PROTEIN METRICS

GETTING STARTED GUIDE – PROTEIN METRICS' PREVIEW™ AND BYONIC™ NODES IN PROTEOME DISCOVERER 1.4

Key Points:

- **Installation: You must install both the node and the corresponding standalone applications**
- **Usage: When running Preview/Byonic, use the templates "PMI-Preview Template" and "PMI-Byonic Template" 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**

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INSTALLATION

Step 0.   You must have a licensed and installed copy of Proteome Discoverer 1.4 SP1 (version 1.4.1.14).

Step 1.   Run the installer for the Protein Metrics' nodes for Proteome Discoverer 1.4. Note that a single installer installs both the Preview node and the Byonic node together.

Step 2.   If not installed already, install standalone Preview and standalone Byonic (**version v2.2.0 or later**), available from http://proteinmetrics.com/. Note that there are separate installers for standalone Preview and standalone Byonic. Make sure the license is activated. (To request a license, launch the standalone application and go to Help -> Register).

INTRODUCTION

These notes describe the basic usage of Proteome Discoverer 1.4 nodes for Protein Metrics' Preview and Byonic search engines. These notes focus on the features that are specific to Proteome Discoverer 1.4. For more details on Preview and Byonic (e.g., setting parameters or interpreting results), please refer to the Preview and Byonic user manuals at http://www.proteinmetrics.com/products/preview/preview-help/ and http://www.proteinmetrics.com/products/byonic/byonic-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 workflow template "PMI-Preview Template" 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 workflow template "PMI-Byonic Template" as a starting point.

RUNNING PREVIEW

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

To load this template, go to Workflow Editor -> Open From Template and select "PMI-Preview Template."
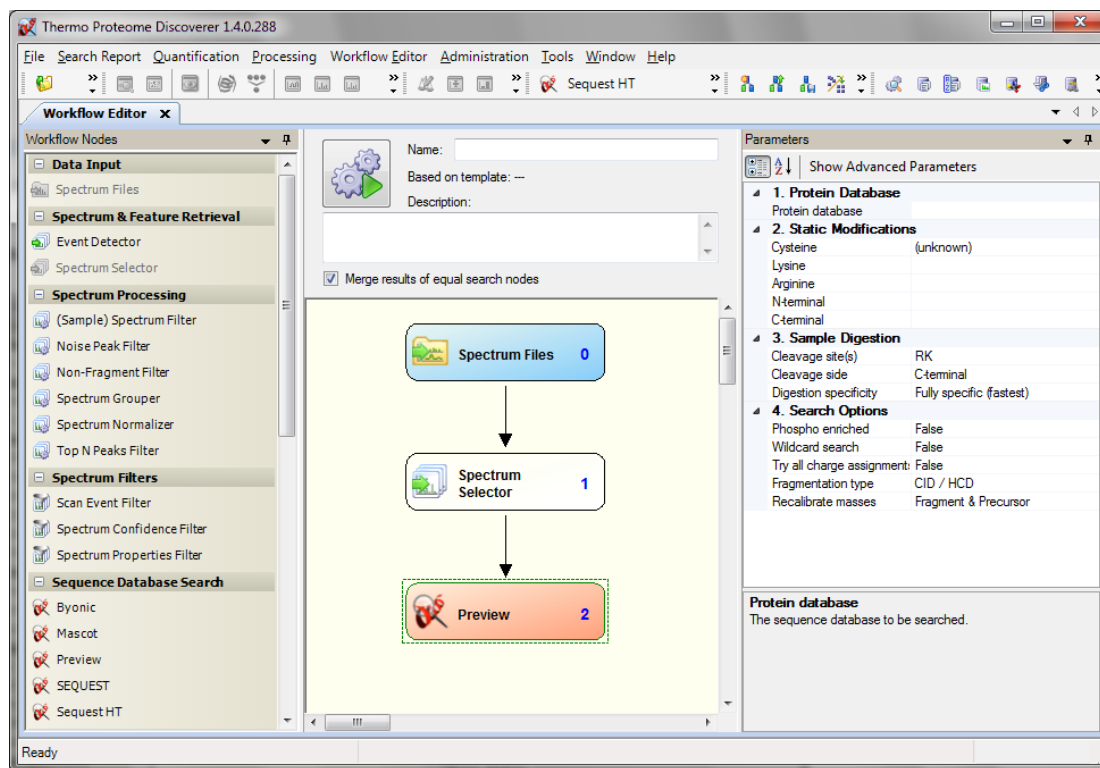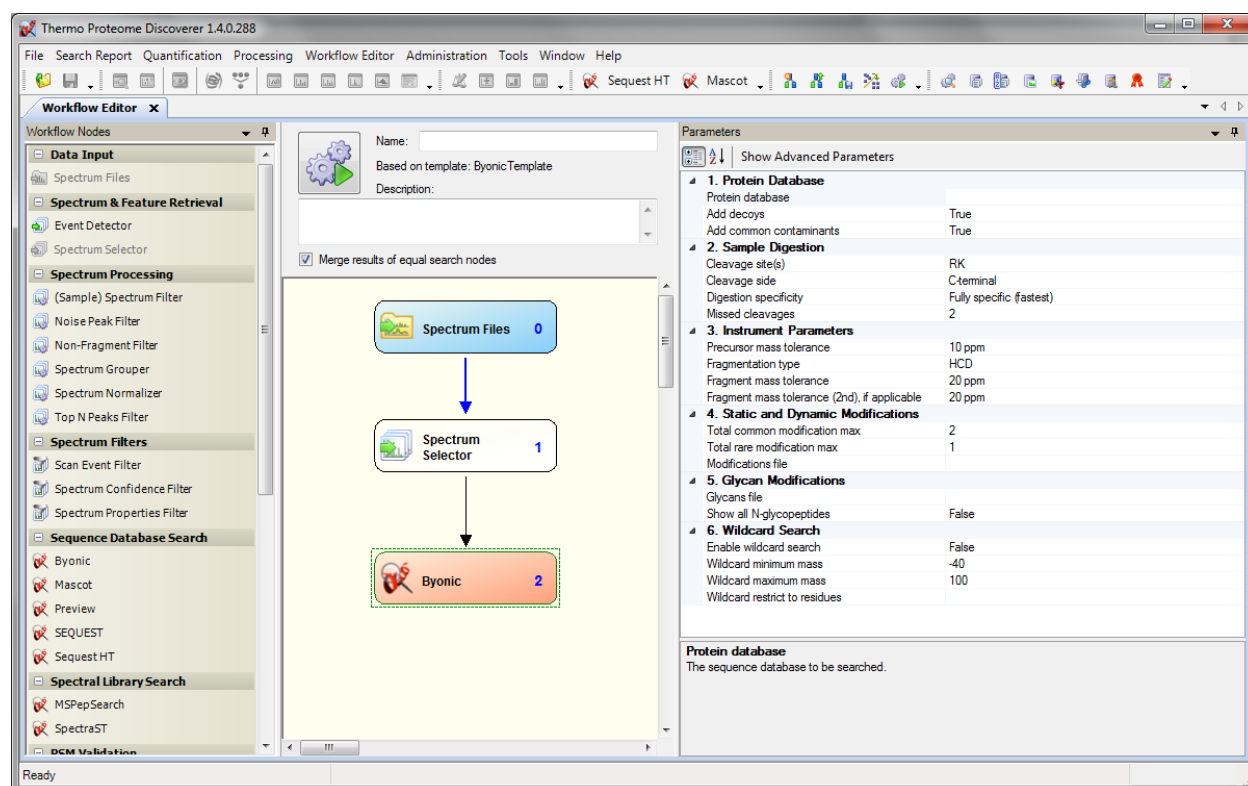

Click on a node to see its parameters.

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

RUNNING BYONIC

We recommend using the workflow template "PMI-Byonic Template" 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).

To load this template, go to Workflow Editor -> Open From Template and select "PMI-Byonic Template."

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:

- Create the modifications file and the glycans file using the "Modifications UI (PD 1.4 Tool)" utility program, which can be launched from the Windows start menu: go to All Programs -> PMi-Byonic -> Modifications UI (PD 1.4 Tool). See screen shot below.
- In the "Spectrum Selector" node, the default "Max. Precursor Mass" for this template has been set to 10000 Da, the highest possible setting.
- Also in the "Spectrum Selector" node, the "MS Order" scan event filter is set to "Is MS1; MS2; MS3; MS4; MS5; MS6; MS7; MS8; MS9; MS10" (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.

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- When viewing search results, you must adjust the result filters if you wish to see protein groups similar to standalone Byonic. In the "Result Filters" tab, click on "Protein Grouping" Settings and change "Consider only PSMs with confidence at least" to "Low" (default setting is "Medium"). See screen shot below.
- When viewing MS/MS fragment ion annotation (double-click on a peptide in the Peptides table), we recommend adjusting the options, especially if you searched multiple fragmentation types. Also see screen shot below.
  1. Uncheck "Use search settings" – Byonic can handle multiple different fragment mass tolerances, but Proteome Discoverer cannot. The workaround is to have match tolerances set based on the mass analyzer (ITMS or FTMS) rather than on the search engine settings.
  2. Lower the "Annotation Threshold" – We find the factory default setting (5% of base peak) is often too high, especially on ETD spectra or glycopeptide spectra.
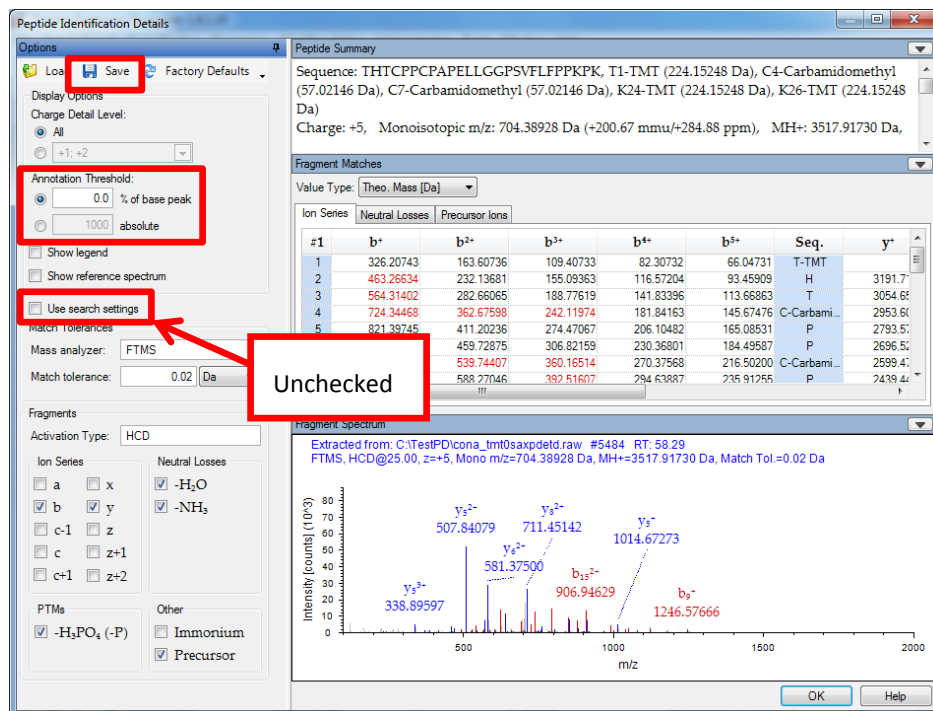 3. Click "Save."

Launching the "Modifications UI (PD 1.4 Tool)" utility program
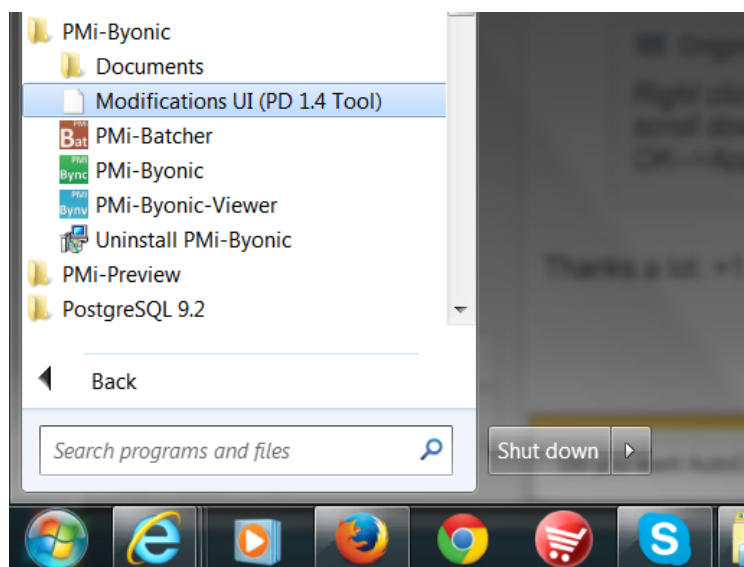
Recommended result filter for viewing Byonic results
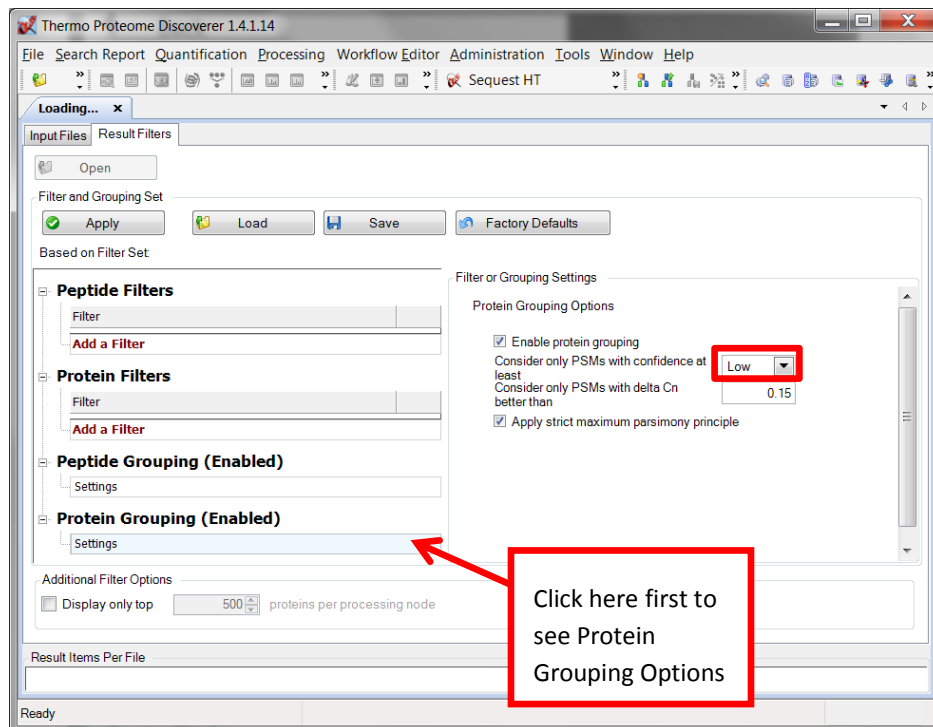
Recommended options for viewing MS/MS fragment ion annotation

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.workflow.xml file, which is named similarly to the Proteome Discoverer .msf file. 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.workflow.xml.

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