Protein Metrics Supernovo™ User Guide

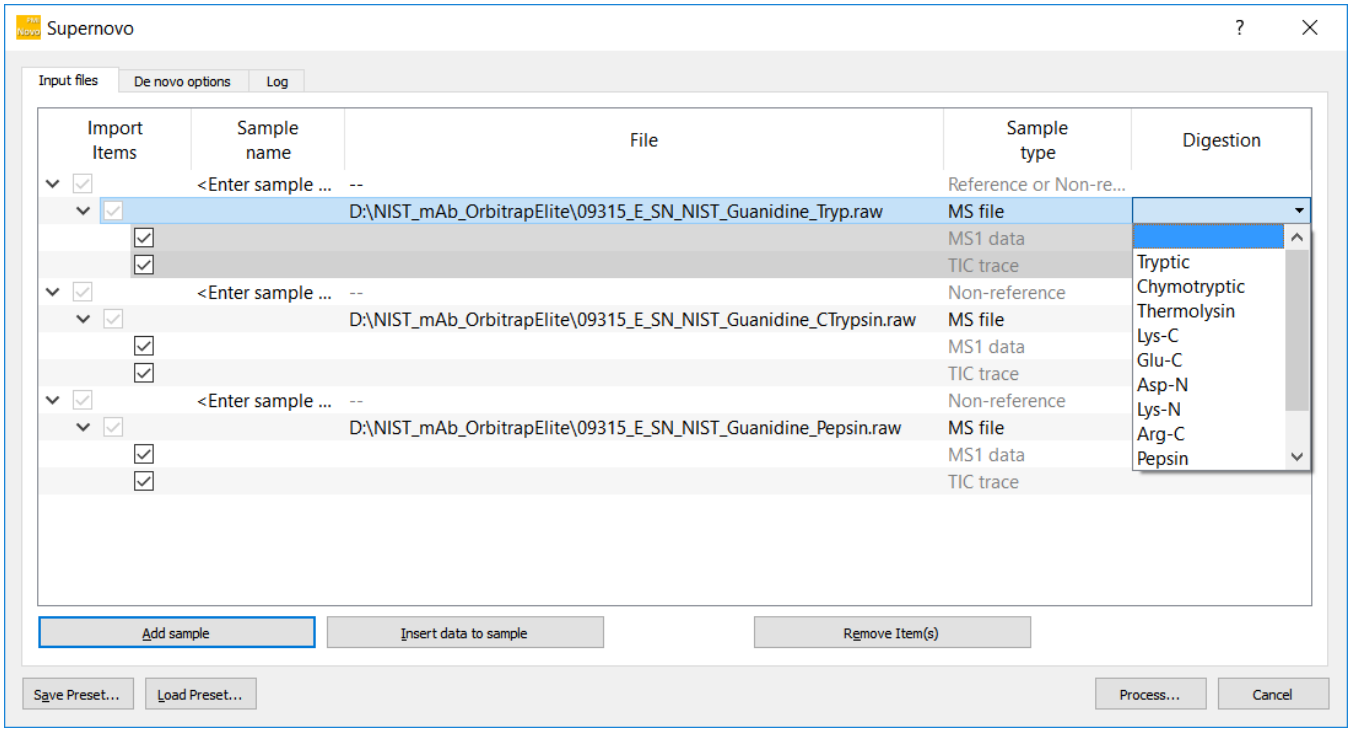
Note: We recommend using Supernovo on a good 64-bit multi-core computer with a solid state drive. A solid state drive improves performance greatly. All Supernovo results (.blgc files) should be saved to the solid state drive.

Running a Supernovo Analysis

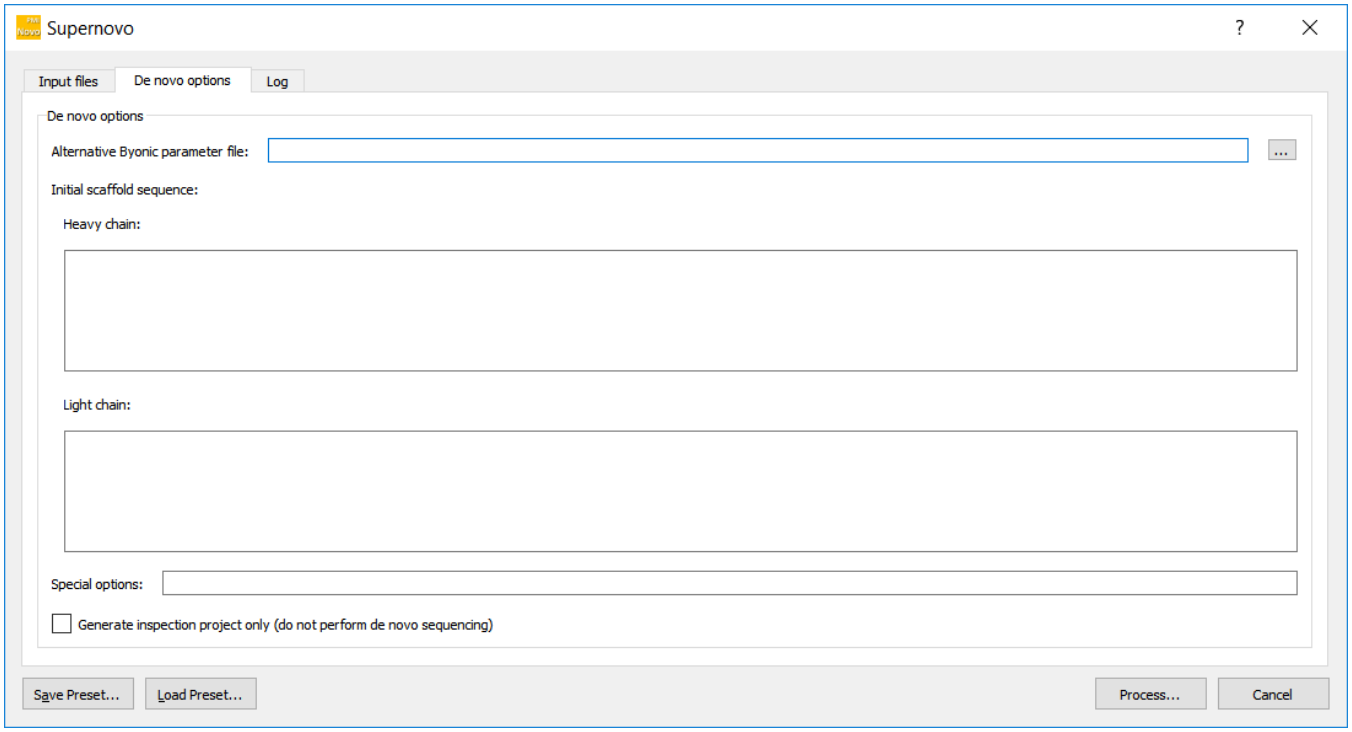
Step 1: Launch PMi-Supernovo. Go to the File menu and select "New Project."

Step 2: Drag-and-drop MS data files onto the "Input files" tab. We recommend that you use at least three to four files, each with a different digestive enzyme. Suggested enzymes include trypsin, pepsin, elastase, and chymotrypsin.

There is a pull-down option under the "Digestion" column in the row for the MS file. If you choose the digestion type (not required), then the sequence coverage map will have color coding for the different enzymes used.



Step 3: Set parameters on the "De novo options" tab. In many experiments, the default settings (leave all entries blank/unchanged) work fine.



Alternative Byonic parameter file

- If left blank, Supernovo uses a default Byonic parameter file, which specifies 10 ppm precursor mass tolerance, 20 ppm fragment mass tolerance, and carbamidomethyl (delta mass +57) for the cysteine alkylation.
- To use an alternative Byonic parameter file, use Byonic to edit and save a .byparms file. Use one of the example .byparms file in the byparms folder as a starting point.

Initial scaffold sequence. If left blank, Supernovo will automatically figure out an antibody scaffold sequence as the starting point for de novo analysis. This works best for antibodies from human, mouse, rat, or related species.

Generate inspection project only. Check this box if you only wish to generate metrics/visualizations for a particular sequence that you specify. Supernovo will not perform de novo sequencing for this inspection project (runs faster).

Special options. Leave this blank generally.

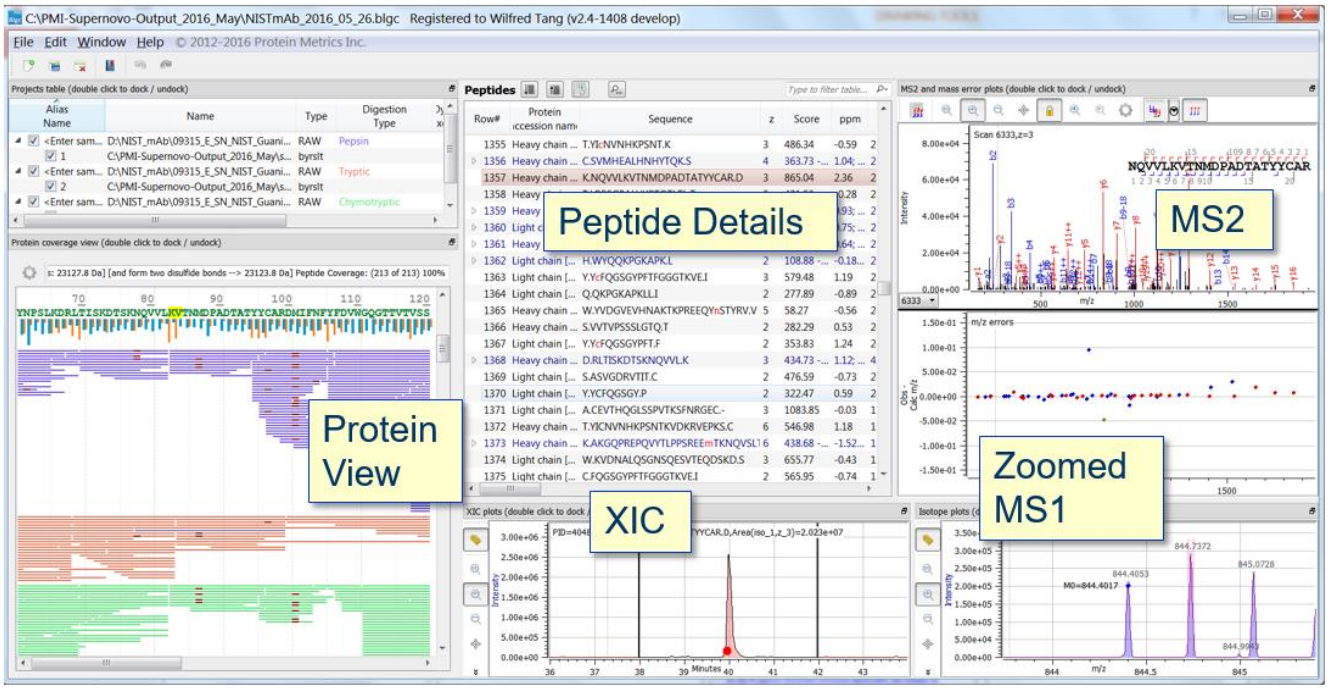
Step 4: Press the "Process" button, and specify a results file name **on the computer's solid state drive**.

The analysis can take hours. Early in the analysis, a constant region depth of coverage is calculated. The number of distinct peptide sequences covering each amino acid residue in the constant regions of the heavy chain and the light chain is counted, and the average is calculated to give the average depth of coverage. The depth of coverage provides an estimate of how likely the full analysis will find the complete sequence accurately and provides feedback to the user on the sequence depth of their data set.

- Greater than 20 – good chance of success
- Between 10 and 20 – moderate chance of success
- Less than 10 – poor chance of success; recommend collecting more data and/or revising data collection procedure

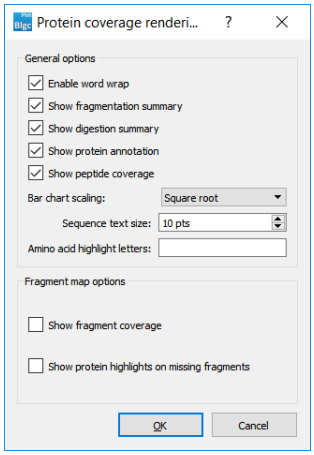
Viewing Supernovo Results

Supernovo's primary output is a .blgc file, which can be opened in Supernovo or Byologic.

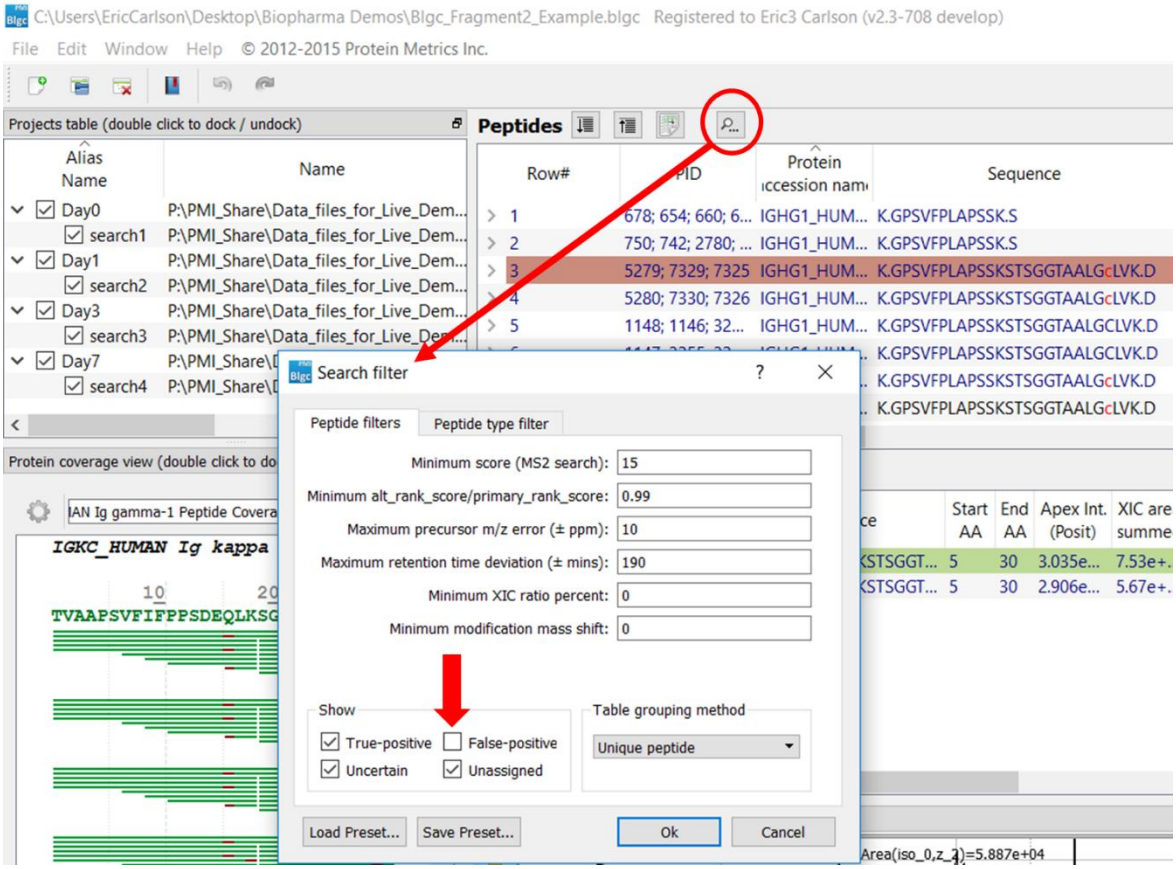


In the protein view:

- Right-click in the protein view pane to export the sequence to a FASTA file
- Amino acid residues that should be inspected are shaded
  - Red – approximately 10% error rate
  - Yellow – approximately 2% error rate
- The bar chart shows a summary of the between-residue evidence accumulated over all peptides from all files
  - Blue – accumulated fragmentation (MS^2) evidence
  - Orange – accumulated digestion (MS^1) evidence
- Click on the gear (upper left of the protein view pane) to adjust settings



- In the peptide details table:
    - The Validation column can be used to help keep track of inspection decisions. To reject a peptide, double-click for a dropdown and select “False positive.”
    - To filter out false positives globally, select the filter option at the top of the Peptides window, go to the “Peptide filters” tab, uncheck “False-positive,” and select “OK.



For further information about using Byologic, please see the Byologic User Manual.

Besides the .blgc file, there is an .html file (with the same base name) which contains some additional information – in particular the alignment between the initial scaffold sequence and the final sequence. Supernovo is sometimes overly aggressive in making changes to the initial scaffold, and this alignment may help you find and fix problems.