Agilent

MassHunter BioConfirm 12.0

Quick Start Guide

What is MassHunter BioConfirm?

MassHunter BioConfirm provides automated and interactive protein and oligonucleotide confirmation for TOF and Q-TOF data, with the following features:

- Automated post acquisition data analysis and report generation.
- Biomolecule-centric navigation for peptides, proteins, released glycans, and oligonucleotides.
- Intact protein UI functionality, most notably the visualization of ion sets and showing deconvoluted spectra in a separate window.
- Protein sequence matching, including target protein and potential modifications for intact protein and protein digest sequence types.
- Finding glycans from the target glycan source.
- Finding targets and impurities for oligonucleotides.
- Confirming sequences for oligonucleotides.
- Letting you assign site specific variable modification for protein and protein digest sequence types.
- MFE algorithm - molecular feature extractor for finding peptides in complex LC MS/MS data.
- Relative protein level can be measured either by height from the deconvoluted spectrum or height/area of EIC using all ion set peaks.
- Protein biomolecule quality filters, which prevent “noise” peaks from the deconvoluted spectrum being considered a biomolecule, by requiring sufficient evidence in the *m/z* spectrum.
- Protein sequence editing/matching.
- Oligonucleotide sequence editing/confirming.
- Viewing fragment confirmation for oligonucleotides analyzed with Sequence Confirmation experiment.
- Linked navigation between biomolecules with assigned protein digest matches and peptide sequence within the Sequence Coverage Map.
- Multiple-enzyme digestion sequence coverage display (where each data file represents a single digestion result).

What's New in 12.0

- BioConfirm has a new workflow to characterize oligonucleotides.
- You select one of the following experiments to use with the oligonucleotide workflow: Target Plus Impurities (TPI) or Sequence Confirmation (SC).
- The Oligonucleotides - Target Plus Impurities workflow is an MS1 analysis of an oligonucleotide and its impurities.
- The Oligonucleotide - Sequence Confirmation workflow is an MS/MS analysis of the full-length product to confirm the sequence of bases in an oligonucleotide.
- The Oligos - Impurity List window shows the impurity profile from running the Oligonucleotide - Target Plus Impurities workflow.
- The Results Compare window has a new tab to show the comparison between data files analyzed with the Oligonucleotide - Target Plus Impurities workflow.
- The Fragment Confirmation Ladder window shows sequence coverage results of the oligonucleotide from the Oligonucleotide - Sequence Confirmation workflow.
- The Sequence Manager allows you to define oligonucleotide sequences and their modifications.
- You can define Building Blocks, Linkers, and Modifications for oligonucleotides in the Chemical Data Dictionary.
- You can print a multi-sample report interactively on samples analyzed with the Oligonucleotide - Sequence Confirmation workflow.
- You can run the DAR calculations as part of the Intact Protein workflow.
- Incorporation of MFE allows for improvements in the detection of deamidation in peptide mapping analysis.

Where to find more information

Resource App

Look for these guides on the Agilent website (**https://agilent.com**.)

- *MassHunter BioConfirm Introduction Guide*
- *This guide (MassHunter BioConfirm Quick Start Guide)*

Online Help

Online Help for BioConfirm is available as part of the *MassHunter Help and Learning*. *Online Help* provides more information and can be displayed in the following ways:

- Click **Contents** from the **Help** menu.
- Press the **F1** key to get more information about a window or dialog box.

Agilent Community

To get answers to your questions, join over 10,000 users in the Agilent Community. Review curated support materials organized by platform technology. Ask questions to industry colleagues and collaborators. Get notifications on new videos, documents, tools, and webinars relevant to your work.

https://community.agilent.com

MassHunter BioConfirm Installation

Installing the software

Refer to the MassHunter Workstation *Installation Guide* for complete installation details. Additional setup tasks are required in the OpenLab Control Panel, which are described in that guide.

1. From the **M6025-10001** installation media, right-click **Setup.exe**, and then click **Run as administrator**.
2. Follow the instructions on the screen to install the software.
3. Get and add the MassHunter BioConfirm license. See the MassHunter Workstation for Agilent LC/MS Installation Guide for details.
4. On a Networked Workstation only, register OpenLab Shared Services.

   For BioConfirm, if OpenLab Settings are not registered as the last step of BioConfirm installation, click Start > Agilent MassHunter BioConfirm > Tools for BioConfirm 12.0. Then, run **Register OpenLab Settings**. All default users/roles/groups are added, and permissions for BioConfirm are loaded into the OpenLab Control Panel.

Removing the software

Use **Programs and Features** in Windows Control Panel to remove **Agilent MassHunter Workstation BioConfirm Software**.

Starting BioConfirm from the OpenLab Control Panel

To start the BioConfirm program, you need to select the project that you are using. If no projects exist, you need to first create the project.

1. Double-click the OpenLab Control Panel icon on the desktop. You can instead click **Start > Agilent Technologies > Control Panel**.
2. Click **Projects** in the left pane.
3. If needed, click **Create > Create Project** in **Projects and Groups** on the ribbon. All of your data files, methods, sequences, report templates, and databases are stored in a project. See the online Help for OpenLab Control Panel for more information.
4. Select the project that you want to use in the left pane.
5. On the ribbon in the **BioConfirm** group, click **BioConfirm > Start BioConfirm**. You can instead click **BioConfirm > Create BioConfirm Shortcut** to create a desktop icon that you can click to start BioConfirm with the selected project.

Migrating Methods from 10.0 or 11.0 to 12.0

Before you use a method in Worklist Automation that was created in a previous release of BioConfirm, open and save the method in the project in BioConfirm 12.0. You only need to import a method in **Networked Workstation** mode.

1. In BioConfirm 12.0, click **File > Import to Project > Method(s)**.
2. In the **Method Import** dialog box, select the methods you wish to import.
3. Click **Import**.

User Interface

Main Functional Areas

The main BioConfirm window consists of three parts: (1) the Menu Bar, (2) the Toolbar, and (3) the Main Window. The main functional areas are shown in **Figure 1**, **Figure 2** on page 10, **Figure 3** on page 11, **Figure 4** on page 12, and **Figure 5** on page 13. Some windows are not shown because they are not in the default layout.

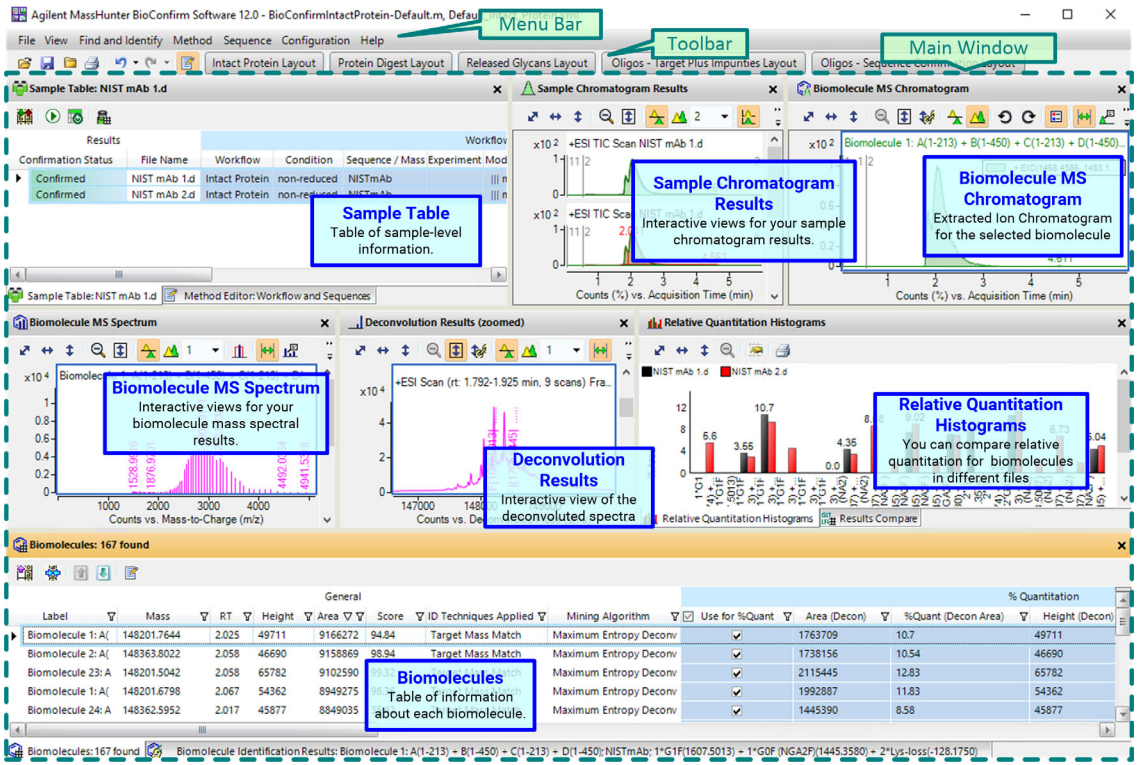


Figure 1. The main functional areas of BioConfirm for the Intact Protein workflow

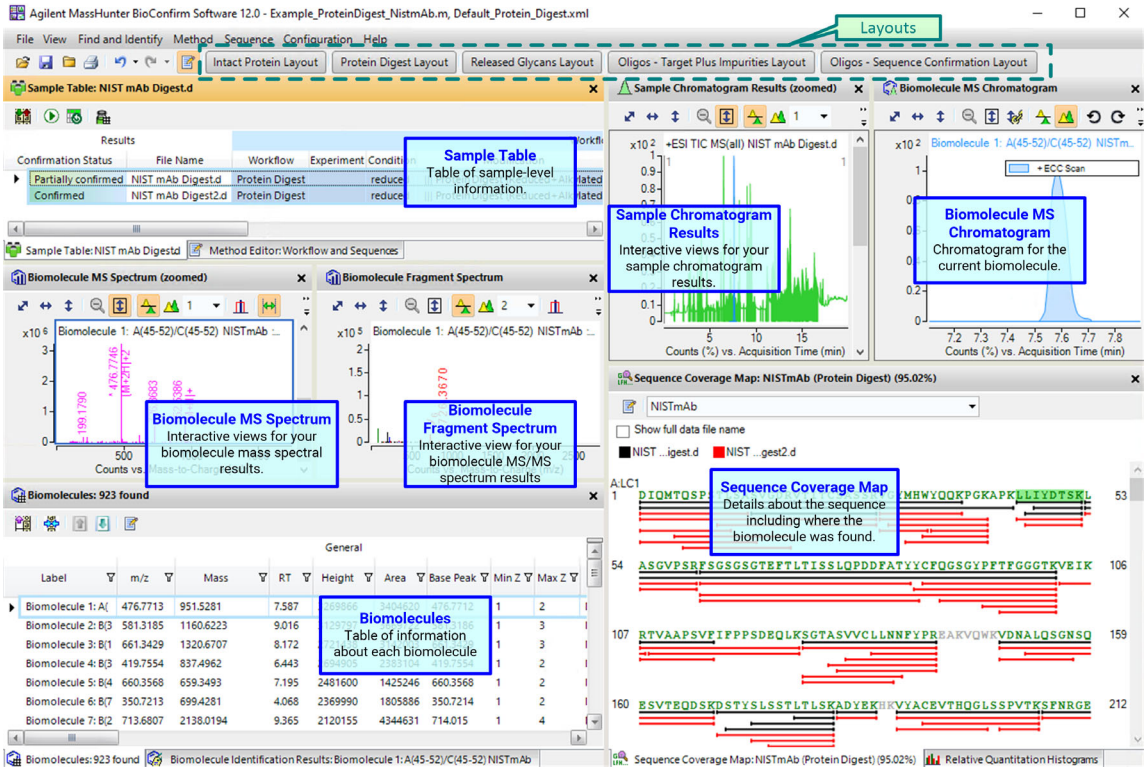


Figure 2. The main functional areas of BioConfirm for the Protein Digest workflow

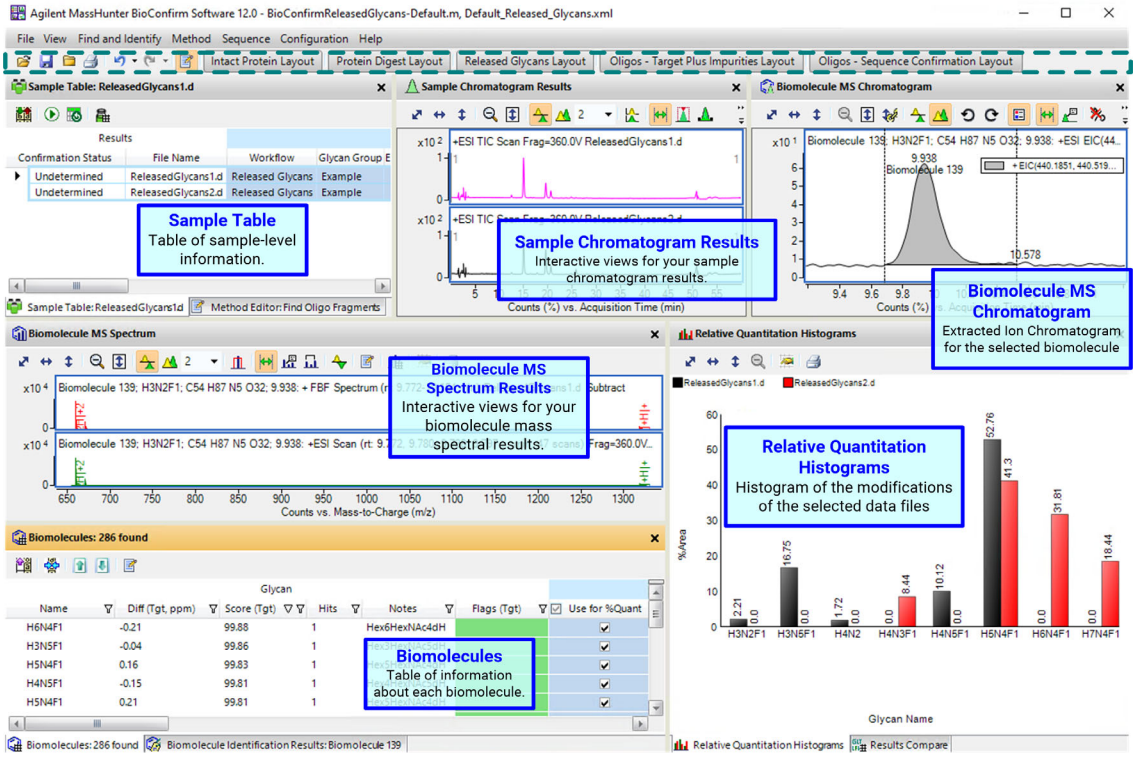


Figure 3. The main functional areas of BioConfirm for the Released Glycans workflow

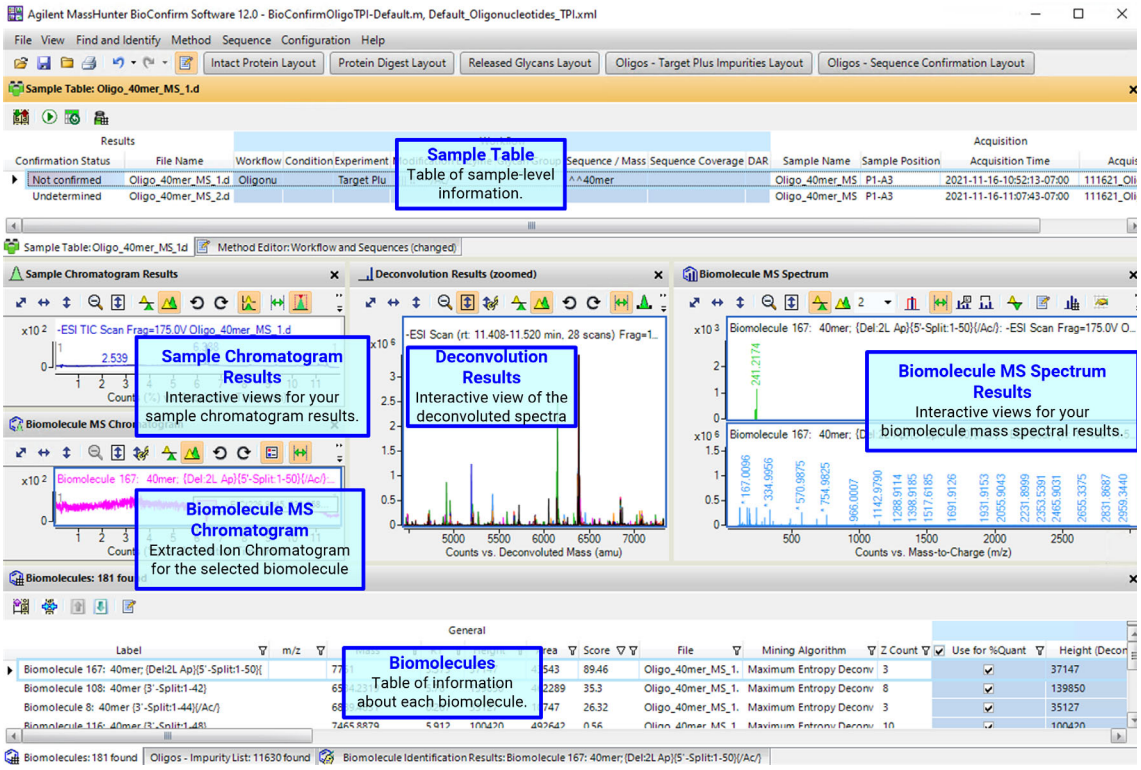


Figure 4. The main functional areas of BioConfirm for the Oligonucleotides workflow when the Experiment is Target Plus Impurities

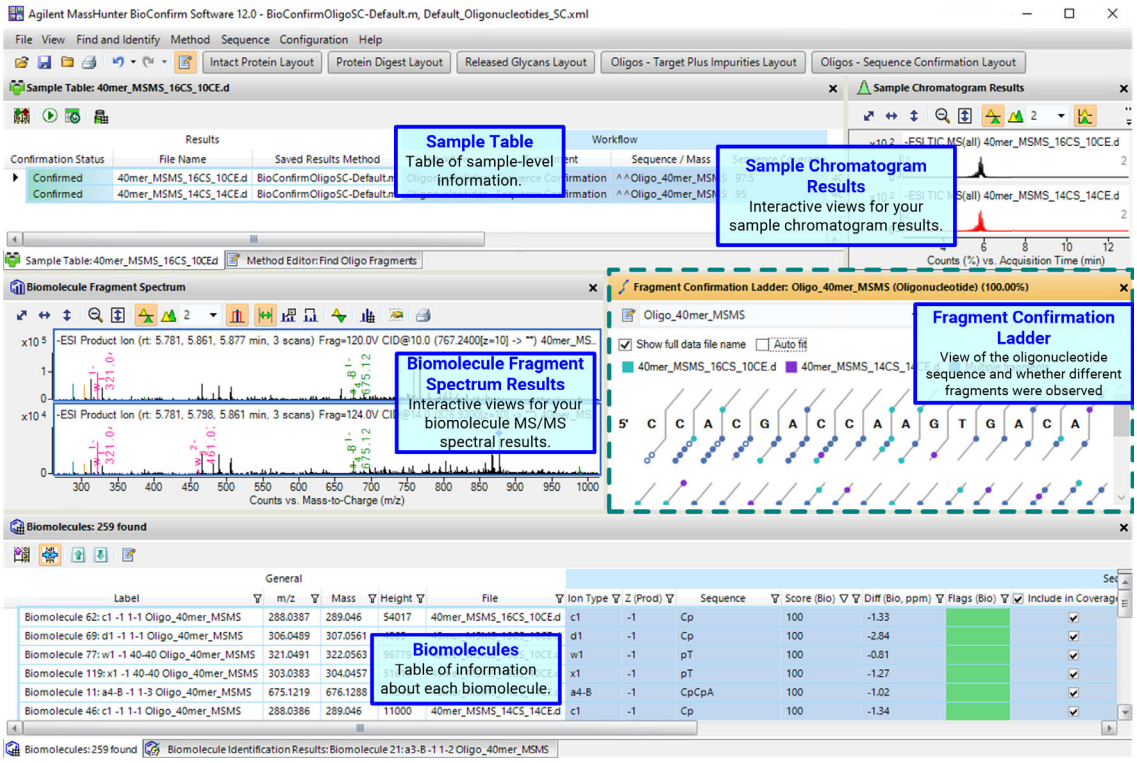


Figure 5. The main functional areas of BioConfirm for the Oligonucleotides workflow when the Experiment is Sequence Confirmation

1. Menu Bar

The menu bar (**Figure 6**) provides actions that are used for opening methods and data files, finding and identifying biomolecules, printing and exporting reports, and accessing the Sequence Manager and Chemical Data Dictionary.

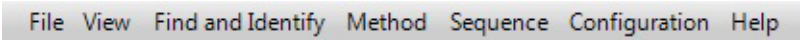
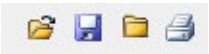
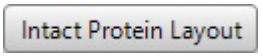
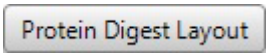
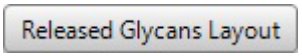
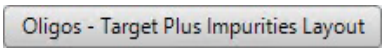


Figure 6. Menu bar

2. Toolbar

The toolbar provides actions that are used for opening data files and closing data files. You can also save results and print a Biomolecule report. Two icons allow you to undo or redo the last actions performed. The last icon toggles whether the Method Editor window is open.

Toolbar Icon	Action
	• File > Open Data File • File > Save Results • File > Close Data File • File > Print > Biomolecule Report
	• Undoes last action performed • Redoes last action undone.
	• Toggles whether the Method Editor window is open.
	• Loads the layout for the Intact Protein workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Protein Digest workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Released Glycans workflow. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Oligonucleotides workflow when the **Experiment** is Target Plus Impurities. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.
	• Loads the layout for the Oligonucleotides workflow when the **Experiment** is Sequence Confirmation. You can change the layout that loads when you click this button in either the **Load BioConfirm Layout** dialog box or the **Save BioConfirm Layout** dialog box.

3. Main window

The main window, see **Figure 2** on page 10, is further divided into many windows: Sample Table, Method Editor, Method Audit Trail, Results Audit Trail, Chemical Data Dictionary Audit Trail, Sample Chromatogram Results, Spectrum Preview, Biomolecule MS Chromatogram, Biomolecule MS Spectrum, Biomolecule Fragment Spectrum, Deconvolution Results, Deconvolution Mirror Plot, MS Spectrum Mirror Plot, Fragment Spectrum Mirror Plot, Biomolecule Chromatogram Mirror Plot, Sample Chromatogram Mirror Plot, MS Actuals, Biomolecules, Biomolecule Identification Results, Results Compare, Relative Quantitation Histograms, Sequence Coverage Map, Fragment Confirmation Ladder, Peptide Relative Quantitation Results, Glycan Structure Viewer, and Oligos - Impurity List. For most of these windows, you toggle whether these windows are visible in the **View** menu.

Sample Table The Sample Table shows information for each sample that is opened. The sample or samples which you select in this window are displayed in the other windows. You can reprocess the selected sample.

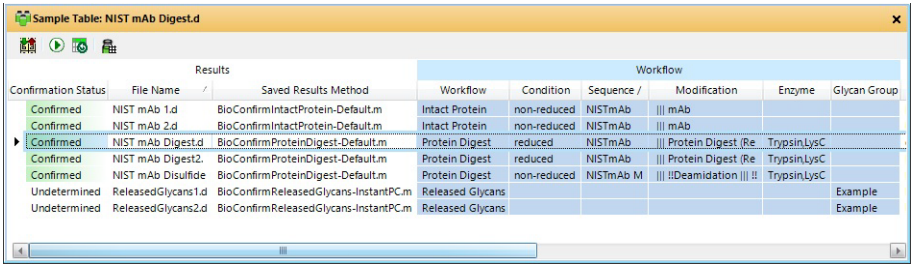


Figure 7. Sample Table window

Method Editor A method is a set of parameters that are associated with the different algorithms that you can run. Methods containing these parameters can be saved using unique file names.

You select the section of the method to display in the left pane. The right pane contains either a single section or multiple tabs. You can get help for each tab or section in the Method Editor when you press **F1**.

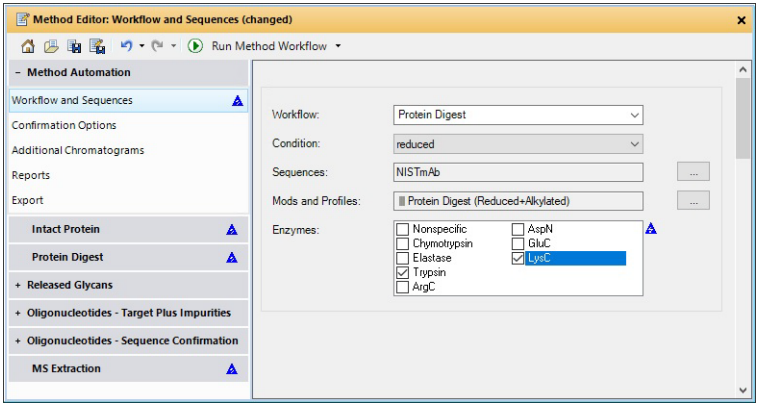


Figure 8. Method Editor window

Sample Chromatogram Results This window shows the chromatograms for each sample that is selected in the Sample Table window. The types of chromatograms include a Total Ion Chromatogram (**TIC**), a Base Peak Chromatogram (**BPC**), an Extracted Ion Chromatogram (**EIC**), and other chromatograms. You can overlay the chromatograms for the selected biomolecule.

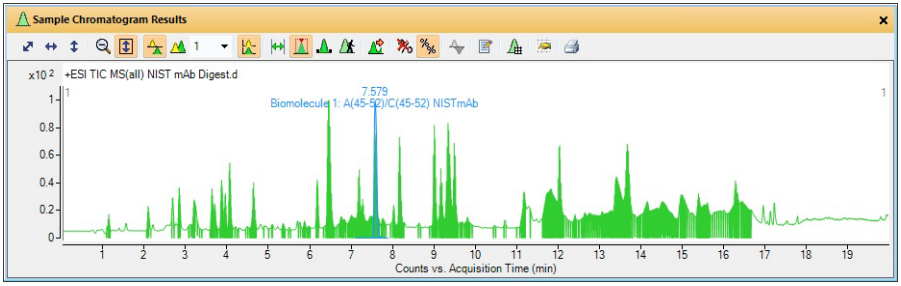


Figure 9. Sample Chromatogram Results window

Spectrum Preview This window is used to quickly scan the spectra in a chromatogram. You start this window either when you click the Walk icon in the Sample Chromatogram Results toolbar or when you click **View > Spectrum Preview**.

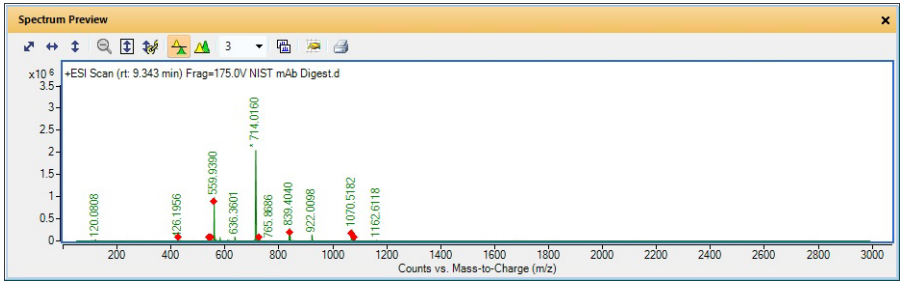


Figure 10. Spectrum Preview window

Biomolecule MS Chromatogram This window shows an Extracted Ion Chromatogram (EIC) for each of the biomolecules you selected in the Biomolecules window. You can display a legend in the upper right corner of the graphic if you select **Overlaid mode** for the chromatograms. You can add annotations to the graphic. You can also export or print the graphic.

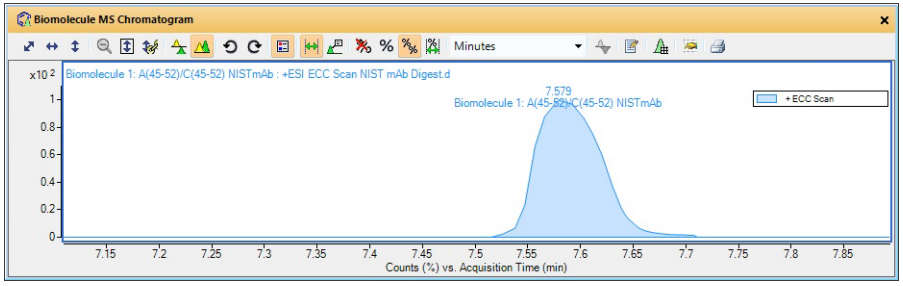


Figure 11. Biomolecule MS Chromatogram window

Biomolecule MS Spectrum This window shows any MS spectrum. MS/MS spectra are displayed in the Biomolecule Fragment Spectrum window. You can add annotations and calipers to a spectrum in this window. You can also see the peak list which is displayed in a table on the right-side of this window. In addition, you can deconvolute, print, and export spectra in this window.

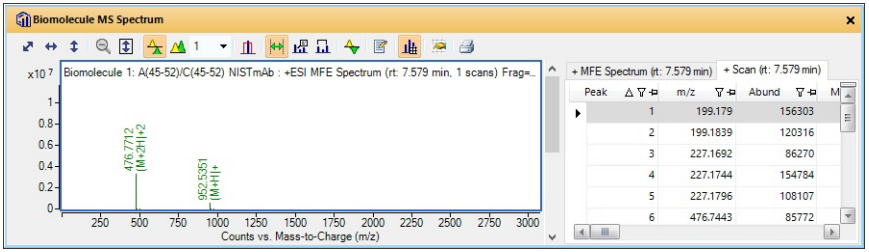


Figure 12. Biomolecule MS Spectrum window

Biomolecule Fragment Spectrum This window shows any MS/MS spectrum. MS spectra are displayed in the Biomolecule MS Spectrum window. You can also see a peak list, annotate, and add calipers to a Fragment Spectrum.

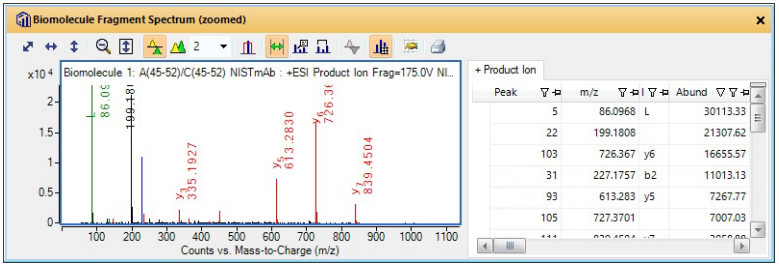


Figure 13. Biomolecule Fragment Spectrum window

Deconvolution Results This window shows deconvoluted mass spectra. You can add annotations and calipers to a spectrum in this window. You can also display the peak list which is displayed in a table on the right-side of this window. You can see peak lists, print spectra, and export spectra in this window.

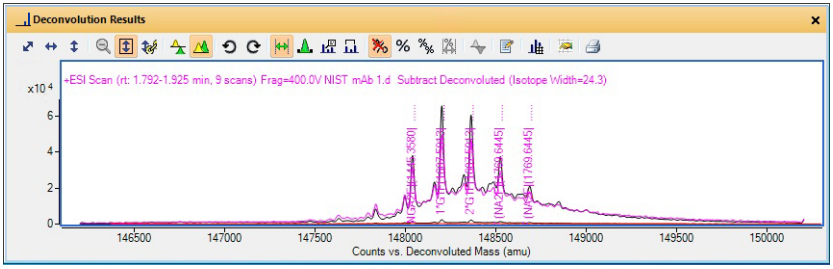


Figure 14. Deconvolution Results window in **Overlaid** mode

Deconvolution Mirror Plot This window displays two deconvoluted spectra selected from the Deconvolution Results window. The first deconvoluted spectra is displayed as the top plot, and the second deconvoluted spectra is displayed as the inverted or bottom plot. You can switch the order of the spectra.

Four additional mirror plots are available for other types of data

- MS Spectrum Mirror Plot
- Fragment Spectrum Mirror Plot
- Biomolecule Chromatogram Mirror Plot
- Sample Chromatogram Mirror Plot

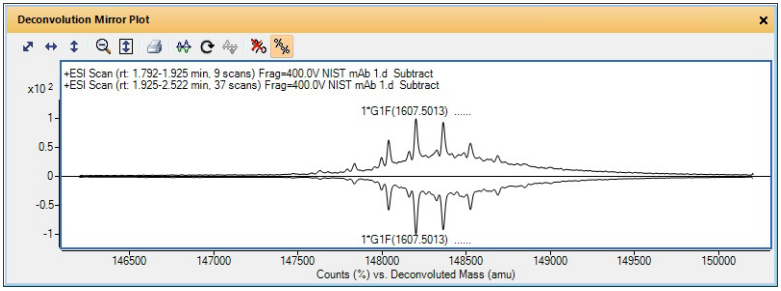


Figure 15. Deconvolution Mirror Plot

MS Actuals This window is used to review the Data Acquisition parameters for the selected spectrum. The four columns in the MS Actuals table are Category, Name, Value, and Unit.

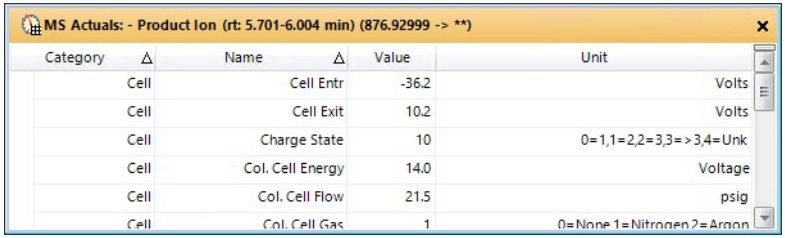


Figure 16. MS Actuals window

Biomolecules This window shows all of the biomolecules which were found for the selected sample files. You can add and remove columns from this table, and you can change the order of the columns.

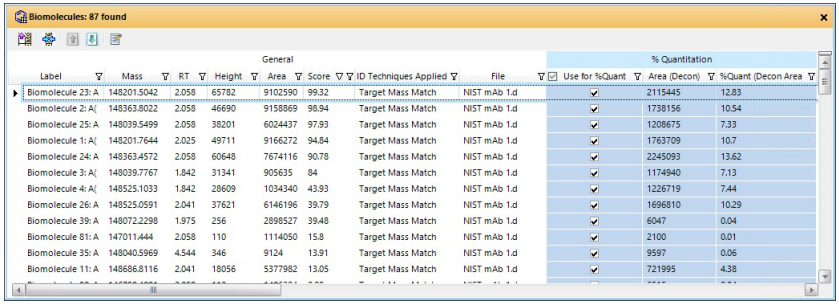


Figure 17. Biomolecules window

Biomolecule Identification Results This window shows the results of running the Match Sequences algorithm on the biomolecules in the Biomolecules table. If you see **Sequence Match** as the value for the **ID Techniques Applied** column, then you can see additional information about that match in this window.

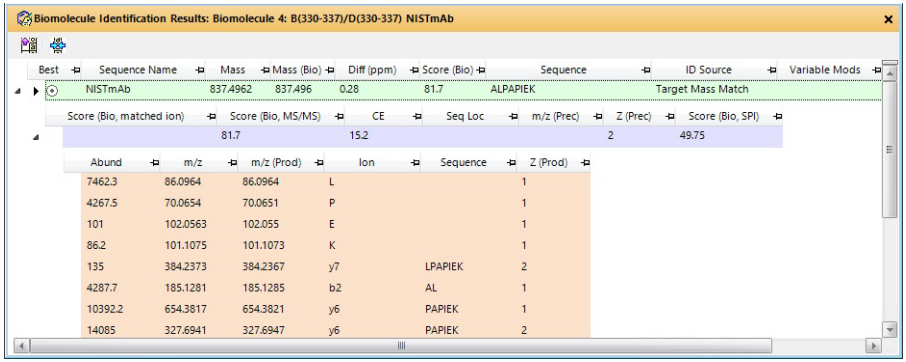


Figure 18. Biomolecule Identification Results window

Results Compare This window displays tables of information that let you compare different data files that have been processed with the same workflow. Results from each workflow are shown in different tabs in this window. The information shown on each tab is different.

- Intact Protein tab
- Protein Digest tab
- Released Glycans tab
- Oligos - Target Plus Impurities tab

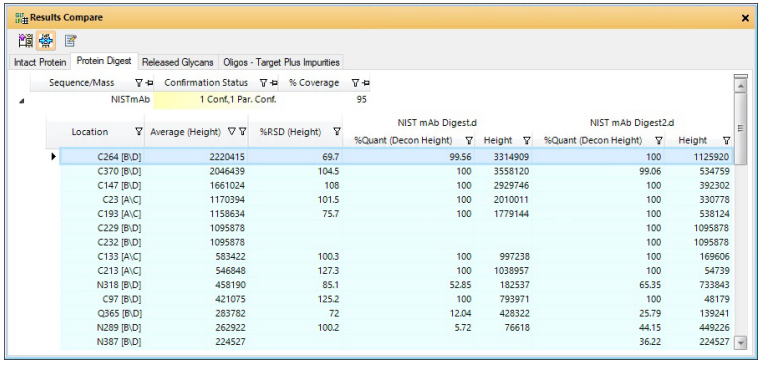


Figure 19. Protein Digest tab in the Results Compare window

Relative Quantitation Histograms This window displays the relative quantitation values for the biomolecules that have the **Use for %Quant** check box marked. The **Use for %Quant** check box is in the Biomolecules table for Intact Protein and Released Glycans biomolecules, and it is in the Peptide Relative Quantitation Results table for Protein Digest biomolecules. You can visually compare the relative quantitation values for up to 10 samples. You can group the biomolecules by either **Biomolecule ID** or by **Sample**. For Intact Protein, this window shows **Pred Mods** vs **%Area** or **%Height**. For Released Glycans, this window shows **Glycan Name** vs **%Area** or **%Height**.

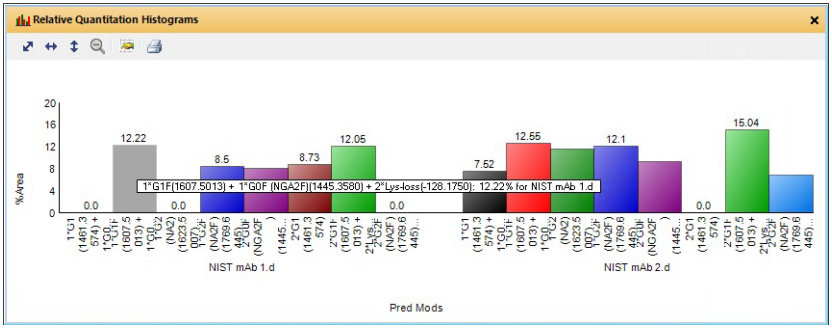


Figure 20. Relative Quantitation Histograms window

For the Protein Digest workflow, this window shows the relative quantitation values for different modifications. You mark the **Use for %Quant** check box in the Peptide Relative Quantitation Results window. The window shows **Modification** on the x-axis, and the plotted value that is clicked in the Histogram Display Options dialog box. You can click **%Area**, **%Height**, or **%Volume (MS-Only Protein Digest)**.

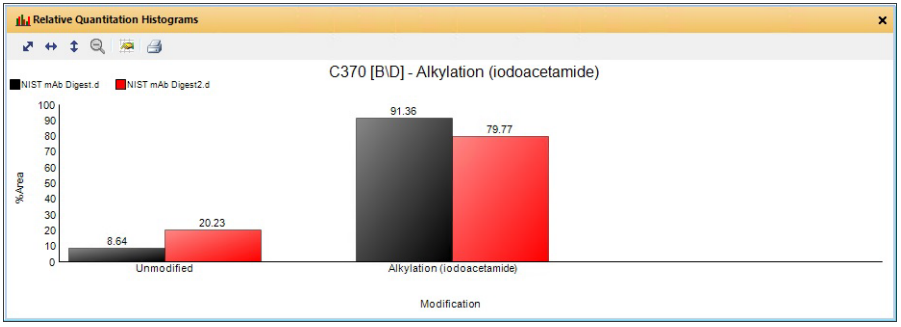


Figure 21. Relative Quantitation Histograms window

Sequence Coverage Map This window displays information for the protein digest sequence selected in the Workflow and Sequences section of the Method Editor window and the biomolecule selected in the Biomolecules window. The highlighted area in the sequence shows where the current biomolecule matches the current sequence. Different lines underneath parts of the sequence show where a biomolecule was matched in the sequence. If you run Match Sequences on multiple sequences, then you can see multiple sequences in this window.

Figure 22. Sequence Coverage Map window

Fragment Confirmation Ladder This window displays a sequence and shows the fragments that were confirmed in the selected data files. This window only displays data for the Oligonucleotides - Sequence Confirmation workflow. The nucleosides are displayed separated by lines. The lines can contain up to 9 dots that represent each of the fragment types expected to be found at each location. The fragments on the bottom limb apply to the nucleoside to the left of the line; the fragments on the top limb apply to the nucleoside to the right of the line.

If you run the Oligonucleotides - Sequence Confirmation workflow on multiple data files, then you can see fragments from multiple data files in the ladder.

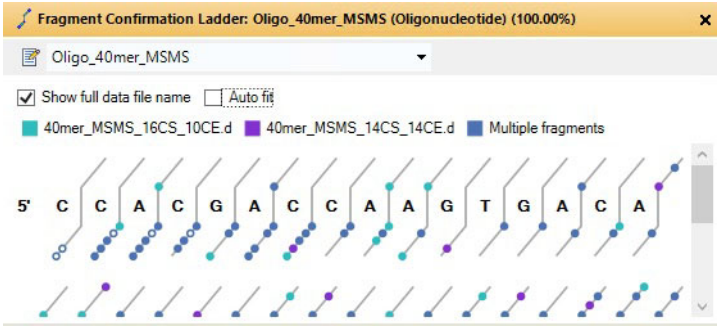


Figure 23. Fragment Confirmation window

Peptide Relative Quantitation Results This window displays only the peptides that were matched and have a predicted modification. If an amino acid at a particular location has been found to have a modification in a particular file, it will be displayed as a row in the table. Each row has a nested level where the included biomolecule information is displayed. For Protein Digest workflow, you mark the **Use for %Quant** check box in the second level of this table.

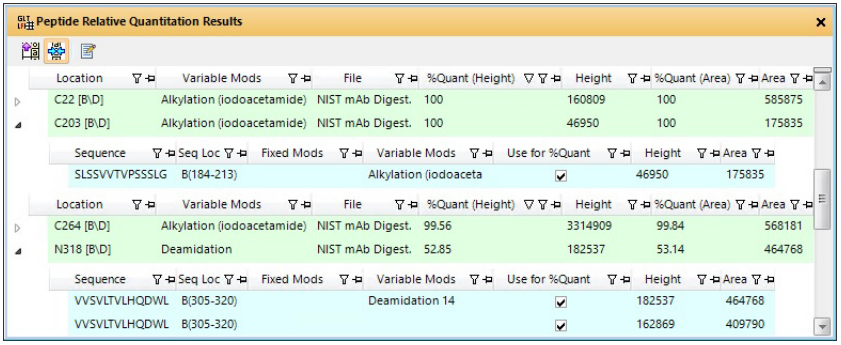


Figure 24. Peptide Relative Quantitation Results window

Glycan Structure Viewer This window displays glycan structures. The structure is also shown in the Biomolecule MS Spectrum window.

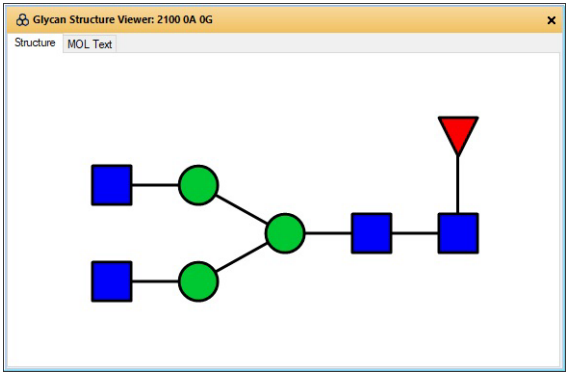


Figure 25. Glycan Structure Viewer window

Oligos - Impurity List This window displays information on the oligonucleotide impurities that are generated from the oligonucleotide sequence, based on the modifications and matching rules used. This window is only available for the Oligonucleotides - Target Plus Impurities workflow. You display this window when you click **Sequence > Oligos - Impurity List**. If you run Match Sequences on multiple sequences, then you can see multiple sequences in this window.

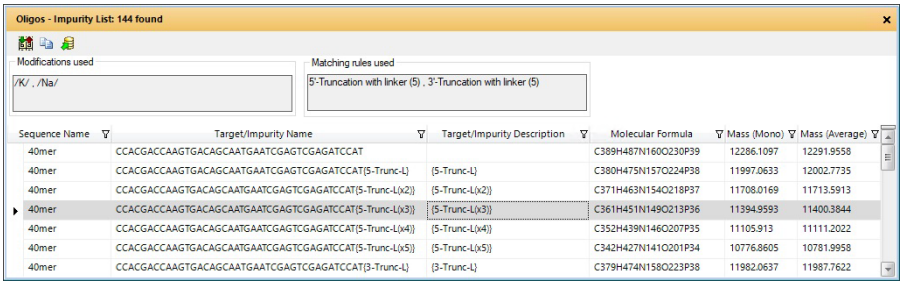


Figure 26. Oligos - Impurity List window

Results Audit Trail This window shows Audit Trail information about the data file that is currently selected in the Sample Table window. The Audit Trail tracks what has changed in the results of the data file. It records who was logged in and when the change was made. On a compliance system, it will also display the version of the data file in which the change was saved.

If a row has a pink background, that row has not been reviewed. To review the results, you click **Review**. Then, you enter a Review comment for the results that have not been reviewed. When you click **OK** on the Audit Trail Review dialog box, the Review comment is added to the Review Comment column. Two rows are added to the audit trail showing that the audit trail has been reviewed and saved and by whom, and the newly reviewed rows will turn white.

Three additional Audit Trail windows are available: **Method Audit Trail**, **Chemical Data Dictionary Audit Trail**, and **Sequence Audit Trail** (in Sequence Manager).

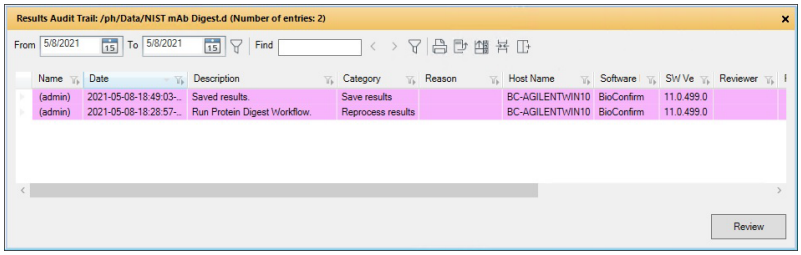


Figure 27. Results Audit Trail window

BioConfirm Dialog Boxes

The following dialog boxes are some of the dialog boxes available in MassHunter BioConfirm. See *online Help* for more information.

Chemical Data Dictionary Dialog Box

Lets you customize proteins and oligonucleotides for use with MassHunter BioConfirm. For proteins, you can update the list of modifications, links, and reagents. The factory-supplied default file cannot be modified, but you can add to it and save to a custom copy. For oligonucleotides, you can update building blocks, linkers, and modifications. You can modify the factory supplied values.

To open this dialog box Click **Open Chemical Data Dictionary Editor** on the Sequence menu. In the Sequence Manager program, you can use the **Open Chemical Data Dictionary Editor** command on the Edit menu.

Show Sequence Description Dialog Box

Lets you review descriptive information that is stored with both method sequences and result sequences.

To open this dialog box Click **Show Sequence Description** on the Sequence menu. In the Sequence Manager program, you can use the **Edit Description** command on the Sequence menu or the Sequence Editor shortcut menu to change the description for a sequence; the **Description** dialog box opens.

Modifications Dialog Box

Lets you view modifications to the protein sequence. This dialog box only applies to Protein sequences.

To open this dialog box Click **Applied Modifications** on either the Sequence menu or the Sequence Editor shortcut menu to open the **Applied Modifications** dialog box. This dialog box contains only the Specified Applied information from the **Modifications** dialog box.

In the Sequence Manager program, you can use the **Edit Modifications** item on the Edit menu or the Sequence Editor shortcut menu to add a modification to a sequence. The **Modifications** dialog box contains three tabs: Local, Global, and Specified Applied.

Applied Links

Lets you review the amino acids linked in the sequence. This dialog box only applies to Protein sequences.

To open this dialog box Click **Specified Applied Links** on the Sequence menu or the Sequence Coverage Map shortcut menu to open the Specified Applied dialog box. This dialog box contains only the Specified Applied information from the **Links** dialog box.

In the Sequence Manager program, you can use the **Edit Links** command on the Edit menu or the Sequence Editor shortcut menu to add a link to a sequence. The **Links** dialog box contains three tabs: Global, Specified Applied, and Unspecified Disulfide Links. See “Applying or editing protein links” on page 86.

Workflows and Sequences

In the Method Editor in the Method Automation > Workflow and Sequences section, you select the workflow and set workflow parameters.

Workflows

You select a workflow in the Method Editor window in the **Method Automation > Workflow and Sequences** section. This workflow decides what operations are to be run and how the sequence match is to be done when you run the method. BioConfirm supports five workflows: **Intact Protein**, **Protein Digest**, **Released Glycans**, **Oligonucleotides**, and **Custom**.

Intact Protein

The workflow runs the Find by Protein Deconvolution algorithm, and then runs Intact Protein matching rules to match sequences.

A report is only generated if you run **Run Method Automation (Workflow + Reports)** or if you generate the report interactively. A Biomolecule report is generated using the **Intact Protein** report template selected on the Method Automation > Reports > Templates tab.

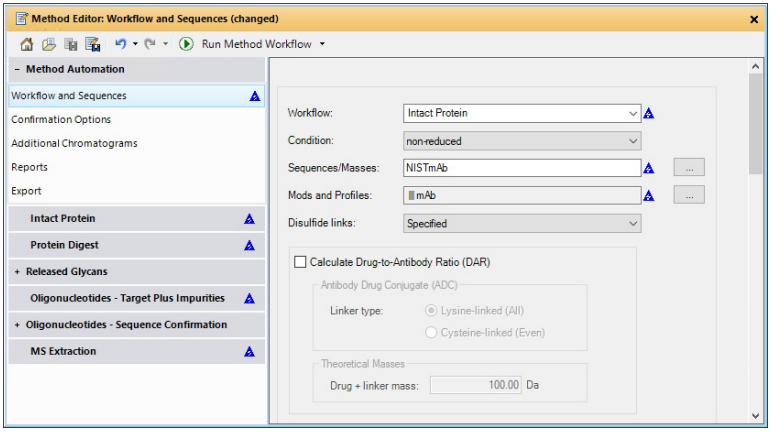


Figure 28. Method Automation > Workflow and Sequences section for Intact Protein workflow

Protein Digest

This workflow runs the Find Peptides algorithm, and uses protein matching rules (Protein Digest, Predicted Modifications) to match sequences. You can select whether or not to use Protein Truncation. The workflow also runs the Match Sequences algorithm with the Sequence or mass that you entered and digests the sequence using the enzyme selected. See **Figure 29** on page 30.

A report is only generated if you run **Run Method Automation (Workflow + Reports)** or if you generate the report interactively. A Biomolecule report is generated using the **Protein Digest** report template selected on the Method Automation > Reports > Templates tab.

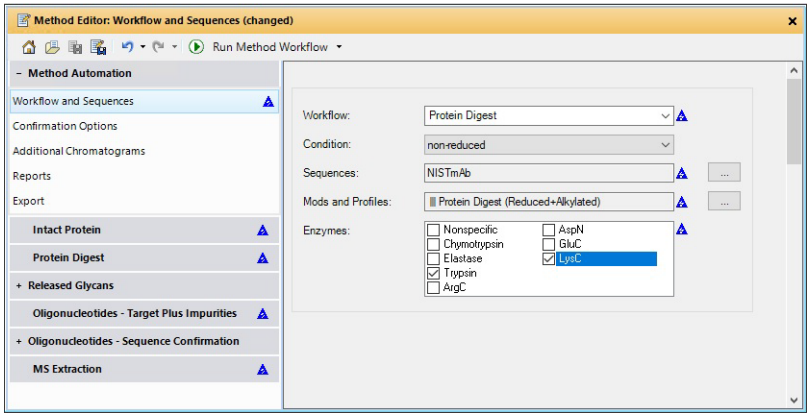


Figure 29. Method Automation > Workflow and Sequences section for Protein Digest workflow

Released Glycans

This workflow runs the Find Glycans algorithm and uses the **Target glycan source** that you entered. See **Figure 30** on page 31. This source by default is in the method but it can also be in the PCDL folder or other folders in the project. You can specify the Glycan Group name for this sample. Results in the Released Glycans tab in the Results Compare window are sorted by Glycan Group.

A report is only generated if you run **Run Method Automation (Workflow + Reports)** or if you generate the report interactively. A Biomolecule report is generated using the **Released Glycans** report template selected on the Method Automation > Reports > Templates tab.

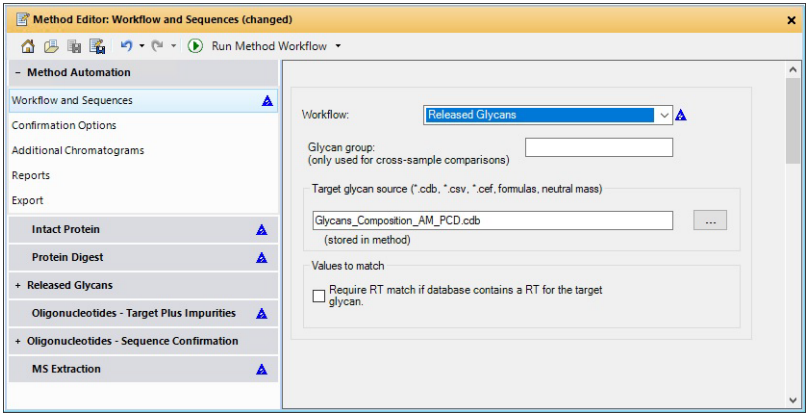


Figure 30. Method Automation > Workflow and Sequences section for the Released Glycans workflow

Oligonucleotides

For this workflow, you also select the **Experiment**: either **Target Plus Impurities** or **Sequence Confirmation**. For the Target Plus Impurities experiment, you enter the Sequence, Modifications, and Matching Rules; for the Sequence Confirmation experiment, you only enter the Sequence. See **Figure 31** on page 31.

A report is only generated if you run **Run Method Automation (Workflow + Reports)** or if you generate the report interactively. A Biomolecule report is generated using the **Target Plus Impurities** report template or the **Sequence Confirmation** report template selected on the Method Automation > Reports > Templates tab.

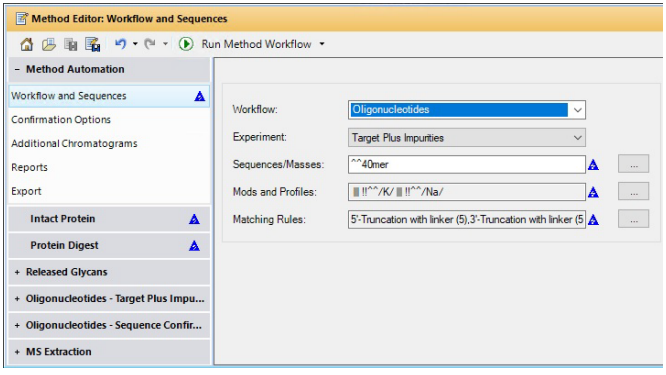


Figure 31. Method Automation > Workflow and Sequences section for Oligonucleotides workflow with Target Plus Impurities experiment

Custom

This workflow runs the actions which you select in the Workflow and Sequences section. You select the actions from the **Available actions** list and place them in the **Actions to be run** list. The order of the actions in the **Actions to be run list** is the order in which the actions are executed. If you generate a biomolecule report, it uses the **Protein Digest report template** if you used the **Find Peptides** algorithm; otherwise, it uses the **Intact Protein report template**.

Sequences

You create and edit sequences in the Agilent MassHunter Sequence Manager program. You can add either Proteins or Oligonucleotides, and they are seen on separate tabs.

You can add a sequence manually, or you can add a sequence from a sequence file (psq), text file (txt), or FAST-A file. You edit sequences in the Sequence Manager program. For Oligonucleotides, you add **Building Blocks**, **Modifications**, and **Linkers** in the **Chemical Data Dictionary** dialog box. For Proteins, you add Modifications, Links, and Reagents.

You specify a sequence in the Method Editor window in the Method Automation > Workflow and Sequences section.

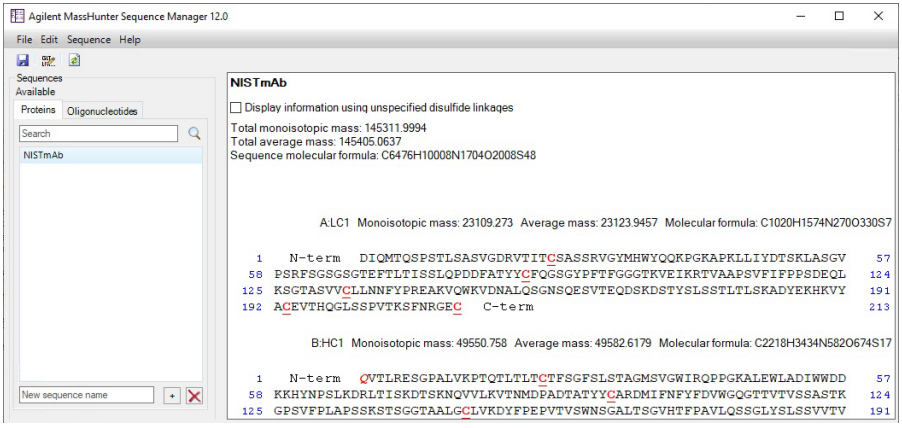


Figure 32. MassHunter Sequence Manager program

Intact Protein Workflow

The topics in this section will help you get started using the Intact Protein workflow features of MassHunter BioConfirm.

What is deconvolution?

The Deconvolution algorithm does charge state deconvolution of mass spectra of large molecules with high charge states. Singly-charged ions with *m/z* values greater than a few thousand Daltons are beyond the mass range of the Agilent TOF instrument. However, multiply-charged ions can be observed if their mass-to-charge ratio (*m/z*) falls within the instrument range. This applies to proteins which typically become multiply-charged. Based on the maximum entropy result, peak modeling (pMod) automatically generates peak models without manual intervention and applies these models through fitting and validating procedures to provide a highly resolved zero-charge spectrum and a set of mass error assessments for each peak. You can select **pMod** as the **Deconvolution algorithm** on the Deconvolution tab. The other Deconvolution algorithms are **Maximum Entropy** and **Large Molecule Feature Extraction (LMFE)**.

Running the Intact Protein workflow

When you run an **Intact Protein** workflow, the workflow automatically does these steps:

- Find by Protein Deconvolution
- Match Sequences

1 Open the data file that contains the biomolecules of interest.

2 Open the **BioConfirmIntactProtein-Default.m** method. Click **Method > Open**.

3 Select Method Automation > Workflow and Sequences in the Method Editor window.

4 Select **Intact Protein** for the **Workflow**.

5 Select either **reduced** or **non-reduced** for the **Condition**.

6 Click the [...] button next to the **Sequences/Masses** parameter to select a sequence. You can instead enter one or more masses.

7 Click the [...] button next to the **Mods and Profiles** parameter to select modifications and profiles.

8 Save the method. Click **Method > Save As** to save to a new name.

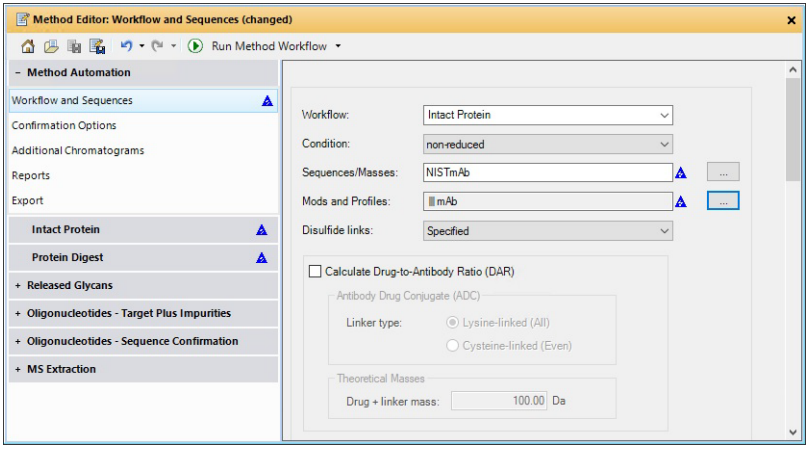


Figure 33. Workflow and Sequences section for an Intact Protein workflow

9 Click the button on the Method Editor toolbar to run the method workflow. You can instead click **Method > Run Method Workflow**.

10 View biomolecules as described in **"Viewing deconvolution biomolecules"** on page 44.

Running the Intact Protein workflow with DAR

For the Intact Protein workflow, you can also calculate DAR values.

1. Open the data file that contains the biomolecules of interest.
2. Open the **BioConfirmIntactProtein-Default.m** method. Click **Method > Open**.
3. Select Method Automation > Workflow and Sequences in the Method Editor window.
4. Select **Intact Protein** for the **Workflow**. Select either **reduced** or **non-reduced** for the **Condition**.
5. Type a mass for the Sequences/Masses parameter. If you are doing DAR calculations, you cannot enter a sequence.
   - If you select **non-reduced**, enter one mass.For non-reduced, you enter the intact DAR 0 mass.
   - If you selected **reduced**, enter two masses for the Reduced DAR light chain and Reduced DAR heavy chain.
   - The **Mods and Profiles** parameter is ignored when running the DAR workflow. You can add them, but they are not considered.
6. Mark the **Calculate Drug-to-Antibody Ratio (DAR)** check box.
7. Select either **Lysine-linked (All)** or **Cysteine-linked (Even)** as the ADC type.
8. Enter **The Drug + linker mass**.
9. Select Intact Protein > DAR Calculation in the Method Editor window.
10. Enter the **DAR 0 Mass(es)**.
11. Save the method. Click **Method > Save As** to save to a new name.

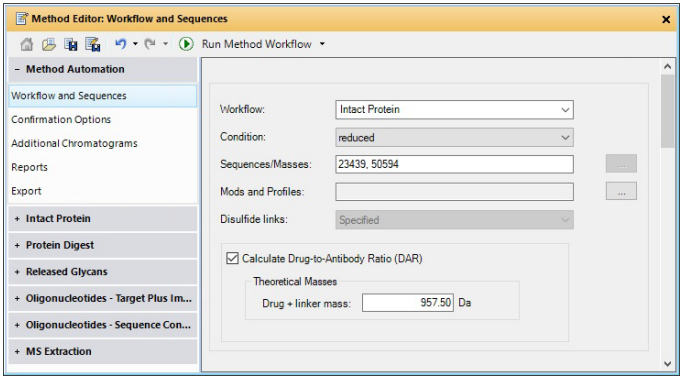


Figure 34. Workflow and Sequences section for an Intact Protein workflow

12 Click the  button on the Method Editor toolbar to run the method workflow. You can instead click **Method > Run Method Workflow**.

13 View biomolecules as described in **"Viewing DAR results for Intact Protein biomolecules"** on page 45.

Finding biomolecules by Protein Deconvolution

Use this procedure to deconvolute proteins and create a biomolecule list.

1. Open the data file that contains the spectra of interest as described in *online Help*.
2. In the Method Editor window, select **Intact Protein > Deconvolute (Protein)** in the left pane.
3. Set parameters in the Method Editor window in the Deconvolute (Protein) > Deconvolution section.
4. Click the  button on the Method Editor toolbar to start processing. You can instead click **Find and Identify > Find by Protein Deconvolution**.
5. Review results in the Deconvolution Results window. If this window is not currently displayed, click **View > Deconvolution Results**.
6. View deconvolution biomolecules as described in **"Viewing deconvolution biomolecules"** on page 44.

Deconvoluting selected spectra

Use this procedure to deconvolute selected *m/z* spectra and create a biomolecule list.

1. Open the data file that contains the spectra of interest. Click **File > Open Data File**. This process is described in *online Help*.
2. Select a spectrum as described in *online Help*.
   - Double-click the Sample Chromatogram Results window. or
   - Select a range in the Sample Chromatogram Results window and double-click that range.
3. In the Method Editor window, select **Intact Protein > Deconvolute (Protein)** in the left pane.
4. Set parameters in the Method Editor window in the Deconvolute (Protein) > Deconvolution section.
5. Click the arrow next to the  button on the Method Editor toolbar and select **Deconvolute Spectrum** to start processing.

Tip You can also initiate deconvolution when you right-click an MS spectrum in the Biomolecule MS Spectrum window and click **Deconvolute > Proteins** from the shortcut menu. You can also deconvolute Oligonucleotides.

6. Review results in the Deconvolution Results window. If this window is not currently displayed, click **View > Deconvolution Results**. You can manually integrate peaks in the Deconvolution Results window.
7. View deconvolution biomolecules as described in **"Viewing deconvolution biomolecules"** on page 44.

Matching sequences for intact protein biomolecules

Use this procedure to match sequences for biomolecules that were found using deconvolution. You need to specify the sequence and modifications and profiles in the Method Automation > Workflow and Sequences section.

1 Create biomolecules through deconvolution. See **"Finding biomolecules by Protein Deconvolution"** on page 37 or **"Deconvoluting selected spectra"** on page 38.

2 Select **Method Automation > Workflow and Sequences** in the Method Editor window.

3 Select **Intact Protein** for the **Workflow**.

4 Select either **reduced** or **non-reduced** for the **Condition**.

5 Click the ... button next to the **Sequences/Masses** parameter to select a sequence.

6 Click the ... button next to the **Mods and Profiles** parameter to select modifications and profiles.

7 In the Method Editor window, select **Intact Protein > Match Tolerances** in the left pane.

   Make sure to choose the Intact Protein > Match Tolerances section.

   Do *not* select the Protein Digest > Match Tolerances section.

8 Review the parameters on the Mass Matching, Matching Rules, and Results tabs.

9 Click the button on the Method Editor toolbar to run the Match Sequences algorithm.

Tip You can also start Match Sequences when you click **Find and Identify > Match Sequences**.

10 View deconvolution biomolecules as described in **"Viewing deconvolution biomolecules"** on page 44.

Viewing protein deconvolution results

Use this procedure to review the results from the following deconvolution process:

- **"Running the Intact Protein workflow with DAR"** on page 35
- **"Finding biomolecules by Protein Deconvolution"** on page 37
- **"Deconvoluting selected spectra"** on page 38

1. (optional) Click the **Intact Protein Layout** icon on the main toolbar.
2. If the Deconvolution Results window is not currently displayed, click **View > Deconvolution Results**.
3. If the Biomolecules window is not currently displayed, click **View > Biomolecules**.
4. Select a biomolecule in the Biomolecules window.
5. Use the following mouse actions to change the display of data:
   - Click to select a single mass in the spectrum.
   - Drag to select a mass range in the spectrum.
   - **Ctrl + drag** to select another area and keeps the previous area/time selected.
   - Drag axes to scroll the axes in the direction you are moving the mouse.
   - Right-drag to expand the selected area. The area you define is shown as a rectangle outlined in black. The Y-scale of the zoomed in display is controlled by the Auto-Scale Y-axis mode.
   - Right-drag axes to scale the axis. Dragging to the right (x-axis) or to the top (y-axis) zooms in on that axis. Dragging to the left (x-axis) or to the bottom (y-axis) zooms out on that axis.

Tip To return to the previous display scale, click the **Unzoom** toolbar button  .

6. Use the following toolbar buttons in the Deconvolution Results window to change the display of data:

Table 1. Toolbar buttons in the Deconvolution Results toolbar

Toolbar button	Action/Meaning
	Scales the x-axis and y-axis automatically to fit the displayed data.
	Scales the x-axis automatically to fit the displayed data.

Table 1. Toolbar buttons in the Deconvolution Results toolbar

Toolbar button	Action/Meaning
	Scales the y-axis automatically to fit the displayed data.
	Returns to the previous display scale (undoes last zoom operation).
	• When this mode is on, the vertical plot range is automatically scaled to fit the data in the selected horizontal range when you zoom in. • When the **Autoscale y-axis during Zoom** mode is off, the vertical plot range is set to the limits you specify by dragging the mouse.
	Toggles whether to scale the y-axis of all deconvoluted spectra to the same scale when you zoom in or out on the x-axis.
	Plots each spectrum separately. The spectra share the same x-axis, but each spectrum has a separate y-axis.
	Overlays all spectra. The spectra are shown with the same x- and y-axes.
	Cycles to previous spectrum. This toolbar button is only available when you have overlaid the spectra.
	Cycles to next spectrum. This toolbar button is only available when you have overlaid the spectra.
	Turns on the Range Select tool.
	Turns on the Manual Peak Integration tool. When you select a peak, two black boxes are drawn at the beginning and end of that peak. You can drag those boxes to change the integration.
	Turns on the Annotate tool, which lets you add various annotations.
	Turns on the Caliper tool, which lets you measure Modifications, Amino Acids, or Delta Mass between peaks or points.
	Stops normalizing the deconvolution spectra.
	Normalizes all deconvoluted spectra. All deconvoluted spectra are normalized to the largest peak in any of the chromatograms.
	Normalizes all spectra. Each spectrum is normalized to the largest peak in itself.
	Normalizes each spectrum to the highest peak within the selected range.

Table 1. Toolbar buttons in the Deconvolution Results toolbar

Toolbar button	Action/Meaning
	Displays the two, selected deconvoluted spectra in the Deconvolution Mirror Plot window. This button is only available if two deconvoluted spectra are selected.
	Displays the Method Editor window as a floating window. This button is not a toggle.
	Toggles the display of the peak list tabs in this window. If this option is on, then the right pane of this window contains a tab for each deconvoluted spectrum. Each tab contains a table of information about a deconvoluted spectrum.
	Opens the **Deconvoluted Spectra Display Options** dialog box.
	Prints an image of the contents of the Deconvolution Results window and puts it in a PDF file. This PDF is opened automatically.

7 Right-click the graph to display the following shortcut menu commands:

- **Copy Deconvolution Settings to Method** - Copies the settings that were used to create the Deconvolution Results the Deconvolute (MS): Maximum Entropy section of the Method Editor window.
- **Include in %Quant** - If you select the Manual Integration icon and you are displaying the results in List mode, then you can specify to include the selected peak in the Relative Quantitation results. The **Use in %Quant** check box for this peak is marked in the Biomolecules table.
- **Delete** - Deletes the currently highlighted spectra.
- **Copy to Clipboard** - Copies spectra that are currently visible in the Deconvolution Results window to the Clipboard for use with other applications.
- **Print** - Lets you print the Deconvolution Results window.
- **Export** - Lets you export data and graphics.
- **Adjust Peak Threshold**- Lets you change the threshold for identifying peaks.

8 (*optional*) To change the number of significant digits in the deconvoluted spectrum, click  in the Deconvolution Results toolbar. Set the **Digits after the decimal** value, and click **OK**.

9 To compare two deconvoluted spectra:

a Highlight the two spectra of interest in the Deconvolution Results window.

b Click the **Deconvolution Mirror Plot Results** button  .

The Deconvolution Mirror Plot Results window appears with the first spectra displayed as the top plot and the second spectra displayed as the inverted or bottom plot. See **"Deconvolution Mirror Plot"** on page 19.

c Click the  button to reverse the two plots.

See *online Help* for more display options in the Deconvolution Mirror Plot Results window.

10 (*optional*) To move the window, drag it to a new position on the screen. To return it to its docked position, right-click the title bar and click the **Floating** command.

Viewing deconvolution biomolecules

Biomolecules are created for proteins that are confidently found. You can view these biomolecules as follows:

1. (optional) Click the **Intact Protein Layout** icon on the main toolbar.
2. If necessary, click **View > Biomolecules**.
3. If necessary, click **View > Biomolecule Identification Results**.
4. Click a biomolecule of interest. Associated data in the following windows is displayed:
   - Deconvolution Results window
   - Biomolecule MS Chromatogram window shows an EIC
   - Biomolecule MS Spectrum window displays all the different peaks in the raw *m/z* spectrum that indicate the presence of the deconvoluted protein mass in the Biomolecule MS Spectrum Results window
   - Biomolecule Identification Results if you also ran the Match Sequences algorithm.
5. Review the results in the Deconvolution Results window. See **"Viewing protein deconvolution results"** on page 40.
6. Click  on the Deconvolution Results toolbar.
7. View the following information for the deconvoluted spectrum in the Deconvolution Results window:

   - Peak
   - *Fit*
   - Charge
   - Max Abund
   - Mass
   - Expected m/z
   - Height
   - Area

8. Review the columns in the Biomolecule Identification Results window.
9. Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

Viewing DAR results for Intact Protein biomolecules

Biomolecules are created for proteins that are confidently found. If you calculated DAR values, you can view the DAR calculations as follows:

1. (optional) Click the **Intact Protein Layout** icon on the main toolbar.
2. If necessary, click **View > Biomolecules**.
3. Click a biomolecule of interest. Associated data in the following windows is displayed:
   - Deconvolution Results window
   - Biomolecule MS Chromatogram window shows an EIC
   - Biomolecule MS Spectrum window displays all the different peaks in the raw *m/z* spectrum that indicate the presence of the deconvoluted protein mass in the Biomolecule MS Spectrum Results window
4. In the Deconvolution Results window, examine the DAR spectrum or DAR spectra. For a reduced data file, find the Light Chain DAR spectrum and the Heavy Chain DAR spectrum. For a non-reduced data file, find the DAR spectrum.
5. Click  on the Deconvolution Results toolbar.
6. View the following information for the DAR spectrum in the Deconvolution Results window:

- Use in Calc
- *% Area*
- DAR Peak
- Max Y
- Peak Mass (Da)
- Expected m/z
- Area
- Mass (Start)
- Mass (End)

7. In the Sample Table window, find the DAR value for the selected Data File. It is in the Workflow section of the table. If you do not see that column, click  in the Sample Table toolbar to add the **DAR** column to the Sample Table.

Viewing relative quantitation results for intact proteins

You can only review relative quantitation results for intact proteins that have the **Use for %Quant** check box marked in the Biomolecules window. Samples are sorted by the Sequence or Mass.

1. Verify that the Sequence for your samples is the same if you want to compare results.
2. Select one or more Intact Protein samples in the Sample Table window.
3. Click the **Intact Protein Layout** icon in the main toolbar.
4. Mark the **Use for %Quant** check box for one or more biomolecules.
5. Compare intact proteins in the Intact Protein tab in the Results Compare window. The Results Compare window shows information for the biomolecules that have the **Use for %Quant** check box marked.
6. Mark or clear the **Use for %Quant** check box in the Biomolecules window if you want to change the results shown in the Results Compare window.

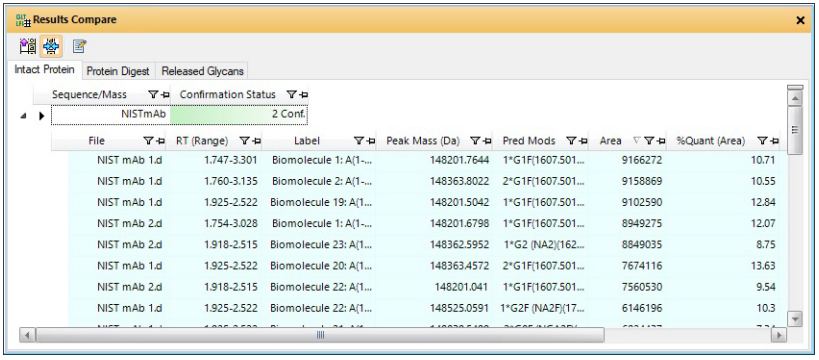


Figure 35. Intact Protein tab in the Results Compare window

7 View the results in the Relative Quantitation Histograms window. You can right-click and drag on the axes to zoom in or zoom out. Once you have zoomed in, you can drag the cursor on the axes to scroll through the results.

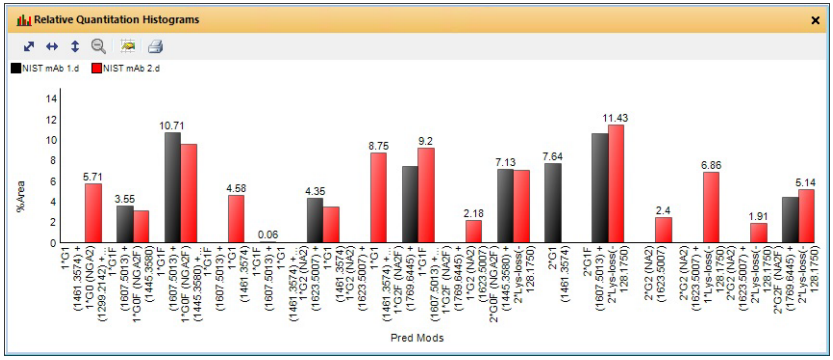


Figure 36. Relative Quantitation Histograms window for an Intact Protein workflow

Printing a report with deconvolution results

1. Select **Method Automation > Reports** in the Method Editor window.
2. Review the options in the **Destination** section.
3. Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Intact Protein,** the **Protein Digest**, the **Released Glycans, the Oligonucleotides -** report templates. By default, the following templates are used:
   - **IntactProteinReport.template.xml** (for intact proteins)
   - **ProteinDigestReport.template.xml** (for protein digests)
   - **ReleasedGlycanReport.template.xml** (for released glycans)
   - **TargetPlusImpuritiesReport.template.xml** (for Oligonucleotides - Target Plus Impurities)
   - **SequenceConfirmationReport.template.xml** (for Oligonucleotides - Sequence Confirmation)
4. To print a report, do one of the following. The report template that is used depends on the workflow used to create the results for the selected sample.
   - Click **Print Report** in the Method Editor toolbar if you are in the Method Automation > Reports section.
   - Click **Biomolecule Report** from the **File > Print** menu to print the report.
   - Click the  button on the main toolbar.

Tip To print deconvolution spectra, right-click the graph area of the Deconvolution Results window and click **Print** from the shortcut menu.

Tip To print the relative quantitation histogram, right-click the graph area of the Relative Quantitation Histograms window and click **Print** from the shortcut menu.

Tip A report is automatically created if you run Method Automation. Method Automation first runs the method workflow and then generates a biomolecule report. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.

Automating protein confirmation

Use this procedure to do protein confirmation automatically for samples in a worklist. See the BioConfirm Introduction Guide for more information.

1. In the Method Editor window, select Method Automation > Workflow and Sequences.
2. Select **Intact Protein** as the **Workflow**.
3. Select either **reduced** or **non-reduced** as the **Condition**.
4. Click the  button to select a sequence. The **Select Sequences** dialog box opens. Select the sequence or sequences, and then click **OK**.
5. Click the  button to select the Mods and Profiles. The **Select Modifications and Profiles** dialog box opens. Select the modifications and profiles, and then click **OK**.
6. Save the method in any of the following ways:
   - Click **Save** or **Save As** from the Method menu.
   - Click  or  in the Method Editor toolbar.
   - Click **Save Method** from Method Editor shortcut menu (right-click).
7. Assign this method to samples of interest when setting up a worklist in the Data Acquisition program as described in the online Help for your instrument.
8. To run this workflow interactively, do the following:
   - Click **Method > Run Method Workflow** *or*
   - Click the  button in the Sample Table.
9. To run this workflow and generate a report interactively, do the following:
   - Click **Method > Run Method Automation (Workflow + Reports)**.

Protein Digest Workflow

The topics in this section will help you get started using the Protein Digest workflow features of MassHunter BioConfirm.

Running the Protein Digest workflow

When you run a **Protein Digest** workflow, the workflow automatically does these steps:

- Find Peptides
- Match Sequences

1 Open the data file that contains the spectra of interest as described in *online Help*.

2 Open the **BioConfirmProteinDigest-Default.m** method. Click **Method > Open**.

3 Select Method Automation > Workflow and Sequences.

4 Select **Protein Digest** for the **Workflow**.

5 Select either **reduced** or **non-reduced** for the **Condition**.

6 Click the ... button next to the **Sequences/Masses** parameter to select a sequence.

7 Click the ... button next to the **Mods and Profiles** parameter to select modifications and profiles.

8 Mark the check box for the appropriate **Enzymes**. You cannot match sequences unless at least one enzyme is marked.

9 Save the method. Click **Method > Save As** to save to a new name.

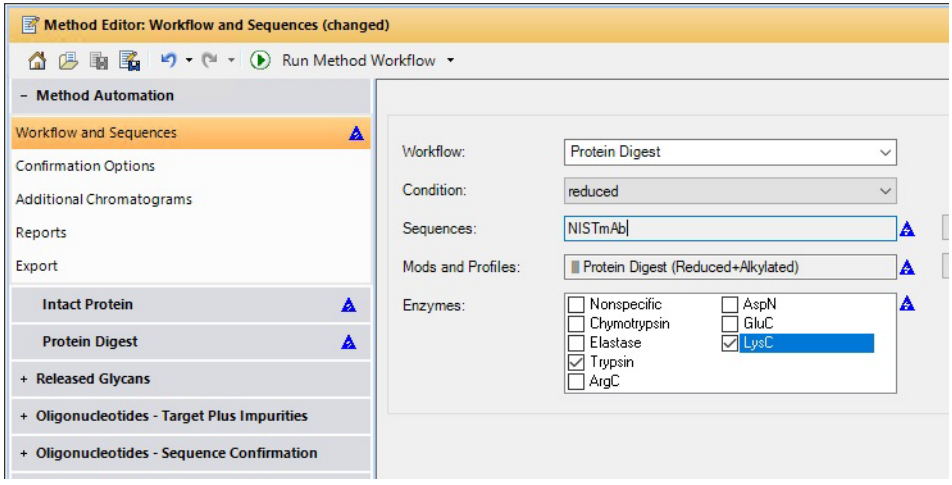


Figure 37. Workflow and Sequences section for a Protein Digest workflow

10 Click the button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.

11 View biomolecules as described in **"Viewing peptide biomolecules"** on page 55.

Finding peptides

Use this procedure to run the Find Peptides algorithm to create a biomolecule list. This algorithm is part of the Protein Digest workflow.

The default method often does not need to be modified. The parameters for the Find Peptides algorithm are in the Protein Digest > Find Peptides section.

1 Open the data file.

2 Click **Method > Open** and open **BioConfirmProteinDigest-Default.m**.

3 Click **Find and Identify > Find Peptides**.

4 View biomolecules as described in **"Viewing peptide biomolecules"** on page 55.

Matching sequences for protein digest biomolecules

Use this procedure to match sequences for biomolecules that were found using Find Peptides. You need to specify the sequence and modifications and profiles in the Method Automation > Workflow and Sequences section.

1 Create biomolecules using Find Peptides. See **"Finding peptides"** on page 52.

2 Select **Method Automation > Workflow and Sequences**.

3 Select **Protein Digest** for the **Workflow**.

4 Select either **reduced** or **non-reduced** for the **Condition**.

5 Click the ... button next to the **Sequences/Masses** parameter to select a sequence.

6 Click the ... button next to the **Mods and Profiles** parameter to select modifications and profiles.

7 Mark the check box for the appropriate **Enzymes**. You cannot match sequences unless at least one Enzyme is marked.

8 In the Method Editor window, select **Protein Digest > Match Tolerances** in the left pane.

   Make sure to choose the Protein Digest > Match Tolerances section.

   Do *not* select the Intact Protein > Match Tolerances section.

9 Review the parameters on the Mass Matching, FDR, Matching Rules, and Results tabs.

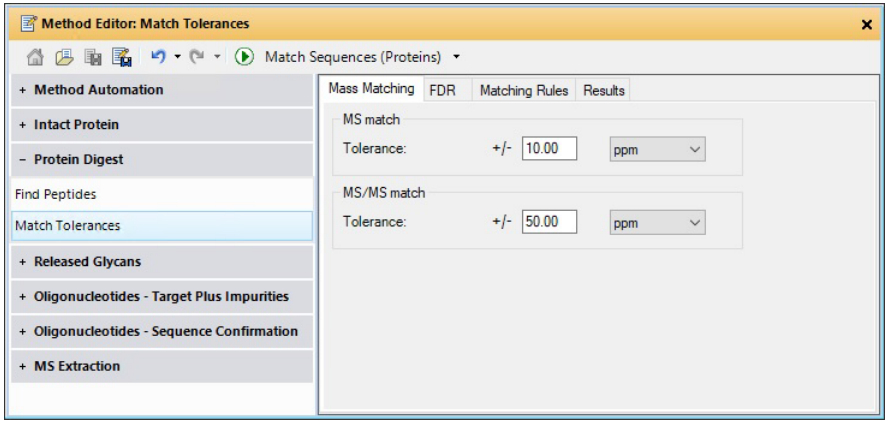


Figure 38. Protein Digest > Match Tolerances section in the Method Editor

10 Click the  button on the Method Editor toolbar to run the Match Sequences algorithm.

Tip You can also start Match Sequences when you click **Find and Identify > Match Sequences > Match Sequences (Proteins)**.

11 View peptide biomolecules as described in **"Viewing peptide biomolecules"** on page 55.

Viewing peptide biomolecules

You can view peptide biomolecules as follows:

1. If necessary, click **View > Biomolecules**.
2. If necessary, click **View > Biomolecule Identification Results**.
3. Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
   - Biomolecule MS Chromatogram window
   - Biomolecule MS Spectrum window
   - Biomolecule Fragment Spectrum window
   - Biomolecule Identification Results if you also ran the Match Sequences algorithm
   - Sequence Coverage Map if you also ran the Match Sequences algorithm
4. To see other information for biomolecules in the list, right-click the table, and then click **Add/Remove Columns** from the shortcut menu.
5. See **"Viewing sequence coverage results for protein digests"** on page 56.
6. Review the columns in the Biomolecule Identification Results window.
7. Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

Viewing sequence coverage results for protein digests

The location in the sequence which was matched is highlighted.

1 To display the Sequence Coverage Map window, click **View > Sequence Coverage Map**.

2 Click the following items on either the Sequence menu or the Sequence Coverage Map shortcut menu to view more information about the current sequence:

- **Applied Modifications**
- **Specified Applied Links**

3 Select another sequence match result to view by selecting a different row in the Biomolecules window which has a value in the **Score (Bio)** column.

Viewing relative quantitation results for protein digests

You can only review relative quantitation results for Protein Digests when you selected one or more modifications and when you mark the **Use for %Quant** check box for at least one biomolecule. By default, the **Use for % Quant** check box is marked, but you can clear any of these check boxes.

1. To display the Peptide Relative Quantitation Results window, click **View > Peptide Relative Quantitation Results**.
2. Select one or more samples in the Sample Table window that has results from the Protein Digest workflow.
3. In the Peptide Relative Quantitation Results window, open a row in the first level of the table. Each row shows an amino acid at a particular location that has a modification in a selected file.
4. In the second level of the table, mark the **Use for %Quant** check box for the sequence that you want to compare.

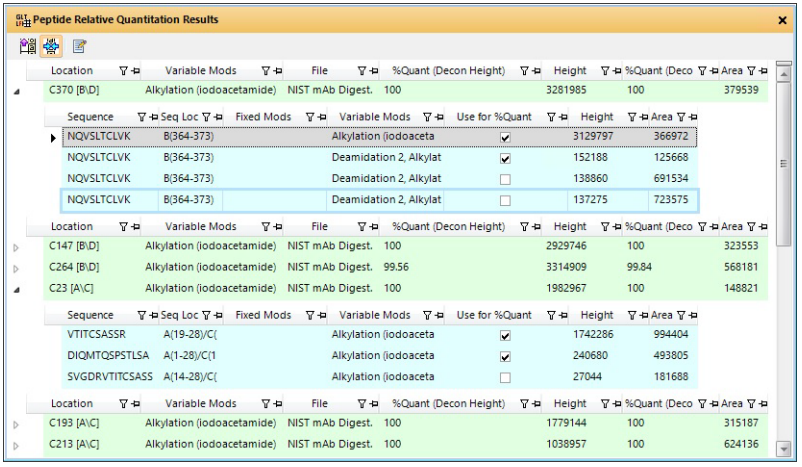


Figure 39. Peptide Relative Quantitation Results window

5 View the results in the Results Compare window. Information on the Sequence/Mass for the selected samples is shown in the same table.

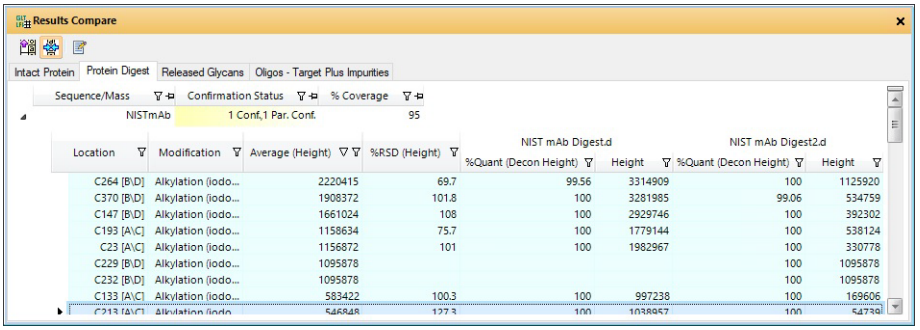


Figure 40. Protein Digest tab in the Results Compare window

6 View the results in the Relative Quantitation Histograms window.

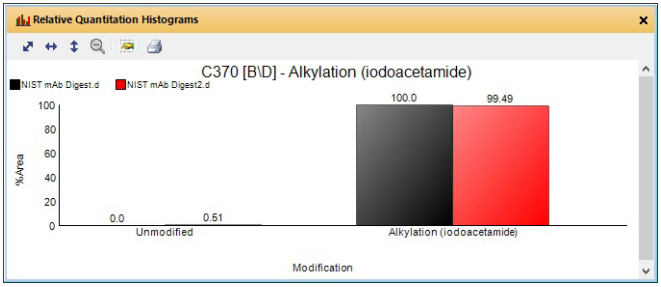


Figure 41. Relative Quantitation Histograms window

Printing a report with peptide results

1 Select **Method Automation > Reports** in the Method Editor window.

2 Review the options in the **Destination** section.

3 Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Protein Digest report template**.

- **ProteinDigestReport.template.xml** (for protein digests)

4 To print a report, do one of the following. The report template that is used depends on the workflow used to create the results for the selected sample.

- Click **Print Report** in the Method Editor toolbar if you are in the Method Automation > Reports section. This method does not show the **Print Biomolecule Report** dialog box.
- Click **Biomolecule Report** from the **File > Print** menu to print the report.
- Click the button on the main toolbar.

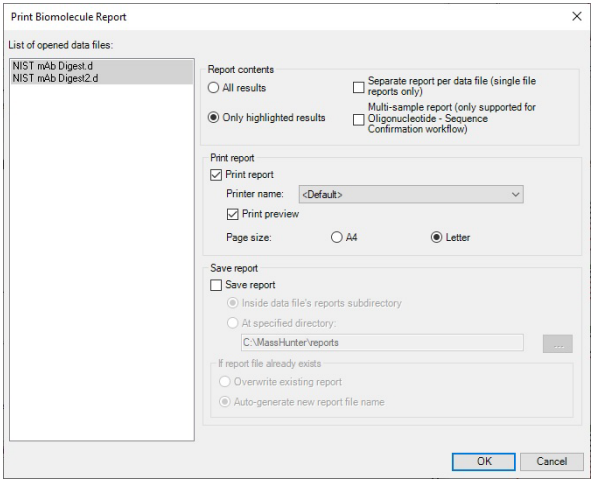


Figure 42. Print Biomolecule Report Dialog Box

Tip To print the relative quantitation histogram, right-click the graph area of the **Relative Quantitation Histograms** window and click **Print** from the shortcut menu.

Tip A report is automatically created if you run Method Automation. Method Automation first runs the method workflow. It then extracts additional chromatograms, and then generates a biomolecule report. Finally, it exports the results. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.

Released Glycans Workflow

The topics in this section will help you get started using the Released Glycans workflow features of MassHunter BioConfirm.

Running the Released Glycans workflow

When you run a **Released Glycans** workflow, the workflow automatically does these steps:

- Find Released Glycans

1 Open the data file that contains the data of interest.

2 (optional) Click the **Released Glycans Layout** icon in the main toolbar.

3 Click **Method > Open** and open the **BioConfirmReleasedGlycans-Default.m** method.

4 Select Method Automation > Workflow and Sequences in the Method Editor window.

5 Select **Released Glycans** for the **Workflow**.

6 (optional) Enter the **Glycan group**.

7 Select the **Target glycan source**.

8 Mark or clear the **Require RT match if database contains an RT for the target glycan**.

9 Select **Released Glycans > Find Glycans**.

10 Click the **Tag** tab in the Released Glycans > Find Glycans section.

11 Select the tag that was used with this data file. 2-AB and InstantPC are defined in the BioConfirm program.

12 Save the method. Click **Method > Save As** to save to a new name.

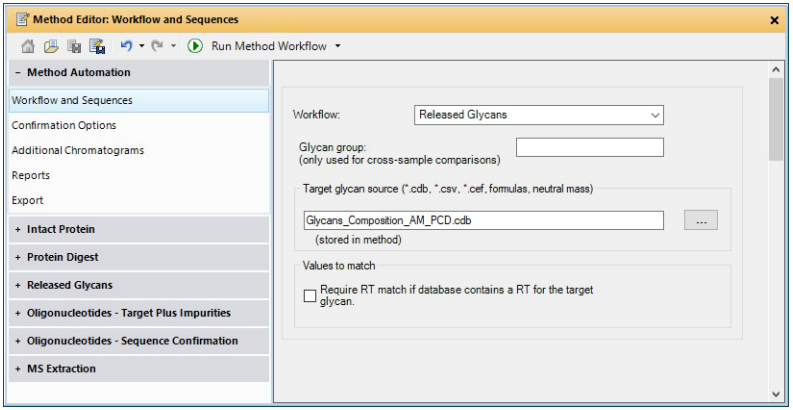


Figure 43. Workflow and Sequences section for a Released Glycans workflow

13 Click the button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.

View biomolecules as described in **"Viewing released glycans biomolecules"** on page 63.

Finding glycans

Use this procedure to run the Find Glycans algorithm to create a biomolecule list. This algorithm is part of the Released Glycans workflow.

The parameters for the Find Glycans algorithm are in the Released Glycans > Find Glycans section. To find glycans, you need to specify the Target glycan source.

1 Open the data file.
2 Click **Method > Open** and open **BioConfirmReleasedGlycans-Default.m**.
3 Click the **Released Glycans Layout** icon in the main toolbar.
4 Select **Released Glycans > Find Glycans** in the Method Editor window.
5 Enter the **Target glycan source**. In this example method the **Target glycan source** is a small database that is part of the method. You can select a different **Target glycan source** that is in the project.
6 Click the **Tag** tab.
7 Select the tag that was used with this data file. 2-AB and InstantPC are defined in the BioConfirm program.
8 Click the button on the Method Editor toolbar to run **Find Glycans**.

View biomolecules as described in **"Viewing released glycans biomolecules"** on page 63.

Viewing released glycans biomolecules

You can view released glycans biomolecules as follows:

1. Click **Released Glycans Layout** in the main toolbar. The Biomolecules window and the Biomolecules Identification Results window are both open and tabbed in the lower left section of the main window. The Relative Quantitation Histograms window and the Results Compare window are both open and tabbed in the lower right section of the main window.
2. Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
   - Biomolecule MS Chromatogram
   - Biomolecule MS Spectrum window
   - Biomolecule Identification Results window
   - Glycan Structure Viewer window (if a glycan structure is available)
3. To see other information for biomolecules in the list, right-click the table, and then click **Add/Remove Columns** from the shortcut menu.
4. Review the columns in the Biomolecule Identification Results window.

Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

Viewing relative quantitation results for released glycans

You can only review relative quantitation results for Released Glycans that have the **Use for %Quant** check box marked in the Biomolecules window. Samples are sorted by the Glycan group.

1. Verify that the **Glycan group** for your samples is the same if you want to compare results. You set the **Glycan group** in the Method Automation > Workflow and Sequences section of the Method Editor. You can edit the **Glycan Group** directly in the Sample Table.
2. Select one or more Released Glycans samples in the Sample Table window.
3. Click the **Released Glycans Layout** icon in the main toolbar.
4. Select the glycans to compare by marking the **Use for %Quant** check box in the Biomolecules window.
5. Compare glycans in the Released Glycans tab in the Results Compare window.
6. Mark or clear the **Use for %Quant** check box in the Biomolecules window if you want to change the results shown in the Results Compare window.

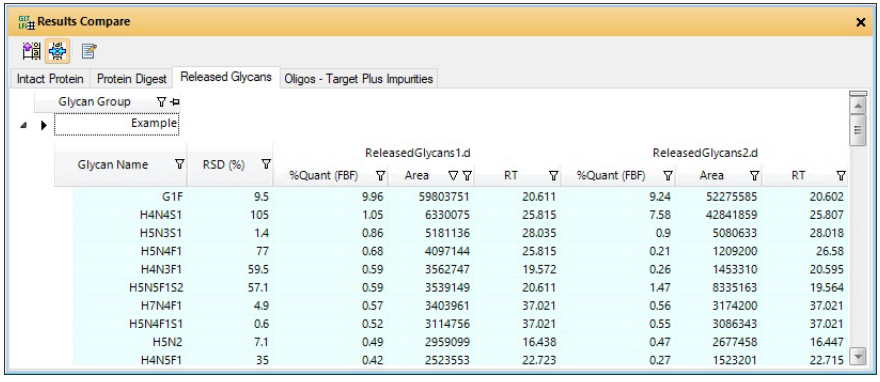


Figure 44. Results Compare window

7 View the results in the Relative Quantitation Histograms window. You can right-click and drag on the axes to zoom in or zoom out. Once you have zoomed in, you can drag the cursor on the axes to scroll through the results.

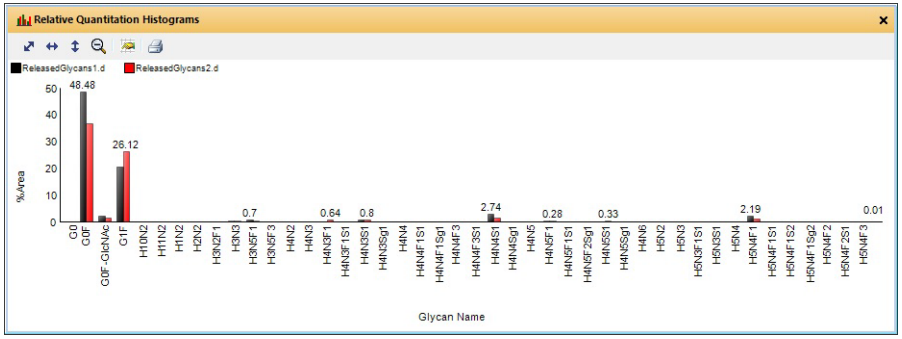


Figure 45. Relative Quantitation Histograms window

Printing a report with released glycans results

1 Select **Method Automation > Reports** in the Method Editor window.

2 Review the options in the **Destination** section.

3 Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Released Glycans report template**.

- **ReleasedGlycansReport.template.xml**

4 To print a report, do one of the following. The report template that is used depends on the workflow used to create the results for the selected sample.

- Click **Print Report** in the Method Editor toolbar if you are in the Method Automation > Reports section. This method does not show the **Print Biomolecule Report** dialog box.
- Click **Biomolecule Report** from the **File > Print** menu to print the report.
- Click the button on the main toolbar.

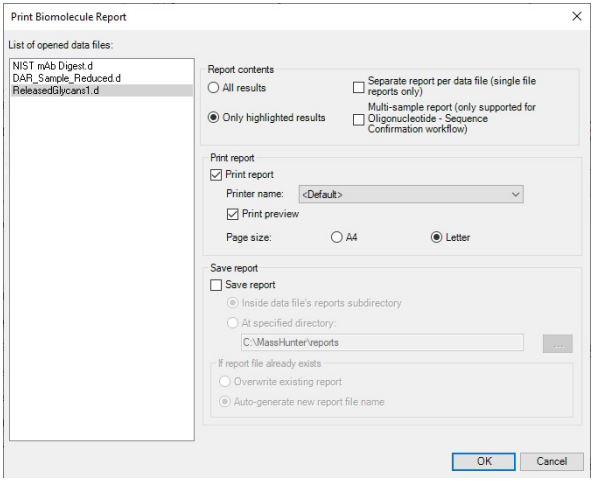


Figure 46. Print Biomolecule Report Dialog Box

Tip A report is automatically created if you run Method Automation. Method Automation first runs the method workflow. It then extracts additional chromatograms and then generates a biomolecule report. Finally, it exports the results. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.

Oligonucleotides Workflow

The topics in this section will help you get started using the Oligonucleotides workflow features of MassHunter BioConfirm.

Running the Oligonucleotides - Target Plus Impurities workflow

When you run an **Oligonucleotides** workflow, the workflow runs one of the following experiment types:

- Target Plus Impurities
- Sequence Confirmation - see **"Running the Oligonucleotides - Sequence Confirmation workflow"** on page 70

1 Open the data file that contains the oligonucleotides of interest as described in *online Help*.

2 Open the **BioConfirmOligoTPI-Default.m** method. Click **Method > Open**.

3 Select Method Automation > Workflow and Sequences.

4 Select **Oligonucleotides** for the **Workflow**.

5 Select **Target Plus Impurities** for the **Experiment**.

6 Click the  button next to the **Sequences/Masses** parameter to select a sequence.

7 Click the  button next to the **Mods and Profiles** parameter to select modifications and profiles.

8 Click the  button next to the **Matching Rules** parameter to select the matching rules to use.

9 (optional) Review parameters in the Oligonucleotides - Target Plus Impurities section of the Method Editor window.

10 Save the method. Click **Method > Save As** to save to a new name.

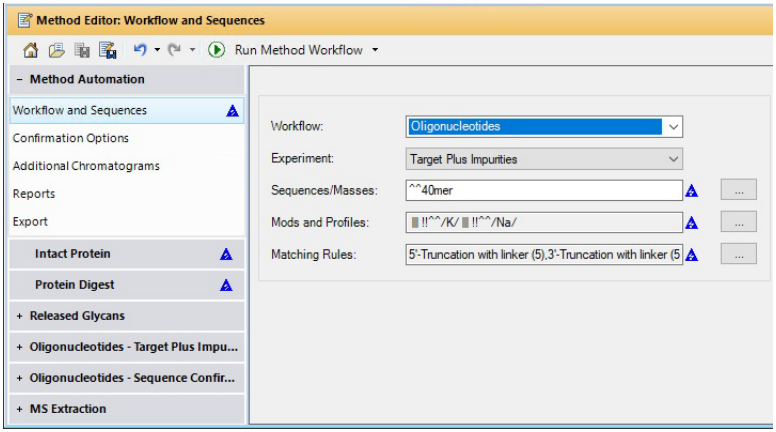


Figure 47. Workflow and Sequences section for an Oligonucleotides - Target Plus Impurities workflow

11 Click the button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.

12 View biomolecules as described in **"Viewing results for Oligonucleotides - Target Plus Impurities"** on page 72.

Running the Oligonucleotides - Sequence Confirmation workflow

When you run an **Oligonucleotides** workflow, the workflow runs one of the following experiment types:

- Target Plus Impurities - see **"Running the Oligonucleotides - Target Plus Impurities workflow"** on page 68
- Sequence Confirmation

1 Open the data file that contains the oligonucleotides of interest as described in *online Help*.

2 Open the **BioConfirmOligoSC-Default.m** method. Click **Method > Open**.

3 Select Method Automation > Workflow and Sequences.

4 Select **Oligonucleotides** for the **Workflow**.

5 Select **Sequence Confirmation** for the **Experiment**.

6 Click the ... button next to the **Sequences/Masses** parameter to select a sequence.

7 (optional) Review parameters in the Oligonucleotides - Sequence Confirmation section of the Method Editor window.

8 Save the method. Click **Method > Save As** to save to a new name.

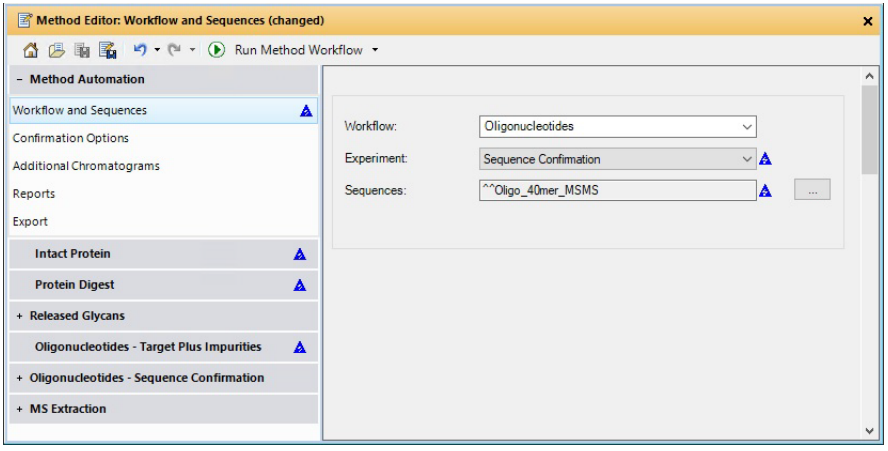


Figure 48. Workflow and Sequences section for an Oligonucleotide - Sequence Confirmation workflow

9 Click the  button on the Method Editor toolbar to run the method workflow. You could instead click **Method > Run Method Workflow**.

10 View biomolecules as described in **"Viewing results for Oligonucleotides - Sequence Confirmation"** on page 74.

Viewing results for Oligonucleotides - Target Plus Impurities

You can view biomolecules for Oligonucleotides - Target Plus Impurities as follows:

1. (optional) Click the **Oligonucleotides - Target Plus Impurities Layout** icon in the main toolbar.
2. If necessary, click **View > Biomolecules**.
3. Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
   - Biomolecule MS Chromatogram window
   - Biomolecule MS Spectrum window
   - Deconvolution Results window
   - Biomolecule Identification Results window
4. To see other information for biomolecules in the list, right-click the table, and then click **Add/Remove Columns** from the shortcut menu.
5. Review the columns in the Biomolecule Identification Results window.
6. Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.
7. Float the Oligos - Impurity List window. Double-click the tab to float the window. By default, it is tabbed with the Biomolecules window. If it is not visible, click **Sequence > Oligos - Impurity List**.
8. Review the impurities in this window.

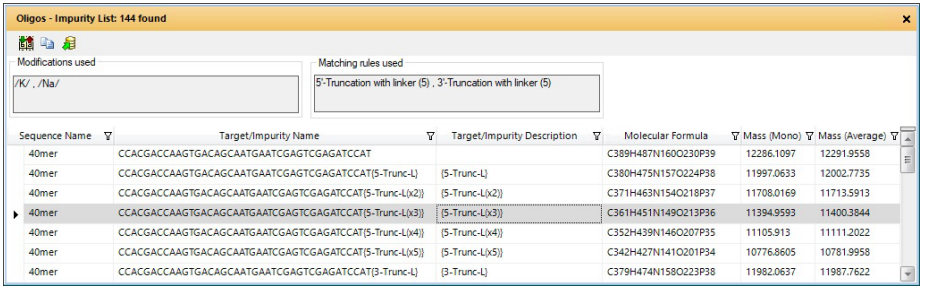


Figure 49. Oligos Impurity List window for the Oligonucleotide Target Plus Impurities workflow

9 If necessary, click **View > Results Compare**.

10 Click the **Oligos - Target Plus Impurities** tab. Review the results of multiple data file in this window.

Viewing results for Oligonucleotides - Sequence Confirmation

You can view biomolecules for Oligonucleotides workflow with Sequence Confirmation as follows:

1. (optional) Click the **Oligonucleotides - Sequence Confirmation Layout** icon in the main toolbar.
2. If necessary, click **View > Biomolecules**.
3. Click a biomolecule of interest. The following windows are updated when you select a biomolecule:
   - Biomolecule Fragment Spectrum window
   - Biomolecule Identification Results
   - Fragment Confirmation Ladder

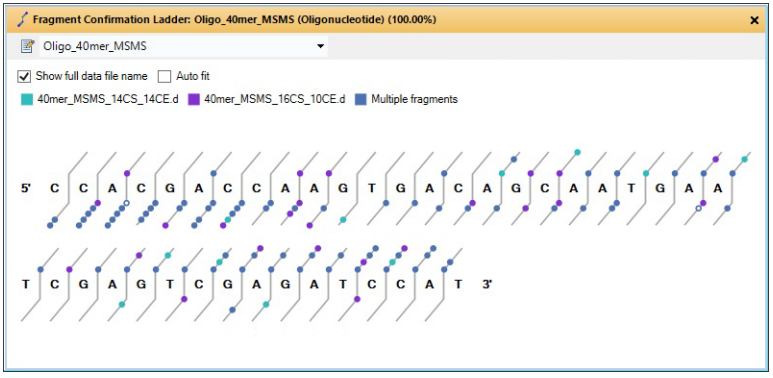


Figure 50. Fragment Confirmation Ladder with two data files selected

Each dot represents a fragment that has been confirmed for the nucleoside. The title bar shows the percentage of nucleosides that have at least one confirmed fragment. See the online Help for more information on the Fragment Confirmation Ladder.

4. To see other information for biomolecules in the list, right-click the Biomolecules table, and then click **Add/Remove Columns** from the shortcut menu.
5. Review the columns in the Biomolecule Identification Results window.
6. Right-click the table in the Biomolecule Identification Results window to see the shortcut menu. You can add and remove columns, copy to Clipboard, print, export and other features.

Printing a report with oligonucleotides results

1 Select **Method Automation > Reports** in the Method Editor window.

2 Review the options in the **Destination** section.

3 Review the parameters in both the **Templates** and **Layouts** tabs. Select the **Target Plus Impurities** report template and the **Sequence Confirmation** report template

- **TargetPlusImpuritiesReport.template.xml** (for Target Plus Impurities)
- **SequenceConfirmationReport.template.xml** (for Sequence Confirmation)

4 To print a report, do one of the following. The report template that is used depends on the workflow and experiment used to create the results for the selected sample.

- Click **Print Report** in the Method Editor toolbar if you are in the Method Automation > Reports section. This method does not show the **Print Biomolecule Report** dialog box.
- Click **Biomolecule Report** from the **File > Print** menu to print the report.
- Click the button on the main toolbar.

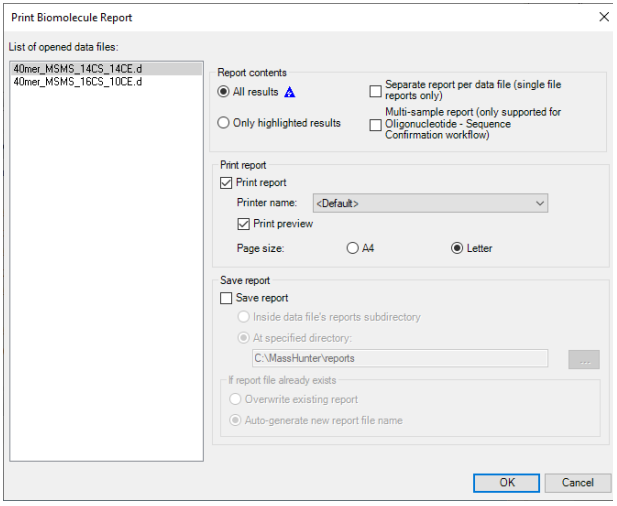


Figure 51. Print Biomolecule Report Dialog Box

5 Review the parameters. For the Oligonucleotides - Sequence Confirmation workflow, see **"Printing a multiple sample report"** on page 77.

6 Click **OK**.

Tip To print the Fragment Confirmation Ladder, right-click the graph area of the **Fragment Confirmation Ladder** window and click **Print** from the shortcut menu.

Tip A report is automatically created if you run Method Automation. Method Automation first runs the method workflow. It then extracts additional chromatograms, and then generates a biomolecule report. Finally, it exports the results. You can run Method Automation when you click **Method > Run Method Automation (Workflow + Reports)**.

Printing a multiple sample report

You can only print a report for multiple samples if the **Workflow** is **Oligonucleotides** and the **Experiment** is **Sequence Confirmation**. This report cannot be generated as part of automation. The multi-sample report is only available from the Print Biomolecule Report dialog box.

You cannot select a different template for the multiple sample report.

1 To print a report, do one of the following. The report template that is used depends on the workflow and experiment used to create the results for the selected sample.
   - Click **Print Report** in the Method Editor toolbar if you are in the Method Automation > Reports section. This method does not show the **Print Biomolecule Report** dialog box.
   - Click **Biomolecule Report** from the **File > Print** menu to print the report.
   - Click the button on the main toolbar.

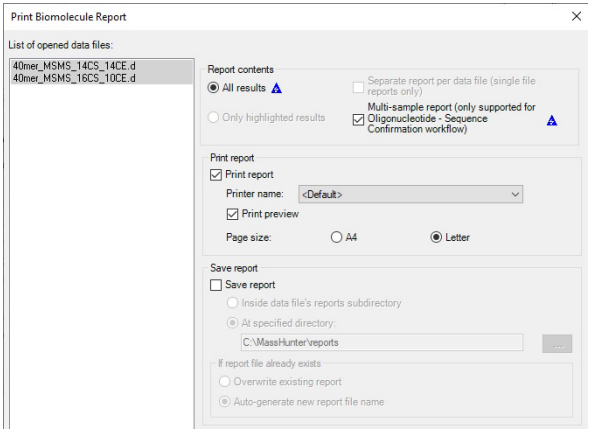


Figure 52. Print Biomolecule Report Dialog Box

2 Mark the **Multi-sample report (only supported for Oligonucleotide - Sequence Confirmation workflow)** check box. The **All results** option is automatically selected. The other options in the **Report contents** section are not available.

3 Review the parameters in the **Print report** section and the **Save report** section.

4 Click **OK**.

Review Results

The topics in this section will help you get started using the Review Results features of MassHunter BioConfirm.

Reprocessing samples

You can reprocess data files using the Sample Table toolbar, and you can review results in the usual windows.

1 Open multiple data files.

2 Examine the Confirmation Status column. The values can be **Confirmed**, **Partially Confirmed**, **Not Confirmed**, and **Undetermined**.

3 Select a row that is **Not Confirmed** or **Undetermined**.

4 (optional) Load a method to use with the sample you selected.

5 Click the  button in the Sample Table toolbar. The **Reprocess Sample** dialog box opens.

6 Select the **Workflow**.

7 If necessary, select the **Condition**.

8 If the Workflow is Oligonucleotides, select the **Experiment**.

9 Select the **Sequences/Masses**. Click the  button to select a different sequence.

10 (optional) If the **Workflow** is **Released Glycans**, enter the **Glycan group**.

11 If necessary, select the **Mods and Profiles**. Click the  button to select different modifications and profiles.

12 If the **Workflow** is **Protein Digest**, mark the **Enzymes** to use.

13 (optional) If the Workflow is Intact Protein, mark the Calculate Drug-to-Antibody Ratio check box. You can only do DAR calculations if you entered a Mass. Review other parameters in this section.

14 If you have a choice, click either **Use current method** or **Use sample result method**. You can reprocess using the current method in the BioConfirm program or you can reprocess using the same method that was used to create previous sample results.

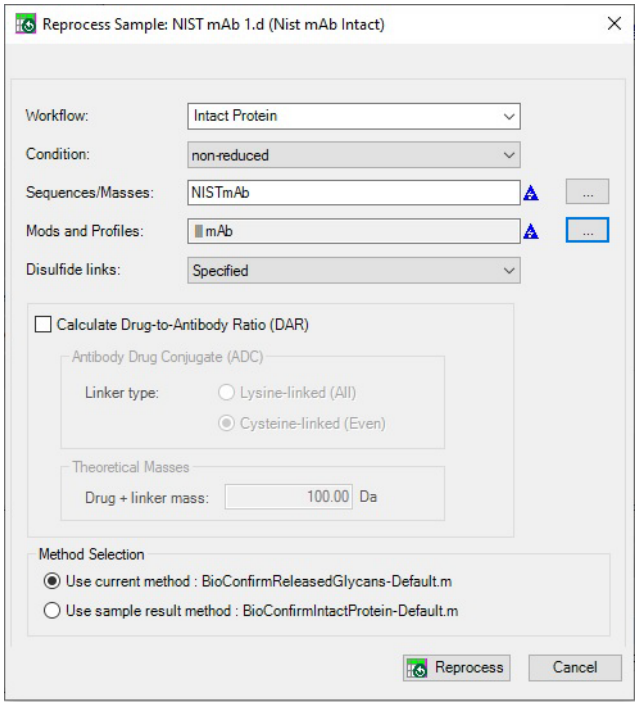


Figure 53. Reprocess Sample Dialog Box

15 Click **Reprocess**.

16 Review results. See one of the following topics:

- **"Viewing deconvolution biomolecules"** on page 44
- **"Viewing peptide biomolecules"** on page 55
- **"Viewing relative quantitation results for protein digests"** on page 57
- **"Viewing released glycans biomolecules"** on page 63
- **"Viewing relative quantitation results for released glycans"** on page 64
- **"Viewing results for Oligonucleotides - Target Plus Impurities"** on page 72
- **"Viewing results for Oligonucleotides - Sequence Confirmation"** on page 74

17 Continue to reprocess other data files.

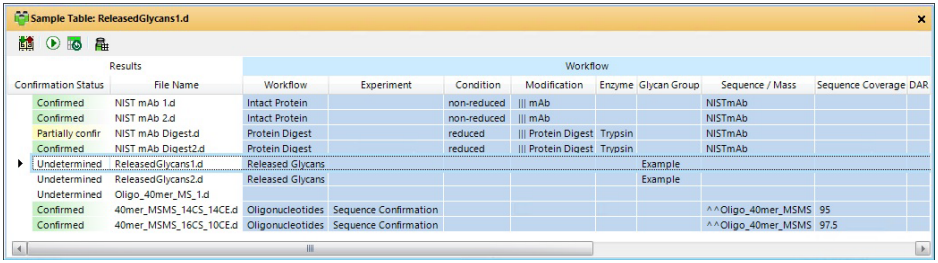


Figure 54. Reprocess samples using the Sample Table

Using Result Review mode

Result Review mode disables the Method Editor window and the **Find and Identify** menu. You can reprocess data files using the Sample Table toolbar, and you can review results in the usual windows.

1. Click **Configuration > Enable Result Review (Disables Method Editing)**. It has a check mark next to the command when it is enabled.
2. Observe that the **Find and Identify** menu is grayed out.
3. The Method Editor window is closed if it was open.
4. Click the **Method** menu. Most commands are grayed out.
5. You can still reprocess samples. See **"Reprocessing samples"** on page 78.
6. To end Result Review mode, click **Configuration > Enable Result Review (Disables Method Editing)**.

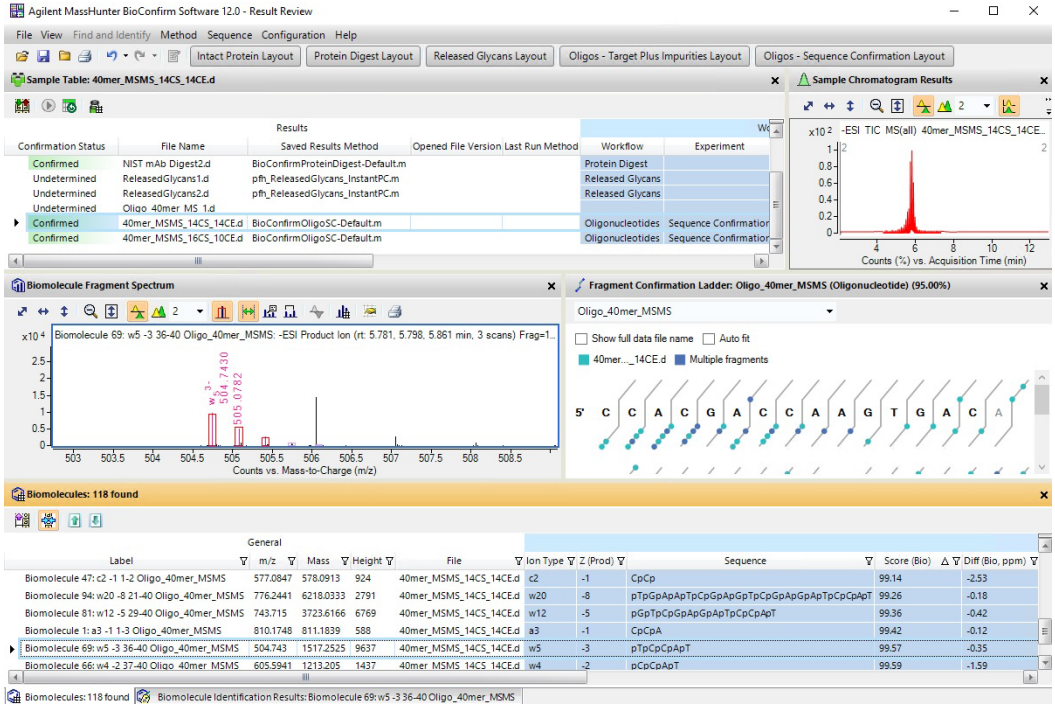


Figure 55. BioConfirm window in Result Review mode

Setting Up Sequences

Topics in this section include:

Creating or editing a protein sequence

You create and edit either a protein or an oligonucleotide sequence in the Sequence Manager program.

1 Open the Sequence Manager program. In the BioConfirm program, click **Sequence > Sequence Manager**.

  Instead, in the OpenLab Control Panel program, you can click 

and then click **Start Sequence Manager**.

2 Click the **Proteins** tab.

3 To add a sequence, type a sequence name and click the  button in the bottom left corner.

4 To edit a sequence, click the sequence in the left pane of the Sequence Manager.

5 In the Sequence Manager:

  a Add or edit the sequence text as described in **"Adding or editing the protein sequence text"** on page 83.

  b Apply or edit modifications as described in **"Applying or editing protein modifications"** on page 84.

c Apply or edit links as described in **"Applying or editing protein links"** on page 86.

6 Click the **Sequence > Export Sequences** to save the sequence as a PSQ file or a Text file.

Adding or editing the protein sequence text

1 In the Sequence Manager program, select the sequence of interest.

2 Select the correct chain to add or edit. Chain A is selected by default.

3 Click the sequence in the right pane.

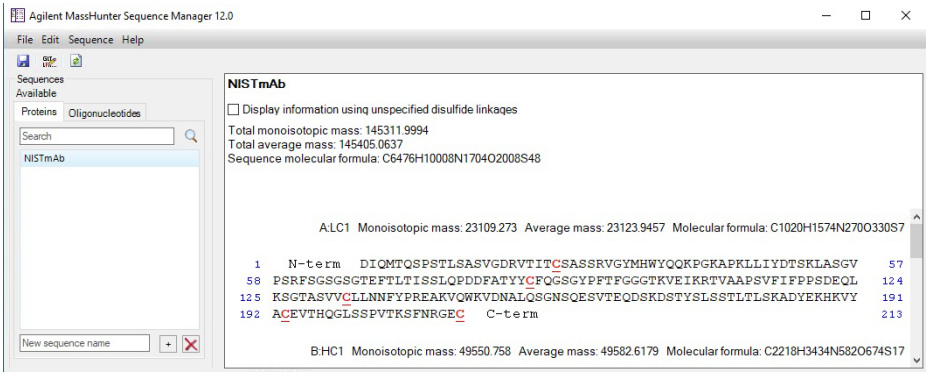


Figure 56. Sequence Manager program

4 Enter or edit the amino acids in the sequence text box in either of the following ways.

- Type in individual amino acids one at a time between the N-term and C-term symbols.
- Copy the sequence from a FASTA-formatted database or a text file. Right-click the sequence in the Sequence Manager and click **Paste** from the shortcut menu. The amino acid sequence will appear in the Sequence box, between the N-term and C-term symbols.

NOTE

Only single-character (letter) amino acids are allowed for the protein sequence parameter. Three character amino acid symbols are not supported.

Applying or editing protein modifications

1. Click the **Proteins** tab.
2. In the Sequence Manager program, select the sequence of interest.
3. Right-click that sequence, and click **Edit Modifications** from the shortcut menu to open the **Modifications** dialog box.
4. To select and apply global modifications:
   - **a** On the Global tab, select the desired modification from the list of **Available modifications**.
     - If desired, you can customize the list of available modifications using the Chemical Data Dictionary; *see the online Help for more information*.
     - Note that many N-linked glycans with numerical abbreviations, such as "2000 0A 0G", are included in the Chemical Data Dictionary; *see online help for more information*.
   - **b** Select the amino acids to modify in the **Apply to all** list. Use **Shift+click** or **Ctrl+click** to select multiple amino acids, if desired.
   - **c** Click **Apply** to apply the specified modification to the selected amino acids throughout the sequence.
   - **d** Repeat **step a** - **step c** to select and apply other global modifications.
5. To select and apply local modifications:
   - **a** On the Local tab, select the location in the sequence to modify: **C-terminus**, **N-terminus**, or the position in the sequence. Use the blue index numbers shown for each row of the sequence in the Sequence Manager program to determine the proper index number for the selected position.
   - **b** Select the desired modification from the **Applicable modifications** list.
   - **c** Mark the **Variable modification** check box if you want to create a variable modification for sequence matching. When marked, sequences that contain that amino acid site are matched both *with and without* this modification.
   - **d** Click **Apply** to apply the selected modification to the specified location (amino acid) in the sequence.
   - **e** Repeat **step a** - **step d** to select and apply other local modifications.
6. Click **OK** to close the **Modifications** dialog box. Note that the molecular weight and formula have been updated in the Sequence Manager.

To remove modifications

1. Click **Edit Modifications** on either the Edit menu or the Sequence shortcut menu to open the **Modifications** dialog box.
2. Click the **Specified Applied** tab and review the list of modifications.
3. Click to select the modifications you want to delete.
4. Click **Delete** to remove the selected modifications.
5. Click **OK**.

Applying or editing protein links

1. Click the **Proteins** tab.
2. In the Sequence Manager program, select the sequence of interest in the left pane.
3. Right-click the sequence in the pane on the right side, and click **Edit Links** from the shortcut menu to open the **Links** dialog box. You can also click **Edit > Edit Links**.
4. Enter the index number for one end of the link in the **From index** box and select the chain (if other than the default Chain A).
5. Enter the index number for the other end of the link in the **To index** box and select the chain (if other than the default Chain A).
6. Select a link from the **Link types** list.

Tip You can customize the list of links types using the Chemical Data Dictionary; *see online help for more information*.

7. Click **Apply** to link the selected amino acids in the sequence.
8. Repeat Steps 3 -6 to create additional links.
9. Click **OK** to close the Links dialog box. Note that the molecular weight and formula have been updated in the Sequence Manager.

To remove links

1. Click **Edit Links** on either the Sequence menu or the Sequence Manager shortcut menu to open the **Links** dialog box.
2. Click the **Specified Applied** tab and review the list of links.
3. Click the links you want to delete.
4. Click **Delete** to remove the selected links.

Creating or editing an oligonucleotide sequence

You create and edit either a protein sequence or an oligonucleotide sequence in the Sequence Manager program.

1 Open the Sequence Manager program. In the BioConfirm program, click **Sequence > Sequence Manager**.

   Instead, in the OpenLab Control Panel program, you can click 

and then click **Start Sequence Manager**.

2 Click the **Oligonucleotides** tab.

3 To add a sequence, type a sequence name and click the  button in the bottom left corner.

4 To edit a sequence, click the sequence in the left pane of the Sequence Manager.

5 In the Sequence Manager, add or edit the sequence text as described in **"Adding or editing the oligonucleotide sequence text"** on page 88.

6 Click the **Sequence > Export Sequences** to save the sequence as a PSQ file.

Adding or editing the oligonucleotide sequence text

1 In the Sequence Manager program, select the sequence of interest.

2 Click the sequence in the right pane between 5’ and 3’.

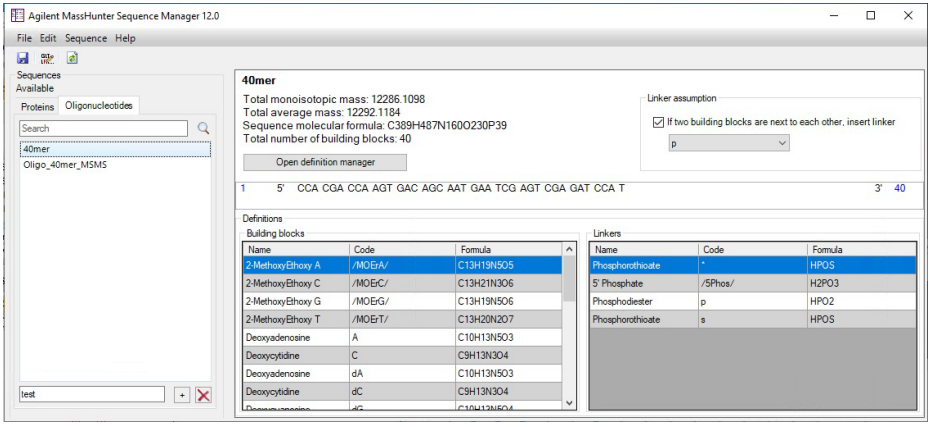


Figure 57. Sequence Manager program

3 Enter or edit the **Code** for the **Building blocks** in the sequence text box in either of the following ways.

- Type in individual building blocks one at a time between the 5’ and 3’ symbols.
- Copy the sequence from a FAST-A formatted database or a text file. Right-click the sequence in the Sequence Manager and click **Paste** from the shortcut menu. The sequence will appear in the Sequence box, between the 5’ and 3’ symbols.

4 Mark or clear the **If two building blocks are next to each other, insert linker** check box.

NOTE

When you enter the **Code**, include the “/” if it is part of the code. Also, be sure to match the exact capitalization for the Building Block.

NOTE

If you want to add another building block, click **Open definition manager** to open the Chemical Data Dictionary dialog box. You can also add **Linkers** and **Modifications** for Oligonucleotides in the Chemical Data Dictionary dialog box.

Adding a building block in the Chemical Data Dictionary

You can add or modify Building Blocks for Oligonucleotides in the Chemical Data Dictionary. You can also add and modify **Linkers** and **Modifications** for Oligonucleotides in the Chemical Data Dictionary.

Modifications and **Modification profiles** can be selected in the Workflow. **Building Blocks** and **Linkers** can be used to create a sequence in the Sequence Manager program.

1 Open the Chemical Data Dictionary.
   - In the BioConfirm program, click **Sequence > Open Chemical Data Dictionary**.
   - In the BioConfirm program, click **Sequence > Sequence Manager**. In the Sequence Manager program, click the **Oligonucleotides** tab. Then, click **Open definition manager**.

2 To add a building block, type a **New building block code**, and click the + button in the bottom left corner.
   - The **Code** must be unique.
   - The first letter of the **Code** needs to be a lower case letter or a special character. Numbers are not allowed.
   - If you want the **Code** to be more than two characters, the **Code** must begin and end with a "/".
   - The **Code** cannot contain the following special characters: /, \, {, }, [,], (,), |, ^, !

3 Optional, edit the **Name** of the new Building block. This name can describe the **Building block**.

4 Enter the **Molecular formula**.

5 (optional) Mark **The code represents a fragment**.

6 If the code is not a fragment, enter the **Base formula**.

7 Click **Close**.

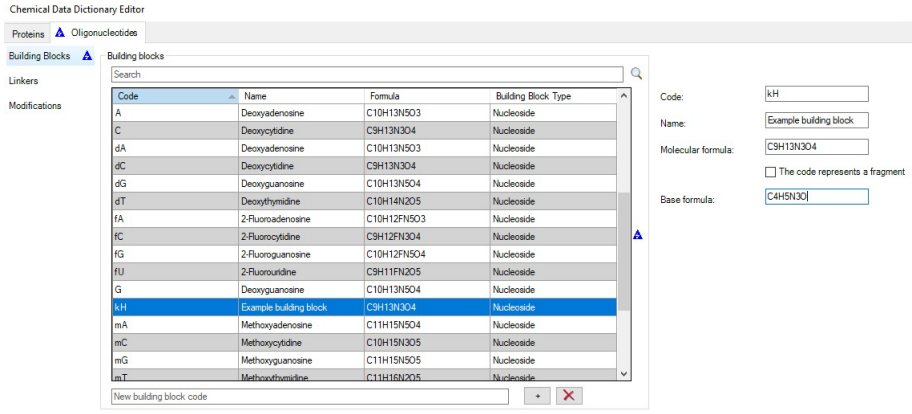


Figure 58. Chemical Data Dictionary with a new Building block - kH

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In This Guide

This guide has instructions for installing and using the MassHunter BioConfirm software.

This guide is valid for MassHunter BioConfirm 12.0.

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