GETTING STARTED GUIDE – PROTEIN METRICS' PREVIEW™ AND BYONIC™ NODES IN PROTEOME DISCOVERER 2.4

Key Points:

- **Installation: You must install both the node and the corresponding standalone application**
- **Usage: When running Preview/Byonic, use the templates "PMI-Preview Template.pdAnalysis" and "PMI-Byonic Template.pdAnalysis" as starting points**
- **Preview and Byonic work well together. Preview is quick (typically runs in less than one minute) and generates suggested parameters for a more thorough search using Byonic**
- **Updates. Please check for software updates periodically**

CONTENTS

INSTALLATION

Step 0. You must have a licensed and installed copy of Proteome Discoverer 2.4.

Step 1. Run the installers for the Preview node and/or the Byonic node. Note that there are separate installers for the Preview node and the Byonic node.

Step 2. If not installed already, install standalone Preview and Byonic (part of the Protein Metrics software suite), available from http://proteinmetrics.com/. Make sure the license is activated.

INTRODUCTION

These notes describe the basic usage of Proteome Discoverer 2.4 nodes for Protein Metrics' Preview and Byonic search engines. These notes focus on the features that are specific to Proteome Discoverer 2.4. For more details on Preview and Byonic (e.g., setting parameters or interpreting results), please refer to the Preview and Byonic user manuals at https://www.proteinmetrics.com/support-information/#preview-help and https://www.proteinmetrics.com/support-information/#byonic-user-manual.

WORKING WITH OTHER NODES

Preview is a very quick and simple search engine

- Preview automatically generates suggested parameters for a subsequent Byonic search – see the "Using Preview and Byonic Together" section below.
- Preview produces text output (formatted as HTML) rather than lists of proteins and peptides that can be used as input to other nodes.
- There is generally no utility in connecting Preview with nodes other than those shown in the workflow diagram under "Running Preview" – see below. We recommend using the analysis template "PMI-Preview Template.pdAnalysis" as a starting point.

A Byonic workflow generally requires fewer nodes compared to other database search engines:

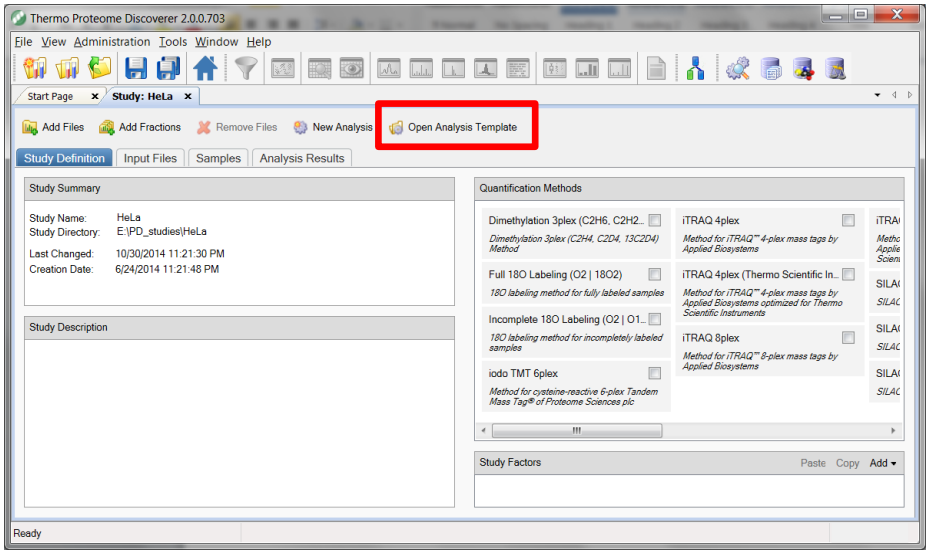
- Though possible, we do not recommend using a PSM Validation node (e.g., Percolator) in a Byonic workflow because Byonic already has its own machine learning optimization and FDR calculation.
- In our experience, spectrum pre-processing generally does not help Byonic and often make results worse. In particular, for ETD data, we do not recommend using the "Non-Fragment Filter" node because Byonic can intelligently handle ETD spectra with large precursor peaks.
- Nodes such as the annotation and quantification nodes are compatible with the Byonic node.
- See the example workflow diagram under "Running Byonic." We recommend using the analysis template "PMI-Byonic Template.pdAnalysis" as a starting point.

RUNNING PREVIEW

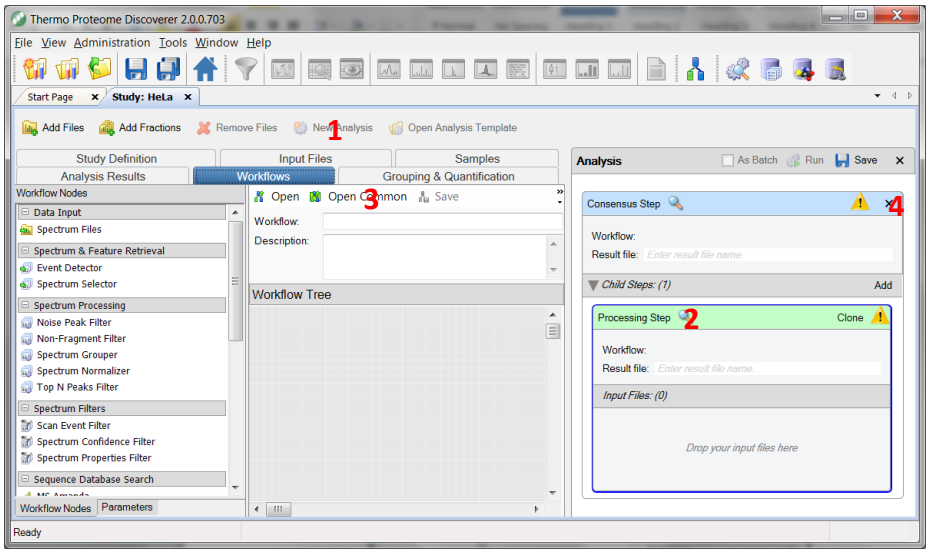
We recommend using the analysis template "PMI-Preview Template.pdAnalysis" as a starting point.

Steps to load this template:

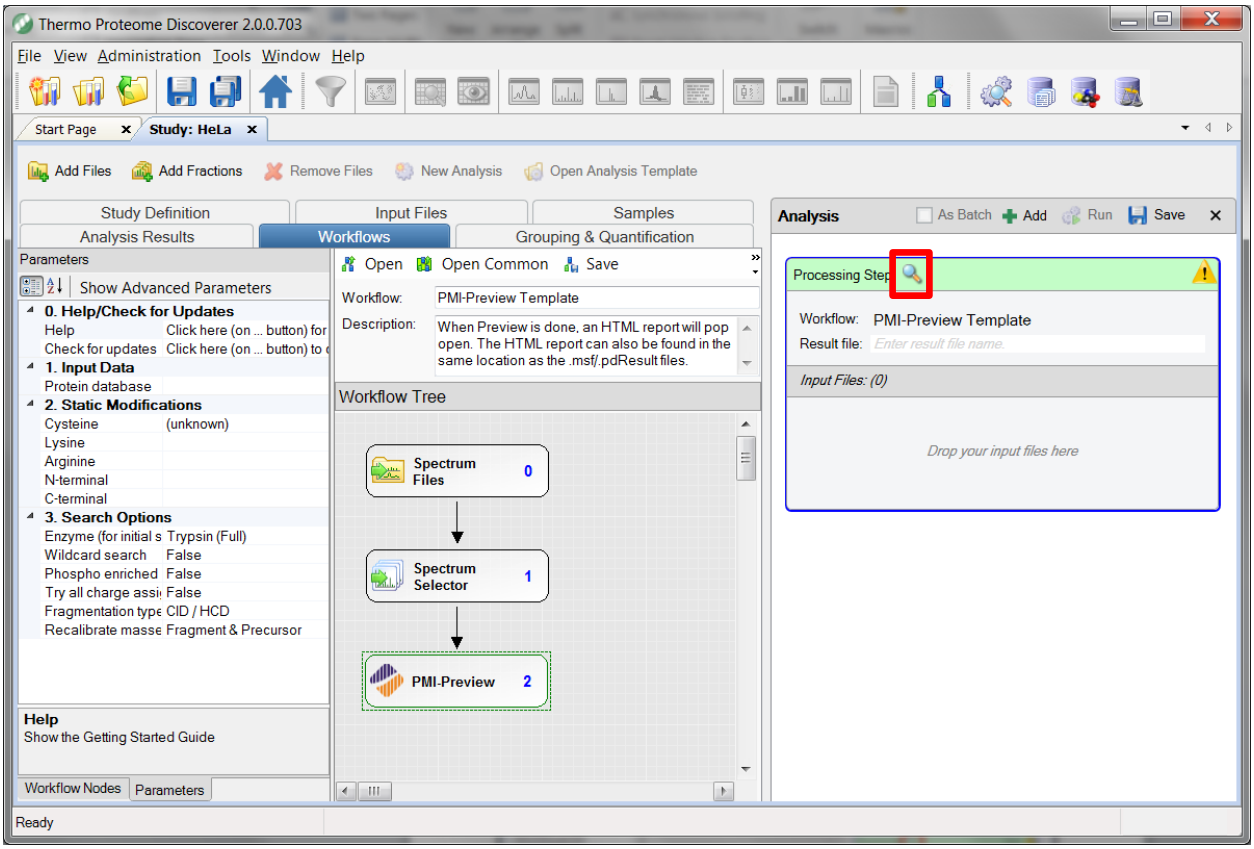
1. Create/open a study.
2. Do ***either:***
    a. Click the "Open Analysis Template" button and browse to find "PMI-Preview Template.pdAnalysis" in the Proteome Discoverer 2.4 standard workflows/templates folder (typically "C:\Users\Public\Documents\Thermo\Proteome Discoverer 2.4\Common Templates")



    b. ***Or*** click the "New Analysis" button, load the processing workflow template "PMI-Preview Template.pdProcessingWF", and delete the consensus workflow (click the buttons in the order given below)



To open the processing workflow, click on the “Show Workflow” icon in “Processing Workflow.”



Click on a node to see its parameters.

When Preview is done, an HTML report will pop open. The HTML report can also be found in the same location as the .msf/.pdResult files.

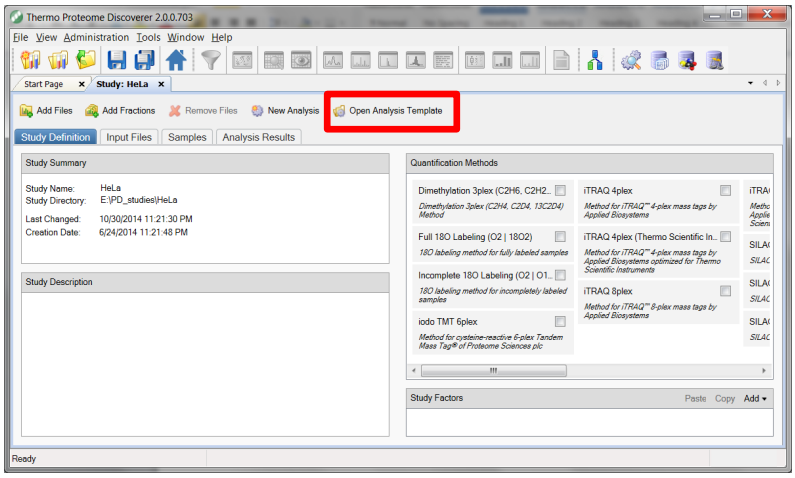
RUNNING BYONIC

We recommend using the analysis template "PMI-Byonic Template.pdAnalysis" as a starting point.

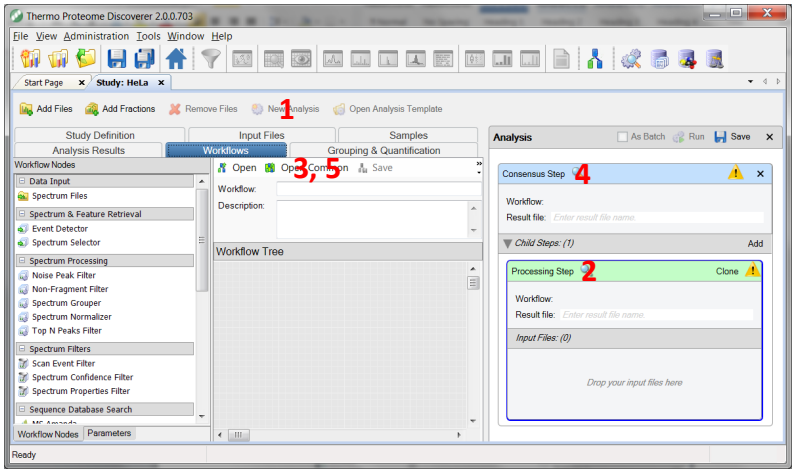
- For the Byonic node – this template uses the Byonic node's default parameters.
- **For other nodes in the workflow – this template sets some parameters that are optimized for Byonic and differ from the Proteome Discoverer default values** (for details, see the notes at the end of this section).

Steps to load this template:

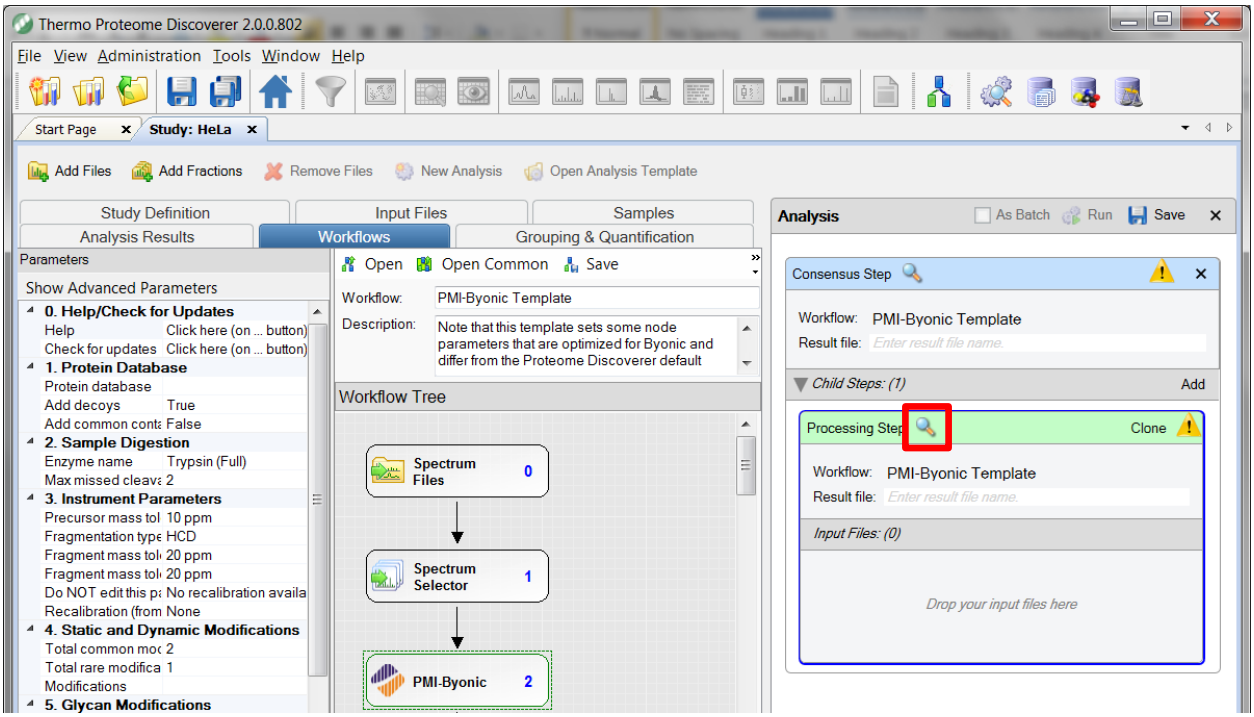
1. Create/open a study
2. Do ***either:***
    a. Click the "Open Analysis Template" button and browse to find "PMI-Byonic Template.pdAnalysis" in the Proteome Discoverer 2.4 standard workflows/templates folder (typically "C:\Users\Public\Documents\Thermo\Proteome Discoverer 2.4\Common Templates")



    b. ***Or*** click the "New Analysis" button and then load the processing workflow template "PMI-Byonic Template.pdProcessingWF" and the consensus workflow template "PMI-Byonic Template.pdConsensusWF" (click the buttons in the order given below)



To open the processing workflow, click on the “Show Workflow” icon in “Processing Workflow.”



Click on a node to see its parameters. To see the advanced parameters, click the “Show Advanced Parameters” button above the list of parameters.

Notes on the processing workflow:

- In the “Spectrum Selector” node, the “Max. Precursor Mass” is set to 0 Da, which means no limit – any precursor mass is allowed. This setting is critical when analyzing topdown data and can be important when analyzing glycopeptide data.
- Also in the “Spectrum Selector” node, the “MS Order” scan event filter is set to “Any.” This allows MS^1 spectra to pass through into the Byonic node. Unlike most other database search nodes, Byonic can re-analyze the MS^1 for precursor charge and m/z. This is particularly important for topdown.

Notes on the consensus workflow:

- In the “Peptide Validator” node, “Validation Mode” is set to “Automatic (Without control of peptide level error rate)” to prevent the consensus workflow from overwriting the confidences set by the Byonic node.
- In the “Peptide and Protein Filter” node, the peptide filters are set to allow through as many peptides as possible:
    1. “Peptide Confidence At Least” is set to low.
    2. “Keep Lower Confident PSMs” is set to true.
    3. “Minimum Peptide Length” is set to 4 (lowest possible setting).
- In the “Protein Grouping” node, parsimony is disabled. Byonic can identify multiple peptides from a single MS/MS spectrum, but parsimony allows only one peptide identification per spectrum.

USING PREVIEW AND BYONIC TOGETHER

Preview generates, in addition to an HTML report, a suggested Byonic search (provided that Preview made a sufficient number of good identifications). **Note that this is a suggested search. In general, the parameters should be reviewed and, if necessary, modified to fit the particular experiment.** Preview is good at finding modifications that are consistently found sample-wide (for example, in vitro modifications from sample handling and processing). However, Preview may miss post-translational modifications that are found only on a few proteins, and Preview does not look for glycopeptides. If you are interested in finding specific post-translational modifications or glycopeptides, you should carefully review and adjust the Byonic parameters suggested by Preview.

To load the suggested Byonic search, open the .suggestedByonicSearch.pdAnalysis file, which is named similarly to the Proteome Discoverer .msf/.pdResult files. As an example, if the data file is named 250ng_Hela.raw, the default .msf file created by the Proteome Discoverer Preview search is named 250ng_hela.msf, and the suggested Byonic search is named 250ng_Hela.suggestedByonicSearch.pdAnalysis.

Also note that the locations of the Preview HTML report and the suggested Byonic search (.suggestedByonicSearch.pdAnalysis file) are shown in the search details in the job queue.