ProSightPC 4.0 Quick Start Guide

The Thermo ProSightPC™ 4.0 application is the only proteomics software suite that effectively supports high-mass-accuracy MS/MS experiments performed on LTQ™ FT™- and LTQ Orbitrap™-based instruments, including the Q Exactive™ and Fusion™ Tribrid™. The ProSightPC 4.0 application builds on the capabilities of the previous versions of the ProSightPC application in several ways.

Use this quick start guide to become familiar with some of the key features of the ProSightPC application. As you go through the procedures, you can use the example data files that were provided on the distributed flash drive. If you did not obtain this flash drive, you can download all files from the following site:

http://proteinaceous.net/prosightpc40-demonstration-data/

For complete details on how to use the ProSightPC application, refer to the *ProSightPC User Guide* or the Help available in the ProSightPC application.

Contents

Starting ProSightPC

❖ **To start the ProSightPC application**

From the Start menu, choose **All Programs > Proteinaceous Inc > ProSight > ProSightPC**, or click the **ProSightPC** icon,  , on your desktop.

If you have never installed a ProSight PC product, you must install the correct font when you first start the application. Perform this procedure one time only.

❖ **To install the ProSightPC font**

1. Choose **Help> Install Font**.
2. In the dialog box that opens, click **Install**.

Importing or Creating a Database

To begin using the ProSightPC application, you must import a database into the proteome warehouse or create a proteome database that you can search to identify the peptides and proteins in your mass spectrometry data. You can download a proteome database from the ProSightPC website to your local computer (see Importing a Database) or manually create your own proteome database (see "Creating a Custom Database" on page 2).

Revision A XCALI-97800

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Importing a Database

You can copy existing proteome databases in PSCW (ProSightPC proteome warehouse) or XML file format.

❖ To download a proteome database from the ProSightPC website

1. Choose **Databases > Download ProSightPC Databases**.

   This step takes you to proteinaceous.net where you can access top-down and bottom-up databases.

2. Click the date of the database that you are interested in, for example, **July 2016**.

3. Click **Archaebacteria**, **Eukaryotes**, **Prokaryotes**, or **Custom**, as appropriate.

   You must know the taxonomy of the species of the database that you want to download.

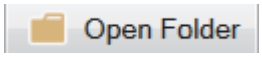
   You can choose from one of the following types of databases:

   - TD Complex PSCW: Includes N-terminal acetylation and initial methionine cleavage. This database contains 12 to 15 modifications per entry.
   - TD Simple PSCW: Includes N-terminal acetylation and initial methionine cleavage. This database contains up to three modifications per entry.
   - Bottom Up PSCW: Includes trypsin digestion using two missed cleavages.
   - UniProt XML File: Recreates a PSCW database in the Database Manager.

   The site automatically downloads the database once you select it. The downloaded database (a PSCW or an XML file) appears in the Downloads folder in the following directory:

   C:\Users > *your_name_folder* > Downloads

4. Choose **Databases > Database Manager**, or click the **View Database Info** icon,  , to open the Database Manager window.

5. Click 

in the toolbar of the Database Manager window.

6. Browse to the Downloads folder at the location just given.

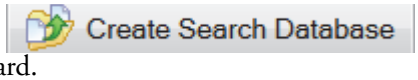
   If you do not want to place the imported PSCW or XML file in the default folder, navigate to the appropriate folder.

7. Right-click the selected folder and choose **Paste**.

Creating a Custom Database

You can create top-down and middle-down/bottom-up proteome databases from an XML file, a UniProtKB XML file, a UniProKB flat file, or a FASTA file.

❖ To create a proteome database with the Database Manager

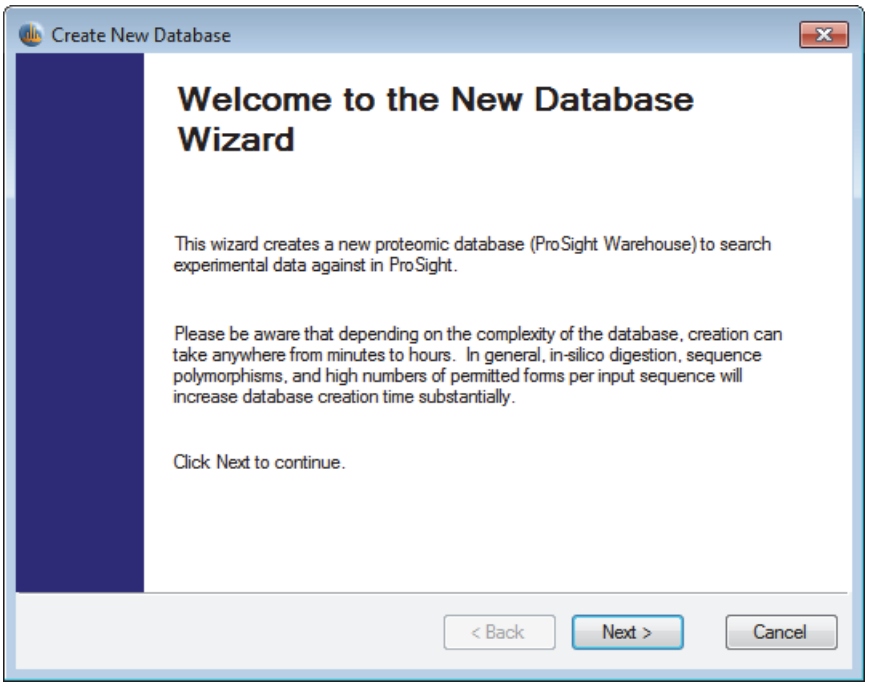
1. Choose **Databases > Create a Custom Database**, or click 

in the Database Manager to open the Create New Database wizard.

   –or–

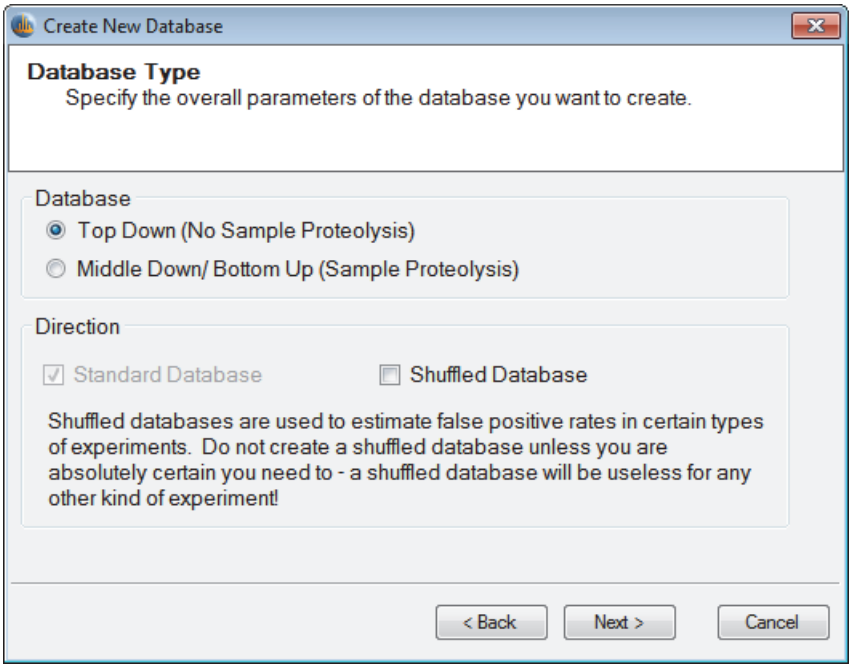
   Choose **Databases > Database Manager**, and then click **Create Search Database**.

The Welcome to the New Database Wizard page opens.



2. Click **Next**.

The Database Type page of the Create New Database wizard appears.



3. Do the following:

   a. Select the type of database to build from these two options:

      - Top Down (No Sample Proteolysis): Builds a database around whole, intact protein sequences and everything that could potentially happen to them in a biological system.

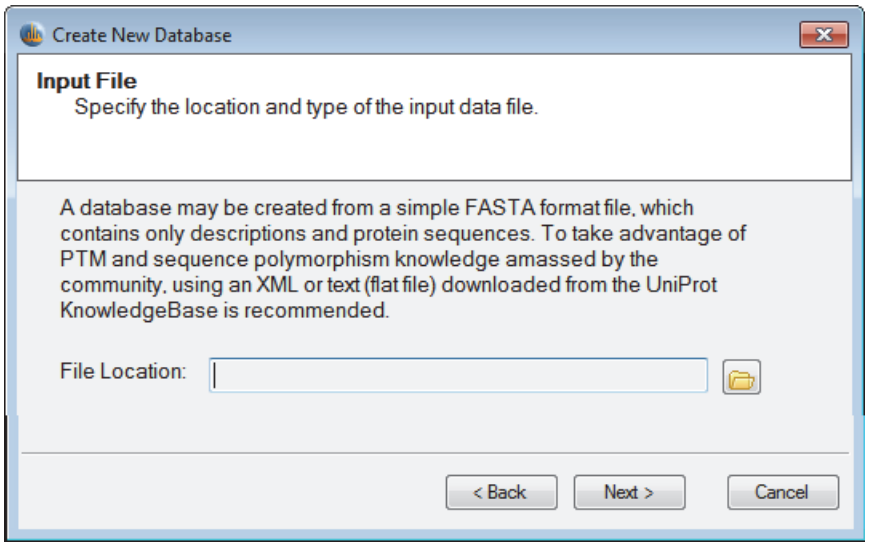
- Middle Down/Bottom Up (Sample Proteolysis): Builds a database around peptide sequences that arose from ex vivo proteolysis. If anything in your sample preparation protocol involves trypsin or Lys-C or any other proteolytic agent, select this option.

b. In the Direction area, select one of two check boxes for the direction of the database to build:

- Standard Database: Creates a database consisting of correct masses and forward sequences. A standard database is a typical protein database.
- Shuffled Database: Creates a nonsense database consisting of correct masses and sequences with randomized letters. Do not select this option unless it is absolutely necessary. You cannot use a reverse database in any other kind of experiment.

c. Click **Next**.

The Input File page appears.



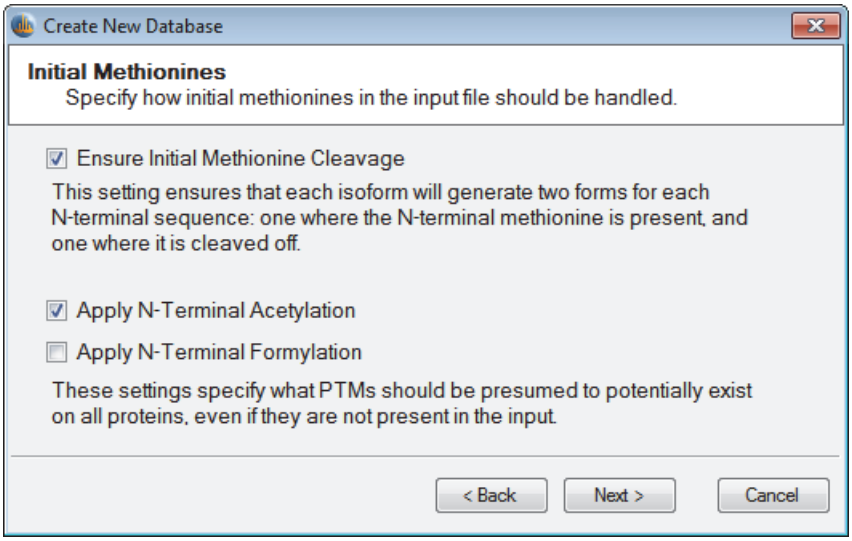
4. Do the following:

a. In the File Location box, enter the name and path of the file containing the sequence information, or browse for it by clicking the **Browse Folder** icon. You can select a FASTA file, a UniProtKB XML file, or a UniProtKB flat file.

To interpret any symbols that might appear on the Input File page, refer to the *ProSightPC User Guide*.

b. Click **Next**.

The Initial Methionines page appears.



5. Do the following:

   a. Select the method of handling initial methionines:

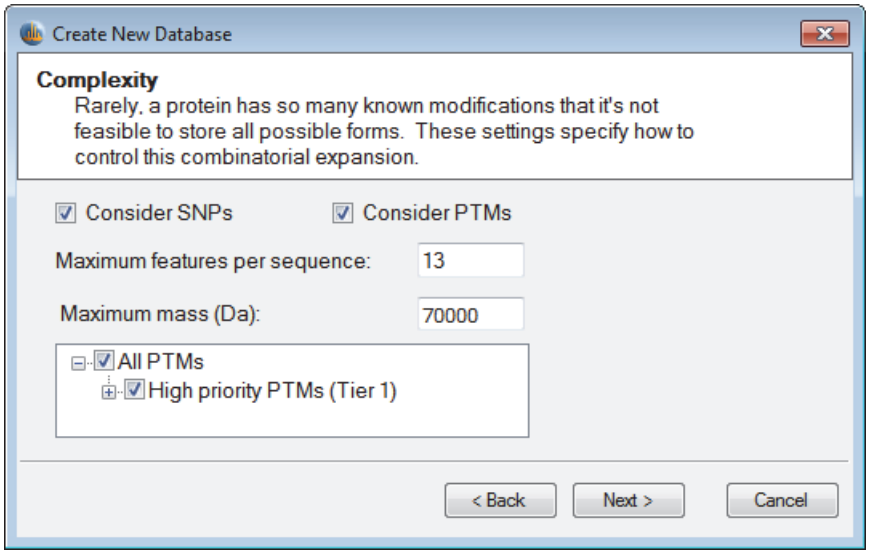
      - (Recommended) Ensure Initial Methionine Cleavage: Ensures that each isoform generates two forms for each N-terminal sequence: one where the N-terminal methionine is present and one where it is cleaved off.

   b. Specify the post-translational modifications (PTMs) that are presumed to exist on all proteins, even if the input does not contain them.

      - Apply N-Terminal Acetylation: Adds N-terminal acetylation whenever it is possible, regardless of whether the input includes it. N-terminal acetylation is a very common PTM in eukaryotes.
      - Apply N-Terminal Formylation: Adds N-terminal formylation. Select this check box if you are building a prokaryotic database. Prokaryotes often use N-formylmethionine to initiate translation.

   c. Click **Next**.

The Complexity page appears.



6. Do the following:

   a. If you want to include potential genetic variation as annotated in the UniProt™ database, select the **Consider SNPs** check box.

      With this option, you can incorporate sequence polymorphisms into the database.

   b. If you want to annotate known PTMs onto a protein, select the **Consider PTMs** check box.

      For an example showing how to use this option, refer to the *ProSightPC User Guide*.

   c. In the Maximum Features Per Sequence box, type the maximum number of features per input sequence.

      As indicated on the Complexity page, a protein may have so many known modifications that it is not feasible to store all possible forms. On this page, you can set options to specify how to control this combinatorial expansion. If you are uncertain about the values to set, use the default values.

   d. In the Maximum Mass (Da) box, enter the upper limit for which PTMs are included in the database.

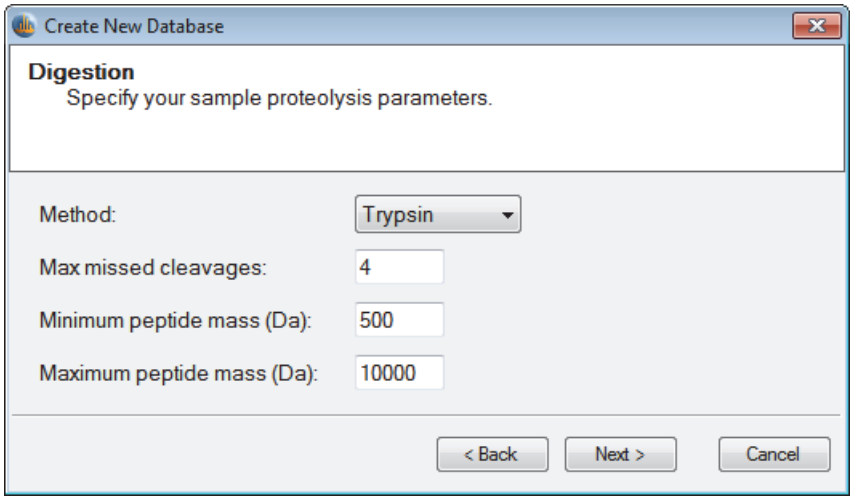
      For more information on this parameter, refer to the *ProSightPC User Guide*.

   e. In the PTM selection box, select the check box for each PTM that you want included in the database.

      If a check box for a PTM (or PTM category) is clear, those PTMs are not put into the database, whether or not they are present in the input data. This option is only available for UniProtKB XML and flat-formatted input data, because the standard FASTA format cannot encode information about PTMs.

   f. Click **Next**.

If you selected the Middle Down/Bottom Up (Sample Proteolysis) option on the Database Type page, the Digestion page of the Create New Database Wizard opens. Use it to specify the parameters for a sample proteolysis.



7. On the Digestion page, do the following:

   a. In the Method list, select the proteolytic method used to catalyze the breakdown of proteins into peptides.

   b. In the Max Missed Cleavages box, type the maximum number of cleavage sites found in the generated peptides.

No (0) missed cleavages indicates that there are no cleavage sites in the generated peptides. One (1) missed cleavage indicates that each peptide has one site in it, two (2) missed cleavages indicate that each peptide has two sites in it, and so on. The Max Missed Cleavages box contains all values up to and including the set parameter. For example, if Max Missed Cleavages is set to 2, peptides with 0, 1, and 2 missed cleavages are generated. For a longer example, refer to the *ProSightPC User Guide*.

c. In the Minimum Peptide Mass (Da) box, type the minimum mass that a peptide must have, in daltons, before it is allowed to be put into the database.

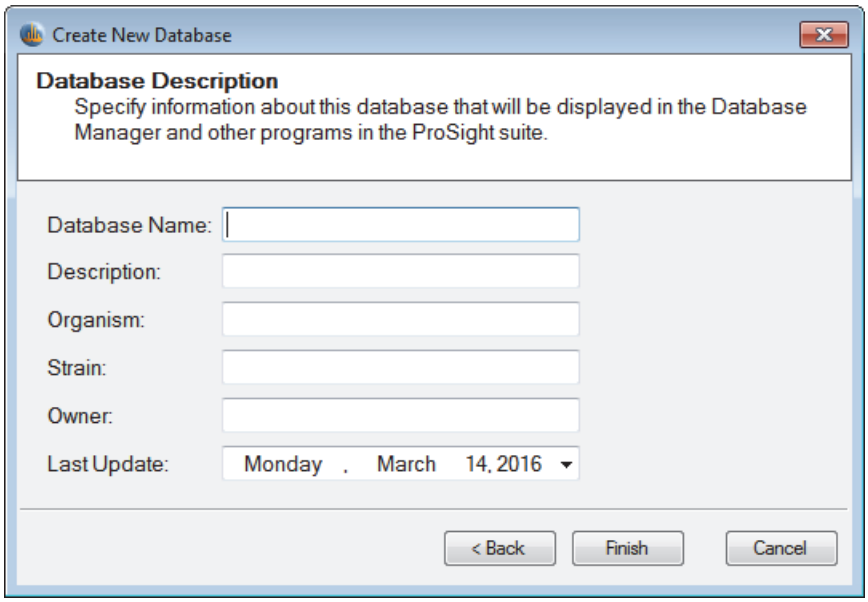
   No peptide less than the minimum peptide mass is put into the database; the ProSightPC application ignores and discards any theoretical peptide less than this mass. This parameter is useful because the application sometimes cannot identify particularly small peptides that have a very strong impact on database size.

d. In the Maximum Peptide Mass (Da) box, type the maximum mass that a peptide can have, in daltons, before it is allowed to be put into the database.

   No peptide greater than this mass is put into the database.

e. Click **Next**.

   The Database Description page appears.



8. Enter identifying information about the database that you want to create:

   a. In the Database Name box, type the name of the database that you want to create. Use only letters, numbers, and underscores.

   b. In the Description box, type a brief description of the database.

   c. In the Organism box, type the name of the organism for the proteome database that you are creating.

   d. (Optional) In the Strain box, type the strain designation for the proteome database that you are creating.

   e. In the Owner box, type either your name or the name of the data source.

   f. In the Last Update box, type the date when the database was last updated or click the down arrow to display a calendar and select a different date.

   g. Click **Finish**.

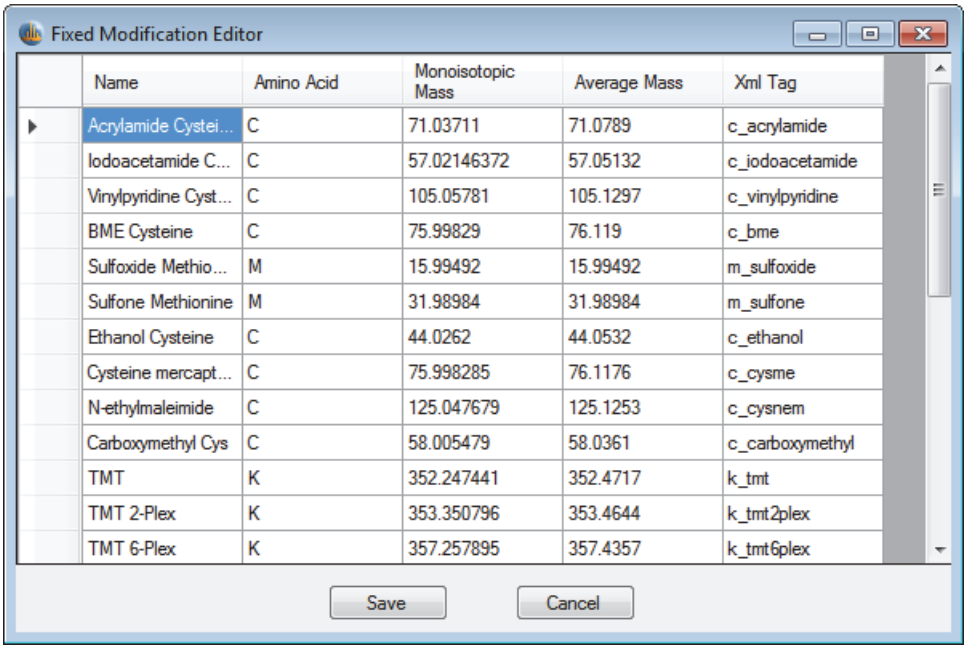
9. On the Ready to Load page, click **Go** to create the new database.

Editing Fixed Modifications

You can customize the chemical modifications that you use to conduct a search by using the Fixed Modification Editor.

❖ To edit fixed modifications

1. Choose **Tools > Fixed Modification Editor** to open the Fixed Modification Editor.



2. Do the following:
   a. Scroll down to the last row, which is marked by an asterisk (*).
   b. In the Name box, type the name of the modification.
   c. In the Amino Acid box, type the symbol of the amino acid being modified.
   d. In the Monoisotopic Mass box, type the monoisotopic mass of the chemical formula of the modification.
   e. In the Average Mass box, type the average mass of the chemical formula of the modification.
   f. In the XML Tag box, type a single word without spaces to indicate the modification.
3. Click **Save**.

The window closes. The modification appears when you create searches.

Setting Parameters for the Search

Specify the type of search to perform and set the parameters for that search. To do this, create a predefined search by following the instructions in "Adding Searches" on page 22.

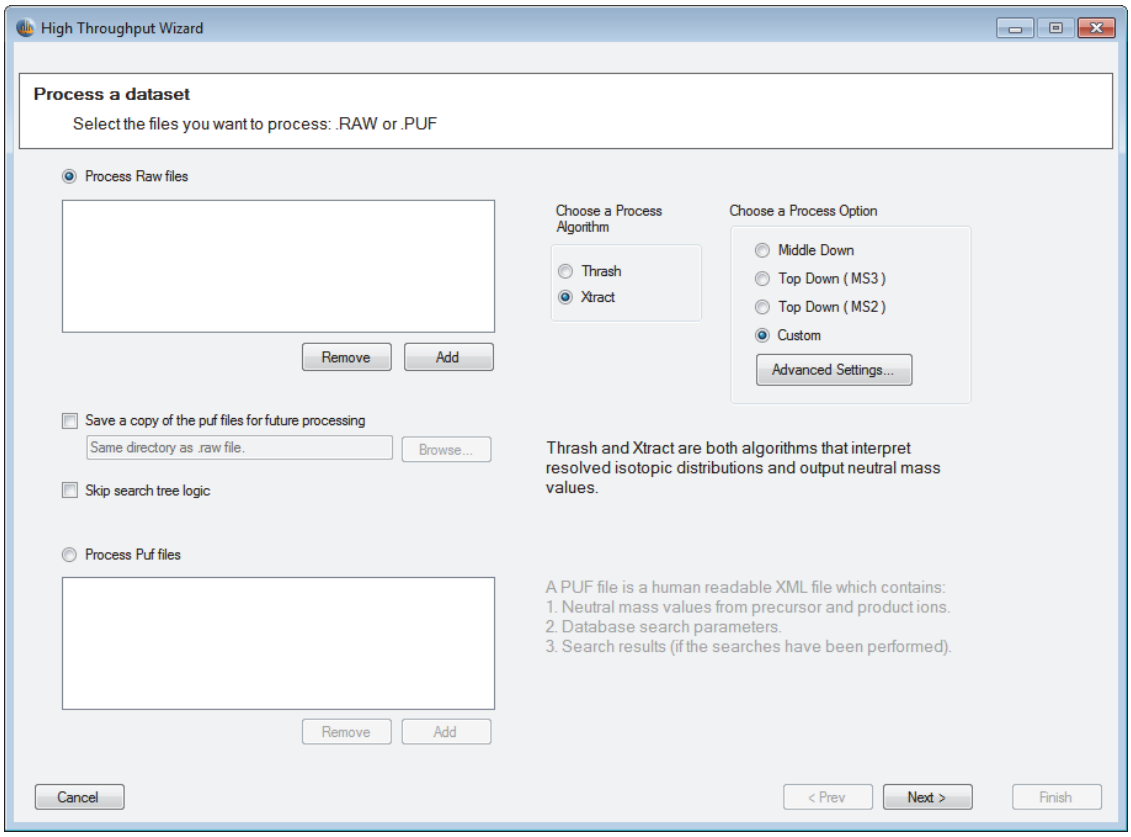
Processing LC/MS/MS Data

To process the LC/MS/MS data and search the imported database, you can use the High Throughput Wizard.

❖ To open the High Throughput Wizard

- Choose **ProSightHT > HighThroughput Wizard**, or click the **High Throughput Wizard** icon,  .

  The Process a Dataset page appears.



Setting Processing Options

❖ To set processing options

1. Select the **Process Raw Files** or the **Process Puf Files** option, depending on the type of data that you want to import.

   - (Default) Process Raw Files: Converts LC/MS/MS raw data files to ProSightPC upload format (PUF) files, using an extension of the THRASH or the Xtract algorithm designed to analyze high-resolution profile LC/MS/MS data collected on Thermo Scientific™ Fourier Transform instruments, such as Orbitrap Elite.

     Example raw data files are available from the following location for demonstration purposes:

     http://proteinaceous.net/prosightpc40-demonstration-data/

   - Process Puf Files: Processes PUF files.

2. To add a RAW or PUF file, click **Add** in the appropriate location, and then browse to the file in the dialog box that opens to select the file.

3. If you choose a raw data file, select one of the following options in the Choose a Process Algorithm area for importing the data files:

   – Thrash: Uses the THRASH algorithm to process the input file.

– (Default) Xtract: Uses the Xtract algorithm to process the input file. This option reduces analysis and search time and, in general, gives better results.

Both Xtract and THRASH are algorithms that interpret resolved isotopic distributions and output neutral mass values. For more information on these algorithms, refer to the *ProSightPC User Guide*.

4. If you choose a raw data file, select a processing option in the Choose a Process Option area for importing the data files. The first three options specify a set of default settings for the Xtract and THRASH algorithms.
   – Middle Down
   – Top Down (MS3)
   – Top Down (MS2)
   – Custom: Click **Advanced Settings** and use the Advanced Settings dialog box to specify your own settings.

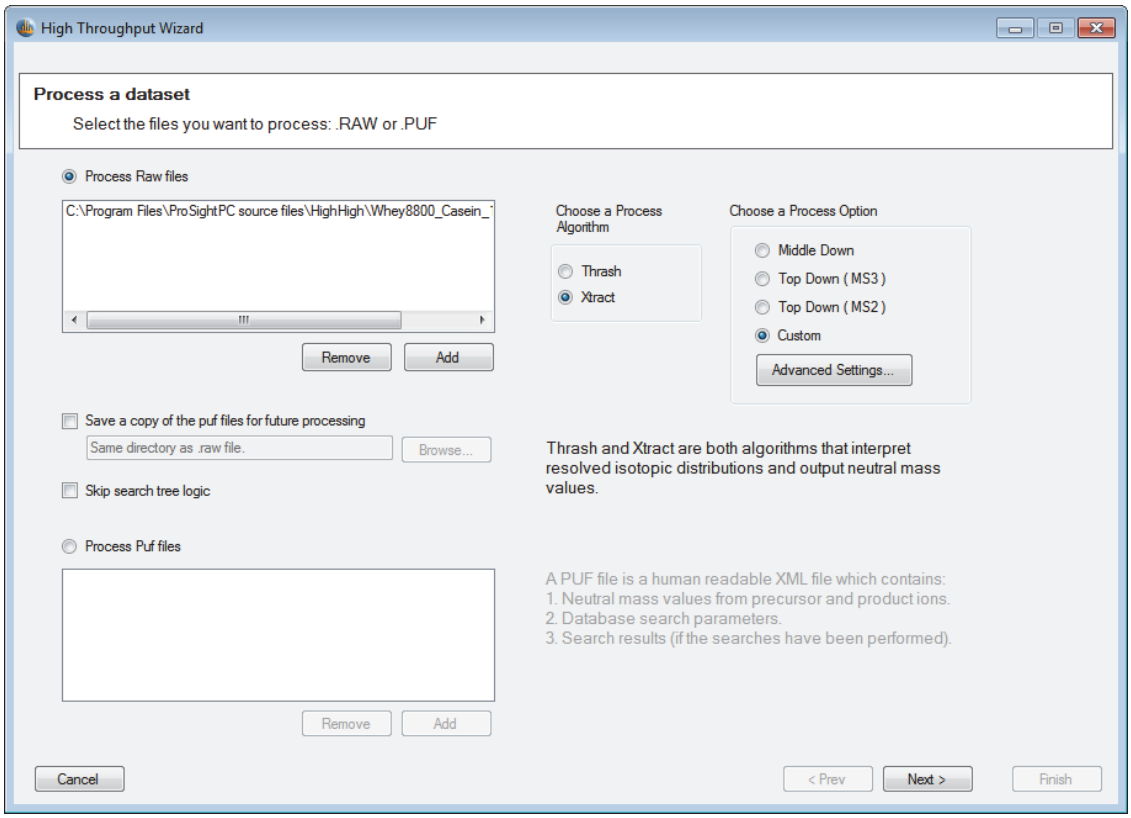
   Refer to the *ProSightPC User Guide* for information on these defaults and for instructions on using custom settings.

5. (Optional) If you selected the Process Raw Files option, select the **Save a Copy of the Puf Files for Future Processing** check box to save a physical PUF file containing the results. Click **Browse** to browse to the directory where you want to save the PUF files.

   This option is useful for rapidly re-searching the data instead of processing the raw data file again. If you do not select this option, the results reside in a ProSightPC repository. You can always import them into the ProSightPC application and save them as a PUF file.

6. (Optional) If you choose not to search the data against a proteome database, select the **Skip Search Tree Logic** check box.

7. On the completed Process a Dataset page of the High Throughput Wizard, click **Next** (see the next figure).



Selecting or Creating a Repository

You must select a repository, which is a database that stores the search results in the ProSightPC application.

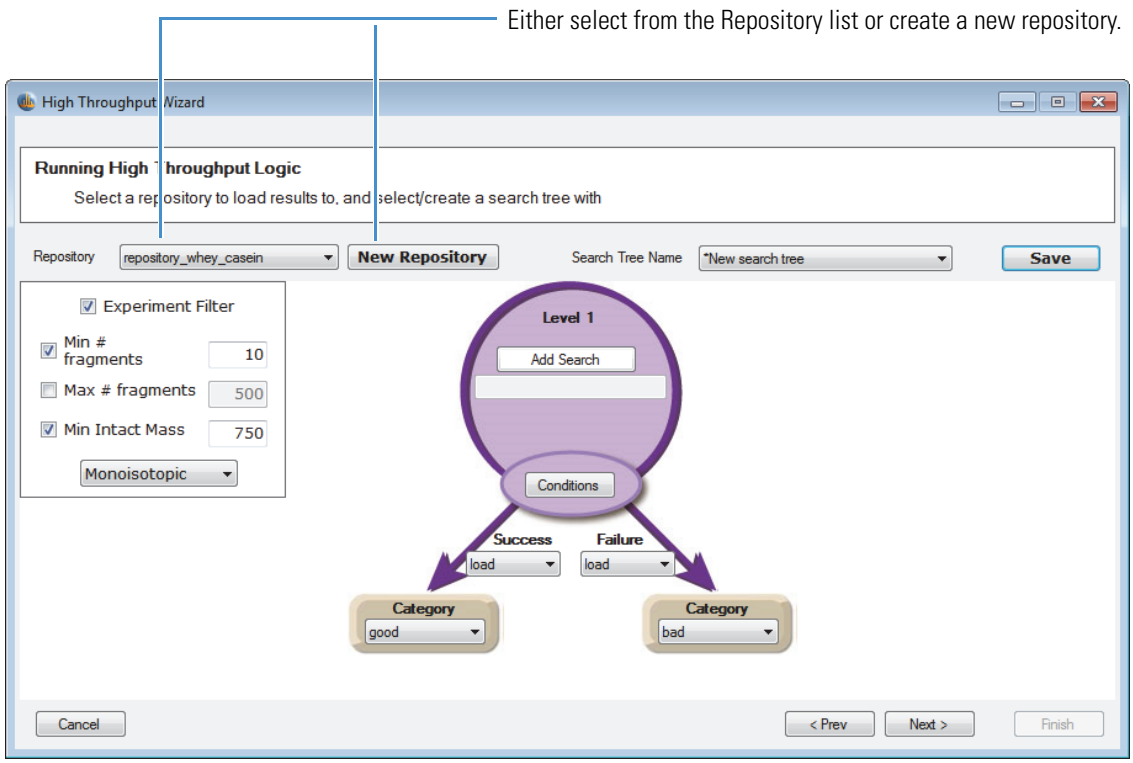
❖ To select or create a repository

1. On the Running HighThroughput Logic page of the wizard (see the next figure), do one of the following:

   - Select an existing repository from the Repository list.

   –or–

   - Create a new repository by clicking **New Repository,** entering a new repository name in the New Repository dialog box—for example, **repository_whey_casein**—and clicking **OK**.



The Edit/Add Repositories dialog box appears, and the new name appears in the Repository list.

2. If you do not want to edit the repository, click **Save**. Otherwise, to edit the repository, follow the instructions in "Editing a Repository" in the *ProSightPC User Guide*, and click **Save** in the Edit/Add Repositories dialog box.

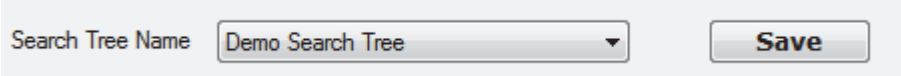
   The name of the repository now appears in the Repository list of the Running HighThroughput Logic page of the High Throughput Wizard.

Selecting or Creating a Search Tree

A search tree can hold one or more branch points. Each branch point in the tree contains one search. The simplest search tree has one branch point, which is the default for a new tree. You can create a new search or select an existing search.

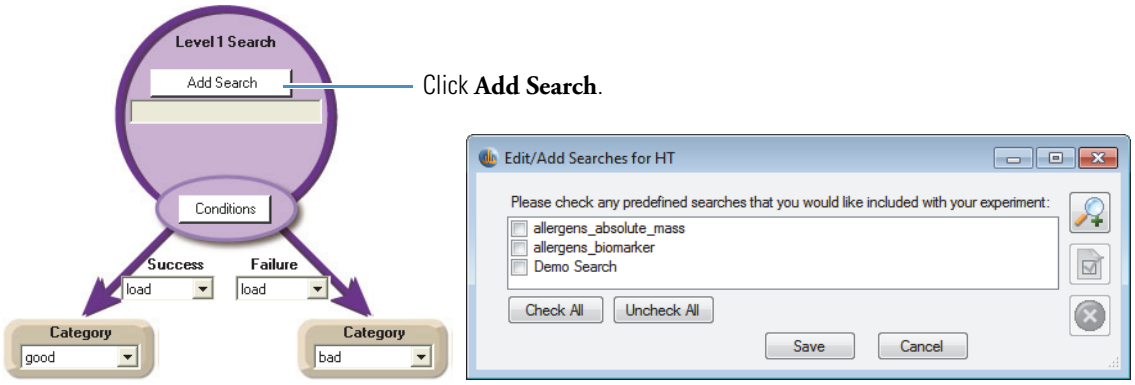
❖ To select an existing search tree

1. If you are running the example files, select **Demo Search Tree** from the Search Tree Name list.
2. Click **Save**.



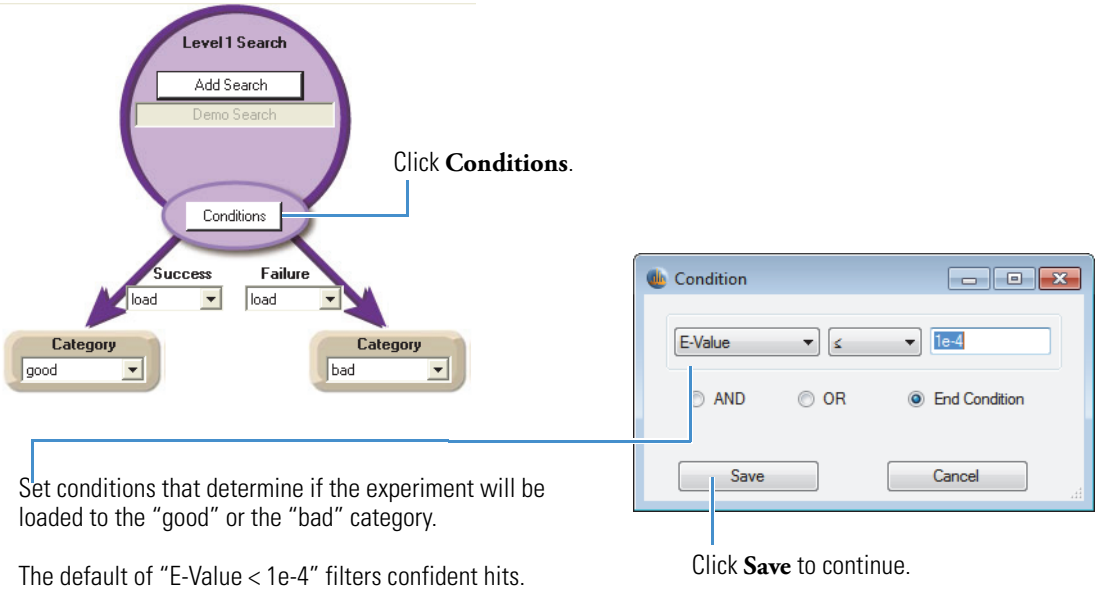
❖ To create a search tree

1. Click **Add Search** to open the Edit/Add Searches for HT dialog box.



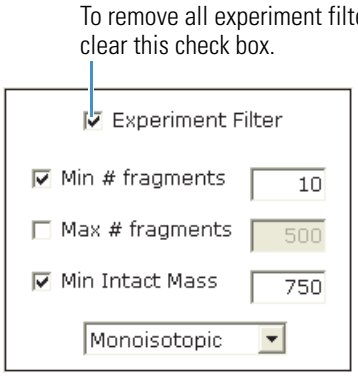
2. (Optional) Add a predefined search or edit an existing predefined search.
3. Click **Save**.
4. To select a condition, click **Conditions** to open the Condition window (see the next figure).
5. Set conditions that determine if the experiment will be loaded to the "good" category representing high-quality data or to the "bad" category representing low-quality data.

   The default of "E-Value < 1e-4" filters confident hits.
6. Click **Save** to continue.

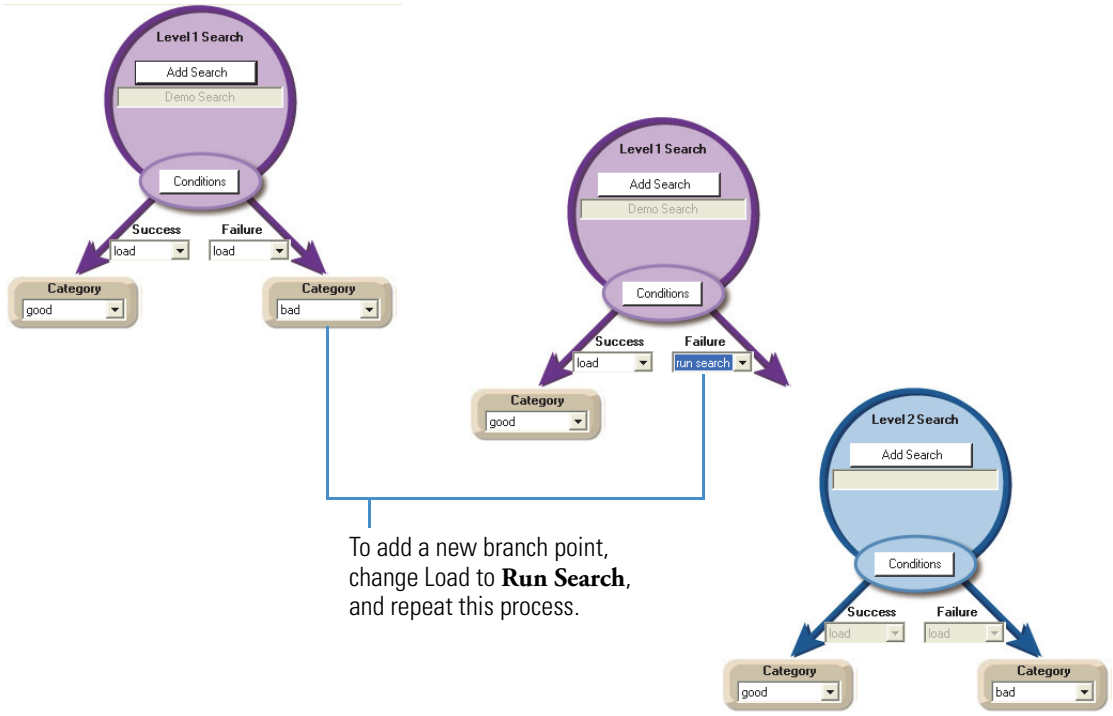


7. (Optional) Select the options in the Experiment Filter box to ignore experiments that are not likely to yield matches.

The default settings are as follows:
* Experiments that contain less than 10 fragments
* Experiments whose intact mass is less than 750 Da

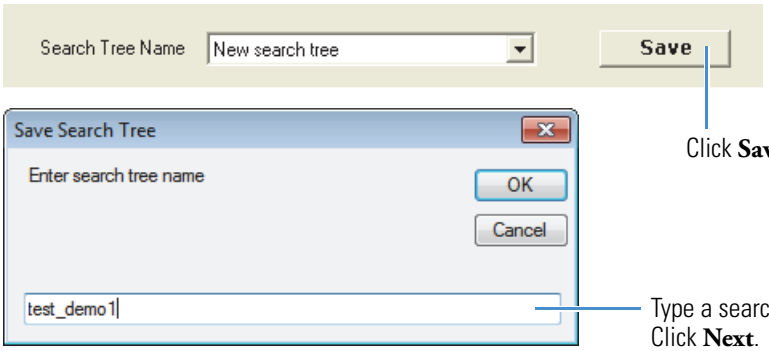


8. (Optional) Specify a two-level search tree by changing Load to **Run Search**, and repeat this process.



9. Click **Save** to save the search tree before continuing.

The Save Search Tree dialog box opens.



10. Type a search tree name and click **OK** twice.

    A search tree is usually used more than once per project.

11. Click **Next**.

Viewing the Summary

❖ **To view a summary of your parameter settings**

Review your parameter settings on the Summary page of the High Throughput Wizard.

Processing the Data

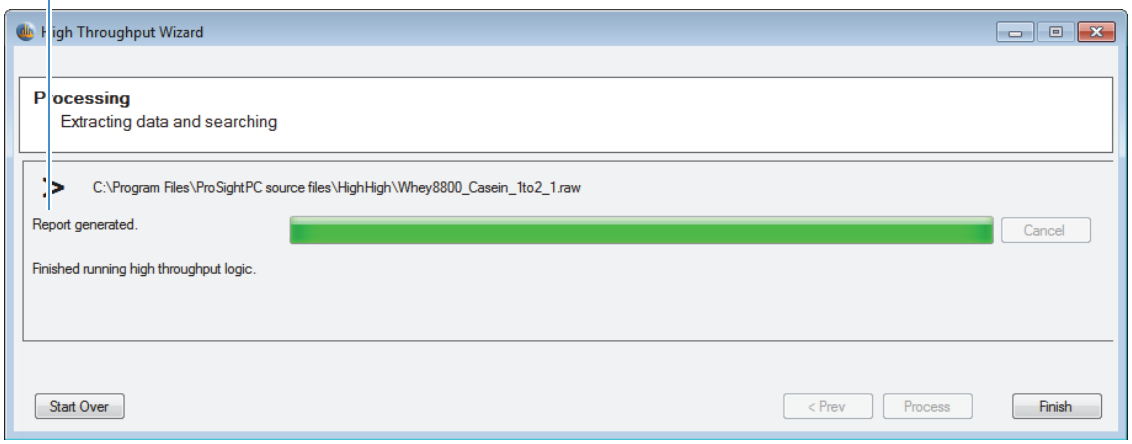
❖ **To process the data**

1. To begin the ProSightPC High Throughput processing, click **Process** at the bottom.
2. When the High Throughput Wizard finishes processing the data, click **Finish**.

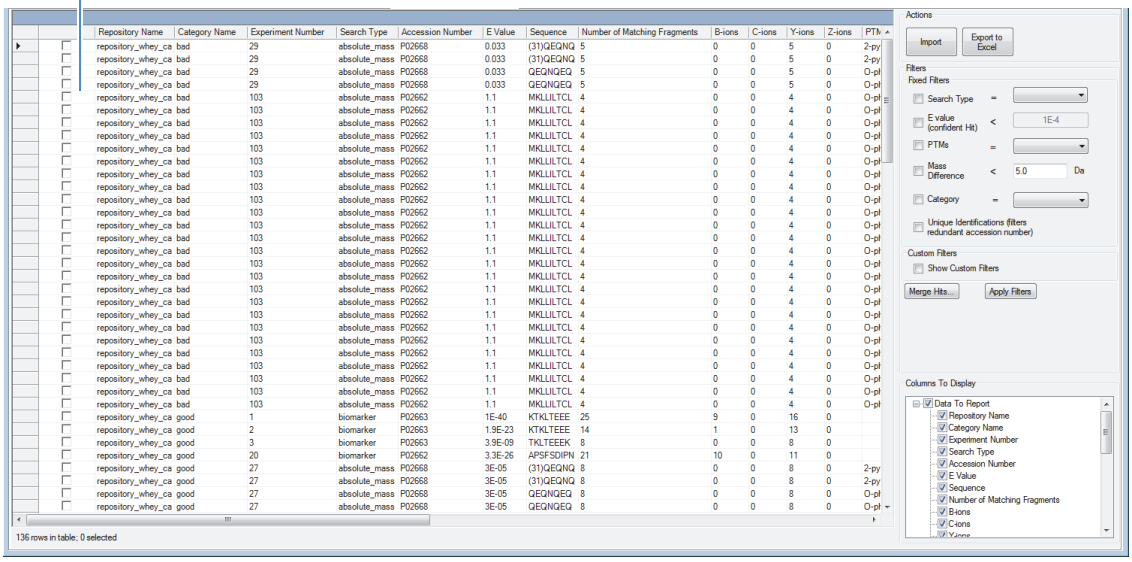
Viewing the Output

Once the search is complete (which might take several minutes, depending on the size of the raw data file, the complexity of the database, and the search parameters), you can view the repository report (see the next figure). You can use filter, import, and export options to view the report. For each raw data file, a table displaying all the matches found appears in the ProSightPC application. Each row represents the best match per MS/MS experiment (see "Generating Reports" on page 16).

The ProSightPC application finishes the first job and generates a report on a new page in the application while continuing to run the other jobs.



Repository report on a new page



Generating Reports

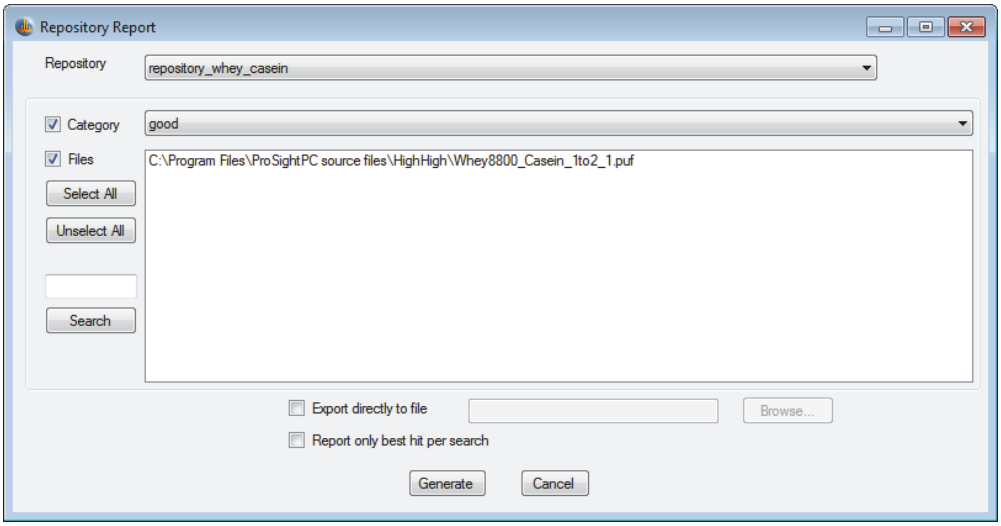
You can generate a report for each repository, per category or per file.

❖ To generate reports

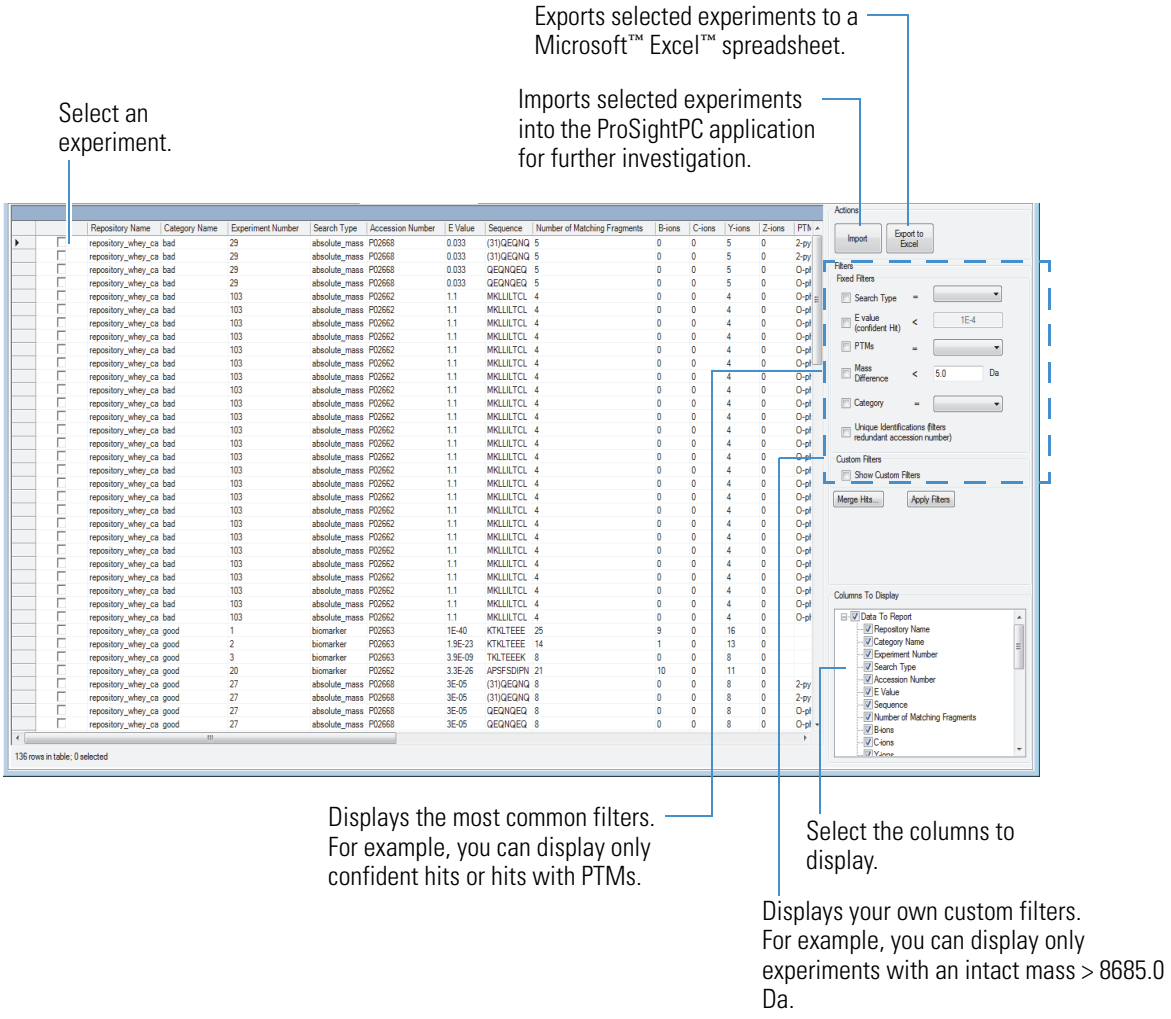
1. Choose **ProSightHT > Repository Report** or **Tools > Reports > Repository Report**, or click the **Repository Report** icon,  , to open the Repository Report window (see the next figure).
2. From the Repository list, select the name of the repository to generate a report for.
3. If you want to generate a report on all the experiments in a category, select the **Category** check box and then select the name of the category from the adjacent list. For example, to view only the "good" experiments, select the **Good** category.
4. If you want to generate a report on all the experiments in a specific file (good and bad), select the **File** check box, and then select the name of the file from the adjacent list or type the name of the file in the box.

5. Click **Generate**.

The application generates a report for each repository, per category or per file.



The Report page displays filter, import, and export information.



Setting Custom Filters

You might want to filter the data in the report. For example, you can set a custom filter to Theoretical Mass > 2000. You can also use any fixed modifications to filter, such as setting Mass Difference to < 5.0 Da.

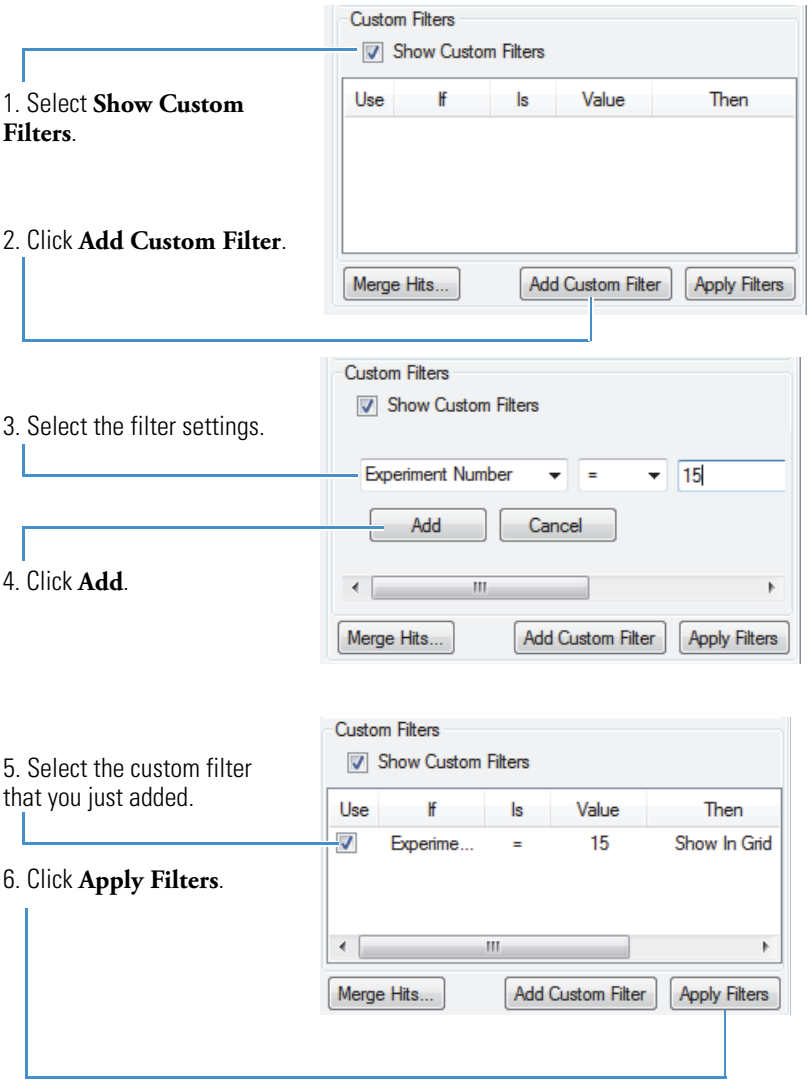
On the right side of the Report page, you can set custom filters to filter the data in the repository report.

❖ To set custom filters

1. Select the **Show Custom Filters** check box.

   The Custom Filters table opens (see the next set of figures).

2. Click **Add Custom Filter**.
3. Select the filter settings, for example, **Experiment Number = 15**.
4. Click **Add**.
5. In the table, select the check box for the custom filter that you just added.
6. Click **Apply Filters**.



Importing and Exporting Experiments

You can import experiments from a repository into the ProSightPC application for manual validation and additional database searching. You can also take a set of results from the experiments that you import to the Sequence Gazer™ tool to see in more detail how your MS/MS data matches a retrieved protein or peptide. For more information on the Sequence Gazer, see "Sequence Gazer" on page 25.

Use any of the following methods to import experiments:

- Select the experiments and click **Import**.
- Right-click the experiments and choose **Import**.
- Double-click an experiment.
- Choose **File > Import Data from Repository**.

Save any changes that you made by exporting the experiments back to the repository or to a different repository.

Use any of the following methods to export experiments:

- Select the experiments that were imported and click **Export to Repository**.
- Right-click the experiments that were imported and choose **Export to Repository**.
- Right-click the experiment in the ProSightPC data grid, and choose **Export Experiment to Repository**.
- Choose **File > Export Data to Repository**.

Sample Processing from a Targeted Run

If you want to import a targeted raw data file as input or if you want to enter data manually into the ProSightPC application, you cannot use the High Throughput Wizard. You must enter data manually or use an analysis to infer mass (AIM) operation, which converts high-resolution mass spectral data from proteins or large peptides to neutral monoisotopic or average masses. The ProSightPC application uses these neutral mass values to identify and characterize proteins.

Importing MS/MS Data

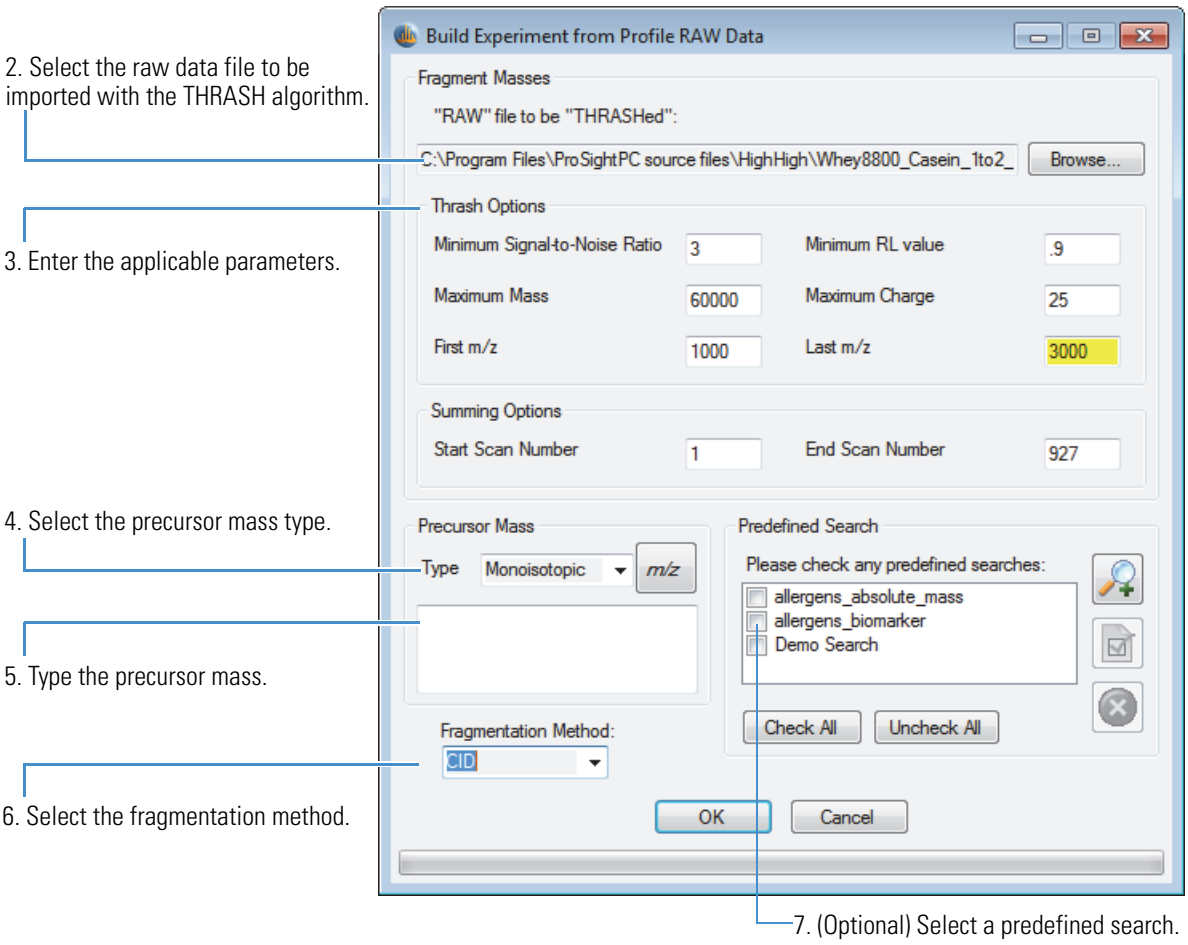
You can use either of two AIMs to import raw data files into a ProSightPC MS/MS experiment: THRASH and Xtract. (For information on these two algorithms, refer to the *ProSightPC User Guide* or the ProSightPC application Help.) You can also enter data manually.

Processing a Raw Data File with the THRASH Algorithm

❖ **To process a raw data file with the THRASH algorithm**

1. Choose **File > Import .raw > Profile**, or click the **Import Profile** icon,  .

   The Build Experiment from Profile RAW Data window opens (see the next figure).
2. In the "RAW" file to be "THRASHed" box, browse to the raw data file to be imported.
3. In the Thrash Options area, enter the applicable parameters.
4. In the Type box under Precursor Mass, select the precursor type.
5. In the box below Type, type the precursor mass.
6. In the Fragmentation Method list, select the fragmentation method.
7. (Optional) Select a predefined search in the Predefined Search box. For the example file, select **Demo Search**.
8. Click **OK**.



The ProSightPC application creates a new MS/MS experiment with the data processed by the THRASH algorithm.

Processing a raw data file with the Xtract Algorithm

Use Xtract to create a raw data file containing the neutral monoisotopic mass values (refer to the *ProSightPC User Guide* or the ProSightPC application Help for more information).

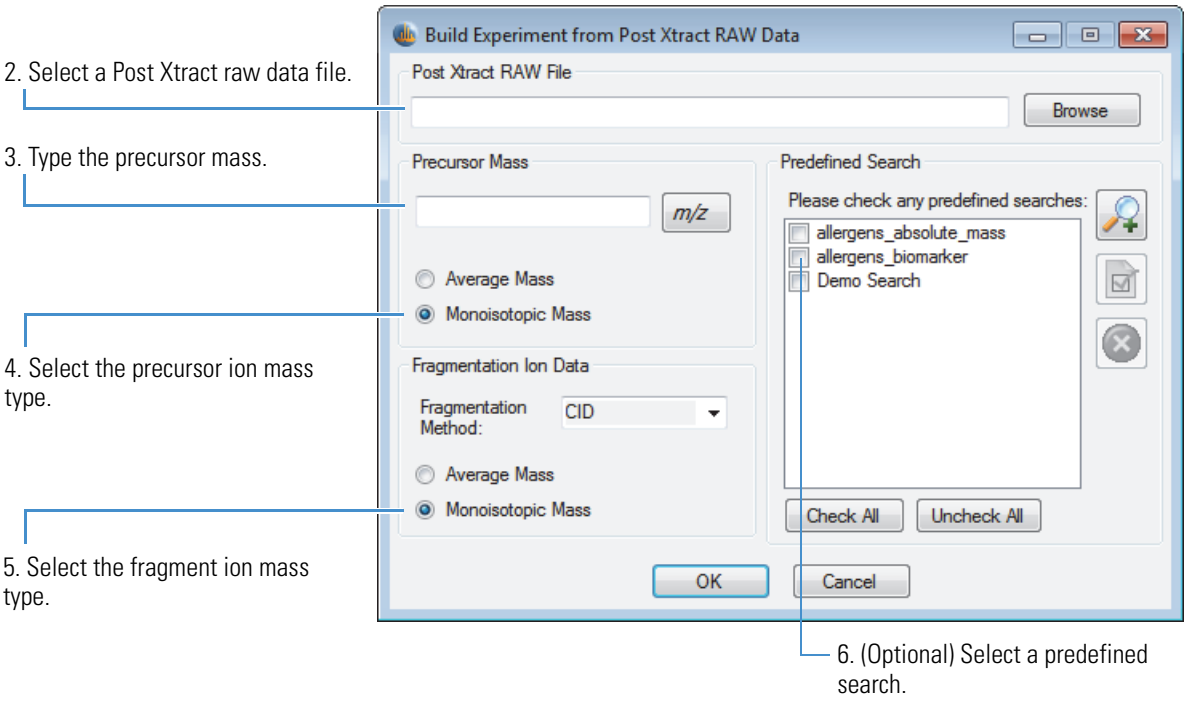
❖ To process a raw data file with the Xtract algorithm

1. Choose **File > Import .raw > Post Xtract**, or click the **Import Xtract** icon, .

   The Build Experiment from Post Extract RAW Data window opens (see the next figure).

2. In the Post Xtract RAW File box, browse to a Post Xtract raw data file.
3. In the Precursor Mass area, type the precursor mass in the box next to *m/z*.
4. Select the mass type of the precursor ions that each Post Xtract file contains, either **Average Mass** or **Monoisotopic Mass**.
5. In the Fragmentation Ion Data area, select the mass type of the fragment ions that each Post Xtract file contains, either **Average Mass** or **Monoisotopic Mass**.
6. (Optional) Select a predefined search in the Predefined Search box.

7. Click **OK**.



The ProSightPC application creates a new MS/MS experiment with the data processed by the Xtract algorithm.

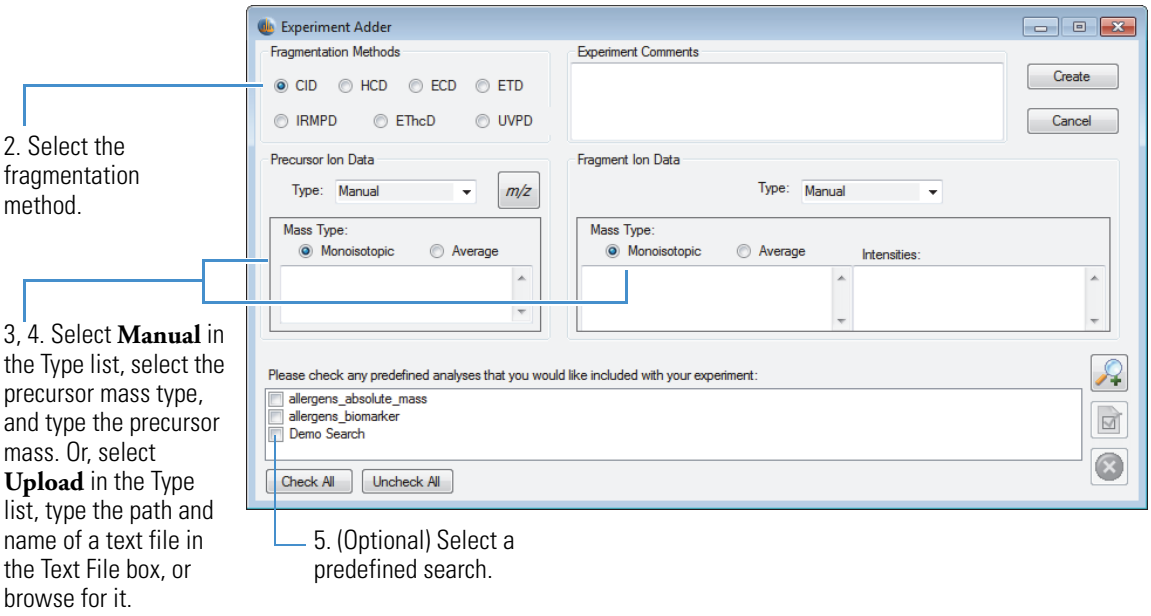
Manually Entering the Data

You can manually enter the data (precursor and fragment masses) if you want to import a targeted raw data file as input.

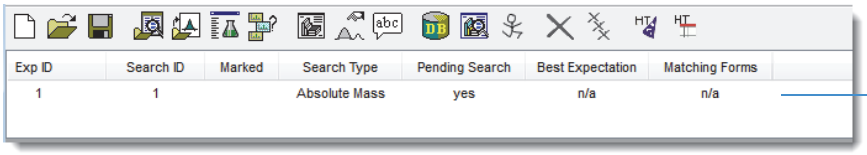
❖ To manually enter the data

1. Click the **Add Experiment** icon, , or choose **Tools > Experiment Adder** to open the Experiment Adder dialog box (see the next figure).
2. In the Fragmentation Methods area, select the fragmentation method.
3. In the Type list under Precursor Ion Data, select the precursor mass type, either **Manual** or **Upload**.
4. If you select Manual in the Type list, select the mass type of the precursor ion in the Mass Type area (either **Monoisotopic** or **Average**) and type the precursor mass.

   If you select Upload in the Type list, a Text File box and a Browse button appear beneath the Type list. Type the path and name of the ASCII text file or files that contain the precursor ion data in the Text File box, or click **Browse** to browse for them.
5. (Optional) Select the appropriate check box for a predefined search at the bottom of the dialog box.
6. Click **Create**.



All three options for importing MS/MS data into the ProSightPC application create new MS/MS experiments in the data grid (with or without searches, depending on whether you added one or more predefined searches).



Adding Searches

The ProSightPC application supports five different search modes. Each search mode represents a specific method used to query a proteome database within the proteome warehouse. For more information on search modes, refer to the *ProSightPC User Guide* or the ProSightPC application Help.

Adding a Predefined Search

Predefined searches are a strategy to simplify repeating identical searches on different sets of MS/MS data.

❖ To add a single predefined search to an experiment

- In the data grid, right-click the appropriate experiment and choose **Append Predefined Search > *search_name***.

  The experiment that the search has been appended to appears in the data grid with the same experiment number in the Exp ID column and a different number in the Search ID column.

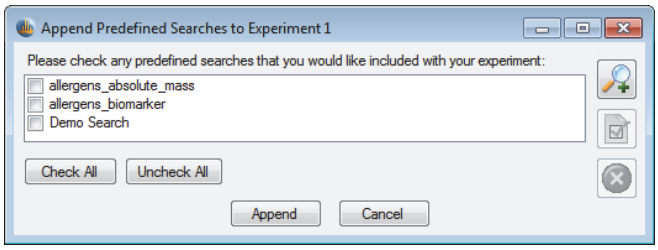
–or–

- Follow the next procedure, "To add multiple predefined searches to an experiment," and select the appropriate search.

❖ To add multiple predefined searches to an experiment

1. Right-click the experiment in the data grid and choose **Append Predefined Searches**. You can also choose **Experiment Tools > Append Predefined Search**.

   The Append Predefined Searches to Experiment *X* dialog box opens (see the next figure).



2. Select the predefined searches to append to the experiment and click **Append**. To select all of the searches listed, click **Check All**.

   You can also execute the search from the Data Manager by clicking Run Search.

> **Tip** To process several predefined searches automatically, see "To perform searches in batch mode" on page 23.

Performing Searches

You have two options for searching: performing searches manually or performing them in batch mode.

Performing Searches Manually

If you have a limited number of searches that you want to perform, you can manually search the database.

❖ To perform searches manually

- Right-click the experiment and choose **Run Search *x***, where *x* is the number of the experiment that appears in the Search ID column.
- If you select more than one experiment or search, right-click and choose **Run Searches**.

Performing Searches in Batch Mode

With batch processing, you can queue and perform a large number of searches over any number of experiments in a single action.

❖ To perform searches in batch mode

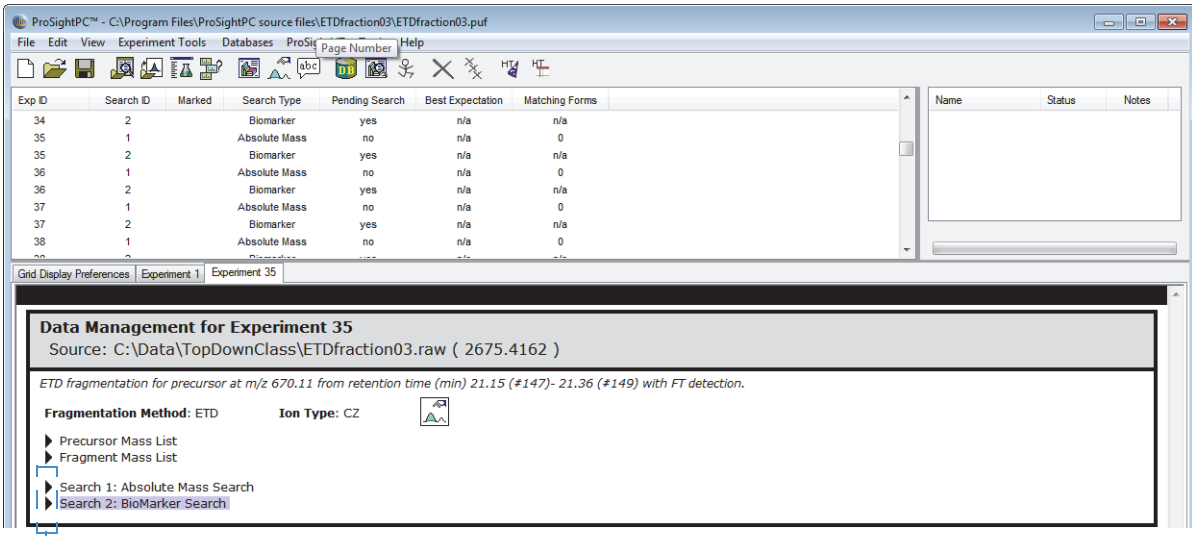
Choose **Tools > Batch Run**, or click the **Batch Run** icon,  .

Interpreting Results

After performing the searches, double-click a search. A new tab opens with the experiment number.

Experiment Information

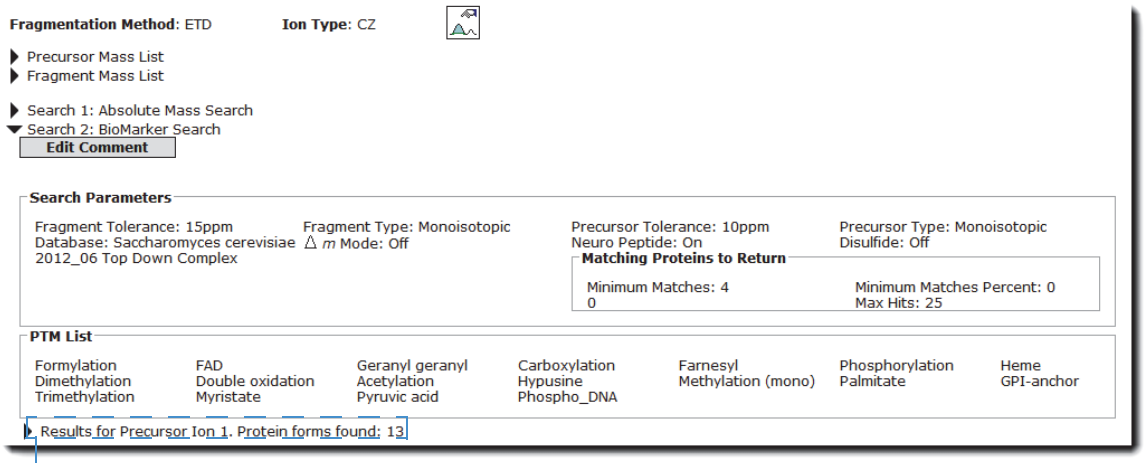
Click the display control arrows to expand or collapse the various displays. These arrows indicate that information related to a search is available. Click the display control arrow to reveal search information (see the next figure).



Display control arrows

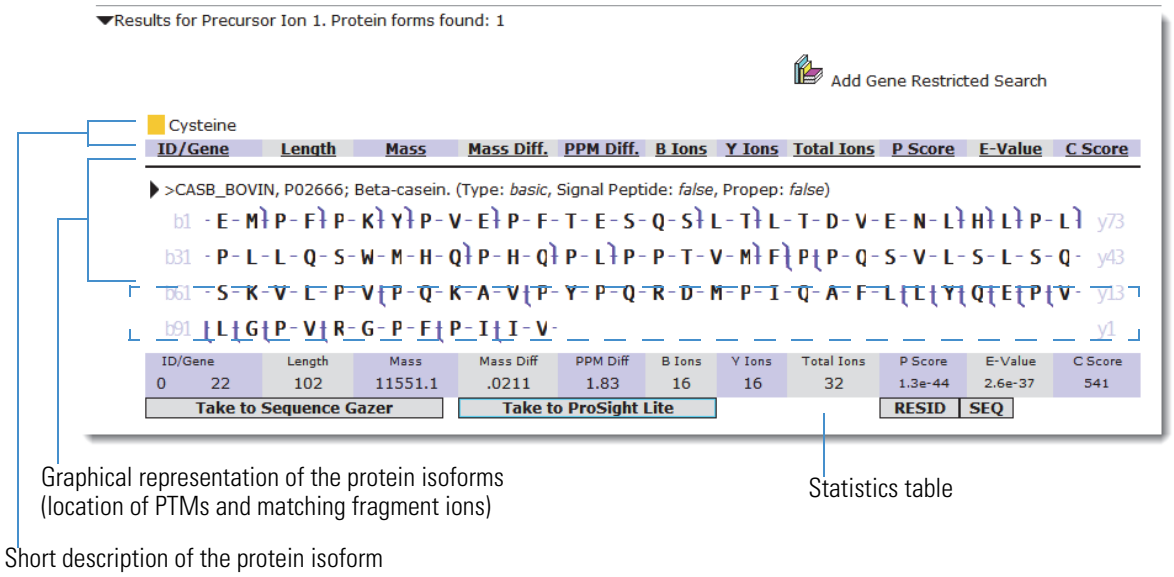
Search Information

Each results list displays the number of protein isoforms found. Click the appropriate arrow to display the results.



Results list

Protein Isoform Information



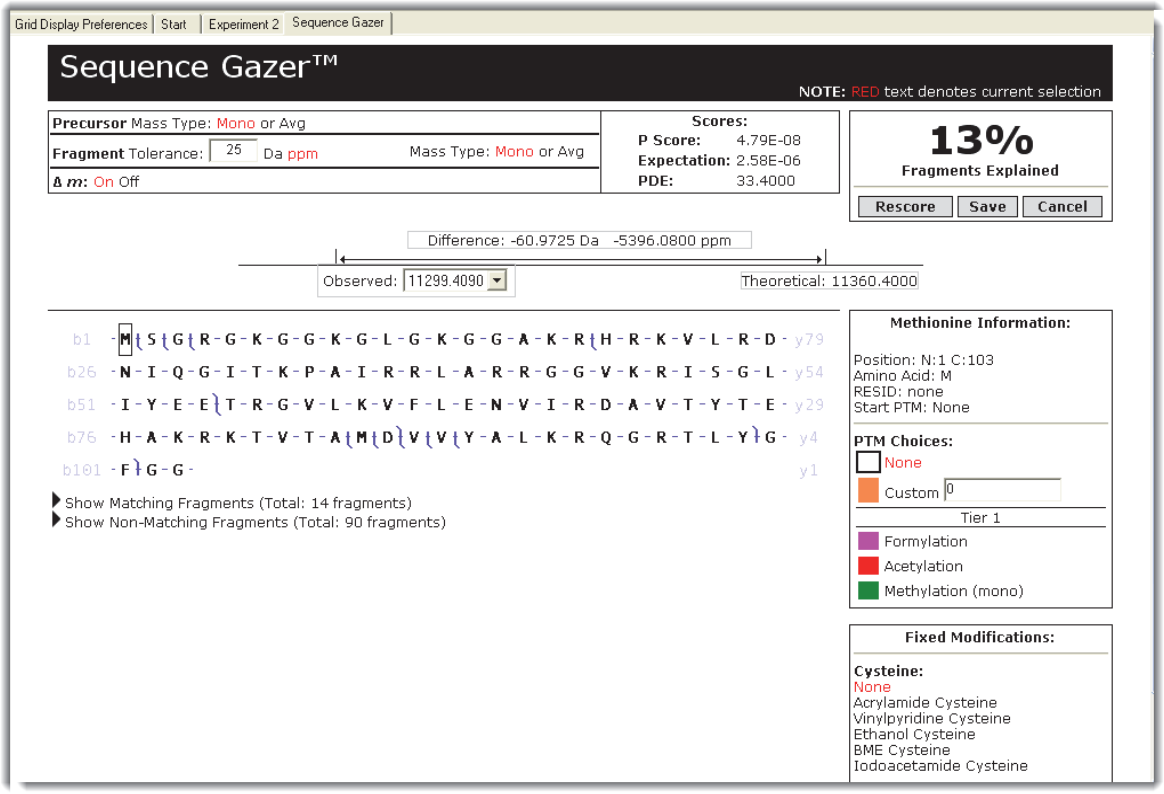
Sequence Gazer

The Sequence Gazer is an interactive environment for querying MS/MS data against a known protein sequence. Use the Sequence Gazer to characterize identified proteins by selectively adding or removing PTMs or custom masses to amino acids within a protein sequence.

❖ To access the Sequence Gazer

Click **Take to Sequence Gazer** (see the previous figure).

In the Sequence Gazer (see the next figure), you can examine the current experiment information (for precursor and fragment masses) to be queried against this sequence.



ProSight Lite

ProSight Lite is a free and simplified version of the ProSightPC application that is mostly used for single protein analysis by infusion. You can use it to view the marked-up spectrum results and, as with the Sequence Gazer feature, edit the sites of modifications. You can also use it to produce publication-ready fragment maps.

Follow this link to access a video containing more information about ProSight Lite and to install the application:

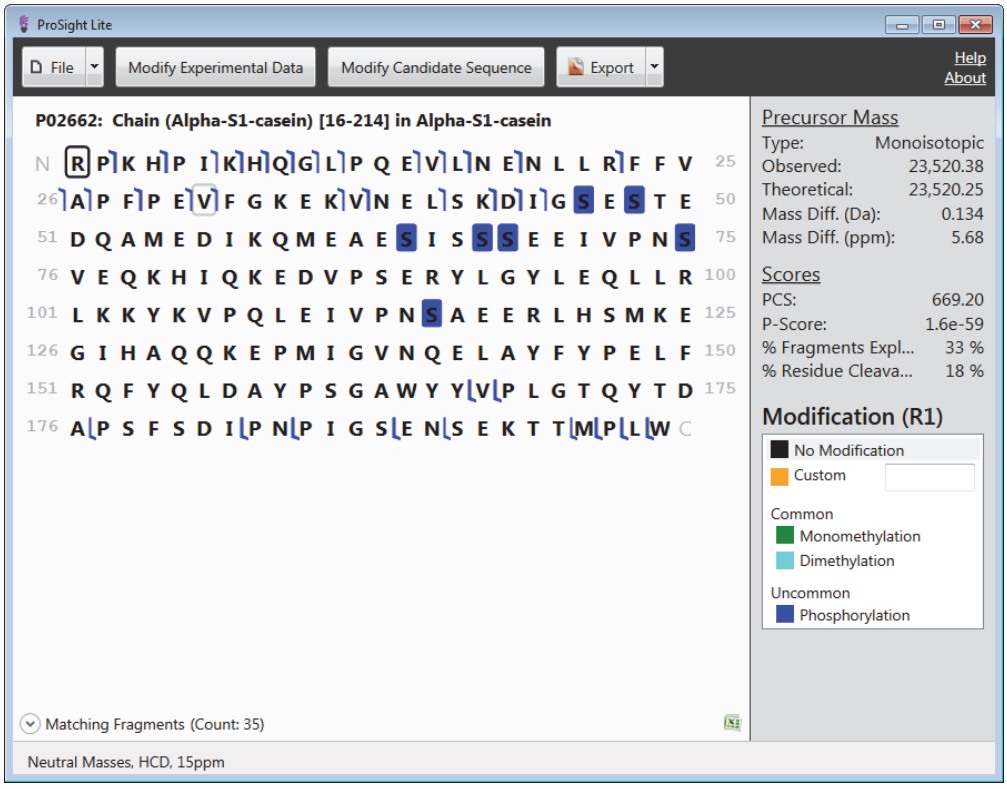
http://prosightlite.northwestern.edu

After installation of the ProSight Lite application is complete, follow these instructions.

❖ To access ProSight Lite

1. Select the appropriate search and click its corresponding arrow in the Data Manager.
2. Locate the appropriate protein identification in the search results and expand the display.
3. Click **Take to ProSight Lite**.

   The ProSight Lite window opens (see the next figure).



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