

MassHunter BioConfirm 12.0

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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

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.	<ol style="list-style-type: none">Double-click the Control Panel icon on the desktop.Click Projects in the left pane.Select a project.On the ribbon in the BioConfirm group, click BioConfirm > Start BioConfirm.	<ul style="list-style-type: none">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.

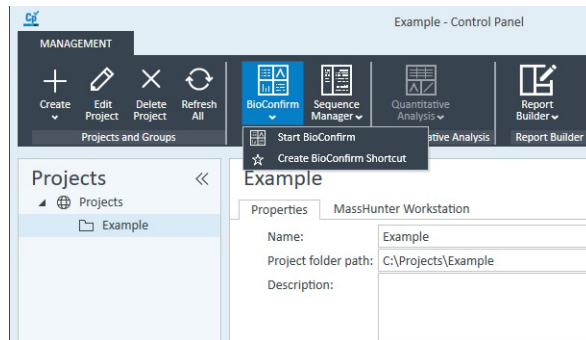


Figure 1. Start BioConfirm from the Control Panel program

Basic Tasks

Task 1. Open the BioConfirm program

Steps	Detailed Instructions	Comments
<p>2 Open these data files:</p> <ul style="list-style-type: none">• NIST mAb 1.d• NIST mAb 2.d• NIST mAb Digest.d• NIST mAb Digest2.d• ReleasedGlycans1.d• ReleasedGlycans2.d <ul style="list-style-type: none">• Make sure that the Use current method button is clicked.• Make sure that the Load result data check box is cleared.	<p>a In the Open Sample dialog box, go to the Data folder in your project.</p>	<ul style="list-style-type: none">• 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.

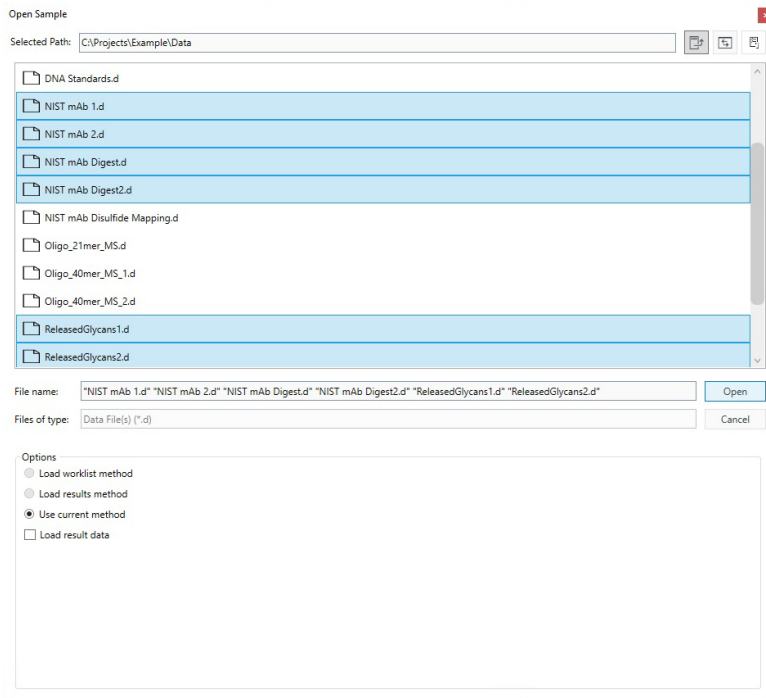





Figure 2. Open data files when opening software

Basic Tasks

Task 1. Open the BioConfirm program

Steps	Detailed Instructions	Comments
	<p>b Click the NIST mAb 1.d file.</p> <p>c Press and hold the Shift key while you click NIST mAb Digest2.d.</p> <p>d Press and hold the Ctrl key while you click ReleasedGlycans1.d and ReleasedGlycans2.d.</p> <p>e Clear the Load result data check box.</p> <p>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.</p> <p>g Click the List Mode button () in the Sample Chromatogram Results toolbar.</p> <p>h Click the NIST mAb 1.d data file in the Sample Table window.</p>	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• Click Intact Protein Layout in the main toolbar.	<ul style="list-style-type: none">• 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.

Basic Tasks

Task 1. Open the BioConfirm program

Steps

Detailed Instructions

Comments

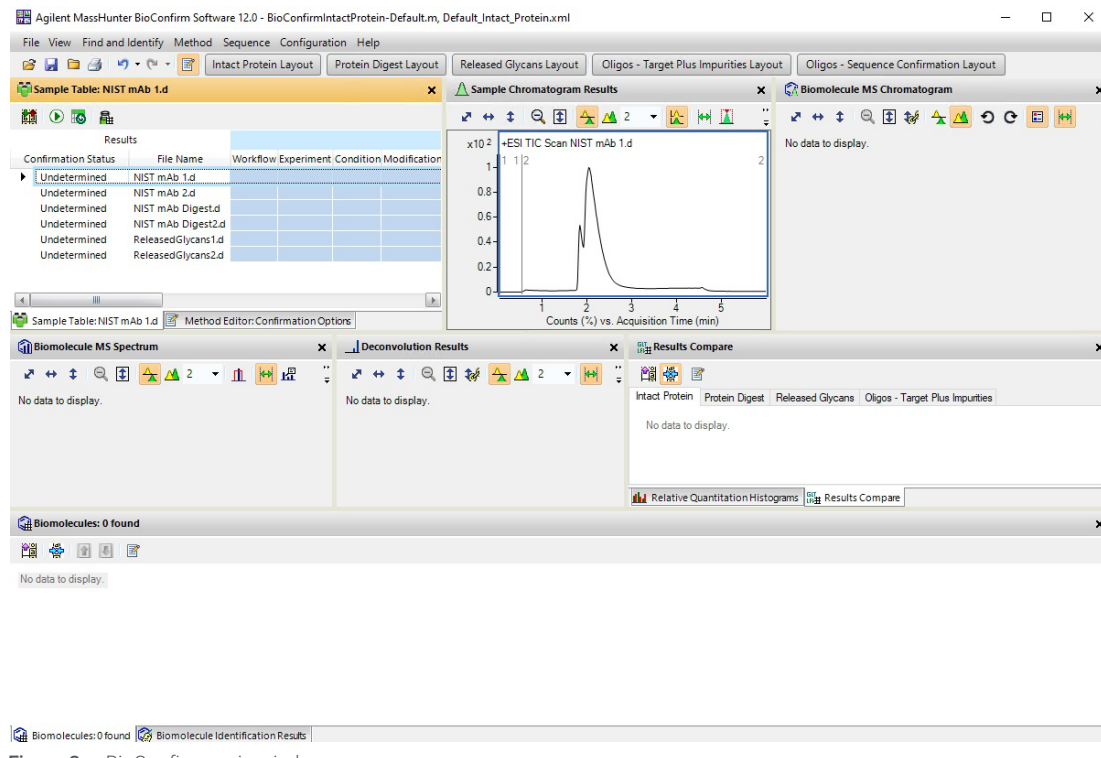







Figure 3. BioConfirm main window

Basic Tasks

Task 2. Zoom in and out of the chromatogram







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
<p>1 Practice zooming in and out on the chromatogram in the Sample Chromatogram Results window.</p> <ul style="list-style-type: none">• 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.	<p>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.</p> <p>b Repeat step a.</p> <p>c Click the Autoscale Y-axis during Zoom button, , in the toolbar.</p> <p>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. </p> <p>e Click the Unzoom button, , to undo the last zoom operation. You can undo the last fifteen zoom operations.</p> <p>f Click the Autoscale X-axis and Y-axis button, , to zoom out completely.</p>	<ul style="list-style-type: none">• 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.

Basic Tasks

Task 2. Zoom in and out of the chromatogram

Steps	Detailed Instructions	Comments
<p>2 Practice zooming in and out on each axis separately.</p> <ul style="list-style-type: none">Zoom in only along the x-axis. <p>Hint: Right-click the x-axis values and move cursor from left to right.</p> <ul style="list-style-type: none">Partially zoom out the x-axis. <p>Hint: Move cursor in the opposite direction.</p> <ul style="list-style-type: none">Completely zoom out of the x-axis.Repeat the previous steps for the y-axis.	<p>a To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears.</p> <p>b Click the right mouse button and drag the new cursor from left to right across the x-axis values.</p> <p>c To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values.</p> <p>d Click the Autoscale X-axis button, , to completely zoom out on the x-axis.</p> <p>a To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears.</p> <p>b Click the right mouse button and drag the new cursor from bottom to top across the y-axis values.</p> <p>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.</p> <p>d Click the Autoscale Y-axis button, , to completely zoom out on the y-axis.</p>	<p> Horizontal Double Arrow</p> <p> New cursor appears when you right-click the x-axis value</p> <p> Vertical Double Arrow</p> <p> New cursor appears when you right-click the y-axis values.</p>

Basic Tasks

Task 3. Change window layouts

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.

Basic Tasks

Task 3. Change window layouts

Steps	Detailed Instructions	Comments
<p>1 Change the window layout:</p> <ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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:<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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:<ul style="list-style-type: none">Default_Intact_Protein.xmlDefault_Protein_Digest.xmlDefault_Released_Glycans.xmlDefault_Oligonucleotides_TPI.xmlDefault_Oligonucleotides_SC.xmlDefault.xml

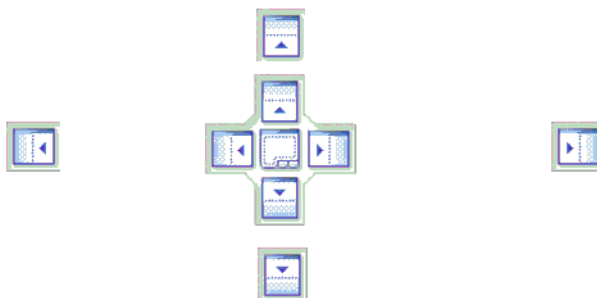


Figure 4. Window repositioning tools

Basic Tasks

Task 3. Change window layouts

Steps	Detailed Instructions	Comments
<p>2 Reposition the Sample Chromatogram Results window.</p> <ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.

Basic Tasks

Task 4. Creating a Protein Sequence File

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.	<ul style="list-style-type: none">Click Sequence > Sequence Manager.	<ul style="list-style-type: none">You can also start the Sequence Manager program from the OpenLab Control Panel.
2 Add a sequence.	<ol style="list-style-type: none">Click the Proteins tab.Click Sequence > Add Sequences > Add Protein Sequence.Select one or more sequences in the list in the Add Protein Sequence(s) dialog box.Click Open. The Sequence Editor pane opens automatically with that sequence.	<ul style="list-style-type: none">Protein is automatically selected for the sequence type.
3 Create a new sequence.	<ol style="list-style-type: none">Type <code>Myoglobin</code> for the name of the Sequence.Click the + button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	<ul style="list-style-type: none">Protein is automatically selected for the sequence type.
4 Enter the amino acid sequence shown below into the Sequence Manager.	<ul style="list-style-type: none">Type in individual amino acids one at a time between the N-term and C-term symbols. <p>GLSDGEWQQVLNVWGKVEADIAGHGQEVLRIRLFTGHPETLEKFDKFKHLKTEAEM KASEDLKKHGTWVLTALGGILKKGHHAEELKPLAQSHATKHKIPIKYLEFISDAIIHV LHSHKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG</p>	<ul style="list-style-type: none">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*.

Basic Tasks

Task 4. Creating a Protein Sequence File

Steps	Detailed Instructions	Comments
5 Save the sequence as the name <i>iii_myoglob.psq</i> , where <i>iii</i> represents your initials.	<ol style="list-style-type: none">Click Sequence > Export Sequence.Type <i>iii_myoglobin</i> in the File name box.Click Save.	<ul style="list-style-type: none">The sequence is saved as a .psq file that can be transferred to other computers or projects.

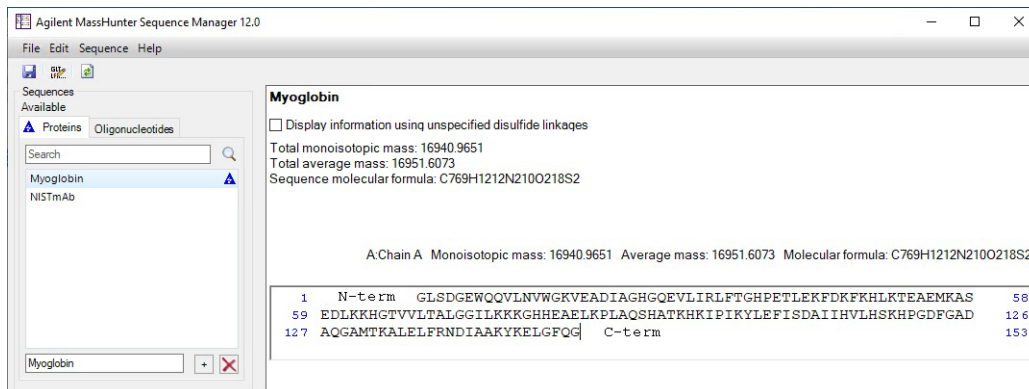


Figure 5. Creating a sequence file of myoglobin in the Sequence Manager program

6 Save changes in the Sequence Manager.	<ol style="list-style-type: none">Click Sequence > Save All Changes.In the Save Sequences dialog box, mark the check box in front of Myoglobin.Enter a description of the change that was made.Click Save.	<ul style="list-style-type: none">A description is always required when you save a sequence.
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Basic Tasks

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

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.	<ul style="list-style-type: none">Click Sequence > Open Chemical Data Dictionary Editor.	<ul style="list-style-type: none">You can also start the Sequence Manager program and click Open definition manager on the Oligonucleotides tab.
2 Create a new building block.	<ul style="list-style-type: none">a Click the Oligonucleotides tab.b Type κH for the Code.c Click the + button.	<ul style="list-style-type: none">See the online Help for rules about naming new Building blocks.
3 Enter the Name , Molecular formula , and Base formula .	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">This example is a duplicate of the Building block Deoxycytidine.

Basic Tasks

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

Steps	Detailed Instructions	Comments
4 Close the program.	<ol style="list-style-type: none">Click Close.In the Save Chemical Data Dictionary dialog box, enter a reason that the change was made.Click Yes.	<ul style="list-style-type: none">If you click No in the Save Chemical Data Dictionary dialog box, the changes are not saved.

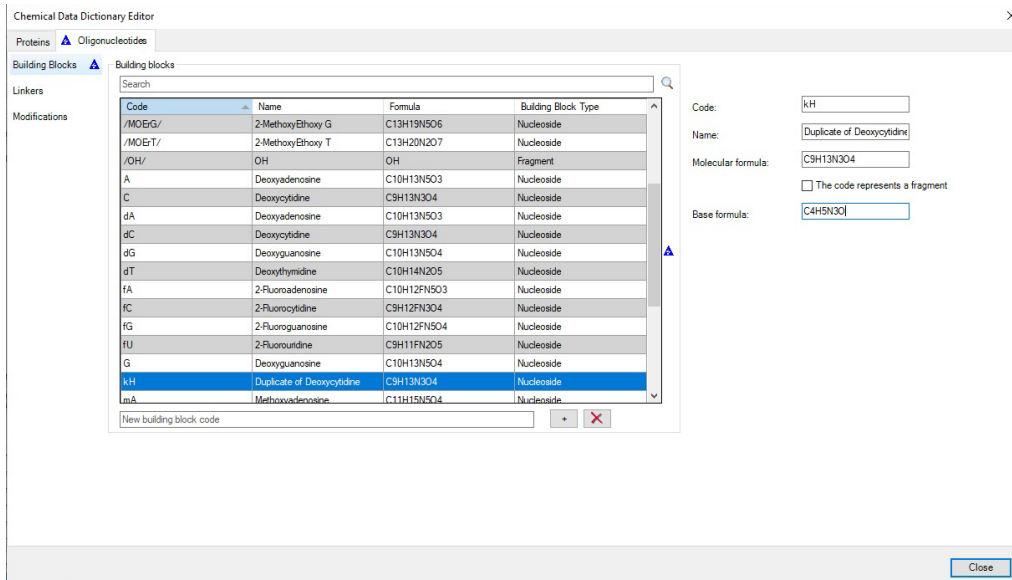


Figure 6. Creating a Building block in the Chemical Data Dictionary Editor dialog box

Basic Tasks

Task 6. Creating an Oligonucleotide Sequence File

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.	<ul style="list-style-type: none">Click Sequence > Sequence Manager.	<ul style="list-style-type: none">You can also start the Sequence Manager program from the OpenLab Control Panel.
2 Add a sequence.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">The Oligo_40mer_MS sequence and the Oligo_40mer_MSMS sequence are used in the exercises in this guide.
3 Create a new sequence.	<ul style="list-style-type: none">a Type <code>Example</code> for the name of the Sequence.b Click the + button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	<ul style="list-style-type: none">Oligonucleotide is automatically selected for the sequence type.
4 Enter the building block sequence shown below into the Sequence Manager. TGACTGTGAACGTTCCGGATGA	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.

Basic Tasks

Task 6. Creating an Oligonucleotide Sequence File

Steps	Detailed Instructions	Comments
5 Save the sequence as the name <i>iii_Example.psq</i> , where <i>iii</i> represents your initials.	<ol style="list-style-type: none">Click Sequence > Export Sequence.Type <i>iii_Example</i> in the File name box.Click Save.	<ul style="list-style-type: none">The sequence is saved as a .psq file that can be transferred to other computers or projects.

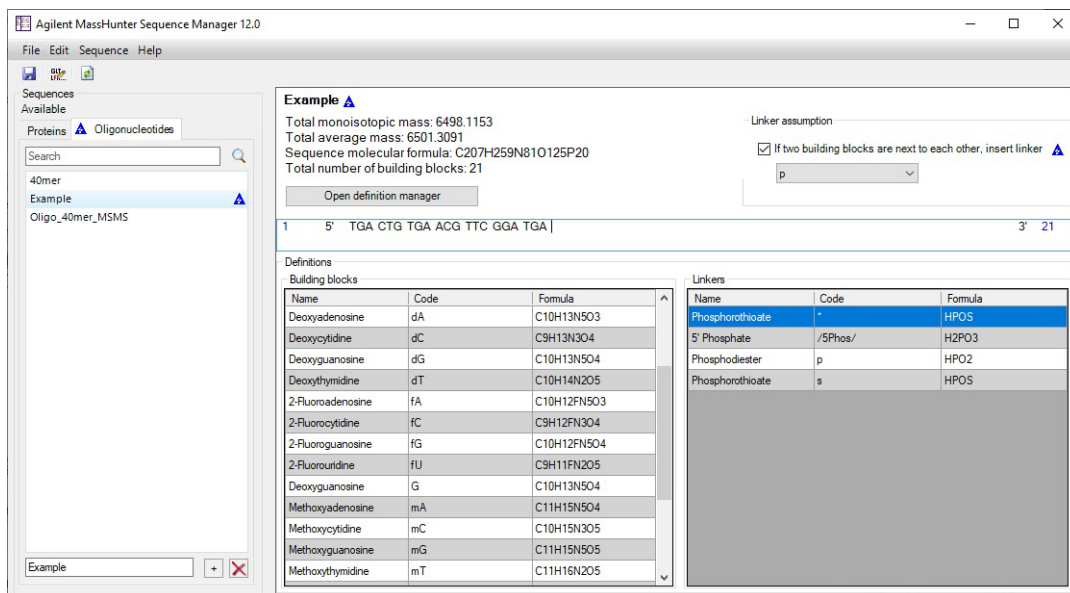


Figure 7. Creating an oligonucleotide sequence in the Sequence Manager program

6 Save changes in the Sequence Manager.	<ol style="list-style-type: none">Click Sequence > Save All Changes.In the Save Sequences dialog box, mark the check box in front of Example.Enter a description of the change that was made.Click Save.	<ul style="list-style-type: none">A description is always required when you save a sequence.
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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


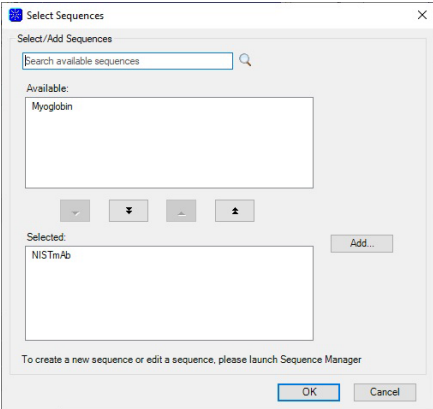


This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the **NISTmAb.seq** sequence file and the **NIST mAb 1.d** data file copied before you started. See **“Before you start”** on page 3.

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.	<ol style="list-style-type: none"> a Click Method > Open. b Select BioConfirmIntactProtein-Default.m. c Click Open. 	<ul style="list-style-type: none"> • 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.	<ol style="list-style-type: none"> a Click File > Open Data File. b Select NIST mAb 1.d. c Click Open. 	<ul style="list-style-type: none"> • The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Select the Intact Protein layout.	<ul style="list-style-type: none"> • Click Intact Protein Layout in the main toolbar. 	<ul style="list-style-type: none"> • You can instead click Configuration > Window Layouts > Load Layout. Then, select <i>Default_Intact_Protein.xml</i>, and click Open.
4 Display the Deconvolute (Protein) section in the Method Editor window.	<ol style="list-style-type: none"> 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.	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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 <i>Quick Start Guide</i> or <i>online Help</i>. • 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.	<ul style="list-style-type: none"> • Click Method Automation > Workflow and Sequences in the Method Editor window. 	

Intact Protein Workflow

Exercise 1. Interactive Intact Protein Workflow

Steps	Detailed Instructions	Comments
7 Import the NISTmAb sequence.	<ol style="list-style-type: none">Select Intact Protein for the Workflow.Select non-reduced for the Condition.Click the  button next to the Sequences/Masses parameter. The Select Sequences dialog box opens.Double-click NISTmAb. If NISTmAb is not available, click Add.Select NISTmAb.psq and click Open.Verify that the NISTmAb sequence is in the Selected list.Click OK.	<ul style="list-style-type: none">You will use the sequence as is. You can add or modify modifications and links to sequences as described in <i>online Help</i> and the <i>Quick Start Guide</i>.
		
8 Select the mAb modification.	<ol style="list-style-type: none">Click the  button next to the Mods and Profiles parameter. The Select Modifications and Profiles dialog box opens.Double-click mAb in the Available list in the Modifications and profiles section.Click OK.	<ul style="list-style-type: none">The mAb sequence has modifications. You can learn how to add modifications in the <i>online Help</i> and the <i>Quick Start Guide</i>.Review the modifications in the Modification Summary.
9 Start the match search.	<ol style="list-style-type: none">Click Intact Protein > Match Tolerances.Click  on the Method Editor toolbar.If necessary, select NIST mAb 1.d and click Match.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Select the Biomolecule 1 row in the Biomolecules table.	<ul style="list-style-type: none">In the BioConfirmIntactProtein-default layout, the Biomolecule Identification Results window is tabbed with the Biomolecules window.

Intact Protein Workflow

Exercise 1. Interactive Intact Protein Workflow

Steps

Detailed Instructions

Comments

The screenshot displays the Agilent MassHunter BioConfirm Software 12.0 interface. The main window is titled "Agilent MassHunter BioConfirm Software 12.0 - BioConfirmIntactProtein-Default.m, Default_Intact_Protein.xml". The interface is divided into several panels:

- Sample Table: NIST mAb 1.d**: Shows a table of results with columns for Confirmation Status, File Name, Workflow, Sequence / Mass Condition, and Modification. The first row is "Confirmed" for "NIST mAb 1.d" using the "Intact Protein" workflow.
- Sample Chromatogram Results**: Displays two chromatograms. The top one is labeled "x10² +ESI TIC Scan NIST mAb 1.d" with a peak at 4.611. The bottom one is "x10² +ESI TIC Scan NIST mAb 1.d" with peaks at 2.051 and 4.561.
- Biomolecule MS Chromatogram**: Shows a chromatogram for "Biomolecule 1: A(1-213) + B(1-450) + C(1-213) + D(1-450)..." with a peak at 4.611.
- Biomolecule MS Spectrum**: Displays two mass spectra. The top one is for "Biomolecule 1: A(1-213) + B(1-450) + C(1-213) + D(1-450)..." and the bottom one is for "Biomolecule 1: A(1-213) + B(1-450) + C(1-213) + D(1-450)...".
- Deconvolution Results**: Shows two deconvolution plots. The top one is for "+ESI Scan (rt: 1.792-1.925 min, 9 scans) Fra..." and the bottom one is for "+ESI Scan (rt: 1.925-2.522 min, 37 scans) Fra...".
- Results Compare**: Shows a table of results with columns for Sequence/Mass, Confirmation Status, and 1 Conf. The table lists three biomolecules: Biomolecule 1... (RT: 2.025, RT Range: 1.747-3.301), Biomolecule 2... (RT: 2.058, RT Range: 1.760-3.135), and Biomolecule 3... (RT: 1.842, RT Range: 1.792-1.925).
- Biomolecules: 87 found**: A table listing 87 biomolecules with columns for Label, Mass, RT, Height, Area, File, Use for %Quant, Area (Decon), %Quant (Decon Area), Height (Decon), %Quant (Decon Height), and Volume.

Label	Mass	RT	Height	Area	File	Use for %Quant	Area (Decon)	%Quant (Decon Area)	Height (Decon)	%Quant (Decon Height)	Volume
Biomolecule 1: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148201.7644	2.025	49711	9166272	NIST mAb 1.d	<input checked="" type="checkbox"/>	1763709	10.7	49711	11.31	
Biomolecule 2: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148363.8022	2.058	46690	9158869	NIST mAb 1.d	<input checked="" type="checkbox"/>	1738156	10.54	46690	10.62	
Biomolecule 3: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148039.7767	1.842	31341	905635	NIST mAb 1.d	<input checked="" type="checkbox"/>	1174940	7.13	31341	7.13	
Biomolecule 4: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148525.1033	1.842	28609	1034340	NIST mAb 1.d	<input checked="" type="checkbox"/>	1226719	7.44	28609	6.51	
Biomolecule 5: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148324.231	2.107	22720	211834	NIST mAb 1.d	<input checked="" type="checkbox"/>	1259231	7.64	22720	5.17	
Biomolecule 6: A(1-213) + B(1-450) + C(1-213) + D(1-450)...	148160.9346	1.859	21543	303055	NIST mAb 1.d	<input type="checkbox"/>	794760		21543		

The bottom status bar shows: "Biomolecules: 87 found | Biomolecule Identification Results: Biomolecule 1: A(1-213) + B(1-450) + C(1-213) + D(1-450); NIST mAb; 1*G1F(1607.5013) + 1*G0F (NGA2F)(1445.3580) + 2*Lys-loss(-128.1750)

Intact Protein Workflow

Exercise 1. Interactive Intact Protein Workflow

Steps

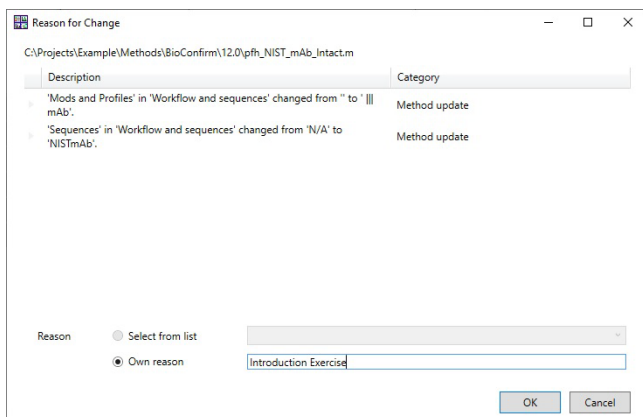
Detailed Instructions

Comments


11 Save the method for use in Exercise 2.

- Click **Method > Save As**.
- Type the **File name iii_NIST_mAb_Intact.m**, where *iii* represents your initials.
- Click **Save**.
- If needed, enter a reason in the Reason for Change dialog box.
- 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).

- In the Biomolecules window, hide all empty columns. Click  in the Biomolecules toolbar.
- Review the values for **Use for %Quant** column for the biomolecules.
- 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.

General					% Quantitation				Sequence Name	
Label	Mass	RT	Height	Area	Use for %Quant	Area (Decon)	%Quant (Decon Area)	Height (Decon)	%Quant (Decon Height)	Sequence Name
Biomolecule 1: A	148201.764	2.025	49711	9166272	<input checked="" type="checkbox"/>	1763709	10.7	49711	11.31	NISTmAb
Biomolecule 2: A	148363.802	2.058	46690	9158869	<input checked="" type="checkbox"/>	1738156	10.54	46690	10.62	NISTmAb
Biomolecule 3: A	148039.776	1.842	31341	905635	<input checked="" type="checkbox"/>	1174940	7.13	31341	7.13	NISTmAb
Biomolecule 4: A	148525.103	1.842	28609	1034340	<input checked="" type="checkbox"/>	1226719	7.44	28609	6.51	NISTmAb
Biomolecule 5: A	148324.231	2.107	22720	211834	<input checked="" type="checkbox"/>	1259231	7.64	22720	5.17	NISTmAb
Biomolecule 6	148160.934	1.859	21543	303055	<input type="checkbox"/>	794760		21543		
Biomolecule 7: A	148486.080	1.859	20465	747503	<input checked="" type="checkbox"/>	716698	4.35	20465	4.66	NISTmAb
Biomolecule 8	148413.753	2.041	19613	6106474	<input type="checkbox"/>	571761		19613		
Biomolecule 9: A	148455.100	1.842	18627	582118	<input checked="" type="checkbox"/>	585354	3.55	18627	4.24	NISTmAb
Biomolecule 10	148258.279	2.074	18210	5413137	<input type="checkbox"/>	628324		18210		
Biomolecule 11: A	148686.811	2.041	18056	5377982	<input checked="" type="checkbox"/>	721995	4.38	18056	4.11	NISTmAb
Biomolecule 12	148576.492	2.058	16416	5651699	<input type="checkbox"/>	654743		16416		
Biomolecule 13	147997.318	1.892	16220	559574	<input type="checkbox"/>	520774		16220		

Intact Protein Workflow

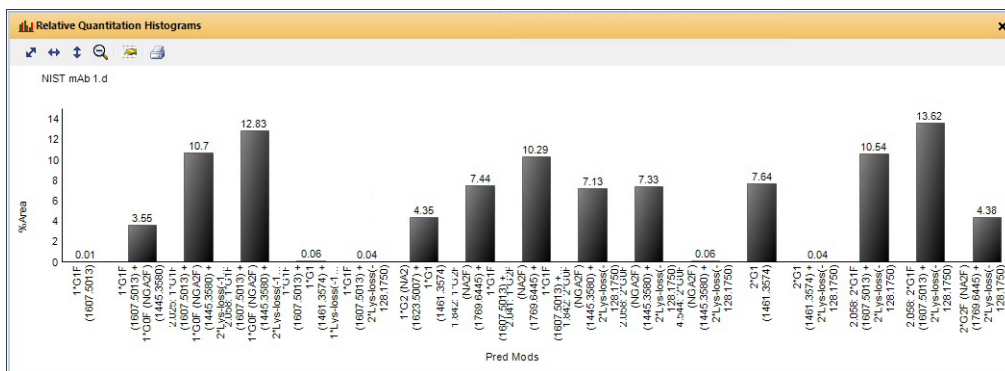
Exercise 1. Interactive Intact Protein Workflow

Steps

Detailed Instructions

Comments

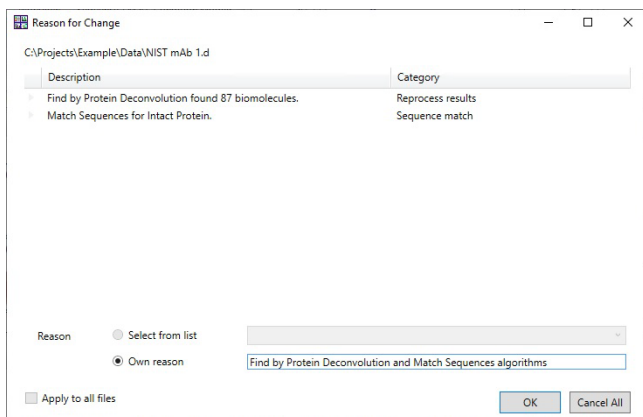
- d Review the results in the Relative Quantitation Histograms window. For an Intact Protein workflow, this window only contains data if the **Use for %Quant** check box is marked in the Biomolecules window for one or more biomolecules and if the sequence has predicted modifications.



Intact Protein Workflow

Exercise 1. Interactive Intact Protein Workflow

Steps	Detailed Instructions	Comments
13 Save the results.	<ol style="list-style-type: none">Click File > Save Results.Select NIST mAb 1.d in the Save Data File dialog box. Click Save.If needed, enter a reason on the Reason for Change dialog box. Click OK.	<ul style="list-style-type: none">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

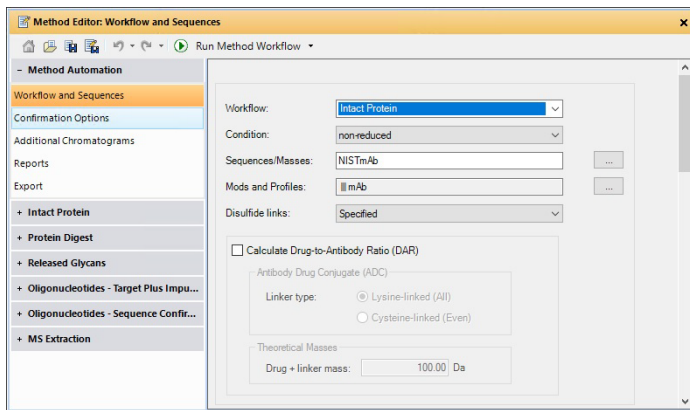
This exercise guides you through the setup of a worklist to automatically confirm the presence of NISTmAb in a previously acquired sample. This exercise uses the **NIST mAb 1.d** data file copied in Exercise 1.

Steps	Detailed Instructions	Comments
1 If not already open, open the method <i>iii_NIST_mAb_Intact.m</i> .	<ol style="list-style-type: none"> Click Method > Open. Select the <i>iii_NIST_mAb_Intact.m</i> folder. 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.	<ul style="list-style-type: none"> Click Method Automation > Workflow and Sequences in the Method Editor window. 	
3 Use the Intact Protein Workflow.	<ul style="list-style-type: none"> Confirm that Intact Protein is selected for the Workflow. 	<ul style="list-style-type: none"> 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 <i>Quick Start Guide</i> or <i>online Help</i>.
4 Select the NISTmAb sequence.	<ol style="list-style-type: none"> Select non-reduced for the Condition. Click the <input type="button" value="..."/> button next to the Sequences parameter. The Select Sequences dialog box opens. If NISTmAb is not available, click Add. Select <i>NISTmAb.psq</i> and click Open. Verify that the NISTmAb sequence is in the Selected list. Click OK. 	<ul style="list-style-type: none"> The NISTmAb.psq sequence file is available on the BioConfirm setup media. You can learn about modifications and links in the <i>online Help</i> and in the <i>Quick Start Guide</i>.
5 Select mAb as the modification and profile.	<ol style="list-style-type: none"> If needed, click the <input type="button" value="..."/> button next to the Mods and Profiles parameter. The Select Modifications and Profiles dialog box opens. Double-click mAb in the Available list. Click OK. 	

Intact Protein Workflow

Exercise 2. Automated Intact Protein Workflow

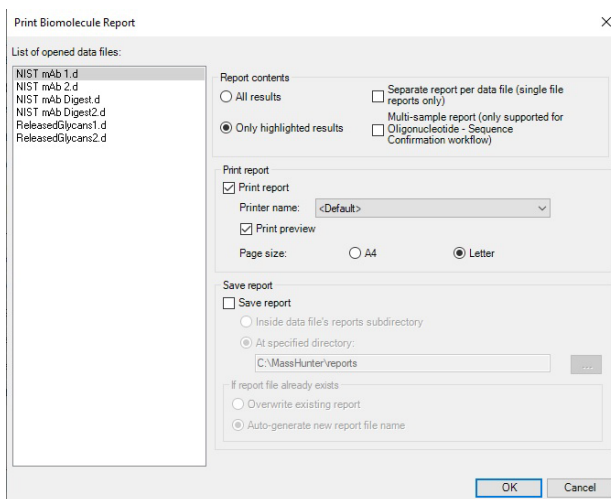
Steps	Detailed Instructions	Comments
6 Save the method.	<ul style="list-style-type: none"> Click Method > Save. If needed, enter the reason for the change. 	<ul style="list-style-type: none"> If you mark the Calculate Drug-to-Antibody Ratio check box, the software will calculate DAR for the data file. See “Exercise 15. Calculate DAR” on page 78 for more information.
7 Run the method workflow or run method automation.	<ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> Method Automation first runs the method workflow, and then extracts additional chromatograms and generates a biomolecule report and exports results.
8 Save the results.	<ol style="list-style-type: none"> Click File > Save Results. Select NIST mAb 1.d in the Save Data File dialog box. Click Save. If needed, enter a reason on the Reason for Change dialog box. Click OK. 	<ul style="list-style-type: none"> You save results before modifying the method again.



Intact Protein Workflow

Exercise 2. Automated Intact Protein Workflow

Steps	Detailed Instructions	Comments
9 (optional) Review the printed Biomolecule reports.	<ul style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> 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.



Report.pdf - Adobe Acrobat Reader DC (32-bit)

File Edit View Sign Window Help

Home Tools Report.pdf x Sign In

BioConfirm Intact Protein Report

Agilent Trusted Answers

Sample Information

Sample Name	Nist mAb Intact	Data File Path	C:\Projects\Example\Data\NIST mAb 1.d
Sample ID		Acq Time (UTC)	2016-08-25-00:11:19-07:00
Instrument	6530 Q-TOF 1	Acq Method Path	D:\MassHunter\methods\desalt_intact_8min.m
MS Type	QTOF (G6530B)	Acq SW Version	6200 series TOF/6500 series Q-TOF 8.06.01 (B6157)
MS SN / FW Ver	5G15362004 / 15.663	DA Method Path	C:\Projects\Example\Methods\BioConfirm\12.0\pfn_NIST_mAb_Intact.m
LC Info	See Instrument Config Report	BioConfirm Version	12.0 (12.0.287.0)
Sample/Plate Position	Vial 1	DA Operator	SYSTEM (SYSTEM)
Inj Vol (ul)	1.0	DA Workstation	TQ-TEST-PH
Acq Operator	I.M. Chemist	Comment	
Acq Workstation		Sequences / Masses	NISTmAb
Condition	non-reduced	Modifications	mAb
Confirmation Status	Confirmed	IRM Status	Success

Matched Sequences

Confirmation Status	Sequence Name	RT	Height	Mass	Tgt Mass	Diff (Da)	Var Mods	Mods
Confirmed	NISTmAb	2.025	49711	148202	148202	0	1*G1F(1607.5613) + 1*G0F (NSA0P(1445.3580) + 2*1ys-loss(-128.1750)	pyroGlu (Q)(B1); pyroGlu (Q)(D1)

Sample Chromatogram List

Additional Chromatograms

8.50 x 11.00 in < >

Protein Digest Workflow

Exercise 2. Automated Intact Protein Workflow

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.	<ol style="list-style-type: none"> a Click Method > Open. b Select the BioConfirmProteinDigest-Default.m folder. c Click Open. 	<ul style="list-style-type: none"> • The parameters in the BioConfirmProteinDigest-Default.m method are a good starting point for Protein Digests.
2 Load the Protein Digest default layout.	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • If available, select NIST mAb Digest.d. Otherwise, do the following: <ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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.	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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 <i>online Help</i>. • 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.	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • You can instead click Find and Identify > Find Peptides.

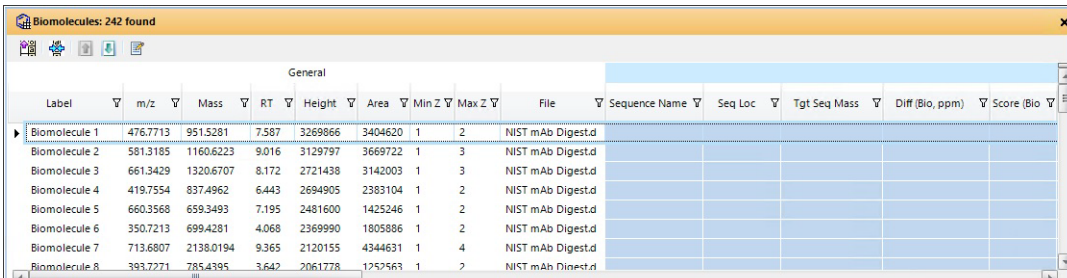
Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

Steps

Detailed Instructions

Comments



Label	m/z	Mass	RT	Height	Area	Min Z	Max Z	File	Sequence Name	Seq Loc	Tgt Seq Mass	Diff (Bio, ppm)	Score (Bio)
Biomolecule 1	476.7713	951.5281	7.587	3269866	3404620	1	2	NIST mAb Digest.d					
Biomolecule 2	581.3185	1160.6223	9.016	3129797	3669722	1	3	NIST mAb Digest.d					
Biomolecule 3	661.3429	1320.6707	8.172	2721438	3142003	1	3	NIST mAb Digest.d					
Biomolecule 4	419.7554	837.4962	6.443	2694905	2383104	1	2	NIST mAb Digest.d					
Biomolecule 5	660.3568	659.3493	7.195	2481600	1425246	1	2	NIST mAb Digest.d					
Biomolecule 6	350.7213	699.4281	4.068	2369990	1805886	1	2	NIST mAb Digest.d					
Biomolecule 7	713.6807	2138.0194	9.365	2120155	4344631	1	4	NIST mAb Digest.d					
Biomolecule 8	393.7271	785.4395	3.642	2061778	1752563	1	2	NIST mAb Digest.d					

6 Add the sequence.

- Click **Method Automation > Workflow and Sequences** in the Method Editor window.
- Select **Protein Digest** as the **Workflow**.
- Select **reduced** as the **Condition**.
- Click the next to the **Sequences/Masses** parameter.
- Double-click **NISTmAb**.
- Click **OK** in the **Select Sequences** dialog box.
- Click the next to the **Mods and Profiles** parameter.
- Double-click **Protein Digest (Reduced+Alkylated)**.
- Click **OK** in the **Select Modifications and Profiles** dialog box.
- 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.

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

8 Review the Matching Rules.

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


9 Save the method for use in Exercise 7.

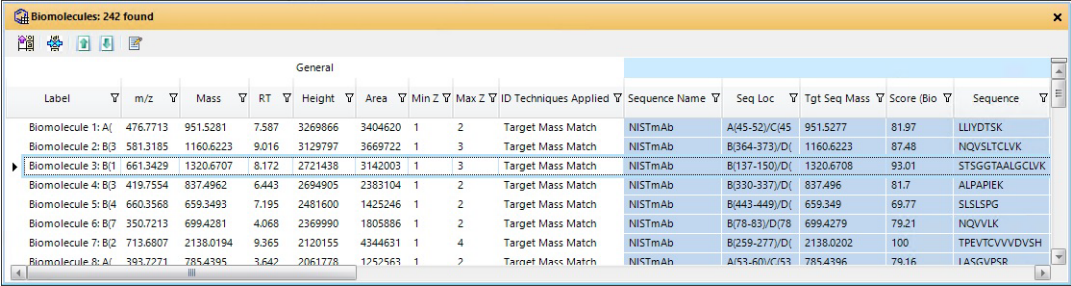
- Click **Method > Save As**.
- Type the **File name** `iii_NIST_mAb_ProteinDigest.m`, where *iii* represents your initials.
- Click **Save**.
- If needed, enter a reason on the **Reason for Change** dialog box and click **OK**.

- 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.

Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

Steps	Detailed Instructions	Comments
10 Start the match search.	<ol style="list-style-type: none"> Click Find and Identify > Match Sequences > Match Sequences (Proteins). Select NIST mAb Digest.d. Click Match. 	<p><i>Alternate methods:</i></p> <ul style="list-style-type: none"> Click  on the Method Editor toolbar. Click Match Sequences on the Method Editor shortcut menu.
11 Review the results.	<ol style="list-style-type: none"> Highlight Biomolecule 3 in the Biomolecules window. Click the Biomolecule Identification Results tab which is tabbed with the Biomolecules window. When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window. Select another sequence match result to view by selecting a different biomolecule in the Biomolecules window. 	<ul style="list-style-type: none"> If the biomolecule was identified, the ID Techniques Applied column contains Target Mass Match.



Biomolecules: 242 found													
General													
Label	m/z	Mass	RT	Height	Area	Min Z	Max Z	ID Techniques Applied	Sequence Name	Seq Loc	Tgt Seq Mass	Score (Bio)	Sequence
Biomolecule 1: A1	476.7713	951.5281	7.587	3269866	3404620	1	2	Target Mass Match	NISTmAb	A(45-52)/C(45)	951.5277	81.97	LLVDTSK
Biomolecule 2: B(3)	581.3185	1160.6223	9.016	3129797	3669722	1	3	Target Mass Match	NISTmAb	B(364-373)/D(1)	1160.6223	87.48	NQVSLTCLVK
Biomolecule 3: B(1)	661.3429	1320.6707	8.172	2721438	3142003	1	3	Target Mass Match	NISTmAb	B(137-150)/D(1)	1320.6708	93.01	STSGGTAALGCLVK
Biomolecule 4: B(3)	419.7554	837.4962	6.443	2694905	2383104	1	2	Target Mass Match	NISTmAb	B(330-337)/D(1)	837.496	81.7	ALPAPIEK
Biomolecule 5: B(4)	660.3568	659.3493	7.195	2481600	1425246	1	2	Target Mass Match	NISTmAb	B(443-449)/D(1)	659.349	69.77	SLSLSPG
Biomolecule 6: B(7)	350.7213	699.4281	4.068	2369990	1805886	1	2	Target Mass Match	NISTmAb	B(78-83)/D(78)	699.4279	79.21	NQVVLK
Biomolecule 7: B(2)	713.6807	2138.0194	9.365	2120155	4344631	1	4	Target Mass Match	NISTmAb	B(259-277)/D(1)	2138.0202	100	TPEVTCVVVDVSH
Biomolecule 8: A1	393.2771	785.4395	3.642	2061778	1752563	1	2	Target Mass Match	NISTmAb	A(53-60)/C(53)	785.4396	79.16	IASGVPSR

12 View sequence coverage results.	<ol style="list-style-type: none"> If necessary, click View > Sequence Coverage Map. Select a different biomolecule in the Biomolecules table to view a different result. 	<ul style="list-style-type: none"> 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.
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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**

Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

Steps

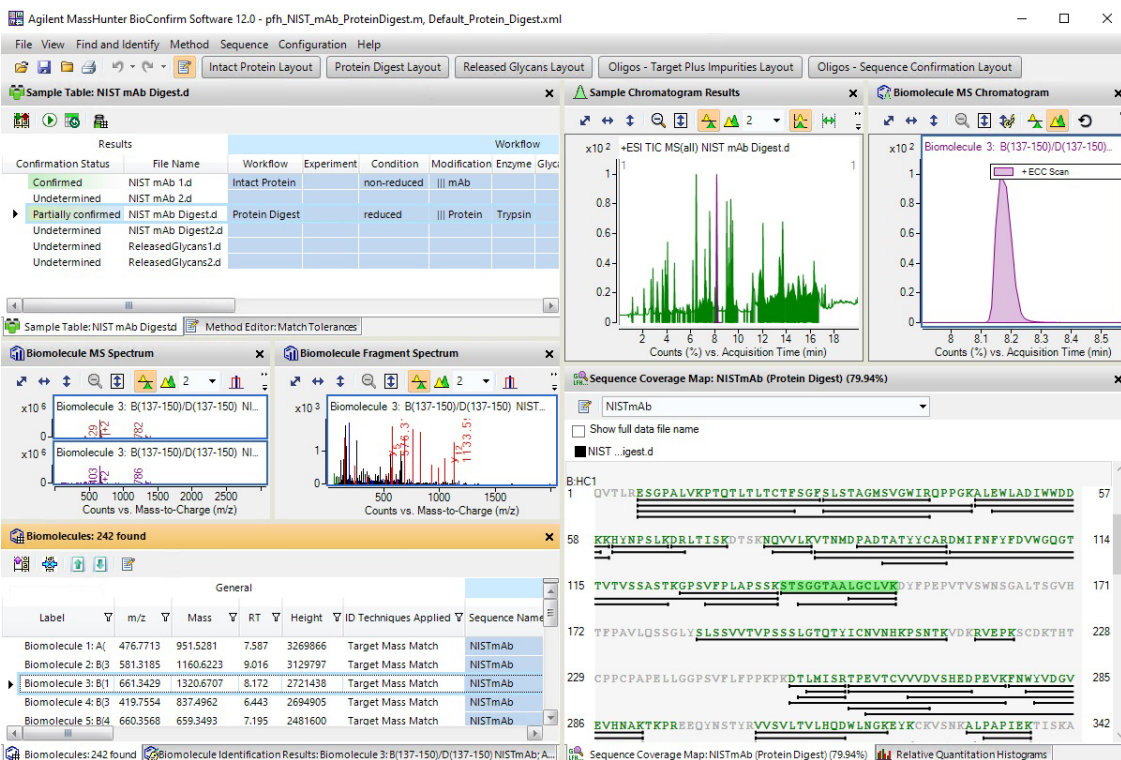
13 Save the method and results

Detailed Instructions

- Click **Method > Save**.
- If needed, enter a reason on the **Reason for Change** dialog box, and click **OK**.
- Click **File > Save Results** to save your results to the data file folder.
- If needed, enter a reason on the **Reason for Change** dialog box and click **OK**.

Comments

- You can also click  to save results.



Agilent MassHunter BioConfirm Software 12.0 - pfh_NIST_mAb_ProteinDigest.m, Default_Protein_Digest.xml

File View Find and Identify Method Sequence Configuration Help

Intact Protein Layout Protein Digest Layout Released Glycans Layout Oligos - Target Plus Impurities Layout Oligos - Sequence Confirmation Layout

Sample Table: NIST mAb Digest.d

Confirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glyc
Confirmed	NIST mAb 1.d	Intact Protein		non-reduced	mAb		
Undetermined	NIST mAb 2.d						
Partially confirmed	NIST mAb Digest.d	Protein Digest		reduced	Protein	Trypsin	
Undetermined	NIST mAb Digest2.d						
Undetermined	ReleasedGlycans1.d						
Undetermined	ReleasedGlycans2.d						

Sample Chromatogram Results

Biomolecule MS Chromatogram

Biomolecule MS Spectrum

Biomolecule Fragment Spectrum

Sequence Coverage Map: NISTmAb (Protein Digest) (79.94%)

NISTmAb

Show full data file name

NIST...igest.d

Label	m/z	Mass	RT	Height	ID Techniques Applied	Sequence Name
Biomolecule 1: A	476.7713	951.5281	7.587	3269866	Target Mass Match	NISTmAb
Biomolecule 2: B	581.3185	1160.6223	9.016	3129797	Target Mass Match	NISTmAb
Biomolecule 3: B(1	661.3429	1320.6707	8.172	2721438	Target Mass Match	NISTmAb
Biomolecule 4: B(3	419.7554	837.4962	6.443	2694905	Target Mass Match	NISTmAb
Biomolecule 5: B(4	660.3568	659.3493	7.195	2481600	Target Mass Match	NISTmAb

Biomolecules: 242 found

General

Biomolecules: 242 found Biomolecule Identification Results: Biomolecule 3: B(137-150)/D(137-150) NISTmAb; A...

Sequence Coverage Map: NISTmAb (Protein Digest) (79.94%) Relative Quantitation Histograms

B:HC1

1 QVTLRESGPALVKPTQTLLTCTCFSGPFLSTAGMSVGIQPPGKALEWLADIWDD 57

58 KKHYNPSLKPRLTISKDTSKNOVVLKVTNMDPADTATYYCARDMIFNFPDVMGQGT

115 TTVSSASTKGPSVFPFLAPSSKSTSGGTAALGCLVDFYFPEPVTVSWNSGALTSVGE

172 TFPVAVLQSSGLYSLSSVTVFPSSLGTQTYICNVNHPKSNTKVDRVEPKSCDRKHT

229 CFPCCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGV

286 EVHNAKTKPREEQYNSTYRIVVSVLTVLHODWLNKGEYKCRVSNRALPAPIEKATISKA

14 Review the results in the Peptide Relative Quantitation Results window.

- 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.
- Click the first triangle next to the first row.
- 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.

Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

Steps

Detailed Instructions

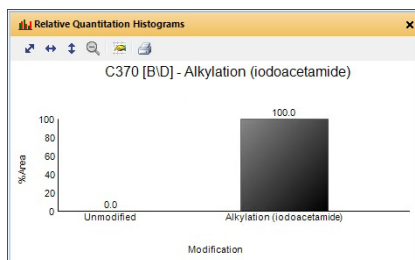
Comments

Location	Variable Mods	File	%Quant (Decon Height)	Height	%Quant (Deco)	Area
C370 [B.D]	Alkylation (iodoaceta	NIST mAb Digest.	100	3558120	100	393690
Sequence	Seq Loc	Fixed Mods	Variable Mods	Use for %Quant	Height	Area
NQVSLTCLVK	B(364-373)		Alkylation (iodoaceta	<input checked="" type="checkbox"/>	3129797	366972
NQVSLTCLVK	B(364-373)		Deamidation 2, Alkylat	<input checked="" type="checkbox"/>	152188	125668
NQVSLTCLVK	B(364-373)		Deamidation 2, Alkylat	<input checked="" type="checkbox"/>	138860	691534
NQVSLTCLVK	B(364-373)		Deamidation 2, Alkylat	<input checked="" type="checkbox"/>	137275	723575
C147 [B.D]	Alkylation (iodoaceta	NIST mAb Digest.	100	2929746	100	323553
C264 [B.D]	Alkylation (iodoaceta	NIST mAb Digest.	99.56	3314909	99.84	568181

15 Review the results in the Relative Quantitation Histograms window.

- Click the **Relative Quantitation Histograms** window.
- Click the first triangle next to the first row.
- 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*.



- If needed, open the data file **NIST mAb Digest2.d** (see [step 3](#)).
- Select Find Peptides in the Method Editor and verify the parameters([step 4](#)).
- Find biomolecules ([step 5](#)).
- Match sequences ([step 10](#)).
- 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.	<ol style="list-style-type: none"> a Click Method > Open. b Select the <i>iii_NIST_mAb_ProteinDigest.m</i> folder. c Click Open. 	<ul style="list-style-type: none"> • This method was created in Exercise 3 (<i>iii</i> represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	<ol style="list-style-type: none"> a If the Method Editor is not visible, click View > Method Editor. b Click Method Automation > Workflow and Sequences in the Method Editor window. 	<ul style="list-style-type: none"> • You can instead click the Method Editor button, , on the main toolbar.
3 Select the appropriate workflow.	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • The Protein Digest workflow automatically runs the following actions: <ul style="list-style-type: none"> • Find Peptides • Match Sequences
4 Save the method.	<ol style="list-style-type: none"> a Click Method > Save. b If needed, enter a reason for the change. 	
5 Run the method workflow or run method automation.	<ul style="list-style-type: none"> • 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. 	<ul style="list-style-type: none"> • 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.	<ul style="list-style-type: none"> • 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. 	<ul style="list-style-type: none"> • 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

Exercise 4. Automated Protein Digest Workflow

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.	<ol style="list-style-type: none"> a Click Method > Open. b Select the BioConfirmReleasedGlycans-Default.m folder. c Click Open. 	<ul style="list-style-type: none"> • The parameters in the BioConfirmReleasedGlycans-Default.m method are a good starting point for Released Glycans.
2 Open the example sample file.	<ol style="list-style-type: none"> a If needed, click File > Open Data File. b Select ReleasedGlycans1.d. c Click Open. 	<ul style="list-style-type: none"> • The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Released Glycans layout.	<ul style="list-style-type: none"> • Click Released Glycans Layout in the main toolbar. 	
4 Review the parameters in the Find Glycans section in the Method Editor window.	<ol style="list-style-type: none"> a Select Method Automation > Workflow and Sequences in the Method Editor window. b Select Released Glycans. c Enter <code>Example</code> 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 <code>Glycans_mAb_AM_PCD.cdb</code> 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. 	<ul style="list-style-type: none"> • 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 <i>online Help</i>. • Some structures are included in the <code>Glycans_mAb_AM_PCD.cdb</code> 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.	<ol style="list-style-type: none"> 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.	<ol style="list-style-type: none"> a Click Method > Save As. b Type the File name <code>iii_ReleasedGlycans_InstantPC.m</code>, where <i>iii</i> represents your initials. c Click Save. d If needed, enter a reason on the Reason for Change dialog box. Click OK. 	

Released Glycans Workflow

Exercise 5. Interactive Released Glycans

Steps

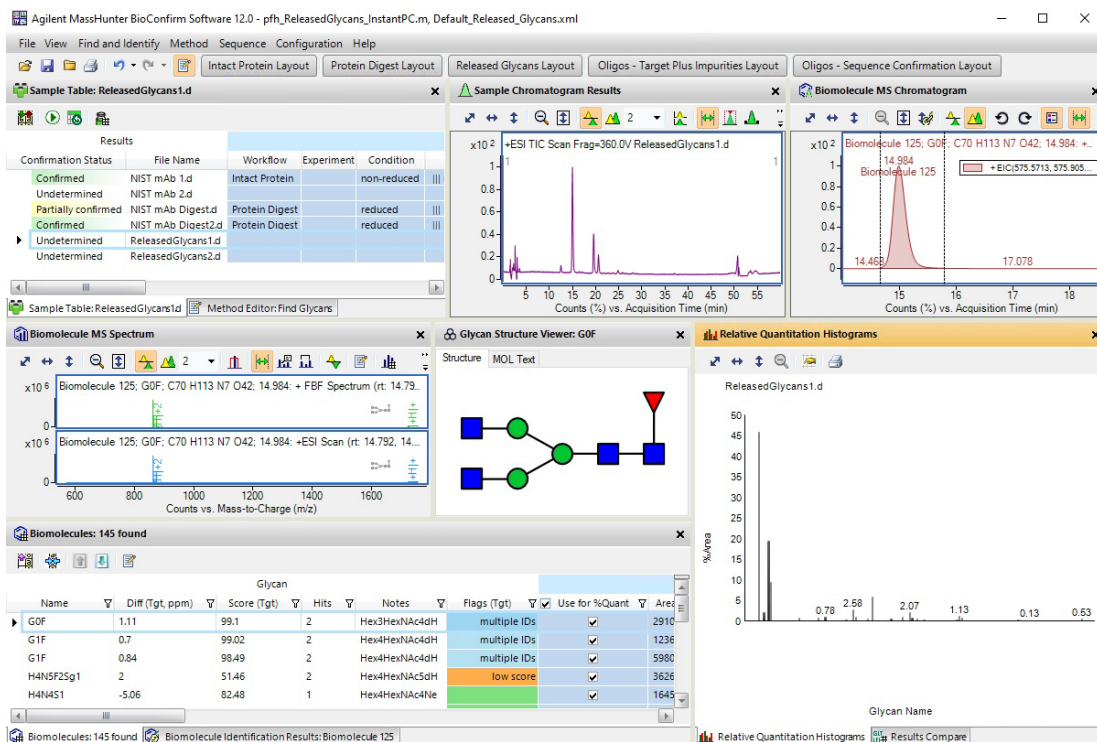
7 Review the results.

Detailed Instructions

- 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.
- Highlight **GOF** in the Biomolecules window.
- Click the **Biomolecule Identification Results** tab. The Biomolecule Identification Results window is tabbed with the Biomolecules window.
- When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window.

Comments

- 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


- Click **File > Save Results** to save your results to the data file folder.
- If needed, enter a reason for the change.

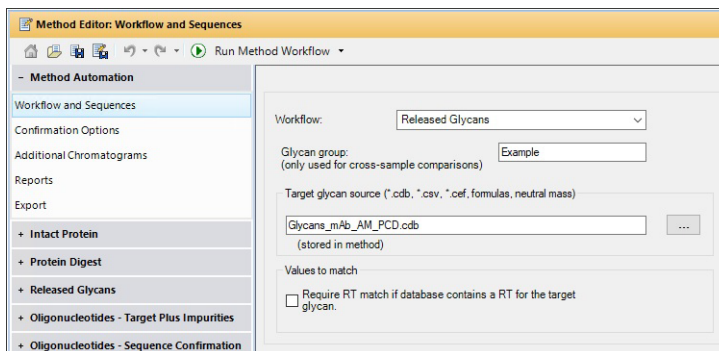
- You can also click  to save results.

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.	<ol style="list-style-type: none"> Click Method > Open. Select the <code>iii_ReleasedGlycans_InstantPC.m</code> folder. Click Open. 	<ul style="list-style-type: none"> This method was created in Exercise 5 (<i>iii</i> represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	<ol style="list-style-type: none"> If the Method Editor is not visible, click View > Method Editor. Click Method Automation > Workflow and Sequences in the Method Editor window. 	<ul style="list-style-type: none"> You can instead click the Method Editor button, , on the main toolbar.
3 Select the appropriate workflow.	<ol style="list-style-type: none"> Select Released Glycans for the Workflow. Enter <code>Example</code> in the Glycan group. Select <code>Glycans_mAb_AM_PCD.cdb</code> as the Target glycan source. Clear the Require RT match if database contains a RT for the target glycan check box. 	<ul style="list-style-type: none"> 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.	<ol style="list-style-type: none"> Click Method > Save. If needed, enter a reason for the change in the Reason for Change dialog box. 	<ul style="list-style-type: none"> The administrator can set up a project to require you to enter a reason for a change to a method



Released Glycans Workflow

Exercise 6. Automated Released Glycans Workflow

Steps	Detailed Instructions	Comments
5 Run the method workflow or run method automation.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.

Released Glycans Workflow

Exercise 6. Automated Released Glycans Workflow

Steps

Detailed Instructions

Comments

The screenshot displays the Agilent MassHunter BioConfirm Software interface for the Released Glycans workflow. The main window is titled 'Sample Table: ReleasedGlycans1.d' and shows a table of results. The table has columns for Confirmation Status, File Name, Workflow, Experiment, Condition, and Modification. The results are as follows:

Confirmation Status	File Name	Workflow	Experiment	Condition	Modification
Confirmed	NIST mAb 1.d	Intact Protein		non-reduced	mAb
Undetermined	NIST mAb 2.d				
Partially confirmed	NIST mAb Digest.d	Protein Digest		reduced	Protein Digest (Reduc
Confirmed	NIST mAb Digest2.d	Protein Digest		reduced	Protein Digest (Reduc
Undetermined	ReleasedGlycans1.d	Released Glycans			
Undetermined	ReleasedGlycans2.d				

The 'Results Compare' window shows a table of glycan data for the 'Released Glycans' group. The table has columns for Glycan Name, RSD (%), %Quant (FBF), Area, and RT. The data is as follows:

Glycan Name	RSD (%)	%Quant (FBF)	Area	RT
H6N6F1S2	0	0.04	909	51.194
H6N5F1S1	0	0.03	1285	50.936
H6N5S1	0.04	0.04	25193	38.16
H6N4F1S1	0.01	0.01	6994	37.303
H5N4F2S1	0	0.02	299	40.662

7 Review the results in the Results Compare window.

- Click the **Results Compare** window.
- Click the **Released Glycans** tab.
- 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.

The screenshot shows the 'Results Compare' window with the 'Released Glycans' tab selected. The table displays the following data:

Glycan Name	RSD (%)	%Quant (FBF)	Area	RT
H6N6F1S2	0	0.04	909	51.194
H6N5F1S1	0	0.03	1285	50.936
H6N5S1	0.04	0.04	25193	38.16
H6N4F1S1	0.01	0.01	6994	37.303
H5N4F2S1	0	0.02	299	40.662

Released Glycans Workflow

Exercise 6. Automated Released Glycans Workflow

Steps	Detailed Instructions	Comments
8 Save the results for the current data file and close all data files.	<p>a Click File > Save Results.</p> <p>b If needed, enter or select a reason in the Reason for Change dialog box. Click OK.</p> <p>c Click File > Close All.</p> <p>d Click No to save results.</p>	<ul style="list-style-type: none">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

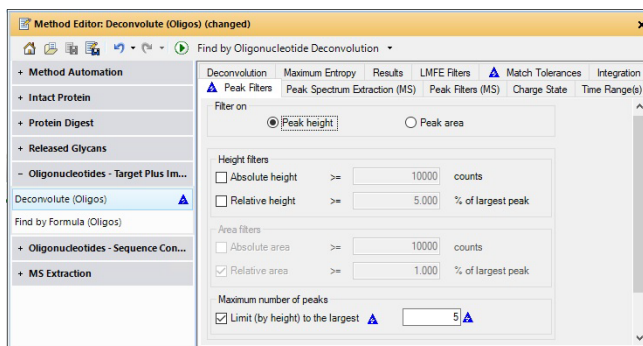
Relative Quantitation Histograms

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.

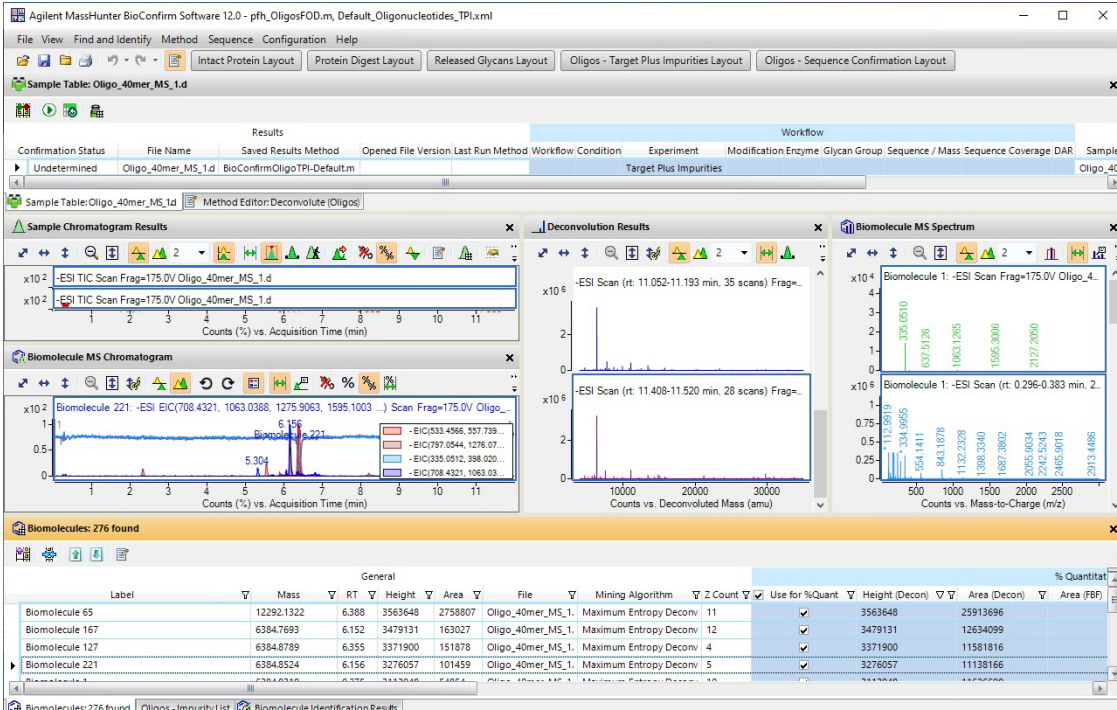
Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	<ol style="list-style-type: none"> Click Method > Open. Select the BioConfirmOligosTPI-Default.m folder. Click Open. 	<ul style="list-style-type: none"> The parameters in the BioConfirmOligosTPI-Default.m method are a good starting point for the Target Plus Impurities experiment.
2 Open the example sample file.	<ol style="list-style-type: none"> If needed, click File > Open Data File. Select Oligo_40mer_MS_1.d. Click Open. 	<ul style="list-style-type: none"> The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Target Plus Impurities layout.	<ul style="list-style-type: none"> 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.	<ol style="list-style-type: none"> Select Oligonucleotides - Target Plus Impurities > Deconvolute (Oligos) in the Method Editor window. Click the Match Tolerances tab. Select Da and enter 1.0 for the Tolerance. Click the Peak Filters tab. Mark Limit (by height) to the largest and enter 5. 	



Oligonucleotide Workflow - Target Plus Impurities


Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
5 Run the Find by Oligonucleotide Deconvolution algorithm.	<ol style="list-style-type: none"> Click  on the Method Editor toolbar to start the biomolecule search. When processing is complete, review the results in the Biomolecules window. 	
6 Save the method.	<ol style="list-style-type: none"> Click Method > Save As. Type the File name <i>iii_OligosFOD.m</i>, where <i>iii</i> represents your initials. Click Save. If needed, enter a reason on the Reason for Change dialog box. Click OK. 	
7 Review the results.	<ul style="list-style-type: none"> When you run the Find by Oligonucleotide Deconvolution algorithm, the Oligos - Impurity List and the Biomolecule Identification Results window are empty. 	<ul style="list-style-type: none"> Several columns have been changed from the default Oligos - Target Plus Impurities Layout.



The screenshot displays the Agilent MassHunter BioConfirm Software 12.0 interface. The main window shows the 'Oligos - Target Plus Impurities' workflow. The 'Biomolecules: 276 found' table is visible at the bottom, listing various biomolecules with their mass, retention time, height, area, and file information.

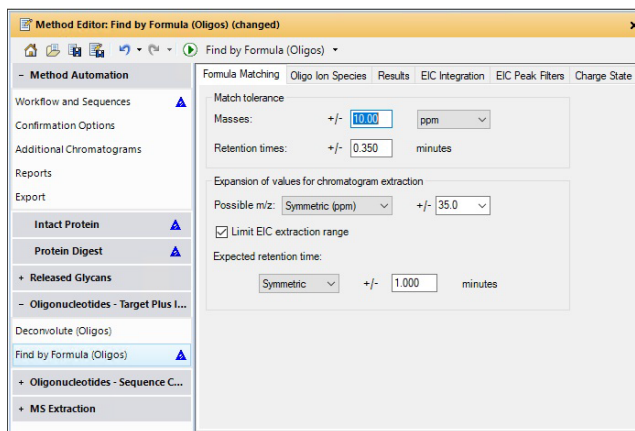
Label	Mass	RT	Height	Area	File	Mining Algorithm	Z Count	Use for %Quant	Height (Deconv)	Area (Deconv)	% Quantit
Biomolecule 65	12292.1322	6.388	3563648	2758807	Oligo_40mer_MS_1	Maximum Entropy Deconv	11	<input checked="" type="checkbox"/>	3563648	25913696	
Biomolecule 167	6384.7693	6.152	3479131	163027	Oligo_40mer_MS_1	Maximum Entropy Deconv	12	<input checked="" type="checkbox"/>	3479131	12634099	
Biomolecule 127	6384.8789	6.355	3371900	151878	Oligo_40mer_MS_1	Maximum Entropy Deconv	4	<input checked="" type="checkbox"/>	3371900	11581816	
Biomolecule 221	6384.8524	6.156	3276057	101459	Oligo_40mer_MS_1	Maximum Entropy Deconv	5	<input checked="" type="checkbox"/>	3276057	11138166	

8 Save the results.	<ol style="list-style-type: none"> Click File > Save Results to save your results to the data file folder. If needed, enter a reason for the change. 	<ul style="list-style-type: none"> You can also click  to save results.
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Oligonucleotide Workflow - Target Plus Impurities


Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

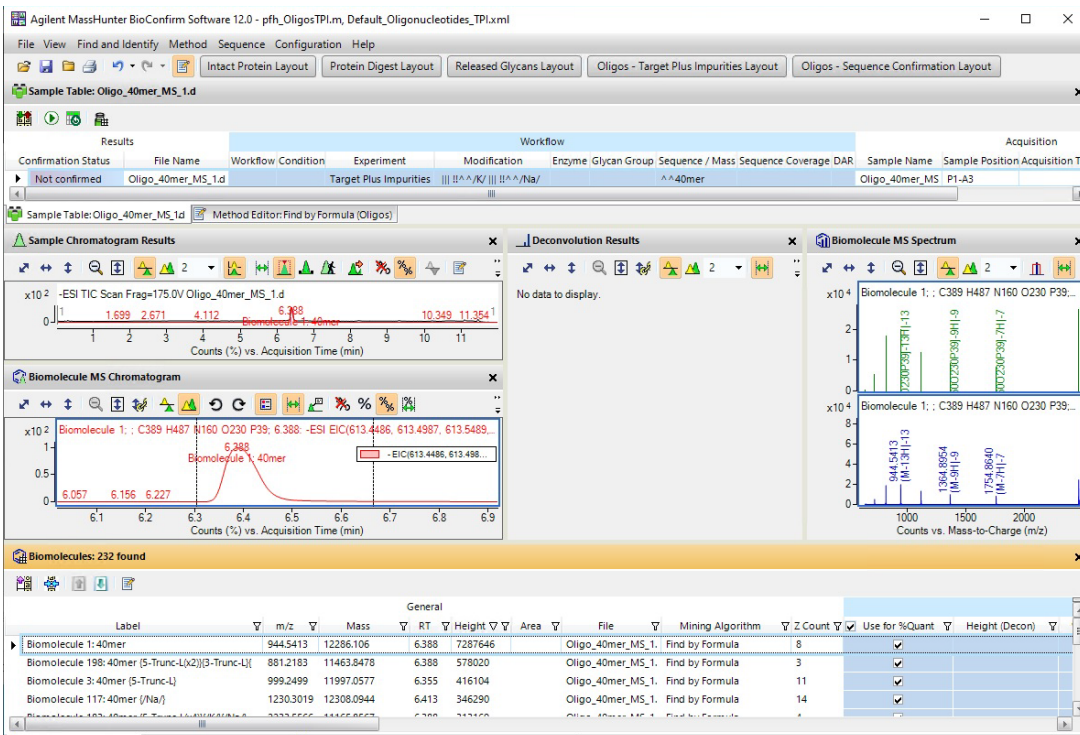
Steps	Detailed Instructions	Comments
9	<p>Select parameters in the Method Automation > Workflow and Sequences section in the Method Editor window.</p> <ul style="list-style-type: none">Select 40mer as the Sequence.Select /K/ and /Na/ for the Mods and Profiles.	<ul style="list-style-type: none">You need 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	<p>Review the parameters in the Oligonucleotides - Target Plus Impurities > Find by Formula (Oligos) section in the Method Editor window.</p>	<ul style="list-style-type: none">You can limit the number of biomolecules found by adding filters.



Oligonucleotide Workflow - Target Plus Impurities


Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
11 Run the Find by Formula (Oligos) algorithm.	<ol style="list-style-type: none"> Click  on the Method Editor toolbar to start the biomolecule search. When processing is complete, review the results in the Biomolecules window. 	<ul style="list-style-type: none"> You can instead click Find and Identify > Find by Formula (Oligos).
12 Save the method for use in Exercise 8.	<ol style="list-style-type: none"> Click Method > Save As. Type the File name <i>iii_OligoTPI.m</i>, where <i>iii</i> represents your initials. Click Save. If needed, enter a reason on the Reason for Change dialog box. Click OK. 	<ul style="list-style-type: none"> 4 compounds and 2 impurities were found when running the Find by Formula (Oligos) algorithm.



The screenshot displays the Agilent MassHunter BioConfirm Software 12.0 interface. The main window shows the 'Results' table with columns for Confirmation Status, File Name, Workflow, Condition, Experiment, Modification, Enzyme, Glycan Group, Sequence / Mass, Sequence Coverage, DAR, Sample Name, and Sample Position. The 'Sample Chromatogram Results' panel shows a TIC scan with peaks at 1.699, 2.671, 4.112, 6.388, 10.349, and 11.354 minutes. The 'Biomolecule MS Chromatogram' panel shows a chromatogram for Biomolecule 1 (C389 H487 N160 O230 P39) with peaks at 6.057, 6.156, 6.227, and 6.388 minutes. The 'Biomolecule MS Spectrum' panel shows a mass spectrum for Biomolecule 1 with peaks at m/z 944.5413, 1394.8954, 1754.8940, and 2002.2349. The 'Biomolecules: 232 found' table lists the following results:


Label	m/z	Mass	RT	Height	Area	File	Mining Algorithm	Z Count	Use for %Quant	Height (Decon)
Biomolecule 1: 40mer	944.5413	12286.106	6.388	7287646		Oligo_40mer_MS_1	Find by Formula	8	<input checked="" type="checkbox"/>	
Biomolecule 198: 40mer (S-Trunc-L)(2)(3-Trunc-L)	881.2183	11463.8478	6.388	578020		Oligo_40mer_MS_1	Find by Formula	3	<input checked="" type="checkbox"/>	
Biomolecule 3: 40mer (S-Trunc-L)	999.2499	11997.0577	6.355	416104		Oligo_40mer_MS_1	Find by Formula	11	<input checked="" type="checkbox"/>	
Biomolecule 117: 40mer (Na)	1230.3019	12308.0944	6.413	346290		Oligo_40mer_MS_1	Find by Formula	14	<input checked="" type="checkbox"/>	

13 Save the results.	<ol style="list-style-type: none"> Click File > Save Results to save your results to the data file folder. If needed, enter a reason for the change. 	<ul style="list-style-type: none"> You can also click  to save results.
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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.

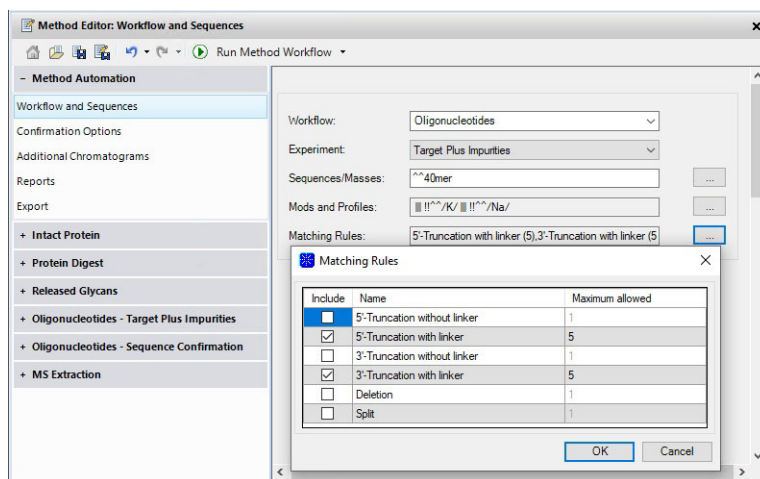
If you select the Oligonucleotides - Target Plus Impurities workflow, the **Find by Oligonucleotide Deconvolution** algorithm or the **Find by Formula (Oligos)** algorithm runs.

Steps	Detailed Instructions	Comments
1 Open the method.	<ul style="list-style-type: none"> a Click Method > Open. b Select the <i>iii_OligosTPI.m</i> folder. c Click Open. 	<ul style="list-style-type: none"> • This method was created in Exercise 7 (<i>iii</i> represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	<ul style="list-style-type: none"> a If the Method Editor is not visible, click View > Method Editor. b Click Method Automation > Workflow and Sequences in the Method Editor window. 	<ul style="list-style-type: none"> • You can instead click the Method Editor button, , on the main toolbar.

Oligonucleotide Workflow - Target Plus Impurities


Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
3	<p>Select the appropriate workflow.</p> <ol style="list-style-type: none"> Select Method Automation > Workflow and Sequences in the Method Editor window. Select Oligonucleotides. Select Target Plus Impurities as the Experiment. Click the <input type="button" value="..."/> button next to the Sequences/Masses parameter. The Select Sequences dialog box opens. Double-click 40mer. If 40mer is not available, click Add. Select Oligo_40mer_MS.psq and click Open. Verify that the 40mer sequence is in the Selected list. Click OK. Click the <input type="button" value="..."/> button next to the Mods and Profiles parameter. Select /K/ and /Na/ from the Available list. Click OK. Click <input type="button" value="..."/> next to Matching Rules. Mark 5'-Truncation with linker and 3'-Truncation with linker. Enter 5 for Maximum allowed for each. Click OK. 	<ul style="list-style-type: none"> The Matching Rules parameter shows the rules that are marked, and it shows the Maximum allowed in parentheses after the rule.



Oligonucleotide Workflow - Target Plus Impurities

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
4 Verify Confirmation Options.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• Click Method > Save.• If needed, enter a reason for the change in the Reason for Change dialog box.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">• 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.

Oligonucleotide Workflow - Target Plus Impurities

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

Steps

Detailed Instructions

Comments

The screenshot displays the Agilent MassHunter BioConfirm Software 12.0 interface for the Oligos - Target Plus Impurities workflow. The main window shows the 'Results' tab with a table of biomolecules. Below the table, there are three panels: 'Sample Chromatogram Results' showing TIC scans, 'Biomolecule MS Chromatogram' showing a peak at 6.308 minutes, and 'Deconvolution Results' showing mass spectra for the peak. A 'Biomolecules: 276 found' table is visible at the bottom.

Label	Mass	RT	Height	Area	File	Mining Algorithm	Z Count	Use for %Quant	Height (Decon)	%Quant (Decon Height)	Area (Dec)
Biomolecule 70	24584.4974	6.388	142950	1052354	Oligo_40mer_MS_1_1	Maximum Entropy Deconv	12	<input checked="" type="checkbox"/>	142950	0.39	1352731
Biomolecule 45	28083.8597	6.388	17328	6220390	Oligo_40mer_MS_1_1	Maximum Entropy Deconv	12	<input checked="" type="checkbox"/>	17328	0.05	94201
Biomolecule 5	7560.8219	6.384	169292	4263834	Oligo_40mer_MS_1_1	Maximum Entropy Deconv	12	<input checked="" type="checkbox"/>	169292	0.46	679522
Biomolecule 6	4720.8233	11.077	215792	3276719	Oligo_40mer_MS_1_1	Maximum Entropy Deconv	9	<input checked="" type="checkbox"/>	215792	0.58	974601

8 Review the results in the Results Compare window.

- Click the **Results Compare** window.
- Click the **Oligos - Target Plus Impurities** tab.
- 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.

The screenshot shows the 'Results Compare' window with the 'Oligos - Target Plus Impurities' tab selected. The table below displays the results for the 40mer sequence.

Oligo ID	Average (Height, MS)	%RSD (Height, MS)	%Quant (Decon Height)	Height (Decon)
CpCpApCpGpAp	3563648		9.62	3563648
ApCpCpApApGpT	3593773		0.97	3593773
CpCpApCpGpAp	77303		0.21	77303
CpGpApCpCpAp	67959		0.18	67959
	15802		0.04	15802

Oligonucleotide Workflow - Target Plus Impurities

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
9 Save the results for the current data file and close all data files.	<p>a Click File > Save Results.</p> <p>b If needed, enter or select a reason in the Reason for Change dialog box. Click OK.</p> <p>c Click File > Close All.</p> <p>d Click No to save results.</p>	<ul style="list-style-type: none">• 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.	<ol style="list-style-type: none"> a Click Method > Open. b Select the BioConfirmOligosSC-Default.m folder. c Click Open. 	<ul style="list-style-type: none"> • The parameters in the BioConfirmOligosSC-Default.m method are a good starting point for the Sequence Confirmation experiment.
2 Open the example sample file.	<ol style="list-style-type: none"> a If needed, click File > Open Data File. b Select 40mer_MSMS_14CS_14CE.d. c Click Open. 	<ul style="list-style-type: none"> • The TIC is automatically displayed in the Sample Chromatogram Results window.
3 Load the Oligonucleotides - Sequence Confirmation layout.	<ul style="list-style-type: none"> • Click Oligonucleotides - Sequence Confirmation Layout in the main toolbar. 	
4 Review the parameters in the Find Glycans section in the Method Editor window.	<ol style="list-style-type: none"> 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 <input type="text" value="..."/> 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. 	<ul style="list-style-type: none"> • 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 <i>online Help</i>.
5 Run the Find Oligonucleotide Fragments algorithm.	<ol style="list-style-type: none"> a Click  on the Method Editor toolbar to start the biomolecule search. b When processing is complete, review the results in the Biomolecules window. 	<ul style="list-style-type: none"> • You can instead click Find and Identify > Find Oligonucleotide Fragments. • By default, all results are selected after you run this algorithm.

Oligonucleotide Workflow - Sequence Confirmation

Exercise 9. Interactive Oligonucleotides - Sequence Confirmation Workflow

Steps	Detailed Instructions	Comments
6 Save the method for use in Exercise 10.	<ul style="list-style-type: none">a Click Method > Save As.b Type the File name <i>iii_40merMSMS_OligosSC.m</i>, where <i>iii</i> represents your initials.c Click Save.d If needed, enter a reason on the Reason for Change dialog box. Click OK.	

Oligonucleotide Workflow - Sequence Confirmation

Exercise 9. Interactive Oligonucleotides - Sequence Confirmation Workflow

Steps

7 Review the results.

Detailed Instructions

- 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.
- Select the first biomolecule in the Biomolecules window.
- Click the **Biomolecule Identification Results** tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window.
- When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window.

Comments

- 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.

The screenshot displays the Agilent MassHunter BioConfirm Software 12.0 interface. The main window is titled "Sample Table: 40mer_MSMS_14CS_14CE.d" and shows a table of results. The "Confirmed" row is selected, showing a file name of "40mer_MSMS_14CS_14CE.d", a workflow of "Oligonucleotides", and a sequence of "40mer_MSMS_95".

Below the table, the "Biomolecule Fragment Spectrum" window is open, showing a mass spectrum plot of "Biomolecule 60: w1 -1 40-40 Oligo_40mer_MSMS -ESI Product Ion (rt: 5.781, 5.798, 5.861 min, 3 scans) Frag1...". The x-axis is "Counts vs. Mass-to-Charge (m/z)" and the y-axis is "x10^4". Several peaks are labeled with their m/z values: 288, 386, 311, 419, 675, and 1219.

To the right, the "Fragment Confirmation Ladder: Oligo_40mer_MSMS (Oligonucleotide) (95.00%)" window is open, showing a ladder diagram of the sequence "G C A A T G A A T C G A G T C G A" with a selected biomolecule represented by an empty circle on one of the limbs.

At the bottom, the "Biomolecules: 118 found" window is open, showing a table of biomolecules. The first row is selected:

Label	m/z	Mass	Height	File	Ion Type	Z (Prod)	Sequence	Score (Bio)
Biomolecule 60: w1 -1 40-40 Oligo_40mer_MSMS	321.0492	322.0565	29543	40mer_MSMS_14CS_14CE.d w1	-1	pT		100
Biomolecule 11: a4-B -1 1-3 Oligo_40mer_MSMS	675.1219	676.1288	11518	40mer_MSMS_14CS_14CE.d a4-B	-1	CpCpA		100
Biomolecule 18: a7-B -2 1-6 Oligo_40mer_MSMS	802.635	1607.2841	11334	40mer_MSMS_14CS_14CE.d a7-B	-2	CpCpApCpGpA		98.41
Biomolecule 46: c1 -1 1-1 Oligo_40mer_MSMS	288.0386	289.046	11000	40mer_MSMS_14CS_14CE.d c1	-1	Cp		100
Biomolecule 48: c2 -2 1-2 Oligo_40mer_MSMS	288.0386	578.0924	11000	40mer_MSMS_14CS_14CE.d c2	-2	CpCp		95.23
Biomolecule 69: w5 -3 36-40 Oligo_40mer_MSMS	504.743	1517.2525	9637	40mer_MSMS_14CS_14CE.d w5	-3	pTpCpCpApT		99.57

8 Save the results


- Click **File > Save Results** to save your results to the data file folder.
- If needed, enter a reason for the change.

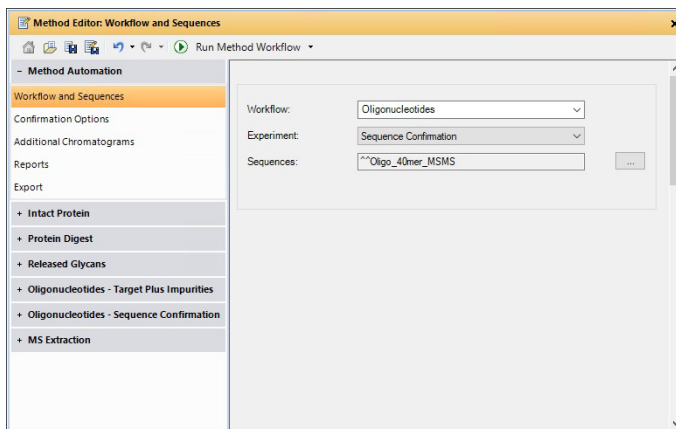
- You can also click  to save results.

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.	<ol style="list-style-type: none"> a Click Method > Open. b Select the <i>iii_40merMSMS_OligosSC.m</i> folder. c Click Open. 	<ul style="list-style-type: none"> • This method was created in Exercise 9 (<i>iii</i> represents your initials).
2 Display the Method Automation > Workflow and Sequences section in the Method Editor.	<ol style="list-style-type: none"> a If the Method Editor is not visible, click View > Method Editor. b Click Method Automation > Workflow and Sequences in the Method Editor window. 	<ul style="list-style-type: none"> • You can instead click the Method Editor button, , on the main toolbar.
3 Select the appropriate workflow.	<ol style="list-style-type: none"> a Select Oligonucleotides for the Workflow. b Select Sequence Confirmation for the Experiment. c Click the <input type="text" value="..."/> next to the Sequences parameter. d Select Oligo_40mer_MSMS and click OK. 	<ul style="list-style-type: none"> • You cannot enter a mass if you select the Sequence Confirmation experiment.
4 Save the method.	<ul style="list-style-type: none"> • Click Method > Save. • If needed, enter a reason for the change in the Reason for Change dialog box. 	<ul style="list-style-type: none"> • The administrator can set up a project to require you to enter a reason for a change to a method



Oligonucleotide Workflow - Sequence Confirmation

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

Steps	Detailed Instructions	Comments
5 Run the method workflow or run method automation.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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.

Oligonucleotide Workflow - Sequence Confirmation

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

Steps

Detailed Instructions

Comments

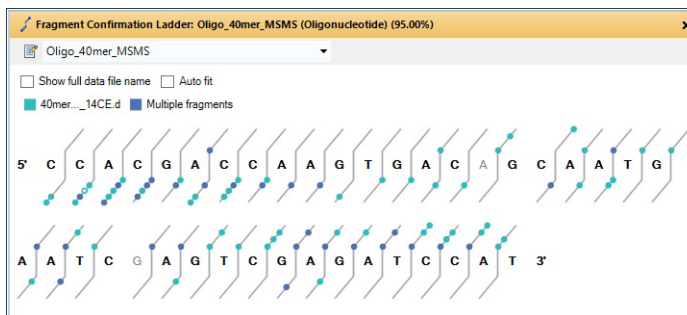
The screenshot displays the Agilent MassHunter BioConfirm Software interface. The main window shows the 'Sample Table' with a 'Confirmed' status for the sample '40mer_MSMS_14CS_14CE.d'. The 'Workflow' column indicates 'Oligonucleotides' and 'Sequence Confirmation'. The 'Sequence / Mass' column shows '40mer_MSMS_95'. The 'Sample Chromatogram Results' panel shows a single sharp peak at approximately 5.8 minutes. The 'Biomolecule Fragment Spectrum' panel shows a mass spectrum with a base peak at m/z 988.1774. The 'Fragment Confirmation Ladder' panel shows the sequence 'G C A A T G A A T C G A G T C G A' with a ladder of peaks corresponding to the sequence. The 'Biomolecules: 118 found' table is also visible.

Label	m/z	Mass	Height	File	Ion Type	Z (Prod)	Sequence	Score (Bio)	Diff (Bio, ppm)
Biomolecule 40: b2 -1-1-2 Oligo_40mer_MSMS	515.1295	516.1374	193	40mer_MSMS_14CS_14CE.d	b2	-1	CpC	93.58	0.74
Biomolecule 110: y8 -2-33-40 Oligo_40mer_MSMS	1195.2249	2392.4517	191	40mer_MSMS_14CS_14CE.d	y8	-2	ApGpApTpCpCpApT	81.12	-1.11
Biomolecule 42: b4 -2-1-4 Oligo_40mer_MSMS	558.1119	1118.238	170	40mer_MSMS_14CS_14CE.d	b4	-2	CpCpApC	98.12	-2.62
Biomolecule 53: c7 -3-1-7 Oligo_40mer_MSMS	702.7732	2111.3492	165	40mer_MSMS_14CS_14CE.d	c7	-3	CpCpApCpGpApCp	85.17	-1.91
Biomolecule 118: z22 -6-19-40 Oligo_40mer_MSMS	1123.3595	6746.1661	164	40mer_MSMS_14CS_14CE.d	z22	-6	ApApTpGpApApTpCpGpApGpTpCpGpApGpApTpCpCpApT	84.14	-0.99

Oligonucleotide Workflow - Sequence Confirmation

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

Steps	Detailed Instructions	Comments
<p>7 Review the results in the Fragment Confirmation Ladder window.</p>	<p>a Click the Fragment Confirmation Ladder window.</p> <ul style="list-style-type: none"> • 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. 	<ul style="list-style-type: none"> • 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.
<p>8 Save the results for the current data file and close all data files.</p>	<p>a Click File > Save Results.</p> <p>b If needed, enter or select a reason in the Reason for Change dialog box. Click OK.</p> <p>c Click File > Close All.</p> <p>d Click No to save results.</p>	<ul style="list-style-type: none"> • 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

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.

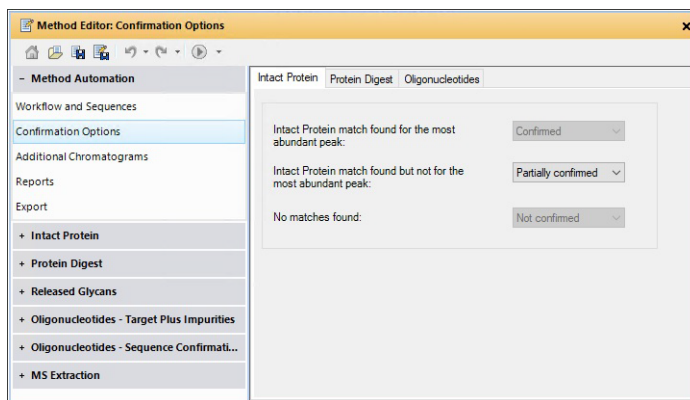
Steps	Detailed Instructions	Comments
1 Open several data files.	<p>a Click File > Open Data File.</p> <p>b Select these example files: NIST mAb 1.d, NIST mAb 2.d, NIST mAb Digest.d, NIST mAb Digest2.d, ReleasedGlycans1.d, and ReleasedGlycans2.d.</p> <p>c Mark the Load result data check box.</p> <p>d Click Open.</p>	<ul style="list-style-type: none">• 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.	<p>a Look at the Confirmation Status column.</p>	<ul style="list-style-type: none">• If you saved results, the table contains information on confirmation.

Results		Workflow							
Confirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glycan Group	Sequence / Mass	Sequence Coverage
Confirmed	NIST mAb 1.d	Intact Protein		non-reduced	mAb				NISTmAb
Undetermined	NIST mAb 2.d								
Partially confirmed	NIST mAb Digest.d	Protein Digest		reduced	Protein Digest (Reduced+Alkylated)	Trypsin			NISTmAb
Confirmed	NIST mAb Digest2.d	Protein Digest		reduced	Protein Digest (Reduced+Alkylated)	Trypsin			NISTmAb
Undetermined	ReleasedGlycans1.d	Released Glycans							Example
Undetermined	ReleasedGlycans2.d								

Review Results


Exercise 11. Reprocess Samples

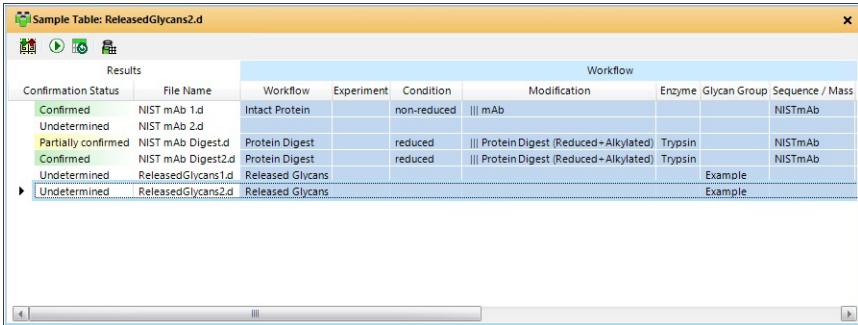
Steps	Detailed Instructions	Comments
3 Review values in Method Automation > Confirmation Options.	<ol style="list-style-type: none">Click View > Method Editor, if necessary.Select Method Automation > Confirmation Options.Click the Intact Protein tab.Review selection for the Intact Protein match found but not for the most abundant peak option.Click the Protein Digest tab.Review selection for the Protein is partially confirmed when sequence coverage is >= option.	<ul style="list-style-type: none">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.



Review Results

Exercise 11. Reprocess Samples

Steps	Detailed Instructions	Comments
4	<p>Reprocess the ReleasedGlycans2.d data file.</p> <ol style="list-style-type: none"> In the Sample Table, click the row containing ReleasedGlycans2.d. Click Method > Open. Select the <i>iii_ReleasedGlycans-InstantPC.m</i> folder. Click Open. Click the  button to open the Reprocess Sample dialog box. Select Released Glycans for the workflow. Enter <code>Example</code> for the Glycan group. Select Glycans_mAb_AM_PCD.cdb for the Target glycan source. Click Reprocess. 	<ul style="list-style-type: none"> 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.



Results		Workflow						
Confirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glycan Group	Sequence / Mass
Confirmed	NIST mAb 1.d	Intact Protein		non-reduced	mAb			NISTmAb
Undetermined	NIST mAb 2.d							
Partially confirmed	NIST mAb Digest.d	Protein Digest		reduced	Protein Digest (Reduced=Alkylated)	Trypsin		NISTmAb
Confirmed	NIST mAb Digest2.d	Protein Digest		reduced	Protein Digest (Reduced=Alkylated)	Trypsin		NISTmAb
Undetermined	ReleasedGlycans1.d	Released Glycans					Example	
Undetermined	ReleasedGlycans2.d	Released Glycans					Example	

5	<p>Save the results for the samples that you reprocessed.</p> <ol style="list-style-type: none"> Click File > Save Results. Click Save. If needed, enter or select a reason in the Reason for Change dialog box. Click OK.
---	--

Review Results

Exercise 12. Use Result Review mode

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.	<ul style="list-style-type: none">Click Configuration > Enable Result Review (Disables Method Editing).	<ul style="list-style-type: none">You can toggle this mode off by clicking this same command again.
2 Review results in Sample Table window.	<ul style="list-style-type: none">All of the options in this window are available except for the Run Method Workflow button. You can still reprocess samples.	

Results		Workflow						
Confirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glycan Group	Sequence / Mass
Confirmed	NIST mAb 1.d	Intact Protein		non-reduced	mAb			NISTmAb
Undetermined	NIST mAb 2.d							
Partially confirmed	NIST mAb Digest.d	Protein Digest		reduced	Protein Digest (Reduced+Alk	Trypsin		NISTmAb
Confirmed	NIST mAb Digest2.d	Protein Digest		reduced	Protein Digest (Reduced+Alk	Trypsin		NISTmAb
Undetermined	ReleasedGlycans1.d	Released Glycans					Example	
Undetermined	ReleasedGlycans2.d	Released Glycans					Example	

Review Results

Exercise 12. Use Result Review mode

Steps

3 Review and compare the *ReleasedGlycans1.d* and *ReleasedGlycans2.d* data files.

Detailed Instructions

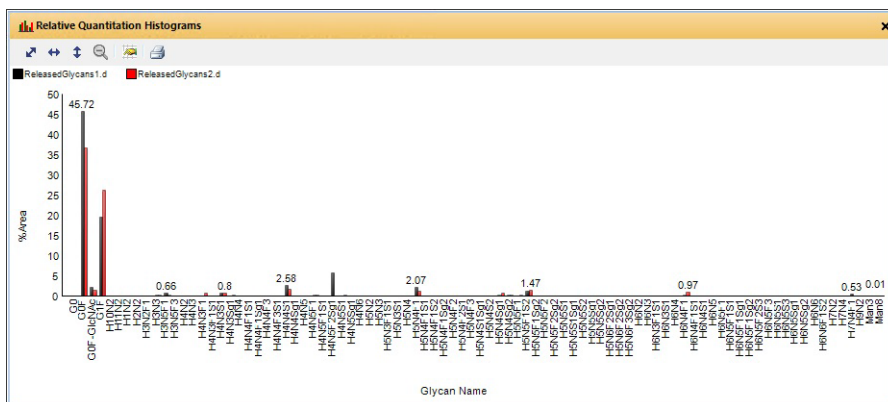
- In the Sample Table, click the row containing ReleasedGlycans1.d.
- Press the **Shift** key and click the row containing ReleasedGlycans2.d.
- Review the results in the Results Compare window. The RSD (%) column has been calculated for glycans that are in both samples.

Comments

Glycan Name	RSD (%)	ReleasedGlycans1.d			ReleasedGlycans2.d		
		%Quant (FBI)	Area	RT	%Quant (FBI)	Area	RT
G1F	9.5	9.39	59803751	20.611	9.24	52275585	20.602
H4N4S1	105	0.99	6330075	25.815	7.58	42841859	25.807
H5N3S1	1.4	0.81	5181136	28.035	0.9	5080633	28.018
H5N4F1	77	0.64	4097144	25.815	0.21	1209200	26.58
H4N3F1	59.5	0.56	3562747	19.572	0.26	1453310	20.595
H5N5F1S2	57.1	0.56	3539149	20.611	1.47	8335163	19.564
H7N4F1	4.9	0.53	3403961	37.021	0.56	3174200	37.021
H5N4F1S1	0.6	0.49	3114756	37.021	0.55	3086343	37.021
H5N2	7.1	0.46	2959099	16.438	0.47	2677458	16.447
H4N5F1	35	0.4	2523553	22.723	0.27	1523201	22.715
G0F-GlcNAc	93.6	0.3	1931147	19.572	1.68	9500305	14.992
H5N4S1Sg1	9.7	0.28	1807009	39.465	0.28	1576053	39.473
H4N4F1Sg1	43.6	0.25	1586348	30.662	0.15	839075	30.645

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.



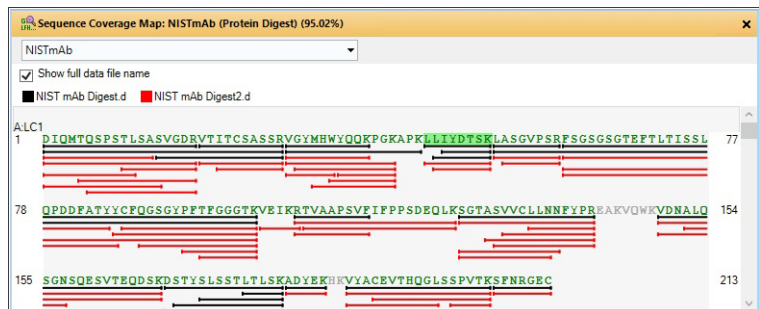
Review Results

Exercise 12. Use Result Review mode

Steps	Detailed Instructions	Comments
4 Review and compare the <i>NIST mAb Digest.d</i> and <i>NIST mAb Digest2.d</i> data files.	<ol style="list-style-type: none"> In the Sample Table, click the row containing NIST mAb Digest.d. Press the Shift key and click the row containing NIST mAb Digest2.d. Click the Protein Digest Layout button in the main toolbar. Review the results in the Results Compare window. The RSD (%) column has been calculated for many modifications that are in both samples. 	<ul style="list-style-type: none"> The Results Compare window is not visible by default in the Protein Digest layout. Click View > Results Compare to open the window.

Location	Modification	%RSD (Height)	%Quant (Decon Height)	Height	%Quant (Decon Height)	Height
C264 [B/D]	Alkylation (iodoa...	69.7	99.56	3314909	100	1125920
C370 [B/D]	Alkylation (iodoa...	104.5	100	3558120	99.06	534759
C147 [B/D]	Alkylation (iodoa...	108	100	2929746	100	392302
C23 [A/C]	Alkylation (iodoa...	101.5	100	2010011	100	330778
C193 [A/C]	Alkylation (iodoa...	75.7	100	1779144	100	538124
C229 [B/D]	Alkylation (iodoa...				100	1095878
C232 [B/D]	Alkylation (iodoa...				100	1095878
C133 [A/C]	Alkylation (iodoa...	100.3	100	997238	100	169606
C213 [A/C]	Alkylation (iodoa...	127.3	100	1038957	100	54739
N318 [B/D]	Deamidation	85.1	62.85	182437	65.35	733843

- 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.



Review Results

Exercise 12. Use Result Review mode

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.

Location	Variable Mods	File	%Quant (Decon Height)	Height
C133 [A/C]	Alkylation (iodoaceta)	NIST mAb Digest.d	100	997238
C133 [A/C]	Alkylation (iodoaceta)	NIST mAb Digest2.d	100	169606
C147 [B/D]	Alkylation (iodoaceta)	NIST mAb Digest.d	100	2929746
Sequence				
STSGGTAALGCLV	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	2721438
LGCLVK	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	180998
SGGTAALGCLVK	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	27310
Location				
C147 [B/D]	Alkylation (iodoaceta)	NIST mAb Digest2.d	100	392302
Sequence				
STSGGTAALGCLV	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	259934
LGCLVK	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	116816
AALGCLVK	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	10772
SGGTAALGCLVK	Alkylation (iodoaceta)		<input checked="" type="checkbox"/>	4780
Location				
C193 [A/C]	Alkylation (iodoaceta)	NIST mAb Digest.d	100	1779144

Other Tasks

Exercise 13. Use Report Builder

Other Tasks

Exercise 13. Use Report Builder

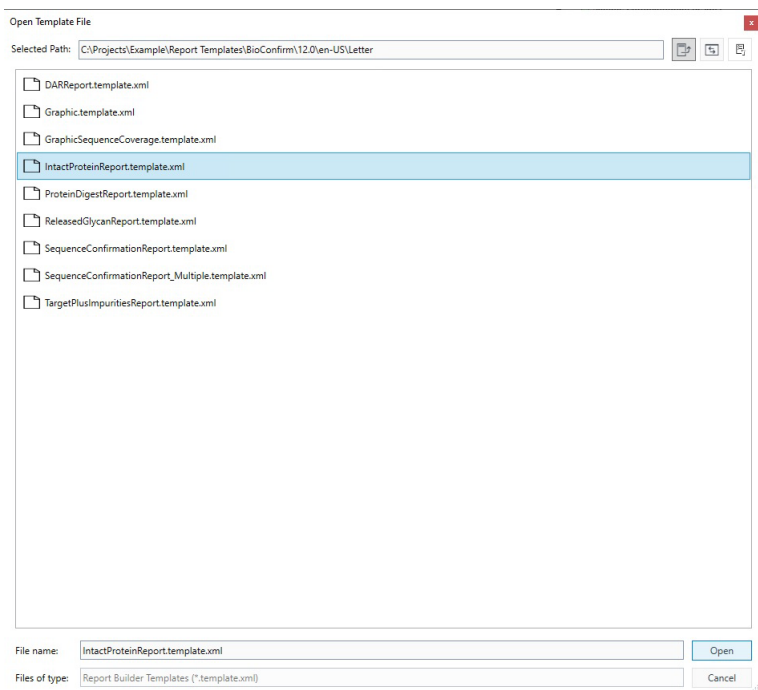
This exercise shows you the program that you use to modify report templates. You can use Report Builder to modify the templates used to generate a Biomolecule report.

Steps	Detailed Instructions	Comments
1 Open Report Builder program.	<p>a Double-click the Control Panel icon on the desktop.</p> <p>b Click Projects in the left pane.</p> <p>c Select a project.</p> <p>d On the ribbon in the Report Builder group, click Report Builder > Start Report Builder.</p> <p>Or you can do the following:</p> <p>a Click Agilent MassHunter Report Builder > Report Builder 11.1.</p> <p>b Select a project to use.</p> <p>c Click OK.</p>	<ul style="list-style-type: none">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.

Other Tasks

Exercise 13. Use Report Builder

Steps	Detailed Instructions	Comments
2 Open an existing template.	<ol style="list-style-type: none">Click File > Open > Browse.Select IntactProteinReport.template.xml and click Open.Click File > Save As > Browse.Enter a file name and click Save.If needed, enter a reason and click OK.	<ul style="list-style-type: none">Report templates are installed in the Report Templates\BioConfirm folder in the project.

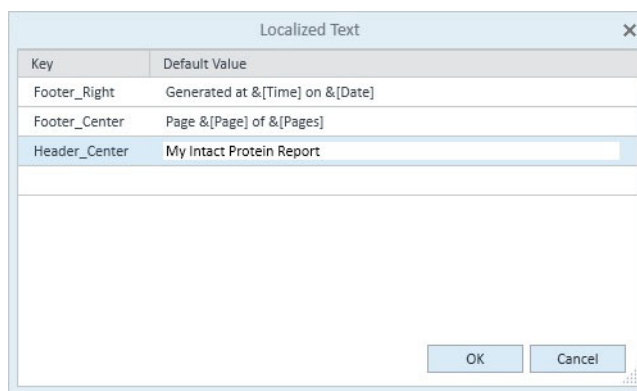


Agilent recommends that you do not modify the default templates. Instead, make a copy of the template and modify the copy.

Other Tasks

Exercise 13. Use Report Builder

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 .	<ul style="list-style-type: none">• 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.



Other Tasks

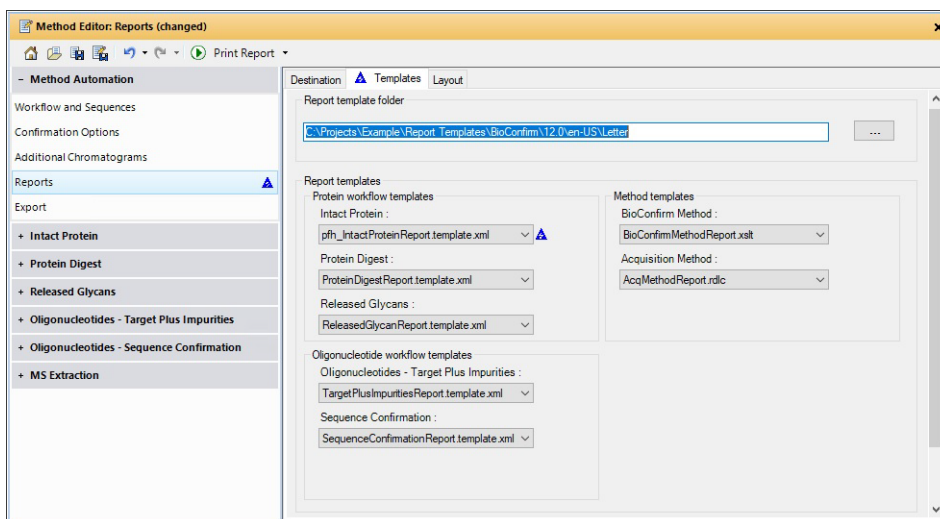
Exercise 13. Use Report Builder

Steps	Detailed Instructions	Comments
4 Save the template.	<ol style="list-style-type: none"> Click File > Save. If needed, enter a reason and click OK. Close the Report Builder program. 	<ul style="list-style-type: none"> 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.

Other Tasks

Exercise 13. Use Report Builder

Steps	Detailed Instructions	Comments
5	Use this new template in a method. <ol style="list-style-type: none">Open the Method Editor window. Click View > Method Editor if it is not visible.Select Method Automation > Reports.Click the Templates tab.Select the changed report for the corresponding report template type. In this example, the Intact Protein report template was modified.	<ul style="list-style-type: none">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.



Other Tasks

Exercise 13. Use Report Builder

Steps	Detailed Instructions	Comments
6	<ol style="list-style-type: none"> Click File > Print > Biomolecule Report. Select a data file that was processed with the Intact Protein workflow. Mark the Print preview check box. Click OK. 	<ul style="list-style-type: none"> When you print a biomolecule report, the report template corresponding to the selected workflow is used.

My Intact Protein Report Agilent | Trusted Answers

Sample Information

Sample Name	Nist mAb Intact	Data File Path	C:\Projects\pjh\Data\NIST mAb 1.d
Sample ID		Acq Time (UTC)	2016-08-25-00:11:19-07:00
Instrument	6530 Q-TOF 1	Acq Method Path	D:\MassHunter\methods\desalt_intact_8min.m
MS Type	QTOF (G6530B)	Acq SW Version	6200 series TOF/6500 series Q-TOF B.06.01 (B6157)
MS SN / FW Ver	SG15362004 / 15.663	DA Method Path	C:\Projects\pjh\Methods\BioConfirm\11.0\pjh_NIST_mAb_Intact.m
LC Info	See Instrument Config Report	BioConfirm Version	11.0 (11.0.507.0)
Sample/Plate Position	Vial 1	DA Operator	SYSTEM (SYSTEM)
Inj Vol (uL)	1.0	DA Workstation	BC-CANYON-MT-VM
Acq Operator	I.M. Chemist	Comment	
Acq Workstation		Sequences / Masses	NISTmAb
Confirmation Status	Confirmed	IRM Status	Success

Matched Sequences

Confirmation Status	Sequence Name	RT	Height	Mass	Tgt Mass	Diff (Da)	Pred Mods	Mods
Confirmed	NISTmAb	2.025	49711	148202	148202	0	1*G1F(1607.5013) + 1*G0F [NGA2F](1445.3580) + 2*Lys-koel(-126.1750)	pyroGlu (Q)(B1); pyroGlu (Q)(D1)

Sample Chromatogram List
Additional Chromatograms

8.50 x 11.00 in < >

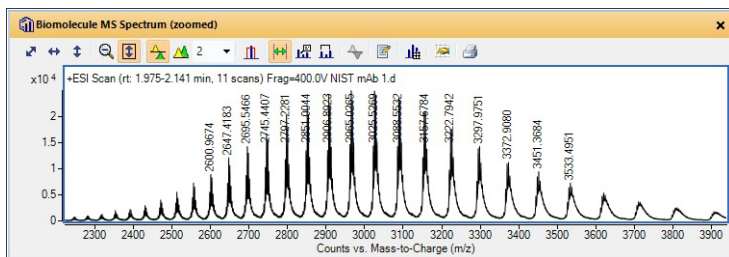
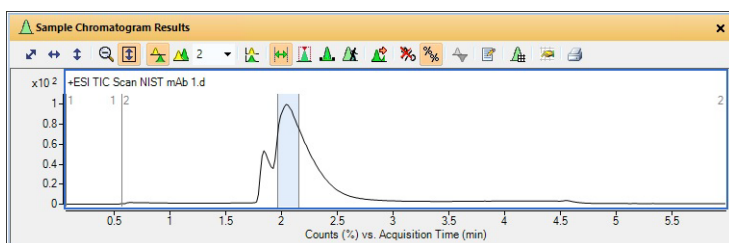
Other Tasks

Exercise 14. Determine Protein Molecular Weight

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.	<ol style="list-style-type: none">Click File > Open Data File.Locate the NIST mAb 1.d folder.Clear the Load Result check box.Click Open.	<ul style="list-style-type: none">The TIC is automatically displayed in the Sample Chromatogram Results window.
2 Extract a peak spectrum.	<ol style="list-style-type: none">Select a range around the peak at 2.1 minutes.Double-click this range.	<ul style="list-style-type: none">To select a range, click one side of the peak and drag to the other side of the peak.

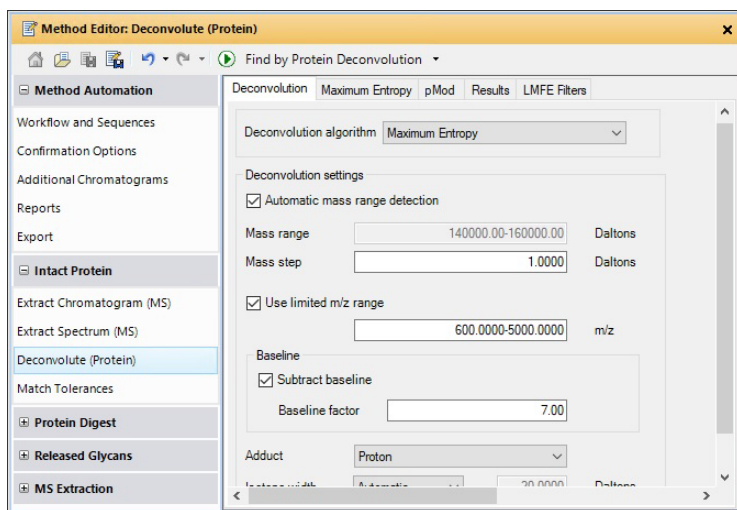


3 Open the default Intact Protein method and open the Deconvolute (Protein) Method Editor section.	<ol style="list-style-type: none">Click Method > Open.Select <i>BioConfirmIntactProtein-Default.m</i>.Click View > Method Editor.Select Intact Protein > Deconvolute (Protein).	<ul style="list-style-type: none">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.
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Other Tasks

Exercise 14. Determine Protein Molecular Weight





Steps	Detailed Instructions	Comments
4 Select Maximum Entropy as the deconvolution algorithm.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Verify that the Automatic mass range detection check box is marked.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">Enter 1 for the Mass step.	

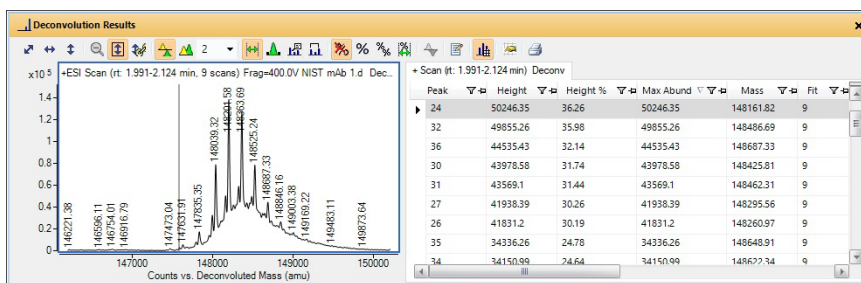


7 Select the extracted MS peak spectrum.	<ul style="list-style-type: none">Click the spectrum in the Biomolecule MS Spectrum window.	
8 Deconvolute the spectrum.	<ul style="list-style-type: none">Right-click the Biomolecule MS Spectrum window and click Deconvolute > Protein to start the deconvolution process.	<ul style="list-style-type: none">You can also click the arrow next to the run button in the Method Editor toolbar and select Deconvolute (Protein).

Other Tasks

Exercise 14. Determine Protein Molecular Weight

Steps	Detailed Instructions	Comments
9 Review deconvolution results.	<ul style="list-style-type: none"> 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 <i>online Help</i>. 	<ul style="list-style-type: none"> 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.	<ol style="list-style-type: none"> Click the spectrum in the Deconvolution Results window to select it. Click the Spectrum Peak List button () . Click the Max Abund column heading to sort results by abundance. Click  in the toolbar in the Deconvolution Results window. Select Mass for the first Peak label and click OK. Click the Spectrum Peak List button () on the Deconvolution Results toolbar to close the peak list tab. 	<ul style="list-style-type: none"> 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.	<ol style="list-style-type: none"> Click File > Save Results. If needed, enter a reason and click OK. Click Save. 	
12 Save the method to iii_Deconvolution_MaxEnt.m where iii are your initials	<ol style="list-style-type: none"> Click Method > Save As. Enter iii_Deconvolution_MaxEnt.m for the method name. 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 <i>DAR_Sample_Intact.d</i> and the <i>DAR_Sample_Reduced.d</i> data files.	<ol style="list-style-type: none"> 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. 	<ul style="list-style-type: none"> • 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.	<ol style="list-style-type: none"> a Click Method > Open. b Select BioConfirmIntactProtein-Default.m c Click Open. 	
3 Open the Deconvolute (Protein) Method Editor section.	<ul style="list-style-type: none"> • 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.

Other Tasks

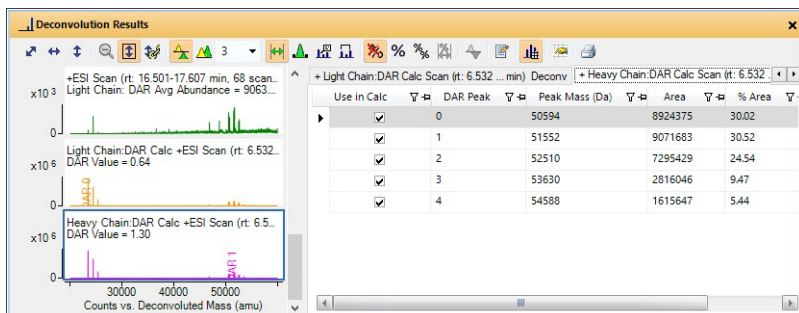
Exercise 15. Calculate DAR

Steps	Detailed Instructions	Comments
<p>4 Select the deconvolution algorithm.</p> <ul style="list-style-type: none"> • Use Maximum Entropy. • Use 20000 - 60000 mass range. • Do not use the limited m/z range. 	<p>a On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select Maximum Entropy as the Deconvolution algorithm.</p> <p>b Clear the Automatic mass range detection check box.</p> <p>c Enter 20000 - 60000 for the Mass range.</p> <p>d Clear the Use limited m/z range check box.</p>	<ul style="list-style-type: none"> • For more information on these parameters, press F1 to open the online Help.
<p>5 Use the default settings for Maximum Entropy deconvolution.</p>	<ul style="list-style-type: none"> • Click the Maximum Entropy tab to review settings. 	
<p>6 Set the time range for extracting spectra to 6 to 8 minutes.</p>	<p>a On the Extract Spectrum (MS) tab of the Intact Protein section of the Method Editor, click Time Range(s).</p> <p>b Enter 6 as the Start and 8 as the Stop.</p>	
<p>7 Set the workflow parameters for the reduced Condition.</p>	<p>a On the Workflow and Sequences tab of the Method Automation section of the Method Editor, select Intact Protein.</p> <p>b Select reduced as the Condition.</p> <p>c Enter 23439, 50594 for Sequences/Masses.</p> <p>d Mark the Calculate Drug-to-Antibody Ratio (DAR) check box.</p> <p>e Enter 957.5 as the Drug + linker mass.</p>	<ul style="list-style-type: none"> • For a method with reduced as the Condition, you enter the Reduced DAR Light Chain, Reduced DAR Heavy Chain as the Sequences/Masses.

Other Tasks

Exercise 15. Calculate DAR

Steps	Detailed Instructions	Comments
8 Save the method.	<ol style="list-style-type: none"> Click Method > Save As. Type the File name <i>iii_DAR_Reduced.m</i>, where <i>iii</i> represents your initials. Click Save. If needed, enter a reason in the Reason for Change dialog box. Click OK. 	<ul style="list-style-type: none"> 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 <i>DAR_Sample_Reduced.d</i> file.	<ol style="list-style-type: none"> Click Method > Run Method Workflow, or click Run Method Workflow in the toolbar in the Method Editor window. Select <i>DAR_Sample_Reduced.d</i> and click OK. 	
10 Review deconvolution results.	<ul style="list-style-type: none"> The results appear in the Deconvolution Results window and in the Sample Table window. 	<ul style="list-style-type: none"> Deconvoluted spectra are added for the light chain and for the heavy chain. The DAR value is added to the Sample table.

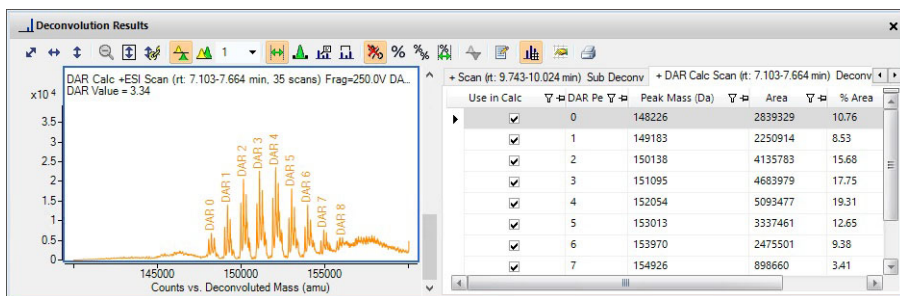


11 Open the method to use as a starting point for the new method.	<ol style="list-style-type: none"> Click Method > Open. Select BioConfirmIntactProtein-Default.m Click Open. 	
12 Select the deconvolution algorithm. <ul style="list-style-type: none"> Use Maximum Entropy. Use 20000 - 60000 mass range. Do not use the limited m/z range. 	<ol style="list-style-type: none"> On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select Maximum Entropy as the Deconvolution algorithm. Clear the Automatic mass range detection check box. Enter 20000 – 60000 for the Mass range. Clear the Use limited m/z range check box. 	<ul style="list-style-type: none"> For more information on these parameters, press F1 to open the online Help.

Other Tasks

Exercise 15. Calculate DAR

Steps	Detailed Instructions	Comments
13 Set the workflow parameters for the non-reduced Condition.	<ol style="list-style-type: none"> On the Workflow and Sequences tab of the Method Automation section of the Method Editor, select Intact Protein. Select non reduced as the Condition. Enter 148057.5 for Sequences/Masses. Mark the Calculate Drug-to-Antibody Ratio (DAR) check box. Enter 974.5 as the Drug + linker mass. 	<ul style="list-style-type: none"> For a method with non reduced as the Condition, you enter the initial Dar 0 mass as the Sequences/Masses.
14 Save the method.	<ol style="list-style-type: none"> Click Method > Save As. Type the File name <i>iii_DAR_NonReduced.m</i>, where <i>iii</i> represents your initials. Click Save. If needed, enter a reason in the Reason for Change dialog box. Click OK. 	<ul style="list-style-type: none"> 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 <i>DAR_Sample_Intact.d</i> file.	<ol style="list-style-type: none"> Click Method > Run Method Workflow, or click Run Method Workflow in the toolbar in the Method Editor window. Select DAR_Sample_Intact.d and click OK. 	
16 Review deconvolution results.	<ul style="list-style-type: none"> The results appear in the Deconvolution Results window and the Sample Table window. 	<ul style="list-style-type: none"> A deconvoluted DAR spectrum is added. The DAR value is added to the table.



17 Save the results.	<ol style="list-style-type: none"> Click File > Save Results. If needed, enter a reason and click OK. Click Save. 	
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Other Tasks

Exercise 16. Use the Mirror Plot window

Exercise 16. Use the Mirror Plot window

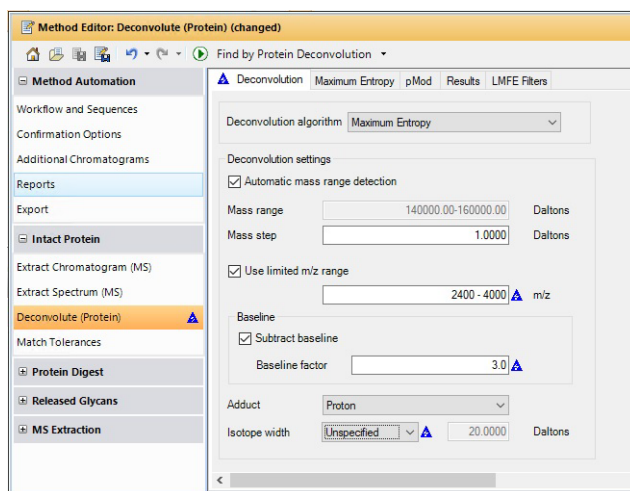
This section shows how to display a Mirror Plot of two deconvoluted biomolecules.

Steps	Detailed Instructions	Comments
1 Open the NIST mAb 1.d data file.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">• 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.	<ul style="list-style-type: none">a Click Method > Open.b Select BioConfirmIntactProtein-Default.mc Click Open.	
3 Open the Deconvolute (Protein) Method Editor section.	<ul style="list-style-type: none">• 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.

Other Tasks

Exercise 16. Use the Mirror Plot window

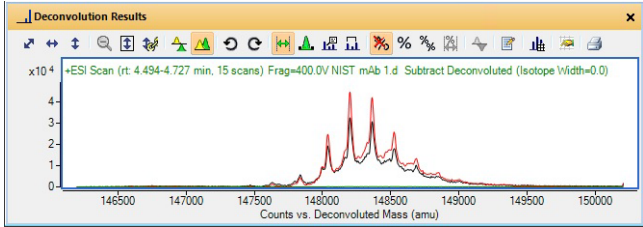


Steps	Detailed Instructions	Comments
<p>4 Select the deconvolution algorithm.</p> <ul style="list-style-type: none">• Use Maximum Entropy.• Use the automated mass range detection.• Use the limited m/z range of 2400 - 4000.• Use 3 for the baseline factor.	<p>a On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select Maximum Entropy as the Deconvolution algorithm.</p> <p>b Mark the Automatic mass range detection check box.</p> <p>c Mark the Use limited m/z range check box.</p> <p>d Enter 2400 - 4000 for the m/z range.</p> <p>e Enter 3 for the Baseline factor.</p> <p>f Select Unspecified for the Isotope width.</p>	<ul style="list-style-type: none">• For more information on these parameters, press F1 to open the online Help.

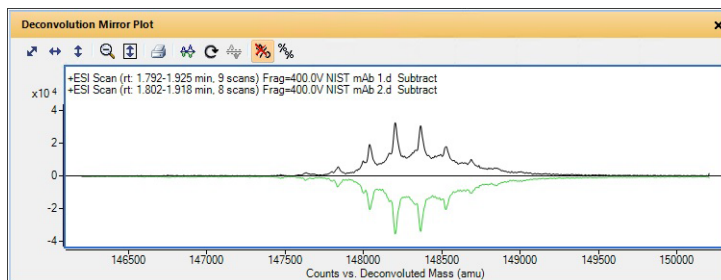


<p>5 Use the default settings for Maximum Entropy deconvolution.</p>	<ul style="list-style-type: none">• Click the Maximum Entropy tab to review settings.
<p>6 Run the Find by Protein Deconvolution algorithm.</p>	<ul style="list-style-type: none">• Click Find and Identify > Find by Deconvolution > Proteins.OR• Click Find by Protein Deconvolution in the toolbar in the Method Editor window.

Other Tasks

Exercise 16. Use the Mirror Plot window

Steps	Detailed Instructions	Comments
7 Review deconvolution results.	<ul style="list-style-type: none"> The results appear in the Deconvolution Results window. 	
		
8 Open the NIST mAb 2.d data file.	<ol style="list-style-type: none"> Click File > Open Data File. Locate the NIST mAb 2.d sample file. Click Open. 	<ul style="list-style-type: none"> The TIC is automatically displayed in the Sample Chromatogram Results window.
9 Run the Find by Protein Deconvolution algorithm on NIST mAb 2.d.	<ul style="list-style-type: none"> Click Find and Identify > Find by Deconvolution > Proteins. 	
10 Review deconvolution results.	<ul style="list-style-type: none"> The results appear in the Deconvolution Results window. 	
11 Select both data files in the Sample Table window.	<ol style="list-style-type: none"> Select one of the sample files in the Sample Table window. Press the Ctrl button and click the other sample file. 	<ul style="list-style-type: none"> 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.	<ol style="list-style-type: none"> Click the  button to show the spectra in list mode. Select a spectra from the Deconvolution window. Press the Ctrl button and select another spectra from the other data file. Click the  button to display the spectra in the Deconvolution Mirror Plot Results window. 	<ul style="list-style-type: none"> Other mirror plots are available: <ul style="list-style-type: none"> MS Spectrum Mirror Plot Fragment Spectrum Mirror Plot Biomolecule Chromatogram Mirror Plot Sample Chromatogram Mirror Plot






Other Tasks

Exercise 17. View Biomolecule Information

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.	<ul style="list-style-type: none">See “Exercise 14. Determine Protein Molecular Weight” on page 75.	<ul style="list-style-type: none">You do not need to repeat the deconvolution steps, if you have already done them in Exercise 1.
2 View the biomolecule list.	<ul style="list-style-type: none">If needed, click View > Biomolecules	<ul style="list-style-type: none">See Figure 8 on page 86.
3 Select the biomolecule with mass around 148039.3.	<ul style="list-style-type: none">Click the row which has a mass around 148039.3 in the Biomolecules window.	<ul style="list-style-type: none">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 <i>m/z</i> 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.	<ul style="list-style-type: none">Click the graphics area for the spectrum selected in step 3.	<ul style="list-style-type: none">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.	<ul style="list-style-type: none">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 ->>	<ul style="list-style-type: none">The following information is displayed for the ion set spectrum:<ul style="list-style-type: none"><i>m/z</i>AbundanceCharge stateSee 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.	<ul style="list-style-type: none">Click  on the toolbar in the Biomolecule MS Spectrum Results window.	<ul style="list-style-type: none">See Figure 10 on page 87.
7 Select Biomolecule 1 in the biomolecule list.	<ul style="list-style-type: none">Click the first line of the Biomolecules table.	<ul style="list-style-type: none">Notice that the spectrum in the Biomolecule MS Spectrum window is updated.
8 Select Biomolecule 2 in the Biomolecules window.	<ul style="list-style-type: none">Click the second line of the Biomolecules table.	

Other Tasks

Exercise 17. View Biomolecule Information

Steps	Detailed Instructions	Comments
9 Print a biomolecule report.	<ol style="list-style-type: none"> Display the Reports section in the Method Editor by selecting Method Automation > Reports. Review the parameters in both the Templates and Layout tabs. Click Biomolecule Report from the File > Print menu to print the report. 	<ul style="list-style-type: none"> 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.

Biomolecules: 14 found										
General										
Label	Mass	RT	Height	Area	Min Z	Max Z	File	Mining Algorithm	Z Count	Sequence Match
Biomolecule 1	148201.5823	2.025	138574	9025919	30	87	NIST mAb 1.d	Maximum Entropy Deconv	53	10
Biomolecule 2	148363.6859	2.058	127009	8956909	30	121	NIST mAb 1.d	Maximum Entropy Deconv	72	10
Biomolecule 3	148039.318	2.074	78986	4985562	30	95	NIST mAb 1.d	Maximum Entropy Deconv	60	9
Biomolecule 4	148525.2449	2.041	78478	6015220	30	95	NIST mAb 1.d	Maximum Entropy Deconv	56	9
Biomolecule 5	148324.012	1.958	59060	1010548	30	65	NIST mAb 1.d	Maximum Entropy Deconv	30	9
Biomolecule 6	148161.8174	2.074	50246	51673	30	54	NIST mAb 1.d	Maximum Entropy Deconv	22	9

Figure 8. Biomolecules window for NIST mAb 1.d

+ Scan (t: 1.991-2.124 min)				
Peak	m/z	Area	Abund	Max Abund
1	2556.1702	10286	7691.49	7671.56
2	2600.9689	11367	9243.91	9235.67
3	2603.8601	11843	8578.05	8510.44
4	2644.4972	12370	7539.82	7533.56
5	2647.425	15942	12633.78	12595.78
6	2650.2955	15867	10801.89	10784.33
7	2692.5761	13863	8992.37	8992.33
8	2695.5486	20768	14838.12	14789.11
9	2698.4734	20499	13379.57	13375.78
10	2701.3391	13980	8537.3	8498.44
11	2742.4017	17838	10649.33	10624.56
12	2745.4441	25296	17405.64	17356.89
13	2748.4238	24077	15976.01	15960.56

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

Other Tasks

Exercise 17. View Biomolecule Information

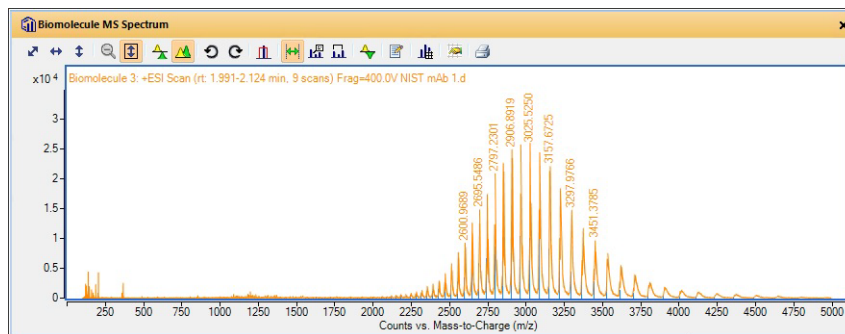


Figure 10. Biomolecule MS Spectrum Results window for NIST mAb 1.d (Overlay Mode)

Other Tasks

Exercise 18. Open results using versions

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.	<ol style="list-style-type: none">Click File > Open Data File.Select the NIST mAb 1.d folder.	<ul style="list-style-type: none">In the previous exercises, results were stored with NIST mAb 1.d multiple times.
2 Select the File version to open.	<ol style="list-style-type: none">Mark Load result data.Select the File version to open.Click Open.	<ul style="list-style-type: none">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.

Open Sample

Selected Path: /p/h/Data

File version: 2021-0614-1828-51054

Name	Size	Date	File version
NIST mAb 1.d		6/14/2021 10:48:57 AM	2021-0614-1828-51054
NIST mAb 2.d		6/14/2021 10:43:29 AM	2021-0614-1823-d1841
NIST mAb Digest.d		6/14/2021 10:45:43 AM	2021-0614-1815-05827
NIST mAb Digest2.d		6/14/2021 10:44:54 AM	2021-0614-1742-42680
NIST mAb Disulfide Mapping.d		6/14/2021 10:45:43 AM	2021-0614-1815-05827
ReleasedGlycans1.d		6/14/2021 10:47:28 AM	2021-0614-1815-05827
ReleasedGlycans2.d		6/14/2021 10:48:57 AM	2021-0614-1828-51054

File name: NIST mAb 1.d (2021-0614-1828-51054) [Open]

Files of type: Data File(s) (*.d) [Cancel]

Options

- Load worklist method
- Load results method
- Use current method
- Load result data

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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