Agilent MassHunter BioConfirm Software

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Where to find more information

- *Agilent MassHunter BioConfirm Software Familiarization Guide*
- Online Help provides in-depth information and can be displayed in the following ways:
  - Click **Contents**, **Index**, or **Search** from the **Help** menu in Agilent MassHunter Workstation Software: Qualitative Analysis.
  - Press the **F1** key to get more information about a window or dialog box.

Agilent Technologies

What is Agilent MassHunter BioConfirm Software?

Agilent MassHunter BioConfirm Software provides the industry-standard software for automated and interactive protein confirmation for TOF and Q-TOF MS-only data and includes the following features:

- Support for MassHunter TOF MS data and Q-TOF MS-only data.
- Automated acquisition and report generation.
- Compound-centric navigation for peptides, proteins, and oligonucleotides.
- Create and search protein, peptide, and oligonucleotide databases by accurate mass and optionally retention time.
- Full integration of intact protein UI functionality into MassHunter Qualitative Analysis, most notably the visualization of ion sets and showing deconvoluted spectra in a separate window.
- Protein sequence matching, including target protein and potential modifications for intact protein and protein digest sequence types.
- Allows you to assign site specific variable modification for protein, protein digest and synthetic peptide sequence types.
- Protein Molecular Feature Extractor for finding protein compounds in complex LC/MS data.
- Two different measures of the relative protein level for more flexibility: height from the deconvoluted spectrum and height/area of EIC using all ion set peaks.
- Protein compound quality filters, which prevent “noise” peaks from the deconvoluted spectrum being considered a compound, by requiring sufficient evidence in the *m/z* spectrum.
- Sequence editing/matching available in MassHunter Qualitative Analysis.
- Synthetic peptide purity calculation.
- Linked navigation between compounds with assigned protein digest matches and peptide sequence within the Sequence Coverage Map.

New feature for B.03.01 Agilent MassHunter BioConfirm Software

- Sequential-enzyme digestion, where a protein digest is first digested with a first reagent, then the resulting digest will be digested with the second reagent, and so on.

Agilent MassHunter BioConfirm Software Installation

If the G6829AA Agilent MassHunter BioConfirm Software is not already installed on your system, install it as follows.

1. Verify that version B.02.00 of Agilent MassHunter Qualitative Analysis software is installed.
2. Insert the disk labeled **G6829AA** into the disk drive.
3. Click the **Start** button and select **Run**.
4. Type **x:\setup.exe**, where **x** is the letter of the disk drive, and click **OK**.
5. Follow the instructions on the screen to install the software.

   Agilent MassHunter BioConfirm Software features are accessible in the Qualitative Analysis software. See "Sequence Matching Features and Parameters" on page 8 and "Deconvolution Features and Parameters" on page 4 for more information.

To *remove* the software

Agilent MassHunter BioConfirm Software becomes part of the Qualitative Analysis software. It is removed when Qualitative Analysis software is removed from the system.

Deconvolution Features and Parameters

The following features are available for deconvolution in Agilent Qualitative Analysis Software when Agilent MassHunter BioConfirm Software is installed:

Method Explorer: Spectrum > Deconvolute (MS): Maximum Entropy,
Method Explorer: BioConfirm Workflow > Deconvolute (MS): Maximum Entropy

Display the Deconvolute (MS): Maximum Entropy section in Method Editor.

Method Editor: Deconvolute (MS): Maximum Entropy section Lets you view and edit the deconvolution parameters. See page 5.

Method Editor: Find Compounds by Molecular Feature > LMFE tab Lets you set parameters for Large Molecule Feature Extraction (LMFE).

Chromatograms menu > Integrate And Deconvolute Peak Spectra,
Actions menu > Integrate And Deconvolute Integrate each selected chromatogram, extract *m/z* peak spectra for each chromatogram peak, deconvolute each *m/z* spectrum separately. See "To integrate chromatograms and deconvolute spectra" on page 15.

Spectra menu > Deconvolute (Maximum Entropy),
Actions menu > Deconvolute Start deconvolution of the selected spectra. See "To deconvolute selected spectra" on page 14.

View menu > Deconvolution Results,
 **toolbar button** Toggle the display of the Deconvolution Results window.

Deconvolution Results window Displays deconvoluted mass spectra. See "To view protein deconvolution results" on page 17.

Plot Display Options dialog box **Deconvoluted Spectra** tab sets the appearance of deconvoluted spectra, such as the number of significant digits.

MS Spectrum Peak List window

Columns for deconvoluted spectra **Peak**, **Mass**, **Abund**, **Abund%**, **Fit**, **Max Abund**, and **Width**. See "To view protein deconvolution results" on page 17.

Columns for compound ion set mass spectra **Peak**, ***m/z***, **Abund.**, **Abund%**, **Charge**, **Max Abund**, **Calc Mass**, **Expected *m/z***, **Cpd Mass**, **Diff (ppm)**, **Diff (*m/z*)**, **Diff (Da)**. See "To view deconvolution compounds" on page 20.

Deconvolution Method Parameters

The following parameters are set on the Method Editor: Deconvolute (MS): Maximum Entropy section.

Deconvolution tab Allows you to set the parameters used in the deconvolution process.

Mass range

Starting and ending values to set an expected range for deconvoluted protein masses. A default range of 10,000 to 17,000 Daltons is supplied.

Mass step

The resolution of the deconvoluted protein mass range (default value is 1.0 Daltons). For small proteins, consider using a lower value for improved mass measurement accuracy.

S/N threshold

Deconvoluted protein peaks that fall below this value are not reported.

Use limited *m/z* range

Marking this option restricts the range used for deconvolution to the specified *m/z* range.

Adduct

The expected ion species (Proton, Ammonium, Potassium, Sodium, or Proton Loss) that acts as the charge carrier for the protein.

Average mass

The % peak height to use for calculating the mass of a peak (25, 50, 75, or 90%). The default value of 90% means that the mass is calculated by averaging the data points along the curve of the top 90% of the peak. This default value works well for low molecular weight, well-separated peaks, such as those in insulin. An Average mass value of 25% works well for

high molecular weight, overlapped mass peaks or when there are many *m/z* ions available to average. When a lower value such as 25% is used for a protein such as insulin, the contribution to the mass from other isotopes is missed. Consider testing other values to get a better estimate of the molecular weight.

Isotope width

The expected full-width at half-maximum height of the target protein or other large molecule mass in Da from the following choices:

Automatic Predicts the isotope width using the mass directly in the middle of the specified Mass range. For example, if the Mass range is set to 10,000-30,000, an estimate of the isotopic width for a protein with mass 20,000 Da is used.

Unspecified Uses the resolution in the mass spectrum to estimate the peak width. This mode always produces wider peaks in the deconvoluted spectrum, but is useful for a very broad mass range, such as 10,000-180,000 Da, since the isotope width varies greatly across this range.

Specified Allows you to enter a specific value in Daltons

Compound Filters

Determine whether a peak in the deconvoluted spectrum produces a compound from the following choices:

Minimum consecutive charge states (default value of 5) Requires that the raw *m/z* spectrum has at least 5 peaks in a row that differ in charge by only 1. For example, if the minimum is set to 5, a compound is created if the 15+, 14+, 13+, 12+ and 11+ charge states are found for a given protein. A compound is *not* created if the 15+, 14+, 13+, and 11+ charge states are found, but the 12+ is not present.

Minimum protein fit score (default value of 8) Sets a fit score threshold for acceptance of a peak as a possible compound. Fit scores between 0 and 10 are assigned to peaks in the deconvoluted spectrum by the maximum entropy algorithm. Fit values are reported in the MS Peak List window for the deconvolution spectrum.

Results Tab The parameters on the Results tab of the Method Editor: Deconvolute (MS): Maximum Entropy section are the same as for other Method Editor sections.

Advanced Tab Allows you to set the Data model for the expected width in m/z of peaks of interest in the raw data (Singlet width) to Automatic, Default, or Specified Peak width and Resolution and to select a Limits for the Number of Iterations. These parameters are explained in more detail in online Help.

Sequence Matching Features and Parameters

The following features for sequence matching appear in Agilent Qualitative Analysis Software when Agilent MassHunter BioConfirm Software is installed:

Menu items and Toolbar buttons

Identify menu > Match Sequences and Sequence menu > Match Sequences Run the match sequence process, using the parameters specified in the Method Editor: Define and Match Sequences section. Results are displayed in the Sequence Match Results window.

View menu > Sequence Editor, Sequence menu > View Sequence Editor, and  toolbar button Toggle the display of the Sequence Editor window.

View menu > Digest List, Sequence menu > View Digest List, and  toolbar button Toggle the display of the Digest List window.

View menu > Sequence Match Results, Sequence menu > View Sequence Match Results, and  toolbar button Toggle the display of the Sequence Match Results window.

View menu > Sequence Coverage Map, Sequence menu > View Sequence Coverage Map, and  toolbar button Toggle the display of the Sequence Coverage Map window.

See online Help for a description of the other items on the Sequence menu.

Tools menu > Open Chemical Data Dictionary Editor Allows you to view and edit the modifications, links, and reagents in the Chemical Data Dictionary.

Method Explorer Window

BioConfirm Workflow > Define and Match Sequences section, Identify Compounds > Define and Match Sequences section Display the Define and Match Sequences section in the Method Editor.

If the BioConfirm workflow is not currently displayed in Method Explorer, you can select it from the **Tools > Configure for Workflow** menu.

Method Editor Window

Define and Match Sequences section Allows you to view and edit sequence matching parameters; see "Sequence Matching Method Parameters" on page 10.

Compound List Window

New columns: Seq Name, Seq Type, Sequence, Seq Loc, Tgt Seq Mass, Mods, Links, Enzyme, Missed, Rule, Pred mods, Hits (BioConfirm), Match Diff (mDa), and Match Diff (ppm).

Data Navigator Window

Matched Sequences data type.

Sequence Editor Window

Allows you to create or edit a method sequence.

Digest List Window

Allows you to view the digest list of the method sequence currently displayed in the Sequence Editor.

Sequence Match Results Window

Displays the sequence matching result for a selected compound.

Sequence Coverage Map Window

Displays sequence information for sequence coverage information for a protein digest sequence.

Sequence Matching Method Parameters

The following parameters are set on the Method Editor: Define and Match Sequences section:

Sequences tab

Allows you to specify sequences and masses to match. You can also create new sequences, edit existing sequences, and delete sequences. Sequences can be saved to and imported from **.psq** files.

Source tab

Allows you to select the match source either from a method or worklist. Both sequences and masses can be matched.

Mass Matching tab

Allows you to specify a **Mass Accuracy** value required for a matching, in **ppm** or **Da**.

Results tab

The parameters on this tab are the same as for other Method Editor sections. See MassHunter Qualitative Analysis online Help.

Sequence Dialog Boxes

The following dialog boxes are available in Agilent Qualitative Analysis Software when Agilent MassHunter BioConfirm Software is installed. See online Help for more information.

Chains

Allows you to add and delete chains and to modify sequence chain names.

To open the Chains dialog box, click **Edit Chains** on either the Sequence menu or the Sequence Editor shortcut menu.

Chemical Data Dictionary

Allows you to customize the list of modifications, links, and reagents for use with MassHunter BioConfirm software. The factory-supplied default lists cannot be modified, but you can add to them.

To open the Chemical Data Dictionary dialog box, click **Open Chemical Data Dictionary Editor** on the Sequence or Tools menu.

Description

Allows you to enter descriptive information that is stored with both method sequences and result sequences.

To open the Description dialog box, click **Edit Sequence Description** on either the Sequence menu or the Sequence Editor shortcut menu.

Digest Reagents

Allows you to select and prioritize reagents to apply to digest the sequence.

To open the Digest Reagents dialog box, click **Edit Digest Reagents** on either the Sequence menu or the Sequence Editor shortcut menu.

Links

Allows you to link amino acids in the sequence.

To open the Links dialog box, click **Edit Links** on either the Sequence menu or the Sequence Editor shortcut menu.

Modifications

Allows you to apply modifications to the sequence.

To open the Modifications dialog box, click **Edit Modifications** on either the Sequence menu or the Sequence Editor shortcut menu.

Rules

Allows you to view or select tests to match the theoretical masses of peptides, proteins, protein digests, and oligonucleotides to those from MS data.

To open the Rules dialog box, click **Edit Matching Rules** on either the Sequence menu or the Sequence Editor shortcut menu.

Deconvolution Workflow

The topics in this section will help you get started using the deconvolution features of Agilent MassHunter BioConfirm Software.

"To deconvolute selected spectra" on page 14

"To integrate chromatograms and deconvolute spectra" on page 15

"To view protein deconvolution results" on page 17

"To view deconvolution compounds" on page 20

"To print a report with deconvolution results" on page 21

"To automate protein confirmation" on page 22

What is deconvolution?

Deconvolution software does charge state deconvolution of mass spectra of large molecules with high charge states. Singly-charged ions with masses greater than a few thousand are beyond the mass range of the Agilent TOF instrument. However, multiply-charged ions may be observed if their mass-to-charge ratio (m/z) falls within the instrument range. This applies to proteins and large oligonucleotides, which typically do become multiply-charged.

To deconvolute selected spectra

Use this procedure to deconvolute selected *m/z* spectra and create a protein list.

1 Open the data file that contains the spectra of interest as described in online Help.

2 Select one or more spectra as described in online Help.

3 Display the Deconvolute section in the Method Editor in *either* of the following ways:

- Select **Deconvolute (MS): Maximum Entropy** from the BioConfirm Workflow section of the Method Explorer.
- Select **Deconvolute (MS): Maximum Entropy** from the Method Items list in the Method Editor.

4 Set parameters on the Method Editor: Deconvolute (MS): Maximum Entropy section. See "Deconvolution Method Parameters" on page 5 for more information.

5 Click the  button on the Method Editor toolbar to start processing.

Tip You can also initiate deconvolution in any of the following ways:

- Right-click on a user spectrum in the Spectrum Results or Navigator window and select **Deconvolute (Maximum Entropy)** from the shortcut menu.
- Select **Deconvolute (Maximum Entropy)** from the Spectra menu.
- Select **Deconvolute** from the Actions menu.

6 Review results in the Deconvolution Results window. If this window is not currently displayed, display it in one of the following ways:

- Select **Deconvolution Results** from the View menu
- Click the **Deconvolution Results** button on the main toolbar 

  See "To view protein deconvolution results" on page 17 for more information.

7 View deconvolution compounds as described in "To view deconvolution compounds" on page 20.

To integrate chromatograms and deconvolute spectra

Use this procedure to integrate a TIC or selected chromatogram, extract peak spectra for each chromatogram peak, deconvolute each *m/z* spectrum separately, display deconvoluted mass spectra, and create a protein list.

1 Open the data file that contain the chromatogram of interest. The TIC is automatically displayed in the Chromatogram Results window.

2 Set the integration parameters:

a Display the BioConfirm Workflow > Integrate (MS) section in the Method Editor in either of the following ways:

- Select **Integrate (MS)** from the BioConfirm Workflow section of the Method Explorer.
- Select **Integrate (MS)** from the Method Items list in the Method Editor toolbar.

b Review and set the integration parameters on the following tabs:

- Integrator
- Peak Filters
- Results

See the Qualitative Analysis online Help for descriptions of these parameters.

3 Set the deconvolution parameters:

a Display the BioConfirm Workflow > Deconvolute (MS): Maximum Entropy section in the Method Editor in *either* of the following ways:

- Select **Deconvolute (MS): Maximum Entropy** from the BioConfirm Workflow section of the Method Explorer.
- Select **Deconvolute (MS): Maximum Entropy** from the Method Items list in the Method Editor.

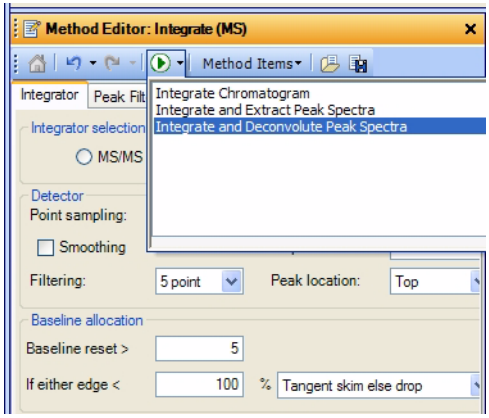
b Review and set the deconvolution parameters as follows:

- Verify that the **Mass range** is appropriate.
- Mark or clear the **Use limited m/z range** check box as appropriate.
- Verify that the Compound Filters are set to appropriate values. The **Minimum consecutive charge states** field and the **Minimum protein fit score** limit the compounds that are created.

c Set other deconvolution parameters as described in "Deconvolution Method Parameters" on page 5.

4 Initiate processing in *any* of the following ways:

- Click in the Chromatogram Results window to select the TIC, then click the Down arrow next to the button on the Method Editor toolbar and select **Integrate and Deconvolute Peak Spectra**, as shown below:



- Right-click the TIC in the Chromatogram Results or Navigator window and select **Integrate and Deconvolute Peak Spectra** from the shortcut menu. You can also select this option from an EIC, if you know what your target *m/z* range is or if the EIC target *m/z* is very broad.
- Click to select the TIC in the Chromatogram results window, then select **Integrate and Deconvolute Peak Spectra** from the **Chromatograms** menu.
- Click to select the TIC in the Chromatogram results window, then select **Integrate and Deconvolute** from the **Actions** menu.

5 Review results in the Deconvolution Results window. See "To view protein deconvolution results" on page 17 for more information.

6 View deconvolution compounds as described in "To view deconvolution compounds" on page 20.

To view protein deconvolution results

Use this procedure to review the results from either of the following deconvolution processes:

- "To integrate chromatograms and deconvolute spectra" on page 15
- "To deconvolute selected spectra" on page 14

1 If the Deconvolution Results window is not currently displayed, display it in one of the following ways:

- Select **Deconvolution Results** from the View menu
- Click the **Deconvolution Results** button on the main toolbar 

Tip There is a layout that automatically opens the Deconvolution Results and Compound List windows and reformats the compound list to show the appropriate information for a deconvolution operation. To select this layout, select **Window Layouts > Load Layout** from the View menu, then select **BioConfirm-IntactProtein-MaximumEntropy-Default** and click the **Open** button.

2 Use the following mouse actions to change the display of data:

- Click to select a single mass in the spectrum.
- Drag to select a mass range in the spectrum.
- **Ctrl+drag** to select another area and keeps the previous area/time selected.
- Drag axes to scroll the axes in the direction you are moving the mouse.
- Right-drag to expand the selected area. The area you define is shown as a rectangle outlined in black. The Y-scale of the zoomed in display is controlled by the Auto-Scale Y-axis mode.
- Right-drag axes to scale the axis. Dragging to the right (x-axis) or to the top (y-axis) zooms in on that axis. Dragging to the left (x-axis) or to the bottom (y-axis) zooms out on that axis. Select **Unzoom** from the shortcut menu to return to the previous display scale.

Tip To return to the previous display scale, click the **Unzoom** toolbar button  or select **Unzoom** from the shortcut menu.

3 Use the following toolbar buttons to change the display of data:

Toolbar button	Action/Meaning
	Scales the x-axis and y-axis automatically to fit the displayed data.
	Scales the x-axis automatically to fit the displayed data.
	Scales the y-axis automatically to fit the displayed data.
	Returns to the previous display scale (undoes last zoom operation).
	When this mode is on and you zoom with the mouse, the vertical plot range is automatically scaled to fit the data contained in the horizontal range you specify. When the **Autoscale y-axis during Zoom** mode is off, the vertical plot range is set to the limits you specify by dragging the mouse.
	Toggles whether to scale the y-axis of all spectra to the same scale when you zoom in on the x-axis.
	Plots each spectrum separately. The spectra share the same x-axis, but each spectrum has a separate y-axis.
	Overlays all spectra. The spectra are shown with the same x- and y-axes.
	Switches to *previous* plot for overlaid spectra.
	Switches to *next* plot for overlaid spectra.
	Sets the number of spectra that are shown together. A scroll bar will appear for additional spectra beyond this number.

4 Right-click in the graph to display the following shortcut menu options:

- **Subtract Any Spectrum** - Subtracts the next spectrum you select from the currently selected spectra. The new spectrum is displayed in the plot and table windows, and the Data Navigator window is updated. Select this menu item again to exit Subtract Any Spectrum mode without subtracting any spectra.
- **Add Any Spectrum** - Adds the next spectrum you select to the currently selected spectra. The new spectrum is displayed in the plot and table windows, and the Data Navigator window is updated. Select this menu item again to exit Add Any Spectrum mode without adding any spectra.
- **View MS Peak List 1** - Toggles the display of the MS Spectrum Peak List window, called MS Peaks One, showing the first peak selected. One of the columns in the peak list is **Fit**. This is a score between 0 and 10 to assess the peak shape in the deconvoluted spectrum. Higher numbers indicate better peak shape.
- **View MS Peak List 2** - Toggles the display of the MS Spectrum Peak List window, called MS Peaks Two, showing the second peak selected.
- **Assign Ranges to > Exclude Masses** - *not used in this release*
- **Assign Ranges to > Find by Molecular Feature** - *not used in this release*
- **Unzoom** - Undoes the last zoom operation (returns to the previous display scale).
- **Assign Random Colors** - Changes the color of the selected spectrum.
- **Choose Defined Color** - Changes the color of the selected spectrum to the specified color.
- **Copy to Clipboard** - Copies spectra that are currently visible in the Deconvolution Results window to the Clipboard for use with other applications.
- **Print** - Allows you to print the Deconvolution Results window.
- **Export** - Allows you to export data and graphics.

5 (*optional*) To change the number of significant digits in the deconvoluted spectrum, select **Plot Display Options** from the Tools menu and click the **Deconvoluted Spectra** tab on the Plot Display Options dialog box. Set the **Digits after the decimal value** and click **OK**.

6 (*optional*) To move the window, drag it to a new position on the screen. To return it to its default position, right-click in the title bar and clear the check mark for the **Floating** menu item.

To view deconvolution compounds

If the correct mass range is selected in the Method Editor: Deconvolute (MS): Maximum Entropy section, compounds are created for proteins that are confidently found. You can view these compounds as follows:

1 Show the Compound List by selecting **Compound List** from the View menu or by clicking the  button on the main toolbar.

Tip There is a layout that automatically opens the Compound List and Deconvolution Results windows and reformats the compound list to show the appropriate information for a deconvolution operation. To select this layout, select **Window Layouts > Load Layout** from the View menu, then select **BioConfirm-IntactProtein-MaximumEntropy-Default** and click the **Open** button.

2 Click on a compound of interest. If linked navigation is turned on, associated data in the following windows are automatically displayed and selected:

- Deconvolution Results window
- An EIC in the Chromatogram Results window
- A compound spectrum that displays all the different peaks in the raw *m/z* spectrum that indicate the presence of the deconvoluted protein mass in the MS Spectrum Results window

3 Display the MS Peak List window in any of the following ways:

- Right-click in the Deconvolution Results graph and select **View MS Peak List 1** from the shortcut menu.
- Click the  button on the main toolbar.
- Select **MS Spectrum Peak List 1** from the View menu.

4 View the following information for the ion set spectrum in the MS Peak List window:

- Peak
- *m/z*
- Abund
- Charge
- Max Abund
- Abund%
- Cpd Mass
- Calc Mass
- Expected *m/z*
- Diff (ppm)
- Diff (*m/z*)
- Diff (Da)

To print a report with deconvolution results

1. Display the Compound Report section in the Method Editor by selecting **Compound Report** from the BioConfirm Workflow section in the Method Explorer or by selecting **Compound Report** from the **Method Items** menu in the Method Editor toolbar.
2. Review the options in this section. Verify that the sections that you want included in the report are marked.
3. Display the Common Reporting Options section in the Method Editor by selecting **Common Reporting Options** from the BioConfirm Workflow section in the Method Editor or by selecting **Common Reporting Options** from the **Method Items** menu in the Method Editor toolbar.
4. Review the parameters in both the **Templates** and **Options** tabs. Select one of the following Compound report templates for match sequence results, depending on the analysis type:
   - **BioConfirmProteinCompoundReport.xltx** (for proteins)
   - **BioConfirmOligonucleotideCompoundReport.xltx** (for oligonucleotides)
5. Click **Print > Compound Report** from the File menu to print the report.

Tip To print deconvolution spectra, right-click in the graph area of the Deconvolution Results window and select **Print** from the shortcut menu.

To automate protein confirmation

Use this procedure to do protein confirmation automatically for samples in a worklist.

1 Display the Assign Actions on Execution from Worklist section in the Method Editor in one of the following ways:

- Select **Worklist Actions** from the Worklist Automation section of the Method Explorer.
- Select **Worklist Actions** from the Method Items list in the Method Editor.

2 Select from the following items for protein deconvolution in the **Available actions** list:

- **Integrate and Deconvolute** - Integrates each selected chromatogram, extracts *m/z* peak spectra for each chromatogram peak, deconvolutes for each *m/z* spectrum separately, displays deconvoluted mass spectra and displays/creates a protein list, according to the parameters set in the Method Editor.
- **Deconvolute** - Deconvolutes all of the selected *m/z* spectra. Displays the deconvoluted mass spectra and creates a protein list, according to the parameters set in the Method Editor.

3 Click the Down arrow to move the selected actions to the **Actions to be run** list. You can also double-click actions to move them to the other list. New items are added to the end of the list.

4 (*optional*) The items in the Actions to be run list can be reordered using the Up and Down arrows to the right of the list. The actions will be executed in the order they appear in the list. Click the **X** button to remove unwanted items from the list.

5 Test the list of actions in either of the following ways:

- Select **Run the Worklist Actions** from the Actions menu.
- Click the  button on the Method Editor toolbar.

6 Save the method in any of the following ways:

- Select **Save** or **Save As** from the Method menu.
- Click the **Save** button in the Method Editor toolbar.
- Select **Save Method** from Method Editor shortcut menu (right-click).

7 Assign this method to samples of interest when setting up a worklist as described in the online Help for your instrument.

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 Oligonucleotides in this version.)
- Apply or edit links. (Not available for Oligonucleotides 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 List and Sequence Match Results windows.

Step 9 - For protein digests only:

- View Sequence Coverage Results in the Sequence Coverage Map window.

Step 10 - Print report.

The topics in this section will help you get started with sequence matching.

Setting Up Match Sequences and Match Sequence Method Parameters

- "To set match sequence method parameters" on page 25
- "To create or edit a sequence" on page 26
- "To add or edit the sequence text" on page 27
- "To apply or edit modifications" on page 27
- "To apply or edit links" on page 29
- "To assign or edit digest reagents" on page 30 (*Protein digest sequences)*
- "To select matching rules" on page 31

Matching Sequences and Reviewing Results

- "To match sequences" on page 32
- "To view match sequence results" on page 33
- "To view sequence coverage results for protein digests" on page 33
- "To print a report with match sequence results" on page 34

Automating Sequence Matching

- "To automate sequence matching" on page 35

Default Methods and Layouts for Sequence Matching

Sequence Type	Default Method	Default Layout
Protein (LMFE)	BioConfirmIntactProtein-Default	BioConfirm-IntactProtein-LMFE
Protein (LMFE >25KDa)	BioConfirmIntactProteinHighMass-Default	BioConfirm-IntactProtein-LMFE
Protein Digest	BioConfirmProteinDigest-Default	BioConfirm-ProteinDigest
Synthetic Peptide	BioConfirmSyntheticPeptide-Default	BioConfirm-SyntheticPeptide
Oligonucleotide (<7 kDa)	BioConfirmOligonucleotideSmall-Default	BioConfirm-Oligonucleotide
Oligonucleotide (>7 kDa)	BioConfirmOligonucleotideLarge-Default	BioConfirm-Oligonucleotide

Setting Up Sequences and Match Sequence Method Parameters

To set match sequence method parameters

1 Display the **Define and Match Sequences** section in the Method Editor window.

Tip If the BioConfirm workflow is not available in Method Explorer, select it from the **Tools > Configure for Workflow** menu.

2 (*optional*) Load an appropriate method and layout for your analysis. See "Default Methods and Layouts for Sequence Matching" on the previous page.

3 If you plan to use the method in a *worklist* or with MassHunter Easy-Access Software, and plan to specify sequences (from **.psq** files) in the worklist:

   a Click **Worklist** on the **Source** tab.

   b Specify masses or sequence files in the worklist as described in "To automate sequence matching" on page 35.

4 To specify the masses or sequences to match in the *method*:

   a Click **Qualitative method** on the **Source** tab.

   b To match sequences, mark the **Sequences** check box, then import the sequences as described in Step 5 or create sequences as described in Step 6.

   c To match masses, mark the **Masses** check box and type in a list of masses separated by commas.

5 To import a sequence:

   a Click the **Import** button on the **Sequences** tab.

   b Select the desired **.psq** file and click the **Open** button. The sequence is added to the list.

6 Create or edit a sequence as described on page 26.

7 On the **Sequences** tab, mark the sequences to match.

8 Click the **Mass Matching** tab and set mass accuracy value and units.

9 Click the **Results** tab and set the result display parameters.

10 Save the method in *one* of the following ways: click **Save** or **Save As** on the Method menu, click the **Save** button on the Method Editor toolbar, *or* click **Save Method** on the Method Editor shortcut menu (right-click).

To create or edit a sequence

1 Click **New Sequence** on the Sequence menu.

   a A default sequence name is assigned and a new sequence is added to the list of sequences on the Sequences tab in the Define and Match Sequences section in the Method Editor window.

   b The new sequence is also displayed in the Sequence Editor window. Enter a **Sequence Name** and select the **Sequence Type** (Protein, Synthetic peptide, Protein Digest, or Oligonucleotide).

   c Edit the sequence as described in Step 3.

2 To edit a sequence:

   a Click to select the sequence of interest on the Sequences tab in the Define and Match Sequences section in the Method Editor window.

   b Click the **Edit** button. The Sequence Editor window opens with the selected sequence displayed for editing.

3 In the Sequence Editor:

   a Add or edit the sequence text as described on page 27.

   b Apply or edit modifications as described on page 27. *Note that modifications are not currently supported for Oligonucleotide sequences.*

   c Apply or edit links as described on page 29. *Note that links are not currently supported for Oligonucleotide sequences.*

   d *For protein digests only,* assign or edit digest reagents as described on page 30.

   e Select matching rules as described on page 31.

4 Click the **Save as** button on the Sequences tab of the **Define and Match Sequences** section in the Method Editor window to save the sequence for use with other methods.

Tip You can also create a new sequence by clicking the **New** button in the **Define and Match Sequences** section in the Method Editor window. This automatically displays the new sequence in the Sequence Editor window. Proceed as described above.

To add or edit the sequence text

1. Select the sequence of interest on the Sequence Editor window.
2. Select the correct chain to add or edit. Chain A is selected by default.
3. Enter or edit the amino acids in the sequence text box in either of the following ways.
   - Type in individual amino acids one at a time between the N-term and C-term symbols. Note that for Oligonucleotide sequences, only A, C, T, and G are allowed as oligonucleotide bases.
   - Copy the sequence from a FASTA-formatted database or a text file. Right-click in the Sequence Editor and select **Paste** from the shortcut menu. The amino acid sequence will appear in the Sequence editor box, between the N-term and C-term symbols.

NOTE Only single-character (letter) amino acids are allowed for the protein sequence field. Three character amino acid symbols are not supported. Use the Amino acid list in the Sequence Editor to select the correct notation for each amino acid.

To apply or edit modifications

1. Select the sequence of interest on the Sequence Editor window. *Note that modifications are not currently supported for Oligonucleotide sequences.*
2. Right-click in the Sequence Editor window and select **Edit Modifications** from the shortcut menu to open the Modifications dialog box.
3. To select and apply global modifications:
   a. On the Global tab, select the desired modification from the list of **Available modifications**.

      If desired, you can customize the list of available modifications using the Chemical Data Dictionary; *see online help for more information.*
   b. Select the amino acids to modify in the **Apply to all** list. Use **Shift+click** or **Ctrl+click** to select multiple amino acids, if desired.
   c. Click the **Apply** button to apply the specified modification to the selected amino acids throughout the sequence.

d Repeat step a through step c to select and apply other global modifications.

4 To select and apply local modifications:

a On the Local tab, select the location in the sequence to modify: **Position**, **C-terminus**, or **N-terminus**.

b If you selected **Position** in Step 4a, enter the index number of the amino acid you want to modify. Use the blue index numbers shown for each row of the sequence on the Sequence Editor window to determine the proper index number for the selected position.

c Select the desired modification from the list of **Applicable modifications**.

d Mark the **Variable modification** check box if you want to create a variable modification for sequence matching. When marked, sequences that contain that amino acid site are matched both *with and without* this modification. If the form of the sequence with the modification is found, the name and the site of modification appears in the Mods column in the compound list.

e Click the **Apply** button to apply the selected modification to the specified location (amino acid) in the sequence.

f Repeat step a through step e to select and apply other local modifications.

5 Click **OK** to close the Modifications dialog box. Note that the molecular weight and formula have been updated in the Sequence Editor.

To remove modifications

1 Click **Edit Modifications** on either the Sequence menu or the Sequence Editor shortcut menu to open the Modifications dialog box.

2 Click the **Applied** tab and review the list of modifications.

3 Click to select the modifications you want to delete.

4 Click the **Delete** button to remove the selected modifications.

To apply or edit links

1. Select the sequence of interest on the Sequence Editor window. *Note that links are not currently supported for Oligonucleotide sequences.*
2. Right-click in the Sequence Editor window and select **Edit Links** from the shortcut menu to open the Links dialog box.
3. Enter the index number for one end of the link in the **From index** box and select the chain (if other than the default Chain A).
4. Enter the index number for the other end of the link in the **To index** box and select the chain (if other than the default Chain A).
5. Select a link from the **Link types** list.

Tip You can customize the list of links types using the Chemical Data Dictionary; *see online help for more information.*

6. Click the **Apply** button to link the selected amino acids in the sequence.
7. Repeat Steps 3 -6 to create additional links.
8. Click **OK** to close the Links dialog box. Note that the molecular weight and formula have been updated in the Sequence Editor.

To remove links

1. Click **Edit Links** on either the Sequence menu or the Sequence Editor shortcut menu to open the Links dialog box.
2. Click the **Applied** tab and review the list of links.
3. Click to select the links you want to delete.
4. Click the **Delete** button to remove the selected links.

To assign or edit digest reagents

Digest reagents apply to protein digest sequences only.

1 Select the protein digest sequence of interest in the Sequence Editor.

2 Right-click in the Sequence Editor window and select **Edit Digest Reagents** from the shortcut menu to open the Digest Reagents dialog box (or select **Edit Digest Reagents** from the Sequence menu).

3 Click to select the reagent to apply to the protein sequence in the **Available** list. You can select multiple reagents by using **Shift+click** or **Ctrl+click**.

   If the reagent you want to apply is not in the list of available reagents, use the Chemical Data Dictionary to customize the list. *See online Help for more information.*

4 Click the right arrow button to move the selected digest reagents from the **Available** list to the **Selected** list.

5 Prioritize the reagents in the **Selected** list by clicking the up and down arrow buttons to set the desired digest order. The sequence will first be digested with the first reagent in the list, then the resulting digest will be digested with the second reagent, and so on.

6 Enter the maximum number of **Missed Cleavages** to allow, then click **OK**.

7 To generate the digest list, right-click in the Sequence Editor window and select **Digest Current Sequence** from the shortcut menu (or select **Digest Current Sequence** from the Sequence menu).

   The digest list for the selected protein digest sequence is displayed in the Digest List window.

To select matching rules

1. Select the sequence of interest on the Sequence Editor window.
2. Right-click in the Sequence Editor window and select **Edit Matching Rules** from the shortcut menu to open the Rules dialog box.
3. Select the tests to use for matching the theoretical masses of peptides, proteins, protein digests, or oligonucleotides to those from MS data. Use **Ctrl+click** to select multiple tests from the list. Only the rules that are appropriate to the selected sequence type are displayed. See *online Help* for a description of the matching tests for proteins, protein digests, synthetic peptides, and oligonucleotides.
4. If you select the rule **Predicted modifications**, then create the list of **Selected modifications** in the lower half of the Rules dialog box. Sequences will be matched with one or more of each predicted modification as long as the sequence has an amino acid site consistent with the modification. If a sequence plus any predicted modifications matches a mass from the compound list, the identity of the predicted modification appears in the Pred Mods column in the compound list row for that sequence match. (*Applies to protein and protein digest sequences only.*)
5. Click **OK** to close the Rules dialog box.

Matching Sequences and Reviewing Results

The topics in this section will help you match sequences and review results.

- "To match sequences" on page 32
- "To view match sequence results" on page 33
- "To view sequence coverage results for protein digests" on page 33
- "To print a report with match sequence results" on page 34

To match sequences

1 Open the data file of interest.

2 Open the method of interest. See "Default Methods and Layouts for Sequence Matching" on page 24 for a list of methods for your analysis.

3 Find compounds by deconvolution *or* molecular feature as follows:

Deconvolution

See "To integrate chromatograms and deconvolute spectra" on page 15.

Molecular Feature

a Review the settings in the **BioConfirm Workflow > Find Compounds by Molecular Feature** section of the Method Editor window. Modify the settings if necessary, keeping the following guidelines in mind.

- For most BioConfirm analyses, set the Target data type on the Extraction tab to **Large molecules (proteins, oligos)**.
- For small oligonucleotides (<7 kDa), set the Target data type on the Extraction tab to **Small molecules (chromatographic)**.
- For oligonucleotides larger than 7 kDa, use either Large Molecule feature extraction or Maximum Entropy Deconvolution.

b Start the compound search in any of the following ways:

- Click **Find Compounds by Molecular Feature** on the Find menu.
- Click the arrow in the  button on the Method Editor toolbar.
- Click **Find Compounds by Molecular Feature** on the Method Editor shortcut menu.

c Review the results in the Compound List window.

4 Set match sequence method parameters as described on page 25.

5 Start the match search in any of the following ways:

- Click **Match Sequences** on the Sequence menu.
- Click the  button on the Method Editor toolbar.
- Click **Match Sequences** on the shortcut menu in Method Editor, Data Navigator Compounds, or Compound List.

6 Review the results in the Compound List and Sequence Match Results windows. See "To view match sequence results" on page 33.

7 (*Protein digests only*) View Sequence Coverage Results for protein digests as described on "To view sequence coverage results for protein digests" on page 33.

To view match sequence results

1 Match sequences as described in the previous section.

2 Display the Sequence Match Results window in one of these ways:

- Click the  toolbar button.
- Click **View Sequence Match Results** on the Sequence menu.
- Click **Sequence Match Results** on the View menu.

3 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). If no sequence match was found for the highlighted compound, then the Sequence Match Results window is empty.

4 Select another sequence match result to view by selecting a different compound in the Data Navigator or Compound List windows.

To view sequence coverage results for protein digests

1 View match sequence results as described in the previous section.

2 Display the Sequence Coverage Map window in one of these ways:

- Click the  toolbar button.
- Click **View Sequence Coverage Map** on the Sequence menu.
- Click **Sequence Coverage Map** on the View menu.

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

- **Applied Modifications**
- **Applied Links**

- **Applied Reagents**
- **Applied Matching Rules**
- **Show Sequence Description**

4 (*optional*) Click **Copy Sequence to Method** on the Sequence Coverage Map shortcut menu to copy the current sequence to the Sequences tab of the **BioConfirm Workflow > Define and Match Sequences** section of the Method Editor window.

5 Select another sequence match result to view by selecting a different **Matched Sequence** result in the Data Navigator window.

To print a report with match sequence results

1 Display the **BioConfirm Workflow > Compound Report** section in the Method Editor in one of two ways:

- Select **Compound Report** from the BioConfirm Workflow section in the Method Explorer. If the BioConfirm workflow is not available in Method Explorer, select it from **Tools > Configure for Workflow**.
- Click **Compound Report** from the **Method Items** list in the Method Editor toolbar.

2 Review the check boxes in this section. Verify that the sections you want to include in the report are marked.

3 Display the **BioConfirm Workflow > Common Reporting Options** section in the Method Editor in one of two ways:

- Select **Common Reporting Options** from the BioConfirm Workflow section in the Method Explorer.
- Click **Common Reporting Options** from the Method Items list in the Method Editor toolbar.

4 Review the parameters on both the **Templates** and **Options** tabs. Select one of the following Compound report templates for match sequence results, depending on the analysis type:

- **BioConfirmProteinCompoundReport.xltx** (for proteins)
- **BioConfirmSyntheticPeptideCompoundReport.xltx** (for synthetic peptides)
- **BioConfirmProteinDigestCompoundReport.xltx** (for protein digests)
- **BioConfirmOligonucleotideCompoundReport.xltx** (for oligonucleotides)

5 Click **Compound Report** from the File > Print menu to print the report.

Automating Sequence Matching

To automate sequence matching

Use this procedure to do sequence matching automatically in a worklist.

1 Set the appropriate options for your analysis in the **Find Compounds by Molecular Feature** section of the Method Editor.

2 Display the **Define and Match Sequences** section of the Method Editor.

3 To specify the masses or sequences to match *in the worklist*:

   a Click the **Worklist** option on the Source tab.

   b Specify masses or sequence files in the worklist as described in Step 11.

4 To specify the masses or sequences to match *in the method*:

   a Click the **Qualitative method** option on the Source tab.

   b To match sequences, mark the **Sequences** check box, then import, create, or select the sequences as described in "Setting Up Sequences and Match Sequence Method Parameters" on page 25.

   c To match masses, mark the **Masses** check box, and enter the masses to search, separated by commas.

5 Set a **Mass accuracy** value on the Mass match tab.

6 Select the following in the **Available actions** list on the **Worklist Automation > Worklist Actions** section of the Method Editor:

   - **Find Compounds by Molecular Feature**
   - **Match Sequences**
   - **Generate Compound Report**

7 Click the button to move the selected actions to the **Actions to be run** list. You can also double-click actions to move them to the other list. The items are added to the end of the list.

NOTE 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.

8 Test the list of actions in either of the following ways:

   - Click **Run the Worklist Actions** from the Actions menu.

- Click the  button on the Method Editor toolbar.

9 Save the method in *one* of the following ways:

- Click **Save** or **Save As** from the Method Menu.
- Click the  button on the Method Editor toolbar.

10 In Agilent MassHunter Workstation Data Acquisition software, set up a worklist for the samples of interest. Assign the method from Step 9 to the samples in the worklist.

11 If you clicked the **Worklist** option on the Source tab in the Define and Match Sequences section of the Method Editor in Step 3, then specify the mass or sequence to use in sequence matching as follows:

a Add a column of Type **Protein**.

b Type in a description of the protein in the **Column name** field.

c In the **Value** field on the **Add Columns** dialog box, either type in a mass or select a **.psq** sequence file name.

d (*optional*) Use multiple Protein columns to search multiple masses or sequences for each sample.

Refer to the online Help for the Agilent MassHunter Workstation Software - Data Acquisition program for more information on setting up worklists.

12 Run the worklist and review the printed Compound reports.

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In This Guide

This guide has instructions for installing and using the Agilent MassHunter BioConfirm Software.

If you have comments about this guide, please send an e-mail to feedback_lcms@agilent.com.

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