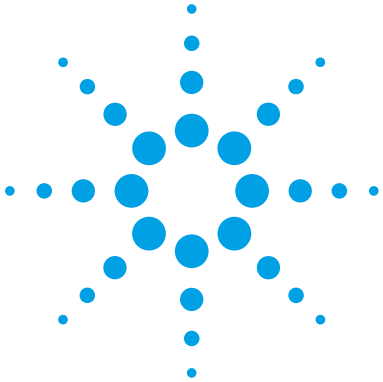


Agilent MassHunter BioConfirm Software

Familiarization Guide

Confirming Protein Molecular Weight 3
- Exercise 1. Interactive Protein Molecular Weight Determination 3
  - Example data for Mirror Plot 9
- Exercise 2. Viewing Compound Information 15

Sequence Matching Workflow 20

Confirming Protein Identity 21
- Exercise 3. Creating a Protein Sequence File 21
- Exercise 4. Interactive Protein Sequence Matching 23
- Exercise 5. Automated Protein Sequence Matching 26

Confirming Protein Digest Identity 28
- Exercise 6. Interactive Protein Digest Sequence Matching 28
- Exercise 7. Automated Protein Digest Sequence Matching 33

Confirming Synthetic Peptide Identity 35
- Exercise 8. Interactive Synthetic Peptide Sequence Matching 35
- Exercise 9. Automated Synthetic Peptide Sequence Matching 38

Confirming Oligonucleotide Identity 40
- Exercise 10. Interactive Oligonucleotide Sequence Matching 40
- Exercise 11. Automated Oligonucleotide Sequence Matching 43

Comparing Protein Digest Files 45
- Exercise 12. Comparing Protein Digest Files 45



Agilent Technologies

Where to find more information

- *Agilent MassHunter BioConfirm Software Quick Start Guide*
- Online Help provides in-depth information and can be displayed in the following ways:
  - Click **Contents**, **Index**, or **Search** from the Qualitative Analysis 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.

Confirming Protein Molecular Weight

Exercise 1. Interactive Protein Molecular Weight Determination

This exercise shows you how to open a data file, integrate the chromatogram, 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 and large oligonucleotides.

Before you start

Copy the data file used for Exercises 1, 2, 4, and 5 onto your hard disk as follows:

1. Copy the **myoglobin.d** data file from the **Data** directory on the Qualitative Analysis setup disk to your computer hard drive.
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 **myoglobin.d** folder 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**, then click **OK**.

Steps	Detailed Instructions	Comments
1 Open the data file.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window.
2 Integrate and extract peak spectra.	Right-click on the TIC and click **Integrate and Extract Peak Spectra** from the shortcut menu. See Figure 1 on page 6.	*Alternate method*: Click **Actions > Integrate and Extract Peak Spectra**.

Steps	Detailed Instructions	Comments
3 Open the Deconvolute (Protein) Method Editor section.	Select **Deconvolute (MS): Protein** from the BioConfirm Workflow section of the Method Explorer.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
4 Select Maximum Entropy as the deconvolution algorithm.	On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **Maximum Entropy** for **Deconvolution algorithm**.	See Figure 2 on page 6.
5 Set the deconvolution range to 16000-18000 Da.	On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, enter 16000-18000 for **Mass range.**	See Figure 2 on page 6.
6 Set the mass step to 0.1 Da.	On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, enter 0.1 for **Mass step**.	See Figure 2 on page 6.
7 Use the default settings for Maximum Entropy deconvolution.	Click on the **Maximum Entropy** tab to review settings.	See Figure 3 on page 7.
8 Select the extracted MS peak spectrum.	Click on the spectrum in the MS Spectrum Results window.	
9 Deconvolute the spectrum.	Click on the Method Editor toolbar to start the deconvolution process.	**Tip:** Steps 2 and 9 can be combined by clicking the **Chromatograms > Integrate and Deconvolute Peak Spectra** menu item.
10 Review deconvolution results.	The results appear in the Deconvolution Results window. See Figure 4 on page 7. For information on changing the display of data in the Deconvolution Results window, see *online Help*.	To compare two deconvoluted spectra, select the spectra of interest, then click the button on the main toolbar. The spectra are displayed in the Deconvolution Mirror Plot Results window. See "Example data for Mirror Plot" on page 9 for more information.

Steps	Detailed Instructions	Comments
11 View peak information.	**a** Click on the spectrum in the Deconvolution Results window to select it. Right-click on the spectrum and click **MS Spectrum Peak List 1** from the shortcut menu. **b** Click on the **Abund**. column heading to sort results by abundance. **c** Click  on the main toolbar to close the peak list window.	Mass (*m/z*), Abundance, and Fit score are listed for each peak in the spectrum. See Figure 5 on page 8.
12 View compound information for the deconvoluted spectrum.	See Exercise 2 on page 15.	

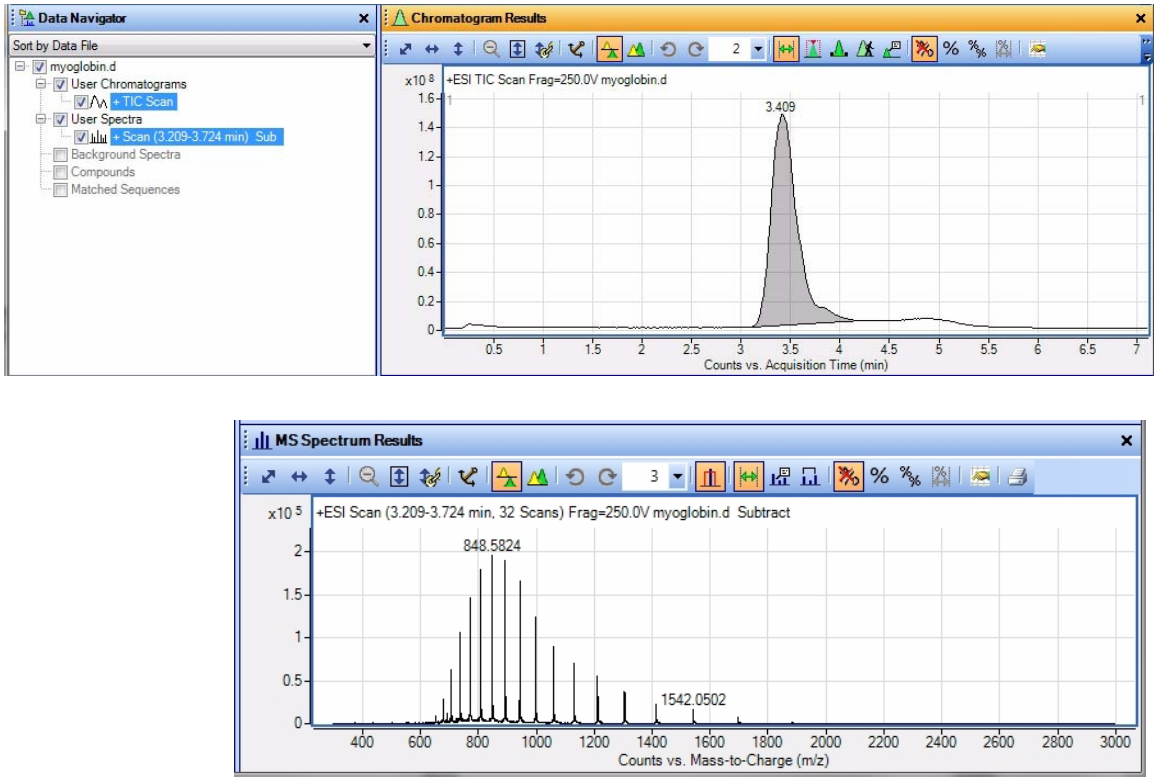


Figure 1 Results of integration and spectra extraction for myoglobin.d

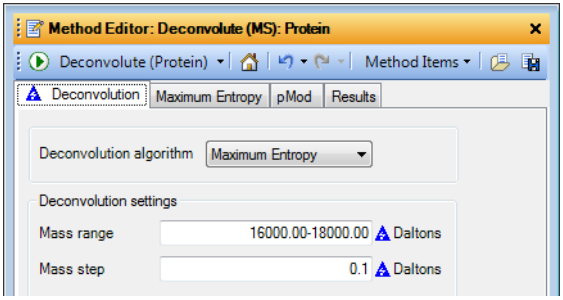


Figure 2 Deconvolution parameters for myoglobin.d

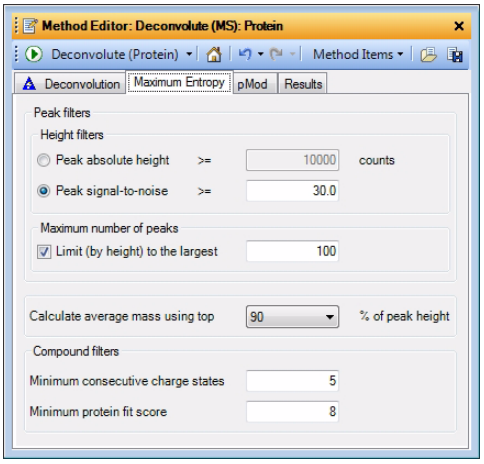


Figure 3 Maximum Entropy deconvolution default parameters

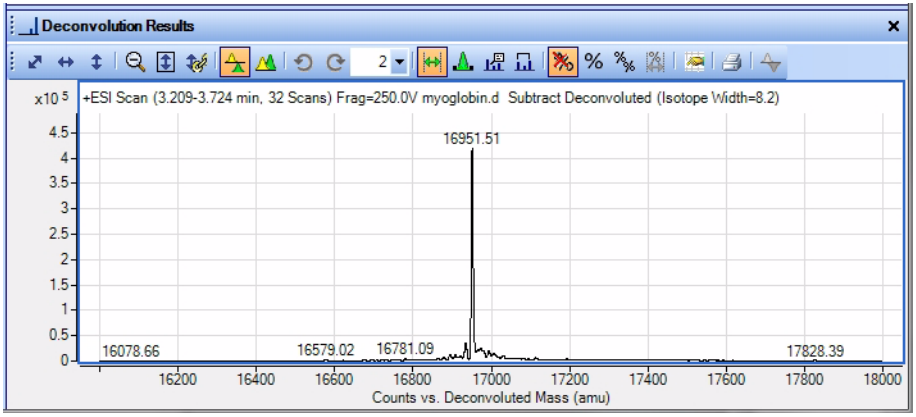


Figure 4 Results of deconvolution for myoglobin.d

MS Peaks One: +Scan (3.209-3.724 min...

Mass	Height	Height % (N
16951.51	421282.97	
16934.65	39372.01	
16973.34	28021.06	
16965.79	25264.27	
16994.55	22483.44	
17006.62	18377.03	
16894.82	14856.71	
16908.9	14731.32	
17030.57	13674.7	
16920.58	13519.25	
16878.79	8904.8	
17049.76	8380.12	
17113.47	7975.46	
17081.96	7376.89	
17059.15	7196.12	
16781.08	6730.52	
17094.89	6360.84	
17071.03	6341.36	
16863.73	5872.07	
17193.45	5069.84	
17131.62	4727.72	
17162.28	4686.13	
17257.35	4490.38	
16849.93	4438.85	

Figure 5 Peak information for the deconvoluted spectrum for myoglobin.d (partial list, sorted by Height)

Example data for Mirror Plot

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

Before you start

1 Copy the following data files from the **Data** directory on the Qualitative Analysis setup disk to your computer hard drive.
   - **mAb1.d**
   - **mAb2.d**

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 on the data file folder of interest, then 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**, then click **OK**.

Steps	Detailed Instructions	Comments
1 Open the 1st data file.	**a** Click **File > Open Data File**. **b** Locate the **mAb1.d** folder. **c** Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window.
2 Integrate and extract peak spectra.	Right-click on the TIC and click **Integrate and Extract Peak Spectra** from the shortcut menu.	*Alternate method*: Click **Actions > Integrate and Extract Peak Spectra**.
3 Open the Deconvolute (Protein) Method Editor section.	Select **Deconvolute (MS): Protein** from the BioConfirm Workflow section of the Method Explorer.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
4 Select pMod as the deconvolution algorithm.	On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **pMod** for **Deconvolution algorithm**.	See Figure 6 on page 11.
5 Use the default settings for pMod deconvolution.	Click on the **pMod** tab to review settings.	See Figure 6 on page 11.
6 Select the extracted MS peak spectrum.	Click on the spectrum in the MS Spectrum Results window.	

Steps	Detailed Instructions	Comments
7 Deconvolute the spectrum.	Click on the Method Editor toolbar to start the deconvolution process.	**Tip:** Steps 2 and 7 can be combined by clicking the **Chromatograms > Integrate and Deconvolute Peak Spectra** menu item.
8 Review deconvolution results.	The results appear in the Deconvolution Results window.	See example in Figure 7 on page 12.
9 Open the 2nd data file.	**a** Click **File > Open Data File**. **b** Locate the **mAb2.d** folder. **c** Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window.
10 Integrate and extract peak spectra.	Right-click on the TIC and click **Integrate and Extract Peak Spectra** from the shortcut menu.	*Alternate method*: Click **Actions > Integrate and Extract Peak Spectra**.
11 Open the Deconvolute (Protein) Method Editor section.	Select **Deconvolute (MS): Protein** from the BioConfirm Workflow section of the Method Explorer.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
12 Select pMod as the deconvolution algorithm.	On the Deconvolution tab of the Deconvolute (Protein) section of the Method Editor, select **pMod** for **Deconvolution algorithm**.	See Figure 6 on page 11.
13 Use the default settings for pMod deconvolution.	Click on the **pMod** tab to review settings.	See Figure 6 on page 11.
14 Select the extracted MS peak spectrum.	Click on the spectrum in the MS Spectrum Results window.	
15 Deconvolute the spectrum.	Click on the Method Editor toolbar to start the deconvolution process.	
16 Review deconvolution results.	The results appear in the Deconvolution Results window.	See example in Figure 7 on page 12.
17 Use Mirror Plot to compare two deconvoluted spectra.	**a** Select the two spectra from Steps 7 & 15. **b** Click the button to display the spectra in the Deconvolution Mirror Plot Results window.	See Figure 8 on page 13. For comparison, Figure 9 is an example of Mirror Plot with Maximum Entropy Deconvolution.

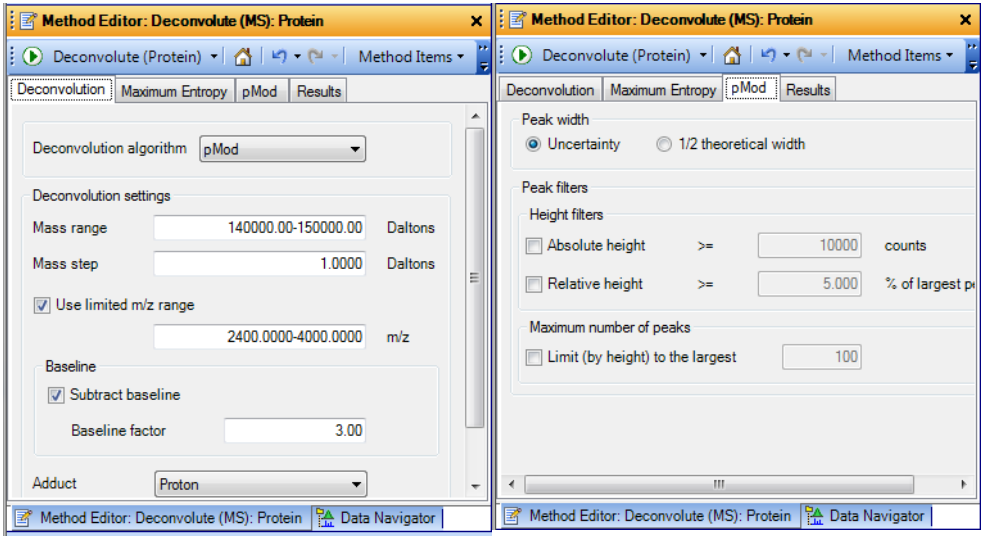


Figure 6 pMod deconvolution parameters

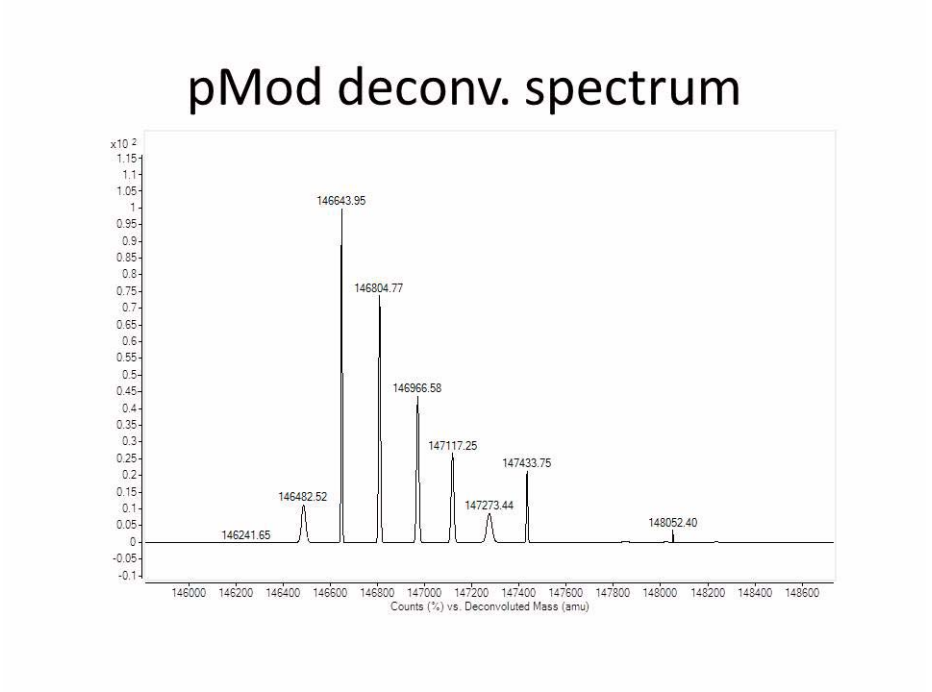


Figure 7 pMod deconvolution spectrum

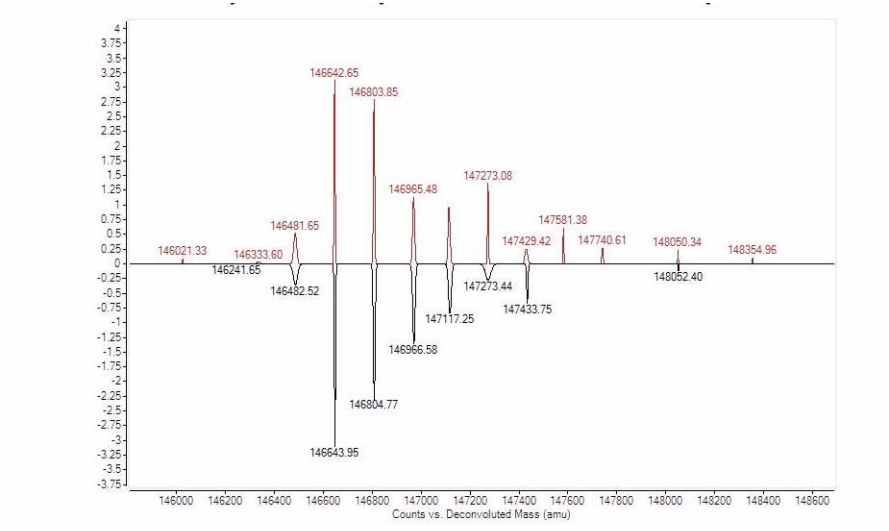


Figure 8 Example Mirror Plot of pMod deconvolution spectra

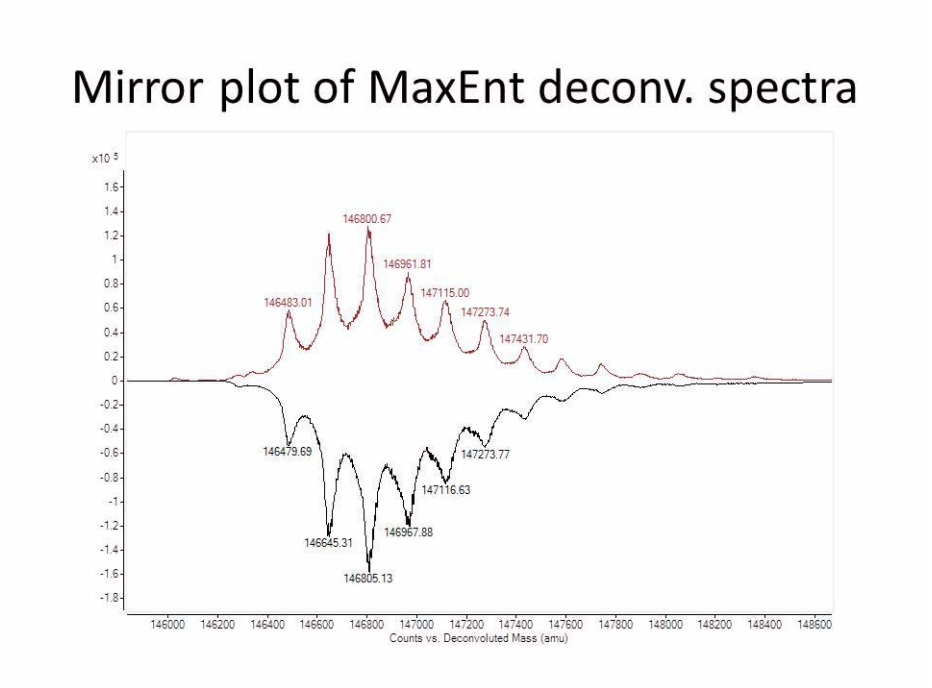


Figure 9 Example Mirror Plot of Maximum Entropy deconvolution spectra

Exercise 2. Viewing Compound Information

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

Steps	Detailed Instructions	Comments
1 Deconvolute **myoglobin.d** spectrum.	See "Exercise 1. Interactive Protein Molecular Weight Determination" on page 3.	You do not need to repeat the deconvolution steps, if you have already done them in Exercise 1.
2 View the compound list.	Click  on the main toolbar to display the Compound List window. Alternate method: Click **Window Layouts > Load Layout** on the Configuration menu, select **BioConfirm-IntactProtein-MaximumEntropy-Default** and click **Open**. This opens and reformats the Compound List to show the appropriate information for a deconvolution operation.	See Figure 10 on page 17.
3 Click on mass 16951.5 in the compound list.		If linked navigation is turned on, associated data in the following windows are automatically displayed and selected: • Deconvolution Results window • A compound spectrum that displays all the charge states from the original *m/z* data for that specific protein mass in the MS Spectrum Results window
4 Select the ion set spectrum in the MS Spectrum Results window for the mass 16951.5.		
5 View the charge states found for the protein along with their ppm error in the MS Peak List 2 window.	Click  on the main toolbar to open the MS Peak List 2 window after clicking on the spectrum to select it.	The following information is displayed for the ion set spectrum: • Mass • Abundance • Charge state • Diff (ppm) See Figure 11 on page 17.

Steps	Detailed Instructions	Comments
6 Switch from List mode to Overlay mode in the MS Spectrum Results window.	Click  on the toolbar in the MS Spectrum Results window.	See Figure 12 on page 18.
7 Zoom in on the *m/z* 848.5 peak in the raw data	Right-drag to expand the area around *m/z* 848.5 in the MS Spectrum Results window.	See Figure 13 on page 18.
8 Select compound 1 in the compound list.	Click on the first line of the Compound List table.	Notice that the ion set peak for that mass shows a peak label. See Figure 14 on page 19.
9 Select compound 2 in the compound list.	Click on the second line of the Compound List table.	Notice that the ion set peak for another peak is highlighted. See Figure 15 on page 19.
10 Print a compound report.	**a** Display the Compound Report section in the Method Editor by selecting **Compound Report** from the BioConfirm Workflow section in the Method Explorer. **b** Review the options in this section. Verify that the sections that you want included in the report are marked. **c** Display the Common Reporting Options section in the Method Editor by selecting **Common Reporting Options** from the BioConfirm Workflow section in the Method Explorer. **d** Review the parameters in both the **Templates** and **Options** tabs. **e** Click **Compound Report** from the **File > Print** menu to print the report.	

Compound List

Show/Hide	File	RT	Mass	Fit	Min Z	Max Z	Height
☑	myoglobin.d		16951.4187	10	8	27	471668
☑	myoglobin.d		16934.9128	9	10	27	38105
☑	myoglobin.d		16973.7846	9	9	23	27258
☑	myoglobin.d		16994.468	8	11	26	21525
☑	myoglobin.d		17006.9	8	16	23	16767
☑	myoglobin.d		16894.7621	8	10	26	13741
☑	myoglobin.d		16908.7668	8	10	26	13443
☑	myoglobin.d		17030.5653	8	9	26	11999

Figure 10 Left half of Compound List window for myoglobin.d

MS Peaks Two: + Scan

m/z	Abund	Abund % (Norm)	Z	Diff (ppm)	Formula 8
654.7071	767.81		26	-97.25	
739.9026	4203.77		23	-2.95	
773.4376	5616.93		22	63.15	
810.2562	8524.92		21	18.42	
850.7023	11393.18		20	37.74	
895.42	12626.79		19	41.16	
945.1271	8969.92		18	22.7	
1000.6583	9120.19		17	27.94	
1063.1504	6681.16		16	14.87	
1133.9707	4915.27		15	5.43	
1214.8899	4197.29		14	10.98	
1308.2785	3067.75		13	1.02	
1417.2628	2403.56		12	-30.72	
1545.9367	1149.39		11	18.87	
1700.5467	714.77		10	-49.96	

Figure 11 MS Peaks Two window for myoglobin.d

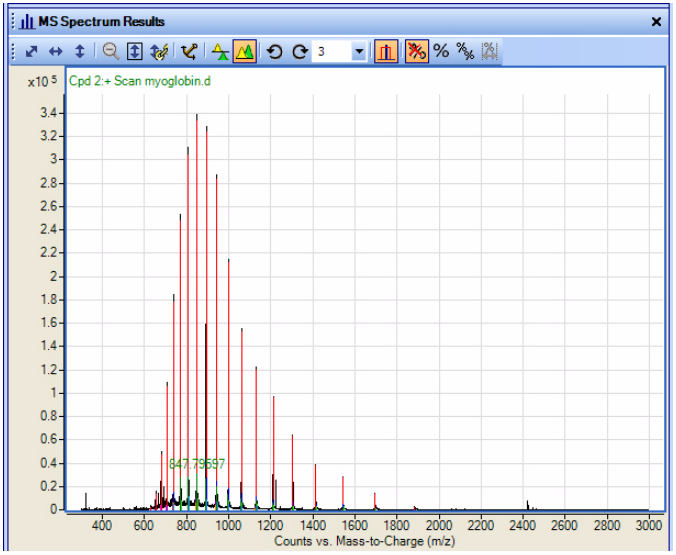


Figure 12 MS Spectrum Results window for myoglobin.d (Overlay Mode)

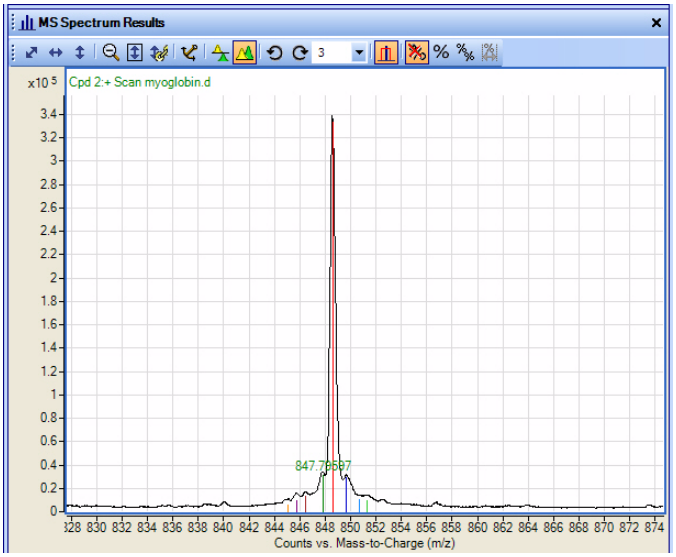


Figure 13 MS Spectrum Results window for myoglobin.d (zoomed)

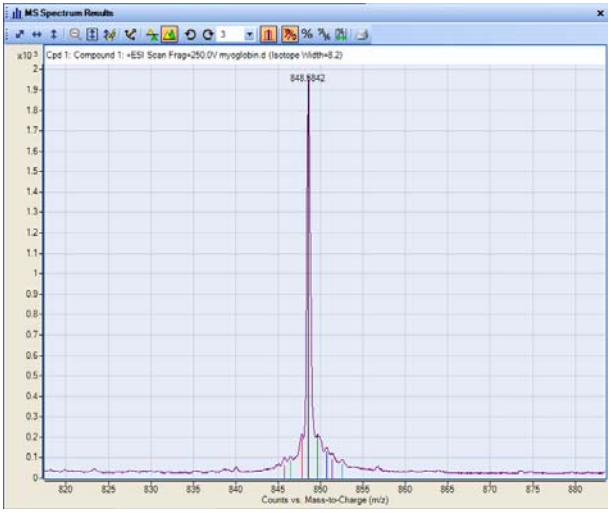


Figure 14 Ion set peak label for Compound 1

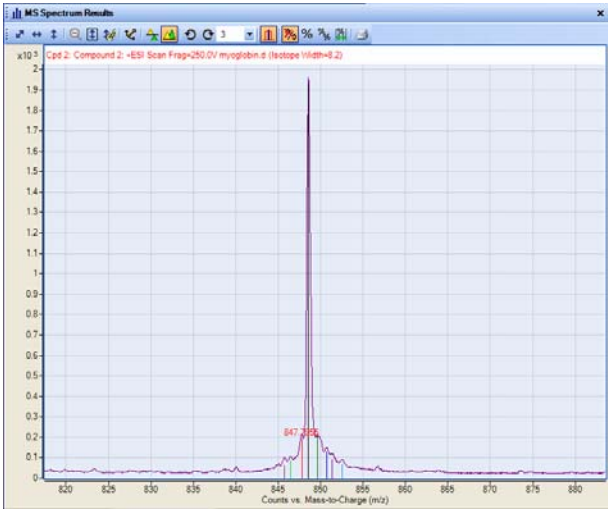


Figure 15 Ion set peak label for Compound 2

Sequence Matching Workflow

The steps outlined below show the workflow for sequence matching with Agilent MassHunter BioConfirm Software.

Step 1 - Open the data file of interest.

Step 2 - Open a Qualitative Analysis Method or create a new one.

Step 3 - Find compounds by molecular feature or by integration and deconvolution.

Step 4 - Select the sequences to match.
If the sequence you want to match is not in the method, then:

- Import or create a sequence.

Step 5 - Edit sequences if necessary:

- Set the sequence type: Protein, Synthetic peptide, Protein Digest, Oligonucleotide.
- Add or edit the sequence text.
- Apply or edit modifications (*not available for Oligonucleotide sequences in this version*)
- Apply or edit links (*not available for Oligonucleotide sequences in this version*)
- Assign or edit digest reagents (*Protein Digest sequences only*).
- Select matching rules.

Step 6 - Review/set other match sequence method parameters.

Step 7 - Start the sequence matching process.

Step 8 - Review the results in the Compound Identification Results windows.

Step 9 - For protein digests only:

- View sequence coverage results in the Sequence Coverage Map window.

Step 10 - Print report.

Confirming Protein Identity

Exercise 3. Creating a Protein Sequence File

This exercise guides you through the creation of a myoglobin sequence file that you will use in "Exercise 4. Interactive Protein Sequence Matching" on page 23 and "Exercise 5. Automated Protein Sequence Matching" on page 26.

Steps	Detailed Instructions	Comments
1 Display the Define and Match Sequences section in the Method Editor window.	Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
2 Create a new sequence.	**a** Click **Sequence > New Sequence**. **b** The Sequence Editor window opens automatically with a new sequence displayed for editing.	The new sequence is also added to the list of sequences in the Sequences tab of the Define and Match Sequences section of the Method Editor window.
3 Enter a **Sequence Name**.	In the Sequence Editor window, type in **Myoglobin** in the Sequence Name box.	
4 Select Protein as the **Sequence Type**.	In the Sequence Editor window, select **Protein** as the Sequence Type.	
5 Enter the amino acid sequence shown below into the Sequence Editor box.	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, as shown in the Amino acid list on the left side of the Sequence Editor window.
GLSDGEWQQVLNVWGKVEADIAGHGQEVLIRLFTGHPETLEK FDKFKHLKTEAEMKASEDLKKHGTVVLTALGGILKKKGHHEAE LKPLAQSHATKHKIPIKYLEFISDAIIHVLHSKHPGDFGADAQG AMTKALELFRNDIAAKYKELGFQG		Tip: If you are reading this document as a PDF file on your computer, you can copy and paste the sequence into the Sequence Editor window.

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

Steps	Detailed Instructions	Comments
6 Save the sequence as the name *iii*_**myoglob.psq**, where *iii* represents your initials.	**a** Click **Save as** on the Sequences tab of the Define and Match Sequences section of the Method Editor window. **b** Type *iii*_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.

Exercise 4. Interactive Protein Sequence Matching

This exercise shows you how to set method parameters, match an intact protein sequence, and view the results. This exercise uses the *iii*_**myoglob.psq** sequence file created in Exercise 3 and the **myoglobin.d** data file copied in Exercise 1.

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 **myoglobin.d** data file is not already open, open it.	**a** Click **File > Open Data File**. **b** Locate the **myoglobin.d** folder. **c** Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window.
3 Display the Find Compounds by Molecular Feature section in the Method Editor window.	Select **BioConfirm Workflow > Find by Molecular Feature** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
4 Find compounds.	**a** Review the settings and modify them if necessary. **b** Click on the Method Editor toolbar to start the compound search. **c** Review the results in the Compound List window.	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
5 Change the layout to BioConfirm-IntactProtein-LMFE.	**a** Click **Window Layouts > Load Layout** on the **Configuration** menu. **b** Select **BioConfirm-IntactProtein-LMFE.xml**. **c** Click **Open**.	
6 Display the Define and Match Sequences section in the Method Editor window.	Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.

Steps	Detailed Instructions	Comments
7 Import the myoglobin sequence.	**a** Click the **Sequence** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Import**. **c** Select *iii*_**myoglob.psq** and click **Open**.	The *iii*_**myoglob.psq** sequence file was created in Exercise 3. For this exercise, we will use the sequence as is, but you can add modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.
8 Select protein matching rules.	**a** Right-click in the Sequence Editor window and click **Edit Matching Rules** from the shortcut menu to open the Rules dialog box. **b** Select the following tests to use for matching the theoretical masses of proteins to those from MS data: • **Intact protein** • **Predicted modifications** **c** Click **OK** to close the Rules dialog box.	Use **<Ctrl>+click** to select multiple tests from the list. Note that these tests are already selected in the BioConfirmIntactProtein-Default method that you loaded in Step 1.
9 Select **Sequence** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Qualitative method** and mark the **Sequences** check box.	Save the method for use in the Exercise 5 as follows: **a** Click **Method > Save As**. **b** Type the **File name** *iii*_**myoglobin.m**, where *iii* represents your initials. **c** Click **Save**.
10 Start the match search.	Right-click in the Compound List window and click **Match Sequences** from the shortcut menu.	Alternate methods: • Click  on the Method Editor toolbar. • Click **Sequences > Match Sequences**. • Click **Match Sequences** on the Method Editor shortcut menu. • Click **Match Sequences** on the Data Navigator Compounds shortcut menu.

Steps	Detailed Instructions	Comments
11 Review the results.	**a** Click  on the toolbar to display the Compound Identification Results window. **b** When you open the window, the window displays the results for the first compound that is highlighted in the Data Navigator (that is marked to show).	Alternate method: • Click **View > Compound Identification Results**.

Exercise 5. Automated Protein Sequence Matching

This exercise guides you through the setup of a worklist to automatically confirm the presence of myoglobin in a previously acquired sample. This exercise uses the *iii*_**myoglob.psq** sequence file created in Exercise 3 and the **myoglobin.d** data file copied in Exercise 1.

Steps	Detailed Instructions	Comments
1 If not already open, open the method *iii*_**myoglobin.m**.	**a** Click **Method > Open**. **b** Select the *iii*_**myoglobin.m** folder. **c** Click **Open**.	This method was created in "Exercise 4. Interactive Protein Sequence Matching" on page 23.
2 Display the Define and Match Sequences section in the Method Editor window.	Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
3 Select **Worklist** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Worklist**.	This selection causes the software to get the sequence from the worklist rather than the method as described in Exercise 4.
4 Display the Worklist Automation > Worklist Actions section in the Method Editor.	Click **Worklist Automation > Worklist Actions** in the Method Explorer window.	
5 Select the appropriate worklist actions.	Select the following worklist actions in the Available actions list: • **Find Compounds by Molecular Feature** • **Match Sequences** • **Generate Compound Report**	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
6 Add the selected actions to the **Actions to be run** list.	Click the  button. The items are added to the end of the list.	Actions will be executed in the order they appear in the list. You can reorder them using the Up and Down arrow buttons to the right of the list
7 Save the method.	Click **Method > Save**.	

Steps	Detailed Instructions	Comments
8 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software.	**a** Display the Worklist pane. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with Agilent MassHunter Workstation Software - Data Acquisition program. In that case, skip Step 12 below.
9 Specify the myoglobin sequence file, *iii*_**myoglob.psq**, in the worklist.	**a** Click **Worklist > Add Column**. **b** When the Add Column dialog box appears, select **Protein** as the Column Type. **c** Enter the Column name as **myoglobin.** **d** Select the *iii*_**myoglob.psq** file as the Value. **e** Click **OK**.	The *iii*_**myoglob.psq** file was created in Exercise 3.
10 Enter *iii*_**myoglobin.m** in the **Method** column of the worklist.	The *iii* represents your initials.	This method was saved in Step 9 above.
11 Enter **myoglobin.d** in the **Data File** column of the worklist.		
12 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the DA method and data file, then click **OK**.	
13 Run the worklist.	Click **Worklist > Run**.	
14 Review the printed Compound reports.		

Confirming Protein Digest Identity

Exercise 6. Interactive Protein Digest Sequence Matching

This exercise shows you how to confirm protein digests interactively.

Before you start

Copy the files used for Exercises 6 and 7 onto your hard disk as follows:

1. Copy the **enolase-Chip-final.d** data file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\Data** folder on your computer hard drive.
2. Copy the **EnolaseDigest.psq** sequence file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\ProteinSequences** folder on your computer hard drive.
3. 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 **enolase-Chip-final.d** folder 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**, then click **OK**.

Steps	Detailed Instructions	Comments
1 Open the method to use as a starting point for the new method.	a Click **Method > Open**. b Select the **BioConfirmProteinDigest-Default.m** folder. c Click **Open**.	
2 Open the demo data file.	a Click **File > Open Data File**. b Locate the **Enolase-Chip-final.d** folder. c Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window.

Steps	Detailed Instructions	Comments
3 Review the parameters in the Find Compounds by Molecular Feature section in the Method Editor window.	**a** Select **BioConfirm Workflow > Find by Molecular Feature** in the Method Explorer window. **b** Review the settings on the various tabs of the Find Compounds by Molecular Feature sections of Method Editor. **c** Click the Extraction tab and set the **Mass range** to 300-1700 *m/z*. **d** In the Extraction tab, set the peak height filter to 500 counts.	• If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu. • 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 *Quick Start Guide* or *online Help*. • A very low peak height filter can result in greater sequence coverage but requires much more time to process.
4 For MS/MS data, set parameters to extract MS/MS spectra.	**a** Click the **Results** tab of Find Compounds by Molecular Feature in the Method Editor. **b** Mark the **Extract MS/MS Spectrum** check box. **c** Mark **Deisotope MS/MS Spectrum**.	
5 Find compounds.	**a** Click on the Method Editor toolbar to start the compound search. **b** When processing is complete, review the results in the Compound List window.	
6 Change the layout to BioConfirm-ProteinDigest.	**a** Click **Window Layouts > Load Layout** on the **Configuration** menu. **b** Select **BioConfirm-ProteinDigest.xml**. **c** Click **Open**.	
7 Import the sequence.	**a** Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window. **b** Click the **Sequence** tab in the Define and Match Sequences section of the Method Editor window. **c** Click **Import**. **d** Select **EnolaseDigest.psq**. **e** Click **Open**.	The enolase digest sequence is automatically displayed in the Sequence Editor window. For this exercise, we will use the sequence as is, but you can add modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.

Steps	Detailed Instructions	Comments
8 Assign or edit digest reagents.	**a** Click the Edit button in the Define and Match Sequence dialog to open the Sequence Editor window. **b** Right-click in the Sequence Editor window and click **Edit Digest Reagents** from the shortcut menu to open the Digest Reagents dialog box. **c** Notice that the Reagent selected is **Trypsin** for EnolaseDigest. **d** Set the maximum number of **Missed Cleavages** to allow to **2**. **e** Click **OK**.	You can customize the list of available reagents using the Chemical Data Dictionary; see *online Help* for more information.
9 View the digest list.	Right-click on the sequence in the Sequence Editor window and click **Digest Current Sequence** from the shortcut menu to digest the sequence and display the results in the Digest List window.	*Alternate method*: Click **Sequence > Digest Current Sequence**.
10 Select protein digest matching rules	**a** To open the Rules dialog box, right-click in the Sequence Editor window and click **Edit Matching Rules** from the shortcut menu. **b** Click **Predicted Modifications**, then under **Selected list**, click **Carbamylation** to move it to the Available list. **c** Select the following tests: • **Complete Digest** • **Incomplete Digest** • **Predicted Modifications** **d** Click **OK** to close the Rules dialog box.	• Use **<Ctrl>+click** to select multiple tests from the list. • The enolase sample was denatured thermally without using urea, so modification by carbamylation is not present. • Note that these tests were automatically selected when you opened the **BioConfirmProteinDigest-Default** method in Step 1.
11 Set Match Sequence parameters.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Qualitative method** and mark the **Sequences** check box.	For MS/MS data, you can adjust the following parameters on the **Scoring** tab: • **MS/MS score** to increase or decrease its contribution to the overall Score (Bio). • **MS/MS scored peak intensity** and **MS/MS matched ion score** contribute to Score (Bio MS/MS).

Steps	Detailed Instructions	Comments
12 Save the method for use in the Exercise 7.	**a** Click **Method > Save As**. **b** Type the **File name** *iii*_Enolase-Chip-Final.m, where *iii* represents your initials. **c** Click **Save**.	
13 Start the match search.	Right-click in the Compound List window and click **Match Sequences** from the shortcut menu	*Alternate methods*: • Click  on the Method Editor toolbar. • Click **Sequence > Match Sequences**. • Click **Match Sequences** on the Method Editor shortcut menu. • Click **Match Sequences** on the Data Navigator Compounds shortcut menu.
14 Review the results.	**a** Click  on the toolbar to display the Compound Identification Results window. **b** When you open the window, the window displays the results for the first compound that is highlighted in the Data Navigator (that is marked to show). **c** Select another sequence match result to view by selecting a different compound in the Data Navigator or Compound List windows.	
15 View sequence coverage results.	**a** Display the Sequence Coverage Map window by clicking  on the toolbar. **b** Click to select a different sequence coverage result in the Data Navigator window (under **Matched Sequences**).	Alternate method: You can also display the Sequence Coverage Map window in these ways: • Click **Sequence > View Sequence Coverage Map**. • Click **View > Sequence Coverage Map**.
16 Save the results	**a** Click  to save your results to the data file folder.	

Steps	Detailed Instructions	Comments
17 Repeat the interactive processing with enolase-oxidized-chip-final.d.	**a** Load the data file **enolase-oxidized-chip-final.d** (see step 2). **b** Select MFE and verify the processing parameters (step 3). **c** Find compounds (step 5). **d** Match sequences (step 13). **e** Save the results to the second data file (step 16).	Most of the processing parameters used for the first data file are the same for the second data file. The data files and results of this exercise will be used in "Exercise 12. Comparing Protein Digest Files" on page 45.
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** • **Applied Links** • **Applied Reagents** • **Applied Matching Rules** • **Show Sequence Description**	

Exercise 7. Automated Protein Digest Sequence Matching

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

Steps	Detailed Instructions	Comments
1 Open the method.	**a** Click **Method > Open**. **b** Select the *iii*_**Enolase-Chip-Final.m** folder. **c** Click **Open**.	This method was created in Exercise 6 (*iii* represents your initials).
2 Display the Worklist Automation > Worklist Actions section in the Method Editor.	Click **Worklist Automation > Worklist Actions** in the Method Explorer window.	
3 Select the appropriate worklist actions.	Select the following worklist actions in the Available actions list: • **Find Compounds by Molecular Feature** • **Match Sequences** • **Generate Compound Report**	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
4 Add the selected actions to the **Actions to be run** list.	Click the  button. The items are added to the end of the list.	Actions will be executed in the order they appear in the list. You can reorder them using the Up and Down arrow buttons to the right of the list
5 Select **Worklist** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Worklist**.	This causes the software to get the sequence from the worklist rather than from the method.
6 Save the method.	Click **Method > Save**.	

Steps	Detailed Instructions	Comments
7 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software.	**a** Display the Worklist pane. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table. **c** Click **Worklist > Add Column**. **d** When the Add Column dialog box appears, select **Protein** as the Column Type. **e** Enter the Column name as **EnolaseDigest.** **f** Select **EnolaseDigest.psq** as the Value. **g** Click **OK**.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with MassHunter Workstation Software - Data Acquisition program. In that case, skip Step 10 below.
8 Enter *iii*_**Enolase-Chip-Final.m** in the **Method** column of the worklist.		The *iii* represents your initials.
9 Enter **Enolase-Chip-Final.d** in the Data File column of the worklist.		
10 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the DA method and data file, then click **OK**.	
11 Run the worklist.	Click **Worklist > Run**.	
12 Review the printed Compound reports.		

Confirming Synthetic Peptide Identity

Exercise 8. Interactive Synthetic Peptide Sequence Matching

This exercise shows you how to set method parameters, import a sequence, match a synthetic peptide sequence, and view the results.

Before you start

Copy the files used for Exercise 8 and 9 onto your hard disk as follows:

1. Copy the **SynPep3.d** data file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\Data** folder on your computer.
2. Copy the **SynPep3.psq** sequence file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\ProteinSequences** folder on your computer.
3. Make sure you have both read and write permissions for the data folder you just created on your computer. This is required if you want to save results.
   a. In Windows Explorer, right-click the **SynPep3.d** folder 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**, then click **OK**.

Steps	Detailed Instructions	Comments
1 Open the method **BioConfirmSyntheticPeptide-Default** to use as a starting point for the new method.	a Click **Method > Open**. b Select the **BioConfirmSyntheticPeptide-Default.m** folder. c Click **Open**.	
2 Open the SynPep3.d data file.	a Click **File > Open Data File**. b Locate the **SynPep3.d** folder. c Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window

Steps	Detailed Instructions	Comments
3 Display the Find Compounds by Molecular Feature section in the Method Editor window.	Select **BioConfirm Workflow > Find by Molecular Feature** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
4 For MS/MS data, set parameters to extract MS/MS spectra.	**a** Click the **Results** tab of Find Compounds by Molecular Feature in the Method Editor. **b** Mark the **Extract MS/MS Spectrum** check box. **c** Mark **Deisotope MS/MS Spectrum**.	
5 Find compounds.	**a** Review the settings and modify them if necessary. **b** Click  on the Method Editor toolbar to start the compound search. **c** Review the results in the Compound List window.	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
6 Change the layout.	**a** Click **Window Layouts > Load Layout** on the **Configuration** menu. **b** Select **BioConfirm-SyntheticPeptide.xml**. **c** Click **Open**.	
7 Display the Define and Match Sequences section in the Method Editor window.	Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
8 Import the sequence.	**a** Click the **Sequence** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Import**. **c** Select **SynPep3.psq**. **d** Click **Open**.	For this exercise, we will use the sequence as is, but you can add modifications and links to sequences as described in *online Help* and the *Quick Start Guide*.

Steps	Detailed Instructions	Comments
9 Select peptide matching rules.	**a** Right-click in the Sequence Editor window and click **Edit Matching Rules** from the shortcut menu to open the Rules dialog box. **b** Select the following tests to use for matching the theoretical masses of oligonucleotides to those from MS data: • **Intact Peptide** • **Extra Amino Acid** • **Missing Amino Acid** • **Fmoc blocking groups** **c** Click **OK** to close the Rules dialog box.	Use **<Ctrl>+click** to select multiple tests from the list.
10 Select **Sequences** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Qualitative method** and mark the **Sequences** check box.	Save the method for use in the Exercise 9 as follows: **a** Click **Method > Save As**. **b** Type the **File name** *iii*_SynPep3.m, where *iii* represents your initials. **c** Click **Save**.
11 Start the match search.	Right-click in the Compound List window and click **Match Sequences** from the shortcut menu.	Alternate methods: • Click  on the Method Editor toolbar. • Click **Sequence > Match Sequences**. • Click **Match Sequences** on the Method Editor shortcut menu. • Click **Match Sequences** on the Data Navigator Compounds shortcut menu.
12 Review the results.	**a** Click  on the toolbar to display the Compound Identification Results window. **b** When you open the window, the window displays the results for the first compound that is highlighted in the Data Navigator (that is marked to show). **c** Select another sequence match result to view by selecting a different compound in the Data Navigator or Compound List windows.	Alternate method: • Click **View > Compound Identification Results**.

Exercise 9. Automated Synthetic Peptide Sequence Matching

This exercise guides you through the setup of a worklist to automatically confirm the presence of SynPep3 in a previously acquired sample.

Steps	Detailed Instructions	Comments
1 Open the *iii*_**SysPep3.m** method.	**a** Click **Method > Open**. **b** Select the *iii*_**SysPep3.m** folder. **c** Click **Open**.	This method was created in Exercise 8 (*iii* represents your initials).
2 Display the Worklist Automation > Worklist Actions section in the Method Editor.	Click **Worklist Automation > Worklist Actions** in the Method Explorer window.	
3 Select the appropriate worklist actions.	Select the following worklist actions in the Available actions list: • **Find Compounds by Molecular Feature** • **Match Sequences** • **Generate Compound Report**	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
4 Add the selected actions to the **Actions to be run** list.	Click the ▼ button. The items are added to the end of the list.	Actions will be executed in the order they appear in the list. You can reorder them using the Up and Down arrow buttons to the right of the list
5 Select **Worklist** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Worklist**.	This causes the software to get the sequence from the worklist rather than from the method.
6 Save the method.	Click **Method > Save**.	

Steps	Detailed Instructions	Comments
7 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software.	**a** Display the Worklist pane. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table. **c** Click **Worklist > Add Column**. **d** When the Add Column dialog box appears, select **Protein** as the Column Type. **e** Enter the Column name as **SynPep3.** **f** Select **SynPep3.psq** as the Value. **g** Click **OK**.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with MassHunter Workstation Software - Data Acquisition program. In that case, skip Step 10 below.
8 Enter *iii*_**SynPep3.m** in the **Method** column of the worklist.	The *iii* represents your initials.	
9 Enter **SynPep3.d** in the Data File column of the worklist.		
10 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the DA method and data file, then click **OK**.	
11 Run the worklist.	Click **Worklist > Run**.	
12 Review the printed Compound reports.		

Confirming Oligonucleotide Identity

Exercise 10. Interactive Oligonucleotide Sequence Matching

This exercise shows you how to set method parameters, import a sequence, match an oligonucleotide sequence, and view the results.

Before you start

Copy the files used for Exercises 10 and 11 onto your hard disk as follows:

1. Copy the **DNA-2ug-r001.d** data file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\Data** folder on your computer hard drive.
2. Copy the **21mer_oligo.psq** sequence file from the **Data** folder on the Qualitative Analysis setup disk to the **MassHunter\ProteinSequences** folder on your computer hard drive.
3. Make sure you have both read and write permissions for the data folder you just created on your computer. This is required if you want to save results.
   a. In Windows Explorer, right-click the **DNA-2ug-r001.d** folder and click **Properties** in 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**, then click **OK**.

Steps	Detailed Instructions	Comments
1 Open the method.	a Click **Method > Open**. b Select the **BioConfirmOligonucleotideSmall-Default.m** folder. c Click **Open**.	
2 Open the data file.	a Click **File > Open Data File**. b Locate the **DNA-2ug-r001.d** folder. c Click **Open**.	The TIC is automatically displayed in the Chromatogram Results window

Steps	Detailed Instructions	Comments
3 Display the Find Compounds by Molecular Feature section in the Method Editor window.	Select **BioConfirm Workflow > Find by Molecular Feature** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
4 Find compounds.	**a** Review the settings and modify them if necessary. **b** In the Extraction tab, change **Restriction retention time to** to 0.5-9.6 minutes, **Restrict m/z to** to 800-2300 m/z, and **Use peaks with height** to 400 counts. **c** In the Charge state tab, change the **Isotope model** to unbiased and limit the charge state to a maximum of 10. **d** In the Results tab, select to extract an MFE spectrum and an ECC for each compound. **e** Click  on the Method Editor toolbar to start the compound search. **f** When processing is complete, review the results in the Compound List window.	These boundary conditions are used with the MFE procedure to avoid long processing times. For guidance to adjust these parameters, see the *Quick Start* Guide or *online Help*.
5 Change the layout to BioConfirm-Oligonucleotide.	**a** Click **Window Layouts > Load Layout** on the **Configuration** menu. **b** Select **BioConfirm-Oligonucleotide.xml**. **c** Click **Open**.	
6 Display the Define and Match Sequences section in the Method Editor window.	Click **BioConfirm Workflow > Define and Match Sequences** in the Method Explorer window.	If the BioConfirm workflow is not available in Method Explorer, select it from the **Configuration > Configure for Workflow** menu.
7 Import the sequence.	**a** Click the **Sequence** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Import**. **c** Select **21mer_oligo.psq**. **d** Click **Open**.	

Steps	Detailed Instructions	Comments
8 Select oligonucleotide matching rules.	**a** Right-click in the Sequence Editor window and click **Edit Matching Rules** from the shortcut menu to open the Rules dialog box. **b** Select the following matching rules to use for matching the theoretical masses of peptides to those from MS data: • **Intact oligonucleotide** • **Oligonucleotide truncation** **c** Click **OK** to close the Rules dialog box.	Use **<Ctrl>+click** to select multiple matching rules from the list. Note that these matching rules are already selected in the **BioConfirmOligonucleotide-Small-Default** method that you loaded in Step 1.
9 Select **Sequence** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Qualitative method** and mark the **Sequences** check box.	Save the method for use in the Exercise 11 as follows: **a** Click **Method > Save As**. **b** Type the **File name** *iii*_Oligo.m, where *iii* represents your initials. **c** Click **Save**.
10 Start the match search.	Right-click in the Compound List window and click **Match Sequences** from the shortcut menu.	Alternate methods: • Click  on the Method Editor toolbar. • Click **Sequence > Match Sequences**. • Click **Match Sequences** on the Method Editor shortcut menu. • Click **Match Sequences** on the Data Navigator Compounds shortcut menu.
11 Review the results.	**a** Click  on the toolbar to display the Compound Identification Results window. **b** When you open the window, the window displays the results for the first compound that is highlighted in the Data Navigator (that is marked to show). **c** Select another sequence match result to view by selecting a different compound in the Data Navigator or Compound List windows.	Alternate methods: • Click **View > Compound Identification Results**.

Exercise 11. Automated Oligonucleotide Sequence Matching

This exercise guides you through the setup of a worklist to automatically confirm the presence of 21mer_oligo sequence in a previously acquired sample.

Steps	Detailed Instructions	Comments
1 Open the *iii*_**oligo.m** method.	**a** Click **Method > Open**. **b** Select the *iii*_**oligo.m** folder. **c** Click **Open**.	This method was created in Exercise 10 (*iii* represents your initials).
2 Display the Worklist Automation > Worklist Actions section in the Method Editor.	Click **Worklist Automation > Worklist Actions** in the Method Explorer window.	
3 Select the appropriate worklist actions.	Select the following worklist actions in the Available actions list: • **Find Compounds by Molecular Feature** • **Match Sequences** • **Generate Compound Report**	In this case we are using the default method parameters. For some data files, you will need to use different parameters as described in the *Quick Start Guide* or *online Help*.
4 Add the selected actions to the **Actions to be run** list.	Click the  button. The items are added to the end of the list.	Actions will be executed in the order they appear in the list. You can reorder them using the Up and Down arrow buttons to the right of the list
5 Select **Worklist** as the match source.	**a** Click the **Source** tab in the Define and Match Sequences section of the Method Editor window. **b** Click **Worklist**.	This causes the software to get the sequence from the worklist rather than from the method.
6 Save the method.	Click **Method > Save**.	

Steps	Detailed Instructions	Comments
7 Create a worklist of one sample in the MassHunter Workstation Data Acquisition software.	**a** Display the Worklist pane. **b** Click **Worklist > Add Sample**. A new sample row is added to the Worklist table. **c** Click **Worklist > Add Column**. **d** When the Add Column dialog box appears, select **Protein** as the Column Type. **e** Enter the Column name as **21mer_oligo.** **f** Select **21mer_oligo.psq** as the Value. **g** Click **OK**.	If you plan to do batch data analysis using the worklist, consider using the DA Reprocessor tool that is installed with MassHunter Workstation Software - Data Acquisition program. In that case, skip Step 10 below.
8 Enter *iii*_**oligo.m** in the **Method** column of the worklist.	The *iii* represents your initials.	
9 Enter **11mer_oligo.d** in the Data File column of the worklist.		
10 Set up to run the worklist for data analysis only.	**a** Click **Worklist > Worklist Run Parameters**. **b** Select **DA Only** as **Part of method to run**. **c** Select the paths for the DA method and data file, then click **OK**.	
11 Run the worklist.	Click **Worklist > Run**.	
12 Review the printed Compound reports.		

Comparing Protein Digest Files

Exercise 12. Comparing Protein Digest Files

This exercise shows you how to compare compounds in two protein digest files.

Before you start

- Do "Exercise 6. Interactive Protein Digest Sequence Matching" on page 28 to get method and results files for this exercise.

Steps	Detailed Instructions	Comments
1 Open the data files to compare. In this exercise, we are using: • **enolase-chip-final.d** • **enolase-oxidized-chip-final.d**	**a** Click **File > Open Data File**. **b** On the Open Data File dialog box, select the **enolase-chip-final.d** folder. **c** Select the following options: • **Load results method** • **Load result data** **d** Click **Open**. **e** Repeat *Steps a - d* to open **enolase-oxidized-chip-final.d**.	The data files used in this exercise have already been processed in Qualitative Analysis to find and identify compounds. These results are loaded when you open the data files.
2 View method parameters.	To view method parameters that were used to find and identify compounds, display the following sections of the Method Editor: • **BioConfirm Workflow > Find by Molecular Feature** • **BioConfirm Workflow > Define and Match Sequences**	• Some of the method parameters are shown in Figure 16 on page 47, and Figure 17 and Figure 18 on page 48. • Note that 95% coverage is obtained for the Enolase digest sequence; see Figure 19 on page 48.

Steps	Detailed Instructions	Comments
3 Start the compare protein digest files wizard.	Click **Wizard > Compare Protein Digest Files: Compare Existing Results**. The Select Reference File and Sample File(s) page is displayed.	For your own data files, you can find and identify compounds in either of the following ways: • Manually as described in "Exercise 6. Interactive Protein Digest Sequence Matching" on page 28, *or* • By clicking **Wizard > Compare Protein Digest Files: Find Results, Identify and Compare** to have the wizard guide you through the process.
4 Select the files to use as Reference and Sample files.	**a** From the list of opened files, select **enolase-chip-final.d** and click **Select reference file**. ◦ The selected file name appears in the **Reference file** text box. ◦ The remaining file, **enolase-oxidized-chip-final.d**, is automatically moved to the list of **Sample files**. See Figure 20 on page 49. **b** Click **Next** to open the next page of the wizard.	For your own data files: • To set the order that the sample files will be processed, use the Up and Down arrow buttons to the right of the list of samples. • To open additional data files, click the Browse (...) button. • To remove a selected Reference or Sample file, double-click on the file or click the Up arrow button. • To remove *all* Sample files, click the double Up arrow button.
5 Set the compound correlation parameters on the Alignment Information page.	**a** Set the retention time **(RT) window** and RT window tolerance values. **b** Set the **Mass window** and mass window tolerance values. See Figure 21 on page 49. **c** When finished setting the parameters, click **Finish**. A progress bar is displayed while the compounds are being compared.	This process may take several minutes to complete, depending on the number of samples and size of the data files.
6 Review the results in the MassHunter Comparative Analysis program.	Results are presented as follows in the Compound List window: ◦ Column 1 (purple shading): correlated compound information ◦ Column 2 (red shading): reference file information ◦ Column 3 (blue shading): sample file information See Figure 22 on page 50.	

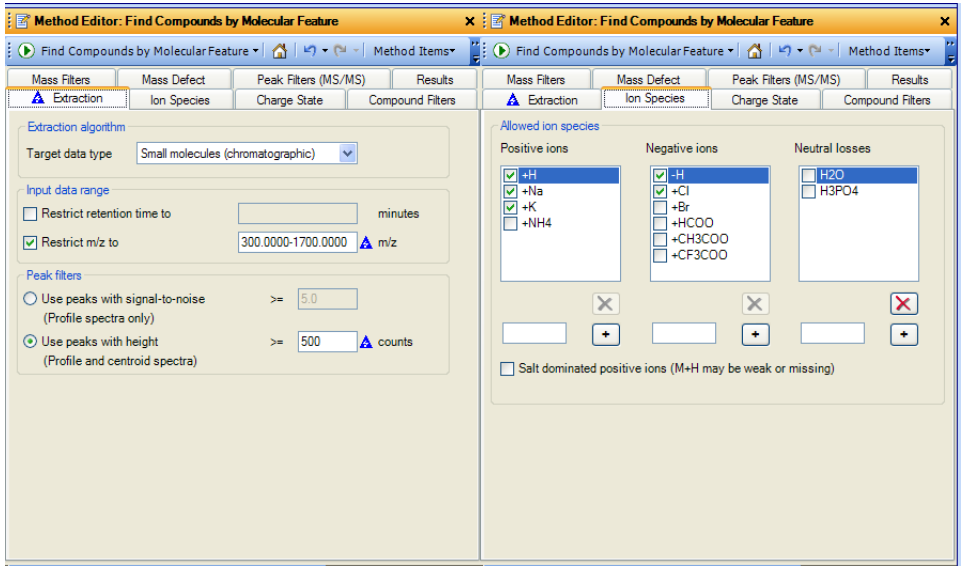


Figure 16 Method Parameters - Find Compounds by Molecular Feature, part 1

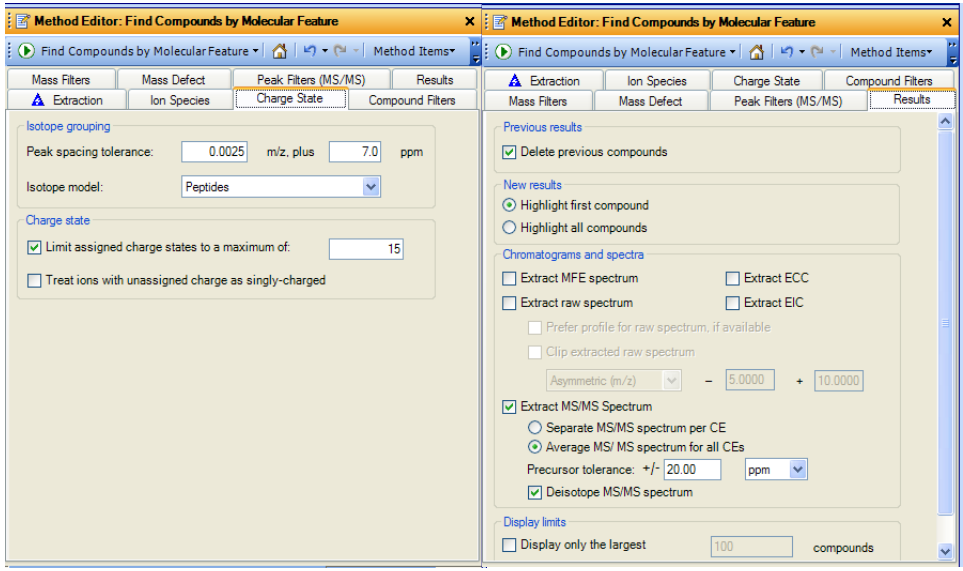


Figure 17 Method Parameters - Find Compounds by Molecular Feature, part 2

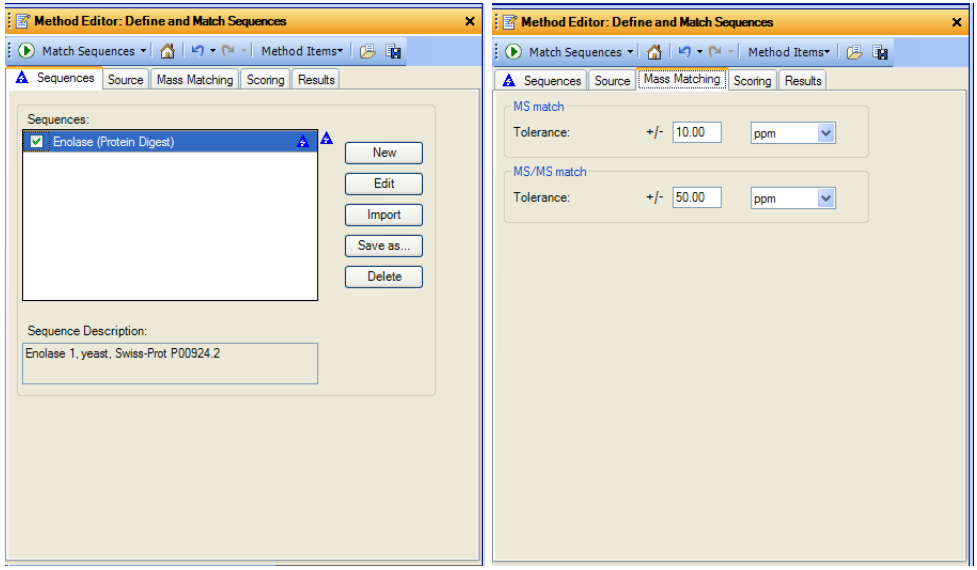


Figure 18 Method Parameters - Define and Match Sequences

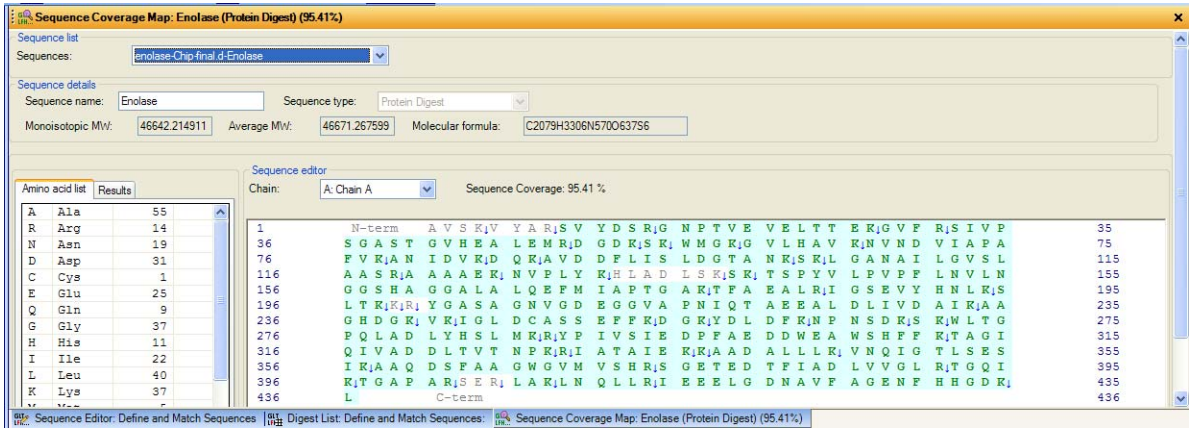


Figure 19 Sequence Coverage Map for Enolase Digest Sequence

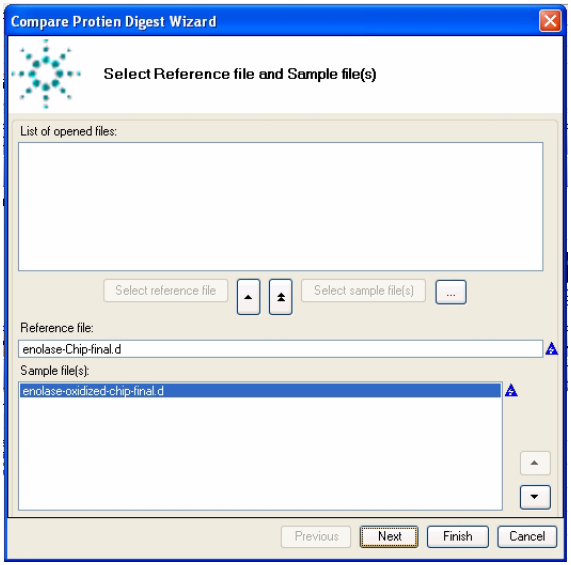


Figure 20 Reference and Sample files selected

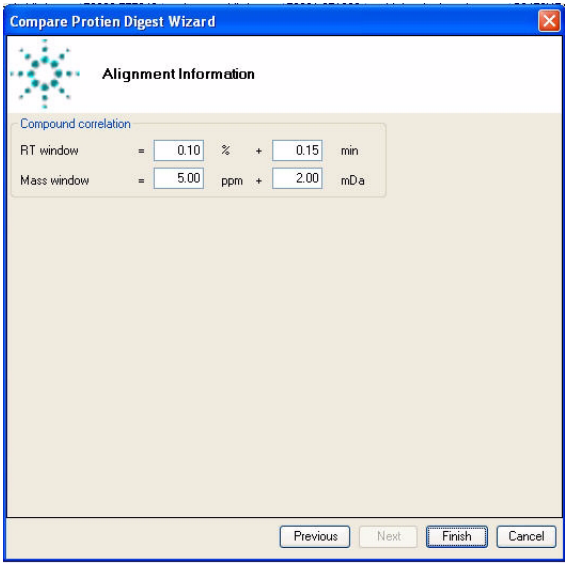


Figure 21 Alignment Information Page with default parameters

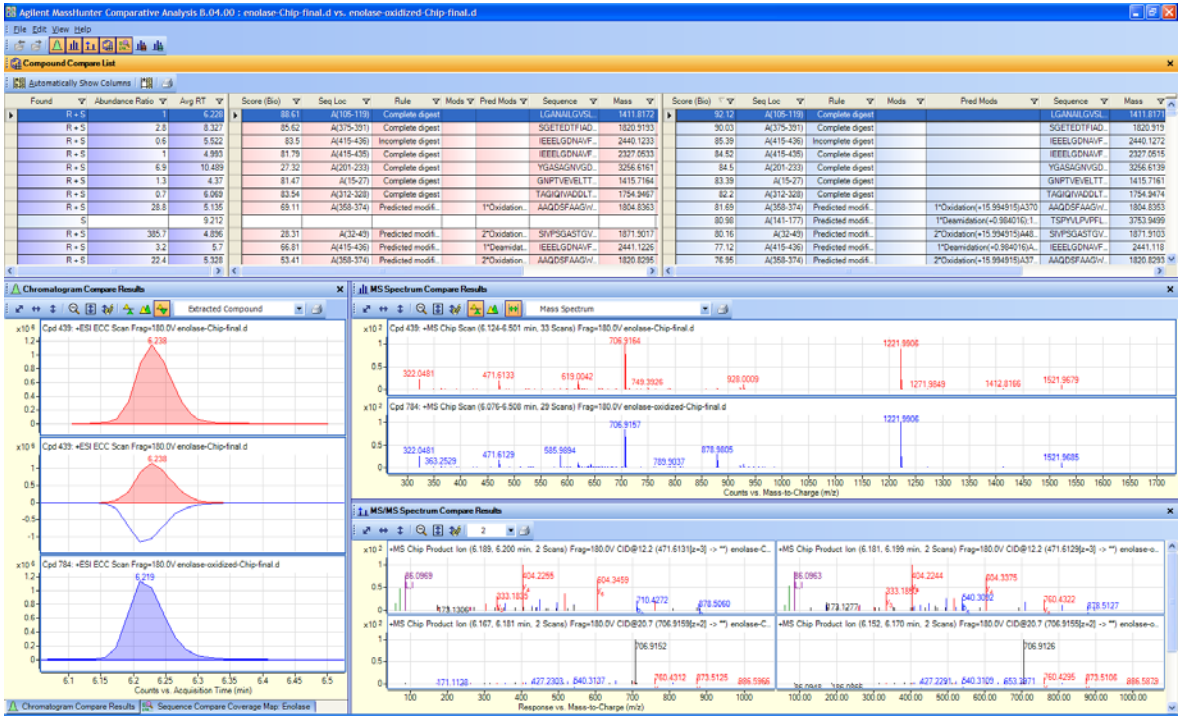


Figure 22 MassHunter Qualitative Compare Program Window

www.agilent.com

© Agilent Technologies, Inc. 2012

Printed in USA
Revision A, October 2012

G6829-90014

Agilent Technologies