

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

Figure 1. Start BioConfirm from the Control Panel program

Task 1. Open the BioConfirm program

 2 Open these data files: NIST mAb 1.d NIST mAb 2.d NIST mAb 2.d NIST mAb Digest.d NIST mAb Digest2.d ReleasedGlycans1.d Open Sample dialog box, go to the Data folder in your project. Only some example files were control to the hard disk for these example. You can get help for any window dialog box, or tab by pressing the key when that window is active. ReleasedGlycans2.d Make sure that the Use current method button is clicked 	pied es.
ReleasedGlyCansz.d Upen sample Make sure that the Use current selected Patr: CAProjects/Example/Data method button is clicked	F1
mathod button is clicked	×
	5 8
Make quire that the Load regult	^
data check box is cleared.	
NIST mAb Digestd	
NIST mAb Digest2.d	
NIST mAb Disulfide Mapping.d	
ReleasedGlycans2d	
Sile stress	Open
	Cancel
new Mer powinch (n)	Current
Options Options Load worklist method	
Load results method	
Use current method	

Figure 2. Open data files when opening software

Task 1. Open the BioConfirm program

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

Task 1. Open the BioConfirm program



Bior	nolecules	:0 found	1	Biomolecu	le Identific	ation R	esults
	-		-				

Figure 3. BioConfirm main window

Task 2. Zoom in and out of the chromatogram

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

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

Task 2. Zoom in and out of the chromatogram

Steps	Detailed Instructions	Comments
 Steps Practice zooming in and out on each axis separately. Zoom in only along the x-axis. Hint: Right-click the x-axis values and move cursor from left to right. Partially zoom out the x-axis. Hint: Move cursor in the opposite direction. Completely zoom out of the x-axis. Repeat the previous steps for the y-axis. 	 a To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears. b Click the right mouse button and drag the new cursor from left to right across the x-axis values. c To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values. d Click the Autoscale X-axis button, ↔, to completely zoom out on the x-axis. a To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears. b Click the right mouse button and drag the new cursor from bottom to top across the y-axis values 	Comments Image: Comments Horizontal Double Arrow Image: Comments Horizontal Double Arrow Image: Comments New cursor appears when you right-click the x-axis value Image: Comments Vertical Double Arrow Image: Comments New cursor appears when you right-click the y-axis
	 c To zoom out on the y-axis, click the right mouse button and drag from the top towards the bottom of the y-axis values. d Click the Autoscale Y-axis button, to completely zoom out on the y-axis. 	0.45- 0.425- 0.4- 0.375- 0.35-

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.

Task 3. Change window layouts

Steps	Detailed Instructions	Comments		
 Change the window layout: Change the window size. Save a window layout. Unlock the layout. Change the Chromatogram Results window to be floating. Move the Chromatogram Results window. Display the tools for repositioning the windows. 	 To change the size of a window, drag the boundary between the windows. To load the default layout for a workflow, click one of the buttons in the main toolbar: Intact Protein Layout Protein Digest Layout Released Glycans Layout Oligos - Target Plus Impurities Layout Oligos - 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. 	 If the layout is locked, the system displays a check mark next to the Lock Layout menu. You can only use the repositioning tools when the layout is unlocked. You can also make a window float by double-clicking the title bar of the window. If two or more windows are tabbed together, you can make one window float by double-clicking the tab for that window. The following layouts are shipped with the software: Default_Intact_Protein.xml Default_Protein_Digest.xml Default_Oligonucleotides_TPI.xml Default_Oligonucleotides_SC.xml Default.xml 		

Task 3. Change window layouts

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

Task 4. Creating a Protein Sequence File

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

Steps		Detailed Instructions			omments
1	Start the Agilent MassHunter Sequence Manager.	•	Click Sequence > Sequence Manager.	•	You can also start the Sequence Manager program from the OpenLab Control Panel.
2	Add a sequence.	a b c d	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.	•	Protein is automatically selected for the sequence type.
3	Create a new sequence.	a b	Type Myoglobin for the name of the Sequence. Click the + button. The Sequence Editor pane opens automatically with a new sequence displayed for editing.	•	Protein is automatically selected for the sequence type.
4	Enter the amino acid sequence shown below into the Sequence Manager.	•	Type in individual amino acids one at a time between the N-term and C-term symbols.	•	Use the single-character (letter) amino acids abbreviations.
	GLSDGEWQQVLNVWGKVEADIAGH KASEDLKKHGTVVLTALGGILKKKGH LHSKHPGDFGADAQGAMTKALELFF	GQEV HEAE NDIA	/LIRLFTGHPETLEKFDKFKHLKTEAEM ELKPLAQSHATKHKIPIKYLEFISDAIIHV AAKYKELGFQG	•	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*.

Task 4. Creating a Protein Sequence File

teps		Detailed Instructions	Comments		
Save th iii _myo g your ini	ne sequence as the glob.psq , where <i>iii</i> itials.	name a Click Sequence > Export Sequence. b Type <i>iii_</i> myoglob in the File name box. c Click Save.	• The sequence is saved as a .psq fi that can be transferred to other computers or projects.		
Agilent Mas	ssHunter Sequence Manager 12.)	- 🗆 X		
File Edit Seq	quence Help				
🔛 👯 🛃					
Sequences Available		Myoglobin			
A Proteins (Oligonucleotides	Display information using unspecified disulfide linkages			
Search	Q	Total monoisotopic mass: 16940.9651			
Myoglobin	A	l otal average mass: 16951.6073 Seguence molecular formula: C769H1212N210O218S2			
NISTmAb		n na har henar santa date men alla varianten salate henali kometala (
		A:Chain A Monoisotopic mass: 16940.9651 Average mass: 16951.60	373 Molecular formula: C769H1212N210O218S2		
		1 N-term GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGH	PETLEKFDKFKHLKTEAEMKAS 58		
		59 EDLKKHGTVVLTALGGILKKKGHHEAELKPLAQSHATKHKIPIKYI 127 AOGAMTKALELFRNDIAAKYKELGEOG C-term	SEFISDAIIHVLHSKHPGDFGAD 126		
			100		

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

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			etailed Instructions	С	Comments	
1	Start the Agilent MassHunter Chemical Data Dictionary.	•	Click Sequence > Open Chemical Data Dictionary Editor.	•	You can also start the Sequence Manager program and click Open definition manager on the Oligonucleotides tab.	
2	Create a new building block.	a b c	Click the Oligonucleotides tab. Type kH for the Code. Click the + button.	•	See the online Help for rules about naming new Building blocks.	
3	Enter the Name, Molecular formula, and Base formula.	а	Type the Name for this Building block. This name is a description of the Building block.	•	This example is a duplicate of the Building block Deoxycytidine.	
		b	Type C9H13N3O4 for the Molecular formula.			
		с	Type C4H5N30 for the Base formula .			

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

Steps			Detailed Instructions 0				Comments		
4 Close the program.			 a Click Close. b In the Save Chemical Data Dictionary dialog box, enter a reason that the change was made. c Click Yes. 				If you click No in the Save Chemical Data Dictionary dialog box, the changes are not saved.		
Chemical Data Dictio	nary Editor						×		
Proteins 🛕 Oligon	ucleotides								
Building Blocks 🔺	Building blocks								
Linkers	Search				Q				
Linkers	Code	Name	Formula	Building Block Type	^	Code:	kH		
Addifications	/MOErG/	2-MethoxyEthoxy G	C13H19N5O6	Nucleoside					
	/MOErT/	2-MethoxyEthoxy T	C13H20N2O7	Nucleoside		Name:	Dupicate of Deoxycytidine		
	/OH/	OH	ОН	Fragment		Molecular formula:	C9H13N3O4		
	A	Deoxyadenosine	C10H13N5O3	Nucleoside			The code represents a fragment		
	С	Deoxycytidine	C9H13N3O4	Nucleoside					
	dA	Deoxyadenosine	C10H13N5O3	Nucleoside		Base formula:	C4H5N3O		
	dC	Deoxycytidine	C9H13N3O4	Nucleoside					
	dG	Deoxyguanosine	C10H13N5O4	Nucleoside	A				
	dT	Deoxythymidine	C10H14N2O5	Nucleoside					
	fA	2-Fluoroadenosine	C10H12FN5O3	Nucleoside					
	fC	2-Fluorocytidine	C9H12FN3O4	Nucleoside					
	fG	2-Fluoroguanosine	C10H12FN5O4	Nucleoside	_				
	fU	2-Fluorouridine	C9H11FN2O5	Nucleoside					
	G	Deoxyguanosine	C10H13N5O4	Nucleoside					
	kH	Duplicate of Deoxycytidine	C9H13N3O4	Nucleoside	<u> </u>				
	LmA	Methoxyadenosine	IC11H15N504	Nucleoside					
	New building block cod	le		· ×					

Task 6. Creating an Oligonucleotide Sequence File

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

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

Ste	eps	D	etailed Instru	uctions		C	Comments					
5	Save the sequence as the name <i>iii_Example.psq</i> , where <i>iii</i> repres your initials.	e a sents b c	Click Seque Type iii_Exa Click Save .	ence > Export S ample in the File	ence. • ne box.	The seque that can be computers	nce is saved as a .psq file e transferred to other s or projects.					
	Agilent MassHunter Sequence Manager 12.0							– 🗆 X				
F	ile Edit Sequence Help											
L.	a 🥦 a											
9 A	equences valiable valiable Search 40mer Example Oligo_40mer_MSMS 1	ample tal monoisotopi tal average ma quence molecu tal number of bu Open definition 5' TGA C1 nitions	c mass: 6498.1153 ss: 6501.3091 lar formula: C207H2 iilding blocks: 21 in manager	59N810125P20 GA TGA		Linker as	sumption o building blocks are i	next to each other, insert linker				
	Na	ame	Code	Formula	^	Name	Code	Formula				
	De	oxyadenosine	dA	C10H13N5O3		Phosphorothioate	•	HPOS				
	De	oxycytidine	dC	C9H13N3O4		5' Phosphate	/5Phos/	H2PO3				
	De	oxyguanosine	dG	C10H13N5O4		Phosphodiester	p	HPO2				
	De	oxythymidine	dT	C10H14N2O5		Phosphorothioate	S	HPOS				
	2-F	luoroadenosine	fA	C10H12FN5O3								
	2-F	luorocytidine	fC	C9H12FN3O4								
	2-F	luoroguanosine	fG	C10H12FN5O4								
	2-F	luorouridine	fU	C9H11FN2O5								
	De	oxyguanosine	G	C10H13N5O4								
	Me	thoxyadenosine	mA	C11H15N5O4								
	Me	thoxycytidine	mC	C10H15N3O5								
	Me	thoxycytidine thoxyguanosine	mC mG	C10H15N3O5 C11H15N5O5								

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

- 6 Save changes in the Sequence Manager.
 - a Click Sequence > Save All Changes.
 - **b** In the **Save Sequences** dialog box, mark the check box in front of Example.
 - c Enter a description of the change that was made.
 - d Click Save.

• A description is always required when you save a sequence.

Task 6. Creating an Oligonucleotide Sequence File

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.

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

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



Steps	Detailed Instruction	ons	Comments				
11 Save the method for use in Exercise 2.	 a Click Method > b Type the File n where <i>iii</i> represent c Click Save. d If needed, enter Change dialog e Click OK. 	> Save As. hame iii_NIST_n sents your initia er a reason in th box.	You may changes if you ma	see additional listed in the dialog box ide other changes.			
Reason for Change		οx					
C/Depierte/Evample/Methode/DisConfirm/120/offs NIST mAb latart m							
Description	Catagoog						
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mAb'.	Method update						
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Steps	Detailed Instructions	Comments				
13 Save the results.	 a Click File > Save Results. b Select NIST mAb 1.d in the Save Data File dialog box. Click Save. c If needed, enter a reason on the Reason for Change dialog box. Click OK. 	 You can save results with the data file. You can select whether to open these results when you open the data file. The administrator can set up the project so that you need to enter a reason when you save results to a data file. You may be able to enter your own reason or you may need to select a reason from a list. If you want to save the results, you need to save them before you change the method. If you do not, then you will need to generate the results again. 				
Reason for Change C\Projects\Example\Data\NIST mAb 1.d	– 🗆 X					
Description	Category					
Find by Protein Deconvolution found 87 biomolecules. Match Sequences for Intact Protein.	Reprocess results Sequence match					
Reason Select from list	v					
Own reason Find by Protein Deconvolution	n and Match Sequences algorithms					
Apply to all files	OK Cancel All					

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.

St	eps	De	etailed Instructions	Comments			
1	If not already open, open the method iii_NIST_mAb_Intact.m.	a b c	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.	•	Click Method Automation > Workflow and Sequences in the Method Editor window.				
3	Use the Intact Protein Workflow.	•	Confirm that Intact Protein is selected for the Workflow.	• In this case you are using the default method parameters. For some data files, you will need to use different parameters as described in the <i>Quick Start Guide</i> or <i>online Help</i> .			
4	Select the NISTmAb sequence.	a b c d e f	Select non-reduced for the Condition. Click the button next to the Sequences parameter. The Select Sequences dialog box opens. If NISTmAb is not available, click Add. Select <i>NISTmAb</i> . pSq and click Open . Verify that the NISTmAb sequence is in the Selected list. Click OK.	 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.	a b c	If needed, 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. Click OK.				

Exercise 2. Automated Intact Protein Workflow

Steps	Detailed Instructions	Comments
6 Save the method.	Click Method > Save.If needed, enter the reason for the change.	
Method Editor: Workflow and Sequer Method Automation Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export Intact Protein Protein Digest Released Glycans Oligonucleotides - Parget Plus Impu Oligonucleotides - Sequence Confir MS Extraction	An Method Workflow •	 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 we method automation	 Click Method > Run Method Workflow. Click Method > Run Method Automation (Workflow + Reports). Click On the Method Editor toolbar. Reprocess the sample. See "Exercise 11. Reprocess Samples" on page 62. 	Method Automation first runs the method workflow, and then extracts additional chromatograms and generates a biomolecule report and exports results.
8 Save the results.	 a Click File > Save Results. b Select NIST mAb 1.d in the Save Data File dialog box. Click Save. c If needed, enter a reason on the Reason for Change dialog box. Click OK. 	• You save results before modifying the method again.

Exercise 2. Automated Intact Protein Workflow

ieps			Det	ailed Instr	uctions		Comments
(optional) Re Biomolecule	I	 If If If If If If If 	f you clicke a report is g Before you you need to Save Resu You can cli Report to g sample.	ed Run I generate can inte o save ti Its to sa ck File : generate	Method Automation, the ed automatically. eractively print a report, he results. You click File ave the results. > Print > Biomolecule a report for the current	 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 these parameters in the Print Biomolecule Report dialog box 	
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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			etailed Instructions	Comments			
1	Open the method to use as a starting point for the new method.	a b c	Click Method > Open. Select the BioConfirmProteinDigest-Default.m folder. Click Open.	•	The parameters in the BioConfirmProteinDigest-Default. m method are a good starting point for Protein Digests.		
2	Load the Protein Digest default layout.	•	Click Protein Digest Layout on the main toolbar.				
3	Select NIST mAb Digest.d. If the data file NIST mAb Digest.d is not open, open the data file.	• a b c d	If available, select NIST mAb Digest.d . herwise, do the following: Click File > Open Data File . Locate the NIST mAb Digest.d sample. Clear the Load result data check box. Click Open .	•	The TIC is automatically displayed in the Sample Chromatogram Results window.		
4	Review the parameters in the Find Peptides section in the Method Editor window.	a b d	Select Protein Digest > Find Peptides in the Method Editor window. Review the settings on the various tabs of the Find Peptides section. Click the Charge State tab. Review the parameters.	•	You can change the default parameters as described in the next steps. You can also use the method without any changes. For some data files, you will need to use different parameters as described in the <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.	a b c	Click () on the Method Editor toolbar to start the biomolecule search. If the Find Peptides dialog box opens, select NIST mAb Digest.d and click Find . When processing is complete, review the results in the Biomolecules window.	•	You can instead click Find and Identify > Find Peptides.		

Protein Digest Workflow Exercise 3. Interactive Protein Digest Sequence Matching

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ŀ	Biomolecule 1	476	5,7713	951,5281	7.5	587	3269866	34	04620	1	2	NIST mAb Digest.d									
í	Biomolecule 2	58	1.3185	1160.6223	9.0	016	3129797	36	69722	1	3	NIST mAb Digest.d									
	Biomolecule 3	661	1.3429	1320.6707	8.1	172	2721438	31	42003	1	3	NIST mAb Digest.d									
	Biomolecule 4	419	9.7554	837.4962	6.4	443	2694905	23	83104	1	2	NIST mAb Digest.d									
	Biomolecule 5	660	0.3568	659.3493 699.4281	7.1	195	2481600	14	25246	1	2	NIST mAb Digest.d									
	Biomolecule 7	713	3.6807	2138.0194	9.3	365	2120155	43	44631	1	4	NIST mAb Digest.d									
4	Biomolecule 8	39	3.7271	785,4395	3.6	542	2061778	12	52563	1	2	NIST mAh Digest.d								•	*
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Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

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

1	Biomolecules: 242	found													×
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c				General											
	Label 🛛 🖓	m/z V	Mass 7	RT V	Height	Area	Min Z 🛛	Max Z 🛛	ID Techniques Applied ヤ	Sequence Name V	Seq Loc 🛛	Tgt Seq Mass ♡	Score (Bio 🛛	Sequence	7
	Biomolecule 1: A(476.7713	951.5281	7.587	3269866	3404620	1	2	Target Mass Match	NISTmAb	A(45-52)/C(45	951.5277	81.97	LLIYDTSK	
	Biomolecule 2: B(3	581.3185	1160.6223	9.016	3129797	3669722	1	3	Target Mass Match	NISTmAb	B(364-373)/D(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(1320.6708	93.01	STSGGTAALGCLV	K
	Biomolecule 4: B(3	419.7554	837.4962	6.443	2694905	2383104	1	2	Target Mass Match	NISTmAb	B(330-337)/D(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(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(2138.0202	100	TPEVTCVVVDVSH	
4	Biomolecule 8: A(393.7271	785,4395	3.642	2061778	1252563	1	2	Target Mass Match	NISTmAh	A(53-60)/C(53	785.4396	79.16	LASGVPSR	ŀ

12 View sequence coverage results.

- a If necessary, click View > Sequence Coverage Map.
- **b** Select a different biomolecule in the Biomolecules table to view a different result.
- Amino acids that are matched are either green (MS/MS) or black (MS-only) in a matched sequence.
- Amino acids that are not matched are gray.
- A line is added below the AA sequence to display where peptides have been identified.
- See the online Help for more information.

To view more information.

Click the following items on the Sequence Coverage Map window shortcut menu to view more information about the sequence:

- Applied Modifications
- Specified Applied Links
- View Digest List

Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching



Protein Digest Workflow Exercise 3. Interactive Protein Digest Sequence Matching

Steps	Detailed Instructions		Comments
해 Peptide Relative Quantitation Results		×	
📫 😤 🖻			
Location ♀+> Variable Mods ♀+> File `	ଟ - ଅ %Quant (Decon Height) ସ୍ଥ - Peight	▼ += %Quant (Deco ▼ += Area ▼ +=	
C370 [B\D] Alkylation (iodoaceta NIST mAb Dige	est. 100 3558120	100 393690	
Sequence X to Seq Loc X to Fixed Mods X to	Variable Mods X + Use for %Quant X	E Height ⊠-b Area ⊠-b	
NOVSLTCLVK B(364-373)	Alkylation (iodoaceta	3129797 366972	
NOVSLTCLVK B(364-373)	Deamidation 2. Alkylat	152188 125668	
NQVSLTCLVK B(364-373)	Deamidation 2, Alkylat	138860 691534	
NQVSLTCLVK B(364-373)	Deamidation 2, Alkylat	137275 723575	
Location ♀+> Variable Mods ♀+> File	ସ୍ଟ ≠ %Quant (Decon Height) ସ୍ସ → Height	⊽-⊨ %Quant (Deco ⊽-⊨ Area ⊽-⊨	
C147 [B\D] Alkylation (iodoaceta NIST mAb Dige	est. 100 2929746	100 323553	
C264 [B\D] Alkylation (iodoaceta NIST mAb Dige	est. 99.56 3314909	99.84 568181 🖵	
15 Review the results in the Relative Quantitation Histograms window.	a Click the Relative C window.	Quantitation Histograms	• For Protein Digest workflow, the software uses the Use for
	b Click the first triang	le next to the first row.	%Quant check box in the Peptide

c Note that **Use for %Quant** is marked for both rows.

In Relative Quantitation Histograms

%Quant check box in the Peptide Relative Quantitation Results window.

×

16 Repeat the interactive processing with <i>NIST mAb Digest2.d.</i>	a If needed, open the data file NIST mAb Digest2.d (see step 3). b Select Find Peptides in the Method Editor and	 Most of the processing parameters used for the first data file are the same for the second
	 verify the parameters(step 4). c Find biomolecules (step 5). d Match sequences (step 10). e Save the results to the NIST mAb Digest2.d file (step 13). 	 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.

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

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

Exercise 5. Interactive Released Glycans

Steps	Detailed Instructions	Comments	
7 Review the results.	 a In the Biomolecules window, click the header of the Area (FBF) column to sort the table by this column. If necessary, click the header again so that the largest areas are at the top of the table. b Highlight GOF in the Biomolecules window. c Click the Biomolecule Identification Results tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window. d When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window. 	 Several changes were made to the default layout for the image below. The Glycan Structure Viewer window is visible. Also, the Flags (Tgt) column was moved The Relative Quantitation Histograms window only contains information when you run a workflow. 	
🚟 Agilent MassHunter BioConfirm Software 12.0 - pfh_	ReleasedGlycans_InstantPC.m, Default_Released_Glycans.xml	– 🗆 X	



Exercise 6. Automated Released Glycans Workflow

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

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

Steps	Detailed Instructions	Comments
1 Open the method.	 a Click Method > Open. b Select the iii_ReleasedGlycans_Insta c Click Open. 	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.	 a If the Method Editor is not > Method Editor. b Click Method Automation Sequences in the Method 	 visible, click View You can instead click the Method Editor button, in the main toolbar. Workflow and Editor window.
3 Select the appropriate workflow.	 a Select Released Glycans f b Enter Example in the Glyc c Select Glycans_mAb_AM_ Target glycan source. d Clear the Require RT mator contains a RT for the targe box. 	 The Released Glycans workflow automatically runs the Find Glycans algorithm. The Glycan algorithm. The Glycan group is used to organize the results in the Results Compare window.
	Method Automation Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export Intact Protein Protein Digest Released Glycans Oligonucleotides - Target Plus Impurities Oligonucleotides - Target Plus Impurities	Workflow: Released Glycans Glycan group: Example (only used for cross-sample comparisons) Example Target glycan source (* cdb, * cav, * cef, formulas, neutral mass) Glycans_mAb_AM_PCD cdb Glycans_mAb_AM_PCD cdb (stored in method) Values to match Require RT match if database contains a RT for the target glycan.
4 Save the method.	 Click Method > Save. If needed, enter a reason for 	The administrator can set up a project to require you to enter a

the **Reason for Change** dialog box.

reason for a change to a method

Exercise 6. Automated Released Glycans Workflow

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

Exercise 6. Automated Released Glycans Workflow



- 7 Review the results in the Results Compare window.
- a Click the Results Compare window.b Click the Released Glycans tab.
- c Note that RSD (%) is empty because only one sample is selected. If you select two or more samples and they belong to the same Glycan Group, then the results are shown in the same table. If the same glycan is in multiple samples, then the RSD (%) is calculated.
- For Released Glycans workflow, the software uses the Use for %Quant check box in the Biomolecules window.
- The Results compare window is tabbed with the Relative Quantitation Histograms window.

	÷ 🖉									
Intact Pr	rotein Protein Diges	st Re	eleased Glyc	ans	Oligos - Target F	Plus Im	purities			
	Glycan Group	7-Þ								
4 1	Exam	ple								
						Deles				
		_		_		Kelea:	sedGiycan	IS L.C		
	Glycan Name	V	RSD (%)	V	%Quant (FBF)	Relea: ₽	Area	si.d V	RT	V
	Glycan Name H6N6f	₽ F1S2	RSD (%)	V	%Quant (FBF)	V 0	Area	V 090	RT 51	⊽ 1.194
	Glycan Name H6N6F H6N5F	₽ F152 F151	RSD (%)	V	%Quant (FBF)	▼ 0 0	Area 12	V 090 2858	RT 5' 50	⊽ 1.194).936
	Glycan Name H6N6F H6N5F H6N	₽ F1S2 F1S1 I5S1	RSD (%)	V	%Quant (FBF)	0 0 0.04	Area 12 25	V 090 2858 1939	RT 51	₽ 1.194 0.936 38.16
	Glycan Name H6N6f H6N5f H6N H6N4f	₽ F1S2 F1S1 I5S1 F1S1	RSD (%)	V	%Quant (FBF)	0 0 0.04 0.01	Area 12 25*	♥ 090 2858 1939 9946	RT 51	₽ 1.194 0.936 38.16 7.303

Exercise 6. Automated Released Glycans Workflow

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

Exercise 6. Automated Released Glycans Workflow

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.

St	eps	Detailed Instructions	Comments
1	Open the method to use as a starting point for the new method.	 a Click Method > Open. b Select the BioConfirmOligosTPI-De folder. c Click Open. 	The parameters in the BioConfirmOligosTPI-Default.m method are a good starting point for the Target Plus Impurities experiment.
2	Open the example sample file.	 a If needed, click File > Open Data File b Select Oligo_40mer_MS_1.d. c Click Open. 	e. • The TIC is automatically displayed in the Sample Chromatogram Results window.
3	Load the Target Plus Impurities layout.	Click Oligos - Target Plus Impurities the main toolbar.	Layout in
4	Review the parameters in the Oligonucleotides - Target Plus Impurities > Deconvolute (Oligos) section in the Method Editor window.	 a Select Oligonucleotides - Target Plu Impurities > Deconvolute (Oligos) in Method Editor window. b Click the Match Tolerances tab. c Select Da and enter 1.0 for the Toler d Click the Peak Filters tab. e Mark Limit (by height) to the largest 5. 	us n the rance. and enter
		Method Editor: Deconvolute (Oligos) (changed)	×
		A Method Automation A Method Automation	youtube V nopy Results LMFE Filters A Match Tolerances Integration m Estraction (MS) Peak Filters (MS) Charge State Time Range(p) O Peak area 100000 counts 5,0000 % of largest peak 100000 counts 1,0000 % of largest peak 2 100000 % of largest peak

Oligonucleotide Workflow - Target Plus Impurities Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

lick on the Method Editor toolbar to start the biomolecule search. //hen processing is complete, review the esults in the Biomolecules window. lick Method > Save As. ype the File name iii_OligosFOD.m, where iii the presents your initials. lick Save. needed, enter a reason on the Reason for thange dialog box. Click OK. hen you run the Find by Oligonucleotide econvolution algorithm, the Oligos - Impurity st and the Biomolecule Identification Results indow are empty. cleotides_TPlxml -
lick Method > Save As. ype the File name iii_OligosFOD.m, where iii presents your initials. lick Save. needed, enter a reason on the Reason for hange dialog box. Click OK. hen you run the Find by Oligonucleotide econvolution algorithm, the Oligos - Impurity st and the Biomolecule Identification Results indow are empty. cleotides_TPLxml -
hen you run the Find by Oligonucleotide econvolution algorithm, the Oligos - Impurity st and the Biomolecule Identification Results indow are empty. Cleotides_TPIxml – — — × Released Glycans Layout Oligos - Target Plus Impurities Layout Oligos - Sequence Confirmation Layout ×
cleotides_TPLxml – C X Released Glycans Layout Oligos - Target Plus Impurities Layout Oligos - Sequence Confirmation Layout X
Workflow Workflow Version Last Run Method Workflow Condition Experiment. Modification Enzyme Glycan Group Sequence / Mass Sequence Coverage DAR Sample Image: Target Plus Impurities Ciligo. 40 Image: Target Plus Impuri
x 2 3
t Q Area Tile Mining Algorithm V Z Count V Use for %Quant V Area (PER) I 18 2755807 Oligo_40mer_MS_1. Maximum Entropy Deconv 11 V 3563648 25915896 V Area (PER) I 10 105027 Oligo_40mer_MS_1. Maximum Entropy Deconv 12 V 3479151 12834099 V Area (PER) I I 0100_40mer_MS_1. Maximum Entropy Deconv 12 V 3479151 12834099 V I I 0100_40mer_MS_1. Maximum Entropy Deconv 12 V 3479150 11581816 I I I 0100_40mer_MS_1. Naximum Entropy Deconv 12 V 3479150 1138186 I I I I I I 1138186 I

b If needed, enter a reason for the change.

Oligonucleotide Workflow - Target Plus Impurities

Exercise 7. Interactive Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
 Select parameters in the Method Automation > Workflow and Sequences section in the Method Editor window. Select 40mer as the Sequence. Select /K/ and /Na/ for the Mods and Profiles. 	 a Select Method Automation > Workflow and Sequences in the Method Editor window. b Select Oligonucleotides. c Select Target Plus Impurities as the Experiment. d Click the button next to the Sequences/Masses parameter. The Select Sequences dialog box opens. e Double-click 40mer. If 40mer is not available, click Add. f Select Oligo_40mer_MS.psq and click Open. g Verify that the 40mer sequence is in the Selected list. h Click OK. i Click the button next to the Mods and Profiles parameter. j Select /K/ and /Na/ from the Available list. k Click OK. l Click next to Matching Rules. m Mark 5'-Truncation with linker and 3'-Truncation with linker. Enter 5 for Maximum allowed for each. Click OK. 	 You need to select a sequence before you can run the Find by Formula (Oligos) algorithm. You need to be very selective with the choices for the Mods and Profiles and for the Matching Rules. These choices can limit the size of the database and significantly affect the processing time.
10 Review the parameters in the Oligonucleotides - Target Plus Impurities > Find by Formula (Oligos) section in the Method Editor window.	 a Select Oligonucleotides - Target Plus Impurities > Find by Formula (Oligos) in the Method Editor window. b Review the parameters. 	• You can limit the number of biomolecules found by adding filters.
	Method Editor: Find by Formula (Oligos) (changed)	×
	Method Automation Formula Matching Olico Ion Species Results E	IC Integration EIC Peak Filters Charge State
	Workflow and Sequences	
	Confirmation Options Masses: +/- 10.00 p	pm 🗸
	Additional Chromatograms Retention times: +/- 0.350 mi	inutes
	Reports	
	Expansion of values for chromatogram extraction	
	Possible m/z: Symmetric (ppm) v +/	/- 35.0 ~

Limit EIC extraction range

Symmetric v +/- 1.000 minutes

Expected retention time:

Intact Protein

Protein Digest

+ Released Glycans

- Oligonucleotides - Target Plus I... Deconvolute (Oligos) Find by Formula (Oligos)

+ Oligonucleotides - Sequence C... + MS Extraction

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.	 a Click () on the Method Editor toolbar to start the biomolecule search. b When processing is complete, review the results in the Biomolecules window. 	 You can instead click Find and Identify > Find by Formula (Oligos). 	
12 Save the method for use in Exercise 8.	 a Click Method > Save As. b Type the File name iii_OligosTPI.m, where iii represents your initials. c Click Save. d If needed, enter a reason on the Reason for Change dialog box. Click OK. 	 4 compounds and 2 impurities were found when running the Find by Formula (Oligos) algorithm. 	
🧱 Agilent MassHunter BioConfirm Software 12.0 - pfh_OligosTPI.m, D	efault_Oligonucleotides_TPI.xml	- 🗆 X	
File View Find and Identify Method Sequence Configuration I	Help		
🔗 🚽 🗎 🦪 🍯 🕆 (* 🕐 🔤 Intact Protein Layout 🛛 Prote	in Digest Layout Released Glycans Layout Oligos - Target Plus Impurities Layout	Oligos - Sequence Confirmation Layout	
Sample Table: Oligo_40mer_MS_1.d		×	
🏥 🕞 🐻 🏯			
Pecults		Acquisition	
Confirmation Status File Name Workflow Condition Not confirmed Oligo_40mer_M5_1.d Targe	Workflow Experiment Modification Enzyme (Glycan Group) Sequence / Mass Sequence Co tt Plus Impurities III II-^/IX/III III-^/INa/ ^^40mer	verage DAR Sample Name Sample Position Acquisition Tin Oligo_40mer_MS P1-A3	
Confirmation Status File Name Workflow Condition Not confirmed Oligo_40mer_MS_11.d Targe Sample Table: Oligo_40mer_MS_11d Method Editor:Find by Formula	Workflow Experiment Modification Enzyme Glycan Group Sequence / Mass Sequence Co t Plus Impurities III III^A/K/I III III^A/Na/ ^A40mer III III IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	verage DAR Sample Name Sample Position Acquisition Tin Oligo_40mer_MS P1-A3	
Confirmation Status File Name Workflow Condition Not confirmed Oligo_40mer_MS_1a Target Sample Table:Oligo_40mer_MS_1a Method Editor: Find by Formula A Sample Chromatogram Results	Workflow Experiment Modification Enzyme Glycan Group Sequence / Mass Sequence Co tt Plus Impurities III II^^//VIII III^^//Na/ ^^40mer (Oligos) III	verage DAR Sample Name Sample Position Acquisition Tin Cligo_40mer_MS P1-A3	
Confirmation Status File Name Workflow Condition Not confirmed Oligo_40mer_MS_1.d Targe Sample Table: Oligo_40mer_MS_1d Method Editor: Find by Formula A sample Chromatogram Results	Workflow Experiment Modification Experime Glycan Group, Sequence / Mass Sequence Co tt Plus Impurities III 1^A/K/ III 11^A/Na/ ^^40mer [Oligos) III III	verage DAR Sample Name Sample Position Acquisition Tin Oligo_40mer_MS P1_A3	
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b If needed, enter a reason for the change.

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

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

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

Steps		Detailed Instructions	Comments	
1	Open the method.	 a Click Method > Open. b Select the <i>iii_</i>OligosTPI.m folder. c Click Open. 	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.	 a If the Method Editor is not visible, click View > Method Editor. b Click Method Automation > Workflow and Sequences in the Method Editor window. 	• You can instead click the Method Editor button, 📑 , on the main toolbar.	

Oligonucleotide Workflow - Target Plus Impurities Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

Steps	Detailed Instructions	Comments
3 Select the appropriate workflow.	 a Select Method Automation Sequences in the Method b Select Oligonucleotides. c Select Target Plus Impurit Experiment. d Click the button ne Sequences/Masses parar Sequences dialog box ope e Double-click 40mer. If 40n available, click Add. f Select Oligo_40mer_MS.p Open. g Verify that the 40mer sequences dist. h Click OK. i Click the button ne and Profiles parameter. j Select /K/ and /Na/ from to k Click OK. l Click import to Match m Mark 5'-Truncation with linker. n Enter 5 for Maximum allow o Click OK. 	 n > Workflow and Editor window. The Matching Rules parameter shows the rules that are marked, and it shows the Maximum allowed in parentheses after the rule. ext to the meter. The Select ens. ner is not usq and click uence is in the ext to the Mods the Available list. hing Rules. inker and wed for each.
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	+ Protein Digest	Matching Rules X
	+ Released Glycans	Include Name Maximum allowed
	+ Oligonucleotides - Target Plus Impurities	5-Truncation without linker 1
	+ Oligonucleotides - Sequence Confirmation	3'-Truncation without linker 1
	+ MS Extraction	3'-Truncation with linker 5
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		Split 1
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Oligonucleotide Workflow - Target Plus Impurities Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

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

Oligonucleotide Workflow - Target Plus Impurities

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow



- 8 Review the results in the Results Compare window.
- a Click the Results Compare window.
- **b** Click the **Oligos Target Plus Impurities** tab.
- c Note that RSD (%) is empty because only one sample is selected. If you select two or more samples and they use the same sequence, then the results are shown in the same table.
- For Oligonucleotides Target Plus Impurities workflow, the software uses the Use for %Quant check box in the Biomolecules window.

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

Oligonucleotide Workflow - Sequence Confirmation

Exercise 8. Automated Oligonucleotides - Target Plus Impurities Workflow

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.

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

Oligonucleotide Workflow - Sequence Confirmation Exercise 9. Interactive Oligonucleotides - Sequence Confirmation Workflow

Steps		Detailed Instructions		Comments
6	Save the method for use in Exercise 10.	a b c d	Click Method > Save As. Type the File name iii_40merMSMS_OligosSC.m, where iii represents your initials. Click Save. 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	Detailed Instructions	Comments
7 Review the results.	 a In the Biomolecules window, click the header of the Height column to sort the table by this column. If necessary, click the header again so that the largest heights are at the top of the table. b Select the first biomolecule in the Biomolecules window. c Click the Biomolecule Identification Results tab. The Biomolecules Identification Results window is tabbed with the Biomolecules window. d When you open the window, the window displays the results for the first biomolecule window. 	 Several changes were made to the default layout for the image below. Several columns were hidden in the Sample Table, and Auto fit was cleared in the Fragment Confirmation Ladder. The Fragment Confirmation Ladder. 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.
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8 Save the results	a Click File > Save Results to save your results	• You can also click 🛃 to save
	to the data file folder.	results.
	b If needed, enter a reason for the change.	

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

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

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

Steps		Detailed Instructions		Comments
1	Open the method.	 a Click Method > Open. b Select the <i>iii</i>_40merMS folder. c Click Open. 	MS_OligosSC.m	• 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.	 a If the Method Editor is in the Method Editor. b Click Method Automatic Sequences in the Method Sequences i	not visible, click View : ion > Workflow and nod Editor window.	 You can instead click the Method Editor button, in the main toolbar.
3	Select the appropriate workflow.	a Select Oligonucleotide b Select Sequence Conf Experiment. c Click the next to parameter. d Select Oligo_40mer_M Method Editor: Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export + Initad Protein + Protein Digest + Released Giycans + Oligonucleotides - Sequence Confirmation + MS Extraction	s for the Workflow. irmation for the the Sequences ISMS and click OK. thod Workflow • Workflow: Oligonuc Experiment: Sequences Sequences: ^^Oligo.4	 You cannot enter a mass if you select the Sequence Confirmation experiment.
4	Save the method.	 Click Method > Save. If needed, enter a reaso the Reason for Change 	on for the change in a dialog box.	 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

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

Oligonucleotide Workflow - Sequence Confirmation

Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow



Oligonucleotide Workflow - Sequence Confirmation Exercise 10. Automated Oligonucleotides - Sequence Confirmation Workflow

S	teps	Detailed Instructions	Comments		
7	Review the results in the Fragment Confirmation Ladder window.	 a Click the Fragment Confirmation Ladder window. If a nucleotide has a fragment that confirms it, the nucleotide is shown in black. If no fragment confirms the nucleoside, it is shown in gray. If no fragments are on a line between two nucleosides, the line is not drawn. Each line can have 9 possible dots to represent each of the fragment types expected to be found at each location. The dots on the bottom limb apply to the nucleoside to the left of the line. If a fragment is found in the results, a dot appears at the correct location on the fragment confirmation ladder. The selected biomolecule or biomolecules in the Biomolecule table are displayed as an open circle in the Fragment Confirmation Ladder. If multiple data files are selected, the dots are shown in different colors. If you hover over a fragment dot, a tooltip shows you which biomolecules show that fragment. 	 If you mark the Auto fit check box, all of the nucleosides are visible in one row, if possible. If you select multiple data files that were processed with the same sequence, the results of all the selected data files are shown in the Fragment Confirmation Ladder (up to a maximum of 10 data files). The dots for each data file are shown on the same fragment confirmation ladder (one color for each data file). If the same fragment is confirmed in multiple data files, then the dot has the color for Multiple fragments. The percentage shown in the title bar for the window shows the cumulative results for all selected data files. 		
		Fragment Confirmation Ladder: Oligo_40mer_MSMS (Oligonucleotide) (95.00 Oligo_40mer_MSMS Oligo_40mer_MSMS Show full data file name Auto fit 40mer14CEd Multiple fragments 5 C C A C G A C C A A G T G A A T C G A G T C G A G A T	A C A G C A A T G $C C A T 3$		
8	Save the results for the current data file and close all data files.	 a Click File > Save Results. b If needed, enter or select a reason in the Reason for Change dialog box. Click OK. c Click File > Close All. d Click No to save results. 	 You need to save results as you process your data files. You can only save results that have been processed with the current method. If you change the method after generating results, then you 		

need to generate results again.

Review Results Exercise 11. Reprocess Samples

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.

S	teps			[Detailed	Instructions		(Comment	S	
1	Open severa	al data files.		a t c c	Click I Select 1.d, N Diges Relea Relea Mark I Click (File > Open Data File. t these example files: N IST mAb 2.d, NIST mA t.d, NIST mAb Digest2 sedGlycans1.d, and sedGlycans2.d. the Load result data cl Open.	lIST r \b 2.d, heck l	nAb	 To sele file. The file. In Network can selet to open "Exerci- version 	ct multiple en, press Sh vorked Wor ect which v when you se 18. Ope s" on page	files, click the first ift and click the last kstation mode, you ersion of the results open a data file. See n results using 88.
2	Review resu window.	Ilts in the Sa	mple Table	e 6	Look colum	at the Confirmation St ann.	atus	•	If you s informa	aved result ation on cor	s, the table contains nfirmation.
	Sample Table: Relea	sedGlycans1.d								×	
	Real Presult	He .				Workflow					
	Confirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glycan Group	Sequence / Mass	Sequence Coverag	
	Confirmed Undetermined Partially confirmed	NIST mAb 1.d NIST mAb 2.d NIST mAb Digest.d	Intact Protein Protein Digest		non-reduced	mAb Protein Digest (Reduced+Alkylated)	Trypsin		NISTmAb		

||| Protein Digest (Reduced + Alkylated) Trypsin

reduced

NISTmAb

.

Example

Confirmed NIST mAb Digest Protein Digest Videtermined ReleasedGlycans1.d Released Glycans

ReleasedGlycans2.d

Undetermined

4

Exercise 11. Reprocess Samples

Steps	Detailed Instructions	Comments
3 Review values in Method Automation > Confirmation Options.	 a Click View > Method Editor, if necessary. b Select Method Automation > Confirmation Options. c Click the Intact Protein tab. d Review selection for the Intact match found but not for the Intact found but not for the Intact found but not for the Protein Digest tab. f Review selection for the Protein partially confirmed when see coverage is >= option. 	 These tabs explain what it means to be Confirmed and Partially confirmed. You are not changing these options. You are only seeing what the software checks to determine if the protein is confirmed. The Confirmation Status is always "Undetermined" if the Workflow is Released Glycans. The Oligonucleotides tab will be reviewed in a different exercise.
	- Method Automation	Protein Protein Diaest Olinopudentides
	Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export • Intact Protein • Protein Digest • Released Glycans • Oligonucleotides - Target Plus Impurities	act Protein match found for the most confirmed undart peak: act Protein match found but not for the Partially confirmed v ast abundant peak: Not confirmed v
	+ Oligonucleotides - Sequence Confirmati	

Exercise 11. Reprocess Samples

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

	Result	ts				Workflow			
Co	nfirmation Status	File Name	Workflow	Experiment	Condition	Modification	Enzyme	Glycan Group	Sequence / Ma
	Confirmed Undetermined	NIST mAb 1.d	Intact Protein		non-reduced	mAb			NISTmAb
	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	
4			Ш						
	Save the res	ults for the s		at a	a Click	File > Save Results.			

c If needed, enter or select a reason in the **Reason for Change** dialog box. Click **OK**.

Exercise 12. Use Result Review mode

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

teps				Detailed Instructions				Comments			
E	Enable Result	Review mode	9.	Click Configuration > Enable Result Review (Disables Method Editing).				• You clic	• You can toggle this mode off by clicking this same command again		
F	Review result: vindow.	s in Sample Ta	able	 All ava Wa rep 	of the op ailable exe orkflow bi	tions in this window a cept for the Run Meth utton. You can still amples.	are nod				
() ()	Sample Table: Relea	sedGlycans2.d							×		
	Sample Table: Relea:	sedGlycans2.d				Wedding			×	•	
	Sample Table: Relea:	sedGlycans2.d				Workflow			×		
Co	Sample Table: Relea: Result ponfirmation Status	sedGlycans2.d ts File Name	Workflow	Experiment	Condition	Workflow Modification	Enzyme	Glycan Group	X Sequence / Mass		
Co	Sample Table: Relea:	sedGlycans2.d ts File Name NIST mAb 1.d	Workflow Intact Protein	Experiment	Condition non-reduced	Workflow Modification mAb	Enzyme	Glycan Group	X Sequence / Mass NISTmAb		
Co	Sample Table: Releas	sedGlycans2.d ts File Name NIST mAb 1.d NIST mAb Digest.d	Workflow Intact Protein Protein Digest	Experiment	Condition non-reduced reduced	Workflow Modification III mAb	Enzyme	Glycan Group	Sequence / Mass NISTmAb		
Cc	Sample Table: Release	sedGlycans2.d ts File Name NIST mAb 1.d NIST mAb Digest.d NIST mAb Digest.d	Workflow Intact Protein Protein Digest Protein Digest	Experiment	Condition non-reduced reduced reduced	Workflow Modification III mAb III Protein Digest (Reduced - Alk III Protein Digest (Reduced - Alk	Enzyme Trypsin Trypsin	Glycan Group	Sequence / Mass NISTmAb NISTmAb		
Cc	Sample Table: Release () () () () () () () () () () () () () (sedGlycans2.d ts File Name NIST mAb 1.d NIST mAb 2.d NIST mAb Digest.d NIST mAb Digest.2.d ReleasedGlycans1.d	Workflow Intact Protein Protein Digest Protein Digest Released Glycans	Experiment	Condition non-reduced reduced reduced	Workflow Modification III mAb III Protein Digest (Reduced+Alk III Protein Digest (Reduced+Alk	Enzyme Trypsin Trypsin	Glycan Group Example	Sequence / Mass NISTmAb NISTmAb NISTmAb		

Exercise 12. Use Result Review mode

Steps		Det	Detailed Instructions				Comments				
3	Review and compare the <i>ReleasedGlycans1.d</i> and <i>ReleasedGlycans2.d</i> data files.	a b c ((n the Sample containing Rel Press the Shif containing Rel Review the res Compare wind column has be glycans that ai	Table, cl easedGl t key and easedGl sults in th low. The een calcu re in botl	ick the row ycans1.d. I click the r ycans2.d. ne Results RSD (%) ulated for n samples.	v row					
		G17 .									
		State F	esults Compare								×
		部 _世 F	esults Compare	elenced Channe	0. T (B						×
		部曲 F 같음 Intac	Iesuits Compare	eleased Glycans	Oligos - Target Plus	Impurities					×
		部曲 F 首第 Intac	lesults Compare ∰ Protein Protein Digest R Glycan Group ▼+P	eleased Glycans	Oligos - Target Plus	Impunties					×
		部世 日朝 Intac	lesuits Compare ♣ IProtein Protein Digest R Glycan Group ♀+= Example	eleased Glycans	Oligos - Target Plus	Impunties					×
		1114 C	lesults Compare	eleased Glycans	Oligos - Target Plus Reid	Impurities		Rele	asedGlycans2.d		×
			iesuits Compare	eleased Glycans RSD (%) V	Olgos - Target Plus Reie %Quant (FBF) 文	Impurities easedGlycans1.d 'Area ⊽⊽	RT Y	Rele %Quant (FBF) 🏾 🍸	asedGlycans2.d Area ♀	RT 🔽	×
			esults Compare	eleased Glycans RSD (%) マ 9.5	Oligos - Target Plus Relia %Quant (FBF) 및 9.39	Impunties easedGlycans1.d Area ⊽ ¥ 59803751	RT 7 20.611	Rele: %Quant (FBF) ∑ 9.24	asedGlycans2.d Area ♀ 52275585	RT ♥ 20.602	×
		intac	esults Compare Protein Protein Digest R Glycan Group ⊽ +a Example Glycan Name ∇ ↓ G1F H414451	eleased Glycans RSD (%) 9.5 105	Oligos - Target Plus Reik %Quant (FBF) ♀ 9.39 0.99	Impunties easedGlycans1.d Area ⊽ ⊽ 59803751 6330075	RT ¥ 20.611 25.815	Rele. %Quant (FBF) ⊽ 9.24 7.58	asedGlycans2.d Area V 52275585 42841859	RT ¥ 20.602 25.807	× =
		Ri H	esuits Compare	eleased Glycans RSD (%) 9.5 105 1.4 77	Oligos - Target Plus Rela %Quant (FBF) 9.39 0.99 0.81	Impurties easedGlycans1.d Area ∇ V 59803751 6330075 5181136 4087144	RT ♥ 20.611 25.815 28.035 28.915	Rele %Quant (FBF) ▼ 9.24 7.58 0.9 0.31	asedGlycans2.d Area ¥ 52275585 42841859 5080633 1106031	RT ♥ 20.602 25.807 28.018 26.58	× =
		Right 관객 Intec	esuits Compare	eleased Glycans RSD (%) 9.5 105 1.4 77 59.5	Oligos - Target Plus Rela %Quant (FBF) 9.39 0.39 0.81 0.64	Impurities easedGlycans1.d Area	RT ¥ 20.611 25.815 28.035 25.815 19.572	Rele: %Quant (FBF) ▼ 9.24 7.58 0.9 0.21 0.26	asedGlycans2.d Area V 52275585 42841859 5080633 1209200 1453310	RT ¥ 20.602] 25.807 28.018 26.58 20.595	× =
		8월 ⁶ 연혁 Intec	esuits Compare	eleased Glycans RSD (%) ▼ 9.5 105 1.4 77 59.5 57.1	Oligos - Target Plus S-Quant (FBF) ¥ 9-39 0-39 0-39 0-39 0-56 0-56	Impurities easedGlycans1.d Area $\nabla \nabla$ 59803751 6330075 5181136 4097144 3562747 3339149	RT ¥ 20.611 25.815 25.815 25.815 19.572 20.611	Rele %Quant (FBF) ¥ 924 758 021 021 026 147	asedGlycans2.d Area V 52275585 42841859 5080633 1209200 1453310 8335163	RT V 20.602 25.807 28.018 26.58 20.595 19.564	× =
		Rate 인행 Intec	esuits Compare	eleased Glycans RSD (%) 95 105 14 77 59.5 57.1 4.9	Olgos - Target Plus %Quant (FBF) 0.39 0.39 0.41 0.64 0.56 0.55 0.53	Impunties easedGiycans1.d Area \no \no \no 59803751 6330075 5181136 4097144 3562747 3339149 3403961	RT ♥ 20.611 25.815 25.815 19.572 20.611 37.021	Rele %Quant (FBF) ♥ 9.24 7.58 0.9 0.21 0.26 1.47 0.56	asedGlycans2.d Area V 52275585 42841859 5080633 1209200 1453310 8335163 3174203	RT V 20.602j 25.807 28.018 26.58 20.595 19.564 37.021	> 4 11
		Right F	esuits Compare	eleased Glycans RSD (%) 9.5 105 1.4 77 59.5 57.1 4.9 0.6	Oligos - Target Plus %Quant (FBF) ▼ 0.99 0.81 0.64 0.56 0.53 0.49	Impunties easedGlycans1.d Area $\nabla \nabla'$ 59803751 6330075 5181136 4097144 3562747 3539149 3403961 3114756	RT 7 20.611 25.815 28.035 25.815 19.572 20.611 37.021 37.021	Retie %Quant (FBF) 77 924 758 09 021 026 147 056 0.55	asedGlycans2.d Area V 52275585 42841859 5080633 1209200 1453310 8335163 3174200 3086343	RT V 20.602 25.807 28.018 26.58 20.595 19.564 37.021 37.021	> 4 III
			esuits Compare	eleased Glycans RSD (%) 9.5 105 14 77 59.5 57.1 4.9 0.6 7.1	Oligos - Target Plus %Quant (F8F) 7 9:39 0.39 0.44 0.56 0.56 0.56 0.56 0.53 0.49 0.44	Impunties assedGlycans1.d Area ♥♥ 59803751 6330075 518113 6330075 518113 562747 3539149 3403961 3114756 2955099	RT 𝔅 20.611 25.815 25.815 25.815 19.572 20.611 37.021 37.021 16.438 26.815	Rele %Quant (FBF) ♥ 9.24 7.58 0.21 0.21 0.26 0.47 0.55 0.47 0.55 0.47	asedGlycans2.d Area V 52275585 42241859 5080633 1209200 1453310 8335163 3174200 3086343 2677458	RT ♥ 20.602 25.807 28.018 26.58 20.595 19.564 37.021 37.021 16.447	> 4 II
		ិធ័រ្យ F	esuits Compare escilits Compare Giycan Group V 4 Giycan Name V Giycan Name V Giycan Name V Giycan Name V H4N451 H5N4515 H5N45152 H7N451 H5N45152 H7N451 H5N45152 H7N451 H5N4551 H5N455 H5N4551 H5N455 H5N45 H5N4	eleased Glycans RSD (%) ∇ 9.5 105 1.4 77 59.5 57.1 4.9 0.6 7.1 35	Oligos - Target Plus Relat %Quant (F8F) 9.39 0.99 0.39 0.45 0.56 0.55 0.49 0.46	Impurties assedGiycans1.d Area ♥♥ 59803751 6330075 5181136 4097144 3562747 3539149 3403961 3114756 295099 2523553	RT ♥ 20.611 25.815 25.815 25.815 25.815 25.815 19.572 20.611 37.021 37.021 16.438 22.723	Rete %Quant (F8F) 924 758 021 226 147 026 047 055 047 027	asedGiycars2.d Area V 52275585 5080633 1209200 1453310 8335163 3174200 3086343 2677459 1523201	RT ♥ 20.602] 25.807 28.018 26.585 19.564 37.021 37.021 16.447 22.715	×
		6월 년 연혁 Intec	esuits Compare Protein Drotein Digest R Glycan Group V + Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V Glycan Name V	eleased Glycans RSD (%) ▼ 95 105 14 77 5955 57.1 49 0.6 7.1 35 93.6	Oligos - Target Plus %Quant (FBF) ♥ 9-339 0-39 0-39 0-56 0-5	Impurities assedGiycans1.d Area	RT 7 20.611 25.815 25.815 19.572 20.611 37.021 37.021 16.438 22.723 19.572	Rete %Quant (FBF) ♥ 9.24 0.28 0.21 0.26 0.47 0.55 0.47 0.27 1.68	asedGlycans2.d Area ♥ 52275585 42841859 5080633 1209200 1453310 8335163 3174200 3066343 2677458 1523201 9500305	RT V 25807 285018 2658 19564 37021 37021 16447 16247 14992	> 4 II
		高量 ⁴ 登離 intac	esuits Compare Security Compare Glycan Group V a Example Glycan Name V Glycan Name V Glycan Name Glycan Name H41451 H51451 H51451 H51451 H51451 H51451 H5145151 H5145151 H5145151 H51451551 H5145155 H5145155 H514515 H514515 H514515 H5145	RSD (%) ▼ 955 105 14 77 595 57.1 49 0.6 7.1 35 93.6 9.7	Oligos - Target Plus %Quant (Ref) %Quant (Ref) 9:39 0:39 0:49 0:46 0:55 0:55 0:49 0:49 0:44 0:33 0:49 0:44 0:33 0:49 0:49 0:44 0:33 0:49 0:44 0:44 0:54 0:45 0:55	Impurties easedGlycans1d Area y y 99803751 6330075 518136 4097144 3662747 3399149 3403961 3114756 2959099 2523553 1931147 1807009	RT ♥ 20.611 25.815 25.815 25.815 19.572 20.611 37.021 19.572 37.021 16.438 22.723 19.572 39.465	Rele %Quant (FBF) ♥ 924 758 021 026 147 055 047 055 047 027 168 028	asedGlycans2.d Area ▼ 5227554 42841559 5080633 1209200 1453310 3066543 33174200 3066543 3174200 3066543 1522201 152505 155053	RT ♥ 20.602 25.807 26.018 26.58 20.595 19.564 37.021 19.544 37.021 16.447 22.715 14.992 39.473	×

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.



Exercise 12. Use Result Review mode

Steps	Detailed Instructions	Comments		
4 Review and compare the <i>NIST mAb</i> <i>Digest.d</i> and <i>NIST mAb Digest2.d</i> data files.	 a In the Sample Table, click the row containing NIST mAb Digest.d. b Press the Shift key and click the row containing NIST mAb Digest2.d. c Click the Protein Digest Layout button in the main toolbar. d Review the results in the Results Compare window. The RSD (%) column has been calculated for many modifications that are in both samples. 	 The Results Compare window is not visible by default in the Protein Digest layout. Click View > Results Compare to open the window. 		
⁶⁰ m∰ Results Compare		×		
Intact Protein Digest Released Glycans Oligos - Target Plu	is Impurities			
Sequence/Mass	Coverage ∀ + 			
NISTmAb 1 Conf.,1 Par. Conf.	95	_		

1.0			Mandification V		NIST mAb Digest.d		NIST mAb Diges	t2.d
LO	cation	Y	Modification V	%RSD (Height Y	%Quant (Decon Height) 🛛 🖓	Height 🖓	%Quant (Decon Height 🖓	Height S
	C264 [B∖D]	Alkylation (iodoa	69.7	99.56	3314909	100	1125920
	C370 [8	B∖D]	Alkylation (iodoa	104.5	100	3558120	99.06	534759
	C147 [8	B∖D]	Alkylation (iodoa	108	100	2929746	100	39230
	C23 [/	A\C]	Alkylation (iodoa	101.5	100	2010011	100	33077
	C193 [/	A\C]	Alkylation (iodoa	75.7	100	1779144	100	53812
	C229 [8	B∖D]	Alkylation (iodoa				100	109587
	C232 [8	B∖D]	Alkylation (iodoa				100	109587
	C133 [/	A\C]	Alkylation (iodoa	100.3	100	997238	100	16960
	C213 [/	A\C]	Alkylation (iodoa	127.3	100	1038957	100	5473
	NR18 (Deamidation	85.1	52.85	182537	65 35	73384

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



Exercise 12. Use Result Review mode

S	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 is the Peptide Relative Quantitation Results window.
	${}^{\rm eff}_{\rm eff}$ Peptide Relative Quantitation Results
	Location △ ∀ + Variable Mods ▽ + File ▽ + %Quant (Decon Height) ♡ + Height ♡ + > C133 (A)(C) Alkylation (lodoaceta NIST mAb Digest.d 100 997238 > C133 (A)(C) Alkylation (lodoaceta NIST mAb Digest.d 100 169606 > C137 (B)(D) Alkylation (lodoaceta NIST mAb Digest.d 100 2292746 Sequence ♡ + Fixed Mods ♡ + Variable Mods ♡ + Use for %Quant ♡ + Height ♡ + SGGTALGCLV Alkylation (lodoaceta ☑ 2721435 LGCLVK Alkylation (lodoaceta ☑ 2721435 LGCLVK Alkylation (lodoaceta ☑ 27310 Location △ ♡ + Variable Mods ♡ + % KQuant (Decon Height) ♡ + Height ♡ + C147 (B)D Alkylation (lodoaceta ☑ 27310 100998 322302 322302 322302 Sequence ♡ + Fixed Mods ♡ + Y = Use for %Quant ♡ + Height ♡ +
	Location △ ▽ +■ Variable Mods ⊽ +■ File ▽ +■ %Quant (Decon Height) ⊽ +■ Height ⊽ +■
	C193 [A\C] Alkylation (iodoaceta NIST mAb Digest.d 100 1779144

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

Exercise 13. Use Report Builder

Steps	Detailed Instructions	Comments
2 Open an existing template.	 a Click File > Open > Browse. b Select IntactProteinReport.template.xml and click Open. a Click File > Save As > Browse. b Enter a file name and click Save. c If needed, enter a reason and click OK. 	 Report templates are installed in the Report Templates\BioConfirm folder in the project.
Open Template File	l	3
Selected Path: C:\Projects\Example\Report Templates\BioConfirm\12.0\en-US\Letter	[] ¹ [5] [5]	Agilent recommends that you
DARReport.template.xml		do not modify the default
Graphic-template.xml		templates. Instead, make a copy
GraphicSequenceCoverage.template.xml		of the template and modify the
IntactProteinReport.template.xml		сору.
ProteinDigestReport.template.xml		
ReleasedGlycanReport.template.xml		
SequenceConfirmationReport.template.xml		
SequenceConfirmationReport_Multiple.template.xml		
TargetPlusimpuritiesReport.template.xml		
File name: IntactProteinReport.template.xml	Open	
Files of type: Report Builder Templates (*.template.xml)	Cancel	

Exercise 13. Use Report Builder

Steps		Detailed Instru	ictions	Comments		
3	Review the template in Report Builder.	 a Click an iter that the rig! b Click the tit c In the right in the Cont d Click the dialog box. e Click the HI f Enter My In g Click OK. 	m in the template. Notice nt pane changes. le of the report. pane, click Localized Text ent section. button. The Localized Text opens. eader_Center. ttact Protein Report.	 The left pane show right pane shows the current selecti You can make ma to the report. This you one possibility the online Help to customizing a rep 	ws the template. The the parameters for on. iny different changes exercise only shows /. Press F1 to access learn more about ort template.	
			Localized Text		×	
		Кеу	Default Value			
		Footer_Right	Generated at &[Time] on &[Date]			
		Footer_Center	Page & [Page] of & [Pages]			
		Header_Center	My Intact Protein Report			
				ОК Са	ancel	

Exercise 13. Use Report Builder

iteps		Det	ailed Instructions	Comments	iments		
Save the template.aClick File > Save.bIf needed, enter a reasoncClose the Report Builder			Click File > Save . f needed, enter a reason and click OK . Close the Report Builder program.	 You can in the file is s template. you do no templates 	stead click File > Save , and saved to the current report Agilent recommends that t modify the default		
File He File Z Y Delete Edit	Font	h_IntactProteinRe s = = = = Fo = = = Alignment	re color Back color Back color Back color Back color Page Textbo Colors	Protein Report) : Image List Insert	? - C X		
		fl Mu Te	Agilent				
GlobalReportPr	operties	LIMY III			✓ Alignment		
PlotOptions					Vertical Alignm Bottom		
Sample Infor	mation				✓ Border		
{C:SampleName}	{V:SampleName}	{C:DataFilePath}	[Expression]		> Paddings 1pt1pt1pt1pt		
{C:SampleID}	{V:SampleID}	{C:AcqTime_UTC}	{V:AcqTime_DTD}		v Content		
{C:InstrumentNa	ne} {V:InstrumentName}	{C:MethodPath_Acquisit	f [Expression]		• content		
{C:MSType}	[Expression]	{C:Version_AcqSW}	{V:Version_AcqSW}		Content lype Localized lext		
{C:MSInfo}	[Expression]	{C:MethodPath_Analysis	[Expression]		Expression		
LC Info	See Instrument Config Report	{C:BioConfirm_Version}	[Expression]		> Field Caption		
{C:SamplePlateP	sition {V:SamplePlatePosition}	{C:DAOperator}	{V:DAOperator}		> Field Value		
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Matched Seq (C.Confination (Seperation.) Sample Chron Sample Chrom	RENCES (C.SequenceName) (V.SequenceName) anatogram List ar Chromatogram bogramPlot	(C:RT) (C:Height) (V:RT) (V:Height)	(CMus) getSeuence) John Di) (CPredicte/Holffication) (V/Kus) eyetSeuence) stDelta Di) (V/Redicte/Holffication) (V/Holffication)	99 	General ID Header_Center1 Outline Level Type Type Text box		
Exercise 13. Use Report Builder

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

 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.

Method Editor: Reports (changed)		×							
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+ Protein Digest	Protein Digest :	Acquisition Method :							
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+ MS Extraction	Oligonucleotides - Target Plus Impurities :								
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Exercise 13. Use Report Builder

Step	s			Γ	Detailed Instr	uctions	;		Comm	ents				
6 F	Print a	a Biomol	ecule report.	a t c c	 Click File > Report. Select a dawith the In Mark the File Click OK. 	• Print > ata file t tact Pro Print pre	Biomolec hat was protein workf eview chec	ule ocessed low. k box.	• Whe the r d the s	n you print a eport templ selected wo	a biomole late corres rkflow is u	cule pon sed.	repo ding	rt, to
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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.



Exercise 14. Determine Protein Molecular Weight

S	teps	Detailed Instructions		Comments			
4 Select Maximum Entropy as the deconvolution algorithm.		On the Deconvolutio Deconvolute (Proteir Method Editor, verify Entropy is selected for algorithm.	n tab of the n) section of the that Maximum or Deconvolution				
5	Verify that the Mass range is automatically detected.	Verify that the Auton detection check box	n atic mass range is marked.	 If you clear this check box, then need to manually enter the Mas which can vary for different inta proteins. 	you s range act		
6 Set the Mass step to 1.		Enter 1 for the Mass Method Editor: Deconvolute	Step.		×		
		6 B h K 9 . C .	Find by Protein Deconv	volution 👻			
		Method Automation	Deconvolution Maximum E	Entropy pMod Results LMFE Filters			
		Workflow and Sequences			^		

	Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export Intact Protein Extract Chromatogram (MS) Extract Spectrum (MS) Deconvolute (Protein) Match Tolerances Protein Digest Released Glycans	Deconvolution algorithm Maximum Entropy Deconvolution settings Automatic mass range detection Mass range Use limited m/z range 600.0000-50 Baseline Baseline Baseline factor Adduct Proton Instructs width A struction	0000 00 Daltons 1.0000 Daltons 00.0000 m/z 7.00 20.0000 Daltons
 7 Select the extracted MS peak spectrum. 	Click the spectrum in MS Spectrum window	the Biomolecule	>
8 Deconvolute the spectrum.	 Right-click the Biomo Spectrum window ar Deconvolute > Proteindeconvolution procession 	lecule MS d click in to start the ss. You can also run button in and select D	o click the arrow next to the o the Method Editor toolbar Deconvolute (Protein).

Exercise 14. Determine Protein Molecular Weight

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

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11 Save the results.

12 Save the method to	a
iii_Deconvolution_MaxEnt.m where iii	Ł
are your initials	

- a Click Method > Save As. b Enter iii_Deconvolution_MaxEnt.m for the method name. c Click Save.
- a Click File > Save Results. **b** If needed, enter a reason and click **OK**. c Click Save.

Agilent MassHunter BioConfirm Software Introduction Guide

Exercise 15. Calculate DAR

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

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

Exercise 15. Calculate DAR

Steps	Detailed Instructions	Comments
 4 Select the deconvolution algorithm. Use Maximum Entropy. Use 20000 - 60000 mass range. Do not use the limited m/z range. 	 a On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select Maximum Entropy as the Deconvolution algorithm. b Clear the Automatic mass range detection check box. c Enter 20000 - 60000 for the Mass range. d Clear the Use limited m/z range check box. 	 For more information on these parameters, press F1 to open the online Help.
	Rethod Editor: Deconvolute (Protein) (changed)	×
	🕼 🥵 🌆 🌠 🧐 🔹 🕐 🔹 💽 Find by Protein Deconvolution 🔹	
	- Method Automation Workflow and Sequences Confirmation Options Additional Chromatograms Reports Export - Intact Protein Extract Chromatogram (MS) Extract Spectrum (MS) Deconvolution Mass range Use limited m/z range Baseline Subtract baseline Baseline factor Adduct Protein Isotope width Automatic Oligonucleotides - Sarget. Oligonucleotides - Sarget. Oligonucleotides - Sarg	pMod Results LMFE Filters intropy 20000 - 60000 Datons 1.0000 Datons 500 0000-5000 0000 m/z 7.00 20 0000 Datons
5 Use the default settings for Maximum Entropy deconvolution.	Click the Maximum Entropy tab to review settings.	
6 Set the time range for extracting spectra to 6 to 8 minutes.	 a On the Extract Spectrum (MS) tab of the Intact Protein section of the Method Editor, click Time Range(s). b Enter 6 as the Start and 8 as the Stop. 	
7 Set the workflow parameters for the reduced Condition.	 a On the Workflow and Sequences tab of the Method Automation section of the Method Editor, select Intact Protein. b Select reduced as the Condition. c Enter 2.34.39, 50.594 for Sequences/Masses. d Mark the Calculate Drug-to-Antibody Ratio (DAR) check box. e Enter 957.5 as the Drug + linker mass. 	 For a method with reduced as the Condition, you enter the Reduced DAR Light Chain, Reduced DAR Heavy Chain as the Sequences/Masses.

Exercise 15. Calculate DAR

Steps	Detailed Instructions	Comments
8 Save the method.	 a Click Method > Save As. b Type the File name <i>iii_DAR_Reduced.m</i>, where <i>iii</i> represents your initials. c Click Save. d If needed, enter a reason in the Reason for Change dialog box. e Click OK. 	The administrator can set up a project to require you to enter a reason for a change to a method
9 Run the method workflow on the DAR_Sample_Reduced.d file.	 a Click Method > Run Method Workflow, or click Run Method Workflow in the toolbar in the Method Editor window. b Select DAR_Sample_Reduced.d and click OK. 	bd
10 Review deconvolution results.	• The results appear in the Deconvolution Results window and i the Sample Table window.	 Deconvoluted spectra are added for the light chain and for the heavy chain The DAR value is added to the Sample table.
Deconvolution Results		×
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+ESI Scan (rt: 16.501-17.607 min, 68 scan + Light	t Chain:DAR Calc Scan (it: 6.532 min) Deconv + Heavy Chain:DAR Calc Scan	(t: 6.532 · · ·
x103 Light chain. DAir Arg Abandance - 5000	se in Calc The DAR Peak The Peak Mass (Da) The Area The	% Area ∵ .
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Light Chain:DAR Calc +ESI Scan (rt: 6.532	2 52510 7295429	24.54
x106 DAR Value = 0.04	✓ 3 53630 2816046	9.47
Heavy Chain DAR Calo +ESI Scan (rt. 6.5. x10 s DAR Value = 1.30	✓ 4 54588 1615647	5.44
30000 40000 50000 Counts vs. Deconvoluted Mass (amu)		
11 Open the method to use as a startin point for the new method.	g a Click Method > Open. b Select BioConfirmIntactProtein-Default.m c Click Open.	
12 Select the deconvolution algorithm.	a On the Deconvolution tab of the	For more information on these

- Use Maximum Entropy.
- Use 20000 60000 mass range.
- Do not use the limited m/z range.
- Deconvolute (Protein) section of the Method Editor, select **Maximum** Entropy as the **Deconvolution** algorithm.
- b Clear the Automatic mass range detection check box.
- c Enter 20000 60000 for the Mass range.
- d Clear the Use limited m/z range check box.

parameters, press F1 to open the

online Help.

Exercise 15. Calculate DAR

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



17 Save the results.

- a Click File > Save Results.
- b If needed, enter a reason and click OK.
- c Click Save.

Exercise 16. Use the Mirror Plot window

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

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

Exercise 16. Use the Mirror Plot window

Steps	Detailed Instructions		Comments			
 4 Select the deconvolution algorithm. Use Maximum Entropy. Use the automated mass range detection. Use the limited m/z range of 2400-4000. Use 3 for the baseline factor. 	 a On the Deconvoluti Deconvolute (Prote Method Editor, sele Entropy as the Dec algorithm. b Mark the Automati detection check box c Mark the Use limite box. d Enter 2400 - 4000 f e Enter 3 for the Base f Select Unspecified width. 	on tab of the in) section of the ct Maximum onvolution c mass range x. ed m/z range check or the <i>m/z</i> range. eline factor. for the Isotope	For more information on these parameters, press F1 to open the online Help.			
	Provide the state of the state	otein) (changed)				
		Find by Protein Deconvolution	•			
	Method Automation	A Deconvolution Maximum Entre	ppy pMod Results LMFE Filters			
	Workflow and Sequences Confirmation Options	Deconvolution algorithm Maxim	aximum Entropy V			
	Additional Chromatograms	Deconvolution settings				
	Reports	Automatic mass range detec				
	Export	Mass range	140000.00-160000.00 Daltons			
	Intact Protein	Mass step	1.0000 Daltons			
	Extract Chromatogram (MS)	Use limited m/z range				
	Extract Spectrum (MS)		2400 - 4000 🛕 m/z			
	Deconvolute (Protein)	Baseline				
	Match Lolerances	Baseline factor	3.0			
	Protein Digest					
	Released Glycans	Adduct Proton	~			
	MS Extraction	Isotope width Unspecified	20.0000 Daltons			
5 Use the default settings for Maximum Entropy deconvolution.	Click the Maximum review settings.	Entropy tab to				
6 Run the Find by Protein Deconvolution algorithm.	Click Find and Ident Deconvolution > Pr or	tify > Find by oteins.				
	Click Find by Protei the toolbar in the Ma	n Deconvolution in ethod Editor				

window.

Exercise 16. Use the Mirror Plot window

Steps	Detailed Instructions	Comments
7 Review deconvolution results.	The results appear in the Deconvolution Results windo	W.
	Deconvolution Results	× M L LT L % % % K ~ IL ~
8 Open the NIST mAb 2.d data file.	 a Click File > Open Data File. b Locate the NIST mAb 2.d sar c Click Open. 	• The TIC is automatically displayed in the Sample Chromatogram Results window.
9 Run the Find by Protein Deconvolution algorithm on NIST mAb 2.d.	Click Find and Identify > Find Deconvolution > Proteins.	by
10 Review deconvolution results.	The results appear in the Deconvolution Results windo	W.
11 Select both data files in the Sample Table window.	 a Select one of the sample files Sample Table window. b Press the Ctrl button and clic other sample file. 	s in the • The results for the sample files selected in the Sample Table are shown in the Deconvolution window and other windows.
12 Use Mirror Plot to compare two deconvoluted spectra.	 a Click the button to show spectra in list mode. b Select a spectra from the Deconvolution window. c Press the Ctrl button and sele another spectra from the oth file. d Click the button to displ spectra in the Deconvolution Plot Results window. 	 Other mirror plots are available: MS Spectrum Mirror Plot Fragment Spectrum Mirror Plot Biomolecule Chromatogram Mirror Plot Sample Chromatogram Mirror Plot ay the
	Deconvolution Mirror Plot	×
	+ESI Scan (rt: 1.792-1.925 min, 9 scans) Frag +ESI Scan (rt: 1.802-1.918 min, 8 scans) Frag	=400.0V NIST mAb 1.d Subtract =400.0V NIST mAb 2.d Subtract

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147000

149000

149500

150000

147500 148000 148500 Counts vs. Deconvoluted Mass (amu)

Exercise 17. View Biomolecule Information

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

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

Exercise 17. View Biomolecule Information

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

Biomolecules: 14	found														×
M 🐇 🗈 🖲	2														
					G	ieneral						% Quantitation	S	equence Ma	at 👗
Label / 5	7 Mass	T RT S	7 Height	V Are	a V	MinZY	Max Z 🏹	File	7	Mining Algorithm	▼ Z Count ▼	Use for %Quant	V	Fit Score	
Biomolecule 1	148201.5823	2.025	138574	902	5919	30	87	NIST mAb 1.d		Maximum Entropy Dec	onv 53		1	0	=
Biomolecule 2	148363.6859	2.058	127009	895	6909	30	121	NIST mAb 1.d		Maximum Entropy Dec	onv 72		1	0	
Biomolecule 3	148039.318	2.074	78986	498	5562	30	95	NIST mAb 1.d		Maximum Entropy Dec	onv 60		9	1	
Biomolecule 4	148525.2449	2.041	78478	601	5220	30	95	NIST mAb 1.d		Maximum Entropy Dec	onv 56		9	1	
Biomolecule 5	148324.012	1.958	59060	101	0548	30	65	NIST mAb 1.d		Maximum Entropy Dec	onv 30		9)	
Biomolecule 6	148161.8174	2.074	50246	516	73	30	54	NIST mAb 1.d		Maximum Entropy Dec	onv 22		9)	-
4															

Figure 8. Biomolecules window for NIST mAb 1.d

Scan + Scan (r	t: 1.991-2.124 min)			_
Peak ∕ ∀+Þ	m/z ⊽+¤	Area ⊽-Þ	Abund ⊽-Þ	Max Abund ▼+	Z TP
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

Exercise 17. View Biomolecule Information



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

Exercise 18. Open results using versions

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

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

Steps	3	tailed Instructions					Comments				
1 Se	Select the NIST mAb 1.d data file.aClick File > Open Data File.bSelect the NIST mAb 1.d folder.				der.	 In the previous exercises, results wer stored with NIST mAb 1.d multiple times. 					
2 Se	Select the File version to open. a b c		 Mark Load result data. Select the File version to open. Click Open. 				 You select the file version based on date and time that the version was saved. You can look at the Results Audit Tr window to look at the Reason colurn or the Review Comment column to help determine which File version to open. 				
Open Sar	nple					×					
Selected	ected Path: /ph/Data										
	Name			Size Date Cre 2021-0614-1828-51054 2021-0614-1823-41841				You need to mark the Load			
	NIST mAb 1.d				6/14/2021 10:- 2021-0614-18 2021-0614-17	5-05827	r	result data check hox before			
	NIST mAb 2.d				6/14/2021 10:45:06 HW10/14/20	21 10.45 28 HW	,	you can calcot which regults to			
	NIST mAb Digest.d			6/14/2021 10:43:29 AM6/14/2021 10:44:53 AM 6/14/2021 10:44:54 AM6/14/2021 10:45:43 AM 6/14/2021 10:45:43 AM6/14/2021 10:47:28 AM)				
	NIST mAb Digest2.d						I	oad. If the list of results is			
	NIST mAb Disulfide Mapping.d						Q	grayed out, verify that the			
L, K	ReleasedGlycans1.d			6/14/2021 10:47:28 AM6/14/2021 10:48:56 AM			(check box is marked.			
[] R	ת RelessedGlycans2.d			6/14/2021 10:48:57 AM/6/14/2021 10:50:30 AM							
File nan	ne: NIST mAb 1.d (2021-0614-1828-51054)					Open					
Files of	type: Data File(s) (*.d)					Cancel					
Option ○ La ○ La ● Us ✔ La	ns ad worklist method ad results method e current method ad result data										

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In This Guide

This guide teaches you how to use MassHunter BioConfirm 12.0.

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