

Agilent MassHunter BioConfirm Software

Familiarization Guide

For Research Use Only. Not for use in diagnostic procedures.

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Where to find more information

- Agilent MassHunter BioConfirm Software Quick Start Guide
- Agilent MassHunter BioConfirm eFamiliarization
- Agilent MassHunter BioConfirm Training Videos
- Online Help provides in-depth information and can be displayed in the following ways:
 - Click Contents or Search from the BioConfirm software Help menu.
 - Press the F1 key to get more information about a window or dialog box.

How to use this guide

Try to do these familiarization 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:

- Copy all of the data files from the **Data** folder on the BioConfirm setup media to your computer hard drive. We recommend copying the data files to the **D:\MassHunter\Data** folder.
- 2 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**.
- **3** Copy all of the sequences from the **ProteinSequences** folder on the BioConfirm setup media to your computer hard drive. We recommend copying the data files to the **D:\MassHunter\ProteinSequences** folder.

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.

Task 1. Open the BioConfirm program with multiple data files

Steps	Detailed Instructions	Comments
 Open the BioConfirm program. Open these data files NIST mAb 1.d Nist mAb 2.d NIST mAb Digest.d NIST mAb Digest2.d ReleasedGlycans1.d ReleasedGlycans2.d 	 a Double-click the Agilent MassHunter BioConfirm 10.0 icon. The system displays the Open Sample dialog box. b Go to the folder D:\MassHunter\Data or to the folder where the example files are located. 	 You can get help for any window, dialog box, or tab by pressing the F1 key when that window is active.
 D:\MassHunter\Data, or in the folder where you copied them. Make sure that the Use current method button is clicked. Make sure that the Load result data check box is cleared. 	Look in: Data Cook in: Cook in: Data Cook in: C	
	File name: "NIST mAb 1.d" "NIST mAb 2.d" "NIST mAb Dget. File name: "NIST mAb 1.d" "NIST mAb 2.d" "NIST mAb Dget. File name: Files of type: Data Flee (".d) Options Octoor Options Sample Information Load results method Sample Name : @ Use current method Sample Position : Description : Description :	Cancel Cancel Help
	Figure 1. Open data files when openin	n software

Task 1.	Open the	BioConfirm	program	with m	nultiple	data files ((continued))
			1 - 2 -					

Steps	Detailed Instructions	Comments
	 c Click the NIST mAb 1.d file. d Press and hold the Shift key while you click NIST mAb Digest.d. e Press and hold the Ctrl key while you click ReleasedGlycans1.d and ReleasedGlycans2.d. f Clear the Load result data check box. g 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. h Click the List Mode button in the Sample Chromatogram Results toolbar. i Click the NIST mAb 1.d data file. 	 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.
2 Return the main window to the default Intact Protein layout.	Click Intact Protein Layout in the main toolbar.	 You click the button in the graphics window to change the display options. 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.

Basic Tasks Task 1. Open the BioConfirm program

Task 1. Open the BioConfirm program with multiple data files (continued)



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.

Task 2. Zoom in and out of the chro	omatogram
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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.

Basic Tasks Task 2. Zoom in and out of the chromatogram

Task 2. Zoom in and out of the chromatogram (continued)

St	eps	De	etailed Instructions	Сс	omments	
2	 Practice zooming in and out on each axis separately. Zoom in only along the x-axis. Hint: Right-click the x-axis values and move cursor from left to right. Partially zoom out the x-axis. Hint: Move cursor in opposite direction. Completely zoom out of the x-axis. Repeat the previous steps for the y-axis. Completely zoom out of the x-axis. Completely zoom out of the x-axis. Click the rew cursor to the double arrow to cursor to the double arrow to the new cursor to the new cursor to the double arrow to the new cursor to the double arrow the new cursor to the double arrow to the new cursor to the double arrow to the new cursor to the new curs	To zoom in on the x-axis, move the cursor to the x-axis values until a horizontal double arrow appears. Click the right mouse button and drag the new cursor from left to right	the a m M S. m M nd drag	M My 8 1 1.2	Horizontal Double Arrow	
		с	 across the x-axis values. To zoom out on the x-axis, click the right mouse button and drag from right to left on the x-axis values. Click the Autoscale X-axis button, +, to completely zoom out on the x-axis. To zoom in on the y-axis, move the cursor to the y-axis values until a vertical double arrow appears. 	V~ 1.8	√ √ 0.9 ⁻¹ 7 1.1 1.	New cursor appears when you right-click the x-axis value
		d a			4.4- 4.2- 1 4- 3.8-	Vertical Double Arrow
		 b Click the right mouse button and drag the new cursor from bottom to top across the y-axis values. c To zoom out on the y-axis, click the right mouse button and drag from the top towards the bottom of the y-axis 	New cursor appears when you right-click the y-axis values.			
		d	Click the Autoscale Y-axis button, 1 , to completely zoom out on the y-axis.			

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 each workflow.

Task 4. Change window layout

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, and Released Glycans Layout. To load a layout, click Configuration > Windows Layouts > Load Layout. To save a window layout, click Configuration > Windows Layouts > Load Layout. To lock or unlock a layout, click Configuration > Window Layouts > Lock Layout. To nake 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 3. 	 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. The following layouts are shipped with the software: Default_IntactProtein.xml Deafult_Protein_Digest.xml Default_Released_Glycans.xml

Figure 3. Window repositioning tools

Basic Tasks

Task 3. Change window layouts

Task 4. Change window layout (continued)

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

Task 4. Creating a Protein Sequence File

This task guides you through the creation of a myoglobin sequence file.

Steps		Detailed Instructions	Comments	
1	Start the Agilent MassHunter Sequence Manager.	Click Sequence > Sequence Manager.		
2	Create a new sequence.	 a Type Myoglobin for the name of the Sequence. b Click the + button. The Sequence Editor pane opens automatically with a new sequence displayed for editing. 	Protein is automatically selected for the sequence type.	
3	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.	
	GLSDGEWQQVLNVWGKVEADIAGHG KASEDLKKHGTVVLTALGGILKKKGH VLHSKHPGDFGADAQGAMTKALELFF	QEVLIRLFTGHPETLEKFDKFKHLKTEAEM HEAELKPLAQSHATKHKIPIKYLEFISDAIIH RNDIAAKYKELGFQG	• 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.	
N	ote: The myoglobin sequence does not	have any links or modifications, but some seque	ences do. In that case, add links and	

modifications as described in the *Quick Start Guide* or *online Help*.

Basic Tasks

Task 4. Creating a Protein Sequence File

Steps		Detailed Instructions	Comments
4 Save the sequence as the na iii_myoglob.psq, where iii rep your initials.	ame presents	 a Click Sequence > Export Sequences. b Type <i>iii</i>_myoglob in the File name box. c Click Save. 	• The sequence is saved as a .psq file that can be imported for use in other methods as described in Exercise 4 or referenced from worklists as described in Exercise 5.
Image: Provide the sequence of			
Sequences	Example_	Myoglobin	
Search Q Enolase Example synthetic peptide Example Myoglobin A Myoglobin (horse) NISTmAb NISTmAb Missing Disulfide link Serotransferrin	Example_Myoglobin Display information using unspecified disulfide linkages Total monoisotopic mass; 16940 9651 Total average mass; 16951 6073 Sequence molecular formula: C769H1212N2100218S2 A:Chain A Monoisotopic mass; 16940 9651 Average mass; 16951.6073 M 1 N-term SLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEKFDKFKHI 69 LTRALGGILKKKGHHEAELKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHPGDFGADF 147 KELGFQG C-term		rage mass: 16951.6073 M TGHPETLEKFDKFKHI IHVLHSKHPGDFGADF

Task 4. Creating a Protein Sequence File

Intact Protein Workflow

Step 1 - Open the data file of interest.

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 **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 Sample Table, 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

Biomolecule Fragment 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 mAb1.d** data file copied before you started. See "**Before you** start" on page 2.

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

Steps		Detailed Instructions	Comments	
1	Open the method to use as a starting point for the new method.	 a Click Method > Open. b Select the BioConfirmIntactProtein-Default.m folder. c Click Open. 		
2	If the NIST mAb1.d data file is not already open, open it.	 a Click File > Open Data File. b Locate the NIST mAb1.d folder. c Click Open. 	 The TIC is automatically displayed in the Sample Chromatogram Results window. 	
3	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. 		
4	Run the Find by Protein Deconvolution algorithm.	 a Review the settings and modify them if necessary. b Click of 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. 	
5	Display the Workflow and Sequences section in the Method Editor window.	 Click Method Automation > Workflow and Sequences in the Method Editor window. 		

Exercise 1. Interactive Intact Protein Workflow

Stens	Detailed Instructions	Comments
6 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 parameter. The Select Sequences dialog box opens. d Double-click NISTmAb. If NISTmAb is not available, click Import. 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.
7 Select the mAb modification.	 a Click the button next to the Modifications 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.
8 Start the match search.	 a Click Intact Protein > Match Tolerances. b Click on the Method Editor toolbar. c Select NIST mAb 1.d and click Match. 	Alternate methods:Click Find and Identify > Match Sequences.
9 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.

Exercise 1. Interactive Intact Protein Workflow



Exercise 1. Interactive Intact Protein Workflow

Steps						Detaile	d Ins	tructions	Comments					
Biomolecules: 32	found													×
🎬 🆀 🗈 🖲	2													
			Gener	al							% Quantitation			
Label V	Mass	V RT V	Height V	Area 🛛	Score	▼ Min Z ▼ I	Max Z 🏹	ID Source V V	Use for %Quant V	Area (MS)	♥ %Quant (Area) ♥	Height (MS) 🔽	%Quant (Height)	7
Biomolecule 1: A(148201.3347	2.025	2488	9166272	92.13	30	101	Sequence Match	2	1765983	11.26	2488	11.88	
Biomolecule 2: A(148363.3865	1.859	2335	1336501	85.49	30	90	Sequence Match	~	1737180	11.07	2335	11.15	-
Biomolecule 3: A(148039.3039	1.842	1567	904188	97.74	30	81	Sequence Match		1039079	6.62	1567	7.48	1
Biomolecule 4: A(148524.599	1.842	1428	1034340	48.52	30	69	Sequence Match		1266461	8.07	1428	6.82	
Biomolecule 5: A(148324.09	2.107	1138	211834	0	118	185	Sequence Match	~	1290098	8.22	1138	5.43	
Biomolecule 9: A(148454.5479	1.842	930	582118	0	30	58	Sequence Match		574872	3.66	930	4.44	
Biomolecule 11: A	148686.4412	1.826	904	802297	3.02	30	70	Sequence Match		716373	4.57	904	431	
Biomolecule 18: A	148201.095	2.058	3294	9106592	71.88	30	115	Sequence Match		2120360	13.52	3294	15.73	
Biomolecule 19: A	148363.0459	2.058	3034	7669524	52.32	30	109	Sequence Match		2242496	14.29	3034	14.48	
Biomolecule 20: A	148039.148	2.058	1911	5087483	89.14	30	98	Sequence Match		1204610	7.68	1911	9.13	
Biomolecule 21: A	148524.6686	2.041	1884	6157593	41.81	30	78	Sequence Match		1704246	10.86	1884	8.99	
Biomolecule 30: A	148348.1728	4.561	17	15640	0.02	30	151	Sequence Match		16080	0.1	17	0.08	
Biomolecule 31: A	148040.2569	4.544	17	9133	37.12	30	156	Sequence Match		9498	0.06	17	0.08	
Biomolecule 6	148160.5959	2.058	1078	1792174		30	106			868267		1078		

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



Agilent MassHunter BioConfirm Software Familiarization Guide

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 mAb1.d** data file copied in Exercise 1.

St	eps	D	etailed Instructions	Comments		
1	If not already open, open the method iii_NIST_mAb_Intact.m.		Click Method > Open . Select the <i>iii_</i> NIST_mAb_Intact.m folder. Click Open .	This method was created in "Exercise 1. Interactive Intact Protein Workflow " on page 13.		
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 Import the NISTmAb sequence.		 a Select non-reduced for the Condition. b Click the button next to the Sequences parameter. The Select Sequences dialog box opens. c If NISTmAb is not available, click Import. d Select <i>NISTmAb</i>.pSq and click Open. e Verify that the NISTmAb sequence is in the Selected list. f Click OK 			The NISTmAb.psq sequence file is available on the BioConfirm setup media. You can learn about modifications and links in the <i>online Help</i> and in the <i>Quick Start Guide</i> .	
5	Save the method.	•	Click Method > Save.			
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. Reprocess the sample. See "Exercise 7. Reprocessing Samples" on page 33.	•	Method Automation first runs the method workflow, and then extracts additional chromatograms and generates a biomolecule report and exports results.	

Exercise 2. Automated Intact Protein Workflow

St	eps	Detailed Instructions	Comments	
7	(optional) Review the printed Biomolecule reports.	 If you clicked Run Method Automation, 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. 	

NISI mAb 1.d	Papat contents
	Gran Fesuits Separate report per data file
	 Only highlighted results
	Print report
	Print report
	Printer name: <default> ~</default>
	Print preview
	Page size: 0 44 (@ Letter
	Save report
	Save report
	Inside data file's reports subdirectory
	At specified directory:
	D:\MassHunter\reports
	If report file already exists
	Overwrite existing report
	Auto-generate new report file name

Exercise 2. Automated Intact Protein Workflow

Protein Digest Workflow

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

Step 1 - Open the data file of interest.

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 **Sequence/Masses** to match on the Workflow and Sequences tab.

If the sequence you want to match is not in the method or Sample Table, 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 8 - 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 9 - 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 2.

S	reps	Detailed Instructions	Comments			
1	Open the method to use as a starting point for the new method.	 a Click Method > Open. b Select the BioConfirmProteinDigest-Default.m folder. c Click Open. 	• The parameters in the BioConfirmProteinDigest-Default. m method are a good starting point for Protein Digests.			
2	Load the Protein Digest default layout.	Click Protein Digest Layout on the main toolbar.	 The TIC is automatically displayed in the Sample Chromatogram Results window. 			
3	Select NIST mAb Digest.d. If necessary, open the example sample file.	 If available, select NIST mAb Digest.d. otherwise a Click File > Open Data File. b Locate the NIST mAb Digest.d sample. c Clear the Load result data check box. d Click Open. 	• The TIC is automatically displayed in the Sample Chromatogram Results window.			
4	Review the parameters in the Find Peptides section in the Method Editor window.	 a Select Protein Digest > Find Peptides in the Method Editor window. b Review the settings on the various tabs of the Find Peptides section. c Click the MS-Only Extraction tab. d Review the parameters. For the example file, you can restrict the mass range to 300 - 1700. e In the MS-Only Extraction tab, enter 500 for the Use peaks with height >= counts. 	 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	Find biomolecules.	 a Click on the Method Editor toolbar to start the biomolecule search. b If the Find Peptides dialog box opens, select NIST mAb Digest.d and click Find. c When processing is complete, review the results in the Biomolecules window. 	 You can instead click Find and Identify > Find Peptides. 			

Protein Digest Workflow Exercise 3. Interactive Protein Digest Sequence Matching

Steps	Detailed Instructions	Comments
Biomolecules: 280 found		×
11 ÷		
G	ieneral	-
Label V m/z V Mass V RT V Heig	ght 文 Area 文 Min Z 文 Max Z 文 File 文 MS/MS Count 文 Seq Name ·	▼ Seq Loc ▼ Tgt Seq Mass ▼ D
▶ Biomolecule 1 952,5351 951,5344 7,579 3784	837 2873301 1 2 NIST mAb Digest.d 1	
Biomolecule 2 1161.6304 1160.629 9.01 3835	631 2010681 1 3 NIST mAb Digest.d 1	
Biomolecule 3 1321.6779 1320.6745 8.164 3337	365 1318007 1 3 NIST mAb Digest.d 2	
Biomolecule 4 838.5037 837.499 6.438 3457 Biomolecule 5 330.6817 659.351 7.197 2510	963 1526813 1 2 NIST mAb Digest.d 1	
Biomolecule 6 700.4356 699.4296 4.068 3532	729 1620004 1 2 NIST mAb Digesta 1	
Biomolecule 7 1070.0165 2138.0212 9.361 2371	596 1372041 2 4 NIST mAb Digest.d 3	
Biomolecule 8 751.8838 1501.7528 9.492 2448	393 8774645 1 3 NIST mAb Digest.d 2	
		×
5 Import the sequence.	 a Click Method Automation > Workflow and Sequences in the Method Editor window. b Select Protein Digest as the Workflow. c Select reduced as the Condition. d Click the next to the Sequences/Masses parameter. e Double-click NISTmAb. f Click OK in the Select Sequences dialog box. g Click the next to the Mods and Profiles parameter. h Double-click Protein Digest (Reduced+Alkylated). i Click OK in the Select Modifications and Profiles dialog box. j Mark the Trypsin check box under Enzymes. 	 For this exercise, you use the sequence as is, but you can add modifications and links to sequences as described in <i>online Help</i>. You can customize the list of available reagents using the Chemical Data Dictionary; see <i>online Help</i> for more information.
7 Review parameters on the Mass Matching tab.	 a Click the Mass Matching tab in the Protein Digest > Match Tolerances section of the Method Editor window. b Review the parameters. 	
8 Review the Matching Rules.	 a Click the Matching Rules tab in the Protein Digest > Match Tolerances section in the Method Editor. b Mark the Allow free cysteines (non-reduced condition) check box. c Enter 2 for the Allow missed cleavages up to. d Review the other parameters. 	
9 Save the method for use in Exercise 7.	 a Click Method > Save As. b Type the File name iii_NIST_mAb_ProteinDigest.m, where iii represents your initials. c Click Save. 	

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. b Select NIST mAb Digest.d. c Click Match. 	 Alternate methods: Click O on the Method Editor toolbar. Click Match Sequences on the Method Editor shortcut menu. 		
11 Review the results.	 a Highlight Biomolecule 3 in the Biomolecules window. b Click the Biomolecule Identification Results tab which is tabbed with the Biomolecules window. c When you open the window, the window displays the results for the first biomolecule that is selected in the Biomolecules window. d Select another sequence match result to view by selecting a different biomolecule in the Biomolecules window. 	 If the biomolecule was identified, the ID Techniques Applied column contains Sequence Match. 		
12 View sequence coverage results.	 a If necessary, click View > Sequence Coverage Map. b Select a different biomolecule in the Biomolecules table to view a different result. 	 Amino acids that are matched are either green (MS/MS) or black (MS-only) in a matched sequence. Amino acids that are not matched are gray. A line is added below the AA sequence to display where peptides have been identified. See the online Help for more information. 		
To view more information.	Click the following items on the Sequence Coverage Map window shortcut menu to view more information about the sequence: • Applied Modifications • Specified Applied Links • View Digest List			

Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching



Protein Digest Workflow

Exercise 3. Interactive Protein Digest Sequence Matching

Step	S				D	etail	ed Instruc	tior	IS		Comments
GLT_P	eptide Relative Qu	antita	tion Results								×
管罰	₩ 🖻										
	Location	₹₽	Pred Mods	\. \. \. \. \. \. \. \. \. \. \. \. \. \	File	\. \. \. \. \. \. \. \. \. \. \. \. \. \	%Quant (Height)	7₽	Height	7₽	*
4	C370 [B\D]		Alkylation (iodoaceta	mide)	NIST mAb Dige	st.d	92.65		3835631		
	Sequence	₹₽	Pred Mods	V₽	Use for %Quan	t 🛛 🕫	Height	⊽+¤			=
	NQVSLTCLVK		Alkylation (iodoaceta	mide) 7			3835631				
	NQVSLTCLVK		Alkylation (iodoacetic	acid) 7			162512				
	NQVSLTCLVK		Alkylation (iodoacetic	acid) 7			141643				
	Location	₹₽	Pred Mods	\. \	File	\. \	%Quant (Height)	∀ ₽	Height	7₽	
>	C147 [B\D]		Alkylation (iodoaceta	mide)	NIST mAb Dige	st.d	96.73		3337365		
Þ	C264 [B\D]		Alkylation (iodoaceta	mide)	NIST mAb Dige	st.d	96.4		2371596		
Þ	C23 [A\C]		Alkylation (iodoaceta	mide)	NIST mAb Dige	st.d	97.51		1829634		
Þ	C213 [A\C]		Alkylation (iodoaceta	mide)	NIST mAb Dige	st.d	97.39		1396248		*

- **15** Review the results in the Relative Quantitation Histograms window.
- a Click the Relative Quantitation Histograms window.
- **b** Click the first triangle next to the first row. c Note that **Use for %Quant** is marked for

both rows.

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



- d Match sequences (step 10).
- e Save the results to the NIST mAb
- Digest2.d file (step 13).
- parameters used for the first data file are the same for the second
- These results are used in "Exercise 8. Using Result Review mode" on page 36.

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	Comments		
1	Open the method.	 a Click Method > Open. b Select the iii_NIST_mAb_ProteinDigest.m folder. c Click Open. This meth Exercise 3 initials). 	od was created in (iii represents your		
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. 	stead click the Method on, 📑 , on the main		
3	Select the appropriate workflow.	 a Select Protein Digest for the Workflow. b Select 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 automatic actions: Find Pe Match S 	n Digest workflow ally runs the following ptides Sequences		
4	Save the method.	• Click Method > Save.			
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 7. Reprocessing Samples" on page 33. Method Automation (Chromatoria) 	utomation first runs the orkflow and then dditional grams, generates a ile report, and exports		
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. If you are of interactive these para Biomolecule 	port options in the ditor window in the utomation > Reports creating a report ly, you specify some of umeters in the Print Jle Report dialog box.		

Exercise 4. Automated Protein Digest Workflow

Released Glycans Workflow

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

Step 1 - Open the data file of interest.

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 9 - Review the results which are shown in these windows: Sample Chromatogram Results Biomolecule MS Chromatogram Biomolecules table Biomolecule Identification Results Biomolecule MS Spectrum Biomolecule Fragment Spectrum Glycan Structure Viewer Results Compare Relative Quantitation Histograms Step 9 - 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 2.

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 BioConfirmProteinDigest-Default. m method are a good starting point for Protein Digests.
2	Open the example sample file.	 a Click File > Open Data File. b Locate the ReleasedGlycans1.d folder. 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 Peptides section in the Method Editor window.	 a Select Method Automation > Workflow and Sequences in the Method Editor window. b Enter Example in the Glycan group. c Clear Require RT match if database contains a RT for the target glycan. d Select Released Glycans > Find Glycans in the Method Editor window. e Select Glycans_mAb_AM_PCD.cdb for the Target glycan source. f Click the Tag tab. g 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>. A very low peak height filter can result in greater sequence coverage but requires much more time to process.
5	Find biomolecules.	 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 in represents your initials. c Click Save. 	ï

Released Glycans Workflow

Exercise 5. Interactive Released Glycans

Steps	Detailed Instructions	Comments		
7 Review the results.	 a In the Biomolecules window, click the header of the Area (Glycan) 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. 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. 		



to the data file folder.

results.

Exercise 6. Automated Released Glycans Workflow

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

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

Steps		Detailed Instructions		Comments		
1	Open the method.	 a Click Method > Ope b Select the iii_ReleasedGlycan folder. c Click Open. 	n. ns_InstantPC.m	• 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 > Method Editor. b Click Method Auto and Sequences in window. 	is not visible, click View omation > Workflow the Method Editor	 You can instead click the Method Editor button, in , on the main toolbar. 		
3	Select the appropriate workflow.	 a Select Released Gly b Enter Example in c Select Glycans_m the Target glycan d Clear the Require contains a RT for check box. 	ycans for the Workflow. the Glycan group. Ab_AM_PCD.cdb as source. RT match if database the target glycan	 The Released Glycans workflow automatically runs the Find Glycans algorithm. The Glycan group is used to organize the results in the Results Compare window. 		
		Method Editor: Workflow and Se	quences	×		
) Run Method Workflow 🔹			
		Workflow and Sequences	Workflow: Released Glv	cans 🗸		
		Confirmation Options Additional Chromatograms Report Export Intact Protein Protein Digest	Gilycan group: (only used for cross-sample comparis Target glycan source (*.cdb, *.csv, *.cr D:\MassHunter\PCDL\Glycans_mAb, Values to match	Example		
		Released Glycans	Require RT match if database con glycan.	ntains a RT for the target		
		MS Extraction				
				~		
4	Save the method.	Click Method > Save	9.			

Released Glycans Workflow Exercise 6. Automated Released Glycans Workflow

St	reps	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 7. Reprocessing Samples" on page 33. 	 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.

Released Glycans Workflow

Exercise 6. Automated Released Glycans Workflow



Released Glycans Workflow Exercise 6. Automated Released Glycans Workflow

Steps			ed Instructions			Comments				
7	Review the results in the Results Compare window.	a Clic b Clic c No on sar sho gly RS	ck the Results Co ck the Released te that RSD (%) e sample is sele more samples a me Glycan Grou own in the same can is in multip D (%) is calcula	by the sample service of the sample window. I Glycans tab. Is empty because cted. If you select and they belong t p, then the result the table. If the samples then ted.	e only ot two to the ts are ne the	 For Release the software Quant che Biomolecule 	d Glycans workflow e uses the Use for eck box in the es window.			
		un∰ Resu	Its Compare				×			
		ři 🐇								
		Intact Pro	tein Protein Digest Release	d Glycans						
		G	ycan Group ♥+ <unassigned></unassigned>							
			Character 10 10 10 10 10 10 10 10 10 10 10 10 10	Releas	sedGlycans1.d					
			Giycan Name Y KSD	%Quant(Glycan) V	Area VV	RT Y				
			G1F	9.96	59803751	20.611				
			H4N4S1	105	6330075	25.815				
			11111121	1100						
			H5N3S1	0.86	5181136	28.035				
			H5N3S1 H5N4F1	0.86	5181136 4097144	28.035 25.815				
			H5N3S1 H5N4F1 H4N3F1	0.86 0.68 0.59	5181136 4097144 3562747	28.035 25.815 19.572				
			H5N3S1 H5N4F1 H4N3F1 H5N5F1S2	0.86 0.68 0.59 0.59	5181136 4097144 3562747 3539149	28.035 25.815 19.572 20.611				
			H5N3S1 H5N4F1 H4N3F1 H5N5F1S2 H7N4F1	0.86 0.68 0.59 0.59 0.57	5181136 4097144 3562747 3539149 3403961	28.035 25.815 19.572 20.611 37.021				

Review Results Exercise 7. Reprocessing Samples

Review Results

Exercise 7. Reprocessing 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	Deta	ailed Inst	ructions			Comme	ents		
1	Open several data files.	a (b S F C M b d (Click File Select the nAb1.d, Digest.d, Released Released Mark the Dox. Click Ope	> Open Sam ese exampl NIST mAb NIST mAb IGlycans1. IGlycans2. Load resul	nple Files. le files: NI 2.d, NIST Digest2.d d, and d. t data cha	ST mAb d, eck	• To se file. T file.	elect multiple files, clic 'hen, press Shift and	ck the fi	rst e last
2	Review results in the Sample Table window.	a L	.ook at th column.	e Confirma	tion Statu	S	 If you information 	u saved results, the ta mation on confirmati	ble con on.	tains
		្រោ ន	ample Table: Rel	easedGlycans2.d						×
			۵ 🖪 🕙							
			Res	ults				Workflow		
		Cont	firmation Status	File Name	Workflow	Condition	Sequence / Mass	Modification	Enzyme	Glycan Group
		Co	onfirmed	NIST mAb 1.d	Intact Protein	non-reduced	NISTmAb	mAb		
		Co	onfirmed	NIST mAb Digest.d	Protein Digest	reduced	NISTmAb	Protein Digest (Reduced+Alkylated)	Trypsin,LysC	
		Ce	onfirmed	NIST mAb Digest2.d	Protein Digest	reduced	NISTmAb	Protein Digest (Reduced+Alkylated)	Trypsin,LysC	
			ndetermined	ReleasedGlycans1.d	Released Glycans	raducad				Example
			nuetermineu	neicaseuoly(dfb2.0	macciriotem	reduced				
								1		
		4								

Exercise 7. Reprocessing Samples

St	teps	Detailed Instruction	s C	Comments		
3	Review values in Method Automation > Confirmation Options.	 a Click View > Meth necessary. b Select Method A Confirmation Op c Click the Intact I d Review selection Protein match f the most abund e Click the Protein f Review selection partially confirm coverage is >= 0 	nod Editor, if • Automation > • otions. • Protein tab. • n for the Intact • ound but not for • ant peak option. • Digest tab. • n for the Protein is • bed when sequence •	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.		
		Method Editor: Confirmation	Options	×		
			•			
		Method Automation	Intact Protein Protein Digest			
		Workflow and Sequences	Destriction of the state of the second	20.00		
		Confirmation Options	coverage is	>= 80.00 %		
		Reports	Protein is partially confirmed when sequence coverage is	>= 60.00 %		
		Export	The communities of			
		Intact Protein				
		∃ Protein Digest				
		□ Released Glycans				
		Find Glycans				
		MS Extraction				

Exercise 7. Reprocessing 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 for the fort the Glycan for 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 ther 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
Both data files that used the Released Glycans workflow have the same Glycan Group.	Sample Table: Released Glycans2.d	Workflow Sequence / Mass Modification Enzyme Glycan Group NISTmAb III mAb III Protein Digest (Reduced + Alkylated) TrypsinLysc NISTmAb III Protein Digest (Reduced + Alkylated) TrypsinLysc Example

Exercise 8. Using Result Review mode

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

Steps			Detailed Instructions			Comments			
Enable Re	esult Review mo	de. • Clic Rev	 Click Configuration > Enable Result Review (Disables Method Editing). 			• You can toggle the clicking this same	• You can toggle this mode off by clicking this same comment again.		
Review re window.	esults in Sample	Table • All c ava Won repr	of the option: ilable except r kflow butto rocess samp	s in this w for the R n. You ca Iles.	vindow are t un Method n still				
and the second second								*	
	â.							*	
1	晶 Results					Workflow		~	
Confirmation Sta	Results tus File Name	Saved Results Method	Workflow	Condition	Sequence / Mass	Workflow Modification	Enzyme	K Glycan Group	
Confirmation Sta	Results tus File Name NIST mAb 1.d	Saved Results Method	Workflow Intact Protein	Condition non-reduced	Sequence / Mass NISTmAb	Workflow Modification	Enzyme	K Glycan Group	
Confirmation Sta Confirmed Undetermined	Results tus File Name NIST mAb 1.d NIST mAb 2.d	Saved Results Method pft_ReleasedGlycans_InstantPC.m pft_ReleasedGlycans_InstantPC.m	Workflow Intact Protein Intact Protein	Condition non-reduced reduced	Sequence / Mass NISTmAb	Workflow Modification mAb	Enzyme	K Glycan Group	
Confirmation Sta Confirmed Undetermined Confirmed	Results File Name NIST mAb 1.d NIST mAb 2.d NIST mAb Digest.d	Saved Results Method pfh_ReleasedGlycans_InstantPC.m pfh_ReleasedGlycans_InstantPC.m pfh_ReleasedGlycans_InstantPC.m	Workflow Intact Protein Intact Protein Protein Digest	Condition non-reduced reduced reduced	Sequence / Mass NISTmAb NISTmAb	Workflow Modification mAb Protein Digest (Reduced + Alkylated)	Enzyme Trypsin,LysC	Glycan Group	
Confirmation Sta Confirmed Undetermined Confirmed Confirmed	Results File Name NIST mAb 1.d NIST mAb 2.d NIST mAb Digest.d NIST mAb Digest.2d	Saved Results Method pfh_ReleasedGlycans_InstantPCm pfh_ReleasedGlycans_InstantPCm pfh_ReleasedGlycans_InstantPCm pfh_ReleasedGlycans_InstantPCm	Workflow Intact Protein Intact Protein Protein Digest Protein Digest	Condition non-reduced reduced reduced reduced	Sequence / Mass NISTmAb NISTmAb NISTmAb	Workflow Modification mAb Protein Digest (Reduced + Alkylated)	Enzyme Trypsin,LysC Trypsin,LysC	Glycan Group	
Confirmation Sta Confirmed Undetermined Confirmed Confirmed Undetermined	Results File Name NIST mAb 1.d NIST mAb 2.d NIST mAb 2.d NIST mAb Digest.d Releasedlycans1.d	Saved Results Method pft,ReleasedGlycans,InstantPCm pft,ReleasedGlycans,InstantPCm pft,ReleasedGlycans,InstantPCm pft,ReleasedGlycansInstantPCm pft,ReleasedGlycansInstantPCm	Workflow Intact Protein Intact Protein Protein Digest Protein Digest Released Glycans	Condition non-reduced reduced reduced reduced	Sequence / Mass NISTmAb NISTmAb NISTmAb	Workflow Modification mAb Protein Digest (Reduced + Alkylated) Protein Digest (Reduced + Alkylated)	Enzyme Trypsin_LysC Trypsin_LysC	Glycan Group Example	

Exercise 8. Using Result Review mode

Steps			iled Instruct	ions		Con	Comments					
3	Review and compare the <i>ReleasedGlycans1.d</i> and <i>ReleasedGlycans2.d</i> data files.	a In ca b P ra c R C C c g	the Sample ontaining Re ress the Sh ow containin eleasedGlyo eview the re ompare wir olumn has b lycans that	Table, cli IleasedGly i ft key ar ng cans2.d. esults in t ndow. The been calc are in bo	ck the row /cans1.d. nd click the the Results e RSD (%) culated for th samples.							
		^{Gt} ∰ Re	sults Compare								×	
		1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1										
		Intact	Protein Protein Digest	Released Glycan	15							
			Glycan Group 🛛 🖓 🛥									
		4	Example									
					Relea	sedGlycans1.d		Releas	sedGlycans2.d		-	
			Glycan Name	7 RSD (%) 7	%Quant(Glycan) V	Area V V	RT V	%Quant(Glycan) V	Area 🛛	RT V		
			► G1	F 9.5	9.96	59803751	20.611	9.24	52275585	20.602		
			H4N4S	1 105	1.05	6330075	25.815	7.58	42841859	25.807		
			H5N3S	1 1.4	0.86	5181136	28.035	0.9	5080633	28.018		
			H5N4F	1 77	0.68	4097144	25.815	0.21	1209200	26.58		
			H4N3F	1 59.5	0.59	3562747	19.572	0.26	1453310	20.595		
			H5N5F1S	2 57.1	0.59	3539149	20.611	1.47	8335163	19.564		
			H7N4F	1 4.9	0.57	3403961	37.021	0.56	3174200	37.021		
			H5N4F1S	0.6	0.52	2050000	37.021	0.55	2677459	16,447		
			HANSE	1 35	0.49	2523553	22,723	0.47	1523201	22,715		
			GOF-GICNA	c 93.6	0.32	1931147	19,572	1.68	9500305	14,992		
			H5N4S1So	1 9.7	0.3	1807009	39,465	0.28	1576053	39,473		
			H4N4F15g	1 43.6	0.26	1586348	30.662	0.15	839075	30.645		
			H5N4S	2 3.9	0.23	1356469	39,473	0.23	1282857	39.482		
						*******		~ * *				

d Review the Relative Quantitation Histograms window.

• You can visually compare the relative quantitation results for different glycans.



Exercise 8. Using Result Review mode

Steps	Detailed Instructions	Comments
4 Review and compare the <i>NIST r</i> <i>Digest.d</i> and <i>NIST mAb Digest2.d</i> files.	 a In the Sample Table, click the containing NIST mAb Digest. b Press the Shift key and clic row containing NIST mAbD c Click the Protein Digest La button in the main toolbar. d Review the results in the Ref Compare window. The RSD column has been calculate many modifications that ar samples. 	 row d. k the igest2.d. yout esults (%) d for e in both
ort International Compare		×
11 🍄 🖻		
Intact Protein Protein Digest Released Glycans		
Sequence/Mass V+ Confirmation Status	▼+ % Coverage ▼+	
A NISTmAb 20	Conf. 96.2 NIST mAb Digest	.d NIST mAb Digest2.d

Leasting 7	Madification 77	Augusta (Halaba)		NIST mAb Dig	jest.d	NIST mAb Dig	est2.d
Location v	Modification ¥	Average (Height) Y	%KSD (Height) Y	%Quant (Height) 🛛	Height 🛛	%Quant (Height) 🛛	Height V
C370 [B\D]	Alkylation (iodo	2124028	114	92.65	3835631	73.3	412425
C147 [B\D]	Alkylation (iodo	1895628	107.6	96.73	3337365	91.07	453891
C264 [B\D]	Alkylation (iodo	1204864	136.9	96.4	2371596	58.96	38132
C23 [A\C]	Alkylation (iodo	1085689	96.9	97.51	1829634	100	341744
C213 [A\C]	Alkylation (iodo	726794	130.3	97.39	1396248	100	57340
C97 [B\D]	Alkylation (iodo	306169	125.4	100	577691	100	34648
C193 [A\C]	Alkylation (iodo	423883	35.3	61.9	318141	100	529624
M255 [B\D]	Oxidation (M)	198278	52.9	12.41	272404	15	124153
C428 [B\D]	Alkylation (iodo	368817	18.9	74.04	319406	87.48	418228
C370 [B\D]	Alkylation (indo	224645	50.1	7 35	304155	25.79	145134

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.



Exercise 8. Using Result Review mode

Detailed Instructions	Comments
f Review the results in the Peptic Relative Quantitation Results window. The Location C147 [B/ has two rows because this loca is in both samples.	 For Protein Digest workflows, you mark the Use for %Quant check box the Peptide Relative Quantitation ation Results window.
UT Peptide Relative Quantitation Results	×
Image: Classical State	File V = %Quant (Height) V = Height V = WST mAb Digest2d 73.29 136442 WST mAb Digest2d 26.71 49714 WST mAb Digest2d 96.73 3337365 WST mAb Digest2d 32.7 112950 Use for %Quant 112950 112950 WST mAb Digest2d 91.07 453891 Use for %Quant V = Height V = WST mAb Digest2d 91.07 453891 Use for %Quant 125893 27665 WST mAb Digest2d 91.07 453891 Use for %Quant 125883 27655 WST mAb Digest2d 91.07 453891 WST mAb Digest2d 91.07 453891
SISEGRAEUCLUK Location / ♥-a Pred Mods ♥-a Pred Mods ♥-a Image: Classification (indexectic acid) N	File T+a %Quant (Height) T+a Height T-a VIST mAb Digest2d 825 41133 41133 41133 VIST mAb Digest2d 619 318141 318141 VIST mAb Digest2d 38.1 195816 55616
	Joetailed Instructions f Review the results in the Peptin Relative Quantitation Results window. The Location C147 [B has two rows because this location is in both samples. Image: Strate St

Exercise 9. Using Report Builder

This exercise shows you the program to modify PDF templates. If you click **Use PDF Report Builder** in the Method Automation > Reports > Templates tab, then you can use Report Builder to modify those templates.

Steps		Detailed In	structions		Comments
I Open Repor	t Builder program.	 a Double-click Report Builder in the Tools for BioConfirm 10.0 folder in the Agilent MassHunter Workstation program folder. b In Windows 10, click Agilent MassHunter Report Builder > Report Builder 10.0. 			 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.
2 Open an exi	sting template.	a Click Filb Select a	e > Open > a template	Browse. and click O	 Report templates are installed in the D:\MassHunter\Report Templates\BioConfirm folder.
🖏 Open				×	
	BioConfirm → 10.0 → en-US → Letter	ٽ ~	Search Letter	م	Agilant recommende
Organize 👻 New fo	lder		l		Agrient recommends
Quick access Config Dropbox OneDrive Config SubReports AnalysisReportemplate.xml CompoundReport.template.xml GraphicSequenceCoverage.template.xml IntactProteinReport.template.xml ProteinDigestReport.template.xml ProteinDigestReport.template.xml ReleasedGiycanReport.template.xml		Date modified 8/3/2018 12:21 PM 8/3/2018 12:21 PM 1/22/2018 5:04 PM 1/22/2018 5:04 PM 1/22/2018 5:04 PM 1/22/2018 5:04 PM 1/22/2018 5:04 PM 1/22/2018 3:04 PM 6/28/2018 3:26 PM	Type File folder File folder XML Document XML Document XML Document XML Document XML Document XML Document XML Document	5ize 6 KB 6 KB 43 KB 17 KB 475 KB 475 KB 475 KB 411 KB 339 KB	the default templates. Instead, make a copy of the template and modify the copy.
File	name: IntactProteinReport.template.xml		 Template files(* 	.template.xml) v	

S	teps	Detailed Instructions				Comments			
3	3 Review the template in Report Builder.		Click an ite that the rig Click the t In the righ Text in the Click the Text dialo Click the H Enter My I Click OK .	m in the template. Notice ht pane changes. itle of the report. t pane, click Localized e Content section. . button. The Localized g box opens. Jeader_Center . Intact Protein Report.	 The left pane shows the template. The right pane shows the parameters for the current selection. You can make many different change to the report. This exercise only show you one possibility. Press F1 to acces the online Help to learn more about customizing a report template. 				
				Localized Text		×			
			Key	Default Value					
			Footer_Right	Generated at & [Time] on & [Date]					
			Header_Center	My Intact Protein Report	-				
						OK Cancel			

Steps	Detailed Instructions	Comments
4 Save the template.	a Click File > Save As > Browse.	• You can instead click File > Save , and

- **b** Enter a file name and click **Save**.
 - c Close the **Report Builder** program.
- You can instead click **File > Save**, and the file is saved to the current method. Agilent recommends that you do not modify the default templates.

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Steps	Detailed Instructions		Comments
5 Use this new template in a method.	 a Open the Method Edit View > Method Edit b Select Method Aut Reports. c Click the Template d Select the changed corresponding rep type. In this examp Protein report tem modified. 	ditor window. Click or if it is not visible. tomation > es tab. d report for the ort template ole, the Intact uplate was	 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.
	Method Editor: Reports		×
) Print Report 🔹	
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Steps	Detailed Instructions	Comments
6 Print a Biomolecule report.	 a Click File > Print > Biomolecule Report. b Mark the Print preview check box. c Click OK. 	• When you print a biomolecule report, the report template corresponding to the selected workflow is used.
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Exercise 10. Interactive Protein Molecular Weight Determination

Deconvolution

Exercise 10. Interactive Protein Molecular Weight Determination

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 2.

Steps			etailed Instructions	Comments		
1	Open the data file.	a b c d	Click File > Open Data File . Locate the myoglobin.d folder. Clear the Load Result check box. Click Open .	•	The TIC is automatically displayed in the Sample Chromatogram Results window.	
2	Extract a peak spectrum.	a b	Select a range around the peak at 3.5 minutes. Double-click this range.	•	To select a range, click one side of the peak and drag to the other side of the peak.	



Exercise 10. Interactive Protein Molecular Weight Determination

St	eps	Detailed Instructions	Comments
	Biomolecule MS Spectrum		×
		. ₩ LΩ II	2000 2000 2000 2000
3	Open the default Intact Protein method and open the Deconvolute (Protein) Method Editor section.	a Click Method > Open. b Select BioConfirmIntactProtein-De c Click View > Method Edito d Select Intact Protein > Dec (Protein).	 The commands in the View menu toggle whether or not a window is visible. If the command is shown in blue and the button has an orange box around it, then the window is currently visible.
4	Select Maximum Entropy as the deconvolution algorithm.	On the Deconvolution tab of Deconvolute (Protein) sectio Method Editor, verify that Ma Entropy is selected for Deco algorithm.	the n of the aximum nvolution
5	Verify that the Mass range is automatically detected.	Verify that the Automatic ma detection check box is mark	ed. If you clear this check box, then you need to manually enter the Mass range which can vary for different intact proteins.

Exercise 10. Interactive Protein Molecular Weight Determination

Steps	Detailed Instructions	Comments								
6 Set the Mass step to 1.	• Enter 1 for the Mass ste	• Enter 1 for the Mass step.								
	Method Editor: Deconvolute (Prote	Method Editor: Deconvolute (Protein)								
	🕼 😕 🖬 🎇 🦻 🔹 🕐 🗉 🕢 Find by Protein Deconvolution 🔹									
	Method Automation De	convolution Maximum Entropy pMod Time Range(s) Results LMFE Filters								
	Workflow and Sequences Confirmation Options	Deconvolution algorithm Maximum Entropy								
	Additional Chromatograms	Deconvolution settings								
	Reports	Automatic mass range detection								
	Export	Mass range 10000.00-17000.00 Daltons								
	Intact Protein	Mass step 1.0000 Daltons								
	Extract Chromatogram (MS) Extract Spectrum (MS)	Use limited m/z range								
	Deconvolute (Protein)	Baseline								
	Match Tolerances	Subtract baseline								
	Protein Digest	Baseline factor 7.00								
	Released Glycans	Adduct Proton ~								
	MS Extraction	Isotope width Automatic V 20.0000 Daltons								
	<	>								
7 Select the extracted MS peak spectrum.	Click the spectrum in the MS Spectrum window.	Biomolecule								
8 Deconvolute the spectrum.	 Right-click the Biomolect Spectrum window and c Deconvolute to start the deconvolution process. 	 You can also click the arrow next to the run button in the Method Editor toolbar and select Deconvolute (Protein). 								
9 Review deconvolution results.	 The results appear in the Deconvolution Results w the Biomolecules window For information on chan- display of data in the Dec Results window, see online 	 To compare two deconvoluted spectra, select the spectra of interest; then, click the Create Mirror Plot button, , on the Deconvolution toolbar. If necessary, click View > Deconvolution Mirror Plot. The spectra are displayed in the Deconvolution Mirror Plot Results window. See "Exercise 11. Using the Mirror Plot window" on page 49 for more information 								

Exercise 10. Interactive Protein Molecular Weight Determination

Steps	Detailed Instructions	Comments
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 the Spectrum Peak List button ()) on the Deconvolution Results toolbar to close the peak list tab. 	 Mass (<i>m</i>/<i>z</i>), 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.



11 Save the method to

iii_Deconvolution_MaxEnt.m where iii are your initials

- a Click Method > Save As.
- **b** Enter *iii_Deconvolution_MaxEnt.m* for the method name.
- c Click Save.

Exercise 11. Using the Mirror Plot window

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

Steps			etailed Instructions	Comments		
1	Open the NIST mAb1.d data file.	a b c d	Click File > Open Data File. Locate the NIST mAb1.d folder. Clear Load Result Data. Click Open.	•	The TIC is automatically displayed in the Sample Chromatogram 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.	lt c	f the Method Editor window is not visible, lick View > Method Editor to display it.	

Exercise 11. Using 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 Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select the Deconvolution algorithm. b Mark the Automatic mass range detection check box. c Mark the Use limited m/z range check box. d Enter 2400 - 4000 for the m/z range. e Enter 3 for the Baseline factor. 	For more information on these parameters, press F1.
	Method Editor: Deconvolute (Protein)	×
	🟠 🕒 🌆 🎇 🦃 🛪 🍽 🔹 💽 Find by Protein Deconvol	lution *
	Method Automation A Deconvolution Maximum	Entropy pMod Time Range(s) Results LMFE Filters
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	Match Tolerances Mass range	140000.00-160000.00 Daltons
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	Released Glycans Use limited m/z range	
	MS Extraction	2400.0000-4000.0000 🔺 m/z
	Baseline	
	Subtract baseline	3.00
	Caseline lactor	5.00 #4
	Adduct Proton	~
	Isotope width Unspec	cified V 🛕 20.0000 Daltons
	<	>
5 Use the default settings for Maximum Entropy deconvolution.	Click the Maximum Entropy tab to review settings.	
 Run the Find by Protein Deconvolution algorithm. 	Click Find and Identify > Find by Protein Deconvolution.	You can also click the arrow next to the run button in the Method Editor window, and select Deconvolute (Protein) .

Exercise 11. Using the Mirror Plot window

Steps		Detailed Instructions	Comments
7	Review deconvolution results.	• The results appear in the Deconvolution Results window.	
		Deconvolution Results	×
		Image: Product of the state of the stat	L LA TA TA 8000 148500 149500 149500 150000
8	Open the NIST mAb 2.d data file.	 a Click File > Open Data File. b Locate the NIST mAb 2.d sam file. c Click Open. 	The TIC is automatically displayed in the Sample Chromatogram Results window.
9	Run the Find by Protein Deconvolution algorithm on NIST mAb 2.d.	Click Find and Identify > Find by Protein Deconvolution.	,
10	Review deconvolution results.	• The results appear in the Deconvolution Results window.	
11	Select both data files in the Sample Table window.	 a Select one of the sample files in Sample Table window. b Press the Ctrl button and click other sample file. 	the The results for the sample files selected in the Sample Table are shown in the Deconvolution window and other windows.

Exercise 11. Using the Mirror Plot window

Steps	Detailed Instructions	Comments
12 Use Mirror Plot to compare two deconvoluted spectra.	 a Click the ★ button to show the spectra in list mode. b Select a spectra from the Deconvolution window. c Press the Ctrl button and select another spectra from the other data file. d Click the ◆ button to display the spectra in the Deconvolution Mirror Plot Results window. 	
	Deconvolution Mirror Piot Results Image: Convolution Mirror Piot Results Im	X 1.4 Subtract 8201.33 149811.10 149811.10 149811.10 149800 149500 150000 involuted Mass (amu)

Exercise 12. Viewing Biomolecule Information

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

Steps		Detailed Instructions	Comments			
1	Deconvolute myoglobin.d spectrum.	 See "Exercise 10. Interactive Protein Molecular Weight Determination" on page 45. 	You do not need to repeat the deconvolution steps, if you have already done them in Exercise 1.			
2	View the biomolecule list.	Click View > Biomolecules	See Figure 5 on page 54.			
3	Select the biomolecule with mass around 16973.1.	• Click the row which has a mass around 16973.1 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</i>/<i>z</i> data for that specific protein mass is shown in the Biomolecule MS Spectrum Results window. 			
4	Select the Biomolecule 3 spectrum in the Biomolecule MS Spectrum Results window.	Click the graphics area for the spectrum for Biomolecule 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 6 on page 54. 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 7 on page 55.			
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.	 Notice that a different spectrum is shown in the Biomolecule MS Spectrum window. 			

Agilent MassHunter BioConfirm Software Familiarization Guide

Exercise 12. Viewing Biomolecule Information

Steps		De	etailed Instructions	Comments				
9	s print a biomolecule report. a biomolecule report. c		Display the Reports section in the Method Editor by selecting Method Automation > Reports . Review the parameters in both the Templates and Layout tabs. Click Biomolecule Report from the File > Print menu to print the report.	 You can use either PDF-based reporting or Microsoft Excel reporting. When you print a Biomolecule Report, it uses the Intact Protein, the Protein Digest, or the Released Glycans template, depending on the workflow selected in the Sample Table window. If the workflow is Custom, then if you use the Find Peptides command, the Peptide Digest report template is used; otherwise, the Intact Protein 				

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omolecule 2		16934.9138		3.409		357004	6	30	myoglobin.d		Maximum Entropy D	23		9
omolecule 3		16974.3351		3.409		238299	6	23	myoglobin.d		Maximum Entropy D	15		9
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Figure 5. Biomolecules window for myoglobin.d

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	2	652.9793	10673.62		10673.26	5261
	3	663,4531	14289.77	1	14094.7	2225
	4	664.4554	7362.35	1	7293.19	1808
	5	678.3782	11343.33		11335.07	9083
	6	679.0638	33580.73		33573.96	15789
	7	679.6011	6808.4		6768.74	1494
	8	685,4358	9048.16		9048.11	3312
	9	693.6207	9546.3	2	9545.37	1886
	10	693.8714	14461.77	2	14176.11	1823
	11	694.1217	12474.57	2	12160.7	1707
	12	694.3714	8719.89	2	8708.22	1388
	13	700.1014	6694.05		6693.59	8414
	14	704.0923	6478.82		6458.52	10830
4						•

Figure 6. Peak information for myoglobin.d displayed in the Biomolecule MS Spectrum window

Exercise 12. Viewing Biomolecule Information



Figure 7. Biomolecule MS Spectrum Results window for myoglobin.d (Overlay Mode)

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