MassHunter BioConfirm 12.0

Introduction Guide

Where to find more information

Online Help

Online Help for BioConfirm is available as part of *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.

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How to use this guide

Try to do these introduction exercises initially using the steps listed in the first column. Then if you need more information, follow the detailed instructions in the second column.

Before you start

Copy the data files used for these tasks onto your hard disk as follows:

1. Create a project. You select a project before you start BioConfirm. See the online Help for OpenLab Control Panel for more information.
2. Copy all of the data files from the **Data** folder on the BioConfirm setup media to your computer hard drive.
   - For **Workstation** mode, copy the data files to the **Data** folder in your project.
   - For **Networked Workstation** mode, import the data files into your project. See **"Import Data Files and Sequences"** on page 4.
3. In Workstation mode, make sure you have both read and write permissions for the folder you just created on your computer. This is required if you want to save results.
   a. In Windows Explorer right-click the folder where you copied the data files and click **Properties** from the shortcut menu.
   b. *Clear* the **Read-only Attributes** check box if it is marked.
   c. In the Confirm Attribute Changes dialog, click **Apply changes to this folder, subfolders, and files**, and then click **OK**.
4. Copy all of the sequences from the **ProteinSequences** folder on the BioConfirm setup media to your computer hard drive. Example protein sequences and oligonucleotide sequences are in this folder.
   - For **Workstation** mode, copy the sequences to the **ProteinSequences** folder in your project.
   - For **Networked Workstation** mode, import the sequences into your project. See **"Import Data Files and Sequences"**.

Import Data Files and Sequences

In **Workstation** mode, you can copy the data files and sequences directly to your project. In **Networked Workstation** mode, you import these files to the project. You can also import methods, report templates, and databases.

1. Copy all of the data files from the **Data** folder on the BioConfirm setup media to your computer hard drive.
2. Start the **OpenLab Control Panel** program.
3. Create a project. See the online Help for the Control Panel program for more information.
4. Select the project.
5. In the Control Panel program on the ribbon in the **BioConfirm** group, click **BioConfirm > Start BioConfirm**. If you are starting BioConfirm with a new project for the first time, you may get a message about default files being automatically imported. If you get this message, wait a few minutes, and then try to open BioConfirm again.
6. Click **Cancel** in the **Open Sample** dialog box.
7. Click **File > Import to Project > Data File(s)**. The **Data File Import** dialog box opens.
8. Select the data file(s) to import. These files are used for exercises in this guide: 40mer_MSMS_14CS_14CE.d, DAR_Sample_Intact.d, DAR_Sample_Reduced.d, NIST mAb 1.d, NIST mAb 2.d, NIST mAb Digest.d, NIST mAb Digest2.d, Oligo_40mer_MS1.d, ReleasedGlycans1.d, and ReleasedGlycans2.d,
9. Click **Import**. It takes several minutes to import data files, so you may have to wait before continuing.
10. Click **File > Import to Project > Sequence(s)**. The **Sequence Import** dialog box opens.
11. Select the sequence(s) to import.
12. Click **Import**.

Basic Tasks

Task 1. Open the BioConfirm program

In this task you open multiple data files using the current method.

Steps	Detailed Instructions	Comments
1 Open the BioConfirm program.	**a** Double-click the **Control Panel** icon on the desktop. **b** Click Projects in the left pane. **c** Select a project. **d** On the ribbon in the **BioConfirm** group, click **BioConfirm > Start BioConfirm**.	• You can get help for any window, dialog box, or tab by pressing the **F1** key when that window is active. • Learn how to create a project in the online Help for Control Panel. • You can create BioConfirm shortcuts on the desktop. These shortcuts are specific to the selected project. See the online Help for more information.

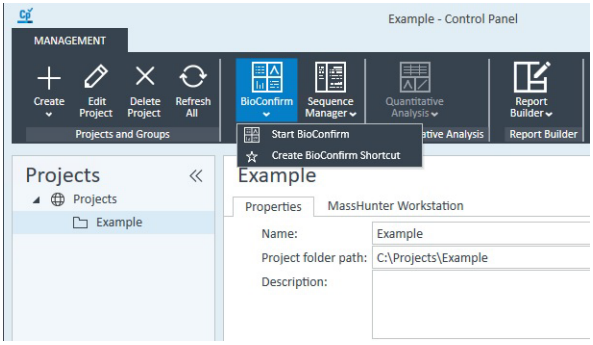


Figure 1. Start BioConfirm from the Control Panel program

Steps	Detailed Instructions	Comments

2 Open these data files:
• NIST mAb 1.d
• NIST mAb 2.d
• NIST mAb Digest.d
• NIST mAb Digest2.d
• ReleasedGlycans1.d
• ReleasedGlycans2.d
  ◦ Make sure that the **Use current method** button is clicked.
  ◦ Make sure that the **Load result data** check box is cleared.
	a In the **Open Sample** dialog box, go to the Data folder in your project.	• Only some example files were copied to the hard disk for these examples.
		• You can get help for any window, dialog box, or tab by pressing the **F1** key when that window is active.

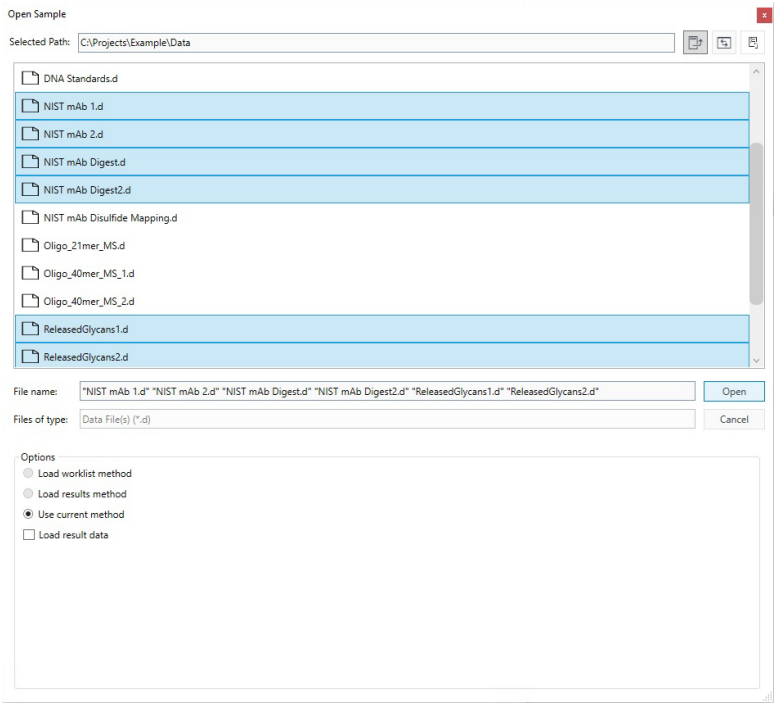


Figure 2. Open data files when opening software

Steps	Detailed Instructions	Comments
	b Click the **NIST mAb 1.d** file. **c** Press and hold the **Shift** key while you click **NIST mAb Digest2.d**. **d** Press and hold the **Ctrl** key while you click **ReleasedGlycans1.d** and **ReleasedGlycans2.d**. **e** Clear the **Load result data** check box. **f** Click **Open**. All the data files are displayed in the Sample Table window. The selected sample in the Sample Table is also shown in the Sample Chromatogram Results window. **g** Click the **List Mode** button ( ) in the Sample Chromatogram Results toolbar. **h** Click the **NIST mAb 1.d** data file in the Sample Table window.	• If you press the **Shift** key, you can pick a group of files that are directly next to each other. • If you press the **Ctrl** key, you can pick files which are not directly next to each other in the list. • What you see in the main window at this point depends on the method, layout, display and plot settings used before you opened these files. • When you click the List Mode button, the background of the button changes to orange.( )
3 Return the main window to the default Intact Protein layout.	• Click **Intact Protein Layout** in the main toolbar.	• If you want to change the display options, you click the button in the graphics window. • You can switch between layouts for the different workflows when you click the buttons in the main toolbar. • You can change the layout if you click **Configuration > Window Layouts > Load Layout**. • Columns in a table can be hidden. Some default columns in the Sample Table are hidden.

Steps	Detailed Instructions	Comments

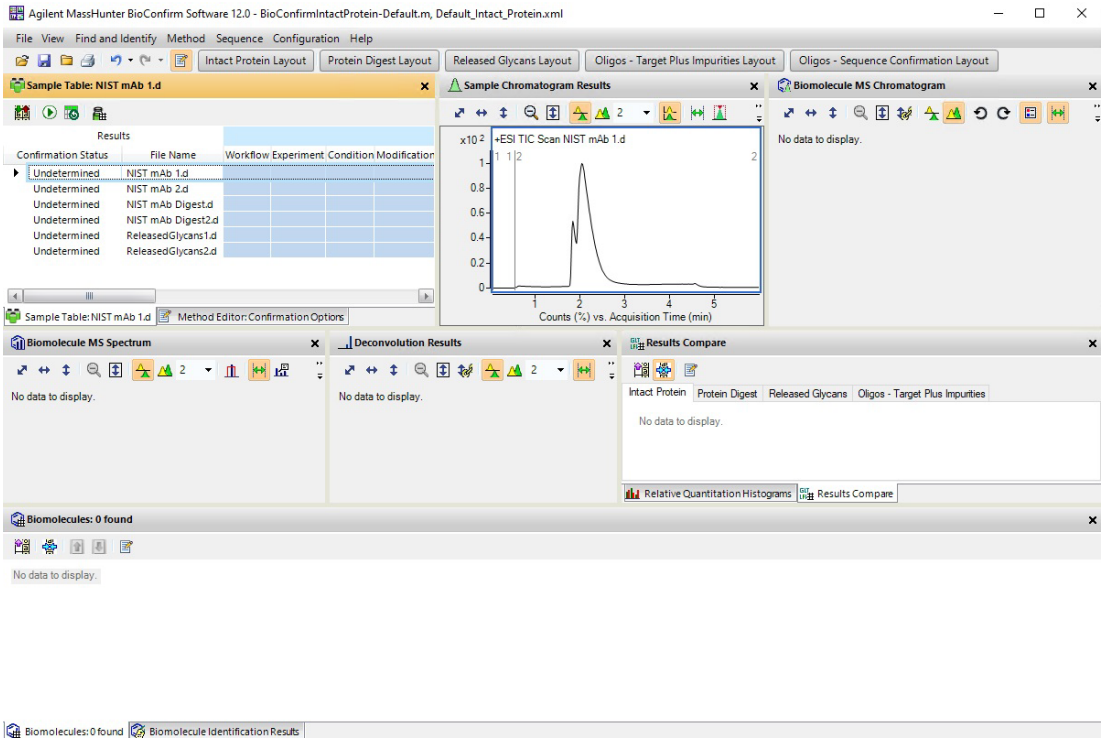
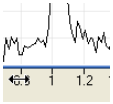
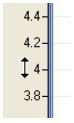
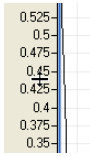


Figure 3. BioConfirm main window

Task 2. Zoom in and out of the chromatogram

In this task, you become familiar with the zoom in and zoom out features of the BioConfirm program.

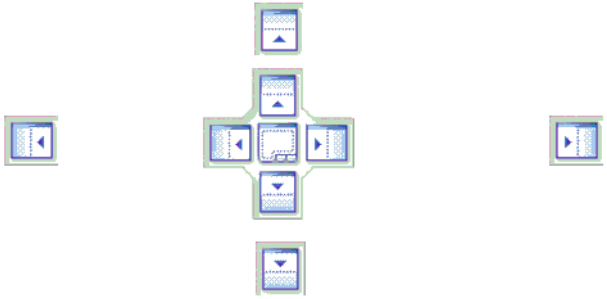
Steps	Detailed Instructions	Comments
1 Practice zooming in and out on the chromatogram in the Sample Chromatogram Results window. • Zoom in twice on the peak. • Zoom in one more time autoscaling the y-axis. • Zoom out once to the previous zoom position. • Completely zoom out to the original chromatogram.	**a** Click the right mouse button and drag over an area on the last peak. Make sure that the Autoscale Y-axis during Zoom button,  , is not selected for this step. **b** Repeat step a. **c** Click the Autoscale Y-axis during Zoom button,  , in the toolbar. **d** Click the right mouse button again and drag over an area of the peak for the third time. The BioConfirm program automatically scales the y-axis to the largest point in the range. **e** Click the Unzoom button,  , to undo the last zoom operation. You can undo the last fifteen zoom operations. **f** Click the Autoscale X-axis and Y-axis button,  , to zoom out completely.	• You can also use these zoom features in the Biomolecule MS Spectrum window, the Biomolecule Fragment Spectrum window, the Deconvolution Results window, the Deconvolution Mirror Plot window, and the Biomolecule MS Chromatogram window. • In addition to those windows, you can also zoom on the x-axis and y-axis and use the toolbar buttons in the Relative Quantitation Histograms window. You cannot drag over an area in the Relative Quantitation Histograms window. • A selected button has an orange background color.

Steps	Detailed Instructions	Comments
2 Practice zooming in and out on each axis separately. • Zoom in only along the x-axis. Hint: Right-click the x-axis values and move cursor from left to right. • Partially zoom out the x-axis. Hint: Move cursor in the opposite direction. • Completely zoom out of the x-axis. • Repeat the previous steps for the y-axis.	**a** To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears. **b** Click the right mouse button and drag the new cursor from left to right across the x-axis values. **c** To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values. **d** Click the Autoscale X-axis button, ↔, to completely zoom out on the x-axis. **a** To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears. **b** Click the right mouse button and drag the new cursor from bottom to top across the y-axis values. **c** To zoom out on the y-axis, click the right mouse button and drag from the top towards the bottom of the y-axis values. **d** Click the Autoscale Y-axis button, ↕, to completely zoom out on the y-axis.	 **Horizontal Double Arrow**  **New cursor appears when you right-click the x-axis value**  **Vertical Double Arrow**  **New cursor appears when you right-click the y-axis values.**

Task 3. Change window layouts

In this task, you move windows within the main view and create various window layouts. Default layouts are available for the Intact Protein workflow, the Protein Digest workflow, the Released Glycans workflow, the Oligonucleotides - Target Plus Impurities workflow, and the Oligonucleotides - Sequence Confirmation workflow.

Steps	Detailed Instructions	Comments

1 Change the window layout:
• Change the window size.
• Save a window layout.
• Unlock the layout.
• Change the Chromatogram Results window to be floating.
• Move the Chromatogram Results window.
• Display the tools for repositioning the windows.
	• To change the size of a window, drag the boundary between the windows.
	• To load the default layout for a workflow, click one of the buttons in the main toolbar:
	  • **Intact Protein Layout**
	  • **Protein Digest Layout**
	  • **Released Glycans Layout**
	  • **Oligos - Target Plus Impurities Layout**
	  • **Oligos - Sequence Confirmation Layout**
	• To load a layout, click **Configuration > Windows Layouts > Load Layout**.
	• To save a window layout, click **Configuration > Window Layouts > Save Layout**.
	• To lock or unlock a layout, click **Configuration > Window Layouts > Lock Layout**.
	• To make a window float, right-click the title bar of the window, and click Floating from the shortcut menu.
	• To move a window, click the title bar of the window and drag the window to the desired location.
	• To display the repositioning tools, drag the window over one of the other windows. When one window is overlapped with another, the program displays several layout tools, as shown in **Figure 4**.
	 
	Figure 4. Window repositioning tools
		• If the layout is locked, the system displays a check mark next to the Lock Layout menu.
		• You can only use the repositioning tools when the layout is unlocked.
		• You can also make a window float by double-clicking the title bar of the window.
		• If two or more windows are tabbed together, you can make one window float by double-clicking the tab for that window.
		• The following layouts are shipped with the software:
		Default_Intact_Protein.xml
		Default_Protein_Digest.xml
		Default_Released_Glycans.xml
		Default_Oligonucleotides_TPI.xml
		Default_Oligonucleotides_SC.xml
		Default.xml

Steps	Detailed Instructions	Comments
2 Reposition the Sample Chromatogram Results window. • Move the window so that it is at the top, to the left, to the right and then at the bottom of the other windows. • Move two windows together so that they are on top of one another and available only through the tabs at the bottom. • Restore the default layout.	• If you drag the cursor over one of the smaller icons, the window you are dragging will be placed above, to the right, below, or to the left of all of the other windows. • Drag the cursor over the larger icon. The window can also be placed above, to the right, below, or to the left of the other window by dragging the cursor over the edges of the larger icon. • To tab two windows together, drag the cursor over the center of the larger icon. You will see a shadow version of the two windows tabbed together. Stop dragging the mouse. The two windows will be tabbed together. • Click **Intact Protein Layout** in the main toolbar.	• The cursor must be over one of the arrows in a box in order for repositioning to occur. • Click the **Configuration > Load Default Layout** command to load the default layout, default.xml.

Task 4. Creating a Protein Sequence File

This task guides you through the creation of a myoglobin protein sequence file. You can also create oligonucleotide sequence files. See **"Task 6. Creating an Oligonucleotide Sequence File"** on page 18

Steps	Detailed Instructions	Comments
1 Start the Agilent MassHunter Sequence Manager.	• Click **Sequence > Sequence Manager**.	• You can also start the Sequence Manager program from the OpenLab Control Panel.
2 Add a sequence.	**a** Click the **Proteins** tab. **b** Click **Sequence > Add Sequences > Add Protein Sequence**. **c** Select one or more sequences in the list in the **Add Protein Sequence(s)** dialog box. **d** Click **Open**. The Sequence Editor pane opens automatically with that sequence.	• Protein is automatically selected for the sequence type.
3 Create a new sequence.	**a** Type `Myoglobin` for the name of the Sequence. **b** Click the **+** button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	• Protein is automatically selected for the sequence type.
4 Enter the amino acid sequence shown below into the Sequence Manager. GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHLKTEAEM KASEDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHV LHSKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG	• Type in individual amino acids one at a time between the N-term and C-term symbols.	• Use the single-character (letter) amino acids abbreviations. • Tip: If you are reading this document as a PDF file on your computer, you can copy and paste the sequence into the Sequence Manager window.

Note: The myoglobin sequence does not have any links or modifications, but some sequences do. In that case, add links and modifications as described in the *Quick Start Guide* or *online Help*.

Steps	Detailed Instructions	Comments
5 Save the sequence as the name *iii*_**myoglob.psq**, where *iii* represents your initials.	**a** Click **Sequence > Export Sequence**. **b** Type *iii*_myoglob in the File name box. **c** Click **Save**.	• The sequence is saved as a **.psq** file that can be transferred to other computers or projects.

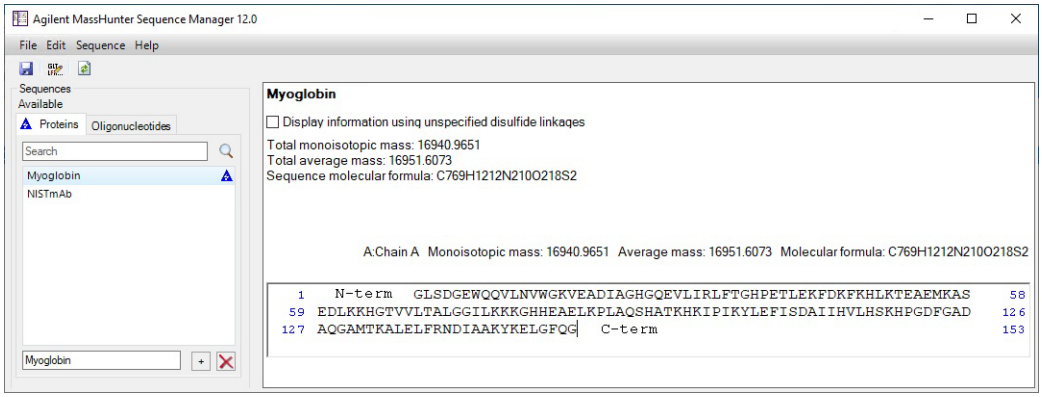


Figure 5. Creating a sequence file of myoglobin in the Sequence Manager program

6 Save changes in the Sequence Manager.	**a** Click **Sequence > Save All Changes.** **b** In the **Save Sequences** dialog box, mark the check box in front of Myoglobin. **c** Enter a description of the change that was made. **d** Click **Save**.	• A description is always required when you save a sequence.

Task 5. Adding a building block to the Chemical Data Dictionary

This task guides you through the creation of a new building block. Building blocks are used when creating an oligonucleotide sequence.

Steps	Detailed Instructions	Comments
1 Start the Agilent MassHunter Chemical Data Dictionary.	• Click **Sequence > Open Chemical Data Dictionary Editor**.	• You can also start the Sequence Manager program and click **Open definition manager** on the Oligonucleotides tab.
2 Create a new building block.	**a** Click the **Oligonucleotides** tab. **b** Type `kH` for the Code. **c** Click the **+** button.	• See the online Help for rules about naming new Building blocks.
3 Enter the **Name**, **Molecular formula**, and **Base formula**.	**a** Type the **Name** for this Building block. This name is a description of the Building block. **b** Type `C9H13N3O4` for the **Molecular formula**. **c** Type `C4H5N3O` for the **Base formula**.	• This example is a duplicate of the Building block Deoxycytidine.

Steps	Detailed Instructions	Comments
4 Close the program.	**a** Click **Close**. **b** In the **Save Chemical Data Dictionary** dialog box, enter a reason that the change was made. **c** Click **Yes**.	• If you click **No** in the **Save Chemical Data Dictionary** dialog box, the changes are not saved.

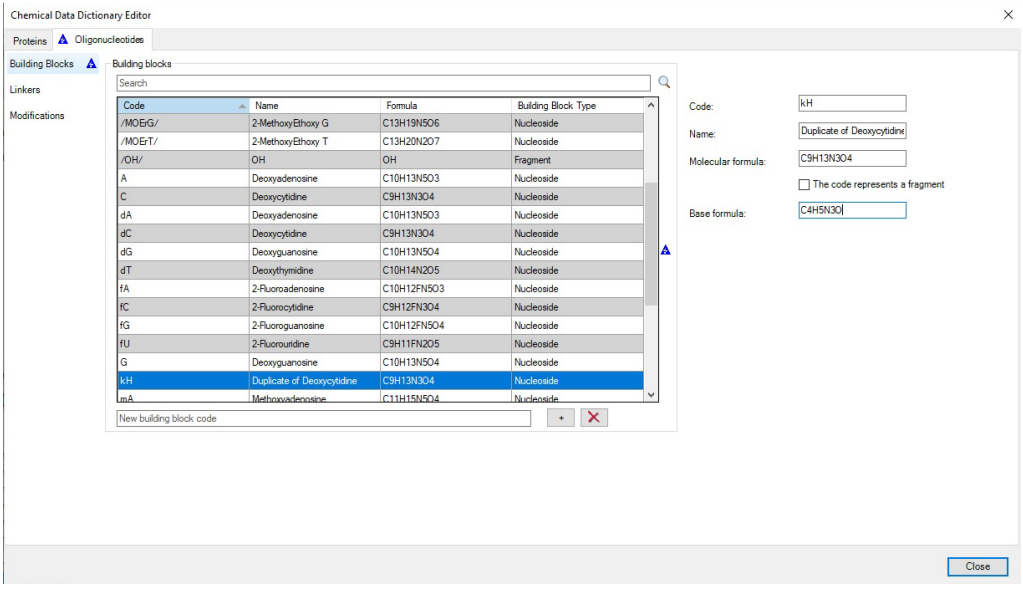


Figure 6. Creating a Building block in the Chemical Data Dictionary Editor dialog box

Task 6. Creating an Oligonucleotide Sequence File

This task guides you through the creation of an oligonucleotide sequence file. You can also create a protein sequence. See **"Task 4. Creating a Protein Sequence File"** on page 14

Steps	Detailed Instructions	Comments
1 Start the Agilent MassHunter Sequence Manager.	• Click **Sequence > Sequence Manager**.	• You can also start the Sequence Manager program from the OpenLab Control Panel.
2 Add a sequence.	**a** Click the **Oligonucleotides** tab. **b** Click **Sequence > Add Sequences > Add Oligonucleotide Sequence**. **c** Select **Oligo_40mer_MS.psq** and **Oligo_40mer_MSMS.psq** sequences in the list in the **Add Sequence(s)** dialog box. **d** Click **Open**. The Sequence Editor pane opens automatically with that sequence.	• The Oligo_40mer_MS sequence and the Oligo_40mer_MSMS sequence are used in the exercises in this guide.
3 Create a new sequence.	**a** Type `Example` for the name of the Sequence. **b** Click the **+** button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	• Oligonucleotide is automatically selected for the sequence type.
4 Enter the building block sequence shown below into the Sequence Manager. TGACTGTGAACGTTCGGATGA	**a** Type in **Building blocks** one at a time between the 5′ and 3′ symbols. **b** Mark the **If two building blocks are next to each other, insert linker** check box. **c** Select **p** for the linker.	• Use the **Code** for the Building block. • The Total monoisotopic mass is 6498.1153 after adding these building blocks. • Tip: If you are reading this document as a PDF file on your computer, you can copy and paste the sequence into the Sequence Manager window.

Steps	Detailed Instructions	Comments
5 Save the sequence as the name *iii*_**Example.psq**, where *iii* represents your initials.	**a** Click **Sequence > Export Sequence**. **b** Type *iii*_Example in the File name box. **c** Click **Save**.	• The sequence is saved as a **.psq** file that can be transferred to other computers or projects.

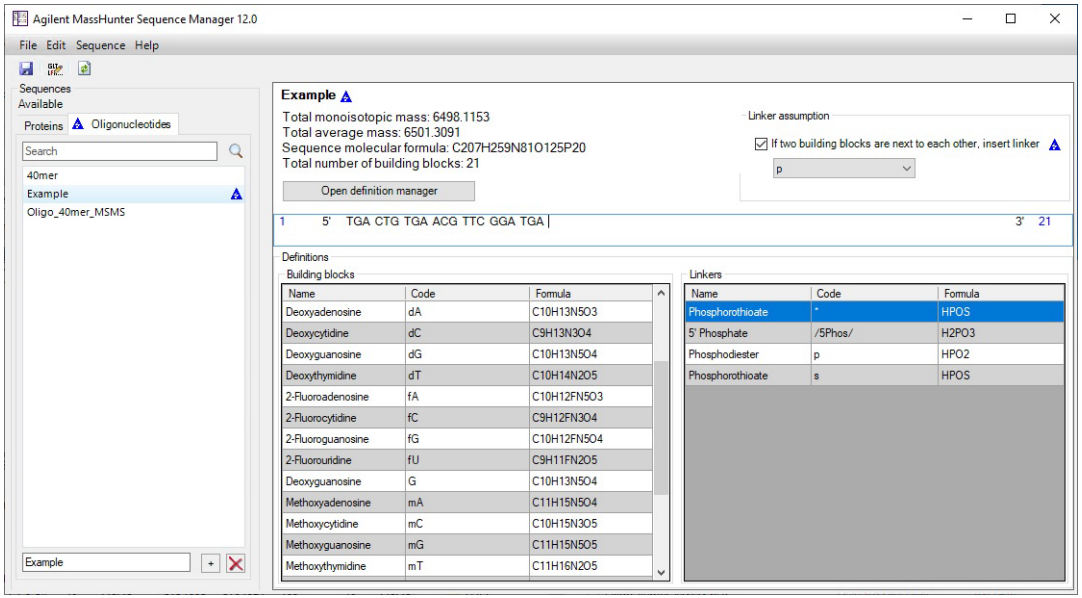


Figure 7. Creating an oligonucleotide sequence in the Sequence Manager program

6 Save changes in the Sequence Manager.	**a** Click **Sequence > Save All Changes.** **b** In the **Save Sequences** dialog box, mark the check box in front of **Example**. **c** Enter a description of the change that was made. **d** Click **Save**.	• A description is always required when you save a sequence.

Intact Protein Workflow

Step 1 - Open the data file of interest and select the Intact Protein layout.

Step 2 - Open a BioConfirm method or create a new one.

Step 3 - Edit protein sequences if necessary in the Sequence Manager program:

- Add or edit the sequence text
- Apply or edit modifications
- Apply or edit links

Step 4 -Select **Intact Protein** for the **Workflow** on the **Workflow and Sequences** tab. Select the **Condition**.

Step 5 - Select the **Sequence/Masses** to match on the Workflow and Sequences tab.
If the sequence you want to match is not in the method or **Select Sequences** dialog, then:

Import or create a sequence.

Step 6 - Select the **Mods and Profiles** on the Workflow and Sequences tab.

Step 7 - Run the Method Workflow.

Step 8 - Review the results which are shown in these windows:
Sample table
Biomolecules table
Biomolecule Identification Results
Deconvolution Results
Biomolecule MS Chromatogram
Biomolecule MS Spectrum
Results Compare
Relative Quantitation Histograms

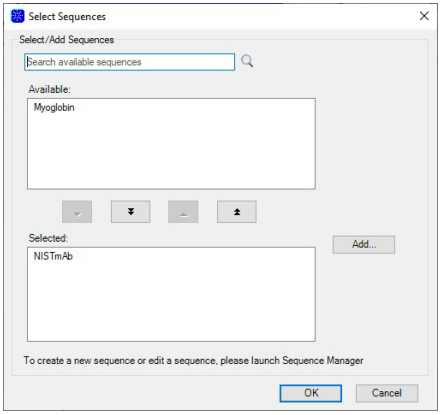
Step 9 - Print report.

Exercise 1. Interactive Intact Protein Workflow

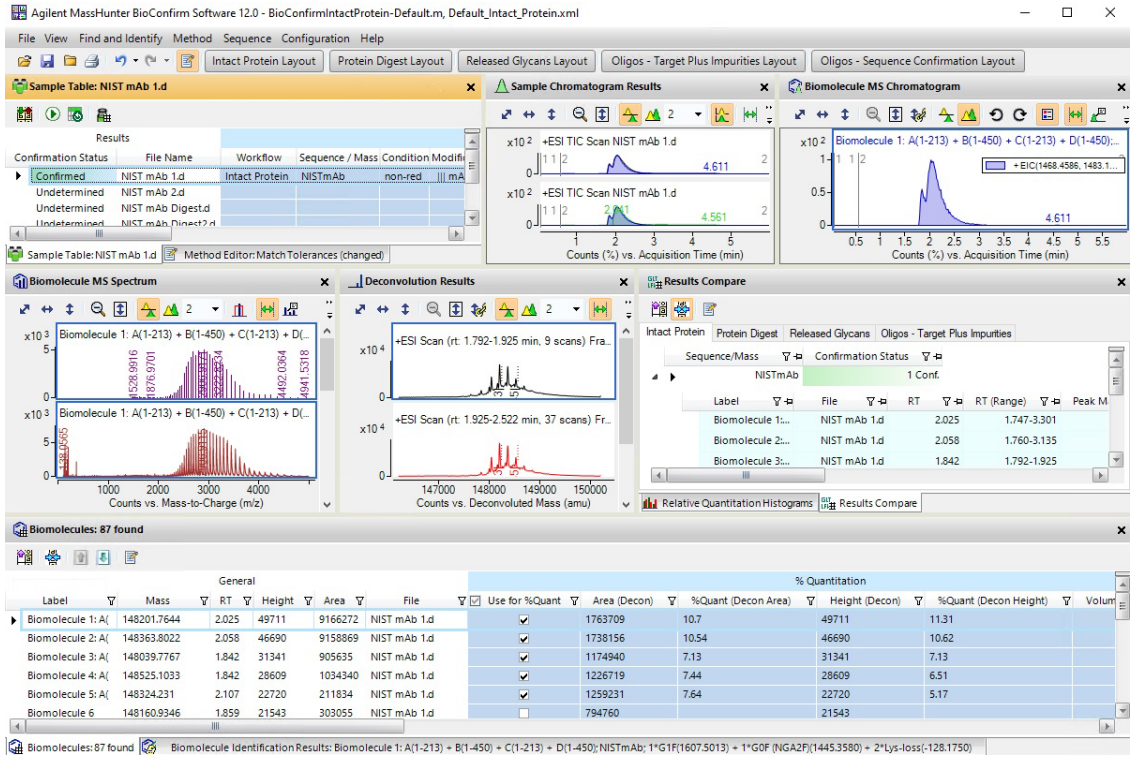
This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the **NISTmAb.seq** sequence file and the **NIST mAb 1.d** data file copied before you started. See **"Before you start"** on page 3.

If you select the Intact Protein workflow, the Find by Protein Deconvolution algorithm runs and uses protein Matching Rules (Intact Protein, and Variable Modifications). You can select whether or not Protein Truncation is done.

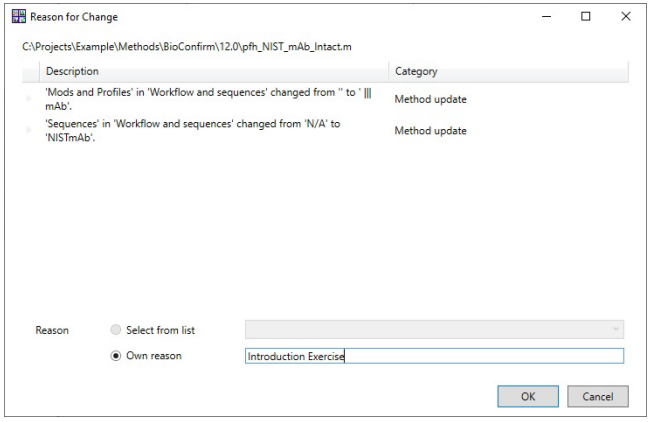
Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point.	**a** Click **Method > Open**. **b** Select **BioConfirmIntactProtein-Default.m**. **c** Click **Open**.	• You may be asked whether or not save Method changes before loading a new method.
2 If the **NIST mAb 1.d** data file is not already open, open it.	**a** Click **File > Open Data File**. **b** Select **NIST mAb 1.d**. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Select the Intact Protein layout.	• Click **Intact Protein Layout** in the main toolbar.	• You can instead click **Configuration > Window Layouts > Load Layout**. Then, select *Default_Intact_Protein.xml*, and click **Open**.
4 Display the Deconvolute (Protein) section in the Method Editor window.	**a** Click **View > Method Editor** if the Method Editor is not visible. **b** Select **Intact Protein > Deconvolute (Protein)** in the Method Editor window.	
5 Run the Find by Protein Deconvolution algorithm.	**a** Review the settings and modify them if necessary. **b** Click on the Method Editor toolbar to start the Find by Protein Deconvolution algorithm. **c** If the Find Proteins dialog box opens, select NIST mAb 1.d and click **Find**. **d** Review the results in the Biomolecules window.	• In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*. • If you have more than one data file open, the Find Proteins dialog box opens.
6 Display the Workflow and Sequences section in the Method Editor window.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	

Steps	Detailed Instructions	Comments
7 Import the NISTmAb sequence.	**a** Select **Intact Protein** for the Workflow. **b** Select **non-reduced** for the Condition. **c** Click the ... button next to the **Sequences/Masses** parameter. The **Select Sequences** dialog box opens. **d** Double-click **NISTmAb**. If **NISTmAb** is not available, click **Add**. **e** Select **NISTmAb.psq** and click **Open**. **f** Verify that the NISTmAb sequence is in the **Selected** list. **g** Click **OK**. 	• You will use the sequence as is. You can add or modify modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.
8 Select the mAb modification.	**a** Click the ... button next to the **Mods and Profiles** parameter. The **Select Modifications and Profiles** dialog box opens. **b** Double-click **mAb** in the **Available** list in the Modifications and profiles section. **c** Click **OK**.	• The mAb sequence has modifications. You can learn how to add modifications in the *online Help* and the *Quick Start Guide*. • Review the modifications in the **Modification Summary**.
9 Start the match search.	**a** Click **Intact Protein > Match Tolerances**. **b** Click on the Method Editor toolbar. **c** If necessary, select NIST mAb 1.d and click **Match**.	• You can instead click **Find and Identify > Match Sequences > Match Sequences (Proteins)**. • If you have multiple data files open, then you select which data files to update.
10 Review the results.	• Select the **Biomolecule 1** row in the Biomolecules table.	• In the BioConfirmIntactProtein-default layout, the Biomolecule Identification Results window is tabbed with the Biomolecules window.

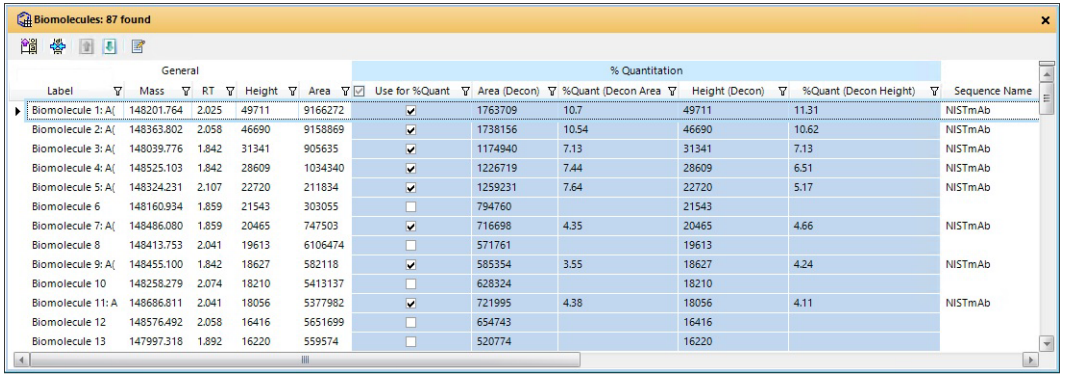
Steps	Detailed Instructions	Comments



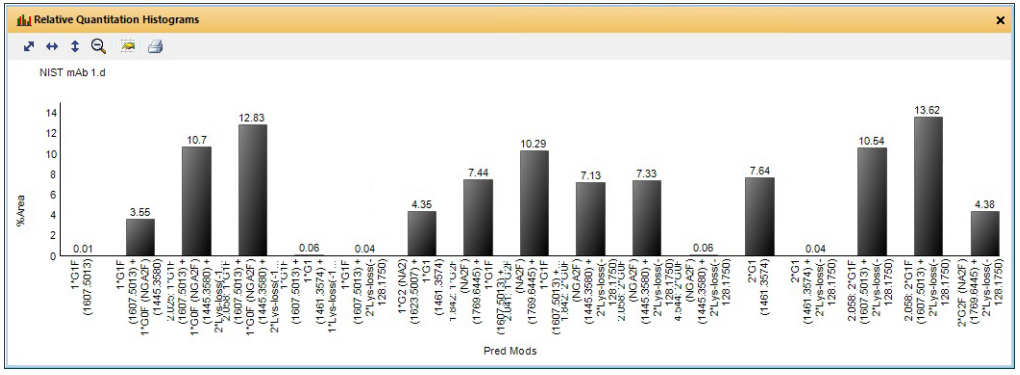
Steps	Detailed Instructions	Comments
11 Save the method for use in Exercise 2.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_**NIST_mAb_Intact.m**, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason in the Reason for Change dialog box. **e** Click **OK**.	• You may see additional changes listed in the dialog box if you made other changes.



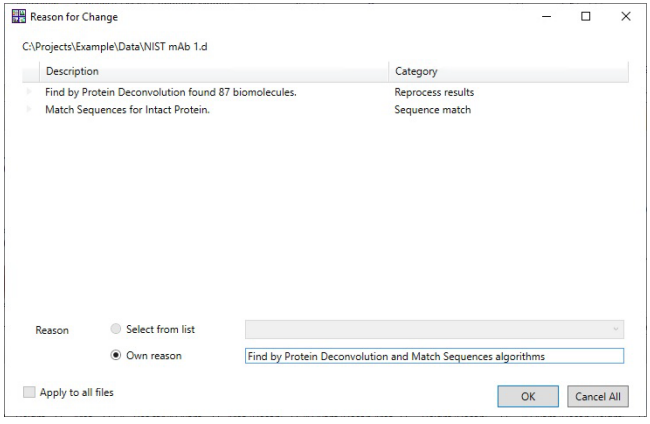
12 Investigate the Relative Quantitation feature. You normally use this feature to quantitate proteoforms (PTMs on the protein).	**a** In the Biomolecules window, hide all empty columns. Click  in the Biomolecules toolbar. **b** Review the values for **Use for %Quant** column for the biomolecules. **c** Review the values for the **%Quant (Decon Height)** and **%Quant (Decon Area)**.	• You can right-click the window and click **Add/Remove** columns to change the columns that are available.



Steps	Detailed Instructions	Comments
	d Review the results in the Relative Quantitation Histograms window. For an Intact Protein workflow, this window only contains data if the **Use for %Quant** check box is marked in the Biomolecules window for one or more biomolecules and if the sequence has predicted modifications.	



Steps	Detailed Instructions	Comments
13 Save the results.	**a** Click **File > Save Results**. **b** Select **NIST mAb 1.d** in the **Save Data File** dialog box. Click **Save**. **c** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	• You can save results with the data file. You can select whether to open these results when you open the data file. • The administrator can set up the project so that you need to enter a reason when you save results to a data file. You may be able to enter your own reason or you may need to select a reason from a list. • If you want to save the results, you need to save them before you change the method. If you do not, then you will need to generate the results again.

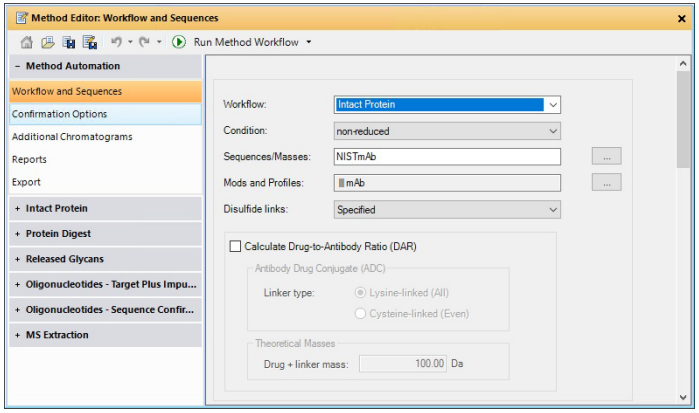


Exercise 2. Automated Intact Protein Workflow

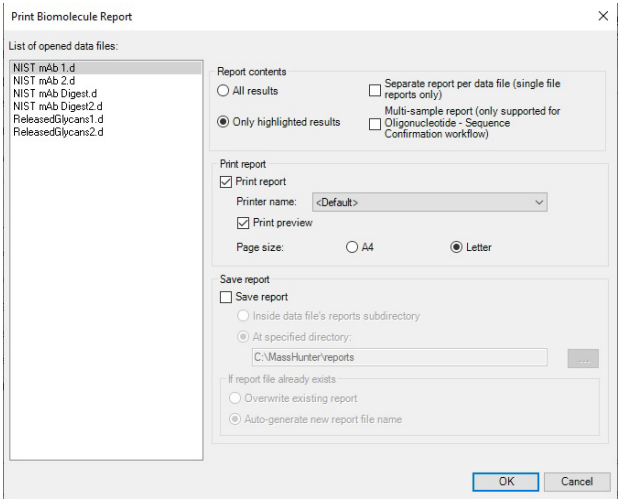
This exercise guides you through the setup of a worklist to automatically confirm the presence of NISTmAb in a previously acquired sample. This exercise uses the **NIST mAb 1.d** data file copied in Exercise 1.

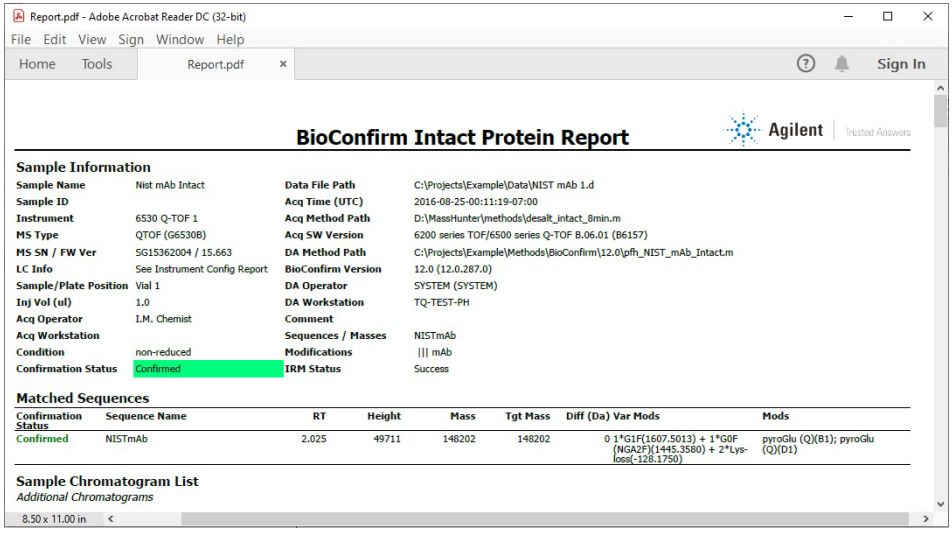
Steps	Detailed Instructions	Comments
1 If not already open, open the method *iii*_**NIST_mAb_Intact.m**.	**a** Click **Method > Open**. **b** Select the *iii*_**NIST_mAb_Intact.m** folder. **c** Click **Open**.	This method was created in **"Exercise 1. Interactive Intact Protein Workflow"** on page 21.
2 Open the automation section in the Method Editor window.	• Click **Method Automation > Workflow and Sequences** in the Method Editor window.	
3 Use the **Intact Protein** Workflow.	• Confirm that **Intact Protein** is selected for the Workflow.	• In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
4 Select the NISTmAb sequence.	**a** Select **non-reduced** for the Condition. **b** Click the ... button next to the **Sequences** parameter. The **Select Sequences** dialog box opens. **c** If NISTmAb is not available, click **Add**. **d** Select *NISTmAb*.**psq** and click **Open**. **e** Verify that the NISTmAb sequence is in the **Selected** list. **f** Click **OK**.	• The **NISTmAb.psq** sequence file is available on the BioConfirm setup media. • You can learn about modifications and links in the *online Help* and in the *Quick Start Guide*.
5 Select **mAb** as the modification and profile.	**a** If needed, click the ... button next to the **Mods and Profiles** parameter. The **Select Modifications and Profiles** dialog box opens. **b** Double-click **mAb** in the Available list. **c** Click **OK**.	

Steps	Detailed Instructions	Comments
6 Save the method.	• Click **Method > Save**. • If needed, enter the reason for the change.	• If you mark the **Calculate Drug-to-Antibody Ratio** check box, the software will calculate DAR for the data file. See **"Exercise 15. Calculate DAR"** on page 78 for more information.
7 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click on the Method Editor toolbar. • Reprocess the sample. See **"Exercise 11. Reprocess Samples"** on page 62.	• Method Automation first runs the method workflow, and then extracts additional chromatograms and generates a biomolecule report and exports results.
8 Save the results.	**a** Click **File > Save Results**. **b** Select **NIST mAb 1.d** in the **Save Data File** dialog box. Click **Save**. **c** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	• You save results before modifying the method again.



Steps	Detailed Instructions	Comments
9 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation**, then a report is generated automatically. • Before you can interactively print a report, you need to save the results. You click **File > Save Results** to save the results. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box.





Protein Digest Workflow

The steps outlined below show the workflow for Protein Digest with MassHunter BioConfirm.

Step 1 - Open the data file of interest and select the Protein Digest workflow.

Step 2 - Open a BioConfirm method or create a new one.

Step 3 - Edit sequences if necessary in the Sequence Manager program:

- Add or edit the sequence text.
- Apply or edit modifications
- Apply or edit links

Step 4 - Select the **Workflow** on the **Workflow and Sequences** tab. Select the **Condition**.

Step 5 - Select the **Sequences/Masses** to match on the Workflow and Sequences tab.
If the sequence you want to match is not in the method or Select Sequences dialog box, then:

Import or create a sequence.

Step 6 - Select the **Mods and Profiles** on the Workflow and Sequences tab.

Step 7 - Mark the **Enzymes** on the Workflow and Sequences tab.

Step 8 - Run the Method Workflow.

Step 9 - Review the results which are shown in these windows:
Biomolecules table
Biomolecule Identification Results
Sequence Coverage Map
Biomolecule MS Spectrum
Biomolecule Fragment Spectrum
Peptide Relative Quantitation Results
Results Compare
Relative Quantitation Histograms

Step 10 - Print report.

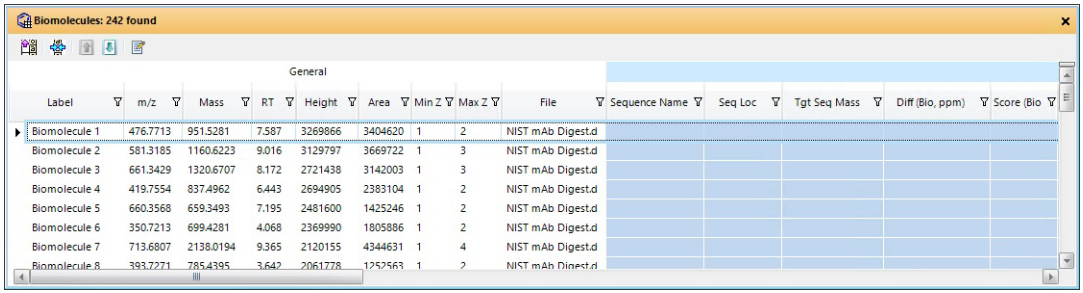
Exercise 3. Interactive Protein Digest Sequence Matching

This exercise shows you how to confirm protein digests interactively.

If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzyme selected in the Workflow and Peptides section and then runs the Protein Digest matching rules. See **"Before you start"** on page 3.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmProteinDigest-Default.m** folder. **c** Click **Open**.	• The parameters in the BioConfirmProteinDigest-Default.m method are a good starting point for Protein Digests.
2 Load the Protein Digest default layout.	• Click **Protein Digest Layout** on the main toolbar.	
3 Select NIST mAb Digest.d. If the data file NIST mAb Digest.d is not open, open the data file.	• If available, select **NIST mAb Digest.d**. Otherwise, do the following: **a** Click **File > Open Data File**. **b** Locate the **NIST mAb Digest.d** sample. **c** Clear the **Load result data** check box. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
4 Review the parameters in the Find Peptides section in the Method Editor window.	**a** Select **Protein Digest > Find Peptides** in the Method Editor window. **b** Review the settings on the various tabs of the Find Peptides section. **c** Click the **Charge State** tab. **d** Review the parameters.	• You can change the default parameters as described in the next steps. You can also use the method without any changes. • For some data files, you will need to use different parameters as described in the *online Help*. • A very low peak height filter can result in greater sequence coverage but requires much more time to process.
5 Run the Find Peptides algorithm.	**a** Click  on the Method Editor toolbar to start the biomolecule search. **b** If the **Find Peptides** dialog box opens, select **NIST mAb Digest.d** and click **Find**. **c** When processing is complete, review the results in the Biomolecules window.	• You can instead click **Find and Identify > Find Peptides**.

Steps	Detailed Instructions	Comments



6 Add the sequence.

- **a** Click **Method Automation > Workflow and Sequences** in the Method Editor window.
- **b** Select **Protein Digest** as the **Workflow**.
- **c** Select **reduced** as the **Condition**.
- **d** Click the [...] next to the **Sequences/Masses** parameter.
- **e** Double-click **NISTmAb**.
- **f** Click **OK** in the **Select Sequences** dialog box.
- **g** Click the [...] next to the **Mods and Profiles** parameter.
- **h** Double-click **Protein Digest (Reduced+Alkylated)**.
- **i** Click **OK** in the **Select Modifications and Profiles** dialog box.
- **j** Mark the **Trypsin** check box under **Enzymes**.

- For this exercise, you use the sequence as is, but you can add modifications and links to sequences as described in *online Help*.
- You can customize the list of available reagents using the Chemical Data Dictionary; see *online Help* for more information.

7 Review parameters on the Mass Matching tab.

- **a** Click the **Mass Matching** tab in the Protein Digest > Match Tolerances section of the Method Editor window.
- **b** Review the parameters.

8 Review the Matching Rules.

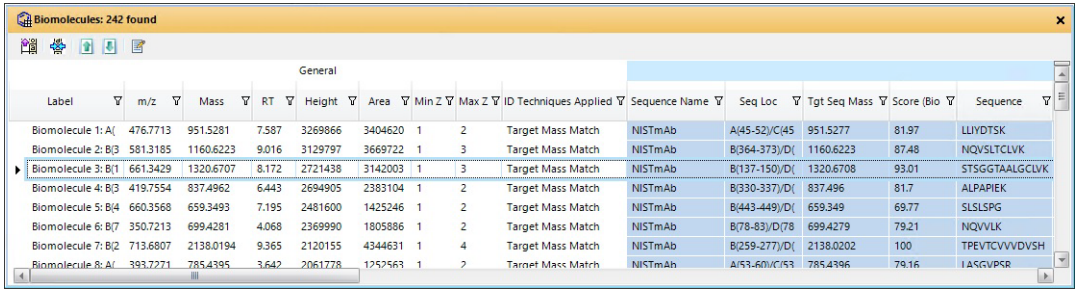
- **a** Click the **Matching Rules** tab in the Protein Digest > Match Tolerances section in the Method Editor.
- **b** Mark the **Allow free cysteines (non-reduced condition)** check box.
- **c** Enter 2 for the **Allow missed cleavages up to**.
- **d** Review the other parameters.

9 Save the method for use in Exercise 7.

- **a** Click **Method > Save As**.
- **b** Type the **File name** *iii*_NIST_mAb_ProteinDigest.m, where *iii* represents your initials.
- **c** Click **Save**.
- **d** If needed, enter a reason on the **Reason for Change** dialog box and click **OK**.

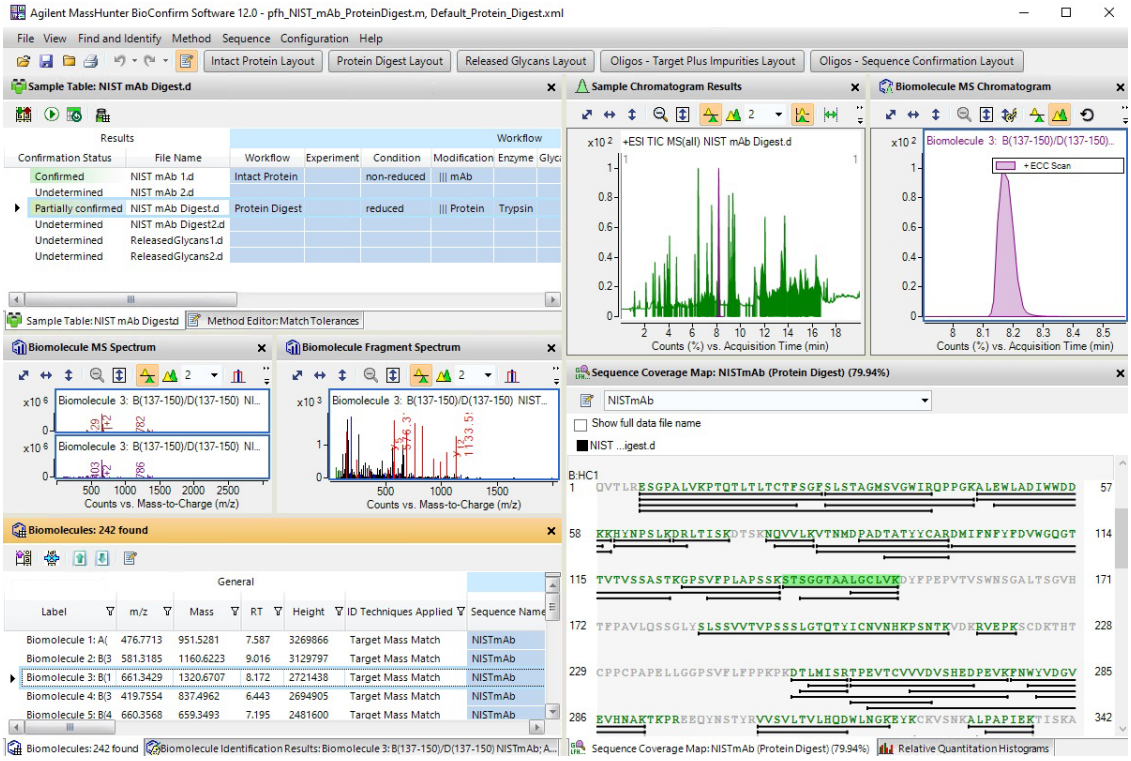
- The administrator can set up the project to require you to enter a reason when you save a method. You may be able to enter your own reason, or you may need to select a reason from a list of possible reasons.

Steps	Detailed Instructions	Comments
10 Start the match search.	**a** Click **Find and Identify > Match Sequences > Match Sequences (Proteins)**. **b** Select **NIST mAb Digest.d**. **c** Click **Match**.	*Alternate methods*: • Click on the Method Editor toolbar. • Click **Match Sequences** on the Method Editor shortcut menu.
11 Review the results.	**a** Highlight Biomolecule 3 in the Biomolecules window. **b** Click the **Biomolecule Identification Results** tab which is tabbed with the Biomolecules window. **c** When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window. **d** Select another sequence match result to view by selecting a different biomolecule in the Biomolecules window.	• If the biomolecule was identified, the **ID Techniques Applied** column contains **Target Mass Match**.



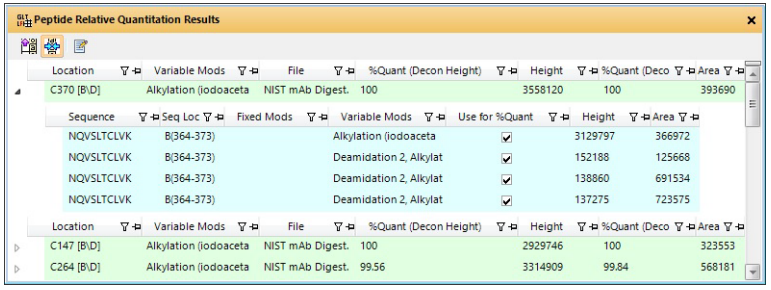
12 View sequence coverage results.	**a** If necessary, click **View > Sequence Coverage Map**. **b** Select a different biomolecule in the Biomolecules table to view a different result.	• Amino acids that are matched are either green (MS/MS) or black (MS-only) in a matched sequence. • Amino acids that are not matched are gray. • A line is added below the AA sequence to display where peptides have been identified. • See the online Help for more information.
To view more information.	Click the following items on the Sequence Coverage Map window shortcut menu to view more information about the sequence: • **Applied Modifications** • **Specified Applied Links** • **View Digest List**	

Steps	Detailed Instructions	Comments
13 Save the method and results	**a** Click **Method > Save**. **b** If needed, enter a reason on the **Reason for Change** dialog box, and click **OK**. **c** Click **File > Save Results** to save your results to the data file folder. **d** If needed, enter a reason on the **Reason for Change** dialog box and click **OK**.	• You can also click  to save results.



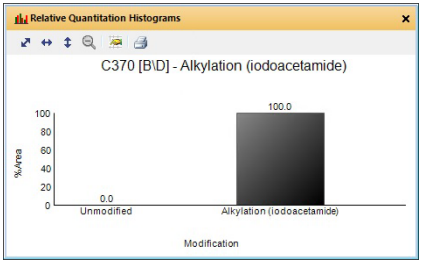
14 Review the results in the Peptide Relative Quantitation Results window.	**a** Click **View > Peptide Relative Quantitation Results**. It is not visible in the default layout. When it is visible, it is tabbed with the Biomolecules window. **b** Click the first triangle next to the first row. **c** Note that **Use for %Quant** is marked for each row.	• For Protein Digest workflow, the software uses the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.

Steps	Detailed Instructions	Comments



15 Review the results in the Relative Quantitation Histograms window.

a Click the **Relative Quantitation Histograms** window.
b Click the first triangle next to the first row.
c Note that **Use for %Quant** is marked for both rows.



- For Protein Digest workflow, the software uses the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.

16 Repeat the interactive processing with *NIST mAb Digest2.d*.

a If needed, open the data file **NIST mAb Digest2.d** (see **step 3**).
b Select Find Peptides in the Method Editor and verify the parameters(**step 4**).
c Find biomolecules (**step 5**).
d Match sequences (**step 10**).
e Save the results to the NIST mAb Digest2.d file (**step 13**).

- Most of the processing parameters used for the first data file are the same for the second data file.
- These results are used in **"Exercise 12. Use Result Review mode"** on page 65.

Exercise 4. Automated Protein Digest Workflow

This exercise guides you through the setup of a worklist to automatically confirm the presence of NIST mAb in a previously acquired sample.

If you select the Protein Digest workflow, the Find Peptides algorithm runs and uses the enzymes selected in the Workflow and Peptides section and then runs the Protein Digest matching rules.

Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**NIST_mAb_ProteinDigest.m** folder. **c** Click **Open**.	• This method was created in Exercise 3 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	**a** If the Method Editor is not visible, click **View > Method Editor**. **b** Click **Method Automation > Workflow and Sequences** in the Method Editor window.	• You can instead click the Method Editor button,  , on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Protein Digest** for the Workflow. **b** Select **reduced** for the **Condition**. **c** Verify that **NISTmAb** is the sequence. **d** Verify that **Protein Digest (Reduced+Alkylated)** is the Mods and Profiles. **e** Mark the **Trypsin** check box.	• The Protein Digest workflow automatically runs the following actions: ◦ Find Peptides ◦ Match Sequences
4 Save the method.	**a** Click **Method > Save**. **b** If needed, enter a reason for the change.	
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar. • Reprocess the sample. See **"Exercise 11. Reprocess Samples"** on page 62.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results.
6 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box.

Released Glycans Workflow

The steps outlined below show the workflow for Released Glycans with MassHunter BioConfirm.

Step 1 - Open the data file of interest and select the Released Glycans layout.

Step 2 - Open a BioConfirm method or create a new one.

Step 3 - Select the **Workflow** on the **Workflow and Sequences** tab.

Step 4 - Select the **Target glycan source.**

Step 5 - Select the tag which you used. 2-AB and InstantPC are listed, and you can create your own.

Step 6 - Run the Method Workflow.

Step 7 - Review the results which are shown in these windows:
Sample Chromatogram Results
Biomolecule MS Chromatogram
Biomolecules table
Biomolecule Identification Results
Biomolecule MS Spectrum
Glycan Structure Viewer
Results Compare
Relative Quantitation Histograms

Step 8 - Print report.

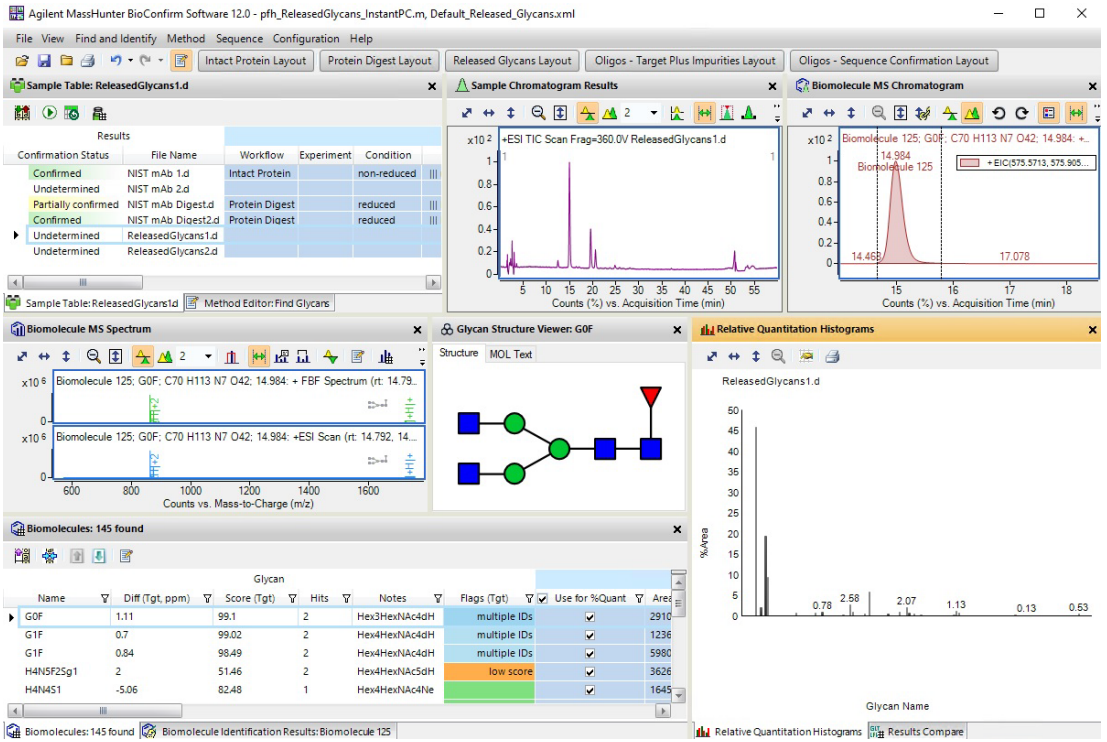
Exercise 5. Interactive Released Glycans

This exercise shows you how to find released glycans interactively.

If you select the Released Glycans workflow, the Find Glycans algorithm runs. See **"Before you start"** on page 3.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmReleasedGlycans-Default.m** folder. **c** Click **Open**.	• The parameters in the BioConfirmReleasedGlycans-Default.m method are a good starting point for Released Glycans.
2 Open the example sample file.	**a** If needed, click **File > Open Data File**. **b** Select **ReleasedGlycans1.d**. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Released Glycans layout.	• Click **Released Glycans Layout** in the main toolbar.	
4 Review the parameters in the Find Glycans section in the Method Editor window.	**a** Select **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Select **Released Glycans**. **c** Enter `Example` in the Glycan group. **d** Clear **Require RT match if database contains a RT for the target glycan**. **e** Select **Released Glycans > Find Glycans** in the Method Editor window. **f** Select `Glycans_mAb_AM_PCD.cdb` for the **Target glycan source**. **g** Click the **Tag** tab. **h** Click the option for the correct tag. For the example data file, click **InstantPC**.	• You can change the default parameters as described in the next steps. • For some data files, you will need to use different parameters as described in the *online Help*. • Some structures are included in the Glycans_mAb_AM_PCD.cdb file. • A very low peak height filter can result in greater sequence coverage but requires much more time to process.
5 Run the Find Glycans algorithm.	**a** Click  on the Method Editor toolbar to start the biomolecule search. **b** When processing is complete, review the results in the Biomolecules window. **c** Click **View > Glycan Structure Viewer**.	
6 Save the method for use in Exercise 6.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_ReleasedGlycans_InstantPC.m, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	

Steps	Detailed Instructions	Comments
7 Review the results.	**a** In the Biomolecules window, click the header of the **Area (FBF)** column to sort the table by this column. If necessary, click the header again so that the largest areas are at the top of the table. **b** Highlight **G0F** in the Biomolecules window. **c** Click the **Biomolecule Identification Results** tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window. **d** When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window.	• Several changes were made to the default layout for the image below. The Glycan Structure Viewer window is visible. Also, the **Flags (Tgt)** column was moved. • The Relative Quantitation Histograms window only contains information when you run a workflow.



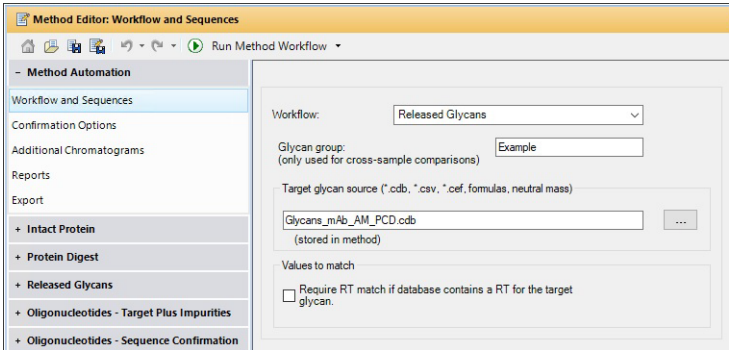
8 Save the results	**a** Click **File > Save Results** to save your results to the data file folder. **b** If needed, enter a reason for the change.	• You can also click to save results.

Exercise 6. Automated Released Glycans Workflow

This exercise guides you through the setup of a worklist to automatically run the Released Glycans workflow.

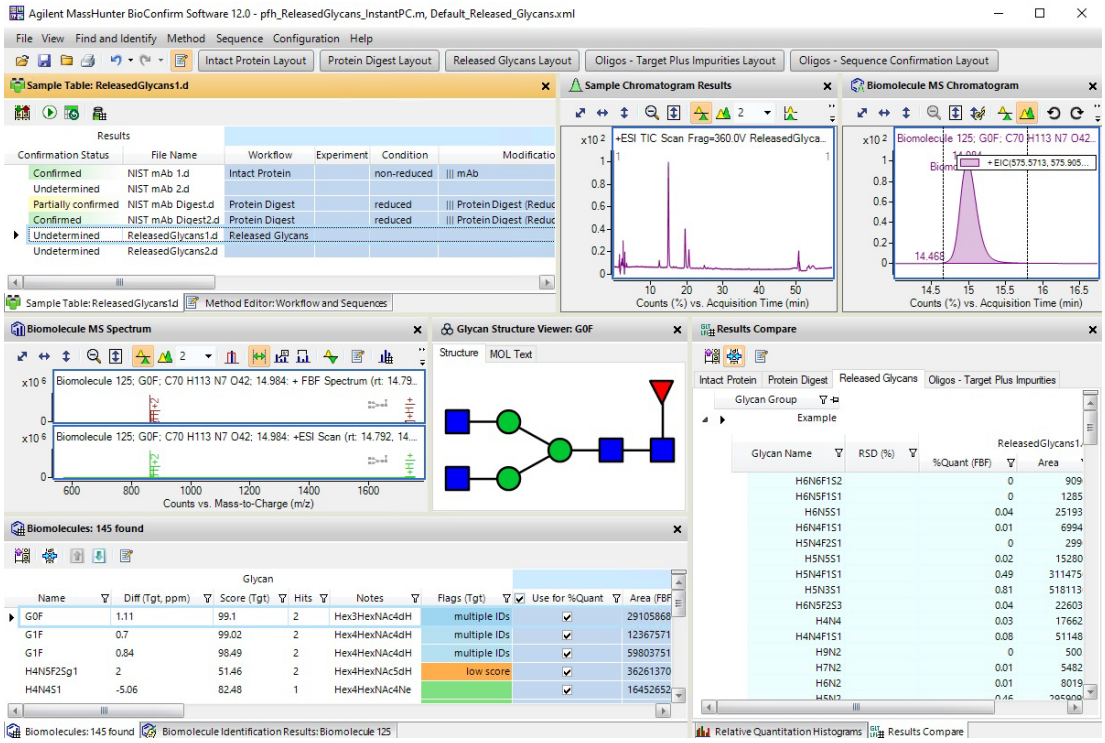
If you select the Released Glycans workflow, the Find Glycans algorithm runs and uses the **Target glycan source** selected in the Workflow and Sequences section.

Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**ReleasedGlycans_InstantPC.m** folder. **c** Click **Open**.	• This method was created in Exercise 5 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	**a** If the Method Editor is not visible, click **View > Method Editor**. **b** Click **Method Automation > Workflow and Sequences** in the Method Editor window.	• You can instead click the Method Editor button, , on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Released Glycans** for the Workflow. **b** Enter `Example` in the **Glycan group**. **c** Select **Glycans_mAb_AM_PCD.cdb** as the **Target glycan source**. **d** Clear the **Require RT match if database contains a RT for the target glycan** check box.	• The Released Glycans workflow automatically runs the Find Glycans algorithm. • The Glycan group is used to organize the results in the Results Compare window.
4 Save the method.	• Click **Method > Save**. • If needed, enter a reason for the change in the **Reason for Change** dialog box.	• The administrator can set up a project to require you to enter a reason for a change to a method



Steps	Detailed Instructions	Comments
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar when the Workflow and Sequences section is showing. • Reprocess the sample. See **"Exercise 11. Reprocess Samples"** on page 62.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results. • The Workflow column is set to "Released Glycans". The Relative Quantitation Histograms window and the Results Compare window contain results.
6 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box. • When you run one of the workflows, the **Workflow** column is updated in the Sample Table. If you entered a Glycan Group You can also see the **Glycan Group** column in the Sample Table.

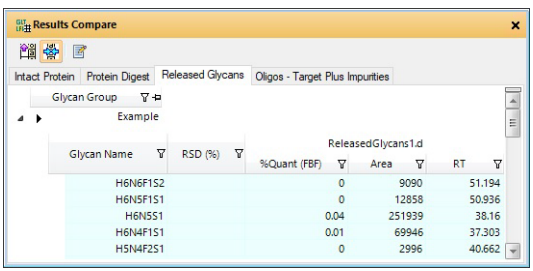
Steps	Detailed Instructions	Comments



7 Review the results in the Results Compare window.

a Click the **Results Compare** window.
b Click the **Released Glycans** tab.
c Note that RSD (%) is empty because only one sample is selected. If you select two or more samples and they belong to the same Glycan Group, then the results are shown in the same table. If the same glycan is in multiple samples, then the RSD (%) is calculated.

- For Released Glycans workflow, the software uses the **Use for %Quant** check box in the Biomolecules window.
- The Results compare window is tabbed with the Relative Quantitation Histograms window.



Steps	Detailed Instructions	Comments
8 Save the results for the current data file and close all data files.	**a** Click **File > Save Results**. **b** If needed, enter or select a reason in the **Reason for Change** dialog box. Click **OK**. **c** Click **File > Close All**. **d** Click **No** to save results.	• You need to save results as you process your data files. You can only save results that have been processed with the current method. If you change the method after generating results, then you need to generate results again.

Oligonucleotide Workflow - Target Plus Impurities

The steps outlined below show the workflow for Oligonucleotides with the Target Plus Impurities experiment with MassHunter BioConfirm.

Step 1 - Open the data file of interest and select the **Oligos - Target Plus Impurities** layout.

Step 2 - Open a BioConfirm method or create a new one.

Step 3 - Edit oligonucleotide sequences if necessary in the Sequence Manager program:

- Add or edit the sequence text

Step 4- Select **Oligonucleotides** as the **Workflow** on the **Workflow and Sequences** tab.

Step 5- Select **Target Plus Impurities** as the **Experiment.**

Step 6- Select the sequence or mass that you want to use.

If the sequence you want to select is not in the method or Select Sequences dialog box, then Import or create an oligonucleotide sequence.

Step 7 - Select the **Mods and Profiles** on the Workflow and Sequences tab.

Step 8 - Select the **Matching Rules**.

Step 9 - Run the Method Workflow.

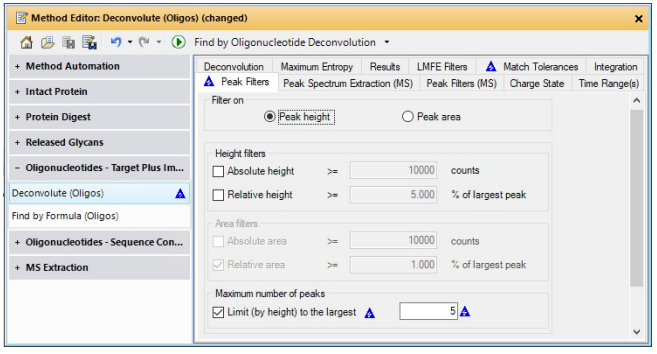
Step 10 - Review the results which are shown in these windows:
Sample Chromatogram Results
Biomolecule MS Chromatogram
Biomolecules table
Biomolecule Identification Results
Biomolecule MS Spectrum
Deconvolution Results
Oligos - Impurity List
Results Compare
Relative Quantitation Histograms

Step 11 - Print report.

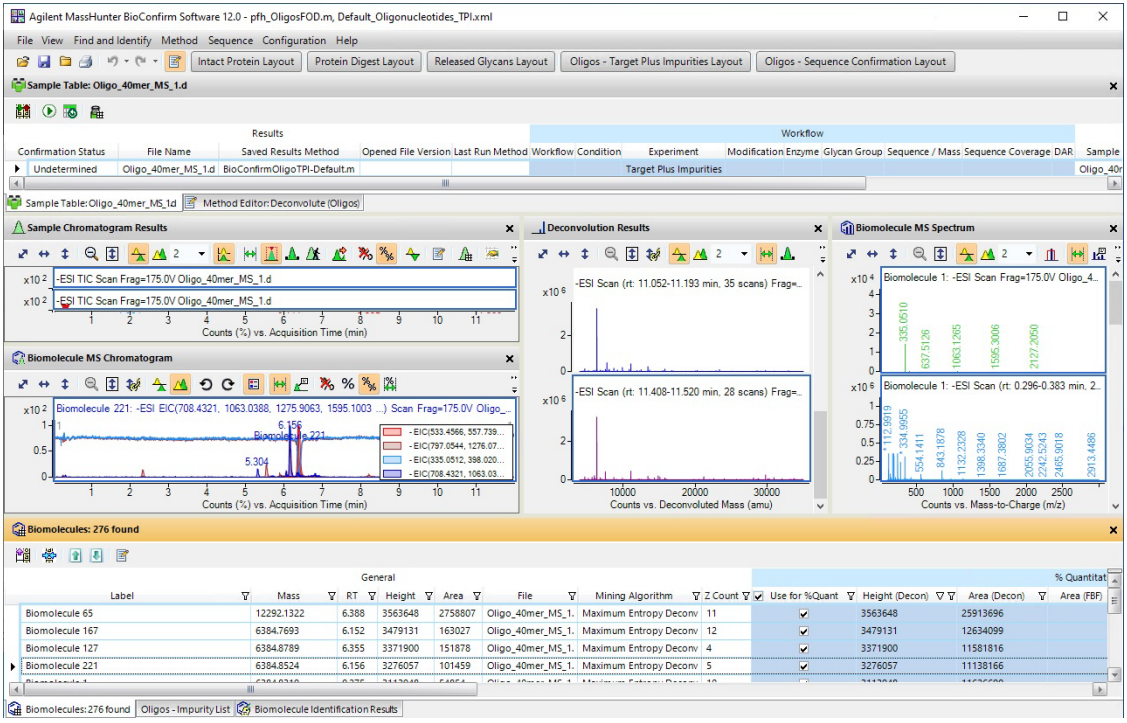
Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

This exercise shows you how to find oligonucleotides interactively with the Target Plus Impurities experiment. If you select the Oligonucleotides workflow with the Target Plus Impurities experiment, the **Find by Oligonucleotide Deconvolution** algorithm or the **Find by Formula (Oligos)** algorithm runs. See **"Before you start"** on page 3. You can run the **Match Sequences** algorithm on results from Find by Oligonucleotide Deconvolution, but that is not shown in this exercise.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmOligosTPI-Default.m** folder. **c** Click **Open**.	• The parameters in the BioConfirmOligosTPI-Default.m method are a good starting point for the Target Plus Impurities experiment.
2 Open the example sample file.	**a** If needed, click **File > Open Data File**. **b** Select **Oligo_40mer_MS_1.d**. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Target Plus Impurities layout.	• Click **Oligos - Target Plus Impurities Layout** in the main toolbar.	
4 Review the parameters in the Oligonucleotides - Target Plus Impurities > Deconvolute (Oligos) section in the Method Editor window.	**a** Select **Oligonucleotides - Target Plus Impurities > Deconvolute (Oligos)** in the Method Editor window. **b** Click the **Match Tolerances** tab. **c** Select **Da** and enter **1.0** for the **Tolerance**. **d** Click the **Peak Filters** tab. **e** Mark **Limit (by height)** to the largest and enter **5**.	

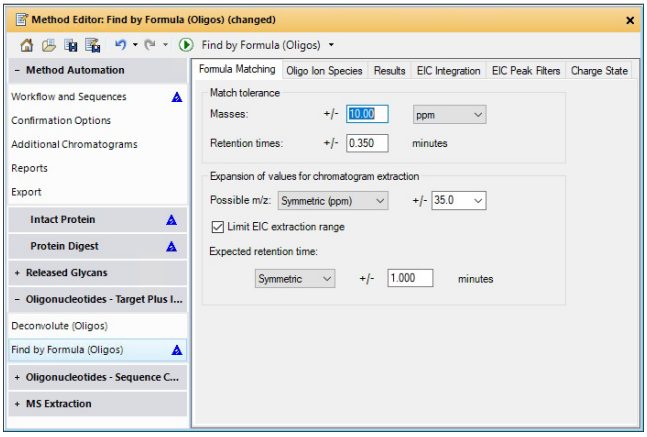


Steps	Detailed Instructions	Comments
5 Run the Find by Oligonucleotide Deconvolution algorithm.	**a** Click on the Method Editor toolbar to start the biomolecule search. **b** When processing is complete, review the results in the Biomolecules window.	
6 Save the method.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_OligosFOD.m, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	
7 Review the results.	• When you run the Find by Oligonucleotide Deconvolution algorithm, the Oligos - Impurity List and the Biomolecule Identification Results window are empty.	• Several columns have been changed from the default Oligos - Target Plus Impurities Layout.

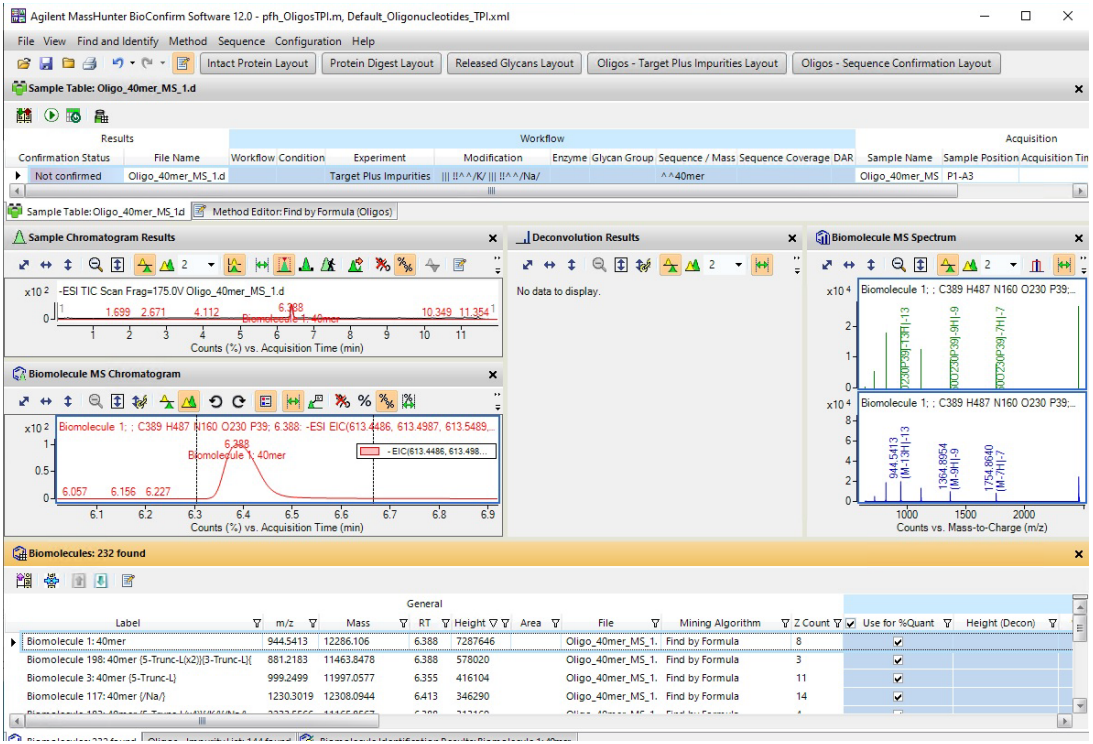


8 Save the results.	**a** Click **File > Save Results** to save your results to the data file folder. **b** If needed, enter a reason for the change.	• You can also click to save results.

Steps	Detailed Instructions	Comments
9 Select parameters in the Method Automation > Workflow and Sequences section in the Method Editor window. • Select 40mer as the Sequence. • Select /K/ and /Na/ for the Mods and Profiles. •	**a** Select **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Select **Oligonucleotides**. **c** Select **Target Plus Impurities** as the **Experiment**. **d** Click the … button next to the **Sequences/Masses** parameter. The **Select Sequences** dialog box opens. **e** Double-click **40mer**. If **40mer** is not available, click **Add**. **f** Select **Oligo_40mer_MS.psq** and click **Open**. **g** Verify that the 40mer sequence is in the **Selected** list. **h** Click **OK**. **i** Click the … button next to the **Mods and Profiles** parameter. **j** Select **/K/** and **/Na/** from the **Available** list. **k** Click **OK**. **l** Click … next to **Matching Rules**. **m** Mark **5'-Truncation with linker** and **3'-Truncation with linker**. Enter **5** for **Maximum allowed** for each. Click **OK**.	• You need to select a sequence before you can run the Find by Formula (Oligos) algorithm. • You need to be very selective with the choices for the Mods and Profiles and for the Matching Rules. These choices can limit the size of the database and significantly affect the processing time.
10 Review the parameters in the Oligonucleotides - Target Plus Impurities > Find by Formula (Oligos) section in the Method Editor window.	**a** Select **Oligonucleotides - Target Plus Impurities > Find by Formula (Oligos)** in the Method Editor window. **b** Review the parameters.	• You can limit the number of biomolecules found by adding filters.



Steps	Detailed Instructions	Comments
11 Run the Find by Formula (Oligos) algorithm.	**a** Click on the Method Editor toolbar to start the biomolecule search. **b** When processing is complete, review the results in the Biomolecules window.	• You can instead click **Find and Identify > Find by Formula (Oligos)**.
12 Save the method for use in Exercise 8.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_OligosTPI.m, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	• 4 compounds and 2 impurities were found when running the Find by Formula (Oligos) algorithm.



13 Save the results.	**a** Click **File > Save Results** to save your results to the data file folder. **b** If needed, enter a reason for the change.	• You can also click to save results.

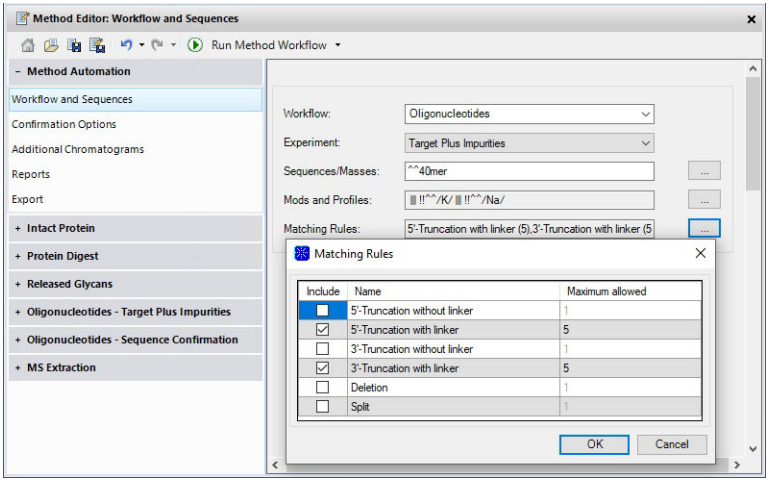
Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

This exercise guides you through the setup of a worklist to automatically run the Oligonucleotides - Target Plus Impurities workflow.

If you select the Oligonucleotides - Target Plus Impurities workflow, the **Find by Oligonucleotide Deconvolution** algorithm or the **Find by Formula (Oligos)** algorithm runs.

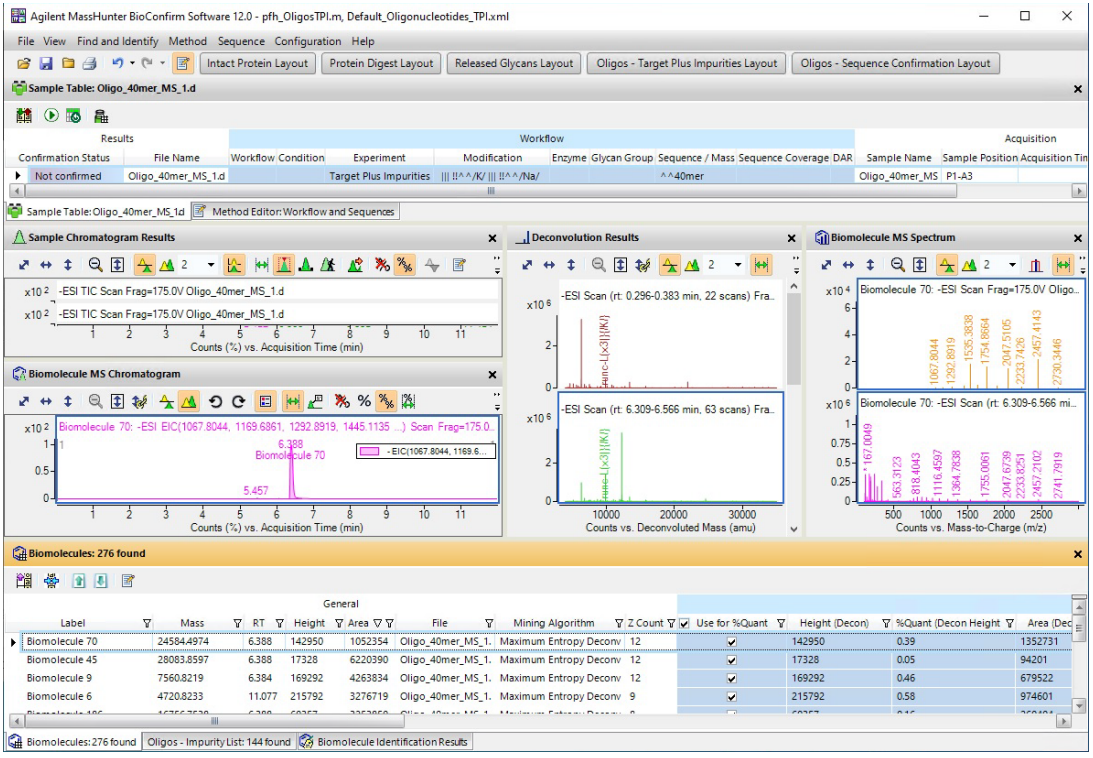
Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**OligosTPI.m** folder. **c** Click **Open**.	• This method was created in Exercise 7 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	**a** If the Method Editor is not visible, click **View > Method Editor**. **b** Click **Method Automation > Workflow and Sequences** in the Method Editor window.	• You can instead click the Method Editor button,  , on the main toolbar.

Steps	Detailed Instructions	Comments
3 Select the appropriate workflow.	**a** Select **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Select **Oligonucleotides**. **c** Select **Target Plus Impurities** as the **Experiment**. **d** Click the ... button next to the **Sequences/Masses** parameter. The **Select Sequences** dialog box opens. **e** Double-click **40mer**. If **40mer** is not available, click **Add**. **f** Select **Oligo_40mer_MS.psq** and click **Open**. **g** Verify that the 40mer sequence is in the **Selected** list. **h** Click **OK**. **i** Click the ... button next to the **Mods and Profiles** parameter. **j** Select **/K/** and **/Na/** from the **Available** list. **k** Click **OK**. **l** Click ... next to **Matching Rules**. **m** Mark **5'-Truncation with linker** and **3'-Truncation with linker**. **n** Enter **5** for **Maximum allowed** for each. **o** Click **OK**.	• The **Matching Rules** parameter shows the rules that are marked, and it shows the **Maximum allowed** in parentheses after the rule.



Steps	Detailed Instructions	Comments
4 Verify Confirmation Options.	**a** Select **Method Automation > Confirmation Options** in the Method Editor window. **b** Click the **Oligonucleotides** tab. **c** Review the **Purity confirmation** values. **d** Verify that the **Workflow transition** is set correctly.	• The Target Plus Impurities experiment switches between two algorithms at the mass that is selected in this tab. See the online Help for more information.
5 Save the method.	• Click **Method > Save**. • If needed, enter a reason for the change in the **Reason for Change** dialog box.	• The administrator can set up a project to require you to enter a reason for a change to a method
6 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar when the Workflow and Sequences section is showing. • Reprocess the sample. See **"Exercise 11. Reprocess Samples"** on page 62.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results. • The Workflow column is set to "Oligonucleotides". The Oligos - Impurity List window, the Deconvolution Results window, and the Results Compare window contain results.
7 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box. • When you run one of the workflows, the **Workflow** column is updated in the Sample Table. You can also see the **Experiment** column in the Sample Table.

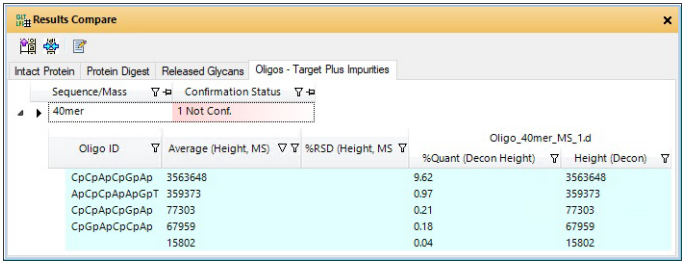
Steps	Detailed Instructions	Comments



8 Review the results in the Results Compare window.

a Click the **Results Compare** window.
b Click the **Oligos - Target Plus Impurities** tab.
c Note that RSD (%) is empty because only one sample is selected. If you select two or more samples and they use the same sequence, then the results are shown in the same table.

- For Oligonucleotides - Target Plus Impurities workflow, the software uses the **Use for %Quant** check box in the Biomolecules window.



Steps	Detailed Instructions	Comments
9 Save the results for the current data file and close all data files.	**a** Click **File > Save Results**. **b** If needed, enter or select a reason in the **Reason for Change** dialog box. Click **OK**. **c** Click **File > Close All**. **d** Click **No** to save results.	• You need to save results as you process your data files. You can only save results that have been processed with the current method. If you change the method after generating results, then you need to generate results again. • If you try to save results, you may get this message "Result set exceeds operating system limits." You get this message if you have too many biomolecules or impurities. See the online Help for more information.

Oligonucleotide Workflow - Sequence Confirmation

The steps outlined below show the workflow for Oligonucleotides with the Sequence Confirmation experiment with MassHunter BioConfirm.

Step 1 - Open the data file of interest and select the Oligonucleotide - Sequence Confirmation layout.

Step 2 - Open a BioConfirm method or create a new one.

Step 3 - Edit oligonucleotide sequences if necessary in the Sequence Manager program:

- Add or edit the oligonucleotide sequence text
- Apply or edit modifications

Step 4- Select **Oligonucleotides** as the **Workflow** on the **Workflow and Sequences** tab.

Step 5- Select **Sequence Confirmation** as the **Experiment.**

Step 6- Select the sequence or sequences that you want to use.

If the sequence you want to select is not in the method or Select Sequences dialog box, then Import or create an oligonucleotide sequence.

Step 9 - Run the Method Workflow.

Step 10 - Review the results which are shown in these windows:
Sample Chromatogram Results
Biomolecules table
Biomolecule Identification Results
Biomolecule Fragment Spectrum
Fragment Confirmation Ladder

Step 11 - Print report.

Exercise 9. Interactive Oligonucleotides - Sequence Confirmation Workflow

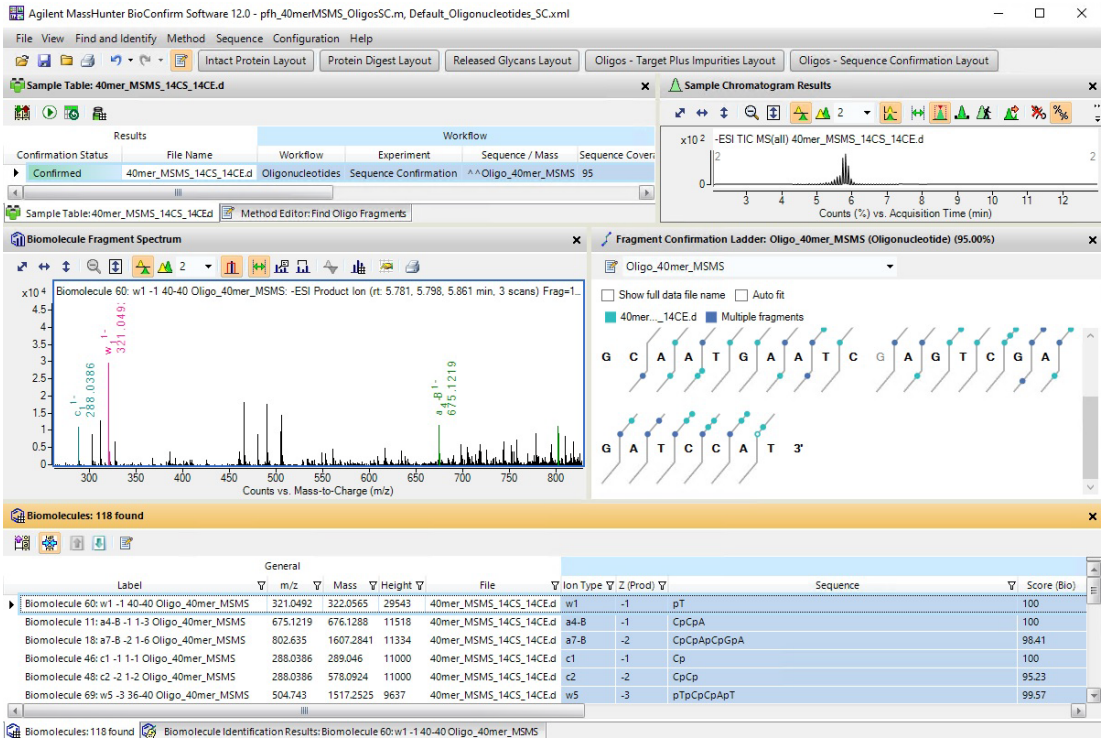
This exercise shows you how to find oligonucleotides interactively with the Sequence Confirmation experiment.

If you select the Oligonucleotides workflow with the Sequence Confirmation experiment, the **Find Oligonucleotides Fragments** algorithm runs. See **"Before you start"** on page 3.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select the **BioConfirmOligosSC-Default.m** folder. **c** Click **Open**.	• The parameters in the BioConfirmOligosSC-Default.m method are a good starting point for the Sequence Confirmation experiment.
2 Open the example sample file.	**a** If needed, click **File > Open Data File**. **b** Select **40mer_MSMS_14CS_14CE.d**. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Oligonucleotides - Sequence Confirmation layout.	• Click **Oligonucleotides - Sequence Confirmation Layout** in the main toolbar.	
4 Review the parameters in the Find Glycans section in the Method Editor window.	**a** Select **Method Automation > Workflow and Sequences** in the Method Editor window. **b** Select **Oligonucleotides**. **c** Select **Sequence Confirmation** as the **Experiment**. **d** Click the ... next to the **Sequences** parameter. **e** Select **Oligo_40mer_MSMS** and click **OK**. **f** Select **Oligos - Sequence Confirmation > Find Oligo Fragments** in the Method Editor window. **g** Review parameters on each tab.	• You can change the default parameters as described in the next steps. • For some data files, you will need to use different parameters as described in the *online Help*.
5 Run the Find Oligonucleotide Fragments algorithm.	**a** Click  on the Method Editor toolbar to start the biomolecule search. **b** When processing is complete, review the results in the Biomolecules window.	• You can instead click **Find and Identify > Find Oligonucleotide Fragments**. • By default, all results are selected after you run this algorithm.

Steps	Detailed Instructions	Comments
6 Save the method for use in Exercise 10.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_40merMSMS_OligosSC.m, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason on the **Reason for Change** dialog box. Click **OK**.	

Steps	Detailed Instructions	Comments
7 Review the results.	**a** In the Biomolecules window, click the header of the **Height** column to sort the table by this column. If necessary, click the header again so that the largest heights are at the top of the table. **b** Select the first biomolecule in the Biomolecules window. **c** Click the **Biomolecule Identification Results** tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window. **d** When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window.	• Several changes were made to the default layout for the image below. Several columns were hidden in the Sample Table, and **Auto fit** was cleared in the Fragment Confirmation Ladder. • The Fragment Confirmation Ladder shows the fragments for each of the nucleosides in the selected sequence. The selected biomolecule is shown as an empty circle on one of the limbs in the ladder. • See the online Help for more information on this window.



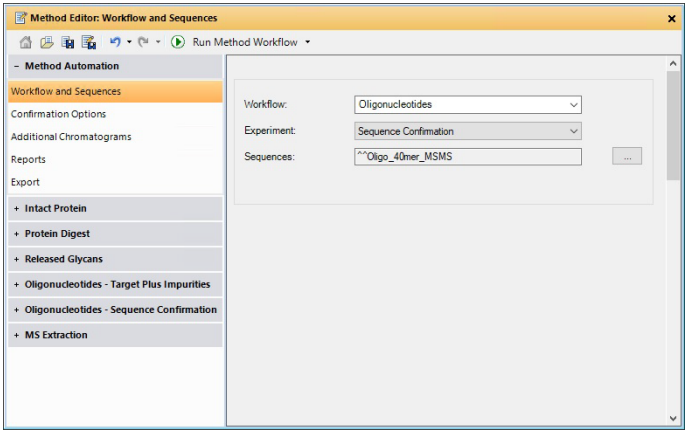
8 Save the results	**a** Click **File > Save Results** to save your results to the data file folder. **b** If needed, enter a reason for the change.	• You can also click to save results.

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

This exercise guides you through the setup of a worklist to automatically run the Oligonucleotides - Sequence Confirmation workflow.

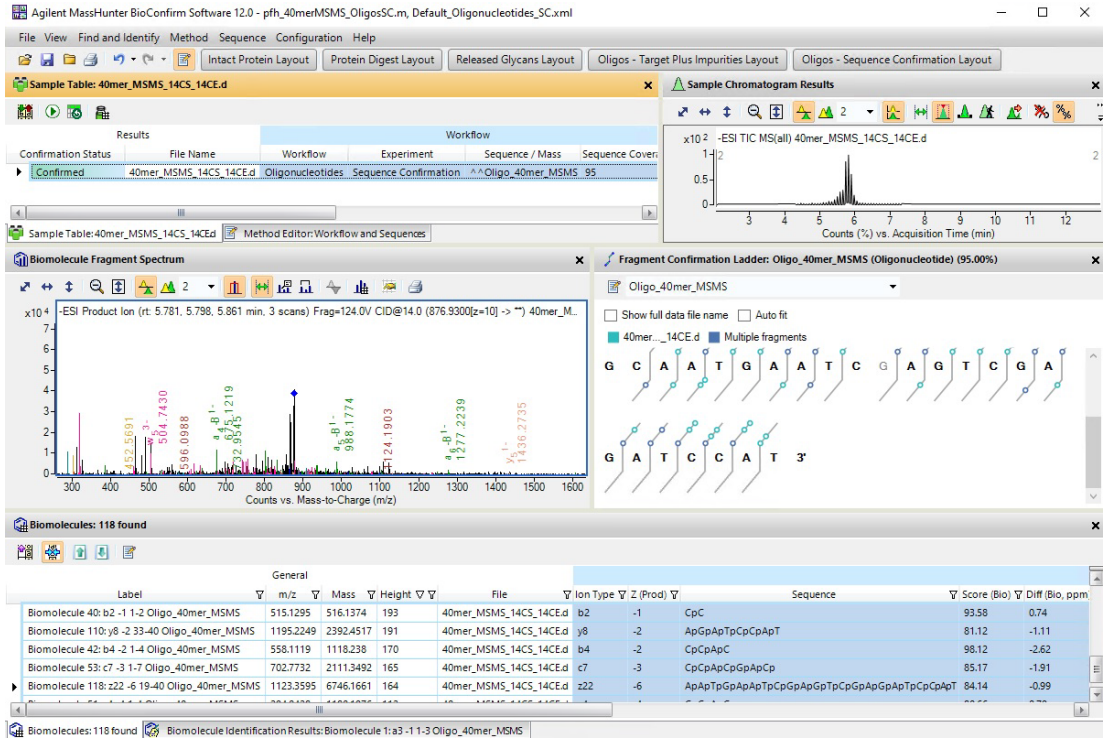
If you select the Oligonucleotides - Sequence Confirmation workflow, the **Find Oligonucleotide Fragments** algorithm runs.

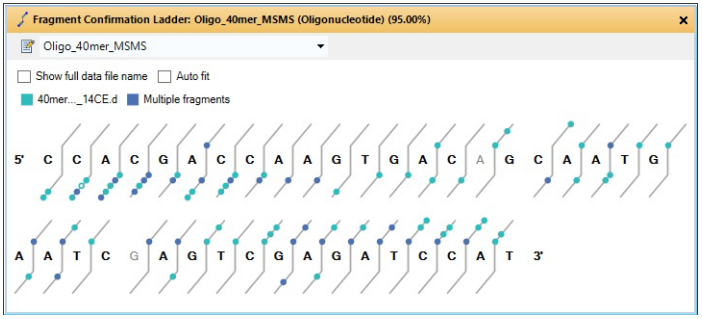
Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**40merMSMS_OligosSC.m** folder. **c** Click **Open**.	• This method was created in Exercise 9 (*iii* represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	**a** If the Method Editor is not visible, click **View > Method Editor**. **b** Click **Method Automation > Workflow and Sequences** in the Method Editor window.	• You can instead click the Method Editor button,  , on the main toolbar.
3 Select the appropriate workflow.	**a** Select **Oligonucleotides** for the Workflow. **b** Select **Sequence Confirmation** for the Experiment. **c** Click the ... next to the **Sequences** parameter. **d** Select **Oligo_40mer_MSMS** and click **OK**.	• You cannot enter a mass if you select the **Sequence Confirmation** experiment.
4 Save the method.	• Click **Method > Save**. • If needed, enter a reason for the change in the **Reason for Change** dialog box.	• The administrator can set up a project to require you to enter a reason for a change to a method



Steps	Detailed Instructions	Comments
5 Run the method workflow or run method automation.	• Click **Method > Run Method Workflow**. • Click **Method > Run Method Automation (Workflow + Reports)**. • Click  on the Method Editor toolbar when the Workflow and Sequences section is showing. • Reprocess the sample. See **"Exercise 11. Reprocess Samples"** on page 62.	• Method Automation first runs the method workflow and then extracts additional chromatograms, generates a biomolecule report, and exports results. • The Workflow column is set to "Oligonucleotides", and the Experiment is set to "Sequence Confirmation".
6 (optional) Review the printed Biomolecule reports.	• If you clicked **Run Method Automation (Workflow + Reports)**, then a report is generated automatically. • You can click **File > Print > Biomolecule Report** to generate a report for the current sample.	• You set report options in the Method Editor window in the Method Automation > Reports section. • If you are creating a report interactively, you specify some of these parameters in the **Print Biomolecule Report** dialog box. • When you run one of the workflows, the **Workflow** column is updated in the Sample Table.

Steps	Detailed Instructions	Comments



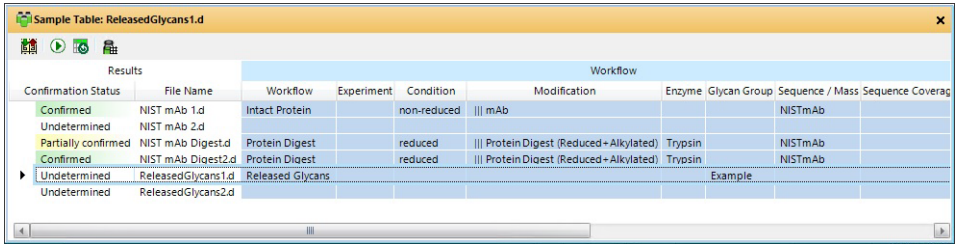
Steps	Detailed Instructions	Comments
7 Review the results in the Fragment Confirmation Ladder window.	**a** Click the **Fragment Confirmation Ladder** window. • If a nucleotide has a fragment that confirms it, the nucleotide is shown in black. If no fragment confirms the nucleoside, it is shown in gray. • If no fragments are on a line between two nucleosides, the line is not drawn. • Each line can have 9 possible dots to represent each of the fragment types expected to be found at each location. • The dots on the bottom limb apply to the nucleoside to the left of the line. • The dots on the upper limb apply to the nucleoside to the right of the line. • If a fragment is found in the results, a dot appears at the correct location on the fragment confirmation ladder. • The selected biomolecule or biomolecules in the Biomolecule table are displayed as an open circle in the Fragment Confirmation Ladder. • If multiple data files are selected, the dots are shown in different colors. • If you hover over a fragment dot, a tooltip shows you which biomolecules show that fragment.	• If you mark the **Auto fit** check box, all of the nucleosides are visible in one row, if possible. • If you select multiple data files that were processed with the same sequence, the results of all the selected data files are shown in the Fragment Confirmation Ladder (up to a maximum of 10 data files). The dots for each data file are shown on the same fragment confirmation ladder (one color for each data file). If the same fragment is confirmed in multiple data files, then the dot has the color for **Multiple fragments**. • The percentage shown in the title bar for the window shows the cumulative results for all selected data files.
		
8 Save the results for the current data file and close all data files.	**a** Click **File > Save Results**. **b** If needed, enter or select a reason in the **Reason for Change** dialog box. Click **OK**. **c** Click **File > Close All**. **d** Click **No** to save results.	• You need to save results as you process your data files. You can only save results that have been processed with the current method. If you change the method after generating results, then you need to generate results again.

Review Results

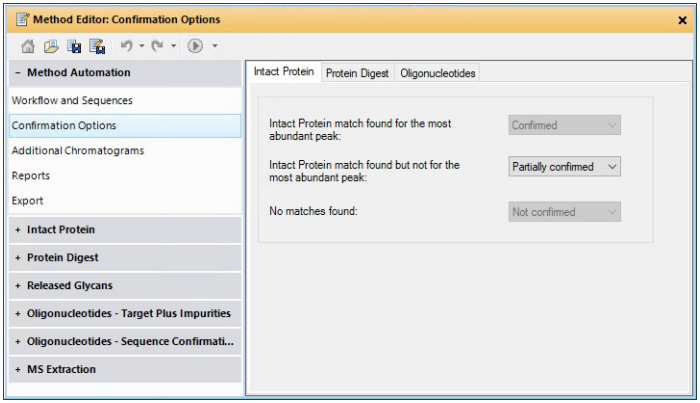
Exercise 11. Reprocess Samples

This exercise shows you how to reprocess samples in the Sample Table. You can quickly check the Confirmation Status of each sample and determine if you need to reprocess the sample.

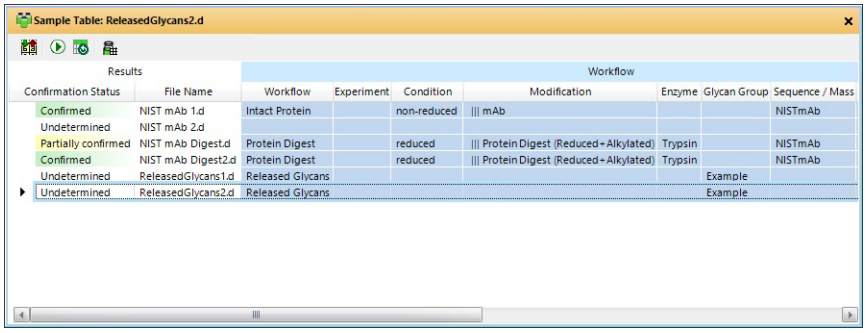
Steps	Detailed Instructions	Comments
1 Open several data files.	**a** Click **File > Open Data File**. **b** Select these example files: **NIST mAb 1.d**, **NIST mAb 2.d**, **NIST mAb Digest.d**, **NIST mAb Digest2.d**, **ReleasedGlycans1.d**, and **ReleasedGlycans2.d**. **c** Mark the **Load result data** check box. **d** Click **Open**.	• To select multiple files, click the first file. Then, press **Shift** and click the last file. • In **Networked Workstation** mode, you can select which version of the results to open when you open a data file. See **"Exercise 18. Open results using versions"** on page 88.
2 Review results in the Sample Table window.	**a** Look at the **Confirmation Status** column.	• If you saved results, the table contains information on confirmation.



Steps	Detailed Instructions	Comments
3 Review values in Method Automation > Confirmation Options.	**a** Click **View > Method Editor**, if necessary. **b** Select **Method Automation > Confirmation Options**. **c** Click the **Intact Protein** tab. **d** Review selection for the **Intact Protein match found but not for the most abundant peak** option. **e** Click the **Protein Digest** tab. **f** Review selection for the **Protein is partially confirmed when sequence coverage is >=** option.	• These tabs explain what it means to be **Confirmed** and **Partially confirmed**. • You are not changing these options. You are only seeing what the software checks to determine if the protein is confirmed. • The Confirmation Status is always "Undetermined" if the Workflow is **Released Glycans**. • The Oligonucleotides tab will be reviewed in a different exercise.



Steps	Detailed Instructions	Comments
4 Reprocess the ReleasedGlycans2.d data file.	**a** In the Sample Table, click the row containing ReleasedGlycans2.d. **b** Click **Method > Open**. **c** Select the *iii*_**ReleasedGlycans-InstantPC.m** folder. **d** Click **Open**. **e** Click the  button to open the **Reprocess Sample** dialog box. **f** Select **Released Glycans** for the workflow. **g** Enter `Example` for the **Glycan group**. **h** Select **Glycans_mAb_AM_PCD.cdb** for the **Target glycan source**. **i** Click **Reprocess**.	• To reprocess a sample, you need to first load the correct method and then complete the Reprocess Sample dialog box. • You can also double-click the Sample Table row to open the **Reprocess Sample** dialog box. • You can either use the current method, or if you have previously saved results, you can use the sample result method.

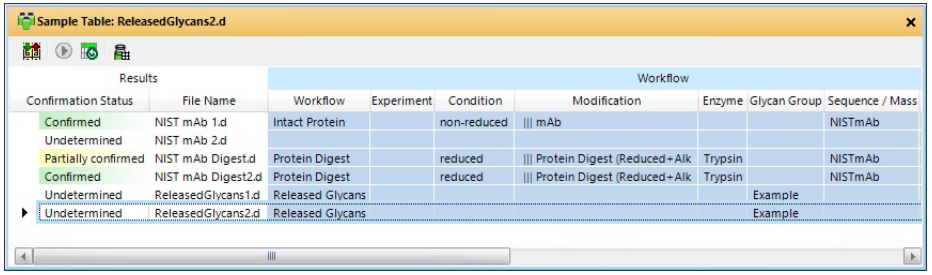


5 Save the results for the samples that you reprocessed.	**a** Click **File > Save Results**. **b** Click **Save**. **c** If needed, enter or select a reason in the **Reason for Change** dialog box. Click **OK**.	

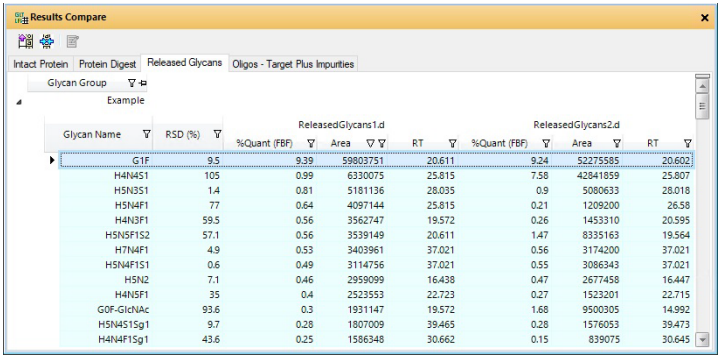
Exercise 12. Use Result Review mode

This exercise shows you how to use the Result Review mode. When this mode is enabled, you cannot edit a method. You also cannot run the algorithms in the Find and Identify menu.

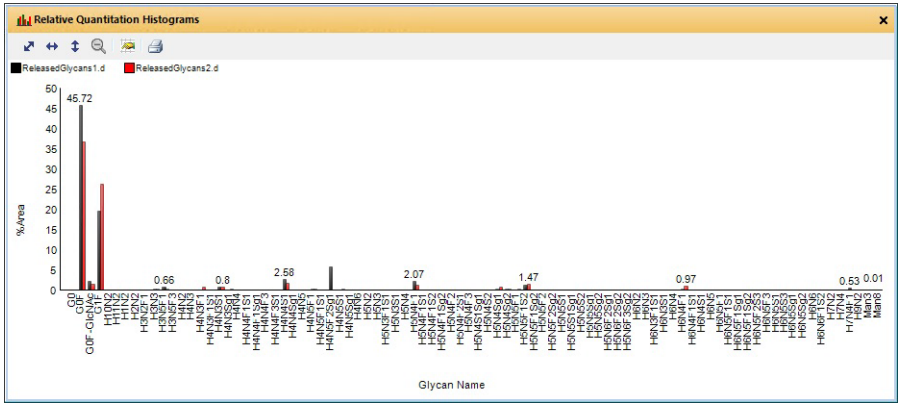
Steps	Detailed Instructions	Comments
1 Enable Result Review mode.	• Click **Configuration > Enable Result Review (Disables Method Editing)**.	• You can toggle this mode off by clicking this same command again.
2 Review results in Sample Table window.	• All of the options in this window are available except for the **Run Method Workflow** button. You can still reprocess samples.	



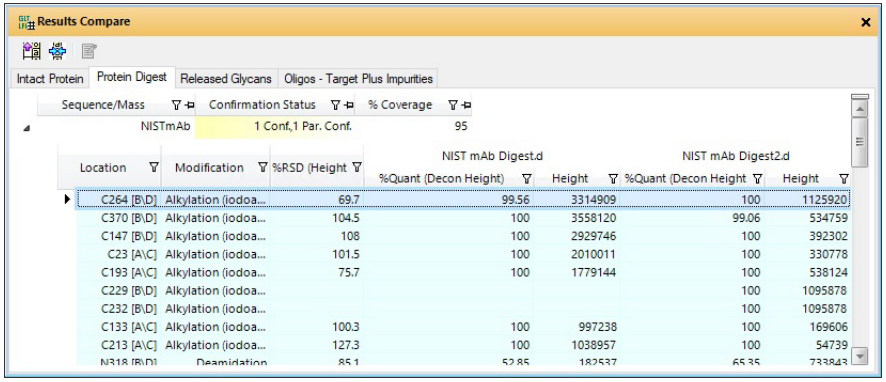
Steps	Detailed Instructions	Comments
3 Review and compare the *ReleasedGlycans1.d* and *ReleasedGlycans2.d* data files.	**a** In the Sample Table, click the row containing ReleasedGlycans1.d. **b** Press the **Shift** key and click the row containing ReleasedGlycans2.d. **c** Review the results in the Results Compare window. The RSD (%) column has been calculated for glycans that are in both samples.	



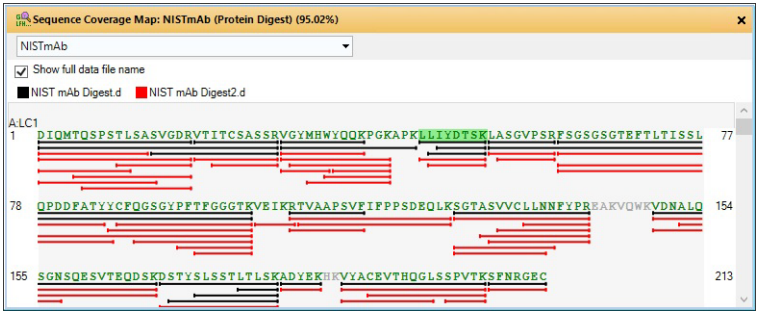
	d Review the Relative Quantitation Histograms window.	• You can visually compare the relative quantitation results for different glycans. • Right-click and drag on either axis to zoom in on that axis. • Click and drag to scroll along the axis after you have zoomed.



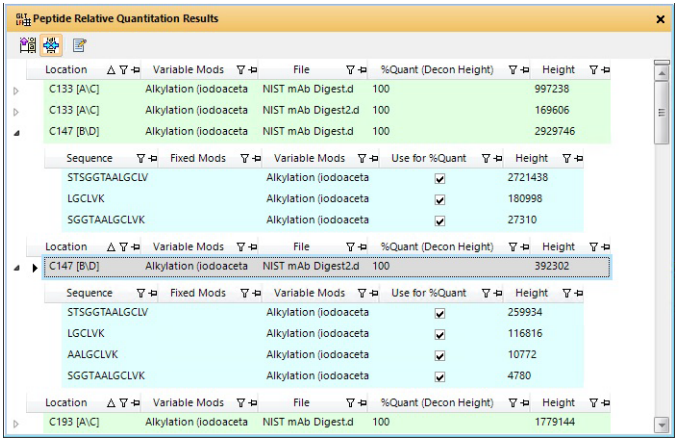
Steps	Detailed Instructions	Comments
4 Review and compare the *NIST mAb Digest.d* and *NIST mAb Digest2.d* data files.	**a** In the Sample Table, click the row containing NIST mAb Digest.d. **b** Press the **Shift** key and click the row containing NIST mAb Digest2.d. **c** Click the **Protein Digest Layout** button in the main toolbar. **d** Review the results in the Results Compare window. The RSD (%) column has been calculated for many modifications that are in both samples.	• The Results Compare window is not visible by default in the Protein Digest layout. Click **View > Results Compare** to open the window.



	e Review the results in the Sequence Coverage Map window. A legend is added to the top of the window and the lines under the sequence are color coded to show which sample is described.	• See the online Help for information on the meaning for the text and for the lines.



Steps	Detailed Instructions	Comments
	f Review the results in the Peptide Relative Quantitation Results window. The Location C147 [B/D] has multiple rows because this location is in both samples.	• For Protein Digest workflows, you mark the **Use for %Quant** check box in the Peptide Relative Quantitation Results window.



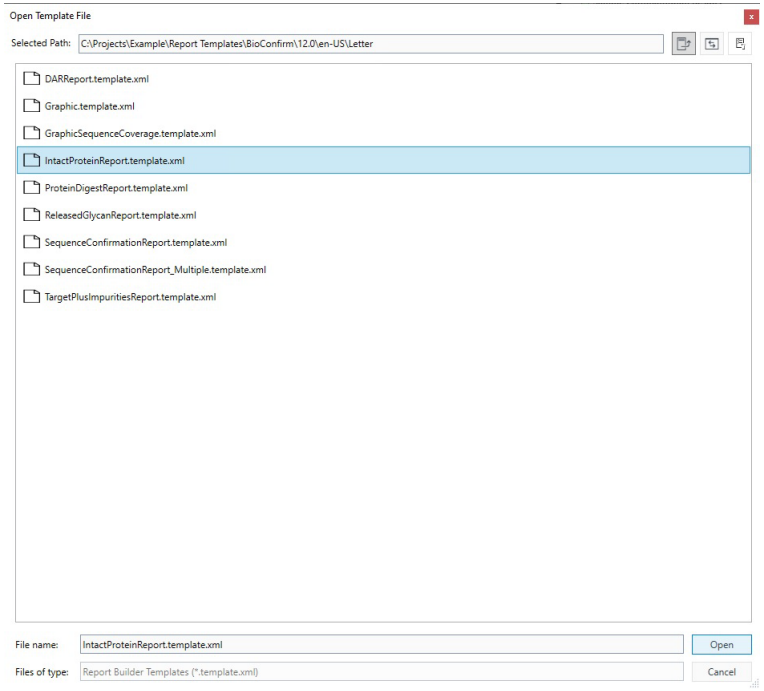
Other Tasks

Exercise 13. Use Report Builder

This exercise shows you the program that you use to modify report templates. You can use Report Builder to modify the templates used to generate a Biomolecule report.

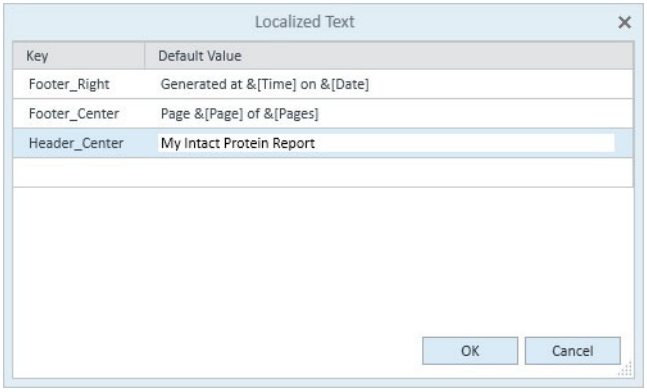
Steps	Detailed Instructions	Comments
1 Open Report Builder program.	**a** Double-click the **Control Panel** icon on the desktop. **b** Click **Projects** in the left pane. **c** Select a project. **d** On the ribbon in the **Report Builder** group, click **Report Builder > Start Report Builder**. Or you can do the following: **a** Click **Agilent MassHunter Report Builder > Report Builder 11.1**. **b** Select a project to use. **c** Click **OK**.	• You can also start the Report Builder program when you click one of the **Edit** button next to a template in the Method Automation > Reports > Template tab. These **Edit** buttons are only available if you click **Configuration > Show Advanced Settings**.

Steps	Detailed Instructions	Comments
2 Open an existing template.	**a** Click **File > Open > Browse**. **b** Select **IntactProteinReport.template.xml** and click **Open**. **a** Click **File > Save As > Browse**. **b** Enter a file name and click **Save**. **c** If needed, enter a reason and click **OK**.	• Report templates are installed in the **Report Templates\BioConfirm** folder in the project.

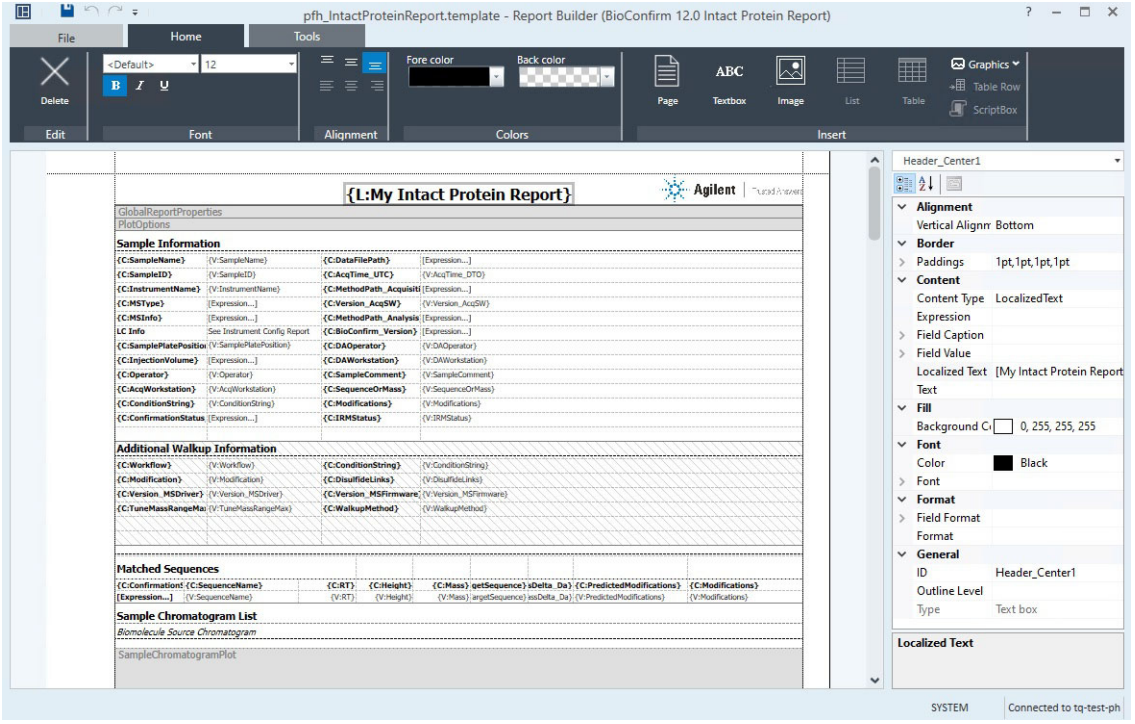


Agilent recommends that you do not modify the default templates. Instead, make a copy of the template and modify the copy.

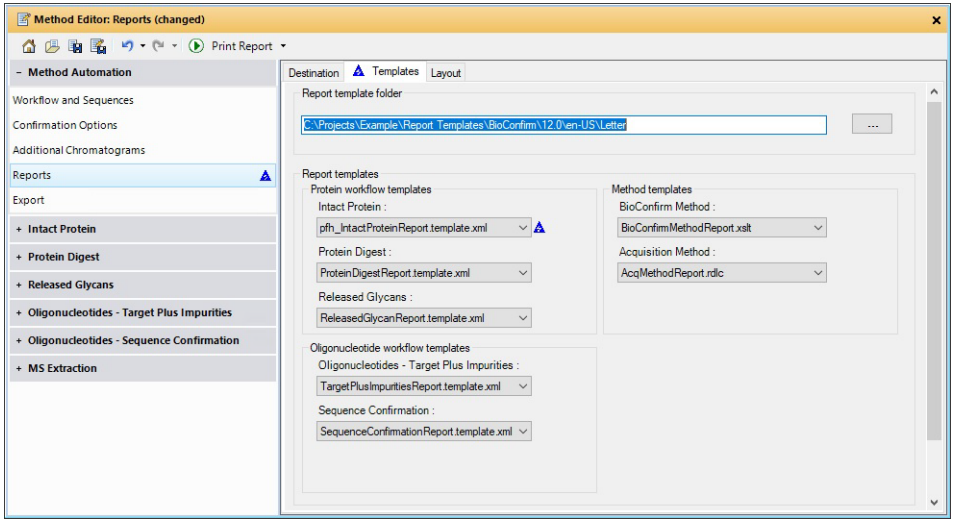
Steps	Detailed Instructions	Comments
3 Review the template in Report Builder.	**a** Click an item in the template. Notice that the right pane changes. **b** Click the title of the report. **c** In the right pane, click **Localized Text** in the Content section. **d** Click the … button. The **Localized Text** dialog box opens. **e** Click the **Header_Center**. **f** Enter **My Intact Protein Report**. **g** Click **OK**.	• The left pane shows the template. The right pane shows the parameters for the current selection. • You can make many different changes to the report. This exercise only shows you one possibility. Press F1 to access the online Help to learn more about customizing a report template.



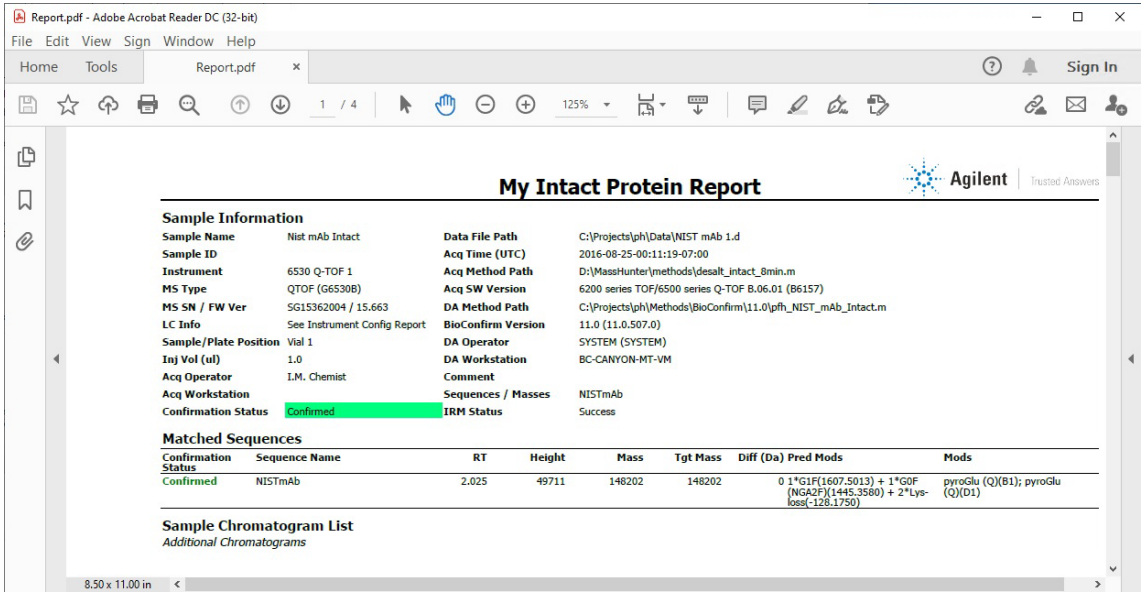
Steps	Detailed Instructions	Comments
4 Save the template.	**a** Click **File > Save**. **b** If needed, enter a reason and click **OK**. **c** Close the **Report Builder** program.	• You can instead click **File > Save**, and the file is saved to the current report template. Agilent recommends that you do not modify the default templates.



Steps	Detailed Instructions	Comments
5 Use this new template in a method.	**a** Open the Method Editor window. Click **View > Method Editor** if it is not visible. **b** Select Method Automation > Reports. **c** Click the **Templates** tab. **d** Select the changed report for the corresponding report template type. In this example, the **Intact Protein** report template was modified.	• Different reports use different report templates. If you modified an Intact Protein report template, then you select the modified template for the **Intact Protein** report template. • When you print a biomolecule report, the report template corresponding to the selected workflow is used. • If the modified template is not shown in the list, you may need to switch to a different section in the Method Editor window and return. This action usually refreshes the list of report templates.

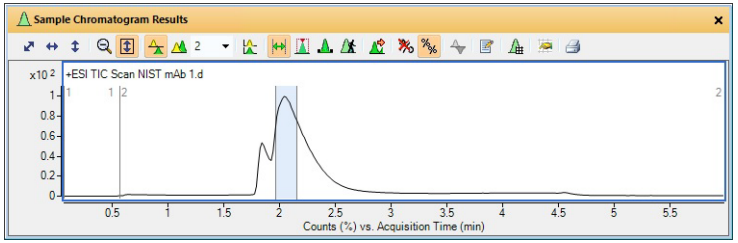
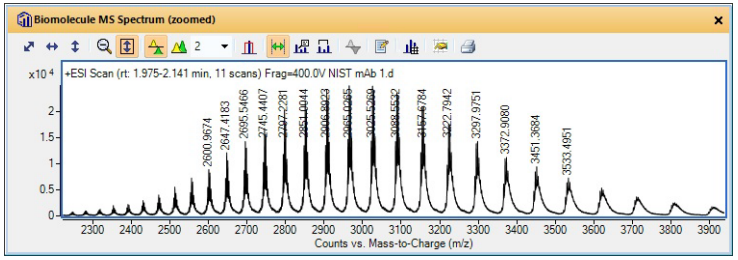


Steps	Detailed Instructions	Comments
6 Print a Biomolecule report.	**a** Click **File > Print > Biomolecule Report**. **b** Select a data file that was processed with the Intact Protein workflow. **c** Mark the **Print preview** check box. **d** Click **OK**.	• When you print a biomolecule report, the report template corresponding to the selected workflow is used.

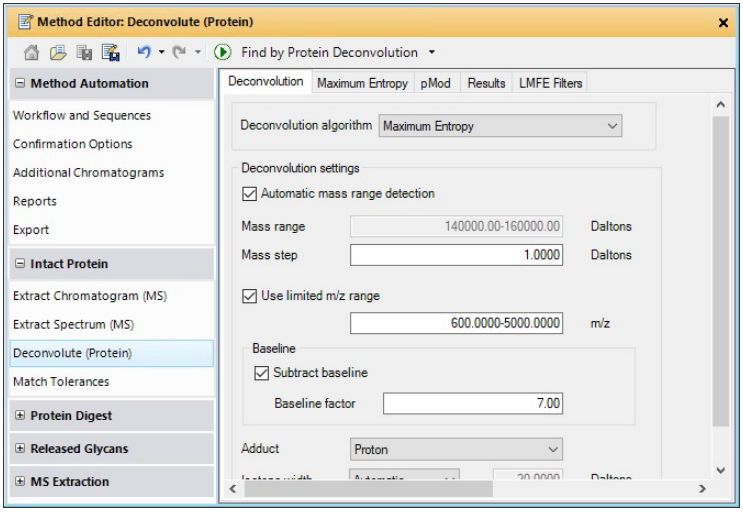


Exercise 14. Determine Protein Molecular Weight

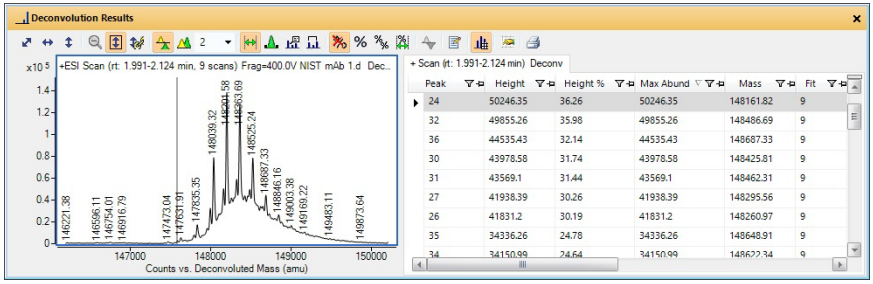
This exercise shows you how to open a data file, extract spectra, deconvolute, and view results. Deconvolution software does charge state deconvolution of mass spectra of large molecules with high charge states, such as proteins. See **"Before you start"** on page 3.

Steps	Detailed Instructions	Comments
1 Open the data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb 1.d** folder. **c** Clear the **Load Result** check box. **d** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Extract a peak spectrum.	**a** Select a range around the peak at 2.1 minutes. **b** Double-click this range.	• To select a range, click one side of the peak and drag to the other side of the peak.
		
		
3 Open the default Intact Protein method and open the Deconvolute (Protein) Method Editor section.	**a** Click **Method > Open**. **b** Select *BioConfirmIntactProtein-Default.m*. **c** Click **View > Method Editor**. **d** Select **Intact Protein > Deconvolute (Protein)**.	• The commands in the **View** menu toggle whether or not a window is visible. If the command is shown in blue and the button has an orange box around it, then the window is currently visible.

Steps	Detailed Instructions	Comments
4 Select Maximum Entropy as the deconvolution algorithm.	• On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, verify that **Maximum Entropy** is selected for **Deconvolution algorithm**.	
5 Verify that the Mass range is automatically detected.	• Verify that the **Automatic mass range detection** check box is marked.	• If you clear this check box, then you need to manually enter the Mass range which can vary for different intact proteins.
6 Set the **Mass step** to 1.	• Enter 1 for the **Mass step**.	
7 Select the extracted MS peak spectrum.	• Click the spectrum in the Biomolecule MS Spectrum window.	
8 Deconvolute the spectrum.	• Right-click the Biomolecule MS Spectrum window and click **Deconvolute > Protein** to start the deconvolution process.	• You can also click the arrow next to the run button in the Method Editor toolbar and select **Deconvolute (Protein)**.



Steps	Detailed Instructions	Comments
9 Review deconvolution results.	• The results appear in the Deconvolution Results window and the Biomolecules window. • For information on changing the display of data in the Deconvolution Results window, see *online Help*.	• To compare two deconvoluted spectra, select the spectra of interest; then, click the Create Mirror Plot button, , on the Deconvolution toolbar. If necessary, click **View > Deconvolution Mirror Plot**. The spectra are displayed in the Deconvolution Mirror Plot Results window. See **"Exercise 16. Use the Mirror Plot window"** on page 82 for more information.
10 View peak information.	**a** Click the spectrum in the Deconvolution Results window to select it. **b** Click the Spectrum Peak List button ( ). **c** Click the **Max Abund** column heading to sort results by abundance. **d** Click in the toolbar in the Deconvolution Results window. **e** Select **Mass** for the first **Peak label** and click **OK**. **f** Click the Spectrum Peak List button ( ) on the Deconvolution Results toolbar to close the peak list tab.	• Mass, Abundance, and Fit score are listed for each peak in the spectrum. • You can change the size of the graphics pane and the table pane in the Deconvolution Results window. Select the line between them and drag it to the right or left. • In the **Deconvoluted Spectra Display Options** dialog box, you can select the labels to use on a deconvoluted spectrum.

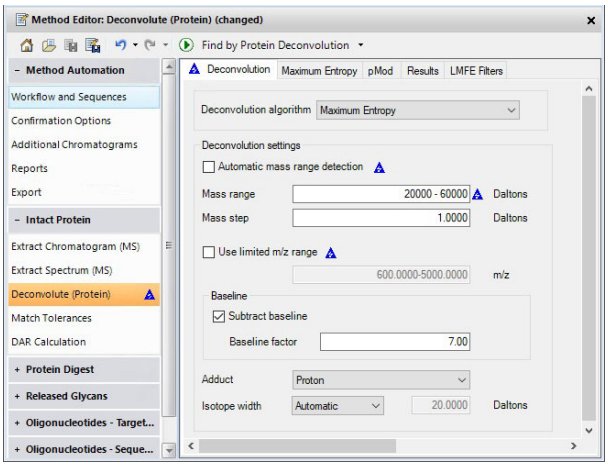


11 Save the results.	**a** Click **File > Save Results**. **b** If needed, enter a reason and click **OK**. **c** Click **Save**.	
12 Save the method to iii_Deconvolution_MaxEnt.m where iii are your initials	**a** Click **Method > Save As**. **b** Enter ***iii*_Deconvolution_MaxEnt.m** for the method name. **c** Click **Save**.	

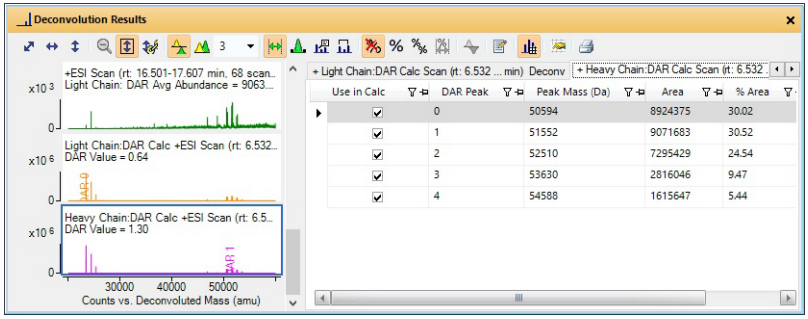
Exercise 15. Calculate DAR

This section shows how to calculate DAR for both a non-reduced and a reduced data file..

Steps	Detailed Instructions	Comments
1 Open the *DAR_Sample_Intact.d* and the *DAR_Sample_Reduced.d* data files.	**a** Click **File > Open Data File**. **b** Locate the **DAR_Sample_Intact.d** and the **DAR_Sample_Reduced.d** folders. **c** Clear **Load Result Data**. **d** Click **Open**. **e** Click **Intact Protein Layout** in the toolbar.	• The TIC is automatically displayed in the Sample Chromatogram Results window. • The Intact Protein layout shows the Deconvolution Results window.
2 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select **BioConfirmIntactProtein-Default.m** **c** Click **Open**.	
3 Open the Deconvolute (Protein) Method Editor section.	• Select **Deconvolute (Protein)** from the Intact Protein section of the Method Editor.	If the Method Editor window is not visible, click **View > Method Editor** to display it.

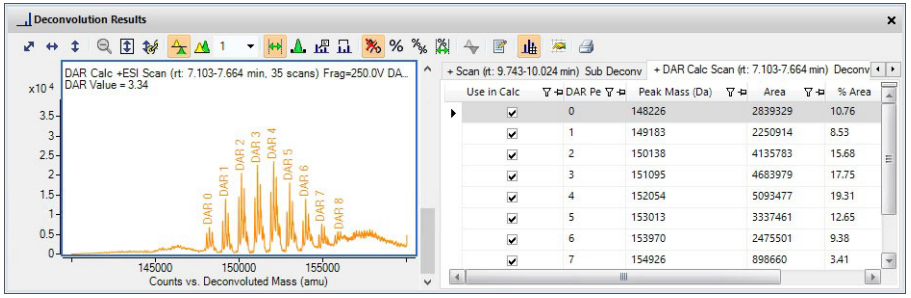
Steps	Detailed Instructions	Comments
4 Select the deconvolution algorithm. • Use Maximum Entropy. • Use 20000 - 60000 mass range. • Do not use the limited m/z range.	**a** On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **Maximum Entropy** as the **Deconvolution algorithm**. **b** Clear the **Automatic mass range detection** check box. **c** Enter `20000 – 60000` for the **Mass range**. **d** Clear the **Use limited m/z range** check box. 	• For more information on these parameters, press **F1** to open the online Help.
5 Use the default settings for Maximum Entropy deconvolution.	• Click the **Maximum Entropy** tab to review settings.	
6 Set the time range for extracting spectra to 6 to 8 minutes.	**a** On the Extract Spectrum (MS) tab of the Intact Protein section of the Method Editor, click **Time Range(s)**. **b** Enter 6 as the **Start** and 8 as the **Stop**.	
7 Set the workflow parameters for the reduced Condition.	**a** On the Workflow and Sequences tab of the Method Automation section of the Method Editor, select **Intact Protein**. **b** Select **reduced** as the **Condition**. **c** Enter `23439, 50594` for **Sequences/Masses**. **d** Mark the **Calculate Drug-to-Antibody Ratio (DAR)** check box. **e** Enter `957.5` as the **Drug + linker mass**.	• For a method with **reduced** as the **Condition**, you enter the Reduced DAR Light Chain, Reduced DAR Heavy Chain as the **Sequences/Masses**.

Steps	Detailed Instructions	Comments
8 Save the method.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_**DAR_Reduced.m**, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason in the **Reason for Change** dialog box. **e** Click **OK**.	• The administrator can set up a project to require you to enter a reason for a change to a method
9 Run the method workflow on the *DAR_Sample_Reduced.d* file.	**a** Click **Method > Run Method Workflow**, or click **Run Method Workflow** in the toolbar in the Method Editor window. **b** Select **DAR_Sample_Reduced.d** and click **OK**.	
10 Review deconvolution results.	• The results appear in the Deconvolution Results window and in the Sample Table window.	• Deconvoluted spectra are added for the light chain and for the heavy chain. • The DAR value is added to the Sample table.



11 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select **BioConfirmIntactProtein-Default.m** **c** Click **Open**.	
12 Select the deconvolution algorithm. • Use Maximum Entropy. • Use 20000 - 60000 mass range. • Do not use the limited m/z range.	**a** On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **Maximum Entropy** as the **Deconvolution algorithm**. **b** Clear the **Automatic mass range detection** check box. **c** Enter `20000 – 60000` for the **Mass range**. **d** Clear the **Use limited m/z range** check box.	• For more information on these parameters, press **F1** to open the online Help.

Steps	Detailed Instructions	Comments
13 Set the workflow parameters for the non-reduced Condition.	**a** On the Workflow and Sequences tab of the Method Automation section of the Method Editor, select **Intact Protein**. **b** Select **non reduced** as the **Condition**. **c** Enter `148057.5` for **Sequences/Masses**. **d** Mark the **Calculate Drug-to-Antibody Ratio (DAR)** check box. **e** Enter `974.5` as the **Drug + linker mass**.	• For a method with **non reduced** as the **Condition**, you enter the initial Dar 0 mass as the **Sequences/Masses**.
14 Save the method.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_**DAR_NonReduced.m**, where *iii* represents your initials. **c** Click **Save**. **d** If needed, enter a reason in the **Reason for Change** dialog box. **e** Click **OK**.	• The administrator can set up a project to require you to enter a reason for a change to a method
15 Run the method workflow on the *DAR_Sample_Intact.d* file.	**a** Click **Method > Run Method Workflow**, or click **Run Method Workflow** in the toolbar in the Method Editor window. **b** Select **DAR_Sample_Intact.d** and click **OK**.	
16 Review deconvolution results.	• The results appear in the Deconvolution Results window and the Sample Table window.	• A deconvoluted DAR spectrum is added. • The DAR value is added to the table.

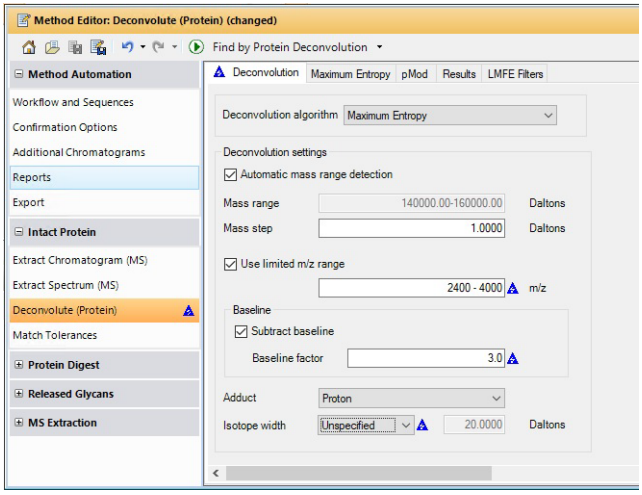


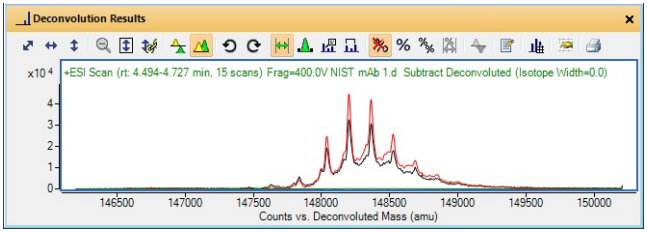
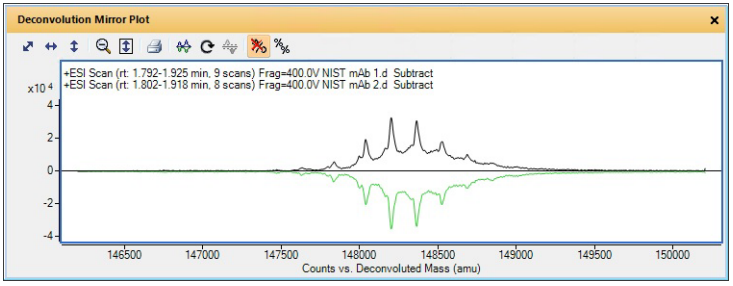
17 Save the results.	**a** Click **File > Save Results**. **b** If needed, enter a reason and click **OK**. **c** Click **Save**.	

Exercise 16. Use the Mirror Plot window

This section shows how to display a Mirror Plot of two deconvoluted biomolecules.

Steps	Detailed Instructions	Comments
1 Open the NIST mAb 1.d data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb 1.d** folder. **c** Clear **Load Result Data**. **d** Click **Open**. **e** Click **Intact Protein Layout** in the toolbar.	• The TIC is automatically displayed in the Sample Chromatogram Results window. • The Intact Protein layout shows the Deconvolution Results window.
2 Open the method to use as a starting point for the new method.	**a** Click **Method > Open**. **b** Select **BioConfirmIntactProtein-Default.m** **c** Click **Open**.	
3 Open the Deconvolute (Protein) Method Editor section.	• Select **Deconvolute (Protein)** from the Intact Protein section of the Method Editor.	If the Method Editor window is not visible, click **View > Method Editor** to display it.

Steps	Detailed Instructions	Comments
4 Select the deconvolution algorithm. • Use Maximum Entropy. • Use the automated mass range detection. • Use the limited m/z range of 2400 - 4000. • Use 3 for the baseline factor.	**a** On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **Maximum Entropy** as the **Deconvolution algorithm**. **b** Mark the **Automatic mass range detection** check box. **c** Mark the **Use limited m/z range** check box. **d** Enter 2400 - 4000 for the *m/z* range. **e** Enter 3 for the **Baseline factor**. **f** Select **Unspecified** for the **Isotope width**. 	• For more information on these parameters, press **F1** to open the online Help.
5 Use the default settings for Maximum Entropy deconvolution.	• Click the **Maximum Entropy** tab to review settings.	
6 Run the Find by Protein Deconvolution algorithm.	• Click **Find and Identify > Find by Deconvolution > Proteins**. or • Click **Find by Protein Deconvolution** in the toolbar in the Method Editor window.	

Steps	Detailed Instructions	Comments
7 Review deconvolution results.	• The results appear in the Deconvolution Results window. 	
8 Open the NIST mAb 2.d data file.	**a** Click **File > Open Data File**. **b** Locate the **NIST mAb 2.d** sample file. **c** Click **Open**.	• The TIC is automatically displayed in the Sample Chromatogram Results window.
9 Run the Find by Protein Deconvolution algorithm on NIST mAb 2.d.	• Click **Find and Identify > Find by Deconvolution > Proteins**.	
10 Review deconvolution results.	• The results appear in the Deconvolution Results window.	
11 Select both data files in the Sample Table window.	**a** Select one of the sample files in the Sample Table window. **b** Press the **Ctrl** button and click the other sample file.	• The results for the sample files selected in the Sample Table are shown in the Deconvolution window and other windows.
12 Use Mirror Plot to compare two deconvoluted spectra.	**a** Click the  button to show the spectra in list mode. **b** Select a spectra from the Deconvolution window. **c** Press the **Ctrl** button and select another spectra from the other data file. **d** Click the  button to display the spectra in the Deconvolution Mirror Plot Results window.	• Other mirror plots are available: ◦ MS Spectrum Mirror Plot ◦ Fragment Spectrum Mirror Plot ◦ Biomolecule Chromatogram Mirror Plot ◦ Sample Chromatogram Mirror Plot
		

Exercise 17. View Biomolecule Information

This exercise shows you how to view biomolecule information for deconvoluted spectra.

Steps	Detailed Instructions	Comments
1 Deconvolute **NIST mAb 1.d** spectrum.	• See **"Exercise 14. Determine Protein Molecular Weight"** on page 75.	• You do not need to repeat the deconvolution steps, if you have already done them in Exercise 1.
2 View the biomolecule list.	• If needed, click **View > Biomolecules**	• See **Figure 8** on page 86.
3 Select the biomolecule with mass around 148039.3.	• Click the row which has a mass around 148039.3 in the Biomolecules window.	• The Biomolecule MS Spectrum window and the Deconvolution Results window are both updated. • A biomolecule spectrum that displays all the charge states from the original *m/z* data for that specific protein mass is shown in the Biomolecule MS Spectrum Results window.
4 Select the biomolecule selected in step 3 in the Biomolecule MS Spectrum Results window.	• Click the graphics area for the spectrum selected in step 3.	• You can right-click the title of the window and click **Floating**. Then, you can make the window wider.
5 View the charge states found for the protein.	**a** Click  on the Biomolecule MS Spectrum toolbar to show the peak information. **b** Right-click the table and click **Add/Remove Columns**. **c** Select the columns in the Available Columns list which you want to see. **d** Click either **Add** or **Add All ->>**	• The following information is displayed for the ion set spectrum: ◦ m/z ◦ Abundance ◦ Charge state See **Figure 9** on page 86. • If you cannot see the graphics when the table is displayed, move the cursor to between the graphics and the table until it looks like  . Then, click and drag to the right to increase the size of the graphics.
6 Switch from List mode to Overlay mode in the Biomolecule MS Spectrum Results window.	• Click  on the toolbar in the Biomolecule MS Spectrum Results window.	• See **Figure 10** on page 87.
7 Select Biomolecule 1 in the biomolecule list.	• Click the first line of the Biomolecules table.	• Notice that the spectrum in the Biomolecule MS Spectrum window is updated.
8 Select Biomolecule 2 in the Biomolecules window.	• Click the second line of the Biomolecules table.	

Steps	Detailed Instructions	Comments
9 Print a biomolecule report.	**a** Display the Reports section in the Method Editor by selecting **Method Automation > Reports**. **b** Review the parameters in both the **Templates** and **Layout** tabs. **c** Click **Biomolecule Report** from the **File > Print** menu to print the report.	• When you print a Biomolecule Report, it uses the **Intact Protein**, the **Protein Digest**, the **Released Glycans**, the **Oligonucleotides - Target Plus Impurities**, or the **Oligonucleotides - Sequence Confirmation** template, depending on the workflow used to create the results. • If the workflow is **Custom**, then if you use the Find Peptides command, the **Peptide Digest report template** is used; otherwise, the **Intact Protein report template** is used.

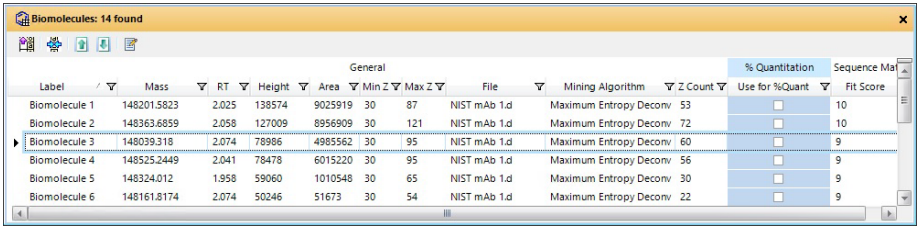


Figure 8. Biomolecules window for NIST mAb 1.d

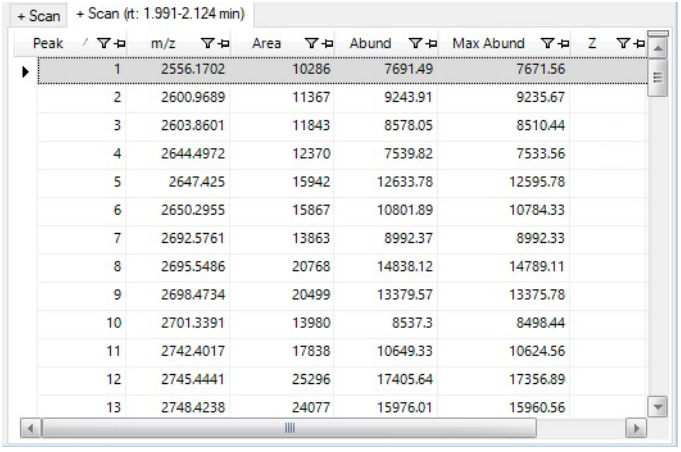


Figure 9. Peak information for NIST mAb 1.d displayed in the Biomolecule MS Spectrum window

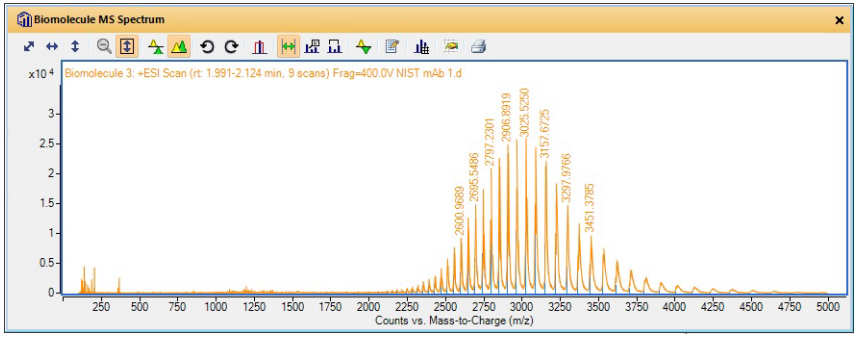


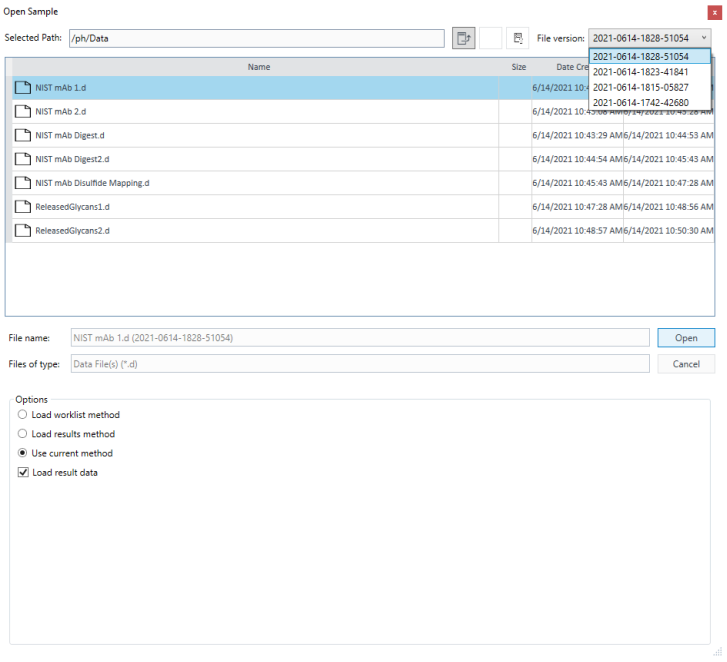
Figure 10. Biomolecule MS Spectrum Results window for NIST mAb 1.d (Overlay Mode)

Exercise 18. Open results using versions

This section shows how to select which results to open when you open a data file. You can only select which results to load in **Networked Workstation** mode. In **Workstation** mode, the **File version** is not available.

If you do not have permission to select results, then this option is grayed out.

Steps	Detailed Instructions	Comments
1 Select the NIST mAb 1.d data file.	**a** Click **File > Open Data File**. **b** Select the **NIST mAb 1.d** folder.	• In the previous exercises, results were stored with NIST mAb 1.d multiple times.
2 Select the File version to open.	**a** Mark **Load result data**. **b** Select the **File version** to open. **c** Click **Open**.	• You select the file version based on the date and time that the version was saved. • You can look at the Results Audit Trail window to look at the **Reason** column or the **Review Comment** column to help determine which **File version** to open.



You need to mark the Load result data check box before you can select which results to load. If the list of results is grayed out, verify that the check box is marked.

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In This Guide

This guide teaches you how to use MassHunter BioConfirm 12.0.

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