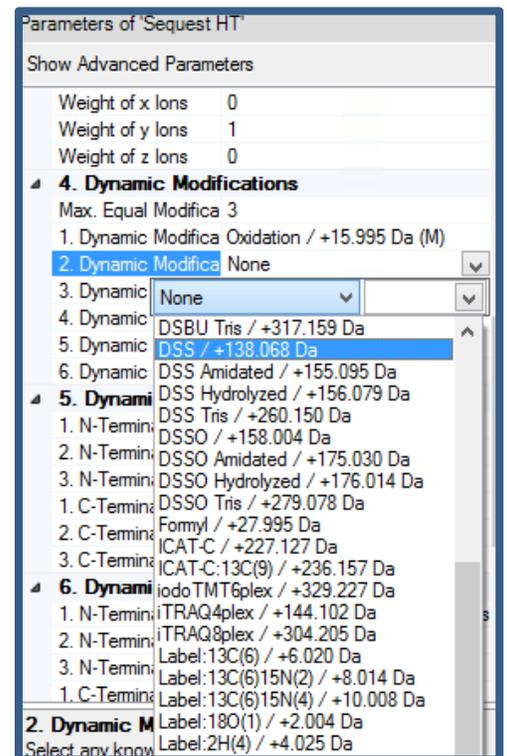


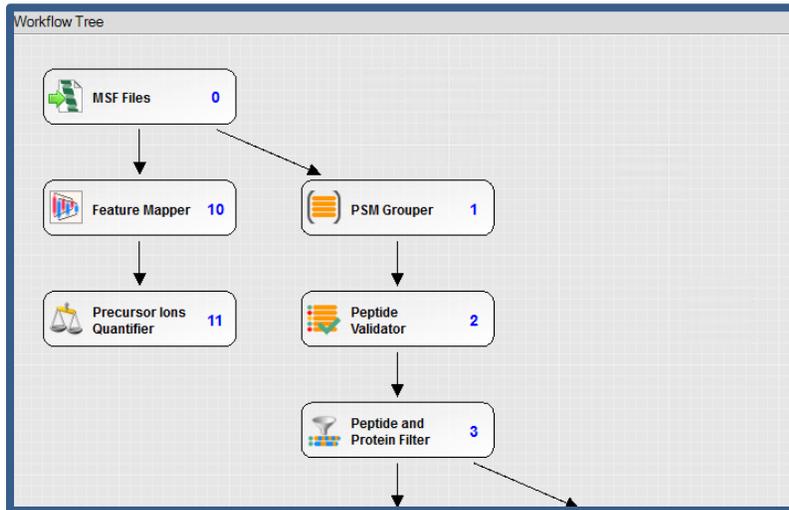
Start by selecting the proper Quant method in the Study Definition tab.

Chose the proper quant method for each file in the Input Files Tab

An example Processing workflow for SILAC data. Note the Minora Feature Detector. Make sure to choose the proper quantitative modifications in the search engine configuration parameters





A portion of the Consensus workflow for SILAC quantitation. Note the Precursor Ions Quantifier.

To set up the workflow above:

1. Use the “PWF_QE_Precursor_Quan_and_LFQ_SequestHT_Percolator” Processing workflow. Remove the Percolator node and use the Target Decoy PSM Validator node instead. Set the DeltaCN, and FDR to 1. Use which ever search engine you like.
2. Set the processing parameters such as modifications and tolerances appropriately for the data that was captured.
3. Use the “CWF_Comprehensive_Enhanced Annotation_LFQ_and_Precursor_Quan” Consensus workflow.
4. If using the Mascot node as opposed the Sequest HT node the iTRAQ Or TMT modifications can be set using the “From Quan Method” dropdown in the Modification Groups section of the search engine parameters. They do not need to be defined as dynamic modifications when this is done.