Configuring Proteome Discoverer 2.1 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 2.1 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.

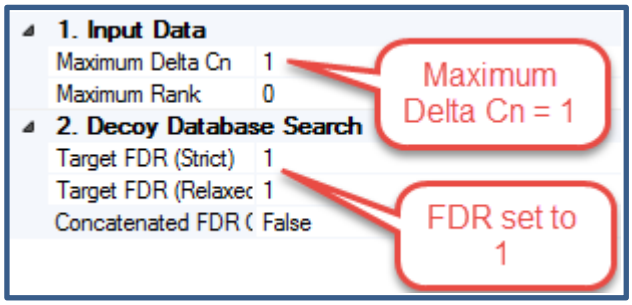
External Document Resources

- 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.

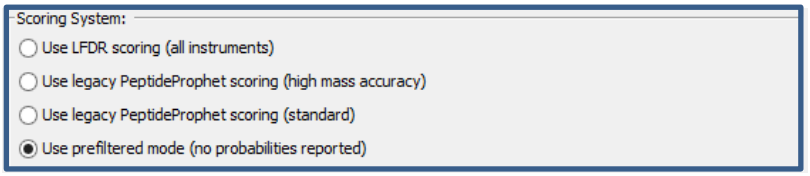
The recommended validation node for use in workflows is the Target Decoy PSM Validator. If loading PD results into Scaffold do not use the Percolator node. The maximum Delta Cn allowed by percolator is 0.1.



Note: 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.

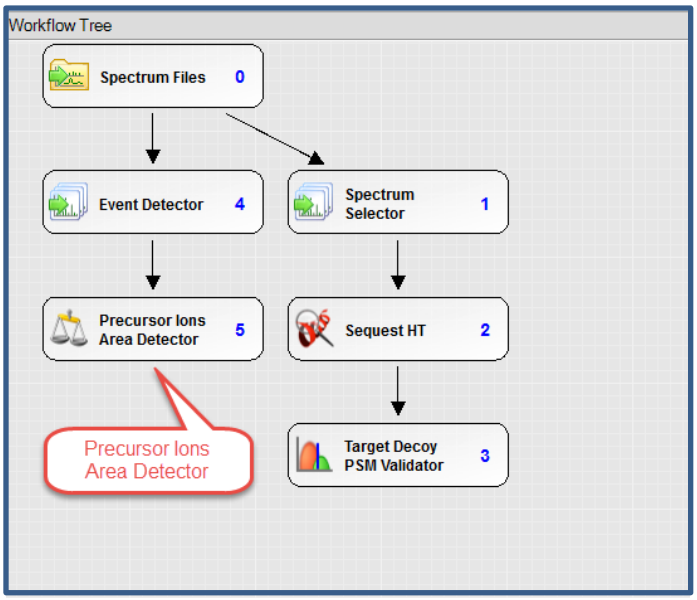


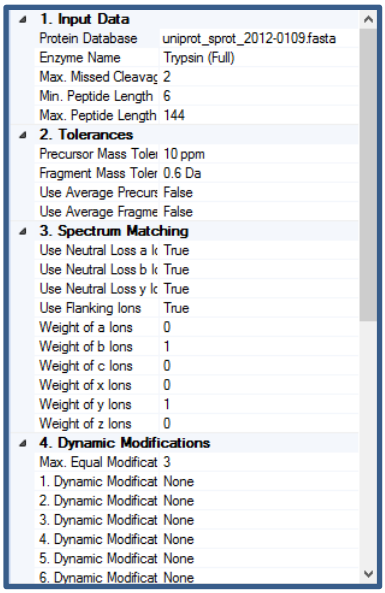
The Pre-Filtered option is found in the Scoring System section of the Load and Analyze Data step of Scaffold's Load Data Wizard

Quantitative Workflows

Scaffold supports multiple types of quantitative experiments when loading files from Proteome Discoverer including iTRAQ and TMT, SILAC, and precursor intensity quantitation. Each type of experiment requires a different quantitative node in the processing workflow:

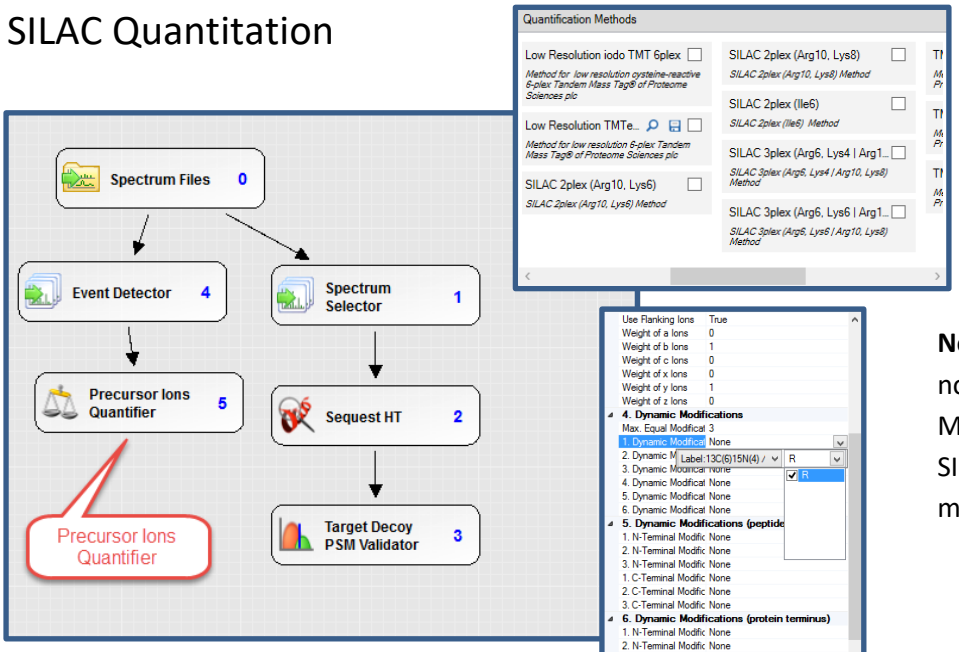
Precursor Intensity Quantitation





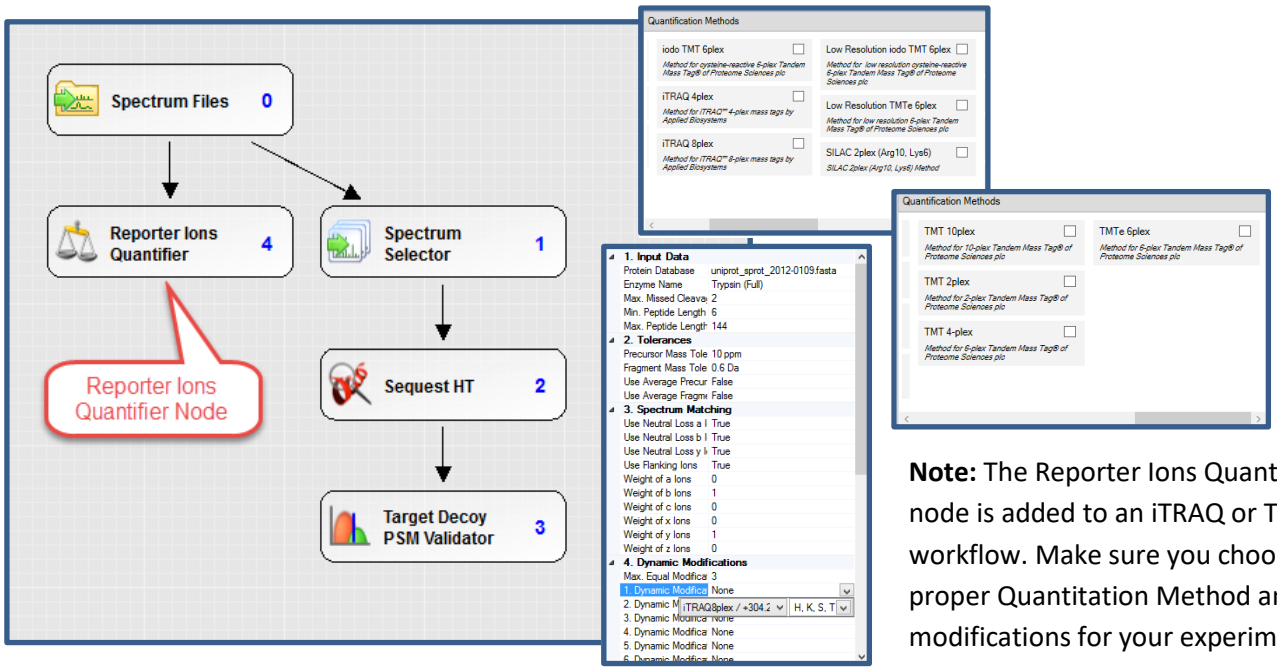
Note: The Precursor Ions Area Detector is added to the workflow for precursor intensity quantitation. The image on the right displays the configuration of Sequest HT parameters. This is where the FASTA file, enzyme and modifications are set.

SILAC Quantitation



Note: The Precursor Ions Quantifier node is added to a SILAC workflow. Make sure you choose the proper SILAC Quantitation Method and modifications for your experiment

Reporter Ion Quantitation



Note: The Reporter Ions Quantifier node is added to an iTRAQ or TMT workflow. Make sure you choose the proper Quantitation Method and modifications for your experiment.