

# Agilent G3835AA MassHunter Mass Profiler Professional Software

# **Familiarization Guide**

Familiarization Tutorial: How do I do a new analysis? 2 Advanced Tasks 25

### What is Agilent Mass Profiler Professional?

Agilent Mass Profiler Professional (MPP) software is a powerful chemometrics platform designed to exploit the high information content of mass spectra (MS) data and can be used in any MS-based differential analysis to determine relationships among two or more sample groups and variables. MPP provides advanced statistical analysis and visualization tools for GC/MS, LC/MS, CE/MS, ICP-MS, and NMR data analysis. MPP also integrates smoothly with Agilent MassHunter Workstation, Spectrum Mill and ChemStation software and is the only platform that provides integrated identification/ annotation of compounds and integrated pathway analysis for metabolomic and proteomic studies. The system also enables Automated Sample Class Prediction that revolutionizes mass spectrometer-based qualitative analysis of unknown samples in many applications. MPP is ideally suited for applications characterized by complex sample matrices such as metabolomics, proteomics, natural products, food, beverages, flavors, fragrances, and environmental analyses.

### How do I get started with Mass Profiler Professional?

MPP comes preloaded with a demonstration experiment to show you the functionality of the product. A project called "Malaria" contains an experiment called "Malaria LCMS ESI+ pH 7." You are encouraged to explore this demonstration project to get to know Mass Profiler Professional. Use the familiarization tutorial to create your first project and experiment with the software.



## Familiarization Tutorial: How do I do a new analysis?

The steps of the familiarization tutorial are (1) Create a new project and experiment for your data, (2) Import and organize your data, (3) Perform your initial differential analysis, (4) Use MassHunter ID Browser to identify your compounds, and (5) Save your project

### Exercise 1. Create a new project and experiment for your data

An easy way to start using the MPP software is to recreate the sample experiment from the demonstration project. The original *Malaria Demo* data you need is provided with the MPP installation. The data files are located in named folders within the main installation folder, usually found in **C:\Program Files\Agilent\ MassHunter\Workstation\Mass Profiler Professional\samples** (Windows 7).

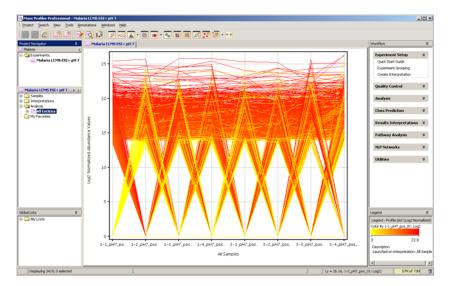
A new project and experiment is created through four sequential dialog boxes: (1) Startup, (2) Create New Project, (3) Experiment Selection, and (4) New Experiment. Follow the steps below to create a new experiment with the *Malaria Demo* data.

Steps	Detailed Instructions	Comments
1 Start Mass Profiler Professional.		
In the Startup dialog box, open a recent project or create a new project.	<ul> <li>If you want to use the <i>Malaria</i> project:</li> <li>a Click Open recent project.</li> <li>b Select Malaria from the Select recent project list.</li> <li>c Click OK.</li> </ul>	<ul> <li>The Startup dialog box helps you quickly set up a new project or continue your analysis with a previous project.</li> </ul>
Startup Welcome to MassProfiler Pro Select what you would like to do from the options below, to continue.	Image: Second Se	• <b>Open recent project</b> opens the project and experiment(s) that are stored in the project.
Options C Greate new project C Open existing project C Open recent project Select recent project Do not show this dialog again Help	OK Cancel	<ul> <li>A project is a "container" for a collection of experiments; each experiment contains your samples interpretations, and analyses. A project can have multiple experiments on different sample types and organisms.</li> </ul>

### Detailed Instructions Comments

- d Select an operation from the Workflow Browser to continue analyzing the *Malaria Demo* project.
- The **Workflow Browser** is found along the right sidebar and is organized in groups of operations.

•



If you instead want to create a new project, click **Create new project** and go to Step 3, otherwise proceed to Exercise 4 on page 21.

3 In the **Create New Project** dialog box, enter your project information.

Steps

- a Type Malaria Demo Familiarization in Name.
- $\label{eq:product} \textbf{b} \quad \text{Type descriptive information in } \textbf{Notes}.$
- c Click OK.

 The project name and notes may be viewed and edited at any time using the Project Inspector by clicking Project > Inspect Project from the menu bar.

🧕 Create New Project	×
New Project Details	
Name	Malaria Demo Familiarization
Note:	One independent variable - Infection Status (1) Uninfected and (2) Infected
Help	OK Cancel

Steps	Detailed Instructions	Comments
In the Experiment Selection Dialog dialog box, create a new experiment.	<ul> <li>a Click Create new experiment.</li> <li>w b Click OK.</li> </ul>	<ul> <li>If you click <b>Open existing</b> <pre>experiment, you are prompted for         the experiment to add to the         project. This is the same as clicking</pre> </li> </ul>
Experiment Selection Dialog     An experiment is an organized collection of     given data source. If you have an experime     project, please choose "Open existing expe experiment with new data or previously imp     Choose Experiment     Create new experiment     Open existing experiment     Help	ent you wish to use from a previous rriment. " You may also create a new	the <b>Add experiment</b> button .
5 In the <b>New Experiment</b> dialog enter and select information th guides your experiment creation	nat experiment in <b>Experiment name</b> .	from the Toolbar, click the New experiment button . g • Experiment type determines how
	e Type descriptive information in Experiment notes. f Click OK.	<ul> <li>Mass Profiler Professional manages the data.</li> <li>You select Unidentified when the</li> </ul>
Neur Experiment	e Type descriptive information in Experiment notes. f Click OK.	<ul> <li>Manages the data.</li> <li>You select <b>Unidentified</b> when the compounds have only been</li> </ul>
statistical significance test and fold change only. "Class Prediction" will guide you throug training data.	e Type descriptive information in Experiment notes. f Click OK.	<ul><li>manages the data.</li><li>You select <b>Unidentified</b> when the</li></ul>
Experiment description Enter a name, analysis type, experiment ty statistical significance test and fold change only. "Class Prediction" will guide you throug training data. Experiment name	e Type descriptive information in Experiment notes. f Click OK. pe and a desired workflow type. "Analysis" will guide you through a analysis. "Data Import" will guide you through experiment creation gh the creation and testing of a prediction model, using imported Malaria Demo samples	<ul> <li>You select Unidentified when the compounds have only been identified by their molecular features of neutral mass and retention time.</li> <li>You select Identified when the compounds have been identified by</li> </ul>
Experiment description Enter a name, analysis type, experiment ty statistical significance test and fold change only. "Class Prediction" will guide you throug training data. Experiment name Analysis type	e Type descriptive information in Experiment notes. f Click OK. pe and a desired workflow type. "Analysis" will guide you through a analysis. "Data Import" will guide you through experiment creation gh the creation and testing of a prediction model, using imported Malaria Demo samples Mass Profiler Professional	<ul> <li>You select Unidentified when the compounds have only been identified by their molecular features of neutral mass and retention time.</li> <li>You select Identified when the compounds have been identified by compound, formula, and/or CAS number.</li> </ul>
Experiment description Enter a name, analysis type, experiment ty statistical significance test and fold change only. "Class Prediction" will guide you throug training data. Experiment name Analysis type Experiment type	e Type descriptive information in Experiment notes. f Click OK. pe and a desired workflow type. "Analysis" will guide you through a analysis. "Data Import" will guide you through experiment creation gh the creation and testing of a prediction model, using imported Malaria Demo samples Mass Profiler Professional Unidentified	<ul> <li>You select Unidentified when the compounds have only been identified by their molecular features of neutral mass and retention time.</li> <li>You select Identified when the compounds have been identified by compound, formula, and/or CAS number.</li> </ul>

### Exercise 2. Import and organize your data

After you set up a project and create an experiment, the **MS Experiment Creation Wizard** immediately guides you through the necessary steps to organize your experiment, import your data, define your experiment variables, and prepare your data for analysis. The **MS Experiment Creation Wizard** flow diagram is illustrated in Figure 1. The data preparation includes grouping, filtering, alignment, normalizing, and baselining.

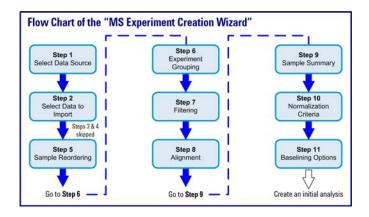


Figure 1 MS Experiment Creation Wizard

Steps	Detailed Instructions	Comments
1 Select the data source in the MS Experiment Creation Wizard (Step 1 of 11).	<ul> <li>a Click MassHunter Qual and select Homo sapiens for the Organism if you are using the <i>Malaria Demo</i> data set.</li> <li>b Click Next.</li> </ul>	<ul> <li>If you are using your own data set, click the source of your sample files, and select the <b>Organism</b> of the sample files or select <b>None</b>.</li> </ul>
MS Experiment Creation Wizard (Step 1 of 11)	×	
Select Data Source Choose the data sources that will be used for the experiment		<ul> <li>Note that selecting an Organism is most important when you use the</li> </ul>
MassHunter Qual		Pathway Analysis features of MPP.
C MassHunter ICP-MS		
C AMDIS		
C Generic		
Organism Homo sapiens 💌		
	< <back< td=""><td></td></back<>	

eps		Detailed Instructions	Comments
	sample data to import in periment Creation ep 2 of 11).	<ul> <li>a Click Select Data Files to display the file selection dialog box.</li> <li>b Select all of the .cef files you want to import into the experiment. The example Malaria data files are: <ul> <li>1-1_pH7_pos_01.cef</li> <li>1-2_pH7_pos_01.cef</li> <li>1-4_pH7_pos_01.cef</li> <li>3-1_pH7_pos_01.cef</li> <li>3-2_pH7_pos_01.cef</li> <li>3-2_pH7_pos_01.cef</li> <li>3-3_pH7_pos_01.cef</li> <li>3-4_pH7_pos_01.cef</li> <li>Click Open to load the selected files.</li> <li>d Click Next.</li> </ul> </li> </ul>	<ul> <li>Note: The <b>Open</b> dialog likely already points to the <b>samples</b> directory in the main Mass Profiler Professional installation directory, but if not, browse to <b>C:\Program</b> <b>Files\Agilent\MassHunter\</b> <b>Workstation\Mass Profiler</b> <b>Professional\samples\Malaria</b> <b>Demo</b>.</li> <li>A progress indicator is displayed while the files are being loaded into MPP.</li> </ul>
MS Experiment Cr	eation Wizard (Step 2 of 11)	X	1
Gelect Data to In			
Data may be impor			
Туре		Selected files and samples	
Туре	1-1_pH7_pos_01.cef	Selected files and samples	
Туре	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef 3-1_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef 3-1_pH7_pos_01.cef 3-2_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef 3-1_pH7_pos_01.cef 3-2_pH7_pos_01.cef 3-3_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef 3-1_pH7_pos_01.cef 3-2_pH7_pos_01.cef	Selected files and samples	
Type	1-1_pH7_pos_01.cef 1-2_pH7_pos_01.cef 1-3_pH7_pos_01.cef 1-4_pH7_pos_01.cef 3-1_pH7_pos_01.cef 3-2_pH7_pos_01.cef 3-3_pH7_pos_01.cef	Selected files and samples	

- **Experiment Creation Wizard (Step** 5 of 11).
- unless an entry is out of sequence. b Click Next.

•	Note: This step presents you with
	the only opportunity you have to
	reorder your samples.

• The sample order shown in this step is used in subsequent views where the results are organized by sample.

order will be used the	I les, select the samples and use the appropriate buttons on the right to move samples up or down. This sample ought out the experiment. that need not be imported.
Select	Sample Name
2	1-1_pH7_pos_01
R	1-2_pH7_pos_01
R	1-3_0H7_006_01
P	1-4,540,505,01
9	3-1_p87_pos_01
P	3-2_pH7_pos_01
R	3-3_p#7_pos_01
P	3-4_pH7_pos_01

Steps	Detailed Instructions	Comments
4 Define the sample grouping with respect to the independent variables and the replicate structure of your experiment in the	a Click Add Parameter.	• In <b>Experiment Grouping</b> , samples with the same parameter values are treated as replicates.
MS Experiment Creation Wizard (Step 6 of 11).		<ul> <li>For the Malaria Demo samples you specify which files contain data from infected samples and which contain data from uninfected</li> </ul>
MS Experiment Creation Wizard (Step 6 of 11)	×	
"Add Parameter" button. You may enter as many parameters a the guided workflow. Other parameters can be used in the adv values here.	re of your experiment. Enter experiment parameters by clicking on the s you like, but only the first two parameters will be used for analysis in anced analysis. You can also edit and re-order parameters and parameter rameter(s). To change, use the button controls below.	• An independent variable is an essential element, constituent, attribute, or quality in a data set that is deliberately controlled in
	Samples	your experiment. An independent
1-1_pH7_pos_01 1-2_pH7_pos_01 1-3_pH7_pos_01		variable is referred to as a parameter and is assigned a
1-4_pH7_pos_01 3-1_pH7_pos_01 3-2_pH7_pos_01		parameter name.
1-4_pH7_pos_01 3-1_pH7_pos_01		<ul> <li>parameter name.</li> <li>The attribute values within an</li> </ul>

Add/Edit Experiment Paramete	r X
Grouping of Samples	
Samples with the same parameter samples. To assign replicate samp the samples and click on the "Assi value for the group. Set the para the parameter values as numbers	les their parameter values, select gn Values" button, and enter the meter type to 'numeric' to interpret
Parameter name Infect	ion
Parameter type Non-N	lumeric 💌
Samples	Parameter Values
1-1_pH7_pos_01	
1-2_pH7_pos_01	
1-3_pH7_pos_01	
1-4_pH7_pos_01	
3-1_pH7_pos_01	
3-2_pH7_pos_01	
3-3_pH7_pos_01	
3-4_pH7_pos_01	
1	
Assign Value.	Clear
Help	OK Cancel

- b Type a name for your Parameter name in the Add/Edit Experiment
   Parameter dialog box. Type Infection for the Malaria Demo.
- c Click your replicate **Samples** that share the same first parameter value in your data. For example:
  - 1-1\_pH7\_pos\_01
  - 1-2\_pH7\_pos\_01
  - 1-3\_pH7\_pos\_01
  - 1-4 pH7 pos 01
- d Select the **Parameter type** for your grouping. **Non-Numeric** is selected for the *Malaria Demo*.
- e Click Assign Value.

- Parameter Type options:
  - Select Non-Numeric if the grouping is not a quantitative value.
  - Select Numeric if the grouping value is quantitative or a value that reflects a degree of proportionality among the samples with respect to an independent variable. A numeric parameter type allows some data plots to be scaled by the parameter values.

Steps	Detailed Instructions	Comments
Assign Value	f Type the value for your first grouping in the <b>Assign Value</b> dialog box. For the <i>Malaria Demo</i> type Not Infected g Click <b>OK</b> .	<ul> <li>In the Malaria Demo example the samples are assigned parameter values representing the Infection parameter name.</li> </ul>
Add/fdit Experiment Parameter  Grouping of Samples  amples with the same parameter values are treated as replaces samples. To assign replace asamples when parameter values, the samples and click on the "Assign Values" button, and enter value for the group. Set the parameter type to "numeric" to in the parameter values as numbers.  Parameter name Infection Parameter type Non-Numeric  Parameter values Parameter Value I:1,0+77,pos.01 Not Infected I:3,0+77,pos.01 Not Infected I:3,0+77,pos.01 Not Infected I:3,0+77,pos.01 Not Infected Assign Value Clear  Help OK	value in your data. For example: • 3-1_pH7_pos_01 • 3-2_pH7_pos_01	<ul> <li>The highlighted samples are assigned the value typed in the Assign Value dialog box.</li> <li>You may change the value of any sample, or group of samples; highlight the sample and click Assign Value or Clear.</li> </ul>
Assign Value X Enter a value for the selected samples Infected OK Cancel	j Type the value for your second grouping in the <b>Assign Value</b> dialog box. For the Malaria data type	

💐 Add/Edit Experiment Par	rameter	×
Grouping of Samples		
samples. To assign replicat the samples and click on th	rameter values are treated as replicate te samples their parameter values, select ne "Assign Values" button, and enter the ne parameter type to 'numeric' to interpret umbers.	
Parameter name	Infection	
Parameter type	Non-Numeric	-
Samples	Parameter Values	
1-1_pH7_pos_01	Not Infected	
1-2_pH7_pos_01	Not Infected	
1-3_pH7_pos_01	Not Infected	
1-4_pH7_pos_01	Not Infected	
3-1_pH7_pos_01	Infected	
3-2_pH7_pos_01	Infected	
3-3_pH7_pos_01	Infected	
3-4_pH7_pos_01	Infected	
	1 Value Clear	
Help	OK Cancel	

- box. For the Malaria data type Infected.
- k Click OK.
- I Review your entries and grouping assignment accuracy in the Add/Edit Experiment Parameter dialog box.
- m Click OK when the grouping for this parameter name is complete.

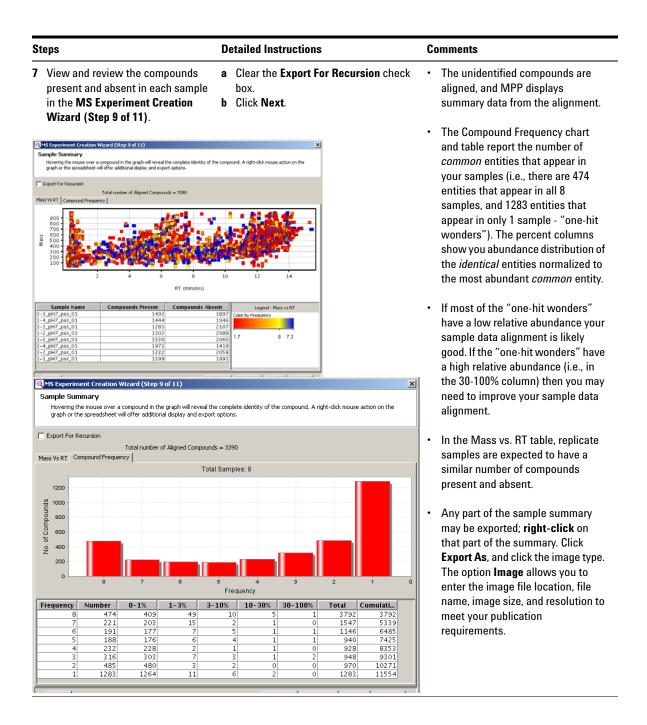
Steps	Detailed Instructions	Comments
	n Click <b>Next</b> when you have completed your experiment grouping.	<ul> <li>You can save your experiment grouping in a tab separated value (TSV) file by using the Save experiment parameters to file button 1.</li> </ul>
MS Experiment Creation Wizard (Step Experiment Grouping Experiment parameters define the grouping	ng or replicate structure of your experiment. Enter experiment parameters by clicking on the	• You can view, edit, and create TSV files using Microsoft Excel or
the guided workflow. Other parameters ca values here. Displaying <b>8</b> sample(s)	Is many parameters as you like, but only the first two parameters will be used for analysis in an be used in the advanced analysis. You can also edit and re-order parameters and parameter with 1 experiment parameter(\$). To change, use the button controls below.	<ul> <li>Windows Notepad.</li> <li>By using the Load experiment parameters from file button 3/200</li> </ul>
the guided workflow. Other parameters ca values here.	an be used in the advanced analysis. You can also edit and re-order parameters and parameter	

Steps		Detailed Instructions		Comments	
5	Select and enter the data filter parameters in the <b>MS Experiment</b> <b>Creation Wizard (Step 7 of 11)</b> .	a b c	Mark the <b>Minimum absolute</b> <b>abundance</b> check box under Abundance filtering. Type a value of 5000 <b>counts</b> . Clear the <b>Limit to the largest</b> and	•	Filtering during the data import process may be used to reject low-intensity data or restrict the mass spectral range of your data.
		d e	Minimum relative abundance check boxes. Mark the Use all available data check box under Retention time filtering. Clear the Use all available data check box and type 50 for Min Mass, and type 1000 for Max Mass under Mass filtering.	•	In Find Compounds by Formula (FbF) generated data files the term abundance actually refers to the feature volume. Whereas, in Find Compounds by Molecular Feature (MFE) generated data files the term abundance actually refers to the feature chromatographic area.
		f g h	Click Minimum number of ions and type 2 for Minimum number of ions under Number of ions. Click Multiple charge states forbidden under Charge states. Click Next.	•	Filtering by mass may improve your statistical analysis by rejecting masses that are not significant to the experiment.

• Filtering works with both GC/MS and LC/MS data.

Keep 7 of 11)	×
	v intensity data or restrict the range of data. After data is imported, requency, Abundance, Variability, Flags and Annotation. For AMDIS
Abundance filtering	
Minimum absolute abundance 5000 counts	
Limit to the largest compounds	
Minimum relative abundance %	
Retention time filtering	Mass filtering
🔽 Use all available data	🗔 Use all available data
Min RT (0.0719) 0.0719	Min Mass (50.0167) 50.00
Max RT (15.4101) 15.4101	Max Mass (2589.16) 1000.00
Number of ions	Charge states
Minimum number of ions     2	C All charge states permitted
	C Multiple charge states required
C Single ion compounds only	Multiple charge states forbidden
Help	

Steps		Detailed Instructions	Comments	
5	Select and enter the retention time and mass alignment parameters in the <b>MS Experiment Creation</b> <b>Wizard (Step 8 of 11)</b> .	<ul> <li>a Clear the Perform RT Correction check box under Retention time Correction.</li> <li>b Type 0.1% and 0.15 min for RT Window under Compound alignment.</li> <li>c Type 5.0 ppm and 2.0 mDa for Mass Window.</li> <li>d Click Next.</li> </ul>	<ul> <li>Alignment parameters are used by MPP to align and group compounds by similar mass and retention time across multiple sample files. The values you enter depend on your experimental conditions and the resolution of your MS instrument.</li> </ul>	
	MS Experiment Creation Wizard (Step 8 of 11) Nignment Parameters Unidentified compounds from different samples are aligned or gr window and the mass spectral similarity as determined by a simp	voped together if their retention times are within the specified tolerance le dot product calculation above the specified level.	<ul> <li>In general, a larger retention time shift may be used to compensate for less than ideal chromatography and a smaller mass window may be used for higher resolution mass</li> </ul>	
-6	etention time Correction		spectrometers.	
M	Perform RT correction           widmum Allowed RT Shift =         0.5         % +           ass Window =         15.0         ppm +           RT Correction Method         © Without Standards         •	0.5 min 2.0 mDa	<ul> <li>It is recommended to inspect the quality of your sample data to determine the best values for your</li> </ul>	
M	Perform RT correction           aximum Allowed RT Shift:=         0.5         % +           sss Window =         15.0         ppm +           RT Correction Method             Without Standards		<ul> <li>It is recommended to inspect the quality of your sample data to</li> </ul>	
	Perform RT correction  sofinum Allowed RT Shift = 0.5 % +  IS.0 ppm +  IC Correction Method  Without Standards  With Standards  No. of Intern	2.0 mDa	<ul> <li>It is recommended to inspect the quality of your sample data to determine the best values for your</li> </ul>	



Steps	Detailed Instructions	Comments
8 Select whether to normalize the data to reduce the variability caused by sample preparation and instrument response in the MS Experiment Creation Wizard (Step 10 of 11).	<ul> <li>a Select None for the Normalization Algorithm in the Normalization tab.</li> <li>b Clear the Use External Scalar check box in the External Scalar tab.</li> <li>c Click Next.</li> </ul>	<ul> <li>You may use normalization and external scalar techniques to reduce the variability caused by sample preparation and instrument response in your data.</li> </ul>
Kine Content Market (Step 10 of 11)	×	1
Normalization Criteria		
The compounds associated with each sample may be normalized scalar.	to an internal standard, percentile shift, quantile and/or an external	
Normalization External Scalar		
Normalization Algorithm None		
· ,	·	
Help	<< Back	
Kenter State (Step 10 of 11)	×	1
Normalization Criteria	<u> </u>	
	to an internal standard, percentile shift, quantile and/or an external	
scalar.		
Normalization External Scalar		
Use External Scalar		
Samples	Scale To Value	
1-1_pH7_pos_01	1.0	
1-2_pH7_pos_01 1-3_pH7_pos_01	1.0	
1-4_pH7_pos_01	1.0	
3-1_pH7_pos_01	1.0	
3-2_pH7_pos_01	1.0	
3-3_pH7_pos_01 3-4_pH7_pos_01	1.0	
5-1_pr/1_p05_01	1.0	
	<< Back	

- ant than compounds with lesser intensities. t sources.	<ul> <li>None: Recommended if only a few features in the samples exist.</li> <li>Z-Transform: Recommended if the data sets are very dense, i.e., with data where very few instances of compounds are absent from any sample, such as a quantitation dat set from recursion.</li> <li>Baseline to of all samples: The abundance for each compound is normalized to its selected</li> </ul>
- ant than compounds with lesser intensities. t sources.	Baseline to of all samples: The abundance for each compoun- is normalized to its selected
se options will treat all compounds equally regardless of	<ul> <li>statistical abundance across all of the samples. This has the effect of reducing the weight of very large and very small compound features on later statistical analyses.</li> <li>Baseline to of control</li> </ul>
Control Samples	samples: The abundance for each compound is normalized to its selected statistical abundance across just the samples selected a the control samples. This has the effect of weighting the compound features to a known value that is considered to be normal in the population while reducing the effect of large and small compound features.
	ilear

10 Continue to improve the quality of your results and produce an initial differential expression for your analysis. Because you chose **Analysis**: **Significance Testing and Fold Change** for **Workflow type** in the **New Experiment** dialog box you are immediately directed to the next exercise.

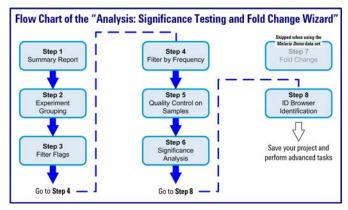
Experiment dialog box you are

the Workflow Browser.

done creating your experiment and can use the operations available in

### **Exercise 3. Perform your initial differential analysis**

The **Significance Testing and Fold Change Wizard** guides you through the steps necessary to enter parameters and values that improve the quality of your results and produce an initial differential expression for your analysis. The **Significance Testing and Fold Change Wizard** flow diagram is illustrated in Figure 2.



**Figure 2** Analysis: Significance Testing and Fold Change Wizard

eps	Detailed Instructions	Comments	
Summary Report (Step 1 of 8): Review your data in the Summary Report workflow step.	<ul> <li>a Verify that you see the Analysis:</li> <li>Significance Testing and Fold Change</li> <li>Wizard as shown below.</li> <li>b Click Next.</li> </ul>	<ul> <li>The Analysis: Significance Testing and Fold Change Wizard shows a profile plot and a series of steps to finish the experiment setup and</li> </ul>	
	X Profile Plac. CMPOURCS experiment, No. of sample(c): 8	your initial analysis. The current step is highlighted.	
ter flog ter frog (c) a savet dd Owge dd Owge ter frog dd Owge dd Owge ter frog dd Owge ter frog ter	A Sampas	<ul> <li>You do not enter analysis parameters at this time. You may review the data, change the plot view, export selected data, or export the plot to a file by a click or right-click at desired features available on the plot.</li> </ul>	

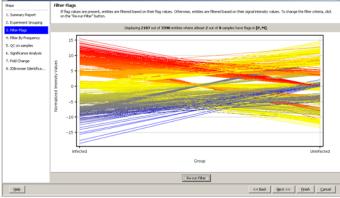
Steps		Detailed Instructions	Comments	
8): Enter the respect to the variables and structure of y	Grouping (Step 2 of sample grouping with e independent the replicate rour experiment in the Grouping workflow	<ul> <li>a Verify that your Experiment Grouping looks like the example below.</li> <li>b Click Next.</li> </ul>	<ul> <li>In Experiment Grouping, samples with the same parameter values are treated as replicates.</li> <li>In the Malaria Demo samples you specify which files contain data from infected samples and which contain data from uninfected</li> </ul>	
Workflow Type - Analysis: Signific	ance Testing and Fold Change (Step 2 of 8)	X	samples.	
Summary Report     Summary Report     Summary Report     Signifi     Signifi	ny parameters as you like, but only the first two parameters v iso edit and re-order parameters and parameter values here. icance analysis step will be skipped if there are no replicates in	your experimer. Enter experiment parameters by diskips on the "Add Parameter" button. You may enter Bis used for analysis in the guided worlflow. Other parameters can be used in the advanced analysis. You any of the condition(d), we ded and if the second parameter increases the number of conditions.	• If you are using your own data set,	
4. Filter Dy Frequency     5. QC on samples     6. Significance Analysis	Displaying & sample(s) with 1	experiment parameter(s). To change, use the button controls below.	click <b>Add Parameter</b> and set up a parameter and enter the parameter	
7. Fold Change 8. Ethnolsen Identifica 1.2. pH7 1.3. pH7 1.3. pH7 3.1. pH7 3.2. pH7 3.	pos.01 pos.01 pos.01 pos.01 pos.01 pos.01 pos.01	Crowp Uninfected Uninfected Uninfected Uninfected Infected Infecte	values for each data file and return to this display.	

- 3 Filter Flags (Step 3 of 8): Enter values that filter entities in your samples based on the quality of their presence in specified samples and conditions in the Filter Flags workflow step.
- a Review your data, change the plot view, export selected data, or export the plot to a file.
- **b** Click **Re-run Filter** to enter parameters in the **Filter Parameters** dialog box.
- c Mark the **Present** and **Marginal** check boxes.
- d Clear the Absent check box.
- e Click at least <u>out of X samples</u> have acceptable values and type 2 in the entry box. By setting this parameter to a value of two or more, "one-hit wonders" are filtered.
- f Click OK.

QFilter Parameters
Acceptable Flags
Present
🔽 Marginal
Absent
Retain Entities in which
C at least 100.0 % of the values in any 0 out of 2 conditions have acceptable values
at least     2 out of 8 samples have acceptable values
OK Cancel

- A flag is used to denote the quality of an entity within a sample. A flag indicates if the entity was detected in each sample as follows: Present means the entity was detected, Absent means the entity was not detected, and Marginal means the signal for the entity was saturated.
- This filter removes irreproducible entities from further consideration as you continue your analysis.
- A major objective of Filter Flags is to remove "one-hit wonders" from further consideration. A "one-hit wonder" is an entity that appears in only one sample, is absent from the replicate samples, and does not provide any utility for statistical analysis.

# Detailed Instructions Comments g Review the profile plot and repeat the Re-run Filter until you obtain the best results for your experiment. h Click Next.



4 Filter by Frequency (Step 4 of 8): Enter values that filter the remaining entities in your samples based on their frequency of occurrence among the samples and conditions in the Filter by Frequency workflow step.

Steps

🔍 Filter Parameters 🛛 🔀
Filtering Conditions
Retain entities that appear in at least 100.0 %
C of all samples
C of samples in only one condition
<ul> <li>of samples in at least one condition</li> </ul>
C of samples within each condition
OK Cancel
OK Cancer

- a Review your data, change the plot view, export selected data, or export the plot to a file.
- **b** Click **Re-run Filter** to enter parameters in the **Filter Parameters** dialog box.
- c Type 100 in the Retain entities that appear in at least box.
- d Click of samples in at least one condition.
- e Click OK.

 Filter Frequency allows you to filter the input data based upon the frequency with which any compound appears in each sample in the experiment. The filter is specified by typing the minimum percentage and selecting the applicable condition.

#### **Detailed Instructions** Steps Comments **f** Review the profile plot and repeat the Re-run Filter until you obtain the best results for your experiment. g Click Next. Wor ficance Testing and Filter By Frequency Entities are filtered based must pass the filter or by on the "Re-run Filter" but 1. Summary Report 2. Experiment Grouping 3. Filter Flags Displaying 917 of 2107 entities where at least 100.0 percent of samples in any 1 out of 2 conditions has flag P 4. Filter Do 15 5. QC on samples 6. Significance Analysis 7. Fold Change 10 0. IDBrowser Identifica

- Scholene Identica. Schole
- 5 OC on samples (Step 5 of 8): Assess the sample quality of your experiment using the OC on samples workflow step.
- **a** Review your data, change the plot view, export selected data, or export the plot to a file.
- b Click Next.

Workflow Type - Analys	sis: Significance Testing and Fold Change (Step 5 of 8)	×					
Steps  1. Summary Report	QC on samples Sample quality can be assessed by examining the values in the PCA plot and other exp	eriment specific quality plots.					
2. Experiment Grouping	Displaying 8 out of 8 sam	Displaying 0 out of 0 samples retained in the analysis,					
3. Filter Flags	Samples	Group					
4. Filter Dy Frequency	1-1_pH7_pos_01	Uninfected					
5. QC on samples	1-2_pH7_pos_01	Uninfected					
	1-3,pH7,pos,01	Uninfected					
6. Significance Analysis	1-4_pH7_pos_01	Uninfected					
7. Fold Change	3-1,pH7,pos,01 3-2,pH7,pos,01	Infected					
8. IDBrowser Identifica	3-3_pH7_p0s_01	Infected					
8. IDbrowser Identifica	3-4 pH7 pos_01	Infected					
	X-AKIS X-X-X-X X-X X-X X-X X-X X-X X-X X-X X-	Legend - 30 PCA Scores Color by Group I brieded Unarfeeted Decropsion Apporten: Propai Components Analysis Parameters: Column index = [1-6] Privile goton = GradintopiaComponents, [4]] Privile goton = GradintopiaComponents, [4] Sola - Tota 3.0 porters = true 3.					
Help		<< Back [gent >> [Drish] Gancel					

Steps		Detailed Instructions	Comments	
8): Asses significat your sam	ance Analysis (Step 6 of ss the differential nce and fold change of aples using the ance Analysis workflow	<ul> <li>a Review your data, change the plot view, export selected data, or export the plot to a file.</li> <li>b Move the p-value cut-off slider or type a value to make adjustments. The default value is 0.05.</li> <li>c Move the Fold change cut-off slider or type a value to make adjustments. The default value is 2.0.</li> <li>d Click Next.</li> </ul>	Significance Analysis are passed on for the Fold Change Analysis.	
Workflow Type - Anal	lysis: Significance Testing and Fold Change (Step 6 of 8) Significance Analysis		compounds that have intensity	
1. Summary Report 2. Experiment Grouping 3. Filter Flags 4. Filter Dy Frequency	Exities are filtered based on their p-values calculated from in the text box. You will not be able to proceed to the nex Test Description Selected Test : T Test unpared	In disticted analysis. To apply the new p-value cut-off, drag the "p-value cut-off" sider or input the new cut-of it step if no entities pass the filter. Ing 81 out of 917 entities satisfying corrected p-value cut-off 0.05.	values that are most           significant among the samples           in the experiment.	
Summary Report     Superiment Grouping     Fitter Flags     Fitter Dy Frequency     QC on samples     Superficience Analysis     Todd Change	$\label{eq:response} \begin{array}{ c c c c c c c c c c c c c c c c c c c$	t shp F no entities pass the filter. Ing B1 out of 917 entities satisfying corrected p-value out-off B.05.	<ul> <li>significant among the samples in the experiment.</li> <li>The Significance Analysis wizard page allows you to adjust the cutoff values for either the p-value or fold change.</li> </ul>	
Summary Report     Experiment Grouping     Filter Flags     Filter Dy Frequency     S. QC on samples	$\label{eq:response} \begin{array}{ c c c c c c c c c c c c c c c c c c c$	t dup f no entides pass the filter. rg 81 out of 917 entides satisfying corrected p-value cut-off 0.05. cont_P < 0.0050 P < 0.0000 extra cut off 0.05. cont_P < 0.0050 P < 0.0000 extra cut off 0.05.	<ul> <li>significant among the samples in the experiment.</li> <li>The Significance Analysis wizard page allows you to adjust the cutoff values for either the p-value or fold change.</li> </ul>	

### 7 Significance Analysis (Step 7 of 8): Because Fold Change was included in the prior step (Step 6 of

8), this separate Fold Change step is skipped.

• Fold Change is included in Step 6 of 11 because the significance analysis is a t-test due to the data consisting of one parameter with two conditions (parameter values).

Steps		D	Detailed Instructions		Comments	
8	Significance Analysis (Step 8 of 8): Identify the entities in your experiment using the IDBrowser	a	Review the table of compounds that remain after all of the previous filtering steps.	•	The compounds remaining are also referred to as entities.	
	Identification workflow step.		Click <b>IDBrowser Identification</b> . Continue with the next exercise.	•	The compounds are identified only by their mass and retention time in the <i>Compound</i> column of the table	

• ID Browser helps you identify the compounds in the table.

teps	IDBrowser Identificatio						
. Summary Report	To identify the Entities that	passed the fold change cut-	off with IDBrowser d	ick on the "IDBrowser Ident	thication" button.		
Experiment Grouping	Identify Entities with IDBrowser	IDBrowser Identification	1				
. Filter Flags			J (				
Filter By Frequency	Compound	p (Corr)	p	Regulation	FC (abs)	FC	Log FC
	649.9687@0.31575	4.03E-10	1.19E-11	down	16.00	-18910.66	-14.2
. QC on samples	693.9807@0.32	1.29E-03	9.68E-05	up	3.08	3.08	1.6
. Significance Analysis	433.9571@0.318	3.48E-03	2.89E-04	up	2.16	2.16	1.1
	611.9776@0.321	6.28E-04	4.65E-05	up	2.60	2.60	1.3
. Fold Change	529.9748@0.321	2.52E-03	2.01E-04	up	2.49	2.49	1.3
IDBrowser Identifica	791.9571@0.321	4.07E-04	2.98E-05	up	2.63	2.63	1.3
autor officient autor for for for	633.9917@0.31725	1.30E-11	5.66E-14	down	16.00	-34984.03	-15.0
	531.9423@0.31775	5.31E-11	6.38E-13	down	16.00	-121746.46	-16.8
	447.972@0.322	1.79E-03	1.37E-04	up	2.01	2.01	1.0
	793.9276@0.31875	3.26E-09	1.48E-10	down	16.00	-41750.41	-15.3
	479.9197@0.327	4.63E-02	5.30E-03	down	2.18	-2.18	-1.7
	217.9361@0.328	2.31E-02	2.34E-03	down	2.00	-2.00	-1.0
	397.9163@0.329	3.91E-02	4.31E-03	down	2.93	-2.93	-1.5
	\$93.8678@0.327	1.30E-11	4.54E-14	down	16.00	-101585.82	-16.0
	315.9133@0.330	1.61E-02	1.58E-03	down	3.16	-3.16	-1.0
	135.9332@0.330	1.49E-02	1.43E-03	down	2.21	-2.21	-1.3
	\$11.8639@0.33075	2.10E-12	2.28E-15	down	16.00	-43339.32	-15.4
	974.3772@0.37175	1.94E-08	1.19E-09	down	16.00	-127095.63	-16.9
	369.1682@0.3785	1.11E-05	7.85E-07	up	2.56	2.56	1.3

### **Exercise 4. Use MassHunter ID Browser to identify your compounds**

Identify the compounds that remain after applying the filtering steps of the **Significance Testing and Fold Change Wizard**.

Steps	Detailed Instructions	Comments		
<ul> <li>Set up your identification method in the first dialog box of the Compound Identification Wizard.</li> <li>Compound Identification Wizard</li> <li>Compound Identification Wizard</li> <li>Compound Identification methods you wish to apply to the comp Compound selection</li> <li>Compound selection</li> <li>Control dentification with the second selection</li> </ul>	a Review the identification method. b Click Next.	<ul> <li>When you launch ID Browser it receives the list of entities (also referred to as compounds) from the last step of the previous exercise.</li> <li>If you started ID Browser from the Workflow Browser, click <b>Run ID</b></li> </ul>		
Identity only unidentified compounds     Identity all compounds     Compound identification methods     Database search (CSV, PCD/METLIN)     Spectral library search     Molecular formulas generator (MFG)     Generate formulas for all compounds     Generate formulas only for unidentified compounds     Help     <	Cancel	<b>Browser</b> on the ID Browser toolbar.		
2 Set the parameters for your identification technique in the second dialog box of the Compound Identification Wizard.           Compound Identification Wizard           Compound Identification Notard           Compound Identification Browser           Please set parameters for identification techniques           Identification method           C: WassHunter/Methods\B.05.00\Default.m           Search Database	a Review the identification techniques. b Click Finish.	<ul> <li>ID Browser provides you with many ways to adjust the identification algorithm.</li> <li>Click <b>Help</b> to get a full description of the options and features available to set up your identification technique.</li> </ul>		
Generate Formulas Values to match C Molecular formula C Mass C Mass and retention tir	ne (retention time optional) ne (retention time required) ppm minutes << Back Next>> Finish Cancel			

#### **Detailed Instructions** Steps Comments 3 Review and adjust your **a** Review the identification technique. •

- identifications.
- **b** Click **Finish**.

ID Browser shows you information about each identified entity and gives you the ability to make adjustments to the identifications.

MS Spectrum Results		×	HS Peaks One	: + MFE Spectru	ım (0.318 min)			×	Structure Viewer		
₹ ↔ ‡  Q, ⊉   <u>n</u>		¢	m/z ≙		Abund % (Norm)	Z	Sat		No data to display.		
10 4 Cpd 8: C14 H16 N2 010 S5; 0.318: + MF	E Spectrum (0.219 min)	<b>-</b>	532.9496	37282.5			1				
532.9496	E opecialit (c.oro min)		533.9518	5115.75			1				
4- (M+H)+			534.9307	7886.5			1				
3.5-			535.9385	1223			1				
3-			1081.9154	1063			1				
2.5-			1082.9102	1043			1				
			1005.3106	1043			-				
2-											
1.5-											
1											
	1082.910	12									
0.5-	1082.910 (2M+NH4	12 ]+									
0-	(2M+NH4	}+-									
0- 500 550 600 650 700 750 800	(2M+NH4 850 900 950 1000 1050 110	}+-									
0	(2M+NH4 850 900 950 1000 1050 110 o-Charge (m/z)	)+	4								
0- 500 550 600 650 700 750 800 Counts vs. Mass-t MS Spectrum Results Spectral Difference Re	(2M+NH4 850 900 950 1000 1050 110 o-Charge (m/z)	}+-	<u>(]</u>					F			
0. 500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 séo gio gió 1000 1050 110 o-Charge (m/z) suits	)+ 		Formula			Mare V	) Ava Man	stal Stal Davi		Marciff
0- 500 550 600 650 700 750 800 Counts vs. Mass+t MS Spectrum Results Spectral Difference Re	(2M+NH4 850 300 950 1000 1050 110 o-Charge (m/z) suits   Label	)+	( Name ⊽		√ Score	7	Mass マ	Avg Mas:	s V SidDev	7	Mass (D
500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 s50 900 950 1000 1050 110 c-Charge (m/2) suite	)+ ) 		C19 H18 N6 O	1 7	77.57	649.9699	Avg Mas:	s V Std Dev	Y	Mass (D
0. 500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 850 900 950 1000 1050 110 c-Charge (m/2) suits	)+ ) ) (55) ; 0.3		C19 H18 N6 0 C23 H10 N4 0	1 7 22 6	77.57	649.9699 693.9802	Avg Mas	s V Std Dev	V	Mass (D
0. 500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 e60 s60 s60 1000 1050 110 cCharge (m/z) sufts	)+ ) S5; ; 0.3 3; 0		C19 H18 N6 0 C23 H10 N4 0 C14 H6 N6 05	1 7 22 6 53 7	77.57 66.93 74.59	649.9699 693.9802 433.9564	Avg Mas:	s ⊽ Std Dev	V	Mass (D
0. 500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 e8o 300 950 1000 1050 110 CTarage (m/z) suite ↓ Label ↓ Cpd 1: C19 H18 N6 010 2 Cpd 2: C23 H10 N6 025 3 Cpd 3: C14 H6 N6 05 S	)+ ) S5) ; 0.3 3; 0 0.321		C19 H18 N6 0 C23 H10 N4 0	1	77.57	649.9699 693.9802	▶ Avg Mas	s ⊽ Std Dev	V	Mass (D
0. 500 550 600 650 700 750 800 Counts vs. Masst MS Spectrum Results Spectral Difference Re Compound List	cbu         950         950         1000         1050         110           cruage         cruate	)+ 0 1 55; 3; 0.3 0.321 ; 0.3		C19 H18 N6 0 C23 H10 N4 0 C14 H6 N6 05 C24 H8 N2 0	1	77.57 66.93 74.59 66.68	649.9699 693.9802 433.9564 611.9779	Avg Mass	s V StdDev	T	Mass (D
0 500 550 600 650 700 750 800 Counts vs. Massh MS Spectrum Results Spectral Difference Re Compound List	(2M+NH4 8/0 9/0 9/50 1000 10/50 110 Charge (m/2) sufte ▼ Lubbel 1 Cpd 1: C19H18 N6 01C 2 Cpd 2: C23H10 NA 025 3 Cpd 3: C14 H6 N6 055 4 Cpd 4: C24 H6 N2 018; 5 Cpd 4: C24 H6 N2 018; 5 Cpd 5: C13 H22 0125	)+ ) (55):- ; 0.3. 3; 0 0.321 ; 0.3. 0.322		C19 H18 N6 0 C23 H10 N4 0 C14 H6 N6 05 C24 H8 N2 0	1 7 22 6 53 7 18 6 55	77.57 66.93 74.59 66.68	649.9699 693.9802 433.9564 611.9779 529.9713	Avg Mass	s 🛛 SidDev	V	Mass (D
o 500 550 600 650 700 750 800 Counte vs. Maset MS Spectrum Results Spectral Difference Re Compound List Cpd	(2M+NH4 e60 s00 s60 1000 1050 110 eCharge (m/z) stults 7 2 2 Cpd 2: C19 H18 N6 010 2 2 Cpd 2: C13 H10 N4 022 3 Cpd 3: C14 H6 N6 055 4 Cpd 4: C24 H8 N2 018; 5 Cpd 5: C13 H2 2012 55 6 Cpd 6: 2 C	)+ ) (1) (2) (3) (3) (3) (3) (3) (3) (3) (3		C19 H18 N6 0 C23 H10 N4 0 C14 H6 N6 05 C24 H8 N2 0 C13 H22 012	1 7 22 6 53 7 18 6 55 5 4 7	77.57 36.93 74.59 36.68 80	649.9699 693.9802 433.9564 611.9779 529.9713 791.9571	Avg Mass	s 文 StdDev	7	Mass (D
0 500 550 600 650 700 750 800 Counts vs. Mass-t MS Spectrum Results Spectral Difference Re Compound List	cb         900         950         1000         1060         100           charge (m/z)         suite         -	)+ 0 1 55: 3; 0 0.321 0.322 0.322 016 55:		C19 H18 N6 0 C23 H10 N4 0 C14 H6 N6 05 C24 H8 N2 0 C13 H22 012 C20 H15 CI N	1 7 22 6 53 7 18 6 55 4 7 1 7	77.57 36.93 74.59 36.68 80 76.85	649.9699 693.9802 433.9564 611.9779 529.9713 791.9571 633.9889	Avg Mass	s V StdDev	7	Mass (DE

- **4** Accept the current identifications and return to Mass Profiler Professional.
- a Click Save and Return on the ID Browser toolbar.
- ID Browser sends all of the information it has generated back to your entity list in Mass Profiler Professional.

🔍 Agilent MassHunter ID Browser B.05.00	
Eile Edit View Identification Method Configuration Help	
🗄 🍤 🗸 🔁 🚽 🕟 Run ID Wizard 🔟 🗰 🗞 Jili 🕼 Save and P	Return
III MS Spectrum Results	Save and Return
	¢

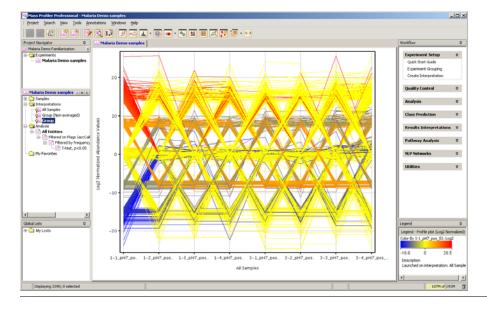
Steps De		Detailed Instructions	Comments
	the results in Mass Professional.	<ul><li>a Study the compounds that are nov identified in the compound table.</li><li>b Click Finish.</li></ul>	<ul> <li>The compound column in the table shown in Significance Analysis (Step 8 of 8) is updated with your identifications from ID Browser.</li> </ul>
Workflow Type - Anal	lysis: Significance Testing and Fold Change (Step 0 of	0)	X
Steps	IDBrowser Identification		
1. Summary Report	To identify the Entities that passed the fold change	ut-off with IDBrowser click on the "IDBrowser Identification" button.	
2. Experiment Grouping	Identify Entities with IDBrowser DBrowser Identification		

iter Flags	Compound	p (Corr)	p	Regulation	FC (abs)	FC	Log FC
iter By Frequency	C19 H18 N6 010 SS	4.03E-10	1.19E-11	down	16.00	-18910.66	-14
C on samples	C23 H10 N4 022	1.29E-03	9.68E-05	up	3.08	3.08	
and the second second second	C14 H6 N6 05 S3	3.48E-03	2.89E-04	up	2.16	2.16	
gnificance Analysis	C24 H8 N2 018	6.28E-04	4.65E-05	up	2.60	2.60	
old Change	C13 H22 O12 S5	2.52E-03	2.01E-04	up	2.49	2.49	
	791.9571@0.321	4.07E-04	2.98E-05	up	2.63	2.63	
Browser Identifica	C20 H15 CI N4 01	1.30E-11	5.66E-14	down	16.00	-34984.03	-1
	C14 H16 N2 010 S5	5.31E-11	6.38E-13	down	16.00	-121746.46	-1
	C14 H12 N2 09 S3	1.79E-03	1.37E-04	up	2.01	2.01	
	793.9276@0.31875	3.26E-09	1.48E-10	down	16.00	-41750.41	-1
	C12 H8 N4 09 S4	4.63E-02	5.30E-03	down	2.18	-2.18	
	C3 H6 O5 S3	2.31E-02	2.34E-03	down	2.00	-2.00	-
	C10 H3 CI 015	3.91E-02	4.31E-03	down	2.93	-2.93	-
	C16 H7 CI N4 011	1.30E-11	4.54E-14	down	16.00	-101585.82	-1
	C6 H8 N2 O3 S5	1.61E-02	1.58E-03	down	3.16	-3.16	-
	135.9332@0.330	1.49E-02	1.43E-03	down	2.21	-2.21	-
	C12 H5 CI N4 09 S4	2.10E-12	2.28E-15	down	16.00	-43339.32	-1
	974.3772@0.37175	1.94E-08	1.19E-09	down	16.00	-127095.63	-1
	C13 H23 N9 02 S	1.11E-05	7.85E-07	up	2.56	2.56	

- 6 Review the profile plot, the Analysis: Significance Testing and Fold Change Wizard is now complete.
- **a** Review the profile plot.
- **b** Click on any entity list in the Experiment Navigator to review the results.

You can follow the results from your analysis in the hierarchical presentation in the Experiment Navigator on the left sidebar.

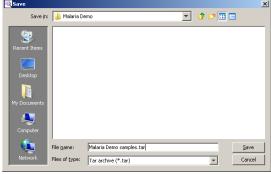
•



### **Exercise 5. Save your project**

Save your current analysis as a TAR file for archiving, restoration of any future analysis to the current results, sharing the data with a collaborator, or sharing the data with Agilent customer support.

Steps	Detailed Instructions	Comments			
Export your project to a TAR file.	<ul> <li>a Click Project &gt; Export Project.</li> <li>b Mark the check box next to the experiment you wish to save</li> <li>c Click OK.</li> </ul>	<ul> <li>You have completed creating you project and analyzing an experiment. It is recommended to archive your progress by exportin your project.</li> </ul>			
🝳 Choose Experiments	×				
Select experiments to export with the project. All the experiment will be exported and can be imported la Malaria Demo samples					
Help	OK Cancel				
	d Select or create the file folder.				
	e Type your file name in File name. f Click Save.				
Save in: Malaria Demo					







## **Advanced Tasks**

The operations available in the Workflow Browser provide the tools necessary for analyzing features from your mass spectrometry data depending upon the need and aim of the analysis, the experiment design, and the focus of the study. This helps you create different interpretations to carry out the analysis based on the different filtering, normalization, and standard statistical methods.

It is recommended that you follow the procedures in the *Mass Profiler Professional Software - Application Guide* for an additional level of detailed information to help you use MPP with your data.

The *Metabolomics Discovery Workflow* and *Mass Profiler Professional User Manual* provide you with techniques and explanations to perform advanced analysis tasks.

### **BioCyc Pathway/Genome Databases**

Includes BioCyc Pathway/Genome databases from the Bioinformatics Research Group at SRI International<sup>®</sup>, used under license.



http://www.biocyc.org/

### Citation based on use of BioCyc

Users who publish research results in scientific journals based on use of data from the EcoCyc Pathway/Genome database should cite:

Keseler et al, Nucleic Acids Research 39:D583-90 2011.

Users who publish research results in scientific journals based on use of data from most other BioCyc Pathway/Genome databases should cite:

Caspi et al, Nucleic Acids Research 40:D742-53 2012.

In some cases, BioCyc Pathway/Genome databases are described by other specific publications that can be found by selecting the database and then going to the Summary Statistics pages under the Tools menu. The resulting page sometimes contains a citation for that database.

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## In this book

The Agilent G3835AA MassHunter Mass Profiler Professional Software -Familiarization Guide presents the functionality of Mass Profiler Professional using a "Malaria" project containing an experiment called "Malaria LCMS ESI+ pH 7."

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